Molecular Machines & Industries

mmi CellCut Plus

User Manual

Molecular Machines & Industries AG
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User Manual: MMI CellCut Plus
Version 4.4

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Contents

1 Safety advice

The system must only be used for microdissection as described in the manual.

Caution – use of controls or adjustments or performance of procedures other than those specified herein may result in hazardous radiation exposure.

Damage due to unauthorized use is not subject to warranties. Only persons who have been properly trained should use the system.

Thoroughly read the security advice in section 1 of the manual before operation.

1.1 Laser safety

This system contains a laser for microdissection. It complies with the following international standard:

IEC 60825-1; Edition 2; 2007; Safety of laser products

A laser of class 3B is incorporated. When the laser casing is open, it presents a potential risk. Any direct or reflected beam must not strike the unprotected eye (do not look directly into the beam).

Laser class 3B: Laser products that are normally hazardous when intra beam ocular exposure occurs including accidental short time exposure. Viewing diffuse reflections is normally safe. Class 3B lasers which approach the AEL (accessible emission limit) for class 3B may cause minor skin injuries or even pose a risk of igniting flammable materials. However, this is only likely if the beam has a small diameter or is focused. The system includes safety devices to prevent laser interference with the user.

1.1.1 Radiation output

Due to the power losses determined by the lenses of the microdissection system and the objectives of the microscopes the average power shall be deemed to be nominal at the microscope work surface (i.e., at the membrane slide/tissue surface).

1.1.1.1 Legacy *CellCut Plus* and *SmartCut Plus*

Laser radiation characteristics systems delivered until 2014 are listed in Table 1.1.

Table 1.1: *Laser radiation data of the CellCut Plus and SmartCut Plus until 2015*

Average power (nominal)	< 10 W
Pulse width	< 1 ns
Repetition rate	> 5 kHz
Wavelength	355 nm
Pulse energy (typical)	> 0.5 µJ

1.1.1.2 CellCut

Starting from 2015 CellCut system are available in with three versions

- standard laser
- universal laser
- high power laser

1.1.1.3 standard laser

Laser radiation characteristics for CellCut systems incorporating a standard laser are listed in Table 1.2.

1.1.1.4 universal laser

Laser radiation characteristics for CellCut systems incorporating a universal laser are listed in Table 1.3.

Table 1.2: *Laser radiation data of the CellCut systems incorporation a standard laser*

Average power (nominal)	< 10 W
Pulse width	< 1 ns
Repetition rate	2 kHz
Wavelength	355 nm
Pulse energy (typical)	> 1 µJ

Table 1.3: *Laser radiation data of the CellCut systems incorporation a universal laser*

Average power (nominal)	< 10 W
Pulse width	< 1 ns
Repetition rate	4 kHz
Wavelength	355 nm
Pulse energy (typical)	> 2 µJ

1.1.1.5 high power laser

Laser radiation characteristics for CellCut systems incorporating a high power laser are listed in Table 1.4.

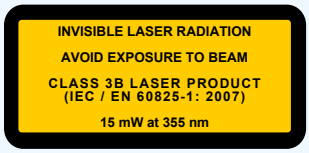
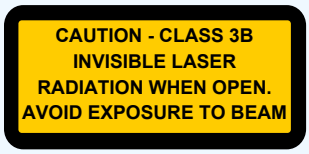
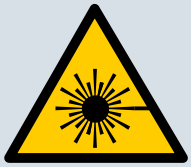
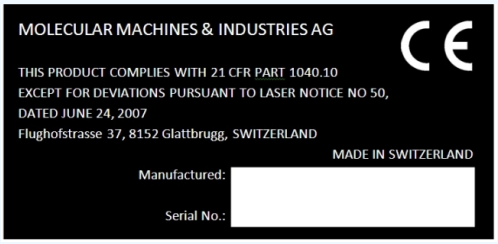
Table 1.4: *Laser radiation data of the CellCut systems incorporation a high power laser*

Average power (nominal)	< 10 W
Pulse width	< 1.5 ns
Repetition rate	500 Hz
Wavelength	355 nm
Pulse energy (typical)	> 70 µJ

1.1.2 Laser safety labels

This system carries the following labels (Table 1.5), which meet the international standard for laser safety IEC / EN 60825-1 and complies with 21 CFR Part 1040.10 except for deviations pursuant to laser notice No 50, dated June 24, 2007.

Table 1.5: *Laser safety labels*

(1)	Explanatory label	
(2)	Aperture label: Indicates accessible laser radiation during operation	
(3)	Caution label: Human access to laser radiation: When housing is opened, class 3B laser invisible radiation is accessible	
(4)	Warning label – Hazard symbol: System and Software (Monitor)	
(5)	Identification label	

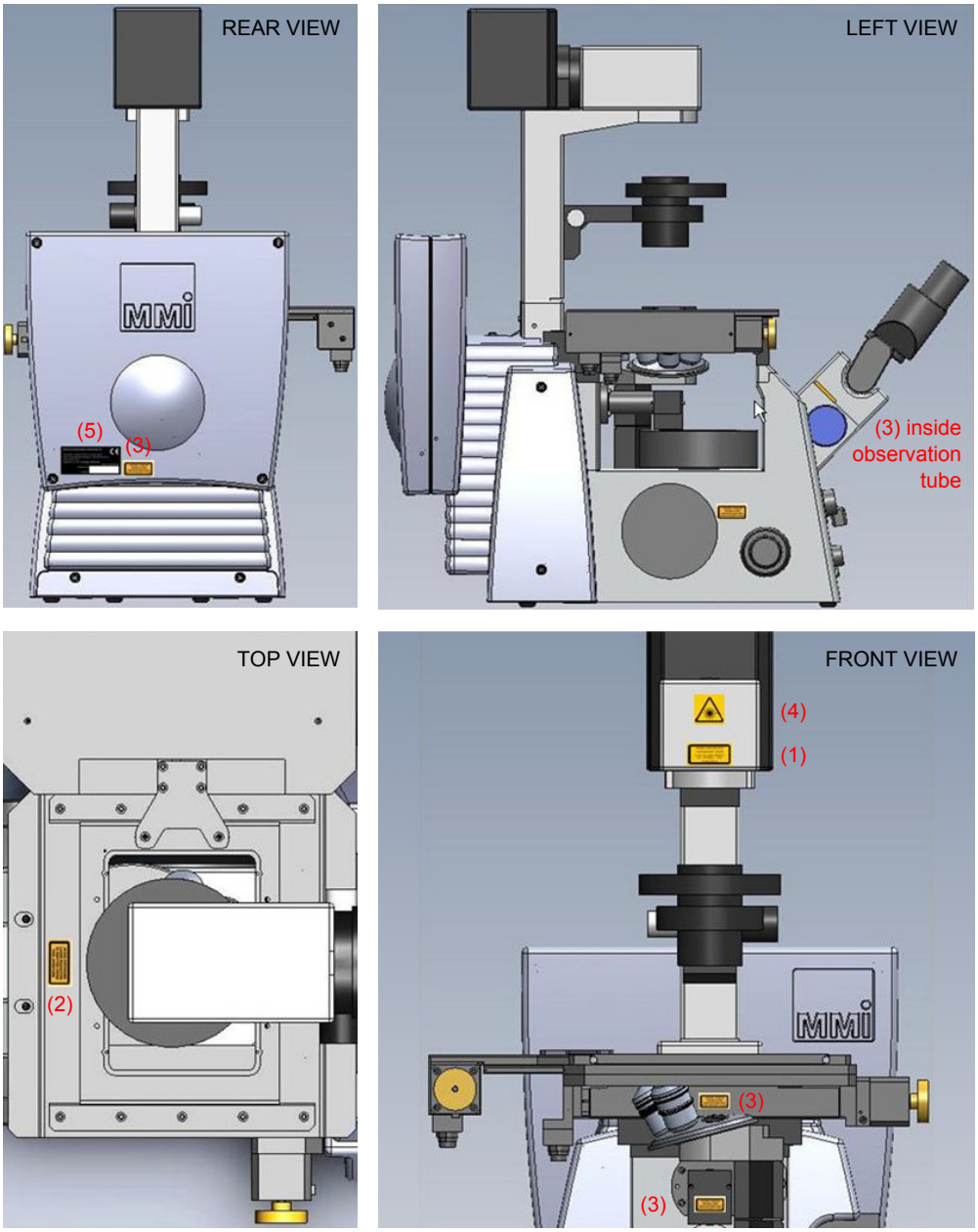
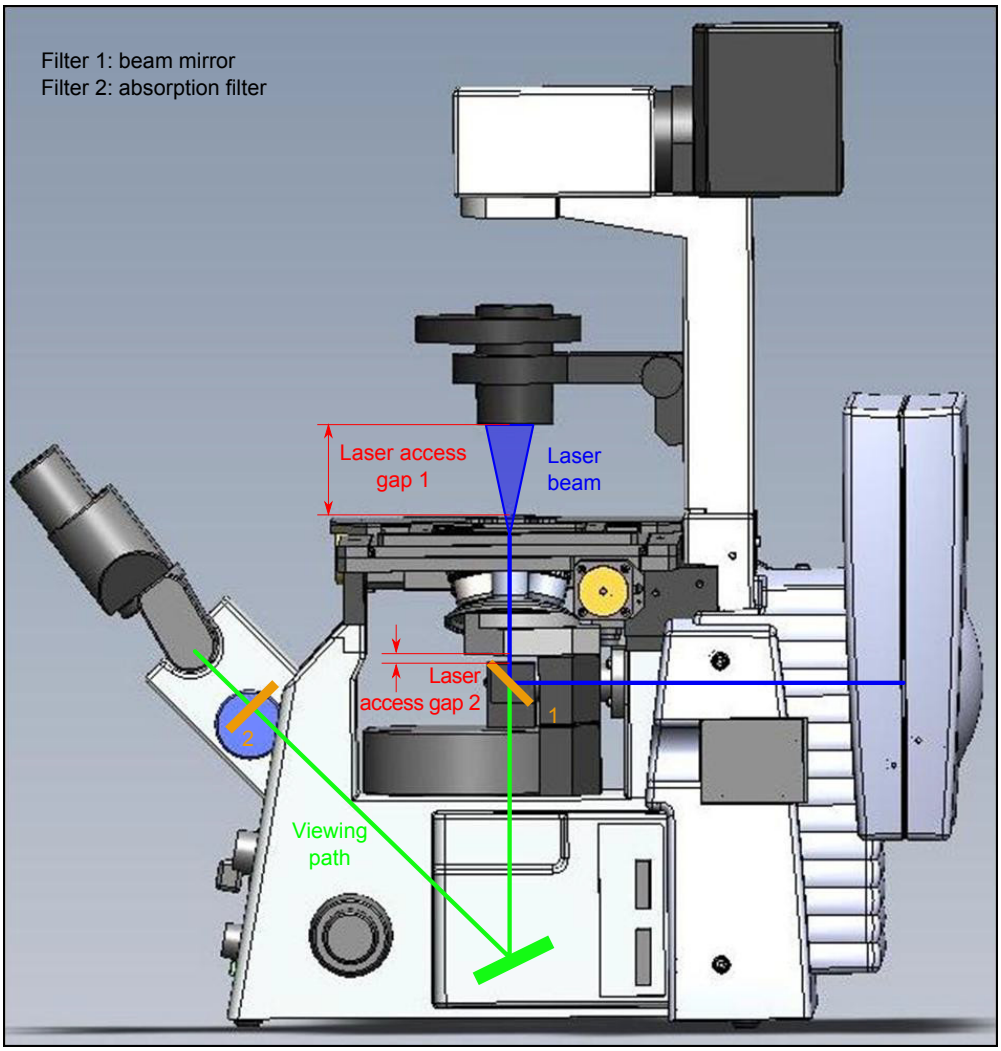


Figure 1.1: *Positions of laser safety labels*



- **Laser access gap 1:** Access to 10 mW UV laser power, gap between 90–97 mm, depends on the z-position of the objective and condenser
- **Laser access gap 2:** access to 12 mW UV laser power, gap between 4–11 mm, depends on the z-position of the objective. This gap does only exists in the legacy versions *CellCut Plus* and *SmartCut Plus* but is covered in *CellCut* systems starting from 2015.

Figure 1.2: *Laser access gaps*

1.1.3 Eye protection

A UV coated beam mirror (filter 1 in Fig. 1.2) and an additional UV absorption filter (filter 2) is used with the eyepiece of the microscope to block and absorb hazardous UV laser radiation. It is contained in the microscope stand. This prevents the user coming into direct contact with laser radiation, even when the binocular tube or eyepieces are removed.

The camera ports and the fluorescence filters in the fluorescence filter turret are protected with the UV coated beam mirror (filter 1).

1.1.4 Microscope interlock

Interlocks are provided to protect you from eye injury resulting from accidental exposure of the eye to the invisible UV laser beam during cutting of dissectates.

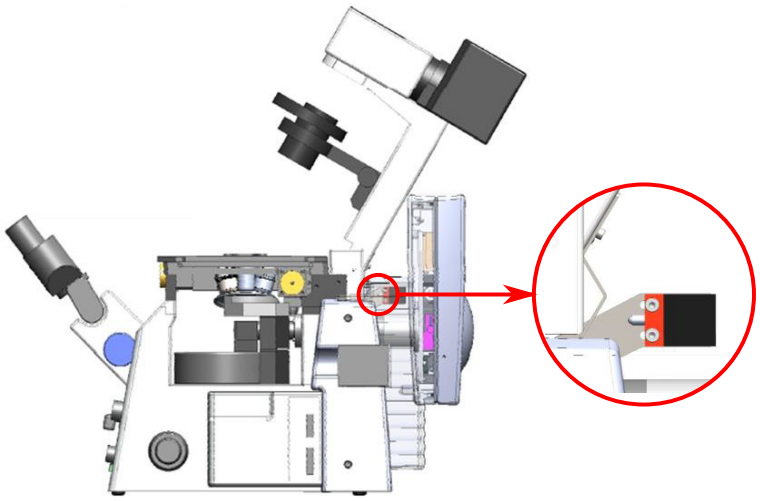


Figure 1.3: *Illumination pillar interlock*

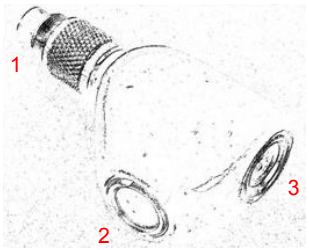
When you tilt the illumination pillar backwards the interlock mechanism switches off the laser (Fig. 1.3).

On *CellCut Plus* and *SmartCut Plus* the LED 1 on the controller changes the color from yellow to red, as shown in table **??**.

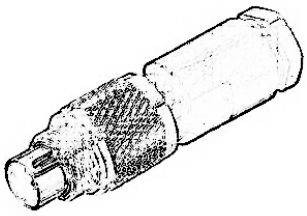
On *CellCut* starting from 2015 the laser will be unpowered. The green, yellow and red LED turn off as indicated in table 1.7a

1.1.5 Remote interlock

1.1.5.1 Legacy *CellCut Plus* and *SmartCut Plus*



(a) Legacy distribution adaptor



(b) Legacy door interlock plug.

Figure 1.4: *Legacy distribution and interlock adaptors. (1) connector to controller. (2,3) connectors to interlocks.*

For the door interlock, connect the laser safety distribution adaptor (Fig. 1.4a) to the rear of the controller. The distribution adaptor has an additional connection for a door interlock. This connector is delivered with a separate plug for the cable of the door interlock.

To connect the door interlock (Fig. 1.4b), consult your laser safety representative.

- Unscrew the door interlock plug.
- Solder the two cables to the door interlock plug.
- Tighten the door interlock plug again.

When you open the door the interlock mechanism switches off the laser. The LED 1 on the controller changes the color from yellow to red.

1.1.5.2 *mmi CellCut*

On the back of the *CellCut* electronics box, see 1.5, a remote internet plug is available. One fitting connector will be delivered with your *CellCut*. You can connect a door interlock and a laser warning lamp using that connector.

To connect a door interlock or a external warning lamp, consult your laser safety representative. The detailed pinning of the connector is available from your MMI service representative.

Figure 1.5: *Remote interlock plug delivered with mmi CellCut*

1.1.6 Laser safety features of CellCut electronics

1.1.6.1 Legacy *CellCut Plus* and *SmartCut Plus*

1.1.6.2 *mmi CellCut*

The *CellCut* electronics consitst of the CellCut controller and the CellCut key switch box. If the key switch box is not properly connected to the controller box, the system will not turn on.

Legacy laser controller					Laser status
Key switch	**Push button**	**Interlock**	**LED 1**	**LED 2**	
Off	Off / On	unlocked	Off	Off	**Off**
On	On	locked	Yellow	Green	**On**
On	Off	locked	Yellow	Off	**Standby**
On	On	unlocked	Red	Green	**Standby**
On	Off	unlocked	Red	Off	**Off**

(a) Laser controller safety logic

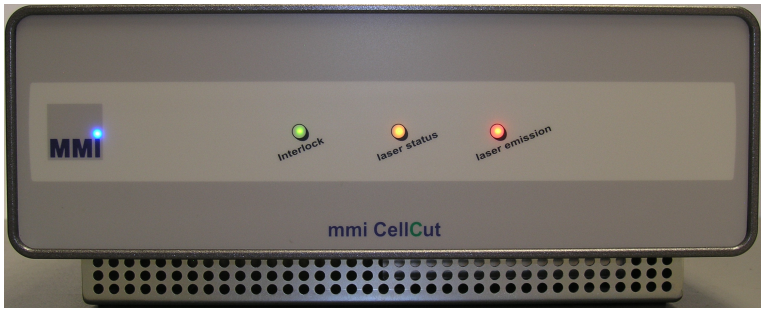
(b) Legacy controller front panel

(c) Legacy controller rear side

Figure 1.6: *Laser safety functions of the legacy laser controller*

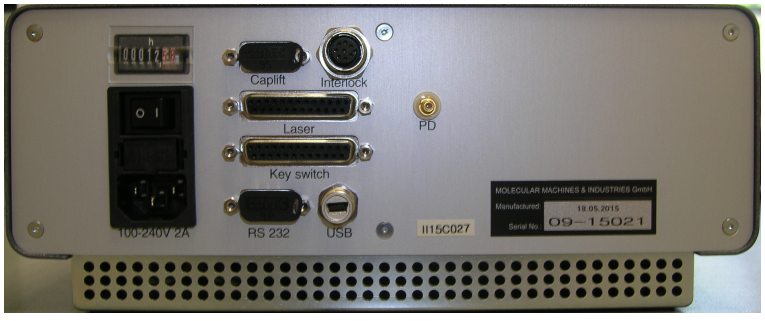
CellCut controller and key switch box						Laser status
Key switch	**Activation button**	**Interlock**	**LED 1**	**LED 2**	**LED 3**	
Off	Off	unlocked	Off	Off	Off	**Off**
Off	Flashing	locked	Off	Off	Off	**Off**
On	Off	locked	Green	Off	Off	**Powered**
On	Off	locked	Green	Slow flashing	Off	**Heating up**
On	Off	locked	Green	Fast flashing	Off	**Error**
On	Off	locked	Green	Yellow	Off	**Standby**
On	Off	locked	Green	Yellow	Red	**On**

(a) *CellCut* safety logic



(b) CellCut front panel

(c) CellCut key switch



(d) Controller rear side

Figure 1.7: *Laser safety functions of mmi CellCut*

1.1.7 Warnings

To ensure full safety of the system please follow the steps below:

- Turn off the laser with the key switch to prevent unauthorized operation of the system.
- Ensure that the laser power is turned off with the key switch if any objectives or blanks are to be removed from the objective turret.
- Ensure that all positions in the objective turret that do not contain an objective lens are covered with blanks.
- Laser beam is invisible to the human eye (λ = 355 nm). Never stare into the microscope objective while the laser is operating. Laser power up to 10 mW could be emitted through the microscope objectives.
- Invisible UV energy could exceed the maximum permissible exposure (MPE) limits for the skin if a person reached into the gap between the stage and the condenser during the cutting process. The exposure time needed to exceed the MPE would depend on the distance above the stage and the magnification of the objective.
- Use only objectives recommended by MMI for laser microdissection.
- Never place reflecting objects in the beam path.
- Viewing the laser output with certain optical instruments (for example eye loupes, magnifiers, microscopes) will likely increase eye hazard.
- The laser source and the optical equipment are enclosed within the MMI housing. Do not attempt to open the laser box or remove beam covers. Laser power of up to 15 mW at 355 nm could be accessible in the interior if a cover is removed.

1.2 Limits for Laser Energy

Skin Exposure Limit The skin Maximum Permissible Exposure (MPE) limit from the IEC 60825-1 standard for irradiance at the laser wavelength is 1 mW/cm^2 for an exposure of 1’000 s–30’000 s. That can also be expressed as 96 µW in a 3.5 mm aperture. Higher levels are allowed for shorter time periods.

There is a potential for a skin exposure that exceeds the MPE in the volume between the stage and the condenser, so the user is cautioned to avoid lengthy skin exposures at that location, particularly for lower magnification objectives. Due to the beam divergence, there is no potential exposure above the MPE beyond the location of the condenser.

Eye Exposure Limit The eye MPE limit from the IEC 60825-1 standard for irradiance at the laser wavelength is 66 µW/cm^2 for an 8-hour exposure at the maximum expected 50% on-time duty cycle. Considerably higher levels are allowed for shorter time periods or lower duty cycles (e.g., MPE = 1 mW/cm^2 for a time period of 1000 s).

When the product is operated per the procedures in this manual, the condenser blocks any laser energy that could cause an eye exposure of persons above the MPE limits during normal operation or routine maintenance. And there is insufficient spacing between the stage and the condenser to allow a direct eye exposure.

The following procedures that could result in an exposure that exceeds the MPE limits should also be avoided: attempting to turn on the laser when there is no slide in the beam path; turning on the laser with the turret at an open position (e.g., without an objective or blank at that position); or inserting a mirror into the narrow gap below the stage where a beam is present.

1.3 General safety

- Do not disassemble the system. The installation of the system is provided by MMI service personnel or a MMI designated representative. Repairs, removal or exchange of components beyond the operations described in this manual may only be carried out by MMI service personnel or persons explicitely authorized by MMI to do so. If you have any problems with the instrument, contact MMI.
- The power supply is installed by MMI. MMI assures that the system is provided with the appropriate voltage. Do not change the power cords.
- Avoid wet or dusty conditions near the system. If liquid gets inside the system, do not attempt to use it. Contact MMI.
- Unplug all electrical supply before cleaning the system. Do not use cleaning fluids or sprays; only use a smooth and dry cloth.
- If the stage control is not calibrated, stage movements can be sudden and fast. Ensure that the working area around the stage is free of clutter and material.
- Read the manual of your microscope for specific microscope precautions. If you do not have the manual, contact your microscope provider or MMI.

2 Installation

The MMI system may only be installed by an MMI service engineer or our designated representative in the laboratory of the customer. After the installation training will be provided in the use and operation of the system. The customer should not change the installation of the equipment.

Should you want to move an installed unit, please contact MMI for assistance. With any malfunction of the device, please contact our service department:

service@molecular–machines.com

After installation or modification of the MMI system, an authorized specialist must perform a thorough check to ensure that the system is in perfect condition. If your system comprises a laser, it must be ensured that the laser safety features are functioning correctly and that the covers to protect against laser radiation are fitted.

