

PHD-THESIS SYNOPSIS

**MUTUAL EXTENSION OF THE APPLICATION  
AND THE TECHNIQUE OF TWO-PHOTON  
POLYMERIZATION AND HOLOGRAPHIC  
OPTICAL TWEEZERS**

AUTHOR:

**VIZSNYICZAI GASZTON**

SUPERVISOR:

**Dr. Kelemen Lóránd**

HAS Biological Research Centre, Institute of Biophysics

**Doctoral School of Physics**

**University of Szeged**

**Faculty of Science and Informatics**

**Szeged, 2019**

# 1. Introduction

My scientific work is mainly focused on the implementation, development, and the experimental application of two, laser-based techniques: two-photon polymerization for 3D microfabrication, and holographic optical tweezers for 3D micromanipulation.

Two-photon polymerization is a three-dimensional photolithographic method, offering the highest resolution as of today. The method, introduced in 1997 [1], allows the fabrication of arbitrary shaped 3D microstructures with sub-micron resolution from light-sensitive photopolymers. Soon after its introduction the fabrication of microstructures operated by the optical micromanipulation technique of laser tweezers was demonstrated [2, 3].

The so-called laser tweezers, or optical tweezers allow the spatial manipulation of microscopic objects with focused laserbeams, through the forces arising from the linear momentum of the electromagnetic radiation. The tweezed objects can be moved in three-dimensions by moving the trapping focus, and with proper calibration procedures the external forces acting on them can be also measured. The method of optical trapping enabled such novel biophysical experiments, as measuring the nanometer scale motion or the exerted force of biological motor proteins [4, 5], and the measurement of the elastic properties of single DNA molecules [6].

One of the most important advancement on the technique of optical trapping was the invention of holographic optical tweezers. It allows the creation of multiple independently moveable optical traps in three-dimensions through the holographic beam shaping of the trapping laser. Holographic optical tweezers makes it possible to grab large and complex micro-objects at multiple points, or to ar-

range live cells in a precise three-dimensional configuration [7].

The photopolymer materials processed in two-photon polymerization have ideal optical properties for optical trapping. Thus, many research groups started to work on the development of two-photon polymerized microstructures that can be operated by optical tweezers, enabling novel experiments to be performed with laser tweezers. The group of Prof. Ormos Pál in the HAS Biological Research Centre was one of the first to work in this field of research. I have conducted my doctoral studies within this group under the supervision of Dr. Kelemen Lóránd. In the two-photon polymerization lab led by him i participated in the development of microstructures that extend the method of optical trapping to novel applications [8–10].

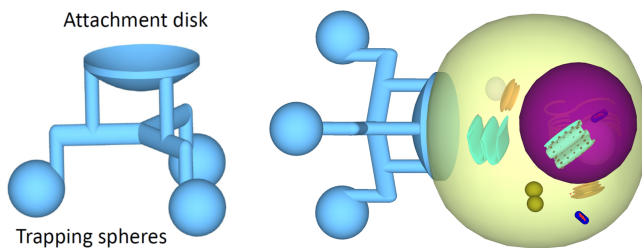


Figure 1.1: The concept of microstructure based indirect optical trapping of cells.

One part of the results presented in my doctoral thesis is from this research direction: we have aimed on improving the optical trapping of biological cells by the application of two-photon polymerized microstructures (Fig. 1.1). Although cells can be directly trapped and manipulated by a laser tweezer, it is hindered by several drawbacks: the high intensity of the trapping focus is harmful for the cell, while the optical properties of cell result in weak trapping forces. To overcome these problems i have developed a microstructure that once attached to a targeted cell, enables its high precision 6 degrees of

freedom spatial manipulation with a holographic optical tweezers, while avoiding the cells exposure to the trapping beams.

Next, we aimed to use this novel indirect optical trapping method of cells [11] for the realization of multiview microscopy. Multiview microscopy enables to overcome the anisotropic, axially low resolution of optical imaging by taking observations from multiple directions, that can be fused together into an isotropic resolution image stack. The key element of the method is the realization of multiple viewing directions of the observed sample, for which the microstructure based 6 degrees of freedom cell manipulation can be well used.

