PALM RoboSoftware
Quick Software Guide
December 2007

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The instruments, instrument components or methods described in this manual are protected by the following patents:

- Laser catapult technology (Laser Pressure Catapulting LPC^{pat})
  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.
- Additional patents pending.

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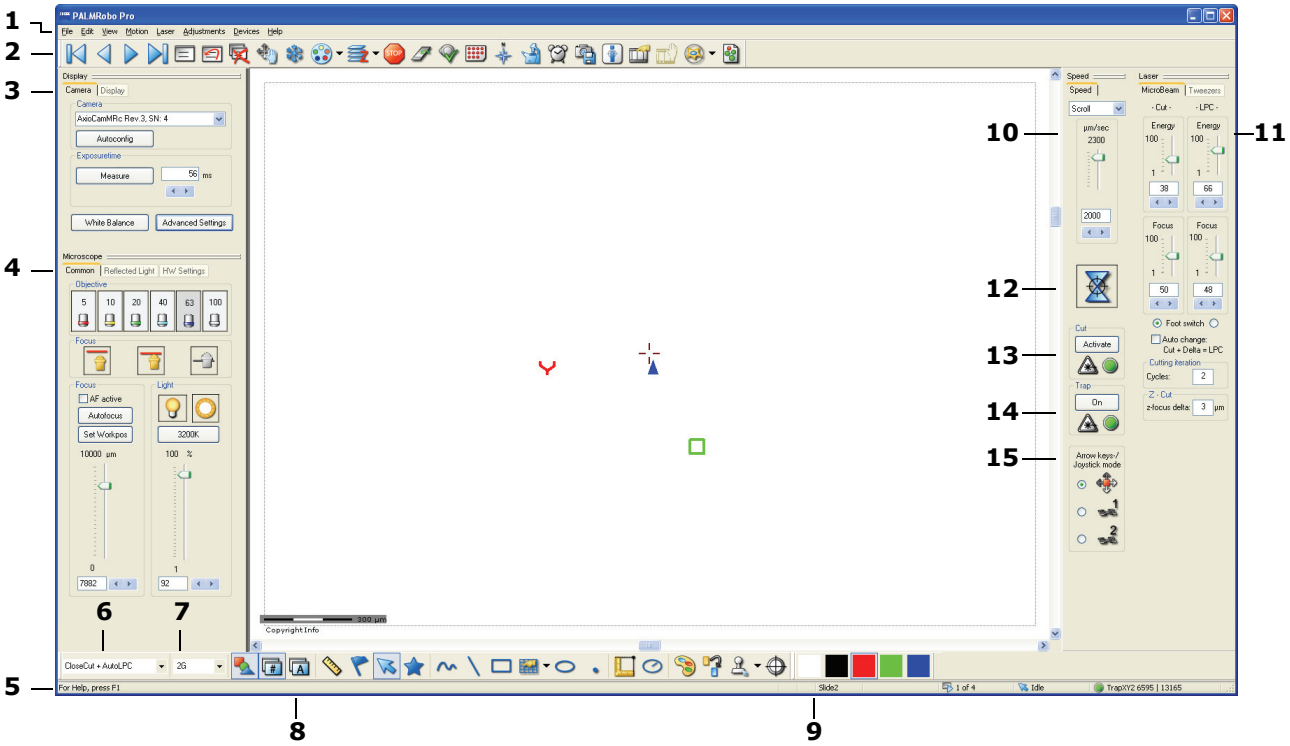
In this quick guide the functions of the PALM RoboSoftware are described in a short way.

1 Program Layout



1 Menus (page 10)

2 Tool Bar (page 5)

3 Camera Tools (Camera and Display Settings; page 20)

4 Microscope Tools (Settings for the microscope, for fluorescence and hardware settings; page 21)

5 Status Bar (page 16)

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10 Speed Tools (page 16)

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13 Cutting Laser status (page 8)

14 Switch on/off Trapping Laser, Trapping Laser status (page 8)

15 Arrow keys/Joystick mode (page 9)

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 set back to default.

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

2 Tool Bar

The Tool Bar contains the following tools:

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

To delete the last drawn element.

Delete all elements

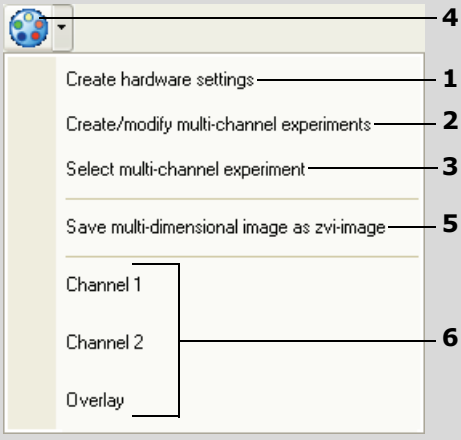
To delete all elements, also when hidden.

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-channel fluorescence images. Proceed step by step as described in the following:

1 - set hardware parameters

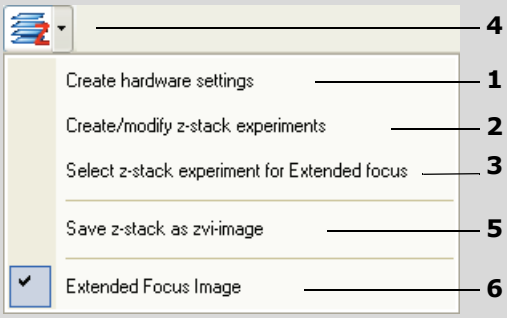
2 - enter and save parameters for your multi-channel experiment

3 - select an experiment

4 - click on this icon to start

5 - save the images

6 - show a single channel image or the multi-channel image (overlay) on the screen (these menu items appear after acquiring the image)



To acquire images with extended focus (z-stack experiments). Proceed step by step as described in the following:

1 - set hardware parameters

2 - enter and save parameters for your z-stack experiment

3 - select an experiment

4 - click on this icon to start

5 - save the images

6 - show the image with extended focus (if you had left the Freeze Mode before; this menu item appears after acquiring the image).

Stop

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

Loadposition

To move the stage to Loadposition.

Capcheck

To position the stage to the Capcheck.

Point of origin

To move the stage from Capcheck back to the point of origin.

Capture device [1]

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.

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.

Microscope window

To open the Microscope Window.
All functions of the microscope are controlled by this window.

