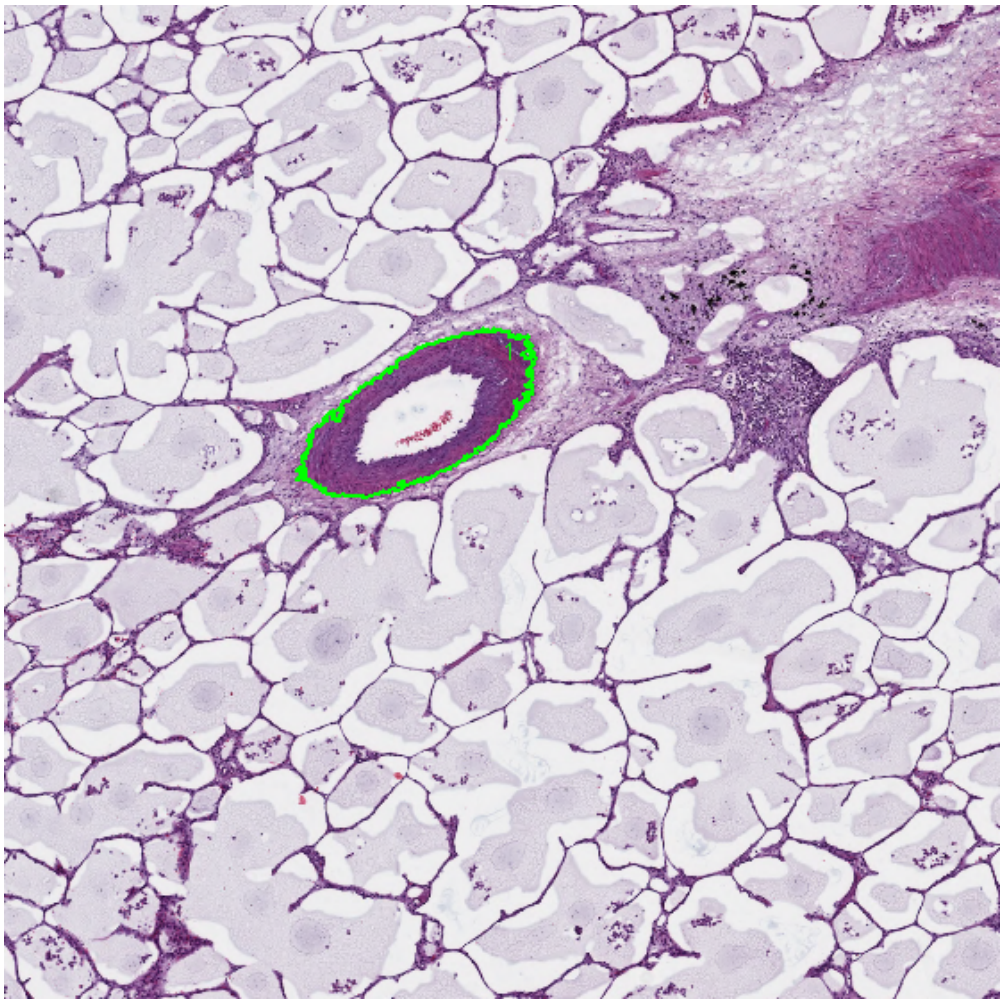
MMI

Microdissection - Micromanipulation - Imaging

mmi CellDetector

User Manual



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

User Manual: MMI CellDetector
Version 6.0

MMI GmbH
Breslauer Strasse 2
D - 85386 Eching
Germany

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1 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*

1.1 mmi CellViewer

The mmi CellViewer 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. 1.1) can be opened via the menu

Setup →Stage insert (Ctrl + I)

