Computer-animated scientific visualizations of tomographic scanned microscopic organic entities

Computeranimierte Scientific Visualizations von tomographisch gescannten mikroskopischen organischen Entitäten

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Ich erkläre hiermit, dass ich die Dissertation selbständig verfasst und keine andere als die angegebenen Quellen und Hilfsmittel benutzt habe, sowie dass diese Dissertation bisher weder im In- noch im Ausland in irgendeiner Form zur Beurteilung vorgelegt wurde.

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Kurzfassung

In dieser Arbeit werden verschiedene technische Lösungsansätze zur Erstellung von Wissenschaftsvisualisierungen mikroskopischer Entitäten vorgestellt. Die untersuchten Beispiele sind sowohl in der Wissenschaftsvisualisierung, als auch in der Computeranimation Spezialfälle. In der Computeranimation ist es nicht üblich, dass tomografisch gescannte 3D-Modelle Einsatz finden, während es in der Wissenschaftsvisualisierung außergewöhnlich ist, jedes digitale Modell einzeln zu bearbeiten und mit Knochengerüstsimulationen zu animieren.

Deswegen soll hier hinterfragt werden, ob diese zeit- und ressourcenintensiven Arbeitsschritte durch die Generierung von zusätzlichem Mehrwert zu rechtfertigen sind. Technische und theoretische Probleme, die den Untersuchungsgegenstand betreffen, wurden zunächst in einer allgemeinen Beschreibung der Bearbeitungsschritte behandelt und anschließend in der Analyse von drei Fallstudien praktisch untersucht. Die Projekte *Two mite gaits*, *CRISPR/Cas9-NHEJ: Action in the nucleus* und *Noise aquarium* wurden beschrieben und diskutiert, um die Angemessenheit der vorgestellten Praktiken zu hinterfragen.

Dies führte zu dem Ergebnis, dass die vorgestellten Pipeline-Prozesse ein zu förderndes Mittel sind, um dreidimensionales, visuelles und zeitabhängiges Denken zu unterstützen. Zusätzlich sind die einzelnen Projektergebnisse in einer visuell orientierten Gesellschaft von erheblichem Nutzen. Die Untersuchung hat ergeben, dass die Authentizität, die durch das vorgestellte Verfahren entsteht, einen großen Mehrwert für Computeranimationen mit wissenschaftlichen Inhalten bringt und daher oftmals hochgeschätzt wird. Folgeuntersuchungen und zukünftige Projekte im hier vorgestellten Subfeld der „Computer-Animated Scientific Visualization“ sollen daher entschieden weiterverfolgt werden.

Abstract

In this thesis, unique techniques for creating scientific visualizations of microscopic entities are presented. The investigated cases are outstanding for the field of scientific visualization as well as for computer animation. Computer animation usually does not include tomographic scanned 3D models, while in scientific visualization it is exceptionally rare to edit every model individually and animate using rigged character computer animation configurations.

Therefore, it was an open question whether the newly introduced time and resource consuming workflows generated sufficient value to justify the effort. Firstly, technical and theoretical problems concerning the subject matter were addressed in a general pipeline description and subsequently investigated with the analysis of three case studies. The projects *Two mite gaits*, *CRISPR/Cas9-NHEJ: Action in the nucleus*, and *Noise aquarium* form a basis to discuss and scrutinize the reasonableness of the practices introduced.

It was found that the presented pipeline steps support important aspects of three-dimensional, visual, and time-dependent thinking. The individual project results are of mind-expanding additional value in a visually oriented society. Furthermore, the extra processing adds highly appreciated authenticity to computer animations involving scientific topics and therefore encourages future investigations and projects within the suggested subfield of computer-animated scientific visualizations.

Chapter 1

Introduction

This thesis deals with microscopic organic entities in scientific visualizations by analyzing three case examples which were the results of projects completed in the last five years. All three projects share the common starting point "data". The main data was acquired through tomographic scanning and the implementation was performed using computer animation. The preparation of the data for computer animations follows an established pipeline, however, the detailed editing steps and techniques to transfer multiple data sets of the here presented organic entities include many new facets which have never been done before as in the projects described here. The applied scanning techniques vary, yet, all the primary input information can be described as volumetric data. Therefore, the main interest of this thesis lies in the methods of transferring volumetric scientific imaging data to computer animations with a focus on the specific cases.

All three case examples are grounded in the field of biology, while the discussion focuses on microscopic organic use cases. However, the outcomes of these projects are not exclusively produced for academia in the field of biology, instead, primarily designed for a broader audience. Furthermore, the projects are not typical representatives of scientific visualizations. The majority of scientific visualizations use scientific data to communicate scientific findings in an automated way and do not utilize the techniques of computer animation. Also, data visualizations that are produced with individually designed styles and prepared for consumption by a larger audience are a rarity. These circumstances might be grounded in the fact that an individually designed computer animation which incorporates scientific imaging data is expensive, both in the complexity of the production process, as well as regarding allotted budget. Computer animation is often employed to convey knowledge and scientific contents in an effective manner. Nevertheless, the joining of computer animation with rigged characters and scientific imaging is rare. This is a reason why this issue should be interrogated in this thesis. The questioning as a driving motif and resulting from the lack of scientif-

ically data-rich computer-animated scientific visualizations, is whether or not it is reasonable to pursue such efforts, and in which cases. This questioning includes discussion about the influence of scientific visualizations on the perception of the reality of scientific findings, although the discourse is foremost executed with the specialized, concrete case examples of this thesis.

After the introduction of terms used and techniques applied, a more general description of the pipeline to transfer primary volumetric, as well as additional scientific imaging data sets into computer animations, is introduced. Then, the three case examples which are the main subjects of interrogation are recorded and analyzed. The detailed implementation is deconstructed for every 3D model of each project separately.

The first project consists of quantitative and qualitative motion studies of the gaits of two different mite species. The project deals with species often neglected by the general public. An extended examination of the subjects, especially their movements, are performed while analyzing and depicting these soil organisms in unique ways.

The second project analyzed is called *CRISPR/Cas9-NHEJ: Action in the nucleus* and deals with the depiction of a gene-editing process in a cell nucleus. For that, data models of macromolecules were animated to convey this complex process which occurs in the nanometer size range.

The third case example is an animated aquarium full of plankton and plastic. The project is an environmental art project to create awareness for pollution in the world's water bodies. It features authentic 3D models of plankton organisms.

The main motivation for this thesis is to visualize entities that are barely or completely invisible to the naked eye using innovative methods. Humans only have rudimentary knowledge about what is going on at microscopic scales, and as a result, are only able to watch these worlds through the use of specialized equipment, experimental data, and their subsequent visualizations. In using tomographic imaging to acquire 3D models, biological imaging can be extrapolated using computer animations to help improve visual thinking of the ongoings in these "microworlds". From the macro size all the way down to the angstrom level—fascinating complexity is to be found everywhere. With computer animation, scientists are able to capture these details and process them for a better holistic understanding of our surroundings. A dive ever deeper into specific details is enabled, thereby providing a glimpse into every structure even down to the atoms.

Scientific imaging technologies are vital research tools for various life sciences. Across all size scales, imaging data is used for state-of-the-art research. One of the starting points for this thesis was the aim of expanding the list of possible applications for detailed scientific imaging. This augmentation of the visualization of the microscopic scale in connecting it with various information, transforming it and particularly showing the data as computer animations, is a crucial objective of the herein presented example

projects. An array of possibilities opens up, to which this text explores how they can be further developed.

Based on the recorded technical implementation of the transfer of tomographic scanned data, including secondary images generated by scientific imaging, along with the corresponding movement data, an analysis and discussion about the primary question is conducted. For all case studies, information regarding raw data sets, as well as the preliminary and final project results is available in this thesis. An in-depth exploration of the making of project examples along with the underlying data sets allows an insight into the pros and cons of using scientific imaging techniques to create computer-animated scientific visualizations. This investigation also leads to a comparison of the introduced tomographic scanned model pipeline in contrast to modeling according to references.

The advancement of knowledge here lies on the one hand in the description and implementation of newly adapted techniques to create animations of organic microscopic entities. On the other hand, their use and meaningfulness are scrutinized and the results are summarized.

There are countless animations of static scans which are created directly by imaging departments of various research facilities. However, animations according to the definition of computer animators using deformation simulations of the 3D model parts, that is, using rigging and skinning, as well as different forms of technical and artistic presentation formats, have not yet been implemented in the here discussed way using the selected microscopic entities in the case example projects.

In particular, the approach using exclusively open-source tools offers an additional, unique feature set and added value for user communities who are interested in incorporating tomographic scanned data in computer animations. The results are provided as reference as well as a basis for discussion.

The description of project-specific workflows is a comprehensive overview of the steps that sometimes required multiple attempts in order to achieve the final desired result. Discussions regarding the application and meaningfulness of computer-animated scientific visualizations will be continued respective to the processed individual projects in the context of existing literature.

In the primary investigation of this thesis, the computer animation implementations of nine microorganisms and twelve protein structural models, all of which were created using scientific visualization methods, are recorded in protocol-like sections. The research introduces a tool for preproduction decisions and especially whether or not it is reasonable to take similar approaches in other project examples.

The topic addressed is important to the field of scientific visualization because it opens up new interdisciplinary opportunities between the different fields of biology, microscopy, and computer animation. Projects such as the herein described ones are a step towards utilizing the full scope of

possibilities for interconnecting these fields.

The application of tomographic scans is a common tool in biology, yet few pieces of research in the field take on the challenge of employing computer animation techniques in order to include more detailed motion information in their renderings. Scientific visualization is the area of computer graphics that illustrates or explains scientific content or outcomes in visualizing data. The main hypothesis here, therefore, is that the more scientific imaging methods and individually designed visualizations are incorporated to create scientific visualization renderings, the more the informative value is increased for various audiences.

An interest in the presented approach exists in various possible application areas such as documentaries, museums, edutainment, art, or expanded scientific visualization. Important application use cases may be found in biology using models especially in zoology. Computer animations may make important contributions towards the detailed description of species, including their motion data as, for example, the later-described attempt to compare the gaits of mites.

In dealing with entities that are invisible to the naked eye, new perspectives may emerge which include an evolved environmental awareness. Persons that are interested in the implementation of projects authentically representing the micro and nano worlds in computer-animated scientific visualizations are the main target group for this text. This primary target audiences are researchers in biology, computer animation, and scientific visualization who are interested in microscopic organic entities.

Chapter 2

Background

Nothing is simple. Complexity is everywhere, when we take a closer look. Only because we humans see something simple, it has not to be, it is only reduced to our cognition capabilities. We tend to underestimate everything that is in our perception small and silent. Although, a source of important knowledge comes from outsider organisms, research tends to focus on the huge, the famous or the striking ones. Of course, this has also practical reasons, as tiny unobtrusive life is harder to capture and research. Digital models and computer animations help to bring these lifeforms into human perception. Computer-animated scientific visualizations will help a lot to gather more knowledge about these essential, yet unseen microscopic entities of our life realities.

In the following subchapters, the main concepts behind the creation of computer-animated scientific visualizations that are created out of tomographic scanned data will be introduced. The topics of the subsections deconstruct and define the main title and give background information to scientific visualizations, scientific imaging and the methods applied in the case studies, micro-tomographic 3D scanning, and the digital depiction of microscopic organic entities.

2.1 Scientific visualizations

In the following section, computer-generated animations in scientific visualizations will be discussed. Stylized computer animations, in the sense of traditional animation, for instance as described in [109], of any type, will be not thematized further. Nevertheless, the title “Computer-animated scientific visualizations” wants to clarify that the animations discussed here in this thesis are animated with techniques of computer animation, hence are more elaborate than simple, over time rotated animations of static 3D models. The application of deformations of 3D models through digital bone system deformations and other user guided simulations should be empha-

sized with the first four words of the title of this text. This is important for the further investigation because the use of simple turnaround and cut through animations in combination with tomographic scanned entities is very common in natural sciences and therefore not part of the discussion in this thesis.

In most of the techniques to transfer motion data to 3D models, a certain amount of human interaction is still needed. Yet, the artistic craft of an animator that does stylized computer animations is something completely different and is to be seen separate from the transfer of motion described in section 3.5.

Scientific visualizations are often perceived as a subcategory of computer sciences. For instance, the ACM (Association for Computing Machinery) lists "scientific visualization" twice as a topic in their classification system[1]. Scientific visualization is classified as a subcategory of "human-centered computing" as well as in "computing methodologies" under "simulation types and techniques". Therefore it is already implied that scientific visualizations are produced with computers. In contrast to scientific illustrations, which are two-dimensional drawings of scientific content, which to this day are either analog or digital. Already the first computer animation by Edward E. Zajac[2] was a scientific visualization of a simulation of how satellites might be stabilized when they orbit earth. In the accompanying paper [221], it is presented as useful to render three-dimensional representations in movies as scientific tool.

One of the main forces in pushing the development of computer graphics was and still is to achieve more insight into complex data sets. The problem of dealing with realistic complexity led to the use of computer graphics and computer simulations in science applications [71].

According to [39], the term "visualization" is used in the fields of computer graphics and image processing since their report in the *Visualization in Scientific Computing* journal in 1987. Even back then, it was already thought of computer graphics as more than just a technology. Digital graphics were seen visionary as a form of communication that transcends use cases and technical restrictions and should be used for research, understanding, communication, and teaching.

More generally, scientific visualization is the digital depiction of all kinds of research data. That data may come from various scientific disciplines and research methods. Ideally, knowledge is created through visualizing data and the process encourages thinking that is not separated into perception and cognition, like [10] found in his widely renowned book titled *Visual Thinking*. Numerous past inventions were possible because of visual thinkers, as [27, p. 20] summarizes:

[1] https://dl.acm.org/ccs/ccs.cfm; 05/04/2018.

[2] https://www.youtube.com/watch?v=RocLdMyUG-4; 18/01/2019.

> The history of science and technology is full of discoveries in which visual thinking played a critical role. Visual thinking from the abstract to the concrete is a powerful strategy. In abstraction, the thinker can readily restructure even transform a concept. Then, the resulting abstraction can be represented in a concrete form and tested in reality. When abstract and concrete ideas are expressed in graphic form, the abstract-to-concrete thinking strategy becomes visible.

Visual thinking is key to innovation, as [174, p. 17] states:

> In the case of the visual arts, in addition to illuminating, imitating, and interpreting reality, a few artists create a language of symbols for things for which there are yet to be words. (...) radical innovations of art embody the preverbal stages of new concepts that will eventually change a civilization. Whether for an infant or a society on the verge of change, a new way to think about reality begins with the assimilation of unfamiliar images.

There is an increase in scientific visualization applications due to the ever increasing availability of computer calculation power to broader audiences, such as smaller studios and institutions who are able to handle more and more data. Cinematic as well as stylized renderings for scientific visualizations are widely used to convey information. Sometimes this is criticized. The difference between visualizations and scientific visualizations is that "scientific visualizations" incorporate scientific data of various origins, while a "visualization" represents something that may be scientifically correct, but has not to be based on data. Still, we have to be aware that there are different approaches to defining the reality of "scientific data" and the ways in which the scientific data ought to be interpreted is not fully defined.

Transformations are unavoidable in scientific visualization. First information is collected, then data might get enriched and enhanced, followed by an information mapping processes and finally the rendering process to obtain displayable pictures is executed [71].

Therefore, within scientific visualization it is expected that the final imagery is an authentic reflection of the processes or theories thematized [178].

Transformations of data should be undertaken with care. Not only are transformations a part of every scientific visualization workflow, but abstraction is also often a necessity, as [27, p. 21] states:

> Complex thinking operations often require imagery that is abstract and Gestalt-like. This is not that abstract imagery is more important than concrete; rather, abstract and concrete imagery are complementary. A flexible visual thinker can move readily back and forth between the two.

In general, visualizations have a long tradition of being a human endeavor– since several thousands of years [35].

Long before the term was defined, images helped humans to think. Therefore image-based representations of scientific research are crucial. There are numerous reasons to create scientific images, as for instance, [50, p. 1] lists:

- As research becomes more interdisciplinary, illumination and intelligent images of your research will become more useful in communicating with those scientists outside your field of expertise.
- Using compelling and accessible pictures is a powerful way to draw the public's interest to the world of research. When the public develops a more intimate association with science the results will be both a richer society and one supporting the important efforts in scientific investigation.
- As you spend more time making these new images of your research to communicate to a larger community, you will see your work differently; you will expand the way you think about your work and therefore the way you envision it.

The knowledge of humankind is expanding and nobody can know everything anymore. It was easier to have an overview of scientific knowledge in the middle-ages or even before 1900 than is the case today. Still, comprehension and knowledge are key and only an educated society can prosper by our means of understanding. So-called "Renaissance Teams", like Donna Cox described in [36], are the geniuses of our time, the times of one individual who "knows it all" are over.

Complex theories and abstract concepts are driving the latest scientific findings. Visualizations can help in understanding and enable faster learning of new concepts. Scientific visualizations are means of human knowledge creation and transdisciplinary as well as interdisciplinary tools.

Scientific visualizations provide visual metaphors and narratives to shape cultural thinking and offer people a scientific view of reality [36].

The goal of scientific visualization should be a deeper level of comprehension of data and the underlying processes with the help of human's ability to visualize [43, p. 5].

Scientific visualization is not trivial and often requires new visualization pipelines, design solutions, and a functional collaborative team. Every project has different constraints and offers new possibilities.

According to [117] the main challenges in creating scientific visualizations are to maintain scientific integrity, by preserving the scientific data while establishing the visualization, the aesthetics, and presenting a narrative that enables the possibility to follow complex information. Framing scientific data as a narrative makes it more interesting and memorable, as

it is easier to remember episodic events rather than sequences of unrelated entities. These narratives are often created to validate scientific experiments, to explore data sets or to communicate findings to other scientists. There should be an interesting context for the target audience. The main narrative impact in scientific visualizations comes from the possibility to see real data that are normally invisible, in which case, scientific visualization acts as an augmentation of the human sensory system.

Impossible scales, perspectives, and time frames may be made visible. Scientific visualization strives for realism and accuracy, details should only be sacrificed to facilitate understanding. Generally, the involved scientists set 'the stage' for the visualization and the 'protagonists' are data items of various sources. Communication between all parties involved is important for the collaborative process.

Visual representations and aids to use the 'Global Information Structure' were already discussed at the beginning of the 1990s. The still nascent Internet was already seen as a benefit and challenge for information distribution through computer graphics and visualizations. In a white paper [59] announcing the usage of information as one of a nation's most critical economic resources this was already emphasized. Research in computer graphics, interactive techniques, visualization, imaging, and design applications were seen as vital for making information sources easily accessible and were acknowledged as useful in promoting and fostering scientific, industrial, and educational collaborations.

Scientific visualizations often show something not visible to the naked eye. It has always been a core task of educational institutions to make invisible things visible. Processes that are imperceptible, not experience-able, unintelligible with the human sensorium, can be made visible, tangible, through scientific and artistic methods apparent, hence finally better understandable [14, p. 1].

This invisible something might be, for example, data correlations or in the case of this thesis, structures and movements of microscopic entities.

According to [179, p. 2], computer graphics, that means also scientific visualization, lack in a comprehensive history because the subject is difficult to approach entirely.

A reason might be the heterogeneous nature of the different backgrounds of artists and scientists who were pioneering in computer graphics. The first computer animation system and the starting point of the multi-billion dollar industry of today were described by Ivan Sutherland in his doctoral thesis about his Sketchpad (cf. [188]). From there on, computer graphics, graphical user interfaces, object-oriented programming languages, and animation evolved, first in university and military facilities because very expensive and large computers were only available there.

Moving and visual innovations can be created with ongoing developments in technology, because of, as put forth by [179, p. 1] the invention of:

> (...) computer graphic imaging, CGI, or just CG, as the artistic edge of the information age, the arts, and entertainment front of the digital revolution.

Most scientific visualizations are a combination of art and science. Of course not every project or piece is artistic or scientific at the same level. For the most straightforward data visualization, someone needs to overcome at least a minor design decision. Also, as the difference between scientific visualization and any other kind of computer animation is the transformed representation of scientific data, the line between "information visualization" as a genre and "scientific visualization" is blurry. While information visualization is transforming data mostly into more or less creative charts, scientific visualization does this transformation process into creatively not limited visual representations. Information visualizations (for more information see [80]) usually depict in graphs and not graphics.

According to [35], data visualization is a broad umbrella term for both information and scientific visualization. In general, data visualization describes computer-related technologies that transform data into a visual model. Data is quantitative information that can have its' origins in all kinds of research. Data visualizations are digital visual metaphors.

In the following, characteristics of these digital visual metaphors out of [35, p. 92] are summarized, because they are a detailed starting point for a reflection about scientific visualizations:

- Digital visual metaphors have a least two parts: a target domain and a source domain.
- They should provide an understanding of the target and the source domain.
- The target and source domains form a concept network.
- These concept networks include difficult to measure parameters like beliefs, concepts, technologies, culture, assumptions, understanding,...
- Data and characteristics should be mapped from the source domain to the target visualization.
- This is not a one-to-one mapping, although...
- ...the mapping has to make sense.
- The mapping enables new meanings through possible new associations and contributes to the target domain concept network.
- Some digital visual metaphors become conventional and embedded into cultures.
- There is a range of established visual metaphors to very creative, new metaphors and the audience interpretation strongly depends on context and communication settings.

When it comes to the question, what exactly is scientific data, a more controversial field of discussion opens up. In the case of the latter in the text analyzed case studies, this data is clearly defined. It comes primarily from tomographic operating scientific imaging techniques in addition to secondary scientific imaging techniques.

Data for scientific visualizations may come from various sources. From scientific simulations of the current ongoings in the universe of physicists, to molecular structures in biochemistry, every data set can theoretically be visualized. Frequently, in natural sciences, these data sets are also density information – such as the main data sets for the case studies described later. When it comes to "big data", human brains can not even comprehend the meanings without some sort of (visual) structuring.

Expanding the sources of data, one might go even further and include all scientific data into the possible sources for scientific visualizations. While in the natural sciences, visualizations are more common, the use in humanities is rare, even though there are data sets of, for example, comparisons of theories or definitions and their arguments. The interpretation and transformation of this, for instance, philosophical data could occur in the same way as is common with the outcomes of experimental physics experiments. The data in natural sciences are derived from simulations and measurements, scientific data in the humanities must often be gathered in a different manner.

In [35, p. 93], a broad sense of 'data' was introduced including:

> (...) systems of numbers, mathematical and scientific models, observations, statistics, assumptions, instrument recordings, and other data (...).

In all the scientific disciplines, the human influence of the researcher is not to be ignored. Without going further into science criticism in this background introduction, it should be mentioned here that there are important relationships between the human perception of the world and scientific facts. The sciences sometimes undergo paradigm shifts and the generation of scientific data cannot be completed without being influenced by the humans who collect them. The challenge of the human factor as an influence on human knowledge production is therefore of great importance to scientific visualization as part of human knowledge production and comprehension.

As already mentioned, scientific visualization is a subfield of computer graphics and if it contains moving images, also of computer animation. The representatives of this comparatively young field see themselves as technicians (programming, coding, hardware specialists), scientists (computer sciences and other sciences), artists (digital art, digital illustration), crafts persons (numerous very specialized crafts persons who are referred to in the film and games industry as 'artists'), designers, or as some hybrid of some or all of these descriptions of professions. That is why, some scholars put computer graphics and animations into a very special position when it comes to

the ‘art and science debate’. Computer-generated images are seen as a possible reunification force between all the different academic disciplines. The “two cultures” [183] debate was academically presented in 1959 and has continued ever since. The split of the arts and the sciences that happened after the renaissance is still present and to question, according to [36], and might be a hindrance for humanity due to mind restricting specialization [54, 33ff.].

People performing creative research using digital technology can be seen as ‘in-between’ and as mediators. As Victoria [203, p. 122] puts it:

> Artists using technology are uniquely positioned in the middle of the scientific and literary/philosophical communities and are allowed a poetic license, which gives us the freedom to reinforce the delicate bridge and indeed contribute to the creation of a new, mutant third culture.

Scientific visualizations are media with roots in numerous historical disciplines. Both 2D and 3D computer graphics are increasingly used in various fields of science and scientific illustration. As a result, they are increasingly being used to visualize scientific content.

Computer graphics have changed the way science is visually represented, in the same way that the development of print has done earlier in history [87, p. xi].

The application of computer-animated and generated images in biology ought to be a progression of historic visualization methods such as drawings and illustrations.

Although, traditional techniques are still used, see for instance [87], and have their own intriguing language and aesthetics, the technique of computer-generated images is available and may be used to enhance the overall representation of research outcomes, especially, but not only when motion is added.

Advances in technology have opened up new possibilities for interactive and three-dimensional imagery. Techniques like scanning electron microscopy, magnetic resonance imaging, computed tomography, confocal laser scanning microscopy, micro X-ray computed tomography, Cryo tomography, and protein X-ray crystallography revolutionized collection-based studies by acquiring morphological data of organic and inorganic material. An increasing number of even extinct specimen studies employ volume data sets. The enabled visibility of important anatomical characteristics leads to the discovery of new species as well as the potential to find problems in taxonomic descriptions. Therein, “cybertaxonomic infrastructure” such as online databases are becoming essential for the classification and description of new species [56].

Knowledge and attention may be created and merged easier than ever before. With scientific visualization, a beneficial tool for every discipline is available. The more researchers in natural sciences, humanities, technology,

and the arts are open to using these tools, the more they may benefit from visualization to support their knowledge creations, regardless of type.

Visualization enables insight into diverse fields like medicine, space and earth science, physics and biology. As a method of computing, it transforms the symbolic into the geometric. The visual output enables scientists to observe their results and data. Effective visualizations leverage existing scientific methods, compare the preface of [76].

As a summary of his motivation to produce scientific visualizations, Alfred Vendl stated in [14, p. 2] as follows:

> Scientific visualizations make the invisible visible because there is an infinite amount of things around us that we humans can not directly perceive with our eyes. For example, if events happen too quickly for our perception of time and we first have to slow them down accordingly. Or, if they run too slowly and we have to accelerate them accordingly to be able to perceive them. The direct perception of too large spatial dimensions is only possible after a corresponding reduction. (...) Above all, however, we miss the universe of tiny micro worlds.

Especially, the last sentence of this citation, translated from German, introduces the microscopic world as an intriguing subject well-suited for scientific visualizations. All three case studies in chapter 4 deal either with the micro worlds of plankton and soil organisms, or protein macromolecules which are only nanometers in size.

2.2 Applied scientific imaging techniques

In general, scientific imaging is the acquisition of images via any type of device for scientific purposes. The resulting scientific image might be edited or not. Every digital image is more or less processed due to the quantification in the digitizing process itself.

While a certain grade of processing is often necessary to unravel the full expressiveness of the scientific image which might be important for the results of a scientific work, thoughtless, undocumented alterations of the visual data are unethical [168, pp. 5–10].

Already as early as in the 18^{th}century, work ethics in microscopy were a topic addressed by Henry [13, p. 52]:

> An Examination of Objects, in order to discover Truth, requires a great deal of Attention, Care, and Patience, together with some considerable Skill and Dexterity (to be acquired by Practice chiefly) in the preparing, managing, and applying them to the Microscope.

Reality has expanded way out of our visual capacity [199].

Uncertainty in data is an important topic and might be evaluated within lab studies, users of data should have a good understanding of the certainty of the data used [180].

The study of internal and external biological structures, which is covered by the disciplines of morphology and anatomy, is important in the study of evolution, ecology, development, and anatomy. Experienced scientists select the best scientific imaging method to answer specific questions. Thereby, certain questions concerning, for example, tissues, cells, biochemical processes, movement patterns, or composition and structure of a whole organism can be explored [17].

In biomedical research, a huge variety of different techniques is currently available for research on tiny entities. Since every imaging method is limited by certain physical parameters, an appropriate combination of two or more methods affords the chance to derive a significantly broader range of information about the investigated specimen [74].

The possibility to approach species digitally offers numerous options for new research disciplines in biology to evolve. Until the mid-twentieth century, morphological descriptions were merely qualitative and typological. The quantitative turn came with new technological possibilities that allowed mathematical descriptions and to form new theoretical concepts as an important starting point for fields like evolutionary developmental biology [73].

Gene-analysis and research on a molecular level using the latest scientific imaging devices has made new findings possible. Custom software enables scientists to publish images and interactive static 3D models directly out of their labs. Scientific illustration applications tend to be replaced by 3D animations [106].

Automated visualizations speed up the communication process, for instance in cell biology, as introduced in [127].

The interest in visualization has a strong tradition in the field of biology, as [19, p. 365] explains:

> Biologists have always visualized their objects to a greater extent than physicists or chemists. Since the early 19thcentury, hand-drawings, professional illustrations, idealized diagrams, micro photography are extensively used, followed by time-lapse motion pictures in the 20thcentury, for visualizing the data and supporting one's own analyses.

Microscopic structures in the micrometer or nanometer range can not be quantified with manual calipers and historic metering tools. For that, hardware and software needed to become capable of processing, visualizing, and quantifying digital imaging data [73].

The importance of imaging was acknowledged by a broader general scientific audience once more in 2017 in awarding the Nobel Prize of physics for work to improve the state of scientific imaging with single-beam gradient radiation pressure laser traps, also called "optical tweezers" [11].

Digital depictions of organisms have become more and more state-of-the-art in the description of and research about organic entities. Particularly the fields of taxonomy and morphology ever since made use of scientific illustrations and visualizations. Large accessible databases like the Protein Data Bank [16], are necessary to enable collaborations in complex fields such as biochemistry. The protein entities used in the case study showing a CRISPR animation in section 4.2 are scanned macromolecules which are available in this database.

In the following subchapters, the scientific imaging techniques chosen for the case examples will be briefly introduced. This will give a summarized insight into the data generation decisions made before beginning the 3D model creation and animation pipeline. The following introductions are not more than an overview of imaging methods for the used data sets. However, they present informative background knowledge for the later workflow as well as the case study analyses and overall relevant terminology.

2.2.1 Light microscopy

The light microscope is the oldest technique to make organic entities perceivable which are not visible to the naked human eye. The basic concept of converging lenses enlarging a specimen when they are in focal distance is simple, but many complex variants of microscopes were developed to enhance resolution and overall imaging quality. Light microscopy uses visible light, therefore its performance is limited by the wavelengths of light which range from about 390 nm to 700 nm.

Reconstruction of serial physical sections using light microscopes is the oldest method of analyzing micro-anatomical data in 3D. These manual reconstruction methods date back to the late 19^{th}century. Optical sectioning with confocal microscopes was invented later. Physical and optical cuts have pros and cons and are still used to acquire geometry of biological structures [73].

Throughout the long history of light microscopy, there were various microscope designs developed. One of these types of light microscopes is the confocal laser scanning microscope that will be discussed in the next section since it was often used to create tomographic stacks of the organisms in the case studies described here.

2.2.2 Confocal laser scanning microscopy

A confocal laser scanning microscope (CLSM) is a special light microscope that scans a sample with a focused laser beam. It is very popular in scientific communities because it brings major improvements in optical resolution and contrast to the captured image. This is done by using a pinhole that blocks out-of-focus light. Today's confocal laser scanning microscopes can gradually shift the focal plane by moving the lens or specimen to produce serial optical sections. It thus eliminates the necessity of actually cutting the sample to be imaged. The light beam penetrates the sample object, the reflected light is detected, and thus image stacks may be generated by recording only the sharp focus plane. Such image series can be used according to the tomographic principle, described in section 2.3, as a basis for digital 3D models.

In optical sectioning, multiple 2D images of the specimen centered in different focal planes are taken. A stack of images is therefore generated by sweeping through the probe along an optical axis [94].

Confocal microscopy of semi-transparent specimen (like some plankton creatures for the case study *Noise Aquarium*, see section 4.3) that are small or thin can be performed non-destructively within minutes. This is an advantage as there might be sources of artifacts like dehydration, fixation, and labeling for fluorescent probes during sample preparation. Additional, during imaging photo-bleaching, might occur [171, p. 2484].

Confocal laser scanning microscopy can be used to image much smaller samples than micro CT at even higher resolutions. Confocal laser scanning microscopy, however, is usually limited to specimen measuring about 300 µm [214].

Thicker samples might lead to artifacts. Moreover, the samples have to be smaller than 1.5–2 mm. Subjects of smaller scale are traditionally imaged using thin sections with conventional wide field light microscopes or transmission electron microscopes. This slicing process is an expensive task that demands trained skills and patience [17].

Block-face imaging is an alternative to save time. With this technique a microtome cuts the sample, this reduces the possibility of artifacts and the result images are aligned automatically. Block-face imaging might be performed with focused ion beam milling and scanning electron microscopy (FIB-SEM) (see for instance [122]) which ablates with an ion-beam and images layer after layer of a sample.

In light microscopy the technique of High Resolution Episcopic Microscopy (H-REM) (for more information see [60]) might be applied to cut and align samples automatically.

An usage of specific dyes in combination with confocal laser scanning microscopy allows data collection of specific tissues or cellular components [74].

Special depictions, for example three-dimensional muscular or neural net-

works were for a long time very time consuming or impossible to acquire. It needed recent developments in computing, data processing, and information storage that these techniques could gain their importance in the life sciences. Software packages that follow guidelines of usability and have lower prices made 3D reconstruction widely reasonable for laboratories that focus on non-applied, basic research. Pre-engineered software tools allow more and more biologists with basic visualization skills to design videos and images of the 3D structures of their research for their presentations [214].

2.2.3 Micro computed tomography

Computed tomography was first used in hospitals in 1972. The technique's existence is widely known because of its usage in human medicine [1].

Unlike clinical computed tomography, micro computed tomography (short micro CT or µCT) or X-ray micro tomography instruments have a revolving stage which rotates the samples between a stable X-ray source and a detector. X-ray photons for microscopic applications might be generated by a laboratory or synchrotron source [171]. The sample of the *Archegozetes* mite was scanned in a synchrotron facility.

In a synchrotron facility elementary particles are accelerated close to the speed of light by a linear accelerator before being injected into a synchrotron ring where the particles are kept in a circular orbit through the use of high energy magnets. As the electrons circle, they emit electromagnetic energy. This energy is funneled down beamlines and used as source [144].

The scanning process time of a micro CT depends on the given radiation. It can take seconds or minutes in synchrotron facilities, or up to hours in laboratories. Potential sources of artifacts are dehydration and fixation, radiation damage, oxidation, and heating [171, p. 2484].

Basically, X-ray detectors (also image intensifier or XRII) convert the X-ray photons into measurable electrical charges and digitize them [111].

In most X-ray detectors, the final image is smaller than the X-ray pattern. Brightness can be regulated by increasing the number of light quanta from a region of the X-ray pattern or by reducing the size of the final image [207].

Computed tomography scanners gather projection images and these are calculated into digital cross-sections of a subject. These slices can be stacked to create 3D volumes. As in all tomographic techniques, the resulting 3D volumes can be used to create virtual 3D models and might be processed accordingly. The internal and external morphology can be depicted with this technique. Computed tomography allows peeking into the inside of fragile, rare, or small probes. However, it can be very time-consuming to create a high quality 3D model on a computer [1].

In using this resulting model, 3D measurements and analyses can be performed. Often, to enhance contrast, specimens are stained with heavy

metals. This should not cause changes to the shape of the specimen but experts are not sure about the effects on the tissue on a cellular level [100].

For samples the size of 2–3 mm, tomographic imaging methods are most frequently used to achieve non-destructive volumetric imaging. The use of X-ray based tomographic imaging like micro CT is constrained by the X-ray absorption of the sample tissue. Successful contrast staining can enhance the range of useful applications, for instance in genomic, functional, mutational, and quantitative studies in developmental biology [125].

Often, numeric values for analyzing computed tomography images are used because the difference between these shades of gray are partly not visible for human eyes. Computed tomography data is usually acquired in 16 bit depth. These data are used for in-depth measurements and calculations. Only for visualization without further processing, 8 bit depth images would be sufficient. This opens up epistemological reflections on the relation of algorithmic and aesthetic characteristics in digital images, as [51, p. 66] points out:

> (...) digital images are both data and visible signs. Taking the relation between these two instances of digital images seriously leads to a deeper understanding of how images become both quantitatively and qualitatively meaningful.

Non-invasive scientific imaging like micro CT increases the amount of data that can be acquired from specimens by handling entire object volumes [56].

According to [73, p. 14], micro CT is the most versatile microscopic imaging technique to visualize accurate 3D geometry for quantitative applications of biological structures without geometric distortions. In the scans for the case examples, optical sectioning techniques like CLSM have more disadvantages and produce not geometrically isotropic and unbiased 3D image volumes because of reduced axial resolution.

Scientists are often satisfied with the possibility to manipulate, measure and analyze static 3D models as outcomes of micro CT scanning. Static images, animations of intersections, or rotations of the 3D models represent the majority of applications in the natural sciences. More advanced scientific visualizations as described in the case examples of this thesis are rare, mostly because of their laborious creation pipelines but also because of certain concerns regarding the transformation and editing necessary.

2.2.4 Scanning electron microscopy

The Scanning Electron Microscope (SEM) is an electro-optic device. The principle within these microscopes is similar to the functionality of light microscopes despite the fact that instead of visible light, electron rays are captured and can be seen with the help of the device sensors instead of a

gaze through the microscopes ocular or the picture of an attached camera. To capture such an SEM image, rays of electrons are generated by an electron source. It needs high energy to accelerate the electrons into the direction of the sample. As in optical microscopy, in electron optics, distortions occur. To reduce the artifacts and to enhance the resolution of the image, the diameter of the electron beam is reduced through a beam limiting device. The recording can be done by exciting the entire sample to emit radiation, or scanning the sample line-by-line. That's why the electron microscope is also called "scanning" electron microscope. Dependent on the device, different sizes of samples up to 30 cm are possible. The classic electron microscope needs a vacuum in its imaging chamber to prevent the irritation of the electrons by gas molecules. That means biological samples have to be prepared for scanning with the SEM [45, pp. 1–3].

In the case of the mites in chapter 4.1, the samples were prepared and sputtered with gold to withstand the vacuum and reflect electrons properly.

Uncoated imaging of hydrated, non-conductive specimens is achievable with the high pressure environmental SEM (ESEM) [218, p. 170].

During imaging, secondary electrons (SE) and back-scattered electrons (BSE) can be recorded. These data can be used to generate displacement, normal and bump maps. As a helper for obtaining size relations, a scale bar is typically provided on SEM images to show the actual size of the depicted specimen. An application for the depiction of accurate surface structures using SEM imaging is, therefore, standing to reason. Subsurface properties can provide additional information for scientific imaging through this back-scattered electron information. Due to the fact that electrons can also be reflected, the detector can gather information from the inside of the sample (approx. 1 nm for metals and up to 10 nm for insulators, compare [45, p. 5]).

The exclusive use of SEM images to define the surface structure of authentic 3D models, as proposed in [53], is to be undertaken with caution. Although it is a good option to use the electron micrographs to generate displacement maps for authentic 3D models, the structures depicted in SEM images are not completely behaving like depictions shot with light-ray capturing devices. Especially, BSE-SEM images resemble a little bit in their visual quality surfaces that are generated using subsurface scattering shading algorithms and are therefore not primarily suitable for the generation of surface-only defining displacement maps.

Also, BSE-SEM images have a lower resolution than the images of the secondary rays. The number of BSE allows conclusions about the chemical composition of the sample. Therefore, chemistry knowledge and knowledge about the chemical composition of the sample and it's behavior when exposed to electrons is necessary for the scientific evaluation of SEM imaging results, compare [45, p. 5].

Furthermore, artifacts occur not only in the recording but also in the preparation of samples and especially in the preparation of biological sam-

ples, for example, shrinkage artifacts related to specimen drying or crystal formation on the surface. After all, however, SEM images are very useful for creating authentic surface structures for 3D models, as they contain accurate details especially from the hard surfaced parts of microorganisms. This was useful for the surface map definition of mites, for instance. In electron microscopy, there are various distinct techniques available to produce scientific imagery. Mainly for additional information on the surface structures of the imaged animals, in the case studies in the sections 4.1 and 4.3, SEM was used.

2.2.5 Transmission electron microscopy

While conventional light microscopy is limited to resolutions of approximately 0.2 µm, and also SEM imaging has a more restricted magnification size, transmission electron microscopy (TEM) opens up the opportunity to investigate subcellular details [74].

In the 1930s, for the first time, the resolution of optical microscopes was undercut. The resolution of TEM images is directly influenced by the radiation source. That means the shorter the wavelength, the higher is the achievable resolution. Atomic resolutions could be attained for biological structures and even higher resolutions are possible within non-organic samples [126].

The TEM microscopy technique is destructive as physical sections are necessary. The speed of the imaging process is taking weeks or months for image stack series and therefore is comparatively slow. Potential sources of artifacts include dehydration, fixation, staining, sectioning induced problems in the sample preparation process, as well as radiation damage and shrinking while scanning [171, p. 2484].

Samples for TEM imaging that consist of ultrathin sections made with special devices called ultramicrotomes, can be irradiated with electrons after cutting. The scattered electrons can be detected to generate graphics-like imaging data (see for instance the imaging data of *Cylindrospermum* in section 4.3.2). Organic samples must be prepared for imaging in TEM. Different methods are available depending on the imaging task. The fixation methods used for the case studies in this work are described in Appendix A.

Cryo EM is a specialized application of TEM and therefore also called "Cryo TEM" or "Cryo EM". In Cryo TEM samples are depicted as they are tilted, resulting in a tomographic image stack. In contrast to other tomographic techniques, samples are prepared in non-crystalline ice and imaged under cryogenic conditions (<-150°C). This has several advantages including the possibility to create imaging data without fixation or dehydration. This is beneficial for the scientific imaging of biological structures [138].

Generally speaking, in the first decade of the 21stcentury a broad range of experimental methods to use Cryo EM were developed. Applications of

Cryo EM in biology cover a wide spectrum ranging from tissue sections to cells, bacteria, viruses, and protein molecules. Different sub-methods of Cryo EM have been used to analyze organic entities in different contexts. All these methods were also used in hybrid approaches in combining data from electron microscopy and Cryo fluorescence microscopy (Cryo CLEM) with X-ray crystallographic and NMR spectroscopic information [126].

Typical reconstruction modes for Cryo EM are single-particle, tomogram or sub-tomogramic averaging, helical reconstructions, or 2D crystals. This allows the generation of 3D models out of the 2D projection images of a tilted biological specimen [212].

Density reconstructions often suffer from distortions and artifacts. The reasons lie in the electron optics in addition to problems in the reconstruction calculations [186, p. 207].

2.2.6 X-ray crystallography

A huge number of entries can be retrieved from the worldwide Protein Data Bank, that means, in preparing for a project like a case example in section 4.2, it can be tedious to decide which is the right data set of the macromolecules that should be part of the planned scientific visualization.

In X-ray crystallography, the proteins are purified, crystallized, and then exposed to intense X-ray beams. This leads to characteristic diffraction patterns which have to be analyzed employing complicated methods to determine phases of the X-ray waves and therefore the distribution of the electrons in the protein. The outcome is a map of electron density which is detected and stored. The electron density map then is used to calculate the location of atoms in the imaged protein[3].

The methods of X-ray crystallography require the development of ever new methods and the combination of techniques because not every protein is in the same way suited for crystallization, as [144, p. 342] explains:

> Crystals are required for X-ray diffraction experiments because scattering from individual molecules is far too weak to measure. Crystals act like an amplifier by increasing the scattering signal due to the multiple copies of molecules within them. Typical protein crystals are about 0.2 mm in size but usable crystals have been reported from tens of microns to a few millimeters.

This might lead to uncertainty in the data sets like [144, p. 360] clarifies:

> Unfortunately, there are a handful of published protein structures that have proved to be partially or even totally wrong.

[3] https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/methods-for-determining-structure; 12/10/2018.

> The most common causes have been incorrect space group assignment and/or over interpretation of a poor quality electron density map. (...) guard against incorrect model building is the free R-factor.

The R-factor is part of the entries in an overview table of all proteins used for the scientific visualization of CRISPR. This table of the chosen data sets for the case study *CRISPR/Cas9-NHEJ: Action in the Nucleus* can be found in section 4.2 and the table in list 3.1.

2.3 Tomographic scanned microscopic entities

Basically, a scanning process records a subject or environment of the real world with a technical device. The process generates data of the surface information, the three-dimensional shape, and/or the interior of the scanned subject. The term 'scan' refers in the use cases here to collections of digital data generated with different scientific imaging devices with the goal to acquire 2D or 3D data sets. Tomographic 3D scanning is a summarizing term to refer to all 3D scanning methods that are performed in the way of the tomographic principle. An amount of these 2D images combined in a stack reveal 3D information of the sample. The visual description in figure 2.1 communicates the principle in a comprehensible way.

The word 'tomography' origins in the Greek words 'tomos' for slice and 'graphein' for to write [1, p. 2].

The tomographic principle is used for numerous scientific imaging applications. Not only the commonly known CT and MRI scans at hospitals, even molecular structures and celestial objects might be investigated in that way [85, p. 1].

The microscopic scale is not a completely defined size range. Roughly, it refers to scales that are not visible without a microscope or with the bare eye. While the word "microorganisms" is mostly reserved for unicellular organisms in biology, microscopic organisms are defined as species in a size range that contain, true to the name, species that can only be seen clearly with a microscope of any kind.

Physicists think of a "microscopic" scale range even between macroscopic (visible to our naked eyes) and quantum scale [151, p. 2].

These definitions are vague, but give an idea of which size the here dealt with organisms and proteins are. Even the larger species discussed in the case examples could be called "microscopic organisms" because limb details are not visible with the bare eye in the animals that are about the size of 2–3 mm. Especially for the research of the biology of microscopic specimen, instrument-based developments are important as these living beings can only be measured and recorded through microscopic equipment.

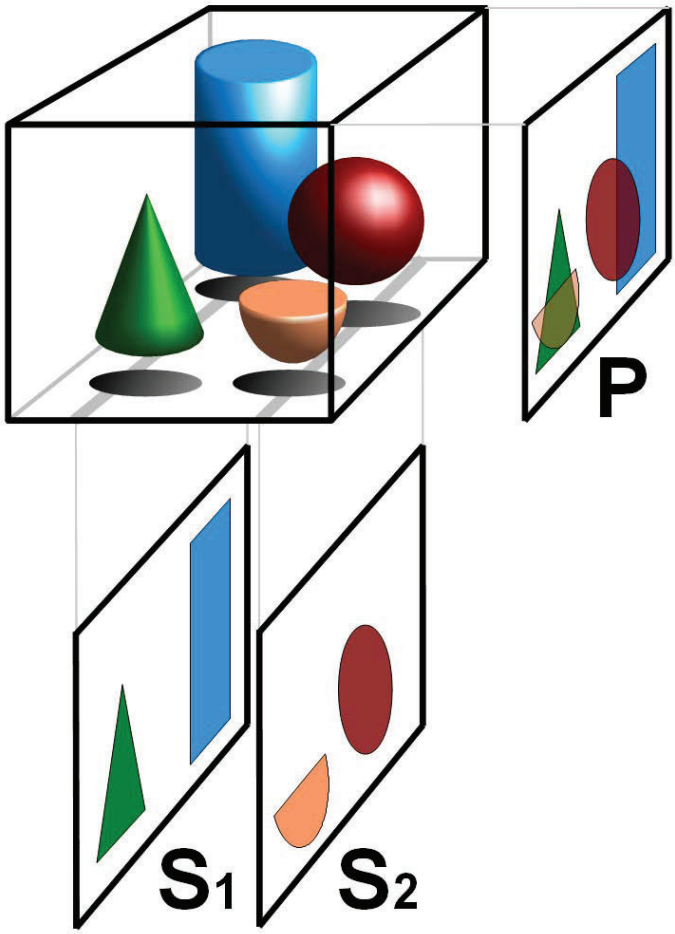


Figure 2.1: The tomographic principle: Cross-sections S1 and S2 were scanned from the sample—here visible in a transparent box; P shows an image of the sample that is not tomographic. Image source: https://commons.wikimedia.org/wiki/File:TomographyPrinciple_Illustration.png.

The outcome of tomographic scanning processes after image reconstruction of any kind are single images or mostly image stacks. The images in these stacks define values which can be calculated into voxel density data. After all, the pipelines described here required calculated polygon models to apply the desired computer animation techniques.

Volume modeling methods represent a subject through solid shapes. These solid shapes are often, and like in the here described cases, voxels (three-dimensional pixels, cubes). Cubic volume models have a regular grid. These voxel models are very common in computational simulations, medical and scientific imaging [89, p. 349].

The creation of polygon models demands a careful segmentation process and application of Marching Cubes algorithms [115] and its iterations (for instance [158]) to get surface coordinates of the three-dimensional data. The intentions and parameters of imaging and the editing steps have to be made clear and well documented. Tomographic techniques for modeling three-dimensional reconstructions like described in the case examples here, allow transformations while still obtaining an authentic shape of the subject. The resulting 3D models are direct representations of the original segmented imaging data.

