Manipulating and studying cellular mechanics and functions in real-time

Cellular mechanics and functions
Application note - C-Trap® Edge

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Manipulating and Studying Cellular Mechanics and Functions in Real-Time

Investigate cellular responses in living cells and organisms.

Cellular responses to cues from the extracellular environment (e.g., receptor activation and filopodia formation) or intracellular space (e.g., small compound organization and transcription) are extremely dynamic and therefore complex to study. Nevertheless, a detailed understanding of these processes is crucial for the future development of therapies aimed at treating aberrant cell functions.

Focused studies of such cellular processes have had a huge impact on our understanding of many different diseases. For example, on the one hand, impaired membrane receptors can cause endocrine diseases, autoimmune diseases, or cancers [**1-3**]. On the other hand, an overactive response to the extracellular environment and subsequent cell migration can lead to metastasis of cancer cells [**4**]. Finally, intracellular or extracellular compounds, such as proteins, may misfold and form protein aggregates, a condition that can lead to various neurodegenerative diseases [**5**].

Common assays like microscopy techniques, immune staining, and content quantification tools can measure phenotypic differences between cells or cell populations. However, these methods cannot capture the dynamic and often short-lived cellular changes in real time while simultaneously measuring or applying forces associated with their biological processes.

LUMICKS' C-Trap® Edge seamlessly combine dynamic and sensitive tools that allow you to study molecular and cellular processes in detail, with high spatiotemporal resolution and in real time. Our instruments integrate three main functions that make them an ideal solution for mechanobiology studies:

- Optical tweezers to measure or manipulate the dynamics of cellular components.
- High-resolution multicolor fluorescence and label-free microscopy.
- An advanced microfluidics system that speeds up the workflow.

In this application note

This application note demonstrates how you can use correlated optical tweezers with fluorescence and label-free microscopy across several areas in the field of mechanobiology to get new insights into cellular mechanics and functions.

We present three different experimental strategies where we used the C-Trap Edge to analyze cellular properties in different contexts:

- How mechanical stimuli or stresses affect cell receptor responses.
- Filopodia formation and functions.
- Cellular droplet fusion and small components in a multicellular organism.

The studies were performed in collaboration with research labs from Columbia University, University of California, Davis, and the University of Minnesota.

Application 1: Study how mechanical stimuli or stresses affect cell receptor responses

The background

Membrane receptors are specialized proteins that respond to extracellular changes or cues by inducing intracellular signals that regulate downstream pathways and cellular responses, such as gene transcription. Apart from antigens, some membrane receptors are sensitive to mechanical cues, such as shearing stresses from the blood flow, the rigidity of the extracellular matrix, or cell–cell contacts (**Figure 1**). Examples of these types of receptors are notch receptors and T-cell receptors [**6, 7**].

The limitations

Current methods that analyze cellular responses to mechanical stimuli rely either on plating and growing cells on surfaces with different rigidities, or in situ force manipulation using atomic force microscopy, magnetic tweezers, or optical tweezers, among others. However, most of the available approaches have technical limitations that may compromise the extent to which we can investigate the effects of mechanical stresses and signals on cellular responses:

Plating and growing cells on on surfaces with different rigidities allow you to emulate changes in the extracellular matrix. However, cells are normally not exposed only passively to a constant and ubiquitous mechanical stimulus; they also experience dynamic and localized mechanical stresses.

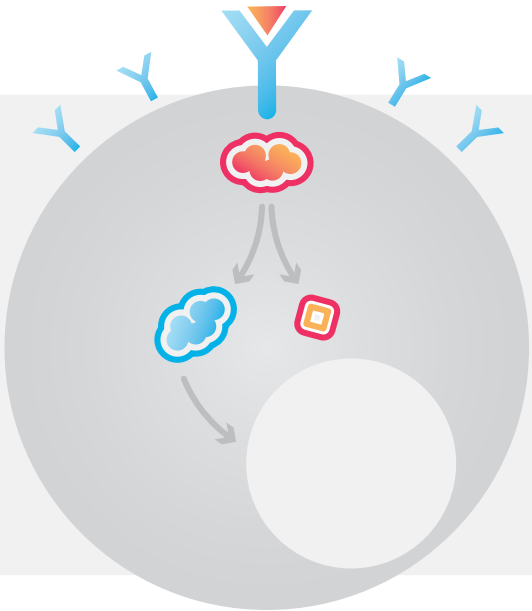
Tools that can apply in situ manipulation through force in a highly controlled manner are increasingly used in mechanobiology studies, allowing researchers to control the cells' exposure to stresses. However, technologies like atomic force microscopy and magnetic tweezers provide limited directions of applied force and force-ranges to study samples.

