Molecular Machines & Industries

mmi CellManipulator

User Manual

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

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Contents

1 Safety advices

The system should only be used for positioning of microparticles like cells, cellular organelles or microbeads as described in this manual. Do not use the system for any other purposes. Damages due to unauthorized use are not subject to warranties. Only properly trained persons are allowed to use the system. Completely read the safety advices in this section and the remainder of the manual before operation.

1.1 Laser safety

This system contains a high power class 4 infrared laser source. The system includes safety devices to prevent laser interference with the user. Due to the power losses determined by the lenses of the optical tweezers system and the objectives of the microscopes the average power shall be deemed to be nominal at the microscope work surface (i.e., inside the sample volume).

Table 1.1: *Nominal radiation output*

Nominal output power	20 W
Operation mode	CW
Wavelength	1070 nm

1.1.1 Laser safety labels

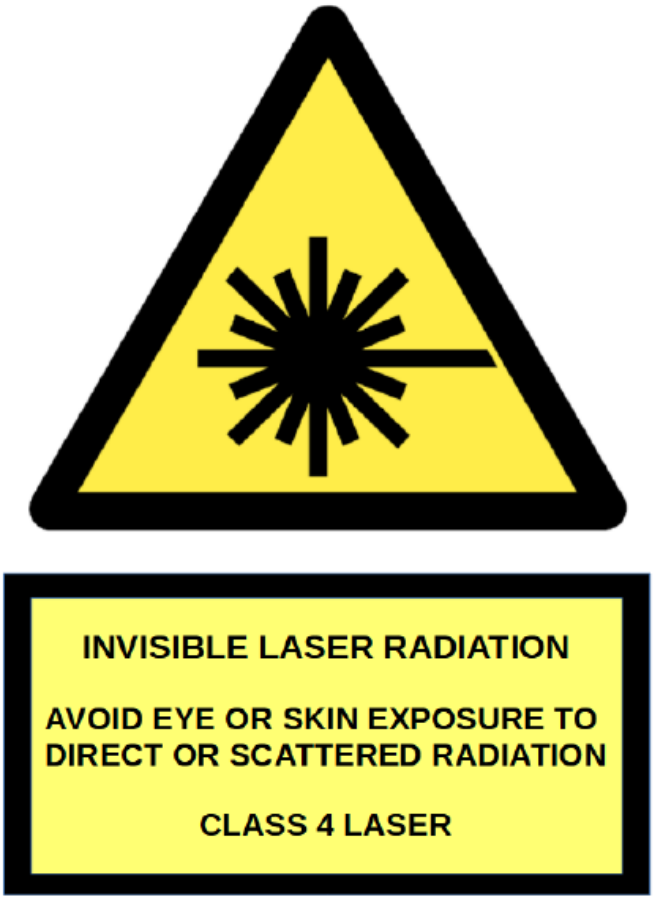


Figure 1.1: *mmi CellManipulator laser safety labels.*

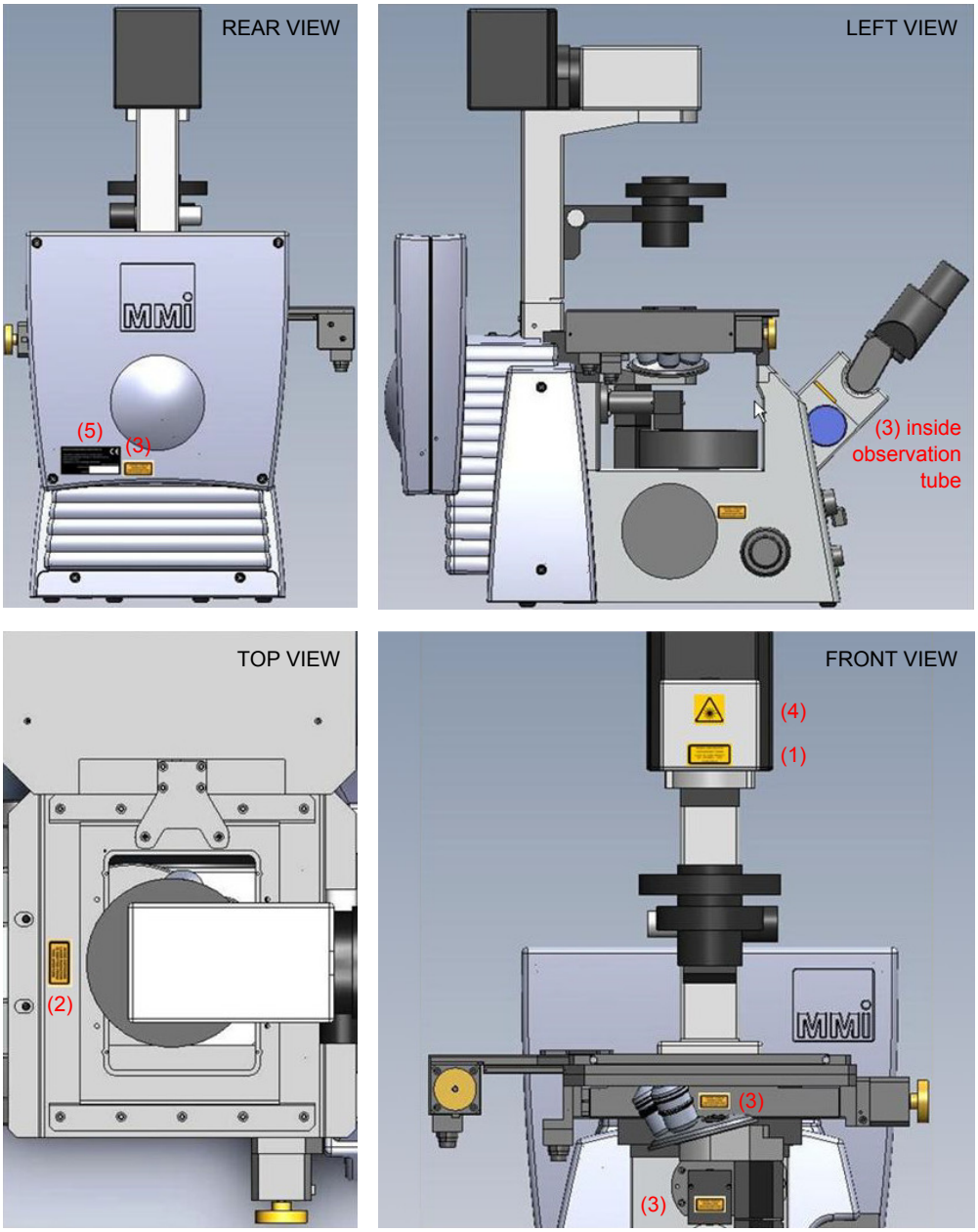
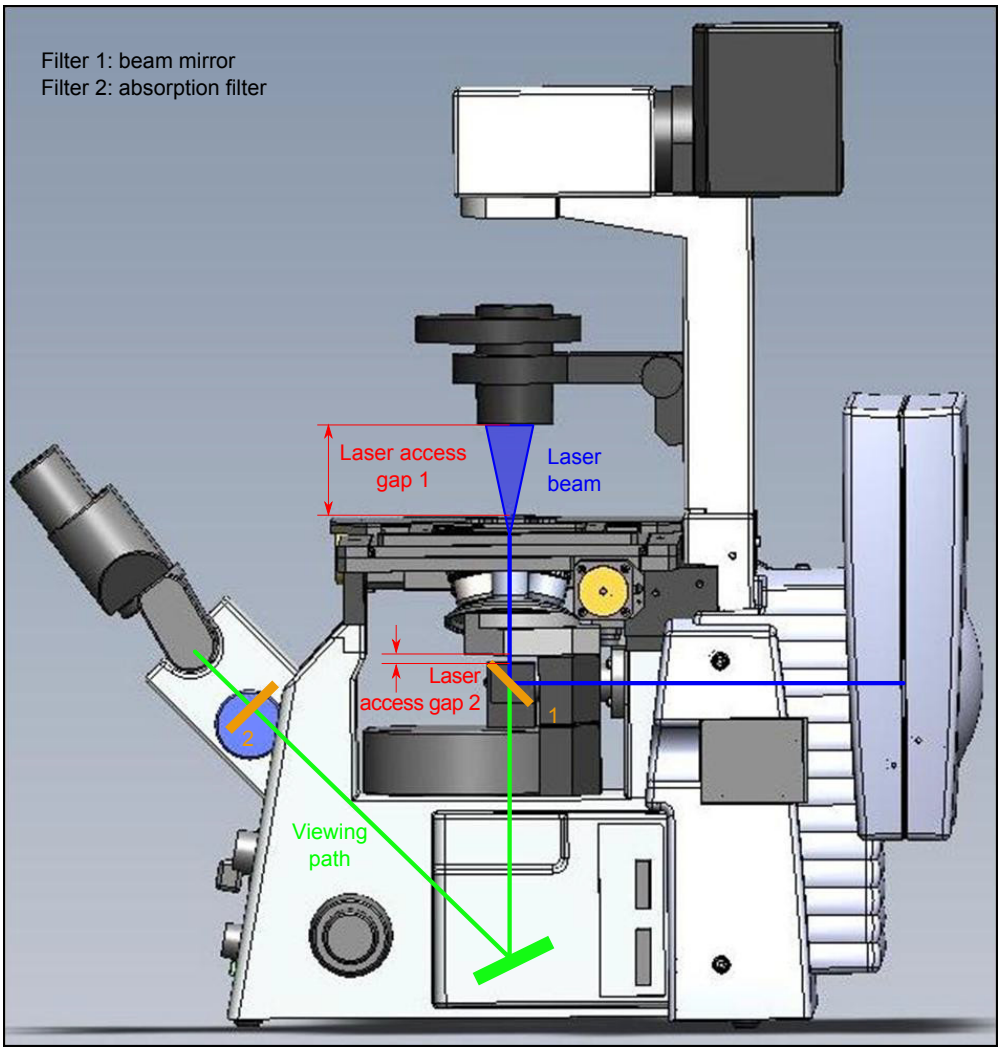


Figure 1.2: *Positions of laser safety labels in mmi CellManipulator*



- **Laser access gap 1:** Access to infrared laser power, gap between 90–97 mm, depends on the z-position of the objective and condenser.
- **Laser access gap 2:** access to infrared laser power, gap between 4–11 mm, depends on the z-position of the objective.

Figure 1.3: *Laser access gaps*

1.1.2 Eye protection

A blocking laser absorption filter inside the microscope provides the necessary eye protection when the oculars may be used. 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.

1.1.3 Miscroscope interlocks

The interlocks are provided to protect you from eye injury resulting from accidental exposure of the eye to the invisible laser beam.

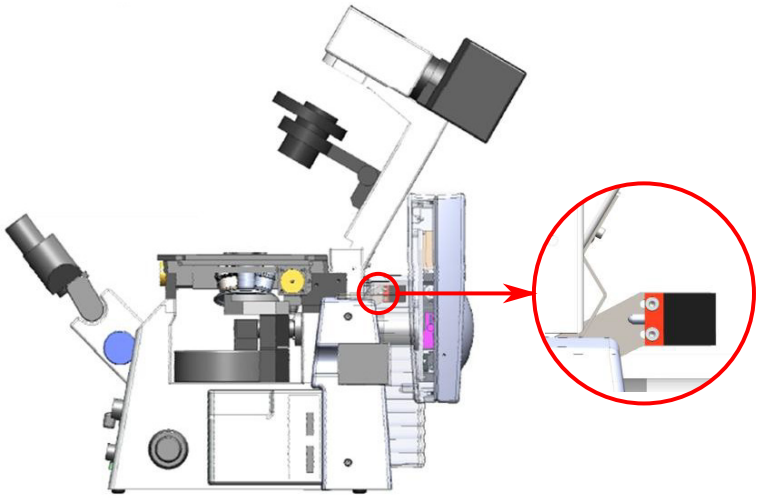


Figure 1.4: *Illumination pillar interlock*

When you tilt the illumination pillar backwards the interlock mechanism switches the laser off (Fig. 1.4). All LEDs on the key switch box will turn off. The laser must be reactivated after the interlock has been closed again.

1.1.4 Remote interlock

A second remote interlock switch should be connected to *mmi CellManipulator* electronics and should be used as door lock. The door lock switches the laser automatically off if someone enters the room.

1.1.5 Key switch

To turn the laser on follow this procedure:

- Put the key switch on the key switch box in horizontal position. If the interlocks are properly closed, the activation buttons starts flashing.
- You are requested to acknowledge the laser start by pressing the activation button. If the interlocks are properly closed, see section 1.1.3, the green “interlock” LED will turn on. If the lasers operates properly also a yellow LED lights up. If the yellow LED flashes, the laser is not operating correctly.
- Wait for about 15 seconds during which the laser will temper. After this the laser is in stand-by mode and ready to use.
- By starting the laser in the software or by creating a trap the laser will start emission. This is indicated by a constant red “laser emits” LED.

The different scenarios indicated by the LEDs are summarized in table 1.2.

Table 1.2: *Laser status indicator of the mmi CellManipulator*

Key switch box						
Key switch	**Activation button LEDs**	**Interlock**	**Green LED**	**Yellow LED**	**Red LED**	**Status Laser**
Off	Off	open	Off	Off	Off	Off
On	flashing	closed	Off	Off	Off	Off
On	Off	closed	On	Off	Off	Powered
On	Off	closed	On	Flashing	Off	Error
On	Off	closed	On	On	Off	Heating up & Ready
On	Off	closed	On	On	On	Emitting

1.1.6 Warnings

To ensure full safety of the system please adhere to the following safety guidelines:

- Turn off the laser with the key switch to prevent unauthorized operation of the system.
- Do not remove any objective while the laser is operating.
- Never stare into the objective turret while the laser is operating.

- Only use objectives recommended from MMI for the optical tweezers system.
- Never remove an objective without immediately closing the thread on the objective turret.
- Never place reflecting objects in the beam path.
- Viewing the laser output with certain optical instruments (for example eye loupes, magnifiers, microscopes) within a distance of 100 mm may pose an eye hazard.
- The laser source and the optical equipment are enclosed within the MMI housing. To avoid laser hazards, do not open the housing.

