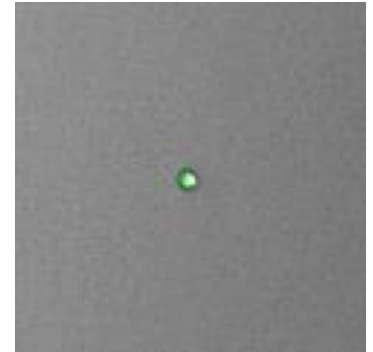


## Experiment Guide – FASCC Fluorescence Activated Single Cell Cloning

The purpose of this document is to guide the user through the experiment setup for Fluorescence Activated Single Cell Cloning (FASCC). It does not contain any procedure of setting up a Fluorescence staining assay itself, i.e. utilizing CellTracker™ or Calcein and seeding the cells into a plate in an appropriate dilution, since this may vary due to customer requirements and internal procedures.



**Technical Note**  
TN-B123-XVII-12

This experiment guide assumes that the user is familiar with running a normal application with the YT-software.

### Content

1. Materials & Procedures .....	2
1.1 Material .....	3
1.1.1 Staining of the Cells .....	3
1.1.2 Sample Carrier.....	3
1.1.3 Centrifuge.....	3
1.2 Procedure.....	3
2. Experiment Setup .....	5
2.1 Experiment Design.....	5
2.1.1 Levels in FASCC Experiments.....	6
2.1.2 Autostart Nanoview.....	7
2.1.3 Sub ROI Creation.....	7
3. The Pre-Scan Measurement.....	8
3.1 Optical Setup and Preparation Pre-Scan.....	8
3.2 Measurement Pre-Scan .....	9
3.3 Evaluation of Pre-Scan .....	10
3.3.1 Result Table in Analyst .....	10
3.3.2 ROI Filter Settings .....	11

4.	The Nanoview Measurement.....	12
4.1	Optical Setup and Preparation Nanoview.....	12
4.1.1	Preparation Nanoview Measurement.....	13
4.1.2	Measurement Nanoview.....	14
4.1.3	Evaluation Nanoview.....	15
5.	The Confluence Measurement.....	15
5.1	Optical Setup and Preparation Confluence.....	15
5.2	Measurement Confluence.....	17
5.3	Evaluation Confluence.....	18
6.	Operator Explanation and Setting for FASCC.....	20
6.1	General Working Principles of FASCC Operators.....	20
6.1.1	Fluorescence Evaluation.....	20
6.1.2	Brightfield Evaluation.....	21
6.2	Show Cases for FASCC Pre Operator.....	21
6.2.1	Too Many Objects Found in Cluster Analysis.....	21
6.2.2	Object Could Not Be Found in Pre-Scan.....	22
6.2.3	Eliminating Lint or Debris.....	23
6.3	Show Cases for FASCC Nanoview Operator.....	24
6.3.1	Erratically Identified Cell Doublet.....	24
6.3.2	Hard to Identify Cell Doublet.....	25
7.	Making a Template for FASCC.....	26
7.1	Template Preparation for FASCC.....	26

## 1. Materials & Procedures

This chapter lists the requirements for setting up the NYONE® or CELLAVISTA® for FASCC: As already mentioned the kind of equipment may vary on individual user requirements. The following items are a suggestion, which have shown to achieve reliable results.

## 1.1 Material

### 1.1.1 Staining of the Cells

For the appropriate staining different kits are commercially available. The following two are the most common and have been successfully used on NYONE® and CELLAVISTA®.

- CellTracker™ (available for different wavelengths and functions)
- Calcein AM

### 1.1.2 Sample Carrier

It is required to use sample carriers with black walls. The format may vary from any number according to the SBS standard. Typical formats are 96- and 384-well plates in standard and half volume size. It should be checked, whether the adhesives of the plates generate autofluorescence at the rims. Depending on the signal strength this might disturb the analysis.

Handling of the sample carrier: The clear bottom of the sample carrier must never be touched with fingers (not even with gloves) at any time before measurement. Place your thumb and fingers at the rim on the longer side of the plate. The transparent bottom is part of the optical path and may lead to erratic measurements if stained with finger prints or dirt.

### 1.1.3 Centrifuge

To ensure that all cells are at the bottom of the sample carrier (e.g. a 384-well plate) we recommend to spin down the cells. A swing out rotor is required to guarantee a fast sedimentation process with uniform distribution of the cells. Setup the centrifuge to ca. 30 x g and 1 minute spinning time. Furthermore, it is required to use acceleration and deceleration (centrifuge brake) at maximum to optimize uniform distribution. Please Note: These centrifugation parameters have to be evaluated by the user in order to reduce ghost wells, it might be necessary to significantly increase the centrifugational force, depending on the cells and mediums densities.

## 1.2 Procedure

In general, you should refer to the standard protocol of the assay provider.

Hereafter you will find a protocol which should work.

An example for CellTracker™ could be:

- Mix well 0.5 µL of CellTracker™ (10 mM) with 800 µL of serum-free medium and 200 µL of your cell suspension
- Incubate at 37 °C for 30 min
- Spin the cells down at 600 x g for 5 min
- Wash the cells and re-suspend with 1 mL of PBS<sup>-</sup>
- Spin the cells down at 600 x g for 5 min
- Wash the cells and re-suspend with 1 mL of PBS<sup>-</sup>
- Spin the cells down at 600 x g for 5 min
- Re-suspend with 1 mL of media and serum

According to your dilution factor mix media with your cell suspension and distribute it into your sample carrier with approximately 0.5 cells per well.

An example for Calcein with adherent cells could be:

1. Aspirate culture medium
2. Wash cells with PBS<sup>-</sup>
3. Aspirate PBS
4. Detach cells with Trypsin
5. Suspend cells in 5 mL serum-free medium
6. Mix 1 mL cell suspension with 1 mL serum-free medium and add 0.1 µL Calcein stock solution ( $\pm$  0.1 µM)
7. Incubate cells 30 min at 37 °C in the incubator on a slowly rotating device (30 rpm) or invert tube 3-4 times during incubation

*Protect stained cells from light during the following steps!*

8. Centrifuge cells 5 min at 300 x g
9. Aspirate supernatant
10. Re-suspend cells in 1 mL serum-free medium
11. Count cells with the suspension cell count operator of the YT-software
12. Calculate how to dilute the suspension to obtain 0.5 cells per well in e.g. 40 µL medium (depending on the well format you are using)
13. Dilute cell suspension in normal growth medium
14. Seed cells into the microplate e.g. 40 µL/well (for optimizing the optical settings seed more cells into one well e.g. A1)
15. Centrifuge plate 3 min at 200 x g to spin down the cells and to remove air bubbles
16. Image cells shortly after staining (30 min to 3 h, depending on staining concentration)

Any rough handling of the filled plate before or after centrifugation may result in inhomogeneous distribution of the cells and should be avoided!

## 2. Experiment Setup

Click on the FASCC icon to start a new FASCC experiment.

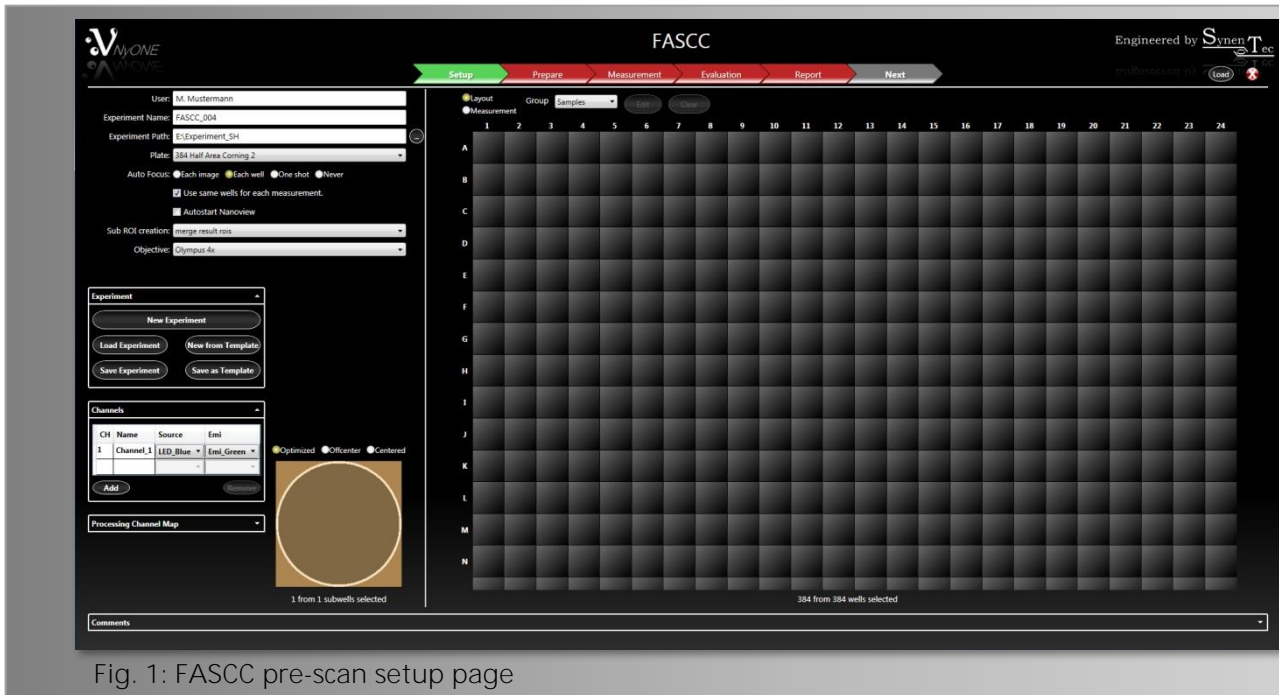


Fig. 1: FASCC pre-scan setup page

### 2.1 Experiment Design

The FASCC experiment consists of 2 - 3 measurement steps in a single experiment on d0 (e.g. seeding day) (Fig. 3).

