

**MMI**

**Microdissection - Micromanipulation - Imaging**

# **mmi CellScan User Manual**



Molecular Machines & Industries GmbH  
[www.molecular-machines.com](http://www.molecular-machines.com)



**User Manual: MMI CellScan**  
**Version 6.0**  
Copyright © 2024 by MMI GmbH

All rights reserved for patents or trademark registration

No part of this document may be distributed or reproduced, utilized or imparted to a third party without the prior written permission of MMI GmbH.  
Non-observance will result in liability for damages.

MMI® is a registered trademark of Molecular Machines & Industries GmbH.

MMI GmbH  
Breslauer Strasse 2  
D - 85386 Eching  
Germany

Technical features are subject to change without notice



# Contents

<b>1</b>	<b>INSTALLATION</b>	<b>1</b>
<b>2</b>	<b>THE MMI CELLTOOLS INSTRUMENTATION FAMILY</b>	<b>3</b>
<b>3</b>	<b>MMI CELLTOOLS – MAIN APPLICATION</b>	<b>5</b>
<b>3.1</b>	<b>Main window and plug-ins</b> . . . . .	<b>5</b>
<b>3.2</b>	<b>User-specific database</b> . . . . .	<b>6</b>
3.2.1	Channel editor . . . . .	7
3.2.2	Objective editor . . . . .	8
3.2.3	Default database reconstruction . . . . .	10
<b>3.3</b>	<b>Camera operation</b> . . . . .	<b>10</b>
3.3.1	Multiple cameras . . . . .	11
3.3.2	Camera settings . . . . .	11
3.3.3	Freeze video / live video . . . . .	13
3.3.4	Saving images . . . . .	13
3.3.5	Recording movies . . . . .	14
3.3.6	Multichannel imaging . . . . .	16
3.3.7	Autofocus . . . . .	16
<b>3.4</b>	<b>Motorized stage control</b> . . . . .	<b>17</b>
3.4.1	Mouse movement . . . . .	17
3.4.2	Keyboard movement . . . . .	17
3.4.3	Stage movement settings . . . . .	18

## CONTENTS

3.4.4 Overview scan . . . . .	19
<b>3.5 Slide viewer . . . . .</b>	<b>22</b>
3.5.1 Drawing tools . . . . .	24
3.5.2 Text annotations . . . . .	29
3.5.3 Hide shape indices . . . . .	29
3.5.4 Move to group . . . . .	29
3.5.5 Reset slides . . . . .	30
3.5.6 Configure scale bar . . . . .	30
3.5.7 Distance measurement . . . . .	31
3.5.8 Slide Navigation . . . . .	31
3.5.9 <i>mmiCellScan</i> 5D channels . . . . .	32
3.5.10 Shape import from third party applications . . . . .	32
3.5.11 Slide viewer plugins . . . . .	34
<b>3.6 Stage inserts configuration . . . . .</b>	<b>34</b>
3.6.1 Stage inserts . . . . .	36
3.6.2 Slides . . . . .	37
3.6.3 Active regions, wells . . . . .	38
3.6.4 Stage inserts first configuration . . . . .	38
<b>3.7 Calibration . . . . .</b>	<b>39</b>
3.7.1 Stage calibration . . . . .	39
3.7.2 Camera alignment . . . . .	40
3.7.3 Lens offset calibration . . . . .	42
<b>3.8 Multi-user report . . . . .</b>	<b>43</b>
<b>3.9 Help . . . . .</b>	<b>44</b>
3.9.1 Help topics . . . . .	44
3.9.2 MMI online . . . . .	44
3.9.3 Version info . . . . .	44
<b>4 MMI CELLSCAN . . . . .</b>	<b>45</b>

<b>4.1 Getting started</b>	45
4.1.1 Quick Start	45
<b>4.2 Slide scanning</b>	46
<b>4.3 Settings panel</b>	47
<b>4.4 Explorer panel</b>	48
<b>4.5 Preview panel</b>	49
<b>4.6 Cell types</b>	49
4.6.1 Definition and editing of groups	50
4.6.2 Regrouping shapes	51
4.6.3 Group statistics	51
4.6.4 Shape list	51
<b>4.7 5D-Scanning</b>	52
4.7.1 Automatic Sample Detection	52
4.7.2 Auto-focus Mapping	52
4.7.3 Multi-Slide Scanning	53
4.7.4 Time-Stack	53
4.7.5 Z-Stack	53
4.7.6 Multi-Channels	53
<b>5 AUTOMATED MICROSCOPE CONTROL</b>	<b>55</b>
<b>5.1 Activating microscope control</b>	<b>55</b>
<b>5.2 Observation methods</b>	<b>56</b>
<b>5.3 Objective control</b>	<b>56</b>
<b>5.4 Z drive control</b>	<b>57</b>
5.4.1 Required calibrations	58
5.4.2 Sample focus map	58
5.4.3 Z drive offset calibration for objectives	59
5.4.4 Automatic Z drive control	60
<b>5.5 Microscope safety parameters</b>	<b>60</b>

## CONTENTS

5.5.1 Objective collision prevention . . . . .	61
5.5.2 Objective-sample collision . . . . .	61
<b>5.6 Fluorescence shutter control . . . . .</b>	<b>62</b>
<b>6 MAINTENANCE . . . . .</b>	<b>63</b>
<b>6.1 System check . . . . .</b>	<b>63</b>
<b>6.2 Cleaning . . . . .</b>	<b>63</b>
6.2.1 Microscope . . . . .	63
6.2.2 Cleaning optical parts . . . . .	63
6.2.3 Cleaning when actually dirty . . . . .	63
<b>6.3 Trouble shooting . . . . .</b>	<b>64</b>
6.3.1 View . . . . .	64
6.3.2 Movement . . . . .	65
6.3.3 Overview . . . . .	66
<b>A LIST OF KEYBOARD SHORTCUTS . . . . .</b>	<b>67</b>
<b>B TECHNICAL DATA . . . . .</b>	<b>71</b>
<b>B.1 Required minimum workspace . . . . .</b>	<b>71</b>
<b>B.2 System components . . . . .</b>	<b>71</b>
B.2.1 Microscope . . . . .	71
B.2.2 Stage . . . . .	71
B.2.3 Digital camera . . . . .	72
B.2.4 Fluorescence Light sources . . . . .	74
B.2.5 Computer . . . . .	74
<b>C SERVICE . . . . .</b>	<b>75</b>

# 1 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](mailto: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.

## 1. INSTALLATION

## 2 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

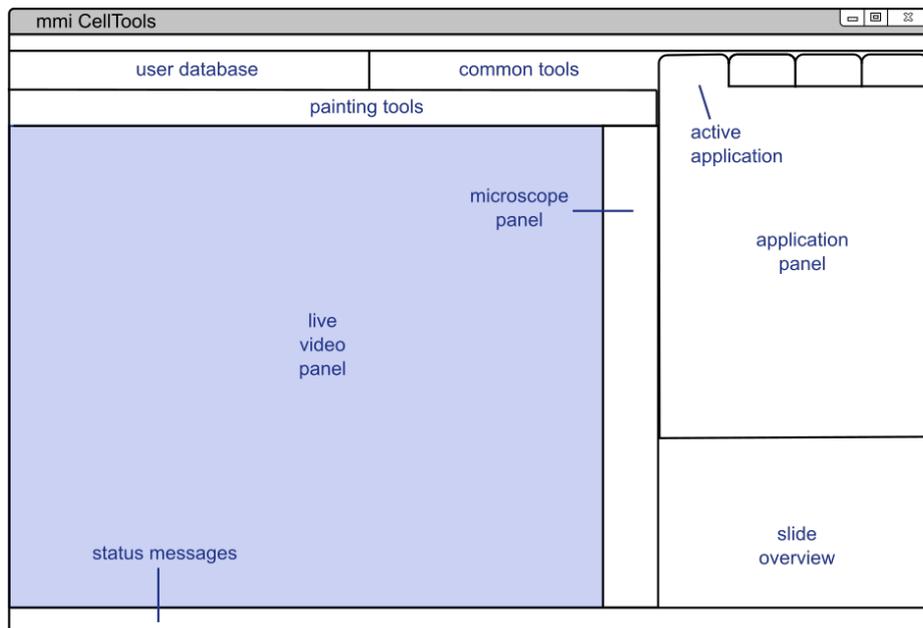
Any or all of these modules can be combined in one microscopic environment. The *mmi CellDetector WSI* and also be used with the stand alone program *mmi CellViewer*

## 2. THE MMI CELLTOOLS INSTRUMENTATION FAMILY

## 3 mmi CellTools – Main application

### 3.1 Main window and plug-ins

Fig. 3.1 shows the extended tool area and explains the main components of the *mmi CellTools* user interface.



**Figure 3.1:** 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 2). 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

### 3. MMI CELLTOOLS – MAIN APPLICATION

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 3.2). The remaining **common tools** on the tool bar provide elements for camera and stage control.

