OLYMPUS

# INSTRUCTIONS

scan^R

## AUTOMATED IMAGE ACQUISITION SOFTWARE

This instruction manual describes Olympus' scan^R Automated Image Acquisition Software for Life Sciences. To ensure safety, obtain optimum performance and familiarize yourself fully with the use of these products, we recommend that you study this manual thoroughly before operation. Together with this manual, please also read the scan^R Hardware and Software manual as well as the manuals of all other devices controlled by this software in order to understand general operation methods. Retain this manual in an easily accessible place near a system for future reference.

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# Imaging Excellence

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Manual version: June 2019

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# Contents

## Contents

# 1 Introduction

Thank you very much for purchasing Olympus Soft Imaging Solutions' High Content Screening System and for your confidence in our products and service.

The scan$^{R}$ Acquisition Software controls the hardware components of the Olympus scan$^{R}$ Screening Station for Life Sciences. The scan$^{R}$ Acquisition Software is designed for automated image acquisition of biomedical samples. The acquired images can be optimally analyzed with the scan$^{R}$ Analysis Software. The software is intended for the use in biomedical research.

> ⚠ The scan$^{R}$ Analysis Software, the scan$^{R}$ Acquisition Software as well as the hardware components of the Olympus scan$^{R}$ Screening Station for Life Sciences are for research use only.

## 1.1 Abstract

This user manual will guide you through the usage of the Olympus Screening Station and will assist you in setting up efficient and reliable screens.

The Screening System scan$^{R}$ is intended to be used for the screening of well-plates and slides. Do not use the system in other ways than described in this manual.

For detailed information on the individual system hardware components please refer to the corresponding manuals delivered with the system.

Special care has been taken to guarantee correct and accurate information, although this is subject to changes due to further development of the Screening System. Thus, the manufacturer cannot assume liability for any possible errors. We would appreciate reports of any mistakes as well as suggestions or criticism.

Please refer to the respective hardware component manuals for regulatory compliances.

## 1.2 Technical support

If you find any information missing in this manual or you need additional support, please contact an Olympus specialist directly.

## 1.3 Safety precautions

Before operating the screening system, make sure that you have carefully read and understood all hardware component manuals and the safety precautions found in the respective manuals.

# 2 Short Step-by-Step Guide

This chapter gives a step-by-step guide on how to use the scan$^{R}$ software to perform a basic scan. Detailed explanations of the entire setup and acquisition procedure can be found in the following Chapters.

# Performing a scan: A short step-by-step guide

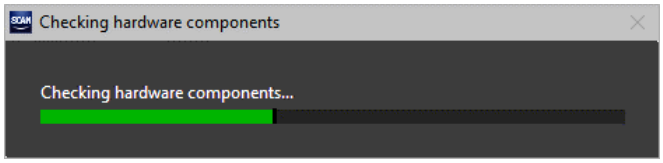
This step-by-step guide assumes you have established scan settings for the type of sample to be screened. If you use new types of well plates, new types of samples, new staining or staining protocols or if you alter the optical hardware configuration, you probably have to set up new scan settings to obtain optimal results. In that case this step-by-step guide will be insufficient for your needs. The necessary information on how to set up new scan settings can be found in the following chapters.

### Before the start

Be sure to have switched on all system devices before starting the scanR software.

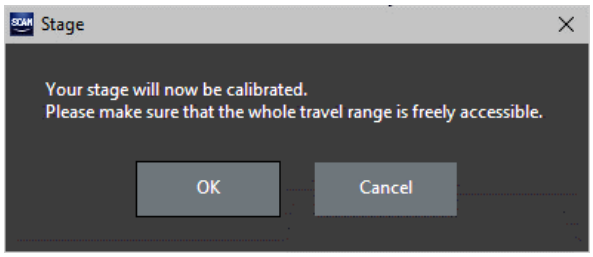
### Start the scanR Acquisition software

Start the scanR Acquisition software by double-clicking the **scanR Acquisition** icon on the desktop.




Upon start, the software will check the availability and status of all hardware components and inform you if one of the components is not available. In that case switch on the missing device and execute the command **Settings ▸ Initialize Devices** in order to initialize all devices without re-starting the software. In case of any problems starting the scanR Screening Station, please refer to Chapter 8 (*System Configuration*).

The software will inform you that it is going to calibrate the stage and ask you to confirm. Remove any obstacles from the stage's operating range and press the **OK** button. The stage calibration will take a few seconds.




### Load or reuse scan settings

Upon starting the software the settings of the previous scan will be loaded automatically. In order to use settings of earlier scans, use the **File ▸ Open Scan** command. By default the file selection dialog will show the settings directory of the user currently logged onto the system. Open one of the files in this

directory or navigate to the location of a previous scan and open the respective *experiment_descriptor.xml* file.

> The *experiment_descriptor.xml* of a previous scan will use the filter and objective positions specified there. If a different hardware configuration is used for the new scan, the settings might have to be adapted accordingly (see following chapters).

### Load the Well Plates

In case of a scan$^R$ system without automatic plate loader (or if the plate loader is not to be used) place the plate manually on the microscope stage.

In order to use the automatic plate loader, place one or multiple plates in the hotels (stacker or shelves).

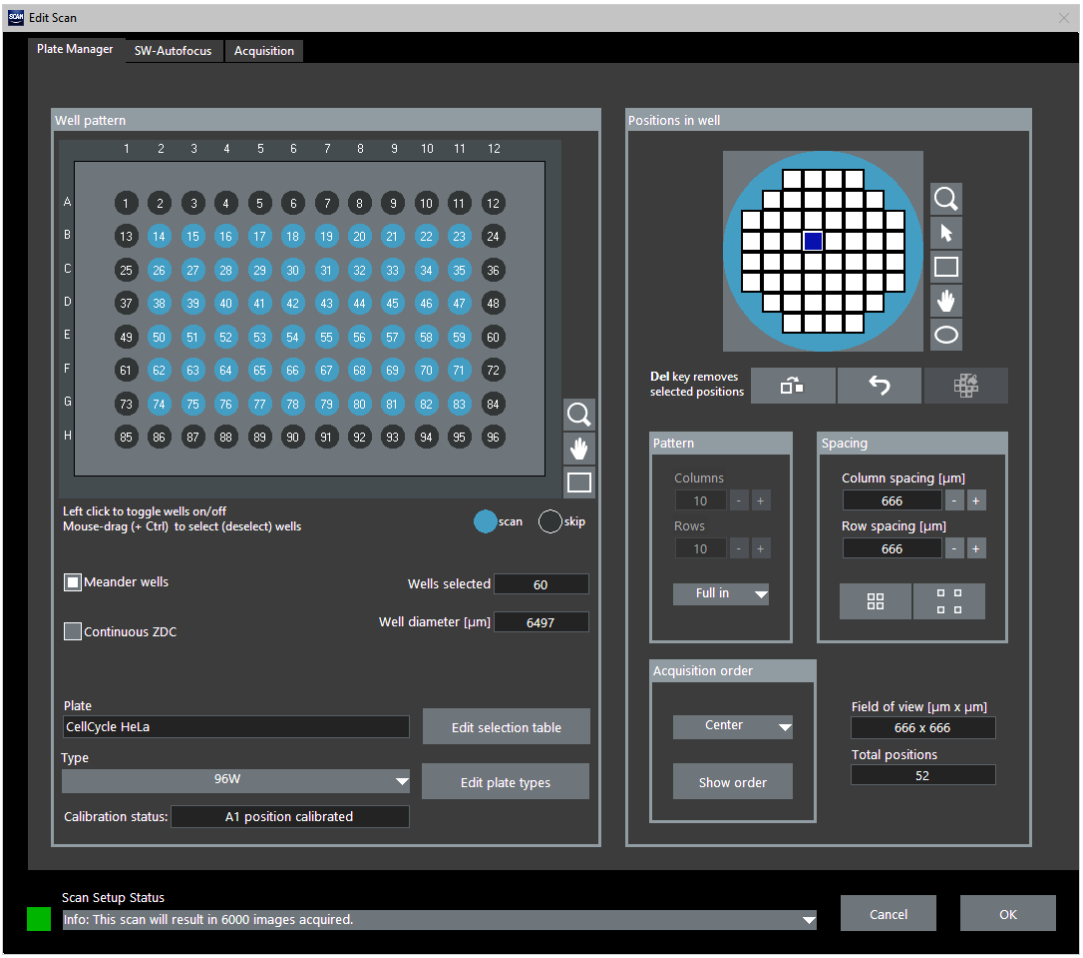
> The automatic plate loader is designed for safe, reliable operation. Please refer to the scan$^R$ Hardware and Software manual for safety requirements. Always wear safety goggles when operating the system with plate loader robot. Never place any items on the base plate in the robot operation range.

### Specify and label the wells and the sample

Click the **Scan: Edit** button (or execute the **File ▸ Edit** command) to open the **Edit Scan** window.

The **Plate Manager** tab of the current scan settings will open.

Enter a name in the **Plate** name box. If the plate name remains unchanged in the course of consecutive scans, the scan directories will be stored with an incremented numeration appended to the name.




Select wells for acquisition by clicking on the circles that represent the wells. Deselect by clicking again.

Multiple wells can be selected by dragging the mouse over them while the mouse button is pressed down. Deselect wells by dragging the mouse while pressing the **Ctrl** -key.

Click **Edit selection table** to get a list of the selected w**ell** numbers and their **Names** or **Descriptions.** The names can be changed to describe the well content for later reference.

Confirm the settings and exit the **Edit Scan** dialog by clicking **OK**.

## Start the Screen

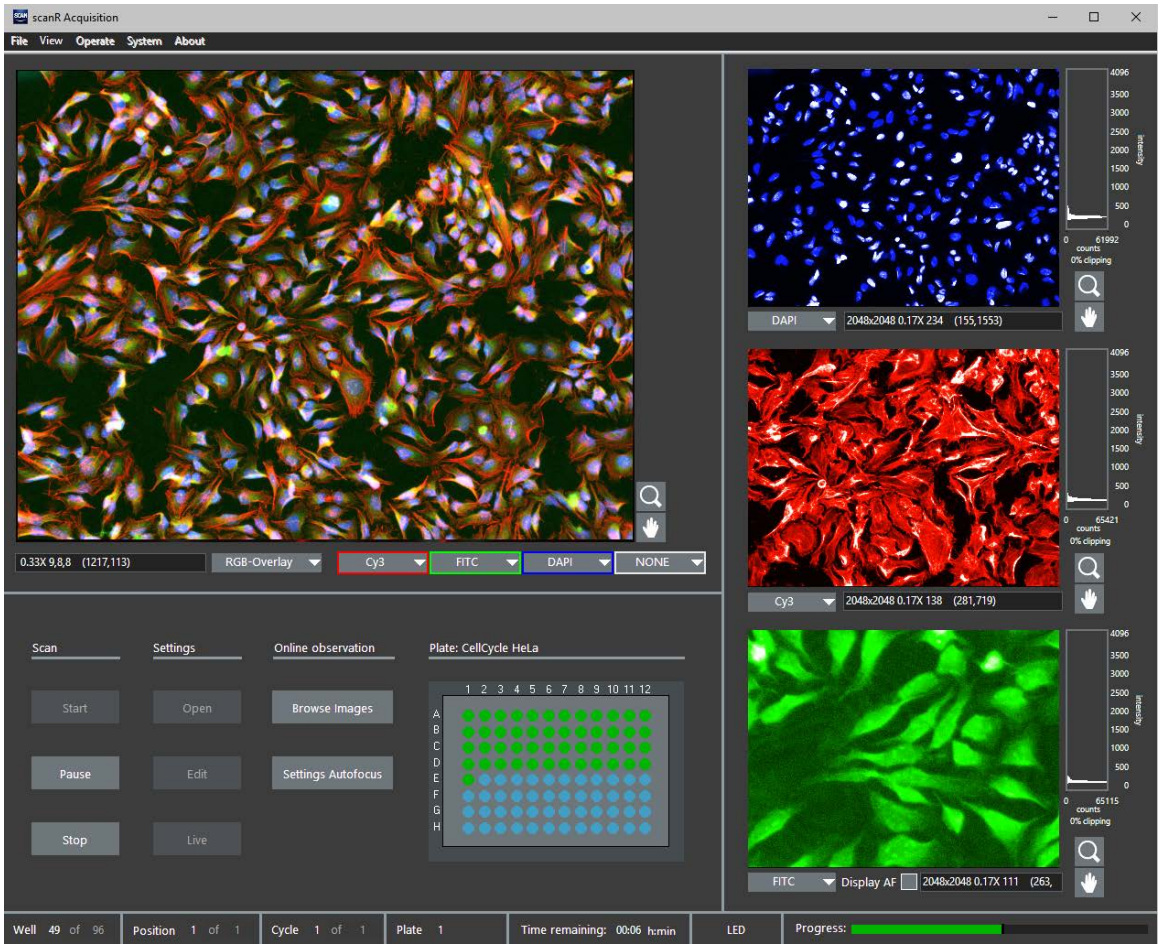
> The burner of the MT20 light source (optional) will be ignited automatically after system and software startup. It is recommended to allow a warm-up time of 10 min to let the light output stabilize before starting the first screening run. If the system is equipped with an LED light source, screening can start immediately.

Start the screen by clicking the **Start** button on the main window. In case of automatic plate loader operation, please select the **Operate ▸ Plate Loader Batch Scan** and press the **Start** button there**.**

The following steps depend on the available hardware and calibration of the well plate.

- If the hardware autofocus option is available for your system or the focus position has been pre-calibrated, and standard plates with a defined position of well A1 are used, scan$^R$ will carry out the scan without further user input.
- If no hardware autofocus is available, scan$^R$ asks the user to set the starting focus position manually. Afterwards the scan will be carried out automatically.
- If spotted slides or other sample holders without defined starting positions are used, scan$^R$ asks the user to move interactively to the center of the first well before the scan can commence.

The Plate Loader Batch Scan requires the use of fully calibrated plates and will always start without further user interaction.

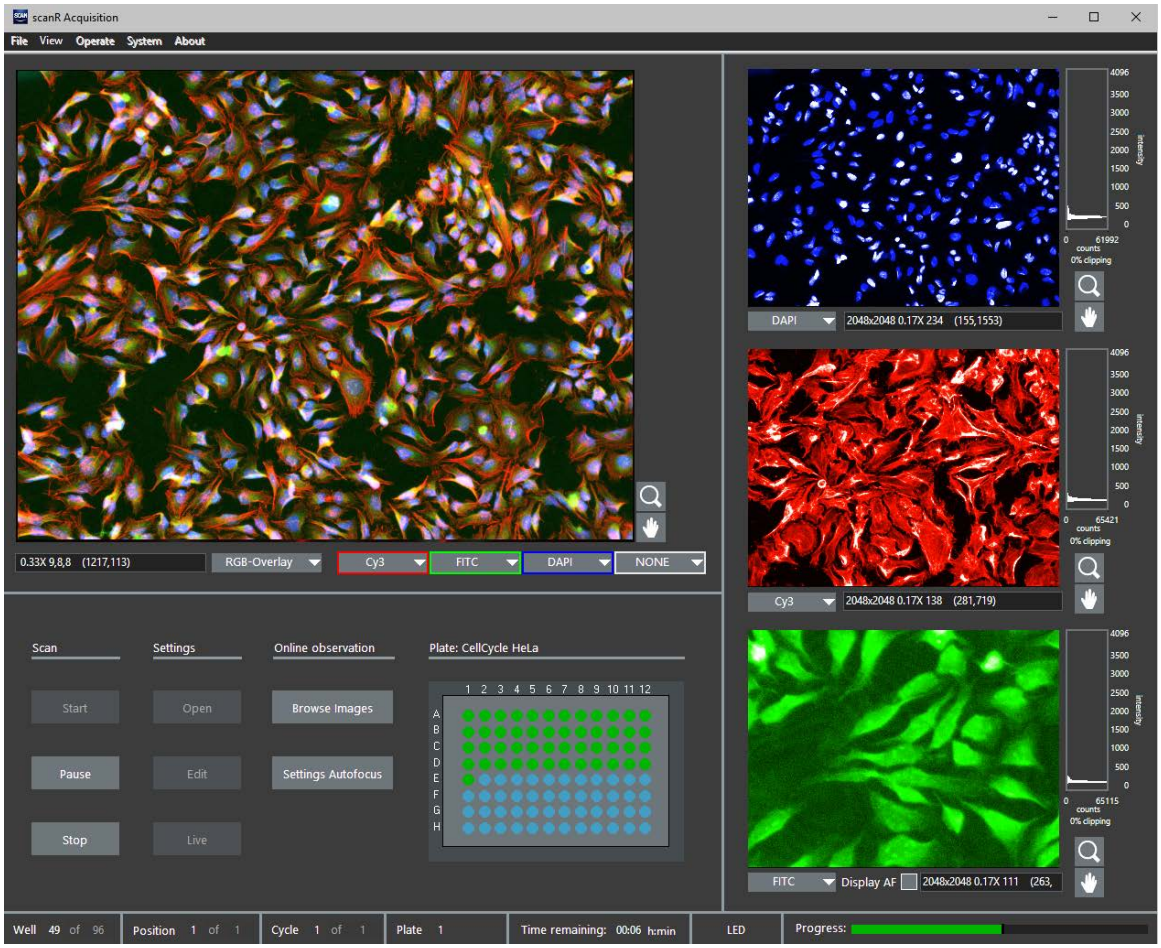



The scan can be paused or canceled by pressing the **Pause** or **Stop** button. The system will finish acquisition of the current position before the scan is paused or stopped. When a scan is stopped in auto loader operation, the user can decide if the system shall continue with scanning the remaining plates or not.

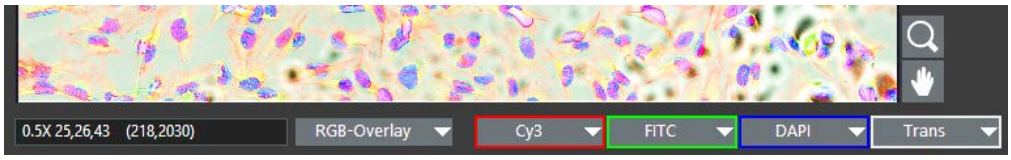
# 3 Main User Interface

This chapter explains the features of the image displays and briefly introduces the different menu points and buttons accessible from the main user interface.

# 3.1 Front-panel displays




The main window features one large and three small live image displays by default. This allows to separately observe different color channels, a multicolor channel overlay (**RGB Overlay + Transmission**) or an online view of the auto-focus procedure (**Display AF**).



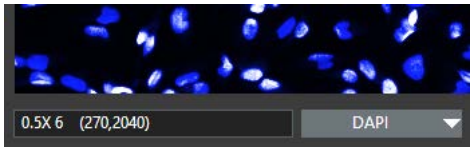

The drop-down menu below the large image display allows selecting **NONE**, single channel or a **RGB-Overlay** (+ transmission) to be displayed in the main panel.

When **RGB-Overlay** is selected, the drop-down menus in the colored boxes become available. The color channels to be displayed in red, green, blue and gray can be selected here. A transmission channel can be selected in the gray drop-down list.

The clipping set for the single image channels will be used for the **RGB-Overlay**. Note however, that the colors may differ from the single image channels depending on the colors selected there (cf. Chapter 3.2, *Display Context Menu*).

When RGB-Overlay is selected in the main image, the image status bar lists the zoom-factor, the intensities in each color channel as 8-bit value and the X/Y pixel-value of the current mouse pointer position.

When a single color channel is selected in the main image, the image status bar lists the image size, the zoom-factor, the intensity as 12- or 16-bit value and the X/Y pixel-value of the current mouse pointer position.




**Color channels**. Select the color channels from the shortlist underneath each display.

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**Zoom** tool

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**Zoom**. Activate the tool and click on a display to zoom into the image by a factor of 2 centering on the click position (if possible) while a **Shift** click will zoom out.

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**Hand** tool

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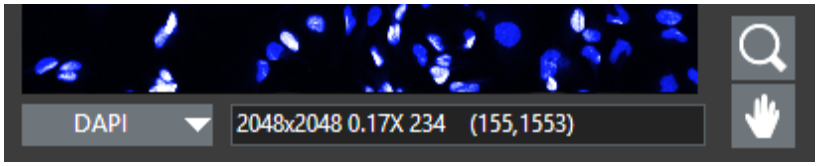
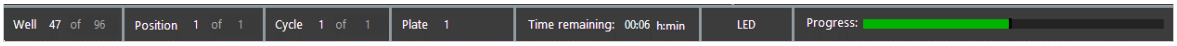
**Hand** tool. Clicking this button in case of a zoomed-in display enables you to move the visible area of the live image via mouse drag.

**Image Display Status Bar.** The status bar below each display lists the image size, the zoom-factor, and the intensity minimum and maximum.

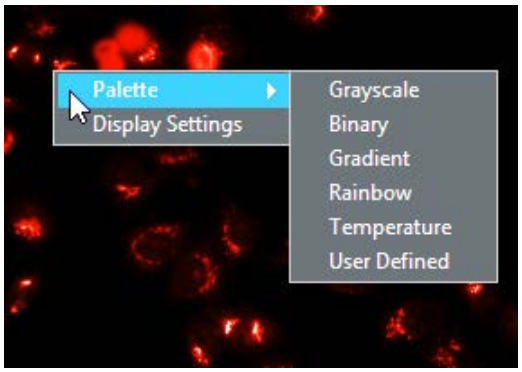



**Display AF**. Check this box in order to display the images of the autofocus process in the bottom image display on the right side. If also an image channel is selected in the drop-down menu the display switches between the autofocus display and the selected image channel. (Note that there may be two different display settings set for the autofocus and the image display, see Chapter 3.2, *Display Context Menu*.) During the autofocus process the autofocus type, Coarse / Fine AF and the autofocus quality is shown in red in the upper left of the autofocus image.

**System Status Bar.** The status bar at the bottom of the main window indicates the progress of the scan and gives an estimate for the remaining scan time. It gives also the actual well number, the total number of wells to be scanned, the current position, the total number of positions to be scanned, the current time-loop and the total number of time-loops to be performed, as well as the current plate number in case multiple plates are scanned using the automatic plate loader. The MT20-icon indicates if the burner of the MT20 is switched on or off (or if the system is equipped with LED light source).



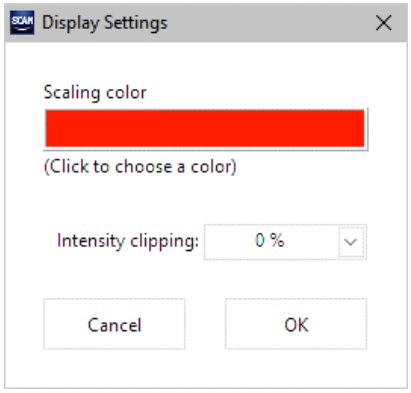
## 3.2 Display Context Menu




A right-click on a display opens a context menu that allows to select additional display settings and to configure the multicolor representation.

**Palette**. Use this command to select the display mode for single channel displays.

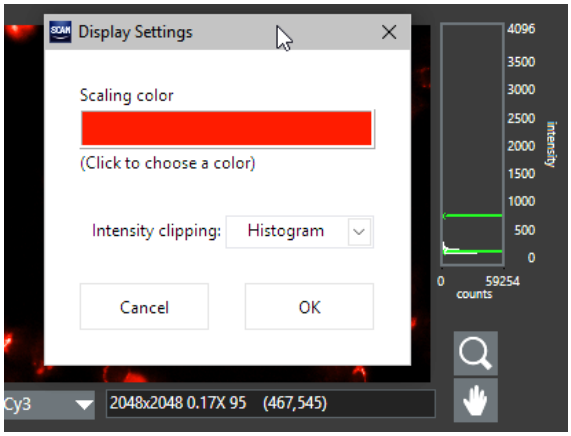
**Display Settings**. Use this command to setup the color display mode. It opens the **Display Settings** dialog.




**Display Settings: Scaling color**. Click on the **Scaling color** box to open a new window that allows you to choose a new image display color (Note that this color is not used for the RGB-overlay).

**Display Settings: Intensity clipping.** To make automatic scaling more effective when noise or bright impurities are present, you can define how many pixels (as a percentage of the total number of pixels) will be displayed uniformly with maximum or minimum brightness. The intensity of the remaining range of pixels will then be scaled linearly according to the selected color palette. Percentage values can be set between **0%** and **5%**. 1% is usually a good value to start with – a slight increase in contrast and brightness results. If the value is set too high, the dynamic range is reduced visibly and the image may look oversaturated. Note, that if you use %-clipping, the intensities of different images will be scaled separately such that you might not be able to visually compare the intensities. In order to get equally scaled intensities for all images use the histogram clipping.

### Display Settings: Intensity clipping / Histogram



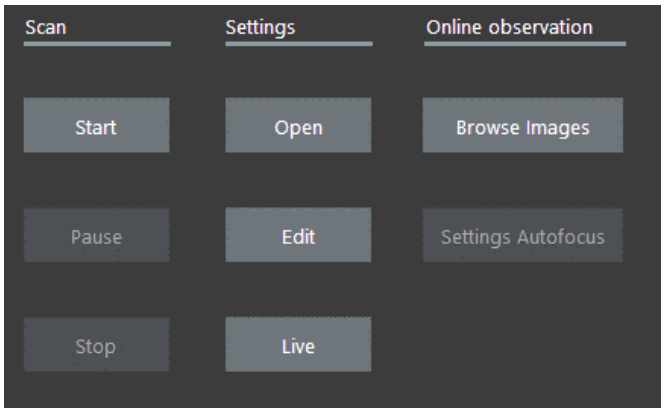

The histogram on the right side of the image displays shows the intensity distribution – in the main display only in case of single channel displays. If the option **Histogram** is selected in **Intensity clipping,** green horizontal lines that signify the clipping limits can be mouse-dragged to adjust the maximum and minimum intensity clipping and thus to optimize the image contrast. Note, that the display values are adjusted for the channels. This means that if you change the channels, the display settings are changed according to the settings last time the channel was used.

## 3.3 Buttons

**Scan Buttons.** A click on the **Start** button starts the scan experiment using the current settings. The **Pause** and **Stop** buttons will become active once a scan is started.

**Settings Buttons**. The **Edit** and **Open** buttons lead to the interfaces needed for the setup of system parameters for a scan experiment.

**Open.** Allows opening an existing experiment settings file. By default the most recently used settings are loaded at startup.[1]




**Edit**. Allows modifying all the settings necessary for setting up a scan like autofocus, acquisition settings and plate types (see Chapter 4, *Setting up a Scan*).

**Live**. Can be used as a preview window and for changing defined color channels. See Chapter 3.4, *Microscope Control and Live View*.

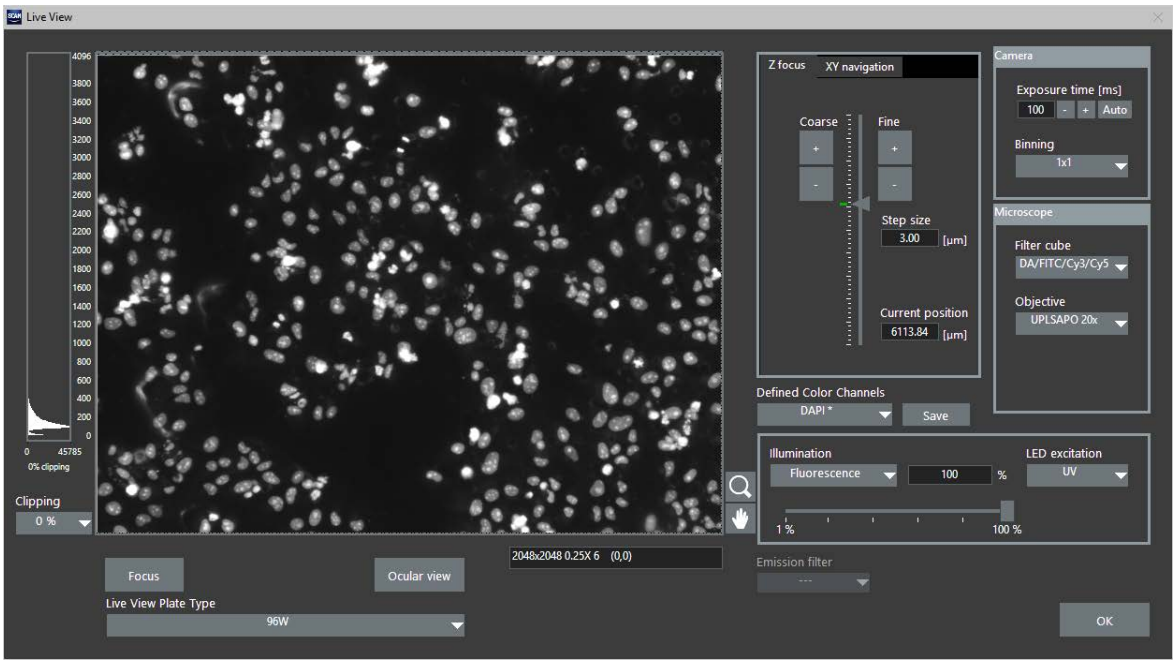
**Online observation Buttons**. The **Browse Images** function enables you to browse through the already acquired images of the current (or a previous) scan. It also allows you to delete entire color channel sets of images that shall not be considered in the analysis and to generate movies from time loop scans.

The **Settings Autofocus** function allows adjusting the autofocus settings during a running scan if necessary.

[1] scanR is designed for multi-user environments and builds on the Microsoft Windows™ user management infrastructure. This means that when multiple (Windows-) users start the system, for each user scanR will load each user's default settings.

# 3.4 Microscope control and Live View

The **Live** button opens the **Live View** dialog that starts live image acquisition and allows to access a selection of hardware settings directly. The device view can be used as a preview to get an impression of the sample and to change defined color channels.




## 3.4.1 Microscope Settings

**Camera: Exposure time [ms]**. The exposure time determines the period of time during which the CCD chip is sensitive to incoming light, in other words, during which photons are collected and converted into charges to be read out afterwards. Enter a value manually or use the **Auto set** button.

**Auto set**. Use the **Auto set** button to obtain a value for the exposure time. The exposure time is set such that the mean intensity of the objects equals a certain value. When the original image was saturated it may be necessary to press the button several times in order to set the exposure time properly. (Note that when using the **Auto set** button in order to set the exposure time for different assays, the value set for the intensity threshold object detection algorithm of the scan$^{R}$ Analysis software will be the same for these assays).

**Camera: Binning**. The camera sensor chip is composed of many light-sensitive units (pixels). These pixels can be read out individually (binning = 1x1) or the signal of neighboring pixels can be combined electronically during data readout (binning > 1x1). Binning reduces the spatial resolution but increases the sensitivity and thus reduces the exposure time required for a good signal-to-noise ratio. It further reduces the amount of data and consequently increases the readout speed. Therefore binning is

recommended if weak signals have to be detected at high acquisition rates or if spatial resolution is of minor importance.

