mmi CellEctor

User Manual

Molecular Machines & Industries GmbH
www.molecular-machines.com

User Manual: MMI CellEctor
Version 6.0
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1 Security advice

1.1 General safety

- Do NOT disassemble the system. The installation of the system is done by MMI service personnel or a designated MMI representative. Repairs, removal or exchange of components except for the operations described in this manual must be carried out solely by the MMI service personnel or persons expressly authorized by MMI. If you have any problems with the instrument, contact MMI.

- The responsibility for the correct functioning of the mmi CellEctor passes onto the owner or user if the system is maintained or serviced improperly by persons not belonging to the MMI service team or if used for a purpose other than that intended. MMI is not liable for damage occurring from non-observance of these notes.

- The power supply is installed by MMI. MMI ensures that the system is provided with the appropriate voltage. Do not change the power cords.

- Avoid wet or dusty conditions near the system. Do not attempt to use the system if liquids get inside it. Contact MMI in such a case.

- Unplug all the electrical supply before cleaning the system. Do not use cleaning fluids or sprays; use solely a smooth and dry cloth.

- If the stage is not calibrated, its movements can be sudden and fast. Ensure that the work area around the stage is free of clutter and material.

- Read the manual of your microscope for microscope specific precautions. If you do not have the manual, contact your microscope provider or MMI.

1.2 *mmi CellEctor* safety

- *mmi CellEctor* uses very thin glass capillaries that may break into splinters and injure eyes or other parts of the human body. Thus, it is compulsory for the user to wear protective glasses, gloves and clothes while working with the *mmi CellEctor*.

- Never point capillaries at persons. Capillaries can suddenly be loosened from their grips under high pressure and become missiles.

- The *mmi CellEctor* is to be used solely for collecting cells in accordance with the appropriate legislative requirements.

- Please ensure that all work is carried out in accordance with this manual.

2 Installation

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

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

service@molecular-machines.com

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

2. INSTALLATION

3 The mmi CellTools instrumentation family

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

- *mmi CellCut*: laser microdissection to isolate single cells or areas of tissue

- *mmi CellManipulator*: optical tweezers to manipulate cells or beads with an optical trap

- *mmi CellEctor*: automated micro-pipetting to mechanically manipulate cells or beads with a capillary and mechanical micromanipulator

- *mmi CellScan*: whole slide imaging scanner to create and store full resolution whole slides images (WSI)

- *mmi CellViewer*: *mmi CellViewer* is a stand alone software package to view and annotate whole slide images created with *mmi CellScan*.

- *mmi CellDetector*: machine learning software for biological image analysis. *mmi CellDetector* is available in two flavours

  - detect objects on whole slide images (WSI)
  - detect objects on the live image

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

3. THE MMI CELLTOOLS INSTRUMENTATION FAMILY

4 Getting started

4.1 System start-up

To start the mmi CellTools® with mmi CellEctor® follow the steps below:

- Start up the PC and allow the boot process to complete.

- Turn on the microscope control box.

- Start *mmi CellTool* software and wait until the software has finished the start up and self-test procedure. For the system to work properly, all the hardware components have to pass successfully.

- Remote control of the microscope is recommended for the *mmi CellEctor* to achieve the highest precision in the capillary vertical positioning with respect to the slide surface.

4.2 System preparation

Procedure

1. Ensure that the following microscope settings are correct. On new systems this configuration was already executed by the service technician:

   - camera orientation
   - objectives calibration
   - illumination settings for each objective
   - camera settings for each objective

2. Prepare the mmi CellCellector® as described in sections 6.15 and 6.14

4.3   System turn off

Procedure

1. Shut down *CellTools* by using the

   Project →Exit

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

2. Switch the microscope off.

3. Shut down the PC by using the standard Windows® command.

4. Let the microscope illumination cool down and protect the system with
   the microscope cover.

5 mmi CellTools – Main application

5.1 Main window and plug-ins

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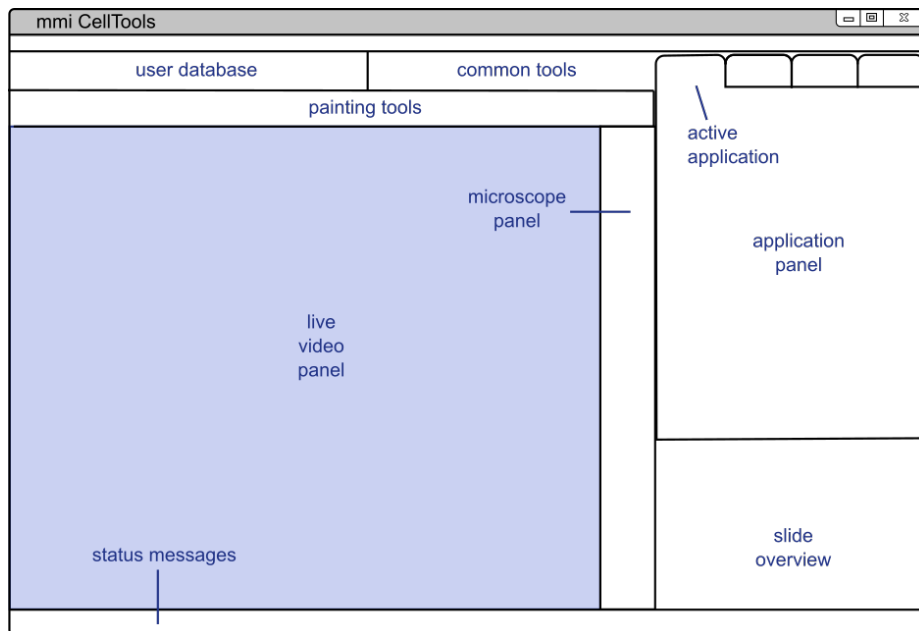
Figure 5.1: *Structure of the mmi CellTools user interface*

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

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

Those user interface controls that are common to all applications are located

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

On systems equipped with an automated microscope, the microscope panel provides access to z-drive (focus) control and other microscope-specific features (see chapter 9). The **slide overview** panel provides controls for slide scanning and navigation (see section 5.4.4). Finally, the **status messages** bar at the bottom displays current stage coordinates and camera frame rate.

CellTools offers three user interface styles.

- gray

- dark

- classic

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

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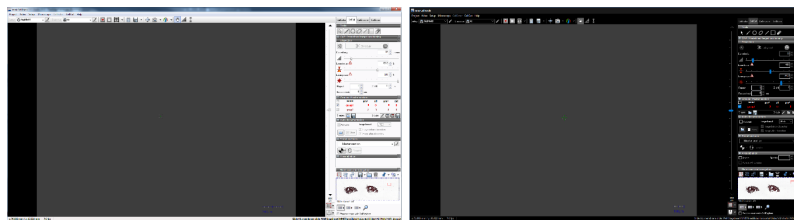
Figure 5.2: *Classic (left) and dark (right) style*

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

Setup → Left-handed UI

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

5.2 User-specific database

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

The database represents a hierarchical structure:

User $\implies$ Channel 1 $\implies$ Objective 1
$\implies$ Objective 2
$\vdots$

$\implies$ Channel 2 $\implies$ Objective 1
$\implies$ Objective 2

$\vdots$ $\quad\qquad$ $\vdots$
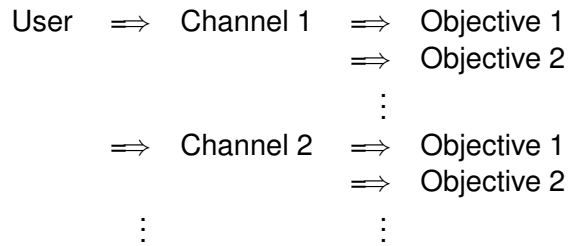
Basically the user can save all optical parameters separately.

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

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

5.2.1 Channel editor

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

The channel selection box contains the defined parameters for each imaging channel. You can add or remove a channel and all corresponding parameters can be edited.
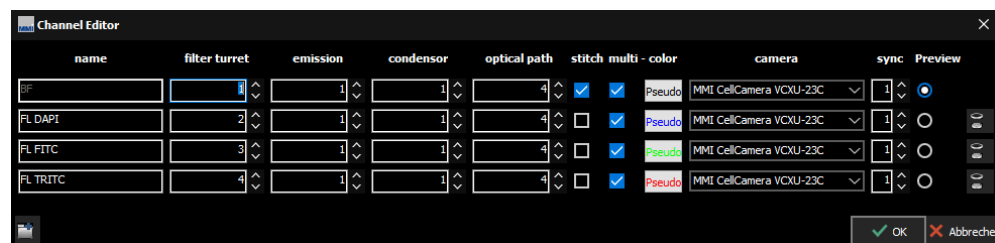
Figure 5.3: *channel editor*

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

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

5.2.1.1 Channel options

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

parameters is explained in sections 9.2 and 5.3.6.

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

- if a supported LED light source with selectable wavelenghts is mounted the channel can be selected by the drop down list **FL Channel**.

- if your system is equipped with more than one camera, different channels can be associated with a certain camera using the drop down list **Camera**. You can then switch to a specific camera by selecting a channel defined for that camera. The camera used should also be reflected in the channel name.

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

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

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

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

5.2.2 Objective editor

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

The objective selection box contains all objectives defined for the active channel. You can add and remove an objective and edit all corresponding parameters. If you create a new channel, all objectives will automatically be copied.
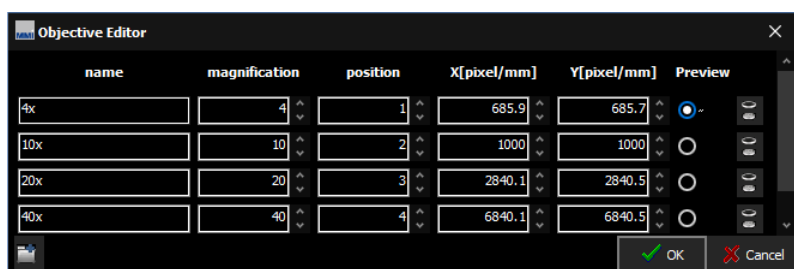
Figure 5.4: *Objective editor*

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

The nosepiece **position** and **lamp voltage** settings are only used with motorized microscopes.

If specified, nominal **magnification** will be used to compute various objective-dependent properties.

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

Setup→Align camera with stage

(see section 5.7.2).

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

- Camera settings (see section 5.3.2)

- Camera alignment (see section 5.7.2)

- Lens offset (see section 5.7.3)

- Z-Focus lens offset (for automated microscopes only, see section 9.4.3)

- 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.

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

5.2.3 Default database reconstruction

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

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

If for some reason, a user's database becomes unusable, the user can recover the default database as follows:

Setup →Restore factory settings

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

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

5.3 Camera operation

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

- The mmi CellCamera range of digital cameras for general microscopy applications
  - DXA285cf
  - MXF285cf
  - VCXU23c
  - VCXU50m
- Hamatsu
  - Fusion
  - Fusion BT
  - Orca Flash IV
- Andor iXon

5.3.1 Multiple cameras

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

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

Do not use the Channel editor to switch between cameras. The Channel editor is only used once to create (at least) one channel

for each camera. Then, change cameras by selecting the corresponding channel. Use different synchroisation groups for different camera channels, so that camera alignments (section 5.7.2), parfocal correction (section 9.4.3) and paraxial corrections (section 5.7.3) only have to be calibrated once for every objective.

5.3.2 Camera settings

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

Figure 5.5: *Camera settings for mmi CellCamera models*

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

The hardware camera amplifier **Gain** can be adjusted to receive brighter images. With high gain the images become noisier. For elder CCD cameras (DXA285cf, MXF285cf) this effect is much more intense than with newer CMOS cameras (VCXU23c, VCXU50m). Higher gain settings will, however, reduce the required exposure time. For high quality images (e.g. for publications) it is preferable to use a longer exposure time and low gain. Note that the allowed gain range is greater when automatic exposure is off.

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

High gamma values reduce noise and improve the black level of the image. The **black level** can be uses to suppress noise, the **white level** to supresss overilluminations.

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

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

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

- remove the sample from the stage

- select an average image brightness

- select the menu

    Setup →Shading correction

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

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

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

    Setup →Save white balance per objective

to enable or disable this function.

Additional camera related option are available in the drop down menu next to the camera button.
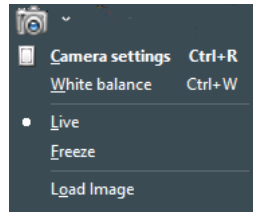
Figure 5.6: *drop down menu to reach camera related options*

5.3.3 Freeze video / live video

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

To freeze the video use the menu item

Freeze

of the drop down next to the camera button.

To go back to live video presentation select

Live

5.3.4 Saving images

To save an image simply press the **Save image** button or press *Ctrl + S*.

The file dialog allows you to specify the image filename and image type (JPG, BMP, PNG and TIF). The image will be saved with maximum pixel resolution. By selecting the **Include drawings** from the drop down next to the **Save image** button, you can save an image including all markers and drawings.
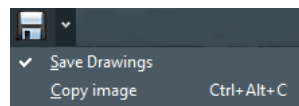
Figure 5.7: *drop down menu for same image options*

Press the **Copy image to clipboard** menu from the drop down next to the **Save image** button or press *Ctrl + Alt + C* to make the current image available to other applications. The image will be copied to the Windows clipboard in order to paste it into e.g. office and image processing applications.

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5.3.5 Recording movies

mmi CellTools allows you to record live camera images into video files (AVI format). It supports compressed and lossless video codecs and allows you to record time-lapse movies.
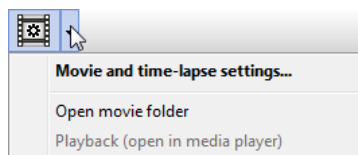
Figure 5.8: *Movie recording functions in the tool bar*

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

By default, the movie folder is located in

```
My Documents\mmiCellTools\Movies.
```

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

Also note that any shapes drawn will not be recorded.

5.3.5.1 Movie settings

Detailed aspects of movie recording can be controlled by opening the **Movie settings** dialog (Fig. 5.9).

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

- Motion JPG

- FFMPEG

- XVID
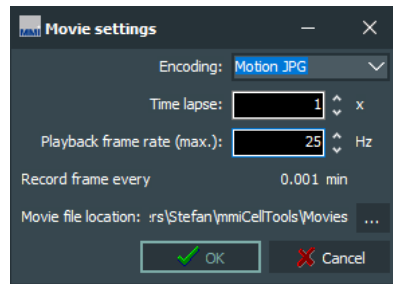
Figure 5.9: *Movie settings*

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

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

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

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

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

5.3.6 Multichannel imaging
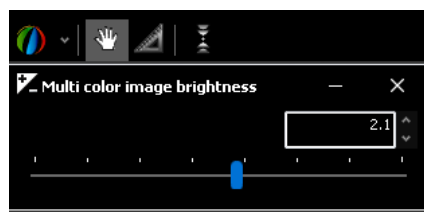
Figure 5.10: *Brightness correction slider for multi channel images*

A **multi channel** image is recorded by pressing the multi channel image button
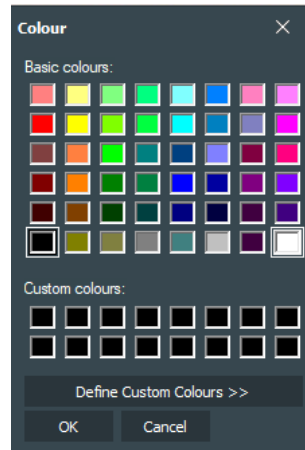
Figure 5.11: *Pseudo color for multi channel images*

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

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

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

- If **black** is slected, the multi-channel image will exclude the image from the selected channel (see Fig. 5.12 right).
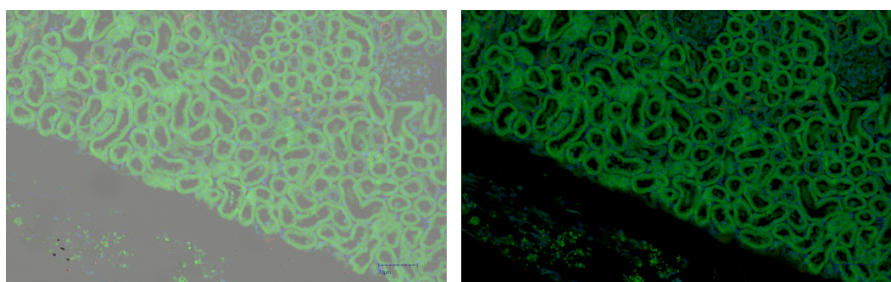
Figure 5.12: *Select white (left) and black (right) as pseudo color for brightfield image*

5.3.7 Autofocus

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

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

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

5.4 Motorized stage control

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

5.4.1 Mouse movement

Choose the move mode by clicking the hand tool button. Additionally, you can quickly switch to moving mode and back again by pressing the *Space* bar.

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

> If stage movement does not exactly follow mouse movement, you may need to carry out the camera alignment procedure (see section 5.7.2).

5.4.2 Keyboard movement

The main arrow keys and numeric pad arrow keys can be used to move the stage in discrete steps or continuously at constant velocity (Fig. 5.13).

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

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

For moving larger distances, hold down the respective key. The velocities for the two sets of movement keys can be set independently (see section 5.4.3). By default, the cursor keys move slowly and the numeric pad keys move fast.
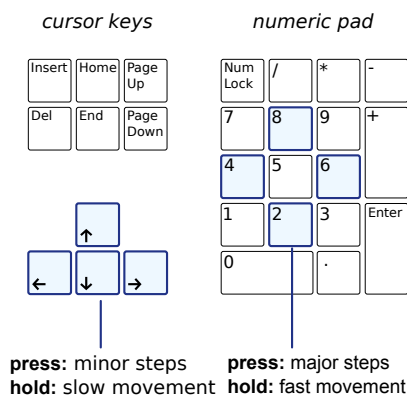
Figure 5.13: *Stage movement using the keyboard*

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

5.4.3 Stage movement settings

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

> *Stage →Movement settings*

or press *Ctrl + P* to get the window in Fig. 5.14.
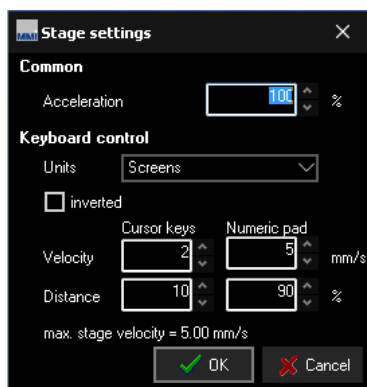
Figure 5.14: *Stage movement settings*

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

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

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

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

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

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

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

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

5.4.4 Overview scan

Fig. 5.15 shows an overview of your sample (the "roadmap image").
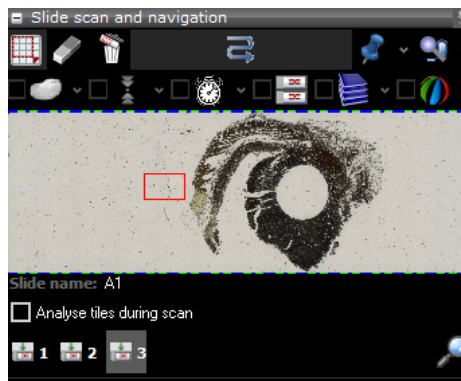
Figure 5.15: *Slide overview*

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

- the inner part of a *mmi MembraneSlide*
- the square around a petri dish
- the square around the cap of a single cap holder
- the square around the well of a custom grid, if only one well is defined.
- the complete slide for all other microscope slides
- the complete multiwell plate

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

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

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

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

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

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

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

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

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

- Show drawings: show or hide the shapes by drawing tools (drawing tools is described in section 5.5.1).

