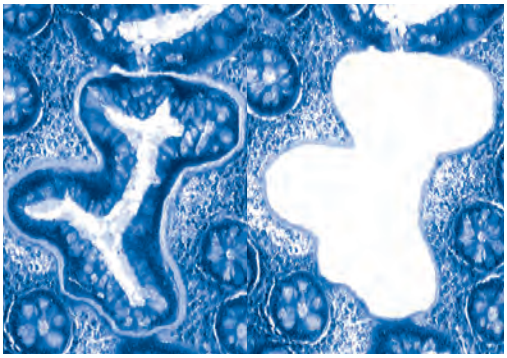
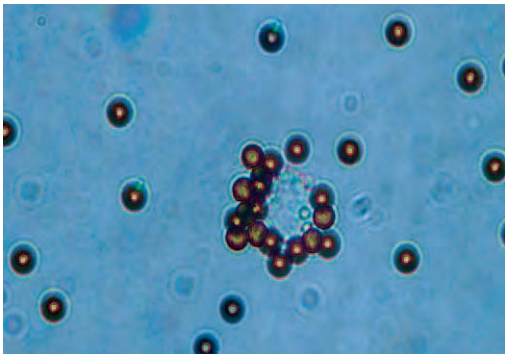
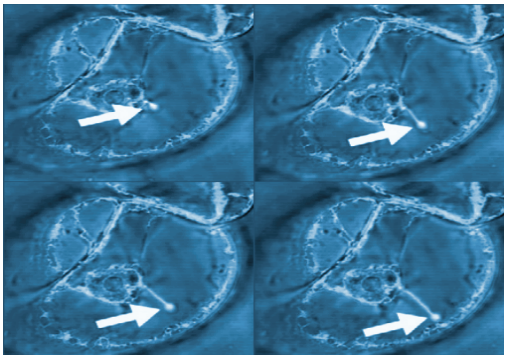
MMI

Microscopy-based Single Cell Isolation

Single Cell Solutions

Cutting-edge technologies for fast and
easy detection and isolation of single cells


Molecular Machines & Industries

Single Cell Solutions

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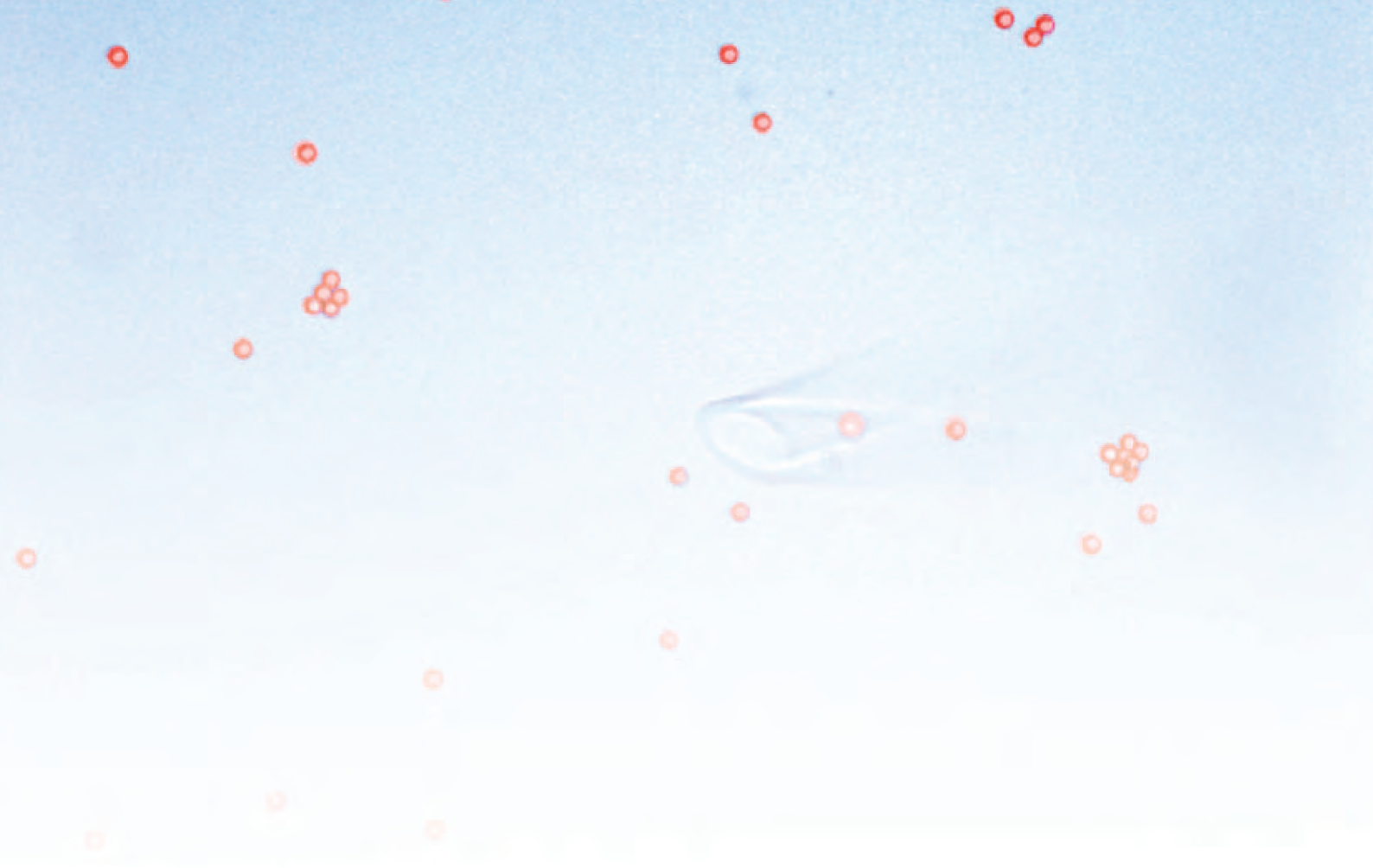


Introduction

In medical diagnostic or life science research, it is crucial to get high quality homogeneous tissue samples, especially in genomics, transcriptomics, and proteomics. Enriched cell populations from tumor, endothelial tissue, histocytes, stem cells, and other materials are a prerequisite for analysis and patient profiling.

MMI's technologies for the detection and isolation of single live cells, stem cells and circulating tumor cells are for example utilized in cancer research and clinical oncology for the enrichment of tumor cells from disaggregated lymphnode blood and bone marrow samples as well as in immunology and virology for the study of monoclonal human antibodies. Furthermore, they are widely used for a range of applications in stem cell research and cellular diagnostics.

A new generation of microscopy based single cell sorting

MMI CellEctor Plus

The MMI CellEctor Plus is a microscope based capillary single cell sorting system for rapid recognition, acquisition and deposition of single or rare cells in suspension. It enables the development of user controlled protocols for aspirating and depositing cells in nanoliter volumes for the isolation of single cells in a format suitable for downstream molecular analysis with nanolitre volume reaction mixtures.