1. Pre-Scan  
With the pre-scan the stained cells are measured with the 4x lens and one fluorescent channel of the appropriate wavelength
2. Nanoview (post scan)  
The Nanoview scan will spot the identified cells (filtered as single cell in a well) to be reviewed in a higher resolution (10x or 20x) in fluorescence and brightfield.
3. Confluence (optionally)  
As a third step a full well confluence measurement in brightfield can be carried out as a last verification that there is no other cell in the well (for manual scan by eye) or as a starting point for a confluence measurement series to monitor either proliferation or size of the colony growth.

After measuring on seeding day, the growth rate of the monoclonal cell lines can be assessed with Confluence Measurements (only the third phase) over time (e.g. d1, d5, d7, etc.).

The page setup differs slightly to the normal experiment designs of the YT-software. This difference will be described in the following chapter more detailed.

## 2.1.1 Levels in FASCC Experiments

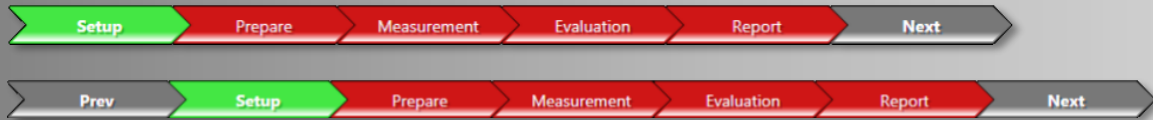


Fig. 2: Experiment level overview

FASCC comprises three Levels: Pre-scan, Nanoview and Confluence. The guiding arrows at the top have either a 'Next' or a 'Prev' step to move to the next or previous level of the experiment. The first two levels are only relevant on seeding day d0, the third level can be measured on d0 and is used from d1 onwards, to generate growth curves for the clones.

The Pre-Scan and the Nanoview level must not be measured after d0. To carry out a measurement on e.g. d1, load the experiment in the setup dialogue (Fig. 1) and go to the Confluence level by clicking 'Next' twice (Fig. 2). Then go to the measurement section and click 'Start'.

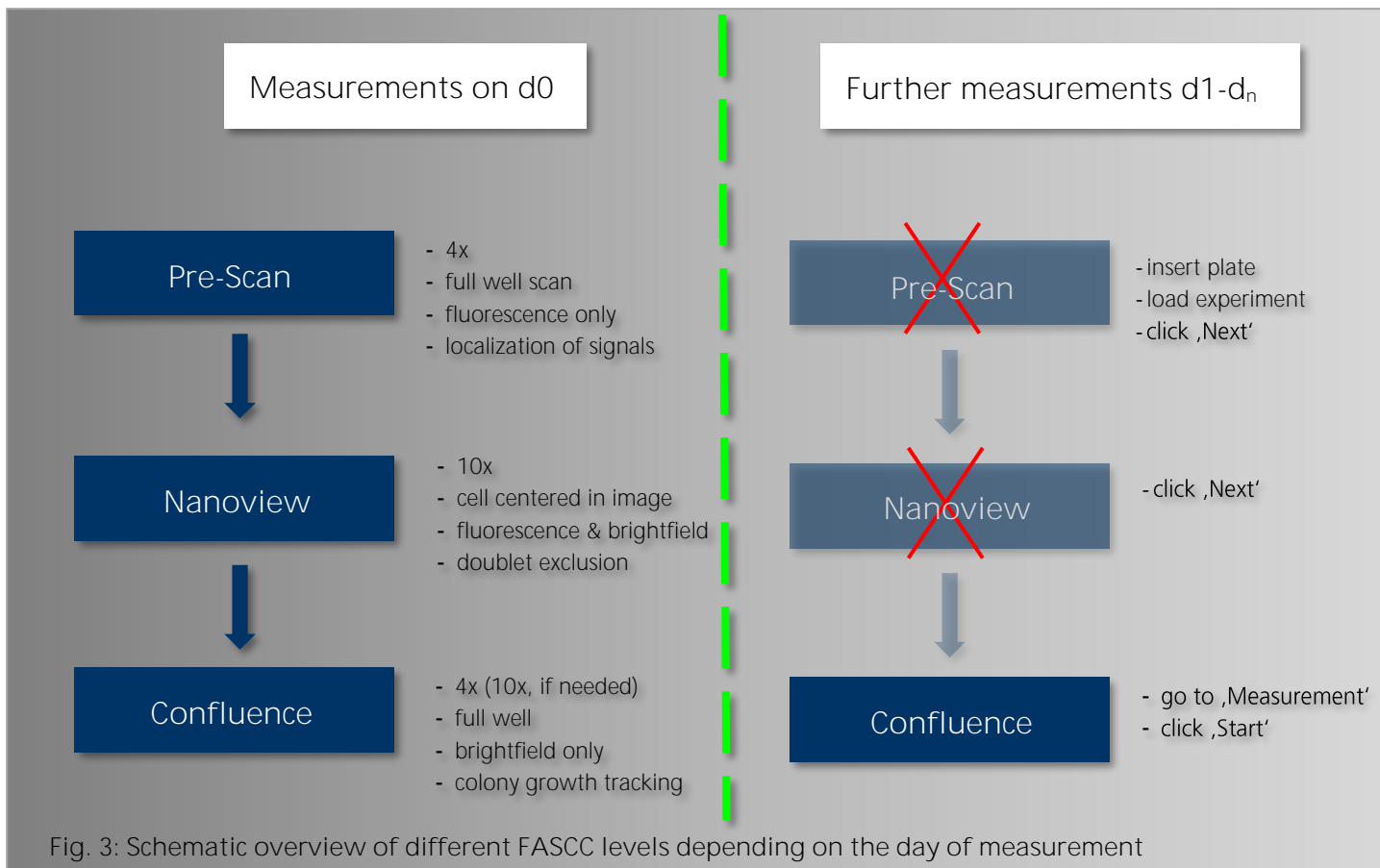


Fig. 3: Schematic overview of different FASCC levels depending on the day of measurement

Fig. 4: Start to set up  
Start with choosing an experiment name, your used plate type, the right autofocus settings and the required objective.

'Autostart Nanoview' and 'Sub ROI creation' are also new features, which are not included in common experiment setups.

### 2.1.2 Autostart Nanoview

Autostart Nanoview automatically enters the next level and starts the next measurement using the pre-defined selection filter. This check box should only be set after you have setup all optical and operator analyzing parameters for your cell line.

### 2.1.3 Sub ROI Creation

Fig. 5: Choose sub ROI creation for nanoview

It can be chosen between 'full well' or 'merge result rois'. This selection becomes active for the next level, i.e. does not have any impact on current measurement.

#### 1. Merge result ROIs:

The pre-scan determines the exact location of the potential clones inside the cells. With this information the Nanoview scan can be done directly on the spot of the cell. If you choose this option the objective lens will be directly positioned at the position of the cell with this single sub-image only.

## 2. Full well:

This option should be chosen in case it is uncertain if a second cell might have been missed for any reason in the pre-scan and you want to ensure that there is no other cell in the well. It is usually only used in a validation phase because it will take a little more time due to the full well scan.

## 3. The Pre-Scan Measurement

### 3.1 Optical Setup and Preparation Pre-Scan

An optical setup and preparation is required - like for any experiment - if the properties of the cells and the fluorescence are unknown or no template is available for such known assay. Therefore it might be advantageous to put some more cells in a well (typically A1) for optical adjustment.

Proceed as you would normally proceed for optical preparation by setting the channels for the optical settings.

For e.g. CellTracker™ Green, LED\_Blue for 'Source' and Emi\_Green for 'Emi'

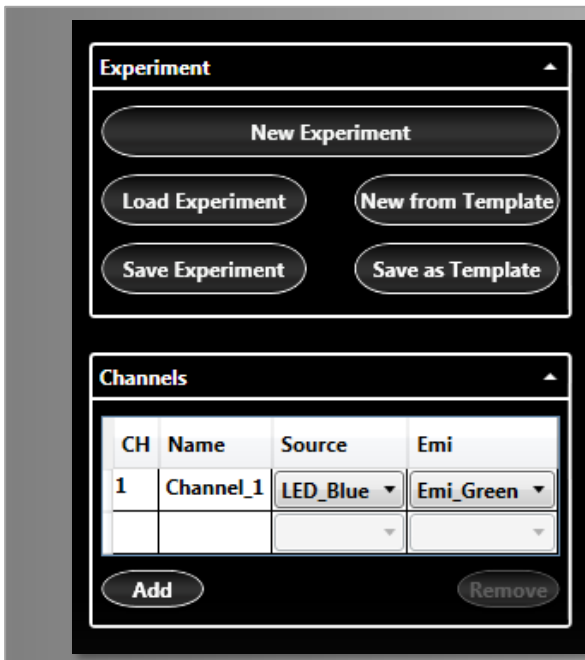


Fig. 6: Fluorescence channel  
Choose your required light source and emission filter.