- Show slide name: show or hide the slide name

- Save scan tiles: all tile images during overview scan can be saved in hard disk
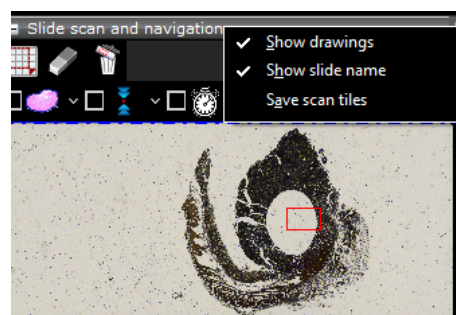
Figure 5.16: *Overview scan configuration*

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

5.4.4.1 Preview image

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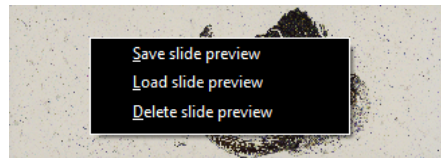
Figure 5.17: *Menu for preview image options*

5.4.4.2 Pin positions

With the **pin** button you can save the current stage position. By clicking on the arrow to the right you obtain the pin positions menu (Fig. 5.18).
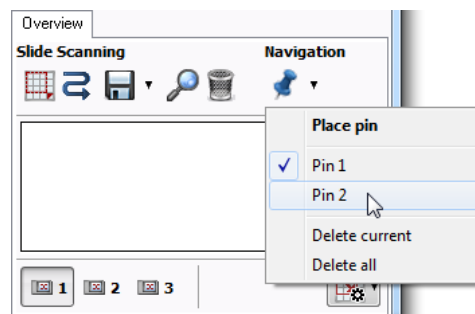
Figure 5.18: *Pin positions*

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

5.4.4.3 Analyse tiles during scan

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

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

5.4.4.4 Shape navigation

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

Once you created shapes you navigate between the shape by using

- move to first shape, *Home* key

- move to last shape, *End* key

- move to next shape, *+* key

- move to previous shape, *-* key

The keyboard keys allways navigate inside the current group, see section 8.2. The shapes can be directly used for further collecting. If we want to collect swimming objects the shape positions will not be very precise are shoud not directly used for cell collection. The intention is, to fast navigate between the found cell candidates by using *Home*, *End*, *+* and *-* keys. Cell collection will be initialised by clicking on the cell of interest, see section 6.10 and 6.11.

5.4.4.5 Drawing tools and measurements

Drawing tools and distance measurements are described in section 5.5.1.

5.5 Slide viewer

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

The slide viewer (Fig. 5.19) can be opened via the menu

> *Setup →Stage insert   (Ctrl + I)*

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

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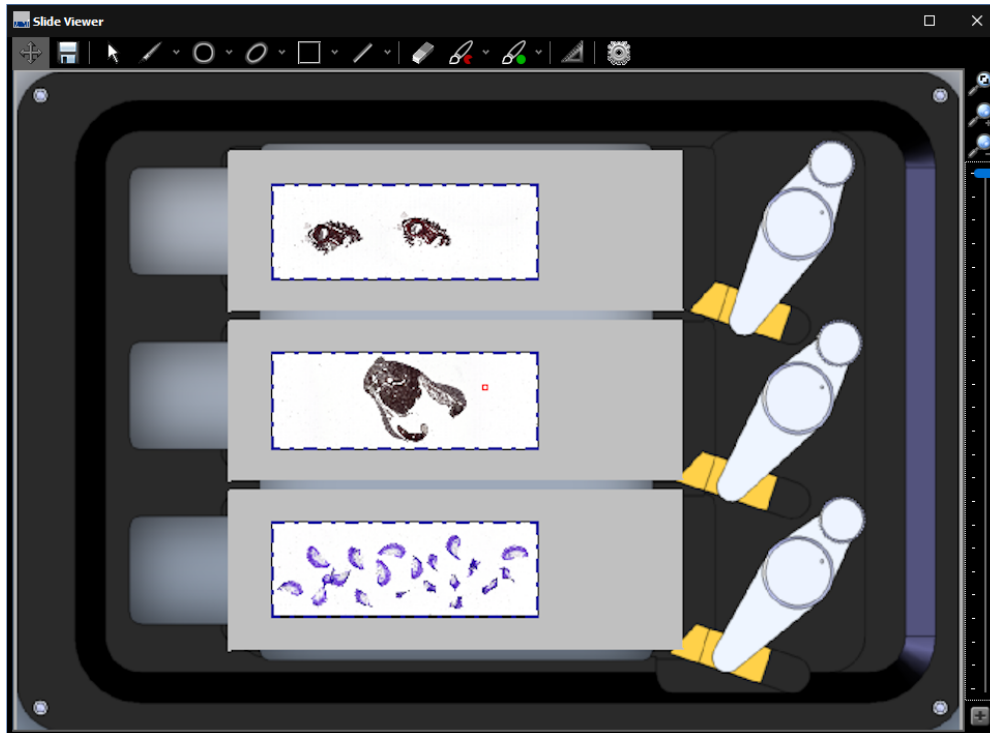
Figure 5.19: *The Slide viewer can be used to display full slide images, draw contours and to configure the stage insert*

- display and zoom whole slide images (WSI)

- draw, display and review shapes around objects of interest

- create and manage groups of objects

- navigate through your samples, by double-clicking on the target position

- configure the geometry of the

  – stage insert and

  – slides or microplates, each containing the active regions called wells.

- assign a role to each well

- exporting and importing stage geometries

5.5.1 Drawing tools

The drawing tools can be selected through the buttons above the main window. Additionally, a quick switch between moving and drawing mode is possible by pressing the *Space* key on the keyboard. As drawing tools you find from left to right
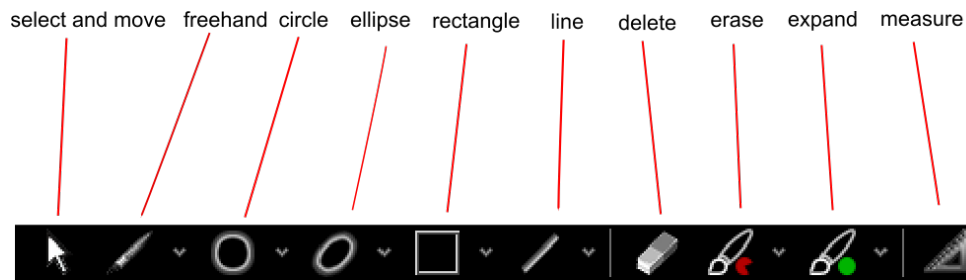
Figure 5.20: *Drawing tools: select, freehand, circle, ellipse, rectangle, line, delete, erase, expand and measure*

- select and move a shape: click on a shape and drag the shape over the field of view. The shape will be highlighted

- freehand: use the mouse as a pencil

- circle: mark a first point of the circle and define a second point by dragging the mouse. Fix diameters can be set via the drop down menu next to the **circle button**

- ellipse: draw the main axis first and than define the minor axis by dragging the mouse. Fix parameters can be set via the drop down menu next to the **ellipse button**

- rectangle: mark the left upper corner and than define the diameter by dragging the mouse. Fix parameters can be set via the drop down menu next to the **rectangle button**

- line: mark the left upper corner and than define the length by dragging the mouse. Fix lengths can be set via the drop down menu next to the **rectangle button**

- delete: delete a shape by clicking on the shape

- erase: remove small pieces of the shape by using this tool as eraser. The erasing size can be set via the drop down menu next to the **erase button**

- expand: add small pieces to the shape by using this tool as brush. The erasing size can be set via the drop down menu next to the **expand button**

Shapes are orginised in groups (see section 8.2).

5.5.1.1 Select and move shapes

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

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

5.5.1.2 Freehand

The **freehand** drawing tool (*Alt + F*) allows you to define arbitrary shapes. Use the left mouse button to draw the contour around the area of interest.
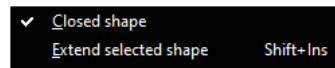
Figure 5.21: *Freehand drawing options*

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

Some objects, especially those larger than the field of view, cannot be traced with a single drawing operation. For such cases multiple segments can be combined into a single shape. Hold the keyboard **shift** key when you want to extend the shape, release the **shift** key to start a new freehand shape.

Procedure

1. Start outlining the object normally using the freehand tool as far as the field of view allows.

2. Move the stage such that the end of the drawing is still visible and you can continue drawing.

3. While holding down the *Shift* key, draw the second segment. (You may release the key while drawing.) Once you have finished drawing, the two segments will be attached.

4. Repeat steps 2–3 as necessary.

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

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

5.5.1.3 Circles

The **circle** tool (*Alt + C*) is suitable for creating circular shapes.
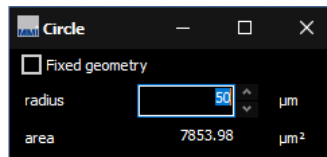
Figure 5.22: *Circle tool*

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

5.5.1.4 Ellipses

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

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

Figure 5.23: *Ellipse tool*

Fixed-size ellipses can be created by checking "Fixed geometry" and typing the values in the corresponding input box (Fig. 5.23)

5.5.1.5 Lines

Cutting straight lines with the **line** tool (*Alt + L*) may be useful for ablation or cell-surgery type experiments, as well as for cutting tests.
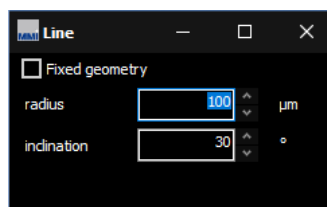
Figure 5.24: *Line tool*

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

5.5.1.6 Rectangles

The **rectangle** tool (*Alt + Q*) provides a quick method to outline objects of interest.
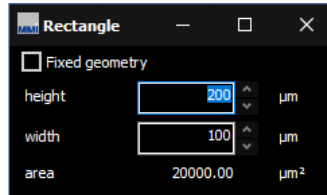
Figure 5.25: *Rectangle tool*

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

5.5.1.7 Deleting shapes

There are several ways to remove shapes:

- Deleting arbitrary shapes using the **eraser** tool (*Alt + Del*)

- Deleting the current (highlighted) shape by pressing *Del*

- Using the context menu (Fig. 5.26)

- Using the group editor (section 8.2.4).
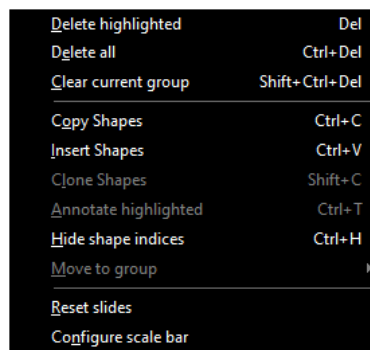
Figure 5.26: *Context menu, open with the right mouse button in the image panel.*

To delete all shapes, or all shapes from the current group, use the context menu (Fig. 5.26) or use the corresponding keyboard shortcuts *Ctrl + Del* and *Ctrl + Shift + Del*.

5.5.1.8 Copying and pasting shapes

To copy the active contour use the menu item

> *CellCut →Shapes →Copy*

Select

> *CellCut →Shapes →Insert*

to insert the copied shape.

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

5.5.1.9 Cloning

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

To start, select the shape you would like to clone and select *Clone...* from the context menu (Fig. 5.26).
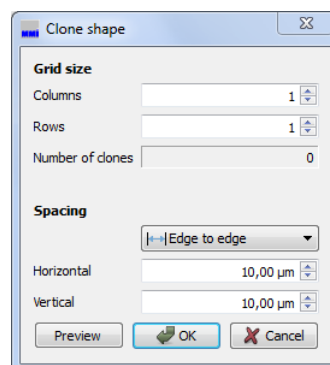
Figure 5.27: *Clone shapes*

The dialog allows you to specify the number of shapes per row and per column, as well as the distances between rows and columns. Depending on your

application, you may choose whether distances are measured between the shapes' edges or from center to center.

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

> Note that, on systems with an automated microscope, the system will focus on each cloned shape using the sample plane focus mechanism (section 9.4.2), if enabled. If clones are out of focus when cutting, either redo the sample plane definition or disable plane tilt focussing.

5.5.2 Text annotations

Highlighte shapes can be annotated with your comments, by using the *Annotate highlighted* menu in the popup menu Fig. 5.26. A small popup window

Figure 5.28: *Free text annotation for shapes*

Fig. 5.28 will allow to enter the text you want to save with the hightlighted shapes.

5.5.3 Hide shape indices

The popup menu shown in Fig. 5.26 or using the shortcut *Ctrl + H* also allows to hide the shape indices. This can be helpfull if many shapes exists. In this case hiding indices also accelerates the drawing.

5.5.4 Move to group

The popup menu shown in Fig. 5.26 also allows to move highlighted shapes into another group. This allows to correct the group assignement in case you forgot to select the correct group before drawing the shapes.

5.5.5 Reset slides

For convienience the popup menu shown in Fig. 5.26 also allows to reset slides. This means

- delete all focus points

- delete all scan regions

- delete all image analysis regions

- delete preview and whole slide images

5.5.6 Configure scale bar

The popup menu shown in Fig. 5.26 also allows to configure the colors and thickens of the scalebar shown in the right lower corner of the image panel.

5.5.6.1 Navigating between shapes

There are several keyboard commands that allow you to locate your shapes and navigate between them. These are illustrated in Fig. 5.29.
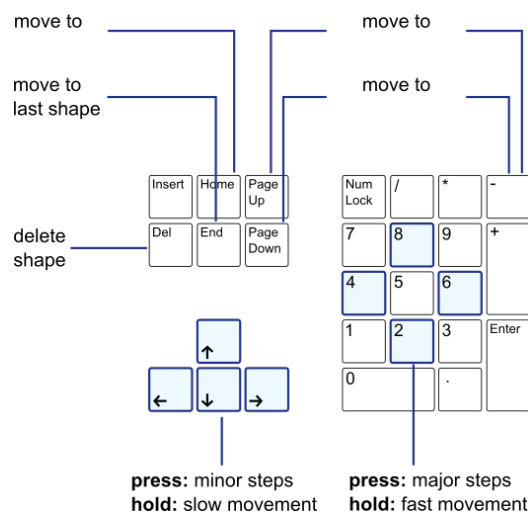
Figure 5.29: *Keyboard navigation*

- *Home* move to the first shape of the highlighted group

- *End* move to the last shape of the highlighted group

- *+* move to the next shape of the highlighted group

- - move to the previous shape of the highlighted group

- *Arrow up* move a small step up

- *Arrow down* move a small step down

- *Arrow left* move a small step left

- *Arrow right* move a small step right

- *Numpad: Up* move a large step up

- *Numpad: Down* move a large step down

- *Numpad: Left* move a large step left

- *Numpad: Right* move a large step right

5.5.7   Distance measurement

Select the measurement tool or press *Alt + M* to measure distances on the sample.
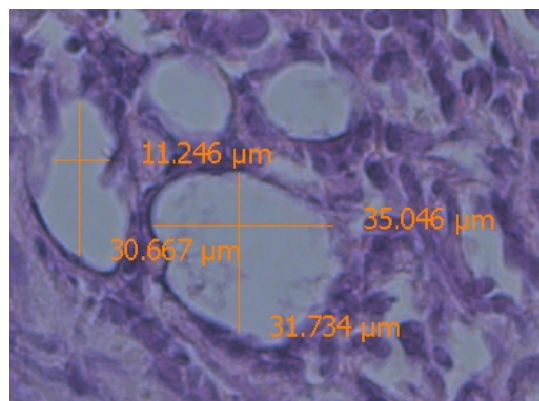
Figure 5.30: *Distance measurement*

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

5.5.8   Slide Navigation

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

- **fit to window** button

- **Zoom in** button

· **Zoom out** button

· **zoom slider** shown in Fig. 5.19 right of the image panel

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

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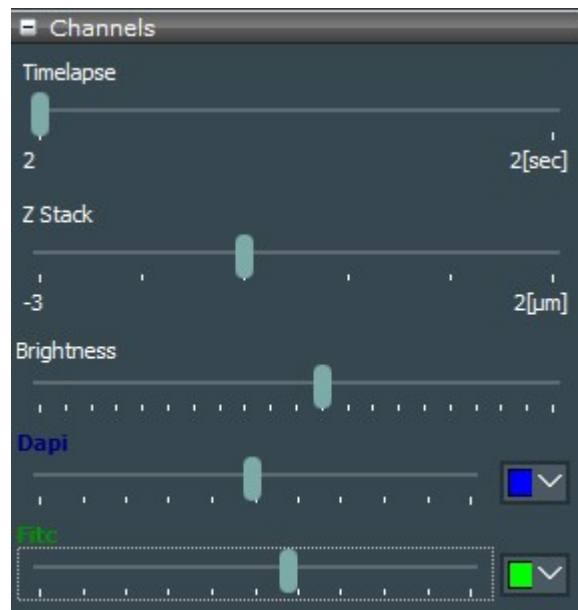
Figure 5.31: *Control the timelapse, zStack and Multi-channels of your 5D WSI.*

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. 5.31. The brightness for merged image or seperate image for each channel can be adjusted. The selection of **pseudo color**, please referto section 5.3.6

5.5.10 Shape import from third party applications

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

· TissueGnostics StrataQuest

· Visiopharm

· Evident ScanR

- 3DHistech SlideViewer

- Generic csv files

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

Select *Import from third party scanner* to select the file you want to import. The file type matching your vendor will be preselected.
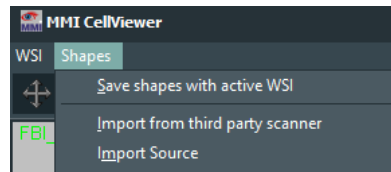
Figure 5.32: *Shape import menu*

5.5.10.1 TissueGnostics StrataQuest

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

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

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

and *mmi CellTools* has following CellTypes (see section 8.2) configured:

Ephitel, Bone, Blood, Muscle

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

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

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

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

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

5.5.10.2 Visiopharm

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

5.5.10.3 Evident ScanR

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

5.5.10.4 3DHistech SlideViewer

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

5.5.10.5 Generic csv files

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

5.5.11 Slide viewer plugins

The Slide viewer is able to host plugins like *mmi CellScan* or *mmi CellDetector* in the right side panel, see Fig. 5.33. This allow you to easily navigate through your whole slide images or use machine learning for image analysis.

5.6 Stage inserts configuration

The slide viewer can be expanded by pressing the **configuration button** to configure the stage inserts. Depending on the stage inserts configuration up to 8 full slide images can be loaded in the slide viewer.
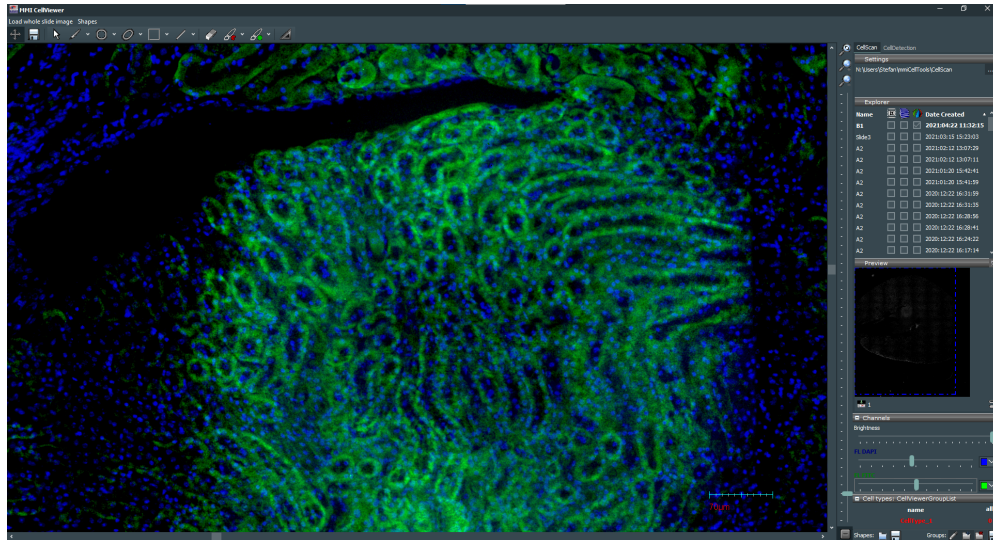
Figure 5.33: *The Slide viewer also allows to host plugins in the right side panel*

In order to properly navigate through your samples

- the origin of the stage (see 5.7.1)

- the geometry of the stage inserts

must be properly defined.

A stage insert is a mechanical holder for slides or multi-well-plates, which simply snaps into the microscope motorized stage. These definitions are a precondition for the use of the overview, see section 5.4.4 scan, and the navigation trough the wells in *mmi CellEctor*. 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

Using

File →Import

the configuration can be reloaded into the stage geometry viewer.