Nevertheless, segmentation might involve extensive post-processing and therefore has to involve exhaustive reporting of post-processing steps in publications to reveal the editing steps [168, pp. 5–12].

In the application of different tomographic methods such as computer tomography or the magnetic resonance imaging, volume data is generated slice-wise. The direct display of image reconstructions like image stacks in a 3D software is possible. For many applications, for instance in medical imaging, direct rendering is a good way to quickly get results and proceed to an evaluation of the image data.

While direct volume rendering shows fine details and realistic structures, specific details are hard to see and need further editing to optimize expressiveness and effectiveness of the visualization [205].

The interest in the investigation of three-dimensional shapes is often a goal in scientific imaging techniques. Hardware and software for processing and visualizing scientific imaging data are constantly evolving and are an active field of research.

We can talk about a true revolution in microscopic imaging techniques since their wider availability in the 1990s [73].

Tomographic techniques became one of the standards to obtaining 3D data in various disciplines and are as well eminently suitable for the depiction of microorganisms [154].

Originally, X-ray computed tomography was developed for human medical imaging [170].

Computer-based quantitative analysis of 3D data has clear advantages to traditional morphometrics, because measurements can be performed in

the micrometer range, directly from the voxel segmentation [73].

The marking process of the parts of the scan that should be included in the 3D model might be done semi-automatically or automatically. Parts that should show up in the 3D model are segmented from the background and not wanted surface parts. Semi-automatic segmentation can be very laborious and often needs an expert in the anatomy of the subject to make sure the outcomes are correct.

Chapter 3

Processing data for scientific visualization

The immediacy with which the 3D models are connected to reality is essential to the following approaches. Real organisms that once lived are taken and depicted as samples of nature. The resulting 3D models have the same basic shape and pose as the original sample, including natural deviations from model organisms. Drawing samples is of importance for the authenticity of the resulting computer animations in the case examples presented here. The direct connection to an unique subject is advantageous and adds various striking aspects to each emerging computer animation. The relation to the "real world" and the "scientific reality" are vital aspects here. A special fascination derives from shapes that are not only modeled according to forms in nature, but which have also been acquired by means of cross-sectional scanned series of one specific organism.

In the following, the primary data sources used for the 3D models in the case studies will be revealed. After that, chosen preview methods as well as model creation will be explained in detail for the discussed project examples. Furthermore, 3D model reshaping and creation, optimization, surface definition, finalization, and animation will be covered. The following subchapters contain descriptions and represent a generalized pipeline, see figure 3.1, whereas, individual deviations are described in the case study chapters of their respective projects. The first subchapters include all the necessary steps on the pathway to animatable 3D models—herein also sometimes referred to as a character.

All tomographic 3D scans may be processed using methods as described in the following sections. These workflows were chosen both because the selected software proved to be beneficial, and the large data sets led to these approaches. The steps regarding editing details might vary in other software applications but meta-methods largely remain the same. The descriptions of the modi operandi should give the reader an insight into the editing steps as

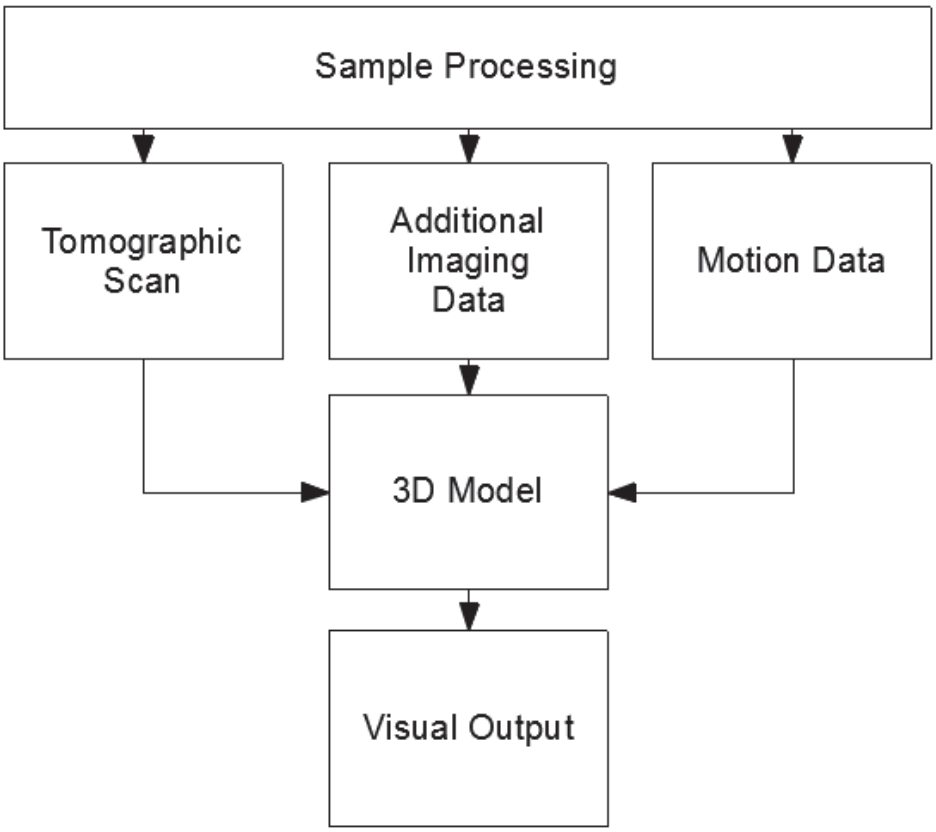


Figure 3.1: Simplified graph of the generalized computer-animated scientific visualization pipeline scheme.

prerequisite for later discussions of the case studies, and in general, place the case examples in the field of scientific visualization. However, they are not tutorials which can be followed without any prior knowledge of the software packages.

3.1 Data collection and preview

The general starting point of the animations in the case examples were image stacks acquired by experienced biologists. The image stacks were obtained using typical editing processes in scientific imaging, for more information see, for instance, [73, pp. 32–33]. These preproduction steps are hereby referred to as part of scientific imaging. Scientific imaging will only be covered marginally since it is the source input for the described scientific visualizations, however, it is not the topic of the present discussion.

Before imaging starts, the samples must be prepared. The sample prepa-

ration for the case examples in section 4.1 and 4.3 is available in Appendix A, the initial steps in the acquisition of the used entries of the Protein Data Bank are retrievable through the cited papers in figure 3.1. Sample preparation steps were taken by the involved experts as described in Appendix A, and the scientific imaging of these samples will be described in the following paragraphs.

For micro CT scanning of the different plankton species, the plastic pipette tips containing the samples were vertically mounted in a micro CT sample holder. Micro CT scans were acquired with an Xradia microXCT-400 (Carl Zeiss X-ray Microscopy, Pleasanton, CA). For the *Tomopteris helgolandica* specimen, the 4x optical magnification lens was used for scanning, while the other samples for the in section 4.3 described *Noise Aquarium* case example (*Phoronis muelleri actinotrocha larvae*, *Oikopleura* sp., *Noctiluca scintillans*) were scanned using the 20x optical magnification lens. Samples were imaged at 40 kVp source peak voltage and 200 µA intensity. Depending on sample contrast, projections were recorded with 30–120 s exposure time and an angular increment of 0.2°. Depending on sample size, isotropic voxel resolution of reconstructed volumes varied between 0.53 µm and 1.98µm. For the acquisition of serial light microscopical sections, the organisms were imaged using an Aperio ScanScope slide scanner with a 20 x objective lens. Single sections were cropped from whole slide images using Adobe Photoshop CC (Adobe Systems Inc., San José, USA).

The scientific imaging of *Amoeba*, *Paramecium*, and *Cylindrospermum* was done with an Axio Imager Z2 (Carl Zeiss, Jena, Germany) microscope equipped with a digital HD camera (1080p). In the case of *Cylindrospermum*, additional transmission electron microscopic images were captured from ultra-thin sections using the FEI Morgagni 268D (FEI Company, Hillsboro, OR).

The mites for the case example in section 4.1 *Parasitiformes* and *Archegozetes* were treated very differently, hence their hard cuticular nature. *Parasitiformes* was, after being stained using 1% elemental iodine in absolute ethanol for 24 hours, scanned using an XRadia microXCT 200 by Stephan Handschuh, as mentioned in [53].

The mite *Archegozetes* was a special case in imaging, collaboration, and depiction. The imaging was done at the European Synchrotron Radiation Facility (ESRF) in Grenoble (France). A short summary of the treatment and processing according to [82, p. 1] follows:

> Synchrotron X-ray tomography was performed at beamline ID19 (ESRF, Grenoble, France, experiment SC-2127). First, the samples were mounted in the sample-holder and adjusted to a central position in the beam. Second, the samples were measured with an energy of 20.5 keV. The radiographs were recorded with a cooled CCD (ESRF FReLoN camera) with a 14-bit dynamic range, 2048

x 2048 pixels and an effective pixel size of 0.7 µm. 1500 projections were recorded over the 180° sample rotation with an exposure time of 0.35 s for each projection. The detector-to-sample distance was 20 mm. Using a certain distance between sample and detector enables a differential imaging of materials with low X-ray attenuation coefficients [31], which would produce insufficient contrast in absorption imaging (where the sample is located directly in front of the detector).

Most biological matters are phase objects, composed of materials with low absorption and/or only small differences in atomic number [17].

However, phase enhanced tomography requires a high spatial coherence of a homogeneous X-ray beam. Therefore, synchrotron radiation is better suited than desktop scanners for these kinds of measurements.

Image stacks from thin sections allow adequate reconstructions of the scanned object, though the given spatial context of adjacent sections might be lost during the sectioning procedure and then has to be restored using a set of methods called image registration or image alignment [73].

After image registration, the scanned specimen were segmented, that means, separated from the background or unwanted details. This can be done automatically or semi-automatically. The output of a segmentation process are usually isosurface meshes.

The models used in the discussed case studies were all acquired from tomographic scans. There are different techniques involved in this process, which requires specialized knowledge and training to correctly use the various instruments. This might be a hindrance, however, when organized as a collaborative project, can lead to unique outcomes such as the examples presented herein. The collaboration with microscopy specialists and biologists opened up the door for professional feedback sessions about the model and animation work in general, as well as inspired new ideas for visualization projects in particular.

Detailed information and technical specifications of the image stack data are put together in figure 3.2 for the case examples *Noise Aquarium* and *Two Mite Gaits* and in list 3.1 for the project *CRISPR/Cas9-NHEJ: Action in the Nucleus*.

The data retrieved from the worldwide Protein Data Bank (wwPDB) have to be listed with different parameters. The uncertainty of scientific imaging outcome in this nanometer scale is considerably higher than in the micrometer scale. The R-value is a measure of the quality of the entry created with crystallography by comparing the consistency of the experimentally

Organism	Size	Imaging Method	Stack Size	Resolution (px)	Voxel Size	Bit Depth/ Channel
Archegozetes	< 1 mm	Synchrotron µCT, LM	1350 tif 1170 tif	1120 x 1040 1080 x 1030	0.7 µm	8
Parasitiformes	~ 1 mm	µCT, LM	756 tif	964 x 1004	-	8
Amoeba	0.2 – 0.5 mm	LM	884 tif	1292 x 969	0.17 µm	24
Paramecium	< 0.25 mm	LM	454 tif/LZW	1292 x 968	0.14 µm	8
Cylindrospermum	0.5 – 1 µm	LM, TEM	365 jpg 6 tif	3208 x 2472 518 x 468	3.605 nm	16 24
Noctiluca	0.2 – 2 mm	µCT, LM	1701 tif	1501 x 1541	0.552 µm	16
Tomopteris	5 – 40 mm	µCT, LM	3342 tif/LZW	1349 x 1388	1.97588 µm	16
Actinotroch	< 1 mm	µCT, LM	1441 tif/LZW	1641 x 1201	0.535 µm	8
Oikopleura	1 – 8 mm	µCT, LM	698 tif/LZW	538 x 764	0.586 µm	16

Figure 3.2: Overview of input data of the microscopic organisms of the projects *Noise Aquarium* in section 4.3 and *Two Mite Gaits* in section 4.1.

observed diffraction pattern with other previously calculated models.[1]

Another quality measurement is the resolution. In contrast to the data in figure 3.2, where the quality was quantified using the parameters' stack size, image resolution, voxel size, and bit depth, the resolution for nanometer size tomographic imaging is typically measured in angstrom (Å).

The resolution shows the level of detail in a computed electron density map of the protein. High resolution values are approximately 1 Å and therefore considerably more accurate.[2]

All data sets are experimental data snapshots, meaning that the data currently online might be different due to snapshots of data being updated frequently. In the online archive, data is also available retrospectively. The data used for the CRISPR project was downloaded on the 6th of June 2017, except for entry "5a9q" which was previously downloaded on the 9th of October 2015.

[1] https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/r-value-and-r-free; 18/10/2018.

[2] https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/resolution; 18/10/2018.

The following list gives an overview of the technical parameters, wwPDB entry codes, names of the papers, and scientific imaging methods of the chosen protein macromolecule structures:

- **Human Nuclear Pore Complex [6], wwPDB entry: 5a9q**
  Experimental Data Snapshot
  Method: ELECTRON MICROSCOPY
  Resolution: 23 Å
  Aggregation State: PARTICLE
  Reconstruction Method: TOMOGRAPHY

- **Composite structure of the inner ring of the human nuclear pore complex [104], wwPDB entry: 5ijn**
  Experimental Data Snapshot
  Method: ELECTRON MICROSCOPY
  Resolution: 21.4 Å
  Aggregation State: PARTICLE
  Reconstruction Method: SUBTOMOGRAM AVERAGING

- **Nuclease Ku: Crystal Structure of the Ku heterodimer bound to DNA [209], wwPDB entry: 1jey**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 2.5 Å
  R-Value Free: 0.280
  R-Value Work: 0.218

- **Polymerase: DNA POLYMERASE BETA/DNA COMPLEX [160], wwPDB entry: 1bpx**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 2.4 Å
  R-Value Work: 0.253

- **Human Ribosome: Structure-function insights reveal the human ribosome as a cancer target for antibiotics [131], wwPDB entry: 5lks**
  Experimental Data Snapshot
  Method: ELECTRON MICROSCOPY

Resolution: 3.6 Å
Aggregation State: PARTICLE
Reconstruction Method: SINGLE PARTICLE

- **XRCC4/XLF-Cernunnos Complex: Crystal structure of xrcc4/xlf-cernunnos complex [157], wwPDB entry: 3q4f**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 5.5 Å
  R-Value Free: 0.309
  R-Value Work: 0.249

- **Ligase IV-Artemis Complex: Crystal Structure of Human DNA ligase IV-Artemis Complex [137], wwPDB entry: 3w1b**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 2.4 Å
  R-Value Free: 0.225
  R-Value Work: 0.176

- **DNA Structure [72], wwPDB entry: 4x18**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 1.05 Å
  R-Value Free: 0.105
  R-Value Work: 0.096

- **Cas9-sgRNA-DNA Complex: Crystal structure of Cas9-sgRNA-DNA complex solved by native SAD phasing [140], wwPDB entry: 5fq5**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 2.136 Å
  R-Value Free: 0.225
  R-Value Work: 0.191

- **Cas9-sgRNA-DNA Complex: Crystal structure of Campylobacter jejuni Cas9 in complex with sgRNA and target DNA [219], wwPDB entry: 5x2g**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 2.4 Å
  R-Value Free: 0.221
  R-Value Work: 0.190

- **PKcs: Crystal Structure of Human DNA-dependent Protein Kinase Catalytic Subunit (DNA-PKcs) [176], wwPDB entry: 5luq**
  Experimental Data Snapshot
  Method: X-RAY DIFFRACTION
  Resolution: 4.3 Å
  R-Value Free: 0.437
  R-Value Work: 0.386

- **Nucleosome Complex: Complex of Snf2-Nucleosome complex with Snf2 bound to position +6 of the nucleosome [114], wwPDB entry: 5x0x**
  Experimental Data Snapshot
  Method: ELECTRON MICROSCOPY
  Resolution: 3.97 Å
  Aggregation State: PARTICLE
  Reconstruction Method: SINGLE PARTICLE

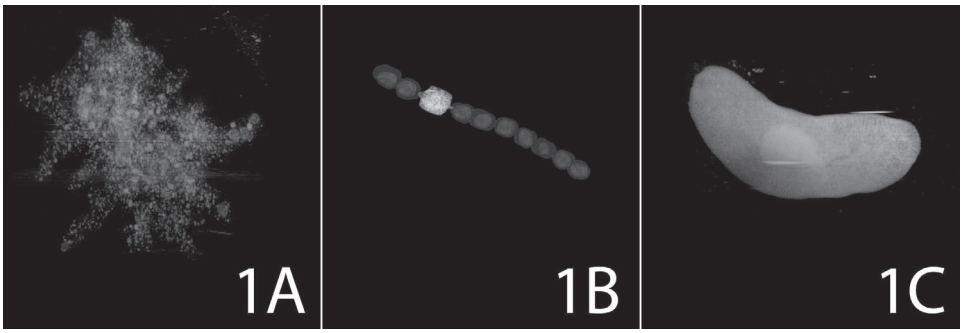


Figure 3.3: Volume renderings to preview data sets three-dimensional. Only automatic segmentation of luminance values was done, and rendered with Blender Internal using serial micrographs of *Amoeba proteus* (1A), *Cylindrospermum* sp. (1B), and *Paramecium multimicronucelatum* (1C).

From both non-segmented and segmented details, renderings can be created using different volume render methods. The image stacks can be imaged as Direct Volume Rendering (DVR), which is particularly useful for the first previews of the density data. For all data sets except protein data, preview volume renderings, as seen in figure 3.3, were created with Blender Internal render engine using volume textures to view the organisms in not edited poses and with unaltered density information.

All stack images have a certain background noise, hence meaningful data must be filtered out. Often, situations occur where the algorithms incorrectly define something as relevant or irrelevant. Thresholds in semi-automatic segmentation generally use luminance values that represent density of the stack image pixels. The higher the magnification factor in microscopy, the more noise that occurs.

The visualization of single atoms is possible, but the noise contained in these recordings must be heavily filtered in order to recognize individual atom positions.[3]

3.2 Model creation

In the context of this work, "model" is most importantly the definition for a three-dimensional representation of the herein investigated entities. Furthermore, the term also includes in this context additional data that was added to the original scanned tomographic image stack data in an augmenting and synergistic way. The term "model" may have different meanings, as [89, p. 2] explain:

[3] https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/methods-for-determining-structure; 6/9/2017.

> In the field of computer graphics, the word "model" can refer to a geometric model or a mathematical model. A *geometric model* is a model of something we plan to have appear in a picture: We make a model of a car, (... and so on). The geometric model is enhanced with various other attributes that describe the color or texture or reflectance of the materials involved in the model. Starting from nothing and creating such a model is called *modeling,* and the geometric-plus-other-information description that is the result is called a *model*. A *mathematical model* is a model of a physical or computational process (...) of how objects move and models of things like the image-acquisition process that happens in a digital camera. Such models may be faithful (i.e., may provide a predictive and correct mathematical model of the phenomenon) or not; they may be physically based, derived from first principles, or perhaps empirical or phenomenological, derived from observations or even intuition.

The first step of the model creation was completed by microscopy technicians and biologists. This step includes acquisition and preparation of a specimen, different scientific imaging and scanning processes, and the segmentation of the scanned image stacks. These tasks must be predominantly accomplished by experienced personnel. In the case of microorganisms, since the anatomic selection of body parts of the organisms requires a trained eye, while the circumstances in the imaging of proteins overall demand experimental research and computing by scientists. The resulting stack images are usually filtered and processed before segmenting the 3D model data out of the acquired data.

The creation of a model for virtual applications is divided into several steps. The goal of the data acquisition is to obtain an approximately isotropic (same resolution in all 3 spatial axes), low noise data set. Approximate isotropy can be achieved by an appropriate choice of scan parameters. The application of high resolution reconstruction algorithms creates increasingly spiky structures. Interpolation can help to avoid these artifacts. Further noise suppression is achieved through the use of filter algorithms. In addition, image processing operators can enhance the contrast between the object to be segmented and the background. The right choice of the segmentation procedure and the editing tools are crucial for accurate segmentation. Before visualization, contour smoothing can be used to create a visually appealing 3D model with reasonable polygon count [173].

Digital reconstructions of tomographic scanned entities face obstacles such as the "banana problem", which posits that a curved 3D object can not be reconstructed from cross-sections without additional information [42, p. 330].

Image segmentation is necessary in order to achieve the desired 3D poly-

gon model since a not segmented data set would only show a cube or cuboid as a polygon model. The image volumes generated by micro CT and serial light microscopical sections were used for segmentation of the whole animal outline and specific features of each animal. The segmentation and transformation for the projects described here were done with Amira (FEI, Hillsboro, USA) by combining intensity-based and manual segmentation tools, with exception of the *Archegozetes* model, which was segmented in ImageJ/FIJI [162] 3D Viewer Plugin [163] automatically with luminance key segmentation. There is also an informative video[4] discussing the practices in [82]. It shows cutting plane and fly-through videos of one of the static *Archegozetes* mite 3D models and their approach to segmentation. After some experiments with various software packages, the polygon geometry for the *Archegozetes* model were created in a different way than described in this video, for more information see section 4.1.

Based on the image segmentation, polygon surface meshes were triangulated. In the software package Amira, an algorithm similar to the historical Marching Cubes surface reconstruction algorithm of [115] is applied to get triangulated meshes. Surface reconstruction is still a major problem in computer graphics, thus why there are ongoing research projects, for instance of [110].

The algorithm promises a result without cracks and holes, without intersecting triangles, and different materials for every region [187].

A typical stack image of a semi-automatic segmentation process of the *Oikopleura* 3D model, one specimen from the later discussed case examples, can be seen in figure 3.4.

After that, the surfaces were smoothed and re-meshed to the desired amount of vertices. Based on image segmentation, triangle meshes (Wavefront Objects) were exported for further processing.

The use of triangle meshes opens up numerous possibilities in computer graphics. They are the dominant modeling technology [89].

The direct 3D visualization of such polygon meshes is called surface rendering, these are widely used to display complex structures in biology [75].

An example of surface renderings of a *Paramecium* is shown in figure 3.5.

Since the new millennium, computed tomography technology has rapidly improved. Still, the main limiting factor in a computed tomography-based analysis is the processing of large data sets [1].

This is an ongoing issue and should be mentioned here. The data sets processed in the case examples were manageable using a contemporary graphics workstation. However, if the Marching Cubes algorithms are set with overly high resolution demands, the models might become too large to load in the system's Random-access memory (at the time of editing 32 GB RAM).

[4] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2583021; 8/12/2017.

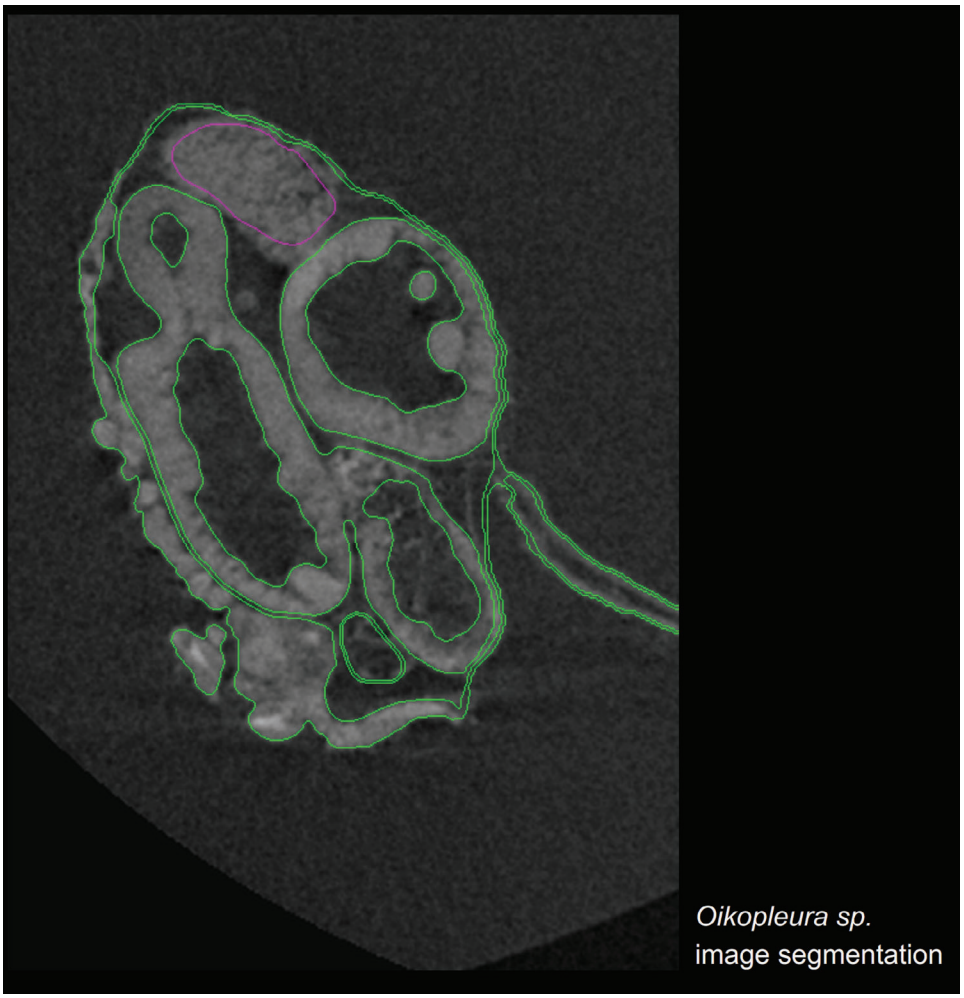


Figure 3.4: One stack image with semi-automatic segmentation masks of the *Oikopleura* scan. The segmentation masks can be seen as green and pink outlines on one image of the stack. Image by Stephan Handschuh.

Thinking and planning before starting the conversion of a density data set can prevent a forced restart. To get as much as possible out of a data set, trial and error tests with different settings while versioning the outcomes followed by evaluating the resulting 3D models are often necessary. Of course, the main factor for the possible level of detail of a segmented 3D model is the resolution and details provided in the original scanned data set.

Final 3D surface reconstruction is done by the computerized processing of collected data. Every pixel of the obtained stack images is assigned a Z-coordinate, as the Z-traveling distance of the microscope lens directly influences the rough fractures of the segmented surfaces [123, p. 149].

Particularly in applications where numerous data sets of similar samples

Figure 3.5: Surface renderings directly out of the Amira software after segmentation. The first (from left to right) shows the surface of the scanned *Paramecium*, the second shows the cell plasma, the third shows the nucleus, and the fourth food vacuoles. Image by Stephan Handschuh.

have to be segmented, recent advances in deep learning technology and pattern recognition show clearly that segmentation is an extremely well-suited field for machine learning. See for instance applications in [146].

Automated segmentation processes are especially important for research in fields with studies using huge amounts of data sets of, for instance, the same species, or in medicine of the same structure, see for example automated tumor segmentation in [99], enhanced error control in segmentation in [149], or automated mitochondria segmentation in the Allen Cell Explorer[5] project [204].

Different areas in the stack images require varying densities to enable semi-automatic segmentation. With machine learning, computers are able to "learn" the expected shape and propose a segmented section.

The workflow for creating static 3D meshes from scientific image data stacks varies from case to case. For a description of these pipeline steps [53], figure 1, is still relevant. Here, the general overview of the process is given, while the case studies in chapter 4 deal with the peculiarities of creating the

[5] https://www.allencell.org; 21/10/2018.

different 3D models of the various organisms.

After model creation, the most important step from the computer graphics side is to choose the right amount of detail for the digitized organisms. The process of planning is key because the level of detail is always related to each individual end-use application and the project's goals. There are huge differences between 3D models that should show detailed processes of the inner life of organisms, the digital organisms that are animated for a comprehensive overview, or an animation of protein structures. Also, there must still be a distinction between 3D models for real-time applications, such as games, virtual or augmented reality applications, or prerendered videos. Real-time editing for 3D artists is still in heavy development and will likely increase in the future, see for instance the Blender EEVEE project[6].

The main challenge described in this thesis is the usage of tomographic and additional scientific imaging information to generate outstanding data sets for use in computer animation. Numerous specialties in generating a 3D model arise due to the specific workflow presented here, thus why various special solutions and the tackling of individual smaller and larger challenges are part of this thesis. Different solutions for every step of the presented approaches are found in chapter 4.

3.3 Optimization for 3D animation

The 3D scanned models must be made editable for the chosen workflow, compare figure 3.1, since, up to now, scientific 3D scanning data does not come cleaned up and ready for the animation process. The data must first be reduced and cleaned of noise and other scanning artifacts in order to be the starting point of an animated scientific visualization, as in the manner of the ones described here.

An important step in the workflow for all projects described herein was the model optimization. It took place in several steps. The editing of the two-dimensional images of the image stacks and their optimization may also be counted as optimization for the later model. In the here described cases, the step of model optimization can be divided into two parts, since a part has been performed by biologists, while the other part is added to the herein described computer animation workflow.

The main mesh optimization steps carried out by the imaging specialists was the Least-Squares algorithm [119] from the Amira software package AlignSlices Tool [41], which performs an automatic image registration by translation and rotation of adjacent slices. This algorithm yields satisfactory results and was verified using prior knowledge of the organisms' shapes [73].

The software Amira also offers in its segmentation editor a number of different filters, for example denoising and smoothing filters as well as mor-

[6] https://code.blender.org/2018/03/eevee-f-a-q; 22/20/2018.

phological filters for erosion, dilation, opening, and closing operations [187].

While Amira is an excellent tool and widely used in multi faceted visualizations of life science research data applications, Blender supports the entirety of pipelines to create computer animation artworks. Blender supports bone animation and physically based rendering with workflows and outputs that are typically needed to generate computer animations for projects like the herein described case studies.

After the import of triangulated polygon mesh models acquired through image segmentation of image stacks, further processing was needed in the case for most microorganisms described here. The software chosen for 3D processing and rendering in the projects was mainly Blender 2.74–2.79 (Blender Foundation, Amsterdam, The Netherlands).

High resolution polygon meshes were extracted directly from the image stacks. During this process, the typical problems in 3D scanning were faced.

Chaotic meshes and loose parts that form Polygon Soups are typical for scan results, hence need to be cleaned up to form consistent models for use in the rest of the graphics pipeline [89].

After generation, re-meshed triangle surface models were imported into Blender for the first review of the three-dimensional shape. For most organisms, due to the following animation workflow, a complete restructuring of the original scanning data was obligatory. Therefore, the scanned data was loaded into the software 3D-Coat 4 (Pilgway, Kiev, Ukraine). In this voxel sculpting software, separated parts of the organisms were edited to make them more suitable for animation workflows, particularly for the initial pose (resting pose) of the organism. This incorporates retopologizing (retopo) and often results in the optional rebuild of the organism in a symmetrical way. These major changes were always made very carefully with feedback from the biologists, especially input about their knowledge of the morphology of the various living beings. Authenticity and close connection to the once living creature's transformation from scientific data is key to the approaches described herein. Depending on whether or not an animation with a rig was planned, the 3D model was retopologized after the various data transformation steps. In every instance, the geometry was verified with Meshmixer 3.2 (Autodesk Inc., San Rafael, USA) to control water-tightness (without holes) and orientation of the polygon faces to avoid problems later on. Also, in Blender, Clean Up Tools[7] were used. Descriptions and images of mesh editing are available in the various case examples in chapter 4.

Due to its critical importance in computer graphics, mesh optimization has been widely studied [89].

Especially if real-time applications are the target output, optimization is extremely important [18].

[7] https://docs.blender.org/manual/en/dev/modeling/meshes/editing/cleanup.html; 12/5/2017.

In the transformation of various tomographic data which record the substance of the organisms bit by bit, many calculation steps are applied. In the discretization of the digital recording using different scanning methods, the first information transformation takes place. Necessary reduction of data is done when choosing mesh density according to the project requirements. For this, the Decimate algorithm of Blender[8], a very effective method of reducing polygons or voxel density when exporting from 3D-Coat, was used. Data reduction can be extremely useful for the clarity of the scientific visualizations if the goal is to show one specific detail of scientific research where the larger context might be given through reduced polygonal detail in order to save render and editing expenses. Naturally, these reductions have to be applied with caution.

Not only data reducing steps were taken. Sculpting was used to add details with stamps and stencils to generate bump and displacement maps from SEM data in order to change or repair the pose of the static mesh, as well as to repair broken topology such as very fine tissue elements. Sculpting is usually done on high resolution meshes. For the microorganisms, predominantly voxel sculpting and occasionally surface/polygon sculpting in 3D-Coat was performed.

While a model should be prepared for animation with retopo processes to ensure a smooth workflow, a rigid model may contain more detail. Models which will not be animated can have a high polygon count, while models that should be animated need workarounds with displacement, bump or normal maps. In the upcoming case examples, workflows for creating a scientific visualization from micro tomographic scans are described.

Often it was necessary to make changes to the pose of the scanned organisms for a better animation starting point. Expert advice was especially important in these cases because of the amount of editing to follow. Also, despite expert preparation of the organism samples for tomographic scanning, artifacts in the fixated specimen and during the scanning process may occur. These must be removed with the utmost care. For that, it had to be determined which types of artifacts occurred. Afterwards we decided together how they might be removed. Also to note, in most of the presented cases different resolution 3D meshes were created. Whole books are written on the topic of Level of Detail of 3D graphics.

An entire field of computer graphics is dedicated to adapt the complexity of meshes according to the circumstances and requirements of a virtual scene [116].

This is a topic that will not lose its popularity soon. The quality standards of computer graphics images are continually on the rise. In the case of the presented projects, manual adaptation through different resolutions

[8] https://docs.blender.org/manual/en/dev/modeling/modifiers/generate/decimate.html; 10/10/2017.

of the chosen 3D meshes to deal with the proximity of the camera, as well as the experimental mode of Blender Cycles (version 2.79) with adaptive sampling was used to render 3D structure information. Specific case dependent information on optimization procedures is available in the respective sections.

3.4 Surface definition

After the 3D meshes were optimized, finely structured details, shaders, and textures were applied to these digital objects. In general, the surface definition can be subsumed into processes of shading and texturing to specify surface behavior for the rendering of computer animations and computer-generated images. Textures and shaders of the microorganisms are based on or taken from references acquired with optical microscopes. Examples of this imaging data can be seen in respective sections of chapter 4. Additional imaging data was commissioned to define the surfaces of the 3D models. For instance, for the *Tomopteris* worm 3D model, very detailed light microscope images were taken by the biologists. For high resolution structures of the 3D models of the mites and two plankton creatures, Scanning Electron Microscopy (SEM) was used in addition to light micrographs.

By means of the tomographic imaging used here, structural information can be obtained from the surface and the inside of the organisms. In contrast to photogrammetry, textures were not generated automatically, thus a texturing method had to be used for colored surfaces of the 3D models. All used image stacks contained no color information due to the applied imaging techniques.

Typical techniques for texturing and shading of 3D surface models were used during the workflows described. What makes the surface design outstanding with common 3D models is the use of scientific imaging techniques such as SEM and light microscopy to obtain extraordinary amounts of details for the computer-animated visualizations. The application of the output of various imaging techniques and the microscopic images available through cooperation with the scientific collaboration partners offered a rich pool of references and base materials for textures. Efforts were made to retain as much as possible from the data through image processing. Stitching of highly detailed close ups led to a gain of additional details in the textures. The textures were applied using the texture projection techniques UV coordinate mapping and generated mapping for procedural textures.

The SEM images were used as displacement and bump maps. No further 3D reconstruction methods were applied, however, this would be a suitable topic for further research, see for instance [190].

Shading and rendering were done in Blender 2.74–2.79 with Cycles render engine and periodically Blender Internal renderer for volume rendering.

Cycles employs physically based shader models and uses the Bidirectional Scattering Distribution Function (BSDF), which defines how light is reflected and refracted from the 3D model's polygon faces.[9]

Synergy as a design principle, which according to [162] is also used in ongoing biological research, was applied to combine multiple imaging data sets and image processing steps. This is a powerful way to enhance the quality of information for each 3D model. In particular, in the case of the micro world examples, the surface structures were combined out of SEM and light microscopic images, in addition to scanned data of the microorganisms.

The texturing, as well as the shading processes, are unique for every 3D model described. They are highly dependent on the references that were made available. Therefore, most of the descriptions are found in the respective case studies in chapter 4.

3.5 Animation

One of the main statements here is, that for a holistic view of our species-rich planet, we should also look closely and carefully analyze living beings invisible to the naked eye. Movement analysis combined with an analytic and close inspection of microscopically small creatures may lead to surprises. The qualitative and quantitative description of the microscopic specimen might include the analysis of motion with computer animation. The pure study of movements, their notation, comparison, and depiction is a topic in art as well as in science. It seems that the outlining of movements has always fascinated people. Even some cave drawings and other ancient artworks are thought of to depict notations of movements.

Animators and biologists both analyze closely when they study natural movements. Both disciplines have utilized as well as created tools and technologies in order to discretize motion. Life sciences use various types of animation to model complex actions [55].

One might argue that more science-oriented men such as Étienne-Jules Marey and Eadweard Muybridge were not the inventors of the art of movies because they were more interested in analyzing movements rather than depicting or questioning reality with their inventions. However, their interest has driven the way we now represent reality and our world in moving pictures [15].

Analyzing movements is an important part of scrutinizing and representing reality in art and science. The reasons for the involvement in studies of movements are numerous. To name a few examples, they can be driven by the beauty of nature [156, pp. 187–196], to produce new design products while considering the various aesthetics of movements [46], analysis, and

[9] https://docs.blender.org/manual/en/dev/render/cycles/materials/surface.html; 15/10/2017.

notation of animal [91] and human movements [107], or for behavior and morphology studies from biology to robotics [133].

The study of motion is the study of life itself because motion is a factor which can separate living beings from static objects. Motion also has a more or less major role in the later presented case examples. For the gait comparison in section 4.1, it is of key importance, for *Noise Aquarium* in section 4.3, motion is the detail we might not expect in plankton floaters, and for the CRISPR motion in section 4.2, it was highly speculative and simplified when created but was nevertheless one of the newest scientific narratives of gene-editing available at that time.

Up until now, animations of tomographic data that are more complex than just rotating, fly-through or intersecting static specimen are only possible with further editing. Described here under the term "computer-animated scientific visualization" are the animated outcomes of the models which were generated as described in this thesis. There is a huge difference between computer animations that statically rotate a 3D model and those which show a fully movable creature. For example, an animated intersection with rotations of a human MRI brain scan can be exported from nearly every scientific imaging software package automatically. Animations that genuinely show the movement of body parts and tissues require considerably more effort. Often for these animations, a digital simple representation of a skeleton (rig) is used to make the creature move. These movements can be purely artistically, or in contrast, as in the following sections, focused on the techniques used to observe motions of organisms in an authentic manner.

The animation techniques using segmented polygon surface 3D models and rigs were chosen for reasons of more flexibility in the overall project designs. Nevertheless, the direct animation of volume data is possible, see for instance [166, 198, 211], however, still requires further development for computer animation design purposes and therefore was not considered in the here discussed case examples.

The first computer animations were created with keyframing, thus every single move was done by hand [109].

This time-consuming technique is still frequently used to create computer animations. Of course, there have been many attempts to speed up the process and partially automate creation of the animation. Due to the importance of computer animation for animated scientific visualizations, various approaches of transferring real-life movements from natural species to digital models will be explored here. The making of creatively constructed computer animations is an art form that needs intense practicing as comparable with acting or puppeteering and will be not addressed here (compare, for example, *Stop Staring: Facial Modeling and Animation Done Right* [141], *The Animator's Survival Kit* [216], ... and other "animation bibles"). Later in the case examples, the computer animation of the individual organisms is described. Depending on the objective of the three projects, different ap-

proaches to computer animating digital models of microscopic entities were applied.

Reference videos from each of the animals have been provided by the collaborating biologists. As part of the meetings with the biologists, some details about shape and motion were discussed using sketches. The quality and amount of reference videos and motion data varied in each individual case. In the gait comparison of the mites in section 4.1, three-dimensional movements were rotoscoped. The other projects incorporated key-framed or rotoscoped approaches to computer animation.

In naturalness of movements, every person who is willing to consciously observe is an expert since we are all confronted daily with natural movements and have learned how to interpret them. Furthermore, most people are since childhood consumers of computer animation products.

The choice of rotoscoping existing references for the computer animation of the scanned organisms in this manner was a deliberate decision. Simple rigs like the ones used here have the disadvantage that the animation process itself requires more time, however, as a result the rig is much more likely to be compatible when software updates are necessary, especially within a Blender workflow. Furthermore, creating a simple rig with fewer constraints is faster. In addition, at the beginning of the projects, it was not yet decided which models might receive data from motion capturing. In the case of using motion capture data, a simple rig would have been advantageous because motion capturing retargeting on rigs with motion constraints is doomed to fail, particularly if not all the pipeline parameters are properly adjusted.

Computer animations provide additional information, which becomes especially clear if, for example, a process animation is available instead of a static 3D model. For instance, the functionality of organs of microscopic creatures might be made visible. In such a case, an animation is considerably more meaningful than mere static 3D models. Additional added value may also be provided using interactive and immersive animations.

The benefits of using animated visualization over static single image depictions are a broader range of storytelling options. Good storytelling enables additional ways to comprehend correlations. In animated storytelling, subject matters are contextualized and this might help to concretize abstract ideas. Animation might reveal logic connections and offer new perspectives on a topic. However, there is evidence in research that animations are more beneficial for teaching students if interactivity is involved. Students might follow the content of animations more effectively if they are able to scroll through and replay them. Animated sequences are generally more engaging and emotionally powerful, more enjoyable, and exciting than single images [49, pp. 333–337]. These circumstances make animations a perfect tool to convey important environmental topics such as the case study *Noise Aquarium* does, as people tend to remember emotional content.

Recording motion data and transferring this data to 3D models still

requires manual inputs, yet, evaluation and creation of movement data is becoming more and more automated. However, more research to provide automated processing of scans and motion data is still required. The following sections and subsections provide an overview of the various methods for generating movement data and transferring them to 3D models of microscopic entities.

3.5.1 Manual approaches

Animation requires the study of motion in depth. This is the case for most presented methods in order to transform motion from nature into 3D animations. While the here described 'Animation with references' is comparable to the scientific drawing of motions, as it requires gazing back and forth, the second manual approach listed here is 'Rotoscoping' and can be described as copying something by tracing. Animations using references are still the most commonly applied 3D computer animation technique, especially if non-scientific applications are included.

There is much ongoing research in the optimization of manual computer animation steps. For instance, skinning simulation algorithms for rigs are to this day under development (e.g. [12]) and are constantly being improved. Motion capturing is commonly used and often over-represented in VFX making ofs for movies since it has an amusing appeal to see actors in tracking suits. However, on closer inspection, these captured motions still need manual input, corrections, and work-overs for realistic perceived movements. Especially, on the scientific side of dealing with computer-generated animation, this is likely going to change. New semi-automatic approaches are improving and increasing the options in computer animation.

Animation according to references

In general, animation according to references is hand-crafted, manual labor. Key poses are saved at defined key frames primarily by hand. A typical method to create manual animations is by moving a virtual object with the help of a digital rig.

Although natural movements hardly contain any exact repeated movements, in animation, walks are often described in so-called walk cycles. These walks cycles loop, nevertheless, in the case examples, cycled motions were mostly avoided as the natural movements taken from the reference videos did not cycle. However, for the real-time installation setup of *Noise Aquarium*, motion cycles were used.

Notation (observation) by experts is one way besides recording with cameras to obtain motion reference data. In morphology, scientific papers that determine for instance the joint movement maxima are common. Also, motion pattern analysis is important for research in this field of biology. All

of this research output combined with papers may be used as a basis for authentic animations or potentially beneficial presentations using computer animations. The observation and notation of motions and movement patterns is the oldest way to investigate locomotion because there are no technical devices required. However, videos as foundation for motion analysis make sense due to the repeatability of the observed motion.

The most common way to approach animation with references is to have several motion references, notations, and videos as patterns for animation. These patterns are intensely studied, yet not completely copied in contrast to rotoscoping. Both rotoscoping and animation with references are time-consuming processes. Rotoscoping permits less freedom in defining the animation, as videos are usually loaded into the viewport of the animation software and then traced. For the case example *Noise Aquarium*, mainly rotoscoping was used. In this case, lack of freedom was not a big issue because plankton drift in bodies of water. This means they might have their natural movements while they still can be animated as they were dragged around by the current to stay in the camera framing.

Summarizing, there is a clear distinction between rotoscoping and animation according to references. In rotoscoping the animator sticks to the movements in the reference videos.

Rotoscoping

Rotoscoping is an animation technique which is used to trace the motions of moving image footage. The name has its roots in the projection device, which was used initially in traditional animation to project individual frames of film in order to provide guides for drawing animations [64].

The "Rotoscope" was invented by Max Fleischer[10] and originally used to draw authentic movements in cartoon animation. However, the herein described technique should not be confused with the manual drawing of masks, nor should it be seen as actual outline tracing. In the case of 3D computer animation, it is not the outline of the model that is copied directly, but rather the rig is deformed according to the outlines of the recorded template. Skinning has to be functional to follow the deformations of the reference. If the recordings are done using X-ray video, or the filmed subject is transparent, tracing of the bone structure is potentially an option.

In scientific cases, defined parameters are applied and therefore the animation technique is called "scientific rotoscoping", for instance in [57]. In joint studies this might, for example, include the option to model the natural shape of the bones of an animal, see for example [136]. Scientific rotoscoping is a pure translation of the motion data captured on video and is often traced by hand. A great deal of planning is necessary to create a final an-

[10] Patent number: US1242674 (A) - 1917-10-09, METHOD OF PRODUCING MOVING-PICTURE CARTOONS.

imation. Rigs and characters must be created according to expectations of the final output. The process of transferring the captured motion data into moving pictures can be extremely time-consuming when long sequences are rotoscoped.

Rotoscoping is to a certain degree restricted to one movement plane if no synchronous video captures taken at different angles are available. This means that either algorithmic or artistic estimations are required if insufficient recording of different view planes exist from which to generate authentic 3D movements. Also, if rotoscoping with purely traced motion is augmented with additional input, it might be referred to as extended rotoscoping. This additional input might include rotoscoped poses, as well as interpolation between poses. Naturally the rotoscope animation is always only as accurate as the captured videos and the amount of effort being made to trace them correctly.

3.5.2 Semi-automatic approaches

Semi-automatic approaches to transfer motion from the real to the digital world should ideally work without much user input. A lot of research is going into this field, especially since Virtual (VR), Mixed (MR), Augmented Realities (AR), and Computer Vision are seen as a lucrative source of income for the big tech companies. Three-dimensional scanned entities and their actions are commonly used in all these XR (X Reality or Cross Reality which summarizes VR, MR, and AR) productions. Multitudes of research fields are currently interested in motion studies and the automatic replication of captured motion in the virtual space. Especially interactive XR applications tend to use real-life motion data captured in real-time to interact with a growing number of virtual worlds.

Motion capturing, tracking and mapping

Motion capture is used in a multitude of fields, from bioengineering to Hollywood blockbuster productions. It means that in general, movements are captured, tracked (converted into digital 2D or 3D data with a time component), and mapped onto a digital character. Initially, movements have to be recorded and interpreted. Following this, the acquired data is then used to create the animation.

While the term motion capture is commonly applied to the whole process, there is the overlooked fact that there is still much more work to be done in order to apply the captured data to the final animation. Working with motion capture data can be difficult. Mainly, motion capture data often contain errors that require extensive clean-up, not to mention that the motion is harder to evaluate and edit because there are typically keys at every frame of the captured motion data [64].

Life sciences and the commercial animation industry share similar technologies for capturing, tracking and remapping motions. In contrast to scientific applications, motion capturing in the movie industry uses the captures often only as inspiration or a guideline to characteristic motion data [55].

Capturing motion can be a tedious process with multiple opportunities for failure to transfer motion from the real world to the digital. Until now, most research has been about motion capturing humans and applying the resulting data on humanoid characters, see for example in [8].

The majority of commercial motion capture systems use the measurements of marker positions to acquire motion data. There are many different systems which use markers: Mechanical systems estimate motion by angles of a fixed tracking skeleton, magnetic systems use an electromagnetic field to find the markers which are coils of electric wire, inertial systems have markers with built-in accelerometers and gyroscopes, and optical systems use either reflecting passive markers or light emitting active markers. Besides these, there are markerless systems which use varying numbers of cameras [150].

In optical tracking systems, occluded markers are a common problem. Occluded markers are not trackable, which leads to interrupted motion curves. Multiple cameras were for many years a prerequisite for capturing three-dimensional motion. Recently, algorithms have evolved and seem to have removed this restriction. Regardless, sufficient data and a proper light setup to create enhanced quality videos, ideally from multiple sides, are highly beneficial to the solving of 3D location points in time.