3 The mmi CellTools instrumentation family

The mmi CellTools are a fully modular instrumentation family, including the following components:

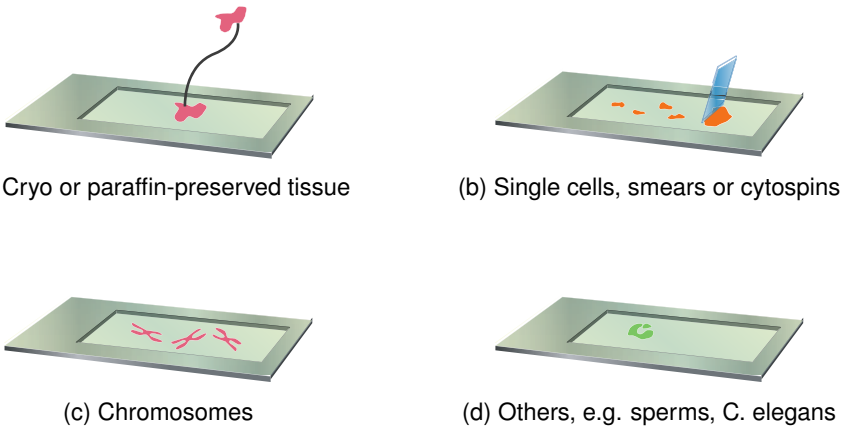
- *mmi CellCut*: laser microdissection to isolate single cells or areas of tissue
- *mmi CellManipulator*: optical tweezers to manipulate cells or beads with an optical trap
- *mmi CellEctor*: automated micro-pipetting to mechanically manipulate cells or beads with a capillary and mechanical micromanipulator
- *mmi CellExplorer*: pattern recognition software for PC based image analysis

Any or all of these modules can be combined in one microscopic environment.

4 Getting started

4.1 Working principles

The *mmi CellCut* is used to isolate, under microscopic view, small areas or single cells from histological sections for further microbiological analysis. Only the cell(s) wanted for further investigation are cut out. DNA, RNA, as well as proteins from undisturbed, pure samples can be investigated. No mechanical contact is necessary for the laser microdissection of the samples. Thus the method avoids contamination of the samples.



(a) Cryo or paraffin-preserved tissue

(b) Single cells, smears or cytospins

(c) Chromosomes

(d) Others, e.g. sperms, C. elegans

Figure 4.1: *Sample preparation*

For sample preparation from any source such as frozen or paraffin embedded sections, smears or cytospins, and chromosomes spreads, the *mmi CellCut* system uses the *mmi Membrane Slides*. This special frame slide is covered with a thin membrane that is completely inert and has negligible auto fluorescence. The different types of samples are prepared on this membrane and covered with a normal glass slide for protection against contamination.

Using the *mmi CellCut* software, the regions of interest are selected on the display using either the mouse, by freehand or predefined geometrical shapes, such as circles and squares (see Fig. 4.2).

Any number of areas across the entire slide can be identified as targets and the sizes of the geometrical shapes can be changed as well as copied and

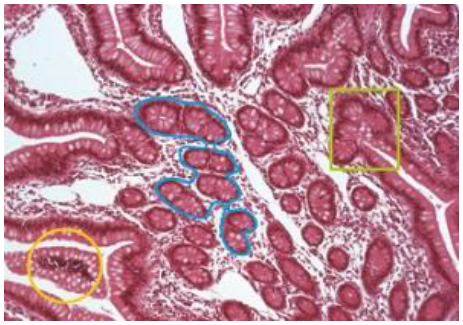


Figure 4.2: *Easy cell selection*

pasted for consistency. A grouping function allows the user to collect an unlimited number of different cells or cell areas within one screening process in different *mmi IsolationCaps*.

The thin (0.3 µm at 100x) cutting path enables the precise and comfortable extraction of the selected areas at an outstanding speed, without affecting its morphology or otherwise negatively affecting the areas of interest. As a result, there is no loss in quality of the material used in subsequent steps. Even the viability of living cells is not affected and, therefore once selected, cells can be recultured. Depending on the sample type, several thousands of cells can be laser dissected in less than a minute.

Several areas of interest can be microdissected in one automated operation and collected in the *mmi IsolationCap*. One *mmi IsolationCap* used for single step collection can collect several dissections, even from different slides.

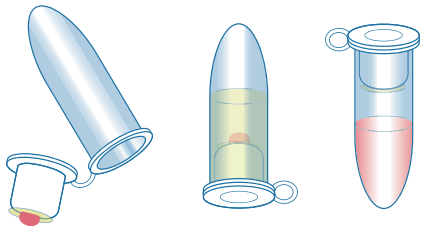


Figure 4.3: *Post-processing: after dissection, the sample is resuspended in a buffer.*

The *mmi IsolationCap* used with the *mmi CapLift* technology allows the collection of target areas across the entire microscope slide.

After microdissection, the *mmi IsolationCaps* are snapped into the micro centrifuge tubes to undergo extraction of the bio molecules (Fig. 4.3). After using the recommended extraction reagents for the desired incubation time, the extracted targets are now ready for further genomic and proteomic processing.

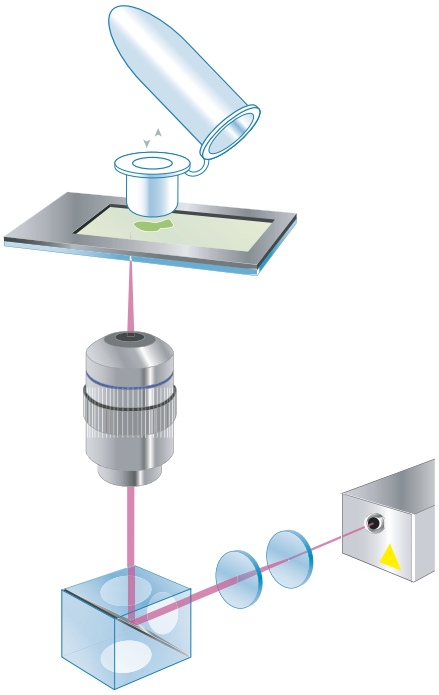


Figure 4.4: *Schematic view of laser microdissection*

4.1.1 System setup

The *mmi CellCut* system consists of a high performance research microscope with motorized scanning stage, an electronically controlled, solid-state laser, laser beam delivery and transfer optics and a high-end workstation with Microsoft Windows and the sophisticated control software *mmi CellTools*.

All usual microscope features are available. The *mmi CellTools* software controls the laser, image capture, and scanning stage actions without blocking any other microscope action.

4.2 Handling of samples

4.2.1 Single-step collection using the mmi IsolationCap

Microdissection for the isolation of cells is only useful if you can remove the parts of the tissue you are interested in from the surrounding tissue and from the slide. The single step collection makes sampling of one or several isolated areas easy and contamination-free.

The single step collection uses a protective membrane and a reaction tube with a special adherent lid.

The purpose of the protective membrane is

- to avoid contamination of the sample; and
- to facilitate easy removal and collection of the cut area.

The laser cuts through the tissue and the membrane from below the stage. The separated tissue together with the membrane is collected on the lid of the reaction tube.

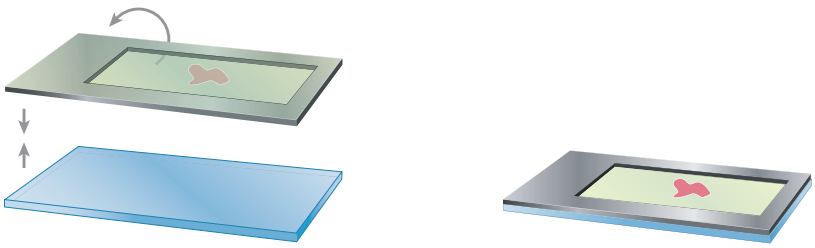
The laser cuts around the selected cells to be isolated. No additional radiation is used for the collection step.

The membrane and the adhesive lid are chemically inert and have no influence on further molecular biological processing. The membrane material is a thin PET membrane. The membrane is transparent and does not perturb the light beam.

The lid of the reaction tube also contains a diffuser insert. The diffuser insert improves the image quality remarkably and can be placed directly on top of the tissue and the membrane during all operations.

4.2.2 Preparation of slides

The Single Step Collection of the dissection requires special slides. The slides are provided with a 1.4 µm thick, clear PET membrane.



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

(b) The assembled sandwich is protected against contamination.

Figure 4.5: *Slide preparation*

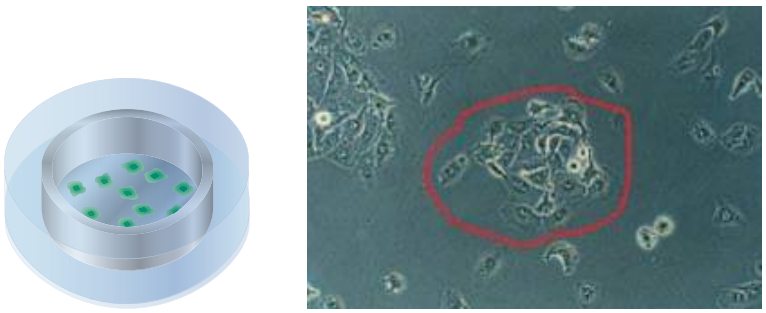
The tissue is mounted on the flat side of the membrane slide, just as on an ordinary slide. After the usual processing the membrane with the tissue is inverted and placed onto a new glass slide and fixed in position on the microscope stage.

Thus the tissue is now under the membrane and protected against contamination.

4.2.3 Preparation of live cells

This section gives a brief overview of options when working with CellCut on live cells. Detailed instructions can be found in the **Live Cell Protocol**, which can be found on the CellTools installation media.

The standard live cell microdissection disposable, the *mmi CellChamber*, consists of an inner stainless steel ring with a membrane at the bottom, in which the live cells can be cultured.



(a) mmi CellChamber (b) Extracting tissue from live cell cultures

Figure 4.6: *Live cell dissection*

Prior to microdissection, the *mmi CellChamber* is placed in a sterile Petri dish, coated with an adhesive bottom. To isolate live cells, the Petri dish is placed on the microscope stage.

Areas of interest can be positively or negatively selected and microdissected by the laser without any need to open the dish or drain the growth medium. The laser quickly and precisely cuts only the membrane to enable easy separation of the adherent targeted live cells.

During this process, the cell cultures can remain in their growth medium with no laser energy focused on them during the isolation. This workflow minimizes the risk of loosing viability, either by stress generated by laser light or by environmental conditions.

There is a new option for high throughput live cell handling with *mmi IsolationCap* technology together with 18-well IBIDI slides.

Through this new method of extracting cell cultures, MMI accomplished an improved sectional view and increased cutting efficiency. At the same time it also allows the use of the *mmi IsolationCap* technology with its known and proven advantages. This guarantees contamination-free and careful handling of the cells.

To receive more information about further applications, please order the mmi Tutorials.

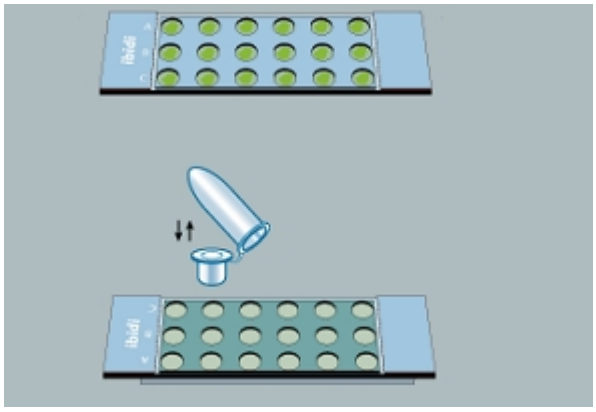


Figure 4.7: *Using 18-well live chamber slides for microdissection*

4.3 Quick start

The *mmi CellTools* software together with the *mmi CellCut* plug-in provide all necessary controls for:

- displaying live video
- saving images and videos
- motorized xy-stage
- adjusting the laser power, speed and focus
- storing the preferred camera settings
- manipulating the automatic cap lift
- manipulating the optional multi cap lift and multislide
- optional handling of motorized microscopes

This results in an easy and user-friendly method for:

- scanning the sample
- documenting the sample
- marking the path for the laser cutting around single cells or cell clusters
- marking several areas to be cut in one operation
- storing the marked cutting paths (for later cutting)
- dissecting the marked sample areas automatically
- collecting the dissected areas without radiation or risking contamination of the isolated material(s)

The operation of all modules is controlled by the easy-to-use *mmi CellTools* software. The main application is responsible for displaying the video, saving the image, adjusting the camera control and basic xy-stage movement.

The *mmi CellCut* plug-in adds the specific *mmi CellCut* functions module. Additional plug-ins for *mmi CellManipulator*, *mmi CellEctor* and *mmi CellExplorer* are available.

4.3.1 System startup

To start *mmi CellCut* follow the steps below:

1. Start up the PC and allow the boot process to complete.
2. Turn on the microscope white light power supply.
3. Switch on the *CellCut* controller by turning the key.
4. Start *mmi CellTools* software and wait until the software has finished the start up and self-test procedure.
5. Power ON the laser by pressing the button on the key switch box (*CellCut II*) or controller electronics box (*CellCut Plus* and *SmartCut Plus*).

Figure 4.8: *The CellTools startup banner*

4.3.2 Handling a new slide

Prepare your sample as described in chapter 4.2 or follow the detailed application notes provided by MMI and mount it on your microscope.

Take care that the stage geometry is properly defined and the stage calibration is valid, see section 5.5.

Use the objective with the lowest magnification (4x on most systems). By clicking on the scan button the software creates an overview of the sample.

By double clicking on the overview you can navigate to the area of interest. You can also use the cursor keys to move the stage or drag the flashing rectangle in the slide overview with the mouse.

Move the cap lift down by clicking on the corresponding button or press *F2*. After lowering the cap lift you should adjust the microscope stage fine focus, as needed.

Activate any button in the toolbar with a mouse click. For example the free-hand drawing tool enables the user to draw a line around the object to be microdissected.

Press the cut button to microdissect the selected object.

Lift the cap holder to collect the microdissected sample.

Now remove the cap and proceed with the application.

4.3.3 System turn off

Shut down *mmi CellTools* by selecting the menu item

Project → *Exit*

or by clicking the main window's close button.

Shut down the laser controller by pressing the button to deactivate the laser and then turn the key to the OFF (vertical) position to switch off the electronic controller.

Shut down the computer.

5 mmi CellTools – Main application

5.1 Main window and plug-ins

Fig. 5.1 shows the extended tool area and explains the main components of the *mmi CellTools* user interface.

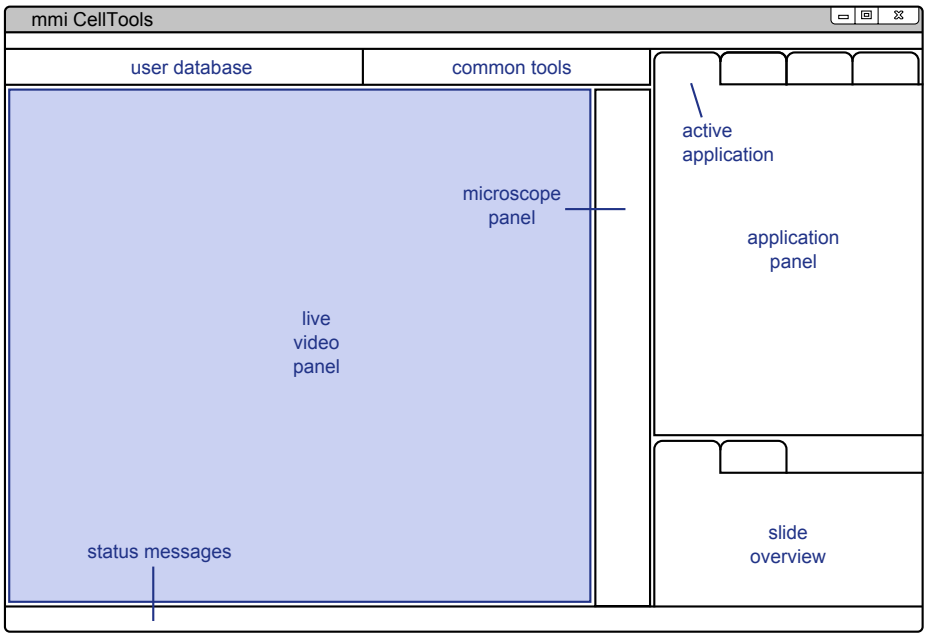


Figure 5.1: *Structure of the mmi CellTools user interface*

The major portion of the window is occupied by the **live video panel**, which displays the current field of view of the microscope and serves as the main area for interaction between the user and the system.

mmi CellTools is a single piece of software that controls all micromanipulation devices of the mmi instrumentation family (see chapter 3). The instrument-specific controls are located in the **application panel**. Switch between applications using the tabs at the top.

Those user interface controls that are common to all applications are located in the tool bar at the top of the window. The **user database** controls provide access to instrument parameters for specific samples, for certain imaging situations, and for each objective (see section 5.2). The remaining **common tools** on the tool bar provide elements for camera and stage control.

On systems equipped with an automated microscope, the microscope panel provides access to z-drive (focus) control and other microscope-specific features (see chapter 7). The **slide overview** panel provides controls for slide scanning and navigation (see section 5.4.4). Finally, the **status messages** bar at the bottom displays current stage coordinates and camera frame rate.

To accommodate for left-handed users, the application and microscope panels can be moved to the left-hand side using the menu item

Edit $\rightarrow$*Left-handed UI*

5.2 User-specific database

All settings saved in the *mmi CellTools* are unique to the current user logged in. *mmi CellTools* fully supports Microsoft Windows user management. During program startup the last settings saved by the active user are loaded.

The database represents a hierarchical structure:

Slide	$\Longrightarrow$	Setup 1	$\Longrightarrow$	Objective 1
			$\Longrightarrow$	Objective 2
			$\vdots$	
	$\Longrightarrow$	Setup 2	$\Longrightarrow$	Objective 1
			$\Longrightarrow$	Objective 2
	$\vdots$		$\vdots$	

Basically the user can save all parameters for each experiment (Slide) he/she runs separately. If the user changes settings for an experiment, settings from recent experiments are still saved and accessible.

The Setup represents all necessary parameters to define an illumination method (bright field, fluorescence, DIC. . .). If you change a parameter in a setup, the change will only be reflected in the current slide.

The Objective represents all objective related settings and calibrations. If you change an objective calibration or objective related parameter, the change only will be reflected in the current setup and slide.

5.2.1 Slide editor

The slide selection box contains all samples you defined in the past. All documentation is saved under this name. To change the database open the slide editor (Fig. 5.2) by pressing the edit button.

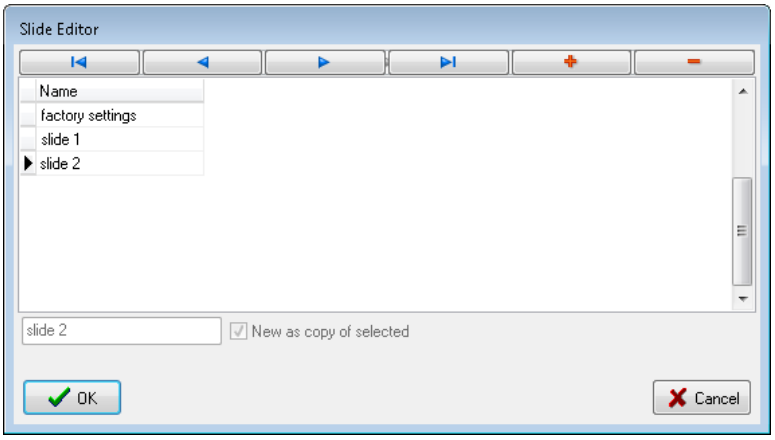


Figure 5.2: *Slide editor*

Use the + button to define a new slide. All parameters from the active slide will be copied. Rename the slide by clicking on the name. Use the − button to remove the active slide.

5.2.2 Setup editor

The setup selection box contains all parameters defined for the active slide. To add, remove or change the database press the edit button. To use this editor follow the same steps as for the slide editor described above. If you create a new slide all setups will automatically be copied from the active slide.

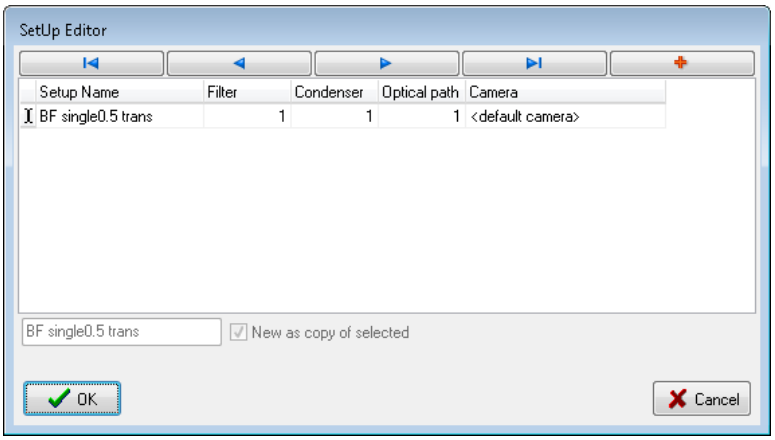


Figure 5.3: *Setup editor*

If you run different types of experiments, e.g., microdissection with bright field, microdissection with fluorescence, or optical tweezers it is recommended to define one setup for each of these experiments.

To rename the defined setups click on the setup name and type in a different name.

The **Filterblock**, **Condenser** and **Opticalpath** settings are only used with motorized microscopes. The use of these parameters is explained in section 7.2.

If your system is equipped with more than one camera, different setups can be associated with a certain camera. You can then switch to a specific camera by selecting a setup defined for that camera.

The camera associated with a setup is displayed in the setup editor (column **Camera**) and can be changed by clicking on the name. The camera used should also be reflected in the setup name. On single-camera systems you should normally select “<default camera>”.

5.2.3 Objective editor

The objective selection box contains all objectives defined for the active setup. To change the database, press the edit button to open the objective editor (Fig. 5.4). To use this editor follow the steps for the Slide Editor described above. If you create a new setup, all objectives will automatically be copied from the active slide.

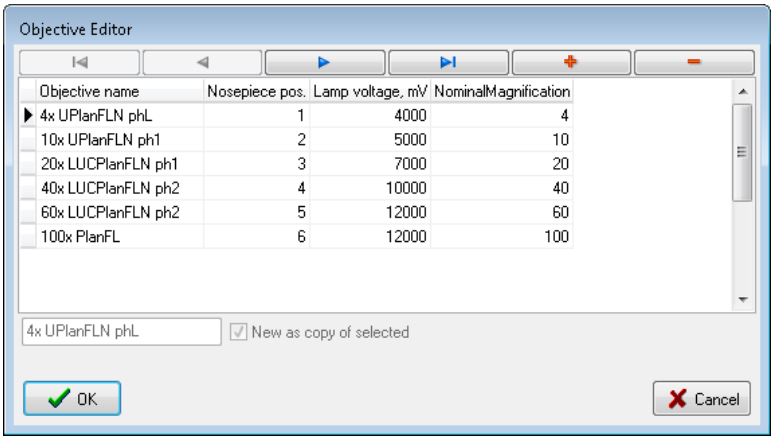


Figure 5.4: *Objective editor*

To rename the defined objective click on the **objective name** and type in a different name.