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

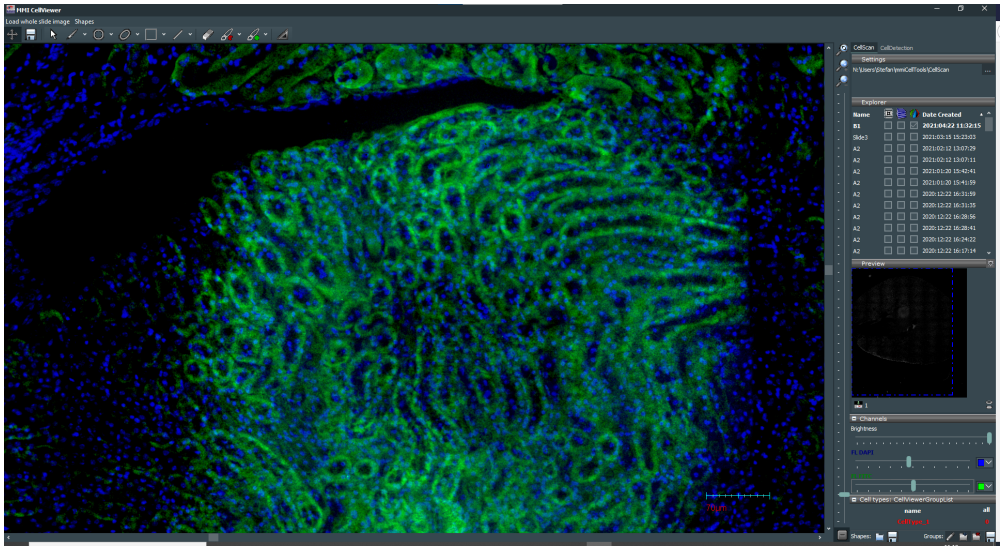


Figure 1.1: *The mmi CellViewer can be used to display full slide images and drawing contours*

The mmi CellViewer has following main purposes:

- display and zoom whole slide images (WSI)
- draw, display and review shapes around objects of interest
- create and manage groups of objects
- navigate through your samples, by double-clicking on the target position
- configure the geometry of the
  - stage insert and
  - slides or microplates, each containing the active regions called wells.
- assign a role to each well
- exporting and importing stage geometries

1.1.1 Drawing tools

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

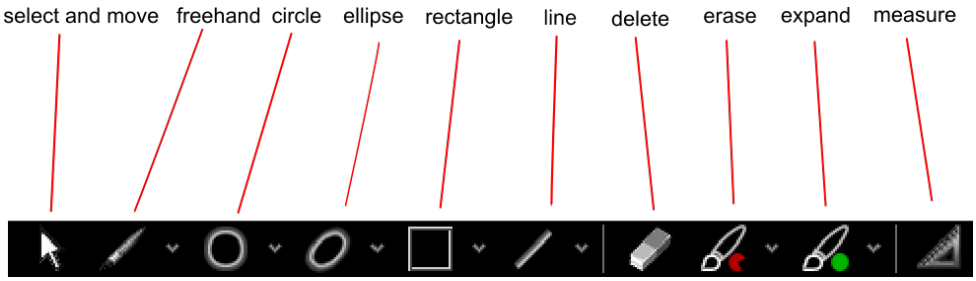


Figure 1.2: *Drawing tools: select, freehand, circle, ellipse, rectangle, line, delete, erase, expand and measure*

to right

- select and move a shape: click on a shape and drag the shape over the field of view. The shape will be highlighted
- freehand: use the mouse as a pencil
- circle: mark a first point of the circle and define a second point by dragging the mouse. Fix diameters can be set via the drop down menu next to the **circle button**
- ellipse: draw the main axis first and than define the minor axis by dragging the mouse. Fix parameters can be set via the drop down menu next to the **ellipse button**
- rectangle: mark the left upper corner and than define the diameter by dragging the mouse. Fix parameters can be set via the drop down menu next to the **rectangle button**
- line: mark the left upper corner and than define the length by dragging the mouse. Fix lengths can be set via the drop down menu next to the **rectangle button**
- delete: delete a shape by clicking on the shape
- erase: remove small pieces of the shape by using this tool as eraser. The erasing size can be set via the drop down menu next to the **erase button**
- expand: add small pieces to the shape by using this tool as brush. The erasing size can be set via the drop down menu next to the **expand button**

Shapes are orginised in groups (see section 2.2).

1.1.1.1 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).

1.1.1.2 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.

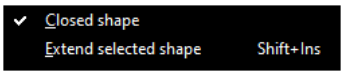


Figure 1.3: *Freehand drawing options*

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).

1.1.1.3 Circles

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

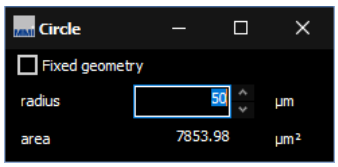


Figure 1.4: *Circle tool*

Fixed-size circles can be created by checking “Fixed diameter” and typing the value in the corresponding input box (Fig. 1.4).