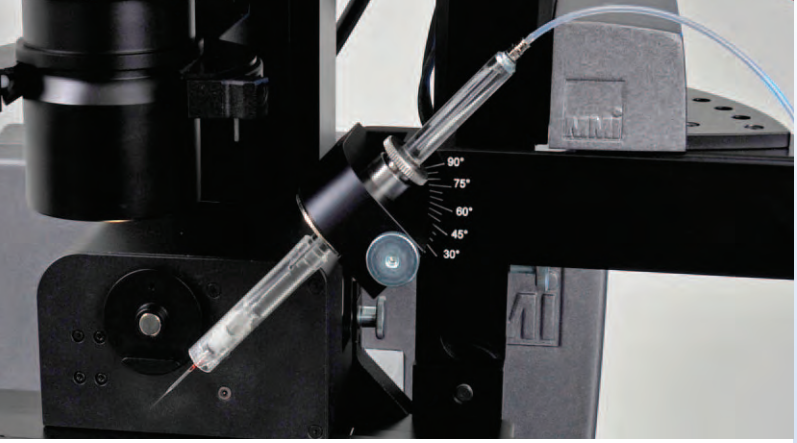
The high precision of the MMI CellPump regulates the acquisition and deposition in a variety of modes, giving the user complete control in manual and automated cell recognition, acquisition and deposition. The completely software manipulated 3D CellRobot controls the precise landing position of the capillary and generates a highly reproducible contact point.

All accessories were directly developed in consultation with MMI CellEctor Plus users to reflect their exacting needs. They allow for the deposition of single cells onto reaction slides or directly into PCR tubes, IBIDI style chambers, microfluidic devices or 96-well plates. User defined liquid handling programmes enable the development of dedicated cleaning and service cycles for capillary cleaning and maintenance.

Features & Benefits

- Microscope based **software controlled** movements of the capillary
- Easy to achieve **accurate and precise** cell isolation and deposition
- **No contamination** from unwanted cells
- **Faster and easier** than manual systems
- Nanoliter pump allows isolation of cells in **small volumes**
- **Brightfield or fluorescence** automated detection of cells
- **Full process control** of cell identification, acquisition and deposition
- Work **manually or automatically** as your workflow demands
- **Compatibility** with a wide range of accessories and microfluidic devices

Designed for fast and reliable detection and isolation of single cells, stem cells and CTCs by fully controllable microscopic collection: MMI CellEctor Plus



MMI SmartCapillary for rapid and ultra-precise recognition, acquisition and deposition of single or rare cells in suspension

Setup

- Based on an inverted or upright manual or motorized high performance research microscope
- High precision motorized scanning stage
- 3-axis motorized software controlled MMI 3D CellRobot for full control of landing position
- High precision MMI CellPump with three independent working modes: manual, semi and fully automated
- MMI SmartCapillary with movable safety cover for protection of the capillary and the operator
- High-resolution MMI CellCamera
- Modular and upgradeable workstation
- MMI CellTools Software combined with MMI CellExplorer Software for automatic cell recognition in fluorescence or bright field
- Interactive LED pen display

Expandability:

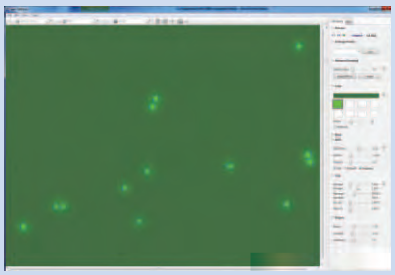
- MMI CellCut Plus for laser microdissection
- MMI CellManipulator Plus optical tweezers
- Range of imaging configurations (Fluorescence, DIC, Phase contrast)
- Others on request

Compatibility:

- Nikon Ti-S, Ti-E, Ni-U, Ni-E, A-1
- Olympus IX73, IX83, FV1000
- Zeiss Axio Observer, LSM 780
- Leica on request
- Others on request

Technology

1. Cell selection and auto recognition



MMI CellExplorer cell recognition software which automatically recognizes cells under fluorescence and in bright field

Place an aliquot of the sample of cells in suspension directly onto a standard glass slide. Visualise the cells in either bright field or fluorescence. Mark the cell of interest by clicking with the mouse. Autodetection can be achieved with the MMI CellExplorer Software designed for the auto recognition of single cells based on their colour, morphology and relative size.

2. Ultra precise cell aspiration

A glass capillary is used to aspirate the cells of interest directly. This is controlled by a high precision 3D robotic arm and ultra precise cell pump. Both can be used in full manual mode, or semi or fully automatically. More adherent cells can be collected by reversing the position of the capillary and lowering it by a few microns to allow the cells to be released by scratching and pumping simultaneously.

3. Automated cell acquistion and deposition with full visual control

Single cells in suspension can be collected manually or automatically onto a wide range of slide based platforms including IBIDI, a PCR tube, a microfluidic or other molecular analysis device. The user gets full optical control throughout the whole workflow.

4. Molecular downstream analysis

For example: FISH, IHC, ISH, immuno fluorescence, Single Cell PCR

Leading the way to single cell research & cellular diagnostics

Applications

The MMI CellEctor Plus technology is widely used for a range of applications in the following fields:

- Detection and isolation of single and rare cells in suspension, e. g. Circulating Tumor Cells (CTC's)
- Cancer research and oncology
- Detection and isolation of monoclonal human antibodies
- Cell sorting on a wide range of biochips
- Lab-on-a-chip technologies
- Stem cell research and cellular diagnostic
- Single Cell PCR and gene expression analysis

Consumables & Accessories

MMI Capillaries

Prod. No. 80105 (40 µm)
Prod. No. 80103 (20 µm)
Prod. No. 80102 (10 µm)

Designed for cell collection with MMI CellEctor Plus, 54 mm long, very sharp 45° beveled tip to collect even adherent cells, available in 3 sizes, 10 pieces per box.

Also available: MMI CapillaryClean (Prod. No.: 80107), keeps the capillary free of dirt and contamination and avoids blockage. Cleaning of capillaries is recommended as part of all workflows.

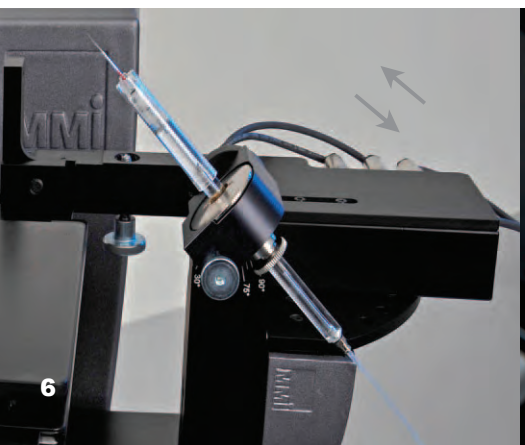
MMI CapStrip with 8 empty caps (0.2 ml per cap)
Prod. No. 50215

For the collection of cells with the MMI CellEctor Plus, 0.2 ml per cap, 5 strips per box.

MMI CellEctor Plus Starter Kit
Prod. No. 70303

Recommended for first application use after instrument purchase. Contains all available consumables for MMI CellEctor Plus.

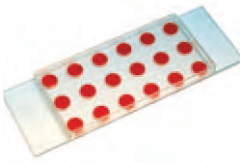
The 3D robotic arm can be rotated to the side for easy and comfortable change of the capillary, the movables protection cap maintains the capillary clean and sterile and ensures optimal work security



The MMI CellEctor Plus can be utilized for a wide range of appllications, for example in cancer research, stem cell science, or immunology and virology

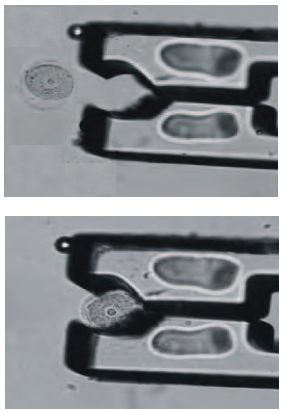
MMI ServiceSlide
Prod. No. 50108

18-well IBIDI® Slide
Prod. No. 50109



MMI ServiceSlide and 18-well IBIDI® Slide are used for liquid handling on the MMI CellEctor Plus.

