New Features in Release 2.2

This short manual gives an overview over the new features in the scanR release v. 2.2 (Acquisition Software v. 2.2.0.8, Analysis Software v. 1.2.0.4).

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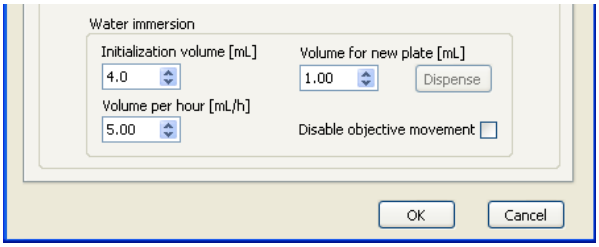
1 Acquisition Software

1.1 Option: Automated Water Immersion System (IX2-AWI)

An automated water immersion system is available as an option or as an upgrade 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. The automated water immersion system IX2-AWI is supported by the scan^R Automated Image Acquisition Software. The settings for the automated water immersion system can be found in **System ▸ System Configurations ▸ Microscope**.



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.

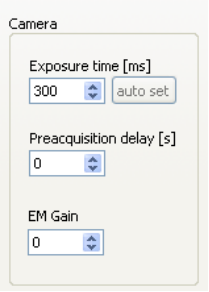
Volume for new plate [ml]. The volume that is dispensed when a plate is changed during acquisition by a plate loading robot. To check this volume during configuration press the adjacent button **Dispense**. If a plate is changed manually press the **Dispense** button at the front of the IX2-AWI control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

Disable Movement: if this checkbox is activated, the movement of the objective turret is disabled.

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

1.2 Support for Hamamatsu EM-CCD 9100-02

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.



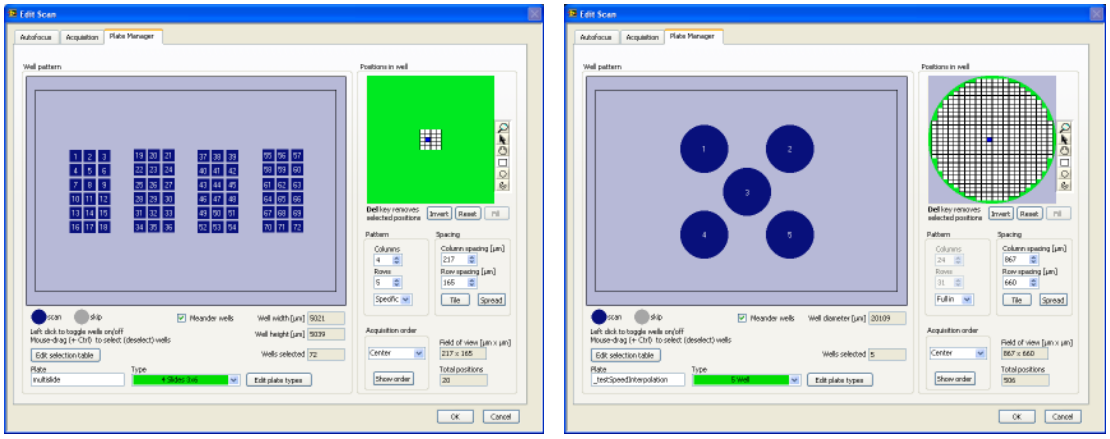
Camera control box

1.3 New Plate Types: multislide plate holder with MTP footprint

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

To create a multislide 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 multiple slides. The number of slides defined will be indicated in the **Repetition** field in the **Pattern** box.

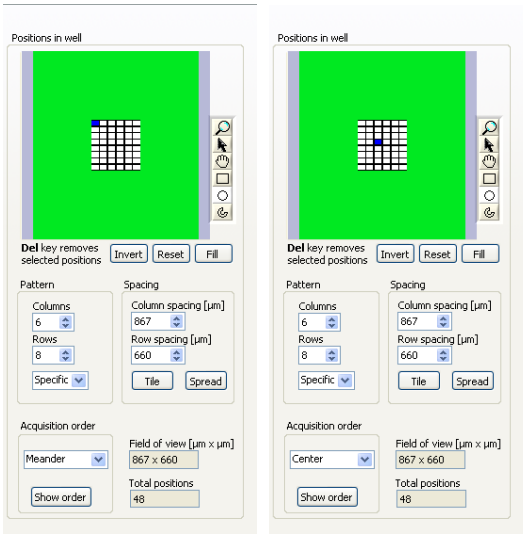
This allows also defining all kinds or regular patterns as plate types.



Example for a multislide plate consisting of 4 individual slides (left) and a plate consisting of 5 wells

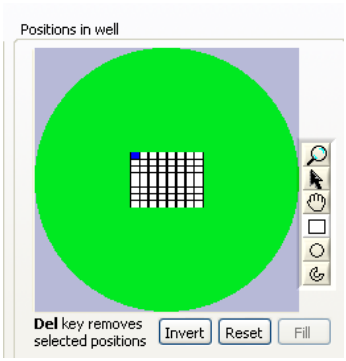
1.4 Position Pattern Improvements

Go to **Edit ▸ Plate Manager**. In the **Pattern** and **Spacing** boxes you can define an arbitrary position pattern. The position that will be scanned first is highlighted blue. In the menu box **Acquisition order** you can select **Center** in order to create a position pattern where the position that is closest to the center of the well is scanned first. Subsequently adjacent positions are scanned.

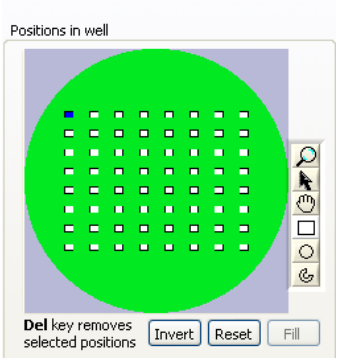


Meander *Center start*

The **Spread** button will distribute the positions in equal distances within the whole well. To reset the pattern to adjacent positions, click on **Tile**.