The solution

The C-Trap Edge is a highly versatile instrument that seamlessly combines optical tweezers with confocal microscopy. While the C-Trap Edge exert forces in three dimensions and with a wide range of forces (0.1–1000 piconewtons), the confocal microscope follows the intracellular responses in real time.

Thus, we can use the C-Trap Edge to apply low forces to the cells and assess the responses of individual receptors (**Figure 2**).

Conversely, we can apply large forces (in the order of hundreds of piconewtons) to deform the curvature of the plasma membrane, a possible process underlying the aggregation of multiple receptors at a specific location.



1 Illustration exemplifying the effects of mechanical forces on receptor proteins: an increasingly studied topic in immunology, vascular mechanics, and developmental biology, among others.

Membrane receptors are specialized proteins that respond to extracellular changes or cues by inducing intracellular signals that, in turn, regulate context-specific cellular responses, such as gene transcription.

The experiment

In this experiment, performed in collaboration with the Javitch Lab at Columbia University, New York, we used the C-Trap to understand the properties involved in the mechanical activation of transmembrane receptors. By applying a controlled mechanical stimulus, we monitored the activation of intracellular responses and signals in real time.

2 Schematic figure showing the optically trapped bead in physical contact with the cell and its membrane receptors.

We prepared the experiment by trapping a 1.76 µm streptavidin-coated bead with an infrared laser using our C-Trap system (**Figure 2**). Once trapped, we guided the bead to the cell surface of a human HEK293 cell (**Figure 3 panel 1**) wand a cytosolic marker (red signal in all panels).

After establishing physical contact between the bead and the cell surface (**Figure 3 panel 1**), we moved the bead away from the cell surface (**Figure 3 panels 2-4**) by applying trap-mediated force. While following changes on the cell membrane and in fluorescence signals using confocal microscopy, we simultaneously measured the forces exerted on the cell membrane and identified four regimes (different shades of colors in **Figure 3 panel A**).

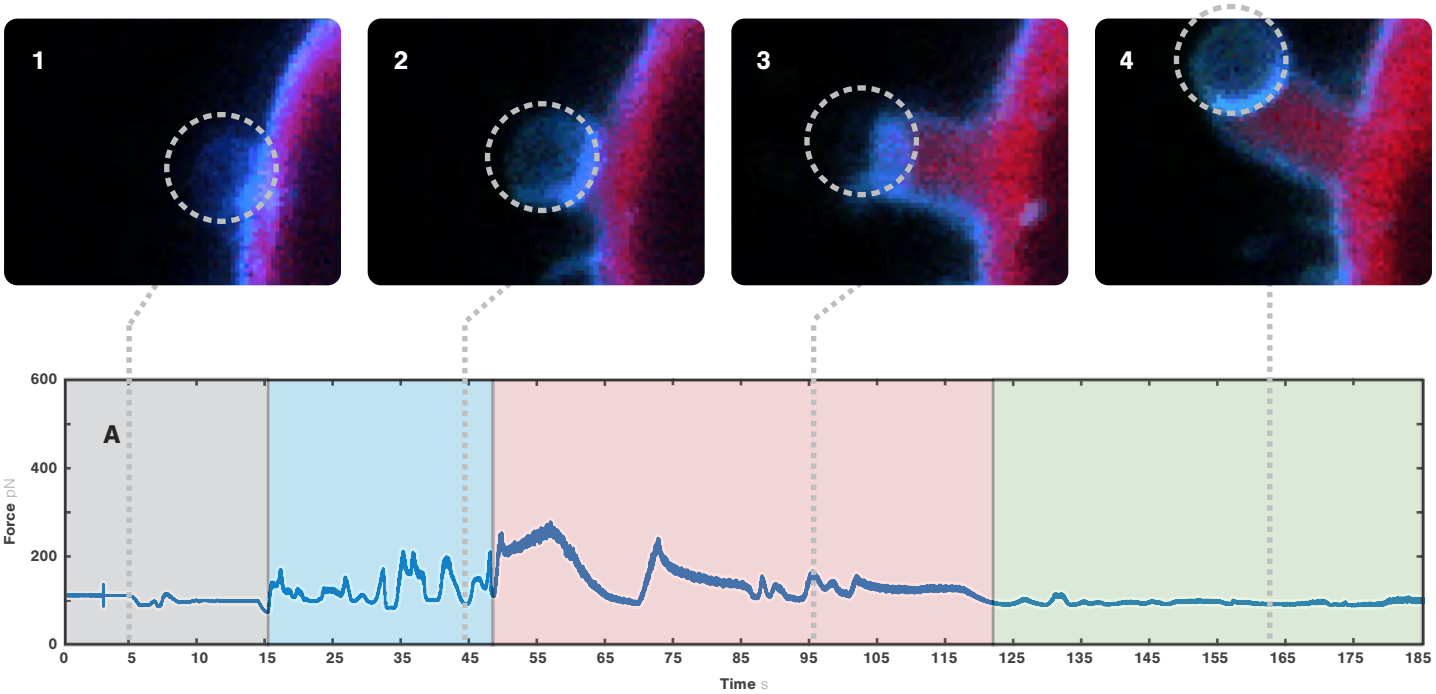
Mere contact between the bead and cell membrane did not elicit large forces to the bead, indicating the absence of cellular pushing or pulling (light gray in **Figure 3 panel A**). Initial application of force on the bead did not cause significant changes in fluorescence associated with the receptors (light blue in **Figure 3 panel A**).

