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Computer-generated images of microscopic soil organisms for documentary films

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Abstract

The depiction of microscopic soil animals in film is complicated. In principle it is possible to capture real film footage of such organisms using microscopes and special camera equipment, but this entails severe limitations in terms of resolution and depth of field. This makes nano-scale close-up details with color and motion impossible. In addition, microscopic soil animals hardly show their natural behavior under the conditions required for filming. Here we describe an alternative approach – based on computer-generated imagery – to portraying the microscopic world in documentary film. In the workflow presented here, we first created high-detail models of various microscopic soil animals by using complementary imaging methods at multiple levels of resolution. These models were then animated based on live observations of motion and behavior. This approach enables photo-realistic digital motion pictures of various soil animals. A broad range of potential applications, the aesthetics, as well as creative advantages justify the efforts to generate such scientific visualizations.

Keywords Acari | Tardigrada | Computer Animation | Micro-Computed Tomography | Workflow

1. Introduction

Tiny soil animals are basically filmable with microscopic camera equipment and sample stages. In practice, however, optical and creative restrictions strongly hamper a cinematic aesthetics. The resolution of light microscopes is restricted by the wavelength of light (Murphy 2001). More details can be seen in the Scanning Electron Microscope (SEM), but these images lack any information on the color of the living animal. The behavior of animals is commonly unpredictable and the scenery for filming the microworld is only minimally amenable to arrangement. These conditions impact the aesthetic image composition and make behavioral studies of microscopic animals difficult. A further problem is the limited depth of field under the microscope. Moreover, neither particles nor illumination effects can be applied to the scene. Computer graphics provide film-aesthetic details that are not visible to the naked eye. This makes the visualization with computer-generated images especially appropriate for habitats that can only be seen through the microscope. Very new points of view are a further benefit. There is a general demand for 3D models and animations in documentaries that deal with tiny animals because animation and color shading information can be achieved at the micro- and nano-level. Computer animation is an important aid 'if the director does not have live action shots – or only insufficient material is available because

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of dramatic and technical aspects' (Hattendorf 1999).

We developed a workflow for creating animated film sequences of microscopic soil animals. It includes sample preparation and live observation of animals, 3D imaging, computer graphics and animation for use in documentary films. Computer-generated animals are an issue in the discussion about the nature of reality in documentaries (Nichols 2001). These discussions began with the invention of motion pictures in the 19th century (Schmidt 2008) and were already a topic for documentary pioneers (Vertov 1922). Documentaries as a medium for the representation of facts is always closely scrutinized (Hohenberger 2012). The special relationship between reality and animation sequences or fully animated documentaries (Khajavi 2011) must support the clarity and authenticity of what is being shown (Odin 2012). The use of computer animations to challenge established conventions of representation, to undermine viewing habits, and to cross generic and genre boundaries is always a challenge in scientific film theoretical discussions (Bartosch 2012). There is popular acceptance of recent hybrid forms that integrate animated imagery to documentaries (Skoller 2011). The mediation and reception of documentary films is being discussed internationally on many platforms (Rosenthal & Corner 2005). Multiple successful examples of computer animation in documentaries show that a critical mass has definitely been reached (Dobson 2012). Therefore, computer-generated images can be considered an option for documentary films about soil animals.

2. Workflow

2.1. Specimens and sample preparation

Specimens of soil animals from groups such as Rotifera, Tardigrada, Oribatida, Gamasida, Uropodina, Pseudoscorpiones were extracted because they are present in the resulting short film:

'The Incredible Tardigrade – Wild Little World' (http://phaidra.univie.ac.at/o:313584, Production: Industrial Motion Art Filmproduktion GmbH, Vienna, Austria and AV Dokumenta, Vienna, Austria).

Turntables of the animals are also available on permanent-links:

Lithobius http://phaidra.univie.ac.at/o:313577,
Pseudoscorpio http://phaidra.univie.ac.at/o:313579,
Phthiracaridae http://phaidra.univie.ac.at/o:313580,
Gamasida http://phaidra.univie.ac.at/o:313581,
Uropodina http://phaidra.univie.ac.at/o:313582,
Tardigrade http://phaidra.univie.ac.at/o:313583.

