Molecular Machines & Industries

mmi CellCut

User Manual

Molecular Machines & Industries GmbH
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User Manual: MMI CellCut
Version 6.0

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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 mW
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)	< 5 mW
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 mW
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.

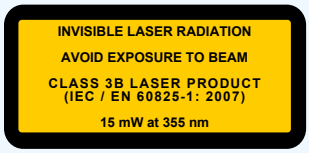
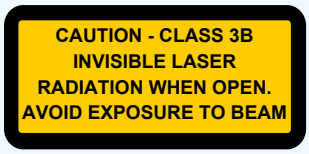
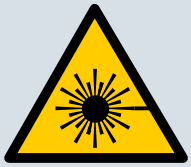
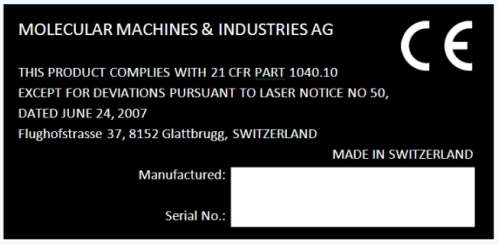
Table 1.4: *Laser radiation data of the CellCut systems incorporation a high power laser*

Average power (nominal)	< 35 mW
Pulse width	< 1.5 ns
Repetition rate	500 Hz
Wavelength	349 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	

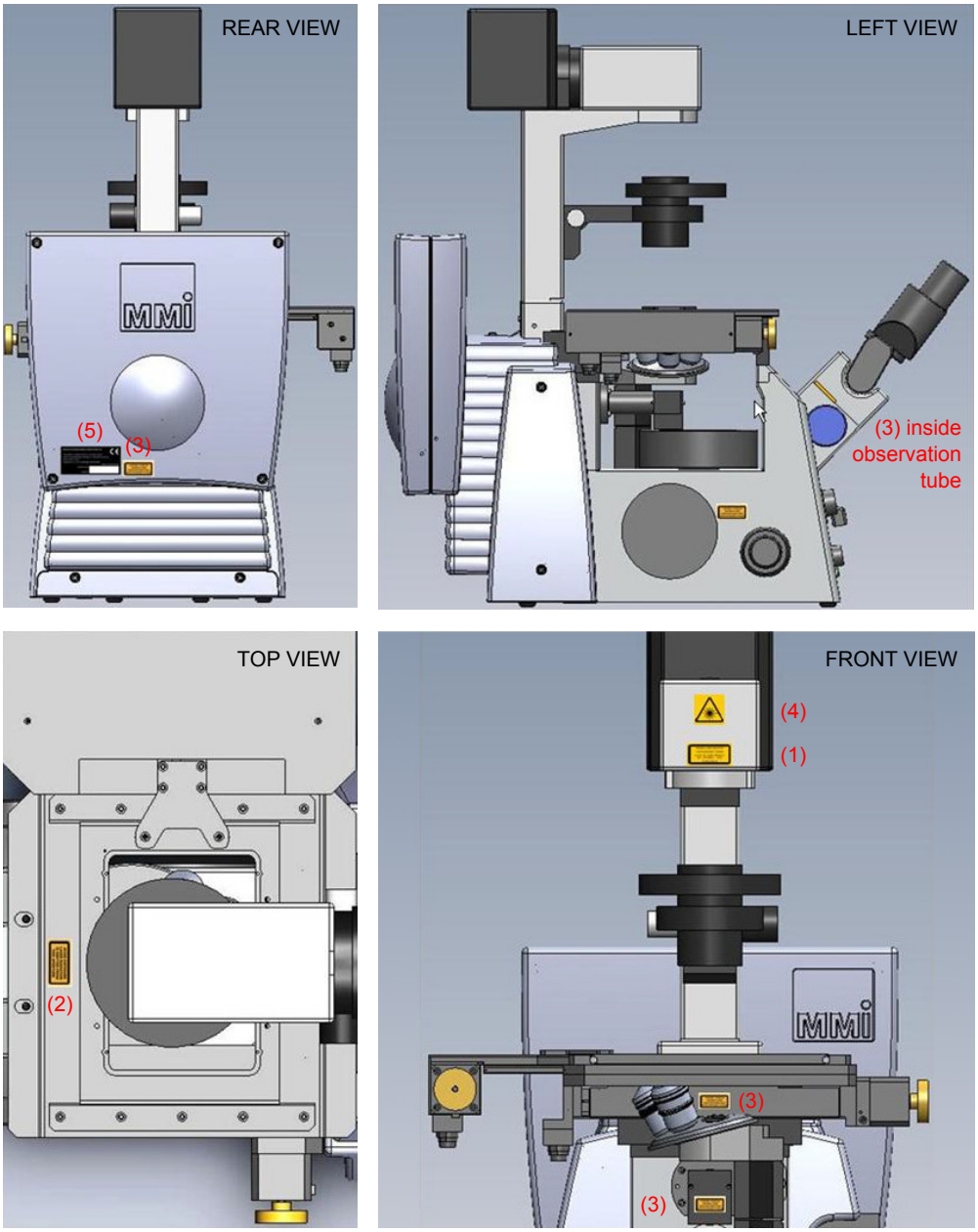
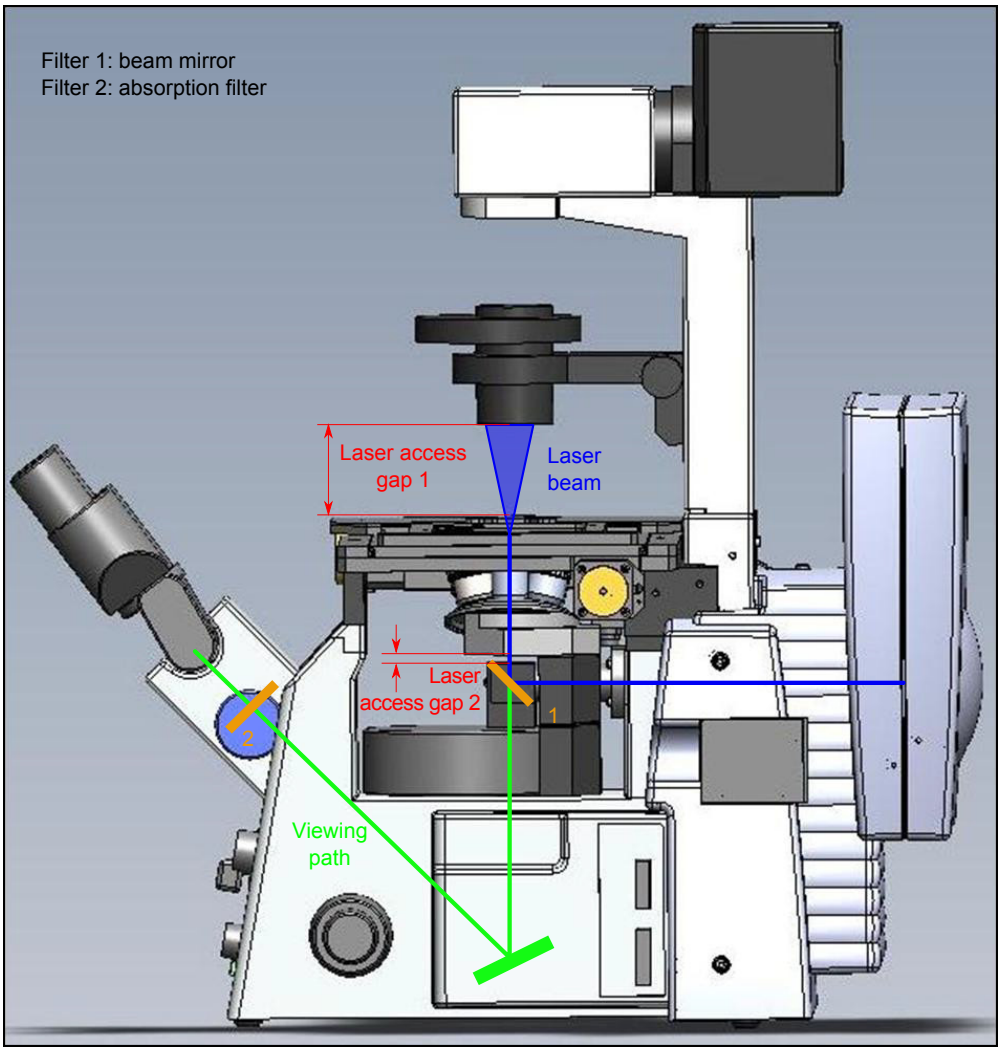


Figure 1.1: *Positions of laser safety labels*



- **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.

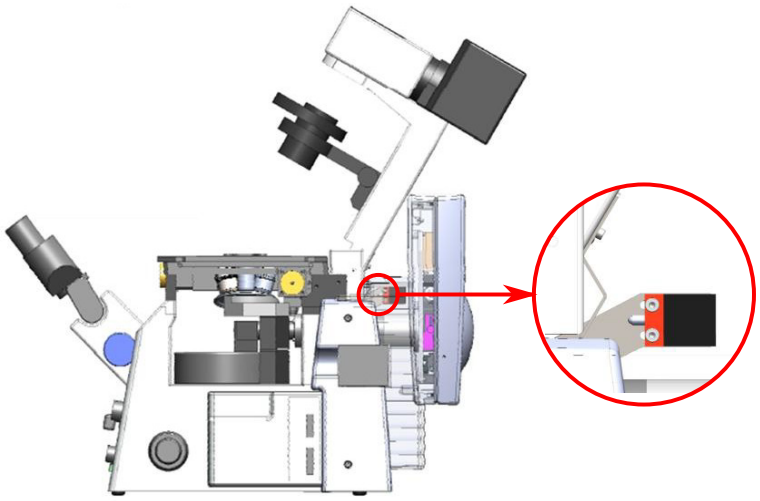


Figure 1.3: *Illumination pillar interlock*

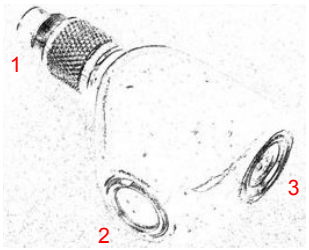
When you tilt the illumination pillar backwards the interlock mechanism switches off the laser (Fig. 1.3).

On *CellCut Plus* and *SmartCut Plus* the LED 1 on the controller changes the color from yellow to red, as shown in table 1.7a.

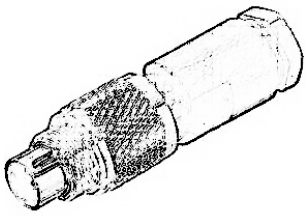
On *CellCut* starting from 2015 the laser will be unpowered. The green, yellow and red LED turn off as indicated in table 1.7a

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

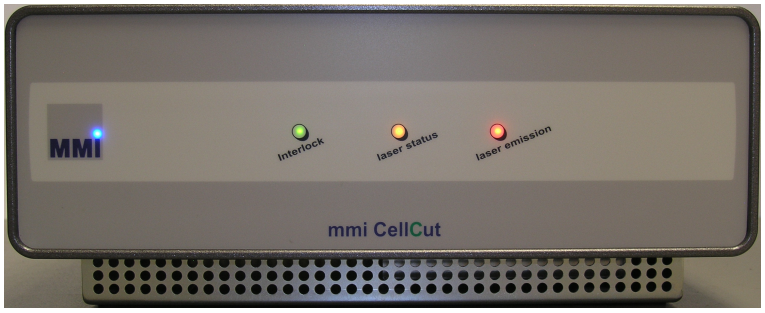
(b) Legacy controller front panel

(c) Legacy controller rear side

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

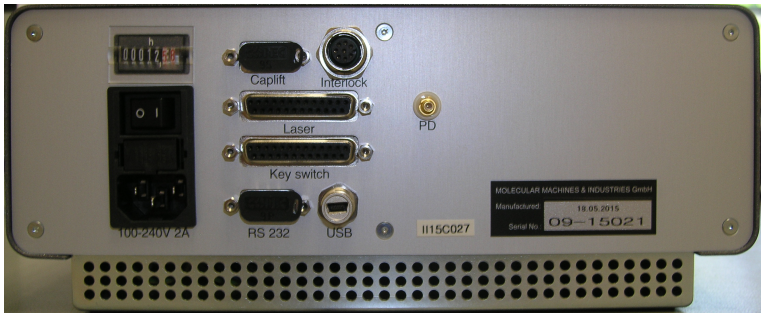
CellCut controller and key switch box						Laser status
Key switch	**Activation button**	**Interlock**	**LED 1**	**LED 2**	**LED 3**	
Off	Off	unlocked	Off	Off	Off	**Off**
Off	Flashing	locked	Off	Off	Off	**Off**
On	Off	locked	Green	Off	Off	**Powered**
On	Off	locked	Green	Slow flashing	Off	**Heating up**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

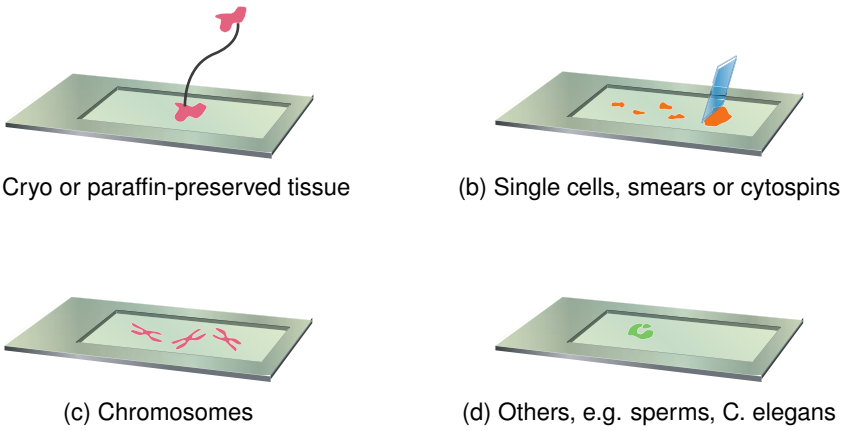
- *mmi CellCut*: laser microdissection to isolate single cells or areas of tissue
- *mmi CellManipulator*: optical tweezers to manipulate cells or beads with an optical trap
- *mmi CellEctor*: automated micro-pipetting to mechanically manipulate cells or beads with a capillary and mechanical micromanipulator
- *mmi CellScan*: whole slide imaging scanner to create and store full resolution whole slides images (WSI)
- *mmi CellViewer*: *mmi CellViewer* is a stand alone software package to view and annotate whole slide images created with *mmi CellScan*.
- *mmi CellDetector*: machine learning software for biological image analysis. *mmi CellDetector* is available in two flavours
  - detect objects on whole slide images (WSI)
  - detect objects on the live image

Any or all of these modules can be combined in one microscopic environment. The *mmi CellDetector WSI* and also be used with the stand alone program *mmi CellViewer*

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

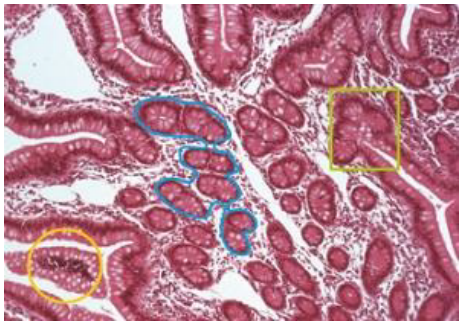


Figure 4.2: *Easy cell selection*

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.