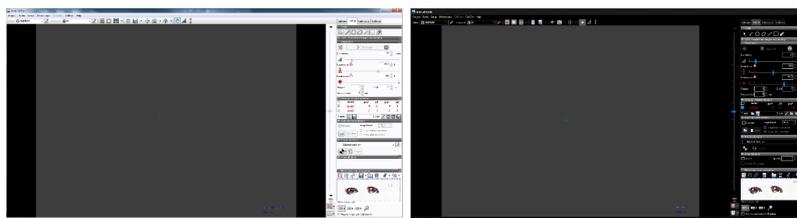
On systems equipped with an automated microscope, the microscope panel provides access to z-drive (focus) control and other microscope-specific features (see chapter 5). The **slide overview** panel provides controls for slide scanning and navigation (see section 3.4.4). Finally, the **status messages** 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 3.2:** 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*

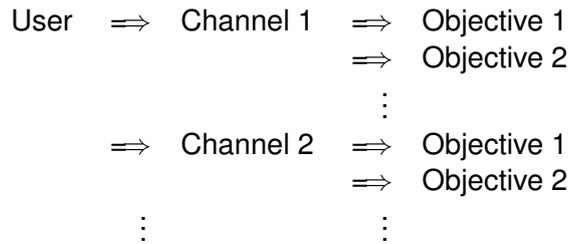


Whenever you see a warning sign at a specific function. Place the mouse over the sign and a text box appears with a description.

## 3.2 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:



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.

### 3.2.1 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.

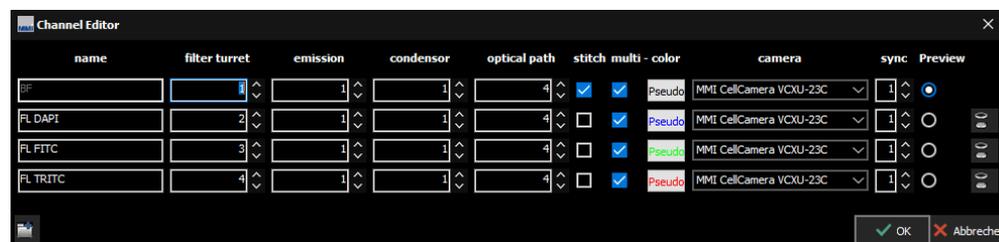


Figure 3.3: channel editor

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.

#### 3.2.1.1 Channel options

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

### 3. MMI CELLTOOLS – MAIN APPLICATION

parameters is explained in sections 5.2, 4.7.6 and 3.3.6.

- if a supported external fluorescence excitation filter wheel is mounted, **emission** filter wheel settings show up additionally.
- 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.

#### 3.2.1.2 Preview channel

One channel can be selected as a **preview** channel. The **preview** channel is used during the automatic sample detection (foreground detection) scan, see chapter 4.7.1. To be able to run a fast automatic sample detection you have to select a channel using short camera exposure times, p. ex. brightfield.

#### 3.2.2 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.



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.

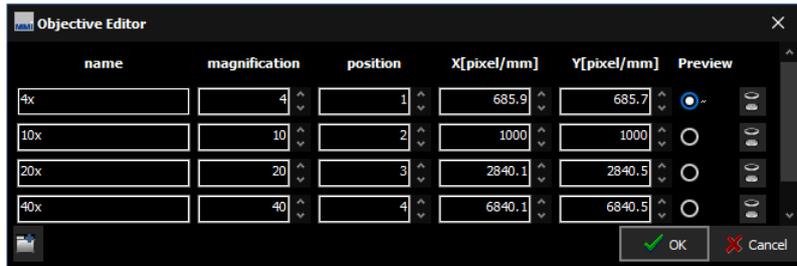


Figure 3.4: Objective editor

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 3.7.2).

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

- Camera settings (see section 3.3.2)
- Camera alignment (see section 3.7.2)
- Lens offset (see section 3.7.3)
- Z-Focus lens offset (for automated microscopes only, see section 5.4.3)
- if a supported LED light source with controlable light intensities is mounted, the intensity can be selected by the parameter **FL intensity[%]**.

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

### 3.2.2.1 Preview objective

One objective can be selected as a **preview** objective. The **preview** objective is used during the automatic sample detection (foreground detection) scan, see chapter 4.7.1. To be able to run a fast automatic sample detection you have to select an objective with low magnification, p. ex. 4x.

### 3. MMI CELLTOOLS – MAIN APPLICATION

#### 3.2.3 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.**

### 3.3 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

### 3.3.1 Multiple cameras

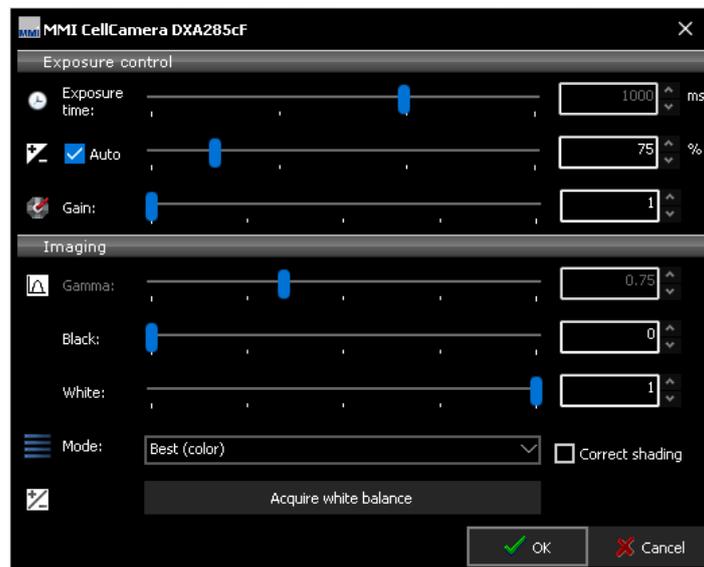
mmi CellTools also supports multiple cameras. If more than one camera is connected to the system, switch between the cameras by selecting a channel dedicated to that camera.

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 synchronisation groups for different camera channels, so that camera alignments (section 3.7.2), parfocal correction (section 5.4.3) and paraxial corrections (section 3.7.3) only have to be calibrated once for every objective.

### 3.3.2 Camera settings

For best imaging results the camera can be controlled through a settings window (Fig. 3.5). To open it, click the camera button in the toolbar (*Ctrl + R*).



**Figure 3.5:** 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.

### 3. MMI CELLTOOLS – MAIN APPLICATION

The hardware camera amplifier **Gain** can be adjusted to receive brighter images. With high gain the images become noisier. For older 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 used to suppress noise, the **white level** to suppress 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 shading 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*

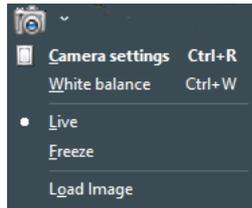
- activate **Correct shading** in the camera control panel (Fig. 3.5)

**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.



**Figure 3.6:** drop down menu to reach camera related options

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

### 3.3.3 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 dissections 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*

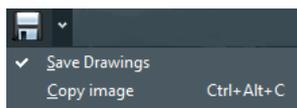
### 3.3.4 Saving images

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.

### 3. MMI CELLTOOLS – MAIN APPLICATION



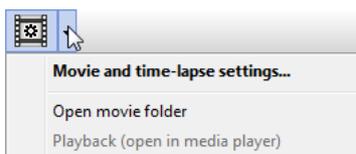
**Figure 3.7:** drop down menu for same image options

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.

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.

#### 3.3.5 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 3.8:** 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

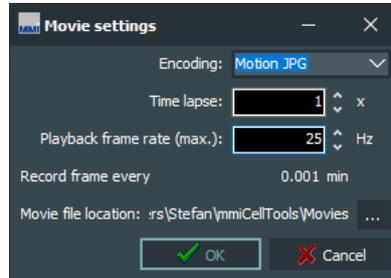
`My Documents\mmiCellTools\Movies.`

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.

### 3.3.5.1 Movie settings

Detailed aspects of movie recording can be controlled by opening the **Movie settings** dialog (Fig. 3.9).



**Figure 3.9:** *Movie settings*

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

- Motion JPG
- FFMPEG
- XVID

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.

### 3.3.6 Multichannel imaging

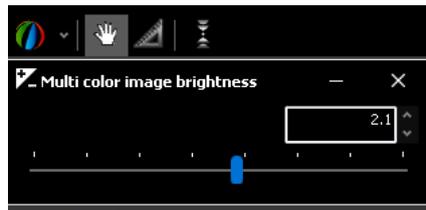


Figure 3.10: Brightness correction slider for multi channel images



Figure 3.11: Pseudo color for multi channel images

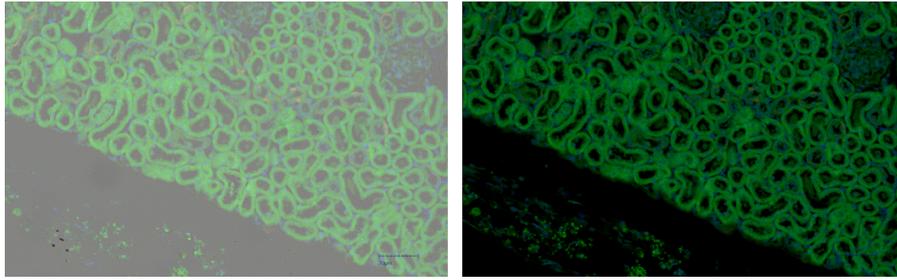
 A **multi channel** image is recorded by pressing the multi channel image button or *Shift + M* (only for motorized microscopes). A multi channel image consists of several images with different camera/filter settings. For example, it can be used to merge brightfield and fluorescence images from different fluorophores (e.g. DAPI and GFP). The brightness of the resulting image can be adjusted in multi channel configuration drop down, Fig. 3.10.

