Live Acquisition Software Manual

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Before you start

Thank you for purchasing the Till Photonics Live Acquisition software. This manual provides information about the operation of the software and how it is configured to work with the hardware. Please familiarize with your hardware setup and manuals where appropriate.

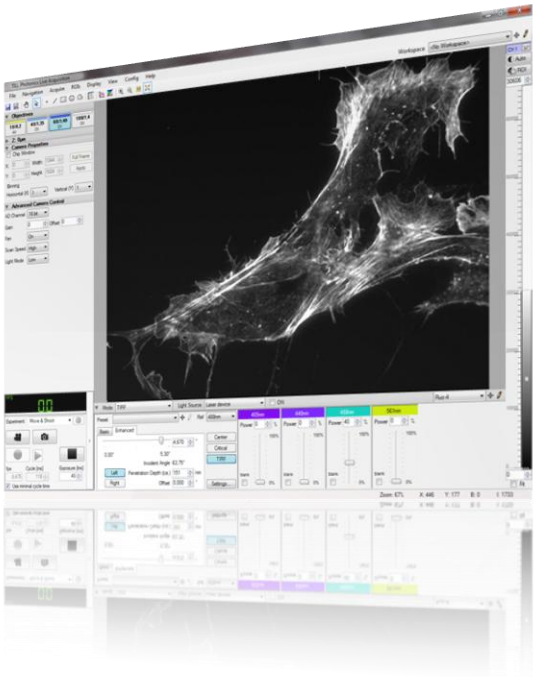
Software protection:
The Live Acquisition software is protected by a USB dongle, which has to be plugged into the computer on which the software is run.

Figure 1: USB Key required for using the Live Acquisition Software

The Live Acquisition Software can be installed without the dongle but cannot be started without it.
If a USB dongle of TILLvisION was installed/used on the computer prior to the Live Acquisition software it might be necessary to reinstall the Dongle driver. (see Trouble shooting: Chapter 14)

Info: The Live Acquisition software is available in many configurations. It might be possible that this manual describes functions that are not available in your purchased version of Live Acquisition.

1 Introduction



Live Acquisition (LA) is more than a software program. It is a part of the LA complete microscopy analysis system. Combined with the robust Offline Analysis application and various software plug-ins and modules for hardware expansions, it is in fact, the only real real-time system you will ever need. As it is both tightly bundled with its ecosystem of hardware (cameras, microscopes, light sources, etc) and it is flexible enough to have software modules added or changed. It is capable of such control and data collection operations as:

- Pre/Post analysis of conducted experiments for Kinetics, RATIO, FRET and FRAP
- Out of the box default experiments such as multi-wavelength experiments, Z Stack, Time Lapse, Tile and Stitch, FRAP and more to facilitate rapid learning curves when first using the software
- Sets a precise, operating system independent timing for very complex measuring protocols due to its built-in intelligent microcontroller
- Commands and responses to external electro-optical devices like cameras, microscopes, mechanical sliders, mechanical stages, galvos, mirrors, and all the other devices that are needed to take and analyze pictures in the visual spectrum
- Fixed and programmable external hardware trigger events (IN/OUT) which allow integration with existing hardware as well as updates as new hardware and hardware features become available
- Intrinsic control of complex screening with Petri dishes, slides, and well plates via easy-to-use navigation controls

For what counts at the end of the day is that you have your experiment completed. The Live Acquisition solution is an invaluable tool to assist you in getting there. And then it allows you to analyze your data in great length both online while collecting more data and offline when you are processing the data.

The out of the box nature with pre-built sample experiments and analytic routines will allow even a novice user to work with a very powerful set of tools that can create and crunch data. Chances are that you will not be aware of many of its features unless you have read the manual carefully as you begin exploring the advanced configuration options. You will be surprised, how many complex experiments you can actually do if you are using the correct approach and using the flexibility in the system to custom tailor your experiment to your needs.

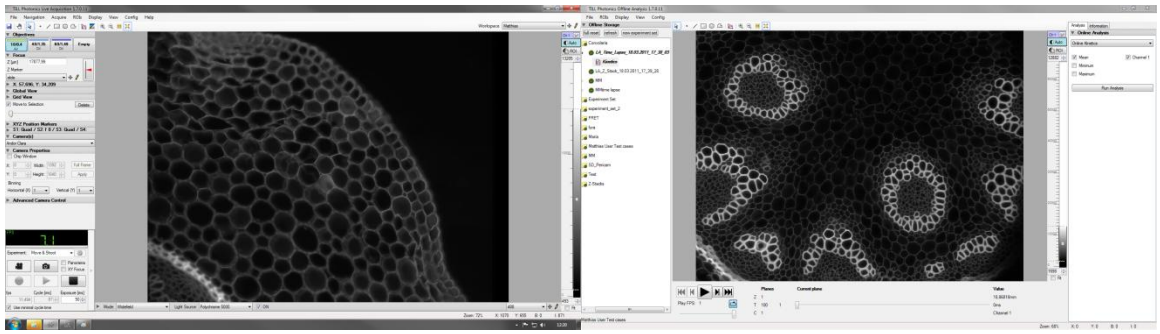


Figure 2: Live Acquisition and the Offline Analysis application

Live Acquisition solution works hard to make the user interface more intuitive so that you don't have to work hard. Getting the experiment running with a minimal investment of time is a reality with Live Acquisition and not just a nice to have. But in those instances where the complexity of the experiment reflects to a complexity in setting up the procedure to execute it, the Live Acquisition solution provides a framework of ready to plug in tools to assist you as well as the ability to craft custom tools to make your work easier. And not to be forgotten is the philosophy behind the company that delivers this solution to you. The philosophy has been to value the flexibility of an experiment as well as the ability to set it up in a quick and intuitive manner.

1.1 Where to Find What - Order of Reading

The documentation found in this manual is divided into the following parts:

- Introduction
- GUI Overview

Also on the installation CD is the complete documentation in electronic form. The following documents are included:

- Live Acquisition Installation and Configuration Guide (pdf)
- Live Acquisition Users Manual (pdf)
- (Optional) LA Browser Help Tool (Windows Help files)

In the 'Installation and Setup', you can find instructions for a complete installation of the Live Acquisition software package. The following add on packages are detailed further in their respective users guides but are installed with the same installation and setup procedure found in the LA package(s) and Bundles.

Tabel 1: Feature list of LA Bundels

	LA Imaging	LA Advanced	LA Premium	Offline Analysis
Main modules	Live Acquisition Protocol Editor Offline Analysis	Live Acquisition Protocol Editor Offline Analysis	Live Acquisition Protocol Editor Offline Analysis	Offline Analysis
Analysis modules	Kinetics RATIO	Kinetics RATIO	Kinetics RATIO	Kinetics RATIO
Additional Analyses/Modules			FRET FRAP Deblur	
Additional Experiments			DynamicFRAP	
Optional Modules	FRET, Deblur	FRET, FRAP, Deblur DynamicFRAP, DualCamera	Dual Camera	FRET, FRAP, Deblur

LA Software Package Optional Components

- Offline Analysis
- Protocol Editor
- Live Analysis FRET (for on- and offline FRET analysis)
- Live Analysis FRAP (offline FRAP analysis with fitting functions)
- Dynamic FRAP three extra FRAP experiments
- Deblur (No-neighbor, nearest neighbor and inverse filter for debluring z-stacks)
- DualCamera (Simultaneous acquisition with two cameras)

Tabel 2: Supported devices in the LA Software Bundles

	LA Basic	LA Imaging	LA Advanced	LA Premium
Cameras	All Cameras	All Cameras	All Cameras	All Cameras
ICU	✓	✓	✓	✓
Polychrome V	✓	✓	✓	✓
Oligochrome	✓	✓	✓	✓
Oligochrome µICU	✓	✓	✓	✓
Lasers (LLC)	✓	✓	✓	✓
TILL Stage	✓	✓	✓	✓
Pifoc	✓	✓	✓	✓
iMIC & uiMIC	✓		✓	✓
Polytrope I&II	✓		✓	✓
Yanus	✓		✓	✓
LLC Fiber switch	✓		✓	✓
Andromeda	✓		✓	✓

Once the installation and configuration manual has been perused and the software loaded and configured, then it is time to become familiar with the acquisition framework.

Once a basic familiarity with the GUI is achieved, the next step of reading and investigation is in the 'Experiment How To Manual.' This is a description from a more practical point of view for the various out of the box experiment setups that can be found in the Live Acquisition software package. Here can be found some more detailed configuration information on how user definable experiments can be created.

1.1.1 Where to find Help

You can get help from the following locations:

- Electronic Manuals
- Online Support

To make it easy to use most parts of the Live Acquisition software package they have online help. Passive popup boxes are prevalent in the software package describing what an individual control does and what an individual result field shows. Simply move the cursor over the item in question to get a tool tip for help. You can also view the online help in a searchable format via the main menu. And when this does not provide a sufficient level of information, you can find pdf files of the complete documentation on the delivery CD. As updates become available and as new sample macros, plug-in, and scripts become available they can be found on our webpage. (http://www.till-photonics.com/Support/) For answers to specific questions, send an e-mail to: **service@till-photonics.com**.

1.1.2 Key Features of Live Acquisition

The Live Acquisition software package provides advanced image acquisition with full control of the acquisition parameters. It allows planning, management and execution of complex experiments that go beyond mere image acquisition. The Live Acquisition solution also includes online and offline data analysis and image processing functionality with the LA Offline Analysis Application and 3rd party software vendors like Fiji (an ImageJ distribution).

1.1.3 Graphical User Interface

The concept behind the Live Acquisition GUI is one of a merger between hardware and standard software interfaces. All the controls that you use on a regular basis are in the same location on the screen and are within easy grouping distance of controls that are used together.

When a second monitor is available it can be used for the Offline Analysis Application (when only one monitor is attached both programs can still run but must be windowed or exchanged). This allows one to see the results of the experiment in a full window directly beside the window controlling the hardware operations. This means fewer keystrokes and changing of focus and minimizing and maximizing of windows. Full control and full overview of the acquired data can be had in a single glance.

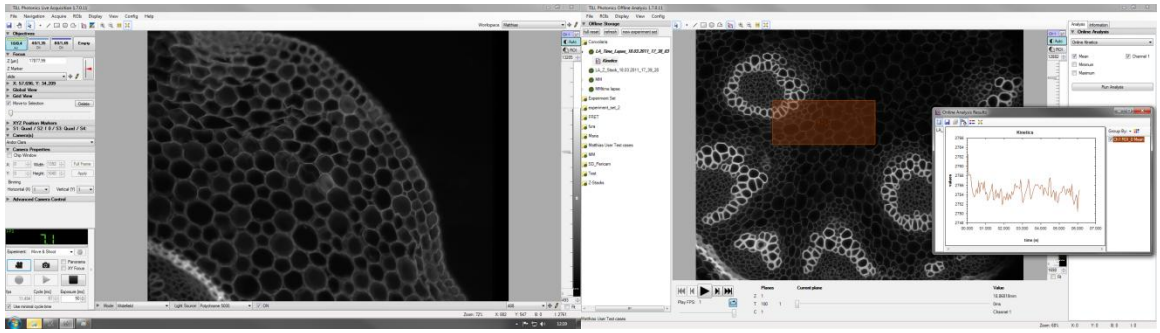


Figure 3: 2 Screen layout of Live Acquisition Software with LA on the left side and Offline Analysis on the right.

Especially when one realizes that to control a microscope without eyepieces and without any direct manual control knobs is a considerable challenge and requires novel concepts, not just a mere projection of manual knobs onto the screen. In the case of the Live Acquisition solution, the user can concentrate exclusively onto the image or onto one or more analysis window(s) and not have to worry about manipulating the buttons, menus, icons, and scroll bars when collecting that all important data.

2 LA GUI Overview

2.1 Starting up: The Welcome Dialog

This chapter assumes that your TILL Photonics system has been pre-configure by the factory or by the technician that installed the system. If a System has to set-up from scratch, maybe because of a computer crash, please read chapter 14.

When Live Acquisition starts-up it shows the "Welcome Dialog", where the user can select his account and then his hardware configurations. In labs with more than one user the settings of each user are preserved by this way.
Going from the top to the bottom of the dialog, there are three main sections:

1. The user and configuration selectors with the "edit/configure" button to the right. Pressing the edit button will take the user to a configuration dialog. In these cases the User management or the Device Configuration dialog for connecting the imaging system devices.
2. The Task chooser section where the user can go directly to LA or open the Protocol editor window directly to load the last used protocol or to restore the last settings and resume where he/she left off the last time. If LA is started for the first time the option "Restore Last Workspace" will not be available.
3. The bottom section has the option to hide this dialog on start-up completely, in case of basic imaging systems, where only one hardware configuration is needed. And the "continue" button is located here where the LA can be started.

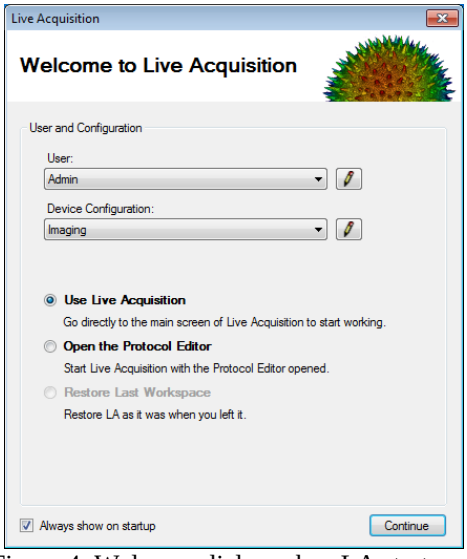


Figure 4: Welcome dialog when LA starts up

2.2 Familiarization with the LA GUI Controls

The Graphical User Interface (GUI) is designed to be easy to use so that you can get the experiment running with a minimal investment of time.
The tools for the inevitable hand grips to maneuver the microscope are placed, clearly arranged, on a panel on the left side of the screen. Additional controls dealing with the actual image under the objective are in a smaller control panel on the right hand side of the main viewing screen. The center area is referred to as the viewing panel.

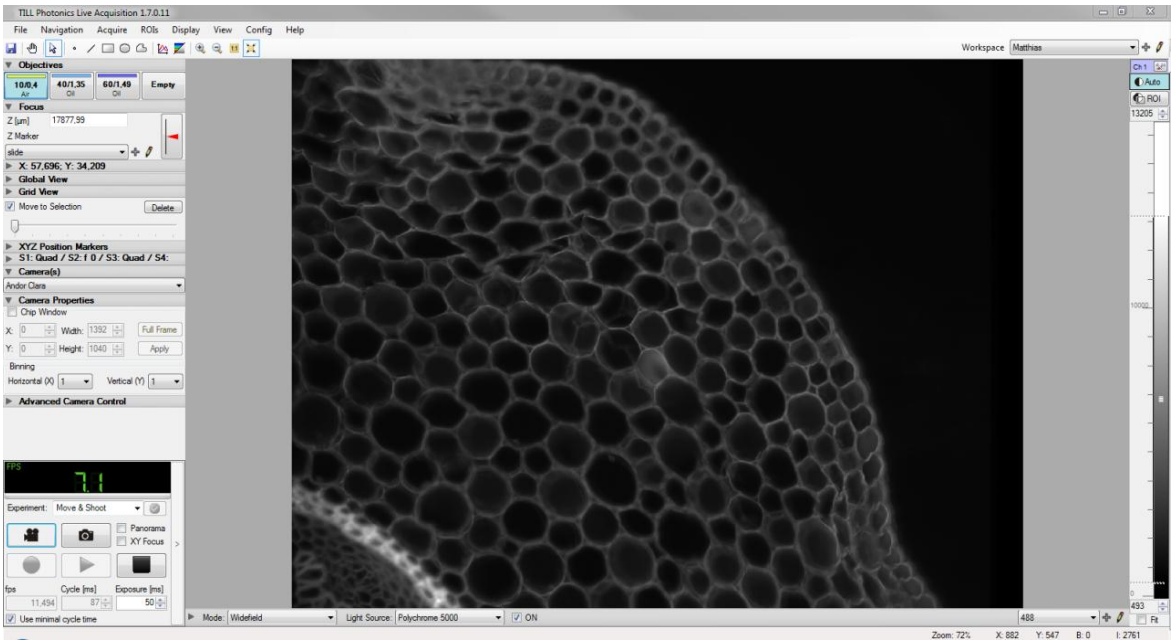


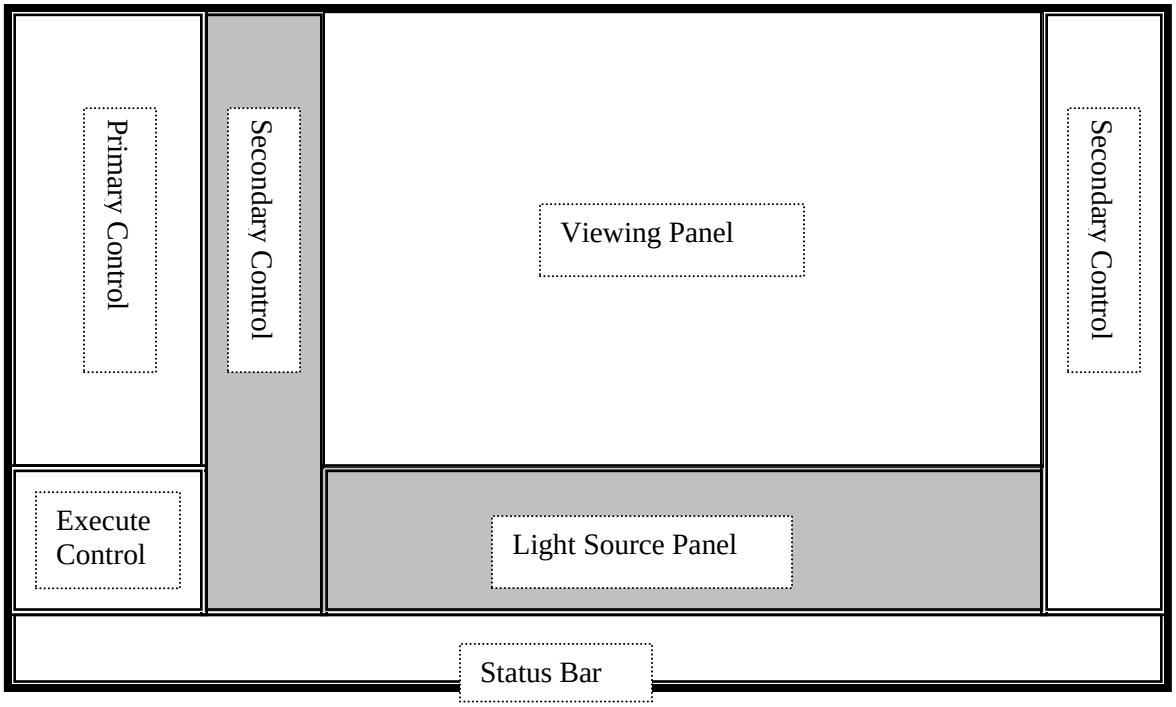
Figure 5 : LA screen after start up on an iMIC system

The GUI is adapted to closely resemble generic hardware interfaces whenever possible. Mostly from the perspective that even when you are changing between various displays and experiments. The critical controls always are in the same location so that you do not have to go hunting for them and can remain focused on what matters most, the Experiment. It is also kept as simple as possible with the controls needed for the experiment being dynamically loaded as you select the experiment.

What you can see from the dashboard concept is that the controls for the stage (x, y, and z axis) as well as the objective are always easily accessible and in the same location. The controls to take a picture in either live or still mode, to launch an experiment, or to save or delete default experiment configurations is also always in the same place and easy to access. This design concept leaves the maximum area available for observation of the experiment. As well as leaving the ‘dashboard’ uncluttered without unnecessary control, menus, and dialogues.

Various experiments require different configurations and settings which is why there is a section left available on the dashboard control for new control windows to be dynamically inserted depending on the experiment currently active. A large number of controls can be accessed with a slide bar on the right hand side of this main hardware control window while leaving the primary

controls always within easy reach and without cluttering the viewing area. Many of the controls are able to be minimized thereby freeing up more of the control window.



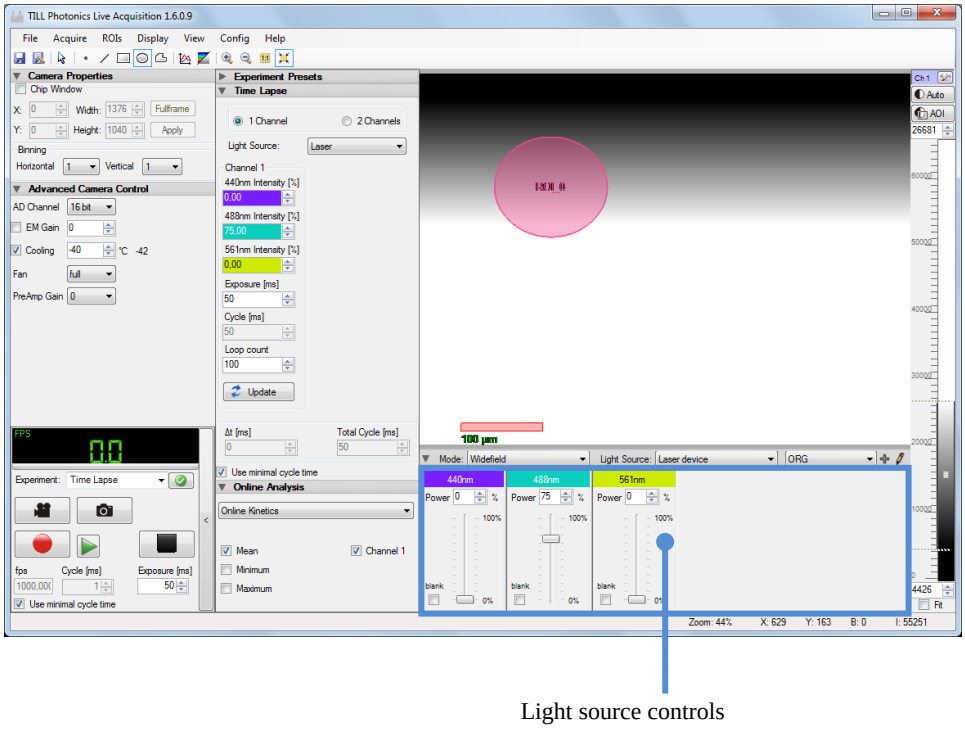


Figure 6: Screen Layout

The LA is designed with ergonometric and ease of utilization in mind. Data visualization in the largest format possible as well as ease of finding the controls without losing concentration on what is most important…the research and investigation of the samples.

All controls are so constructed that various input devices can be used and you are not tied to a keyboard on your lab desk. In its default configuration, the GUI supports keyboard, mouse, USB HID as well as3D Connexion Joysticks. When heavy data entry tasks are required the keyboard can be the most efficient input device. But in a darkened laboratory and by using the intuitive and programmable interface of the mini keyboard, joystick and a standard mouse, the keyboard can be put away and the full functionality of the Live Acquisition solution is still at a finger click away and your focus can always remain on the experiment window without having to hunt down keys on the keyboard.

In keeping with this concept the control windows are hidden when they are not needed thereby keeping the viewing panel large and robust. The above figure shows two examples of this in that the grayed out panels can be hidden thereby increasing the size of the viewing panel but at the same time allowing the most important controls to always be visible and at your fingertips.

2.2.1 Description of the Hardware Interfaces for the LA GUI Controls

In keeping with the ergonometric approach and the design methodology that the most important activity is watching what is going on with the sample, various hardware input techniques have been developed to keep the focus on the sample.

In this regards the various keyboard keys, mouse buttons, and 3D Connexion Space Navigator controls have been worked into a unified whole to ease the navigation of the software without having to move the mouse, keystrokes or eyes repeatedly to hunt for and find buttons, controls, menus, or pull downs.

2.2.1.1 Mouse Controls

The mouse is the main control for manipulating the LA GUI.

Tabel 3: Mouse functions in LA

Motion / Button	Action	(Needed in Combination)
Move the mouse	Moves the cursor	None
Press left button of the mouse	When in a text control sets the modal focus allowing the text to be changed. When on a button activates the button. When on a slider control allows the slider to be manipulated.	None
Right click of the mouse	Opens the context menu of a control if available.	None

Mouse Scroll Wheel	Changes the focus when the mouse is over the viewing panel. When rolling up increases the focus height and when rolling down decreases the focus height.	None
Press Mouse Wheel	Allows changing the rotation of ROIs by moving the mouse up and down	

Table 1: Mouse Controls

2.2.1.2 Keyboard Controls – Short cuts

Various keyboard shortcuts have been set aside to control various features of the software. The goal of these shortcuts are to keep the eyes focused on the viewing panel and the mouse over the image without having to refer back to any other controls thereby breaking eye contact with what the specimen is doing.

Tabel 4: Keyboard short cuts for accessing LA functions

Key Stroke	Action	(Needed in Combination)
SPACEBAR	Will set a time marker in an active experiment. The imaging database can then be referenced to see what the user found at an interesting point in time without the need to interfere with the experiment execution.	None
+	Will set an image marker in both SNAP, LIVE, and EXPERIMENT. Both the imaging database and the LA Acquisition primary controls will be updated. The imaging database and/or the acquisition controls can then be referenced to see what the user found at an interesting point in space without the need to interfere with the experiment execution. This allows rapid return to an Area of Interest (AOI).	None NOTE: Works with both the larger numpad button as well as the smaller standard key
SHIFT	Will change the Viewing Panel so that an overlaid secondary viewing pane is created. This allows re-centering the viewing panel by placing the mouse and making a left click.	The mouse movement & the mouse left button

CTRL	Will open an overlaid step control for focusing in the view panel. Use of the mouse to select and left click a new step control changes the default focus steps.	The mouse movement & the mouse left button
ALT	Temporarily disable the image acquisition if the z position is changed or the light source settings like the wavelength is changed.	None
X	Will open an overlaid virtual joystick control in the viewing panel. This allows fine movement joystick control by using the mouse as a joystick.	The mouse movement
ALT-Enter	Switching to full screen image view or back to windowed mode	
Ctrl + cursor left	Got to the previous xyz position marker in the position marker list	
Ctrl + cursor left	Got to the next xyz position marker in the position marker list	
Cursor keys	Moves the stage on frame into the given direction	
Page up and page down	Changes the focus with the last set step size	
Ctrl-T	Initiates a test run of the currently open/edited protocol (this does not save the images)	
Ctrl-R	Runs the currently selected protocol and saves the acquired images	
Ctrl-S	Save the currently displayed image to the disk	
Ctrl-Shift-S	"Save as…" short cut. Saves the currently displayed image to the disk and will prompt for a filename and directory.	
Crtl-1	Show/Hide the protocol parameter panel	
Ctrl-2	Show/Hide the light source control panel	
Ctrl-3	Minimize/maximize the Protocol Editor	Protocol Editor needs to be open/active
ALT-F4	Quits the LA Software	
Del	Deletes a ROI if it is selected in the image display.	

Table 2 : Keyboard Controls

2.2.1.3 3D Connexion Space Navigator Controls (in LA)

The secondary mouse/joystick has been set aside to control various features of the software. The goal of these shortcuts is to speed navigation and the saving of data.