They were extracted using the MacFadyen technique (Koehler 1993) from litter samples from the Vienna woods and collected alive in plastic containers whose bottom was filled with a wetted plaster of Paris: charcoal mixture (10:1). Tardigrada and Rotifera were sampled by pressing moss cushions wetted with water, collected from a shingle-covered farmhouse in Lower Austria. First the selected species were filmed alive with a digital camera for color references and live observation, then they were prepared for micro-computed-tomography (microCT) or confocal laser scanning microscopy (CLSM) and scanning electron microscopy (SEM).

Our exemplary workflow (Fig. 1) concentrates on the making of a predatory mite; only the preparation and low-detail 3D model acquisition show some described special treatment. Animals with hard cuticula required a different treatment in sample preparation and scanning than soft sclerotized ones.

Hard sclerotized animals were fixated in CARNOY-fluid (60% ethanol, 30% chloroform and 10% glacial acetic acid) for 2 h at room temperature, and soft-bodied specimens microwaved in 4% formaldehyde for 8 s in a 600W microwave oven (Walzl 1991), cooled down to room temperature and stored in 70% ethanol. Specimens selected for microCT were washed after fixation in 96% ethanol and then deposited in 1% iodine in absolute ethanol solution for at least 24 h. Specimens selected for CLSM were transferred in glycerine. The SEM samples were chemically dehydrated with 2,2-dimethoxypropane (Johnson et al. 1976), washed in acetone and air-dried after treatment with hexamethyldisilazane (Nation 1983).

2.2. Scanning electron microscopy

For imaging in the SEM, a FEI Quanta 250 FEG, several individuals of each species were mounted on stubs with TEMPFIX thermoplastic glue in different positions to allow imaging from all sides (dorsal, ventral, lateral). To achieve best quality, all samples were sputter-coated with gold and the imaging was done in the conventional high-vacuum mode of operation of the SEM.

For each side of the individuals, several images (4096 × 3536 px, 16 bit gray-scale) at different positions along the body were taken at magnifications in the range of 500× to 1000×. These images, when stitched together, yield an image of the whole animal at high resolution. From various parts of the animals (head, legs etc.), additional images were taken at higher magnification up to 10000×.

At each position, three images were taken simultaneously: a secondary-electron image (SE) providing the best resolution and finest detail, and two back-scattered-electron images (BSE) having lower

intrinsic resolution but information about depth due to different shading. The BSE detector is subdivided in two sectors and, in normal operation, the signals of both sectors are combined either additively to enhance atomic number (compositional) contrast or subtractively to enhance topographical contrast. Taking the signal of each sector individually provides a set of two images comparable to optical images with direct lightning from different sides.

2.3. 3D image acquisition and image segmentation for low-detail animal models:

Depending on specimen size, we used both microCT and CLSM to acquire 3D image data from fixed animal samples. Current lab-based microCT systems can be used to image samples in a size range of 500 µm to 2 mm (such as many mites and other small arthropods) at optical resolutions slightly below one micron (for examples see e.g. Metscher 2009a, Metscher 2009b, Wilhelm et al. 2011). Confocal laser scanning microscopy can be used to image much smaller samples at even higher resolutions. The latter technique, however, is usually limited to specimens measuring ~150 µm (thicker specimens are problematic because laser penetration into the tissue is limited) (e.g. Wanninger 2007, Hama et al. 2011). In general, we recommend using tomographic techniques (such as microCT) to acquire geometrically correct models of microscopic samples rather than optical sectioning techniques. The latter might show significant geometric distortions and anisotropies (non-uniform properties of the image (XY) axes in respect to the optical (Z) axis) related to refractive index mismatches and the optical properties of the system. In the current study, all mites as well as the pseudoscorpion were scanned using an XRadia microXCT 200 (after being stained using 1% elemental iodine in absolute ethanol for 24 h). Confocal image stacks of tardigrades were taken with a Leica SP5 II confocal laser scanning microscope (Leica, Wetzlar, Germany) using the autofluorescence of tissues.

