Quick user-guide for the IncuCyte S3

Molecular Imaging Center

General rules for operating the IncuCyte

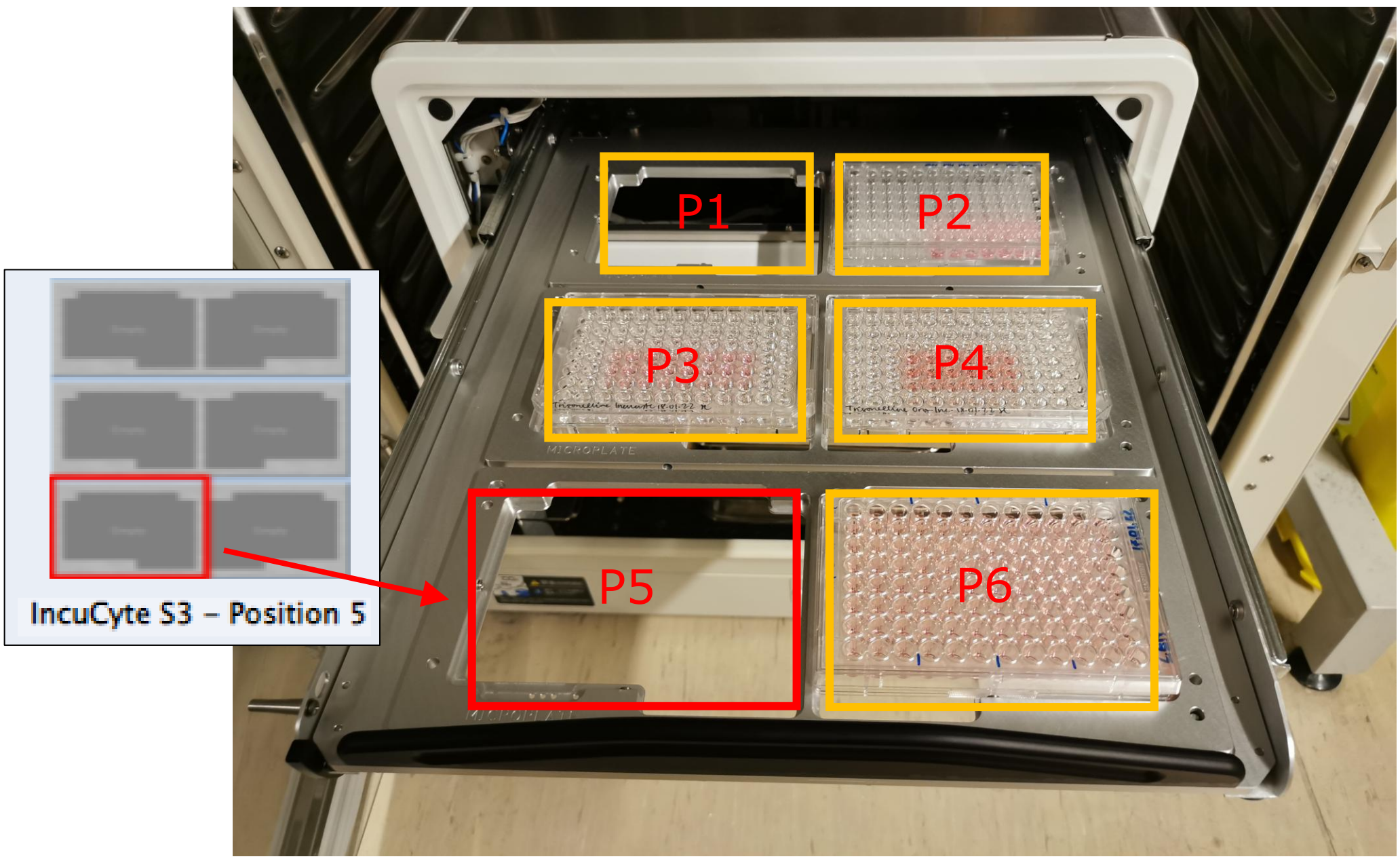
- Training is mandatory for all users, please ask if you are not yet comfortable on the system (MIC personnel). Once trained, you can get access to the S3 software and connect remotely from your own work pc or from your home computer through VPN. The software is not compatible with mac, but you can connect though the uib portal (se more info on page 31) with your mac. There is also a workstation available in the Em lab.

- Every user will have his/her own user account (First name-Last name).

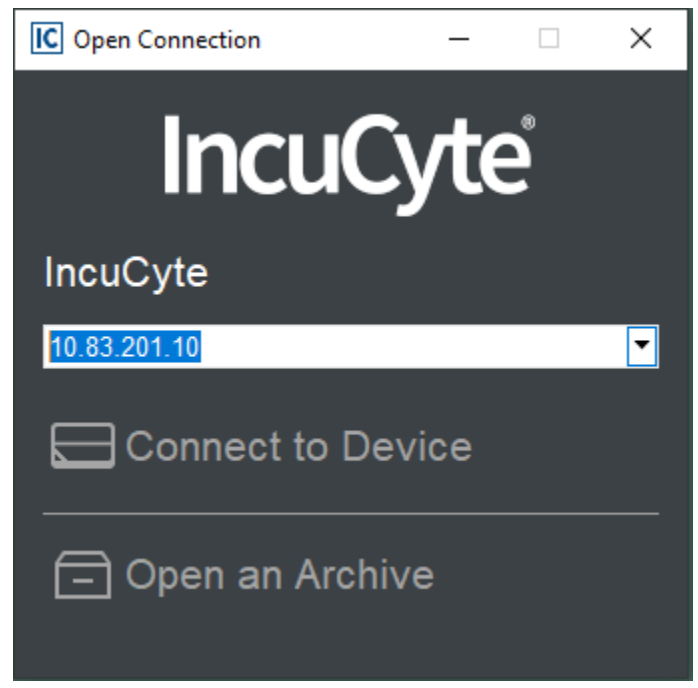
- Booking in advance is compulsory. Make sure you insert your plate in the correct position. If your booked position is occupied, discard the plate before placing your own vessel.

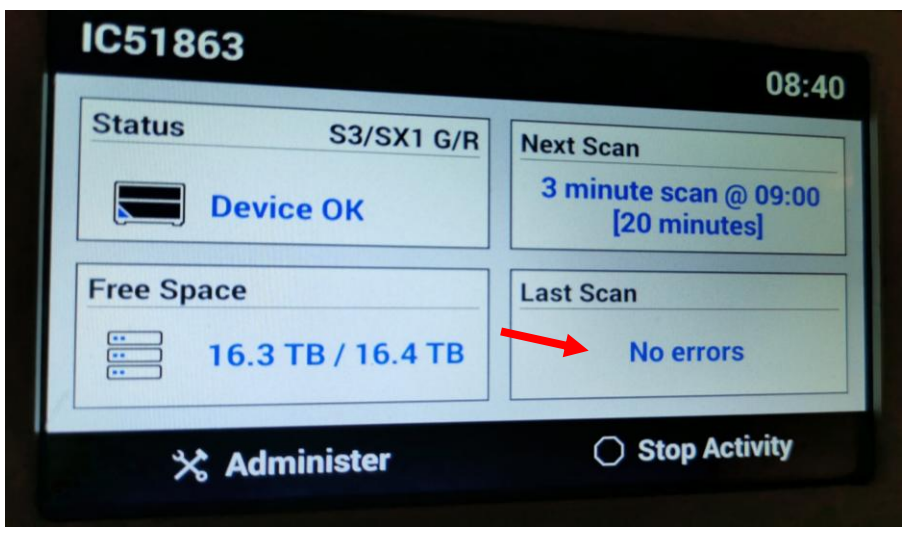
- If you are doing scratch wound healing, book the first hour as “supervised” and notify MIC personnel in advance. The wound-maker is only handled by MIC personnel as this involves special cleaning procedure and special care.

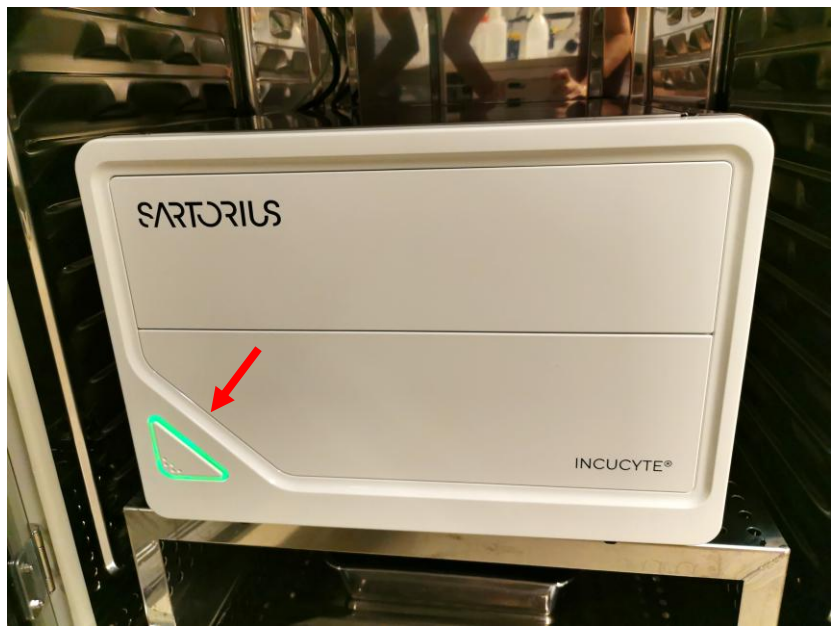
- It is mandatory to use gloves when handling the IncuCyte. It is **not** recommended to bring plates from the IncuCyte back to your own cells lab.

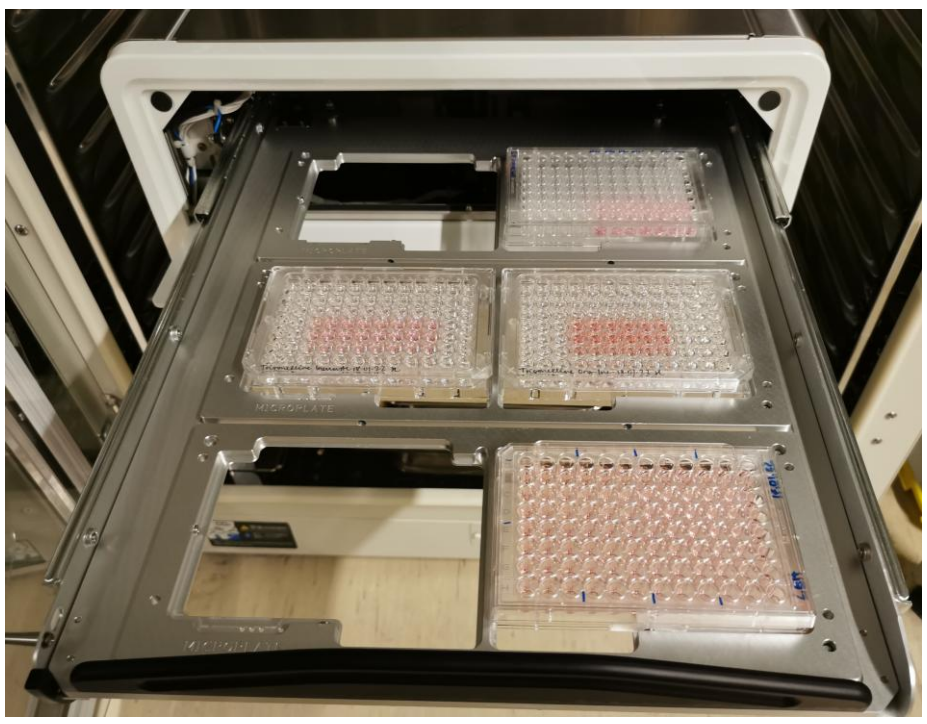


Installing the IncuCyte S3 software on your personal pc









- The S3 software can be installed on your personal PC. It is not compatible with macOS unless running Windows. Mac users can connect through the uib portal (more info on page 31)

- IncuCyte software is also available on a workstation in the EM Lab (6th floor), which can be booked free of charge through the MIC booking system.

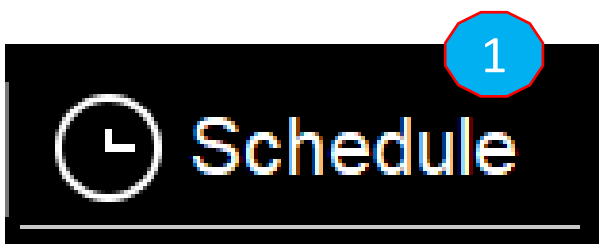
- Contact MIC personnel to obtain software access. Log in to the controller at 10.12.33.12 using the username and password provided after training.

- Before opening the drawer, ensure the system is not scanning. The status light must be green.

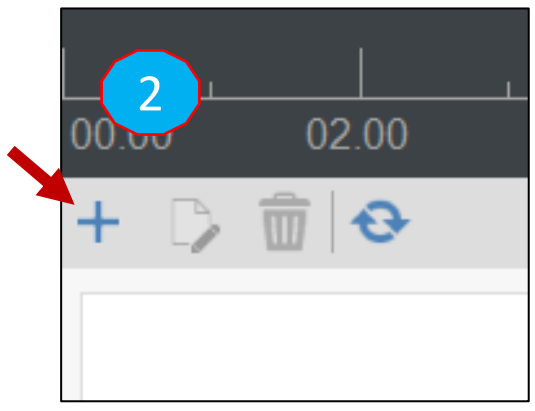
- To load a plate, first remove the metal rack and place it on the clean bench. Do not insert the plate directly into the drawer.

- After closing the incubator, verify that no error messages are displayed on the control panel. Contact MIC personnel if you have any doubts.

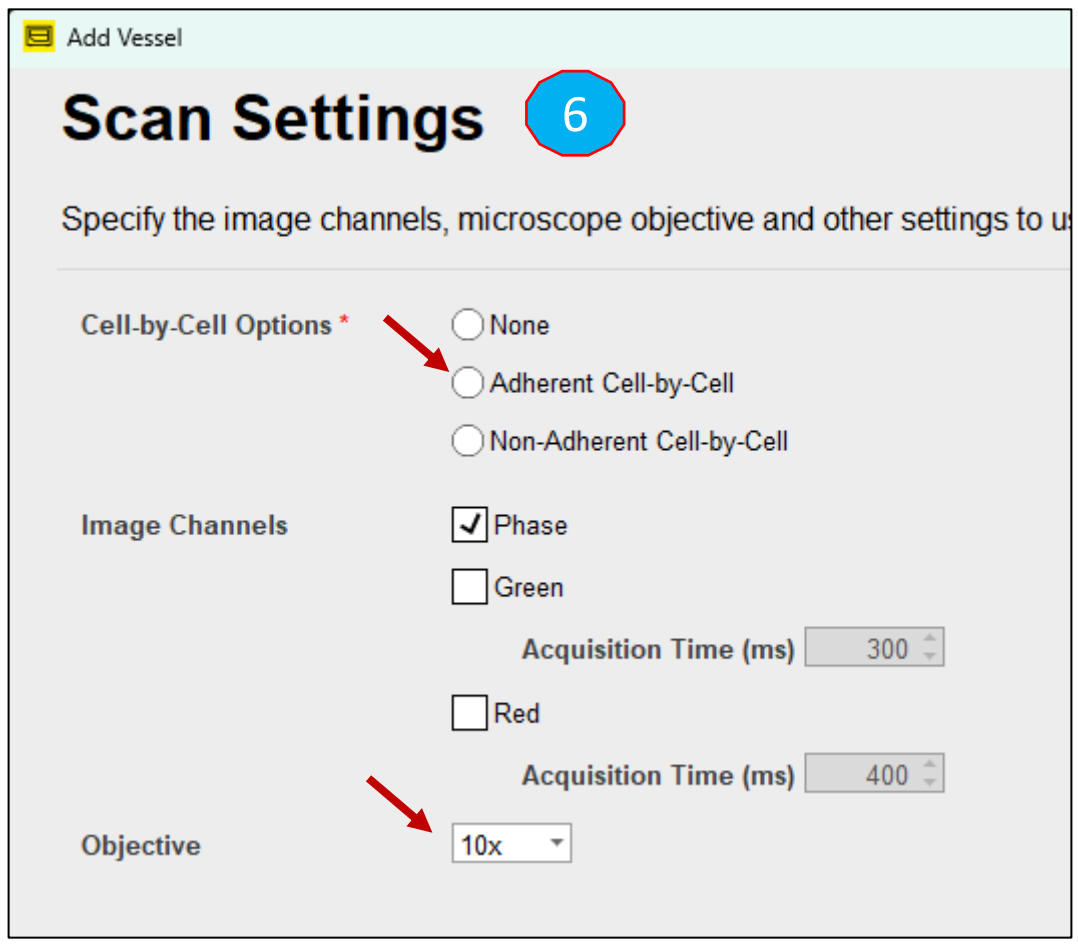
Scheduling a scan



1. Open the **Schedule** window and verify that your reserved position is available.
2. Click **+** to add a new plate.
3. The guided setup wizard will open. For timelpase imaging with repeated imaging at define intervals, select **Scan on Schedule**.
4. Create a **new** vessel og copy a current or existing experiment plate.
5. Select the appropriate **Scan Type** for your assay (page 8 for more info). The **Standard** scan type is suitable for a wide range of assays, including cell health, phagocytosis and immune cell killing experiments.

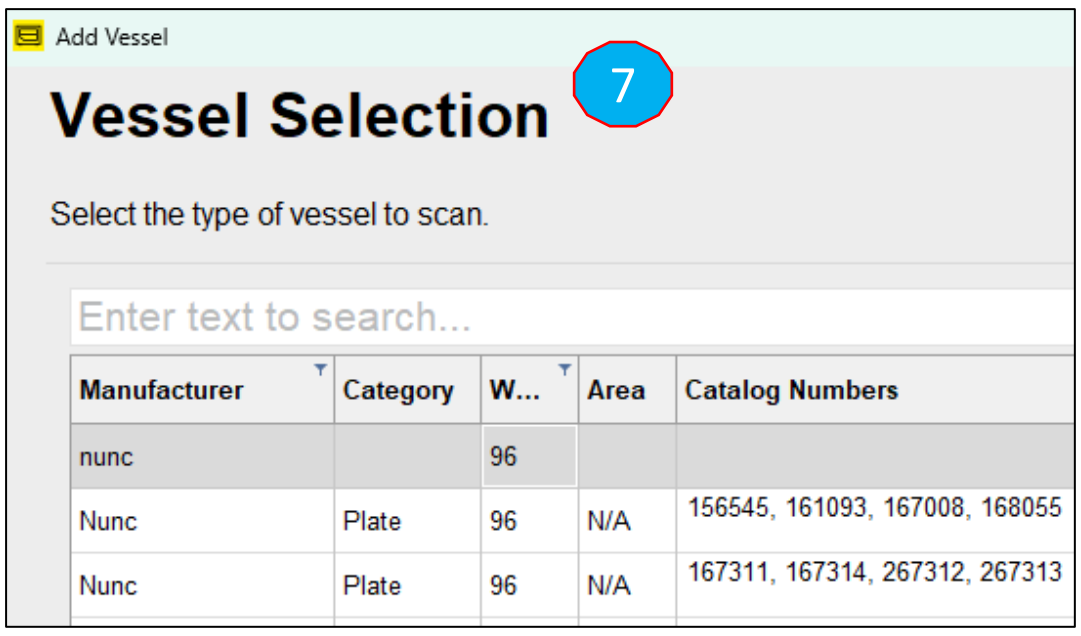


Scheduling a scan - continues

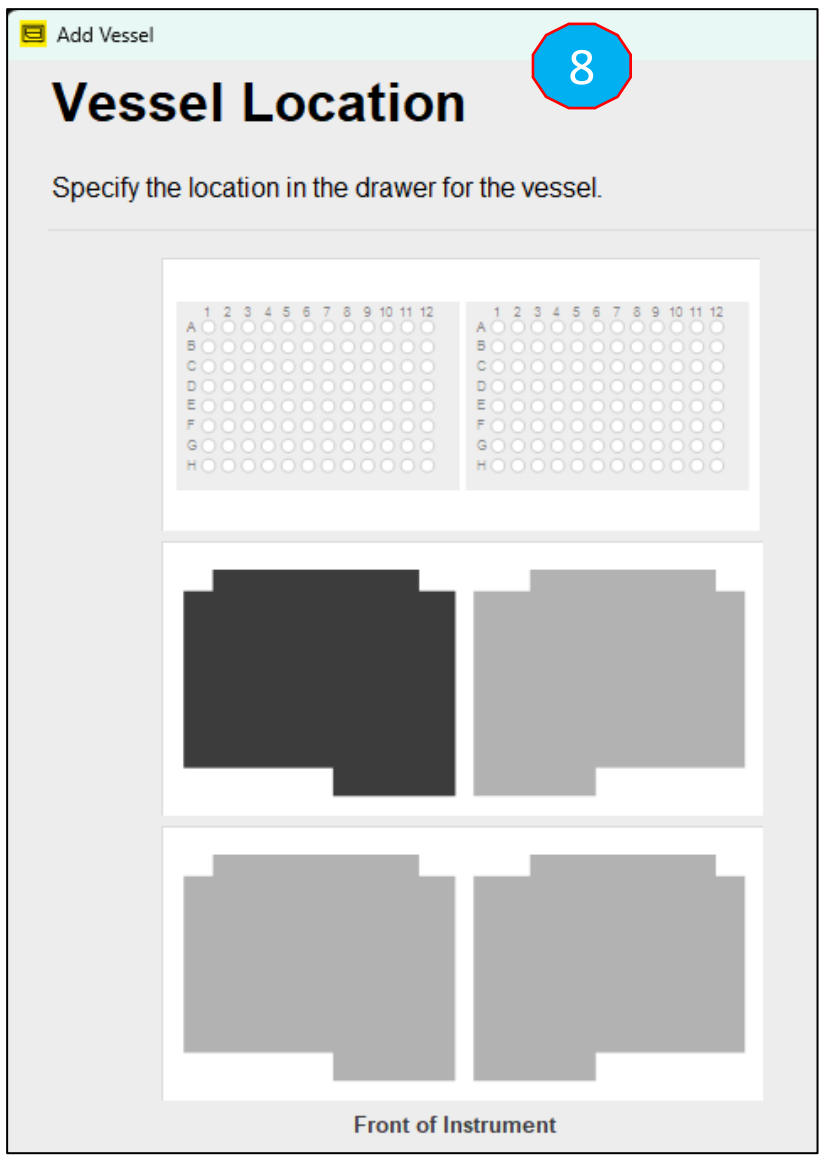


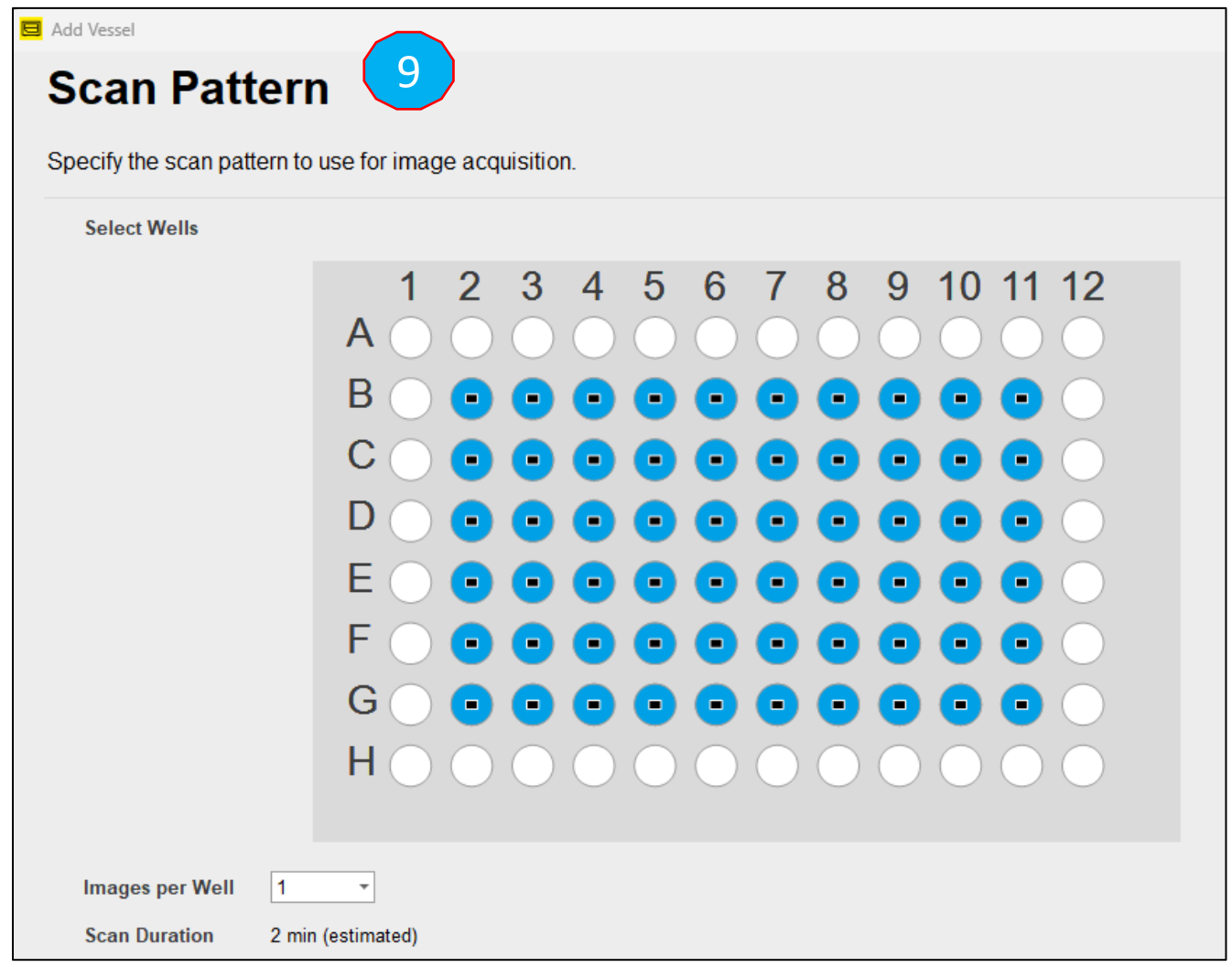
6. Select **Adherent Cell-by-Cell** and select the appropriate image channels and objective. The available options will depend on the scan type selected in the previous step.

Green channel:	Ex: 440-480 nm Em: 504-544 nm
Red channel	Ex: 565-605 nm Em: 625-705 nm

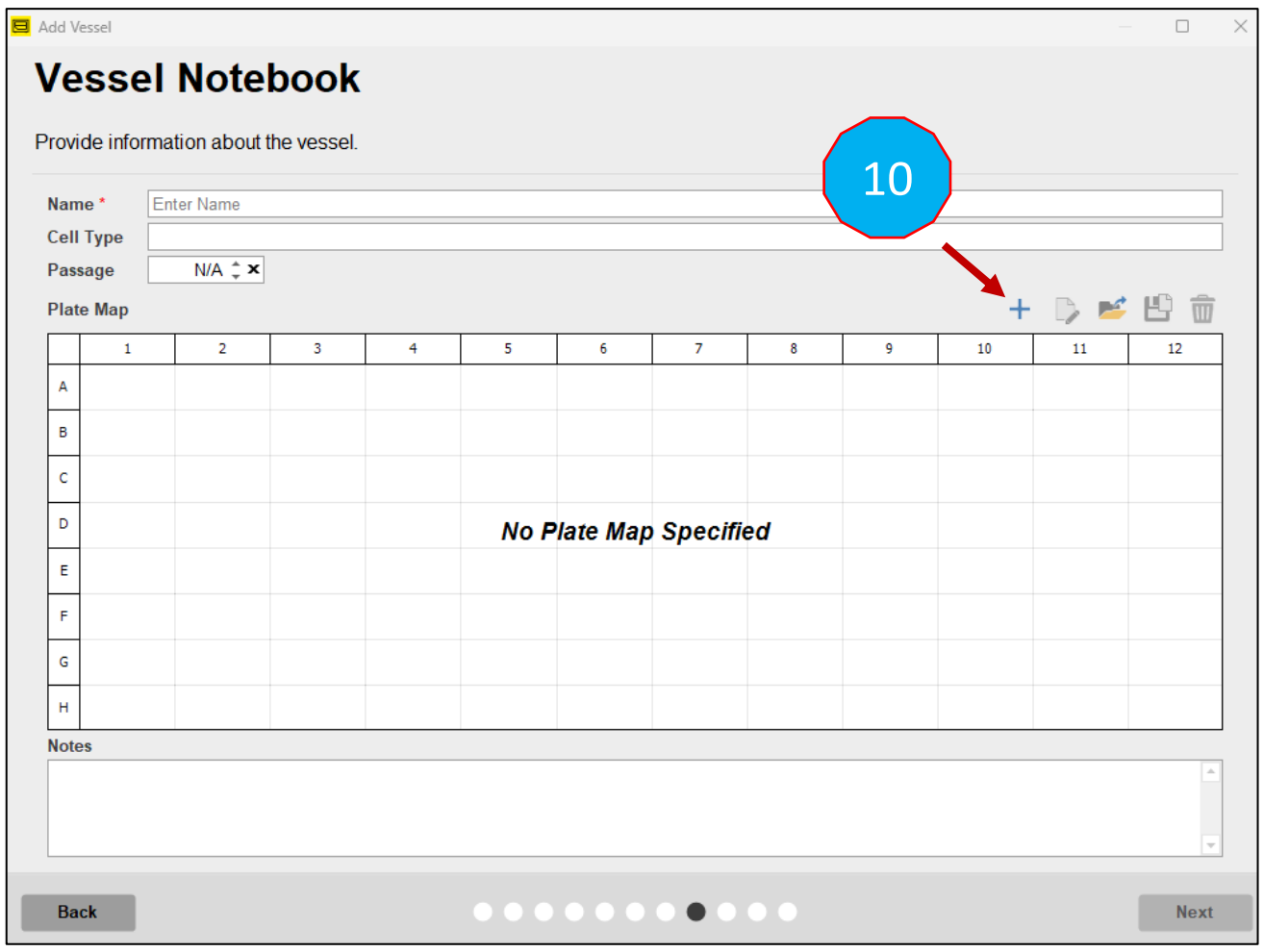


6. Choose the appropriate vessel. The list of compatible plates will be long, so filtering by manufacturer, well format or catalog number can be helpful. Using a compatible vessel is essential to ensure accurate x, y and z coordinates for well positions.
8. Specify the plate location within the incucyte instrument.
9. Select the appropriate scan pattern and specify the number of images per well.