Alarm Bell

Opens the Alarm Bell window. In this window you can select a time and a message for the alarm, and you can start the alarm.

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 "Adjustments > PALM Robo ...", page 14). 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.

Information Center

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

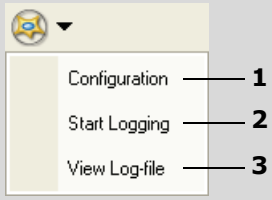
Time Lapse [2]

Opens the Time Lapse window. In this window you can adjust parameters for the Time Lapse function and determine the trigger point. Depending on the chosen trigger point one of the following icons appears in the Tool Bar:

Time Lapse will be started manually:
Click on this icon to start the Time Lapse function.

Time Lapse will be started together with the next Cutting Laser function start:
Foot switch (Cut or LPC), or
LPC Laser function.

Time Lapse will be started scheduled.



To work with incubation [3]

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 "Adjustments>PALM Robo ..." event controlled or time controlled)
After having started logging the menu item changes to "Stop Logging"

3 - open the log-file

Field of View Analysis [2]

To start the function Field of View Analysis which will find elements on your specimen in an interactive way.

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

2) Only available in systems with Pro Licence. Contact palm-info@zeiss.de for further information.

3) Only available in systems equipped with Incubation module.

3 Graphic Tools

Display functions

Graphic on/off

To show or hide all elements.

Number on/off

To show or hide the numbers of the elements.

Element areas

To display the size of an element area in µm².

Select elements

Pointer

To select elements, which then can be changed (e.g. right mouse button > "Change"), moved or deleted.

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 other elements to be selected.

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

Ruler, flag, comment

Ruler

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

Flag

To set a flag into the image. You can add a comment to the flag.

You can change the comment in the dialog "Edit > Change".

Create and edit elements

Reference Point

To set a reference Point.

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.

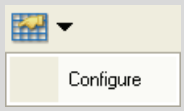
If the quadratic attribute is selected, you draw horizontal and vertical lines.

Rectangle

To draw rectangles.

If the quadratic attribute is selected, you draw quadratic elements.

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



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.

Circle

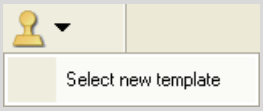
To draw ellipses.

If the quadratic attribute is selected, you can only 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).



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 you now can select the element to copy.

Click on the element to copy.
Click at the desired position on the screen to copy the element.

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.

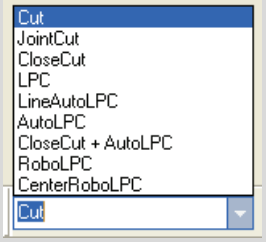
The colors displayed in the Color Palette can be chosen via icon "Colors".

Center

Centre

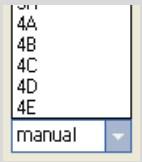
Click on an arbitrary point in your microscope image to center this point on the screen.

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

The Cutting Laser is deactivated.

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

The Cutting Laser is activated.

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

The Cutting Laser is activated and has been started.

Trapping Laser

The Trapping Laser is switched off.

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

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.

Click into the first button to control the stage with Arrow keys resp. Joystick.

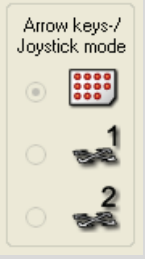
Only possible when the stage is not positioned at the Capcheck.

Click into the second button to control Trapping Laser beam 1 with Arrow keys resp. Joystick.

Click into the third button to control Trapping Laser beam 2 with Arrow keys resp. Joystick.

If both buttons are activated you can control both Trapping Laser beams simultaneously with Arrow keys resp. Joystick.

Only possible when the stage is not positioned at the Capcheck.

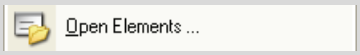


When the stage has been positioned at the Capcheck you move PALM RoboMover resp. PALM CapMover with the Arrow keys resp. the Joystick. In this case the buttons are deactivated.

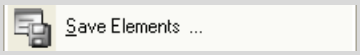
7 Menus

Menu "File"

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



To open elements from a file (only File Mode):
Open the elements with "Open Elements...".

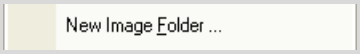


To save the drawn elements in a file (only File Mode).
The file can be called up any time with PALM RoboSoftware.
The elements are saved in the default directory (see "Adjustments > PALM Robo ..." page 14).

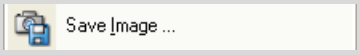
To enter or select data in Database Mode (only Database Mode).

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.



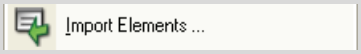
To select or create a document folder for saving pictures in File Mode (in Database Mode this folder is of no meaning).



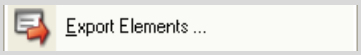
To save an image as a file (with or without the drawn elements).
In File Mode the images are saved in the default directory (see "Adjustments > PALM Robo ..." page 14).
The folder for saving can be changed.

In Database Mode the images are saved in the database.

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

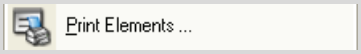


To import the element properties shown in the element list (color, number, type etc.) and the coordinates of the anchor points of the elements from a text file.



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.



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.

To open one of the last four files with saved elements (only File Mode).

To quit PALM RoboSoftware.

Menu "Edit"

Undelete Alt+BackSp

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

Select all (all positions)

To select all elements for all slides.

Select All (current position only) Ctrl+A

To select all elements for the current slide.

DeSelect All Ctrl+A

To deselect all elements.

Change Alt+Enter

To show and change the properties of the selected element.

Restore

To restore a selected element that has been edited previously.
If more than one element is selected, all selected elements are restored.

Renumber all

To renumber the remaining elements after deletion of elements.

Copy Ctrl+C

Paste Ctrl+V

To copy the selected elements to clipboard.

To paste the elements from clipboard.

Delete selected elements Del

To delete the selected elements.

Delete last element BackSp

To delete the last drawn element.

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

Delete all elements Ctrl+Y

To delete all elements, including hidden elements.

Create Group

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

Add to Group

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

Remove from Group

To remove one or more selected elements from a group.

Define Group-Reference-Figure

To define up to three elements as Group Reference Figures.

UnDefine Group-Reference-Figure

To undefine an element as Group Reference Figure.

Match Serial Section Group

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

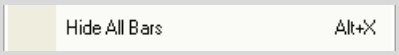
Match Reference Point manual

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

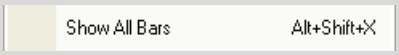
Update selected or all Elements

To refresh the presentation of elements on the screen.