After selecting the appropriate optical setting proceed with the preparation.

The settings for the fluorescence are determined using the same criteria as for any fluorescence experiment. Having several cells in a well for adjustment allows better distribution of the



potential fluorescence signals to be expected. Saturation of the fluorescence signal is not desired but must be accepted if the weakest cell cannot be detected.

Otherwise try not to have too many cells in signal saturation (i.e. 255 counts). Increase exposure, gain and intensity accordingly. Therefore, the used exposure and gain intensity should be set to 100%. There should be no fear of bleaching because the source will only illuminate the probe while exposing with an automatic electronic shutter.



Fig. 7: FASCC pre-scan 'Prepare' page

Under normal circumstances the default settings of the operator will work properly. You can check the function and the correct identification of the cells by clicking on 'Process Image'.

### 3.2 Measurement Pre-Scan

Click on the arrow for measurement and start the measurement by clicking the 'Start' button on the lower left side.

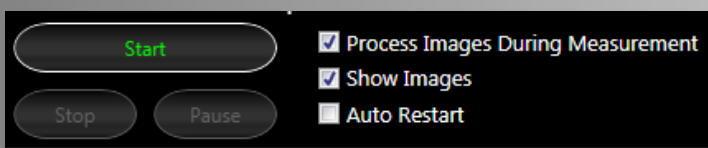


Fig. 8: Options for measurement

'Process Images During Measurement' and 'Show Images' are set as default and should be left in that state.

## 3.3 Evaluation of Pre-Scan

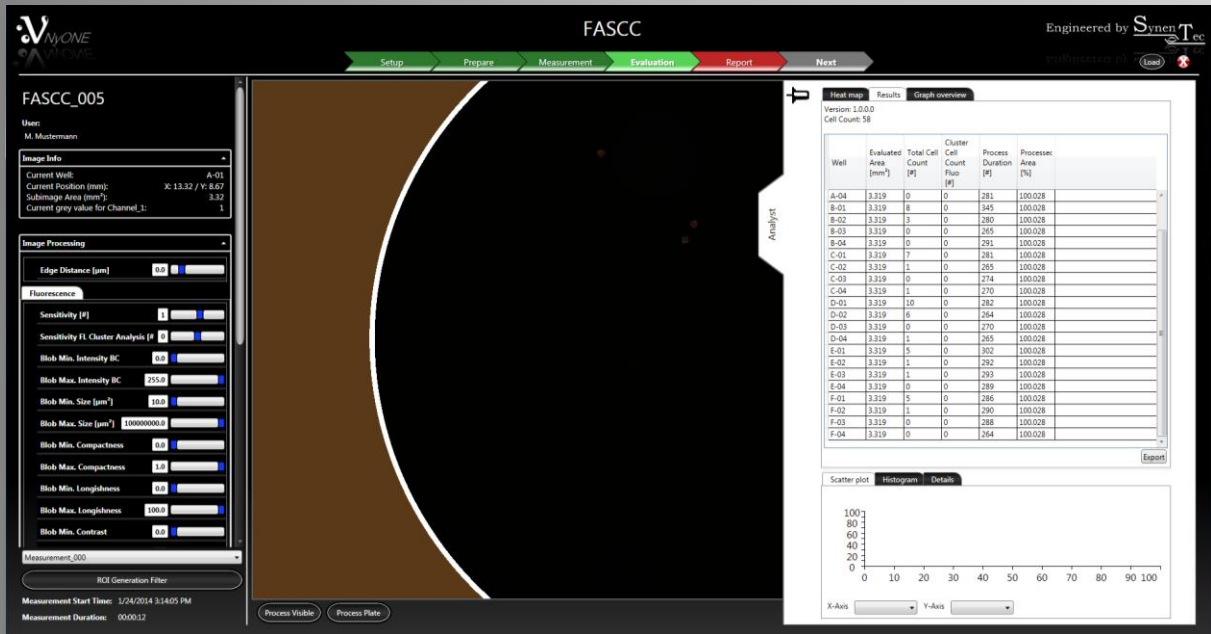


Fig. 9: FASCC pre-scan 'Evaluation' page

### 3.3.1 Result Table in Analyst

The result table gives the best overview of the results. The total cell count will be displayed. It is now possible to cross check the results and alter operator settings and reprocess as required by the circumstances.

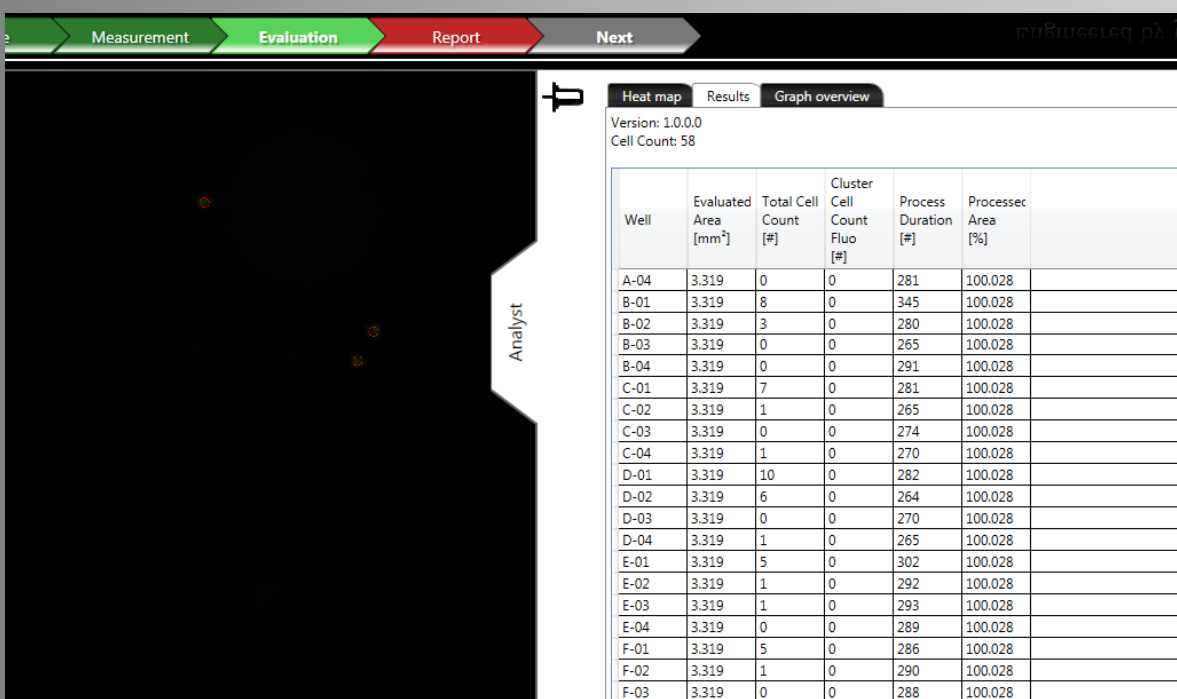


Fig. 10: Analyst flag: Result table

### 3.3.2 ROI Filter Settings

The ROI Filter is used for selecting only the wells with potential single cells. Other numbers may be set but are not recommended for FASCC.

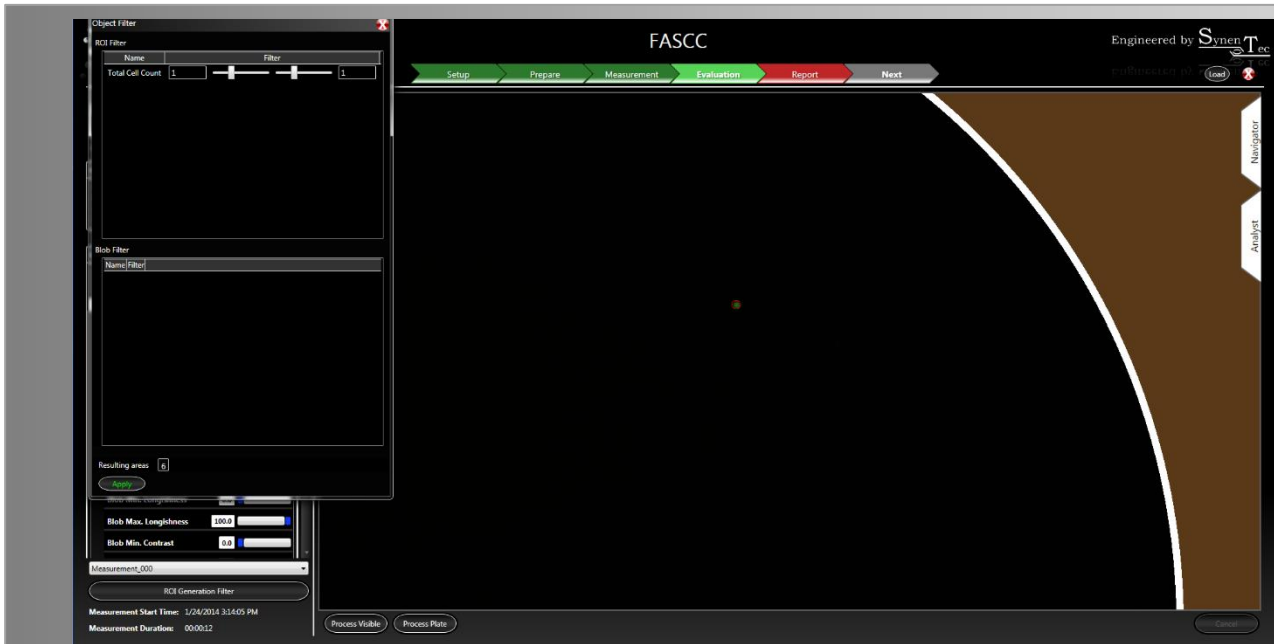


Fig. 11: Use the ROI filter to choose the right wells for the 'Nanoview' step

Object Filter settings for Total Cell Count:



Fig. 12: 'Object Filter' window

After clicking the 'Apply' button the areas of single cells will be set. Close the 'Object Filter' window and proceed to the next level → Nanoview.