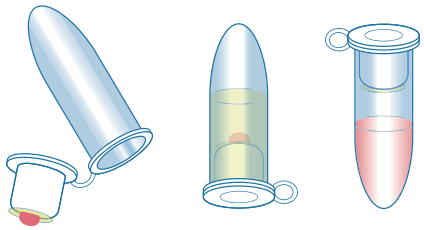


Figure 4.3: *Post-processing: after dissection, the sample is resuspended in a buffer.*

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.

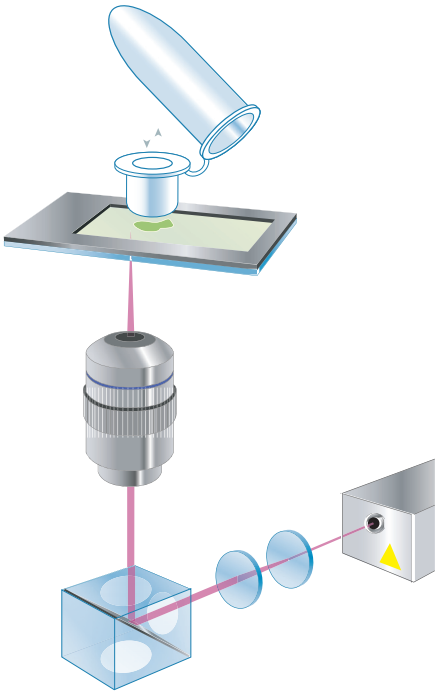


Figure 4.4: *Schematic view of laser microdissection*

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.

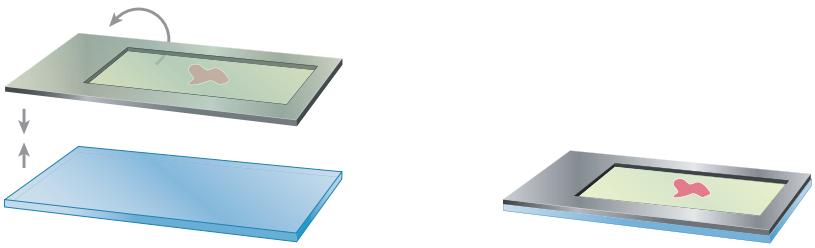
The laser cuts around the selected cells to be isolated. No additional radiation is used for the collection step.

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*

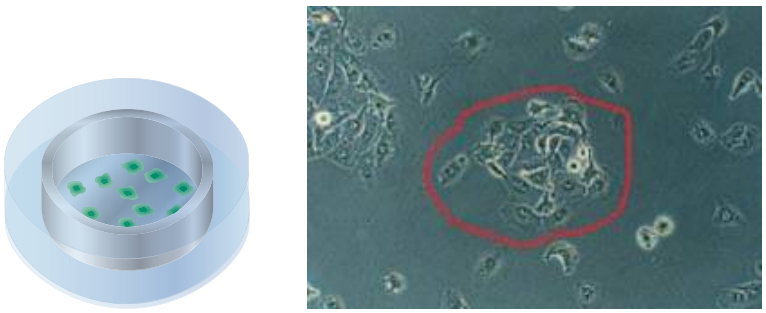
The tissue is mounted on the flat side of the membrane slide, just as on an ordinary slide. After the usual processing the membrane with the tissue is inverted and placed onto a new glass slide and fixed in position on the microscope stage.

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.

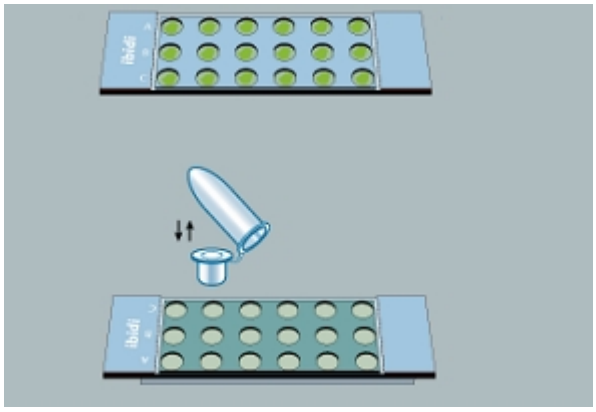


Figure 4.7: *Using 18-well live chamber slides for microdissection*

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*) 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 5.6.

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.

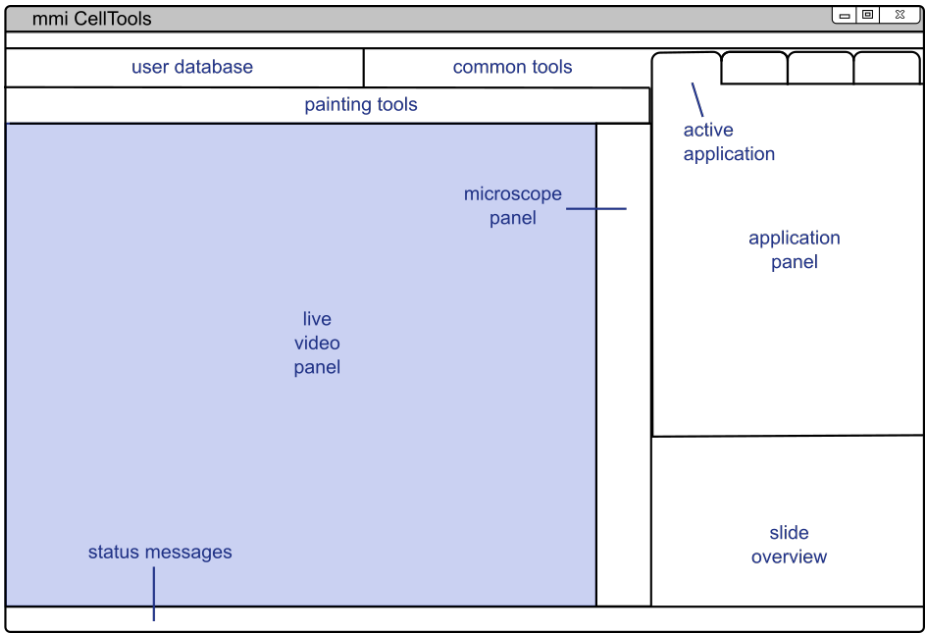


Figure 5.1: *Structure of the mmi CellTools user interface*

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.

CellTools offers three user interface styles.

- gray
- dark
- classic

The dark style is optimized for fluorescence and light sensitive applications. To switch between styles select:

Project →*Style*

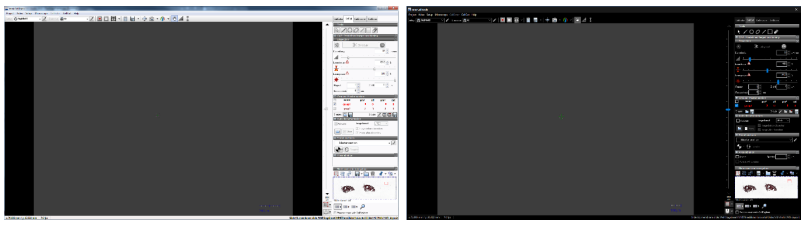


Figure 5.2: *Classic (left) and dark (right) style*

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

Setup →*Left-handed UI*

Whenever you see a warning sign at a specific function. Place the mouse over the sign and a text box appears with a description.

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:

User ⟹ Channel 1 ⟹ Objective 1
⟹ Objective 2
⋮
⟹ Channel 2 ⟹ Objective 1
⟹ Objective 2
⋮ ⋮

Basically the user can save all optical parameters separately.

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 for the current user.

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 for the current setup and user.

5.2.1 Channel editor

To open the Channel editor press the edit button next to the selected channel.

The channel selection box contains the defined parameters for each imaging channel. You can add or remove a channel and all corresponding parameters can be edited.

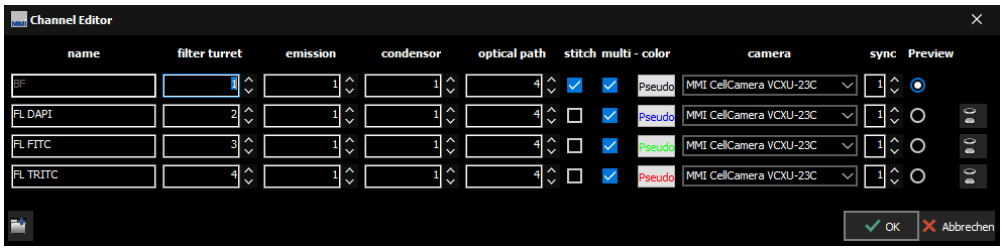


Figure 5.3: *channel editor*

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 channel for each of these experiments.

To rename the defined channels click on the channel name and type in a different name.

5.2.1.1 Channel options

- the **filter**, **condenser**, **optical path**, **stitch** and **multi channel** imaging settings are only used with motorized microscopes. The use of these

parameters is explained in sections 7.2 and 5.3.6.

- if a supported external fluorescence excitation filter wheel is mounted, **emission** filter wheel settings show up addtionally.
- if a supported LED light source with selectable wavelenghts is mounted the channel can be selected by the drop down list **FL Channel**.
- if your system is equipped with more than one camera, different channels can be associated with a certain camera using the drop down list **Camera**. You can then switch to a specific camera by selecting a channel defined for that camera. The camera used should also be reflected in the channel name.

The camera associated with a channel is displayed in the Channel editor (column **Camera**).

Channels are orginzed in synchronisation groups. If two channels are in the same group, calibrations will apply not only to the currently active channel, but to all channels in the same synchronisation group.

It is highly recommended to use dedicated synchronisation groups for different cameras.

Options like automatic sample detection (**preview**) and scanning with stitiching (**stitch**) - consider the *mmi CellScan* module.

5.2.2 Objective editor

To open the Objective editor press the edit button next to the selected objective.

The objective selection box contains all objectives defined for the active channel. You can add and remove an objective and edit all corresponding parameters. If you create a new channel, all objectives will automatically be copied.

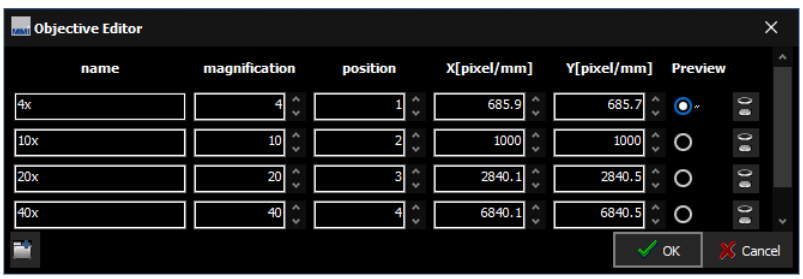


Figure 5.4: *Objective editor*

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

The nosepiece **position** 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 6.2.2.2).

The **X** and **Y [pixel/mm]** values define the field of view and are dependant of the used objective and camera. These values are calculated automatically with

Setup→Align camera with stage

(see section 5.7.2).

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)
- Camera alignment (see section 5.7.2)
- Lens offset (see section 5.7.3)
- Z-Focus lens offset (for automated microscopes only, see section 7.4.3)
- if a supported LED light source with controlable light intensities is mounted, the intensity can be selected by the parameter **FL intensity[%]**.
- Cutting parameters (see section 6.2.2)
- Laser position (see section 6.4.1)

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

The **preview** objective is used during the automatic sample detection (foreground detection) scan - consider the *mmi CellScan* module.

5.2.3 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. 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 as follows:

Setup →Restore factory settings

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

You will lose all slide, setup and group data contained in the user database.