To perform multiview microscopy on microstructure manipulated biological cells i have built a holographic optical tweezers system that is combined with a fluorescence microscope. I have also written a dedicated software that can control both the holographic optical traps and the imaging of the microscope. With the built system we could successfully record fluorescence image-stacks of single cells in a set of different orientations that were realized with the microstructure based optical manipulation. By fusing the recordings of various orientations we could reconstruct the three-dimensional structure of a given cell with isotropic resolution.

In the other half of my doctoral thesis i present results about advancing the technique of two-photon polymerization (TPP). The fabrication of microstructures with TPP can be very time consuming: depending on the complexity and size of the microstructure it can take from a few minutes to several hours. Thus fabricating a large number of microstructures with TPP can be especially long. In light of this it is worth to look for solutions for speeding up the fabrication process. I have examined the applicability of dynamic holographic beam shaping (the basis of holographic optical tweezers) in TPP. I

have successfully developed a novel version of two-photon polymerization, where the fabrication of single microstructures is speeded up by exposing them with multiple holographically created and translated foci [12].

## 2. Materials and methods

For the realization of holographic two-photon polymerization i have inserted a spatial light modulator into the TPP system of our group. I have written a dedicated software that controls the holographic foci in the fabrication process by controlling the holograms displayed on the spatial light modulator. For the calculation of the necessary holograms i have used an optimized algorithm [13], that can create foci with highly uniform intensities and precise three-dimensional positions. To reduce the calculation time of the hologram algorithm i have coded it to run on an NVIDIA CUDA technology GPU.

Microstructures made when testing the holographic TPP, and the micromanipulator structure were fabricated from the photopolymer SU-8.

I have performed the microstructure bases indirect optical manipulation experiments on a Nikon Eclipse Ti-U inverted fluorescence microscope, that i extended with a holographic optical tweezers setup. For the user friendly operation of the setup i have written a software with a graphical interface. To enable the real-time control of the holographic optical traps i have applied the GPU based hologram calculation that i have written for the holographic TPP.

In multiview microscopy i have recorded epifluorescence image-stacks of K562 white blood cells decorated with fluorescent beads or stained with a mitochondria dye. For processing the recorded image-

stacks (deconvolution, spatial registration, multiview fusion) i have coded small MATLAB routines.

## 3. Summary

In my doctoral thesis i have shown, that the technique of two-photon polymerization, and the application of holographic optical tweezers can be mutually extended: i) the method of holographic beam shaping, used in holographic optical tweezers, can be very well applied in two-photon polymerization; ii) the application of holographic optical tweezers can be extended by the usage of two-photon polymerized microstructures.

**[T1]:** I have implemented a novel mode of two-photon polymerization that enables the fabrication of single microstructures with multiple foci, thereby speeding up the fabrication process. For this i have applied the method of holographic beam shaping: using a spatial light modulator i split the laser beam of the TPP system into multiple holographic beams, each of which is projected into a distinct focus by the focusing objective of the system. For the coordinated movement of the holographic foci i have written a control software that updates the beam splitting hologram on the SLM in synchrony with its internal refresh rate. For the calculation of the holograms i have applied an optimized algorithm that can create multiple foci with highly uniform intensities on precise three-dimensional coordinates. I have tested the holographic TPP method by fabricating test microstructures that were exposed by 5 holographic foci. From the evaluation of the fabricated microstructures i conclude that holographically created and translated foci can be very well used in two-photon polymerization. The achieved scanning speed of  $9\mu\text{m/s}$  can

be easily increased with the application of a higher refresh rate SLM.

**[T2]:** I have developed a microstructure, produced by two-photon polymerization that can greatly enhance the method of optical trapping of biological cells. This micromanipulator structure can be trapped with holographic optical tweezers, and its biochemical surface functionalization enables it to be easily attached to a targeted cell. Through its optimal shape and refractive index, the micromanipulator enables the high precision, 6 degrees of freedom spatial manipulation of the trapped cell, while avoiding the cell's exposure to the high intensity beams of the holographic optical traps. The trapped cell's lateral spatial fluctuation is low enough (x: 90 nm, y: 150 nm) to allow the unblurred imaging of the trapped cell, even with long exposure times. Overall, this is a great improvement over the previous modes of optical manipulation of biological cells.