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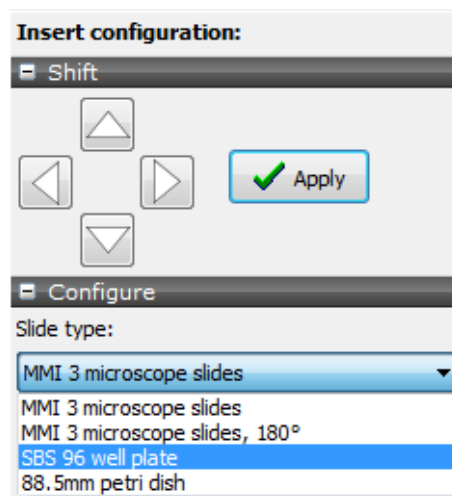
Figure 5.34: *Slide viewer: Insert configuration panel. You can select different insert types and shift the whole insert to adapt it to overview images.*

In the configuration panel (Fig. 5.34) 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 it's stage insert position. If the overview image of slide 1 does not exatly fit into the slide shift the insert as described in chapter 5.6.4.

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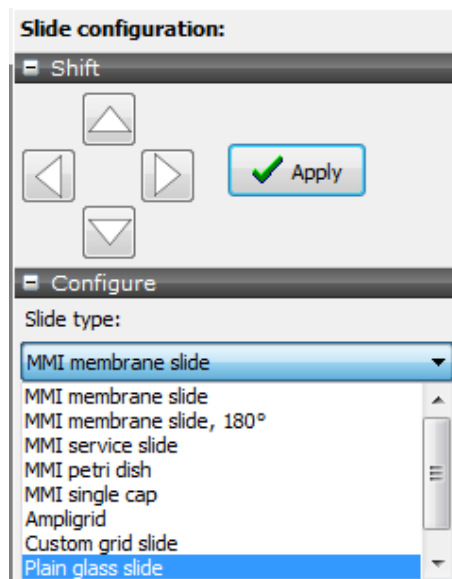
Figure 5.35: *Slide viewer: Slide configuration panel. You can select different slide types and shift the whole slide to adapt it to it's overview image.*

Depending on the stage insert selected in section 5.6, the configuration panel for slides (Fig. 5.35) offers a selection of different slide types. If your slide type is not listed, you can define and configure a custom slide.

- MMI membrane slide

- MMI membrane slide, mounted roted by 180°

- 8 well slide

- 18 well slide

- MMI petri dish holder

- MMI single cap holder

- MMI double cap strip holder

- plain glass slide

- empty

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

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

If overview scans in slide 1 are shifted against the geomtetry displayed, first calibrate the stage origin, see section 5.7.1. If this is not sufficient follow the procedure described in section 5.6.4

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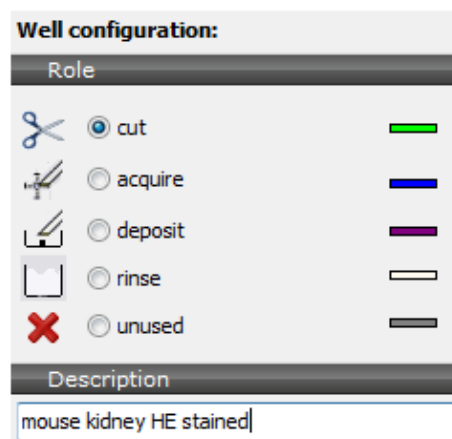
Figure 5.36: *Slide viewer: Well configuration panel. You can select different well rules and add descriptions to each well.*

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.

5.6.4 Stage inserts first configuration

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

Procedure

1. select a mounted insert in the insert configuration panel

2. select the mounted slides in the slide configuration panel

3. shift the first slide to it's position in the stage insert, see section 5.6.2

4. calibrate the stage origion, see section 5.7.1

5. scan an overview image in slide 1, see 5.4.4 and adjust the image to the slide position by shifting the whole insert. To get a precise positioning we recomment to adjust the image of the left upper corner of an membrane slide (Fig. 5.37) or the image of wells of an ampligrid to the displayed slide.

6. press **OK**

7. adjust the other slides to their overview images, see section 5.6.2

This procedure only has to executed for a new stage insert. If the configuration is properly set, a mismatch between the scanned image positions and the slide configruations indicates a corrupt stage origin calibration. Recalibration of the stage origin, see section 5.6.2, will be sufficient.

You are now ready to use slide navigation and scanning. The field-of-view indicator and the dimensions of the slide overview will adapt during calibration.
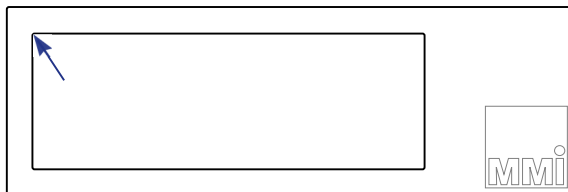
Figure 5.37: *Suggested positions on a mmi Membrane slide to precisely adjust the stage insert position.*

5.7   Calibration

5.7.1   Stage calibration

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

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

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

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

- press the **Calibrate origin** button in the Slide viewer.

- *Setup →Calibrate Stage origin*

- *Ctrl + O* in the main CellTools window.

During the calibration process the microscope objective will move down and the stage will move to its limit switches. If the stage geometry is still not matching a scanned overview image, the stage geometry configuration needs to be adjusted, see sections 5.6.2 and 5.6.4.

5.7.2 Camera alignment

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

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

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

Setup →Align camera with stage   (Ctrl + A)

from the main menu.

If automatic alignment was successful, a dialog (see figure 5.38) displays the results, which include:

- The measured width and height of the field of view,

- The relative standard error of the measured width and height, and
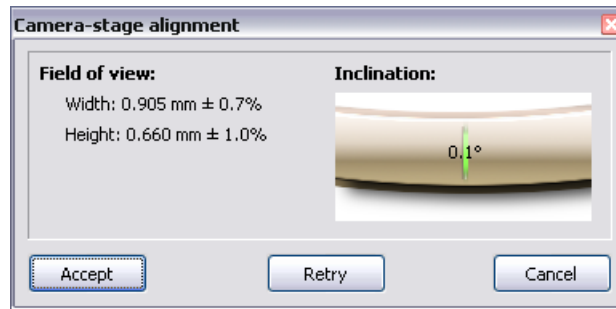
Figure 5.38: *Camera alignment results*

- The measured inclination of the camera.

Ideally, camera inclination should be very close to zero ($< 0.3°$). If it is larger, eliminate camera tilt before proceeding (see section 5.7.2.1). The lower the standard error of width and height, the more accurate positioning can be achieved. The standard errors can be reduced through optimal focussing and by viewing an area of high contrast at optimal illumination. (Also ensure that microscope illumination is set up correctly.)

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

When finished, check that the stage exactly follows mouse movement when using the Move stage tool. If not, repeat the procedure.

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

5.7.2.1 Eliminating camera tilt

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

Procedure

1. Start automatic stage alignment as described above

2. If camera inclination is larger than $0.3°$, slightly loosen the camera's mounting screw using the supplied hex wrench, turn the camera by a very small amount and retighten screw (lightly).

3. Repeat alignment until inclination is optimal

4. Fully tighten mounting screw.

5.7.3 Lens offset calibration

Because of mechanical and optical tolerances two objectives never have exactly the same optical axis. You see this effect by observing pixel shifts in the video when you change an objective. The **Paraxial Lens Offset** function is introduced to ensure that a marker points to the same object for different objectives.

When you install a new objective into your microscope you have to calibrate the Lens Offset. Over the time of operation it may be necessary to recalibrate, when a shape is no longer fitting the same object by changing the objective.

Procedure

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

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

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

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

3. Mount a slide with a sample.

4. Select the start (higher magnification) objective and the corresponding objective in the software

5. Move an easily noticeable object to the centre of the video screen.

6. Start the paraxial lens offset calibration with the menu item

   Setup →Paraxial Lens Offset   (Ctrl + O)

7. Draw a line around that object

8. Change the objective at the microscope and in the software

9. Move the shape exactly over the selected object
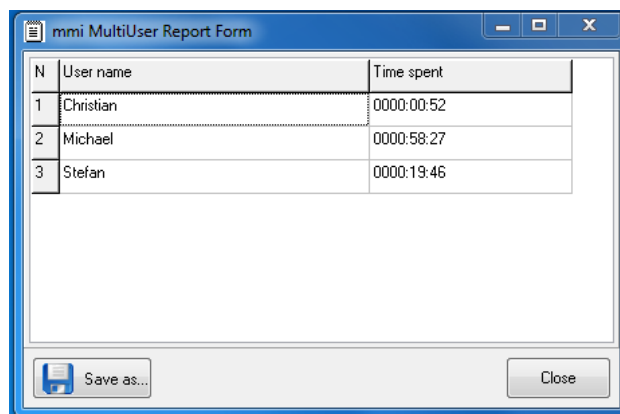
You are now asked whether you would like to calibrate further objectives. If you answer "yes" you are asked to repeat steps 8 and 9 for another objective.

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

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

5.8 Multi-user report

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

Figure 5.39: *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.

5.9   Help

5.9.1   Help topics

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

5.9.2   MMI online

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

Help →MMI online

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

Help →Online Support

5.9.3   Version info

Information about the currently installed software version can be found under

Help →Version info

6 mmi CellEctor

6.1 Principles

The molecular analysis of pure enriched cell populations or even of single cells is an important prerequisite of medical genomics and proteomics and may become essential for molecular patient profiling in the future. Up to now this goal is often very difficult and tedious to accomplish. Therefore, MMI developed a new analysis and cell sorting device: the mmi CellEctor. The isolation of single and rare cells from tiny sample sources is required for specific genotypic and phenotypic characterization. The mmi CellEctor is the starting point for a new integrated work flow. It facilitates the automated isolation of any type of single and rare cell in three steps:

- cell recognition

- cell acquisition

- cell deposition

Additionally rinse procedures for the used capillaries can be defined. The *mmi CellEctor* is based on:

- automated microcapillary

- automated pump

- motorized stage

To increase the automation level the *mmi CellEctor* can be upgraded with:

- motorized microscope

- *mmi CellExplorer*, the MMI cell recognition solution

Acquisition and deposition are realised using a high precision pump. This allows the use of protocols with nanoliter volumes for the separation process and provides the basis for molecular downstream analysis in tiny volumes.
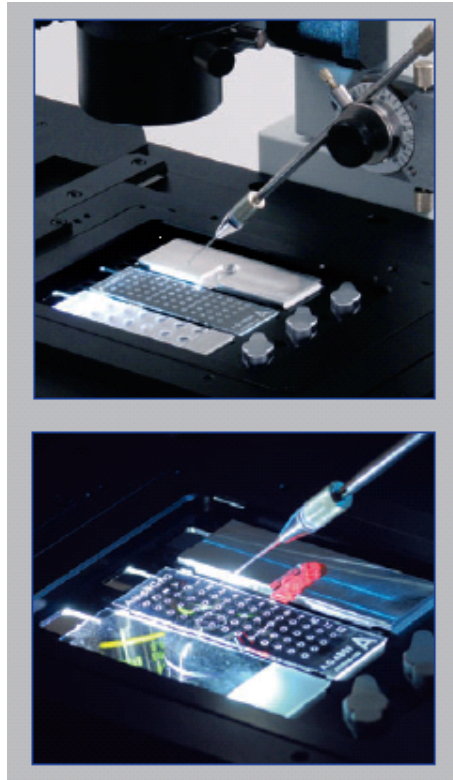
Figure 6.1: *Sample area of the microscope with a ampligrid and microcapillary.*

There is no mechanical stress to (non-adherent) cells: the acquisition occurs solely by flow of the liquid surrounding a cell. Under optimal conditions, partly adherent cells can be acquired in this way. However, the *mmiCellEctor* offers possibilities to mechanically detach adherent cells from the surface.

The capillary diameter (10, 15, 20, 40 or 80 μm) may significantly exceed that of the cell. For example, cells of 6 μm diameter can be acquired with a capillary of 40 μm diameter efficiently.

The *mmi Collector* is compatible with a variaty of slides or well plates. The most widely used types will be described in section 6.2. The classification of the wells of your disposables is desribed in the stage geometry viewer section, 5.6.3.

The acquisition and deposition of cells can processed in a variety of modes:

- step-by-step mode: the user launches each work step separately.

- semi-automted mode: the user marks the cell and the system automatically acquires and deposits the cell

- fully-automated mode: automatic cell recognition by *mmi CellExplorer* followed by automatic cell acquisition and deposition.

However, even in the step-by-step mode, there is no need to manually operate

the hardware components of the system. All the operations are launched via the *mmi CellTools* software.

The *mmi CellEctor* is an adjustable, open platform for any assay format. It closes the gap between frequent and rare cell sorting. It is dedicated for the use in:

- cancer research & oncology

- immunology & virology

- stem cell sciences

- molecular and cellular biology

- single cell analysis

as well as many other applications in biomedical research, cellular diagnostics, microbiology & virology, environmental ecology, forensics. MMI is committed to establish specified and detailed application-related protocols in a close cooperation with the users.

All the methods applied to measure molecular changes require 100% pure cell populations to ensure unbiased results. For the analysis of DNA, copy number variations or single nucleotide polymorphisms are being measured. Changes of RNA expression are analyzed by microarrays or massive parallel sequencing approaches. Protein patterns are analyzed with spectroscopic or biochemical protocols, and epigenetic changes are detected by changes in methylation patterns. FACS-sorting and bead-based protocols are the major state-of-the-art methods to isolate single cells. Also a wide variety of physical and immunological methods are employed. The *mmi CellEctor* helps to overcome the hurdles and drawbacks of the above mentioned methods.

The *mmi CellEctor* technology has been used for the enrichment of tumor cells and immune cells from disaggregated lymphnode material, blood and bone marrow of cancer patients. For the outlined workflow of an application example, the following purification steps were established: Lymphnode metastasis is disaggregated by the use of scalpels and filtered through a 70 micron filter to get a cell suspension. Blood and bone marrow is treated by erythrocyte lysis, to remove present erythrocytes, Tveito et. al. 2007 [1]. In a first enrichment step, paramagnetic beads coated with an appropriate surface-antibody were used for the immunomagnetic enrichment. In the second step the mmi CellEctor technology is used for individual cell picking.

---

[1]Reference: Tveito, S., Maelandsmo, M. G., Hoifodt, K. H., Rasmussen, H., Fodstad, O.:"Specific isolation of disseminated cancer cells: a new method permitting sensitive detection of target molecules of diagnostic and therapeutic value", Clin. Exp. Metastasis 5 (24), 317 (2007).

(a) Preparation of cell suspension: disaggregate lymphnodes and filter cells

(b) Lyse erythrocytes from blood or bone marrow samples
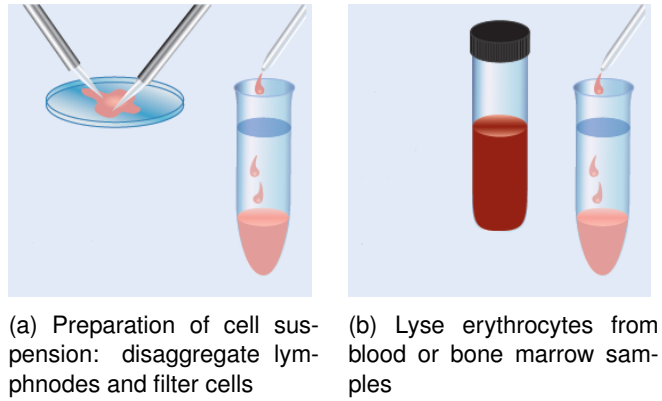
Figure 6.2: *Preparation of the patient material*

(a) specific immunomagnetic beads and incubate under rotation

(b) magnetic separation of immunomagnetically rosetted cells, microscopic evaluation and *mmi CellEctor* mediated cell picking
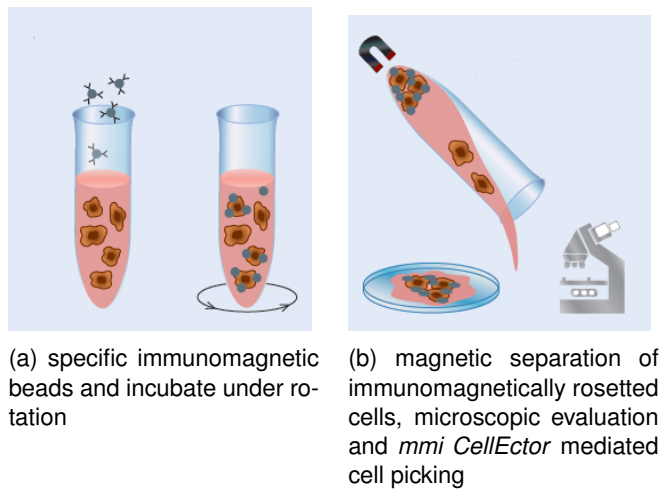
Figure 6.3: *Pre-enrichment of target cells by immunmagnetic selection via targeting a cell specific surface marker and mmi CellEctor mediated cell picking.*

(a)   (b)
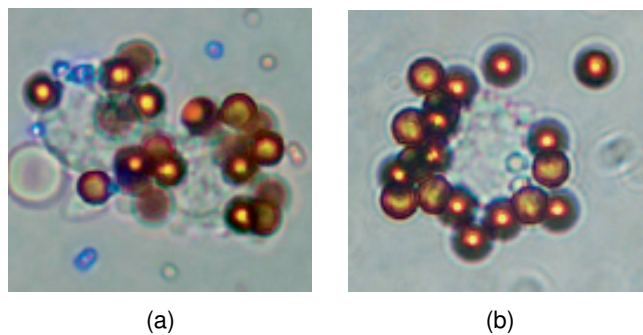
Figure 6.4: *Lymphnode metastatic cells targeted with an anti EP-CAM antibody*

6.2   Wells for acquisition, deposition and rinsing
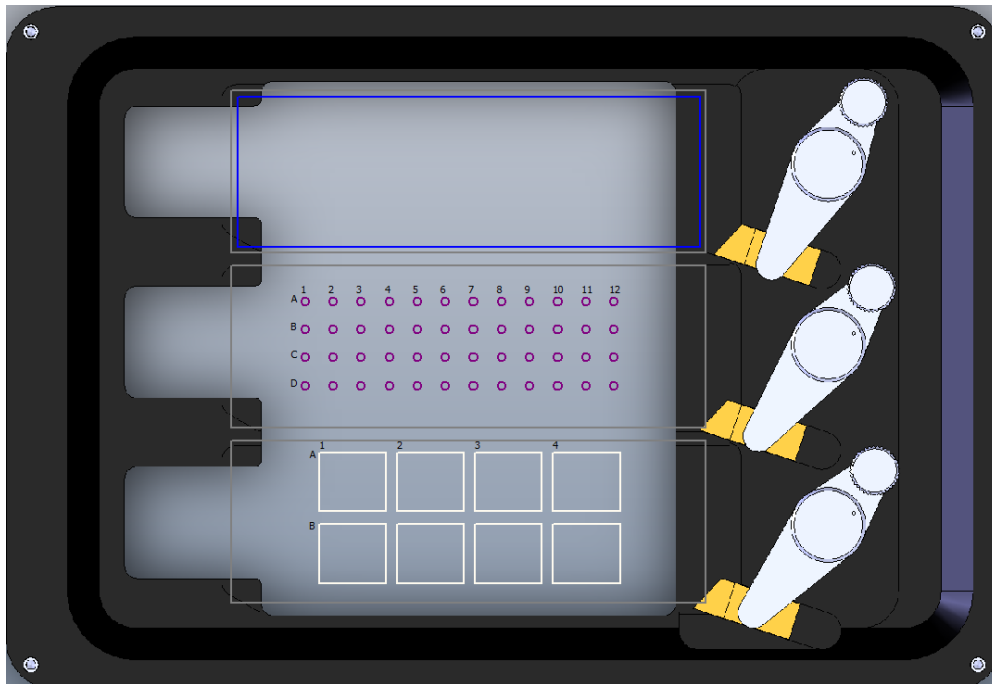
Figure 6.5: *A typical stage insert configuration consist of a plain glass slide as acquisition (blue) vessel, an Ampligrid with deposit (purple) wells and a mmi ServiceSlide to rinse (cream) the capillary .*

The *mmi Cellector* requires to define wells for acquisition, deposit and rinse procedures. The classification of the wells can be done in the stage insert viewer, see section 5.6.3. The rule of each well can freely be defined by the user. A typical configuration is shown in Fig. 6.5. Also multiwell plates can be used and wells for all purpused can be freely distriuted on the multi well plate. An example is shown in Fig. 6.6.