However, upon multiple trials, we found that forces above 300 piconewtons (light pink in **Figure 3 panel A**) caused deformation of the cell membrane (**Figure 3 panel 3**) and an accumulation of blue signal at the site of manipulation. As we proceeded, minimal applied forces on the cell membrane were enough to pull out a thick membrane tube (**Figure 3 panel 4** and light green in **Figure 3 panel A**).

These observations suggest a correlation between cell membrane deformation and local accumulation of receptor protein at the site of manipulation (blue signal in panels 2-4).

What can you do with the C-Trap® Edge?

- Apply forces in the hundreds of piconewton range to manipulate the cell surface.
- Follow multiple cellular components simultaneously with multi-color confocal microscopy.



3 Cell membrane deformation and receptor accumulation at the site of manipulation. Panels 1-4: Confocal microscopy images of a cell expressing fluorescently labeled biotinylated receptors (blue) and a cytosolic marker (red) showing changes in cell membrane, receptor and cytosol-associated activity, upon manipulation of the polystyrene bead with a laser (grey dashed circle). Panel A: Force measurements depicting four distinct regimes (grey, blue, pink, and green) based on forces exerted on the cell membrane over time.

Application 2: Track forces linked to filopodia formation and functions

The background

Filopodia are cellular protrusions that certain cell types use to interact with their surrounding environment [**8**]. Cells use filopodia to sense, migrate, and communicate with other cells through external and internal pulling forces (**Figure 4**). The protrusions grow through actin polymerization, a process driven by a variety of proteins, including actin-mobilizing motor proteins belonging to the myosin superfamily and the actin-polymerizing formin family of proteins.

Defects in filopodia formation alter cell spreading, adhesion, and mobility. For example, overproduction of filopodia or filopodia-like protrusions is associated with increased invasiveness of metastatic cancer cells [**4**].

To further understand the functions of filopodia and filopodia-associated diseases, we need to study the roles of proteins involved in protrusion formations and their functional features, such as the exerted forces of the structures.

The limitations

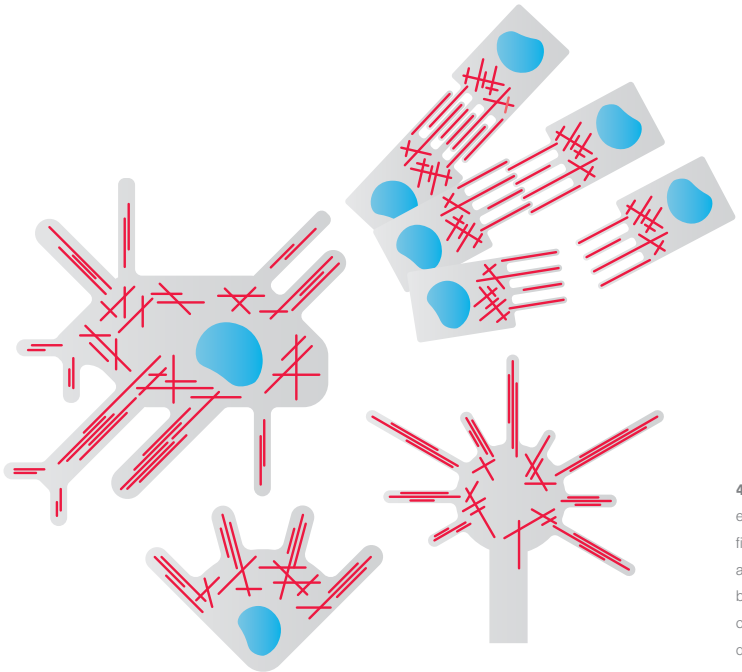
Until now, researchers have relied on traditional light microscopy and fluorescence microscopy approaches to study filopodia. This is typically done by tracking beads or proteins at the tips of the cell protrusions. However, these methods cannot determine the force exerted by the filopodia formation and retraction.