Motion / Button	Action	(Needed in Combination)
Joystick Move (left, right, up, down)	Rapid screening of a sample. Allows panoramic movement or joystick movement depending on preferences settings so that the viewing panel shows neighboring CCD images from the camera.	None
Push joystick down	Saves the current image	None
Left joystick button	Will set an image marker in both SNAP, LIVE, and EXPERIMENT. Both the imaging database and the LA Acquisition primary controls will be updated. The imaging database and/or the acquisition controls can then be referenced to see what the user found at an interesting point in space without the need to interfere with the experiment execution. This allows rapid return to an Area of Interest (AOI).	None
Right joystick button	Allows the configuration of the sensitivity of the controls.	None

Table 3: Space Navigator Controls

2.2.1.4 USB Joystick (HID compatible)

Connecting as USB Joystick, digital or analog, will allow controlling the xy-movement of a motorized stage.
Switching between a (camera) frame wise and continuous xy-movement is done by deselecting or selecting the Joystick move.
Activating the joystick in LA is done with the menu item *Config > Start HID joystick*. LA does not need to be restarted.

3 Acquiring images

The Live Acquisition software offers many possibilities to acquire images and also complex experiments in multiple dimensions, time, multiple channels, xy and z. Some functions described

in this chapter may need optional available modules of the LA Software, like the Offline Analysis application and the Protocol Editor.
The image acquisition is primarily controlled with the controls shown in Figure 7.

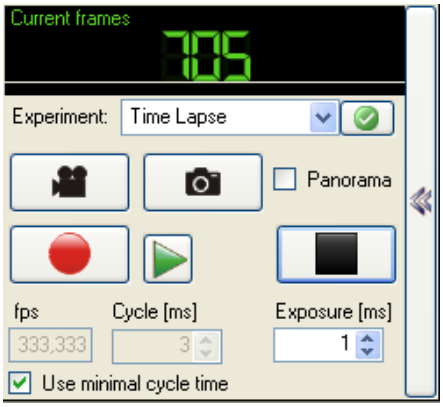


Figure 7: Acquisition controls

3.1 Snapshot

The *Snapshot* function acquires one image with the current exposure settings and with the currently selected light source and keeps it in memory. The light source will be triggered in this mode, like in any protocol, thus minimizing the photo toxicity and photo bleaching. The image can be saved by pressing the *Save/Disk* icon in the toolbar.

3.1.1 Panorama

The *Panorama* option is only valid for the *Snapshot* function and is only available on systems with a motorized stage like the *iMIC* or imaging systems with the *TILL Stage*.
If this option is enabled, by setting the check mark, a series of adjacent images either 2 by 2 or 3 by 3 images is taken. This allows a larger field of view without changing the objective. The size of the Panorama can be set in the *Acquisition* > *Panorama Size* menu.
All navigation features like *Move and shoot* are available in the *Panorama* mode. In the Grid view the grid spacing will also be adjusted to represent the larger field of view.

3.2 Live

The *Live* function displays a series of images with the current exposure and light source settings. In the *Live* mode the light source in use will be triggered to avoid unnecessary exposures that would otherwise lead to photo bleaching of the samples and photo toxicity in the sample. The acquisition of images in *Live* mode is stopped by pressing the stop button. The last displayed image can be saved by pressing the *Save/Disk* icon in the toolbar (see chapter 3.3.1.)

Hint: To save a series of images the *Time lapse* protocol (see chapter 3.9) needs to be used.

Information:
Before taking images with either Snapshot or Live the following device settings should be checked:

- Exposure time

- Selected camera
- Camera settings like gain, binning chip window
- Selected light source
- Light source settings like filter position/wavelength, intensity, bandwidth
- Filter slider position

3.3 Saving images

Pressing the *Disk* icon  in the toolbar saves the currently displayed image to the disk. The image that will be saved can be an image taken by using the *Snapshot* function or the last image of a *Live* image sequence.

3.3.1 Saving options

Saving options for the images depend on image streamer that is used to save images. Selecting and changing the image streamer is described in detail in chapter xyz.

3.3.2 Offline Analysis saving option

The acquired data is send to the Offline Analysis application and is stored in one of the available *Experiment Sets*. It is possible in LA to select one existing Set where the newly acquired data should be stored and it is also possible to create new Experiment Sets.

3.3.3 LA Browser saving options

The acquired image is sent to the LA Browser and displayed in a window. The image is also stored in the default folder set in the LA Browser. The default folder in the LA Browser can be set in the *Extra* > *Preferences* > *General* menu. Change the value of the *Working path* panel.

3.3.4 LA TIFF and TIFF ImageJ saving options

If the user selected the TIFF or TIFF ImageJ streamer the acquired image will be either saved to the disk using the default path or the user will be asked to select the destination folder in a dialog. Toggling the behavior, using the working directory or asking for a destination folder to save the image, is done in the *Config* > *TIFF ImageJ streamer* or *Config* > *TIFF Streamer* dialog.
If *Ask for save directory* is set to "*true"* the user will be prompted to select the directory when the *Disk* Icon was pressed. If the value is set to *false* the directory in *Autosave path* will be used to save the images.

3.4 Experiments

The experiment list contains items in up to three categories (Figure 8), depending on the hardware configuration (e.g. TIRF and/or FRAP experiments) and the purchased software modules.

If the Protocol Editor module was purchased the first entry in the list is the *Protocol Editor* item. Selecting this item will open the Protocol Editor window where workflows built from multiple experiment items can be created and edited.

The second category holds basic experiments, like *Time Lapse*, *Tile* or *Z-Stack*. The items of this category can also be found in the *Protocol Editor* and used there to build more complex experimental workflows.

In the third category are the advanced protocols, which are partially built from multiple basic experiments or are special protocols only available with special hardware (e.g. DynamicFRAP).

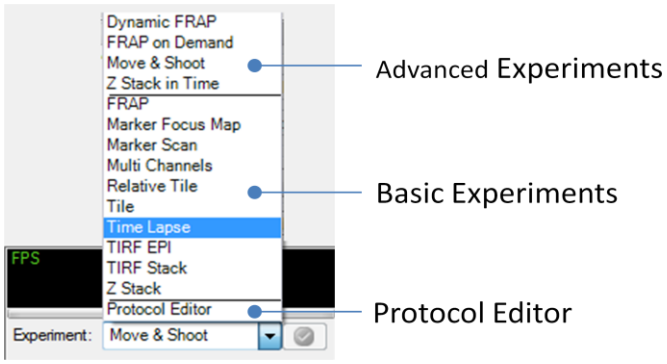


Figure 8: Example of available experiments

Selecting one of the items in the list will open the secondary experiment panel or in case of the Protocol editor an additional floating window.

3.4.1 Show and hide the experiment panel

The secondary experiment panel can be hidden or shown by pressing the button with the double arrow on the right side in the acquisition control panel. This is particular useful if the settings of an experiment are done and acquisition should start to maximize the viewing area for the acquired images.

3.4.2 Experiment parameter check button

To validate the parameters of an experiment (basic and advanced) or a workflow created with the Protocol Editor the green check button to the right of the experiment list can be clicked (Figure 9). The experiment or workflow will then be check and the minimum time for certain operations, like the cycle time, will be optimized.

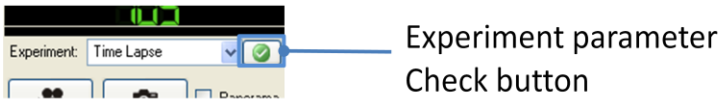


Figure 9: Location of the Experiment parameter check button.

3.5 Acquire

The *Acquire* button  starts the execution of an experiment or custom workflow created in the *Protocol Editor* and **saves** the acquired images do the disk using the currently selected streamer. The *Acquire* button changes its appearance during the acquisition into a *Pause* button, which allows suspending the current experiment by the user and resume it when needed.

3.6 Play/Test run

The *Play* or *Test*  button with the green triangle also starts the execution of an experiment or workflow but in contrast to the *Acquire* button the acquired images are NOT saved to the disk! This mode is useful to test the experiment settings before running the real experiment.

3.7 Stop

The *Stop* button is used to stop the acquisition of images in *Live* mode or to stop the execution of experiment and workflows early.

3.8 Selecting and controlling a light source

The controls for the light sources are located at the bottom of the LA application window.
The light source control has up to three areas, where the user can adjust the parameters for the selected illumination mode.
In the top area is the title bar of the control. In the title bar are several control located from left to right
:

- The collapse/expand triangle for showing or minimizing the light source control
- The "Mode" list, where the user can switch between different available illumination modes (Widefield, TIRF,..)
- The "Light sources" if multiple are available for the selected illumination mode, e.g. PolychromeV, Lasers, LEDs.
- The "On" switch where the currently selected light source can be switched on permanently. The light source then will be turned on independent of any experiment or protocol.
- The light source "Preset"-control on the far right allows switching between multiple, user defined presets and managing them.

3.8.1 Polychrome V

Changing the Wavelength, Intensity (optional) and Bandwidth (optional) of the Polychrome V from the Live Acquisition software is done in the Light Source control panel.

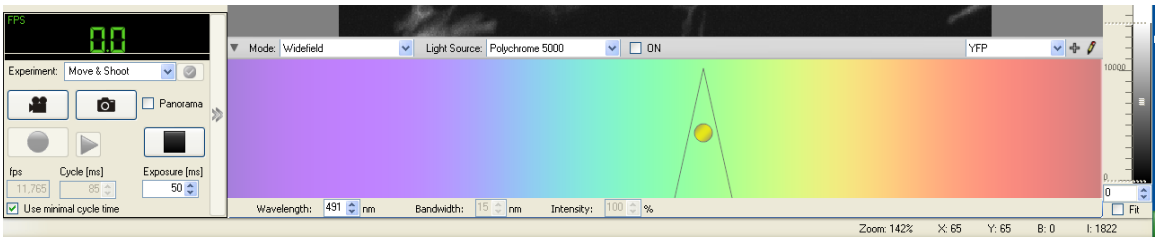


Figure 10: Light source control panel for the Polychrome V

The graphically control can be moved by a left mouse click and a drag. There are three yellow circles in the graphical spectrum slider.

- Clicking and dragging in a vertical fashion on the topmost one will change the intensity.
- Clicking and dragging in a horizontal manner on the middle one will change the wavelength.
- Clicking and dragging in a horizontal fashion on the lowermost one will change the bandwidth.

Intensity, wavelength, and bandwidth can also be edited directly with the roll bar.

3.8.2 Laser Line Combiner

The Laser Line Combiner Control consist of sliders for changing the intensity of each laser line individually. Each laser line can be “blanked” by clicking on the checkmark “blank”, by this the user can turn individual laser lines on and off without changing the intensity.

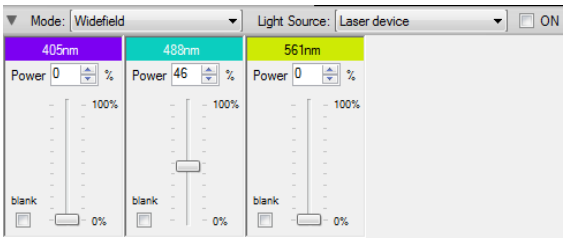
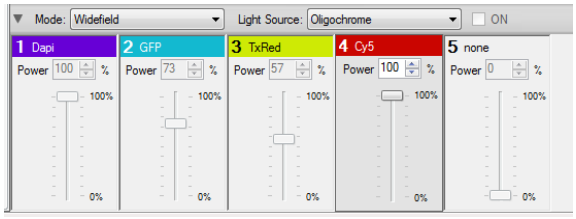


Figure 11: Laser line combiner control for Live view and Snapshots

3.8.3 Oligochrome



3.9 Basic acquisition: A “Time Lapse” experiment

The Live Acquisition Software provides some pre-defined experiments that can be started right away without utilizing a protocol editor.
In this chapter an introduction is given on how-to acquire a time lapse experiment with the following parameters:

- one channel
- a series of 100 images
- With a frequency of 1Hz or an image every second
- a Polychorme V as light source

Opening the panel for the parameters of a time lapse experiment can be done by going to the menu *Acquisition* > *Time Lapse* (Figure 12: Selecting the Time Lapse experiment in the “Acquire” menuFigure 12).

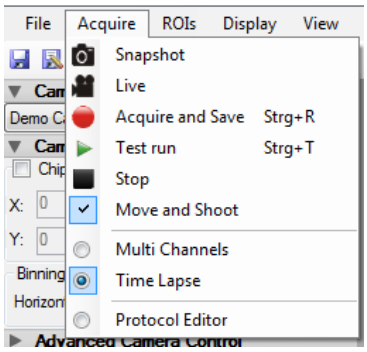


Figure 12: Selecting the Time Lapse experiment in the "Acquire" menu

A second row of panels, located to the right side of hardware control panels, the will open in the LA software (Figure 13). This panel is divided into three sections from top to bottom:

- **Experiment Presets**: Manage presets of experiments for frequently used workflows for more details see chapter (3.10).
- **Time Lapse**: This is the area where the parameters for the image acquisition will be set.
- **Online Analysis**: Depending on the parameters set in the experiment, LA will show different options for online evaluation like Kinetics, RATIO and FRET (please see chapter 6 for more information).

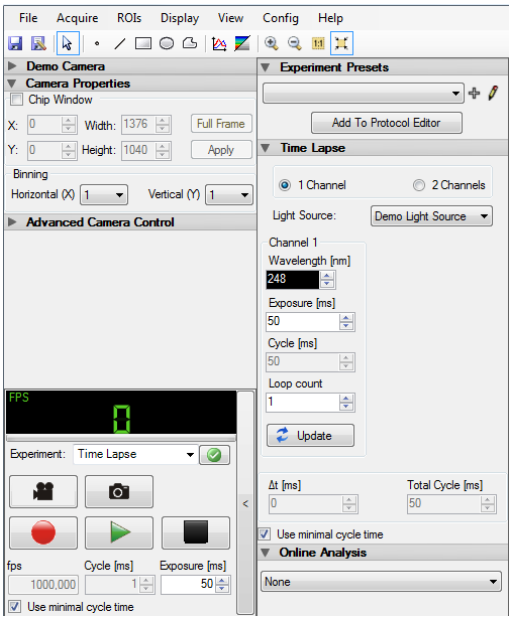


Figure 13: Experiment parameter panel open

The Time Lapse section provides the input for entering the parameters of our Time Lapse experiment. Going from the top of the Time Lapse area to the bottom we have a selector for:

- Switching between a single (**1 Channel**) and a dual (**2 Channel**) acquisition. By default the single channel option is selected and we leave it like this.

- The "**Light Source"** selector. Here we can choose a light source from the installed ones on our system. For our planned experiment we select the Polychrome V (or the Oligochrome) from the list.
- Below the light source selector on the left side we can enter the parameters for first channel. For two channel experiments a second column will be displayed with the same parameters.
- If we have chosen the **Polychrome V** as light source the first parameter for Channel 1 from the top is the "**Wavelength"** that we want the Polychrome to generate when an image is acquired. This wavelength is depended on the fluorescent dye, which is used in the experiment (e.g. Fluo-4 and Oregon Green BAPTA can be excited at 488nm).

OR

- If we have chosen the **Oligochrome** as light source the first parameter in for channel 1 is the filter position of the Oligochrome. With this filter the excitation wavelength is chosen.
- The next parameter is the **"Exposure time"** for the image. This parameter applies for both the Polychrome and the Oligochrome again. By default the *exposure time* is taken from the *Live/Snapshot* setting but can be changed. Longer exposure times mean brighter images but can lead to saturation if chosen too long.
- The next parameter is the "**Cycle Time".** The cycle time is the time between the start of two images in contrast to a delay which is the time between the end of one image acquisition and the start of the next. In our example a frequency of one *Hertz* is desired, which means an image is taken every second.

Hint: If the cycle time control is grayed out and cannot be edited, please have a look at the bottom of the Time Lapse panel if the option "Use minimal cycle time" is checked. To edit the cycle time this option has to be un-checked.

- The last parameter that needs to be set is the "**Loop Count**". With this parameter we tell Live Acquisition how many image we want to acquire. In our case we want to acquire 100 images.

To acquire the one channel Time Lapse experiment the "**Record**" button  has to be pressed.

In Figure

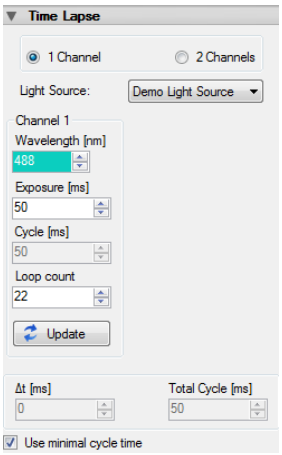


Figure 14: Screen shot of the example time lapse experiment with a Polychrome V light source

3.10 Toggle in the Image View

In RATIO experiments it is often useful to show the calculated RATIO image instead of the individual channels. The LA software allow switching between the normal display where the images are displayed in their order in that they are acquired or the RATIO image display where the calculated RATIO image will be shown.
Switching between normal and RATIO image display is either done by selecting the menu item *View > Switch to/from Float View* or the respective icon in the toolbar.

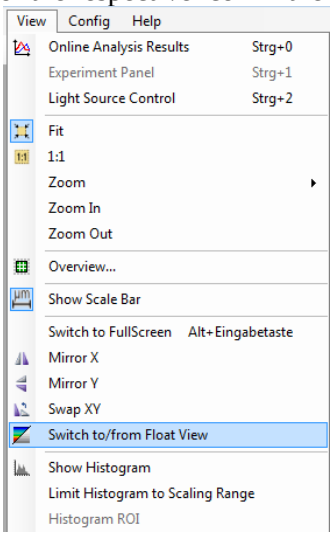


Figure 15: View options for toggleing the image display from and to the RATIO view

3.11 Experiment Presets

The *Experiment Presets* allow the users to save and recall frequently used experiment settings. This allows recalling experiment presets for RATIO, multi-channel or Z-Stacks. The saved presets are linked to the experiment; this means you will only see the presets in the experiment where they are created.

3.11.1 What is saved in an Experiment Preset?

A Preset saves all parameters of the active experiment like:

- The selected light source (if it is selectable in an experiment)
- Light source parameters (Wavelength, intensities,…)
- Cycle times
- Loop count
- Number of z-planes
- Step size of the z-stack
- Type of analysis (Kinetic, RATIO or FRET)
- FRAP ROIs and their parameters (bleach time, line count etc.)
- Parameters of the analysis like thresholds, algorithms and channel assignment

However, a preset does not save the "normal" regions of interest (ROIs), only the FRAP ROIs.

3.11.2 Example 1: Saving a single channel Time-Lapse experiment.

3.11.3 Example 2: Saving a two channel Time-Lapse for FURA-2 experiments.

3.12 Finding the sample on an iMIC system

The TILL Photonics iMIC is a digital microscope that has no eye-pieces anymore. Therefore the procedure of finding the sample is different from traditional microscopes.

The eye pieces are replaced by the camera and the software and the manual z-drive is replaced with the mouse-wheel on iMIC microscopes.
There are several advantages to this approach, one being the ability to turn the light source on only if an image is taken. This exposes the cells to less light than the approach on traditional microscopes, where the user will turn on the light source permanently while browsing the sample by looking through the eye pieces.

Here's a short list of the advantages of using a camera and software instead of eye pieces for browsing the sample:

- Less photo bleaching
- Color mapping (applying different palettes)
- Adjustment of brightness and contrast in software manually or with auto contrast
-

Having all axes motorized has several advantages for the user like setting position markers to mark interesting spots in the sample and to run experiments/protocols on each of the position markers.
However, as there are no physical knobs on the microscope anymore to move the axes of the stage, the software needs to provide at least equivalent functionality.
This is what the LA software was made for from the very first release, it provides a broad range of tools for different workflows in navigating the sample:

- Move and Shot
- Overview Scan with the option to do sub scans
- Stage Navigator with Overlays and Grid view
- Software Joystick mode
- Click and Drag – Mode
- Support for USB Joysticks
- Using the keyboard

3.12.1 Recommended frame rate for browsing the sample

To have a smooth impression while browsing the sample we recommend a frame rate of at least **10Hz** while browsing the sample.
There are cameras that cannot achieve this frame rate in full frame mode. We recommend using binning, vertical and horizontal, to increase the frame rate. This also allows reducing the exposure time and in turn bleaches the cells even less. The xy-resolution is decreased a bit but should in normal cases still be more than good enough to find the sample.
Bleaching is usually not an issue with this method as the LA software will turn the light off is no image is acquired by the camera. In the end there is less photo bleaching that browsing the sample with oculars where the light is permanently on.

3.12.2 Bringing the sample into the field of view

There are multiple ways in bringing the sample in the field of view. To travel large distances, the easiest way is to use the Navigator. By clicking into the navigator the xy-stage will move there. Other ways are the keyboard cursor keys or one of the supported joysticks.

3.12.3 Adjusting the Z-Position: The Z-Control

Like on conventional microscopes one of the hardest parts is to find the focus position. If the standard insets for the iMIC Prior stage are used or the integrated stage, the focus is usually found between 18 and 20mm of the iMIC z-drive.
Using a lower magnification objective (10x or below) makes it easier to find the focus.

3.12.4 Changing filters/filter cubes

On iMIC microscope systems the user can change the filter cubes from the software. There are button or list controls provided for this in the *Filters* panel of LA (). Here the user can also find controls for additional filter wheels or the Andormeda filter wheels and dichroic slider.

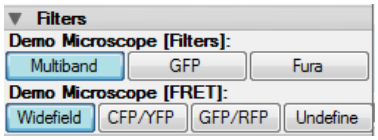


Figure 16: Filter and dichroic slider control in LA

3.12.5 Overview Scan

Once the focus position is found, LA offers a unique and very convenient way of browsing the sample with the Overview Scan.
The Overview Scan acquires images in a rectangular previously defined scanning area and creates one large tiled image from the acquired ones.
The Overview Scan allows zooming into the image and to inspect the acquired images without acquiring new ones and thereby preventing the sample from bleaching while browsing the sample.
An Overview Scan is started by going to the Menu: *Navigation > Overview Scan…*
If an Overview Scan has already been acquired and the window was close in the meantime, it can be re-opened again without rescanning the area by the *View > Overview Scan* menu item.
Zooming is done with the Computer mouse wheel.
Moving the images in x and y direction in the window by clicking into the image area and holding down the mouse button and moving the mouse in x and y. The movement will stop once the mouse button is released.

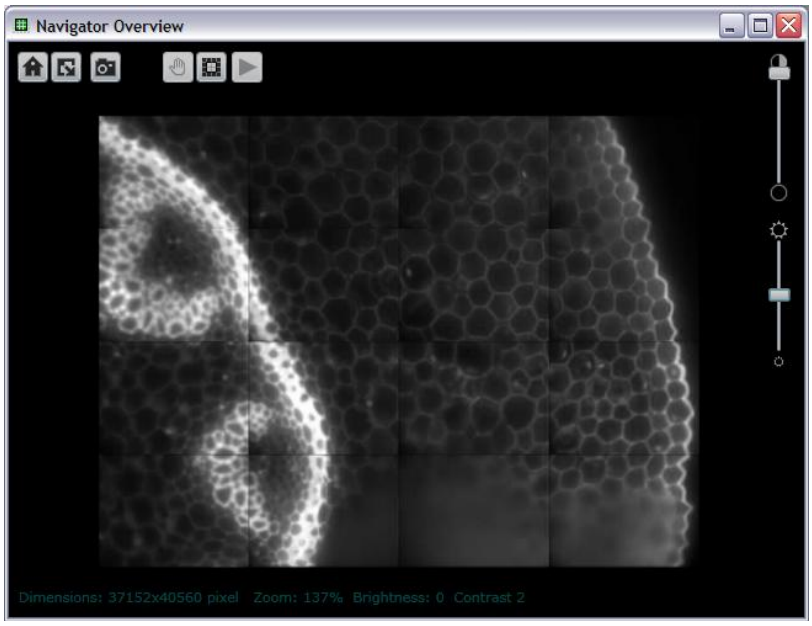


Figure 17: Overview Scan window.

The controls in the Overview Scan window allow executing the following functions:

- zooming out to view the full width of the scanned images area
- Toggling between full screen and windowed display mode
- Saving an image of the currently displayed area
- Toggle between navigation and sub scan mode
- Run a sub scan on the selected area
- Change contrast and brightness of the displayed images in the Overview scan window

The scan area can be limited by creating new Navigator calibrations, please refer to chapter 10.

Furthermore the Overview Scan allows scanning sub areas of the sample. This can be done with the same magnification or if needed also with a higher magnification, to create higher resolution sub scans with a low resolution overview scan.

3.12.6 Changing Objectives

Changing objectives is done by clicking on the desired objective button in the Objectives panel of LA. This panel is the top most in the hardware control section. The buttons show the internationally standardized color coding for the magnification on top, then in the next row the magnification and NA and in the last row of the button caption the immersion medium.

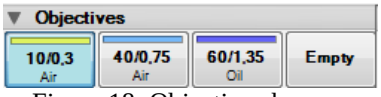


Figure 18: Objective changer

The parameters of the objectives can be changed in the *Config* menu. In case of an iMIC system the objectives parameters can be found in *Config > iMIC > Objectives Configuration.* If a manual objective changer has been configured in an imaging system the objectives parameters can be changed in the menu *Config > Manual Objective Changer.*

3.12.7 Using the Keyboard for navigation

The LA Software allows using the arrow keys to move the stage frame wise in the corresponding direction. Additionally to change the z-Position can be changed with the *Page-Up* and *Page-Down* keys. For a list of all available keyboard short cuts please refer to chapter 2.2.1.2

3.12.8 Moving the stage with the mouse

In the LA Software it is possible to navigate with the computer mouse only. Changing the Z-Position is done with the mouse wheel. Be reminded that the image display needs to be “active” and the mouse needs to be hovering above the image display. Otherwise other graphical controls might react to the mouse wheel and change their values.

3.12.9 Virtual Joystick

The keyboard <X> key combined with the Mouse will act as virtual joystick. As long as the ‘X’ key is held down when the mouse is in the Viewing Panel will ‘anchor’ a joystick icon on the Viewing Panel. If the ‘X’ cursor is continued to be held down while the mouse is moved then the mouse movements will act like a joystick centered on the ‘anchor’ position.

The longer the mouse is held and the farther it is moved speeds up the rate at which the XY position is changed. Releasing either the left mouse button or the ‘X’ key will deactivate the joystick mode.

3.12.10 Using a Joystick to move the stage

If a HID (Human Interface Device) USB Joystick is connected to the computer, this Joystick can be used to move the xy-stage. Enabling the USB joystick in the software requires to activate the driver in LA in the menu *Config > Start HID Joystick.*

The user can then select between two modes, continuous movement or camera frame wise, by checking or un-checking the menu item *Navigation > Joystick mode*

3.12.11 Full Screen mode

The Full Screen mode maximized the image display by hiding the hardware, light source controls as well as the menu bar.
Entering the Full Screen Mode can be done by two ways:

- either with the key combination "*Alt-Enter*"
- By the Menu item *View > Full Screen.*

Leaving the Full Screen mode is achieved by:

- pressing the *Alt-Enter* key combination
- the *Exit* button in floating palette window.

In Full Screen mode it is possible to use many navigation functions like the mouse wheel, the joysticks and the keyboard for browsing the sample.
The context menu is also available, where the z-step size can be change or the Auto focus can be invoked. ROIs can also be modified but not created.

4 Camera controls

4.1 General

Most cameras come with advanced controls for a fine grained control of camera acquisition parameters. Depending on the camera manufacturer and camera model different options are provided by the Live Acquisition software.