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
  - DXA285cf
  - MXF285cf
  - VCXU23c
  - VCXU50m
- Hamatsu
  - Fusion
  - Fusion BT
  - Orca Flash IV
- Andor iXon

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 channel dedicated to that camera.

To define the camera used by a channel, open the Channel editor and click on the camera field. A drop down list shows up, in which you simply select the camera related to that channel.

Do not use the Channel editor to switch between cameras. The Channel editor is only used once to create (at least) one channel for each camera. Then, change cameras by selecting the corresponding channel. Use different synchroisation groups for different camera channels, so that camera alignments (section 5.7.2), parfocal correction (section 7.4.3) and paraxial corrections (section 5.7.3) only have to be calibrated once for every objective.

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 (*Ctrl + R*).

Figure 5.5: *Camera settings for mmi CellCamera models*

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.

The hardware camera amplifier **Gain** can be adjusted to receive brighter images. With high gain the images become noisier. For elder CCD cameras (DXA285cf, MXF285cf) this effect is much more intense than with newer CMOS cameras (VCXU23c, VCXU50m). 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 **black level** can be uses to suppress noise, the **white level** to supresss overilluminations.

The camera **Mode** provides color settings optimized for various applications. For bright field, the color quality can be optimized with the **Best color** mode. The camera transfers a high quality data stream with full pixel resolution. By selecting **Fast** imaging mode, the image rate (frames per second, fps) will be maximized. In this mode up to 55 fps (depending on your camera model) with full pixel resolution are displayed. For fluorescence applications, the contrast can be optimized with the **Fluorescence** option. The camera transfers a high quality 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 by combining pixels, which yields in brighter black and white images at a very high frame rate. Black and white modes are also available for the VCXU23c camera.

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.

MMI CellTools also supports automatic shaping correction. To get a well corrected image please display a gray image without any structures

- remove the sample from the stage
- select an average image brightness
- select the menu

  Setup →Shading correction

- activate **Correct shading** in the camera control panel (Fig. 5.5)

The Set white balance option is not available for fluorescence and monochromatic imaging modes.

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

Setup →Save white balance per objective

to enable or disable this function.

Additional camera related option are available in the drop down menu next to the camera button.

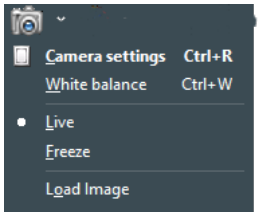


Figure 5.6: *drop down menu to reach camera related options*

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

Freeze

of the drop down next to the camera button.

To go back to live video presentation select

Live

5.3.4 Saving images

To save an image simply press the **Save image** button 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** from the drop down next to the **Save image** button, you can save an image including all markers and drawings.

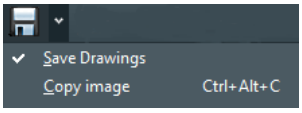


Figure 5.7: *drop down menu for same image options*

Press the **Copy image to clipboard** menu from the drop down next to the **Save image** button or press *Ctrl + Alt + C* 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.

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.

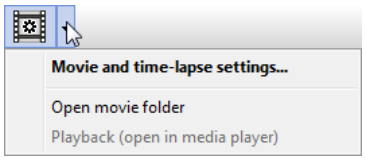


Figure 5.8: *Movie recording functions in the tool bar*

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. 5.9).

The **Encoding** specifies the format (codec) to store the video frames. Following formats are available:

- Motion JPG
- FFMPEG
- XVID

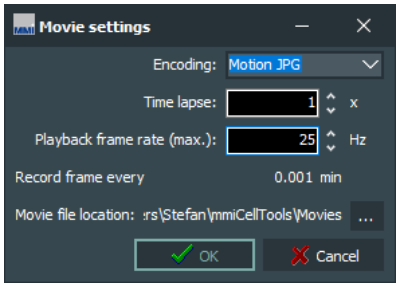


Figure 5.9: *Movie settings*

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.

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.

5.3.6 Multichannel imaging

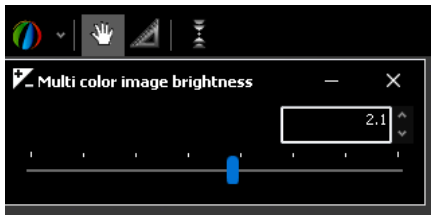


Figure 5.10: *Brighness correction slider for multi channel images*

A **multi channel** image is recorded by pressing the multi channel image button 

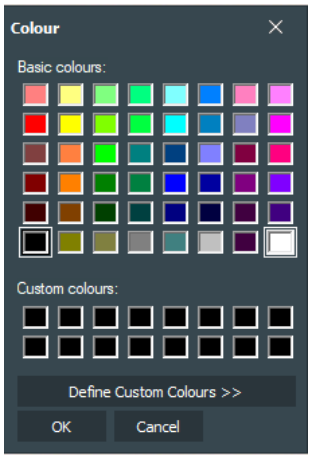


Figure 5.11: *Pseudo color for multi channel images*

or *Shift + M* (only for motorized microscopes). A multi channel image consists of several images with different camera/filter settings. For example, it can be used to merge brightfield and fluorescence images from different fluorophores (e.g. DAPI and GFP). The brightness of the resulting image can be adjusted in multi channel configuration drop down, Fig. 5.10.

To enable an imaging channel for the multi-channel image select **enable** in the **Channel editor** (see section 5.2.1). These recorded images are then merged to one image with the selected **pseudo color** for each channel. The image color can be selected or defined by custom in the **pseudo color**, Fig. 5.11.

- If **white** is selected, a original image will be merged to the multi-channel image (see Fig. 5.12 left).
- If **black** is slected, the multi-channel image will exclude the image from the selected channel (see Fig. 5.12 right).

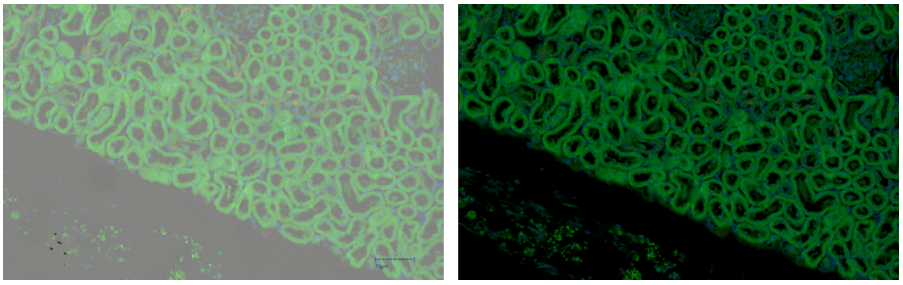


Figure 5.12: *Select white (left) and black (right) as pseudo color for brightfield image*

5.3.7 Autofocus

The **Autofocus** can be enabled/disabled by pressing the autofocus button or

Ctrl + F (only for motorized microscopes). With activated autofocus the microscope focus the plane with the sharpest image after each movement, automatically.

For safety reasons and speed opitmization the autofocus search range can be limited by using the **focus range** option in the drop down near the **focus button**.

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. Additionally, you can quickly switch to moving mode and back again by pressing the *Space* bar.

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 5.7.2).

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. 5.13).

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.

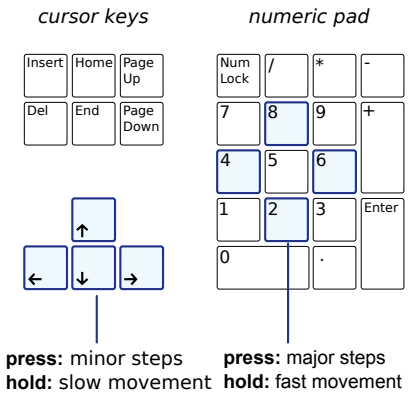


Figure 5.13: *Stage movement using the keyboard*

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. 5.14.

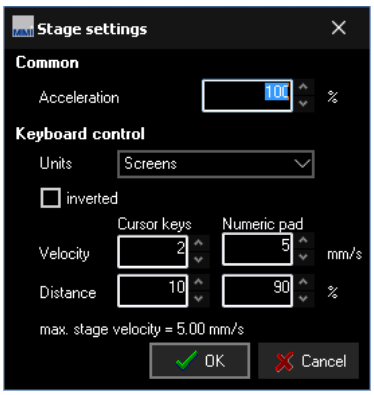


Figure 5.14: *Stage movement settings*

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:

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

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

inverted determines the movement direction using the arrow keys. By default (unchecked) the stage moves in the direction of the arrow keys. By inverting the movement, the field of view is shifted in the direction of the arrow keys.

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

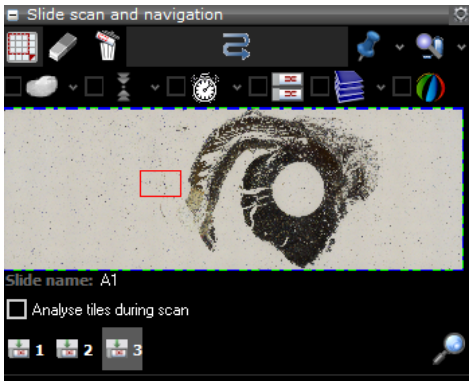


Figure 5.15: *Slide overview*

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

Press the **Delete scan region** button to delete selected scan area with the left mouse button.

Press the **Delete scan regions** button to delete all manually selected scan areas. The maximal scan area will be used instead.

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.

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.

You can set the name of the slide in the corresponding field (Fig. 5.15). The slide name is used to save the scaned image.

The overview scan configuration can be setted by pressing the **configuration button** in Fig. 5.16.

- Show drawings: show or hide the shapes by drawing tools (drawing tools is described in section 5.5.1).
- Show slide name: show or hide the slide name
- Save scan tiles: all tile images during overview scan can be saved in hard disk

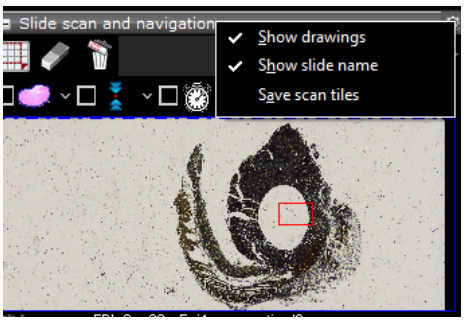


Figure 5.16: *Overview scan configuration*

For full resolution whole slide scanning - including options like multi slide scanning, time-lapse scanning, z-stack scanning, multi-channel scans - consider the *mmi CellScan* module.

5.4.4.1 Preview image

To save, load or delete the scaned preview image by simply clicking into the overview area with the right mouse button, see Fig. 5.17.

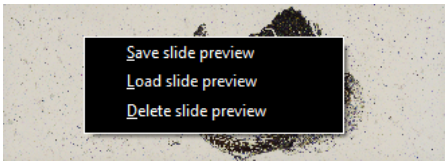


Figure 5.17: *Menu for preview image options*

5.4.4.2 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. 5.18).

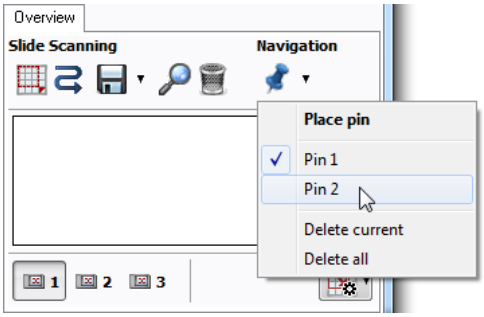


Figure 5.18: *Pin positions*

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.

5.4.4.3 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.

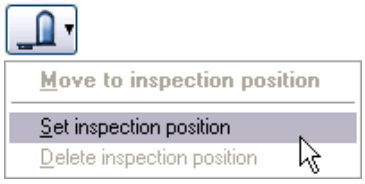


Figure 5.19: *Setting the inspection position*

To define the inspection position click on the triangle at the right edge of the **inspection button** (Fig. 5.19) 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.

5.4.4.4 Analyse tiles during scan

To automatically analyse all overview scan tiles with the image analysis software *mmi CellDetector* check the **Analyse tiles during scan** checkbox in the **Overview** panel.

> You need to train the *mmi CellDetector* before **Analyse tiles during scan** will work properly, see the separeted **mmi CellDetector** manual.

5.4.4.5 Shape navigation

Objects in the sample, like cell areas or cells, are displayed as shapes. These shapes can be created manually or by using *mmi CellDetector*.

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

The keyboard keys allways navigate inside the current group, see section 6.3. The shapes can be directly used for further cutting.

5.4.4.6 Drawing tools and measurements

Drawing tools and distance measurements are described in section 5.5.1.

5.5 Slide viewer

The Slide viewer is a tool to display full slide images, to draw shapes around regions of interest and to annotate them. Images acquired with the **mmi CellScan** can be accessed at any magnification.

