

**September 2010**

**PALM RoboSoftware 4.5**  
**Quick Guide**

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- Laser catapult technology (Laser Pressure Catapulting LPC<sup>pat</sup>)  
Patents: US 5,998,129, EP 879408 B1 and others.
- Three-dimensional laser beam positioning system  
Patents: US 5,689,109, EP 679325 B1 and others.
- Element List  
Patent: US 6,930,764.
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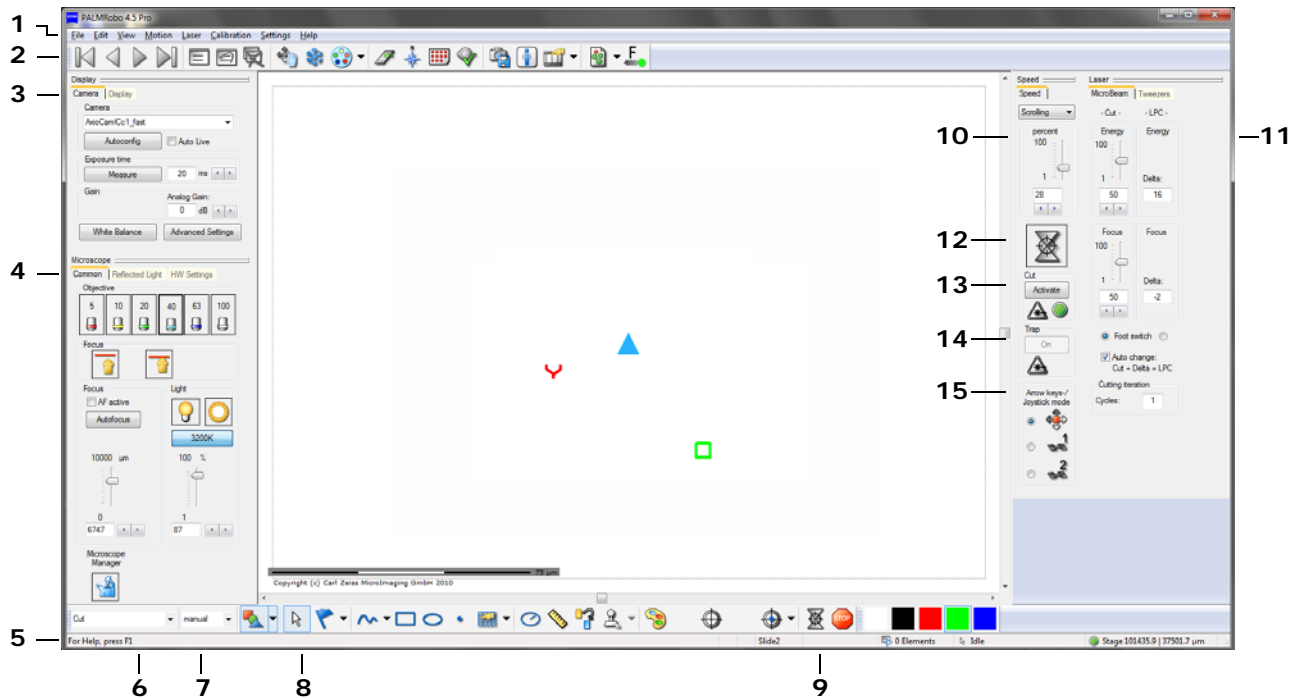
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<b>Chapter</b>	<b>Page</b>
1	Program Layout . . . . . 4
2	Toolbar . . . . . 5
3	Graphic Tools . . . . . 7
4	Cut Tools . . . . . 9
5	Start Laser and Laser indications . . . . . 10
6	Arrow keys/Joystick mode . . . . . 10
7	Menus . . . . . 11
8	Status Bar . . . . . 17
9	Speed Tools . . . . . 17
10	Laser Tools . . . . . 18
11	Camera Tools . . . . . 21
12	Microscope Tools . . . . . 22
13	Element List . . . . . 24
14	Basic Mode and Pro Mode . . . . . 27
15	Shortcuts . . . . . 28
16	Laser Functions – an Overview . . . . . 29

This quick guide gives you a short description of the functions of the PALM RoboSoftware.

## 1 Program Layout



- |  |   |
|--|---|
| <ul style="list-style-type: none"> <li>1 Menus (page 11)</li> <li>2 Toolbar (page 5)</li> <li>3 Camera Tools<br/>(Camera and Display Settings; page 21)</li> <li>4 Microscope Tools<br/>(Settings for the microscope, for fluorescence and hardware settings; page 22)</li> <li>5 Status Bar (page 17)</li> <li>6 Cut Tools</li> <li>7 To select a well for catapulting with PALM RoboMover (page 10)</li> </ul> | <ul style="list-style-type: none"> <li>8 Graphic Tools (page 7)</li> <li>9 Color Palette</li> <li>10 Speed Tools (page 17)</li> <li>11 Laser Tools (page 18)</li> <li>12 Start Cutting Laser (page 10)</li> <li>13 Cutting Laser status (page 8)</li> <li>14 Switch on/off Trapping Laser, Trapping Laser status (page 8)</li> <li>15 Arrow keys/Joystick mode (page 10)</li> </ul> |
|--|---|


The site of each Toolbar resp. tool on the screen can be changed: with the cursor on the dashed stroke and while pressing the left mouse button you can move it. Via menu item "View > Default Bar Configuration" changes of their sites can be reset to default.

In the window "Preferences and Configuration" each Toolbar resp. tool can be hidden or shown (open the window via menu item "Settings > Preferences ..." and click on tab "Appearance").



## 2 Toolbar


 First Element	 Last Element
 Previous Element	 Next Element

The stage is moved so that the desired element is centered on the screen.


 Element List

To show the "Element List". See also page 24.

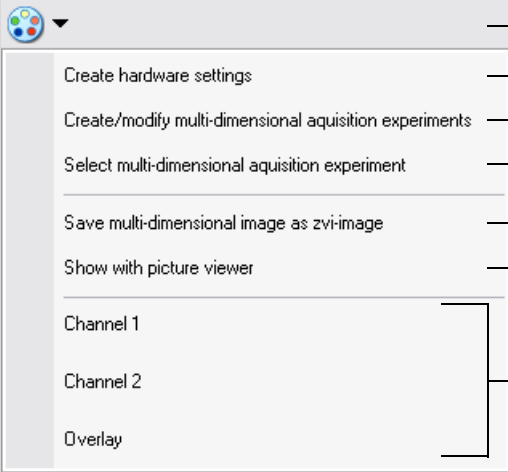
 Delete last element	 Delete all elements
To delete the last drawn element.	To delete all elements, also when hidden.

 Stage Mode

To switch to the Stage Mode. In the Stage Mode you move the stage with mouse. To exit the Stage Mode click left mouse button once.

 Freeze Mode

To switch to the Freeze Mode (the stage cannot be moved anymore and the video image is frozen).




To acquire multi-dimensional acquisition experiments.

- Dimensions can be:
- fluorescence channels (to acquire multi channel fluorescence images)
  - z-layers (layers of different focus to acquire images with extended focus: z-stack experiments)
  - time (to acquire time lapse experiments)


Proceed step by step as described in the following:

- 1 - set hardware parameters
- 2 - enter and save parameters for your multi-dimensional acquisition experiment
- 3 - select an experiment
- 4 - click on this icon to start the selected experiment
- 5 - save the images
- 6 - show a single channel image (e.g. a single fluorescence channel or the image with extended focus, if you had left the Freeze Mode before) or the multi channel image (overlay) on the screen
- 7 - show the zvi multi channel image in the picture viewer dialog


Menu items 5 to 7 appear after acquiring the image.

 Loadposition

To move the stage to Loadposition.



 Navigator


Opens the PALM Navigator Window. With PALM Navigator you can scan your slide or certain parts of it and easily move the stage to points defined by a mouse click.

 Capture device <sup>1)</sup>

Opens the PALM RoboMover resp. the PALM CapMover II window. With PALM RoboMover you can use collectors with one or more target vessels and position them manually or automated. With PALM CapMover II you can position one target vessel.


1) Only available in systems equipped with PALM RoboMover resp. PALM CapMover II. Please contact palm-info@zeiss.de for further information.

 <p><b>Cap Check</b></p> <p>To position the stage to the Cap Check.</p>	 <p><b>Point of origin</b></p> <p>To move the stage from Cap Check back to the point of origin.</p>
--	--



**Field of View Analysis <sup>1)</sup>**

To start the function "Field of View Analysis" which will find elements on your specimen in an interactive way (see PALM RoboSoftware Manual chapter 18).




**Save Image**

To save the current image.

In File Mode the image will be saved under the default name with an image number added in the default directory (see "Settings > Preferences ...", page 16). The image numbers will be increased automatically.

In Database Mode the image will be saved in the connected database. The name will be created by the program.

You can save the image with or without the drawn elements.




**Incubation <sup>2)</sup>**

Configuration	—	<b>1</b>
Start Logging	—	<b>2</b>
View Log-file	—	<b>3</b>


To work with incubation

- 1 - set incubation parameters
- 2 - start logging: a log-file will be created. In this file the actual settings will be written (depending on your settings via menu item "Settings > Preferences ..." event controlled or time controlled) After having started logging the menu item changes to "Stop Logging"
- 3 - open the log-file




**Information Center**

To start the Information Center to display and organize stored pictures.



