OLYMPUS

Your Vision, Our Future

Laser Microdissection System

CellCut

for IX2 Microscopes

A cut above the rest: Olympus CellCut microdissection system

NEW-GENERATION TECHNOLOGY

Flexible, high-precision laser microdissection

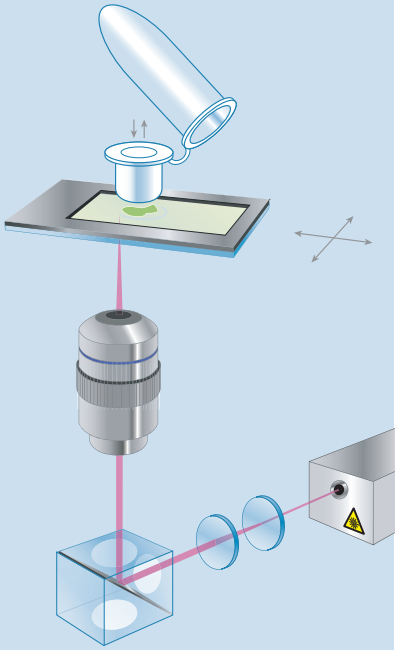
The primary goal of a researcher is to quickly isolate target samples uncontaminated by neighbouring cells or tissue. Ascertaining what went wrong in a malignancy, for example, may depend upon the ability to separate tumour cells from precancerous neoplasm and supporting stroma. Similarly, a single cell or homogeneous starting population may ultimately be required for a researcher to determine if a gene therapy protocol has had the intended outcome.

Traditional protocols can be frustrating and although newer laser techniques can achieve all of the researcher's goals, they too are often limiting, complicated and time-consuming processes. The next generation of laser microdissection tools from Olympus, though, is designed to meet and exceed all of these exacting requirements.

The new, superior Olympus CellCut laser microdissection system utilises high-precision, solid-state UVa laser technology, providing easier and quicker contamination-free isolation. Furthermore, the flexible, modular design provides extremely precise extraction of tissue cells and cell components from almost any starting material.

A Principle of microdissection

Fast, precise and contamination-free



Computer-controlled movements

UV-Cut software-controlled laser focus and energy

B CapLift technology

CUTTING-EDGE TECHNOLOGY

The CellCut system is the ultimate laser microdissection tool for molecular biology researchers wanting to isolate groups of cells, single cells and cell components carefully, for analysis across a wide range of fields.

For example, CellCut is the perfect target isolation system for research into general cell biology, genomics and proteomics, immunology, and cancer as well as live cell-handling studies (e.g. stem cells). Moreover, clinical-related applications such as molecular pathology, microbiology, virology and forensic medicine will also find CellCut essential.

Principles of laser microdissection

A CellCut combines several proven, leading-edge technologies to provide very fast, precise and, above all, absolutely uncontaminated target isolation from a wide range of microscopical samples. More importantly, targets are extracted without any vigorous physical or chemical disruption, and therefore high quality for subsequent "downstream" analysis is provided.

High-speed ultra fine laser cutting

The CellCut system is fully controlled through the intuitive UV-Cut software which provides a live view of the microscopic sample on the monitor and allows the user to identify, mark and isolate the target cells.

For target isolation, the maintenance-free, solid-state UVa laser (~355 nm) is focussed by the UIS2 objective onto a microscopical sample and produces a cutting spot of less than 1 µm. This, combined with a picosecond pulse duration and high (5 kHz) repetition rate, provides ultra precise and very quick target excision. The UV-Cut software automatically controls the focus and energy of the laser. To cut out a target on the Olympus IX2 inverted research microscope, the laser remains aligned to the centre of the optical axis whilst the highly precise motorised stage is used to accurately move the sample.

Cold ablation

Due to the very low (< 1 µjoule) pulse energy, the CellCut's "cold ablation" process leaves the target unaffected with no impact on subsequent protein analysis, DNA or RNA extraction. This means that cells and cell components from cryo or paraffin-preserved tissues, archived material, smears, cytospins and living cells from cell cultures can be marked on-screen in the microscopic image and extracted without any negative impact on quality for the subsequent steps.