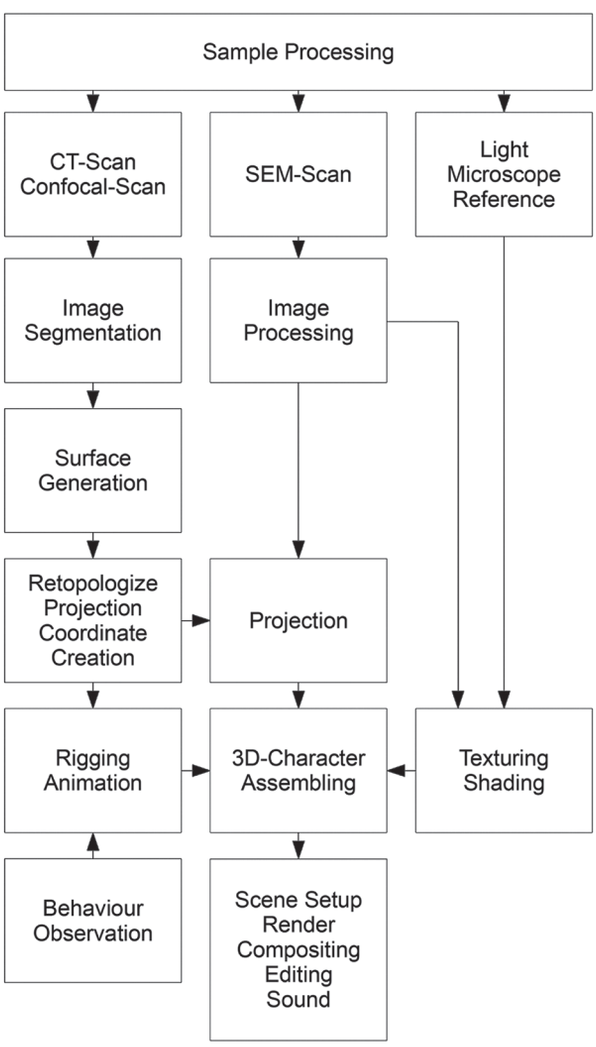

Figure 1. Three different scanning devices are used and combined to create high-detail 3D models. Each scanning result is processed to combine them into a single model. The 3D models with animations are staged in a digital scene and rendered.

Based on 3D image data acquired by microCT and CLSM, we used voxel- (volumetric pixel) based segmentation tools to create polygon models of the animal samples. All image segmentation procedures were done using AMIRA 5.3 (FEI Visualization Sciences Group, Burlington MA). First, we used automatic threshold segmentation to segment structures that show sufficient (hyper-intense) contrast in comparison to the background (Fig. 2A). For microCT images of stained arthropods this includes the cuticle, muscles, and many other soft tissues. Second, we used manual segmentation tools to segment delicate structures with less contrast such as intersegmental membranes in the legs and the abdomen, and to remove dirt particles. Finally, for each animal the voxel segmentation dataset (AMIRA label field) contained one material consisting of contiguous voxels and without any holes. From this segmentation file, we triangulated a polygonal surface mesh. Subsequently, this surface was smoothed and the number of vertices was reduced to approximately 30k-100k for each animal model. Then, the surface was re-meshed to obtain isotropic triangle surfaces (Fig. 2B).

2.4. Computer animation workflow

After the low-detail model was created, fine details (that were not included in the low-detail model due to