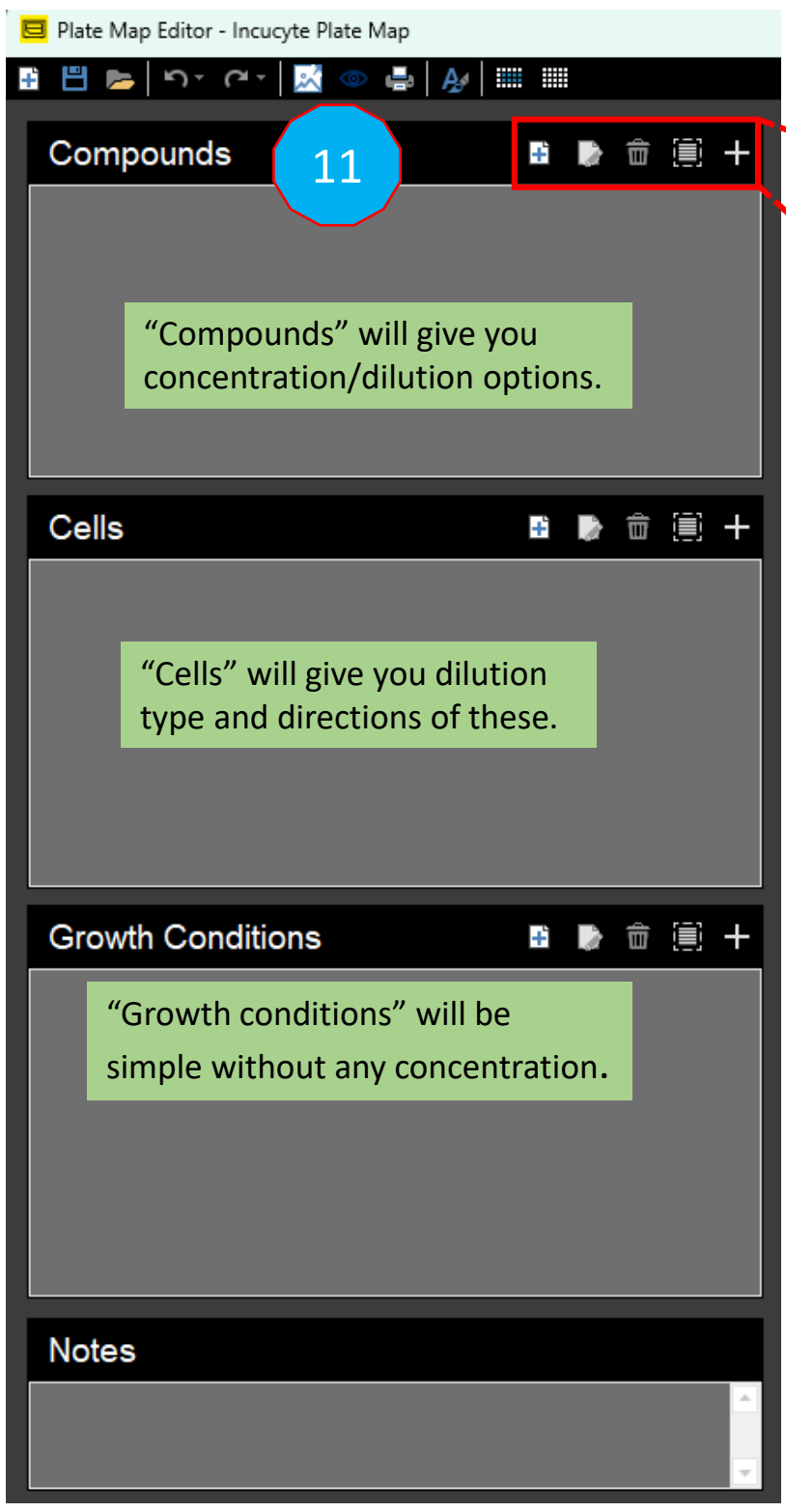


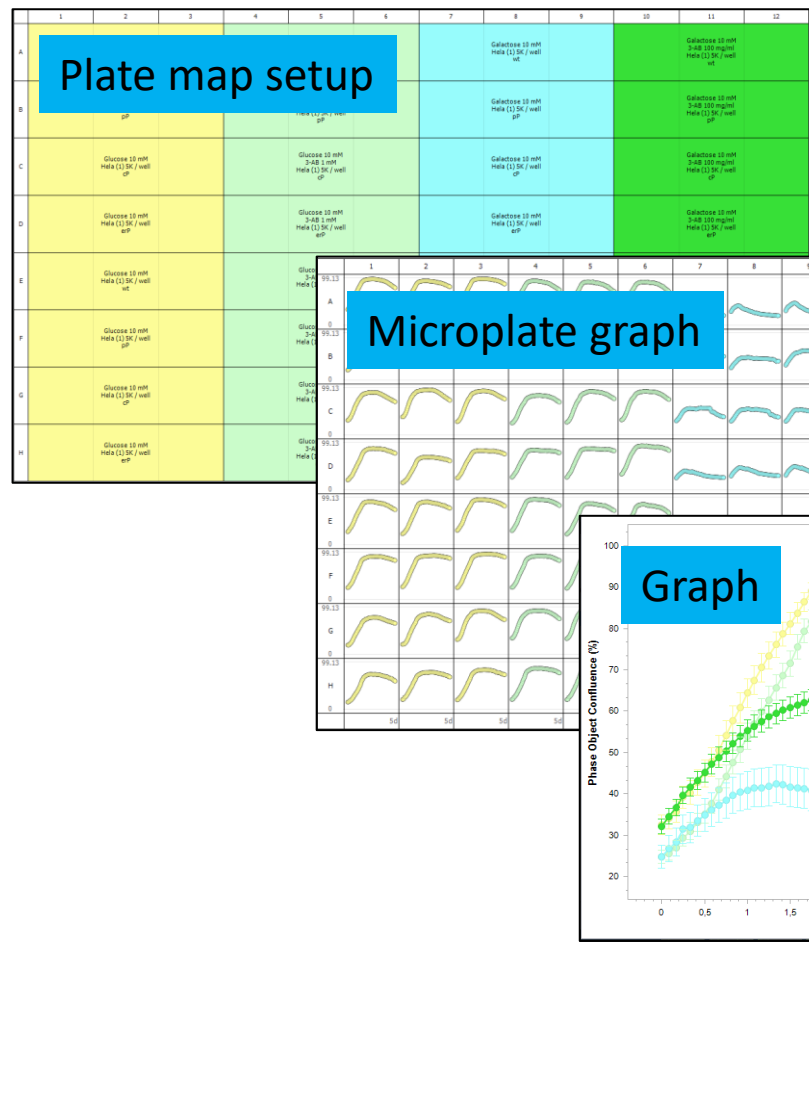


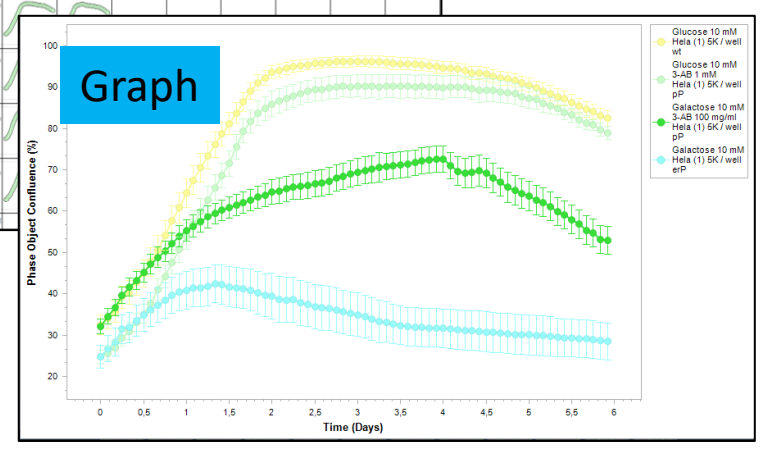
Scheduling a scan - continues



10. To add a plate map, select +.
11. Define the relevant experimental parameters, such as compounds, cell types and growth media. The plate map can be saved, exported and imported and is required for the final analysis.



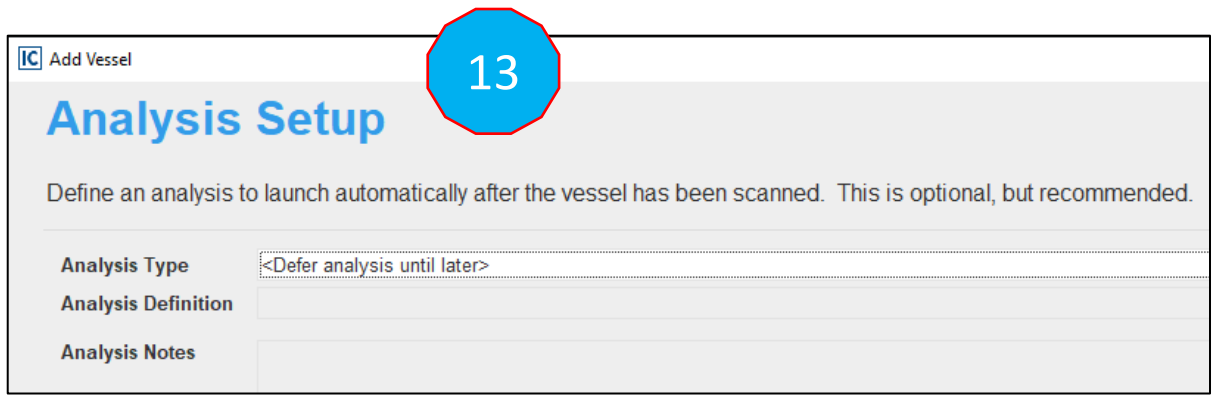




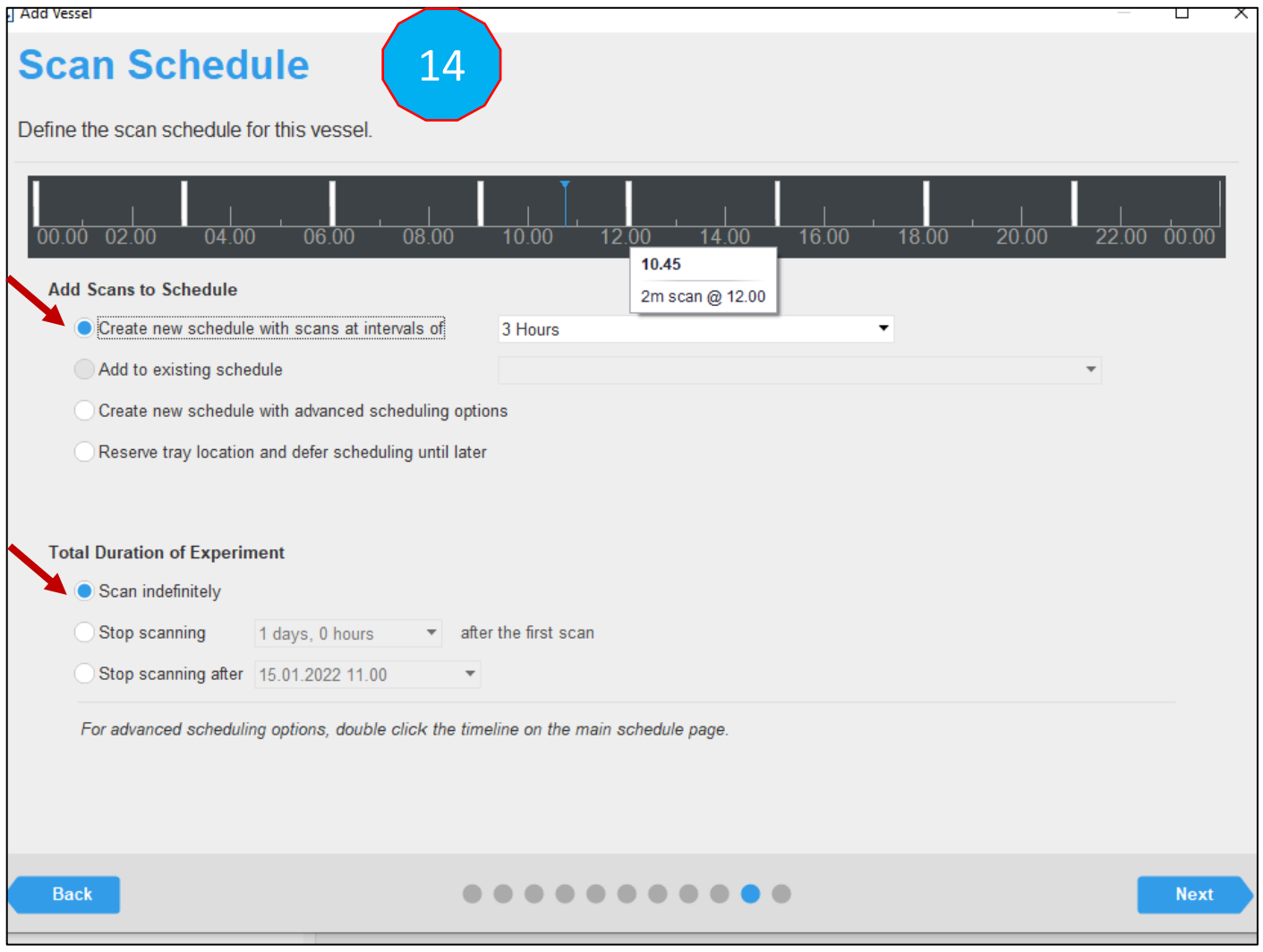
Why do I need a plate map?

A plate map organizes your experimental conditions across the plate and enables automatic grouping of replicated during data analysis, simplifying comparisons and data visualization.

Scheduling a scan - continues



13. You can skip the **Analyze setup** step (recommended) unless you have an analysis template from a previous identical experiment.



14. Define the **Scan Interval** for your experiment. Ensure that the selected time points do not conflict with other scheduled scans. If they do, try moving your scheduled scans.

Define the **total duration of experiment.** You can leave the duration set to indefinitely (recommended!) and stop the experiment by removing the plate once you have your images.

Important: Always remember to physically remove your plate from the incucyte when your experiment has been completed.

How often should I scan?

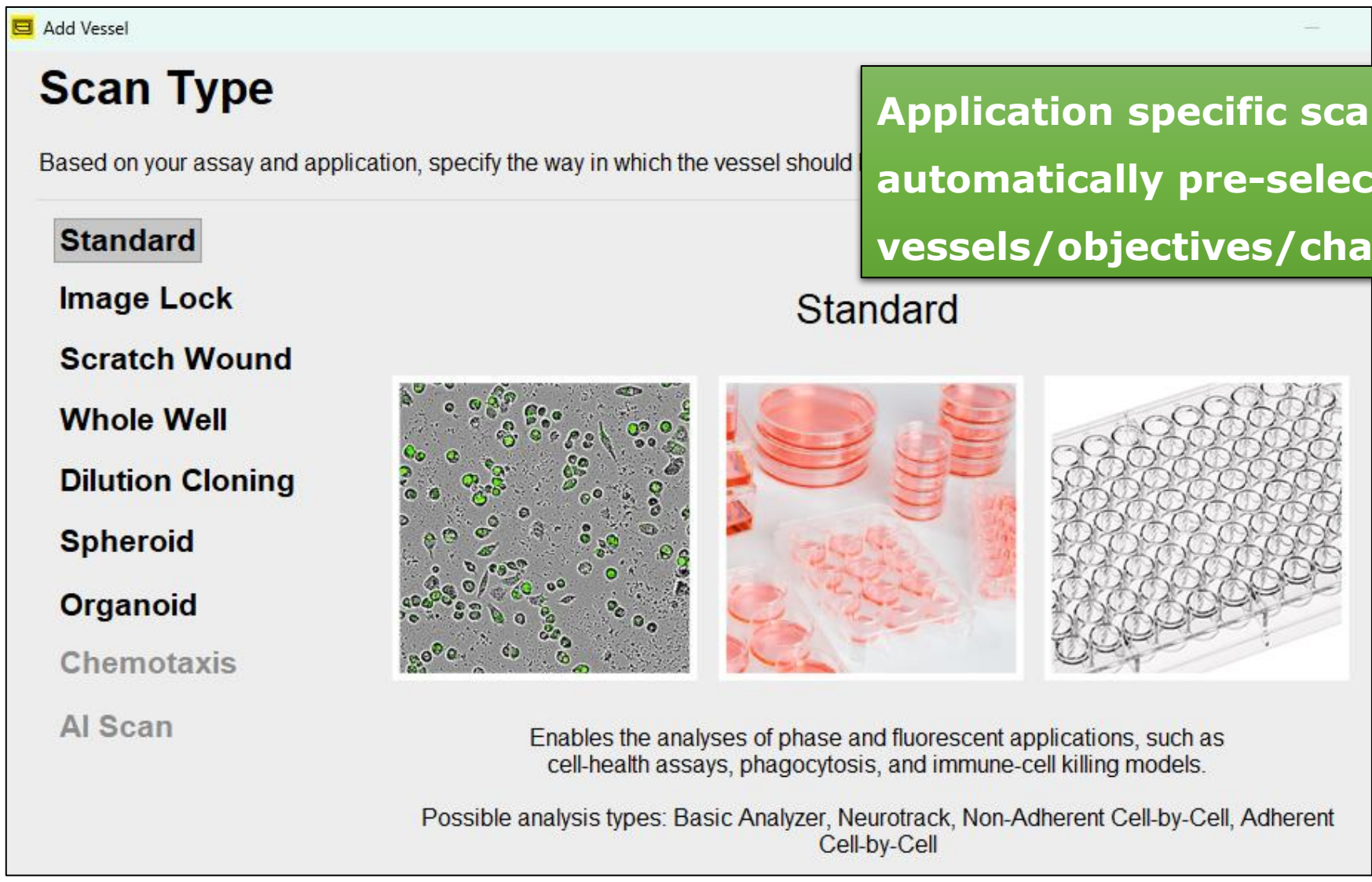
Scan frequency depends on the type of assay and the speed of the biological process being measured.

Avoid over-sampling and choose an interval that captures meaningful changes without generating unnecessary data.

This table indicates recommended scan intervals for common applications.

Common experiments	Typical sampling interval
Phagocytosis or Immune Cell killing	15-30 min
Scratch wound	0.5-2 hours
Proliferation and Cell Health	2-4 hours
Colony formation, spheroids, organoids	4-6 hours

Scheduling and scan types

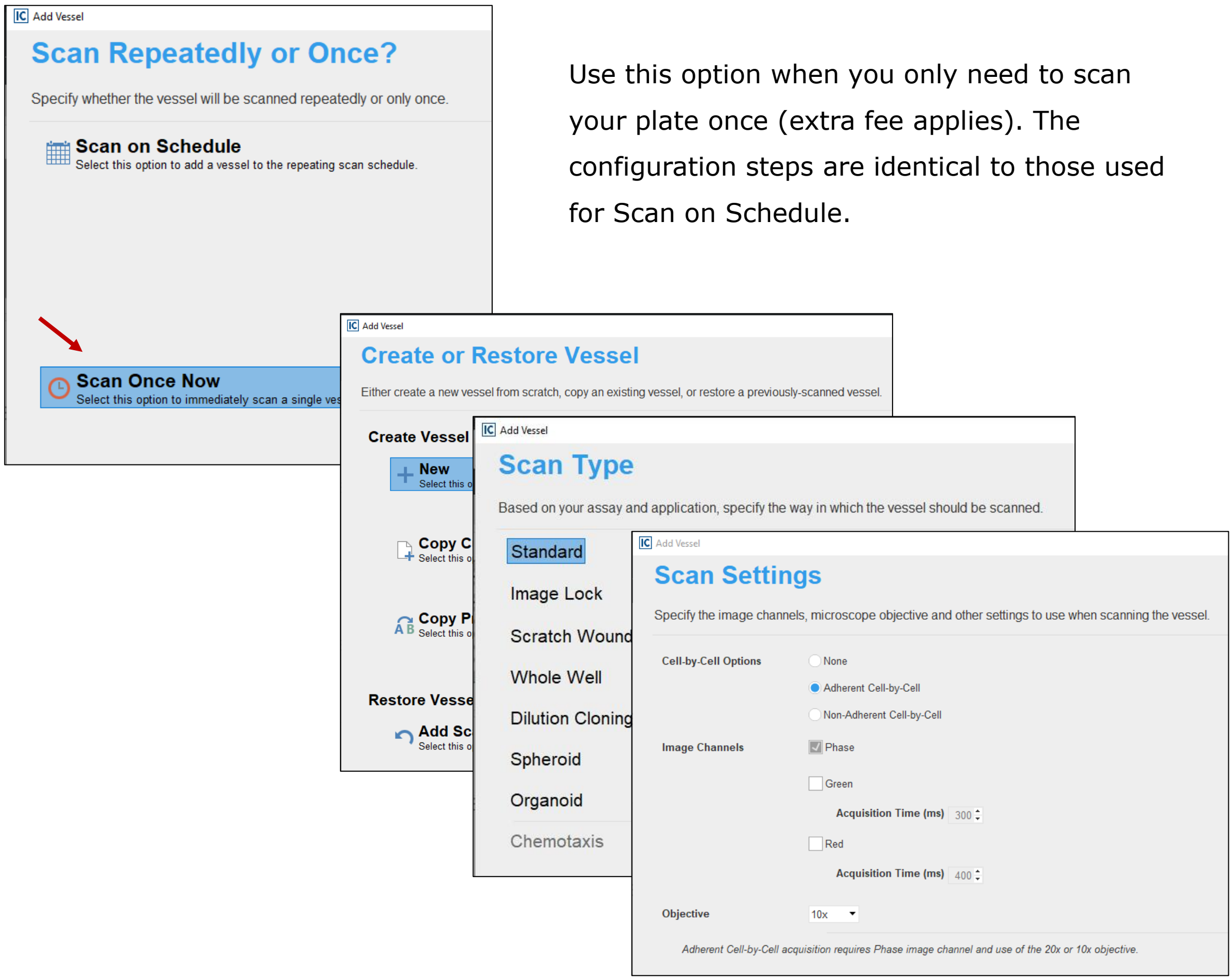


Application specific scan types will automatically pre-select appropriate vessels/objectives/channels.

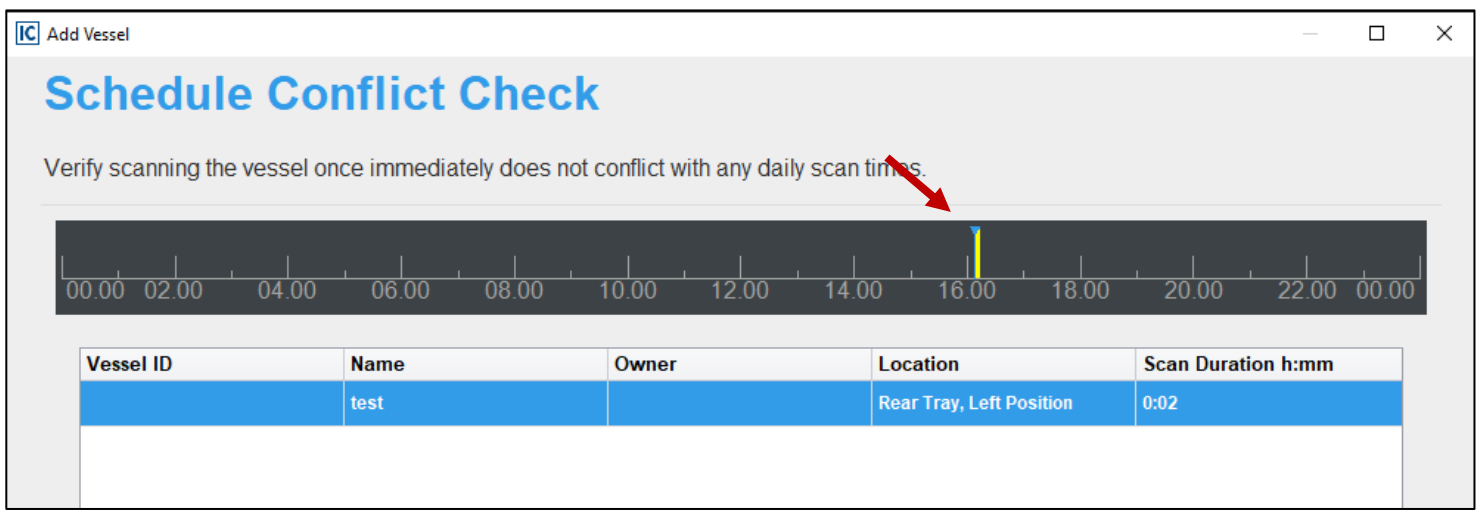
Scan type	Objectives	Plates available	Comment
Standard	4x, 10x, 20x	100s of plates available	Commonly used for assays including cell health, phagocytosis and immune cell killing experiments.
Image Lock	4x, 10x, 20x	Only 2 plates available	Especially designed for accurate xy positioning and export of movie. For advanced analysis, “use scratch wound.”
Scratch Wound	4x, 10x, 20x	Only 2 plates available	Wide mode not available for 4x and mandatory for 20x
Whole Well	4x	Corning (6-384 wells), TPP	For usage of 35 mm petri dish (from TPP), you need a special metallic rack (which we do not have).
Dilution Cloning	4x	Corning plates	96 and 384 wells. Imaging is done over the whole well.
Spheroid	Single spheroid (4x, 10x) Multi spheroid (10x)	Brand, Corning, S-Bio	Single spheroid (96-384 wells), Multi spheroid (only 96 well from Corning). Embedded multi spheroid (4x) with Corning 96 well.
Organoid	4x	Corning 24-48 and 96 wells	QC with Corning 24 or 48 well plate. Assay with Corning 96 well plate.

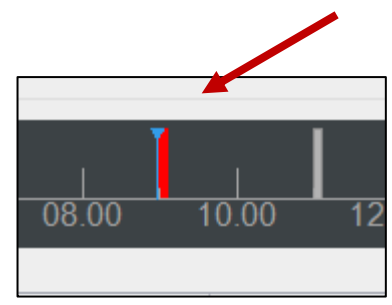
It is important that to seed you cells, spheroids or organoids in the appropriate plate format. Before planning your experiment, use the software to search for and identify the most suitable plates for your experiment.