No impurities with automated CapLift technology

B The unique CapLift technology provides automated transfer of untainted targets into microtubes from the entire microscope slide. They are therefore immediately ready for subsequent "downstream" molecular biological analysis.

High-clarity imaging

The fine excision produced by the CellCut system is complemented by the excellent imaging capabilities of Olympus's advanced UIS2 optics in combination with the latest digital camera technology. These produce extremely clear images through both the binocular eyepiece and the digital imaging system, enabling much more accurate target identification and cut analysis.

Application examples

This cutting-edge technology is incorporated into the advanced Olympus IX71 or motorised IX81 inverted research microscopes. As a result, the CellCut system is ready to be used for a wide range of applications.

Multiple cells in pathological samples

C For example, transverse sections of intestinal glands from colon tissue can be identified and quickly isolated to study specific genes and the corresponding hormone response. Further on, differences in gene expression or protein translations of locally distinct areas, with virulent, malignant or benign tissues or between species, can be compared easily.

Single cells in haematology or cytology

D Any single cell such as an atypical plasmoblast from a blood smear or other relevant cytological cells can be easily identified, cut and isolated with the CellCut system. With the sample remaining securely attached to the membrane, it is very easy to match the isolated target and the remaining sample to document the precision and the efficiency of the extraction.

Fluorescence-labelled cell components

E The precision of the CellCut system also enables structures smaller than single cells to be quickly and cleanly extracted. For example, even chromosomes with detectable fluorescence *in situ* hybridisation (FISH) signals can be isolated for subsequent investigation by using oil immersion objectives and 0.17 mm slides.

Forensic applications

F The CellCut 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 downstream genetic analysis, possibly leading to conviction.

Other samples

G Further on, living specimens like *C. elegans* can also be examined with the CellCut system. Here, the living organism can be fixed between the membrane and glass slide in order to isolate and extract areas of interest based on the specific customer experiment.

Live cells

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 and exact. With CellCut, there is the perfect balance between speed and precision, allowing the target stem cells to be isolated and re-cultured without any deleterious effects, such as karyotype changes.

Versatile laser micromanipulation

The CellCut system can also be used as a laser micromanipulation system. Single, short laser shots produce small self-sealing holes in the plasma membrane of a living sample, which improves protoplast fusion or increases the transfection rates of exogenous substances.

* Images courtesy of MMI GmbH, Glattbrugg, Switzerland.

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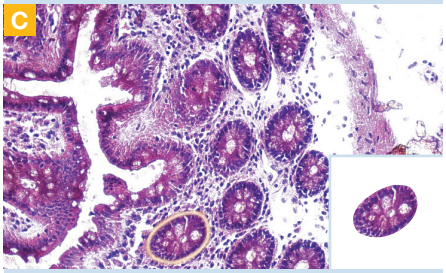

Intestinal gland isolated from transverse tissue sections of colon*

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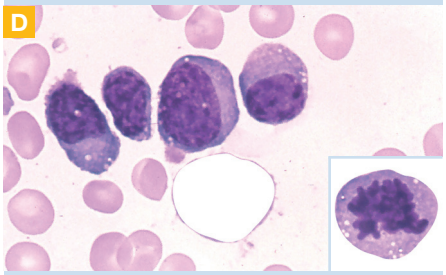

Blood smear with atypical plasmoblasts*

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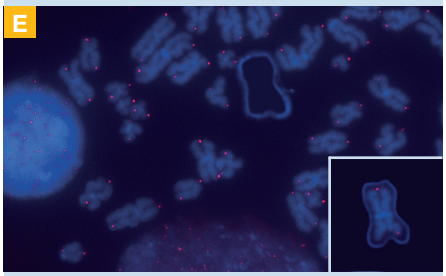

Microdissection of fluorescence stained chromosomes*

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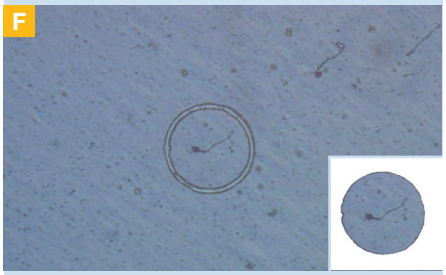

Single sperm isolated from a gynaecological smear

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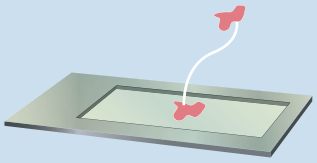
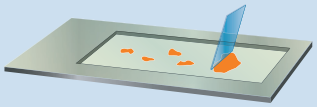
Live *C. elegans*, fixed onto a membrane and isolated.

