



Molecular Machines & Industries

Laser Microdissection System mmi SmartCut Plus



LEAD THE WAY FOR LCM REDUCED TO THE MAX

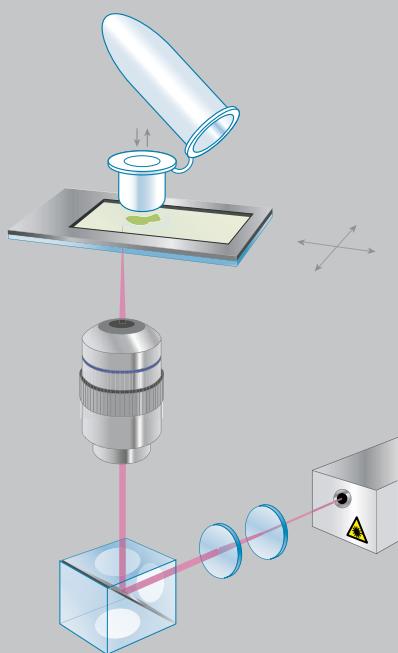
Nature provides us with a huge variety of precise and fast dissection systems. For example the Leaf Cutter Bee - the first bee seen in early spring. She darts to a suitable tree, and within two minutes, selects a leaf, cuts a hole out, and then quietly departs.

MMI Switzerland developed and optimized solid state laser technology for the ultra-precise cutting of samples from tissue and live cell cultures. Today, this technology is emerging as a prime technology for the rapid isolation of single cells or multicellular structures.

Hereby, we introduce our latest & smartest innovation: the mmi SmartCut Plus system - a compact and high-precision laser capture microdissection system (LCM) that will impress with it's fast and easy handling and not least with it's cost effectiveness.



A Principle of microdissection Fast, precise and contamination-free



Computer-controlled movement



CellTools software-controlled laser focus and energy

AT THE CUTTING EDGE

The mmi SmartCut Plus system consists of a comfortable inverted microscope with a motorized scanning stage, a solid state laser with a requisite laser beam delivery, transfer optics and a high-end computer which runs under MS Windows. The system is operated via MMI's especially developed software mmi CellTools, which controls the laser, image capture, all scanning stage actions and automated mmi CapLift options.

A In microdissection a "live view" image of a microscopic sample is displayed on the monitor, allowing the user to easily identify and mark the target area e.g. a cell or a group of cells. During microdissection, the UV laser is focused by the objective onto the microscopic sample and remains aligned at the centre of the optical axis whilst the high-precision motorized stage is used to accurately move the sample.

High speed and ultra fine laser microdissection is achieved via

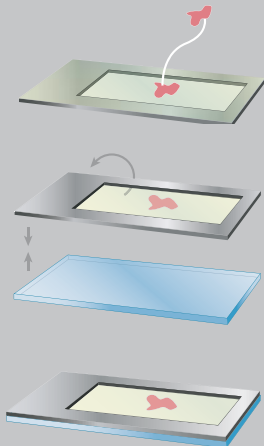
- A maintenance free UV solid state laser ✓
- Optical laser focusing to a cutting line $< 1 \mu\text{m}$ ✓
- Short laser pulse duration in picoseconds and high repetition rate of $>5 \text{ kHz}$ ✓

Furthermore the very low laser pulse energy ($<1 \mu\text{joule}$), the mmi SmartCut's Plus "cold ablation" process leaves the target material unaffected with no impact on subsequent protein analysis, DNA or RNA extraction. Even live cells can be isolated and cultured after microdissection. And the unique and patented mmi CapLift technology provides the contamination-free and targeted collection of microdissected sample material.

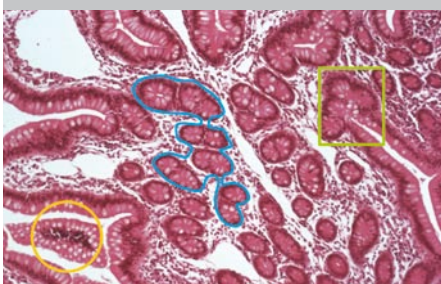
The mmi SmartCut Plus is easy and convenient to handle, requires no extensive technical training and does even fit under a laminar flow hood. The mmi SmartCut Plus is especially developed for all routine applications and researchers in cell biology, molecular pathology, forensic medicine prenatal diagnostics and many other fields will greatly benefit from it's unique features.