The slide viewer (Fig. 5.20) can be opened via the menu

Setup →Stage insert (Ctrl + I)

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

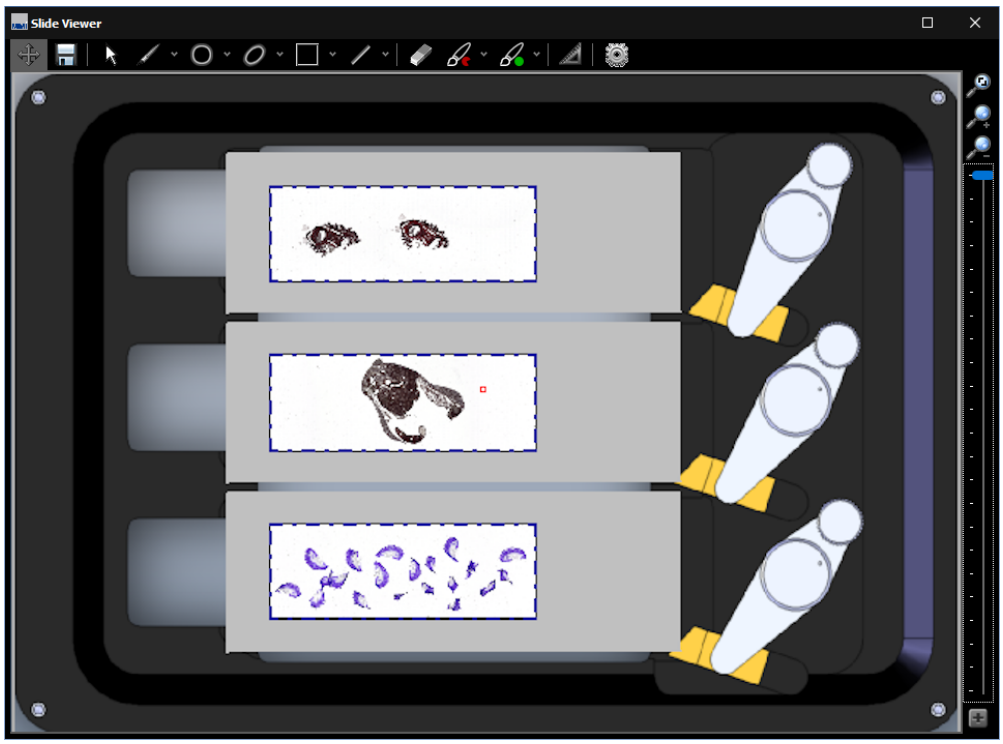


Figure 5.20: *The Slide viewer can be used to display full slide images, draw contours and to configure the stage insert*

The Slide viewer has following main purposes:

- display and zoom whole slide images (WSI)
- draw, display and review shapes around objects of interest

- create and manage groups of objects
- navigate through your samples, by double-clicking on the target position
- configure the geometry of the
  - stage insert and
  - slides or microplates, each containing the active regions called wells.
- assign a role to each well
- exporting and importing stage geometries

5.5.1 Drawing tools

The drawing tools can be selected through the buttons above the main window. Additionally, a quick switch between moving and drawing mode is possible by pressing the *Space* key on the keyboard. As drawing tools you find from left

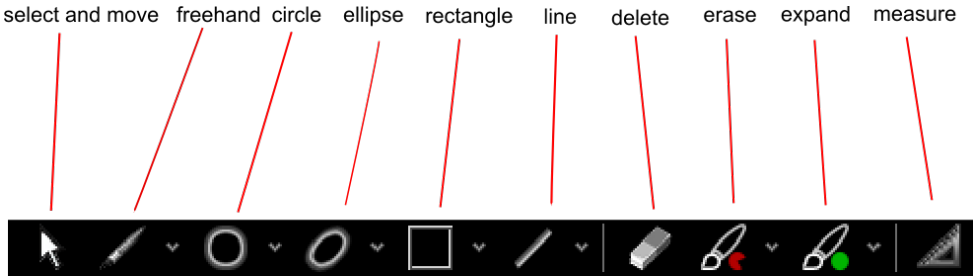


Figure 5.21: *Drawing tools: select, freehand, circle, ellipse, rectangle, line, delete, erase, expand and measure*

to right

- select and move a shape: click on a shape and drag the shape over the field of view. The shape will be highlighted
- freehand: use the mouse as a pencil
- circle: mark a first point of the circle and define a second point by dragging the mouse. Fix diameters can be set via the drop down menu next to the **circle button**
- ellipse: draw the main axis first and than define the minor axis by dragging the mouse. Fix parameters can be set via the drop down menu next to the **ellipse button**
- rectangle: mark the left upper corner and than define the diameter by dragging the mouse. Fix parameters can be set via the drop down menu next to the **rectangle button**

- line: mark the left upper corner and than define the length by dragging the mouse. Fix lengths can be set via the drop down menu next to the **rectangle button**
- delete: delete a shape by clicking on the shape
- erase: remove small pieces of the shape by using this tool as eraser. The erasing size can be set via the drop down menu next to the **erase button**
- expand: add small pieces to the shape by using this tool as brush. The erasing size can be set via the drop down menu next to the **expand button**

Shapes are orginised in groups (see section 6.3).

5.5.1.1 Select and move shapes

With the **select and move** tool (*Alt + R*) you can select and activate contours with a left mouse click. All highlighted shapes are activated. To reposition the activated shapes, drag and drop the shapes with the cursor.

By holding down the *Shift* key all shapes can be repositioned simultaneously (e.g. to compensate for a shifted sample).

5.5.1.2 Freehand

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

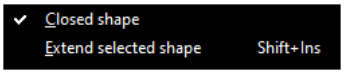


Figure 5.22: *Freehand drawing options*

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. Hold the keyboard **shift** key when you want to extend the shape, release the **shift** key to start a new freehand 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 drawing may be easier when “Closed shape” is turned off (unchecked).

5.5.1.3 Circles

The **circle** tool (*Alt + C*) is suitable for creating circular shapes.

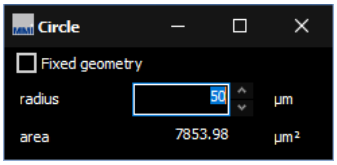


Figure 5.23: *Circle tool*

Fixed-size circles can be created by checking “Fixed diameter” and typing the value in the corresponding input box (Fig. 5.23).

5.5.1.4 Ellipses

Certain shapes can be approximated as an ellipse. Select the **ellipse** tool (*Alt + E*) to draw an ellipse. Drawing an ellipse is done in two steps:

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

Fixed-size ellipses can be created by checking “Fixed geometry” and typing the values in the corresponding input box (Fig. 5.24)

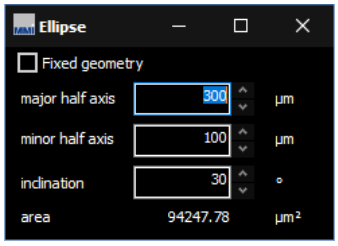


Figure 5.24: *Ellipse tool*

5.5.1.5 Lines

Cutting straight lines with the **line** tool (*Alt + L*) may be useful for ablation or cell-surgery type experiments, as well as for cutting tests.

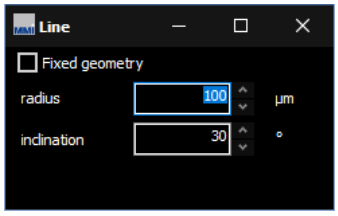


Figure 5.25: *Line tool*

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°.

5.5.1.6 Rectangles

The **rectangle** tool (*Alt + Q*) provides a quick method to outline objects of interest. Combined with the area ablation option (section 6.6) rectangles are useful for ablation type experiments.

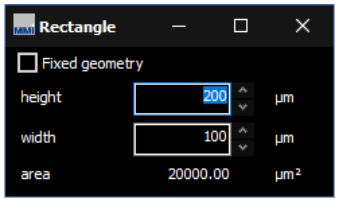


Figure 5.26: *Rectangle tool*

Fixed-size rectangles can be created by checking “Fixed dimensions” and typing the values in the corresponding input box (Fig. 5.26).

5.5.1.7 Deleting shapes

There are several ways to remove shapes:

- Deleting arbitrary shapes using the **eraser** tool (*Alt + Del*)
- Deleting the current (highlighted) shape by pressing *Del*
- Using the context menu (Fig. 5.27)
- Using the group editor (section 6.3.4).

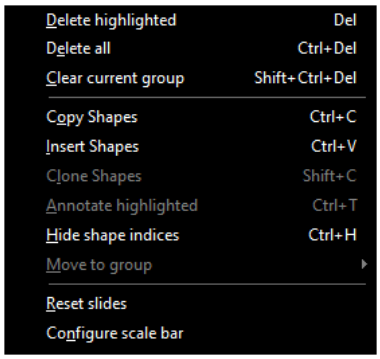


Figure 5.27: *Context menu, open with the right mouse button in the image panel.*

To delete all shapes, or all shapes from the current group, use the context menu (Fig. 5.27) or use the corresponding keyboard shortcuts *Ctrl + Del* and *Ctrl + Shift + Del*.

5.5.1.8 Copying and pasting shapes

To copy the active contour use the menu item

CellCut → *Shapes* → *Copy*

Select

CellCut → *Shapes* → *Insert*

to insert the copied shape.

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

5.5.1.9 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 *Clone...* from the context menu (Fig. 5.27).

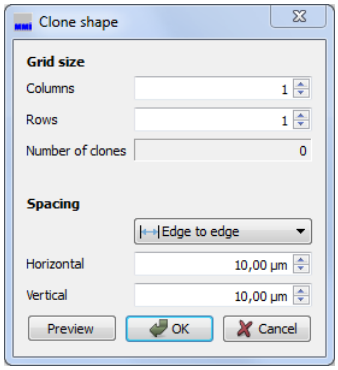


Figure 5.28: *Clone shapes*

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.

> 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.

5.5.2 Text annotations

Highlighte shapes can be annotated with your comments, by using the *Annotate highlighted* menu in the popup menu Fig. 5.27. A small popup window Fig. 5.29 will allow to enter the text you want to save with the hightlighted shapes.

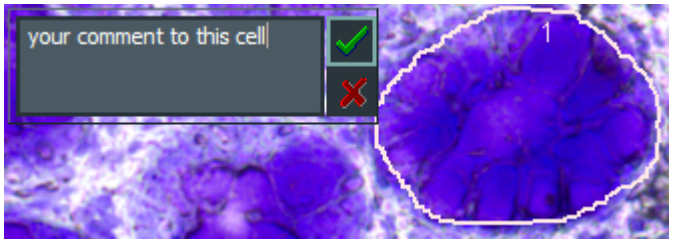


Figure 5.29: *Free text annotation for shapes*

5.5.3 Hide shape indices

The popup menu shown in Fig. 5.27 or using the shortcut *Ctrl + H* also allows to hide the shape indices. This can be helpfull if many shapes exists. In this case hiding indices also accelerates the drawing.

5.5.4 Move to group

The popup menu shown in Fig. 5.27 also allows to move highlighted shapes into another group. This allows to correct the group assignement in case you forgot to select the correct group before drawing the shapes.

5.5.5 Reset slides

For convienience the popup menu shown in Fig. 5.27 also allows to reset slides. This means

- delete all focus points
- delete all scan regions
- delete all image analysis regions
- delete preview and whole slide images

5.5.6 Configure scale bar

The popup menu shown in Fig. 5.27 also allows to configure the colors and thickens of the scalebar shown in the right lower corner of the image panel.

5.5.6.1 Navigating between shapes

There are several keyboard commands that allow you to locate your shapes and navigate between them. These are illustrated in Fig. 5.30.

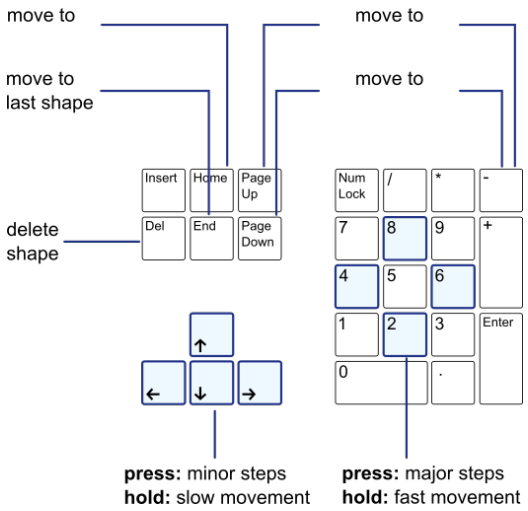


Figure 5.30: *Keyboard navigation*

- *Home* move to the first shape of the highlighted group
- *End* move to the last shape of the highlighted group
- *+* move to the next shape of the highlighted group
- *-* move to the previous shape of the highlighted group
- *Arrow up* move a small step up
- *Arrow down* move a small step down
- *Arrow left* move a small step left
- *Arrow right* move a small step right
- *Numpad: Up* move a large step up
- *Numpad: Down* move a large step down
- *Numpad: Left* move a large step left
- *Numpad: Right* move a large step right

5.5.7 Distance measurement

Select the measurement tool or press *Alt + M* to measure distances on the sample.