**Force Measurement <sup>3)</sup>**

Opens the Force Measurement window. In this window you can calibrate the trap stiffness, adjust parameters, start force measurement experiments and view logged data.








**Recorder <sup>1)</sup>**

Configuration
---------------

With the Recorder function you can acquire video sequences and/or sequences of images.

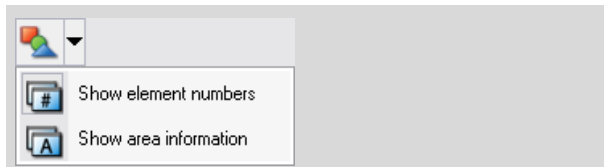
Click on Menu item "Configuration" to open the "Recorder and Time Lapse Configuration" window. In this window you can adjust parameters for the Recorder and Time Lapse function and determine the trigger point. Depending on the actual state resp. the chosen trigger point one of the following icons appears in the Toolbar:



	No recorder experiment configured.
	Recording will be started manually: Click on this icon to start the Recorder function.
	Recording will be triggered by the next Cutting Laser function start: Foot switch (Cut or LPC), or LPC Laser function.
	Recording will be started scheduled.
	Recording is running and can be stopped by a click on this icon.




- 1) Recorder function and Field of View Analysis are only available in systems with Pro Licence. Please contact palm-info@zeiss.de for further information.
- 2) Only available in systems equipped with Incubation module.
- 3) Only available in systems with Force Measurement licence.

### 3 Graphic Tools


#### Display functions



 Show element numbers  
 Show area information

 To show or hide all elements.  
 To show or hide the numbers of the elements.  
 To display the element areas and sizes in  $\mu\text{m}^2$ .

#### Select elements

 Pointer
 

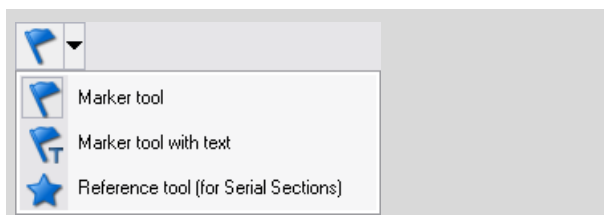
To select elements to change (e.g. via menu item "Edit > "Element Properties"), move or delete them.




To select one element: Click on the number of an element to select it.




To select more than one element:  
Click and draw a rectangle which contains the elements or parts of them, or  
Click on first element, then press "Shift" and click on the elements to be added to the selection.

If several elements are positioned one above the other, press "Ctrl" and click several times until the desired element is selected.

#### Marker, comment, Reference Point

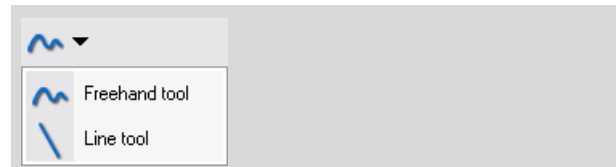




 Marker tool  
 Marker tool with text  
 Reference tool (for Serial Sections)


 To position a marker into the image.  
 To position a marker with a comment into the image.  
 You can change the comment in the dialog "Edit > Change".  
 To position a Reference Point for Serial Sections.


In the Toolbar always the last used tool is shown.

#### Create and edit elements




 Freehand tool  
 Line tool


 Freehand  
 To draw freehand lines.  
To draw a line press left mouse button and move the mouse.  
  
To correct an element of type "figure" (Line, Freehand, Rectangle or Circle), press "Ctrl" and move the mouse to the part to be corrected. The nearest anchor point of the element will be shown. Click and draw the correction line.  
  
To connect two elements, press "Ctrl" and draw a line from the end of one element to the end of the other element.


 Line  
 To draw straight lines.  
You start drawing with the first mouse click, after another mouse click you can change the direction, with a double-click you finish the element.  
  
Press the shift key to draw horizontal and vertical lines.

In the Toolbar always the last used tool is shown.


 Rectangle
 


To draw rectangles.  
Press the shift key to draw quadratic elements.

 If the centric attribute is selected, you draw the elements from their center.


 Circle
 

To draw ellipses.  
Press the shift key to draw circles.

 If the centric attribute is selected, you draw the elements from their center.

 Dot
 


To mark single cells for catapulting. These dots are used for the Laser Pressure Catapulting function (LPC).


 Grid rectangle

You can draw a rectangle using the Grid Rectangle Tool; this rectangle will be automatically divided into a number of smaller rectangles you have defined. Click on menu item "Configure" to define the parameters (number of lines and rows, orientation).

You can now catapult the elements into PALM RoboMover wells such that the morphology is retained, i.e. the individual elements are catapulted such that their arrangement in the wells is exactly the same as the arrangement in your samples.


Press the shift key to draw quadratic elements.

 If the centric attribute is selected, you draw the elements from their center.

 Stamp

To copy an element and to place the copy with one mouse click at the desired position.


Click on menu item "Select new template".

 Select Stamp Template

The icon changes to indicate that now you can select an element to copy.


Click on the element.  
Click at the desired position on the screen to paste the copied element.

**Ruler**

 Ruler


To measure.  
You measure with mouse moving while pressing the left mouse button.

**Change element attributes**


 Colors

To determine a display-color for each element.  
To determine line, dot and ruler thickness.  
The color and thickness of the drawn elements will not be changed.

To determine colors to be displayed in the Color Palette and to assign names to the colors.

 Change Figure Color

To change the color of a drawn element. Click first on this icon, then select a color in the Color Palette, and then click on the element to be changed.

 Color Palette

To chose the color for the next element to be drawn. Click first on the desired tool to draw an element. Then select a color in the Color Palette, and then draw your element.


The colors displayed in the Color Palette can be chosen via icon "Colors".

**Center stage/Position Trapping Laser**

The function of this tool depends on the setting of the Arrow keys/Joystick tool (see chapter 6).


Note: When the stage has been positioned at the Cap Check position the buttons of the Arrow keys/Joystick tool are deactivated. In this case you can use the Arrow keys or the Joystick to position PALM RoboMover resp. PALM CapMover. But you cannot use the Center stage/Position Trapping Laser tool.


If stage is chosen:


 Center stage

Click on an arbitrary point in your microscope image to center this point on the screen.

If Trapping Laser beam 1 resp. 2 resp. 1 and 2 is chosen:

 Position Trapping Laser beam 1

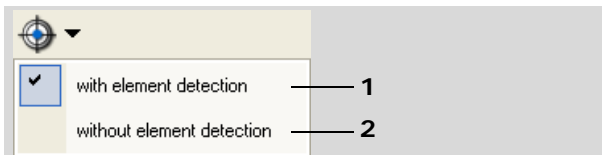
 Position Trapping Laser beam 2

 Position Trapping Laser beam 1 and 2 simultaneously

Click on an arbitrary point in your microscope image to position Trapping Laser beam 1 resp. 2 resp. 1 and 2 simultaneously at this point on the screen.



## Oneclick-LPC



With one click, a designated element will be located in the screen center and lifted up by a single laser pulse.

Depending on the selected menu entry the tool has different functionalities:

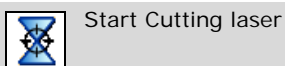
- 1 - Menu entry "with element detection"
 

If an element was not lifted up properly (e.g. because of too less energy) you can use this tool to lift up the element again. Select the tool and then click with the mouse pointer on the point within the element laser shot. The PALM System recognizes the element automatically, positions the capture device correctly (if a well has been assigned), performs the laser shot and increases the shot counter of this element.

Note: If the PALM System cannot identify an element (e.g. you click on a position where no element is located) no laser shot will be released. The stage will be directed to this point only.
- 2 - Menu entry "without element detection"
 

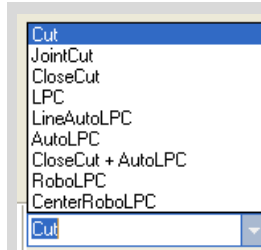
If the menu entry is activated the stage will be centered on the clicked point and the laser shot will be released independently of drawn elements or the position of the capture device. You can use this functionality to collect small pieces from a glass slide on a fast way.

## Start/Stop Cutting Laser



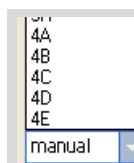
To stop all laser functions and movements immediately (in case of emergency).

## 4 Cut Tools



To select the Cutting Laser function for the next element to be drawn.


For an overview of the Laser functions see page 29.



To select a well in a PALM RoboMover collection device manually for the next element to be drawn. In this well the element will be catapulted (you can select the well also in the Element List (see page 27)).

## 5 Start Laser and Laser indications


### Cutting Laser


 Start Cutting Laser

See also page 9 "Start/Stop Cutting Laser".

**Cut**


The Cutting Laser is deactivated.


 Click on the button "Activate" to activate the Cutting Laser.



**Cut**



The Cutting Laser is activated.

 Click on the button "Deactivate" to deactivate the Cutting Laser.



**Cut**


The Cutting Laser is activated and has been started.


 

### Trapping Laser

**Trap**


The Trapping Laser is switched off.


 Click on the button "On" to switch on the Cutting Laser.




**Trap**


The Trapping Laser is switched on.

 Click on the button "Off" to switch off the Cutting Laser.



### Additional Laser indications

 The laser interlock has been tripped, please check microscope (support).