1.1.1.4 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)

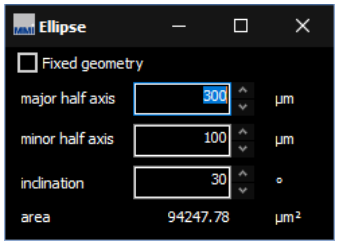


Figure 1.5: *Ellipse tool*

Fixed-size ellipses can be created by checking “Fixed geometry” and typing the values in the corresponding input box (Fig. 1.5)

1.1.1.5 Lines

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

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

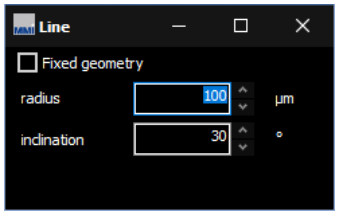


Figure 1.6: *Line tool*

1.1.1.6 Rectangles

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

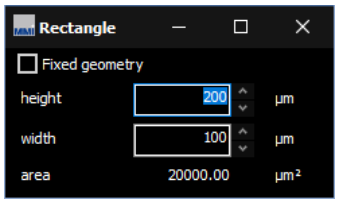


Figure 1.7: *Rectangle tool*

Fixed-size rectangles can be created by checking “Fixed dimensions” and typing the values in the corresponding input box (Fig. 1.7).

1.1.1.7 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. 1.8)
- Using the group editor (section 2.2.4).

To delete all shapes, or all shapes from the current group, use the context menu (Fig. 1.8) or use the corresponding keyboard shortcuts *Ctrl + Del* and *Ctrl + Shift + Del*.

1.1.1.8 Copying and pasting shapes

To copy the active contour use the menu item

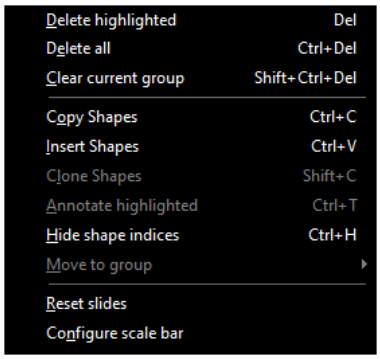


Figure 1.8: *Context menu, open with the right mouse button in the image panel.*

CellCut → *Shapes* → *Copy*

Select

CellCut → *Shapes* → *Insert*

to insert the copied shape.

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

1.1.1.9 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. 1.8).

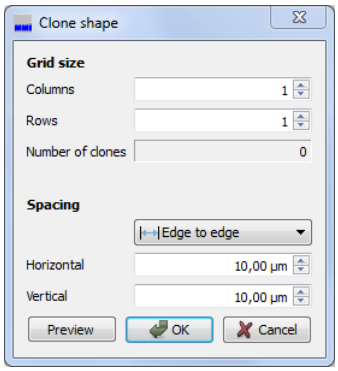


Figure 1.9: *Clone shapes*

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

1.1.2 Text annotations

Highlighte shapes can be annotated with your comments, by using the *Annotate highlighted* menu in the popup menu Fig. 1.8. A small popup window

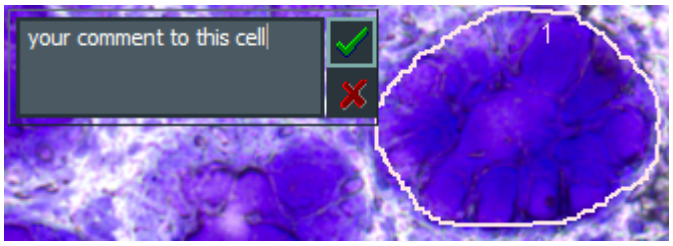


Figure 1.10: *Free text annotation for shapes*

Fig. 1.10 will allow to enter the text you want to save with the hightlighted shapes.

1.1.3 Hide shape indices

The popup menu shown in Fig. 1.8 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.

1.1.4 Move to group

The popup menu shown in Fig. 1.8 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.

1.1.5 Reset slides

For convienience the popup menu shown in Fig. 1.8 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

1.1.6 Configure scale bar

The popup menu shown in Fig. 1.8 also allows to configure the colors and thickens of the scalebar shown in the right lower corner of the image panel.

