Quick user-guide for the IncuCyte S3

- General rules for operating the instrument page 2
- Installing the S3 software on your personal pc page 3
- Scheduling a scan page 4-7
- Scheduling and scan types page 8
- Scheduling a scan once now (“scan on demand”) page 9
- Viewing your data set page 10
- Analyzing a confluency experiment page 11-14
- Normalizing your data and graph page 15
- Analyzing fluorescence imaging page 16
- Analyzing a scratch wound page 17-18
- Analyzing a spheroid experiment page 19
- Analyzing an organoid experiment page 20
- How to export image sets and movies page 21
- Archiving your data and deleting it from remote ctrl page 23
- Scratch wound maker page 24

Trouble shooting

- The position you reserved is not free page 25
- Red and orange warnings when scan scheduling page 26
- I do not have the recommended plate page 27
- How much of the well area are we scanning page 28
- Connecting remotely page 29

MIC
Molecular Imaging Center

UNIVERSITAS BERGENSIS

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 (not compatible with mac). If you need to access the system from home, you need to access through the uib portal (ask MIC for help).

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

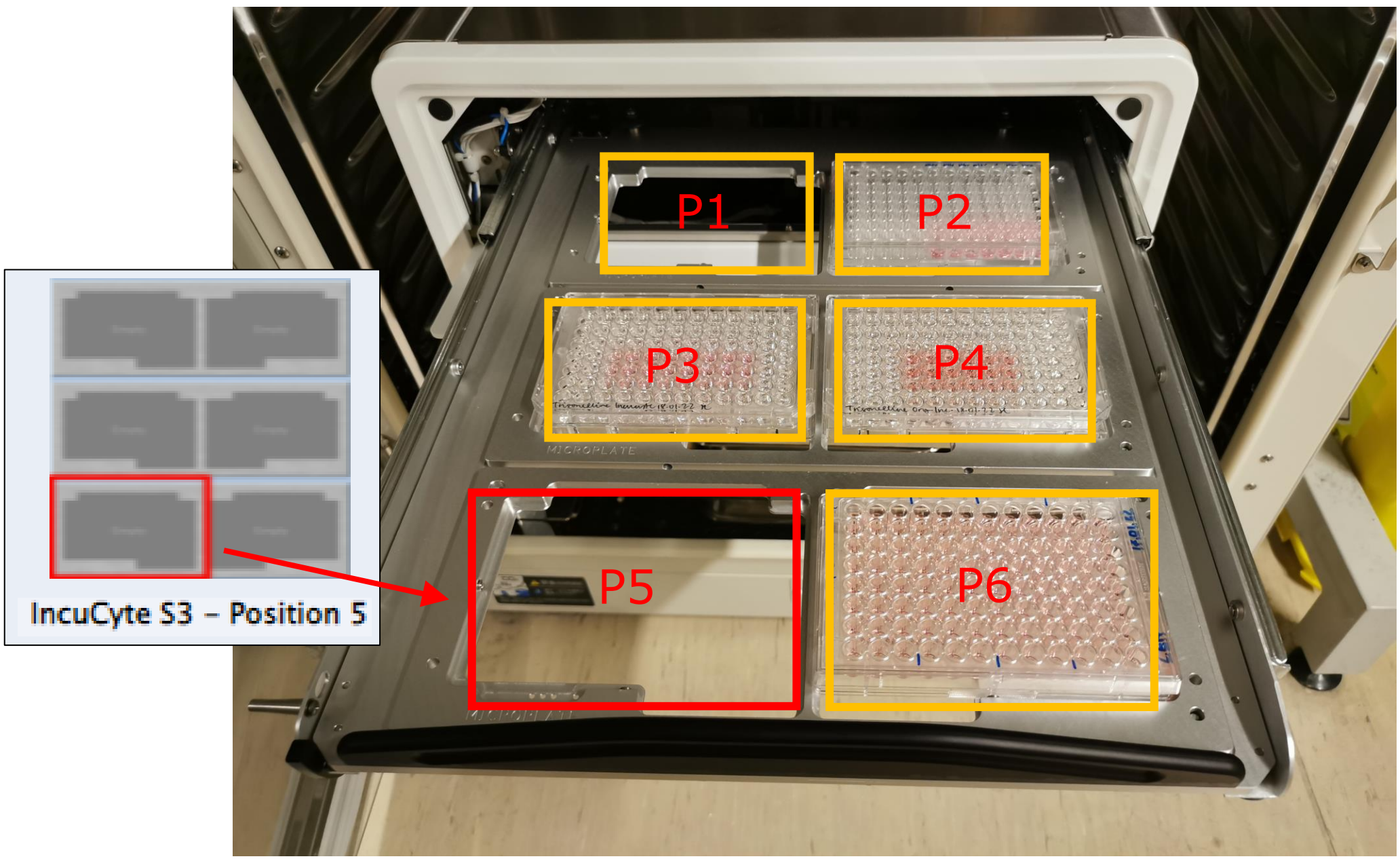
- Please save your files in the IncuCyte in the following format:

  yyyymmdd_scientist name_experiment name

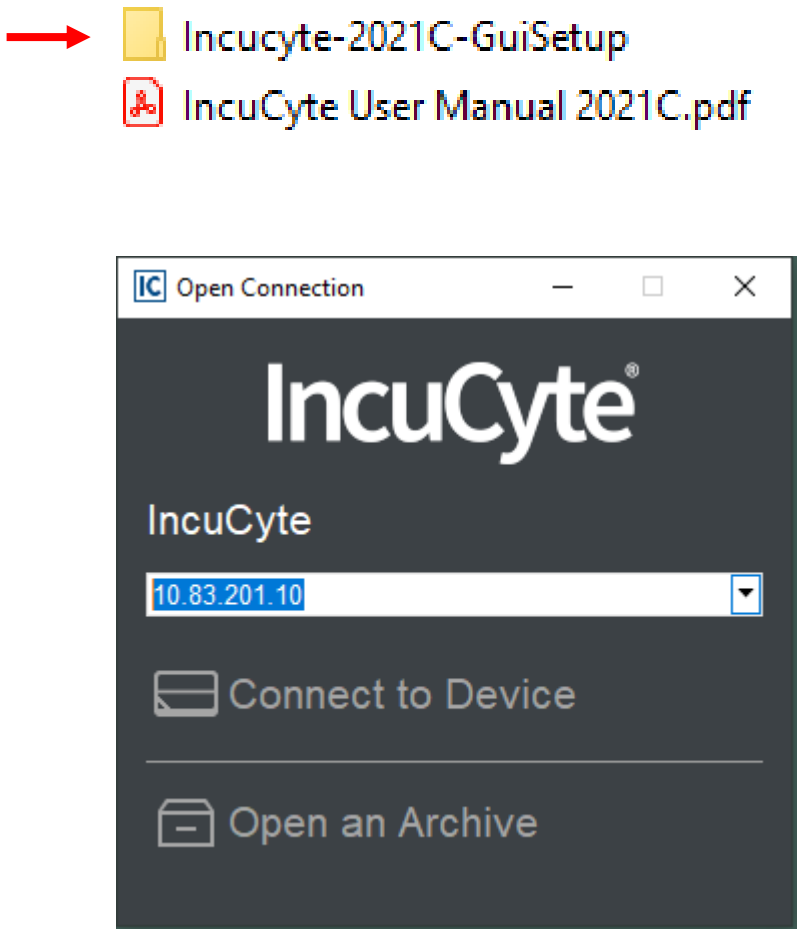
- Booking in advance is compulsory. Make sure you insert you plate in the correct position.

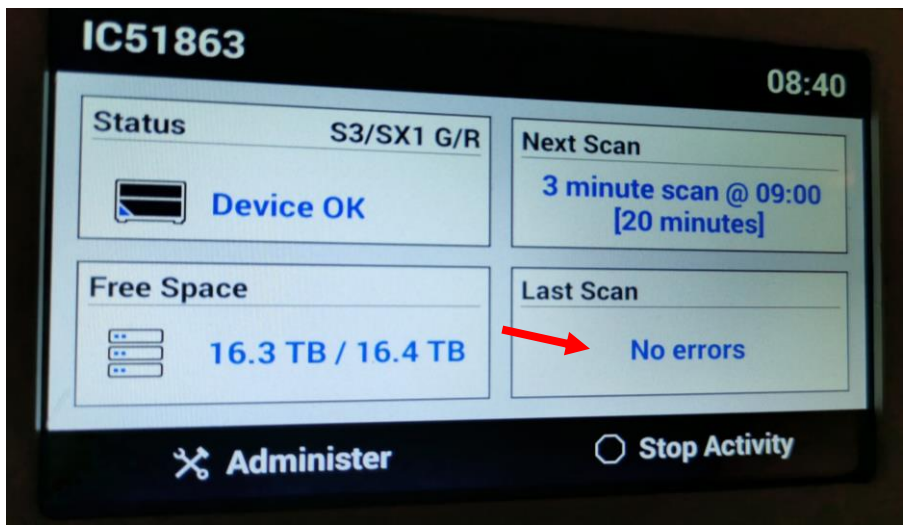
- If you are doing scratch wound healing, book the first hour as “supervised” and notify MIC personnel in advance. The wound-maker is only handled by MIC personnel as this involves special cleaning procedure and special care.

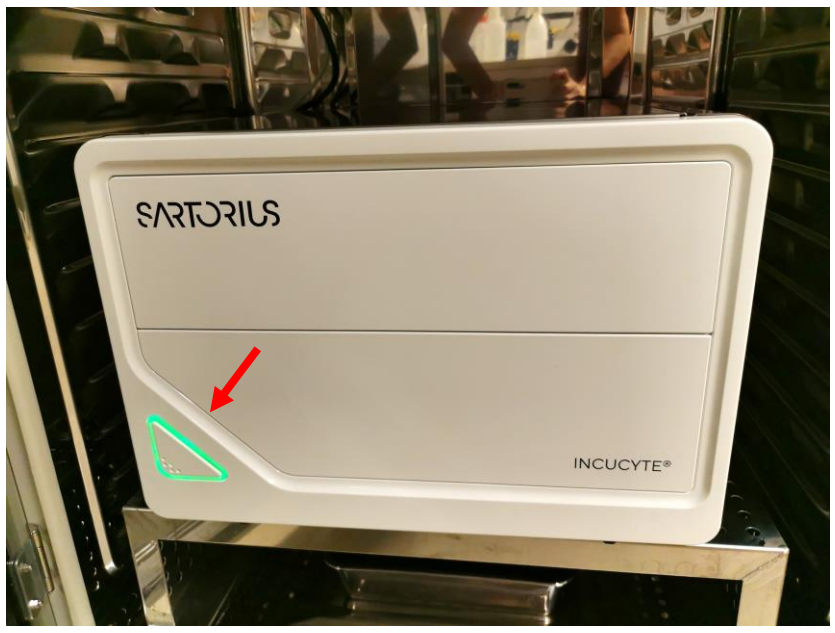
- It is mandatory to use shoe protection when entering the cell lab. Use gloves when handling the IncuCyte.

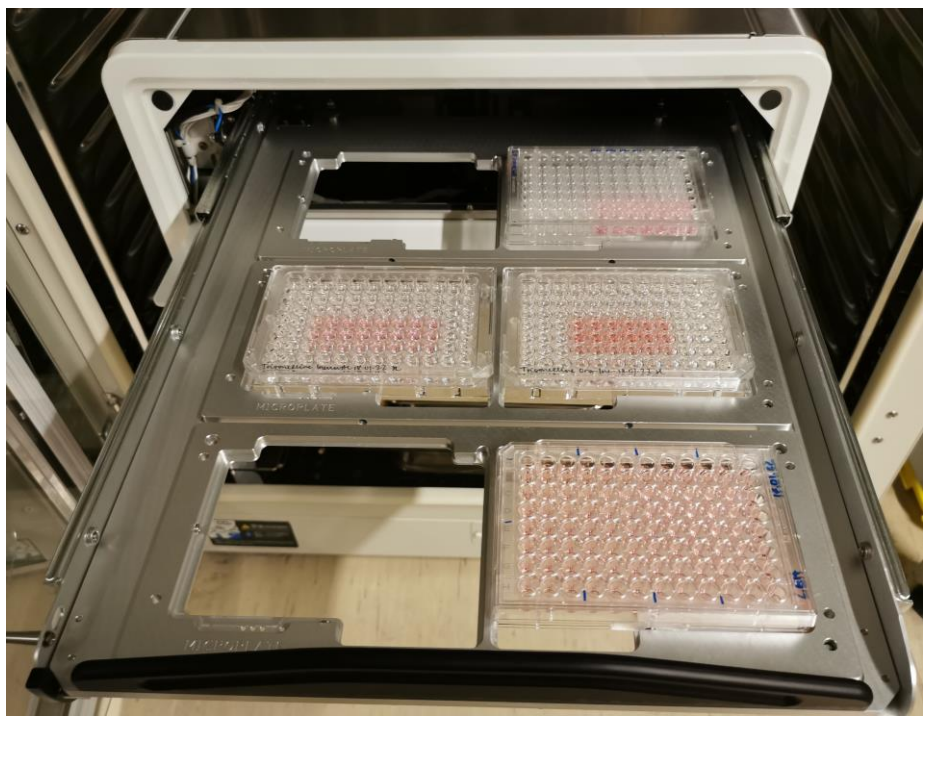


Installing the IncuCyte S3 software on your personal pc









You can install the S3 software on your personal pc. The software is not compatible for mac (except with windows).

The IncuCyte software has also been installed on a workstation in the MIC PC room on the 6th floor. This can be booked free of charge through the MIC booking system.

Get the "heart" usb from the MIC office or connect to the Biomic server and download the software from the "free software-Incucyte" folder.

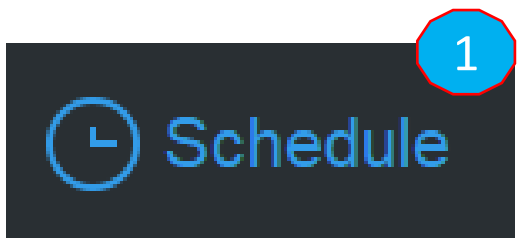
Log on to the controller with the IP address:
10.83.201.10
Put in your username and password. You can get this from Endy, Hege or Halala once you have done the training.

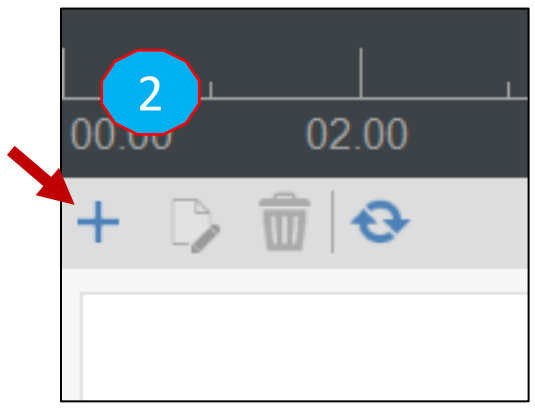
Make sure the system is not actively scanning (check the control panel) before opening the drawer (light will be green if you can open).

Do not insert your plate directly into the drawer. Remove the metallic rack, place it on the clean bench and then insert your plate.

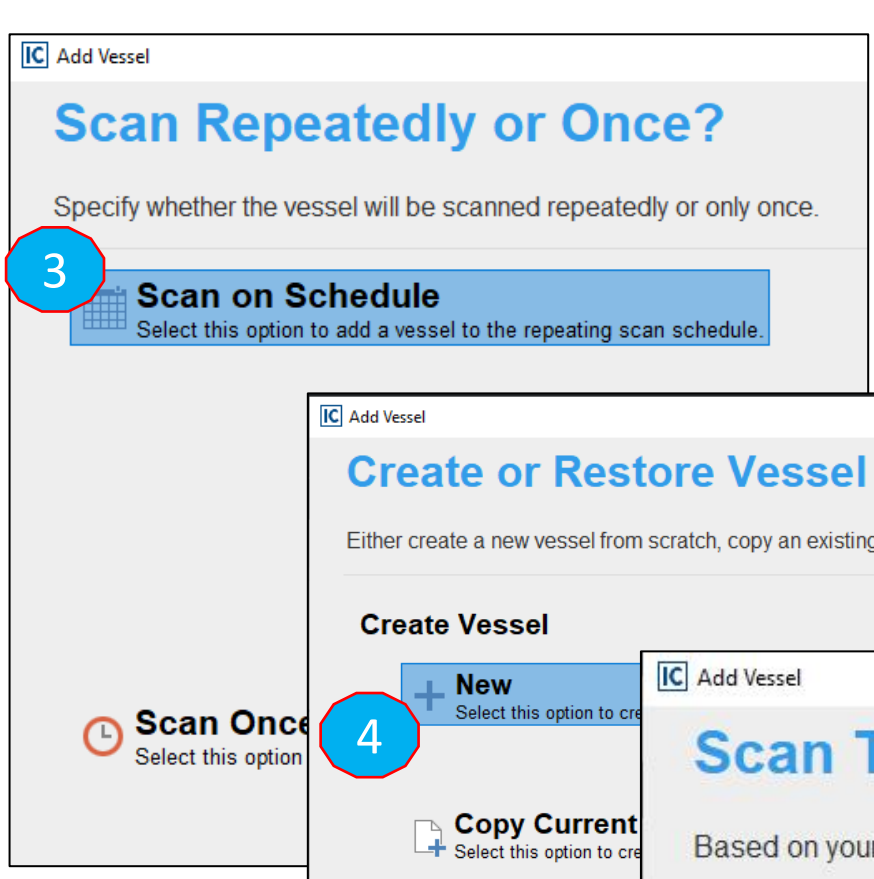
Make sure there are no error messages displayed on the control box after you have closed the incubator.

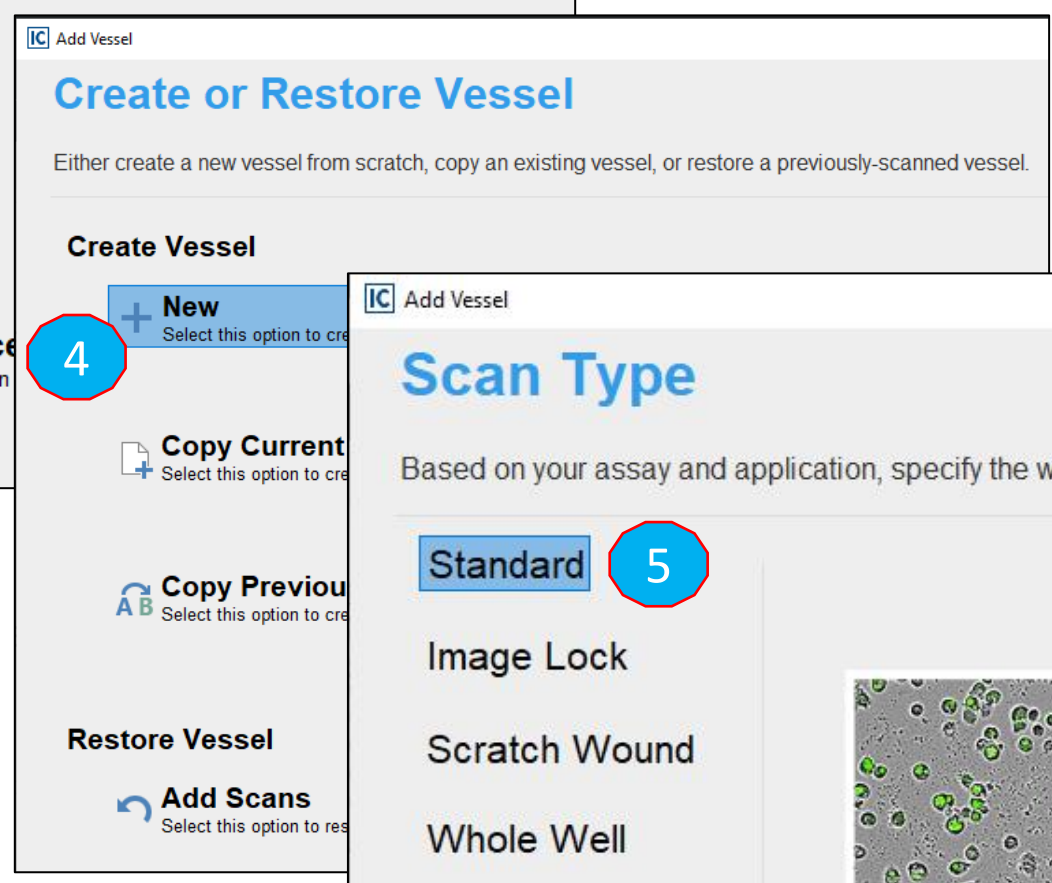
Scheduling a scan

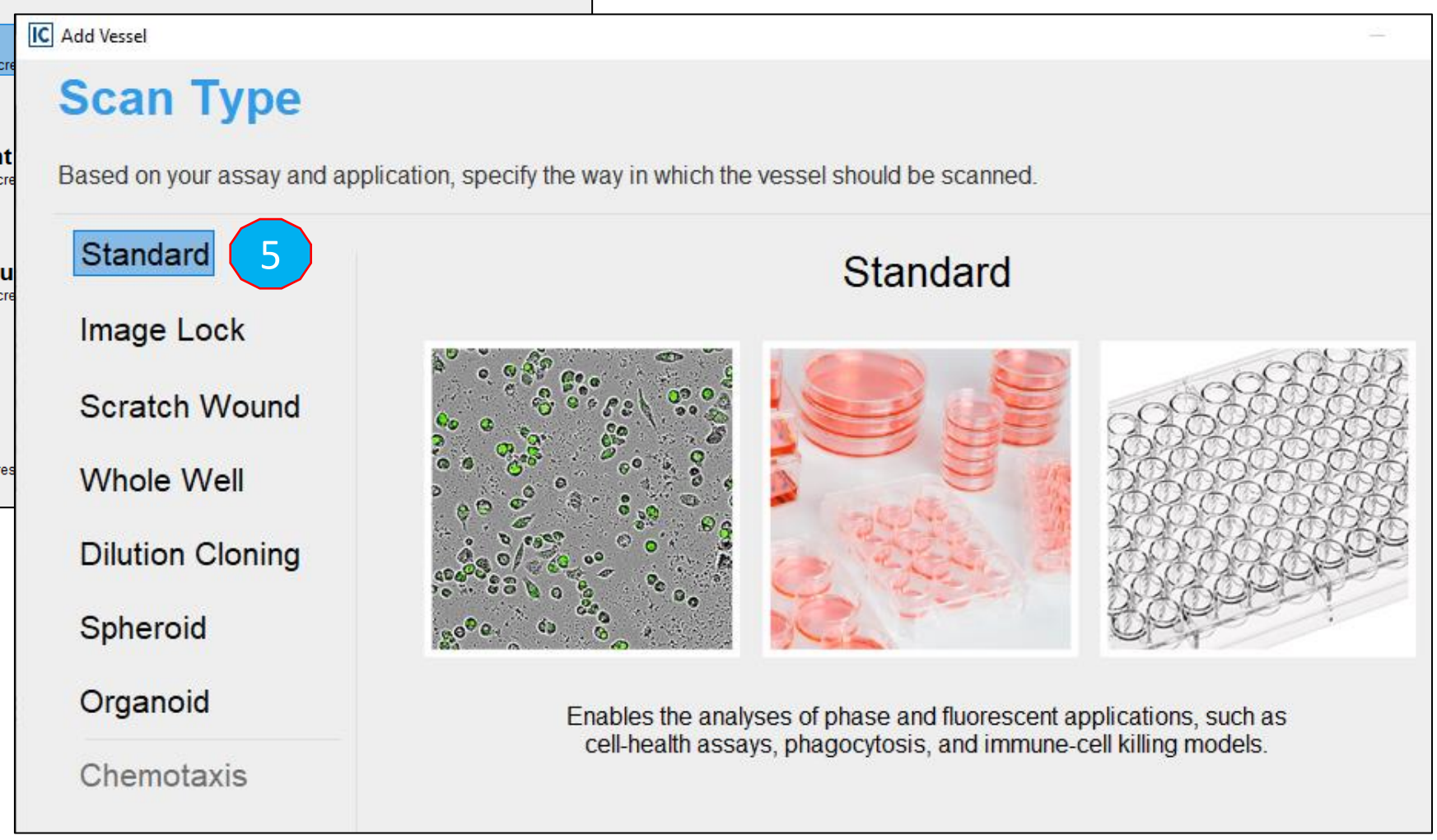




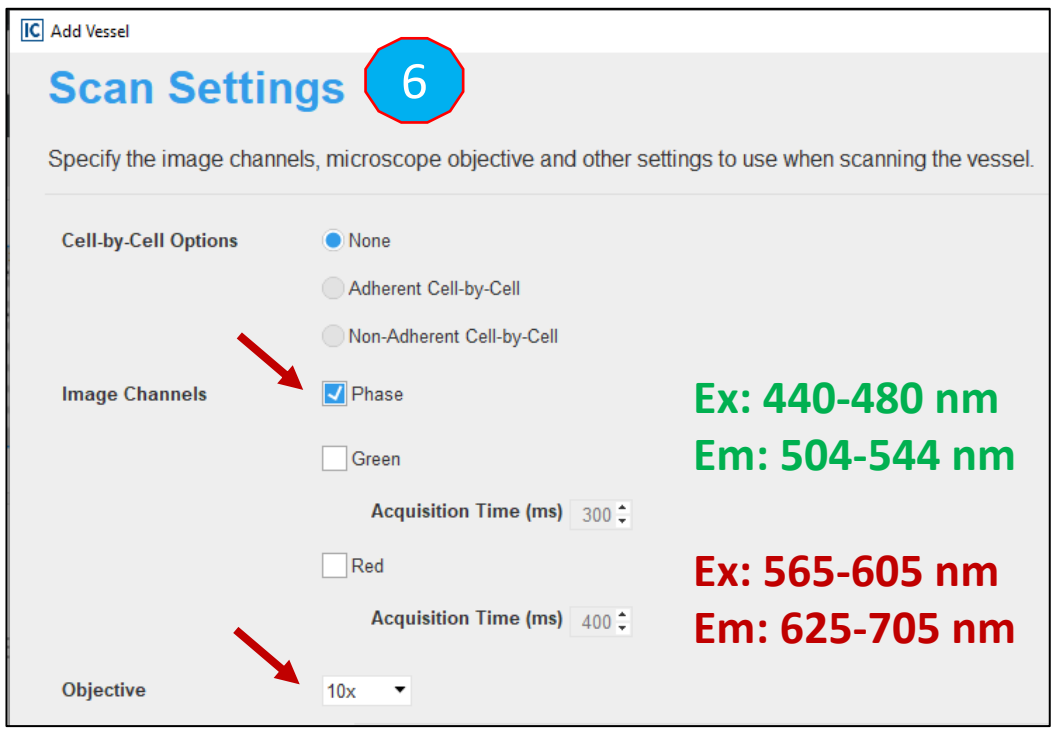
1. Open the "**Schedule**" window. Here you will see plates already in the system. Make sure the position you reserved is free.
2. To add a new plate, press the "**+**" sign.
3. You will now go through a guided assistant wizard. Select "**scan on schedule**" for timelapse imaging (repeated imaging every x hours).
4. Create a "**new**" vessel. If you are repeating a similar vessel to previous experiment, you can select one of the other options.
5. Select a scan type based on your assay, see page 8 for more details. "**Standard**" is used for a large number of assays including cell health, phagocytosis and immune cell killing experiments.