 The laser is not ready for use (this indication appears during the laser warm-up phase or if the interlock is tripped).

## 6 Arrow keys/Joystick mode

With the Arrow keys-/Joystick mode control you can chose the unit which will be controlled by the arrow keys resp. the joystick resp. the "Center" tool (see page page 8).

Click into the first button




Arrow keys-/ Joystick mode

-  to control the stage with arrow keys resp. joystick
-  1 to center an arbitrary point in your microscope image on the screen with the "Center" tool by clicking on this point (see page 8)
-  2



Only possible when the stage is not positioned at the Cap Check.

Click into the second button



Arrow keys-/ Joystick mode

-  to control Trapping Laser beam 1 with arrow keys resp. joystick
-  1 to move Laser beam 1 to an arbitrary point in your microscope image on the screen with the "Center" tool by clicking on this point (see page 8)
-  2

Click into the third button

-  to control Trapping Laser beam 2 with arrow keys resp. joystick
-  1 to move Laser beam 2 to an arbitrary point in your microscope image on the screen with the "Center" tool by clicking on this point (see page 8)





If both buttons are activated

-  you can control both Trapping Laser beams simultaneously with arrow keys resp. joystick
-  2 you can move Laser beam 1 and 2 simultaneously to an arbitrary point in your microscope image on the screen with the "Center" tool by clicking on this point (see page 8)

Only possible when the stage is not positioned at the Cap Check.

When the stage has been positioned at the Cap Check you move PALM RoboMover resp. PALM CapMover with the arrow keys resp. the joystick. In this case the buttons are deactivated.

Arrow keys-/ Joystick mode

-  
-  1
-  2

## 7 Menus

### Menu "File"

**New Elements / Delete All Elements**    **Ctrl+N**

To prepare the software for drawing new elements (only File Mode). Existing elements can be saved previously.

**Open Elements ...**

To open elements from a file (only File Mode).

**Save Elements ...**

To save the drawn elements in a file (only File Mode). The file can be called up any time with PALM RoboSoftware. The elements are saved in the default directory (see "Settings > Preferences ..." page 16).

**Enter / Select Data ...**

To enter or select data in Database Mode (only Database Mode).

In Database Mode you can only draw elements after creating entries in the database.

With selecting data from the database you open elements from the database.

**New Image Folder ...**

To select or create a document folder for saving pictures in File Mode (in Database Mode this folder is of no meaning).

**Save Image ...**

To save an image as a file (with or without the drawn elements). In File Mode the images are saved in the default directory (see "Settings > Preferences ..." page 16). The folder for saving can be changed.

In Database Mode the images are saved in the database.

**Import Elements ...**

To import the element properties shown in the element list (color, number, type etc.) and the coordinates of the anchor points of the elements from a text file.

**Export Elements ...**

To export the element properties shown in the element list (color, number, type etc.) and the coordinates of the anchor points of the drawn elements into a text file.

The text file can be opened with PALM RoboSoftware and any software that can handle \*.txt files.

**Print Elements ...**

To print the element properties into an Excel import file or into a text file or to print on a printer. You can choose which elements and which properties of the elements will be printed.

1 testslide.palm on Position 2  
 2 testslide.palm on Position 1  
 3 3324yElements.palm  
 4 19344yElements.palm

Last Elements: to open one of the last four files with saved elements (only File Mode).

**Exit PALMRobo**                      **Alt+F4**

To quit PALM RoboSoftware.

**Menu "Edit"**

**U**ndelete Alt+BackSp

To undo the last command "Delete ...".

---

Select All Elements (all Slides)

To select all elements for all slides.

---

Select All Elements (current slide) Ctrl+A


To select all elements for the current slide.

---

**U**nselect All Elements Ctrl+A


To unselect all elements.

---

 **E**lement Properties Alt+Enter

To show and change the properties of the selected element.

---

 **R**enumber All Elements

To renumber the remaining elements after deletion of elements.

---


**C**opy Ctrl+C

**P**aste Ctrl+V

To copy the selected elements to clipboard.


To paste the elements from clipboard.

---

 **D**elete Selected Elements Del

To delete the selected elements.


---

 **D**elete **L**ast Element BackSp

To delete the last drawn element.

If the last drawn element is already processed, the menu item is deactivated.

---

 **D**elete **A**ll Elements Ctrl+Y

To delete all elements, including hidden elements.

**S**erial Section ▶

---

**C**reate **G**roup

To create a group of elements from the currently selected elements.

---

**A**dd to Group

To add one or more selected elements to an existing group.

---

**R**emove from Group

To remove one or more selected elements from a group.

---

**D**efine Group-Reference-**F**igure

To define up to three elements as Group Reference Figures.

---

**U**ndefine Group-Reference-**F**igure

To undefine an element as Group Reference Figure.

---

**M**atch Serial Section **G**roup

To transfer elements automatically from one slide to another for serial sections.

---

**M**atch Reference Point **M**anual

To transfer elements manually from one slide to another for serial sections.

## Menu "View"

Hide All Bars	Alt+X
Show All Bars	Alt+X

To hide resp. show all Toolbars and Tools.


Note: There is only one menu item. Depending on the current state the entry switches into the other state. The menu item description toggles between "Hide All Bars" and "Show All Bars".

Default Bar Configuration
---------------------------


To show all Toolbars and Tools in the default configuration.

Laser Marker ...
------------------


To open the window "Laser-Marker Definitions". In this window you can for each laser separately define the appearance of the Laser-Marker (type, style, color and size), and you can show or hide the Laser-Marker.

 Incubation Device ...	F2
---	----


To open the window "Incubation" where you can select and change settings for your incubation device. <sup>1)</sup>

 Information Center ...	F3
--	----


To start the program Information Center to display and organize stored pictures.

 Navigator Window	F4
--	----


To open the PALM Navigator Window.

 Element List	F5
--	----


To show or hide the Element List (see "Element List", page 24).

 Microscope ...	F6
--	----


To open the Microscope window.

 Capture Device ...	F12
--	-----

Opens the PALM RoboMover resp. the PALM CapMover II window. With PALM RoboMover you can use collectors with one or more target vessels and position them manually or automated. With PALM CapMover II you can position one target vessel. <sup>2)</sup>

 Show Elements
---

To show or hide all drawn elements.

 Show Numbers
--

To show or hide the numbers of the elements.

Show Points
-------------

To show or hide the anchor points of drawn elements

Scroll Rectangle
------------------

To show or hide the scroll rectangle (dashed frame).

Scale Bar
-----------


To show or hide the Scale Bar.

Copyright Information
-----------------------

To show or hide the copyright info.


- 1) Only available in systems equipped with incubation device. Please contact palm-info@zeiss.de for further information.
- 2) Only available in systems equipped with PALM RoboMover resp. PALM CapMover II. Please contact palm-info@zeiss.de for further information.

**Menu "Motion"**

 Stop ESC

To stop the Cutting Laser function, to switch off the Trapping Laser, to stop the movement of the stage and to stop PALM RoboMover immediately in case of emergency.


---

 Stage Mode F7


To switch to Stage Mode (the stage can be moved with mouse motion).

Invert Stage Motion


With "Invert Stage Motion" activated, the stage moves opposite to mouse motion.

 Trap-1 Mode

To switch to Trap-1 Movement Mode. In Trap-1 movement Mode you move the variable Trapping Laser beam 1 with mouse. To exit this mode click left mouse button once.

 Trap-2 Mode

To switch to Trap-2 Movement Mode. In Trap-2 movement Mode you move the variable Trapping Laser beam 2 with mouse. To exit this mode click left mouse button once.

 Freeze Mode Alt+F





To switch to Freeze Mode (the stage cannot be moved anymore and the video image is frozen).

---

Increase Speed Ctrl+S  
Decrease Speed Ctrl+F


To modify the current speed of the movement.

---


 Goto First Element  
 Goto Prev Element  
 Goto Next Element  
 Goto Last Element

To center a certain element on the screen.

---




 Goto Cap Check Ctrl+K

To move the stage to the Cap Check (to examine the sample in the cap).

 Goto Load Position


To move the stage to the Load Position.

---



Goto Center  Stage  
 Trap 1  
 Trap 2

To move the stage resp. the Trapping Laser beam 1 resp. 2 to the center.

---


Goto Reference  Group  
Ref-1  
Ref-2  
Ref-3

To move the stage to the elements previously defined as Reference 1 resp. 2 or 3 for a group of elements.

 Trap 1  
 Trap 2



To move the Trapping Laser beam 1 resp. 2 to their Reference Position.

---

Initialize Unit  Stage

To move the stage to the end position in x and y direction.  
This position is set as zero point for element coordinates.  
Then the stage moves back to its initial position.


The stage is automatically initialized at every start of the program. Therefore "Initialize Unit > Stage" must only be used in case of error of referencing elements.

 Trap 1  
 Trap 2

To initialize the Trapping Laser beam 1 resp. 2.

The Trapping Laser beam 1 resp. 2 is automatically initialized when starting the program. Therefore "Initialize Unit > Trap 1" resp. "... Trap 2" must only be used in case of error.

### Menu "Laser"

 Start Laser Function

To start Cutting Laser function after all settings are done.

Trap Laser on/off

To switch on/off Trapping Laser.

