PROTEUS - A virtual reality platform for live micromanipulation of cells with holographic optical tweezers

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ABSTRACT
For more than three centuries we have been watching and studying microscopic phenomena behind a microscope. Project PROTEUS can virtually shrink the user by a million times and take him inside a microscope slide where he can use his own hands to interactively grab, move and assemble microscopic systems composed of synthetic objects and living cells. This is achieved by integrating holographic techniques for microscopy and micromanipulation through a virtual reality system.

Keywords: virtual reality; optical tweezers; holographic microscopy.

1. INTRODUCTION

In the science fiction movie *Fantastic Voyage*, a team of scientists is miniaturized to "about the size of a microbe and sent into the body of a scientist to remove a blood clot from his brain. Although miniaturization of a human body is clearly impossible, the dream of touching single cells has become partially true after the invention of optical tweezers, for which Arthur Ashkin was awarded the Nobel Prize in Physics in 2018. Optical tweezers can hold microscopic particles or cells in the focus of a laser beam using optical forces arising from radiation pressure. Using optical tweezers we can move organelles inside larger cells to probe intracellular environment, we can diagnose cancer by mechanical phenotyping individual cells, we can stretch a single DNA filament to study the dynamics of DNA binding proteins. All this is usually done while sitting at a desk, watching a 2D projection of the system through the window of a computer display and moving traps with a mouse or joystick.

Project PROTEUS moves a significant first step towards the Fantastic voyage vision: if we cannot miniaturize a man to size of a microbe we can use Virtual Reality technologies to reproduce a live virtual replica of a microscopic system, send the user in this virtual microscopic world where he can use his hands to manipulate real cells that are simultaneously present under the microscope. Thanks to our algorithm for the computer generation of holographic optical trap arrays we can quickly generate multiple optical traps in 3D which can smoothly follow, as tiny light hands, the real movements of the user hands as detected by hand tracking devices. At the same time, using our 3-axis digital holographic microscopy, we obtain 3D volumetric reconstructions of the system under the microscope, so that the virtual scene that we see in front of us is the actual simultaneous configuration of the real system we are manipulating. By integrating advanced optical hardware with cutting-edge computing and 3D graphics, we bring optical tweezers into the virtual reality era, enabling immersive Lab on a Chip technologies for biomedical applications, physics and engineering of micro-system and even for education.

2. STATE OF THE ART

A focused laser beam can trap and move small objects with sizes ranging from a few nanometer (viruses and nanoparticles) to few microns (colloids and cells). This is called single beam optical tweezers [1]. In holographic optical tweezers [2], the wavefront of a laser beam is sculpted by a spatial light modulator using a computer generated phase mask. The mask is engineered in such a way that, after propagation through a microscope objective, laser light is focused into a tiny 3D hologram made of multiple focal spots, each one serving as a computer controllable optical trap. Traps can be created, destroyed and moved around interactively by translating user input into corresponding phase masks. Using optical tweezers we can trap individual cells, measure their mechanical stiffness or arrange them in precisely controlled 3D micro-environments. A few advanced interfaces have been proposed to replace the computer mouse with a multi-touch interface or force-feedback devices. However the visual feedback of the system that is being manipulated has always been limited to 2D projections viewed through the window of a computer display. Today we have extraordinary tools to tackle one of the oldest challenges in life science. Can we explore and interact with individual cells as if they were large macroscopic objects in front of us? Can we touch them, move them and assemble them in complex structures using our own hands?

3. BREAKTHROUGH CHARACTER OF THE PROJECT

For the first time, with project PROTEUS, we can send the user in a virtual microscopic world where he can use his own hands to manipulate real cells that are simultaneously present under the microscope. Thanks to our algorithm for the computer generation of holographic optical trap arrays [3] we can quickly generate multiple optical traps in 3D which can smoothly follow, as tiny light hands, the real movements of the user hands as detected by hand tracking devices. At the same time, using our 3-axis digital holographic microscopy [4], we can obtain 3D volumetric reconstructions of the system under the microscope, so that the virtual scene that we see in front of us is the actual simultaneous configuration of the real system we are manipulating. By integrating advanced optical hardware with cutting-edge computing and 3D graphics, we bring optical tweezers into the virtual reality era, enabling immersive Lab on a Chip technologies for biomedical applications, physics and engineering of micro-system and even for education.