In evaluation: The MMI Micrograpper

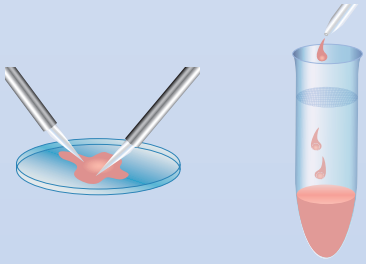


Manipulation experiment where the micrograpper is able to detach a mouse oocyte (diameter 100µm approx.)

MMI is currently in the development of a micrograpper. The aim is the incorporation onto the MMI CellEctor Plus as a compliment to the micro capillary.

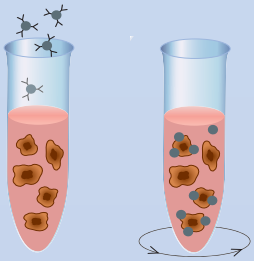
From user, for user: workflow example cancer research

Lymphnode metastasis is disaggregated into small pieces and passed through a 70 micron filter to produce a cell suspension. Blood and bone marrow samples are erythrocyte lysed, to remove erythrocytes. The sample is enriched using paramagnetic beads coated with an appropriate surface-antibody. The MMI CellEctor Plus can now be effectively used for single cell picking for all downstream applications.

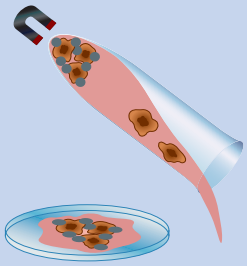


1. Preparation of a cell suspension: patient material as disaggregated lymphnodes and filter cells...

... or erythrocyte lysis from blood or bone marrow

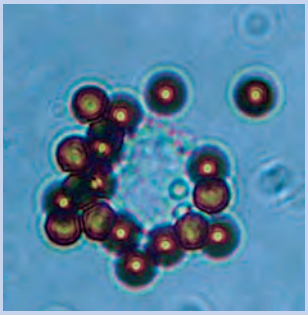


2. Pre-enrichment of target cells by adding specific immunomagnetic beads and incubating under rotation



3. Magnetic separation of immunomagnetically rosetted cells and microscopic evaluation

4. MMI CellEctor Plus mediated single cell isolation



5. Lymphnode metastatic cells targeted with anti EP-CAM antibody

Single cell isolation by laser microdissection

About the need

Single cell isolation in combination with laser microdissection is of growing interest. Mainly, laser microdissection systems are widely used for analysis of tissue invading cells like immune cells, vascular cells, invading metastatic cells and tissue or cancer stem cells. Likewise, single cells from blood or bone marrow smears or any other aspirate can be microdissected prior to downstream analysis in cytogenetics and biomedical research.

MMI CellCut Plus

The MMI CellCut Plus laser microdissection system combines proven cutting-edge technologies for the precise isolation of single and groups of cells. The ultraprecise system is fast and easy to use for a wide variety of sample types including fresh frozen or paraffin embedded tissues, archived slides, cytospins, smears and live cells.

The MMI CellCut Plus delivers outstanding image quality and enables the selection and isolation of single and groups of cells quickly and easily directly on the touch screen.
The cells of interest are marked and cut automatically using the precisely focused UV-Laser.

The microdissected samples are collected in an adapted PCR tube for downstream analysis. This results in a pure cell population for reliable gene expression analysis.

The ideal tool for the detection and isolation of single cells or group of cells from tissue or live cell cultures: MMI CellCut Plus

Fast and easy isolation of cells from up to three slides simultaneously

Setup

- Based on an inverted or upright manual or motorized high performance research microscope
- High precision motorized scanning stage
- Electronically controlled solid-state laser (standard or optional with high power for thick, hard, or wet tissue), laser beam delivery and transfer optics
- MMI CapHolder with MMI Single CapLift, MMI MultiSlide insert for parallel us of up to 3 slides, MMI LiveCell insert for applications with living cells, optional MMI Multi CapLift for the parallel use of up to 8 caps
- High-resolution MMI CellCamera
- Modular and upgradeable workstation
- MMI CellTools Software for full control of the laser, image capture, and scanning stage actions combined with MMI CellExplorer Software for automatic cell recognition in fluorescence or bright field
- Interactive LED pen display

Expandability:

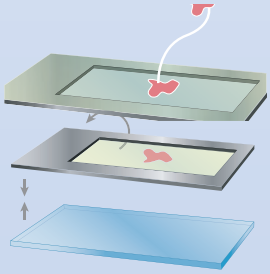
- High power laser for microdissection of harder material, e. g. plants, bone (marrow), teeth, forensic tapes
- MMI CellEctor Plus for single cell sorting
- MMI CellManipulator Plus optical tweezers
- Range of imaging configurations (Fluorescence, DIC, Phase contrast)
- Others on request

Compatibility:

- Nikon Ti-S, Ti-E, Ni-U, Ni-E, A-1
- Olympus IX73, IX83, FV1000
- Zeiss Axio Observer, LSM 780
- Leica on request
- Others on request

Technology

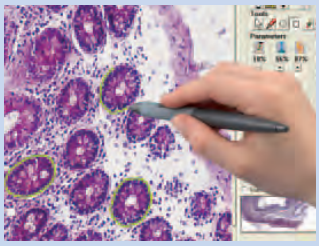
1. Sample preparation



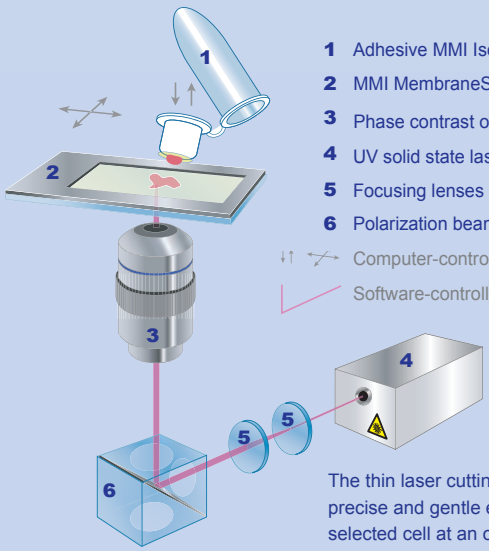
The section is placed on the MMI MembraneSlide, a frame slide covered with a thin membrane that is inert and has negligible auto fluorescence. Afterwards the MMI MembraneSlide is inverted and placed onto a glass slide for protection against contamination. Now the sample is sandwiched between the membrane and the glass.

2. Easy cell selection

The cells of interest can be selected on the display using either the mouse, by freehand or predefined geometrical shapes which can be modified. Any number of cells across the slide can be identified as targets within one screening process. The stage is moving to trace the path drawn and the laser is fixed and focused from below.



3. Automated laser cutting

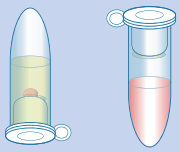


1 Adhesive MMI IsolationCap
2 MMI MembraneSlide and glass slide
3 Phase contrast objectives
4 UV solid state laser
5 Focusing lenses
6 Polarization beam combiner
Computer-controlled movements
Software-controlled laser focus and energy

The thin laser cutting path enables a precise and gentle extraction of the selected cell at an outstanding speed. The isolated target cell is collected by lowering and lifting of the adhesive cap held from above. The sample morphology remains 100% intact.