It is possible to stick magnetic or optical markers on ants to acquire motion from their natural locomotion, see [61].

However, using markers on even smaller organisms proved unfeasible, particularly with the equipment available for the tracking in the mite case study in chapter 4.1. Capturing the microorganisms from two sides simultaneously was the first step on the way to a better solution.

There are relatively simple to use motion capture setups available for the capturing of humans and eventually larger animals, for instance, Microsoft Kinect or Vicon tracking systems. Still, these are not applicable to microorganisms.

Procedural motion simulation

Procedural animations are animations that are defined automatically by equations which define how certain entities move under given circumstances or forces. These kind of simulated animations are mostly calculation intensive, yet humans must define the rules and goals in order to achieve the desired results.

Furthermore, complicated motion and subtle nuances in movements are difficult to describe [64].

The research on generating synthetic actors, i.e. digital models that move according to predefined equations and parameters, involves various disciplines such as mechanics, physics, robotics, artificial intelligence, artificial life, biology, and cognitive sciences [148].

The simulation of synthetic actors which may be used for the type of scientific visualizations described herein, is questionable. It would on one hand be advantageous since the motions can be standardized, while on the other hand, the individual movements and the claim of transferring the once living creature into the virtual world would be unfulfilled. The uniqueness of one organism is lost in a standardized, database-derived model, see for example [9].

Additionally, many approaches only use motion capture as a starting point for synthesized animations and thus are often more simulated than recorded, as the impact of the generalization can be enormous, whereas the influence of the capture is marginal, such as in [78].

Physical simulations are more or less based on real-world physics. An organism is simulated according to movement patterns, which are influenced by physics and body anatomy. However, simulations are not required to follow the laws of physics. In the virtual world, everything is possible, hence it is important to remember when using simulations that the representation of the real world is only as good as the equations involved.

Already in [194], artificial fish were generated, simulated, and promoted as an artificial life form. Nevertheless, due to the complexity of the topic, much research is still required until complex organisms can be actual virtual agents. Some researchers of physical simulations with animals think that it will be possible to have digital artificial life in the near future. Simulations in research are important to investigate and visualize real-world systems or processes. Up to now, highly biased systems are prerequisite to simulate sensory systems of organisms and appropriate reactions to their virtual environment.

Autonomous virtual robots which are situated in a dynamic 3D virtual world should include whole functional design, including motor control, behavioral simulation, and perceptual modeling. The creation of such virtual autonomous agents presents a challenging research field, as even the simulation of simple animals is complicated. Various interdisciplinary research approaches including fields like physics-based graphics modeling, biomechanics, and behavioral animation have to be considered in the research of virtual autonomous agents [200].

An end to the growth of this field of research is not in sight. First and foremost because there are constant improvements and iterations of agent-based systems, meaning that animations are based, more or less, on complex computational reaction systems. Furthermore, the creation of interactive content requires more and more simulations of life. An animation based on real-life physics is especially useful for the animation of phenomena such as crowds, particles, fluids, smoke, and the like.

Subsequently, the subfields of computer graphics dealing with procedural animations are extensive [5].

In humanoids or computer-animated animals, simulations are on the rise due to the possibility of saving precious animator's time with, for instance, task-based approaches such as in [33].

Furthermore, the growing field of real-time animations demands generated reactions and movements. Procedural animations for humanoid characters are persistently explored, for instance in [62, 97, 220], likewise, for animal animation simulations, for example in [98].

Although research already began in the early 1990s, the following enlisted features proposed by [200, p. 3] are still not the majority of use cases in simulated animal computer animation:

> (...) create self-animating, autonomous agents which emulate the realistic appearance, movement, and behavior of individual animals, as well as the patterns of social behavior evident in groups of animals.

Nevertheless, numerous tools to speed up animation processes were developed in research as cited above. Animators prefer to work with skinning, simulated muscle systems, spring systems, and other physics simulation helpers when they are necessary for a project. The main reason for semi and not fully-automatic approaches is the possibility to have controllable actions of the performing agents. One of the major advantages of computer animation compared to real-life recording is, (besides the obvious usage herein described for not filmable scale sizes using normal camera equipment) the possibility to stage animals and actors. In real-life, directing animals is only possible with trained animals to a certain extent.

Various fields of research such as experimental biology, computer animation, and robotics benefit from simulated gait patterns of virtual insects [70].

In the case examples in this work, no procedural animation was used, yet elements of the models were animated through simulations. For particles in several organisms, fluid simulations were used. These were defined by fluid simulation rules and algorithms in the Blender software package. Furthermore, cilia of the organisms were created using hair particle simulations. Different techniques used to animate the 3D models are described in the respective sections in chapter 4.

Structure from motion and motion-photogrammetry

Structure from motion and motion-photogrammetry are the most automatic solutions for cases in which it makes sense to digitize the moving reality unaltered.

In structure from motion techniques, animations, 3D shape information, and textures are captured from image sequences. The computer graphics community has since the beginning of research in this field attempted to further automate these processes [197].

Structure from motion is more commonplace in computer vision research. In this field, it is a vital part of such topics as scene reconstruction, object recognition, and 3D shape extraction. In structure from motion approaches processed videos or image sequences use analysis algorithms to acquire 3D motion data.

In general, there is a multitude of techniques for scanning movements and 3D shapes simultaneously. The dominant techniques are based on image sequences, laser-scanning technology, or structured light approaches. Photogrammetry is also available in the form of mobile phone applications and varying approaches using low-quality microscopes.

The extraction of motion and structure from videos or image sequences struggles with various, commonly occurring artifacts, for example, noise [90] and rolling shutter [79].

Until now it has not been widely used in computer animation due to limited accuracy, high amount of calculation power required, and restricted creative freedom. The 3D structure acquired through 'structure from motion' is usually not very detailed, since the resulting resolution is quite low, however, that will likely change in the near future with higher resolution cameras.

Humans are often the topic of automated tracking and retargeting approaches, see for instance in [4, 142, 177].

Exciting use cases might come up in the future with the progression of computer vision developments, some of which may also be used for creating computer animations.

Nevertheless, using computer vision concepts such as motion photogrammetry requires a certain fidelity and quality provided by the applications that are often not designed to be used in an animation configuration, compare [64].

An example of future developments which may be interesting for the depiction of microscopic entities is to find in [152]. In this example, they were able to extract motion, 3D shape, and textures from singular wildlife videos of mammals. This method could be adapted to microscopic scenarios as discussed here. Although there might be problems with microscopic lens distortion, transparency, reflections, and the lack of fine details.

3.6 Rendering and visual decisions

Visual decisions and the final look are the outcome of planning, design, and production. All technical and creative aspects of the computer animation production process, which include surface materials, 3D geometry, scene setup, and motions, are part of the final style of the artistic and technical result. All of the presented projects have differing styles, yet a particular look is always inherent. The approach with Cycles render engine and the chosen output workflows provides a creative basis for the case examples. The decision to use Blender and Cycles was a creative, political, and deliberate solution.

Crowdfunding campaigns are presently quite popular and are widely renowned, however, back in 2002, Blender Foundation was the first successful campaign. The community wanted to save the software from the "buy-and-kill" trap it had fallen into. As a result, the people worked together to purchase the then closed-source project in order to open-source it. Currently, it is maintained and supported by the Blender Foundation[11], with the first Blender developer Ton Roosendaal[12] as its head. It is open-source, free (as in speech), and should continue as a prospering 3D software for creative people without the threats that come with publicly traded companies.

In scientific visualization and academic environments, open-source software is more commonly used than in commercial computer animation studios. However, Blender is steadily becoming more and more renowned for its usage in commercial computer graphics, yet it still primarily has more users among academics, as well as hobbyists due to its free price tag.

One should consider the background of products we use daily. Like every tool, Blender has its limitations, hence it is a crucial part of the creative process to overcome or incorporate these restrictions. The "Cycles look" might be visible to a trained eye in the case examples and may be seen as part of the look as, for example watercolors have a different look as oil colors. When

[11] https://www.blender.org/foundation; 19/10/2018.
[12] https://www.blender.org/foundation/history; 19/10/2018.

it comes to the discussion regarding the effect which different render engines have on the output look of a production, there is definitely a lack thereof within the Blender community. One problem of the Blender community (and the computer graphics community in general) is the lack of research into aesthetics. Technical feasibility, render problems, and performance are widely discussed, while hardly any effort is invested into comparing the visual qualities of render engines. The reasons for this might be related to the historical development of computer graphics, which is generally often considered a technical challenge, as well as the general difficulty of measuring the quality of visuals. The looks of the productions discussed here are defined by the sum of all decisions made during production with Cycles renderings and After Effects compositing.

Here, Cycles render engine was chosen to effectively fulfill the task of authentically representing entities taken from reality. The described procedures are seen as a continuation of the process of capturing reality using technical devices. Computer graphics simulate the behavior of our environment for our perception system. A proposition here is that we should more frequently push farther and begin simulating our surroundings and universe (e.g. [155]) as it is perceived by different perception systems (e.g. insects, animals, etc.), or other completely unique visual parameters. Whereas computer graphics renderings often aspire to depict physically accurate images, stylized renderings are also suitable for scientific visualizations. It is more common to render graphics and animations "realistically" in visual arts and effects than in visualization. This might have to do with the fear of presenting research output in a manner where it may be interpreted as normal photographs by laypersons.

In general, Physically Based Rendering (PBR) does not mean the resulting imagery must look realistic, since with physically based render engines such as Cycles, stylized pictures may also be created. See for instance the list of so far created animated Blender short films[13] or the latest Disney/Pixar productions, compare for example [23]. These animated film productions are hyper-realistically, in the sense that their roots are only loosely based on reality, however, they are rendered according to biased physically based light simulations. Whereas, the biological structures in the case examples are generated uber-realistically, as they contain the data of real entities and still are rendered with the Cycles render engine. The renderings are abstracted, still they depict portions of reality. Every detailed involvement with the scanned tomographic data sets is an attempt to see more than which is possible for human nature without technological transcendence. The scanned samples are taken out of nature's reality and presented in an amplified depiction of it.

[13] https://www.blender.org/about/projects; 19/10/2018.

World shader texture maps were combined with lighting setups to generate light in the scenes for the case examples, this is often called environment lighting. The world environment can emit light in all colors or textures may be used to define the scenes basic mood.[14]

The default color space of a Blender scene is 'scene linear', as rendering in this color space produces more physically accurate computations it was applied to the scenes for the case examples. The linear method to treat simulations of visible light is convenient for photo-realistic rendering and compositing[15].

For the three presented case examples, physical properties of surfaces were simulated were it was beneficial to the desired look. Overall, stylized and realistic renderings of the tomographic scanned entities are presented here.

[14] https://docs.blender.org/manual/fi/dev/render/cycles/world.html; 12/09/2017.

[15] https://docs.blender.org/manual/en/latest/render/post_process/color_management.html; 03/02/2019.

Chapter 4

Applications and case studies

The first step into the world of microscopic organic entities was the production of the short movie *The Incredible Water Bear*[1] (AV-Dokumenta GmbH and Industrial Motion Art GmbH, 2013). In this production, an already established usage of scientific imaging devices like micro CT, light microscopes, and scanning electron microscope were combined and used to depict the adventures of a tardigrade in the soil layer. The tardigrade was the only creature in the short film that was not textured with original scanning electron microscopic images stitched together to increase resolution and therefore accuracy. The production of a team of 27 persons won prizes and became renown among the local computer animation community and international tardigrade lovers. It was originally a pitch project for an IMAX production and the work on this project also initiated a paper with a detailed description of the authentic visualization of computer-animated soil organisms for documentary films, for more see [53].

Proceeding from the fascination for the possibility to scan microscopic organisms, to analyze and render them with amazing details, other projects were started with a similar focus on authentic biological details. The short film *The First Greed* (AV-Dokumenta GmbH, University of Applied Arts Vienna, 2016) and the interactive installation *Noise Aquarium* (University of Applied Arts Vienna, AV-Dokumenta GmbH, University of California Los Angeles, 2017) came to follow. Also, the research on scientific visualizations on mites continued and was extended by data that could be used through the collaboration with the Zoological Institute at the University of Tuebingen.

The biological entities depicted in the case examples were chosen because of specific reasons. The most important selection criteria were the intriguing shapes of the selected species or, in the case of the CRISPR animation, the importance of the macromolecules for the animated process. For most of the plankton choices, the scientific imaging experienced biologists presented a list of outstanding species, then the decision was made because of visual

[1] https://www.youtube.com/watch?v=cp1WwNE6Lms; 01/11/2018.

features and availability of the samples. The procedure was slightly different for the mite gaits comparison example. The two mites were selected due to the availability as samples. The proteins for the CRISPR animation were selected as agreed by the scientific supervisors.

The cooperation with most scientists derived from the fact that the Technical Chemistry department at the University of Applied Arts Vienna was the first institution in Austria to set up an ESEM scanning unit as a demonstration facility. On the one hand, this increased the confidence of the scientists in the documentary productions of the head of the department Alfred Vendl and, on the other hand, facilitated contacts with other institutions for the emerging Science Visualization Lab Angewandte. The project *CRISPR/Cas9-NHEJ: Action in the Nucleus* was commissioned by rector Gerald Bast to be shown as part of the 150 years jubilee exhibition in the Museum of Applied Arts in Vienna. It was shown in the *Future Room* installation as part of the show. The starting point of *Noise Aquarium* was a request of a producer who was at that time in preproduction of the Terrence Malick documentary *Voyage of Time: Life's Journey*. The Science Visualization Lab Angewandte had already gathered experience in depicting authentic microorganisms in the soil organisms project *The Incredible Water Bear* and the paper according to it, as mentioned above. Therefore, the team of the documentary requested 3D models from the Science Visualization Lab Angewandte[2]. In *Voyage of Time: Life's Journey*, the evolution should be described and the first time an organism fed on another organism. In the end, the scanned 3D models were only used as references for the documentary, but the Science Visualization Lab Angewandte started to produce a digital animated short film production with this topic called *The First Greed*. The scanned 3D models of *Amoeba*, *Paramecium* and *Cylindrospermum* species were created and later also used in the *Noise Aquarium* project.

In the following sections, details of the projects *Noise Aquarium*, *CRISPR-/Cas9-NHEJ: Action in the Nucleus*, and *Comparison of two mite gaits* are described. All these projects have in common that their base 3D models are generated out of tomographic scanned imaging data. Furthermore, microscopy and other scientific imaging techniques played a crucial part as input parameters. The selected projects will be analyzed, the main focus will be on the technical implementation and the project objectives.

[2] http://www.angewandte.at/institute/bildende_und_mediale_kunst/digitale_kunst/science_visualization; 04/11/2018.

4.1 Comparison of two mite gaits

For the first case example, computer animation as an analysis tool for motion studies of mite gaits was the topic. It resulted in a video[3] for a scientific visualization exhibition and the accompanying publication *Behind the curtain; science visualization—das Unsichtbare sichtbar machen* [14] along with a detailed analysis of the mites *Parasitus coleoptratorum* and *Archegozetes longistosus*. One key feature of this project is the golden thread throughout this thesis, which is that all entities were scanned tomographic and computer-animated for different applications of scientific visualizations. Mites are notably important for Earth's ecosystems, however, research about them is underrepresented. Computer graphics make it possible to observe microscopic animals and their surrounding scenery in computer-animated documentaries. The use of computer animation for biological visualizations enables a completely new perspective of animals that, at first sight, may not seem interesting to laypersons. In this work, two types of mites are compared in terms of their motion cycles. The outcomes were both of an experimental-visual and analytic-quantified nature.

Mites offer an extremely species-rich diversity. They make up the second most diverse array of animals on Earth after insects. Mites are biologically fascinating, however, most of the research done in the field focuses on agricultural importance of, or medico-veterinary conditions caused by mites.

They are optimal organisms for comparative studies and manipulative experiments which might question some established biological theories [210].

The soil's fertility depends on soil organisms such as mites and a balanced equilibrium of the species represented there. Soil organism communities with a higher diversity decompose organic debris at considerably lower energy costs [118].

Spreading the knowledge of the importance of soil organisms for our well-being and the health of our planet should be emphasized. Mites are often associated with disease because of bloodsucking ticks and the illnesses they might spread. Even biologists who work with them have at times mixed feelings when it comes to particular mite species. However, most of these small arthropods in the subclass "Acari" are helpful and harmless. Computer animation can help to promote this fact and potentially increase awareness for Earth's holistic ecosystems. Mites often are found in accumulations and such a bundle of crawling specks might be associated with dirt and may even cause some people to become nauseous. Though, when we pick one individual animal and look at it in detail, the perception changes dramatically.

[3] https://youtu.be/0TiAhNQjMHw; 05/11/2018.

They are worthwhile creatures and can even be presented in a beautiful, hence still true-to-life manner. Computer-generated animated scientific visualizations help to find alternative perspectives on microscopic organisms such as these.

4.1.1 The mites

The gaits of the oribatid mite *Archegozetes longistosus* (Oribatida) and the predatory mite *Parasitus coleoptratorum* (Gamasina) were compared. They were taken from wild collections (*Parasitus*) and lab culture (*Archegozetes*) and were adequately prepared for the various technical imaging procedures, for more details see Appendix A. As they show different feeding behavior, their body shape and walk locomotion are substantially varied. These notable differences were registered and compared with techniques described in the accordingly named following chapters. Through the light microscope, live cultures were observed for behavioral studies. Like all microorganisms discussed in this thesis, the animals were scanned with various tomographic methods to obtain accurate 3D models of the subjects. Videos filmed from two sides with macro lenses were simultaneously taken to obtain the primary motion data sets. Additional micrographs of the animals were taken for texturing and as references.

Parasitus coleoptratorum

Parasitus mite species of the Gamasina order have predominantly predatory lives, some families are known to parasite on other arthropods or vertebrates. There are small species which have approximately 300 µm in body length and other relatively large species measuring up to 2 mm in length. These predominantly predatory animals use only three of the four pairs of legs for progressive motion. The fourth pair of legs is used as a tactile organ and can feel the finest vibrations caused by potential prey[4].

The predatory mite *Parasitus coleoptratorum*, see light microscopic micrograph in figure 4.1, hereby referred to as "*Parasitus*" moves quickly, particularly in comparison to the second mite in this project which is no hunter and has a plant-based diet.

Their functional role in the soil ecosystems as predators, their high species richness, robustness towards sampling and extraction methods, and good identifiable features make Gamasina good bio-indicators [102, p. 43].

In its immature nymph stage, *Parasitus* uses dung beetles as a means of transport. This kind of phoresy is typical for this mite[5].

[4] http://www.senckenberg.de/root/index.php?page_id=14561, 02/10/2013.

[5] http://www.arthropods.de/arachnida/acari/parasitidae/parasitusColeoptratorum01.htm; 15/12/2017.

Figure 4.1: Micrograph of a *Parasitus coleoptratorum* mite.

A visualization of phoresy with a pseudo-scorpion on a stag beetle was depicted as the final scene in the above mentioned short film *The Incredible Water Bear*.

The *Parasitus* mite was likewise one of the subjects of our paper *Computer-generated images of microscopic soil organisms for documentary films*, [53]. In that paper, the scanning, preparation, surface generation, and general workflow were already described in detail. Regardless, the steps will be described later in this chapter since the model was completely redone for the herein described gait analyses, with the exception of the micro CT imaging process. Additionally, for the comparison of the two mite gaits, the walk of the *Parasitus* mite was further investigated.

Without preparations and scans of the animals, the further workflow would be impossible. State-of-the-art scans of the mites were taken to ensure the best results possible. After the isosurface model was exported from the primary registration, labeling, and segmentation software package Amira, the editing process of the scanned animal started. Mites cramp when they die and therefore the tomographic depicted specimen did not have a healthy walking pose to start with. As the relaxation of the pose in sculpting had major advantages, the mite was edited and retopologized. The 3D model

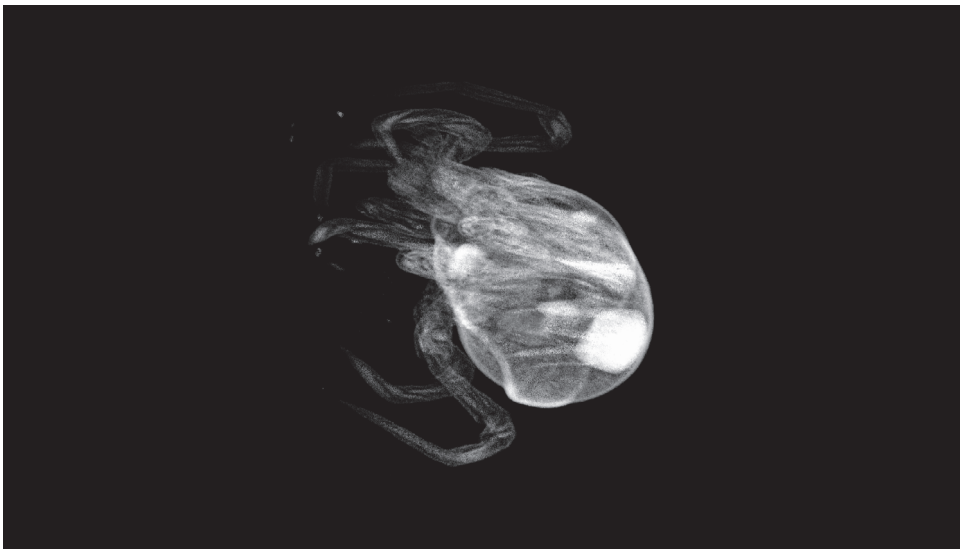


Figure 4.2: Volume rendering of a cramped *Parasitus* mite.

was prepared to have a pose that is more like a natural standing pose. Such a more neutral pose is a much better starting point for rigging and later on, animation. Also, for the model of *Parasitus*, the decision was made to convert the model into a symmetrical representation to ease the animation workflow as in computer animation, it is often the case to have mirrored geometry and rigs. Of course, one can argue that important details are lost when the scan is reduced to half of the original data, however, it helped to focus on motion experiments and deliver the animations for the exhibition described in [14] on time. A non-symmetrical animation would have taken considerably more time and would have left no room for visual motion experiments which were sought as part of the visualization to apply an artistic approach to the scientific topic.

The scanned triangulated model was first edited in the voxel sculpting software 3D-Coat to repair the cramped pose of the chosen scan, compare figure 4.2. After this process, the model needed retopologizing because the polygon flow of a scanned model, as well as the polygon arrangement of a model exported from a sculpting application, is not appropriate for animation. Additionally, in the case of this mite, the animal was segmented into multiple parts for animation. This was a decision made due to the hard cuticular nature of these creatures and the possibility to save weighing time in rigging separate parts. Also, the extremities of the animals appear somewhat separated by nature as they are stiff and only connected through soft joints. The overall separate parts of the *Parasitus* mite 3D model led to satisfying limb movement animations.

Starting from the same data set as in [53], texture coordinates, surface shaders, and textures were completely redone, because unlike the project in 2014 which was rendered in V-Ray (Chaos Group, Sofia, Bulgaria) for 3Ds Max (Autodesk, Inc., San Rafael, CA, USA), the mite gait comparison animations were completely rendered with the Blender Cycles render engine and therefore needed completely new shader definitions. The decision to use Cycles render engine was made due to the use of Blender as primarily software package. Cycles render engine is much better integrated into Blender than the option of using the not open source render engine V-Ray.

For the additional imaging of the *Parasitus* mite with the SEM, a FEI Quanta 250 FEG was used. All samples were sputter-coated with gold and the imaging was done in the conventional high vacuum operation mode of the SEM. Multiple images at different positions along the surface of the animal bodies were taken at magnifications in the range of 500x to 1000x. When stitched together, these images yield a map of the whole animal in ultra-high resolution, see figure 4.3.

This gives the possibility to use different SEM mode images (SE, BSE, and BSA) to acquire three-dimensional surface data as described in [53]. Secondary and two back-scattered electron images may be used as a displacement map to enrich the surfaces of scanned animals with fine details. A final rendering of one frame of the video that was shown in the exhibition can be seen in figure 4.4.

While the plain 3D model was created symmetrically, the textures were asymmetrical. This had the benefit that micrographs of the mite could be used instantly as color textures which in turn gave the final mite 3D model an overall more authentic appearance. The micrographs were taken as texture map in Blender Texture Painting mode and applied to the body of the digital 3D model.

Archegozetes longistosus

The second animal described in the project is the moss mite *Archegozetes longisetosus* Aoki (Acari, Oribatida). A light microscopic micrograph of the mite can be seen in figure 4.5. The soil organism is according to [83] a tropical mite that feeds on living and dead plant material such as bark and algae. It has five times higher pulling strength relative to its weight and than is theoretically expected of organisms of this size. An adult *Archegozetes* mite is less than one millimeter in size and weighs about one microgram.

The genetic strain used was established by Roy A. Norton in 1993 from a single gravid female collected from coconut debris at Lucillo in Puerto Rico and since then it has reproduced through parthenogenesis [81].

The here described mite was chosen for the gait comparison because it was available in huge quantities due to dedicated breeding by Manfred Walzl at the University of Vienna. He also invited us to submit a contribution to

Figure 4.3: Multiple details of mite SEM images stitched together into a high resolution image.

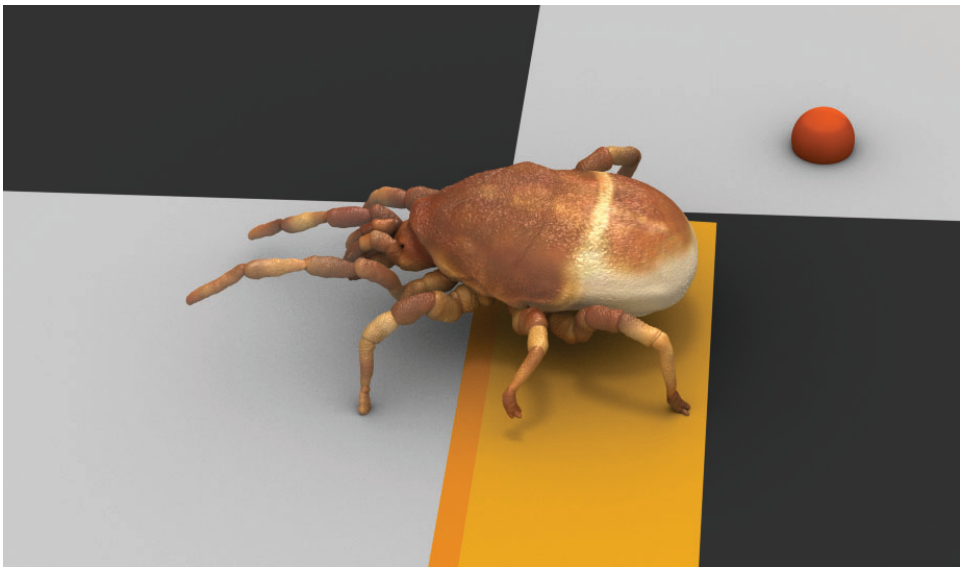


Figure 4.4: Rendering of the 3D model of the *Parasitiformes* mite in a virtual course.

the 9thColloquium on Acarology 2013 in Graz. Furthermore, a fortunate coincidence led to a meeting with Michael Heethoff at this colloquium while he happened to be working with this species. The board of the colloquium invited us to present the paper [53], a poster (compare figure 4.6), and the corresponding computer-animated video material. Throughout this conference, a collaboration was established which led to working with scanning data of the scientific group from Michael Heethoff. This data included two data sets produced in the European Synchrotron Radiation Facility, ESRF located in Grenoble, France.

The use of Synchrotron radiation makes a significantly higher resolution, better signal-to-noise ratio, and short acquisition times possible [17].

The availability of huge data sets made detailed insights into the mite possible. Details are available in the video supplement to the paper [82], wherein the authors suggest the use of Synchrotron radiation for imaging whenever non-destructive imaging of internal fine detailed structures of small samples is of interest.

The two data sets were high resolution and were not pre-segmented, therefore the starting point for the 3D model were raw image stacks. That means segmentation was tested with various semi-automatic segmentation methods. The experiments with suitable software included mostly freeware, shareware and/or open-source packages. The most effective tested software should be mentioned here: ImageJ [165] /FIJI [162], Spiers [189], Drishti [113], Meshlab [29], CloudCompare [63], and UCSF Chimera [147]. The final

Figure 4.5: Light microscopic micrograph of *Archegozetes longistosus*.

choice was ImageJ/FIJI for the segmentation of the *Archegozetes* model. The other models used in the case examples came pre-segmented from Stephan Handschuh as a raw 3D model to the Science Visualization Lab of the University of Applied Arts Vienna.

The semi-automatic segmentation with ImageJ/FIJI along with luminance threshold selection in the 3D Viewer plugin[6] led to very high resolution 3D models which were then carefully reduced in resolution to make them editable. This resulted in an authentic 3D model of the animal. This worked for the segmentation of the whole organism, however, for a repre-

[6] https://imagej.nih.gov/ij/plugins/3d-viewer; 03/11/2018.

Figure 4.6: Poster presented at the 9thColloquium on Acarology 2013 in Graz. It shows a turtle mite in a scenic soil scenery. Image by Industrial Motion Art GmbH.

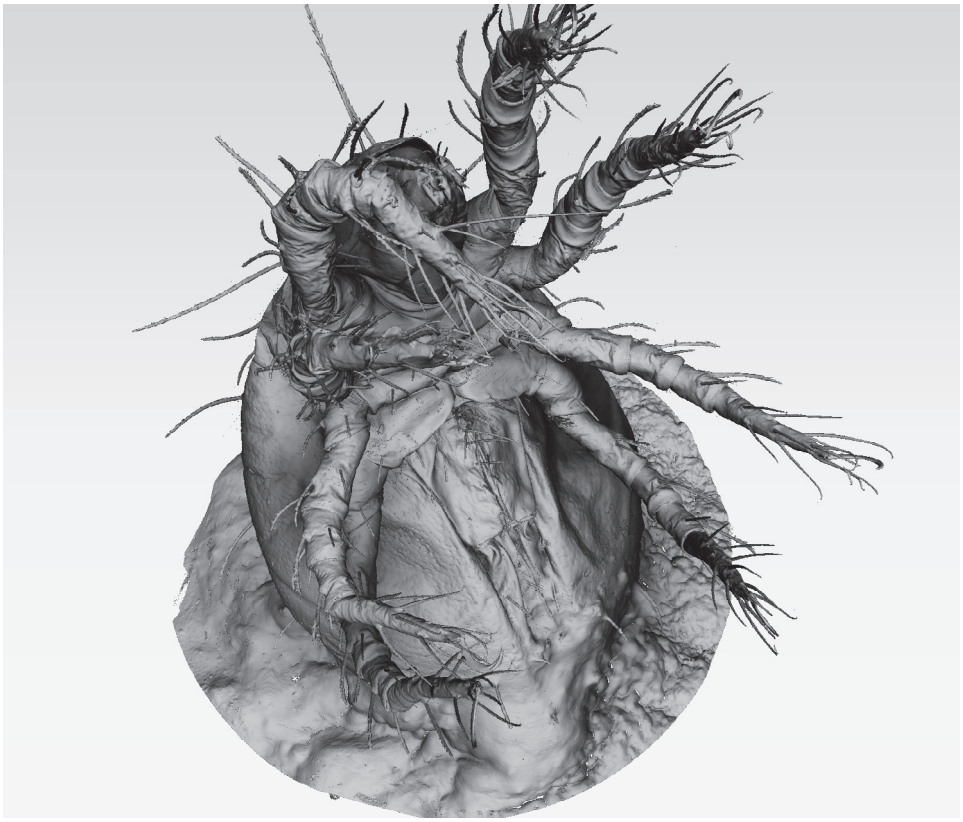


Figure 4.7: High detail tomographic scanned 3D model of the *Archegozetes* mite. The glue site at the bottom is clearly visible.

sentation of specific details of the body, such as the digestion system, it is necessary to consult experienced biologists for segmentation because body parts of interest must be manually selected. The cleaned up 3D models were processed further and became the starting point for the computer animation pipeline. The creation of the 3D model included reconstruction of lost data due to larger glue sites which occurred during scanning preparation since for one data set glue was applied on the rear side and the other had glue applied to the bottom, therefore data were combined into one large data set to restore the lost 3D information of the glue sites. The merging and editing were done in 3D-Coat in Voxel Sculpting Mode[7]. In figure 4.7 the unedited 3D model of the mite that was glued at the bottom is visible in high resolution.

The raw outcome of a simple luminance threshold segmentation, as done with the *Archegozetes* data sets, is generally highly detailed. All details that are in the range of certain grey values become part of the 3D model and

[7] http://3dcoat.com/manual/sculpt/81-voxels; 02/11/2018.

are visible as either model details or noise spikes. Spikes that are the result of noise in the images of a stack are reduced using basic image processing techniques if possible. When it comes to the desired details of a scanned organism, it is far more problematic to choose the correct amount and type of reduction. Features such as bones or hard cuticular structures have clearly distinguishable density and it is possible to segment them with thresholding.

In contrast to the *Parasitiformes* mite 3D model, the *Archegozetes* 3D model included a hair simulation to show the bristles of the animal. The bristles could not be used directly from the scans and had to be modeled and simulated. The focus was on the locomotion of the mites, therefore, there was no major focus on restoring the bristles.

In general, color textures and shading references were acquired with the light microscope. There was the possibility to gather microscopy experience, as the author received mite cultures of *Archegozetes* and a mixed soil organism sample and could therefore use the stereo light microscope from the Science Visualization Lab Angewandte. An outcome of these stereo microscopy sessions were, for instance, the "mite safari" videos[8].

4.1.2 Motion analysis of two mites

The recording of microscopic mites in videos for motion analysis is a challenge. In principle, it is possible to capture videos of such animals using various microscopy techniques, but this is associated with severe limitations in terms of magnification and depth of field. Close details of organisms in this size range can only be displayed with monochrome SEM electron micrographs. Color details and specific movements are therefore hard to capture with conventional methods. In addition, it is unlikely that the animals will present their natural movements on microscope stages with bright lighting specially prepared for micrographs or macro videos. The comparison of the mite gaits presents an approach for using computer animation and various scientific imaging techniques to produce videos and high resolution models of two specific mites to compare their distinct gaits. The focus was on high resolution textures, the natural walks of the animals, and a targeted quantified comparison of two types of mite gaits. The mites should be tracked and the gaits compared in computer animations, both analytically and artistically. The first step to achieve this goal was to acquire the necessary data. For that, a custom tracking setup was developed.

In capturing videos of microscopic-scale animals, the surface of the stage was of great importance. The stage had to be prepared with a surface on which the animals could move naturally. Due to static charge, slickness and hardness of microscope glass slides, the walking movements of mites which should include clawing their claws into the ground, the first attempts to film

[8] https://vimeo.com/72893336 and https://vimeo.com/72815251; 03/11/2018.

the animals failed. Other teams doing such video capturing used adhesive tape for the mites to walk on. We avoided that method because it might influence the natural movements of the animals.

We did some tests with different materials to coat slides for use under the microscope and in other recording configurations. Finally, a custom-made stage using plaster of Paris was prepared. We also had to moisten the plaster, because if it became too dry the static charge occurred again. With wet plaster the shooting worked successfully and the animals were able to walk on a smooth, and still in principle, natural surface. The living mites were brought to the center of the stage with a very fine painting brush to not injure the animals before recording. The image sequences for analyzing the motions could be obtained by recording the mites with this custom setup.

The resulting videos served as the basis for the motion analysis techniques applied, thereby enabling conclusions to be drawn about the movement patterns of the studied animals. The custom setups for mite tracking can be seen in figure 4.8. The setup developed for this project was enthusiastically supported by microscopy technician Helmut Goldammer. We used two Nikon J1 models with an AF Micro NIKKOR 60 mm lens using an aperture 20 and 1/200s exposure time as the primary settings. The videos were shot with HQ settings of the cameras in HD (1920 x 1080 pixel resolution) and 29.97 frames/second. The video recording was started simultaneously with wireless remote control and the live videos could be watched on two HD computer screens while recording for quality control. One camera was positioned above the stage and the other one in the front of the stage in order to record the walking animal from two sides.

The use of computer animation to create motion analysis opens up completely new perspectives. Movements of various kinds and recording methods can be qualitatively and quantitatively transferred. Video sequences of both mites were available. Selected images of the recorded sequences are visible in figure 4.9.

With the acquired data, analyzing and tracking experiments were possible. Image sequences were loaded into the software packages ImageJ and After Effects to segment the silhouette of the mites from the background. These segmented image sequences were helpful in determining the outline shape of the walking mites. Pictures of the experiments to automatically segment the recorded mite video sequences are visible in figure 4.10.

Although we found a way to capture them on a more natural surface, the mites could only be recorded in a setup using a flat surface. That means natural movements involving obstacles could not be retrieved. The project outcomes therefore only show the comparison of two mites walking on a straight surface. For detailed biological locomotion details in their natural habitat soil, a detailed study and follow-up digital simulation of the whole musculoskeletal system would be necessary. One interesting circumstance is the scaling of physical forces and live realities, as [83, p. 3037] pointedly

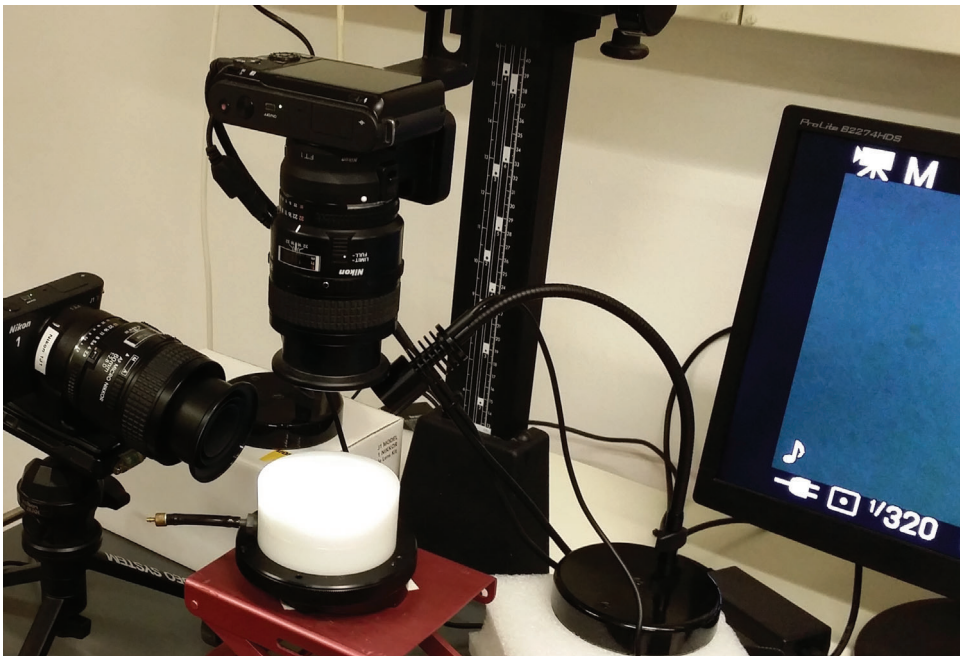
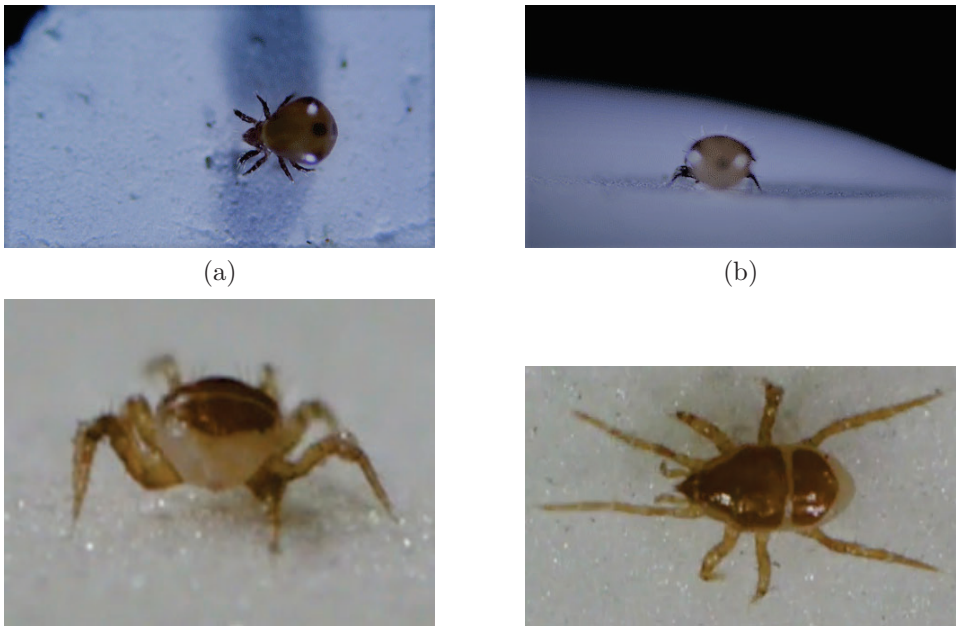


Figure 4.8: Custom mite recording setup with two cameras, macro lenses, and a platform.

describe it:

> Micro arthropods experience their environment as a three-dimensional habitat of interstices and gaps. In this context, small organisms are thought to walk 'through' rather than 'over' a landscape.

Typically, even experts only investigate certain joints of interest and not a whole organism for locomotor system studies. The focus on details of organisms can lead to astonishing facts, and yet holistic knowledge about a single organism might be a lifelong challenge. For further processing, the videos were subjected to image processing in order to create optimal starting material for the motion analysis experiments. First, tracking was attempted with the application of background subtraction. Despite the custom setup and image processing experimentation, the limits of the recording possibilities became quickly apparent, especially with regards to depth of field. In order to guarantee progress of the project, the movement data were determined following these first tracking and motion analysis tests with rotoscoping. Moving passages in which the reference videos provided usable movements for rotoscoping were traced precisely.



(a) (b) (c) (d)

Figure 4.9: *Archegozetes* recording from the top (a) and behind (b). *Parasitus* mite recording from the behind (c) and top (d). Both recordings were acquired simultaneously and used for the gaits analysis.

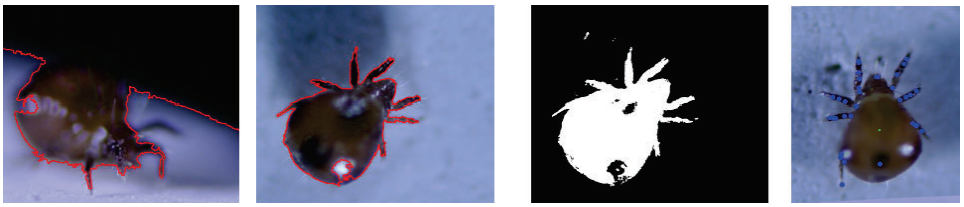


Figure 4.10: Different approaches to segment the *Archegozetes* mite footage.

4.1.3 Visual experimental depiction of mite gaits

The motion analysis should be done in a scientific and an experimental-artistic way. The goal was to open up different viewing angles to the locomotion of mites and present possible solutions for the depiction of tiny animals. The history of zoological motion analysis shows, that one never knows which discoveries might be made possible by scientific visualization. The cognitive aid and trigger of different types of visualization should never be underestimated. The famous horse motion studies of Eadweard Muybridge were interesting not only because of the insights into horse walks.

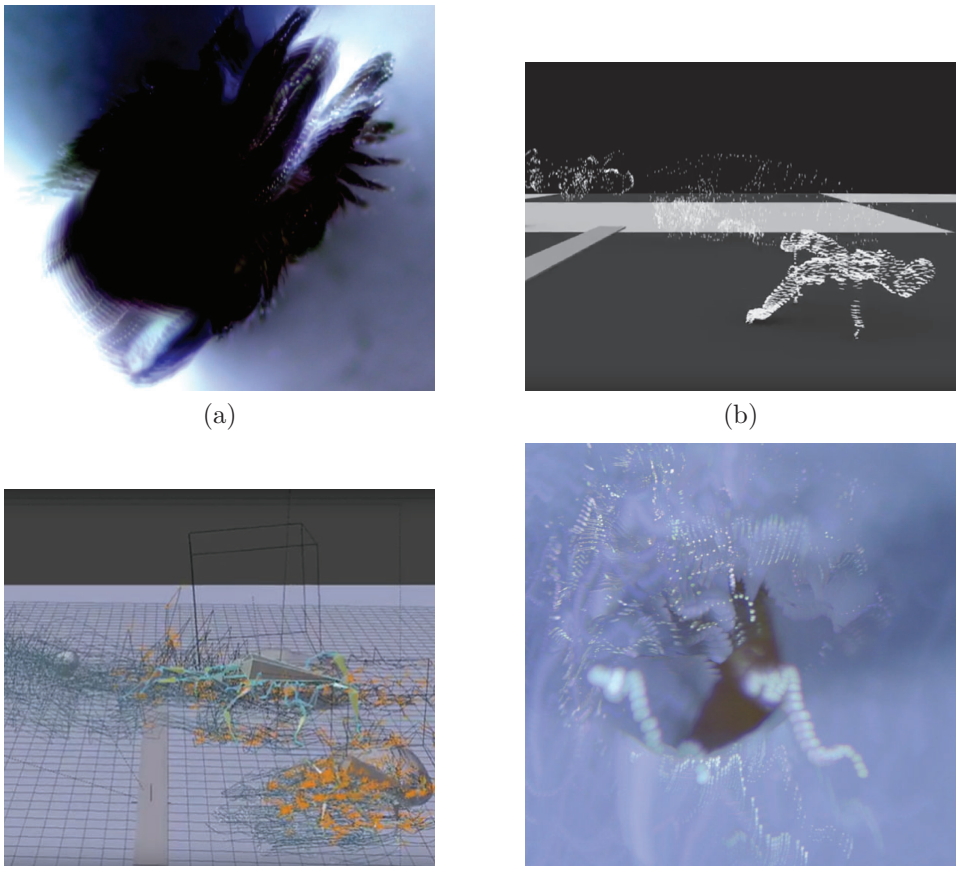
These photographs were also an intermediate step to modern cinematography, thus an important predecessor for computer animation. The aim of this project was to create movement cycles of the mites and to compare them using scientific visualization. For that, the animals should move through a previously defined virtual course. For this purpose, special scripts available for Blender called the Motiontrail scripts[9] for visualizing the movements have been adapted to visualize the different mite gaits in line graphs in 3D space, an image of the application is visible in figure 4.11 (c). The results were transferred to computer-animated models, providing additional perspectives on scientific considerations and the awareness for these species. A selection of the developed visual representations was presented in the video for the exhibition in 2014[10]. While the quantitative analysis output was mainly a table with spatio-temporal coordinates of the manually tracked joints, the qualitative representation included different visual outputs such as acceleration graphs, line graphs for viewing the whole temporal displacement of the animal in one frame, and experimental depictions of the movements through particle traces. The main hypothesis for the visual exploration of the mite gaits were experimental thoughts: What if there is no such thing as time? What if our biological systems simulate time to make it easier for us to perceive reality and space? If so, there would be no movements, there would only be poses in spatial dimensions. Three visual representations of this thought experiment are visible in figure 4.11.

As a continuation of the project described in [53], the *Two Mite Gaits* project continued the investigation of computer-animated scientific visualizations of mites. The animations of the mites were captured and transferred to the 3D models via rotoscoping. The new outcomes are important for understanding the differences in movement in a visual way, however, they do not present new results for the specialized field of mite research. Nevertheless, the animations add new value in terms of computer-animated scientific visualization of different mite gaits. It is the first scientific visualization that prepared these two different mite groups in this manner for a general audience. Research time was mainly invested in the capturing and representation of movements in virtual 3D space. The various movement systems of arachnids, such as hydraulic and muscle-driven, are far from being fully explored, and research about them is underrepresented.

There are, for example, various patterns of movements for different velocities in the gaits of terrestrial arthropods [38], not to mention the motions and joint loads are heavily dependent on the substrate in which the animals live [164].

Due to the complexity of each individual discipline of accumulated scientific knowledge, it is necessary to read into each topic as a creator of scientific

[9] https://sites.google.com/site/bartiuscrouch/scripts/motion_trail; 25/11/2016.
[10] https://youtu.be/0TiAhNQjMHw; 21/11/2018.



(a) (b) (c) (d)

Figure 4.11: Image (a) shows the key frames of the top view tracking video overlaid into one frame, (b) makes the animated models visible through particles that are regularly born on the surface of the animated model over time, (c) shows the output of the adapted Motiontrails script in one viewport camera view over time, and (d) shows the highlights of the recorded tracking sequence from above, again merged into one frame.

visualization. However, this does not mean that scientific visualization creators must become subject matter experts in the respective fields they are working on. An important factor is to exchange ideas with the respective scientists. Open collaboration is critical to present the topics and implement complex visualizations that are beneficial for the specialized field of research or the general audience.