1.2 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 expressly authorized by MMI to do so. If you have any problem 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 but only smooth and dry cloth.
- If the xy-stage control is not calibrated, table movements can be sudden and fast. Assure that the work area around the table 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 System Setup

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

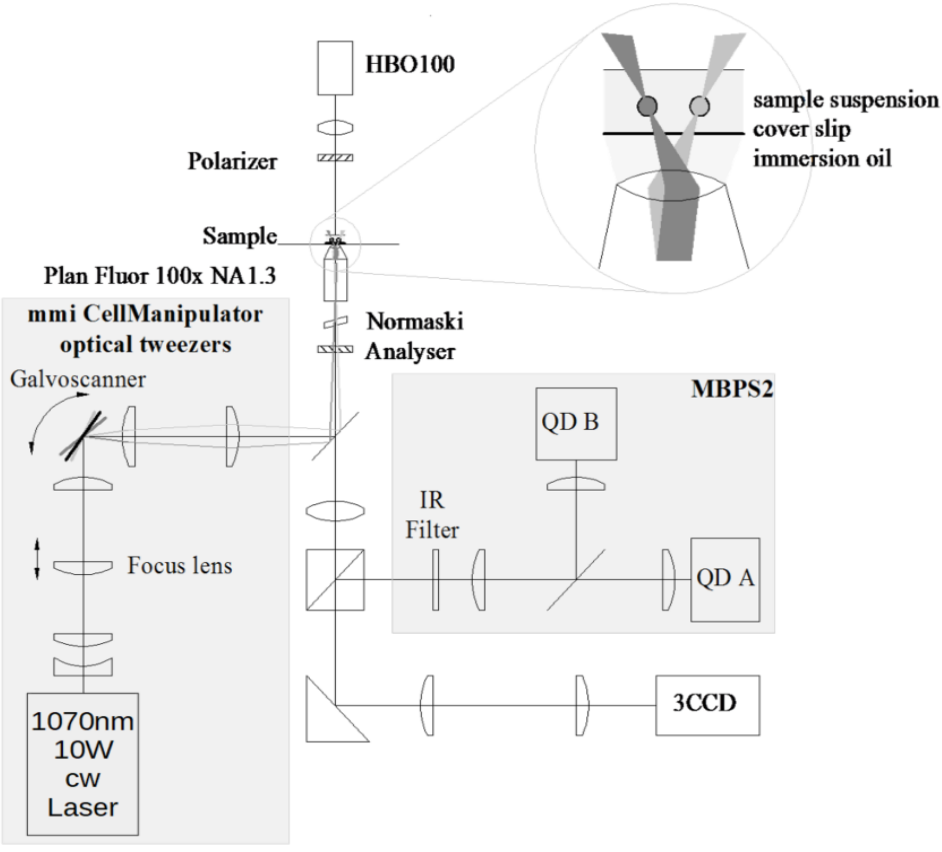


Figure 4.1: *Setup of the mmi CellManipulator with a one level optical trap, a standard inverted scientific microscope with DIC illumination and the microbead position sensor.*

4.2 Principle of operation

The *mmi CellManipulator* is a high-tech optical tweezers instrument for ultra-precise microprocessing and micromanipulation. Laser technology in combination with microscopy allows the manipulation of microscopic particles without contact. The *mmi CellManipulator* is based on the mechanical forces arising from a strongly focused infrared (IR) laser beam:

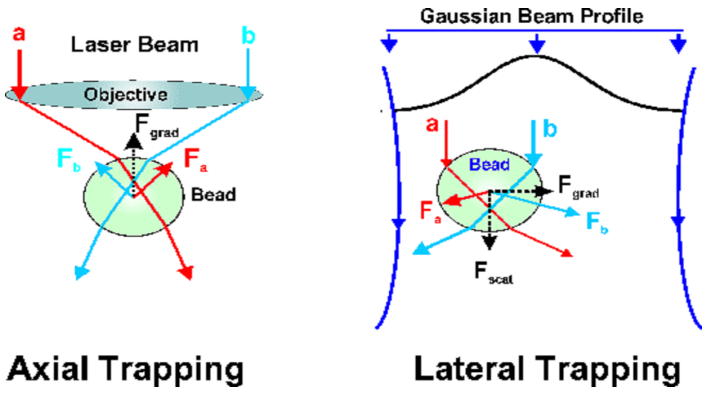


Figure 4.2: *Forces exerted during trapping of particles*

Typically infrared laser radiation causes no harm to biological tissue because the IR absorbance of most biological materials is extremely low.

Details of the optical trapping principles are described in: Neumann and Block: “Optical trapping”, Rev. Sci. Inst., Vol 75, 9, 2004.

The advantages over conventional micromanipulation are

- working inside cells without opening them
- no needles or capillaries needed
- no risk of contamination
- sterile processing in hermetically closed receptacles
- easy to use and very precise through software control

Up to 10 traps can be used quasi-simultaneously. In the optional dual-level configuration, up to 20 traps are possible. Groups of tweezers can be rotated around their centre or scaled up and down. To move traps in the field of view and to create multiple quasi-simultaneous traps, a very fast galvanic scanner system is used. The dual-level configuration allows for two independent, stationary traps with two separate beams. Additional traps are created by time-sharing.

In combination with the UV laser microdissection system *mmi CellCut*, cutting and ablation of biological and technical material is available. Optionally, the

mmi CellManipulator can be equipped with the extremely sensitive position detection system MBPS or MBPS2. The system allows to measure positions of micrometer scale objects with a precision down to the nanometer scale.

4.3 Quick Start

4.3.1 System start-up

To start the *mmi CellManipulator*, proceed as follows:

- Start up the PC and log into your account.
- Turn on the microscope white light power supply.
- For *mmi CellManipulator* (pre 2010) turn the instruments power supply on. The new *mmi CellManipulator* is switched on electronically. The hardware automatically starts up when *mmi CellTools* is started.
- Start the *mmi CellTools* software and wait until the software has finished the startup procedure.

4.3.2 Preparation of a test slide

- Dilute 1 drop of 3–6 µm polystyrene beads in 5 ml water
- Remove membrane from membrane slide (e.g. #50102)
- Fix the coverslip on a metal frame with super glue or vacuum grease
- Pipette 1 drop of diluted beads on slide and cover with a second cover slip to prevent drying

4.3.3 Basic trapping

Select a suitable setup for your illumination conditions. In most cases, this will be “brightfield”. Select the objective you would like to work with. For optical tweezing the standard objective is a 100x oil immersion lens with a NA $\geq$ 1.3.

Carefully read the laser safety instructions in chapter 1 before you continue.

- Never activate the laser without wearing laser goggles
- Make sure the brightfield illumination pillar is in upright position

- Close the door and ensure that nobody enters the room
- Turn the key switch in ON position
- Push the activation button on the key switch box

4.3.4 Microbead manipulation

Up to 2 $\times$ 10 tweezers can be placed on the sample by activating the “add trap” tool and clicking (and dragging) with the left mouse button on the desired position on the screen. If you cannot trap your beads please check the following, more detailed sections. You can delete traps using the eraser tool. Traps can be moved individually or in groups. *mmi CellManipulator* offers straightforward documentation of your samples by saving images or capturing video sequences.

4.3.5 System turn off

Shut down *mmi CellTools* by using the

Project $\rightarrow$ *Exit*

command. Or press the close button in the top right corner of the program window.

If you use *mmi CellManipulator* the hardware shuts down automatically. In older setups you need to turn off the separate power supply.

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.

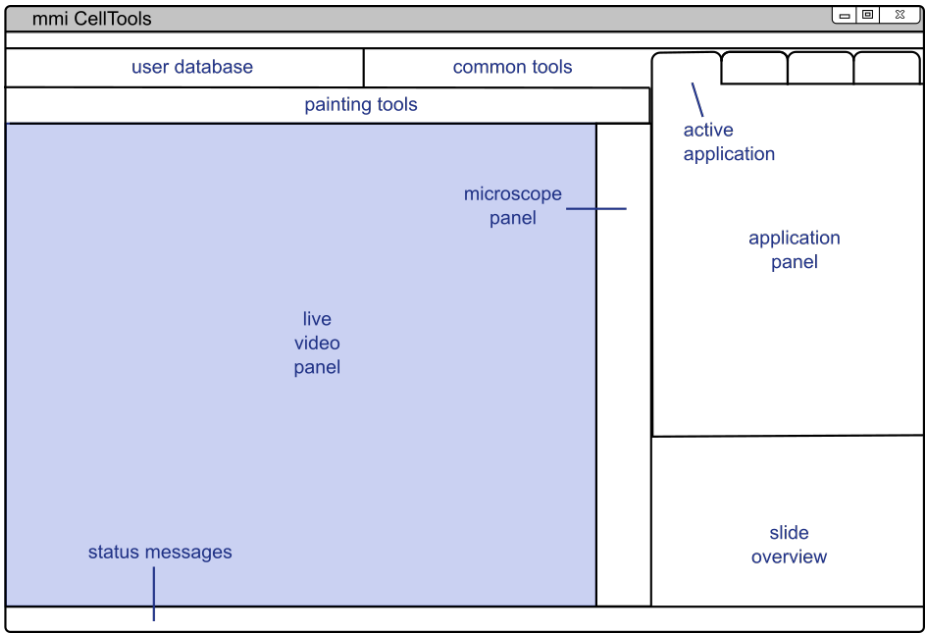


Figure 5.1: *Structure of the mmi CellTools user interface*

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

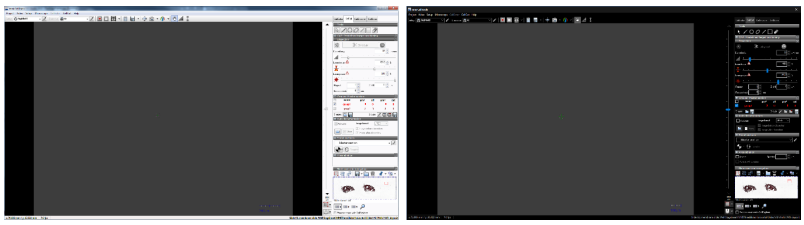


Figure 5.2: *Classic (left) and dark (right) style*

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.

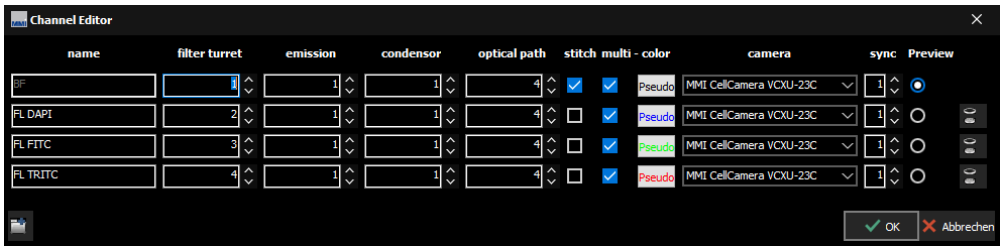


Figure 5.3: *channel editor*

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.

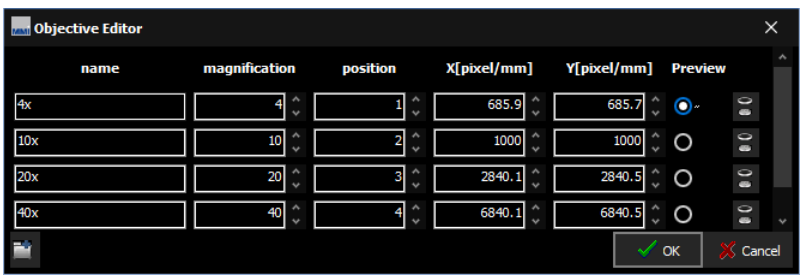


Figure 5.4: *Objective editor*

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.

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[%]**.
- Laser power and focus (see sections 6.1.2 and 6.1.3)
- Scanner offset and amplitude (see sections 6.4.6.1 and 6.4.6.2)

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.

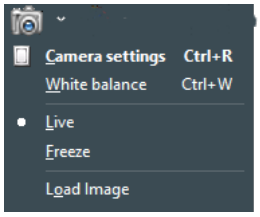


Figure 5.6: *drop down menu to reach camera related options*

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.

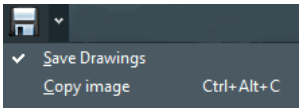


Figure 5.7: *drop down menu for same image options*

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.

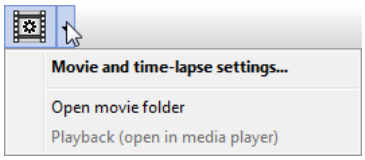


Figure 5.8: *Movie recording functions in the tool bar*

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

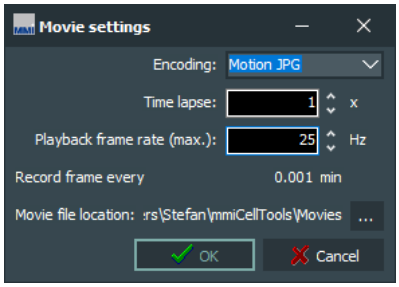


Figure 5.9: *Movie settings*

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

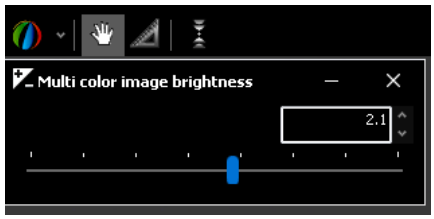


Figure 5.10: *Brighness correction slider for multi channel images*

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

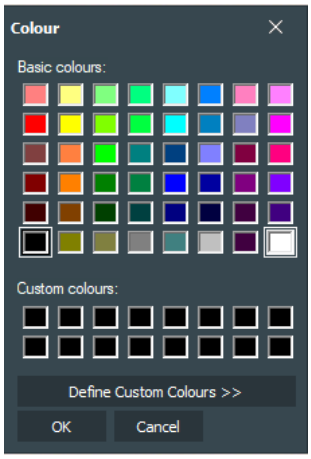


Figure 5.11: *Pseudo color for multi channel images*

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).

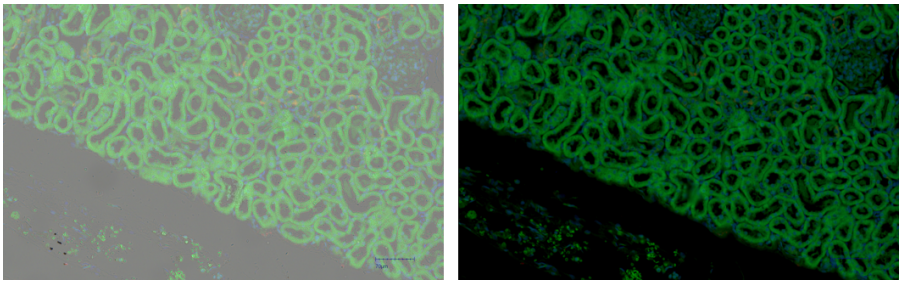


Figure 5.12: *Select white (left) and black (right) as pseudo color for brightfield image*

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.

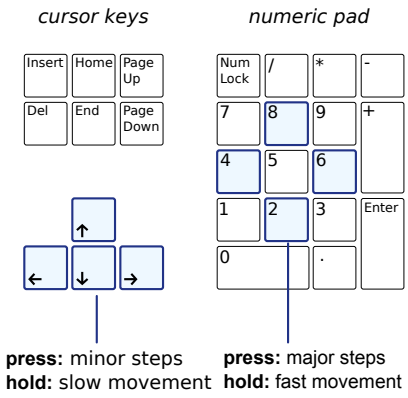


Figure 5.13: *Stage movement using the keyboard*

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.

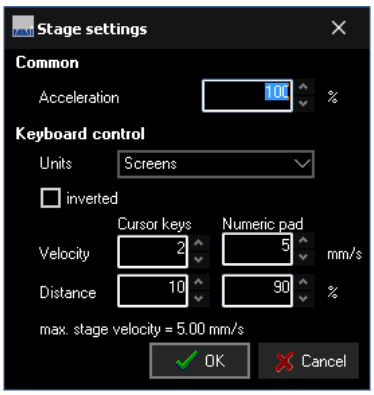


Figure 5.14: *Stage movement settings*

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”).

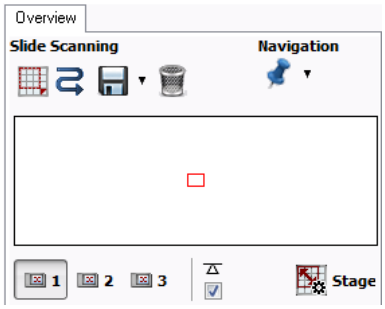


Figure 5.15: *Slide overview*

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

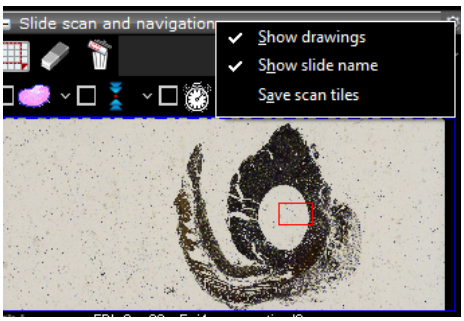


Figure 5.16: *Overview scan configuration*

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.

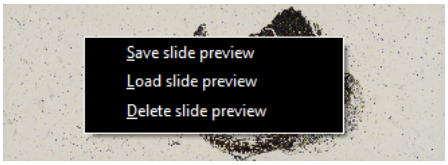


Figure 5.17: *Menu for preview image options*

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).