**[T3]:** I have successfully applied the micromanipulator based cell manipulation to perform multiview imaging. For this i have built a holographic optical tweezers system combined with a fluorescent microscope. I have written a dedicated software for the control of the holographic optical manipulation and the multiview imaging. I have characterized the multiview imaging, and shown that it is capable of isotropic resolution reconstruction of the three-dimensional structure

of a biological cell.

# List of publications

<https://scholar.google.hu/citations?user=hcgq8uwAAAAJ>

1. Lóránd Kelemen, Pál Ormos, and Gaszton Vizsnyiczai. Two-photon polymerization with optimized spatial light modulator. *Journal of the European Optical Society-Rapid publications*, 6, 2011.
2. R Di Leonardo, András Búzás, Lóránd Kelemen, Gaszton Vizsnyiczai, László Oroszi, and Pál Ormos. Hydrodynamic synchronization of light driven microrotors. *Physical Review Letters*, 109(3):034104, 2012.
3. Darwin Palima, Andrew Rafael Bañas, Gaszton Vizsnyiczai, Lóránd Kelemen, Pál Ormos, and Jesper Glückstad. Wave-guided optical waveguides. *Optics Express*, 20(3):2004–2014, 2012.
4. Anrdás Búzás, Lóránd Kelemen, Anna Mathesz, László Oroszi, Gaszton Vizsnyiczai, Tamás Vicsek, and Pál Ormos. Light sailboats: Laser driven autonomous microrobots. *Applied Physics Letters*, 101(4):041111, 2012.
5. Darwin Palima, Andrew Rafael Bañas, Gaszton Vizsnyiczai, Lóránd Kelemen, Thomas Aabo, Pál Ormos, and Jesper Glück-



- stad. Optical forces through guided light deflections. *Optics Express*, 21(1):581–593, 2013.
6. [T1] Gaszton Vizsnyiczai, Lóránd Kelemen, and Pál Ormos. **Holographic multi-focus 3d two-photon polymerization with real-time calculated holograms.** *Optics Express*, 22(20):24217–24223, 2014.
  7. Gaszton Vizsnyiczai, Tamás Lestyán, Jaroslava Joniova, Badri L. Aekbote, Alena Strejčková, Pál Ormos, Pavol Miskovsky, Lóránd Kelemen, and Gregor Bánó. Optically trapped surface-enhanced raman probes prepared by silver photoreduction to 3D microstructures. *Langmuir*, 31(36):10087–10093, 2015.
  8. László Oroszi, András Búzás, Péter Galajda, Lóránd Kelemen, Anna Mathesz, Tamás Vicsek, Gaszton Vizsnyiczai, and Pál Ormos. Dimensionality constraints of light-induced rotation. *Applied Physics Letters*, 107(20):204106, 2015.
  9. Badri L Aekbote, Tamás Fekete, Jaroslaw Jacak, Gaszton Vizsnyiczai, Pál Ormos, and Lóránd Kelemen. Surface-modified complex su-8 microstructures for indirect optical manipulation of single cells. *Biomedical Optics Express*, 7(1):45–56, 2016.
  10. Silvio Bianchi, Riccardo Pruner, Gaszton Vizsnyiczai, Claudio Maggi, and Roberto Di Leonardo. Active dynamics of colloidal particles in time-varying laser speckle patterns. *Scientific Reports*, 6:27681, 2016.
  11. [T2] Gaszton Vizsnyiczai, Badri L Aekbote, András Buzás, István Grexa, Pál Ormos, and Lóránd Kelemen. **High accuracy indirect optical manipulation of**

