



# Live and Interactive 3D Photomanipulation under the Microscope using Virtual Reality

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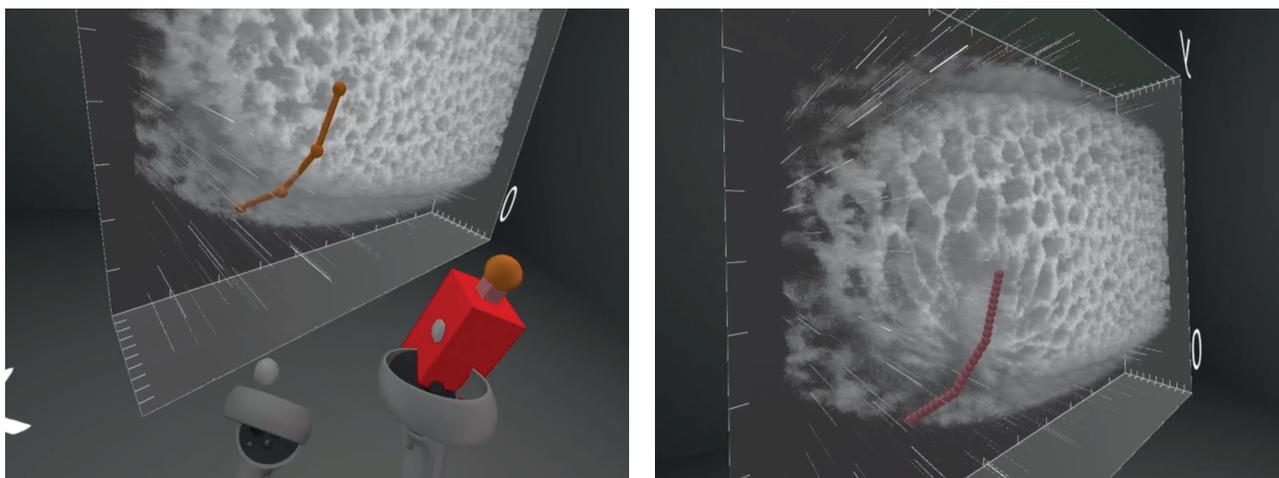
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**Figure 1: A successful 3D ablation of a *Drosophila melanogaster* (fruit fly) embryo using our system connected to a confocal spinning-disk fluorescence microscope. *Left*: Using VR controllers to define the ablation path in the 3D volume by placing points along the curved surface of the sample (shown in orange color). *Right*: The 3D volume data showing disruption of the cellular surface structure, with the interpolated ablation points shown in light red. Both images were taken from a session of our expert review study.**

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## ABSTRACT

State-of-the-art microscopes, as used in cell biology, are not only capable of capturing 3D images, but also permit manipulation of (sub-)cellular structures using techniques such as optical traps, optogenetics or laser ablation. However, such microscopes are still controlled using 2D interfaces, prohibiting actual 3-dimensional manipulation.

We present *microscenery*, a virtual reality (VR) microscope control software, designed to facilitate 3D laser ablation experiments. We combine microscopy automation with VR rendering and intuitive controller-based input to empower biologists with the precision of laser-based techniques while providing the full 3D spatial context of their sample.

We describe the design goals and architecture of the software and illustrate the potential of the system by conducting a brief expert review study for 3D ablation experiments. Our results suggest VR is not only an effective interface for microscopic manipulations, but can enable novel experiments which are either impossible with traditional 2D interfaces, or prohibitively time-consuming.

## CCS CONCEPTS

• **Human-centered computing** → **Human computer interaction (HCI)**; **Scientific visualization**; • **Computing methodologies** → **Virtual reality**; • **Hardware** → *Biology-related information processing*; • **Applied computing** → *Systems biology*.

## KEYWORDS

virtual reality, microscope control, visualization, human-computer interaction

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## 1 INTRODUCTION

Microscopy technology has advanced to a point where real-time 3D imaging is possible [19, 23]. Further, various photomanipulation techniques enable the experimenter to not only view a sample, but also to (optically) interact with it. This interaction happens at cellular and subcellular scale – a scale not accessible with simple mechanical tools.