Scheduling a **Scan Once Now**



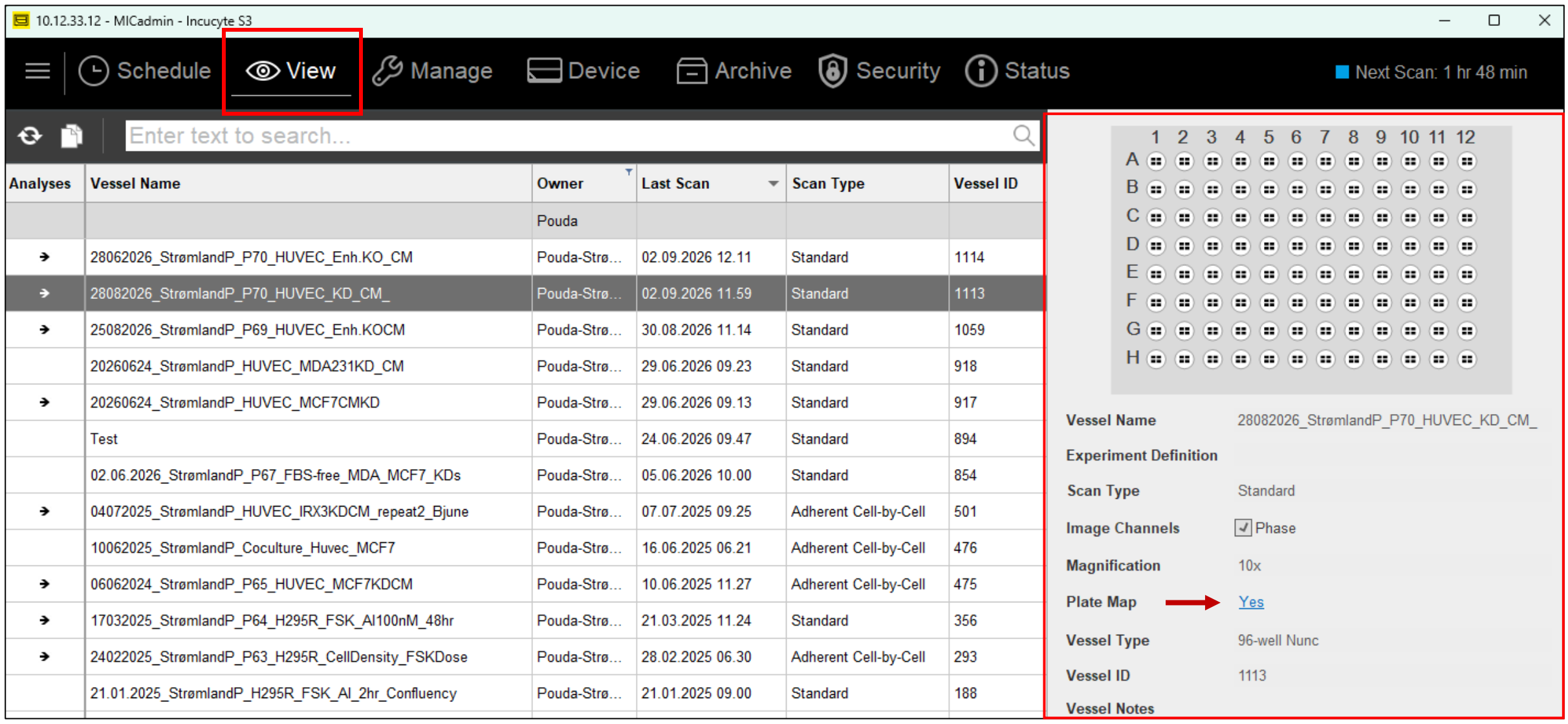
Use this option when you only need to scan your plate once (extra fee applies). The configuration steps are identical to those used for Scan on Schedule.



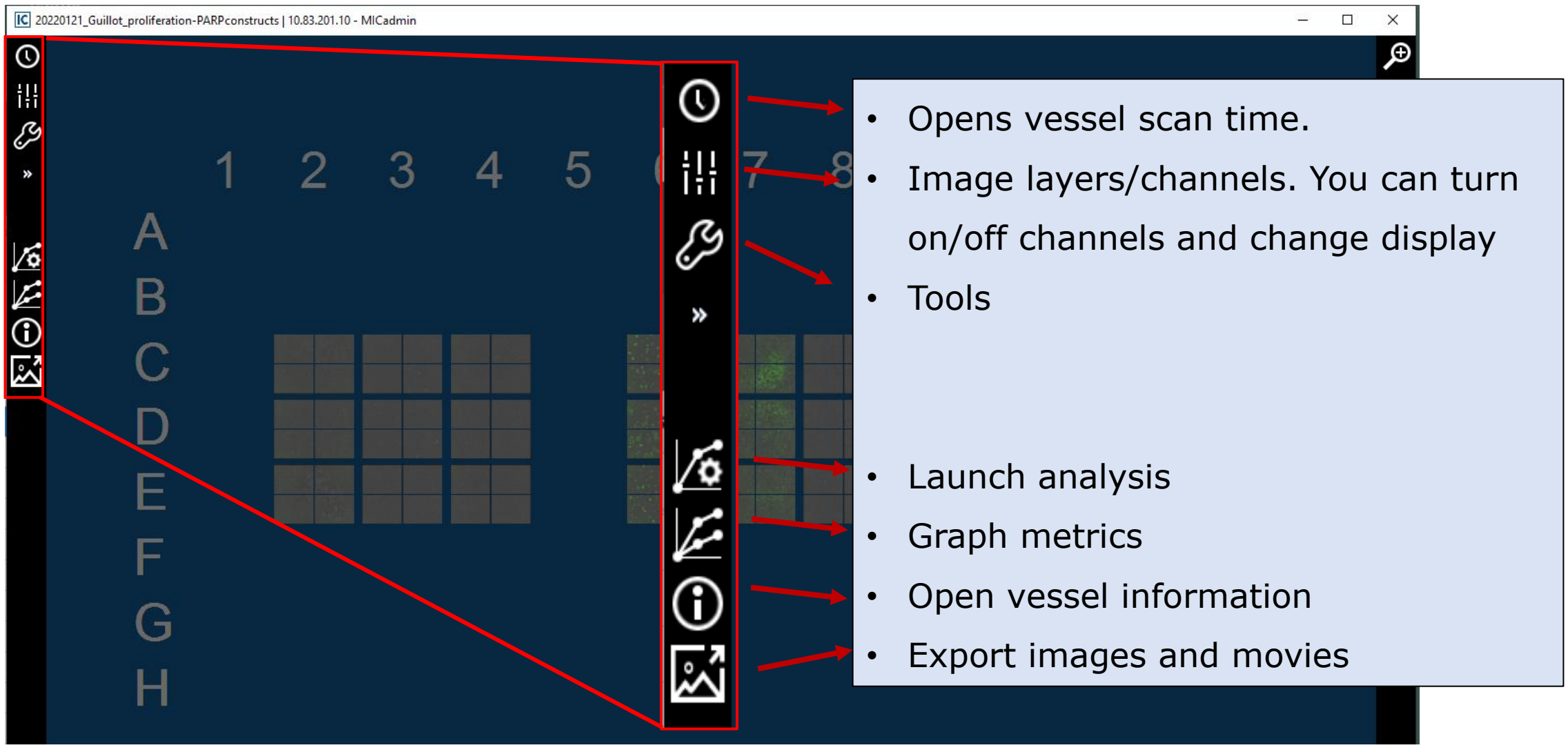


Before starting, the software checks for schedule conflicts. If no conflict is detected, the schedule bar will be highlighted in yellow and the Next button will become available. Conflicts are displayed as red bars, indicating that the scanner is unavailable during that time. Please wait for an open slot before launching the scan.

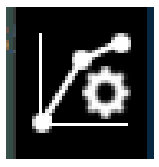
Viewing your data set



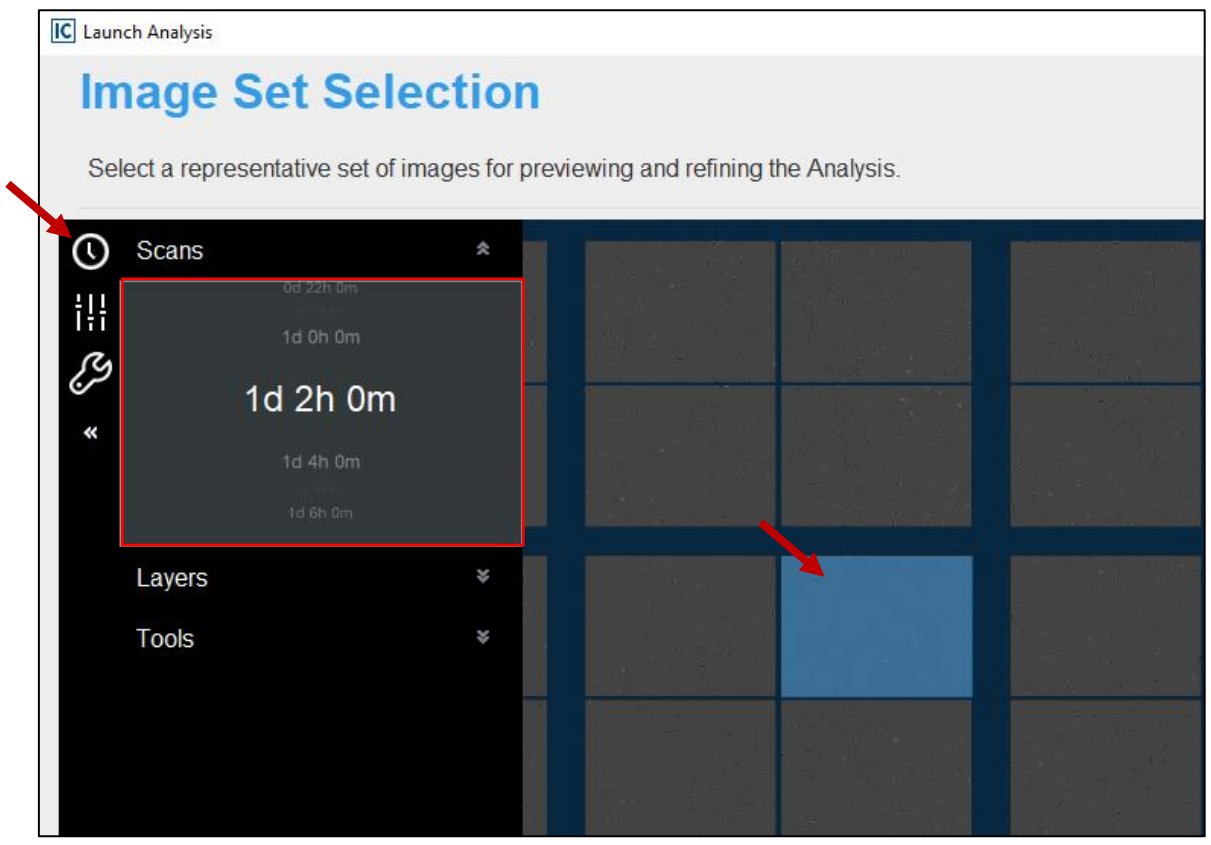
- Click **View** to display the list of scanned vessels. To find your experiment, sort the list by your username in the **Owner** list.
- To view additional experiment details, select a vessel. Relevant information will appear in the panel on the right. The plate map can also be accessed from this panel.
- To open an experiment, double-click the corresponding experiment line.



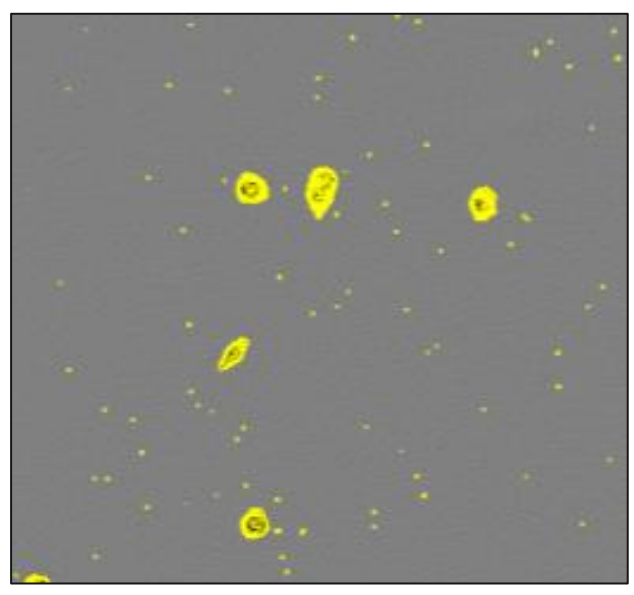
Analyzing a confluency experiment – BASIC Analyzer



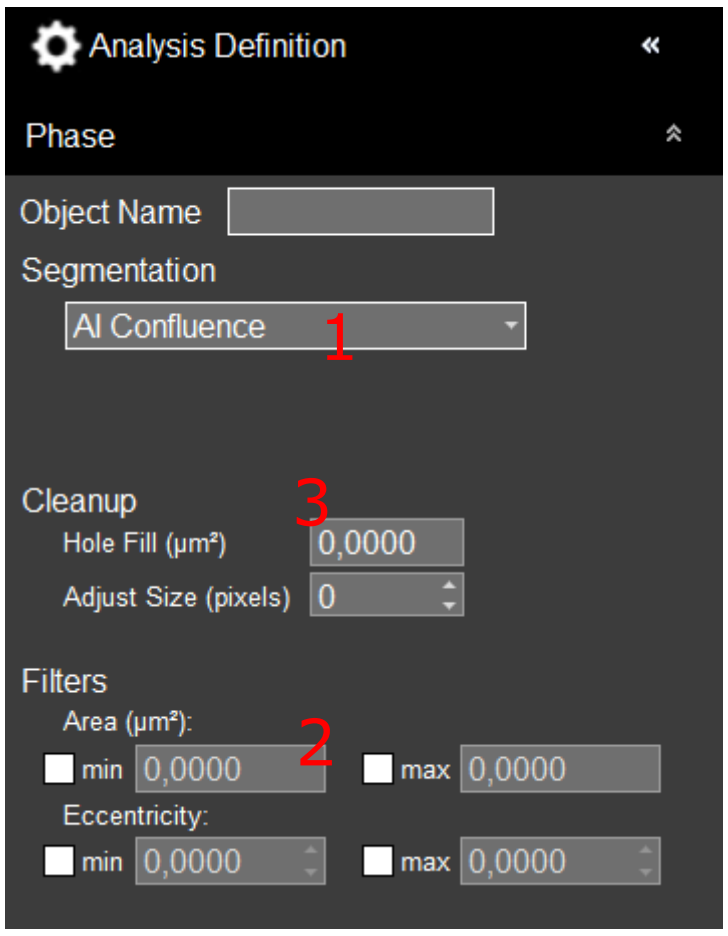
- Open your experiment and start the analysis by following the guided wizard step by step.
- Select **Create New Analysis Definition,** then choose **Basic Analyzer**.
- Select the image channels you want to include in the analysis.
- In **Image Set Selection**, choose 4-8 representative images from different time points. Once you have selected the images, click Next.



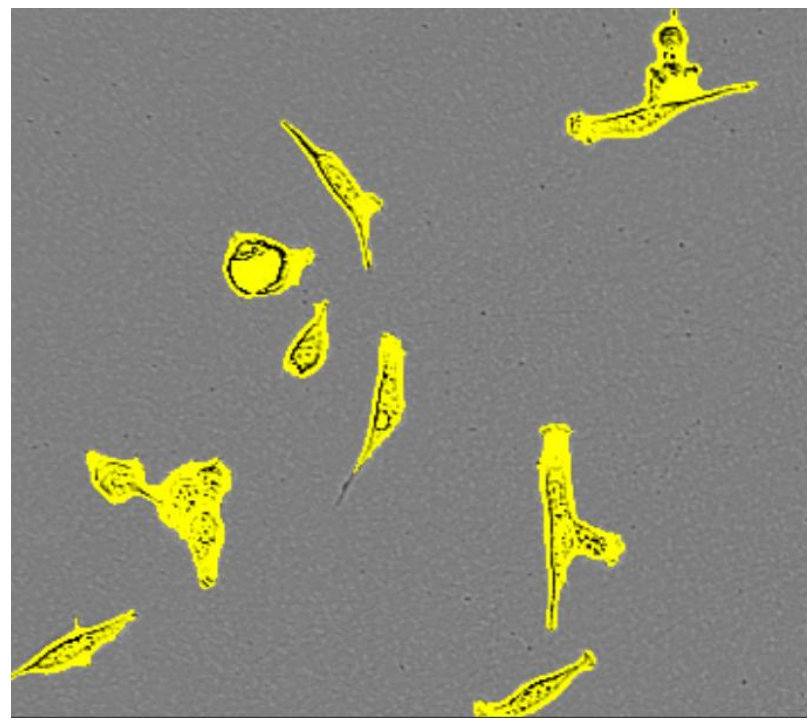
- The selected images will now be downloaded to your computer.
- To begin the masking process, click **Preview Current**. A default mask will be generated and displayed on the image. The next section explains how to optimize the mask settings to improve the segmentation results.

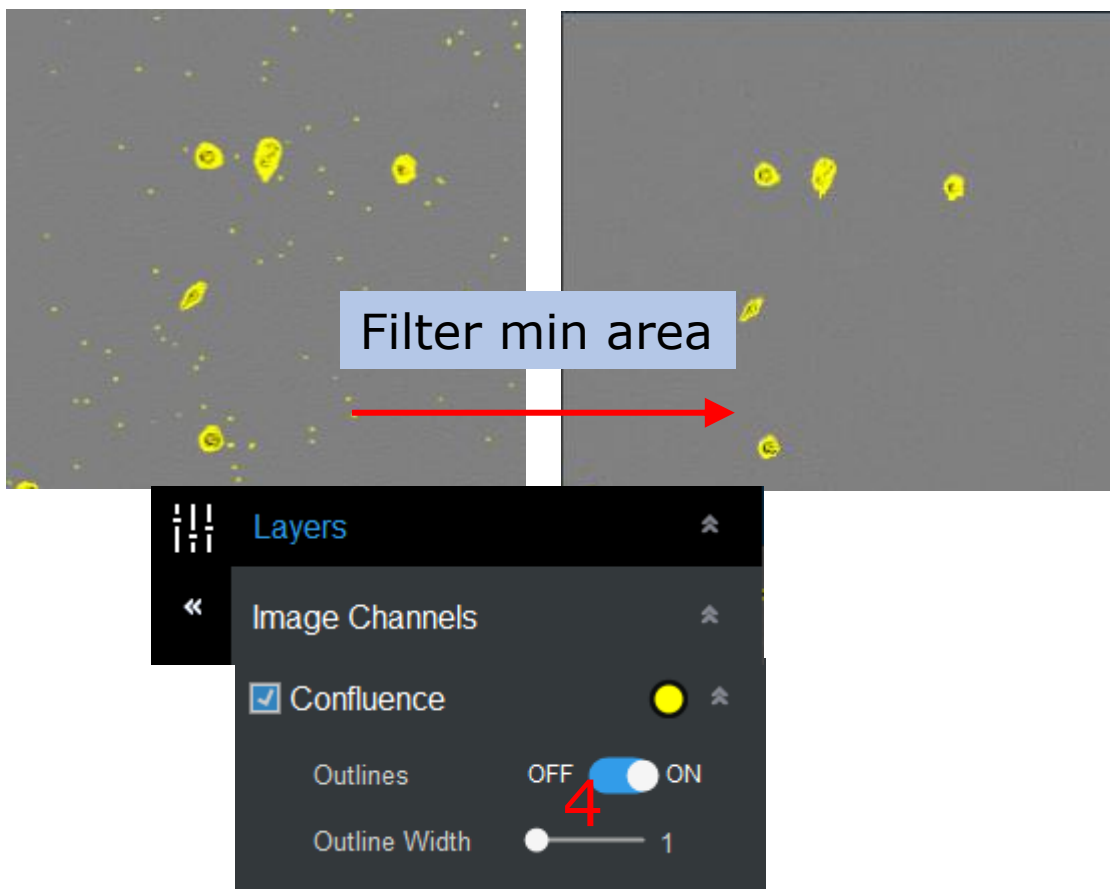


Optimizing and improving the masking



- **Segmentation:** Use the new **AI Confluence** feature(1) to generate cell segmentation masks.

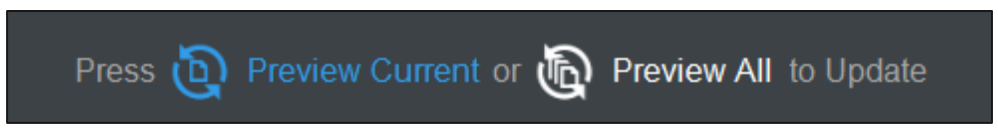




- To remove background artifacts, use the minimum **Area** filter (2).

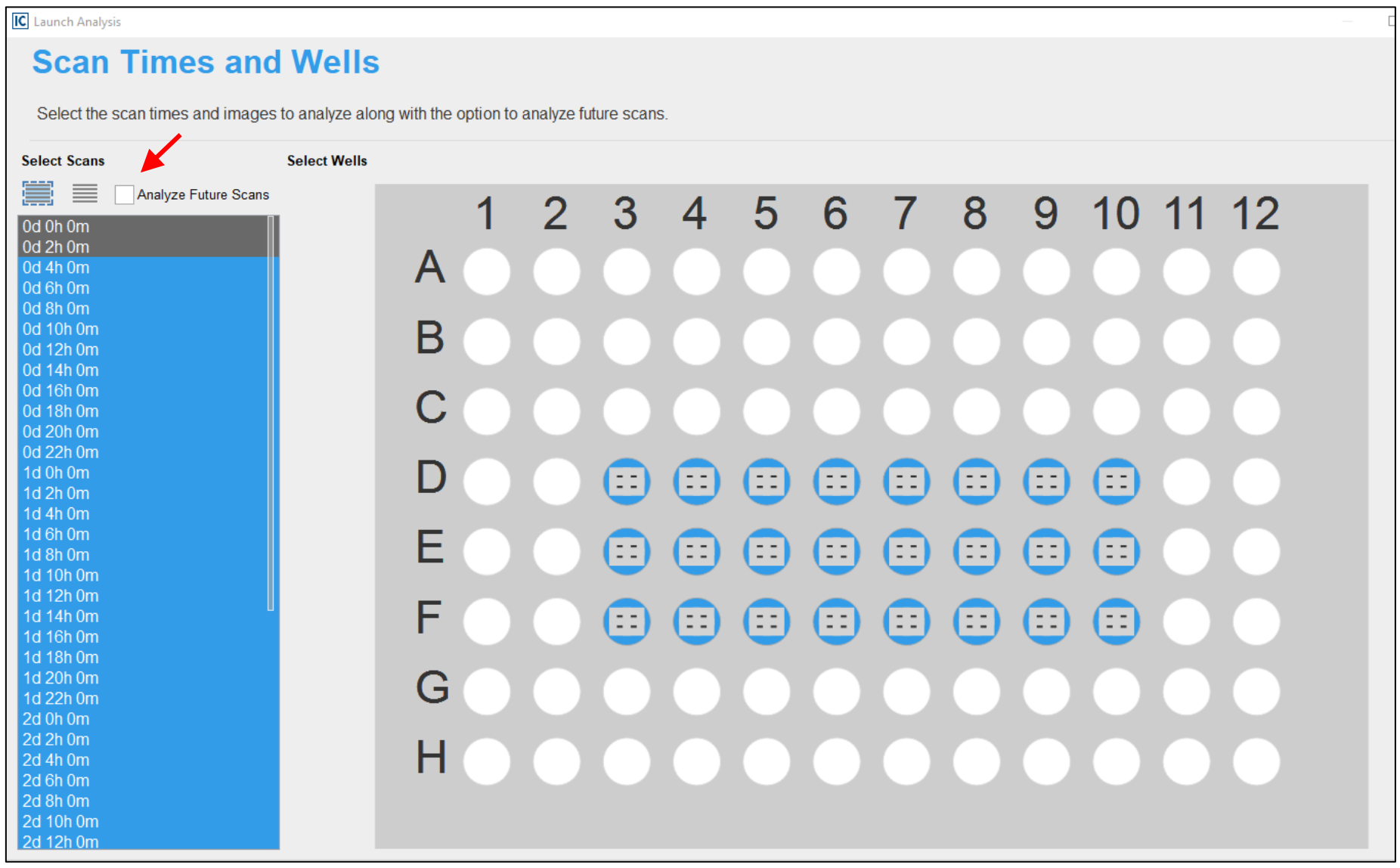
- If the mask contains holes, apply the **Hole Fill** option (3) to create more continuous cell segments. If the mask obscures the cells and make evaluation difficult, switch the display mode to **outlines** (4).

Analyzing a confluency experiment - continues

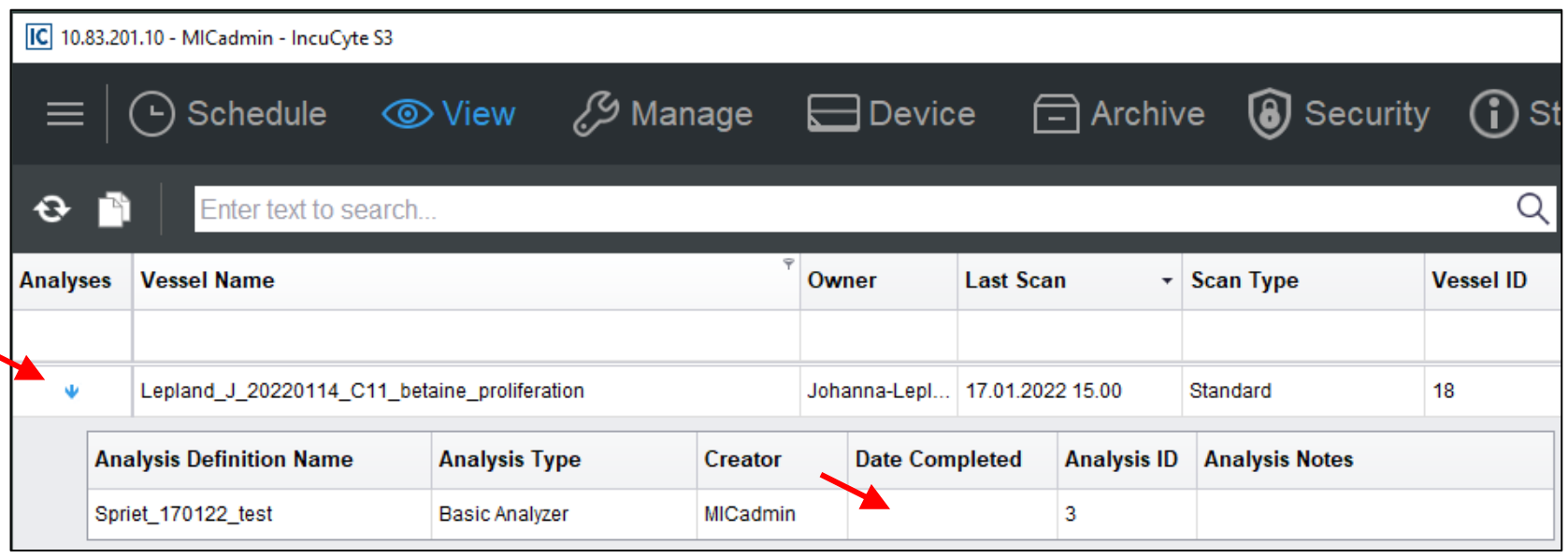


- Once you are satisfied with the masking result, click **Next**.

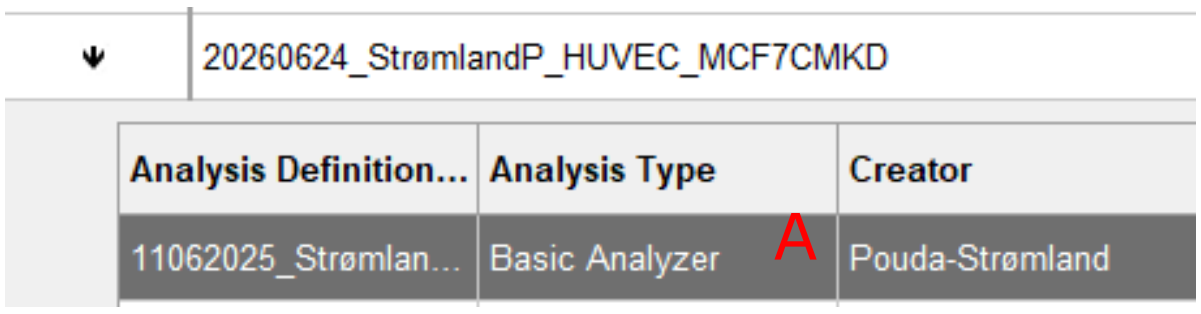
- Select the wells to be analyzed and choose the relevant time points. If the experiment is still running, also enable the **Analyze Future Scans** option.
- Click Next, enter a name for the analysis definition, and launch the analysis.
- You can monitor the progress by clicking the blue arrow next to the experiment in the View window.