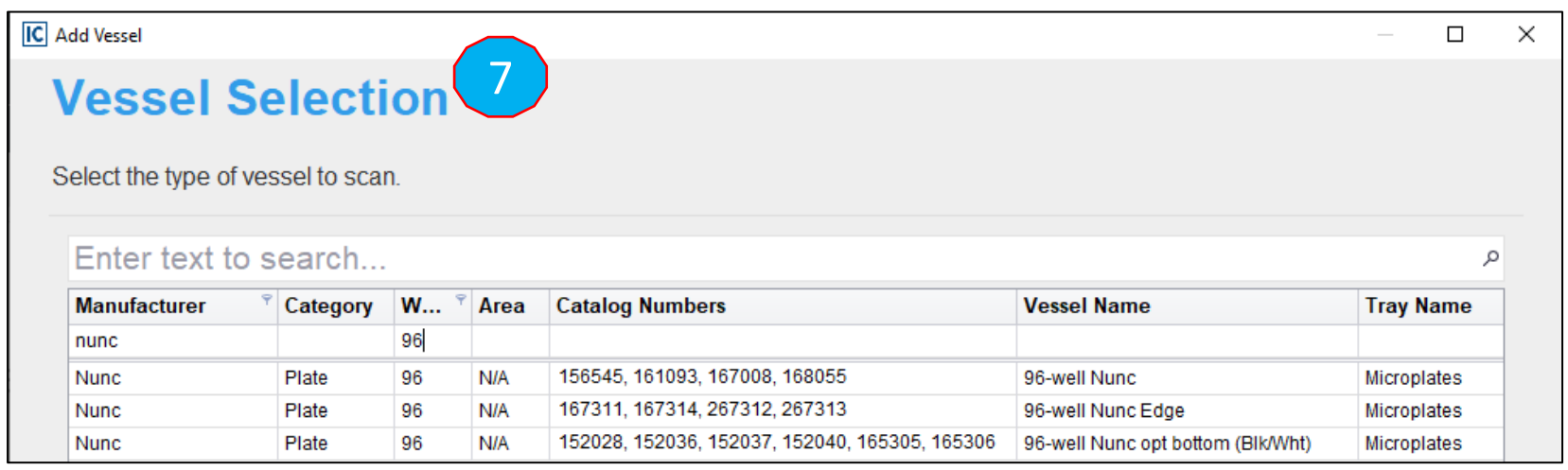


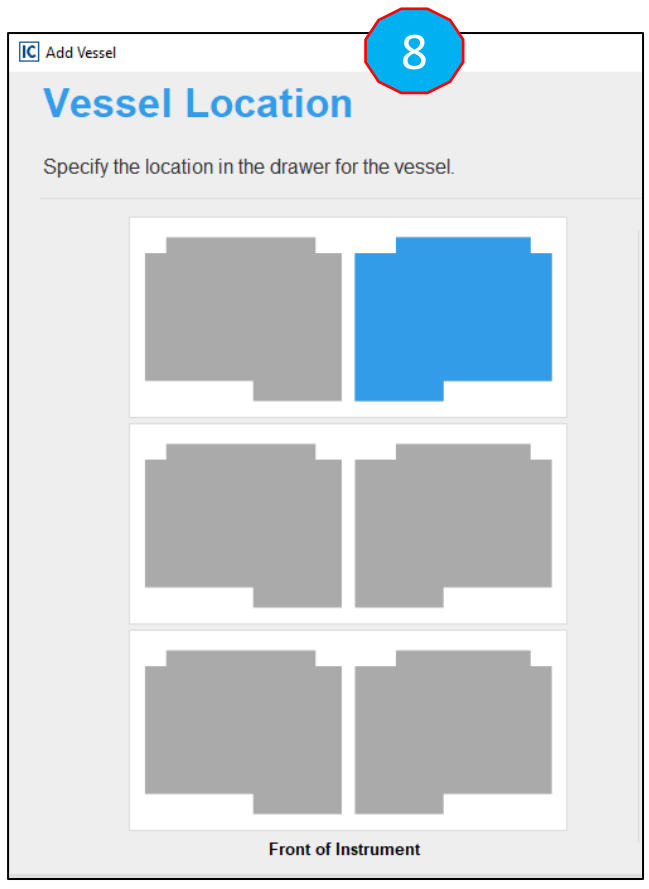


Scheduling a scan - continues

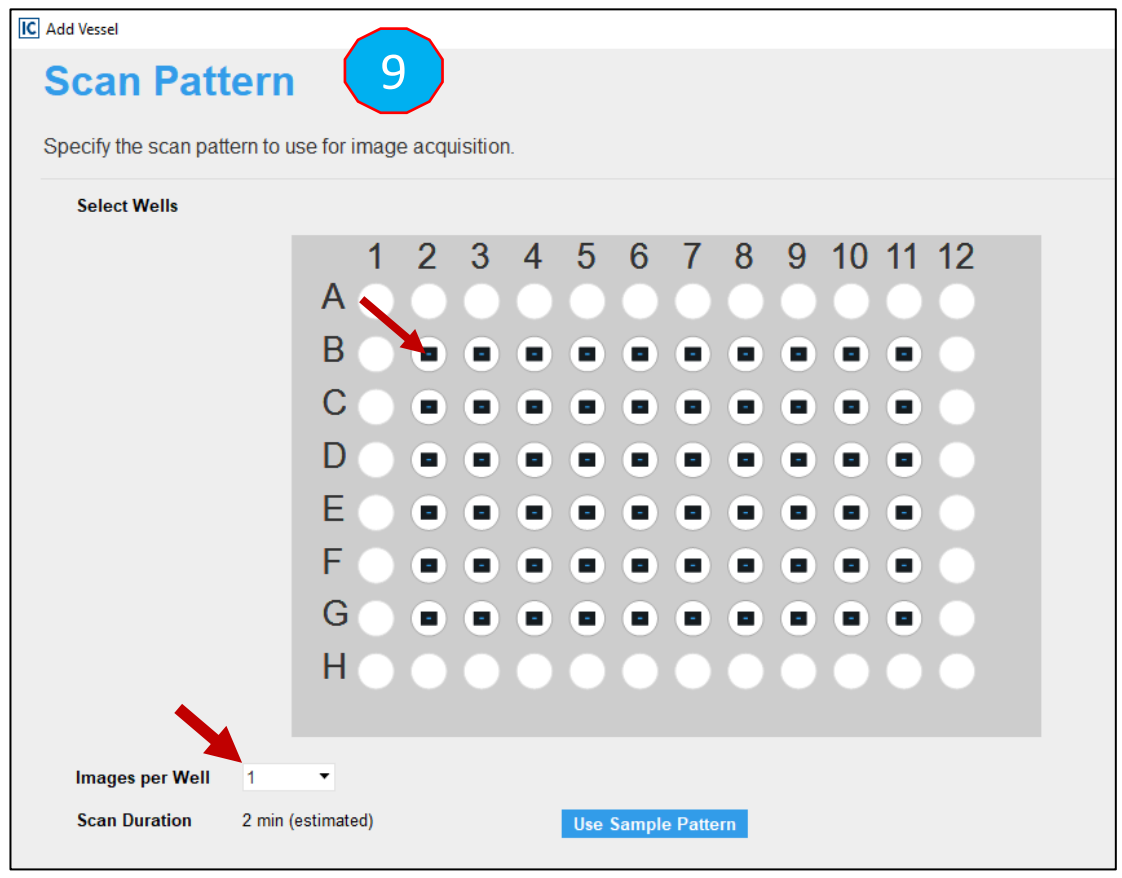


6. Specify Image channels and objective (this will vary depending on the "scan type" chosen in the previous step.
7. Select the correct vessel. The list of compatible plates will be long. Sorting for manufacturer, well or catalog number is very useful. It is important to use a compatible vessel in order to get correct xyz values for well positions.

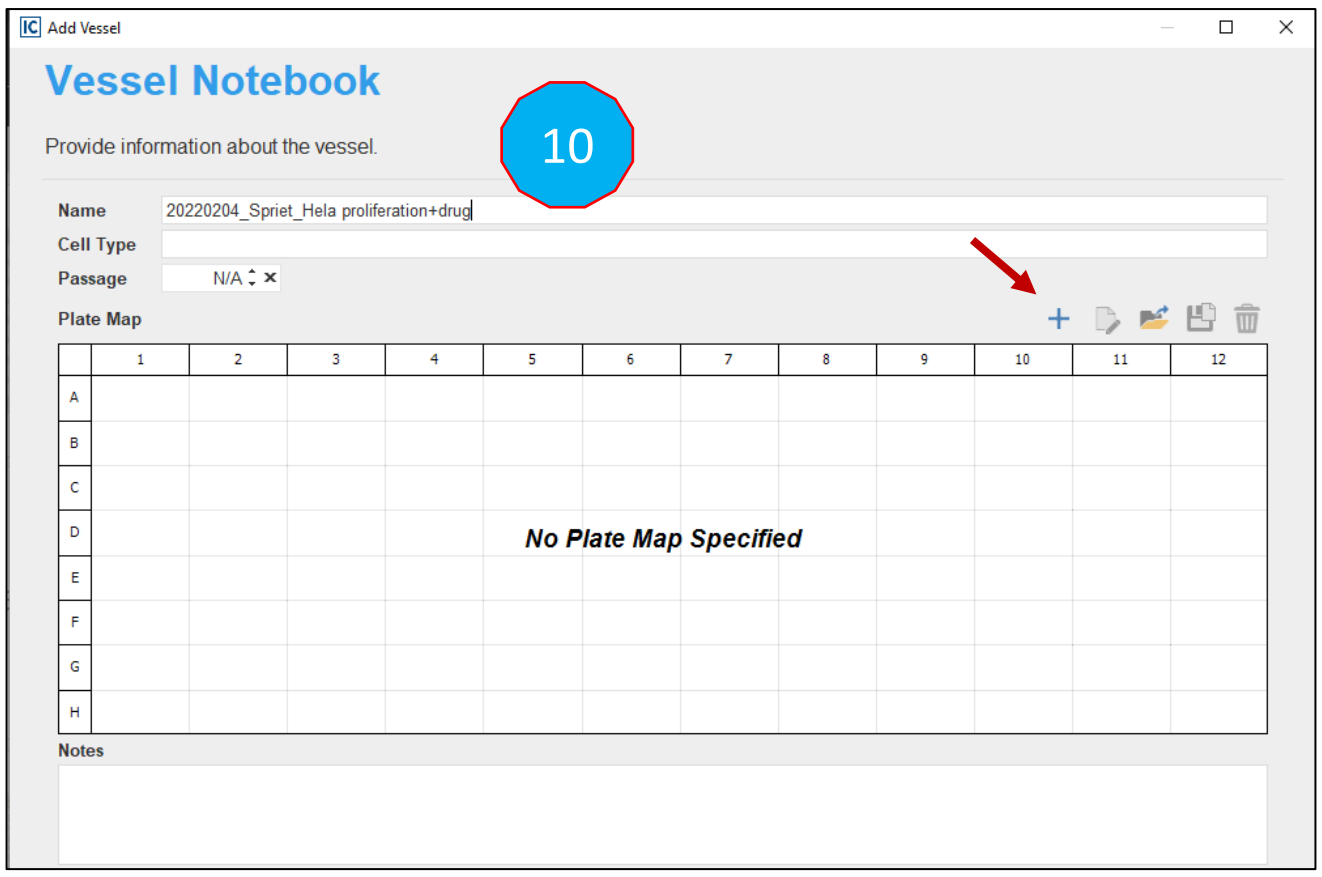




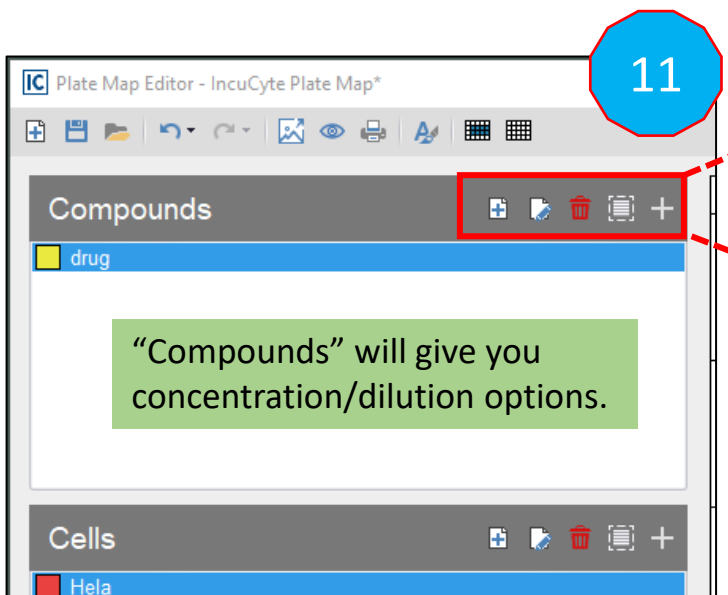
8. Specify the location in the incucyte for your plate.
9. Specify the scan pattern and number of images per well.

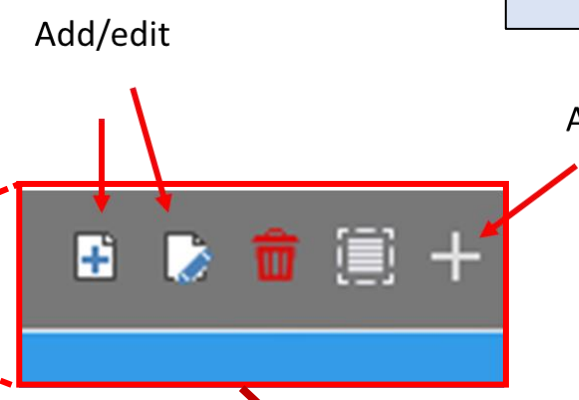


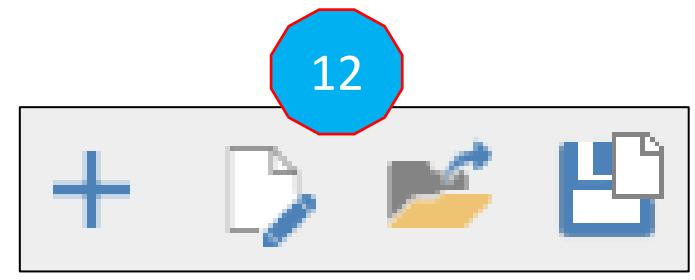
Scheduling a scan - continues

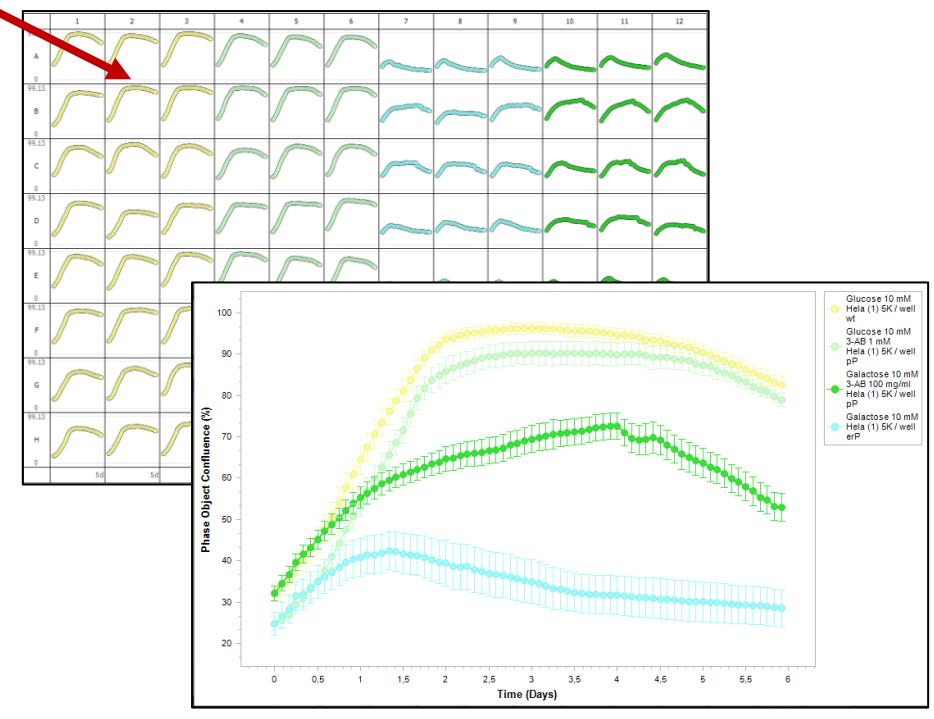


10. Save your plate information as following: **yyyymmdd_scientist name_experiment name**
11. Add a plate map. You can choose between compounds, cells and growth conditions.
12. The plate map can also be exported and imported for other plates later.



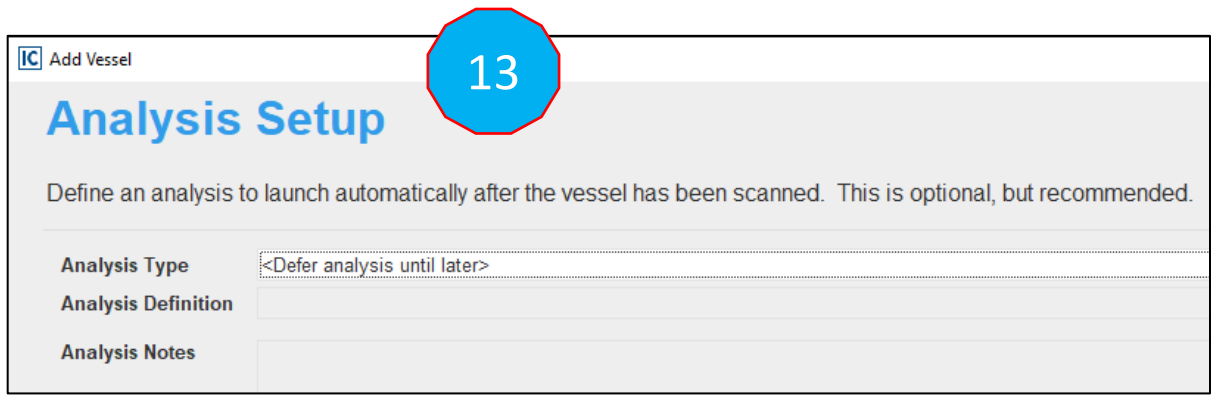




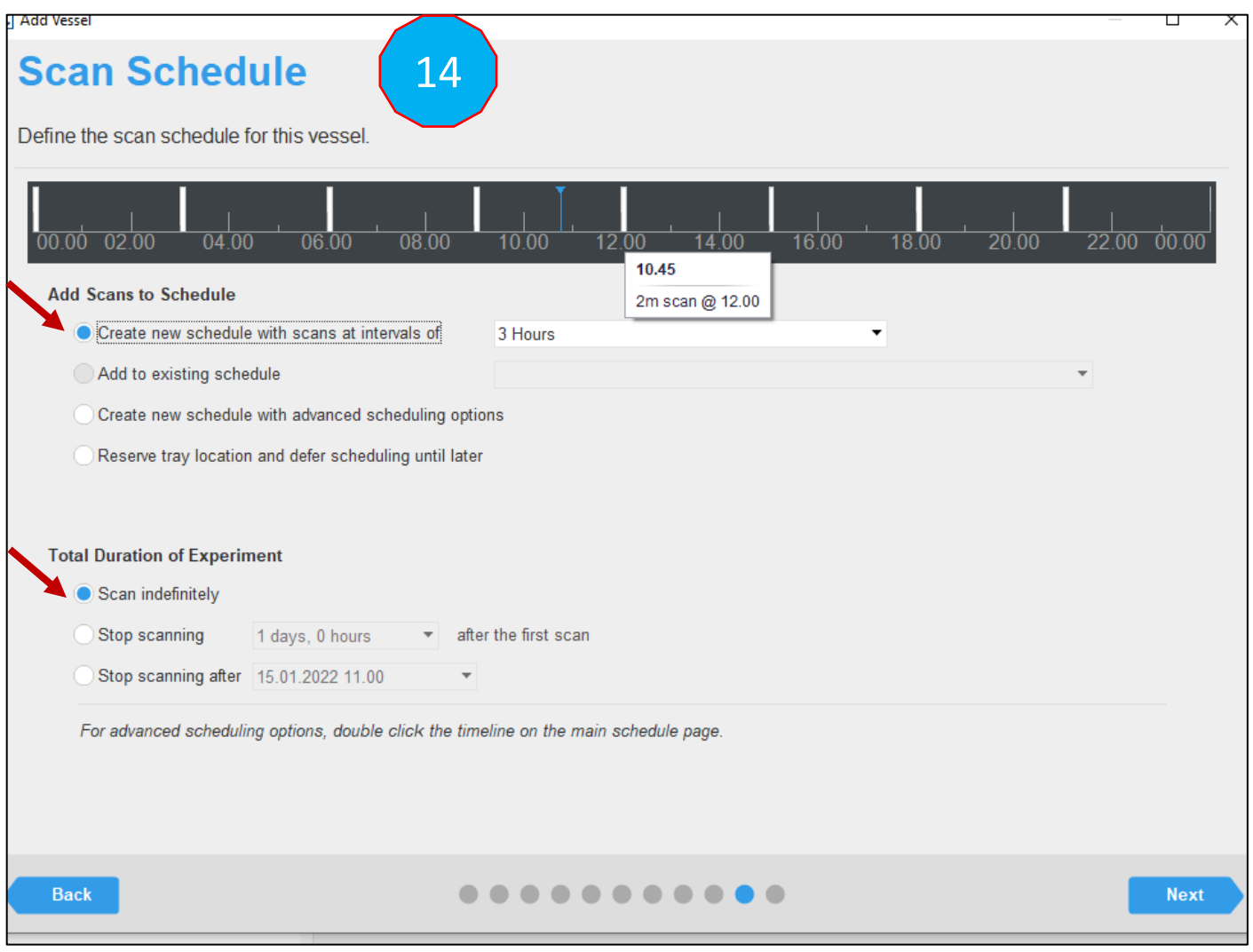


Why do I need a plate map?
It is very useful as this is your custom-design map helping you in having the overview of the experiment. When analyzing the data you will be able to pool the replicates together for the graph and histogram.