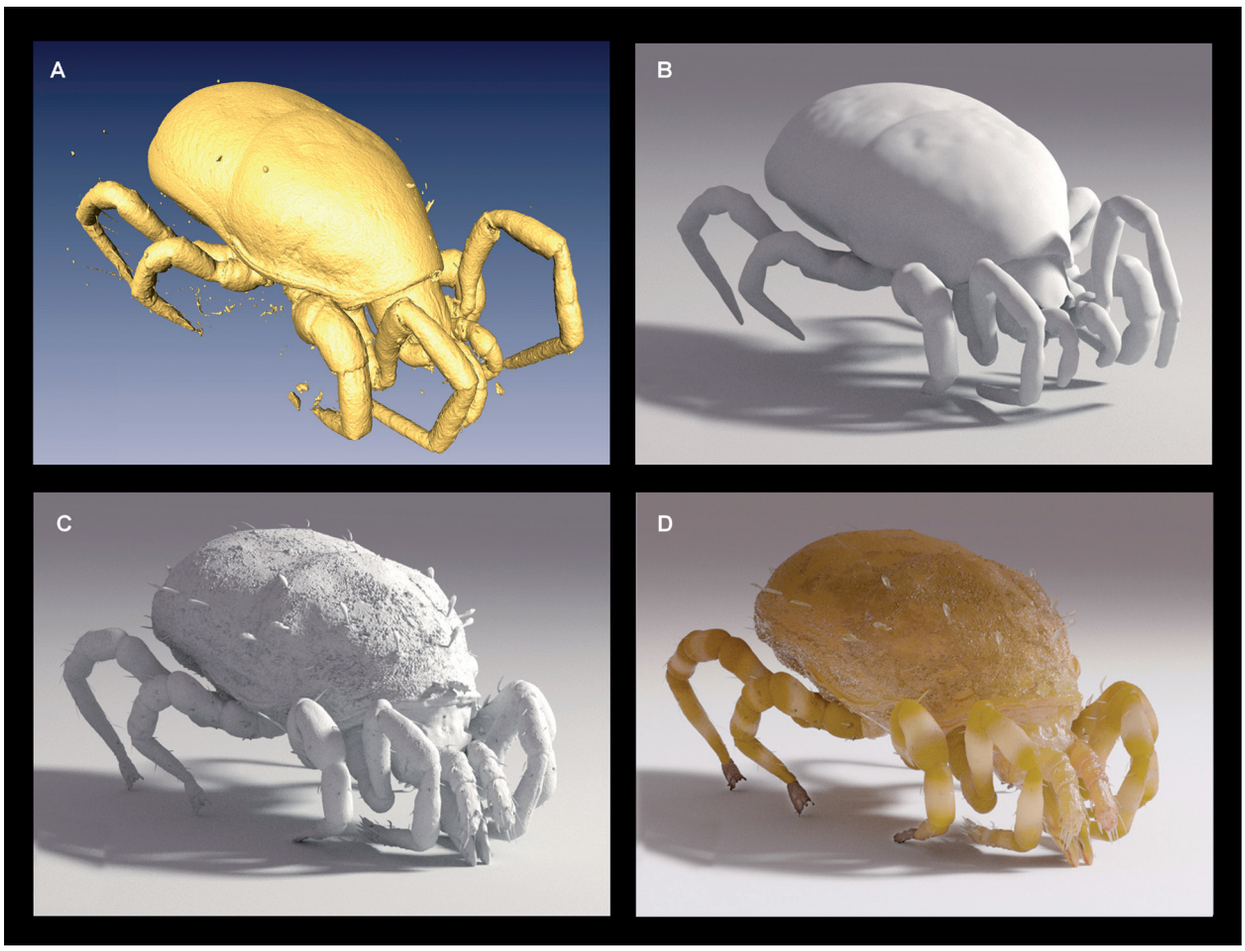

Figure 2. To create a model with fine details, the re-meshed microCT scan of the creature is imported (**A**), cleaned up and prepared for projection (**B**). (**C**) Shows the creature with the image-processed SEM, and (**D**) shows the final texture and shading set-up in a neutral light situation.