To enable an imaging channel for the multi-channel image select **enable** in the **Channel editor** (see section 3.2.1). 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. 3.11.

- If **white** is selected, a original image will be merged to the multi-channel image (see Fig. 3.12 left).
- If **black** is selected, the multi-channel image will exclude the image from the selected channel (see Fig. 3.12 right).

### 3.3.7 Autofocus

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



**Figure 3.12:** Select white (left) and black (right) as pseudo color for brightfield image

*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 optimization the autofocus search range can be limited by using the **focus range** option in the drop down near the **focus button**.

## 3.4 Motorized stage control

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

### 3.4.1 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.

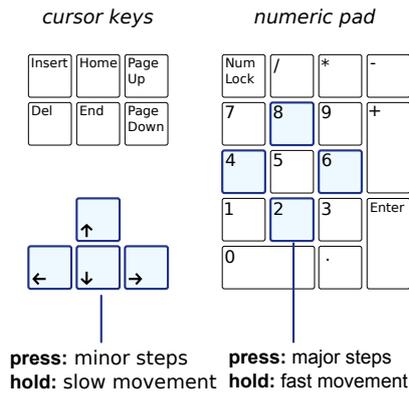
If stage movement does not exactly follow mouse movement, you may need to carry out the camera alignment procedure (see section 3.7.2).

### 3.4.2 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. 3.13).

A single press of a cursor key moves the stage by a defined distance. By

### 3. MMI CELLTOOLS – MAIN APPLICATION



**Figure 3.13:** Stage movement using the keyboard

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 3.4.3). By default, the cursor keys move slowly and the numeric pad keys move fast.

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

#### 3.4.3 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. 3.14.

**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)

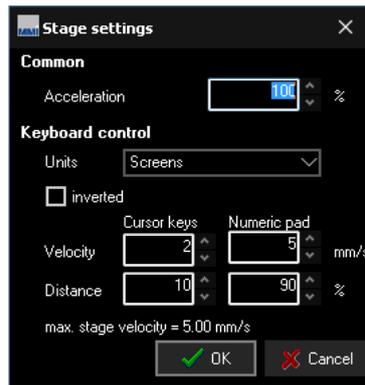


Figure 3.14: Stage movement settings

- Micrometers ( $\mu\text{m}$ )

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.

### 3.4.4 Overview scan

Fig. 3.15 shows an overview of your sample (the “roadmap image”).



Figure 3.15: Slide overview

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



### 3. MMI CELLTOOLS – MAIN APPLICATION

- 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 **Scan slides** button. You can always interrupt the scan with the **Stop scan** button. Also pressing the *Esc* key will interrupt the scan.



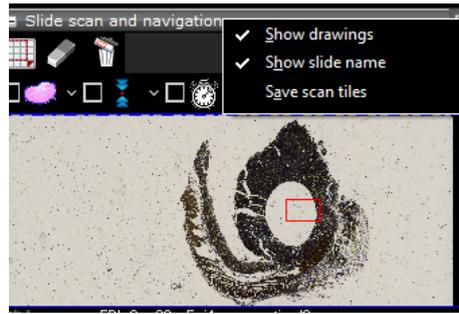
To move from one slide to another you can directly select the **target slide** button. Alternatively you can move to another slide with the keyboard arrow keys. The slide number indicator will automatically adjust to the current slide.

You can set the name of the slide in the corresponding field (Fig. 3.15). The slide name is used to save the scanned image.



The overview scan configuration can be setted by pressing the **configuration button** in Fig. 3.16.

- Show drawings: show or hide the shapes by drawing tools (drawing tools is described in section 3.5.1).
- 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 3.16:** 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 see section 4.7.

#### 3.4.4.1 Preview image

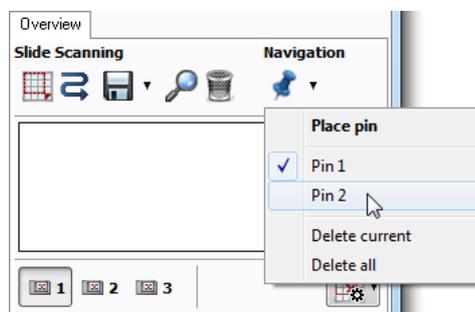
To save, load or delete the scanned preview image by simply clicking into the overview area with the right mouse button, see Fig. 3.17.



**Figure 3.17:** Menu for preview image options

#### 3.4.4.2 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. 3.18).



**Figure 3.18:** Pin positions

### 3. MMI CELLTOOLS – MAIN APPLICATION

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.

#### 3.4.4.3 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 separated **mmi CellDetector** manual.

#### 3.4.4.4 Shape navigation

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

Once you created shapes you navigate between the shape by using

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

The keyboard keys always navigate inside the current group, see section 4.6. The shapes can be directly used for further .

#### 3.4.4.5 Drawing tools and measurements

Drawing tools and distance measurements are described in section 3.5.1.

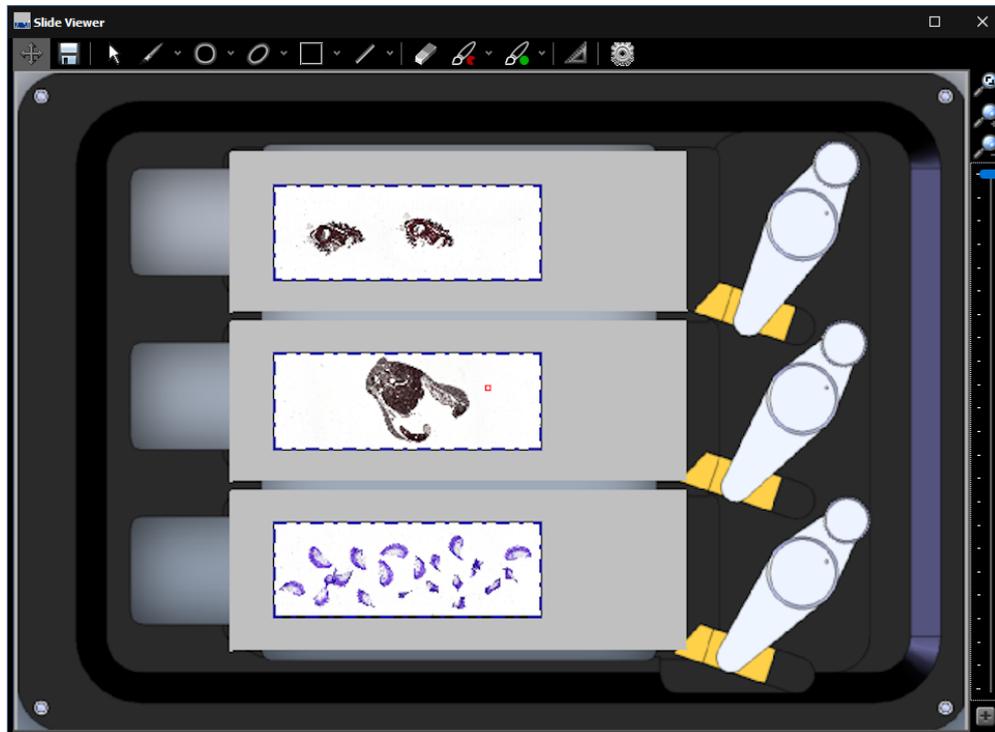
### 3.5 Slide viewer

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. 3.19) can be opened via the menu

*Setup* → *Stage insert* (Ctrl + I)

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



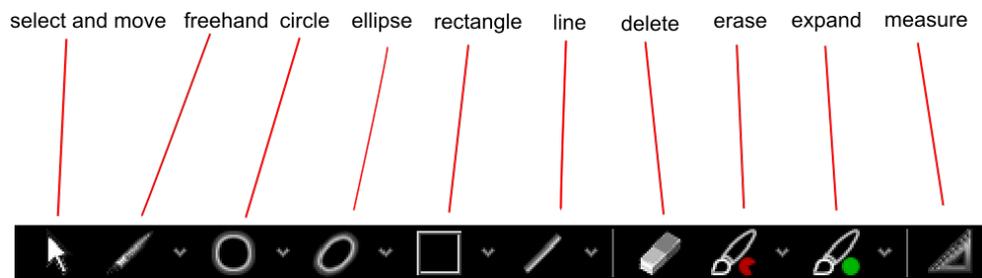
**Figure 3.19:** The Slide viewer can be used to display full slide images, draw contours and to configure the stage insert

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

### 3.5.1 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 3.20:** 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 then 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 then 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 then 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 organised in groups (see section 4.6).