1.1.6.1 Navigating between shapes

There are several keyboard commands that allow you to locate your shapes and navigate between them. These are illustrated in Fig. 1.11.

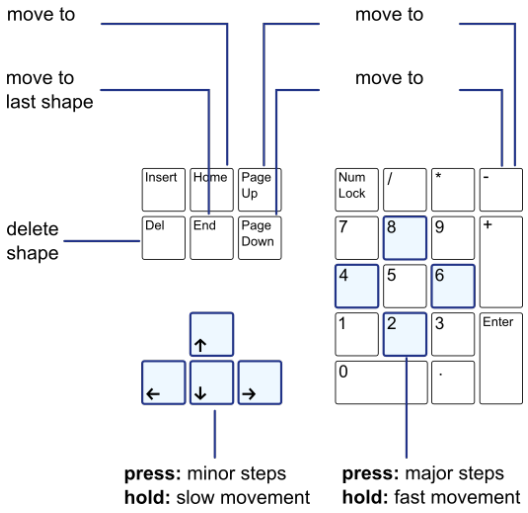


Figure 1.11: *Keyboard navigation*

- *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

1.1.7 Distance measurement

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

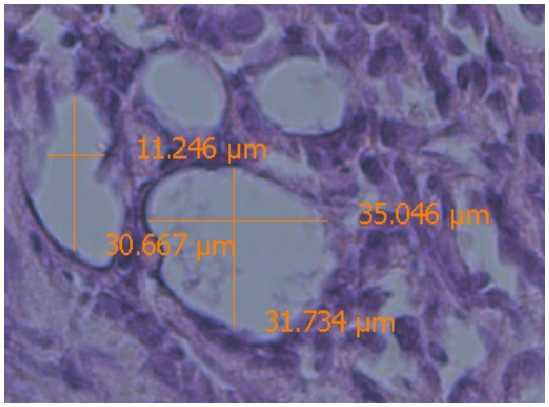


Figure 1.12: *Distance measurement*

Press the left mouse button and drag the mouse. After releasing the mouse button you can see the measured distance (Fig. 1.12)

1.1.8 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. 1.1 right of the image panel

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

1.1.9 *mmiCellScan* 5D channels

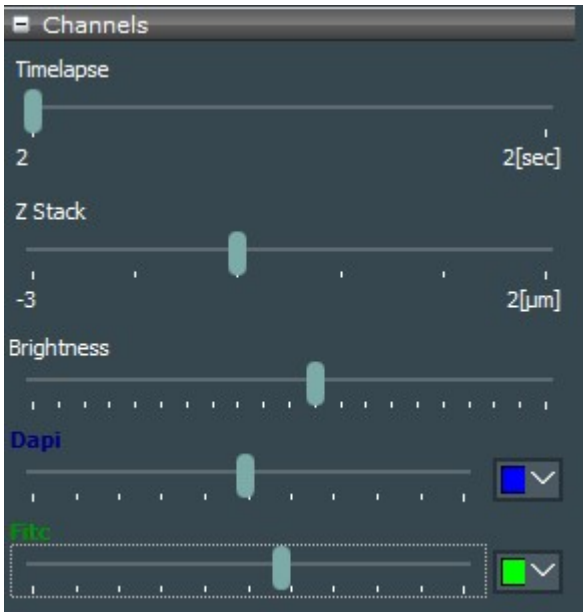


Figure 1.13: *Control the timelapse, zStack and Multi-channels of your 5D WSI.*

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

1.1.10 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.

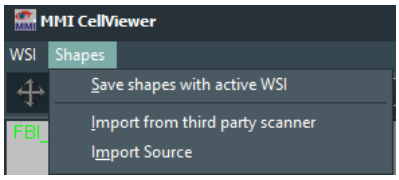


Figure 1.14: *Shape import menu*

1.1.10.1 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 2.2) 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.

1.1.10.2 Visiopharm

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

1.1.10.3 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.

1.1.10.4 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.

1.1.10.5 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.

1.1.11 mmi CellViewer plugins

The mmi CellViewer is able to host plugins like *mmi CellScan* or *mmi CellDetector* in the right side panel, see Fig. 1.1. This allow you to easily navigate through your whole slide images or use machine learning for image analysis.

2 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.