## 4. The Nanoview Measurement

The layout of the experiment page looks very similar to the pre-scan page. The major difference is that you cannot choose any wells to be measured as this has been determined automatically by the 'Object Filter' setting in the previous level.

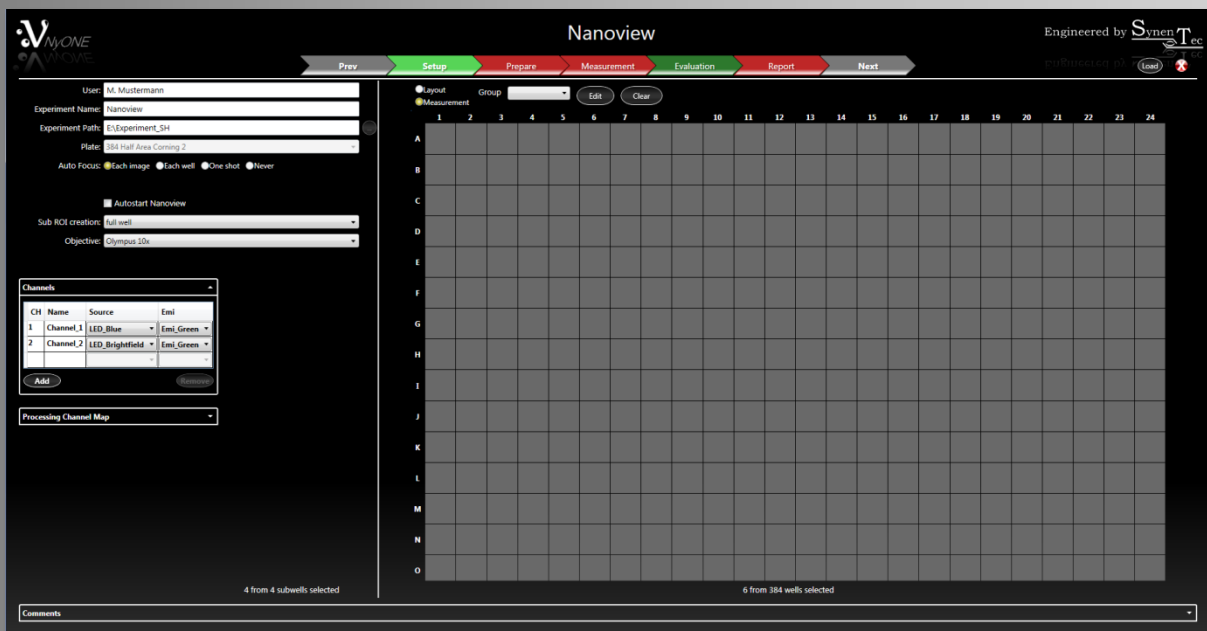


Fig. 13: FASCC 'Nanoview' 'Setup' page

### 4.1 Optical Setup and Preparation Nanoview

The Sub ROI will be set to full well for a subsequent full well confluence measurement. Objective default is 10x but other resolutions can be chosen, e.g. 20x, depending on what is most suitable. In case that the Objective is changed, it is necessary to go back to the Evaluation page of the pre-scan and set the ROI Filter again before continuing with the channel selection.

The channels should be set as follows: The Fluorescence channel as channel 1 and the Brightfield channel as channel 2. Ensure that the 'Emi' is set to the same filter for both channels otherwise unnecessary changes of the filter will be carried out.

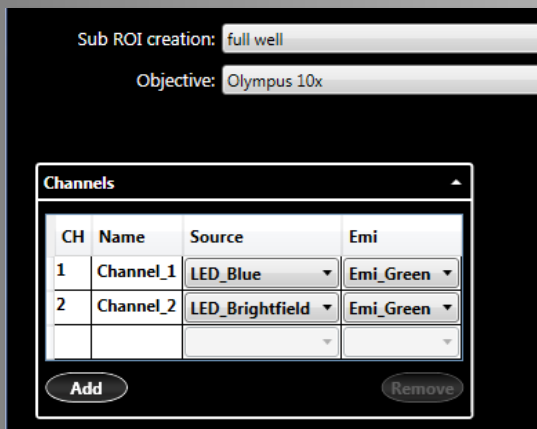


Fig. 14: Channel selection

#### 4.1.1 Preparation Nanoview Measurement

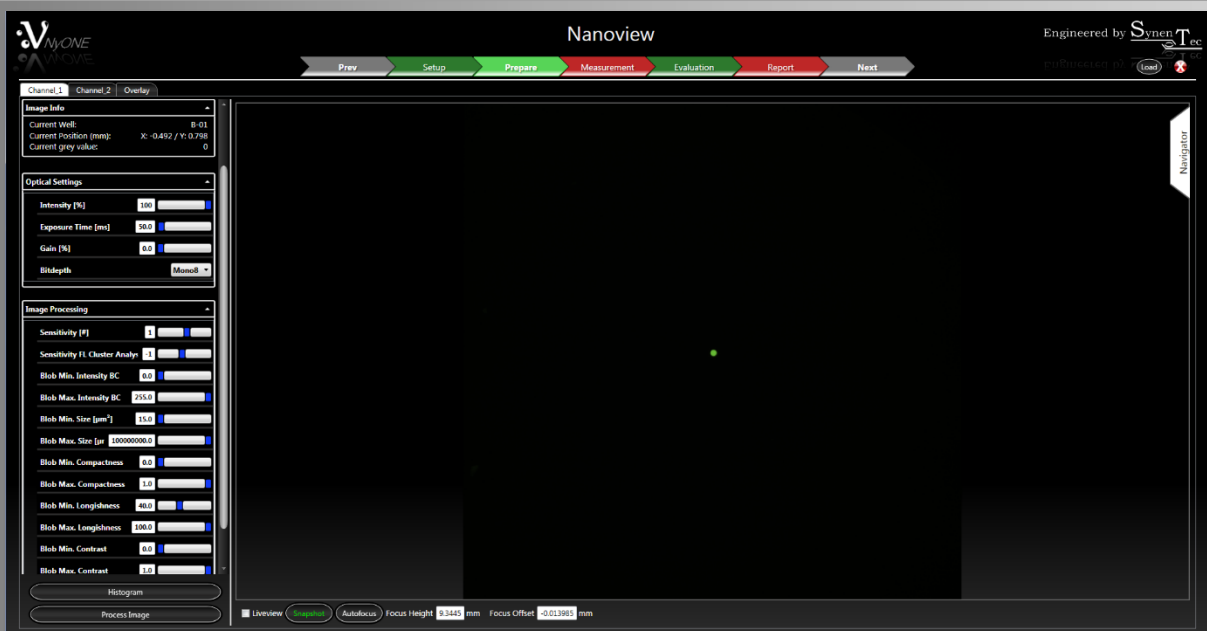


Fig. 15: FASCC 'Nanoview' 'Prepare' page

When you click on the preparation arrow the lens will be positioned exactly over the first cell found in the pre-scan. From here on you can setup the optics for the fluorescence and brightfield channel.

The operator settings should only be altered if a doublet has been found and was not resolved. This can only be done when the whole plate has been measured and the probability of finding a doublet is higher. Another possibility in the evaluation phase would be to seed out more cells per well and to search for doublets to ensure that the operator parameters are set properly. But nevertheless the default values should work in most cases.