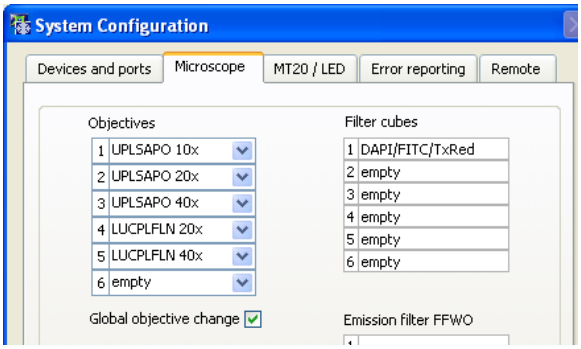
Tile



Spread

1.5 Global objective change

In each channel (AF, color channels) the objective can be set independently. In order to facilitate the operation of scanR, the objectives can be changed for all channels simultaneously. This mode of operation can be set in **System ▸ System Configuration ▸ Microscope**. 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 (cf. scanR Automated Image Acquisition Software, Chapter 4.1 *Autofocus settings*) is automatically adapted to the selected objective.



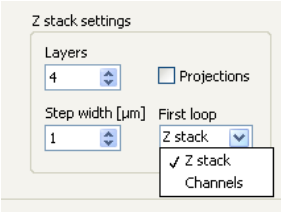
1.6 Color channels z inner loop

Go to **edit ▸ Acquisition**. In the **z stack settings** box you can set the number of **Layers** for the z stack as well as the **Step width**. In the drop down menu **First loop** you can select what is to be performed first when a z stack is acquired:

- 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.

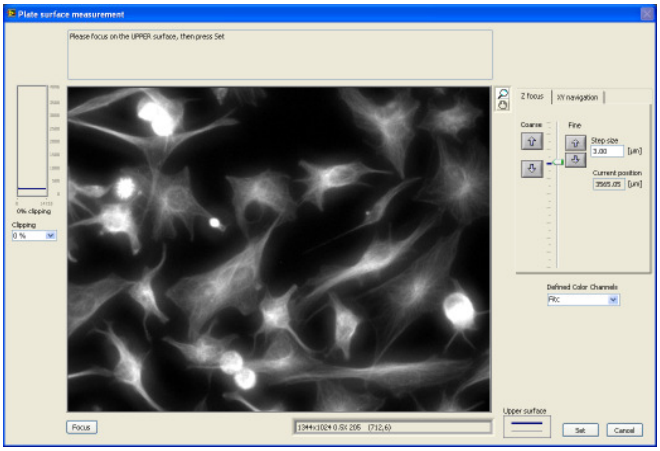
- Channels: When this option is selected for every layer of the z stack all selected color channels are scanned consecutively. Only when all color channels are acquired, the objective moves to the next z position and then all color channels at this position are acquired, and so on.



1.7 Improved measurement functions

For z calibration of the plate an own dialog for manual/ZDC z plate adjustment is now available. In the **Edit ▸ Plate Manager ▸ Edit plate type** menu click on the **Measure** button.

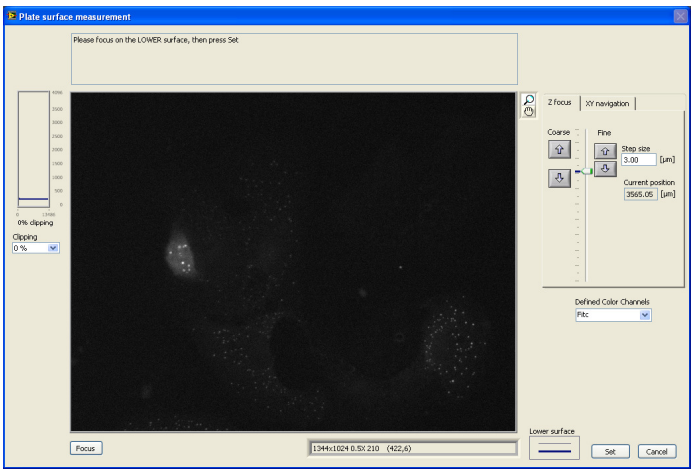
If the system is equipped with a ZDC the acquisition software will now detect the lower surface of the sample automatically. This value will be entered as **Distance from plate frame bottom**. After that the **Plate surface measurement** window will appear.



Define upper surface

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 sample. The **Thickness** of the sample will thus be calculated.

If no ZDC 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.

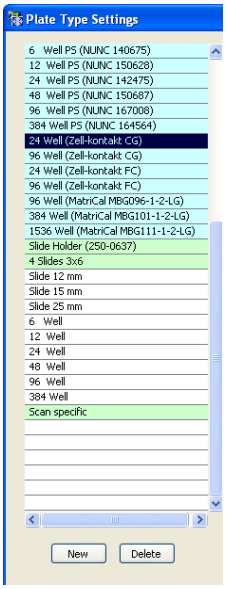


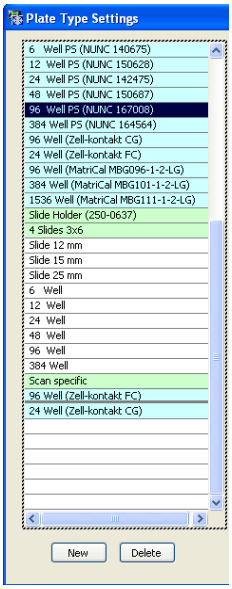
Define lower surface (optional)

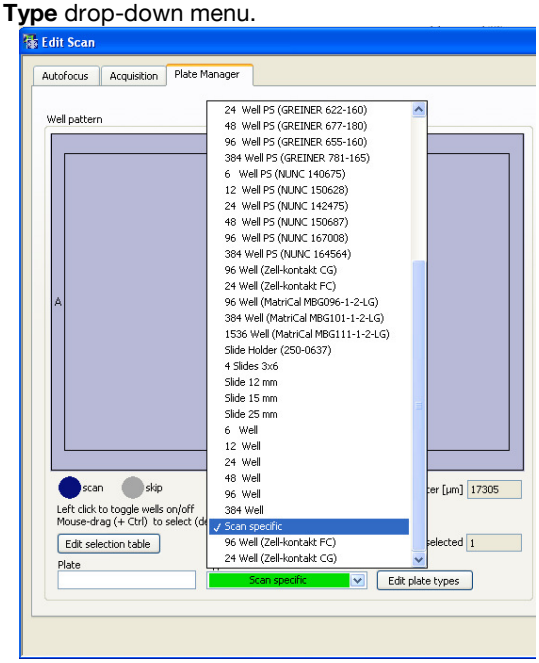
If these measurements have been performed successfully, in both cases the upper surface of the sample will be indicated in all **z focus** sliders by a blue bar.