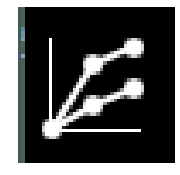
- The analysis results will only be available once a completion date is displayed.



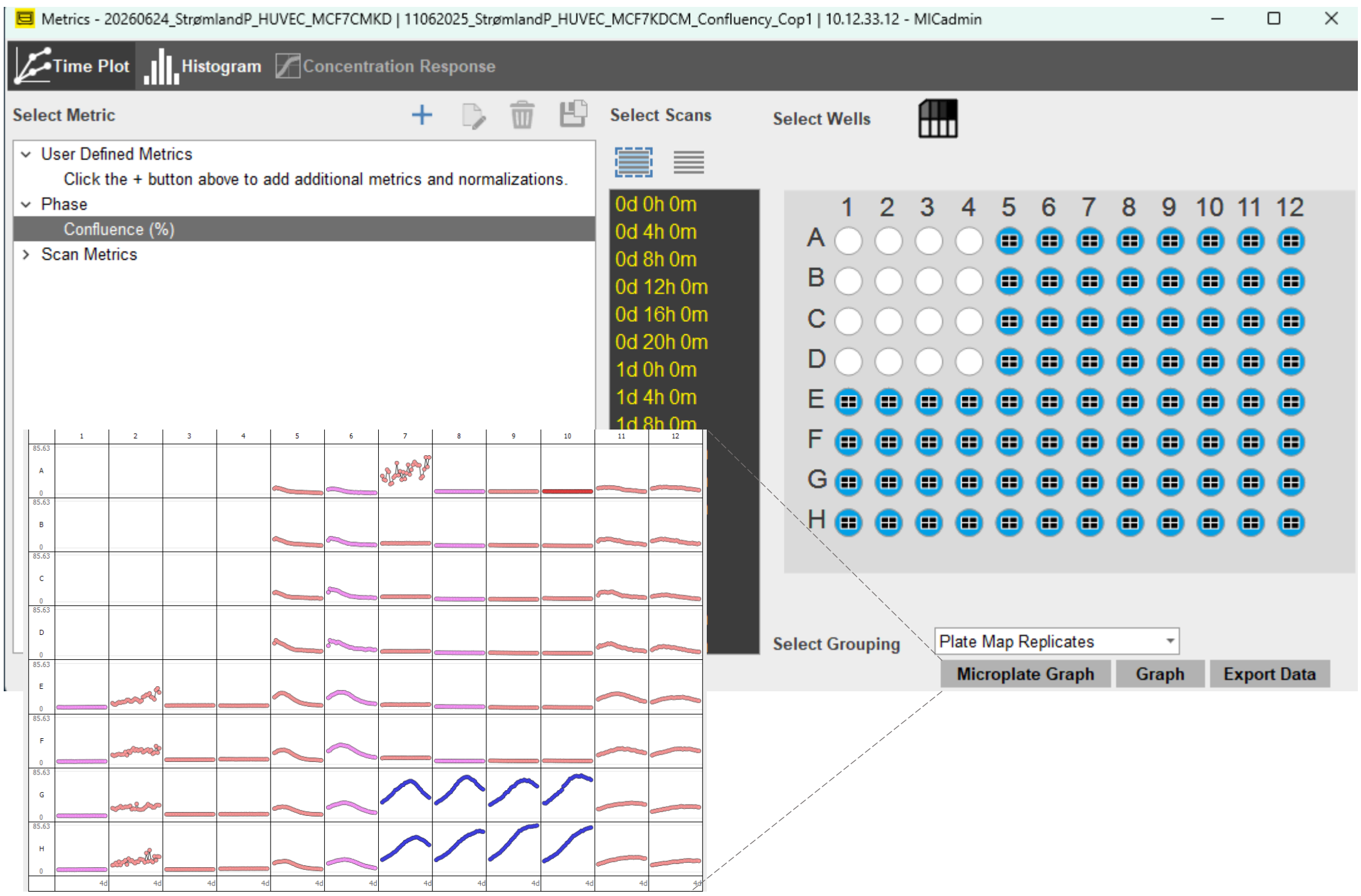
Analyzing a confluency experiment continues



B

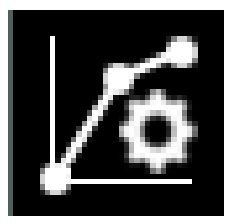


- Open the final analysis by clicking the arrow next to your experiment under View and open the analysis definition (A).
- Click the Graph Metrics icon (B).
- Check the microplate graph to get a quick overview of all the wells on the plate.
- Create a graph and select **Grouping** - **Plate Map Replicates**. This option is available only if a plate map has already been created for the experiment.



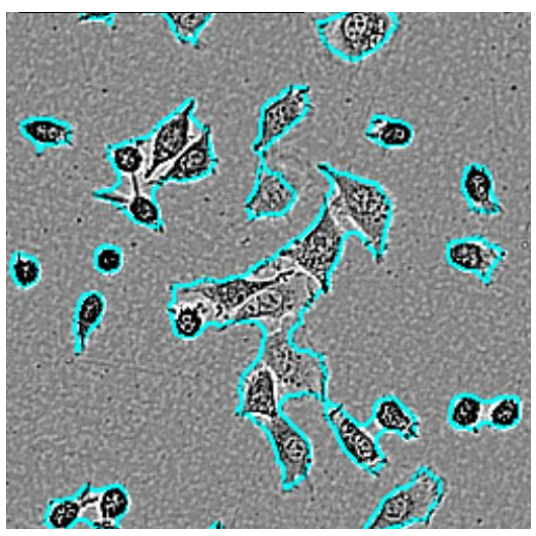
The Microplate Graph provides a visual overview of all the wells on the plate allowing you to quickly assess treds and potential problematic wells.

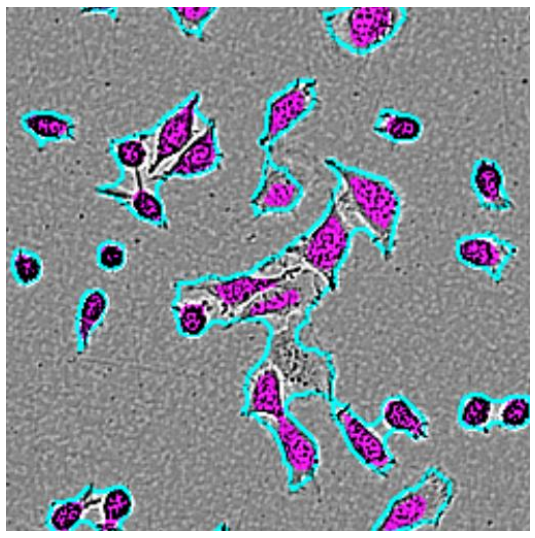
Analyzing a confluency experiment – **Adherent Cell-by-Cell**

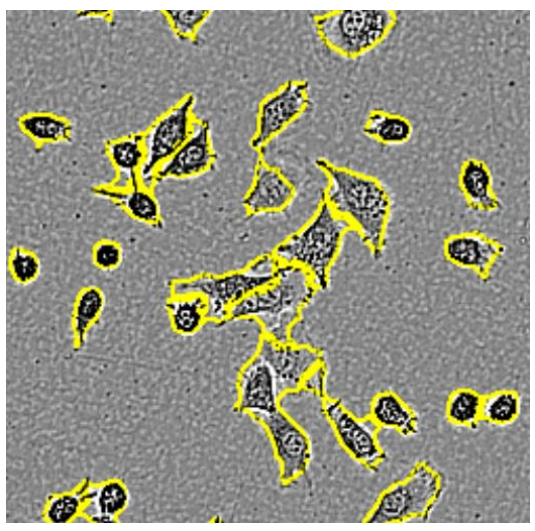


Adherent Cell-by-Cell

- Open your experiment and start the analysis by following the guided wizard step by step.
- Select **Create New Analysis Definition,** then choose **Adherent cell-by-cell**.
- Select 4-5 images and press **Preview**.







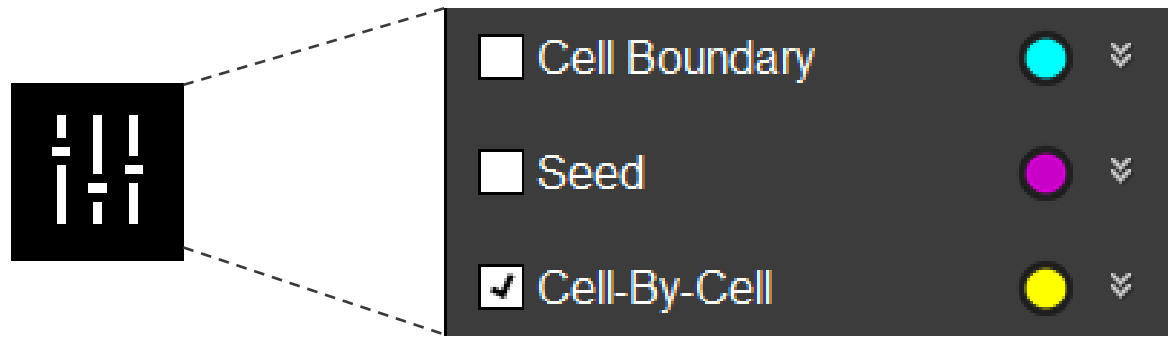
Three key parameters need to be defined:

- **Cell Boundary** – adjust the **Segmentation** slider to control wheather the algorithm detects more cells or places greater emphasis on the background. You can also adjust the Size setting to improve how closely the mask matches the cell boundaries.

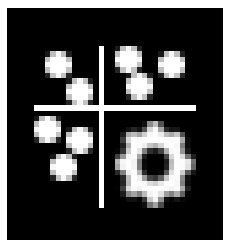
- **Seed** – the goal is to generate one seed per cell. Optimize the seed detection by adjusting **Cell Detection Sensitivity**, **Cell Contrast** and **Cell Morphology** until the seeds accurately represent your cells.

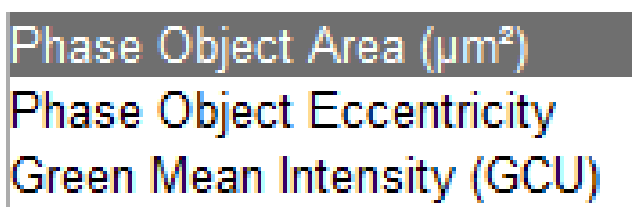
- **Cell-by-Cell** – use this option to filter objects based on size and eccentricity, allowing you to exclude objects that are too small/large/round/elongated.

- Throughout the optimization process, it is recommended to toggle the channel mask on/off to compare the segmentation results with the original image and ensure accurate cell detection.

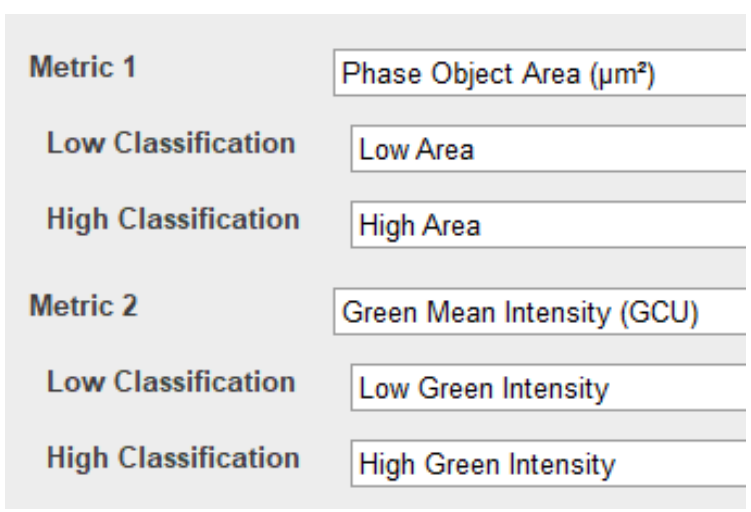


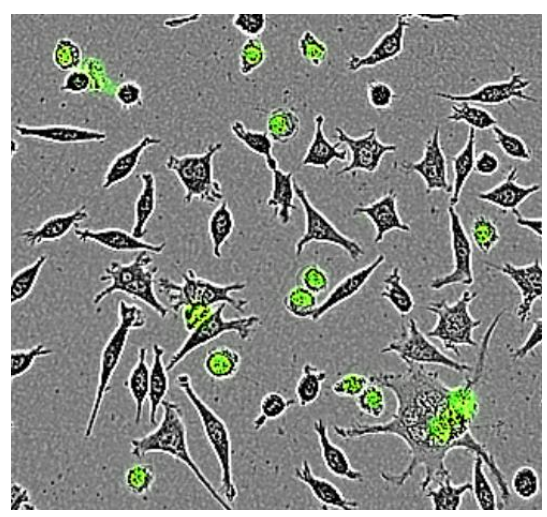
How to run the **Cell-by-Cell Classification**

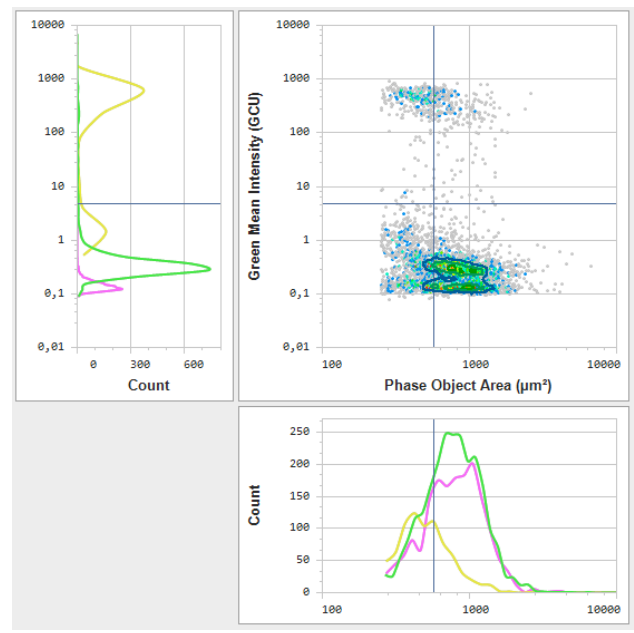




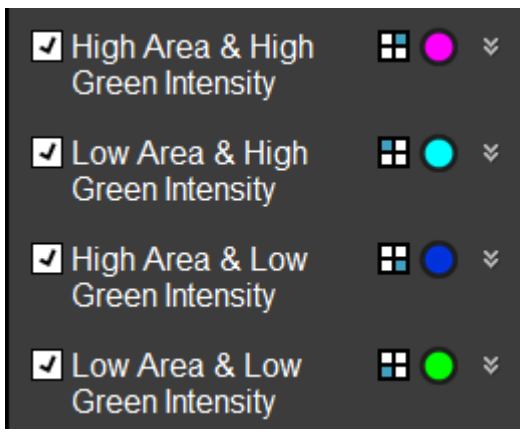
- In this example, two imaging channels are used: a phase contrast channel and a green fluorescence channel.
- Cell classification is based on two selected metrics. With phase-contrast and green images, up to three classification parameters can de defined. Alternatively, the analysis can be performed using data from 2 fluorescent channels.
- Use the resulting classifications to identify and quantify distinct cell subpopulations within your dataset.

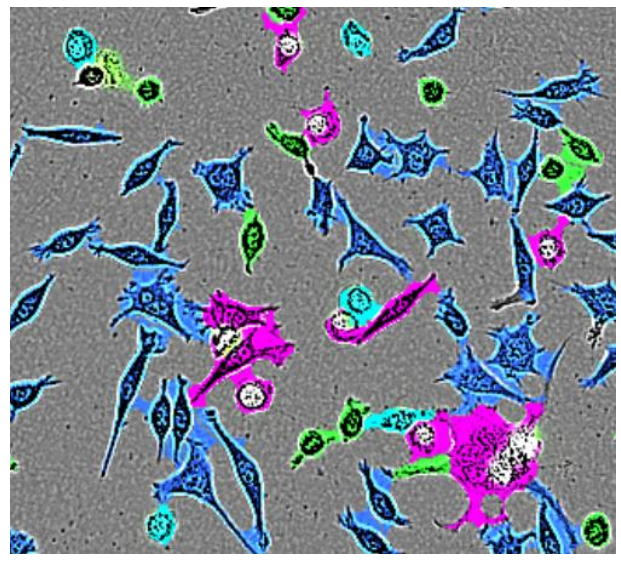


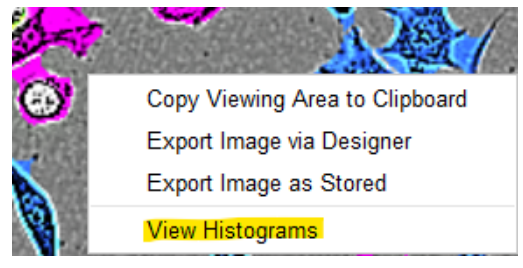




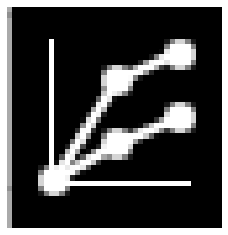
- Visualize the identified subsets using color-coded mask overlays. To further explore the data distribution, right-click an image to display the corresponding histogram or dot plot



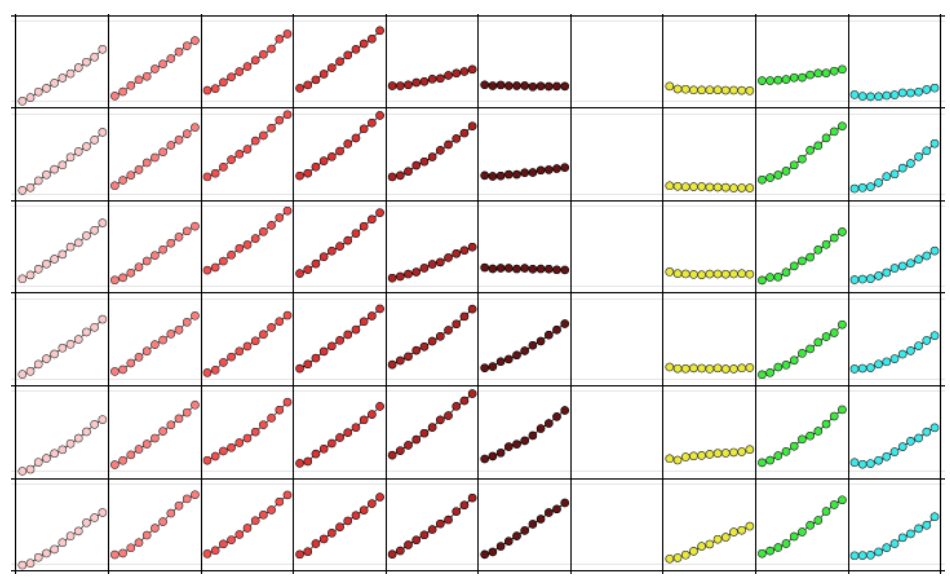




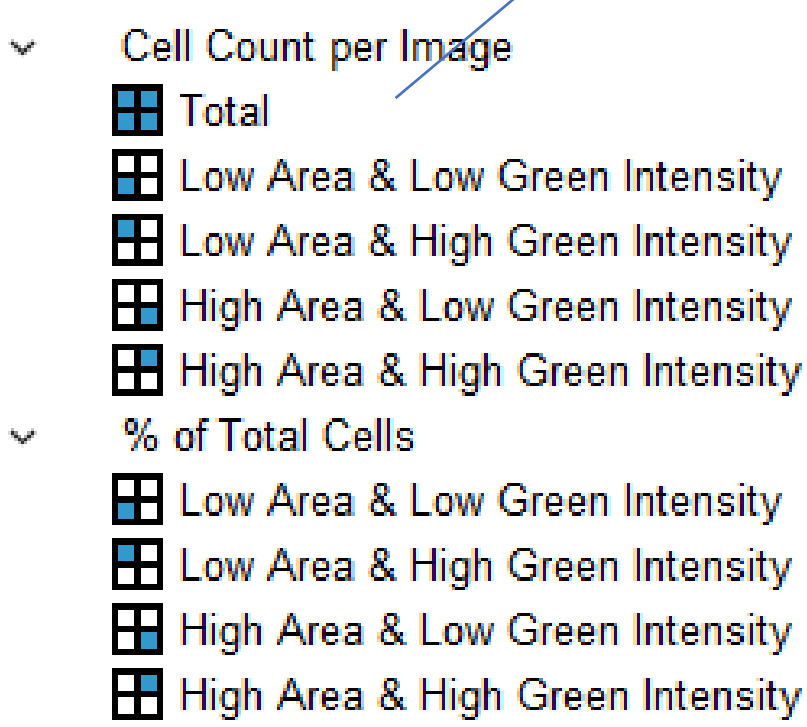
Cell-By-Cell classification – Graph metrics:

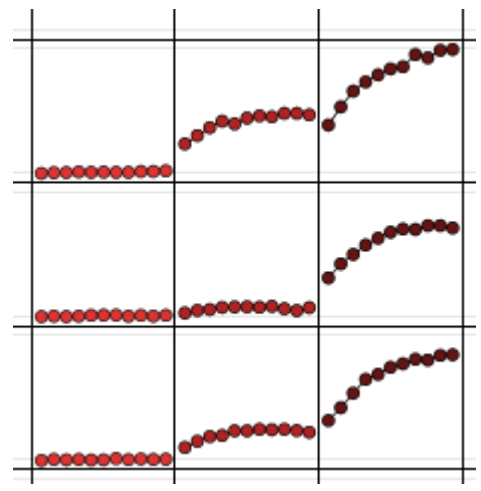


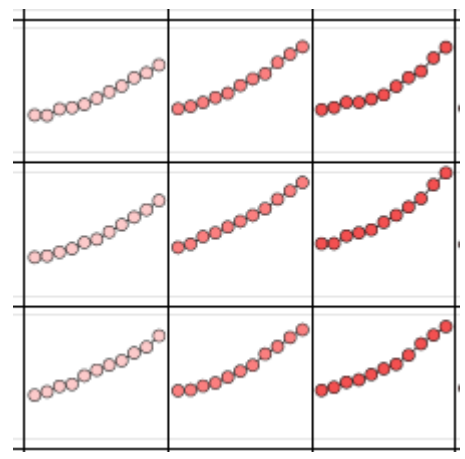
- You can visualize the classes of cell populations using phase and/or fluorescent metrics.
- Open the graph metrics and select two parameters to visualize at a time.
- It’s possible to view histograms per well at individual time points by right-clicking on an image (view histogram).



Total cell count







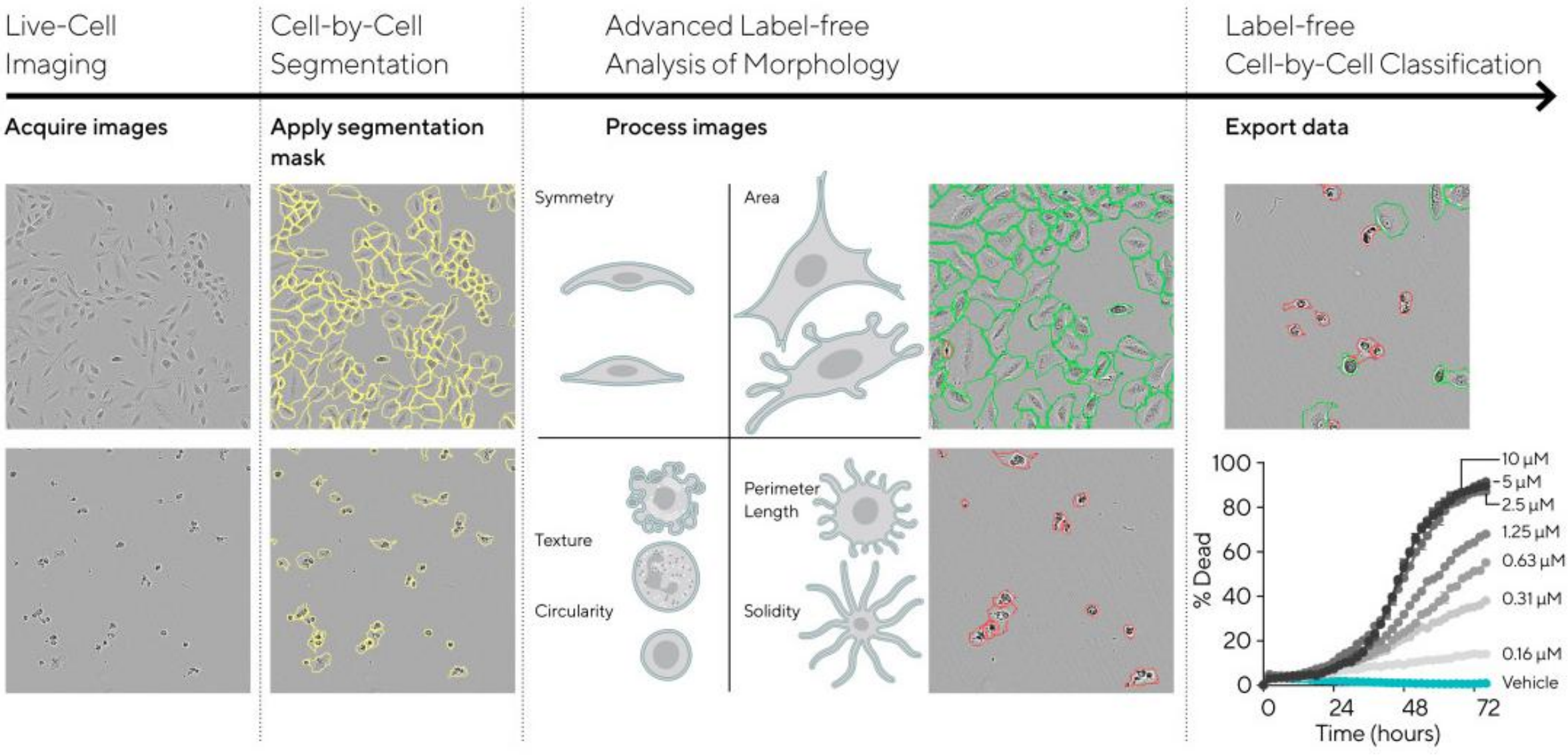
Advanced Label Free Module

The Advanced Label-Free Module is a software package that enables live-cell, non-invasive image analysis without the need for fluorescent labels, dyes, or reporters. Instead of detecting fluorescence, it uses sophisticated image processing and machine-learning-based segmentation to identify and quantify cells directly from standard brightfield or phase-contrast images.

This approach allows researchers to follow cellular behavior over long periods while avoiding potential effects of dyes or labels on cell health and function.

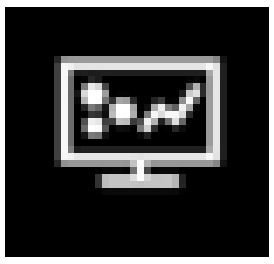
Examples of what Advanced Label Free can be used for:

Application	Class A	Class B
Live/Dead	Live	Dead
Differentiation	Monocyte	Macrophage, dendritic cell
Activation	Immune cell	Activated immune cell
Mitotic Index	Non-mitotic	Mitotic
Anything!	Anything with distinct shape and well-segmented	Anything with distinct shape and well-segmented

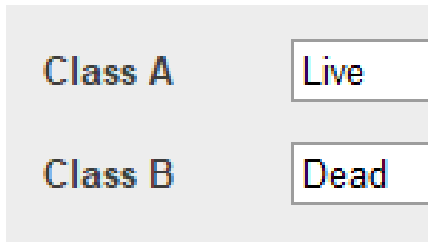


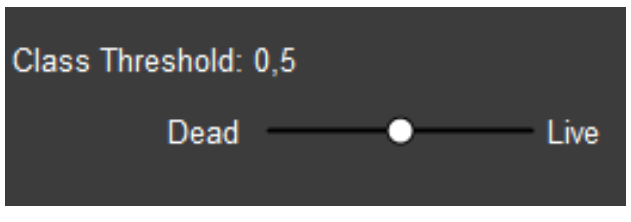
Keep in mind that you need to run your experiment in Cell-by-Cell module.