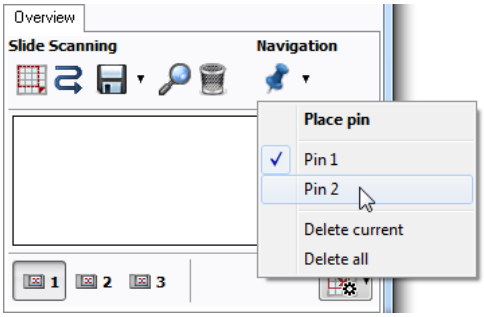


Figure 5.18: *Pin positions*

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 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.

5.4.4.4 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. 5.19) can be opened via the menu

Setup →Stage insert (Ctrl + I)

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

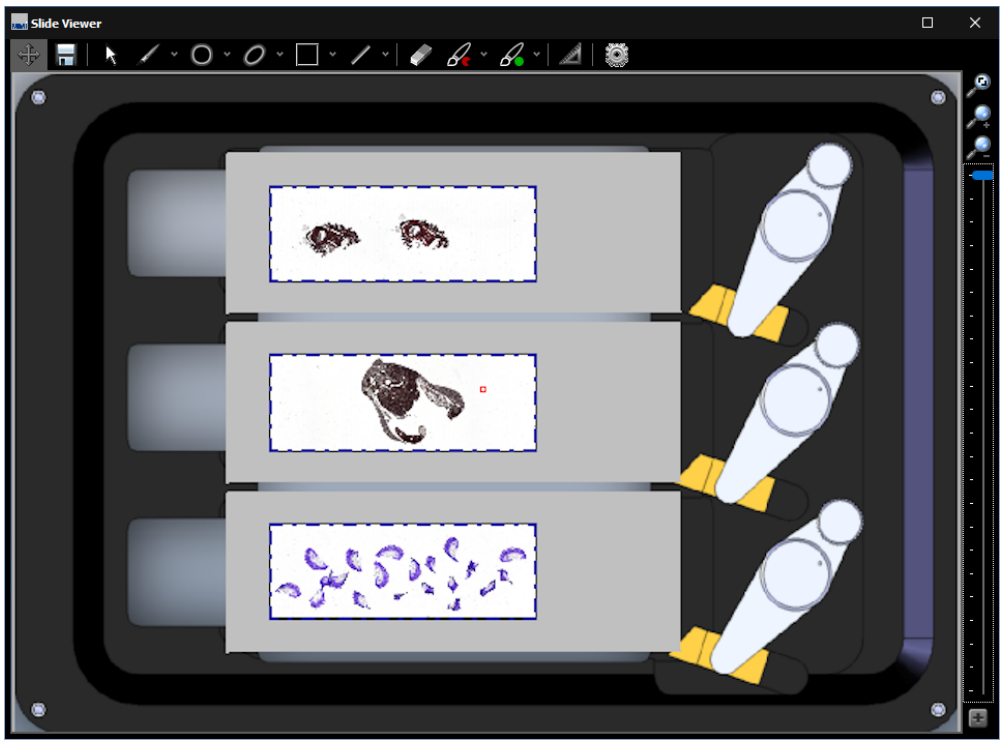


Figure 5.19: *The Slide viewer can be used to display full slide images, draw contours and to configure the stage insert*

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

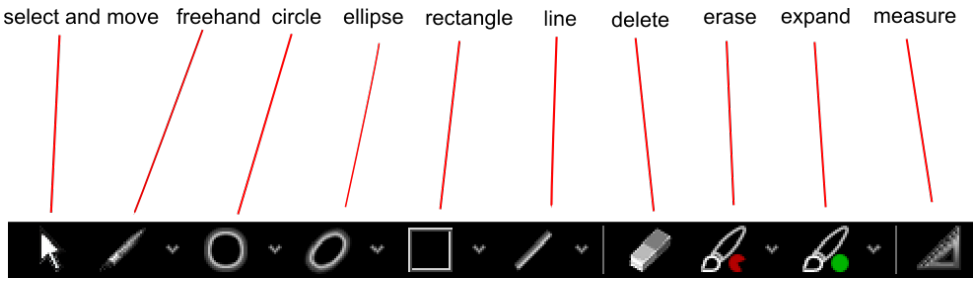


Figure 5.20: *Drawing tools: select, freehand, circle, ellipse, rectangle, line, delete, erase, expand and measure*

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.

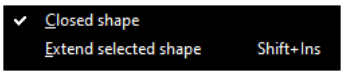


Figure 5.21: *Freehand drawing options*

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.

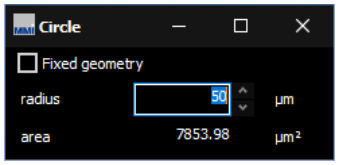


Figure 5.22: *Circle tool*

Fixed-size circles can be created by checking “Fixed diameter” and typing the value in the corresponding input box (Fig. 5.22).

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. 5.23)

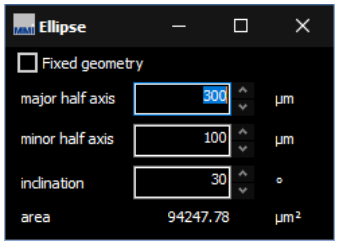


Figure 5.23: *Ellipse tool*

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.

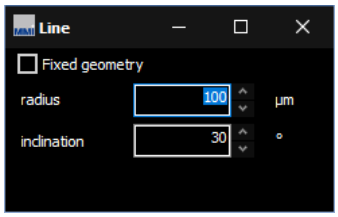


Figure 5.24: *Line tool*

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.

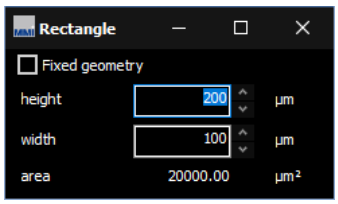


Figure 5.25: *Rectangle tool*

Fixed-size rectangles can be created by checking “Fixed dimensions” and typing the values in the corresponding input box (Fig. 5.25).

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. 5.26)
- Using the group editor (section **??**).

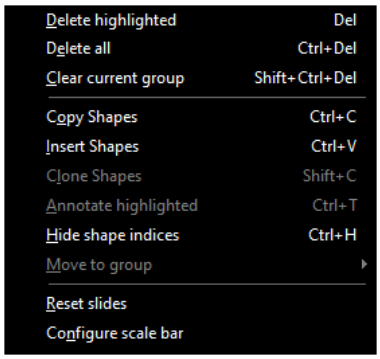


Figure 5.26: *Context menu, open with the right mouse button in the image panel.*

To delete all shapes, or all shapes from the current group, use the context menu (Fig. 5.26) 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. 5.26).

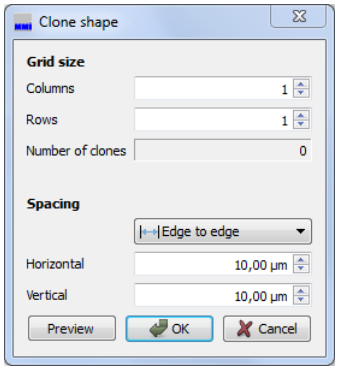


Figure 5.27: *Clone shapes*

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. 5.26. A small popup window Fig. 5.28 will allow to enter the text you want to save with the hightlighted shapes.

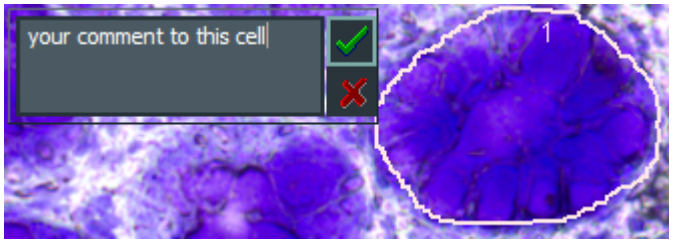


Figure 5.28: *Free text annotation for shapes*

5.5.3 Hide shape indices

The popup menu shown in Fig. 5.26 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. 5.26 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. 5.26 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. 5.26 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. 5.29.

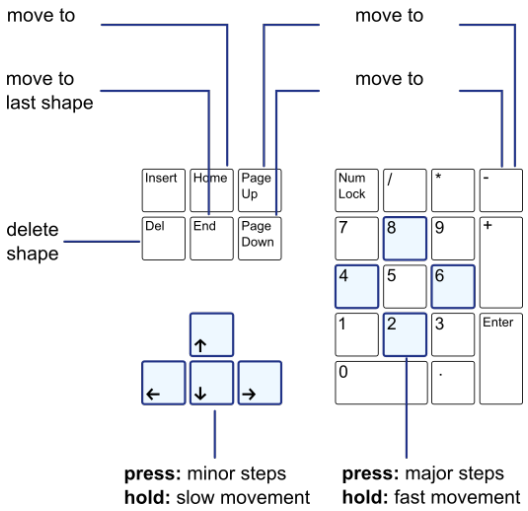


Figure 5.29: *Keyboard navigation*

- *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.

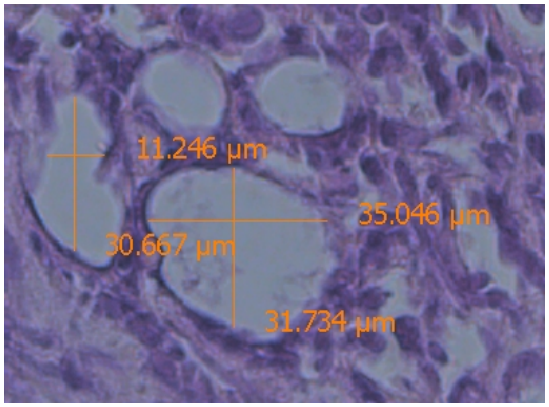


Figure 5.30: *Distance measurement*

Press the left mouse button and drag the mouse. After releasing the mouse button you can see the measured distance (Fig. 5.30)

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. 5.19 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. 5.31. 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

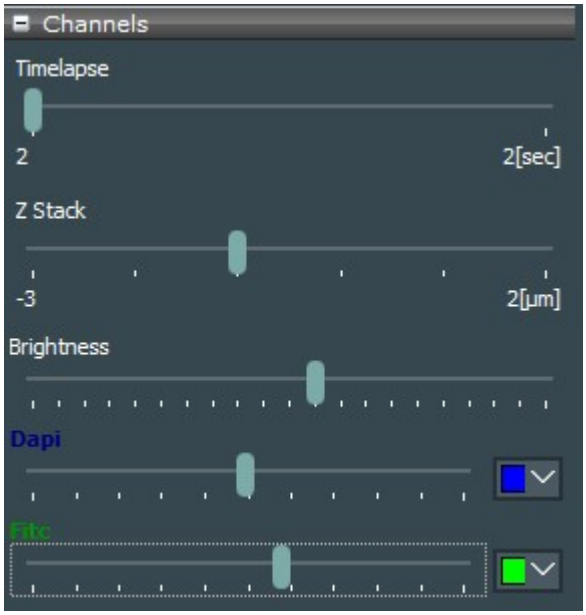


Figure 5.31: *Control the timelapse, zStack and Multi-channels of your 5D WSI.*

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.

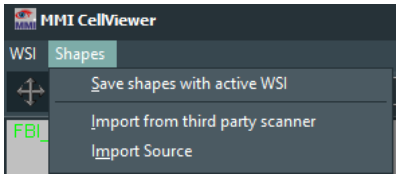


Figure 5.32: *Shape import menu*

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. 5.33. This allow you to easily navigate through your whole slide images or use machine learning for image analysis.

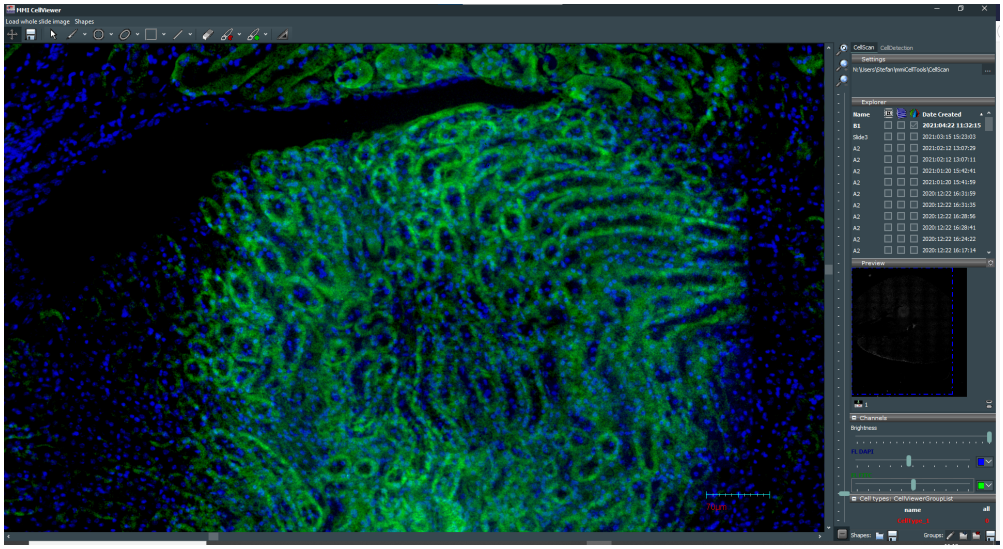


Figure 5.33: *The Slide viewer also allows to host plugins in the right side panel*

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. 5.34) different types of slide inserts can be selected:

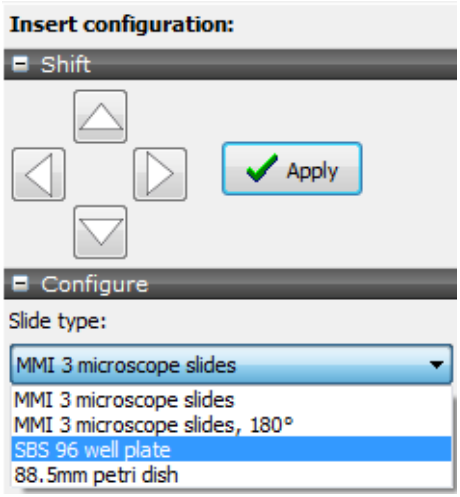


Figure 5.34: *Slide viewer: Insert configuration panel. You can select different insert types and shift the whole insert to adapt it to overview images.*

- 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. 5.35) 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

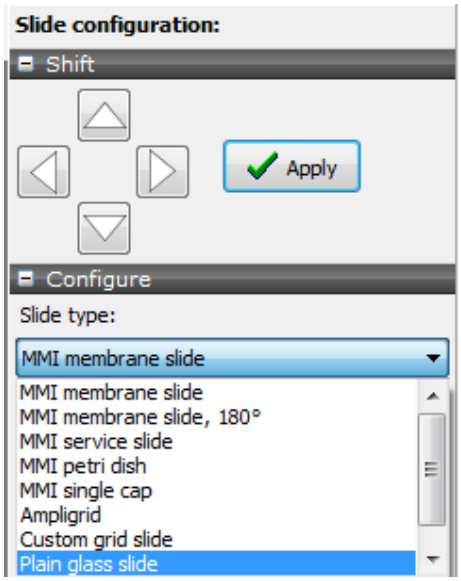


Figure 5.35: *Slide viewer: Slide configuration panel. You can select different slide types and shift the whole slide to adapt it to it's overview image.*

- 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

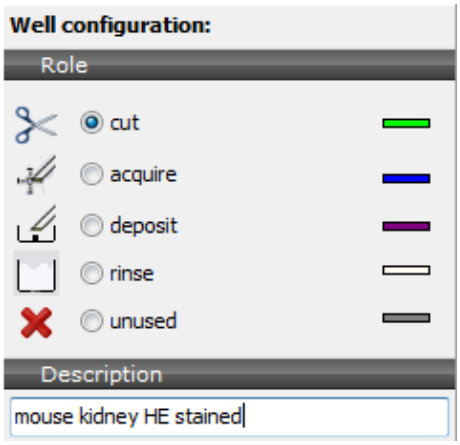


Figure 5.36: *Slide viewer: Well configuration panel. You can select different well rules and add descriptions to each well.*

- 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. 5.37) 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.