The **Nosepiece** and **Lamp Voltage** settings are only used with motorized microscopes.

If specified, **Nominal magnification** will be used to compute various objective-

dependent properties (e.g. laser focus, see section 6.2.3.2).

In addition to the parameters displayed in the Objective Editor window, the following information is stored separately for each objective:

- Camera settings (see section 5.3.2)
- Field of view dimensions from camera alignment (see section 5.7.1)
- Lens offset (see section 5.7.2)
- Z-Focus lens offset (see section 7.4.3) (for automated microscopes only)
- Cutting parameters (see section 6.2.3)
- Laser position (see section 6.7.1)

If one of these settings is not correct, please refer to the corresponding chapter.

5.2.4 Default database reconstruction

Each user handles his/her own database. Changes in this user-specific database will not be visible for any other user.

A default database is always stored separately in a central file location. This default database is specific for your instrument and will be set up and handled only during installation and service.

If for some reason, a user's database becomes unusable, the user can recover the default database by the following procedure:

Procedure

1. Close *mmi CellTools*
2. Delete the following folder from the hard disc:

   `C:\Users\`*username*`\AppData\Roaming\MMImCUTDataBase`

   or for Windows XP Systems:

   `C:\Documents and Settings\`*username*`\Application Data\MMImCUTDataBase`
3. Open *mmi CellTools*

The default database will automatically be recovered and is directly visible in *mmi CellTools*.

You will lose all slide and setup data contained in the user database. You will need to recalibrate the entire system.

5.3 Camera operation

mmi CellTools supports a range of scientific digital cameras, supplied by MMI or third parties:

- The mmi CellCamera range of digital cameras for general microscopy applications
- The Andor iXon^{EM} EMCCD camera for ultra-sensitive detection

5.3.1 Multiple cameras

mmi CellTools also supports multiple cameras. If more than one camera is connected to the system, switch between the cameras by selecting a setup dedicated to that camera.

To define the camera used by a setup, open the setup editor and click on the camera field showing `<default camera>` as default. A drop down list shows up in which you simply select the camera to connect to that setup.

Do not use the setup editor to switch between cameras. The setup editor is only used once to create (at least) one setup for each camera. Then, change cameras by selecting the corresponding setup.

5.3.2 Camera settings

For best imaging results the camera can be controlled through a settings window (Fig. 5.5). To open it, click the camera button in the toolbar or select

Video → *Camera settings*

from the menu (*Ctrl + R*).

Image exposure is controlled through the top elements in the dialog. Unless **Automatic exposure** is selected, the exposure time can be controlled through the **Exposure time** slider or the adjacent input field. In **Automatic exposure** mode the slider is inaccessible. If the auto-exposed images are too bright or too dark, use the **Exposure correction** slider to compensate.

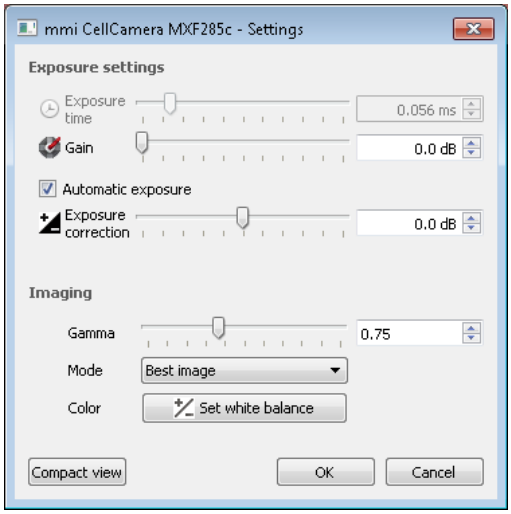


Figure 5.5: *Camera settings for mmi CellCamera models*

Finally, the amplifier **Gain** can be adjusted to yield brighter, but noisier images. Higher gain settings will, however, reduce the required exposure time. For high quality images (e.g. for publications) it is preferable to use a longer exposure time and low gain. Note that the allowed gain range is greater when automatic exposure is off.

The controls in the bottom part of the dialog allow control over color and contrast. Use the **Gamma** slider to control image contrast. The default Gamma value is 0.75, and it may be adjusted over a range of 0.01 to 2.0. Low gamma values are recommended especially to brighten low light fluorescence images. High gamma values reduce noise and improve the black level of the image.

The camera **Mode** provides color settings optimized for various applications. For bright field, the color quality can be optimized with the **Best image** mode. The camera transfers a high quality YUV4:4:4 data stream with full pixel resolution. By selecting **Fastest rate**, the image rate (frames per second, fps) will be maximized. In this mode up to 20 fps (depending on your camera model) with full pixel resolution are displayed. For fluorescence applications, the contrast can be optimized with the **Low light** option. The camera transfers a high quality RGB data stream with full pixel resolution. This setting is used mainly in combination with the **Gamma** and **Gain** setting. If you select **Binning**, the camera operates at a lower resolution, but yields brighter black and white images at a very fast frame rate.

Color shifts, mainly caused by changes in lamp brightness, can be corrected using the white balance function. To set the white balance, first locate an empty, transparent part on the sample slide, then click the **Set white balance** button. If the image is too bright or too dark the white balance fails.

The white balance function is also available in the drop down menu next to the camera button, in the main menu under

Video →Adjust white balance

or using the keyboard shortcut *Ctrl + W*.

Do not use the Set white balance option in fluorescence imaging.

All camera settings, including white balance, are stored separately for each objective. This eliminates the need to adjust the camera after each objective change. Optionally, the software uses a single white balance setting for all objectives. This is recommended for non-automated microscopes. Select

Video →Save white balance per objective

to enable or disable this function.

5.3.3 Freeze video / live video

In fluorescence applications it makes sense to freeze the video when you have acquired a good image. After freezing the video you can close the fluorescence shutter and go ahead with drawing and cutting your dissectates without further photo bleaching of the fluorescence dye.

To freeze the video use the menu item

Video →Video freeze

To go back to live video presentation select

Video →Video live

5.3.4 Saving images

To save an image simply press the **Save image** button, use the menu item

Video →Save Image

or press *Ctrl + S*.

The file dialog allows you to specify the image filename and image type (JPG, BMP, PNG and TIF). The image will be saved with maximum pixel resolution. By selecting the **Include drawings** check box you can save a screen shot from the current video image including all markers and drawings.

When saving images with drawings, the system will produce a screenshot from the live video area, resulting in reduced pixel resolution of the saved image.

Press the **Copy image to clipboard** button to make the current image available to other applications. The image will be copied to the Windows clipboard in order to paste it into e.g. office and image processing applications. The same result can be seen with the menu item

Edit →Copy Image

The **Include drawings** does not affect the **Copy image to clipboard** function. The clipboard always receives the original camera image without drawings.

5.3.5 Recording movies

mmi CellTools allows you to record live camera images into video files (AVI format). It supports compressed and lossless video codecs and allows you to record time-lapse movies.

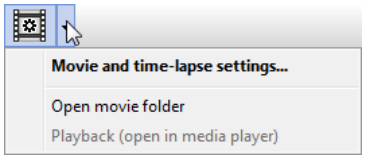


Figure 5.6: *Movie recording functions in the tool bar*

Click the **Record** button to start recording a new movie. CellTools will automatically create a new AVI file in the movie folder and record video until you press the **Stop** button. If **Auto-open** is enabled (see below), the movie will open in Media Player immediately after recording. Otherwise, you can access recorded movies through the drop-down menu items *Playback (open in media player)* and *Open movie folder*.

By default, the movie folder is located in

`My Documents\mmiCellTools\Movies`.

Movies are compressed with a video codec, currently either Windows Media Video 9 or XVID. To play back those movies, your computer will require installation of the same codec. Codecs are found on the mmi CellTools installation media.

Also note that any shapes drawn will not be recorded.

5.3.5.1 Movie settings

Detailed aspects of movie recording can be controlled by opening the **Movie settings** dialog (Fig. 5.7).

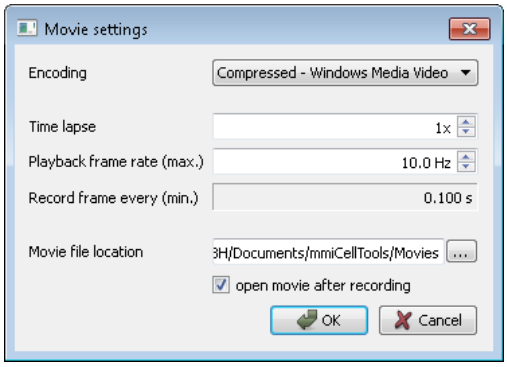


Figure 5.7: *Movie settings*

The **Encoding** specifies the format (codec) to store the video frames. Table 5.1 gives an overview of available encodings and their application.

Table 5.1: *Movie encoding options*

Encoding method	Image quality	Recommended for
Uncompressed	Lossless	Short videos Extracting frames in full quality
Compressed – Windows Media Video (default)	Lossy	General use Playback primarily on Windows systems.[1]
Compressed – XVID	Lossy	Color video (not usable for black and white cameras) Playback on any system with XVID codec installed

To create basic time lapse movies only adjust the **Time lapse** factor as desired. This factor defines the speed at which the movie will play back. Enter “1x” to turn off time lapse (standard time).

The **Playback rate** specifies at how many frames per second the final video should be played back. (This is identical to the recording rate unless using time lapse.) The default playback rate is 10 Hz and should be suitable for general use.

The **Recording interval** specifies the minimum time interval between two recorded video frames. The recording interval is computed from time lapse factor and playback rate and is for information only.

[1] To play back Windows Media Video files on Mac OS X, install Windows Media Player for Mac OS (Flip4Mac), which is downloadable from the Microsoft website.

> Depending on the current camera settings and available computer hardware it may not be possible to achieve very short recording intervals.

To control the rates at which frames are recorded and played back, change the **Playback frame rate**. If time lapse is off, the movie is played back at the same rate at which it was recorded. When using time lapse, the recording rate is automatically adjusted. The dialog displays the recording interval, i.e. the time elapsed between two frames. This interval significantly influences the size of the resulting video file.

The **Movie folder** input field allows you to specify the destination folder where movie files are saved. For maximum performance, this should not be a network folder. Check the **Open movie after recording** option to have movies automatically open and playback in Media Player after recording.

5.4 Motorized stage control

The movement of the motorized stage is controlled by the *mmi CellTools* software.

5.4.1 Mouse movement

Choose the move mode by clicking the hand tool button. You can reach the same functionality by pressing the right mouse button with the cursor in the video window. A menu pops up and you choose *Move stage* with a left mouse click.

In the move mode the cursor in the video panel always appears as a hand. By clicking and dragging the left mouse button, the stage directly follows the mouse movement.

> If stage movement does not exactly follow mouse movement, you may need to carry out the camera alignment procedure (see section 5.7.1).

You can quickly switch to move mode and back again by pressing *Space*.

5.4.2 Keyboard movement

The main arrow keys and numeric pad arrow keys can be used to move the stage in discrete steps or continuously at constant velocity (Fig. 5.8).

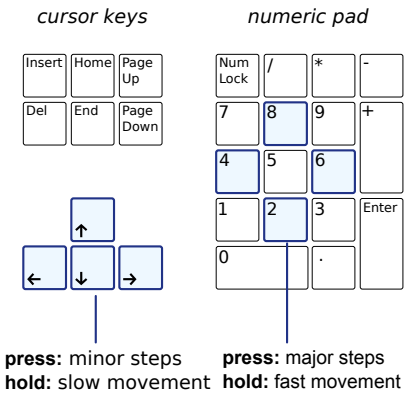


Figure 5.8: *Stage movement using the keyboard*

A single press of a cursor key moves the stage by a defined distance. By default, this is 10% of the screen for the cursor keys and 90% of the screen for numeric pad keys.

> *NumLock* must be turned on for numeric cursor keys to work as expected.

For moving larger distances, hold down the respective key. The velocities for the two sets of movement keys can be set independently (see section 5.4.3). By default, the cursor keys move slowly and the numeric pad keys move fast.

In applications where you need movement by well-defined distances only, the continuous movement can be suppressed by enabling *Caps Lock*.

5.4.3 Stage movement settings

To change stage movement settings, press the **Stage movement settings** button, click the menu bar item

> *Stage →Movement settings*

or press *Ctrl + P* to get the window in Fig. 5.9.

Acceleration This value determines the stage motors' acceleration for both keyboard and mouse movement.

Reducing this value may facilitate the handling of liquid suspension samples. For all other samples we recommend using 100%.

Units For the arrow keys on the keyboard you can set the stage settings in two different units:

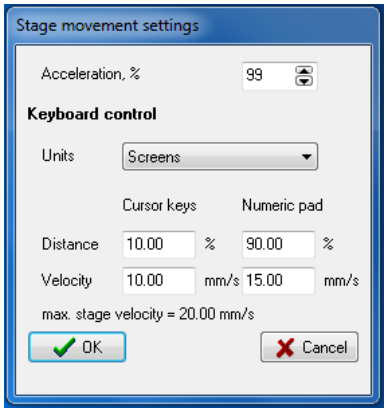


Figure 5.9: *Stage movement settings*

- Screens (or percentage of screen)
- Micrometers (µm)

When using micrometer units, note that you may have to adapt distances with every objective change.

Distance and velocity These values can be chosen independently for cursor and numeric pad keys. By default, cursor keys are used for minor steps and numeric pad keys for major steps.

Values that are outside the allowable range will be shown in red.

5.4.4 Overview scan

Fig. 5.10 shows an overview of your sample (the “roadmap image”).

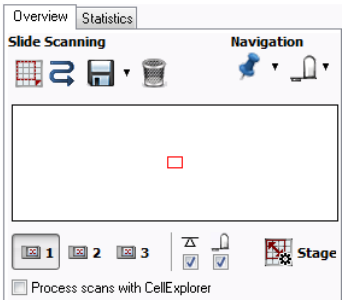


Figure 5.10: *Slide overview*

The overview scan is started by pressing the **Start scan** button. If no area of interest is defined, the maximal scan area will be used. The maximum scan area is:

- the inner part of a *mmi MembraneSlide*
- the square around a petri dish

- the square around the cap of a single cap holder
- the square around the well of a custom grid, if only one well is defined.
- the complete slide for all other microscope slides
- the complete mulitwell plate

The current field of view is indicated with a red blinking rectangle or point. You can move this red frame by clicking and dragging the left mouse button. The motorized stage moves automatically to the chosen detail. With this navigation method you always see the position on the slide.

You can also move to the position of interest by double clicking into the overview area with the left mouse button.

Define the area of the scan with the **Select area** tool. After pressing the button you can select the area of interest in the overview window using the mouse. Only the area of interest will be scanned.

Begin the scan by pressing the **Start Scan** button. You can always interrupt the scan with the **Stop scan** button. Also pressing the *Esc* key will interrupt the scan.

The scanned image can be made available to other applications. Use the **Save** button or the corresponding menu item to save the overview image to disk. Alternatively select *Copy image to clipboard* from the popup menu next to the **Save** button to copy the overview image into the Windows clipboard and paste it in other applications.

To erase the scanned image, click on the **Clear overview image** button.

To move from one slide to another you can directly select the **target slide** button. Alternatively you can move to another slide with the keyboard arrow keys. The slide number indicator will automatically adjust to the current slide.

If slide number indicator does not fit with the position of the stage, please refer to section 5.5.

5.4.5 Pin positions

With the **pin** button you can save the current stage position. By clicking on the arrow to the right you obtain the pin positions menu (Fig. 5.11).

You can select a pin, which moves the stage to the respective position. The last two entries enable you to delete either the currently selected or all pin positions.

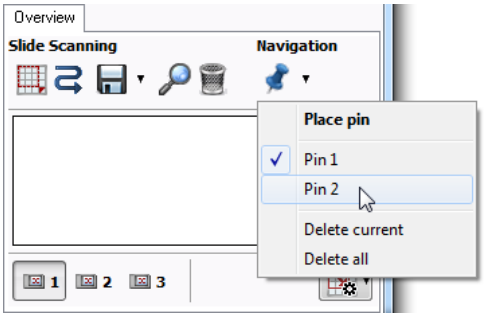


Figure 5.11: *Pin positions*

5.4.6 Inspection position

Objects collected on the cap can be easily viewed when the cap is set down in an empty location on the slide. For this purpose you can define an inspection position.

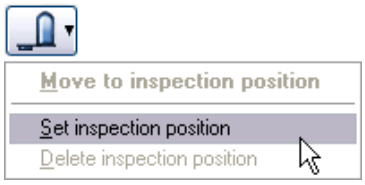


Figure 5.12: *Setting the inspection position*

To define the inspection position click on the triangle at the right edge of the **inspection button** (Fig. 5.12) and select *Set inspection position*. Click on the desired location in the slide overview. A cap marker will appear in the slide overview if **Show inspection position** is activated.

After the position has been set, you can quickly move between your current working position and the inspection position by clicking the inspection button. The cap lift will be lowered automatically.

To delete the cap marker, select *Delete Inspection Position* from the popup menu.

5.4.7 Process scan with CellExplorer

To automatically analyse all overview scan tiles with the image analysis software *mmi CellExplorer* check the **Process scans with CellExplorer** checkbox in the **Overview** panel.

> You need to switch in the *mmi CellExplorer* panel and define a parameter set once, before **Process scans with CellExplorer** will

work properly, see chapter 8. After switching back to *mmi CellCut* the calculated shapes are automatically available.

5.4.8 Shape navigation

In *mmi CellCut* objects in the sample, like cell areas or cells, are displayed as shapes. These shapes can be created manually or by using *mmi CellExplorer*, see section 5.4.7 and 8.

Once you created shapes you navigate between the shape by using

- move to first shape, *Home* key
- move to last shape, *End* key
- move to next shape, *+* key
- move to previous shape, *-* key

In *mmi CellCut* the keyboard keys allways navigate inside the current group, see section 6.3.4. The shapes can be directly used for cutting.

5.5 Stage geometry and stage viewer

In order to properly navigate through your samples the geometry of the stage insert must be properly defined. The stage insert is the mechanical holder of your slides or multi-well-plate, which usually simply snaps into the microscope motorized stage. These definitions are a precondition for the use of the overview, see section 5.4.4 scan.

The stage viewer Fig. 5.13 can be opened via the menu

Stage →Show insert (Ctrl + I)

or alternatively by the **Show stage insert** button in the overview panel.

The window has following main purposes:

- configure the geometry of the
  - stage insert and
  - slides or microplates, each containing the active regions called wells.
- assign a role to each well
- display and zoom the overview images

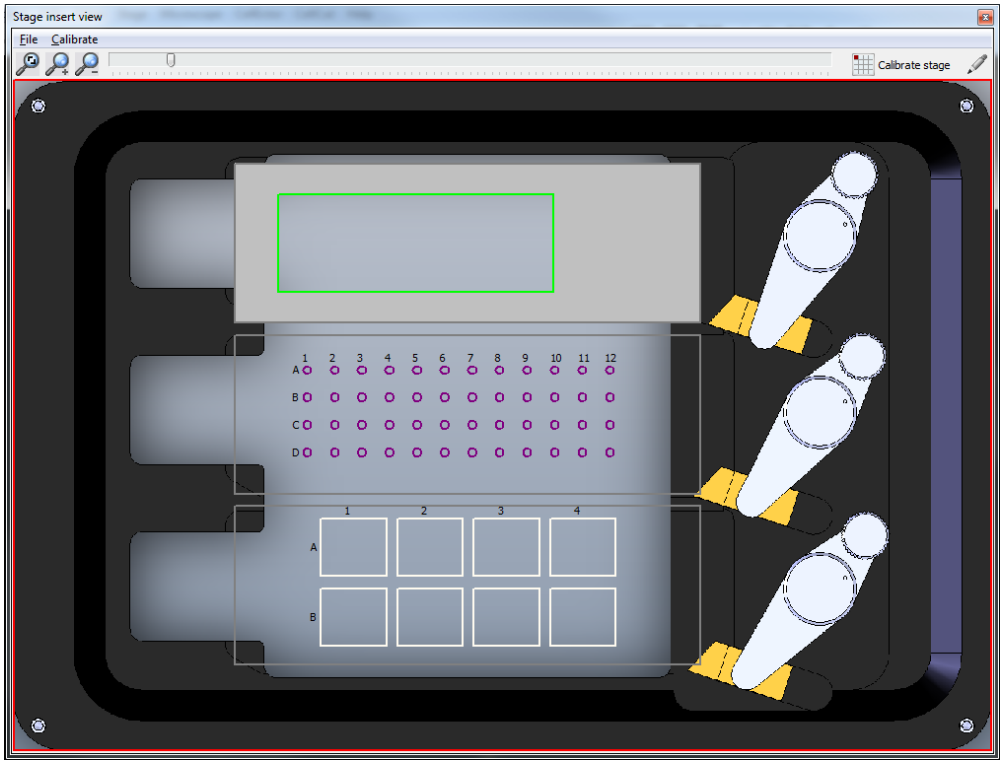


Figure 5.13: *The stage viewer shows the geometrical configuration of your stage insert. The position the insert and the slides can be adjusted so that overview images exactly match the stage configuration.*

- navigate through your samples, by double-clicking on the target position
- exporting and importing stage geometries
- calibrate the stage

The stage insert is divided in following regions:

- stage insert
- slides or microplates
- active regions, called wells

By moving the mouse over the stage insert, the current region (the stage insert itself, a slide or a well) will be highlighted. That region can be selected via a **left mouse click**. Selected regions will be displayed with a red border. The editor button will now open the configuration panel of the selected region. For wells a multi-selection is possible. The configuration of the regions is explained in the following sections 5.5.1, 5.5.2 and 5.5.3.

The full geometry configuration can be exported into a file by

File →Export

Using

File →Import

the configuration can be reloaded into the stage geometry viewer.

5.5.1 Stage inserts

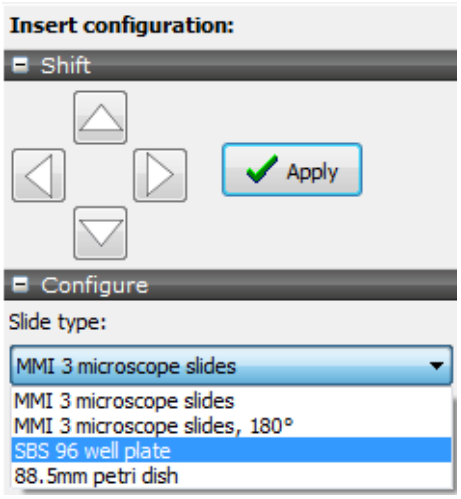


Figure 5.14: *Stage viewer: Insert configuration panel. You can select different insert types and shift the whole insert to adapt it to overview images.*

In the configuration panel (Fig. 5.14) different types of slide inserts can be selected:

- insert for three microscope slides, clamps on the right hand side
- insert for three microscope slides, clamps on the left hand side
- insert for multiwell plates in SBS format
- insert for 88.5 mm petri dish

Second you get the option to shift the whole insert. This feature allows you to precisely overlay a scanned overview image with the displayed slides. It is recommended to first shift slide 1 to fit it's stage insert position. If the overview image of slide 1 does not exatly fits into the slide shift the insert as described in chapter 5.5.5.

