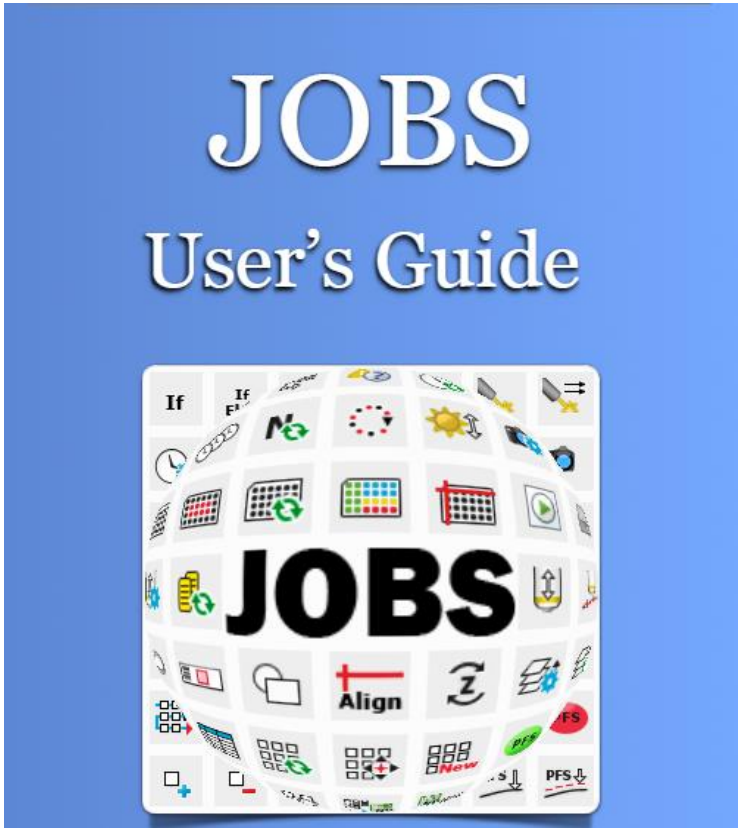
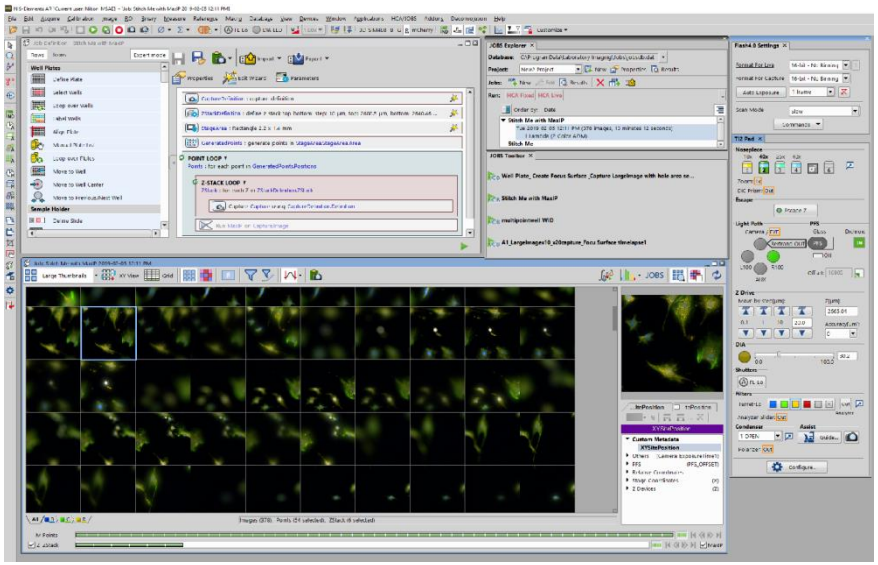
Nikon NIS-Elements
[Experiment sequence construction tool]

JOBS User's Guide Vol.1



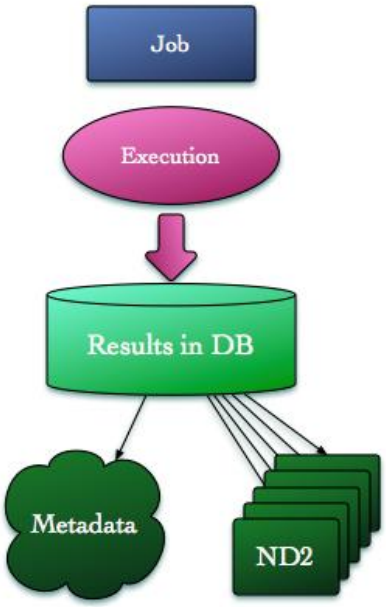
Main Concepts

JOBS is an environment aiming at complex acquisition and data analysis with easy to use and comprehensive user interface.



Overview

DIAGRAM 1.1 Jobs



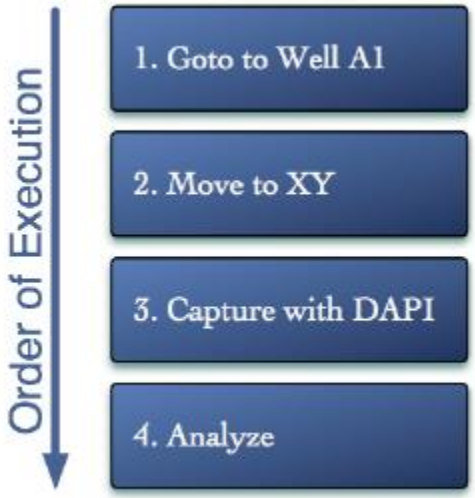
Jobs are automated procedures for complex image acquisition and data analysis. Typically it is a conditional acquisition on well plates, dishes or slides for long times. It may include Confocal and widefield camera acquisition combined with photo-activation or bleaching.
The strength of Jobs is their flexibility. They relay on building blocks that can be freely assembled into arbitrary procedures in order to fulfill any particular needs.
As all this is done visually using Drag&Drop it is called Visual Programming.

This approach combines advantages of programming – flexibility – and limits the drawbacks: having to learn how to program. When such procedures – Jobs – are executed, images and analysis data are produced. The images are stored in ND2 files. Metadata and analysis results are stored in the database together with links to the ND2 files. Since the complete acquisition history is stored in the database as well as the whole organization and structure of Jobs and Projects they appear in and Analysis Recipes it is called Asset Management.

About Tasks

Jobs are composed of tasks Task Reference. The order of tasks in a job and their layout is essential. Tasks are executed in the same order in which they appear in the job: from the top to the bottom. There are two kinds of tasks: simple tasks and tasks that have blocks with other tasks nested inside them (e.g. loops and conditions). If there were only simple tasks the job would be a mere chain of tasks, but on the other hand it would be difficult to write a simple time-lapse with 100 repetitions, as one would have to copy the tasks 100 times. In order to avoid this copying there are tasks which have an inner block that can embed other tasks. Loops for instance can embed other tasks. When a loop is run, it repeatedly executes the whole inner block of tasks one after another. Next good example of tasks that can embed other tasks is the Condition (Condition IF, Condition (if-else)). Conditions are used for branching. Embedded tasks in a condition are executed only if that

DIAGRAM 1.2 Order of Execution



condition is valid. One may want to take more images on a well only if there is some signal otherwise go on the successive.

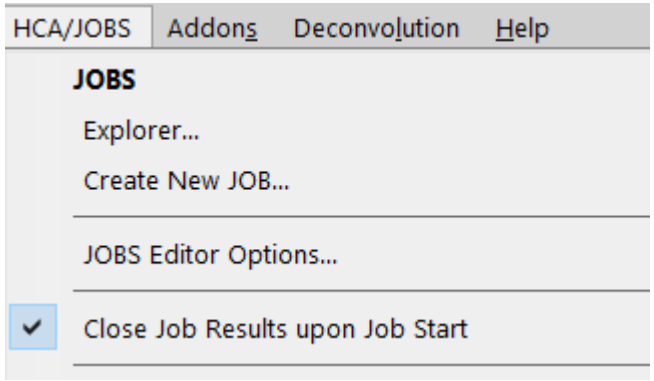
- Tasks are executed in order.
- If a task has any inner block with tasks, all tasks in that block are executed in order one zero (condition is not met), one (condition is met) or many times (e.g.: loop 100 times).

Some of the tasks require other tasks to be present in the job. They depend on them. For instance a well loop that goes over wells requires a selection of wells on a plate in order to loop over it. Well selection requires a plate definition in order to know if it is a 24-well, 96-well or a plate.

Some operations like Capture or Z-Stack acquisition have separate tasks for definition and Execution. This may be useful in situations where a single definition is to be executed in different places in the program.

Tasks have different parameters depending on their kind. These parameters are set mainly in design time (e.g. in Job definition) or may be changed in runtime. This can be done interactively (by showing a window) or automatically using expression.

Menu and Explorer

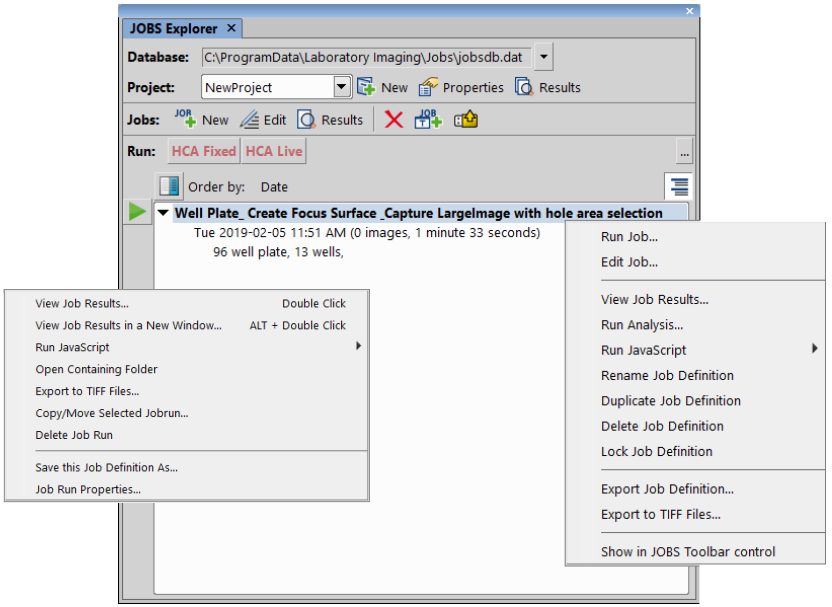


The Menu

Jobs Menu gives basic framework for working with Jobs. It is accessible from the Top Menu Bar where it groups fundamental settings and managing tools.

Jobs Explorer

Before creating a job, you need to open the Jobs Explorer window, where all jobs and all acquired data are viewed and managed.



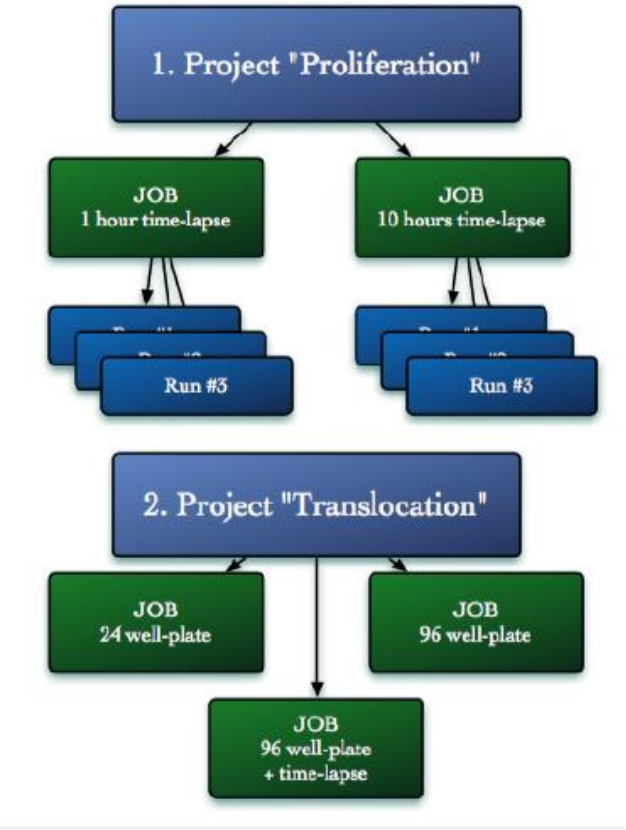
Jobs Explorer is the window for managing Jobs, Individual Runs with their Image data and Metadata. Most common actions are done from toolbars. Jobs related operations are done from context menus.
At the top there is an information line with current database location together with Database backup and properties. Below

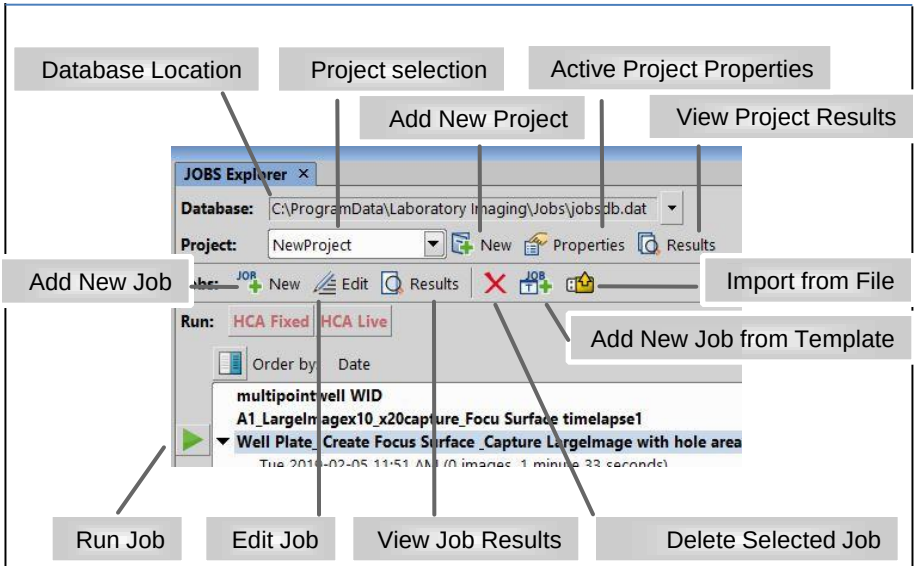
is current project selection with related tools and Jobs toolbar. The Job list is organized into a shallow tree view with Jobs as nodes and Individual runs as leaves. Runs are shown by date and time with the number of images acquired and duration.

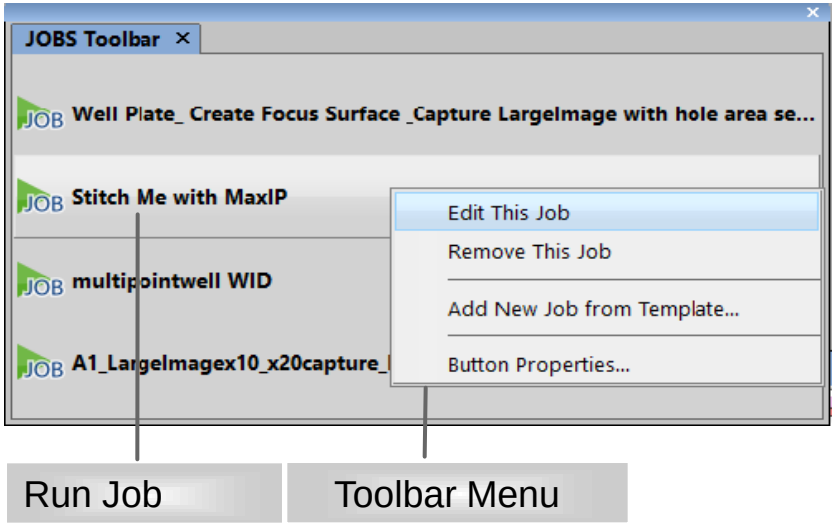
Managing Projects and Jobs

Jobs are organized into Projects. In the top row of the JOBS Explorer window, there is a pull-down menu containing all created projects.
A project is no more than a named container for storing / sorting of jobs. Create your own project or use the default one. It is up to the user and his needs to choose the criterion for grouping jobs into projects. It can be either by an assay (e.g. “cell proliferation”, “translocation”, etc.) or by users (e.g. “Catherine”, “John”, etc.).
The Jobs Explorer shows the contents of one project at a time as a list of jobs. Each job can be unfolded to disclose all instances of when it was run. The job represents the definition which can be viewed and modified in an editor or a wizard and run. The run instances can be opened to reveal a view on acquired images and data.
The structure of Projects, Jobs and individual job Runs is designed to be flexible. The user chooses what the projects and jobs will be. There can be completely different Job runs under a single job, but the usefulness of such an organization is very low. Therefore it is a good idea to keep similar jobs together. For instance it may be useful to have the 10 hour proliferation job together with 5 hour one. But it may not be wise to put 6 well-plate data together with 96 well-plates because of later visualization.
Projects are managed from the Project toolbar from Jobs Explorer.

IMAGE 1.1 Projects, Jobs and Runs





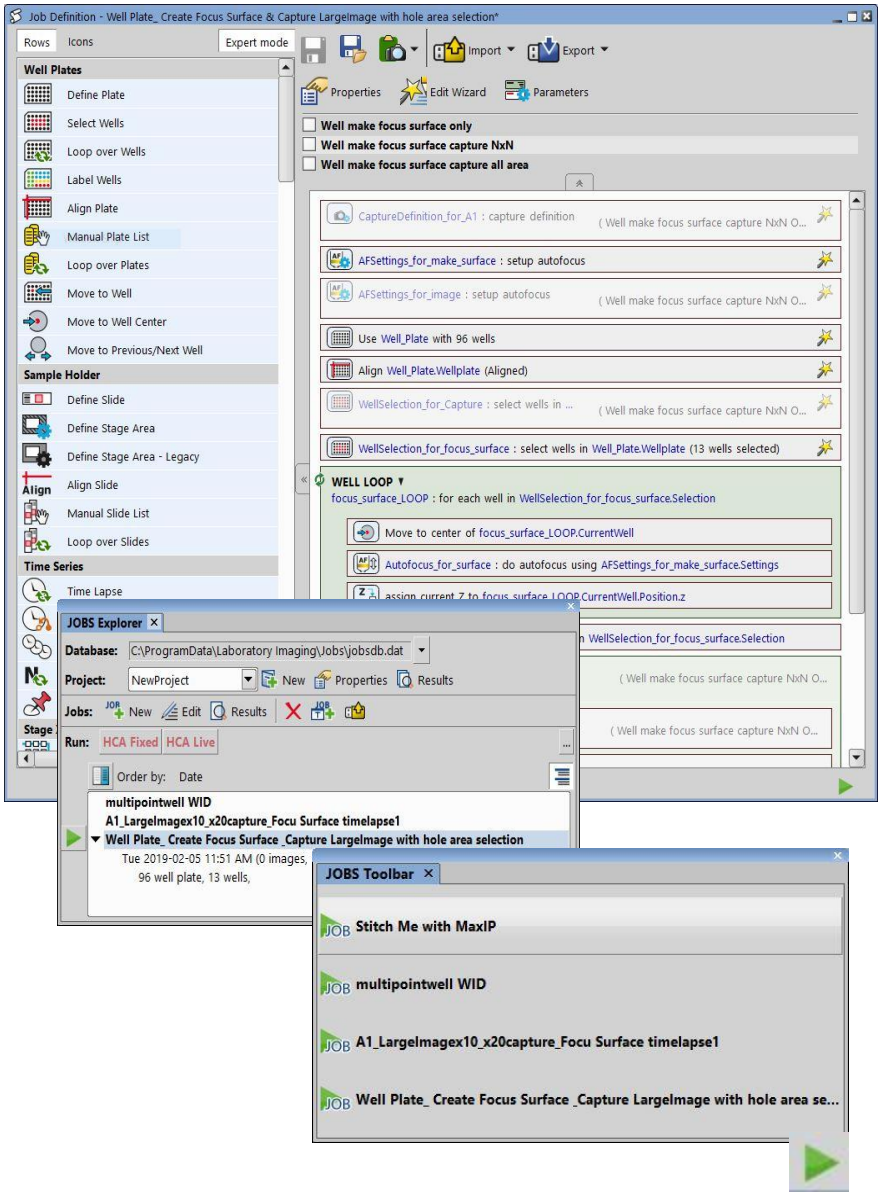


JOBS Toolbar

The JOBS Toolbar has some common features with the Jobs Explorer. It enables organizing, editing and running single Jobs or Job Wizards. A job can be added to the toolbar from within the Jobs Explorer Window – right click on the job name and select Show on Toolbar or just drag and drop Jobs from the Job Explorer.

Getting Started

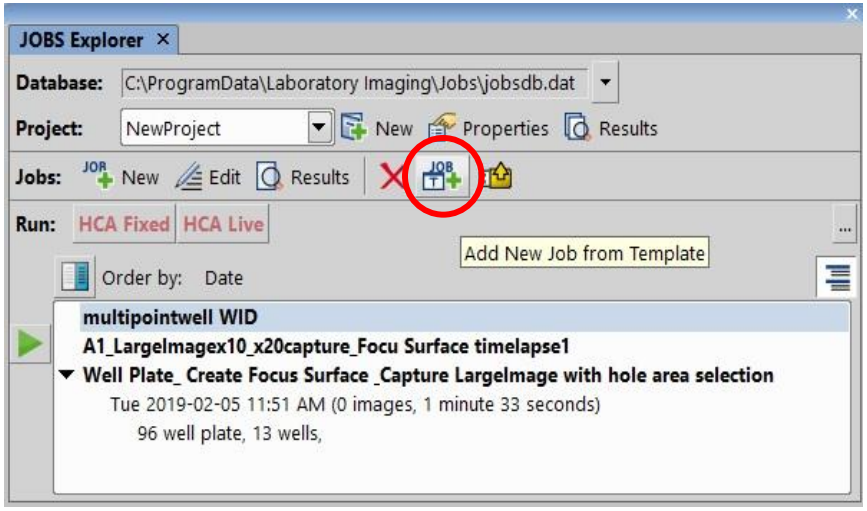
There are many ways how to create a job. The best way is to start by loading a predefined template and adjusting its parameters immediately or later in the Definition wizard. You can also start a new job from scratch.



Creating Jobs

Using Job Templates

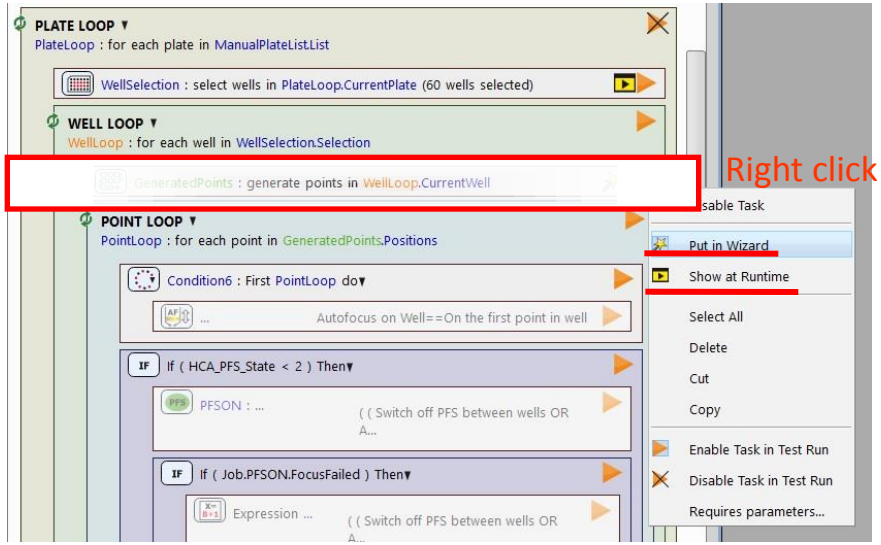
Easiest way to start using Jobs is to choose from pre-existing Job templates created for most common tasks. Either by clicking the Add New Job from Template button in Jobs Explorer or the Load Job Templates button in the Job Definition window you can access the Job Templates.



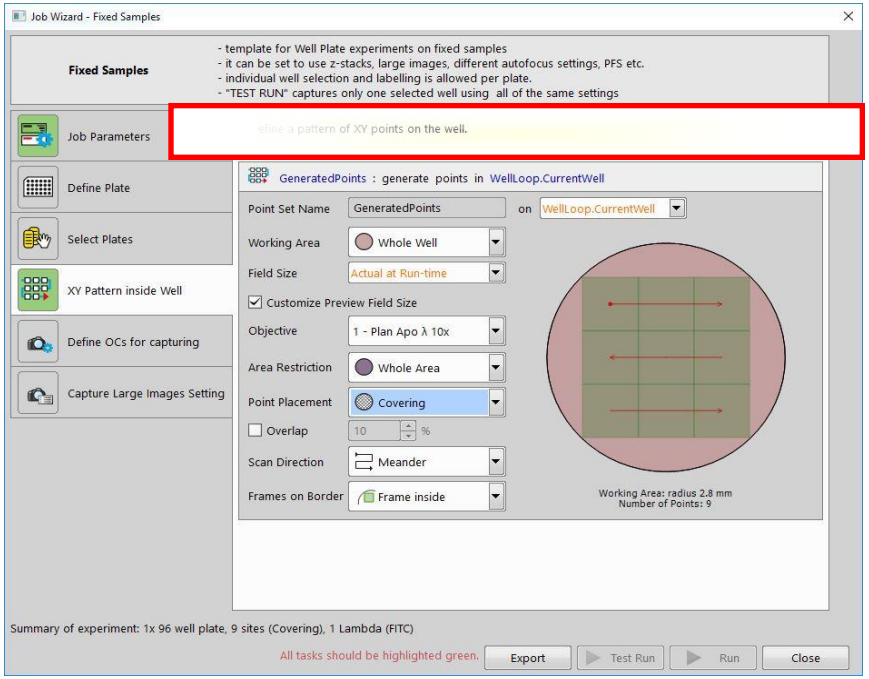
A template is a predefined set of tasks (see Task reference) which you simply insert to your current job definition. The template can be used either “out of the box” or you can select a template closest to your objective and add other tasks to it. To insert the selected template to the job definition window, press the Import button.

Using Definition Wizard

A Job is usually run multiple times to acquire data in different conditions. It happens that few parameters have to be changed before each run (e.g. length and interval of a timelapse, well-plate metadata, like drug concentrations or labels, or even threshold intensity level). For this reason it is possible to mark some tasks for reviewing in a Wizard. In this case it is possible to run a Wizard where only the tasks that were marked are presented for definition. This is also useful for specialist / operator role division. The specialist creates complex Jobs with decisions and analyses. He does not want the operator to see all the complexity of the task but still needs to let him change a few parameters. The definition wizard enables this.



When inserting a wizard, a question column is displayed during execution.



Creating a New Job from Scratch

1.Go to Job Explorer window.

2.Here you can select an existing project or create a new one by clicking the Add New Project button.

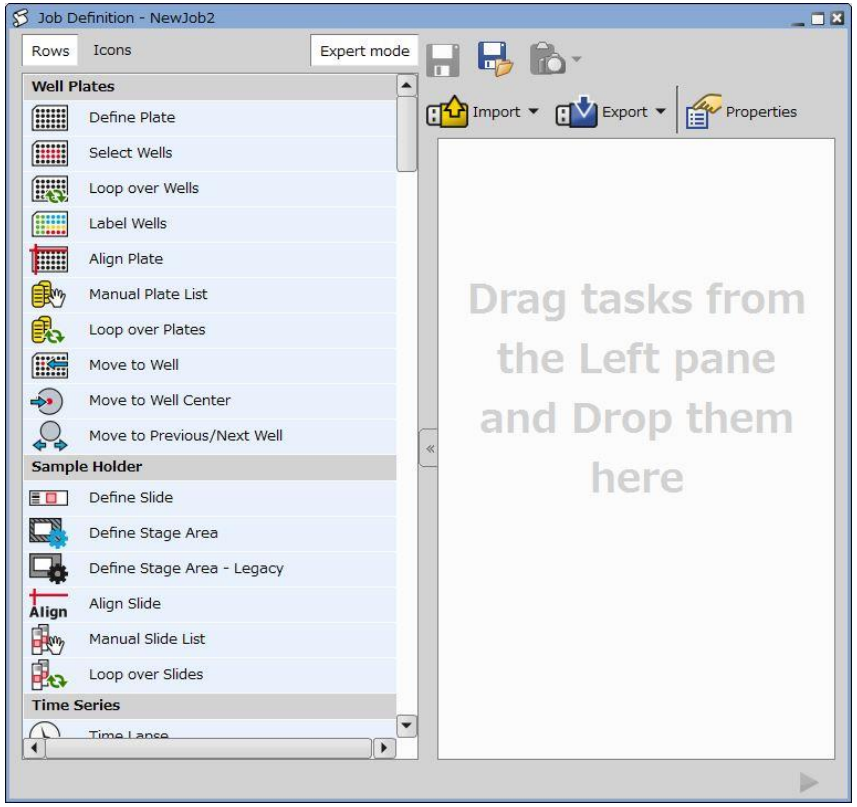
3.After choosing a project click on the Add New Job button.

4.The Job Definition window opens. Here you can combine all different tasks to create a new job fulfilling your research needs together with all available devices compatible with NIS Elements.
Just drag and drop tasks from the left into the main area of the Job Definition window.

5.The tasks inside a job definition can be reorganized by dragging them up or down. Tasks which are not needed at the moment can be placed in the temporary area (Show/Hide Deposit button) or deleted by dragging the task out of the job window.

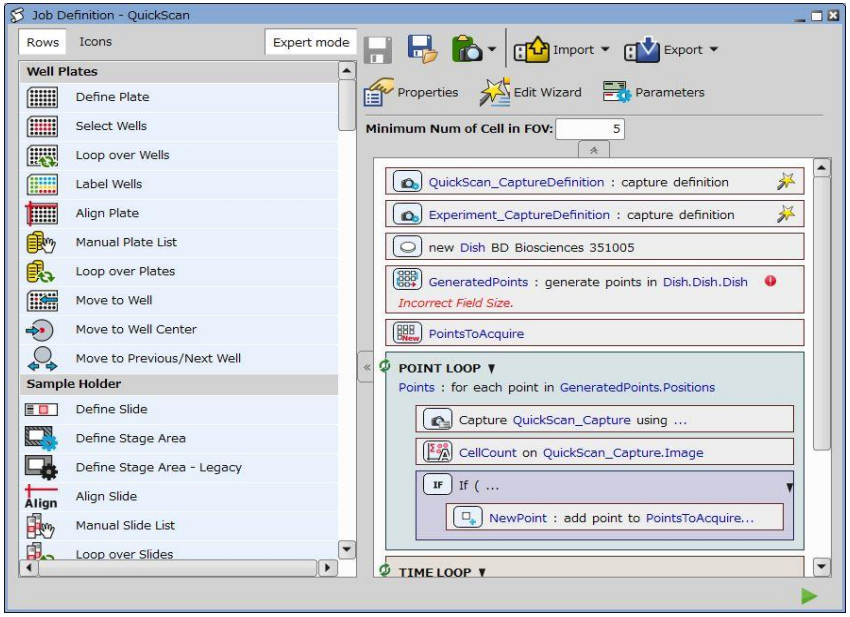
6.To set the task parameters, double click the task.

7.Don't forget to save your job before closing the Job Definition window.