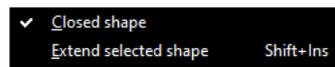
### 3.5.1.1 Select and move shapes

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).

### 3.5.1.2 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 3.21:** 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.

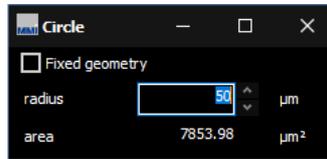
Multi-segment drawing may be easier when “Closed shape” is turned off (unchecked).

### 3. MMI CELLTOOLS – MAIN APPLICATION

#### 3.5.1.3 Circles



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



**Figure 3.22:** *Circle tool*

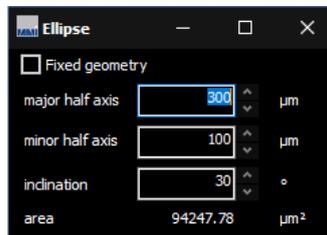
Fixed-size circles can be created by checking “Fixed diameter” and typing the value in the corresponding input box (Fig. 3.22).

#### 3.5.1.4 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)



**Figure 3.23:** *Ellipse tool*

Fixed-size ellipses can be created by checking “Fixed geometry” and typing the values in the corresponding input box (Fig. 3.23)

#### 3.5.1.5 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.

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°.

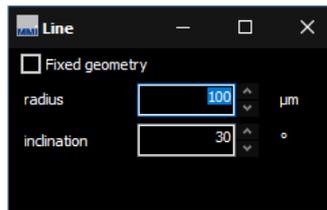


Figure 3.24: Line tool

### 3.5.1.6 Rectangles

The **rectangle** tool (*Alt + Q*) provides a quick method to outline objects of interest.

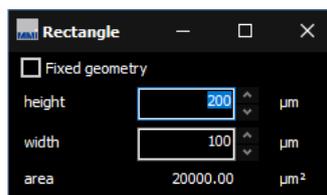


Figure 3.25: Rectangle tool

Fixed-size rectangles can be created by checking “Fixed dimensions” and typing the values in the corresponding input box (Fig. 3.25).

### 3.5.1.7 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. 3.26)
- Using the group editor (section 4.6.4).

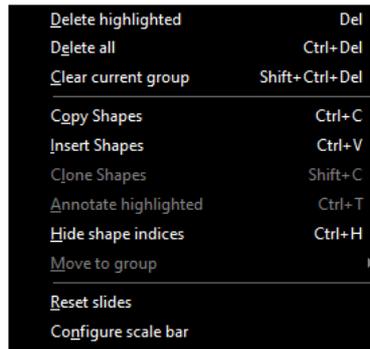


To delete all shapes, or all shapes from the current group, use the context menu (Fig. 3.26) or use the corresponding keyboard shortcuts *Ctrl + Del* and *Ctrl + Shift + Del*.

### 3.5.1.8 Copying and pasting shapes

To copy the active contour use the menu item

### 3. MMI CELLTOOLS – MAIN APPLICATION



**Figure 3.26:** Context menu, open with the right mouse button in the image panel.

*CellCut* → *Shapes* → *Copy*

Select

*CellCut* → *Shapes* → *Insert*

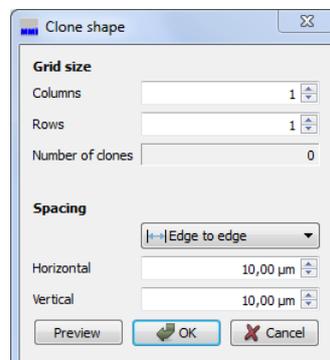
to insert the copied shape.

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

#### 3.5.1.9 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. 3.26).



**Figure 3.27:** Clone shapes

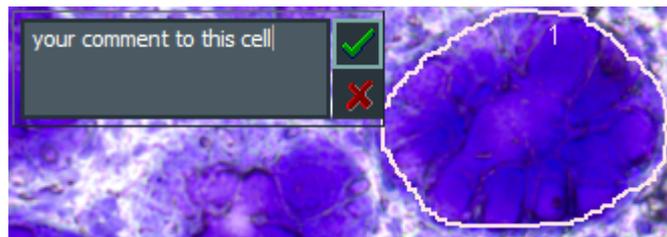
The dialog allows you to specify the number of shapes per row and per column, as well as the distances between rows and columns. Depending on your application, you may choose whether distances are measured between the shapes' edges or from center to center.

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 5.4.2), if enabled. If clones are out of focus when cutting, either redo the sample plane definition or disable plane tilt focussing.

### 3.5.2 Text annotations

Highlighted shapes can be annotated with your comments, by using the *Annotate highlighted* menu in the popup menu Fig. 3.26. A small popup window



**Figure 3.28:** Free text annotation for shapes

Fig. 3.28 will allow to enter the text you want to save with the highlighted shapes.

### 3.5.3 Hide shape indices

The popup menu shown in Fig. 3.26 or using the shortcut *Ctrl + H* also allows to hide the shape indices. This can be helpful if many shapes exists. In this case hiding indices also accelerates the drawing.

### 3.5.4 Move to group

The popup menu shown in Fig. 3.26 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.

### 3.5.5 Reset slides

For convenience the popup menu shown in Fig. 3.26 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

### 3.5.6 Configure scale bar

The popup menu shown in Fig. 3.26 also allows to configure the colors and thicknesses of the scalebar shown in the right lower corner of the image panel.

#### 3.5.6.1 Navigating between shapes

There are several keyboard commands that allow you to locate your shapes and navigate between them. These are illustrated in Fig. 3.29.

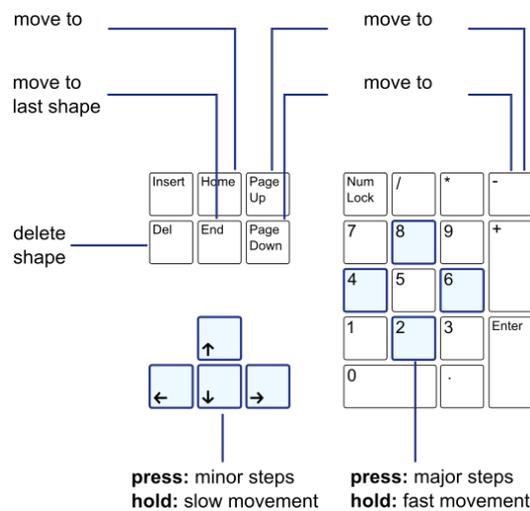


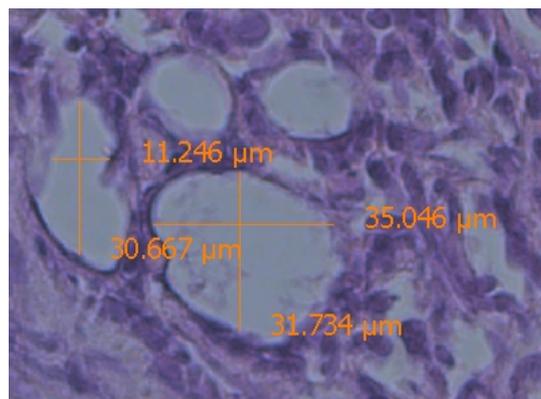
Figure 3.29: Keyboard navigation

- *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

### 3.5.7 Distance measurement

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



**Figure 3.30:** *Distance measurement*

Press the left mouse button and drag the mouse. After releasing the mouse button you can see the measured distance (Fig. 3.30)

### 3.5.8 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



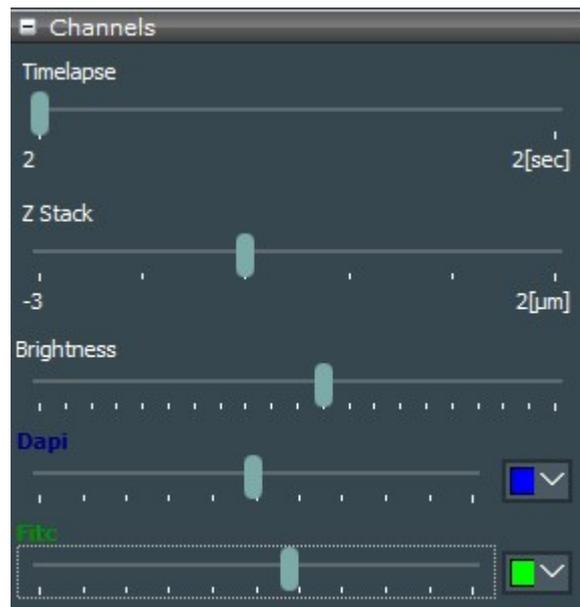
### 3. MMI CELLTOOLS – MAIN APPLICATION

- **Zoom out** button
- **zoom slider** shown in Fig. 3.19 right of the image panel



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

#### 3.5.9 *mmiCellScan* 5D channels



**Figure 3.31:** Control the timelapse, zStack and Multi-channels of your 5D WSI.

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. 3.31. The brightness for merged image or separate image for each channel can be adjusted. The selection of **pseudo color**, please refer to section 3.3.6

#### 3.5.10 Shape import from third party applications

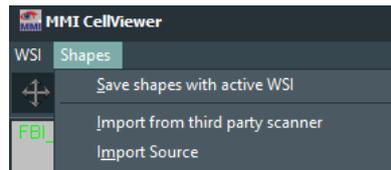
In case you ordered the *Shape Import* function, *mmi CellTools* enables you to load shapes created in following third party applications:

- 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 3.32:** Shape import menu

### 3.5.10.1 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 4.6) 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 white 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.