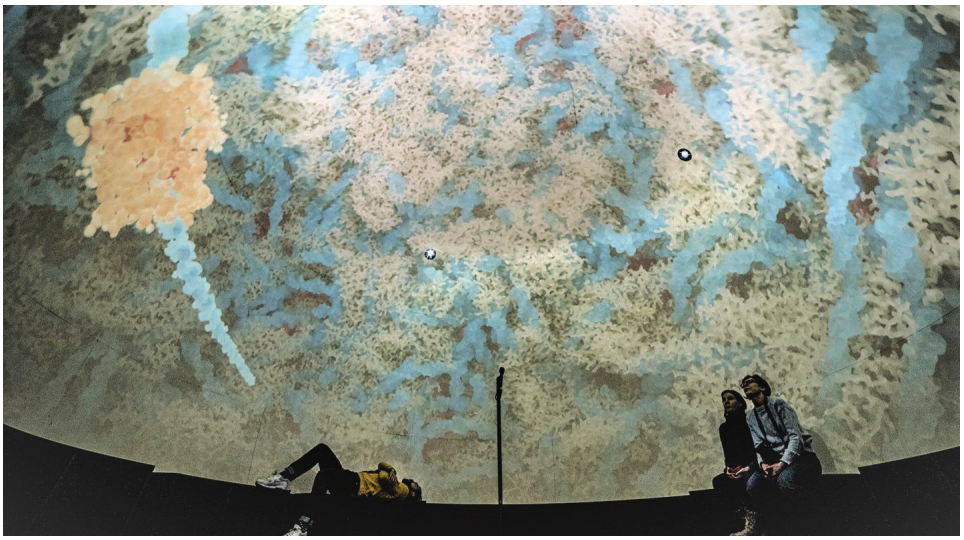


Figure 4.12: Photo by Glenn Bristol of the installation *Future Room* in the 150 years Angewandte jubilee exhibition from 15thDecember 2017 to 15thApril 2018. *CRISPR/Cas9-NHEJ: Action in the Nucleus* was shown as one video in the interactive full dome installation.

4.2 CRISPR/Cas9-NHEJ: Action in the Nucleus

The project started in 2017 and derived from an interdisciplinary creative process. In the following paragraphs, the creation of the scientific computer animation, the usage of data from the worldwide Protein Data Bank (wwPDB, [16]), and the audio-visual representation are explained. Genetic engineering is thought to be one of the most influential technologies of the near future, therefore, the contribution for this topic was carefully elaborated in cooperation with researchers in this field. *CRISPR/Cas9-NHEJ: Action in the Nucleus* is the outcome of a collaboration process between the Vienna BioCenter and the Science Visualization Lab at the University of Applied Arts Vienna. The first showing was exhibited over a period of four months in the Museum of Applied Arts (MAK) Vienna[11]. A photograph of the exhibition can be seen in figure 4.12. Prior to starting, there were already several scientific visualizations of CRISPR/Cas9 available, however, none of them depict the organic structures and chaotic organization of the CRISPR/Cas9 process, nor the NHEJ repair pathways as in the here described animation.

[11] https://www.mak.at/aestheticsofchange; 15/01/2018.

4.2.1 Visualizing data of the wwPDB

The data used for the *CRISPR/Cas9-NHEJ: Action in the Nucleus* project was retrieved with the latest techniques available in scientific imaging. The models used show the representations of single atoms and are therefore noticeably smaller than the details of the scanned plankton and mites in the other case examples. The depiction of molecules requires different scientific imaging strategies than with micrometer sized entities, as described in section 2.2. Up until now, the nanometer size range can not be imaged as accurately as the micrometer range. For scanning the insides of the smallest organism for the *Noise Aquarium* project, the *Cylindrospermum* (cell size 0.5–60 µm), cryo-electron microscopy was utilized. The scanning of macromolecules requires even more elaborate strategies. Structural biologists and biochemists apply methods such as cryo-electron microscopy, X-ray crystallography, and nuclear magnetic resonance (NMR) spectroscopy to scan proteins and retrieve atomic models of them. These techniques typically use a projection to calculate an atomic coordinate 3D model. From the model they add the probability of atom locations and data of previous scans to verify the data sets of entries for the wwPDB. As a result, scientists have experimental data of parts of or the whole molecule structure. For X-ray crystallography they detect a X-ray diffraction pattern, for NMR spectroscopy they collect data on the local conformation and gaps between the atoms, and for cryo EM they determine the basic structure of the molecules[12].

Information about the two used methods for the data sets in the scientific visualization described here, is a topic in chapter 2. As there are massive amounts of structures and differing models of molecular data in the wwPDB, it can be a challenge to find the desired information.

As already mentioned in section 2.2.6, factors such as the R-value describe the uncertainty in identifying the missing portions of the molecule. For the project described here, an overall shape was important for connecting to the reality of the involved molecules of gene alteration in the cells. However, missing atoms were not as problematic for the animation as for the scientists. Accordingly, the collaboration partners sought the best fitting models for the planned animations. It was decided to use the representations of the thus far known major macromolecular proteins involved in the process for visualizing the actions. Three representations are available in most software packages for visualizing molecules. These are wireframe (where lines are drawn for each bond, essentially a digital ball and stick model), Space-filling (which shows the relative size of the atoms with spheres), and Backbone and Ribbon (which highlights abstracted protein chain folds and the typical ladder diagrams for nucleic acids). All three are compared on the

[12] https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/methods-for-determining-structure; 02/11/2018.

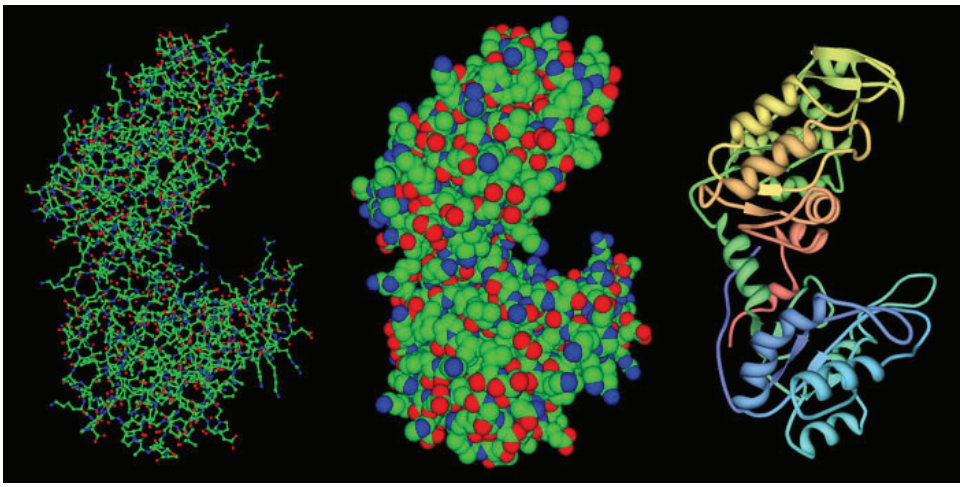


Figure 4.13: Wireframe (left), Spacefilling (middle), and Backbone and Ribbon model (right) of the 3pgk entry in the wwPDB, image source: https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/molecular-graphics-programs.

PDB101 website[13]. These representations are typical in biochemistry and can be seen in figure 4.13. In the described project, Spacefilling models were used directly for the 3D representations of the repair mechanisms, or indirectly converted to an isosurface model as a particle emitter for DNA and Cas9 in the computer animations. Simulations of atom dynamics, chemical reactions, and efficient automatic depiction of new findings in biochemistry are popular research fields. An overview of biomolecular visualizations is given for example in [105]. For the CRISPR animation, no dynamics simulation, visualizing automatism, or helpers for animating the processes, for example as described in [58], were used. This has the disadvantage that the depicted processes may no longer be altered automatically if new findings are available. Then again, neither mass-produced follow-up videos nor chemical simulations were the goals of the project. The objective was rather to depict the gene-editing process in an individually designed abstracted way with a narrative that still conveys the main recent state of research on the processes depicted. Automated visualizations are more common and they serve the mass output of biochemical labs well, while supporting communication of the findings with their real-time output possibilities. Nevertheless, the CRISPR approach combines the opinions of researchers, the unique design of an animation, and experiments in the depiction of protein molecules in a labor intensive animation using sound with mise-en-scène. It has the charm of an incomplete, imperfect, unpolished, hand-crafted, showing of life

[13] https://pdb101.rcsb.org/learn/guide-to-understanding-pdb-data/molecular-graphics-programs; 15/10/2018.

as it is. For the effort to visualize the CRISPR/Cas9 NHEJ process with data of the wwPDB, Protein Data Bank files (.pdb) were imported using the Atomic Blender 1.7 addon[14] from Clemens Barth. This allowed a smooth integration of the data into the computer animation.

4.2.2 The biochemical topic

Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) can be used to modify the genetic code of organisms effectively, especially since the process utilizing nuclease Cas9 was discovered as described in [84]. For the discussed animation, Non-Homologous End Joining (NHEJ) was chosen from the varying repair methods cell nuclei possess to heal broken DNA strands. It is a process that happens naturally multiple times a day in every cell to prevent damage to the cell. The main goal of this project was to create computer animations of the CRISPR/Cas9 process including the latest findings in biochemistry in using the appropriate experimental scientific imaging data. In cell biology, it is particularly difficult to clearly define the locomotion of protein macromolecules for visualization. If these were actually filmed, the individual parts would not be distinguishable and the resulting video would contain mostly noise. Nevertheless, only recently has such a video from the CRISPR/Cas9 process been recorded, as described in [172]. This was only possible due to recent major developments in the field of scientific imaging together with a comprehensive preparation of the nucleases beforehand.

There are still many unexplored processes in cellular protein movements [139], even with the the above-mentioned video recording of Cas9 cleaving a DNA strand, it is likely that there are, for example, several ways in which Cas9 approaches the DNA strand to determine appropriate cutting sites.

Various atomic models of macromolecules are available in the wwPDB. In this database, published crystal structures of the protein macromolecules of various organisms are available. The IDs of the PDB entries, their shape, and color coding in the present visualization are visible in figure 4.14.

The discussed video itself contains no text or voice-over explanations of the visible processes. For somebody who does not at least vaguely know what CRISPR is, only the philosophical voice samples might provide some insight. The basic storyline of the short animation shows the camera approaching the cell nucleus through a rather schematic cell model. The camera then flies through a nuclear pore directly into the nucleus. Inside the nucleus we see the Cas9 complexes forming in combination with RNA pieces. With this information, the Cas9 endonucleases in a complex with RNA pieces are programmed to go to specific sites of the DNA strand and in this case, knock out the predefined (red) sequences. The climax is the "cut" of the DNA strand by

[14] https://en.blender.org/index.php/Extensions:2.6/Py/Scripts/Import-Export/Atomic_Blender_Panel; 05/11/2018.

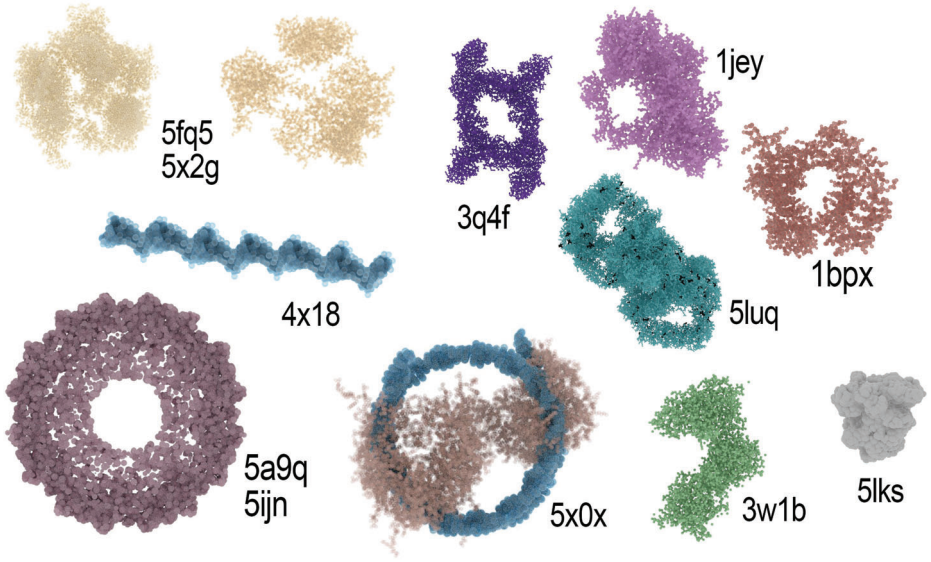


Figure 4.14: Structures of the worldwide Protein Data Bank, 5a9q [6] and 5ijn [104]: Human Nuclear Pore Complex, 1jey [209]: Nuclease Ku, 1bpx [160]: Polymerase, 5lks [131]: Human Ribosome, 3q4f [157]: XRCC4/XLF-Cernunnos Complex, 3w1b [137]: Ligase IV-Artemis Complex, 4x18 [72]: DNA Structure, 5fq5 [140] and 5x2g [219]: Cas9-sgRNA-DNA Complex, 5luq [176]: PKcs, 5x0x [114]: Nucleosome Complex.

the Cas9 proteins. After the main protagonists (Cas9 endonucleases) complete their operation and knock out the marked DNA sequence strand, they leave the "stage". As soon as the DNA is broken, the cell's own repair proteins, nucleases, polymerases, and ligases come into play to rejoin the altered DNA strand and prevent the cell from further damage. The whole process is simplistically visualized, but the unimaginably more complex reality is hinted at by the dense walls of nucleosomes in the background.

4.2.3 Visual concept

The actual density of proteins and the proximity of the chemical reactions that take place inside cells are as intriguing as they are difficult to understand. In order to experience something of this complexity, simplification is necessary. Therefore, creating an animation which shows a particular process might be done in a similar way to scientific micrographs or videos, whereupon in creating a "real" video of biochemical processes, a reduction to the proteins of interest for the depicted process supports the intelligibility, overview, and might be necessary.

The use of color coding for different units in scientific visualizations is advantageous for distinguishing separate components. Colors were not contextually or scale-dependently generated as in [208] but rather picked to achieve a specific, subjective mood that comes into my mind when I think about a human cell. There are no major standards for the color coding of whole protein models in biochemistry visualization, only the colors for often occurring atoms of the elements are defined. The selected colors are personally associated with the relaxed feeling I get when thinking of translucent orange with rosé shining through. The environment map in the Blender scene was an adapted image of a gastroscopy because this was thought of giving the light impression of being inside a meaty cave. The discussed CRISPR/Cas9-NHEJ animation used this method along with visually engaging shading and lighting. The initial data was obtained from the wwPDB, similar to the animations of Drew Berry and Janet Iwasa, or visualizations of Maria Voigt or David Goodsell. In contrast to depictions in Goodsell's Machinery of Life [65], the project described represents something resembling a 'goo of life'. The intention was to try a more organic approach while still obtaining a comprehensible insight into the actual biological processes in action. Therefore, the protein parts are designed akin to jelly clouds with floating locomotion behavior in order to present the organized chaos of life visible and tangible. A pinch of noise and flicker indicates the density of the visible atoms, their movements, and the uncertainty of the positions of the atoms within quantum processes. The visual style was developed using the software package Blender and rendered using the Cycles render engine. Blender gives the freedom necessary for a new visual approach as introduced with this scientific computer animation.

4.2.4 The interdisciplinary communication process

For a sophisticated scientific visualization, an active communication process between the scientists and the visualization team is usually necessary. Of course there are scientists who create their own visualizations, but more elaborate visualizations, such as the one presented here, are usually too time-consuming and training-intensive for working scientists. Therefore, it is more appropriate for various professional reasons to start an interdisciplinary cooperation. Two scientists involved in gene-editing research and the CRISPR/Cas9 process were convinced to contribute their knowledge and advice to the animation *CRISPR/Cas9-NHEJ: Action in the Nucleus*. The involved scientists were Renée Schröder, who leads the department of biochemistry at the University of Vienna and has written inspiring books, and, Krzysztof Chylinski, who contributed to multiple impactful papers, see for instance [95] and [40], and directs a lab helping Vienna BioCenter researchers with successful CRISPR/Cas9 utilization. They shared their knowledge in several sessions and were available for feedback on the anima-

tions. Interdisciplinary communication in such projects is always an exciting challenge.

4.2.5 Presentation of CRISPR

The discussed visualization was commissioned by the rector of the University of Applied Arts Vienna, Gerald Bast, for the *Future Dome* installation during an exhibition of the 150thanniversary of the University of Applied Arts Vienna at the Museum of Applied Arts Vienna. The full dome installation was conceptualized and implemented by Gerald Bast, Martin Kusch, Ruth Schnell, and Peter Weibel. The installation was realized by researchers and artists of the Department of Digital Art of the University of Applied Arts Vienna, who have completed numerous projects using full dome projections. For more information read [26]. In the installation, visitors were invited to choose a tag from an initially visible tag cloud by speaking into a microphone. After selecting a tag, the related video is projected inside the dome. The tag cloud consisted of catchphrases from recent topics of interest and global challenges. A voice track overlaid each video and recited sophisticated texts matching the chosen theme. The soundtrack of the scientific visualization served primarily as ambient sound to enhance immersion. The noise was deliberately chosen in order to indicate the connection between chaos, organization, and life, and to communicate omnipresent intricacy. Complexity in the protein processes was reduced to increase comprehension while remaining noticeable in a subliminal manner via sound. The experimental soundtrack based on pink noise attempted to amplify both the mood and the message.

The scientific animation *CRISPR/Cas9-NHEJ: Action in the Nucleus* should be experienced by the largest possible audiences. The reactions of the recipients in the exhibition were consistently positive, although many people wanted to know what exactly was shown in the animation. Despite the scientific visualization approach in the making of the video, it was the wish of the artistic supervisors not to be educational in the sense of explaining everything using texts or voice-overs, since there should be no distractions from the abstract processes and the complete experience of being inside a cell nucleus. Therefore, the animation gives more of a feeling and visual of what happens in the CRISPR process rather than explaining itself in detail and it is a linear video experience. Nevertheless, a scientific animation should be able to convey the underlying research results. Therefore, a folder could accompany the animation describing the processes in detail as well as explaining the color codes of the crystal structures of the proteins, as shown in figure 4.14. Comparable to a program folder for a ballet, both the undisturbed experience of the animated CRISPR/Cas9 “dance” in all its visual imagery in the cell nucleus, and the possibility to obtain information about the scientific background could be achieved.

The video and concept were selected for presentation at SIGGRAPH 2018 in the poster section, compare [52]. The topic and the animation are extraordinarily well-suited for further development, for instance in a participative performance.

Content is perceived differently in a dome compared to watching it on a flat screen. Inside the dome, movement and depth are able to create the perception of three dimensions while only being a two-dimensional projection. The recipients are not able to perceive the whole picture at once because some parts of the projection always happen behind them. This requires delicate consideration of camera perspective and movements. For a journey into the cell nucleus, the feeling of "being in a warm soft cave" is a factor in increasing the tacit perception that should ideally be associated with the experience of the video. This conveyance of tacit knowledge might be better achieved with a projection in a comfortable enclosed space. The subject of being inside of a cell nucleus is particularly well-suited to be projected in a hemispherical projection space. Entering through a small gate and sitting in a domed "cave" increases the feeling of actually being in an environment such as a cell nucleus. Nevertheless, the versions for flat screens, both the stereoscopic, as well as the regular HD resolution video have their own advantages. They can be shown to even larger audiences because they do not need a specialized full dome setup. That is why after the dome presentation, custom 2D and 3D stereoscopic versions were produced. The sound from the installation was used, however, the framing and animations had to be adapted. Both the 2D version of *CRISPR/Cas9-NHEJ: Action in the Nucleus*, as well as a linear version of project *Noise Aquarium* were shown at a curated exhibition in Singapore at the School of Art, Design and Media at the Nanyang Technological University in November 2018[15].

4.3 Noise Aquarium

The underwater world is fascinating and a popular video and film motif. However, rarely are aquatic creatures smaller than two millimeters depicted. In the following, earlier described general methods to create authentic three-dimensional models of the organisms will be the topic as they were applied to the animals for the case study *Noise Aquarium*. Also, for this project, in cooperation with the University of Vienna and the Veterinary Medical University Vienna, plankton samples were processed. For this purpose, individual animals were prepared and then imaged using tomographic scanning methods. The resulting image stacks yielded a three-dimensional volume model. From this data, the calculation of digital geometry was possible, cf. chapter 3, which in turn after elaborate edits were processed into computer

[15] http://zentrumfokusforschung.uni-ak.ac.at/index.php/understanding-art-research; 15/11/2018.

animations. The peculiarities of creating the individual 3D models and animations as well as information about the project and its presentations can be found in the following subsections.

4.3.1 Objective of the project

The project *Noise Aquarium* deals with the realm of noise pollution in the ocean and the possible effects on plankton. There is a project website available[16] with the latest details and presentation dates of the project. The project team aspires to spotlight tiny species which are tremendously important to the whole ecosystem of Earth and present them as large as possible in installations, on screens and projections all around the world. The creatures can be enlarged enormously for the project, depending on the desired projection size. Through computer animation, the plankton were scaled so that they could be shown side-by-side in one large plankton aquarium.

The *Noise Aquarium* team[17] chose the biome of "floaters in the water" because plankton serves as one of the primary basis of the marine food chain, and are as a result a crucial component of the Earth's ecosystem. In the long run, considering our dependence on natural resources, it is essential that plankton survives. This importance is not fully reflected in the amount of scientific research being performed about the plankton biome up until now.

Noise Aquarium has presented and will present tiny plankton creatures on big screens in numerous international locations in order to create awareness about these essential life forms and the threats they currently face. In order to establish a connection to the reality of the actual organisms, a scientific collaboration was established to use tomographic scans to acquire data of actual plankton for the computer animations of the project. The newly refined methods to transform the gathered information and data into scientific visualizations enabled the presentations to become more than artistic happenings. They were showings of both art and science. The continuous process of content creation for the various presentations was meant to lead to a steady growth of the project. The team members contributed their expertise and will continue to support the presentations of artifacts of actual living organisms. This happened and will happen in various extents and setups such as linear computer animations as well as in interactive settings. The topic is a major environmental issue that is sadly often overlooked by the general public. This was pointed out by [2, pp. 1–2]:

> The ocean dominates the surface of our planet and plays a major role in regulating the biosphere. For example, the microscopic

[16] http://noiseaquarium.com; 01/06/2017.
[17] http://noiseaquarium.com/collaborative-team; 08/05/2017.

> photosynthetic organisms living within provide 50% of the oxygen we breathe, and much of our food and mineral resources are extracted from the ocean. In a time of ecological crisis and major changes in our society, it is essential to turn our attention towards the sea to find additional solutions for a sustainable future. Remarkably, while we are overexploiting many marine resources, particularly the fisheries, the planktonic compartment composed of zooplankton, phytoplankton, bacteria, and viruses, represents 95% of marine biomass and yet the extent of its diversity remains largely unknown and underexploited.

Plankton calcifying organisms maintain the total alkalinity in seawater and the ocean acts as a major CO_2 sink to store anthropogenic emissions [28].

The ocean cannot be considered a flat blue surface that serves as dumping zone where we can let all of our anthropogenic remains vanish. There are a vast amount of organisms living down there who suffer due to our waste and noise pollution. Generally, anthropogenic xenobiotic pollution of the seas is widely known. The project *Noise Aquarium* deals with unnatural noise in the ocean as a further environmental issue.

When it comes to studies demonstrating the impact of the anthropogenic noise on the ocean, mostly striking examples of largely visible ocean fauna are used, for example, by [121, 181] or more generally, the whole ecosystem was addressed by [21, 217].

However, not many studies have investigated the possible impact on marvelous microscopic organisms such as plankton, for example, [185]. *Noise Aquarium* highlights animated 3D models of the extremely diverse plankton spectrum obtained utilizing expensive scientific imaging techniques. The project offers a species-rich bizarre idyll as well as visual attractiveness, thus evoking interest and attention for these important creatures and their disturbance through noise pressure waves. *Noise Aquarium* aims to awaken awareness for biodiversity and introduces a collection of accurate 3D models as a resource for research.

The project's past and future presentations have occurred and will take place in multifaceted ways, since varied locations and time add context for project presentation parameters that influence the events. The project team aimed to show the content in various interactive and linear installations and presentations. The species depicted in *Noise Aquarium* and the making of their digital models will be topic of the following sections.

4.3.2 Creating 3D models of plankton

Generally speaking, plankton is a group that includes megaplankton up to sizes of two centimeters, for instance, some types of jellyfish, to pico and

femtoplankton in sizes as small as 0.2 µm including viruses and phages [184, p. 9].

The etymology describes the property of this divergent group. The term "Plankton" is a loanword from German and has its roots in the Greek word "planktos" which stands for "wandering, drifting"[18]. The organisms shown in *Noise Aquarium* are all part of this varied group.

Like most computer animations, an art installation is always both a technical achievement and an artistic piece. Many people were involved in the realization of the different *Noise Aquarium* versions and showings. The computer animations and all pipeline steps after the sample preparation, scanning, and raw model export were done by the author.

Organism selection involved discussions with international experts on plankton. Particularly *Amoebae* and *Paramecium* were the reason for discussions since these species might not be present in the ocean. Nevertheless, the artistic and scientific heads of the project decided it would not be an issue for the scientific visualization background of the artistic installation to show *Amoebae* and *Paramecia* as part of the plankton flock. The selection for the flock was always meant to be a selection of outstanding and diverse plankton organisms rather than a depiction of a realistic mix of species of a certain habitat. At the beginning of the project, there were already three species (*Paramecium*, *Amoeba*, and *Cylindrospermum*) finished. As the workflows are very laborious and costly, we discussed with the collaborating biologists if we could include them into *Noise Aquarium* or if it would be totally scientific wrong to do so.

We found evidence that *Paramecia* can indeed survive in salt water (compare [182] and *Amoebae* are found nearly everywhere on the planet[19] including in the Pacific Ocean [206], not to mention the *Cylindrospermia* in the seas [143].

Based on the above mentioned points, it was therefore decided to include these three organisms into *Noise Aquarium*. Furthermore, the project should be seen to be about all bodies of water and not only the ocean.

The first steps in the generalized pipeline described in section 3.2 referred to as "preproduction" are sample processing and image segmentation. The samples of the subjects for *Noise Aquarium* had some uniquely specific steps in their processing. The preparation of the living specimen was performed with workflows for soft-bodied species. The preparation chemicals and techniques, as well as the origin of the samples, can be found in Appendix A. Preparation was done according to [167] and micrographs were taken by Thomas Schwaha. Scanning and segmentation were done by Stephan Handschuh. Much of his expertise used for the scans can be looked up in [73].

[18] https://www.etymonline.com/word/plankton; 02/02/2018.

[19] http://www.bms.ed.ac.uk/research/others/smaciver/Ecology%20of%20the%20Amoebae.htm; 24/07/2018.

The connection to the organisms from our environment through scanning, as well as the special position in depicting reality via computer animations is rare to find in the art scene and seldom done in scientific inquiries. Not only do scientists appreciate the value of scanned 3D models, the advantages of using them in scientific visualizations are numerous. In making microscopic videos, only very restricted camera movements are possible. Photogrammetry of animals is gaining traction as a tool to generate 3D models for free camera movements, as well as the possibility to view species in three-dimensional space, for instance, see [130]. Micro photogrammetry is also becoming increasingly useful, as [128], [195], and [135] describe. Fully or partially automated 3D modeling approaches are a major field of research. Micro photogrammetry can be used for the depiction of the surface of organisms, see for example [134]. Though, for our approaches, it was always of interest to scan the innards, which is not yet possible with photogrammetry. Furthermore, there are some problems with reflections, small details like for example hair, resolution, and transparencies in photogrammetry.

Photogrammetry works for some fields of research but for small species recognition and determination it is often not precise enough [20].

The microorganisms depicted in the present case studies have both reflective surfaces, as well as they are partly or fully transparent, tiny, and have intricate body parts. Therefore, a micro tomographic scanning process for modeling was more suitable. The here introduced usage of micro CT scans in combination with other scientific imaging methods was a successful approach. Also of note, the intestines of each animal needed to be recorded. For this purpose, the internal organs of the organisms processed were also modeled precisely using micro CT or confocal microscopy scans. This opened up unique possibilities for representing internal processes such as how particles flow through the body.

For a project with micro tomographic scans, there are several steps needed to edit the acquired volumetric data and to prepare them for use in computer animations. If the animal is to be processed as a high resolution model, the goal is to preserve as much data as possible while still creating an animatable polygon model.

The scientific imaging devices used for the case studies in this thesis were confocal laser scanning microscope, transmission electron microscope, and micro computed tomography to obtain tomographic data sets, as well as light microscope and scanning electron microscope for additional scientific imaging data. An overview can be seen in figure 4.15. The image stacks used for the here described pipelines were generated with a combination of optical and virtual sectioning techniques. Micro computed tomography stacks are derived from virtual sections, as described in [124], while confocal microscopic stacks were optically sectioned as [175] describes. The diverse techniques were introduced in section 2.2.

An important part of a project like this is the collaboration, constant

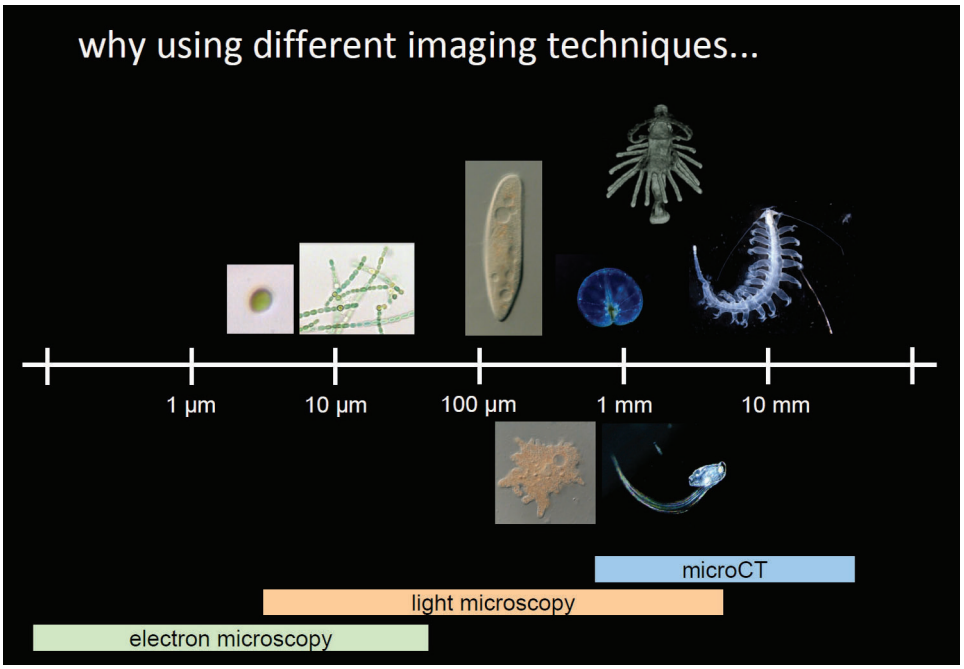


Figure 4.15: Various imaging techniques that were used for different specimen sizes for the case example *Noise Aquarium*. Image by Stephan Handschuh.

feedback, and communication with the field experts in the project. This has resulted in the paper [53]. The interdisciplinary approach with scans obtained from subject matter experts has many advantages and guarantees the most authentic results possible.

All creatures in *Noise Aquarium* are soft-tissued organisms. Therefore, threshold segmentation can only be a starting point for a semi-automatic approach to isolate specific animal parts. That means an expert who has knowledge and experience in anatomy should manually or semi-automatically segment the image features of interest from the ones that should not be in the resulting 3D model.

Another example, in which collaboration is of enormous importance to this project, are all cases in which scan artifacts needed to be corrected. In biology, it is generally assumed that the scientific publications and results are consumed primarily by subject matter experts, so they often do not go into detail about various phenomena such as drying artifacts or crystal formation on surfaces. However, if the scans are to be processed by computer graphics engineers or designers, it is important to clarify these peculiarities. In our project, this was accomplished with a number of feedback sessions.

Trained biologists might label individual organs selectively and export portions of organisms separately in 3D models. This has the additional ad-

vantage that body regions are provided as individual 3D models by the imaging experts with experience in segmentation of imaging data. The first steps performed in the Science Visualization Lab were the repair of broken features of the organism, removal of artifacts, and then retopologization. These editing steps were taken with care and had to be often carried out manually.

In the pipeline, samples of one organism arrived as digital data set at the computer graphics designers workplace. Then, the data was reviewed with the imaging experts to clarify what might be deformed or otherwise not correctly imaged in any manner. Additional light microscopic reference material offers additional clues to the original look of the imaged organism.

In the following sections, some key features of the portrayed microscopic animals will be described. These subchapters only reflect a brief presentation of the animals discussed in *Noise Aquarium* and the creative process of creating the 3D models. They try not to give a thorough description, but rather serve to primarily introduce the most interesting aspects of the animals, as well as the creation process of their virtual 3D representations. Many body parts, behavioral patterns, and biological processes of plankton are at most scarcely explored or completely unexplored by biologists. The chapters about the plankter summarize known remarkable details about the organisms which were the reasons why the project team chose these species in particular for the projects. There were also plans to visualize complicated processes in the innards of the organisms. Detailed biological information, new findings, and detailed visual descriptions might be a topic for future projects and publications.

Actinotroch larva

The first sample for the *Noise Aquarium* was the so-called *Actinotroch* larva, see a micrograph in figure 4.16. It is a planktonic free drifting larva. In fact, *Actinotroch* is one of the largest larvae in the ocean (0.6 mm to over 2.5 mm) and belongs to the horseshoe worm family (Phoronida). It has an umbrella-like upper body region, a middle body collar, and a ring of tentacles. The metamorphosis is unique and swift, it lasts 15–30 minutes, before this transformation an *Actinotroch* lives among plankton[20].

The Phoronida larva *Actinotroch* is a remarkable creature among marine invertebrates. *Actinotroch* live among plankton for a period of 17 to 20 days and are constantly growing new tentacles in this period of their life [34]).

After that, they undergo dramatic metamorphosis, in which according to [192], parts of the larval body being eaten by the developing worm.

The longish pear-shaped body with a cap-like upper part and radial arranged cilia [86] might remind some people of squids.

[20] http://www.imas.utas.edu.au/zooplankton/image-key/phoronidae; 21/07/2018.

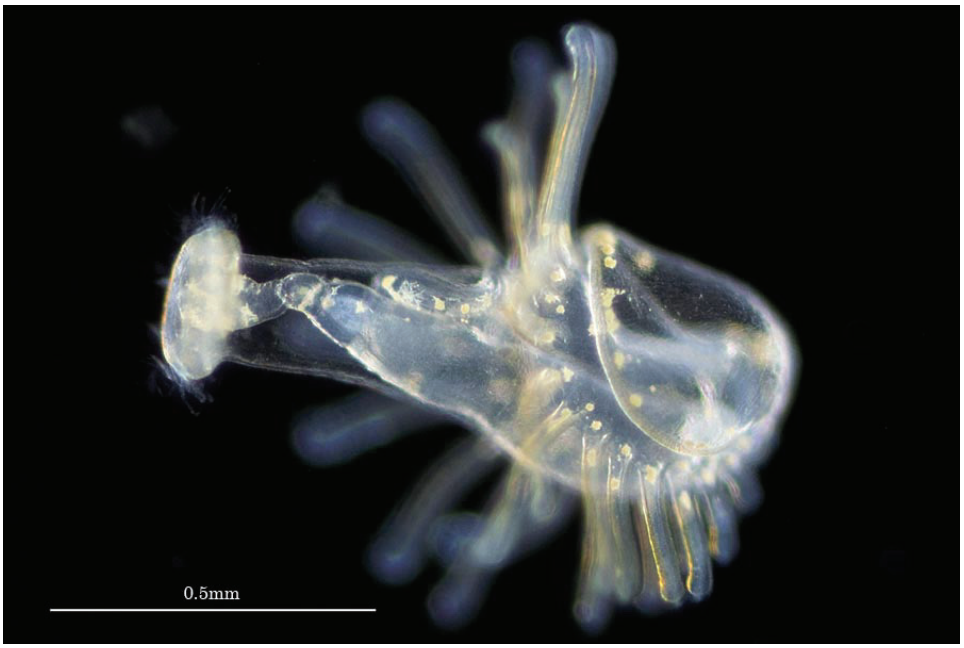


Figure 4.16: Micrograph of *Actinotroch* larva by Thomas Schwaha.

In [192], the Phoronida life cycle and the metamorphosis are described in detail and an additional time-lapse video[21] is available. An animation of this metamorphosis would be a motivating challenge and might give extra insights into the transformation process of this organism, but has to be reserved for projects with more focus on that individual animal.

In the here presented case study, an *Actinotroch* that had reached a developmental stage with a trunk, the cap-like hood, and the distinctive tentacle ring is depicted. It already had a fully developed intestine and would feed on small algae but did not enter the metamorphosis stage yet.

The main data from *Actinotroch* larva received from the scientific imaging experts were two image stacks. One image stack was the outcome of a micro CT scanning session which resulted in 1441 images with a voxel size of xyz 0.535 µm and the other one of light microscopic imaging resulted in an optical image stack of 654 images with a voxel size of xyz 0.4 µm, one image example of each can be seen in figure 4.17.

For the *Actinotroch* model, 3D data exported as Wavefront Objects (.obj) were available. The Wavefront Object data was split into two files, see figure 4.18. The imaging expert and collaboration partner Stephan Handschuh scanned, segmented, and preprocessed the data of the image stacks to pro-

[21] https://figshare.com/articles/Additional_file_2_of_Metamorphic_remodeling_of_morphology_and_the_body_cavity_in_Phoronopsis_harmeri_Lophotrochozoa_Phoronida_the_evolution_of_the_phoronid_body_plan_and_life_cycle/4417646; 23/22/2018.

Figure 4.17: Single image out of micro CT stack (left) and one of the optical image stack (right) of *Actinotroch* larva scanning by Stephan Handschuh.

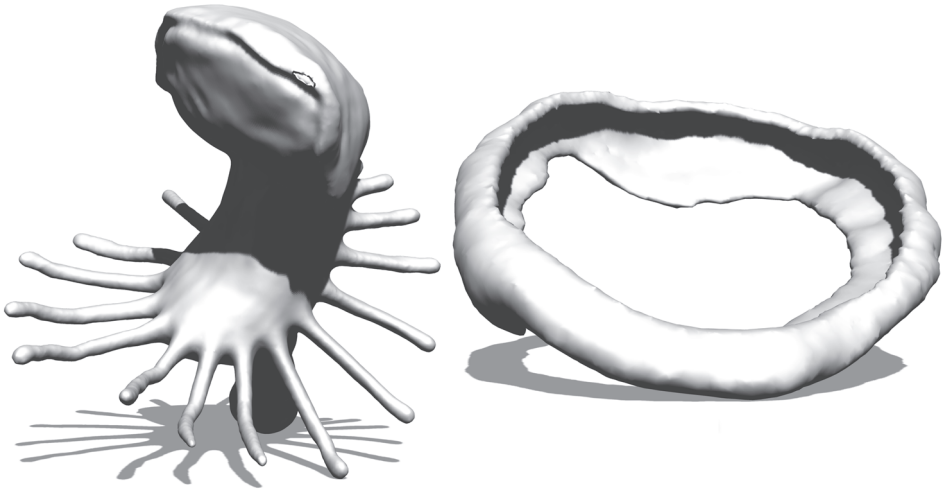


Figure 4.18: Images of Wavefront Object files rendered with Photoshop, *Actinotroch* at the left, *Actinotroch* cilia ring on the right.

duce this 3D data set.

Video references taken with a light microscope to see how the larva moves were provided by Thomas Schwaha. Three videos represented by frames in figure 4.19 of different larval stages were used. Since *Actinotroch* is a larva, there were only samples of slightly different stages of development as motion references available. One video of Phoronid *Actinotroch* is also available at Thomas Schwaha's YouTube channel[22].

The throughout informative video *Phoronis muelleri* (*Tentaculata*) –

[22] https://www.youtube.com/watch?v=9UQ-r3Jho1s; 05/10/2018.

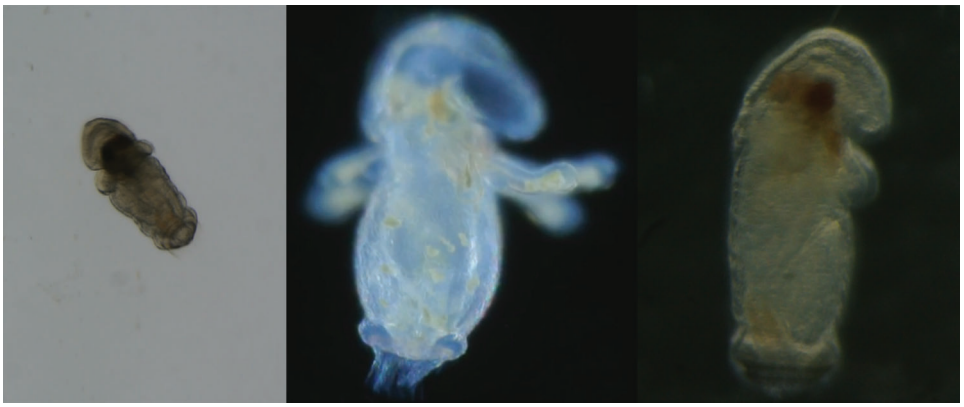


Figure 4.19: Selected cropped frames of light microscopic video references of different larva stages of *Actinotroch*. The references show different larval stages than the scanned subject, with the middle image being closest to the developmental stage of the probe. The videos were taken by Thomas Schwaha

Embryonalentwicklung[23] was also a resource for knowledge about the larva.

Scientific papers consulted for references about the *Actinotroch* larva were [193], [192], and [86] on the metamorphosis, plus [153] on the tentacles and their cilia. In the beginning, for general reference and an overview of the 3D data sets, volume renderings of both image stacks were created. Frames of these renderings can be seen in figure 4.20.

As described in chapter 3, the 3D models had to be corrected due to various scanning artifacts. A comparison of the model before and after corrections can be seen in figure 4.21.

Feedback sessions with experts are necessary to maintain an accurate model of the imaged subject after corrections are completed. In figure 4.22 detailed feedback for the head and symmetry of the animal are visible.

In figure 4.23, notes from an important review session concerning the anatomical features of *Actinotroch* can be seen. The sketch refers to a problem out of the field of Discrete Tomography called “banana curvature problem” in the sketch in German. See also chapter 3.2 for a description. The sketch contains notes from the paper [153] on *Actinotroch* cilia. The scribbles note anatomical features of the animal and indicate that it feeds on phytoplankton. These quick notes might seem trivial but they also represent something critical in the creation process of authentic 3D models using tomographic scans. The condensed meeting notes are valuable documents of the interdisciplinary thinking process two people of different disciplines achieved together.

Preparation shriveling of the subject sample had to be removed to create an authentic model ready for animation. In figure 4.24 the overall structural

[23] https://av.tib.eu/media/11362; 22/11/2018.

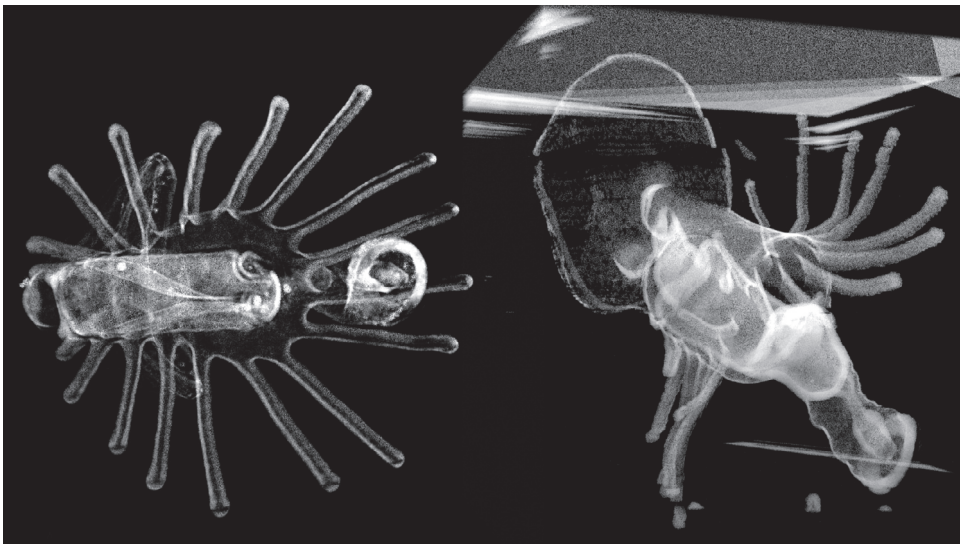


Figure 4.20: Direct volume renderings created with Blender Internal Renderer of two different tomographic stacks showing scans of the same sample. While the left image shows one frame of the rendering of micro CT scanning data, the right image shows one frame of the volume rendering of the optical stack.

changes can be seen in direct comparison. Repairing of the 3D geometry was done with 3D-Coat and Blender. The main restructuring was necessary for remodeling the deformed umbrella-shaped head and the crooked trunk.

As described in the general description of the 3D modeling process in chapter 3, the topology that comes with automatically meshed 3D models is not optimized for computer animation through rigs. Retopologization has to be done in order to achieve the desired polygon topology that will allow the geometry to deform as desired under the weighted deformation with rigs (Armature Modifier in Blender) in the 3D animation software. Sometimes guided or automatic retopologization[24] can produce useful topology and therefore save some hours of work. In the case of *Actinotroch*, the algorithms behind semi-automatic retopologization did not achieve the desired geometry, in particular, the tentacles were not generated correctly. That is why a second attempt had to be made. The first retopo version was an automatic trial with the retopo tool in 3D-Coat. This tool is generally very helpful to retrieve useful topology after a digital sculpting process. The first and the second attempt of retopologization can be seen in figure 4.25. The automatic retopo did not work because of the high detailed shapes of the tentacles and the innards. For the *Actinotroch* model, no texture and UV Texture Coordinates were created since the animal did not require skin struc-

[24] http://3dcoat.com/manual/retopo/248-autopo; 03/11/2018.

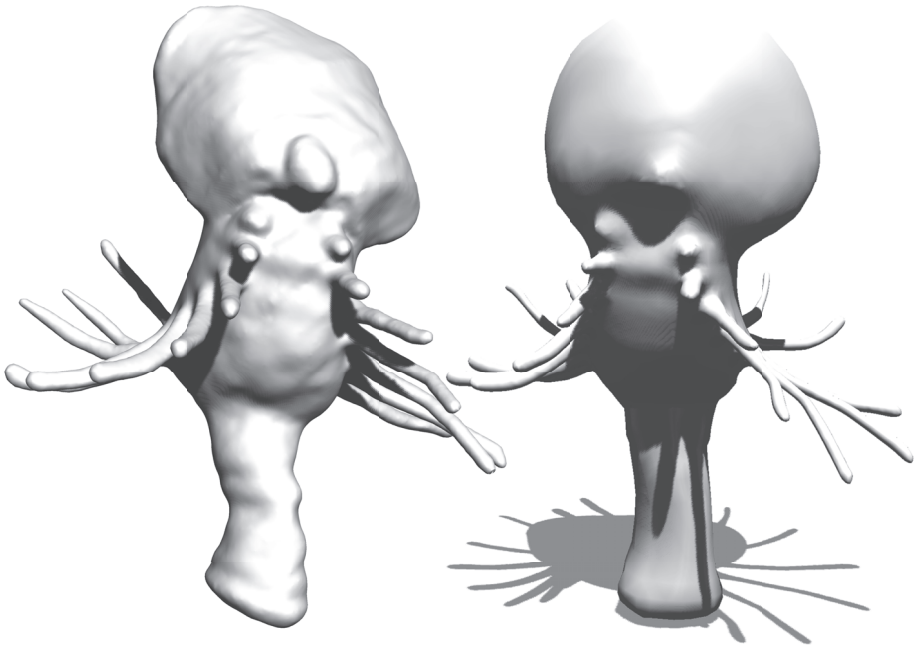


Figure 4.21: Screenshots of the models before sculpting on the left, and after on the right. The necessary corrections of the geometry were mostly performed in the software 3D-Coat.