Simultaneously, advanced and user-friendly virtual reality (VR) hardware is now commercially available. This includes the headset itself, and the rendering hardware – usually a dedicated computer – that is able to stereoscopically render 3D scenes with a high-enough sustained frame rate. Therefore, we hypothesize that the combination of these two inherently 3D technologies might enable users – in our case biologists – to be able to conduct new kinds of experiments, taking full advantage of the third spatial dimension.

To validate our hypothesis we have developed the VR microscopy software *microscenery*. As exemplar use case for photomanipulation, we choose laser ablation (performing microscopic cuts with a high-powered laser), as it produces fast and visible results, and is both a widely-used and widely-available tool in cell biology. Our design goals were:

- (1) **Plan and execute 3D ablation experiments in VR.** The tool should be easy to learn and use the capabilities of VR to support the user in performing novel 3D ablation experiments.
- (2) **Use existing microscopy hardware.** Current photomanipulation hardware and software is built with 2D experiments in mind. The goal is to create a setup that extends existing hardware to support 3D ablation experiments.
- (3) **Easy integration into existing systems.** Following the paradigm *Don't break what we have to give us the new* [29], adding VR capabilities to existing microscopy systems should happen in the least invasive way possible.

- (4) **Reproducibility of experiments.** In order to make experiments reproducible, all data – including but not limited to raw image data – must be stored and accessible for future reference and repeatability.

In this work, we detail how we have implemented those goals, and evaluate them using an expert review study. We will start by introducing the microscopy techniques relevant for this work, then detail background and related work, before describing our implementation, study, and results.

## 2 FLUORESCENCE MICROSCOPY AND PHOTOMANIPULATION BASICS

### 2.1 Fluorescence Microscopy

In fluorescence microscopy, the sample under investigation features either a fluorescent dye, or some kind of genetic manipulation to express fluorescent proteins in cells or subcellular structures. When illuminated with photons that correspond to their excitation wavelength, these fluorescent markers emit longer wavelength, lower-energy photons, making the structure they are attached to visible to an observer, such as a photodiode or camera. For example, the popular marker *mCherry* has an excitation wavelength of 540 – 590 nm and emits light at 550 – 650 nm [28]. One particular kind of fluorescence microscope is the confocal microscope, where the sample is not illuminated whole, as in so-called bright-field imaging, but scanned in a point-wise manner. This method has the benefit that only light emitted from the focal plane is collected by a photodiode, thereby enabling optical sectioning. Via optical sectioning, a full 3D volume of the sample can be reconstructed by stacking 2D images on top of each other (called a *z stack*, with *x* and *y* being the axis of a single image, and *z* being the stacking dimension). The downside of confocal microscopy is speed – the collection of a single *z stack* might take upwards of 30 minutes, and therefore it is not viable for actual live imaging. Two improvements on the confocal microscope fix this issue:

- *Spinning-disk confocal microscopy*, where a so-called Nipkow disk is used to illuminate multiple pinholes in parallel, greatly enhancing speed. Acquisition of a single stack is done in approximately 10 seconds (for more details see [22, Chapter 10]).
- *Lightsheet fluorescence microscopy/Selective Plane Illumination Microscopy* [13, 26], where a cylindrical lens, or scanned laser is used to illuminate an entire plane of the sample at once, enhancing speed even more. In the fastest microscopes, acquisition of a single stack can be done multiple times per second.

Both methods also reduce phototoxicity and bleach the fluorescent markers at a lower rate, making them suitable for long-term and live imaging. In this work, we use a spinning-disk confocal microscope for image acquisition. We will now continue to describe the methods used for live manipulation of the sample.

### 2.2 Photomanipulation

With photomanipulation, cellular and subcellular structures with a size on the order of micrometers can be manipulated optically. Many different techniques exist, such as:

- *Optogenetics* [7], where light-sensitive genetic switches are incorporated into the DNA or other subcellular structures of the model organism, which can then be controlled using light.
- *Optical traps/tweezers* [4, 24], where multiple crossed light paths are used to trap microscopic objects, such as bacteria.
- *Fluorescence recovery after photobleaching (FRAP)* [17], where mobile fluorescence markers are illuminated so strongly that they lose their fluorescence. Then, the recovery time until fluorescence returns is measured, as new, still fluorescent particles have diffused or been transported into the region of interest.
- *Laser ablation* [21], where a laser is used to disrupt structures in a point-wise manner. Usually a high-powered, pulsed UV laser, or an infrared Titanium-Sapphire laser is used. Depending on the exact laser used, even positions deep inside the sample can be targeted 3-dimensionally.