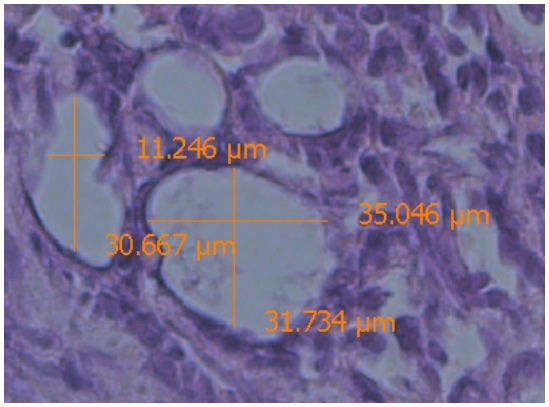


Figure 5.31: *Distance measurement*

Press the left mouse button and drag the mouse. After releasing the mouse button you can see the measured distance (Fig. 5.31)

5.5.8 Slide Navigation

Using the **mouse wheel** you can zoom into the slide image and back. Alternatively you can use:

- **fit to window** button

- **Zoom in** button

- **Zoom out** button
- **zoom slider** shown in Fig. 5.20 right of the image panel

To move the image select the **shift image**

5.5.9 *mmiCellScan* 5D channels

The *mmi CellScan 5D* can create multichannel whole slide images (5D WSI's). To navigate through the timepoints, the z-Positions and the to colorize the different channels appropriate sliders and color selection options will be provided, see Fig. 5.32. The brightness for merged image or seperate image for each channel can be adjusted. The selection of **pseudo color**, please referto section 5.3.6

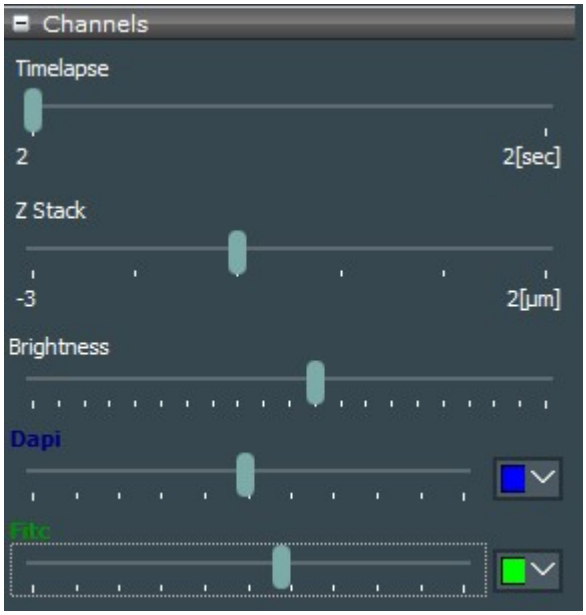


Figure 5.32: *Control the timelapse, zStack and Multi-channels of your 5D WSI.*

5.5.10 Shape import from third party applications

In case you ordered the *Shape Import* function, *mmi CellTools* enables you to load shapes created in following third party applications:

- TissueGnostics StrataQuest
- Visiopharm
- Evident ScanR
- 3DHistech SlideViewer
- Generic csv files

The mmi Service will configure your system to select the requested vendor.

Select *Import from third party scanner* to select the file you want to import. The file type matching your vendor will be preselected.

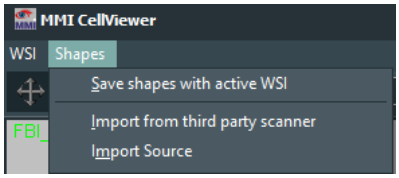


Figure 5.33: *Shape import menu*

5.5.10.1 TissueGnostics StrataQuest

StrataQuest can provide black and white mask files in the *bigtiff* format. The regions of interest (ROI) show up as white blobs on black background.

mmi CellTools can find these ROI's and import them as shapes into all cell types defined in *mmi CellTools*. If you select a filename with the format:

FilenameGroup1.tif (p. ex. xyzEpithel.tif ....)

and *mmi CellTools* has following CellTypes (see section 6.3) configured:

Ephitel, Bone, Blood, Muscle

CellTools will search the folder containing xyzEpithel.tif file for following additional files:

xyzEpithel.tif xyzBone.tif xyzBlut.tif xyzMuscle.tif xyzREFERENCE.tif

from each of these files Celltools finds, it imports the shapes given as withe dots on black background (mask), into the related group.

If *mmi CellTools* does not find any related cell type, it imports the ROI's from the selected mask file in the active (highlighted) cell type.

If CellTools finds XihangREFERENCE.tif CellTools imports (up to 3) reference points. Having reference points the user can use the serial section functionality to fine tune the shape positions.

5.5.10.2 Visiopharm

Visiopharm can provide xml files containing the shape information. These shapes will be loaded into the active (highlighted) cell type.

5.5.10.3 Evident ScanR

In *Evident ScanR* you can define so called gates to select different objects types. The found objects for all gates can be exported into a text file. The *mmi CellTools* will compare the gate names and the cell type names defined in *mmi CellTools*. *mmi CellTools* will load the centers of objects into the related cell type.

5.5.10.4 3DHistech SlideViewer

SlideViewer can export shapes into comma separeted files ("*.csv"). These can be imported from *mmi CellTools* into the active (highlighted) cell type. Shapes only having on point will be interpreted as reference point.

5.5.10.5 Generic csv files

Shapes from generic comma separeted files ("*.csv") can be imported. *mmi CellTools* will load these shapes into the given cell type. If the cell type is not defined the shape will be loaded into the active (highlighted) cell type. Shapes only having on point will be interpreted as reference point. The generic csv import also supports shape z coordinates and the use case (cut or meander). Example files can be provided by MMI.

5.5.11 Slide viewer plugins

The Slide viewer is able to host plugins like *mmi CellScan* or *mmi CellDetector* in the right side panel, see Fig. 5.34. This allow you to easily navigate through your whole slide images or use machine learning for image analysis.

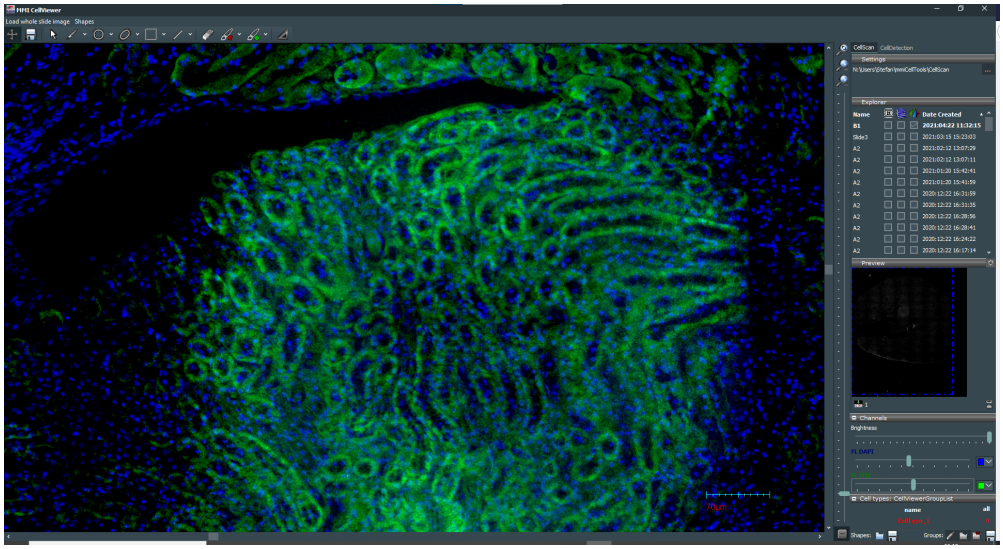


Figure 5.34: *The Slide viewer also allows to host plugins in the right side panel*

5.6 Stage inserts configuration

The slide viewer can be expanded by pressing the **configuration button** to 

configure the stage inserts. Depending on the stage inserts configuration up to 8 full slide images can be loaded in the slide viewer.

In order to properly navigate through your samples

- the origin of the stage (see 5.7.1)
- the geometry of the stage inserts

must be properly defined.

A stage insert is a mechanical holder for slides or multi-well-plates, which simply snaps into the microscope motorized stage. These definitions are a precondition for the use of the overview, see section 5.4.4 scan. For long travel distance stages two inserts are supported.

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.

The full geometry configuration can be exported into a file by

File $\rightarrow$ *Export*

Using

File $\rightarrow$ *Import*

the configuration can be reloaded into the stage geometry viewer.

5.6.1 Stage inserts

In the configuration panel (Fig. 5.35) different types of slide inserts can be selected:

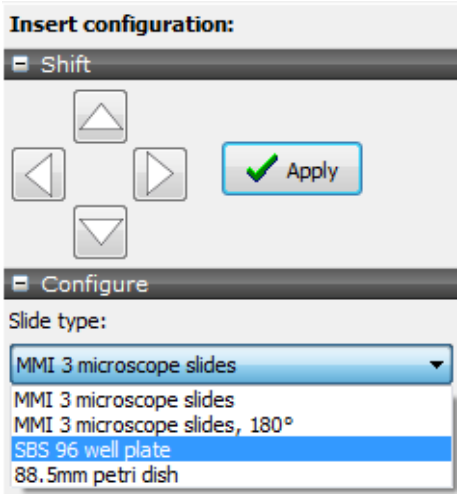


Figure 5.35: *Slide viewer: Insert configuration panel. You can select different insert types and shift the whole insert to adapt it to overview images.*

- insert for three microscope slides, clamps on the right hand side
- insert for three microscope slides, clamps on the left hand side
- insert for four microscope slides
- 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 fit into the slide shift the insert as described in chapter 5.6.4.

5.6.2 Slides

Depending on the stage insert selected in section 5.6, the configuration panel for slides (Fig. 5.36) 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 slide
- 18 well slide

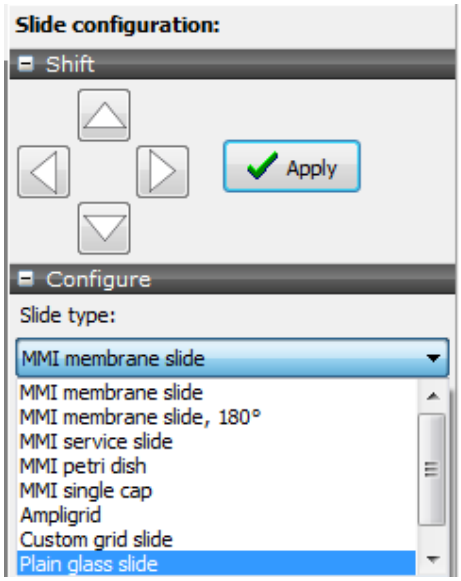


Figure 5.36: *Slide viewer: Slide configuration panel. You can select different slide types and shift the whole slide to adapt it to it's overview image.*

- MMI petri dish holder
- MMI single cap holder
- MMI double cap strip holder
- plain glass slide
- empty

Additionally you can 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 5.7.1. If this is not sufficient follow the procedure described in section 5.6.4

5.6.3 Active regions, wells

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

- region for microdissection

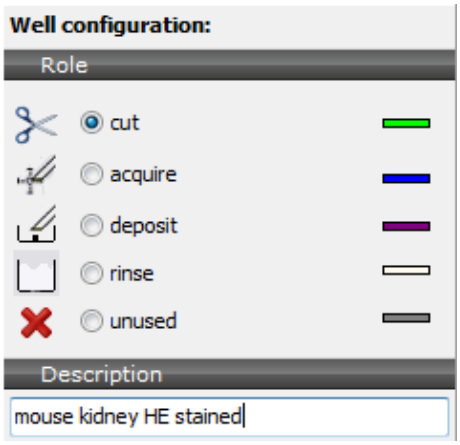


Figure 5.37: *Slide viewer: Well configuration panel. You can select different well rules and add descriptions to each well.*

- collection well for CellEctor
- deposit well for CellEctor
- rinse well for CellEctor
- unused well

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

5.6.4 Stage inserts first configuration

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

Procedure

1. select a 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 5.6.2
4. calibrate the stage origion, see section 5.7.1
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. 5.38) 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 5.6.2

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 5.6.2, 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.

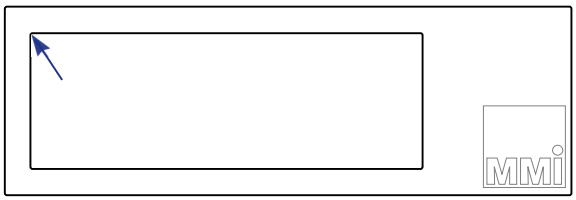


Figure 5.38: *Suggested positions on a mmi Membrane slide to precisely adjust the stage insert position.*

5.7 Calibration

5.7.1 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 one of the following options:

- press the **Calibrate origin** button in the Slide viewer.
- *Setup →Calibrate Stage 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 5.6.2 and 5.6.4.

5.7.2 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

Setup →Align camera with stage (Ctrl + A)

from the main menu.

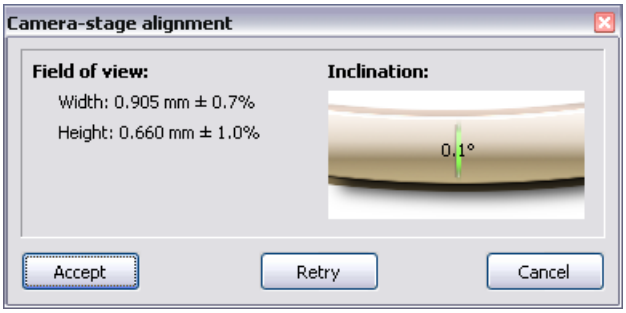


Figure 5.39: *Camera alignment results*

If automatic alignment was successful, a dialog (see figure 5.39) 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 5.7.2.1). 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).

5.7.2.1 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.

5.7.3 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 **Paraxial Lens Offset** function is introduced to ensure that a marker points to the same object for different objectives.

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 a shape is no longer fitting the same object by changing the objective.

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 paraxial lens offset calibration with the menu item

   Setup →Paraxial Lens Offset (Ctrl + O)

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

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.

Select the menu item

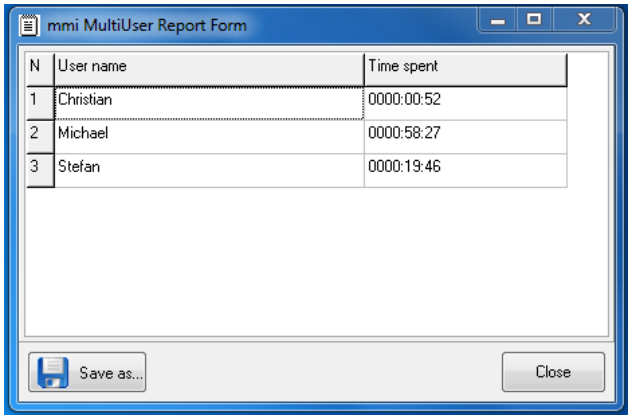


Figure 5.40: *Multi-user report*

Project →Usage report...

or start it from

Start →All Programs →mmi CellTools →mmi MultiUser Report

The 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 CellDetector*, the *mmi CellScan* 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).