2.1 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. 2.1). 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.

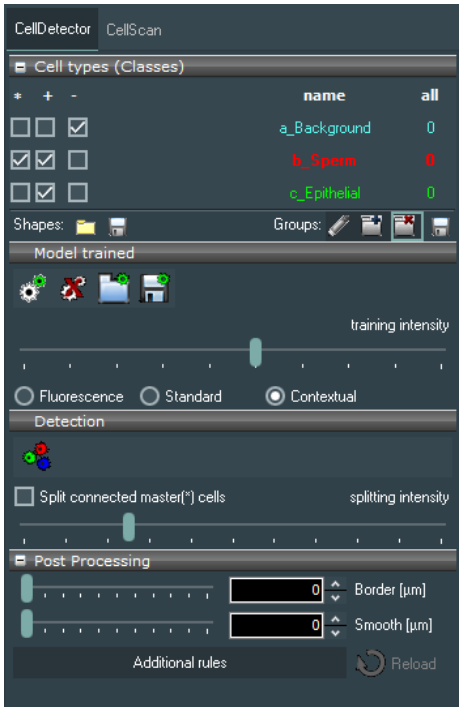


Figure 2.1: *Main panel*

2.2 Cell types

Any object drawn will always be assigned to the highlighted cell type group shown in the **Cell types** panel (Fig. 2.2).

> 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 checkboxes on the left side of Fig. 2.2, groups (classes) can be selected for different use:

- one class has to be selected as master class *: cells in this class can be combined with overlapping cells from other classes. Additionally for these cells the split function can be applied
- several groups can be selected as detectable +: annotations drawn in

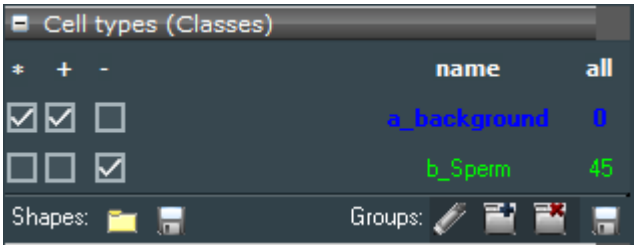


Figure 2.2: *Cell type group selection.*

this group will be used as training shapes to teach the *mmi CellDetector* model. Cells of this type are indented to be found by *mmi CellDetector*.

- one class has to be selected as background **-**: the areas of that class should not be found by *mmi CellDetector*. The detection result is pretty sensitive to the precision of drawing areas close to wanted cells.

  It is highly recommended to always add training annotations to the background class **-**.

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.

2.2.1 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. 2.3) using the edit button.

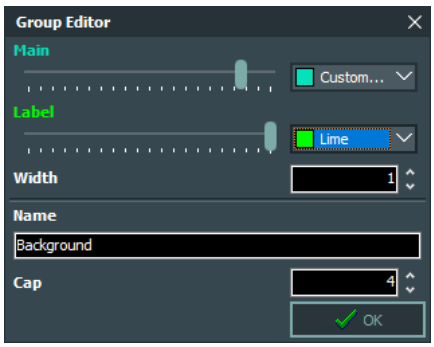


Figure 2.3: *Group editor*

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

2.2.2 Regrouping shapes

To move individual shapes from one cell type group to another, open the context menu (Fig. 1.8) by right-clicking on video panel and select the target group from sub-menu (*Move into group →GroupX*).

2.2.3 Group statistics

The statistics can be seen directly in the group panel (Fig. 2.2).

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

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

2.2.4 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. 2.4).

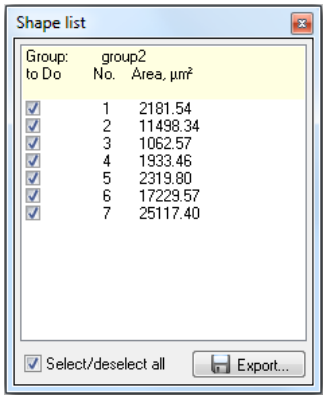


Figure 2.4: *Shape list*

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 1.1.1

2.3 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*.

2.3.1 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. 2.5. When the detection is executed (see 2.5), the current field of view is analysed for target cells.