Increase Energy/Power	Page Up
Decrease Energy/Power	Page Down
Focus Up	Home
Focus Down	End

To change the values for laser energy/power and laser focus. The set values are valid for the currently in the Laser Tools selected laser only (Cutting Laser or Trapping Laser; see page 18 and page 19). During laser action these values can be changed via keyboard (see page 28) or Laser Tools (see page 18 and page 19).

### Menu "Calibration"

Calibration Wizard ...

To start the Calibration Wizard. With the Calibration Wizard you can perform the following calibrations:


- Camera adjustment
- Stage calibration
- Objective Offset
- Reflector Offset

Trap Laser Calibration Wizard ...


To start the Trap Laser Calibration Wizard. With the Trap Laser Calibration Wizard you calibrate the movable Trapping Laser units.

Cut Laser Adjustment Wizard ...


To start the Cut Laser Adjustment Wizard. With the Cut Laser Adjustment Wizard you can find suitable settings for cutting laser energy and focus.

 Position Cut-Laser


To align the position of the Cut-Laser-Marker with the actual UV-laser position.

 **1** Position Trap 1-Laser


To align the position of the Trap-1-Laser-Marker with the actual IR-laser position.

 **2** Position Trap 2-Laser

To align the position of the Trap-2-Laser-Marker with the actual IR-laser position.

 **1** Set Reference Trap 1

To define a Reference Position for the Trapping Laser beam 1.

 **2** Set Reference Trap 2

To define a Reference Position for the Trapping Laser beam 2.

## Menu "Settings"

**Preferences ...**

General settings for configuration (operating mode, stage, joystick, metric, saving settings) and appearance, settings for saving elements, saving images, copyright information, laser function, motorized microscope, Trap-Footswitch, and (if installed) settings for Recorder function, incubation logging, Field of View Analysis, Force Measurement Experiments.

**Hardware Settings ...**

To open the window "Settings editor". In this window you can define Hardware Settings which can be activated via "Microscope Tools" / tab "HW Settings" (page 23).

**Fluorescence ...**

Opens the window "Fluorescence adjustments". In this window you can

- create a new or change an existing set of fluorescence settings.

Defined fluorescence settings are activated via Microscope Tools, Tab "Reflected light" (see page 23).

- define significant names for the fluorescence filters instead of filter numbers 1...8 (e.g. Rhodamin, DAPI, FITC etc.).
- select a fluorescence filter.
- get information about the installed filter wheel.
- open and close the shutter.
- get information about the shutter type.
- open or close the fluorescence shutter to activate or deactivate the fluorescence beam (if your system is equipped with a filter wheel).
- get information about the reflector type.
- calibrate the reflector (i.e. set the Reflector Offset).
- define significant names for the reflector colors.
- select a reflector.

**Save Settings ...**

**Load Settings ...**

To save and load preset speed settings for: Stage mode, Arrow keys, Scrolling, Positioning, Cutting and Trap Mode, and to save and load laser settings (Cutting Laser and Trapping Laser) for: energy/power, focus, lens value, LPC distances, Auto-change and balance.

**Load Factory Defaults**

To reset all settings to default settings.

## Menu "Help"

**PALMRobo Help F1**

To list help topics.

**About PALMRobo ...**

To get information about the program (version number, license information and manufacturer) and to show Live image information (resolution, frame rate).



## 8 Status Bar

The Status Bar at the lower margin of the program window contains six fields which are described from left to right.

For Help, press F1

- Short descriptions for tools in Toolbar or Graphic Tools, when moving the cursor over the buttons. Doubleclick into the field to open the "PALMRobo Information" window.

1 2

- Shows that you can control the Trapping Laser beams with the Joystick. If these fields are empty you control the stage or (when the stage is in Cap Check position) PALM RoboMover with the joystick.

Slide2

- Shows the current object holder, or indicates that an element is being calculated at the moment. Doubleclick into the field to open the "Navigator" window.

Exp061110\_2.set

- Currently used setting file.

9 Elements

- Number of elements, or number of the centered element (x of ...) and of all elements (... of y) (shown after using any function for centering an element). Doubleclick into the field to open the "Element List" window.

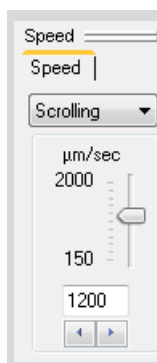
Idle

- Shows active mode: Laser ON, Stage Mode, Cursor Mode, Scrolling, Positioning, Continuous, Calibration, Reference Position, Trap 1, Trap 2. During drawing an element, the current size of the element will be shown. While no action takes place, "Idle" is shown.

Stage 106376.3 | 46662.0 µm

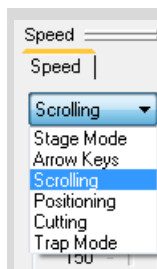
- Coordinates (x|y) of the current moving resp. the last moved unit (Stage, Trapping Laser, PALM RoboMover, PALM CapMover II). Doubleclick into the field to open the "States" window (shows the current coordinates and status of all installed units).

## 9 Speed Tools



To display and to change the preset speed for the parameters described below.

The unit can be set via menu item "Settings > Preferences ..." > Tab "Configuration" to µm/sec. or to percent.



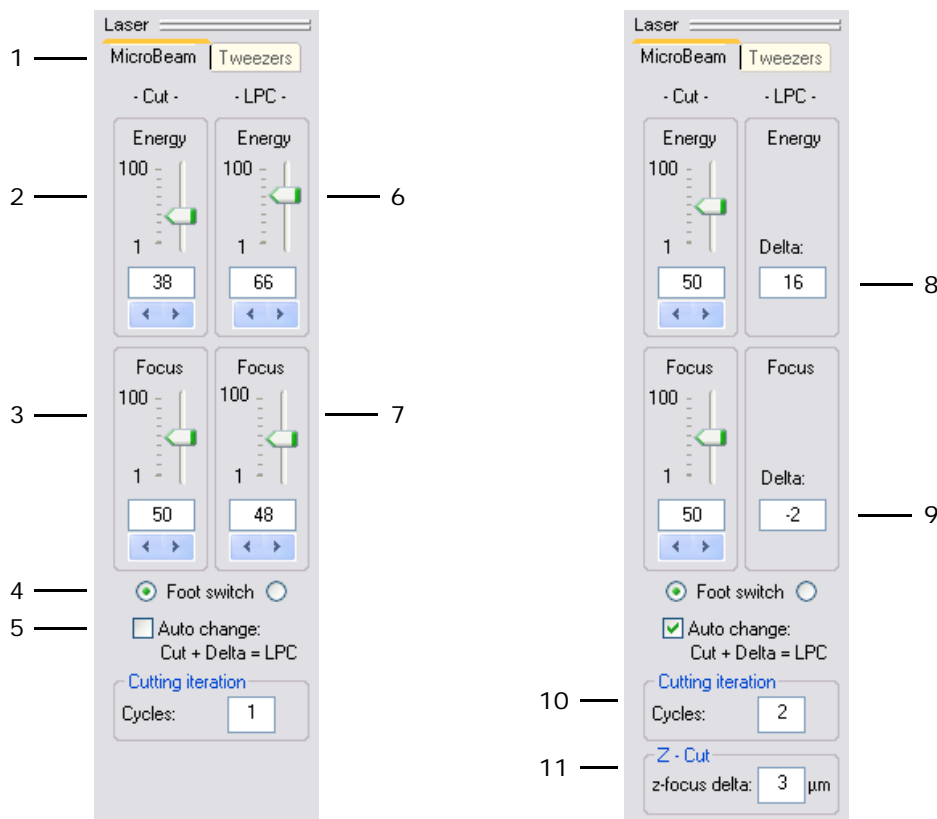
Stage Mode	Speed setting which relates mouse movement to stage movement.
Arrow keys	Speed setting for the movement of stage with arrow keys.
Scrolling	Speed setting for scrolling in cursor Mode.
Positioning	Speed setting for stage movement from element to element, to Cap Check or to Reference Position.
Cutting	Speed setting for stage movement during Cut and AutoLPC function.
Trap Mode	Speed setting for movement of the Trapping Laser beam.

## 10 Laser Tools

With the Laser Tools you set values for energy resp. power and focus for the Cutting Laser (MicroBeam) resp. Trapping Laser (MicroTweezers).  
The preset values for energy resp. power, focus and balance can be changed before each laser operation.

In this way you optimize the parameters for each operation to obtain a precise cut and an effective catapulting resp. trapping.  
For fine adjustment the values can be changed even during cutting resp. trapping.