The laser power used in those techniques is often orders of magnitude higher than the laser power used for illumination. For more details on the physics and biology of laser ablation, we refer to Niemz [21]. We will now continue to explain how fluorescence microscopy experiments are set up, and how they are combined with laser ablation in state-of-the-art systems.

### 2.3 Laser Ablation Workflow

The setup for microscopy experiments can differ widely, as experiments are often built around a specific sample. A common denominator is that the process is based on the definition of experiment configurations which are then executed by the hardware — similar to a musical score played by an orchestra. For imaging experiments, first the sample has to be mounted on the microscope and put into focus. This is usually done using regular 2D imaging, in a manner similar to a classic microscope. Then, parameters like imaging laser wavelength, laser intensity, number and position of the 2D images acquired for a 3D stack, and the timing of the imaging is configured. In terms of time, an experiment can be as short as the capture of a single image, taking only a few milliseconds, or it can last days or weeks with repeated capture of image stacks at set intervals.

Ablation experiments are an extension of the imaging experiments just described. For ablation experiments targeting tissue regeneration or mechanical properties, at least one “before” image (or stack) is captured before the ablation is executed, after which one or several result images (or stacks) are captured, depending on whether only the final tissue state is of interest, or the entire dynamics. The imaging resolution of the microscope and the number of images per stack are chosen such that the structures for ablation are well visible. While the resolution of the ablation itself depends mainly on the ablation system, the imaging resolution determines how well the user can plan and evaluate the experiment. If ablation and imaging are required to be tightly coupled, the imaging and ablation systems can communicate directly (e.g. via TTL triggers), to avoid the latency of a round-trip over the software. In state-of-the-art systems, the ablation points or areas are planned by the experimenter on a regular 2D view, using mouse and keyboard, as seen in Fig. 2.

## 3 RELATED WORK

As laser ablation is a widely-used and versatile tool in biology, we list only a few highlights for its application: Li et al. [16] combined laser ablation with optogenetics to disrupt specific neurons of *C. elegans* (a nematode worm and important model system in cell biology) to examine a specific neuron’s importance for locomotion. Saha et al. [27] used laser ablation to disrupt the cellular cortex, a part of the cell membrane, of *C. elegans* embryos in order to determine mechanical properties of the cortex. These examples however only used 2D ablations.

The first 3D ablations were demonstrated in 2007 by Engelbrecht et al. [8], where they were able to perform multiple difficult ablations with high precision. Further, Brugués et al. [3] used 3D ablation to manipulate mitotic spindles — the biological apparatus facilitating DNA separation during cell division — and infer information about its mechanics and the length distribution of sub-segments of it.

Visual analysis of 3D data in VR may bring new insights [5, 31] and has therefore already been widely explored (see reviews of the use of VR/AR in the biomedical context e.g. in [34] and [32]). The next step was to use the 3D input modalities of VR for sample manipulation: The first VR-controlled microscope was presented in 1993 [30], where a Scanning Probe Microscope (SPM, a special kind of electron microscope) was controlled using VR with force feedback to measure and manipulate the elevation profile of a sample with close to atomic resolution. In 2015, VR was again used to control a SPM, this time to manipulate single molecules [15]. Further examples of SPM in conjunction with VR can be found in a review [18].

To our knowledge there are only two published VR interaction systems for optical and fluorescence microscopy that feature a (close to) live data feed from the microscope to VR and/or instrument control: First, Ferretti et al. [9] reconstructed 3D positions of free-floating particles or bacteria from bright-field microscopy and used optical tweezers to trap or move those objects in real time in 3D. Second, Yokoe et al. [33] developed a VR pipetting system to aid in performing artificial insemination and showed that using a pen or glove outperformed manual pipetting. Both systems however only use a simple geometric representation of the situation under the microscope, impose restrictions on sample geometry, and only solve a very specific task. In contrast, our software aims to be more general, works directly on a volume rendering of the 3D data acquired from the microscope, and allows to perform many kinds of laser ablation experiments without restrictions on the sample geometry.