Scheduling a scan - continues



13. You can skip the "analyze setup" unless you already have one setup from a previous identical experiment.



14. Define the scan interval. Make sure there is no conflict with other scans (will show up as orange and red columns). Your intervals show up as white lines and you can move them around.

Define the total duration or leave it on "indefinitely" (recommended!) and remove the plate when you see that you have your results.

Remember to physically remove the plate from the incucyte when your experiment has ended.

How often should I scan?

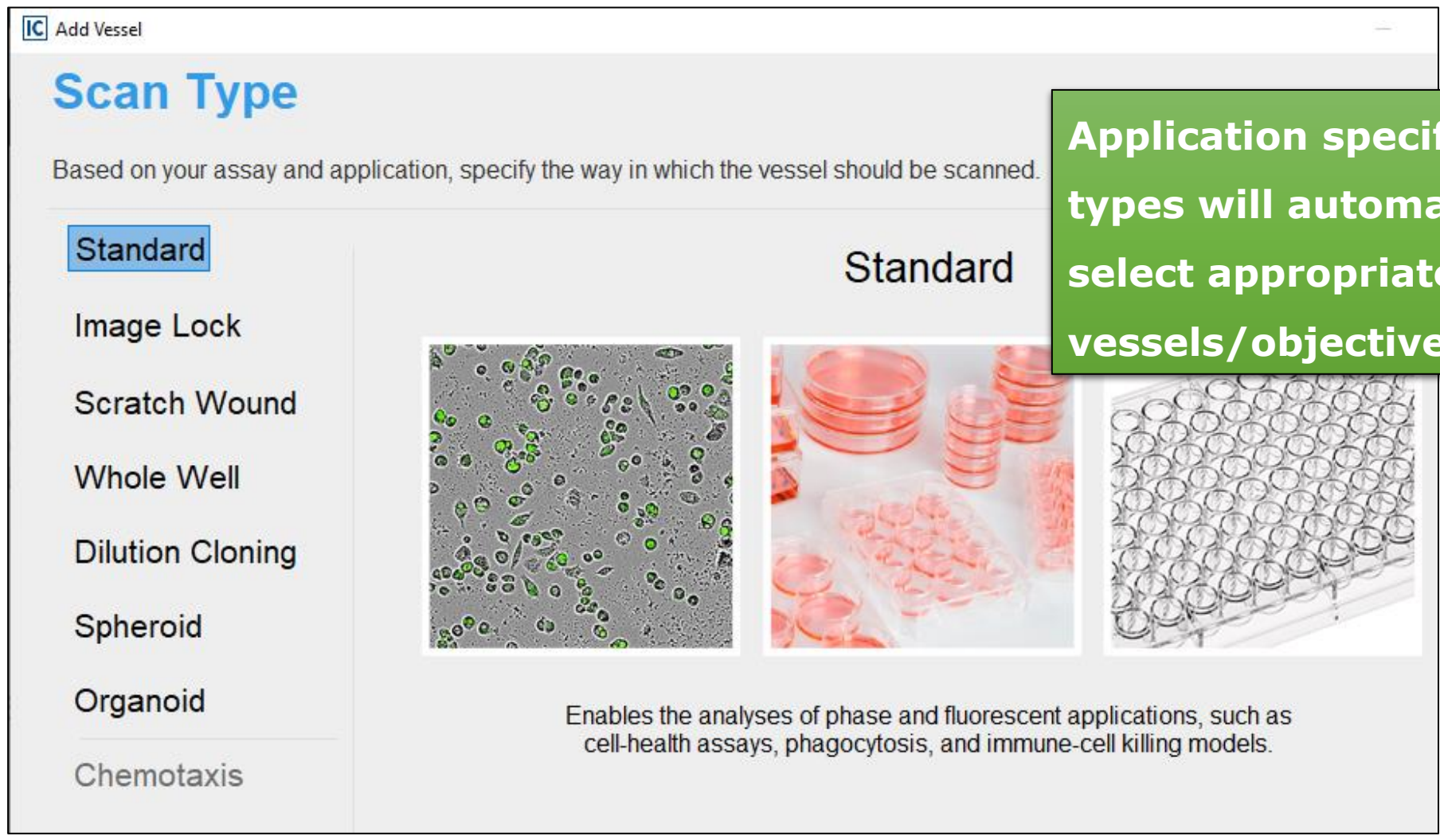
This will depend on the type of imaging you are doing.

Do not over-sample. How fast is the process you want to quantify?

This table indicates common scan intervals.

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 type



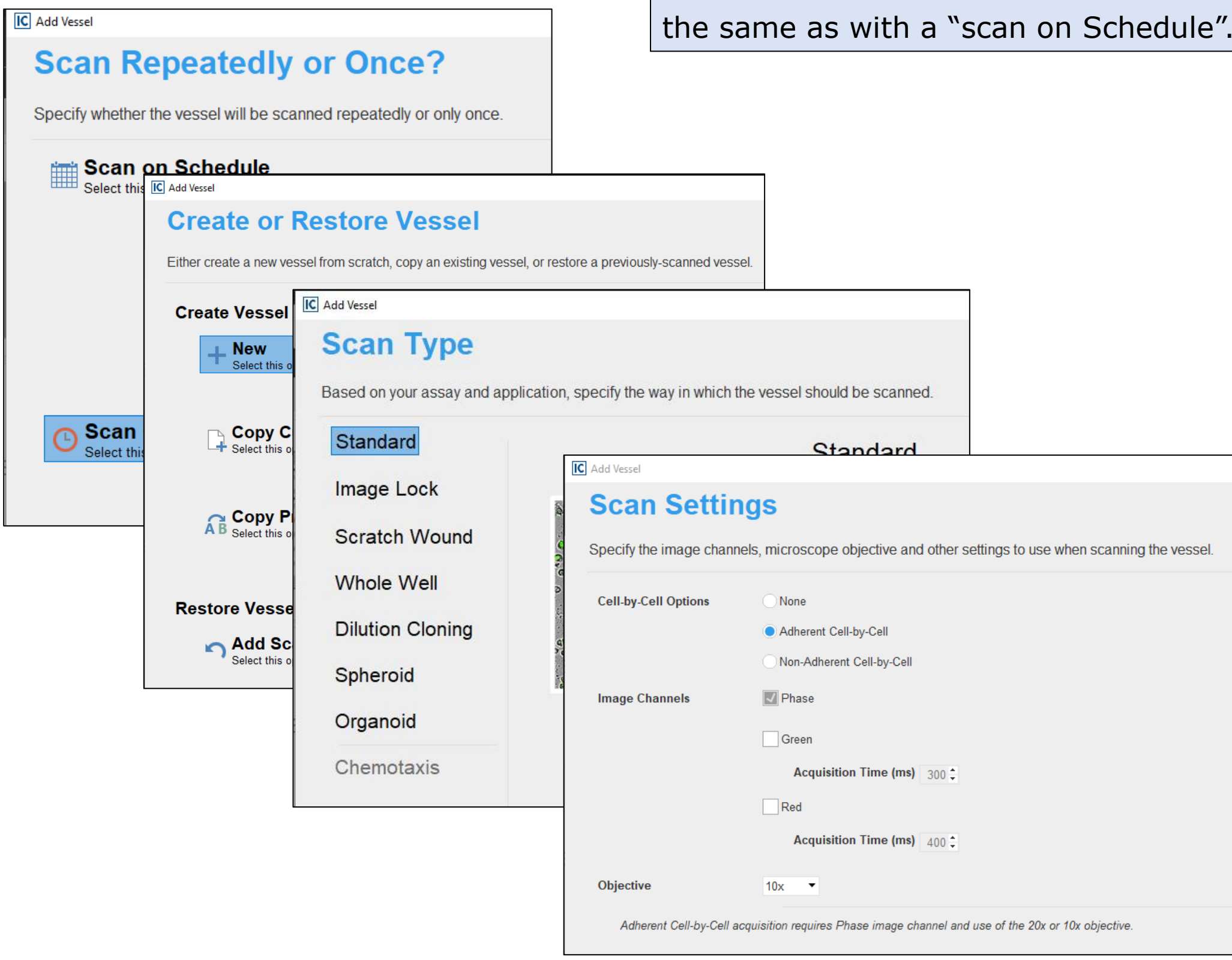
Application specific scan types will automatically pre-select appropriate vessels/objectives/channels.

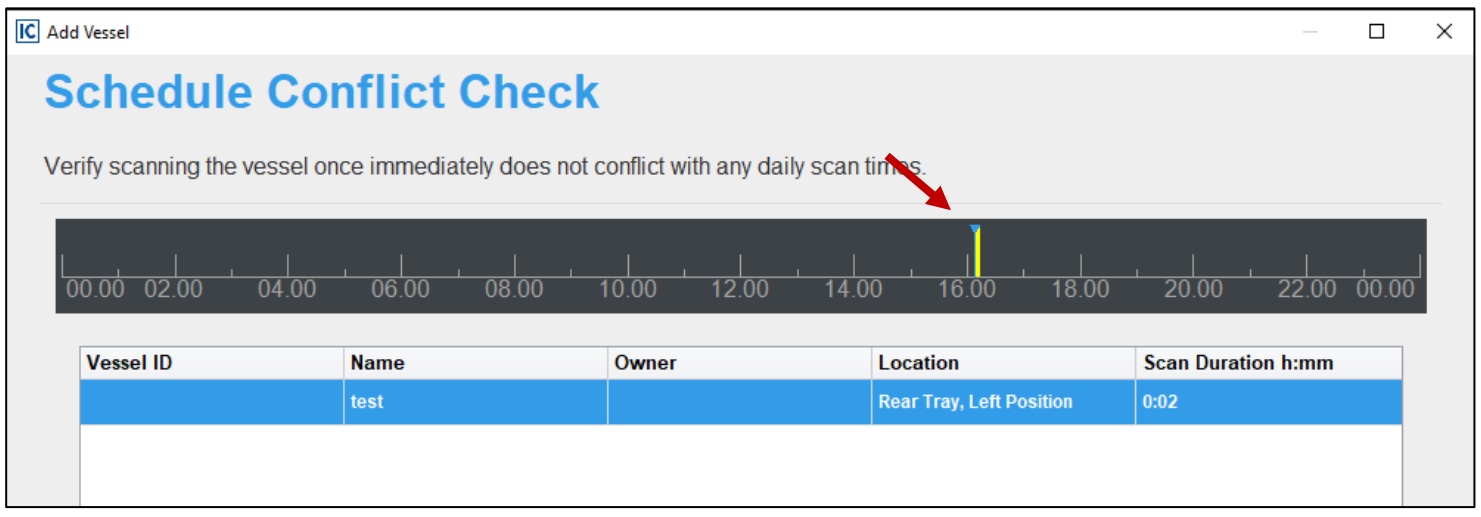
Scan type	Objectives	Plates available	Comment
Standard	4x, 10x, 20x	100s of plates available	Commonly used for assays including cell health, phagocytosis and immune cell killing experiments.
Image Lock	4x, 10x, 20x	Only 2 plates available	Especially designed for accurate xy positioning and export of 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 in 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.

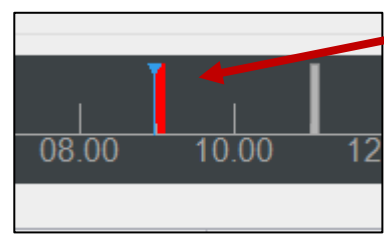
It is important that you seed out your cells, spheroids or organoids in the proper plates. Open the software and make a search as you plan your experiment.

Scheduling a “scan once now”

You can use this option when you want to only scan over your plate once. The setup is exactly the same as with a “scan on Schedule”.



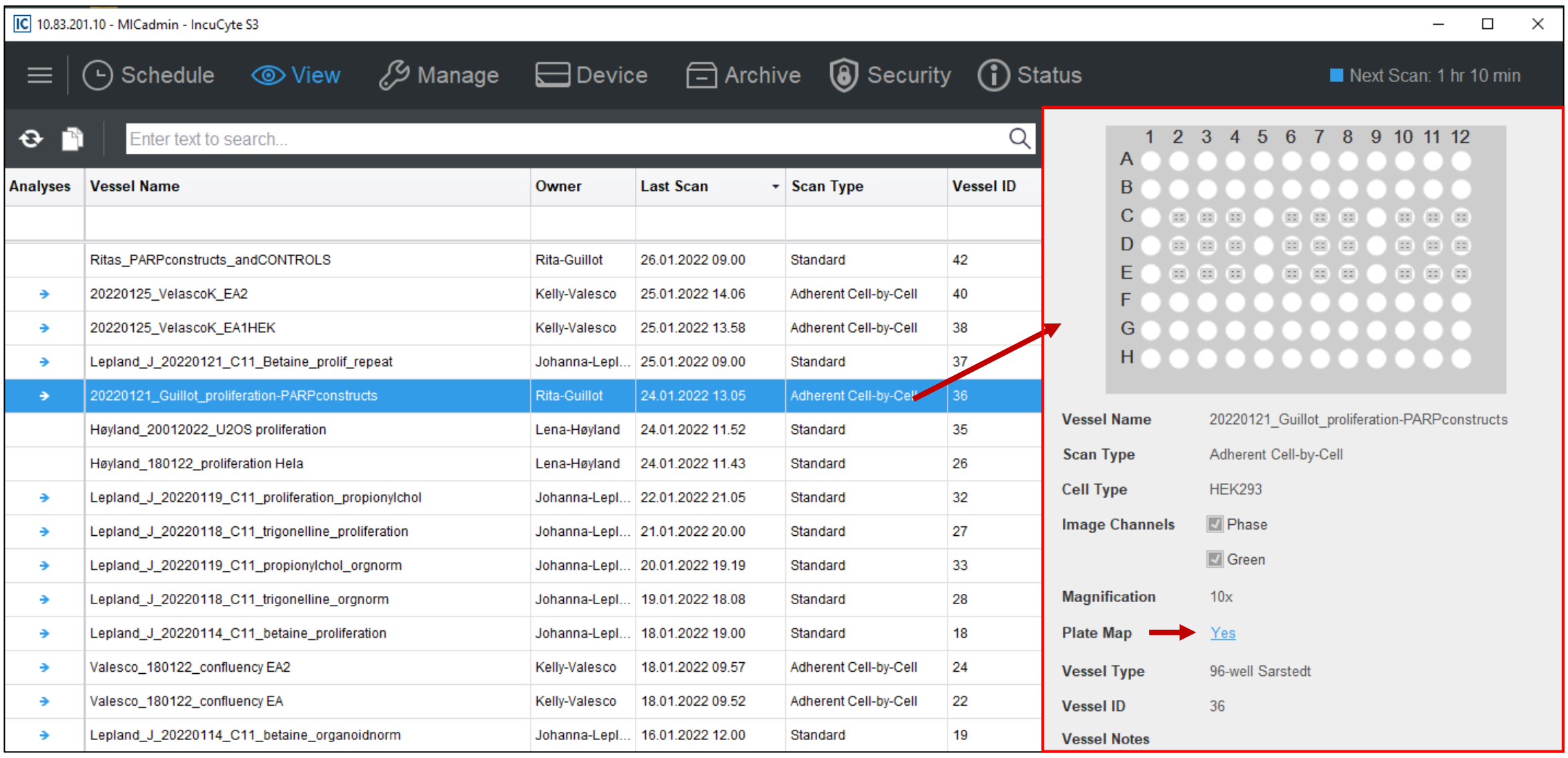




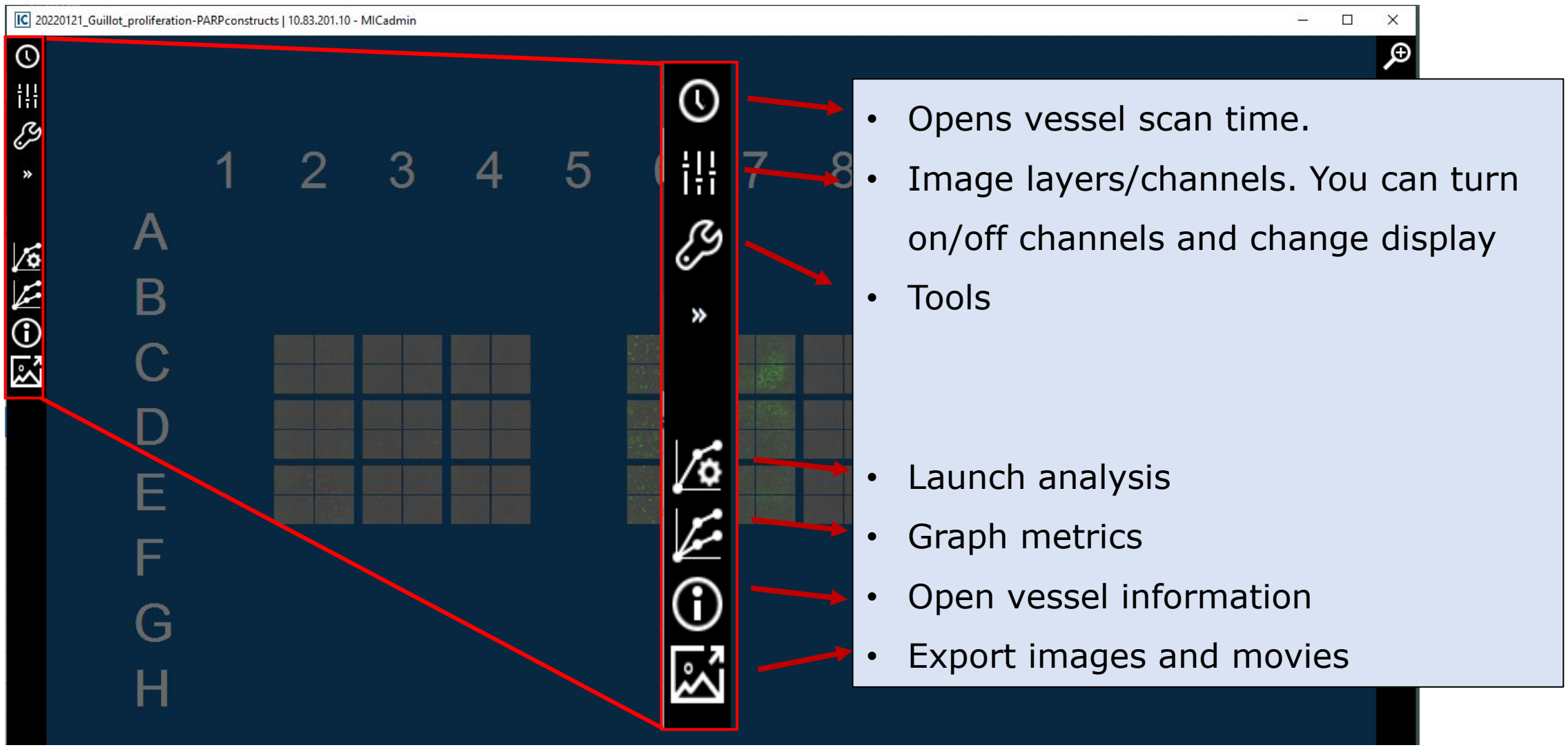
You will only be able to launch the scan if there is no schedule conflict. If there is no conflict, bar is yellow and you can click on “next”.

Conflicts show up as red bars. You need to wait until there is an opening in the schedule.

Viewing your data set

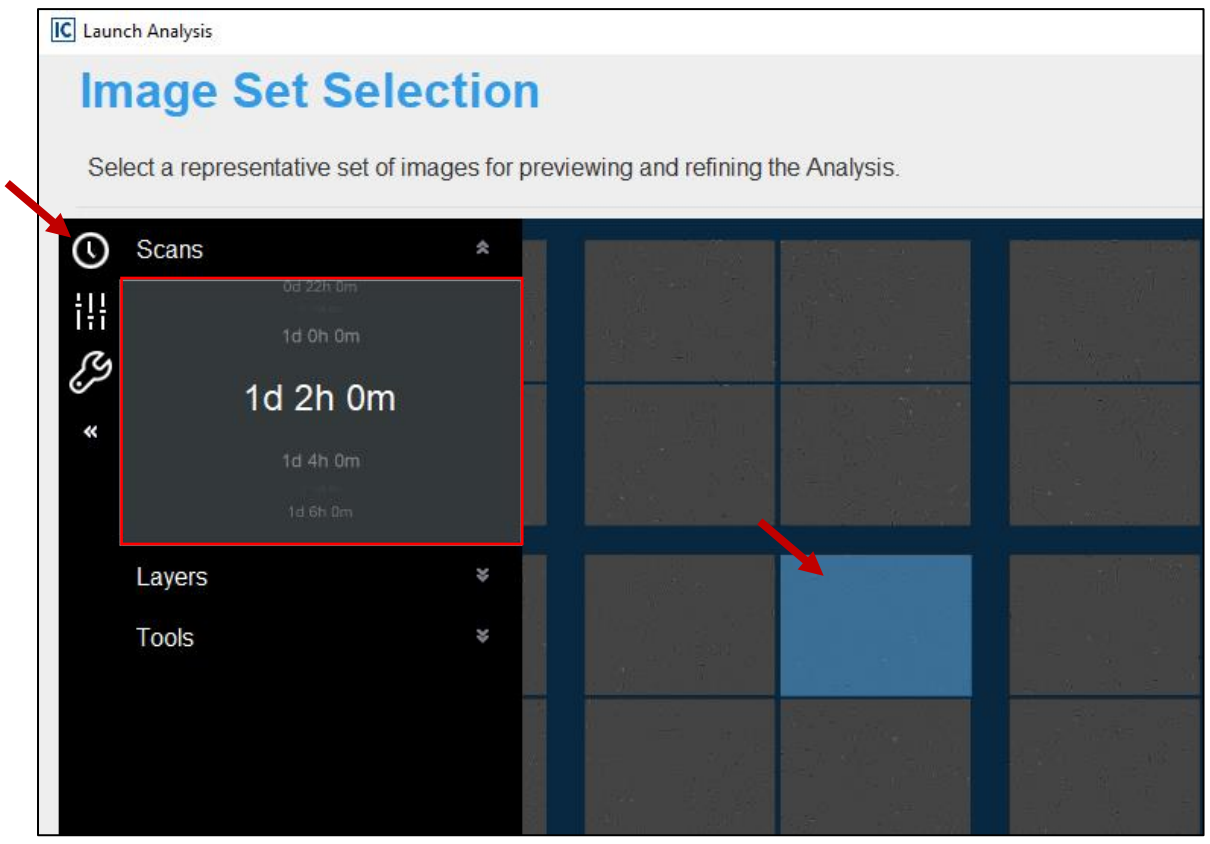


- Click on “view” to see the list of vessels scanned. Sort with your username under “owner”.
- To see more details on the experiments, click on the vessel and info will show up on the left side. You also have access to the plate map from here.
- To open the experiment, double-click on the experiment line.