Job Definition

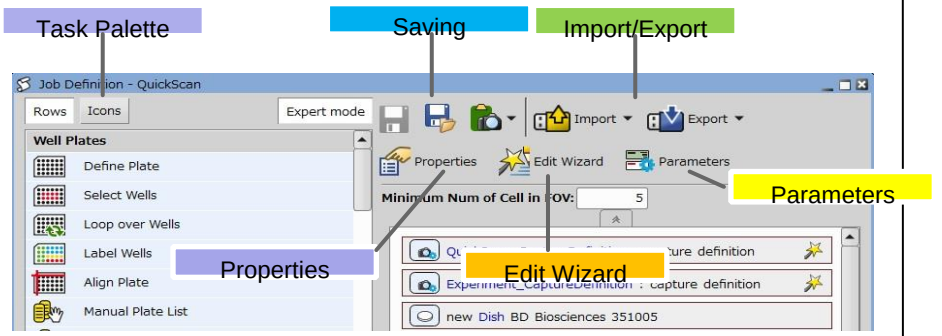
Job Definition window is a main place for editing the Job program flow and changing task parameters.



The window features two panes below a toolbar: The Task Palette, The Main Program. Below are two buttons for Running the Job or Invoking a Wizard.

Toolbar

The top toolbar row controls whole job operations like Saving, Importing, Exporting and loading from Template. Second line controls appearance.

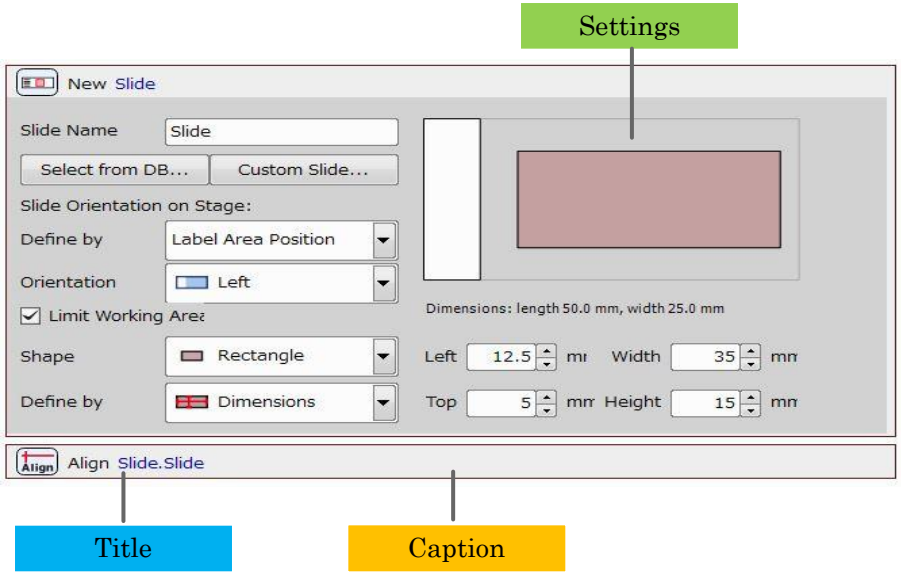


Task Palette

All available tasks are displayed in the Task Palette in a form of icons or icon with description. Tasks are organized into logical groups. Some Tasks depends on the presence of some devices. They may not appear in the palette until that device is added in the device manager.

Main Program Area

This pane holds the tasks that form the program. Each Task has an Icon and text that describes what the task will do when executed. Every task has different parameters which are revealed by double-clicking on the task itself. Tasks can be marked as to be shown in the Wizard or in Runtime.



Deposit

The deposit pane is used to hold snippets of job programs in order to have them stored together with current Job for later use.

Operation

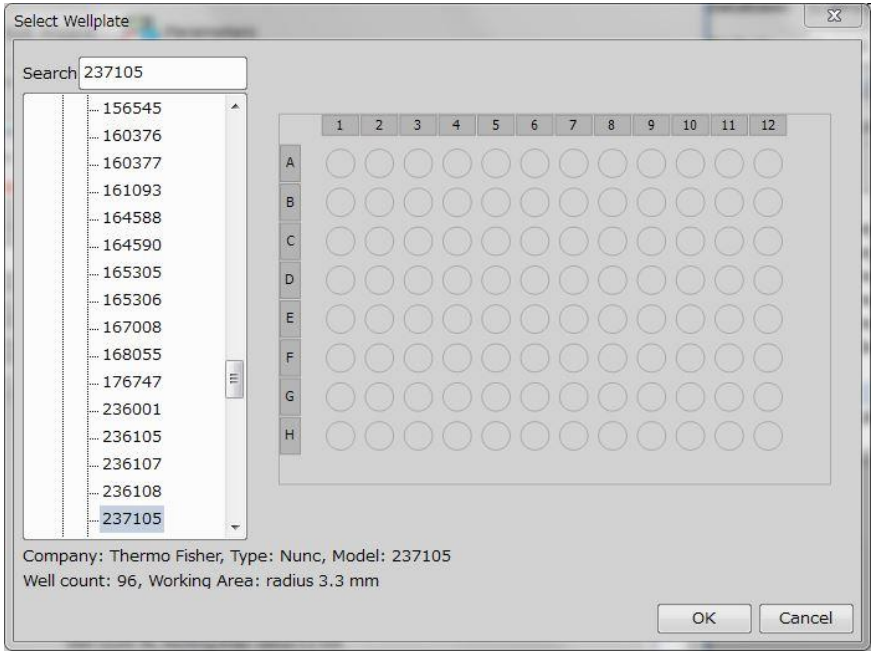
Most of the editing operations are done by simply moving the Tasks around. This is done by Drag&Dropping tasks.

- To insert a task into the job program, drag the icon to the main program area which contains the job definition.
- To move a single task or multiple tasks to different place hold the task in the main area and drag it to another place in the program.
- To delete a task from program drag it simply out of the job main program area.
- To move single or multiple tasks between the program and deposit, drag and drop them there.

Building Jobs

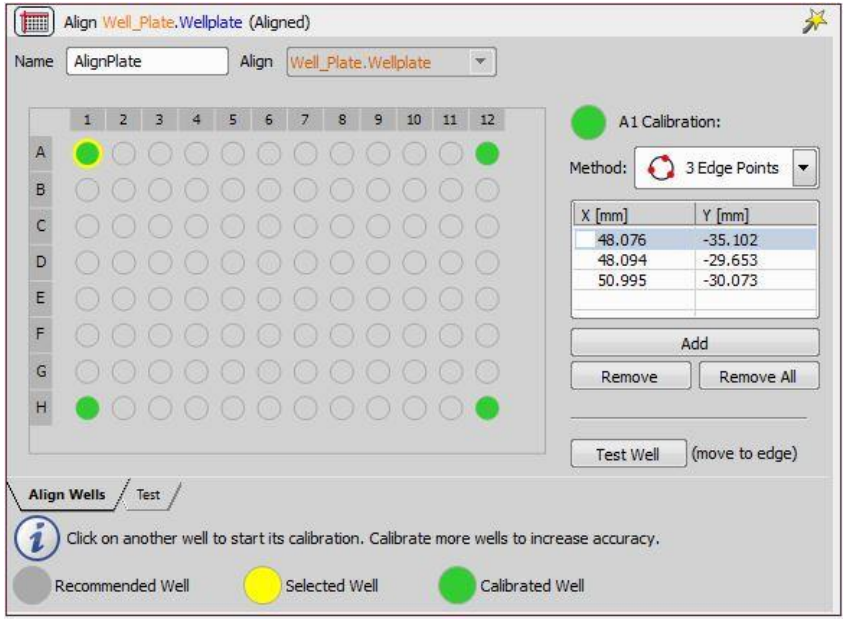
Defining samples

First step of any job procedure is to define your samples. You can choose between well plates, dishes, slides or free shapes.
If defining standardized sample holders (plates, dishes, slides), it is recommended to select the specific product model you're using from the database. This can be done by clicking the Select from DB... button.

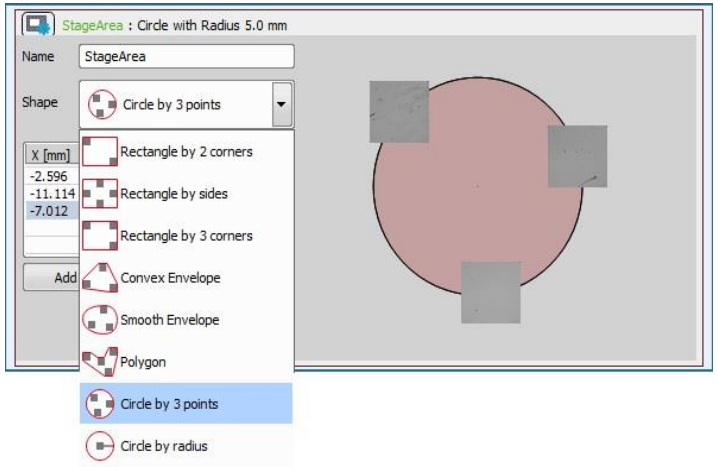


After choosing the proper sample holder, click OK and set the Working Area in the define task menu. Set single plate / dish / slide by defining the threshold distances from the border of the holder as needed. When using slides or custom shapes, you have to define the slide / shape width and height (Define Slide/ Define Free Shape).
Any sample holder that was selected in previous steps has to be properly aligned. This can be done using the Align Plate or Align Sample buttons. In case of well plates, you have to define the well plate orientation and a position of at least one selected well. You can choose between the center setting, 3 o'clock edge position or any other three edge positions.

For more information about well plate aligning please see Align Plate.

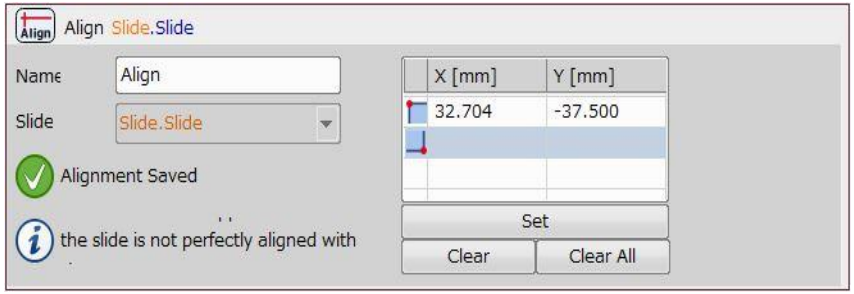


When using dishes, the only parameter you have to set in the Define Stage Area window is the dish position. You can use some methods mentioned below.



When working with slides, you have to set the slide top left corner and define the Bottom right corner position.

In the case of free form, it can also be manually positioned in an analog manner by the shape created using the "Define Stage Area" task in the above figure.

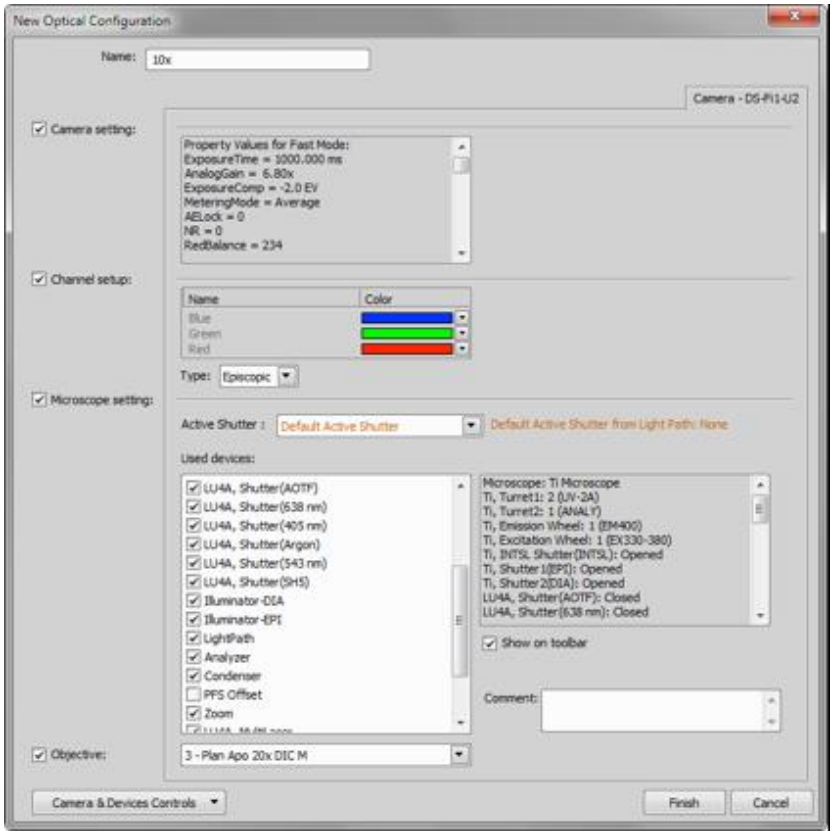


Defining Capture

Before capturing any images using the Capture task you have to define the capture settings in the Capture Definition task window, where you select your optical configuration to be used to capture each of the channels. Typically, laboratory computer image analysis systems consist of a computer, a camera, and a microscope equipped with certain accessories (objectives, filters, shutters, illumination, rotary changers, etc).
Most of the mentioned microscopic hardware is often motorized and therefore can be controlled via NIS Elements. In addition, it is possible to integrate single settings of all these devices into one compact set called Optical Configuration. It is recommended to create several optical configurations containing particular devices settings. Then a single click can completely change the current hardware configuration.

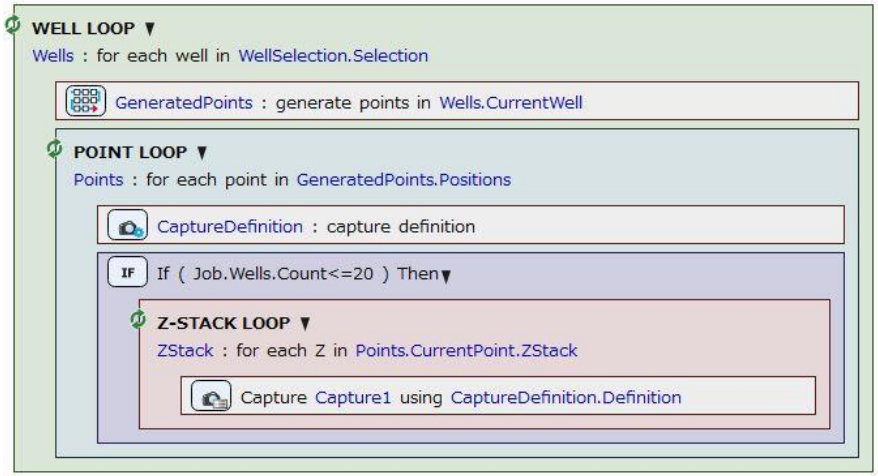
Creating New Optical Configuration

1) Please check that all the devices (microscopes, cameras, etc.) which you want to associate with the new optical configuration are properly attached to the system and working.
2) Choose Calibration > New Optical Configuration from the top menu bar. The following window appears. Adjust the settings of the devices to match the intended state which will be saved to the optical configuration.



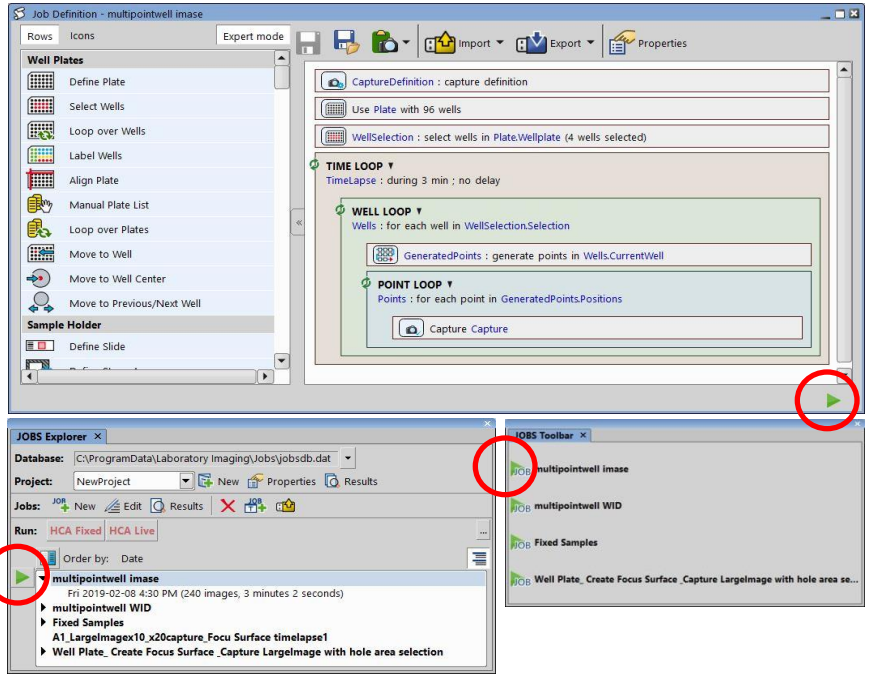
4) In the left column, select which device settings to associate with the Optical configuration. List of the current camera properties of the Camera setting appears on the right. Channel setup determines how channels of newly captured images will be named and what color will be assigned to them. If there is more than one shutter available and you would like to associate a shutter with the optical configuration, select which one is the Active Shutter from the pull-down menu in the Microscope setting. Select which parts of the microscope shall be included in the configuration by checking them in the Used devices dialog box. An objective mounted to a motorized nosepiece can be included in the configuration. Select the objective from its pull-down menu. Objectives which are currently assigned to any position of the nosepiece are listed.
5) If some of the device settings still need to be adjusted, click the Camera & Devices Controls button and select the appropriate control window.
6) Click Finish to save the new optical configuration and to close the window.
7) You can create more optical configurations by repeating this procedure.

Defining Loops



Loops are special repetitive tasks which contain an inner block. When you fill this block with tasks and run the loop, it repeatedly executes the whole inner block of tasks one after another. You can then define whenever you want to stop the repetition (after a certain amount of time, number of loops, after a condition is true, etc.). You can also loop tasks inside another loop to make building of repeated processes even more effective.

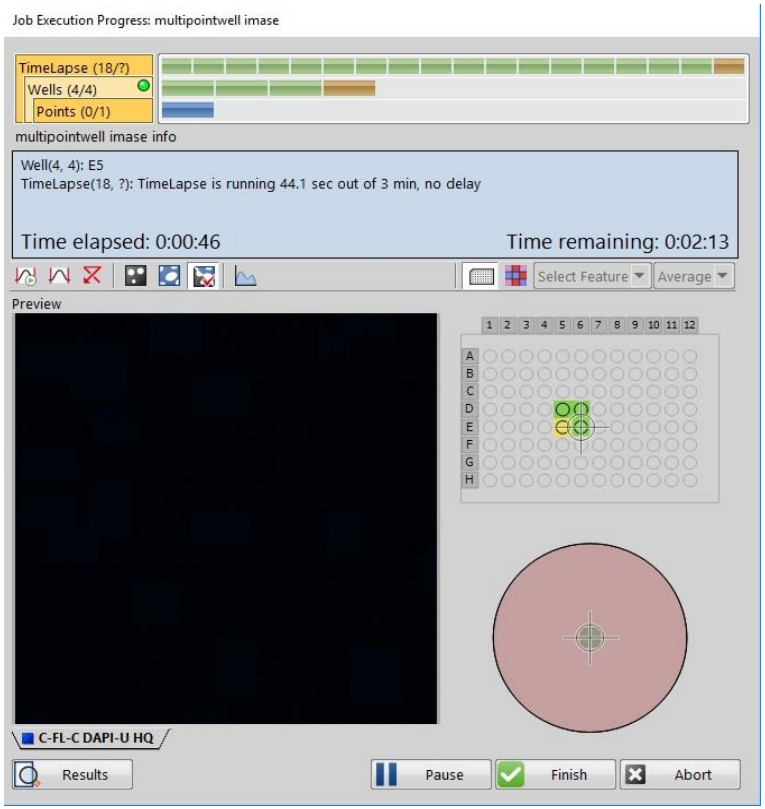
Running Jobs



Once the job is run (after clicking the green triangle Run button) a window is displayed showing the progress. Similarly to ND2 acquisition blue rectangles representing dimensions or frames turn green when captured. Some tasks require user interaction in runtime – during the job progress (e.g. Add / Edit Points Manually and other tasks can be marked to show up their definition dialog in runtime (Job Definition Window).

Once the task definition dialog shows up, the Job execution is paused until user confirms settings modification by pressing Next button.

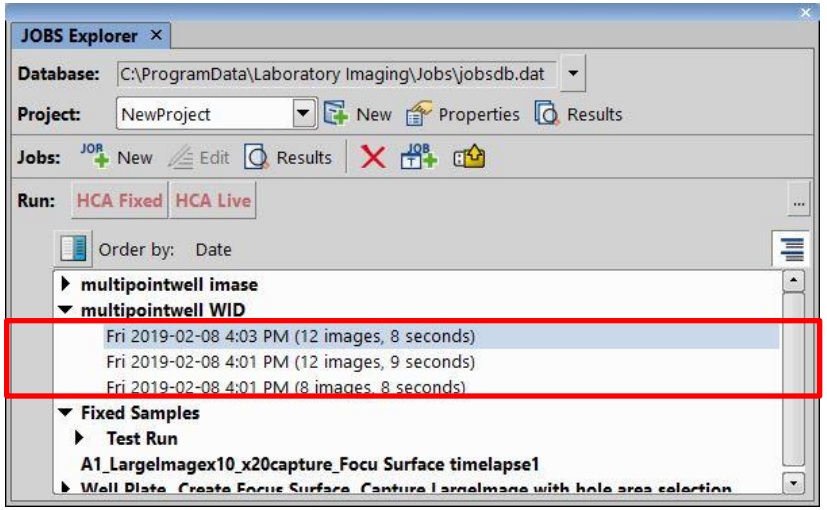
The following picture shows a well plate selection of 4 wells being scanned, a time lapse is captured on each well. Green color indicates wells which have been already captured, Yellow color indicates the well being scanned at the moment (E5).



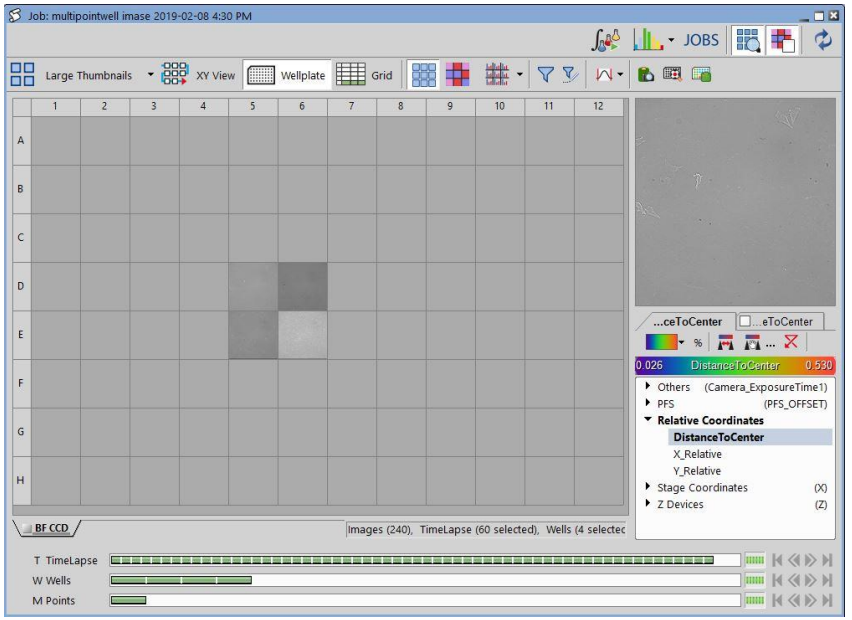
If your job does not perform auto-focus before each frame but you see that the preview image is getting blurry, you may use the pause button to pause the acquisition, re-focus and then start it again by clicking the Continue button. If you click Finish, only images captured so-far will be saved and the experiment will be ended. Clicking Abort ends the experiment without saving any data.

Viewing Results

Once the job is finished, results are saved automatically and the Job results window opens. To open some other saved job results, select the particular job-list in the JOBS Explorer window and double click the list.

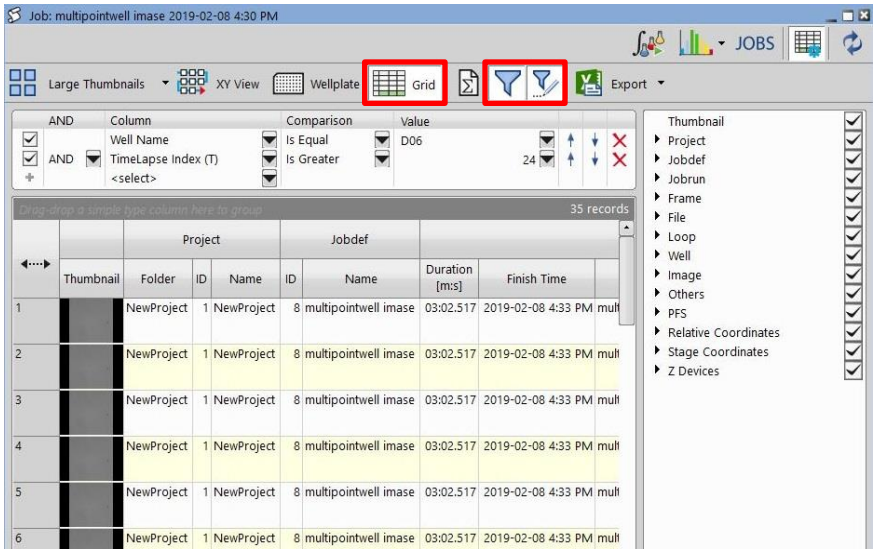


Jobs results window

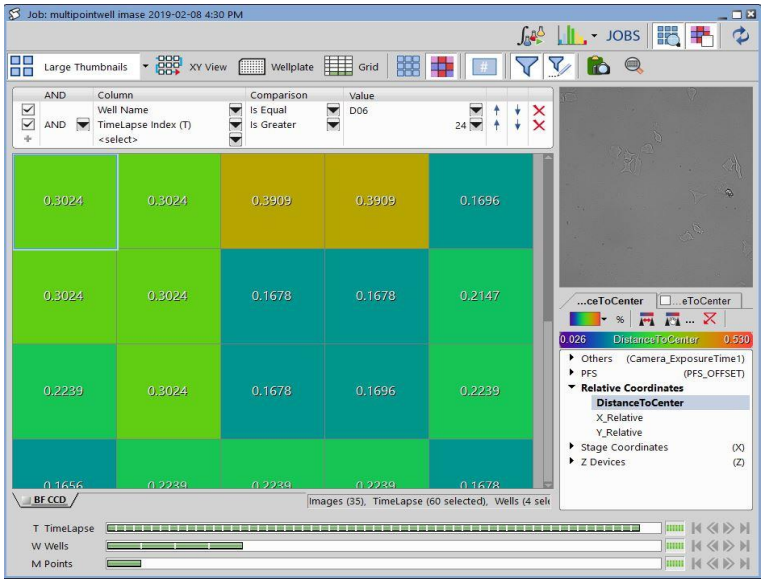


This picture shows results of a well-plate experiment performed on a 2 x 2 selection displaying preview of the captured images in each well (Well Plate View). The Job result window shows images and analysis data of a single job run. In the bottom part you can choose what you see with ND navigation bars. On the right you can switch to the Preview, Data browser with heat-map LUT control and a Label View. On the right you can switch on the Preview, Data browser with heat-map LUT control and a Label View.
The browser enables to view the results “from various angles”. There are three basic views: Thumbnail view, Well Plate view (if it was well-plate acquisition) and a Grid view. In Thumbnail and Well Plate view you can view Images, Heat-maps of selected data values (Quantities, Acquisition metadata and Analysis results)

and labels (in case of well-plate). In Grid view you see everything in a tabular form. Double-clicking on any image opens the underlying ND2 file on that particular image frame.
The thumbnail view (large, medium, small, tiny) displays all acquired images in the order of acquisition.
If you click the Well-plate icon a real layout of the plate is displayed. Grid button displays images along with meta-data. Filtering and grouping is supported (you can sort and group by any column). Statistics can be turned on.
Independently on the current view (thumbnails, well plate, grid), you can select whether to display thumbnails of the captured image data or a heat-map which would represent one of meta-data values (e.g. gas concentration, temperature, etc.).
In the heat-map view you see the selected colored squares instead of images. The color is based on the value and can be tuned. It is possible to aggregate (calculate min/max/mean/ sum/SD) in order to get single value per each well.
A filter may be also applied to images displayed in the result view. Click the Define Filter button to display a table with the filter definition. Toggle the Use Filter button to turn the filter ON and OFF.
The Analysis button runs further analyses on captured images (see: How to Run Analysis on Captured Data).



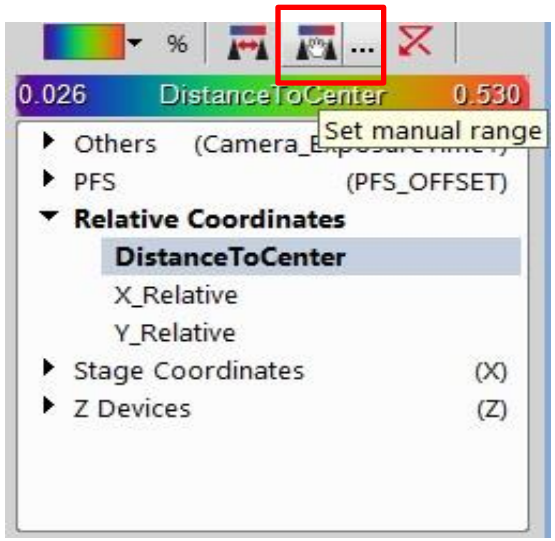
This picture shows a filter applied to a grid view. The filter definition says: “Display only images captured in the well D6 and, at the same time, display only images following the 25th frame of the time sequence” (TimeLapseIndex starts from 0).

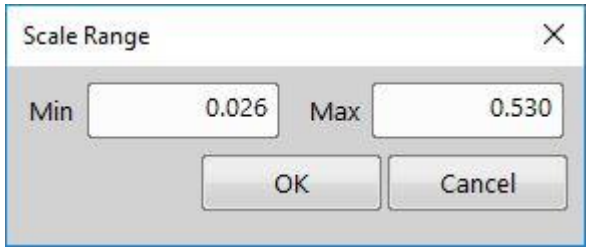


This picture represents an example of a heat-map displayed as thumbnails. Each image is overlaid by a color and a value both of which carry information about the metadata selected in the bottom-right corner of the window. In this picture, Distance To Center is indicated. The color scale range and colors can be modified by the user.

The Set range from current view button assigns the left color to the lowest value and the right color to the highest value present in the selected meta-data.
Click the ... button and insert the minimum and maximum values to be used instead of the default ones.

Then click the Set manual range button to apply the manual scale.





Advanced Workflows

In many situations it is necessary to make decisions during the experiment run: branch it automatically based on some conditions or simply ask the operator how to continue.

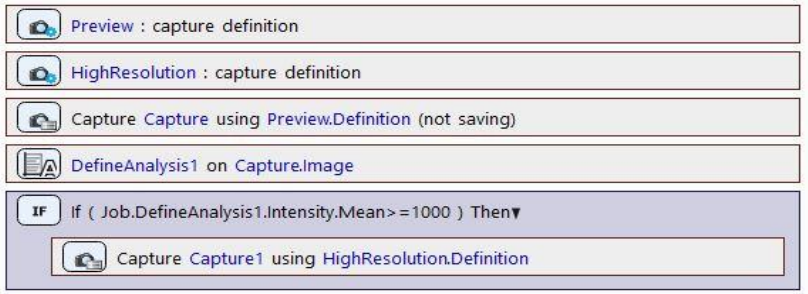
Using Conditions

Condition (Condition IF) is a great tool useful in all sorts of laboratory situations where we define which parameters influence the task execution. Conditions are entered as texts or using the "Define..." button. Condition is a special kind of expression (see: Using Expressions) that evolves into logical True (any non-zero number) or False (zero number).
Deciding whether the condition is True or False is called "evaluation" and it is computed by NIS Elements when the task is executed at runtime.
Almost every task exposes number of variables reflecting its settings and current state. These variables are available within the Expression task. They are organized in a tree-like objective way delimited by a dot ".": The root is the current job – "Job" followed by the unique task name ("Capture" in this case). Then the task variables depend on its type and settings.
Specifically, the Capture task exposes a variable Analysis (among others) containing a list of defined analyses.

