Developing a Next Generation Biophotonics Workstation

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Developing a Next Generation BioPhotonics Workstation

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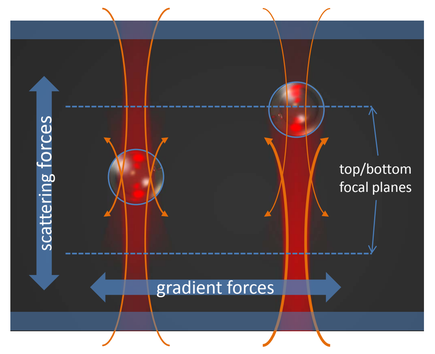
Abstract

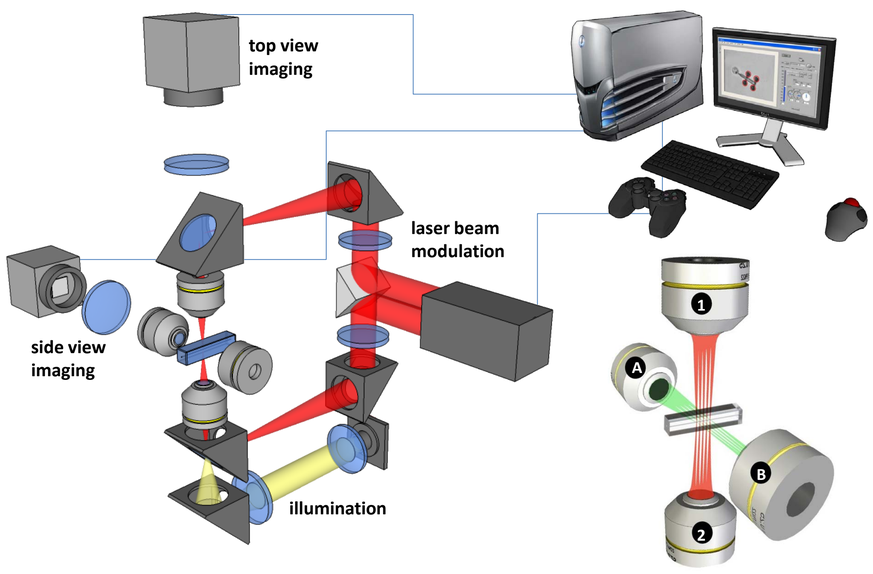
Optical trapping have established a track record for cell handling in small volumes. However, methods like fluorescent labeling are often utilized to measure single-cell properties in the trapping experiments. These methods require extra steps in the cell preparation process, and might influence the experimental outcome. To circumvent these issues, we are pursuing a novel idea; applying microscopic tools in the sample volume, which enable direct probing of specific cell properties. Here we present the initial experiments, simplifying introduction of microtools to the sample and precision positioning of several microtools simultaneously near one single cell. The experiments are performed in our BioPhotonics Workstation with a counterpropagating beam geometry. This geometry provides a large manipulation area and allows realtime manipulation of a plurality of traps (currently 100 independently reconfigurable traps), facilitating precise control and a rapid response of the optically manipulated microtools. The microtools are prefabricated by two-photon polymerization. The tools consist of a tip with sub-micron features, connected to three spheres functioning as trapping handles. The separation of handles provides leverage enabling submicron positioning accuracy of the tip. The tip can be joystick positioned in 3D with full rotational freedom, as close to the cell as desired. Using microtools allows experiments on cells without requiring extensive sample preparation. Furthermore, each tip of the microtools can be chemically activated; this provides an abundance of new opportunities, e.g. by applying enzymes that allows the tip to penetrate the cell walls or utilizing a Ph-sensing fluorochrome to measure on specific sites in or around biological cells.

Forces in a counter-propagating trap



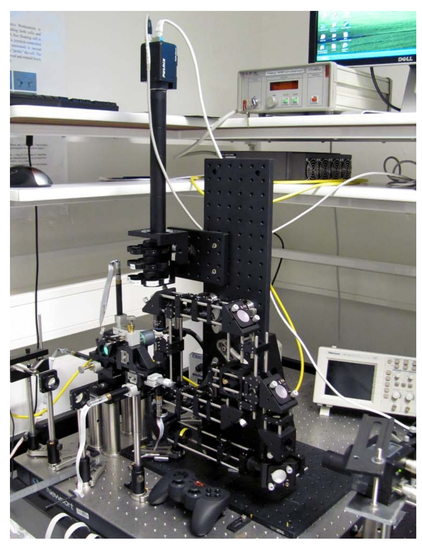
The counterpropagating based workstation geometry achieves particle trapping in the x-y (transverse) plane due to gradient forces and z (axial) trapping due to equilibrium between the scattering forces caused by a set of counterpropagating beams. Changing the top and bottom beams' relative intensities causes axial translation. Axial positions can be stabilized via dynamic feed back based on machine vision.