1.8 Change plate list order by drag&drop

Go to **Edit ▸ Plate Manager**. Click on **Edit plate types** to enter the **Plate Type Settings** menu. 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 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.







1.9 Significant Software improvements & changes in software behavior

Exit confirmation in Acquisition

The camera gain is set to 0 for new setups: this setting gives a better comparability between Hamamatsu C8484 and ORCA R2

Change Plate selection: When changing the set plate in the **Type** drop down menu, (**Edit ▸ Acquisition ▸ Plate manager**) all wells will be automatically selected. When changing the plate while **Full In** or **Full out** is set, this pattern is still used for the new plate instead of switching to square pattern.

Liquid com interface: command "GetTimeloopType" added, cf. scanR Automated Image Acquisition Software, Chapter 7.4 *Remote Control*)

z update in live views: When in live view the sample is focused using the microscope wheel, it is no more necessary to press the **update** button. Instead, the current z position from the microscope wheel will be updated in the software automatically.

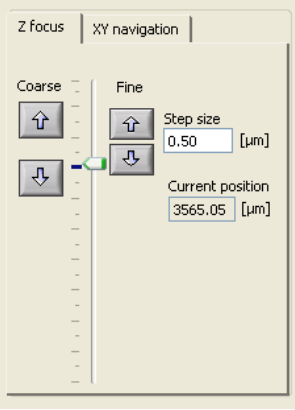
Selected channel in the live view of the Devices **window is stored**

Support for cell 3.2 (1631-1700) and RTC(PC104) v.3.2.2.2: the scanR version 2.2 is therefore not compatible with older versions of the cellR software.

Support for Hamamatsu ORCA R2: for an update of the scanR Acquisition software and simultaneous change of the camera a new DCAM API is required.

Grayed out time loop and z settings if t,z deactivated (Edit ▸ Acquisition). As long as in the **Z stack** settings and in the **Time-lapse** settings control boxes only 1 **Layer** or 1 **Cycle**, respectively, is set, the other options will be grayed out in order to indicate that no z stack and no time-lapse experiment is acquired.

Displays for plate z location: 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).

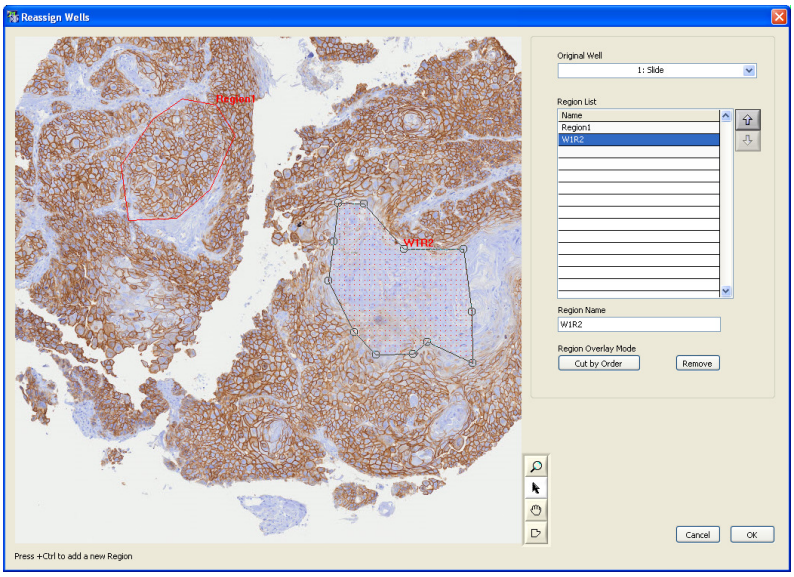


Z focus

2 Analysis Software

2.1 Reassign Wells

In some cases, especially for histological samples, it may be of interest to define special regions within one well (e.g. tumor and healthy tissue). The regions to be analyzed can be defined in the **Reassign Wells** window. To open this window, go to **Scan ▸ Reassign Wells…**. On the left side of the menu the image of the currently selected well will be displayed.

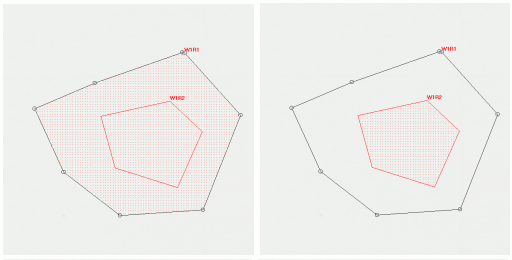


To change the well that is displayed on the left, select another well in the **Original Well** drop down menu. To navigate in the well image use the **Zoom** and **Move** mouse tools. Use the **polygon** tool to create a polygon around the region of interest, and close the polygon with a double-click. A new region will appear in the **Region list**. The default name is W1R1 (Well 1, Region 1). You can enter a meaningful name in the **Region Name** field instead. It is possible to assign the same name to two regions in order to group these regions.

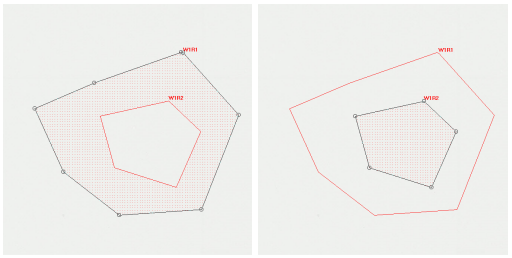
To create further regions use the **polygon** tool and press the **Ctrl** key on the keyboard at the same time. Otherwise the first region will be replaced by the new region. With the **Selection** tool, the nodes of the existing regions can be displaced (indicated by a double arrow cursor) or the whole region can be moved (indicated by the crossed arrow cursor).