4. Selected target

After cutting the sample can be visualised on the cap. Lysis buffer is added and the tube inverted for approx. 10 mins. The cells are now in suspension ready for downstream processing.



MMI CellCut Plus: Features & Benefits

Contamination free cutting

Sample sandwiched between a membrane and glass so it is not exposed to the air. This ensures a safer working environment and better sample integrity.

Maximum collection efficiency

Passive collection of samples using isolation cap for maximum collection efficiency and minimal sample damage.

Contamination free collection

MMI Isolation cap is only in contact with the membrane and never in direct contact with the tissue.

The thinnest cleanest cut

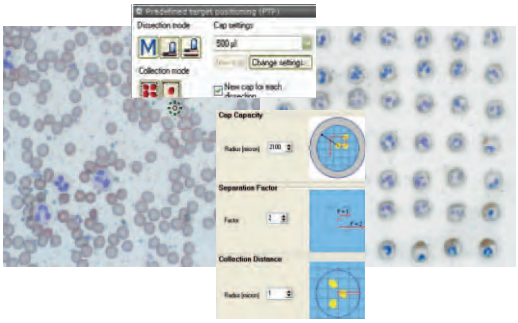
Ultra precise high pulse rate, low power fixed UV laser for the thinnest cleanest cut of all commercially available laser microdissection systems. Cuts as narrow as 0.3 microns for accurate and precise cutting everytime.

Positive sample inspection

This feature ensures you have collected your sample after every laser cut. The isolation of the target area occurs in the same relative position as on the slide. So the morphology of the sample remains 100% intact and damages of the surrounding tissue will be prevented. Reproducible results are achieved easily and effectively. This is excellent for auditability and accurate quantitative results.

Predefined Target Positioning (PTP)

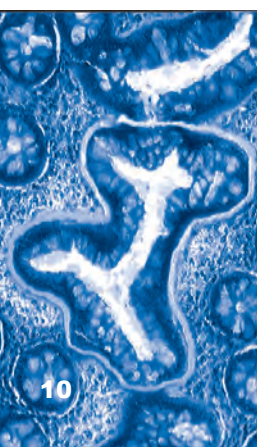
PTP is a patented product feature that allows for the precise, predefined, contamination free visually controlled collection of individual cells on the cap. PTP is excellent to maximize the number of samples collected into a single reaction tube, guarantees isolated samples are not compromised by subsequent cutting and essential for auditability.

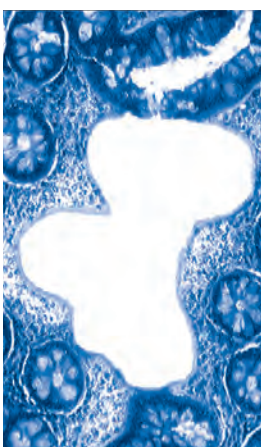


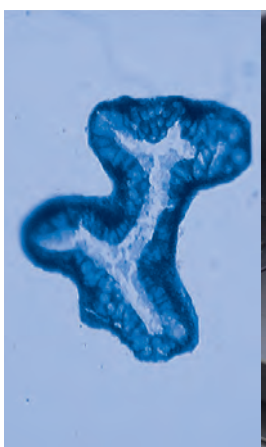
Firstly an overview of the entire sample is produced. Secondly visual inspection and selection of the target cells is performed. This step can be fully automated, using the MMI CellExplorer Software. Switch to PTP mode and cells are dissected and collected on the adhesive cap in a spiral pattern starting from the centre of the cap.

MMI MultiCap and MMI MultiSlide

MMI MultiSlide for the isolation of single cells from up to three slides and the MMI MultiCap for isolation of single cells into up to eight individual caps without the need to reload. Essential for single cell workflow involving RNA isolation, where speed is of the essence.







Live cell dissection from specially designed MMI LiveCell Chambers for the isolation of cells in living culture

Autodocumentation

The only laser microdissection system that allows full auditability of every cut. The autodocumentation feature records images showing the position of the cut and of the collection (PTP) on the cap together with the time, date and method details.

Serial section

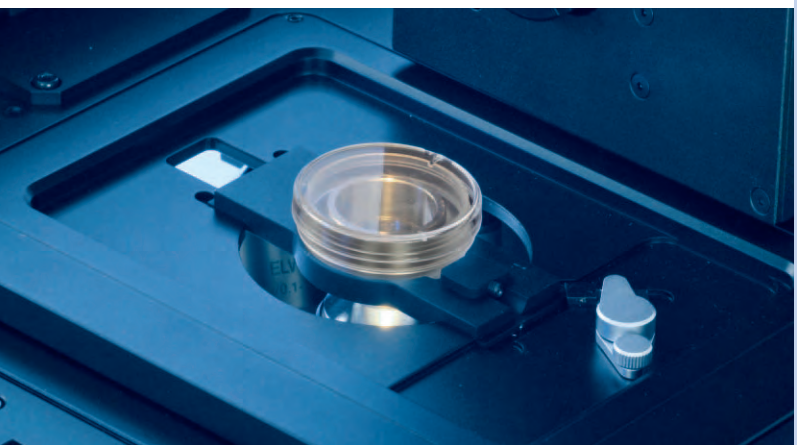
Maximise RNA recovery by minimising the use of staining. Use the visible position of target cells on a stained section to detect, mark, cut and isolate regions of interest on an unstained section.

Z-drill

Cut thick and wet tissue without the use of a high power laser by the z-drill feature. Refocus the laser on different z-levels whilst cutting to minimise the use of excessive laser power and so preserve your sample and maximize its usability for downstream analysis. An alternative high power laser is available for working with harder to cut material such as teeth, bones, or forensic tapes.

MMI LiveCell Chamber

Special MMI LiveCell Chamber for positive and negative isolation of live cells for removal of contamination and isolation of target cells for unrivalled re-culturing and cloning.

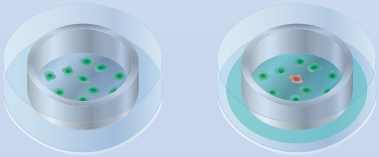


In vitro cultures

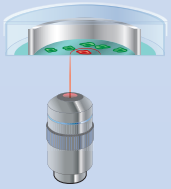
The isolation and enrichment of individual live cells for culture and differentiation experiments, or for their proteomic and genomic analysis is of increasing interest in stem cell research, cancer research and tissue engineering.

The MMI CellCut Plus in combination with the approved MMI LiveCell Chamber enables contamination free isolation of live cells in living culture. Enzyme treatments (i.e. trypsin), other potentially harmful selective reagents as well as time consuming repetitive enrichments can be avoided. The benefit is an increase in reliability, effectiveness and accuracy of your research.

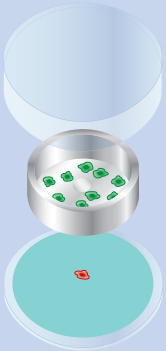
The workflow



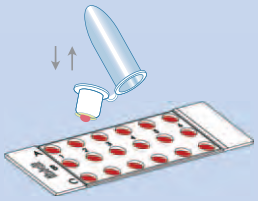
1. Seed and cultivate cells to the desired density on the membrane surface of the metal culture ring. Visualise cells of interest (e.g. phase contrast, immuno labeling, etc.). Transfer the membrane ring with cells to the adhesive area of the microdissection chamber and place it on the microscope stage.