Menu "View"



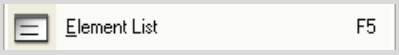
To hide all Tool bars and Tools.



To show all Tool bars and Tools.

To show all Tool bars and Tools in the default configuration.

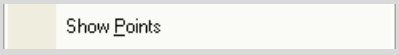
To open the PALM Navigator Window.



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

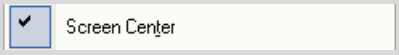
To show or hide all drawn elements. Newly drawn elements are not shown if the display of elements is switched off.

To show or hide the numbers of the elements.

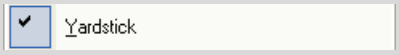


To show or hide the anchor points of drawn elements

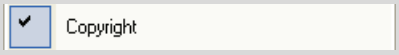
To show or hide the scroll rectangle (dashed frame).



To show or hide the screen center mark (red reticule).

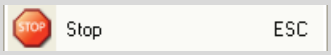


To show or hide the yard stick.



To show or hide the copyright info.

Menu "Motion"



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.

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

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

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

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

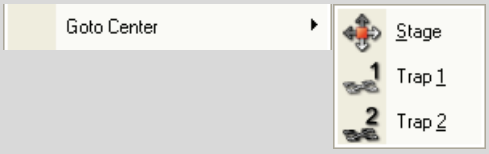
To switch to TrapXY 2 movement mode. In TrapXY 2 movement mode you move the variable Trapping Laser beam 2 with mouse. To exit this mode click left mouse button once.

To modify the current speed of the movement.

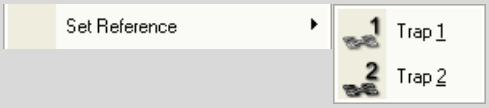
To center a certain element on the screen.

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

To move the stage to the Loadposition.

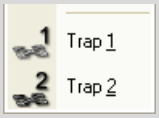


To move the stage resp. the Trapping Laser beam 1 resp. 2 to the center.

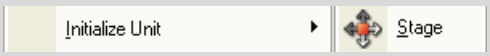


To define a Reference Position for the Trapping Laser beam 1 and 2.

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



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



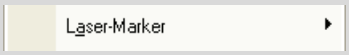
To move the stage to the end position in x and y direction.
This position is set as zero point for element coordinates.
Then the stage moves back to its initial position.

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.

To initialize the Trapping Laser beam 1 resp. 2.

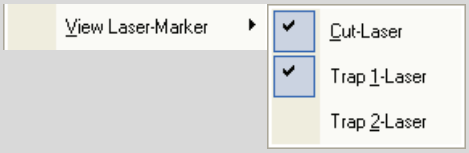
The Trapping Laser beam 1 resp. 2 is automatically initialized at every start of the program. Therefore "Initialize Unit > Trap 1" resp. "... Trap 2" must only be used in case of error.

Menu "Laser"



To align the position of Laser-Marker with the current position of laser focus (for each laser separately).

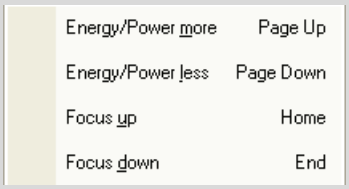
With "Layout Laser-Marker" the color, size, type and style of the Laser-Marker can be defined (for each laser separately).



With "View Laser-Marker" the Laser-Marker can be shown or hidden (for each laser separately).

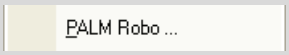
To start Cutting Laser function after all settings are done.

To switch on/off Trapping Laser.



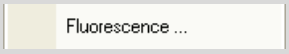
To change the values for laser energy/ power and laser focus. The set values are valid for the currently in the Laser Tools selected laser only (Cutting Laser or Trapping Laser; see page 17 and page 18). During laser action these values can be changed via keyboard (see page 28) or Laser Tools (see page 17 and page 18).

Menu "Adjustments"



General settings for configuration (operating mode, stage, metric, saving settings), saving elements, saving images, laser function, autofocus of a motorized microscope, Trap-Footswitch and Time Lapse function.

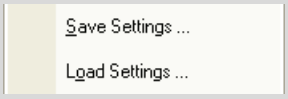
To open the window "Settings editor". In this window you can define Hardware Settings which can be activated via "Microscope Tools" / tab "HW Settings" (page 23).



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

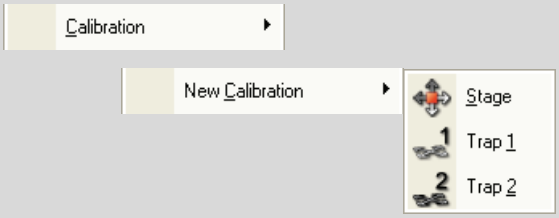
- create a new or change an existing set of fluorescence settings.

  Defined fluorescence settings are activated via Microscope Tools, Tab Reflected light (see page 22).
- 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.



To save and load preset speed settings for: stage, arrow keys, cutting, LPC, positioning, scrolling, TrapXY and TrapXY Arrow), and to save and load laser settings (Cutting Laser and Trapping Laser) for: energy/ power, focus, lens value, LPC distances, Auto-change and balance.

To reset all settings to default settings.



To calibrate the software with respect to the microscope setup.
This is necessary for each kind of movement separately to obtain exact cuttings and measurements resp. exact trapping.
The calibration is stored with respect to lens magnification.

To call up the default setting for the calibration.

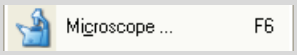
To compensate the offset of the microscope objective (chromatic aberration).

To call up the default setting for the Objective-Offset.

To compensate the offset of the reflector.

To call up the default setting for the Reflector-Offset.

Menu "Devices"



To open the Microscope window.

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)]

To open the window "Incubation" where you can select and change settings for your incubation device. [2)]

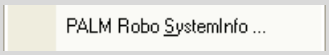
1) Only available in systems equipped with PALM RoboMover resp. PALM CapMover II. Contact palm-info@zeiss.de for further information.

2) Only available in systems equipped with incubation device. Contact palm-info@zeiss.de for further information.

Menu "Help"

The "PALM Robo Instructor" gives help for various subjects.

To list help topics.



To show system information.

To get information about the program (version number and manufacturer).