If more than one region is created and some of the regions are overlapping, you can choose the **Region Overlay Mode**:

- **Order Independent**: The overlapping area will not be cut. The complete area inside a defined region belongs to this region. Overlapping areas will belong to two regions.
- **Cut by Order**: The overlapping area of a region further down the **Region List** will be cut from the higher region. By this, doughnut-like regions can be defined.

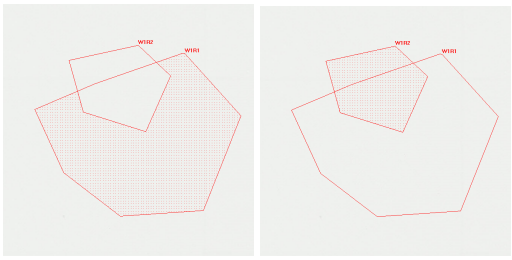


Order Independent

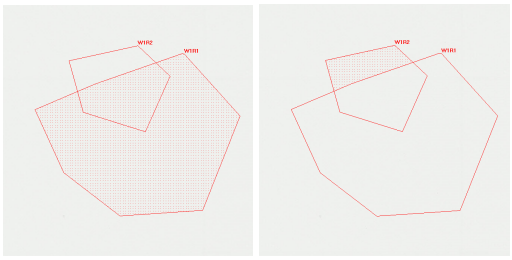


Cut by Order

The **Cut by Order** mode depends on the order of the regions in the **Region List**. To change the order of the regions, mark the region to be moved in the **Region List** and use the arrow icons to move this region up or down in the list. The region that is lower in the list will cut the overlapping area from the region that is higher in the list.



Cut by Order W1R1 above W1R2

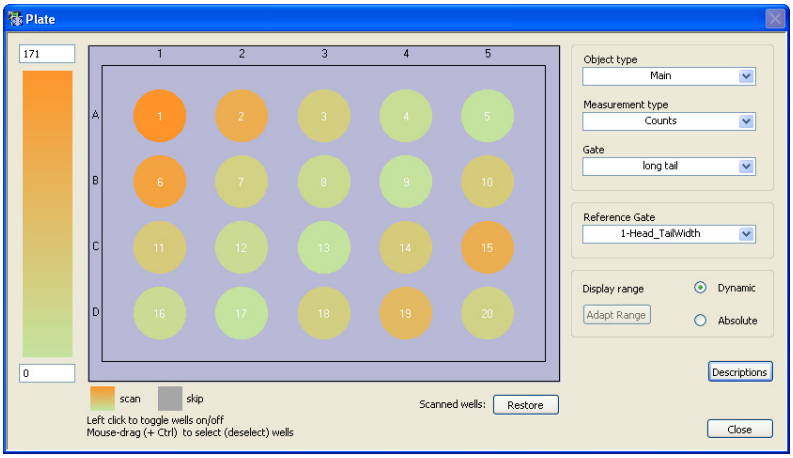


Cut by Order W1R2 above W1R1

The new “virtual” wells that are defined in the **Reassign Well** menu will fully replace the original wells in the analysis. This means they replace the original wells in the **Assay Setting** menus (cf. scanR Automated Image and Data Analysis Software, Chapter 3 *Assays*), the front panel and the **Well Results** (cf. scanR Automated Image and Data Analysis Software, Chapter 4.3 *Well Results*).

2.2 Interactive plate result view

The results of all measurements can be displayed interactively in the **Plate** window. To open this window, go to **Scan ▸ Plate…**. In the center of the window you will find a graphical representation of the screened plate. When the window is opened for the first time, the wells that were skipped during acquisition are shown in gray, the scanned wells show a different color.



By default all screened wells will be taken into account for analysis. The well selection is operated according to the well selection in the scanR acquisition software, i.e. to change the selection you can click on the wells. To deselect multiple wells you can press Ctrl on the keyboard and draw a rectangle with the mouse around the wells to be excluded. When a well is deselected, the corresponding data points will interactively be removed from all histograms. To include a deselected well again, click on the well. It will again be displayed in a color other than gray and the corresponding data points will be shown in the histograms.

The **restore** button will restore the initial well pattern.

A **well overview** of each well can be shown by right-clicking on one of the wells and selecting **well overview** (cf. scanR Automated Image and Data Analysis Software, Chapter 2.7 *Selecting Wells for Analysis*).

To display a heat map of the measurement results you have to run the analysis first and then proceed as follows:

Select the object type (main object or sub-object) from the first drop-down menu (**Object type**) on the right. The drop down-menu **Measurement type** allows you to switch between **Counts**, **Counts%**, **Mean**, **Error**, **Error%** and **CV%.**

When **Counts** is selected as **Measurement type**, you can select the population you are interested in the **Gate** drop-down menu. Here all gates that you have created in the histograms can be selected. You can select the population, you want to use as reference (e.g. a gate “good nucs” that contains all nice, round cells) in the **Reference Gate** drop-down menu. Here also all gates that you have created in the histograms can be selected. The wells will now be displayed in a color that indicates how many cells in every well are found in the selected gate. When a well is displayed in red, many cells of this well can be found in the selected gate, whereas a green color indicates, that only few cells can be found in the selected gate.

The same applies when **Counts%** is selected as **Measurement type**, in this case however, the color will indicate the percentage of cells that are in the selected gate.

When **Mean**, **Error**, **Error%** or **CV%** are selected as **Measurement type**, again the **Gate** drop-down menu contains all gates that you have created in the histograms. However, the next drop-down menu now allows you to select the **Measurement** to be performed. Here all parameters that you have previously defined in **Edit Assay ▸ Measurement Parameters** (cf. scanR Automated Image and Data Analysis Software, Chapter 3.5 *Measurement Parameters*) and in **Derived Parameters** (cf. scanR Automated Image and Data Analysis Software, Chapter 3.5 *Derived Parameters*) can be selected from the drop-down list. For example if the parameter **Area** is selected as **Measurement** and **Mean** is selected as **Measurement type**, the mean **Area** of all cells per well will be color encoded. This means that wells with very large cells will be displayed red, whereas wells with smaller cells will be displayed green.