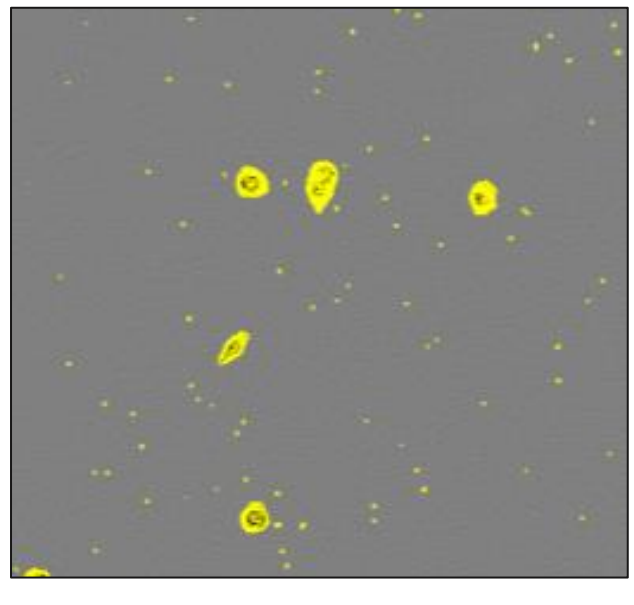


Analyzing a confluency experiment

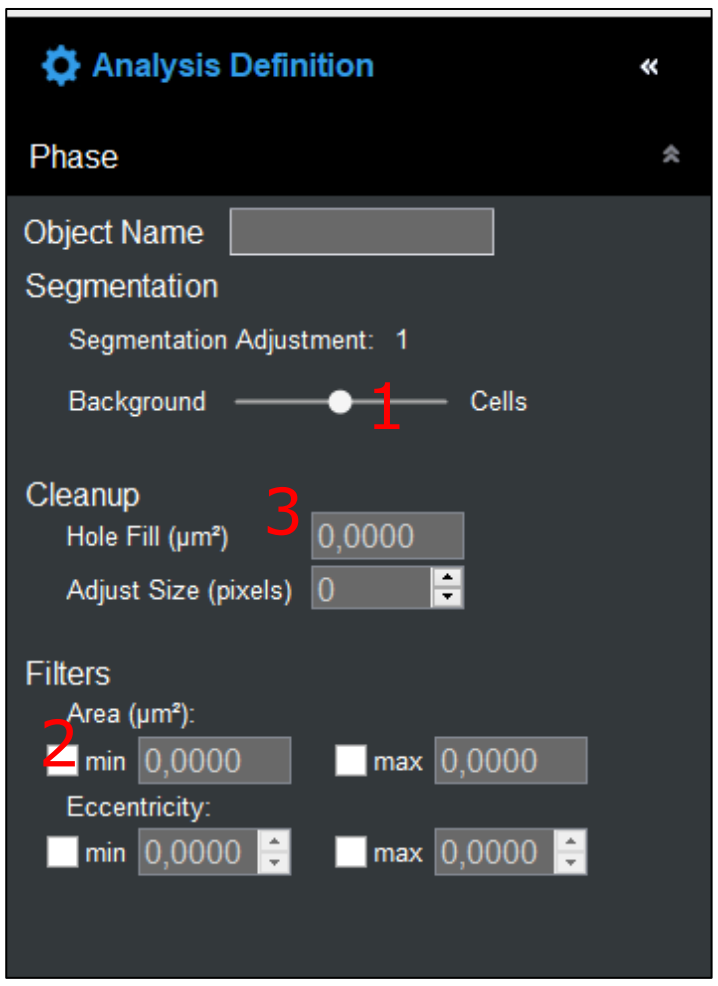
- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select "**create new analysis definition**" and select "**basic analyzer**".
- Select the Image channels you want to analyze.
- Select a few representative images in the "image set selection" by clicking on the image. Scroll through the "vessel scan times" and select different time points. When you have 4-8 images, click next.



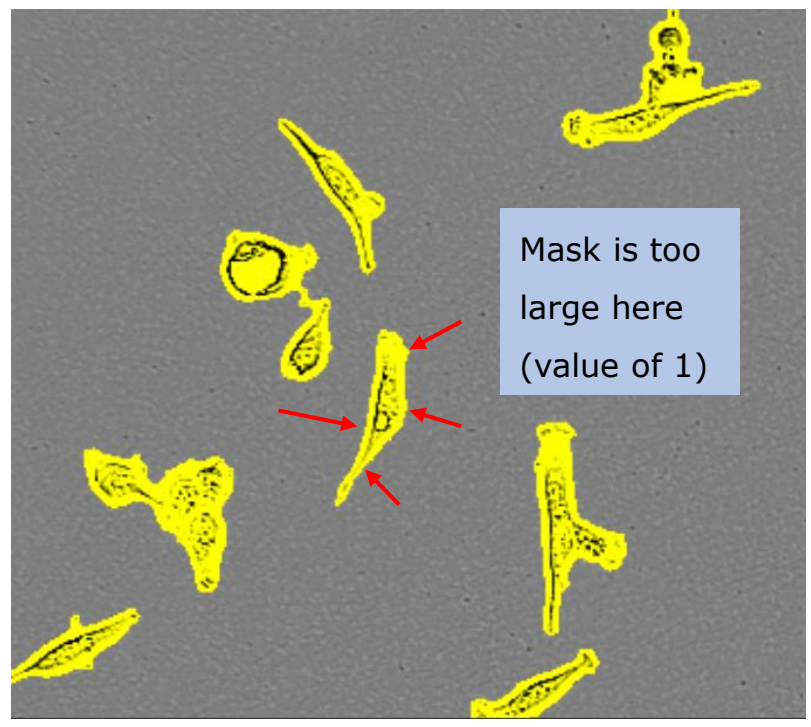
- The image selection will now be uploaded to your computer.
- To start the process of masking, you need to first press "preview current. A default masking will then appear on your image. On the next page you will see how we can improve the masking.

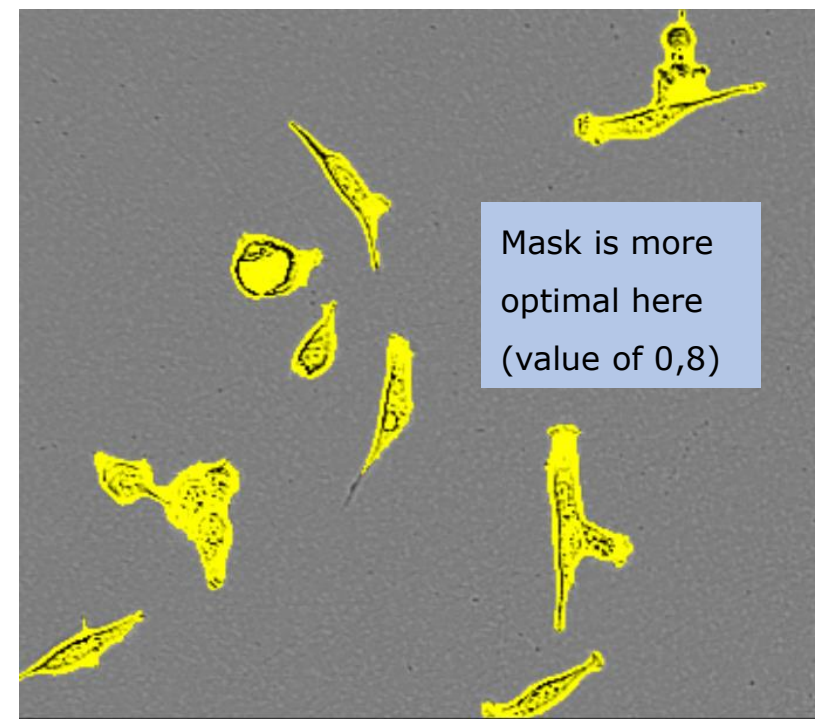


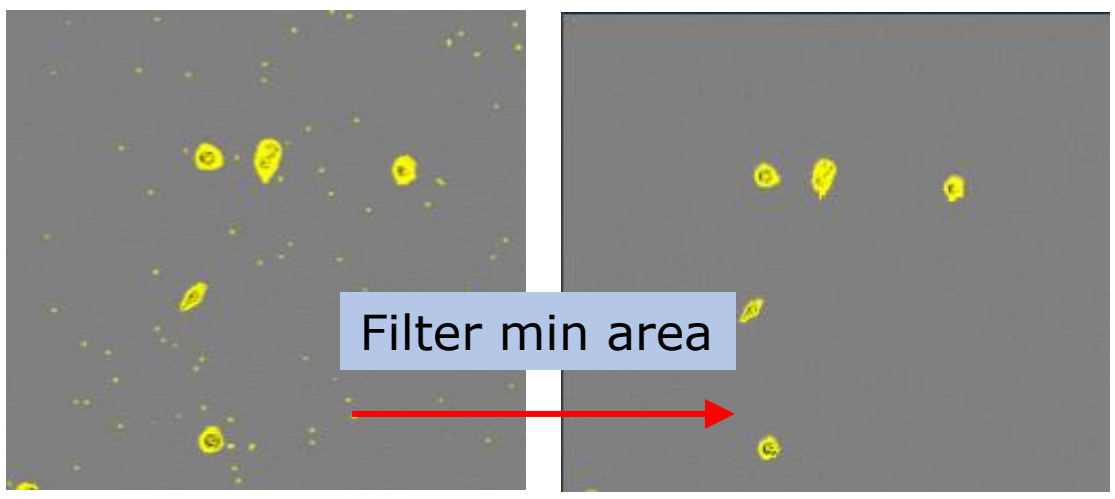
Analyzing a confluency experiment continues



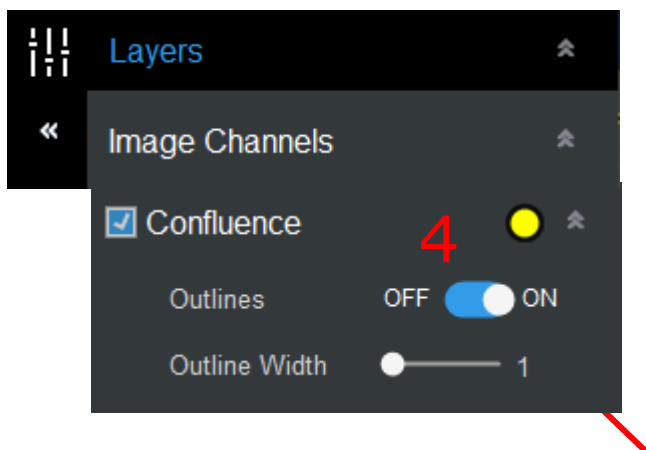
- Segment adjustment (1): if cells are very transparent, move slider towards cells (more sensitive to pick up cells). If cells have a good contrast on the background, move slider more towards background. It can be useful to turn on/off the mask to check the phase image underneath.

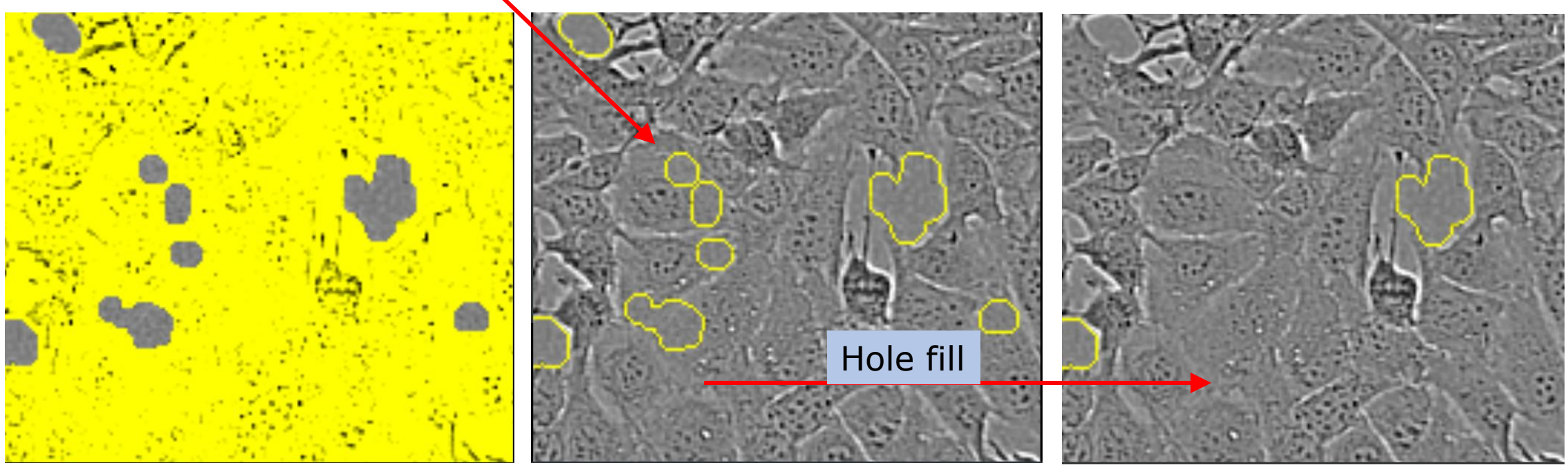




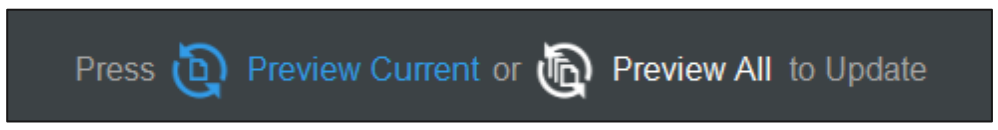


- You can easily clean up the background by filtering on minimum area (2). Image to the right has a min filter of 160 µm2.
- If there are holes in the mask, you can use the "hole fill" option (3). If it's hard to see the cells underneath the mask, change to outlines (4). Image to the far right has a value of 450 µm2 of Hole fill.

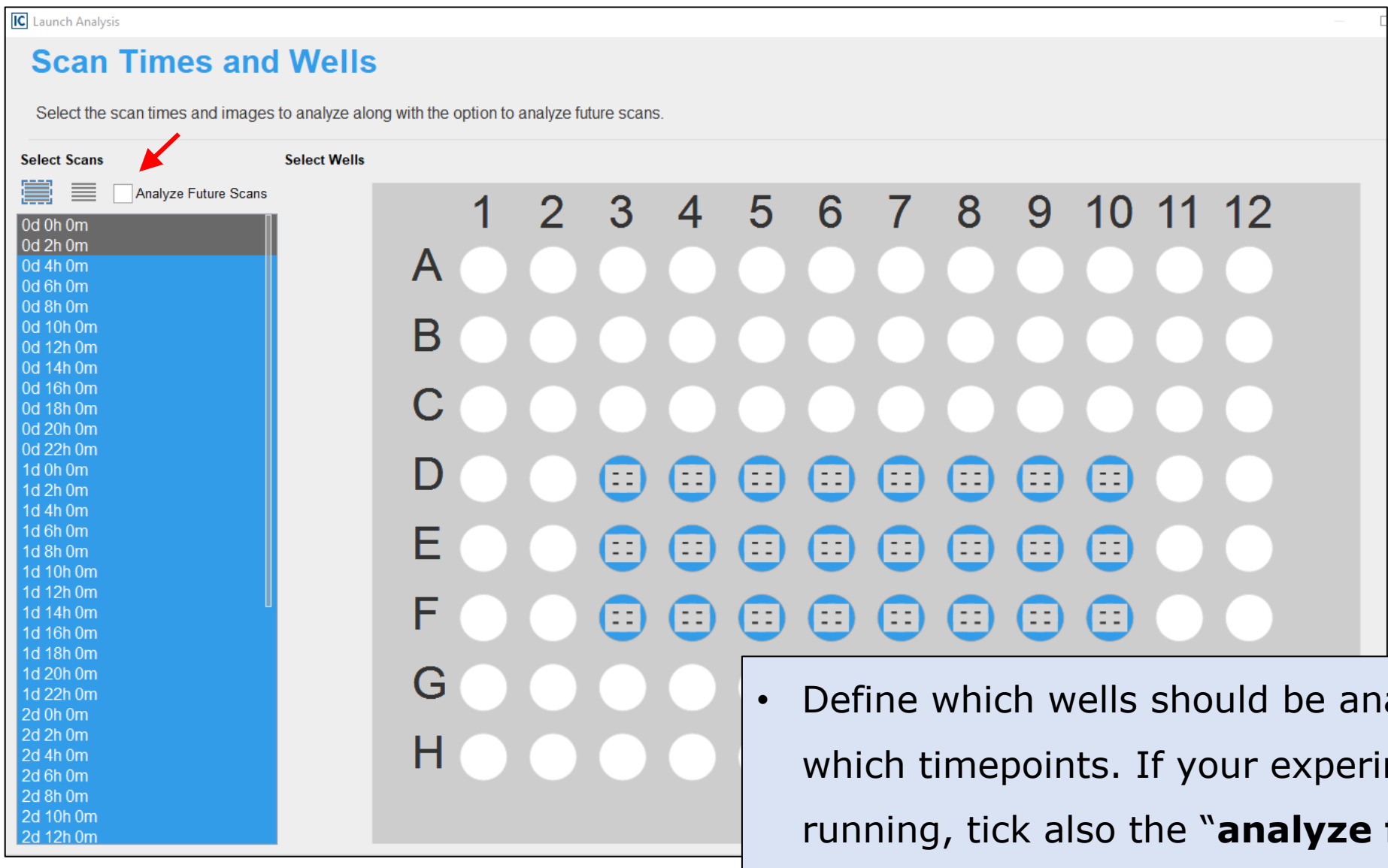




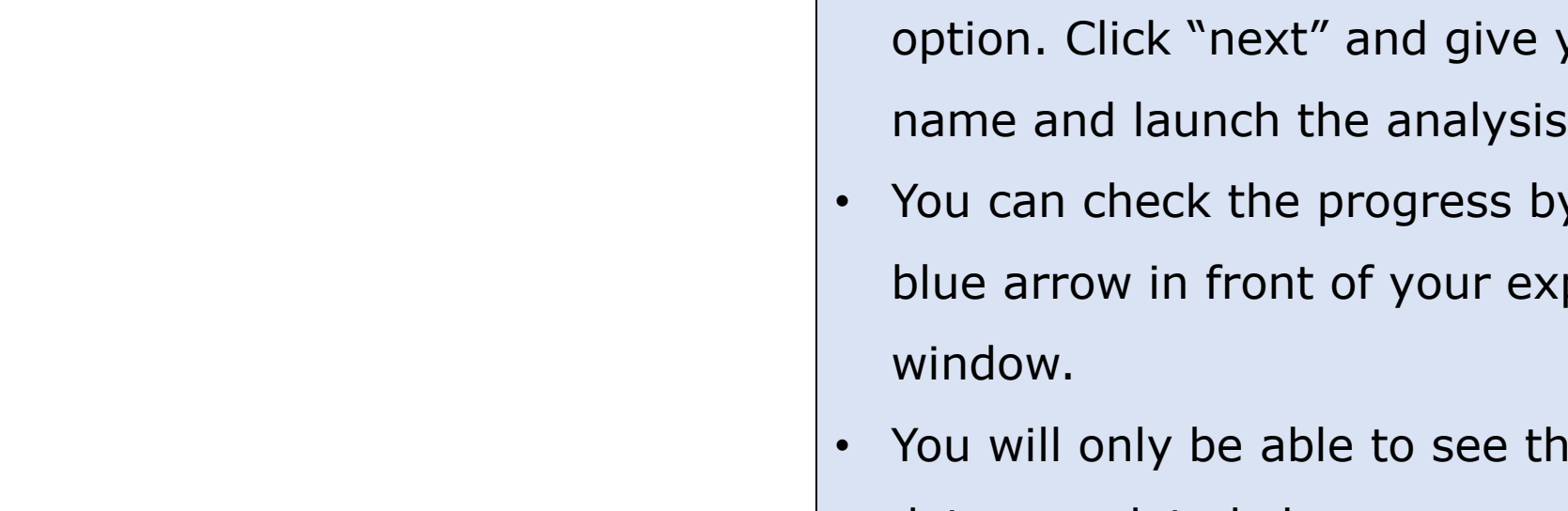
Analyzing a confluency experiment - continues



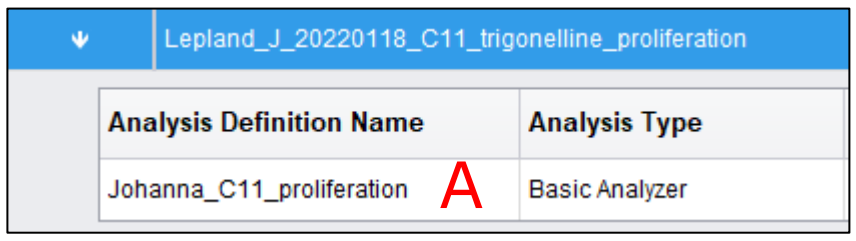
- Check all the images and click preview all to see the mask on alle the images.
- When you are happy with the result of the masking, click “next”



- Define which wells should be analyzed and select which timepoints. If your experiment is still running, tick also the “**analyze future scan**” option. Click “next” and give your definition a name and launch the analysis.
- You can check the progress by clicking on the blue arrow in front of your experiment in the View window.
- You will only be able to see the analysis once the date completed shows up.