8 Status Bar

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

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

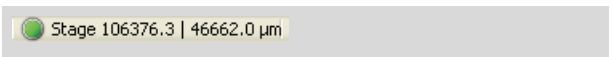
- Shows that you can control the Trapping Laser beams with the Joystick. If these fields are empty you control the stage or (when the stage is on the Capcheck) PALM RoboMover with the joystick.

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

- Currently used setting file.

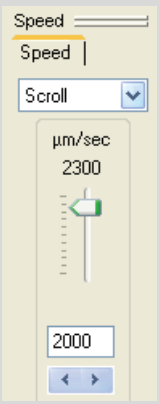
- Number of elements, or number of the centered element (x of ...) and of all elements (... of y) (shown after using any function for centering an element).
  Doubleclick into the field to open the "Element List" window.

- Shows active mode: Laser ON, Stage Mode, Cursor Mode, Scrolling, Position, 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" will be shown.



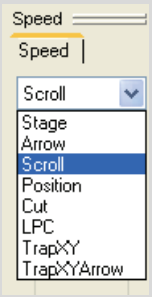
- Coordinates (x|y) of the current moving resp. the last moved unit (stage, Trapping Laser, PALM RoboMover, PALM CapMover II).
  Doubleclick into the field to open the "States" window (shows the current coordinates and status of all installed units).

9 Speed Tools



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

The unit can be set via menu item "Adjustments > PALM Robo... > Tab Configuration" to µm/sec. or to percent.



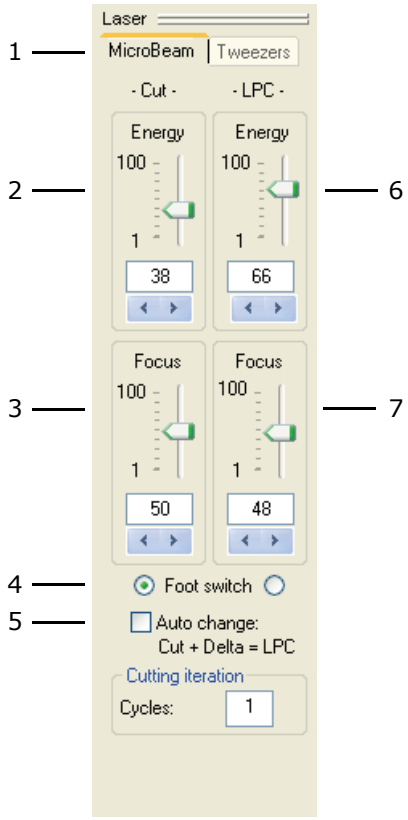
Stage	Setting of the relation between mouse movement and movement of stage.
Arrow	Speed setting for the movement of stage with arrow keys.
Scroll	Speed setting for scrolling in cursor mode.
Position	Speed setting for stage movement from element to element, to Capcheck or to Reference Position.
Cut	Speed setting for stage movement during Cut function.
LPC	Speed setting for stage movement during AutoLPC function.
TrapXY	Speed setting for movement of the Trapping Laser beam with mouse and track function.
TrapXYArrow	Setting of speed for the movement of the Trapping Laser beam with joystick in Trapping mode.

10 Laser Tools

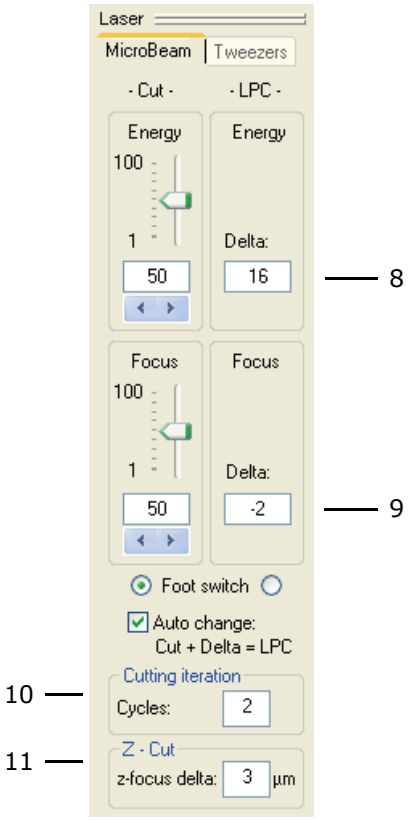
With the Laser Tools you set values for energy resp. power and focus for the Cutting Laser (MicroBeam) resp. Trapping Laser (Tweezers). The preset values for energy resp. power, focus and balance can be changed before each laser operation.

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

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).

2 Energy setting for laser function "Cut".

3 Focus setting for laser function "Cut".

4 To select the footswitch function. Click into the left button to select "Cut"; click into the right button to select "LPC".

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.

6 Energy setting for laser function "LPC".

7 Focus setting for laser function "LPC".

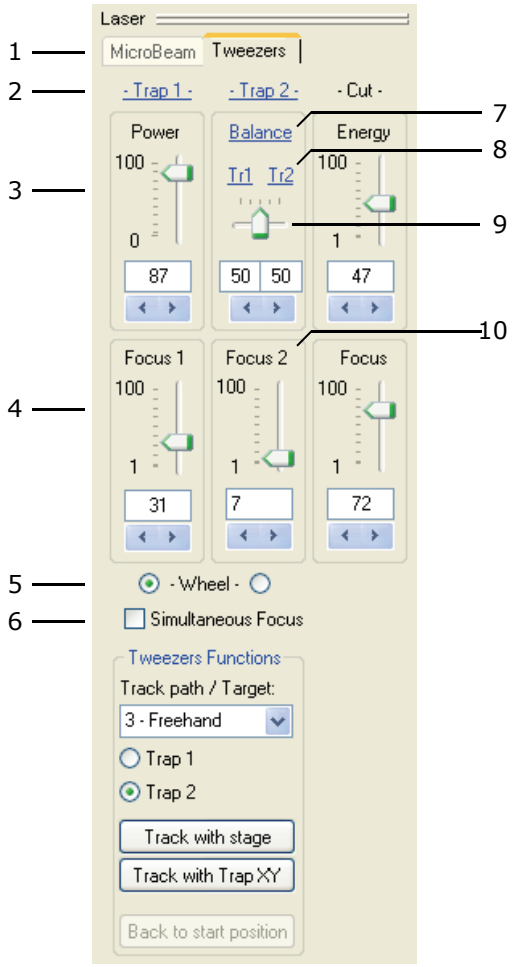
8 To enter a Delta value for energy when Auto change is activated.