## 4 MICROSCENERY IMPLEMENTATION

In this section, we briefly describe the implementation of our system, both architecturally, and from the UI perspective (to see the system in action, please refer to the supplementary video). In order to achieve *Goal 3*, our architecture is based on the use of existing APIs of the microscope/device manufacturers’ software. This enables us to outsource most of the heavy lifting to pre-existing, tested software, and avoids having to re-implement the actual microscope control code. Microscope and ablation unit are specially orchestrated in a way that enables to perform 3D ablations even on

ablation units that are usually restricted to 2D. VR rendering and input is performed on a separate dedicated VR computer connected to the microscope control computer over the network. The microscope control computer hosts a server that relays commands to the microscope control software and streams acquired microscope images to the VR computer. This is implemented using separate *ZeroMQ* TCP sockets and the commands are encapsulated using Protocol Buffers. In addition to separation of concerns, this architecture has the advantage that the user can utilise VR control in a separate room, which will likely be less regulated and cramped than a microscopy lab room. For more details on the architecture, please see Appendix B.

#### 4.1 Rendering and Interface with VR hardware

*microscenery* is built using the rendering framework *scenery* [10], which provides support for VR rendering and interaction. Images for VR are rendered asynchronously using the Vulkan API, and submitted to the VR headset using SteamVR. While an Oculus Quest 2 was used in this work, our software is usable with any SteamVR-compatible headset, and also with room-scale systems like CAVEs. Volume rendering is implemented via alpha blending, with front-to-back compositing. How the single-channel voxel data from the microscope is rendered on screen is determined by a user-defined and adjustable transfer function. Any time points that have been acquired will be stored on the rendering machine, allowing the user to both sift through all acquired time points during the experiment, and to perform post-hoc analysis. We do not perform any post-processing (such as denoising or deconvolution) on the volume data – only the raw data is shown to the user. The data can be 8-bit or 16-bit (un)signed integer pixel values that need to fit into the GPU’s memory, which is usually the case for raw data coming live from the microscope<sup>1</sup>.

#### 4.2 VR User Interface

The user interface is shown in Fig. 3. Several tools are offered to the user, such that individual point ablations or full 3-dimensional paths can be created. Ablations are first drafted in planning mode, and can still be modified or deleted before being sent to the microscope. To move around the scene, the user can either physically move, use the controller’s joystick to fly around freely, or use hand tracking to move the scene around (*grab-the-world*). The volume rendering representing the microscope’s scan volume can be freely scaled, rotated or moved, and all those properties can be locked individually. In addition, the user can choose to either show the full volume, or use a cropping tool to crop or slice the volume with an arbitrary number of planes. With the cropping tool, the volume will be either cut off at the slicing plane, or the voxel data on the slicing plane will be shown. This interface extends the possibilities of traditional 2D interfaces: While navigation is vastly simplified by using physical movement or controller movement, the 3D volume rendering allows the experimenter to better judge the surroundings of a planned cut and more easily define ablation paths that extend over many 2D image slices. With the cropping tool in turn, the experimenter is still

<sup>1</sup>While the framework also supports out-of-core volume rendering for data that does not fit into the GPU memory, this requires pre-processing and can not therefore be used for live viewing.

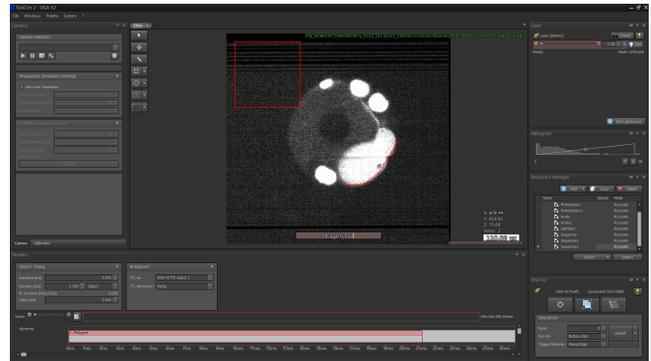


Figure 2: The user interface of the state-of-the-art photomanipulation software Rapp SysCon2. The red line at the bottom-right of the round structure defines an ablation path, while the timeline at the bottom of the window can be used to define multiple ablations over time.