The indivudal configurable properties can be selectively accessed via the properties button displayed in each sub-panel.

6.1 Drawing the cutting contour

6.1.1 Basics

For laser microdissection you need to define a cutting contour. You can choose between different drawing tools (Fig. 5.21) to create a contour with the mouse in 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 area of interest, that you want to microdissect. The following calibration and settings need to be done correctly before you can start cutting:

1. Camera alignment, section 5.7.2
2. Cutting velocity, section 6.2.2.1
3. Laser focus, section 6.2.2.2
4. Laser power, section 6.2.2.3
5. Laser position, section 6.4.1

After drawing the contours of the shapes press the **Cut active group** button and all defined contours of the active group will be cut Fig. 6.2.

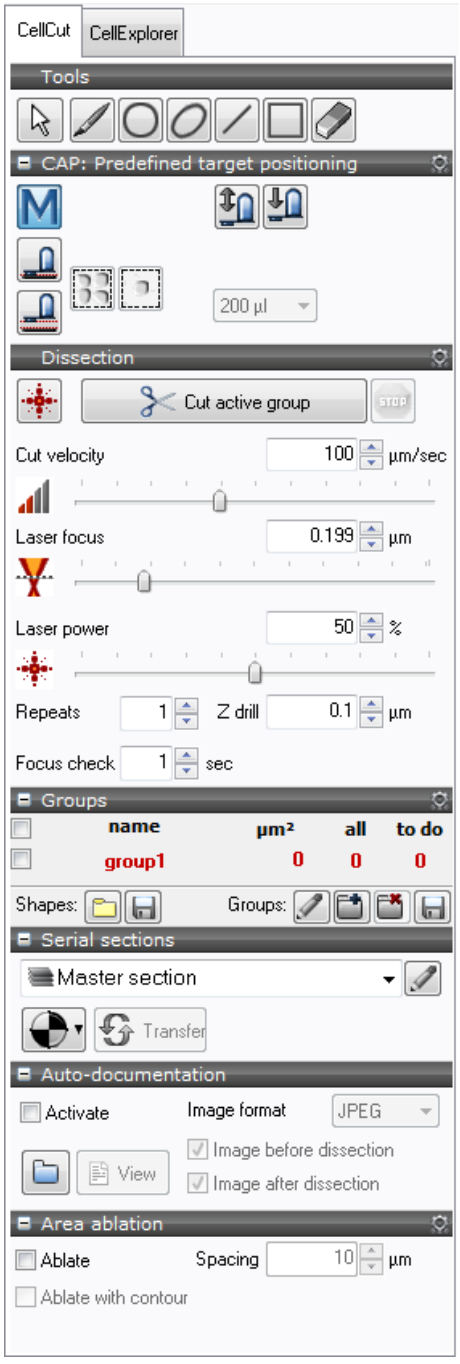


Figure 6.1: *The CellCut plug-in panel*

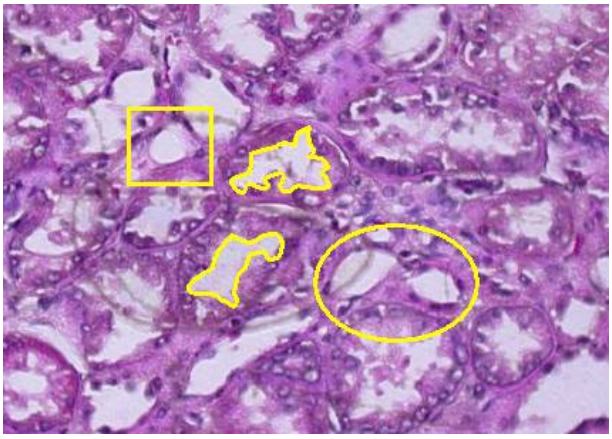


Figure 6.2: *Defining contours to cut*

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. 6.3). The default setting of the single laser shot time is 50 ms. A range of 50–100 ms is typical.

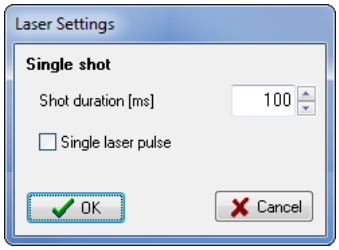


Figure 6.3: *Single shot laser settings*

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* →*Fire 50 ms laser shot* or via the keyboard shortcuts *ALT + I* (timed interval) and *ALT + S* (single pulse).

6.2.2 Dissection parameters

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. 6.4).

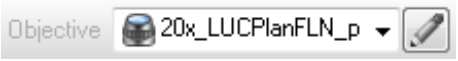


Figure 6.4: *Objective chooser*

The laser setup includes the following parameters (Fig. 6.5).

- Cut velocity (of stage movement)
- Laser focus
- Laser power

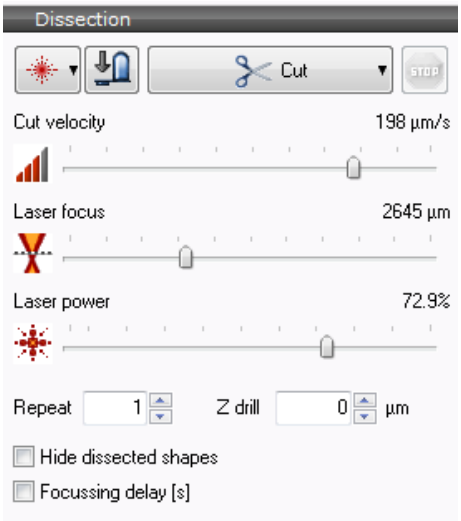


Figure 6.5: *Dissection panel with laser parameters*

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

6.2.2.1 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.

6.2.2.2 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 5.2.2).

6.2.2.3 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 is based on measurements performed at the MMI factory site. The actual laser power is not monitored in the device.

6.2.2.4 Iterative dissection

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

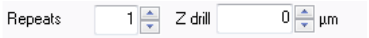


Figure 6.6: *Iterative dissection user interface. Z drill is only available on systems with an automated microscope*

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 parame-

ters are optimal (see section 6.2.4).

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.

6.2.2.5 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.

Dissection 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 entering a value greater than zero in the **Focus check** field. Enter the number of seconds you need to adjust the focus before the dissection is started.

6.2.3 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 the shape with 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. 6.7 shows a typical example of a cut.

6.2.4 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.

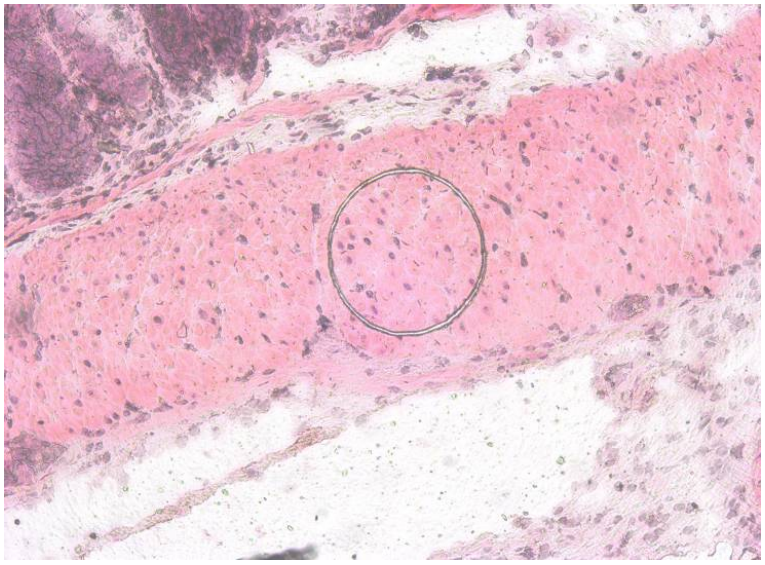


Figure 6.7: *A cutting test on a piece of biological tissue*

6.2.5 Laser safety

When the laser is turned on a small window with a laser warning label always pops up (Fig. 6.8). When the software turns off the laser, the symbol disappears.

Figure 6.8: *Laser safety warning sign*

6.2.6 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.

6.2.7 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 Cell types

Any object drawn will always be assigned to the highlighted cell type group shown in the **Cell types** panel (Fig. 6.9).

> In image analysis applications these cell type groups are commonly called classes. Anyway cell type groups or classes define different cell types.

The most common application for using more than one cell type 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 cell type group are marked with the same contour color. The number of groups is not limited.

The active cell type group is highlighted. To switch between groups, click on the group of interest from the group list. If the *mmi MultiCap* is installed and active, it will immediately rotate such that the designated cap is in position.

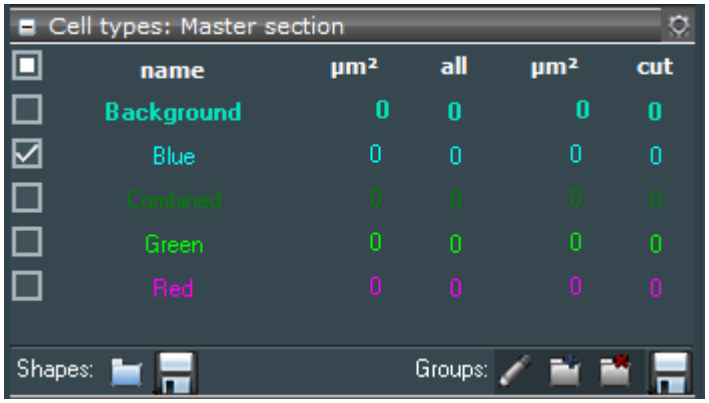


Figure 6.9: *Cell type group selection.*

Using the checkbox on the left side of Fig. 6.9 the group can be selected as **cuttable**. If you have a *mmi CellEctor* and the group is aslo selected as **collectable** the *mmi CellEctor* will automatically try to collect cut shapes.

The shapes of all cell type groups can be exported as xml–file. In return such a file can be imported to replace the current groups and shapes.

6.3.1 Definition and editing of groups



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

In order to deal with groups invoke the **Group editor** (Fig. 6.10) using the edit button.

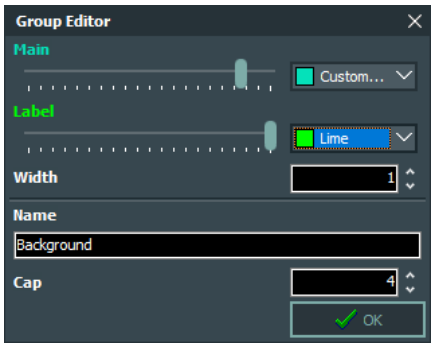


Figure 6.10: *Group editor*

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 6.5.4).

6.3.2 Regrouping shapes

To move individual shapes from one cell type group to another, open the context menu (Fig. 5.27) by right-clicking on video panel and select the target group from sub-menu (*Move into group* →*GroupX*).

6.3.3 Group statistics

The statistics can be seen directly in the group panel (Fig. 6.9).

For each group, the list displays the number of shapes and how many of them are not cutted, yet. In the properties menu the total area of all shapes (*Show total area*) and the total uncut area (*Show total cuttable area*) can be displayed.

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

6.3.4 Shape list

If you click on the number of shapes in a group, a list with all shapes defined in that group pops up (Fig. 6.11).

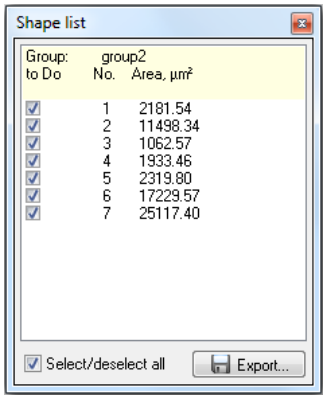


Figure 6.11: *Shape list*

The list displays the area in µm of each shape and an editable tick mark for all shapes that will be processed with future actions.

To quickly deselect or select all shapes, use the checkbox at the bottom. By holding down *Shift* multiple shapes can be selected or deselected.

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.

6.4 Calibration routines

6.4.1 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 5.7.2).

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.

6.5 Sample 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.

6.5.1 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.

6.5.2 mmi CapLift

6.5.2.1 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 can be equipped with cap holders for 200 µl, 500 µl or 1500 µl caps.

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 6.5.4.

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.

6.5.2.2 Mounting cap

To mount the cap, simply move the closed cap into the cap holder and then open it. The cap 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.

6.5.2.3 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.

6.5.3 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.

6.5.3.1 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. 6.12).