ⓘ The **Plate** view is fully interactive. When a gate is modified in the front panel, the results will automatically updated in the **Plate** view.

In the **Display range** box you can set how the range of the color display will be adapted, when the same assay is run on a new data set. The display range can then be either adapted in **Dynamic** mode, which means the min and max values of the display range will be adapted according to the new data set. Alternatively, the range can be adapted in **Absolute** mode, which means the range that you have set in the first place will be used for all other analyses.

In order to change the display range manually, you can directly enter min and max values at the bottom and the top of the color bar, respectively. When you change the range manually, the **Display range** mode will automatically change from **Dynamic** to **Absolute**. The **Adapt Range** button can then be used to adapt the min and max ranges of the color display to the current data set and when a new experiment is analyzed, also this display range will be used. Switching back to **Dynamic** will also restore the min and max values of the display, but when a new experiment is analyzed, the min and max ranges will be adapted to the results of the new dataset.

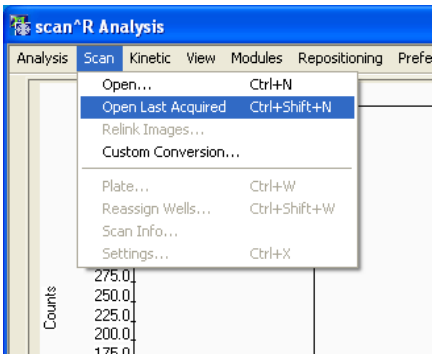
A click on the **Descriptions** button opens **Name/Description** list. This list displays all selected wells. By default, the wells will be named A1, A2,… etc. The names of the wells can already be changed to a meaningful name when setting up the acquisition (cf. scanR Automated Image Acquisition Software, Chapter 4.4.1 *Well pattern*). Alternatively, the names can be changed now in the **Plate** window. Click in the **Name/Description** field you want to change and enter a new name.

ⓘ Note: when the same name is used for multiple wells, these wells can be grouped. In the **Measurement Results** and **Populations** tabs of the **Well Results** window (cf. scanR Automated Image and Data Analysis Software, Chapter 4.3 *Well Results*) you can then switch between **Wells** and **Groups** to have every well listed individually or to show the results of the created groups.

A second click on **Descriptions** hides the **Name/Description** list.

2.3 Open last acquired

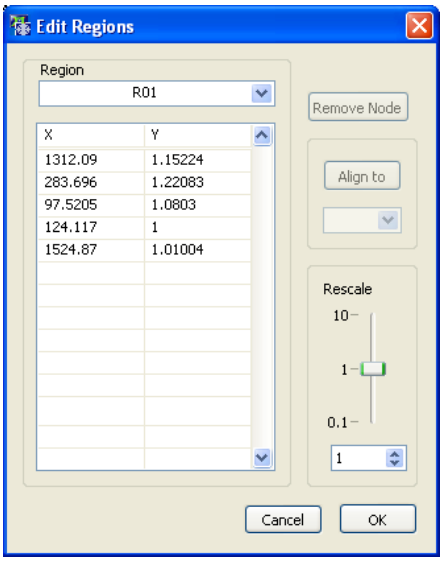
Go to **Scan ▸ Open Last Acquired**. This opens the experiment_descriptor.xml file from the scan that was started last.



2.4 Adjustment and alignment of regions

The regions in one histogram can be numerically adjusted and if multiple regions are created in one histogram, these regions can be aligned with respect to each other.

First draw a region in the first histogram by selecting the polygon tool. Close the region with a double-click. To numerically adjust the created polygon, go to **Analysis ▸ Assay Gating** and highlight in the **Gate application** list the histogram in which you have created the region. Then click on **Edit Regions**. The **Edit Regions** window opens.

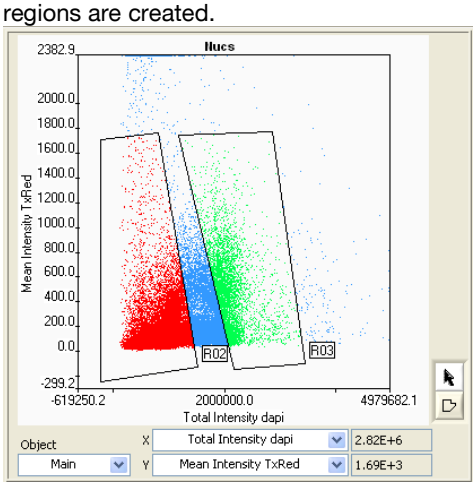


Edit Regions menu

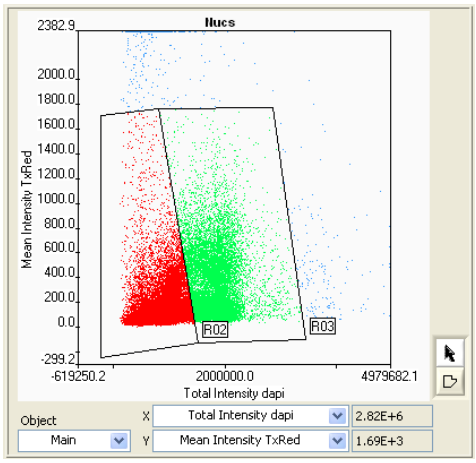
In the **Region** drop-down menu you can select the region that you want to change numerically. The list will then show the x and y positions of the nodes of this region. You can change these values by entering numbers in the list. When you click on one of these values the **Remove Node** button will become accessible. With this button, single nodes of the polygon can be removed. Similarly, a new node can be created by entering the x and y values in a new line of the list.