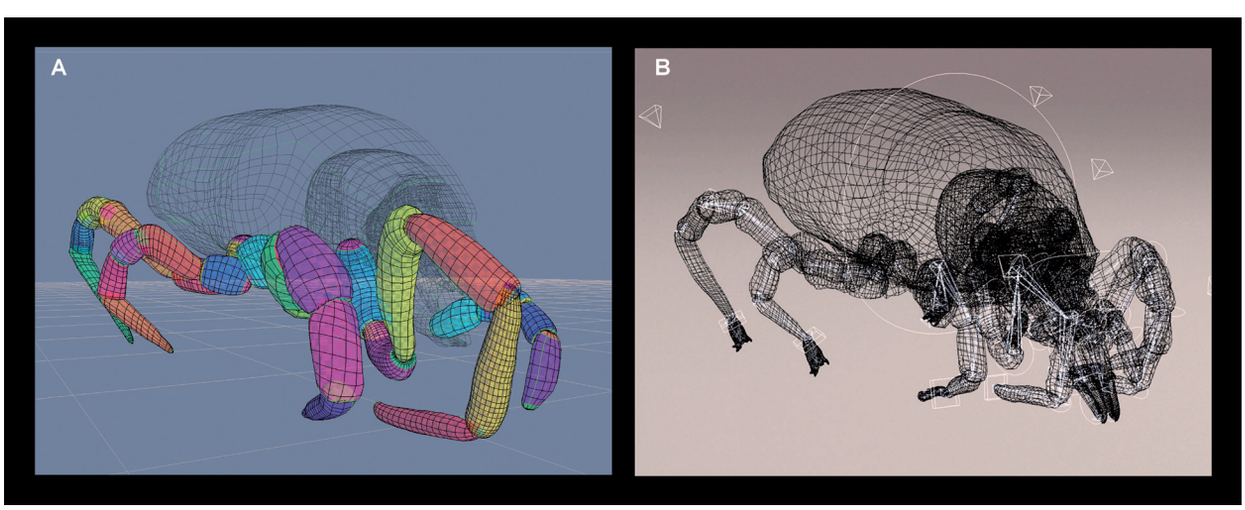

Figure 3. Wireframe illustration of the cleaned-up creature with optimized edge flow- retopologizing (**A**). The so-called Rig set-up is a virtual bone-set that helps the animator control the extremities in the animation process of the 3D character (**B**).

limited resolution of the applied imaging techniques) were repaired or added using digital sculpting. Digital sculpting has become one of the most important tools for 3D content creation in the film industry, special effects and computer games. It currently replaces traditional modeling methods that use individually controlled points to adjust surface patches or create a simple mesh that will be later globally subdivided (Stanculescu et al. 2011). To create high-level details, we used data from the three channels of the SEM images combined to maps (Meka & Merchant 2011) for fine structures of the 3D models. Because of the different angles of the SEM detectors, as described earlier, these two back-scattered channels could be used to calculate height and normal maps with 'Shape from Shading' algorithms (Zhang et al. 1999) (Fig. 2C). Colors were taken from light microscopic images and videos.

Parallel to adding fine structure details, the low-detail 3D mesh was prepared for computer animation by reduction of polygons and appropriate distribution of polygon edge loops. The process of adjusting the mesh edge flow is termed Retopologization and is a prerequisite for animation. In contrast to various automatic surface re-mesh algorithms, in Retopologization the polygons are rearranged manually or semi-automatically. The edges have to follow the surface appropriate to the deformations during the animation process (Fig. 3A). In this stage it is crucial to have knowledge on the objects shape and its movements and to transfer this information into the key shape of the 3D model, in order to render animation without artefacts (Osipa 2010). For the animation process a Rig or deformation skeleton is generated in the retopologized low-detail 3D model (Fig. 3B).

When the 3D model shape is finished, fine structure details, shader and texture are applied to the 3D models and their surroundings (Fig. 2D). Generally, shading and texturing are the processes of specifying surface behavior for the final rendering of the computer-generated images. For our workflow, different shading and texturing techniques were combined and individually optimized for each digital animal, as well as for backgrounds and scenery. Obtaining maximum detail requires handling a huge amount of projection data. For better editing and faster feedback, the textures were sliced into many tiles and saved in three different resolutions. This allows more rapid tuning of projections and shaders. The software packages for projection and texturing were Mari 2 (The Foundry, London, United Kingdom) and 3Dcoat 4 (Pilgway, Kiev, Ukraine). A script, which was developed and written in Visual Studio 2012 (Microsoft, Redmond, USA) and in MAXScript 2013 (Autodesk Inc., San Rafael, USA), creates, optimizes and loads the different tiles and resolutions of texture and projection maps automatically.