Schematics of workstation



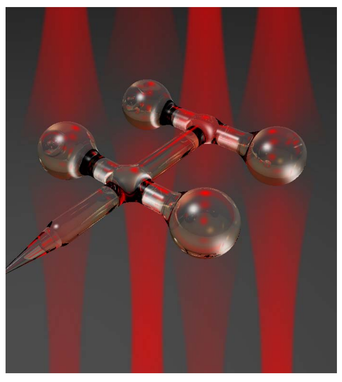
The long working distance allows an extra microscope to be mounted perpendicular for side view or for an independent optical setup. The laser source is modulated and shaped by a single spatial light modulator; the upper and lower parts of the beam are separated and projected into the sample from opposite directions. A periscopic design, with two mirrors in each arm, simplifies the necessary optical alignment.

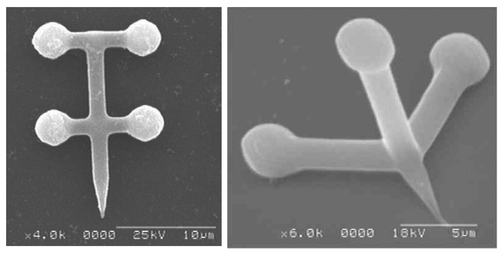
Photographs of workstation



Close-up of the sample mount surrounded by three long-working objectives (Olympus LMPL 50xIR NA:0.55/WD:6.0). Two cuvettes (Hellma 131.050, od: 4x4 mm, id: 0.25x0.25 mm) used as sideview chambers are shown on the mount.

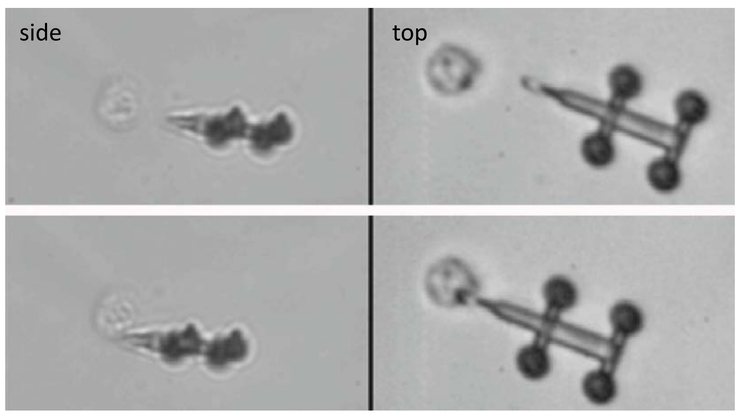
Probing cells with light driven microtools



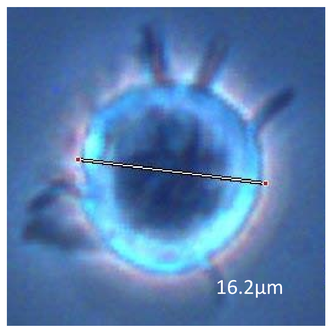


Scanning electron microscope image of a microtool produced by 2-PP photopolymerization of SU-8. This technique can be used to make tools with down to 80 nm feature sizes.

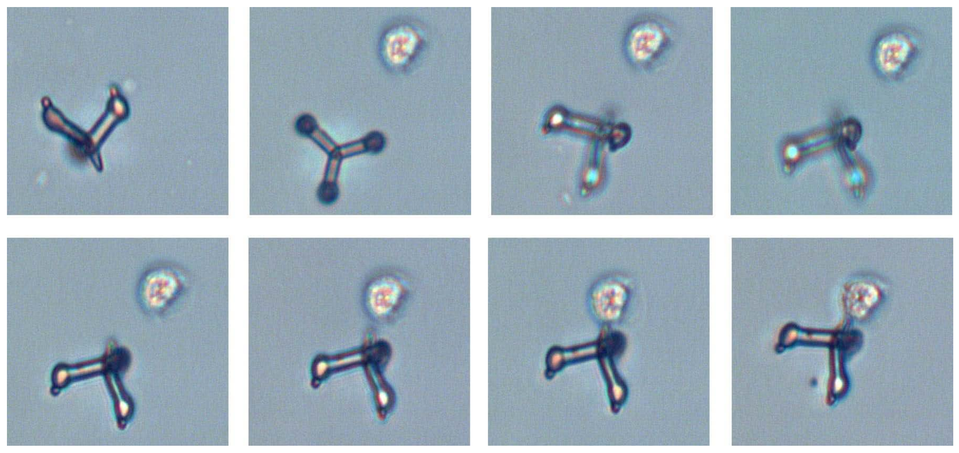
Through optical handles, these microtools can be manipulated over a large volume with 6 degrees of freedom. These tip can be positioned anywhere in biological samples for probing and sending stimuli.