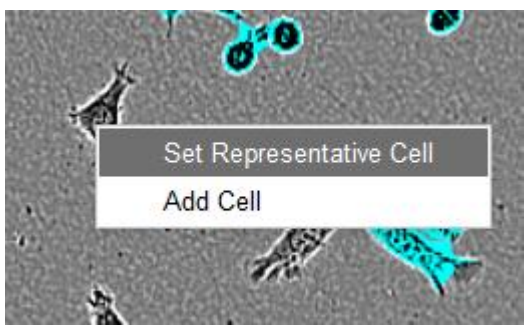
Advanced label-free Classification:

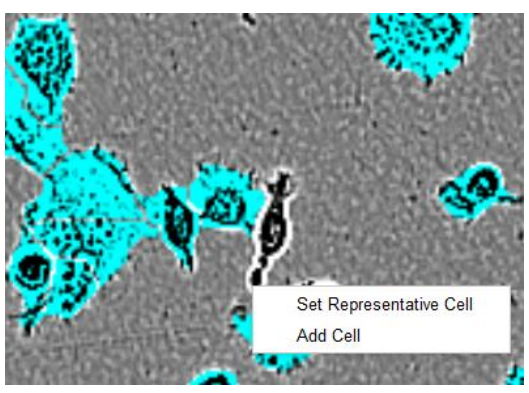


This module allows you to classify adherent cells into two user-specific groups based on their morphology. It requires you to have control wells showing as much as possible each morphology classes.



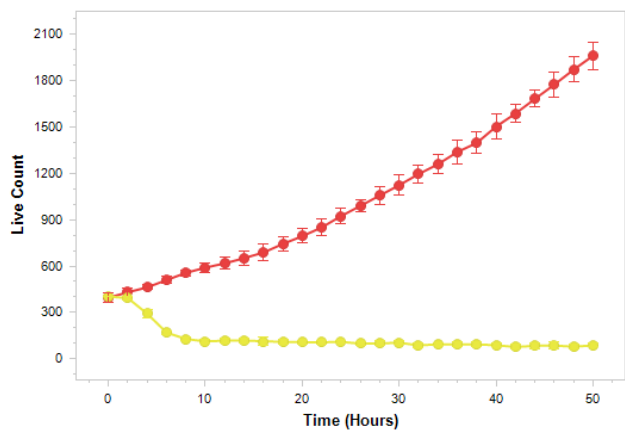


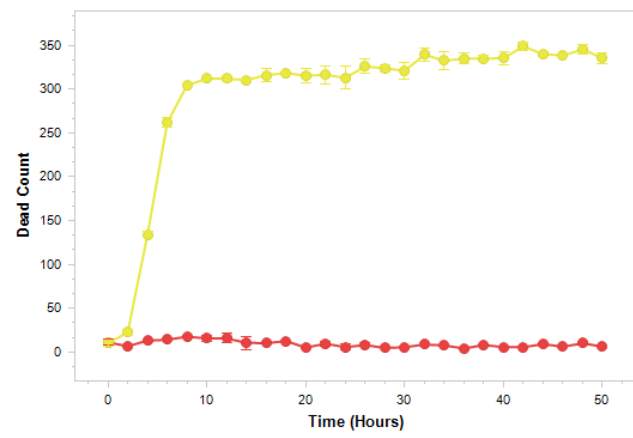




- Provide the name to your two classes and select wells (1-2) and timepoints (1-2) where these type of cells are is abundance.
- Select a few experimental images for the image set selection.
- Estimate the threshold between the two classes. If you don’t agree with what you see, then you might have to refine your two training sets: advanced training set selection.
- **Refinement:** Estimate how many cells are similar.

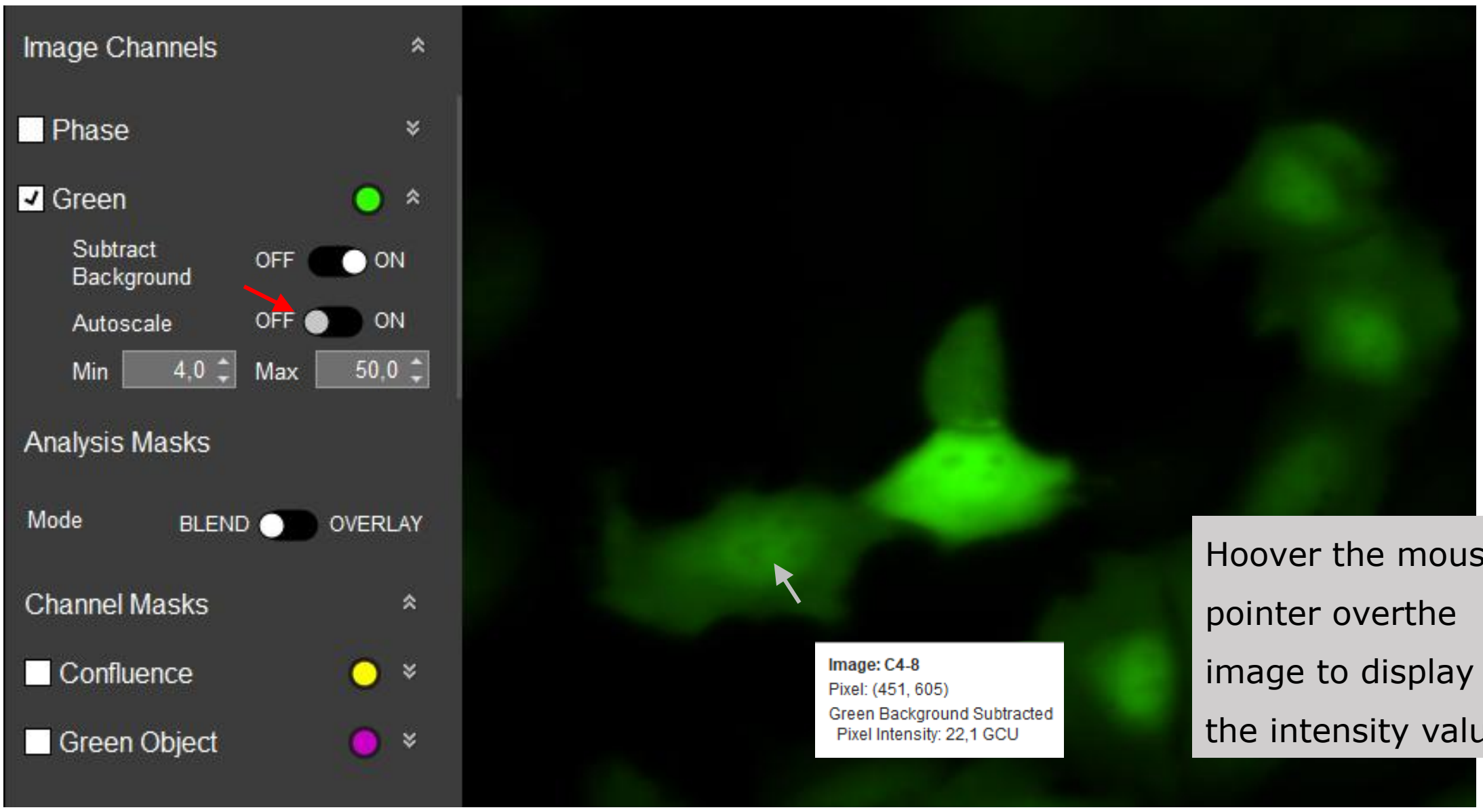
  Set a representative cell. Add cells and remove cells to train your analysis definition.
- Do the refinement for the other channel as well.
- You now have two populations based on the change of cell morphology.

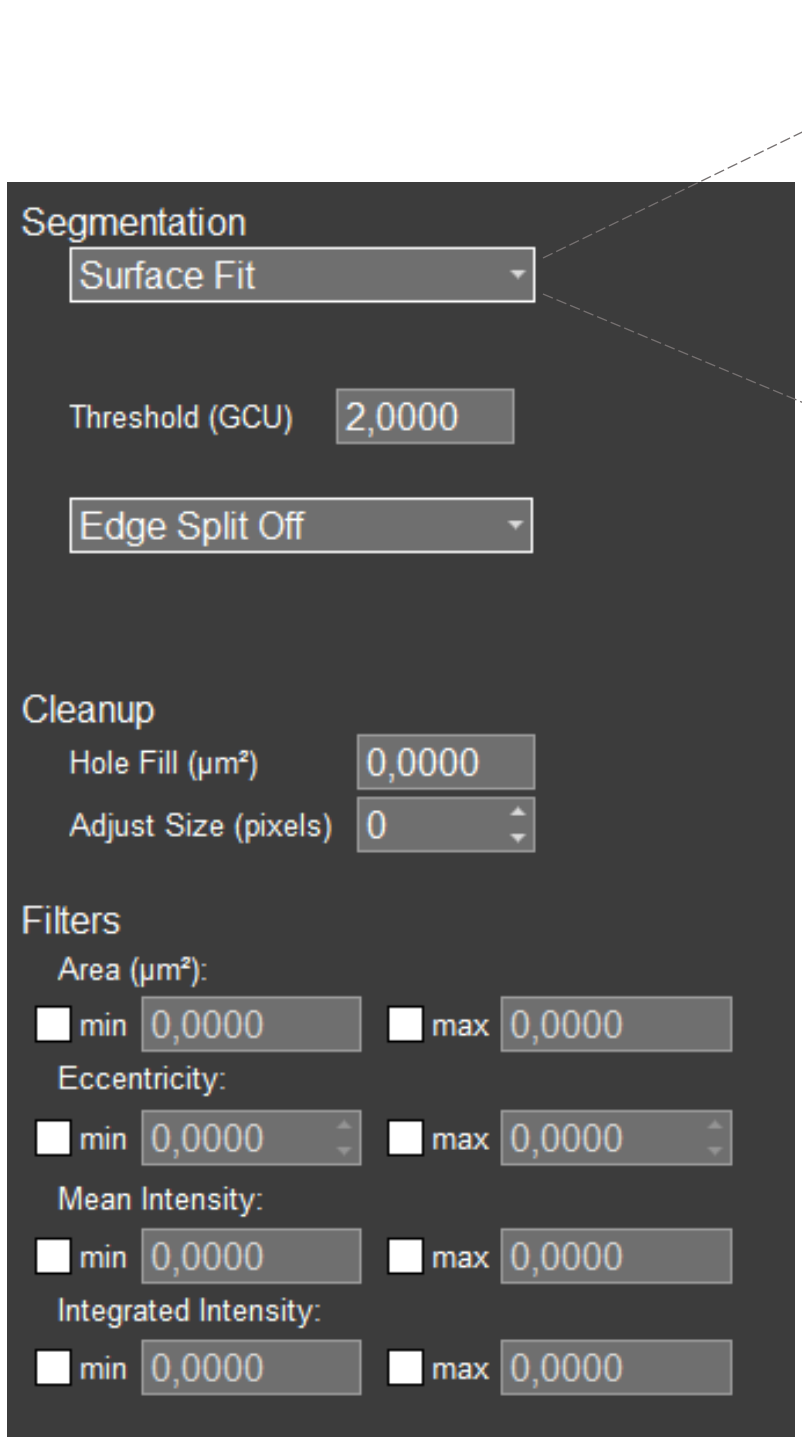


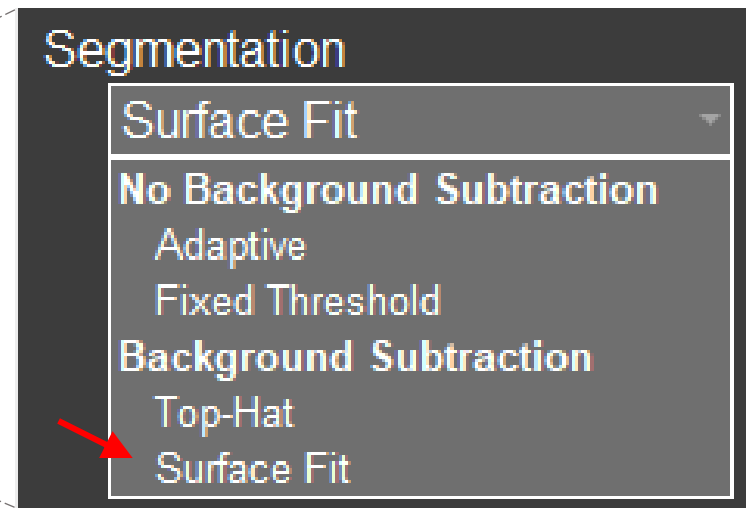


Analyzing a fluorescence channel

- Be aware that you can remove the autoscale in order to better visualize the florescence display in your image. Set the min and max values (GCU - green calibrated units)



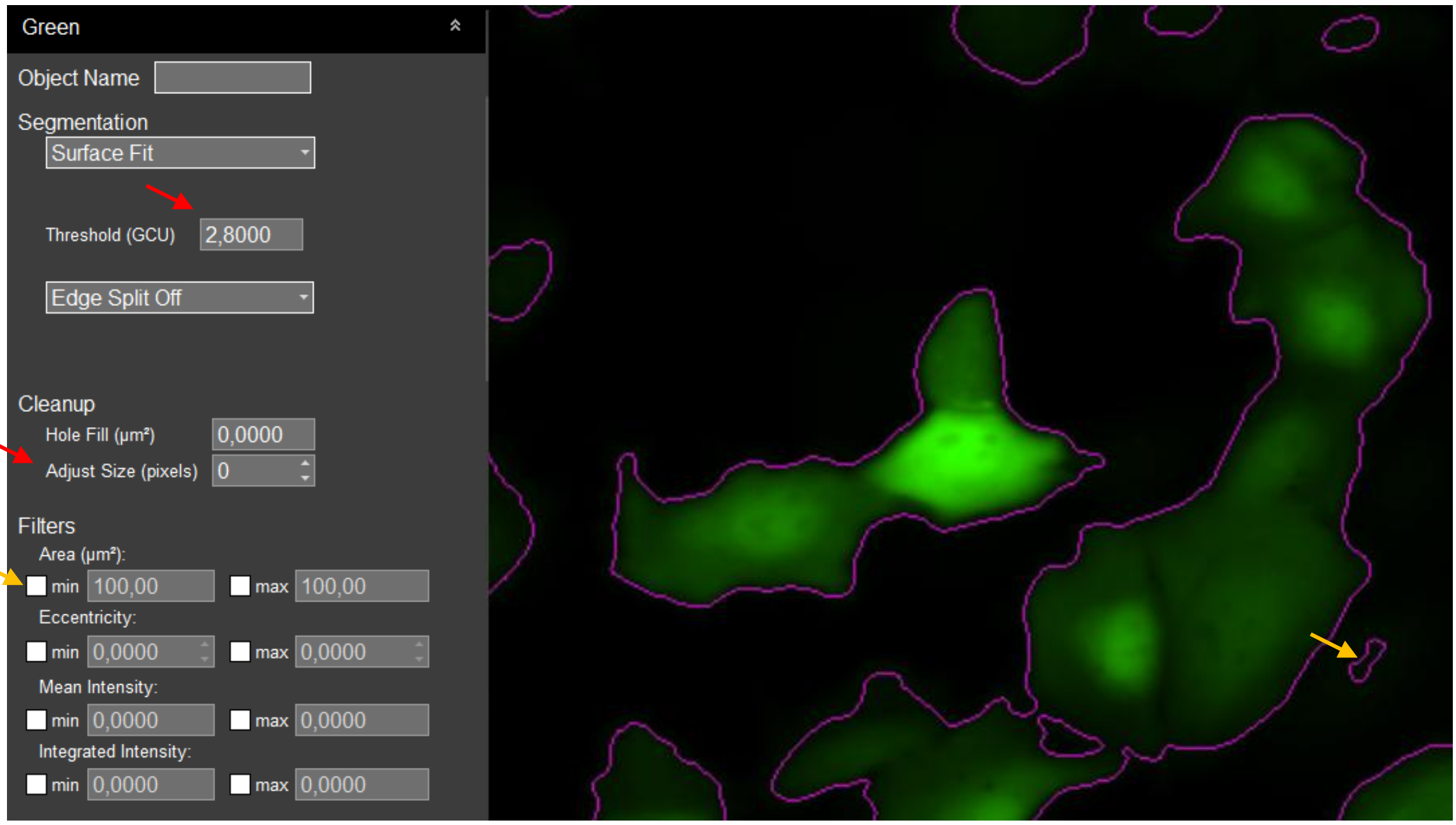




Surface fit is recommended and works for most applications

Assay type	Recommended starting point
Nuclear marker (GFP, RFP, mCherry nuclei)	Top-hat + adaptive threshold
Cell counting of fluorescent cells	Top-hat + adaptive threshold
Bright, clean fluorescence	Fixed threshold
Heterogeneous fluorescence	Adaptive threshold
Whole-cell fluorescence intensity	Surface fit
Confluent cultures with uneven illumination	Surface fit + adaptive threshold

Analyzing a fluorescence channel

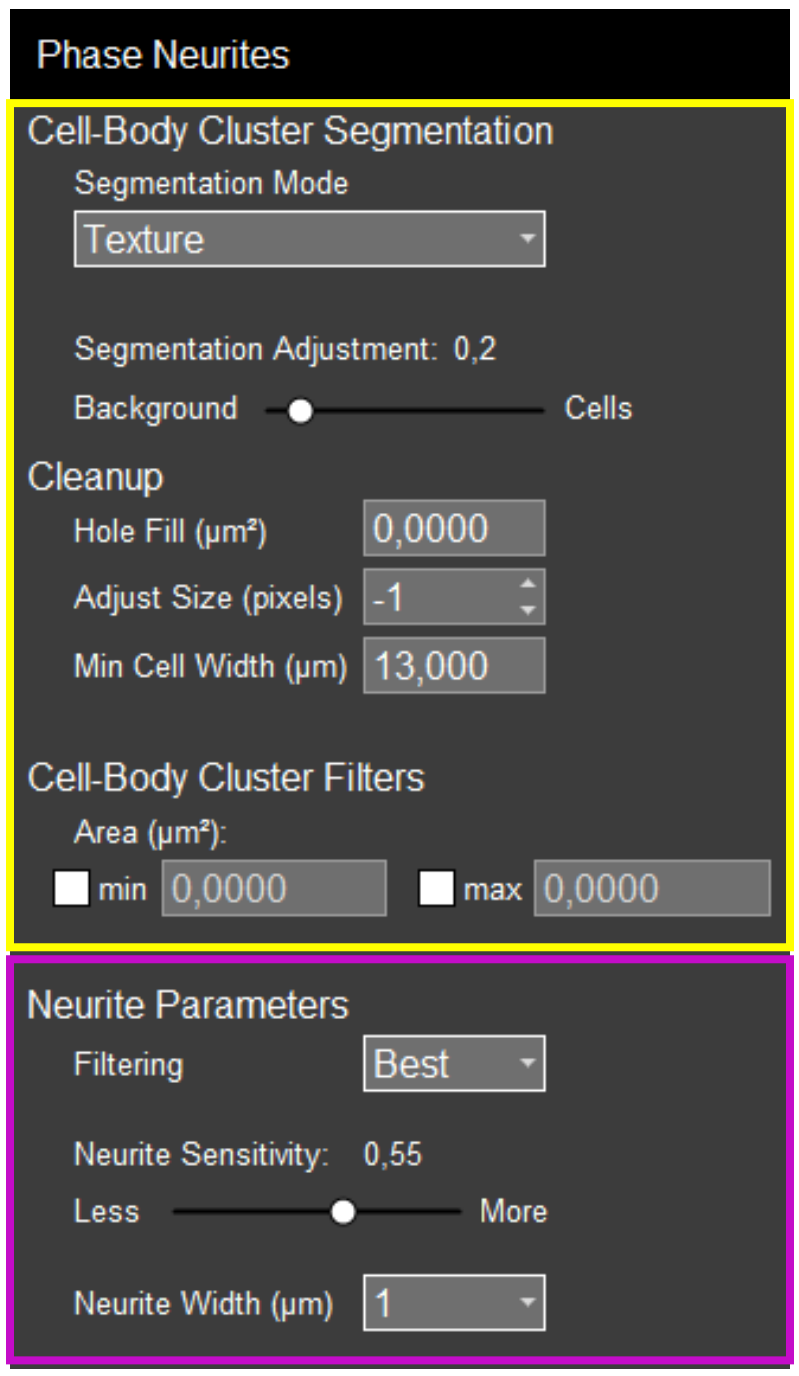


- Start by selecting **Surface Fit** and define a threshold to accurately mask your fluorescent signal.
- If the mask needs to be slightly tighter or less inclusive, adjust it using the Cleanup – **Adjust Size**.
- To exclude small objects, apply the **Area** Filter.
- The **Mean Intensity** (the average brightness of the pixels within the mask) and **Integrated Intensity** filter (the sum of the brightness value of all the pixels within the mask) can be useful for removing objects that are either too dim or exhibit unusually high signal levels.

Use Mean Intensity if you want to compare the fluorescent level per cell with different sizes. Use Integrated Intensity if you want to measure the total fluorescent content

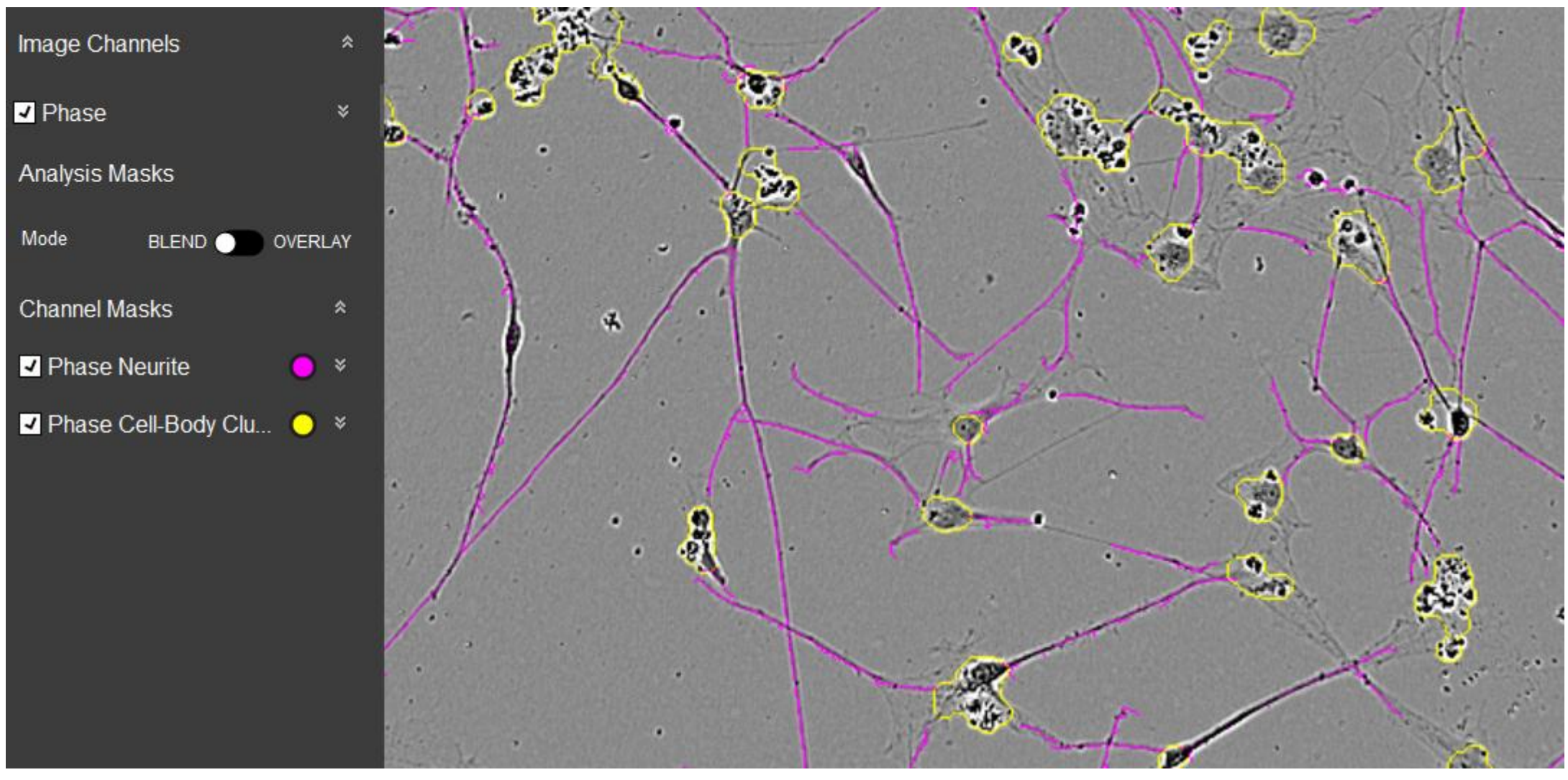
Neurotrack Analysis module

- The Neurotrack Analysis software module is specifically engineered to dynamically quantify neurite outgrowth, branching and cell body clustering over time. You can use the phase-contrast images or fluorescent labels.
- Run a **Standard** imaging setup to acquire the images. Select the Analysis Type called **Neurotrack** and follow the wizard to analyse your images.



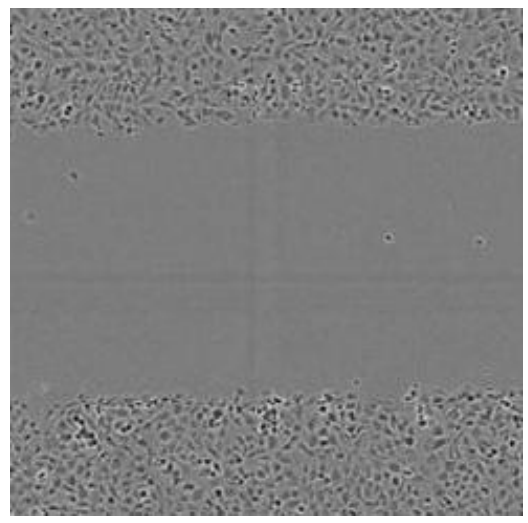
- Configure **cell body segmentation**: use segmentation mode *texture* if using phase contrast images and *brightness* for fluorescent images.
- Set **Min Cell width** (12-15 µm) and use the **Adjust Size** if needed. The goal is to mask just the cell body (here in yellow).

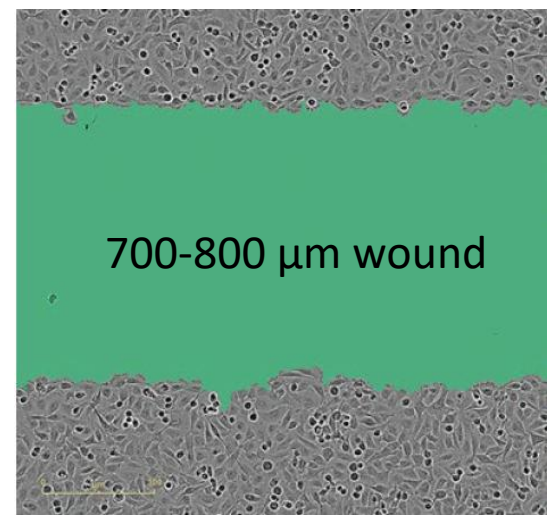
- Configure the **Neurite parameters**: use *Best* filtering, test out different **neurite sensitivity** and leave neurite width on 1 or 2.

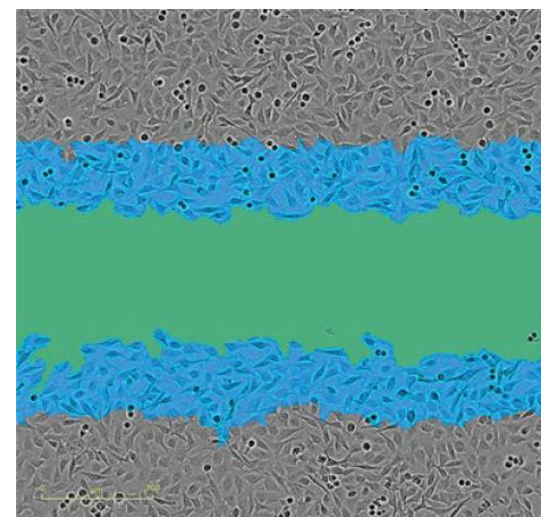


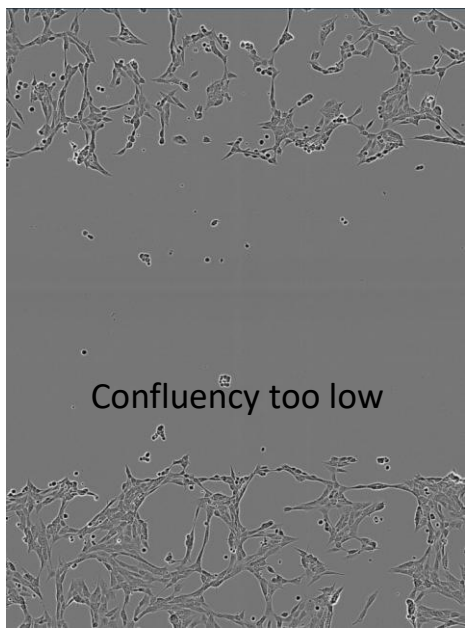
Scratch Wound – Migration and Invastion assay

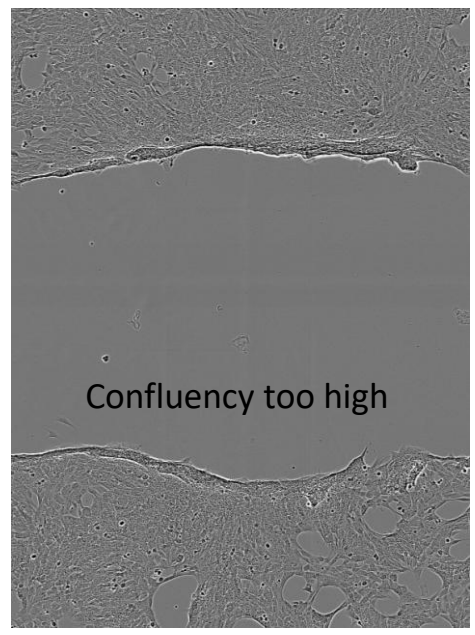
- 96 well ImageLock plates from Sartorius are recommended (can be purchased through Bookitlab). These plates have lines on the bottom for accurate xy imaging.
- The scratch wound maker (operated by MIC personnel) produces wounds of 700-800 µm.