- Binning
- Chip-Window
- (EM) Gain settings
- Temperature settings
- Vertical and/or horizontal shift speeds
- Voltage clocks of Camera ADCs
- Fan speed control

4.2 Camera properties

The camera properties panel holds the most frequently used controls for camera operation. These are the option to change the chip-window of a camera, the binning value and the EM-Gain setting for EM-CCD camera.
With the chip-window feature the user can restrict the area where the camera is acquiring only a sub frame/area. By this the user can increase the speed of the acquisition and reduce the amount of data generated during the experiment. Usually the speed increase is only dependent on the height of the chip-window and not on the width.
The binning feature of the camera has two advantages. The first advantage is that by using the binning the amount of image data is reduced and thus leads to a profound speed increase of the acquisition rate of the cameras.
The second advantage is that by using the binning multiple pixels of the camera are read out in one step. Without binning each pixel would have been read out by the camera electronics and each readout process would add some noise. With binning multiple pixels are read out once and therefore only adding once the readout noise to the pixel value!
The only drawback of binning is the reduction in resolution. Depending on the camera model the user might change the binning values independently (e.g.: 1x2, 2x8) or symmetrical (2x2, 4x4,…).

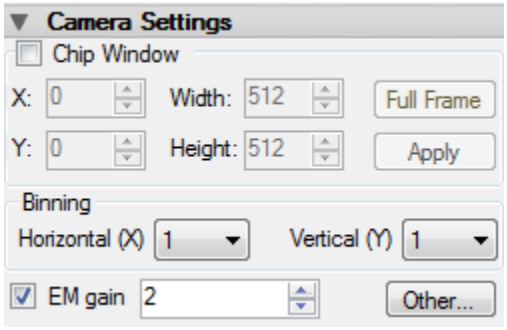


Figure 19: Camera properties panel

4.3 Advanced Camera Controls

In the advanced camera controls the user will find camera specific controls and options. A typical example is the EM-gain setting on the EM cameras, that is not available on interline chip cameras. Other features include control for the cooling system if available or selection of the read-out speed and register.

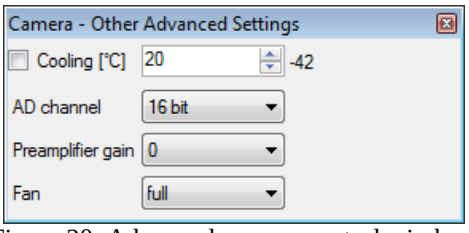


Figure 20: Advanced camera control window

4.4 Camera selector

The camera selector control is only available if multiple cameras have been configured in the device configuration. Changing the camera by selecting it from the drop down list (Figure 21: Camera selector) will make its properties and advanced properties available.

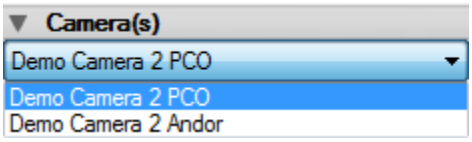


Figure 21: Camera selector

Additionally the tube factor in the iMIC configuration menu (*Config > iMIC with Imaging Control Unit > Filter and Axis*) will change. This allows having different camera port magnifications if needed.

Hint: to change the tube factor for a camera, the desired camera has to be selected first.

If the camera selector is collapsed it will show the name of the selected camera in the title (Figure 22).

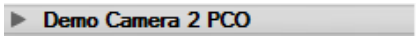


Figure 22: Camera selector showing the name of the selected camera when collapsed

4.5 Assigning multiple camera to the ports on the iMIC

If the LA software detects a camera port switch in the iMIC it will offer a dialog where the user can assign the available cameras on the system to the three available positions of the iMIC camera port switcher.
The dialog for the camera to port assignment is located under *Config > iMIC with Control Unit.* Switching between the installed cameras is done by selecting the desired one in the Camera selector (Figure 21).

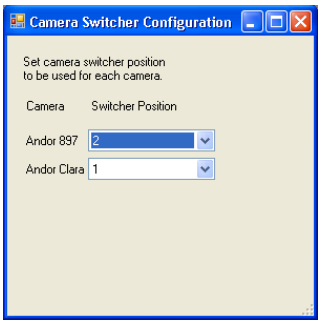


Figure 23: iMIC Camera Switcher dialog

4.6 Simultaneous acquisitions of images with two cameras (DualCam)

This feature is an optional module for the Live Acquisition software that allows acquiring images from two cameras simultaneously. Both acquired images are displayed in a split, overlay or ratio view. These view options are the same as for FRET images when using an iMIC Dichrotome.
In LA it is possible to select the individual cameras or to use them simultaneous. This selection is done with the camera selector (Figure 21).

To configure a DualCam setup in LA the user has to use the "Multi Camera" device in the device configuration dialog.
Opening the properties window of the Multi Camera Device (Figure **24**) requires the user to enter the trigger connection (usually to the ICU "Camera sync Out" connector).

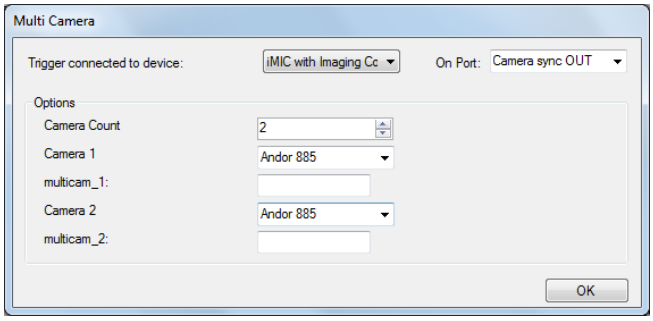


Figure 24: Multi Camera device properties dialog

5 Image processing

5.1 Applying color palettes to gray scale images

In Live Acquisition the user can change the color palettes by selecting the desired palette in the menu *Display* > *Palette*.

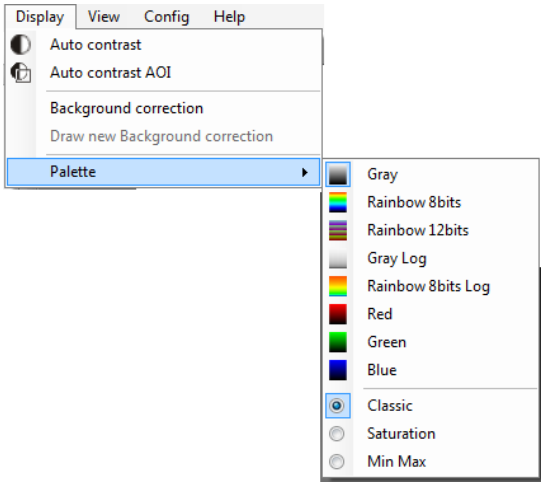


Figure 25: Menu to change the color palette

Alternatively the palette can be changed by "right clicking" onto the color bar on the right in the Live Acquisition window.

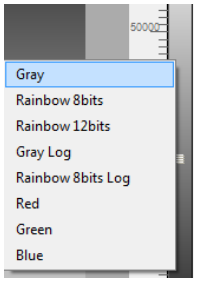


Figure 26: Context menu to change the color palette

5.2 Advanced color palettes options

Additionally to the different color palettes the user can choose between three palette options:

Classic: This is the standard mode for the color mapping. The color palette is unmodified and covers the full dynamic range of the camera or the adjusted dynamic range set by the user manually (brightness and contrast) or by using the Auto Contrast functions.

Saturation: This mode will display the maximal and minimal value of the dynamic range from the camera in different colors. This allows to observe if the camera chip is saturated or in case of cameras with baseline clamping if the clamp value is too low and therefore cuts of signals from the sample.

Min Max: Here the minimum and maximum value of the palette will have different colors to make the values stand out. This min max also works with manual adjustment of brightness and contrast as with the Auto Contrast features. If the background correction feature is enabled the mean intensity values of the background region will be taken into account for the minimum calculation, too.

5.3 Changing contrast and brightness

Contrast and brightness are controlled with the control on the right to the displayed image (Figure 27).

Figure 27: Auto scale - Auto Contrast

These scales are each composed of three controls.

- Maximum – Appears at the top of the scale
- Auto scale – The stylized ‘Auto’ Button in the top of the scale
- Minimum – appears at the bottom of the scale

Maximum and minimum each consist of the following component(s):

- A roll bar for sequential increment/decrement of the parameter
- A limit control (a dotted line in the intensity scale) to change the values of the parameter

The vertical scaling bar has a box on its upper and lower end, in which one can manually enter the max and min value to be displayed.

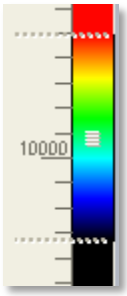


Figure 28: Interactive intensity range/brightness scaling

Within the spectrum there is a horizontal line on both ends. Clicking into the spin buttons moves up and down stepwise; continuously clicking into a spin button causes a gradually accelerating continuous move. Dragging the horizontal line to a given position in the spectrum chooses this position as the upper/respective lower limit. There are two ways to extend the reduced display limits:

The auto scale button is a configurable button. With a right click on the button you have the ability to set the new auto scaling min and max values.

- Clicking on auto scale sets max min values to the current ones. Double Clicking turns a continuous auto scale on. Clicking again freezes the current value and turns auto scale off;
- Single Clicking on auto scale executes a single auto scale and scales all subsequent images within the one time determined boundaries;

The parameters in the in the scaling controls can be reset by right clicking the mouse in their respective windows.

There is also a button labeled 'Area' which when clicked allows the mouse left button to set a rubber band bounded border inside the Viewing Panel. When the left mouse button is released then the auto contrast will be triggered and will use the area inside the bounding box as the determination criteria of how to set the contrast scale.

This 'ROI' button has a second option. If it is double clicked before the bounding box is drawn then the bounding box remains semi-permanent and will continue to be used for contrast calculations.

5.4 Auto contrast

The Auto Contrast feature will optimize the image quality for the display. The original image data will not be modified when saved to the hard disk. There are three ways to enable the Auto Contrast:

- The first way is to go to the menu Display > Auto contrast (Figure 29), that will enable the feature permanently until the item in the menu is deselected again.
- The second method is to enable the feature by using the context menu by right-clicking onto the currently displayed image.

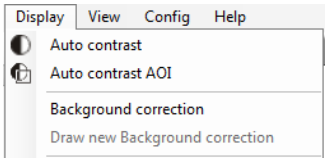


Figure 29: Enable Auto Contrast from the menu bar

- The third way to enable the Auto Contrast feature with the button above the color bar control on the right side of the LA window. This button has three states: off, once and permanently.

5.5 Background correction

Background correction is meant for wide-field microscopy where a certain level of background fluorescence is unavoidable. Background correction in the Live Acquisition software reduces the overall intensity of an image by the mean value of the background correction ROI.

Drawing a ROI for the Background correction feature is done either form the context menu of the image display or from the menu *Display > Draw new Background (*Figure 30*).* Alternatively the drawing of a new background correction region can be invoked from the context menu;

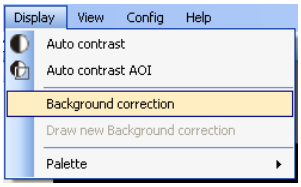


Figure 30: Menu item for drawing a background correction ROI

5.6 Scale bar

The scale bar can be enabled from the View menu View > Sow Scale Bar. To display the correct length the configuration and calibration of the system has to be done first. The Scale bar uses the properties of the currently selected Objective (magnification and correction factor), the pixel size of the camera and the tube factor from the microscope configuration to calculate the correct values.
The Scale bar can be configured by the *Config > Image Scales configuration* menu. The Image Scales dialog allows to enable/disable the scale bar and also to change its size and orientation (Figure 31).

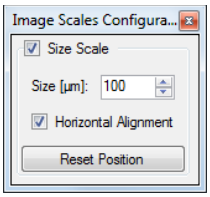


Figure 31: Scale bar options dialog

Hint: to make proper use of the scale bar in imaging systems without an iMIC, a manual objective changer can be configured.

5.7 Pixel value

The Intensity value of a given pixel in the image can be inspected by moving the mouse cursor over the desired pixel. The intensity value of the pixel under the mouse cursor is then displayed right hand side in the status bar at the bottom of the LA window (Figure 32).

Figure 32: Pixel intensity value und the mouse cursor in the image

5.8 Histogram

The Histogram can be shown by using the menu item *View > Histogram* or the histogram icon in the toolbar. I show a normalized gray-value distribution and displays it besides the color-bar.

Optionally the histogram calculation can be restricted to a ROI. The ROI can be created by using the menu *View > Histogram ROI*. After selecting the menu item the user can draw a rectangular ROI on the image display without further actions.

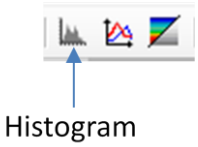


Figure 33: Toggle the histogram display from the toolbar

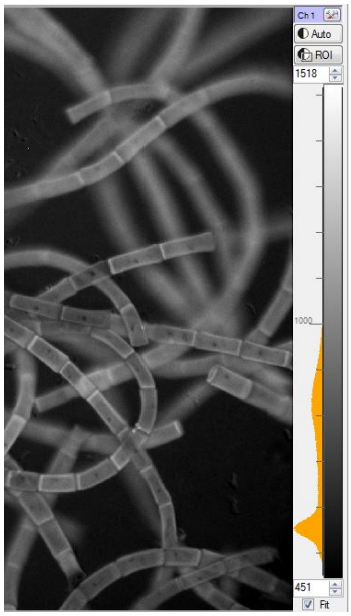


Figure 34: Histogram display between the image and the color bar.

6 Offline Analysis

The Offline Analysis Application allows displaying and managing data and to run analyzes offline. It also features an extensive Meta data info panel where a relevant data about the acquired images and sequences can be displayed. There are also the options to export the images and/or open a selected file in ImageJ/Fiji.

We preserved the workflows and the look and feel of the Live Acquisition software as far as possible and where applicable to keep the learning curve for this application as flat as possible. You will find yourself familiar with the application if you already know the Live Acquisition software. This applies especially for the menu structure, the tools in the toolbar and the analysis panels.

6.1 Screen layout

There are four main areas in the Offline Analysis application.

- On top there's the menu bar.
- On the left is the "Set" management where all the image data can be organized
- In the center of the application window the display area for images and the related controls for navigating in the image data set and the toolbar for ROIs, the graph window and zooming.
- Located on the right in the main window are the panels for analysis functions and information/meta data display.

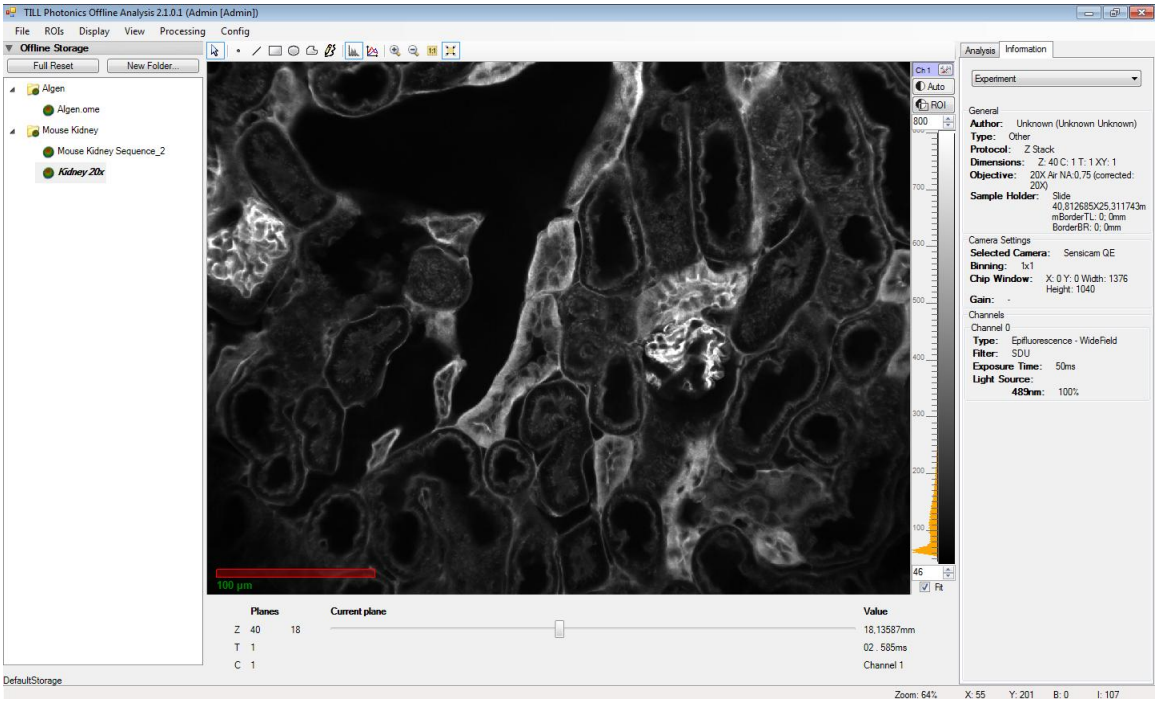


Figure 35: Main window of the Offline Analysis application

6.2 Data management with "Experiment Sets"

The Offline Analysis application provides functions for grouping image sequences into data sets. This allows grouping of multiple acquired image sequences that belong to a series/type of (biological) experiments. The Groups/Sets can be collapsed or expanded by clicking on the small triangle in front of the name. This behaves like the directory tree view in Microsoft's Windows 7. Analysis data, here graphs, are stored as child item of an image sequence and can be recalled with the used ROIs and the graph by double clicking on them.

6.2.1 Creating a new Experiment Set

Creating a new Experiment Set is done with the button "New Experiment Set" on top of the Experiment Set tree view.
All *Experiment Sets* in the Offline Analysis Applications are also visible from the Live Acquisition application and can also be created there before an experiment is acquired.

6.2.2 Deleting an Experiment Set

Deleting or renaming an *Experiment Set* is done through the context menu.

Warning: Deleting an Experiment Set is done through the context menu item "Delete".
Be aware that this will also delete all enclosed image data!

6.2.1 Renaming an Experiment Set

An *Experiment Set* can be renamed by using the *Edit* command from the context menu. To rename an *Experiment Set* it must be selected. The *Edit* command will open a dialog where the name of the *Experiment Set* can be changed. To apply the name change press "OK".

6.2.2 Moving (Cut&Paste) acquired image sequences between Experiment Sets

The acquired images or image sequences can be moved from one Experiment Set to another with the "Cut" and "Paste" commands from the context menu.
Selecting a sequence by clicking on it allows using the "Cut" command in the context menu. The context menu is opened by pressing the right mouse button.
Hint: It is not necessary to open the file that should be moved by double-clicking on it.
The cut image sequence can then be "pasted" to another Experiment set by selecting the experiment set and selecting the "Paste" command from the context menu.

Summary for moving an image sequence:

- Select the desired image sequence by clicking on it (left mouse button)
- Open the context menu for this item by pressing the right mouse button
- Select the "Cut" item in the context menu
- Select the Experiment Set where the image sequence should be moved
- Open the context menu for the Experiment Set by pressing the right mouse button
- Select "Paste" from the context menu

→ The image sequence is then moved to the other Experiment Set

6.3 Data display and Movie player

The center of the Offline Analysis application window is used to display the image data. This area can be divided into three sub regions. On top resides the tool bar which holds icons for creating and managing Regions-Of-Interest (ROIs; see chapter 7.1), toggling the Graph window and icons for zooming in and out of the images.
In the middle is display for the images and there are also the same controls for adjusting the brightness and contrast of the image as well as applying color transfer functions (color mapping through false color palettes) like in Live Acquisition. For further reading please refer to chapter (0).
At the bottom of the center area the control for navigating the image data are located (Figure 36).

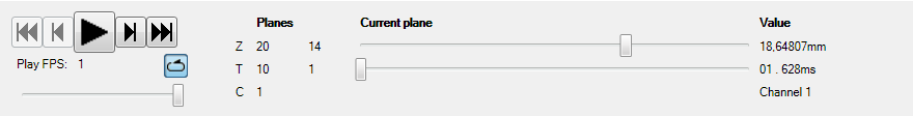


Figure 36: Movie Player and image navigation controls

The Movie player controls allow replaying a time series at different speeds. Further options are to move to the next or previous frame or to move to the beginning or end of a sequence.

6.4 Toolbar

The toolbar is located on top of the image display area. The toolbar contains functions for creating and editing ROIs, displaying the Offline Graph window and options to zoom in and out of the image

6.4.1 Drawing ROIs

The Offline Analysis Application provides the same ROI functionality as the Live Acquisition application. For a detailed description of the ROI managing capabilities please refer to chapter 7.1ff.
By default all ROIs that where draw in the Live Acquisition software are also available in the Offline Analysis application if the image data is opened there.

6.4.2 Show Analysis Graph window

With the Graph button it is possible to toggle the visibility of the Offline Graph window. The offline Graph window has the same options like the Graph window of the online analyzes. A detailed description of the Graph window functionality can be found in chapter 7.5

6.4.3 Zoom options

The toolbar has a bunch of buttons to zoom in and out of the displayed images or to fit the image to the available space in the application window. A detailed description of the functions can be found in chapter

6.5 Analysis Tab – Offline analyzes

Here are all the controls that are needed for running an analysis, kinetics, RATIO or FRET, on an existing image data set. As the layout of the control and all options are the same as in Live Acquisition, one will find itself in a familiar place. At this point please have a look at the

6.6 Information Tab

The Information Tab shows the Meta data of the currently open image sequence. The Meta data of the sequence is divided in to four categories:

- **Experiment:** Here the parameters of the experiment can be found. This includes used camera, light source, number of frames, channels, planes.
- **Images:** In this category the data of all images is shown as a table. The shown parameters for each image include x,y,z position, running number, time stamp. This data can be selected either with the mouse or with Ctrl-A (selects all cells) and copied to the clipboard via the Ctrl-C shortcut for further processing.
- **Hardware:** This category shows the system configuration (hardware), which was used to acquire the currently open image sequence. This includes information like the vendor specific device version strings.
- **LA:** This category shows the LA software specific data: This includes calibration data for devices like the Polytrope and Yanus.

An example on how the information tab looks like is given below (Figure 37).

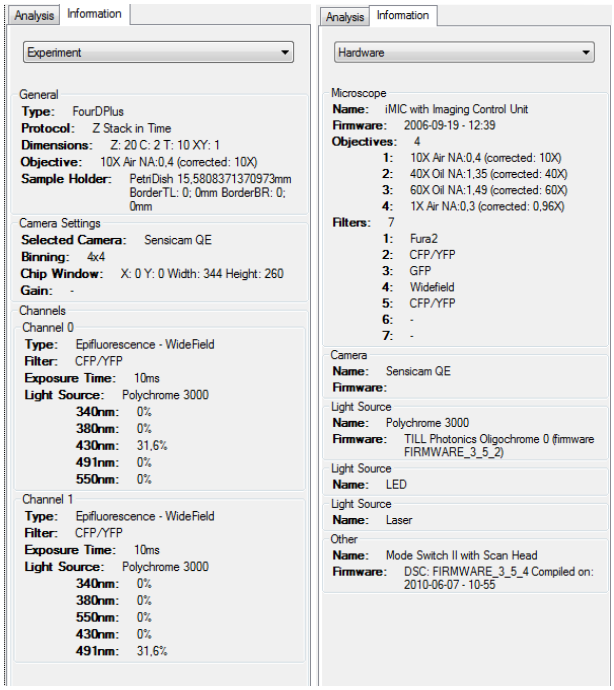


Figure 37: Information tab examples for the *Experiment* and *Hardware* subcategory

7 Advanced image analysis

7.1 Pixel Value

These values can be found on the right side of the status bar at the bottom of the main window. For more information, please refer to chapter (5.7).

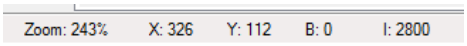


Figure 38: Pixel intensity display in Offline Analysis

7.2 Regions Of Interest (ROI)

Regions Of Interest are user defined smaller parts of the acquired image. These parts or regions can be ellipses, rectangles or polygons.

7.2.1 Drawing ROIs

To define or draw a ROI the user can select different shapes from the tool bar. For analysis functions the *rectangle, ellipse, polygon and freehand* tool can be used (from left to right). The *spot* and *line* tools can only be used in FRAP experiments.
Once the desired ROI shape has been selected the user can draw multiple ROI of the selected shape in a row. To stop creating ROIs the user has to select the *Arrow/Mouse* cursor icon in the tool bar.

Figure 39: ROI drawing tools in the main toolbar

When the Arrow tool is selected the user can manipulate the dimensions of the ROIs by dragging the outline.

7.2.2 Region (ROI) Manager

The ROI manager allows manage ROIs and to change their properties. The ROI Manager window is opened from the menu *ROIs* > *ROI manager*… .

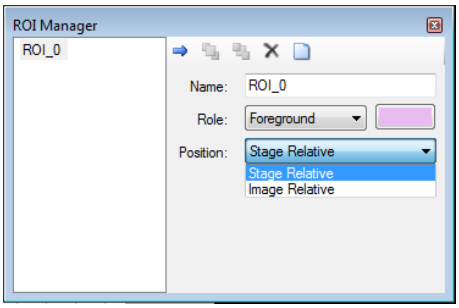


Figure 40: ROI Manager Window

On the left hand side of the ROI Manager window the list of all currently drawn ROIs is shown. On the top of the window besides the ROI list a toolbar (Figure 41: Toolbar in the ROI Manager window) is located that holds some icons for managing the ROIs.

Figure 41: Toolbar in the ROI Manager window

Tools available for managing ROIs:

Go to: Pressing this button will move the stage to the position where the ROI was drawn. This feature is only available on systems with a motorized xy-stage. The ROI position needs to be *Stage Relative*.

Send to back & Send to front: Allows changing the order of the ROIs. This is usefull if one smaller ROI is hidden by a bigger one that was draw later. In this case it is possible to move the "hidden" ROI to the front an make it selectable again. The order f the ROIs has no effect on the analysis features in Live Acquisition.

Delete: This deletes the currently selected ROI. Selecting the ROI can be done in the ROI list of the ROI Manager window or directly in the image display area of Live Acquisition.

Delete All: This will delete all ROIs form the list after a safety dialog is show to confirm this action.

Editing the properties of a ROI is also possible in the ROI Manager window.

The following ROI properties can be edited:

Role: For analysis purposes it might be necessary to assign different Roles to the ROIs. Foreground is the default Role for all newly created ROIs. Background is used by analysis functions to subtract a mean value in some calculations.

Name: This allows editing the ROI name. By default the ROI name is created with a prefix "ROI" and the running number.

Color: Each ROI gets a random color when it is created by the user. To change the color, click on the Color-button and select a new color for the ROI.

Position: This option allows on systems with a motorized xy-stage to change the relative position of ROIs in the system. Selecting the *Stage relative* option will make the ROI position relative to the xy-stage which is useful if analyzes (Kinetics, RATIO, FRET,..) at multiple positions should be done. Selecting the *Image relative* option will position the ROI relative to the image coordinates; this is the default on systems without a motorized stage (e.g. Imaging Systems). This also allows user to create ROIs that are used in each position in multi-position experiments

Hint: If the position option "Stage relative" is selected the ROIs will disappear if the stage is moved. Use the "Go to"-Button to move the stage to the ROI selected in the list.

7.3 ROI roles and how to change them

In the LA software it is possible to assign different roles/functions to the ROIs. Changing the role of a ROI is done either in the context menu or in the ROI Manager.

There are two default roles for ROIs in LA depending on the selected experiment:

1) By default each ROI is assigned the "foreground" role if the experiment is not any of the FRAP experiments (Time Lapse, Multi channel, Snapshot, TIRF Epi,…).
2) If the user has selected one of the FRAP experiments the default role of the newly created ROI will be "FRAP" and the ROIs are labeled accordingly.

Available ROI roles:

None: If this role is selected the ROI will not be used for an analysis function nor in a FRAP experiment as bleaching region.

Foreground: This is the default for many experiments and with this role the ROI will be used to calculate the mean intensity of the pixels covered by its outline.

Background: Setting the role of a ROI to "Background" will tell the analysis functions (RATIO, FRET and FRAP) to use this ROI for subtracting the mean intensity value of this ROI from certain parameters in the calculation of the RATIO and FRET values.
If multiple "Background" ROIs are used Live Acquisition will use the pixel values of all background ROIs to calculate the mean intensity. This means that LA will sum up all pixel values of the background ROIs first and then divide the sum by the total number of the pixel in the background ROIs.