Considering that cell protrusions are highly dynamic structures (e.g., tips can move at velocities ranging between 0.8 µm/s to 1 µm/s) and possibly exert forces in the low piconewton range, we need a highly dynamic and sensitive method to determine these parameters.

The solution

Optical tweezers, combined with imaging, fulfill these requirements since we can measure forces at low piconewton ranges and couple these with cellular processes through live imaging. The approach provides us real-time measurements of otherwise hard-to-capture processes like filopodia formations and properties.

We can combine the optical tweezers with a variety of fluorescence microscopy methods, such as confocal and TIRF1 for background rejection, widefield for high temporal resolution, and IRM2 for label-free studies. Since sample manipulation and visualization are seamlessly correlated in the system, we achieve the highest sensitivity, resolution, and stability while imaging data collections in real time.



4 Schematic figure showing examples in filopodia structures and their interactions with the surrounding environments through the polymerization of actin filaments (here shown in red). The examples show filopodia in:
a) migrating neuro.
b) epithellal sheets.
c) migrating cell.
d) neuronal growth cone.

[1]TIRF Total internal reflection fluorescence microscopy: A type of microscope that can excite fluorophores within a thin region of a sample

[2]IRM Interference reflection microscopy: Optical microscopy technique used to study cell mobility or adhesion on a glass surface

The experiment

The following experiment highlights the forces and kinetics associated with filopodia and their formation (**Figure 5**). In collaboration with the Titus Lab at the University of Minnesota, Minneapolis, we studied Dictyostelium discoideum cells, a eukaryotic amoeba cell and model system that is commonly used to understand human cell processes.