4. PROJECT RESULTS

We built a VR application that connects to a remote server (holographic engine) controlling the optical hardware in the lab. Hand tracking data is continuously streamed to the holographic engine to compute phase masks in real time and project optical traps that follow user gestures (**Fig.1**). At the same time, using our three-axis implementation of holographic microscopy [4], the main geometric features of the sample can be extracted and sent back to the VR-engine for rendering on the VR headset a virtual replica of the 3D scene that is being manipulated. As a first application example we show the interactive assembly and manipulation of a system of four silica microspheres (radius 1 μm) that are quickly and easily arranged on the vertices of a tetrahedral structure that is then translated, rotated and scaled using simple and direct hand gestures (**Fig.2 a**).

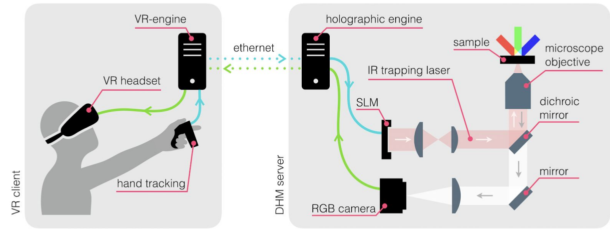
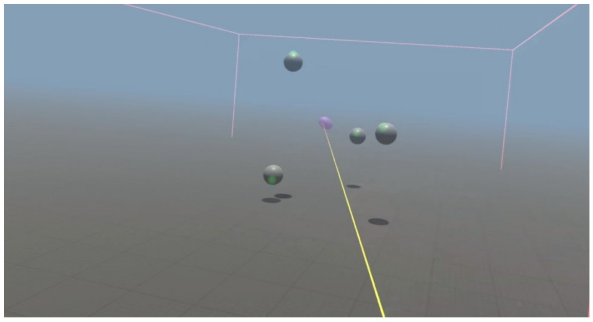


Fig. 1. Schematic view of the optical and computer hardware setup. The sample is illuminated by three tilted RGB LEDs and a 100x magnified image is captured by a color camera. The holographic engine, running on a control PC in the lab, performs 3D numerical reconstructions and identifies the objects in the field of view. Position and shape parameters of the objects are sent to the VR-engine that renders the objects on a VR headset. Hand tracking input data is transferred back to the holographic engine for the computer generation of digital holograms to be displayed on the SLM. The SLM shapes the wavefront of an IR laser beam that is then focused by the same imaging objective to generate the desired 3D traps arrangement.

a)



b)

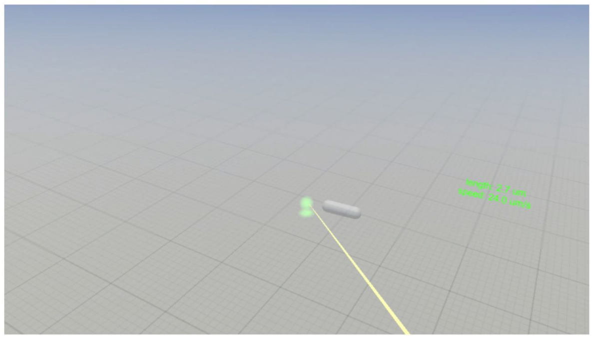


Fig. 2. a) Interactive assembly of 3D microstructures made of colloidal building blocks. **b)** Live “fishing” of swimming bacteria using a virtual laser pointer to move an optical trap over the targeted cell.

The second application example highlights the fluidity and real time capabilities of our system by showing that we can even trap an *E. coli* cell that swims nearby at a speed of approximately 20 μm/s (**Fig.2 b**). We also implemented automatic feature extraction algorithms such that when the user looks at a swimming cell, an info panel appears showing the cell length and speed (**Fig.2 b**).

5. FUTURE PROJECT VISION

5.1. Technology Scaling

The system is composed of a VR-software application and a custom microscope. The VR application is developed using the Unity game engine and requires commercial hardware to run: 1) a VR headset and tracking system (Oculus Rift or HTC Vive), 2) a GPU on the host computer. The optomechanical hardware needs to be engineered as an add-on for commercial microscopes. Reconstruction algorithms will also need to be optimized for the different and specific optical properties of the main cell types of research and clinical interest.