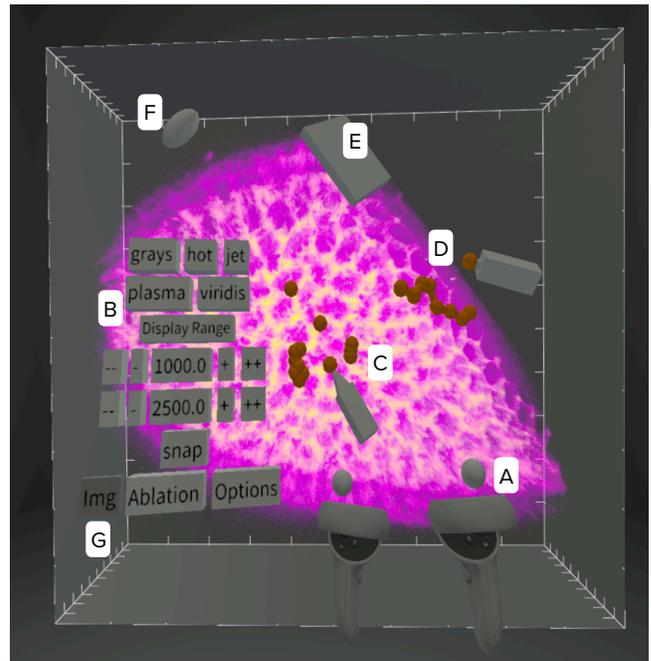


Figure 3: The VR user interface with cropped volume data and the following user-interface components: A Controllers with selection spheres; B menu; C point ablation tool with defined ablation points; D path ablation tool with path drawn; E volume slicing tool; F mover tool; G *Img* tab active in the menu. See Appendix A for a details.

able to revert to a 2D view, which they might be more accustomed to, which is remaining embedded in the spatial context of the sample. For more details on the user interface and the menu system, please refer to Appendix A.

## 5 EVALUATION: EXPERT REVIEW STUDY

Our study is based on the principles of semi-structured qualitative studies [1, 2]. We chose this type of qualitative study for three reasons: (1) Time at a microscopy facility is an expensive and limited resource, (2) the time of the domain experts is limited, and (3) the number of experienced domain experts is relatively small.

Each individual study session was conducted with one expert. Before the session, we prepared the microscope, sample, and VR system. Tasks like adjusting the microscope focus, finding the sample or adjusting the laser power, which in a regular experiment would also have to be performed by the user and are already well known by the experts, were handled beforehand to keep the focus on the VR interaction.

At the beginning of the session the expert was asked to fill out the pre-study questionnaire, plus consent forms for recording and data use and privacy. Then the VR hardware was introduced and the recording was started. The first scenario was a demo scene with pre-recorded data, in order to familiarise the expert with the system. The VR user interface (see Appendix A) was introduced and the expert could try out each element in the demo environment. Once the expert was familiar with the controls, the scenario with the live microscope connection and ablation was started.

The expert was asked to start an image acquisition, adjust the display range of the resulting image, plan an ablation path along the curvature of the embryo along the z-direction (in order to create an actual 3-dimensional ablation path), execute the ablation, and finally to assess the outcome. Afterwards, more ablation paths could be placed, or a different sample could be selected as the expert desired. Note that the participants were not given particular targets, but could decide on the ablation paths based on their own experience. As described in Section 4.2, ablation paths could also be corrected by the expert before being sent to and executed by the microscope. Once the expert decided they had sufficiently explored the system and its possibilities, they were asked to fill out the post-study questionnaire, which concluded the study session. Each session lasted around 60 minutes, for which both audio and VR view were recorded. The time was divided into 20 minutes for a hands-on tutorial of the system, 30 minutes of using the system independently, and 10 minutes for filling out the questionnaires. A member of our team was always present to guide the expert, should questions arise, or problems occur, or to help out if any adjustments to the microscopy setup were necessary. To evaluate load and well-being, we used the Simulator Sickness Questionnaire (SSQ) [14] and the NASA Task Load Index (TLX, [12]).