### Laser Tools for Cutting Laser (MicroBeam)

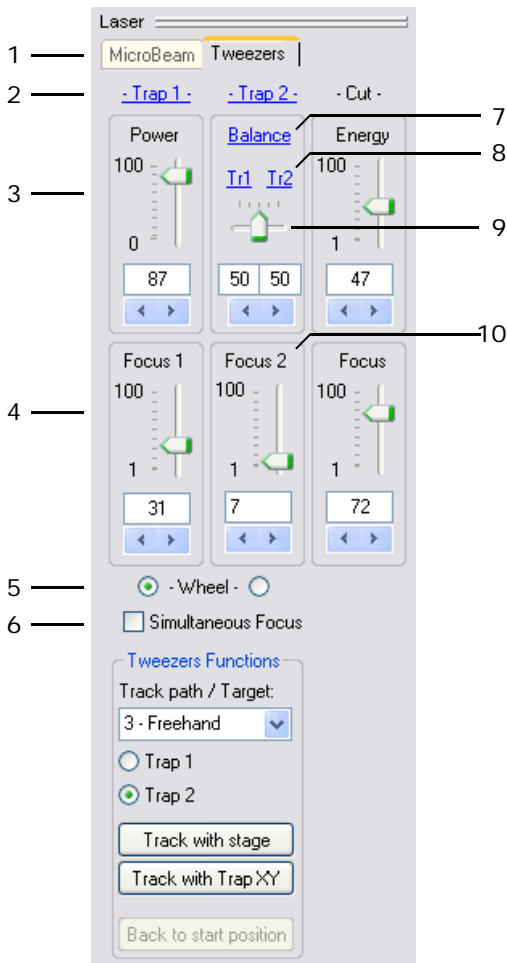


Auto change: deactivated  
Cutting iteration: Cycles = 1

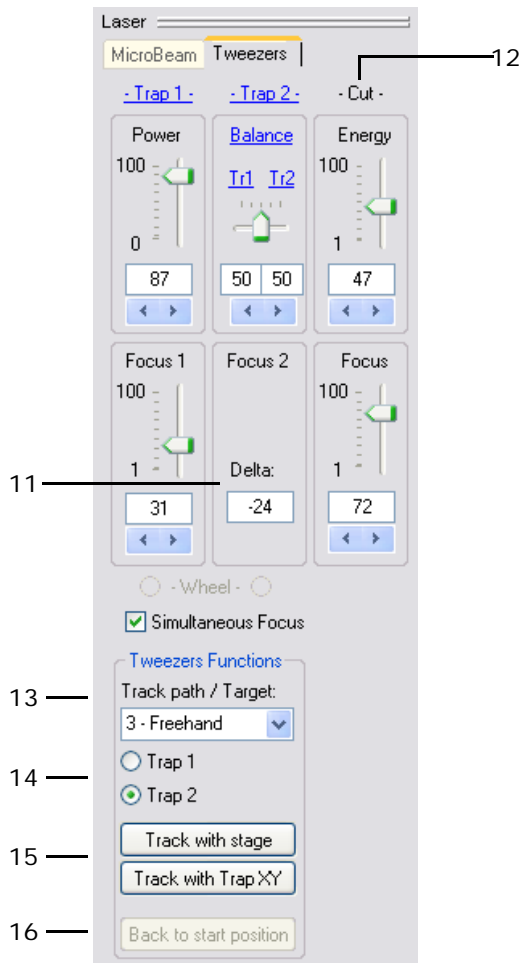
Auto change: activated  
Cutting iteration: Cycles > 1

- |   |   |
|---|---|
| <p>1 To select the laser for which you want to set values for energy and focus. Click on the left tab to show the Laser Tools for the Cutting Laser (MicroBeam).</p> <p>2 Energy setting for laser function "Cut".</p> <p>3 Focus setting for laser function "Cut".</p> <p>4 To select the footswitch function. Click into the left button to select "Cut"; click into the right button to select "LPC".</p> <p>5 To activate resp. deactivate the coupling of energy and focus settings for "Cut" and "LPC". If activated, the values for energy and focus will be changed simultaneously.</p> <p>6 Energy setting for laser function "LPC".</p> | <p>7 Focus setting for laser function "LPC".</p> <p>8 To enter a Delta value for energy when Auto change is activated.</p> <p>9 To enter a Delta value for focus when Auto change is activated. The focus value for LPC will be Focus for Cut + Delta.</p> <p>10 To enter the number of laser operations "Cut" to be performed on each element.</p> <p>11 3-dimensional cutting (appears only when "Cutting iteration Cycles" &gt; 1): To enter a value for z-focus delta. For each cutting cycle the focus will be changed by the z-focus delta value. So you can easily cut thicker specimen.</p> |
|---|---|

**Laser Tools for Trapping Laser (MicroTweezers)**



Simultaneous Focus: deactivated



Simultaneous Focus: activated

**Trapping Laser power and focus settings:**

- 1 To select the laser for which you want to set values for energy and focus. Click on the right tab to show the Laser Tools for the Trapping Laser (MicroTweezers).
- 2 Click on "Trap 1" resp. "Trap 2" to switch to the Trap 1 resp. Trap 2 Movement Mode. In this modes you move the variable Trapping Laser beam 1 resp. 2 by mouse. To exit it click the left mouse button once.
- 3 Power setting for Trapping Laser (sum of energy for both beams).
- 4 Focus setting for Trapping Laser beam 1.
- 5 You can also change the Trapping Laser focus by turning the mouse wheel. Click into the left button to change the focus of Trapping Laser beam 1 with the mouse wheel.

- 6 When the check box is active, the focuses of both beams are coupled. If you change the focus of beam 1, the focus of beam 2 will also be changed, and vice versa (see also No. 11).
- 7 Click with the mouse on "Balance" to set the Trapping Laser power to 50% for each beam.
- 8 Click with the mouse on "Tr1" to set the Trapping Laser power for beam 1 to 100% and for beam 2 to 0%. Click with the mouse on "Tr2" to set the Trapping Laser power for beam 2 to 100% and for beam 1 to 0%.
- 9 Power balance setting for beam 1 and 2.
- 10 Focus setting for beam 2 (only when "Simultaneous Focus" is deactivated).

- 11 To set a Delta value for the focus of Trapping Laser beam 2. When "Simultaneous focus" is activated, the focus of Trapping Laser beam 2 will be Focus beam 1 + Delta.

With changing the focus you can move a trapped specimen in z-direction.

Cutting Laser energy and focus settings:

- 12 Energy and focus setting for Cutting Laser function "Cut" (same as No. 2 and 3 of Laser Tools for Cutting Laser, see page 18. So you can change these settings without switching to tab "MicroBeam").

MicroTweezer Functions:

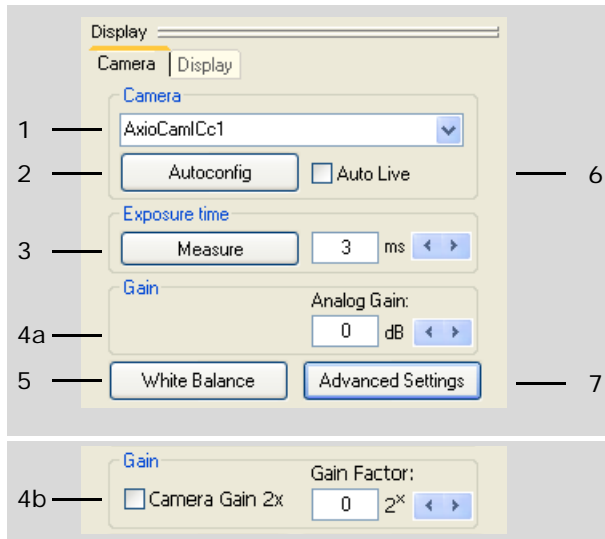
You can move the Trapping Laser along a pre-defined way:

Draw the figure (freehand, line, rectangle, circle; refer to page 7) along which the laser beam is to be moved.

- 13 To chose the figure.
- 14 Click on button "Trap 1" or "Trap 2" selecting the laser beam you want to move along the path.
- 15 Click on "Track with stage" if you want to move the stage under the laser beam while the laser beam covers the selected figure. The laser beam is not moved during this process.  
Click on "Track with Trap XY" if the laser beam is to be moved. The stage is not moved during this process.
- 16 To move the stage or the trapping laser beam back to the start position (after the movement the stage or the laser beam remains stationary at the end-point for the movement).

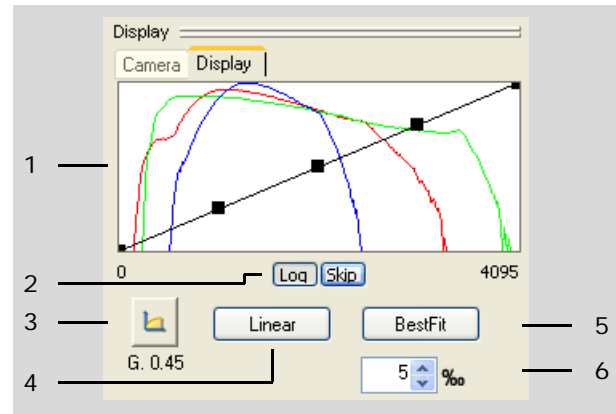
## 11 Camera Tools

Tab "Camera": settings for the camera



- 1 To choose the camera to be used.  
Depending on the camera type, the controls of the supported features are visible in the Camera Toolbar.
- 2 To set the following parameters automatically: exposure time, white balance, gamma, brightness and contrast.
- 3 To measure resp. set the exposure time for the video camera.
- 4 Gain adjustments for fluorescence samples.
- 4a AxioCam ICc1  
To set the analog gain for the AxioCam ICc1; this camera supports an analog gain from 0 ... 24 dB.
- 4b AxioCam MRc/m Rev.3  
To switch on or off the additional camera gain of the AxioCam MRc/m Rev.3.  
To set the gain factor for the AxioCam MRc/m Rev.3. This camera uses a gain factor (0 ... 5) as power of 2 for the gain adjustment. This way gain values of 1, 2, 4, 8, 16, 32 are possible.
- 5 To set the white balance.
- 6 To switch on or off the automated and continuous adaption of camera exposure times of the live image during e.g. objective change or move of the sample.
- 7 To open the window "Live Image". In this window you can adjust parameters for the camera, parameters of the camera picture on the screen (contrast, brightness, gamma) and you can measure color and brightness of a chosen point of your image.