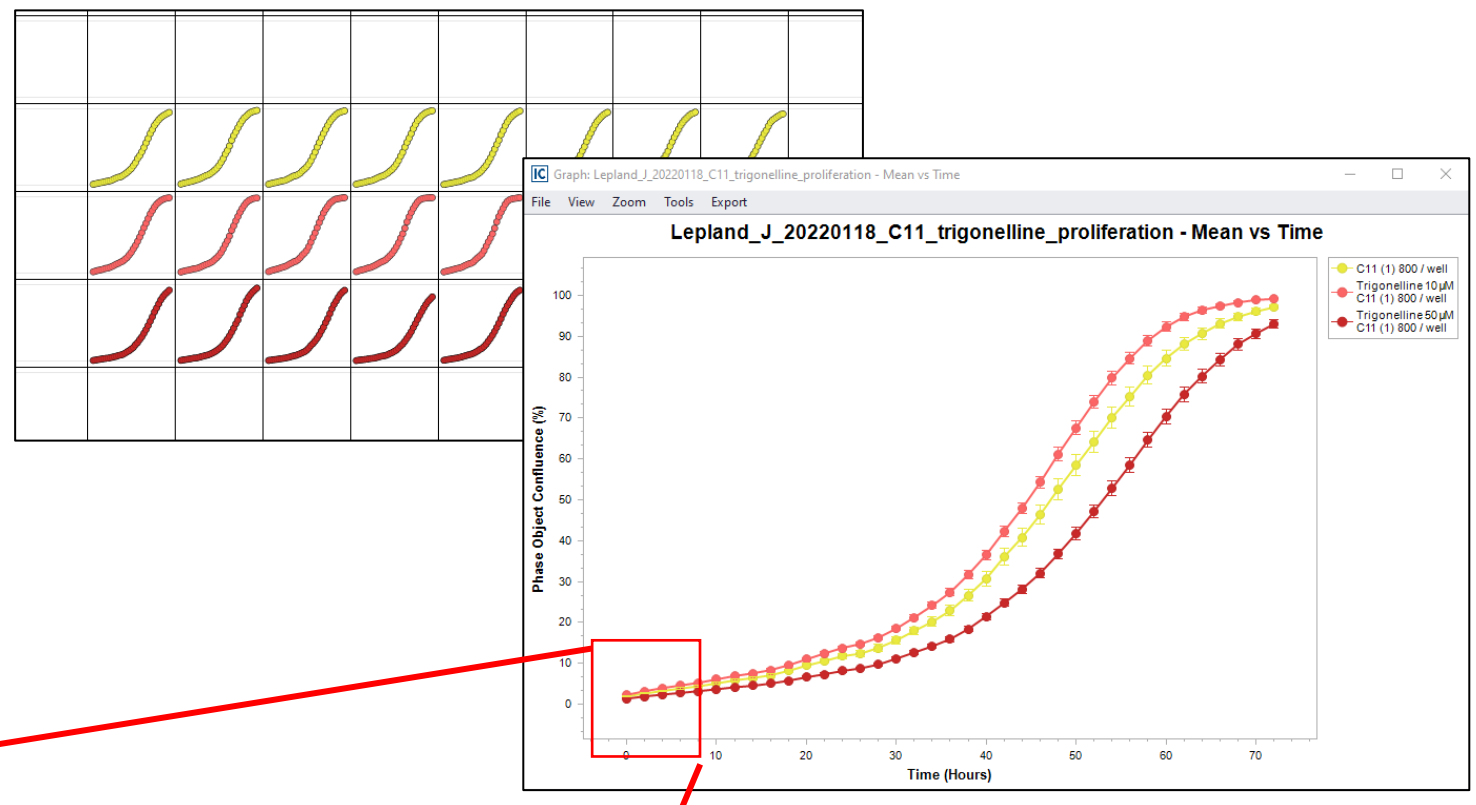


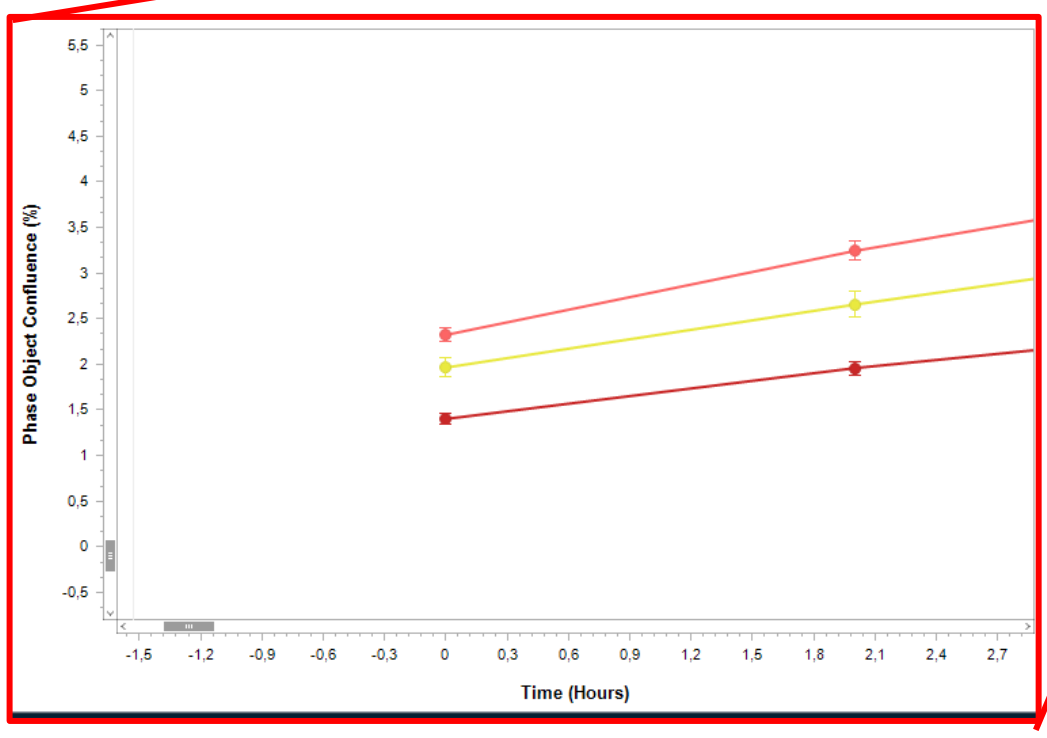
Analyzing a confluency experiment continues



B

- Open the final analysis by either clicking on the blue arrow next to your experiment under "view" and open the analysis definition (A) or simply open your experiment click on the "graph metrics" icon (B).
- Check the microplate graph to have a quick overview over all the wells.
- Create a graph and "**select grouping**" with "**plate map replicates**". This only works if you have created already a plate map.

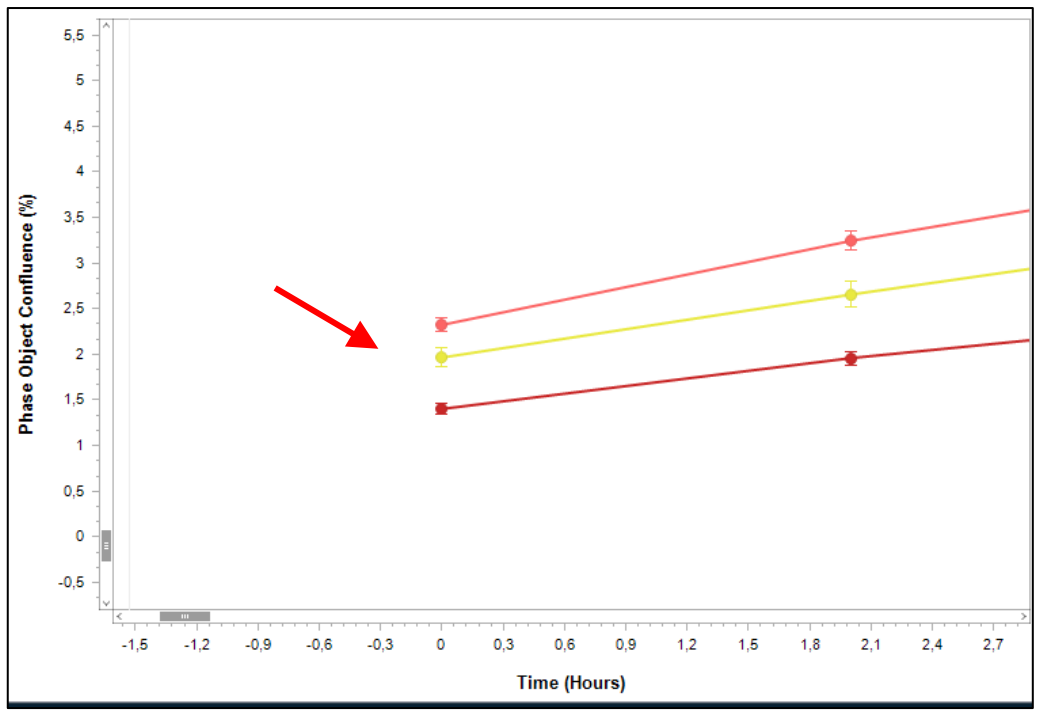


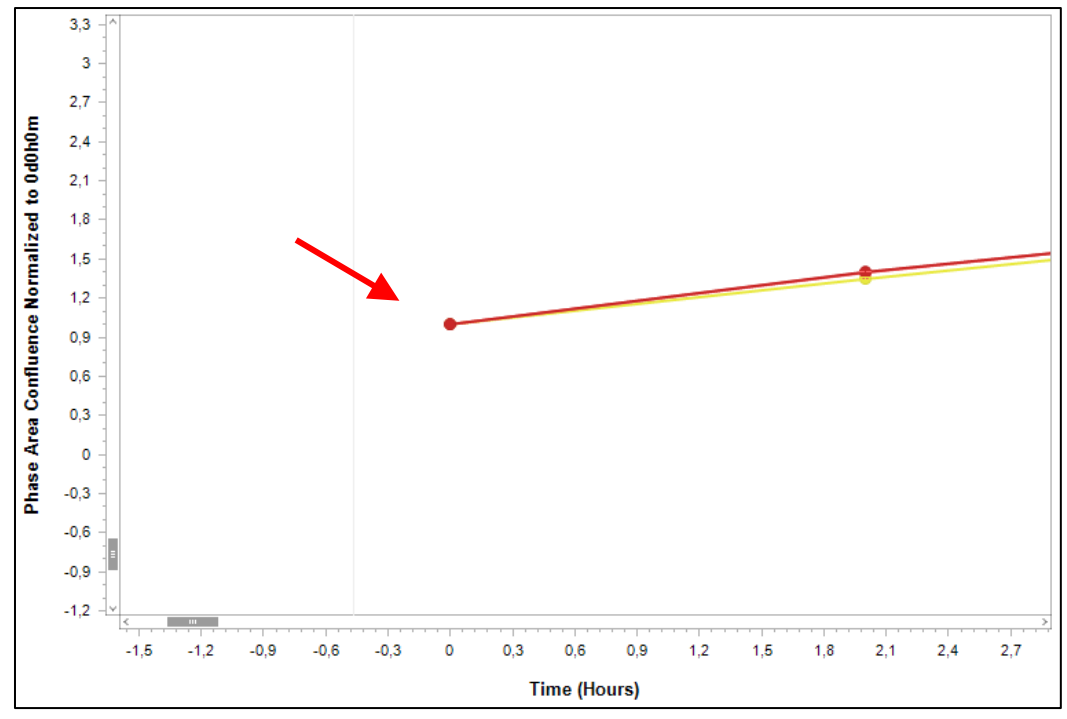


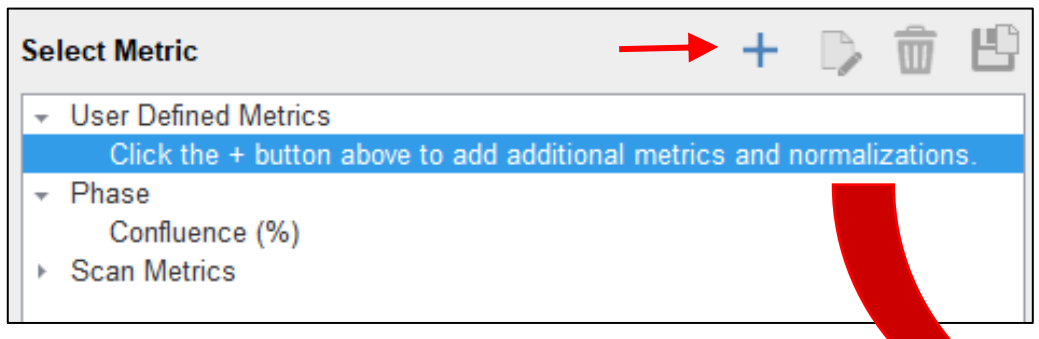
Most results will need to be normalized. In this example we seeded the same number of cells in each well, but the starting points show different values. See next page for how to normalize the results.

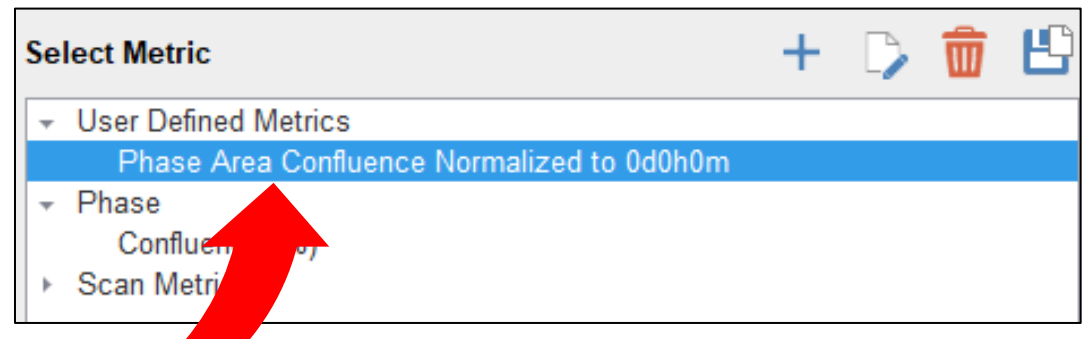
Normalizing your data and graph

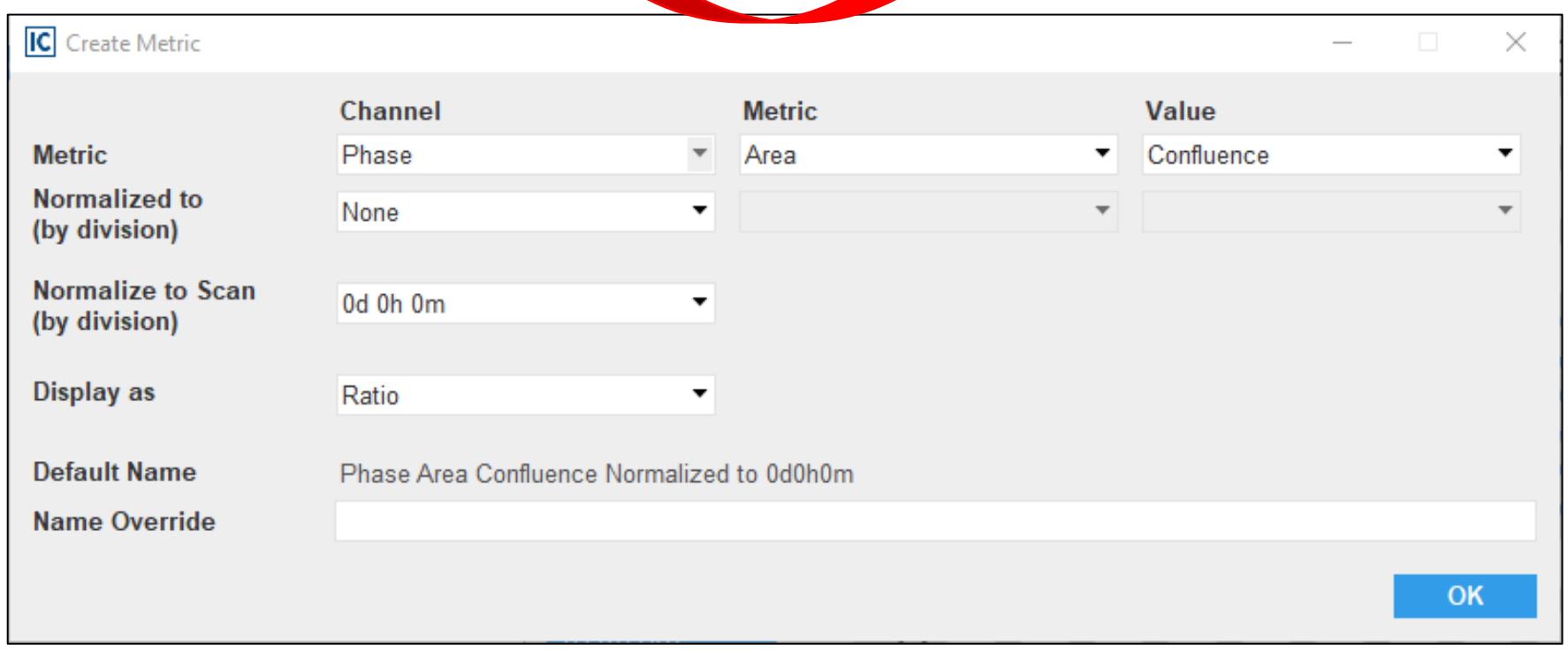
- If you want to normalize the starting point (because all wells should in principle contain the same number of cells), you can do this by creating a "**user defined metrics**".
- Click on the + and define the channel mask over confluency with normalization towards the first timepoint.







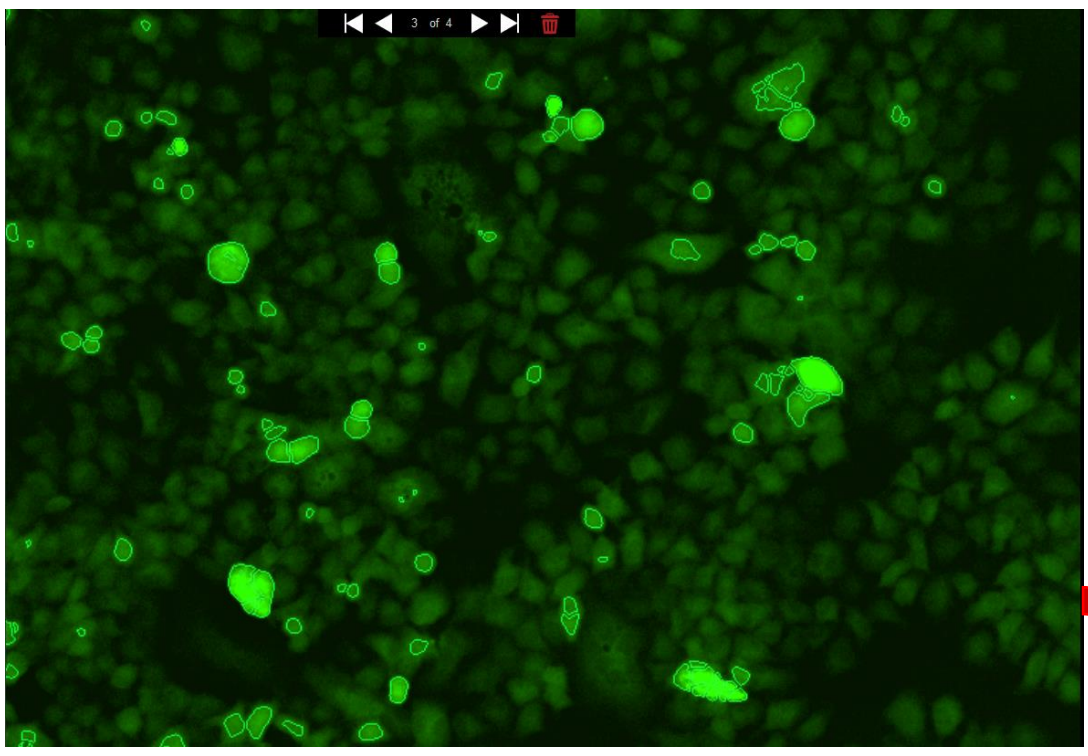




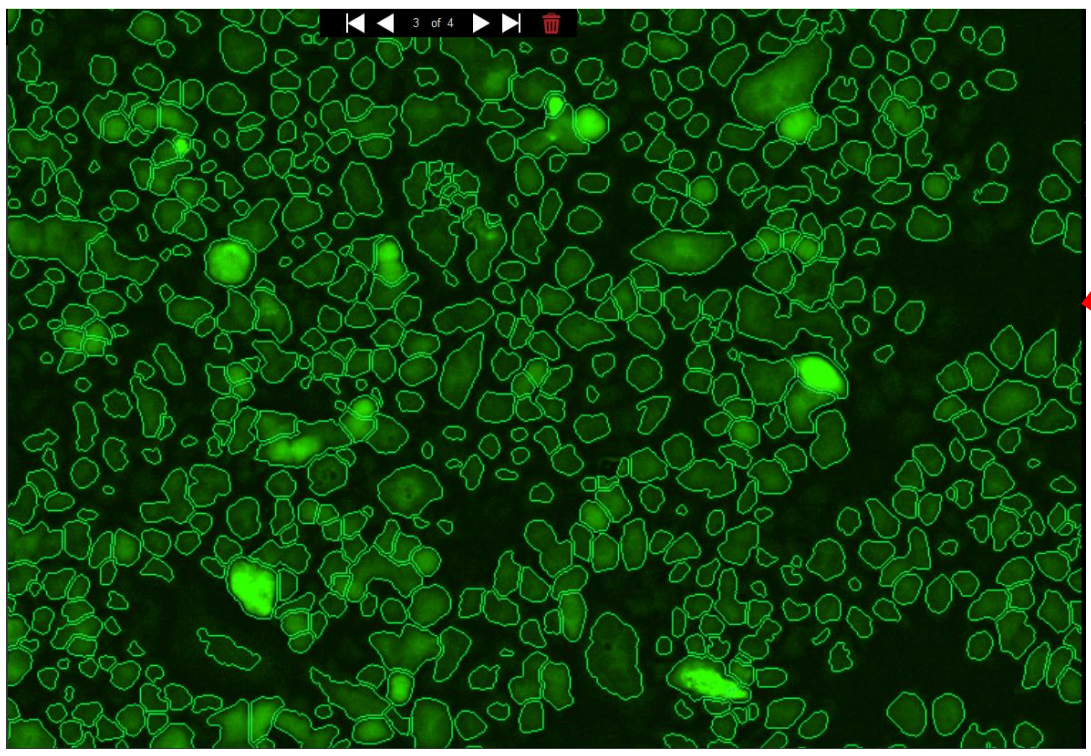
- Now, run the new user defined metrics and see the results in the graph. You can also export your normalized data to excel. Timepoint will then be 1 and the rest of the timepoints show up in relation to this.

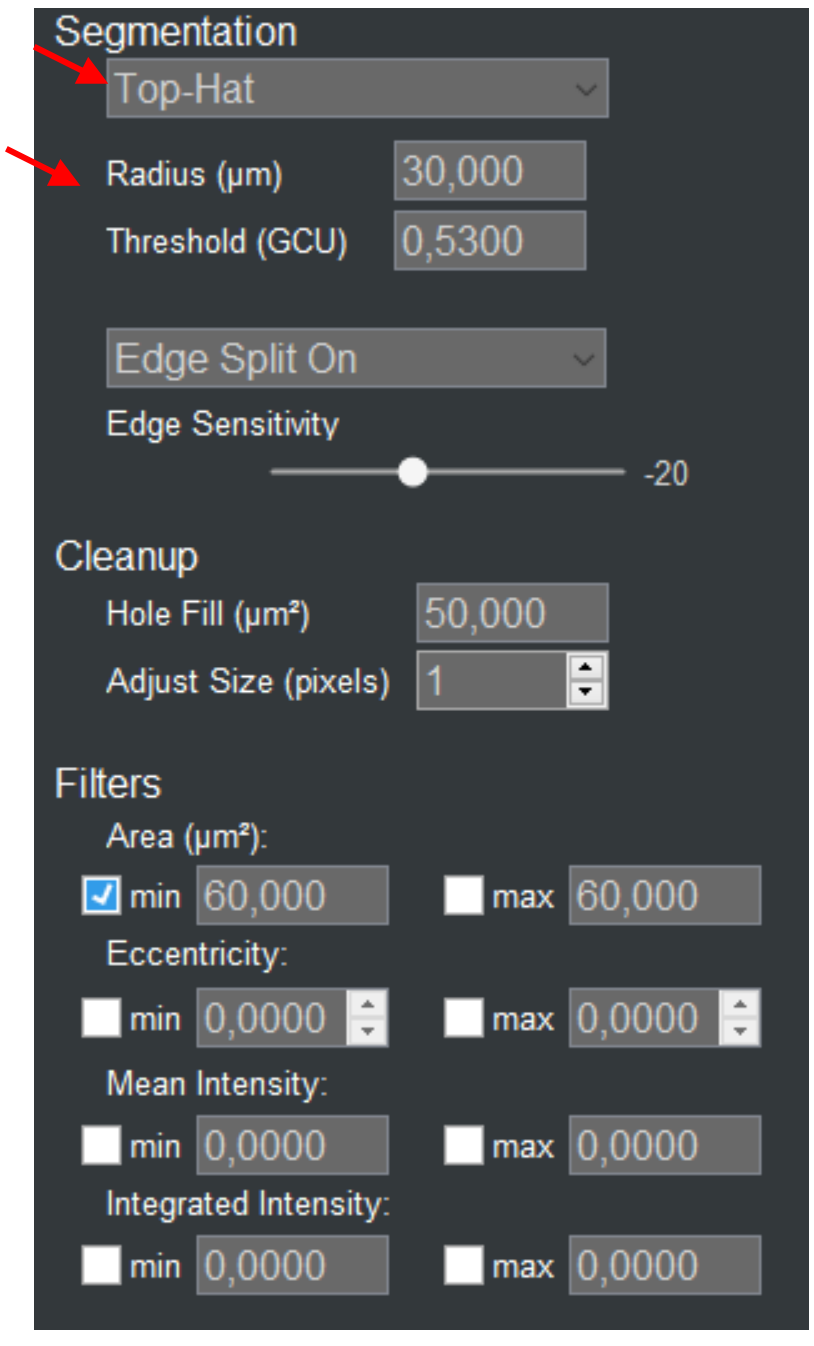
Analyzing a fluorescence channel

- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select "**create new analysis definition**" and select "**basic analyzer**".
- Select the Image channels you want to analyze.
- Select a few representative images in the "image set selection" by clicking on the image. Scroll through the "vessel scan times" and select different time points. When you have 4-8 images, click next.
- "Preview image" in order to get access to the masking.