### 3. MMI CELLTOOLS – MAIN APPLICATION

#### 3.5.10.2 Visiopharm

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

#### 3.5.10.3 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.

#### 3.5.10.4 3DHistech SlideViewer

SlideViewer can export shapes into comma separated 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.

#### 3.5.10.5 Generic csv files

Shapes from generic comma separated 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.

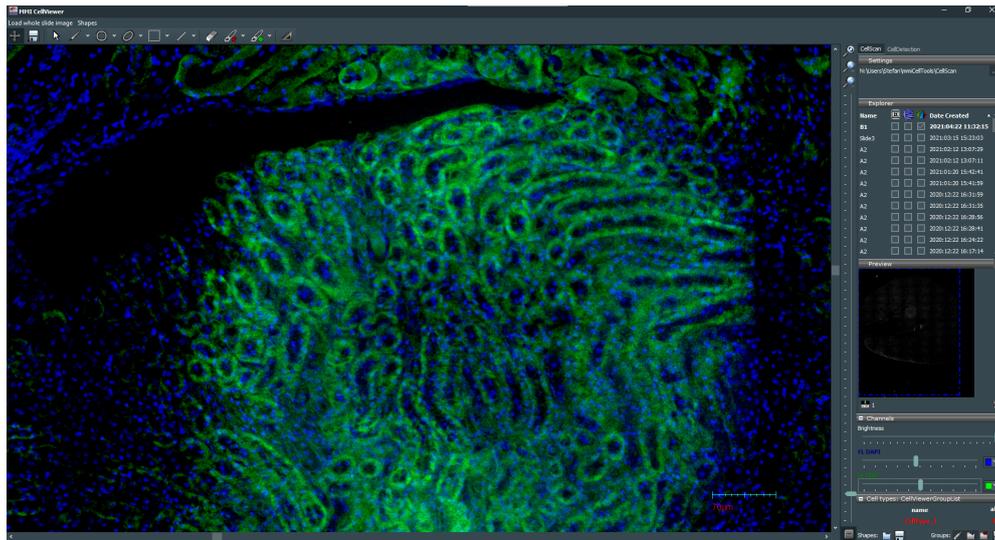
#### 3.5.11 Slide viewer plugins

The Slide viewer is able to host plugins like *mmi CellScan* or *mmi CellDetector* in the right side panel, see Fig. 3.33. This allow you to easily navigate through your whole slide images or use machine learning for image analysis.

### 3.6 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.



**Figure 3.33:** The Slide viewer also allows to host plugins in the right side panel

In order to properly navigate through your samples

- the origin of the stage (see 3.7.1)
- 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 3.4.4 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* → *Export*

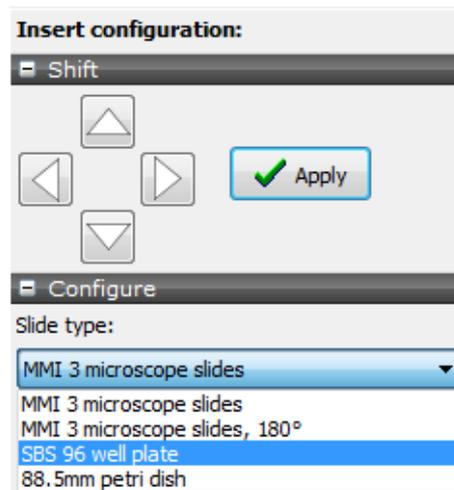
### 3. MMI CELLTOOLS – MAIN APPLICATION

Using

*File → Import*

the configuration can be reloaded into the stage geometry viewer.

#### 3.6.1 Stage inserts

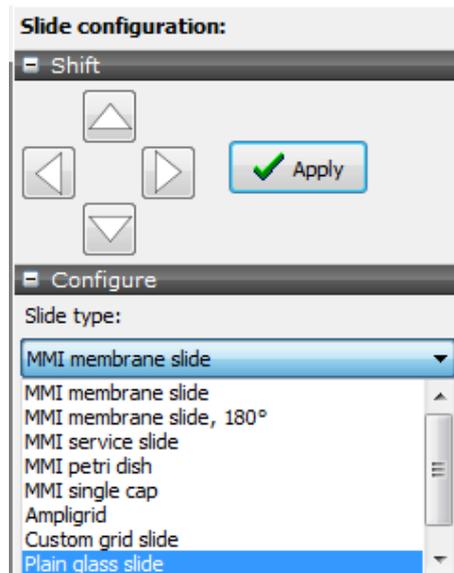


**Figure 3.34:** Slide viewer: Insert configuration panel. You can select different insert types and shift the whole insert to adapt it to overview images.

In the configuration panel (Fig. 3.34) different types of slide inserts can be selected:

- insert for three microscope slides, clamps on the right hand side
- insert for three microscope slides, clamps on the left hand side
- insert for 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 its stage insert position. If the overview image of slide 1 does not exactly fit into the slide shift the insert as described in chapter 3.6.4.



**Figure 3.35:** Slide viewer: Slide configuration panel. You can select different slide types and shift the whole slide to adapt it to its overview image.

### 3.6.2 Slides

Depending on the stage insert selected in section 3.6, the configuration panel for slides (Fig. 3.35) 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 rotated by 180°
- 8 well slide
- 18 well slide
- MMI petri dish holder
- MMI single cap holder
- MMI double cap strip holder
- plain glass slide
- empty

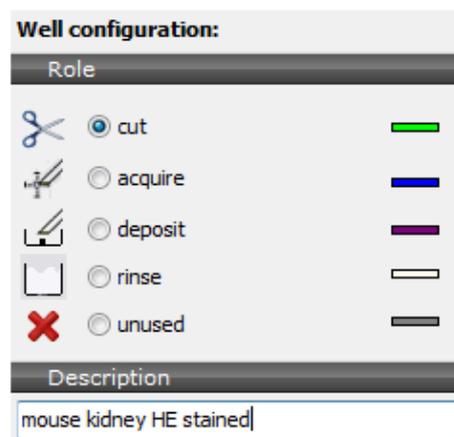
Additionally you can shift the slide. This feature allows you to precisely overlay a scanned overview image with the displayed slide. By the slide shifting procedure you can correct the geometry of your stage insert to precisely reflect the hardware.

### 3. MMI CELLTOOLS – MAIN APPLICATION

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 geometry displayed, first calibrate the stage origin, see section 3.7.1. If this is not sufficient follow the procedure described in section 3.6.4

#### 3.6.3 Active regions, wells



**Figure 3.36:** Slide viewer: Well configuration panel. You can select different well rules and add descriptions to each well.

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

-  • region for microdissection
-  • 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.

#### 3.6.4 Stage inserts first configuration

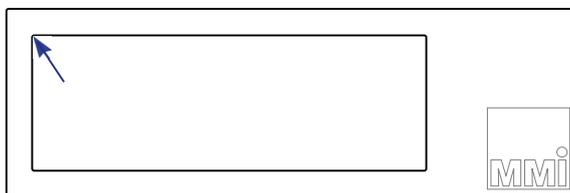
To adapt the insert position to the scanned overview images proceed as follows:

## 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 3.6.2
4. calibrate the stage origion, see section 3.7.1
5. scan an overview image in slide 1, see 3.4.4 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. 3.37) 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 3.6.2

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 configurations indicates a corrupt stage origin calibration. Recalibration of the stage origin, see section 3.6.2, 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 3.37:** Suggested positions on a mmi Membrane slide to precisely adjust the stage insert position.

## 3.7 Calibration

### 3.7.1 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.

### 3. MMI CELLTOOLS – MAIN APPLICATION

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 3.6.2 and 3.6.4.

#### 3.7.2 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.

If automatic alignment was successful, a dialog (see figure 3.38) 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



**Figure 3.38:** Camera alignment results

- The measured inclination of the camera.

Ideally, camera inclination should be very close to zero ( $< 0.3^\circ$ ). If it is larger, eliminate camera tilt before proceeding (see section 3.7.2.1). 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 3.2).