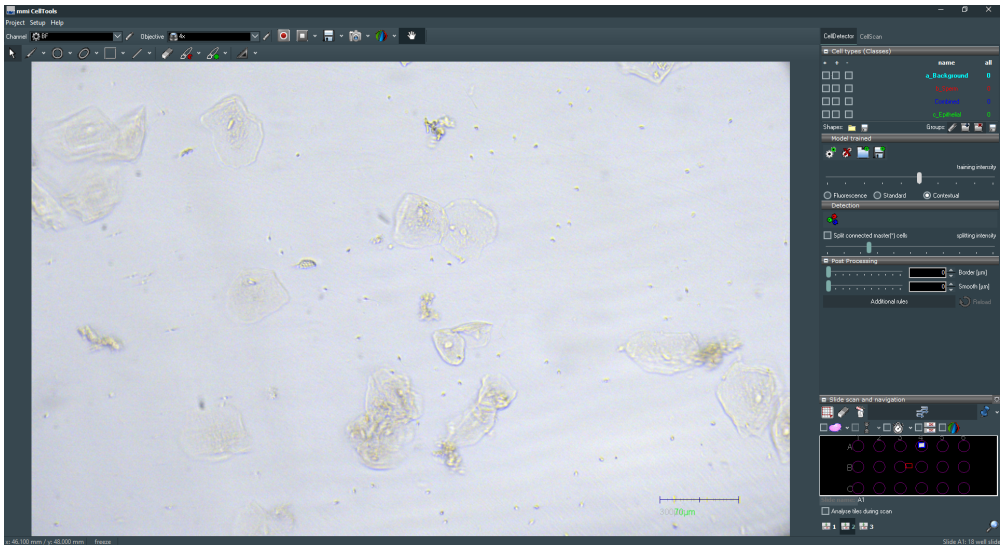


Figure 2.5: *mmiCellTools with mmi CellDetector*

2.3.2 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 2.4), 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 2.5), the selected image analysis (see 2.5.1) of the loaded whole slide image is analysed for target cells.

2.4 Training

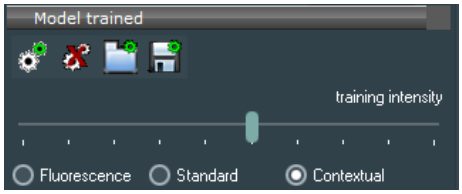


Figure 2.6: *Training intensity control in the main panel*

2.4.1 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.

2.4.2 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 2.2
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 2.4.1 and section 2.4.2
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 2.4.5).

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 2.5.

> 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.

2.4.3 Live cell *mmi CellDetector*

During the training process (see 2.4), 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.

2.4.4 Whole slide image *mmi CellDetector*

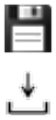
When the model is trained (see 2.4), the loaded whole slide image image and the drawn shapes are used as input to train the image anlysis algorithm.

2.4.5 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.

2.4.6 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.

2.5 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 2.3) either the image analysis area or the current field of view is analyzed for target cells.

2.5.1 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 2.5) that area is analysed for target cells. When the detection is executed

(see 2.5), the selected image analysis area(see 2.5.1) of the loaded whole slide image is analysed for target cells.

2.5.2 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.

2.6 Post Processing

The post processing panel allows additional refinement of the detected cells.

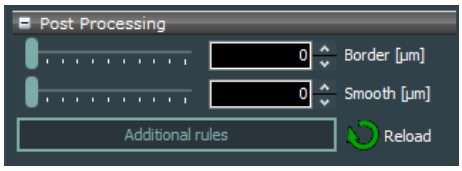


Figure 2.7: *Post Processing panel*

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.

2.6.0.1 Class rules

Figure 2.8: *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 circulatity** 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.