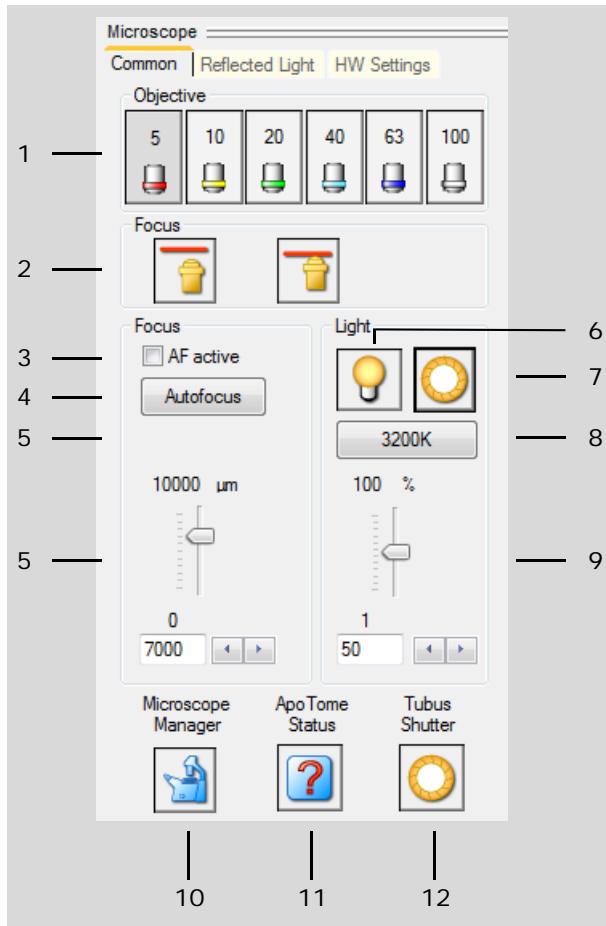
Tab "Display": settings for the display



- 1 Histogram of the current microscope picture on the screen.  
Click on one of the black squares, hold the mouse button and move the mouse to change gamma resp. brightness and contrast of your picture.
- 2 Log: to switch between logarithmic and linear display.  
Skip: ignores the gray or color values for black when displaying the histogram. Useful for images with a predominantly black background.
- 3 To set the gamma value to 0,45.
- 4 To display the entire range of possible values on the screen and sets gamma = 1.0.
- 5 To set brightness and contrast automatically to the best values.
- 6 To set the percentage of pixels to be shown as totally white resp. totally black: the histogram is set in such a way that (in this example) 0.5% of the brightest pixels in the image are shown as completely white, and 0.5% of the dark pixels in the image as completely black.

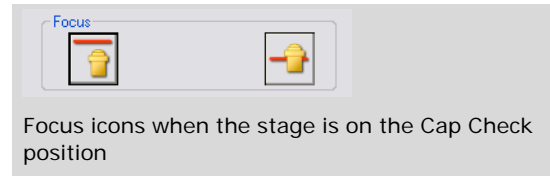
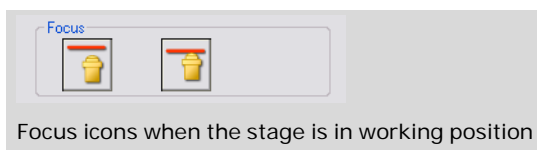
## 12 Microscope Tools




Tab "Common": common settings for the microscope



1 To select the required magnification on the microscope.  
For a correct display of your drawn figure elements and for correct laser functions it is important, that the setting of this menu matches with the set lens on the microscope. For use with Trapping Laser, only the Trapping-specified objective lenses can be selected. Please make sure that the selected objective corresponds to the microscope magnification.

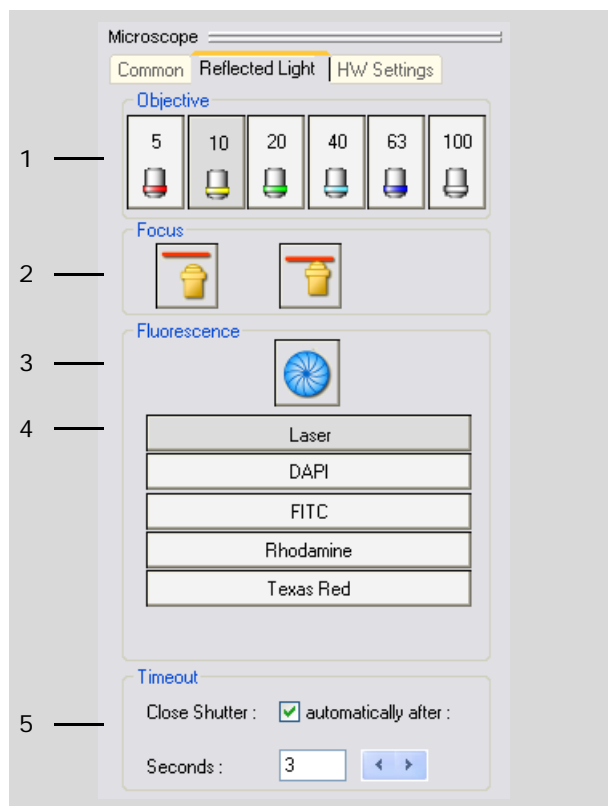
2 To set the microscope focus



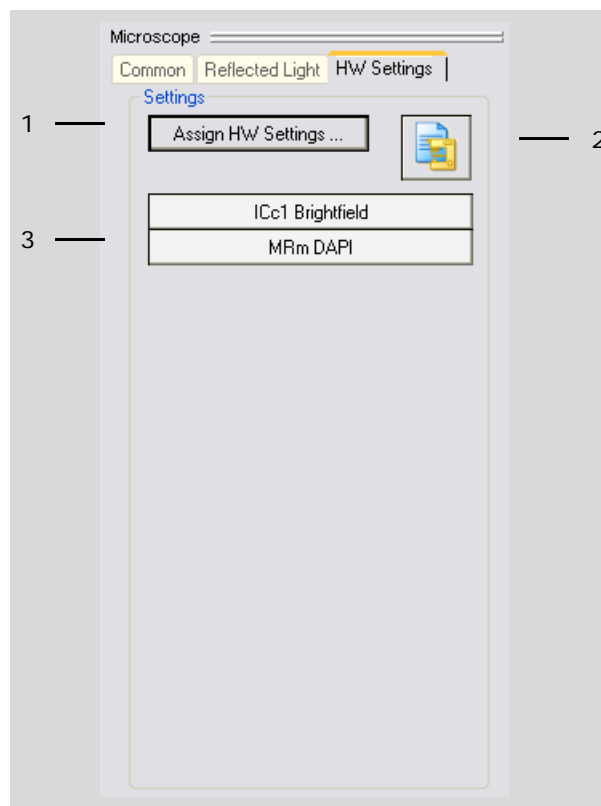
-  To set the microscope focus to Load Position
-  To set the microscope focus to Work Position
-  To set the microscope focus to Check Position

- 3 To switch on/off the Autofocus (only active when your microscope is equipped with Autofocus).  
If the Autofocus is switched on, the focus will always be adjusted when the objective is changed and when the stage is moved to an element during a laser function.
- 4 To release the automatic focusing. Click on the button, and the image will be focused (not active when the stage is positioned at the Cap Check).
- 5 Focus setting.  
You can also set the focus with the mouse wheel.  
For rough setting press the right button of the mouse and turn the wheel.  
For fine setting press the left button of the mouse and turn the wheel.
- 6 To switch the microscope lamp on and off.
- 7 To open and close the microscope transmitted light shutter.
- 8 To set the color temperature of the microscope light to 3200 K.
- 9 Light setting.
- 10 To open the Microscope Window.  
All functions of the microscope are controlled over this window.
- 11 Shows the actual state of the Apotome (appears only if your system is equipped with an Apotome)
- 12 Shows the actual state of the tubus shutter (appears only if your system is equipped with a tubus shutter)

**Tab "Reflected light": settings for fluorescence experiments <sup>1)</sup>**



**Tab "HW Settings": To work with hardware settings**



- 1 To select the required magnification on the microscope (same as Tab "Common"; see No. 1 on page 22).
- 2 To set the microscope focus (same as Tab "Common"; see No.2 on page 22).
- 3 Opens or closes the fluorescence shutter to activate or deactivate the fluorescence beam (If your system is equipped with a filter wheel).