**Microscope: Filter cube**. Select the filter cube to be used. Note that a filter cube switch is time-consuming. Imaging speed can be increased considerably by using multi-band filter sets instead of several single-band sets.

**Microscope: Objective**. Select the objective to be used. Note that a switch of objectives during an experiment is possible but rather time-consuming. In order to maintain the same focus position for different objectives use the **Objective Parfocality** setup (see Chapter 8.3, *Objective Parfocality*).

**Defined Color Channels**. Select predefined color channels. The objective, filter cube, illumination pathway, excitation filter/ contrast inset and intensity of the illumination are set according to the settings of the predefined color channel. You can change the settings individually. This is indicated by a '*' behind the name of the color channel. Click on **save** to store the new settings.

**Illumination: Fluorescence / Transmission**. Select one of the two possible illumination modes: epi-fluorescence illumination with the MT20 or transmission illumination.

**Illumination: Excitation filter / LED excitation / Contrast insert**. Select a MT20 excitation filter or LED excitation channel for a **Fluorescence** image or a transmission condenser contrast insert for a **Transmission** image, respectively. It has to match with the **Microscope: Filter cube** selection.

**Illumination: Light intensity**. Use the slider to set the illumination intensity.

**Focus**. Performs an autofocus procedure according to the current settings.

**Ocular view**. Enables the eyepiece. As long as the eyepiece is enabled, the image is not updated and *Ocular view enabled* is shown in the upper left of the image. Click again to disable the eyepiece.

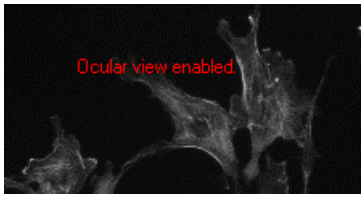
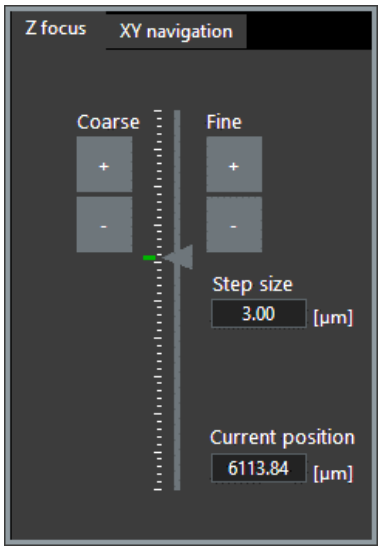


Image display when ocular view is enabled.

**Live View Plate Type**. Allow to select other plates than defined in the current experiment. This is intended for quick review of different plate types. The selected plate will not influence the experiment definition but will be used only within the Live View.

### 3.4.2 Z focus



Z focus

In live views the Z position of the plate is indicated by a blue bar (provided that the stage and the plate are properly calibrated).

**Z focus: Slider**. Use the slider for larger movements of the microscope Z-drive.

**Z focus: Coarse** buttons. Click on the arrows on the left to move the Z-drive in steps 10 times as large as set in **Step size**.

**Z focus: Fine** buttons. Click on the arrows on the right to move the Z-drive in steps as set in **Step size**.

**Z focus: Z-drive steps [µm]**. Set here the step size for the Z-drive when using the Z-drive buttons.

### 3.4.3 XY navigation

**Row**, **Column**. Use these boxes to move the stage to the well of interest. Make sure that the correct well plate type is selected and that the stage is calibrated. See Chapter 8.2, *Stage Calibration*.

**Navigation: Interactive Scheme**. The blue circle represents the well currently placed under the objective. The white rectangle represents the field of view of the camera. Click anywhere in the scheme and the stage will move this spot to the center of the field of view. When **XYZ Limits** (cf. Chapter 9.2.2, *LimitSetup.exe*) is activated, white lines represent the limits of navigation for X and Y.

## 3.5 Menus

**File ▸ Open**. This command loads scan settings from *experiment_descriptor.xml* files. It can also be executed with the **Open** button.

**File ▸ Save as**. This command saves the current scan settings to an *experiment_descriptor.xml* file. Note that this is performed automatically upon starting the scan.

**File ▸ Edit**. This command allows to edit the current scan settings and to define new types of formats (see Chapter 4, *Setting up a Scan*). It can also be executed with the **Edit** button.

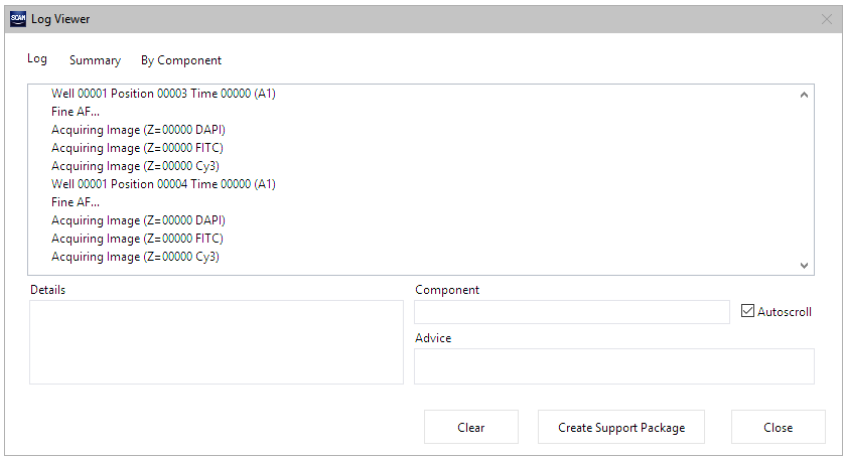
**File ▸ Exit**: This command shuts down the scanR software.

**View ▸ Status Bar**: This command minimizes the **scanR** window to display only the **Status Bar**.

**View ▸ Main Window**: This command minimizes the **scanR** window to display only the largest of the four displays.

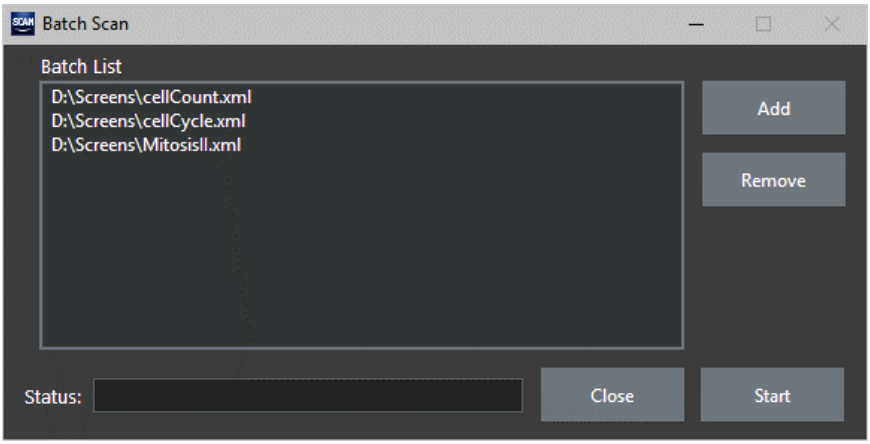
**View ▸ Maximized**: This command causes the entire **scanR** window to be shown.

**View ▸ Log File**. This command opens the **Log Viewer**. The Log Viewer shows all executed steps of the scanR system which are relevant to review the current screen. Deactivate the **Autoscroll** checkbox in order to navigate through all steps. The **Summary** view shows on a glance if there were any warnings or errors during the screen. The **By Component** view is mainly used by Olympus service to diagnose potential hardware issues.




**Operate ▸ Live View**. This command starts live image acquisition and opens the **Device Control** window that allows to access a selection of hardware settings directly. It can also be executed with the **Live** button.

**Operate ▸ Batch Scan**. This command opens the **Batch Selection** window to create a list of experiment descriptor .xml files and then start a series of scans with different settings.




**Operate ▸ Plate Loader Batch Scan**. This command opens the **Plate Loader Batch Scan** window which allows defining and executing an automated screen of multiple well plates using the automatic plate loader (See Section 6.6, *Multi-level* ).

**Operate ▸ Multi-level Scan**. This command opens the **Multi-level Scan** wizard which allows defining and executing an advanced screen with the aim to automatically re-screen a sample based on the results of a first screen. (See Chapter 7, *Multi-level* ).

**Operate ▸ Start**. This command starts a scan. It can also be executed with the **Start** button.

**System ▸ Initialize Devices**. This command performs a hardware component check. It is necessary, for example, if a device is switched on after the software has been started.

**System ▸ System Configuration**. This command opens the window that allows configuring the hardware modules. (See Chapter 8*, System Configuration*).

**System ▸ Import Plate Types**. This command allows importing new plate type definitions that are not available in the plate type list.

**System ▸ Calibrations ▸ Stage Calibration**. This command starts the stage calibration (See Chapter 8.2, Stage Calibration).

**System ▸ Calibrations ▸ Shading Correction**. This command opens the Shading Correction Image Manager. It is the central location to review, manager, and acquire correction images for the shading correction (See Chapter 8.5, Shading Correction)

**System ▸ Calibrations ▸ Objective Parfocality**. This command allows you to set an offset for the Z-position for the used objectives, so that when changing the objective, the focus is maintained. Therefore enter a value for **Parfocality [µm]** to correct the focus shift for the objectives relative to the objective which you used for the autofocus (See Chapter 8.3, *Objective Parfocality*).

**System ▸ Calibrations ▸ HW-AF Parfocality**. This command allows you to calibrate the ZDC hardware device such that its measurements become independent of the selected objective lens.

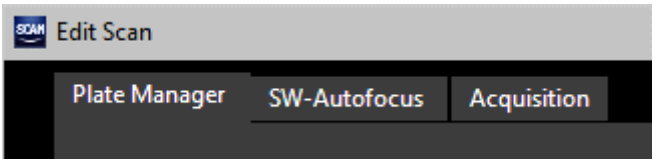
**About**. This command gives system and software information. A support package can be generated which helps the Olympus service in trouble-shooting in case of any issues.

# 4 Setting up a Scan

This chapter gives a detailed description on how to customize scan settings and how to setup the system for maximum performance and reliability. The necessary settings encompass the selection of appropriate microscope, illumination and camera settings, the autofocus setup, the parameterization of the well plates and the details of the imaging to be performed at each position.

The scan$^{R}$ Screening Station is a complex system with a number of hardware devices and a wide range of experimental possibilities. In order to perform a scanning experiment, a complete set of parameters is required with information that allows the controller to synchronize the motorized microscope modules, the illumination source and the motorized stage with the camera, that configures the autofocusing procedure and, last not least, defines the type of imaging experiment to be executed at a precise pattern of positions on the plates and within the wells.

The **Edit Scan** dialog window allows you to modify and verify all settings. To open it, click the **Edit** button or select **File ▸ Edit**.




All modifications in the settings persist until they are changed again. When starting a scan, the complete settings will be stored in the resulting data directory in a file named *experiment_descriptor.xml*. Note that by default the current user settings are stored when the scanR software is exited and these setting will be restored on next startup. This means that when multiple (Windows-) users start the system, for each user scan$^{R}$ will load their default settings.

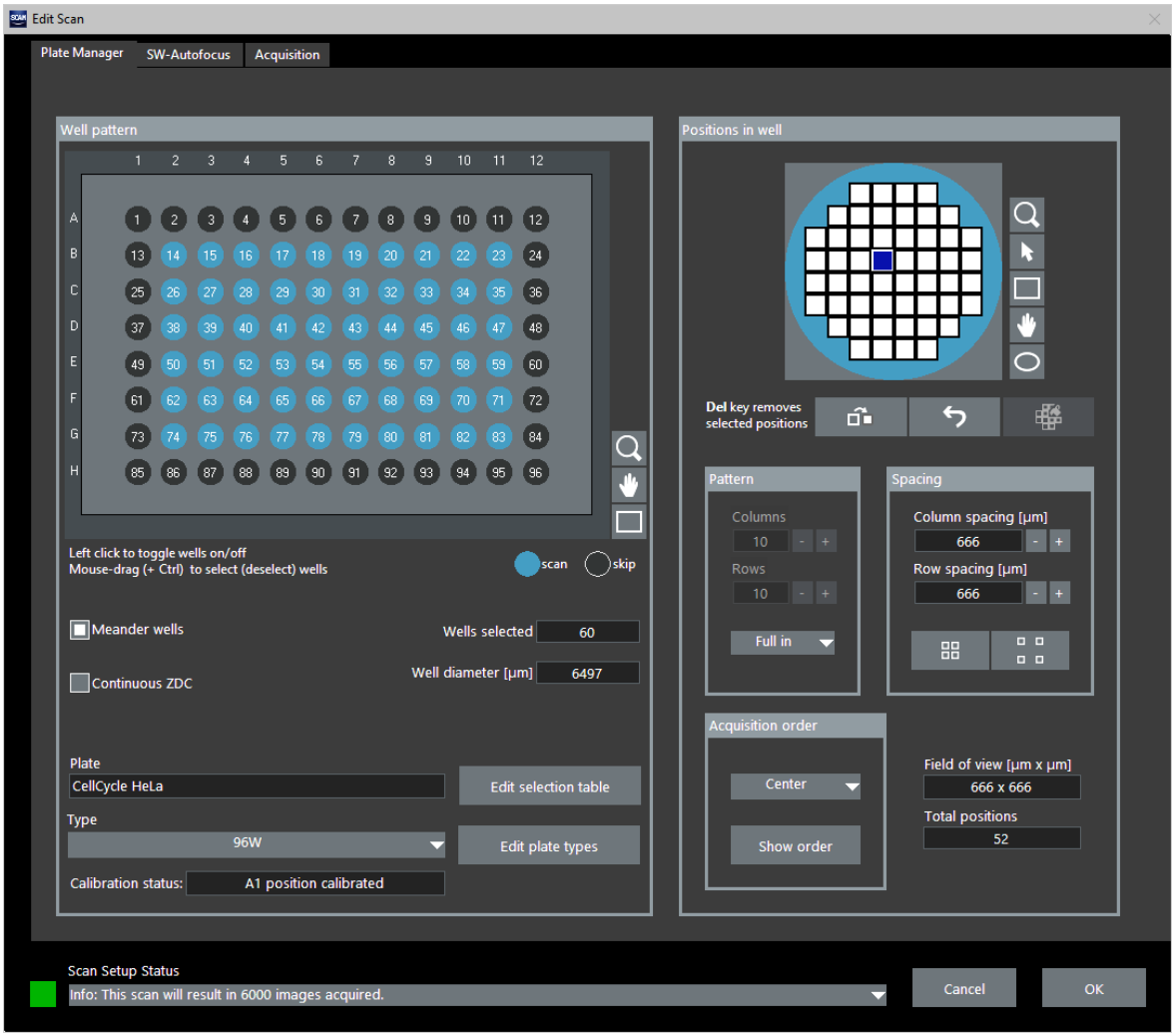
The current user settings can be stored at any time via **File ▸ Save as** (or a click on the **Save** button). User settings can be loaded via **File ▸ Open** (or a click on the **Open** button) from the manually saved files or from any previous experiment by loading the *experiment_descriptor.xml* file saved with the experiment.

The **Edit Scan** dialog window groups the settings on three tabs:

- **Plate Manager** to choose the well plate format, and select the wells and the positions within wells to be imaged.
- **SW-Autofocus** to define and optimize the autofocus procedure
- **Acquisition** to define and visually control the microscope, camera and illumination settings, to set cycle times for scan repetitions and to setup the acquisition of z-stacks

## 4.1 Plate Manager

The **Edit Scan ▸ Plate Manager** tab provides the functions needed to define the pattern of wells and the pattern of positions within each well to be imaged during the scan.




### 4.1.1 Well pattern

The circles (or squares) in the scheme represent the wells of the selected well plate type. Blue circles are wells activated for scanning, gray circles are wells to be skipped. When changing the set plate in the **Type** drop down menu, all wells will be automatically selected. In order to exclude wells from being imaged, deselect these by clicking on individual wells or press the Ctrl-key and drag a region around the wells to be deselected.

**Plate**. Enter a name in the text field. If the plate name is unchanged in the course of consecutive scans, the scan directories will be stored with an incremented numeration appended to the given name.

**Type**. Select the well plate format from the shortlist of standard plate types. It is possible to add custom plate types via **Edit plate types** and the **Plate Type Settings** window; see Section 5, *Well Plate Library*.

**Edit selection table**. This opens the **Well Description** window with the list of selected wells. You can enter a name or description for the selected wells. It is also possible to **Import** a well selection.

**Wells selected**. Shows the number of currently selected wells. All wells are selected by default. Click on a well to deselect it. Click again to select it. To select a group of wells, drag a rectangle around the wells. To deselect a group of wells, press the **Ctrl** key and drag a rectangle around the wells.

**Meander Wells**. Check this option if the wells are supposed to be scanned in meandering order rather than in numeric order (row by row from left to right).

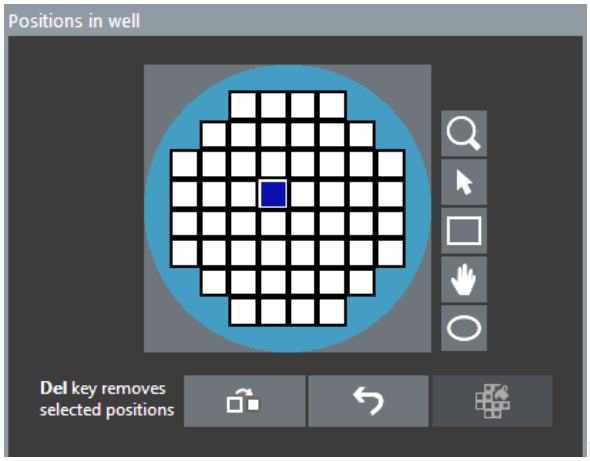
**Continuous hardware autofocus**. Activates a special autofocus mode which will only be available under certain conditions. It is prerequisite the system is equipped with the IX3-ZDC2 revision of the hardware autofocus device. See Section 4.5 *Continuous Hardware Autofocus* for further details.

### 4.1.2 Position patterns

scanR is able to scan a series of positions within each well. The pattern of positions to be scanned is defined in the **Positions in well** box on the right side of the **Plate Manager.** To specify the pattern to be scanned within each well, use the **Positions**, **Spacing** and **Selection** fields. The pattern is based on a basic rectangular pattern and can then be refined by manual selection of a sub-set of positions. The position that will be scanned first is highlighted blue. In order to show a simulation of the order in which the positions are scanned click on **show order**.

The number of **Columns** and **Rows** as well as their distances can directly be entered in the corresponding fields. Alternatively a special **Pattern** can be selected from the shortlist. By default the spacing between adjacent positions is set so that neither an image overlap nor a gap results. It is calculated from objective magnification and camera chip size.

**Pattern.** Set the number of **Columns** or **Rows** that are to be scanned within one well. The dropdown box contains also special patterns like **single**, **2*2**, **full in** and **full out**.

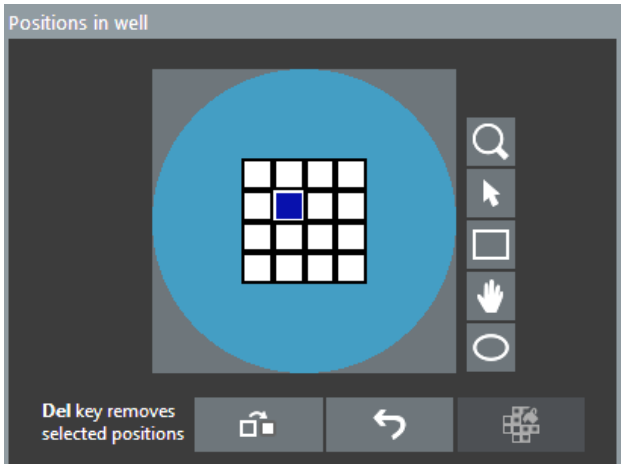


Full in Pattern

**Spacing: Column spacing [µm]**. Set here the distance between (the centers) of two adjacent **Columns** of positions.

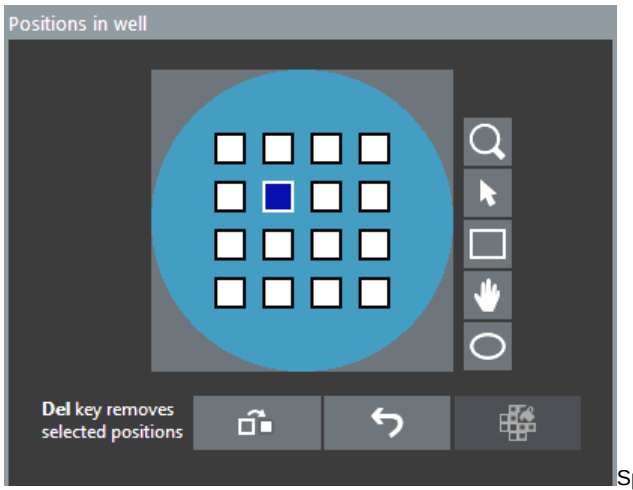
**Spacing: Row spacing [µm]**. Set here the distance between (the centers) of two adjacent **Rows** of positions.

**Spacing:**  **(Tile)**. Click here to reset the spacing between columns and rows to the default (without gaps or image overlap).



Tile

**Spacing:**  **(Spread)**. A click on this button will distribute the positions in equal distances within the whole well. To reset the pattern to adjacent positions, click on **Tile**.

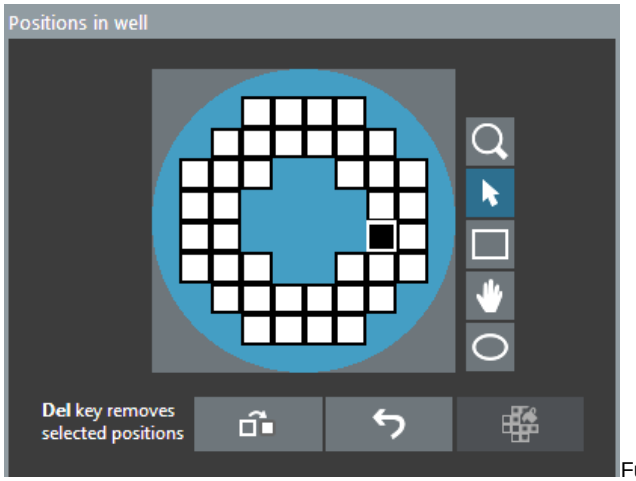


Spread

**Field of view**. This is the specimen area covered by a single image. It depends on objective magnification and camera chip size.

**Total positions**. Shows the number of positions per well.

The scheme shows the basic rectangular positions pattern relative to the entire well (the green circle). By default all positions will be scanned. To modify this pattern use the **tool buttons** on the left to select a sub-set of positions. Remove the selected positions from the well pattern by pressing the **Del** key.



Full in pattern with removed central positions.

**Selection:** **(Invert).** Click here to invert the pattern of selected positions.

**Selection:** **(Reset position defaults)**. Click here to return to the basic positions pattern (as defined by **Columns/Well** and **Rows/Well**) and the default autofocus settings for each position (see the **Selection context menu**).

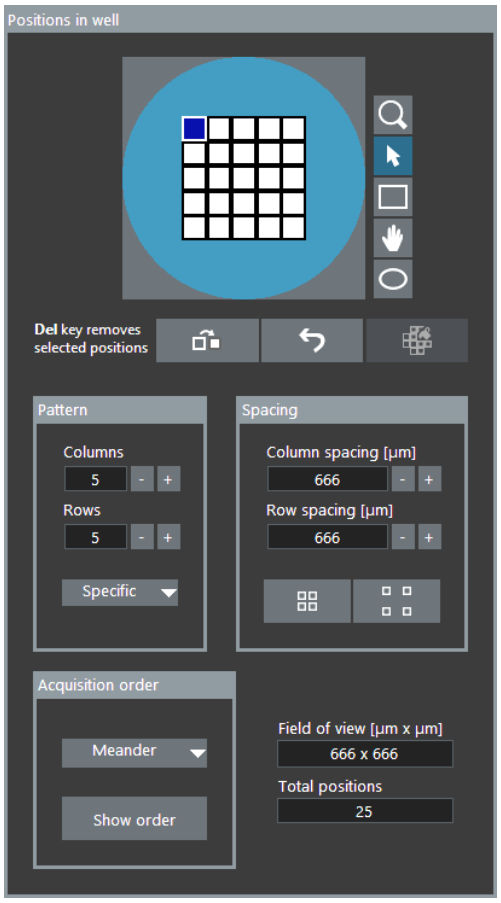
**Selection:** **(Fill)**. Click here to fill a ROI (e.g. a drawn circle or rectangle) with sub-positions.

**Selection context menu**. A right-click on a position opens its context menu. This context menu allows selecting the kind of autofocus to be carried out at every position individually. Depending on the sample it may or may not be useful to carry out the hardware autofocus and/or the coarse software autofocus only at the first position and only the fine software autofocus at each position.

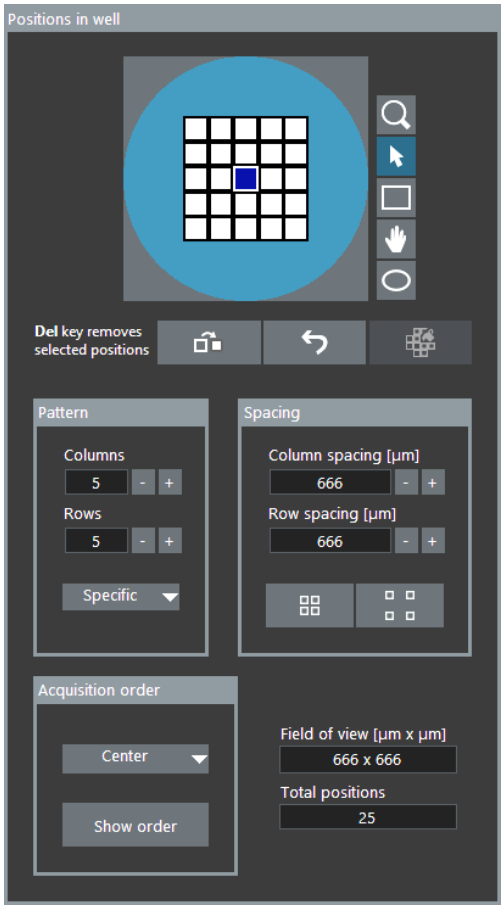

**Acquisition order: Meander**. Click here if the positions are to be scanned in meandering order rather than in numeric order (row by row from left to right).

**Acquisition order: Center**. For arbitrary patterns the position next to the center is selected as starting position. Subsequently adjacent positions are scanned.

**Acquisition order: Unidirectional**. Click here if the positions are to be scanned in numeric order (row by row from left to right).



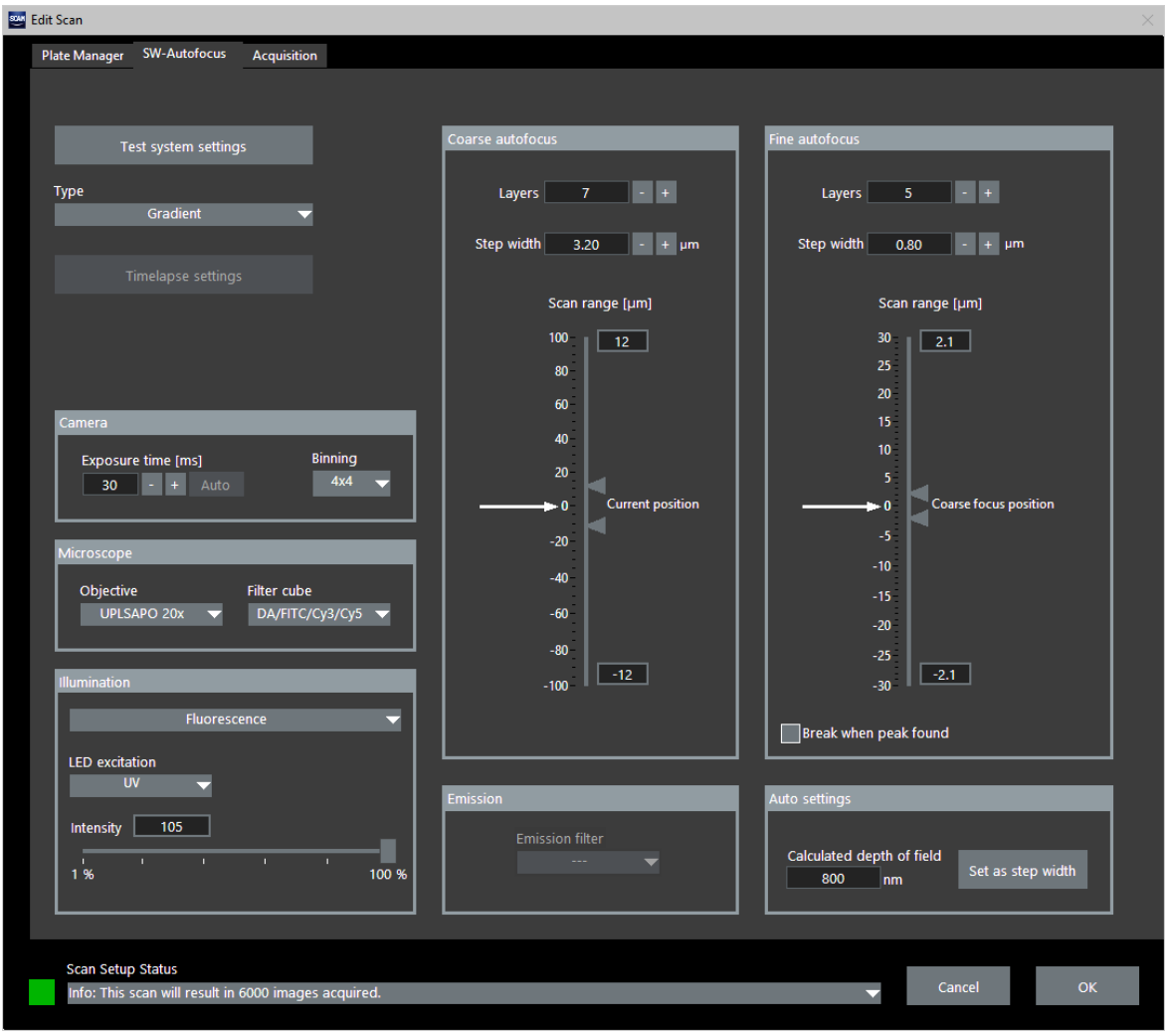

Meander




Center start

**Acquisition order: Show order**. Click here to display an animation of the optimized position acquisition order. Click again to stop the animation.