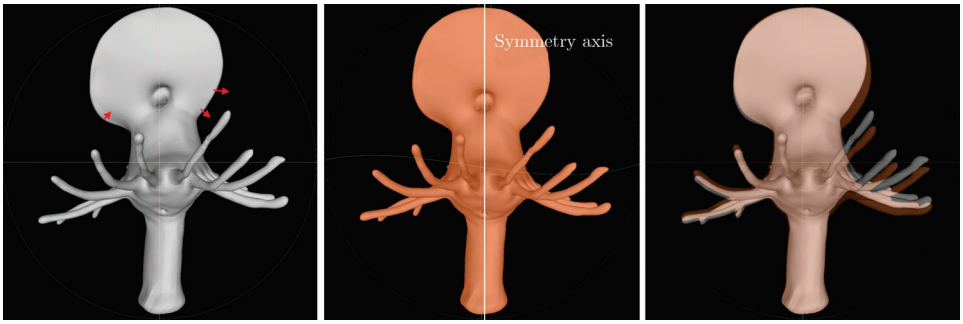


Figure 4.22: Visual feedback on the corrections of the overall shape and symmetry of the corrected *Actinotroch* model. On the left is the state before the feedback; middle and right image show how it should look corrected.

tures or patterns to look authentic. Also, highly detailed skin tissue scans were not available as references.

After determining the flexibility and general movements according to the references, a bone rig was created to animate the 3D model true to the videos. The rig is simple and has few special bone constraints. The umbrella-shaped head section has Copy Location Constraints to allow more efficient

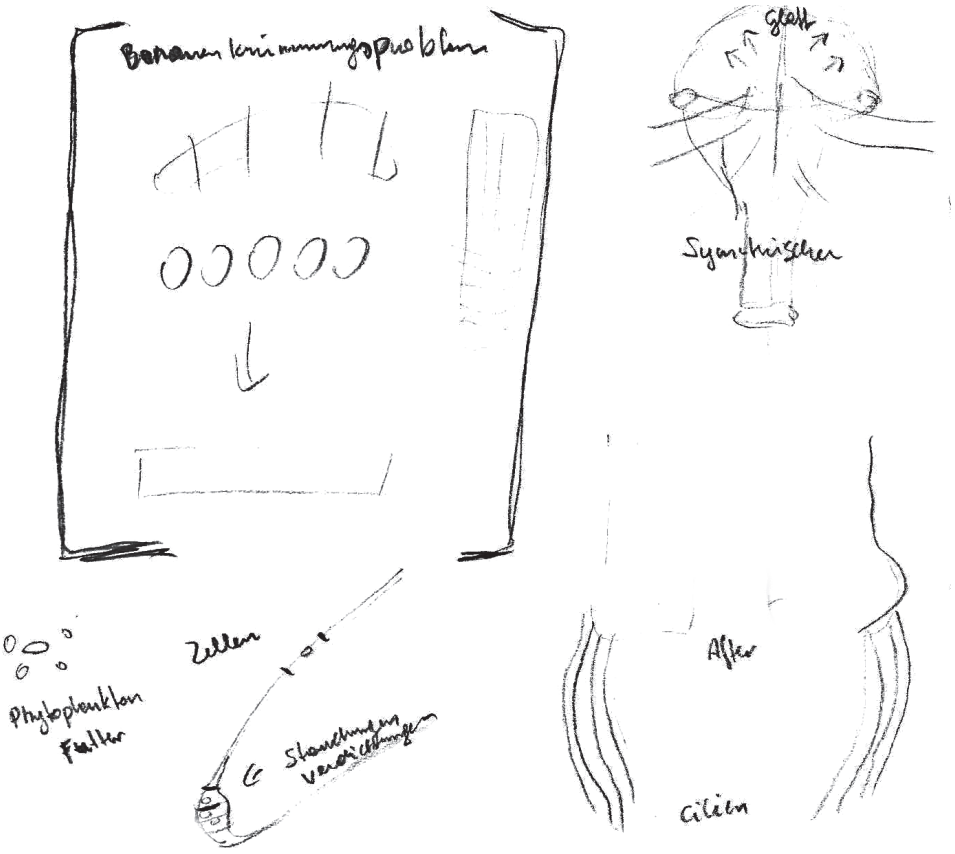


Figure 4.23: Scribbles of an important meeting discussing anatomical features of *Actinotroch* larva and general problems with tomographic imaging.

animation of this part of the model. In figure 4.26, the created digital bone setup can be seen. No Inverse Kinematics spline constraints were used for this bone rig, only a hierarchy of the above mentioned constraints to copy the offset location.

According to the video references, all the *Actinotrochs* have typical convulsions and their cilia move in patterns. In figure 4.27, a flat rendering of an image sequence with cilia moving according the paper [153] is visible. An animated texturized (generated Marble Texture with Soft Noise, Parameter Size: 3, Depth: 24, Turbulence 23) Force Field was used to make the cilia move in wave patterns towards the oral grove to create a stream that transports nutrient particles into the digestion tract of the animal. The animations for *Noise Aquarium* were created through a rotoscopy animation process (cf. section 3.5.1) using a selected partial passage out of the video available on Thomas Schwaha's YouTube channel.[25]

[25] https://www.youtube.com/watch?v=9UQ-r3Jho1s; 22/11/2018.

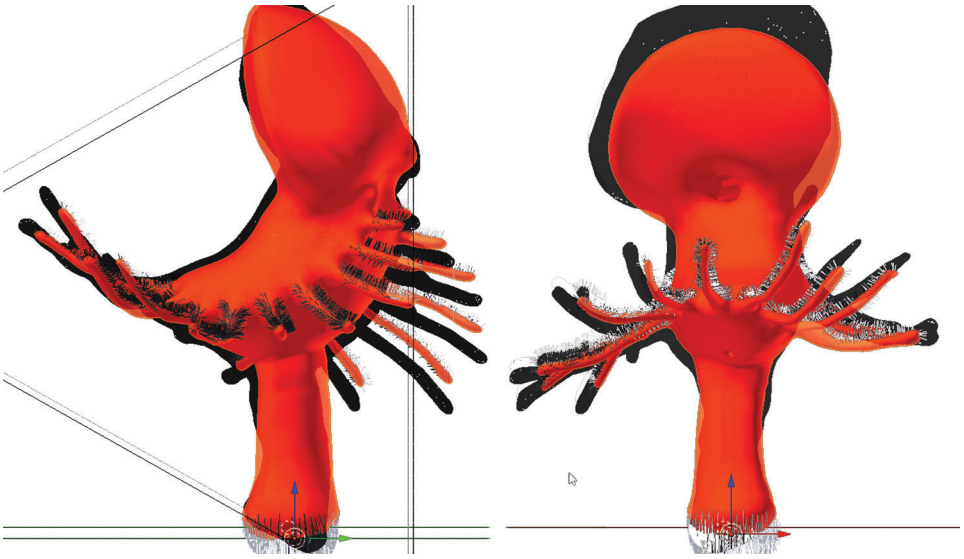


Figure 4.24: Direct comparison of two 3D models, frontal and side view, in Blender. Screenshots of the original Wavefront Object exported from Amira (black) and the final repaired 3D model (orange).

The shading of the final 3D model was done according to the light microscopic references. Several shading tests with various materials were performed while the environments for the use case were selected. The shading configuration in the node tree of Blender for the Cycles render engine consists of varying combinations of glass, emission, glossy, and transparent shaders. For *Noise Aquarium*, a lossless encoded image sequence (PNG) containing the alpha channel of the animation for the compositing of various versions of the project was rendered.

Amoeba

Amoeba is a single-celled organism that moves in a typical amoebic way. It is usually 220 to 760 µm in size. The cytoplasm is granular and often contains crystalline particles. The nucleus is discoid or ovoid. There are also *Amoebae* with several nuclei to be found in nature.[26]

The organisms live in fluids or wet places and are frequently found in suitable habitats.

Amoebae were chosen for the project *Noise Aquarium* for numerous outstanding reasons. One main research interest of *Amoebae* is the possibility that mitochondria evolved from ingested bacteria that did not die when they were indulged, instead starting a symbiotic existence with their former predator, cf. [68]. Also, *Amoebae* are model organisms for research about

[26] https://www.arcella.nl/amoeba-proteus; 22/11/2017.

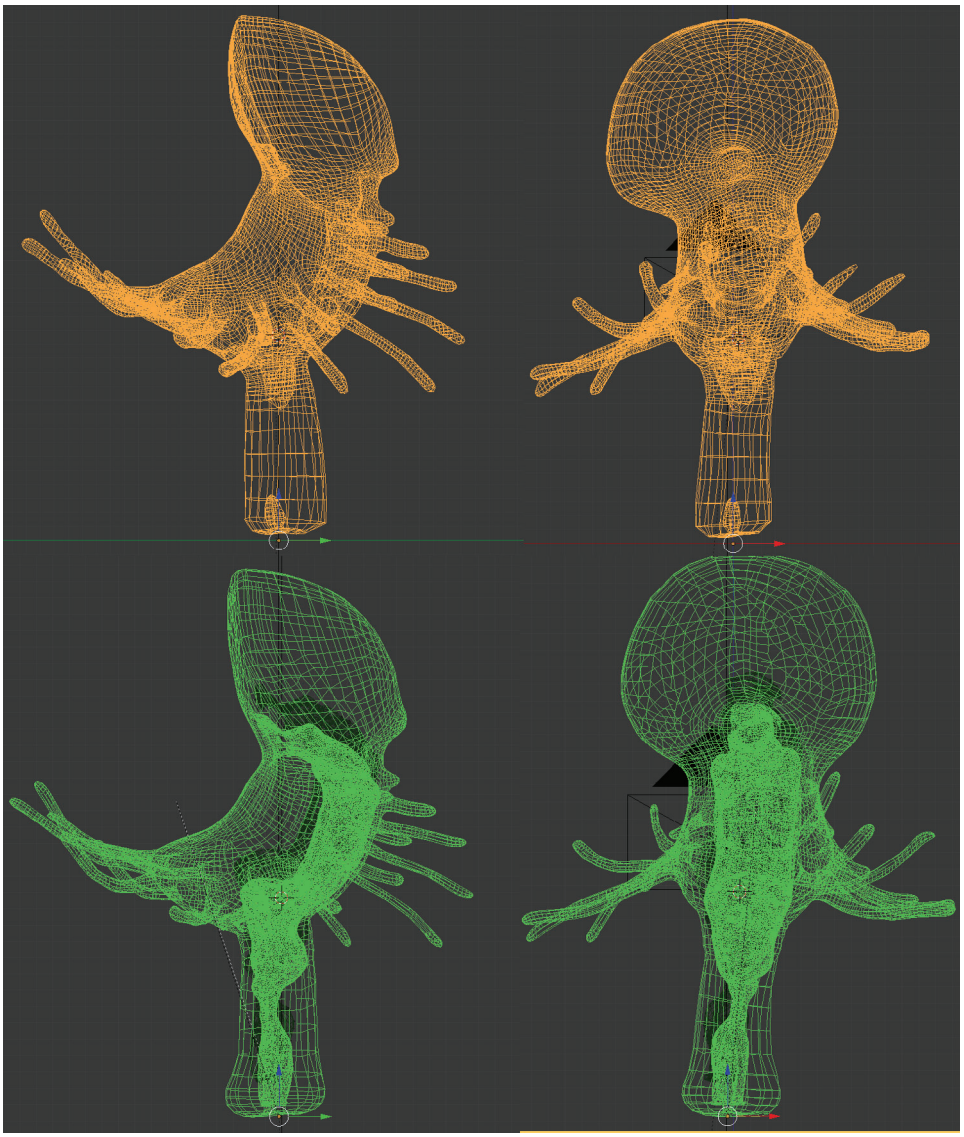


Figure 4.25: The first, above, and the second attempt, below, of the retopologization of the *Actinotroch* 3D model. Clearly, in the first version, there were some problems in the geometry, especially with the edge loops of the innards.

cooperative behavior and altruism, cf. [112]. One of the species of the family *Amoebae*, *Entamoeba histolytica*, causes the disease Amebiasis. In German, *Amoebae* are also called 'shape shifters' because of their shape shifting properties and ability to form pseudopods. There is ongoing research about *Amoeba* movements for example, as [3, 32, 129, 213] have published. The simulation of *Amoeba* cytoplasm and membranes is a complex topic.

The *Amoeba proteus* model is, like all 3D models presented in this thesis,

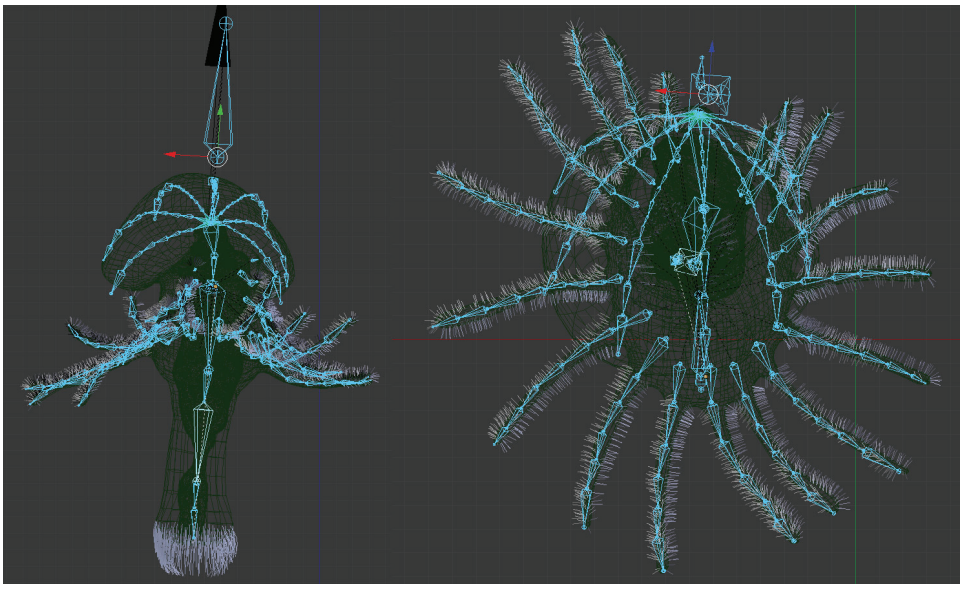


Figure 4.26: Screenshots of the back view, on the left, and the top view, on the right, of the final *Actinotroch* 3D model with the digital bone setup in Pose Mode with blue "digital bones".

based on tomographic scans. In the case of an *Amoeba*, the shape is very fluid and flexible, therefore the outer shape of the scanned animal is to be understood only as a template. In the light microscopic image stack, typical details of the organism are visible. One representative image of the stack shows a cross section containing many inner details of the animal (cf. figure 4.28).

The nucleus and vacuoles of varying sizes were adopted from the scans and were additionally filled with generated particles since the insides of the scanned organelles were too small for the required scanning resolution. Due to the undefined shape of *Amoeba*, the scan can only be understood as one of many shapes the organism can form. The original scanned model was used as the starting material for a variety of simulations of generated plasma particles. The implementation in Blender turned out to be a challenge. The number of particles at the beginning of the project in 2015 was the limit of the calculable particles with the available workstation. Consequently, only slow progress was possible and the handling needed undue patience. The setup of the scene had to be therefore designed according to the software and hardware restrictions. The initial model supplied by the biologists consisted of the individual Wavefront Objects of the scanned animal's outer membrane, as well as the inner organelles and can be seen in figure 4.29. In order to achieve a particular density and the amoebic flow of granular particles as authentically as possible, particle systems were combined to simulate

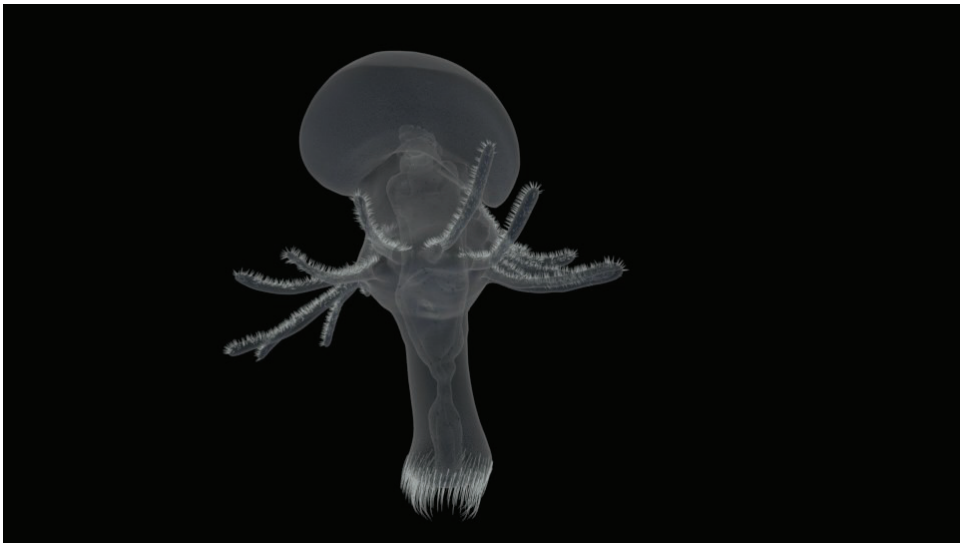


Figure 4.27: This figure shows a flat rendering of the *Actinotroch* 3D model for Noise Aquarium with strong visible cilia.

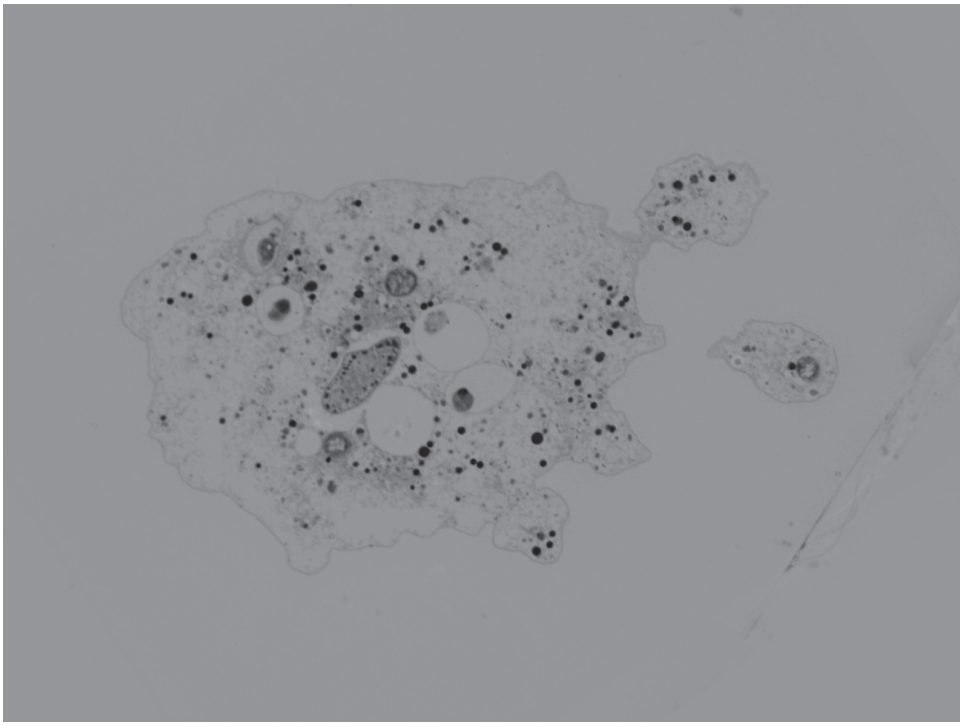


Figure 4.28: Image of the light microscopic stack of the *Amoeba* scan. It shows a cross section of the scan with the main organelles. Image by Thomas Schwaha.

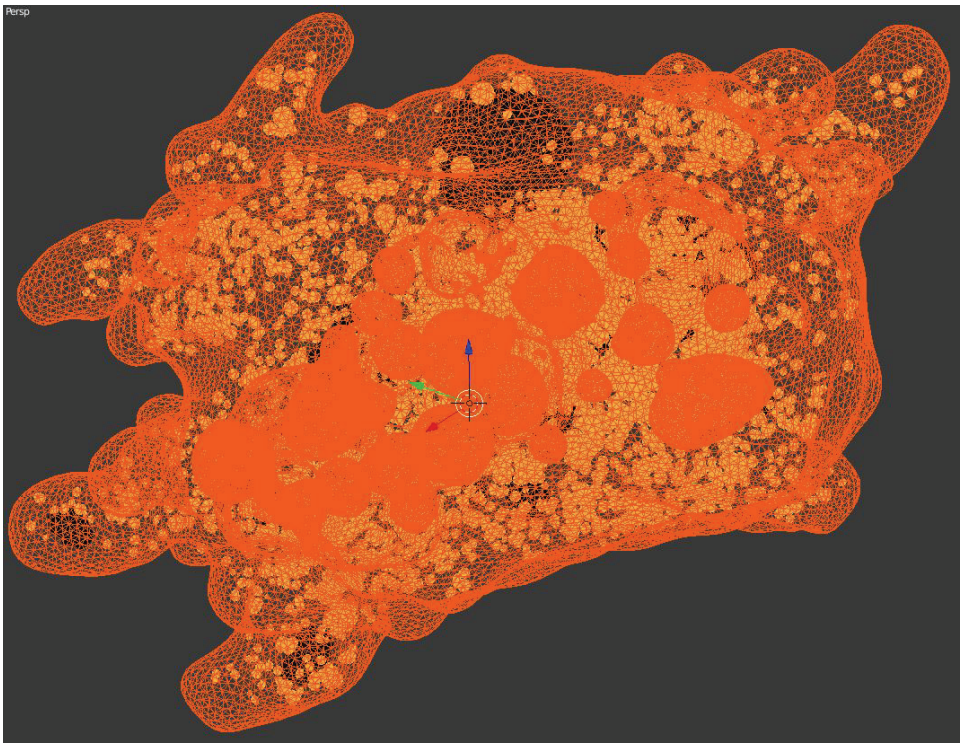


Figure 4.29: Screenshot of the imported raw separate scanned parts of the *Amoeba*. The image contains exported and segmented data by the biologists as an isotropic triangulated mesh.

plasma particles in the 3D model.

The initial 3D model by the biologists shows an informative overview of the anatomy of an *Amoeba*. The inner parts (brain, crystals, and vacuoles) were used from the scanned data as they move with the fluid shape of the *Amoeba*, yet still retaining their shape. All parts were used for the later animations, with the exception of the plasma particles.

The reference videos and micrographs (figure 4.30) show different shapes of flat *Amoeba* since they were imaged with the help of coverslips. The *Amoeba* animations are all expecting free floating *Amoebae*, therefore literature and descriptions of free floating *Amoebae* were more important than the reference videos, though the reference videos were still of great importance for the particle movements. The particle simulations and movements of the organism's membrane were thus animated and simulated as accurate as possible according to reference videos together with descriptions of movement habits from the historic paper [120, pp. 383–386].

Additionally, the data of *Amoeba* was supplemented by online information. In particular, one graph (figure 4.31) was eminently helpful. It shows how *Amoebae* feed (Phagocytosis) on other organisms. This feeding was an-

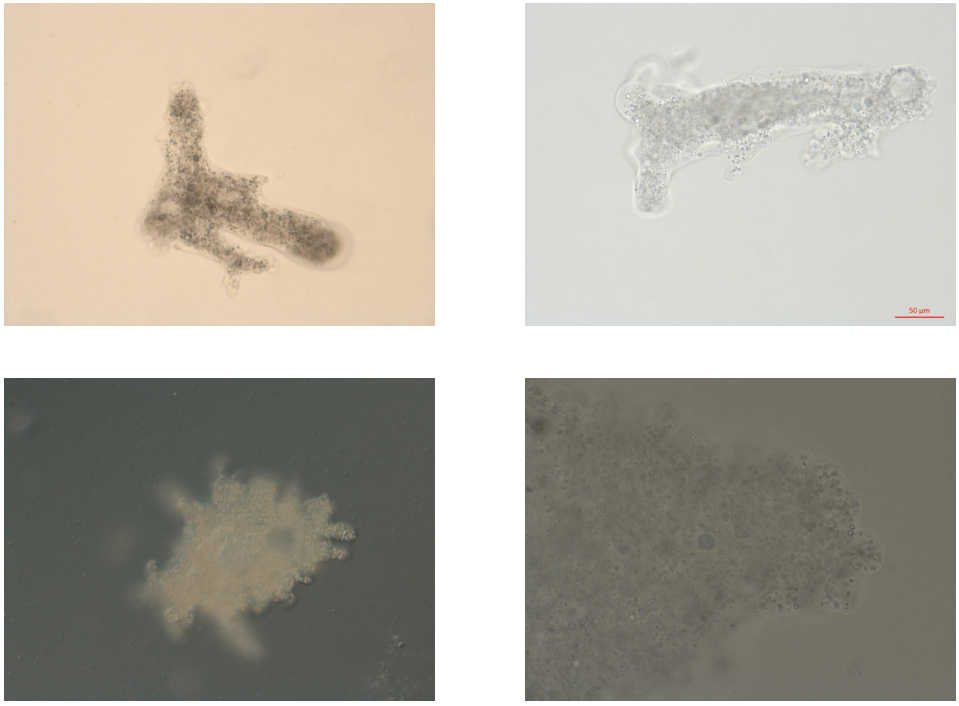


Figure 4.30: Light microscopic video frames of the *Amoeba* references by Stephan Handschuh and Martin Glösmann.

imated according to this reference. In some of the animations used in *Noise Aquarium*, an *Amoeba* can be seen feeding on a *Paramecium*. This indulging of another organism was also thematized in another animation project called *The First Greed* and produced by the Science Visualization Lab Angewandte prior to *Noise Aquarium*.

For a better understanding of the particle motions of the *Amoeba*, particle analysis was performed. Analysis of the flow movements from a short part of *Amoeba* reference videos shot with a stable camera and processed with ImageJ/FIJI [162], Build v1.51) was performed. These analyses were done with the TrackMate plugin [196], which operates in four main stages and can be seen in figure 4.32. The outcomes showed various movements in every body region of the organism *Amoeba proteus*. A volume render sequence of the primary optically scanned *Amoeba* stack was produced for an overview with Blender Internal render engine together with the included volume texture feature. All scanned organelles and some larger plasma grains can be seen in frame one of the turntable animation, see figure 4.33.

The cited paper [120] contains scientific illustrations of the movements which also helped to simulate and animate the behavior of *Amoeba* in a generalized way. In addition to the videos that were taken as a reference, some

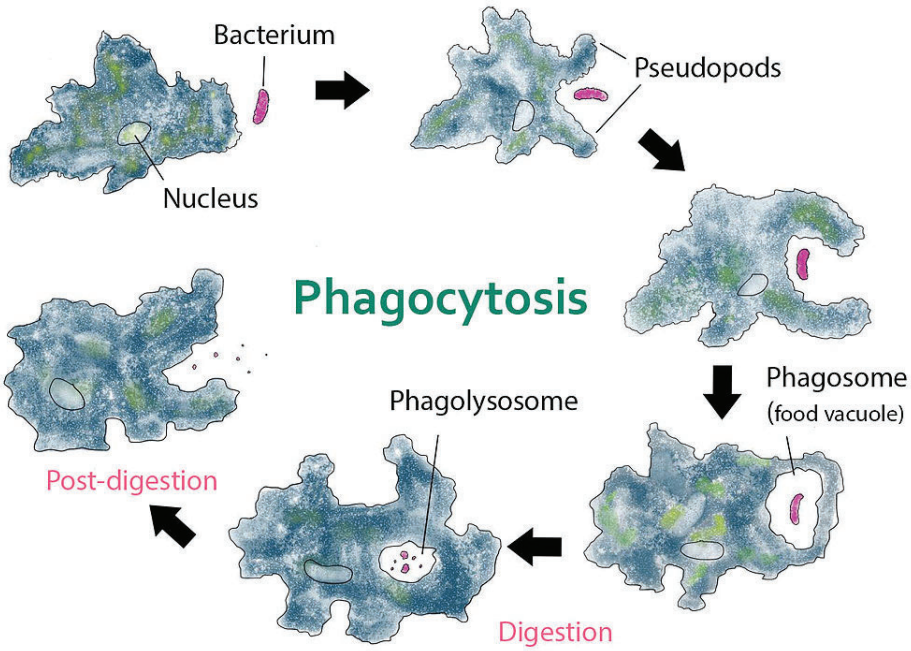
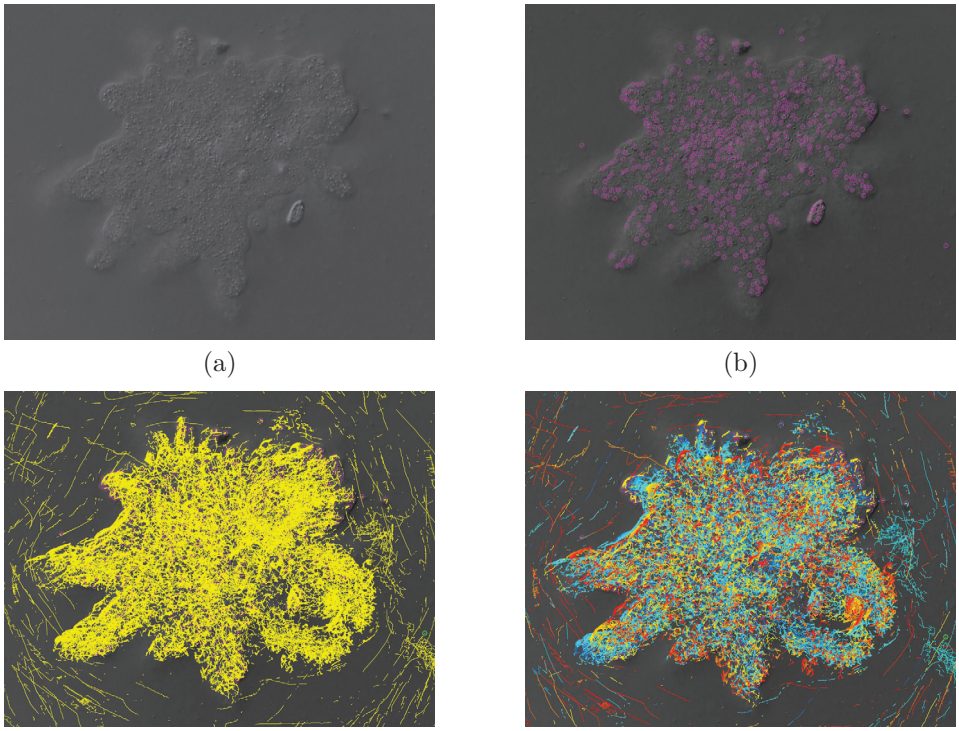


Figure 4.31: Phagocytosis—the feeding process of *Amoebae*. This was, alongside microscopic videos provided by the collaboration partners, one of the main references for the animation of the *Amoeba* eating a *Paramecium*. Image source: https://commons.wikimedia.org/wiki/File:Phagocytosis_--_amoeba.jpg.

key facts were given as a collection of information by the biologists involved. These facts included details about the cell organelles nucleus, food vacuoles, contractile vacuole, and other aspects that were visible in most images and videos. Videos were taken in different contrast modes to highlight diverse features. Enlarged video frames show the movement of particles in the endoplasma best while other videos show the whole *Amoeba* and formation of pseudopodia. The *Amoeba* feeding on a *Chilomonas* species can also be seen in one of the reference videos. The videos show how the content of a contractile vacuole is released and the enlargement of the contractile vacuole itself. Contractile vacuoles regulate the quantity of water inside freshwater *Amoebae*.

For fluid animation, different approaches were taken to achieve a natural looking result. Numerous tests with various setups of Blender particles and fluids, different physics and the CubeSurfer addon[27] did not perform adequately. Predominantly, the particles did not stay within their containers, even after utilizing different baking routines or the animation was not

[27] http://pyroevil.com/cubesurfer-documentation; 07/21/2018.



(a) (b)

(c) (d)

Figure 4.32: Images a–d show the tracking steps of the TrackMate Plugin in FIJI.

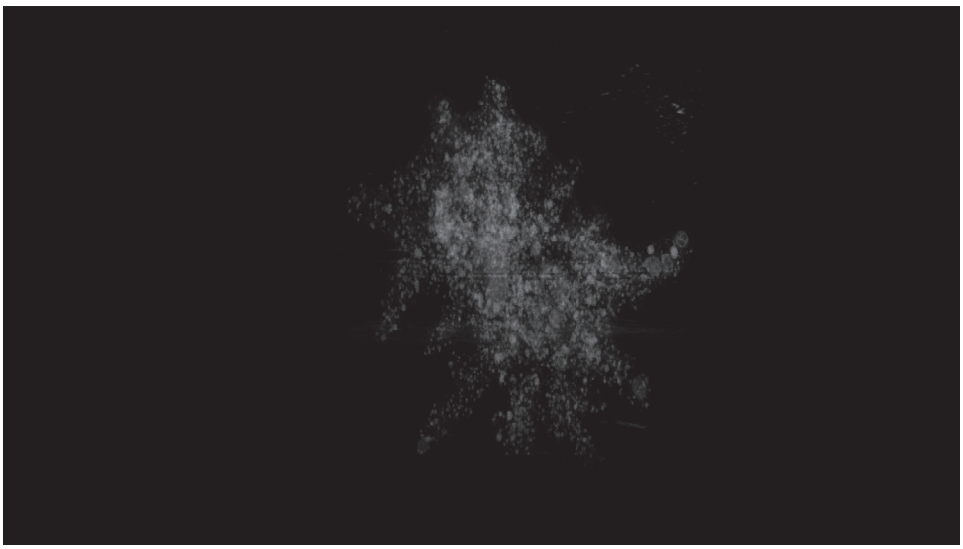


Figure 4.33: Volume rendering output of Blender Internal render engine with a volume texture of the *Amoeba* image stack.

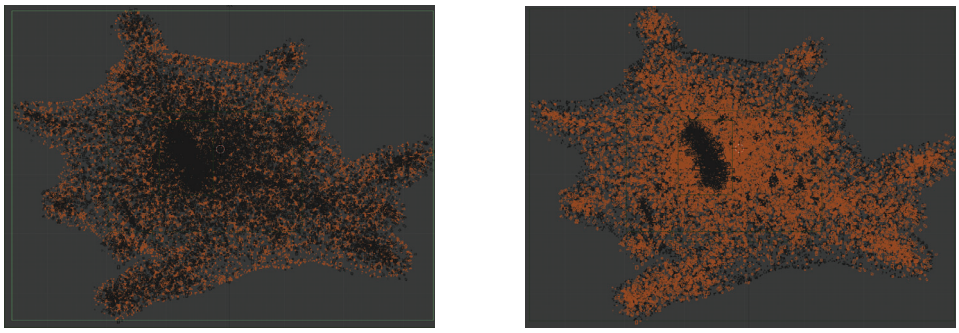


Figure 4.34: The solution used for the animation of an authentic looking amount of plasma particles was a combination of noise displacement for animating the *Amoeba* membrane and particle systems with 8k particles at the left side and one particle system with 15k particles on the right.

as controllable as needed. With CubeSurfer, the structure of *Amoeba* could potentially have followed the particles, since the CubeSurfer addon builds geometry around particles. Unfortunately there were severe problems with rendering the inner particles, not to mention the unnecessary overlaps of geometry which can lead to rendering artifacts. Therefore, the final solution used three particle systems with two times 8k and one time 15k amount of particles. They are not using physics simulation and the particles are moved by the baked animation of the *Amoeba* model, together with an animated displacement of the *Amoeba* hull that has an effect on the particles inside the digital *Amoeba*. Figure 4.34 shows images of the solution for the *Amoeba* animation and simulation.

Shading was also a challenging task, as all the parts of *Amoeba* are transparent and shiny. The shading of the *Amoeba* should be transparent, while at the same time not overly translucent or reflective. All the organelles should be clearly visible despite the vast amount of plasma grains. Renderings of *Amoeba* can be seen in figure 4.35.

Due to the reflectiveness of the *Amoeba* components, environment lighting with an environment texture for the Blender World was used and projected equirectangular. The inside view of the *Amoeba* was impressive because it revealed numerous reflective and semi-transparent materials, as well as opening up close ups of the *Amoeba* organelles such as the nucleus, as seen in figure 4.36.

The aspect of being able to swim through the interior of an *Amoeba* and capturing the abstract views of the vacuoles, nucleus, and plasma grains opened up visuals of the insides of the organism. This type of "traveling" through organisms is something that is only possible using computer animations and is useful for edutainment productions and artworks. For the insides, using Cycles glass shader led to attractive reflections along with the

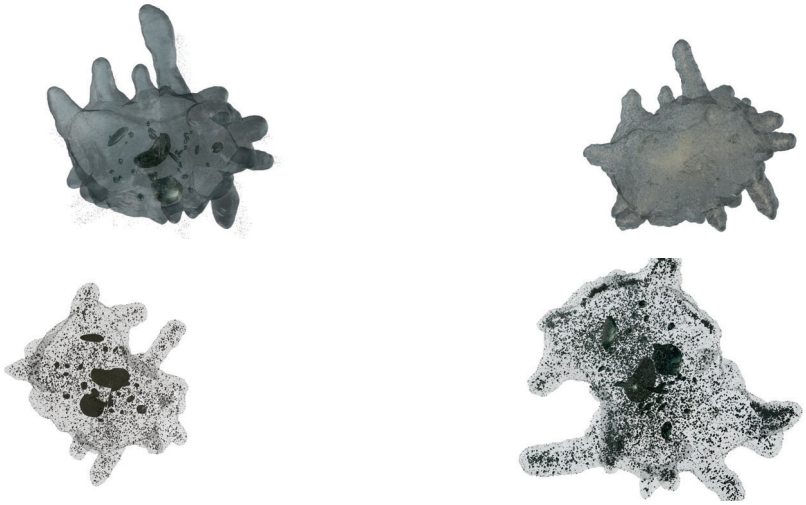


Figure 4.35: Different configurations using different settings and combinations of shaders led to diverse visibility levels of the inner organelles of the *Amoeba* model.

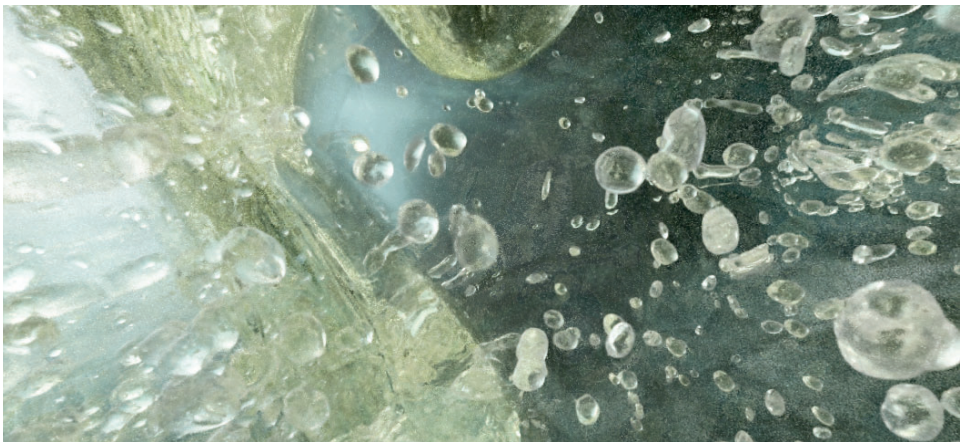
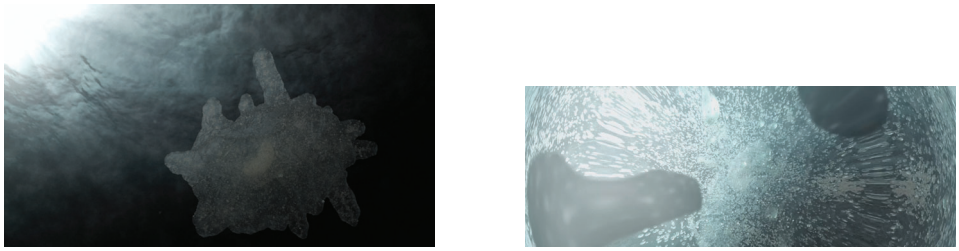


Figure 4.36: Rendering of the interior of the 3D model of *Amoeba* offers views that are not filmable with cameras attached to microscopes.

feeling of volume, yet had to be reduced as it easily led to artifacts in the application on the different geometry components.

Slight changes to color and compositing setups were made to meet the various expectations of outputs for *Noise Aquarium*. Different final rendered images can be seen in figure 4.37.



(a) (b)

Figure 4.37: Final rendering of *Amoeba* for an unpublished paper (a), and (b) shows an *Amoeba* animation output frame of the version which was shown in Hangzhou, China, at the opening of the Powerlong Art Center.

Cylindrospermum

Due to new genetic sequencing methods, it might be possible to solve the long-running uncertainty, whether *Cylindrospermum* is part of the animal or plant kingdom. It is expected that there will be changes in the future, as [103, p. 295] put it:

> The whole classification of *Cylindrospermum* (species, genera, families, orders) has undergone extensive restructuring and revision in recent years with the advent of phylogenetic analyses based on molecular sequence data. Several recent revisionary and monographic works initiated a revision and it is anticipated there will be further changes in the future.

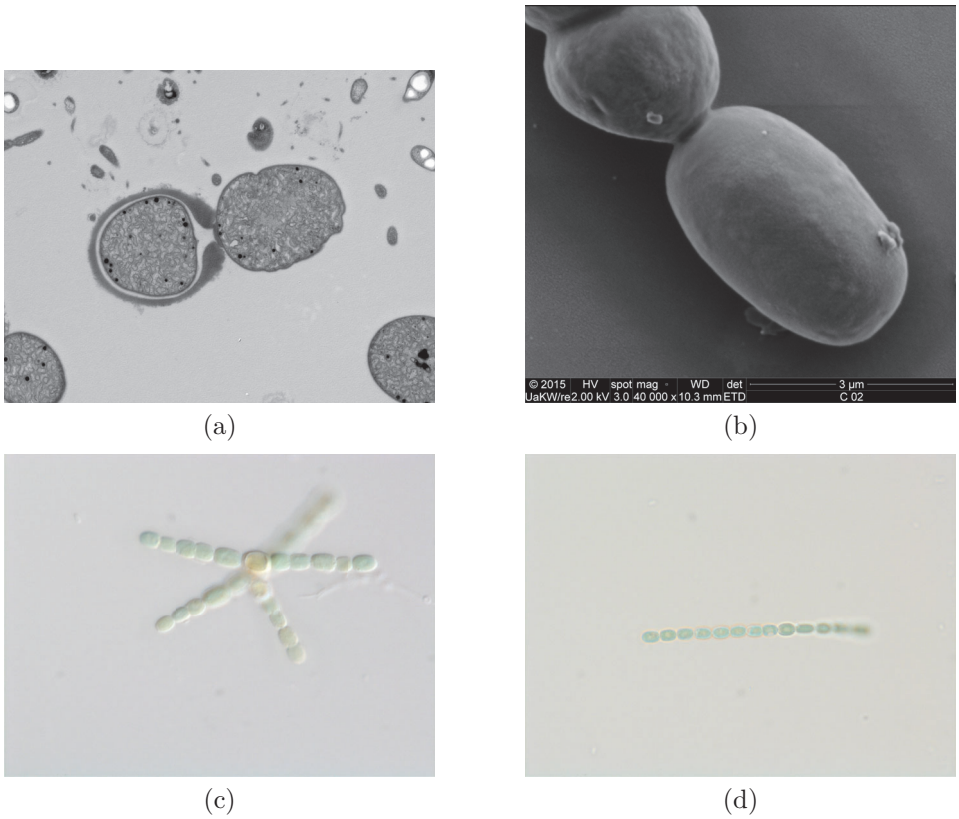
Here, the processed organism is referred to as *Cylindrospermum*. *Cylindrospermum* is the smallest organisms depicted in the project *Noise Aquarium*. Reasons against the classification of *Cylindrospermum* into the plant kingdom despite their photosynthetic properties is explained in [77, p. 277]:

> Before 1960, the organisms we know today as cyanobacteria were called blue-green algae. They were classified along with the green algae, the red algae, and the brown algae as photosynthetic microbes. It was universally agreed that all of these carried out green plant photosynthesis, fixing CO^2 and generating O^2 from water. But in the 1960s it became apparent, from new biochemical evidence, that the blue-green algae, unlike the other algae, are really bacteria: they are sensitive to penicillin because of their peptidoglycan cell walls; they have bacterial-sized ribosomes sensitive to the usual antibiotics, and they do not contain organelles such as chloroplasts and mitochondria. A major consequence of the name change was to remove this vast array of organisms from the realm of botany and to put them into the microbial world.

In the case of *Cylindrospermum*, scanning the inner membrane was an ambitious task. For the making of the *Cylindrospermum* 3D model, a new technique of combining TEM, SEM, and light microscopic stacks to achieve representations of the photosynthetic interior of the organism was used. This was possible in cooperation with the imaging facilities of VBCF Vienna Biocenter Core Facilities[28] through the contacts of the collaborating biologists. Samples of the variations of imaging data are depicted in figure 4.38. Additional pictures from algae and *Cylindrospermum* were gathered from the Internet to use as shading references.

The organism was only partly scanned, then assembled from different scanning data. The original 3D model sent by the biologists consisted of the components: *Cylindrospermum* lipid droplets cell 1–3, membranes cell 1–3, heterocyst inner and outer, and normal cells as a Wavefront Object. The interior of the other cells was copied from the cells 1–2. At the beginning of the project, Cycles rendering and volume texture shader of Blender Internal were combined through compositing to depict the details of the interior structures. For that, two render passes were rendered separately and combined in post processing. This process had the advantage to use the direct TEM originated ultrastructure of the cell, but the disadvantage of two-pass rendering and compositing. Also, for the needed stereoscopy aspect, the volume texture shader rendering of Blender Internal was not suitable. Therefore, to create the project *Noise Aquarium*, an entirely new configuration and workflow was used. To start, it had the heterocyst generated as geometry, and then combined with the regular cells of the scan.

[28] https://www.vbcf.ac.at; 07/10/2018.



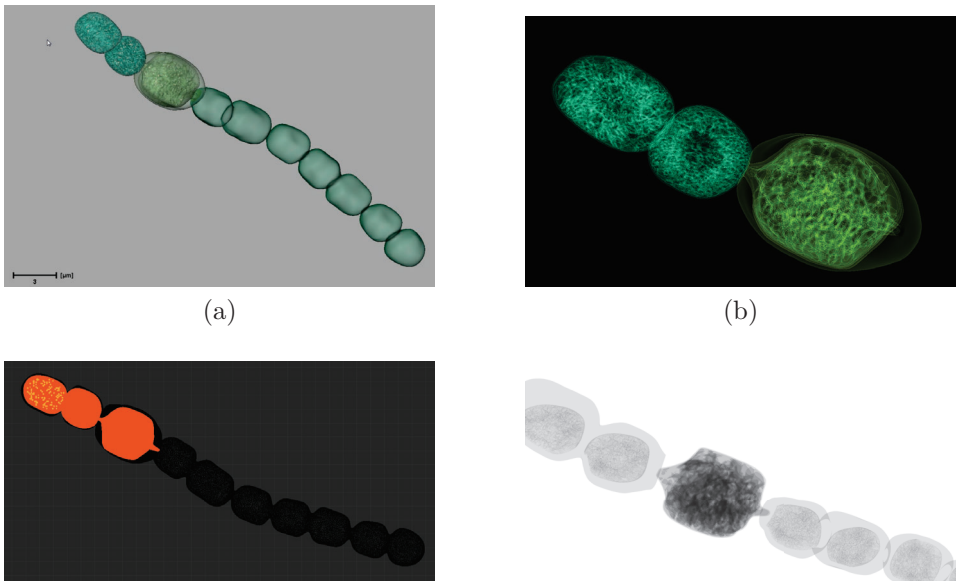
(a) (b)

(c) (d)

Figure 4.38: Reference and image data that was the basis for the *Cylindrospermum* 3D model. Image (a) by Nicole Fellner shows a TEM image (Levels altered in Photoshop), (b) is a SEM image by Rudolf Erlach, while (c) and (d) by Stephan Handschuh are light microscopic image samples.

The generated geometry is high resolution, yet was still manageable with 512.000 polygonal faces. Even the particle system instances of the grouped *Cylindrospermum* 3D model could be processed in this way. In figure 4.39, the exported model as fast preview rendering out of the software Amira, a close up of the scanned fine details (ultrastructure), a wireframe model in the Blender viewport, and the volume render pass out of Blender Internal render engine can be seen.

