Developing a Next Generation Biophotonics Workstation

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Abstract

Optical trapping has 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 counter-propagating beam geometry. This geometry provides a large manipulation area and allows routine 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 fabricated 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-sensitive fluorochrome to measure intracellular concentrations.

Schematics of workstation

Probing cells with light driven microtools

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 perisopic design, with two mirrors in each arm, simplifies the necessary optical alignment.

Photographs of workstation

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.

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.

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References


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.