# 4.2 Autofocus settings




**Test system settings**. Click here to open the **Autofocus Settings** window. It allows you to focus manually and to control if the autofocus channel settings lead to images suitable for autofocusing.

> When opening the **Autofocus settings** window, the **SW-Autofocus** tab remains active. Move the **Autofocus** settings windows aside to access the **SW-Autofocus** tab and change the settings (e.g. exposure time).

**Type**. Select the method to quantify the image focus from the following options:

- **Gradient**. The focus is determined by analyzing mean intensities and intensity gradients of image structures.
- **Object-based**. The software first detects the objects in each image and then determines the focus by analyzing their mean intensities and intensity gradients. The result is weighted with the number of detected objects. The objects have to be defined in the **Autofocus Settings** window, which can be accessed through the **Test system settings** button. See Chapter 4.2.5, Object-based Autofocus).

Note that image acquisition is skipped if no objects are detected to save disk space.

- **Object-based: coarse on A1**. This is the same method as above with the difference that only on the first position of the first well both a coarse and a fine autofocus scan are carried out. On all other positions only the fine autofocus is performed. This leads to an increased imaging speed, however is only suitable for plane samples.
- **Object-based coarse, gradient fine**. This method combines the other methods to determine the focus. The object-based focus quantification is used for the coarse autofocus, while the gradient-based quantification is used for the fine autofocus.

For ease of use it is recommended to select the **Gradient** mode if possible.

**Timelapse settings**. Click here to define the autofocus settings for time lapse acquisitions. If **Repeat autofocus in each time-lapse cycle** is activated, autofocus scans will be repeated in each time-lapse cycle. This reduces the overall speed of the experiment considerably but may be necessary if focus drifts are to be expected. Alternatively, **Repeat only fine autofocus with *n* layers** can be selected or, using **Do not repeat autofocus**, the autofocus procedure can be restricted to the first time lapse cycle.

**Camera**, **Microscope** and **Illumination** settings. (See Chapter 3.4, Microscope Control and Live View)

**Exposure time: auto set.** Use the **auto set** button to obtain a value for the exposure time. The auto **set button** is available only when the **Autofocus settings** window is opened via the **Test system settings** button. The exposure time is set such that the mean intensity of the objects equals a certain predefined value. Note that this value may be much lower than the value specified for the image acquisition channels (see Chapter 3.4.1, *Microscope Settings* and Chapter 4.3, *Acquisition Settings*) to reduce the time necessary for the autofocus procedure. When the original image was saturated it may be necessary to press the button several times in order to set the exposure time properly.

The SW-Autofocus tab will be disabled completely when the **continuous hardware autofocus** (cf. Section 4.5 *Continuous Hardware Autofocus*) is enabled.

### 4.2.1 Coarse autofocus

The following settings are interdependent and the software will adjust the controls using the equation Scan range = Layers x Step width.

**Scan range [µm]**. Use one of the sliders or type in a value to set the search range for the coarse autofocus. Make sure that the range is large enough to encompass all possible variations of focal positions caused by the unevenness of the substrates.

If the scan range is set too large, it may be possible that the autofocus focuses on the wrong surface, for example on dust particles underneath the plate.

**Step width [µm]**. The step width between neighboring Z-positions is set automatically to four times the **Calculated depth of field** (see next Chapter 4.2.2, *Fine Autofocus*). However, it can be changed at will here.

**Layers**. This is the number of layers to be imaged during the coarse autofocus scan. It is automatically calculated from **Step width** and **Scan range**.

**Interpolate AF start positions**. This function is deprecated and probably will be removed in future versions of the scan$^R$ software.

**Collect reference positions**. This function is deprecated and probably will be removed in future versions of the scan$^R$ software

### 4.2.2 Fine autofocus

**Calculated depth of field**. This is the theoretical depth of field as determined by the NA of the selected objective. It is not necessary to set the step width significantly smaller than this value. An approximation (450/NA$^2$ nm) is used for the calculation of the depth of field.

**Set as step width**. Click here to set the **Calculated depth of field** as step width between neighboring Z-positions of the fine autofocus scan. The number of **Layers** will be adjusted automatically.

This function additionally sets the step width of the **Coarse autofocus** to four times the **Calculated depth of field** and adjusts the number of **Layers** accordingly.

The following settings are interdependent and the software will adjust the controls using the equation Scan range = Layers x Step width.

**Scan range [µm]**. Use one of the sliders or type in a number to set the imaging range for the fine autofocus. Reasonable values to start are +10 µm and -10 µm.

**Step width [µm]**. You may set here a value different from the **Calculated depth of field** as step width to be made between neighboring Z-positions during the fine autofocus scan.

**Layers**. This is the number of layers to be imaged during the fine autofocus scan. It is automatically calculated from **Scan range** and **Step width**.

**Break when peak found.** When setting this option, the focus search finishes *n* layers (**Layers after peak**) after a possible focus position has been found. This behavior is particularly useful for transmission images to increase accuracy of the focus search

### 4.2.3 Gradient Autofocus Adjustment: Step-by-step

The **Gradient Autofocus** algorithm determines the focus by analyzing local intensity gradients of image structures.

1. Select the **Gradient** autofocus procedure.

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2. Select suitable Camera, Microscope and Illumination settings.

3. Click the **Fine autofocus: Set** as step width button to automatically set the **Fine autofocus: Layer and Step width Step width**.

4. Set the **Fine autofocus: Scan range** to +/-10 µm, for example.

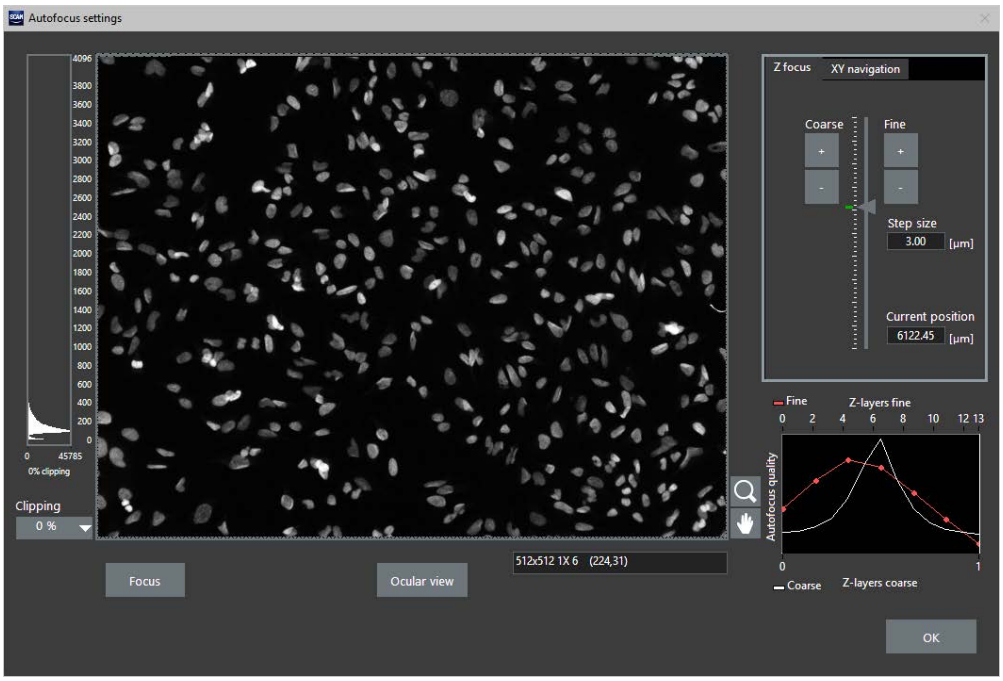
5. Set the **Coarse autofocus: Step width** to 10 µm. It should not be larger than half the **Fine autofocus: Scan range**.

6. Set the **Coarse autofocus: Scan range** to +/-50 µm, for example.

7. Once finished, click the **Test system settings** button.

8. The **Autofocus settings** live image window will open and display a live image acquired with the settings of the **SW-Autofocus** tab...

9. Focus manually using the Z-focus wheel on the microscope or use the Z-focus tools (see Chapter 3.4.2, *Z Focus*) in the software to focus the sample roughly. You can use **Ocular View** to switch the microscope from camera view to ocular view when using the Z-focus wheel on the microscope to set the focus.




10. In order to change the illumination or microscope settings, you can move the **Autofocus settings** window aside to have access to the **Edit scan ▸ SW-Autofocus** tab. The changes made in the **SW-Autofocus** tab will immediately be applied.

11. Click **Focus** to run the autofocus. First the coarse AF is performed and afterwards the fine AF. Afterwards the focused image is displayed in the main panel and on the graph on the right

the quality of the AF procedure is displayed. The focusing was successful when both curves (for fine and coarse AF) show a pronounced peak.

12. To optimize coarse and fine range change the corresponding settings in the **SW-Autofocus** tab and click again **Focus** in the **Autofocus settings** window to run the AF procedure. (You can also navigate to other positions on the plate using the **XY navigation** tool, (see Chapter 3.4.3, *XY Navigation*)

13. When you are satisfied with the result close the **Autofocus settings** window by clicking **OK**.

### 4.2.4 Object-based autofocus: step-by-step

The **Object-based Autofocus** algorithm first detects the objects in each image and then determines the focus by analyzing their local intensity gradients.

The weighting function of the coarse autofocus is calculated from the sum of all objects' sharpness functions and is very sensitive to the number of objects detected. The **Object-based Autofocus** properties must be adjusted carefully to ensure that the number of objects is maximal when the image is focused.

The **Object based** autofocus may be suitable if a certain structure placed in a certain Z-layer within the sample is to be focused (e.g. small cells growing upon larger cells). By setting min / max cell size accordingly, it can be ensured that the desired objects are used for autofocus.

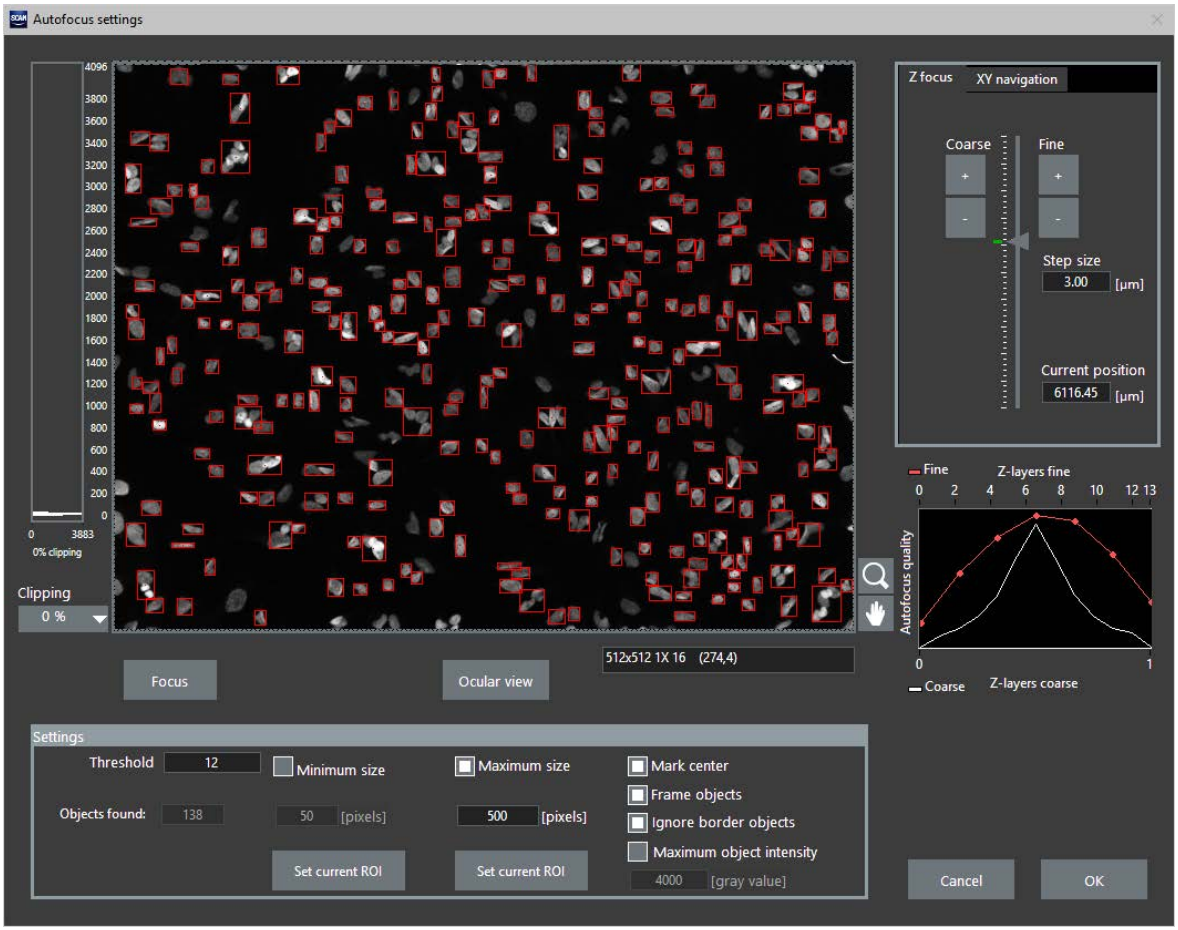
> Note that the **Object-based Autofocus** properties are important also because images free of detected objects will not be stored on hard disk during the scan.

Click the **Test system settings** button on the **Edit Scan ▸ SW-Autofocus** tab to open the **Autofocus Settings** window. The functions needed to adjust the object recognition are shown in the **Autofocus Settings** window.

**Threshold**. The threshold causes all image areas – better said all image pixels – of lower intensity to be ignored for object detection. This prevents that background noise or low-intensity artifacts are falsely detected as objects. The value should be somewhat higher than the background intensity but lower than that of any cell or structure of interest.

**Minimum size**. Activate this option if you want to set a minimal size for detected objects. Enter the minimal number of pixels in the box below.

**Maximum size**. Activate this option if you want to set a maximal size for detected objects. Enter the maximal number of pixels in the box below.




It is possible to use regions of interest (ROIs) to set the maximum and minimum size. To do so, click the **ROI** button (rectangle icon next to the bottom right corner of the live image) and draw a (green) rectangle to define the respective ROI. Click **Set current ROI** to adopt the values of the ROI.

**Minimum cell size: Set current ROI**. Click here if you have defined a ROI that determines the minimal size of the objects.

**Maximum cell size: Set current ROI**. Click here if you have defined a ROI that determines the maximal size of the objects.

**Mark center**. Activate this option in order to mark the center of each detected object with a red dot.

**Frame object**. Activate this option in order to mark each detected object with a red bounding frame.

**Ignore border objects**. Activate this option in order to have objects touching the image borders ignored.

**Maximum object intensity.** Activate this option to have objects ignored which exceed the provided gray value

1. Select the **Object-based** autofocus procedure.

2. Select suitable Camera, Microscope and Illumination settings.

3. Click the **Test** button on the **Edit Scan ▸ SW-Autofocus** tab to open the **Autofocus Settings** window

4. Focus manually using the Z-focus wheel on the microscope or use the Z-focus tools (see Chapter 3.4.2, Z Focus) in the software to focus the sample. (You can use **Ocular View** to switch the microscope from camera view to ocular view).

5. Set a value for **Threshold**. To determine a meaningful value, activate the pointer tool by clicking the **arrow** button in the tool bar at the bottom right side of the image window. Move the cursor over a background area towards the border of an object. The image information bar below the image window list the intensity at that point. For an initial try you may set the threshold to such a borderline value.

6. Activate **Minimum cell size** and click the **ROI** and draw a rectangle which is somewhat smaller than the smallest object to be detected.

7. Click the **Set current ROI** below **Minimum cell size**. The value is entered in the corresponding pixel field. Alternatively you can directly enter a value in the pixel field.

8. Activate **Maximum cell size** and click the **ROI** and draw a rectangle which is somewhat larger than the largest object to be detected.

9. Click **Set current ROI** below **Maximum cell size**. The value is entered in the corresponding pixel field. Alternatively you can directly enter a value in the pixel field.

10. Check **Mark center** and **Frame object**.

11. Click **Focus** to run the autofocus. First the coarse AF is performed and afterwards the fine AF. Afterwards the focused image is displayed in the main panel and on the graph on the right the quality of the AF procedure is displayed.

12. The detected objects are indicated by a red box.

# 4.3 Acquisition settings

**Selected Color Channels** list. This lists all currently defined color channels in the order of acquisition. Select a channel by mouse click for modification. Drag a channel up or down to alter the channel acquisition order.

**New**. Click here to add a new color channel to the list. The new channel will be given the name *Blank* by default and can be changed in the **Name** box. It will be used for the image file name upon storage.

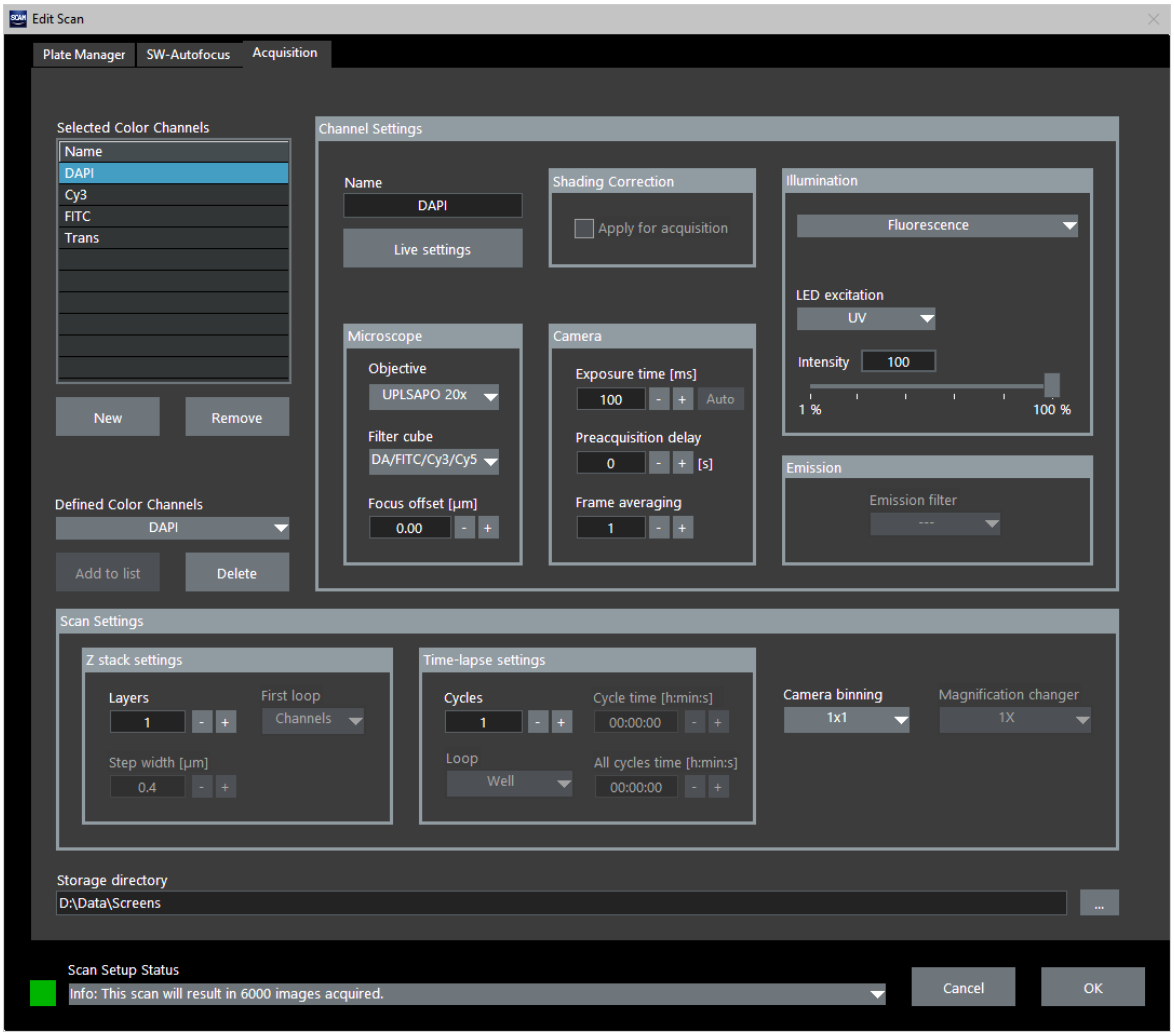
**Remove.** Removes a channel from the selected color channels. The channel will be stored in the **Defined Color Channels** list.

**Defined Color Channels List** of predefined color channels. The predefined color channels can be changed in the **Device** window (**Settings ▸ Devices**).

**Add to list.** Adds a selected color channel from the **Defined Color Channels** to the **Selected Color Channels**. The color channel will be added at the bottom of the list.

**Delete**. Click here to delete the listed **Image type** from the **Defined Color Channels** list.

**Live settings**. Click here to start a live image acquisition in a new window that opens. This allows observing the influence of the settings changes online. See Chapter 4.4, *Live Settings*. Note that first an autofocus scan will be carried out. When no ZDC is available, a starting position has to be selected for the autofocus procedure. After that an autofocus is performed and the **Live settings control** window opens.




**Microscope: Objective**. Select the objective to be used. Note that a switch of objectives during an experiment is possible but rather time consuming. In order to maintain the same focus position for different objectives use the **Objective Parfocality** setup (see Chapter 8.3, *Objective Parfocality*). If the **Global objective change** option is activated in the System Configuration (see Section 8.1.2, *Microscope*), the objective will be kept synchronized between all Color Channels and the autofocus settings.

**Microscope: Filter cube**. Select the filter cube to be used. Imaging speed can be increased considerably by using multi-band filter sets.

**Microscope: Z offset [µm]**. This allows setting a Z-position offset for the image acquisition relative to the position found by the autofocus. The offset is useful for imaging structures that are located in a

different Z-position within the sample than the position found by the autofocus. The Z-offset is most conveniently set interactively in the **Live Settings** window (see Section 4.4, *Live Settings*).

**Shading Correction: Apply for acquisition**. This option can be activated if the necessary correction images for the selected color channel and the current objective are available (see Chapter 8.5, *Shading Correction*). Activating this option will apply the shading correction to the acquired images before they are saved to the disk. Independent of this option, all available and relevant correction images will always be stored with the acquired data.

**Camera: Exposure time [ms]**. The exposure time determines the period of time during which the camera sensor chip is sensitive to incoming light, in other words, during which photons are collected and converted into charges to be read out afterwards. Enter a value manually or use the **Auto set** button. The type of camera attached to the system will be automatically detected. For the Hamamatsu EM-CCD camera the **camera** control box contains also a field to set the **EM Gain**. Set 0 for no gain and 255 for max gain.

**Camera: Auto**. Use the **Auto** button to obtain a value for the exposure time. The **Auto set** button is available only when the **Live settings control** window is opened via the **Live Settings** button. The exposure time is set such that the mean intensity of the objects equals a certain value. When the original image was saturated it may be necessary to press the several times in order to set the exposure time properly. (Note that when using the **Auto set** button in order to set the exposure time for different assays, the value set for the intensity threshold object detection algorithm of the scan$^R$ Analysis software will be the same for these assays).

**Camera: Preacquisition delay [s]**. You may set a delay to be applied before the color channel is acquired. Enter a preacquisition delay, e.g. if transmission light intensity is to be changed during acquisition to wait for the light intensity to reach a constant value.

**Camera: Frame Averaging**. If the number of frames set here is larger than 1, multiple frames will be acquired for each position and Color Channel and the images will be averaged to reduce noise. This is particularly useful for transmission images with high intensity but low contrast. For performance reasons, the averaging will only be applied during a scan and not in live view.

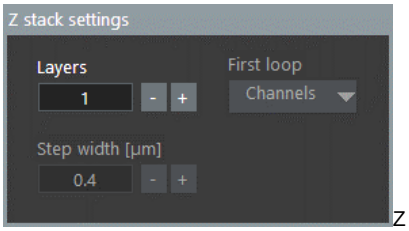
**Illumination: Fluorescence / Transmission / Fluorescence (CSU)**. Select one of the two possible wide field illumination modes: epi-fluorescence illumination with the MT20/LED, or transmission illumination. Optionally, if the system is equipped with the CSU confocal option, a third mode for confocal imaging can be selected. Special settings for the CSU option are described in Section 4.6, *Confocal Scanning*.

**Illumination: Excitation filter / Contrast insert / LED excitation**. Select a MT20 excitation filter or LED channel for a **Fluorescence** image, or a transmission condenser contrast insert for a **Transmission** image, respectively. It has to match with the **Microscope: Filter cube** selection.

**Illumination: Light intensity.** Use the slider to set the illumination intensity of the MT20, the LED, or the transmission light, respectively.

**Storage Directory**. Set here the general scan data directory. The data for each individual plate will be stored here in a subdirectory with the same name as the plate.

### 4.3.1 Z stack settings



Z stack settings disabled

In the **Z stack settings** box you can set the number of **Layers** for the z stack as well as the **Step width**.

**Z-stack settings: Layers**. Set the number of layers of the Z-stack.

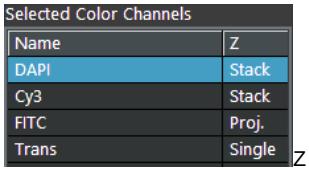
**Z-stack settings: Step width [µm]**. Set the distance between the layers of the Z-stack.

In the drop down menu **First loop** you can select the order of image acquisition for the z stack. With the setting **First loop: Z stack**, the system will first acquire the whole stack for one color channel and then repeat the z stack acquisition for the next color channels. With the **First loop: Channels** setting, the system first acquires images for all color channels at one z position and then goes to the next z position until the z stack is complete.

Note that the Z stack settings are grayed as long as 1 layer is set. The other options are grayed out in order to indicate that no z stack experiment is acquired. As soon as more than 1 **Layer** is set, the **Z stack settings** will be enabled.

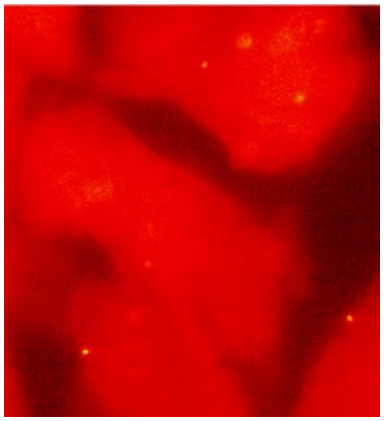
Z stack settings enabled

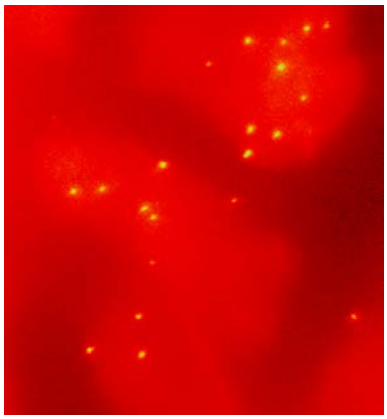
Furthermore, the **Selected Color Channels** list box will offer additional settings per channel. Using the context menu of the list box (by right-clicking on a channel), it is now possible to select for each active channel if a z-stack is acquired, or a projection or only the in-focus image.

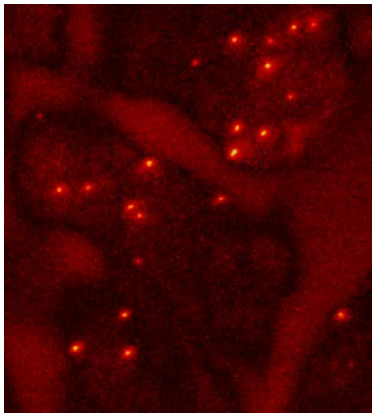


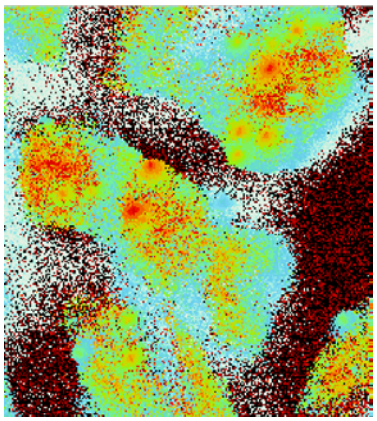
Z stack settings

**Z-stack settings: Projections**. If this option is selected for a channel, a Z-stack will be acquired but not saved as such. The software calculates a *maximum intensity projection* (*fitc_zMax.tif), a *maximum-minus-mean intensity projection* from Z-stack (*fitc_zMaxBkg.tif) and a *layer-of-origin image* of the maximum intensity projection (*fitc_zMaxZ.tif) and only keeps the central layer (i.e. the layer found by the AF procedure) of the original stack (*fitc.tif). All four images are then being stored as a four-channel image. (Recommended to use with high Numerical Aperture (NA) objectives; use *_zMaxBKG.tif for object segmentation in the Analysis).


fitc.tif


fitc_zMax.tif


fitc_zMaxBkg.tif


fitc_zMaxZ.tif

**First loop: Z stack.** First all layers of the z stack for the first color channel are acquired, then a z stack for the next color channel is acquired, and so on. This has the advantage that the time consuming steps such as filter cube change and excitation filter change are performed only once at the beginning of a z stack.

**First loop: Channels.** When this option is selected for every layer of the z stack all selected color channels are scanned consecutively. Only when all color channels are acquired, the objective moves to the next z position and then all color channels at this position are acquired, and so on.

### 4.3.2 Time-lapse settings

**Time-lapse settings: Loop**. Select the kind of time-lapse experiment: for the whole **plate**, for a **well** or on each single **position** of a well.

**Time-lapse settings: Cycles**. Set the number of times the entire plate scan is to be repeated.

**Time-lapse settings: Cycle time**. Set the repetition time. If this time is smaller than the time required for a single run, the repetition will be performed as fast as possible without delay.

Note that the time-lapse settings are grayed as long as in the **time-lapse** control boxes only 1 **cycle** is set. The other options will be grayed out in order to indicate that no time-lapse experiment is acquired

### 4.3.3 Other scan settings

**Camera binning**. The camera sensor chip is composed of many light-sensitive units (pixels). These pixels can be read out individually (binning = 1x1) or the signal of neighboring pixels can be combined electronically during data readout (binning > 1x1). Binning reduces the spatial resolution but increases the sensitivity and thus reduces the exposure time required for a good signal-to-noise ratio. It further reduces the amount of data and consequently increases the readout speed. Therefore binning is recommended if weak signals have to be detected at high acquisition rates and if spatial resolution is of minor importance.

**Magnification Changer.** This option is available for systems equipped with an encoded magnification changer. The software will take care that the actual magnification changer position is consistent with this scan setting.