Various imaging data for the *Cylindrospermum* model came from light microscopy and TEM. For the structure of the different cells of the bacteria, TEM image stacks and exported models were provided by the biologists. One image of these TEM sections of the cells by the biologists can be seen in figure 4.40a. TEM images were acquired, optimized, and delivered by the collaborating institutions. A TEM section can be seen in figure 4.40b. The interior of the other "normal" cells was reused for the two scanned cells to create the finished 3D model. The lipid droplets were generated with a



(a) (b)

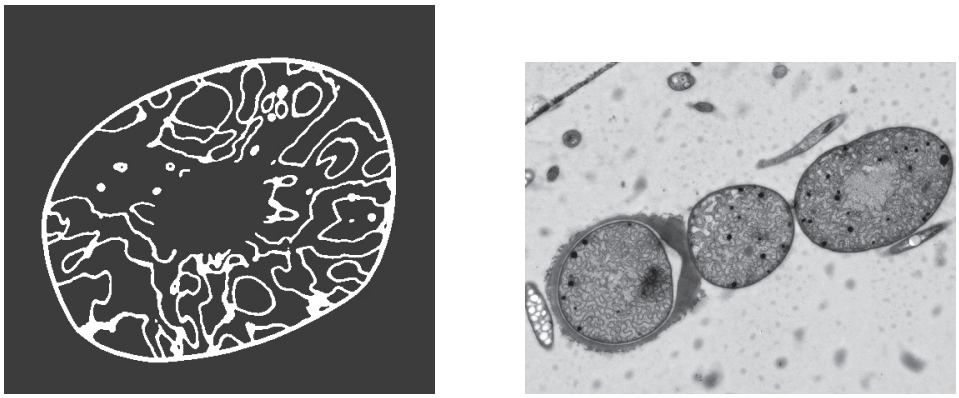
(c) (d)

Figure 4.39: Fast preview rendering of the acquired data of *Cylindrospermum* (a) and a close up of the "ultra-structures" that were scanned with TEM (b) both by Stephan Handschuh. A wireframe representation of the data in Blender viewport (c) and the volume render pass of the scanned interior of *Cylindrospermum* (d) are visible in this image.

particle system for the final 3D model as this had advantages in terms of low polycount.

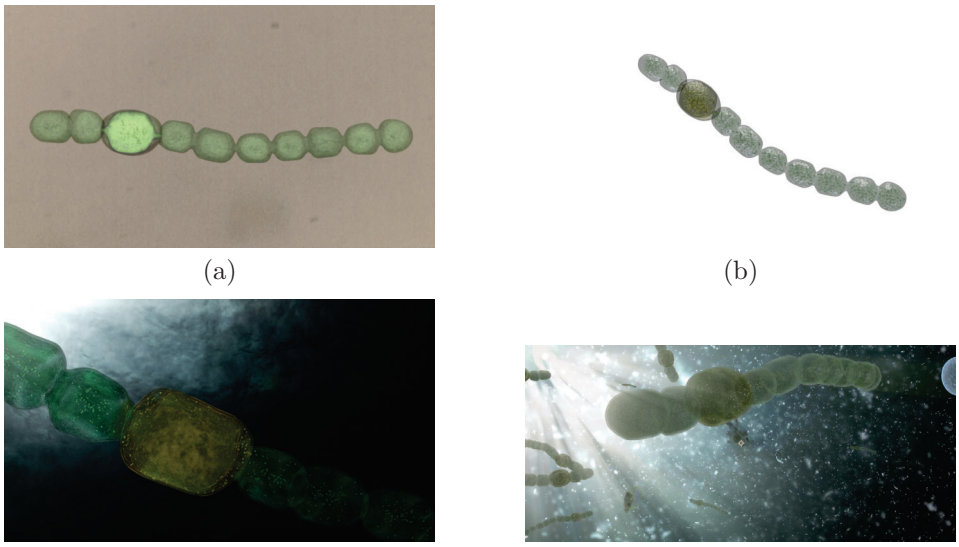
For the movements of *Cylindrospermum*, no animation with digital bones was needed at the presented scale. For most animation sequences, multiple *Cylindrospermum* 3D models were instantiated with a particle system, while fluids or physics simulation was used to move the generated flock of organisms. To prevent the 3D model from appearing too static in the animated sequences, a Wave Deformer was added. To move the whole compound of bacteria uniformly, as it would while drifting in water, a group for the bacteria cells was created.

The shading of the *Cylindrospermum* 3D model was done using simple semi-transparent shaders. The main challenge was finding the right amount of transparency to see all the scanned structures, while simultaneously not allowing the bacteria to appear too dense. In the references, the transparent nature of the bacteria is clearly visible. Due to the different projects and respective requirements, each of the final renderings varied visibly. Figure 4.41 shows different final images of various applications.



(a) (b)

Figure 4.40: One slice of the model structure according to cross-sectioned cell data of a *Cylindrospermum* (a), cell imaging obtained with TEM (b) by Nicole Fellner.



(a) (b)

(c) (d)

Figure 4.41: There are different rendered version available of *Cylindrospermum*. Some of them combine a polygon surface rendering with a volumetric rendering in a composition like (a), (b), and (c). For that, the TEM image stack was used as a volumetric shader in Blender and rendered for composting in a separate pass. The image (a) shows a rendering in an illustrative textbook look. Depiction (c) shows a composited frame and close up of the hetereocyst of the *Cylindrospermum* 3D model, and (d) shows a frame of one *Noise Aquarium* version, in which the organisms were depicted in a flock. In (d) the interior of the *Cylindrospermum* model is a decimated polygon model of the TEM ultrastructure.

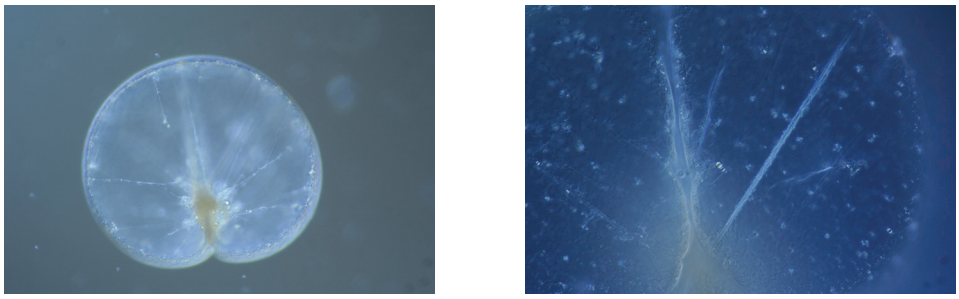


Figure 4.42: Samples of reference micrographs of a *Noctiluca*, additional to the scanning data material of the collaboration partners, information on the surface structure and plasma networks inside was gathered from various images like these by Thomas Schwaha. The left micrograph shows the whole organism, the right image a close up of the inside structures.

Noctiluca scintillans

A *Noctiluca scintillans* organism can be seen in figure 4.42. They vary in size from 0.2 to 2 mm, though on average they are roughly 0.5 mm in size. The organism has a balloon-shaped cell with an oral pouch, a short flagellum, and a tentacle. The balloon-shaped cell is filled with cytoplasm and can cause spectacular bioluminescence. It lives mostly in coastal waters. It digests organic matter, yet can also synthesize own nutrients. It is prey for numerous species of animals and feeds on diatoms and various eggs[29].

For the depiction in *Noise Aquarium*, *Noctiluca* species was chosen because of its intriguing shape and light emission. Bioluminescence occurs in some groups of animals and is more often seen in creatures in the open sea. There are different wavelengths of light animals are able to emit, and in the case of *Noctiluca*, there are blue light wavelengths (490–450 nm) that are produced by the animal enabling it to glow in the dark. Fascinating phenomena of whole beaches that glow blueish can happen with large populations of *Noctiluca* in the water.

The organism has a simple anatomy with a spherical floating vacuole, which makes up most of the cellular volume, as well as radial cytoplasmic strands and one tentacle. The cytoplasmic strands are irregular in shape and are fused together in vein-like networks. These networks could not be scanned by the biologists due to their fragility. The image stacks were generated with micro CT and light microscopy. The imaging data did not convey much information because *Noctiluca* samples are by nature very fragile. In figure 4.43, one slide of the micro CT imaging can be seen. In combination with different reference resources, an authentic 3D model could be completed.

[29] http://www.imas.utas.edu.au/zooplankton/image-key/noctiluca-scintillans; 18/02/2017.

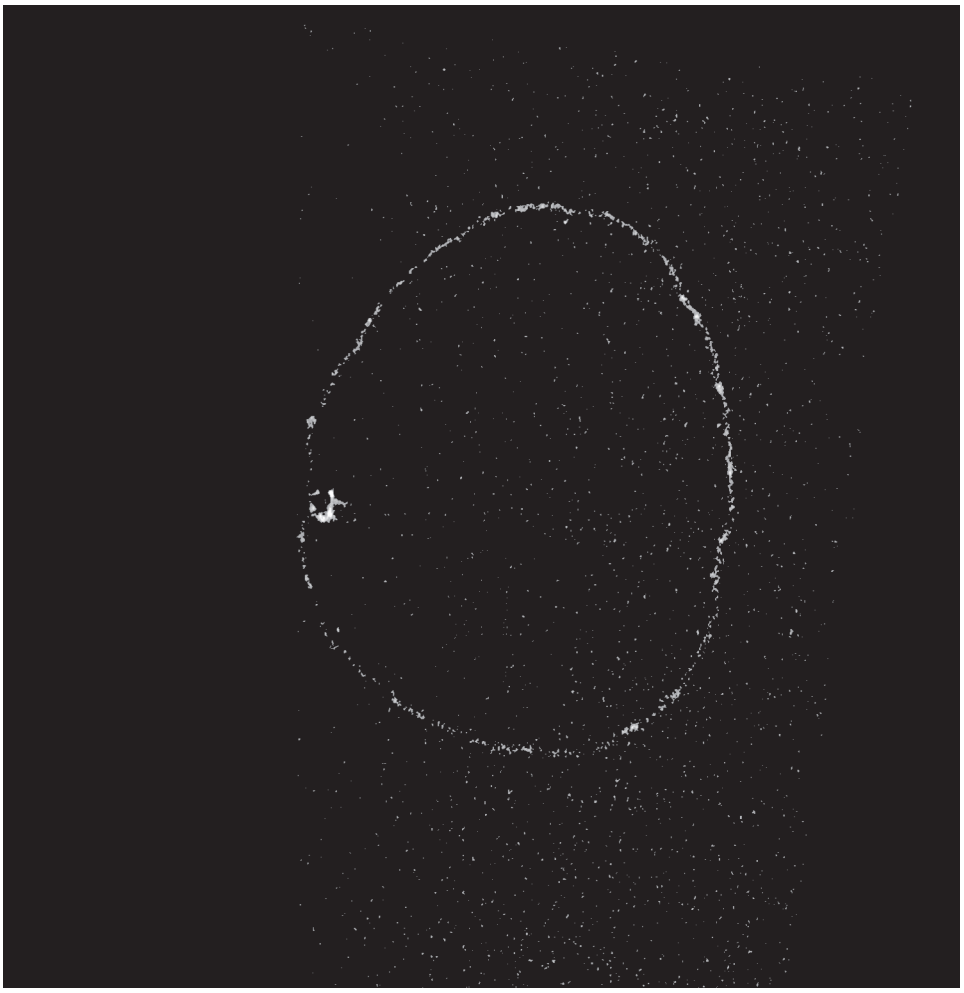


Figure 4.43: Micro CT stack image of the scan used for the base mesh of the 3D model of a *Noctiluca* by Stephan Handschuh.

The organism scans led to intriguing 3D models because the inside of its cytoplasm contains radiant structures, while the skin of the "body balloon" has uniform patterns. The imaging expert had a difficult time to segment more than the hull of the balloon-shaped floatation vacuole of the *Noctiluca*. Regardless, a basic model was acquired using micro CT scanning. The Wavefront Objects from the biologists can bee seen in figure 4.44.

With both reference and scientific imaging data, the model data of the *Noctiluca* for the 3D model could be assembled. It was necessary to model the inner plasma networks of *Noctiluca* according to references, since the individual radial cytoplasmic strands were too thin to be scanned with the initially chosen methods. There were some initial tests using generated hair particles to simulate the missing cytoplasmic strands. In the end, it was

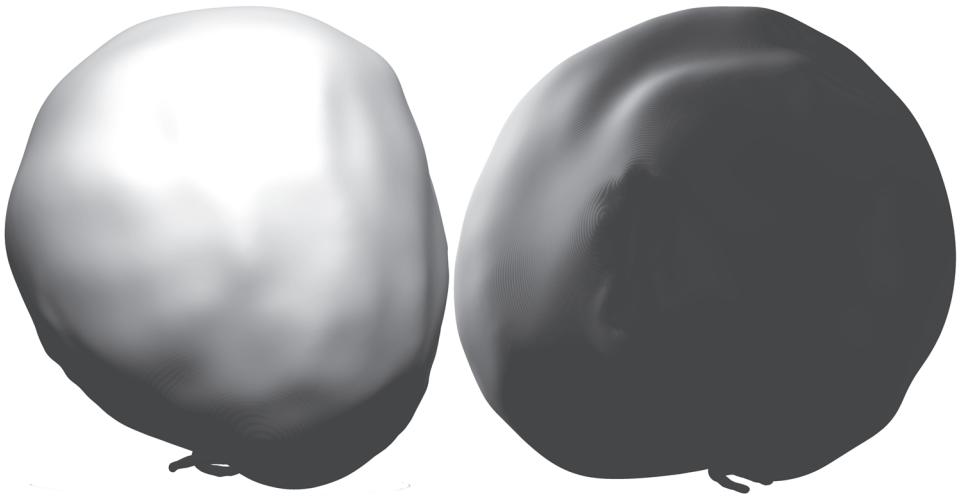


Figure 4.44: Renderings of the raw Wavefront Objects derived from the segmentation of *Noctiluca*.

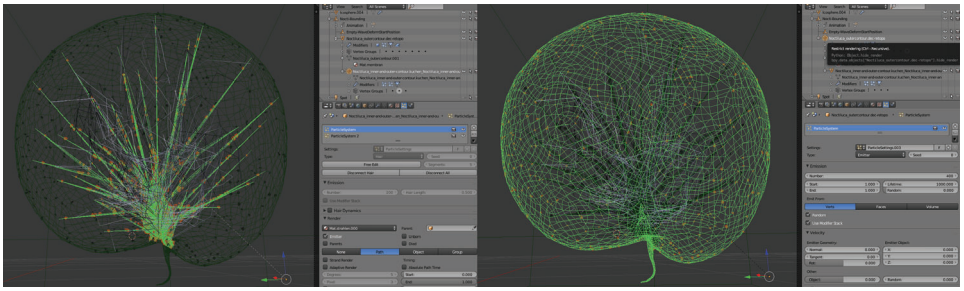


Figure 4.45: A screenshot of a wireframe depiction of the *Noctiluca* 3D model. In the left image, the strand geometry is selected and the particles that were used to simulate lumps are visible colored orange, while in the right image, the outer membrane geometry is selected.

more natural to model the strands due to diverse thicknesses and irregular clumping and then extend the modeled strands using hair and particles. The outer membrane of the floatation vacuole was extended using a particle system to visualize the highlights in the bioluminescent pattern. In figure 4.45, a wireframe of the *Noctiluca* with modeled inner structures can be seen, as well as the three particle systems, one for fine cytoplasmic strands, one for lumps on these strands, and one for the fluorescent source spots. The main references for this modeling process were light microscopic images with various depths of field, see the middle image of figure 4.46.

The scanning process caused deformations of the round shape of the floating vacuole. This had to be repaired in sculpting and the firm surface of

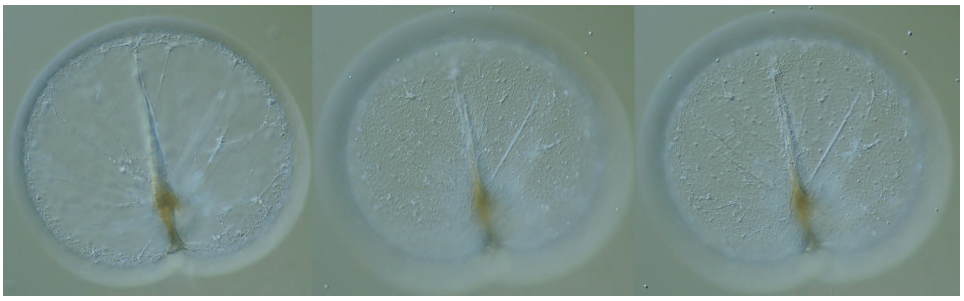


Figure 4.46: Light microscopic images with different depth of field to convey the structural differences of the strands inside the *Noctiluca* floating vacuole. Images by Thomas Schwaha.

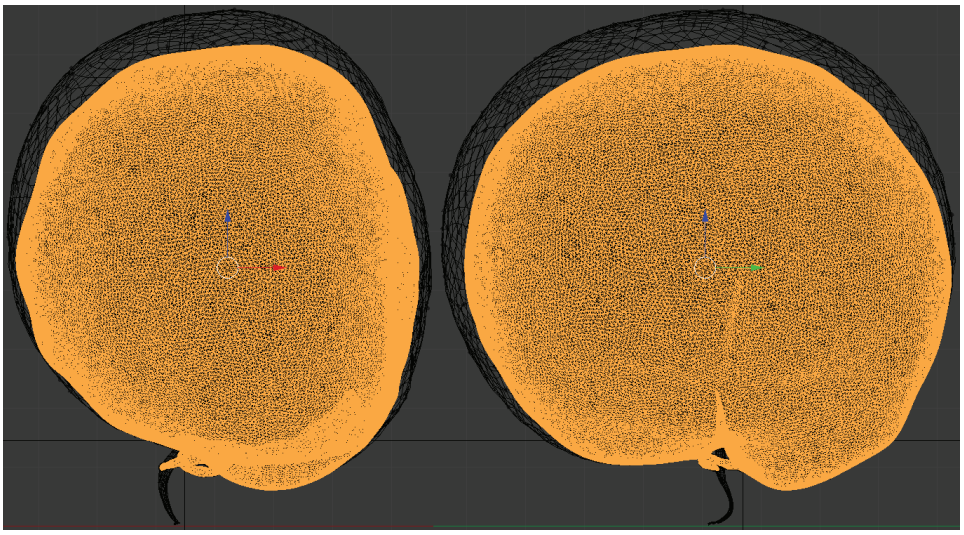


Figure 4.47: Side view at the left and front view at the right of *Noctiluca* raw mesh (orange wireframe mesh) and the final (black), according to references and feedback repaired mesh.

a living *Noctiluca* had to be restored according to references. In figure 4.47 the original model is overlaid with the final repaired sculpted version.

The bioluminescence of the *Noctiluca* organism was taken into account in shading. The shader contains a configuration including the structured texture of [44, figure 1], along with a material that includes an emission shader. In figure 4.48, the shader tree of the used Cycles material overlaying a non-transparent textured rendering is visible.

The movements of *Noctiluca* species are rather simple. The organism has a tentacle that influences the direction it will drift. This tentacle was rigged with a simple set of bones. Every other animation were translations

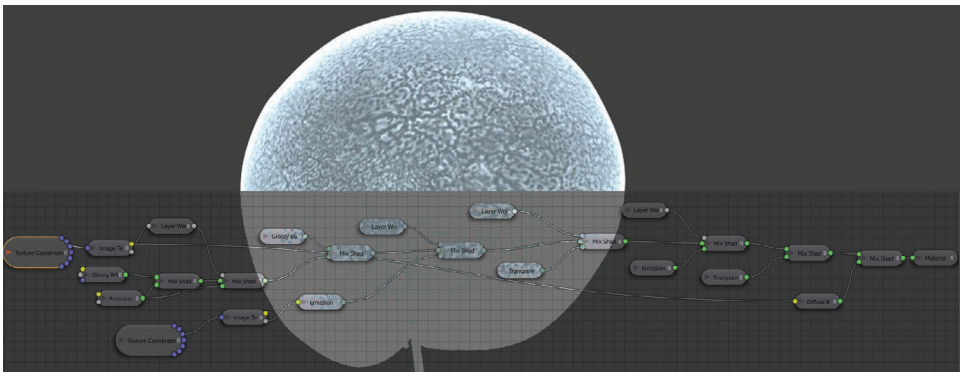


Figure 4.48: Shader Tree setup of the used Cycles material overlaying a non-transparent textured rendering of the *Noctiluca* 3D model.

or rotations of the whole model. Figure 4.49 shows the digital bones of the *Noctiluca* 3D model. Later in the project *Noise Aquarium*, the *Noctiluca* tentacle was animated with a wave deform modifier and a vertex group selection.

Figure 4.50 shows a rendering with the chosen material configuration in a frame taken from a version of a linear *Noise Aquarium* video.

Oikopleura

Oikopleura species are common in marine zooplankton communities [159].

The species belong to the planktonic Tunicates and posses a specialized, external filtering system that extracts food particles below Micrometer size. The digestive system of an *Oikopleura* consists of three different primary cell types. Ciliated cells transport the food and absorb nutrients. *Oikopleura* secretes a complex, gelatinous cave called "house", which they inhabit and use for creating food particle streams. These filter houses are of special interest for researchers and have been thoroughly studied for different species of the genus. The house functions when the animal beats its tail to concentrate a nutrition particle stream. Food is trapped by a mucous net and thereafter digested [22].

The animal has genes of the vertebrate forebrain, hindbrain and spinal cord, hence it became a study object for evolutionists in determining the origins of vertebrate brains. Especially with genetic analysis of the larval brain, new insights have been made [24].

Additionally, evolutionary thyroid development can be studied in *Oikopleura* species [25].

Oikopleura eventually discards the gelatinous filter houses full of fecal pellets. These discarded *Oikopleura* houses full of excrement are of importance for pelagic ecosystems as they provide protection and are full of nu-

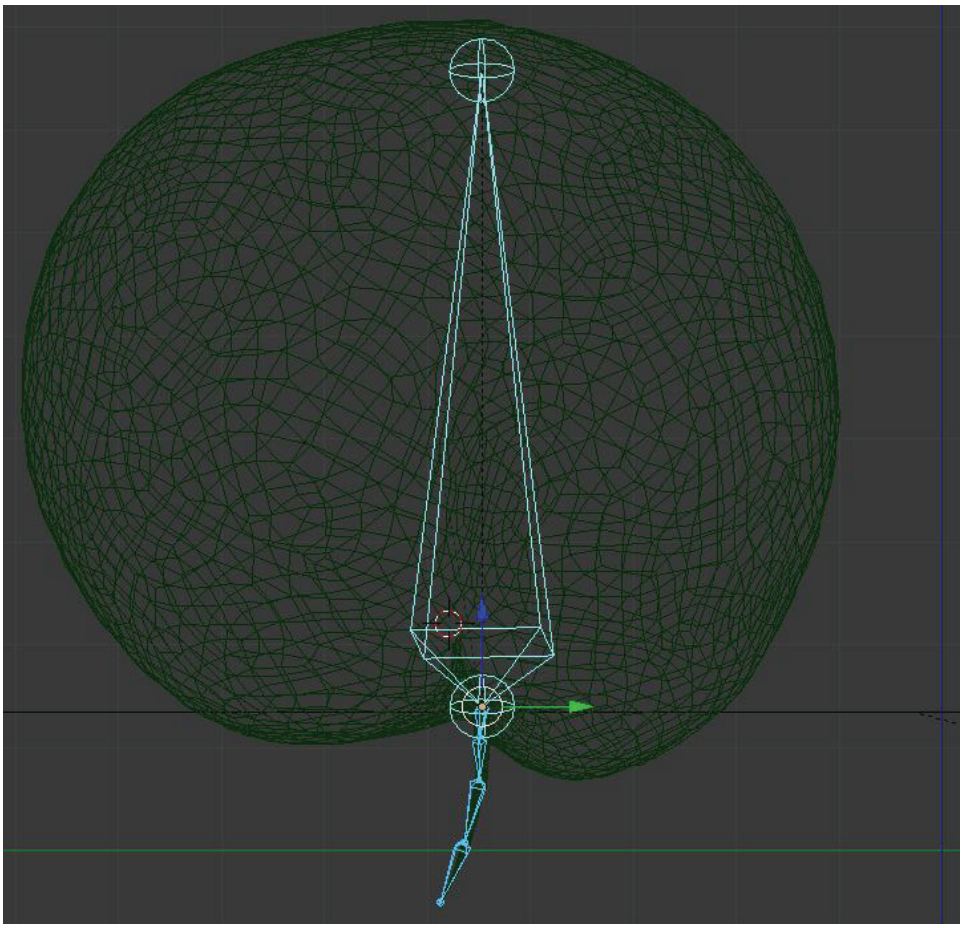


Figure 4.49: Digital bone setup which was used to animate the *Noctiluca* 3D model.

trients for other plankton [30].

Oikopleura and its slimy house are also of interest to researchers outside the field of biology. The structure of the gut is simple, yet the whole animal and its compound with the "house" are the subject of numerous research projects. For instance, during a guided tour through the Max Planck Institute Potsdam in Germany, the interdisciplinary working researchers there showed their research regarding the house membranes for material sciences.

Particularly in *Oikopleura*, the intestinal system was a difficult job for the biologist to prepare and capture due to extremely thin structures. The animal has fascinating anatomic aspects which could be the topic of detailed close up scientific visualizations. Nevertheless, with the focus on a more general environmental topic, the detailed animations of certain bodily functions of the animals were neglected, though they could be resumed at any time.

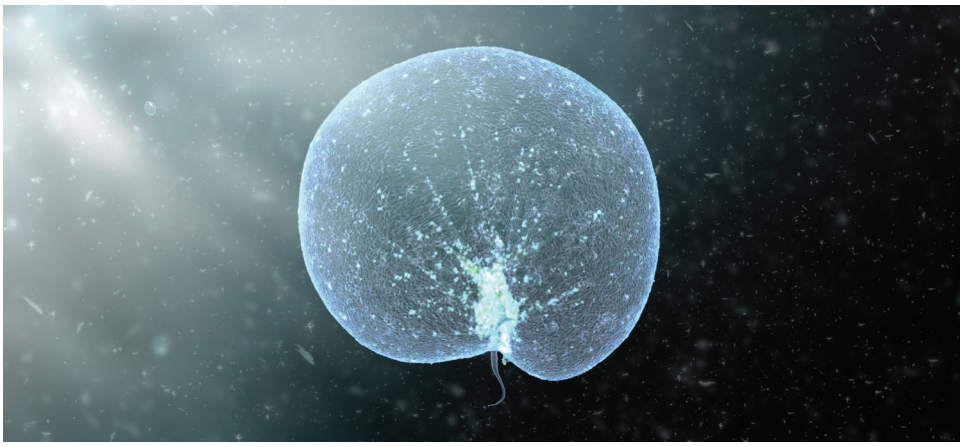


Figure 4.50: Final rendering of the *Noctiluca* 3D model in the project Noise Aquarium.

The primary scientific data sets and references were micro CT images and light microscopic micrographs. The main light microscopic references were two videos, both of which are available on the video streaming platform YouTube, see figure 4.51 for one frame of each of these videos.

The main scientific imaging data for the *Oikopleura* 3D model came from a light microscopic and a micro CT image stack. One representative image from each stack can be seen in figure 4.52.

The segmented micro CT image stack of *Oikopleura* resulted in a detailed 3D model of the organism which was then exported into a Wavefront Object to be edited for the scientific visualization. The Wavefront Object figure 4.53a and a volume rendering figure 4.53b were provided by the collaborators. The information came in separate parts, each containing the data of the body wall and gut, buccal glands, ganglion, and gonad separately. Further, the more detailed scan of the head came without the tail, as seen in figure 4.53c. The Wavefront Objects were extremely high detailed geometry, while the tail of the animal was cramped and distorted due to sample preparation. That means, particularly to animate the tail (see figure 4.53d), a sculpting and retopo workflow, as described in general in chapter 3.2 was necessary. The tail was detached from the body and edited separately. The head from the micro CT scan had too detailed bumps, therefore, the intensity of the structure was reduced for the final 3D model. A main "before" and "after" comparison of the Wavefront Object segmented by the collaborating biologists and the edited 3D model is displayed in figure 4.54.

The animation of the *Oikopleura* model was done with a rig and skinning simulation. The focus of the animation lay on the motion of the tail, as the head as a whole moves very little. The animation was rotoscoped according

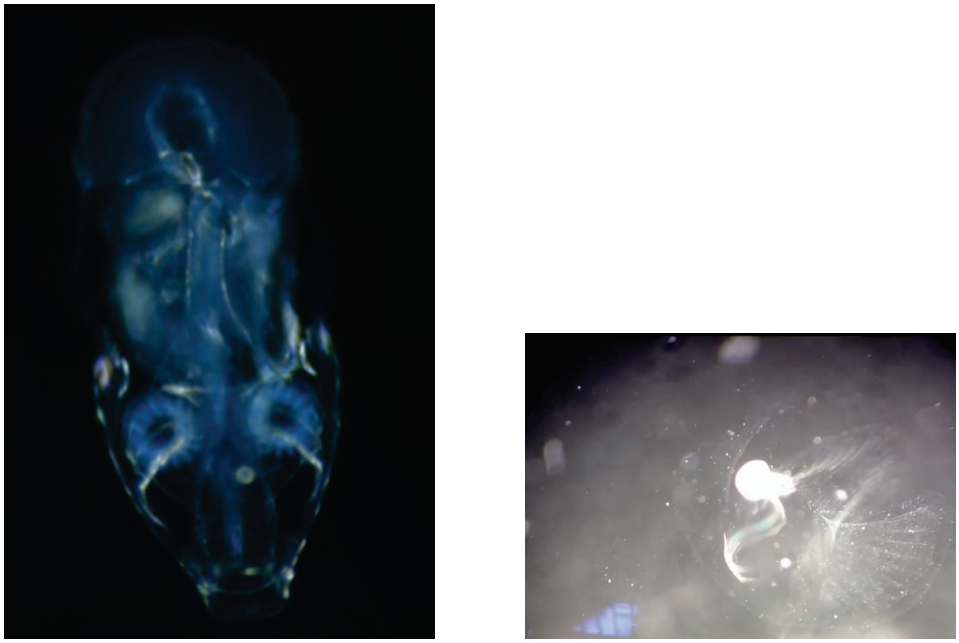


Figure 4.51: *Oikopleura* video references by Thomas Schwaha. The left image is a video frame from https://www.youtube.com/watch?v=Yq4PHnCDeBk, and the right is a still image from https://www.youtube.com/watch?v=pS6EqU2JE_k.

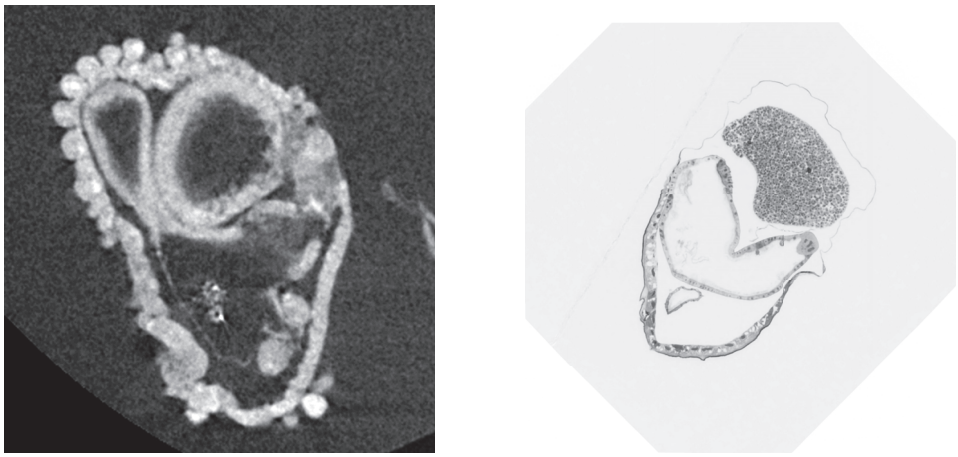


Figure 4.52: *Oikopleura* stack image examples—the left image shows one image of the micro CT image stack by Stephan Handschuh, while the right image shows an image of the light microscopic image stack by Thomas Schwaha.

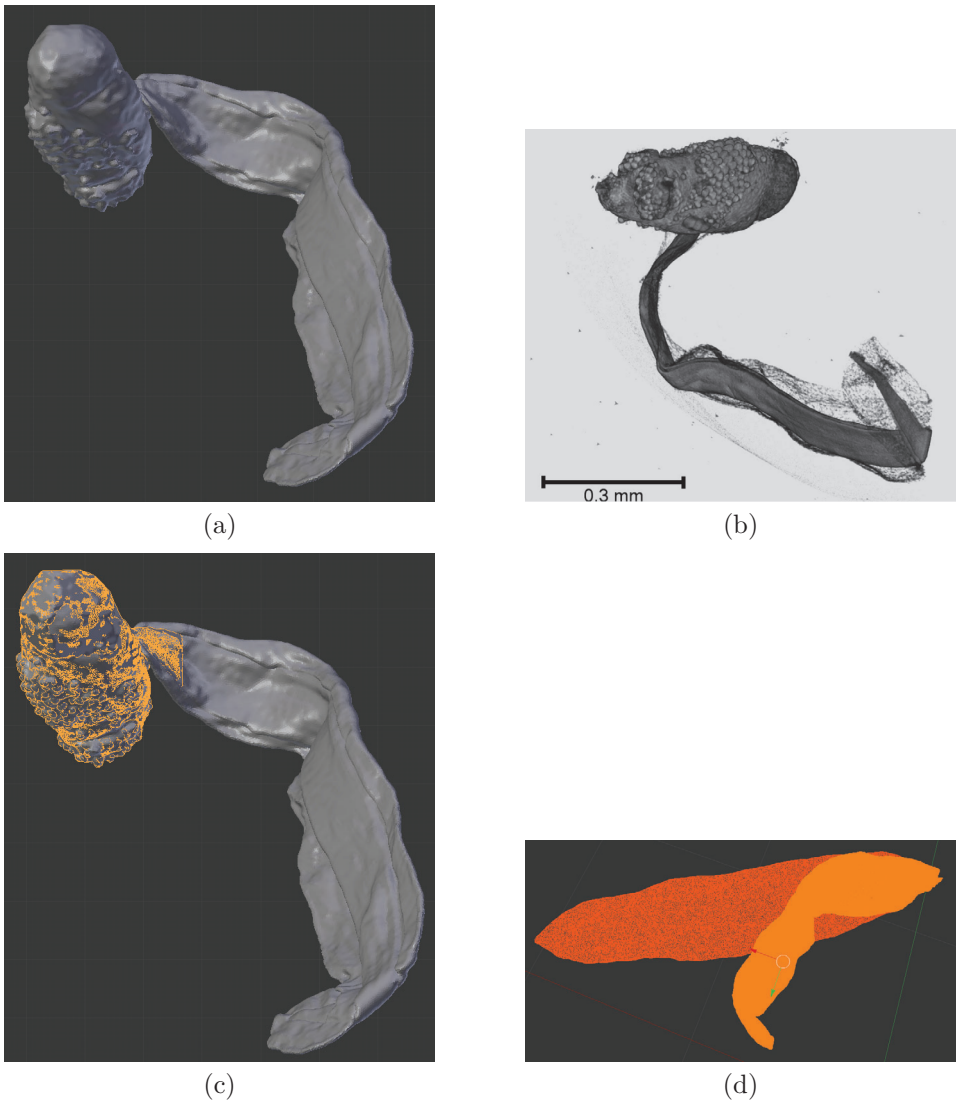


Figure 4.53: The Wavefront Object (a) and a volumetric rendering (b) were provided by the collaborator Stephan Handschuh. The head came as separate data set (c). Image (d) shows the raw mesh of the tail in bright orange and the flattened geometry prepared for animation in darker orange.

to a reference video sequence[30]. The video sequence was exported as a contact sheet as visible in the left image of figure 4.55, while the armature can be found in the right image of figure 4.55.

Shading was challenging for the *Oikopleura* model. *Oikopleura* has an iridescent tail that shimmers if light is shining onto it at a certain angle.

[30] https://www.youtube.com/watch?v=pS6EqU2JE_k; 20/07/2018.

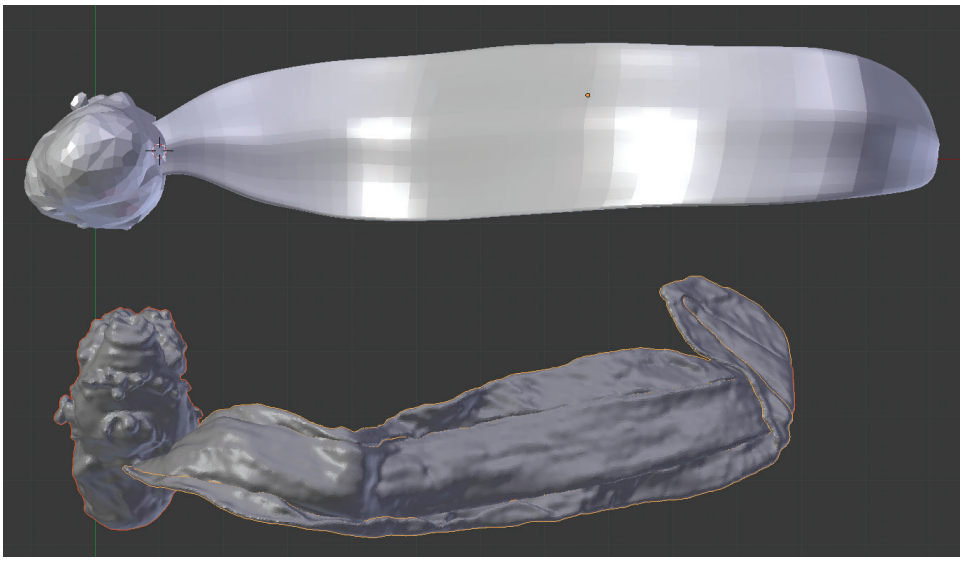


Figure 4.54: Top view of the *Oikopleura* 3D model before the preparation process for animation (below) and the final geometry ready for animation (above).

Additionally, the head is very dense and contains a lot of structures which should be visible in the final rendering. Therefore, the *Oikopleura* model was shaded in multiple ways to achieve a natural look. In figure 4.56 varied shading variants can be seen.

The *Oikopleura* 3D model was mainly created to be used in the project *Noise Aquarium*. A final rendering was composed into one version of the linear visual videos for this project and is visible in figure 4.57.

Oikopleura is of increasing interest for a wide range of scientific fields, in part due to the slime house it resides in, and also due to the interesting step of evolution it represents. As the feeding of *Oikopleura* is also a popular research topic, fluid simulation tests to visualize the digestion of the animal were performed, as seen in figure 4.58.

The visualization of inner biological processes for all the plankton creatures depicted in the case example *Noise Aquarium* might be interesting for follow up science as well as art projects. Creating scientific visualizations involving scientific imaging is a promising future direction for the depiction and study of inner processes of microscopic organisms.

Paramecium

The *Paramecium* is an oval, slipper-shaped microorganism which is rounded at the front/top and pointed towards the back/bottom. It has a stiff yet elastic membrane which gives the *Paramecium* a definite shape, while allowing

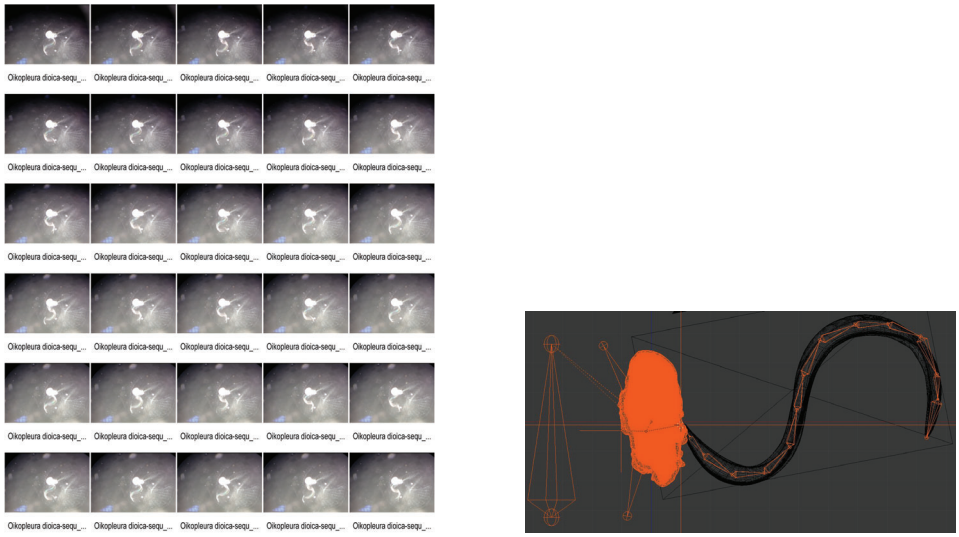


Figure 4.55: A contact sheet of the reference video for the *Oikopleura* animation on the left and the armature of the 3D model is shown on the right.

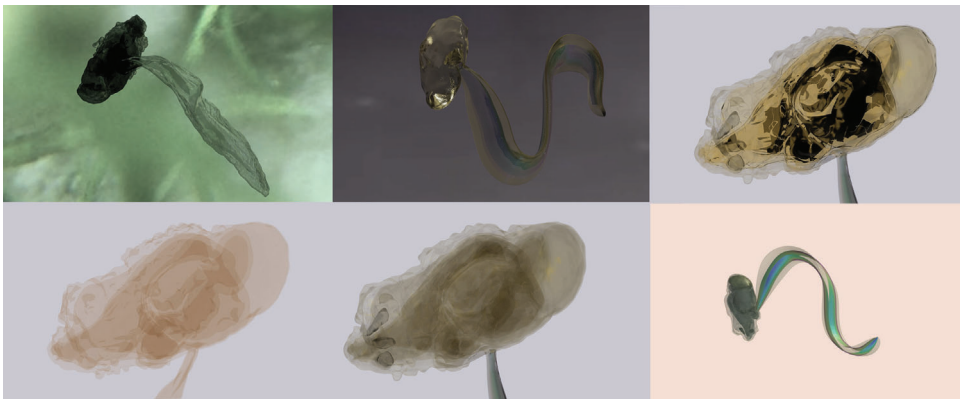


Figure 4.56: Different shading versions and preview tests for the *Oikopleura* 3D model.

some minor deformations to adapt to the environment. The membrane is covered by many tiny hairs called cilia. On the sides, beginning near the front end and continuing halfway down is the oral groove. The rear opening is called the anal pore. A contractile vacuole and radiating canals are also found on the outside of a *Paramecium*. Inside is cytoplasm, the gullet, food vacuoles, and the nucleus.[31]

[31] http://101science.com/paramecium.htm; 23/07/2018.

Figure 4.57: Final composed rendering frame of one linear visual video version of *Noise Aquarium*, depicting the *Oikopleura* 3D model prominently in the middle.

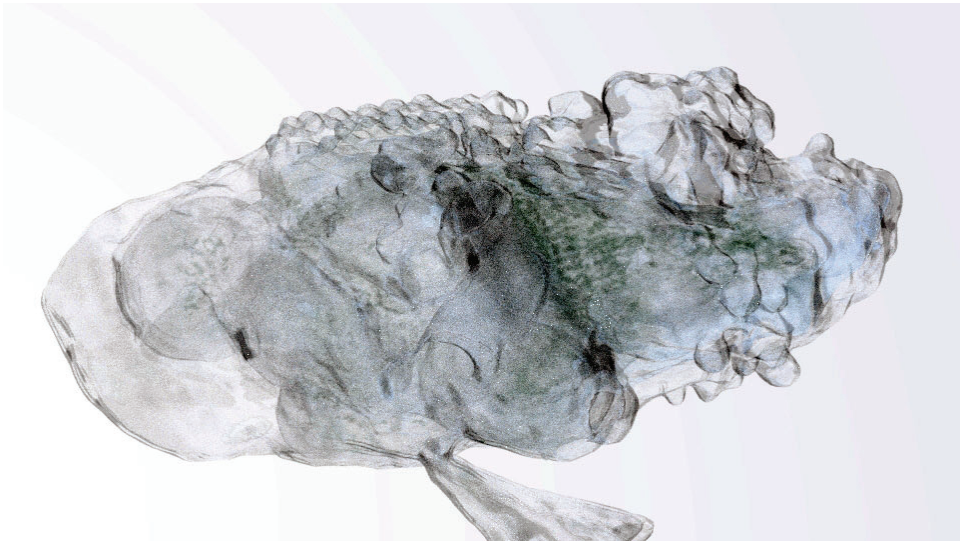


Figure 4.58: Fluid simulation in the intestine of the *Oikopleura* 3D model.

The *Paramecium* model was the starting point for a short film project called *The First Greed*, as well as the plankton flock for *Noise Aquarium*. It had several appearances in both projects. The starting point for the 3D model was a light microscopic stack. Furthermore, SEM images were taken to define the cilia, while light microscopic videos were taken for references of movements, behavior, and the outside structure. Additionally, light microscopic images in various lighting modes were taken to obtain more details, see for example figure 4.60. The making of the *Paramecium* 3D model and

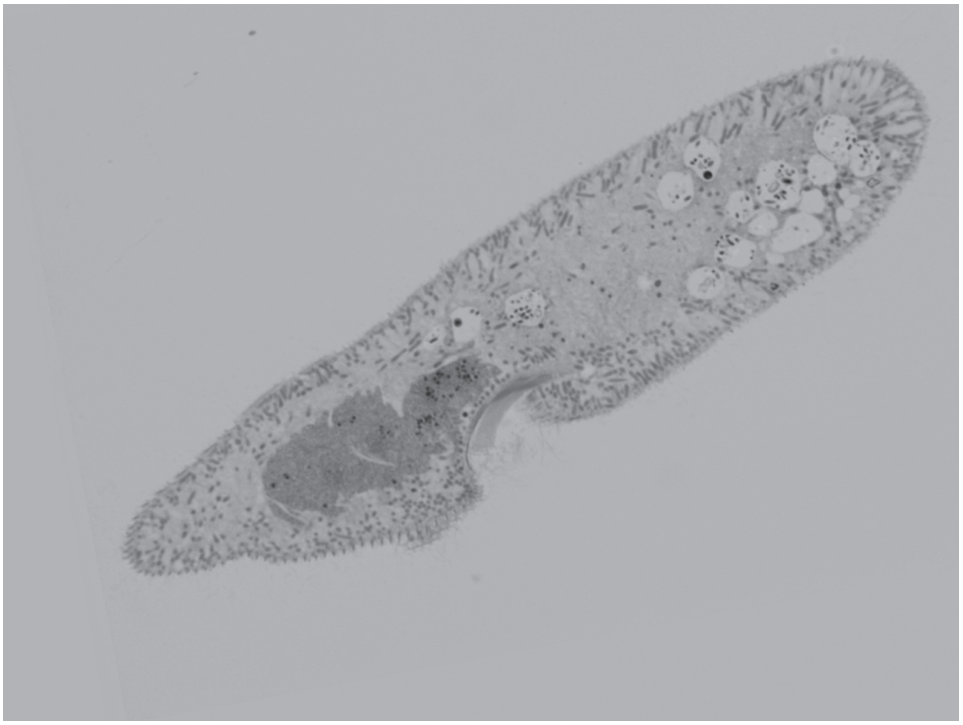


Figure 4.59: One image of the light microscopic image stack that was the basis for the raw 3D model of *Paramecium* by Stephan Handschuh.

animation was particularly compelling due to multiple aspects of the discussed tomographic workflows being used. The model contains a displacement map with scientific imaging origin, particle and hair simulations, and animated interior organelles. The main imaging stack was derived from light microscopy and is represented by one image as seen in figure 4.59. Typical membrane structures of *Paramecium* could be observed in additional light microscopic images and additional input from the SEM imaging confirmed the look of the rutted outside structures, as seen in figure 4.61.

Paramecia are found under certain lighting conditions when using color accents. These are the results of intracellular crystals according to [69]. The first versions of the *Paramecium* sp. visualizations contained these colored particles. Later on in the project the inner particles were shaded similar to the organelles of *Paramecium*. The interior parts are therefore less visible than in typical light microscopic two-dimensional images that reveal more inner details because of special microscope lights and between coverslips squeezed specimen. The 3D model of *Paramecium* is fully equipped with animated organelles according to the reference videos. Also, the cilia movements were simulated according to reference videos chosen for the project and the paper [191]. Movements for the *Paramecium* 3D animation were

Figure 4.60: One example of a light microscopic reference for the *Paramecium* 3D model by Stephan Handschuh and Martin Glösmann.

analyzed with rotoscoping but mostly animated according to the references. A good summary of organism movement behavior is given through 101science[32]:

> A *Paramecium* is a one-celled living organism that can move by beating the cilia. The *Paramecium* moves by spiraling through the water on an invisible axis. For the *Paramecium* to move backward, the cilia simply beat forward on an angle. If the *Paramecium* runs into a solid object the cilia change direction and beat forward, causing the *Paramecium* to go backward. The *Paramecium* turns slightly and goes forward again. If it runs into the solid object again it will repeat this process until it can get past the object.

The movements of food vacuoles from the oral groove in cytoplasm and crystals were animated with shape keys (also called morph targets in other software applications) according to reference videos. A contractile vacuole was animated according to videos showing dying *Paramecia* trying to main-

[32] http://101science.com/paramecium.htm; 30/8/2016.