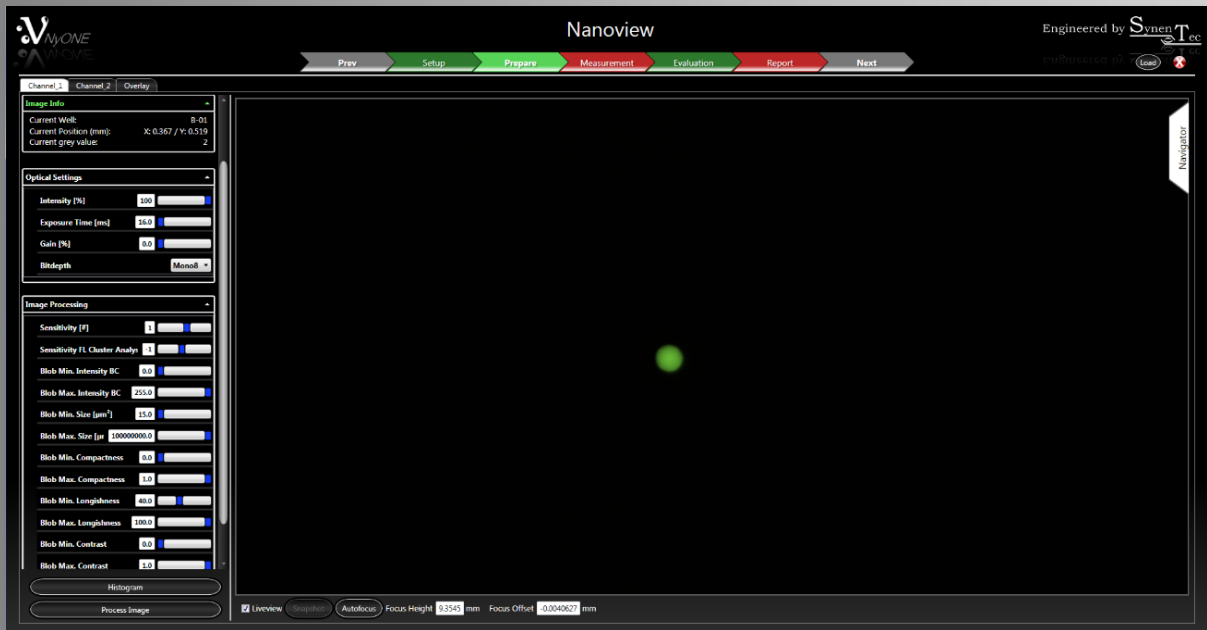


Fig. 16: FASCC 'Nanoview' 'Prepare' page

## 4.1.2 Measurement Nanoview

When you switch to the measurement arrow you may observe the little rectangles, which show the ROI (region of interest) to be measured in Nanoview. To start the experiment, click on the 'Start' button.

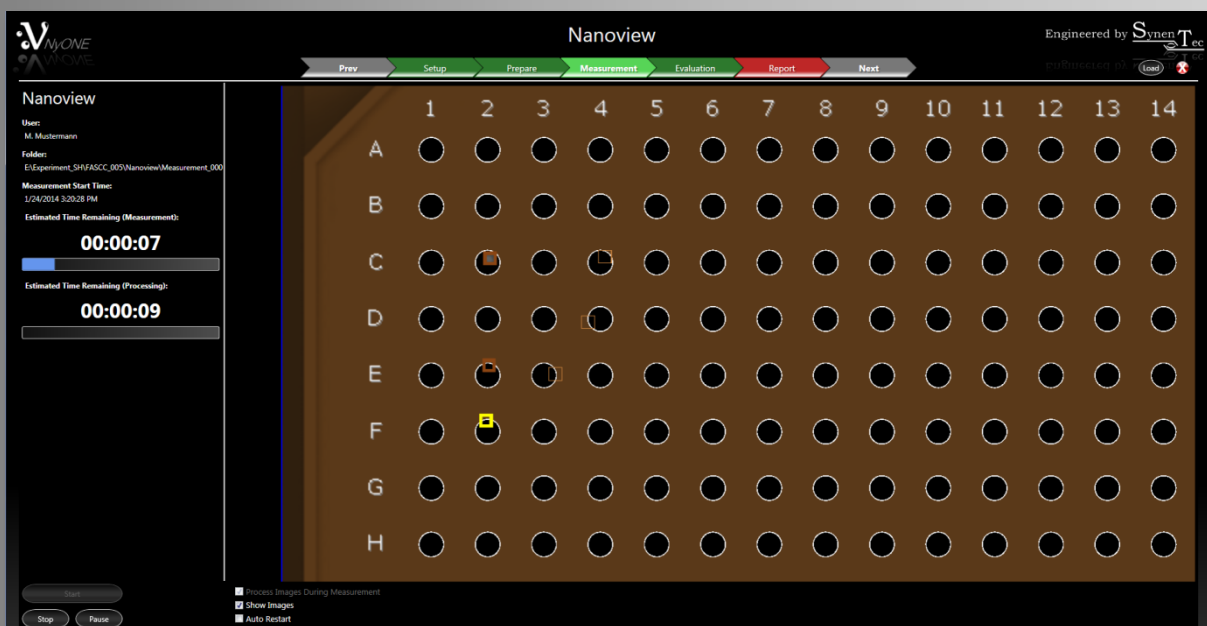


Fig. 17: FASCC 'Nanoview' 'Measurement' page

### 4.1.3 Evaluation Nanoview

After the measurement you can use the object filter in the same manner as for the pre-scan. The Nanoview operator will separate doublets in either fluorescence or brightfield.

It is important to set the object filter to ensure that only 'real' single clones will be monitored in the subsequent confluence measurement.

## 5. The Confluence Measurement

As a last verification that there is only one cell in the well or as a starting point for a confluence measurement series to monitor either proliferation or size of the colony growth, a full well confluence measurement can be carried out.

If the confluence measurement of day 0 is already done and you want to carry out an e.g. daily confluence measurement to observe the growth of the colonies, you do not have to consider the Pre-Scan or the Nanoview. Open the experiment in the setup of the 'FASCC' dialog, click on the 'Next' arrow (Fig. 19) and the 'Nanoview' dialog will appear. Click on 'Next' again to open the 'Confluence' window (Fig. 19). Now you can proceed with 'Prepare' of the confluence measurement as on day 0.

### 5.1 Optical Setup and Preparation Confluence

The layout of the setup page looks very similar to the Nanoview setup page. The well selection has been determined automatically by the 'ROI Filter' setting in the previous level.

The default objective is the 4x but other resolutions can be chosen, e.g. the 10x, depending on what is most suitable. In case that the objective is changed, it is necessary to go back to the Evaluation page of the Nanoview and set the ROI Filter again.

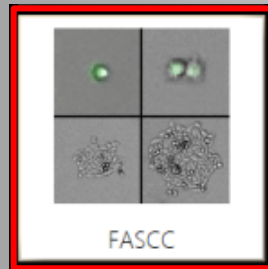
The next step is to go to the prepare page and to set up the optical and autofocus settings. If you click on the 'Prepare' tab the microplate will proceed automatically to the first well with one cell.

Before an image can be obtained an autofocus must be carried out followed by a snapshot to get an overview of the current image for the right optical settings (Fig. 18).



Fig. 18: Start with an autofocus

Continuous Confluence Measurements after Seeding Day (d0)



Open the FASCC application



Load your experiment



Click on 'Next'



Click on 'Next'



Click on 'Prepare' to check your optical settings

Fig. 19: How to proceed with a confluence measurement after the seeding day (d1 - d<sub>n</sub>)

Over an incubation period evaporation of media can influence optical conditions.

**TIP:** Check and readjust the optical settings before measuring again!

In case that the image is either too dark or bright, activate the 'Histogram' on the lower left side and use 'Liveview' while adjusting 'Exposure Time' and 'Gain' accordingly.

Once the result is satisfactory deactivate 'Liveview' as its warmth will affect the condition of the cells and hide the histogram by clicking on the appropriate button. To cross check your settings, move the mouse cursor directly over the image while watching the 'Current grey value' in the upper left corner (Image Info – Fig. 20). A value at abt. 130 counts reflects good settings. Press 'Process Image' (lower left side of screen) to get an actual overview of your settings.



Image Info	
Current Well:	---
Current Position (mm):	X: 50.35 / Y: 22.07
Current grey value:	129

Fig. 20: Image Info – current grey value

To adjust the focus offset activate 'Liveview' first and either position the mouse cursor into the focus offset box and move the mouse wheel up/down or position the mouse cursor right into the image and press CTRL while turning the mouse wheel. A good focus offset has been achieved when suspension cells appear bright inside and dark at the rim, which is called the 'Lens Effect', and adherent cells are rich in contrast.

Once all optical settings have been completed and the required image quality was found, the image processing should now be tested. Click on 'Process Image' to test your settings and view the results. It is recommended to start using the Default parameters which in general ensure to meet the desired results.

In case the result is not satisfactory the Image Processing Parameters can either be re-adjusted or previously saved parameters may be used or the parameters can be reset to default.

Now proceed with the Measurement.

## 5.2 Measurement Confluence

There are two further options which may be chosen before you simply click on the 'Start' button on the lower left side to start the measurement.

It is recommended to tick both: 'Process Image During Measurement' (results will then also be available immediately in the 'Evaluation' mode) and 'Show Images' while the measurement will be carried out. These options allow to zoom into the appropriate wells and to watch the imaging process.

Proceed with 'Evaluation' to view the results of your experiment by clicking on the corresponding arrow (Fig. 21).

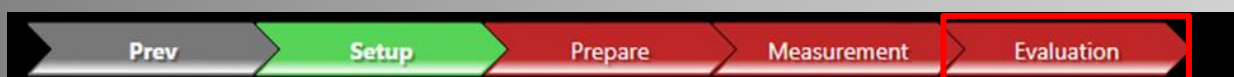


Fig. 21: workflow arrows

## 5.3 Evaluation Confluence

You can now check your results by scrolling through the result table, the Heat map, a timed chart graph, the Scatter plot and Histogram to obtain an overview. Therefore, click on the 'Analyst' flag on the right. For each type of result presentation many different properties like 'Cell Area' or 'Cell Confluence' can be chosen.