2. The cells of interest are selected and cut via laser microdissection. Unwanted cells can be destroyed or ablated by individual laser shots.



3. After removing the cell chamber the cells of interest remain either on the ring or in the cell chamber. Sufficient medium is added and either set of cells can be re-cultivated.



4. The use of the MMI IsolationCap technology together with 18-well IBIDI Slides enables an higher throughput for working with live cells as well as an improved sectional view and increased cutting efficiency.

MMI LiveCell Chamber Prod. No. 50301

Components of the MMI LiveCell Chamber are a membrane ring for the initial cultivation of cells, a cell culture dish to house the membrane ring with seeded cells, and a UV-permeable microdissection chamber. All components can be sterilized ready to use.

Leading the way to life science research and clinical applications

Applications

Laser microdissection is a widely used technology in life sciences research and for clinical applications for the accurate dissection of diseased cells from biopsy material. Thus, it is an essential tool for early diagnosis of cancers and neurological disorders, and it is established as a step towards personalised medicine.

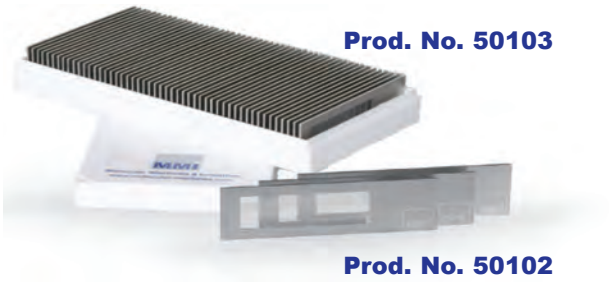
The main advantage of laser microdissection over other methods lies in the unsurpassed high precision and visual control of the selection and isolation process.

The MMI CellCut Plus is used for a wide range of applications in the following fields:

- Cancer research and oncology
- Stem cell research
- Neuroscience
- Immunology
- Forensic Science

Consumables & Accessories

MMI MembraneSlides



Prod. No. 50103

Prod. No. 50102

The 26 mm x 76 mm metal frame is coated with a 1.5 µm thick PEN membrane. You can choose between standard MMI MembraneSlides that are packed into a slide rack (Prod. No. 50103). Or, If you need the highest standards of purity the MMI MembraneSlides are available RNAse and nucleic acid free (Prod. No. 50102). They are packaged into boxes of five for better contamination control. Every sales unit comes with 50 MMI MembraneSlides in total.

MMI IsolationCaps

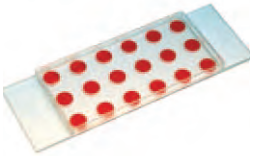
Transparent caps
Prod. No.
50208 (0.2 ml)
50204 (0.5 ml)
50212 (1.5 ml)

Diffuser caps
Prod. No.
50206 (0.2 ml)
50202 (0.5 ml)
50210 (1.5 ml)

For the isolation and collection of the target cells from the MMI MembraneSlide. Available in three sizes (0.2 ml, 0.5 ml and 1.5 ml) and two types (either filled with nontransparent silicone, diffused, or with transparent silicone). Diffuser caps are recommended for stained or coloured samples. Transparent caps are used for fluorescent applications and recommended for working with the MMI SmartCut Plus. Available in boxes of 50 pieces.

MMI SupportSlide
Prod. No. 50105

To support the transfer process from a cryo section to a membrane slide. The MMI SupportSlide is designed to give rigidity to the membrane during sample preparation. It also acts as a heat sink to aid reproducibility and ensure you correctly place the sample on the correct side of the membrane.

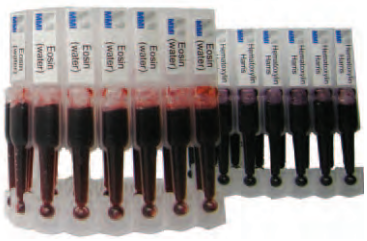


18-well IBIDI® Slide
Prod. No. 50109

If you are working with small cell populations an alternative to the MMI LiveCell Chamber is the 18-well membrane IBIDI slide. The cells are cultivated within the wells. Afterwards, the slide is turned upside down and placed on the stage. The cells can now be cut and isolated with a standard MMI IsolationCap (0.2 ml recommended). If stilled filled with liquid the MMI CellEctor Plus can, instead, be used to transfer the cells directly from the wells.

The MMI CellCut Plus with the MMI CellTools Software for easy and convenient laser microdissection is widely used in traditional research, for cancer and personalized medicine

MMI H&E Staining Kit Plus
Prod. No.: 70302



Designed for users who need to quickly stain only a few samples and need to ensure that the result is clean, clear of RNAse and contamination free. Allows quick handling without the need for pipetting or the preparation of staining jars. Guarantees a uniform drop size, and ensures the solutions remain contamination free. Each kit contains 2x15 MMI SafeStain ampoules, designed for 30-60 stainings. Ampoules are designed for the staining of 2-4 slides.

MMI 8-CapStrip (0.2 ml per cap)
Prod. No.
50214 (transparent)
50213 (with diffuser)

Used together with the MMI Multi CapLift for the collection of cells in up to 8 different caps simultaneously. A box per type contains 5 strips.

MMI LiveCell Chamber
Prod. No. 50301

Cells can be cultivated in a membrane coated metal ring. Unwanted cells can be shot, cut out, or be left on the membrane. Afterwards, the cells of interest can be recultivated.

MMI CellCut Plus Starter Kit
Prod. No. 70304

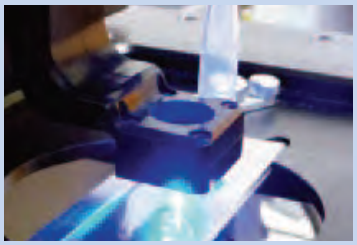
Recommended for first application use after instrument purchase. Contains all available consumables for MMI CellCut Plus.

From user, for user: workflow example

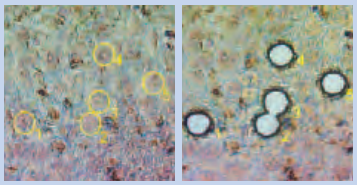
"[...] One of the projects that we have been working on is a study of amyotrophic lateral sclerosis (ALS), a neurodegenerative disorder characterized by loss of motor neurons resulting in progressive paralysis. [...]The ALS study clearly demonstrates the feasibility of using the MMI LCM to perfectly capture single cells consistently during the course of a project that may last for several years."

Erik Cabuy MEng, MSc, PhD
Single Cell Genomics
Friedrich Miescher Institute
for Biomedical Research
Basel, Switzerland

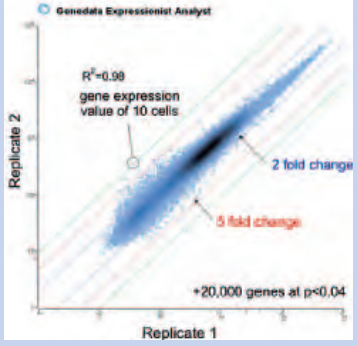
1. Frozen brain embbeded in Tissue-TEK on a Cryostat ready for sectioning.



2. A single dehydrated brain tissue section mounted on top of a MMI MembraneSlide.



3. View onto the MMI MembraneSlide: before and after capturing of single cells.



4. Experimental results of microarray data representing the gene expression profiles of two replicates containing each 10 laser-dissected neurons.