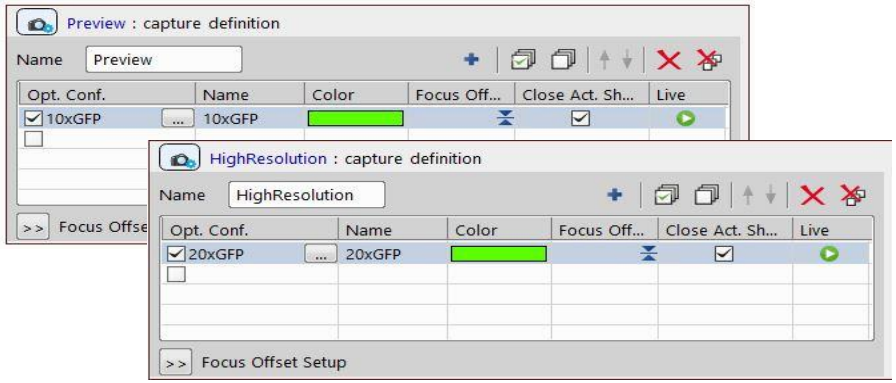
Conditions contain relational operators (e.g.: =, < and >).

Condition Tasks have blocks with tasks that are executed or skipped based on the result (True or False) of the condition.

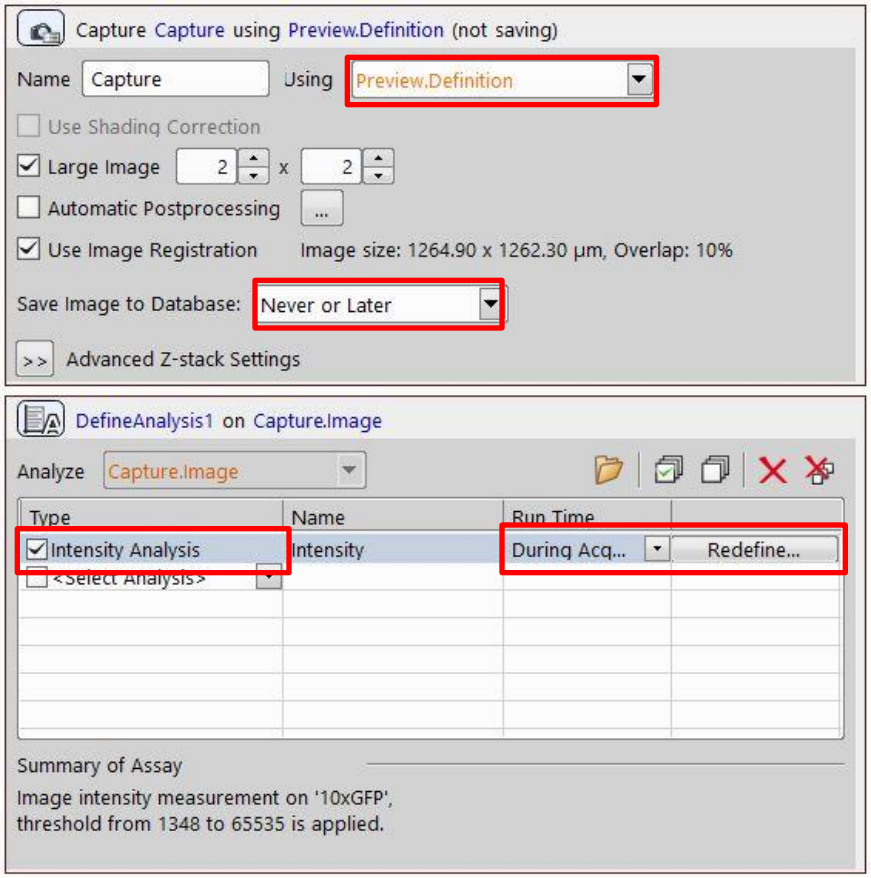
Let us show the usage of a condition on a simple job.
We want to capture a large image of a well if there are some cells inside. To do this, we will capture a large image in low resolution and measure its mean intensity. Only if the mean intensity is above the defined threshold, we will capture a much larger image with higher magnification objective. The resulting job will look like this in the job definition window:



1.Create two Capture Definitions
Define two Capture Definition tasks, each using a different optical configuration. Assign some simple names to the tasks.



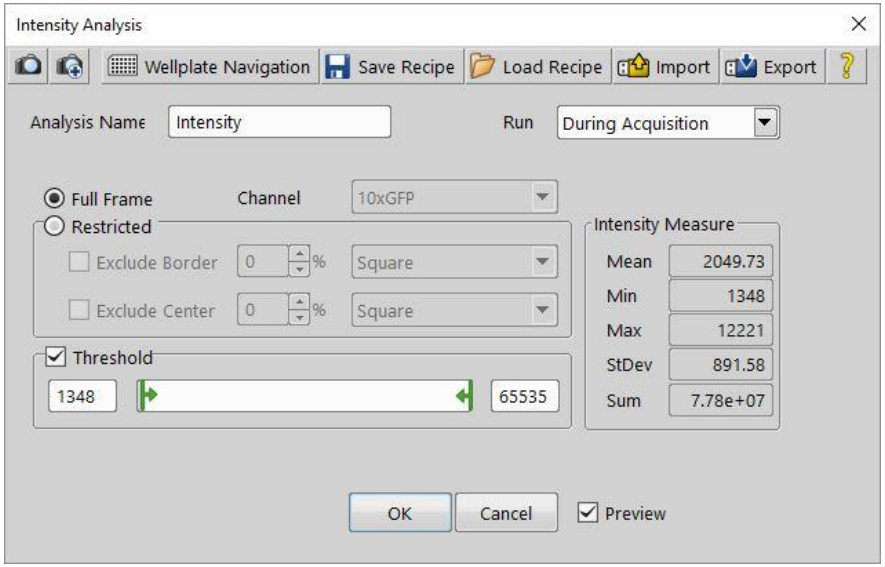
2.Capture the preview image and measure its intensity
Insert the Capture task and set it so that it will use the low-magnification capture definition.



Set everything as it is in the picture. Please select Intensity Analysis in Define Analysis. Then click Define (Redefine) to set-up the analysis.

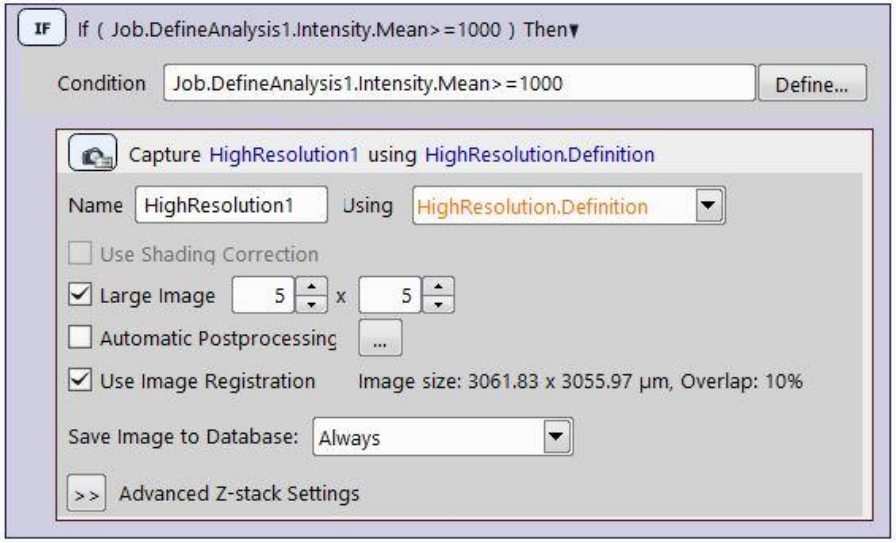
Since purpose of this task is to decide whether to capture the high-magnification image or not, there is no need to save the images, so leave the Save Image to Database pull-down menu set to Never.

3.Analysis Definition
Specify parameters for the selected Analysis (after you clicked the (Re)define button):



4.Conditional Capture

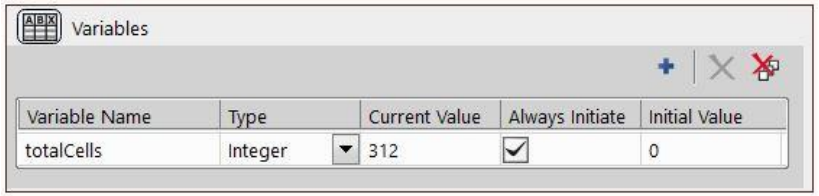
By now, everything is prepared to make the decision whether to capture the high-resolution image or not. We captured a preview image, measured its intensity and now we set the resulting value into the Condition IF task and add another Capture task within.



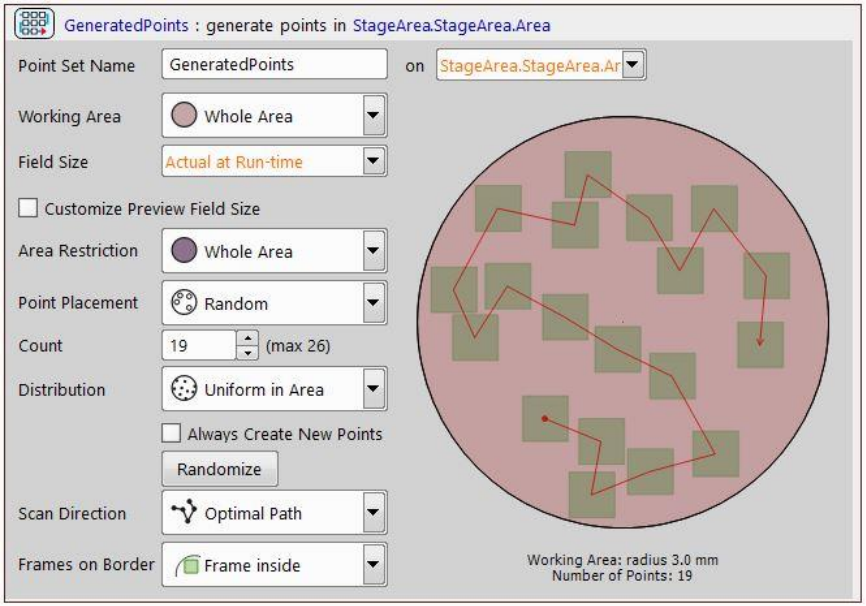
Specify the conditional expression. If the evaluated result of this expression is True, the capture will be performed. Use the Define button to specify the conditional expression as described in Using Expressions.

Using Expressions

An expression in Jobs is a combination of explicit values, variables, operators and functions (see Wikipedia). It is typically in the form of an assignment (variable = expression) whereby a value is computed (by evaluating the expression on the right side) and assigned to a variable (on the left side). Expression is evaluated and assigned to the variable when the task is executed.
In the following text we show an example that uses an expression.
Imagine we want to capture random positions on a dish.
We want to finish capturing when we gather 3000 objects.
First of all we have to define our capture using the Capture Definition. We choose the optical configuration and insert the Variables task which we set as follows:



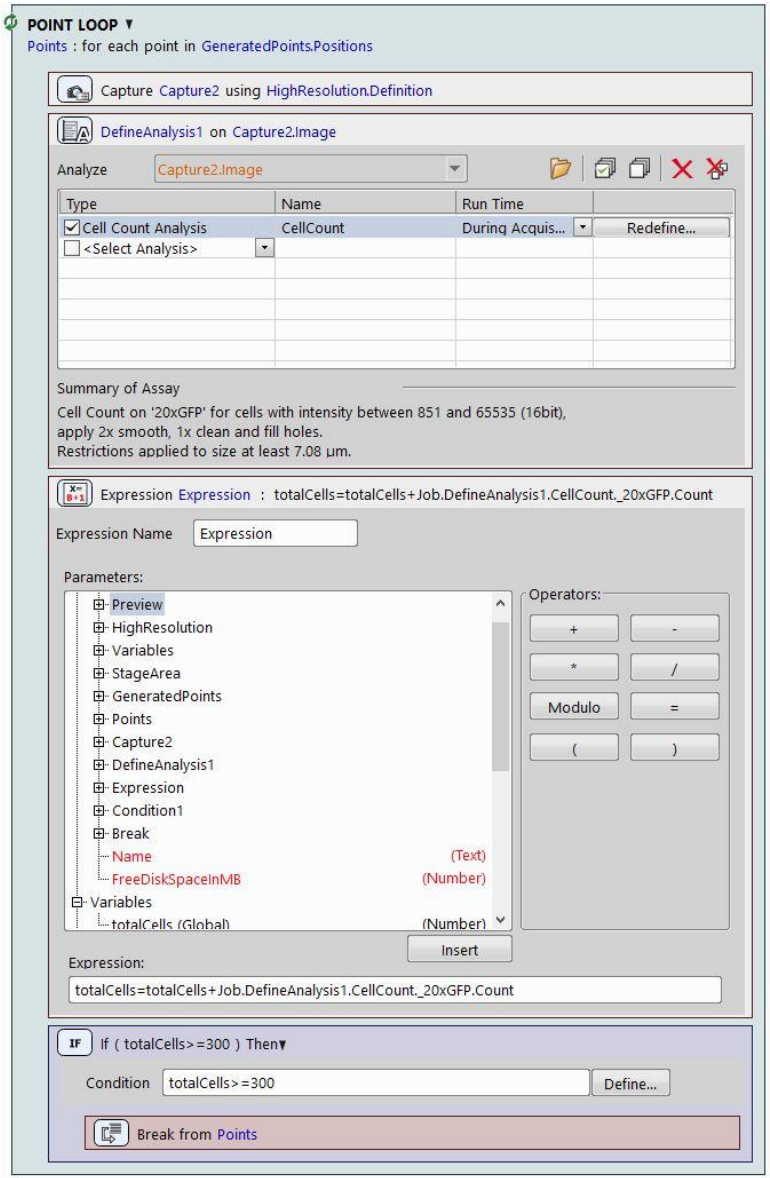
Then we insert a new Stage Area and Generate Points task and set their parameters as on the following picture.



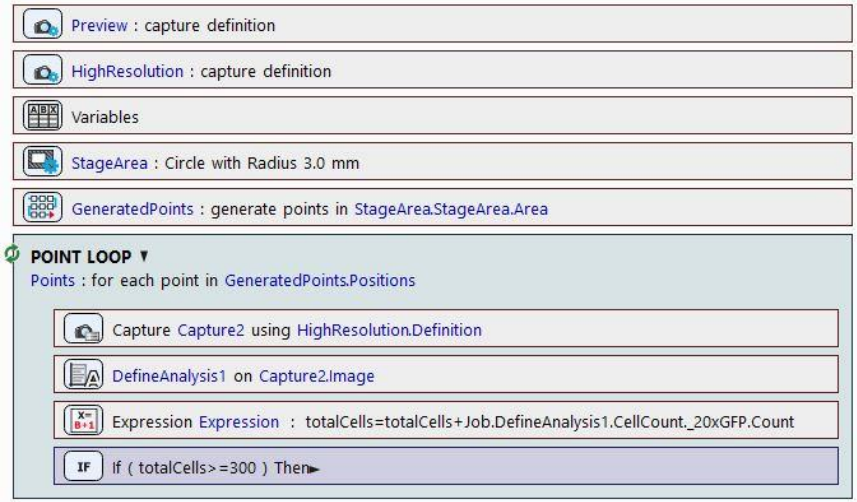
Finally we insert the point loop (Points) and fill it with Capture, Define Analysis, Expression and Condition IF.
In the Capture task we choose our analysis – Cell Count and further define its parameters (see: Cell Count).
In the task expression we enter the following expression:

totalCells=totalCells+
Job.DefineAnalysis1.CellCount._20xGFP.Count

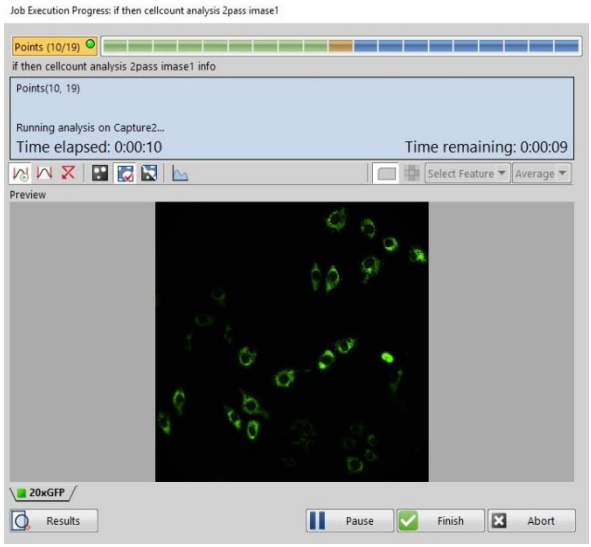
Into the Condition task we enter the following condition:
totalCells > 300
Inside the condition we insert a Break task. Thus if the condition is True (we accumulate 300 objects), the whole job stops.



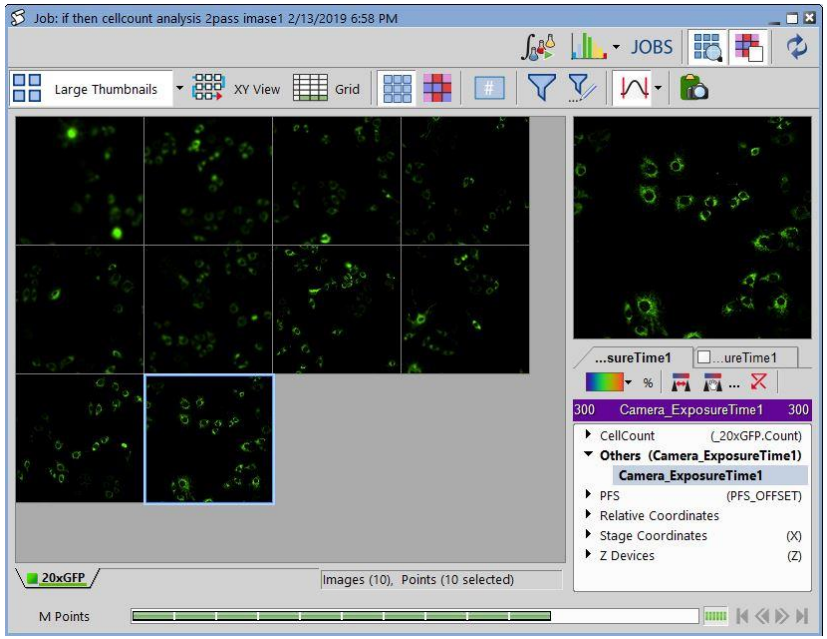
The final job looks as follows:



Job execution progress window:



Final results:

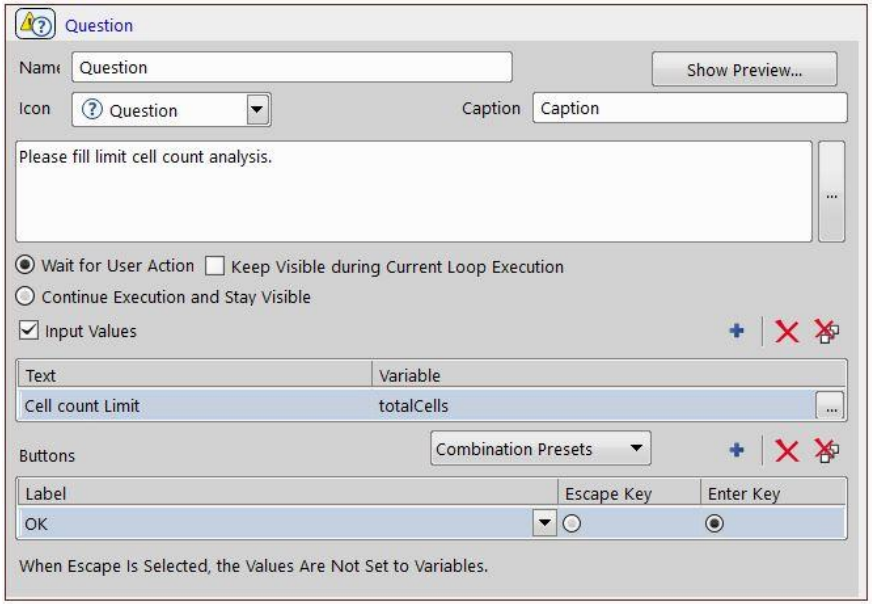


For more information about Jobs Progress and Results window (see: Running Jobs).

SECTION 3

Using Questions

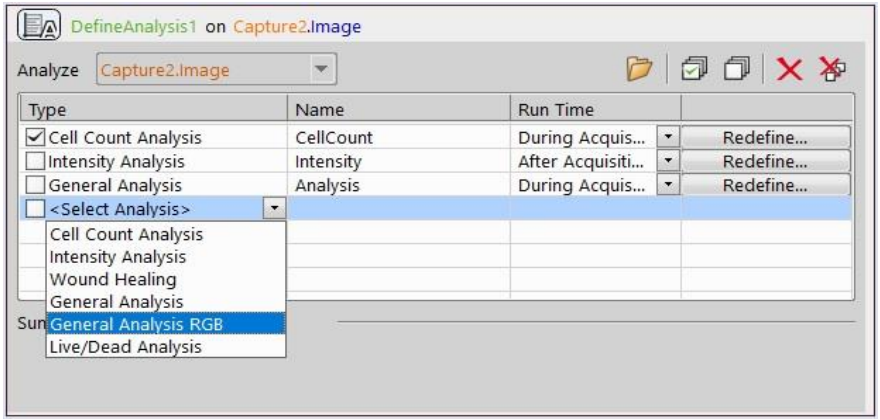
Question is a useful task if you want to stop your job in a specified part of the Job sequence and let the user decide what to do next. Start by dragging the Question task into the main area of the Job Definition window. Then double click the task to maximize it and set up your question.



Choose an icon which will be displayed together with your question and type a text to be used in the dialog window heading into the Caption box. Wait for user action checkbox stops the job sequence until the user answers the question. If the Keep visible during current loop execution option is selected, the question stays visible during the loop execution even after user input. If you check continue execution and stay visible, the question shows up and the job continues even without user interaction. Check if you want to input any user data. Insert the name of the required input into the "Text" column and add variables into the "Variable" column. Define the button names to be displayed when the question is to be answered.

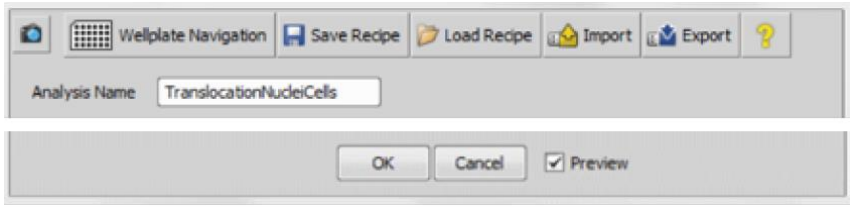
Using Analyses

The Define Analysis task in the Job Definition window contains a special feature that enables defining and running analyses. Target usage of this feature is to run a simple analysis “on the fly” and make a decision based on its results. The same Analyses can be run after the data is acquired.



Start by selecting the type of your analysis and naming it. Choose “During Acquisition” if you want to have the partial results available “During Acquisition” or “After Acquisition” if you want to run the analysis after capturing all the images.
Redefine button opens the redefinition window according to the analysis type selected.

All Analyses share the same top and bottom part of the definition dialog. They are designed for setting up and training the analysis.



At the bottom there usual OK and Cancel buttons for ending the definition together with the preview check box. It is enabled if the currently active image is compatible with the analysis.
In the top toolbar there is a Capture button that acquires and image according to the current Capture definition settings.
Well-plate navigation shows the dialog for interactive navigation on the plate. This is useful to find proper spots for capturing different images for training.
Whereas Save and Load store and retrieve definitions from the Database Import and Export save it to the file making it possible to share between workstations.
Analysis name will denote the particular analysis in the result view. Analyses with the same name are considered to be the same and thus available for aggregation.

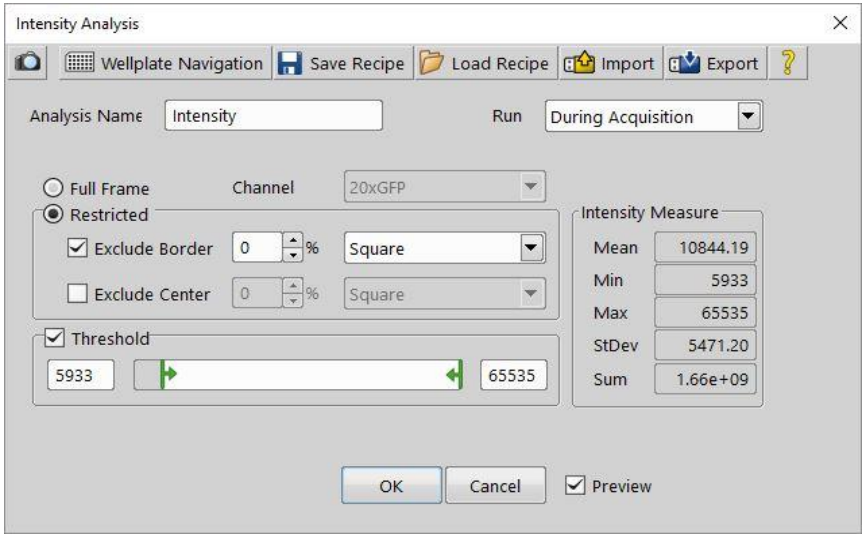
Six types of analyses are available:

- Cell Count Analysis
- Intensity Analysis
- Wound Healing
- General Analysis
- General Analysis RGB
- Live/Dead Analysis

Image Intensity

The Image Intensity analysis evaluates the intensity of the whole Field of View. It is the simplest analysis available. The example of usage for this analysis is when deciding whether there is a signal in the field or not. If the field is the whole well, there are some options to restrict the well outline (rectangular or circular) and center portion if necessary. It is also possible to restrict the range of intensities.

Image Intensity analysis window enables defining the Image Intensity as shown on the following picture. If you click the first Preview icon you can see your changes directly on the active image. After defining the parameters, click OK and now you can use parameters resulting from this analysis to define an expression. The parameters are then available in the “Expression window” under the “name of your Capture task” / Analysis / “name of your Image Intensity”.



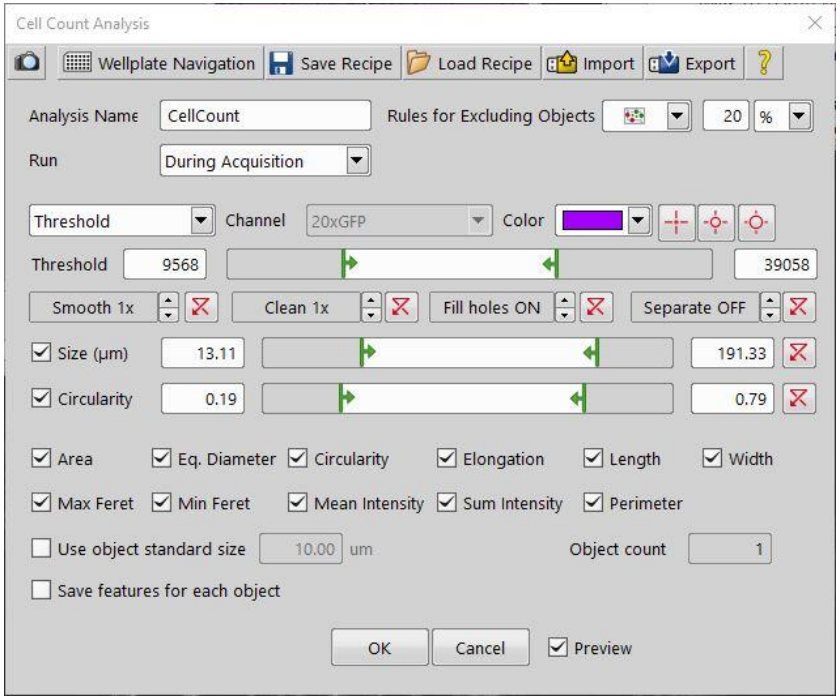
Available features measuring the image intensity are:

NAME	TYPE	DESCRIPTION
Mean	Field	Mean image intensity
Min	Field	Minimal image intensity
Max	Field	Maximal image intensity
StDev	Field	Standard deviation
Sum	Field	Sum image intensity
Area	Field	Area of all objects under the mask
AreaFraction	Field	Ration of Area to the whole Field Area

Cell Count

Overview

This analysis is designed for counting organic cells. It operates on a single channel and produces one binary mask and a set of predefined features. It has similar functionality as the Object Count in NIS Elements.



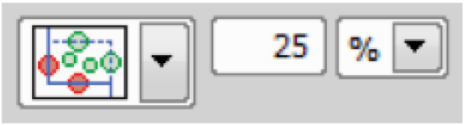
You should start by choosing the channel and selecting a color that is well visible on the preview image.

Thresholding

The main control here is the Threshold bar whereby you elect the low and high intensity thresholds. Only the pixels within this interval will be taken to make objects visualized as a binary mask. Under the main Threshold bar there are four post-processing controls to further tweak the objects. If the objects are too rough it is a good idea to try some smoothing, but not too high values in order to avoid merging of nearby objects. Then use little bit of Clean to get rid of the smallest objects. Set Fill holes if it makes sense in your sample. And finally play with Morphological separate (higher numbers separate less – produce bigger objects).

You can filter the objects based on their size (EqDia – Equivalent Diameter: calculated from object area as if it was a circle) or Circularity.

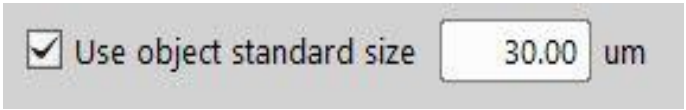
Objects Touching the Border



For sound statistics it is wise to exclude objects touching border. If adjacent images are captured and one Cell may appear in more than one field of view it is advised to use the measurement frame that excludes objects touching the bottom and left borders of the frame.

The offset of the frame from border should be roughly the mean object size (EqDia). It can be set using percentage of the image or in microns.

Standard Object Size



In some circumstance it is not possible to consistently threshold individual objects. If it is priori known that the object size does not vary significantly, it is possible to use the Standard object size (EqDia) feature to determine consistent count. The count is then calculated as a ratio of total area under mask and the Standard object area (calculated from its size).

Generated Results

Cell Count analysis generates one binary mask accessible after opening acquired images. Following set of features is always saved into the database.

NAME	TYPE	DESCRIPTION
Count	Field	Object count
Area	Field	Area of all objects under the mask
AreaFraction	Field	Ratio of Area to the whole Field Area
ObjectArea	Object	Individual Object Area*
ObjectEqDia	Object	Individual Object EqDia*
ObjectMeanIntensity	Object	Individual Object Mean Pixel Intensity*
ObjectSumIntensity	Object	Individual Object Sum Pixel Intensity*

All the object features (ObjectArea, ObjectEqDia, ObjectMeanIntensity and ObjectSumIntensity) produce following aggregated statistics: Mean, Min, Max, Sum, StDev (as member variables).