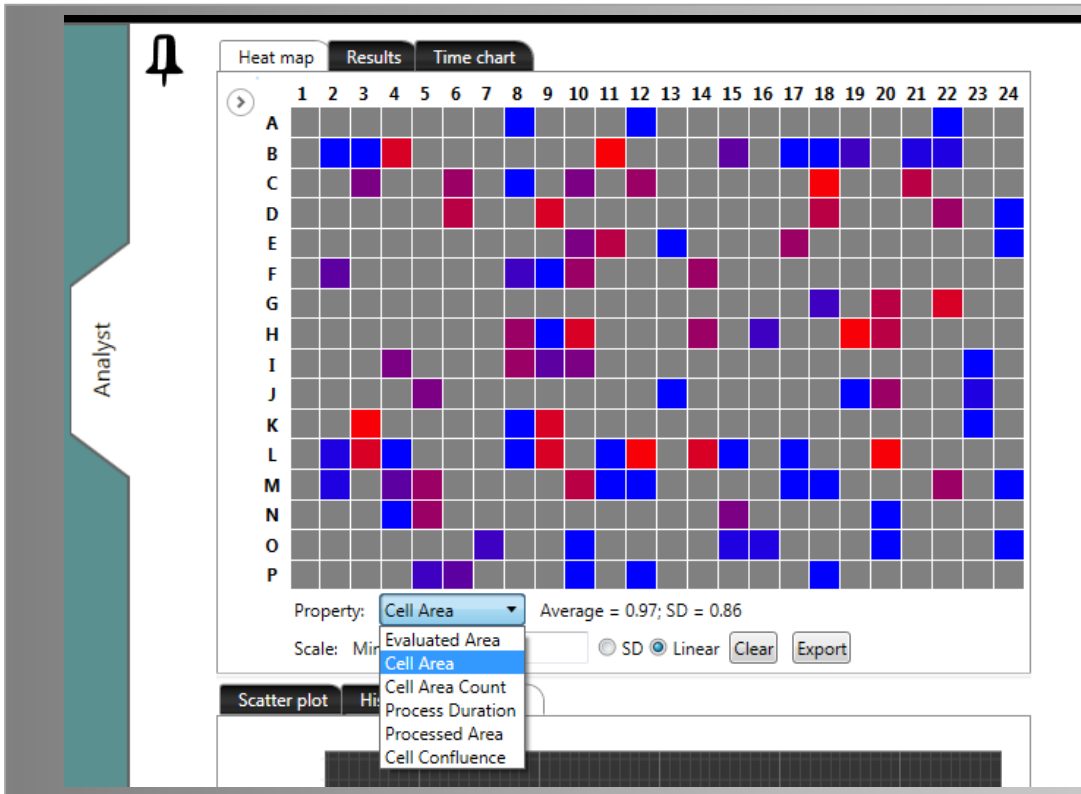


Fig. 22: Analyst with the variety of result presentations

The above seen different colors of the Heat map represent the cell confluence according to the covered area within each well. Blue represents a low and red a high cell confluence.

The result table as well as required images can be exported as a CSV file using the 'Export' buttons in the 'Results' tab.

The Time chart allows the measurement of cells over several days and also presents details for each well by simply clicking on the appropriate curve (Fig. 23).

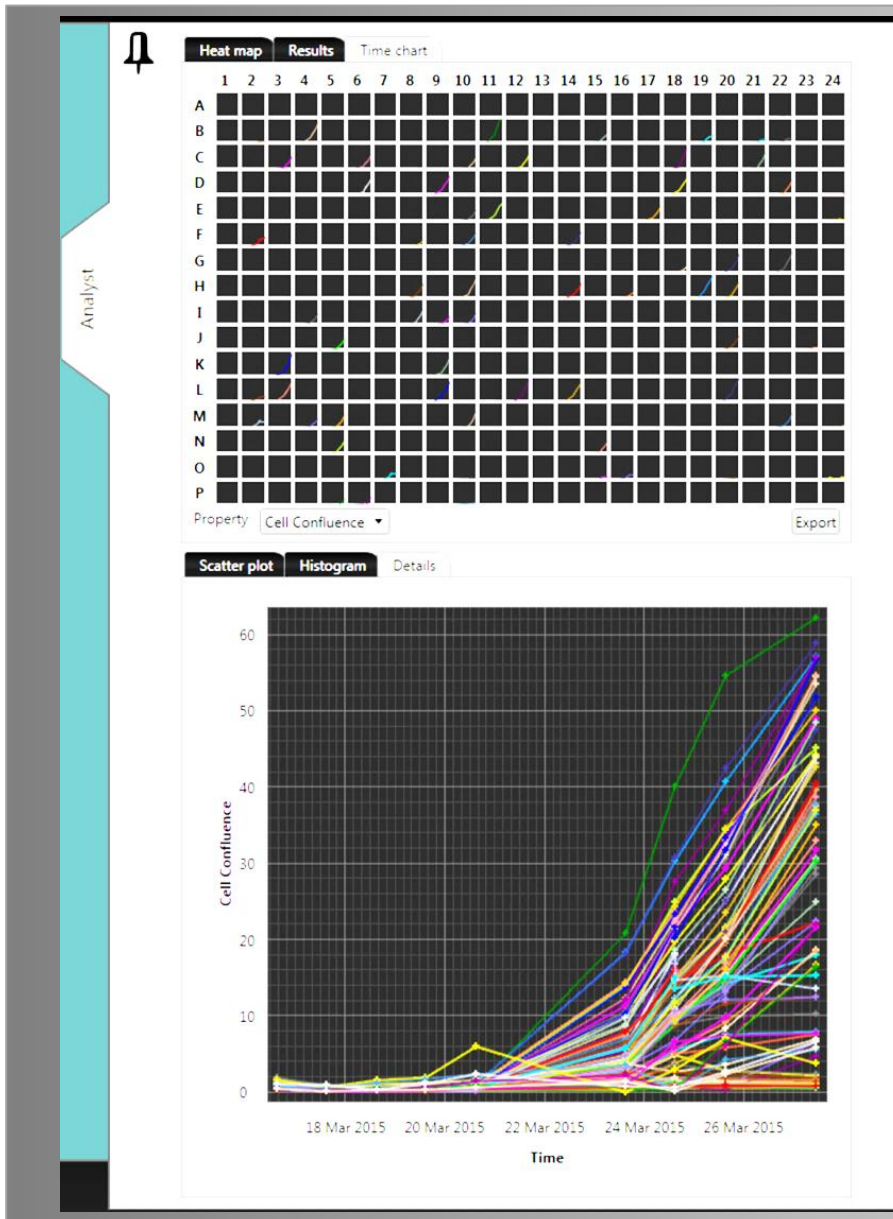


Fig. 23: Time chart  
Single or several curves and fields may be selected by pressing 'Ctrl' and a) clicking on the required curve/field or b) dragging the mouse cursor to mark several fields at once.

If you are satisfied with the results you may 'Export' either just the results from the actual experiment or click on 'Export all' to export the data from previously carried out experiments (Fig. 24).



Fig. 24: Possible options after measurement is done

In case you are not satisfied with your results you can alter the image processing parameters accordingly, check same by clicking 'Process Visible' and further reprocess the whole plate by clicking on 'Process Plate' (Fig. 24).

## 6. Operator Explanation and Setting for FASCC

### 6.1 General Working Principles of FASCC Operators

The FASCC operators are divided into the FASCC Pre- and the FASCC Nanoview operator. Both work similar except for the FASCC Nanoview which additionally utilizes a brightfield image for evaluation. Thereby you can see that the parameter tables differ. For the pre-scan you will only find parameters which are effective on fluorescent images.

If you take a closer look at the table for Nanoview there are parameter settings in the upper section which are designed to set the parameters for the cluster analysis. In the lower section it is possible to toggle between 'Fluorescence' and 'Brightfield' to adjust the parameters for the different channels accordingly.

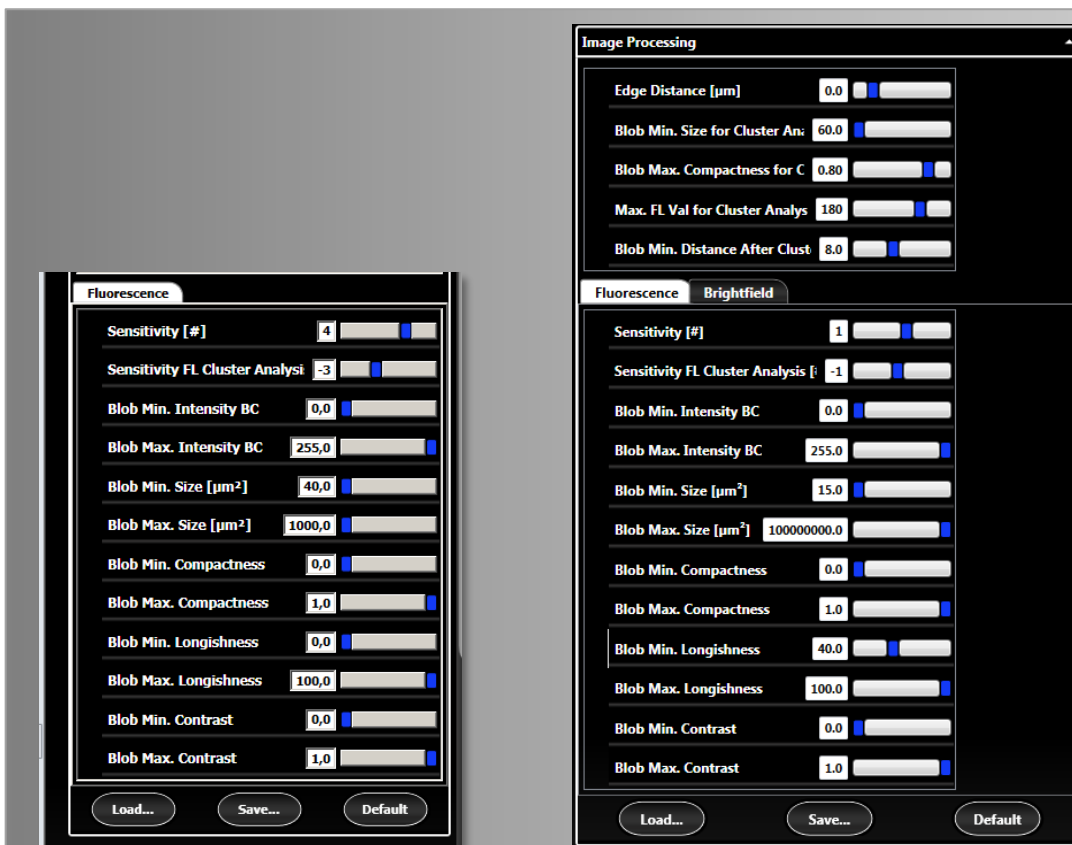


Fig. 25a: Parameter table for 'FASCC Pre'