For the whole customer report please have a look on pages 4 and 5 of the MMI Bibliography Vol3 (Issue on Laser Microdissection for Transcriptomics).

Manipulation of single cells by optical trapping

MMI CellManipulator Plus

The MMI CellManipulator Plus is a powerful optical multibeam tweezers system based on the mechanical forces arising from a strongly focused laser beam. It enables comfortable, ultra-precise and contact-free manipulation of microscopic particles, single or living cells, or subcellular organisms and the measurement of intracellular activities.

Thus, it can hold, move, rotate, join, separate, stretch or otherwise manipulate up to 2x10 microscopic objects simultaneously or separately in three dimensions. The wavelength of the laser does not interfere with the integrity of living specimens.

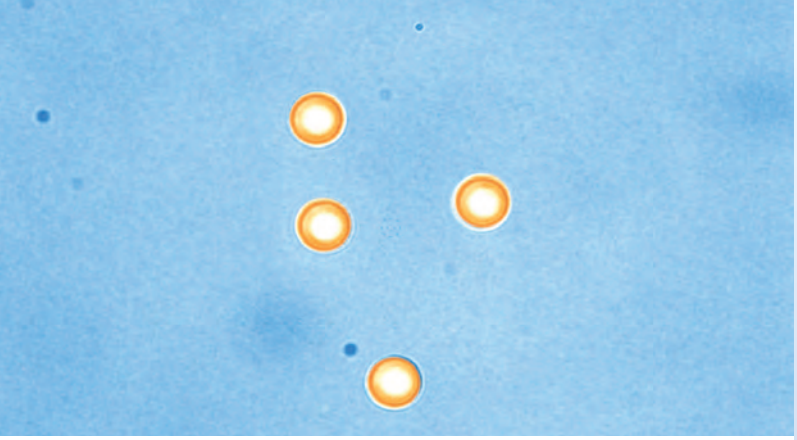
Cell sorting and cell positioning can also be accomplished together with position detection enabling the measurement of binding forces or viscosities at sub cellular level. The use of ultra sensitive quadrant detectors allows to sense extreme low forces down to 0.2pN without compromising in speed. The detector can be used in imaging mode or is also available as back focal plane version allowing the user to adapt the sensor always to his experimental needs.

Due to multiple ports and dual-level laser integration, the seamless use of different modules and imaging technologies is possible.

Features & Benefits

- **Strongest trap** with > 800 pico Newton
- Excellent **longterm stability**
- One or two tweezer levels for **up to 2 x 10 traps**
- **Full control** of laser power and focus
- **Ultra-precise positioning** of each single trap
- **High modularity** for a wide range of applications
- **Extremely compact** laser box and controller
- **Force detection** 0.2 - 800 pico Newton
- **Fully automated** data acquisition and live data displays
- Up-to-date **laser safety** concepts

Our powerful optical multibeam tweezers system for cell manipulation, extremely customizable for a wide range of applications: MMI CellManipulator Plus



Comfortable, ultra-precise and contact-free manipulation of cells or other microscopic particles

Setup

- Based on an inverted or upright manual or motorized high performance research microscope
- High Power fibre laser with 8W maximum power, excellent longterm stability, and a wavelength of 1070 nm
- Second level tweezers up to 2 x 10 traps
- High-resolution MMI CellCamera or EMCCD Andor Camera
- Modular and upgradeable workstation
- MMI CellTools Software for simple creation, organization, and manipulation of traps; full control of laser power, focus, image capture, and scanning stage actions for ultra-precise positioning
- Interactive LED pen display
- Anti-vibration table

Expandability:

- MBPS quadrant detector for ultra-precise position detection, used for force calculations
- High precision XYZ Piezo scanning stage
- High end imaging (Fluorescence or Bright field scanning, Confocal, TIRF)
- RAMAN Spectoscropy
- Spinning Disc
- MMI CellCut Plus for laser microdissection
- MMI CellEctor Plus for single cell sorting
- MMI CellExplorer cell recognition software
- Customized adapations to individual needs

Compatibility:

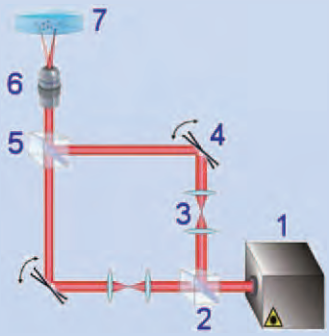
- Nikon Ti-S, Ti-E, Ni-U, Ni-E, A-1
- Olympus IX73, IX83, FV1000
- Zeiss Axio Observer, LSM 780
- Leica on request
- Others on request

Technology

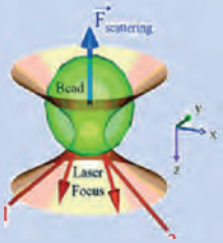
Optical Tweezers are capable of manipulating micrometer-sized dielectric particles, living cells, or subcellular organisms by exerting pico Newton forces via a highly focused laser beam. The beam is focused by sending it through a microscope objective. The narrowest point of the focused beam, known as the beam waist, contains a very strong light gradient. Dielectric particles are attracted along the gradient to the region of brightest light, the center of the beam. Using an infrared laser an invisible optical trap is created.

The principle of MMI CellManipulator Plus:

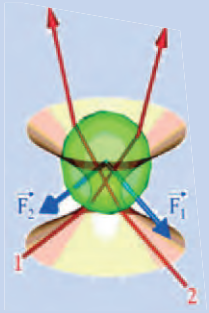
1 Ytterbium fiber laser 1070 nm, 8 Watt
2 Beam splitter (second level)
3 Two focusing lenses
4 Two galvo scanners 2 kHz
5 Polarization beam combiner
6 Objective with high NA
7 Cells, particles in solution



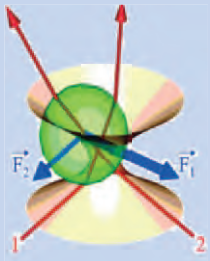
3D ray optics model is illustrating the scattering and the gradient force:



A: Scattering force: The reflection of rays produces momentum in the opposite direction, resulting in a net force along the direction of laser propagation.



B: Gradient force: When the bead or cell is not in the beam's center, the larger momentum change of the more intense rays causes a net force that pulls the bead back towards the center of the trap.



C: Gradient force: When the bead or cell is literally centered in the beam, the net force points toward the focal point of the beam.

Single cell manipulation: A wide range of applications

Applications

Optical Tweezers are highly accurate instruments. When combined with the extremely sensitive quadrant detector technology, they are capable of the manipulation and detection of sub-nanometer displacements for sub-micrometer dielectric particles.

Thus, they are often used to manipulate and study single molecules such as DNA, proteins, and enzyme interactions. The MMI CellManipulator Plus is the only tool of its kind to offer customized and complete solutions in the following areas.