SAMPLE PREPARATION CUTTING & RECOVERY

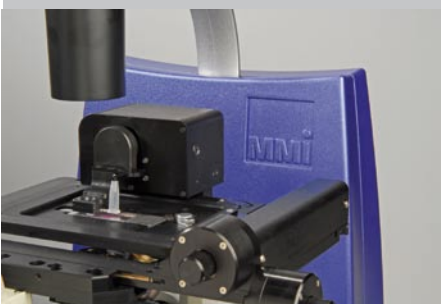
B Sample preparation On frame slide with membrane



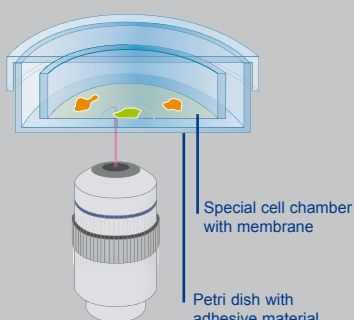
C Easy cell selection



D automatic mmi CapLift



E Excision of live cells



Unique contamination-free sample preparation

B MMI's unique sample preparation technique effectively protects the sample by forming a complete barrier to contaminants, such as environmental impurities, which is essential for laser microdissection steps. To isolate targets from any source, including cryo or paraffin-preserved tissues, smears and cytopins, the mmi SmartCut Plus uses a metal frame slide with a 1.4 μm PET-membrane. The different types of samples are placed directly on the membrane, which is then inverted and a standard glass slide placed below. In order to optimise the preparation of delicate samples, standard protocols – such as the use of poly-L-lysine coating or UV exposure to increase cell adhesion – can still be used. Thus, the sample is ready for the subsequent, highly pure isolation via the adhesive cap of the mmi IsolationCap.

Selection and cutting

C Targets of interest to be extracted are selected on-screen using either free-hand or predefined geometric shapes, such as circles, squares and ellipses. Any number of areas across the entire section can be identified and the sizes of the geometric shapes can be changed as well as copied and pasted for consistency. A group function allows the user to collect an unlimited number of different cells or cell compartments within one screening process in different microtube caps. The $<1 \mu\text{m}$ cutting line enables the precise excision of the selected targets at an outstanding speed, without affecting the target. As a result, there is no loss in quality of the material used downstream. Even the viability of living cells is not affected and therefore they can be re-cultured once selected.

With such fine control over cutting, mmi SmartCut Plus can be used on a wide range of sources, making it a highly flexible tool for most laboratories. Target cells are extracted without any damage to their proteins, DNA or RNA, and as a result, downstream analyses can be carried out with total confidence.

Target recovery with the automatic mmi CapLift technology

D The unique mmi CapLift technology has been developed to maintain the ease of use and contamination-free nature of the mmi SmartCut Plus system. Sample uptake after microdissection is carried out automatically with the newly designed single mmi CapLift technology. The mmi IsolationCap is centred to the optical axis of the system to enable user-friendly collection of the selected targets. With the cap kept centred in the optical axis, the motorised stage is moved to enable collection of target cells from across the entire slide. This plug in module does not only allow for a targeted and traceable sample uptake of dissected samples it even allows to adjust the cap contact pressure for sample uptake.

Isolated targets, placed on the cap of the mmi IsolationCap, are ready for subsequent downstream processes such as DNA and RNA extraction, as well as protein analysis. Isolation buffer is added and the tube closed for centrifugation to keep the recovery process as short and delicate as possible. This unique feature ensures full control of the work flow and thus provides safety and reliability for tricky application e.g. single cell analysis

A Lens offset function ensures convenient orientation on the slide after changing the objective as the selected area stays centered.