A Sample preparation

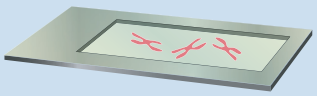
On frame slide with PET membrane



Cryo or paraffin-preserved tissue



Single cells, smears or cytospins



Chromosomes

Others, e.g. sperms, *C. elegans*

QUICK AND CLEAN TARGET ISOLATION

Unique sandwich system for sample preparation

A B To isolate targets from any source, for example cryo- or paraffin-preserved tissues, smears or cytospins, and chromosomes, the Olympus CellCut system uses a special frame slide covered with a 1.4 µm PET membrane. This is completely inert and has very low autofluorescence. The different types of sample are placed directly on the membrane, turned upside down, and a standard glass slide is placed below.

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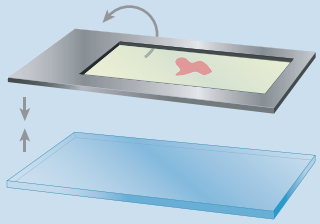
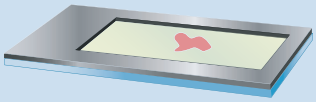


Assembly of the reversed metal frame and a glass slide (1.0 or 0.17 mm)



Unique sandwich with contamination-free and protected sample

This unique sandwich effectively protects the sample by forming a complete barrier to contaminants, such as environmental impurities, which is essential for professional laser microdissection steps. The sample is thus ready for automated, highly pure isolation via the adhesive cap of the CapLift microtube. 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.

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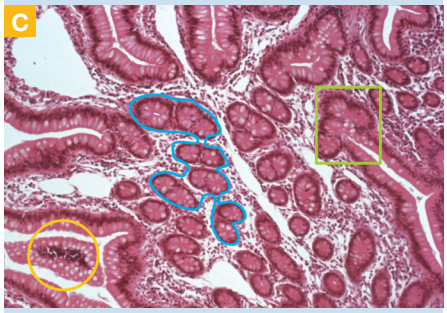


Selection and cutting

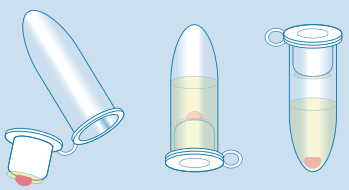
C Targets to be extracted are selected on-screen using either freehand or predefined geometrical shapes, such as circles, squares and ellipses. Any number of areas across the entire section can be identified as targets and the sizes of the geometrical shapes can be changed as well as copied and pasted for consistency. A grouping function allows the user to collect an unlimited number of different cells or cell compartments within one screening process in different microtube caps.

Fast, precise and clean excision

The >1 µm cutting spot 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.

D Selected target

Incubation and centrifugation



Ready for "downstream" analysis

D The specially supplied IsolationCaps used with the CapLift technology allow the collection of target areas across the entire microscope slide. The tube, including the required isolation buffer, can then be closed and microcentrifuged to keep the extracted targets in an isolated environment ready for protein analysis or DNA and RNA extraction.

Microdissection of live cells

E For live cell isolation from cell cultures, a different recovery method is used. A specialised cell chamber enables the CellCut system to microdissect living cells under fully sterile conditions. Different cell types are cultured under standard conditions in the chamber containing a poly-L-lysine-coated membrane.

F Prior to microdissection, the chamber is placed in a sterile Petri dish with an adhesive bottom. To isolate live cells, the Petri dish is placed on the microscope. Target areas are positively or negatively selected and microdissected by the laser without any need to open the dish. Here, fluorescence labels are very helpful for identification. The laser quickly and precisely cuts only the membrane to enable easy separation of the target live cells.