## 4.4 Live settings

Click the **Live settings** button in the **Edit Scan ▸ Acquisition** tab to come to the **Live settings control** window that allows you to observe the changes of the settings on the image acquired by the camera.

1. If a ZDC hardware autofocus is available the system will first perform a complete hardware and software autofocus run as defined in the **Edit Scan ▸ SW-Autofocus** tab; see Chapter 4.2, *Autofocus Settings*)

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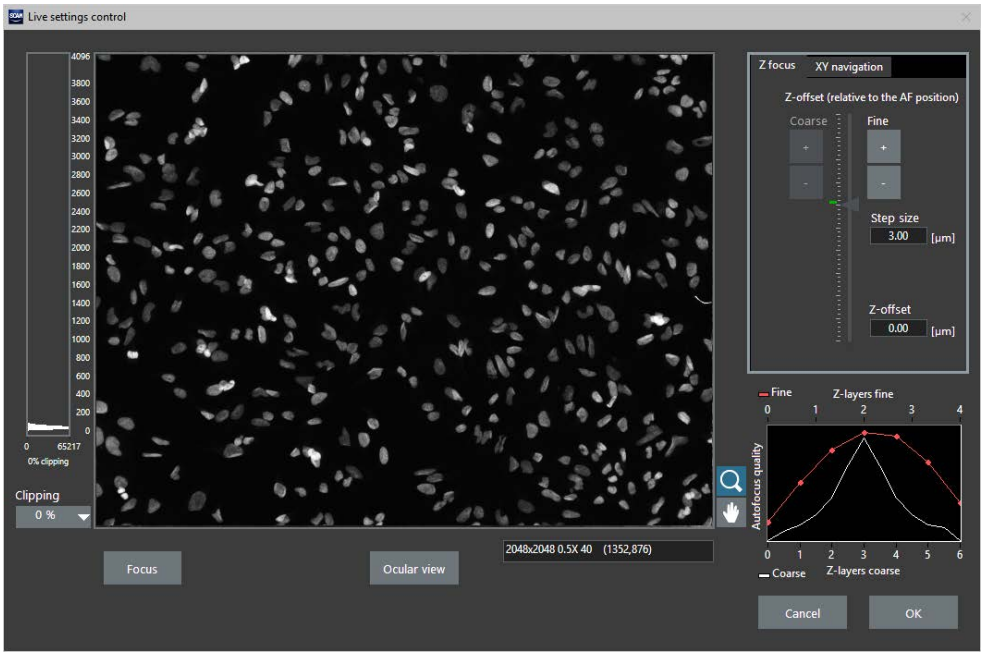
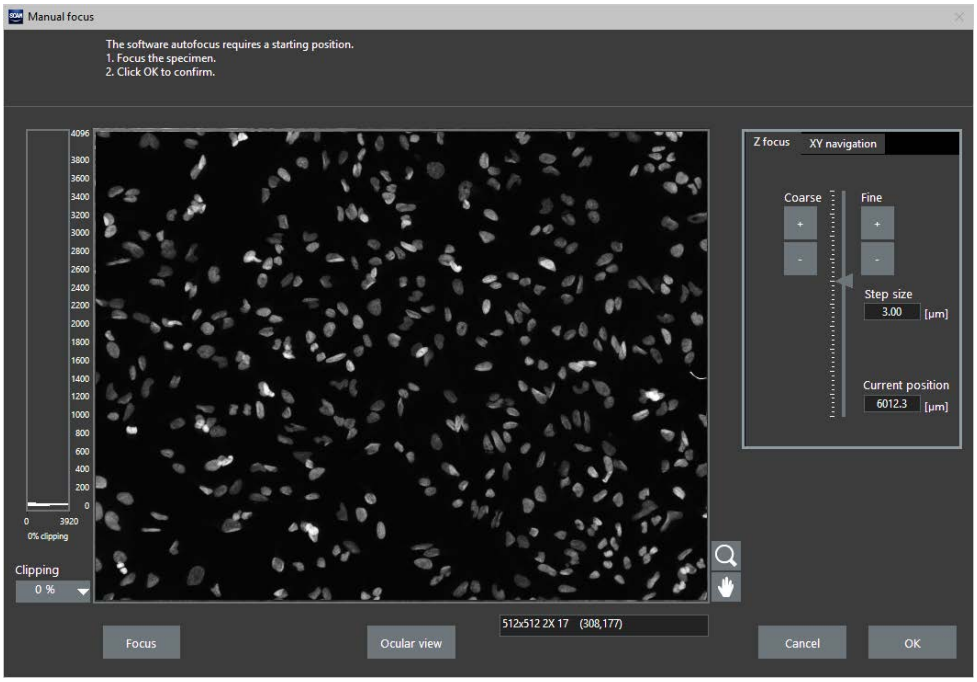
2. When the AF procedure is completed, the **Live settings control** window opens. The quality of the autofocus is shown as a graph.



1. If no ZDC is available and the focus of the active plate type has not been calibrated, the starting position for the software Autofocus has to be set first. In this case the **Manual Focus** window opens. The settings of the **SW-Autofocus** tab are used for this display (i.e. the color channel used for autofocus is shown).



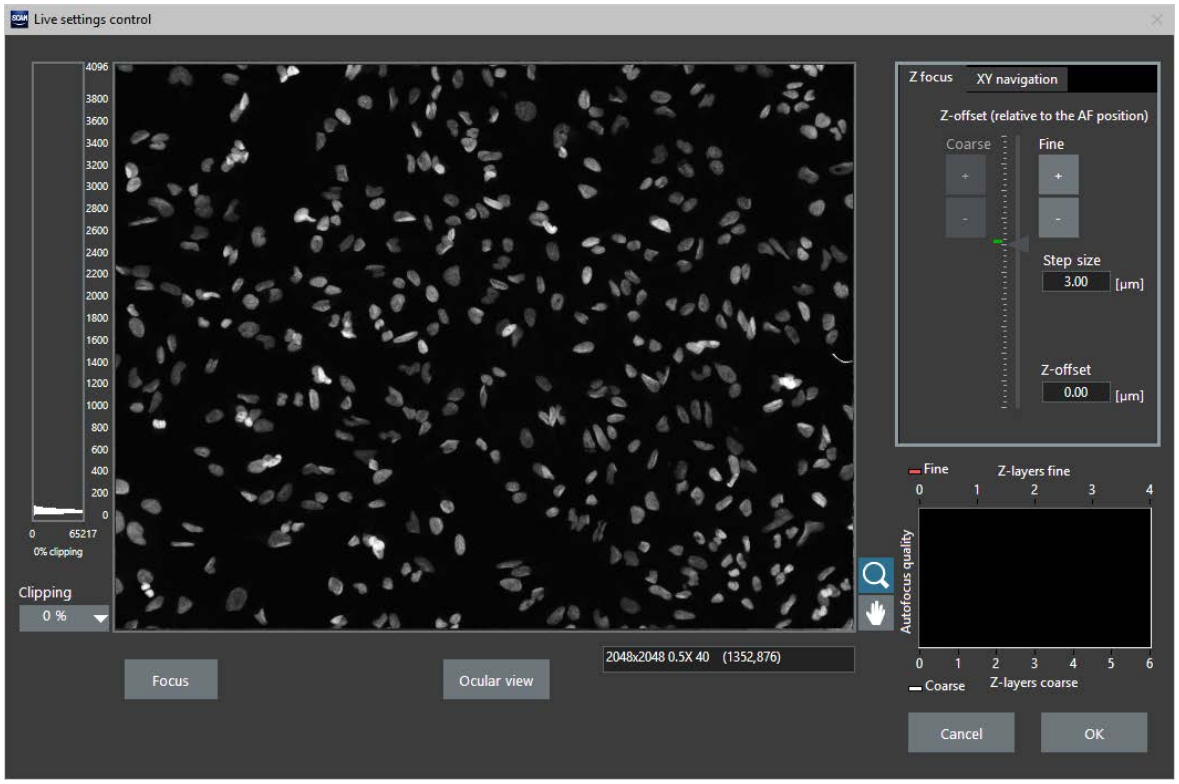

2. Focus manually using the Z-focus wheel on the microscope or use the Z-focus tools (see Chapter 3.4.2, *Z Focus*) in the software to focus the sample. (You can use **Ocular View** to switch the microscope from camera view to ocular view).

3. Click **OK** to start the software autofocus.

4. Once the autofocus run is completed the **Live Settings control** window will open. The quality of the autofocus is shown as a graph.

> When opening the **Live settings control** window, the **Acquisition** tab remains active. Move the **Acquisition settings** windows aside to get access to the **Acquisition** tab and to change the settings (e.g. color channels, exposure time, etc.).

You can now change and check the settings in the **Edit scan ▸ Acquisition** tab and have control over these settings in the **Live settings control** window simultaneously. The status bar below the live image display gives information about the image size, the display zoom factor, the image type as well as the intensity and the coordinates of the cursor. Check the exposure time for all color channels and avoid over-saturating the camera by keeping the maximum intensity well below 4,000 or 65,000 counts (for a 12-bit camera or 16-bit camera, respectively). Adjust the **Camera: Exposure time** on the **Edit Scan ▸ Acquisition** tab accordingly.




Depending on the model, the camera can have bit depths of 12 or 16 so that saturation is reached at pixel values of 4,095 or 65,535, respectively. However, the pictures are alwys stored with 16 bit depth for reasons of computer efficiency.

**Z-offset (relative to the AF position)**. Use the arrow buttons to determine the focus offset of the different channels relative to the position found by the autofocus. The offset is useful for imaging structures that are located in a different Z-position within the sample than the position found by the autofocus. The **Z-offset** set in the **Live settings control** window is also entered in the **Z-offset** field of the **Edit Scan ▸ Acquisition** tab. Color channels are switched by selection on the **Edit Scan ▸ Acquisition** tab. (Note that in the **Live settings control** window you can only change the **Z-offset**, but not the Autofocus properties.)

**Autofocus quality: Coarse** and **Fine**. These two graphs enable you to judge the performance of the software autofocus. If either graph shows a pronounced peak, the focus has been found and the settings in the **Edit Scan ▸ Autofocus** tab are suitable. If the maximum of one or both curves is located at the left or right border of the scan range, the settings have to be adjusted.

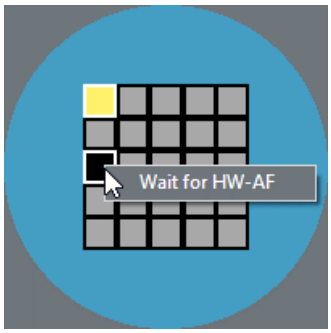
If the **Autofocus quality** functions do not show a pronounced peak, the focus has likely not been found. Enlarge the **Coarse autofocus: Scan range** in the **Edit Scan ▸ SW-Autofocus** tab or try to find a more reasonable starting position.

# 4.5 Continuous Hardware Autofocus

The continuous mode is a special mode for hardware autofocusing. It is only available if the scanR system is equipped with the IX3-ZDC2 hardware revision of the hardware autofocus device. The basic concept is that the sample always is kept in focus, even when moving from position to position or from well to well.

While the continuous focusing mode has limitation regarding flexibility, and high demands regarding the well plate bottom quality, it will be really fast if possible to use. Continuous mode is restricted to scanning a single z-plane (no combination with inter-channel offsets, z-stacks or software autofocus possible) and requires a high-quality well plate bottom which has low variations in thickness, depending on the depth-of-field of the used microscope objective lens.


Activate **Continuous hardware autofocus** in **Edit Scan ▸ Plate Manager** tab. This will automatically de-activate all options which cannot be combined with continuous mode, including the complete **SW-Autofocus** tab.

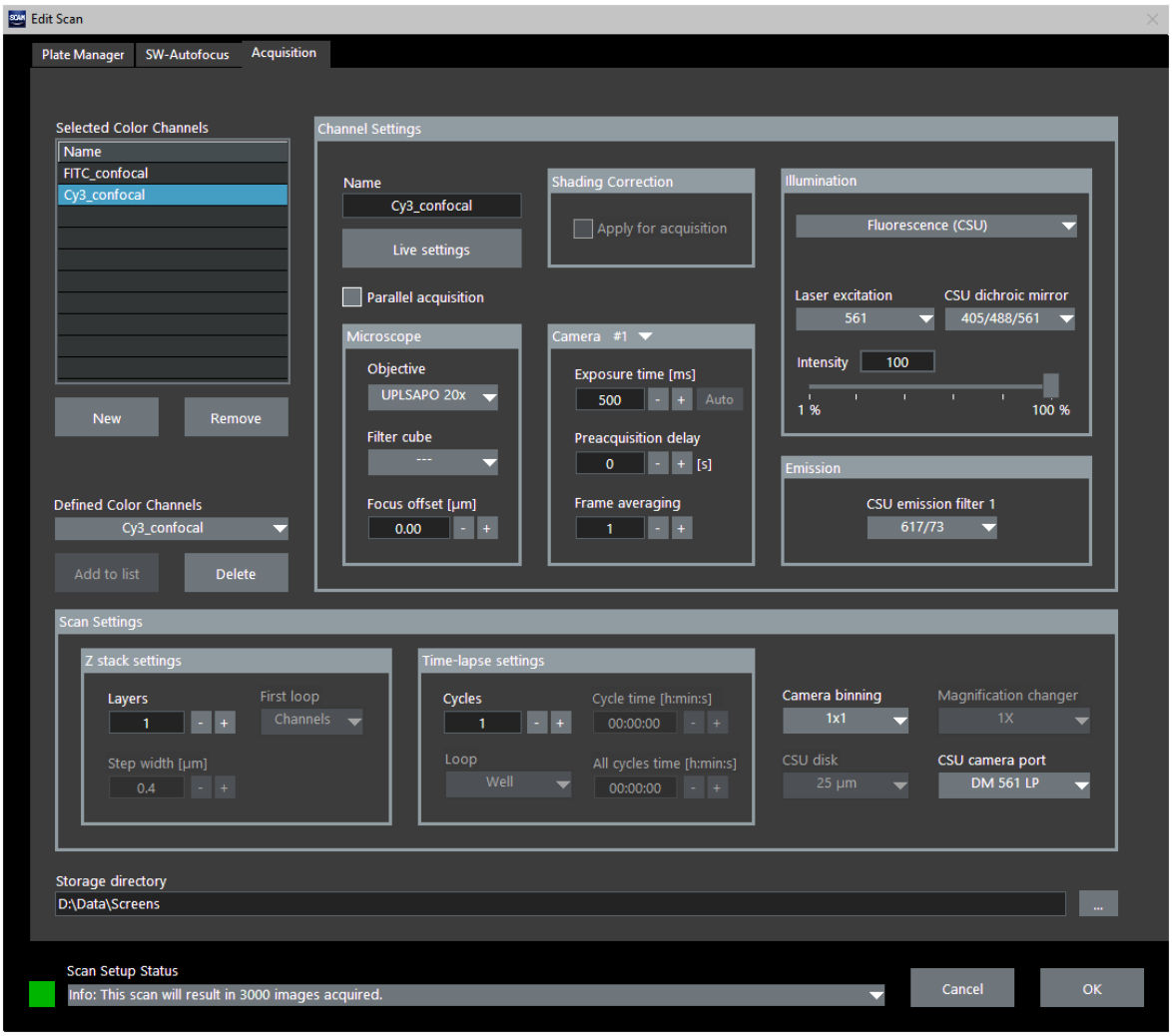



In the **Positions in well** configuration tool, individual positions can be configured to **Wait for HW-AF** which will verify actively that the sample is still in focus there. Depending on the plate properties and settings, the effective focusing time per position can be << 100 ms.

# 4.6 Confocal scanning

The optional Confocal Scanner Unit (CSU) adds the capability of high-speed confocal imaging to the scanR screening station. When this option is available, there are additional control elements visible in the workflow of setting up a scan. In particular, the **autofocus settings** and the **acquisition settings** have

the additional confocal imaging mode **Fluorescence (CSU)** in addition to the wide field modes **Fluorescence** and **Transmission**.




**Illumination: Laser excitation**. A laser light source is used for confocal imaging. Depending on the configuration, one or multiple different laser colors can be used for fluorescence excitation.

**Illumination: CSU dichroic mirror**. The suitable dichroic mirror within the CSU device.

**Emission: CSU emission filter 1**. The suitable emission filter within the CSU device. In case of the dual camera option, the CSU emission filter 2 can be selected for camera 2.

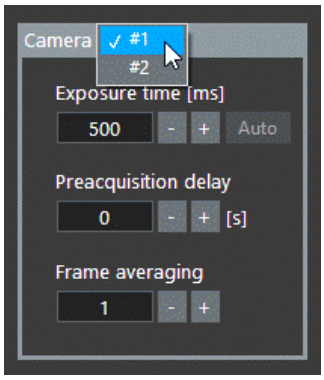
**Scan Settings: CSU disk**. Select a different spinning disk for this scan (optional).

**Scan Settings: CSU camera port**. Select the camera port splitter (optional; only in combination with dual camera option).

The optional CSU specific control elements are also visible in Live View when the confocal scanning option is available for the scan$^{R}$ system.

# 4.7 Dual camera acquisition

The dual camera option enables the acquisition of two color channels with best possible speed. The dual camera option requires the confocal scanning option which includes the necessary image splitting optics and two independent emission filter wheels for the two cameras.




When the dual camera option is available, the **Camera** configuration in the **autofocus settings**, the **acquisition settings** and the **Live View** enables the selection of the camera (**#1** or **#2**) to be used for the autofocusing or for the current color channel.

## 4.7.1 Acquisition settings

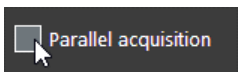
Dual camera acquisition can increase the scanning speed significantly if it is used in such a way that two color channels which are acquired successively fulfil the following requirements:

1. The two successive channels are assigned to the two different cameras. For example, the first channel uses the camera **#1**, the second channel uses the camera **#2**.
2. The two channels share the same imaging mode, for example both channels are **Fluorescence (CSU)** channels
3. The two channels are only different in the **excitation wavelength** and the **CSU emission filter**

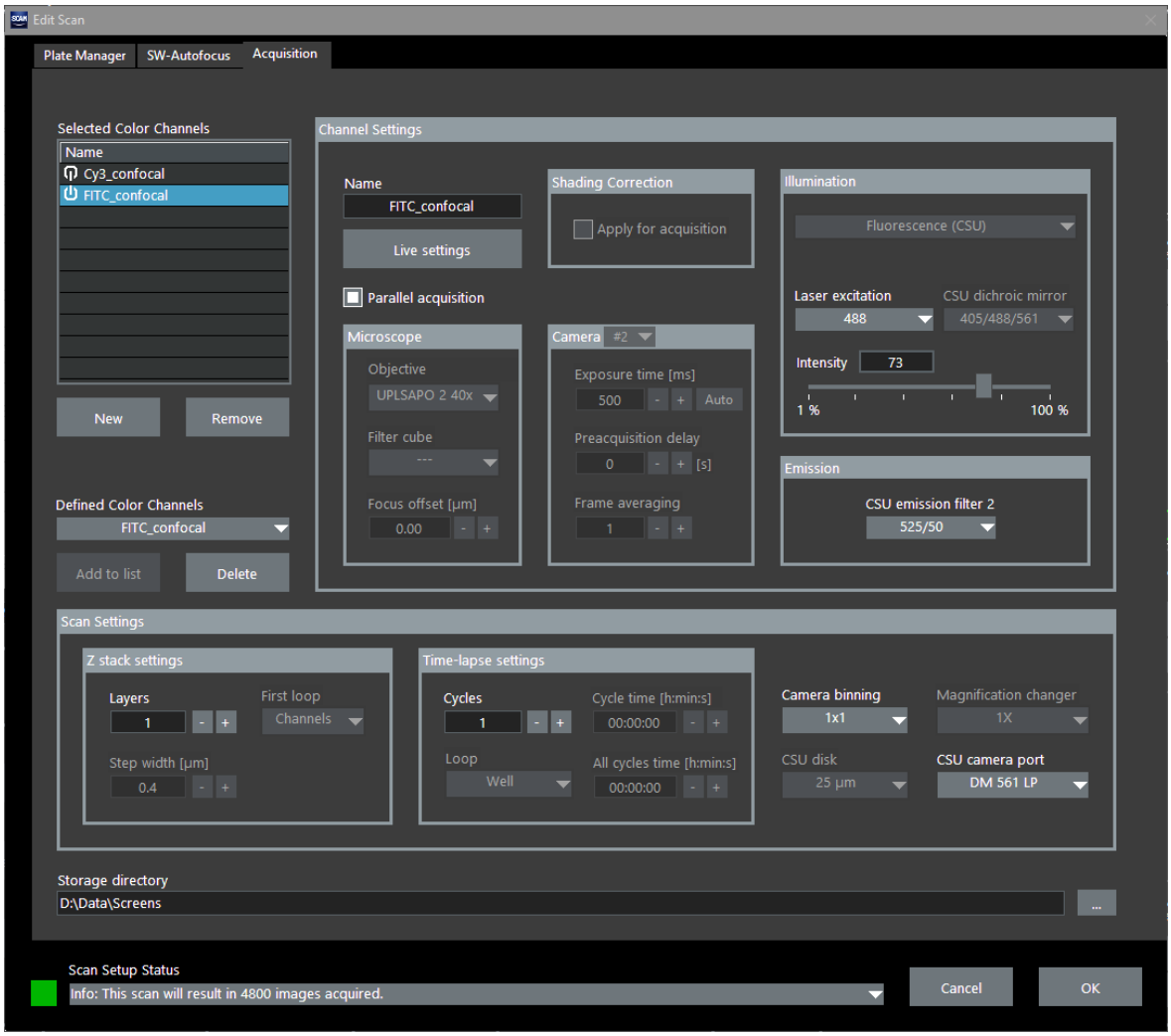
If these prerequisites are fulfilled, the two color channels can be acquired without the need for any mechanical movement in between.

## 4.7.2 Parallel acquisition

If the prerequisites for maximum scanning speed (see Section 4.7.1, *Acquisition settings*) are fulfilled, two channels can even be acquired at the very same time. In order to enable **Parallel acquisition** for two color channels, the option has to be activated for the second channel of a pair.



As a result, the two color channels will be linked and acquired as a pair at the very same time. This will increase the acquisition speed most because both cameras are exposed simultaneously. On the other hand, cross-talk (bleed-through) between the two color channels might be increased.




Two linked channels are indicated by a connecting symbol in the **Selected Color Channels** list. For the second channel of a linked pair, all control elements which cannot be different for a pair of linked channels are disabled.

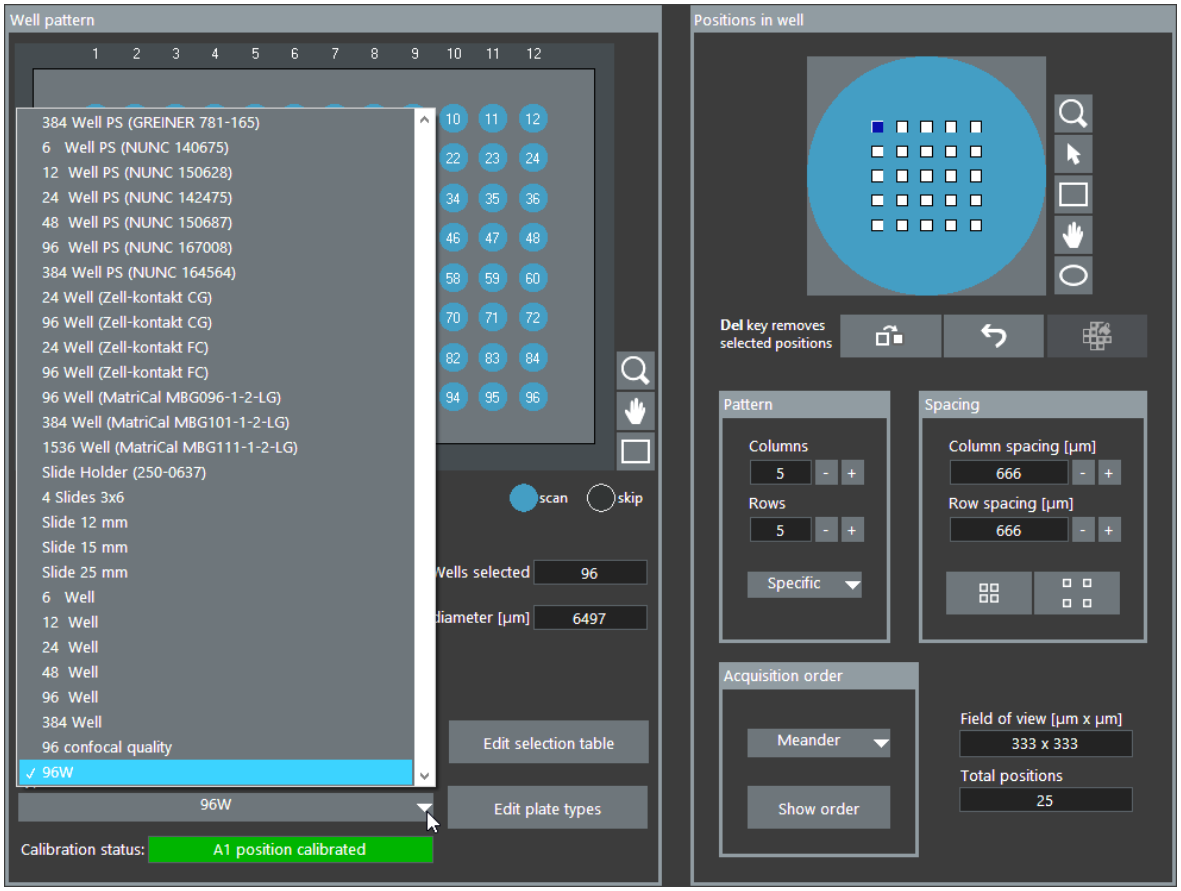
A pair of linked channels can be split at any time by unchecking the **Parallel acquisition** option for the second channel. This will result in normal, successive (sequential) acquisition with less increased scanning speed but minimal crosstalk (bleed-through) between the two channels.

# 5 Well Plate Library

scanR features a well plate library which allows easy management of different well plate types. A number of typical well plates are predefined and can be simply selected when setting a new scan. The software, however, is very flexible and enables defining even unusual and non-standard well plate and other dish formats for easy use in various scans.

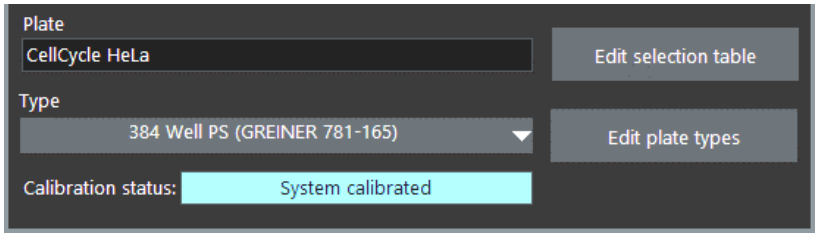
# 5.1 Well plate types

There are three different classes of well plate types which can be selected when setting up a scan.



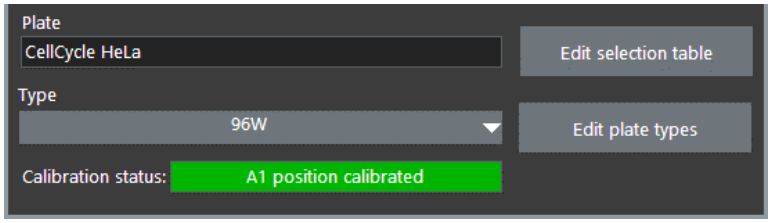

## 5.1.1 System standard well plates

These are certain commercial plates with hard-coded dimensions using the specifications of the manufacturers. Their parameters are not editable in the **Plate Manager**. The A1 position of these plates is always determined after stage initialization upon software start – and thus does not have to be determined prior to the start of a scan.
Standard well plates are highlighted blue in the **Plate Manager**.



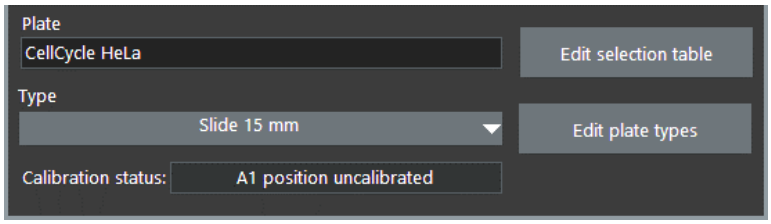
### 5.1.2 User calibrated well plates

This can be any type of well plate in principle. Once such a plate type is calibrated by the user, the A1 position of such a plate is always determined after stage initialization upon software start – and thus does not have to be determined prior to the start of a scan.
Calibrated well plates are highlighted green in the **Plate Manager**.