- Default segmentation might not be very optimal for your images.
- Try using "**Top-Ha**t" to clean up background. "**Radius**" should be larger than the smallest object radius.
- "**Threshold**" is where you define which objects brightness should be included in the mask.

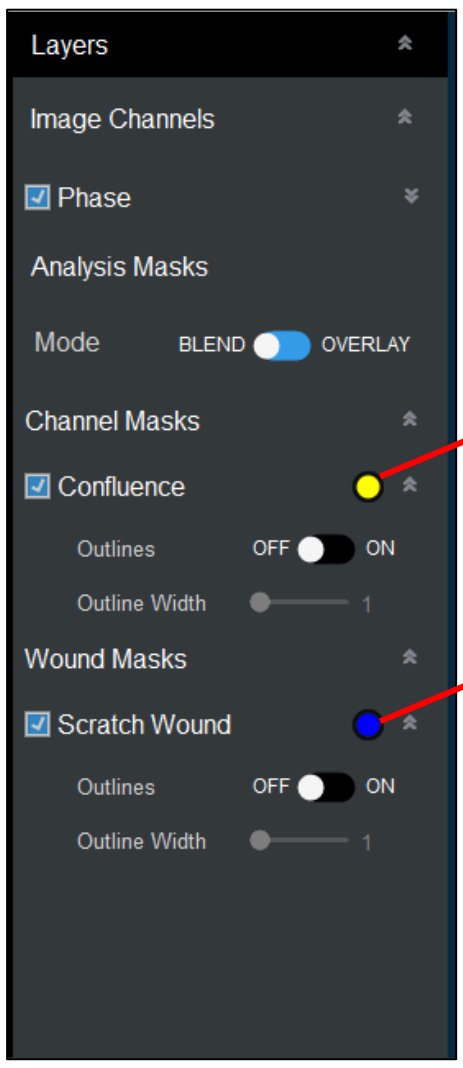


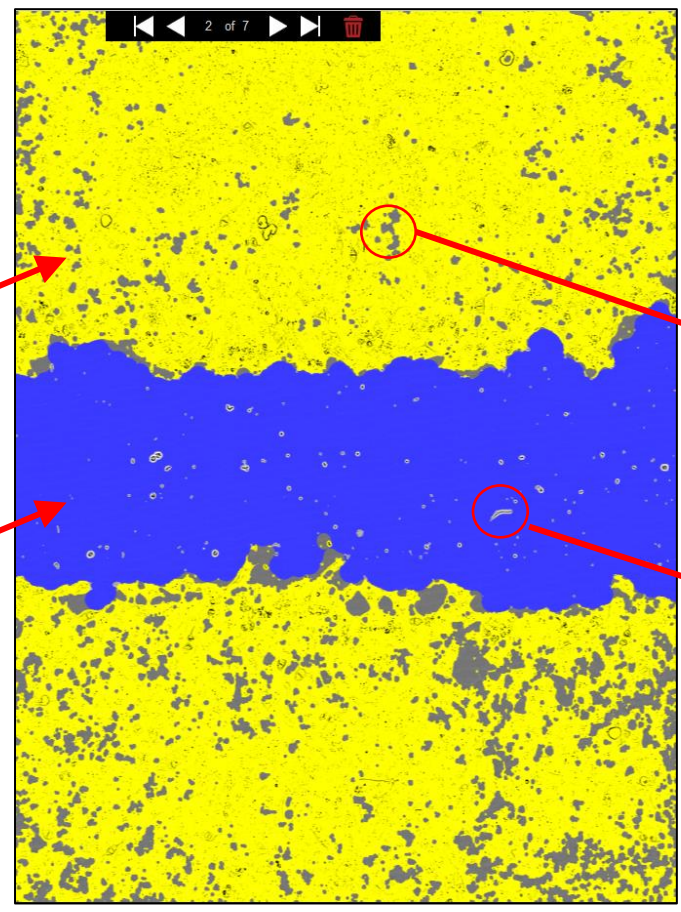


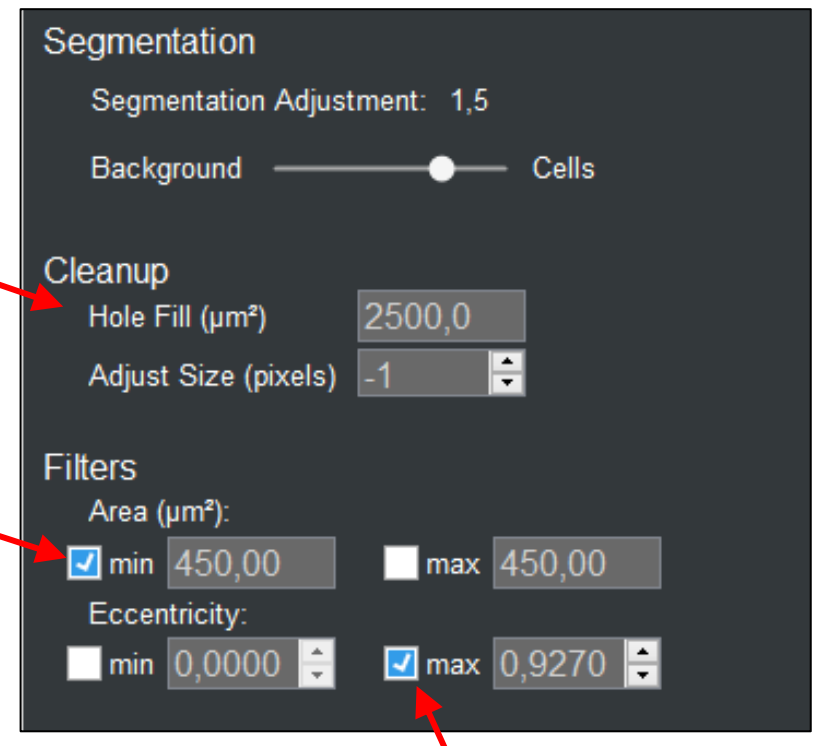
- You can use "hole fill" if there are dimmer signal inside a larger stronger signal.
- "Adjusting size" will make the mask shrink (- values) or grow (+ values).
- "Filter" is used to remove objects from the mask. Here we removed smaller objects than 60 µm2.
- Launch the analysis.

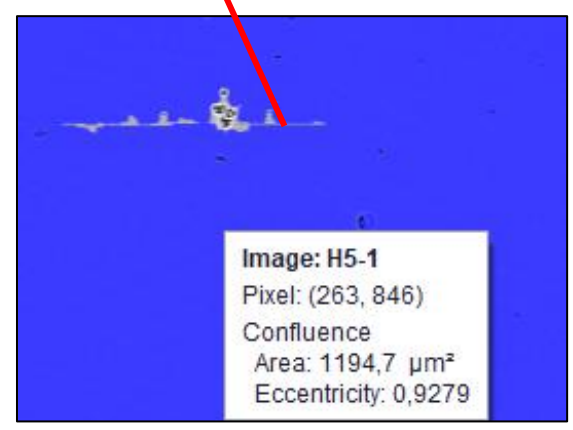
Analyzing a scratch wound

- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select "**create new analysis definition**" and select "**scratch wound**".
- Select the Image channels you want to analyze.
- Select a few representative images in the "image set selection" by clicking on the image. Scroll through the "vessel scan times" and select different time points. When you have 4-8 images, click next.
- Click "preview current" and wait for "confluence" and "scratch wound" mask to load and start adjusting the segmentation/cleanup and filters.

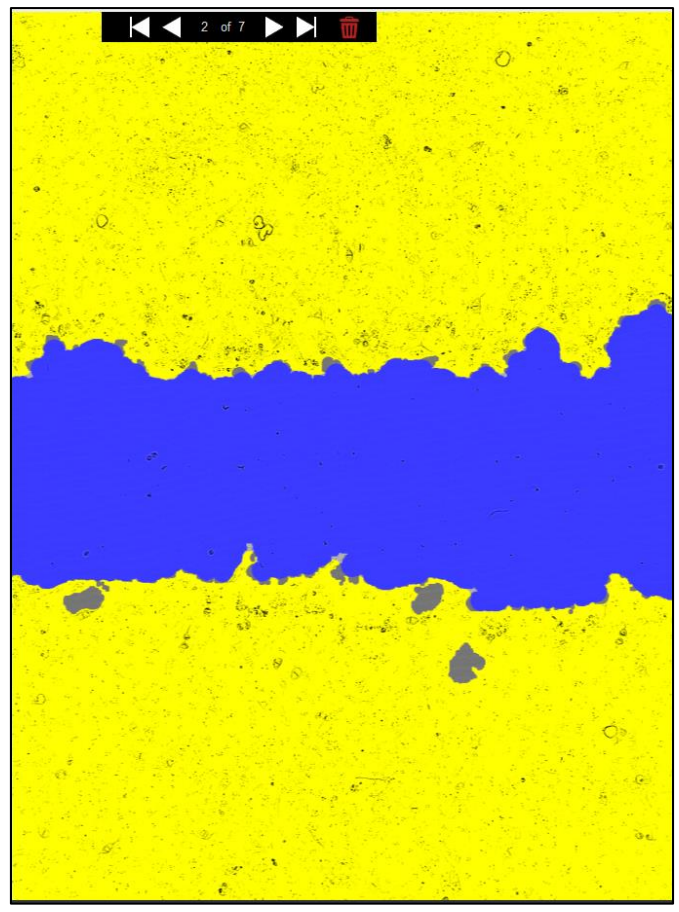








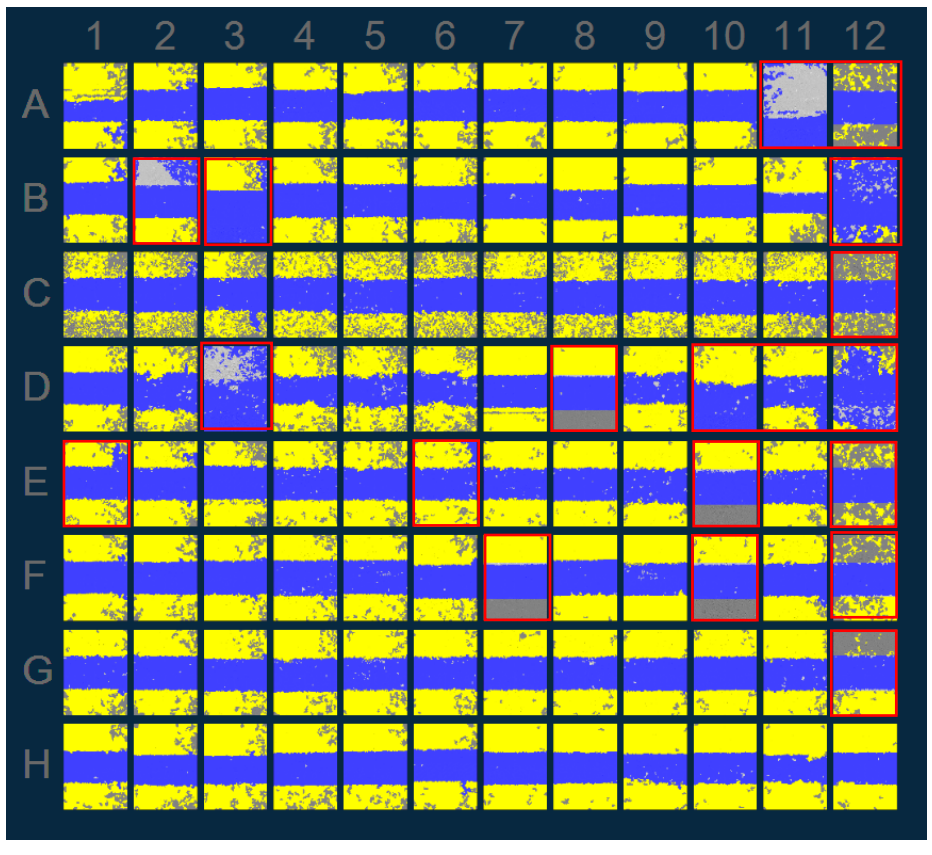
- In this experiment segmentation of 1,5 towards cells with a -1pixel adjustment seem to pick up cells accurately.
- We choose to filter away particles and free cells in the wound.
- We also used "eccentricity" to remove some minor scratches in one of the wells.

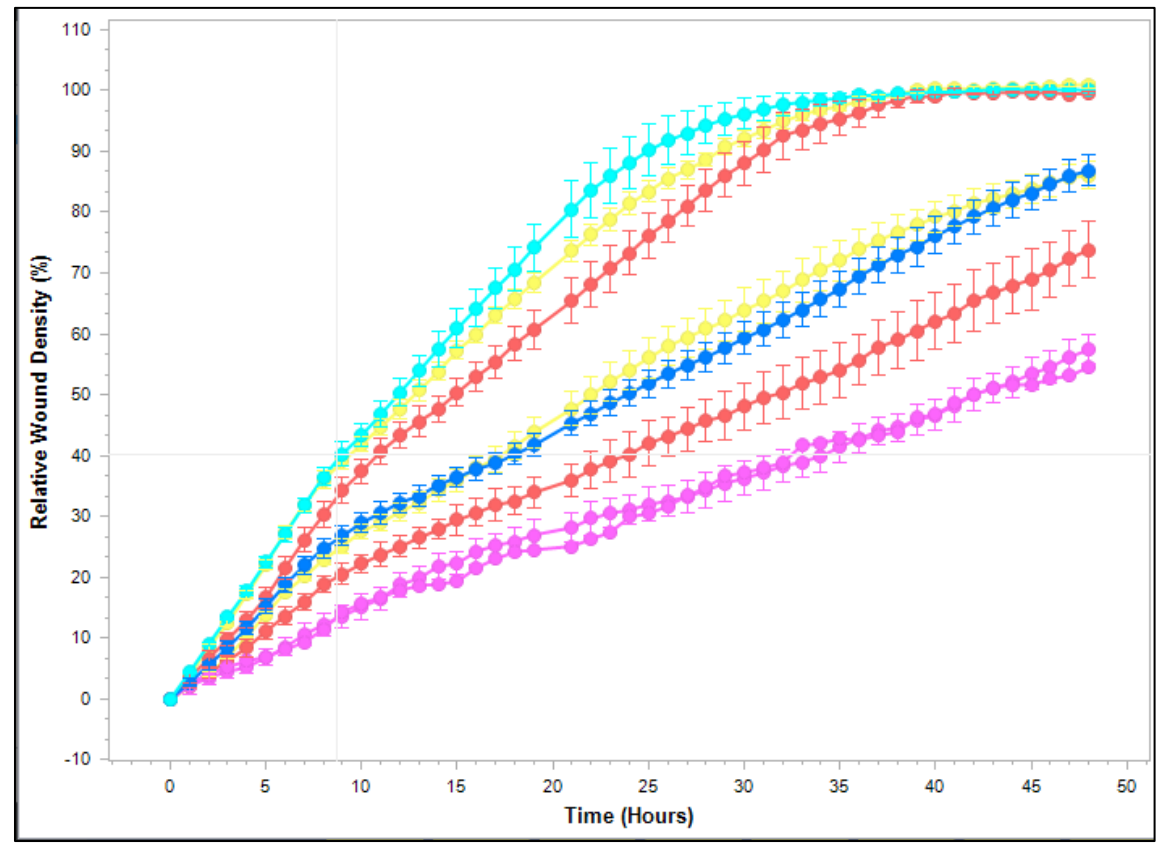


- Continue to "launch analysis". Select wells and time points to be analyzed.

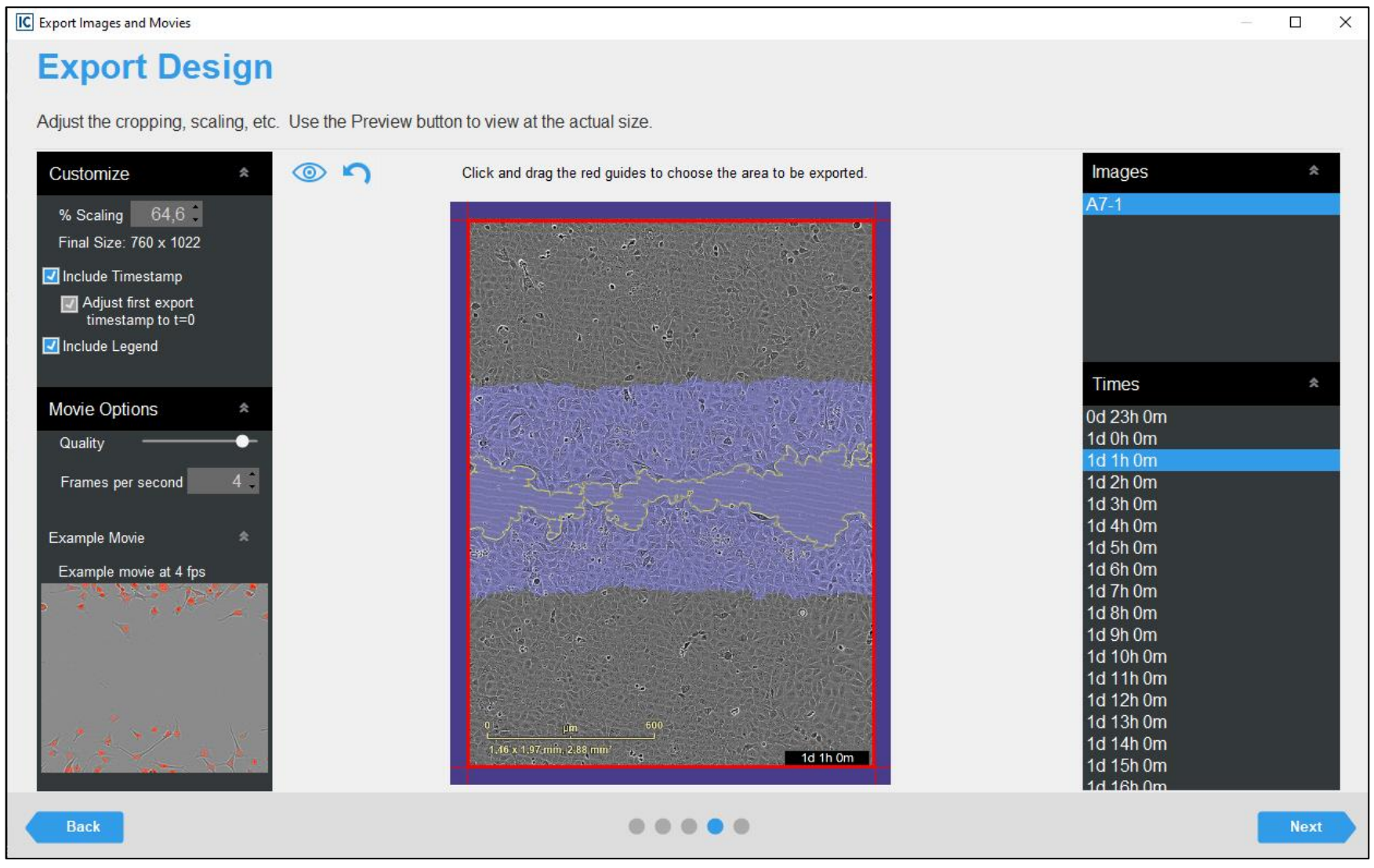
Analyzing a scratch wound continues

- Open your experiment and click on "**graphics metrics**".
- Check the relative wound density/wound confluency of the microplate graph to get an idea of how good the masking was. Remove wells which are problematic or consider running a new analysis definition.



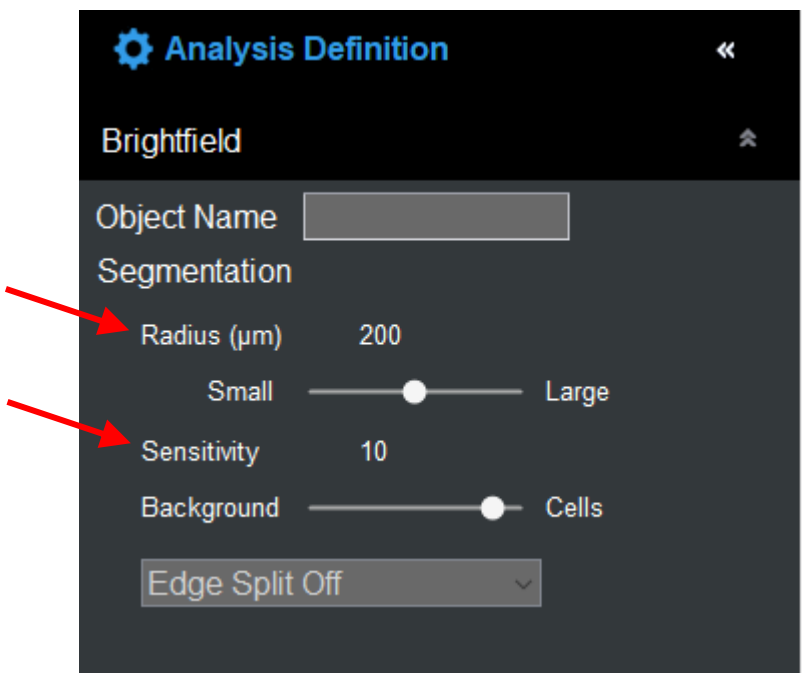


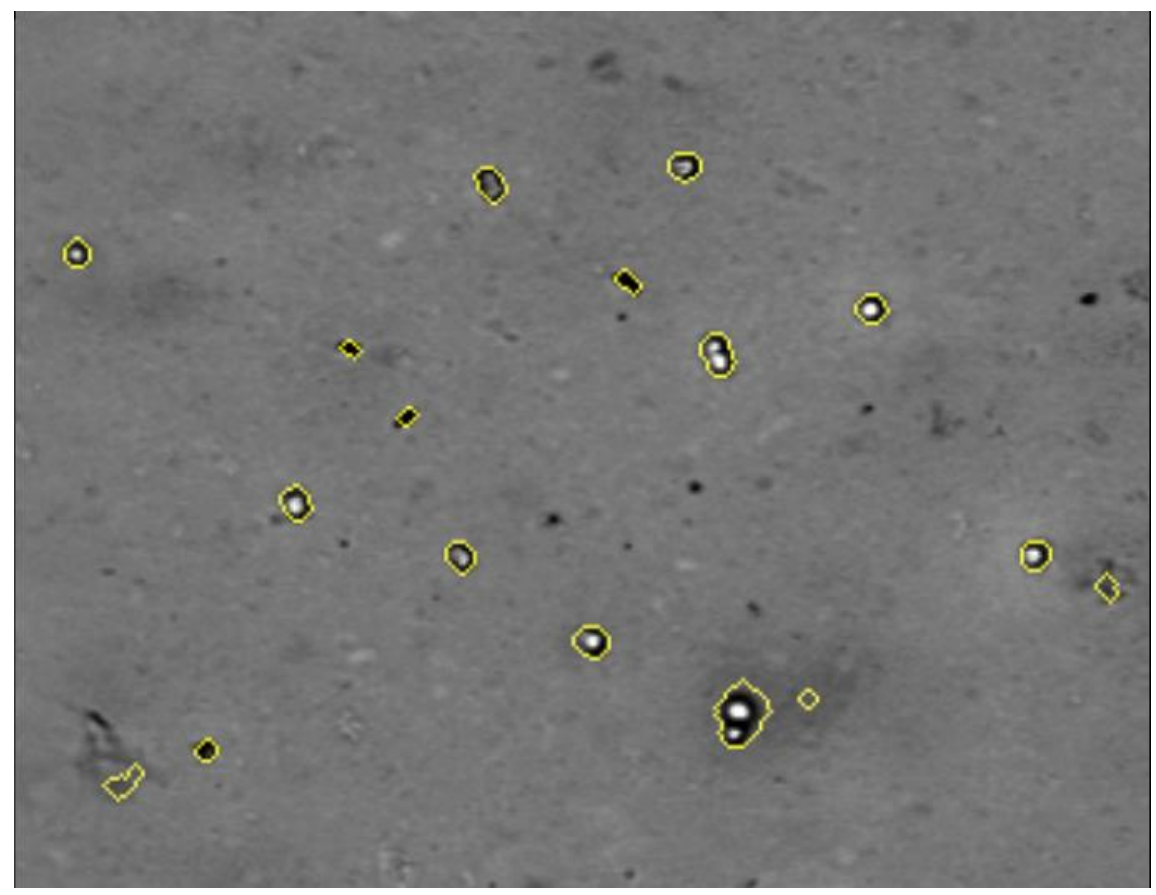
- You can for example export a movie from a representative well and leave the "confluency" and "initial scratch wound" masks on.