### 3.7.2.1 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  $0.3^\circ$ , 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

### 3. MMI CELLTOOLS – MAIN APPLICATION

4. Fully tighten mounting screw.

#### 3.7.3 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

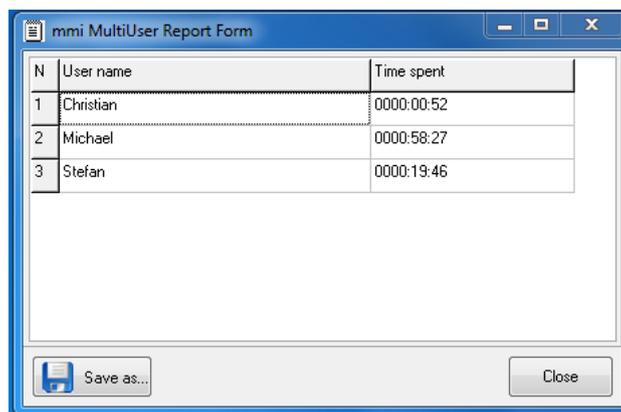
You are now asked whether you would like to calibrate further objectives. If you answer “yes” you are asked to repeat steps 8 and 9 for another objective.

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.

### 3.8 Multi-user report

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



N	User name	Time spent
1	Christian	0000:00:52
2	Michael	0000:58:27
3	Stefan	0000:19:46

**Figure 3.39:** Multi-user report

Select the menu item

*Project* → *Usage report...*

or start it from

*Start* → *All Programs* → *mmi CellTools* → *mmi MultiUser Report*

The time format is hhhh:mm:ss.

## 3.9 Help

### 3.9.1 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.

### 3.9.2 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*

### 3.9.3 Version info

Information about the currently installed software version can be found under

*Help → Version info*

## 4 mmi CellScan

The *mmi CellScan* offers the ability to scan and store microscope slides in full resolution. The *mmi CellScan* is fully compatible with all other mmi products and is designed with optimized speed, precision and data processing to allow fast and reliable scans with huge amount of data.

The CellScan can be accessed by selecting the CellScan panel.

### 4.1 Getting started

#### 4.1.1 Quick Start

##### 4.1.1.1 System startup

To start *mmi CellScan* follow the steps below:

1. Start up the PC and allow the boot process to complete.
2. Turn on the microscope white light power supply.
3. Start *mmi CellTools* software and wait until the software has finished the start up and self-test procedure.



**Figure 4.1:** *The CellTools startup banner*

## 4. MMI CELLSCAN

### 4.1.1.2 Calibration and Configuration

1. Calibrate Stage Origin by selecting menu item

*Setup* → *Calibrate Stage Origin*

or press *Ctrl+O*

2. Configure what type of Stage insert you are using

*Setup* → *Configure Stage Insert*

### 4.1.1.3 Manual Scanning

1. Switch to 4x objective and make an overview scan of your whole slide(s) of interest.
2. Change to the objective you want to scan with
3. Focus on the object or structures you are interested in
4. Set Interpolation points by pressing *F8*. The number of Interpolation points depends on your sample and the area you want to scan but is at least three. They should cover all differences in focus planes.
5. Press *Scan*

### 4.1.1.4 System turn off

Shut down *mmi CellTools* by selecting the menu item

*Project* → *Exit*

or by clicking the main window's close button.

Shut down the computer.

## 4.2 Slide scanning



To enable the creation and storage of full resolution scans check **Create WSI's** in the settings panel (Fig. 4.2). Every time a scan is started now (see section 3.4.4), a full resolution image is automatically created and stored. The recorded scans are saved as *tagged image file format* (TIFF) in the active folder, which is displayed in the lower part of the settings frame. After the scan is finished the image can be inspected using the slide viewer (see section 3.5).

The slide viewer is updated with the most recent image by default. It is displayed at the position of the active slide.



**Figure 4.2:** *The CellScan settings*

If the option **Stitching** is checked CellScan will use an active stitching procedure, so that no seams between the tiles will appear. The scanning speed can be compromised using this option.

The scan of a full microscope slide with a 20x objective can have the size of more than 40 GB. The output size can be decreased by selecting a compression option found in the left part of the settings view. You can choose between:

- **None:** the image is saved as raw data
- **LZW:** the image is compressed with Lempel–Ziv–Welch algorithm
- **PackBits:** the image is compressed with PackBits

Note: A compression unequal to None reduces the scanning speed and its visualization in the slide viewer.

With any whole slide image created an additional XML-file will be save to the same folder. This XML-filse stores the geometrical information of the slide used for the slide scan.

### 4.3 Settings panel

With the **mmi CellScan** you can scan slides in all available imaging modes and objectives. Simply select your desired settings and start the overview scan. Additionally, if your microscope is equipped with fluorescence you can perform scans in fluorescence mode (see section 3.2.1).

The speed of the **mmi CellScan** is mainly dependent following parameters:

- **exposure time** of the camera: the exposure time can be set in the camera settings (see 3.3.2), with an longer exposure time the acquisition of each frame increases, which leads to an elongated scan time

## 4. MMI CELLSCAN

- **acceleration** of the stage: the stage acceleration can be set in stage settings (see 3.4.3), with a faster acceleration the stage movement increases and reduces the total scan time
- activation of the **enhanced image quality** feature
- activation of image compression and compression mode

To ensure that the sample is in focus across the scanned area it is recommended to set the focus map properly (see section 5.4.2). If the focus map is pre-defined, the microscope changes its focus during scanning accordingly.

Note: Before using the CellScan make sure the camera is aligned properly (see section 3.7.2)

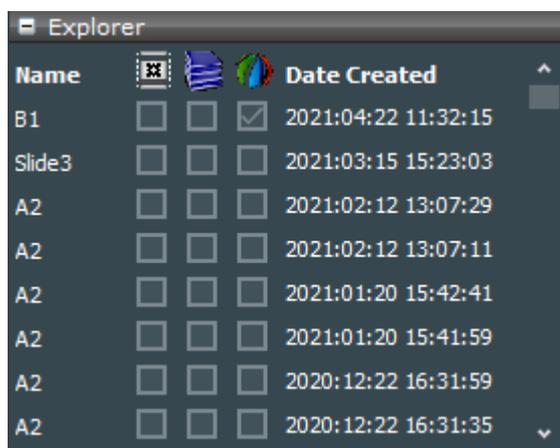


In the *mmi CellScan* settings panel you can set the folder where your whole slide images are saved.



Also you find a **refresh** button to reload the whole slide images in the selected folder.

### 4.4 Explorer panel



Name	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	Date Created
B1	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2021:04:22 11:32:15
Slide3	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2021:03:15 15:23:03
A2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2021:02:12 13:07:29
A2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2021:02:12 13:07:11
A2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2021:01:20 15:42:41
A2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2021:01:20 15:41:59
A2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2020:12:22 16:31:59
A2	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	2020:12:22 16:31:35

**Figure 4.3:** *The CellScan explorer*

In the explorer panel (Fig. 4.3) you can see all recorded scans. Whenever a scan is finished the explorer panel is updated. By pressing on the name of a file in the explorer panel a preview is displayed in the preview panel below the explorer panel (4.5). By holding the mouse over an image name the image details are displayed. You also see three checkboxes for every whole slide image, indicating



- time lapse: several timepoints are recorded

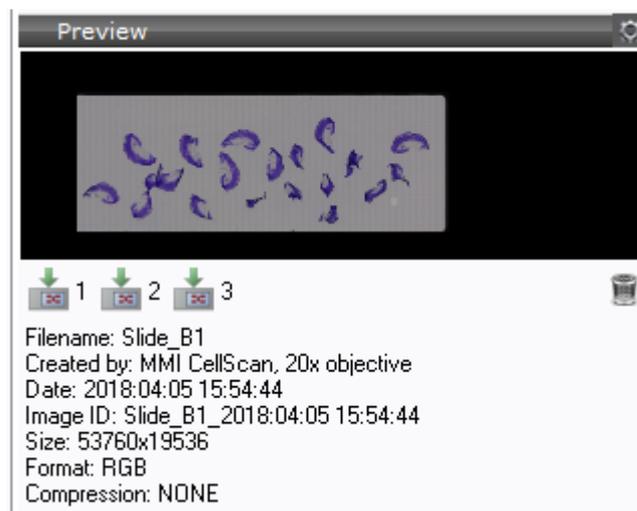


- z Stack: several focus positions are recorded
- multi channel: several imaging modes are recorded



The images can be sorted by **name** or **date** by clicking on the corresponding button.

## 4.5 Preview panel



**Figure 4.4:** *The CellScan preview*

An image that was selected in the explorer panel is displayed in the preview frame Fig. 4.4. The corresponding file info can be shown by selecting **Show Fileinfo** in the settings menu. To load a file in the slide viewer press the **load slide** button with the desired slide number. By pressing the **delete** button the selected slide image can be deleted.



If an appropriate xml-file containing shapes exists, it will be automatically loaded with the whole slide image.

## 4.6 Cell types

Any object drawn will always be assigned to the highlighted cell type group shown in the **Cell types** panel (Fig. 4.5).