With the **Rescale** slider or the corresponding numerical box below the slider, you can scale the polygon you have created. Values greater than 1 will increase the size of the polygon, values smaller than one will decrease the size of the polygon. The x and y values in the list will be adapted accordingly.

In the case that more than one region is created in one histogram, these Regions will become available in the **Region** drop down menu and the **Align to** button will become active. For example, the following regions are created.



Regions not aligned

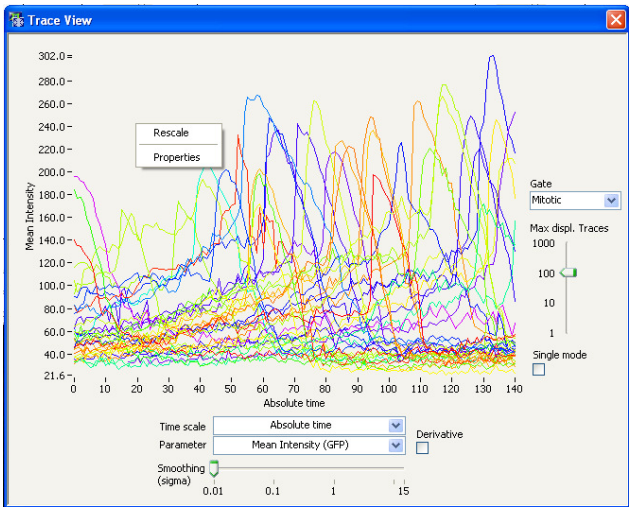


Regions aligned

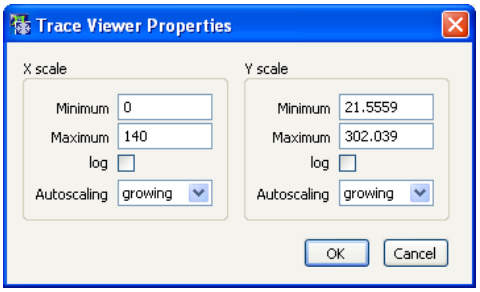
Now region R02 and region R03 will be aligned. In the **Edit regions** window Select R03 in the **Region** drop down menu and select **Align to** R02. Click on **Align to**. This will update the x, y coordinates of the Region R03. Click **OK** to leave the menu. The Regions R02 and R03 will now be aligned.

2.5 Set TraceViewer scale

In tracking data sets the traces of the particles can be displayed in the **Trace Viewer** (cf. scanR Automated Image and Data Analysis Software, Chapter 3.9.3 *Trace Viewer*). Open the **Trace Viewer** by selecting **Tracking ▸ Show Traces** in the main menu. A right-click in the plot opens a context menu, where you can select **Properties**.



The **Trace Viewer Properties** window opens. Here you can set the **X** and **Y scale** of the histograms.



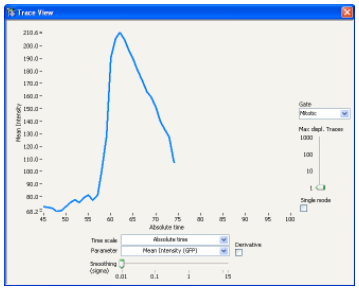
Minimum: Sets the minimal value of the x- and y-axis, respectively.

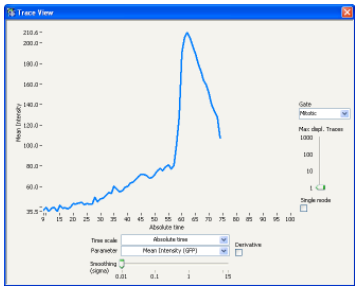
Maximum: Sets the maximal value of the x- and y-axis, respectively.

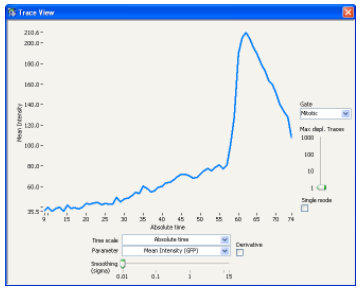
Log: Check this box for logarithmic scaling of x- and y-axis, respectively.

Autoscaling: Choose between three autoscaling methods:

- **off**: no autoscaling. The scale remains fixed when different curves are displayed
- **growing**: the scale will increase if the current scale is not large enough to display all data points of the selected curve(s) but it will not decrease if the selected curves do not fill the complete histogram.
- **compact**: the scaling is optimized to display fit the selected curves optimal into the histogram, i.e. the display is chosen such that the max and min values of the curves are also the maximal and minimal values of the histogram.


off (45..100)

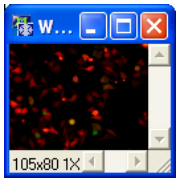

growing


compact

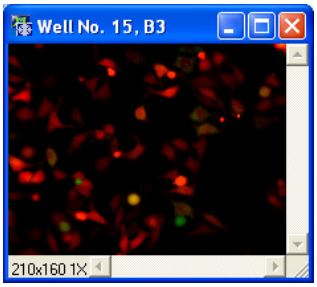
A right-click in the **Trace Viewer** gives also the option **Rescale**. This option can be used to scale the currently selected curves optimal in the histogram without changing the settings of the autoscaling when further curves are added.

2.6 Set well overview resolution

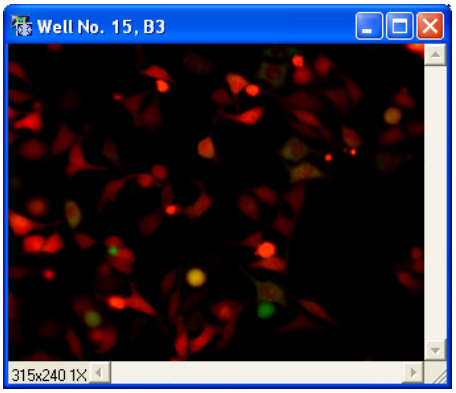
The pixel size of a single position for the well overview can be set to 80, 160, 240 pixels. To change the overview size of an image, go to **Preferences**. The size can be set in the **Overview Size** drop down menu. It can be useful to decrease the resolution of the well overview for large overviews in order to increase the speed of the display.