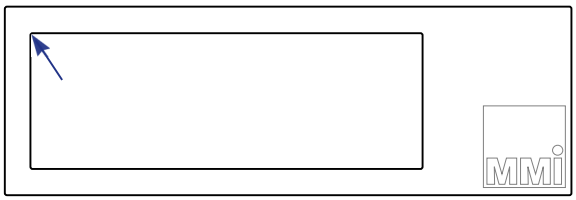


Figure 5.37: *Suggested positions on a mmi Membrane slide to precisely adjust the stage insert position.*

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.

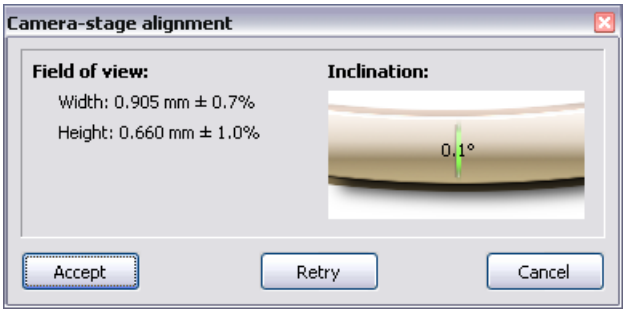


Figure 5.38: *Camera alignment results*

If automatic alignment was successful, a dialog (see figure 5.38) 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. 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

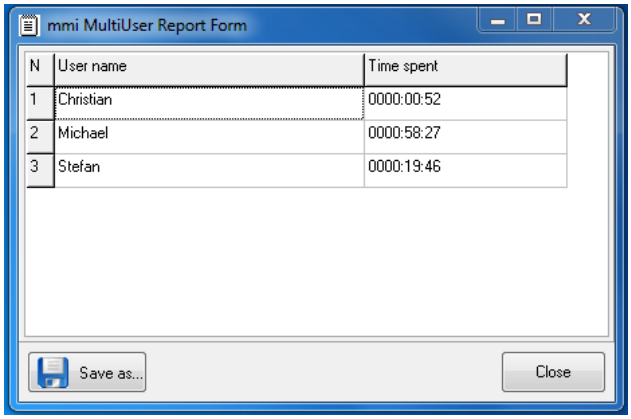


Figure 5.39: *Multi-user report*

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 – CellManipulator plug-in

As described in section 5.1, the special features of the *mmi CellCut*, *mmi CellEctor*, *mmi CellExplorer* and *mmi CellManipulator* are installed as separate plug-ins (software modules). The *mmi CellManipulator* 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).

Pressing one of the buttons **Groups**, **Position and force measurement**, **Trap oscillation** or **Hardware control** opens additional functionalities.

6.1 Laser controls

6.1.1 Laser operation

Carefully read the laser safety instructions in chapter 1 before you continue.

- Never activate the laser without your laser safety goggles.
- Put the brightfield illumination pillar in upright position
- Turn the key switch in ON position
- Push the activation button on the key switch box:
  - the green LED on the key switch box turns on
  - the yellow LED on the key switch box turns on if no error occurs
  - 5 seconds later the laser is ready to operate.

The laser can be manually switched on or off by clicking the IR laser Power button or using the hotkey *Ctrl + L*.

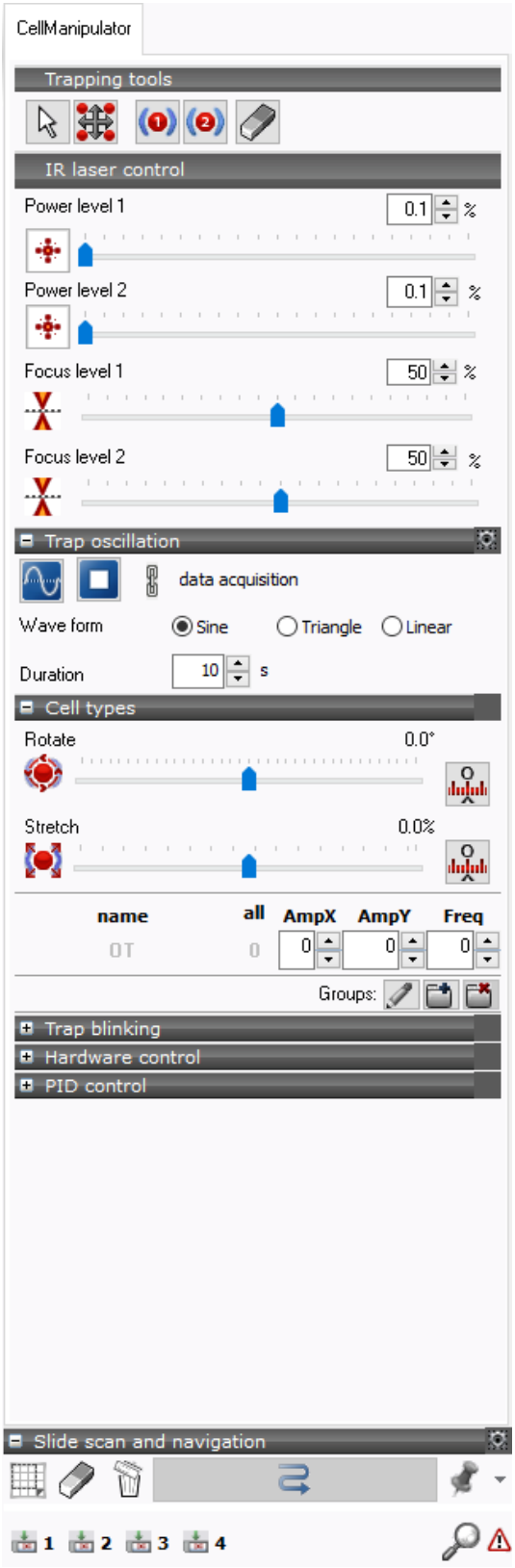


Figure 6.1: *The CellManipulator plug-in panel*

In day-to-day use, manual laser operation is rarely needed. The laser will be switched on automatically when creating a trap, and switched off after the last trap is deleted.

When the laser is emitting radiation, the laser button will appear depressed, and a warning sign will appear on the screen (see right).

6.1.2 Adjusting laser power

Laser power can be adjusted to the needs of the application. This is done by dragging the sliders next to the IR laser Power button in the *mmi CellManipulator* panel (Fig. 6.2).

Figure 6.2: *Power controls for the IR laser*

If particles escape the traps frequently the laser power may be too low. If the laser is too strong its power can be reduced by adjusting the slider.

6.1.3 Adjusting laser focus

The trapping laser beam (or laser beams, for dual-level configuration) can be moved in z-direction in order to adjust focus. In most applications it is desirable to let the laser focus coincide with the focal plane of the microscopic image. This will ensure that trapped particles remain visible and in focus. It is however possible to move particles vertically by adjusting the laser focus. This is especially interesting in the dual-level configuration, where the two trapping beams can be focused independently.

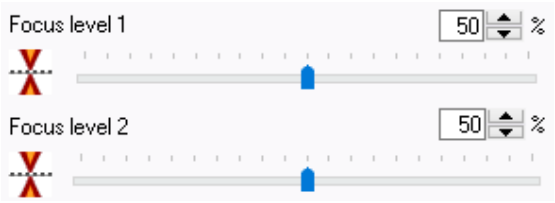


Figure 6.3: *Focus controls for the IR laser*

Depending on your hardware configuration, one or two focus sliders will be visible. To adjust the focus use the appropriate slider. Note that changing the

microscope objective will also affect the laser focus. Therefore, the focus must be adjusted separately for each objective.

6.2 Trap handling

6.2.1 Creating traps

Up to 10 traps per level can be placed in the field of view using the add trap tool. After clicking the tool button, create a trap by clicking a position on the main screen. A trap target symbol will appear on the screen, the laser switches on and a trap will be created on the selected position. The newly created trap can be moved to its final position by holding down the mouse button and dragging.

The trap number and group number are written next to the trap symbol. In a dual-level configuration, the level to which the trap corresponds is added.

In a single-level configuration you can use the hotkey *Alt + T* to switch to this tool. In a dual-level configuration use *Alt + T* for the first level and *Alt + Z* for the second level. You can quickly switch between the stage movement tool and the add trap tool by pressing *Space*.

When more than one trap is active, time-sharing mode is started: the laser is quickly moved from one position to the next using the galvanometric scanners, producing the illusion of multiple, simultaneous laser spots.

> When time-sharing is active, the red light on the *CellManipulator* controller may flash. This is normal behavior.

6.2.2 Single trap manipulation

6.2.2.1 Move a single trap

Activate the select tool (hotkey *Alt + R*), then click and drag the respective trap to its new position. The laser beam will follow the mouse movement. To move a trap with your keyboard, see section 6.2.3.

6.2.2.2 Deleting a trap

To delete a single trap click on the delete trap tool button (eraser) and then on the traps to delete. Alternatively you can delete the selected trap, all traps in the current group, or all traps using the context menu. Right-click on

any point in the main screen and select the corresponding item in the popup menu. Pressing *Del* also removes the selected trap, *Ctrl + Del* removes all traps from the currently active group, *Shift + Del* removes all traps.

6.2.3 Step movement

Single traps or groups can be moved in steps of defined length. While holding down the Ctrl key, press the cursor keys or the numeric pad arrow keys to move the currently selected trap. (Note that the NumLock switch on the keyboard must be activated).

By default, the following step sizes are used:

Numeric pad arrow keys	10	µm
Regular arrow keys	1	µm

Step sizes can be customized via the menu item

CellManipulator →Step movement settings...

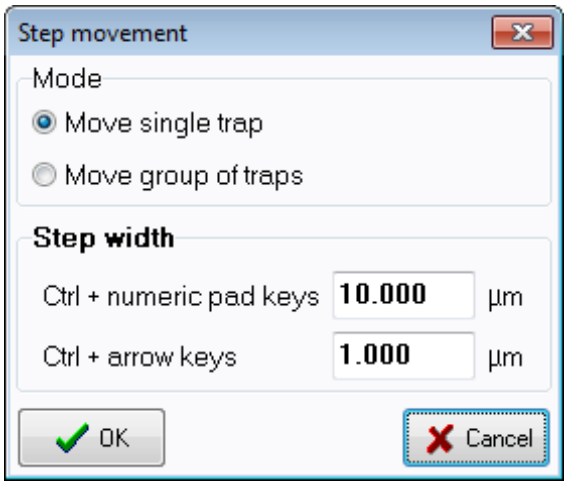


Figure 6.4: *Step movement settings*

The minimum recommended step size is 25 nm. Use the controls in the section **Mode** to switch between moving a single trap or all traps within the active group (Fig. 6.4).

6.3 Cell types

Any object drawn will always be assigned to the highlighted cell type group shown in the **Cell types** panel (Fig. 6.5).

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

.

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.

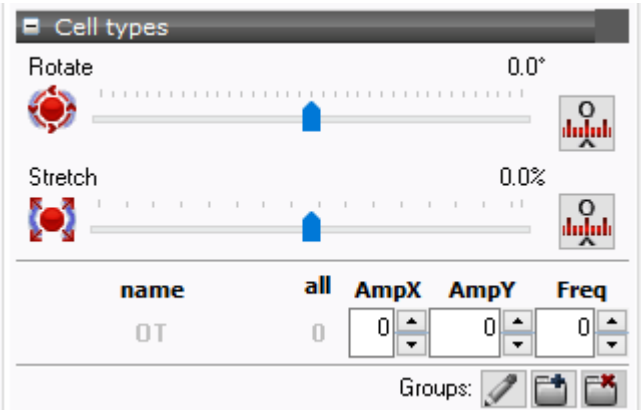


Figure 6.5: *Cell type group selection.*

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. 6.6) using the edit button.

You can adjust the group name and drawing attributes (color and line thickness) in the lower part of the editor.

6.3.2 Regrouping shapes

To move individual shapes from one cell type group to another, open the context menu (Fig. 5.26) by right-clicking on video panel and select the target group from sub-menu (*Move into group* →*GroupX*).

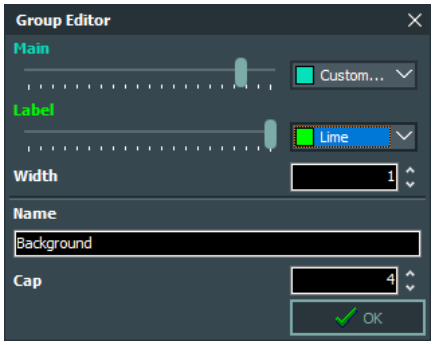


Figure 6.6: *Group editor*

6.3.3 Move a group of traps

Activate the move group tool, then click and drag anywhere on the main screen to move all traps within the active group.

6.3.4 Rotate and stretch groups of traps

To scale or rotate groups of traps use the corresponding sliders in the Groups section. Move these sliders to rotate, stretch or shrink the trap distance within the active group. Clicking in the groove next to the slider will rotate in steps of 10° or scale in steps of 2%. The rotation slider allows rotations from –180° to +180°. The stretch slider allows distance of traps to be doubled (+100%) or halved (–100%). To continue a transformation past the end of the slider range, click the reset button. The slider will return to center position, while the traps remain unaffected. Rotations and stretch/shrink operations will use the common center (position average) of the traps in the group.

6.3.4.1 Deleting groups of traps

If you click with the right mouse button on the main video panel a popup menu as in Fig. 6.7 shows up

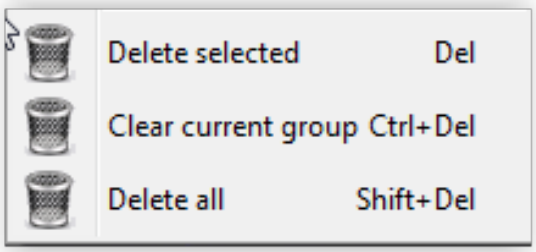


Figure 6.7: *Menu entries for deleting traps*

To delete all traps or all traps of the active group, select the appropriate menu entry.

6.3.5 Oscillation experiments

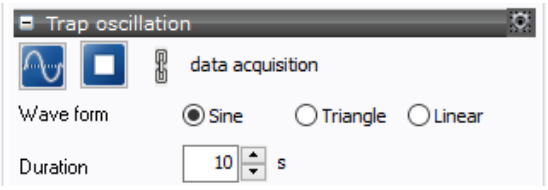


Figure 6.8: *Tools for oscillating traps*

mmi CellTools allows to execute oscillation experiments oscillating

- the trap itself, if you have created any
- a connected piezo manipulator.

In both cases you can oscillate

- in a harmonic sinusoidal wave by selecting **Sine**
- in a triangle wave by selecting **Triangle**
- using constant movement velocities by selecting **Linear**

The oscillation properties can be defined for the groups as shown in figure 6.5, meaning that each trap in that group will be oscillated with those properties.

In case you have created one trap you can use oscillation frequencies up to 2 kHz. In case you created two traps per level, the timesharing requires reducing the maximum oscillation frequency to 100 Hz.

An example of a bead oscillating with a horizontal amplitude of 10 µm and a frequency of 500 Hz is depicted in Fig. 6.9.

If you oscillate a trap in a sinusoidal harmonic matter, the software calculates the minimum force the trap has to exert to keep the bead captured during oscillation. This force is equivalent to the *Stokes force* induced by the oscillation movement through liquid.

If you like to estimate the maximal possible trapping force on that bead, ramp up the oscillation frequency and determine the threshold freqency where the trap is losing the bead.