In the expression they appear this way:

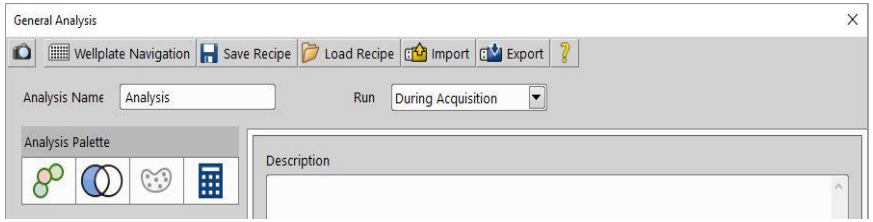
Job.*CaptureName*.Analysis.*CellCountName*.*FieldFeature*
Job.*CaptureName*.Analysis.*CellCountName*.*ObjectFeature.*Mean
Job.*CaptureName*.Analysis.*CellCountName*.*ObjectFeature.*Min
Job.*CaptureName*.Analysis.*CellCountName*.*ObjectFeature.*Max
Job.*CaptureName*.Analysis.*CellCountName*.*ObjectFeature.*Sum
Job.*CaptureName*.Analysis.*CellCountName*.*ObjectFeature.*StDev

Where CaptureName is the name of the capture task, CellCountName is the name given to the CellCount analysis, FieldFeature is Count, Area or AreaFraction and ObjectFeature is ObjectArea, ObjectEqDia, ObjectMeanIntensity, ObjectSumIntensity.

General Analysis

Overview

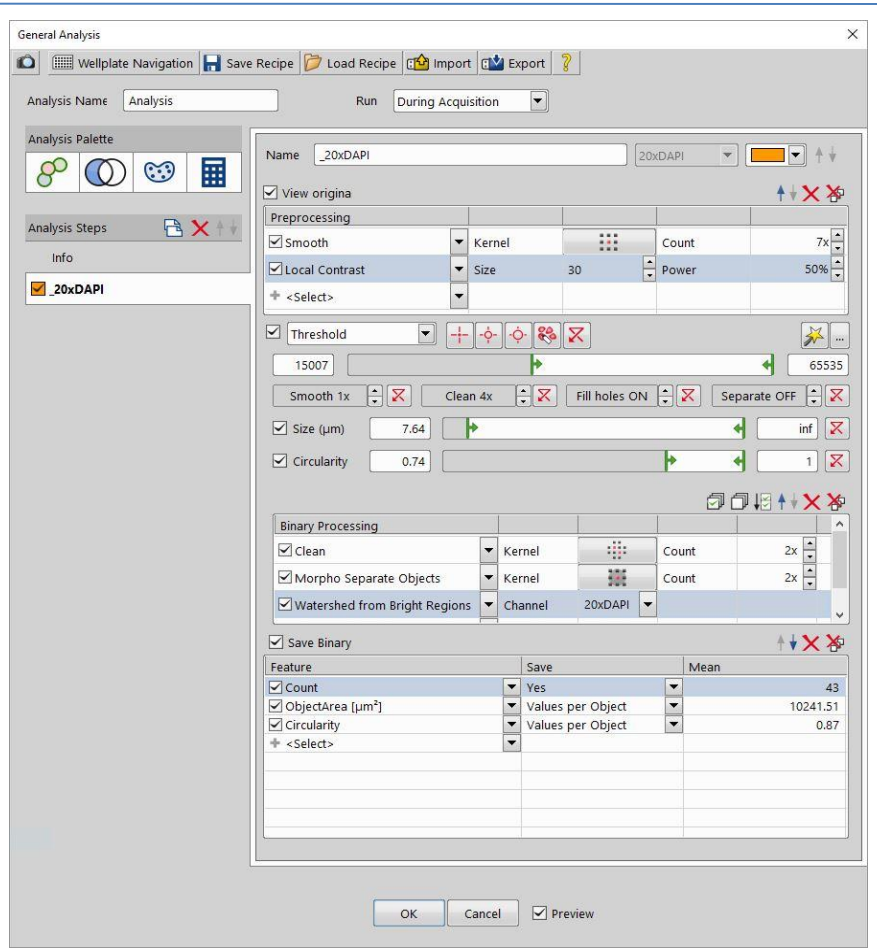
The General Analysis is the most complex and flexible of all of the analyses available. It is possible to make multiple binary masks on a single channel as well as to combine multiple masks by means of generic expression with powerful operators. It is also possible to calculate custom features.



In general analysis you start with an empty dialog. You must add one or more channel analysis tabs and optionally some combined analysis tab.
Every tab has a colored check box and a name. The check box turns ON or OFF the binary mask in the preview image. Its color corresponds to the color of the binary mask for easy identification.

Channel Analysis Tab

Channel analysis tab is used to measure features of one selected channel. There are four steps: preprocessing, threshold, binary processing and feature selection. They are performed in that order and have the same functionality as in NIS Elements.



Start by properly naming the tab, set the color and ensure that it is operating on a correct channel. Then switch the preview ON and start tuning the threshold. In many cases doing preprocessing is a good idea.

The preprocessing is helpful for improving the color image for easier later thresholding. These are examples of available : Auto-contrast, Close, Detect Peaks, Detect Regional Minima, Detect Regional Maxima, Detect Valleys, General Convolution, Morphological Gradient, Local Contrast, Median, Open and Smooth etc. For instance in case of uneven illumination a local contrast may help. To improve object detection try Regional Maxima or Detect Peaks.

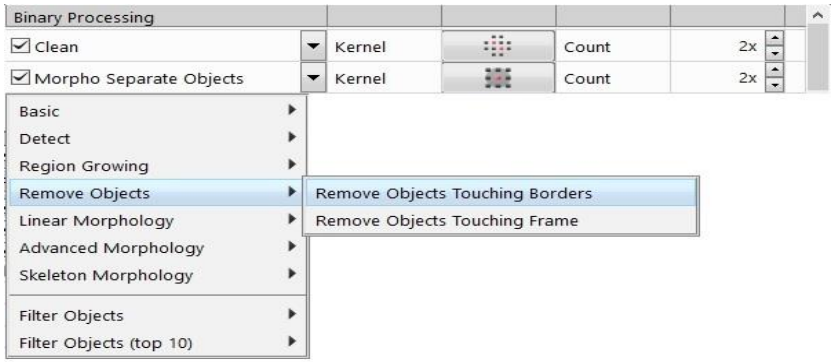
Each preprocessing type has its dedicated parameters. Kernel defines the shape of neighborhood and count affects the size of objects. Percentage(%) defines the strength of the processing.

Note that any change made to the color image is temporary (for thresholding only) and all measurements are made on the original (unaffected) image data as well as saving.

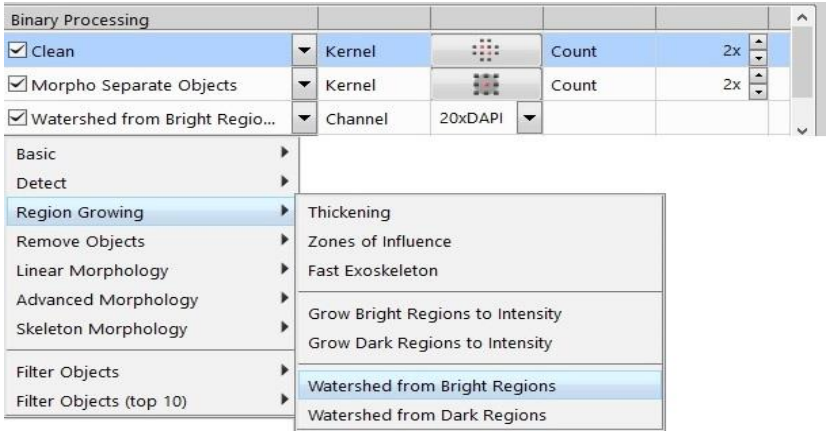
Thresholding works the same as in the Cell Count Analysis. However in General Analysis it is optional. If it is skipped, only intensity features on whole field of view are available. If the threshold is performed, it may be chosen not to save the binary image which is useful for temporary binary masks (see the Translocation example below).

Binary mask can be further modified in Binary Processing. These operations are available: Clean, Close, Close Holes, Dilate, Erode, Fill Holes, Medial Axis, Morphological Separate, Open, Remove Objects, Skeletonize, Smooth, Thickening and Watershed. Most of the processing methods have kernel and count as parameters, where kernel defines the neighborhood and count defines the size of an object.

”Remove Object Touching Borders” handles unwanted objects touching the image borders (see Objects Touching Border).



Watershed grows the binary objects following the intensity gradient.



When the Binary mask is fine-tuned it is time to set the features to be measured. Available features are: Area, AreaFraction, Count, ObjectArea, ObjectCircularity, ObjectElongation, ObjectEqDiameter, ObjectMaxFeret, ObjectMeanIntensity, ObjectMinFeret and ObjectSumIntensity and so on.

FIELD FEATURES	DESCRIPTION
Area	Area of all objects under the mask
AreaFraction	Ratio of Area to the whole Field Area
Count	Object Count

OBJECT FEATURES	DESCRIPTION
ObjectArea	Individual Object Area
ObjectCircularity	Individual Object Circularity
ObjectElongation	Individual Object Elongation
ObjectEqDiameter	Individual Object EqDiameter

All the object features (ObjectArea, ObjectCircularity, ObjectElongation, ObjectEqDiameter, ObjectMaxFeret, ObjectMeanIntensity, ObjectMinFeret and ObjectSumIntensity) produce following aggregated statistics: Mean, Min, Max, Sum, StDev (as member variables).

In the expression they appear this way:

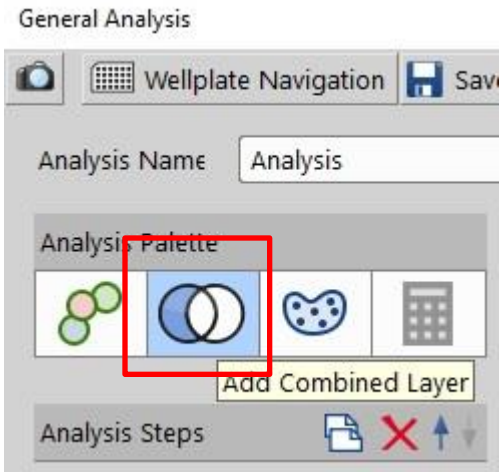
Job.*CaptureName*.Analysis.*AnalysisName*.TabName.*FieldFeature*
Job.*CaptureName*.Analysis.*AnalysisName*.TabName.*ObjectFeature.*Mean
Job.*CaptureName*.Analysis.*AnalysisName*.TabName.*ObjectFeature.*Min
Job.*CaptureName*.Analysis.*AnalysisName*.TabName.*ObjectFeature.*Max
Job.*CaptureName*.Analysis.*AnalysisName*.TabName.*ObjectFeature.*Sum
Job.*CaptureName*.Analysis.*AnalysisName*.TabName.*ObjectFeature.*StDev

Where CaptureName is the name of the capture task, Analysis-Name is the name given to the analysis, TabName is the name given to the Tab, FieldFeature is Area, AreaFraction or Count and ObjectFeature is ObjectArea, ObjectCircularity, ObjectElongation, ObjectEqDiameter, ObjectMaxFeret, ObjectMeanIntensity, ObjectMinFeret or ObjectSumIntensity.

For every feature it may be selected not to save it into the database.
This may be useful if the feature is used only for subsequent calculation.
If there is a document with a preview, the features are measured and preview value is displayed in the table for each row.

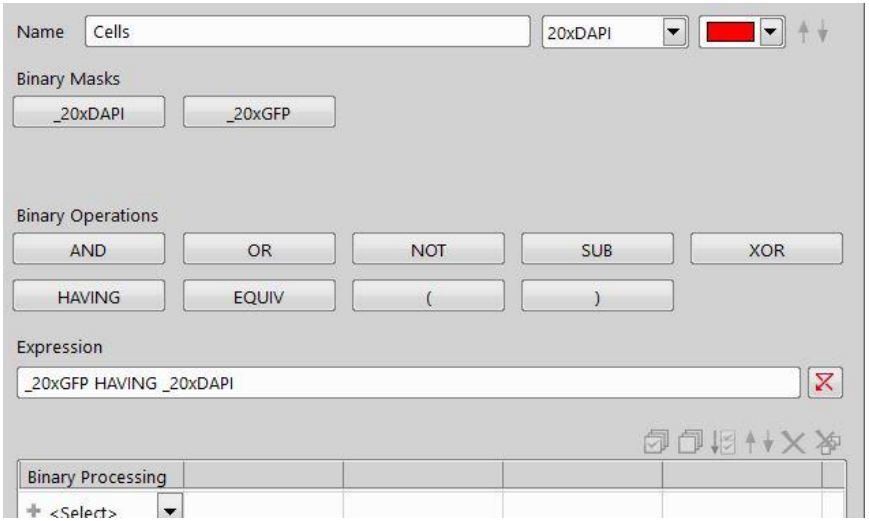
Combined Analysis Tab

The Combined Analysis Tab has different dialog window appearance. It is displayed by "Add combined layer" button.



Instead of preprocessing and threshold there is an Expression that defines how existing binary masks are combined.
The first row of buttons contains operands – the binary masks (from the tabs to left of the current tab). Below are the operators: AND – Intersection, OR – Union, NOT – Complement, SUB – Subtraction, XOR – Exclusive OR, HAVING, Equivalence(EQUIV) and parentheses. AND, OR, SUB, XOR and HAVING, EQUIV are all binary operators: the are placed between operands. NOT is an unary operator placed before one operand. Parentheses should be used to dictate the order of operations.

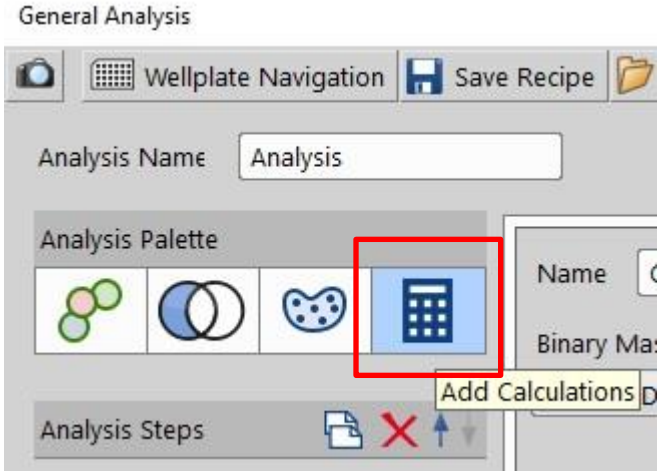
The expression " _20xGFP HAVING _20xDAPI" used to get only the _20xGFP objects that are having at least one pixel from _20xDAPI (see Translocation Example).



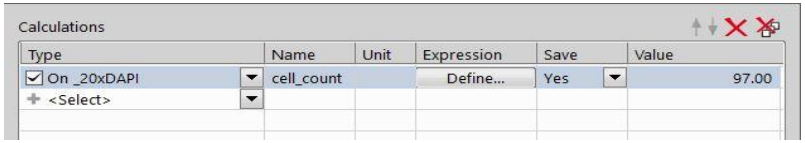
The remaining of the dialog is the same as the Channel Analysis Tab.

Calculations

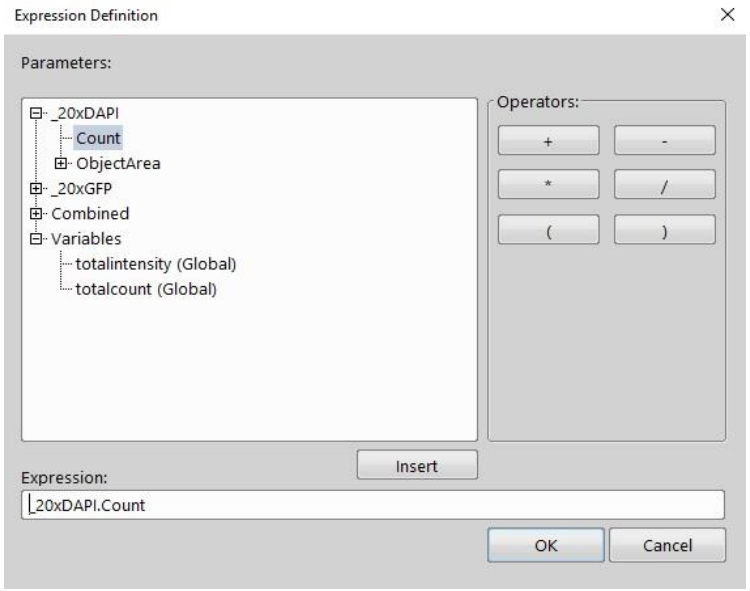
It is displayed by "Add combined layer" button.



The top part of the dialog contains a table with Calculation features which are defined by a freely editable expression, feature name and unit. There is an option to save the feature into the database and a preview of the value.



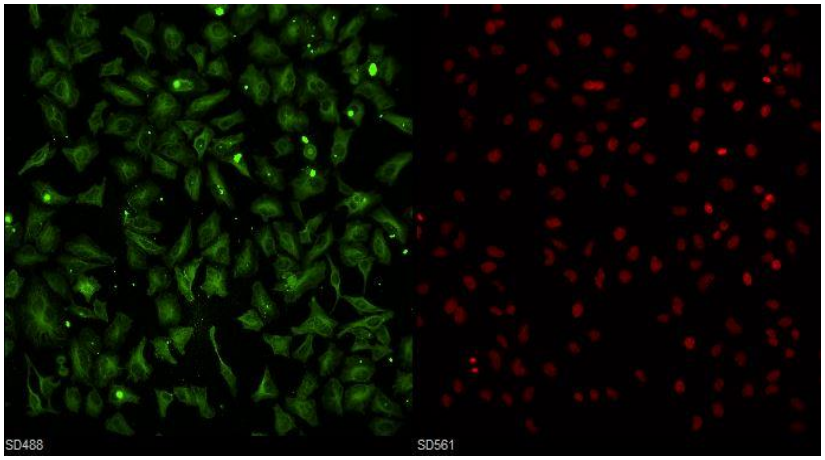
For easier expression editing a special dialog is available by clicking the Define... button.



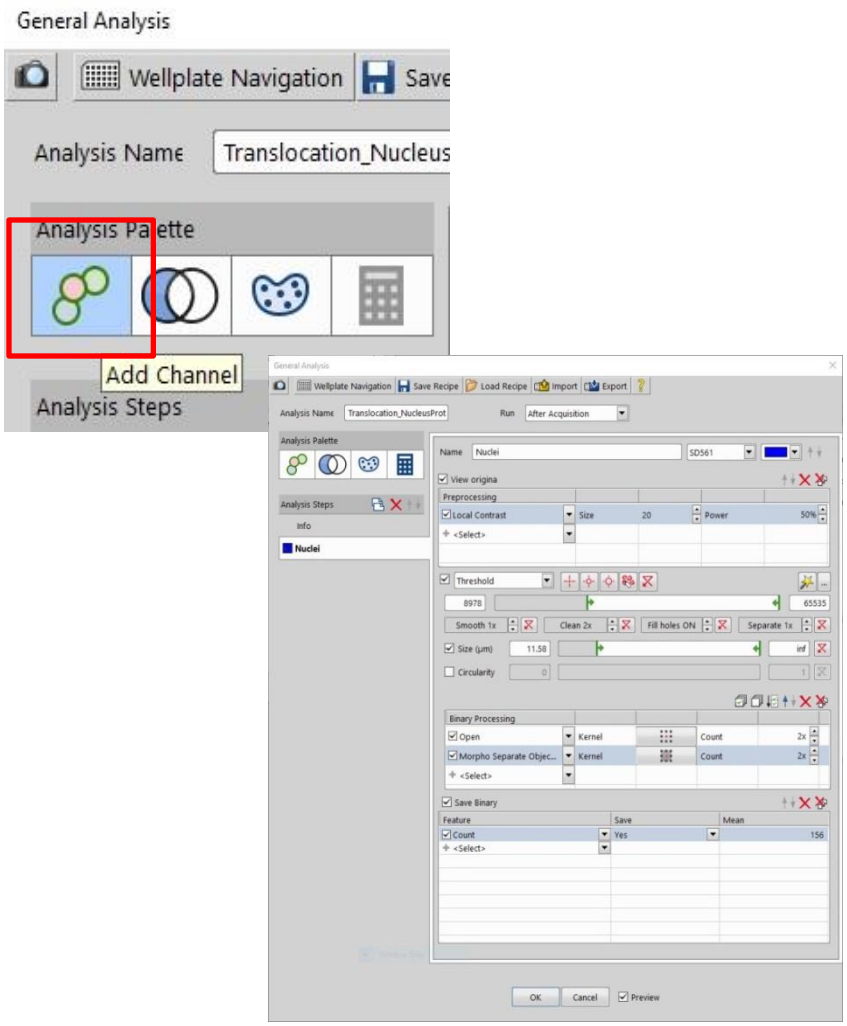
Variables available for the calculation of expressions are features measured from all the tabs plus the global variables populated by the Job.

Translocation Example

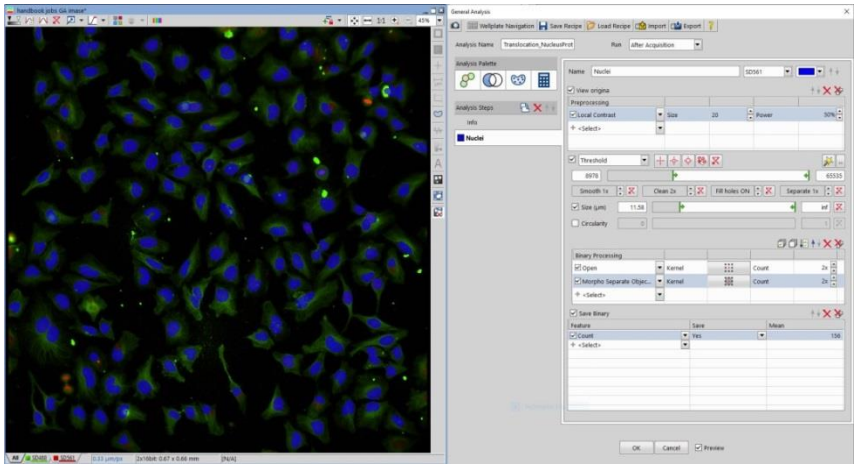
The possibilities of the General Analysis are best shown on a real example. In this case we are trying to find percentage of cells having a protein in nucleus. We are having a two-color image.



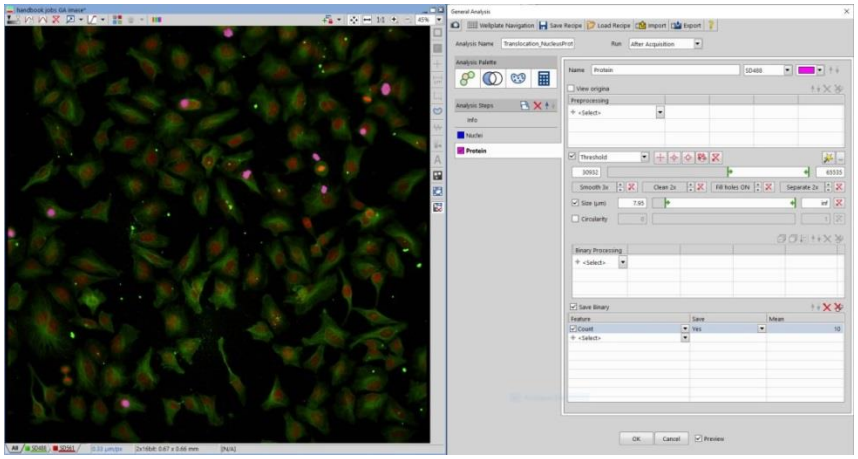
The left green channel “SD 488nm” contains cells and proteins.
The right red channel “SD 561nm” contains nuclei.
Let’s setup the “Add Channel” for the nuclei detection.



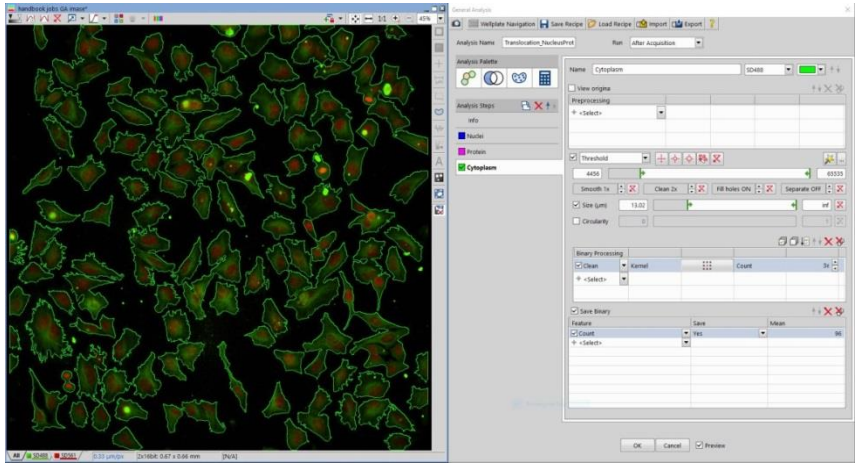
Here is the result: Nuclei channel SD 561nm (red –> blue)



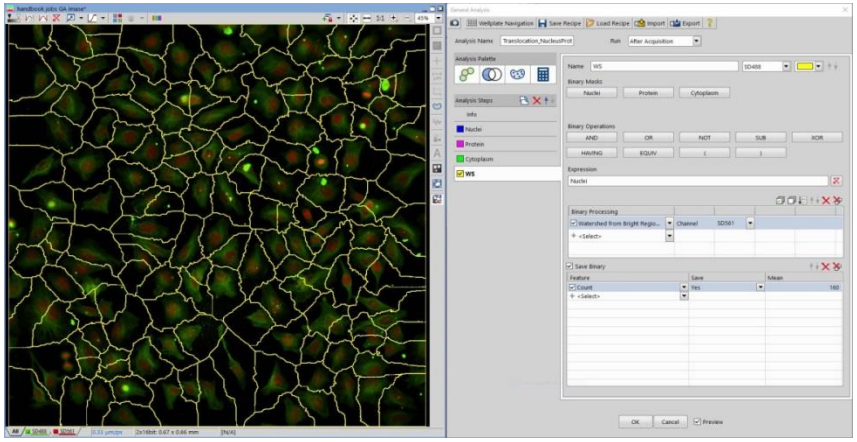
Another “Add Channel” for the protein detection. High intensity green spots: Protein channel SD 488nm (green –> pink).



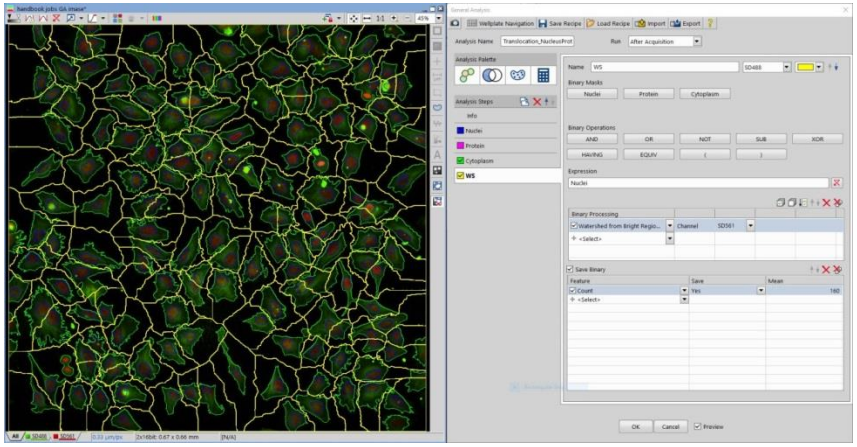
And the third one for cells which are usually difficult to threshold. Let’s do a best threshold of cytoplasm on the green channel expression. Cytoplasm channel SD 488nm (green) overview.



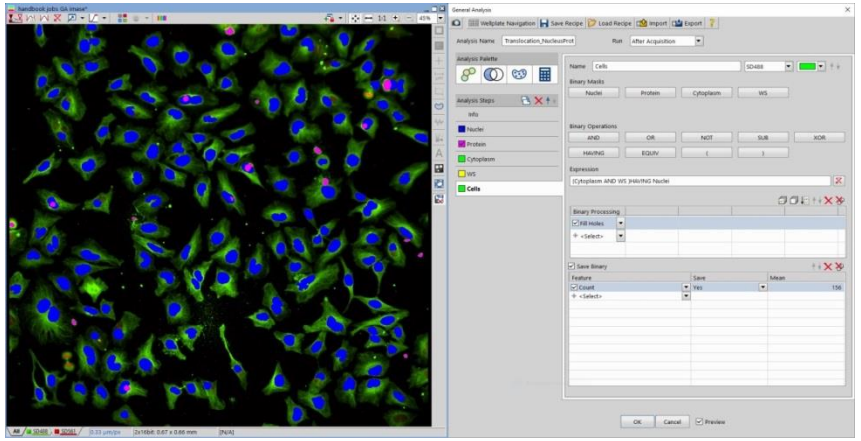
In many biological applications it is correct to assume, that each cell has exactly one nucleus. Thus we can get individual cell outlines by growing all nuclei simultaneously and following the intensity gradient of channel with cytoplasm SD488nm. The regions (initial Nuclei) are growing simultaneously until they touch or the image ends. This is called Watershed. For this we first make Layers with "Add Combined Layer".
Basically we take Nuclei and modify it with processing (WS –> yellow).

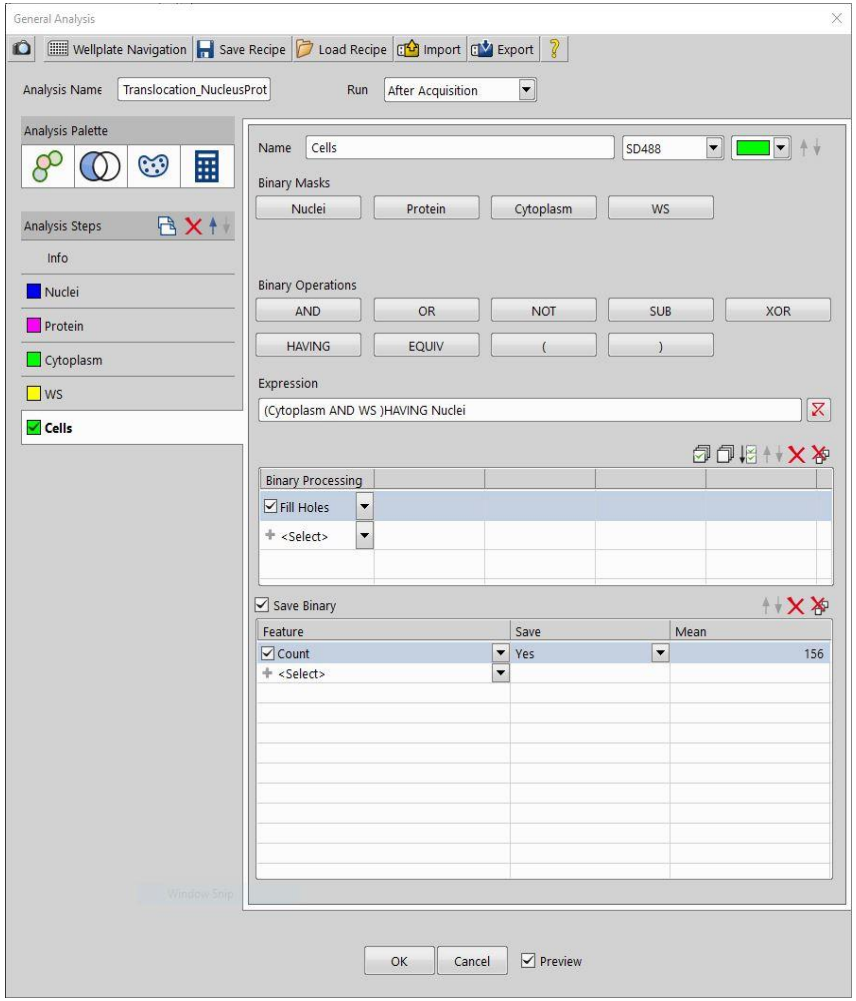


This is not yet what we wanted. In the following image we can see three objects: Nuclei (blue), Cytoplasm (green) and watersheds (yellow).