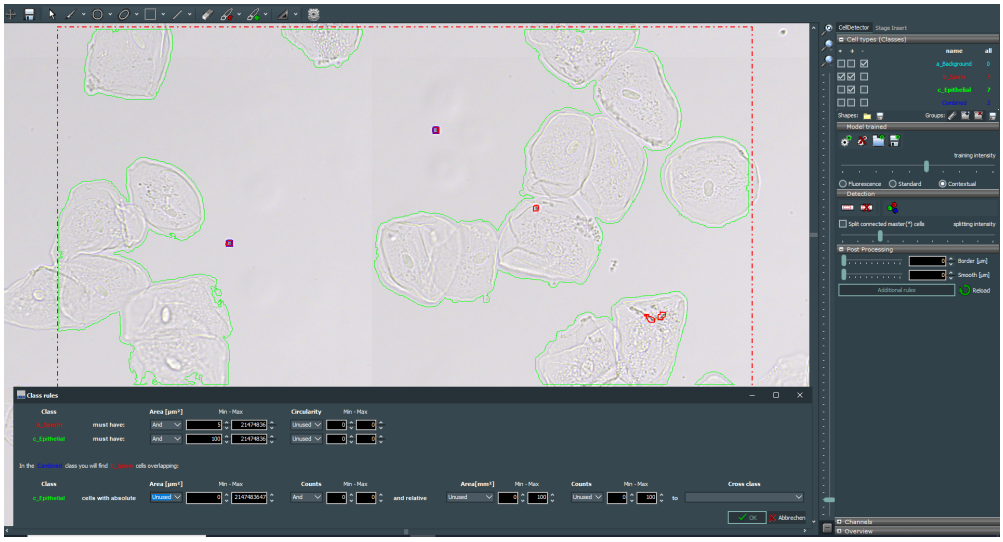


Figure 2.9: *Class rule example: With the **area** and **cicularity** sperm and epithelial cells can be indentified. Select the sperm cells, which arenot co-located with epithelial cells.*

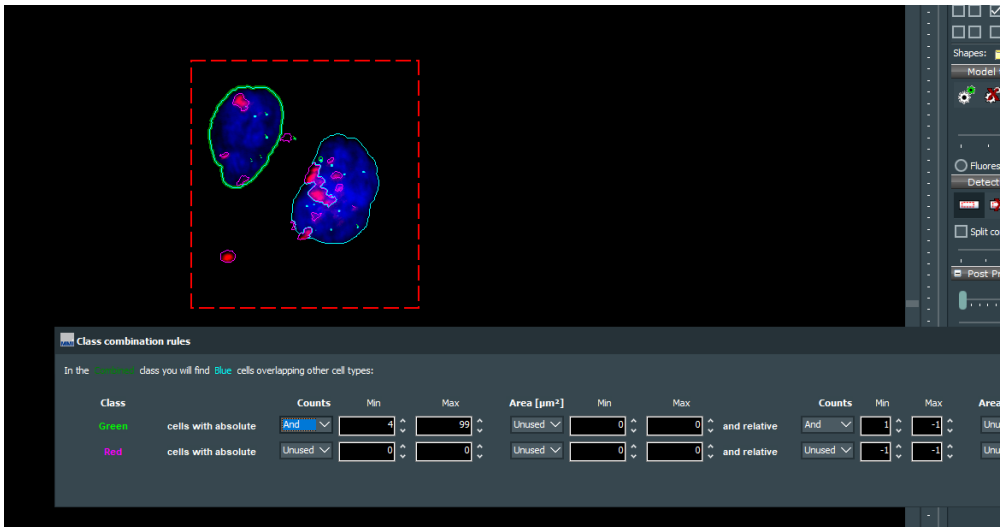


Figure 2.10: *Class combination rule example: Select the blue cells with at least 4 green cells and at least as many green as red cells. The found cell shows the bold contour.*

A List of Keyboard Shortcuts

General

+	Move to next shape	9
-	Move to previous shape	10
Alt + C	circle tool	5
Alt + Del	delete shape tool	6
Alt + E	ellipse tool	5
Alt + F	freehand tool	4
Alt + L	line tool	5
Alt + M	Measure distance	10
Alt + Q	rectangle tool	6
Alt + R	select and move tool	4
Arrow down	Move small step down	10
Arrow left	Move small step left	10
Arrow right	Move small step right	10
Arrow up	Move small step up	10
Ctrl + C	Copy shape	7
Ctrl + Del	Delete all shapes	6
Ctrl + H	Hide shape indices	8
Ctrl + I	Show stage insert	2
Ctrl + Shift + Del	Delete all shapes from current group	6
Ctrl + V	Paste shape	7
Del	Delete shape	6
End	Move to last shape	9

Home	Move to first shape	9
Numpad: Down	Move large step down	10
Numpad: Left	Move large step left	10
Numpad: Right	Move large step right	10
Numpad: Up	Move large step up	10
Space	Toggle between moving and drawing mode	3

Navigation