Live cell culture microdissection in our mmi CellChambers

E Using a mmi CellChamber, the mmi SmartCut Plus can be used to dissect living cells under sterile conditions. The cells grow in the chamber on a membrane coated with poly-L-lysine and, prior to microdissection, are placed in a Petri dish with an adhesive base. Both parts can be sterilised. After microdissection, a different recovery method is used. Here the culture chamber is removed from the Petri dish under aseptic conditions, leaving the isolated cells behind.

mmi SMARTCUT PLUS OPTIONS

F PenScreen
For on-screen target identification



G mmi CellExplorer
For cell identification



H manual mmi CapLift



I Toolbar and navigation overview



mmi customized features

The mmi SmartCut Plus can be customized with the following choice of features:

F A PenScreen, which enables the selection of target cells and components directly on a touch-sensitive screen using a special pen. This allows the user much greater accuracy in selection, especially for free-form shapes.

G The mmi SmartCut Plus is also available with the mmi CellExplorer, a cell identification software based on colour recognition, which automatically identifies and cuts-out cells,

H Some application may require the work with our manually adjustable mmi CapLift. Isolated targets, placed on the cap of the microtube, are ready for subsequent downstream processes such as DNA and RNA extraction, as well as protein analysis. Isolation buffer is added and the tube closed for centrifugation to keep the recovery process as short and delicate as possible.

INTUITIVE CONTROL

The mmi CellTools software is used to control the mmi SmartCut Plus system. Its graphical user interface allows precise and intuitive identification of the target areas to be excised for the subsequent isolation processes. It also provides full control over the laser cutting parameters, the objective used and specific camera settings in respect of different application-specific requirements. Moreover, all system functions are structured in a way to make them self-explanatory, providing easy operation without any sophisticated technical knowledge or training. Some examples are given below.

Overview and easy navigation

I Using the 4x objective, an overview scan of the entire microscope slide can be made, allowing the user to operate and navigate the system more easily

Cutting laser control

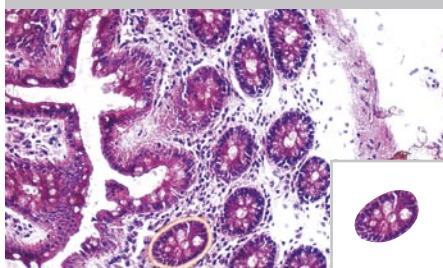
To maximise the laser microdissection process, all laser parameters, such as speed, focus and energy, can be changed to match the individual needs of the application, sample and objective. Once set, the parameters are stored for each process and can be easily recalled for use or editing

Autodocumentation

All the data relevant to a session are stored via the autodocumentation mode. This includes the number and size of the cut-out areas, as well as instrument settings and parameters. The isolated targets are documented by the mmi SmartCut Plus system during the cutting and isolation process, and pictures of the remaining sample are automatically acquired and saved – all with multi-user access. This enhances traceability and is proof of a clear, clean and quick isolation process.

ONE TOOL: VARIOUS APPLICATIONS

J Intestinal gland isolated from transverse tissue sections of colon



K Single sperm isolated from a gynaecological smear



L Cell culture in phase contrast observation



mmi SmartCut Plus is an extremely versatile instrument, across different applications. Whether you are isolating specific cells from an entire tissue, maintaining a pluripotent stem cell line, or extracting a telling foreign cell for forensic investigation, mmi SmartCut performs perfectly.

Pathological, haematological and cytological samples

J Today, targeting specific cells in healthy as well as in diseased tissues is of particular importance in elucidating the molecular mechanisms that lead to cancer and other life threatening diseases. The group of Tomlins et al. published recently a molecular concept of prostate cancer progression after laser microdissection and individual profiling of 101 specific cell populations with the mmi CellCut, (Ref.1), further Buckanovich et al. used Immuno-LCM for the expression profiling of cell populations in the tumor microenvironment of human ovarian cancer (Ref.2.) Figure J showed a colon tissue section stained with hematoxylin/eosin where the insert displays intestinal glands from this section after microdissection. The easily dissected area is now ready for any downstream analysis such as molecular profiling for proteomics or genomics means.