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

## 4. MMI CELLSCAN

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 4.5: 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.

### 4.6.1 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. 4.6) using the edit button.

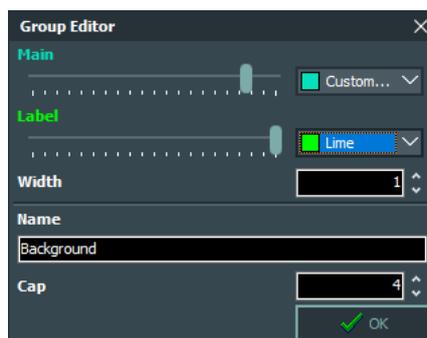


Figure 4.6: Group editor

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

#### 4.6.2 Regrouping shapes

To move individual shapes from one cell type group to another, open the context menu (Fig. 3.26) by right-clicking on video panel and select the target group from sub-menu (*Move into group* → *GroupX*).

#### 4.6.3 Group statistics

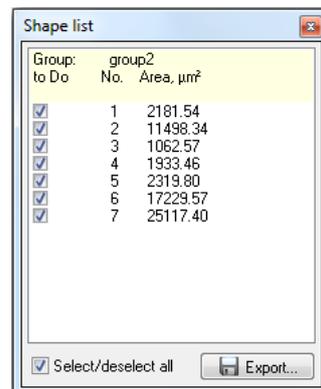
The statistics can be seen directly in the group panel (Fig. 4.5).

For each group, the list displays the number of shapes .

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

#### 4.6.4 Shape list

If you click on the number of shapes in a group, a list with all shapes defined in that group pops up (Fig. 4.7).



**Figure 4.7:** *Shape list*

The list displays the area in  $\mu\text{m}$  of each shape and an editable tick mark for all shapes that will be processed with future actions.

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

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

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

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

## 4. MMI CELLSCAN

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

### 4.7 5D-Scanning

CellScan offers you a variety of scanning options to get the best result for your Slide-Scanning. Besides the option for automatic sample detection and auto-focus mapping, you can scan your sample in 5 dimensions: XY-Scan of whole slides, time-stacks, z-stacks and Multi-Fluorescence.

#### 4.7.1 Automatic Sample Detection



When checking this option, CellScan will automatically scan for samples on your slide and create an area of interest around them. This is particularly useful if you want to reduce scanning time by not scanning the whole dish but only the relevant parts that contain samples.

An automatic sample detection scan will use the selected **preview** channel and the selected **objective** channel, see chapter 3.2.1 respectively 3.2.2. If the required microscope parts are not motorized, **CellTools** will show a message what you have to change. After the automatic sample detection **CellTools** will switch back to the previous channel and objective.

A separate sample detection scan can be triggered by a click on the **sample detection** button.

Automatic sample detection starts with a camera autoexposure on the start position. Make sure the slide positioning is well configured to not start on an intransparent slide position resulting in a very slow scan speed, see chapter 3.6.2.

#### 4.7.2 Auto-focus Mapping



This option will automatically create a focus map (see 5.4.2) of your slides. 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.

A separate generation of the focus map of the active slide can be triggered by a click on the **create focus maps** button.

### 4.7.3 Multi-Slide Scanning

Normally CellScan will only scan the active Slide Position. With this option checked it will scan all the selected slides at once.



### 4.7.4 Time-Stack

With the Time-Stack option you can perform multiple scans over a defined time. With the drop-down menu next to the icon you can set the two main parameters:



- Total Time: The overall time the scanning will take in minutes.
- Time Increment: The time between individual scans in minutes.

### 4.7.5 Z-Stack

The Z-Stack option will let you scan your samples in different focus planes. With the drop-down menu next to the icon you can define the three main parameters:



- z Min: The minimal position below the current focus position where the Z-Stack will start.
- z Step: The step-size between the single pictures.
- z Max: The maximal position above the current focus position where your Z-Stack ends.

The CellScan will start at the z Min position and work upwards to the z Max position.

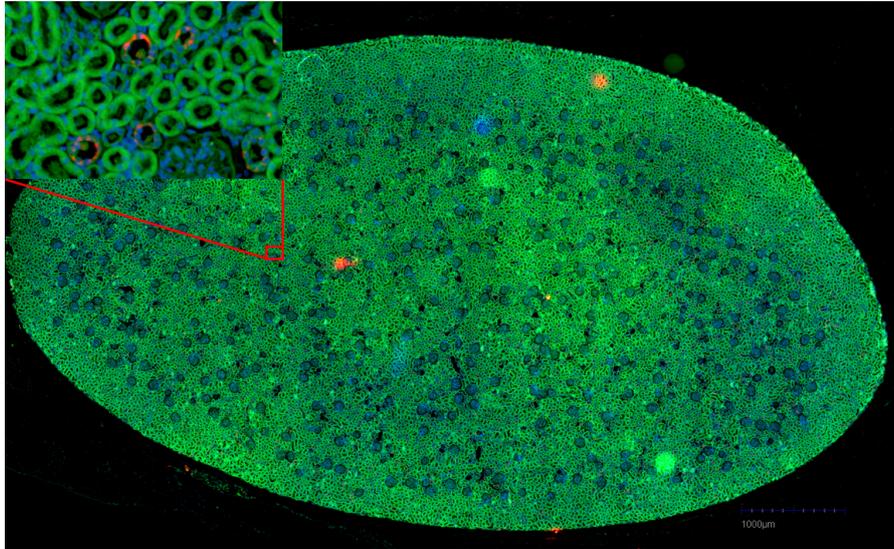
### 4.7.6 Multi-Channels

With this option you can make multicolor image scans. The multi channels and its pseudocolors can be selected in the Channel editor (see section 3.2.1). If the option **Stitching** in Fig. 4.2 is checked, only one channel can be selected as **stitch** channel in the channel editor. This **stitch** channel will be used for stitching procedure, other selected multi channels will copy the stitching information, so that all separate images have identical stitching result Fig. 4.8. You should select the channel, which has best image signal for stitching, e.g. if the sample has too low fluorescence signal in fluorescence channel, you can stitch the multi-channel image as well by selecting the bright field channel as



#### 4. MMI CELLSCAN

stitch channel. If the scan is finished the merged multi-channel image can be displayed using the slide viewer (see section 3.5).



**Figure 4.8:** *multi-channel image in full resolution with stitch*

## 5 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.

### 5.1 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.

## 5. AUTOMATED MICROSCOPE CONTROL

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

### 5.2 Observation methods

The Channel editor is used to define the observation methods, see section 3.2.1.

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 5.1).

**Table 5.1:** *Optical path settings for Olympus and Nikon microscopes*

	Olympus IX-83	Olympus IX-81	Nikon T1	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*.

### 5.3 Objective control

In the objective editor, see section 3.2.2, 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 3.3.2).

## 5.4 Z drive control

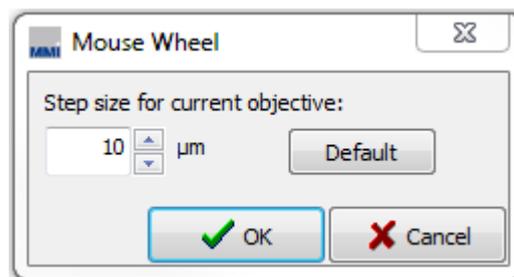
CellTools features built-in focusing aids and functions that rely on motorized z drive control. These are:

- 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. 5.1).



**Figure 5.1:** *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.

## 5. AUTOMATED MICROSCOPE CONTROL

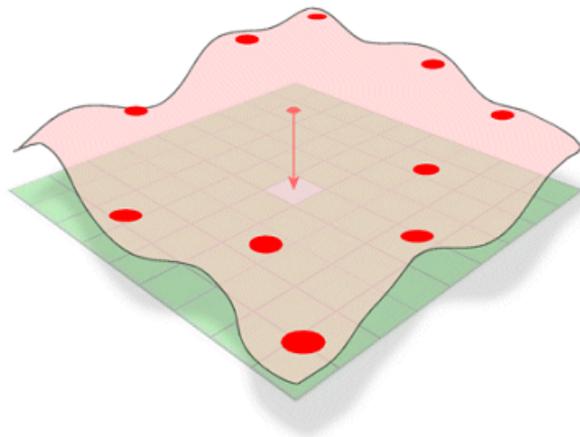
### 5.4.1 Required calibrations

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

### 5.4.2 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.



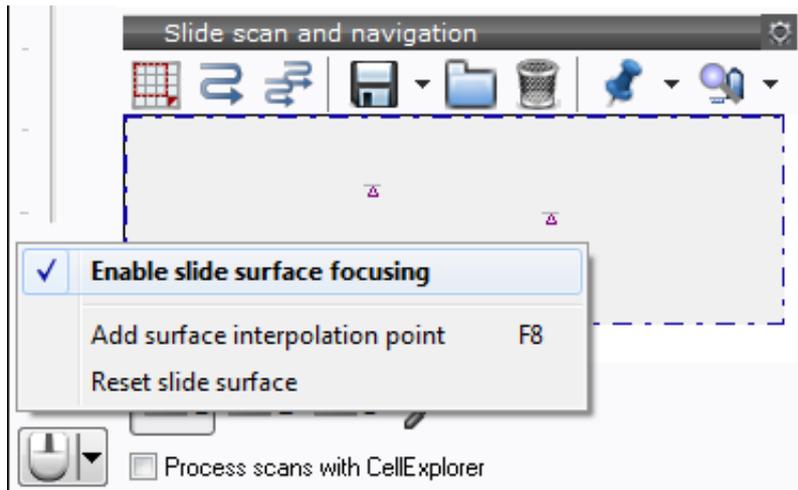
**Figure 5.2:** *Sample surface with defined surface points*

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 5.3:** *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.