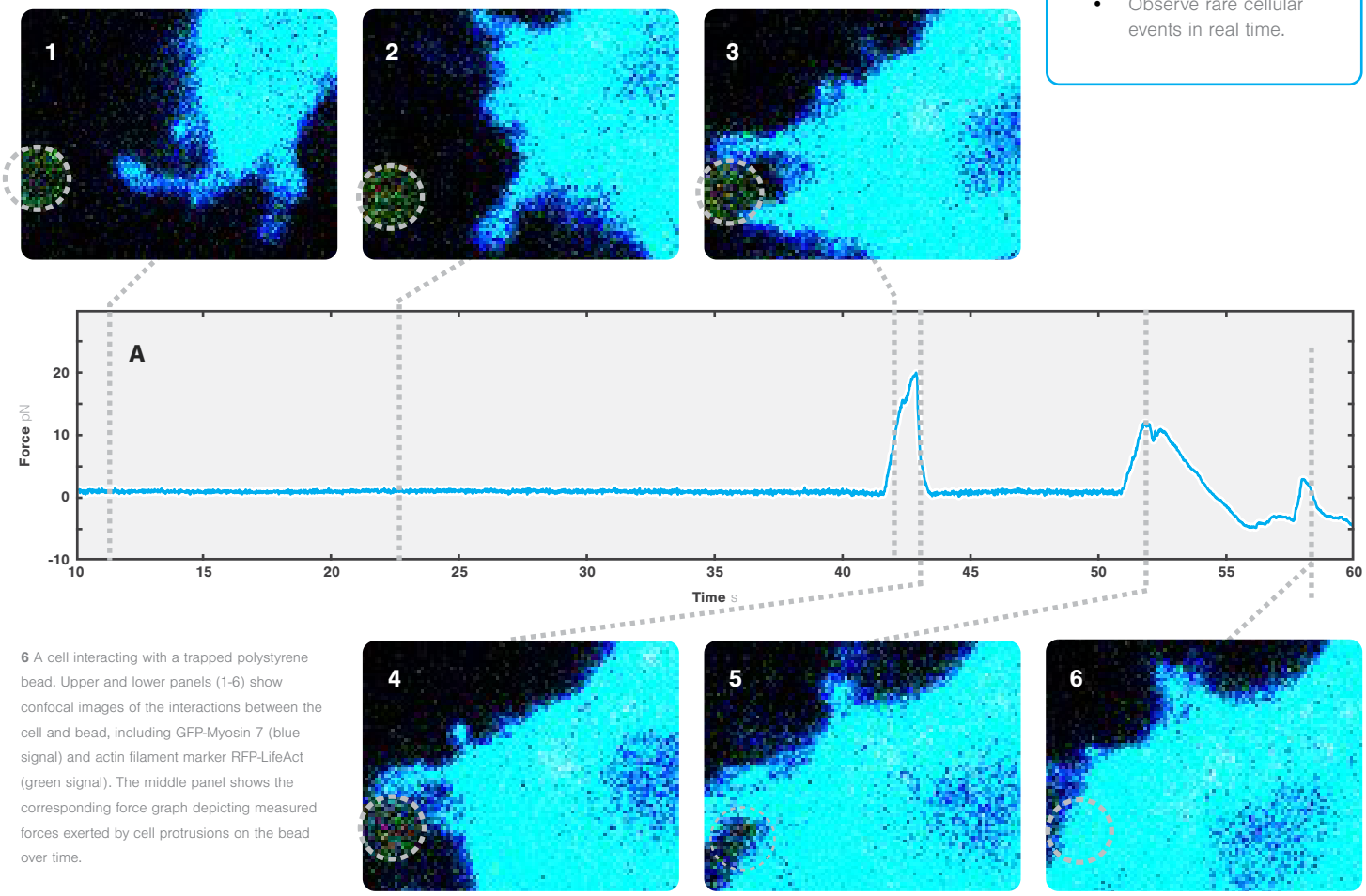
5 Illustration of a cell interacting with a trapped polystyrene bead through formed cell protrusions.

We first trapped a 1.76 µm streptavidin-coated bead (**Figure 6 panels 1-6**) with a laser. Next, we moved the bead in proximity to the D. discoideum cell, ectopically expressing GFP-Myosin 7 (blue signal) and actin filament marker RFP-LifeAct (green signal; **Figure 6 panels 1 and 2**). Confocal microscopy allowed us to visualize the dynamics of the filopodia protrusions as they searched their local environment. Upon sensing the presence of the bead, the cell moved in the direction of the trapped object and extended multiple protrusions to interact with it.

While tracking the forces exerted on the bead (**Figure 6, panel A**), we observed a characteristic signal every time the cell engulfed the bead (**Figure 6, panels 5 and 6**). Interestingly, we also found an occasional force spike right before bead engulfment (**Figure 6, panels 3 and 4**). Since we observed the formation and retraction of one of the formed filopodia touching the bead, we suggest that this peak possibly is caused by the interaction between the cell protrusion and the bead. Thus, the maximum value shown on the graph (~20 pN; **v**) corresponds to the force that a single protrusion exerted over the foreign object (the bead).

What can you do with the C-Trap® Edge?

- Follow filopodia formation and actions in real time using fluorescence.
- Track forces associated with cellular interactions and environment.
- Observe rare cellular events in real time.



6 A cell interacting with a trapped polystyrene bead. Upper and lower panels (1-6) show confocal images of the interactions between the cell and bead, including GFP-Myosin 7 (blue signal) and actin filament marker RFP-LifeAct (green signal). The middle panel shows the corresponding force graph depicting measured forces exerted by cell protrusions on the bead over time.

Application 3: Study cellular droplet fusion and small components in a multicellular organism

The background

Living cells and organisms are composed of cellular components, which are biomolecules and structures comprising intracellular and the extracellular compartments (**Figure 7**). Since cell components carry out essential biochemical reactions, contain the genetic material, and develop or maintain cellular structures, they are crucial for proper cell function. Manipulation of small cell-components can, hence, provide an extensive understanding of their organization and properties in both health and disease.

We can study small cell components like lipid droplets and their fusion through external manipulation, which offers us a unique approach to analyze cellular fat storage in vivo [**9**]. We can apply the same assay in different cellular compartments to analyze other organelle structures, such as protein droplets, which may aid our understanding of the underlying causes of, for example, certain neurogenerative diseases [**10**].