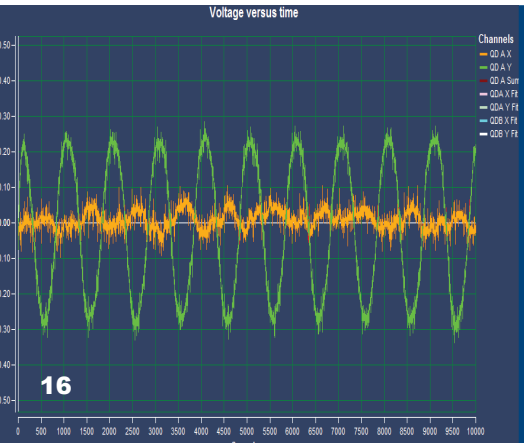
Cell-based studies:
- Cell fusions and cell-to-cell interactions
- Implant studies
- Intracellular manipulations
- Study of neuronal networks
- Drug effects on cells
- Ca2+-channel studies

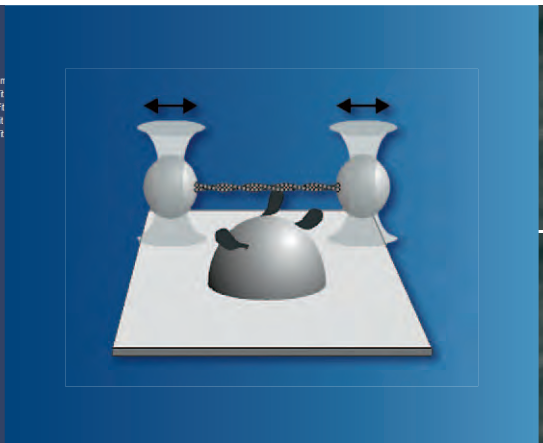
Measurements of Binding Forces:
- DNA studies
- Viscosity measurements
- Antibody, antigen binding forces
- Bacterial adhesion studies
- Virus to cell adhesion studies
- Protein Folding Forces
- Microrheology Experiments
- Interactions Experiments

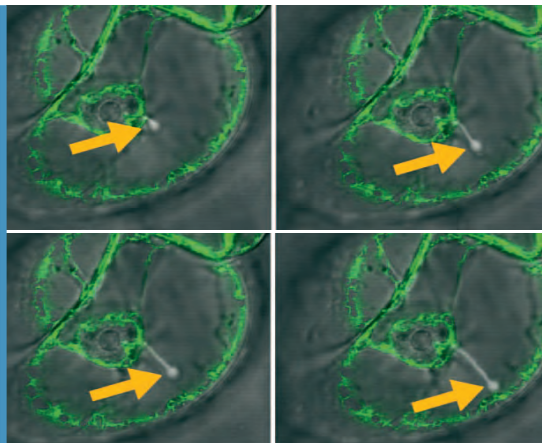
Molecular Motor Studies:
- Actin, Myosin interactions
- Kinesin Motors
- Dynein Motors

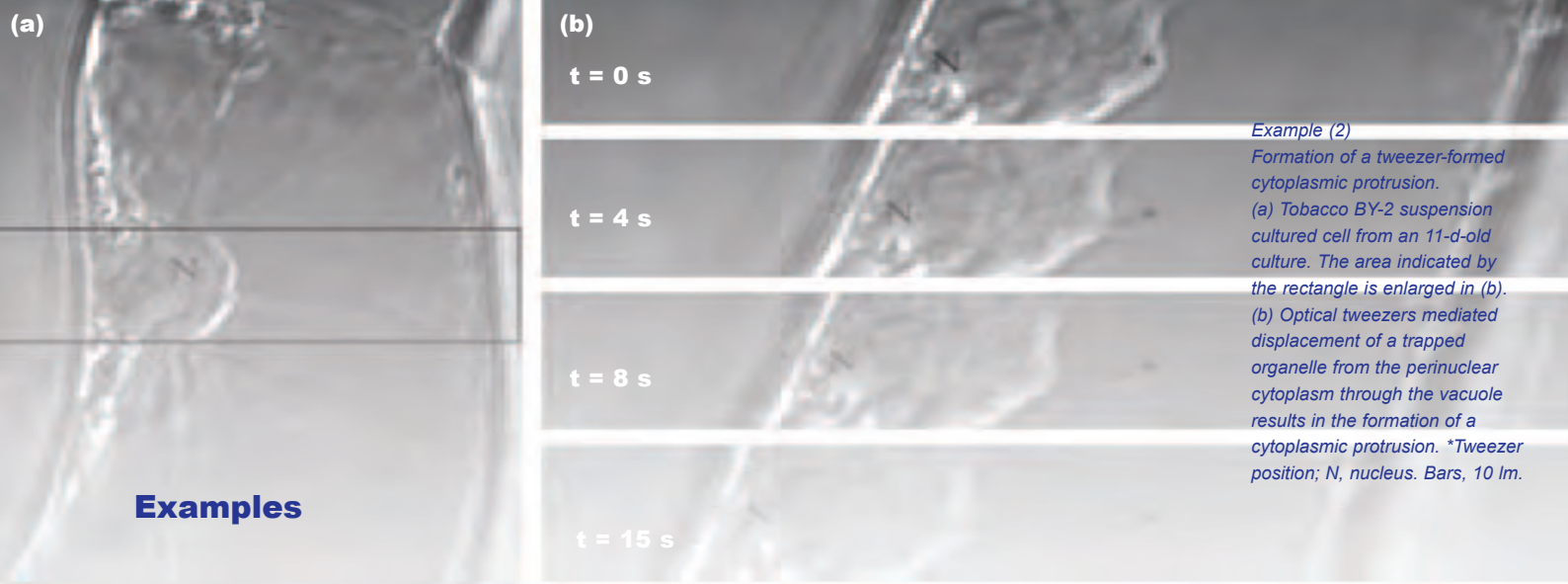
Laser Raman Tweezers:
- Identification and isolation of cells and single living micro-organisms
- Cancer Research

Lab-on-a-Chip Device:
- Biosensor Assays
- Single cell isolation and sorting









*Example (2) Formation of a tweezer-formed cytoplasmic protrusion. (a) Tobacco BY-2 suspension cultured cell from an 11-d-old culture. The area indicated by the rectangle is enlarged in (b). (b) Optical tweezers mediated displacement of a trapped organelle from the perinuclear cytoplasm through the vacuole results in the formation of a cytoplasmic protrusion. *Tweezer position; N, nucleus. Bars, 10 lm.*

Examples

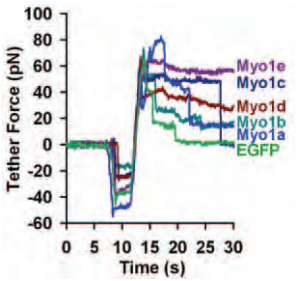
(1) Control of cell membrane tension by myosin-I

Department of Cell and Developmental Biology, Vanderbilt University Medical Center, Nashville, TN 37205

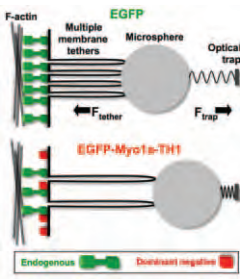
All cell functions that involve membrane deformation or a change in cell shape (e.g., endocytosis, exocytosis, cell motility, and cytokinesis) are regulated by membrane tension. While molecular contacts between the plasma membrane and the underlying actin cytoskeleton are known to make significant contributions to membrane tension, little is known about the molecules that mediate these interactions.

We used an optical trap to directly probe the molecular determinants of membrane tension in isolated organelles and in living cells. Here, we show that class I myosins, a family of membrane-binding, actin-based motor proteins, mediate membrane/cytoskeleton adhesion and thus, make major contributions to membrane tension.

These studies show that class I myosins directly control the mechanical properties of the cell membrane; they also position these motor proteins as master regulators of cellular events involving membrane deformation.