The session recordings were processed with an offline speech-to-text software [25]. The transcripts were then validated against the recording, remarkable statements or actions concerning our goals were highlighted, and the transcript was corrected if there were mistakes from the speech-to-text software. Then, notes on the performed actions were added if applicable. Finally, the tagged sections were thematically grouped across sessions.

### 5.1 Participants

We invited four experts for the study (3 female, 1 male; age 27 to 54; all right-handed). Two of them (E1 and E4) were members of local institute’s microscopy facilities, with up to decades of microscopy

experience. The two other experts (E2 and E3) were PhD students in molecular and cell biology, who regularly use both microscopy and photomanipulation in their research. Besides those general qualifications, each expert had individual, specific experience with photomanipulation. E1 has experience with optical traps. E2 uses laser ablation on *Drosophila* (fruit fly) embryos in their research. Similarly, E3 uses laser ablation, but with *Danio rerio* (zebrafish) as a model organism. E4 is long-term member of the microscopy facility and instructs new users in the use of the laser ablation. All experts were familiar with the concept of VR, but had only limited (though mostly positive) prior experience with using actual VR systems.

### 5.2 Hardware

For VR rendering, a Razer Blade 15 Advanced (Early 2021) notebook with a Nvidia Geforce RTX 3080 Mobile GPU (driver version 537.42) and an Intel Core i7-10875H with 2.30 GHz base clock/ 5.10 GHz turbo clock and 32 GiB RAM was used. The machine was running Windows 10 22H2 (build 19045). A Meta Quest 2 was used as head-mounted display, connected with a USB 3.0 cable using Oculus Link. The VR computer was connected to the network using 5GHz 802.11ac Wifi, while the microscope control machine was connected using gigabit ethernet. For details on the microscope hardware used and on sample (mounting), together with a photo of the actual microscope setup, please refer to Appendix C.

## 6 RESULTS

In this section, we describe the results from the expert review study described in the previous section. We want to emphasize here that while we have collected data for both SSQ and TLX, we do not present a statistical analysis, due to the small sample size. Instead, we highlight some results from these questionnaires that were consistent between participants, while the remainder of the results originates from the audio recordings collected during the experiments. All questionnaires, transcripts, and SSQ/TLX results are provided as part of the supplementary material.

### 6.1 General Impression and Feature Requests

In general, the experts were mostly satisfied with *microscenery*, commending the ease-of-use, with E2 saying “I’m surprised, this is quite easy to use!”, and further “There’s nothing right now like [it]”. E1 agreed that the software is engaging, stating “Please let me continue to play with this for a bit”. E4 compared the application to a video game. E4 emphasized with specific structures, “This looks very three-dimensional.” Navigation was perceived as easy as well, though slightly imprecise – the default speed for flying was too fast; users overshot their target sometimes and had to correct using the precise but more strenuous *grab-the-world*. All experts selected minimal scores in the SSQ (see Supplementary Material), indicating overall well-being.

While we aimed for a complete feature set to be able to perform 3D ablations (*Goal 1*), our software does not completely replicate the feature set of state-of-the-art 2D ablation tools. Commonly requested features were: Rulers and measurement tools, a histogram, and a 2D ortho slice view (while our slicing tool can slice the volume at arbitrary angles, this request aims at providing an option

to view the stack at hand by x/y/z slice, as microscopists are used to). E3 deemed the 2D slice view to be of high importance, as “[...] I find it difficult to judge if the cut happened correctly [from the volume rendering]”, and highlighted that especially the occurrence of cavitation bubbles is difficult to see without it. Both E3 and E4 found the VR volume rendering to be unusual, as they were used to viewing the data by slice. They suggested to additionally inspect the performed cut as 2D x/y/z slices. In the questionnaire, all users agreed that the quality/fidelity of the volume rendering was high. E1 requested that ablation paths can be created and manipulated as in vector graphics software – currently, ablation path editing is not possible. In addition, E1 suggested that online segmentation of the volume data could be done in order to generate ablation paths semi-automatically, or snap ablation points/paths to structures.