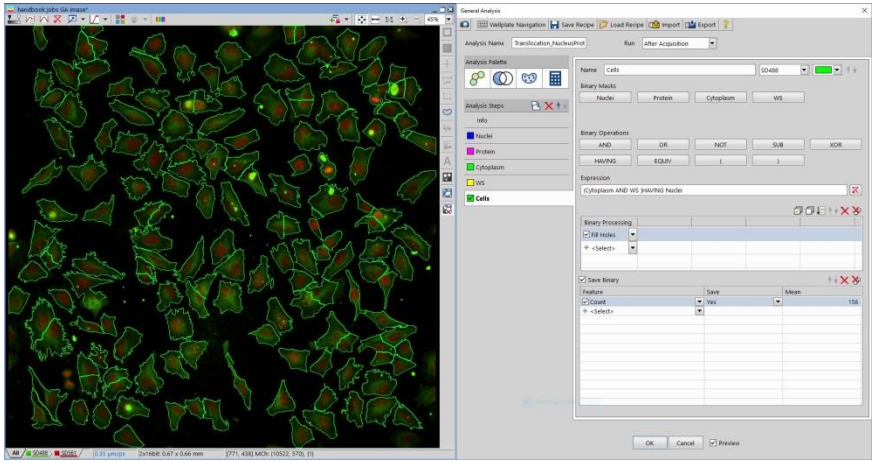


Now we have to intersect Cytoplasm with Watersheds and take only the objects that are having nuclei. In a new Combined Layer, we use the binary operation buttons or write the following expression manually: (Cytoplasm AND WS) HAVING Nuclei

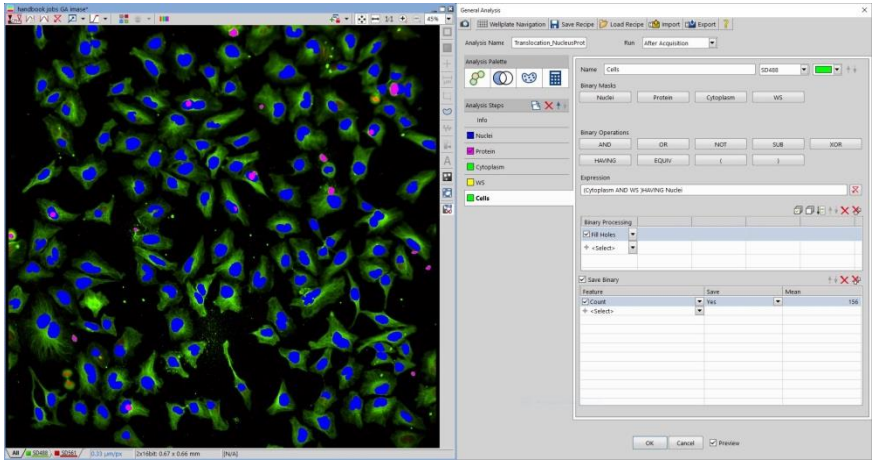




These are the resulting cells (green)



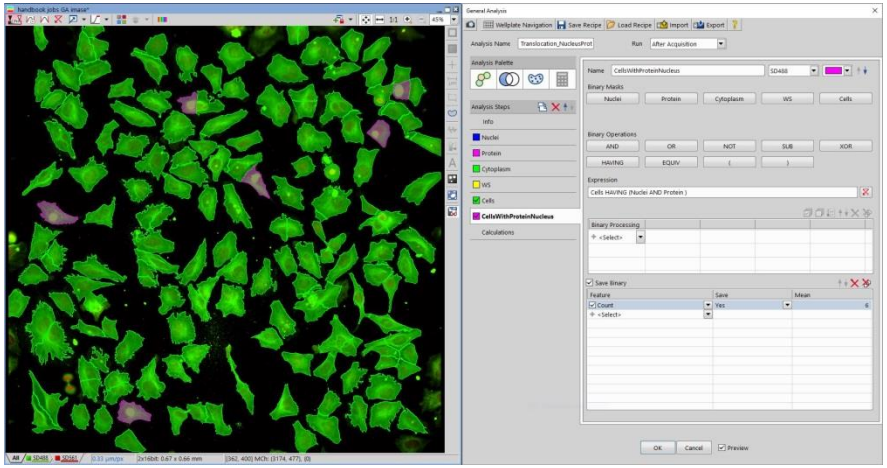
And all objects (with zero transparency): Cells (green), Nuclei (blue), Proteins (pink).



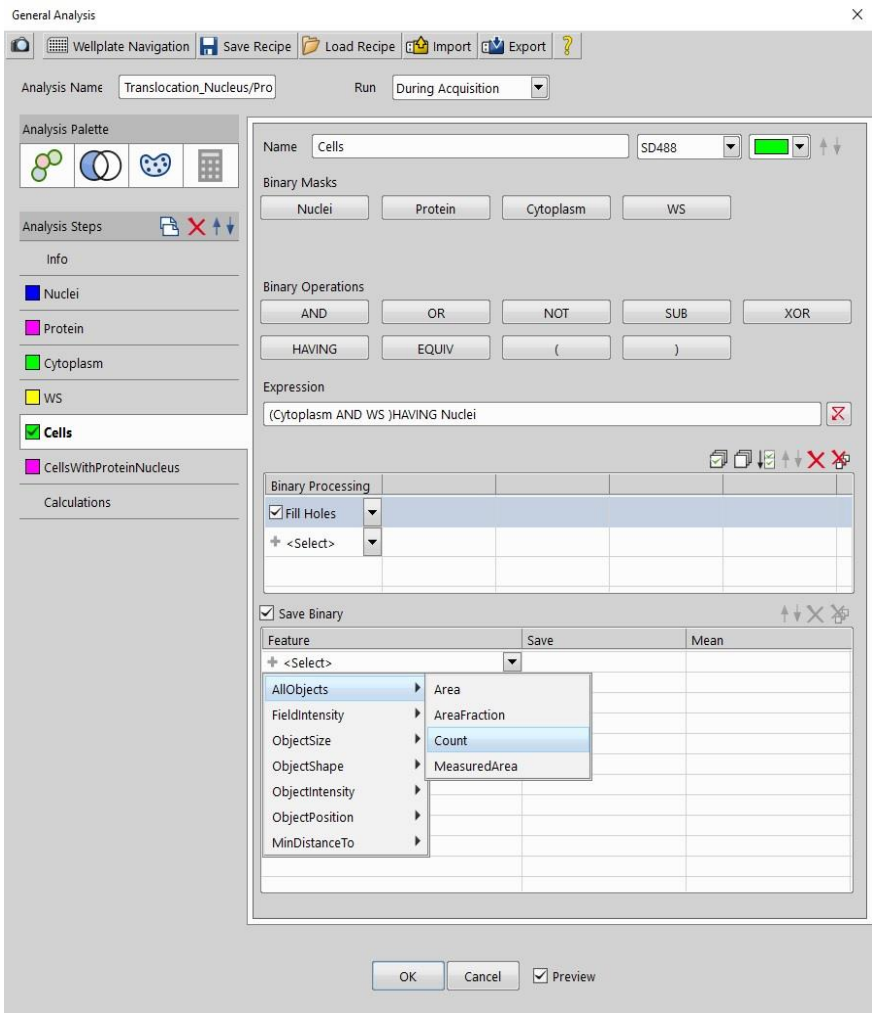
Going back to our task: We wanted to get the ratio of cells having protein in nucleus. For this we need to count all cells.

Then we need to count the cells with protein in nucleus. For this we will make a new Combined channel- cellsWithProteinInNucleus（Name example）with the following expression (pink):

Cells HAVING (Nuclei AND Protein)

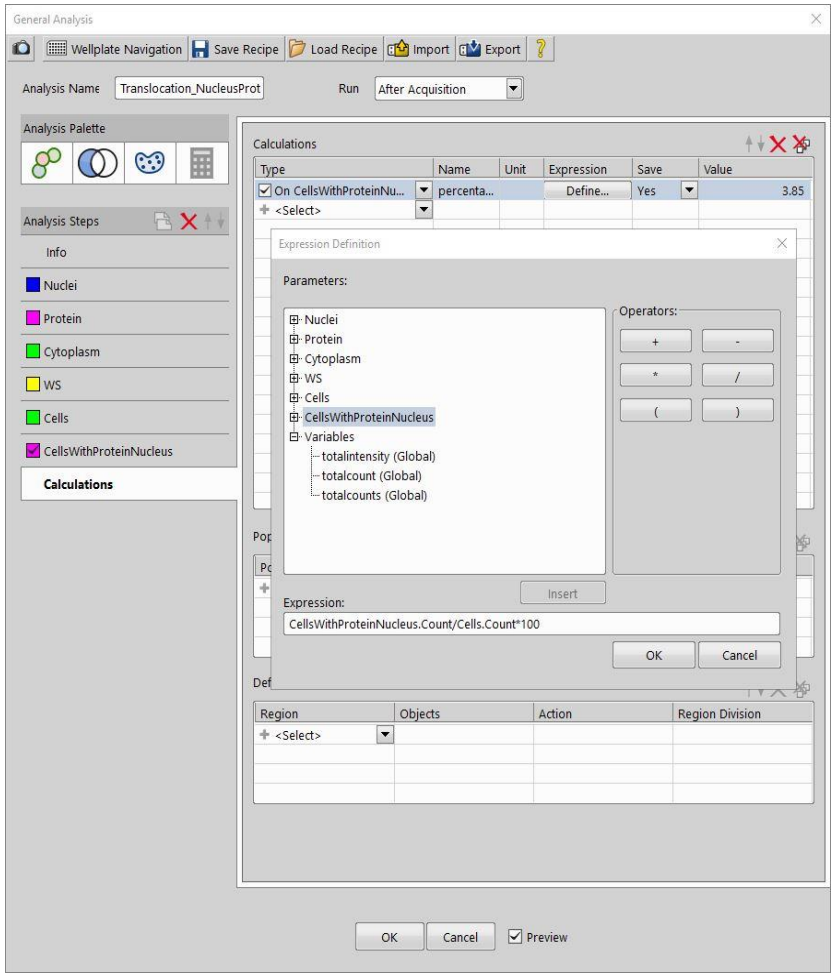


In order to get the percentage, we first have to add count feature into both Tabs: Cells and CellsWithProteinInNucleus.



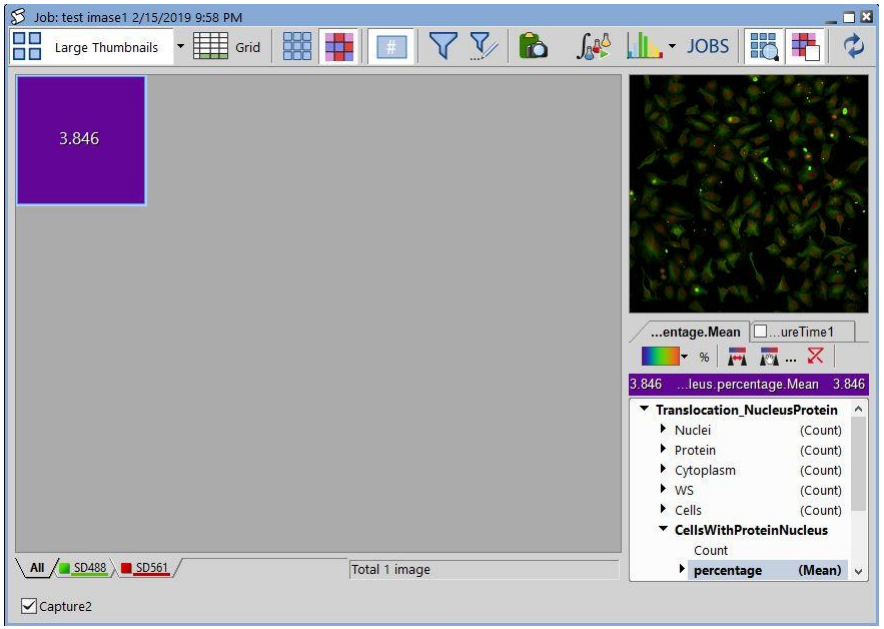
The last step is to add the formula for the percentage:

*PercentageOfCellsWithProteinInNucleus = CellsWithProteinInNucleus.Count / Cells.Count *100*

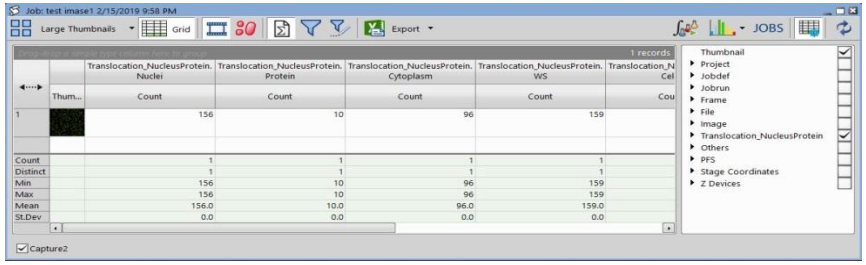


We can observe the value in the preview: 3.85 %. And in the result window: in the heat-map.

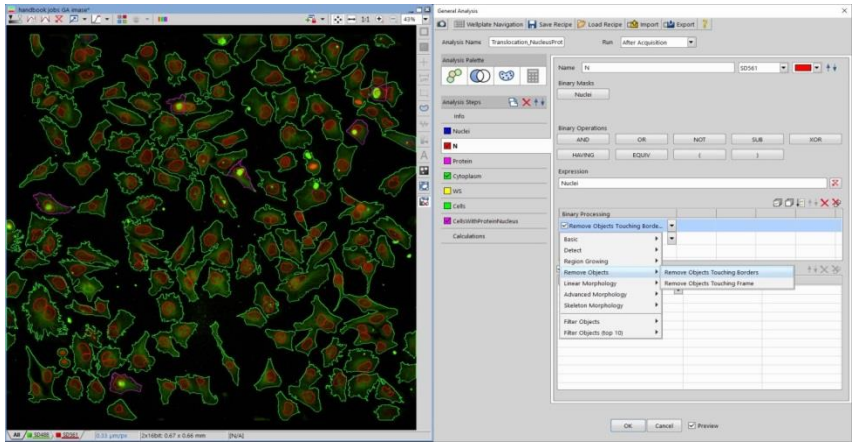
...



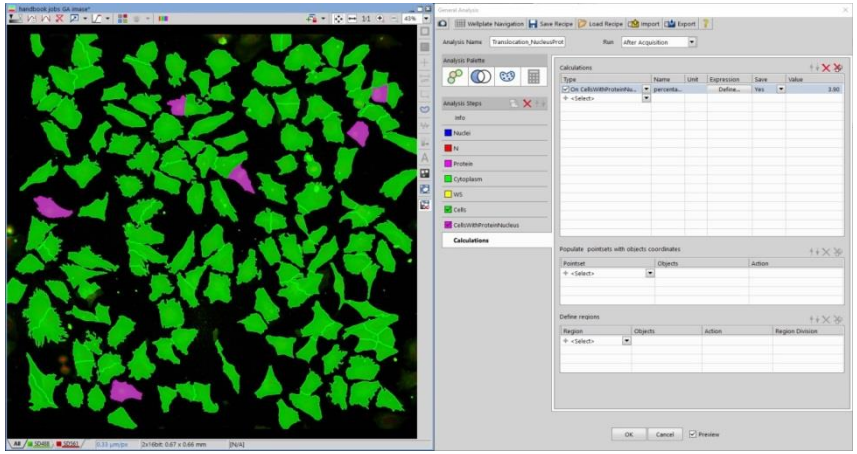
... and in the Grid View together with the counts.



In order to get more correct results we may improve the workflow to exclude objects whose nuclei are touching the borders. Here we made a new Combined Analysis Tab for the accepted nuclei called simply N (red) and modified all depending tabs to reference the N instead of Nuclei (blue).



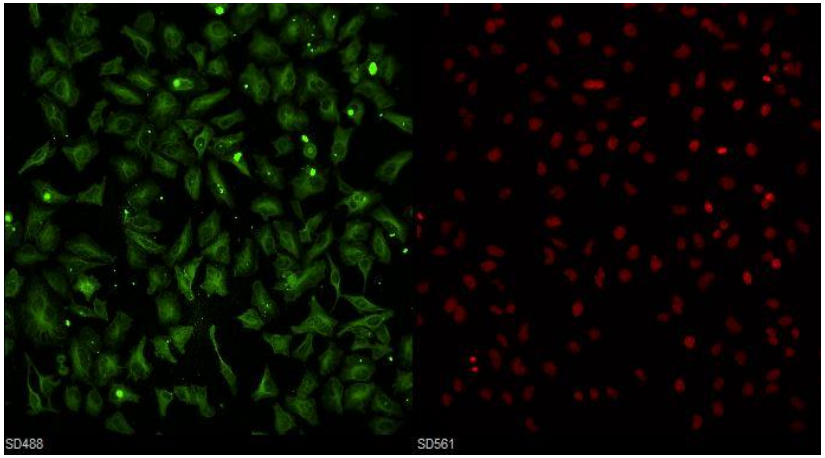
There is a slight difference now 3.9% of cells are having protein in nucleus instead of 3.85%



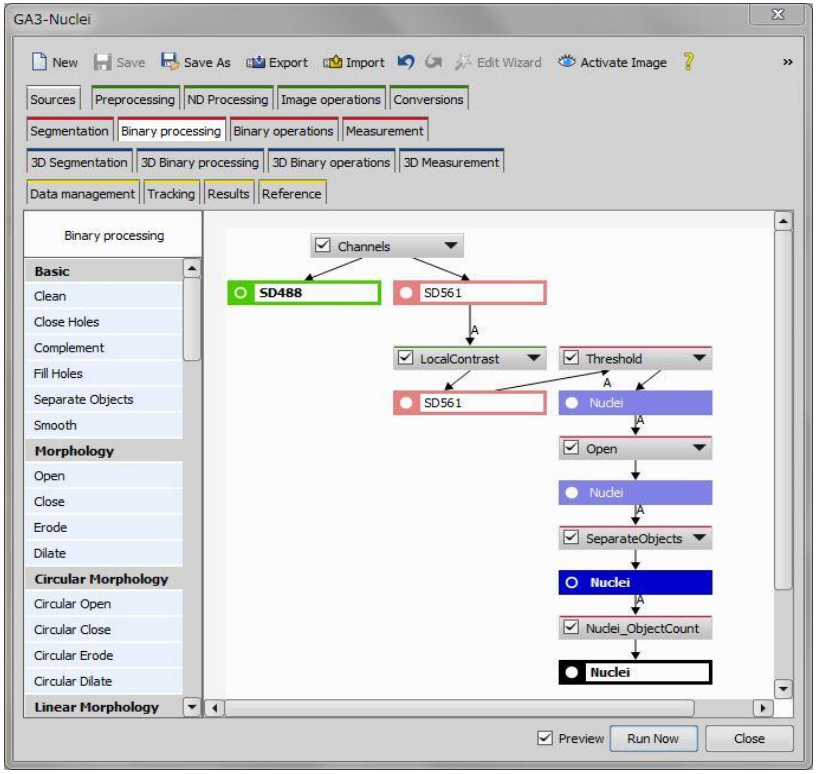
Translocation Example

(Example of analysis performed by GA3)

The case of General Analysis is analyzed by GA3 in the same way. Examine cells in which proteins in the nucleus are present. We are having a two-color image.

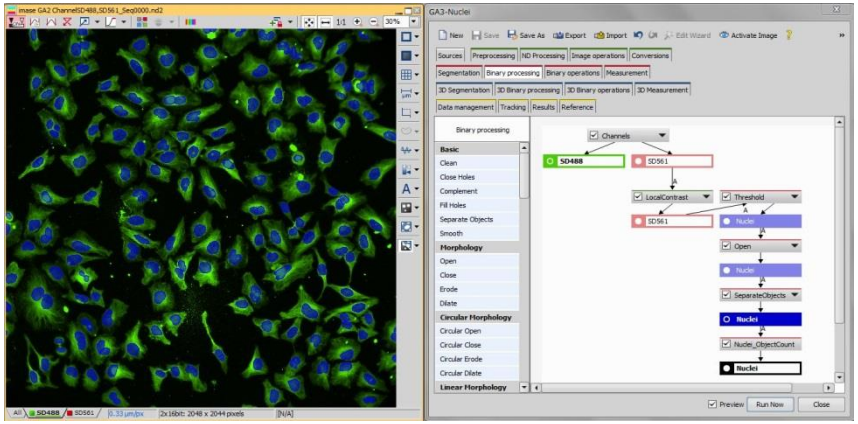


The left green channel “SD 488nm” contains cells and proteins. The right red channel “SD 561nm” contains nuclei.

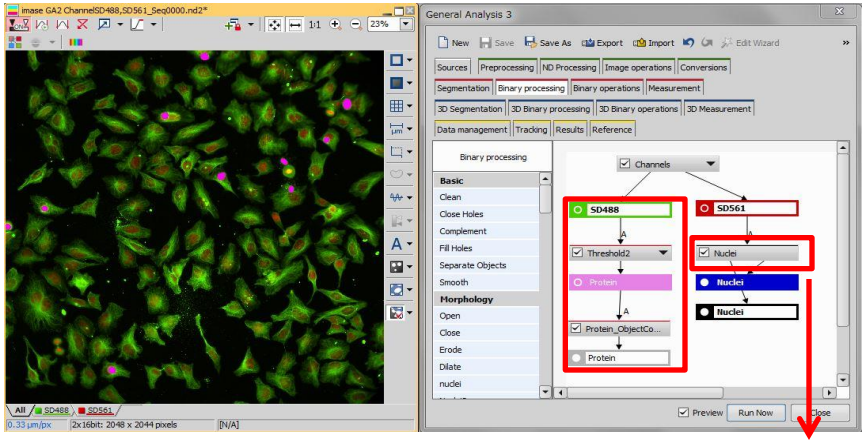


First, to detect nuclei, highlight the contrast of nuclei with LocalContrast in the Preprocessing tab, and use the Threshold in the Segmentation tab to determine the threshold.

Here is the result: Nuclei channel SD 561nm (red –> blue)

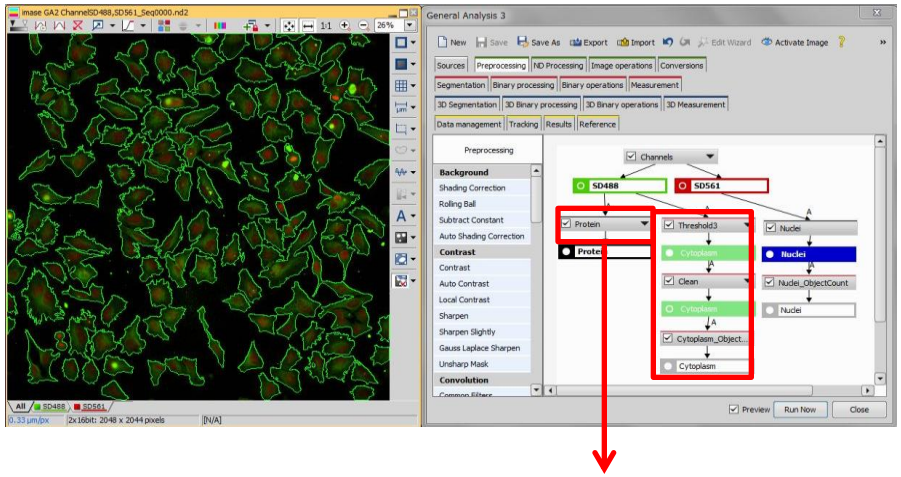


Another example of protein detection at SD 488 nm. High intensity green spots: Protein channel SD 488nm (green –> pink).



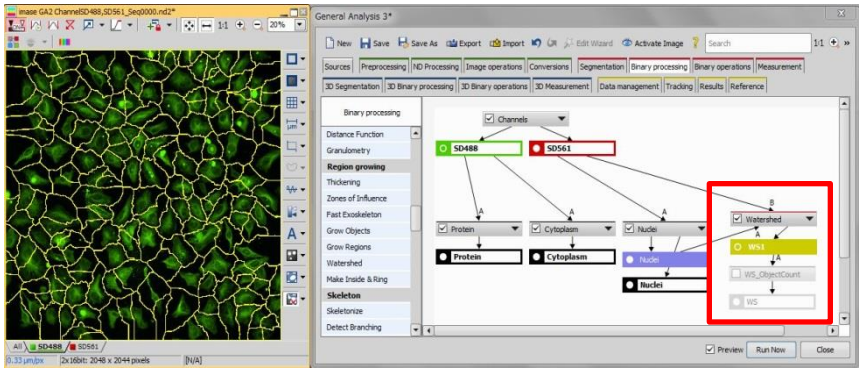
Nuclear detection is grouped.

And the third one for cells which are usually difficult to threshold. Let's do a best threshold of cytoplasm on the green channel expression. Cytoplasm channel SD 488nm (green) overview. This example again uses "Threshold" to determine the threshold, and then uses "Clean" on the Binary processing tab to remove the garbage.

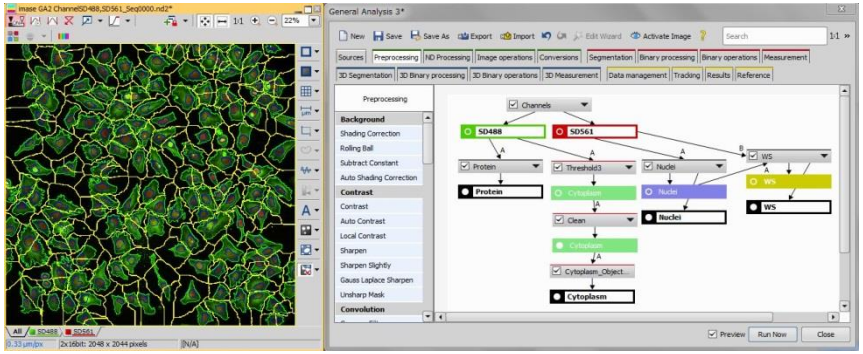


Protein detection is grouped.

In many biological applications it is correct to assume, that each cell has exactly one nucleus. Thus we can get individual cell outlines by growing all nuclei simultaneously and following the intensity gradient of channel with cytoplasm SD488nm. The regions (initial Nuclei) are growing simultaneously until they touch or the image ends. This is called Watershed. To do this, first use "Watershed" in the Binary processing tab. Basically we take Nuclei and modify it with processing (Watershed(WS) –> yellow).



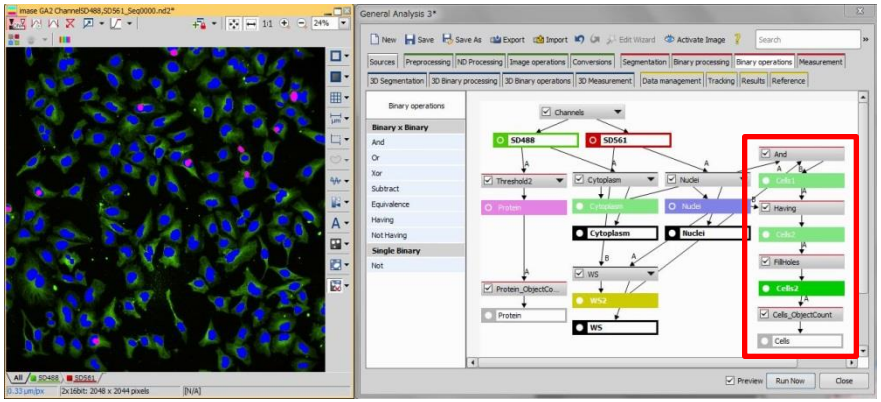
This is not yet what we wanted. In the following image we can see three objects: Nuclei (blue), Cytoplasm (green) and watersheds (yellow).

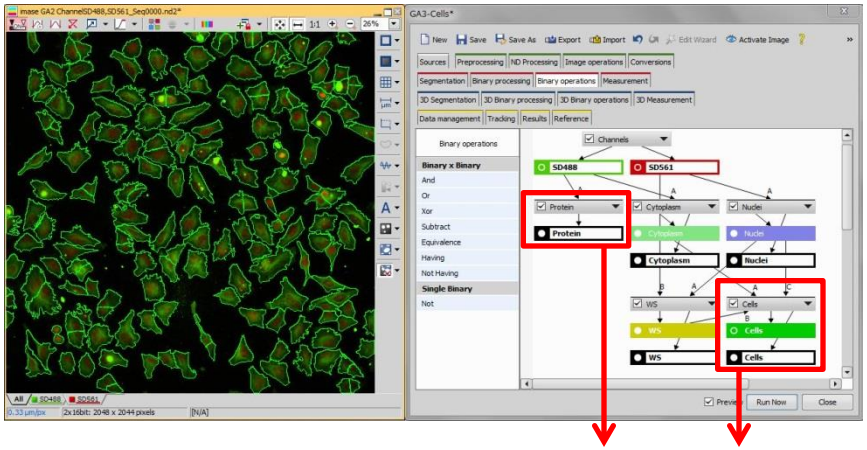


Now we have to intersect Cytoplasm with Watersheds and take only the objects that are having nuclei. So use "AND" and "HAVING" in the Binary operations tab. In GA, I wrote the following expression.

(Cytoplasm AND WS) HAVING Nuclei

GA3 uses "AND" and "HAVING", and realizes by connecting Cytoplasm, WS, and Nuclei with a line.

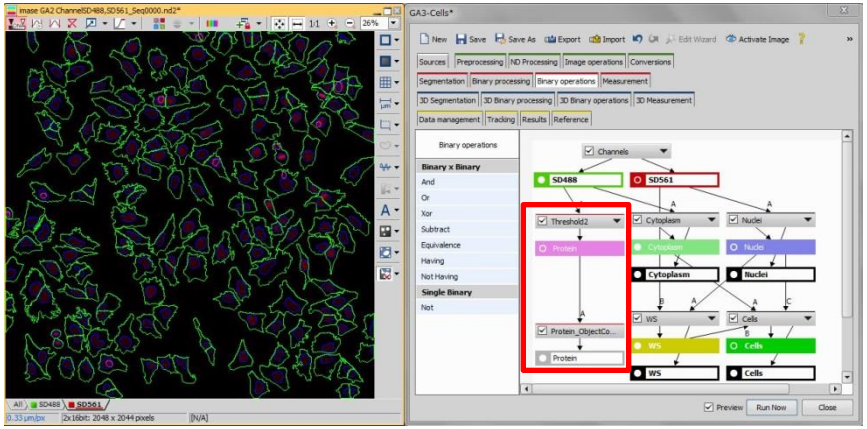




These are the resulting cells (green)

It is grouped.

And all objects (with 100% transparency): Cells (green), Nuclei (blue), Proteins (pink).