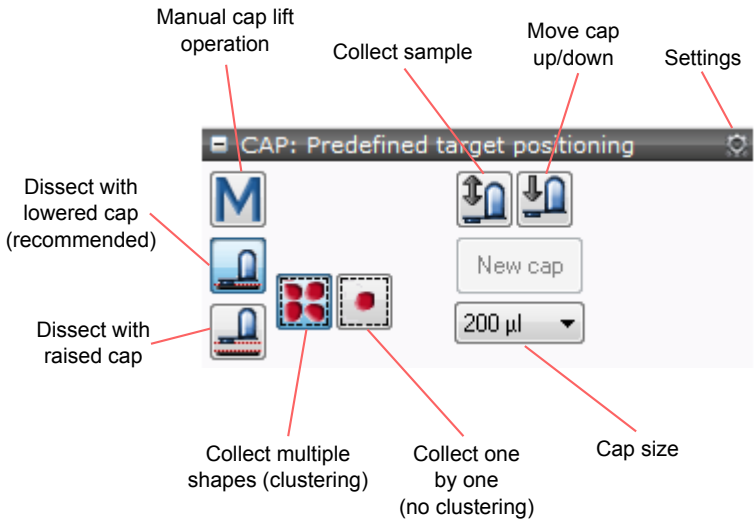


Figure 6.12: *Predefined target positioning (PTP) panel*

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 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. Use:
  - **Collect** to lower and subsequentially lift the cap lift to collect potential dissectates.
  - **Move cap up/down** to move the cap lift to the up or down position depending on its current position.

- 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** in settings, the system assumes that you use a new cap each time you press the **Cut active group** 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 unselect the option 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. 6.12). 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.

6.5.3.2 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 *Properties* →*Configure* (Fig. 6.13).

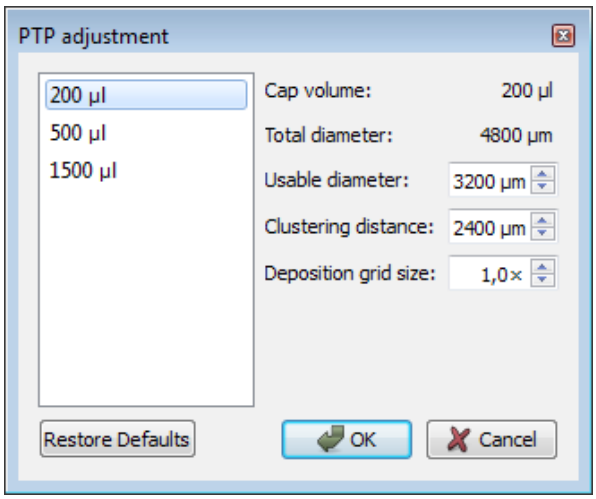


Figure 6.13: *Adjusting PTP cap parameters (advanced)*

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.

6.5.4 mmi MultiCap

The *mmi MultiCap* system can be equipped with a cap holder for an eight adhesive cap strip for up to eight 200 µl tubes. With the Multi cap it is possible to cut dissectates and automatically subdivide samples to different test tubes.

If your system supports the *mmi MultiCap* you can switch between

CellCut → *Cap Lift* → *Single Cap*

and

CellCut → *Cap Lift* → *Multi Cap.*

6.5.4.1 Using the mmi MultiCap

To use the *mmi MultiCap* it is important to work with groups (see 6.3). Each group can be assigned to a specific cap of the multi cap. By default a new group is assigned to the next free cap.

Users can cut one, several or all of the groups in one single step. Groups can be selected with the check box next to the group name (Fig. 6.9).

After selection of the groups, press the **Cut** button to initiate the cutting process. Automatically the *mmi MultiCap* cuts all selected groups and collects the dissectates of each group with the assigned cap.

If a single cap holder is installed all shapes from all groups will be cut as if they were in one group.

6.5.4.2 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

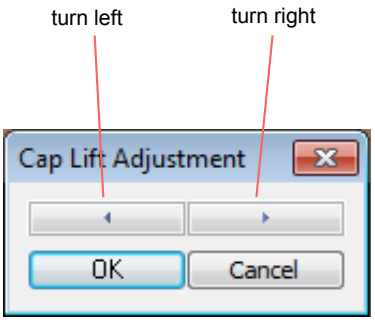


Figure 6.14: *Cap lift adjustment*

After adjustment recalibrate the cap holder.

6.6 Area ablation

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

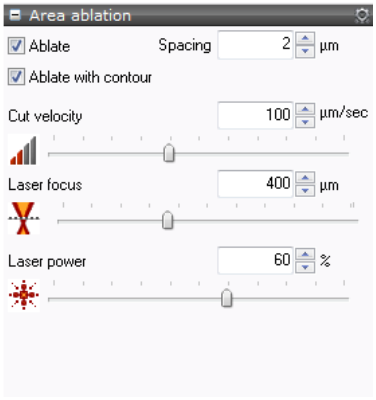


Figure 6.15: *Area ablation panel*

Area ablation is activated through the check box in the **Area ablation** panel (Fig. 6.15). When checked, the laser will not trace the contour, but instead follow a meandering path, covering the shape's entire area. Additionally, the contour of the shape can be traced with the laser by checking **Ablate with contour** to get a smooth border of the shape. By setting *Properties →Cut before ablate* the contour is cut before the ablation process.

For the ablation process all laser settings (**Cut velocity, Laser focus, Laser power**) can be set independently of the standard dissection parameters (see 6.2.2). Typically, the best ablation results can be achieved, when the laser focus is slightly below the sample.

> 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 **Spacing** parameter to eliminate gaps between cutting lines. This value should be equal to the width of the cutting line.

Open free-hand drawings are treated as closed shapes.

6.7 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.

6.7.1 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.

6.7.2 Basic usage

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.

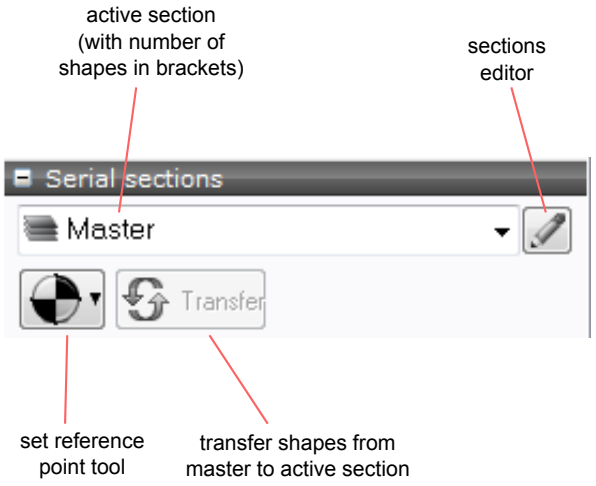


Figure 6.16: *Serial sections panel*

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 **Create a landmark** 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. 6.17) 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.

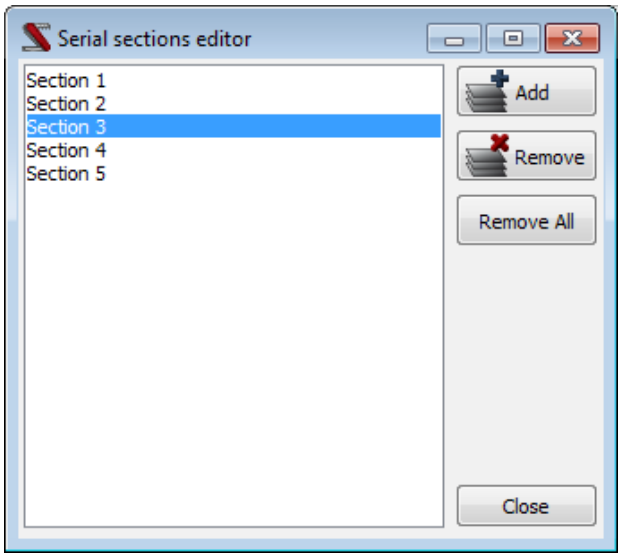


Figure 6.17: *Managing sections*

6. Locate the reference points on the section slide, again using the **Create a landmark** 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.

   All shapes of the active section will be deleted and replaced by the shapes of the master 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. 6.18). Clicking on one of the menu items will move the stage so that the reference point is centered in view.

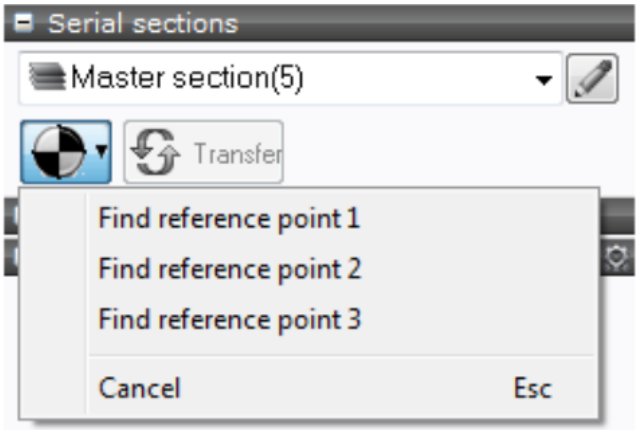


Figure 6.18: *Locating reference points*

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

6.7.3 Using shapes to define reference points

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

To do so, simply select a drawing tool (see 5.5.1) after you pressed **Create a landmark** and outline the shape you want to use as reference.

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

6.7.4 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 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.

6.7.5 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 (see section 6.3).

All groups are transferred using the **Transfer** button. The group structure of the target section is inherited from the master section.

6.7.6 Using mmi CellDetector to identify shapes

mmi CellDetector can be used together with serial sections in a straightforward manner. CellDetector 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 6.7.2.

6.8 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.

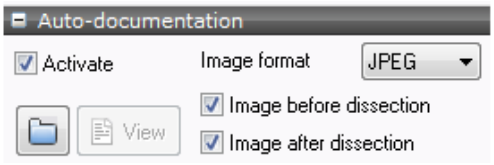


Figure 6.19: *Auto documentation*

6.8.1 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.

6.8.2 Settings

The settings are displayed in the Auto-documentation section of the CellCut panel (Fig. 6.19). 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.

6.9 Shape import from external scanners

mmi CellTools can support the import of shape data from external scanners. If this option is activated by an MMI dongle, following data files can be imported:

- from 3D Histech scanners: Shapes can be annotated and exported in the 3D Histech Pannoramic viewer software. To be able to export reference points a specific dongle from 3D Histech is required on the PC running Pannoramic Viewer. The 3D Histech exchange format supports x and y coordinates.
- Generic data files can be imported. The generic data file supports x,y coordinates plus the microsope z position.

The active file format will be set by an MMI service technician.

6.9.1 Import

File imports work on comma separated file (csv). If the import is successful the numer of imported shapes will be displayed. Automatically a serial section, see 5, will be created. If the scanner offset is defined proberly, see 6.9.2, the referene markers appear in the same field of view as the physical reference position:

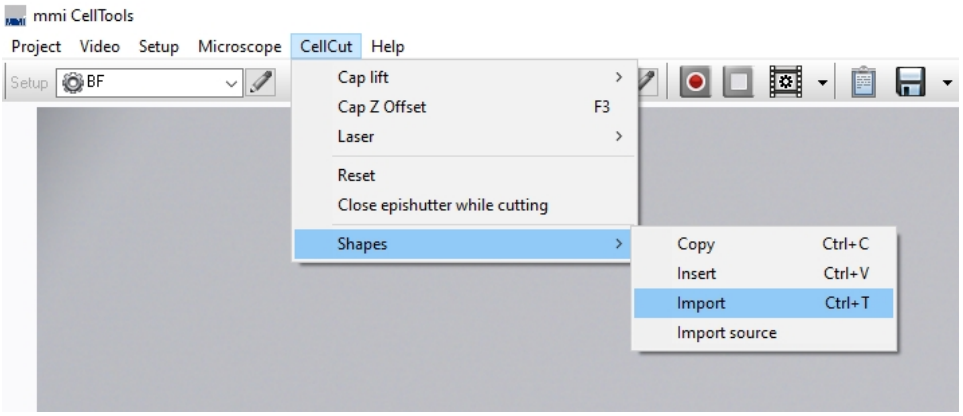


Figure 6.20: *Import of shape data from external scanners*

Procedure

1. Select the

   CellCut → *Shapes* → *Import*

   to receive a file selection dialog
2. open the serial sections panel, see 6.7.2
3. move to the reference markers and shift them to the physical reference position
4. press **Transfer**

6.9.2 Import Source

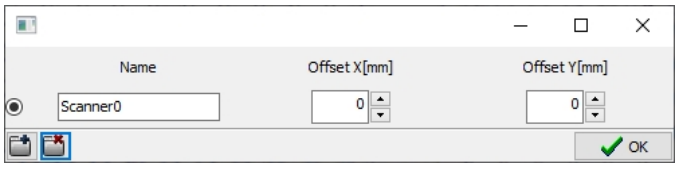


Figure 6.21: *Define external scanners as import sources. You can define the name and coordinate offsets for each scanner. Press the **plus** icon to add a scanner, press the **minus** icon to delete a scanner*

By selecting the

CellCut → *Shapes* → *Import Source*

several scanners with their scanner specific offsets can be defined. The offset should be set so that after importing shape data the imported reference markers show up in the same field of view as the pysical reference position:

Procedure

1. select the 4x objective
2. import a data file
3. open the serial section panel, see 6.7.2 and move to the first reference point
4. measure the distance between the marker and the reference position
5. correct the scanner offsets with the measured values
6. import the data file again and correct the offsets until the reference marker and the physical reference position show up in the same field of view
7. if required set the offsets more precise in higher magnifications

7 Automated microscope control

mmi CellTools supports the following automated microscope types:

- Olympus IX-81
- Olympus IX-83
- Nikon TE2000 E
- Nikon Ti
- Nikon Ti2

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*

or by press *CTRL + M*.

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*.

7.2 Observation methods

The Channel editor is used to define the observation methods, see section 5.2.1.

In the Channel editor you define for each channel the position of the

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

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

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

	Olympus IX-83	Olympus IX-81	Nikon TI	Nikon Ti2
Left side port	1	1	5	4
Binocular	3	2	1	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 → *Binocular*

or by pressing *F7*.