9 To enter a Delta value for focus when Auto change is activated. The focus value for LPC will be Focus for Cut + Delta.

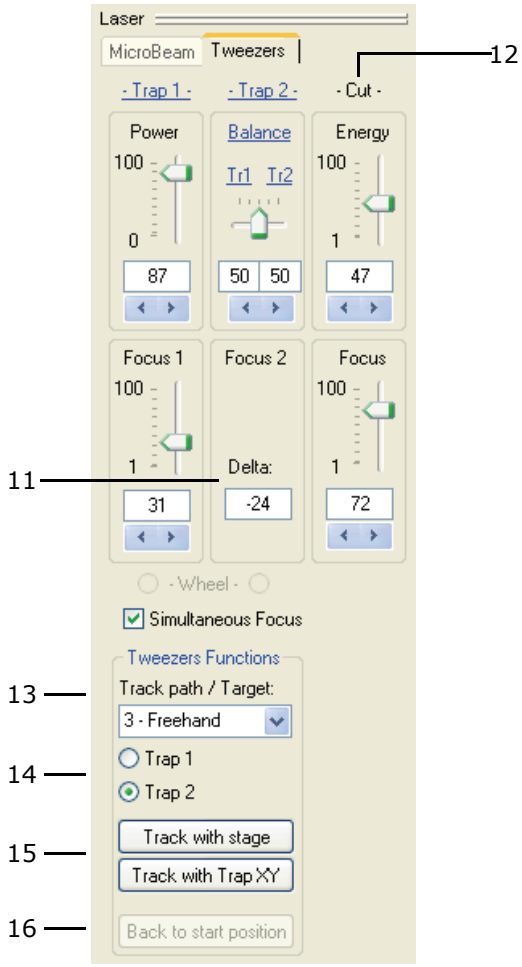
10 To enter the number of laser operations "Cut" to be performed on each element.

11 3-dimensional cutting (appears only when "Cutting iteration Cycles" > 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.

Laser Tools for Trapping Laser (Tweezers)



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 (Tweezers).

2 Click on "Trap 1" resp. "Trap 2" to switch to the Trap 1 resp. Trap 2 Movement Mode. In the Trap 1 resp. Trap 2 Movement Mode you move the variable Trapping Laser beam 1 resp. 2 with the mouse.
To exit this mode 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.
Click into the right button to change focus of Trapping Laser beam 2 with the mouse wheel.

6 When the check box is activated, the focus of both beams is coupled to each other. 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 17. So you can change values for energy and focus without changing to tab "MicroBeam").

Tweezer Functions:

You can move the Trapping Laser along a predefined 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" depending on which laser beam you want to move along the path.

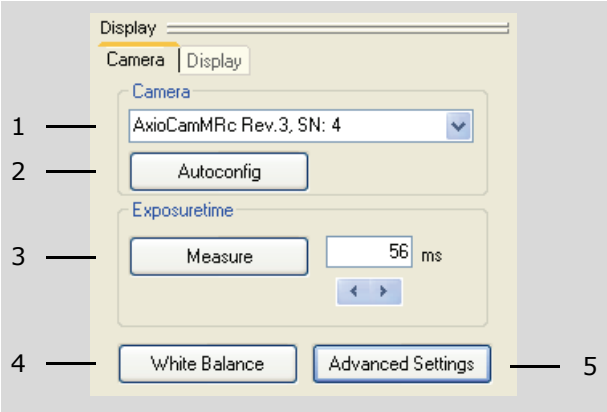
15 Click on "Track with stage" if you want to move the stage under the laser beam such that the laser beam covers the selected figure. The laser beam is not moved during this process.

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 dot for the movement).

11 Camera Tools

Tab "Camera": settings for the camera



1 To chose the camera to be used.

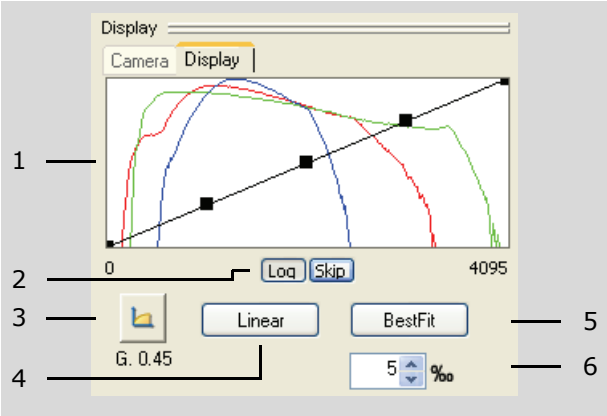
2 To set the following parameters automatically: exposure time, white balance, gamma, brightness and contrast.

3 To measure resp. set the exposure time for the video camera.

4 To set the white balance.

5 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 change 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.

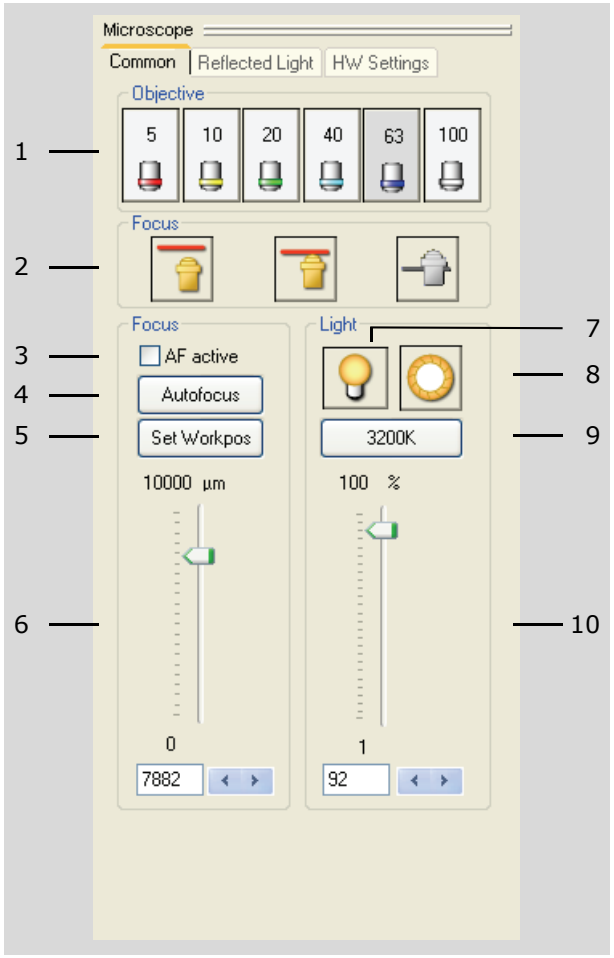
4 To display the entire range of possible values on the screen and sets gamma = 1.0.