Wells assigned to deposit or rinse will be used in the following order, respectively:

- starting with the most upper left well

- using all wells in the row from left to right

- going to the next row containing a well

- using all wells in the row from left to right

- ...

MMI's current range of slides is available on our website:

www.molecular-machines.com/products/consumables/various_slides
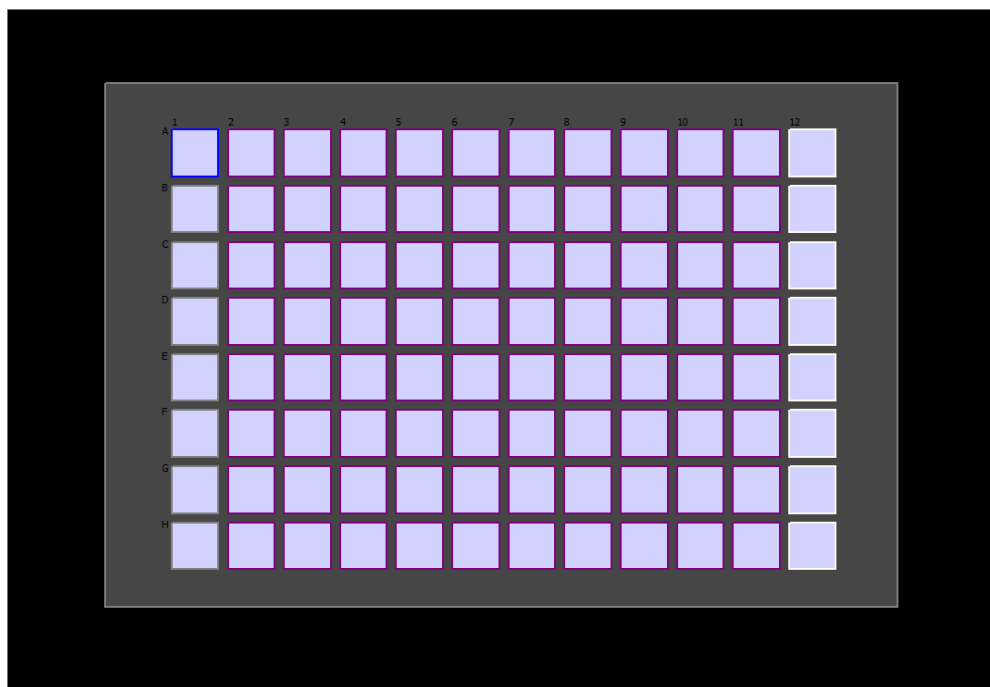
Figure 6.6: *A typical well plate configuration consist of a acquisition (blue) wells, deposit (purple) wells, rinse (cream) wells and unused (gray) wells.*

6.3 Service slide

The capillary can be treated with various solutions for cleaning, filling with additional reagents or diluting the reagents already present in the capillary. Typically the *mmi ServiceSlide* Fig. 6.7 is used for this purpuse.

6.4 Capillaries

6.4.1 Capillary types

mmi CellEctor standard glass capillaries are straight with the outer diameter of 1 mm and an inner tip diameter of 10, 15, 20, 40 or 80 μm. The capillary tip is bevelled at 45 deg. Any other capillary shapes and sizes that fit the capillary holder may also be used, however, in such a case the handling, the calibration and the standard usage procedures might differ from those described in this manual.

MMI's current range of capillaries is available on our website:

www.molecular-machines.com/products/consumables/mmi_capillaries

Figure 6.7: *The mmi CellEctor 8 well service slide*

6.4.2   Capillary handling

Please observe the safety precautions described in the first chapter of this manual.

mmi CellEctor is delivered with a ready to use capillary placed in the capillary holder of the 3D manipulator (illustrated below).

The following capillary replacement procedure is recommended:

Procedure

1. Place the 3D manipulator in the position shown in the figure 6.9.

2. Slide the capillary cover tube along the transparent capillary holder down to the lowest position.

3. While rotating the capillary cover tube, gently press it down until it locks in the screwing position.

4. In this position, slightly loosen the o-rings that tighten the capillary by rotating the capillary cover tube.

5. Pull the old capillary out of the holder.

6. Pump out oil until it reaches the outer o-ring that tightens the capillary. Take care than there are no air bubbles in the oil.
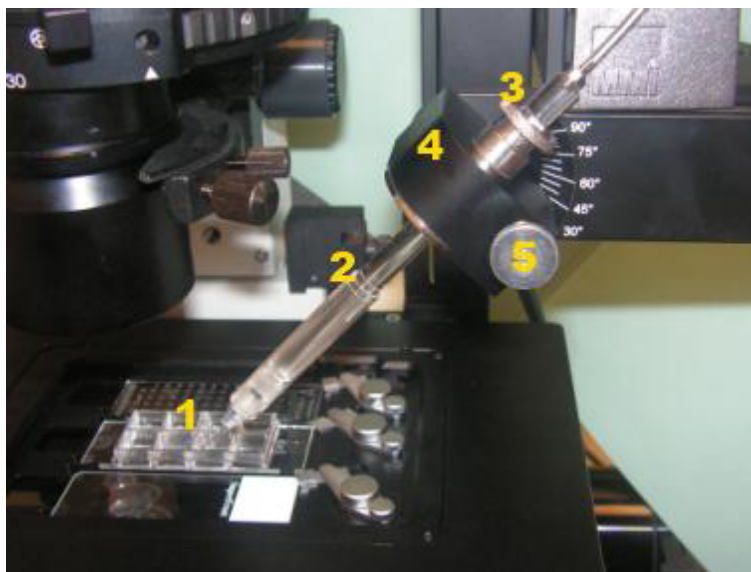
Figure 6.8: *The capillary (1) is mounted in a transparent capillary holder (2) that connects to the pump. The capillary holder is fixed in the manipulator holder (4) by a knob (3). An additional knob (5) allows changing the rotational position of the capillary holder. Furthermore, there is a screw at the rear side of the manipulator holder (4) that allows adjusting the capillary tilt. The standard tilt is 45 deg.*
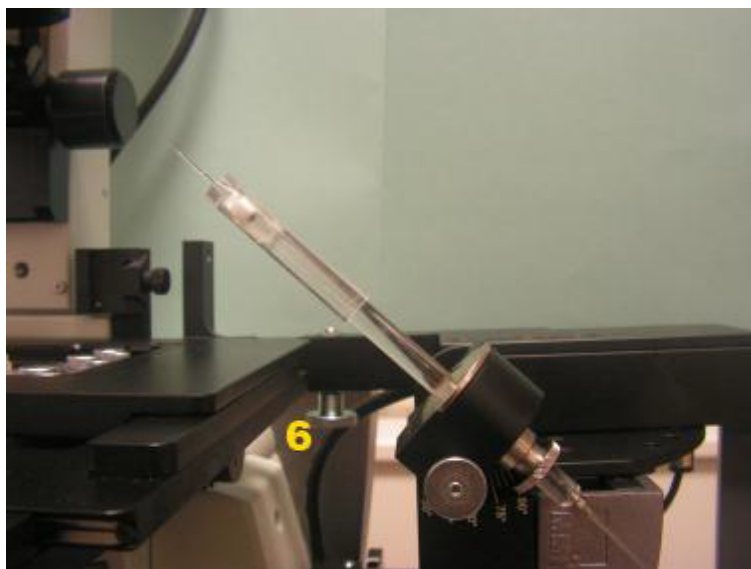
Figure 6.9: *The entire 3D manipulator can be tilted by 90 deg down to a position at which the replacement of the capillary is facilitated. For this purpose, unscrew the knob (6) and gently rotate the manipulator to the required position.*

7. Insert the new capillary. While inserting, ensure that the capillary passes both o-rings.

8. Tighten the capillary by rotating the capillary cover tube.

The capillary can be filled either before or after it is mounted in the capillary holder. To fill the capillary before mounting use the following procedure:

Procedure

1. Fill a small vessel with buffer solution. For easy capillary handling the walls of the vessel should be smaller than the capillary length.

2. Place the capillary with the tip to the top inside the filled vessel. Due to capillary forces the capillary is slowly filled with the liquid.

3. Wait until the capillary is filled to about half of its volume. Note that the filling time decreases with the tip diameter.

4. Pump oil to the forefront of the capillary holder and loosen the front part of the capillary holder with the cover tube.

5. Insert the capillary in the capillary holder and fix the front part with the cover tube. Take care not to touch the tip of the capillary.

6. Pump out the air in the front part of the capillary until the buffer reaches the tip. You should see a phase shift from aqueos buffer to oil in the capillary.

To fill the capillary after it is mounted in the capillary holder or to refill it, use the following procedure:

Procedure

1. Fill almost the entire capillary with oil. The oil boundary must not enter the narrow part of the capillary, i.e. leave some air in the conical part of the capillary, see Fig. 6.10.

2. Stop pumping out and wait approx. 30 seconds for the pressure in the capillary to equilibrate.

3. Immerse the capillary into the buffer solution placed on a slide.

4. Pump in the buffer solution up to the moment the air gap between it and the oil moves into the transparent part of the grip head, see Fig. 6.11.

5. Stop pumping in and wait for the pressure in the capillary to equilibrate.

6. Move the capillary up from the buffer solution.
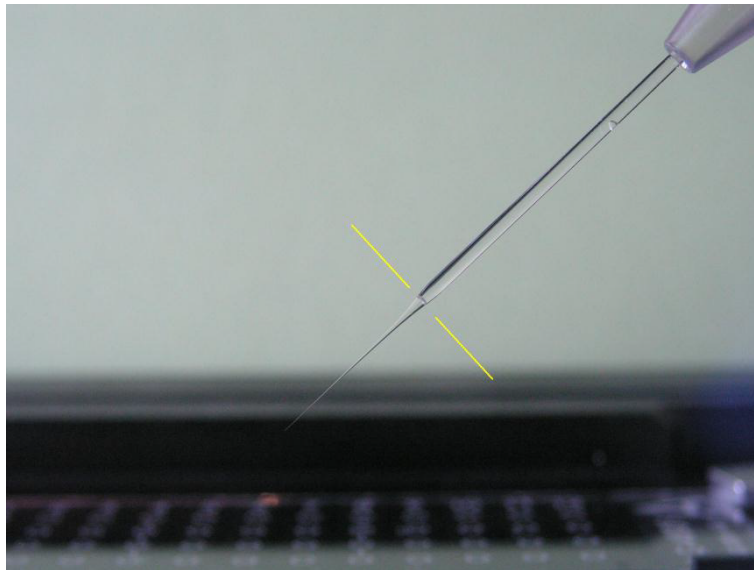
Figure 6.10: *Fill almost the entire capillary with oil. The oil boundary must not enter the narrow part of the capillary, i.e. leave some air in the conical part of the capillary.*
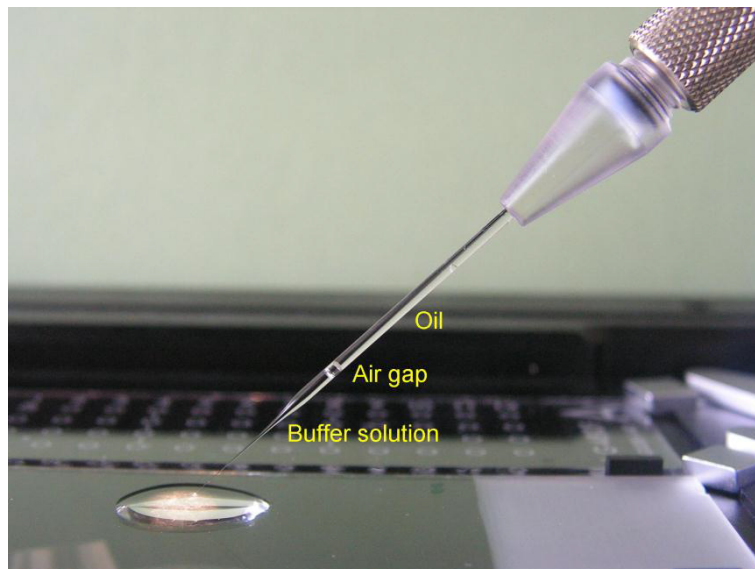
Figure 6.11: *Pump in the buffer solution up to the moment the air gap between it and the oil moves into the transparent part of the grip head.*

The air gap between the oil and the buffer solution works well for capillaries with a tip diameter of at least 30 µm. The narrower the capillary tip is, the longer time it takes to equilibrate the over- or underpressure after each pumping step.

6.5 The *mmi CellPump*

Figure 6.12: *The mmi CellPump allows to pump in and out by manually turning the rotation knob.*

Manual pumping   To operate the *mmi CellPump* manually, raise the rear cover of the pump and turn the knob, see Fig. 6.12. Manual pumping is preferably used to fill the pump or the newly inserted capillary with the oil. While pumping manually take care that you don't move the piston outside the allowed range (see below).

> **While using capillaries with a small opening, quick manual pumping may increase the pressure in the pump so that the capillary may burst.**
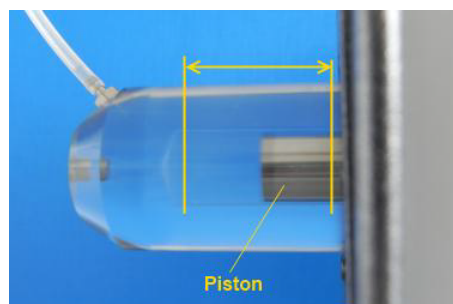
Figure 6.13: *The mmi CellPump allows to pump the shown range.*

Pumping range   The piston has a limited range of movement as indicated in Fig. 6.13. If a limits has been reached, the software issues a warning icon in the pump panel. In such a case, you have to move the piston in the opposite

direction to get out of the end switch.

6.6 Use 3D manipulator with joystick

In *mmi CellEctor*, the joystick can be used to move the capillary. The horizontal XY position of the capillary can be changed by tilting the joystick to the left or right (X coordinate) or forth and back (Y coordinate). Its vertical Z position can be changed by turning the joystick clockwise for lowering (Z value increases) or anticlockwise for raising (Z value decreases) the capillary.

> The speed with which the capillary moves is proportional to the deviation of the joystick from its equilibrium position. Please test the movement without capillary first to get a feeling for the movement velocity.

6.7 Typical workflow for cell sorting

6.7.1 Positions used in cell sorting

For the different tasks like acquisition, deposition and rinsing *mmi CellTools* need to know following positions:

- **Idle**: The safety position of the capillary above the sample (i.e. when you move the capillary up).

- **Down**: This is the working position of the capillary for collecting and depositing a cell or to rinse the capillary.

- **Screen**: The position on the video screen where the lowered capillary is visible.

- **Well target**: The position inside the well, where acquisition, deposition or rinsing should take place.

6.7.2 Setting up the slides, the capillary and the pump

Type of slides or multiwell plates Decide what kind of slides will be used for the acquisition, deposition and rinsing. The stage geometry viewer, see section **??**, will offer you a selection of diffent disposables. If you do not find your disposable you also can define a "Custom slide". Mount the slides on the stage insert and configure the stage insert geometry accordingly.

Capillary tilt   Insert a new capillary into the capillary holder, adjust its tilt and set the tilt value in the software, see section 6.4.2.

Slide plane tilt (motorised microscopes only)   Select the working objective and determine the plane tilt of the slides, see section 9.4.2.

Pump   Ensure that

- the pump is filled with oil

- the pump piston is away from its limit positions, see section 6.5

- there are no air bubbles and no leaks between the pump and the capillary, see section 6.4.2

6.7.3   Routine working procedure

While sorting cells, a cell is collected from an acquisition well and deposited in a deposition well:

Procedure

1. Ensure that the objectives are calibrated and the slide planes are defined (for automated microscope).

2. Set up the *mmi CellEctor* major components
    - Ensure the stage insert geometry is correct.
    - Set the rule of the wells. Wells you do not want to use should be marked as unused.
    - Mount and fill a capillary.

3. Define the down positions for acquisition and deposition.

4. Define the capillary tip position on the screen for acquisition and deposition.

5. Move the capillary down in an acquisition well and adjust the capillary spacing and the pumping parameters so that the cells can efficiently be aspirated.

6. Move the capillary down in a deposit well and adjust the capillary spacing and the pumping parameters so that the cells can efficiently be deposited.

7. Collect cells using the semi-automatic mode.

8. Set up the image recognition parameter in *mmi CellExplorer* to find the cells of interest in an acquisition well

9. Collect cells using the fully-automated mode.

The following chapters describe these steps in more detail. In a routine work under the same experimental conditions (same type and size of cells, buffer solution, capillary size, etc.), steps 1, 2, 5 and 6 can usually be skipped.

6.7.4 Finding the optimum conditions for collecting cells

Physiological solutions containing cells exhibit a broad range of physicochemical and biological properties. Thus, the optimum conditions have to be found for the particular cells of interest. While experimenting with new cells and buffer solutions, one can vary the following parameters:

Size of the capillary tip   In principle, it should be larger than the size of the cells to be collected. If the concentration of cells is not too large, even relatively large capillaries (tip diameter of 40 µm) can efficiently collect small cells (approx. 5 µm).

Orientation of the capillary tip bevel   Rotating the capillary around its axis may affect the efficiency of catching and depositing the cells.

Spacing   The distance between the capillary tip and the slide surface (spacing) affects both the volume of the liquid necessary to be pumped in order to pick a cell as well the volume to be pumped out at the deposit to get the cell out of the capillary. It should be adjusted for the sample and the deposit slide separately.

Pumping parameters   These parameters have the major influence on the efficiency of collecting the cells. In most cases it is enough to adjust the main volume to be pumped in or out. In some cases one has to use the extended pump parameters like pre- and over-pumping, relaxation time or speed and acceleration of the pump, see section 6.15.4.

6.7.5 Performing cell sorting

Cells can be collected using either the step-by-step, semi-automatic or fully-automatic mode.

step-by-step mode   Moving the stage, moving the capillary up or down and pumping are separately launched by the user. This mode is suitable to learn the function of the *mmi CellEctor* and to find the best parameters, see section 6.10.

semi-automatic mode   Cell sorting is devided in the following actions:

- cell acquisition

- cell deposition

- capillary rinsing and cleaning

These actions are separately launched by the user. Most customers will mainly work in the semi-automated mode, see section 6.10.3.

fully-automatic mode   Routine work is preferably done in the fully-automated mode that combines these actions to a complete cell sorting workflow, see section 6.11.3. Even the cell recognition can be automated, if the *mmi CellExplorer* is installed.

6.8 Menu and control panel

As described in section 5.1, the special features of the MMI products *mmi CellCut*, *mmi Cellector*, *mmi CellScan*, *mmi CellExplorer* and *mmi CellManipulator* are installed as separate plug-ins (software modules). The plug-ins appear as separate tool panels on the right side of the program window. Each plug-in adds its own menu (e.g. *CellEctor*) to the main menu and provides its own control panel.

The *mmi CellEctor* control panel always displays the calibration status of the system. If a calibration is invalid, the header of the corresponding panel will be displayed in red. Additionally warning icons will be shown to clearify the reason of the problem detected. Moving the mouse cursor over a warning icon will bring up a hint with detailed explanations.
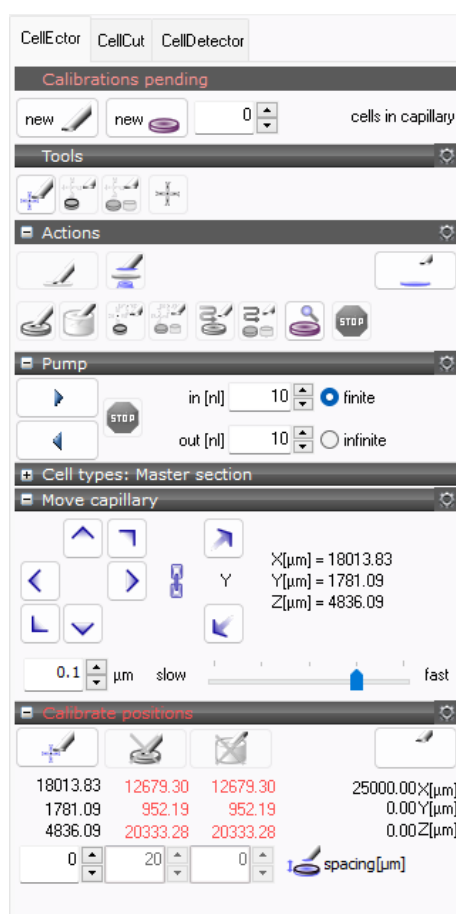
Figure 6.14: *The mmi CellEctor panel. The panel is devided in several fixed or collabsible subpanels. If calibration procedures are pending they are clearly indicated in red.*

The CellEctor menu is shown in Fig. 6.15. It provides access to all configuration windows and several functionalities.