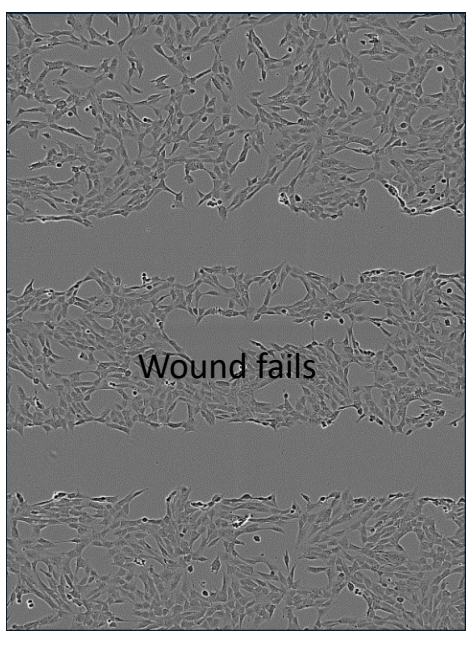


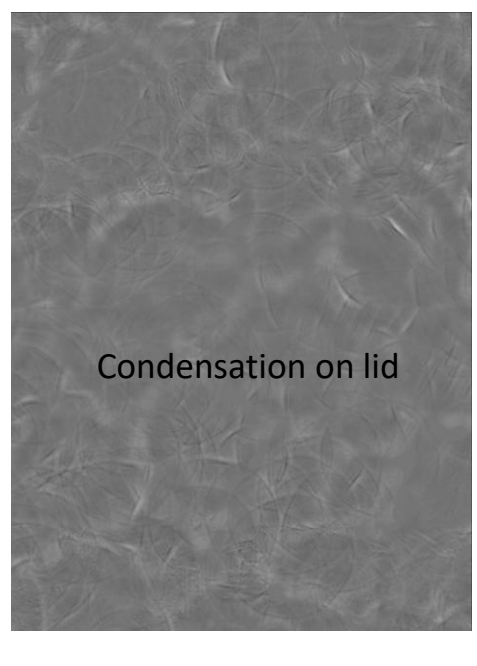


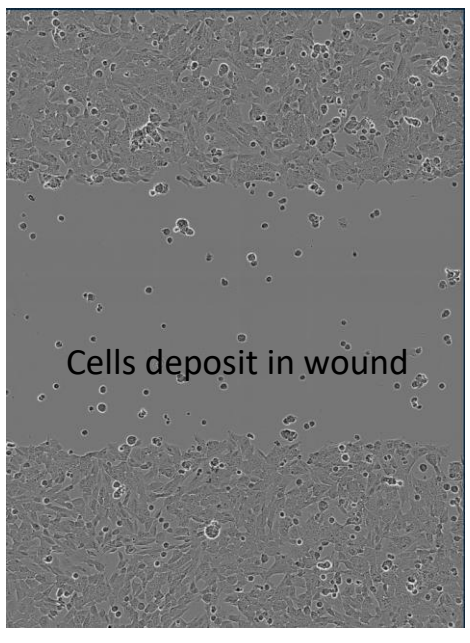


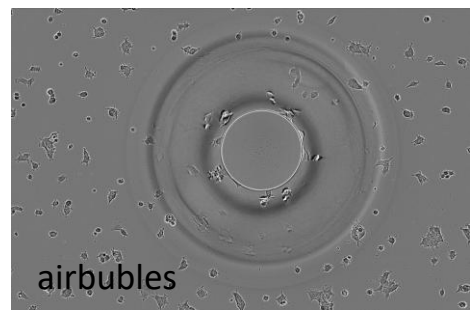


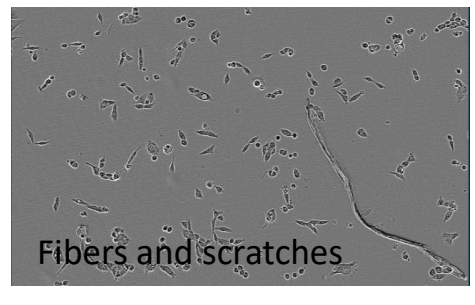










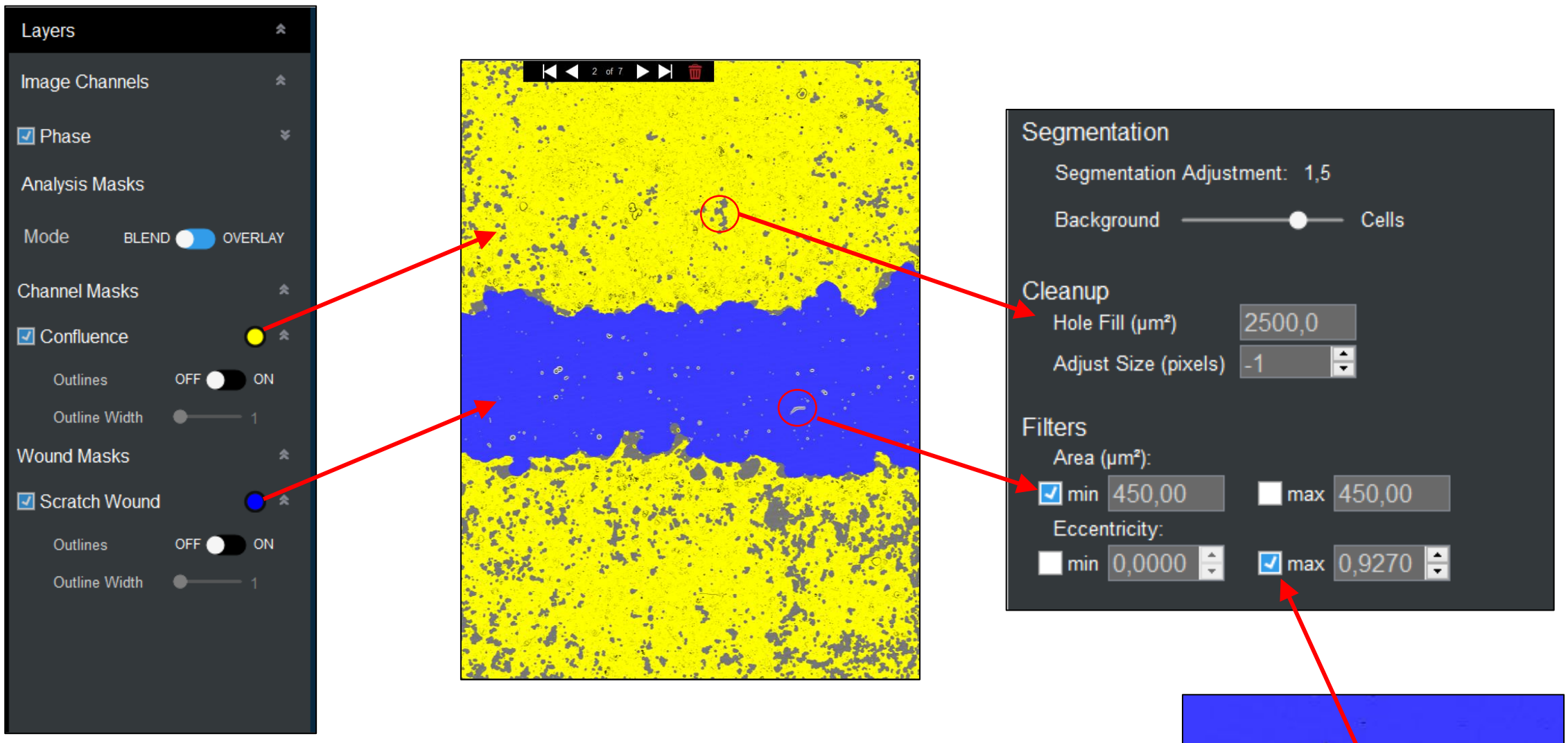


Wound healing assay are tricky but beautiful when successful.

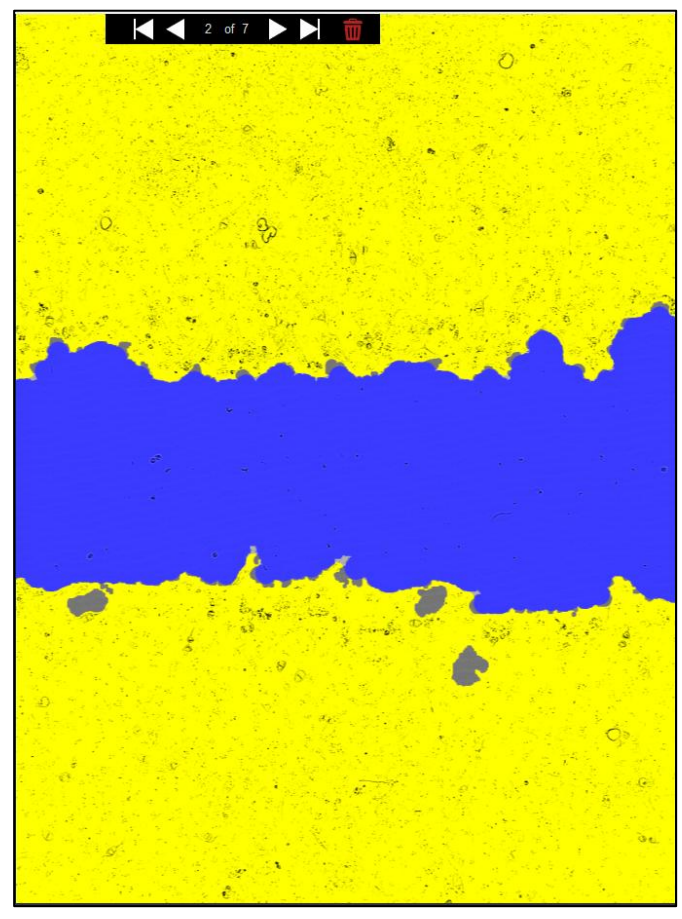
- Aim for 90-95% confluency, overnight or 24 hours (longer incubation = matrix).
- Remove air bubbles (bubble maker).
- Scratches/fibers will disrupt focusing.
- Don’t write on the plate!
- Sometimes woundmaker fails. Replicates!
- Condensation on lid due to temperature changes. Keep lid warm before imaging.
- After wound, remove floating cells.
- Woundmaker requires: No dry wells, max 100 µl volume per well upon scratching! You can increase the volume after scratching.

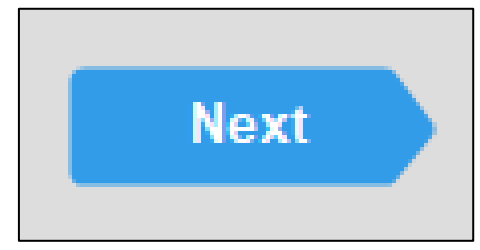
Analyzing a **Scratch Wound**

- Open your experiment and launch the analysis. Follow the guided assistant wizard step by step.
- Select **Create New Analysis Definition** and choose **Scratch Wound**.
- Select the Image channels you want to analyze.
- In the **Image Set Selection** step, choose several representative images from different time points. Once you have 4-8 representative images, click **Next**.
- Click **Preview Current** and wait for the Confluence and Scratch Wound masks to load. Begin optimizing the **Segmentation Adjustment**, **Cleanup** and **Filter settings.**



- In this experiment **Segmentatio Adjustment** value of 1,5 towards cells, combined with a -1 pixel **Adjust Size**, provided accurate cell detection.
- To exclude debris and individual cells located within the wound aread, apply appropriate size ad morphology filters.
- The **Eccentricity** filter was used to remove minor scratch artifacts present in one of the wells.

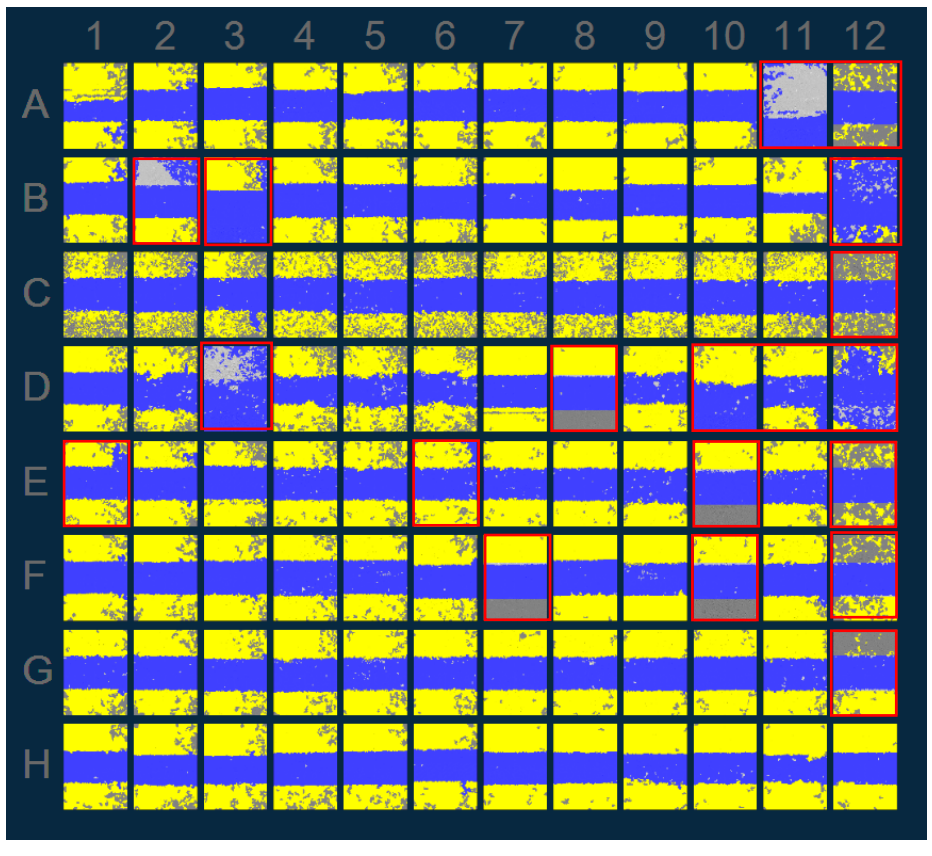


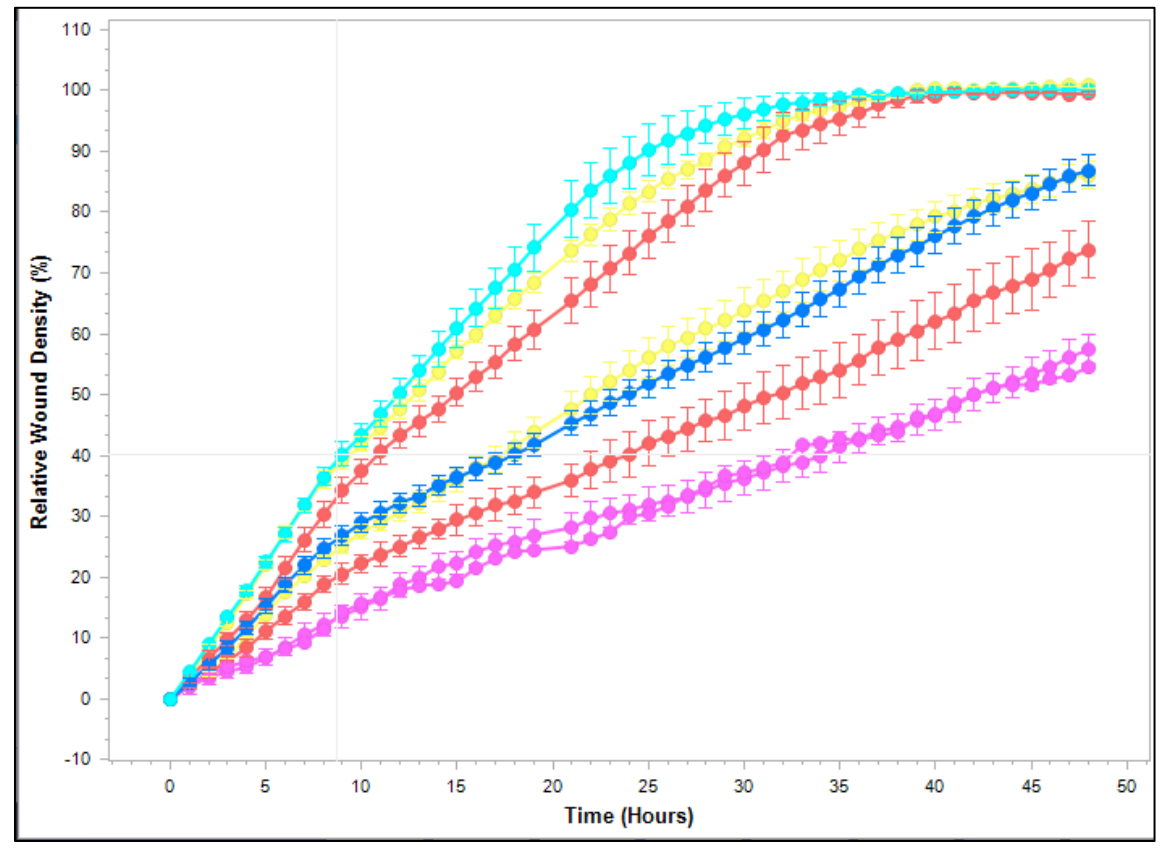


- Select the wells and time points to be analyzed, then start the analysis.

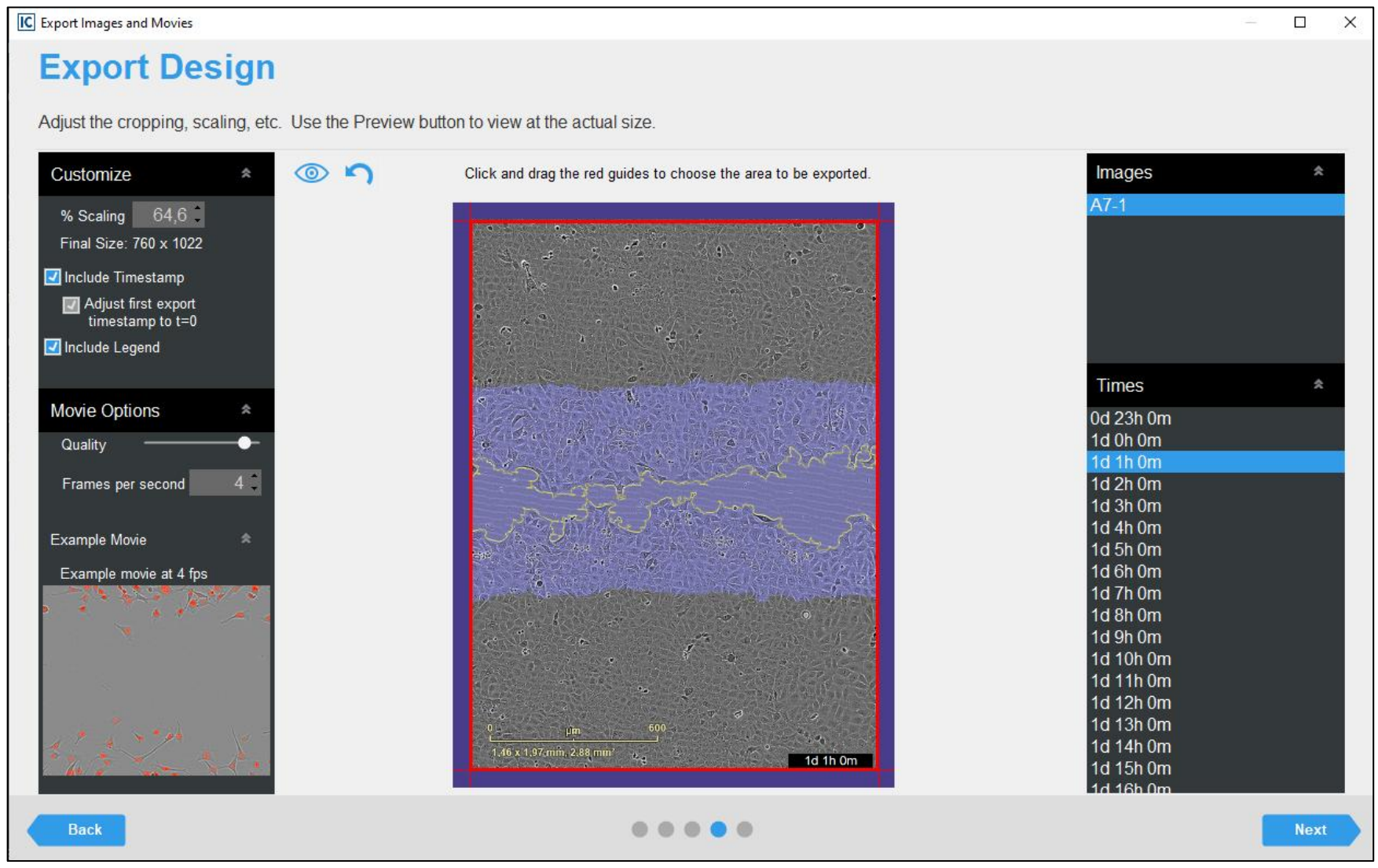
Analyzing a Scratch Wound continues

- Open your experiment and click **Graphics Metrics**.
- Review the relative wound density/wound confluency graph for the microplate to assess the quality of the masking. Exclude problematic wells from the analysis, if necessary, create and run a new analysis definition with optimized settings.



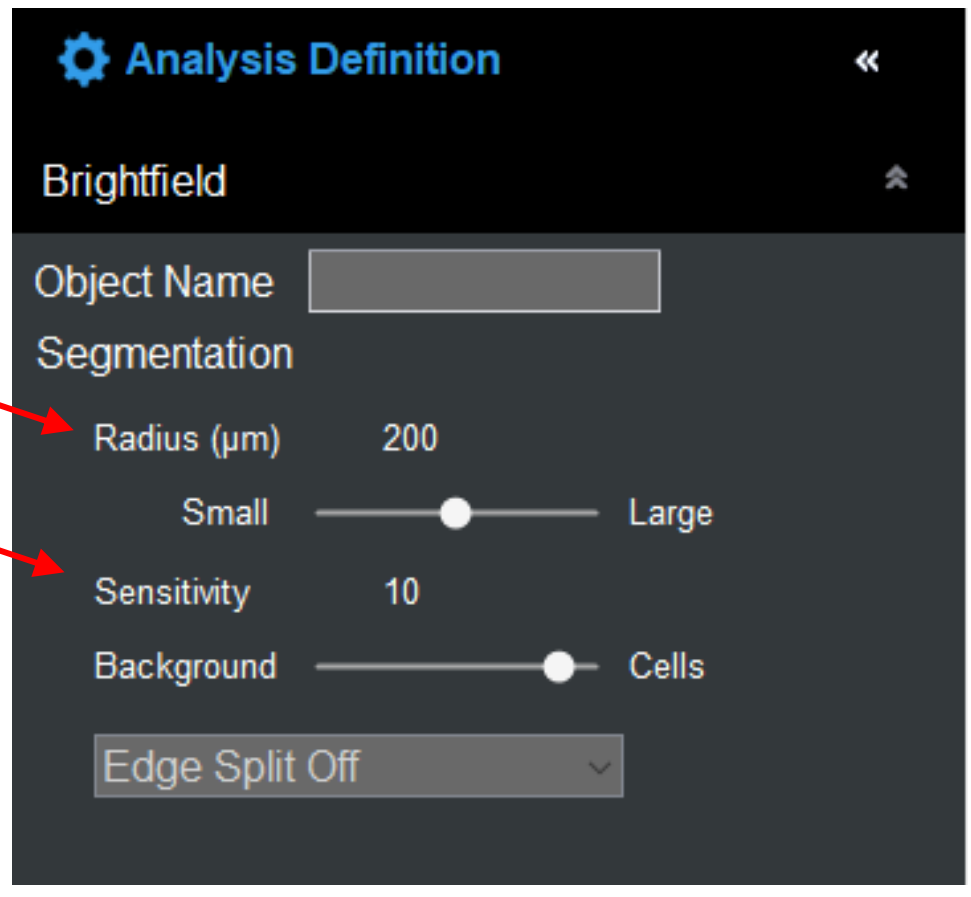


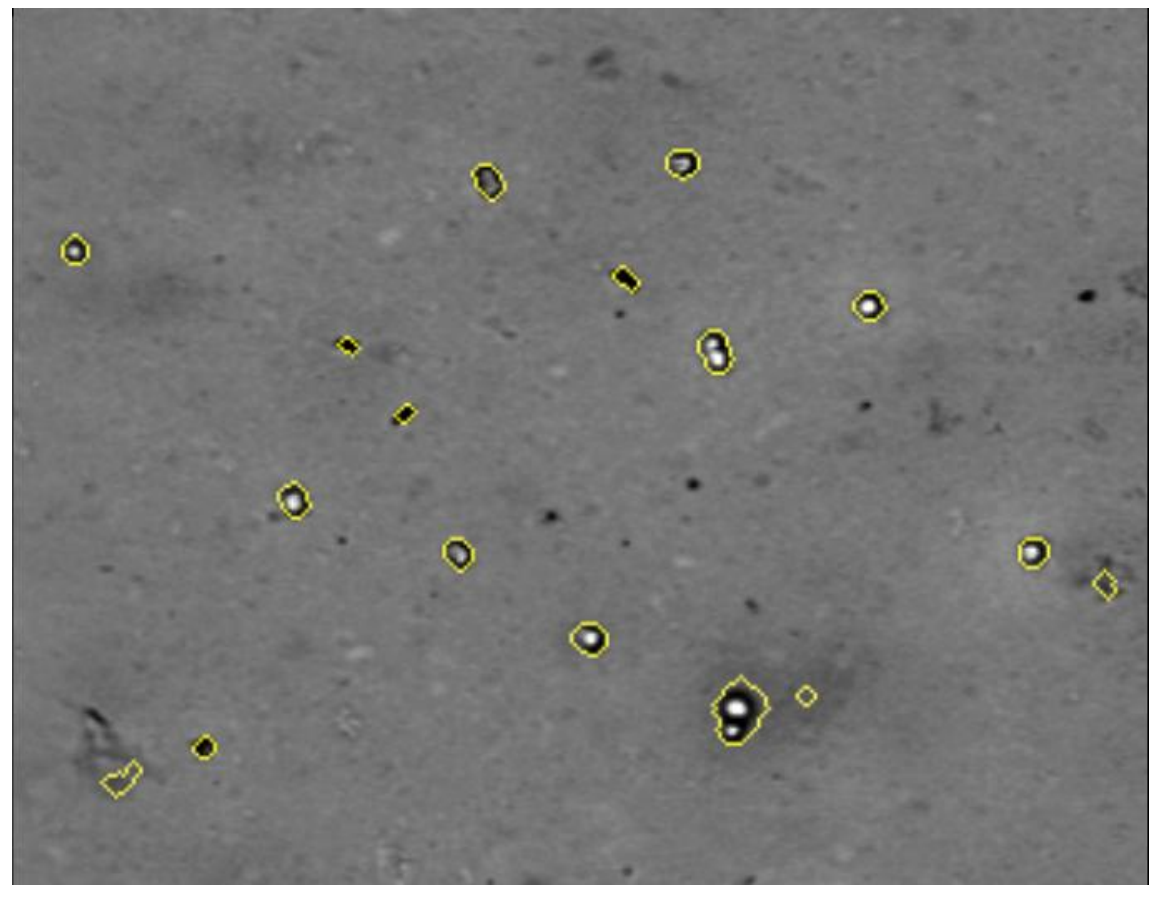
- To visually verify the analysis, export a movie from a representative well and display the Confluency; Wound and Initial Scratch wound mask throughout the time course. This will help confirm that the masks accurately track wound closure over time.