The limitations

Several cell biological approaches are available to study cell components, including various forms of cell imaging techniques, immunostaining, or chromatography. However, while the available techniques can identify, quantify, and localize the components of interest, they do not allow to visualize and manipulate the structures in real time.

Manipulation of intracellular components in vivo, for example to study droplet fusion, often requires non-invasive tools. Apart from optical tweezers, biophysical tools like atomic force microscopy (AFM) and magnetic tweezers are among the most commonly used tools for in situ manipulation. However, to study intracellular components, both techniques present certain limitations:

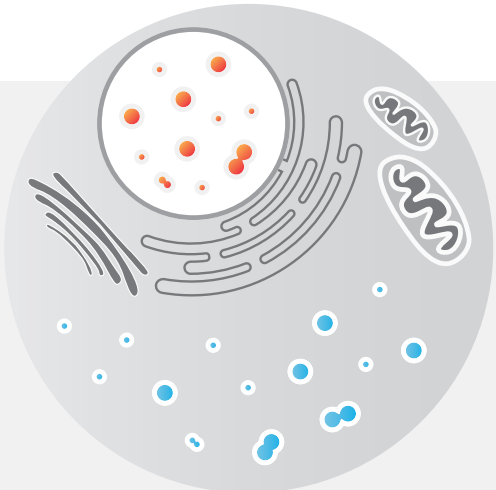
- AFM is often limited to surface scanning as a force-transducer to directly probe the elastic response or adhesion properties of a membrane.
- Magnetic tweezers require magnetic structures, which means that we need to introduce a permanent magnet or magnetizable object, such as beads with a paramagnetic core, in vivo through endocytosis or microinjections to study the molecules.

The solution

Because of its multiple functionalities, C-Trap Edge is a non-invasive and comprehensive tool that can manipulate cellular components and processes in vivo without the need of introducing ectopic components.

With the C-Trap Edge, we can trap droplets inside the cell and visualize them using a bright-field camera or a variety of fluorescence microscopy techniques (**Figure 8**). We can furthermore analyze the cells in controlled temperature environments and provide physiologically relevant conditions.

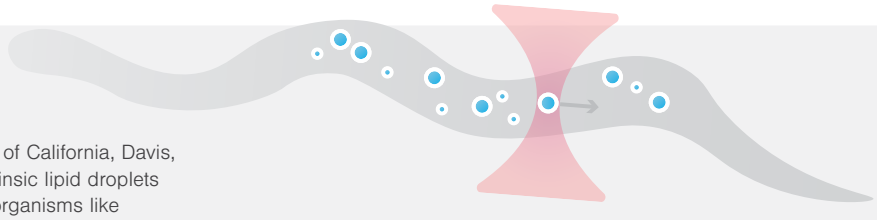
We can combine the optical tweezers with a variety of fluorescence microscopy methods, such as confocal and TIRF1 for background rejection, widefield for high temporal resolution, and IRM2 for label-free studies. Since sample manipulation and visualization are seamlessly correlated in the system, we achieve the highest sensitivity, resolution, and stability while imaging data collections in real time.



7 Schematic figure of a cell with small components (red and yellow) distributed in different compartments of the cell (here: nucleus and cytosol).

The experiment

In collaboration with the Starr Lab at the University of California, Davis, we used C-Trap optical tweezers to manipulate intrinsic lipid droplets and study droplet fusion inside of living cells and organisms like Caenorhabditis elegans (roundworm).