For the final computation of 3D images, animated 3D models are positioned in the digital scene. The digital scene needs camera and light setup, comparable to a real film set. Then, the whole programmed scene is rendered to single frames that can be combined to animation films. The main software packages for this workflow step were Autodesk 3Dsmax 2013 (Autodesk Inc., San Rafael, USA) and Blender 2.6. (Amsterdam, Nederlands) The main renderer was Vray 2.3 (Chaos Software Ltd., Sofia, Bulgaria).

2.5. Video post-production or compositing

To further improve the results, additional video post-production steps and algorithms were applied after rendering. Video post-production, or so-called composting, is the backbone of visual effects work and the final step in creating the desired imagery. Rendered image sequences were improved by 2D effects and other compositing tools to enhance the rendered 3D images (Brinkmann 2008). The final images (Fig. 4), after compositing, were rendered to single frame image sequences and edited with narrator, sound effects and music.

3. Discussion

We present a workflow for the specific use of computer animation in documentary film that aims to show highly realistic models in their microscopic environments that are scientifically correct and aesthetically pleasing. This project was realized in a fruitful cooperation of various institutions including scientists and computer animators. We believe that the soil habitat can serve as a subject for general education about the ecology of our world and is therefore an important topic for documentary film. In our opinion, an appealing representation of the associated flora and fauna, as in our short film example, should attract great interest. We argue that the presented process can be used for various projects that aim for photo-realistic computer animation. This interdisciplinary approach is a case study, and we believe that various disciplines will prosper from such co-operation. Highly realistic visualizations are of general relevance because images are important for the human thought process in every discipline (Wittgenstein 1953).

There is an ongoing discussion about the use of 3D computer animations in science visualization and documentaries. An important issue here is the accuracy of 3D models and computer animations. Based on

Figure 4. A predatory mite attacks a prey mite. Selected single frame from the short film 'The Incredible Water Bear'. It is a high-detail shot of the soil habitat. Close-ups are available of every animal and can visualize more details than a light microscope set-up could ever show. Cinematic effects and camera set-up enhance the mood of the scene.

complementary imaging methods at different levels of resolution (like microCT, stereomicroscopy, and SEM), the geometry, color, and texture of the models are highly realistic and should not face serious criticism. Nonetheless, there is a need for further investigations on the motion and behavior of animals in order to improve animation accuracy. Motion capturing of microscopic animals seems to be a promising scientific approach to digital animation (proposed by Gibson et al. 2005), and we may use motion capturing in further projects. Computer animations depicting realistic motion patterns of animals can then also be used to quantitatively analyse arthropod kinematics (e.g. Weihmann 2013). This adds high scientific value beyond visualization.

As Ward (2008) noted: 'The relationship between reality, documentary and animation could be described as 'creating the real', if we argue that live action documentary attempts to 'claim' the real by virtue of its immense mimetic potential. The clear differences between the 'real world' and its animated counterparts, therefore needs to be understood not necessarily as a drawback but also as a strength of animation: its ability to move beyond naturalistic, surface representation and ideally embraces real relations between things in all their magnitude.' Computer animation is a tool for visualizing non-filmable details and processes. So far, many nano- and micro-level details including motion and color can only be visualized this way. As a consequence of new techniques for hyper and photo-realistic rendering, the use of computer animation in documentary films is increasing. (http://www.documentary-campus.com/v2/page/symposia/13/, 12.10.2013) Computer animation aids the presentation and understanding of scientific findings to non-scientific audiences. New possibilities and challenges open up with the use of film-aesthetic representations of scientific results.

4. Conclusions

Computer animation is currently the best tool for visualizing non-film-able details and processes. Computer-generated image sequences help the presentation and understanding of scientific outcomes for all audiences. New possibilities and challenges arise with the use of film-aesthetic representations of scientific results. The use of computer animation in various types of documentary films requires a discussion about the appropriate degree of accuracy because budget restrictions usually apply for scientific references and animation. Documentary film is, of course, only one of numerous fields of application for cinematic computer-generated images showing scientific facts. We present a valuable workflow for the specific interdisciplinary challenge of depicting animals from the soil habitat. Importantly, the use of computer

animation in documentary films is increasing because of new techniques for hyper- and photo-realistic rendering. Currently, nano- and micro-level details including motion and color can only be visualized this way.

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