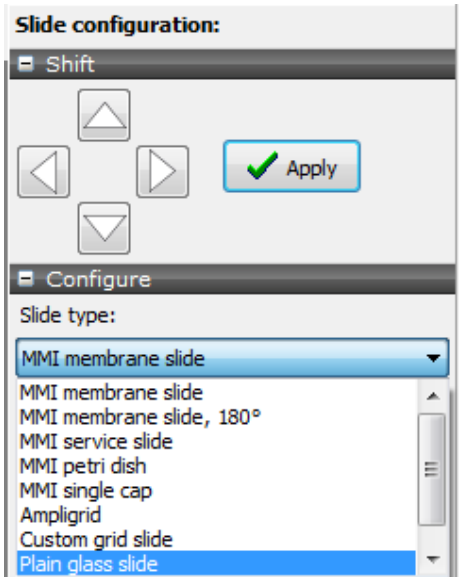


Figure 5.15: *Stage viewer: Slide configuration panel. You can select different slide types and shift the whole slide to adapt it to it's overview image.*

5.5.2 Slides

Depending on the stage insert selected in section 5.5.1, the configuration panel for slides (Fig. 5.15) offers a selection of different slide types. If your slide type is not listed, you can define and configure a custom slide.

- MMI membrane slide
- MMI membrane slide, mounted roted by 180°
- 8 well MMI service slide
- MMI petri dish
- MMI single cap holder
- Ampligrid
- plain glass slide

Additionally you shift the slide. This feature allows you to precisely overlay a scanned overview image with the displayed slide. By the slide shifting procedure you can correct the geometry of your stage insert to precisely reflect the hardware.

> If overview scan in slide 1 match the slide configuration, it is recommended only to shift slide 2 and 3. It is acceptable that slide 2 and slide 3 do not perfectly match the displayed stage insert positions.

If overview scans in slide 1 are shifted against the geomtetry displayed, first calibrate the stage origin, see section 5.5.4. If this is not sufficient follow the procedure described in section 5.5.5

5.5.3 Active regions, wells

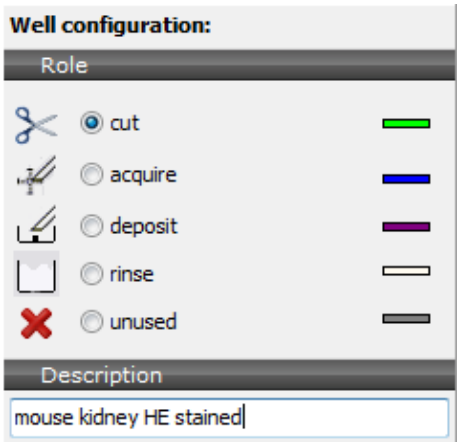


Figure 5.16: *Stage viewer: Well configuration panel. You can select different well rules and add descriptions to each well.*

In the well configuration panel you can define follwing rules for each well:

- region for microdissection

- collection well for CellEctor

- deposit well for CellEctor

- rinse well for CellEctor

- unused well

Currently thess rules are exclusively used by *mmi CellEctor*. Additionally you can add own descriptions to each well.

5.5.4 Stage calibration

Under normal circumstances, the stage calibration remains valid unless the stage is moved manually or using a software other than *mmi CellTools*.

If the software detects an invalid stage calibration, slide scanning will be disabled and the overview scan will show a red warning sign.

In this case or if the overview scans are shifted against a correct stage geometry stage recalibration is required.

To recalibrate the stage start the calibration procedure by on of the following options:

- press the **Calibrate origin** button in the stage viewer.
- *Stage →Calibrate origin* *(Ctrl + O)* in the main CellTools window.

During the calibration process the microscope objective will move down and the stage will move to its limit switches. If the stage geometry is still not matching a scanned overview image, the stage geometry configuration needs to be adjusted, see sections 5.5.2 and 5.5.5.

5.5.5 Stage geometry first configuration

To adapt the insert position to scanned overview images proceed as follows:

Procedure

1. select the mounted insert in the insert configuration panel
2. select the mounted slides in the slide configuration panel
3. shift the first slide to it's position in the stage insert, see section 5.5.2
4. calibratre the stage origion, see section 5.5.4
5. scan an overview image in slide 1, see 5.4.4 and adjust the image to the slide position by shifting the whole insert. To get a precise positioning we recomment to adjust the image of the left upper corner of an membrane slide (Fig. 5.17) or the image of wells of an ampligrid to the displayed slide.
6. press **OK**
7. adjust the other slides to their overview images, see section 5.5.2

This procedure only has to executed for a new stage insert. If the configuration is properly set, a mismatch between the scanned image positions and the slide configruations indicates a corrupt stage origin calibration. Recalibration of the stage origin, see section 5.5.2, will be sufficient.

You are now ready to use slide navigation and scanning. The field-of-view indicator and the dimensions of the slide overview will adapt during calibration.

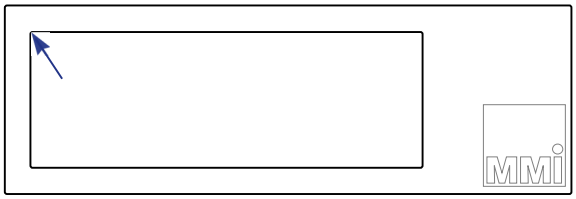


Figure 5.17: *Suggested positions on a mmi Membrane slide to precisely adjust the stage insert position*

.

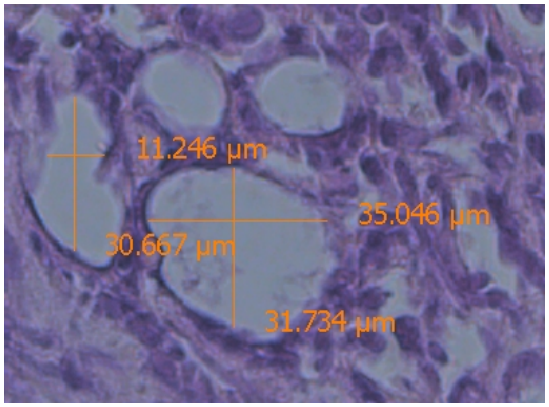


Figure 5.18: *Distance measurement*

5.6 Distance measurement

Select the measurement tool to measure distances on the sample.

Press the left mouse button and drag the mouse. After releasing the mouse button you can see the measured distance (Fig. 5.18)

5.7 Calibration

5.7.1 Camera alignment

For the software to correctly overlay objects onto the camera image, and to correctly measure distances, the exact width and height of the field of view in millimeters must be known for each objective and optical setup. *mmi CellTools* offers a fully automated calibration procedure that makes it easy to measure these dimensions. The procedure also allows to minimize positioning errors due to a tilted camera mount.

Camera alignment must be carried out independently for each objective, as each objective has a differently sized field of view.

To prepare automatic alignment, place an arbitrary sample on the microscope, navigate to an area that yields an image of good contrast and focus. A slide with printed text on its surface is also suitable as long as it can be optimally focussed. Start alignment by selecting

Stage →Align camera

from the main menu.

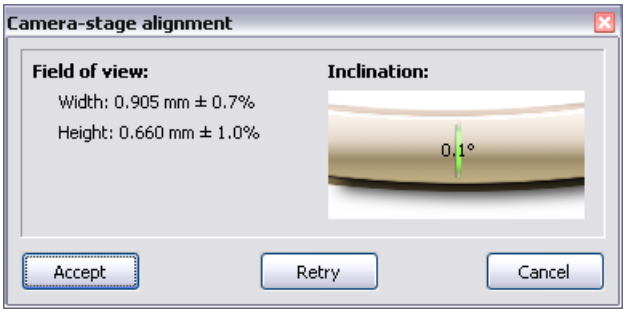


Figure 5.19: *Camera alignment results*

If automatic alignment was successful, a dialog (see figure 5.19) displays the results, which include:

- The measured width and height of the field of view,
- The relative standard error of the measured width and height, and
- The measured inclination of the camera.

Ideally, camera inclination should be very close to zero ($< 0.3°$). If it is larger, eliminate camera tilt before proceeding (see section 5.7.1.1). The lower the standard error of width and height, the more accurate positioning can be achieved. The standard errors can be reduced through optimal focussing and by viewing an area of high contrast at optimal illumination. (Also ensure that microscope illumination is set up correctly.)

If satisfied with the results, click **Accept** to use the measured values. In some cases, the automatic procedure does not yield an acceptable result and displays a failure message. In this case you may adjust lighting, view a different area on the sample and retry.

When finished, check that the stage exactly follows mouse movement when using the Move stage tool. Also verify that any shapes that you draw match the objects as close as possible after you move the stage. If not, repeat the procedure.

When working with a motorized microscope, *mmi CellTools* will ask whether you would like to use the results for all objective items at this nosepiece position. If you choose yes, the measured values will be used in all other setups as well (see section 5.2).

5.7.1.1 Eliminating camera tilt

If the camera was unmounted or accidentally pushed out of its position, the following procedure will help restoring the upright mounting position.

Procedure

1. Start automatic stage alignment as described above
2. If camera inclination is larger than 0.3°, slightly loosen the camera's mounting screw using the supplied hex wrench, turn the camera by a very small amount and retighten screw (lightly).
3. Repeat alignment until inclination is optimal
4. Fully tighten mounting screw.

5.7.2 Lens offset calibration

Because of mechanical and optical tolerances two objectives never have exactly the same optical axis. You see this effect by observing pixel shifts in the video when you change an objective. The **Set lens offset (paraxial)** function is introduced to search and draw your dissectates in one objective and cut with a second objective.

When you install a new objective into your microscope you have to calibrate the Lens Offset. Over the time of operation it may be necessary to recalibrate, when contours and cutting lines are no longer exactly fitting.

Procedure

1. Please note the Lens offset calibration procedure should go from the objective with highest magnification to the objective with the smallest magnification. The standard order is:

   (a) 40x to 20x
   (b) 20x to 10x
   (c) 10x to 4x

   If your system is set up with other magnifications please select the order of calibrations in the same manner.

2. Verify that the camera is aligned with the stage (movement follows mouse) and mounted in an exactly upright position.
3. Mount a slide with a sample.

4. Select the start (higher magnification) objective and the corresponding objective in the software
5. Move an easily noticeable object to the centre of the video screen.
6. Start the Lens Offset calibration procedure with the menu item

   CellCut →Objective →Lens Offset (paraxial)
7. Draw a line around that object
8. Change the objective at the microscope and in the software
9. Move the shape exactly over the selected object
10. Complete the calibration with a right mouse click.

You are now asked whether you would like to calibrate further objectives. If you answer “yes” you are asked to repeat steps 8 and 9 for another objective.

Ensure that the shape is sufficiently large to start with. If at any point during the calibration, the shape becomes too small to be moved accurately, you may simply delete the shape and proceed with a new, larger shape. Alternatively, you may stop the calibration procedure at a mid-range objective and later restart the calibration procedure, starting from that objective downwards.

You can cancel the calibration procedure with the *Esc* key.

5.8 Multi-user report

Users can get information about time spent using *mmi CellTools* for each Windows user account.

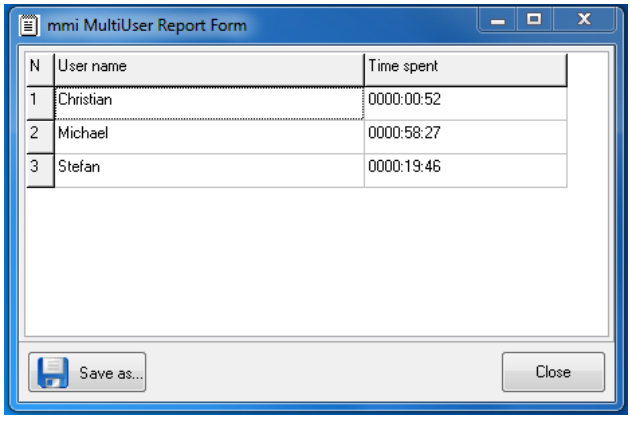


Figure 5.20: *Multi-user report*

Select the menu item

Project →Usage report

or start it from

Start →All Programs →mmi CellTools →mmi MultiUser Report

A time format is hhhh:mm:ss.

5.9 Help

5.9.1 Help topics

The user manual can be opened directly inside the *mmi CellTools* software. By pressing *F1* on the keyboard the PDF file of the User Manual shows up. You also launch the user manual by selecting the respective item in the Help menu.

5.9.2 MMI online

If your PC is connected to the internet you can launch the MMI web page by the menu item

Help →MMI online

Questions about the system can be sent to MMI service staff by the item

Help →Online Support

5.9.3 Version info

Information about the currently installed software version can be found under

Help →Version info

6 mmi CellTools – CellCut plug-in

As described in section 5.1, the special features of the *mmi CellCut*, the *mmi CellExplorer* and *mmi CellManipulator* are installed as separate plug-ins (software modules). The *mmi CellCut* plug-in appears as a separate tool panel on the right side of the program window.

To switch from one plug-in to the other you only have to click on the appropriate tool panel (Fig. 6.1).

6.1 Drawing the cutting contour

6.1.1 Basics

You can draw using the mouse on the video panel. If your system is set up with an interactive pen display you can draw directly on the screen using the pen.

The laser cuts along the contour which you have drawn around the interesting area you want to microdissect. The following calibration and settings need to be done correctly before you can start cutting:

1. Camera alignment, section 5.7.1
2. Cutting velocity, section 6.2.3.1
3. Laser focus, section 6.2.3.2
4. Laser power, section 6.2.3.3
5. Laser position, section 6.7.1

After drawing the contours of the shapes press the **cut** button and all defined contours will be cut. 

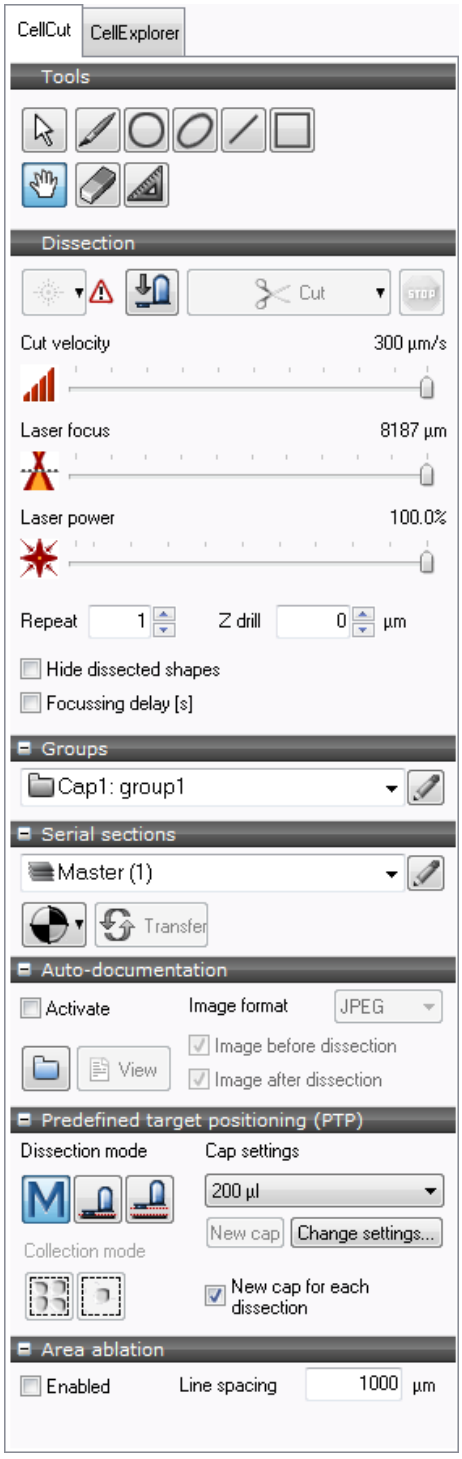


Figure 6.1: *The CellCut plug-in panel*

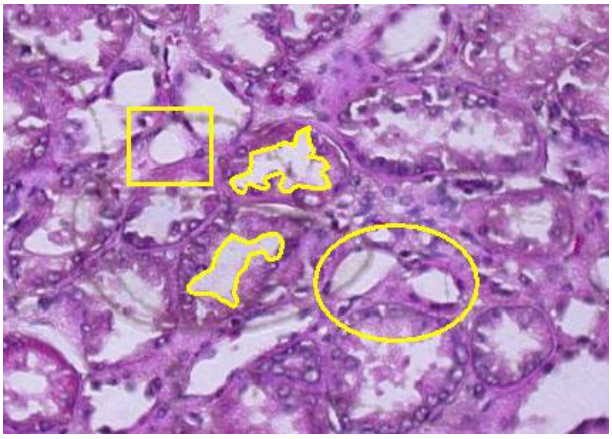


Figure 6.2: *Defining contours to cut*

6.1.2 Drawing tools

The drawing tools can be switched using the buttons in the tools panel

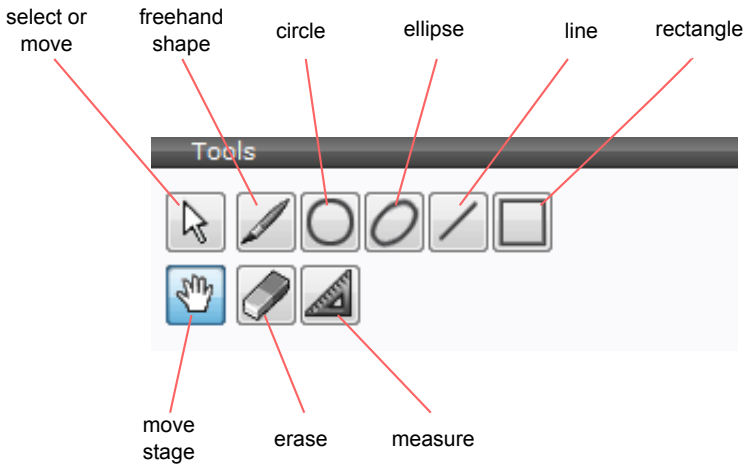


Figure 6.3: *Drawing tools*

The drawing mode can be activated through the buttons in the *mmi CellCut* plug-in or in the context menu (Fig. 6.4). The context menu can be activated by pressing the right mouse button (with the cursor in the video window) and by choosing *Draw/edit shape*. The last used drawing tool will be activated.

A quick switch between moving and drawing mode is possible by pressing the *Space* key on the keyboard.

6.1.2.1 Select and move shapes

With the **select or move** tool you can select and activate single contours with a left mouse click. To adjust the positions of individual shapes, click and drag

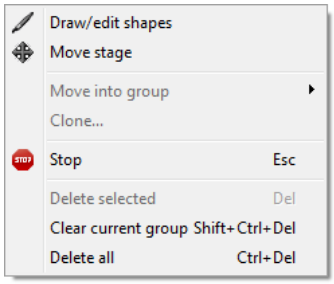


Figure 6.4: *CellCut context menu*

on a shape.

To adjust the positions of all shapes (e.g. to compensate for a shifted sample) use the move stage tool (section 5.4) while holding down the *ALT* key.

6.1.2.2 Freehand

The **freehand** drawing tool allows you to define arbitrary shapes. Use the left mouse button to draw the contour around the area of interest.

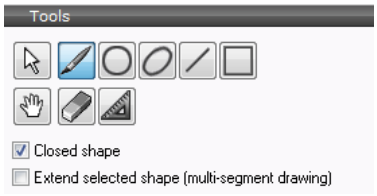


Figure 6.5: *Freehand drawing options*

CellTools normally closes the contour automatically when you release the left mouse button. If this is not desired, you can turn it off by unchecking the tick box “Closed shape”.

Some objects, especially those larger than the field of view, cannot be traced with a single drawing operation. For such cases multiple segments can be combined into a single shape.

Procedure

1. Start outlining the object normally using the freehand tool as far as the field of view allows.
2. Move the stage such that the end of the drawing is still visible and you can continue drawing.
3. While holding down the *SHIFT* key, draw the second segment. (You may release the key while drawing.) Once you have finished drawing, the two segments will be attached.

4. Repeat steps 2–3 as necessary.

As an alternative, you can check the tick box “Extend selected shape (multi-segment drawing)” instead of holding the *SHIFT* key. Remember to uncheck it when you have finished with the last segment.

Multi-segment drawings may be easier to do when “Closed shape” is off (unchecked).

6.1.2.3 Circles

The **circle** tool is suitable for creating circular shapes.

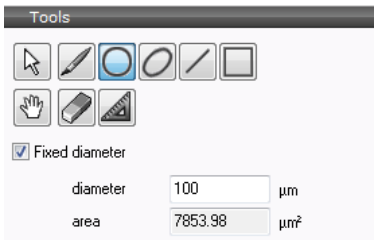


Figure 6.6: *Circle tool*

Fixed-size circles can be created by checking “Fixed diameter” and typing the value in the corresponding input box (Fig. 6.6).

6.1.2.4 Ellipses

Certain shapes can be approximated as an ellipse. Drawing an ellipse is done in two steps:

- define the major axis (longest distance)
- define the minor axis (width)

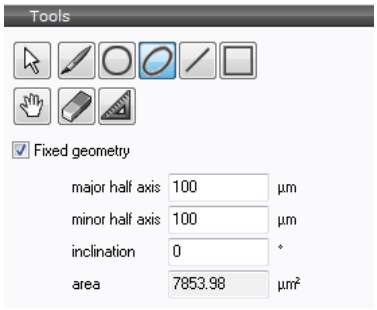


Figure 6.7: *Ellipse tool*

Fixed-size ellipses can be created by checking “Fixed geometry” and typing the values in the corresponding input box (Fig. 6.7)

6.1.2.5 Lines

Cutting straight lines may be useful for ablation or cell-surgery type experiments, as well as for cutting tests.

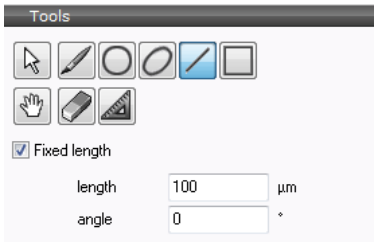


Figure 6.8: *Line tool*

Fixed lines can be created by checking “Fixed length” and typing length and angle in the input boxes. To reverse the cutting direction, enter an angle of 180°.

6.1.2.6 Rectangles

Rectangles provide a quick method to outline objects of interest. Combined with the area ablation option (section 6.4) they are useful for ablation type experiments.

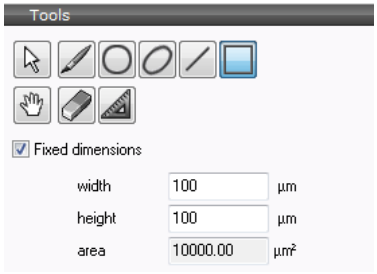


Figure 6.9: *Rectangle tool*

Fixed-size rectangles can be created by checking “Fixed dimensions” and typing the values in the corresponding input box (Fig. 6.9).

6.1.2.7 Deleting shapes

There are several ways to remove shapes:

- Deleting arbitrary shapes using the eraser tool
- Deleting the current (highlighted) shape by pressing *Del*
- Using the context menu (Fig. 6.4)
- Using the group statistics window (section 6.3.6).