80 pixels



160 pixels



240 pixels

2.7 Image Processing: Smooth image

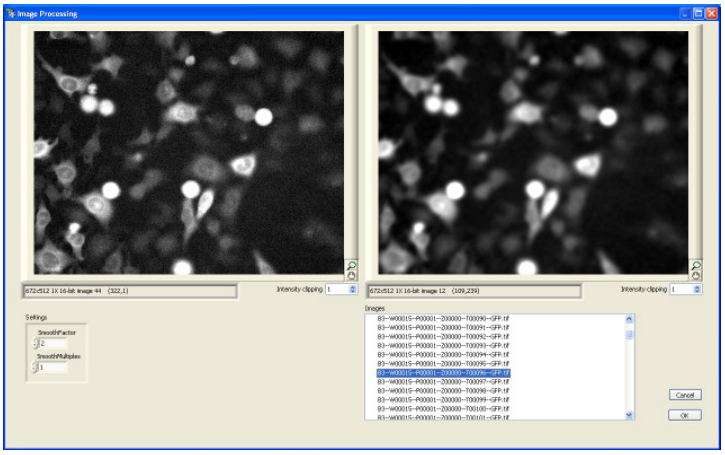
Loading the Smooth Image IP:

The **smooth image** Image Processing (IP) module is by default not selectable in the corresponding drop down list of the **Assay Settings**. In order to make this IP module available go to **Modules ▸ Image Processors…** and click on the **Add** button. A new line in the **Image Processing Modules** list is created. Then click on the folder icon 🗁 next to the **Module Location** field. Select **SmoothImage.vi**

and confirm with **OK**. The **SmoothImage.vi** is added to the **Image Processing Modules** list. Close the menu with **OK**. The **Smooth Image** IP will now be available in the **Assay Settings** menu like other IP Modules and can be selected in the **Image Processing** (cf. scanR Automated Image and Data Analysis Software, Chapter 3.7 *Image Processing*) tab as well as in the **Virtual Channels** tab (cf. scanR Automated Image and Data Analysis Software, Chapter 3.8 *Virtual Channels*).

Using the Smooth Image IP:

In order to use **Smooth image** go to the **Image Processing** tab or the **Virtual Channels** tab and press **New**. Then select **SmoothImage** from the **Module** drop down list. Click on **Adjust** in order to change the settings of the IP. The **Image Processing** menu opens. On the left the original image and on the right the processed image is displayed. In the **Images** list on the right, the acquired image that is to be shown in the displays can be selected. In the **Settings** field two parameters are available that allow you to adjust the smoothing.

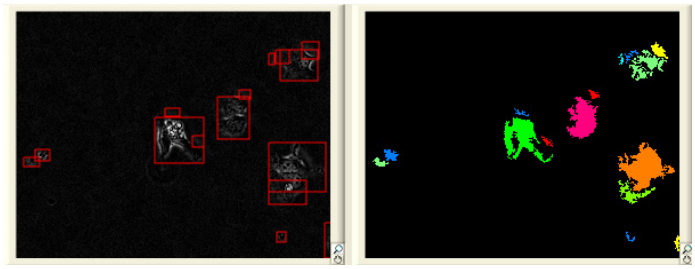


The SmoothImage Menu

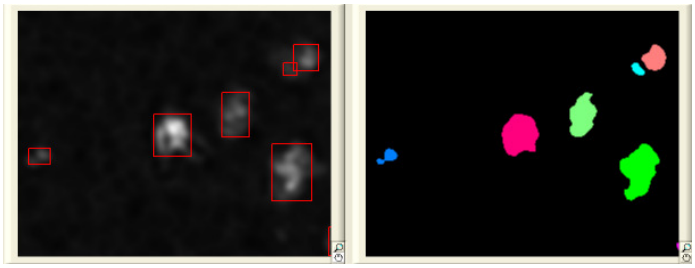
Applications

In some cases it may be necessary to smooth the image before object detection. This is the case for

- noisy images,
- when the border of the objects is fuzzy,
- if the object detection algorithm splits one object in many small fragmented objects.



Object detection without smoothing



Object detection with smoothing

2.8 Significant software improvements & changes in software behavior

Improved memory management allows significantly larger particle lists

Support for .slide raw format

Initial software start is 3x faster than in older versions

Improved custom conversion: it is not necessary any more to use regular expressions. Simply enter the strings preceding the numerical values for **Well/Position/Layer/Time**. New version is backward compatible, i.e. old conversion rules are compatible with new software version.

ShiftXY - Image Splitter support: the image processing (IP) module **XY shift** (cf. scanR Automated Image and Data Analysis Software, Chapter 3.7.2 *XY Shift*) allows to set arbitrary shifts in x,y-direction, which allows now to analyze images taken with an image splitter with **scanR.**

3 Supplementary Information

3.1 Pixel size and optical resolution for supported cameras

3.1.1 Hamamatsu Orca R2 / C8484 / ER

The chip of the camera has 1344 x 1024 pixels with a cell size of 6.45x6.45µm. The pixel size of the image is given by pixel size of the chip, divided by the magnification factor of the objective. E.g.

Objective	Magnification	Pixel size
UPLSAPO	10X	645 nm
UAPO	20X	322,5 nm
UPLSAPO	40X	161,25 nm

3.1.2 Hamamatsu EM-CCD 9100-02

The chip of the camera has 1000 x 1000 pixels with a cell size of 8.0x8.0µm. The pixel size of the image is given by pixel size of the chip, divided by the magnification factor of the objective. E.g.