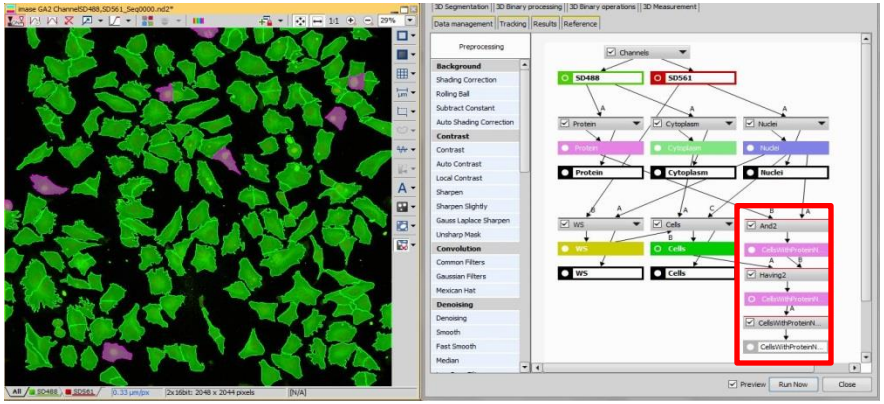
Display the protein (pink) by setting the purple radio button to "NO" at ungrouping.

Going back to our task: We wanted to get the ratio of cells having protein in nucleus. For this we need to count all cells.

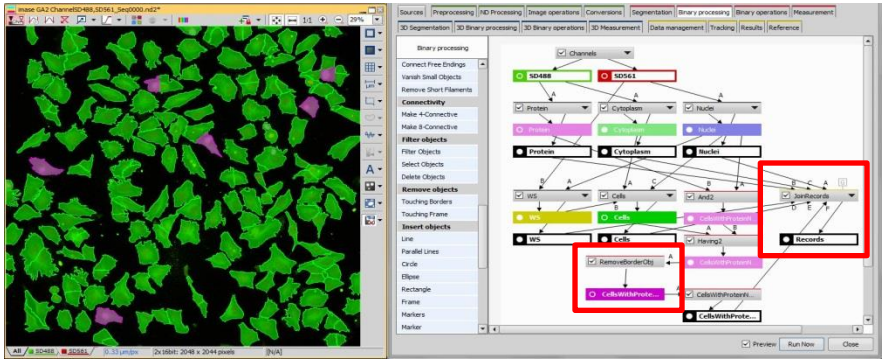
Then we need to count the cells with protein in nucleus. GA created a new composite channel by the following "expression (pink)". Similarly, GA3 is realized by connecting "Cell", "Nuclei" and "Protein" by using "AND" and "HAVING" in the Binary operations tab.

As a new name, this function is registered with “CellsWithProteinInNuclues”.

Cells HAVING (Nuclei AND Protein)

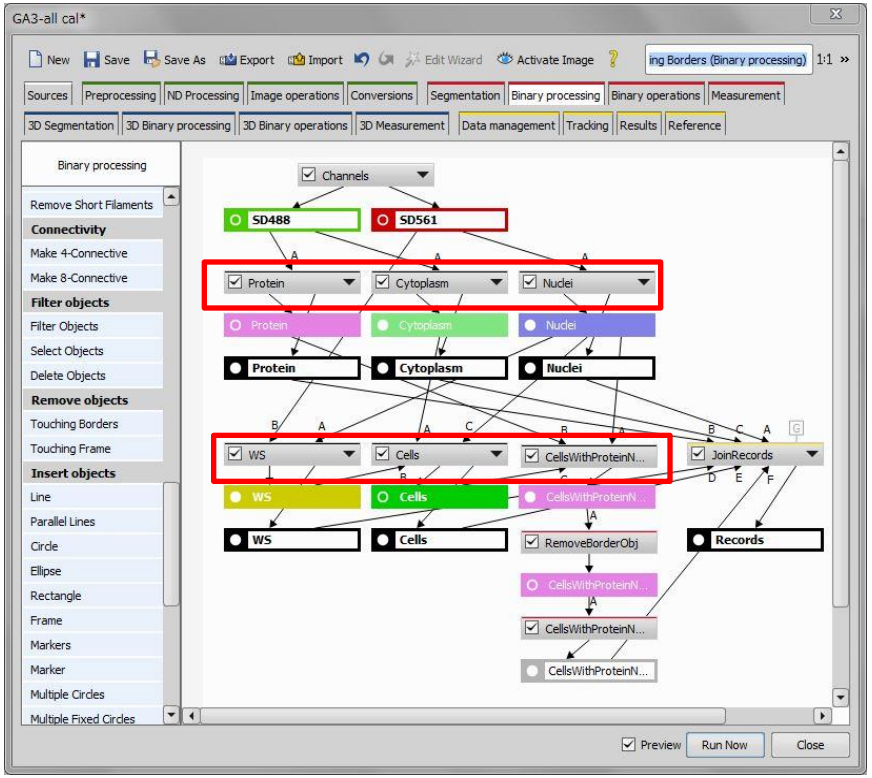


In order to get more correct results we may improve the workflow to exclude objects whose nuclei are touching the borders. Here, use "Touching Borders" in the Binary processing tab and modify it to select "Nucleus" in the frame.

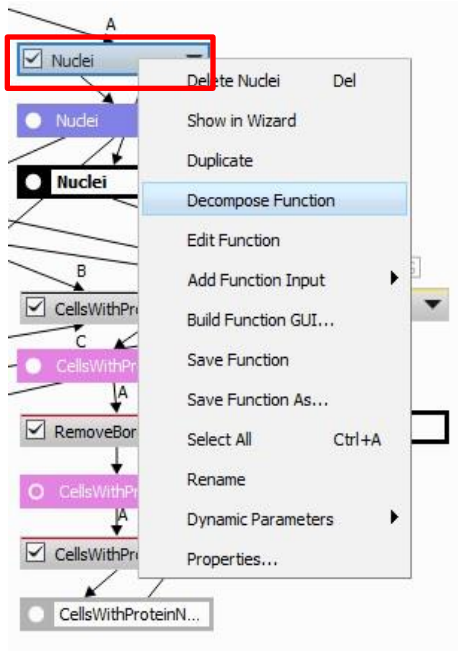


Finally, the count data of each object can be collected and displayed collectively by "Join Records" on the Data management tab.

The end result is the GA3 analysis sequence as shown below.



The red box shows the contents grouped, so expand the sequence with "Decompose Function" and check if necessary.

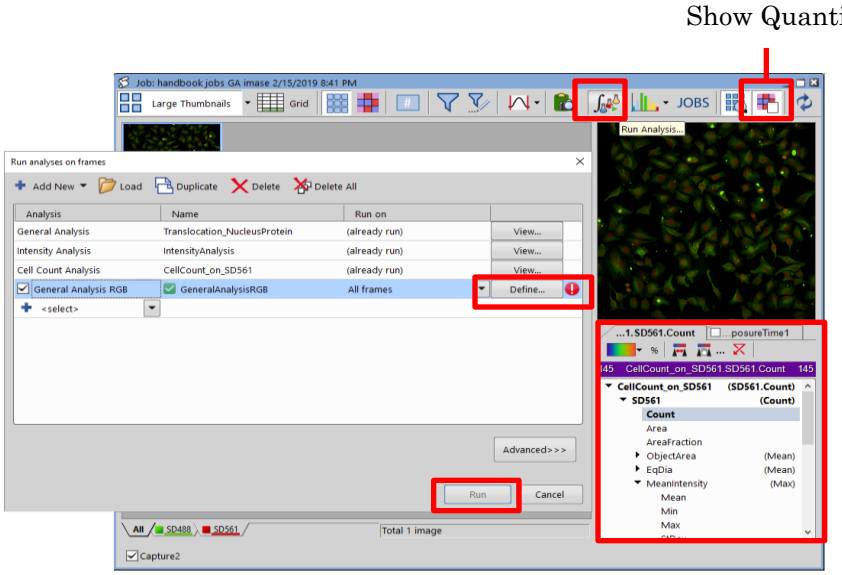


Example:

Click on Nuclei to activate and right click. The contents expand when "Decompose Function" is selected.

How to Run Analysis on Captured Data

After running your job, there is a possibility to execute more analyses on captured frames. Go to the Job Results window and click the Run Analysis button. The Run Analysis dialog window opens.



Click the Add New Analysis button and choose the type of your analysis in the pull down menu. Continue setting up your analysis as described in their particular sections (Image Intensity, General Analysis, Cell Count, etc). After (re)defining your analysis click the Run button. Your analysis is then processed and the resulting variables are added to the Show Quantities panel.

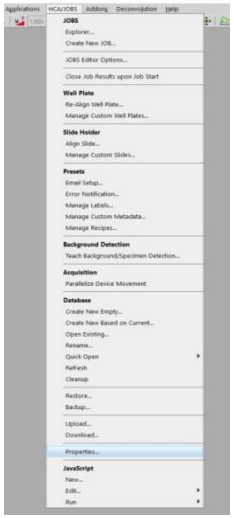
Tip: Hide your preview by clicking the Show Preview button to see more of your analysis results.

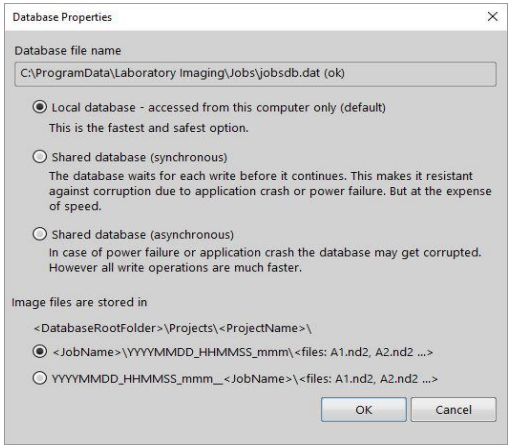
Database Managing

Projects, jobs and job results are stored in a database. This database contains all meta-data except images acquired by jobs. Images are not stored in the database file. This is for two reasons: efficiency and data security. As images are ordinary .ND2 files they are independent of the database and can be opened by NIS-Elements directly. Images are marked as readonly and should not be modified in NIS-Elements. On the other hand, the database can be opened and accessed without the images. Therefore images are stored in folders and the database contains relative paths to them. Images and database files exist in a folder and make a bundle. This bundle can be copied as a whole. However it is not safe to copy the folder while it is accessed by NIS-Elements. Use the **Database Backup** / **Database Restore** functions instead.

Database Properties

You can adjust the following database settings after you click the **Database Properties** button:





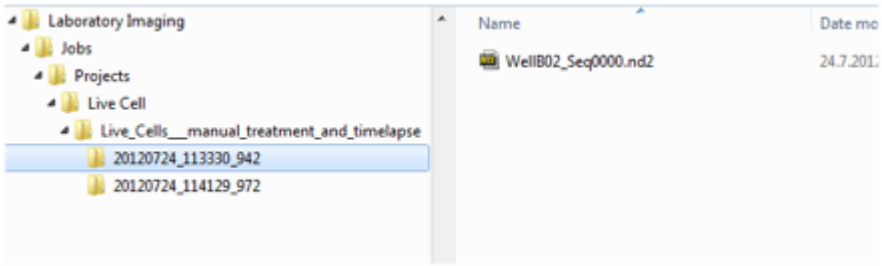
Database root folder
Define the path to your database files and select the type of access to the data. You can choose between:

- Local database - recommended, fast, safe.
- Shared database (synchronous) - opt this if access from multiple PCs is required. This setup is safe and sufficient for long-lasting experiments (e.g. 1 frame per minute).
- Shared database (asynchronous) - if access from multiple PCs is required along with the speed of access. Regular back-ups are absolutely necessary with this setup.

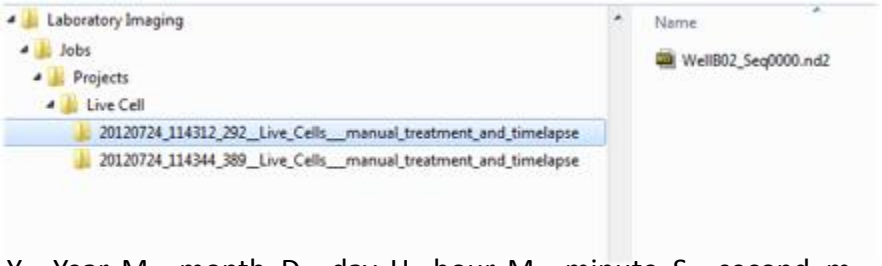
Warning
If an unexpected error such as power failure occurs while the database is being accessed, the database may get corrupted (lost).

Image files are stored in
Choose in which form you want to save your Image files. The following options are available:

• <JobName>¥YYYYMMDD_HHMMSS_mmm¥<files: A1.nd2, A2.nd2 ...>



YYYYMMDD_HHMMSS_mmm__<JobName>¥<files: A1.nd2, A2.nd2 ...>



Y = Year, M = month, D = day, H =hour, M = minute, S = second, m = millisecond

Backing up the Database

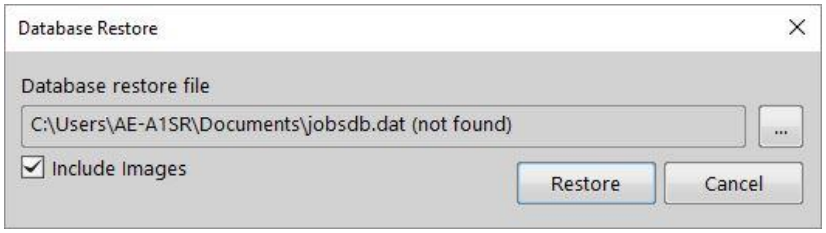
This dialog box enables saving all files you have created using Jobs into a desired folder. The backup routine copies the database file to a specified folder. If image files are also backed up (recommended) they are copied as well. The backup folder is a regular database bundle and can be accessed from NISElements. If the backup folder already contains a previous backup, it is merged in the following way:

• The previous database file is renamed (current date is appended) and a new file is created.
• New image files are added to the backup folder.

Please see Database Properties chapter on how to set the database.

Restoring the Database

This operation replaces the content of the current database folder with the contents of the backup folder. Restoring the Database destroys the original content of the current database.



Press the **...** button to browse to your database folder. After Selecting it, click **OK** and **Restore** to finish the restoration process. You can uncheck the **Include Images** checkbox in case you don't want to restore image files.

Database files

The database file contains the project and the job hierarchy, all the definitions (jobs, analysis recipes, labels, tags, etc...) acquisition data like plate or well information, loop indexes, time analysis results, links to the image files and much more. All this information is stored in a single file. The database file is usually much smaller than the size of an image file. The database is contained in the “jobsdb.dat” file in the database folder. Other temporary files (typically jobsdb.dat-shm, jobsdb.dat-wal or jobsdb.dat-journal) which indicate that the database is in use may also appear in this folder.

All temporary files should disappear after all NIS-Elements applications accessing that particular database are closed. If NIS-Elements did not finish properly these files may remain. They must not be deleted as they contain a valid database state. In this case NIS-Elements should be launched, database connected and NIS-Elements closed again. Note that it is not safe to copy the .DB file at that moment. NIS-Elements is connected to a single JOBS database at any given time – it is the current database. The current database setting can be changed in the [Database Properties]. NIS-Elements stores the current database folder on per user basis, which means that every user can have his own database.

Program Upgrades

The database file format may change to support new features with new versions of NIS-Elements. An upgrade of the database is required before it can be used by a new version of NIS-Elements. However, the upgrade renders the database inaccessible from any previous version of NIS-Elements. It is a good practice to make a backup of the database before an upgrade of NIS-Elements is made. When copying a database and accessing from multiple NIS-Elements, Please be sure to read it from the version of NIS-Elements that created the database.

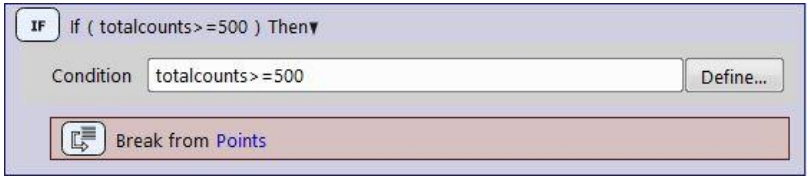
Task Reference

Tasks are building blocks of the whole Job. A detailed description of each task can be found in the following sections.

Execution Flow

Condition IF

If the specified conditional expression is evaluated to TRUE, the contained tasks will be run. Otherwise the contained tasks are skipped.



Example of an expression. See also Using Conditions.

Condition (if-else)

If the specified expression is evaluated to TRUE, the contained tasks will run. Otherwise the script proceeds to "Else".
Tasks contained in the Else section will be run only if the specified conditional expression is evaluated to FALSE.
See also Using Expressions.

Break

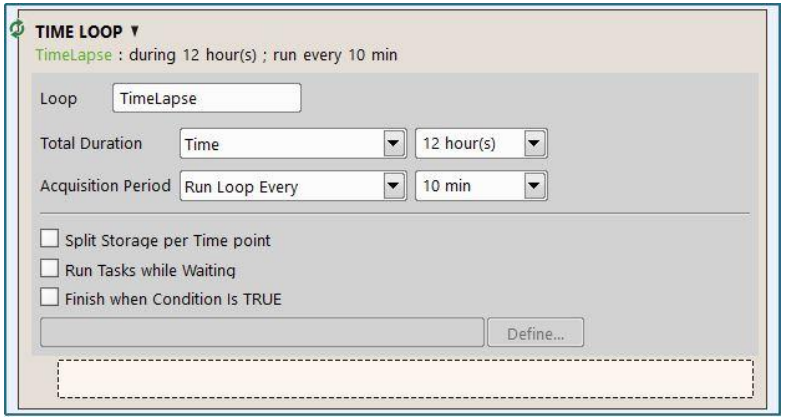
Exits execution of the selected loop and continues to subsequent tasks. Break from selects one of the parent loops to exit. The job will continue with the task following the selected loop.

Question

Displays a pop-up window requiring user interaction. After "answering" by clicking one of the buttons, the job continues.

Time Lapse

Repeats the contained tasks according to the specified parameters (total duration / number of loops, interval...). This task typically contains just the Capture task, but can contain anything else of course.



Type the name of your loop and select one of the following time lapse modes: Fixed duration - set a precise amount of time to the Timelapse [msec / sec / min / hour(s)]. Number of loops - define the number of loops after which the time lapse runs the following tasks. Use Unknown duration (User or Program Break) function if you don't know when to stop the time lapse. With this setting it can be stopped manually or automatically after a program break.

Then you can define the speed of acquisition. Select No-delay to let the task run as fast as possible. The other two options specify intervals between two adjacent repetitions of the task with the difference that the Run loop every option specifies how fast the tasks are started, but the Wait between loops option specifies the time between the end of the current loop and the beginning of the following loop.

Other tasks can be performed repeatedly if there is a spare time between two adjacent loops. When you select the Run Tasks while Waiting box, an additional space named “Run while waiting” appears at the end of the task. You can place the tasks to be run there.

Especially if a loop with Unknown duration is defined, you can specify a conditional expression which will cause the loop to be exited. Check Finish when condition is TRUE and click Define to specify the conditional expression. See also Using Expressions

Redefine Time Lapse

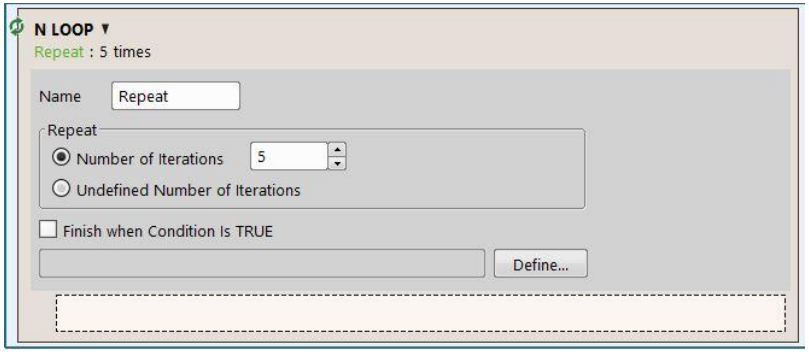
Modifies an existing Time Lapse. This task is usually used within a “Condition if” something happened, redefine Time Lapse (e.g. increase frame rate). See Time Lapse for description of the task options.

Time Sequence

Chains acquisition time-phases and phases of different types (temperature / gas control, perfusion control).

Repeat

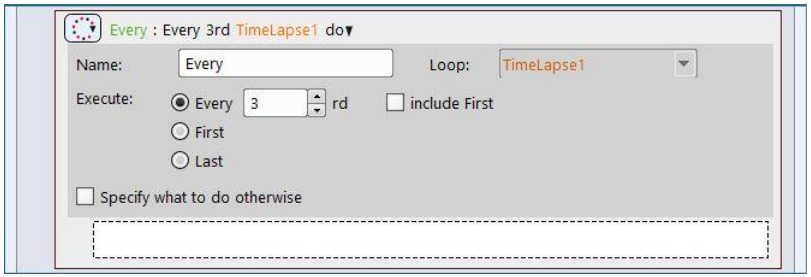
Repeats the contained tasks for the specified number of iterations or indefinitely until Break.



In the repeat checkbox menu choose from the following options: Number of iterations - insert a number to define how many times the tasks will be repeated. Use Undefined number of iterations if the loop will be exited either by the Break task or by the Finish when condition is TRUE option. If using the second option you can specify a conditional expression which will cause the loop to be exited. Click Define... button to specify the conditional expression. See also Using Expressions.

Every Nth

When using repetitive tasks (e.g.: repeats, well loops,...), you can create a new task sequence that is triggered by every first / last / nth loop using the [Every nth] function.



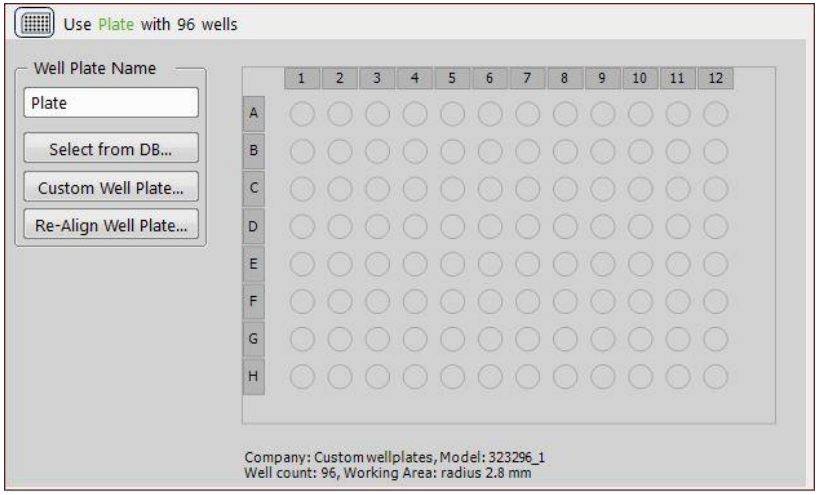
Enter the desired number of the repeated task in the Every field.
The First option executes after the first repetitive task selected.
The Last option executes after the last repetitive task selected.
Choose the loop you would like to modify by this task (only parent loops are available).

SECTION 2

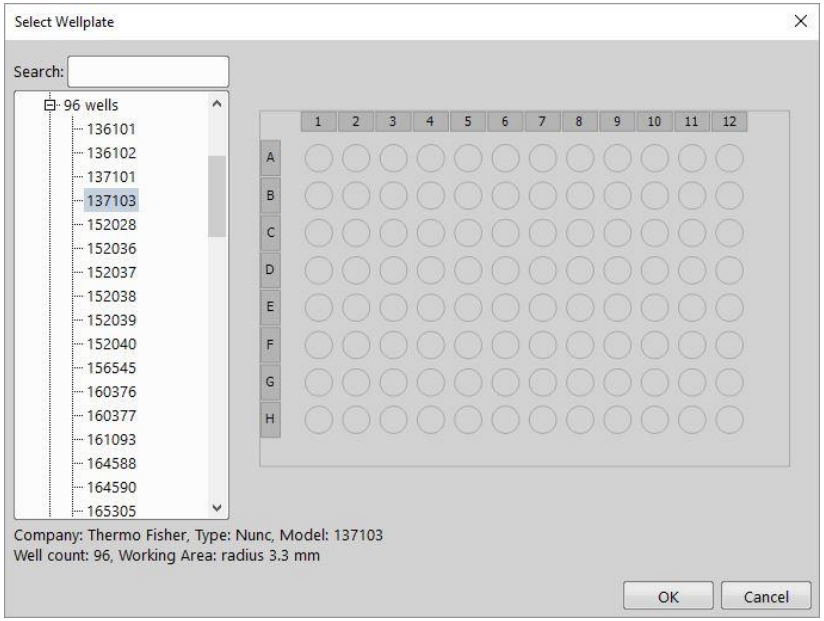
Well Plates

Define Plate

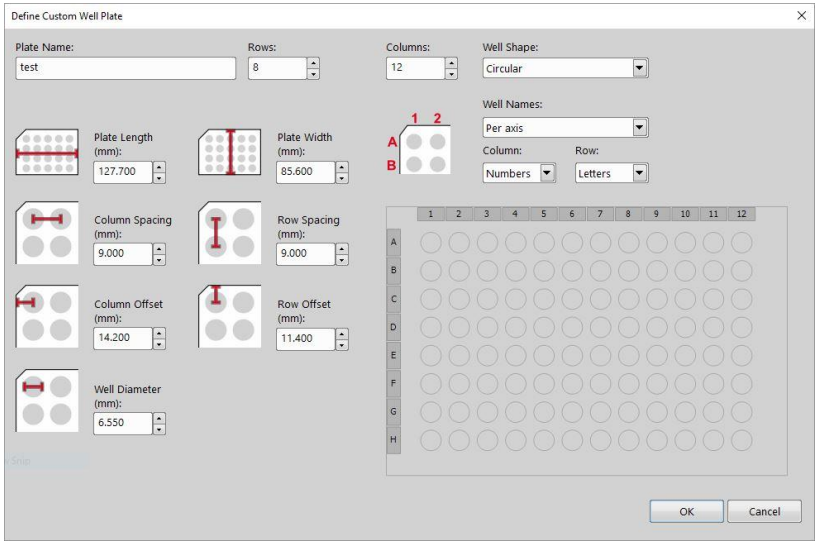
This task specifies shape and size of a well plate as well as the working area of single wells.



Type the name of your well plate and click the Select from DB... button to open the well plate database, where you can find many standardized well plates.

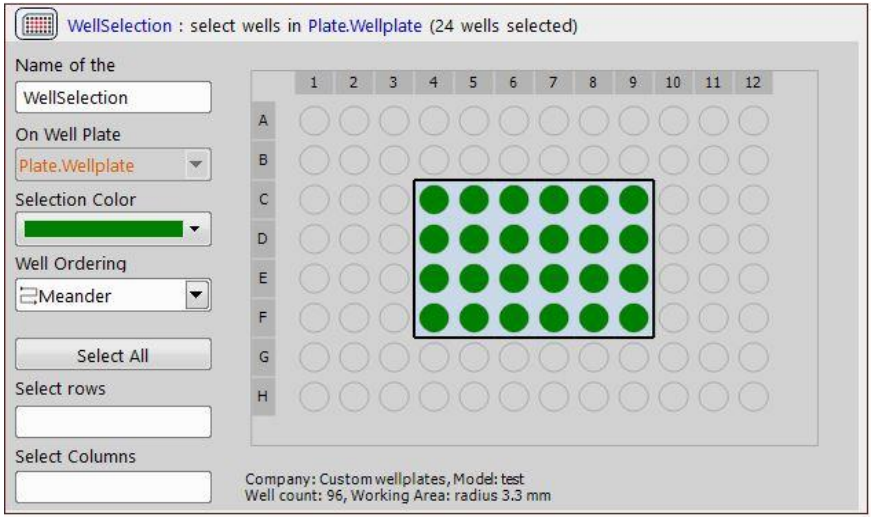


If you can’t find your well in the database you can customize your own well plate by clicking the Custom Well Plate... button. Now adjust all necessary parameters of your nonstandard well plates.

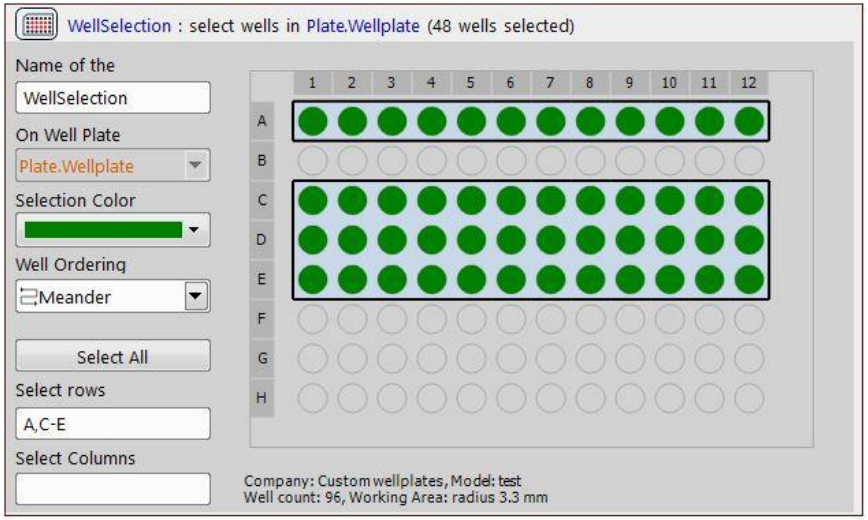


Select Wells

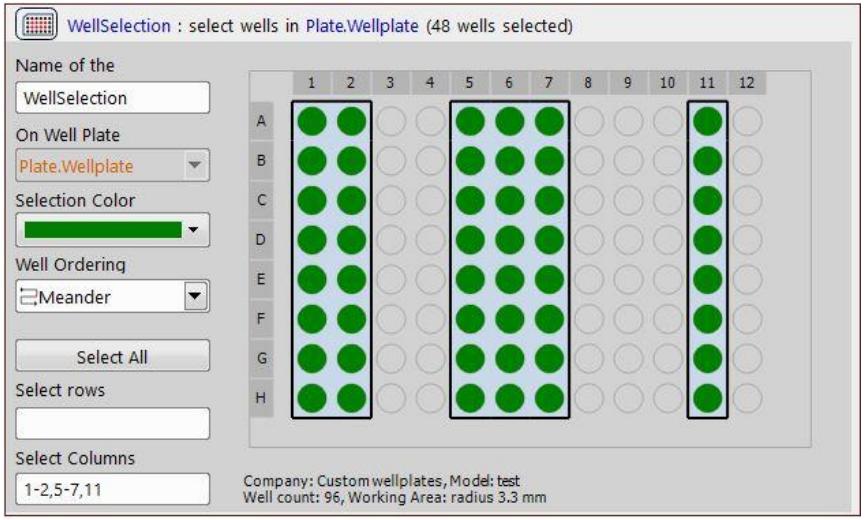
This tool specifies which wells of the selected well plate are actually used in the experiment. Before selecting any wells you need to define the well plate (see Define Plate). After the plate definition, you can select the wells using the Select Wells task. The wells can be easily selected by clicking and dragging over the well board. You can select additional parts of the well plate with Shift + click. If you hold down the control key and drag over wells already selected, you exclude them from your actual selection. You can also select single rows / columns by clicking on their letter / number.



Select Rows



Select Columns



Loop Over Wells

Runs the contained tasks on each well of the selected well selection.

Type the name of your loop and switch between different well selections available within your job. Split storage per well influences how the system stores the captured data on the disk. If checked, separate ND2 file will be created for each well. If you select the Advanced option, the Loop over wells will be performed only on wells with defined class typed in the Loop over wells in class field.