Analyzing a **Spheroid** experiment

- Open your experiment and launch the analysisFollow the guided assistant wizard step by step.
- Select **Create New Analysis** definition and choose **Spheroid** analysis module.
- Select the Image channels you want to analyze.
- In the **Image Set Selection** step, choose several representative imgaes throughout scan times. Click **Next**.
- Enable the **Confluence** mask and begin optimizing the **Radius** and **Sensitivity** settings. If needed, further refine the mask using **Cleanup** and **Filter** options.

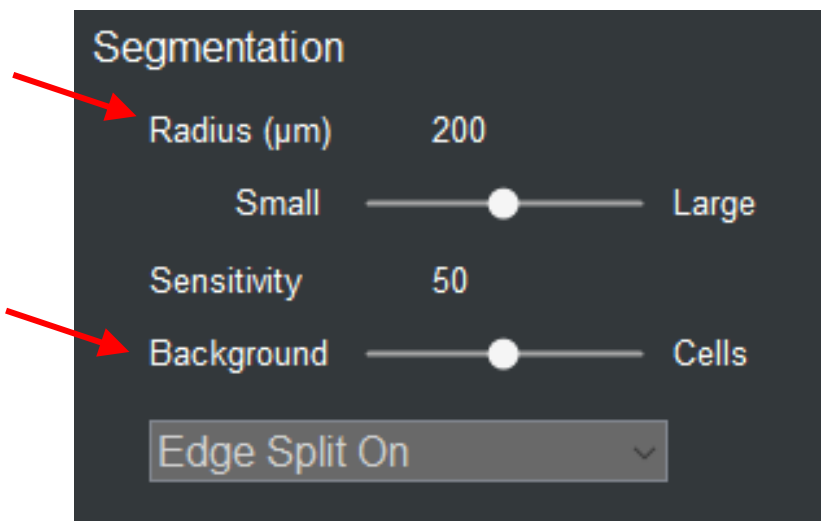


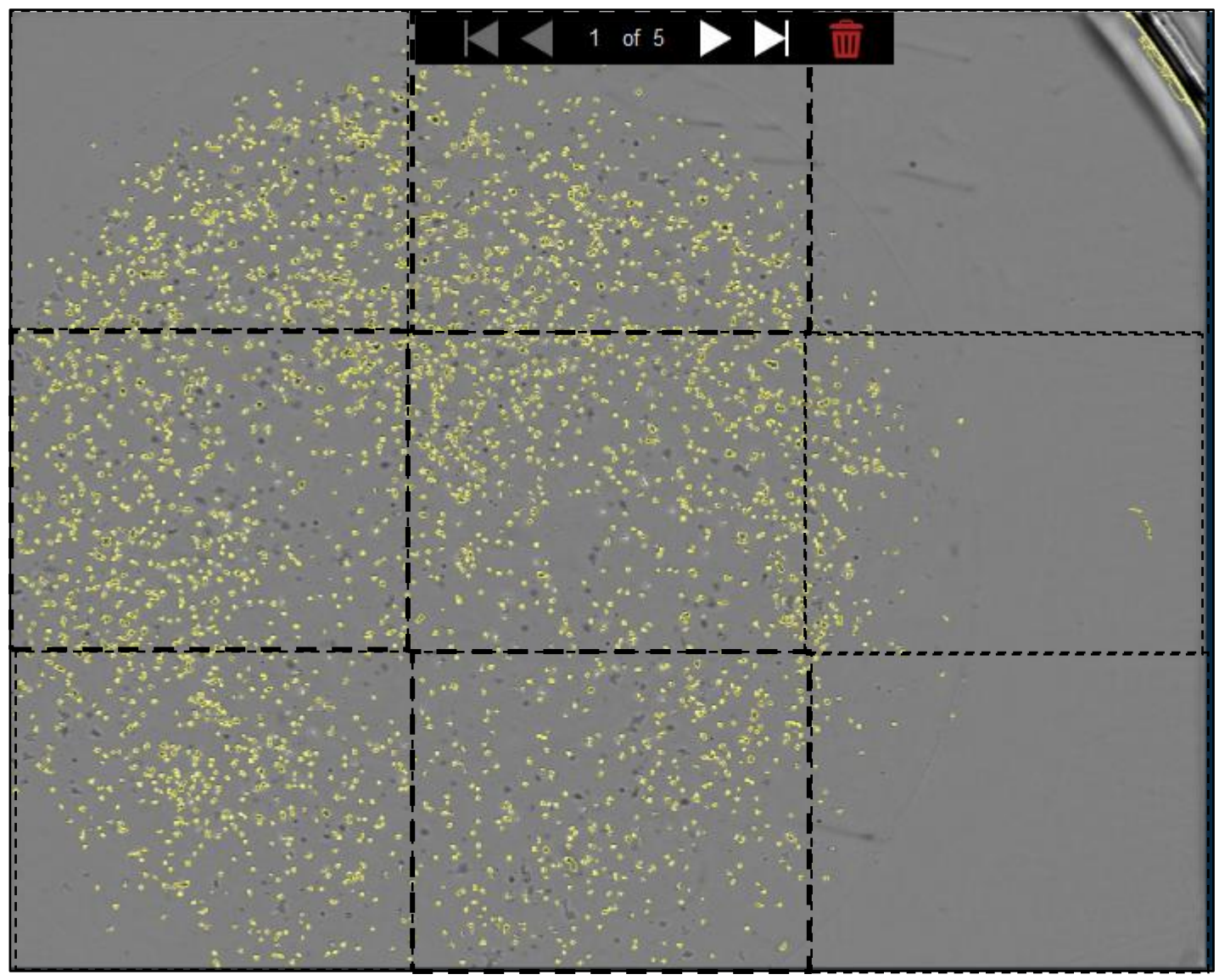


- **Radius** controls background removal. Move the slider towards **Large** to increase background subtraction and improve spheroid detection.
- **Sensitivity** determines how much contrast is required for an object to be identified as a spheroid. Move the slider towards **Cells** to detect spheroids with lower contrast relative to the background.

Analyzing an **Organoid** experiment

- Open your experiment and launch the analysis. Follow the guided assistant wizard step by step.
- Select **Create New Analysis** definition and choose **Organoid** analysis module.
- Select the Image channels you want to analyze.
- In the **Image Set Selection** step, choose several representative images throughout scan times. Click **Next**.
- Enable the Confluence mask and begin optimizing the **Radius** and **Sensitivity** settings to accurately identify the organoids.
- Review the mask performance across all selected images and time points to ensure that organoids are detected consistently and accurately.
- Once you are satisfied with the segmentation, proceed to Launch Analysis, select the wells and time points to analyze and start the analysis.



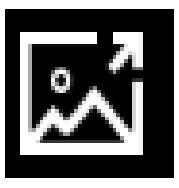


- Changes to the mask and image refreshes will take longer than usual because each images is composed of nine individual fields.

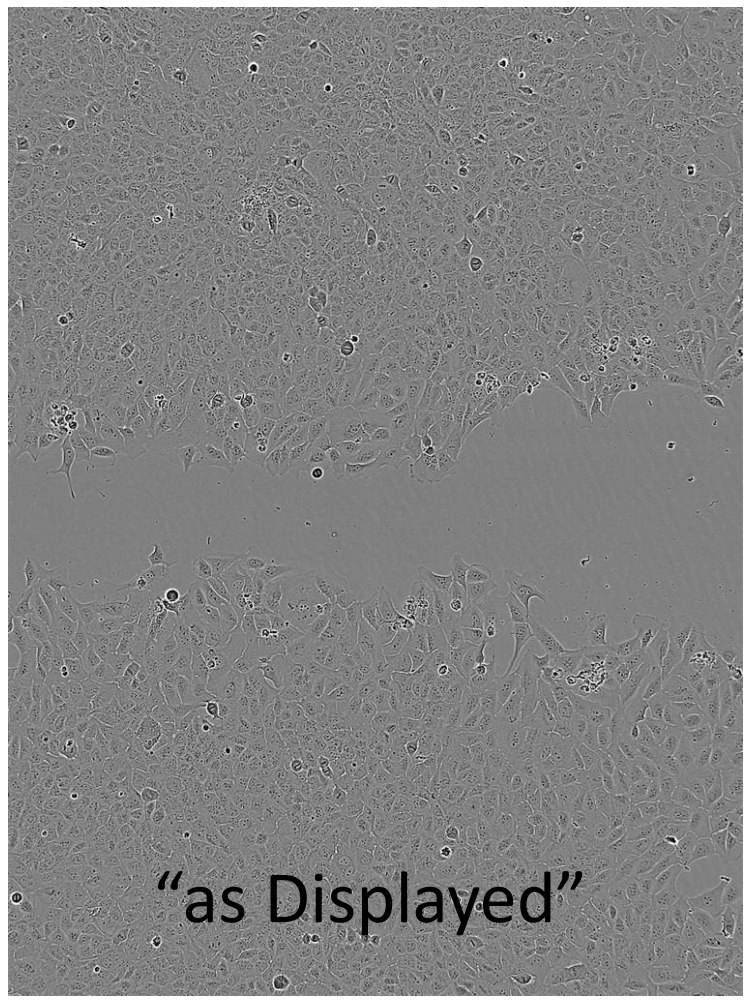
- **Radius** controls background removal. Move slider towards **Large** to increase background subtraction.

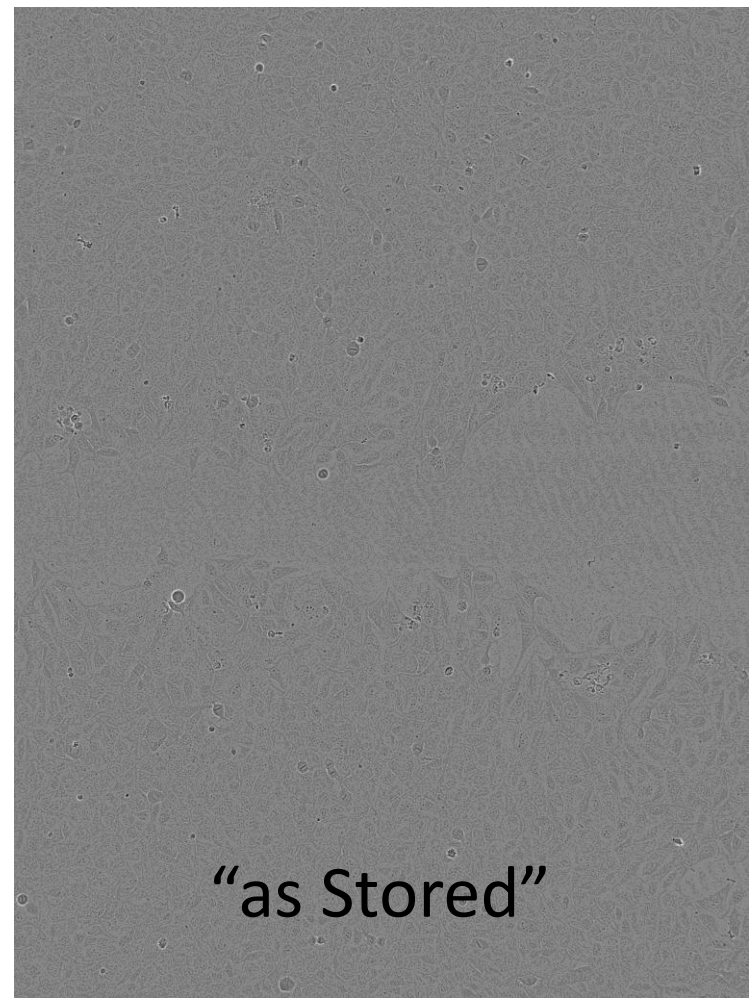
- **Sensitivity** controls how much contrast is required for an object to be detected relative to the background. Move the slider towards **Cells** to identify spheroids with lower contrast and weaker boundaries.

How to Export Image Sets and Movies

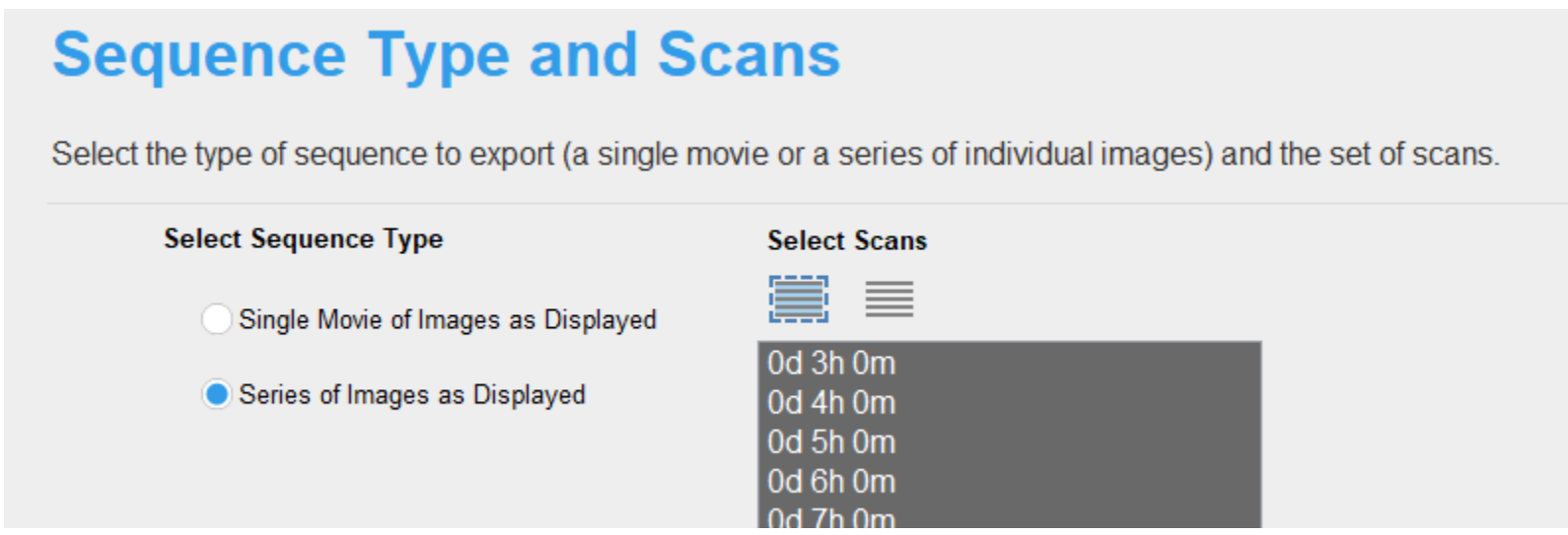


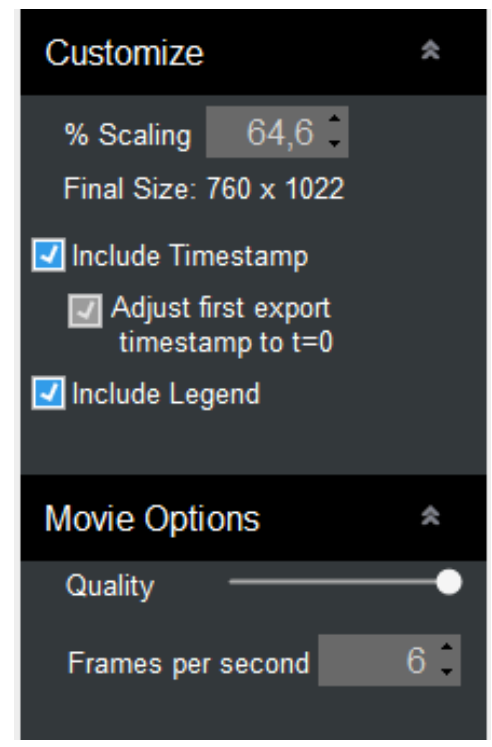
- Open your experiment and click on **Export Images and Movies** icon.
- Choose whether to export images **As displayed** or **As stored**. The latter exports raw image data which typically appears with low contrast. As Displayed exports the images with any display adjustments applied, including changes to brightness and contrast made in the image channel (Layers) settings.



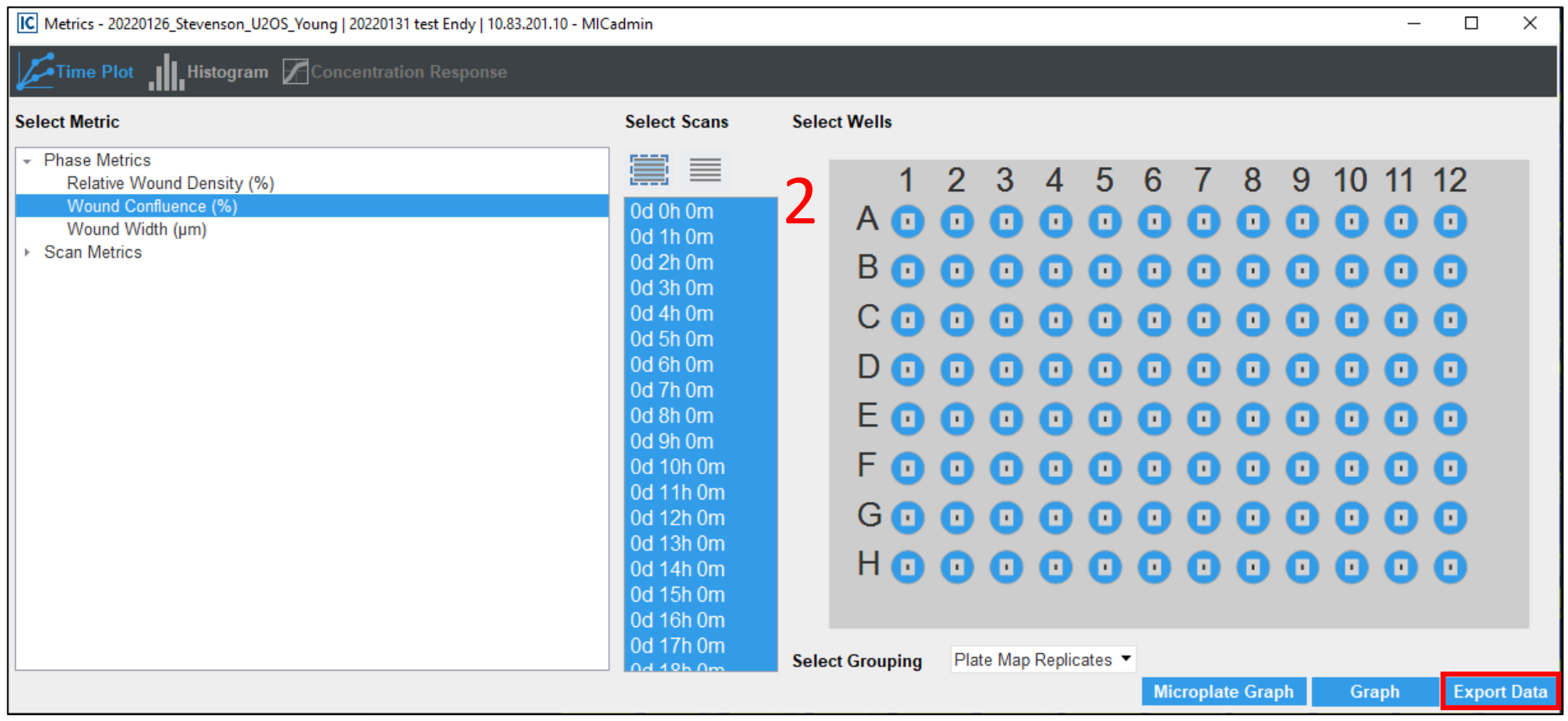


- Select the S**equence Type: Single Image/Series** of images or a **Movie**.
- Adjust the export settings, including **Image Quality** and playback **Speed** (frames per second).
- You can also choose whether to include timestamps and a scale bar in the exported image/movie.

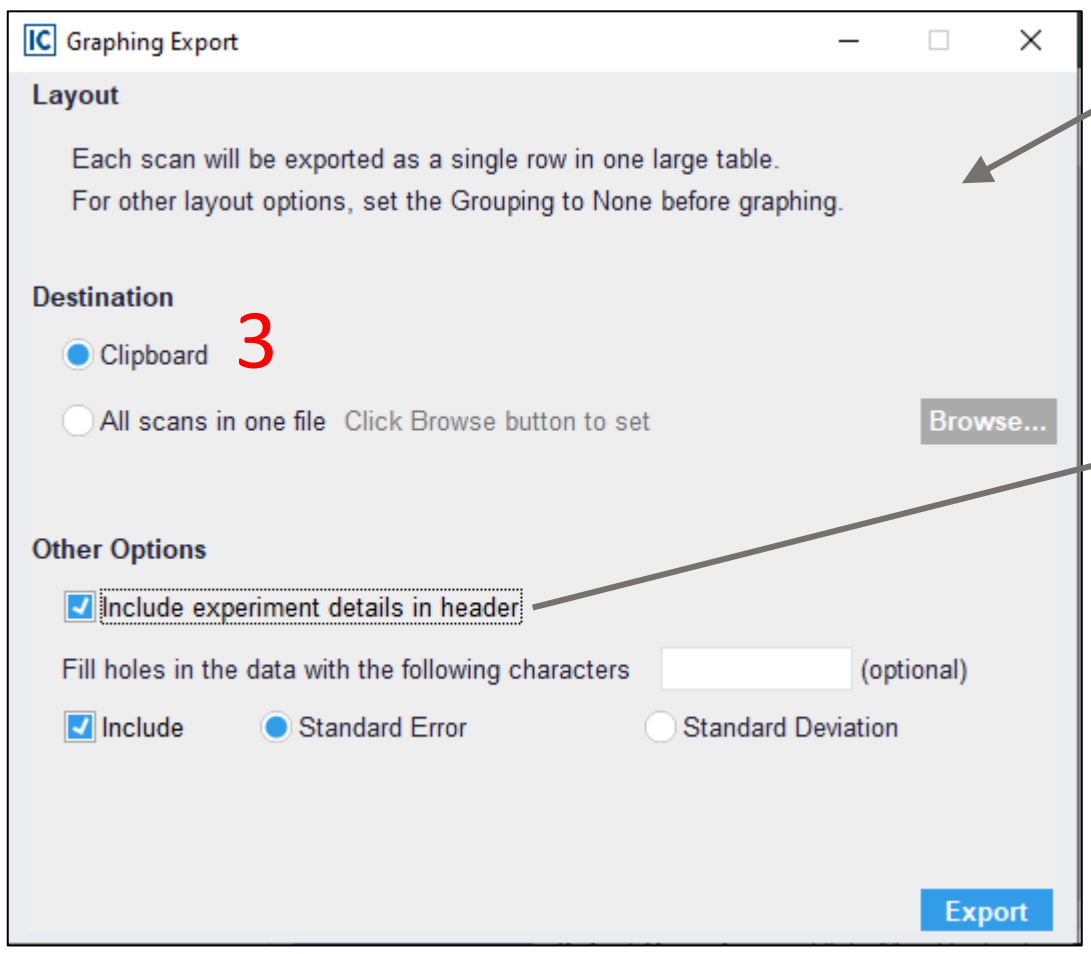


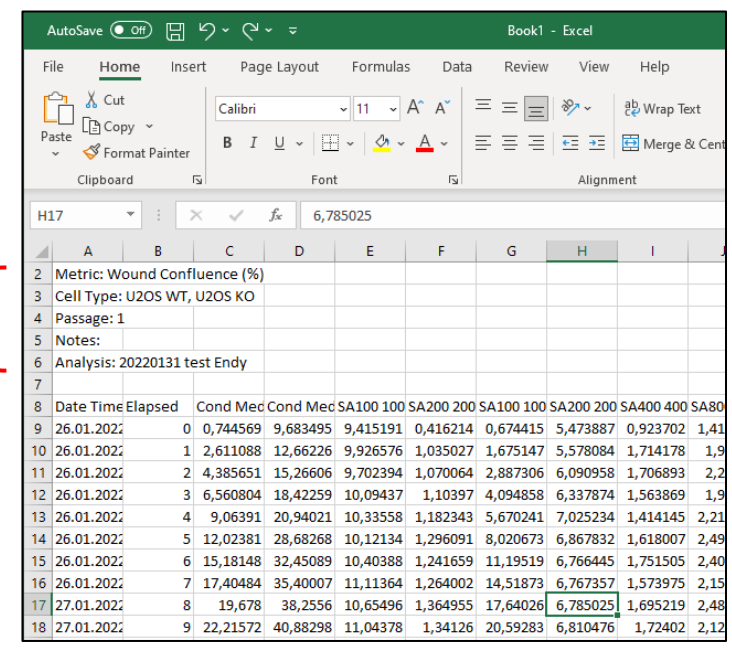


How to Export Data



Choose the **Metric** you want to export (1). Select the wells and timepoint (2) you want to export. Click on **Export Data**.





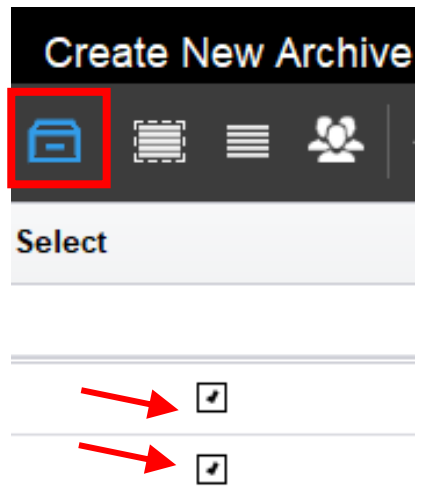
If you select Clipboard (3) as the destination, simply open excel and paste the data (ctrl v).

Selecting **Include experiment details in header** can be useful for later.

Enabling Standard Error/Standard Deviation will add additional columns at the end og the data table.

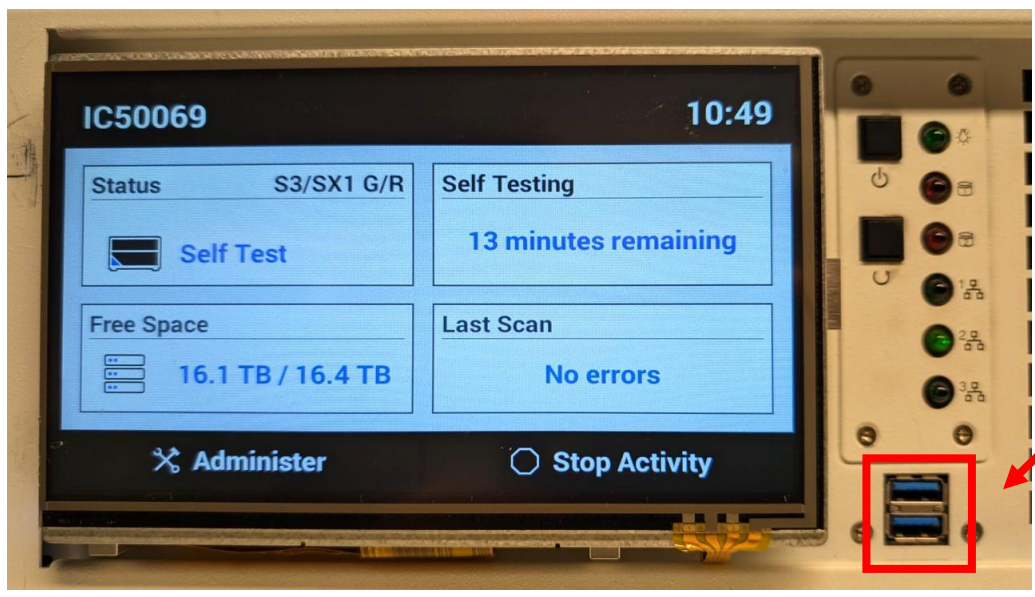
To export data from individual images when multiple images have been acquired within the same well, set *Grouping – None*. This enables the *Break data down into individual images*.