The configurations can also be selectively accessed via the configuration but-

ton displayed in each configurable panel.
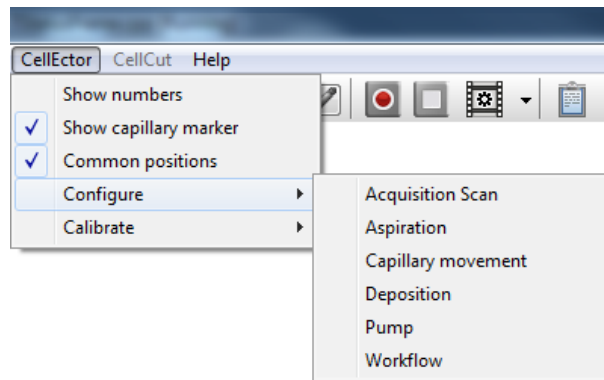
Figure 6.15: *The mmi CellEctor menu*

With

   Cellector →Show numbers

the 3D manipulator positions will be displayed in the control panel. The numbers can be helpfull to understand the behaviour of the 3D maniplator and also if very precise positioning is required.

In many applications the same position information can be used for acquisition, deposition and rinsing. Common positions can be turned on in

   Cellector →Common positions.

6.9   Status panel

6.9.1   Status indicator

The panel indicats the calibration status of *mmi CellEctor*: **Ready** or **Calibration pending**.

6.9.2   New capillaries and deposit wells

Figure 6.16: *The status panel summarizes the calibration status of the of the mmi CellEctor. If a calibration is pending "calibration pending" will be displayed in the header.*

In the status panel the user can inform the system about the use of a **new capillary** or a **new deposit** well. If a new capillary is mounted the system will lift all down positions by the value configured in the capillary movement configuration, see section 6.15.2. After inserting new deposit wells all deposit wells will be assumed as empty and depositing will restart in the first deposition well.

Cells in capillary shows the current number of cells in the capillary, which is editable.

6.10 Tools panel
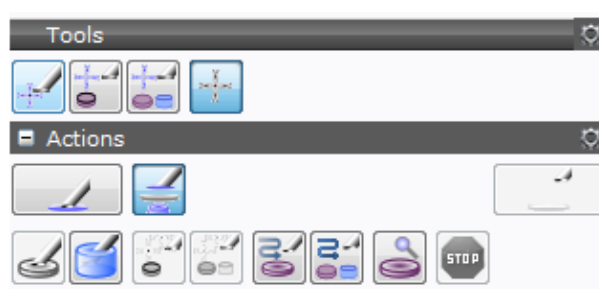
Figure 6.17: *The tools and actions panels provide all features to use the mmi CellEctor in routine work. If a tool is disabled a required calibration is missing.*

Routine work with the *mmi CellEctor* is executed using the functions in the **Tools** or **Actions** panel. By pressing an action button the system immediately will execute the requested workflow. The tool buttons will mostly define the workflow executed by klicking into the video panel.

In the upper tool line you can select between following workflows:

6.10.1 Aspirate cells

If the button **aspirate a cell** (*Alt + A*) is selected, the *mmi CellEctor* will move the capillary tip to the selected cell by a left mouse click and aspirate the cell into the capillary.

6.10.2 Acquire and deposit

If the button **acquire and deposit** (*Alt + E*) is selected, the *mmi CellEctor* will acquire the selected cell and deposit it to the next free deposit well.

6.10.3 Acquire and deposit cells and rinse the capillary

If the button **acquire, deposit and rinse** (*Alt + F*) is selected, the *mmi CellEctor* will acquire and deposit the selected cell, followed by the rinse procedure defined by the rinse steps set up in the workflow editor, see chapter 6.15.7.

6.10.4 Mark a cell

If the button **mark a cell** (*Alt + C*) is selected, you can mark cells by a left mouse click. By a right click on the related marker a popup menu will provide access to all available workflows for the marked cells, see Fig. 6.18. Alternatively, you can use shortcuts to execute the related workflows (acquire and deposit: *Ctrl + Alt + E*; acquire, deposit and rinse: *Ctrl + Alt + F*). If multiple cells are marked all cells will be processed sequentially.

Delete highlighted shapes	Del
Delete all	Ctrl+Del
Clear current group	Shift+Ctrl+Del
Back to sample	
Acquire and Deposit	Ctrl+Alt+E
Acquire, deposit and rinse	Ctrl+Alt+F

Figure 6.18: *By a right click on a marked cell a popup menu will provide access to all available workflows for that cell.*

6.10.5 Moving the capillary up or down

The idle and down positions of the capillary will be defined in the **Capillary positions** panel. For the different well rules - acquire, deposit and rinse - different down positions can be defined. For details see section 6.14.3.

- **Capillary up** (*Alt + U*) moves the capillary to its idle position.

- **Capillary down** (*Alt + D*) moves the capillary to its down position.

6.10.6 Toggle microscope focus between capillary tip and slide surface

The **Toggle focus between capillary tip and slide surface** toggles the microscope focus between the capillary tip and the slide surface.

6.11 Actions panel

In the **Actions** panel routine actions can be lauched. All actions can be interrupted using the **Stop** button.

6.11.1 Deposit

Deposit (*Ctrl + Alt + D*) launches a cell deposition. The system will move to the next free deposition well and dispose a acqurired cell into that well.

6.11.2 Rinse

Rinse (*Ctrl + Alt + R*) launches the rinse steps rinse steps set up in the workflow editor, see chapter 6.15.7.

6.11.3 Scan for cells

Scan for cells, acquire and deposit starts with scanning of the predefined sample area followed by automatic cells recognition using *mmi CellExplorer*, cell acquisition and cell deposition.

> **Scan for cells, acquire and deposit** is usually applied under fluorescence conditions, since these are optimal conditions for automatic cell recognition.

In detail the following procedure will be executed recurrently:

Procedure

1. A snapshot of the video screen containing the cells is made at the starting position of the stage and is then transferred to the image processing software *mmi CellExplorer*.

2. *mmi CellExplorer* identifies cells, marks them and sends the set of markers back to *mmi CellEctor*.

3. *mmi CellEctor* selects a single cell to be aspired.

4. acquisition

5. deposition

6. stage moves to next scan field

In order to scan through the sample, the user can define the number of horizontal and vertical fields of view in the acquisition configuration dialog, see chapter 6.15.5. Since usually there are several cells of interest within one snapshot, the recognition and selection of cells can be repeated several times in the same field of view.

For the fully automatic scanning to work correctly, it is compulsory to load the correct set of parameters to *mmi CellExplorer* software before you start the scan. Please refer to the *mmi CellExplorer* manual (section **??**).

6.11.4 Scan for cells and run user defined workflow

Scan for cells and run workflow starts with scanning of the predefined sample area followed by cells recognition and running all steps defined in the workflow editor (see chapter 6.15.7). This enables an individual, user defined workflow for automatic cell recognition.

6.11.5 Inspect deposit wells

Inspect deposit inspects the deposit wells. Dependent from the deposit configuration (see section 6.15.6), *mmi CellEctor* is waiting for user inspection of the displayed deposit well or is using the *mmi CellExplorer* and shows the number of collected cells.

Reports If the automatic deposit inspection by *mmi CellExplorer* is actived in the configuration dialog (see section 6.15.6), a snapshot of each inspected spot is stored in the JPG format for documentation purposes. The image files are named RxCy.jpg where R, C represent row and column, and x, y represent their number, respectively. The images are stored it the folder

```
My Documents\mmiCellTools\AutoDoc\
\CL_InspectData_yyyy-mm-dd_hh-mm-ss\RxCy.jpg
```

6.12 Pump panel

The pump is delivered ready for use, i.e. it is filled with oil and with the piston set in the backward position.

The buttons **Pump in** (*Alt + I*) and **Pump out** (*Alt + O*) launch pumping of liquid, see Fig. 6.19:

Figure 6.19: *Set the pump volumes and launches pumping by presseing **Pump in** or **Pump out***

- **Pump in** will pump in fluid
- **Pump out** will pump out fluid

Dependent from the check boxes **finite** or **infinite** a predefined volume will be pumped or the pump moves until the **Stop** button will be pressed. The pump speed and acceleration and some advanced pumping parameters can be configured as described in chapter 6.15.4. Pumping allways can be stopped by pressing the **Stop** button.

6.13 3D manipulator panel
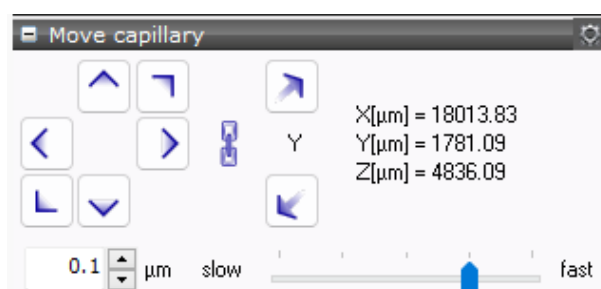
Figure 6.20: *Manual movement of the 3D manipulator with different speeds or fixed distances. The manipulator will be locked, so that it can not move far below the down position. The numbers can be hidden in by unchecking the menu CellEctor →Show numbers.*

The 3D manipulator can be moved by using the arrow buttons in the software. The speed can be selected by using the speed slider. Two different modes are available:

- continuous move by keep pressing the arrow button
- move with fixed distance by a single click of the arrow button

The fixed distance can be edited in the manipulator panel.

To protect your capillary the manipulator will be locked, so that it can not be moved far below the down position. The distance of the capillary below the down position can be configured in section 6.15.2.

In case of a motorized microscope and an active plane tilt, the downposition and the 3D manipulator lock will be automatically adapted to the plane.

The lock can be released using **(un)lock 3D manipulator** button .

If you like to use the joystick to move the 3D manipulator you can activate it anytime by pressing the **Joystick on/off** button . The 3D manipulator lock mechanism will also be active in combination with the joystick.

6.14 Calibration routines

The software can handle different capillary positions for each well type. However, in general the down position can be shared for different well types and only the distance to the sample surface needs to be defined. To use a common down position for different well types select *CellEctor →Common positions*.

Whenever a new capillary is inserted, the capillary has to be calibrated in a single step procedure. Following positions will be calibrated:

- Define the down position of the capillary in the contact with the well. (On which 3D manipulator position the capillary touches the well)

- Define the XY position of the capillary tip on the screen (where you see the capillary tip on the screen)

- Define the well target position (where in the well we want to work)

The well target position is most important for the deposition of cells and for rinsing the capillary. It may deviate from the well's geometrical center in order to take care for well walls or to compensate for the asymmetry of the droplets formed during the pumping of liquid out of the capillary.

Wherever you see a red cross the corresponding calibration is not valid and must be executed to get work-flows depended from that calibration active. Additionally the text in the panel header will be displayed in red.

6.14.1 Calibrate positions

6.14.2 Capillary positions panel

6.14.2.1 Idle position

First the idle position of the capillary should be set. The idle position is the safety position of the capillary to which it returns after each workstep.
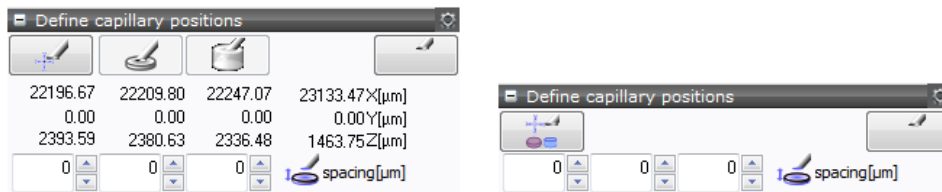
(a) position numbers displayed and different positions for different well types

(b) position numbers hidden and common positions for different well types

Figure 6.21: *All specific positions of the capillary can be defined in the **Define capillary positions** panel. To use a common down position for deposition, collection and rinsing select CellEctor →Common positions. Displaying number can be set up in CellEctor →Show numbers.*

Procedure

1. Move the capillary tip to a save position just above all slides or multi-well-plate. A collision with the slide by moving the stage must be excluded. Easiest you just move the capillary upwards from a down position.

2. Press the **idle** button .

6.14.2.2 Down positions

To define the capillary down positions two methods are available:

- Manual: Move the capillary in contact with the upper well surface, press the down button for **acquire**, **deposit** or **rinse** and click on the tip of the capillary inside the video. A detailed description is given in this section.

- If the microscope plane tilt is active the **calibration guide** becomes available, see section 6.14.3 .

The calibration can always be cancelled with the **stop** button.

For each down position an additional spacing can be defined. The spacing will define the vertical distance between the capillary tip and the well surface after moving the capillary down. For detailed recommendations, how to set the spacing value see section 6.16.2

In the case of active microscope focus surface (section 9.4.2), additional check-box allow to define, if the microscope focus on the well surface or the capillary tip after moving the capillary down.

Be aware of two independent coordinate systems used by *mmi CellEctor*:

- coordinate system of the capillary 3D manipulator

- coordinate system of the microscope stage and the microcope z-drive.

Their relative orientation is shown in the Fig. 6.22a and Fig. 6.22b.

(a) 3D manipulator   (b) stage and microscope focus
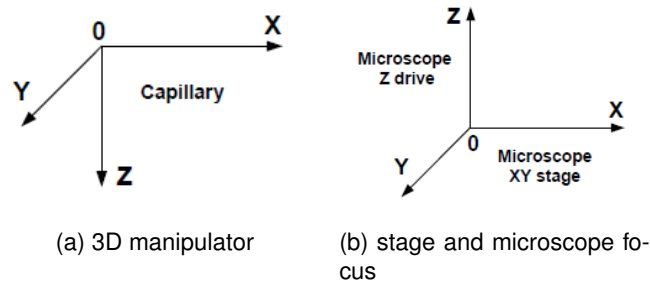
Figure 6.22: *Coordinate systems used in mmi CellEctor.*

The XY planes of the capillary and of the stage work fully independently. On the other hand, the relative position of the of the capillary and the microscope must be determined whenever a new capillary is inserted or its position is changed. For this purpose, the capillary is moved down to the contact position with the slide and the microscope is focused on both the capillary tip and the slide surface at the same time.

> If you enable the surface focus map (9.4.2), the down position will be altered depending on the position on the sample surface.

Procedure

1. Focus on the well surface

2. Move the working position of the well close to the screen center

   > The working position of a well is where the capillary should touch in the well when it comes down for acquisition, deposition or rinsing

3. Move the capillary to the idle position

4. Switch to 4x objective

5. Close the condensor iris lens to encrease the field of depth in z-direction

6. Move the capillary Y axis towards the optical axis of the microscope.

7. Totally move the capillary to the left (x-axis)

8. Lower the capillary down to approx. 2–3 mm above the slide plane.

9. You now should see the the capillary shadow

10. Center the capillary tip on the screen.

11. Move the capillary just above the well surface

12. Switch to your working objective

13. Focus on the slide plane.

14. Slowly lower the capillary down to the slide until it touches the latter. The contact position can be recognized by the onset of the sideward movement of the capillary as shown in Fig. 6.23.
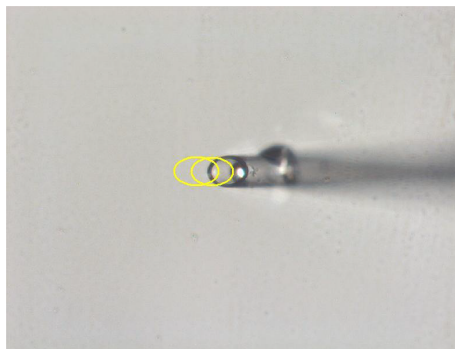
Figure 6.23: *Capillary starts moving sidewards, when touching the slide by moving the capillary down.*

15. Click the button for **acquire**, **deposit**, **rinse** or **all** in the **capillary position panel**

16. Mark the capillary tip

> The marker for the capillary tip indicates the position the cell is moved to before the capillary comes down for acquisition

> If common positions is selected you can set an offset between the down position for different well types with the spacing parameter (Fig. 6.21b)

6.14.3 Calibration guide for capillary positions

If the microscope focus surface is active a down position can also be calibrated by using the **down position guide** in the **Capillary position** panel. The guide window is shown in Fig. 6.24. A step-by-step wizard guides you through the calibration.

The guide runs through six steps described below and can be used for all down position types, independend if you use common down positions or not. The wizard takes into account, if the well has a wall or not. Walls exists p.ex. on 96 well plates, on single caps or on petridishes.
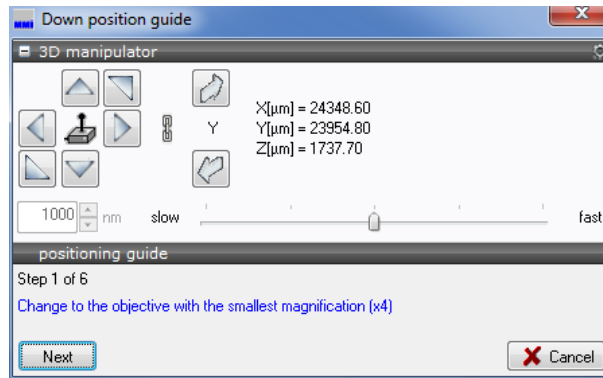
Figure 6.24: *Capillary down position guide window.*

Procedure

1. Once the down position guide is opened, a large target marker is shown in the center of the screen facilitating the 3D positioning. At this mo-

Figure 6.25: *Center of the screen indicated in the video panel.*

ment, the capillary stays in the idle position (the **Capillary up** button in the **Tools** panel is disabled). The system is now ready to move the capillary towards the optical axis of the objective and to lower it towards the slide. The first step is to change to the objective with the smallest magnification, usually 4x. Then press **Next**.

2. Move the stage so that the anticipated capillary landing point in the well is placed on the screen center.

3. The capillary is lowered so that its shadow should already be seen on the screen, see Fig. 6.26a. If it is not, you have to adjust its horizontal position first.

4. If the well has a wall please check that the capillary can move down by the wall height.

5. The system has now set the focus from the capillary tip back to the slide. Change to the objective you anticipate to work with during collection of cells.

(a) Capillary moved over the center marker but is still above the optical plane.

(b) Capillary lowered to the optical plane.

(c) Capillary positioned exactly on the center marker and to the optical plane.
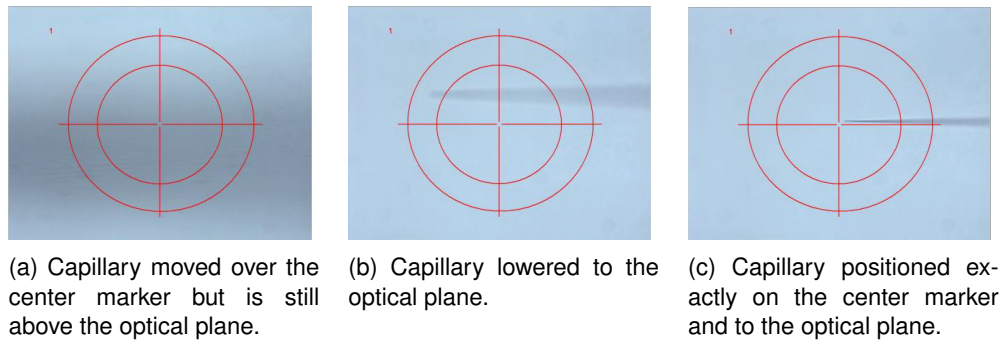
Figure 6.26: *Step 2: Capillary vertical positioning.*
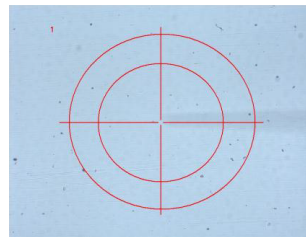
Figure 6.27: *Step 5: The system has now set the focus from the capillary tip back to the slide.*

6. The system further lowered the capillary, thus it is roughly in focus again, see Fig. 6.28a. Fine tune the position of the capillary tip. The tip can be moved out of the screen center if required. The vertical position of the capillary should be adjusted so that its tip gets in a gentle contact with the slide. To protect your capillary use slowest speed for the movement or fixed small distances to change the vertical position. At the contact position, both the slide and the capillary tip should be in focus, as illustrated in Fig. 6.28b. Click **Finish** to set the contact point of the capillary tip with the slide and to complete the capillary positioning.