During a trap oscillation data acquisition can be performed (see section 6.5.2). The system uses a hardware trigger to synchronize the data acquisition with

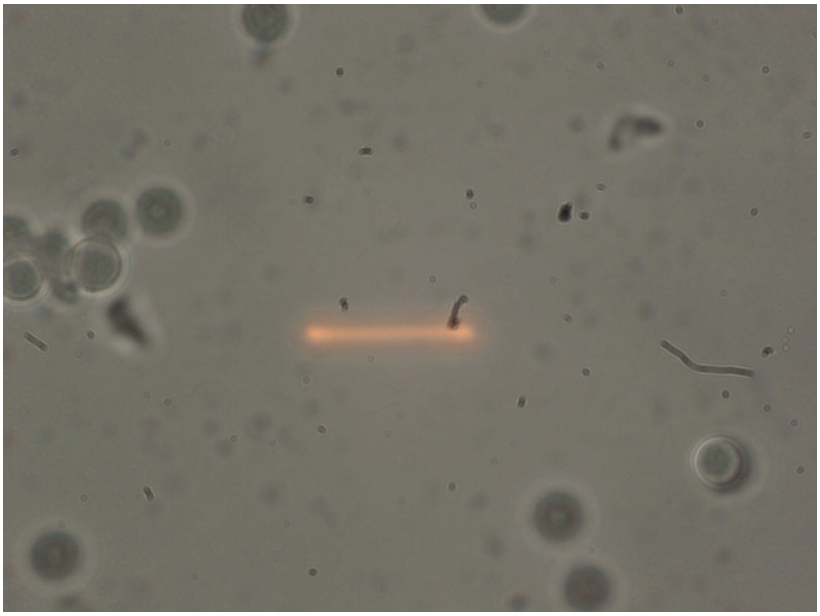


Figure 6.9: *An oscillating bead*

the oscillation process. This allows the measurement of phase shifts between the trap oscillation and the bead position.

If the data acquisition should run free, that means independent from the oscillation parameters, unlock the data acquisition .

6.3.6 Calculation parameter

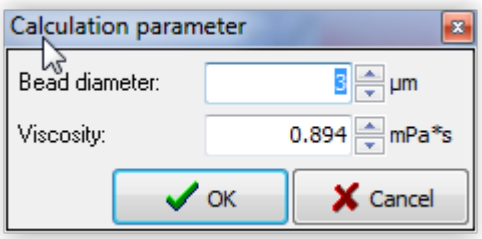


Figure 6.10: *Configuration window for bead diameter and fluid viscosity.*

To calculate the stokes forces and the stiffness of the trap, the bead position and the fluid viscosity must be known. These parameters you can change in the **Calculation paramters** window. The window is accessible via the configuration button in the oscillation and the force spectroscopy panel.

6.4 Calibration

6.4.1 Reflective light calibration setup

First, the trapping laser must be made visible on your camera. If your camera is mounted on the left or right side port of the microscope use the following procedure to optimize the laser focus position:

Procedure

1. Move the stage to an empty area of the cover slip
2. Focus the microscope on the upper surface of the cover slip
3. Turn the microscope illumination off
4. Turn the laser on (e.g. by creating a trap)
5. Set the laser power to 3%
6. Increase the camera exposure time until you see the laser as white scattered light (Fig. 6.11)

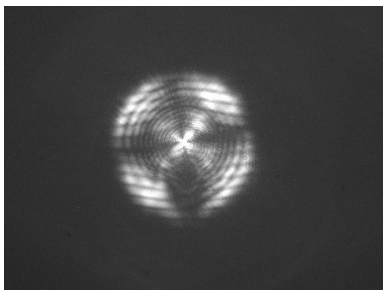


Figure 6.11: *The image of a trap on the video camera*

6.4.2 Black ink calibration setup

If your camera is mounted on the camera trinocular, the reflected light on an empty cover slip cannot be used to adjust the focus position, since the eye protection filter blocks all IR laser reflections. In this case use the following procedure:

Procedure

1. Draw a line or spot with a black felt tip pen on the cover slip
2. Insert the cover slip and move the stage to view the ink

3. Create a trap
4. Focus the microscope on the ink until the laser burns a hole in the ink
5. Reduce the laser power to minimize the hole size. Different laser intensities (20%, 10%, 5%, 2.5%, 1%) are shown in Fig. 6.12. The asymmetry is caused by the laser getting into the right position after activation

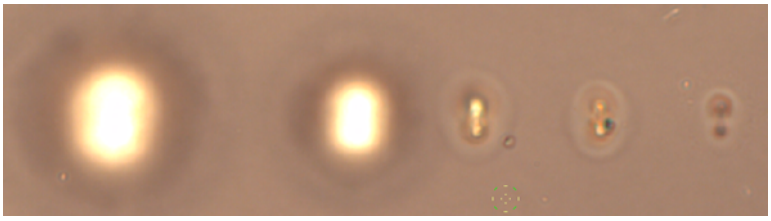


Figure 6.12: *Results of black ink calibration*

6.4.3 Laser focus and laser power

In order to achieve optimal trapping efficiency, laser focus and power calibration must be carried out **for each objective** to be used with the optical tweezers. As detailed in sections 6.1.2 and 6.1.3, focus and power can be adjusted using the sliders at the top of the *CellManipulator* panel (see Fig. 6.13).

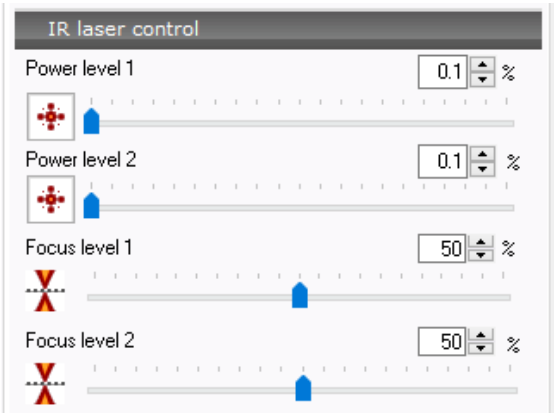


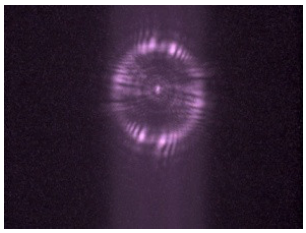
Figure 6.13: *Controls for the IR laser*

The following are common procedures used to optimize the trapping force.

6.4.4 Coarse laser focus adjustment

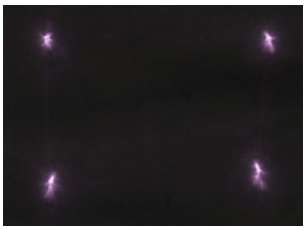
If you use the reflective light calibration setup (see section 6.4.1) move the focus slider. You will see the laser focus grow or shrink. Make sure the cover glass top surface is in focus and move the slider until the laser focus becomes

as small as possible. If the image of the laser focus is too bright, reduce the laser power. You should get an image similar to the images in Fig. 6.14.



(a) laser focus above coverslip

(b) laser focus on coverslip



(c) laser focus below coverslip

Figure 6.14: *Focus adjustment using reflected laser light*

If you use the black ink calibration setup (see section 6.4.2) adjust the focus until the spots of removed ink are smallest, while keeping laser power to a minimum. You may need to iterate several times between power and focus adjustment.

6.4.5 Fine laser focus and laser power adjustment by target objects

After the coarse focus adjustment carry out a fine adjustment with the particles of interest for your application to maximize trapping efficiency.

Procedure

1. Prepare your sample
2. Focus on the specimen
3. Trap an object of interest by creating a new trap. When trapped, the object will move into the focus of the beam
4. Move the focus slider until the trapped object appears in focus
5. Reduce the laser power to the lowest possible setting that keeps the object trapped

6.4.6 Trap positioning calibrations

In order to keep the laser beam aligned with the trap symbols on screen it is necessary to calibrate the positioning system in certain intervals. Due to mechanical and thermal influences, after moving an instrument, or after installing an objective, recalibration of the galvanometric scanners may be required.

If the laser trap no longer follows the trap positions on screen, you should calibrate the scanners for each objective used for the *mmi CellManipulator* and for each scanner installed in your system.

Scanner calibration consists of three separate procedures:

- Scanner offset calibration
- Amplitude calibration
- Rotation calibration

You can run all three calibrations in reflected light setup (see section 6.4.1) or black ink setup (see section 6.4.2). They must be run separately for the second laser level, if installed.

These calibrations can be accessed from the menu entries

CellManipulator →*Calibration* →*Scanner1/2*

6.4.6.1 Scanner offset calibration

The scanner offset calibration is used to align the laser beam to the center of the screen. If the Center marker is not visible, select *CellManipulator* →*Show center*

First insert a slide following the reflected light setup (section 6.4.1) or black ink setup (section 6.4.2). Create a single trap exactly on the Center marker. (Alternatively, in a single level system, switch on the laser by clicking the power button.) The laser should show up exactly on the Center marker position. If not do the following:

Procedure

1. Select the menu item *CellManipulator* →*Calibration* →*Scanner 1/2->Scanner offset*. The window shown in Fig. 6.15 opens.
2. To adjust the beam position, drag the horizontal and vertical sliders until the laser beam aligns with the symbol on the screen (Fig. 6.16).
3. When finished, click the **OK** button and switch the laser off by either deleting the trap or clicking the power button again.
4. Repeat the procedure for any other objective you would like to use.

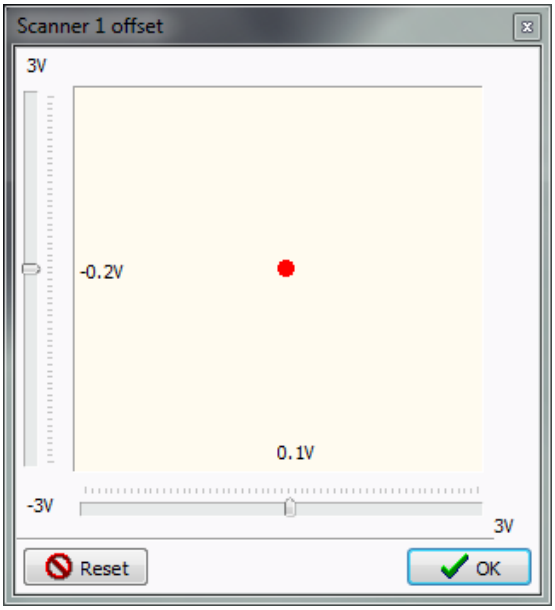


Figure 6.15: *Scanner offset calibration tools*

Figure 6.16: *Adjusting the scanner offset*

6.4.6.2 Scanner amplitude calibration

If the scanner amplitude calibration is not valid, the software will misjudge distances when placing traps, causing the beams to appear too far from or too close to the center of the screen. Before you carry out the amplitude calibration, ensure that the scanner offset is correct (see section 6.4.6.1). First insert a slide following the reflected light setup (section 6.4.1) or black ink setup (section 6.4.2). Then

Procedure

1. Select the menu item

   CellManipulator → *Calibration* → *Scanner 1/2* → *Amplitude*

2. Create a trap somewhere near the upper left corner of the screen. The laser beam will show, possibly at an incorrect position.

3. Click a second time on the actual laser position, to renew the calibration.

4. Repeat the procedure for any other objective you wish to use.

The procedure can be interupted any time by pressing *Esc*. In the dual-level configuration, both scanners must be calibrated. Choose scanner 1 or scanner 2 in the menu, depending on which scanner requires calibration.

> If the calibration is far off the laser might not be visible in step 2. You may need to create the trap closer to the screen center.

6.4.6.3 Scanner rotation calibration

If the scanner rotation calibration is not valid, all traps will appear offset by a certain angle. Such a situation can arise due to thermal and mechanical influences, but also if the camera has been tampered with.

It is sufficient to perform this calibration once for each scanner. It is *not* necessary to calibrate each objective, as this calibration is global. Before you carry out the rotation calibration, ensure that the laser position is correct (see section 6.4.6.1) and the camera is mounted properly.

> The scanner rotation calibration will not work if the camera is not mounted upright (see 5.7.2.1).

Procedure

1. Select the menu item

   CellManipulator → *Calibration* → *Scanner 1/2* → *Rotation*

2. Place a trap at the center. CellTools will bring that trap to the center if your trap is not exactly at the center. CellTools will create a horizontal line and will start to oscillate the trap.

Figure 6.17: *Rotation calibration tool*

3. Drag the rotation slider figure 6.17 until the laser beam projection appears over the line. If this is not possible, try to eliminate any angular deviation first and repeat the amplitude calibration afterwards.

4. When finished, press the button next to the slider. Press the Esc key to cancel.

6.5 Microbead position detection and force measurements

The *mmi CellTools* software features a unique, integrated position monitoring and quantitative force measurement tool for working with microbeads or cellular structures (vacuoles, growth cones, etc). The object of interest is monitored using the optional micro-bead positioning sensor (MBPS), a quadrant detector system that can measure particle positions with nanometer range precision.

> Force measurement functions require the optional microbead positioning sensor.

The following section briefly reviews the theory underlying force measurement.

6.5.1 Theory: force measurement using optical tweezers

6.5.1.1 Harmonic oscillation method

The force measurement procedure established in *mmi CellTools* follows the ideas of Anderson et al. 2007[1].

First the basic equations that are valid for micrometer sized objects that are moved around in a Newtonian fluid will be given. The equation explaining the forces in play for particle motion in liquids is given by the Langevin relation $F(x,t) = m\ddot{x} + \mu\dot{x} - f(t)$ where m is the particle mass, μ is the kinetic friction coefficient, $f(t)$ is the thermal motion, and $F(x,t)$ is the external particle force. This relation can be highly simplified for the case of micron-sized objects moved over relatively long distances. The term $m\ddot{x}$ is relative to the viscosity of the liquid and can be neglected due to the relatively low Reynolds numbers obtained. Also the term associated with thermal force can be neglected due to the long time scale of the forced oscillations, even though it will contribute to an increased noise level. These modifications have proven to be valid in previous work[2]. For a single particle at infinite dilution in a Newtonian fluid, the friction is given by the Stokes equation $\mu = 6\pi\eta a\dot{x}$, where a is the particle radius and μ is the dynamic viscosity. Combining the simplified Langevin equation with the Stokes equation gives

$$6\pi a\dot{x} = F(x,t) \tag{6.1}$$

In the case of oscillating the particle, the position of the trap x_t can be written as

$$x_t = a\sin(\omega t) \tag{6.2}$$

[1] M. Anderson, A. Madgavkar, M.Sterndahl, Y. Wu, W. Tan, R.Duran, S. Niehren, K. Mustafa, K. Arvidson, A. Wennberg, Rev. Sci. Instrum. 78, 074302, 2007

[2] C.D. Mellor, M.A. Sharp, C.B. Bain, and A.D. Ward, J. Appl. Phys. 97, 103114, 2005

and the position of the particle x as

$$x = a_p \sin(\omega t - \theta), \tag{6.3}$$

where a_t is the amplitude of the oscillating trap and a_p is the amplitude of the oscillating particle. θ is the phase lag between the trap and the particle. This phase lag is a result of the viscous drag that is felt by the trapped object. The trap force can be expressed as a harmonic spring that follows Hook's law,

$$F = kd, \tag{6.4}$$

where k is the spring constant or, as it will be referred to here, the trap stiffness, and d is the object's distance from the trap center. In this oscillation case $d = x_t - x$ and by combining Eqs. 6.1–6.4 the following equation is obtained:

$$k = 6\pi\eta a\omega / \tan\theta \tag{6.5}$$

From Eq. 6.5 it is seen that the trap stiffness can be acquired if the phase lag θ is measured as a function of oscillating frequency ω. The harmonic oscillation method is used by *CellTools* to measure the stiffness of the trap (see section 6.5.5). If the stiffness is known, the forces can be easily calculated using Hook's law in equation 6.4.