An H-shaped 4-lobed microtool is brought into position near a T cell. Using sideview for observing orthogonally at the trapping plane. This can be used for fine-positioning the microtool to the cell. Another use of the side-geometry is as port for optical measurements of cells, unobstructed by the optical components necessary for the trapping and manipulation.

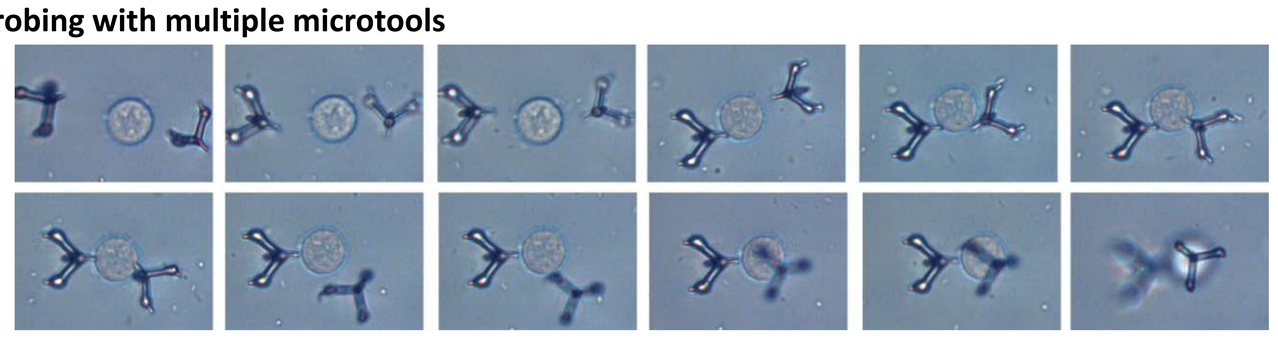


The cells used are Jurkat cells which are an immortalized line of T lymphocyte cells. They are ~16µm in diameter.



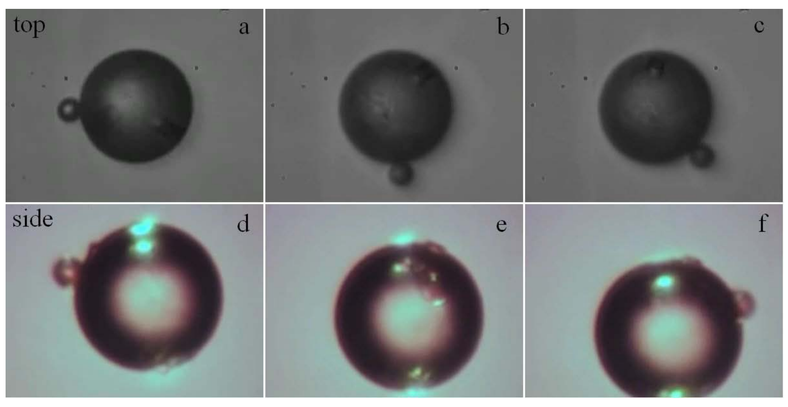
The BioPhotonics Workstation is capable of handling both cells and tools at once. A free floating cell is trapped while a joystick-controlled tripod shaped microtool is moved into position to "probe" the cell. The tool can be moved and rotated freely around the cell.

Probing with multiple microtools



Many cells will attach themselves to surfaces. The tools maintain their maneuverability near the surface. Frames 1–6: Two tools are brought into position for probing along the border of the cell. Only the two upper handles of the left tool are trapped, the glass surface, combined with a beam downwards on the tip is used to tilt it towards the cell. Frames 7–12: One tool is released from the surface and brought into position above the cell. Frame 12: The focus plane is shifted to focus on the tool.

Rotation of grouped particles



Particles having different sizes are trapped simultaneously and rotated about an axis.. ***Top row:*** Looking at the particles from above as they are simultaneously rotated..***Bottom row:*** Viewing the particles orthogonally to the top view.

Conclusion

The BioPhotonics Workstation can handle both cells and microtools in a medium required for viable cells. Microtools can be positioned freely in 3D along the border of a cell, with the tip as close to as desired. Future functionalized tools might even penetrate cell walls, allowing handling of organelles etc.

Acknowledgements

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