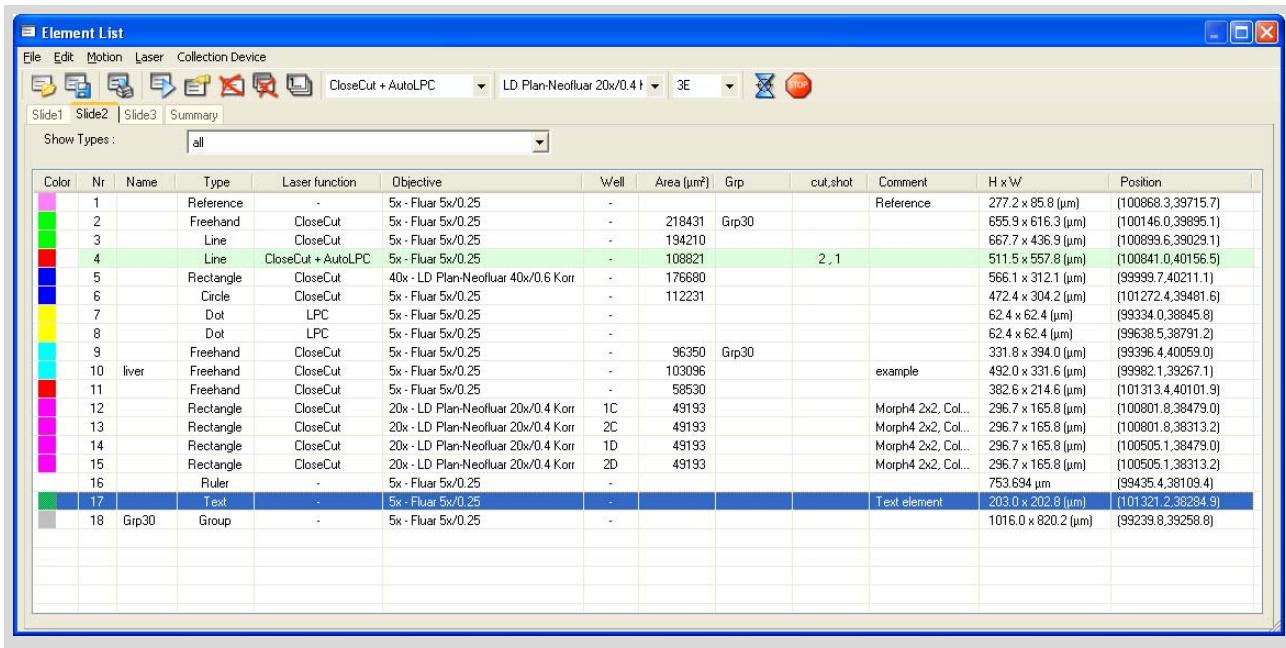


- 4 To select a set of fluorescence settings defined via menu item "Settings > Fluorescence ..." (see page 16).
- 5 Allows to set a timer to close the shutter automatically after a preset time (only when operating manually).

1) Only available in systems equipped with fluorescence Unit. Please contact palm-info@zeiss.de for further information.

- 1 To open the window "Hardware setting adjustments". In this window you chose the hardware settings to be listed below (see No. 3).
- 2 To open the window "Settings editor". In this window you can create different hardware settings.
- 3 To activate pre-defined hardware settings with one mouse click.

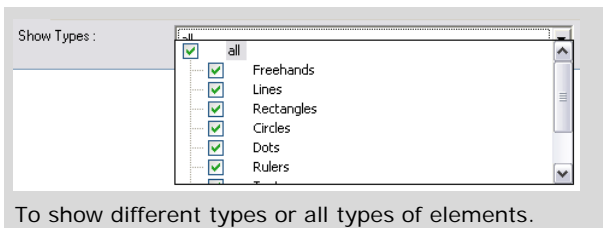
### 13 Element List



The element list displays information about all drawn elements and allows performing operations on them.

Depending on the object holder, there are shown at least two tabs: one or more for the object holders and one for the display of summaries.

#### Tab "Object Holder"

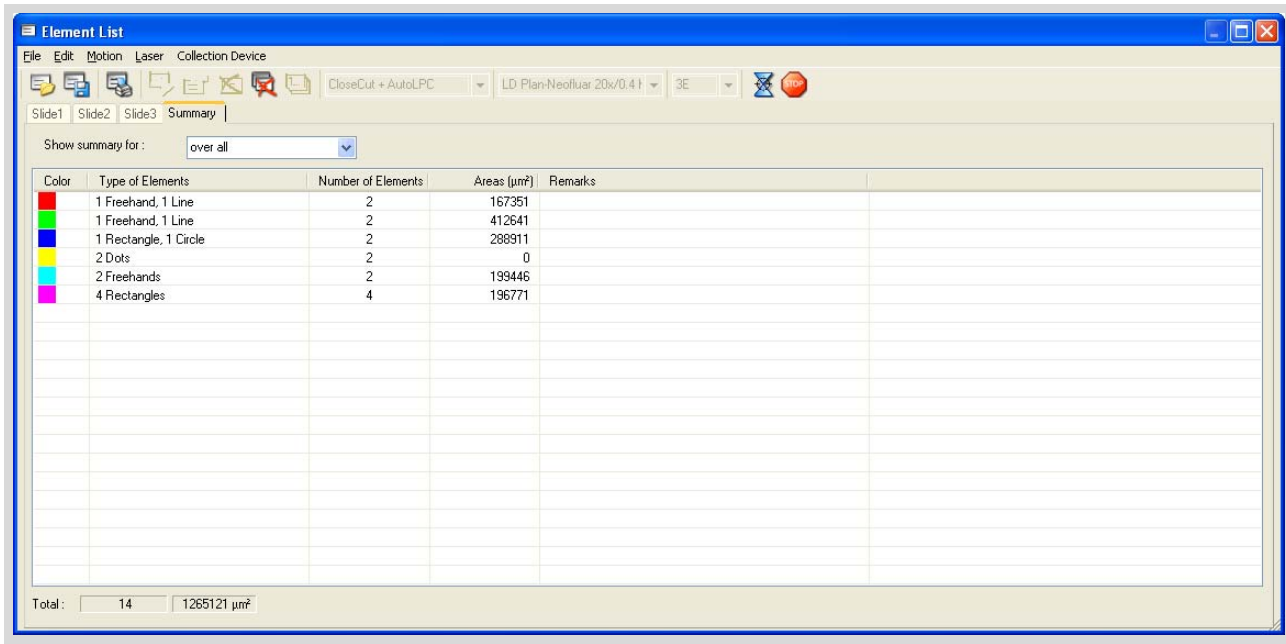


The columns in the table contain the following information about each element:

- 
- Color: color
  - Nr: number
  - Name: name
  - Type: type
  - Laser function: Laser function selected for the element (you can change the laser function in the Cut Tools)
  - Objective: Objective that is used to process the element with the laser (as a rule, the objective that was used when the element was drawn; you can select a different objective)
  - Well: Coordinates of the well which the element is to be catapulted into when a laser function is triggered.
  - Area: area of elements of type "Figure" (Freehand, Line, Rectangle, Circle) (in µm<sup>2</sup>)
  - Grp: group name of grouped elements
  - cut,shot: number of performed laser cuttings or catapultings
  - Comment: a possibly added text
  - H x W: height and width
  - Position: the position (X,Y) relative to the Reference Position
- 

Elements processed with the laser are highlighted in green. Selected elements are highlighted in blue.





**Tab "Summary"**

Show summary for : over all

- over all
- Slide1
- Slide2
- Slide3

To show summary information of a single position or of all possible positions.  
(Elements of types "Ruler", "Text", "Reference" and "Group" are not shown on Tab "Summary").

The columns in the table contain the following information:

Color:	reports the used colors for all types of elements
Type of Elements:	type of elements for the color shown in the first column
Number of Elements:	total number of elements for each color and type
Areas (µm²):	total area of all elements of type "Figure" for each color (in µm²)
Remarks:	remark

Below the table are displayed the sums:

Total:	total number of elements
	total area of all elements of type "Figure"

**Menus of Element List**

Menu "File" (see also page 11, Menu "File")

- New Elements / Delete all Ctrl+N  
To prepare the software for drawing new elements (only File Mode).
- Open Elements ...  
Save Elements ...  
To open and save elements (only File Mode).
- Enter / Select Data ...  
To enter or select data (only Database Mode).
- Import Elements ...  
Export Elements ...  
To import resp. export the element properties (color, number, type etc.) and the coordinates of the anchor points of the elements from resp. to a text file (\*.txt).
- Print Elements ...  
To print the element properties into an Excel import file (\*.csv).
- Close  
To close the element list.

Menu "Edit" (see also page 12, Menu "Edit")

Select All Elements	
Unselect All Elements	

To select resp. deselect all elements.

Element Properties
--------------------

To change the properties of the selected element.

Renumber All Elements
-----------------------

To renumber the remaining elements after deletion of elements.

Copy
Paste

To copy the selected elements to clipboard.  
To paste the elements from clipboard.

Delete Selected Elements	Del
Delete All Elements	Ctrl+Y

To delete the selected resp. all elements.

Create Group
Add to Group
Remove from Group

To create a group of elements from the currently selected elements.  
To add elements to a group.  
To remove elements from a group.

Define Group-Reference-Figure
UnDefine Group-Reference-Figure

To define resp. undefine up to three elements as group reference figures.

Match Serial Section Group
----------------------------

To transfer elements from one slide to another for serial sections automatically.

Menu "Motion" (see also page 13, Menu "Motion")

Go to Element
---------------

To center the selected element on screen.

Menu "Laser" (see also page 15, Menu "Laser")

Start Laser Function	F11
----------------------	-----









To start Cutting Laser function after all settings are done.

Menu "Collection Device"

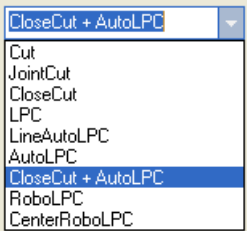
Calculation
-------------

To open the window "Distribution Calculation". In this window you choose an operating mode for PALM RoboMover (only possible and appropriate if a capture device with several capture positions is fitted).