FRAP: This option is only available if a FRAP experiment (*FRAP* or *FRAP on Demand*) is selected. Additional parameters for the FRAP ROI are set in the FRAP experiments them self, like bleach time and loop counts. During online analyzes (e.g. Kinetics) the FRAP ROIs can be used for analyzes, too. They will be available in the ROI manager and an Online Analysis panel is show below the FRAP experiment panels

Hint: FRAP Regions will only show up in the ROI Manager if a FRAP experiment is selected!

7.4 Starting an Online Analysis

The available analysis functions can be found below the experiment parameters on a separate panel called "Online Analysis" (Figure 42) that is located below the experiment panel.
Depending on the experiment parameters the list of available Online Analysis functions changes.

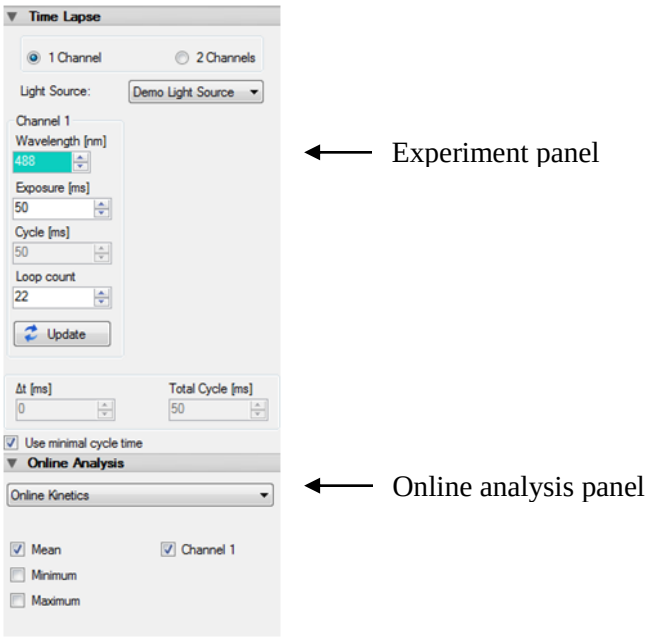


Figure 42: Online Analysis panel below an experiment panel

7.4.1 Selecting the type of the analysis

Depending on the number of channels in an experiment the LA software will offer a variable list of analyzes.

Number of channels:

- ONE: Always available is the Kinetic analysis that analyses the mean pixel value of the ROIs and plots these values vs. time.

- TWO: If two channels are selected in an experiment the LA software will additionally offer a RATIO analysis for the experiment. The Kinetics analysis will then allow the user to select which channels should be analyzed.

- THREE or more: If three or more channels are used in an experiment the software will also list the FRET analysis. However, if no license for the FRET analysis has been obtained a warning will be displayed no online analysis is possible. However, it is possible to edit the names/labels for the channels in the FRET analysis panel.

7.5 The Graph-Window and its options

This chapter describes the functionality and layout of the Graph window. The Graph window has the same feature set in the Live Acquisition and in the Offline Analysis application.

7.5.1 Graph window layout

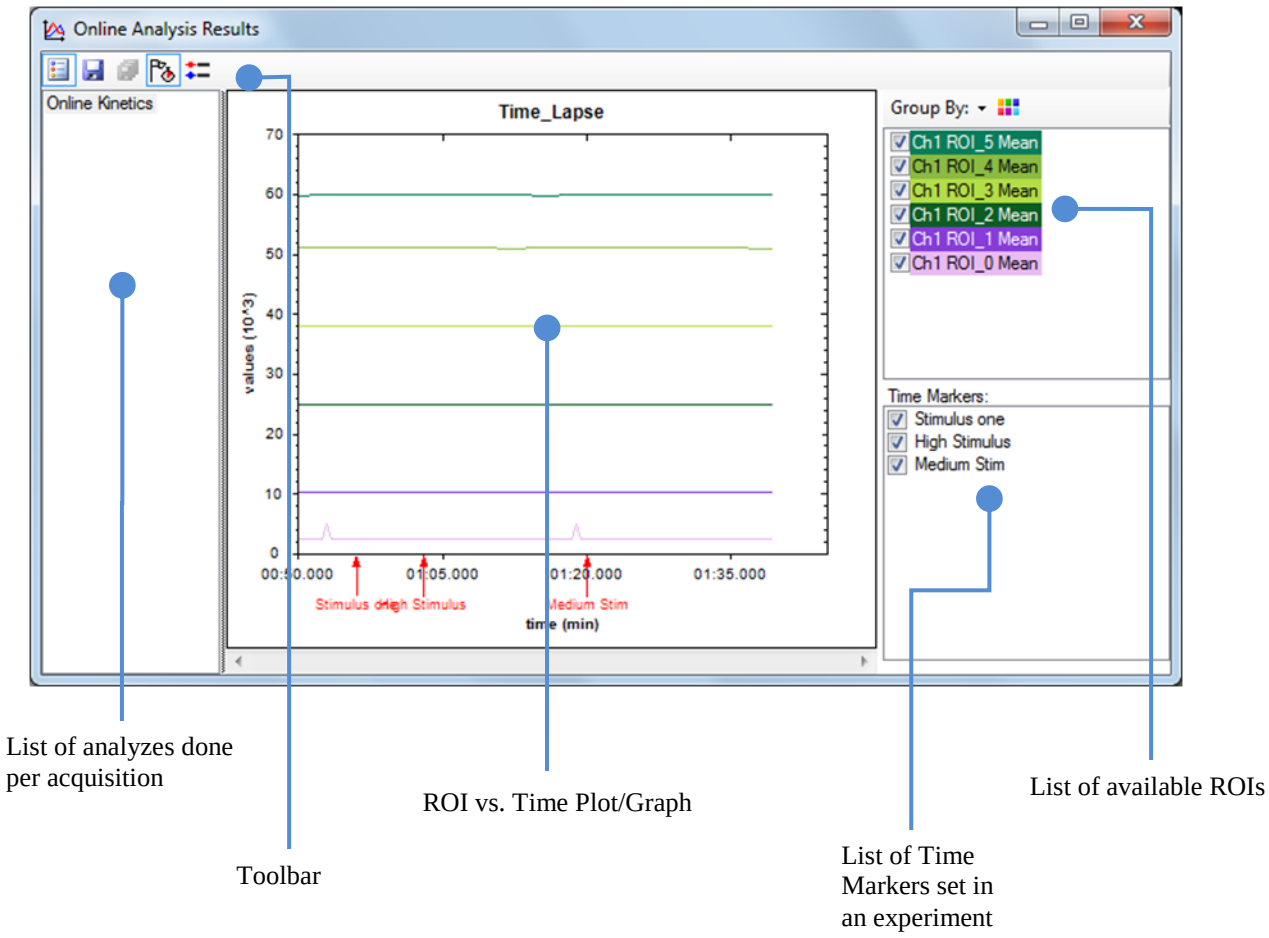


Figure 43: Online analysis graph window layout.

The Online Analysis Results Window is divided into five areas:

1) **Toolbar:** The Toolbar is located at the top of the window an contains buttons for saving the results and toggle some views (Time Markers, Captions of the Graph,…) and to zoom out of the graph.
2) **List of analyses**: List of analyses done during an experiment. Usually this list has only one analysis listed, but if a "Marker Scan" experiment is done, the list will show the analyzes of each position from the scan.
3) **ROI vs. Time Plot**: The Plot/Graph shows the mean values of the ROIs plotted versus time. If Time Markers are set during an experiment it will be displayed below the x-axis. Moving the mouse over a Time Marker will show a vertical line in the plot.
4) **List of available ROIs**: All ROIs that were used for the analysis are shown here. Checking and un-checking will toggle the visibility of the ROI traces in the Plot.
   a) **Group by**: Drop down list allows changing the way the ROIs are grouped, e.g by channel (Figure 44) or function.

b) The **color palette** icon allows toggling between the original coloring and a new coloring schema where every ROI in each channel has a distinct color. This is usefull in multichannel experiments, where the color of the ROI is the same in each channel.
   a. **Time Marker**: List of the *Time Markers* set during an experiment are listed here and can be enabled/disabled by using the checkmark.

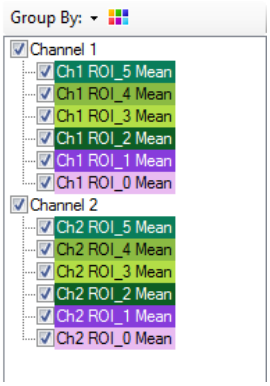


Figure 44: ROIs grouped by channels in the *Online Analysis Results* window

7.5.2 The toolbar buttons

7.5.2.1 List of Analyzes

This is a toggle button that allows showing or hiding the list of analyzes available. This is useful if image sequences are acquired that are done in one location.

7.5.2.2 Save

With this button the user can save the ROI mean values as CSV (comma-separated-values) or as native XLS (Excel) –files. This will only save the selected analysis from the list of available analyzes in case of multiple analyzes are available.

7.5.2.3 Save All

If multiple analyzes are available pressing this button will save all of the available analyzes regardless of the state (selected or not).

7.5.2.4 Time Marker display

With this button the visibility of the time markers, set during an experiment, in the graph can be toggled.

7.5.2.5 Graph legend

Turning the legend on an off is done this icon in the toolbar of the *Online Graph*-Window

7.5.2.6 Zoom out

If the user has zoomed in to a particular region of the graph, he or she can un-zoom the graph fully by pressing this button without going to the context menu of the graph.

7.5.3 Saving, coping, Printing of the Graph and Data

The data in the graph window can be saved, copied to the clipboard or printed (Figure 45). The options are available from the context menu when “right-clicking” in the plot area. Saving the data point of the plot is done by using the Save icon and multiple Save icon in the Toolbar of the *Online Analysis Result* – window.

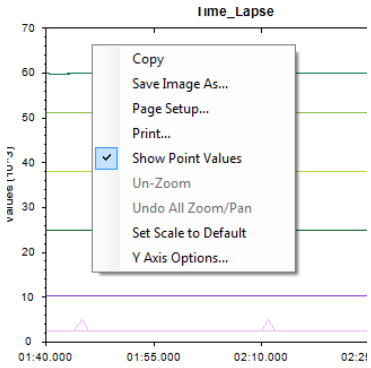


Figure 45: Context menu items in the *Online Analysis Results* window

7.5.4 Time Marker display

The Time Markers are displayed as red arrows below the x-axis of the graph. If the mouse cursor hovers over one of the markers a vertical red line appears in the graph area. This makes it visually easier to see what happened at this Time Marker if multiple traces are shown in the graph. Time Markers can be shown or hidden by checking or un-checking them in the *Time Markers* - List in bottom-right corner of the *Online Graph*-Window.

7.5.5 Plot axis scaling

The scaling of the y-Axis can be change from the context menu item *Y Axis Options…* of the graph (Figure 45). In the appearing dialog the user can select between manual scaling or automatic scaling. Choosing manual scaling allows setting the minimum and maximum of the y-Axis. Choosing automatic will determine one of the values, minimum or maximum, automatically.

7.6 Kinetics analysis

This chapter describes how-to analyze the mean value of ROIs over time. The LA software can analyze the mean value of ROIs that are assigned as foreground regions. Regions that are assigned as Background will not be used for the calculation. Regions draw are for the first time are by default foreground ROIs.

7.7 Ratio analysis

The LA and OA software allows the analysis of RATIO experiments. It offers two algorithms for calculating the RATIO that are described in this chapter.

7.7.1 Ratio algorithm

By selecting the algorithm RATIO algorithm the LA and OA software calculate a RATIO image (32bit floating point) by dividing the pixel values from the same xy position from each channel.

Before the division is done the optional background values from each channel is subtracted from the pixel values of the corresponding channel.
The mean value of the ROIs is calculated on the RATIO image.
Pixels in the RATIO image with the value of zero will not be counted when the mean value of the ROIs is calculated. All values that might be below zero during the calculation of the RATIO values will be clipped to zero. This might happen when using background ROIs.

Hint: Running a Kinetic analysis on an RATIO image (sequence) may result in different intensity values for the ROI mean values as the Kinetic analysis will count the zero intensity pixels for the ROI mean value calculation!
This can be changed if the thresholds for the RATIO image calculation are set to zero!

7.7.2 Ratio (integral) algorithm

This variation of the RATIO calculation resembles the way that RATIO is calculated in Photometry setups. This algorithms is useful when working with samples where the signal to noise ratio is not optimal → the signal is relative weak.
This algorithm first calculates the background value from the background ROIs in each channel and then subtracts this mean value from the mean value of the ROIs in each channel. In the next step the RATIO is calculated from the corrected (background subtracted) mean ROI values from both channels. The division is done with the mean values of the ROIs and not on a pixel basis! This works like having multiple photometry systems in the field of view.
Optionally an RATIO image is calculated where the RATIO is calculated pixel by pixel like in the "normal" RATIO algorithm.

7.8 FRET analysis

This module is made for analyzing three channels FRET experiments where two excitation wavelengths are used to alternate excite the donor and acceptor expressed in one specimen. Donor and acceptor can be a pair for matching proteins like CFP (cyan fluorescent protein) and YFP (yellow fluorescent protein) or others (GFP, RFP, ...).
Typically for a FRET experiment are two excitation wavelengths that excite both fluorescent proteins in a sequential manner as fast as possible to obtain three images by using the Dichrotome image splitter in the iMIC. The three channels that are used for the calculation of the FRET signal are the Donor, Acceptor and FRET channel.

7.8.1 Description of available NFRET algorithms

The LA software offers three algorithms for calculating a normalized FRET image. The algorithms differ in the way the denominator is calculated.
The Live Acquisition (LA) and Offline Analysis (OA) application calculate a FRET image pixel by pixle using one of the below formulas. When calculating the FRET image, the LA and OA software takes the xy-offset an background values (if assigned) into account.
The mean value of the ROIs is calculated from the pixel values of the FRET image. All calculations are done with 32bit precision.

$$NFRET = \frac{(I_{fret} - background) - bt_{don} * (I_{don} - background) - bt_{acc} * (I_{acc} - background)}{N}$$

I_{fret} = Intensity of the FRET signal (excitation 440nm) of channel 2

I_{don} = Intensity of the Donor-Emissionen (excitation 440nm) of channel 1

I_{acc} = Intensity of the Acceptor-Emissionen (excitation 490nm) of channel 2

bt_{don} = “Bleed-through” correction factor for the Donor

bt_{acc} = “Bleed-through” correction factor for the Acceptor

N = correction-factor for the normalization of the FRET signal

The following formulas are used to calculate the denominator:

Xia et al. :

$$N = \sqrt{(I_{don} - background) * (I_{acc} - background)}$$

Gordon et al.:

$$N = (I_{don} - background) * (I_{acc} - background)$$

Youvan et al. (FRET in relation to the acceptor):

$$N = (I_{acc} - background)$$

7.10 FRAP analysis

7.10.1 Online FRAP analysis

Live Acquisition allows to analyze the ROI mean value of FRAP ROIs during an experiment. However, this analysis does not allow running any fitting methods during the experiment; this has to be done offline in the Offline Analysis application.

7.10.2 FRAP Analysis offline

The FRAP Analysis module in the Offline Analysis application allows to calculate diffusion coefficients and a curve fitting with various formulas.
The majority of the fitting formulas and other calculations are bases on the publication of *Kota Miura from the EMBL in Heidelberg, Germany*.

It allows running extensive analysis functions on the acquired FRAP data that include bleaching correction, background subtraction and multiple fitting functions for calculating the important FRAP parameters.

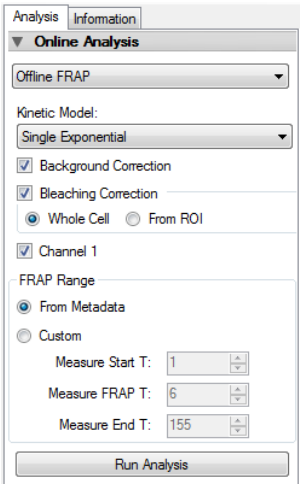


Figure 46: Parameters for the offline FRAP analysis

7.11 Cropping of Image Sequences

The Offline Analysis allows cropping new image sequences/stacks with the *Crop Dimensions* function. The *Crop Dimensions* allows copying parts of a multi-dimensional image sequence into a new sequence. This can be useful if only part of a z-stack is needed for visualization or deconvolution.
The *Crop Dimensions* function can be found in the menu *Processing > Crop Dimensions.*

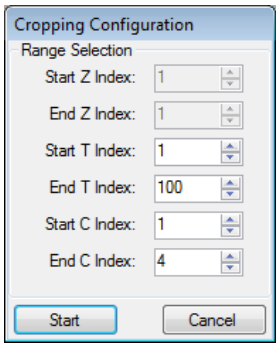


Figure 47: Sequence cropping dialog

New created image sequences by the Crop Dimensions will have a generic name "*Cropped*" which is extended by a number at the end of the sequence name like "*Cropped_2*".
These new sequences can then be exported via the *Export* function in the *File* menu.

8 Hardware Control Panels

8.1.1 Primary Control Panel

There are a handful of controls that are always needed and provided with the LA Dashboard. The defaults provided are for Setting Storage Control, Z-Axis Focal Control, Objective Changer, XY Stage Control, 2-D Navigation Control, Image Marker,
Camera, Camera Properties, Light Selector, Display Options, Filter, and Streaming Controls.

8.1.2 Secondary Control Panel

On the right side of the main viewing area is a secondary hardware control. This control is composed of a pair of combo-text boxes with roll bars and a pair of buttons. From this control maximum and minimum color correction can be established as well as an auto contrast correction.

8.1.3 Status Bar Control Panel

At the bottom of the viewing area is a status bar indicating current state of hardware and protocol execution.

8.1.4 Primary Control Enhancement Panels (Collapsible Panels)

As mentioned earlier the Live Acquisition solution is dynamic and designed to be updated and expanded as the needs of your experiments change or is adapted. Various default views and experiments dynamically load the necessary controls into the dashboard and unnecessary controls are removed.

8.1.4.1 Lighting and Filter Control Panel

At the bottom of the viewing are above the status bar is a dock able control panel showing the current filter and lighting settings. Opening and closing this window adjusts the overall display of the viewing pane.

8.1.4.2 FRET Secondary Control Panel

In FRET configurations a second secondary control window appears directly to the right of the primary control window so that the main viewing window is sandwiched between the two secondary control windows.

8.1.5 Execution Control Panel

Directly beneath the primary control window is the execution window. It contains buttons to control Experiment, Live and Still modes of operation. These three provide out of the box control for hardware so that you can quickly examine a sample or series of samples in a rapid manner and quickly determine if it is worthwhile to continue running experiments on the samples. One of the advantages of the Live Acquisition solution is its ability to be used in screening applications and this is built directly into the core concept of the user interface. As it is constantly being manipulated here can also be found the exposure time control.

8.2 Hardware Positioning Controls

8.2.1 Z Axis and Objective Focal Controls

The Z Axis and Focal Controls directly manipulate the hardware as you change the settings on the graphical display. This corresponds to an equivalent movement of the focus axis. Every time this is done a new picture is made.

The Objective Focal Control allows you to change different magnification ratios as well as different focal forms (air, oil, etc) based upon what is available in the configured hardware from the hardware configuration. The control is seamlessly updated with the right click context menus to select the focal setting desired. With a left click the objective is lifted up and down into focus position.

Fine tune focusing is accomplished either with the text box, roll bars, or directly with the mouse and Space Navigator.

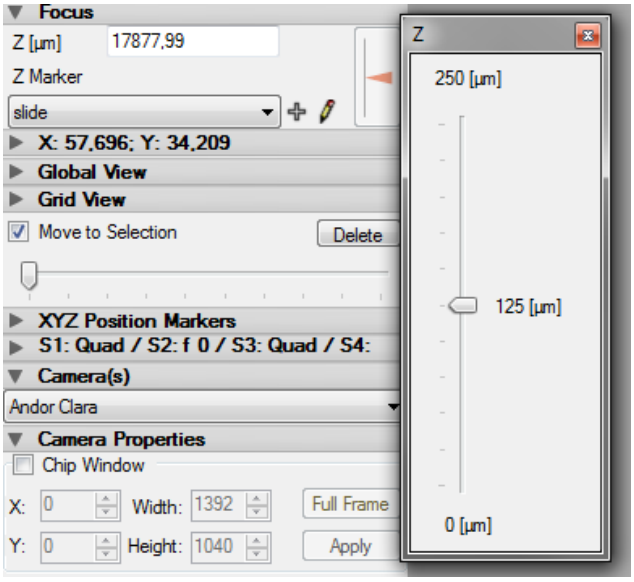


Figure 48: Z and Object Changer Control

Finding the Right Focus is the one most important feature of any microscope.
Live Acquisition allows multiple ways of configuring and doing this as each experiment and each user has different needs.

Focusing can then be achieved by:

- use of the Mouse Scroll Wheel (Manual Focus)
- the keyboard <CTRL> key combined with the Mouse (Autofocus)
- directly entering absolute values in the Focus Control GUI (Manual Focus)
- using the Mouse to drag the slider of the Focus Control GUI (Manual Focus)
- Right Mouse Click in the Viewing Panel to open a context menu (Autofocus)
- Returning to Z Home or a ZMarker Position

8.2.1.1 Mouse Scroll Wheel Manual Focus

To change the focus one must roll the mouse wheel up increases the absolute position of the Z drive thereby increasing the focus level. One can also roll the mouse wheel down lower the Z drive thereby decreasing the focus level using the last used step width. This facilitates fine-tuning when approaching the final position.
When one right-clicks onto the Display area, a context menu opens that contains the step sizes for the focus controls (Mouse wheel and Page up/Page Down keys).

8.2.1.2 Z Focus Text Box Manual Focus

A first order focus can be achieved by typing in an approximate focal level in the text box.

8.2.1.3 Fine Motion Z Focus Slider Manual Focus

One can achieve sub micrometer focus control without the need to adjust default focal step sizes by using the Z Focus slider. This slider however is limited in focal ranges of +/- 250 micrometers.

8.2.1.4 Autofocus

The Autofocus action will both set a new focal step size and perform an autofocus with the currently configured autofocus algorithm.
Invoking auto focusing is done by using the mouse right button in the viewing window and selecting the autofocus action item.

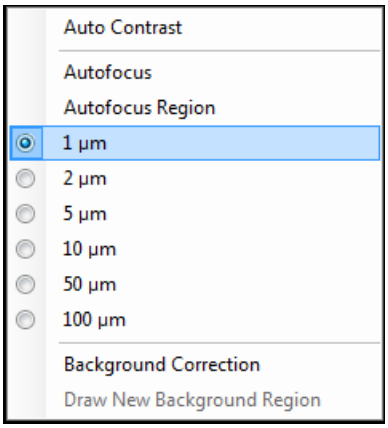


Figure 49: Autofocus and Auto Contrast

This can be combined with selecting new focus steps as well as setting an autofocus region. Setting an autofocus region is achieved by selecting this option from the context menu and then using the mouse left button to rubber band highlight a ROI in the viewing panel.

8.2.1.5 Z Marker Manual Focus

The Z Marker is similar to the Z Home but it allows multiple Z Positions to be marked and then to select them from a pop up list. Selecting one of the list entries will return the focus to this position.

8.2.1.6 Setting the Focus Steps

To configure the focus Steps for the objectives in a system the user has to open the Step configuration dialog (Figure 50) from the menu bar *Config > iMIC > Focus Control Configuration.*

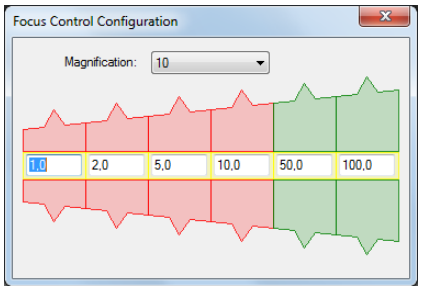


Figure 50: Dialog for configuring the step sizes for the focus controls.

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8.2.2 XY Axis Stage Controls

The XY Axis stage controls directly manipulate the hardware as you change the settings on the graphical display. Every time this is done a new picture is made.

Finding an AOI is the second most important feature of any microscope. Live Acquisition allows multiple ways of configuring and doing this as each experiment and each user has different needs.

The following feature allows display of 2x2 or 3x3 images simultaneously thereby allowing Snapshot & Live mode to provide a rapid manual screening.

- Panorama Viewing
- Panoramic Move

The following experiments aid finding AOIs

- Tile and Stitch

The different methods that LA has of navigating in XY and finding and marking AOIs are:

- 2D Navigator Control
- the keyboard <SHIFT> key combined with the Mouse to re-center
- the keyboard <X> key combined with the Mouse to act as virtual joystick
- directly entering absolute/relative values in the Stage Control
- using the Image Markers to return to a previous marked AOI
- Space Navigator Joystick &/or Mouse based Panoramic Move
- Returning to XY Home Position

8.2.2.1 Panorama Viewing

From the Live Acquisition menu, selecting *Acquisition* > *Panorama Size* one is able to select between a multi-image viewing modes whereby 2x2 or 3x3 images can be displayed simultaneously on the Live Acquisition viewing panel.

In the execution panel is a checkbox which turns and turn off the panoramic viewing mode. When this mode is activated it allows a peripheral view of what lies next to the region where the stage is currently centered.

When the 2x2 mode is active the hardware moves in the following pattern displaying the neighboring sample areas and then returning back to the original stage position. This all happens with a single click on the '*Snapshot*' button. What are displayed in the Viewing Panel are a 2x2 Tile and Stitch Image of the surrounding areas.

When the 3x3 mode is active the hardware moves in the following pattern displaying the neighboring sample areas and then returning back to the original stage position. This all happens with a single click on the '*Snapshot* 'button. This all happens with a single click on the '*Snapshot*' button. What are displayed in the Viewing Panel are a 3x3 Tile and Stitch Image of the surrounding areas.

8.2.2.2 Panorama Movement (Full Screen and Half Screen)

Just as Panoramic Viewing aids in seeing what lies around the centre point of the stage the panoramic movement allows rapid movement to both quickly manually scan a sample as well as to prevent the sample from being overexposed by light. The two features are capable of being used together.

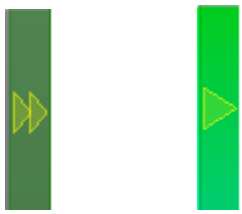


Figure 51: Panoramic Movement Borders

The fastest and easiest way to activate the panoramic motion is to use the Space Navigator Joystick. Moving the joystick left, right, up or down will trigger the full screen panoramic movement. This is indicated by the double arrow appearing on the border of the viewing panel as the stage moves a full screen in the desired direction.

It is also possible to use the mouse and keyboard in panoramic mode. Holding the <SHIFT> key down and moving the mouse cursor to one of the four edges of the Viewing Panel until the border changes. If the border is a single arrow then a half frame motion will be activated with a left mouse click. If the border is a double arrow then a full frame motion will be activated with a left mouse click.

8.2.2.3 Re-centering the XY Stage

A hidden control is also available to move the XY stage. By pressing and holding the <SHIFT> key on the keyboard with the mouse in the viewing window, the XY stage has a floating region displayed in the GUI. When the mouse left button is pushed the stage will re-center on this position.

As the XY position is changed the Global and Zoom Viewers and the XY Axis Control are updated accordingly.