6.5.1.2 Brownian motion method

Another frequently applied method is the Brownian motion method to measure trap stiffness. This method is well covered in scientific literature, see for example Neuman and Block, 2004[3]. A bead in solution is subject to Brownian (random) motion that can be quantified using a suitable detector (such as the MBPS optionally supplied with *mmi CellManipulator*). Under the influence of an optical trap, this Brownian motion is reduced to a degree that depends on the trap's strength. The trap's stiffness is calculated by determining the cutoff-frequency, at which the trap starts losing the object (see Fig. 6.18). The analysis requires Fourier transformation of the measured position data. For details refer to the literature cited.

The experimental steps required for the Brownian motion method can easily be carried out in *CellTools*. External analysis tools can be used to analyze the acquired data.

6.5.2 Force spectroscopy

Data plotting, calibration and force measurement routines are implemented in the *CellManipulator* plugin (see Fig. 6.19). The related windows can be opened via the **Force spectroscopy** section.

[3] K.C. Neuman, S.M. Block, Rev. Sci. Instrum. 75, 2787, 2004

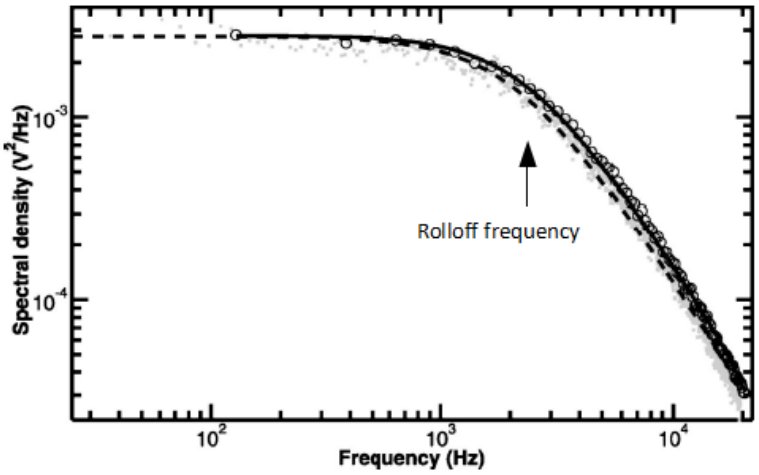


Figure 6.18: *Power spectrum obtained from the Brownian motion of a trapped particle*

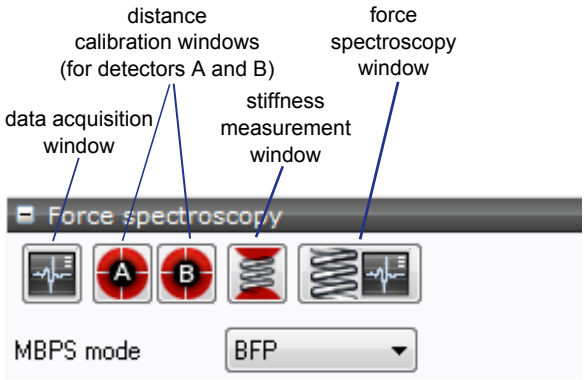


Figure 6.19: *Controls for position and force measurement: A) The data acquisition window is working like an oscilloscope and displaying data acquired by the MBPS. B) Distance calibration windows implement the routines to calibrate distances against measured voltages. The results a displayed. C) The stiffness calibration window implements the routines to measure the trap stiffness. D) Finally the force spectroscopy window represents all needed tools to run force measurement experiments with the MBPS detector.*

Beside the experimental windows the **Force spectroscopy** panel allows to set the working mode of the MPBS. To modes are supported:

- Imaging mode, the MBPS is mounted on a camera port and analysing the images of the traped bead
- Back focal plane (BFP) mode, the MBPS is mounted above the condensor and analysing the interference patterns created by the laser transmitting the trapped bead.

All four windows contain a customizable plotting area. This area can be customized using the graph controls in the top right corner of the graph.

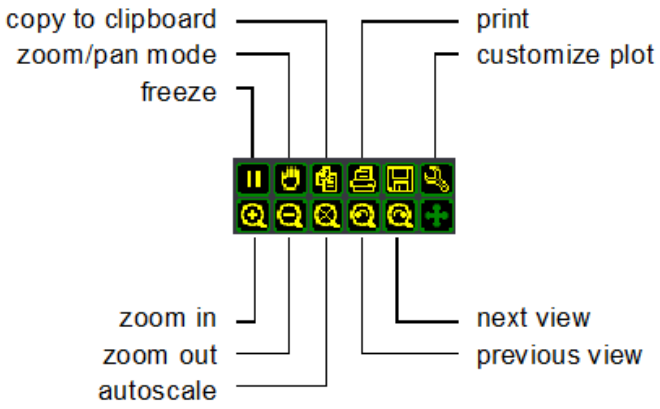


Figure 6.20: *Customizing the graphs*

By default, the plot window auto-adjusts to fit the entire data in the graph. You can manually adjust zoom using the zoom buttons or by selecting zoom mode (lens symbol) and drawing the desired viewing rectangle on the graph. This automatically activates the freeze function. In pan mode (hand symbol) you can shift the viewing rectangle by dragging the mouse on the graph.

You can quickly switch back and forth between different zoom settings using the next/previous view buttons. The graph controls also offer quick clipboard copying, printing and a number of customizations through a customization dialog.

> If the plot area doesn't display current data check that the freeze mode is not active in the graph controls.

6.5.3 Data acquisition

Open the **data acquisition** tool by clicking on the respective button in section Position and force measurement.  This will open the force measurement window (Fig. 6.21). All data acquisition operations are carried out in this window.

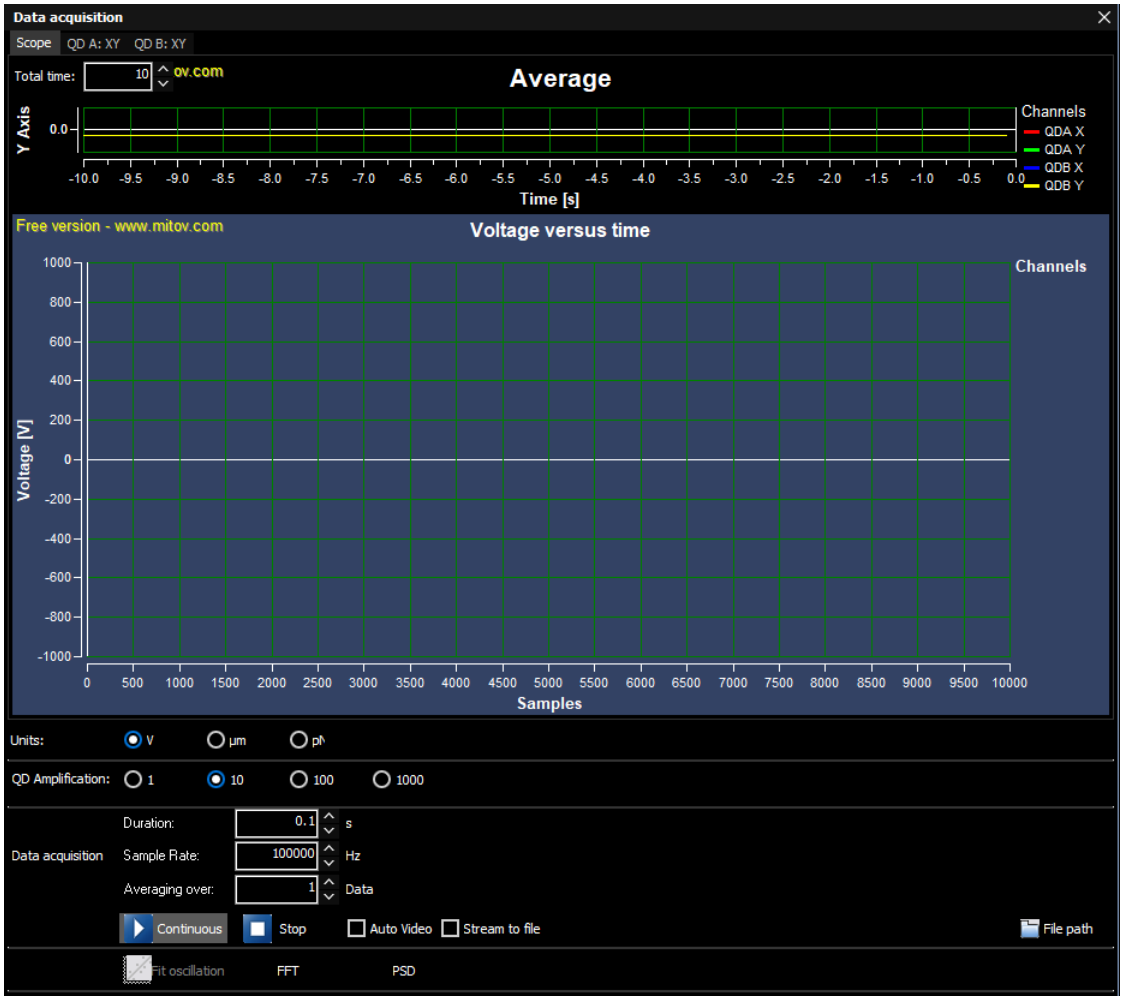


Figure 6.21: *The data acquisition window*

The uppper **Average** plot displays the long term behaviour of the data acquired. The maximum time displayed in the panel can be selected **Total time** edit box.

The lower data acquisition window offers three main plotting modes, which can be accessed via the tabs at the top

- The Scope displays measured data versus time.
- The XY plot (one for each quadrant detector) is used to trace particle positions in two dimensions.

All plots offer direct monitoring of detector voltage or metric positions in µm. Use the unit selector below the plot to switch.

> Display of micrometer positions requires calibration prior to use. See section 6.5.8.3.

To continuously acquire data press the **Continous** button. If the data in the oscilloscope window will not be updated according the parameter set, please make sure the oszilloscope is not paused. Anytime you can press **Stop** button to stop the acquisition.

The checkbox **Stream to file** allows you to stream your aquisition data as a CSV (comma-separated values) file to your harddrive. The file name will be created automatically by date and time and the folder, where the file will be saved can be selected by pressing the **File** button. The header section of the CSV file contains information about the data columns and the experiment parameters.

If you want to save a time synchronised video with your CSV file, check **Auto video**.

The sampling rate and the duration are adjusted in the respective entry fields in the “data acquisition” section. In the case of a continuous data acquisition the duration controls the refresh rate of the plotted data. The displayed data can be averaged over a floating data window with settable window size. The averaging will also apply to the data streamed to disk.

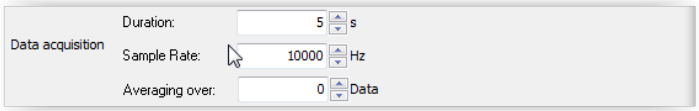


Figure 6.22: *Parameters for data acquisition window*

To synchronize data acquisition with saving a video the **Auto Video** feature can be checked.

When monitoring trap oscillations (section 6.3.5), the recorded signal can be automatically fitted to a sine function by clicking **Fit oscillation**. This button is only available after an oscillation has been performed. The fitted result of the four input signals (x and y for detectors A and B respectively) is displayed in the plot area. Additionally, the fit parameters are shown in a separate window.

You can translate the signal into the frequency-domain signal by performing Fast Fourier Transform method. This is done by pressing the **FFT** button. The energy of those frequency is calculated by using its Power Spectral Density (**PSD** button).

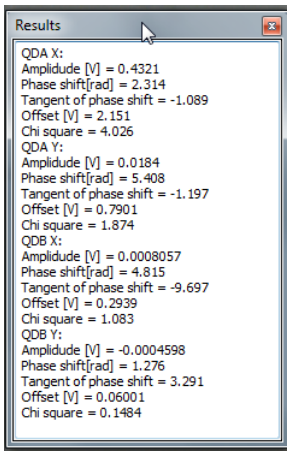


Figure 6.23: *Fit parameters for harmonic oscillation*

6.5.4 Calibrating distances

Before you can measure distances, the MBPS must be aligned properly (see section 6.5.8). The distance calibration tool can be invoked from the Position and force measurement panel (Fig. 6.19). Bead displacement calibration allows *mmi CellTools* to measure object displacements in µm units. Most of the calibration procedure is automated. The procedure is working slightly different for the **Imaging** and the **BFP** mode.

6.5.4.1 MBPS in imaging mode

The observation position of the two quadrant detectors can be shown on screen. To enable the QD markers, check *CellManipulator* → *Calibration* → *Show QD marker*. The QD position calibration procedure needs to be carried out regularly, as calibration is subject to drift.

Following is the procedure to calibrate the position of one 4QD detector. This can be done only if the microscope has the main camera mounted on the trino (20%) and the optomechanics of 4QD imaging is mounted on the left port (80%).

Procedure

1. The image from the microscope port is divided (50:50) by a beam splitter. One half goes into the 4QD detector. Mount a C-mount camera to the other half. The image observed on this camera is additionaly magnified around 13x.

2. Adjust XY micrometric translator in front of the 4QD detector and the camera to be at similar values in X and Y. Also make sure that both the 4QD detector and the camera are in the center of the micrometric translator.

3. Put sample (3 μm beads in suspension) and wait until some bead settle on the surface.

4. Use the trino mounted camera to see the FOV. Open the "Data Acquisition" window. Choose the "V" as Units. Set the duration = 0.1 s, the sample rate = 10000 Hz, and the average over = 10 Data.

5. Open the external software for the C-mount camera (e.g. μEye Cockpit if using the IDS camera).

6. Focus on the sample and monitor the observation C-mount camera (The Kohler illumination must be done prior to this as the C-mount camera needs lot of light. Also one might need to adjust camera gain to the max).

Try to locate on the trino camera the position of the bead observed in the center of the C-mount camera FOV.

7. Open the "QD A Calibration" in the imaging mode. Click **Place** to mark those position. This will be roughly (not yet accurate) the center of 4QD detector.

8. Trap a 3 μm bead somewhere in FOV and oscillate (X=1 μm, 100 Hz, 200 s). The acquisition will freeze after clicking the "Oscillate" button. Click the "Continous" button on the "Data Acquisition" window once to observe the real-time 4QD signal.

9. Drag the oscillating trap close to the marker. Observe the signal on the "Data Acquisition" window. Fine-adjust the position (CellManipulator - Move trap - Move single trap) until X component resembles a nice, symmetric (the center of the waves in y axis are at 0 V) sine waves. If the waves are nice but not symmetric, carefully adjust the X-potentio at the back of the 4QD detector as described in chapter 6.5.8.

10. Observe the Y component. If it is not almost straight line and close as possible to 0 V as shown in (see Fig. 6.24), fine-adjust the trap up or down till 0 V. If the signal still cannot reach 0 V, carefully adjust the Y-potentio at the back of the 4QD detector as described in chapter 6.5.8.

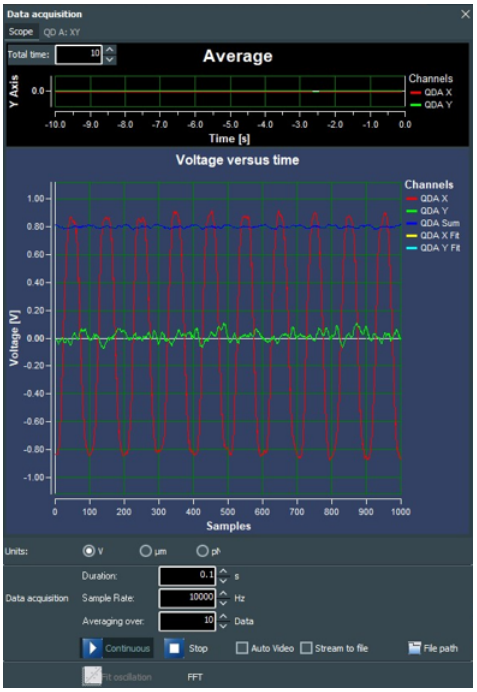


Figure 6.24: *Response curve of a quadrant detector when correctly adjusted, X oscillation*

11. Stop the oscillation. Click "Move to Trap" on the "QD A Calibration". This is now the accurate center of 4QD detector.