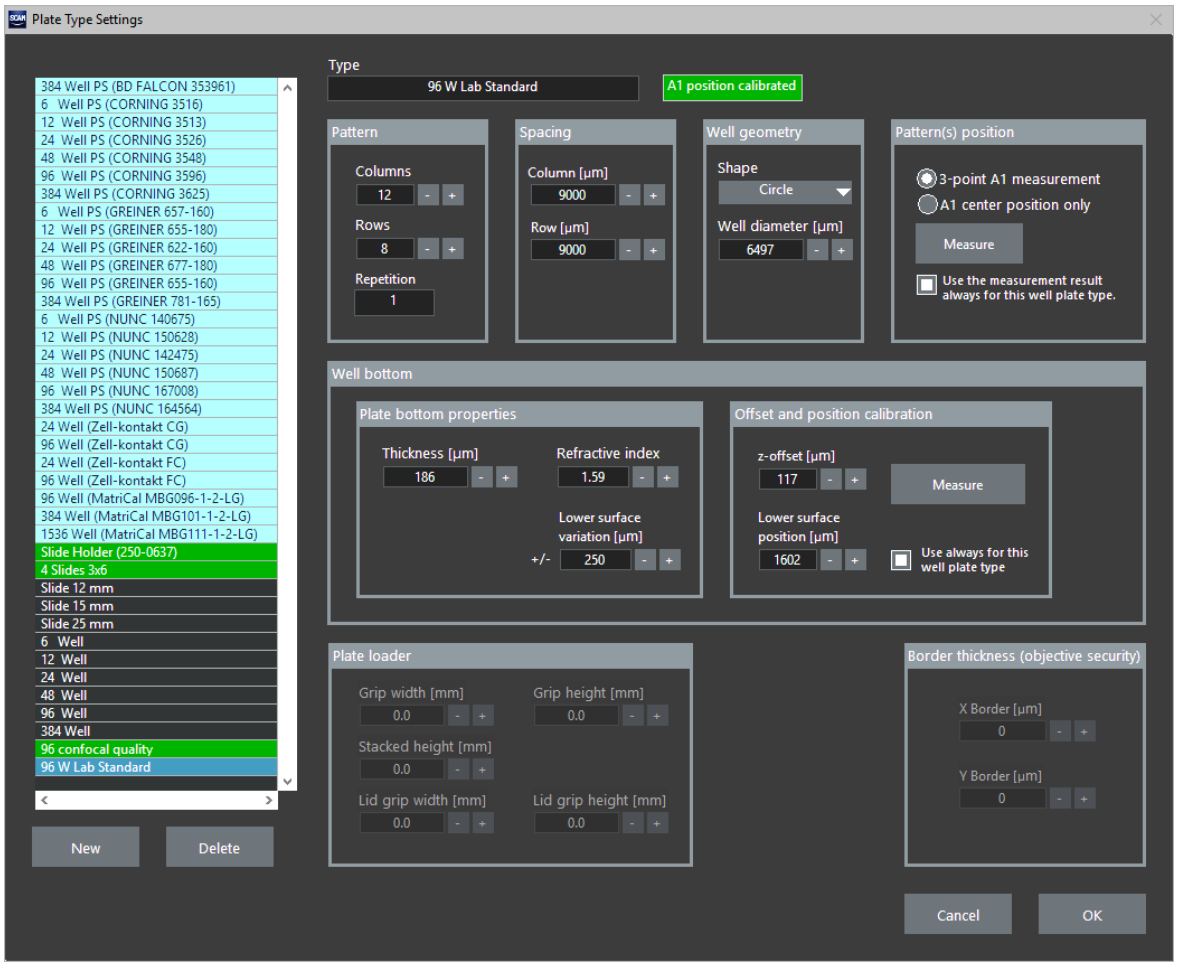



### 5.1.3 Non-calibrated well plates

Before the start of a scan the user is asked to move the stage to the A1 position. Non-calibrated well plates are not highlighted in the **Plate Manager**.



# 5.2 Plate type settings




The button **Edit plate types** in the **Edit Scan ▸ Plate Manager** tab to open the **Plate Type Settings** window. Here, new plates can be created and existing plates can be changed (geometry of the plate and A1 position). The well bottom related settings can also be found here (**Well bottom** box); these values are used as starting parameters for the hardware and software autofocus. When a plate loader is used for automated delivery of plates to the microscope, the relevant parameters can be selected in this window as well (**Plate loader** box). The **Plate loader** parameters define the way the plate is optimally gripped by the loading robot.

On the left side of the window, the currently available plates are listed. New plate type definitions that are not available in the plate type list can be imported with **System ▸ Import Plate Types**. You can customize the order of the plate list by clicking on one of the plates in the list and dragging it to another position in the list. By this means, a customized plate list can be created. This modified plate list will then be available in **Edit ▸ Plate Manager** in the **Type** drop-down menu.

The blue marked plates are system calibrated plates. The settings of the system calibrated plates cannot be changed. The message “System calibrated format, Press “new” to modify the format” is shown right to the plate type. The settings for green and white plates (“custom plates”) can be changed. For green plates the A1 position has already been calibrated (see Section 5.4, *Well Measurement*), whereas for plates that are not calibrated the A1 position has to be set when the scan is started.

**Delete**. Click here to remove the selected custom plate type from the shortlist. Standard plate types cannot be removed.

**New.** Creates a new plate type based on the selected plate type.

**Type**: Enter here the name of the well plate type as it is to be listed in the shortlist.

**Plate geometry settings**

**Pattern: Columns, Rows**. Set here the number of columns and rows of wells on the plate, respectively.

**Pattern: Repetition**. Indicates if a standard well plate has been defined (i.e. a well plate with a single A1 position) or if a special format has been defined with multiple A1 positions (e.g. multi slide plate holder)

**Spacing: Column [µm], Row [µm].** Set here the distance between the centers of two adjacent well columns and rows, respectively.

**Well geometry**. Select here the **Shape** (circle or rectangle) and the **diameter** of the wells.

**Pattern(s) positions:** The position of the well plate pattern is defined by the position of the A1 well. It is possible to have multiple A1 wells in order to design plate geometries with multiple sub-patterns (e.g. 4 slide holder. See Section *5.3 Defining Multi-Slide Plate Holder*)

**Pattern(s) positions: 3-point A1 measurement.** See Section *5.4 Well Measurement***.**

**Pattern(s) positions: A1 center position only**. See Section *5.4 Well Measurement.*

**Pattern(s) positions: Use the measurement result always for this well plate type.** Uncheck this option if you want to have the plate marked as “A1 position uncalibrated” in order to set the A1 position individually on scan start.

**Pattern(s) positions: Measure.** Starts the A1 calibration process. Please make sure a matching well plate is available on the microscope sample holder.

**Well bottom**

**Well bottom: Plate bottom properties**: **Thickness [µm]**. Set here the thickness of the plate bottom. This is only necessary for systems with hardware autofocus. The value usually can be taken from the well plate’s data sheet. Alternatively, it will be calculated from the **Offset and position calibration** values once they have been measured.

**Well bottom: Plate bottom properties**: **Refractive index:** Set here the refractive index of the well bottom material; only necessary for systems with hardware autofocus. This value will be used to calculate well plate bottom **Thickness** from **z-offset** and vice versa.

**Well bottom: Plate bottom properties**: **Lower surface variation [µm]**. Set here a value for the variation of the z-position of the well plate bottom. This will be used as search range for the hardware autofocus. In general this value should not be too small for successful autofocusing. If the hardware autofocus tends to find the upper surface instead of the lower surface for a given well plate, the value should be adjusted to be smaller than the z-offset.

**Well bottom: Offset and position calibration: z-offset [µm].** This is the distance the microscope z-drive has to travel from the lower surface of the plate bottom to the upper surface. It is strongly related to the plate bottom **Thickness** value: **Thickness [µm]** = **Refractive index** * **z-offset**. When an accurate value for **Thickness** has been entered, this value does not need to be measured. If high accuracy is required, the **z-offset** can be measured by the system and the value for **Thickness** is also updated then.

**Well bottom: Offset and position calibration: Lower surface position [µm].** Set here the distance from the bottom corner of the plate to the well bottom. If the **Use always for this well plate type** checkbox is activated, this z-value is used as starting position for hardware or software autofocus. If the checkbox is unchecked, the system will ask for the starting position on scan start.

**Well bottom: Offset and position calibration: Measure.** The values for **z-offset** and **Lower surface position** can be determined by pressing this button. The actual measurement is done manually or supported by the hardware autofocus, depending on the system configuration.

**Plate border settings**

**Border thickness [objective security] X/Y Border [µm]**. Enter a value only when the plate or slide holder extends over the insert. Otherwise use default values.

**Plate loader settings**

**Plate loader: Grip width [mm]**. Defines the width of the well plate at the height where it is gripped by the plate loader.

**Plate loader: Grip height [mm]**. The height at which the plate loader fingers will grip the well plate.

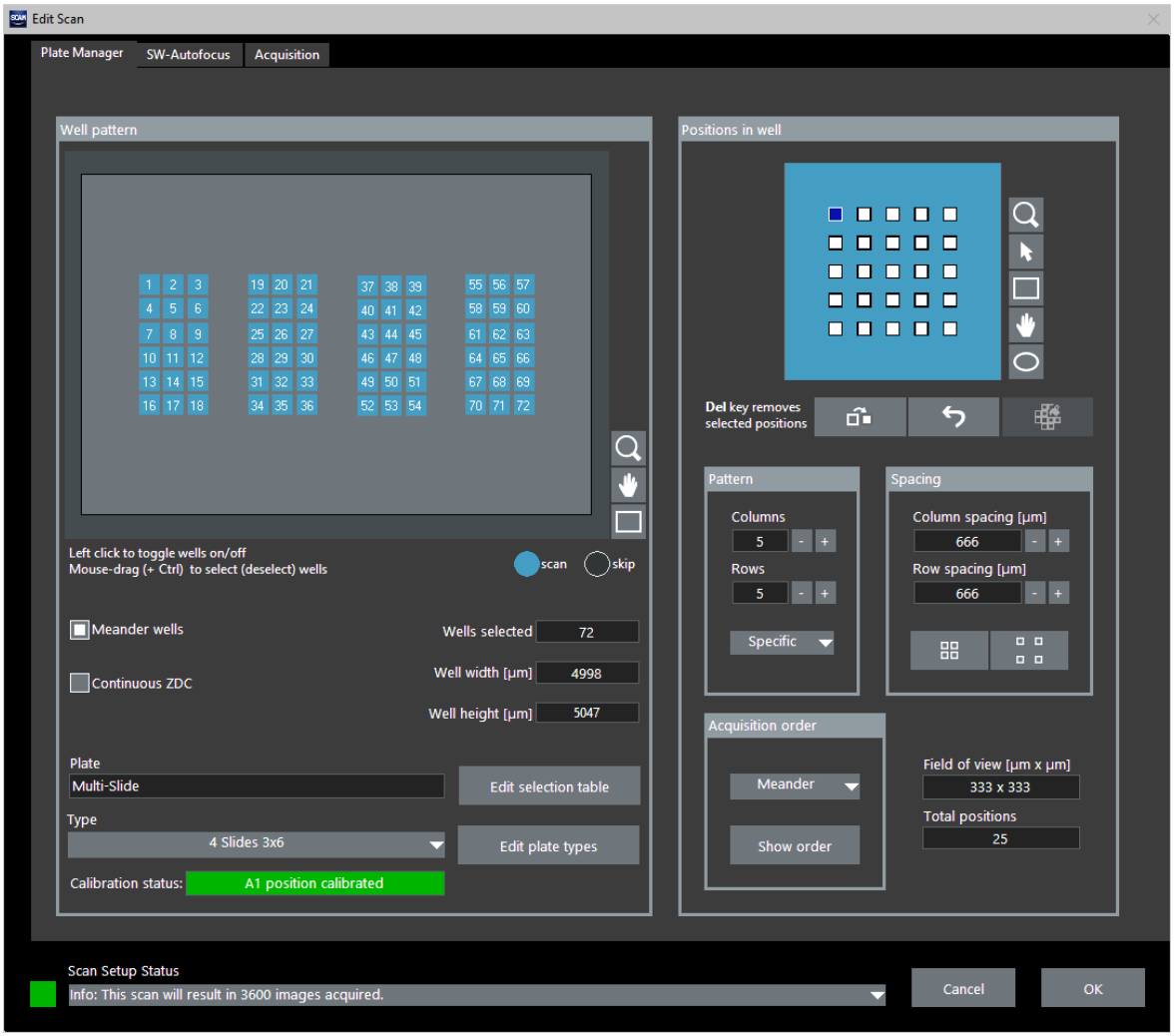
**Plate loader: Stacked height [mm]**. The effective height of the well plate when multiple plates are stacked.

## 5.3 Defining multi-slide plate holder

**Edit ▸ Plate Manager ▸ Edit plate types** opens the **Plate Type Settings** menu. Here also well-patterns for multi-slide plate holders can be defined. These multi-slide plates can be loaded via robot and are supported by the analysis software. One multi-slide consisting of several individual slides can be analyzed in a single run.

To create a multi-slide plate, the pattern of a single slide has to be defined using **Pattern**, **Spacing** and **Well geometry**. The A1 position can be defined by **2-/3-point measurement** or **A1 center position only**. The definition of A1 has then to be repeated for each of the multiple slides. The number of slides defined will be indicated in the **Repetition** field in the **Pattern** box.

This allows also defining all kinds or regular patterns as plate types.




Example for a multi-slide plate consisting of 4 individual slides with wells.

# 5.4 Well Measurement

scanR requires information about the position of the first well (A1) of the plate in order to start the automated scan. All other positions are then defined by the values given in the **Plate Type Settings**. Often it is sufficient to determine the position of the well center roughly, but in some cases a more precise measurement is necessary. The two different procedures are described in the two following sections.

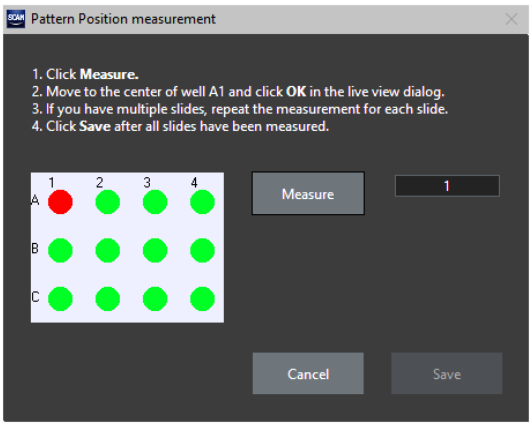
**Use the measurement result always for this well plate type**. Check this check box if you do not want to repeat the A1 well measurement (see below) for each scan. The stored position will then be used each time this plate type is selected. Once the measurement has run successfully, a message in light green will be displayed right of the **Type** box in the plate type settings window: “A1 position calibrated”. Plate loader operation is only possible with calibrated plates.


For convenience and reproducibility it is recommended to select **Types** with defined first well positions (blue / green background) whenever possible even if no plate loader robot is used. This avoids having to set the scan starting position before each scan.

## 5.4.1 Well measurement: A1 center position only

**A1 center position only**. Choose this option if, for all relevant experiments for this plate type it is sufficient to roughly set just the center position of the first well (e.g. if only central positions of each well are to be measured).

**Measure**. Click here to open the **Pattern Position Measurement** window and proceed as follows.




1. Click **Measure** to open a live image window.

2. Move the center of the first well into position.

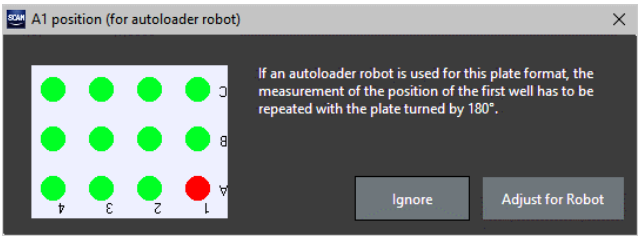
3. Confirm by clicking **OK**.

4. Repeat steps 1 to 3 if more than one A1 position is required (see Section *5.3 Defining Multi-Slide Plate Holder*)

5. Confirm by clicking **Save**

If the scan$^R$ system is equipped with an automatic plate loader, the **A1 position (for autoloader robot)** window will open. Click **Ignore** if no autoloader robot is used for this plate type.

The plate loader handles the plates by turning them by 180°. This has to be accounted for in the measurement. Proceed as follows if a plate loader robot is to be used.




1. Mount the plate turned by 180° as depicted in the scheme.

2. Click **Adjust for Robot** to open a live image window.

3. Move the center of the first well into position.

4. Confirm by clicking **OK**.

### 5.4.2 Well measurement: 2/3-point A1 measurement

**2/3-point A1 measurement**. Choose this option if the experiment and the plate type require a precise determination of the first well position (e.g. when using full-in to measure all positions of one well). Depending on the well shape (rectangular/circular), two or three points have to be set. In case of a rectangular well shape, the two opposite corners of the well have to be set. In case of a circular well, three points on the border of the well have to be determined.

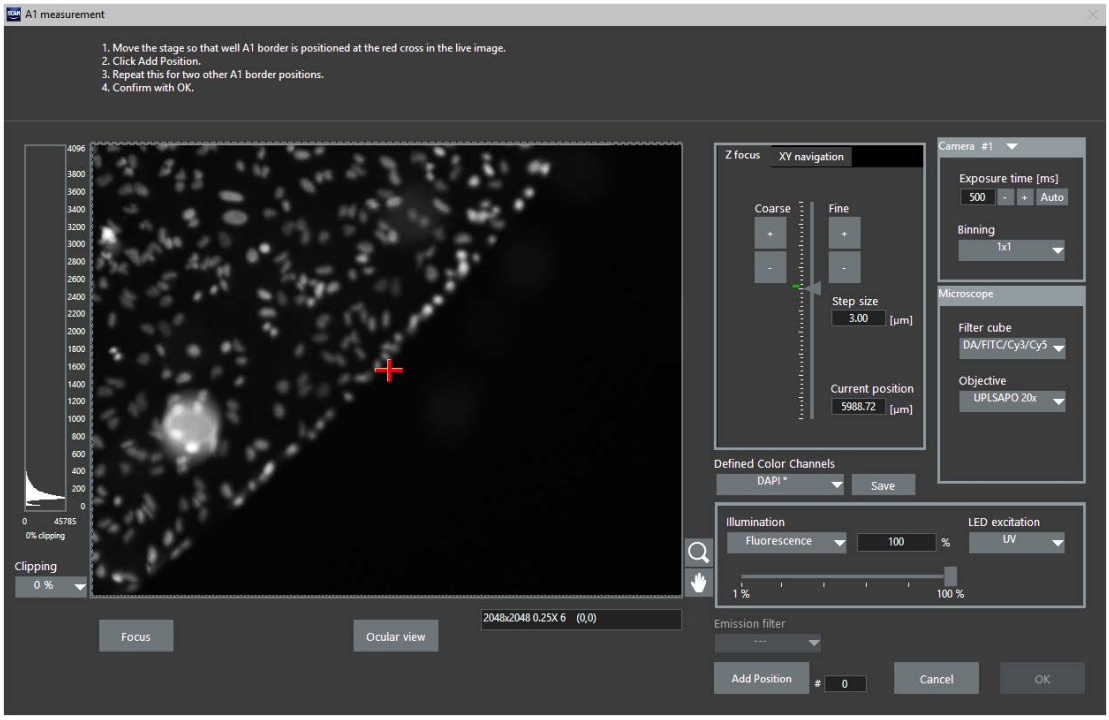
**Measure** Click here to open the **Pattern Position Measurement** window and proceed as follows.

In case of circular wells:

1. Click **Measure** to open a live image window.

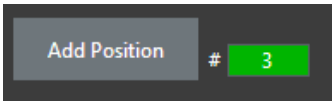
2. Move the border of the first well to the red cross (center) in the live image window.

3. Click **Add Position**.




4. Repeat this for two more well-border positions.

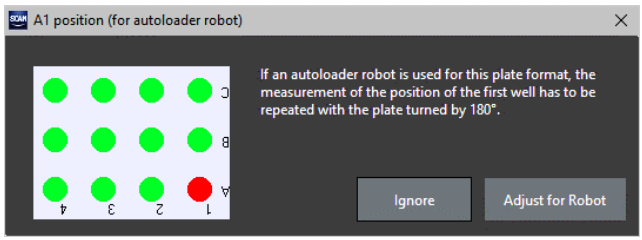
5. After the third position has been added, the numbers field next to the **Add Position** button turns green to indicate that the measurement is completed. Confirm by clicking **OK**.




6. Repeat steps 1 to 5 if more than one A1 position is required (see Section *5.3 Defining Multi-Slide Plate Holder*)

The three positions per well are used to determine the **Well diameter** (that will be set automatically in the **Plate Type Settings** window) and the center position of well A1.

If the scanR system is equipped with an automatic plate loader, the **A1 position (for autoloader robot)** window will open. Click **Ignore** if no plate loader robot is to be used with this plate. Click **OK** to repeat the measurement with the plate rotated by 180°.




In case of rectangular wells:

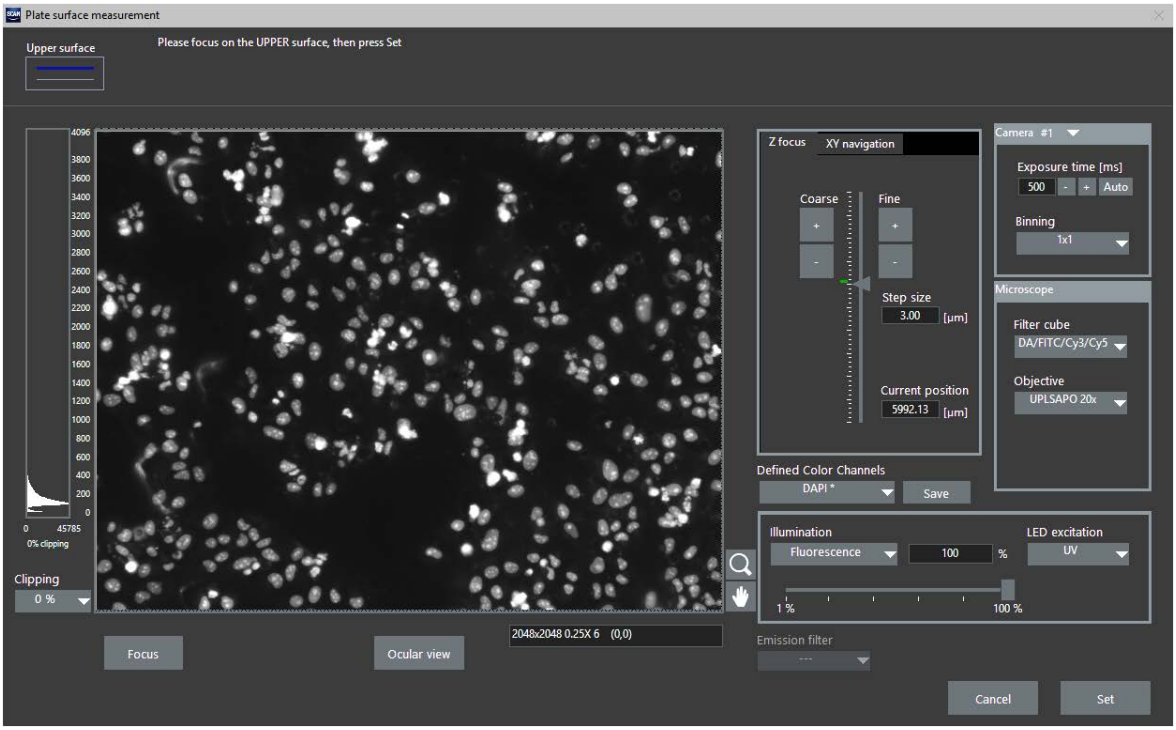
1. Click **Measure** to open a live image window.
2. Move to a corner of the first well (A1) to the red cross.
3. Click **Add Position**.
4. Repeat this for the opposite corner of the well (the numbers field next to the **Add Position** button turns green to indicate that the measurement is completed).
5. Confirm by clicking **OK**.
6. Repeat steps 1 to 5 if more than one A1 position is required (see Section *5.3 Defining Multi-Slide Plate Holder*)

The two positions are used to determine the well height, well width and the center position of well A1.

## 5.5 Measure plate bottom offset and position

For fast automated focusing, at least the position of the “upper surface”, e.g. the plastic-buffer interface of the well bottom where the cells are adherent, needs to be known. For hardware autofocus operation, also the position of “lower surface” should be known to increase speed of the autofocusing procedure.

If the system is equipped with the hardware autofocus option, the acquisition software will detect the lower surface of the sample automatically. Therefore go to the **Edit ▸ Plate Manager ▸ Edit plate type** window and click on the **Measure** button in the **Well bottom** area. The measured value for the lower surface will be entered as **Lower surface position [µm]**. After that the **Plate surface measurement** window will appear.




Define upper surface of a plate

Here you have to focus the sample manually, either by using the up/down buttons for **Coarse** and **Fine** or by using the microscope wheel. Press the **Set** button to define the upper surface of the well bottom. The **z-offset** and indirectly also the **Thickness** of the well bottom (coverslip) will be calculated from the measured values.

Note that if no hardware autofocus is present, two windows will appear after the **Measure** button is pressed: one window to measure the upper surface of the sample and a second window to optionally also measure the lower plate surface.

If these measurements have been performed successfully, in both cases the upper surface of the sample will be indicated in all **Z focus** sliders by a blue bar.

# 6 Running a Scan

This chapter explains how to start a scan and the steps that are necessary once a scan is started and the actions and online observations possible in the course of an experiment.

For scan^R to perform a proper scan, it is required that all **Autofocus**, **Acquisition** and **Plate Manager** settings in the **Edit Scan** window are properly set; see Chapter 4, *Setting up a Scan*.

After starting the scan by clicking **Start**, there are different scenarios depending on

- the availability of a ZDC hardware autofocus
- the usage of plates with or without predefined starting position

These different scenarios are explained in the following chapters.

# 6.1 Systems with hardware autofocus

The hardware autofocus enables fast and reliable detection of the well plate focus position, even if there is a stronger variation from plate to plate. This means the system can detect the focus fully automatically and no user interaction is required at scan start. If the **Offset and position calibration** has been performed (see Section 5.1, *Well Plate Types*) and the **Use always for this well plate type** option is checked, the system hardware autofocus will use the calibration and can be much faster for the first position.

## 6.1.1 Plates with predefined A1 position

This is the simplest scenario. A system with hardware autofocus and a plate type with predefined starting position, i.e., either system standard or A1 position calibrated plate types.

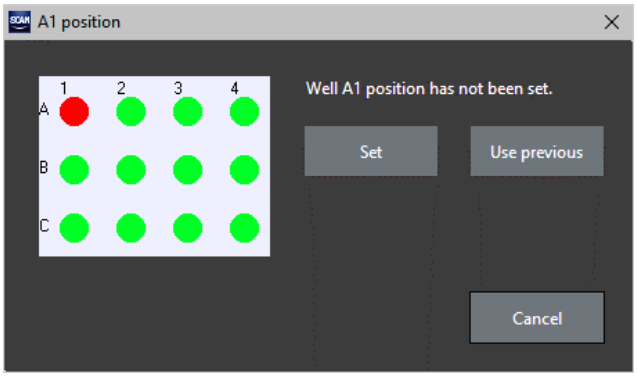
Upon a click on the **Scan: Start** button scan^R conducts the entire scan automatically as set up in **Edit Scan** and stores the focused images in the predefined folder.

## 6.1.2 Plates/Slides without predefined A1 position

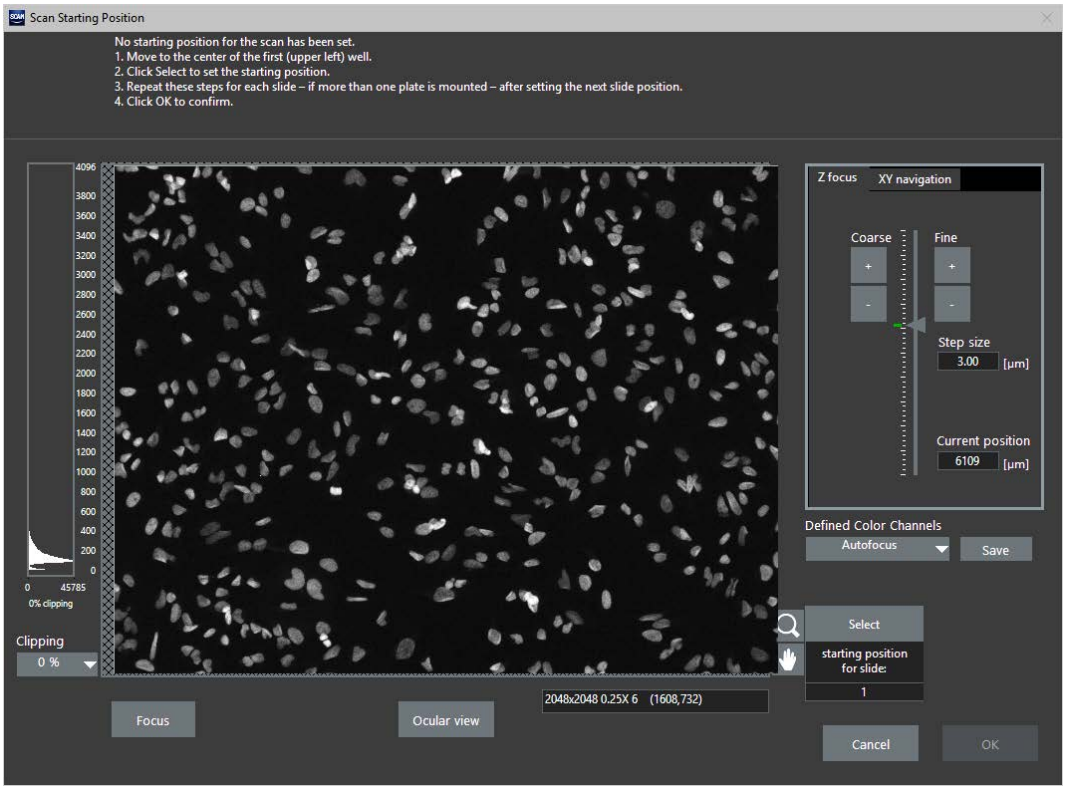
In case of commercial plates of one and the same type, one can in most cases assume that upon mounting on the stage the position of well A1 will always be at the same position, and the following scenario will not apply.

In case of spotted slides and non-calibrated plates, however, scan^R needs to be taught the starting position each time anew.

1. Click **Scan: Start** to start the scanning routine. The following message will appear:




**Use previous**. This option is useful if a scan is to be repeated with the same slide immediately.




2. Click **Set** to open the **Scan Starting Position** live image window.

3. Move to the center of the first (upper left) well.

4. Focus the specimen using the **Z focus** tools.

5. Click **Select** to set the starting position for **slide 1**.

6. Repeat these steps for each plate – if more than one plate is mounted. Make sure to set the correct **slide number** each time.

7. Click **OK** to confirm.

8. scanR conducts the entire scan automatically as set up in **Edit Scan** and stores the focused images in the predefined folder.

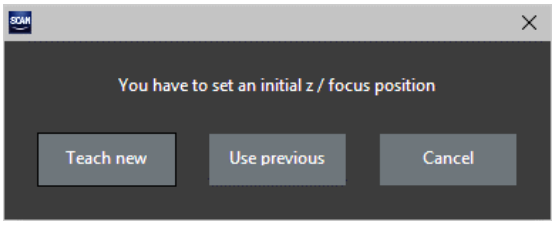
> Note that the positions you define refer to the center of well A1 of the well pattern and thus not necessarily to the first well activated for acquisition in the **Edit Scan ▸ Plate Manager** window!

# 6.2 Systems without hardware autofocus

System without hardware autofocus usually require user interaction on start of a scan because the variations of the bottom of typical well plates can vary from plate to plate. In this case it usually is faster to provide the focus for the first position manually in contrast to using exhaustive software autofocus. If the **Offset and position calibration** has been performed (see Section 5.1, *Well Plate Types*) and the **Use always for this well plate type** option is checked, however, the system will start with the focus values from the well plate library. This can be useful for very precise (highest quality) well plates or batches of plates with low focus variation.