Representative tether force records for NIH 3T3 fibroblasts expressing EGFP (green) or 1 of 5 different EGFP-tagged class I myosins (as labeled on the plot).



Model of the mechanism underlying the formation of multiple tethers in cells expressing EGFP (negative control) or EGFP-Myo1a-TH1 (dominant negative). These cartoons represent "snap shots", taken at the beginning of the tethered phases at the time point indicated by the black arrows.

(2) Actin and myosin regulate cytoplasm stiffness in plant cells: a study using optical tweezers

Laboratory of plant cell biology, Wageningen University, The Netherlands

Here, we produced cytoplasmic protrusions with optical tweezers in mature BY-2 suspension cultured cells to study the parameters involved in the movement of actin filaments during changes in cytoplasmic organization and to determine whether stiffness is an actin-related property of plant cytoplasm.

Optical tweezers were used to create cytoplasmic protrusions resembling cytoplasmic strands. Simultaneously, the behavior of the actin cytoskeleton was imaged.

After actin filament depolymerization, less force was needed to create cytoplasmic protrusions. During treatment with the myosin ATPase inhibitor 2,3-butanedione monoxime, more trapping force was needed to create and maintain cytoplasmic protrusions. Thus, the presence of actin filaments and, even more so, the deactivation of a 2,3-butanedione monoxime-sensitive factor, probably myosin, stiffens the cytoplasm.

During 2,3-butanedione monoxime treament, none of the tweezer-formed protrusions contained filamentous actin, showing that a 2,3-butanedione monoxime-sensitive factor, probably myosin, is responsible for the movement of actin filaments, and implying that myosin serves as a static crosslinker of actin filaments when its motor function is inhibited. The presence of actin filaments does not delay the collapse of cytoplasmic protrusions after tweezer release.

Myosin-based reorganisation of the existing actin cytoskeleton could be the basis for new cytoplasmic strand formation, and thus the production of an organised cytoarchitecture.

Development, software, service and application support

Combined solutions for integrated workflows

MMI's fully modular platforms can be used either alone or in combination with other technologies or micromanipulation tools. As an example, the MMI CellEctor Plus can be combined with the MMI CellCut Plus laser microdissection instrument or the MMI CellManipulator Plus optical tweezers. This creates unique flexibility for a wide range of applications and for the development of integrated workflows for single cell isolation.

MMI CellTools software for full on screen control

All MMI instruments come with the MMI CellTools software package which generates a live view of the entire sample. The MMI MultiSlide function allows the navigation on up to three slides. High quality components such as the MMI CellCamera ensure high resolution and real time application success when working with delicate sample material.

The MMI CellTools software and corresponding Plug-Ins provide full control of the system. Adjusting xy-stage, camera, laser and other parameters is easy and convenient. Even the automated microscope functions such as objective or fluorescence turret changes can quickly be initiated directly from the software making full remote control possible.

MMI CellExplorer cell recognition software

The MMI CellExplorer is the only cell recognition software package specifically designed for working with single or rare cells. It has chiefly been developed to find, count, sort and measure microscopic objects. The recognition process first screen using colour, either brightfield or in fluorescence. Fine tuning is then achieved using a range of morphological related factors.

Round shapes (undifferentiated cells), for instance, can be automatically distinguished from oval forms (differentiated cells). Objects lying adjacent to each other are automatically separated and counted. Finally, the found object shapes can automatically be optimized for further application steps, for example laser cutting.

A carefully selected and application adapted set of functionalities guarantees a high find throughput with comfortable level of automation. Nevertheless, a high degree of flexibility is supported.

The MMI CellExplorer software comprises complex image pre-processing that can be performed prior to object finding. For this purpose many different filters are available such as image sharpness, color intensity, brightness, background separation or gamma corrections.

The MMI CellCut Plus laser microdissection instrument, MMI CellEctor Plus single cell sorting solution, and MMI CellManipulator Plus optical tweezers combined in one system

The MMI software packages are intuitive and easy-to-use

Consider the benefits of service contracts, regular upgrades and application trainings to ensure you always get the best from your instrument and longterm continuous use

Service contracts, upgrades, trainings

We recommend all customers consider the benefits of service contracts, regular upgrades and application trainings to ensure you always get the best from your instrument and longterm continuous use.

Keep your system running smoothly, reliably and ready for use: Choose between different service contracts over a time frame to fit your needs. They can include annual preventive maintenance by one of our service engineers and emergency visits per year. Please contact MMI or your local distributor for availability and options in your country.

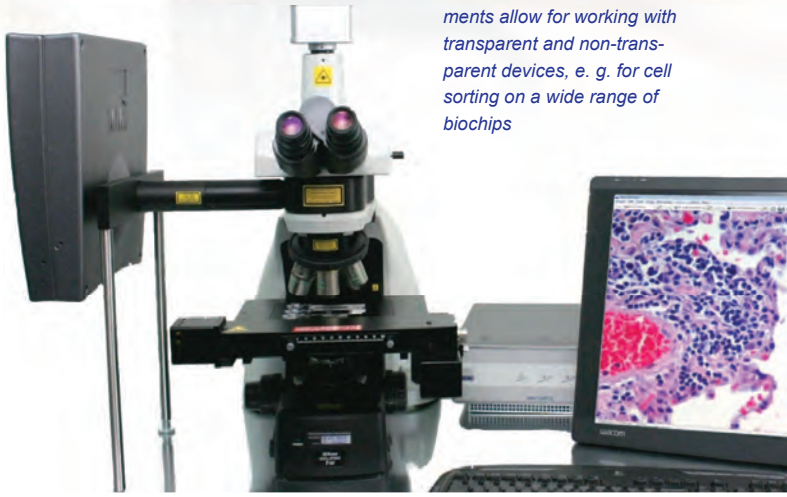
Stay up-to-date with new developments and upgrades: We regularly provide various upgrades for all our instrument platforms, for example a more powerful workstation with interactive pen display, a new laser or camera, the latest software version with new features and benefits.

Ensure trouble-free daily operation: We also offer application support and instrument trainings either in-house or on-site with your own equipment.

Remote support via TeamViewer

Fast, easy and cost effective troubleshooting and software updates: allows our service engineers to remotely control your system, worldwide. All you need is the TeamViewer Software and an internet connection. You can easily download the MMI Customer TeamViewer from our website. Please call us after the download.

Cell sorting on non-transparent biochips



Based on an upright microscope, these MMI instruments allow for working with transparent and non-transparent devices, e. g. for cell sorting on a wide range of biochips

All standard MMI systems are based on inverted research microscopes. However, advances in single cell analysis have seen a shift towards the use of a wide range of non-transparent sample platforms (ie microfluidic devices & chip based systems). Utilisation of an upright microscope platform has enabled us to now develop the first upright micromanipulation systems on the market. Currently, both the MMI CellEctor Plus for single Cell sorting and the MMI CellManipulator Plus optical tweezers have been designed to meet the specific needs of customers wanting to work with non-conventional devices.

MMI systems are compatible with the Nikon Eclipse Ni-E and Ni-U. These instruments are the best choice when working with microfludic or chip based cell systems where the manipulation of cells in or out of non-transparent test systems is required. We have commercial customers using these systems as an intermediate step for the production of pure cell populations. These upright systems incorporate all the key features of a standard inverted system, utilizing reflected and transmitted light making it possible to work with both transparent and non-transparent devices.

Please note that some illustrated products or features might be unavailable in some countries. Please contact MMI or your local distributor for availability and options in your country.

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