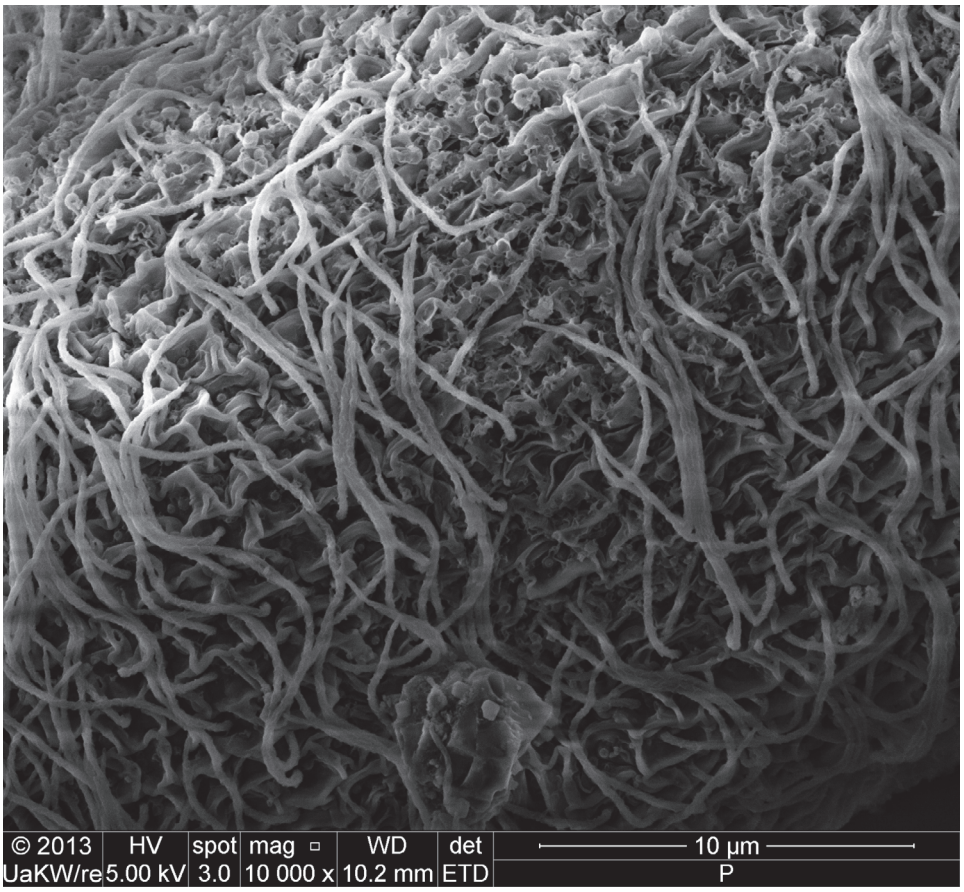


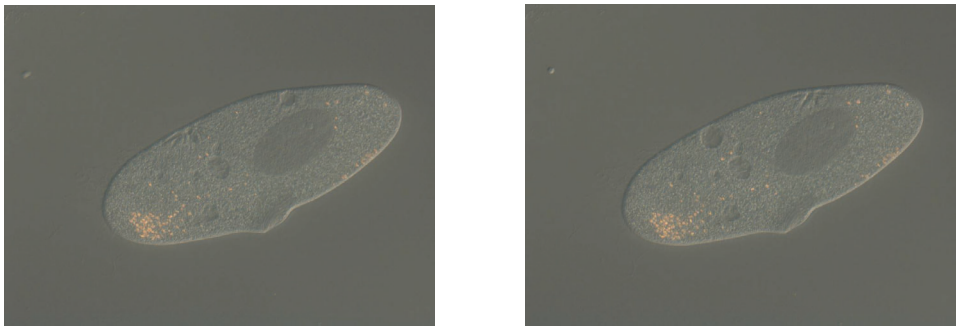
Figure 4.61: SEM image of the typical ridges of the *Paramecium* membrane. The cilia are cluttered together. SEM image by Rudolf Erlach.

tain balanced osmotic pressure within their cells through faster contractions of the vacuole, see figure 4.62.

As with all 3D models for *Noise Aquarium*, the *Paramecium* 3D model starting point was the exported Wavefront Object data set received from the collaborating biologists. Often, as in the case of the *Paramecium* data, it came with a simple overview turntable rendering. One frame of this turntable rendering can be seen in figure 4.63a, and one frame of a raw volumetric rendering of the *Paramecium* image stack is in figure 4.63b.

The typical surface structure of the organism was created using a detailed normal map (figure 4.64a). The normal map was generated in 3D-Coat. The ridges of the membrane of *Paramecia* are not very pronounced.

The Wavefront Object delivered from our collaboration partners was of high resolution, especially regarding the fine particles in the plasma. This was very helpful for the generation of an accurate normal map in order

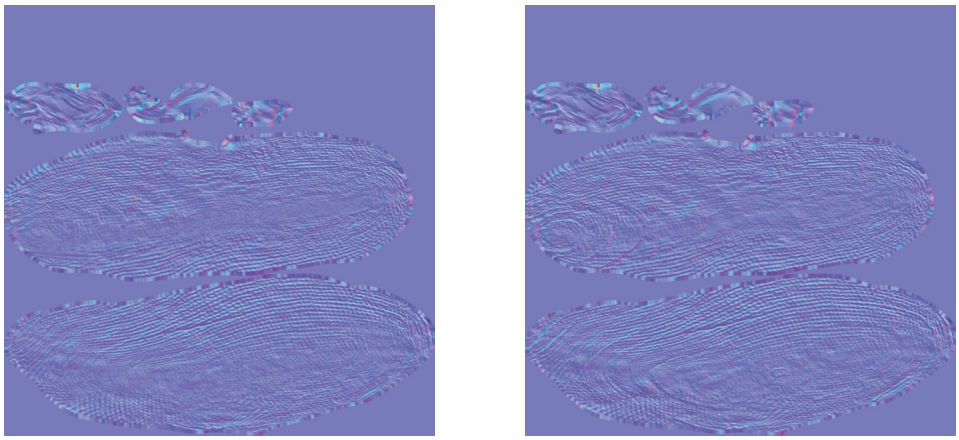


(a) (b)

Figure 4.62: Frames of a reference video by Stephan Handschuh and Martin Glösmann. Frame (a) shows the contracted vacuole and frame (b) shows the opened contractile vacuole of this *Paramecium*.

(a) (b)

Figure 4.63: Frame of turntable rendering by Stephan Handschuh (a), and a frame of a volume rendering of the raw image stack rendered in Blender Internal (b).



(a) (b)

Figure 4.64: Normal map texture for the *Paramecium* 3D model. Figure (a) shows the corrected normal map, while figure (b) shows the map extracted from the unaltered high resolution scanned geometry.

to achieve fine ridges and details of the *Paramecium* surface in the final renderings. And it allowed the usage of the actual surface structures of the scanned organism but had to be corrected due to some visible scan lines (figure 4.64b). The surface had numerous ridges forming a polygonal ridgework. Each center carries a cilium. Close to the mouth opening, there is a suture in the ridgework. For the animation and the normal map baking process in 3D-Coat, a retopo low polygon geometry version was needed. That is why the whole scanned 3D data were reworked. In figure 4.65 two stages of the editing process are recorded. In the process of retopo in 3D-Coat, texture coordinates (UV map) were also created and later reimported and reassigned to the Blender scene.

The animation and the cilia simulation incorporated various references, see for example figure 4.60 and figure 4.66. For the inner parts, a contractile vacuole was animated with shape animation. The cilia simulation is based on the descriptions of the collaborating biologists and was done with an animated textured force field that influences the hair simulation. This generated beating cilia as seen in figure 4.67.

Hair simulations for cilia had to be baked after the animation process and the Shape animation process. The scanned plasma had to be replaced by two particle systems, while the other intestine organelles (crystals, vacuoles, and nucleus) were used as scanned and animated with shape animations according to the video references. Animations should be finished and baked before particle or hair simulations. For flocks of *Paramecia*, either particle instances were created while smaller groups and single organisms were hand animated.

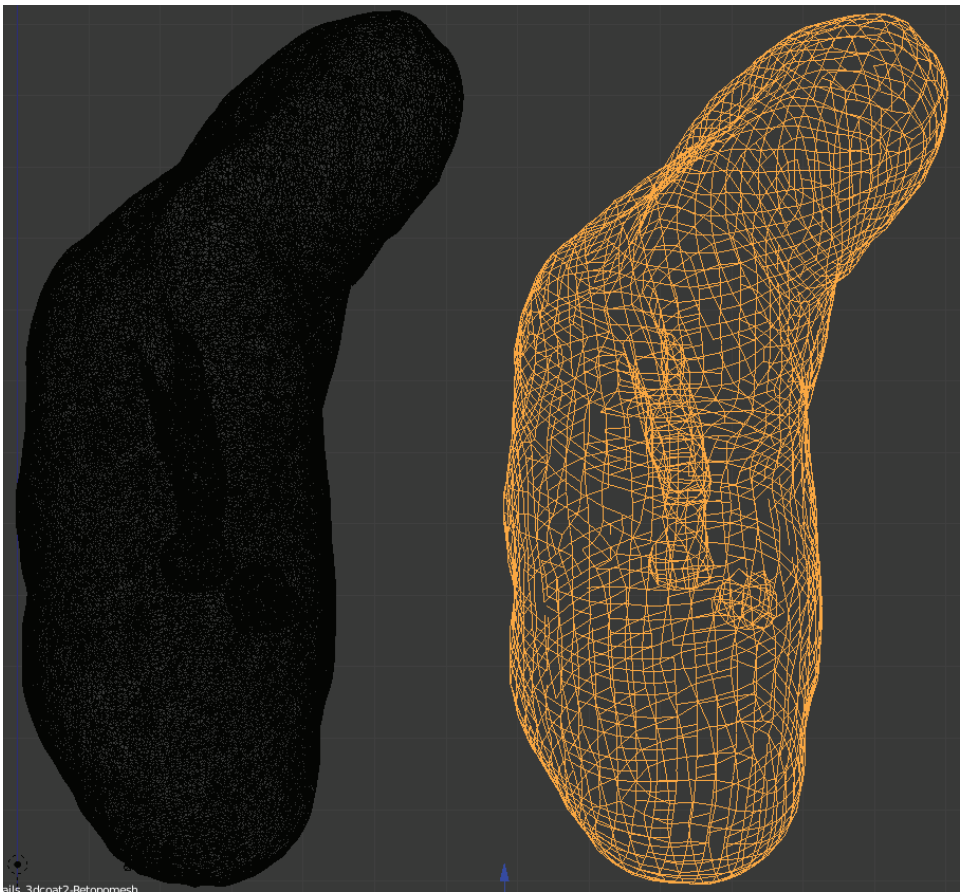
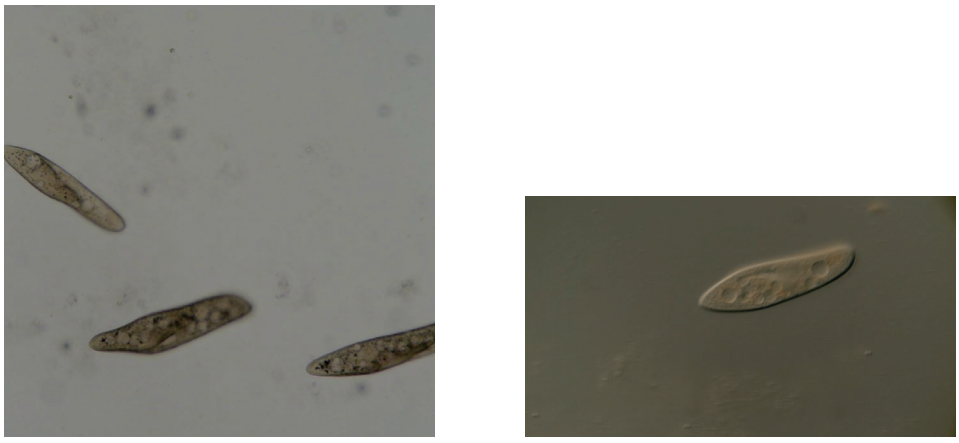


Figure 4.65: Two stages in the editing process in the creation of *Paramecium* using tomographic 3D scanning data. On the left side is the raw triangulated ultra-high resolution mesh and on the right side, a lower resolution mesh with quads only.

Crystals (see for more information [69] and organelles in *Paramecium* move through the organism. Light microscopically visible structures and cell organelles such as the mouth opening, nucleus, food vacuoles, contractile vacuoles, crystals, and others, were visible in most reference images and videos. The swimming behavior could also be observed in the reference videos. *Paramecium* organisms spin around their longitudinal axis while swimming. These movements are elegant and can be presented in the scientific animation more three-dimensionally than in the microscope since microscopic imaging of *Paramecia* restricts their movements through the use of coverslips. The *Paramecia* in the reference videos rotate with and against the clockwise direction with the oral groove generally in the front. The beating cilia of *Paramecium* draw food to the oral groove opening. *Paramecium* organisms feed on microbes such as bacteria. These microbes are accumu-



(a) (b)

Figure 4.66: Three *Paramecia* in one frame of a light microscopic video reference (a) by Stephan Handschuh and one frame with a single organism of one of a better video references (b) by Stephan Handschuh and Martin Glösmann.

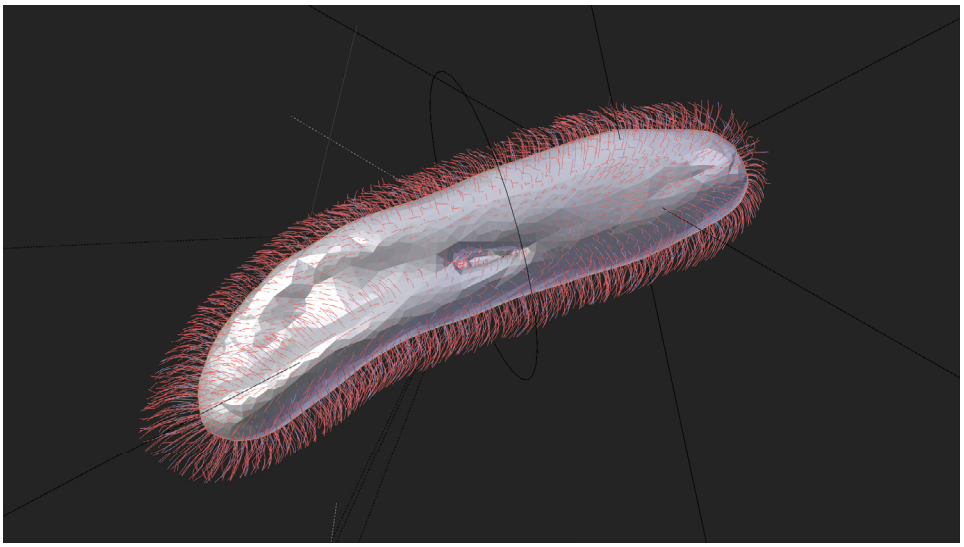
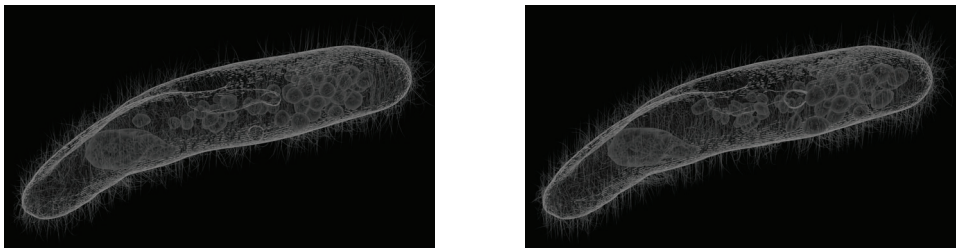


Figure 4.67: The *Paramecium* 3D model with hair simulation to depict the motion of the cilia.

lated and ingested by newly forming food vacuoles, which detach from the mouth as soon as the vacuole is filled, see figure 4.68.

The surface material of the *Paramecium* 3D model consists of the normal map, an occlusion map and a combination of shaders. In particular, the reflectivity was altered depending on the demands of each rendering.



(a) (b)

Figure 4.68: Shape animations of the organelles of *Paramecium*. Image (a) shows the beginning of the simulation, while frame (b) shows a later stage. In image (a) the contractile vacuole is filled and in (b) it is contracted.

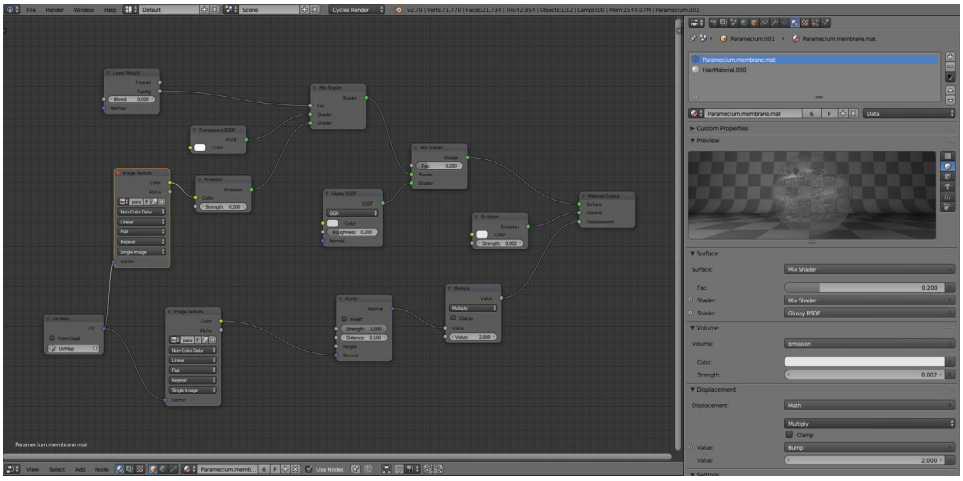


Figure 4.69: Shader setup of the *Paramecium* 3D model. Node configuration was slightly altered to show or partially hide the organelles of the organism depending on the requirements of the final output.

Sometimes it was necessary to remove reflection or the glass shader completely to make the interior of the *Paramecium* visible, while in other cases the outer hull was reflective and the insides were only distinctively visible. The shading configuration for the main shader of the *Paramecium* can be reconstructed through figure 4.69.

The shader of the cilia is a combination of Glossy BSDF, Emission, and Transparent BSDF Cycles render engine shaders. A final rendered image of the *Paramecium* 3D model in a composed final image of a look test is documented in figure 4.70.

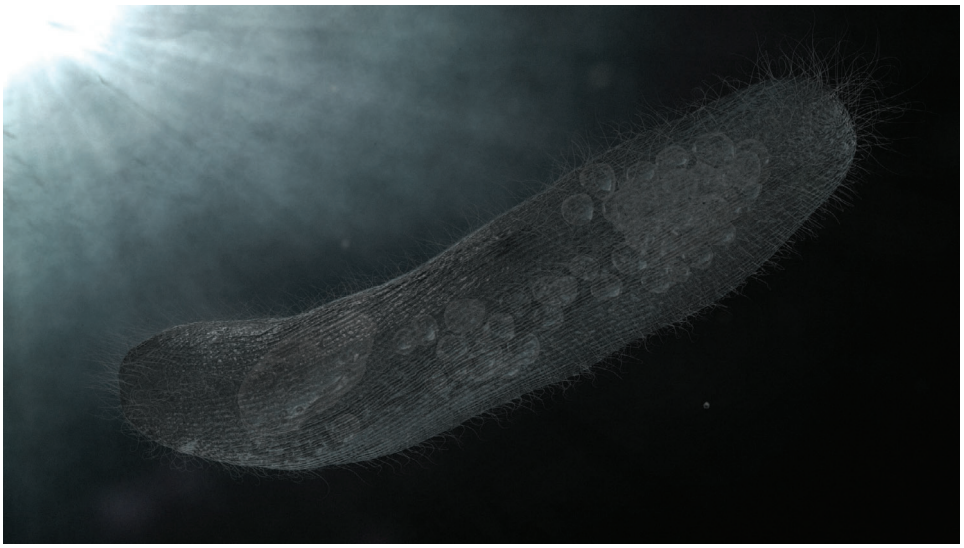


Figure 4.70: Compositing of a rendering of the *Paramecium* 3D model.

Tomopteris helgolandica

Tomopteridae grow to sizes of up to 100 mm and approximately 60 species have been discovered. They have deeply divided, forked, fin-like pods, a pair of lensed eyes, and long front tentacular cirri. *Tomopteridae* are among the few marine animals with yellow bioluminescence. They have a predatory lifestyle and mostly feed on *Chaetognaths*, *Tunicates*, and fish larva[33].

Tomopteridae never stop growing their entire life and the chosen specimen for imaging was approximately 5 mm long. During one of the lab visits to the Veterinary Medicine University of Vienna, photos of the *Tomopteris* samples could be taken. *Tomopteris* can be seen free floating in a petri dish in figure 4.71 and as ready for imaging in figure 4.72.

Tomopteris helgolandica is a transparent light emitting planktonic organism that belongs to the *Polychaetes*. These oceanic worms are often described as yellow emitters due to the fact that they emit yellow light in contrast to most bioluminescent pelagic animals [66].

There are yellow glowing spots on every fin. The multi-legged animal is thought to produce glow and light flashes in order to communicate [67].

These light flashes were not animated because there was mostly only one *Tomopteris* in frame and the light is a means of communication. While the glands of the legs of *Tomopteris* glow yellow, the rest of the body appears bluish in specific lighting situations. It was chosen for the cabinet of planktonic curiosities representing all planktonic species of the world in the

[33] http://www.imas.utas.edu.au/zooplankton/image-key/annelida/tomopteridae; 20/12/2018.

Figure 4.71: Various *Tomopteris helgolandica* specimen in a petri dish.

project *Noise Aquarium* because of its elegant body shape with fin-like pods and of course for its special bioluminescence. It moves in wave-like patterns and is very intriguing to observe.

Despite the assumption that *Tomopteris* is an insatiable predator which feeds on other planktonic organisms, often, the transparent guts are found entirely empty in caught specimens. Some species might even dominate planktonic communities and therefore must be of considerable importance as fish nutrition [47].

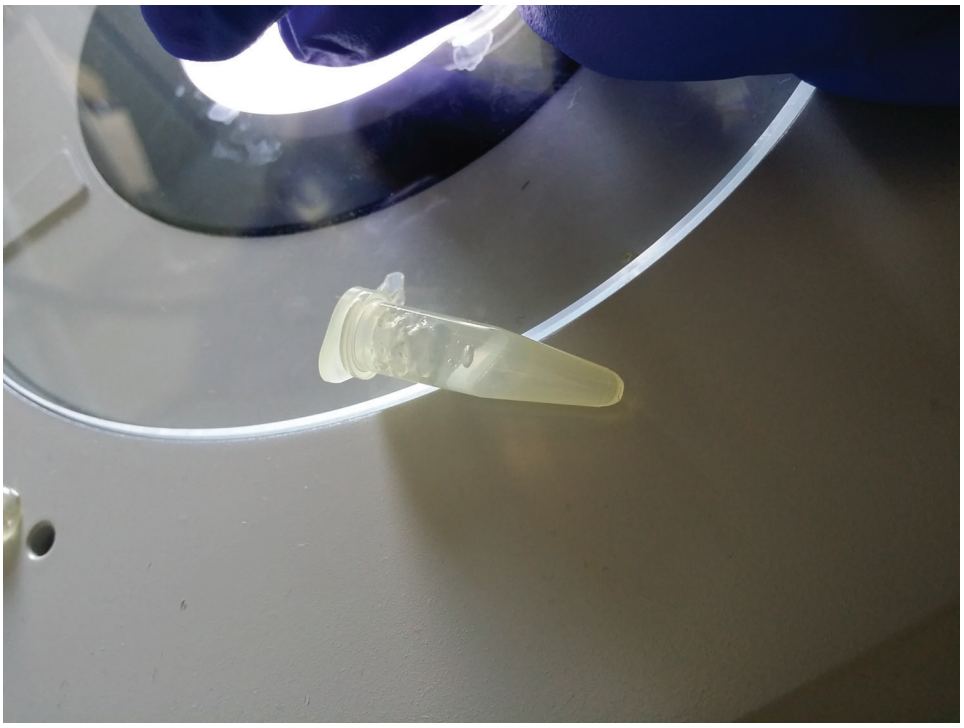


Figure 4.72: Prepared *Tomopteris helgolandica* sample.

There is an amazing variety of *Polychaete* worms, also called bristle worms on the seafloor as well as in the water column. Marine biologists have named over 8,000 species, and yet there are many more left to discover, especially in the deep sea[34].

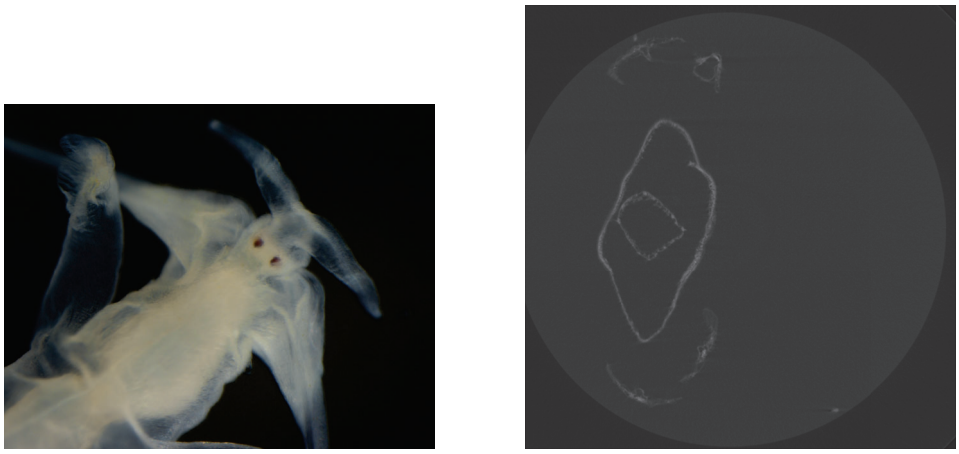
There were plenty of reference micrographs for the 3D model of *Tomopteris* available. The main data received from the collaboration partners were light microscopic and micro CT images (for instance see figure 4.73).

Starting with the initial tomographic scanned data from the micro CT stack of the scanned *Tomopteris*, a volume rendering in Blender Internal render engine was calculated. One frame of this rendering can be seen in figure 4.74.

In the raw 3D surface model that was exported immediately after segmentation, the details of the scanning data can be seen (figure 4.75a). One of the pods was additionally scanned separately (figure 4.75b). This gave additional information on the anatomy of the individual fin pods.

The micro CT scanned data of *Tomopteris* needed some mesh repair. The data had some artifacts but was already segmented upon arrival for further processing in the animation pipeline. The body of the animal is by

[34] https://www.mbari.org/bristle-worms-get-their-turn; 01/05/2016.



(a) (b)

Figure 4.73: Light microscopic reference of the head of a *Tomopteris* specimen, image by Thomas Schwaha (a) and a stack image of the micro CT scan (b) by Stephan Handschuh.

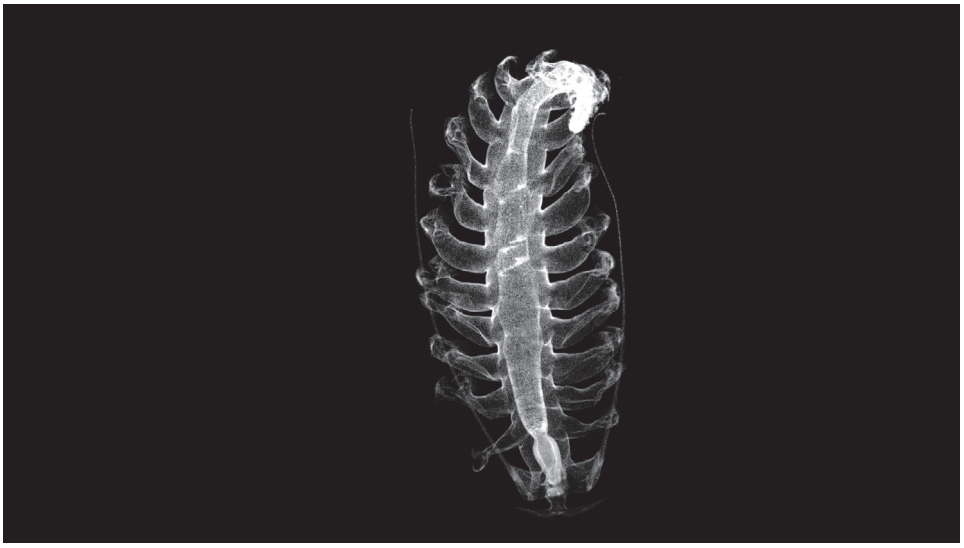
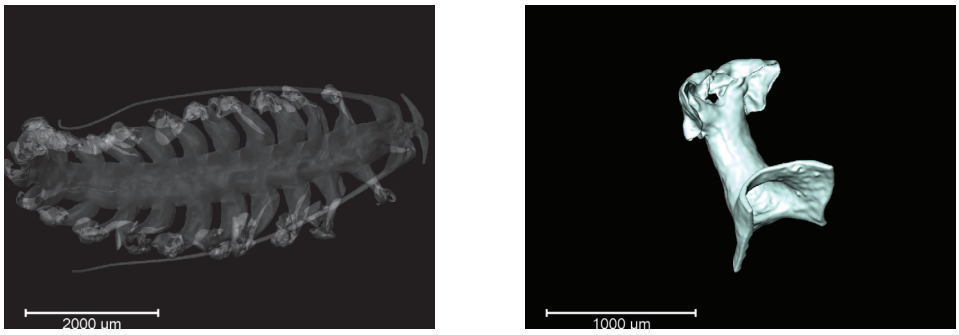


Figure 4.74: One frame of a preview volume rendering of *Tomopteris helgolandica*.

nature roughly symmetrically. At the beginning of a 3D model pipeline using tomographic scans, there is often the consideration whether the finished character should be complete, meaning unnaturally symmetrical or not. It is easier to animate and rig a symmetrical animal, which is why it is an often employed technique in computer animation. In the case examples herein, for



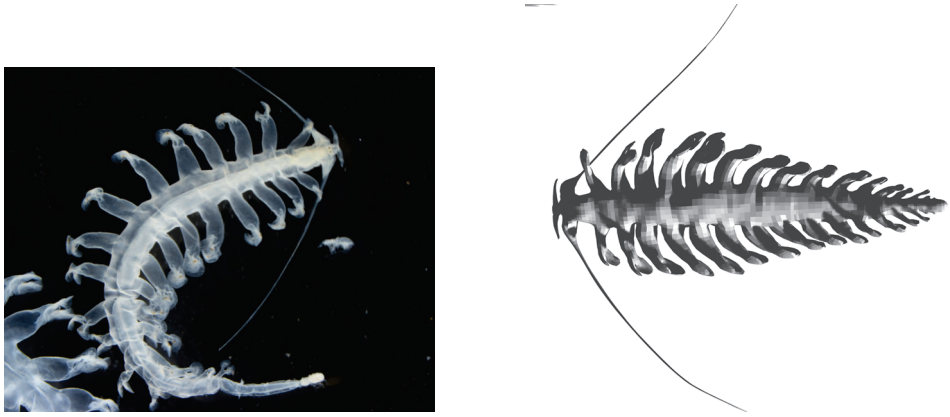
(a) (b)

Figure 4.75: *Tomopteris* digital 3D model rendered with transparency as preview (a) and a rendering without transparency of a separately scanned pod (b). Images by Stephan Handschuh and rendered in Amira.

most 3D models, a decision against total symmetry was made since it does not correspond to the naturally scanned animals. The *Tomopteris* case is particularly demanding because it has many paired limbs, each of which also have finned pods. Symmetrical limbs change the scanned data enormously. As a result, it was decided to use symmetry in the creation of the 3D model, since the scanned animal was broken (compare figure 4.76a) and needed numerous repairs to show a healthy looking representation of the once living animal. Therefore, the data of the *Tomopteris* was first repaired and then mirrored, but in the end not entirely symmetrically in order to maintain the authentic look (see figure 4.76b). The repair process was done with the voxel sculpting software 3D-Coat. One of the most important decisions in voxel sculpting is the selection of the appropriate voxel resolution. Otherwise, the digital sculpture might become unmanageable when the voxel resolution is either set too high or contain holes and artifacts when set too low. The innards of the *Tomopteris* were also scanned and segmented. The *Tomopteris* sample had a broken tail that means it had to be repaired. Internal and double walled cavities of the organism also posed an extra challenge in the making of this 3D model.

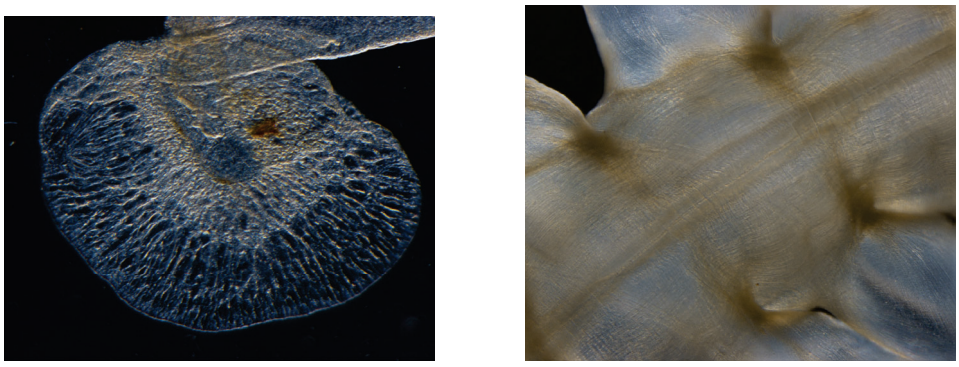
Additional light microscopic images were available as references. This was particularly important for the detailed reconstruction and repair of the scanned pod fins. Highly detailed micrographs with out-flattened fins or separated body parts were taken with a focus on the generation of textures. In figure 4.77a, a micrograph of a fin used for the 3D model can be seen as one example of several available images of this type. In figure 4.77b one micrograph that was used to texture the skin of the 3D model is depicted.

In order to use the texture which was painted using a combination of the available micrographs, a detailed texture coordinate layout was necessary (figure 4.78). Texture Paint Mode in Blender with self-created stencils



(a) (b)

Figure 4.76: Broken sample of *Tomopteris helgolandica* that was scanned (a) and the repaired mesh ready to rig (b).



(a) (b)

Figure 4.77: Light microscopic micrograph of a *Tomopteris* pod fin (a) and detailed micrograph of the skin of a specimen (b). Micrographs by Thomas Schwaha.

created from close up light microscopic references was used to produce the final texture for the 3D model.

The shading of the *Tomopteris* 3D model required several vertex groups to define the parts of the animal that glow along with the rest of the non-glowing surfaces. The material with texture inputs and shaders can be seen in figure 4.79.

Video references for the animation of the *Tomopteris* model were unfortunately lacking. There was only one video of a dying *Tomopteris* available from the collaboration partners. Additional material taken from the Internet were either not in sufficient resolutions, or showing completely other

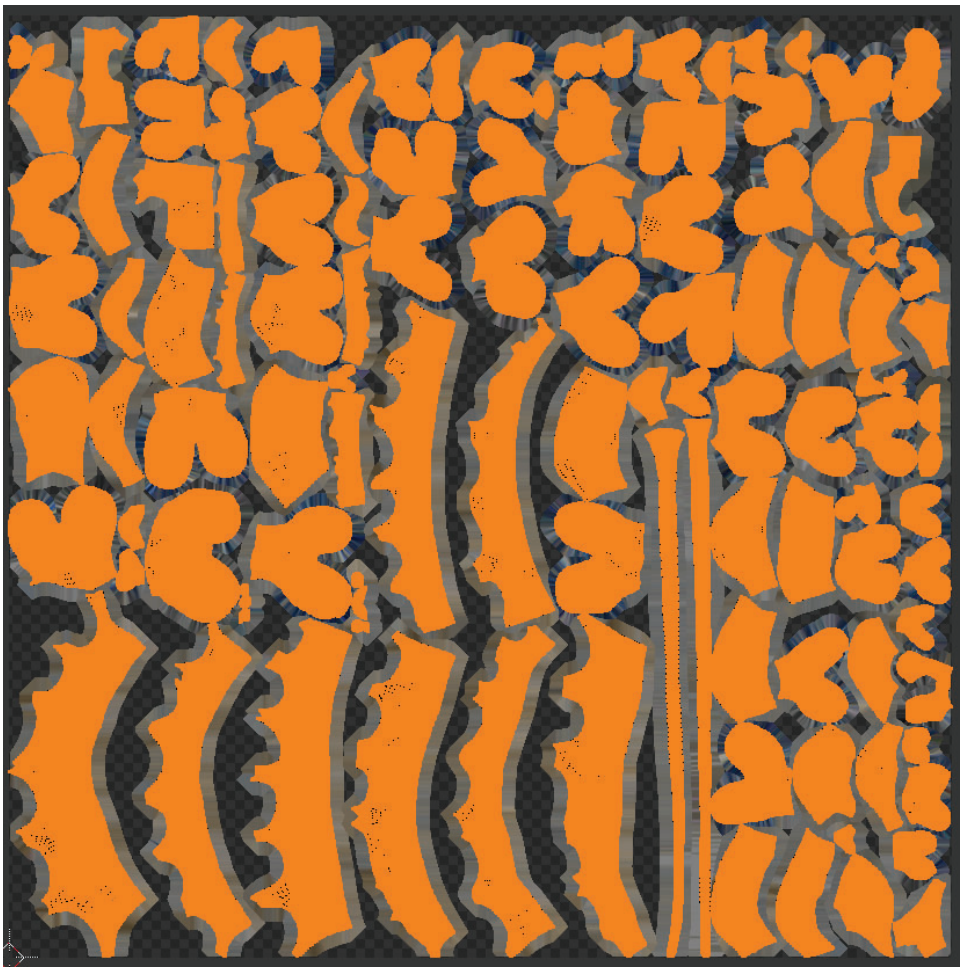


Figure 4.78: The UV layout of *Tomopteris* 3D model.

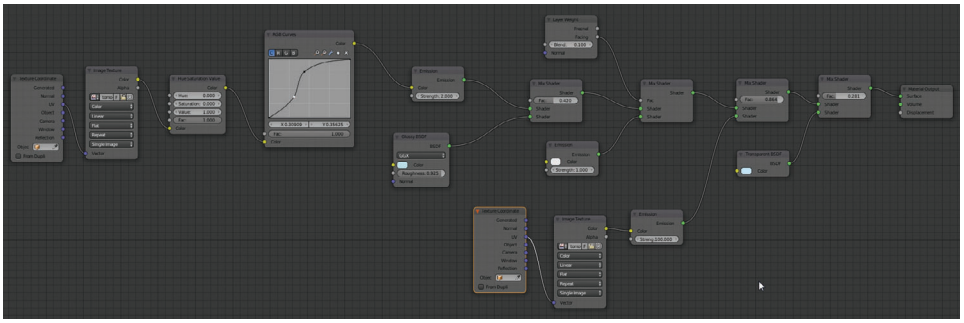


Figure 4.79: Shading setup for the 3D model of *Tomopteris*.

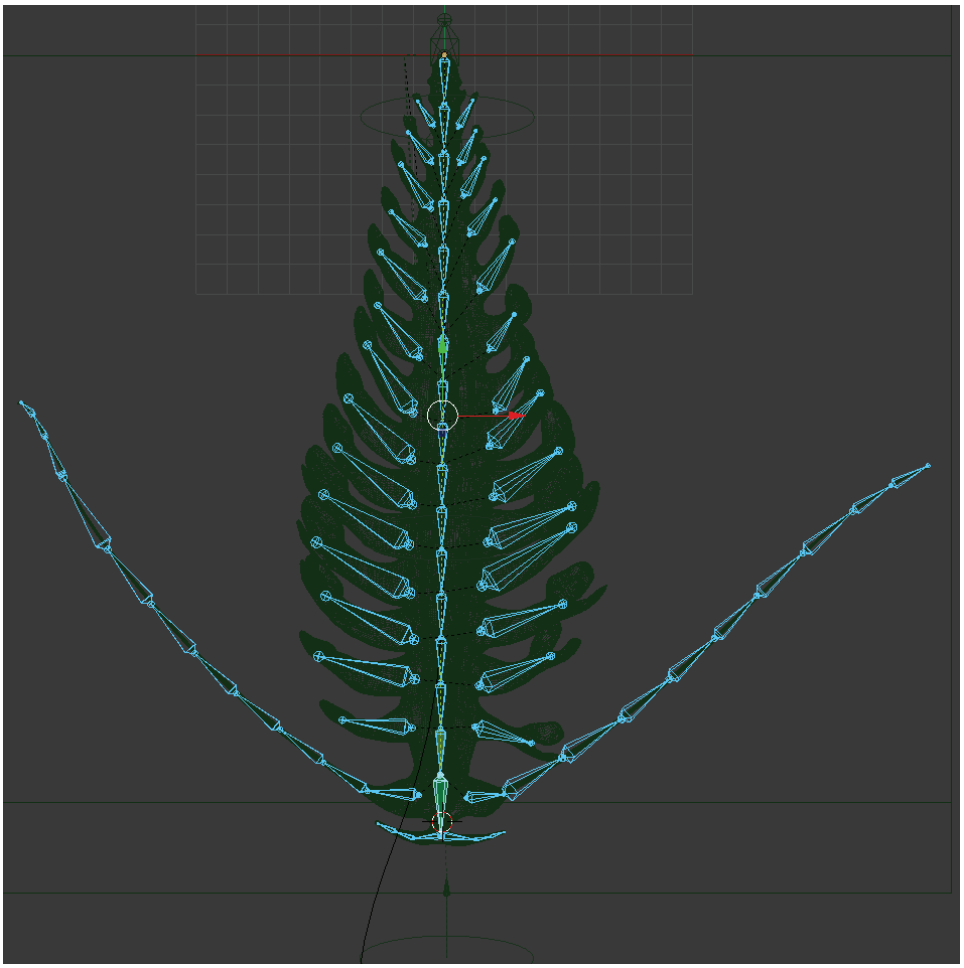
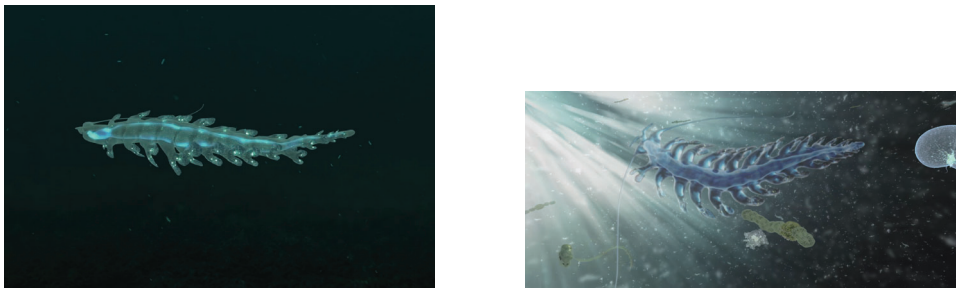


Figure 4.80: Rig for the 3D model of *Tomopteris*. A hierarchical order and copy location constraints were used to animate the digital animal.

types of bristle worms. The paddling motion was animated with a simple rig (figure 4.80). There is ongoing research on the behavior and locomotion of *Tomopteris*. We were in contact with researchers at the Smithsonian Washington Osborn Lab[35] in order to form a collaboration. Their research focuses primarily on the study of the organisms taken from midwater (ocean water) and they expressed interest in using our 3D model to visualize their motion data analysis of the *Tomopteris*. Nevertheless, to this date, additional motion data could not be acquired from this collaboration.

The first final rendering, visible in figure 4.81a, was shown at the Blender Conference 2016, from which a making of talk titled *Microscopic Data Pro-*

[35] http://invertebrates.si.edu/osborn/index.html; 04/06/2018.



(a) (b)

Figure 4.81: First rendering of the *Tomopteris* 3D model which was shown at BCon 2016 (a) and one of the later renderings for the project *Noise Aquarium* (b).

cessing in Blender may be viewed online.[36] For the project *Noise Aquarium*, several renderings were produced, one of which can be seen in figure 4.81b.

4.3.3 Visualizing the realm of a polluted ocean

Seas and the ocean are the original sources of all life on Earth as we know it, and they have a major impact on the duration and speed of climate change [48].

Plankton is a critical component of these important ecosystems, for instance as the basis of the food chain. A multitude of threats arises from anthropogenic influences for plankton.

Various experts calculate that approximately 50–70 percent of the oxygen in our atmosphere is formed by plankton (compare [37], and [169], yet the total number of plankton organisms decreases significantly and the biodiversity is minimizing [37].

The project *Noise Aquarium* deals with another possible threat to microscopic-sized organisms in Earth's bodies of water: the strain of anthropogenic noise in the ocean. The impact of various noise sources on large marine life is widely known through striking examples in the media of stranded whales.

Only a handful of studies also address the influence on microscopic life forms in plankton, such as scallop larva [185] or planktonic living coral larva [201].

Sound waves travel at different speeds in water than air. Many marine animals use sound for orientation, perception, and communication since sound waves are perceptible underwater much further than light waves. Sound can propagate in the water with less resistance and there are more echoes as sound waves are reflected repeatedly. At the same time, the propagation of sound in water is five times faster than in the air. For life in the ocean,

[36] https://www.youtube.com/watch?v=0hnT_aoHuEg; 04/07/2017.

acoustic communication is more important than visual. The noise levels in the seas are continually rising. A variety of anthropogenic noise sources such as airguns, acoustic tomography, bubble curtains, dredging, explosives, icebreakers, motors, pile driving, sonars, torpedoes, wind turbines and even underwater breathing apparatuses for diving are known to disturb natural life underwater[37].

As a result, living beings underwater start to communicate louder. Similar to humans, animals in loud environments start to increase volumes and pitch, such as, for instance [161] found in St. Lawrence River beluga, or [145] investigated in North Atlantic right whales, meaning the so-called Lombard Effect comes into play.

In the first linear video version of *Noise Aquarium*, the organisms disintegrate into small pieces when exposed to loud noise. This was an overly dramatic visualization of the topic and probably unrealistic except for situations where plankton are directly exposed to threats such as marine propellers and explosions. Later in the project, we were able to find a more realistic form of presentation by showing tumbling and descending affected plankton. Both methods of reacting are used in the different visual versions of *Noise Aquarium* and presented on various occasions[38].

The representation of the pressure waves was developed through many experiments. During this development phase, much emphasis was put into the representation of the actual sound waves. Gradually, various visual presences of *Noise Aquarium* were developed. One challenge was to authentically visualize the densification of the water and therefore visualize the sound waves in an understandable manner. The first publicly presented video version utilized the Blender Wave Deformer controlled by sound samples. The chosen solution shows planes which are deformed by waves. The sound waves have a common origin which adds to the underwater view a type of fake central perspective. This has qualities of a "sonic rosette", see figure 4.82, which pulls the viewer into the visual. Sound only becomes visible through deformation of the particles emitted by the invisible surfaces. The dimensions are represented by aligned virtual planes at various angles relative to each other in the digital 3D space. The noise matches the visuals because the particle system deformations were controlled by the sound. For later versions of the linear *Noise Aquarium* videos, this sound control of the deformation was implemented with the CC Ripple Pulse Effect[39] from the compositing software After Effects (Adobe, Inc., San Jose, CA, USA).

There are only a few studies on the impact of noise on marine microscopic organisms, but the *Noise Aquarium* team believes strongly in the importance of drawing attention to the topic and continuing researching

[37] https://dosits.org/decision-makers/tutorials/science; 26/11/2018.

[38] Visit the project website www.noiseaquarium.com for more information.

[39] http://www.cycorefx.com/downloads/cfx_hd_std/CycoreFX%20HD%201.7.1%20Manual.pdf; 27/11/2018.

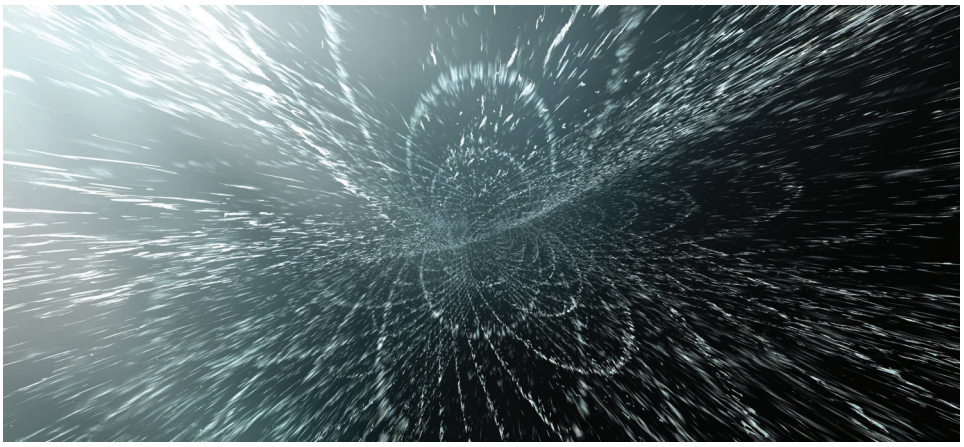


Figure 4.82: Visualization of noise pollution through multidimensional "sound rosettes" for *Noise Aquarium*.

and awareness building. The influence on scallop larvae [185], and coral larvae [201] appears to be confirmed and thus it stands to reason that other pelagic microscopic organisms in plankton are also influenced. This indicates that all topics covered in the *Noise Aquarium* project are scientifically relevant. Even though the impact of noise pollution is thus far not confirmed to be as severe as with large ocean mammals, drifting microorganisms can be still seen as a metaphor for general problems of noise sources in water bodies of the world. Therefore, it is of great importance to create awareness using authentic scientific visualizations of these ecologically important living beings. Additionally, in the interactive and later linear versions of *Noise Aquarium*, the topic of plastic pollution was included into the project since plastic pollution in water bodies of the world has recently become an important topic. There can be never too much awareness creation for such pressing environmental issues.