(a) The system further lowered the capillary, thus it is roughly in focus again.

(b) At the contact position, both the slide and the capillary tip should be in focus.
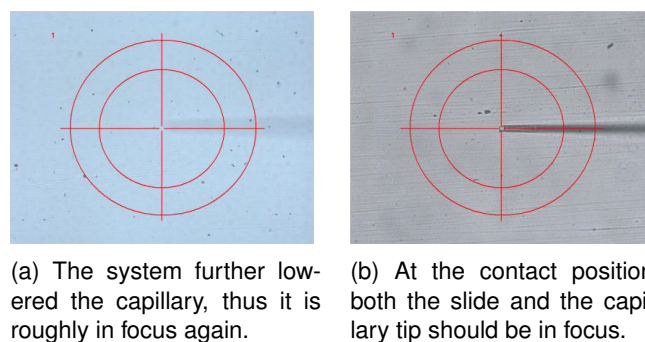
Figure 6.28: *Step 6: Down position guide*

6.14.4 Objective calibrations

In order to efficiently perform the acquisition and deposition of cells, *mmi CellEctor* requires the microscope objectives be set up properly. The objective setup includes the following

Procedure

1. calibration of the parfocal lens offset (automated microscope only, see section 9.4.3)

2. calibration of the paraxial lens offset (see section 5.7.3)

Before performing these calibrations, make sure the camera is aligned with the current objective (see section 5.7.2).

6.14.5 Calibrate park position

In some cases it might happen that the motors of the 3D manipulator loose track of their positions. Use

CellEctor →Calibrate →Park position

to recalibrate the positions of the 3D manipulator.

This calibration is automatically performed on software startup and shutdown. Usually, it is not necessary to execute the calibration during daily operation. If the software detects a position error, a warning and a **calibrate park position** button appears in the **Move capillary** panel.

6.15 Configuration routines

Before you start using *mmi CellEctor*, some preliminary work is necessary to make the system ready. Most of this work has to be done only once and is usually done by the MMI service technician during the installation of the system. Some activities are recurrent, like capillary positioning of a freshly inserted capillary.

6.15.1 Well types

The configuration of the stage insert including its slides and wells is described in detail in section **??**. A correct configuration of the slides, wells and slide

positions is essential for the *mmi CellEctor* to properly use the wells. The *mmi Cellector* is using wells of the following types:

- acquire: a source well, out of which the cells are collected.

- deposit: a target well, used to deposit acquired cells.

- rinse: a cleaning well, used to rinse the capillary.

How to define well types is descibed in section 5.6.3.

6.15.2 Capillary movement configuration

The capillary tip moves from the idle position towards the destination point along the three vectors shown in Fig. 6.29:
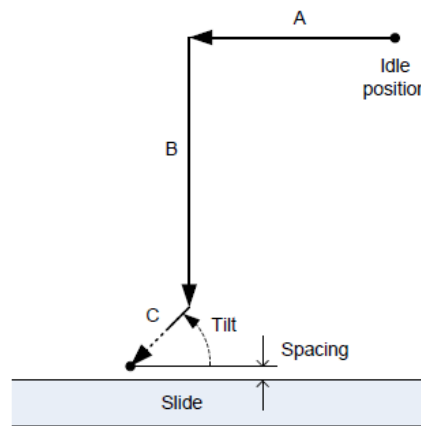
Figure 6.29: *Movement pathway of the capillary from idle to down position: A – horizontal movement; B – vertical movement; C – axial movement (immerse distance)*

- A – horizontal movement

- B – vertical movement

- C – axial movement (immerse distance)

The user can adjust the movement characteristics in the following dialog (Fig. 6.30) that can be opened from the main menu

    CellEctor →Configure →Capillary Movement

In many cased the **tilt** of the capillary can be set equal to the value read from the capillary holder. If the capillary should approch straight down to the

Figure 6.30: *Capillary movement configuration dialog.*

well surface set the **tilt** to 90°. **Immerse distance** corresponds to vector C in the above figure. **Slow down distance** is the last portion of the immerse distance (indicated as a dashed line on vector C in the above figure) where the capillary moves with a highly reduced speed in order to minimize pushing away the cells.

> While moving up out of the sample, the capillary moves with the normal speed.

Both immerse as well as the slow down distance can be set for acquisition, deposit and rinse wells independently. The numbers at the right hand side of the dialog (in [mm]) are calculated from a given capillary tilt and a given immerse distance. They help to adjust the immerse distance if the capillary has to be immersed in liquids confined to container walls.

Additionally the following parameters can be set:

- **slow down speed** defines the velocity in % of the maximum velocity of the capillary when moving the slow down distance

- **raise down position:** lifts the down position by the value in mm for a new capillary (section 6.9.2)

- **lock 3D manipulator:** the distance in µm below the down position at which the capillary movement is disabled

- **save stage travel:** an executed stage movement with a distance longer than the entered value results in a movement of the capillary to the idle position to prevent damage of the capillary

There are several other safety mechanisms for capillary movement (moving outside a well, changing the slide and others), which are unaffected by that value.

6.15.3 Aspiration configuration

With the *mmi CellEctor* it is possible to detach adherent cells mechanically or chemically. The aspiration configuration dialog (Fig. 6.31) can be open with:

$CellEctor \rightarrow Configure \rightarrow Aspiration$

or press the **properties** button in the **Actions** panel.
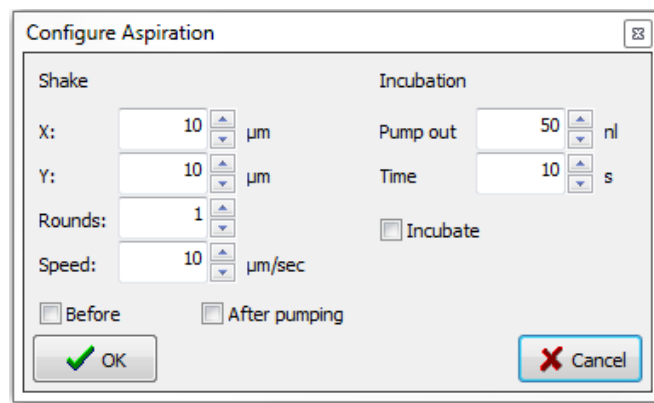
Figure 6.31: *Aspiration configuration dialog.*

The aspiration configuration enables a 'shaking' procedure that relocates the stage against the capillary to mechanically scrape off an adherent cell. Different parameters can be set:

- **X:** distance in x-direction in μm

- **Y:** distance in y-direction in μm

- **Rounds:** number of repeats

- **Speed:** velocity of the stage μm/sec

- **Before**/**After pumping:** shaking can be enabled before and/or after pumping

Additionally, an incubation can be set to detach cells chemically with a detergent loaded in the capillary:

- **Pump out:** incubation volume in nl pumped out. The same volume is pumped in before cell acquisition.

- **Time:** incubation time in s, which is maintained after the incubation volume was pumped out

- **ncubate:** activate/deactivate incubation step

6.15.4 Pump configuration
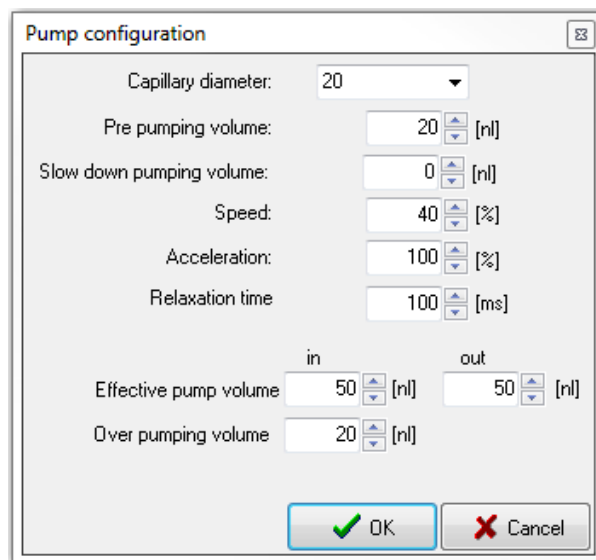
Figure 6.32: *Pump configuration dialog.*

All the pump parameters can be set in the pump configuration dialog that can be opened either from the main menu:

CellEctor →Configuration →Pump

or by clicking the **properties** button in the **Pump** panel. The following pump parameters can be changed by the user:

- **Volume in** main volume that is pumped in if the user presses the button Pump in or Acquire in the action panel

- **Volume out** main volume that is pumped out if the user presses the button Pump out or Deposit in the action panel

- **Slow down pumping volume** part of the main volume that is pumped in when the capillary approaches the target, i.e. before reaching the down position. Useful to collect floating cells.

- **Pre-pumping volume** volume that is added to the main volume whenever the pump changes the direction (from in to out or vice versa)

- **Over-pumping volume** volume that is pumped in prior to the main volume to be pumped in and then pumped out after the main volume has been pumped in. This value has no effect on the pumping out process.

- **Relaxation time** time waiting for the pressure to equilibrate after the pumping process is finished

- **Speed pump** speed (in percent of the maximum speed)

- **Acceleration pump** acceleration (in percent of the maximum acceleration)

- **Capillary diameter** the current capillary size for which the pump parameters are displayed. Click on it opens the list with available capillaries (as shown in the figure above).

All the pump parameters are specific for a given capillary. Up to five parameter sets can be stored for capillaries with different sizes. You can retrieve a stored parameter set for a given capillary by selecting the corresponding capillary size.

> Whenever the pump parameters are modified, the changes are saved automatically for that capillary setting.

6.15.5 Acquisition configuration
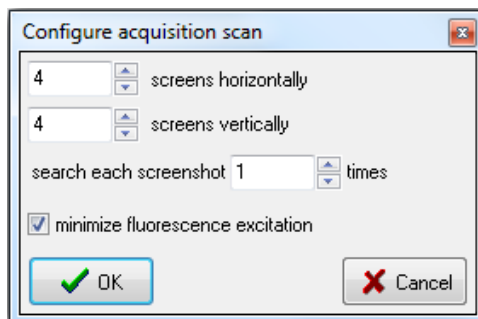
Figure 6.33: *Cell acquisition configuration dialog.*

For fully automated cell acquisition the cell searching process needs a few parameters to be set. The configuration window, Fig. 6.33, can be opened from the main menu:

CellEctor →Configure →Acquisition scan

In the fully automated mode **Scan for cells** acquisition is preceded by making a snapshot of the current field of view and automatic cell recognition and selection. The collection well is scanned horizontally and vertically by a user

defined number of screenshots. For each scan a single cell is detected, acquired and deposited. To scan a screen multiple times you can edit the **search each screenshot** parameter.

Minimize fluorescence excitation option is relevant only for the case that the automatic cell recognition is performed under fluorescence observation and is used to prevent bleaching of cells. If this option is checked, the motorized fluorescence shutter is opened just before a screenshot is made and closed immediately afterwards.

6.15.6 Deposition configuration

Several parameters of the deposition process can be defined in the deposit configuration (Fig. 6.34), that can be opened from the main menu:

CellEctor →Configure →Deposition

The number of cells per well defines how many cells can be collected in one deposit well. The software assumes that in one deposition procedure exactactly a single cell is deposited. No further deposition is possible if all deposit well are full.
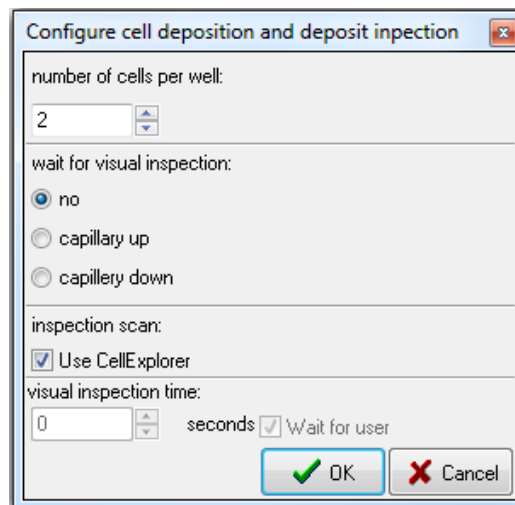
Figure 6.34: *Cell deposit and deposit inspection configuration.*

If you need to get time for a visual control of the deposition result, you can configure the system to wait for the **visual inspection time** in the capillary idle position or the capillary down position. In the standard deposition process, the capillary is moved up as soon as the pumping out is completed (including the pump relaxation time). After the inspection the microscope stage will be moved back to the acquisition well.

The scanning scan always includes all deposit wells with at least one deposited cell. For automatic inspection of the deposit wells, check **use Cell-**

Explorer. In this case the microscope stage waits until the recognition of cells is completed by *mmi CellExplorer*. Otherwise the system waits for the **visual inspection time**. A report will be created automatically (see also chapter 6.11.5).

Prior to starting the deposit inspection with the image processing, the correct image processing parameters have to be loaded to *mmi CellExplorer*.

6.15.7 Workflows editor

In *mmi CellEctor* a workflow is a combination of acquisition, deposition and the capillary treatment on the service slide that is launched by a single mouse click as a continuous series of actions. The workflow can be customized in the workflow editor (Fig. 6.35).

CellEctor →Configure →Workflow

In the example shown in the above figure, the workflow consists of three consecutive steps:

Procedure

1. acquisition

2. deposition

3. cleaning the capillary in well #4 of the service slide
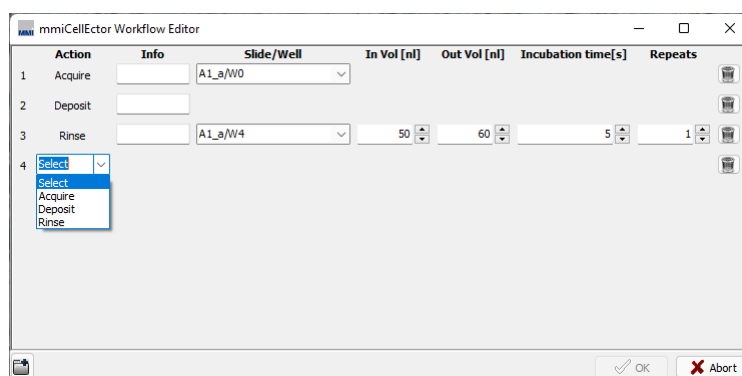
Figure 6.35: *The workflow editor*

The parameters of a single step can be modified by editing the values in a single row of this table. The entire rows can be deleted or inserted.

Once the workflow has been edited and the workflow editor is closed, it becomes the active workflow in *mmi CellEctor*. Pressing **Workflow** or **Scan for cells and run workflow** in the action panel, the workflow or *mmi CellExplorer* scan in combination with the workflow is launched, respectively.

6.16   Optimization of the cell collection

mmi CellEctor can be used in a variety of modes starting with the step-by-step mode, via the semi-automatic mode ending with fully automated workflows.

In the manual mode, the basic steps of acquisition and deposition like moving the microscope stage, moving the capillary or pumping are not combined in a sequence of actions. Thus, the user can perform each single step separately. The manual mode is used mainly for the tuning of the system to new experimental conditions (type of cells and buffer solution).

Once the optimum conditions for the movement of the capillary and pumping have been found, either the semi-automatic mode or the fully automated workflows can be used.

In the semi-automatic mode, the work is divided in three steps: acquisition, deposition and rinse.

In fully automatic workflows, these three steps are combined in a series of actions (workflow). The simplest workflow is Acquire and deposit, the most advanced is Scan for cells. If required, the rinse step can be included.

> While working in the semi-automatic mode or executing fully automated workflows with automated microscopes, it is compulsory to determine the plane tilts for all slides.

There are two key steps in the process of collecting rare cells:

- getting a cell into the capillary at the sample

- getting the cell out of it at the target

Since there is a large variety of cells and of buffer solutions, the working parameters of *mmi CellEctor* have to be optimized for the given experimental conditions. The major optimization parameters are:

- pump parameters

- spacing between the capillary and the slide surface

- size, tilt and orientation of the capillary

Please note, that prior to the tuning of the system, one has to determine the tilt of the slide plane(s) (see section 9.4.2).

6.16.1 Pump parameters

In order to understand the meaning of the pump parameters, let us go through an example of the following series of the user's actions:

Procedure

1. Acquisition of a cell.

2. Deposition of the cell.

3. Acquisition of another cell, however, the cell could not be pumped in the first attempt.

4. Retrial to pump the cell in.

The Fig. 6.36 shows how the pump behaves during these four actions under assumption that the following pump parameters have been set:
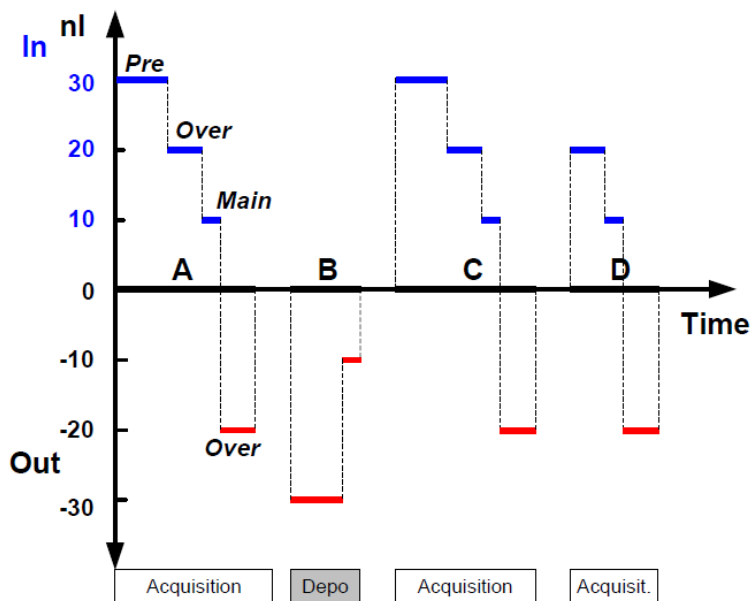

Figure 6.36: *Panel representing the workflow functions of mmi CellEctor*

- Main volume 10 nl for both in and out

- Pre-pumping volume 30 nl

- Over-pumping volume 20 nl

During the acquisition (1.) of the cell, 30 nl (pre-pumping) are pumped in at first. We assume here that pumping out took place just prior to it, thus the pump changes the pumping direction and 30 nl are necessary for the compensation of the overpressure remaining after pumping out. In the second step, 20 nl are pumped in (over-pumping) that will be then pumped out in the fourth step. Thus, over-pumping does not add net volume to the main volume. However, it helps to pump in cells that adhere to the slide surface. In the third step, the main volume of 10 nl is pumped in. Thus, totally 40 nl is pumped in during the acquisition process.

During the subsequent deposition (2.) of the cell, 30 nl are pumped out first (pumping direction changed again) and then additional 10 nl of the main volume, resulting in a total of 40 nl. The next acquisition action (3.) proceeds exactly the same way as the first one (1.). Afterwards, the user launches an extra pump in process (4.) in order to get the cell into the capillary. Since the pump did not change its direction (the over-pumping volume that is pumped out at the end of the pumping in does not count), no pre-pumping volume is added to the main volume this time.

There are no universal rules for choosing the values of the pump settings, however, several practical hints can be given:

- The smaller the diameter of the capillary tip, the slower are the dynamics of the fluid movement through the tip and the larger is the required under/over-pressure to pump in/out. If the dynamics are slow, one has to increase the pre-pumping volume, the relaxation time as well as the speed and the acceleration of the pump.