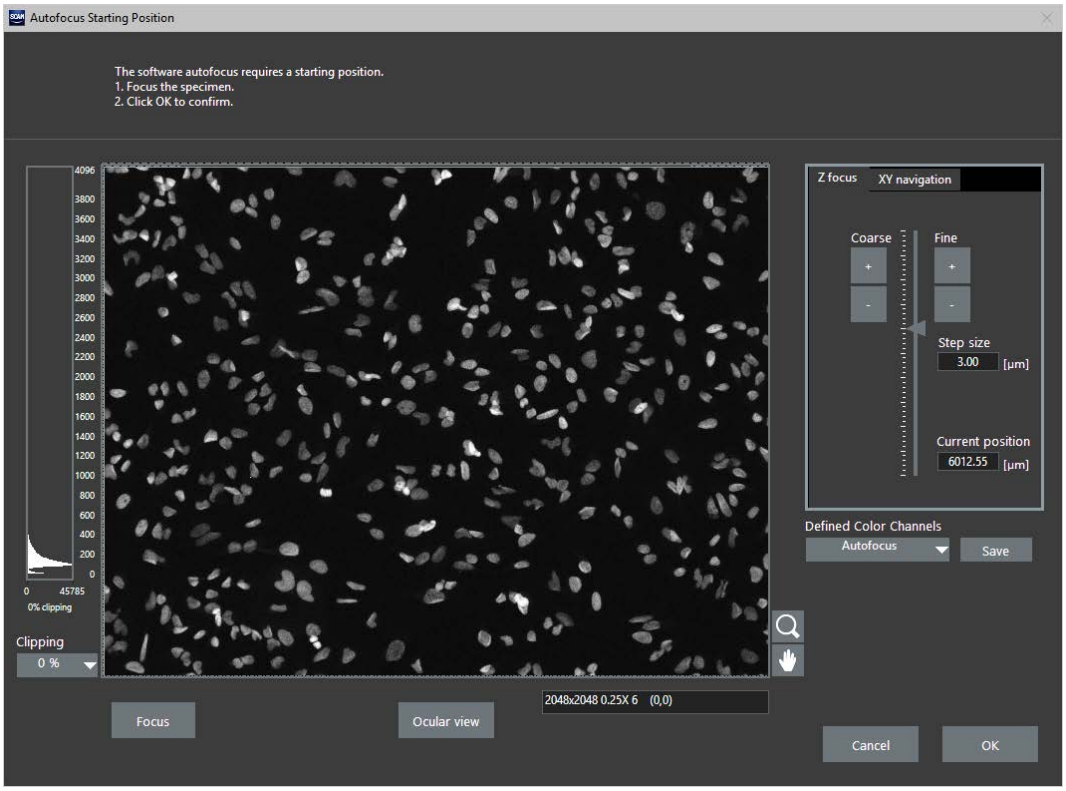
## 6.2.1 Plates with predefined A1 position

1. Click **Scan: Start** to start the scanning routine. The following message will appear:




**Use previous**. This option is useful if a scan is to be repeated with the same plate immediately.

2. Click **Teach new** to open the **Autofocus Starting Position** live image window.

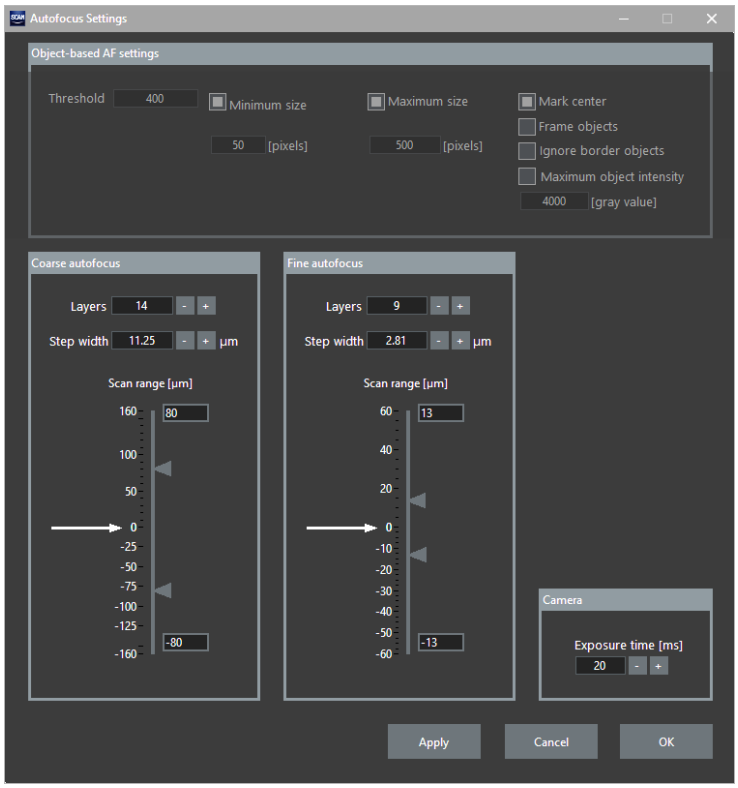



3. Focus the specimen using the **Z focus** tools.

4. Click **OK** to confirm.

## 6.3 Autofocus adjustment during a scan

Click the **Autofocus Settings** button on the main window in order to optimize the software autofocus during a scan. The **Autofocus Settings** window opens. The upper box affects only the settings of the object-based autofocus. The options of coarse and fine autofocus can be tuned and the exposure time of the autofocus channel can be changed.



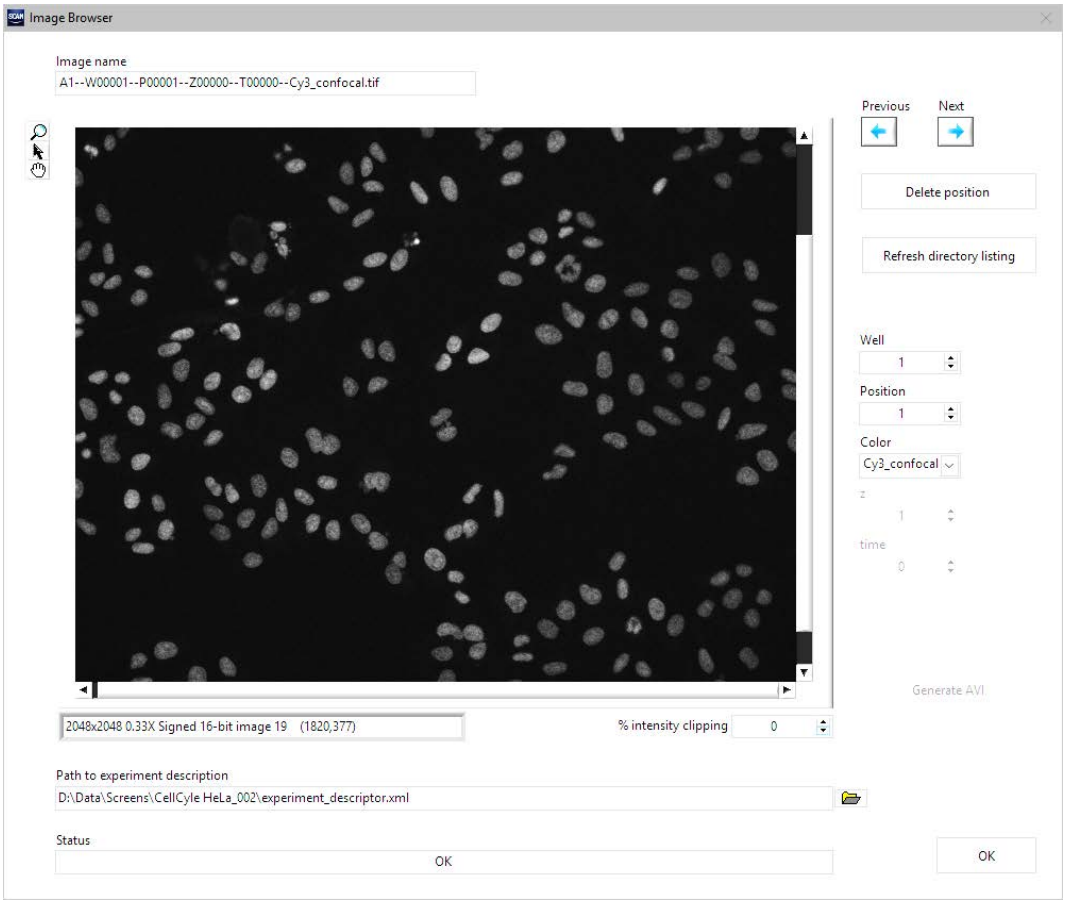

The controls are explained in detail in Chapter 4.2, *Autofocus Settings*.

## 6.4 Online image browsing

Click the **Online Observation: Browse Images** button to browse through stored images of the current scan. It is also possible to open previous scans for quick review.

Use the **Previous** and **Next** buttons to navigate through the images.

**Delete associated images.** Removes associated images, e.g. the images of one color channel.




**Refresh directory listing.** Updates the list of available images (in cases where acquisition is still ongoing)

**Well/ Position / Color/ Z/ Time.** Use these buttons to navigate through the images.

**Generate AVI.** Set a file to export an .avi file for time-lapse experiments.

**Path to experiment description.** Displays the path to the current scan or sets the path to an experiment descriptor file to load other data that were previously acquired.

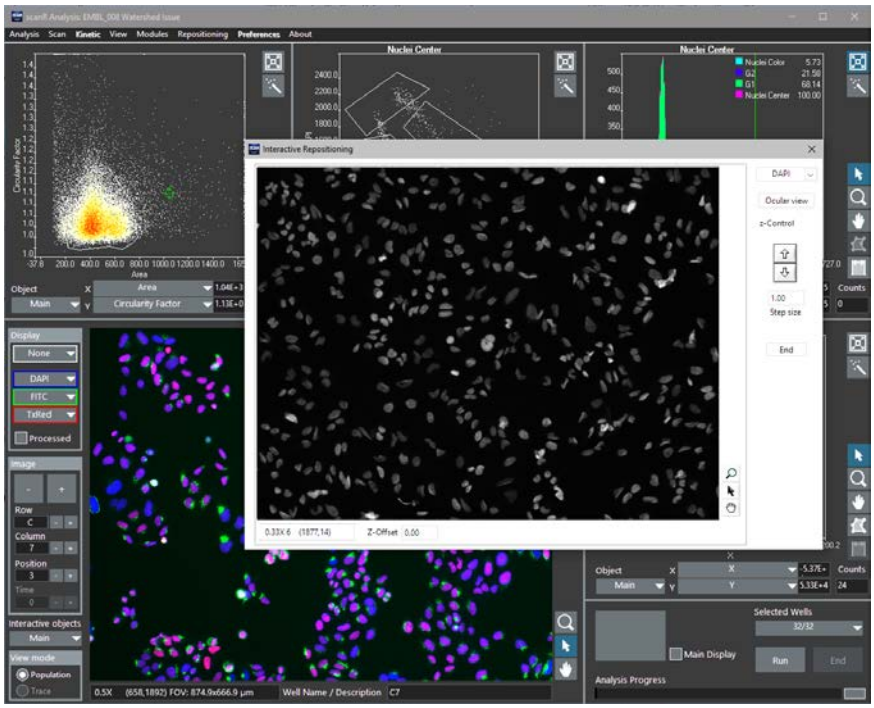
## 6.5 Online analysis

After starting the acquisition it is possible to directly start the analysis of the experiment. To do so, start the scan$^R$ Analysis software and load the *experiment_descriptor.xml* file manually by executing **Scan ▸ Open…** or use **Scan ▸ Open Last Acquired** to open the experiment currently running. Set up a new analysis or load an existing assay file as described in the scan$^R$ Analysis Software manual.

After the acquisition and analysis are completed, it is possible to revisit the objects that were detected during analysis. Therefore go to **Repositioning ▸ Interactive** in the analysis software.

The **Interactive Repositioning** window opens. Now the Analysis software will work together with the Acquisition software in order to control the microscope. Select an object in the scan$^R$ Analysis software, i.e. click either on one of the data points or on a detected object in one of the image displays (main

image panel, galleries). After selecting an object, the stage moves back to the position and shows a live view. Thereby the object selected in the scanR Analysis software is centered.




Select the color channel to be displayed in from the short list. The sample can be focused with the **Z-control** in case a Z-drift occurred in between the first measurement and the repositioning. The **Ocular view** allows to inspect the 'hits' detected by the analysis by eye.

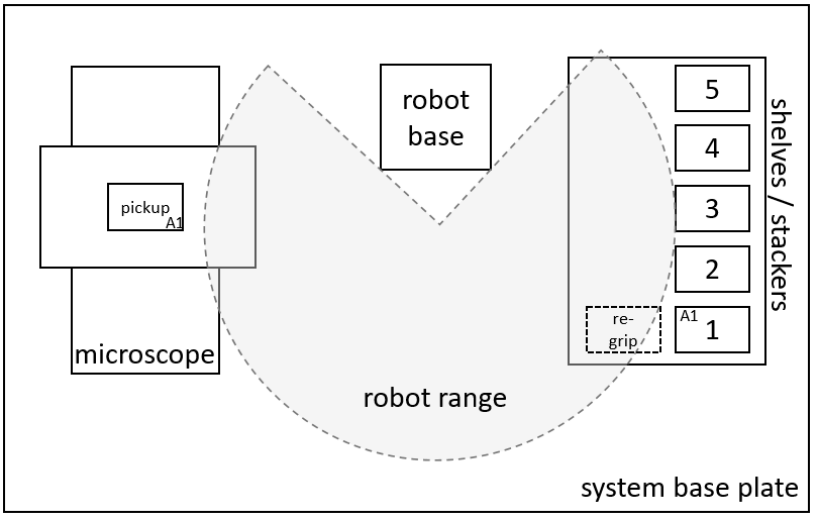
In the case that the scanR analysis software is running on a different computer than the acquisition software, it is necessary to set the IP address (**Allowed hosts**) and the **port** (use default 2055) of the computer, where the analysis is running (see Chapter 8.1.4, *Error Reporting / Remote*). In the scanR Analysis software, open **Preferences** and in the **Repositioning & Reclassification** box enter the **Port** of the **Acquisition Server** (2055) as well as its IP **Address** (see also scanR Analysis Instructions, Chapter *Preferences*).

# 6.6 Systems with automatic plate loader

A scan$^R$ system with automatic plate loader allows screening several well plates within a single run. Up to five stackers or shelves are available and will be positioned on the right of the robot. In stacker operation, one of the stackers has to be empty at the start of the scan and will serve as swap stacker. Optionally, there is an additional position (re-grip station), which is used for fine positioning of the plates. Keep this position empty. You can remove the stackers/shelves from their positions to make loading and transportation easier.

When loading the stackers, take care of the following:

- Start with filling stacker 1, then 2, etc.
- Orientate the plates with the first column pointing towards the microscope.
- Make sure that each stacked plate fits accurately on the plate below.
- Stack the plates as well aligned as possible.
- Leave a minimum gap of at least 10 mm from the last plate to the top of the stacker to allow the robot to lift the uppermost plate from the stack.

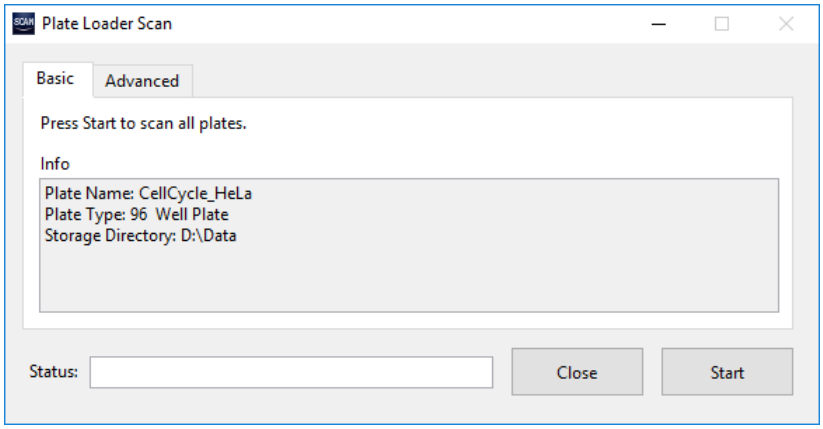



When loading the shelves take care of the following:

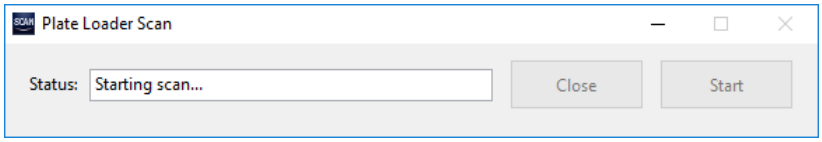
- Start with filling shelf 1, then 2, etc.
- Start filling from bottom to top
- Orientate the plates with the first column pointing towards the microscope.
- Make sure that each plate fits accurately on its floor.

## 6.6.1 Plate Loader Scan

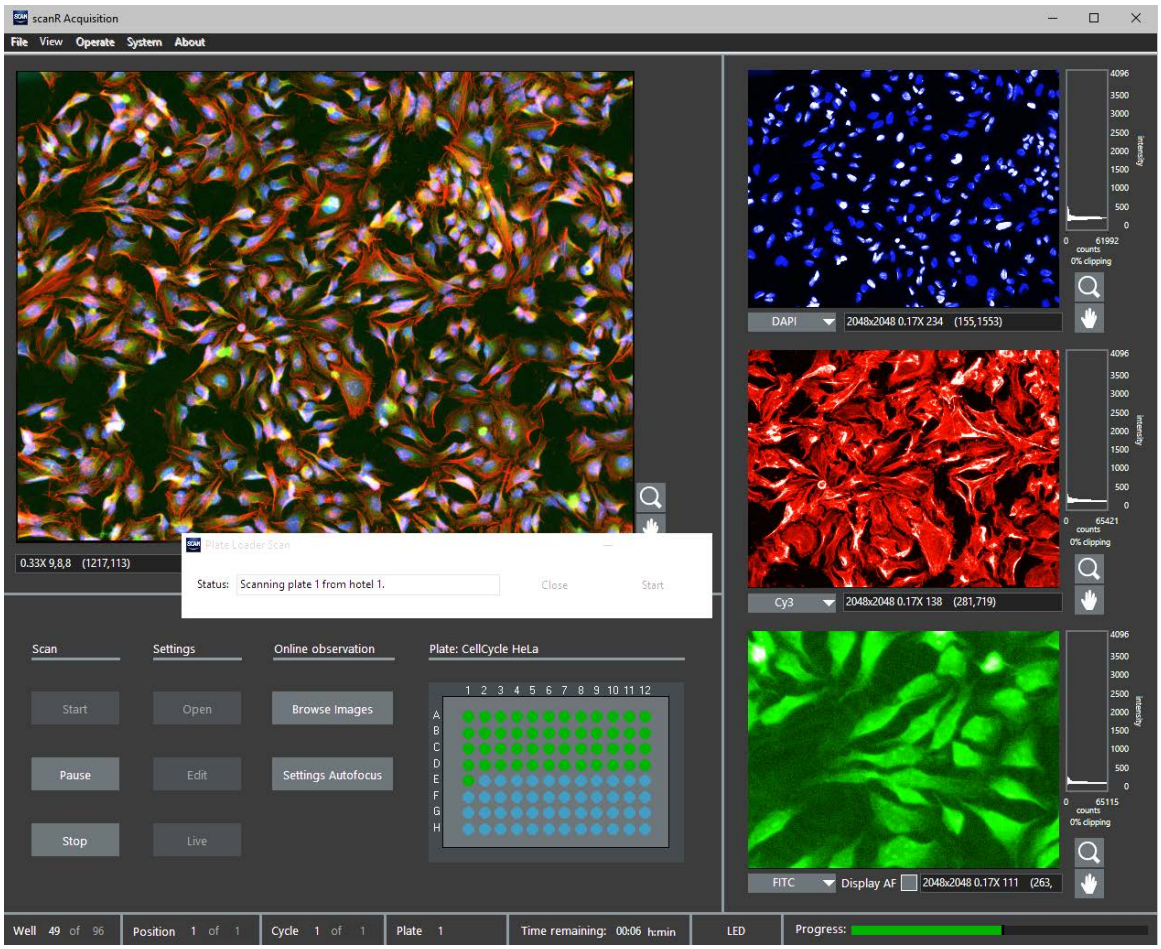
Select **Operate ▸ Plate Loader Batch Scan** to start a screen using the automatic plate loader. The current experiment settings will be used to scan each plate.



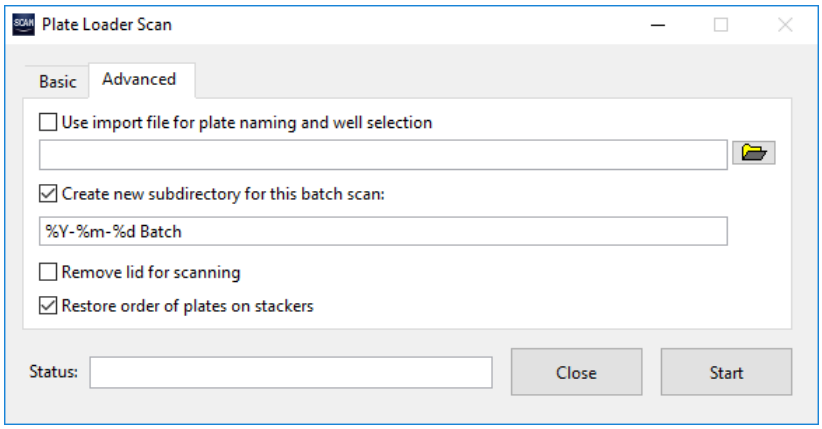

The **Plate Loader Scan ▸ Basic** tab gives a brief overview of the **Plate Name** and **Plate Type** settings, and of the **Storage Directory** which will be used for the automatic scan. Press **Start** to start the screen of all plates on the shelves or stackers.




This will initialize the automatic plate loader, deliver the first plate the microscope, start a scan with the current experiment settings and store the images at the specified **Storage Directory**. During the screen, the **Status** field will continuously provide information about the current status and progress.



The **Plate Loader Scan** ▸ **Advanced** tab provides options for more advanced configuration.




**Advanced: Use import file for plate naming and well selection**. Allows selection of a file with well plate names, and well selections and descriptions to better customize the documentation and selection of wells to be scanned for each plate.

**Advanced: Create new subdirectory for this batch scan**. When checked, this option will create a new subdirectory in which all scans collected for better documentation and data handling. The directory name provided here can include special variables which will be replaced by the current date or time.

| **Special variable example** | **Resulting directory name example** |
|---|---|
| %Y | 2017 (year) |
| %m | 10 (month number, i.e. October) |
| %d | 26 (day of month) |
| %Y-%m-%d Batch | 2017-10-26 Batch |
| %Y-%m-%d_started-%H:%Mh Batch | 2017-10-26_started-13:15h Batch |

**Advanced: Remove lid for scanning**. For well plates covered with lids. The lid will be removed after the plate has been delivered to the microscope and returned to the plate when the scan has finished. Using this functionality ensures the well plate is protected most of the time and only left without lid for the actual scanning process.

**Advanced: Restore order of plates on stacker**. This option is enabled by default. If disabled, the system will not use the empty stacker as swap stacker and restore the plate order afterwards. It rather will store them as time efficient as possible. This means, the top plate scanned first from stacker 1 will be the bottommost plate on the originally empty stacker and so on.

# 7 Multi-level Screening

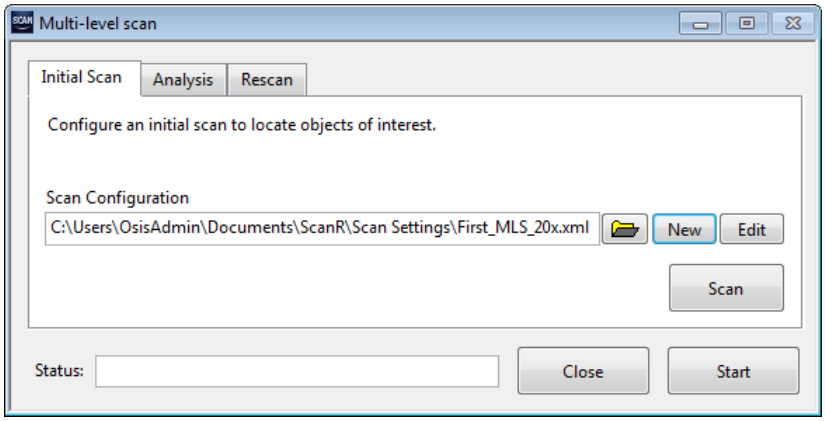
This chapter explains how to set up and perform a multi-level scan.

# 7.1 Overview

Multi-level Acquisition is a powerful means of screening all interesting views of a sample with the best possible parameters in a minimum of time and with maximum flexibility. Based on an initial pre-scan, the scanR Analysis software determines all interesting objects with the complete image processing, image analysis, and cytometric data exploration engine available. In an automated workflow, the analysis results are employed to scan selectively only the objects-of-interest in a second, targeted screen. Typical scenarios where Multi-level Screening excels are large area sample with few cells which require high resolution or single cell events.

# 7.2 Setting up a multi-level scan

Select **Operate ▸ Multi-level scan** in the main window. This command opens the **Multi-level Scan** wizard.




A multi-level scan consists of three steps. These steps correspond to the three tabs of the wizard.

1. The **Initial Scan**. This, for example, can be a fast low-resolution scan of the whole sample

2. The **Analysis** of the initial scan. In this step, the interesting objects or positions in the sample are discovered by the scan$^R$ Analysis.

3. The second scan, or **Rescan**, of the same sample using information from the previous analysis. This, for example, can be a targeted high-resolution scan of the objects of interest only.

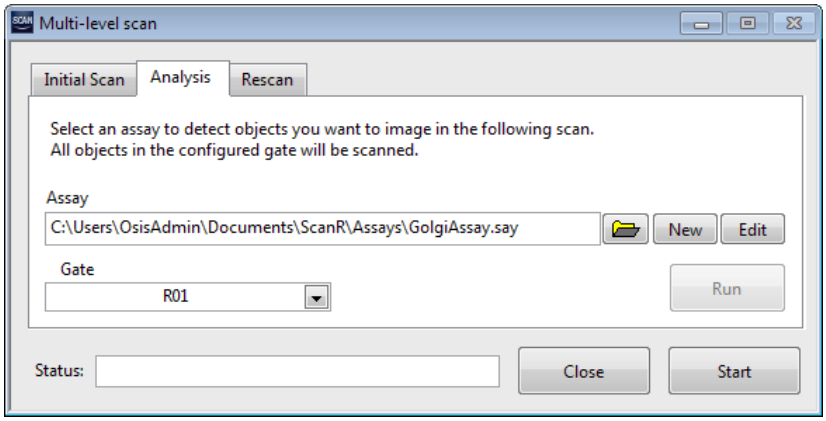
Each step can be set up and tested individually using the respective tab of the wizard. Once the multi-level scan has been set up correctly, it can be started using the **Start** button. The Status line will show information about the progress of the multi-level scan as well as information about the result of individual steps during the set-up process.

### 7.2.1 The initial scan

On the **Initial Scan** tab the configuration for the initial acquisition is defined. There are two options to start with setting up this step. By selecting the folder icon , any *experiment_descriptor.xml* file from previous scans can be loaded from disk. Alternatively, the **New** button generates a copy of the scan settings currently active in the scan$^R$ Acquisition software. Using the Edit button, the initial scan can be modified as usually (see Chapter *4, Setting up a Scan*). By clicking the **Scan** button, the defined initial scan will be started directly. In contrast to using the **Start** button, which executes all three steps of the multi-level scan automatically, starting only the initial scan manually can help setting up the multi-level scan and finding the best possible parameters for the initial scan.

### 7.2.2 The analysis

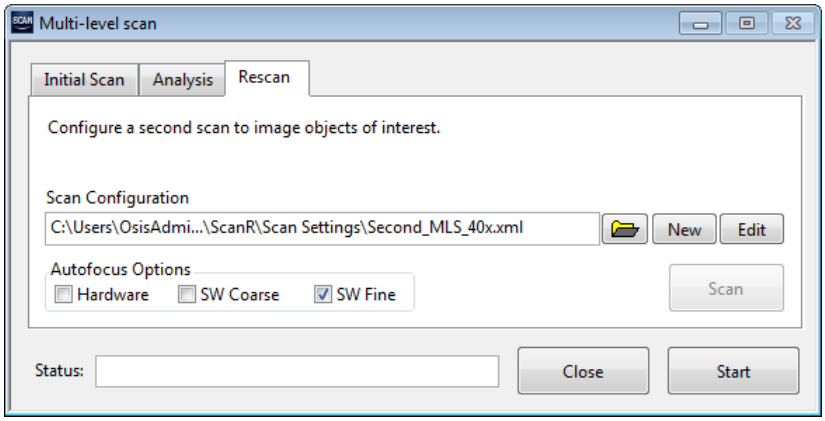
The **Analysis** tab is used to set up the analysis step of the multi-level acquisition.




There are two options to start with setting up this step. By selecting the folder icon , any scanR Analysis assay file (*.say) can be loaded. Alternatively, the **New** button generates an empty template for setting up a new assay for analysis. Using the Edit button, the assay will be loaded in the scan$^R$ Analysis software and can be modified there as usually. It is important that the selected analysis assay file contains at least one gate. Using the **Gate** dropdown box, a gate can be selected. All objects which fall in this gate when analyzing the initial scan will be re-scanned when the multi-level scan is started. The settings can be tested using the **Run** button if an initial scan has been performed in the first step of setting up the multi-level scan. As soon as the test run has been finished, the **Status** line will show the number of objects found during analysis. These are the candidates for the rescan.

### 7.2.3 The rescan

The **Rescan** tab is used to define template experiment settings for the rescan of objects found during the analysis of the initial scan.




As in the other steps, there are two options to start with setting up this step. By selecting the folder icon , any *experiment_descriptor.xml* file from previous scans can be loaded from disk. Alternatively, the **New** button generates a copy of the scan settings used in the **Initial Scan** tab.

Using the Edit button, the rescan scan can be modified as usually (see Chapter *4, Setting up a Scan*) with the exception that the plate settings cannot be modified. This is the case because the plate definition cannot change between initial and rescan, of course. Only the autofocus options applied at each position during rescan can be selected in the tab directly.

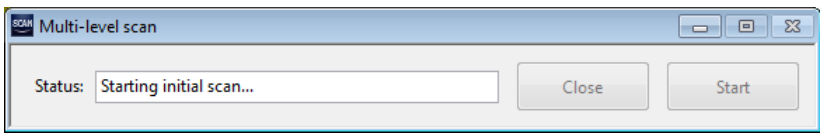
When the first two steps (initial scan and analysis) of the multi-level scan have been performed during set-up of the multi-level scan, the **Scan** button can be used to start the rescan with the defined settings directly. In contrast to using the **Start** button, which executes all three steps of the multi-level scan automatically, starting only the rescan manually can help setting up the multi-level scan and finding the best possible parameters for the rescan.