8.2.2.4 Joystick

The LA Software supports almost any HID (Human Interface Device) compatible USB Joystick for controlling the xy-stage. Nowadays all available USB Joysticks are HID compliant.
Alternatively the Space Navigator Device can be used for XY movements.
Both, the HID USB Joysticks and the Space Navigator, can be easily configured between normal joystick mode and panoramic movement mode by a checkbox in the menu: *Navigation* > *Joystick Mode*.
A HID compatible joystick can be attached and used with LA without restarting LA. After a Joystick has been connected to the computer and Windows finished the installation of the joystick, the Joystick can be activated in LA by going to the menu *Config* > *Start HID Joystick.*

8.2.2.5 XY Stage Text Box Manual Motion

It is possible to enter the absolute/relative values into the Stage Control by using the edit fileds. A movement is carried out when the “return” or “enter” key on the keyboard is pressed.

8.2.2.6 XYZ Position Markers (User defined)

A Position Marker is similar to the XY Home but it allows multiple positions to be marked and then to select them from a drop down list. Selecting one of the list entries will return the focus to this position.

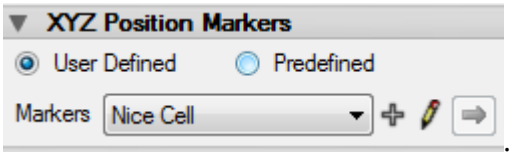


Figure 52: Position Marker

The Position Marker is similar in to how the Z Reference works. But it not only stores a position but all the parameters necessary to exactly recreate the image including camera and light settings.

Position Markers can be set by depressing the '+' key either thru the numlock or the normal keyboard. This allows markers to be set while performing manual screening and then to easily return to the interesting positions.
Position markers can also be added and removed by using the buttons right to the Position Marker list. The '+' button will add a user defined Position Marker to the list.

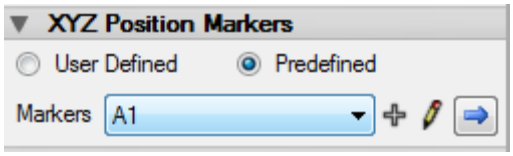


Figure 53: Position Marker Panel buttons

The edit icon, depicted by the "pen" icon, will open a dialog for managing the position markers. Available functions for managing position the markers and their properties include renaming, deleting and changing xyz-parameters (**Figure 54**).
Multiple markers can be selected by either holding down the "shift" or "Ctrl" modifier key. This allows deleting multiple markers at once. The behavior is the same as in the Windows Explorer.

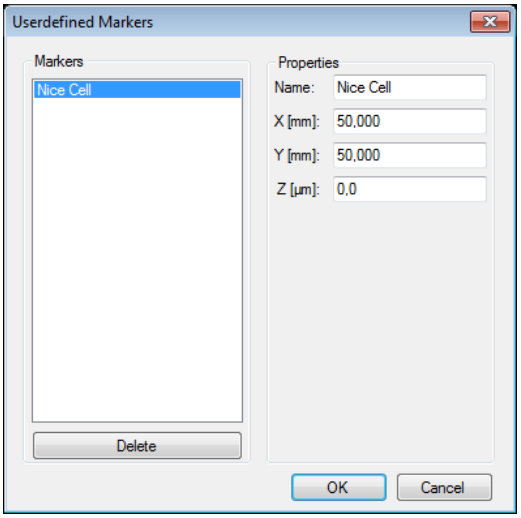


Figure 54: Dialog for editing xyz-markers and their properties.

8.2.2.7 XYZ Position Marker (Pre-defined)

The Position markers can be switched from User defined to pre-defined if a Well plate is configured in the navigator. In this mode the User is presented with a list of the center positions of the wells in the Well plate.

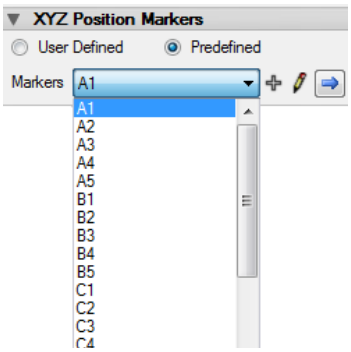


Figure 55: Pre-defined Position Marker list

In contrast to the user defined Position Markers the pre-defined markers do not have a z-position by default. They only have the x and y position of the well center. The current z-position is used if the Well is changed. Depending on magnification of the objective and the flatness of the well plate it might be necessary to adjust the focal position after the stage has moved to a new well. The user can not add markers to this list, they are given by the dimensions of the well plate. To adjust the z-positions the User can run the "Marker Focus Map" protocol. This protocol will try to find the best focus for each position, starting at the current z-position.

8.2.3 Navigator Controls

The navigator control is designed similar to a 'Heads Up Display' in a cockpit. It allows rapid navigation of a sample in a manner in a Random Walk format as well as providing a scratchpad of highlighting regions of interest (ROI) and points of interest (POI). The navigator is divided into two regions which can be hidden and resumed separately depending on your current needs.

- Global View
- Zoom View

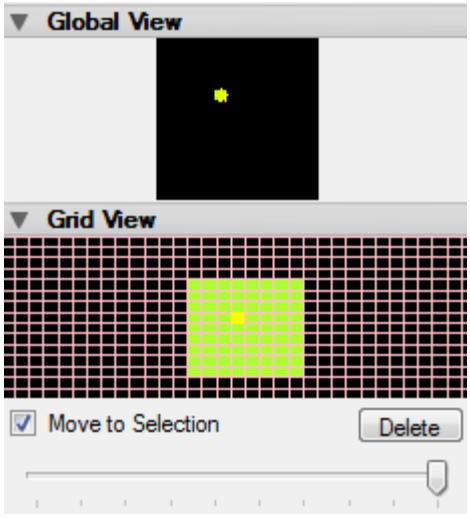


Figure 56 : XY and Navigator Control

There is a check box here to either allow or disallow hardware movement as you are selecting regions and points. When it is selected every new point will be moved and an immediate image will be made. This is very useful for rapid scanning. Otherwise it can be disabled to allow a more complicated pattern of regions and points to be developed to be later used in a scanning protocol.

Note that it is possible to select points in both the global view and the zoom viewer. But only points and regions will be displayed in the zoom view. Selecting points is simply a matter of left clicking in the control with the mouse. Dragging the mouse cursor over a region in the Grid View with the left button depressed will select a ROI. Single clicking into any grid position with the left mouse button will select a POI. Depressing the right mouse button on any selected point removes it from the list to be tiled. The yellow cursor highlights the current hardware position of the stage.

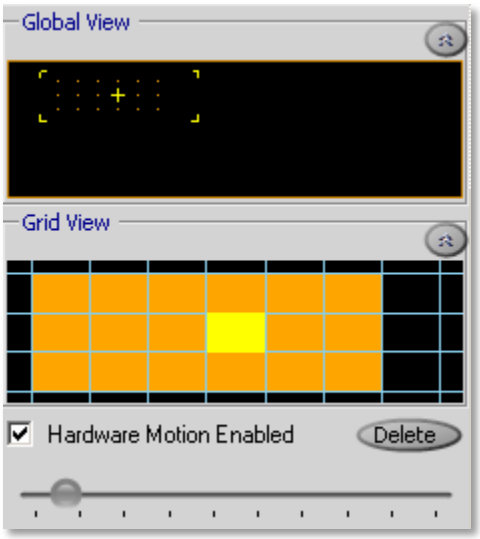


Figure 57: ROI Enabled Navigator

As the XY position is changed the Global and Zoom Viewers and the XY Axis Control are updated accordingly.

There is a configuration tool available under Navigator Menu that allows setup of the stage sample holder. It allows a rapid selection of slide, Petri, or well plate. As well as allowing calibration of the GUI and hardware so that the GUI remains in sync with actual hardware even after changing the sample dishes. In some configurations of samples it is necessary to reverse the viewing angle of the sample (top down vs. bottom up) or to reverse the motion of the X, Y axis for better data collection. This is also accomplished with this configuration tool. Please refer to the setup and installation manual for details.

8.2.3.1 Global View

The global view is at the top of the navigator window. It provides a virtual view of the entire stage, attached slides, attached Petri dish, or attached wells. With a left click in the navigator, the stage moves to center the point chosen under the objective. It is also possible to drag and drop a rectangle with the left mouse button to select a larger area. Any POIs and ROIs which have been selected are shown in this window in a scale corresponding to the stage size. The global view can be hidden / resumed using the arrow in the top right corner of the control. The current zoom view and location of the image currently under the objective is shown in the global view as a small set of corners marking the location of the smaller rectangle of the Zoom Viewer.

8.2.3.2 Zoom View

The zoom view is found immediately below the global view. It can be hidden / resumed using the arrow in the middle right corner of the control. This view shows the same view that the camera has as a background image and allows the user to select ROIs and POIs by using the right mouse button. Note that the zoom and what is in the camera CCD delivers is always in sync. A note here is that the pixels displayed in the zoom view directly correspond to the CCD images of the camera. Selected regions can also be erased by a second left click on the same location. A global erase button is also provided that erases all regions in both global and zoom windows. To select a POI simply move to the location on the grid and click. To select a ROI move to a grid, point, click, and drag and drop to the desired location of the opposing corner. A highlighted region will be drawn. A corresponding ROI / POI will be displayed in the Global View. The zoom view can also be used in a scrolling mode to move the stage. There are four arrows at the edges of the Zoom Viewer for this. The Global view is shifted as is the stage. And as the name states the Zoom view can be zoomed with the use of the mouse roll bar.

8.3 Primary Hardware Configuration Controls

Certain other hardware controls are used almost as often as the positioning controls and they are always available in the Primary Hardware Control Panel. Others are configured less often and are hidden from the main hardware control and appear as a docking window to the right of the primary control panel. The following is a short description of the primary controls for configuration.

8.3.1 Light Source

Changing the Wavelength, Intensity (optional) and Bandwidth (optional) of the Polychrome V from the Live Acquisition software is done in the Light Source control panel.

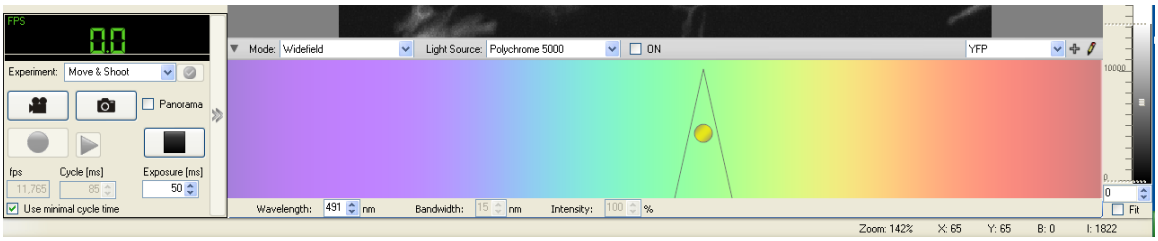


Figure 58: Light source control panel for the Polychrome V

The graphically control can be moved by a right mouse click and a drag. There are three yellow circles in the graphical spectrum slider.

- Clicking and dragging in a vertical fashion on the topmost one will change the intensity.
- Clicking and dragging in a horizontal manner on the middle one will change the wavelength.
- Clicking and dragging in a horizontal fashion on the lowermost one will change the bandwidth.

Intensity, wavelength, and bandwidth can also be edited directly with the roll bar.

8.3.2 FRET Mode

If the FRET module is configured then an additional control appears. From here there are two options. When using the FRET filters 2, 3, and 4 it is possible to mirror the images. It is also possible to overlay the two images for direct comparison.

8.4 Move and Shoot Mode

When first started, the default mode of operation for the Live Acquisition solution is a ‘Move and Shoot’ mode for manual scanning. In this mode there is little to nothing that must be configured and one can start investigating samples within minutes of beginning to use the Live Acquisition solution.

In ‘Move and Shoot’ mode samples can be recorded in either a still snapshot mode or in a live mode.

The default mode of the ‘Move and Shoot’ is a non-persistent picture. This means that even though a picture is taken at the end position of every move it is not immediately saved to the hard drive or sent to the Offline Analysis application. The current image can be sent to the Offline Analysis application or saved to the hard disk for later analysis by clicking on the ‘Save’ Button.

9 LA Protocol Editor

The LA Protocol Editor allows the user to create and manage complex workflows with ease. The user can build workflows with the pre-defined Protocols from the Live Acquisition and simple commands, like '*Snapshot*' and '*Trigger*'. The items in the workflow can be easily rearranged via drag and drop or with standard key shortcuts. The workflow is presented as a hierarchical tree view (Figure 60). Workflows generated with the Protocol Editor are optimized for the best timing before they are sent to the Imaging control unit where they are executed in real-time.

9.1 Exploring the interface

The Protocol Editor is started by selecting it from the *Experiment* button in Live Acquisition. The Protocol Editor is the first item at the bottom of the list, see Starting the Protocol Editor.

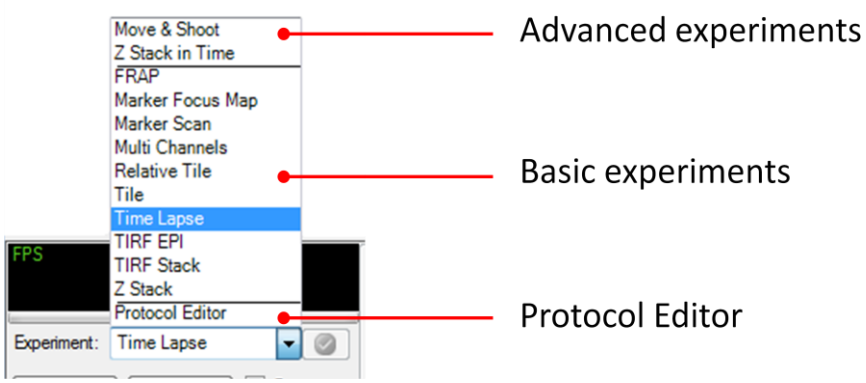


Figure 59: Starting the Protocol Editor from the Experiment list.

Selecting the Protocol Editor item will then open an additional window that floats above the other Live Acquisition interface elements. The first look at this window will present the interface elements of the Protocol Editor seen in Figure 60.

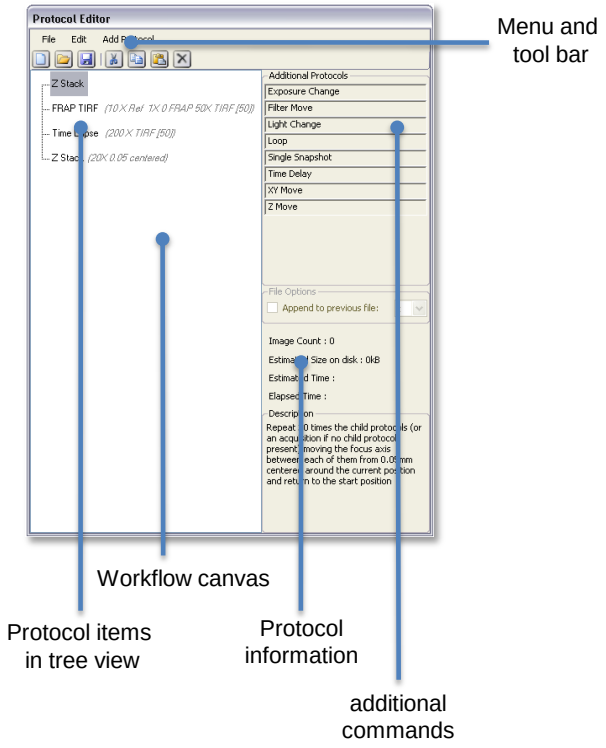
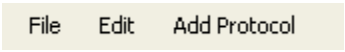


Figure 60: Protocol Editor Window

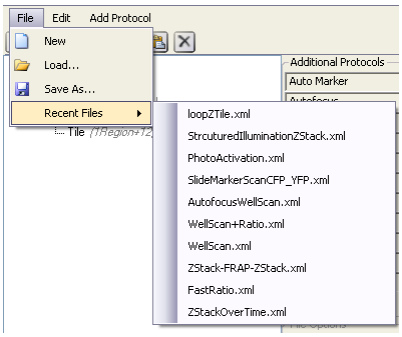
9.1.1 The menu bar

The menu bar consists of three items, *File*, *Edit* and *Add Protocol*.



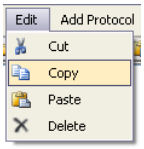
The *File* menu contains options to manage protocols, to load save, and create them. In the *File* menu the following options are available:

- *New* : Clears the current Protocol without saving!
- *Load*: Opens a dialog where the user can select a protocol to be loaded
- *Save as…*: Saves the current protocol to a specified location; a name can be given to the file
- *Recent files*: a list of recently used protocols, allows fast access to frequently used protocol files

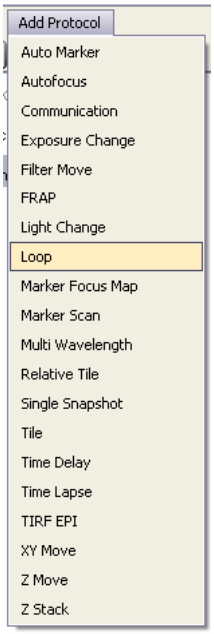


Functions for editing the protocols can be found in the *Edit* menu. The options in the *Edit* menu are:

- *Cut*: copies the current selected protocol item to the clipboard and deletes it from the protocol
- *Copy*: copies the current selected protocol item to the clipboard
- *Paste:* pastes the protocol item from the clipboard to a protocol
- *Delete*: deletes the currently selected item in a protocol

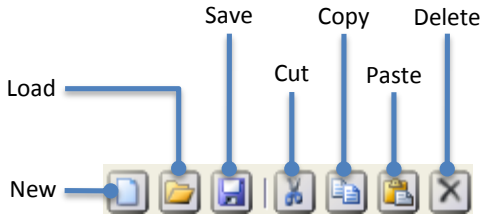


In the *Add Protocol* menu item all available protocol items for the configured hardware are listed. Selecting one of these items in the menu will add it to the protocol tree in the workflow canvas.



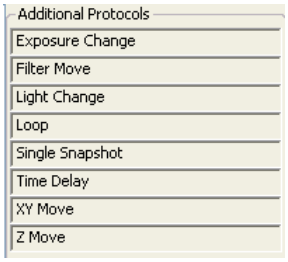
9.1.2 The Tool Bar

The tool bar contains buttons with icons that allow fast access to functions of the Protocol Editor, without going to the menus in the menu bar.



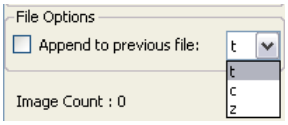
9.1.3 Additional Protocols panel

On the right side of the Protocol Editor Window the *Additional Protocols* are kept. These are so called Simple Commands, which allow a finer control in building a protocol if needed.
Depending on the hardware configuration some items are not accessible to all users. A list with a description of the Simple Commands functions can be found in chapter 16.
The Simple Commands can be dragged with the mouse to the Protocol Canvas and thereby added to the Protocol. Note: Dragging *of items to an empty Protocol Canvas is not allowed.*



9.1.4 File Options panel

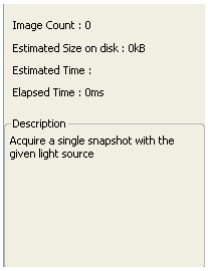
The *File Options* panel holds controls, which allow influencing the way how the acquired image data is stored in the files and if channels are stored in separate or in one file.



The options *Append to previous file* is only active if two or more items that acquire image data are following each other. These could be two *Snapshots* or two *Time Lapse* items. It is also possible that a *Snapshot* follows a *Time Lapse* item, but in this case both items need to have the same "dimensions"! A dimension is not only x,y and z but also the number of channels. In other words this means that a *Snapshot*, which is a single/one channel acquisition, cannot be appended to *Time Lapse* item with a two channel acquisition!

The second option in the *File Options* is the selection to what dimension the acquired image(s) will be added. Images can be appended in time – t -, as another channel – c – or in the z-direction – z- to a file.

9.1.5 Protocol Information



9.2 Protocols: Getting started

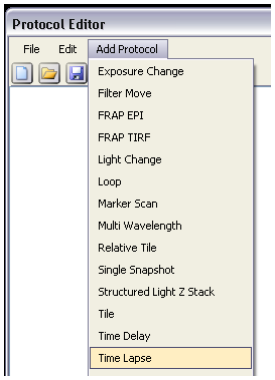
The first Protocol that will be built in this tutorial consists of two *Time Lapse* acquisitions and a *Trigger* item. The Time lapse items will be configured to acquire a series of 100 images at one wavelength. Between those two *Time Lapse* items the *Trigger* item is placed to trigger an external device like an electrical stimulator.

Required hardware:
- TILL Photonics imaging system
- Light source (Polychrome V or Oligochrome)
- Imaging Control Unit
- Camera

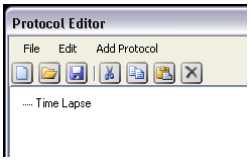
Protocol items described in this tutorial:
- Time Lapse
- Trigger

Recommended chapters:
4.1, 4.1.1, 4.1.

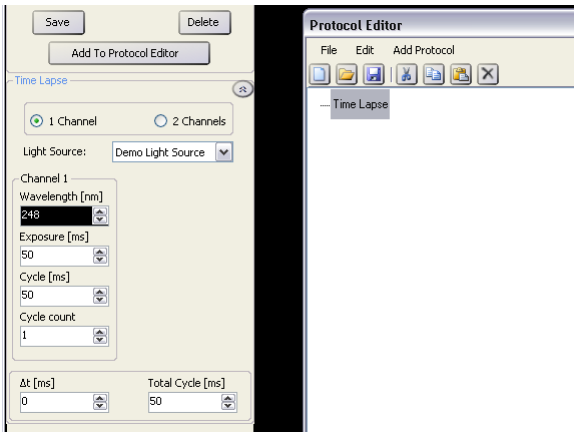
The first step is to add a protocol item to the workflow canvas in the Protocol Editor. This is done by clicking the *Add Protocol* menu in the Protocol Editor. This menu consists of a list with all available protocol items appropriate for the installed hardware.
To add the first *Time Lapse* item to the sample protocol select the *Time Lapse* item from the list.



This action will add a *Time Lapse* item to the Workflow canvas.



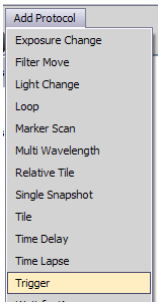
To edit the properties of this *Time Lapse* experiment, it needs to be activated by selecting it with a mouse-click. When the *Time Lapse* experiment in the Protocol Editor is selected, the panel with its settings is opened on the left side of the LA Acquisition window.



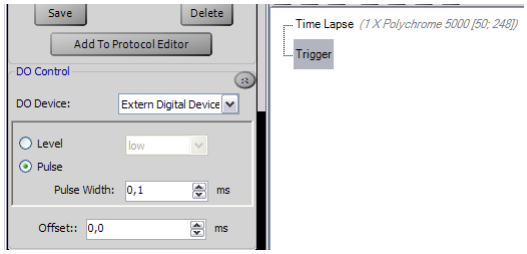
In this panel the properties of the *Time Lapse* experiment can be edited like in the pre-defined Experiments. The *Time Lapse* properties reflect the current setting from the *Live* and *Snapshot* settings; this includes the exposure time and light source settings like the wavelength.
This shows the tight integration of the LA Protocol Editor with Live Acquisition. Usually these settings where used before by the User in *Live* or *Snapshot* mode as he was searching for a good spot in the specimen to make an experiment.

However, if the User needs to make changes to properties, these changes are made immediately, there is no need to explicitly save them when switching between multiple protocol items or adding new ones to the workflow canvas.

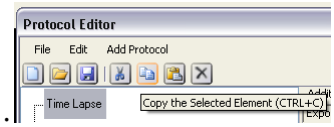
The next step is to add the *Trigger* item to the protocol by selecting it from the *Add Item* > *Trigger*.



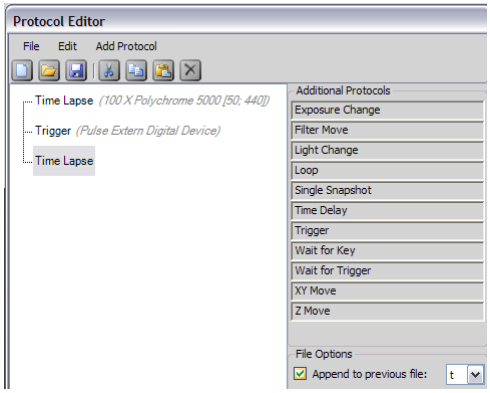
Selecting the *Trigger* item will show its properties in the experiment panel on the left side of the Live acquisition window labeled as *DO Control*.
Setting the property "Pulse" in the *Trigger* items will generate a Pulse of a given duration, here 0,1ms.



To add the second *Time Lapse*, two possible routes can be taken. One route is to add another *Time Lapse* item from the *Add Item* menu like in the first case.
The second route is to copy and paste the first *Time Lapse* item. To copy the *Time Lapse* item, it first needs to be selected by clicking on it. Then it can be copied either by using the "Copy"-icon in the tool bar of the Protocol Editor window or by using the Shortcut keystroke "ctrl-c". Pasting is done by either the "Paste"-Icon form the tool bar or by the "ctrl-v" key combination.

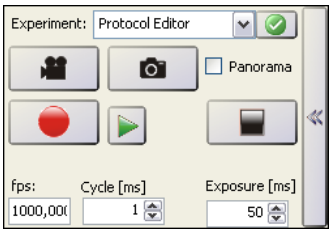


To add the *Time Lapse* item below the *Trigger* item we first have to select the *Trigger* item, or in general the item after which the new one should be added. This step is independent of whether the item is added via copy and paste or via the "Add Item" menu.
Adding/Pasting the *Time Lapse* into the protocol is done either by the paste icon or by the shortcut keystroke "ctrl-v".

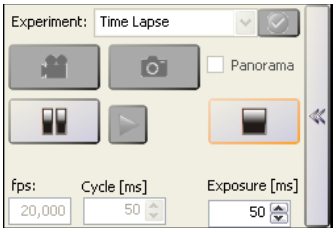


For later evaluation the images of both Time Lapse sequences should be combined in one file. To achieve this, the *Append to previous file* option in *File Options* has to be checked. Otherwise the two *Time Lapse* items will create a file for each own image sequence.
To save the protocol go to the *File > Save as…* . This will open a dialog asking you to give the protocol a file name under that you can reload it for later re-use.

To save the data press the *Acquire* button (the one with the red circle).
To run the protocol just built; press the *Play* button (the one with the green triangle) in LA Acquisition. Custom protocols built with the Protocol Editor are controlled in the same way that the pre-defined Protocols are.



While the experiment is running, the *Acquire* button changes to a *Pause* button. Clicking the *Pause* button will temporarily stop the protocol. To resume the protocol execution, press the button again.



To terminate the protocol execution early, press the *Stop* button. Pressing *Stop* does not discard any data already recorded.

Try this:

Feel free to try out the interface.

- Rearrange the Protocol by dragging and dropping items.
- Add a second Trigger item at the beginning of the Protocol
- Delete items by pressing the “Del” key
- Create a new Protocol by pressing the “New” icon; add all three items first before editing their properties

9.3 Protocols: Nesting items in a loop

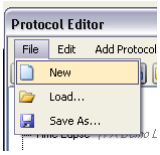
The protocol that will be created in this chapter shows the use of the *Loop* item and another Simple Command, the *Snapshot.* The nesting of items will be shown, by using the *Loop* and *Snapshot* items to create a time lapse experiment from Simple commands instead of using the pre-defined *Time Lapse* item.
The goal is to build a time lapse experiment with 100 repeats and to take two snapshots in this loop. One Snapshot should take a fluorescent image and the other snapshot a transmission image. Another goal is to introduce the usage of the *File Options* in the protocol editor, here the *Append to previous file*.