Fig. 25b: Parameter table for 'FASCC Nanoview'

#### 6.1.1 Fluorescence Evaluation

For both operators the fluorescence evaluation is done in two sequences. In a first step the fluorescence image is evaluated and checked for fluorescent objects according to the parameter settings. In a second step there is a so called 'Cluster Analysis' which will be automatically

performed on the initially identified objects. This closer look tries to separate two or more cells from each other (if there are any) by taking a closer look into that relevant object. Depending on the parameters these additional objects could be resolved. This analysis cycle is done for both operators, the Pre and Nanoview.

## 6.1.2 Brightfield Evaluation

After its 'Fluorescence Cluster analysis' the FASCC Nanoview operator uses the 'Brightfield cluster analysis' to double check for additional cells.

## 6.2 Show Cases for FASCC Pre Operator

### 6.2.1 Too Many Objects Found in Cluster Analysis

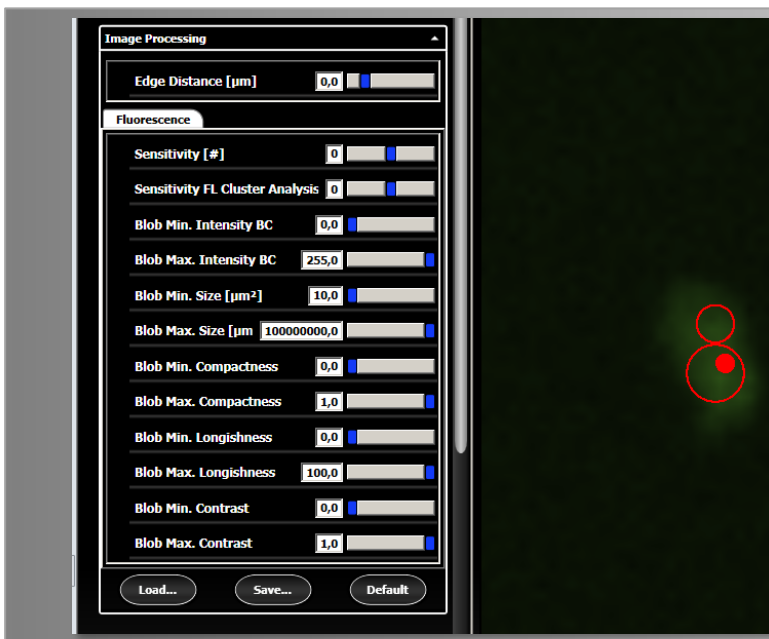


Fig. 26:  
In this example the set default parameters erroneously resolved this cell into three objects using a 4x magnification.

In the following you will see how altering the parameters generate different results.

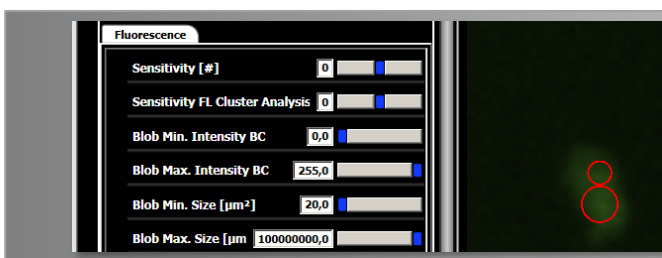


Fig. 27:  
Increasing the 'Blob Min. Size' removed the very little blob. But the result is not satisfactory.

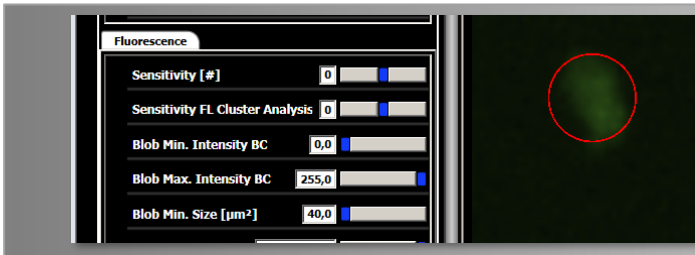


Fig. 28:  
By further increasing 'Blob Min. Size' a single object is now found.

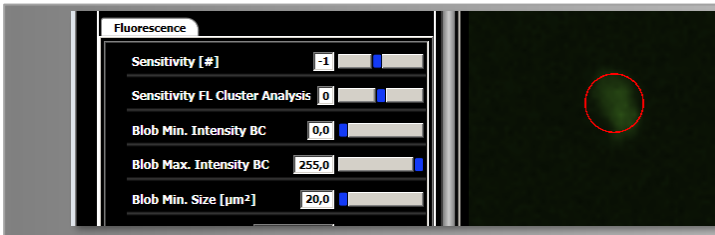


Fig. 29:  
Alternatively it could also be corrected by changing the 'Sensitivity' or 'Sensitivity FL Cluster Analysis' from '0' to '-1', which would have the same effect.

## 6.2.2 Object Could Not Be Found in Pre-Scan

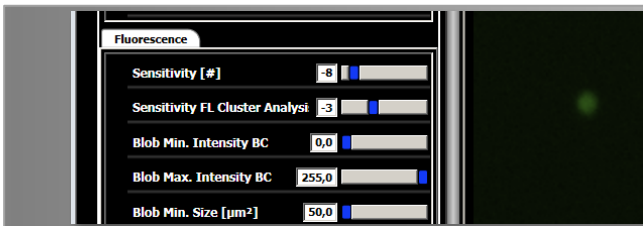


Fig. 30:  
'Sensitivity' is set to a value of '-8' in this example which is very insensitive. The sensitivity is used to evaluate the background and to normalize it compared to the signals.

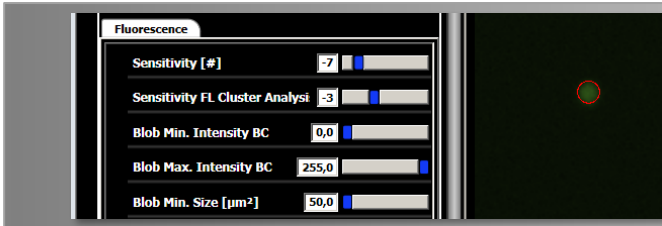


Fig. 31:  
This value was changed by one tick i.e. '-7' and the object was found.

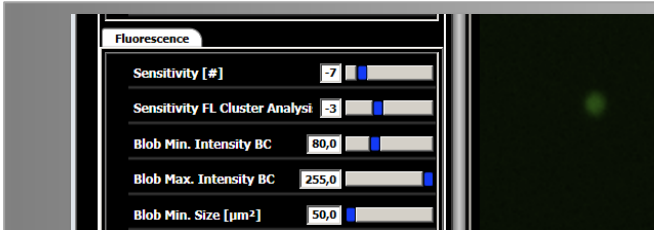


Fig. 32:  
By increasing the 'Blob Min. Intensity BC', which means the signal is higher than background, we can eliminate this object from detection. The same applies for limiting the maximum signal.

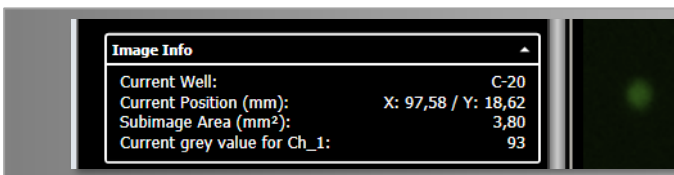


Fig. 33:  
The signal of the cell is 93 and the background approximately 20 therefore it would be excluded in the previous example.

### 6.2.3 Eliminating Lint or Debris

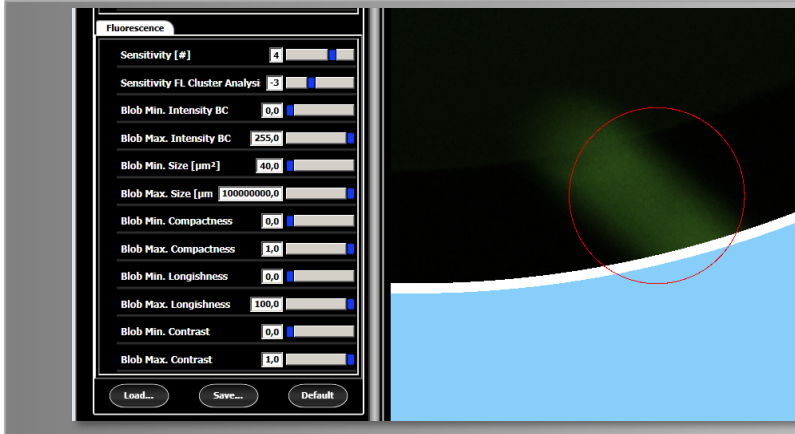


Fig. 34: Detected lint

Obviously one can see that this is not a cell. It looks more like lint or some dirt inside the well. It is by far larger than any cell. This can be eliminated by confining the cell size through alteration of 'Blob Max. Size'.