> When using different microscope objective lenses for the initial scan and the rescan, respectively, and using hardware autofocus for the rescan, it is important that hardware autofocus parfocality has been calibrated carefully (cf. Section 8.4)!

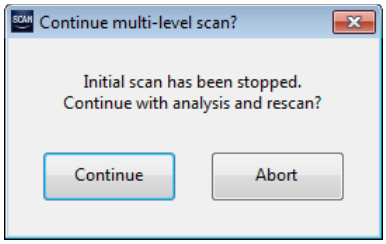
## 7.3 Running a multi-level scan

Setting up a multi-level scan requires some care and accurateness because all three steps need to execute successfully and consecutively with the desired intermediate results. For this reason, each of the three steps (initial scan, analysis, rescan) can be run individually during the set-up and optimization phase as described above until they are optimally configured.

Once the multi-level scan is set up successfully, the **Start** button can be used to run the complete multi-level scan for a given sample. When the multi-level scan has been started, the wizard window will be reduced to show only the status line.



Without user interaction, the multi-level scan will run completely until all three steps are finished. If desired, the process can be interrupted using the **Stop** button in the scanR Acquisition main window as usually. In case the initial scan is stopped by the user, a dialog will pop up with two options to choose from.




By clicking **Continue**, only the initial scan will be stopped but the multi-level scan will be continued, i.e. the data acquired so for will be used for the analysis and rescan steps. Selecting **Abort**, on the other hand, will stop the multi-level scan completely.

When the multi-level scan has been completed, the results of the rescan will be stored as subfolder in the directory defined and used for the initial scan. The name of the subfolder always is rescan_001 where the number will be increased for each rescan if multiple rescans are performed during set-up phase. For analysis, the rescan can be opened from scan$^{R}$ Analysis software as usually.

# 8 System Configuration

This chapter explains how the system devices have to be configured in order for scan$^{R}$ to work properly.

# 8.1 System configuration

Upon installation of the scan$^{R}$ screening station all hardware components have to be configured for the system to work properly. The configuration has to be adjusted if changes are made, for example, if new filters or objective lenses are added.

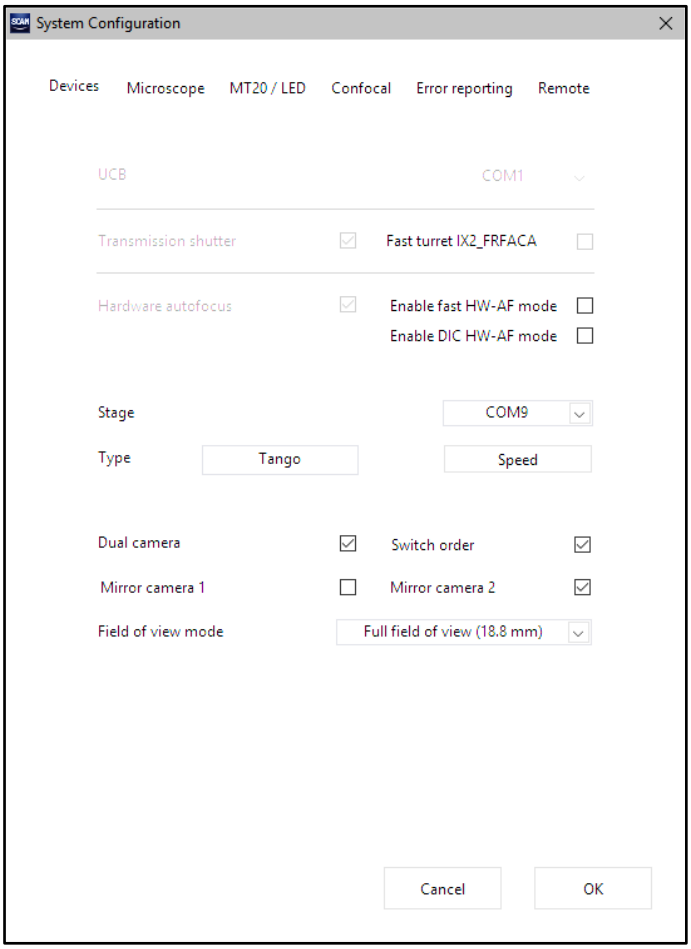
The execution of the **System configuration** command is password protected.

The **System ▸ System Configuration** command gives access to the **Hardware configuration** window with different tabs (see following chapters):

- **Devices and ports** to select the available hardware components and configure the PC ports
- **Microscope** to view the configured objectives, filters and contrast inserts
- **MT20** to view the configured excitation filters of the fluorescence illumination system
- **Error reporting**
- **Remote**

The **System ▸ Calibrations** menu provides a number of calibration wizards to set up the system optimally. For example, the **Calibrations ▸ Stage Calibration** command allows an absolute calibration of the stage with a system calibrated well plate type as reference. This is not to be confused with the stage initialization that is being carried out each time the software is started (see Chapter 8.2, *Stage Calibration*).

### 8.1.1 Devices




**UCB**. Select the serial port (RS232) of the imaging PC that is connected with the universal control box (UCB) (IX81 only).

**Transmission shutter**. Check this box if a shutter for transmission mode is connected. Some shutter types will be auto-detected and the box checked automatically.

**Fast turret IX2_FRFACA**. If a fast filter turret is available, this is automatically checked (IX81 only).

**Hardware autofocus**. Check this box if a ZDC hardware autofocus device is connected and should be used by the system. For newer system configurations, this box is checked automatically.

**Enable fast HW-AF mode**. Activates fast hardware autofocus mode (available only with the IX3-ZDC2 hardware revision of the ZDC autofocus device).

**Enable DIC HW-AF mode**. Activates DIC optimized hardware autofocus mode (available only with the IX3-ZDC2 hardware revision of the ZDC autofocus device).

**Stage**. Select the serial port (RS232) which is connected to the stage controller. Older systems might have other connection types which do not need to be configured here.

**Stage: Speed**. Click here to open the **Stage speed** window if you do not want to use the default settings for stage speed and acceleration. Change the **Velocity** and the **Acceleration** using the respective sliders for movements during a scan (**System Control**) and for movements under **Joystick control**.

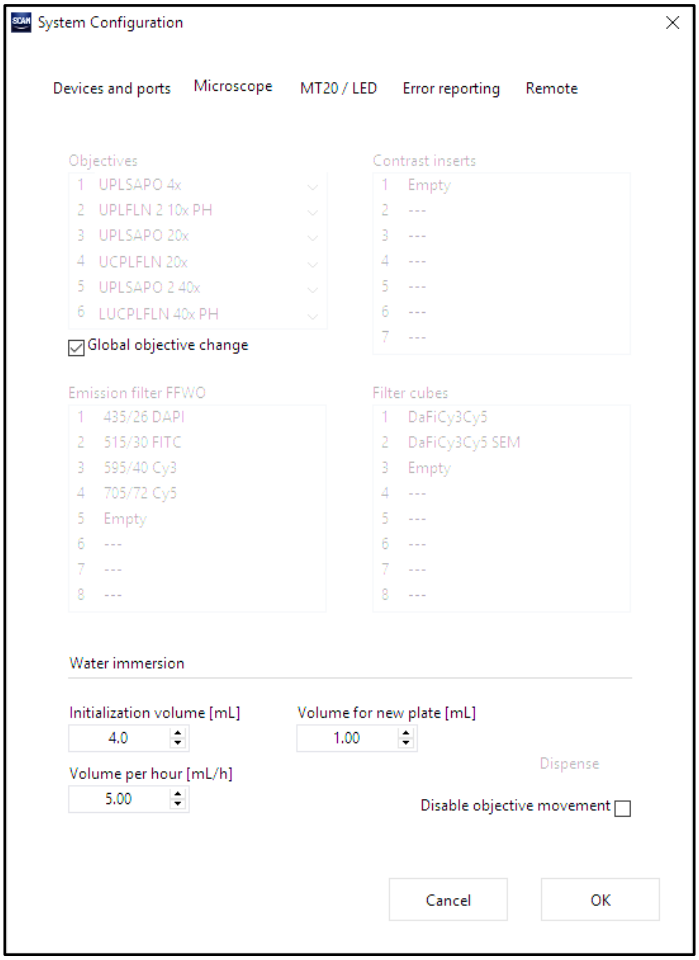
**Dual camera**. Activates dual camera mode if two cameras are connected to the system (optional).

**Switch order**. Reverses the order of camera #1 and camera #2 if two cameras are available.

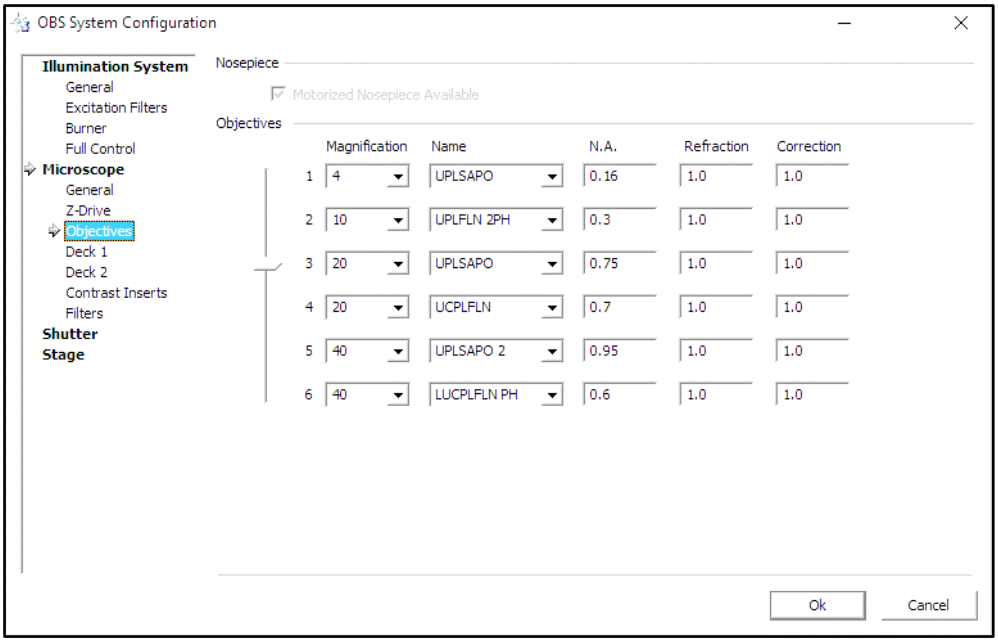
**Mirror camera 1/2**. Applies vertical flip to the respective camera image. This is required for beam slpitter configurations, for example with the CSU (optional).

**Field of view mode**. For systems equipped with a large field of view camera, the field of view can be reduced in order to reduce possible shading effects or to be backward-compatible to the 2/3” sensor industry standard.

### 8.1.2 Microscope




These settings mainly are displayed here only for reference. They are configured in the OBS System Configuration. Settings for the water immersion module can be done directly in scanR.




**Objectives**. Shows the microscope objective lenses configured in the OBS System Configuration for each nosepiece position.

**Filter cubes**. Shows the filter cube names for all positions in the filter cube turret as configured in the OBS System Configuration.

**Global objective change.** Generally, in each channel (AF, color channels) the objective can be set independently. In order to facilitate the operation of scan$^R$, the objectives can be changed for all channels simultaneously (this checkbox is activated by default for all new scan$^R$ installations). If the checkbox **Global objective change** is activated, the change of the objective in one channel will lead to a simultaneous change of the set objective in all other color channels. In this case also the fine range step width of the software autofocus (See Chapter 4.2.1, *Fine Autofocus*) is automatically adapted to the selected objective.

**Contrast insert**. Shows the names of the transmission condenser inserts as configured in the OBS System Configuration.

**Emission filter FFWO**. Shows the filter names for all position in the emission filter wheel as configured in the OBS System Configuration.

**Automated Water Immersion System IX2-AWI (optional, IX81 only).** An automated water immersion system is available as an option for scan$^R$. The system supports either the 40x objective UAPO40X340W/1.15 or the 60x objective UPLSAPO60XW/1.2. With this device high resolution and very low light screening applications can be performed with scan$^R$.

**Initialization Volume [ml]**. The volume of water that is dispensed during startup.

**Volume per hour [ml/h]**. The volume that is dispensed within one hour. From time to time the pump will start to dispense a part of this volume. The maximal value is limited by the pump rate. The **Volume per hour** may have to be adapted depending on plate types (flat glass bottom or foil plate with dents) and on the type of pattern that is used to screen the plate.

**Volume for new plate [ml]**. The volume that is dispensed when a plate is changed during acquisition by a plate loading robot. To check this volume during configuration press the adjacent button **Dispense**. If a plate is changed manually press the **Dispense** button at the front of the IX2-AWI control box.

**Disable objective movement**: if this checkbox is activated, the movement of the microscope objective revolver is disabled.

For a more detailed description of the automated water immersion and its operation in scanR please refer to the IX2-AWI manual.

### 8.1.3 MT20 / LED

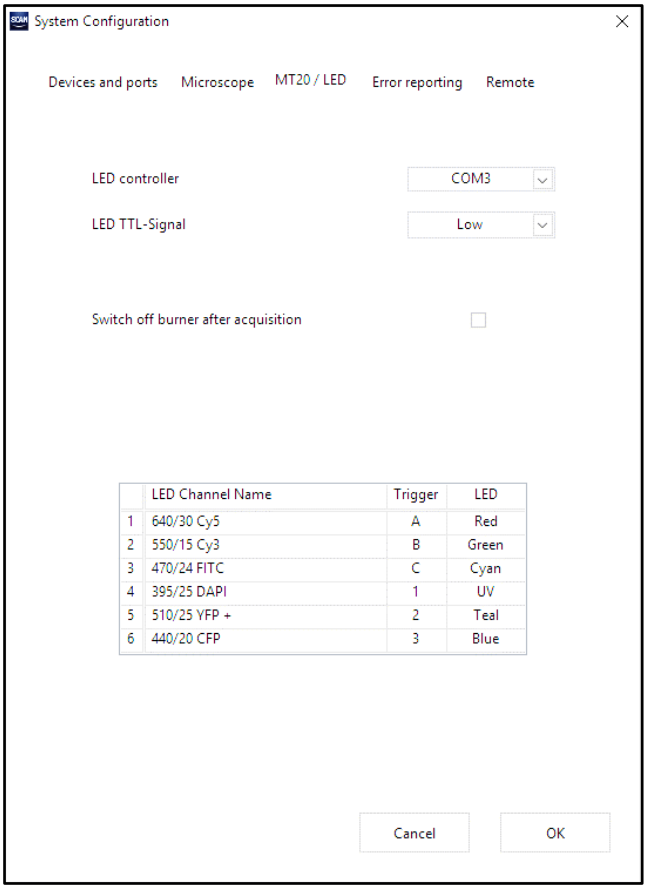
**LED controller**. This option is available only for scanR systems equipped with an LED light source. Select the serial port where the LED light source is connected to.

**LED TTL-Signal**. This option is available only for scanR systems equipped with an LED light source. It determines if the LED operates in TTL **Low** or TTL **High** mode. Switch this option if LED channels appear to be always on.

**Switch off burner after acquisition**. This option is available only if the scanR system is equipped with an MT20 light source. Check this box to cause the arc burner to be switched off automatically after the scan is completed.

> This is a useful option for weekend or overnight operation because it saves burner lifetime. Mind, however, that the burner needs about 10 min on restart to reach full and stable intensity. Also, switching the burner on and off frequently reduces the lifetime. It is not recommended to activate this switch if expected idle time of the system is less than an hour.

**Excitation filter / LED excitation channels**. Shows the filter names for all positions of the MT20 filter wheel as configured in the OBS System Configuration, or the definition of the LED excitation channels.




The settings for LED allow definition of the LED channel Names

### 8.1.4 Error Reporting / Remote

**Error reporting**

**Error reporting**. Check this box if you want error messages that may be generated during a scan to be sent automatically as emails.

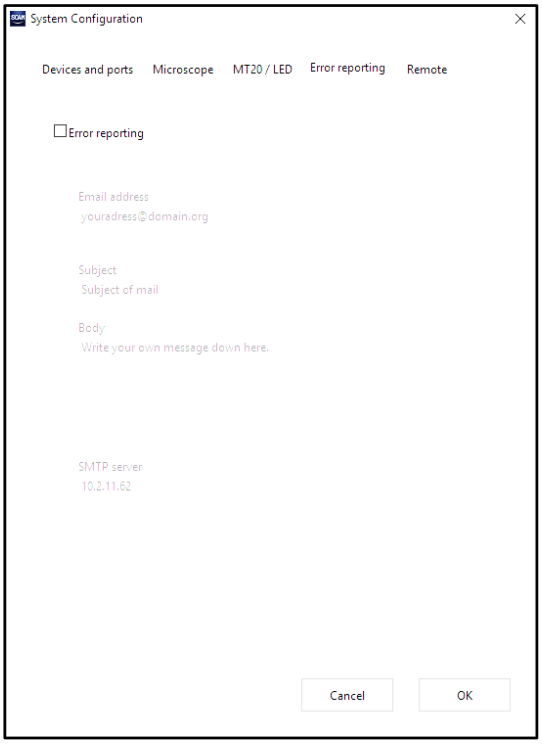
**Email address** and **SMTP**. Make sure to set the correct addresses here.

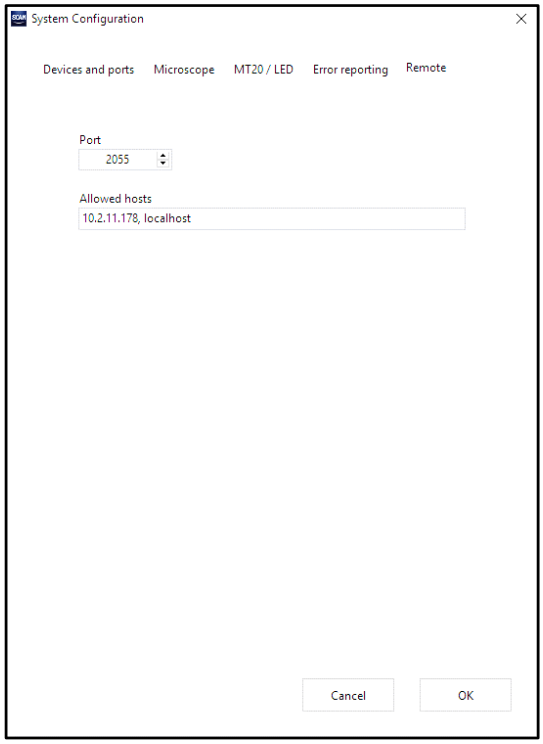
**Subject** and **Body**. You may enter default content here to be sent in addition to the error message.

**Remote**

The information here is needed to allow communication between the scan$^{R}$ Analysis software and the system from a remote PC or to control scan$^{R}$ by another software (see Section 9.3, *Remote control*).

Make sure that remote access is enabled in the corresponding settings of the operating system.



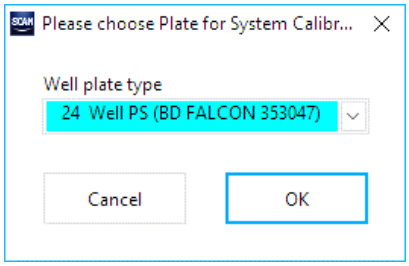

**Port** and **Allowed hosts**. Make sure to set the correct addresses here to allow remote access to the system.

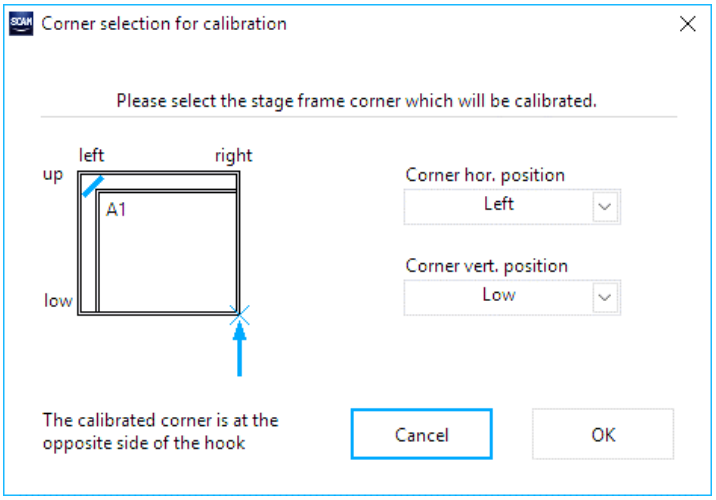
## 8.2 Stage Calibration

When the scanR Acquisition software is started the stage **initializes** automatically to determine its maximum limits of movements.

When a system is set up initially or maintained later, the stage needs to be **calibrated**. More precicely, the position of the well plates relative to the absolute stage coordinates is calibrated and saved. This is not a procedure that has to be undertaken routinely, it suffices to do it once upon installation of the system. Furthermore is has to be done very accurately because it will affect all plates defined in the Plate Library. Thus, the access to this routine is password protected.

### 8.2.1 Stage Calibration Procedure




1. Place a system standard well plate on the stage and select the well plate **type** in the **Plate Format** window.

---

2. Execute **System ▸ Stage Calibration**.

---

3. Perform the **3-point A1 Measurement** as described in Section *5.4 Well Measurement***.**

Once the procedure is completed the stage is calibrated and the A1 position of system standard as well as calibrated well plates will always be located at a known position.

## 8.3 Objective Parfocality

This command allows you to set an offset for the Z-position for the used objectives, so that when changing the objective, the focus is maintained.

1. Execute **System ▸ Calibrations ▸ Objective Parfocality**.

---

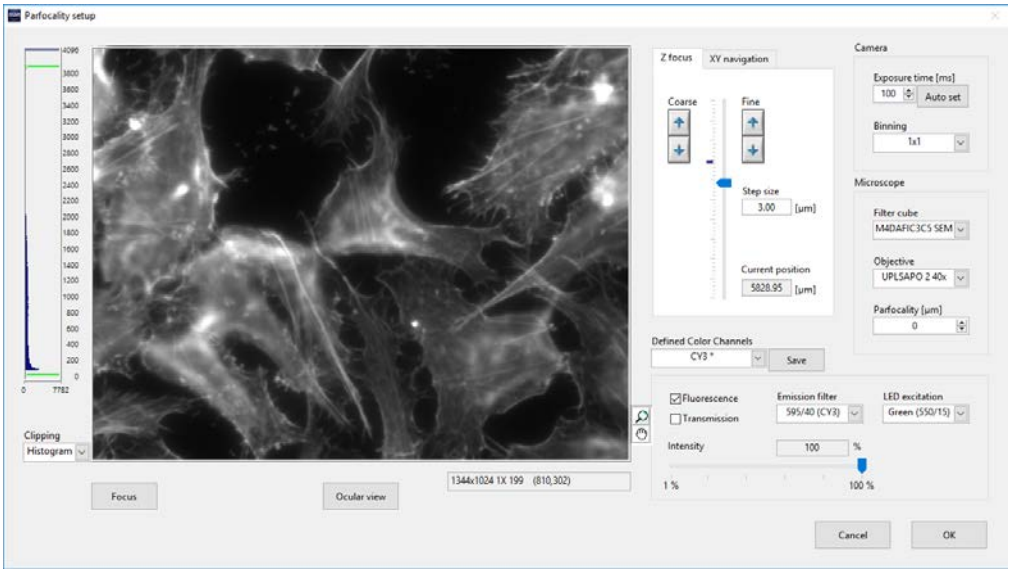
2. Select the first objective to be used from the **Objective** short list (e.g. UPLSAPO 40x). (It is convenient to start with the objective which has the highest magnification).

---

3. Set the focus by using the Z-focus wheel on the microscope until the sample is focused or by using the **Z-focus** arrows/slider.

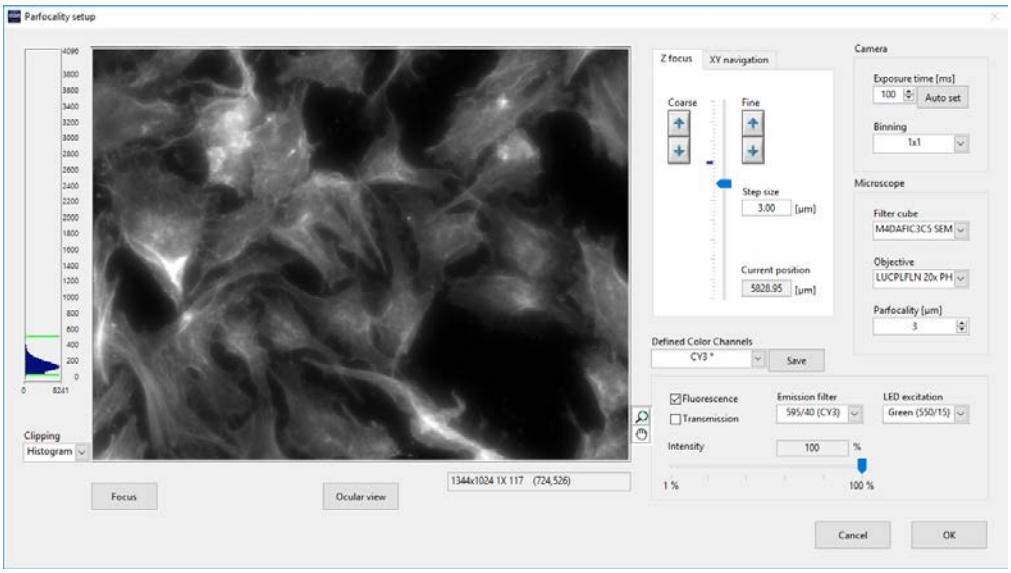
---

4. Make sure that the **Parfocality** value for the first objective is set to 0.




5. Select the next objective from the **Objective** short list (e.g. UPLSAPO 10x). At first the image might be blurred.

6. Set the focus for the second objective by using the up and down arrows in the **Parfocality** field.




7. Repeat steps 5 and 6 for all other objectives for which **parfocality** is to be set.

The focus position will now be maintained when changing the objectives.

# 8.4 HW-AF Parfocality

Similar to the objective lens itself, the ZDC device (which is used for hardware autofocus in scan$^R$) might also have some variation in the determined focus position for different objective lenses. To compensate for this, the hardware autofocus parfocality can be calibrated.

1. Before calibration of HW-AF Parfocality it is important to have Objective Parfocality calibration completed.

---

2. Execute **System ▸ Calibrations ▸ HW-AF Parfocality**. The **HW-AF Parfocality** overview opens which can be used to review the calibration, start the calibration procedure, or reset the calibration.


---

3. Press the **Measure** button to start the calibration procedure. If the IX3-ZDC2 revision of the ZDC device is installed in the scan$^R$ system, the procedure can be started for thin (plastic or glass, ~170µm) plates or thick (plastic, ~1mm) plates separately.

---

4. For the calibration procedure you will need a well plate (or two different well plates in case of IX3-ZDC2). It is recommended to use the plate type which will be used later for the actual measurements. If multiple plate types will be used, it is best to choose the plate with the highest quality for the calibration procedure.

---

5. A Live View window is displayed as preparatory step for the automatic calibration. Here it is necessary to focus manually on the lower surface of the plate to provide the reference measurement. Usually it is possible to focus on the lower surface even of an empty plate because there are tiny scratches or dust particles. If the lower surface is not visible, it can be marked with a felt pen to find it more easily. It is important to focus on the lower surface as accurately as possible using the objective lens with the highest magnification.

---

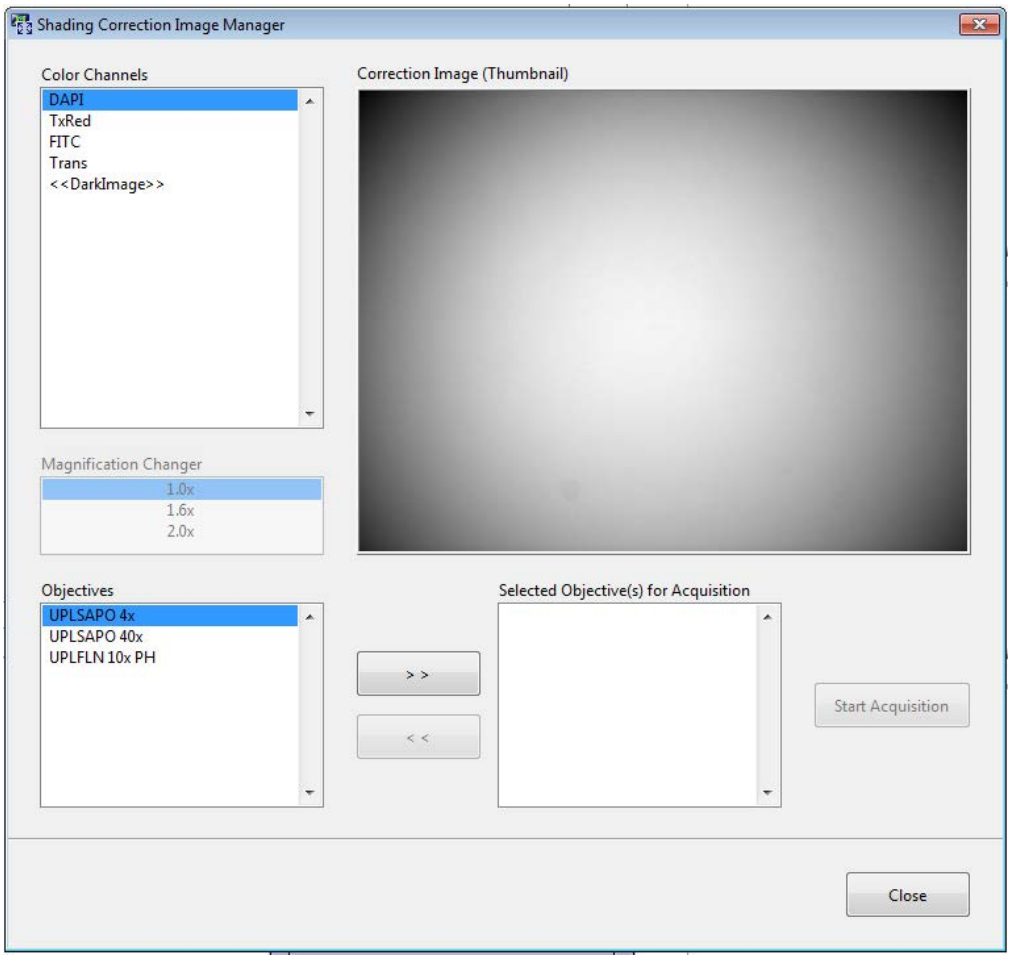
6. Press **OK** to start the automatic calibration process. The system will select all objective lenses compatible with the hardware autofocus and measure the focus position, thereby determining the optimal correction value (if any).