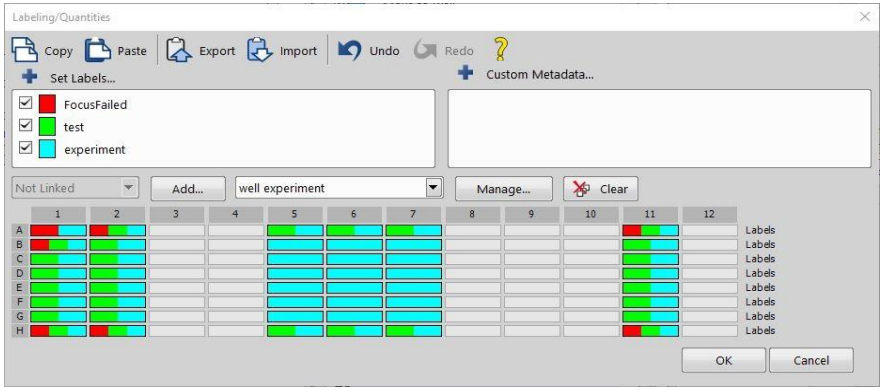
The class number can be assigned using an expression to each well.The Class can be defined in the Expression task - find a parameter named "Class" under Job / Wells / CurrentWell. Insert this expression by double-clicking on it and use given operators to define your class e.g.:
Job.Wells.CurrentWell.Class=2
In our case "2" is the Class we fill into the Loop over wells in class field.

Label Wells

Enables to label, add a description or quantity to each well or a group of wells. If you are well labeling for the first time and don't have any saved labels, start by clicking on the Define... button in the Labeling/Quantities window. Here click on the Select Labels... button and the Labeling Tags window opens.

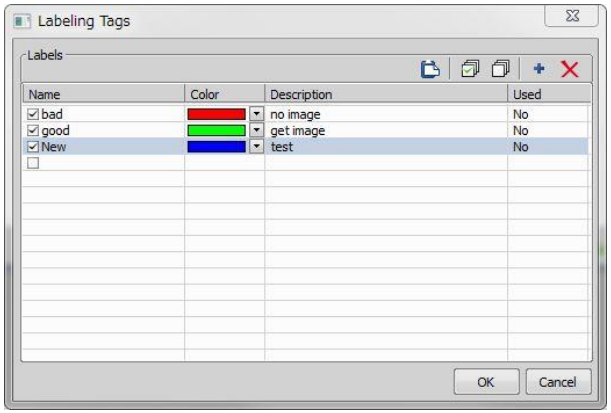
Using the Insert new Keyword button you can add a new label, name it and give it a description.

After setting up your labels and clicking OK you are back in the [Labeling/Quantities] window and ready for the actual labeling. Click (select) the well you want to label and then drag the required labels to the well table. You can use the shift key to select a range of cells or the control key to select remote parts of the well plate. By right-clicking on any cell, you get more selecting options - i.e:. select all, invert all, select row, invert row, select column and invert column. When you are satisfied with your well selection, right-click on one of the selected wells and choose a labeling tag you predefined in the Labeling Tags window. The color of each label can be adjusted in the "Labeling Tag" window. Grey colors in the column "12" presented in the following figure were not selected in the Select Wells.

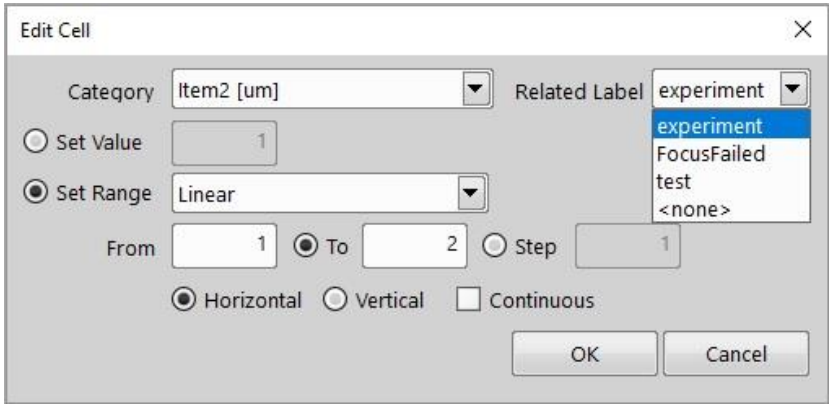


Quantities

Labeling wells with quantity information can be done by clicking the Select Labels... button. The Quantities window appears. Here you can add new quantities, select units and edit the description as discussed when using the Labeling Tags window.

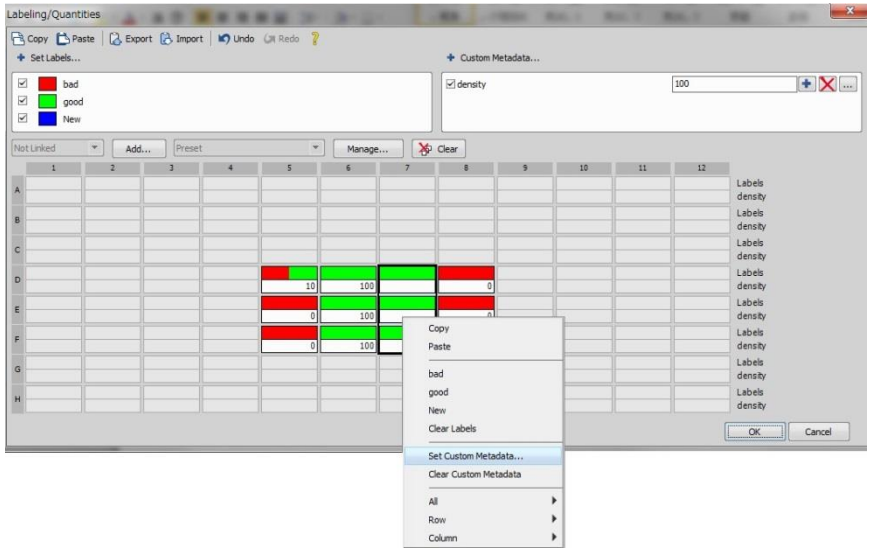


After choosing defining your quantities, click the OK button and select wells with known quantity. Right-clicking on the cell selection and choosing the Set Custom Metadata… you enter the Edit Cell window.



Here you specify the actual number. If you select "Related Label", the same number will be assigned to the related label.

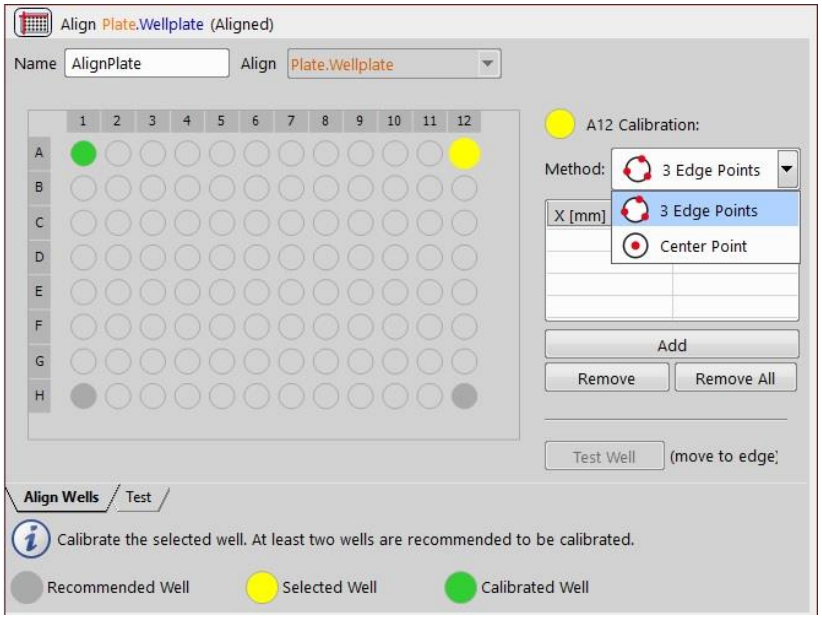
Here you can set the exact numeral value, set a range of values (with steps) and choose the cell counting order. If you choose Horizontal, the cells will be labeled from left to right independently in all rows selected. If you choose Vertical, the cells will be labeled from top to bottom independently in all rows selected. If you check Continuous, all the cells selected will be labeled from left to right and top to bottom.



After labeling your well plate, Click OK in the "Labeling /Quantities" window. In the Label Wells window, press the [Save to file] button to save as an XML file. You can also import your saved labels by clicking the Load from File button. You can always clear the label with the Clear button in the "Labeling / Quantities" window.

Align Plate

This function determines the exact position of a well plate on the XY stage. Typically, this task is run on runtime.

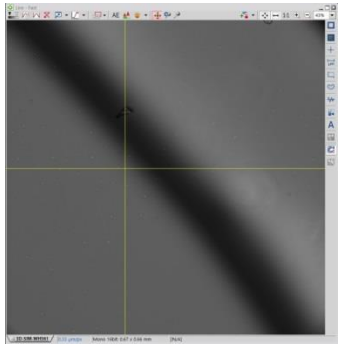


First enter the name of the alignment and select the actual plate with “Align”. Then select "Method" on the screen. This is because it is necessary to accurately define the position of different wells.

- 3Edge Points :
  Determine at least three different boundary positions in the well.
- Center Point:
  Determine the center point in the well.

There are two ways to determine the exact position of the well. The procedure goes as follows:

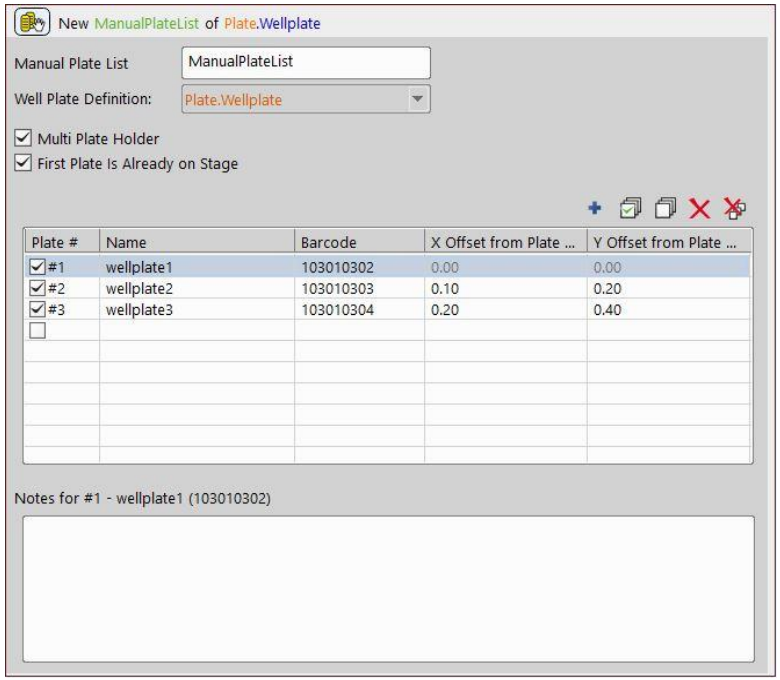
1. Select one of the methods.
2. Display live image from the camera. A cross will be displayed in the image.
3. Move the XY stage so that the significant part of the well (edge) is in the center of the cross.
4. 1 When Center Point is selected: Click the "Set" button.
4. 2 When 3 Edge Points is selected: Click the Add button to add 3 new points to the well alignment table.
5. In case of the 3-point circle, click the Add point button and repeat this for at least 3 points. To ensure good precision, find points on opposite sides of the well.



Example of selecting Edge Points.

Manual Plate List

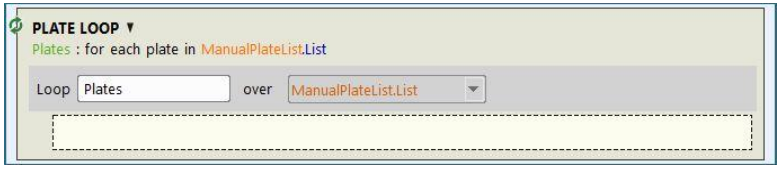
Specifies the list of well plates to be used in a loop over well plates. These well plates are changed manually.



Name your manual plate list and select a well plate to be looped. If using a well plate holder, you can adjust the well plate offset from the first well plate in the table.

Plates

Runs the contained tasks on each well plate of the specified list of plates. The list of plates is defined by the Manual Plate List.



Name your plate loop and choose a plate list from your current job.

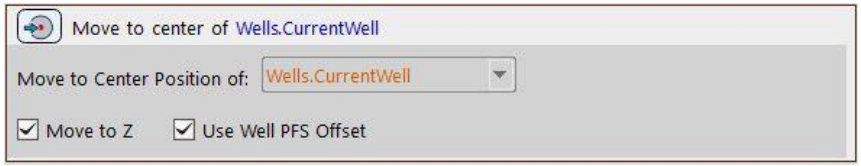
Move to Well

Moves the XY stage to a particular well of the specified well plate.

Name your move task and select a well plate for the move definition. Define a row / column position where to be moved. Last two options tell the system to set the Z drive to a position previously assigned to the current well.

Move to Well Center

Moves the XY stage to the center of the current well. This task can be used with a dish, slide or inside a loop over wells.

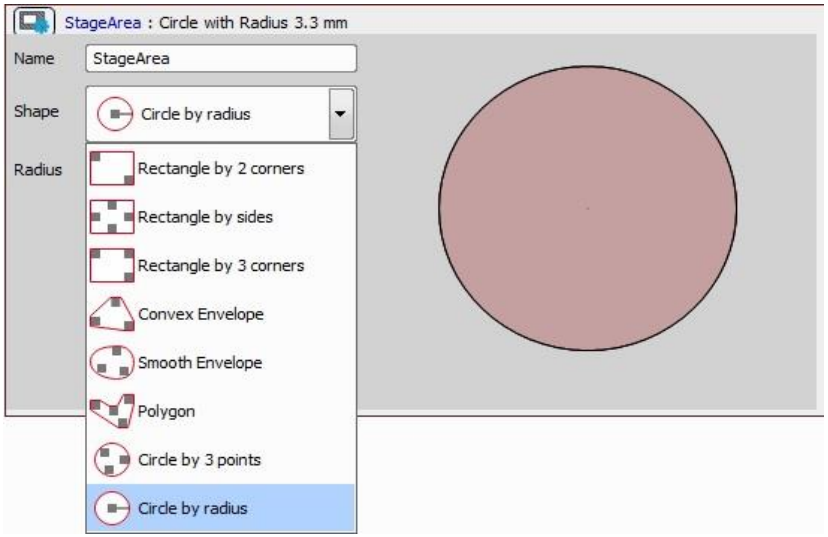


Sample Holder

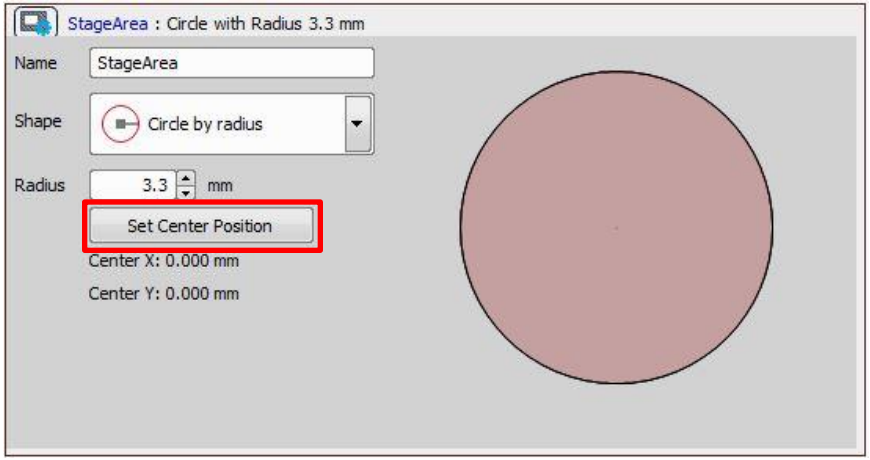
Define Stage Area

You can select the area you want to acquire from 8 modes according to the sample.

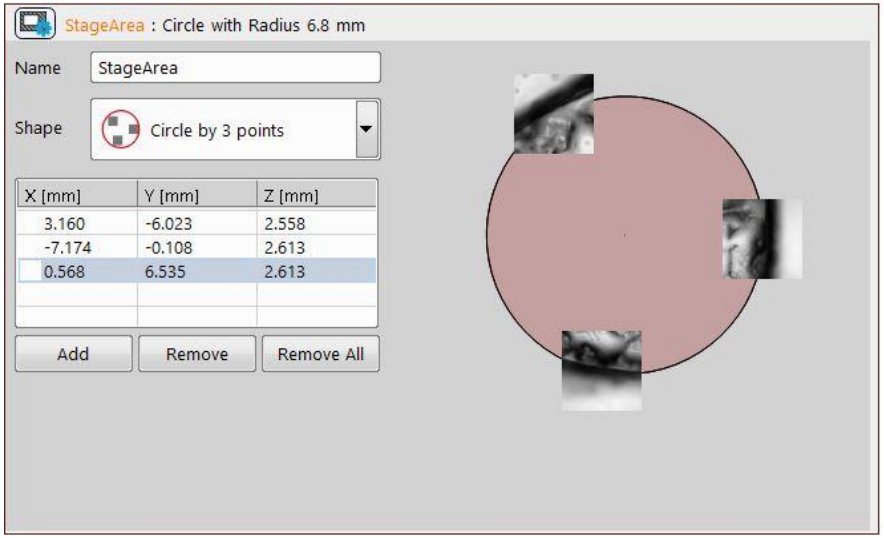
Specifies the shape, size and the working area of a dish.



Here, the size of the dish is defined as an example. In addition to the method of determining the center position, the dish calibration can be performed by registering three points.

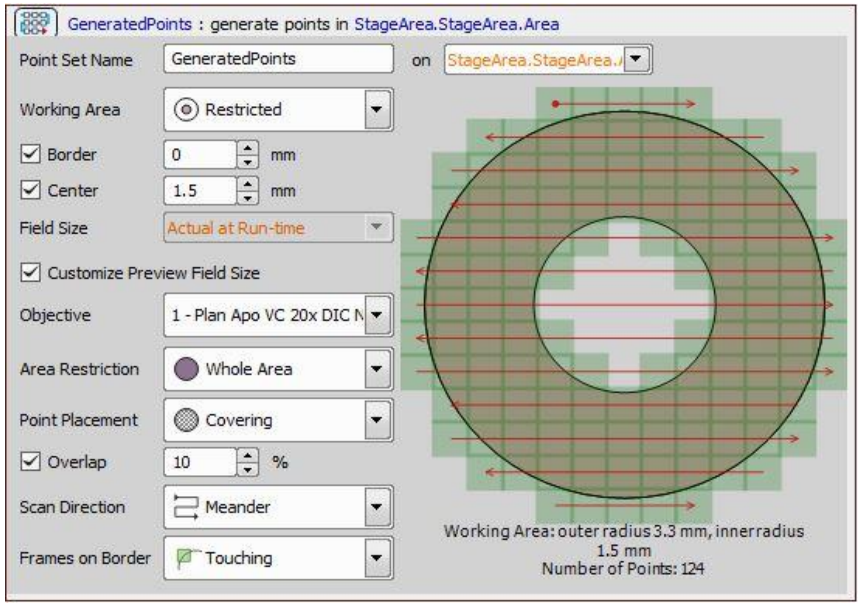


Calibration at center position
(The center position is recorded with “Set Center Position”)



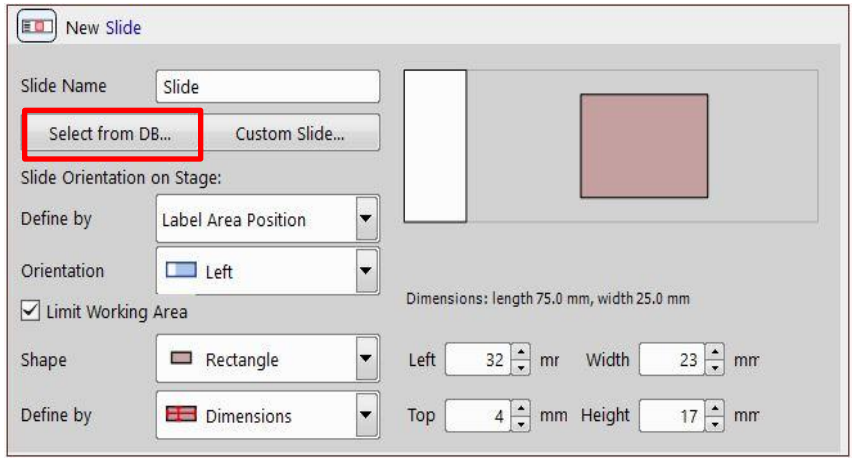
Register 3 points and calibrate.

Next, add a dish name to “Point Set Name” in the Genarate Points function and select a work area. If you want to maximize your working area, select Whole bottom. To restrict your dish working area, select Restricted and enter the Distance from border and / or Exclude center dimensions.

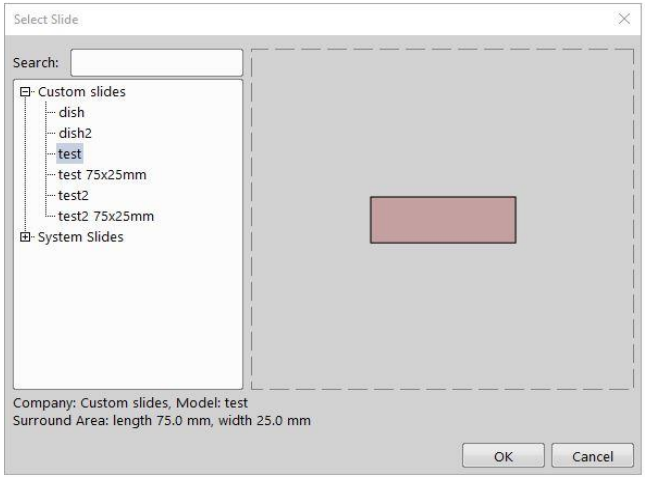


Define Slide

Specifies the shape, size and the working area of a slide.



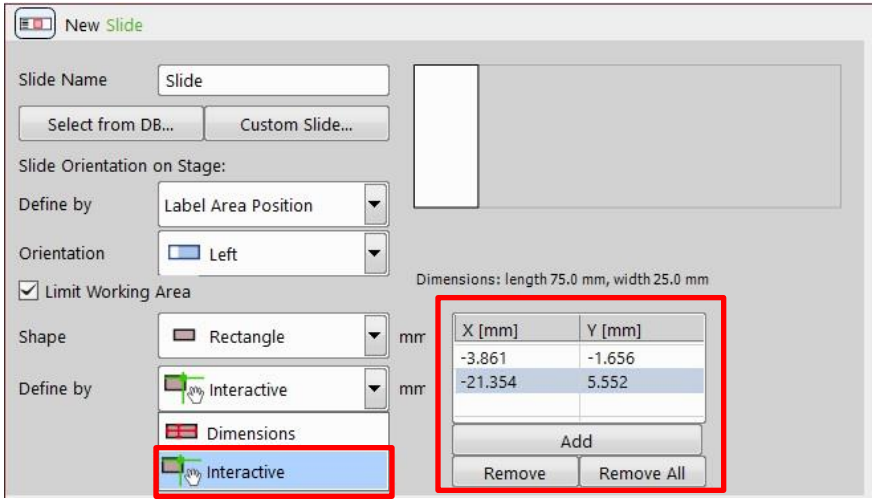
Type the name of your slide and limit its working area if necessary.



Select from DB ... screen

Work area setting

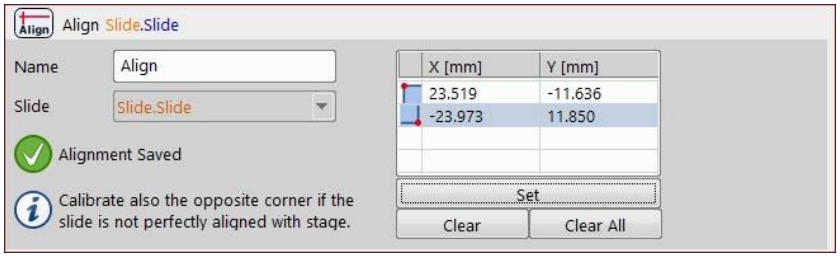
If you want to set the work area, check "Limited Working Area" and set "Define By" to "Interactive" to move the XY stage and specify the shape and size of the area.



Enter the two diagonal XY positions with the "Add" button. In the case of a circle, set the radius or specify 3 points of the area boundary.

Align Slide

This task determines the exact position of the selected sample on the XY stage.

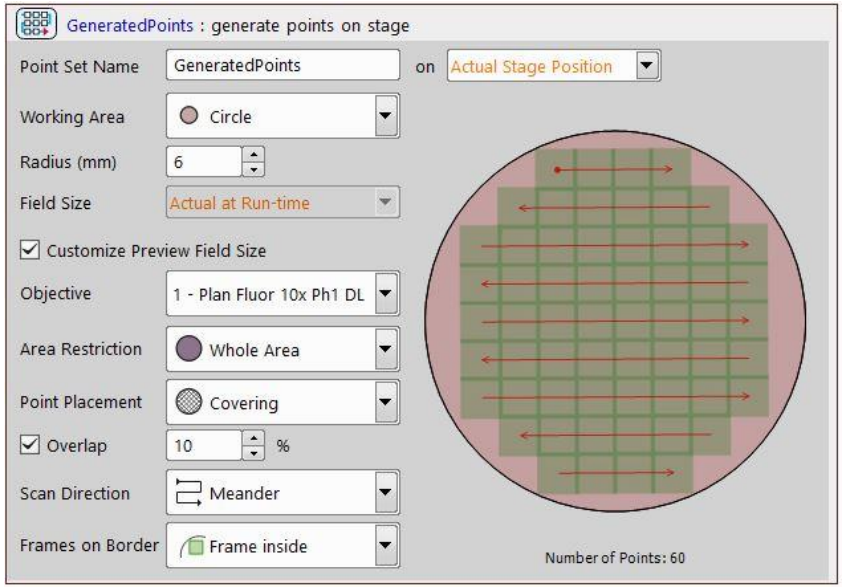


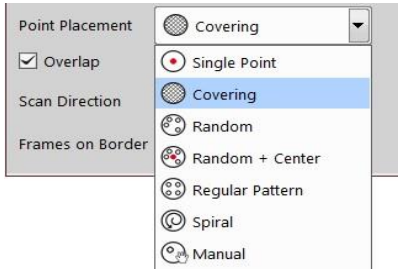
Enter and define XY positions of two diagonal corners in free form.

Stage XY Points

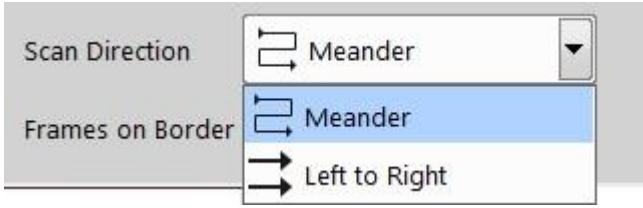
Generate Points

Creates a pattern of XY points on the selected sample.





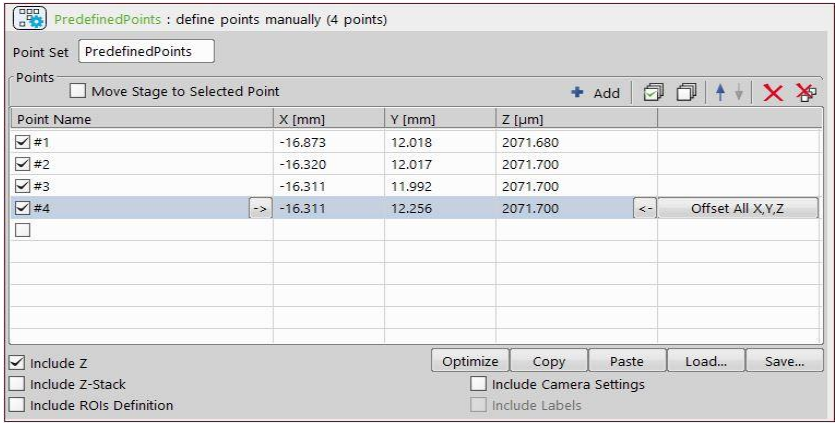
Type the name of your point set and select one of your active well / slides / Stage Area. Choose one of the seven placement methods listed. All of the methods except Single Point enable further settings. At that time, with the Scan Direction function, you can select the scanning method from the predetermined pattern. In this example, it is setting when "Covering" of "Point Placement" is selected.



In the Covering mode you can select the Overlap amount in percent. In "Random" mode, you can set the number of points you want to generate in "Well / Slide / Stage Area" with "Count". The maximum number is indicated in the bracket. In “Frames on Border”, you can use “Frame inside” to make points outside of a single sample holder area. Whenever you use "Always Create New Points", new points will be created. The "Randomize" function generates points randomly.

Predefined Points

Using Predefined Points, you can specify a list of XY points on the selected sample manually in a tabular way.



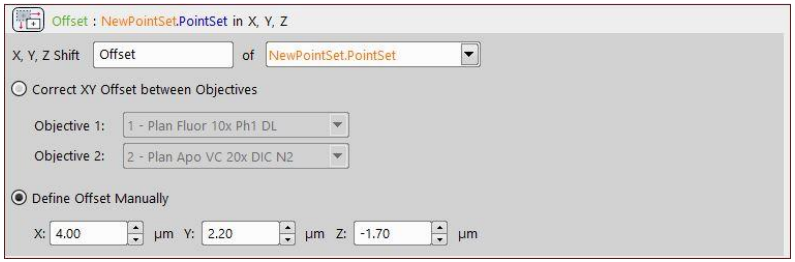
Points

Runs the contained tasks on each XY point of the selected pointset.

Name your point loop and select one of your active point tasks to be looped (Generate Points, Predefined Points, New Point Set). Split storage per point option influences how the system stores the captured data on the disk. If checked, separate ND2 file will be created for each point. Each point can be assigned to a class (by an expression). If you select the Advanced option, the Loop over points in class field appears. Entering a class number applies to the current loop and only executes that class number. Define classification in advance in the "Expression" task.

Offset Point Set

Offsets all XYZ coordinates of the selected point set.



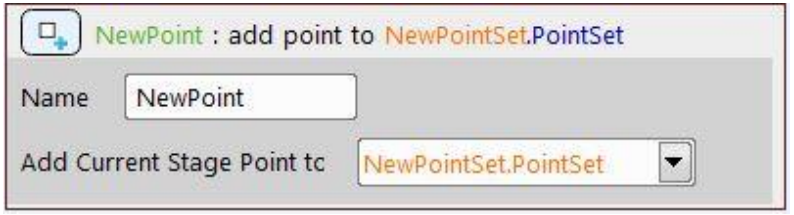
Specify distances of the shift in all three axes.

New Point Set

Creates an empty point set. Points shall be added to it later either by Add Point or Add/Edit Points Manually.

Add Point to Point Set

Adds a named XY point to the selected point set. Current XY(Z) position is used.



Select the point set you want to add in “Add Current Stage Point to”.

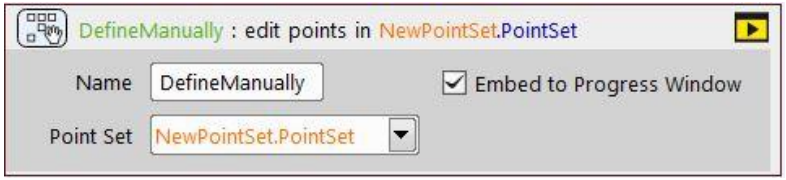
Remove Point from Point Set

Removes the active XY point from the selected point set. For example, you can use this task with “Loop over Points” to remove points that might be useful for further analysis.(e.g. do not produce any image signal).

Select an active point loop from which your point will be removed.