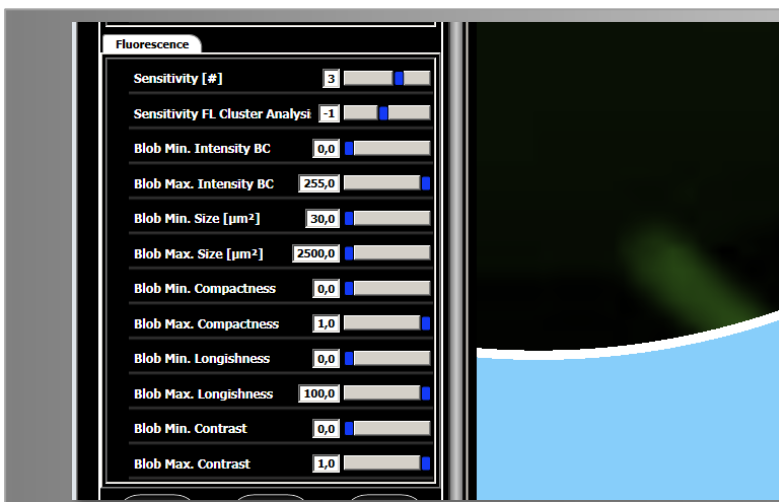


Fig. 35: Change the '**Blob Max. Size**' to exclude lint

## 6.3 Show Cases for FASCC Nanoview Operator

### 6.3.1 Erratically Identified Cell Doublet

In the following example (Fig.36-38) a light reflex was identified as a cell. This can be eliminated by either reducing the 'Blob Min. Size' or by taking geometrical aspects of the cell into account.

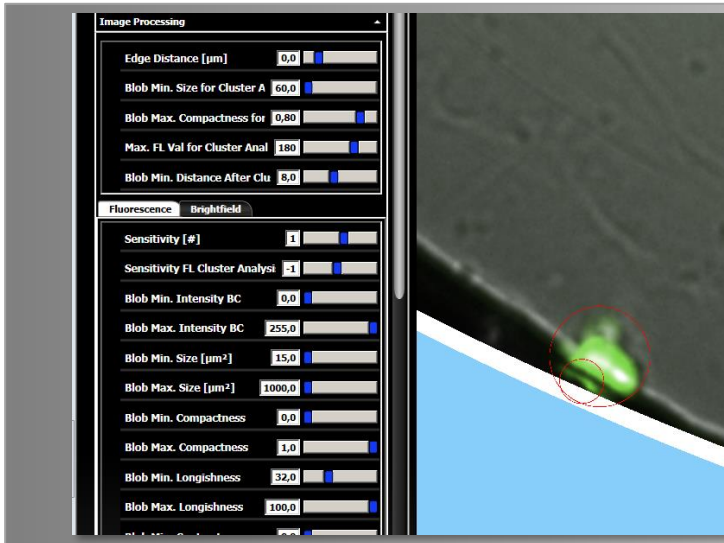


Fig. 36: Detected light reflex

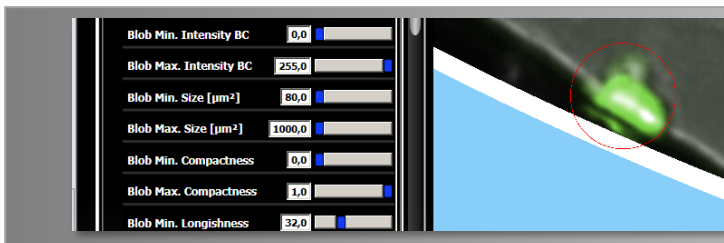


Fig. 37: Size change eliminated the reflex

If we take the size as the only option we might end up losing a small cell somewhere else. Another parameter change could help to avoid this. A critical parameter is also the so called 'Longishness' which describes to what extent the object reaches a perfect circle. A longishness of 100 means a perfect circle and a longishness of 0 means a line.

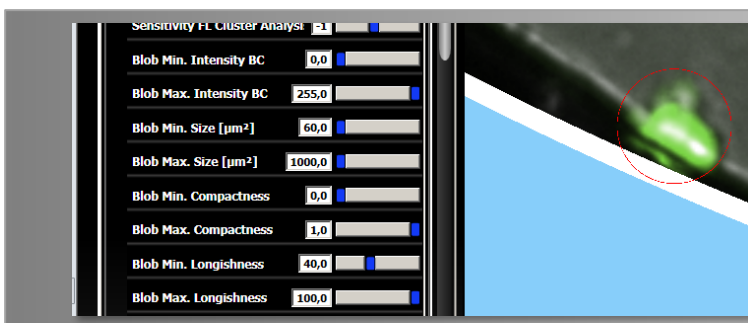


Fig. 38: **'Longishness' change** eliminated the reflex

With a value of 40 for longishness we could also eliminate the reflex.



### 6.3.2 Hard to Identify Cell Doublet

In the following case the second cell (if it is one) is smaller and much fainter than the first (bigger) one. By observing the brightfield image this becomes clearer.

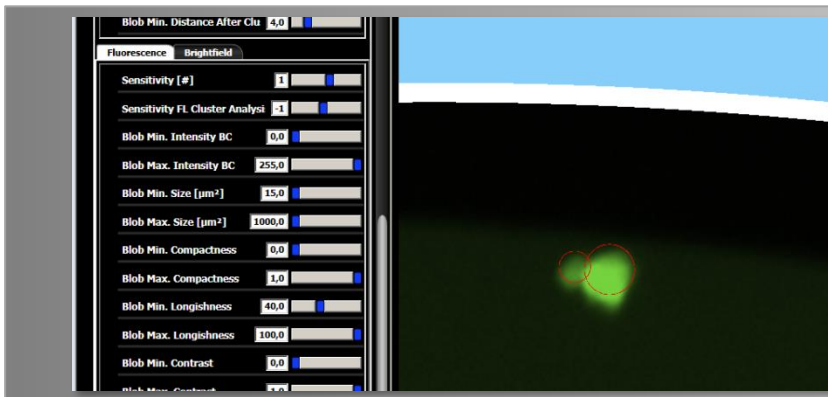


Fig. 39: Cell doublet in the fluorescence channel

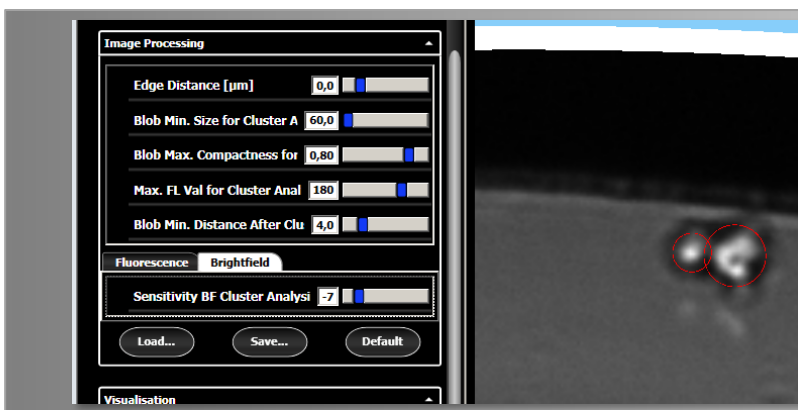


Fig. 40: Cell doublet in the brightfield channel

The image processing could use the brightfield image to identify them as two cells. On the left side of the image processing table the sole parameter for the brightfield analysis is shown. It is the sensitivity for the BF Cluster analysis

## 7. Making a Template for FASCC

Saving a template in FASCC differs from the conventional template creating. It is not possible to use the 'Save as Template' button in the 'Experiment' setup.

### 7.1 Template Preparation for FASCC

You need a defined experiment and have already carried out all steps through the levels from pre-scan, Nanoview and confluence (which could be omitted). When the measurement in the confluence level has been completed go back to the Nanoview and the pre-scan level and set the checkboxes to 'Autostart Nanoview'

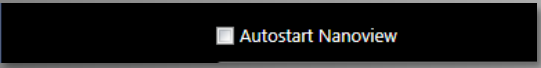
A screenshot of a software interface showing a checkbox labeled 'Autostart Nanoview' which is currently checked (indicated by a small square).

Fig. 41: Check the 'Autostart Nanoview' box

Instead of 'Save as Template', save the experiment.

If the 'Save Experiment' is not applicable please toggle between one of the Autofocus options, e.g. from 'one shot' to 'each well' and back. Then the button should become active and you can save the experiment.

Now generate a new experiment by pressing the button 'New Experiment from Template' and use the previously saved experiment as template. Ensure that the 'Autostart Nanoview' mode is set and [save this experiment](#). This is now your new template without any imaging data, which can be used as a template.

SYNENTEC GmbH

Otto-Hahn-Str. 9a

25337 Elmshorn/Germany

Phone +49 (0)4121 46311-0