5.2. Project Synergies and Outreach

Scaling to TRL 5-7 will require the identification of at least one partner lab, possibly in the biomedical area, to develop a customized prototype to be tested in an operational environment. A possible candidate could be a fertility clinic where our system may assist sperm motility analysis and sorting.
Virtual, Augmented and Mixed Reality will transform the way we explore and interact with the world around us. There are already a few ATTRACT projects exploiting VR/AR/MR technologies for surgical guidance (H3D-VISIOnAiR, MIIFI, SUGAR, MRbrainS) plus other projects developing highly correlated technologies such as displays (GIMOD, InGaN-FULL-SPECTRUM), detectors (VISIR) and software (DEBARE). All these projects, however, address macroscopic length scales (>1 mm). Within a larger consortium addressing VR/AR/MR applications to life sciences and medicine, project PROTEUS will synergically address the completely different world of micron sized objects, and provide technologies for the immersive analysis, manipulation and diagnosis at the level of individual cells.

5.3. Technology application and demonstration cases

In Phase 2 project PROTEUS will customize the technology towards a specific challenges for healthcare. Possible demonstration cases could be:

1. An immersive platform, integrating microfluidics, optical micromanipulation and holographic microscopy, for the interactive analysis of sperm motility and sorting. The user will be immersed in a lab on a chip structure where sperm cells will swim around him like in a big fish tank and she will have the chance of exploring this environment, identify interesting cells based on automatically displayed geometric and dynamic features, isolate those cells and transfer them into a fluid stream for collection.
2. Integrate PROTEUS technology in a multimodal microscope where the sample could be immersively explored, interesting cells isolated on the basis of morphological or mechanical response to deformation and then subsequently analyzed with complementary microscopies such as confocal fluorescence microscopy.

A recent MIT document ("The Third Revolution: The Convergence of the Life Sciences, Physical Sciences and Engineering") addressed to the Life Sciences community highlights how the convergence of the physical, engineering and life sciences is a necessary goal to address the challenges of public health in the 21st century. According to the authors of this report, promoting convergence means not only supporting highly interdisciplinary projects but also training, expanding and supporting a new generation of researchers in an area of convergence. We are convinced that the research activities proposed in PROTEUS can also provide entirely new learning tools. Our platform would allow "direct experience" of the microscopic world making possible an "intuitive knowledge" of microscopic phenomena (i.e. constant thermal agitation, strong hydrodynamic couplings, micro-gravity, enormous surface tension) governed by physical laws that can be in stark contrast to our natural intuition gained through observation and action in macroscopic reality. A PROTEUS system could be installed for educational purposes in our university permanently, or temporarily on the occasion of scientific festivals (Rome Science Festival, European Researchers' Night, etc.).

5.4. Technology commercialization

VR/AR/MR applications to life science are so wide and different that the main commercial potential probably lies in providing customized solutions that integrate the pieces of technology and know-how developed by a Phase 2 Consortium focusing on VR/AR/MR technologies. Individual hardware and software products could be released with an open license in the spirit of the Open Science approach fostered by EU.

5.5. Envisioned risks

A possible risk for Phase 2 could be that algorithms for the reconstruction of larger cells (we used bacteria in our tests) could run too slowly to provide a real time feedback. In that case we may think of defining different resolution layers. One does not need to see a cell in full detail to measure its size or speed when you move it around. More detailed and slower scans could be performed only on interesting cells after isolation and immobilization and maybe also using the manipulation capabilities for providing controlled rotation and tomographic projections.

5.6. Liaison with Student Teams and Socio-Economic Study

As one of the oldest and largest Universities in Europe, Sapienza University offers a unique opportunity to engage MSc students from all different fields that converge into PROTEUS project: physics, engineering, computer science, biology and medicine. In particular students from the Sapienza School for Advanced Studies are constantly encouraged to team up to provide novel ideas to be tackled in interdisciplinary projects. As a former Fellow of the school the PI may act as contact person to promote PROTEUS as an extremely stimulating platform to build creative projects that will encourage MSc students to address Societal Challenges.

Also, ATTRACT Phase 2 will undertake an expert-driven socio-economic study of the ATTRACT initiative and ecosystem. Both Sapienza and NANOTEC-CNR have dedicated offices where highly experienced managers owning a long-standing experience in technological innovation could contribute to the assessment of socio-economic impact of ATTRACT.

6. ACKNOWLEDGEMENT

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