G Back in the clean bench and thus under fully aseptic conditions, the culture chamber is removed from the Petri dish, leaving the positively selected and isolated cells behind. The chamber, placed in a new Petri dish, can therefore be used a number of times to isolate different cells from the same culture if required.

The positively selected cells in the Petri dish are ready for subsequent analysis or further cell culturing.

Advanced yet easy to use

It is clear that the Olympus CellCut system is a highly advanced instrument with exceptional flexibility. Yet, due to the careful integration of the laser, microscope and imaging components, controlling the system is simple and intuitive. All functions can be controlled by the UV-Cut software, which also offers enhanced features such as automated image capture, ready-to-go routine reports or high-quality images to be used in scientific papers.

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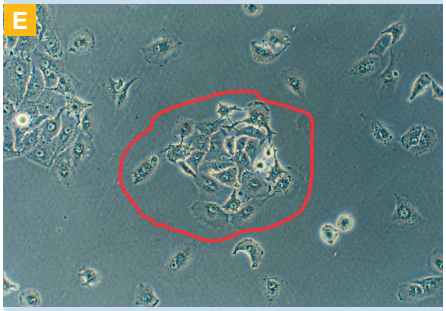
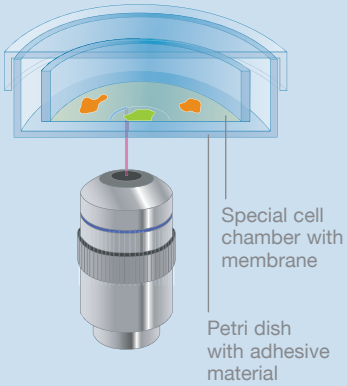


Cell culture in phase contrast observation

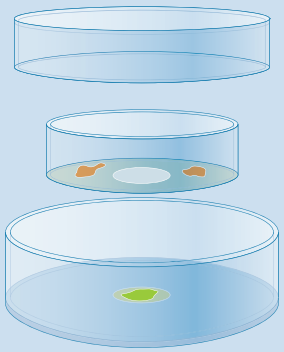
F **Excision of live cells**

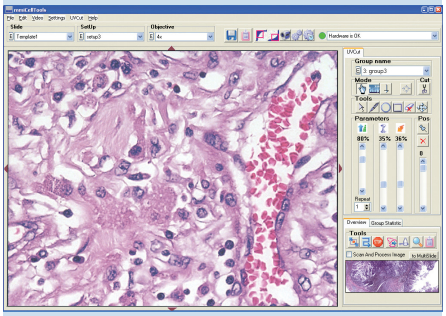
On a microscope

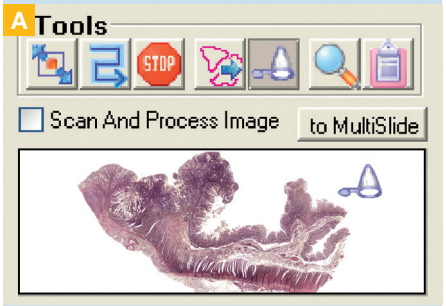


G **Positive selection**

Under clean bench conditions



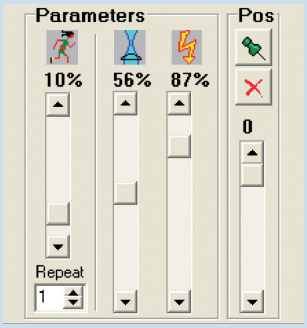




Toolbar and navigation overview

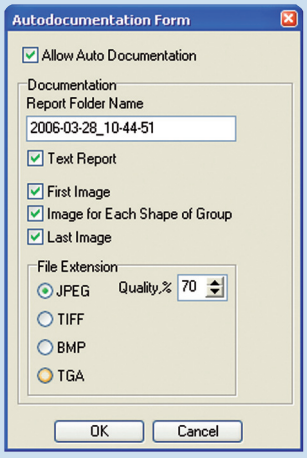
B Laser parameter control

Clear and intuitive setting



C Automatic documentation

Options



INTUITIVE SOFTWARE CONTROL

The UV-Cut software is used to control the CellCut system. Its graphical user interface allows precise and intuitive identification of the target areas to be excised for the subsequent isolation processes. Moreover, it also provides full control over the cutting laser parameters, the objective used and specific camera settings with respect to different application-specific requirements.