Required hardware:
- TILL Photonics imaging system
- Light source (Polychrome V or Oligochrome)
- Imaging Control Unit
- Camera

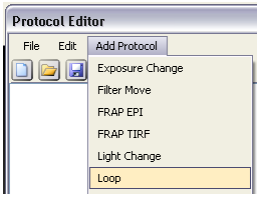
Protocol Editor Items described in this tutorial:
- Loop
- Snapshot
- Append to previous file

Recommended chapters:
- 4.1, 4.1.1, 4.1.

Open the Protocol Editor and if another is still there, save it and then press and then press the *New* icon in the tool bar or select by going to the menu *File > New* in the Protocol Editor window.

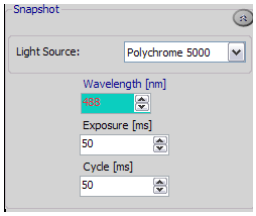


The next step is to add a *Loop* item to the protocol. This is done by the going to the menu *Add Protocol > Loop*.

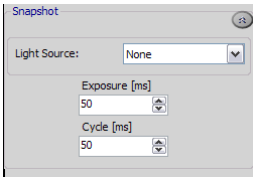


Select the *Loop* item and go to the property panel and change the *Loop Count* to 100.

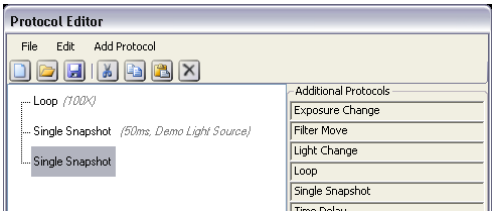
A *Snapshot* item is added by *Add Protocol* > *Snapshot*. This *Snapshot* item should be configured to take a fluorescent image. Therefore select the first *Snapshot* item in the Workflow canvas and change the *Light Source* in the Snapshot panel to Polychrome V or Oligochrome depending on your setup. If the User was using the Polychrome light source before in Live- or Snapshot-mode, usually no change to the parameters in necessary.



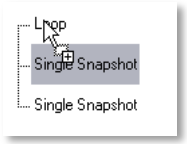
Now the second *Snapshot* item should be added and configured to be a transmission. The change is needed here as this item also defaults to the *Live*- and *Snapshot*-mode settings. Changing to transmission is done by setting the *Light Source* to *None*. It might be necessary to adjust the exposure setting.



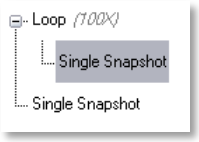
The protocol now has all three items sitting in line to each other. If the protocol would now be run, only two images would be taken as the two *Snapshots* are not nested in the *Loop* item. The dotted line to the left of the protocol items shows that all three items are on the same level.



To get the two Snapshots executed within the *Loop* item, they have to be dragged onto the *Loop* item one after another. If a protocol items is dragged over another protocol item, the mouse cursor will change and display an additional plus right below the arrow of the mouse pointer.



If the mouse button is now released, the dragged *Snapshot* item will become a "child" of the *Loop* item.

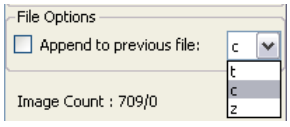


Hint: If a protocol item has child/nested items, it will display a "plus" or "minus" in a small box left to it. By clicking onto this "plus" or "minus" the child/nested items will be shown or hidden. This behavior is consistent with that from the Windows Explorer when navigating the Folders.

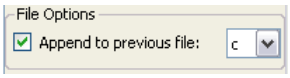
The next step is to move the other *Snapshot* to the *Loop* item, too. This is done by dragging it to over the *Loop* item as previously described.

If the protocol is now run, we will get two files, one containing the images with the fluorescent excitation and the other containing the transmission images only. This is independent of the streaming option currently in use, Offline Analysis application, TIFF or ImageJ.
If the Offline Analysis or ImageJ is used, two windows will be opened, each containing a complete sequence of 100 images, one the fluorescent images and the other the transmission images.

If the User wants to combine both image types (fluorescent and transmission) in **one** file, the second Snapshot has to be selected in the protocol. This will enable the *File Options* on the right side in the Protocol Editor window.



In this example the fluorescent image should go into channel 1 and the transmission image into channel 2 of the same file. To do this, the option *Append to previous file* needs to be checked and from the drop down list the "***c***" – for **channel** – has to be selected.



Hint: The other options in this drop down list are "t" – append images in **time** – and "z" – for appending images in **z-direction.** See chapter **9.1.4**

Now the protocol will create one file containing both sequences. If the Offline Analysis application is used switching between the two channels can be done easily. For further details please refer to the Offline Analysis chapter 6.
If the image data of the protocol is streamed to TIFF files or the ImageJ TIFF streamer, the images will be "multiplexed" in the TIFF-file. This means they are stored in an alternating sequence: Channel1, Channel2, Channel1,….

Try this:
Feel free to try out the interface.

- Re-arrange the two Snapshots, to first take the transmission and then the fluorescent image
- Test what happens if "z" or "t" is selected in the *File options*
- Add a snapshot with transmission before and after the loop
- Add snapshot fluorescence

9.4 Multi-channel Z-Stack

The goal of this chapter is to demonstrate how to build a z-stack with two channels over time. This is sometimes referred as a 4D experiments.
This tutorial also shows how to replace the standard acquisition, a snapshot, of a protocol item, here the *z-stack*.

Required hardware:
- iMIC series microscope
- Light source (Polychrome V or Oligochrome)
- Camera

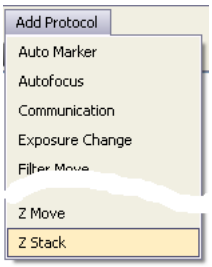
Protocol items described in this chapter:
- Loop
- Z-Stack
- Time Lapse
- Nesting of items

Recommended chapters:
- 4.1, 4.1.1, 4.1.

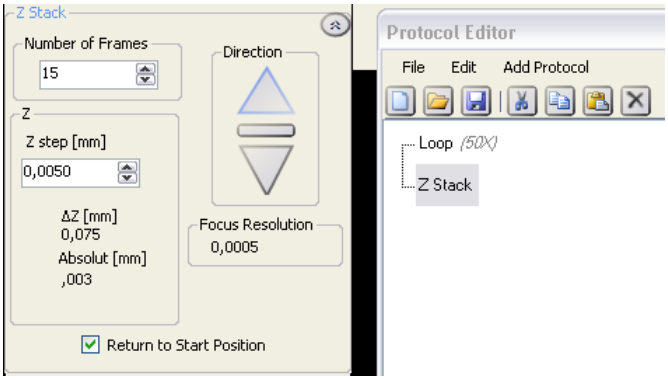
The first item we add to the empty working canvas is the *Loop* item; *Add Protocol > Loop*

Select the *Loop* item and change the counter of the loop to 50.

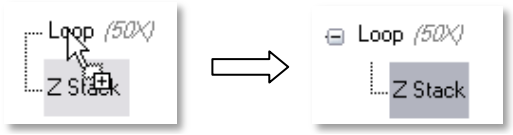
Now we add a *Z-Stack* item to the protocol; *Add Protocol* > *Z Stack*



To edit the *Z-Stack* properties, select the *Z-Stack* item in the Protocol Editor and change the properties to appropriate values. For this example a *Z-Stack* with 15 frames, a *Z-step* size of 5 µm is chosen and the Direction of acquiring is upwards from its current position (select the up pointing triangle).



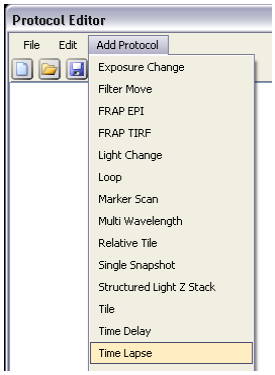
To repeat the *Z-Stack* 50 times, as we have it specified in the *Loop* item, it has to become a child of the Loop item. This is done by dragging the *Z-Stack* item onto the *Loop* item. The mouse cursor will then change a seen in the following picture.



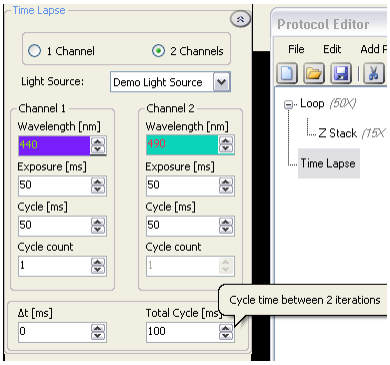
If the protocol would be run now, fifty *Z-Stacks* each containing 15 frames or z-planes would be acquired with the current camera and light source settings.
The standard behavior of items like the *Z-Stack* or *(Relative) Tile* and *Marker Scan* is to use the current settings from the LA Acquisition, which were used in *Live* or *Snapshot* mode.

Important! The goal of this tutorial is to acquire a two channel z-stack over time, so the standard behavior of the z-stack, a single Snapshot, needs to be replaced.

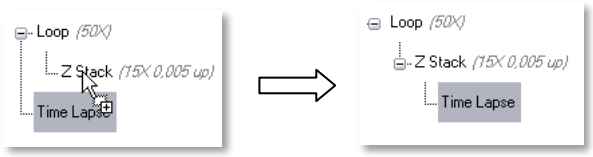
The standard Snapshot feature of the *Z-Stack* will be replaced by adding a *Time Lapse* item. However, the *Time Lapse* item has to added to the protocol in a first step; *Add Protocol* > *Time Lapse*



The next step is to activate the *Time Lapse* item in the Protocol Editor and set its properties to be a two channel experiment with for example a 440nm excitation on channel 1 and 490nm on channel 2.
The *Cycle count* property in the *Time Lapse* item is left at 1 (one) as only one pair of images should be taken in one plane of the z-stack before going to the next plane. The Time Lapse now basically behaves like two Snapshots. The Advantage of using the Time Lapse item instead of two Single Snapshots is that the User doesn't have to bother about the *File Options* settings. Both channels will be stored in one file.



Next up is the placement of the *Time Lapse* item as a child of the *Z-Stack* by dragging it onto the *Z-Stack* item.



This was the final step to build a two channel *Z-Stack* that is acquired over time. Now the protocol can be started by pressing the *Play* button in LA Acquisition. To save the acquired data, the user has to press the record button. If the user wants to test the protocol before acquiring data the play button can be use.

Try this:

- Replace the *Time Lapse* item with two *Single Snapshots*
- Replace the *Time Lapse* with a *Multi channel* item and acquire three channels
- Replace the *Time Lapse* with a *Relative Tile* item and acquire a 3x3 tiles per plane

9.5 Advanced Protocols: Multi position (Wellplate) scan

The goal of this section is to record an image at the center of certain wells of a well plate, with two wavelength. Additionally the acquisition for each well is repeated 10 times.
This will demonstrate how to replace the standard snapshot functionality of the *Marker Scan* protocol item with a two wavelength acquisition.

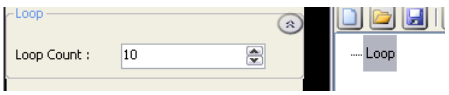
Required hardware:
- iMIC series microscope with a Prior Stage
- Light source (Polychrome V or Oligochrome)
- Camera

Protocol items described in this tutorial:
- Marker Scan
- Loop
- Time Lapse

Recommended chapters:
- 4.1, 4.1.1, 4.1.

If necessary save any previous protocol in the Protocol Editor and then clear it by using the *New* command either from the File-menu, *File* > *New*, or by using the *New* icon in the Toolbar.

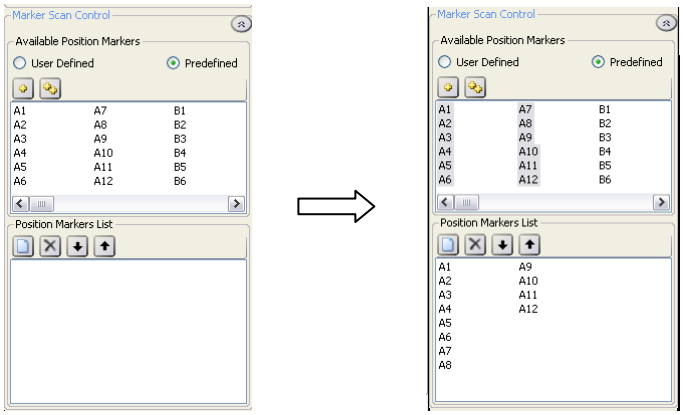
The first item to add to the Protocol is the *Loop* (*Add Item* > *Loop*) and we set its *Loop count* property to 10.



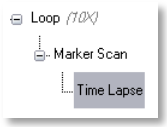
Now the *Marker Scan* item should be added to the protocol canvas (*Add Item* > *Marker Scan)* and then dragged onto the *Loop* item to make it a child of the *Loop.*

The next step is to edit the list of wells that should be scanned by the *Marker Scan* item.
Therefore the *Marker Scan* item needs to be selected. Make sure that the *Predefined* option is selected on top of the panel. If this option is selected a list of all available well center positions (A1, A2, …C11, C12…) is shown in the upper list view of the panel.
In this example only the row A should be selected, in case of a 96 well plate this would be A1 to A12. To do this in the *Marker Scan* Protocol properties the first item, A1 needs to be selected.

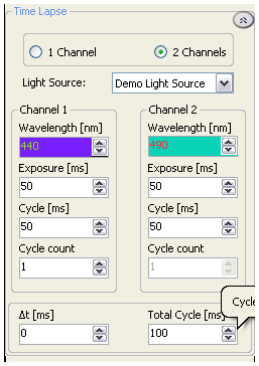
Now hold down the *Shift*-key and click on A12 in the list. Now the *Shift*-key can be released. To add the selected wells from the *Available Position Markers* list to the *Position Marker List* the Plus-icon needs to be clicked.



Now that the desired wells are added to the list of positions that will be scanned in the protocol, the standard Snapshot functionality of the *Marker Scan* item will be replaced with a two wavelength *Time Lapse* item, that has only one repetition . To achieve this, a *Time Lapse* item needs to be added (*Add Item* > *Time Lapse*) and dragged onto the *Marker Scan* item to make it a child of it.



Now the properties of the *Time Lapse* item needs to be set. The screen shot shows an example configuration with two excitations wavelength (440 and 490 nm). For this tutorial the *Cycle count* should be left at the value of one.



Leaving the *Cycle count* value at one, will take two images at two wavelengths (440/490nm). The protocol then moves on to the next well in the *Position Marker List*.

If this Protocol is now run, a total of 12 sequences each with two channels are generated. The two images of each *Time Lapse* will be appended to the previous one.

Try this:

- Take a *Time Lapse* of 100 frames at each well, therefore remove the *Loop* item. **Hint:** move the child items of the *Loop* out of it before removing the *Loop* item.

10 Stage calibration for sample holders

This chapter describes how stage calibrations for a variety of sample holders, like slides, dishes and well plates can be create and saved easily with the Calibration Wizard. The described process is the same for all supported stages available on the iMIC and uiMIC, making it easy and consistent across all devices.

Required hardware:
iMIC series microscope with any of the available stages or a uiMIC microscope
Transmission light source or any epi light source
A CCD Camera
Recommended chapters:
3.2.1, 3.2.2, 3.2.4

To start a calibration, open the Calibration Wizard from *Navigation* > *Navigator Calibration Wizard*

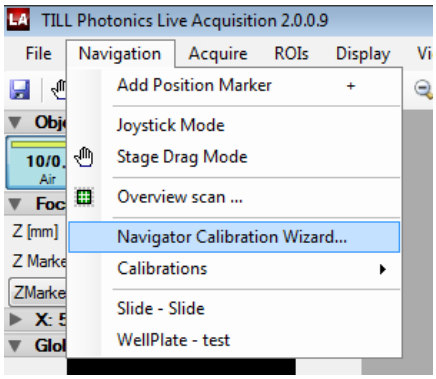


Figure 61: Open the Navigator Calibration Wizard

The Calibration Wizard opens and will ask to select the basic sample holder type that will be calibrated. Choices are Slide for rectangular sample holders, Petri dish for round sample holder and Wellplate for sample holder with multiple wells.

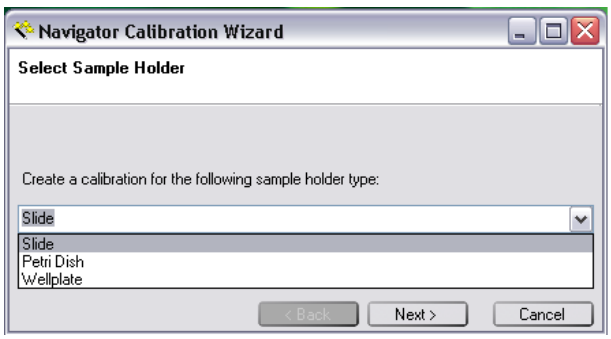


Figure 62: Selecting the basic sample holder type for a calibration process

After selecting one sample holder type, a warning will be shown before starting the calibration process (Figure 63). The area of the stage should be cleared, as during the calibration process the stage might travel its full range. During the calibration the user is able to use the whole, unrestricted travel range of the stage!

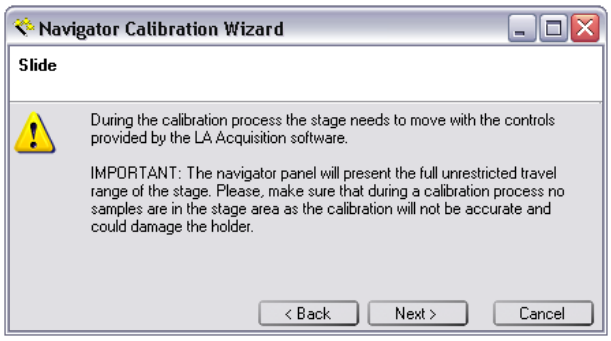


Figure 63: Warning before starting a calibration

If Slide of Petri Dish was chosen as a sample holder type the calibration starts by pressing the "Next" button. The user is then asked to move to different calibration points.
Moving the stage can be done by using all navigation features available in the Live Acquisition software.

- Move and Shoot
- Software Joystick
- Clicking into Global- and Grid view
- USB-Joystick
- Direct input of x- and y-coordinates using the *Stage* panel
- If Wellplate was the selected sample holder type, an additional step is needed before the calibrations start. In this step the user selects the shape of the wells (Figure 64). Depending on the selected well shape the appropriate calibration process is started by pressing "Next".

Figure 64: Selecting the appropriate well shape

Navigate to the calibration points by using the above mentioned navigation tools in LA Acquisition. Press the “Next” button when the calibration point is under the red cross-hair (Figure 65), which is displayed in the LA image view area during the calibration process.

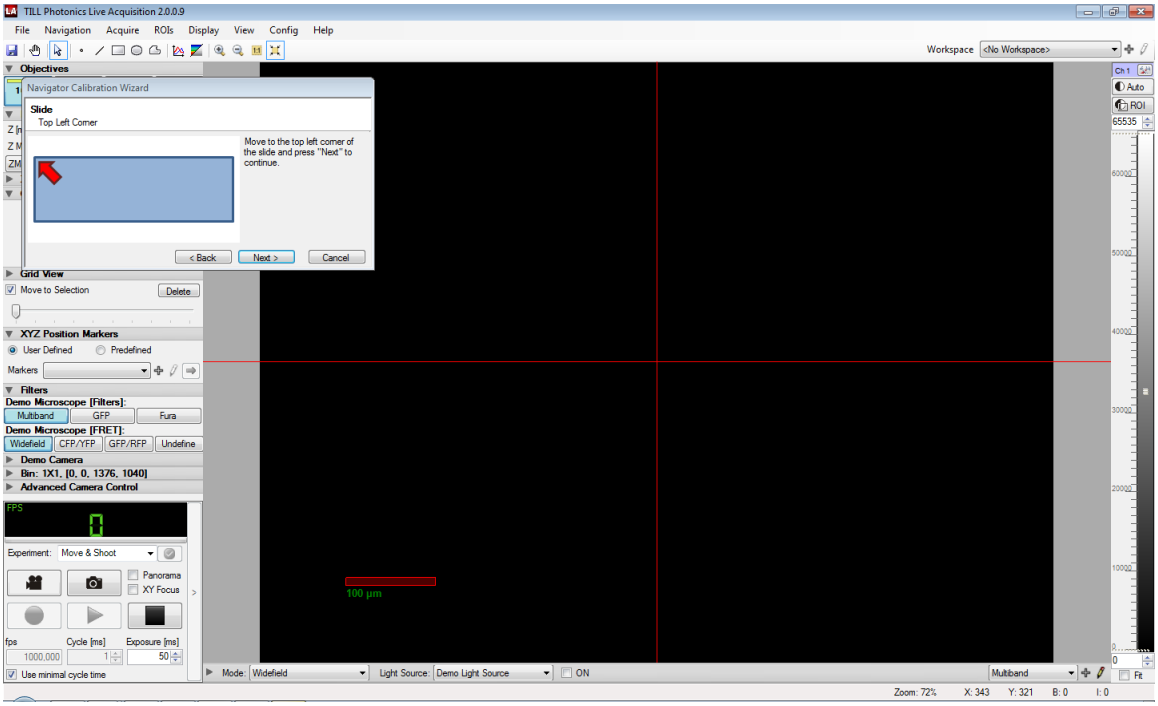


Figure 65: LA main screen with Navigator calibration wizard open

After setting all required calibration points the Calibration Wizard asks for a name under which the calibration will be saved.

Figure 66: Saving a navigator calibration

The saved calibrations can be selected from the *Navigation* > *Calibrations* menu. Thre recently used five calibrations are directly listed in the *Navigation* menu.

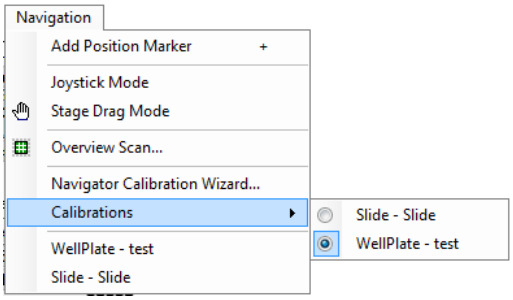


Figure 67: Recall a saved navigator calibration

11 Objective Alignment (xyz Translation)

This chapter describes how the parfocality of the objectives can be set in the Live Acquisition software. This chapter describes the steps necessary to calibrate the objectives in the microscope system. As a result all objectives will "see" the center same area of the sample.
The objective alignment feature in the Live Acquisition software will correct the x, y and z-translation of all installed objectives in the system.
Starting an objective alignment is done from the Objective Alignment dialog. This dialog is opened from the menu *Config > iMIC and Imaging Control Unit > Objectives Alignment.*

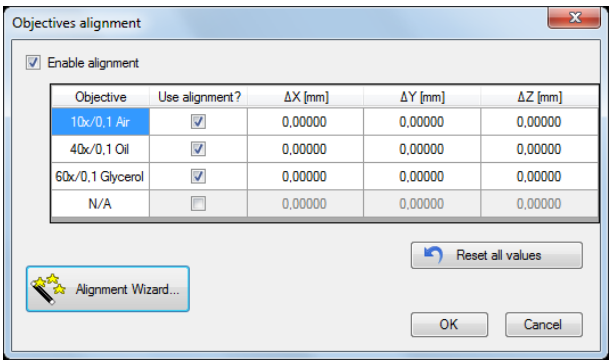


Figure 68: Objective Alignment dialog

From this dialog the Alignment Wizard can be started which guides the user through the calibration process step by step.

Hint: If there are objectives which need an immersion medium like Oil or Water, this has to be added onto the objectives in advance (before starting the Alignment Wizard)!

The Alignment can be enabled or disabled globally or for each objective individually in the list. Manual modification of the alignment parameters is also possible in the list but is usually not necessary.
When starting the alignment Wizard a red cross-hair is shown as overlay over the camera image. To adjust the objectives alignment you should chose a relive simple structure that is easy to find and has a high contrast.
We recommend using a micrometer calibration slide and to use one of the corners of a number on the scale as structure.

For the calibration you have to move the same structure under the cross-hair for each objective. You can use all navigation features available in the Live Acquisition software like, the USB or Software Joystick, the Click-to-Center function or the cursor keys on the keyboard.
Once all objectives have been aligned the Wizard will return to the dialog where it was started from.

12 FRAP calibration

The FRAP calibration in LA 2.0 and later has been reworked to be wizard based and therefore guides the user through the calibration process. The calibration requires setting three calibration points.
The new FRAP calibration also allows to manage more than one calibration which is needed on systems where more than one objective and/or filter is used for FRAP experiments. The FRAP calibration manager, where FRAP calibrations can be created and managed is located in the *Config > Polytrope I / II and Yanus> FRAP Calibration* or in *Config > Yanus > FRAP Calibration.*

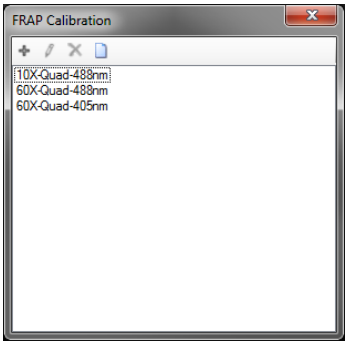


Figure 69: FRAP Calibration management dialog

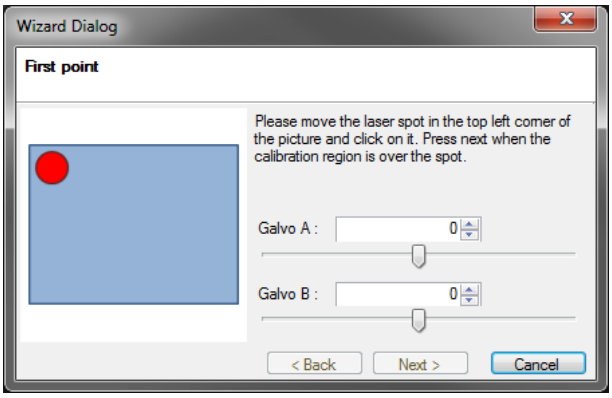


Figure 70: Calibrating FRAP in LA

This control is very similar to the FRAP Hardware configuration control in that it has two sliders. To use this tool one must position the FRAP spot in three separate locations in the sample (top left corner, middle right side, and middle bottom side) to establish a FRAP calibration.

Starting at the upper corner select a FRAP region and turn the laser on and then off. Adjust the sliders where the top slider represents X Axis calibration and the bottom slider represents the Y

Axis calibration and repeat the FRAP process by turning the laser on and off. Repeat this process until the FRAP region and the fluorescence depleted region overlap.

Move to the middle left position and repeat the procedure until the FRAP region and the fluorescence depleted region overlap.

Move to the bottom center position and repeat.

To complete the FRAP calibration set three FRAP regions at each of the three previous positions and turn the laser on and off one time. Fine tune the FRAP regions with the sliders until all three regions are correctly located with the fluorescence depletions.

Check the calibrated box and save experiment to save the FRAP SW calibration. In future experiments as long as the check box is still active the same calibration values will be used.

This control builds on the primary calibration of the HW. If after following this process a calibrated FRAP is not able to be achieved then it will be necessary to return to the hardware calibration and repeat both the HW and SW calibration process.

13 External device controls

This chapter describes how to control external devices and setup custom GUI controls in LA.
It allows for example to set multiple external devices like TTL, analog outputs and serial devices with one button click. Besides the controls management, there's also a direct control of the configured external devices.
To use this control, at least one TTL-Out, Trigger-In, Analog Out or RS232 has to be configured in the *Device Manager*. Of course any configuration combination of these devices is possible.

13.1 Direct control of external devices

With the Direct control it is possible to change the state of the external devices on the ICU. The controls are grouped into tabs (*Digital*, *Analog* and *RS232*). The changes made to a device state take immediate effect.