To delete all shapes, or all shapes from the current group, use the context menu (Fig. 6.4) or corresponding keyboard shortcuts *Ctrl + Del* and *Ctrl + Shift + Del*.

6.1.2.8 Copying and pasting shapes

To copy the active contour use the menu item

Edit →Copy shape

Select

Edit →Insert shape

to insert the copied shape.

The corresponding keyboard shortcuts are *Ctrl + C* for Copy and *Ctrl + V* for Paste.

6.1.2.9 Cloning

Cloning is a more controlled way of replicating shapes. This function is especially useful for laser ablation experiments, or when you need to collect multiple pieces of tissue at regular distances. The clone function allows you to create multiple copies of a shape, where copies are arranged in a rectangular grid.

To start, select the shape you would like to clone and select

Edit →Clone...

or use *Clone...* from the context menu (Fig. 6.4).

The dialog allows you to specify the number of shapes per row and per column, as well as the distances between rows and columns. Depending on your application, you may choose whether distances are measured between the shapes' edges or from center to center.

Click the **Preview** button to see the result, or click **Ok** to generate the clones.

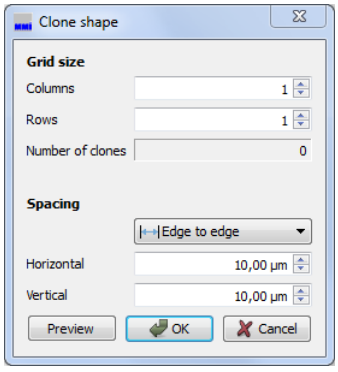


Figure 6.10: *Clone shapes*

Note that, on systems with an automated microscope, the system will focus on each cloned shape using the sample plane focus mechanism (section 7.4.2), if enabled. If clones are out of focus when cutting, either redo the sample plane definition or disable plane tilt focussing.

6.1.2.10 Navigating between shapes

There are several keyboard commands that allow you to locate your shapes and navigate between them. These are illustrated in Fig. 6.11.

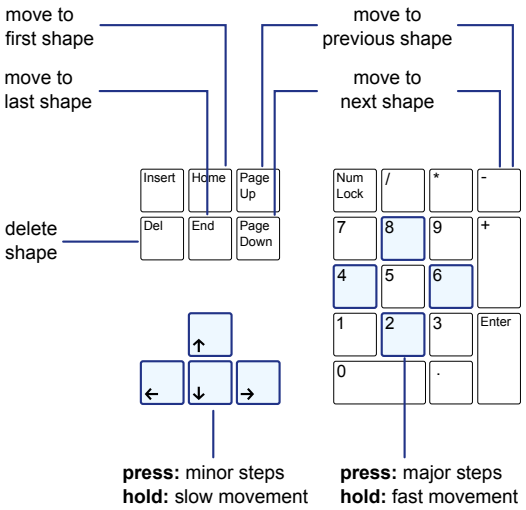


Figure 6.11: *Keyboard navigation*

6.2 Cutting dissectates

6.2.1 Single laser shot

The laser shot button is used to activate the laser for a short time period, or to generate a single laser pulse (available on CellCut II systems only).

The time duration can be adjusted from the drop-down menu next to the button (Fig. 6.12). The default setting of the single laser shot time is 50 ms. A range of 50–100 ms is typical.

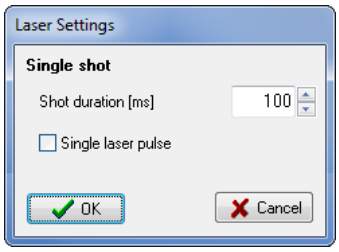


Figure 6.12: *Single shot laser settings*

CellCut II systems can be configured to produce a single laser shot instead of timed activation. This will achieve the shortest possible laser activity and therefore applies the minimal achievable energy to the sample. To change the default mode, activate the checkbox **Single pulse**.

Both laser shot modes can be triggered independently of the above setting using the commands in menu *CellCut →Laser* or via the keyboard shortcuts *Ctrl + M* (timed interval) and *Ctrl + Shift + M* (single pulse).

6.2.2 Overview

The automated cutting along the contours makes your work easy and fast. You can decide if you want to cut one area or a group of areas.

For most applications the best cutting results are observed with the 20x or the 40x objective. The user must select the appropriate objective in the objective selection box (Fig. 6.13).

Figure 6.13: *Objective chooser*

The laser setup includes the following parameters (Fig. 6.14).

- Cut velocity (of stage movement)

- Laser focus
- Laser power

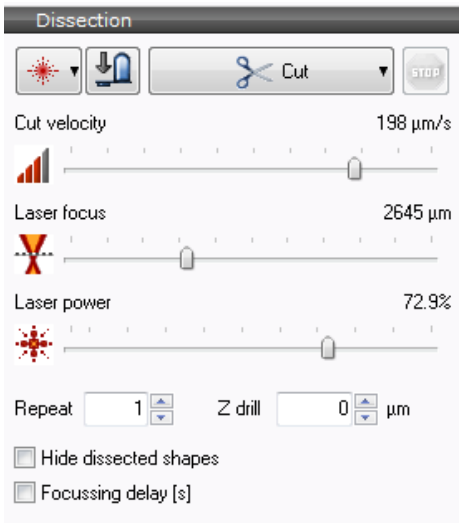


Figure 6.14: *Dissection panel with laser parameters*

It is essential for optimal cutting performance to properly adjust these parameters for each objective and tissue type.

6.2.3 Dissection parameters

6.2.3.1 Cutting velocity

The velocity slider defines how fast the stage moves during the cutting procedure itself (unit: µm per second). It does not affect any other movement parameters of the stage.

6.2.3.2 Laser focus

The focus slider adjusts the focus position of the laser beam in the z-direction within the tissue sample. The cutting performance is very sensitive to this parameter. For example, the user would expect to use different focus settings for soft tissue vs. bone.

Focus position is normally given in µm or mm units. Note that the range of the focus depends on the current objective. Also, the displayed value cannot be taken as an absolute distance – the 0 mm position simply denotes that the laser focus position is as close to the objective as possible for the objective.

If your system displays percentages instead of metric values, ensure that the magnification values for the objectives are set (see section 5.2.3).

6.2.3.3 Laser power

The power slider defines the laser power. The power needed to cut the sample normally will be proportional to the sample thickness. Choose the power setting which enables clean laser cutting.

Depending on your system configuration, power is given as a percentage, or in milliwatts (mW).

If available, the milliwatt value refers to the average laser output power before passing through the optical componentes inside the *mmi CellCut* optics and the microscope. Typical microscope objectives for fluorescence applications have UV transmissions between 80% and 95%. Note that the milliwatt power value value is based on measurements performed at the MMI factory site. The actual laser power is not monitored in the device.

6.2.3.4 Iterative dissection

Iterative dissection can be used for tissue that is difficult to cut, e.g. very hard or thick tissue.

Figure 6.15: *Iterative dissection user interface. Z drill is only available on systems with an automated microscope*

The **Repeat** field indicates the number of times that the laser should process each shape. Increase if a single iteration does not completely cut through the tissue.

> Before increasing the **Repeat** count, make sure you laser parameters are optimal (see section 6.2.5).

The **z-drill** option (only available with motorized microscopes) can be used in conjuction with the **Repeat** field. After each iteration, the microscope's focus drive is moved down by this amount, effectively drilling through the tissue from top to bottom.

6.2.3.5 Options

Viewing or hiding dissected shapes By default, shapes that have been processed, are drawn with thin, dashed lines for quality control. In some application this may be distracting. To turn off display of dissected shapes, check the **Hide dissected shapes** option.

Focussing delay Some thick or uneven samples may require manual focussing prior to dissection. Therefore it can be convenient to allow some time for focussing before the laser is switched on and dissection starts.

This option is turned on by checking the **Focussing delay** option. Enter the number of seconds to wait in the entry field that appears next to the check box.

6.2.4 Cutting tests

Before dissecting the tissue of interest, test the laser performance in another area of the sample or on a test slide with the same tissue. Draw a line or a circle and start cutting this figure with a low speed. Change the focus and power parameters during cutting to observe the effects. The sharpest cutting line is obtained with power as low as possible and with exact focus.

Fig. 6.16 shows a typical example of a cut.

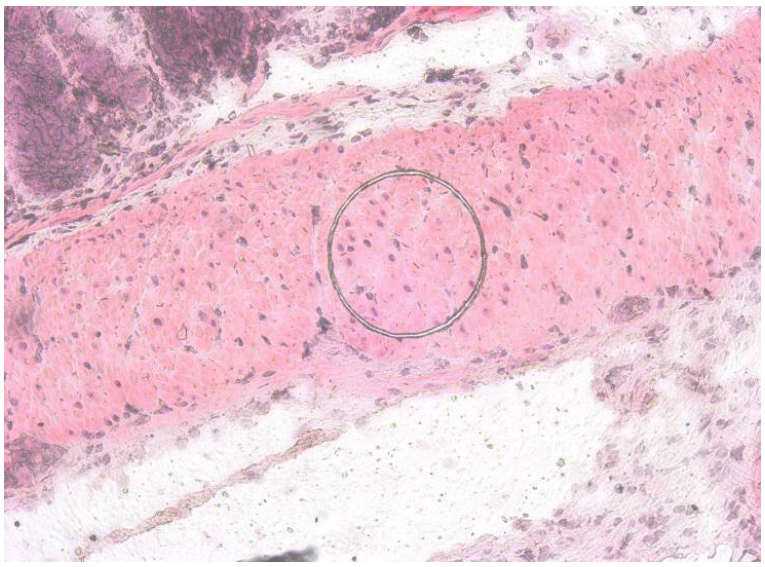


Figure 6.16: *A cutting test on a piece of biological tissue*

6.2.5 Adjustment procedure

To adjust the parameters start with a high power value. The focus can be adjusted easier with high power. Make several cuts with different focus values and compare the results. With a reduced power the focus will be in a more defined area. Decrease the power step by step and repeat focusing until the cutting line is continuous, fine and clear.

You can store as many of the laser parameter settings as necessary for each objective. To define new settings see section 5.2.

6.2.6 Laser safety

When the laser is turned on a small window with a laser warning label always pops up (Fig. 6.17). When the software turns off the laser, the symbol disappears.

Figure 6.17: *Laser safety warning sign*

6.2.7 Hiding the laser position icon

By deselecting the menu item

CellCut →*Laser* →*Show laser position*

the laser position icon can be hidden. After selecting the item again the laser position icon becomes visible again.

6.2.8 Focus memory

On systems with an automated microscope, *mmi CellTools* automatically saves the current position of the microscope's focus control with each shape that you draw. Before cutting, that focus position is restored.

For focus memory to work correctly, microscope remote control must be switched on (see section 7).

6.3 Single-step collection using the mmi IsolationCap

The *mmi IsolationCap* is a standard Eppendorf™ tube with a special adhesive material filling the cap. By lowering the cap onto the membrane a contact will be established. If you lift the cap after cutting, the dissectate will be fixed to the cap by adhesive force.

6.3.1 Available IsolationCap consumables

The mmi IsolationCaps are available in the following sizes

- 200 µl
- 500 µl
- 1500 µl
- strips of 8 caps for 200 µl tubes

The standard *mmi IsolationCap* for a single step collection contains a diffuser inside the adhesive material of the cap. If you position the cap over the area of interest you will receive the maximum image quality during microdissection operations.

You can also order transparent caps. The adhesive in the cap does not contain a diffuser material and is suited to fluorescence applications.

6.3.2 mmi CapLift

6.3.2.1 Versions

The *mmi CapLift* system is available in two versions. The standard version gives you the option to automatically lower and lift a single cap. The standard CapLift system is able to hold a 500 µl cap.

The *mmi MultiCap* version of the *mmi CapLift* can additionally handle eight-cap strips. With this option you can auto sample eight different types of dissectates. The *mmi Multi Cap* is described in section 6.3.7.

In some life cell applications where you need more workspace on top of the stage and where you don't need the *mmi CapLift*, you can easily unplug the complete mechanism.

6.3.2.2 Mounting cap

To mount the tube, simply move the closed tube into the cap holder and then open it. The tube holder can easily be fixed to the automatic cap lift. The lift has an easy to use auto positioning system. Bring the cap in front of the lift, and slide the cap holder towards the holder counterpart. The holder will be fixed automatically by a small magnet.

6.3.2.3 Adjusting cap pressure on membrane

The cap pressure on the membrane can be changed by a knurled screw on the left side of the *mmi CapLift* mechanism. If you use thin cover slips instead of 1mm glass slides the correct cap pressure allows extraction of the dissectates without adversely bending the cover glass.

6.3.3 Cap lift settings

You can cut an object with the *mmi CapLift* in top (up) and bottom (down) position. But the *mmi IsolationCap* works most reliably if microdissection is done in the cap down position.

The stage can also be moved when the cap is down. The maximum moveable distance, without lifting the cap, will be limited to avoid tearing or other damage to the sample membrane.

A sophisticated software algorithm, the mmi “Predefined Target Positioning” (PTP) function, optimizes the use of the mmi IsolationCap when many dissectates are sampled over a large slide area.

The software automatically identifies when there is no more usable area on the cap. A message will pop up, requesting the user to mount a new cap in the cap holder.

6.3.3.1 Basic settings

For standard applications the standard settings should be used and not changed. Only the cap type in use must be selected.

The options are displayed in the PTP section of the CellCut panel (Fig. 6.18).

If you use an eight-cap strip, select the 200 µl option.

If the cap is down on the tissue the cap will automatically be lifted whenever you leave the collection distance area by moving the stage. The cap will be

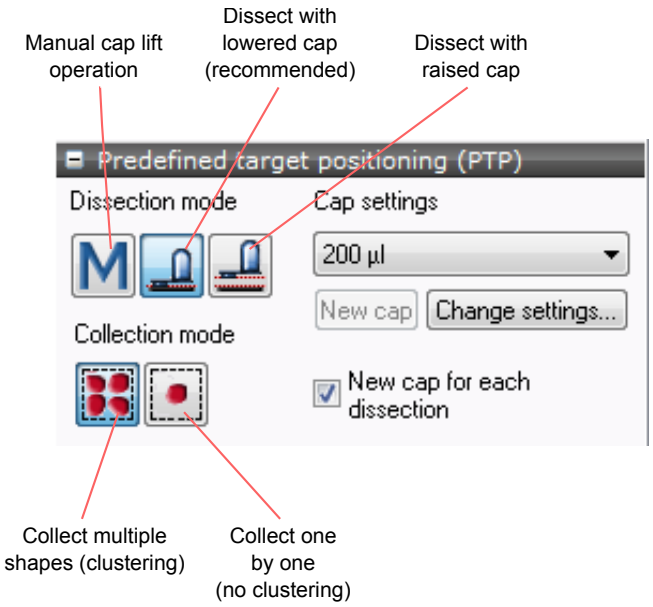


Figure 6.18: *Predefined target positioning (PTP) panel*

repositioned automatically.

You can choose between three dissection modes using the buttons:

- **Manual mode** means that you have to operate the cap lift yourself, PTP is switched off.
- In **Dissect with lowered cap** mode the dissectates are cut with the cap in the lower position and will be collected automatically. This mode is recommended for most applications.
- In **Dissect with raised cap** mode the dissectates are cut with the cap in the upper position and will be collected automatically.

If you check the **New Cap for each dissection** tick box the system assumes that you use a new cap each time you press the **Cut** button. This will affect the placement of dissectates on the cap's adhesive surface. If you use one cap for more than one cutting procedure deselect the check box and either use the menu item

CellCut →Cap Lift →New Cap

or press the **New cap** button each time you change the cap in the cap holder.

Two clustering modes are available using the lower left buttons in the PTP-area (see Fig. 6.18). The default behavior is to simultaneously pick up multiple objects whenever possible (clustering). Alternatively you can decide to pick the dissected objects one by one (no clustering). The latter will be slower, but produce more accurate placement on the cap's adhesive surface.

6.3.3.2 Advanced settings

To be able to optimize the *mmi CapLift* functionality several advanced settings can be adjusted by the user. These settings are only suggested for experienced users and can be selected by the **Change settings...** button (Fig. 6.19).

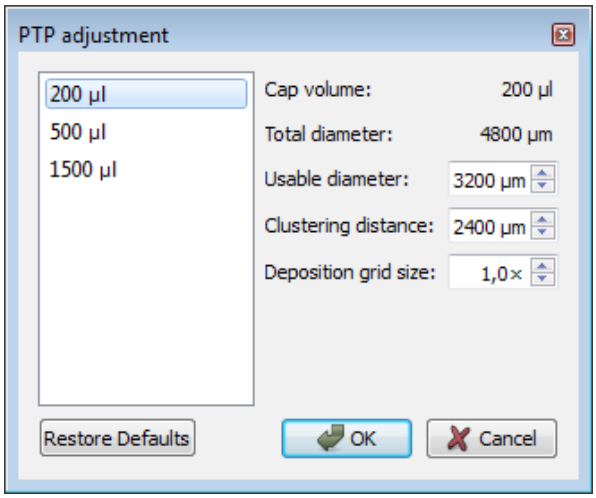


Figure 6.19: *Adjusting PTP cap parameters (advanced)*

Usable diameter The usable diameter depends on the *mmi CapLift* version installed on your system and the diameter of the *mmi IsolationCap*.

For older *mmi CapLift* systems (cable connected to the *mmi CellCut* controller box) the diameter is limited to 2000 µm.

For new versions of *mmi CapLift* (cable connected directly to the PC) the maximum diameter is 6000 µm. The usable diameter must be restricted only for the small cap sizes.

If the cap lift is down you can only cut objects fitting into the usable diameter area. If you work with a 500 µl cap it is recommended to set a diameter of about 4200 µm.

Deposition grid size Default value for the grid size is 1.

If you want to dissect clusters of single dissectates the mmi Predefined Target Positing (PTP) algorithm must be tuned by setting the grid size to the average size of the clusters. The average size is measured in multiples of the average dissectate size.

As an example if the normal cluster size is about 10 times the size of the dissectates, set the grid size to 10.

Clustering distance The clustering distance describes the maximum distance the stage can move after lowering the cap.

The standard setting for the clustering distance is between 1000 µm and 2000 µm. The clustering distance always has to be smaller than the usable radius.

If you set the clustering distance to values smaller than the normal dissectate size then the cap will be lifted before each cut of the dissectates.

If you set the clustering distance equal to the usable diameter then all dissectates inside this radius will be cut and collected. The user gets the message that the cap is filled up.

The smaller the clustering distance the more frequently the cap will be lifted.

6.3.4 Group Handling

Any object drawn will always be assigned to the current group shown in the group selection box (Fig. 6.20).

Figure 6.20: *Group selection*

The most common application for using more than one group is to collect different types of cells on different *mmi IsolationCaps* (e.g. Group A with Cap A, Group B with Cap B, etc. or using the *mmi MultiCap*).

All objects of the same group are marked with the same contour color. The number of groups is not limited.

To switch between groups, select the group of interest from the group list. If the *mmi MultiCap* is installed and active, it will immediately turn such that the selected cap is in position.

6.3.4.1 Definition and editing of groups

In order to deal with groups invoke the **Group editor** (Fig. 6.21) using the edit button next to the group selection box.

To define a new group press the **Add** button. To delete the selected group press the **Remove** button.

You can adjust the group name and drawing attributes (color and line thickness) in the lower part of the editor. The cap number can only be set if the *mmi MultiCap* is installed and activated (see section 6.3.7)

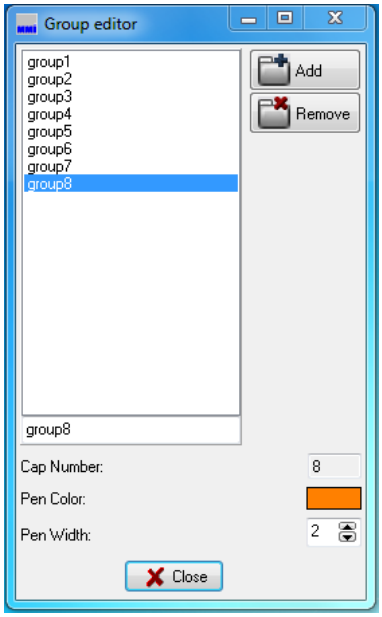


Figure 6.21: *Group editor*

6.3.4.2 Regrouping shapes

To move individual shapes from one group to another, open the context menu (Fig. 6.4) by right-clicking on the drawing area and select the target group from sub-menu *Move into group*.

6.3.5 Group statistics

The statistics page is located in the lower right panel (Fig. 6.22).

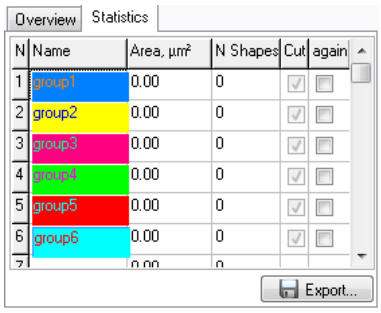


Figure 6.22: *Group overview*

This page shows a list of all defined groups. For each group the list displays the total shape area (sum of the areas of all shapes), the number of shapes and whether the shapes are already cut. With the checkbox in the last column you can specify whether the group will be cut again. By default the shapes drawn since the last cutting operation will be cut again; see the next section

how to change this.

To export the entire data sheet to a file, click **Export**. Data is saved as character-separated values (CSV) file, which can be opened by most data visualization and spread-sheet programs.

6.3.6 Shape list

If you click on a group name in the group statistics page, a list with all shapes defined in that group pops up (Fig. 6.23).

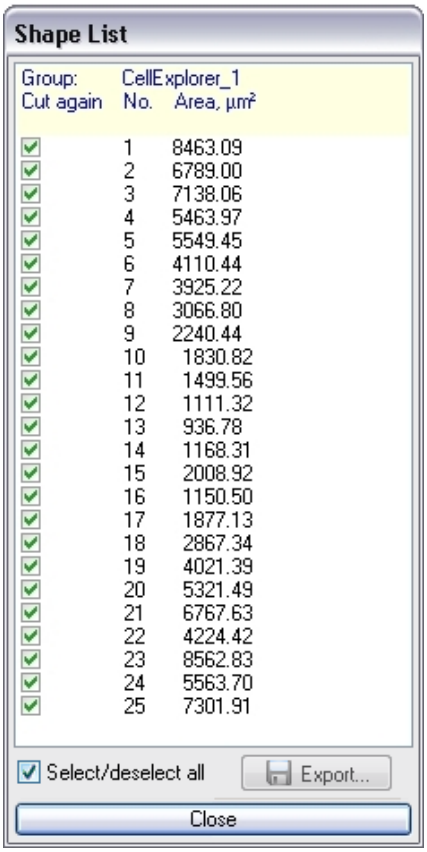


Figure 6.23: *Shape list*

The list displays the area of each shape and a tick mark for the cut shapes. By clicking the Cut again checkbox each shape can be cut again.

To quickly deselect or select all shapes, use the checkbox at the bottom.

By a double click with the left mouse button on the area field in the shape list the stage is navigated to the selected shape.

The selected shape can be deleted by pressing the keyboard *Del* key.

To export the entire data sheet to a file, click **Export**. Data is saved as

character-separated values (CSV) file, which can be opened by most data visualization and spread-sheet programs.