When used with the fully motorised IX81, even the automated microscope functions, such as Z-focus, objective and fluorescence turret changes, are controlled via UV-Cut. With such user-friendly software in conjunction with the unique optional PenScreen operation, the CellCut system makes even the most complex experiments easy.

Overview, easy navigation and positive target identification

A Using a low-magnification objective, an overview scan of the entire microscope slide can be acquired, allowing the user to operate and navigate the system more easily. Users can thus find areas of interest as well as positions to place the IsolationCap for the inspection mode. Moreover, when using the MultiSlide option, the overview and easy navigation modes are extended to incorporate all slides.

Inspection mode

A The UV-Cut software can move the CapLift system to an empty area of the slide so the user can easily inspect the cap and verify the complete isolation of the micro-dissected areas. The user-defined inspection position is easily set without difficult adjustments – just by pressing a button on the navigator screen.

Cutting laser control

B 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 and can be edited easily. The precise nature of the controls enables the same laser settings to be used repeatedly on the same sample, with each position marked precisely.

Automated full-slide CapLift technology

Since many target selections can be made over the entire slide area, the software automatically moves the CapLift over the slide to pick up the microdissected areas, saving a significant amount of time in the screening and isolating process. In addition, with the MultiSlide and MultiCap options, the system automatically extracts targets from up to three slides and collects them in up to eight different IsolationCaps.

C 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 CellCut 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.

Modular design

The UV-Cut software is designed to allow the easy addition of other options to the CellCut system, such as CellExplorer for image analysis-based automatic target detection, MultiCap, MultiSlide and other system upgrades, including an optical tweezer called CellManipulator.

IX2 SYSTEM COMPONENTS

The Olympus IX2 inverted microscopes are designed to provide researchers with the high performance and versatility needed for a wide range of research activities.

Optical bench flexibility

C D With multiple ports and dual-level laser integration, the microscopes offer "optical bench" flexibility, enabling different modules and imaging technologies to be used without any limitation or extensive equipment changes. The system-integrated digital camera is connected to the standard left side port, whereas other cameras may be added at different ports.

Standard fluorescence without limits

E In particular, the dual-level coupling of the UVa cutting laser ensures the full use of the IX2 microscope for a wide range of fluorescence applications. For example, six fluorescence cubes can be used and therefore no additional or specialised dichroics or external filter wheels are necessary.

In conjunction with the UV-Cut software's freeze mode for fluorescence images, the CellCut system provides a perfect and customer-tailored combination for fluorescence applications and laser microdissection.

The L-shaped fluorescence condenser is attached for user-friendly handling of the 100 W mercury lamphouse. Moreover, all available IX2 options, including the fibreguide illumination or other external lamphouses, can also be used.*

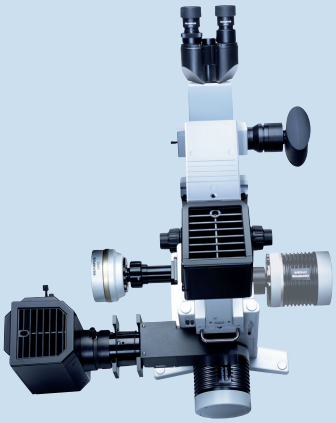
F All relevant controls of the IX2 microscope are easily accessible from the front of the microscope, meaning that additional peripheral items, such as optical tweezers, can be placed close by.

The motorised IX81 microscope as a fully automated platform can be used as a CellCut system basis. This allows the system software to control motorised Z-focus movements, objective change and fluorescence filter cube selection as well as multi-user settings for cameras, light intensity and several other operations.

* Please also refer to our IX71/81 brochure for details and additional options.