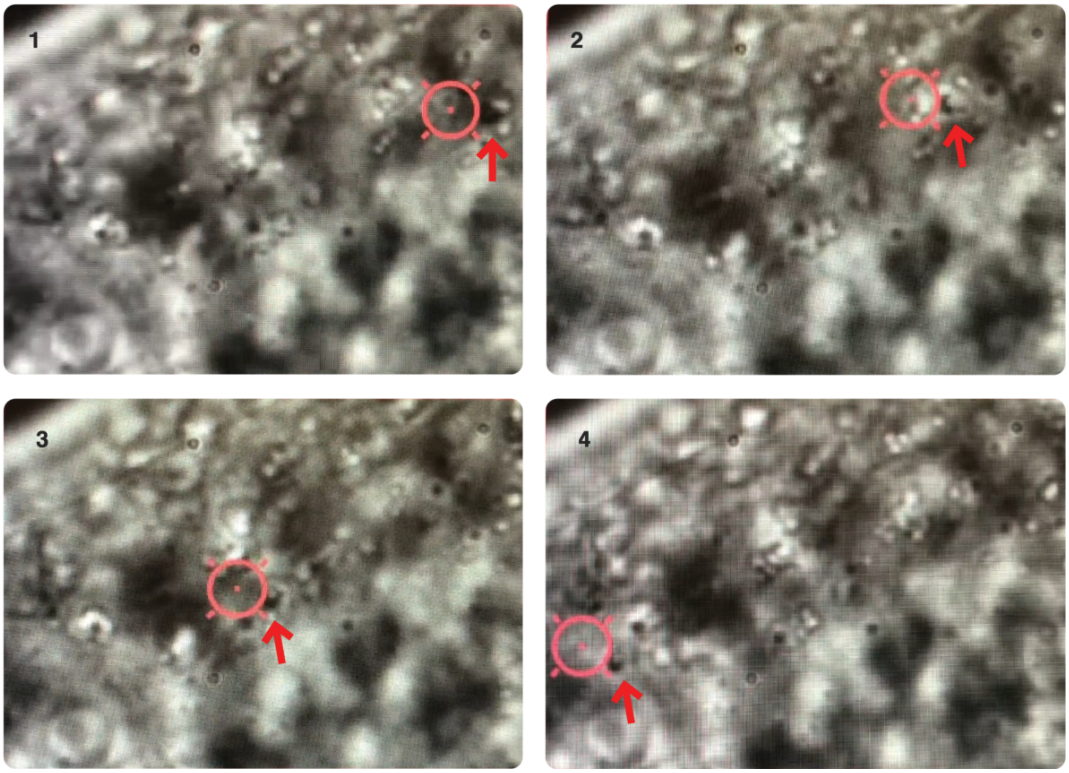


8 Illustration of lipid droplets (yellow circles) in C. elegans (roundworm). One droplet is trapped and moved along the ringworm with a laser.

For this experiment, we mounted C. elegans (late larva or adult organisms) on a 2% agarose pad (agar bed) and paralyzed them with the antiparasitic agent tetramazole. Using brightfield illumination, we could localize the lipid droplets inside the organism and subsequently trap the molecules with optical tweezers (**Figure 9**). To avoid photodamage while trapping the droplets, we exposed the body of the worm with a low laser intensity (red circle).

We can quickly scan cell samples with a micrometer-precision stage and, on top of that, obtain fine movements when needed using a nanometer-precision positioning system. These features allow us to optically trap droplets in two ways: dragging the droplets around the body, thus exposing them to each other, or keeping them at the same position and letting the stage bring other droplets into the proximity of the trapped molecule.

The approach allowed us to follow the small components in vivo and in real time and assess forces associated with the fusion process.



9 Imaging from brightfield microscopy showing directional manipulation of a lipid droplet (red arrow) inside the C. elegans with a laser (focused in the center of the red circle).

What can you do with the C-Trap® Edge?

- Study live multicellular organisms.
- Localize small components using brightfield illumination microscopy.
- Trap and manipulate organelle structures to understand their properties.
- Control the temperature of the sample to provide relevant physiological conditions.
- Finetune the laser intensity to avoid photodamage to the cells.

Conclusions

Here, we have highlighted the use of C-Trap Edge to study cell functions. The system gives you a detailed insight into the specific cellular processes and interactions that you want to assess – both in single cells and multicellular organisms.

The C-Trap Edge seamlessly combines highly dynamic and sensitive measurements with a variety of microscopy techniques. You can choose between confocal microscopy, total internal reflection fluorescence (TIRF), label-free interference reflection microscopy (IRM), and widefield imaging to visualize cell functions and interactions. On top of that, the system supports up to three channels of fluorescent excitation, which allows you to identify different proteins and their co-localization in a cell. Finally, the C-Trap Edge allows you to perform non-invasive studies on living cells and organisms in optimal temperature conditions.

The integrated optical tweezer and high-resolution microscopy system offered by the C-Trap® Edge make them a powerful tool to study dynamic and force sensitive cellular processes in real time.

Collaborators

Javitch Lab, University of Columbia, New York

Titus Lab, University of Minnesota, Minneapolis

Starr Lab, University of California, Davis

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