6.3.7 Cutting with mmi MultiCap

If the *mmi MultiCap* system is installed, the group name will always start with the word “Cap” followed by the number of the corresponding cap (Fig. 6.24a).

(a) MultiCap operation (b) single cap operation

Figure 6.24: *MultiCap vs. single cap mode*

If the mmi MultiCap is not installed or the single cap mode is selected, the group name will always start with the word “Single:” (Fig. 6.24b).

To switch from a single cap holder to MultiCap holder select the menu item

CellCut →*Cap Lift* →*Single Cap*

or

CellCut →*Cap Lift* →*Multi Cap*

6.3.7.1 Using mmi MultiCap

Users can cut one, several or all of the shape groups in one single step.

Click on the arrow to the right of the **Cut** button and select the desired group or groups.

After selection of the groups, press the **Cut** button to initiate the cutting process. Automatically the *mmi MultiCap* selects the corresponding cap and starts cutting of all dissectates in the active group. After finalizing the active group, the next selected group and the corresponding cap will be selected and cut. If a single cap holder is installed all shapes from all groups will be cut as if they were in one group.

The maximum number of separate caps using the *mmi MultiCap* is 8. By creating a new group the next available free cap is assigned to that group.

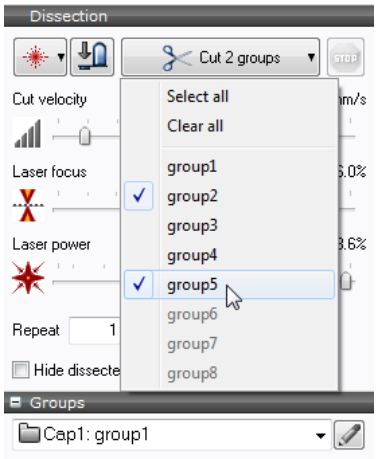


Figure 6.25: *Cutting multiple groups in one operation*

6.3.7.2 Calibration

The *mmi MultiCap* system can be calibrated if the caps are not aligned in the light path correctly. Mainly this happens if the motor axes are turned accidentally when changing the cap holder. Calibrate the mmi MultiCap by selecting the menu item

CellCut →Cap Lift →Calibrate Multi Cap lift

In some cases it is necessary to adjust the cap holder after calibration. Select the menu item

CellCut →Cap Lift →Cap lift adjustment

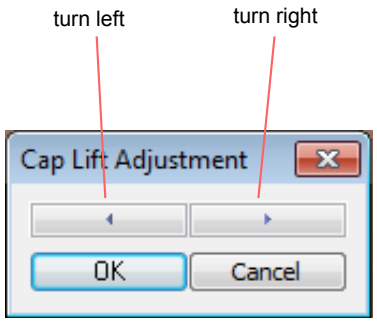


Figure 6.26: *Cap lift adjustment*

After adjustment recalibrate the cap holder.

6.4 Area ablation

mmi CellTools includes the ability to ablate the area of arbitrarily shaped objects.

Figure 6.27: *Area ablation panel*

Area ablation is activated through the check box in the **Area ablation** panel (Fig. 6.27). When checked, the laser will not trace the contour, but instead follow a meandering path, covering the shape's entire area.

> Depending on the object's shape CellTools may not be able to find a single consecutive path that covers the entire area. In this case, the operation is automatically split into multiple paths which are then cut one after the other.

For optimal results, adjust the **Line spacing** parameter to eliminate gaps between cutting lines. This value should be equal to the width of the cutting line.

Open free-hand drawings and shapes create using the **Line** tool and are not affected by the ablation option.

6.5 Serial sections

The serial sections functions permits dissection of the same objects on more than one slide. Objects can be marked on a master slide and subsequently copied to one or more section slides. In order to locate the objects on the section slides, you provide a set of reference points (at least one and up to three).

The serial sections function allows you to dissect samples without dye. In this case, the staining procedure is only applied to the master slide in order to identify objects, and not to the remaining sections. This is useful in cases where the dye may create artifacts in the sample.

6.5.1 Mode of operation

CellTools uses geometric transformations to map object positions and shapes. Reference points are used to define these transformations. For most applications, using at least two reference points is recommended. These should be clearly visible points in your section that are easy to locate precisely.

Depending on the number of reference points used, CellTools will apply different types of changes to the objects' original shape and thus accommodate variations in shape that were introduced in the sample preparation process.

Table 6.1: *Transformation types with 1, 2 and 3 reference points*

Reference points	Types of transformation	Transformation preserves
1	Translation	size, orientation and angles
2	Translation, rotation and scaling	angles
3	Translation, rotation, scaling and shearing	neither of the above

Depending on the process used, you should decide on the number of reference points that works best for your application. For example, when samples may have been subjected to shearing during slide preparation, it is imperative to have three reference points per section. If you can ensure that a 90° angle on one slide will remain rectangular on another (that is, no shearing occurred), two reference points are sufficient.

6.5.2 Basic usage

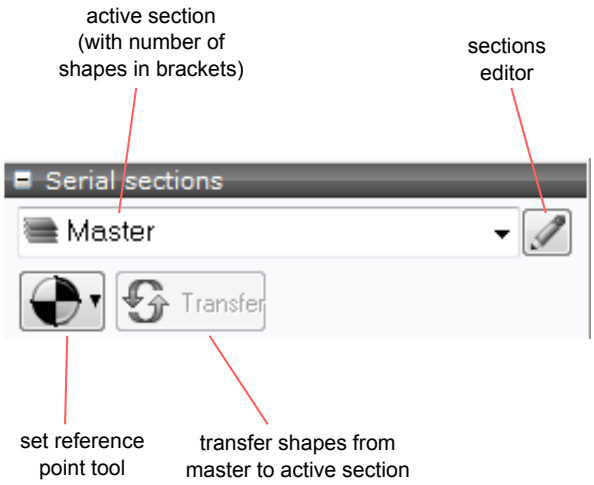


Figure 6.28: *Serial sections panel*

Procedure

1. Insert the master slide. Mark objects as usual.

   If desired, objects on the master slide can be cut, too. To do so, click on the **Cut** button as usual.

2. In the Cellcut panel, open the serial sections panel. The serial sections panel shows the currently active section, tools to mark reference points and the transfer button.
3. Select the **Set reference point** tool to mark the locations of your reference points. Define between one and three reference points.
4. Remove the master slide and insert the first section slide.

   If your section is located on the same slide, ignore this step.
5. Open the serial sections editor (Fig. 6.29) by clicking on the button to the right of the active section display. Add a new section by clicking on the **Add** button. Close the editor. Your newly created section is now active and your marked objects are no longer visible.

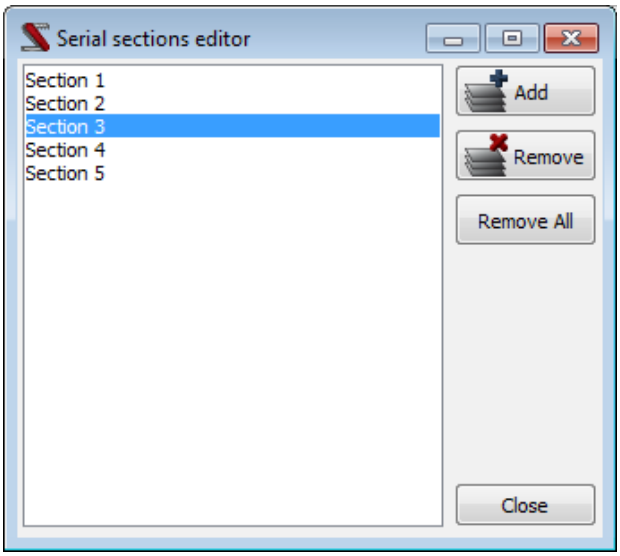


Figure 6.29: *Managing sections*

6. Locate the reference points on the section slide, again using the **Set reference point** tool.

   > Reference points points must be entered in the same order on each section.
7. When finished, click the **Transfer** button to transfer the objects from master slide to the active section.
8. If satisfied, use the **Cut** button to start dissection as usual.

   You can refine the transformation by moving reference points and clicking on **Transfer** again.
9. Repeat steps 4–8 for subsequent sections.

At any time you can switch forth and back between the master slide and the defined sections using the drop-down list in the serial sections view. Do not

forget to switch back to the master slide to commence work on a different sample.

Once the reference points are defined, you can move a particular reference point into view by clicking on the arrow to the right of the **Set reference point** tool. A pop-up menu will appear, listing all available reference points for the currently selected section (Fig. 6.30). Clicking on one of the menu items will move the stage so that the reference point is centered in view.

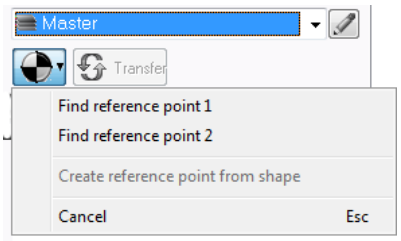


Figure 6.30: *Locating reference points*

Reference points can be moved and deleted in the same way as other shapes.

6.5.3 Using shapes to define reference points

Instead of marking reference points directly by clicking, you can use the outline of a shape to define reference points. If you use landmarks that are rather large or of irregular shape, this is the preferred method.

Procedure

1. Outline your landmark shape using the regular drawing tools, such as the freehand tool. Alternatively, select an already existing object using the select tool.
2. Click on the arrow to the right of the reference point tool and select *Create reference point from shape* from the popup menu. The reference mark now appears inside the shape, indicating that the shape is now used as a reference point.

As above, take care to follow the same order of reference points on each slide.

6.5.4 Using multiple-slide trays

The multiple-slide tray allows you to insert the master slide and one or two section slides at the same time. If you are working with more than three sections, you can exchange slides when needed.

Procedure

1. On the master slide, mark reference points and objects as detailed above in steps 1–3.
2. Switch on the next slide using the controls in the overview panel.
3. Mark reference points and transfer the objects from the master slide as detailed above in steps 5–7.
4. If desired, press the cut button, or first proceed to transfer shapes to another slide.

   To cut multiple sections, select the first section to cut in the drop-down menu ("active section") and press the cut button. When finished, switch to the next section and press the cut button again. The stage will automatically move to the correct slide.

6.5.5 Using multiple groups

The serial sections function works seamlessly with the multiple groups and the *mmi MultiCap* features. Just like on individual slides, you can sort your objects into separate groups (e.g. according to cell type) and process them separately in each section (see section 6.3.4.

Procedure

1. On the master slide, mark objects in different groups.

   You may need to switch back to the groups view to do so.
2. Switch to the serial sections view and define reference points and sections as detailed in section 6.5.2.
3. When you click **Transfer**, all of your groups will get transferred to the active section.
4. You can cut combinations of groups as described in chapter 6.3.7.1.

   Alternatively you can switch groups using the drop-down list in the groups view, or by clicking on an object within the desired group. After that, a single left click on the **Cut** button will process only that group.

6.5.6 Using mmi CellExplorer to identify shapes

mmi CellExplorer can be used together with serial sections in a straightforward manner. CellExplorer can be used to identify objects on your master slide. You can then transfer these objects to subsequent sections by just adding reference points. Use the procedure described in section 6.5.2.

6.6 Auto documentation

The auto documentation feature produces a report for each dissection operation, documenting your experiments with statistical information and images. The documents include all relevant parameters, main features of the cut dissectates and images of all cutting processes.

The report is saved in html format. It is viewable with a standard web browser or can be imported with word processing tools. All images are saved as separate files.

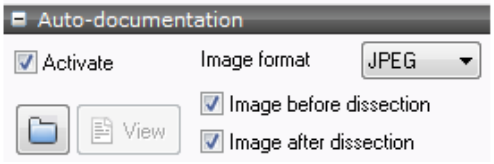


Figure 6.31: *Auto documentation*

6.6.1 File and folder structure

mmi CellTools automatically organizes reports in a dedicated folder in your user documents directory. A separate folder is created for each dissection operation.

To open the autodoc folder select

CellCut →Auto documentation →Browse autodoc folder

or click the folder symbol in the Auto documentation panel. To view the most recent report in a web browser, select

Cellcut →Auto documentation →Show last report

or click on the **View** button.

6.6.2 Settings

The settings are displayed in the Auto-documentation section of the CellCut panel (Fig. 6.31). You can activate auto documentation by checking the **Activate** tick box.

By default, images are saved in JPEG format (compressed). If you wish to save images in a lossless format, choose either PNG or TIFF in the **Image format** drop-down.

Depending on your browser and operating system, TIFF images may not be displayed correctly in the report. If you experience problems, you may use the PNG format instead.

If **Image before dissection** is selected the program saves an image before each cut. If **Image after dissection** is selected the program saves an image after each cut.

6.7 Calibration routines

6.7.1 Laser position

Precise cutting requires that the laser beam position is exactly marked on the viewing screen. Over time and due to vibration, temperature extremes in the laboratory environment or accidental moving of the instrument, recalibration of the laser position may be required.

If the cutting no longer follows the marked line you should adjust the laser position. This should be done for each magnification separately.

Correct camera alignment is a prerequisite for accurate contour tracing. In addition, please test whether mouse and stage movement match, and carry out the camera alignment procedure if needed (see section 5.7.1).

Procedure

1. Locate an empty area on a membrane slide and focus on the membrane
2. Cut a hole in the sample by clicking the **Single shot** button (see section 6.2.1).
3. Select the menu item *Cellcut* →*Laser* →*Set Laser Position*.
4. Locate the center of the hole with the mouse pointer and confirm with a right mouse button click.

This procedure must be carried out for every objective that will be used for dissection.

7 Automated microscope control

mmi CellTools supports the following automated microscope types:

- Olympus IX-81
- Olympus IX-83
- Nikon Eclipse Ti
- Nikon TE2000 E

The automated microscope control functionality must be installed by an authorized MMI technician.

7.1 Activating microscope control

To enable automated microscope control, the microscope's controller hub must be connected to the PC (COM port, USB or IEEE1394 FireWire).

> **The Olympus IX-83 must not be connected to the same FireWire interface as is used for the *mmi CellCamera*. When reconnecting cables, observe the labels next to the computer's interface plugs, otherwise camera and/or microscope may cease to operate correctly.**

To make *mmi CellTools* connect to the microscope, activate the menu item

Microscope → *Remote control*

This activates control of objectives, filter block, condenser cassette, fluorescence shutter and lamp brightness. The focus knobs on the microscope body will still be useable.

mmi CellTools will remember these settings and restore them at startup. Make sure the microscope is connected and switched on before starting *mmi CellTools*.

Table 7.1: *Optical path settings for Olympus and Nikon microscopes*

	Olympus IX-83	Olympus IX-81	Nikon
Left side port	1	1	5
Binocular	3	2	1

7.2 Observation methods

The setup editor is used to define the observation methods, see section 5.2.2.

In the setup editor you define for each setup the position of the

- Filter block
- Condenser
- Optical path (Camera port, Binocular)

When you change the setup, the motorized microscope will set these three items to the positions defined in the new setup automatically. The optical path settings are specific to microscope vendors (see Table 7.1).

Filter wheel position numbers and condenser turret position numbers are clearly indicated on the microscopes.

A fast switch from any camera port to binocular and backwards is performed in the menu

Microscope $\rightarrow$ *Binocular*

or by pressing *F7*.

7.3 Objective control

In the objective editor, see section 5.2.3, you can save nosepiece position number and the lamp voltage used.

When changing the objective the corresponding nosepiece position and lamp voltage will be established automatically by the microscope. Nosepiece position numbers are clearly indicated on the microscopes.

The lamp voltage setting may be different for different objectives. Therefore it is recommended to enable the per-objective camera white balance setting (see section 5.3.2).

7.4 Z drive control

CellTools features built-in focusing aids and functions that rely on motorized z drive control. These are:

- Sample plane definition
- Automatic focus adjustment objective and slide change and caplift operation (CellCut only)
- Focus memory for shapes (CellCut only) and pin positions (see section 6.2.8)
- Focus memory for pin positions

mmi CellTools offers three ways to manually focus the z drive:

1. Using the vertical slider on the right-hand side for coarse focussing;
2. Using the two arrow buttons at each end of the slider for fine focusing and
3. Using the mouse wheel.

In order to use the mouse wheel for focusing, click the **mouse wheel focus** button. While the button is activated, the mouse wheel is linked to the microscope z-drive.

Of course, it is possible to refocus using the microscope's built-in focusing controls.

> When using the manual focus wheel on the microscope, some microscope models may not feedback the changed focus values into the software. In order to notify CellTools that the focus has changed, click on the numerical focus display below the slider to update its value.

7.4.1 Required calibrations

To estimate the correct focus position requires:

- Correctly measured location of the membrane slide in three dimensions → Sample plane definition
- Correctly measured z-drive offsets for
  - *mmi IsolationCap* up / down
  - different objectives

7.4.2 Sample plane definition

The sample plane focus mechanism is a simple focussing aid based on the assumption that the sample is planar, but slightly tilted against the horizontal plane of the stage. As a consequence, the focus always lies on this plane, which can be defined by three points on the slide (see Fig. 7.1).

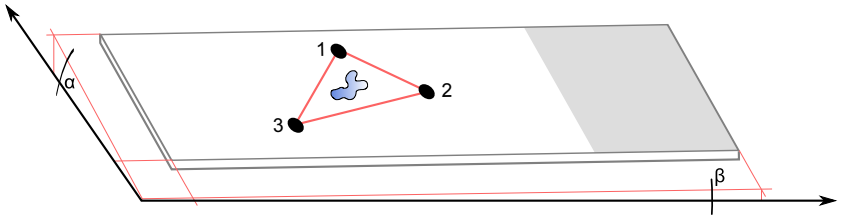


Figure 7.1: *Definition of a tilted sample plane. Given three arbitrary base points (1, 2 and 3), the software computes the tilt angles α and β with respect to the horizontal plane.*

When moving to a different location on the slide, the focus is automatically adjusted according to the sample plane definition.

> While plane tilt focussing is a useful focussing aid on glass slides and for small areas of interest, the approach is limited. By principle, this method cannot compensate for rugged and non-planar surfaces. For such samples, it is recommended to switch plane tilt focussing off (see below).

To properly define a plane in three dimensions, three points must be set. These points should not be on a line. To get most precise results use a high magnification objective for this procedure (20x or higher).

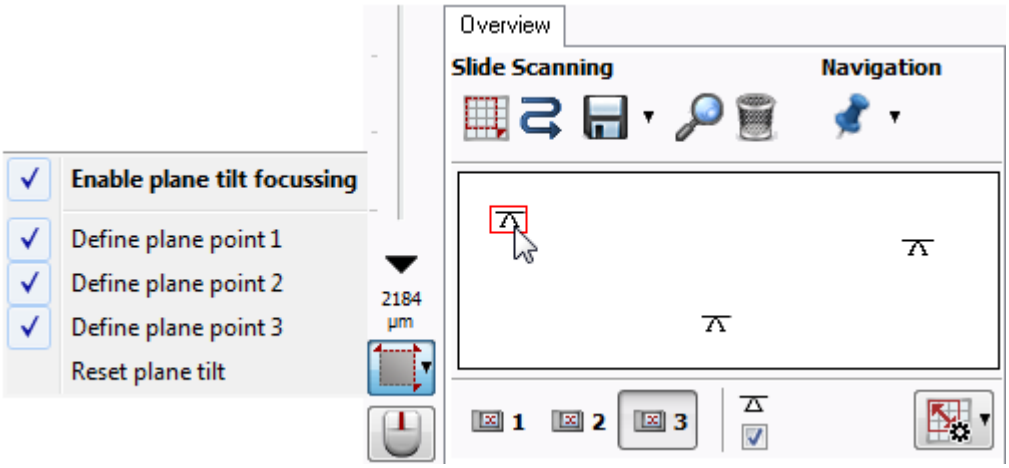


Figure 7.2: *Plane tilt user interface*

Procedure

1. Move to a location near the edge of your sample and focus the microscope.
2. Select *Define plane point 1* from the plane tilt menu (Fig. 7.2) or press *Ctrl + 1*.
3. Move to a second suitable location and focus.
4. Select *Define plane point 2* or press *Ctrl + 2*.
5. Move to a third suitable location and focus.
6. Select *Define plane point 3* or press *Ctrl + 3*.
7. Repeat this procedure for any other slide on the tray.

Note that the positions of the plane points are displayed in the overview scan.

After the plane is defined, the sample plane focussing aid can be switched on and off through the plane tilt button or menu (*Enable plane tilt focussing*). Any of the three points can be redefined individually afterwards to refine the plane definition.

> The orientation of the sample plane is stored independently for each slide. When changing to a slide, for which no sample plane has been defined, plane tilt focussing will be disabled.

You may use *Reset plane tilt* to remove all points and start defining a plane from scratch.

7.4.3 Z drive offset calibration for objectives

Each objective needs a different position of the Z drive. To calibrate the objectives use the following procedure:

Procedure

1. Start with the objective with highest magnification
2. Focus microscope
3. Select another objective with closest magnification factor
4. Focus microscope
5. Select menu item

Microscope →Z Drive Offset →Objective (parfocal)

6. go to step 3) until you calibrated all objectives.

After successful calibration the scope does not lose focus when you change the objective.

7.4.4 Z drive offset calibration for cap up/down

When you lower the cap on the membrane, the cap will press down the membrane by a few microns. This distance can be measured and corrected automatically:

Procedure

1. Lower cap on membrane
2. Focus microscope
3. Lift cap up
4. Focus microscope
5. Select menu item

   Microscope →Z Drive Offset →Cap

   or press the keyboard function button *F3*.

After successful calibration the scope does not lose focus, when you lower or lift the cap.

7.4.5 Automatic Z drive control

Each time you move the stage with the arrow keys or mouse the best Z drive position will be calculated and the Z drive motor moved to that position. With flat samples you immediately receive a sharp image. The more uneven the sample is, the more you need to correct the Z position manually.

If you draw a shape or if you set a pin position the software saves the z position. Each time you go back to the shape or the pin position, the saved Z position will be recalled. You never need to refocus during cutting.

7.4.6 Z drive offset for slide

In the case of a slide which is non-planar, it is necessary to define a Z drive offset for the current area. This distance can be measured and corrected automatically.

Procedure

1. Focus microscope
2. Select menu item

   Microscope →Z Drive Offset →Slide

   or press *F8*.

After successful calibration the scope does not loose the focus, when you change the position of the stage slightly.

7.5 Microscope safety parameters

mmi CellTools provides functions to help prevent physical damage to the microscope objectives. These functions are always in place and do not require manual intervention. However, under certain circumstances, they can be configured through the safety parameters dialog.

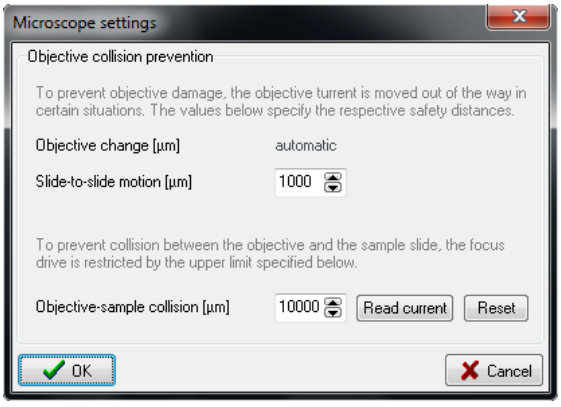


Figure 7.3: *Microscope safety parameters*

Open the dialog using the menu item

Microscope →Safety parameters. . .