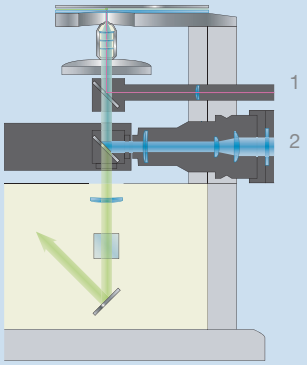
C IX2 multiport concept

For increased flexibility



D IX2 dual-level laser coupling

Scheme of different light paths



1 UVa Cutting Laser coupling

2 Fluorescence light path

E IX2 fluorescence

Up to six fluorescence filter cubes

F IX2 front operation

With easily accessible controls

A LUCPlanFLN
With 20x magnification

A LUCPlanFLN
With 40x magnification

A LUCPlanFLN
With 60x magnification

B UPlanFLN
With 4x magnification

B UPlanFLN
with 10x magnification

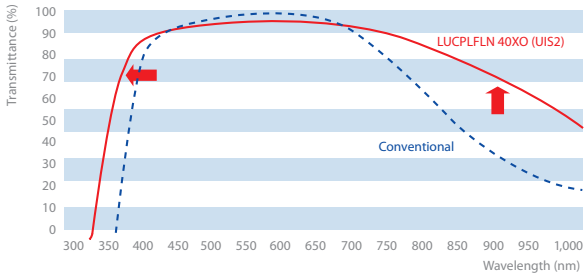
PEERLESS IMAGE QUALITY

The unique Olympus UIS2 objectives are produced from a new type of glass that provides low autofluorescence and excellent signal to noise ratios. This enables them to produce superior image quality with peerless optical clarity.

The CellCut system also utilises another novel property of the integrated UIS2 objectives – high transmission rates in the UV and IR ranges. This allows the CellCut system to work very precisely with the 355 nm solid-state laser and to integrate an optional optical tweezer system based on a YAG-type infrared laser (1,064 nm).

The CellCut system-integrated UIS2 objectives have outstanding numerical apertures (NA) and working distances (WD), making them the most important prerequisite for laser microdissection of small organisms, tissues, single cells or even subcellular structures.

Transmittance of UIS2 objectives for microdissection



A The long working distance LUCPLFLN objective series, with 20x, 40x and 60x magnification, have correction collars to adjust for different sample thicknesses. They are thus the most suitable objectives for the majority of microdissection techniques. This increased flexibility allows the uncomplicated, contamination-free use of the system for both sandwich slides and cell culture chambers.

B The UPLFLN objectives (4x, 10x, 20x, 40x, 60x and 100x) are designed to work with 0.17 mm cover glass combined with the contamination-free sandwich frames. The larger numerical apertures of the UPLFLN objectives series provide ideal conditions for more specialised applications. For example, the 40x/O and 60x/OI have very high resolving powers due to NAs of 1.3 and 1.25 respectively. This is indispensable for selective ablation, cutting of chromosomes or cell fusion work, for example. Both objective ranges are available in oil immersion or dry versions.

	Objective	NA	WD (mm)	Cover glass correction (mm)	Microdissection technique
A	LUCPLFLN 20x (20x/PH; 20x/RC)	0.45	6.6 – 7.8	0-2	LCut, LAbl, LCH, LMan
	LUCPLFLN40x (40x/PH; 40x/RC)	0.6	2.7 – 4	0 – 2	LCut, LAbl, LCH, LMan, LTwz
	LUCPLFLN60x (60x/PH)	0.7	1.5 – 2.2	0.1 – 1.3	LCut, LAbl, LCH, LMan, LTwz
B	UPLFLN4x	0.13	17	-	Overview, navigation
	UPLFLN10x	0.3	10	-	LCut, LAbl
	UPLFLN20x	0.5	2.1	0.17	LCut, LAbl
	UPLFLN40x	0.75	0.51	0.17	LCut, LAbl
	UPLFLN40xO	1.3	0.2	0.17	LCut, LAbl, LMan,
	UPLFLN60x	0.9	0.2	0.11 – 0.23	LCut, LAbl, LMan,
	UPLFLN60xOI	0.65 – 1.25	0.12	0.17	LCut, LAbl, LMan, LTwz
	UPLFLN100xO	1.3	0.2	0.17	LCut, LAbl, LMan, LTwz
	UPLFLN100xOI	0.6 – 1.3	0.2	0.17	LCut, LAbl, LMan, LTwz

LCut = laser cutting, LAbl = laser ablation, LCH = “live cell” handling, LMan = laser manipulation, LTwz = laser tweezing

FURTHER SYSTEM OPTIONS

For more advanced functions and different requirements, the CellCut system based on the inverted microscopes of the Olympus IX2 series can be upgraded with a number of useful additions.