13.2 Configure custom User Interface elements for controlling devices from LA

The LA software allows the user to create custom interface elements for controlling external devices. The control will show up in a separate panel on the left side of the LA main window below the camera control panel.
The process of defining the user interface (UI) elements is started by selecting the menu *Config* > *External Devices* > *User Controls Manager ()*.Pressing the *'New'* – button will start the Wizard for creating new UI controls for external devices.

14 Configuring LA for the first time

Usually the imaging and iMIC systems form TILL Photonics come pre-configured and the user can start right away after the system has been installed by a TILL Photonics technician. However, sometimes a system is setup from the scratch and the user(s) will have to start over again.

This chapter describes briefly how-to setup a device configuration and how-to import an old configurations. This chapter goes through the start up process step by step on a fresh installed computer. If LA is re-installed on a computer some of the steps might not be necessary.

14.1 The Configuration Import dialog

When LA is started for the first time and no configuration file is found it will show a dialog where the user can directly import configuration files from a directory. After selecting a directory LA will import all configuration files. If a user wants to select only a few hardware configurations from all available configurations in the config files, he has to skip the import at this point and do this in the Device Configuration dialog.

14.2 The Welcome Dialog

After the configuration Import dialog LA will open the Welcome Screen. This is usually the first dialog a user will see if LA has configurations file in place. A description of all options of the Welcome Dialog can be found in chapter 2.1.

For a single user system only a valid hardware configuration needs to be created. If no configuration file was imported before LA will display a message “Missing device configuration. Please press edit to create a device configuration” right below the configuration list control. The

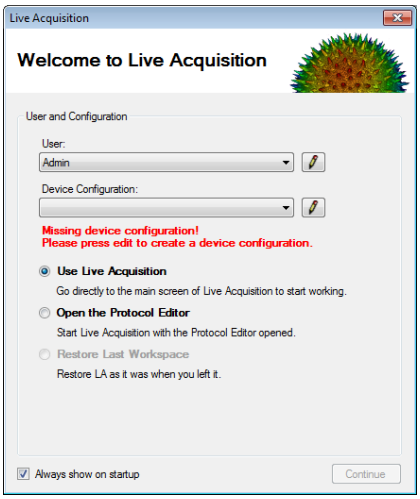


Figure 71: Welcome Dialog at first start of LA without any configuration

14.3 The Device Configuration dialog

After the Welcome screen, the first step is to go to the hardware configuration dialog where the devices have to be connected. If no configuration is present LA will ask on the first start to import an older configuration. However, if no older configuration is available the user has to cancel the import dialog and continue to the Welcome screen. In the Welcome screen the user will find a message printed in red that no configuration is found and that he needs to create one first before he can continue. The Device Configuration dialog is opened by clicking on the Edit-Button to the right of the (still empty) configuration list control.

The hardware configuration supports a drag and drop interface to add new hardware to the hardware desktop. Simply click on a desired hardware item from the list on the left and drag it over to the desktop white space on the right. Note that double clicking is not sufficient to add the hardware.

If this is a new system then notice that the configurations already reflect the factory default settings. The only thing that is required at this point is to simply ensure that the cabling is correctly connected.

If however this is a new system or a system update it will either be necessary to restore to factory defaults via the CD provided or to manually reconfigure the hardware configurations with the use of the configuration utility.

Most devices require additional configurations. As every device is different this is achieved in the hardware configuration by right clicking on and selecting the hardware component in question once it has been dragged onto the configuration desktop. Control windows capable of uniquely configuring the chosen hardware will pop up. This is where the “Sample Hardware Connectivity Diagram” comes into play. It is a generic representation of a very extensive hardware configuration on how to connect the various cables of the system. By simply following the diagram, one can quickly and easily connect the system hardware components together. The hardware configuration tool is the software representation of these physical connections. It allows the software to know which COM ports, which triggers, which digital/analog signals have been connected to which devices. This configuration tool should match the physical connectivity of the system for the software to function correctly.

There is also an important pull down box at the bottom of this screen which requests the default method for saving and transmitting stored images. This sets the default path for the transmission of acquired images. When set to the Offline Analysis Streamer the captured images will be sent to the Offline Analysis application for archival. When set to TIFF all images will be stored as OME compliant metadata TIFF images in the default data folder of the LA installation.

The following flowchart shows the hardware configuration process.

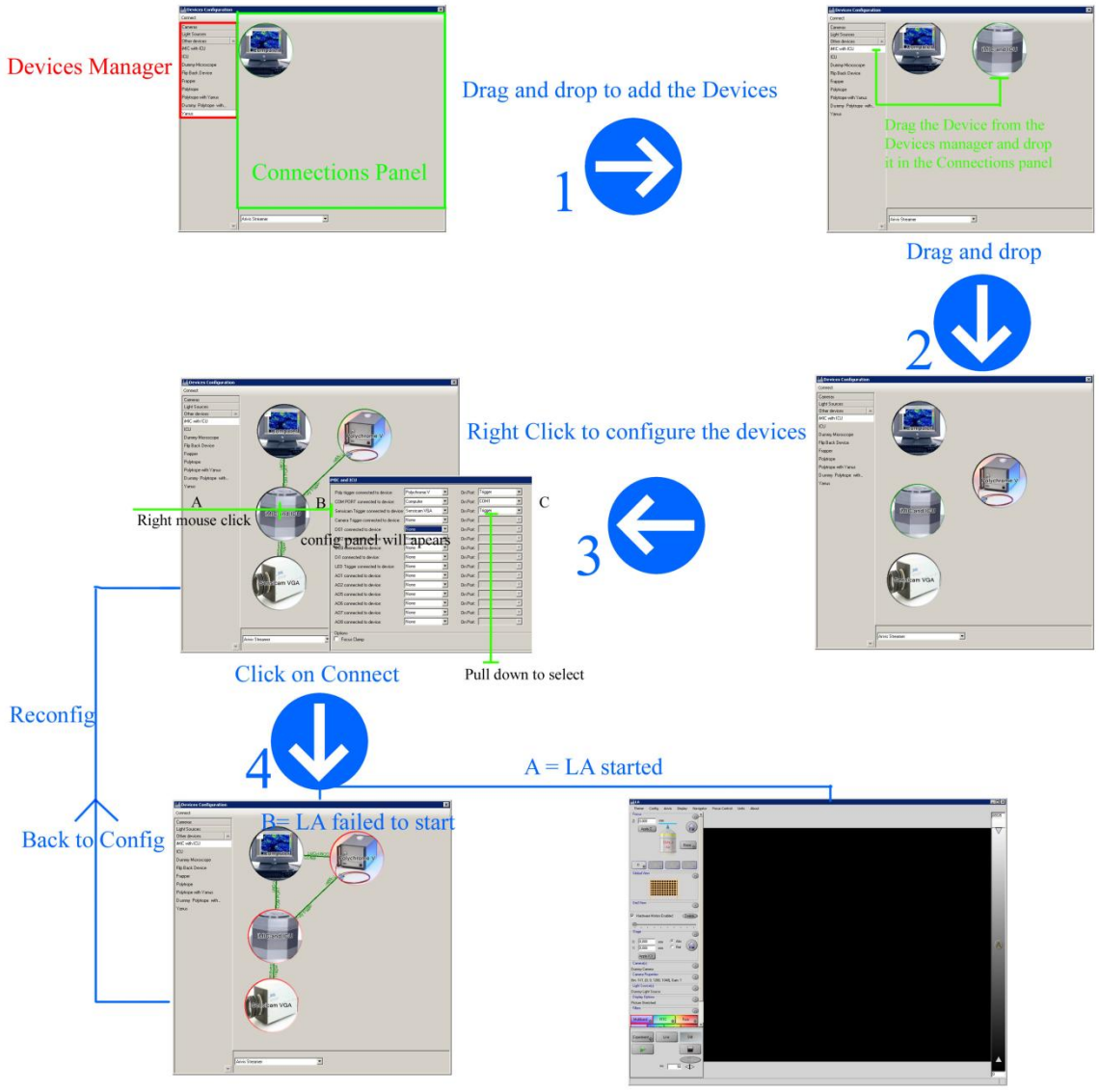


Figure 72: Hardware Configuration Workflow

Once all of the hardware has been selected and placed on the hardware desktop and checked for secondary configuration options, it is time to begin using the Live Acquisition solution. Simply click on connect and the LA system is started and configured for you based upon your hardware selection. This means that when the program executes it only has the controls, menus, and parameters of the associated hardware making it easier for you to control the devices without having to negotiate complex and lengthy menus for hardware and options that are not present on the system.

If after connection one of the selected devices is highlighted in a red circle it means that the system could not connect the configured hardware devices and the highlighted element needs to have its configuration proofed.

Once the system has been configured it is no longer necessary to reconfigure it with every new start. The last known system configuration will be used at startup. This allows the possibility to change configurations if the hardware has been changed but in most cases simply click on connect to begin using the software.

If you are updating a previously installed LA version the older configuration settings will be saved and will not need to be reconfigured.

15 User management

The Live Acquisition software features a sophisticated user management independent of the host operating system (e.g. Windows). This allows labs/facilities/departments to manage multiple users on one setup without requiring the IT department to set-up User accounts on the operating system level.
To manage the list of users the button with the pen icon left to the user list control has to be pressed. If LA is started for the first time and no configuration is available or was imported, a new configuration has to be created to continue.

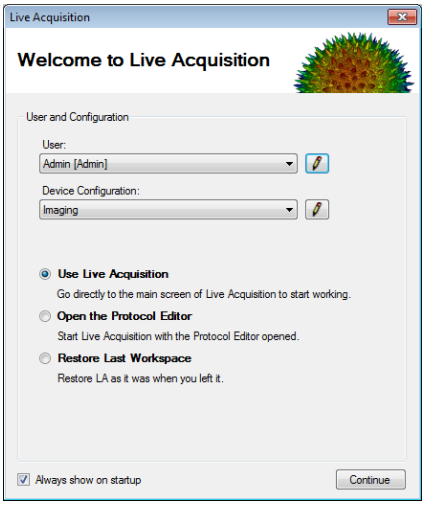


Figure 73: Welcome Dialog with user management

The *Welcome* dialog differs between Live Acquisition (LA) and Offline Analysis (OA) as in the latter one no hardware configuration controls are needed (Figure **74**). If LA and OA are used on one computer, all user accounts are shared between the both programs. This means that if a new user is created in on of the applications it is automatically available in the other one, too.

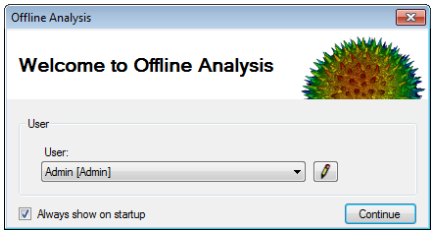


Figure 74: Welcome dialog of Offline Analysis

15.1 Add, rename and delete users

Adding user is done by clicking on the “Edit” button to the right of the user list control. This opens the “User Management” dialog.

To add a new user to the list of users, simply press the “New …” button . LA with then ask for a user name.
Once a user is added, additional properties can be edited by selecting the user in the list.
This dialog allows adding additional metadata about the user like first and last name as well as the email address and Institution. These additional data will be added to the OME (TIFF and XML) image files created by the Live Acquisition software.

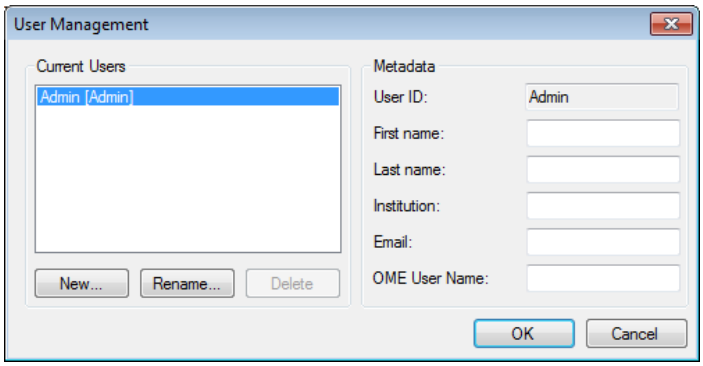


Figure 75: User management dialog

By default a new user will inherit all hardware configurations of the Admin(-istrator).
To rename or delete users, they have to be selected and can then be deleted or renamed by pressing the buttons.

16 Experiment references

16.1 Time Lapse

The Time Lapse Experiment and the 2 Wavelength Experiment are closely related. For this reason the two experiments are able to switch between each other as well as being launched from the same Experiment control.

The Experiment Window list has the following four controls added:

- Mode of Operation Button: Time Lapse or 2 Wavelength
- Light Source
- Wavelength
- Exposure
- Cycle(s)
- Cycle Count
- Delta Time
- Total Cycle(s)

Here one can independently set the wavelength, exposure time, cycle, and cycle count for the two separate wavelengths. The wavelengths will be switched and images acquired one directly after the other as fast as the hardware can switch the image exposure time/ cycle for each wavelength and read out the image from the camera. When the cycle count triggers a new pair of images the process is repeated. The independent cycle count of the second wavelength is at the moment not a feature that can be used. It is prepared for future expansion so that the two can be separated and set at different wavelength harmonics.

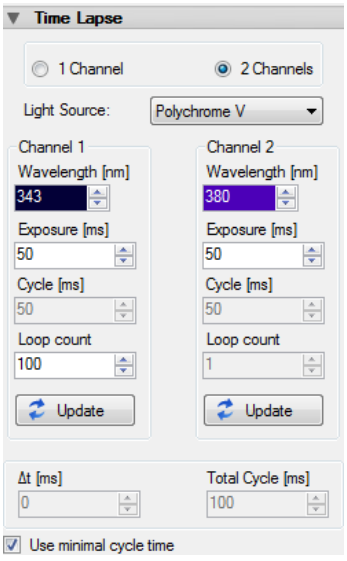


Figure 76 : Time Lapse / 2 Wavelength Experiment Controls

Switching between Time Lapse and 2 Wavelength is achieved with the button at the top of the Experiment Window. The Time Lapse controls are similar to those of the 2 Wavelength experiment but with only a single wavelength. The only difference between experiments is that in a 2 Wavelength mode 2 images at 2 different wavelengths are taken every time the experiment repeats instead of a single image which is the case with the Time Lapse mode.

16.2 Multi Wavelength

The Multi Wavelength Experiment window list has the following four controls added:

- Light Source
- Number of Wavelengths Selections
- Wavelength
- Exposure
- Cycle
- Filter Selection
- Cycle Count
- Delta Time
- Total Cycle(s)

The Multi-wavelength experiment is a more advanced version of the 2 Wavelength experiment but operates under the same principles. The major difference is that each of the wavelength selections is associated with:

  - Light Source
  - Wavelength
  - Exposure
  - Cycle
  - Filter Selection

This means that each of the wavelength selections needs to be configured at system start. At the execution time of the selected wavelength the filter will be changed as needed and the other parameters set.

Each selection will receive its own configuration information and is not dependent on the settings of the others. Simply click on one of the selection buttons and configure the rest of the fields. The information will be saved with that wavelength selection. Clicking on another selection button will allow one to configure the remaining selections accordingly. Clicking on a previously configured wavelength selection shows the values it has been configured to. Note that this also allows unique combinations of light sources.

If filter changes are needed then this experiment is not perfectly suited for time lapse / ratio experiments because of the time required to change the hardware filters but may still be used to deliver useful analysis. If no filter changes are needed then the experiment is a fast and very useful one for doing rapid spectral analysis applications.

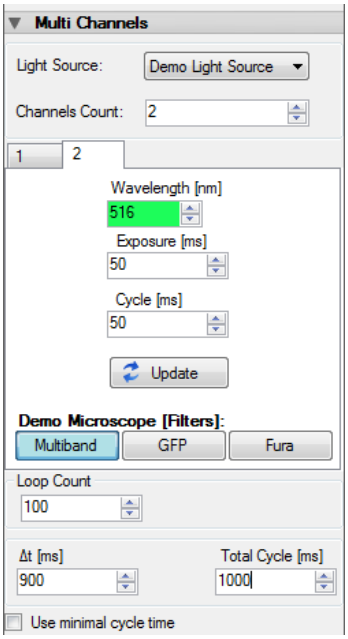
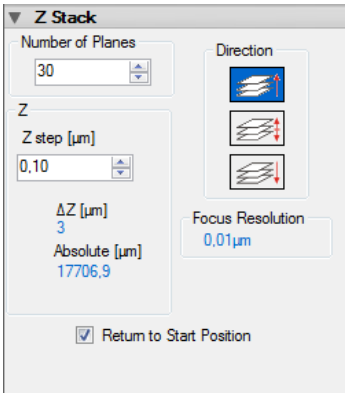


Figure 77: Multi Wavelength Experiment Controls

16.3 Z-Stack

The Z-Stack experiment allows taking relative z-Stacks at the current position. The user can set the number of planes, the step size and the direction



16.4 Absolute Z-Stack

The Absolute Z Stack experiment allows the user setting the top and bottom of a Z Stack and to calculate a optimal z step size according to the Nyquist criteria. The step size is calculated according to the following formula:

The calculated step size is then ideal for image data that will be deconvolved with deconvolution software.
The Step Size and Slices controls are dependent on each other and will change is one or the other is changes. The defining/limiting parameters for Step Size and Slices are the top and bottom position

Further the Absolute Z Stack has controls for navigating in the defined Z-Stack. First of all are the “Go” buttons with which a user can go to the previously set top or bottom positions. Additionally the Slice Explorer can be used to inspect the defined Z Stack by dragging the slider.

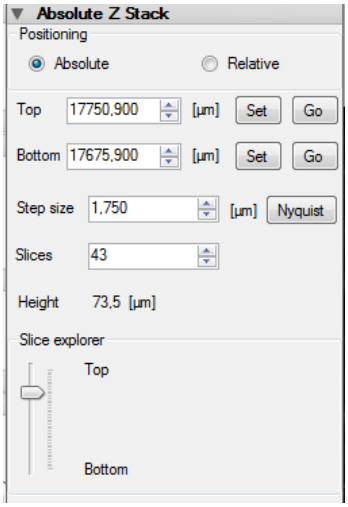


Figure 78: Absolute Z-Stack control

The *Absolute Z-Stack* can also be used as a relative Z-Stack. Setting the *Positioning* to “*Relative*” will allow acquiring Z-Stacks relative to the current z-position. The current position is then the center of the Z-Stack. In case a Z-Stack of 10 Planes/Slices should be acquired, five planes will be acquired below the current z-position and five above the current z-position. The controls for setting the top and bottom position are disabled in this mode.

16.5 Z Stack in Time

The Z Stack in time has the same controls as the Z Stack experiment with the addition of the controls from the Time Lapse / 2 Wavelength experiment.

This experiment is provided as a learning tool on how one takes the various sample experiments and merges and modifies them into something tailored for the application workflow of the researcher.

The workflow of this experiment is as follows.

- A plane from the Z Stack will have either 1 or 2 Wavelength images taken.
- The next plane of the Z Stack will then repeat the process until all planes are completed.
- The cycle count will then be decremented and the process will be repeated across the entirety of the Z Stack.

What is important to note about this DEFAULT experiment is that no data sorting is made at the LA Browser or TIFF streamer and that the images have to be manually regrouped by matching the image capture metadata.

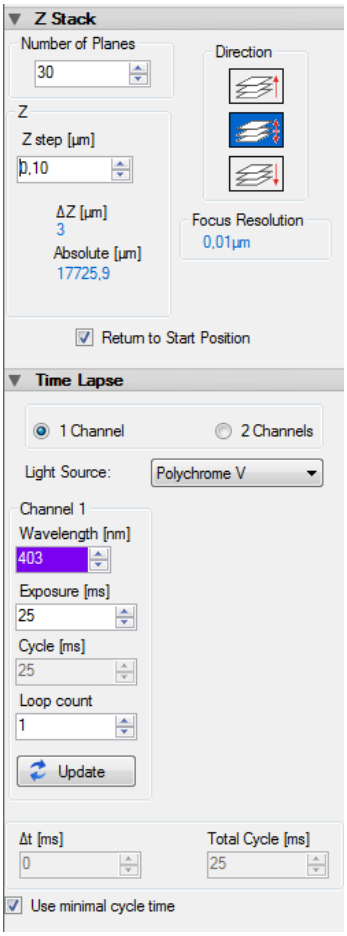


Figure 79: Z-Stack in Time Experiment Controls

This process can be automated by replacing the default provided Z Stack in Time experiment with the Z Stack in Time Serialized. The details on how to do this can be found in Appendix B and describe the process needed to update and modify C# plug-in.

After the updated plug-in has been installed then the LA Browser now has 1 (or 2) separate stacks. The first (and second) are default Z Stacks in Time with a single wavelength represented. This allows a 3-D/4-D model of the data to be analyzed per wavelength. The two separate Z Stacks can then be overlaid such that the Ratios of the wavelength data can be analyzed in an offline manner.

16.6 Tile and Stitch

The Tile experiment is both a calibration tool and a manual screening application. It allows a large region to be mapped with individual images and then later stitched together into a complete image.

The Tile and Stitch Experiment window list has the following two controls added:

- Overlapped Pixels X
- Overlapped Pixels Y

In the Navigator control ROIs and POIs (regions and points of interest) are chosen. The only other control is a correction factor on the number of pixels that need to be overlapped to correct any image imperfections in hardware calibration. This is based on a camera pixel overlap so that the tile and stitch algorithms of the LA Browser or TIFF Streamer can be used for visual inspection of the accuracy of the tiling movement.

Dragging the mouse cursor over a region in the Grid View with the left button depressed will select a ROI. Single clicking into any grid position with the left mouse button will select a POI. Depressing the right mouse button on any selected point removes it from the list to be tiled. The yellow cursor highlights the current hardware position of the stage. Disabling hardware motion allows selection of POIs and ROIs without the hardware moving during the setup. Enabling the Hardware causes the XY stage to move to each selected point thereby giving the user an overview image as the experiment is being configured.

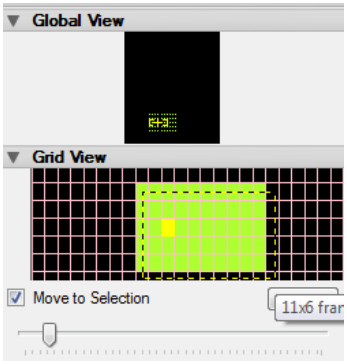


Figure 80: Navigator Control

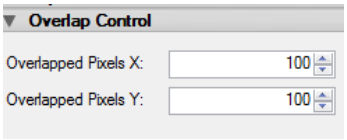


Figure 81: Tile and Stitch Experiment Controls

16.7 Relative Tile

The Relative Tile experiment allows to acquiring a tile of n by m images around the current position. The current position will be the center of the Tile scan.

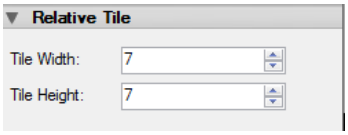


Figure 82: Relative Tile experiment parameters

Using this experiment with a MarkerScan experiment allows acquiring Tile experiments at each position in the list of the MarkerScan.

16.8 FRAP

The FRAP experiment is one that allows use of a laser for point, line, and polygon based photo bleaching/activation of a sample. In the supplied FRAP experiment it has been combined with an optional 2 Wavelength / Time Lapse experiment as these three experiments tend to be used together. The FRAP experiment is available as experiment in LA and as a building block in the Protocol Editor for larger protocols.

The workflow of this experiment is as follows.

- A number of reference images will be taken (with the same light/exposure setting as the Time Lapse)
- Each ROI image will be FRAPed (optionally multiple times)
- Each ROI will then be revisited and FRAPed until all loop count FRAP cycles have been completed
- The Time Lapse / 2 Wavelength experiment will then begin to execute

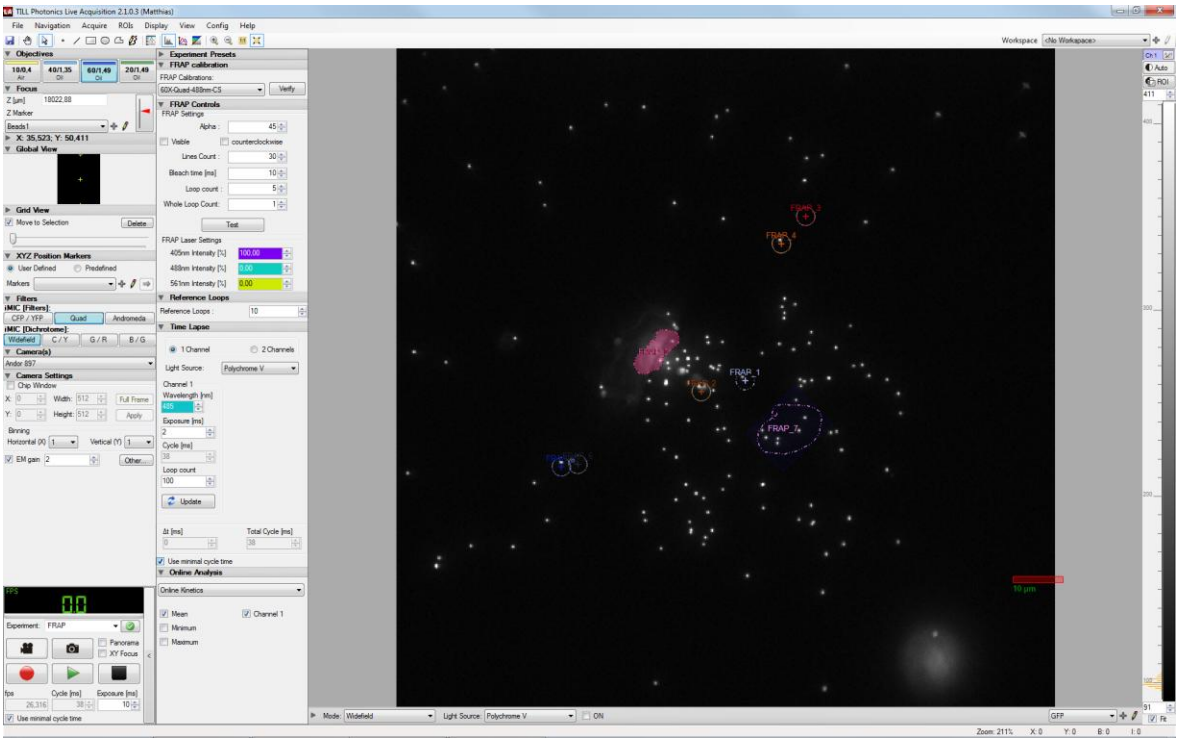


Figure 83: FRAP Experiment

The FRAP Experiment window list has the following controls added:

- FRAP region color selection
- Alpha value
- Display Option for the Alpha value (Visible)
- Alpha counterclockwise vs. clockwise rotation selection
- Line count
- Bleach time
- Loop count
- Whole Loop Count
- Reference cycles

The FRAP controls are found at the top of the Experiment Window.