- Light objects can be drawn into the capillary with a small main volume and no over-pumping.

- To catch heavy objects, e.g. cells labeled with paramagnetic beads, reduce the main pump-in volume to nearly zero and work with over-pumping volume solely. The pump speed and acceleration should also be reduced in this case.

- Whenever possible, use capillaries with a large tip diameter, even if the cells are relatively small. The optimal pump parameters can be adjusted much easier for such capillaries and the pumping process is faster.

6.16.2 Spacing and capillary orientation

The distance between the capillary tip and the surface of the slide affects the efficacy of the cell acquisition and deposition significantly. There are different practical rules of thumb for setting the spacing value depending on the orientation of the capillary bevel with respect to the slide surface:

6.16.2.1 Capillary bevel parallel to the slide surface

If the capillary bevel is oriented parallel to the slide surface, the cells get into the capillary by the vertical flow forces. Following rules apply:

- The smaller the distance of the capilary and the sample, the smaller the volume of the liquid that has to be pumped in to get a cell into the capillary. However, one has to take into account the size of the cells compared to the diameter of the capillary tip and accordingly the height of the cells compared to the spacing.

- The above rule does not apply for pumping the cells out on the deposit. If the deposition takes place on a dry surface, the surface tension of the liquid has to be considered, which, at small spacing, may prevent the liquid from flowing out of the capillary.

- The optimum spacing on the deposit depends on the amount of liquid to be pumped out and vice versa. Both parameters should be chosen in a way that the liquid touches the deposit surface and flows out of the capillary before a droplet is formed and falls down.

- For deposition into a single cap, spacing should in principle be set to almost zero. Other values may still be used to keep the capillary tip in the liquid to correct changes in the liquid level caused by evaporation.

6.16.2.2 Capillary bevel vertical to the slide surface and cell scratching

If the capillary bevel is oriented vertically to the slide surface, the flow forces (parallel to the slide surface) can be combined with a scratching action of the capillary. Scratching is achieved by sliding the capillary tip along the slide surface. The capillary will move in this way if the spacing is set to a negative value since the capillary narrow end part will be deflected by the slide surface.

Be aware that the capillary may break if the negative value exceeds -10 μm. Please observe the safety precautions described at the beginning of this manual (section 1).

6.16.3 Capillary size and tilt

Some hints concerning the size have already been mentioned above. Generally, acquisition is easier with a bigger capillary tip diameter. However, a larger capillary tip pushes away more liquid and is less precise and selective. Whenever you have a high cell density or you need high precision use a smaller tip diameter.

The tilt can be chosen arbitrary unless the tip bevel should be parallel to the slide surface; in that case the tilt angle should be equal to the bevel angle. By changing the tilt of the capillary holder the working area of the capillary tip is changed, too. Take care that you can reach the sample surface in the field of view with the capillary tip. A tilt angle of 45 degree is usually a good choice. Remember to enter the tilt angle in the software (section 6.15.2).

6.16.4   Collecting cells to a matrix of spots (grid)

Since there is a variety of commercially available grids, we will describe collecting of cells to AmpliGrid as an example. Most of the steps apply, however, to all other grids as well.

If a new grid is placed under the microscope, the following operations have to be performed in order to make the grid ready for cells:

Procedure

1. Notify the software about the new grid: Open the **deposit configuration** dialog, select the type of the grid, set its parameters if necessary (custom grid) and check the option **New grid inserted**, if it has not automatically been checked.

    This deletes all the data stored for the previous grid.

2. Set the number of cells you want to deposit at each spot. Please note that this number is used to determine whether or not the spot is full, i.e. whether the next cell can be added to the current spot. Close the deposition configuration dialog.

3. Determine the tilt of the grid plane.

4. Optionally, determine the contact point of the capillary on the new grid. This is necessary only if the thickness of the new grid significantly differs from the previous one and/or a high accuracy of the spacing between the capillary and the grid is required.

5. Calibrate the position of the spot located in the first row, first column (R1C1).

6. Perform the first deposition at the first spot of choice leaving the capillary down after pumping out. If the buffer solution with a cell could not leave the capillary after having pumped out the preset volume, increase the latter or decrease the capillary spacing to the deposit.

Continue with filling all other spots with cells. The software registers the number of deposition actions in each spot and assumes that one cell is deposited

Table 6.1: *Standard settings for beads*

Capillary	
Bevel orientation	Parallel to slide
Tip diameter	40 µm
Spacing to sample slide	5 - 10 µm
Spacing to AmpliGrid	50 µm

Pump	
Pump in volume	10 nl
Pump out volume	100 nl
Pre-pump volume	0 nl
Over-pump volume	0 nl
Relaxation time	0 ms
Speed	100%
Acceleration	100%

each time. Once every spot in the grid is filled with the preset number of cells, the entire grid is full, no further deposition is possible and the user is prompted to replace the grid.

> One can also fill a selected part or every second spot on the grid only. To do the former, simply define the current and the end row/column in the deposit configuration dialog. To do the latter, define a custom grid with spacing between the columns that is double the spacing and the number of columns (rows) that is a half of the number of columns (rows) on the actual grid.

6.16.5   Standard settings for beads

The settings in the Table 6.1 apply to 10 µm polystyrene beads in aqueous solution. Concentration of the beads should be approximately 20 beads in the field of view of a 20X objective.

7 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.

7.1 Getting started

7.1.1 Quick Start

7.1.1.1 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 7.1: *The CellTools startup banner*

7.1.1.2 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

7.1.1.3 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*

7.1.1.4 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.

7.2 Slide scanning

To enable the creation and storage of full resolution scans check **Create WSI's** in the settings panel (Fig. 7.2). Every time a scan is started now (see section 5.4.4), 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 5.5).

The slide viewer is updated with the most recent image by default. It is displayed at the position of the active slide.
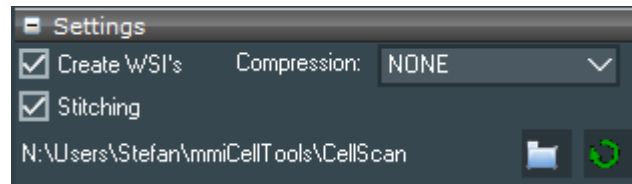
Figure 7.2: *The CellScan settings*

If the option **Stitching** is checked CellScan will use an active stitching procedure, so that no seems 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.

7.3 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. Additionially, if your microscope is equipped with fluorescence you can perform scans in fluorescence mode (see section 5.2.1).

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 5.3.2), with an longer exposure time the acquisition of each frame increases, which leads to an elongated scan time

- **acceleration** of the stage: the stage acceleration can be set in stage settings (see 5.4.3), 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 recomended to set the focus map properly (see section 9.4.2). If the focus map is predefined, the microscope changes its focus during scanning accordingly.

Note: Before using the CelScan make sure the camera is aligned properly (see section 5.7.2)

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.

7.4 Explorer panel

Figure 7.3: *The CellScan explorer*

In the explorer panel (Fig. 7.3) 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 (7.5). 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.

7.5 Preview panel
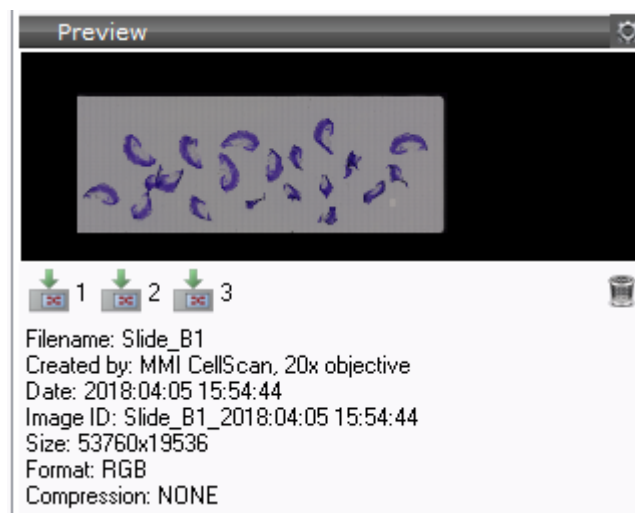
Figure 7.4: *The CellScan preview*

An image that was selected in the explorer panel is displayed in the preview frame Fig. 7.4. 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.

7.6 Cell types
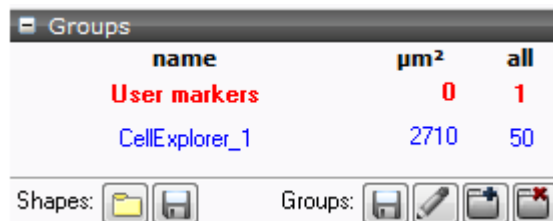
Any object drawn will always be assigned to the highlighted cell type group shown in the **Cell types** panel (Fig. 8.2).

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

.

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

The active cell type group is highlighted. To switch between groups, click on the group of interest from the group list.

Figure 7.5: *Cell type group selection.*

Using the checkbox on the left side of Fig. 8.2 the group can be selected as **collectable**. If you have a *mmi CellCut* and the group is aslo selected as **cuttable** the *mmi CellEctor* will automatically try to collect cut shapes.

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

7.6.1 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. 8.3) using the edit button.
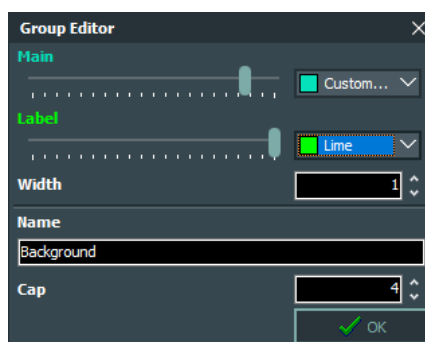
Figure 7.6: *Group editor*

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

7.6.2 Regrouping shapes

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

7.6.3 Group statistics

The statistics can be seen directly in the group panel (Fig. 8.2).

For each group, the list displays the number of shapes and their total area.

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.

7.6.4 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. 8.4).
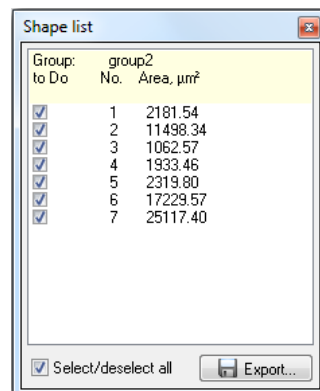
Figure 7.7: *Shape list*

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

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

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

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

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

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

7.7   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.

7.7.1   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 particulary 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 5.2.1 respectively 5.2.2. If the required microscope parts are not motorized, **CellTools** will show a message what you have to change. After the automatic sample detection **Cell-Tools** 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 5.6.2.

7.7.2   Auto-focus Mapping

This option will automatically create a focus map (see 9.4.2) 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.

7.7.3 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.

7.7.4 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.

7.7.5 Z-Stack

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

- z Min: The minimal position below the current focus position where the 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.

7.7.6 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 5.2.1). If the option **Stitching** in Fig. 7.2 is checked, only one channel can be selected as **stitch** channel in the channel editor. This **stitch** channel will be used for stithing procedure, other selected multi channels will copy the stitching imformation, so that all seperate images have identical stitching result Fig. 7.8. 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

stitch channel. If the scan is finished the merged multi-channel image can be displayed using the slide viewer (see section 5.5).
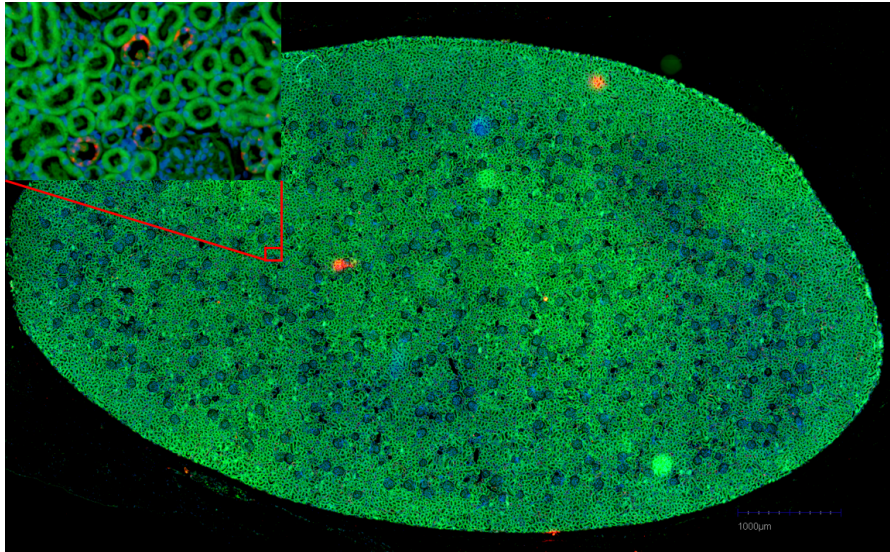
Figure 7.8: *multi-channel image in full resolution with stitch*

8 mmi CellDetector

The *mmi CellDetector* is an image analysis software to automatically detect and classify target cells. The resulting cells shapes can not only be used to isolate the cells using *mmi CellCut* or *mmi CellEctor* but also can be measured or count. The software is using models to detect the specified cells and returns a pixel precise boundary.

To create a *mmi CellDetector* model, the *mmi CellDetector* has to be trained with user created cell annotations (shapes). With its ability to learn by user input the software offers a high degree of flexibility and countless applications. The more cell annotations are used for the training the more specific and precise the cell finding works.

The *mmi CellDetector* is available in two flavours.

- detect objects on whole slide images (WSI)

- detect objects on the live image

The *mmi CellDetector* can be used in perfect harmony with all other mmi products and enables the automation of scanning (*mmi CellScan*), detection (*mmi CellDetector*) and isolation (*mmi CellEctor* or *mmi CellCut*) of cells.

8.1 Basics

Switch to the CellDetector panel by pressing the corresponding tab button.

The primary functionality of the *mmi CellDetector* can be found in the *mmi CellDetector* panel (see Fig. 8.1). This panel controls the cell types, the training process, the cell detection as well as post processing of the found cells. Moreover, options to split connected areas and to combine overlapping cells are being made available.
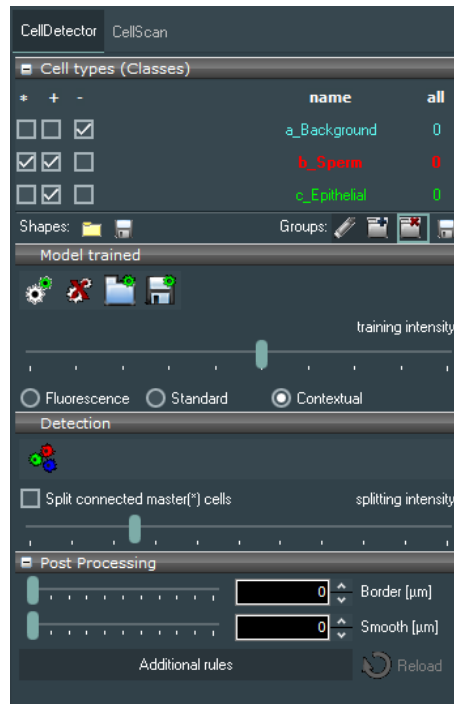
Figure 8.1: *Main panel*

8.2   Cell types

Any object drawn will always be assigned to the highlighted cell type group shown in the **Cell types** panel (Fig. 8.2).

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

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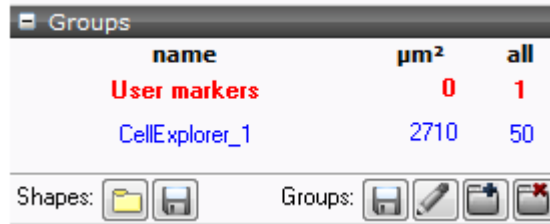
All objects of the same cell type group are marked with the same contour color. The number of groups is not limited.

The active cell type group is highlighted. To switch between groups, click on the group of interest from the group list.

Using the checkbox on the left side of Fig. 8.2 the group can be selected as **collectable**. If you have a *mmi CellCut* and the group is aslo selected as **cuttable** the *mmi CellEctor* will automatically try to collect cut shapes.

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

Figure 8.2: *Cell type group selection.*

8.2.1 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. 8.3) using the edit button.
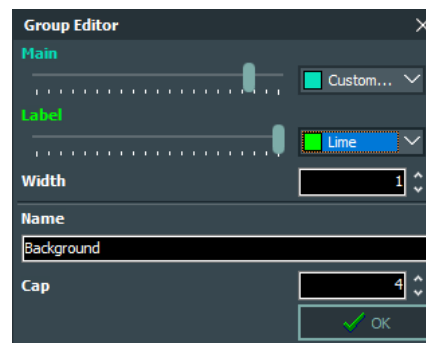
Figure 8.3: *Group editor*

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

8.2.2 Regrouping shapes

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

8.2.3 Group statistics

The statistics can be seen directly in the group panel (Fig. 8.2).

For each group, the list displays the number of shapes and their total area.

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.

8.2.4 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. 8.4).
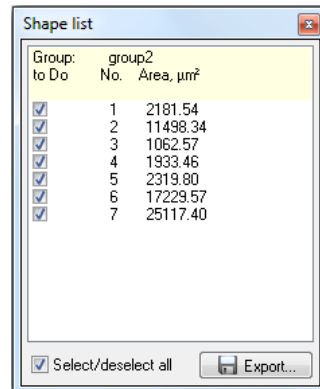
Figure 8.4: *Shape list*

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

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

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

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

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

The order of the cell type groups is important for *mmi CellDetector*. If you want to save and reload anntations, make sure that the group names starts with a sorted letter (a_xxxx, b_xxxx, c_xxxx ....) to keep the order unchanged. If the group list, including the class order, differs between the training and the detection phase, the detected cells will show up in wrong classes.

To learn how to draw and change training objects, refer to 5.5.1

8.3 Modes of Operation

The *mmi CellDetector* can be used in two different operating modes. The *live cell detection mode* and the *whole slide detection mode*.

8.3.1 Live cell detection

The live cell detection mode operates on the live image of the microscope. No additional scanning feature is required. The *mmi CellDetector* panel can be found in the main window of CellTools, see Fig. 8.5. When the detection is executed (see 8.5), the current field of view is analysed for target cells.
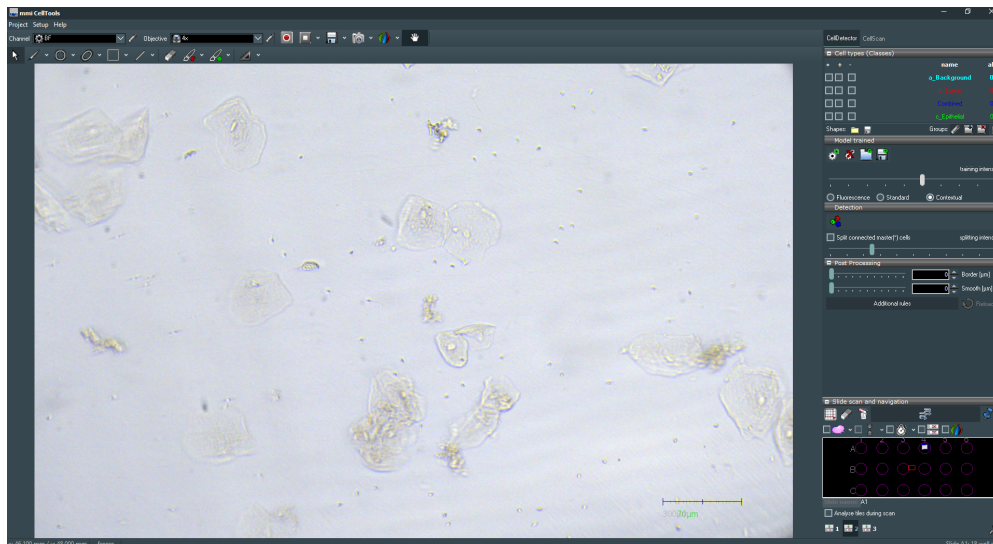
Figure 8.5: *mmiCellTools with mmi CellDetector*

8.3.2 Whole slide detection

The whole slide detection mode operates on a scanned whole slide image (e. g. scanned with the *mmi CellScan*). The *mmi CellDetector* panel can be found in the imageviewer window. When the model is trained (see 8.4), the loaded whole slide image image and the drawn shapes are used as input to train the image anlysis algorithm. When the detection is executed (see 8.5), the selected image analysis (see 8.5.1) of the loaded whole slide image is analysed for target cells.
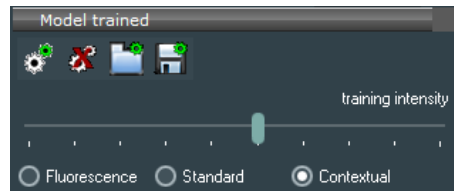
Figure 8.6: *Training intensity control in the main panel*

8.4 Training

8.4.1 Training Intensity

With the **Intensity** slider the training intensity can be changed. the higher the training intensity, the more complex the underlying model and the longer the training duration.