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 Left icon: to set the microscope focus to Load Position.
Center icon: to set the microscope focus to Work Position.
Right icon: to set the microscope focus to Check Position.

Caution!
Set the focus only to the Check Position, if the stage has been moved to the Capcheck before. Otherwise the objective will collide with the object holder or the stage and may be damaged.

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.

5 Depending on the current position of the stage one of the two buttons shown below appears:

If the stage is not at the CapCheck, you can use the button to define the focus setting "Work Position".

If the stage is at the CapCheck, you can use the button to define the focus setting "Check-Position".

6 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.

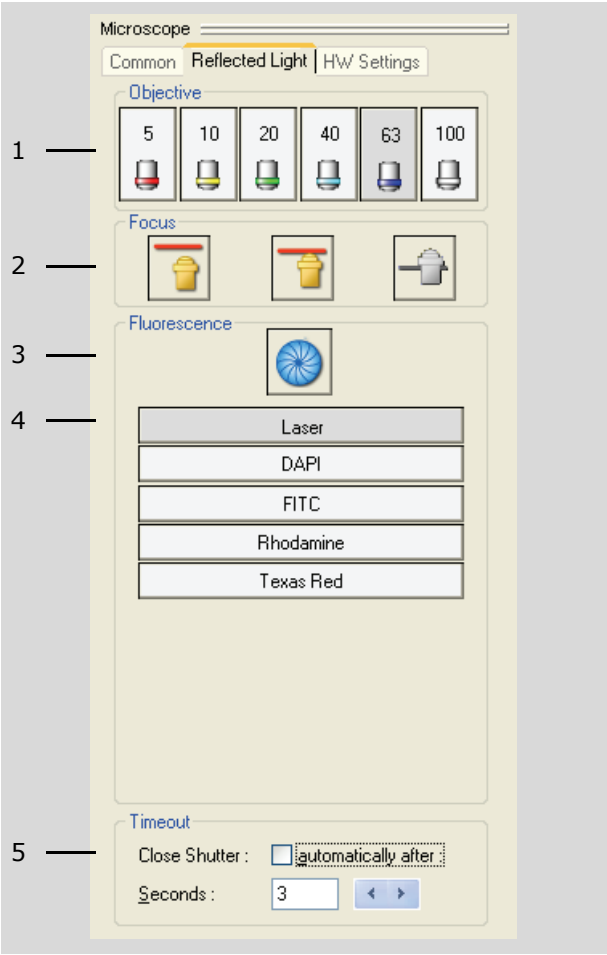
7 To switch the microscope lamp on and off.

8 To open and close the microscope transmitted light shutter

9 To set the color temperature of the microscope light to 3200 K.

10 Light setting.

Tab "Reflected light": settings for fluorescence experiments [1)]



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 Left icon: to set the microscope focus to Load Position.

Center icon: to set the microscope focus to Work Position.

Right icon: to set the microscope focus to Check Position.

3 Opens or closes the fluorescence shutter to activate or deactivate the fluorescence beam (If your system is equipped with a filter wheel).

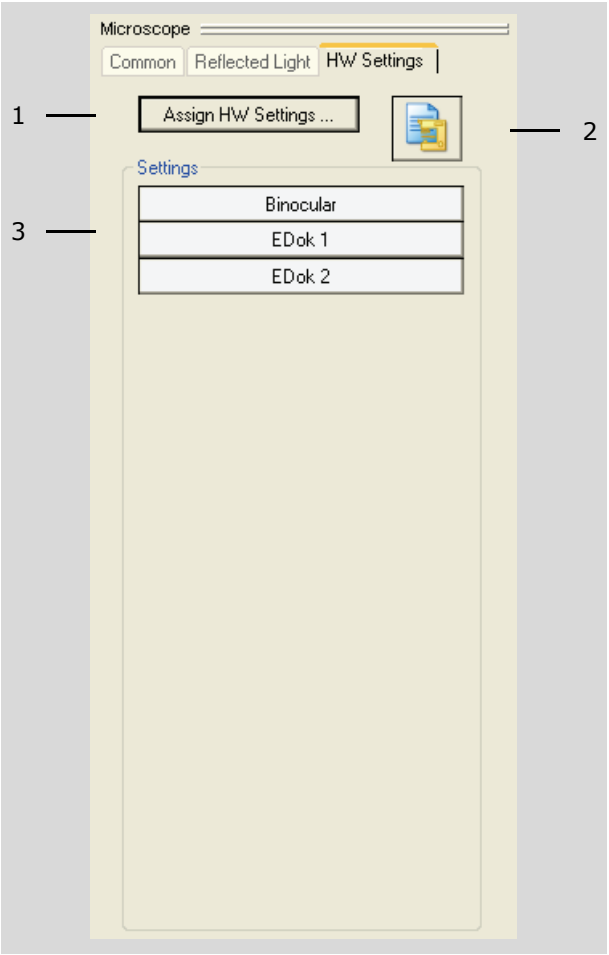
 Fluorescence Shutter (closed)	 Fluorescence Shutter (open)