7.5.1 Objective collision prevention

There are two situations in which mmi CellTools automatically lowers the objective turret to prevent potential damage to the objectives:

The first situation is when switching to another objective. This function is carried out automatically and does not require configuration.

The second situation is when moving the stage to a different slide. If the two slides are not perfectly aligned the surface of the objective may scratch against the slide's lower surface. To reduce the risk of damage, the objective turret is lowered by a certain amount, which can be specified in the safety parameters dialog (Slide-to-slide motion). If the slides used in your setup exhibit very high tolerances you may wish to increase this value.

When you are using the small step arrow keys or the mouse the z drive escape function is switched off.

7.5.2 Objective-sample collision

To prevent contact between the objectives and the specimen and to protect the microscope itself, the range of the focus drive is automatically limited to a certain maximum value, which can be specified in the safety parameters dialog.

To use the current focus setting as objective-sample collision limit, press the Read current button. To reset the limit to its default value, press Reset.

The recommended procedure for setting the objective-sample collision limit is:

Procedure

1. Select the objective with the shortest working distance (normally the objective with the highest magnification)
2. Focus the microscope
3. Open the safety parameters dialog:

   Microscope → *Safety parameters. . .*
4. Press the **Read current** button
5. Add the working distance in µm to the displayed value and go to step 8, or if the working distance is unknown proceed with step 6.
6. Carefully move the objective as close as possible to the specimen without touching it.

7. Write down the value displayed under Objective-sample collision.
8. Press the Read current button again.
9. Now enter an Objective-sample collision limit half-way between the two positions.
10. Click OK

7.6 Fluorescence shutter control

If installed, the microscope's epifluorescence shutter can be opened and closed via software. Select

Microscope → *Shutter*

or press *F6*.

8 Image analysis using mmi CellExplorer

mmi CellExplorer is an optional image analysis software module for *mmi CellTools*. It was developed to find, count and measure microscopic objects for subsequent extraction or manipulation. A carefully selected and application-adapted set of functionalities guarantees a high throughput at a comfortable level of automation. Nevertheless, a high degree of flexibility is supported.

The *mmi CellExplorer* finds, counts and sorts objects primarily based on the object colors. Secondly, the automatic distinction of various object shapes is a particularly innovative feature. Round shapes (undifferentiated cells), for instance, can be automatically distinguished from oval forms (differentiated cells). Objects adjacent to each other are automatically separated and counted by the software. Finally, the object shapes found can automatically be optimized for laser cutting.

For advanced users the *mmi CellExplorer* comprises complex image preprocessing that can be performed before the object finding. For this purpose the software has many different filters like image sharpness, color intensity, brightness, color space transformations, background separation by fast Fourier transformations, gamma corrections.

8.1 Getting started

Switch from *mmi CellCut* or *mmi CellEctor* to the *mmi CellExplorer* by choosing the *mmi CellExplorer* tab. The live image is loaded to the *mmi CellExplorer*.

8.1.1 Encircling objects of one uniform color

By clicking on the plus left to the **color** label the small color panel (Fig. 8.1) opens.

This panel shows eight fields representing eight different object types you can search for.

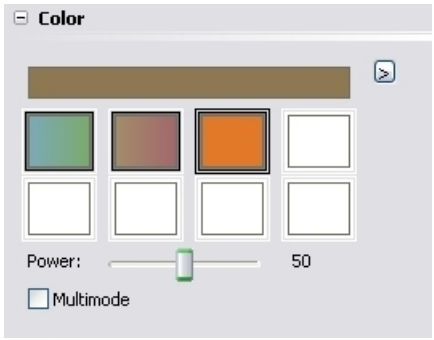


Figure 8.1: *Color panel*

Move the mouse cursor onto the displayed image and left-click on the color of the object you would like to count. The color will be displayed on the left side of the first of the eight color fields in the settings panel.

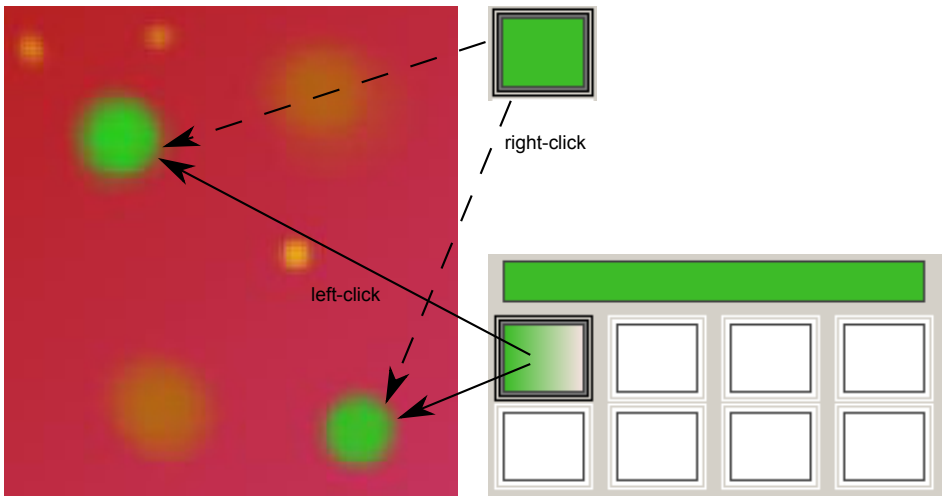


Figure 8.2: *Detecting objects of uniform color*

With a **left mouse click**, the color under the cursor will be entered into the left side of the first color field. The computer would search for all objects with color ranging from green to white. The gradient in this color field displays the "search color".

A **right mouse click** will choose the "end-color" of the color gradient to search for. In our case of a unicolored object select the same object with a left and the right mouse click. The color field will now be filled with green only. Only green objects will be searched for.

A **left and right mouse click** onto a unicolored object searches unicolored objects with the same color (Fig. 8.3).

If the objects are small, it can be tricky to hit the right color. By using the zoom options of the *mmi CellExplorer* (section 8.2.2) the selection will be very easy and precise.

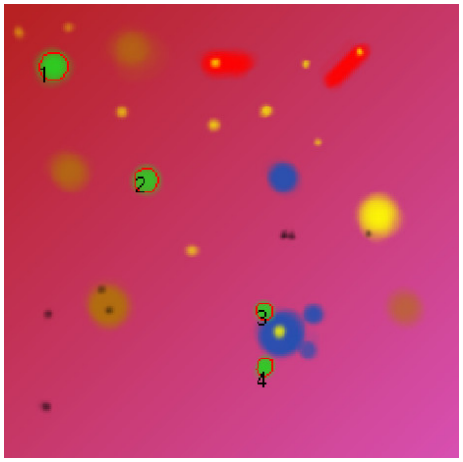


Figure 8.3: *Detecting objects of uniform color: Resulting objects*

8.1.2 Encircling Objects with different uniform colors

In order to search for objects with different colors you need more than one color field. Corresponding to the eight color fields, you can define up to eight object types for your search. In order to define the colors for those objects activate the respective color field:

Activate the field for color editing with a left-click. Only one color field can be edited at once. The outer frame of the color field indicates the selection status (black for active, light gray for inactive)

Activate/deactivate the field for object search with a right-click. The inner frame of the color field indicates the status (see section 8.2.3.4).

You can find, count and calculate objects with different colors separately with the *mmi CellExplorer* software (see Fig. 8.4).

8.1.3 Encircling Objects with color gradients

Many objects show a color gradient. The *mmi CellExplorer* can detect them as well as uniform colored objects. Select the start color of your gradient with a left-click and the end color with a right-click. Differing brightness or intensity of the same color can form the gradient too.

Note: Only activated color fields will be used for object search.

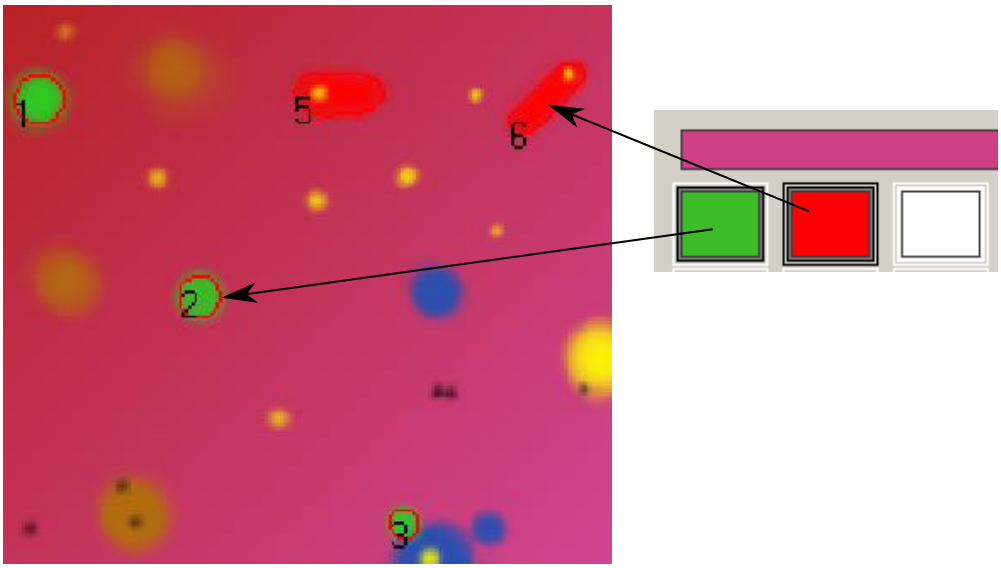
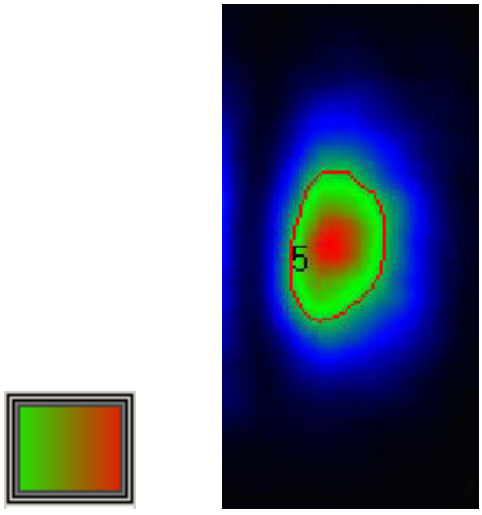


Figure 8.4: *Two colors selected*



(a) Color field (b) Result

Figure 8.5: *Green was selected with the left mouse click, red with the right mouse click. The selection is displayed in the color field as a gradient. All colors contained in the gradient will be searched for during a calculation.*

8.1.4 Transfer to other mmi CellTools modules

After selecting your colors, click on the calculate button (green arrow) in the process toolbar. The software will now search and mark your objects. Please wait until the process has been completed. This can take a while for large images.

To return to the main application, click the **Return to application** button at the top of the *CellExplorer* panel. After switching back all detected cells are lined out and stored in the group statistics. Now you can start the cutting or collecting process of all marked specimens.

8.1.5 Optimization of results

If your result is not totally satisfactory you can optimize each step of the object detection. Please refer to section 8.2.

The settings for optimizing the object detection procedure described herein can be executed directly in the respective section of the Settings panel.

8.1.6 Result lists

8.1.6.1 Object list

The following information is available via the menu item

View $\rightarrow$ *Object list*

after each object detection. It displays the calculated parameters like diameter, total area, circumference and roundness for each detected object.

8.1.6.2 Count list

Under the menu

View $\rightarrow$ *Count list*

the count list shows a summary of the detection results. The table lists the number of objects as well as relative area, sample information and respective calculated objects per unit in your sample.

	ImageNo	ObjectNo	ColorNo	Unit	Diameter	TotalArea	Circumference	Roundness	RelativeArea %	DateTime	ImageFile
	1	1	1	µm	120.5660	10306.7551	409.4428	1.1077	6.1333	9/17/2009 1:35:52 PM	Img___1
	1	2	1	µm	106.9294	7978.2835	350.6021	1.1256	4.7477	9/17/2009 1:35:52 PM	Img___1
	1	3	1	µm	95.8472	6349.4134	305.8837	1.1364	3.7784	9/17/2009 1:35:52 PM	Img___1
	1	4	1	µm	158.0753	14469.0305	474.1817	1.3564	8.6102	9/17/2009 1:35:52 PM	Img___1
	1	5	1	µm	80.8497	4353.0759	253.4891	1.1794	2.5904	9/17/2009 1:35:52 PM	Img___1
	1	6	1	µm	124.2890	8529.4847	370.8822	1.4224	5.0757	9/17/2009 1:35:52 PM	Img___1
	1	7	1	µm	141.2918	11550.4910	414.7095	1.3574	6.8734	9/17/2009 1:35:52 PM	Img___1
	1	8	1	µm	67.9304	3141.1400	208.7576	1.1538	1.8692	9/17/2009 1:35:52 PM	Img___1
	1	9	1	µm	105.5324	6529.6138	311.5219	1.3396	3.8856	9/17/2009 1:35:52 PM	Img___1
	1	10	1	µm	94.0611	4886.6103	267.0753	1.4220	2.9079	9/17/2009 1:35:52 PM	Img___1
	1	11	1	µm	71.5282	2982.1396	206.1013	1.3475	1.7746	9/17/2009 1:35:52 PM	Img___1
	1	12	1	µm	72.5581	3077.5398	222.2597	1.3436	1.8314	9/17/2009 1:35:52 PM	Img___1
	1	13	1	µm	73.8612	3063.4065	222.3631	1.3987	1.8230	9/17/2009 1:35:52 PM	Img___1
	1	14	1	µm	93.1931	4964.3438	294.6811	1.3740	2.9542	9/17/2009 1:35:52 PM	Img___1
	1	15	1	µm	94.3985	4890.1437	286.6229	1.4312	2.9100	9/17/2009 1:35:52 PM	Img___1
	1	16	1	µm	108.5039	6815.8144	345.5217	1.3566	4.0559	9/17/2009 1:35:52 PM	Img___1
	1	17	1	µm	127.6272	9317.4197	391.4636	1.3730	5.5446	9/17/2009 1:35:52 PM	Img___1
	1	18	1	µm	106.3661	6681.5474	319.9865	1.3299	3.9760	9/17/2009 1:35:52 PM	Img___1
	1	19	1	µm	139.5050	11875.5584	434.9877	1.2871	7.0669	9/17/2009 1:35:52 PM	Img___1
	1	20	1	µm	159.2555	14631.5642	497.4977	1.3614	8.7069	9/17/2009 1:35:52 PM	Img___1
	1	21	1	µm	125.0543	9356.2864	384.0695	1.3128	5.5677	9/17/2009 1:35:52 PM	Img___1
	1	22	1	µm	146.3044	12296.0259	435.5593	1.3672	7.3171	9/17/2009 1:35:52 PM	Img___1

Figure 8.6: *Object list*

	ImageNo	Objects	RelativeArea %	Colonies/ml	SampleVolume	DilutionFactor	DateTime	ImageFile	SettingsProfile
	1	22	1.6649	22000.0000	1.0000	1.0000	9/17/2009 1:35:52 PM	Img___1	white4x

Figure 8.7: *Count list*

8.2 Reference

Once the CellExplorer tab is selected in the component control panel of the *mmi CellTools* main window, CellExplorer becomes the active component. It adds its own menu items group in the main menu and shows its own control panel.

8.2.1 Menu reference

The CellExplorer menus consists of four main-menu groups:

- File
- Edit
- View
- Tools

The main menu “Tools” is not used yet. It is reserved for future use.

8.2.1.1 File Menu

Open Opens the window for opening a saved counting session

Save Quick save of the current counting session, when it has already been saved before

Save as . . . Opens the window for saving a counting session

Printer Setup Opens the window for printer setup

Open Image file Opens the window for loading a photo

8.2.1.2 Edit Menu

Copy Copies the current photo to the clipboard of your PC

Undo Cancels the last change of settings

Reset Resets the current photo to its initial state

Calculate Starts the analysis

8.2.1.3 View Menu

Image View Shows the current photo

Object List Shows the object list

Count List Shows the count list

Panel/Settings Switches the panel between settings and projects (see section 8.2.4)

8.2.1.4 Tools Menu

The main menu “Tools” is not used yet. It is reserved for future use.

8.2.2 Zoom function

The *mmi CellExplorer* contains a comfortable zoom algorithm. Moving the slider on the right hand side of the displayed image zooms the image in or out.

With the **zoom in** function the *mmi CellExplorer* zooms into a specific part of your image. Draw a rectangle over the respective area with the mouse button held down. The mouse pointer will change to a cross shape.

With the **zoom out** function the *mmi CellExplorer* zooms out to the original image size.

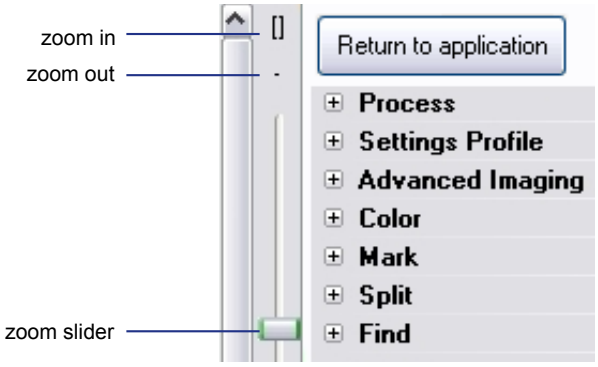


Figure 8.8: *Zoom controls*

8.2.3 Control panel

The *mmi CellExplorer* controls are subdivided into control panels that can be opened and closed on demand by clicking on the button to the left of the panel's title (Fig. 8.9).

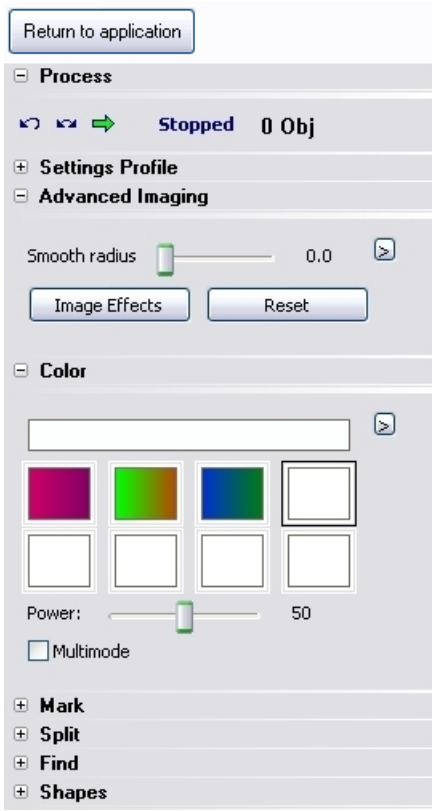


Figure 8.9: *The mmi CellExplorer control panel for cell recognition*

Most panels contain a **preview** button. When clicked, CellExplorer will perform all calculations defined on the respective tool panel on the original image. Use the button to check the settings you have defined so far.

8.2.3.1 Process tool panel

The process panel (Fig. 8.10) contains the following controls (from left to right):

Figure 8.10: *Process panel*

Undo Undo the last change when editing settings

Reset Reset the current photo to its initial state

Calculate Performs a complete analysis on the current image using all parameters defined in the control panel. After this calculation, the objects can be transferred to *mmi CellCut* or *mmi CellEctor*.

Objects Displays the number of counted objects.

8.2.3.2 Settings profile panel

Save your settings by entering a name in the settings profile toolbar and clicking **Save**.

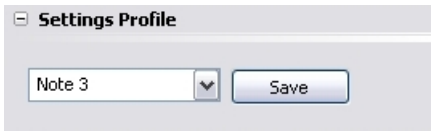


Figure 8.11: *Settings profile panel*

8.2.3.3 Advanced imaging panel

The advanced imaging functionalities allow preprocessing and optimizing the image before an object search is performed.

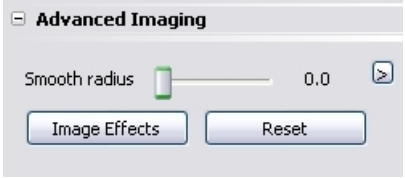


Figure 8.12: *Advanced imaging panel*

Smoothing You can smooth your image with the smoothing function in order to round over edges and achieve a better counting and splitting result. By adjusting the **Smooth radius** slider, you can define the level of smoothing.

The radius used for the calculation of the smoothing can be set with the slider:

- 0 is a small radius
- 10 is a large radius

By clicking the **Check** button, you can verify the effect.

Image Effects For manipulation of digital images, the *mmi CellExplorer* contains additional image editing algorithms. Pressing the **Image Effects** button opens a dialog (Fig. 8.13). You can see two small versions of your image. The left image shows a copy of the original, while the right one directly displays the effects of the changes made by adjusting the controls.

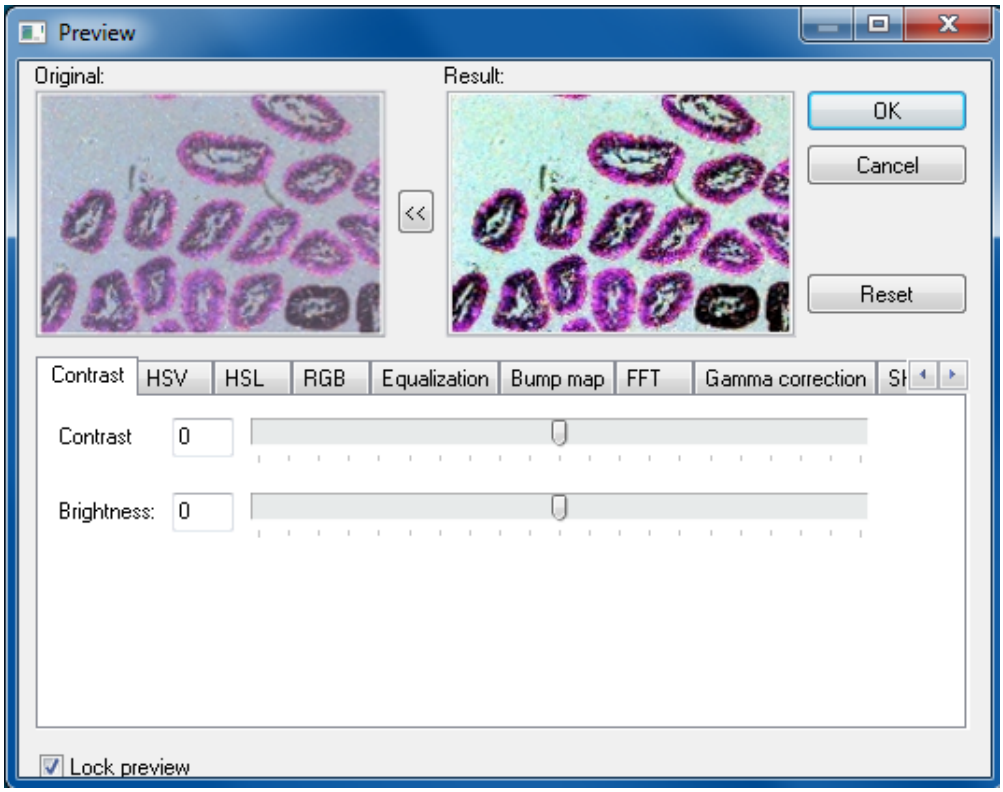


Figure 8.13: *Image effects dialog*

How to use the combination of several image effects:

Evaluate the best set of image effect parameters:

Procedure

1. Open the Image Effect panel
2. Change a parameter and press **Copy Results to Original**
3. Change as many effect parameters and press **Copy Results to Original** until you found the best set of parameters
4. Close

Set the best set of image effect parameters for the use in combination with the green arrow key

Procedure

1. Open the Image Effect Panel
2. Set the best set of parameters without using the **Copy Results to Original** buttons and close the Image Effect Panel with the **OK** button.