Ref. 1 Tomlins, S.A. et al. NATURE GENETICS 39, 41-48 (2007)

Ref. 2 Buckanovich, RJ et al. CANCER BIOLOGY & THERAPY, 5:6 635-642 (2006)

Forensic investigations

K The mmi SmartCut Plus system is an extremely useful tool in forensic medicine, since it can be used, for example, to isolate a single sperm from a vaginal smear for genetic analysis, leading to traceable DNA fingerprints. In a recent work published by Anslinger et al. the sex-specific isolation of cells from mixtures by means of combined in-situ hybridization and single cell isolation via laser microdissection has been judged as a tool that will greatly facilitate forensic work.

Ref. 3 Anslinger, K. et al. INT J LEG MED 121 (1), 54-56 (2007)

Live cells

L Most stem cell lines are presently grown at high densities on mouse fibroblast "feeder layers". Therefore, isolating specific pluripotent cells from the surrounding fibroblasts and differentiating cells needs to be rapid, exact and easy. mmi SmartCut Plus offers you the perfect balance between speed, precision and ease of use.



mmi SmartCut Plus SPECIFICATION

Item	Specifications
Samples	For all application-relevant samples; cryo or paraffin-preserved tissues, single cells, cytopins, cell compartments, chromosomes, etc.
Microscope	System integrated Nikon TS100-F with CF160 infinity optics, illumination pillar with 30 W halogen lamphouse, long working distance condenser NA 0.3 WD = 75mm,
CF160 objectives	a) CFI PLan Fluor objective with 4x magnification, NA 0.13 and WD of 17.1 b) CFI PLan Fluor ELWD objective with 20x magnification, NA 0.45, WD from 7.0 to 8.1
Picosecond UV, solid-state laser	Computer-controlled; Wavelength: 355 nm Pulse duration: <500 psec Pulse energy/average energy: <1 µjoule/approx. 4 mW Repetition rate: > 5 kHz System complies with IEC 60825-1 Am. 2: 2001. Class 1M laser product (invisible laser radiation. Do not view directly with optical instrument)
CapLift technology	Covering full slides; unique and contamination-free sandwich technology
Digital camera with high sensitivity	Digital colour: 1032 x 776 pixels with integrated 1/3" interline progressive CCD Compact housing and FireWire connection
mmi CellTools software basic functions	Laser energy and focus control Full slide and Petri dish control Saving multi-user profiles MultiGroup function across entire sample/slides Autodocumentation for sample, images and parameters
PC and monitor	Windows XP, 19" LCD monitor Specifications will be continuously updated according to market development
Motorised stage	Computer-controlled for high-precision movement/cutting Travelling range: 120 x 100 mm; step width: 0.075 µm; repositioning accuracy: 2 µm
Options	
PenScreen system operation	Sensitive 17" or 21" touch screen monitor for user-friendly system operation and to allow direct target identification with a special pen
mmi CellExplorer	A cell identification software based on colour recognition which automatically identifies and cut's out cells
manual mmi CapLift	manual mmi CapLift available upon request

mmi SmartCut Plus can be delivered optionally with a Nikon TS100-F or a Olympus CKX 41 microscope.



Swiss Precision

The manufacturer reserves the right to make technical changes without prior notice.



MMI AG
Flughofstrasse 37
8152 Glattbrugg, Switzerland
Phone: +41 44 809 10 10
Fax: +41 44 809 10 11
Mail: info@molecular-machines.com
www.molecular-machines.com

MMI GmbH
Breslauer Strasse 2
85386 Eching, Germany
Phone : +49 89 319 048 40
Fax : +49 89 319 048 59
Mail : info@molecular-machines.com
www.molecular-machines.com

MMI Inc.
P.O. Box 560728
Rockledge, FL 32956-0728
Phone: +1 603 629 9536
Fax: +1 321 978 0304
Mail : sales_us@molecular-machines.com
www.molecular-machines.com