Analyzing a spheroid experiment

- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select "**create new analysis definition**" and select "**spheroid**".
- Select the Image channels you want to analyze.
- Select a few representative images in the "image set selection" by clicking on the image. Scroll through the "vessel scan times" and select different time points. When you have 4-8 images, click next.
- Put on the "confluence" mask and start adjusting the "**radius**" and "**sensitivity**".
- You can also use the "**cleanup**" and "**filter**" if needed.

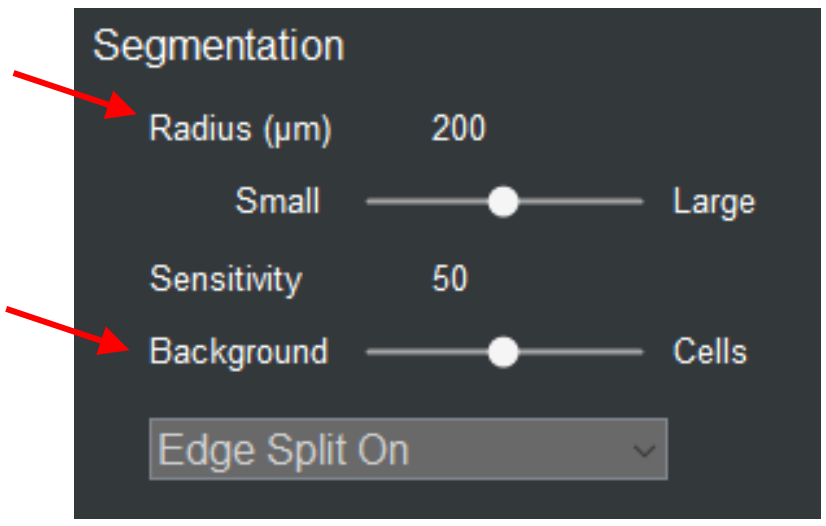


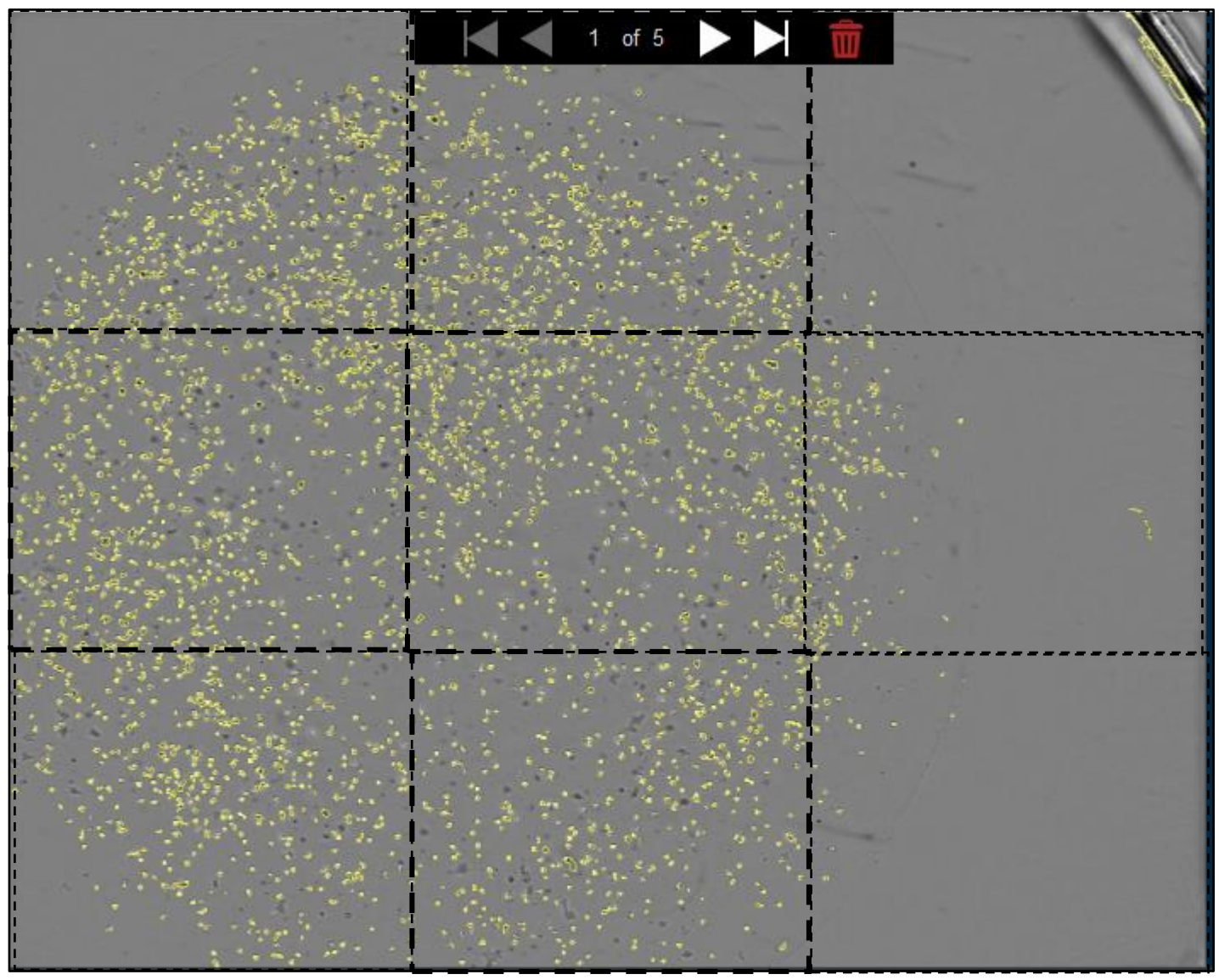


- **Radius** = background remover. Move slider towards "large" to remove more from the background.
- **Sensitivity** = spheroid brightness relative to the background. Move towards "cells" if you want to pick up spheroids which have lower contrast.

Analyzing an organoid experiment

- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select "**create new analysis definition**" and select "**organoid**".
- Select the Image channels you want to analyze.
- Select a few representative images in the "image set selection" by clicking on the image. Scroll through the "vessel scan times" and select different time points. When you have 4-8 images, click next.
- Put on the "confluence" mask and start adjusting the "**radius**" and "**sensitivity**".
- You can also use the "**cleanup**" and "**filter**" if needed (to remove bobbles and edged).



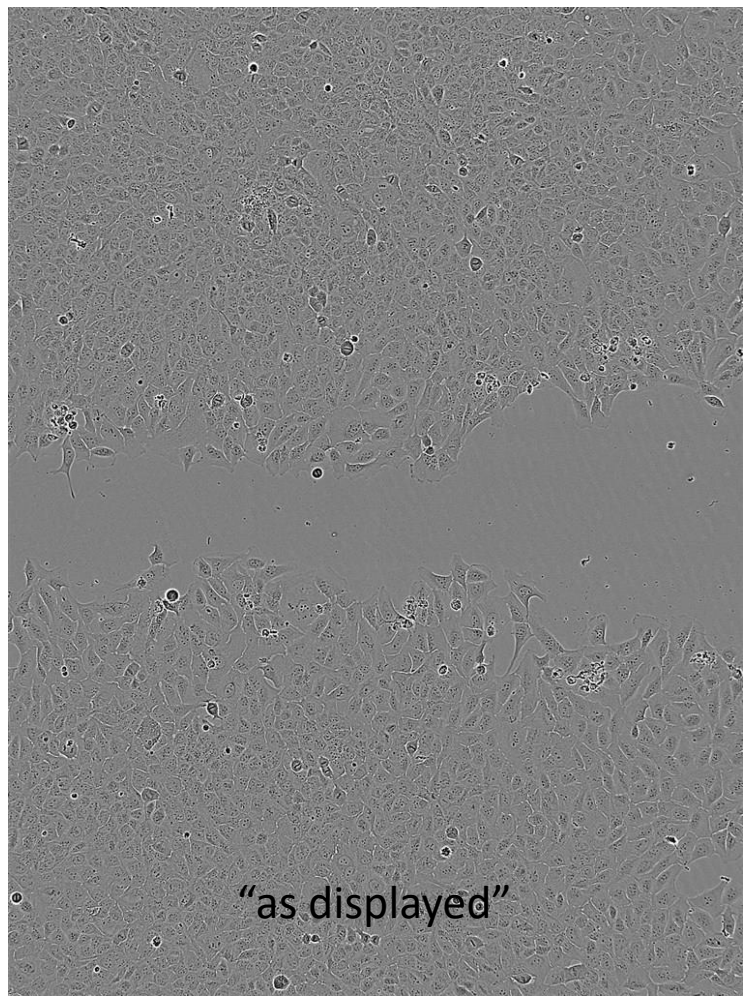


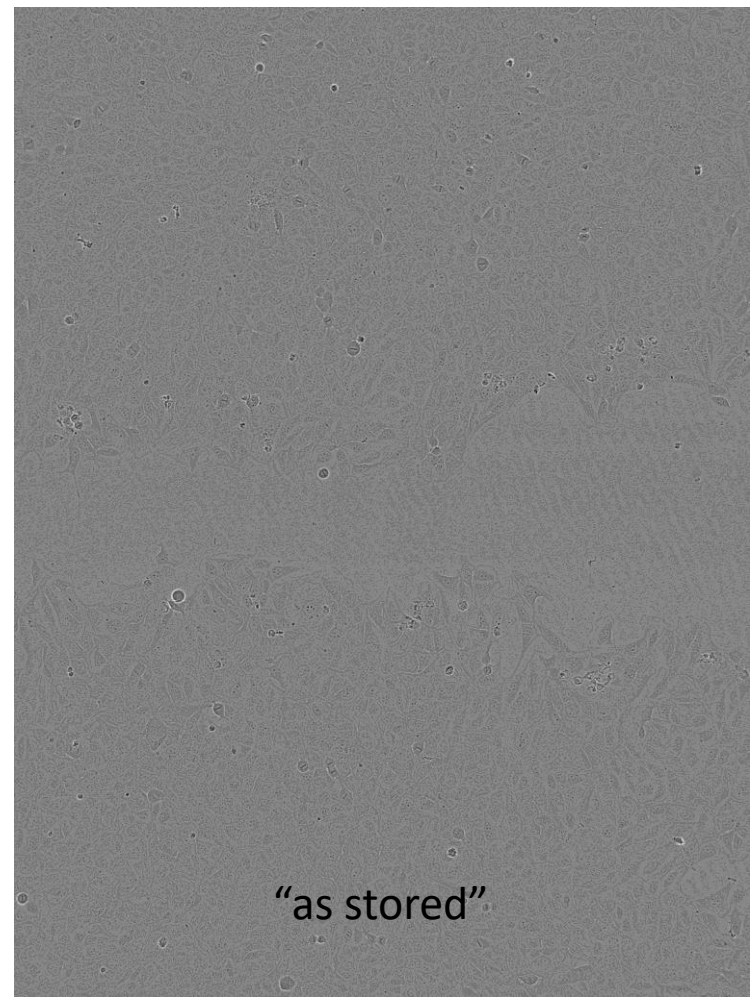
- Making changes to the mask and refreshing the image takes a longer time because your image consists of 9 images (if using 12 well plate). Be patient!

- **Radius** = background remover. Move slider towards "large" to remove more from the background.
- **Sensitivity** = spheroid brightness relative to the background. Move towards "cells" if you want to pick up spheroids which have lower contrast.

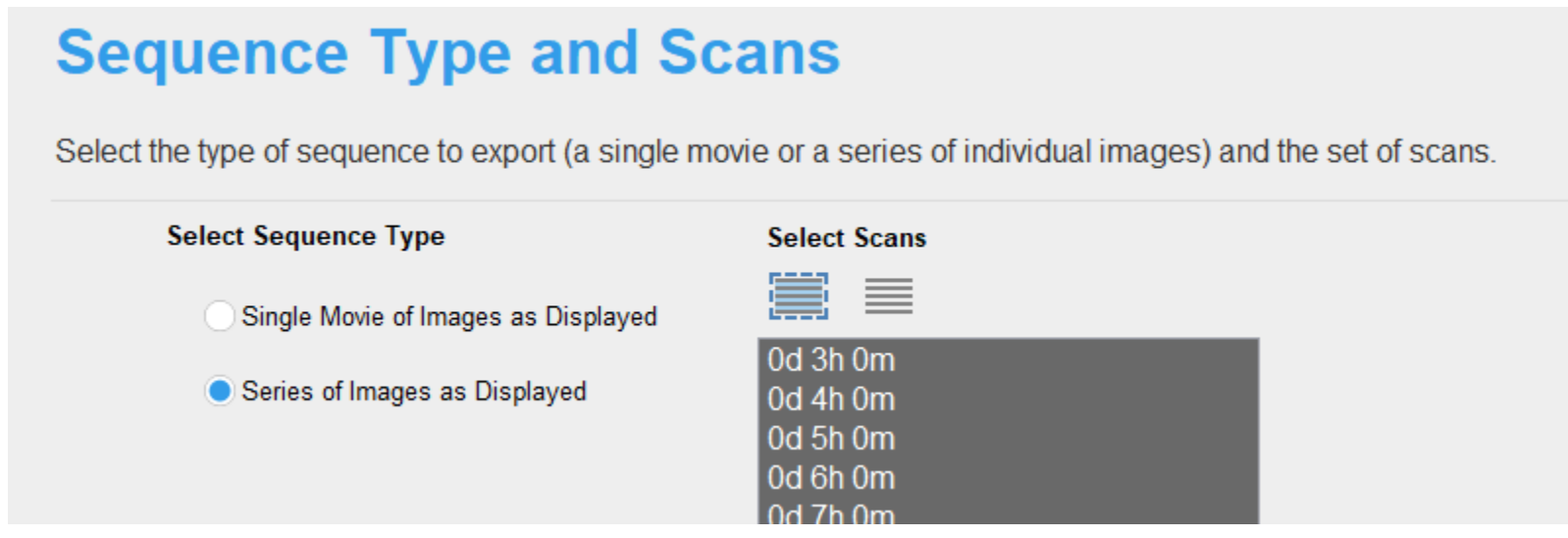
How to export image sets and movies

- Open your experiment and click on “export images and movies” icon.
- Choose if you want to export images “**as displayed**” or “**as stored**”. The latter are as raw data and images have very low contrast. “as displayed” will take into account the display changes applied after changing brightness and contrast under the image channel (layers).

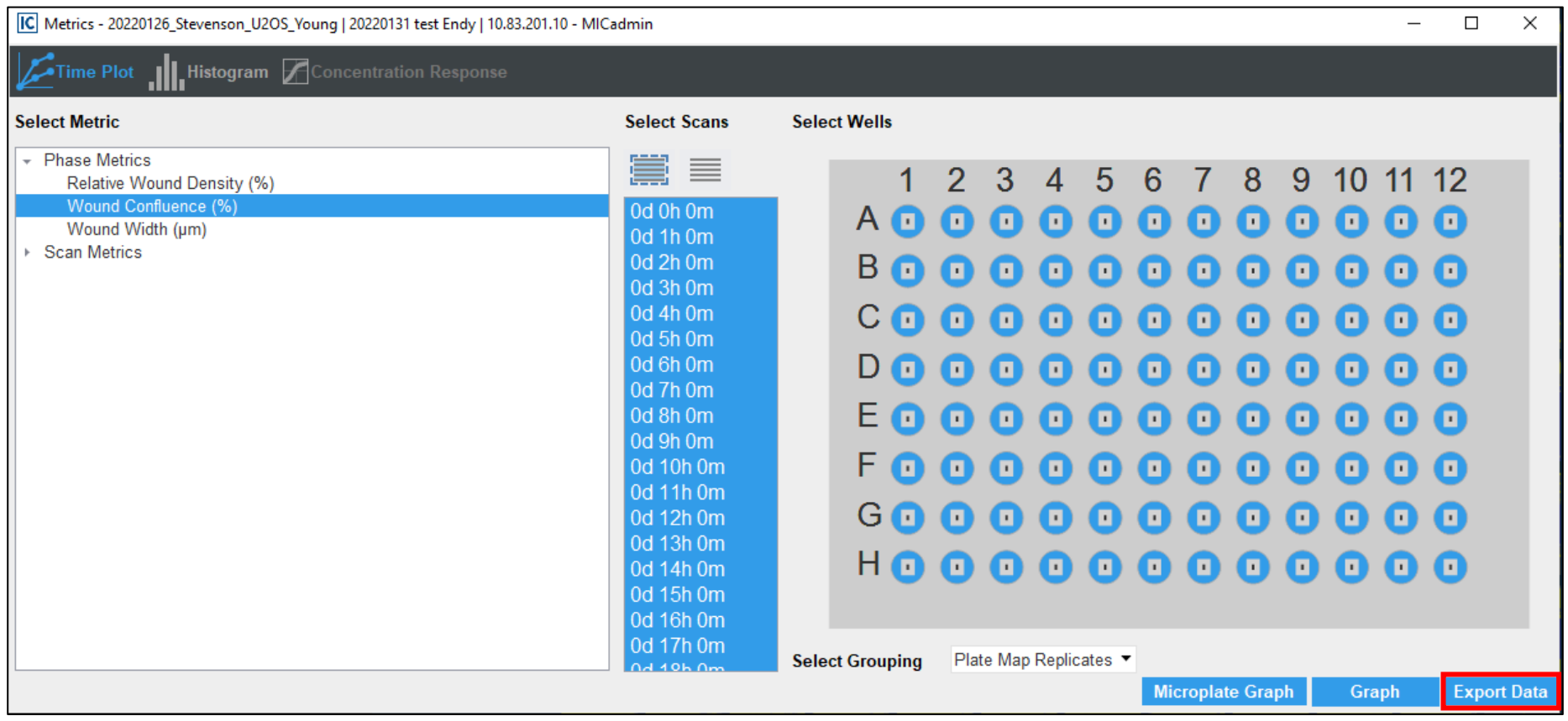


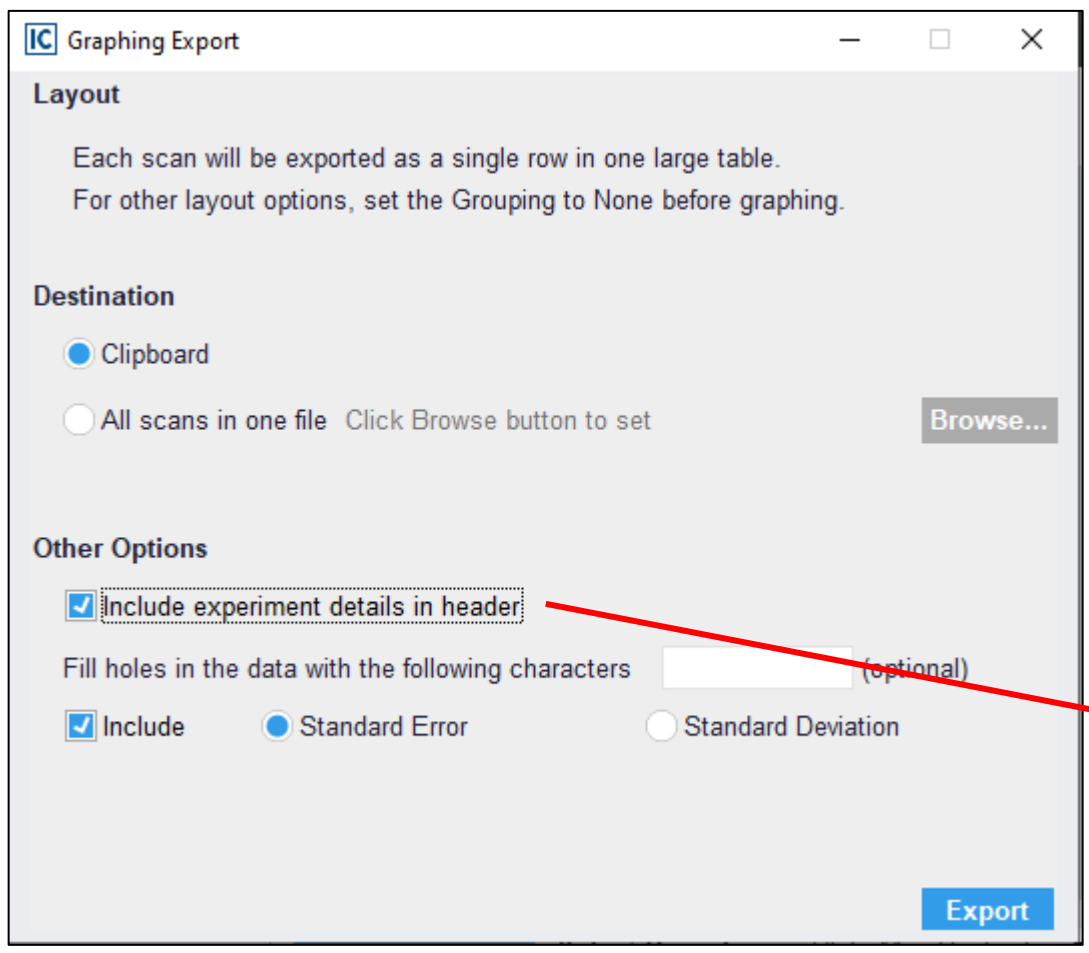


- Select the “**sequence type**”, either a **single image/series** of images or a **movie**.
- Set the quality and speed (frames per second) and decide if you want the timestamps and scale bar included.

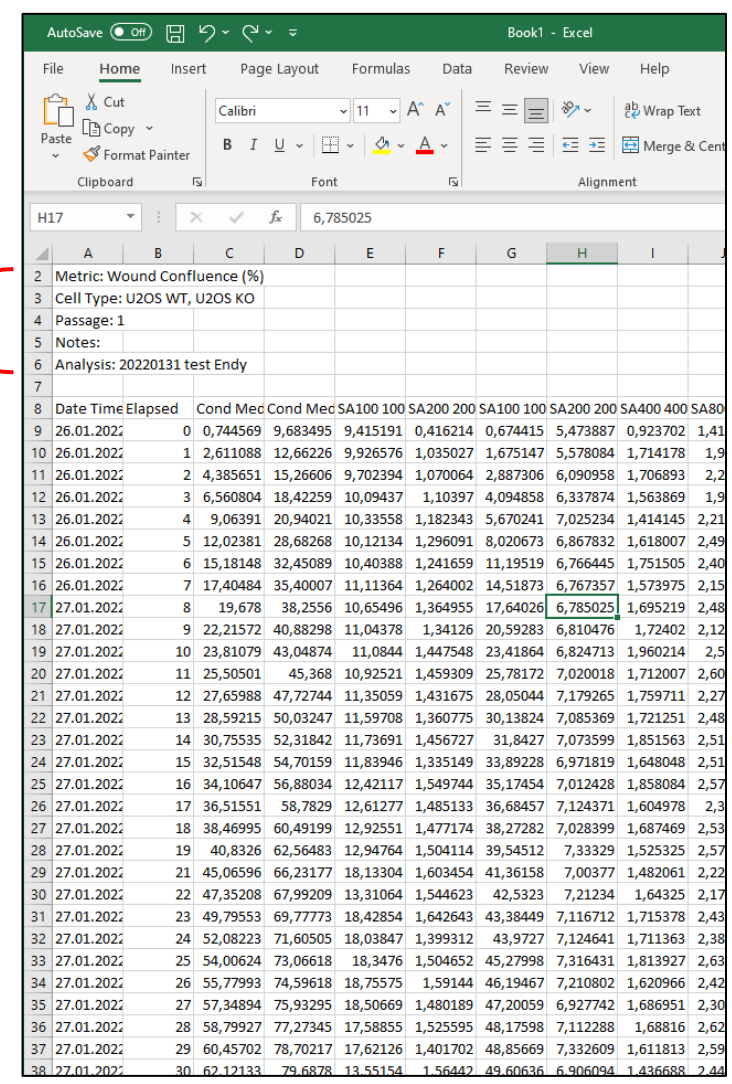


How to export data sets



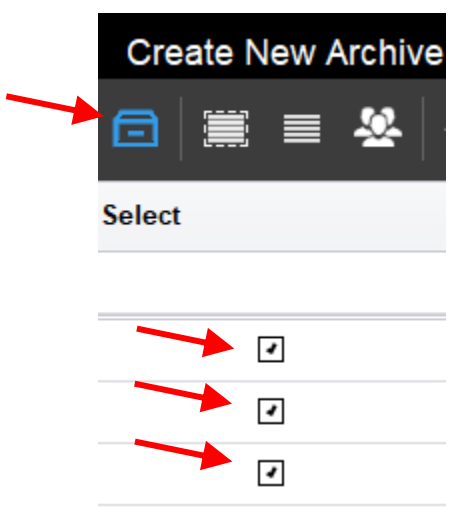


- Select wells and timepoint and click on “export data”.