### 5.4.3 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*

## 5. AUTOMATED MICROSCOPE CONTROL

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

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

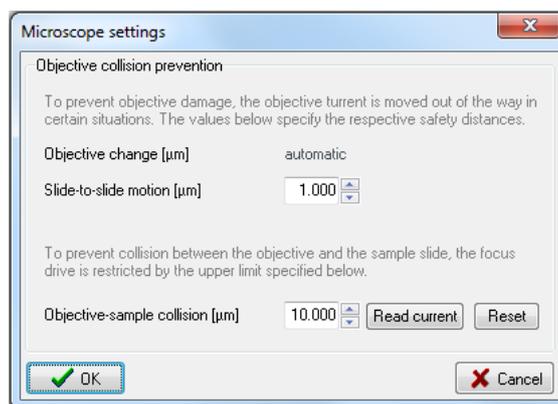
### 5.4.4 Automatic Z drive control

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.

## 5.5 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 5.4:** *Microscope safety parameters*

Open the dialog using the menu item

*Microscope* → *Safety parameters...*

### 5.5.1 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.

### 5.5.2 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  $\mu\text{m}$  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.

## 5. AUTOMATED MICROSCOPE CONTROL

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

### 5.6 Fluorescence shutter control

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

*Microscope → Shutter*

or press *F6*.

# 6 Maintenance

## 6.1 System check

- Verify the correct operation of the LEDs by simulating their functions.

## 6.2 Cleaning

### Caution

- Before cleaning the system, disconnect all system components from the mains.
- 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.

### 6.2.1 Microscope

For further details, see microscope manual.

### 6.2.2 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%

### 6.2.3 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

## 6. MAINTENANCE

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

## 6.3 Trouble shooting

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

### 6.3.1 View

No image on the monitor

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

The image is not clear, too dark, too bright

- check the camera setup
- Check that there is sufficient illumination from the white light and that the light path is not obstructed
- Check that the correction ring of the objective is on the value 1 (corresponding to glass thickness)

- Use the diffuser for improved image quality
- Calibrate the camera in the z-axis

The colors are wrong

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

### 6.3.2 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 3.7.2)
- The camera is not mounted in an exactly upright position

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

- Wrong objective selected
- Camera alignment not correct (see section 3.7.2)
- The camera is not mounted in an exactly upright position

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

- Parfocal lens offset not correct.

Displacement of markers after objective is changed:

- Paraxial lens offset not correct.

Overview images not matching stage geometry.

- Stage geometry not properly defined, see section 3.6
- Stage origin calibration invalid, see section 3.7.1

## 6. MAINTENANCE

### 6.3.3 Overview

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

- Camera alignment not correct (see section 3.7.2)
- The camera is not mounted in an exactly upright position

# A List of Keyboard Shortcuts

## General

<b>+</b>	Move to next shape . . . . .	22, 30
<b>-</b>	Move to previous shape . . . . .	22, 31
<b>Alt + C</b>	circle tool . . . . .	26
<b>Alt + Del</b>	delete shape tool . . . . .	27
<b>Alt + E</b>	ellipse tool . . . . .	26
<b>Alt + F</b>	freehand tool . . . . .	25
<b>Alt + L</b>	line tool . . . . .	26
<b>Alt + M</b>	Measure distance . . . . .	31
<b>Alt + Q</b>	rectangle tool . . . . .	27
<b>Alt + R</b>	select and move tool . . . . .	25
<b>Arrow down</b>	Move small step down . . . . .	31
<b>Arrow left</b>	Move small step left . . . . .	31
<b>Arrow right</b>	Move small step right . . . . .	31
<b>Arrow up</b>	Move small step up . . . . .	31
<b>Caps Lock</b>	Suppress continuous stage movement . . . . .	18
<b>Ctrl + A</b>	Align Camera . . . . .	40
<b>Ctrl + C</b>	Copy shape . . . . .	28
<b>Ctrl + Del</b>	Delete all shapes . . . . .	27
<b>Ctrl + H</b>	Hide shape indices . . . . .	29
<b>Ctrl + I</b>	Show stage insert . . . . .	23
<b>Ctrl + O</b>	Set stage origin . . . . .	40, 42
<b>Ctrl + P</b>	Stage movement settings . . . . .	18

## List of Keyboard Shortcuts

<b>Ctrl + Shift + Del</b>	Delete all shapes from current group . . . . .	27
<b>Ctrl + V</b>	Paste shape . . . . .	28
<b>Del</b>	Delete shape . . . . .	27
<b>End</b>	Move to last shape . . . . .	22, 30
<b>Esc</b>	Interrupt current process . . . . .	20, 43
<b>F1</b>	Display user manual . . . . .	44
<b>Home</b>	Move to first shape . . . . .	22, 30
<b>Numpad: Down</b>	Move large step down . . . . .	31
<b>Numpad: Left</b>	Move large step left . . . . .	31
<b>Numpad: Right</b>	Move large step right . . . . .	31
<b>Numpad: Up</b>	Move large step up . . . . .	31
<b>Space</b>	Toggle between moving and drawing mode . . .	17, 24

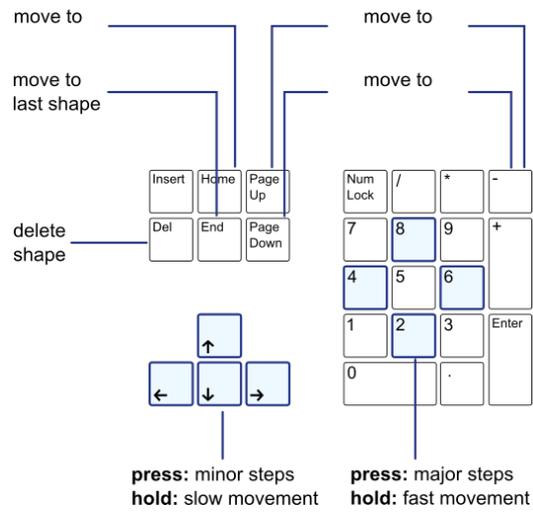
### Camera control

<b>Ctrl + Alt + C</b>	Copy image . . . . .	14
<b>Ctrl + R</b>	Open camera settings . . . . .	11
<b>Ctrl + S</b>	Save an image . . . . .	13
<b>Shift + M</b>	multi channel image . . . . .	16

### Microscope control

<b>Ctrl + F</b>	autofocus . . . . .	17
<b>CTRL + M</b>	Remote control On/Off . . . . .	55
<b>F6</b>	Open/close fluorescence shutter . . . . .	62
<b>F7</b>	Switch between camera and binocular . . . . .	56
<b>F8</b>	add surface point . . . . .	58

## Navigation





## 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 × 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
- Nikon TS2R

#### B.2.2 Stage

##### Standard stage

Scanning stage with stepper motors.

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

### Long travel scanning stage for two inserts

Long travel scanning stage with stepper motors.

<b>Scanning area:</b>	280 × 82 mm <sup>2</sup>
<b>Repositioning accuracy:</b>	< 1 μm
<b>Step resolution:</b>	0.075 μm
<b>Speed:</b>	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 μm x 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 μm x 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™: Absolute EMCCD gain selectable directly from a linear and quantitative scale.
- TE Cooling to  $-100^{\circ}\text{C}$ : 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 Power 500mW per color band  $\pm 10$

## **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.

## C Service

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

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

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

### **Head office Germany:**

✉ Molecular Machines & Industries GmbH (MMI)  
Breslauer Strasse 2  
85386 Eching  
Germany  
☎ +49 – (0)89 319 048 40  
☎ **FAX** +49 – (0)89 319 048 59

### **Switzerland:**

✉ MMI Molecular Machines & Industries AG  
Flughofstrasse 37  
8152 Glattbrugg  
Switzerland  
☎ +41 – 44 522 19 36

### **North America:**

✉ MMI Molecular Machines & Industries Inc.  
P.O. Box 348  
Haslett, MI 48840  
USA  
☎ +1 – 331 307 0273  
☎ **FAX** +1 – (321) 978 0304

## APPENDIX C. SERVICE

 [www.molecular-machines.com](http://www.molecular-machines.com)

 [service@molecular-machines.com](mailto:service@molecular-machines.com)