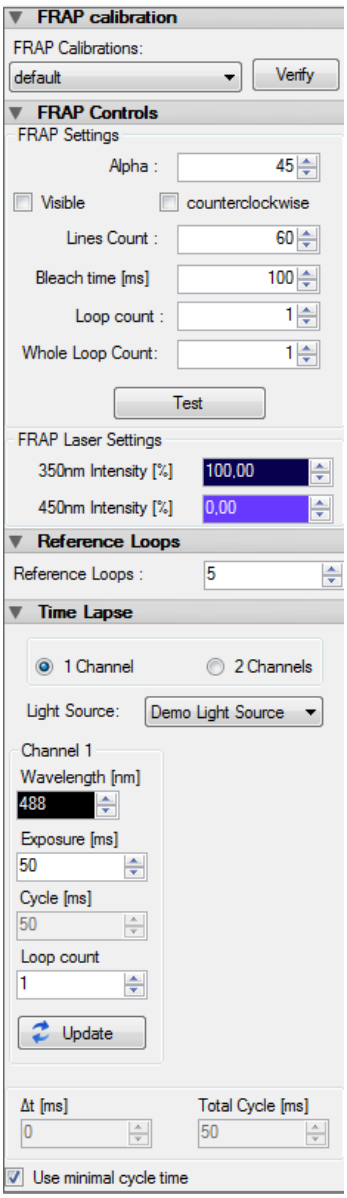


Figure 84: FRAP Experiment Controls

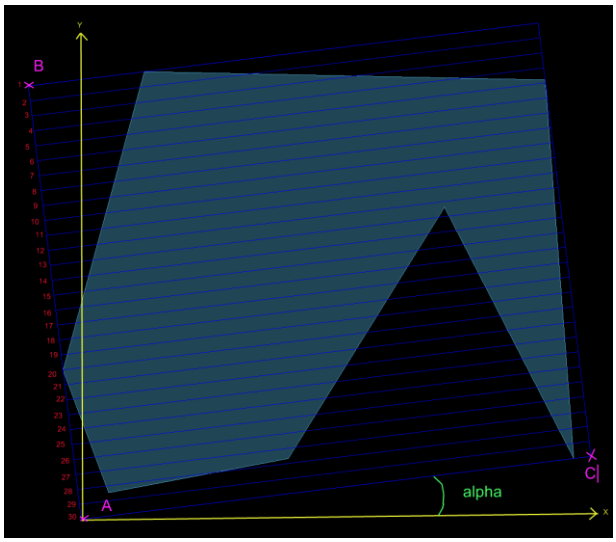


Figure 85: FRAP Parameters

The above figure represents the way the FRAP parameters are handled. The Alpha combo-text box is the angle from the bottom left corner of the region of interest to the horizontal plane. The checkbox for visible displays the FRAP raster lines on the GUI. The line count combo-text box is used to determine how many lines will be populated in the chosen region of interest. The more lines the better the FRAP detail but the larger the bleaching time on the sample. Bleach time is the amount of laser exposure time for the complete region of interest. This does not include the time that it takes the hardware to move to a new line as the laser is blanked during this time. The last control is a counter that allows re-bleaching of the same selected region of interest in an iterative fashion. This is particularly useful when doing time lapse analysis of FRAP regions.

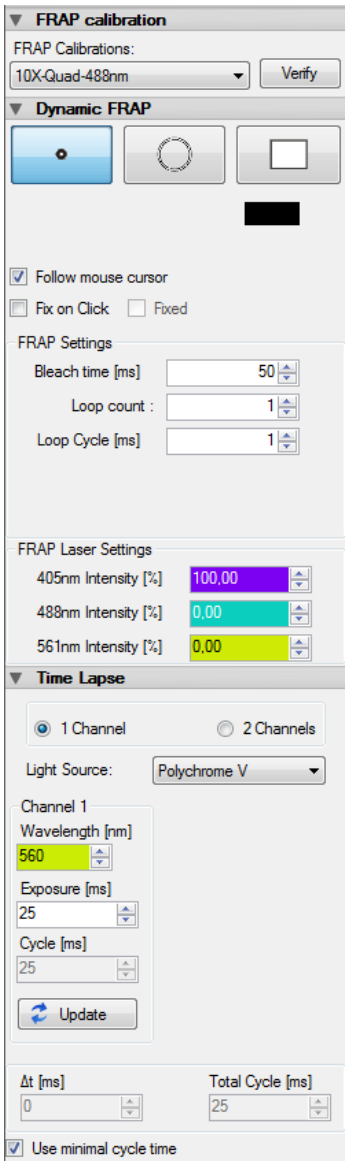
The last parameter in the control is the number of reference FRAP images to take before switching over to 2 Wavelength / Time Lapse aspect of the experiment. This allows a FRAP analysis to be made and then an Epifluorescent movie to be made directly afterwards in either 1 or 2 separate wavelengths.

The lower half of the Experiment Window possesses the same controls as that detailed in the section for Time Lapse 2 Wavelength and their usage can be found there.

When all the parameters have been entered and the experiment setup has been saved then simply press the ‘Record’ button to begin execution of the acquisition.

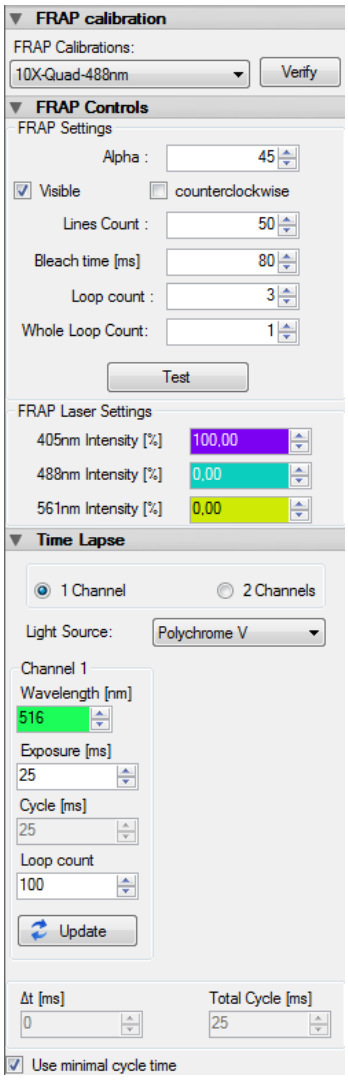
16.9 DynamicFRAP

The DynamicFRAP experiment is made for applications where the time point and location of the FRAP ROI is not known. Application where the loading of vesicles is observed or other transport activities are monitored this FRAP experiment can help.
The DynamicFRAP experiment allows choosing between three shapes of FRAP ROIs, a point, an ellipse and rectangle type shape.



16.10 FRAP on Demand

The FRAP on Demand experiment allows the user to set the Time point when the previously defined FRAP ROIs should be “frapped”. In contrast to the normal FRAP protocol there is not option to edit the number of reference cycles as this protocol will start with an endless loop and therefore acquires images until the user presses the “FRAP Now!” button. The images acquired before the FRAP use the same parameters as in the Time Lapse part are shown.



16.11 FRAP on Demand (PE)

The “FRAP on Demand (PE)” is only available in the Protocol Editor of the LA software. It has the same parameters as the “FRAP on Demand” experiment, but is capable of being combined in a Protocol Editor workflow.

16.12 TIRF EPI

The TIRF / Epifluorescence experiment is similar to the 2 Wavelength experiment. In this case a single wavelength epifluorescence and multiple wavelengths (depending on laser line combiner settings) TIRF image are acquired.

- TIRF image(s) will be taken
- A time lapse of epifluoresent images will be taken
- The process will repeat until all loops have executed

The first is a control allows entry of exposure time and cycle time for the TIRF image(s).

The second is a fluorescence control that allows the exposure time and cycle time to be set as well as the wavelength. At the bottom of these two controls is cycle count with no delay being provided between cycles. It cycles between two images at multiple wavelengths as fast as possible.

The third section of the control panel allows two separate focus(es) to be established as TIRF and epifluorescence are at slightly different focus depths. The search triggers the corresponding light source until set is pressed storing the local focus position. During this calibration time all the primary control panel functions are available to determine proper focus depth.

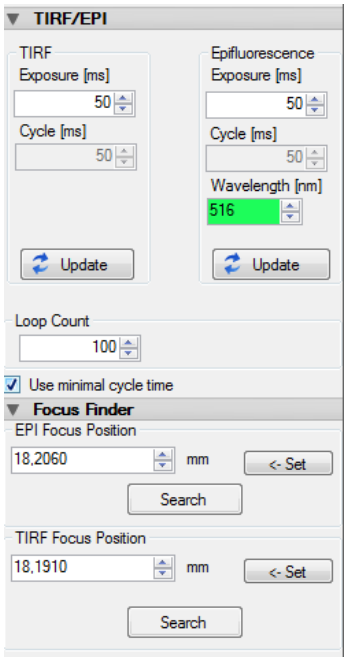


Figure 86: TIRF EPI Experiment Control

16.13 Marker Scan

The Marker Scan protocol item allows managing a list of xyz position markers. The user can select markers from a list and add them to the list of markers that will be addressed during a protocol. Users can add child items to the Marker Scan item to execute for example a Z-Stack at each marker position. For more detail please have a look at the Protocol Editor chapter.

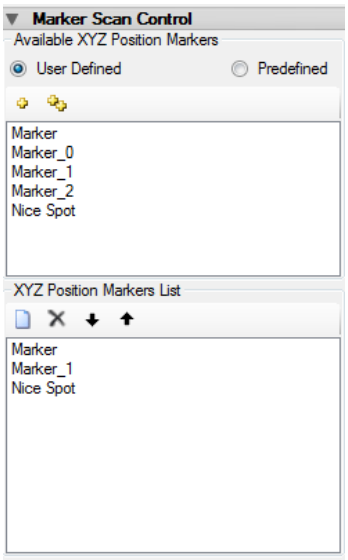


Figure 87: Marker Scan protocol item

16.14 Marker Focus Map

The *Marker Focus Map* protocol item allows correcting the Z-position of user defined and pre-defined marker prior to a Markers Scan item in a Protocol Editor workflow. It allows modifying the number of focus steps and the step width. The second step count and step width is for a fine focus on the best found position with the coarse movement.

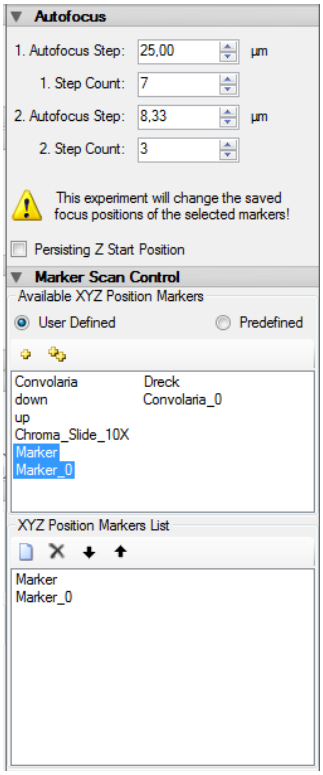
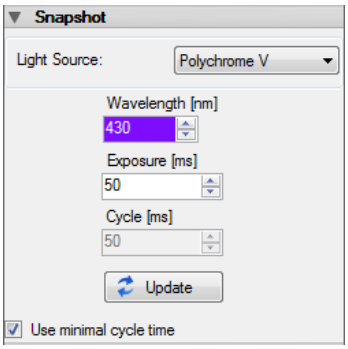


Figure 88: Marker Focus Map

The “Persist Z Start Position” will start the search for the best focus position always from the last z-position the user has set either by moving to a position in the protocol. If this is the first item in a protocol the start position will be the current z-position from the live view.
Under Marker Scan Control the user can select the markers, user defined or pre-defined, where the focus position should be corrected. This works in the same way as in the Marker Scan Item.

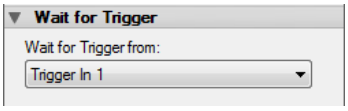
16.15 Snaphot

The Snapshot item takes one image with the selected light source for illumination. If used in a Loop the user can build a time lapse experiment or multichannel experiment from scratch. However, putting a Snapshot item in a Loop will not allow overlapped readout-exposure modes of the camera even if the camera supports it, as each Snapshot item will re-program the camera.



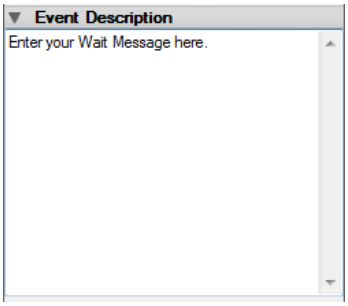
16.16 Wait for Trigger

Wait for Trigger is a control item for Protocols as it allows to control the workflow of the protocols by wait for an external event. This item can be used to synchronize the start of two setups like imaging and electrophysiology. Where one of the two setups would be the “master” as it would send a trigger to the other as a start signal.



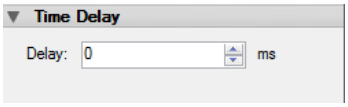
16.17 Wait for Key

Here the protocol workflow can be interrupted to wait for user input. The user has the option to enter customized text message that will be displayed until a key is pressed.



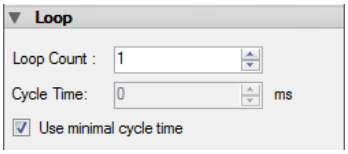
16.18 Time Delay

The Time Delay item allows delaying the execution of protocols by a certain time. This item is usually used in conjunction with loop constructs.



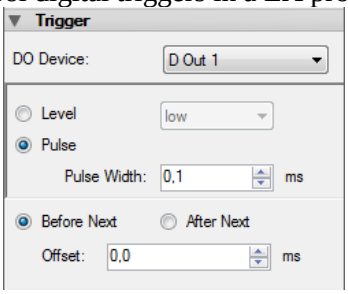
16.19 Loop

The Loop item allows building simple time lapse experiments and also complex protocols by nesting multiple loop items.
It allows setting the number of repetitions (*Loop Count*) and *a Cycle Time*. However, the cycle time will not be used if a PC controlled experiment is nested in the loop. PC controlled items are the '*AutoFocus'* and '*Wait for Key*' experiments. These two protocols need commands from the PC to continue, like a key pressed on the keyboard or the analysis of the focus algorithm. As the time point, when the results from these items are ready, is undefined, the cycle time will be ignored in this cases.



16.20 Trigger (Digital Out)

With the Trigger protocol item the user can send TTL triggers from the ICU to other devices. There are multiple options to control digital triggers in a LA protocol.

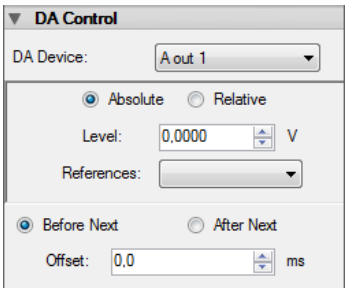


The user can select between multiple digital outs that are available on the ICU by selecting them from the drop-down-list. The analog out ports have to be configured in the LA device configuration dialog on start of the LA.

The next option available to the user is whether the user wants to set the Digital out to one of the two levels, zero or plus five volts, or if the user wants to generate a pulse to +5V.
The time point when the TTL will be modified can be changed by the user with the options *Before Next* and *After Next*. Entering a time in the offset control will execute the TTL modification the entered time before or after the next item in a protocol tree.

16.21 Analog out

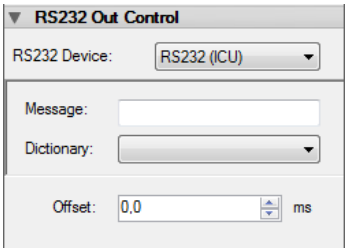
With the Analog Out item the user can set one of the analog outs of the ICU to a certain level in the protocol execution. The voltage can be set to an *absolute* value or is used in a loop increased or decreased from loop to loop iteration when selecting the *relative* option.
References created with the External Control feature (Chapter 13) can also be used here by selecting them from the drop-down-list labeled "References".



The time point when the analog voltage will be modified can be changed by the user with the options *Before Next* and *After Next*. Entering a time in the offset control will execute the analog voltage modification for the given analog out port the entered time before or after the next item in a protocol tree.

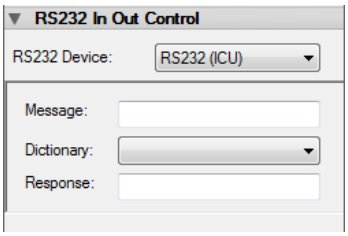
16.22 RS232 Out

With this item a RS232 command can be sent to a connected device. The time offset relative to the succeeding command can be adjusted.



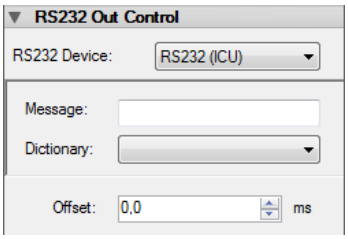
16.23 RS232 In Out

This item allows sending a command to an RS232 device and shows the response from the corresponding device.



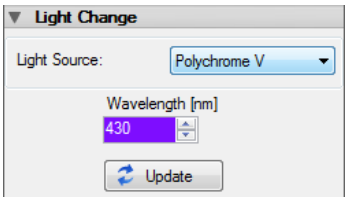
16.24 RS232 Acknowledge

This item will wait for a defined text string defined by the customer. Once the defined string has been received the workflow will continue. This works much like a “wait for trigger” – item. This only works with serial ports (Com-Ports) on the PC, not on the ICU. The time offset relative to the succeeding command can be adjusted.



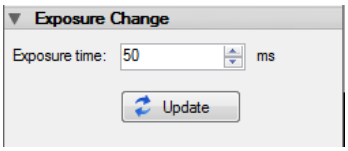
16.25 Light change

This item is used if a change of the light source is required in a protocol. Usually this is used if protocols are built with Snapshots and Loop items from scratch.
If used before a Time Lapse or Multi-Channel item, this will change the light source and its parameters accordingly.



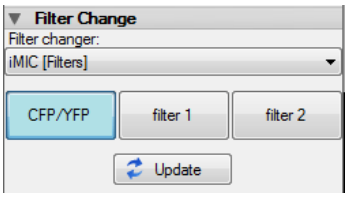
16.26 Exposure change

The “Exposure Change” allows changing the exposure time in succeeding experiments in a protocol workflow. This works with experiments like the Z-Stack, Absolute Z-Stack, Tile and Relative Tile where no controls for setting the exposure time are present.
The “Exposure Change” has therefore no influence on experiments like Time Lapse, Multi Channel, FRAP or TRIF-EPI.



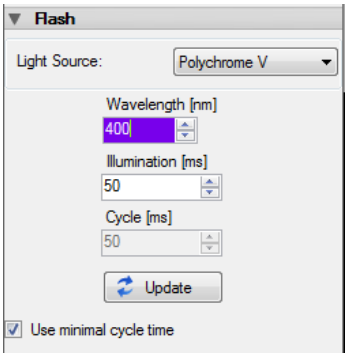
16.27 Filter Change

The “Filter change” allows changing the filter position in succeeding experiments in a protocol workflow. This works with experiments like the *Time Lapse, Z-Stack, Absolute Z-Stack, Tile and Relative Tile* where controls for setting the changing filters are not present.
The “Filter Change” has therefore no influence on experiments like the Multi Channel.



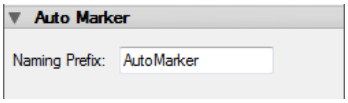
16.28 Flash

The Flash item is made for experiments, where the user needs to expose the sample to light but does not want or need to take an image with a camera. This is usually the case when working with caged compounds or other photo activated substances.

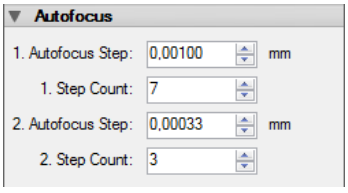


16.29 Auto Marker

The Auto Marker adds a xyz-Position Marker to the xyz Position marker list in the LA control panels.

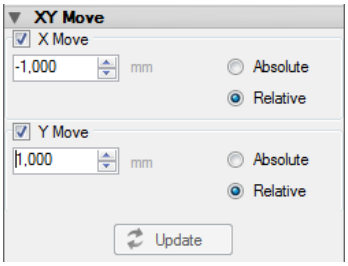


16.30 Auto Focus



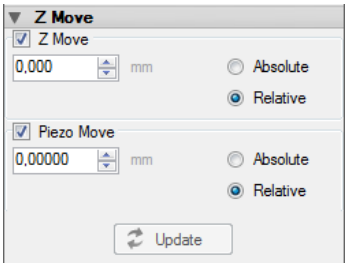
16.31 Move XY

If a motorized xy-stage is installed on the system it can be moved with this item. The user can select between absolute and relative movement.



16.32 Move Z

When working with an iMIC or an imaging system with a Pifoc, the Z-Move experiment allows changing the z position with the Z-Move experiment.



17 Supported Hardware and general features

17.1 Supported motorized Microscopes

- iMIC series
- uiMIC
- MORE microscope

17.2 Supported TILL Hardware

- ICU (with CCM and CCM2)
- SCU/SPU
- Polychrome V
- Oligochrome
- iMIC Dichrotome Image Splitter
- iMIC Polytrope I and Polytrope II Mode Switch
- Yanus Scan Head (digital control with DSC)
- iMIC Fixed Stage
- iMIC Large Stage (Prior)
- iMIC Integrated Stage

- iMIC Transmitted Light

17.3 Cameras

- AVT Stingray F-145B & Pike F-145B
- Andor Clara & Clara E
- Andor Luca S & R
- Andor iXon 885, 888, 897
- Hamamatsu Orca R2
- Hamamatsu Orca 03G
- Photometrics Evolve 512
- QImaging Retiga 2000 RV & DC, EXI Aqua & Blue
- TILL Imago QE / PCO Sensicam QE

17.4 Supported Light Sources

- Polychrome V
- Oligochrome
- LED(s) (up to 5)
- LLC - Laser Line Combiner with Pegasus AOTF
- IR Illumination
- Trans-Illumination

17.5 Supported Expansion I/O Devices

- 3D Connexion Space Navigator Joystick
- USB HID Joysticks

17.6 Real Time Execution and Control Unit specifications

- µSec resolution in protocol response times to external triggers
- 1 mSec resolution between camera and light source timing
- Protocol timing can be structured for timing optimization in a dynamic fashion with support for start at start, end at end, and start at end whereby each protocol action can be timed to any other protocol action in various formats
- 1µSec galvo/laser control timing
- Guaranteed timing generated by OS independent Imaging Controller Unit (ICU)
- Zero-position focus map correction
- Real time hardware monitoring preventing hardware device collisions
- More than 300 full frame image readout with current fast overlap readout cameras are possible
- Intelligent acquisition to memory or direct to disk (depends on timing requirements)
- Real time (PC Connectivity speed) FRAP/TIRF region of interest changes
- Parallel real time protocol execution allowing pre-built protocols to be paused, started, and resumed when other protocols are active

- Loop with in loop complex protocol execution / decision matrix(es)
- Digital outputs and inputs to control other devices
- Control external devices by TTL pulses
- "Wait for" Triggers

17.7 Software Extensibility

- Common data exchange (OME-TIFF, OME-XML)
- Import of OME-TIFF compatible files
- Driver level SDK API encapsulates core functionality of the iMIC microscopes

18 FAQ and Trouble shooting

In this chapter solutions and trouble shooting for known issues are described.

Q: LA crashes after the devices have been initialized and I can see the main window

A: This looks like a driver issue either with the graphics card or with a camera. In both cases a driver update is recommended. Typical is that the LA main window has a plain gray background and no black area is shown where the camera image would be displayed.

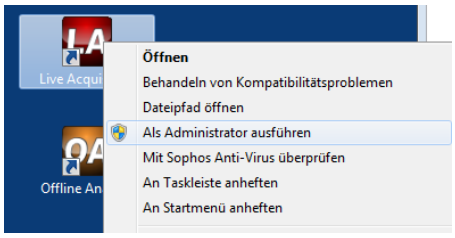
Graphic card drivers can be downloaded from the nVidia or AMD/ATI websites.
If the computer was setup from the scratch the first thing to do before or after the Installation of LA is to update the graphics card driver.
If problems remain a too old camera driver version could be the cause for the crash. In this case please contact the service at TILL Photonics.

Q: I cannot update my dongle with the V2C-File I have received.

A: Check your settings of the Anti-Virus software they might be too strict and will not allow the LARUS.EXE to update your USB-dongle. Please contact your IT department to change this.

Q: LA won't start on a newly created user account, but it starts on other accounts.

A: If you are running Windows 7 (or Vista) this might be the User-Access-Control (UAC) that interferes. Try running the LA software as Administrator by right-clicking on the LA or OA Icon on the Desktop and start LA/OA by "Run as Admin…".
If this works you can set the UAC control in the Windows Control panels to a lower security level.

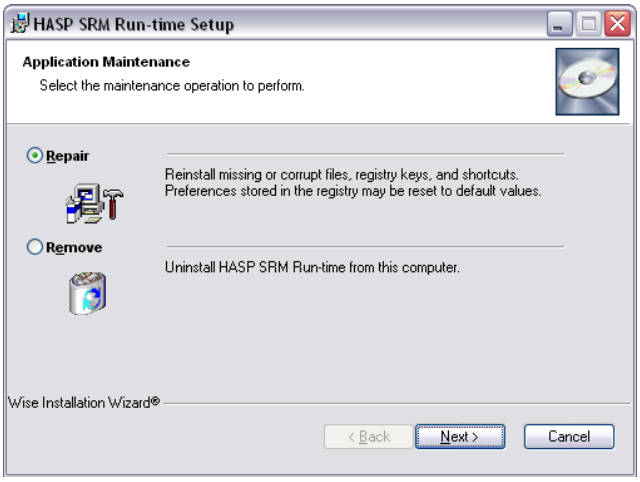


Q: LA Software says that the Dongle is required but it is already plugged in.

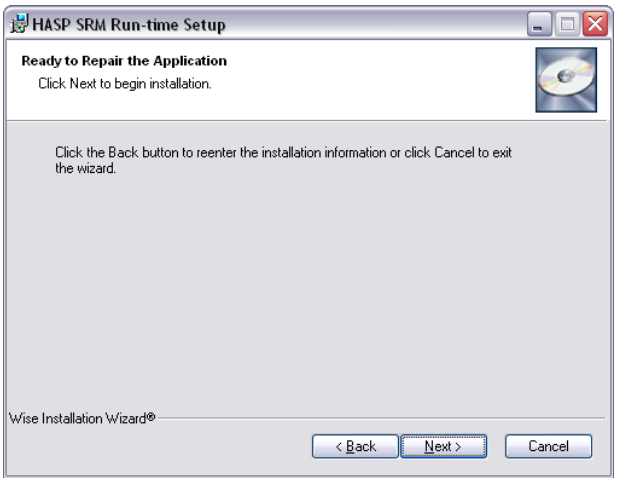
A: If you have installed other software that needs a dongle, it might be necessary to reinstall/repair the Dongle driver. To do this, follow the instructions:

If you have an Anti-Virus software running on your computer you might first check if the security settings are too strict. This can prevent the installer from copying the driver files to the windows directory. In this case please contact your IT department.

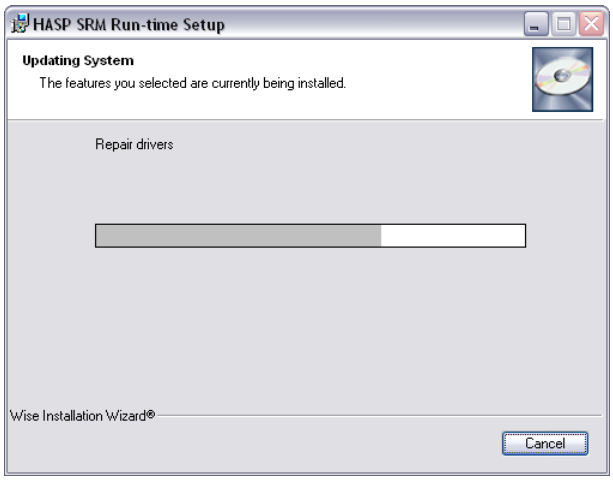
Go to the Drivers folder e.g.(c:\program files\TILL Photonics\Live Acquisition\Drivers) of the Live Acquisition software and run the HASPUserSetup.exe. Then follow the on screen instructions.



Select the “Repair” option and press “Next”.



Simply press “Next”



Wait until the software has fixed the drivers. Then close the program. If problems still persist check if the security setting of your virus scanner are set to high. In this case please check first with your IT department.

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