## 6.2 Further observations from study sessions

The modality of the ablation planning mode was one aspect that seemed confusing to the experts – in multiple cases, the experts had already created an ablation plan, and deleted the original control points. The confusion aspect here was that the plan still remains visualized, which the users did not expect.

During the study sessions, it was visible that the experts’ grasp and command of the system was solid within about 30 minutes. Indications for this were independent use of the tools offered by the software without asking further questions, and that metaphors introduced by a specific tool were transferred to other tools as well. All experts made extensive use of rotation of the sample, inspecting it from all sides. Locking of scaling and rotation functionality without blocking the other one was heavily used, too.

During E4’s session, direct interaction with the microscope was required in order to improve the microscope scanning quality, which necessitated temporary removal of the VR headset.

The experts also reported some minor usability issues, such as the volume’s display range being difficult to adjust at the moment, the menu positioning being suboptimal, or affordances for toggles and switches being unclear. As those are issues that are easy to solve and do not significantly hamper the applicability of our software, we consider them to be rather minor points that will be fixed in the next iteration of the software.

## 6.3 Perspective on novel experiments

One of the goals of our software is to enable experiments that were not possible before. E2 stated, “It is super cool to do these [3D] surface ablations.” E3 started to suggest novel experiments involving three-dimensional microtubule structures or cortical structures while using the system<sup>2</sup>. Similarly, E4 mentioned a certain fun factor, “If you really have a longer experiment series [...] the fun factor could help.” E3 deemed even more novel experiments to be possible if the software offers filled ablation shapes (e.g. an ellipse bent over a surface, where not only the outline is ablated, but also the interior). All experts selected the two highest options answering the post-study questionnaire items *The presented technique would*

*allow me to perform new kinds of experiments* and *The presented technique provides an improvement over current techniques*.

## 7 DISCUSSION AND FUTURE WORK

Based on the results of the evaluation, we would argue that we have fulfilled all the goals stated in the beginning of this work: *microscenery* enables VR-controlled 3D laser ablation (*Goal 1*), while being compatible with existing systems (*Goal 2* and *Goal 3*), being user-friendly, and opening possibilities for novel experiments involving more complex 3D (sub-)cellular structures not accessible to laser ablation so far. By writing all resulting images and configurations to disc, therefore providing a full record of the experiment, we are fulfilling *Goal 4*.

The experts saw high potential for novel experiments, which also outweighs the added complexity in the system. In the future, a tighter integration with the existing microscopy and ablation software packages will also decrease the complexity of the system.

Further considering that one of the experts needed to remove the VR headset during the experiment to adjust the microscope, we want to investigate how augmented reality (AR) headsets compare to VR headsets in such a setup. Being able to see both the environment and microscope, while simultaneously having a stereoscopic 3D rendering of the sample available might be the close-to-perfect solution. Most of the insights gained in this work will likely be applicable in an AR setup.

Another future extension possible with our software is the operation of the microscopy and ablation setup in fully remote and collaborative sessions, as in, over the internet with multiple experimenters telepresent at the same system. This could enable more researchers to access state-of-the-art hardware, and also limit necessary travel. While most volumetric data is too large to transmit over the internet quickly, approximate representations of volumetric data, such as Volumetric Depth Images [11], or adaptive resolution methods [6, 20] could be investigated for close-to-realtime remote visualization.

On the microscopy hardware side, the use of a two-photon laser for ablation would enhance the precision of the system, making cuts into deeper layers of tissue possible – without disturbing layers above the target area.

Finally, we believe that most of our results are transferable to other photomanipulation techniques such as FRAP or optogenetics, and we will investigate those possibilities in the future.

## 8 SOFTWARE, CODE, AND DATA AVAILABILITY

The software is available as open-source software under the MIT license for non-commercial purposes at [github.com/scenerygraphics/microscenery](https://github.com/scenerygraphics/microscenery). All forms and questionnaires used in the expert review study can be found in the supplementary material.

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<sup>2</sup>While the participants usually went into more detail with their description, we are unable to replicate those here, as the ideas involved ongoing, unpublished biology research.

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