4 To select a set of fluorescence settings defined via menu item "Adjustments > Fluorescence ..." (see page 14).

5 Allows to set a timer to close the shutter automatically after a preset time (in case of manually usage only).

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

Tab "HW Settings": To work with hardware settings

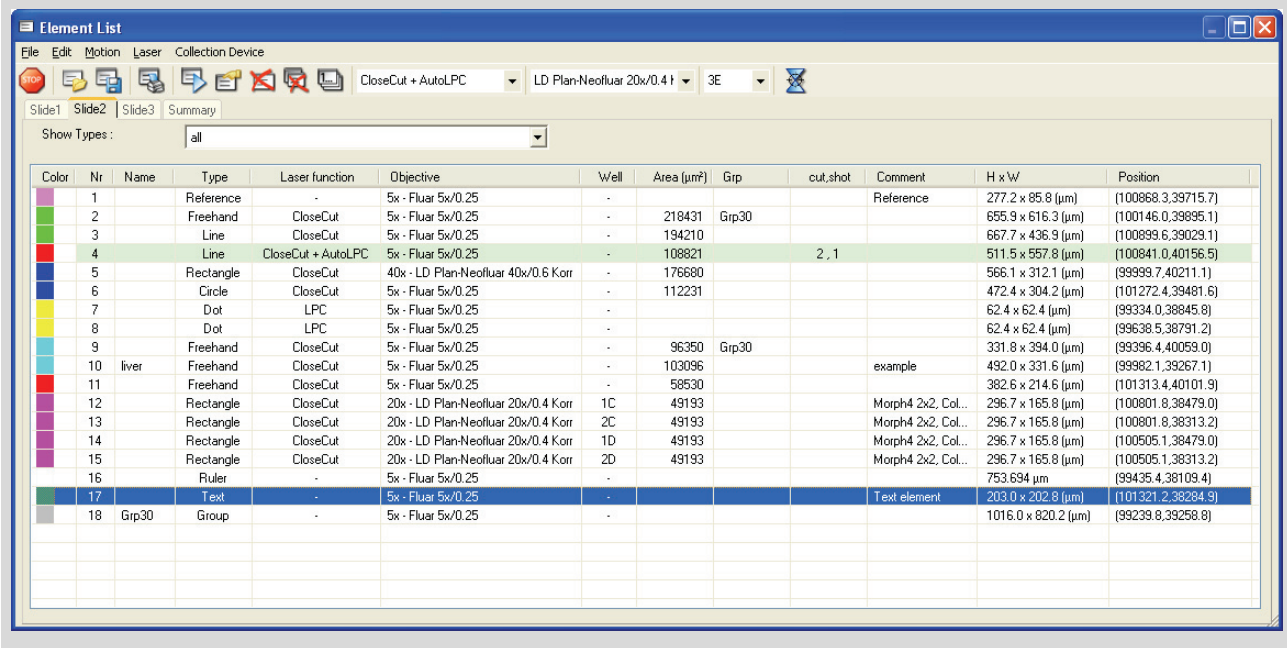


1 To open the window "Hardware setting adjustments". In this window you chose the hardware settings to be listed below (see No. 3).

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.

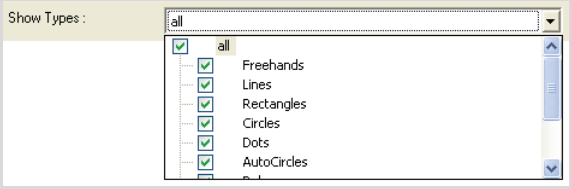
13 Element List



The element list displays information about all drawn elements and allows 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"

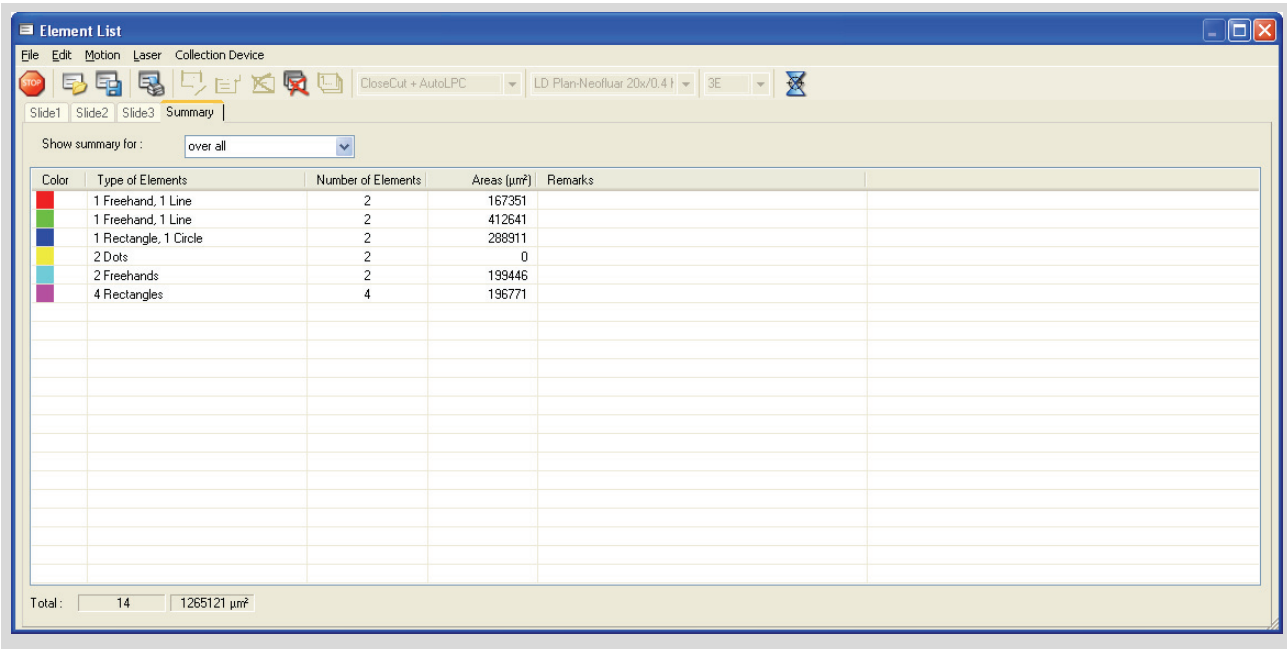


To show different types or all types of elements.

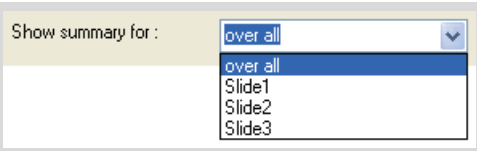
The columns in the table contain the following information about each element:

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 into which the element is to be catapulted when a laser function is triggered.
Area:	area of elements of type "Figure" (Freehand, Line, Rectangle, Circle) (in μm^2)
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 green. Selected elements are highlighted blue.



Tab "Summary"



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 (μm^2):	total area of all elements of type "Figure" for each color (in μm^2)
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 10, Menu "File")

To prepare the software for drawing new elements (only File Mode).

To open and save elements (only File Mode).

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

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).

To print the element properties into an Excel import file (*.csv).

To close the element list.