8.4.2 Input Features

The *mmi CellDectection* uses different input features for the cell recognition. The features encopass color, texture and contextual features. The most important characteristic of image data sets is that they have a large number of variables. These variables require a lot of computing resources to process them. In order to effectively reduce the amount of data we need to select and combine image characteristics which are easy to process and describing the actual data set with the accuracy and originality. There are three options to specify which type of image characteristic are taking to the account.

- Fluorescence: When the fluorescence mode active, only different color values of each pixel in image is taking to the consideration.

- Standard: When the standard mode is active, only the value of pixels in the image are utilize to get image information.

- Contextual: When the contextual mode is active, besides considering the content features like color values of each pixel(visual information), it focuses on context features(annotations) such as neighborhood information for each pixel in the image.

To train the image analysis algorithm do:

Procedure

1. Define the classes used for training annotations and background, see section 8.2

2. Define the master class

3. Define the background class

4. Draw sufficient number of training annotations ind the detectable classes (wanted cells)

5. Draw sufficient number of training annotations in the background shapes (not wanted areas)

6. Define the model complexity, see 8.4.1 and section 8.4.2

7. press the **Training** button.

The *mmi CellDetector* uses the user input to optimize the underlying model in that way, that the model predicts cells similar to its input. The training status can be monitored in the status panel (see 8.4.5).

The training time depends on the number of input cells, the training intensity and the selected input features. More shapes, higher training intensity and more input features result in longer traning times but better detection results.

When the training is finished a region of interest can by analyzed for target cells, see chapter 8.5.

Training can be repeated using the current input each time to continuously improve recognition accuracy.

It is possible to delete the trained model by pressing the **reset model** button. By resetting the model, it is possible to apply further changes to the model and re-train the model. However, it should be noted resetting the model bring us to the start point. All the information about the current shapes, the training intensity, and type of image is going to be lost.

8.4.3 Live cell *mmi CellDetector*

During the training process (see 8.4), the microscope moves to the position of each selected cell and uses the live image and the corresponding drawn shape as input to train the *mmi CellDetector* model.

8.4.4 Whole slide image *mmi CellDetector*

When the model is trained (see 8.4), the loaded whole slide image image and the drawn shapes are used as input to train the image anlysis algorithm.

8.4.5 Training Status

The training status are as follows:

- Model not trained: Before starting the training.

- Training percentage: During the training process show how many percentage of training completed.

- Model trained: When the training is finished successfully.

8.4.6 Reusing Models
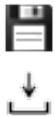
By pressing the **Save** button models can be saved. Each saved model can be reused and loaded with the **folder-group-icon-24x24** button.

8.5 Detection

After the Training is finished the trained model can be used to detect target cells by pressing the **Detect** button. Depending on the operation mode (see 8.3) either the image analysis area or the current field of view is analyzed for target cells.

8.5.1 Select image Analysis area for whole slide image detection

The image analysis tool is used to mark the area used for cell recognition. Draw a rectangle around the region of interest. By pressing the detect button (see 8.5) that area is analysed for target cells. When the detection is executed (see 8.5), the selected image analysis area(see 8.5.1) of the loaded whole slide image is analysed for target cells.

8.5.2 Split connected cells

By activating the split the connected cells check box, an algorithm will calculate and identify connected areas. Through using image processing techniques, it tries to split connected areas in seperated cells. With respect to the fact that different images might have different cell types, the split intensity would be different. It needed to be adjusted to get more efficient result.

8.6   Post Processing

The post processing panel allows additional refinement of the detected cells.
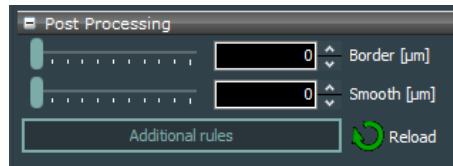
Figure 8.7: *Post Processing panel*

The **Border** slider adds a border around the detected cells. This might be important if the cells are processed with the **mmi CellCut** to cut outside the detected of the detected cells.

The **Smooth** slider smoothes the contours of the detected cells. The higher the value the more the contours are simplified.

With the **Reload** button the most recent detected contours can be reloaded with current post processing settings. So, when changing a postprocessing parameter, it is not necessary to run a full detection, but instead the detected contours can be reloaded with the new settings, quickly.

8.6.0.1   Class rules

Figure 8.8: *class rules*

The **Class rules** window allow to define several rules which conditions detectable cells have to fullfil.

With the **Min** and **Max** sliders cells can be sorted out by their size. The sliders are logarithmic to map the size from single cells up to complete tissue parts.

With the **Min circularity** and **Max circularity** sliders cells can be sorted out by their roundness. If you want to discriminate elongated cells encrease the **Min circulatity**.

The **Combination rules** allow to define several rules which conditions overlapping cells have to fullfil, so that a cell from the master group is copied into to **comined** group.

Following rules are available:

- the master cell overlaps with a minimum area of cell of the defined class

- the master cell overlaps with a maximum area of cell of the defined class

- the master cell overlaps with a minimum number of cell of the defined class

- the master cell overlaps with a maximum number of cell of the defined class

- the master cell overlaps with a minimum area of cell of the defined class relative to the defined cross class (p. ex. minimal 2 times bigger area of green cells than blue cells)

- the master cell overlaps with a maximum area of cell of the defined class relative to the defined cross class (p. ex. maximal 8 times bigger area of green cells than blue cells)

- the master cell overlaps with a minimum number of cell of the defined class relative to the defined cross class (p. ex. minimal 3 times more green cells than blue cells)

- the master cell overlaps with a maximum number of cell of the defined class relative to the defined cross class (p. ex. maximal 10 times more green cells than blue cells)

Rules can be combined using an **AND** or **OR** operator. Selecting **UNUSED** will deactivate the rule. Allways use **AND** to activate the first rule.

A parameter set to a negative value will deactivate the rule.
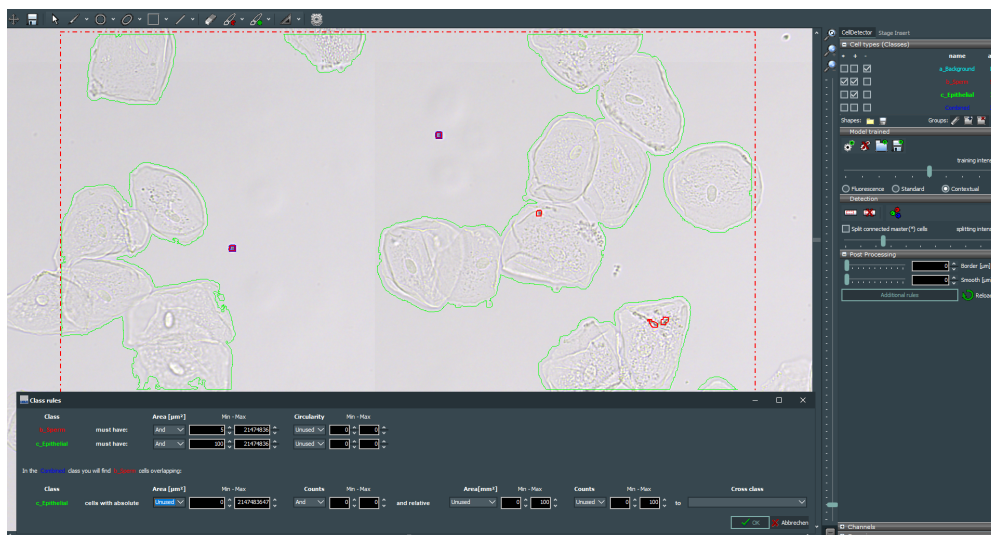
Figure 8.9: *Class rule example: With the **area** and **cicularity** sperm and epithelial cells can be indentified. Select the sperm cells, which arenot co-located with epithelial cells.*
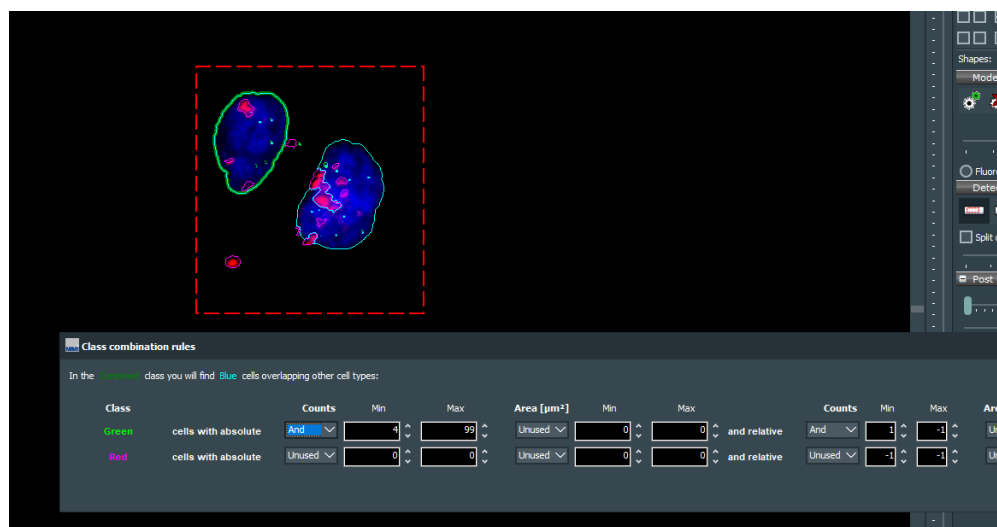
Figure 8.10: *Class combination rule example: Select the blue cells with at least 4 green cells and at least as many green as red cells. The found cell shows the bold contour.*

8. MMI CELLDETECTOR

9 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.

9.1 Activating microscope control

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

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

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

Microscope →Remote control

or by press *CTRL + M*.

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

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

9.2 Observation methods

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

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

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

When you change the channel, the motorized microscope will set these three items to the positions defined in the new channel automatically. The optical path settings are specific to microscope vendors (see Table 9.1).

Table 9.1: *Optical path settings for Olympus and Nikon microscopes*

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

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

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

> *Microscope →Binocular*

or by pressing *F7*.

9.3 Objective control

In the objective editor, see section 5.2.2, you can save nosepiece position number and the lamp voltage used.

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

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

9.4 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. 9.1).
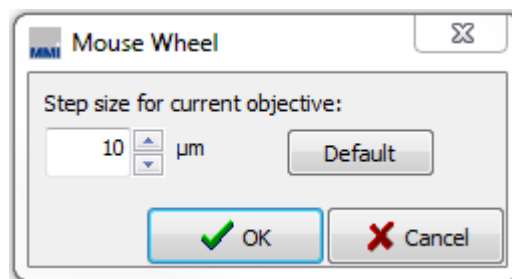
Figure 9.1: *Mouse wheel settings*

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.

9.4.1 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

9.4.2 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.
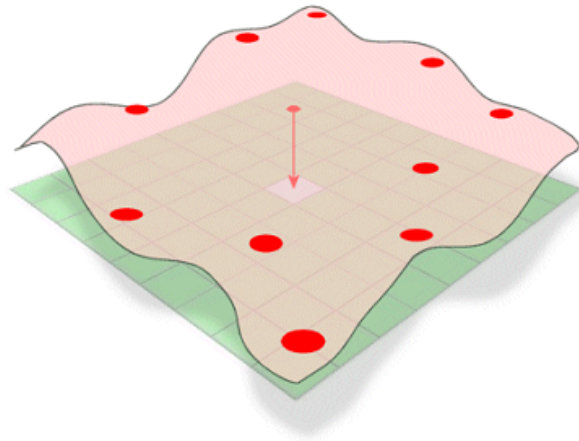
Figure 9.2: *Sample surface with defined surface points*

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.
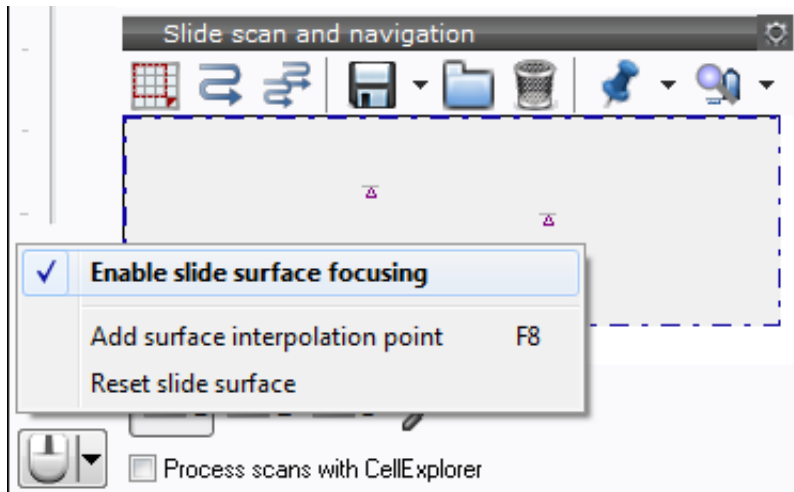
Figure 9.3: *Surface map user interface*

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.

> Neither the single cap nor the service slide require the determination of the plane tilt.

9.4.3 Z drive offset calibration for objectives

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

Procedure

1. Start with the objective with highest magnification

2. Focus microscope

3. Select another objective with closest magnification factor

4. Focus microscope

5. Select menu item

> *Setup →Parfocal Lens Offset*

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

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

9.4.4 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.

9.5 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.
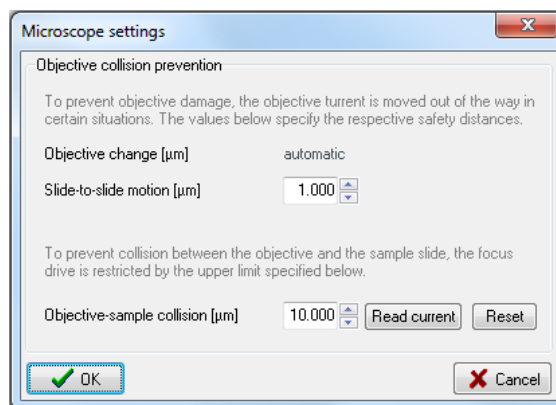
Figure 9.4: *Microscope safety parameters*

Open the dialog using the menu item

> *Microscope →Safety parameters. . .*

9.5.1 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.

9.5.2 Objective-sample collision

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

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

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

Procedure

1. Select the objective with the shortest working distance (normally the objective with the highest magnification)

2. Focus the microscope

3. Open the safety parameters dialog:

    Microscope →Safety parameters. . .

4. Press the **Read current** button

5. Add the working distance in μm to the displayed value and go to step 8, or if the working distance is unknown proceed with step 6.

6. Carefully move the objective as close as possible to the specimen without touching it.

7. Write down the value displayed under Objective-sample collision.

8. Press the Read current button again.

9. Now enter an Objective-sample collision limit half-way between the two positions.

10. Click OK

9.6 Fluorescence shutter control

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

Microscope →*Shutter*

or press *F6*.

10 Maintenance

10.1   System check

- Visually inspect the housing periodically to verify that no panels are loose or distorted.

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

10.2   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.

10.2.1   Microscope

For further details, see microscope manual.

10.2.2   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%

10.2.3   Cleaning when actually dirty

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

- Lightly moisten a cleaning tissue with pure alcohol (70-80%) or spirit and wipe the dirty parts very carefully

- Clean dry objectives and oculars gently with a cotton bud or a cotton wool wrapped around a toothpick; Only use surgical cotton from the pharmacy

- Remove cotton lint and fluff by blowing, e.g. with an enema syringe

Caution

- Moisten cleaning tissue only slightly, do not soak; Excessive solvent might dissolve the cement of the lenses

- Do not use acetone for cleaning under any circumstances

10.3   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.

10.3.1   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

10.3.2   Movement

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

- Wrong objective selected

- Camera alignment not correct (see section 5.7.2)

- The camera is not mounted in an exactly upright position

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

- Wrong objective selected

- Camera alignment not correct (see section 5.7.2)

- The camera is not mounted in an exactly upright position

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

- Parfocal lens offset not correct.

Displacement of markers after objective is changed:

- Paraxial lens offset not correct.

Overview images not matching stage geometry.

- Stage geometry not properly defined, see section 5.6

- Stage origin calibration invalid, see section 5.7.1

10.3.3   Overview

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

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

A Service

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

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

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

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B List of Keyboard Shortcuts

General

+	Move to next shape	. . . . 26, 34
-	Move to previous shape	. . . . 26, 35
Alt + C	circle tool	. . . . 29, 67
Alt + Del	delete shape tool	 31
Alt + E	ellipse tool	. . . . 30, 66
Alt + F	freehand tool	. . . . 29, 67
Alt + L	line tool	 30
Alt + M	Measure distance	 35
Alt + Q	rectangle tool	 31
Alt + R	select and move tool	 28
Arrow down	Move small step down	 35
Arrow left	Move small step left	 35
Arrow right	Move small step right	 35
Arrow up	Move small step up	 35
Caps Lock	Suppress continous stage movement	 22
Ctrl + A	Align Camera	 44
Ctrl + C	Copy shape	 32
Ctrl + Del	Delete all shapes	 32
Ctrl + H	Hide shape indices	 33
Ctrl + I	Show stage insert	 26
Ctrl + O	Set stage origin	. . . . 44, 46
Ctrl + P	Stage movement settings	 22

Camera control

CellEctor

Microscope control

Navigation

move to

move to

move to
last shape

move to

delete
shape

Insert | Home | Page Up

Del | End | Page Down

Num Lock | / | * | –

7 | 8 | 9 | +

4 | 5 | 6

1 | 2 | 3 | Enter

0 | .

press: minor steps
hold: slow movement

press: major steps
hold: fast movement

C Disposables

Our current range of disposables is available on our website:

www.molecular-machines.com/products/consumables

D Technical data

D.1 Required minimum workspace

The table top for the microscope with 3D manipulator, optical equipment, mmi pump, 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.

D.2 System components

D.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

D.2.2 Stage

Standard stage

Scanning stage with stepper motors.

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

Long travel scanning stage for two inserts

Long travel scanning stage with stepper motors.

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

D.2.3   Digital camera

mmi CellCamera VCXU23c

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

mmi CellCamera VCXU50m

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

mmi CellCamera MXF285c

- IEEE1394b (FireWire) color CCD camera
- 2/3" interline transfer frame readout CCD
- Temperature controlled active Peltier cooling

- signal to noise ratio: > 62 dB
- On board integrated color processor for high quality color calculation
- 1392 × 1040 pixels with up to 20fps
- Ultra high sensitivity

mmi CellCamera DXA285cF

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

mmi CellCamera DXA285F

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

Hamamatsu Orca Flash4.0 V3

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

Hamamatsu Fusion BT

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

D.2.3.1 Andor iXonEM+ EMCCD Camera 897

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

D.2.4 Fluorescence Light sources

D.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 ± 10

D.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.

D.2.6 3D Manipulator

Travel range:	25 mm
Resolution:	0.025 µm

D.2.7 *mmi CellPump*

Precision	2 nl

E Declaration of Conformity

C E

Declaration of Conformity

In accordance with:

DIRECTIVE EMC 2014/30/EU

Manufacturer
Molecular Machines & Industries GmbH
Breslauer Strasse 2
85386 Eching

Apparatus	**MMI CellEctor**
Production country	Germany
Type of product	Capillary Cell Sorting Instrument
Technical documentation	CellEctor User Manual

Summary of test results

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

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

Molecular Machines and Industries GmbH
85386 Eching
June 04th, 2020

MMI