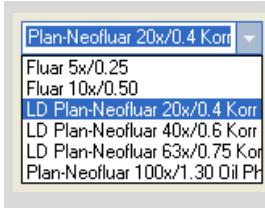
Toolbar of Element List

	Load elements To load previously saved elements.
	Save Elements To save the drawn elements in a file (only File Mode).
	Print Elements To print the element properties into an Excel import file (*.csv).
	Goto To center the selected element on screen.
	Element Properties To change the properties of the selected element.
	Delete selected To delete the selected elements.
	Delete all To delete all elements.
	Renumber All To renumber the remaining elements after deletion of elements.

Cut Tools of Element List

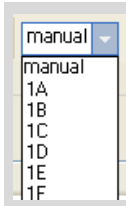
	<p>To select a laser function for the elements currently selected.</p> <p>For an overview of the Laser functions see page 29.</p>
--	---

### Objective Tools of Element List



To select an objective under which the currently selected element is to be handled by the Cutting Laser.

### Well Tools of Element List



To select the well into which the currently selected element is to be catapulted.

### Start/Stop Cutting Laser



Start Laser



Stop








To stop the Cutting Laser function and the stage movement immediately in case of emergency.

## 14 Basic Mode and Pro Mode

PALM RoboSoftware is available as Basic version and as Pro version. The Basic version provides you with all basic functions for your work with PALM MicroBeam and PALM MicroTweezers. The Pro version is licensed for more functionalities: Pro-Mode (with Autofocus, Recorder, Field of View Analysis).

Please contact [palm-info@zeiss.de](mailto:palm-info@zeiss.de) for further information.

## 15 Shortcuts

Shortcut	Icon/Menu
<b>Menu Help</b>	
F1	Help > PALMRobo Help
<b>Menu File</b>	
Ctrl+N	File > New Elements / Delete All Elements
Alt+F4	File > Exit PALMRobo
<b>Menu Edit</b>	
Ctrl+A	Edit > Select All Elements (current slide) / Edit > Unselect All Elements
Ctrl+C	Edit > Copy
Ctrl+V	Edit > Paste
Backspace	 Delete Last Element Edit > Delete Last Element
Alt+Backspace	Edit > Undelete
Ctrl+Y	 Delete All Elements Edit > Delete All Elements
Del	 Delete Selected Elements Edit > Delete Selected Elements
Alt+Enter	Edit > Element Properties
<b>Menu View</b>	
Alt+X	View > Hide All Bars / View > Show All Bars
F2	 Incubation View > Incubation Device ...
F3	 Information Center View > Information Center ...
F4	 Navigator Window View > Navigator Window
F5	 Element List View > Element List

Shortcut	Icon/Menu
F6	 Microscope Window View > Microscope ...
F12	 Capture Device Window View > Capture Device ...
<b>Menu Motion</b>	
Alt+F	 Freeze Mode Motion > Freeze Mode
Ctrl+F	Motion > Increase Speed
Ctrl+K	Motion > Goto Cap Check / Return
Ctrl+S	Motion > Decrease Speed
Esc	 Stop Motion > Stop
F7	 Stage Motion > Stage Mode
<b>Menu Laser</b>	
End	Laser > Focus Down
Home	Laser > Focus Up
Page up	Laser > Increase Energy/Power
Page Down	Laser > Decrease Energy/Power
<b>other</b>	
B	Scroll through the multichannel fluorescence images backwards
F	Scroll through the multichannel fluorescence images forwards
Alt+P	Toggle between Standard Pointer and Group Reference Pointer
Ctrl+U	Microscope focus up (1 µm)
Ctrl+D	Microscope focus down (1 µm)

Turnig the mouse wheel while pushing the left mouse button      Microscope focus up resp. down (1  $\mu\text{m}$ )

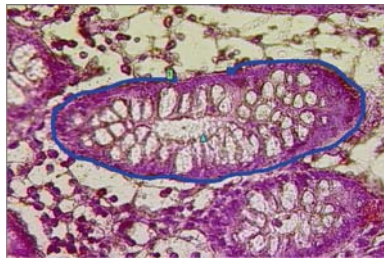
Turnig the mouse wheel while pushing the right mouse button      Microscope focus up resp. down (10  $\mu\text{m}$ )

Turnig the mouse wheel      Trap laser focus 1 or Trap laser focus 2 up resp. down

The focus of which laser beam is changed depends on the setting of the wheel radiobuttons in the Trapping laser Tools – see page 19.

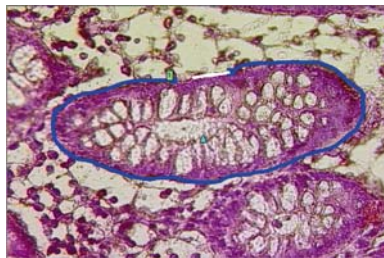
## 16 Laser Functions – an Overview

**Cut**  
Cutting along the pre-defined line



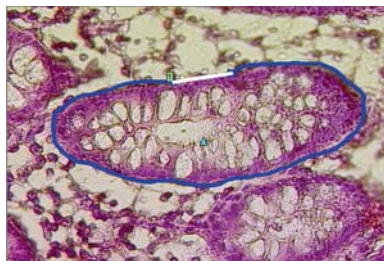
The laser cuts precisely along the predefined line yielding a clear-cut gap between the selected and non-selected material. Thus pure sample preparation is possible without danger of contamination.

**JointCut**  
Close the line but leaving a small connecting piece to cut membrane-mounted preparations, living cells and moist tissue samples.



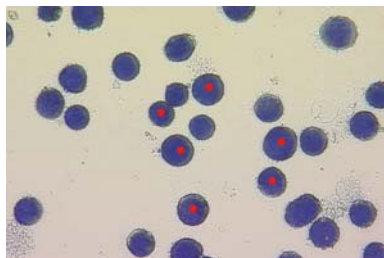
A cutting function where the marked line leaves a small connecting piece. The entire area can be catapulted later with one single shot. This function is dedicated for cutting automatic geometric figures to avoid unintentional movement.

**Close & Cut**  
Close and cut the line. For membrane-mounted preparations; living cells on membranes and moist tissue samples.



The enhanced cut function will close the incompletely drawn figure by connecting the start point and the end point with a straight line.

**LPC**  
Laser Pressure Catapulting



Only LPC dot-marked specimens are catapulted. The catapult point can be set manually, to individually catapult samples out of tissues after laser cutting. This function is of special benefit for cytocentrifuged specimen and for isolated cells within a histological preparation.

**LineAutoLPC**

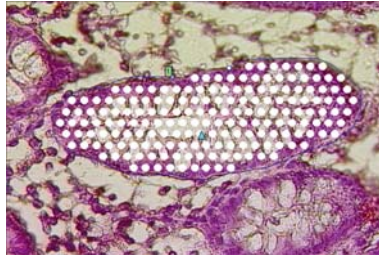
This function is designed to extract line-shaped routes.



A line-shaped structure is catapulted into your collection vessel using this function. The line is therefore not catapulted in one piece, but with several laser pulses. The original structure of the material is not preserved when using this function.

**AutoLPC**

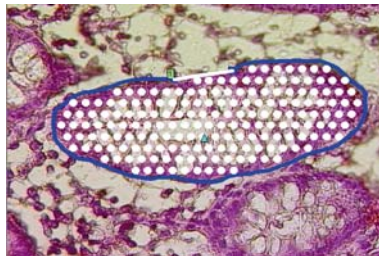
Automatic catapulting of larger areas from glass-mounted preparations only.



With glass-mounted preparations only a small amount of cellular material can be catapulted with each single shot. Therefore larger areas have to be catapulted with multiple shots. The user circumscribes the area to be catapulted and defines the laser shot grid in the Settings menu (how many shots per  $\mu\text{m}^2$ ).

**Close & Cut + AutoLPC**

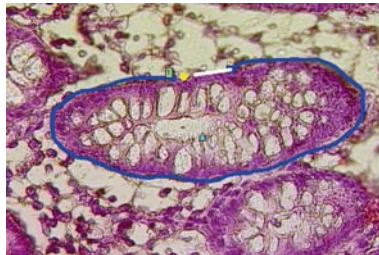
Glass-mounted preparations: An open figure is closed and subsequently cut and catapulted.



Prior to AutoLPC the selected material is completely separated by cutting a closed line around the area of interest. Used for critical preparations, where contamination with neighboring tissues definitely has to be avoided.

**RoboLPC**

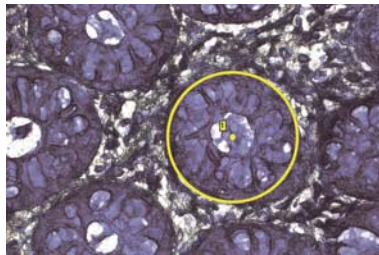
Cutting and catapulting in a single step! Only possible with membrane-mounted specimen.



The marked line is entirely closed leaving a small connecting piece from where the entire area is immediately catapulted with one single shot. The size of the connecting piece can be pre-selected in the Settings menu and displayed together with the RoboLPC-dot.

**Center RoboLPC**

Similar to the "RoboLPC" function, only the element is cut completely and the laser pulse for catapulting is placed in the center of the figure.



With the "Center RoboLPC" function, defined structures are cut out and catapulted intact into the cap in one work step.