- live cells with functionalized microtools. In *Optical Trapping and Optical Micromanipulation XIII*, volume 9922, page 992216. International Society for Optics and Photonics, 2016.
12. Gaszton Vizsnyiczai, Giacomo Frangipane, Claudio Maggi, Filippo Saglimbeni, Silvio Bianchi, and Roberto Di Leonardo. Light controlled 3d micromotors powered by bacteria. *Nature Communications*, 8:15974, 2017.
  13. Giacomo Frangipane, Dario Dell’Arciprete, Serena Petracchini, Claudio Maggi, Filippo Saglimbeni, Silvio Bianchi, Gaszton Vizsnyiczai, Maria Lina Bernardini, and Roberto Di Leonardo. Dynamic density shaping of photokinetic E. coli. *Elife*, 7:e36608, 2018.
  14. Silvio Bianchi, Gaszton Vizsnyiczai, Stefano Ferretti, Claudio Maggi, and Roberto Di Leonardo. An optical reaction micro-turbine. *Nature Communications*, 9(1):4476, 2018.
  15. Giacomo Frangipane, Gaszton Vizsnyiczai, Claudio Maggi, Romolo Savo, Alfredo Sciortino, Sylvain Gigan, and Roberto Di Leonardo. Invariance properties of bacterial random walks in complex structures. *Nature Communications*, 10(1):2442, 2019.
  16. **[T3] Gaszton Vizsnyiczai, András Buzás, Badri L Aekbote, Tamás Fekete, István Grexa, Pál Ormos, and Lóránd Kelemen. Multiview microscopy of single cells through microstructure-based indirect optical manipulation. Available on the arXiv preprint server (arxiv.org).**

# Bibliography

- [1] Shoji Maruo, Osamu Nakamura, and Satoshi Kawata. Three-dimensional microfabrication with two-photon-absorbed photopolymerization. *Opt. Lett.*, 22(2):132–134, Jan 1997.
- [2] Péter Galajda and Pál Ormos. Complex micromachines produced and driven by light. *Applied Physics Letters*, 78(2):249–251, 2001.
- [3] Satoshi Kawata, Hong-Bo Sun, Tomokazu Tanaka, and Kenji Takada. Finer features for functional microdevices. *Nature*, 412(6848):697–698, August 2001.
- [4] Karel Svoboda, Christoph F. Schmidt, Bruce J. Schnapp, and Steven M. Block. Direct observation of kinesin stepping by optical trapping interferometry. *Nature*, 365:721–, October 1993.
- [5] J. E. Molloy, J. E. Burns, J. Kendrick-Jones, R. T. Tregear, and D. C. S. White. Movement and force produced by a single myosin head. *Nature*, 378:209–, November 1995.
- [6] Steven B. Smith, Yujia Cui, and Carlos Bustamante. Overstretching b-dna: The elastic response of individual double-stranded and single-stranded dna molecules. *Science*, 271(5250):795–799, 1996.

- [7] G M Akselrod, W Timp, U Mirsaidov, Q Zhao, C Li, R Timp, K Timp, P Matsudaira, and G Timp. Laser-guided assembly of heterotypic three-dimensional living cell microarrays. *Biophysical journal*, 91(9):3465–3473, November 2006.
- [8] D. Palima, A. R. Bañas, G. Vizsnyiczai, L. Kelemen, P. Ormos, and J. Glückstad. Wave-guided optical waveguides. *Opt. Express*, 20(3):2004–2014, Jan 2012.
- [9] Darwin Palima, Andrew Rafael Bañas, Gaszton Vizsnyiczai, Lóránd Kelemen, Thomas Aabo, Pál Ormos, and Jesper Glückstad. Optical forces through guided light deflections. *Opt. Express*, 21(1):581–593, Jan 2013.
- [10] Gaszton Vizsnyiczai, Tamás Lestyán, Jaroslava Joniova, Badri L. Aekbote, Alena Strejčková, Pál Ormos, Pavol Miskovsky, Lóránd Kelemen, and Gregor Bánó. Optically trapped surface-enhanced raman probes prepared by silver photoreduction to 3D microstructures. *Langmuir*, 31(36):10087–10093, 2015. PMID: 26292094.
- [11] Gaszton Vizsnyiczai, Badri L Aekbote, András Buzás, István Grexa, Pál Ormos, and Lóránd Kelemen. High accuracy indirect optical manipulation of live cells with functionalized microtools. In *Optical Trapping and Optical Micromanipulation XIII*, volume 9922, page 992216. International Society for Optics and Photonics, 2016.
- [12] Gaszton Vizsnyiczai, Lóránd Kelemen, and Pál Ormos. Holographic multi-focus 3D two-photon polymerization with real-time calculated holograms. *Opt. Express*, 22(20):24217–24223, Oct 2014.

- [13] Roberto Di Leonardo, Francesca Ianni, and Giancarlo Ruocco. Computer generation of optimal holograms for optical trap arrays. *Opt. Express*, 15(4):1913–1922, Feb 2007.