Add/Edit Points Manually

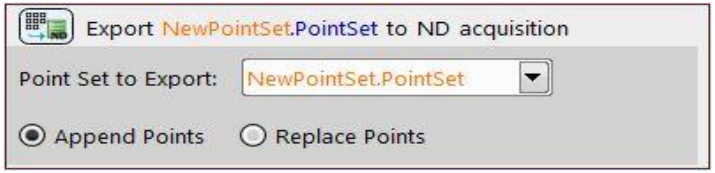
Waits for the user to add or edit points to the selected point set manually during the runtime.



If "Embed to progress window" is checked, the Edit Points window will be inserted at the bottom of the JOB execution window. You can also select the points you want to edit manually.

Export Point Set to ND

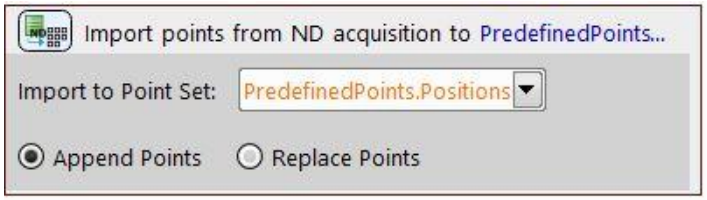
Exports the selected point set definition(Generate Points, Predefined Points, new Point Set, etc.) to the ND Acquisition window.



Select the point set you want to export to the XY tab of the ND Acquisition window. Append points function adds new generated points to the the XY tab of the ND Acquisition window right behind already existing points. You can also use "Replace Points" to replace all existing points in the XY tab of the ND Acquisition window with newly created points.

Import Point Set from ND

Imports the multi point definition from the ND Acquisition window to the selected point set. "Replace Points" will overwrite the existing point set settings.



Select a point set to which data from the ND Acquisition window will be imported. Append points adds newly generated points to your selected point set behind already existing points.

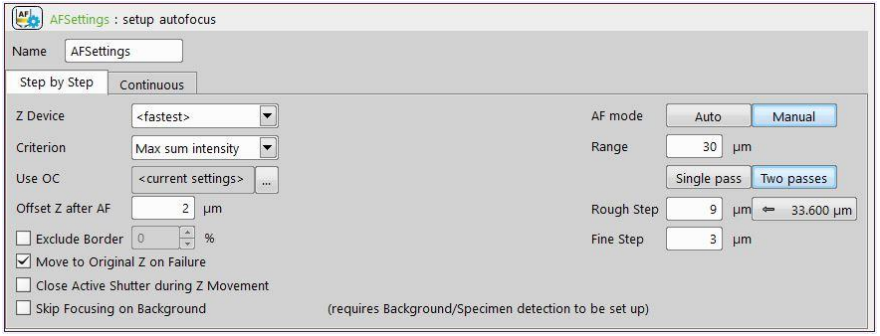
SECTION 5

Z + Piezo Z

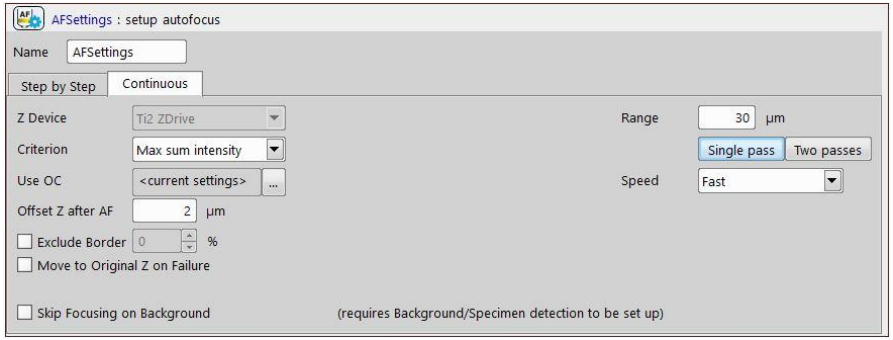
Define Auto Focus

Defines Z device settings to be used by the Auto Focus task. Different AF settings may be defined for different purposes. Select a method you will use:

1.Step by Step
This method moves the Z drive in steps within the defined range, captures images and evaluates their focus criterion. The Z position with the best focus criterion is used as focused.



2.Continuous
This method is similar to Step by Step except that in this case, the step size depends on the camera frame rate. The camera takes pictures as fast as possible while the Z drive is moving within the defined range.



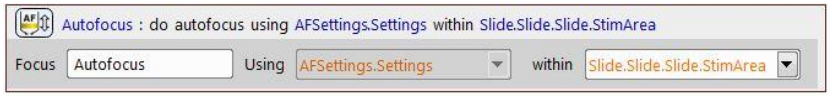
Name your autofocus setting. Next,Choose the Z drive to be used for focusing (two-Z drives systems are quite common). Criterion defines which focus criterion is used:

- Brightfield - standard contrast based criterion.
- Fluorescence - suitable for fluorescence microscopy.
- Max sum intensity - criterion based only on the intensity values. Can be useful in confocal microscopy.
- Yeast, Bacteria (Ph): criteria optimized for yeast under phase contrast microscopy.

Then select the optical configuration (OC) to be used for focusing. Automatic focus mode requires only the range. The system calculates optimal step and whether 1 pass or 2 passes will be performed. Manual focusing mode enables further settings.
If 1 pass is selected, Step size is set, and if 2 pass, “Rough Step” is also set.

Auto Focus

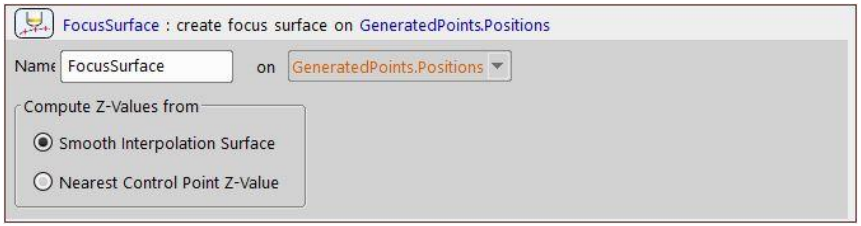
Performs autofocus using the selected Auto Focus settings defined in Auto Focus Setting.



Name "Focus" and select the required setting in "Using" or "within."

Create Focus Surface

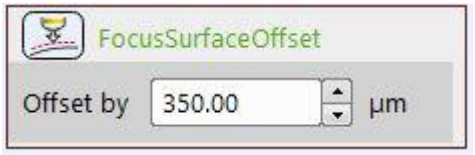
Calculate the focus surface from the existing Z position. The Z position must be predetermined.



Name your focus surface and select the source Z point set from which the surface will be calculated. Any position on the resulting surface is a result of interpolation.

Offset Focus Surface

Modifies an existing Focus Surface by adding / subtracting an offset distance. You can use the focus surface offset on each point for pre-focus, check every N-th point whether the focus has not shifted. If it has, shift (offset) the whole focus surface to match the current focus.



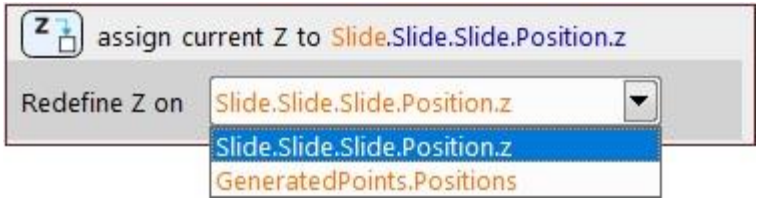
Offset your focus surface by a defined numeric value.

Move to Focus Surface

Moves the Z drive to the position defined by the Create Focus Surface task (or Offset Focus Surface). Use it on each XY position within a point loop to get the image into focus (or prefocus).

Assign Current Z to Point/Well /Point Set

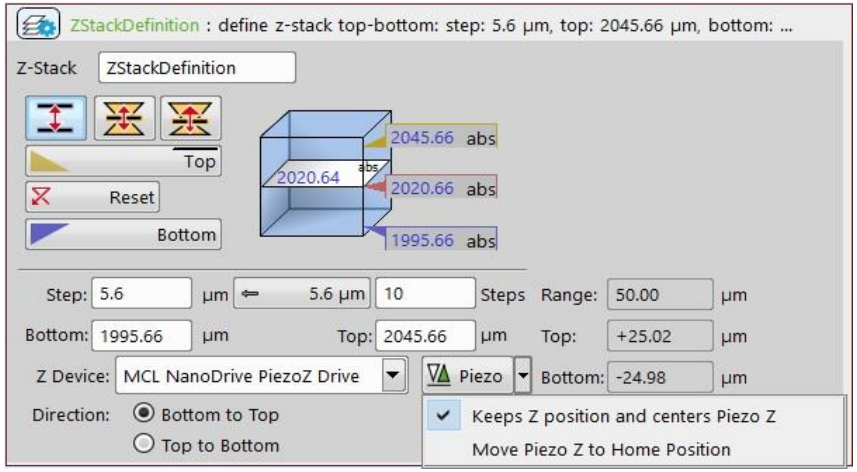
Assigns the current Z position to the selected XY position of a sample. You can use this task inside a loop after Auto Focus to pre-define Z positions for fast scanning.



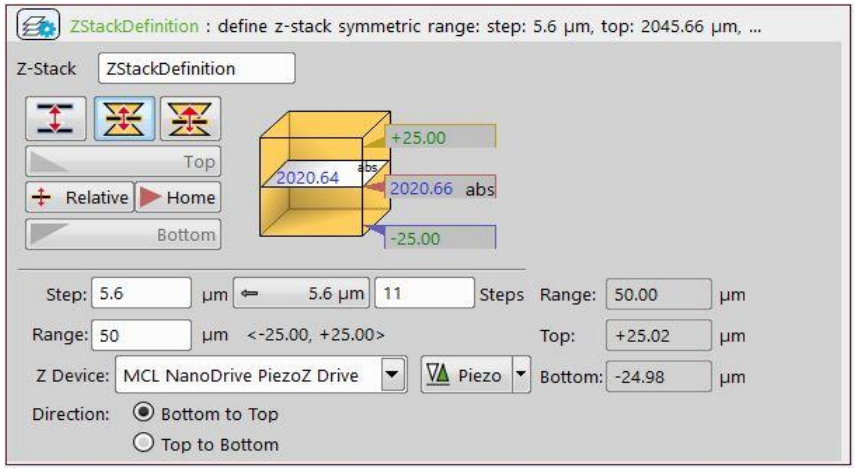
Select a sample holder on which you want to set the current Z position.

Define Z-Stack

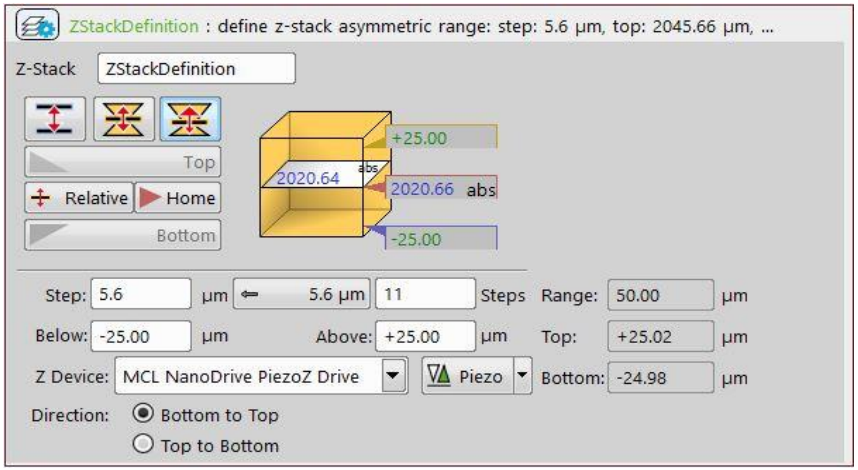
Specifies the distances and other parameters for scanning by the Define Z-Stack task. When scanning thick samples, set the focused image as a home position and define the Z - range. Z-stack can then be used to create all-infocus images or 3D models regardless of your depth of field parameters.



Defined by top bottom mode.



Symmetric mode defined by range .



Asymmetric mode defined by range.

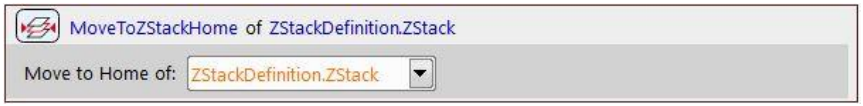
Z-Stack Loop

Repeats the contained tasks for each Z position of the selected Z Stack. This task can be used inside any other loop (timelapse, point loop) with Capture. It captures Z stacks to extend spatial resolution of the image sequence.

Name your Z-Stack loop and select an existing Z-Stack definition (Define Z-Stack).

Move to Z stack home

Moves the Z drive to the central position of the selected Z Stack Definition. When capturing multi-channel Z-stacks, not all the channels are required to contain the Z dimension. These are usually captured in the home / center position.



Choose your Z-stack definition from your current job.

PFS

PFS On and Focus

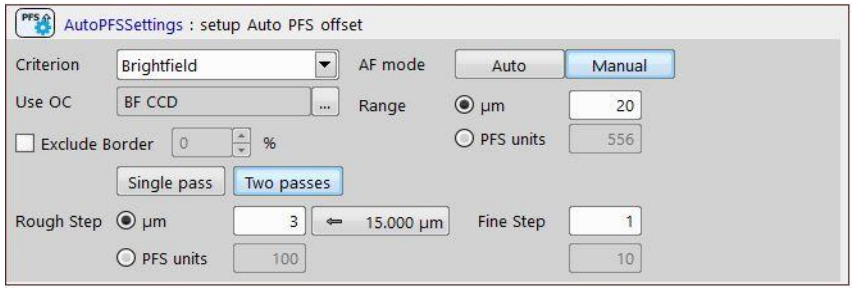
Turns the Perfect Focus System on and tries to find the PFS focal plane. Make sure the Z drive focal plane is not too far from the PFS focal plane otherwise the task may fail.

PFS Off

This task switches the PFS Off.

Auto PFS Focus Setup

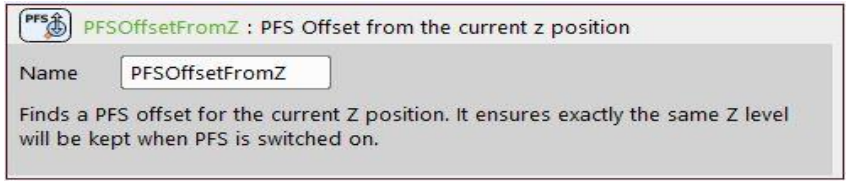
This task defines settings to be used by the Auto PFS Focus task. Select the optical configuration to be used. As with autofocus, areas are defined in automatic mode or in manual mode either single pass or two pass.



Select the focus criterion based on the nature of your specimen.

PFS Offset form the current z position/ Assign PFS to Point/Well

The current Z position is specified as the PFS offset amount. (Eg in one well)
It can be used in "Loop over Points" after Auto PFS focus.

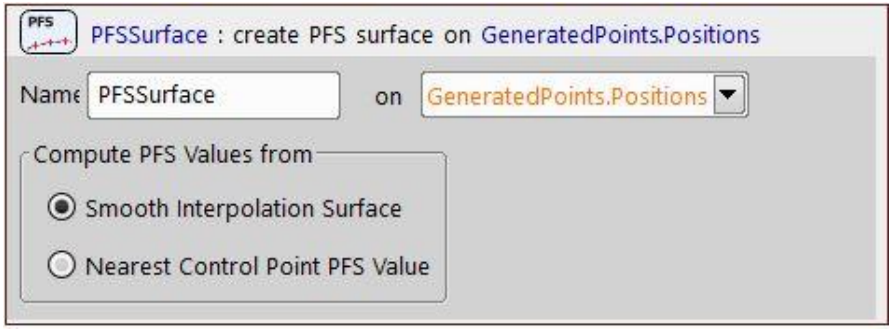


Register the current PFS Offset with “Assign PFS to Point / Well”.

How to use:
Calculate and register the appropriate PFS Offset amount in the first loop, apply it in the subsequent loops, and capture.

Create PFS Surface

Calculates the global PFS surface from an existing set of PFS positions. To XY positions without the PFS position defined, the PFS position of the closest XY point (which includes PFS position) is copied.



Move to PFS Surface

Changes the current PFS offset value to match the calculated PFS Surface.

Offset PFS Surface

Modifies an existing PFS Surface by adding / subtracting an offset distance.

Auto PFS Focus

The PFS system searches for the most in-focus plane and sets the PFS offset accordingly. This task uses settings defined by the Auto PFS Focus Setup task.

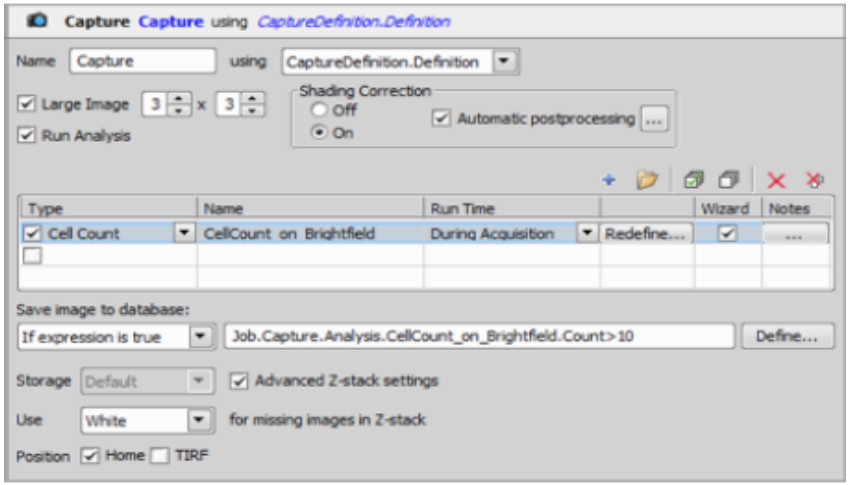
Acquisition

Capture Definition

This task defines the single-channel or multi-channel acquisition settings. Select an optical configuration to be used to capture each channel. In addition user can control active shutter individually for each channel. Check "Close Act. Shutter“ check box to minimize bleaching or uncheck it to leave the shutter opened and thus speed up the acquisition. Sometimes when using multiple channels for acquisition, not all channels have the same Z positions with the best focus. Using one channel as reference, user can specify focus offsets for individual channels. Therefore all channels are captured in focus automatically.

Capture

Performs acquisition according to the selected Capture Definition. Large images can be acquired and an analysis can be run upon capture.



Name your capture task and select a capture definition previously set in the Capture Definition task.

Large image is acquired as a series of overlapping images taken one by one in regular grid of given dimensions (3 by 3 in previous picture). Overlap is 10%. Images are than stitched together into one big image.
Select "Always" in "Save Image to Database" to save the image in Database. Also, by selecting the Storage task, specification of the save destination folder becomes available.
Advanced Z-stack settings is useful when combining multiple captured images together. Not all frames have to be captured in the image file. For example: Capture Z-stack with Capture1 and than capture only home position with Capture2. Images from Capture2 will be missing in other than home position of the Z-stack. You can use a color for missing images in Z-stack.
Select black for missing images to be black, similarly select white. Select duplicate to duplicate captured image to all missing images in Z-stack.

ND Acquisition

Acquires an ND2 image according to the definition specified within the task.

Sequential Stimulation

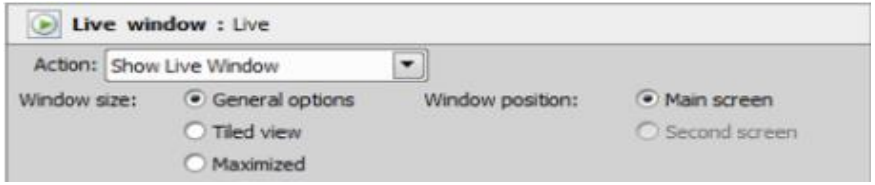
Chains acquisition time-phases and stimulation / bleaching / waiting phases.

Simultaneous Stimulation

Runs a time-lapse acquisition and stimulation simultaneously.

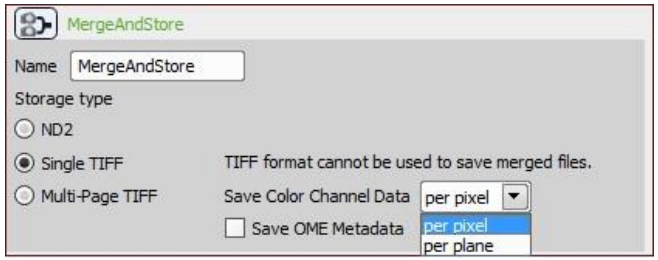
Live Window

In this task, you can "Show the Live window" / "Close" / "freezes ". Use this tool whenever a preview of what is happening under the microscope is needed.



Marge And Store Captured Images

Define in advance in "Marge And Store Captured Images", and specify where to save the image in the Capture task. Recommended (default) is ND2 format. (If possible, the system will try to merge into a single file.) The save format is ND2. If your image is to be processed by other software, set it to TIFF (single or multi page) format.

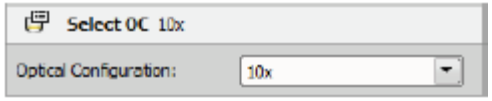


“Save color channel data” enables choosing the color channel storage method - Pixel or Plane. This options may be useful for advanced users who plan to further process the images in other applications (such as MatLab). You can ignore this if you use NIS-Elements AR only.

Optical Configurations

Select OC

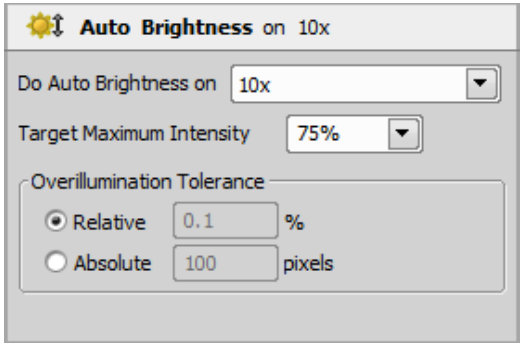
Switches the system to the selected optical configuration. You can make arbitrary changes on automated devices such as changing the camera settings, switching objectives, etc...



Choose one of your optical configurations to be activated.

Auto Brightness

Automatically adjusts the brightness settings (exposure, gain...) on the selected Optical Configuration.



Target Maximum Intensity determines intensity of the lightest pixel in the resulting image. It is computed as a percentage of the camera bit-depth. For 8-bit cameras, the Target Maximum Intensity of 75% would be 255 x 0.75 ~ 191.

Overillumination Tolerance specifies the number of pixels which will be ignored when computing the Target maximum intensity. For example, if your camera has one or more defective pixels which gives constantly the maximum value (white), these pixels shall be excluded from the calculation. Define the tolerance either as a percentage of all pixels (Relative) or Absolutely by specifying their number.

Close Active Shutter

Closes the shutter specified within the task. Select one of the shutters available in the system.

Open Active Shutter

Opens the shutter specified within the task. Select one of the shutters available in the system.

System

Macro

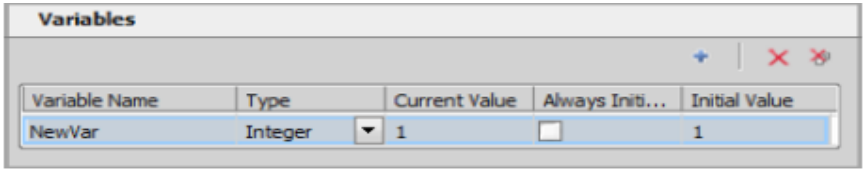
This task enables the user to write and run a macro. The whole set of available macro-commands can be used. Write sophisticated C-like scripts for tasks which cannot be done by the Job or reuse your existing macro.

Expression

Assigns values to variables available within the job. It enables to modify settings of any tasks, manipulate motorized devices, etc. For more information about working with expressions please see Using Expressions.

Variables

Declares global variables within NIS-Elements.



Add a new variable to the Variables list and select its numeric type. You can choose between: Integer (integral number), Double (real number), String (sequence of characters). Current value assigns a value to the variable within this task or within Expression. Each variable keeps its value after the job is finished. If "Always Initiate" is checked, the value of the variable is reset with the value of "Initial value" at the start of the job.

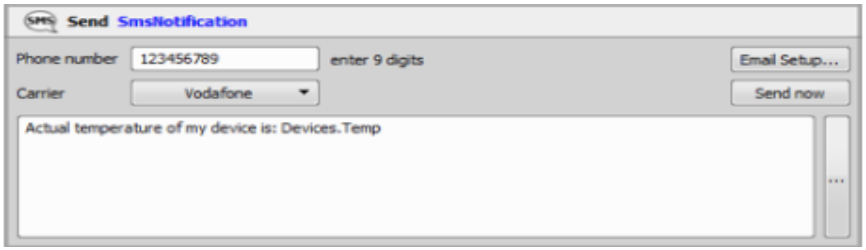
Send E-mail Notification

Sends an email to the specified address. SMTP server configuration (within the task) must be correct. You can send results of the experiment, messages about the experiment progress (failed, finished, in progress,...), etc.

The ... button on the right side adjacent to the text box enables inserting Job variables. You can also use your custom variables defined in the Variables task.
Examples: Notification about the Job experiment end, notification to exchange well plates, notification that auto focus failed, etc.

Send SMS Notification

Sends a text message to the specified phone number if the particular mobile phone operator provides such services (sending SMS over email).

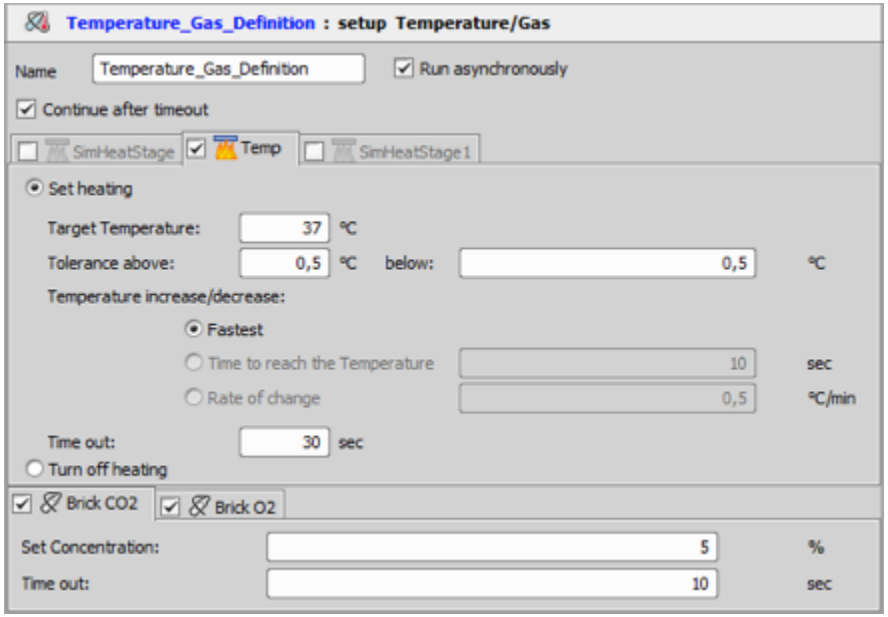


Type the recipient's phone number and select the local mobile operator (Carrier). The ... button on the right side adjacent to the text box enables inserting Job variables. You can also use your custom variables defined in the Variables task.

Device Control

Temperature and Gas Control

Sets the target temperature and gas concentration.



Name your Temperature and Gas Control task. If you check Run asynchronously, the subsequent tasks will be run immediately - the system will not wait till the time-out is reached or the temperature/concentration achieved. Continue after timeout overrides the Timeout settings which are ignored. The task waits until the defined temperature/concentration is reached. Check Set heating if you want to adjust the temperature. Set the target temperature of your selected device and enter the tolerance interval above and below your Target Temperature. If any temperature within this interval is reached, the system considers the task to be finished successfully.

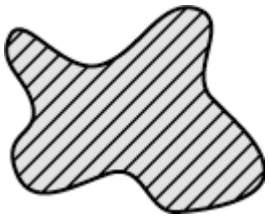
Temperature increase/decrease enables adjusting the speed of heating. Three options are available: Fastest, Time to reach the Temperature and Rate of change (choose a change rate in degrees per minute). Only some devices support this feature. Time out specifies the time given to reach the target temperature. If the device does not reach it in time, the job will continue nevertheless. If the temperature is reached before the time-out, the job will continue immediately. Turn off heating holds the temperature of the device within the given tolerance once the task reaches a specified temperature. Select this option if you want to turn the heating off in another part of the job. Set the target Concentration of the selected gas and specify the Time out - time given to the task to reach the concentration.

Calculation method of feature quantity (reference material)

Area (feature)

Area is a principal size criterion. In a non-calibrated system, it expresses the number of pixels; in a calibrated one, it expresses the real area.

Measurement Type: object, field, manual
Image Type: binary



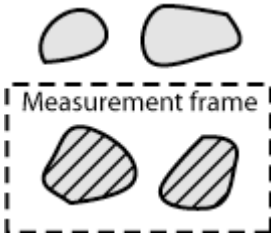
Related Glossary Terms
AreaFraction (feature), EqDiameter (feature)

AreaFraction (feature)

AreaFraction is the ratio of the segmented image area and the MeasuredArea. It has a strong stereological interpretation: in the case of isotropic uniform random sections it is equal to the volume fraction.

AreaFraction = Area/MeasuredArea

Measurement Type: field
Image Type: binary



Related Glossary Terms
Area (feature)

Circularity (feature)

Circularity equals to 1 only for circles; all other shapes are characterized by circularity smaller than 1. It is a derived shape measure, calculated from the area and perimeter.
This feature is useful for examining shape characteristics.

Circularity = 4* π *Area/Perimeter2

Measurement Type: object
Image Type: binary

Elongation (feature)

Elongation is determined as a ratio of MaxFeret and MinFeret features. This feature is useful for shape characteristics.

Elongation = MaxFeret/MinFeret
Measurement Type: object
Image Type: binary

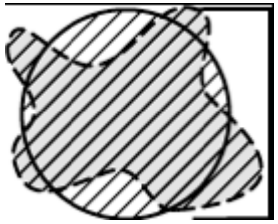
EqDiameter (feature)

Abbreviation for the measurement object feature “Equivalent Diameter”. It is calculated
from object Area as if the object was perfect circle.

$$EqDiameter = 2\sqrt{\frac{Area}{\pi}}$$

In many circumstances it is better than quadratic Area.

Measurement Type: object,manual
Image Type: binary



Related Glossary Terms
Area(feature)

Job

Sophisticated tool for creating and managing automated procedures composed of tasks.

Related Glossary Terms
Task

MaxFeret (feature)

The MaxFeret is the maximal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle α equals the projected length of an object at angle
α, α (0, 180); NIS-Elements AR calculates Feret's diameter for α =0, 10, 20, 30, ..., 180.

Measurement Type: object, manual
Image Type: binary

Related Glossary Terms
MinFeret (feature)

Mean Intensity (feature)

MeanIntensity value is derived from the intensity histogram. It is the usual statistical mean of intensity values of pixels.

Measurement Type: object, field, manual
Image Type: color/binary

Measurement features

Elements that can be measured within NIS-Elements AR software.

MinFeret (feature)

The MinFeret value is the minimal value of the set of Feret's diameters. Generally (for convex objects), Feret's diameter at angle α equals the projected length of an object at angle α, α (0, 180); NIS-Elements AR calculates Feret's diameter for α =0, 10, 20, 30, ..., 180.

Measurement Type: object, manual
Image Type: binary

Related Glossary Terms
MaxFeret (feature)

Task

Tasks substitute selected functions of the connected devices in accordance with the software. Ordered in a sequence they create a Job procedure.

* Please refer to NIS Elements HELP for details.

Related Glossary Terms
Job