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

Select All
DeSelect All

To select resp. deselect all elements.

Change

To change the properties of the selected element.

Renumber all

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 12, Menu "Motion")

Go to Element

To center the selected element on screen.

Menu "Laser" (see also page 14, 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).

Tool Bar of Element List

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

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.

Change
To change the properties of the selected element.

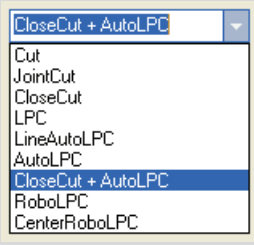
Delete selected
To delete the selected elements.

Delete all
To delete all elements.

Renumber All
To renumber the remaining elements after deletion of elements.

Start Laser

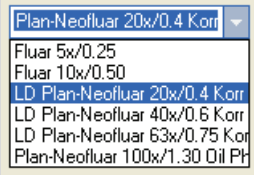
Cut Tools of Element List



To select a laser function for the elements currently selected.

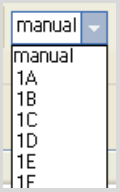
For an overview of the Laser functions see page 29.

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.

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 und PALM Tweezers. The Pro version is licensed for more functionalities: Pro-Mode (with Autofocus, TimeLapse, Field of View Analysis).

Contact palm-info@zeiss.de for further information.

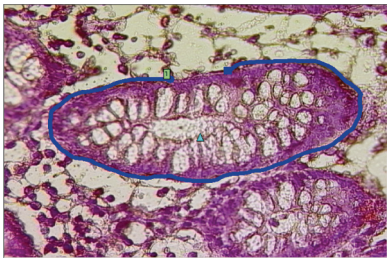
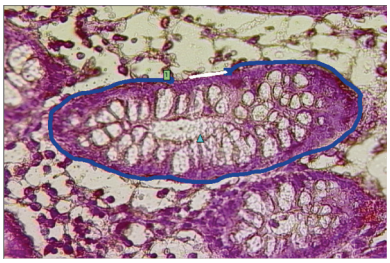
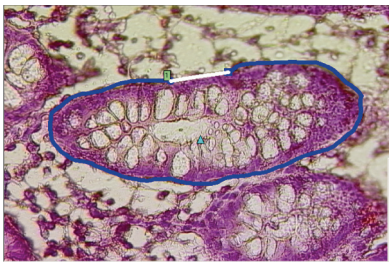
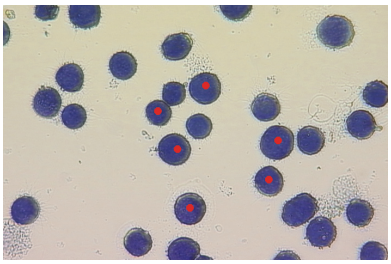
15 Shortcuts

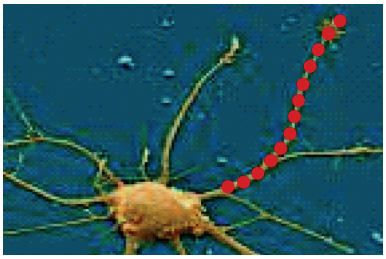
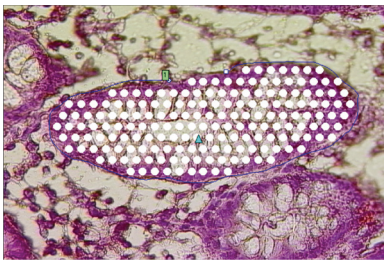
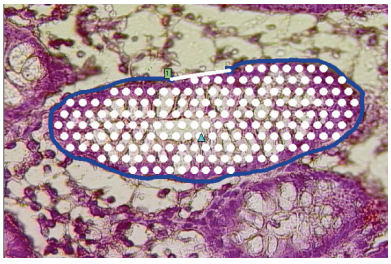
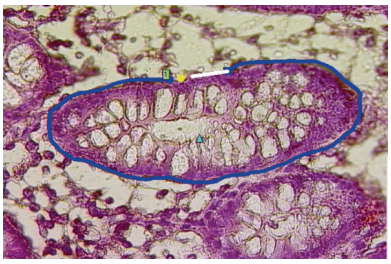
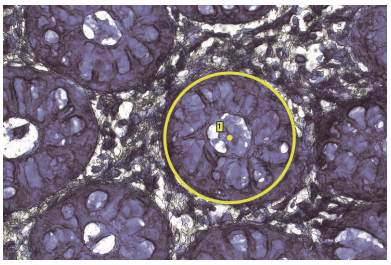
Shortcut	Picto/Menu
Menu Help	
F1	Help > PALM RoboHelp
Menu File	
Ctrl+N	New Elements
	File > New Elements
F3	 Information Center
	File > Open Information Center ...
Alt+F4	File > Exit PALM Robo
Menu Edit	
Ctrl+A	Edit > Select all / Edit > DeSelect all (Current position only)
Ctrl+C	Edit > Copy
Ctrl+V	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 > Change
Menu View	
Alt+X	View > Hide All Bars
Shift+Alt+X	View > Show All Bars
F4	 Navigator Window
	View > Navigator Window
F5	 Element List
	View > Element List

Shortcut	Picto/Menu
Menu Motion	
Alt+F	 Freeze Mode
	Motion > Freeze mode
Ctrl+F	Motion > Speed faster
Ctrl+K	Motion > Goto Capcheck / Return
Ctrl+S	Motion > Speed slower
Esc	 Stop
	Motion > Stop
F7	 Stage
	Motion > Stage mode
Shift+F7	 TrapXY 1 Mode
	Motion > TrapXY 1 Mode
Ctrl+F7	 TrapXY 2 Mode
	Motion > TrapXY 2 Mode
Menu Laser	
F11	 Start Laser function
	Laser > Start Laser function
End	Laser > Focus down
Home	Laser > Focus up
Page up	Laser > Energy/Power more
Page Down	Laser > Energy/Power less
Menu Devices	
F2	 Incubation
	Devices > Incubation Device ...
F6	 Microscope Window
	Devices > Microscope ...
F12	 Capture Device Window
	Devices > Capture Device ...

Shortcut	Picto/Menu
other	
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

16 Laser Functions - an Overview

Cut Cutting along the predefined 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 end point and the start 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 retained 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 circumcises the area to be catapulted and defines the laser shot grid in the Adjustments menu (how many shots per µm^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 Adjustments 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.