Objective	Magnification	Pixel size
UPLSAPO	10X	800 nm
UAPO	20X	400 nm
UPLSAPO	40X	200 nm

3.1.3 Optical Resolution

The optical resolution of an objective in the x, y direction is given by 0,61 x λ / N.A., with λ being the wavelength of the light and N.A. the numerical aperture of the objective. For a wavelength of λ = 500 nm this yields:

Objective	Magnification	N.A.	optical resolution
UPLSAPO	10X	0,4	763 nm
UAPO	20X	0,7	436 nm
UPLSAPO	40X	0,9	339 nm

3.2 PC Specifications for Analysis Software

RAM: min. 2 GB

CPU: min. 2 GHz, dual core processor recommended if other applications are executed in parallel

Hard drive 1: min. 80 GB for system and programs

Hard drive 2: min. 500 GB for data and results

Network: 1 Gbps

OS: Microsoft Windows XP professional

Video controller: minimum resolution 1280x1024, dual view recommended but not required

Monitor(s): minimum resolution 1280x1024, 2 monitors recommended

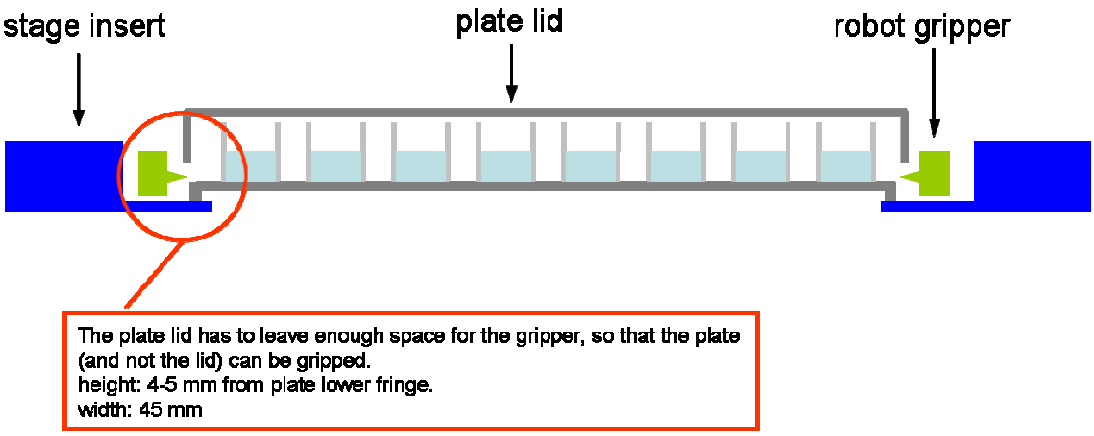
3.3 Power Consumption of Hardware

Microscope (UCB):	max. 360W
PC:	max. 840W
MT20:	cont. 380W
Robot:	max. 200W
Monitor:	240W, each
STC/FFWO/FRFACA:	40W, each
Hamamatsu C8484:	powered by PC
Hamamatsu ORCA:	90W
Total:	max. 2500 W

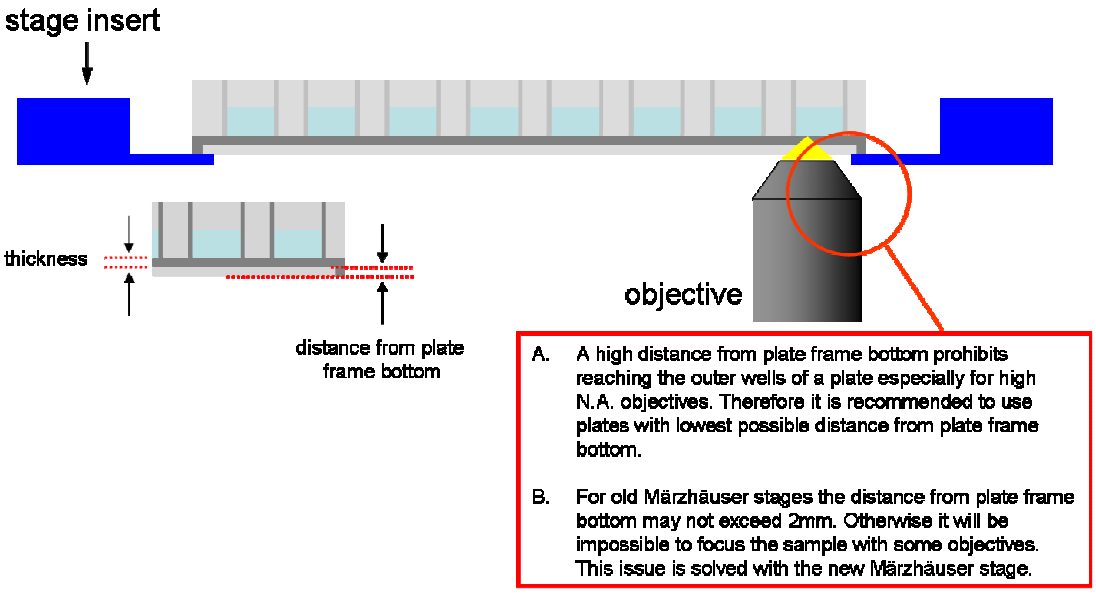
3.4 Plate restrictions

The sketch below gives an overview about the geometrical situation of objective, robot gripper and plate. From this situation several restrictions regarding the plate selection have to be considered:

- The plate lid has either to be half as high as the plate or to leave a sufficiently large window on both sides of the plate so that the gripper has access to the plate. Several plate manufacturers provide low profile plate lids.
- For microscopes equipped with a Märzhäuser stage of type Scan IM IX2 112 x 74 the **distance from plate frame bottom** may not exceed 2mm to permit focusing of the sample with any objective.



- It is recommended to choose plates with a minimum **distance from plate frame bottom** so that also the outer wells can be imaged completely. This becomes more important with increasing numerical aperture and decreasing working distance of the objective and especially if an automated water immersion (IX2-AWI) is mounted to the objective.
- An interim solution to allow focusing also for plates with a **higher distance from plate frame bottom** is to use spacers that lift the objective by 6 mm and the stage by 4 mm. This increases the z-range by 2 mm (Art. Order nr. E0431727)
- The new generation of the Märzhäuser stage SCAN IM IX2 allows focusing plates with a **distance from plate frame bottom** of up to 4 mm and therefore will not require the spacers any more. Nevertheless it is recommended to use “flat” plates so that the outer wells can be accessed by high N.A. objectives.



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