7.3 Objective control

In the objective editor, see section 5.2.2, 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 surface definition
- Automatic focus adjustment objective and slide change and caplift operation (CellCut only)
- Focus memory for shapes (CellCut only) and pin positions (see section 6.2.7)
- 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. To open the mouse wheel settings panel press *Configure wheel step* in the menu of the mouse wheel button. The stepsize of the mouse wheel can be set in the mouse wheel settings panel (Fig. 7.1).

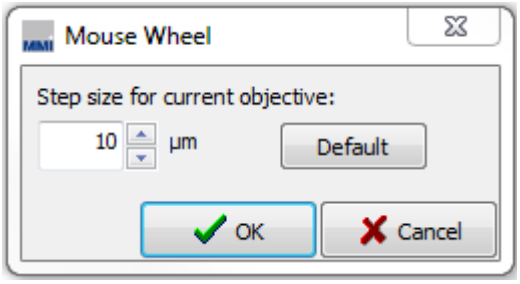


Figure 7.1: *Mouse wheel settings*

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 focus map

The sample on a microscope or membrane slide has a specified topographical surface. To avoid permanent manual re-focussing, CellTools offers the ability to specify a focus map that represents the surface of the sample.

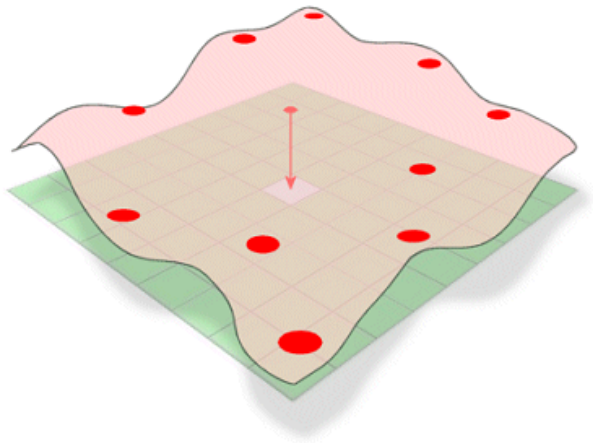


Figure 7.2: *Sample surface with defined surface points*

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

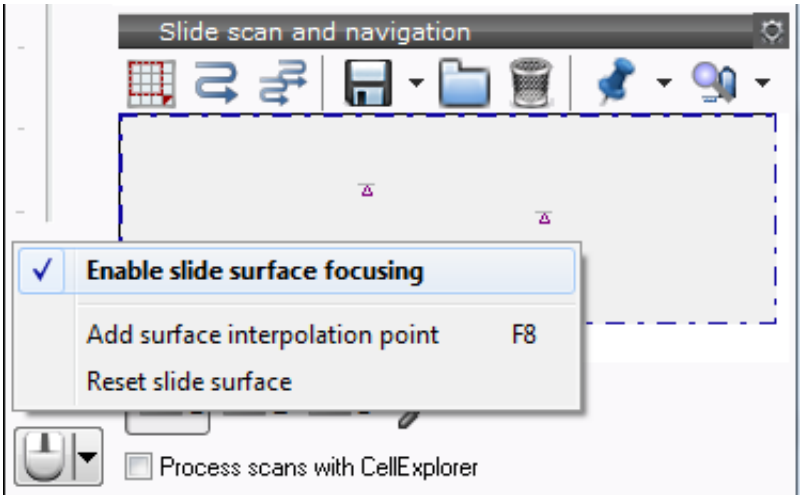


Figure 7.3: *Surface map user interface*

Procedure

1. Focus the sample and define a surface point by pressing *Add surface interpolation point* or *F8*
2. You can place as much surface points as you like but a minimum of 3 surface points is required
3. in general the more points are defined the better the surface is mapped

   Note that the plane surface can be set with any objective but the precision increases with magnification.

After the focus map is defined, the sample focussing aid can be switched on and off through the **focus surface** button or menu (*Enable slide surface focussing*).

The focus map is stored independently for each slide. When changing to a slide, for which the focus map has not been defined, surface focussing will be disabled.

You may use *Reset slide surface* to remove all points and start defining a surface 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

   Setup →Parfocal Lens Offset

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

   CellCut →Cap Z Offset

   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.

7.5 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.

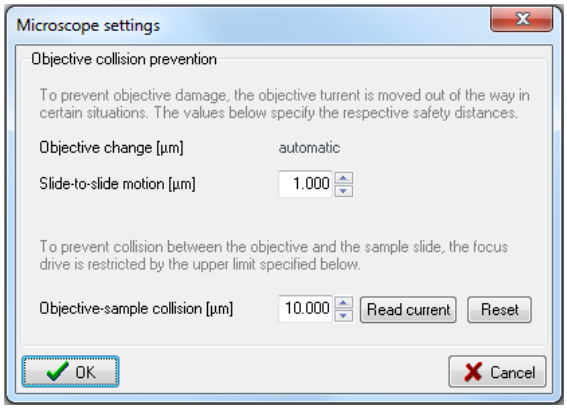


Figure 7.4: *Microscope safety parameters*

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 Maintenance

8.1 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.

8.2 Cleaning

Caution

- Before cleaning the system, disconnect all system components from the mains.
- The user should not remove or open the following parts for cleaning:
  - beam path covers
  - laser box
- 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.
- Hazardous laser light 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.

8.2.1 Microscope

For further details, see microscope manual.

8.2.2 Cleaning optical parts

- Clean all easily accessible optical parts once a week
- Carefully wipe the optical parts with a cleaning tissue moistened with alcohol of 70% to 80%

8.2.3 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

8.3 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.

8.3.1 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

8.3.2 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 5.7.2)
- 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 5.7.2)
- 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 5.6
- Stage origin calibration invalid, see section 5.7.1

8.3.3 Overview

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

- Camera alignment not correct (see section 5.7.2)
- The camera is not mounted in an exactly upright position

8.3.4 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

8.3.5 Cutting

8.3.5.1 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 are 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

8.3.5.2 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 too 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

8.3.6 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 List of Keyboard Shortcuts

General

+	Move to next shape	44, 53
-	Move to previous shape	44, 53
Alt + C	circle tool	48
Alt + Del	delete shape tool	50
Alt + E	ellipse tool	48
Alt + F	freehand tool	47
Alt + L	line tool	49
Alt + M	Measure distance	53
Alt + Q	rectangle tool	49
Alt + R	select and move tool	47
Arrow down	Move small step down	53
Arrow left	Move small step left	53
Arrow right	Move small step right	53
Arrow up	Move small step up	53
Caps Lock	Suppress continous stage movement	40
Ctrl + A	Align Camera	63
Ctrl + C	Copy shape	50
Ctrl + Del	Delete all shapes	50
Ctrl + H	Hide shape indices	52
Ctrl + I	Show stage insert	45
Ctrl + O	Set stage origin	62, 65
Ctrl + P	Stage movement settings	40

Ctrl + Shift + Del	Delete all shapes from current group	50
Ctrl + V	Paste shape	50
Del	Delete shape	50
End	Move to last shape	44, 53
Esc	Interrupt current process	42, 65
F1	Display user manual	66
F2	Lower or raise cap lift	26
Home	Move to first shape	44, 53
Numpad: Down	Move large step down	53
Numpad: Left	Move large step left	53
Numpad: Right	Move large step right	53
Numpad: Up	Move large step up	53
Space	Toggle between moving and drawing mode	39, 46

Camera control

Ctrl + Alt + C	Copy image	35
Ctrl + R	Open camera settings	33
Ctrl + S	Save an image	35
Shift + M	multi channel image	38

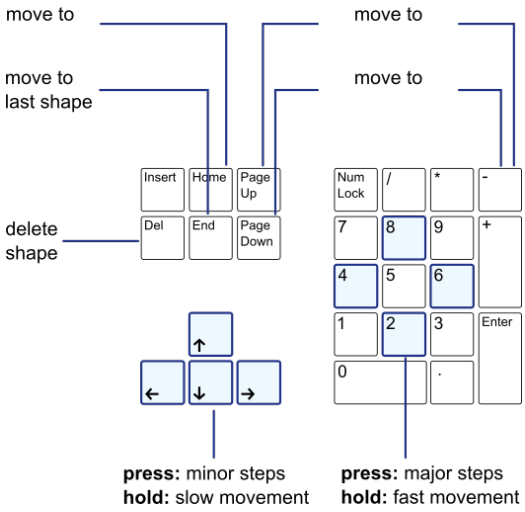
CellCut

ALT + I	Fire laser for timed interval	71
ALT + S	Fire single laser pulse	71

Microscope control

Ctrl + F	autofocus	39
CTRL + M	Remote control On/Off	95
F3	Define Z drive offset	100
F6	Open/close fluorescence shutter	103
F7	Switch between camera and binocular	96
F8	add surface point	99

Navigation


move to
move to
last shape
delete
shape
move to
move to
Insert
Home
Page
Up
Del
End
Page
Down
Num
Lock
/
*
-
7
8
9
+
4
5
6
1
2
3
Enter
0
.
press: minor steps
hold: slow movement
press: major steps
hold: fast movement

B Technical data

B.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 $\times$ 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.

B.2 System components

B.2.1 Microscope

Inverted or upright research microscope.

Supported microscope models:

- Olympus IX-71/81
- Olympus IX-53/73/83
- Nikon Ti (S, U, E)
- Nikon Ni (U, E)
- Nikon TE 2000 (S, U, E)
- Nikon Ti2 (U,E)
- External z-Drive motorisations for different manual inverted microscopes

B.2.2 Stage

Standard stage

Scanning stage with stepper motors.

Scanning area:	120 × 100 mm^2
Repositioning accuracy:	< 1 µm
Step resolution:	0.156 µm
Speed:	50 mm/sec

Long travel scanning stage for two inserts

Long travel scanning stage with stepper motors.

Scanning area:	280 × 82 mm^2
Repositioning accuracy:	< 1 µm
Step resolution:	0.075 µm
Speed:	25 mm/sec

B.2.3 Digital camera

mmi CellCamera VCXU23c

- Chip type: Sony IMX174
- 1 /1.2“ progressive scan CMOS
- Native Resolution 1920 x 1 200 pixels
- Pixel Size 5.86 µmx 5.86 µm
- Dynamic Range 72 dB
- USB3 interface
- >45 fps, full resolution, live view, best color calculations

mmi CellCamera VCXU50m

- Chip type: Sony IMX174
- 2/3“ progressive scan CMOS
- Native Resolution 2448 x 2048 pixels
- Pixel Size 3.45 µmx 3.45 µm
- USB3 interface
- >50 fps, full resolution, live view, best color calculations

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 × 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 × 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 × 1040 pixels with up to 15 fps
- Ultra high sensitivity

Hamamatsu Orca Flash4.0 V3

- Digital CMOS camera with sCMOS sensor designed for scientific research use.
- quantum efficiency 82% @ 560 nm
- dynamic range 36000:1
- TE Cooling to –10 °C

Hamamatsu Fusion BT

- Digital CMOS camera with sCMOS sensor designed for scientific research use.
- quantum efficiency 95% @ 550 nm
- dynamic range 21400 :1
- 2304 × 2304 pixels with up to 89.1fps
- Air-Cooling to –8 °C
- Water-Cooling to –15 °C

B.2.3.1 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 ℃: Critical for elimination of dark current detection limit.

B.2.4 Fluorescence Light sources

B.2.4.1 Lumencor Spectra III

- Sources: 8 solid state sources including LEDs, lasers and proprietary luminescent light pipes
- Wavelengths: 380 - 750 nm
- Bandpass Filters: Integrally installed bandpass filters for spectral output refinement
- Output Power5̃00mW per color band ± 10

B.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 Windows10, 64bit.

B.2.6 CapLift

Classic CapLlift

- ± 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

C Service

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

It is strongly recommended to execute an annual system service to maintain the system performance.

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

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D Declaration of Conformity

CE

Declaration of Conformity

In accordance with:

DIRECTIVE EMC 2014/30/EU

Manufacturer

Molecular Machines & Industries GmbH
Breslauer Strasse 2
85386 Eching

Apparatus	MMI CellCut
Production country	Germany
Type of product	Laser Microdissection
Technical documentation	CellCut User Manual

Summary of test results

Test	Standard	Results
ESD immunity	*IEC 61000-4-2*	*Test passed*
Radiated electromagnetic field immunity	*IEC 61000-4-3*	*Test passed*
Power frequency magnetic field immunity	*IEC 61000-4-8*	*Test passed*
Voltage dips	*IEC 61000-4-11*	*Test passed*
Short interruptions	*IEC 61000-4-11*	*Test passed*
AC power immunity to electrical fast transient (EFT) / burst transients	*IEC 61000-4-4*	*Test passed*
Immunity to Mains surge	*IEC 61000-4-5*	*Test passed*
Conducted RF immunity of AC power	*IEC 61000-4-6*	*Test passed*
I/O cable immunity to electrical fast transient (EFT) / burst transients	*IEC 61000-4-4*	*Test passed*
Conducted RF immunity of I/O cable	*IEC 61000-4-6*	*Test passed*
AC/ DC Power Emission	*CISPR 55011*	*Class B*
Housing Emission	*CISPR 55011*	*Class B*

Accordingly, based on the test results (that are performed following the international standards), the "MMI CellCut" conforms with the immunity requirements of Directive EMC 2014/30/EU.

Molecular Machines and Industries GmbH
85386 Eching
February 08th, 2021

MMI