C PenScreen – A sensitive touch screen can be used to easily operate the CellCut system and to allow targets to be selected and marked on-screen with a special pen.

D CellExplorer – Image analysis software which automatically identifies and cuts-out cells based on specific morphological parameters such as fluorescent markers.

MultiCap – Allows automatic collection of targets in up to eight different Isolation-Caps for different targets to be kept separately (e.g. MultiGroup function) or to increase throughput together with MultiSlide.

MultiSlide – For simultaneous microdissection from up to three slide assemblies to increase throughput, or improve the isolation rate of rare cells.

E CellManipulator – This optical tweezer is a complete system upgrade including laser box and optics. In addition to laser microdissection applications, it enables the ultra precise, contact-free manipulation of microscopic particles with a high-quality, YAG-type infrared laser.

Up to ten different microscopic objects can be held, rotated, joined, separated or streched by the intensely focussed laser beam. Together with a 4-quadrant micro-bead position sensor, even binding forces or viscosities can be measured.

* Image courtesy of N. de Ruijter, T. Ketelaar, Plant Cell Biology, Wageningen University, the Netherlands.

C PenScreen

For on-screen target identification

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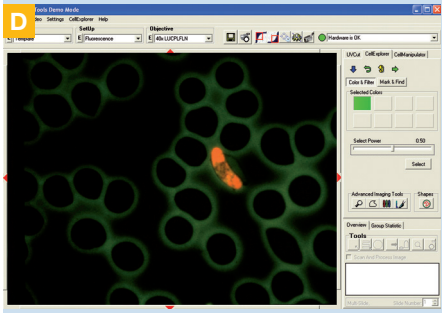

GUI of CellExplorer

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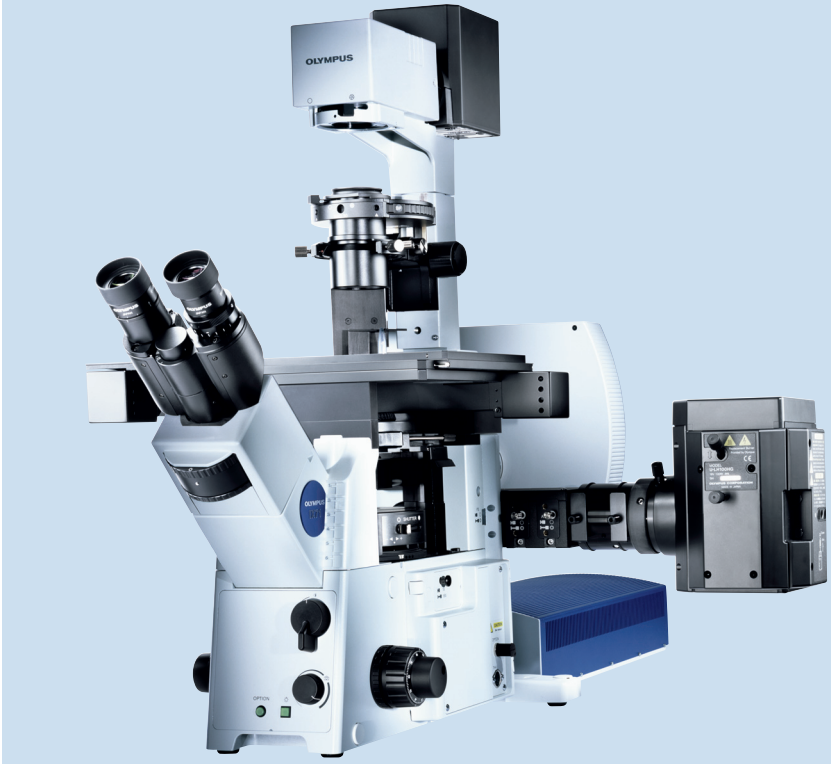