When HW-AF calibration has been done successfully, the autofocus measurements will be independent of the selected objective lens. This is prerequisite to use one plate definition with all objectives together with hardware autofocus.

# 8.5 Shading Correction

The Shading Correction Image Manager is the central location to review and acquire the reference images used for the shading correction. At least one image per color channel is required for correction of inhomogeneities of the illumination and shading of acquired images by the optical properties of the microscope system. This reference image can be taken individually for each color channel and objective setting. Additionally, a dark image can be acquired to correct for dark noise induced by the camera.

1. Execute **System ▸ Calibrations ▸ Shading Correction**.




2. Select a color channel from the list of available **Color Channels**.

3. Select an objective from the **Objectives** list to review the available shading correction data for this combination of color channel and objective. A thumbnail of the correction image will be shown if available.

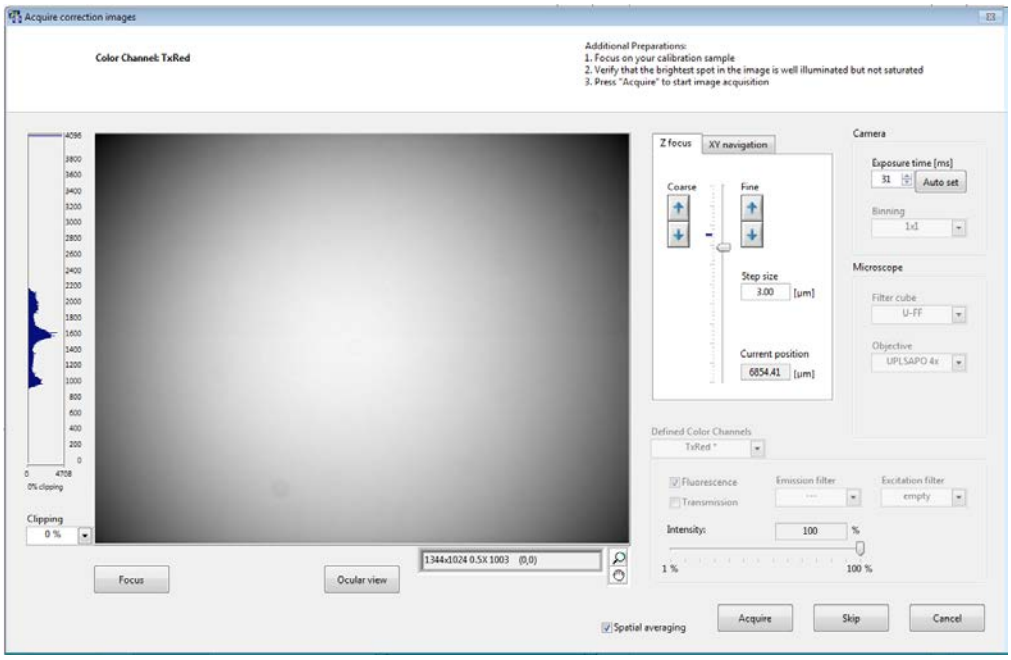
4. Select one or more objectives for acquisition of new correction image by adding them to the **Selected Objective(s) for Acquisition** list.

5. Press **Start Acquisition** to initiate acquisition of correction images for the selected combination of color channel and objective(s).

---

6. For each selected objective, follow the instructions provided in the live view window. A good reference image should reflect the shading properties of the optical system and exhibit as little distortions as possible. A homogeneous calibration sample is prerequisite for good reference images. The optional **Spatial averaging** function can help to suppress small distortions of the sample by acquiring multiple images at slightly different positions around the current field of view and averaging these images.

---




---

7. Select <<DarkImage>> from the list of color channels to acquire a dark image.

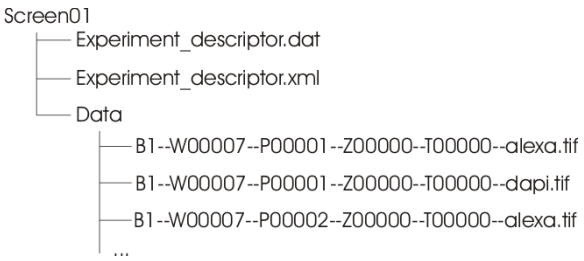
If correction images are available for a particular combination of color channel and objective, the shading correction can be activated in the acquisition settings (see Chapter 4.3).

# 9 Appendix

The following chapter provides additional technical information.

# 9.1 Data Storage Format

All the data for a specific scan is stored in the respective storage directory defined on the **Acquisition** tab of the **Edit scan** window. The storage scheme is as follows:




**Screen01**: storage directory; **Experiment_descriptor.dat**: position file; **Experiment_descriptor.xml**: scan settings file.

The system parameters are stored in the .xml file, the stage positions in the .dat file.

A **Data** subdirectory contains the actual image files in .tif format. Individual fields separated by a double dash compose the image file names. These fields denote in the order of appearance the well comment as shown in the table of the format manager, the well index, the position index, the Z-stack index, the time loop index, and the channel name.

The names are assembled systematically as a systematic train of parameters separated by double dashes in the following order: well name (**Comment**), well index, position index, Z-layer index, time loop index and the channel name.

If shading correction images are available for the data, they will be located in the **ShadingData** subdirectory.

# 9.2 Factory Tools

The programs described here are stored in the scan$^R$ program folder in the *Acquisition* subdirectory. They are only required for system installation and configuration and neither meant nor required to be used for daily operation.

## 9.2.1 LidPickup.exe

**Pick-up position**

The Pickup position is used when performing a scan with an autoloader. This is the position at which the stage will move when the autoloader adds or removes the plate.

Follow these steps to set-up the pick-up position:

1. Select the frame type.

2. Move the stage to the desired pick-up position

3. Press **Set as Pickup**

**Pipetting position**

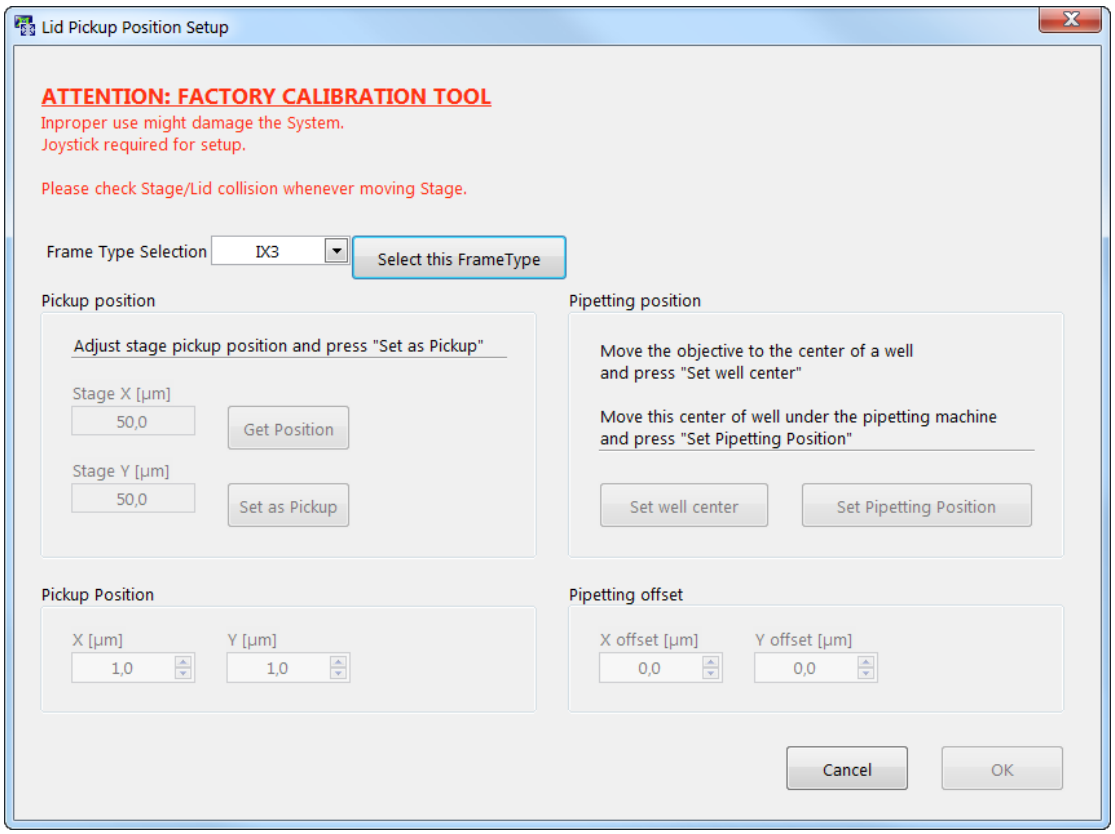
The pipetting position is only used by the liquid handling interface, (see Chapter 9.3, *Remote control*)

Follow these steps to set up the pipetting position:

1. Move the objective to the center of a well and press **Set well center**.

2. Move the same well under the pipetting machine and press **Set Pipetting position**.

When the offsets are many centimeters large, it will not be possible to move every well under the pipetting machine.




## 9.2.2 LimitSetup.exe

The options described in this chapter are only available with a motorized stage controlled by a STC controller or Tango controller.

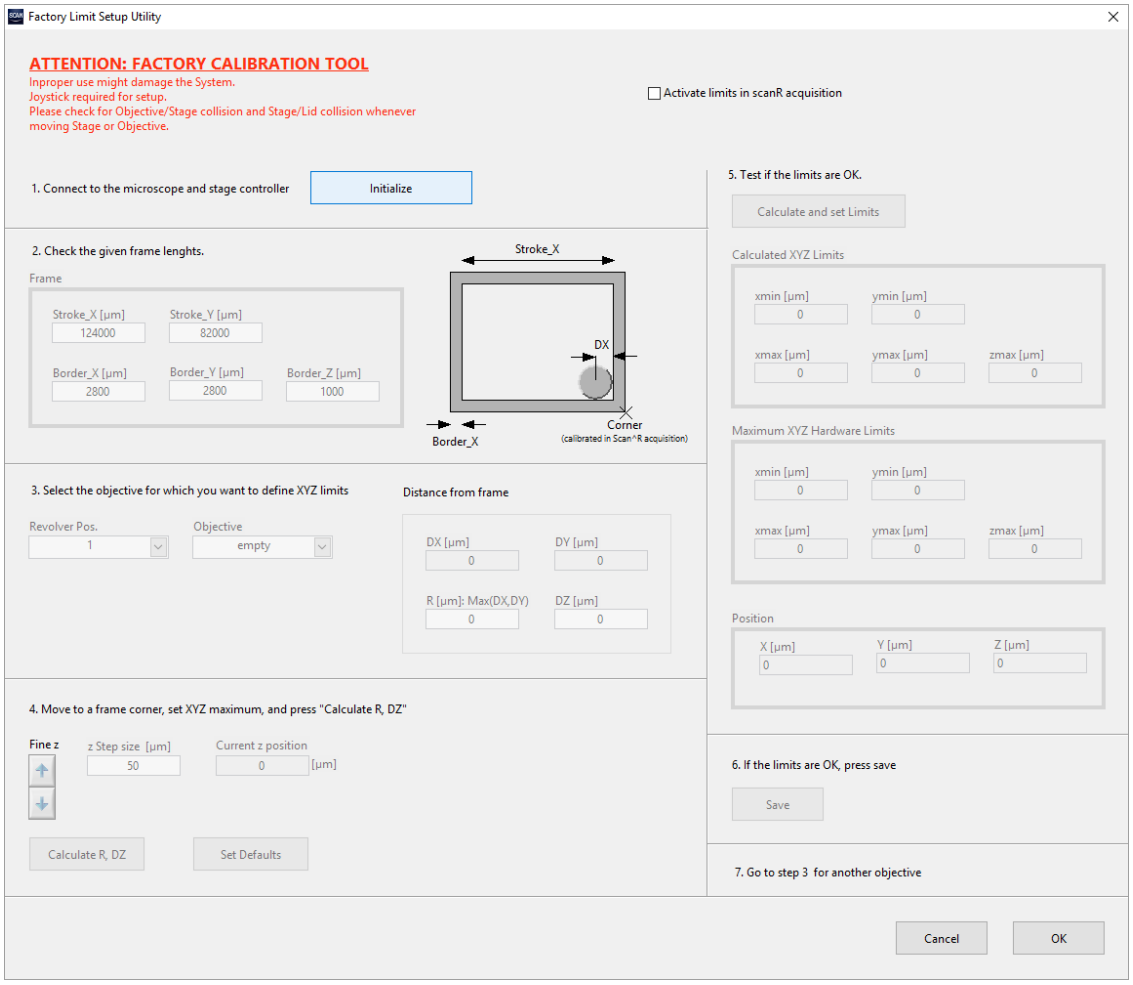
With this program it is possible to define separate XYZ limits for each objective.

To activate or deactivate the limits in the Scan$^R$ acquisition program, check or uncheck the box **Activate the limits in Scan$^R$ acquisition**.

If the stage is not calibrated, run a stage calibration using scan$^R$ Acquisition.exe (see Chapter 8, *System Configuration*)

The defaults might be used depending on the accuracy required.

Start the Program *LimitSetup.exe* and follow the steps as indicated on the dialog.




1. Press the **Initialize** button to connect to the microscope and stage controller.

---

2. Check the given frame lengths: When the dialog appears the first time it will show the defaults values for Stroke and Border. Check if these defaults are the correct ones when you compare them with the construction drawings of the used frame in scan$^R$. If you have more precise values in your drawing, correct these values.

---

3. Select the objective for which you want to define the XYZ limits. Start with one objective for which you want to define XYZ limits. To do that select the revolver position where the objective is installed and select the corresponding objective from the list. You may physically unscrew the objective and exchange it by another one if required. This step will define the objective for which the limits are set.

4. Move to a frame corner. This is the adjustment step for the objective. You can modify Z with the cursors and XY with the joystick to adjust the limit position for the objective. For control reasons the distances DX, DY are also displayed and for safety MAX(DX,DY) is taken as Objective Radius for the limit setup.

---

5. Test …This allows checking if the limits are OK. To do this, press the button **Test Limits**. The hardware limits will then be applied. You can now carefully move with the joystick to check if the objective stops at the required positions.

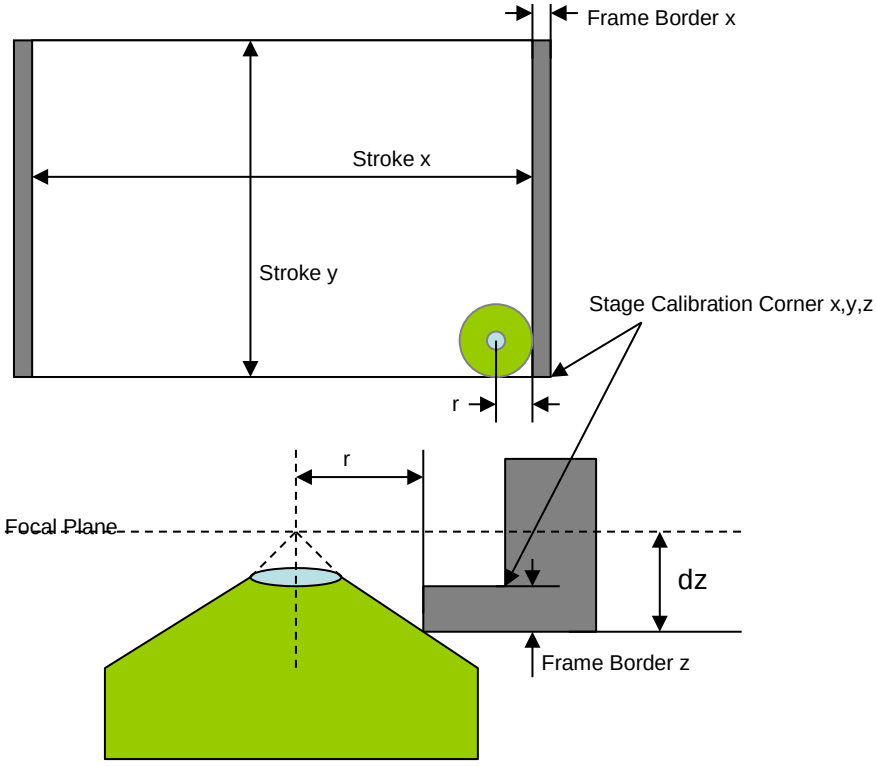
---

6. Click **Save** if the limits are OK. This will store the frame settings and the values R,dZ for the specified objective in the objective list (%ProgramData%\Olympus\OSIS\scanR\Acquisition\config\ObjectiveSettings.xml).

---

7. Continue at point 3 for the next objective

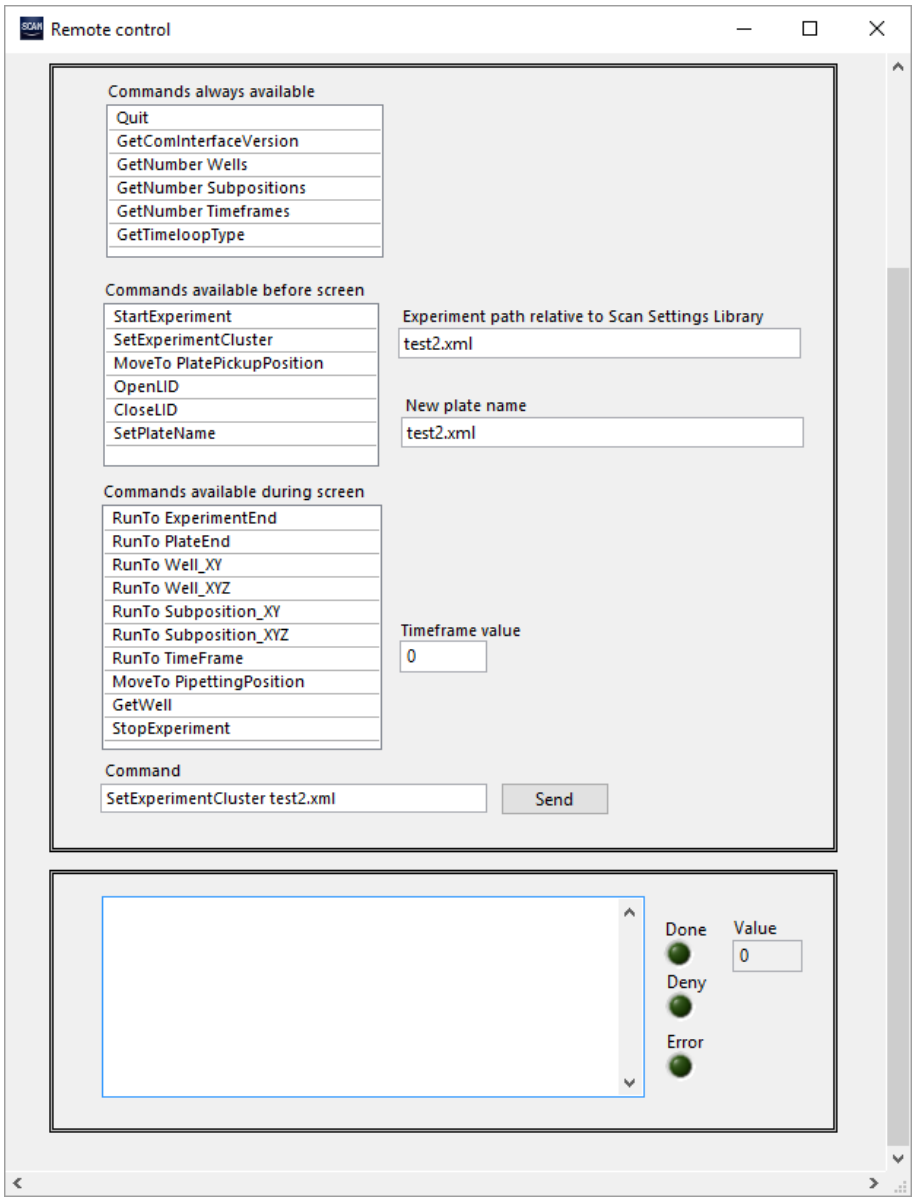
If an additional limit is required because of a frame or plate inserted this can be modified in the **Border Thickness** Fields of the Plate format (see **Edit Scan** ▸ **Plate manager** ▸ **Edit Plate Types**; Chapter 0, ).




Representation of the frame and objective. When setting up the limits, the objective parameters r and dZ are evaluated and stored.

## 9.3 Remote control

The scanR screening system can be controlled remotely by external software, for example to synchronize an external hardware device (e.g. a liquid handling system) with the scan$^{R}$ Acquisition. The program *Remote control.exe*, which can be found in the scan$^{R}$ program folder in \Aquisition\Remote control, allows testing the remote control functionality easily.



### 9.3.1 Connection to the server

The acquisition software is the server. The client connects to the server with the TCP/IP protocol on the port defined in **System ▸ System Configuration ▸ Remote**

Only one client can connect to the acquisition software.

When not in standalone mode, and when the **Edit Scan** window is not opened, the server is waiting for connection.

When a client connects, all buttons of the scan$^{R}$ main interface are deactivated, except for the **Browse Images** button.

### 9.3.2 Communication interface concept

**Syntax**:

Client Request: [Command_name + arguments + carriage return + linefeed]

This has to be sent within 6 seconds

Server Answer: [Command_name + arguments + (OK or ERROR or DENY) + (response or error description) + carriage return + linefeed]

**DONE.** The command was successfully executed

**DENY.** The command cannot be executed (the syntax is not correct or the time point is not correct). When a previous command is being executed, it continues to run. When no command was being executed, it waits for the next command.

**ERROR**. An error occurred while executing the command

Error descriptions:

- Command not known
- Argument not known
- Argument over range
- Screen is running
- No screen is running
- Waiting for end of previous command
- RunTo "Argument" not reached until end of experiment
- File not found
- Empty message: others errors are written in the logfile
- screen continues
- screen ended

During a screen, when an error occurred, the error descriptions “**screen continues”** or “**screen ended”**

indicate if the screen is aborted or not.

The communication is logged in the scan$^{R}$ Acquisition logfile, with following preceding Server/Client

TAG:

SERVER:

CLIENT:

### 9.3.3 Commands

**Quit.** The connection is closed by the server.

When a screen is running:

**GetComInterfaceVersion.** Returns the interface version number

Response:

**GetComInterfaceVersion DONE 3**

**StartExperiment *UseZPrevious*.** Starts the experiment like the start button.

***UseZPrevious***:

0 -> Z dialog will pop up (if no z-position has been calibrated in plate manager)

1 -> Use previous Z, like use previous Z button

2 -> Use previous taught Z (independently of the loaded experiment file)

It returns the path of the image data directory

Responses:

**StartExperiment DONE** F:\Data\BDFalcon_fullwell_002

**StartExperiment DENY** Screen is running

**StartExperiment ERROR** (cancelled by user, wrong settings for example, not enough hard disc space)

When not enough hard disc space is left, the screen is aborted.

**StartExperimentInversePlate *UseZPrevious***

Same function as **StartExperiment** except the plate will be expected to be rotated by 180° on the stage. This is useful sometimes if the plate is supplied by a robot.

**StopExperiment.** It stops the experiment like the stop button.

Responses:

**StopExperiment DONE**

**StopExperiment DENY** No screen is running

**SetExperimentCluster *filename*.** Changes the current scan settings like the open button when no screen is running

The experiment is specified by the filename. The filename is relative to the scan settings directory of the current user, for example: %userprofile%\Documents\ScanR\Scan Settings\test.xml

**Examples:**

CLIENT: SetExperimentCluster test.xml
SERVER: SetExperimentCluster test.xml DONE

CLIENT: SetExperimentCluster test_not_existing.xml
SERVER: SetExperimentCluster test_not_existing.xml ERROR File not found

CLIENT: SetExperimentCluster test.xml
SERVER: SetExperimentCluster test.xml DENY Screen is running

**FilterWells list**

List is a list of wells to be included, based on the wells marked as selected in the current scan settings.

**Examples:**

FilterWells 2 3 4 5 6 7 8 9 10

Screening is restricted to the wells 2-10, even if more wells have been selected in the scan settings. Wells not selected in the scan settings will never be screened even if listed here.

**SetPlateName *name***

Set a different name for the current experiment than defined in the current scan settings. This also influences the directory name where the scan is stored.

**Examples:**

SetPlateName Exp2153

**GetNumber *Argument*.**

Argument is one of the following:

- **Wells**
- **Subpositions**
- **Timeframes**

**Examples:**

CLIENT: GetNumber Subpositions
SERVER: GetNumber Subpositions DONE 4

CLIENT: GetNumber Timeloops
SERVER: GetNumber Timeloops DENY Argument not known

**GetWell**

Returns the number of the current well (1…n)

**Example:**

CLIENT: GetWell

SERVER: GetWell DONE 5

**RunTo *Argument*.** Argument might be one of the following:

- **ExperimentEnd**
- **PlateEnd**
- **Well_XY**
- **Well_XYZ**
- **Subposition_XY**
- **Subposition_XYZ**
- **TimeFrame Argument: number**

**Examples:**

CLIENT: RunTo ExperimentEnd
SERVER: RunTo ExperimentEnd DONE

CLIENT: RunTo TimeFrame 6
SERVER: RunTo TimeFrame 6 DENY Argument over range

**MoveTo *Argument*.**

Argument might be one of the following:

- **PlatePickupPosition.** Only available before screen has started. Moves to the Plate Pickup Position (used for Robot) and returns to last position when the next command is received
- **PipettingPosition.** Only available after screen has started. Moves the Plate by a fixed predefined XY-offset and returns to last position when the next command is received. The Pipetting offset position is defined in the utility ./FactorySettings/LidPickup.exe . This can be useful for transmission light imaging.

**Example of responses:**

MoveTo PlatePickupPosition DONE

MoveTo PlatePickupPosition DENY Screen is running

MoveTo PipettingPosition DONE

MoveTo PipettingPosition DENY No screen is running

MoveTo PipettingPosition ERROR (pipetting position cannot be reached for example)

**GetProgress**

Returns the progress of the currently running screen in terms of percentage.

**Example:**

CLIENT: GetProgress

SERVER: GetProgress DONE 10

The **RunTo** commands are executed synchronously and new commands will not be accepted before the **RunTo** command has finished. In order to query the progress for example when **RunTo PlateEnd** has been started, the connection has to be closed by using the **quit** command first (this will only close the connection and not influence the running screen) and re-established then. Once re-connected, the **GetProgress** command will be accepted.

### 9.3.4 Known use cases and corresponding communication samples

General: all setting clusters can be used in normal user mode as well. Thus they can be tested and setup independently from the server/client mode. All data resulting from a client/server mode screen is fully compatible and analyzable with the analysis.

**A) scan$^R$ is to be fully integrated into a plate sample preparation line**

1. Test the different plates and assays in scan$^R$. Save the different setting files in the directory 'Scan Settings Library'. Every kind of scan available in the user mode will also be available in the server mode.

2. The user activates once the Server Mode in the GUI of scan$^R$

3. Sample Workflow of sent messages:

:

| **Server** | **Client** |
|---|---|
| | SetExperimentCluster PrefinedExperiment**1** |
| SetExperimentCluster DONE | |
| | MoveTo PlatePickupPosition |
| MoveTo PlatePickupPosition DONE | |

| | |
|---|---|
| | StartExperiment |
| StartExperiment DONE | |
| | RunTo ExperimentEnd |
| RunTo ExperimentEnd DONE | |
| | SetExperimentCluster PrefinedExperiment**2** |
| SetExperimentCluster DONE | |
| … and so forth | |

4. Analyze separately each scan in scan$^R$ analysis, i.e. no automation integration. The analysis has to be run in batch mode or in parallel. The batch has to be fed manually.

**B) Well-Time-lapse acquisition using a liquid handling automate trigger substance for starting the time-lapse (Analogous for Subpositions or Plate Time-lapse). The liquid insertion changes the AF layer position. Therefore AF should be performed after liquid insertion.**

Example:

1. In the scan$^R$ Acquisition software execute **Edit scan ▸ Acquisition** and select **Loop: Well** in the **Time-lapse settings** box and adjust the settings.

---

2. Activate once the Server Mode in the GUI of scan$^R$ and communicate with the server like the following workflow (3 wells have been selected in the scan$^R$ Acquisition GUI)

| **Server** | **Client** |
|---|---|
| | GetNumber Wells |
| GetNumber Wells DONE 3 | |
| | StartExperiment |
| StartExperiment DONE | |
| | RunTo Well_XY |
| RunTo Well_XY DONE | |
| | “The automate provides the liquid” |
| | RunTo Well_XY |
| RunTo Well_XY DONE | |
| | “The automate provides the liquid” |
| | RunTo Well_XY |
| RunTo Well_XY DONE | |
| | RunTo ExperimentEnd |

| | |
|---|---|
| RunTo ExperimentEnd DONE | |

**C) Well-Time-lapse acquisition using a liquid handling automate trigger substance for starting the time-lapse. The trigger point is time sensitive and insertion of the liquid does not change the AF plane. It is required to make time-lapse images of the same position before and after the insertion to observe the changes due to the insertion.**

Example:

1. In the scan$^R$ Acquisition software execute **Edit scan ▸ Acquisition** and select **Loop: Well** in the **Time-lapse settings** box and adjust the settings. Only one subposition should be selected for the settings

---

2. Activate once the Server Mode in the GUI of scan$^R$ and communicate with the server like the following workflow (2 wells have been selected in the scan$^R$ Acquisition GUI, the number of time-lapse frame has been set to 40)

| **Server** | **Client** |
|---|---|
| | StartExperiment |
| StartExperiment DONE | |
| | RunTo Well_XYZ |
| RunTo Well_XYZ DONE | |
| | RunToTimeFrame 20 |
| RunToTimeFrame 20 DONE | |
| | “The automate provides the liquid” |
| | RunTo Well_XYZ |
| RunTo Well_XYZ DONE | |
| | RunToTimeFrame 20 |
| RunToTimeFrame 20 DONE | |
| | “The automate provides the liquid” |
| | RunTo Well_XYZ |
| RunTo Well_XY ERROR RunTo Well_XY not reached until end of experiment | |
| | RunTo ExperimentEnd |
| RunTo ExperimentEnd DENY No screen is running | |

**Not supported with the given specification are:**

- Multiple liquid insertions at a single Well/Position with subsequent interrupted time-lapse acquisition **AND** AF after the second insertion step.

- Automated Analysis takeover of time-lapse ranges (time-lapse liquid insertion points have to be remembered and set manually in the analysis software).
- Feedback loops are only possible with considerable client programming as scans have to be stopped and newly prepared settings have to be issued.
- Within a single scan no sequences are possible that are different from the regular sequences. In order to change the sequence the scan has to be stopped and restarted.