4.3.4 Presentations and interdisciplinary collaboration

Noise Aquarium is an internationally exhibited art project. It was developed in close cooperation between the principal collaborators Victoria Vesna and Alfred Vendl. The digital artwork and animation for the project were done by the author of this thesis. The project evolved in close collaboration with the biologists and scientific imaging experts Stephan Handschuh and Thomas Schwaha. Additional advisors and support helped the project thrive even further. The academic and creative potential of a cooperation, as it was developed for the project, is not typical. All participants first had to learn to go beyond their field of expertise and communicate with experts from other fields.

In multidisciplinary projects, the main challenge is often to start communication processes with trust and respect. Most interdisciplinary collaborations are more innovative than projects concerning only one academic field. That is one reason why more and more universities encourage interdisciplinary projects. Once such a collaboration has begun, the project benefits from the intellectual contributions of all involved partners. Every example of the recognizable trend to form interdisciplinary project teams intends to start creative processes without field boundaries. The *Noise Aquarium* collaboration partners of the ArtSci Center at the University of California Los Angeles, the Science Visualization Lab of the University of Applied Arts Vienna, and the Universities of Vienna and Veterinary Medicine Vienna forged a path together to create something valuable and unique for the project *Noise Aquarium*.

The first public version[40] of *Noise Aquarium* is a nine and a half minute long visual experience. It deals with anthropogenic noise pollution in the ocean. Sound samples were first used from the dosits website[41], then underwater recordings by Artist Robertina Šebjanič, later in the project mixed sounds by Paul Geluso were used. In the first version, all planktonic organisms are represented individually and each creature is destroyed by a different noise source. The sound waves are displayed as a representation of sounds in all possible directions starting from one point. Almost symmetrically they create a pattern that is a visual sonic shape, coming from the background and traveling towards the planktonic creature to eventually overwhelm them. The populated swarms in the background are the first to be affected. All creatures are visually perturbed and shaken. One minute we could admire an enormously enlarged microscopic organism, the next it symbolically dissolves under the intense sound pressure. This stress becomes visually and acoustically perceivable. Disease and healing is a part of all living systems in our environment. This constant struggle for survival, forthcoming, or an equilibrium is part of systems surrounding the human presence on Earth. If the stress becomes too high, many ecosystems die. This is hinted at by fading colors at the end of the video, which itself is designed as a loop.

Unconventional collaborations lead to completely new approaches, both in artistic and technical research. Art-science collaborations can be conducted in various ways. Many research teams have to be interdisciplinary due to the complexity of the research topic. However, disciplines which are unrelated generally only rarely collaborate, even when it would be highly beneficial for the outcomes. There are few true interdisciplinary collaborations in which people from natural sciences, technical sciences, and the arts work together. The *Noise Aquarium* collective is one example where

[40] https://www.youtube.com/watch?v=fWlH66uqMEQ; 08/02/2019.
[41] https://dosits.org/galleries/audio-gallery/#manmade, 02/02/2019.

such a true collaboration occurs. Humanity as an information retrieving and understanding community should overcome conservative structures to accommodate such unconventional collaborations. There should be a basis for understanding a topic at various levels which might be emotional, physical, rational, and analytical. A nice example of intellectual and emotional understanding, for instance, within the field of biochemistry are the ongoing collaborations between different departments of the University of Applied Arts Vienna and the Vienna Biocenter, to name a few examples: the collaboration between the Angewandte Innovation Lab and the Vienna Biocenter,[42] a project between the department of Applied Photography and Time-based Media and Vienna Biocenter named *Looking Glass*,[43] and the case study described here *CRISPR/Cas9-NHEJ: Action in the Nucleus*. Hybrid methods in computer animation augment the spectrum of digitally created motion pictures by applying additional tools and procedures. These with scientific data sets expanded animations are pushing the boundaries of what defines a digital animation and a computer-animated scientific visualization. The hybrid methodology applied in the *Noise Aquarium* project utilizes scientific imaging techniques within digital animation workflows and was the first animation project created using the laborious process described in this text. This uncommon integration of scientific imaging into computer animations benefits from a fruitful collaboration of diverse institutional settings.

Various invitations for exhibitions confirmed the arising interest in the project, as well as the general topicality of the matter. In 2017, the collaborative project was presented at the California NanoSystems Institute in Los Angeles, at the Art Gallery of the Web3D conference at the Queensland Technical University in Brisbane, at the symposium for Fluid Visualizations and Sound Matters at Angewandte Innovation Lab of University of Applied Arts Vienna, at the Media Art Nexus Screen at Nanyang Technical University Singapore, as part of the Vis Matters Conference exhibition at the EPICenter Sydney, and in an art lounge at TEDx Manhattan Beach. The project grows organically, meaning that every presentation is unique and adapted for the location. For future exhibitions and presentations, the team wishes to further evolve the *Noise Aquarium* project. The ongoing installations and screenings should create awareness for topics on which the survival of all humans might depend. The installation conspicuously places the destructive nature of sound pollution effects of the Anthropocene into large images and as a result questions global authorities who hesitate to initiate actions to intervene against the destruction of our environment. One might argue, every individual can potentially do something, thought it generally requires a certain amount of influencers to form a critical mass. The project

[42] https://www.imba.oeaw.ac.at/about-imba/general-news-press/art-and-science-presentation-at-the-vienna-biocenter; 01/03/2019.

[43] https://www.imba.oeaw.ac.at/science-society/events-outreach/looking-glass; 01/03/2019.

tries to create awareness of the importance of our water-based ecosystems and hopefully inspire great minds from all over the world to find solutions for environmental issues.

In the year 2018, the Ars Electronica Festival in Linz, Austria, and the Paseo Project festival in Taos, New Mexico, USA, invited the *Noise Aquarium* team to exhibit the project. This was the opportunity to create the long-anticipated interactive version of the project. All previous presentations were linear video projections of the scientific imaging originated computer animations. Although the international screenings were very successful and there was a huge interest to make linear visual versions, the goal has always been an interactive, participative version.

For the interactive version of *Noise Aquarium*, it was clear from the start that considerable attention to visual details, as well as the highest possible fidelity obtained from scanned organisms and the resulting computer animation should be maintained. Therefore, the decision was made to use prerendered 2D image sequences with matching normal maps. With this solution, the geometry did not need to be reduced and a real-time stereoscopic version was possible. The interactivity was developed by Glenn Bristol and uses the Unity game engine for the visual and audio rendering while communicating wirelessly to a Python program running on a Raspberry Pi mounted underneath a custom built platform as an input device, see Figure 4.83. The platform has a Nintendo Wii Balance Board embedded inside and is connected to the Raspberry Pi via Bluetooth. The interactive installation initially shows a flock of numerous plankton creatures drifting around, with pieces of plastic also visible. One participant is asked to step onto the custom octagonal platform, whereby the interface triggers one random plankton organism to come closer until it fills nearly half of the virtual screen. The participant must then balance the chosen organism for a short time period in the center of the screen. If the person is able to keep the plankton stable and balanced, a metaphorical balancing of the whole ecosystem occurs, thereby causing an auditory symphony of whales who will have enough to eat. In the other case, if the person does not manage to stabilize the ecosystem, the number and intensity of destructive underwater sounds increases, while the plankton sink dead to the ground leaving only micro plastics behind. During the approximately one minute interaction phase, the movements of the interacting person are also interpreted through directional sound to give the participant additional cues for the visual movement of the enlarged plankton to indicate which sides are unbalanced. The sounds triggered by movements during the interaction phase are anthropogenic underwater noises.

The title *Noise Aquarium* already imparts the importance of sound for this project. For the media installation, the sound was mixed in 5.1 and travels through the room according to the balancing movements of the player on the platform. The sound samples were provided and mixed by Paul Geluso at NYU Steinhardt. The older linear visual versions featured real underwater

Figure 4.83: *Noise Aquarium* showing in the Deep Space of Ars Electronica 2018. A person interacts with the projection using the custom platform. Image by Tom Mesic.

sound samples recorded by artist Robertina Šebjanič and were mixed by the author. The huge depictions of plankton with their authentic details, paired with immersive presentations in either 2D or stereoscopic 3D led to memorable experiences for visitors. A Virtual Reality version for HTC Vive Pro headsets will be available in May 2019 and presented at the Angewandte Innovation Lab during the Vienna Biennale for Change. The locations for the presentations were highly diverse, as were the projection types and screen formats, each leading to unique, multifaceted experiences. One thing that all of these visual representations have in common is that the plankton organisms were enlarged enormously, such that they were sometimes several meters in height and considerably bigger than the visitors. Some people felt very alienated by these huge representations of usually microscopic-sized creatures. In most exhibitions, the projections were so immersive that some recipients even described the large creatures as uncanny. We have not so far conducted any surveys, however, we talked to many people during and after each presentation. The overall feedback was that the enlarged creatures fascinated the spectators simply due to the unfamiliarity. People were definitely attracted to the unusual visual experience. The huge projections made people think of living beings which they normally can not observe.

Chapter 5

Discussion

5.1 Positioning in the field of data visualization

Scientific visualization is the computational creation of visual representations of scientific data. It is a broad field, with a diverse range of applications. Whereas the majority of scientific visualizations are generated automatically with the major design implementations predefined in the initial writing of the software application, there is also the smaller group of visualizations that are animated, conceptualized, designed, and implemented manually and individually for every topic that should be depicted. As scientific visualization is a subfield of "data visualization" and a sibling field of "information visualization" a further subcategory might be introduced which provides a distinction between automatically and individually produced scientific visualizations. The projects in the discussed case examples are distinctly and individually produced for every use case, therefore the term "computer-animated scientific visualization" is proposed to name them explicitly.

Another distinct feature for scientific visualizations might be the use of narration. Computer-animated scientific visualizations may be subdivided into those with and without major narrative efforts. Naturally every picture tells a story, nevertheless, within video sequences using dramaturgy and sequential storytelling, the narrative approach is more apparent. Sequential storytelling approaches, for example in the case examples in section 4.3 and 4.2, are common amongst individually produced scientific visualizations. These storytelling approaches originated in the Science Visualization Lab Angewandte's history of producing computer animations for documentary productions. The pipeline used in the case examples may be generally reproducible, however, every project required custom steps and techniques to fully incorporate scientific data and their respective structured narratives inclusive sound design.

While numerous universities have established visualization labs or simi-

larly facilities, few produce the same level of customized computer-animated scientific visualizations which are the main topic here. Many of these facilities are computer graphics labs which exist primarily to invent and design new applications for automated data visualizations rather than dedicated animation studios. Software development is essential for digital visualization, nevertheless, the intense research in art and design perspectives should not be overlooked in scientific visualization. Automated approaches have the advantage of being economically feasible for mass production. Among studios and labs producing individualized scientific visualizations are often private companies with connections to research facilities who receive assignments because someone wants a high polished, or at least a unique representation of their current research outcomes, often to impress the public and therein attract potential sponsors. Larger studios and labs usually employ or contract personnel from both computer animation and computer graphics fields to unleash the full potential of their computer-animated scientific visualizations.

Due to the heretofore small scope of the Science Visualization Lab Angewandte, it would not be appropriate to compare our work with institutions such as the Advanced Visualization Laboratory at NCSA (National Center for Supercomputing Applications, Champaign, Illinois, USA), the NASA SVS (National Aeronautics and Space Administration Scientific Visualization Studio, Washington, USA), including CI Lab (Conceptual Image Lab, Greenbelt, Maryland, USA) and Goddard Media Studios, or the animation team at WEHI (The Walter and Eliza Hall Institute of Medical Research, Melbourne, Australia), nevertheless, they produce computer-animated scientific visualizations of the kind which are the main topic of this thesis. Taking a closer look, this placement within a certain niche of data visualization places the herein discussed animations into a subgenre, in which not all discussed productions fit equally. In particular, *Noise Aquarium* has been developed continually in the direction of an art installation, yet the underlying data and the technical processing were scientific visualization pipelines. Hardly anyone questions the value of MRI and CT scans in medicine, biology, and biochemistry—tomographic scans are widely used and valued. In all of these fields, computer graphics are used to illuminate and present processes, yet use cases are rare where scanned data also become computer-animated using rig manipulations, or artistically set up simulations as in some of the projects from the above mentioned institutions.

Most researchers, both artistic and scientific, are generally interested in the limits of perception within the confines of our physical world. The experiments that each perform may differ drastically, yet the fundamental questions are similar. In this mutuality among research targets, there are artists who specifically focus on utilizing scientific data. Artists who are interested in incorporating the rigid, restrictive digital measurements of scientific institutions into their artistic practice are, for instance, the British

artist duo *Semiconductor*[1]. In their artworks they explore how our immediate environment is researched using scientific data and how this information is communicated with or without artifacts. Artistic expressions like these are presenting scientific data at a meta level using computer animation because the process of recording and of data phenomena are discussed in such pieces. The editing steps for the case studies were documented in the previous chapter. For example, for the presentation of *Noise Aquarium* at Ars Electronica 2018, a booklet was produced which introduced an overview of the editing steps to the audience. It is a difficult task to convey the right amount of background information regarding production to general audiences. It is definitely simpler to clarify the data processing background, and therefore the uncertainty of the visualized data to domain field experts of scientific imaging and scientific visualization.

5.2 Impact of the case studies

The case examples in chapter 4 show clearly that there are many technical steps necessary to produce scientific visualizations of tomographic scanned microscopic entities. New technical approaches and therefore innovative projects have been introduced. Here it should be discussed whether or not it is reasonable to produce computer-animated scientific visualizations with the introduced laborious technical approaches. This work deals with this question by analyzing three case examples, all of which came into existence within the last six years. All three projects have in common that their initial "data" is acquired through tomographic scanning. The scanning techniques vary, yet the input data taken from the examples in the previous chapter are technically similar.

The case study that examined two different mites species and their gaits was the most scientific project of the three examples. It was planned as an intensive study of the locomotion of two separate mite species. Starting point was an invitation to a symposium and the first results could be presented in a scientific visualization exhibition. The 3D models were scanned and edited to acquire two representational models of the respective species. The benefits for mite biology could have been greater, had a mite expert used the animatable models in closer collaboration in order to study and visualize the latest theories of mite movements, or even to depict previously researched mechanics and motion patterns. This science project depended strongly on the involvement of field experts in order to exploit the full scope of scientific possibilities. For the use case of a presentation in the scientific visualization exhibition, the usage of tomographic scanned animated 3D models and the slightly higher expenditure of time, energy, and resources was reasonable. The 3D models are stored and the research part could be resumed.

[1] https://semiconductorfilms.com; 01/03/2019.

For the CRISPR animation, the usage of data sets was essential, even though it was produced with the intention to be a scientific visualization as part of an art project. The video *CRISPR/Cas9-NHEJ: Action in the Nucleus* mixes data sets with purely schematic 3D geometry. Nevertheless, the main molecules involved in the depicted processes all have their experimental shapes derived from the scientific data taken from the wwPDB. This connection to authentic scientific data was the reason that the animation could achieve international attention at SIGGRAPH 2018.

Although it started as a scientific visualization project for documentary films, *Noise Aquarium* eventually became an interactive media installation project, due to the artistic collaboration with Victoria Vesna, head of the UCLA's ArtSci Center. It is a project that has thus far been and will be in the future presented at major media arts festivals world wide. We have not collected questionnaire data nor recipients studies until now, however, all project members had interesting conversations with persons who experienced the project firsthand. Nearly all viewers and participants pointed out that the project is extraordinary due to the authentic approach to generate the plankton computer animations. As the project's main objective is to deal with environmental issues, further collaborations with environmental scientists, and therefore simulations of the influence of plastic particles and noise on the plankton, are desirable.

Arguments to consider when using tomographic scanned entities in computer-animated scientific visualizations which emerged while working on the case examples can be seen in the following list:

- Scanned data offers accurate representations, although scanning artifacts must be corrected.
- A scan is a depiction of one individual and might not represent a generalized model of the species.
- Sample preparation influences the results.
- Image and geometry distortions may occur in scanning while misinterpretations or modeling mistakes may happen while modeling 3D geometry according to references.
- Additional reference images are mostly still necessary even with tomographic scanned entities, especially for motion and texture data.
- Experiments and measurements with volume raw data are possible in tomographic scanned models.
- For computer animating scanned 3D models, retopologizing and mesh repair are almost always necessary.
- Segmentation is laborious if specific parts of the scanned subject should be extracted.
- The innards are available as tomographic scanned data.

The computer animations of the case studies were not only produced for presentation as a work of art, nevertheless, they have been able to achieve the greatest publicity to date using this approach. Feedback from visitors and participants of the exhibitions was mostly that of pure amazement, especially when revealing the authentic background of the computer animations. Through authentic 3D models, added value is clearly given to most visitors of the exhibits. It is a more complex case for scientific presentations because in science, the resistance is greater to recognize computer animations as transformations of scientific data. Even if editing steps are clearly defined, computer-animated scientific visualizations are still sometimes viewed critically. However, the literature discussed here suggests that experiments in 3D virtual space are utmost beneficial for science communication, understanding, and scientific thought processes.

5.3 Discussion of the practice

Critics of the described practices presented in the case examples approached the topic from different angles. In general, computer animation as in-between art, science, and technology is sometimes criticized for being too artistically pretentious from the scientist's point of view, while being too commercialized from the art world's point of view. For instance, [215] mentioned two years after the paper, [53], that they see a particular problem in depicting animated mites in a realistic way, despite the fact that experts still scarcely understood the movement dynamics of arthropods. Of course if we would acknowledge this argument, nobody would be allowed to use scientific resources to visualize any locomotion because who should decide when subject matter is researched thoroughly enough to visualize the first hypothesis? Or, in examples of biochemical viz, no one would be allowed to simulate or visualize proteins until all movements are without any uncertainty researched. Experimental research regarding movement patterns could not be performed with animations in publications, as for instance [70] did with ants. Furthermore, such notions hinder attempts to undertake interdisciplinary projects for the greater understanding and interest in fields which may not have received much attention otherwise. Contrasting this notion, other biologists, for example [7], see computer animation as a legitimate tool to empower and present their research in the same way as a scientific illustration does. Computer animation is, on the one hand, criticized as superficial if stylized, while on the other hand, has been used continuously to stand in for the actual depiction of reality, for instance the computer animations produced for television to show the Voyager 2 mission[2]. In any case, computer-generated imagery should be declared as such in all scientific papers to avoid misunderstandings.

[2] https://www.youtube.com/watch?v=SQk7AFe13CY; 09/11/2018.

There is a tendency to blur the line between entertainment and scientific cinema [108].

This blur is generally viewed in a critical light. In the discussion of influences of computer animation on science communication, the distinction between entertainment and scientific video might be unnecessary, if people, in general, are able to scrutinize the representation of reality in film and media productions of any topic they consume. First, any video may be seen as entertainment, after that, additional information might be presented. The respective situation and context in which a visualization emerged should be clearly evident. It is necessary to examine whether such visualizations offer a solution, not only to communicate with people in the same scientific field, but also specifically to enable a better understanding between sciences and the general public.

The demonstration of, for instance, magnification or acceleration in films augments human vision and perception, and are particularly useful to convey scientific findings, while at the same time providing fascination and inspiration for artistic film making due to the use of visual effects. This is the reason why Walter Benjamin found it hard to pinpoint what is more fascinating for viewers—the artistic or the scientific value of science films [108].

Cinematography was a looking glass to study movements, as digital visualization devices have a similar function to provide insights into new types of motion. The judgment over objectivity in science is mainly influenced by the customs of a field [55].

That is why well-documented and technically verified data transformations in the ethical frameworks of a field do not damage the visualization value. Scientific information which is represented in data visualization is subject to many factors which lead to information transformation. That does not mean that digital visualizations are fundamentally implausible, however, it means that any uncertainty should be made apparent.

Critics of the introduced practice were also brought forward during the Fluid Visualisation and Sound Matters: Bridging Art, Science, and Visualisation Symposium on the 6th of July, 2017 in the Angewandte Innovation Laboratory Vienna (AIL). Questions were asked by attending natural scientists. The main questions were: Why should science resources be used for art? Is it not enough to merely model plankton creatures according to references to convey the messages? Are these visualizations not more of a hindrance than assisting in understanding complex coherence? This thesis exclusively describes 3D models and computer animations which utilized primarily science resources. Of course nothing was “stolen” by the project teams, as critics tend to taunt. Instead, it would be considered giving rather than taking. Also, the imaging data was contracted, open source, or acquired as a byproduct of research. The outcomes of the projects provided additional value, at least from a visual and transdisciplinary viewpoint. Imaging and

3D data were combined into videos in a manner which scientists, without special training or sufficient extra time to spend in solving design questions, are not able to produce.

It is difficult to acquire an overview of all of the factors that influence visual results. That does not immediately imply that the underlying data may not be authentic. Nevertheless, some scientists sometimes feel attacked when this problem is mentioned. Artists are often allowed more leeway to exploit insecurities and work with them—this does not mean that there should not be defined anything. Work ethics should be straightforward as well as transparent and information about them should be included in presentations.

Artistic and creative involvement might assist in opening up completely new modes of thinking, perception, and perspectives. Scientific and artistic communities still hold gifted polymaths, however, the real geniuses are rarely a single person, even when society with cult-like worshipped celebrities might prefer a different conclusion. The real contemporary geniuses are interdisciplinary and transdisciplinary research groups and think tanks who might enable revolutionary synergies. This thesis would not have been possible without the believe in mutual symbiotic inspiration from art, science, and technology.

Most visualizations in science nowadays are generated through the use of computer graphics. This connection puts computer-generated images into a special position concerning the representation of scientific realities. This relationship of scientific visualizations and how we as humans perceive reality through them, should be discussed due to the importance for the main research interest of this thesis. When computer animations are used to interpret or present scientific research or findings, they start a relationship with the scientific reality of their respective field. This relationship can be interpreted as either highly beneficial or counterproductive in its influence on our perception of reality. In the following paragraphs, this relationship between scientific reality and computer animation will be discussed.

Most computer animations are created according to references taken from various sources. There are different types of scientific images, for instance online—often free to use. The 3D models of the here discussed case examples are unique because the scientific imaging data was commissioned exclusively for the projects or there was at least a focus on feedback to evaluate the necessary editing steps. The process of selecting which entities should be depicted, as well as the discussion surrounding the selection of primary (tomographic scans) and secondary imaging techniques opens up a different approach, in contrast to just downloading whatever is available online for usage as references. In underlying interdisciplinary projects as described here in the case examples, there should be a continual process of back-and-forth between the collaboration partners. Every team member has the opportunity to learn from the others. For example, scientists can learn to use computer animation tools to empower their three-dimensional thinking,

as Janet Iwasa promotes in [92], and digital engineers might learn about the respective field they engage in to visualize. Reciprocal learning and new perspectives of one's own work are major benefits of close cooperation. If there is an open, positive social climate in such a project team, new findings, innovative questions, and their answers may be discovered.

Conventional modeling may potentially be a less laborious process for making 3D models of organic entities. Nevertheless, if the whole animal, potentially also including innards, should be modeled, the scanning, optimization, and cleaning processes will still be comparably fast and, most notably, more accurate due to having scans of the actual structure.

The 3D scanned subject shapes are a direct record of the actual organism. Real three-dimensionality can be acquired using different scanning methods. A 3D model may look correct if it has been carefully created using references taken from different views, yet it never has a direct relationship through the immediate contact via the scan of the original entity. If a biologist, for example, wants to compare body parts based on 3D geometries and chooses only to use images of the side, front, top, and bottom, as is common in 3D modeling, this may eventually lead to the creation of a 'realistic' model. However, details may still be lost or the model may become 'boxy' (not fully round natural looking shape), especially if the modeler is inexperienced.

However, even in scientific visualization, 3D scanning techniques are far from obligatory. The study of morphology can be supported not only by scanned 3D models but also by exact recreation through the use of scientific imaging with a modeling software. For instance, [7] performed detailed studies for the morphology and biomechanics of Sepsid flies. Functional reflections and visual thinking were principally supported through the modeling processes therein described.

For research, laboratories or scientific work protocols should be opened up to artists. There should be spaces where young researches can meet as Victoria Vesna suggests in [202, p. 99]. This leads to collaborations, as for instance Sonja Bäumel[3] is pursuing. The first institution to enable artists and researchers to engage in biology practices in a natural sciences department was *SymbioticA*[4]. Research laboratories hosting residents and events are a platform to enable fruitful regular meetups amongst artists and scientists. Computer graphics help combine fields that are often perceived as separated—most researchers use computers and many visualizations. For scientific visualization, the most important quality aspect is the input data. These data preferably are provided via close collaborations with experts in their respective fields.

[3] http://www.sonjabaeumel.at; 05/03/2019.
[4] http://www.symbiotica.uwa.edu.au; 05/03/2019.

In the history of science, there are various examples of scientists referred to as polymaths, who were successful because they were trained in various fields of human knowledge and creation. Since the rise of imaging technology, it is no longer necessary for biologists to illustrate, nevertheless, they should draw and animate their subjects to understand and communicate [93].

When it comes to investigations into the nature of reality, art and science share the same interests [174, p. 16].

A high potential for information retrieval lies in the use of motion data as an additional source of knowledge or inspiration. Completely new insights are possible from emerging techniques, in which, for example, one can sit in a virtual environment representing the inside of a cell to watch the chemical processes within, or inspect the organs of a microscopic creature animated in detail. The presence and immersion that may be achieved with, for instance, Virtual Reality, are factors to enable the understanding of scientific outcomes in a holistic manner. Emerging and existing Mixed Reality techniques may be applied to enable new thinking processes. In the near future, it will be possible to capture animals and their movements fully automatically without any knowledge of image processing or special capturing equipment. However, design and artistic research approaches will not likely be automated soon.

The creative staging of the tomographic scanned entities can be seen negatively, since it has the potential to blur the distinction between fact and fiction. Editing and compositing should be declared for the audience, however, there is a certain naivety to thinking that there is something such as unedited reality in any type of visualization. Especially in cases with a lack of distinction between “true” and “false”, in times where humans realize that they actually do not know much, considering for example the universe or quantum physics. It is a strange notion to believe in digital depictions without feeling any level of uncertainty about their authenticity. There are fewer reservations regarding the use of computer animation to depict the protein building blocks of life than in the field of microbiology. A reason for that might be the vagueness with which reality is apperceptive in the nanoscale and there might be higher budgets from both private and public funding in nanobiology to afford individually evolved visualizations.

Researchers should be involved in animation processes since they may develop new ideas while working on or supervising scientific visualizations. Many scientific animations are more of a visualization of a hypothesis, thus everything may not be supported by experimental evidence, yet that is the case for any type of hypothesis. Researchers should be enabled to use animation as a tool for experimentation on 3D models [92].

Another important issue regarding the discussion of the relation of computer-animated scientific visualizations and the representation of scientific outcomes, is the question of how scientific visualizations are perceived.

Visualization researchers produce appealing pictures, however, effectiveness in conveying the embedded information has been a minor research field

so far [88].

Nevertheless, it is a reciprocal situation with creating understandable pictures because the audience may also adapt to various types of visual representations. Science visualization can help demystify science for laypersons. Especially in current times of increasingly abstract expert knowledge, it is important to keep colleagues and society holistically informed. Even simplistic or stylized scientific visualizations can help to accomplish this goal, in particular, carefully researched scientific topics using animations which can provide people with key pieces of information concerning complicated topics.

An increasing number of scientific imaging and video data is shared alongside their respective papers on open data platforms for reuse, for instance, at Dryad Digital Repository[5], or the data sets of the wwPDB, which was subsequently used for the CRISPR animation. Much of this data are publicly available and could be the basis for computer-animated scientific visualizations, or inspiration for computer animation practitioners and students to engage in the field of computer-animated scientific visualization.

The field of scientific visualization is growing, particularly since scientific issues and outcomes are becoming continually more complex and are often in scales humans are unable to perceive without technological aid. Thankfully, technology to visualize scientific findings digitally is improving constantly.

This helps with the understanding of critical and complex issues such as environmental challenges [178].

In foundational research, it should be possible to gather knowledge without methodological limitations, commercial research objectives, or other restrictions. In this thesis, scientific visualizations are presented that are in many ways a type of visual foundational research. In the same vein, as morphology and locomotion were originally documented and analyzed through hand-drawn pictures, and later through the use of video recordings, computer animation has become a contemporary medium of analysis and recording for science. The digitization of once living organisms taken directly from nature along with their unique features is a way of directly transferring examples from their natural environment into virtual space.

Computer-animated scientific visualizations of microscopic entities lead to better knowledge and understanding for wider audiences than only the scientific community. Transdisciplinarity should be key for addressing global challenges of humanity. Therefore, further upcoming projects in the manner of the discussed case examples are of importance. The depiction of entities that are invisible to the naked eye enable fascinating new experiences for audiences of projects presented similarly to the case examples and offer the possibility of exploring other emerging presentation media using digital content.

[5] http://datadryad.org; 27/12/2017.

The question of the reasonable applicability of tomographic data in computer-animated scientific visualizations was posed here. In general, it is difficult to define or measure how scientific imaging data increases the authenticity of the outcomes. On the one hand, multiple recordings for the same model increase the authentic connection to reality. On the other hand, too many recordings may lead to confusion in the processing pipeline. This is particularly the case if the scientific imaging data are acquired from different living subjects of the same kind of microorganisms and are subsequently combined into one single model of a particular species. However, in most cases, a reasonable amount of collected scientific imaging data combined into one model results in a fascinating and authentic 3D model.

Ever since humankind has acquired the technical ability to enlarge and view microscopic worlds, people have been fascinated by them. This fascination derives from important findings on the existence of bacteria, cells, parasites, viruses, phages, algae, fungi, and so on, which directly affect every person. The look into smaller dimensions brought about a plethora of knowledge about ourselves and our place in this world and will most likely reveal further surprises in the future. Computer-animated scientific visualizations will continue to support and convey these new findings.

The arts and sciences should work together in a holistic and symbiotic manner. Expertise is indispensable, however, as strenuous as it may be, the larger picture of investigating our human reality holistically should not be forgotten. The presented case examples and their documented production are intended to be an inspiration and encouragement to experiment more within different areas of scientific visualization, as well as to test and create new workflows and to help overcome any remaining reservations between art, science, and computer animation.

Chapter 6

Conclusion and outlook

Computer graphics are currently used predominantly to visualize scientific results. In animated visualizations simple motions, for example, cross-section animations or rotations of scientific 3D model data sets are widely applied. Animations are in the majority of cases created automatically utilizing software tools to quickly prepare the latest outcomes for the presentation of 3D data.

This thesis discusses scientific visualizations which are animated computer graphics and a subgenre of data visualization. The term "computer-animated scientific visualizations" is incorporated into the title in order to emphasize the exceptional characteristics of the subject matter. More specifically, for these computer-animated scientific visualizations, tomographic scanned entities were edited in individually adopted pipelines to transfer scientific data into computer-generated results. The main fields of research were digital 3D models of organic entities of the micro and nano world and their movements. Therefore, the primary data sets were scientific imaging data from the field of biology and were available as scientific visualization source data material.

The investigated case studies are outstanding for both the fields of scientific visualization and computer animation. For the field of computer animation, the described work steps are unusual due to the application of tomographic scanned data, whereas in scientific visualization, it is exceptional to design every project individually, and to animate foremost with rigged 3D model computer animation configurations. In computer animation, rigged characters often supplemented by, for example, hair simulations are common, especially when representing the movements of living beings.

6.1 Question and methods

The main question here was, how reasonable are labor and financially costly workflows using tomographic scanned data in accomplishing specific com-

puter-animated scientific visualization projects. A hypothesis was that it is legitimate to employ the more elaborate scientific imaging techniques of various tomographic scanning techniques for computer-animated scientific visualizations, since it adds to the authenticity of the digital depictions. When multiple scientific imaging methods are included to create one realistically rendered 3D model, the overall production becomes more complicated, yet the final computer animation becomes more valuable for various audiences.

This thesis is scrutinized and the main question is addressed in covering the aspects of transferring digital scientific data into computer animations in the context of three projects using authentic 3D models of plankton, mites, and macro molecules. Generalized pipeline steps offered an overview of methods to transfer tomographic scanned entities into computer-animated scientific visualizations for various applications. Therein, the underlying imaging and preparation techniques were made comprehensible.

The method of recording and analyzing the making of the case studies led to insights into specific work processes. These interdisciplinary projects provided the groundwork to address theory and production practice in the creation of authentic computer-animated organic entities. Particular emphasis was placed on highlighting special cases in the workflow from the subfields of modeling, sculpting, shading, texturing, animation, rigging, and rendering in order to allow insights into the specialties in the production pipeline.

Every introduced project includes several aspects that have never been conducted before, as in the manner described in this thesis. The three case examples were the basis to gain detailed insight into computer animation productions which show transformed scientific imaging data in individually designed ways.

Primarily, unique cases of using scientific computer animations to visualize microscopic entities based on tomographic 3D scanned data were approached. In that respect, the transfer of the scanned data, in combination with secondary data which originated from non-tomographic scientific imaging processes and corresponding movement data, was analyzed. In every generation process of the described 3D models, new challenges emerged, as every entity had disparate requirements. The case example projects offer a variety of individual solutions for partial aspects of the production of computer-animated scientific visualizations. The practical work and the protocols of these case studies offered innovative strategies to solve challenges in the production.

6.2 Relevance

There are noticeably few computer-animated scientific results using rigs in microbiology. Interestingly, the most comparable productions to the projects presented in the case studies are to be found in biochemistry, in particular

nanoscale biology, where the visualization of data might even allow observation of single atoms. Reservations regarding computer animation to depict the protein building blocks of life seem to be fewer than in microbiology. Nanoscales are harder to visually comprehend than microscales, therefore, abstractions, reductions, and locomotion hypothesis might be more scientifically acceptable. One case study analyzed here explores the nanoworld while the other two examples are scientific visualizations of the microworld. Examples of computer-animated scientific visualizations are mostly produced for documentary films, whereas only a handful for research in biology. Likewise, the here introduced projects dealing with microbiology were only planned as scientific research in their early stages. During implementation, the projects developed progressively in predominantly artistic directions. However, this statement is not conclusive since a more scientific orientation is already planned for the work with plankton organisms and the visualization of their detailed inner processes and their ingesting of micro plastic particles.

The in-depth investigation of computer-animated scientific visualization generation processes allows for easier decision making by teams who are considering integrating raw tomographic data into computer animations. Making such a decision before starting a new project is beneficial, as financial and work investments must be considered. Pre-project decisions in terms of interdisciplinarity and data usage might be supported by this thesis. The efforts to produce the here described computer-animated scientific visualizations can be in certain cases tremendous thus why this thesis interrogates their reasonableness. Various challenges were overcome and are described in the protocols. The practical section is especially of interest for teams who prefer to work with open-source software in their scientific visualization workflows.

6.3 Outcomes

Most scientific animations present their topics using a variety of comprehensive methods, however, still fail to combine scientific data and the possible mind-expanding visual presence of an individually produced computer-animated scientific visualization.

At first glance, the techniques and workflows introduced here might be considered overly expensive and complicated compared to other approaches used to convey scientific topics to various audiences. In scientific visualization, computer animations using individual rigs together with narrative animations are only found in rare instances. The difference between “computer animation with scientific topics” and “computer-animated scientific visualizations” is that the latter transforms and utilizes scientific imaging data to generate the visualizations. Computer animations with scientific topics may also make use of various authentic references, yet they do not directly use

scientific data in their 3D models.

In further consideration, it became clear that the direct connection to the depicted entities through tomographic volumetric scan data added a multitude of additional values. However, the question about the reasonability of using tomographic scanned data for computer-animated scientific visualizations can not be answered in a general manner, as it depends on different factors of the planned project. Nevertheless, the primary factors required to answer the question individually for future projects became clear during the investigation in this thesis. The primary decision factors are: the expectations of the project team and the audience concerning the authenticity of the presented scientific realities, the target platform for which the scientific visualization will be produced for, and the resources available for the project. For the selected case examples, the questions which were the focus of the interrogations here could be answered. These answers will be summarized briefly in the next three paragraphs.

The comparison of two different mite gaits was created as interdisciplinary research project. Due to the lack of time to further investigate mite locomotion in detail the results were experimental depictions of time in space, along with the comparison in a predefined animation through a course using rotoscoped individual specimens. These were shown in a scientific visualization exhibition, for which the increased efforts were reasonable. The original plan was to perform further investigations, therefore, the animation video in the exhibition was presented as a work-in-progress with computer animations of the mites. Furthermore, both scientific imaging data sets of the mites were not created exclusively for the computer animations, rather, they already existed and were repurposed, only the motion data was newly captured. This drastically reduced the costs of the project. The 3D models are still available for further projects and collaborations.

The CRISPR animation depended on wwPDB data, which are freely available online. The project was originally commissioned for the 150th year jubilee exhibition of the University of Applied Arts Vienna in order to depict the global challenge to deal with "gene-editing". The usage of scientific data sets was essential because an computer-animated scientific visualization was explicitly commissioned. The authentic scientific data, as well as the collaboration with experts were the primary reasons that the animation could achieve international attention.

As not originally anticipated, the *Noise Aquarium* project turned out to be primarily an art project. However, the authentic tomographic data of the plankton organisms were the leading reasons why the project received a lot of international attention. It is an extraordinary case example that demonstrates the additional value achieved through using authentic data for art-science projects.

During the detailed analyses of the recorded case examples, it became clear that it is not the overall quantity of data that is important for an au-

thentic depiction of scientific outcomes and research in computer animation, rather the quality of the data and the appropriate kind of information for each specific use case. For example, on the Internet, one might find additional information, yet these data may not be suitable for the target scientific visualization. Furthermore, one key aspect in accomplishing a successful computer-animated scientific visualization project is not solely dependent on the project budget but rather on the social skills and the interdisciplinary capabilities of the project members involved. Therefore, a general recommendation for computer animators with ambitions to engage in the area of computer-animated scientific visualizations would be to search for collaboration partners who may already have imaging data and are willing to share, or even better, explore this data together, since the joint exploration opens up feedback and insight possibilities. The 3D models and animations were created with constant feedback processes with experts to optimize the biological correctness of the outcomes.

The representation of authentic data is the goal of all scientific visualizations. The communication of the core issue with transparency, clarity, and accuracy is crucial. This critical quality of perceptual content can also be achieved through modeling according to references, however, the resulting 3D model will then be foremost an artificial work. In contrast, in the case of the scan of, for instance, an organism, the directly applied and transferred data into a computer animation has a different quality, thereby providing an immediate connection to nature and therefore reality. There is not only the mere form and data that is recorded and conveyed—in the projects samples taken from nature are processed which cause the willing and open-minded viewer to see more than a digitized recording of an organic entity.

An increasing number of teams are publishing imaging data in online databases alongside their research papers. Therefore, working with such data is not always tied to budgets for acquiring new imaging data. An interdisciplinary collaboration project on a specific topic is in most cases feasible with already existing data. Accordingly, this text should be of meaning to all animators drawn to science and scientists interested in animation, as the herein described field of computer-animated scientific visualizations opens up intriguing areas of activity, not least, ideas and solutions are enabled to contribute to the common good.

Any computer-animated scientific visualization project should be planned and tested before production because software and hardware are constantly evolving and the processing of sometimes huge scientific data sets is a challenge even for experienced digital engineers. Data sets of immense sizes which once required mainframe computers can now be mastered on a mid or high level CG-artists workstation. Nevertheless, expectations of computer-generated imagery continually rise as available processing power and computers are always pushed to their limits.

This thesis can be particularly beneficial for persons working with pro-

jects which attempt to transfer scientific tomographic imaging data into interdisciplinary as well as transdisciplinary comprehensible computer animations. Computer-generated images are technology as well as art, thus implying that they are suitable to address various scientific topics using a holistic approach. Computer animations incorporating tomographic scanned data should not be solely considered as animated scientific visualization, since their creation process is able to augment the scientific and artistic research practice.

6.4 Outlook

Ideally, the practical and theoretical consequence of this work will be an increased interest in working with microscopic organic data sets in computer animation. Furthermore, an increased interest for scientists to see their data presented in expanded ways should be emphasized. Through computer animations, micro and nano worlds may be experienced in an understandable, and if requested, entertaining way. These visual representations of reality may increase the general interest in under-explored topics. There are a multitude of factors to consider when implementing an animation workflow enriched with scientific image data. Concerning the micro-scaled organisms of plankton and mites, a database project might be initiated which offers a collection of high quality 3D models that are direct digital portrayals of once-living organisms. This immediate representation of organisms leads to accurate, thus sample-like data of the subjects which then may be used in an artistic, technological, or scientific context. As automation progresses, some of the manual steps described here will soon be redundant, yet it will take much longer for newly developed looks, narrative elements, and creative areas of application to be completely automated.

Ongoing negotiations of the Science Visualization Lab Angewandte with international art and science experts anticipate further investigations. Future projects will hopefully exploit the full scope of intellectual and visual possibilities while transferring them into comprehensible computer-animated scientific visualizations. The media will not be restricted to digital videos, rather, all emerging options for the presentation of digital content should be considered. In general, the presented case studies showed that the outcomes here were not only about the individual pipeline results and their different representations. The projects are more than visualization productions, as they additionally reflect the elaborate thinking processes of all project partners involved. The discussion of possible impacts of the outcomes of computer-animated scientific visualizations and their relation to the depiction and presentation of scientific generated realities found that we can not stress enough the notion that visualizations have a huge impact on and are important for most communication processes, not least, in the discipline of

biology. Therefore, the imaging and representation of microscopic entities will provide better knowledge and understanding in even broader audiences than the scientific community.

Scientific visualization has led and will lead to a new perception of the world. There are numerous phenomena we are not able to see, thus they are existing. Especially in terms of scaling, both vast and microscopic entities are far from being fully understood. Scientific visualization is an ever-growing field of data visualization which might increase the influence on diverse audiences if expanded by individually designed computer animations. There are critics from both the art and science communities. Individually designed computer-animated scientific visualizations are often seen as a by-product or merely decoration in the sciences—they are rarely treated as a source of knowledge. The majority of people are visual thinkers, therefore, it is beneficial to combine the fields of art and science. Computer-animated scientific visualization is a path to pursue this goal from different angles. Interdisciplinarity and transdisciplinarity are key to expanding the human knowledge basis and computer-animated scientific visualizations were found to be important tools for this path.

Appendix A

Sample preparation

The following paragraphs give information about the acquisition, preparation and fixation of the specimen. Stephan Handschuh and Thomas Schwaha provided the information of eight specimen: *Amoeba proteus*, *Cylindrospermum* sp., *Paramecium multimicronucelatum*, *Actinotrocha* larvae, *Oikopleura* sp., *Noctiluca scintillans*, *Tomopteris helgolandica*, and the *Parasitiformes* mite. The very high resolution image stacks of *Archegozetes longisetosus* were provided by Michael Heethoff and his team.

The living specimens of *Amoeba proteus*, *Cylindrospermum* sp., and *Paramecium multimicronucelatum* were acquired through Carolina Biological Supply (Carolina Biological Supply Company, Burlington, U.S.A.). In order to analyse locomotion in living specimens, they were observed under an Axio Imager Z2 (Carl Zeiss, Jena, Germany) microscope equipped with a digital full HD camera. Specimens were fixed and embedded using routine tissue processing protocols as described by protocol modified from [167]. Serial micrographs were taken from semi thin sections using an Axio Imager Z2 (Carl Zeiss, Jena, Germany) microscope. Digital image stacks based on serial 0.5 µm sections were aligned using the software Amira 6 (FEI Visualization Sciences Group, Mérignac Cédex, France). In the case of Cylindrospermum sp., additional transmission electron microscopic images were captured from ultrathin sections using the FEI Morgagni 268D (FEI Company, Hillsboro, OR). Based on image segmentation, polygon models (Wavefront Objects) were exported for further processing.

Actinotrocha larvae of *Phoronis muelleri* were obtained from the Swedish Coast (near Lysekil) and fixed with 2% glutaraldehyde in sodium cacodylate buffer at a pH value of 7.4. Subsequently, specimens were post-fixed using 1% OsO4. For micro CT scanning, a specimen was stained with I_2KI (0.1% (w/v) elemental iodine (I_2) and 0.2% (w/v) potassium iodide (KI) in distilled water) and embedded in 1.5% low melt Agarose in a plastic pipette tip. For light microscopic investigation, a specimen was embedded in Agar low viscosity resin (Agar Scientific, Stansted, UK) and serially sectioned using

a Jumbo diamond knife (Diatome AG, Biel, Switzerland) on a Reichert UltraCut-S microtome with a section thickness of 0.5 µm.

Specimens of *Oikopleura* were obtained from the Swedish Coast (near Lysekil) and fixed with 2% glutaraldehyde in sodium cacodylate buffer at a pH value of 7.4. Subsequently, specimens were post-fixed using 1% OsO4. For micro CT scanning, a specimen was stained with I_2KI (0.1% (w/v) elemental iodine (I_2) and 0.2% (w/v) potassium iodide (KI) in distilled water) and embedded in 1.5% low melt Agarose in a plastic pipette tip. For light microscopic investigation, a specimen was embedded in Agar low viscosity resin (Agar Scientific, Stansted, UK) and serially sectioned using a Jumbo diamond knife (Diatome AG, Biel, Switzerland) on a Reichert UltraCut-S microtome with a section thickness of 0.5 µm.

Specimens of *Noctiluca scintillans* were obtained from Biologische Anstalt Helgoland (BAH) and were fixed with 4% buffered formalin. For micro CT scanning, a specimen was stained with I_2KI (0.1% (w/v) elemental iodine (I_2) and 0.2% (w/v) potassium iodide (KI) in distilled water) and mounted in distilled water in a plastic pipette tip. For light microscopic investigation, a specimen was post-fixed using 1% OsO4 and embedded in Agar low viscosity resin (Agar Scientific, Stansted, UK) and serially sectioned using a Jumbo diamond knife (Diatome AG, Biel, Switzerland) on a Reichert UltraCut-S microtome with a section thickness of 0.5 µm.

Specimens of *Tomopteris helgolandica* were obtained from Biologische Anstalt Helgoland (BAH) and were fixed with 4% buffered formalin. For micro CT scanning, a specimen was stained with I_2 KI (0.1% (w/v) elemental iodine and 0.2% (w/v) potassium iodide (KI) in distilled water) and mounted in distilled water in a plastic pipette tip.

Parasitiformes mites were obtained and fixated as described in [53, p. 96]:

> Specimen of *Parasitiformes* mites were extracted using the MacFadyen technique [101] from litter samples from the Viennese woods and collected alive in plastic containers with wetted mixture of plaster of Paris and charcoal (10:1). These hard sclerotized animals were fixated in Carnoyfluid (60% ethanol, 30% chloroform, and 10% glacial acetic acid) for two hours at room temperature. Before imaging with micro CT, they were washed after fixation in 96% ethanol and then deposited in 1% iodine in absolute ethanol solution for at least 24 hours. For *Parasitiformes* also SEM imaging was done. For that, samples were chemically dehydrated with 2,2-dimethoxypropane [96], washed in acetone and air-dried after treatment with hexamethyldisilazane [132].

Archegozetes mites were obtained and fixated as described in [82, p. 1]:

> Specimens of the parthenogenetic oribatid mite *Archegozetes longisetosus* (Acari, Oribatida) were taken from our laboratory culture. The culture grows on a plaster of Paris/charcoal mix (9:1)

in plastic jars, in constant dark at 20–23°C with approximately 90% of air humidity. (...) 1. Specimens were taken from the culture, cleaned with a fine brush and placed in a 6:3:1 mixture of 80% ethanol, 35% formaldehyde and 100% acetic acid for 24 hours.

2. Afterwards, specimens were dehydrated in a graded ethanol series of 70%, 75%, 80%, 85%, 90%, 95%, and 100% with 3 changes at each concentration, and 10 min between the steps.

3. Finally, samples were placed in fresh 100% ethanol overnight and critical point dried in CO2 (CPD 020, Balzers). Dried specimens were attached to the tip of plastic pins (1.2 cm long; 3 mm in diameter).

List of Figures

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