Contrast and Brightness Optimize the image contrast and brightness by adjusting the sliders.

The HSV register The HSV register contains further important functions for image editing. It mainly helps with manipulating color images with a color depth of up to 32 bits. The three sliders modify the hue, saturation and intensity/value. New colors are defined with the cursor in the color field located at the lower part of the register. The primary colors are defined with the rainbow tool displayed in the larger part of the window.

The HSL register In the HSL register, you can further adjust the colors, applying the same principles as described for the HSV register.

The RGB register In the RGB register, you can control the individual primary color values. All true colors can be modified by adjusting the three sliders for red, green and blue.

The Equalization register In the Equalization register, halftones can be controlled exactly. The distribution and frequency of the halftones (0–255 for 8-bit images, 0–65535 for 16-bit images) is displayed in the histogram. By adjusting the sliders, halftone ranges can be shielded from the image. Just move the slider with the left mouse button held down. You can also edit color images.

Just click into the small windows below the word “histogram”. The respective primary color will be displayed. Now the individual distribution of the colors red, green and blue (RGB) can be filtered.

Bump Map filter The Bump Map Filter produces a surface structure like those of freeze-cuts. It simulates a metallic surface where objects show high contrasts. You can control the illumination from punctual to flat by adjusting the sliders left/right (Left) and top/down (Top). You can also dye the surface. In order to do so, just move the mouse pointer within the color window.

In case you would like include the original colors of your object into the metallic surface, increase the percentage value below the Source image quantity.

Fast Fourier Transformation The fast fourier transformation searches for edges in the image. This transformation can mainly be used to reduce background noise.

Gamma correction The Gamma correction is a special brightness and contrast Special function. Especially very low light images can be improved by using the gamma correction.

Sharpen With the sharpen function you sharpen the contour of the objects.

8.2.3.4 Color tool panel

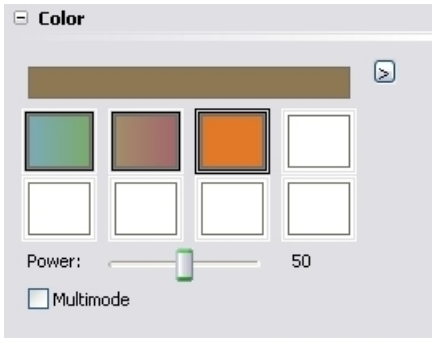


Figure 8.14: *Color panel*

Color fields for different object types Open the color panel to choose up to eight color gradients as settings for counting eight different object types. This enables you to find, count and measure object types with different colors simultaneously; the counting results are shown separately for each color. The color fields are characterized in Fig. 8.15.

(a) Color field inactive for editing and inactive for object searching

(b) Color field active for editing (only one color field can be edited at once), but inactive for object searching

(c) Color field active for object search but inactive for editing.

Figure 8.15: *Color fields state indicators*

Standard color selection With a left mouse click on the displayed image, the color under the cursor will be entered into the left side of the first color field.

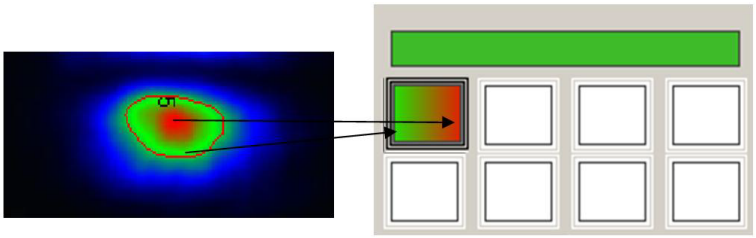


Figure 8.16: *Standard color selection*

A right mouse click on the displayed image will choose the “end-color” of the color gradient to search for.

If the objects are small, it can be tricky to hit the right color. By using the zoom options of the *mmi CellExplorer* as described in section 8.2.2 the selection will be very easy and precise.

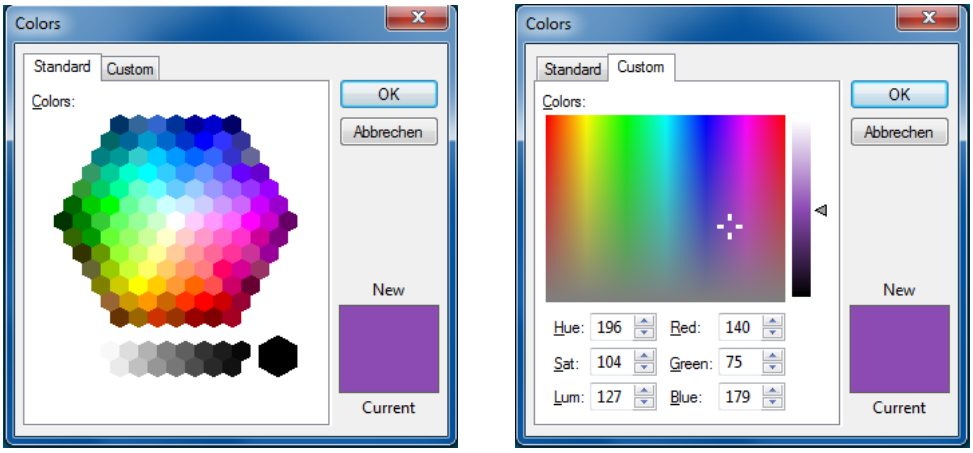
Advanced color selection With a double left mouse click in the right or left part of the color field a standard color menu is opened to select a new colour for the right or left part of the color field. Select the Custom file card in the Color menue to customize the favoured color.

The **Power** slider (Fig. 8.14) adjusts the intensity of the marking. You should try to get homogeneous dark objects and a light background

- 100 is the lowest intensity
- 0 is the strongest intensity

Important: Please try to obtain objects as dark as possible!

Multimode If multimode (Fig. 8.14) is selected the parameters in the lower setting panels defining the object shape, like object area, object roundness. . .



(a) Standard colors (b) Custom colors

Figure 8.17: *Advanced color selection*

will be handled separately for each color set. If multimode is not selected the set of shape parameters will be applied to all color sets.

8.2.3.5 Mark tool panel

After selection of the colors the image will appear as a black and white image. The darker the pixels are the better will the color match the selected color.

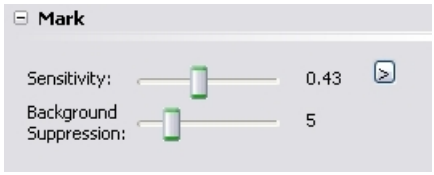


Figure 8.18: *Mark tool panel*

In the **Mark** tool panel (Fig. 8.18) objects can be separated from the surroundings of similar color by adjusting **Sensitivity**:

- 0.01 is the lowest intensity
- 1.00 is the strongest intensity

The **Background suppression** filters the background:

- 1 means low filter suppression
- 20 means high filter suppression

For small objects high filter suppression is recommended, while large objects should be selected with a low filter suppression.

By applying the Mark function the candidate pixels for an object will be labeled red.

> Important: Please try to mark all objects in red with a white background.

8.2.3.6 Split tool panel

The mmi CellExplorer contains a mode for automatic splitting of objects. In microscopic images of cells or structures the relevant objects are often located next to each other in a way that the computer detects those objects as one single structure. The toolbar split is used for separating these overlapping objects:

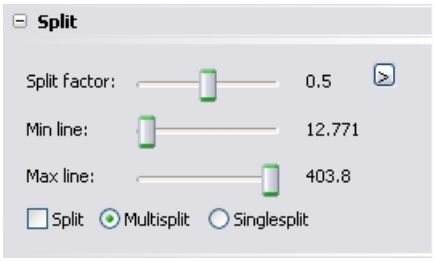


Figure 8.19: *Split tool panel*

For the separation of overlapping objects activate the box **Split**.

Adjusts the intensity of the split factor:

- 0 is a low split factor
- 1 is a high split factor

The **Min line** slider selects the shortest length of the split line. If you select a very small value, many small segments of larger objects are separated as individual objects. If you enter a very large value, overlapping objects are no longer separated.

The **Max line** slider selects the longest length of the split line. The value is preset to maximum. If large individual objects are separated into two parts, you have to decrease the value.

The split feature allows the separation of overlapping objects. The feature **Singlesplit** separates objects once, while the feature **Multisplit** cross-checks after separating whether another separation of the objects is necessary. Separating with **Multisplit** is time-consuming!

8.2.3.7 Find tool panel

With the find tool panel you may adjust the size and roundness of the selected objects.

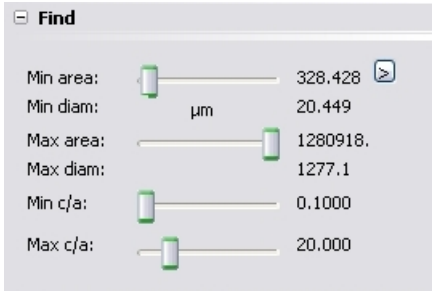


Figure 8.20: *Find tool panel*

Min area and **Min diam** define the smallest object area and diameter, which are counted. **Max area** and **Max diam** defines the largest object size and diameter, which are counted.

Use **Min c/a** and **Max c/a** to filter by roundness: All objects with roundness between the **Min c/a** and **Max c/a** value are counted.

Definition of the roundness in *mmi CellExplorer*:

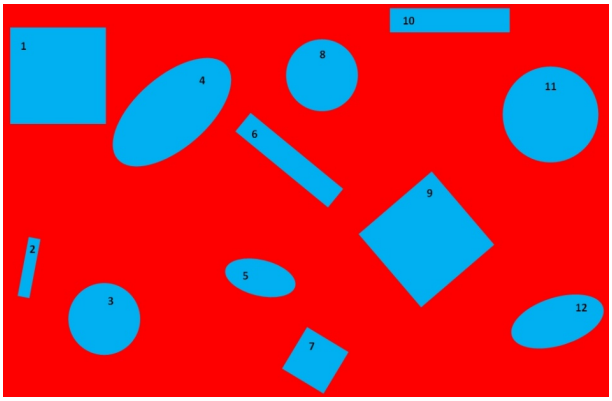
$$\frac{c}{a} = \frac{\text{circumference}^2}{4\pi \cdot \text{total area}} \tag{8.1}$$

The roundness will separate different geometrical shapes such as circles, squares, rectangles and ellipses. The results are independent from shape size and allow you to separate shapes by geometry.

> Attention: In binary images, objects appear as a collection of pixels; therefore, objects are never entirely round or rectangular.

Table 8.1: *Roundness of simple objects*

geometry	roundness
circle	≈ 1.0
square	≈ 1.5
rectangle	≈ 2.0
ellipse, 1:2	≈ 4.0



(a) Original image

	ObjectNo	Unit	Diameter	TotalArea	Circumference	Roundness
	1	µm	1184.9754	702083.3185	3351.6165	1.5708
	2	µm	534.9537	54970.8773	1331.2057	4.0887
	3	µm	632.2779	311343.6148	2088.5129	1.0085
	4	µm	1260.5487	622489.1109	3213.6582	2.0048
	5	µm	630.9560	155816.8586	1611.3605	2.0067
	6	µm	1071.0476	221631.1996	2606.0141	4.0651
	7	µm	600.6331	181413.0935	1819.0820	1.5619
	8	µm	632.4457	311378.9932	2084.1057	1.0089
	9	µm	1192.8757	714720.4644	3475.2633	1.5637
	10	µm	1066.2970	219678.3148	2511.0524	4.0650
	11	µm	841.7502	553172.3134	2779.7572	1.0060
	12	µm	840.5515	276778.9687	2144.4515	2.0049

(b) Results

Figure 8.21: *Example for calculating the roundness of different objects independent from size*

8.2.3.8 Shapes toolbar

With the shape toolbar the contours of the selected objects can be optimized.

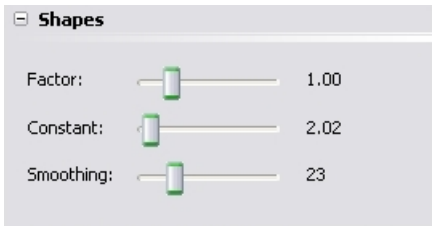


Figure 8.22: *Shapes tool panel*

The **Factor** defines a percentage correction of the shape size:

- Factor < 1.00: reduction of the shape size (Min: 0.8)
- Factor = 1.00: no correction
- Factor > 1.00: increase of the shape size (Max: 1.2)

The **Constant** parameter adds a pixel correction to the shape size:

- Constant = 0.00: no pixel correction
- Constant > 0.00: shape size increases by “constant” pixels (Max: 50)

The pixel correction can be used to compensate for the laser cutting width independent from the object size.

With the **Smoothing** feature the contours of the selected objects can be smoothed:

- Smoothing = 0: no smoothing
- Smoothing > 0: increase smoothing of object contours (Max: 100)

8.2.4 Project panel

The *mmi CellExplorer* project panel shows all images contained in the current session. You can choose whether the image is displayed in the work image panel using the checkboxes on the left of the images. You can also delete an image from the current session by marking it with the mouse and clicking the **Delete** button.

9 Maintenance

9.1 System check

- Visually inspect the housing periodically to verify that no panels are loose or distorted so as to allow access to laser or electrical energy in the interior.
- Verify the correct operation of the LEDs by simulating their functions.

9.2 Cleaning

Caution

- Before cleaning the system, disconnect all system components from the mains. OR
- Before connecting all system components to the mains again, ensure that all positions in the objective turret that do not contain an objective lens are covered with blanks and that the beam path covers and the laser box are not disconnected.
- Laser power up to 15mW at 355nm is accessible in the interior, if a cover, an objective, a blank to cover an unused hole in the objective turret or the laser box is removed or opened.

9.2.1 Microscope

For further details, see microscope manual.

OR

9.2.2 Cleaning when actually dirty

Actual dirt, e.g. caused by fingerprints or immersion oil, must be removed immediately or the optical functions will be permanently impaired. Dirt that has been burnt onto the surface of lenses or filters by laser light can no longer be removed.

- Lightly moisten a cleaning tissue with pure alcohol (70-80%) or spirit and wipe the dirty parts very carefully
- Clean dry objectives and oculars gently with a cotton bud or a cotton wool wrapped around a toothpick; Only use surgical cotton from the pharmacy
- Remove cotton lint and fluff by blowing, e.g. with an enema syringe

Caution

- Moisten cleaning tissue only slightly, do not soak; Excessive solvent might dissolve the cement of the lenses
- Do not use acetone for cleaning under any circumstances

9.3 Trouble shooting

This section provides support for problems that can occur when working with the mmi CellTools. Most of the problems can easily be solved by the user.

9.3.1 View

No image on the monitor

- The microscope camera port switch should be set towards the camera (default is left side port)
- The camera exposure time is much too low
- The camera cables are not connected properly

The image is not clear, too dark, too bright

- check the camera setup

- Check that there is sufficient illumination from the white light and that the light path is not obstructed
- Check that the correction ring of the objective is on the value 1 (corresponding to glass thickness)
- Use the diffuser for improved image quality
- Calibrate the camera in the z-axis

The colors are wrong

- Set white balance
- A fluorescence filter is still turned into the light path

9.3.2 Movement

The stage moves a large distance very fast when using the mouse, or stage movement does not follow mouse.

- Wrong objective selected
- Camera alignment not correct (see section 5.7.1)
- The camera is not mounted in an exactly upright position

Live image does not follow the hand, if moving stage with the mouse:

- Wrong objective selected
- Camera alignment not correct (see section 5.7.1)
- The camera is not mounted in an exactly upright position

Image not in focus after objective is changed (for automated microscope only)

- Parfocal lens offset not correct.

Displacement of markers after objective is changed:

- Paraxial lens offset not correct.

Overview images not matching stage geometry.

- Stage geometry not properly defined, see section 5.5
- Stage origin calibration invalid, see section 5.5.4

9.3.3 Overview

The stitched image is patchy. Tiles do not match correctly.

- Camera alignment not correct (see section 5.7.1)
- The camera is not mounted in an exactly upright position

9.3.4 Drawing

The drawing line is difficult to see

- Choose dark line colors for bright samples and bright line colors for dark samples
- Increase the thickness of the line if necessary

9.3.5 Cutting

9.3.5.1 Standard test procedure

Procedure

1. Mount a MMI membrane slide with a supporting microscope slide into the slide holder.
2. Set the cutting speed, the focus and the power to the values given in your system test report delivered with your *mmi CellCut*
3. Cut 10 circles

Following actions could be required:

- The circles a well cut: no further action required
- The cutting is not homogeneous or not complete: Probably the laser power dropped down, please contact your service representative
- The cutting line is not closed on the left edge: Probably the stage accuracy is critical, please contact your service representative

9.3.5.2 Cutting problems

No cutting can be observed

- The laser has not been switched on (key and button)
- The magnification chosen does not correspond to the objective in use.
- The laser power is too low
- The laser focus is not well adjusted

The cutting is not perfect; bridges remain in the cutting path

- Repeat the cut if necessary
- The cutting speed is to high
- The illumination pillar is not set upright and the interlock switch can't close

The cutting is not clean; The cutting width and the laser spot can be seen on the video image during the cutting process

- The sample is too wet

9.3.6 Collection

The dissected objects are not collected.

- The cap is lowered onto the metal frame and does not contact the membrane properly.
- Cutting was not complete and successful. Adjust laser settings or select repetitions to achieve full penetration of the beam through the membrane and tissue.

A Service

Service should only be performed by qualified MMI personnel or our designated representative(s). The MMI system contains no user-serviceable parts.

For questions about your instrument (technical, consumables, warranties) please contact:

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B List of Keyboard Shortcuts

General

+	Move to next shape	42
-	Move to previous shape	42
Caps Lock	Suppress continous stage movement	38
Ctrl + C	Copy shape	59
Ctrl + Del	Delete all shapes	59
Ctrl + I	Show stage insert	42
Ctrl + M	Fire laser for timed interval	61
Ctrl + O	Set stage origin	47
Ctrl + P	Stage movement settings	38
Ctrl + Shift + Del	Delete all shapes from current group	59
Ctrl + Shift + M	Fire single laser pulse	61
Ctrl + V	Paste shape	59
Del	Delete shape	59
End	Move to last shape	42
Esc	Interrupt current process	40, 51
F1	Display user manual	52
F2	Lower or raise cap lift	26
Home	Move to first shape	42
Space	Switch between moving and drawing mode	37, 55

Camera control

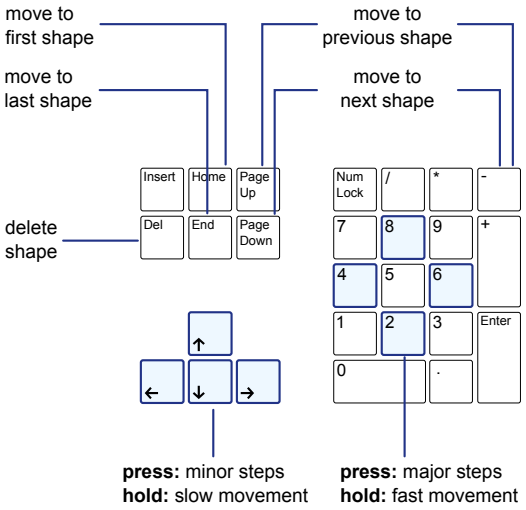
Ctrl + R	Open camera settings	32

Ctrl + S	Save an image	34
Ctrl + W	Set white balance	34

Microscope control

Ctrl + 1	Define first sample plane point	87
Ctrl + 2	Define second sample plane point	87
Ctrl + 3	Define third sample plane point	87
F3	Define Z drive offset	88
F6	Open/close fluorescence shutter	91
F7	Switch between camera and binocular	84
F8	Define z drive offset for current slide	89

Navigation



C Technical data

C.1 Required minimum workspace

The table top for the microscope, laser, optical equipment, computer monitor and keyboard requires a minimum workspace of 1.20 m × 0.90 m.

The computer should be positioned under or near the table. The camera–computer connection cable is 2 m long to ensure reliable data transfer.

C.2 System components

C.2.1 Microscope

Inverted or upright research microscope with fluorescence port.

Supported microscope models:

- Olympus IX-71 (non-motorized)
- Olympus IX-81
- Olympus IX-83
- Nikon Ti (S, U, E)
- Nikon TE 2000 (S, U, E)

C.2.2 Stage

Scanning stage with stepper motors.

Scanning area:	120 × 100 mm^2
Repositioning accuracy:	< 1 µm
Step resolution:	0.078 µm (CellCut Plus)
	0.039 µm (SmartCut Plus)

C.2.3 Laser system

- Fixed beam
- Extremely sharp focus due to computer simulated optics
- Computer controlled laser power
- Computer controlled laser focus (z-position of the beam)
- Safety devices and eye protection filters

Wavelength:	355 nm
Line voltage:	200–240 VAC or 100–110 VAC, 50–60 Hz, 1,0 A

C.2.4 Digital camera

mmi CellCamera MXF285c

- IEEE1394b (FireWire) color CCD camera
- 2/3" interline transfer frame readout CCD
- Temperature controlled active Peltier cooling
- signal to noise ratio: > 62 dB
- On board integrated color processor for high quality color calculation
- 1392 $\times$ 1040 pixels with up to 20fps
- Ultra high sensitivity

mmi CellCamera DXA285cF

- IEEE1394a (FireWire) interline transfer color CCD camera
- 2/3" interline transfer frame readout CCD
- Super HAD technology
- signal to noise ratio: > 56 dB
- 1392 $\times$ 1040 pixels with up to 15 fps
- Ultra high sensitivity

mmi CellCamera DXA285F

- IEEE1394a (FireWire) interline transfer CCD camera
- 2/3" interline transfer frame readout CCD
- Super HAD technology
- 1392 $\times$ 1040 pixels with up to 15 fps
- Ultra high sensitivity

Andor iXonEM+ EMCCD Camera 897

- EMCCD Technology: Even single photon signals are amplified above the noise floor. Full QE of CCD chip is harnessed (no intensifier).
- RealGain^{TM}: Absolute EMCCD gain selectable directly from a linear and quantitative scale.
- TE Cooling to −100 °C: Critical for elimination of dark current detection limit.

C.2.5 Computer

Suitable computer workstation models are preselected and tested by MMI. Only use computer hardware supplied through MMI. Before performing hardware modifications, contact service.

mmi CellTools supports Microsoft Windows XP, Windows 7 and Windows 8 in 32-bit and 64-bit configuration.

C.2.6 Cap lift

Classic cap lift

- ± 1 mm moving distance
- Single cap holder

mmi CapLift

- ± 3 mm moving distance
- adjustable cap pressure
- high repositioning accuracy
- Single cap holder

mmi MultiCap

- ± 3 mm moving distance
- adjustable cap pressure
- high repositioning accuracy
- Single cap holder
- Multicapholder