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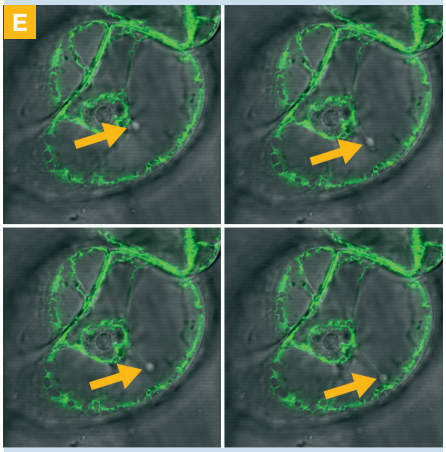

Sequential confocal images (2 sec. intervals) of a living tobacco suspension cell. The endoplasmic reticulum (ER) is tagged with GFP.

A trapped organelle (arrows) is pulled through the vacuole away from the nucleus, resulting in the formation of a new thread of cytoplasm that remains present and connects the trapped organelle with the cytoplasm surrounding the nucleus.

Simultaneous optical trapping and confocal imaging reveals that ER is present in this new thread of cytoplasm.*

CellCut specifications

Item	Specification	(with Olympus microscopes)
System components		
Samples	For all application-relevant samples (cryo or paraffin-preserved tissues, single cells,	
	cell compartiments, cytospins, chromosomes, etc.)	
“Live cell” handling	Positive and negative cell selection/Isolation possible	
Picosecond UVa, solid-state laser	Computer-controlled	
	Wavelength: 355 nm	
	Pulse duration: < 500 psec	
	Pulse energy/average energy: < 1 µjoule/appr. 4 mW	
	Repetition rate: > 5 kHz	
CapLift technology	SW-controlled, covering full slides; unique and contamination-free sandwich technology	
Nosepiece, UIS2 objectives	6-position nosepiece, NA and WD specified to be selected according to application requirements	
	Excellent UV/IR transmission	
Digital camera with ultra high sensitivity	Digital colour: 2,088 x 1,550 pixels or	
	Digital monochrome: 1,392 x 1,040 pixels	
	Compact housing and FireWire connection	
UV-Cut software basic functions	Laser energy and focus control	
	Full slide and Petri dish control	
	Inspection mode with positive target identification	
	Saving multi user profiles	
	MultiGroup function across entire sample/slides	
	Autodocumentation for sample, images and parameters	
PC and monitor	Specifications will be continuously updated according to market development,	
	Windows XP, 20” LCD monitor	
Motorised stage	Computer-controlled for high-precision movement/cutting	
	Travelling range: 120 x 100 mm,	
	Step width: 0.075 µm	
	Repositioning accuracy: 1 µm	

Differences between systems based on IX71 and IX81		**IX71**	**IX81**
Motorised nosepiece		-	+
Z-focus	10 nm step size and 1 µm reproducibility independent of movement	(manual)	+
	Direction, fine/coarse movement with 3 mm/sec max. speed	-	+
Condenser/Contrast methods	BF, phase- and RC contrast and DIC, application-oriented	manual	+
Fluorescence	6-cube turret, dual-level laser coupling	manual	+

Options	
PenScreen system operation	Sensitive 20” touch screen monitor for user-friendly system operation
	and to allow direct target identification with a special pen
CellExplorer image analysis software	Identifies and cuts out automatically defined targets based on user settings
MultiCap (motorised)	Allows automatic collection of targets in up to 8 different IsolationCaps
MultiSlide (motorised)	For microdissection of up to 3 slide assemblies

Possible CellCut system upgrade	
CellManipulator optical tweezer system	Ultra precise, contact-free manipulation of microscopic particles
	with up to 10 independent beams based on a high-quality, YAG-type infrared laser.

OLYMPUS

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