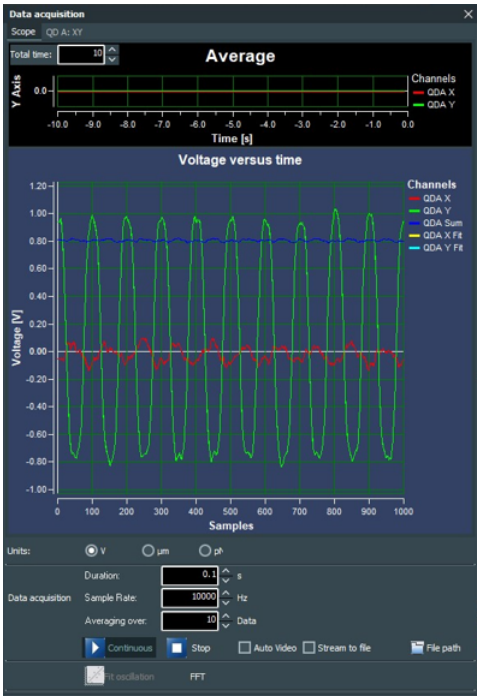


Figure 6.25: *Response curve of a quadrant detector when correctly adjusted, Y oscillation*

12. Oscillate the bead (Y=1 μm, 100 Hz, 200 s). The X component should be close to 0 (see Fig. 6.25).

In the calibration procedure for the bead displacements, a bead is moved along one axis using an optical trap, and the QD response is measured and fitted to a curve (see Fig. 6.26). The curve model is subsequently used to translate QD voltage response to µm displacement.

Procedure

1. Trap a bead on the QD marker (Right click-Create trap at QDA).

2. In the calibration window, click the **Calibrate** button 

3. Calibration starts with the horizontal (X) axis. Data is acquired and displayed in the graph. This should take approx. 10 seconds.

4. The graph shows the data acquired and the fitted curve. To obtain an optimal fit, adjust the smoothing parameter and the fit region by using the smooth fit slider and the vertical bars in the plot window (see Fig. 6.26). Then click the **Accept** button.

5. Calibration will continue with the vertical (Y) axis. Adjust smoothing, then click **Accept** to finish.

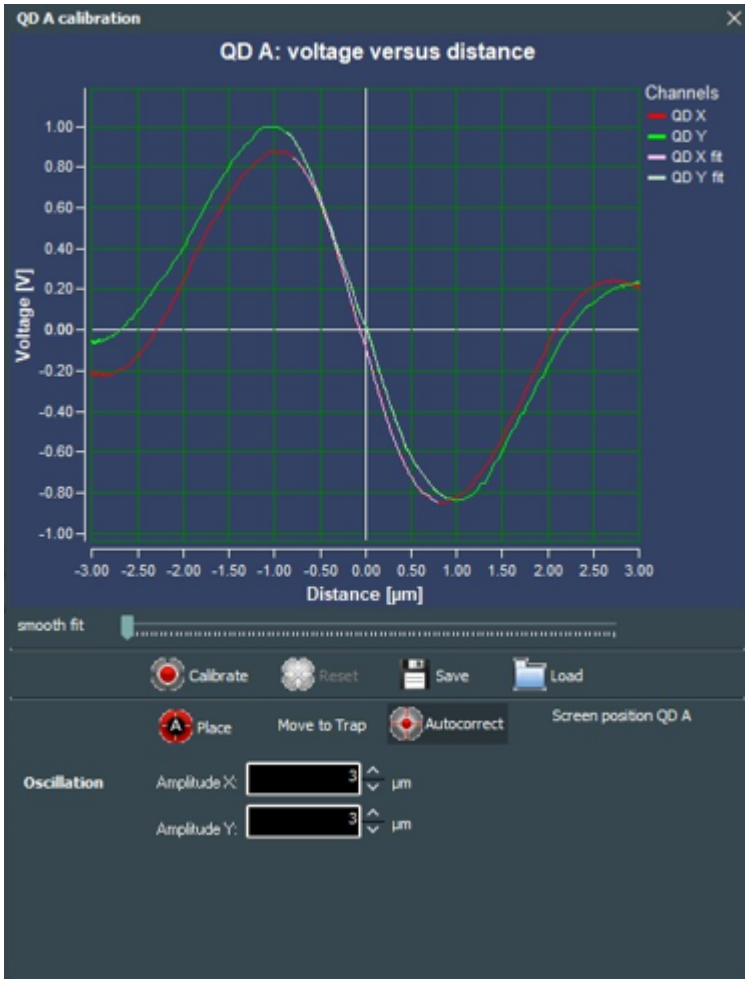


Figure 6.26: *Response curve of a quadrant detector in imaging mode during calibration*

Also a successful calibration can show the zero volt crossing not exactly at zero distance position. The **Correct** will reset the quadrant detector position, so that a following calibration will show the voltage zero crossing very close to the zero distance. After a successful distance-voltage calibration the data acquisition plot can be used to directly show bead displacements in µm.

6.5.4.2 MBPS in Back focal plane (BFP) mode

During the calibration procedure, the bead will be oscillated multiple times with a fixed frequency of 32 Hz. The time-domain signals will be converted to the frequency domain and subsequently the Power Spectral Density will be calculated. The curve will be fitted with a Lorentzian function.

Procedure

1. Trap single 1 μm bead. If necessary, adjust the hardware as described in chapter 6.5.9

2. In the CellManipulator Plugin, switch the QD mode to BFP and open the QD Calibration Window (see Fig. 6.27). On the bottom of the graph, enter the oscillation parameters (e.g., Amplitude = 0.5, Repeat = 10, Duration = 1). Click **Calibrate**. The calibration starts with the horizontal (X) axis.

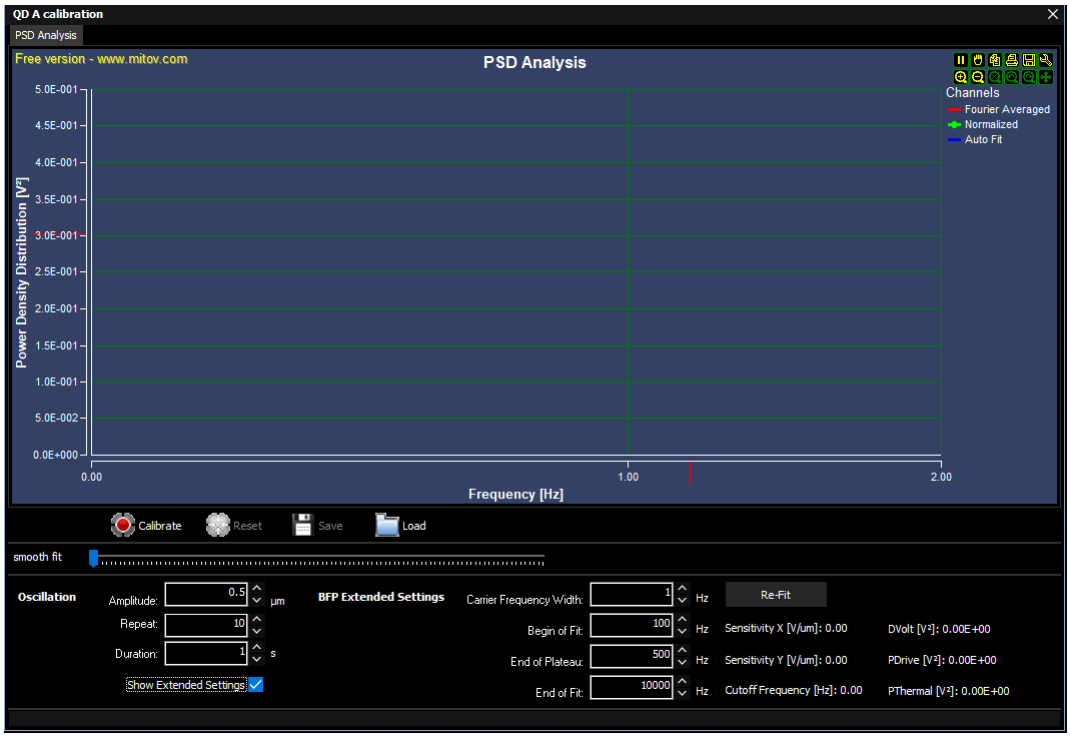


Figure 6.27: *QD calibration window*

3. Monitor the oscillation on the Data Acquisition window. The response should resemble a sine wave.

4. After the calibration is done, the graph will show the Power Spectral Density of the signal together with the Lorentzian fit.

5. When the fitting curve fails, Click **Show Extended Settings** and adjust

   - **Carrier Frequency Width**: The frequency of the oscillation (32 Hz) results in a spike in the PSD, which needs to be excluded in the fitting. Ideally, the width is 1, but in some cases it may be beneficial to increase the width to exclude power leakage in the spectrum.
   - **Begin of Fit**: The start of the fitting curve. Ideally the curve has a plateau of constant height, which begins to drop off at a specific frequency, in reality the plateau may show increased values at very

low frequencies. Increase this value to exclude these values. Typically between 10 and 100 Hz.

- **End of Plateau**: An estimate of the end of the plateau, used as starting value for the automated fitting. Adjust this to be roughly at the end of the plateau, typically around 1 to 2 kHz.
- **End of Fit**: The end of the fitting curve. It should be placed where the drop in the PSD has approached zero or slightly beyond, typically 10 to 20 kHz
- The Extended settings show the calculated sensitivity values for both X and Y direction, as well as the detected cutoff frequency.

6. Calibration will continue with the vertical (Y) axis. Click **Accept** to finish.

6.5.5 Stiffness

The harmonic oscillation method permits determination of the system's stiffness by measuring particle displacement phase shift at various oscillation frequencies (see section 6.5.1.1).

> Before you can measure stiffness, the MBPS must be aligned properly (see section 6.5.8).

mmi CellTools provides an automated stiffness measurement tool. The stiffness button opens the calibration window shown in Fig. 6.28

The stiffness measurement tool will sequentially ramp up the oscillation frequency and measure particle displacement using the parameters given under "Data acquisition".

Before you can start the measurement you need to trap a single bead and move it to the QD-position defined in chapter 6.5.4.1. If the position is slightly off, the stiffness calibration routine automatically corrects it at the beginning of the measurement.

In section "Oscillation" you can control the frequencies and amplitudes used:

max. trap frequency f_{max}	Highest oscillation frequency used
frequency steps Δf	frequency increase per measurement
amplitude X	horizontal oscillation amplitude

During measurement, the current oscillation frequency is displayed under trap frequency. When all parameters are set, click the **Calibrate Stiffness** button to start. The bar at the bottom indicates the experiment's progress.

After completion, the results are displayed in the graph window along with a linear fit from which the stiffness is determined. The resulting stiffness value is displayed in section "Stiffness".

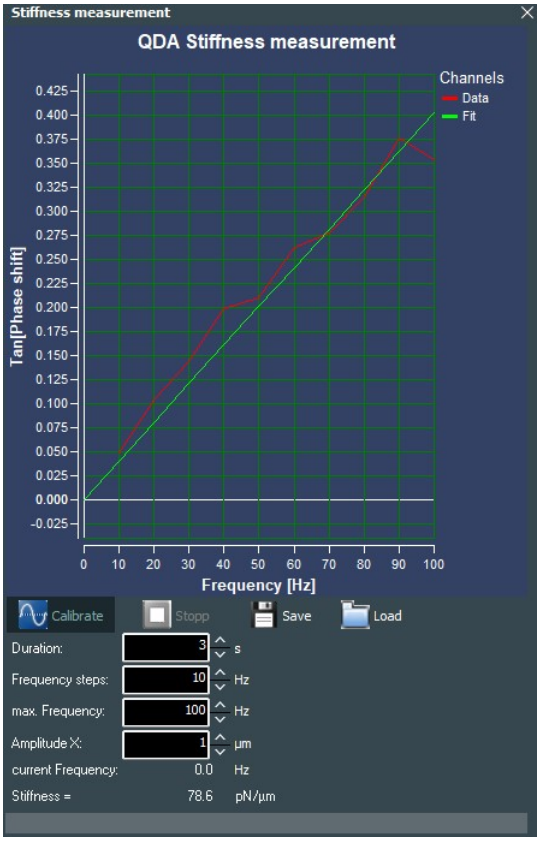


Figure 6.28: *Stiffness measurement window*

6.5.6 Force spectroscopy experiment

After calibrating distances and the trap stiffness the system is ready for force spectroscopy experiments. When running a force spectroscopy experiment the trapped object will be forward-backward moved with constant velocity. Forces will be measured continuously and the averaged force - position plots will be calculated and displayed.

The In the BFP mode an experimental setup can be as follows:

Procedure

1. immobilise one object chemically
2. trap a second object and move it 10µm to the right of the immobilised object
3. set the force spectroscopy parameters to move the trapped object 9 µm to left (negative). Execute the movement for 10sec with 2Hz.
4. the trapped object will move 10 time towards the first object and back to the origin

5. distance dependent forces will be plotted with distances between 1 µm and 10 µm.

Using the imaging mode the measured object must be hold in a fixed trap and the second object must be moved. To run a force spectroscopy experiment a piezo driven stage insert is recommended so that the movement of the hole sample can be precisely realised:

Procedure

1. immobilise one object chemically
2. trap a second object and move it 10 µm to the right of the immobilised object
3. set the force spectroscopy parameters to move the piezo stage 9 µm to right (positive). Execute the movement for 10sec with 2Hz.
4. the fixed object will move 10 time towards the trapped object and back to the origin
5. distance dependent forces will be plotted with distances between 1 µm and 10 µm.

These experiments will be executed in the **Force spectroscopy** window.

As explained with oscillation experiments, see section 6.3.5, the experimental parameters for an oscillation can be set. The system allays will use constant oscillation velocities (triangle position signal) and finally plot the measured forces against the position.

Additionally you can select, if the oscillation will be directed to

- the left side of the current position
- the right hand side of the current position
- centric around the current position

The measured data can be saved as comma separated text files using the **Save** button.

6.5.7 PID control

Systems that are equipped with the optional PID optical trap controller permit a closed-loop control of trap positioning in response to the microbead position detection system.

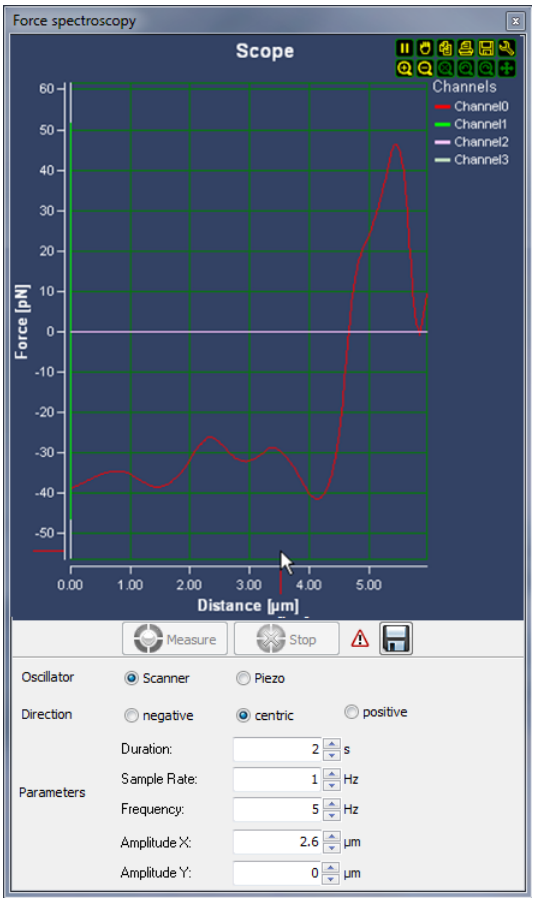


Figure 6.29: *Force spectroscopy window*

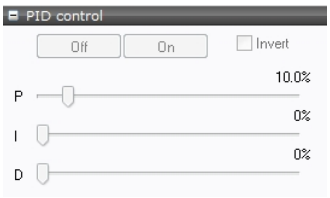


Figure 6.30: *The PID control section appears for systems equipped with the PID optical trap controller.*

6.5.7.1 Theory

The PID controller, when activated, continually monitors the quadrant detector's signal. When the bead is displaced from the center, the controller can adjust the position of the optical trap in order to eliminate the displacement. As a result, the bead's position is kept constant, even when external forces are applied to it.