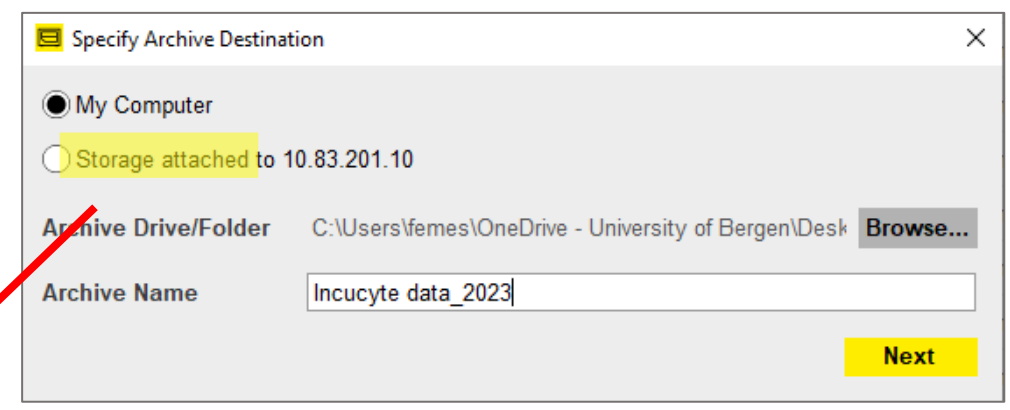
How to Archive and Delete your data from the remote controller

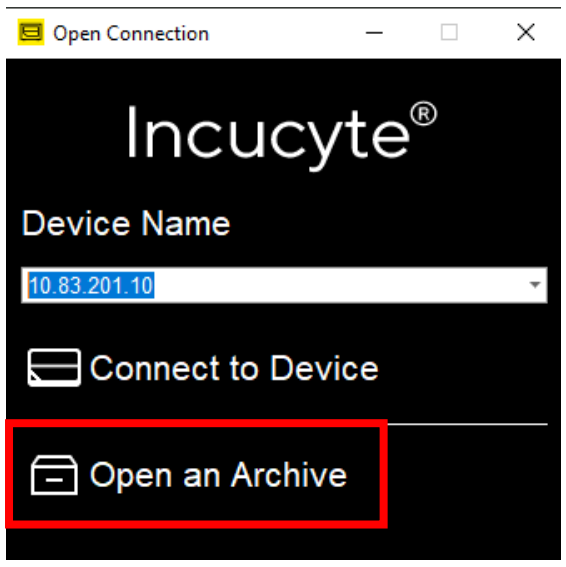


ARCHIVING

- Go to **Archive**, use the **Owner** column to filter and locate your experiments.
- Select the data you want to archive (arrows) and click the **Create Archive** icon.
- Choose the archive destination: **My Computer** or **Storage Attached** (for external Harddisk attached to remote controller - FASTEST option!).







OPENING AN ARCHIVE

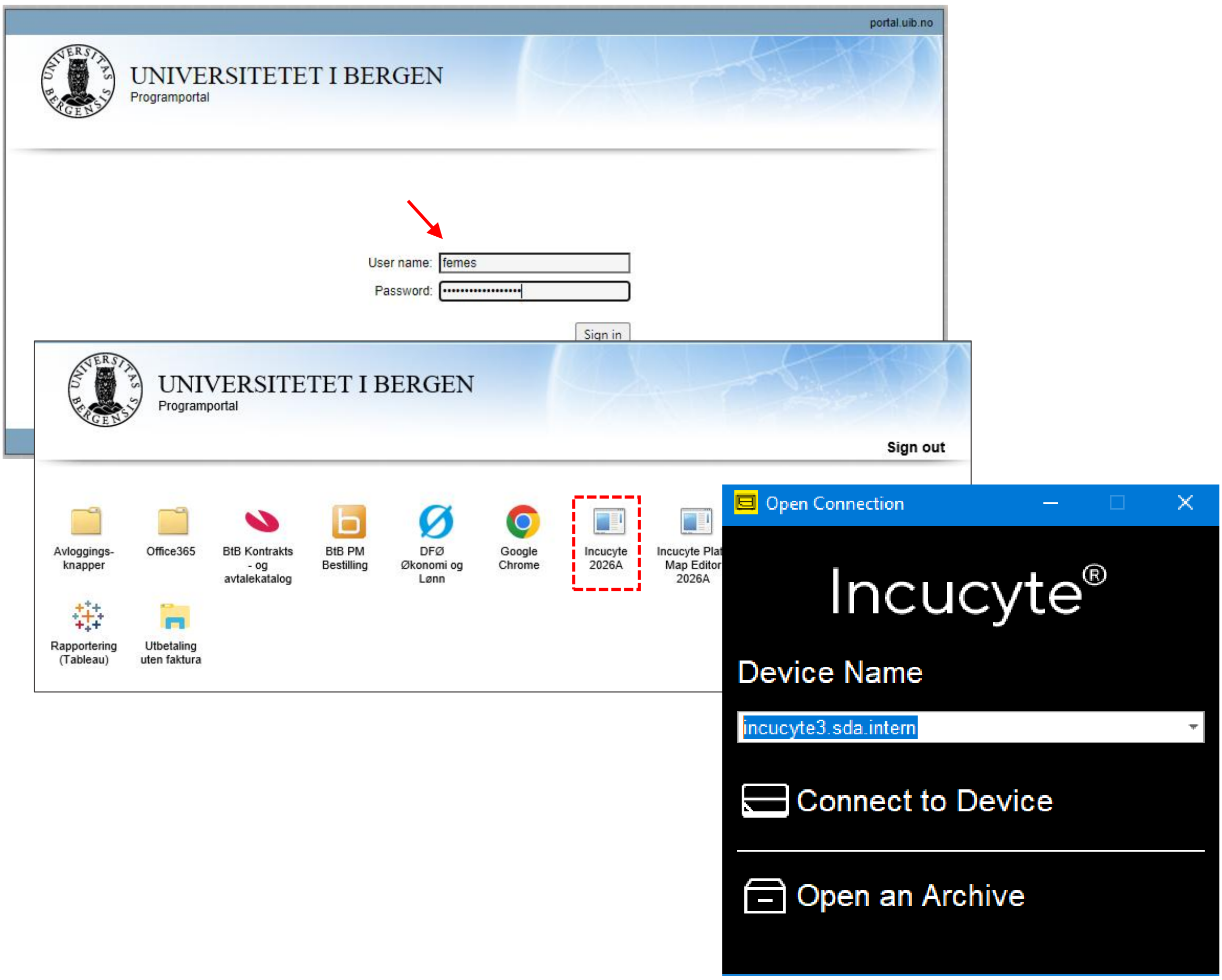
- When you next launch the Incucyte software, select the **Open an Archive** option. You can browse, review and analyze your archived experiments just as you would with data stored on the controller.

DELETING DATA FROM CONTROLLER

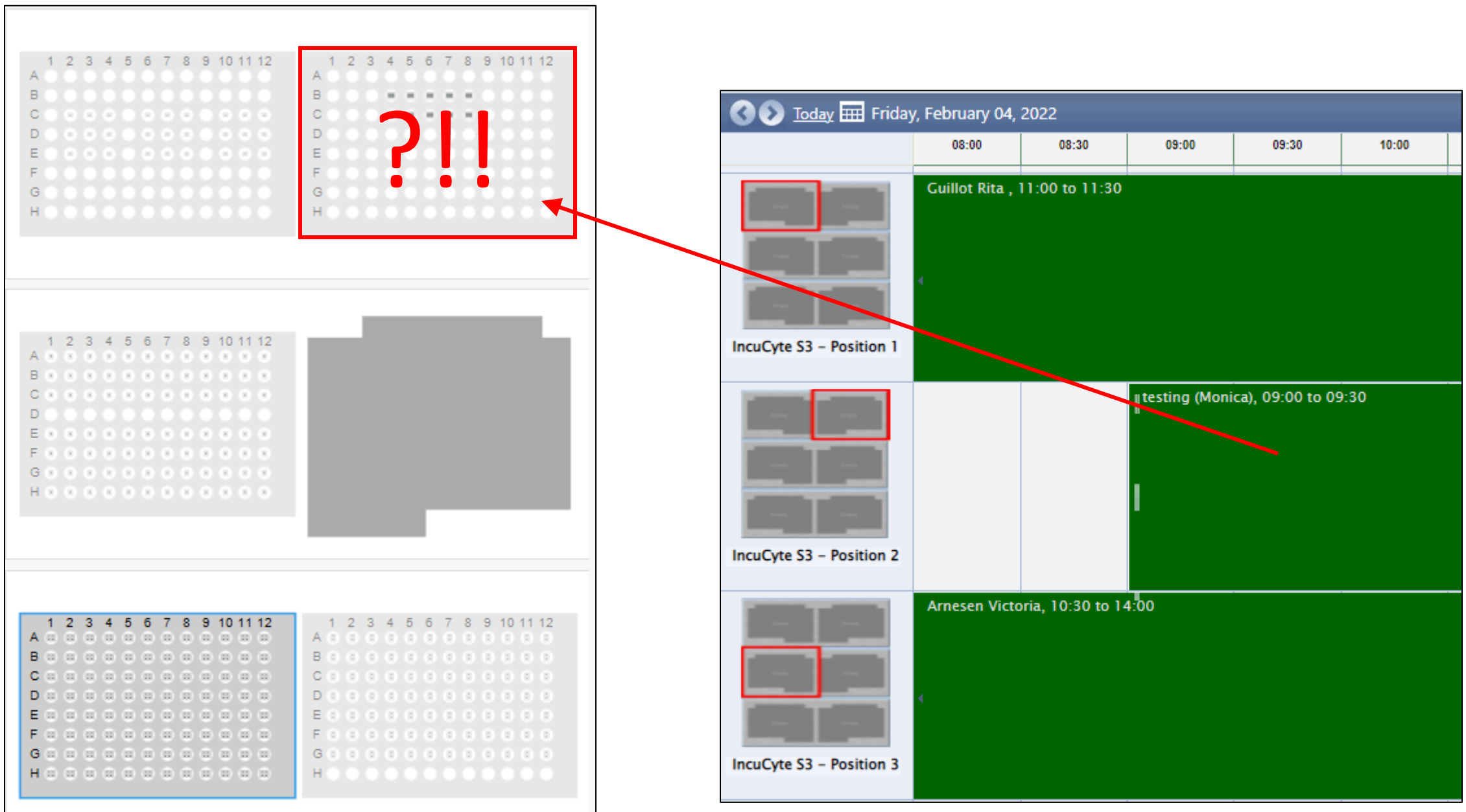
- Go to **Manage**, filter to locate your experiments. Select the checkbox next to each dataset you want to delete. Click the Trash icon and permanently remove the data.

How to connect to the Incucyte S3 from a pc/mac outside uib

1. Contact MIC personnel to request access to the portal (mic@uib.no).
2. Open a web browser and navigate to **https://portal.uib.no**
3. Log in using your UiB username and password.
4. Once connected, launch IncuCyte 2026A software.
5. When prompted to connect to the instrument, enter ***incucyte3.sda.intern*** instead of the IP address.



Troubleshooting – the position you reserved is not free

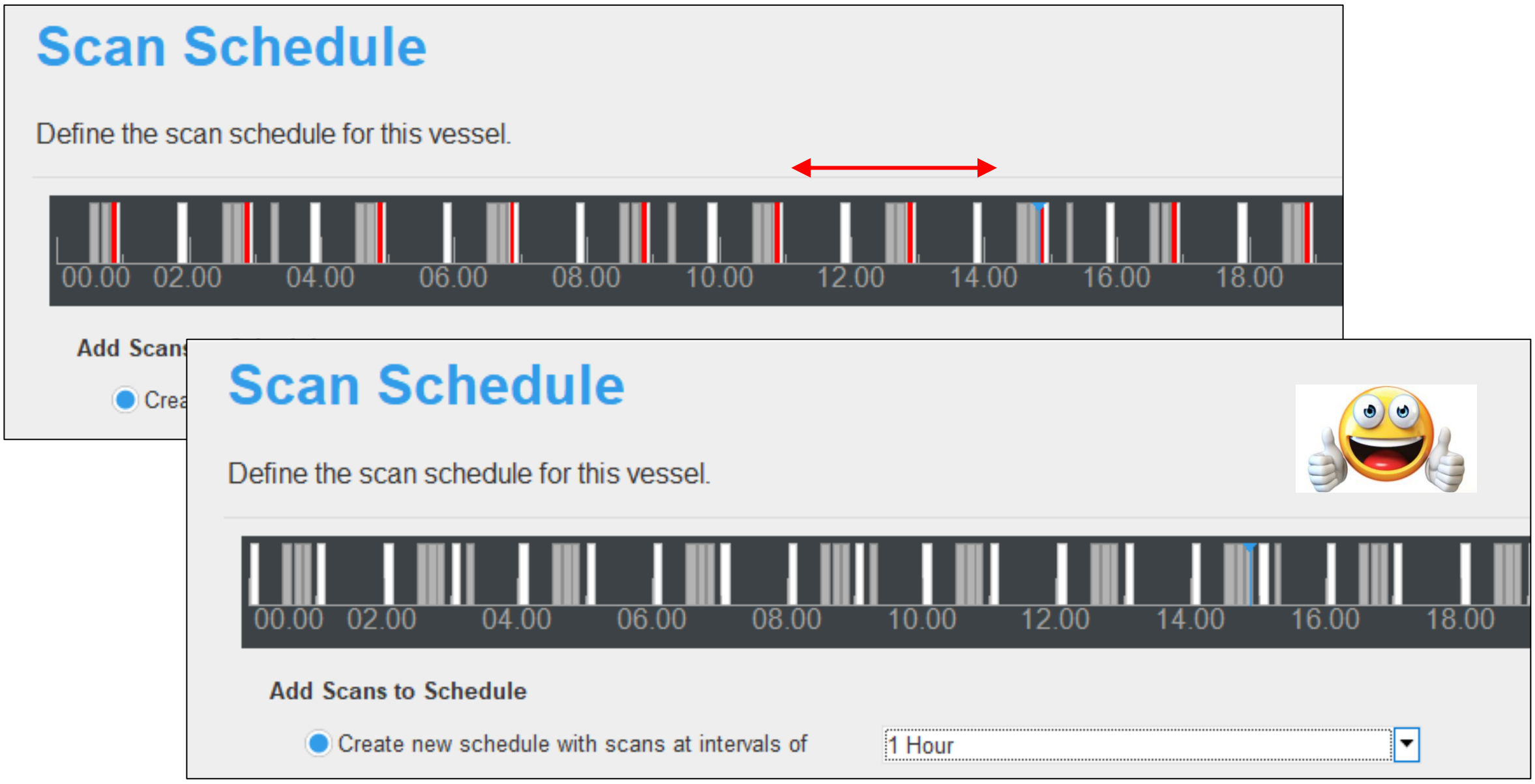


- **Mandatory Booking:** You must always reserve your IncuCyte position in advance. Always double-check and verify your specific assigned position before starting.

- **Clearing Old Plates:** If you find an existing plate in your reserved position, you are authorized to clear it:

  - **Terminate the job** in the software by clicking on the vessel icon and selecting the trash bin.
  - **Physically remove** the vessel from the machine.
  - **Dispose of it** immediately according to standard cell lab protocols.

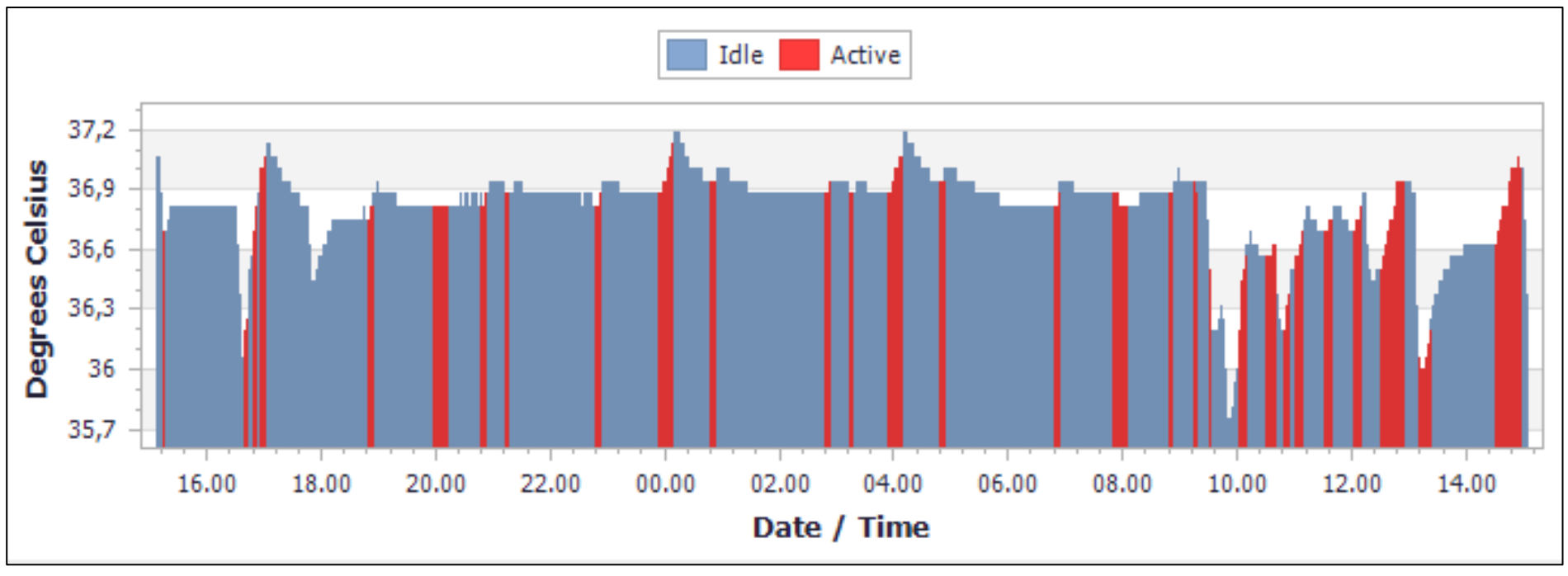
Troubleshooting – there are red/orange warning recommendations popping up when scheduling scan



- **Conflict Blockers:** If there is a scheduling conflict with another user, the system will block you from clicking **Next** or launching your job.

- **Resolving Conflicts:** Click and drag the timeline slider with your mouse arrow until the conflict warning disappears.

- **Cooling Intervals:** Always aim to leave a buffer between scheduled scans. This cooling time ensures the internal environment remains stable and optimal for cell health.



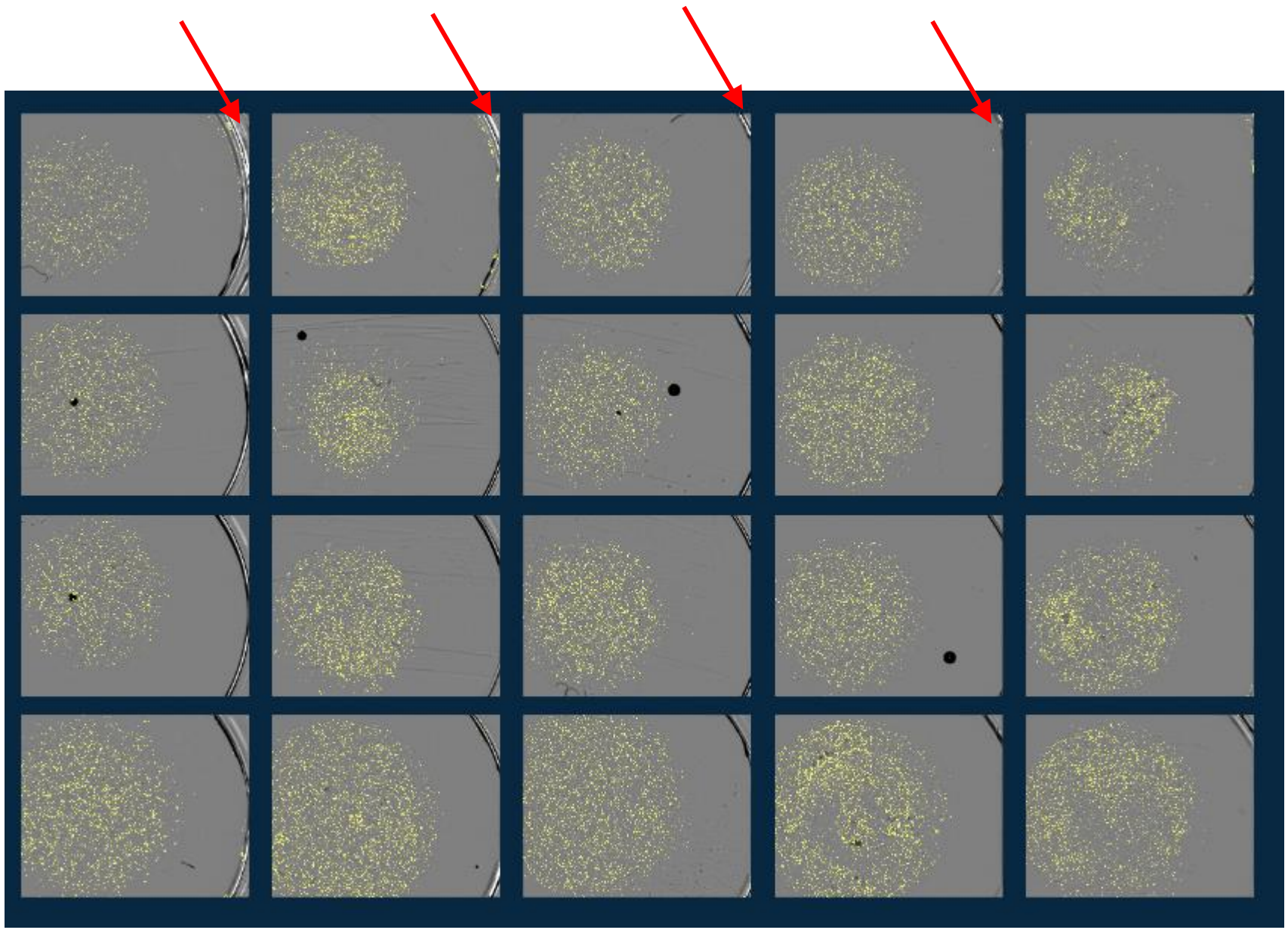
Troubleshooting – I do not have the recommended plate

- **Use Recommended Culture Vessels:** Always seed your cells, spheroids, or organoids in the specified brands. Different brands use distinct **XYZ well coordinates**, which can cause alignment errors. While "off-brand" plates occasionally work, using them risks experiment failure.

- **Wound Healing Plates:** Standard 96-well regular Nunc plates may cause autofocusing issues and minor XY alignment drift. For reliable, accurate focusing, you must use specialized wound healing plates featuring calibration crosses on the bottom of the wells (**ImageLock plates**).

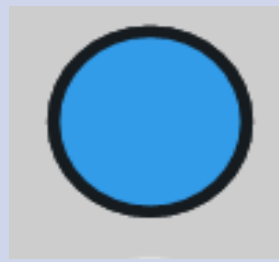
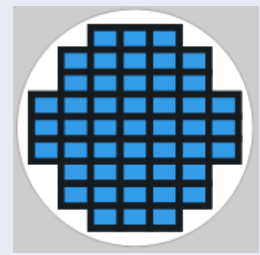
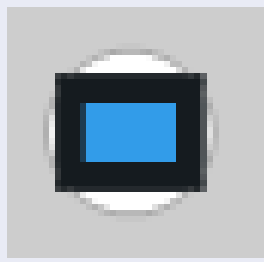
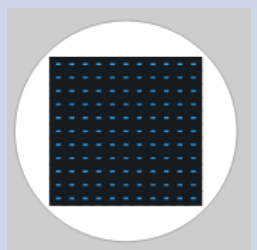
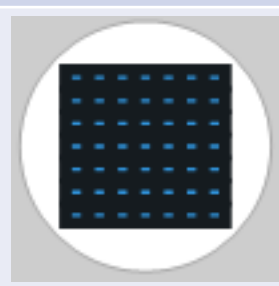
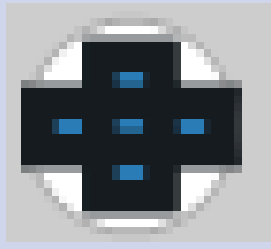
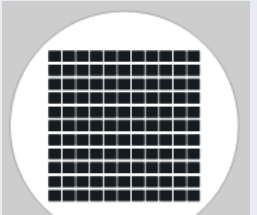
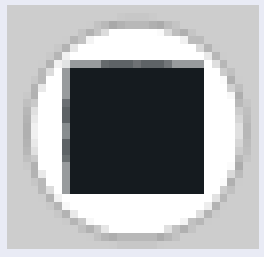
- **Spheroid & Organoid Experiments:** These must be run exclusively in **Corning plates**. Utilizing Nunc plates for these assays exposes the well edges—particularly on the right side—which frequently disrupts downstream data analysis.

Manufacturer	Category	Wells	Area	Catalog Numbers
Corning	Plate	24	N/A	3337, 3473, 3524, 3526, 3527
Corning	Plate	48	N/A	3338, 3548



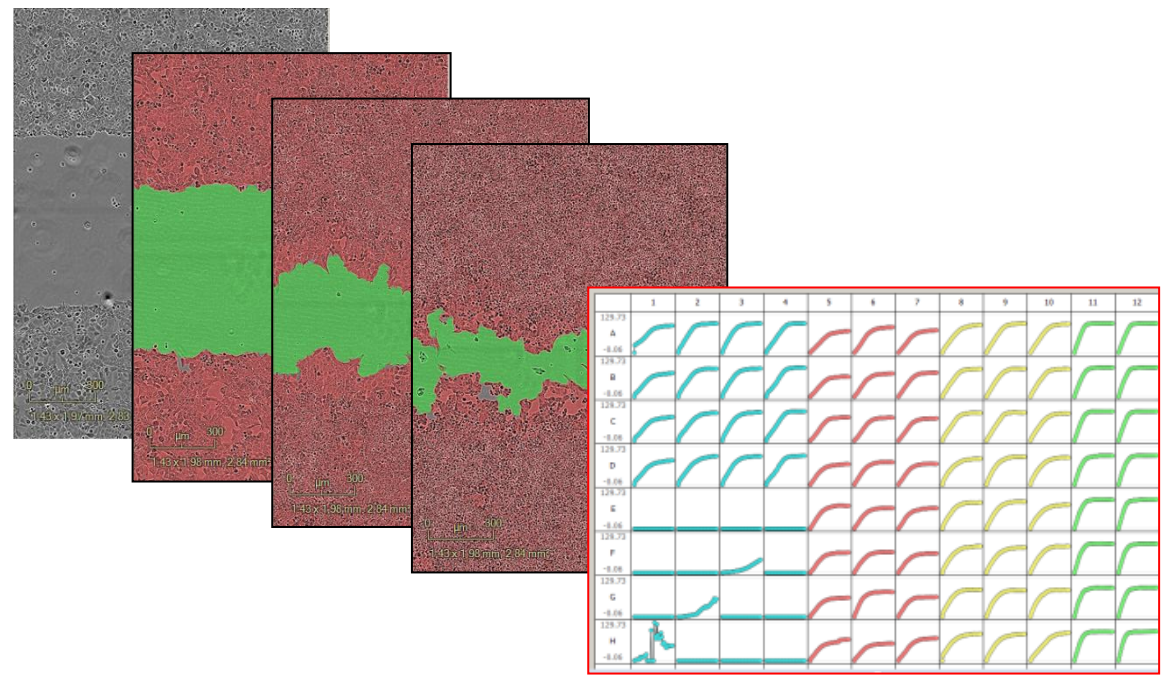
24 well plate from Nunc

Troubleshooting – How much of the well area are we scanning?

Objective	Vessel	Images pr well	~ Area scanned
4x	6-12-24-96-384 well plate	“whole well”	
4 x	6 well plate	47	
4 x	12 well plate	12	
4 x	96 well plate	1	
10 x	6 well plate	121	
10 x	12 well plate	49	
10 x	96 well plate	5	
20 x	6 well plate	121	
20 x	12 well plate	49	
20 x	96 well plate	1	

Scratch wound maker (only used by MIC personnel)

The wound maker is a very useful and very expensive toy for creating homogenous, 700-800 µm wounds in 96 wells.



Users must remember the following:

- Preferably user **ImageLock plates** from (Essenbio) from MIC (booking system-supplies).
- Seed cells overnight or 24h in advance to a **90-95% confluency (10-40.000 cells/well)**. If cells are left growing longer there will be a lot of matrix left on the bottom surface disrupting the wound healing. If cells do not attach well, coat the bottom with collagen or poly L-lycine.
- Do not write on the lid, this might disrupt the focusing and later the analysis.
- Take care not to scratch the bottom of the plate, nor the bottom of the wells, this will disrupt the focussing and analysis.
- Never leave any wells **dry**, this will damage the pins creating the wounds.
- Before creating the wounds, the wells should not contain more than **100µl** of solution!
- Wash away suspended cells in order to have a nice and clean wound.
- If you want to run a invasion assay, you will need to coat the cells and wound with an extracellular matric (please ask for more information or read the application note and protocol).

Admins must remember the following:

- Before usage, soak pins in sterile water for 5 min and then 70% ethanol, and let air dry.
- Perform the wound. If you are wounding multiple plates of the same cell line, simply soak pins in 45 ml sterile distilled water between wounding.
- After the last wound: 45 ml of 0,5% Alconox for 5 min,
  45 ml of 1% Virkon for 5 min,
  45 ml of sterile distilled water for 5 min,
  2x with 70% ethanol for 5 min.