PID is a technical term that stands for a common type of control circuitry that works well with various types of control tasks, such as the one described above. A PID control circuit is controlled by three parameters: a proportional (P) component, an integral (I) component and a differential (D) component.

1. The P component determines the general strength of the response. Increasing the P value means the trap will make larger movements and thus will help to eliminate bead displacements quickly. However, large P values can result in overshooting, and may cause the trap to lose the bead.

2. The I component eliminates a displacement in response to static forces.

3. The D component reacts to quick changes in the bead's position. It can help to keep its position constant if a sudden force is applied. Again, setting this value too high may cause exaggerated responses to sudden forces.

For more information about PID control, and for advice on how to find optimal PID values, please refer to standard technical literature, p.ex. K. Åstrom and T. Hägglung, "PID Controllers: Theory, Design and Tuning", 1995.

6.5.7.2 Usage

To activate PID control, you will first need to create a single trap (see section 6.2.1). In the PID control frame (Fig. 6.30), activate or deactivate PID closed-loop control using the **On** and **Off** buttons.

The sliders below can be used to adjust the three control parameters as outlined above. The three parameters are indicated as percentage values. In order to find ideal parameters, start with a low value of P and set I and D to zero. Then gradually increase their values, according to the parameter description above, until you are satisfied with the result.

Once you have obtained ideal parameters for a certain application, it is advisable that you take notes of them. *mmi CellTools* automatically saves the last PID parameters used and restores them when you restart the application.

One prerequisite for closed-loop control is that the detector's sum signal is sufficiently large. Set the QD amplification in the data acquisition window to get signals between 1 and 9 Volt.

6.5.8 MBPS hardware adjustments in imaging mode

To ensure correct operation of all force measurement functions the MBPS system must be aligned with the main camera. All of the following calibrations are carried out by MMI staff at system installation. However, due to thermal and mechanical influences, the alignment may be subject to drift, and recalibration may become necessary. To calibrate the MBPS, you will need the following tools

- A calibration slide (see below)
- A C-mount observation camera to connect to the MBPS

In the following procedures, the observation camera replaces one of the detector heads in order to determine position and focus of the quadrant detectors (see Fig. 6.31).

Figure 6.31: *Mounting a camera at the MBPS location*

Please also consult the instructions provided with the microbead positioning sensors.

6.5.8.1 Preparation of a calibration slide

You will need a suitable solution of microbeads with a diameter of around 1 µm (e.g. Polysciences, Polybead 3.0 µm microspheres, Cat #17134-15). The beads can be prepared on a standard MMI membrane slide (MMI Cat #50103)

Procedure

1. Dilute 1 drop of microbeads in 5 ml of water

2. Remove membrane from membrane slide

3. Fix a cover slip (at least 25x60 mm) to the metal frame (e.g. using super glue)

4. Pipette one drop of diluted beads onto the slide

5. Dry the slide

6.5.8.2 Ensuring parfocality

For correct operation, parfocality between the main camera and the MBPS must be ensured. Parfocality means that the focal planes of these two systems are aligned such that an object that appears in focus also lies in the focal plane of the quadrant detector.

This procedure is carried out by MMI personnel at installation. Recalibration is only required in rare circumstances or after tampering with the optical setup.

Parfocality can be achieved by adjusting the length of the tube between C-mount adapter and the beam splitter cube.

Procedure

1. Unfasten the adjustment ring (first ring next to the c-mount adapter, Thorlabs mount)

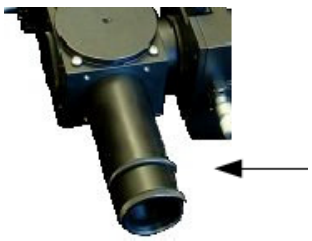


Figure 6.32: *Location of the adjustment ring*

2. Unmount one detector head and hook up the observation camera instead.

3. Monitor the observation camera's image, as well as the main camera's image in *mmi CellTools*. Adjust the tube length until both images are in focus at the same time.

4. Lock the adjustment ring.

6.5.8.3 Rough XY Position alignment

Both detector heads need to be calibrated independently.

Procedure

1. Insert the calibration slide (see section 6.5.8.1).
2. Mount the observation camera to the detector position to be calibrated.
3. Adjust lighting and imaging to obtain clear, bright beads on a dark grey background. (Background should not be totally black, as this will increase noise). Best results are obtained with DIC illumination.
4. By moving the stage, position a well recognizable bead (best a double or triple) in the center of the observation camera.
5. Using the stage, slowly move the observed bead to the position where you like to measure bead displacement (do not move too far from the center of the screen). Keep the observed bead in the field of view of the observation camera with the translation screws of the detector mount.

6.5.8.4 Detector head settings for quadrant detectors delivered before 2014

To obtain an optimal signal, the gain and offset settings of the detector must be set properly. The gain switch on the detector head should be set to 3 or 4, depending on the illumination intensity of the microscope. The dials on the MBPS controller should be set as described in the following procedure for basic setup. They can be adjusted to your individual experiment once the system is aligned.

Procedure

1. Remount the detector head
2. Set proportional gain (dial on MBPS controller box) for x- and y-axis to 2
3. Set integral gain for x- and y-axis to 0
4. Open the data acquisition window (see figure 6.21) and start continuous data acquisition
5. If you are working with a *mmi CellManipulator*, make sure that the sum signal of your detector is between 1 and 9 V. Change the illumination intensity or the gain switch at the detector head if necessary
6. Position the stage such that there is no bead or object at the detector position

7. Adjust the offsets on the MBPS controller to get the x- and y-signals to 0V

   The last two steps should be done every time the illumination of the microscope is changed.

6.5.8.5 Fine xy position adjustment

To fine-adjust the position, open the scope view in the position and force measurement section. Start continuous data acquisition by clicking on the **Continuous** button.

Procedure

1. Using the stage, move the observed bead horizontally in small steps of about 0.1 µm (see section 5.4.3).
2. Check the voltages of QD A/B X for the appropriate response. The x signal should show distinct steps of equal size within a range of about 1 µm, the y signal should not change. If the signals are interchanged or not independent, adjust the rotation of the detector accordingly.
3. Move the observed bead to the 0V center position
4. Repeat the previous step with the y axis
5. Switch the data acquisition to the XY plot view
6. The measured position will appear as a dot, likely off-center. Move the stage until the displayed position is exactly centered
7. Set the QD marker as described below

Set QD A/B position markers in software Open the quadrant detector calibration tool. The QD A calibration window opens. Click on the **Place** button, then right-click on the center of the observed bead on the main screen. The QD marker will show at the new position.

6.5.9 MBPS hardware adjustments in BFP mode

Procedure

1. Unmount one detector head and hook up the observation camera instead.

2. Check the condensor to be in Köhler illumnination, see the microscope manual.

3. Close the condensor apertur diaphragm until you see in on the observation camera.

4. Sharpen the diaphragm image by turning the adjustment device

Figure 6.33: *Parfocal adjustment of the BFP detector*

5. Center the aperture by using the adjustment screws in BFP mounting minting block.

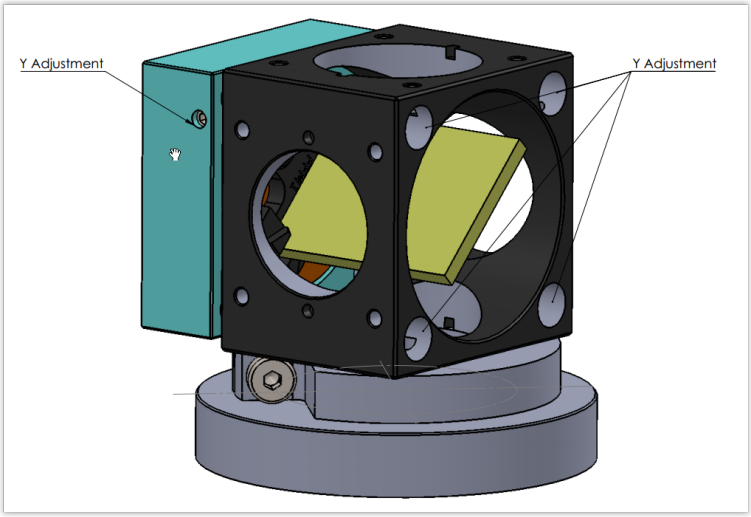


Figure 6.34: *BFP detector mounting block with lateral adjustment screws*

6.6 Controlling external devices

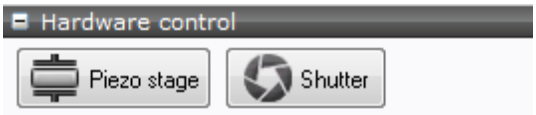


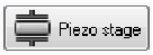
Figure 6.35: *Tools for external devices*

6.6.1 Piezo control

The *mmi CellManipulator* can control piezo devices that operate with 0–10 V signals. The piezo controller can be used for

- vertical (z) movement of an objective
- sample stage movement in two or three dimensions using a piezo stage

For questions on recommended models and setup please contact MMI service.



The piezo control window is opened by clicking on the Piezo button in the Hardware control section of the *CellManipulator* panel.

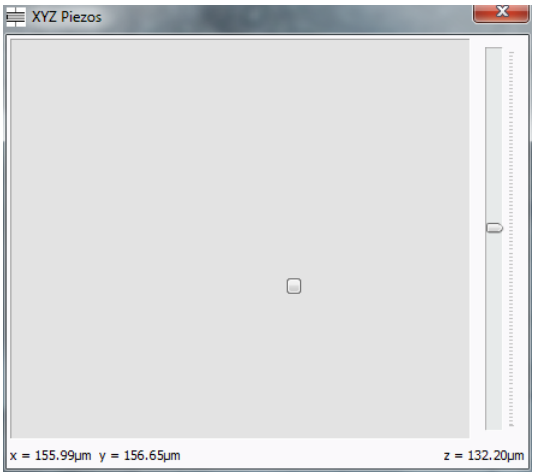


Figure 6.36: *Piezo controller window*

Depending on your configuration, the piezo window offers controls for manipulating x, y and z piezo positions. Drag the respective sliders to operate the piezo controller.

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
- 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).

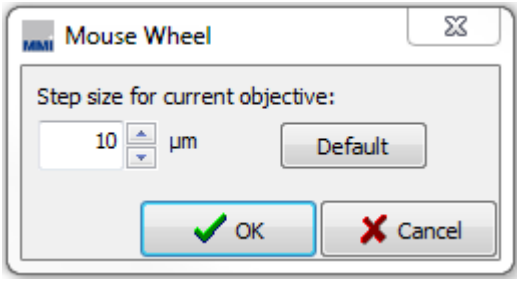


Figure 7.1: *Mouse wheel settings*

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
  - 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.

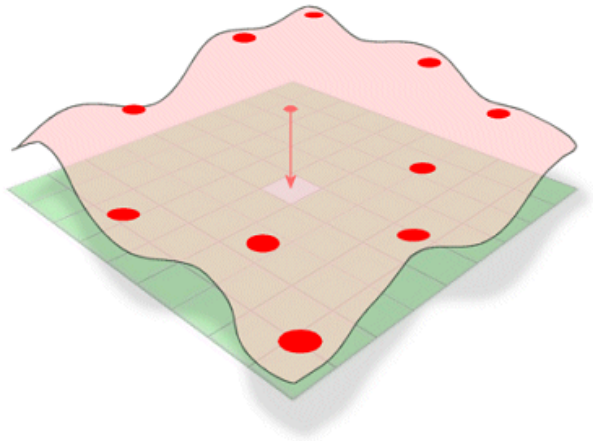


Figure 7.2: *Sample surface with defined surface points*

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

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.

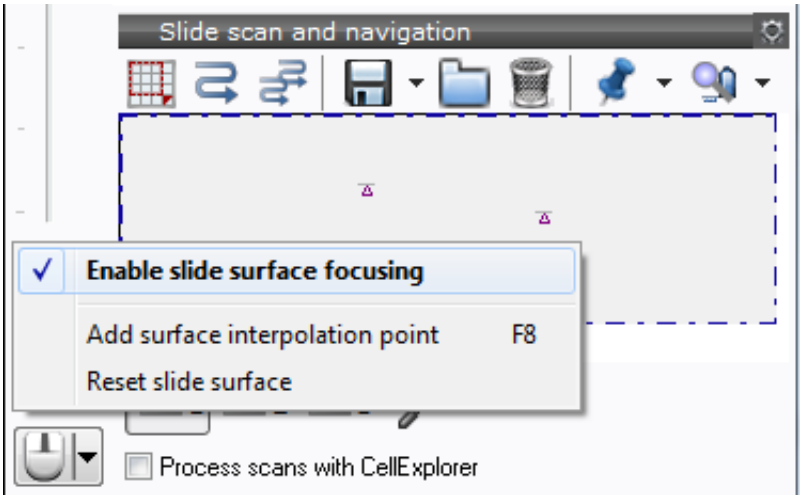


Figure 7.3: *Surface map user interface*

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 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.

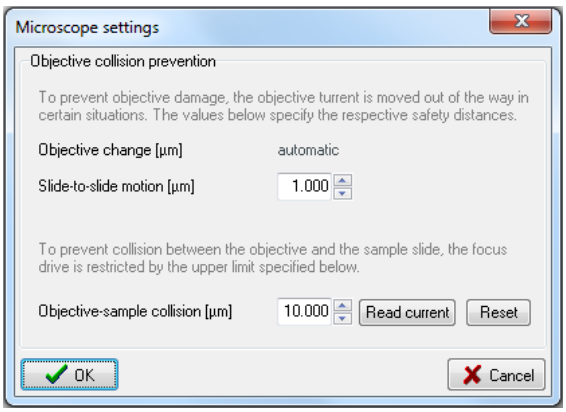


Figure 7.4: *Microscope safety parameters*

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 $\rightarrow$ *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.
- Laser power up to 8 W at 1070nm 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 CellCut. 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

- set the camera exposure to automatic
- 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 0,17 for coverslips or 1 for microscope slide. The value always needs to correspond to glass thickness)

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

8.3.3 Overview

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

- Stage - Camera alignment not correct (see section 5.7.2)
- The camera is tilted against the stage xy-axes. If you adjust the camera rotation, you also need to check the galvo-scanner rotation calibration.

8.3.4 Tweezers

No tweezing can be observed.

- The laser has not been switched on (interlock, key switch and activation 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 illumination pillar is not set right upwards and the interlock switch can’t close
- The galvo scanner alignment (offset, amplitude or rotation) is off
- The microscope focus is to far away from the coverslip upper surface

A 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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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°C: 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 Laser system

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

Wavelength:	1070 nm
Laser power:	10 W
Line voltage:	200–240 VAC or 100–110 VAC, 50–60 Hz, 4.0 A

B.2.7 Galvanic scanner

X-Y scanning system

max. update rate:	2 kHz
typical resolution:	25 nm