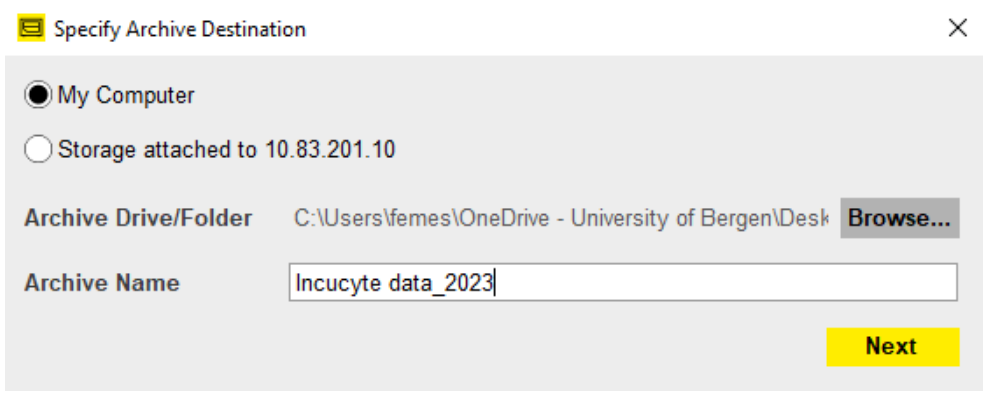


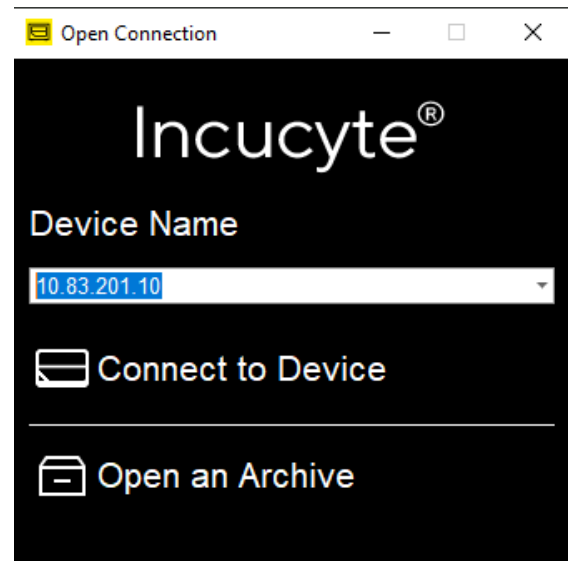
- If you select “clipboard” as the destination, simply open excel and paste the data (ctrl v).
- Include “experiment details in header” can be useful for later.
- Including standard error/standard deviation will add additional columns at the end.

How to archive and delete you data from the remote controller

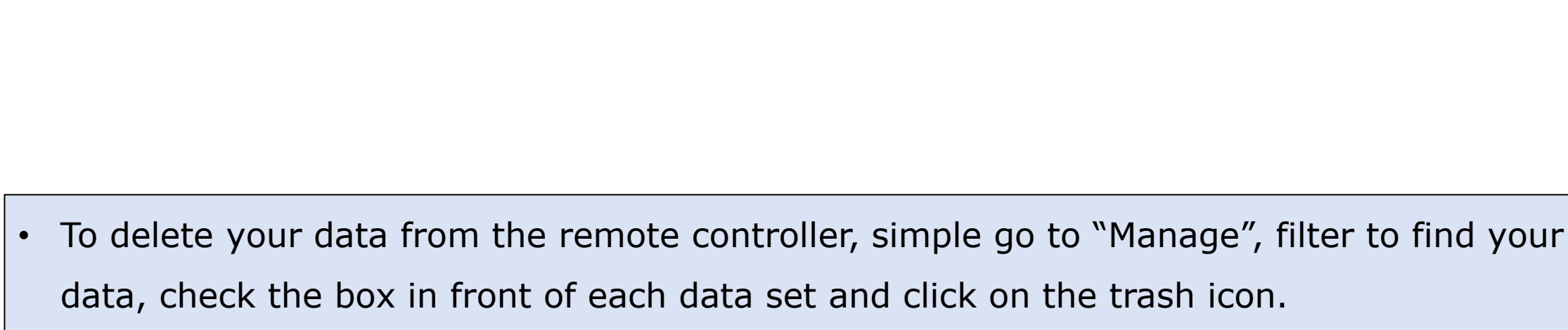


- Go to **Archive**, filter out your data by using the owner column.
- Select your data and press the create archive icon.
- Define where you want to save your data on your Harddisk/external Harddisk/server etc.
- Next time you open the Incucyte software, choose the "**Open an Archive**" option. You may now investigate your data and also start analyzation as before.



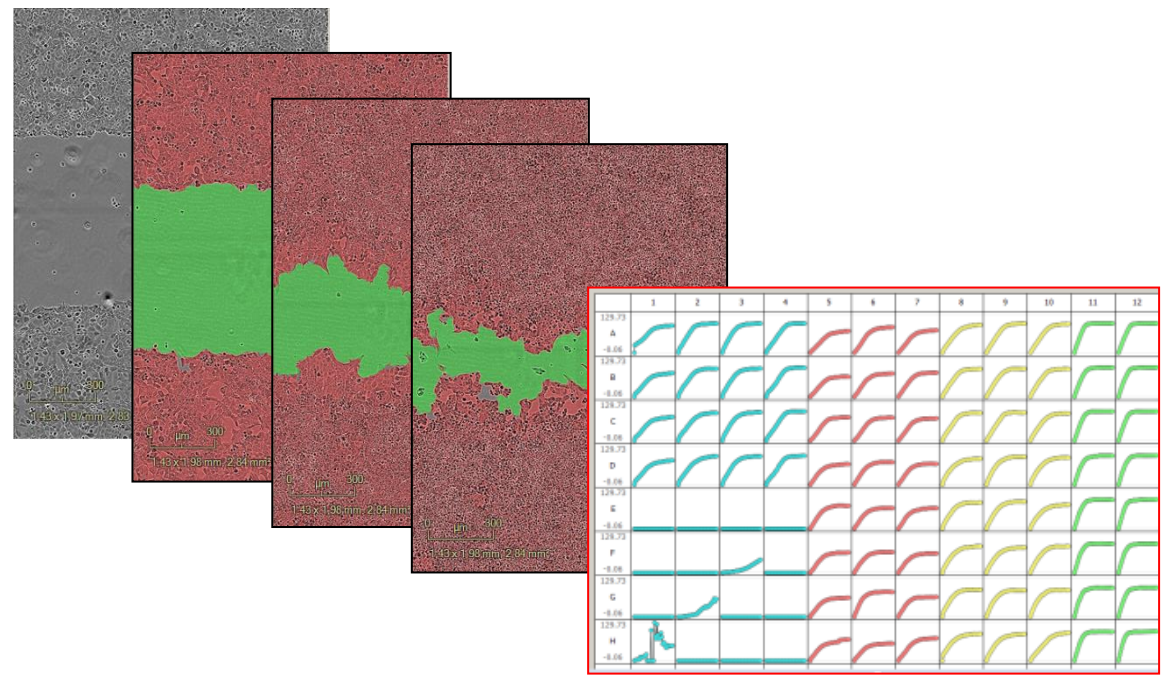


- To delete your data from the remote controller, simple go to "Manage", filter to find your data, check the box in front of each data set and click on the trash icon.



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.



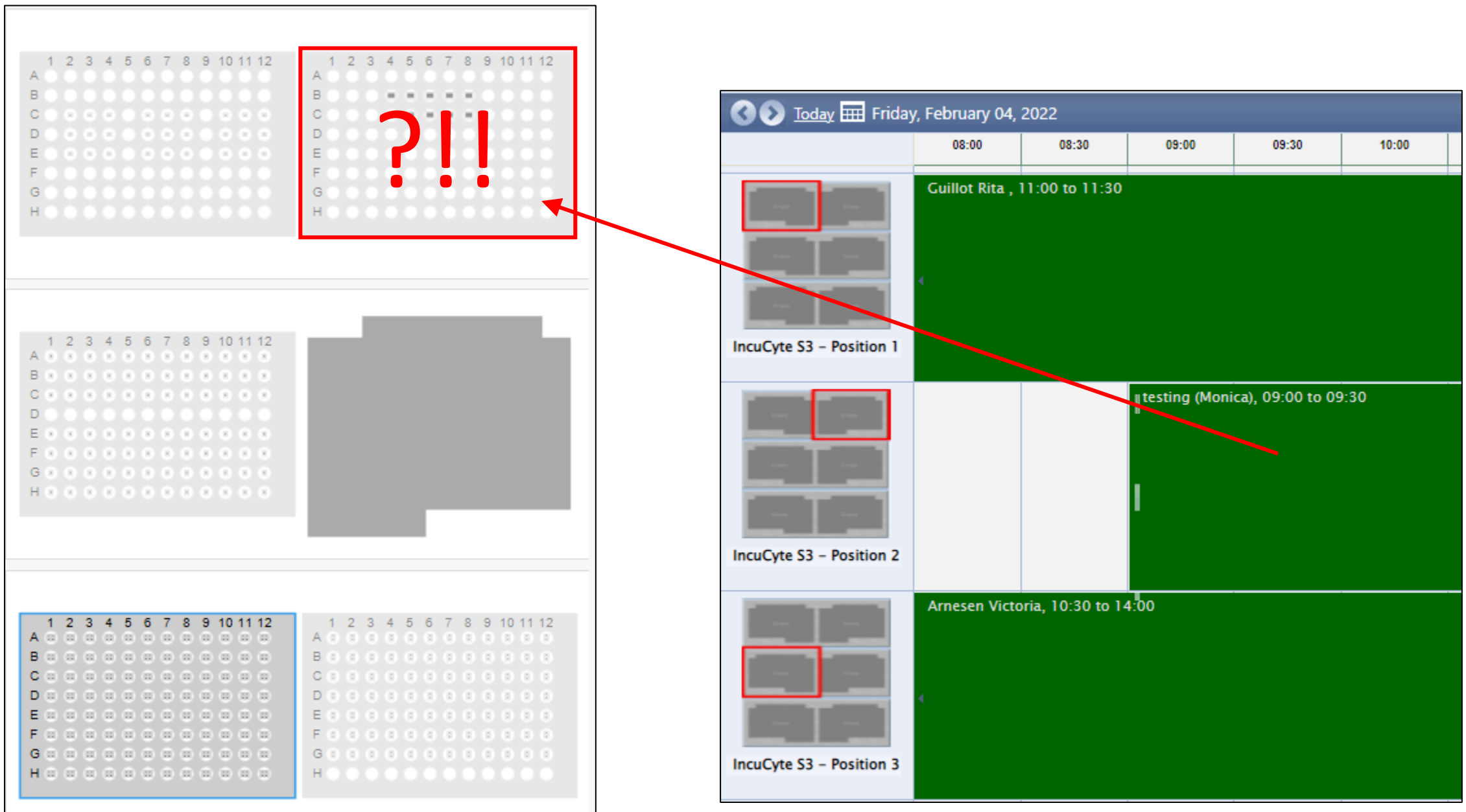
Users must remember the following:

- 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:

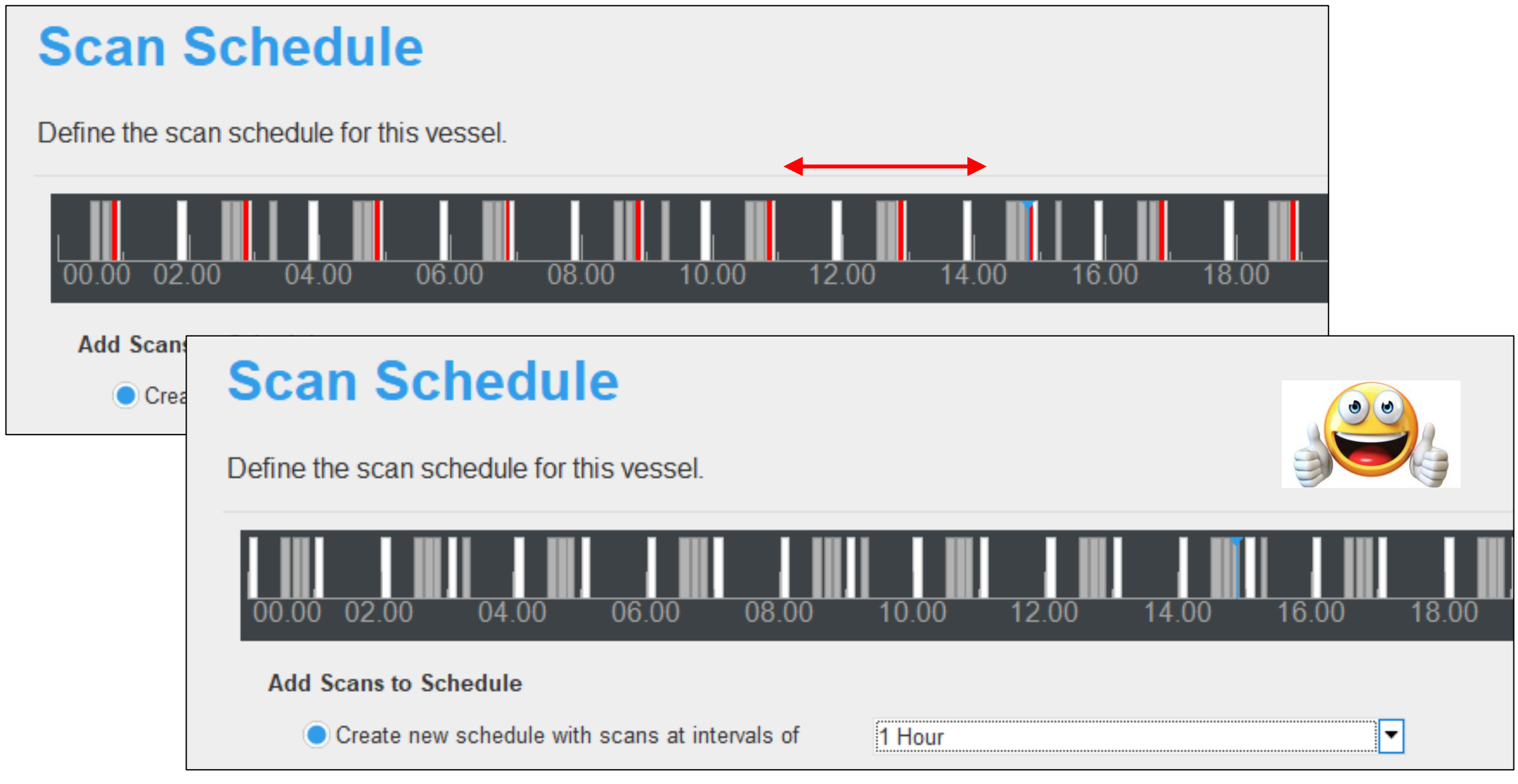
- Before usage, soak pins in sterile water for 5 min and then 70% ethanol, and let air dry.
- Perform the wound. If you are wounding multiple plates of the same cell line, simply soak pins in 45 ml sterile distilled water between wounding.
- After the last wound: 45 ml of 0,5% Alconox for 5 min,
  45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

Trouble shooting – the position you reserved is not free

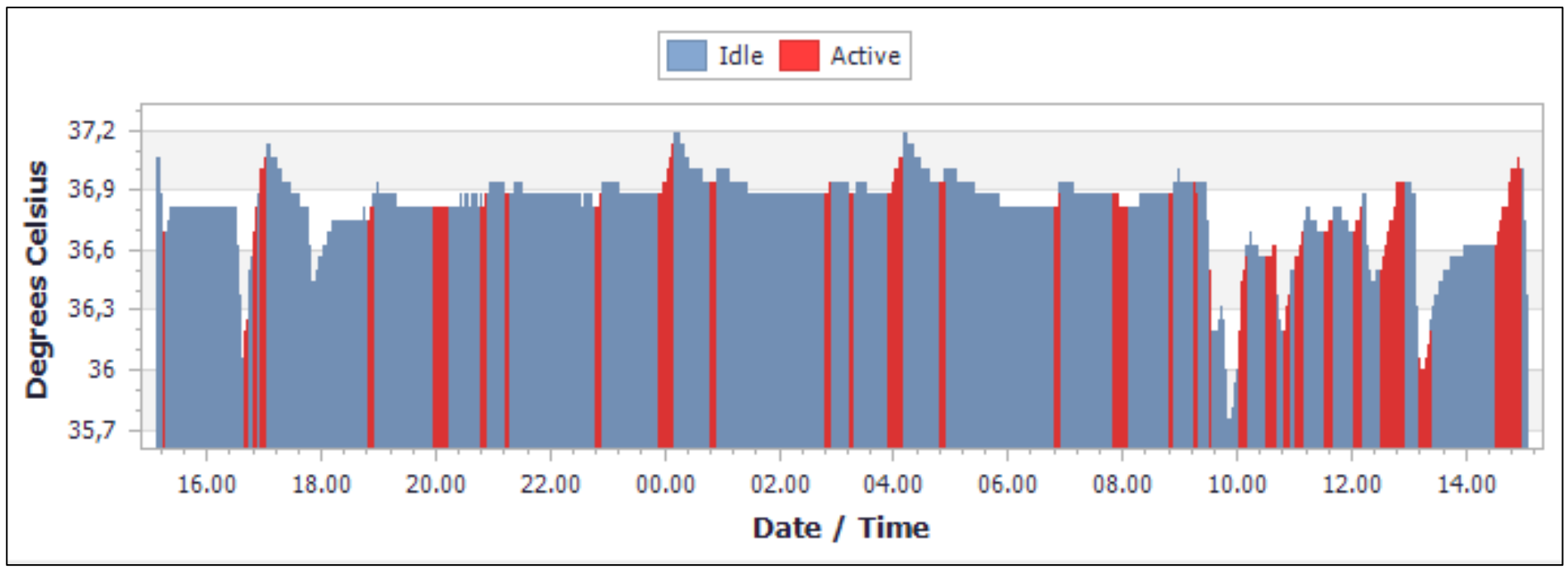


- Booking is mandatory! You always need to book a position in advance and please make sure that you know which position you have booked.
- If there is still a plate in the IncuCyte in the position that you have booked, then you are allowed to terminate the job (click on the vessel and then the trash bin) and physically remove the vessel from its position and dispose of it following cell lab routines.

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



- If there are conflicts in the time schedule, you will not be able to click next and launch the job.
- Use the mouse arrow and drag the schedule till it is not showing conflict anymore.
- Keep in mind that it is optimal to keep some cooling time in between scans in order for the cell environment to be as stable as possible.



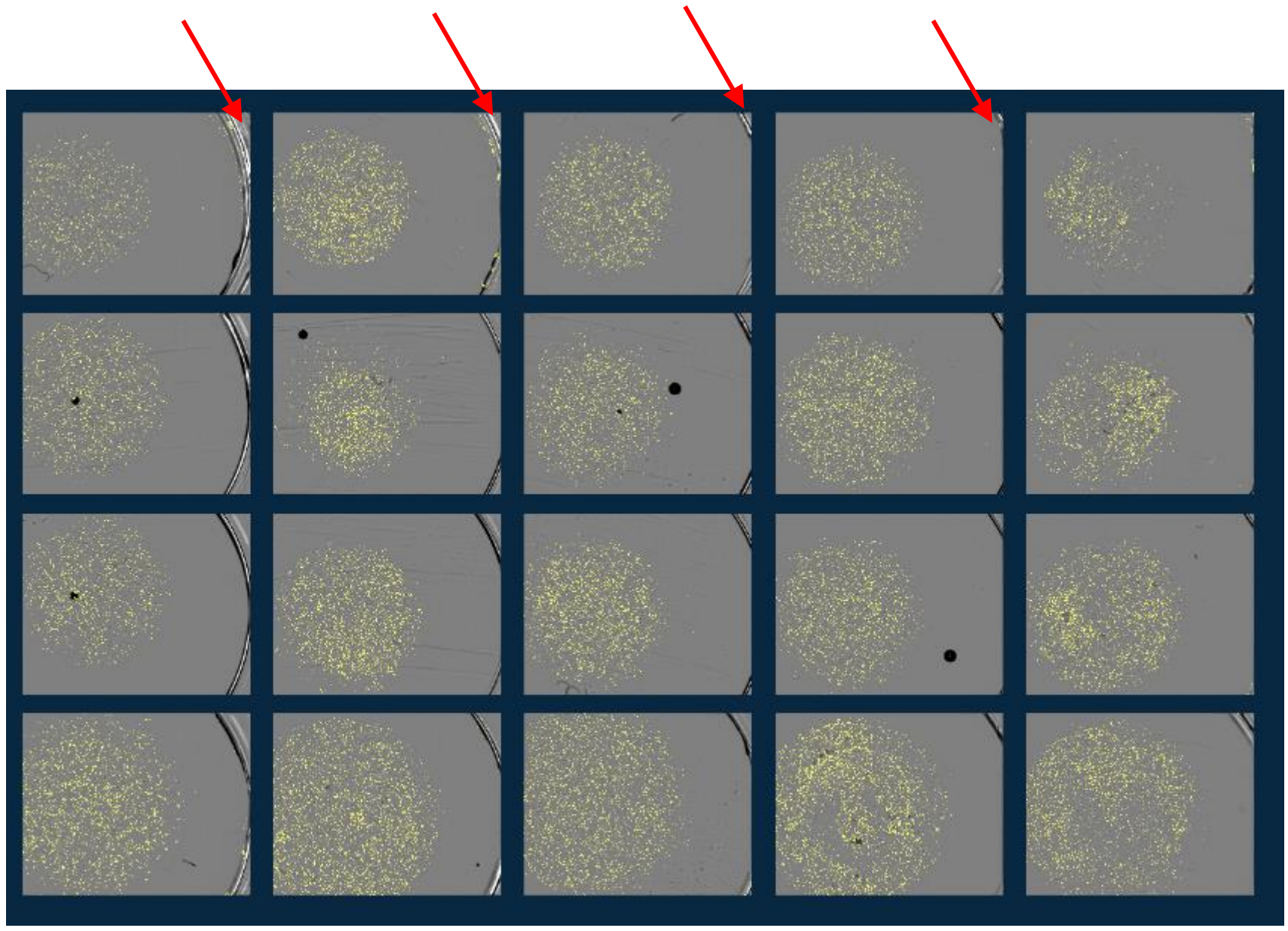
Trouble shooting – I do not have the recommended plate

- It is recommended that you seed your cells/spheroids and organoids in the recommended vessels. This is because different brans have different xyz positions for their wells. In some cases we have been able to run an experiment in a "wrong" brand.

- Wound healing plates have special crosses marked on the bottom of the wells in order to get accurate xy focusing. You can try running a 96 well regular Nunc plate, but you might see focusing problems and some variable movement in xy.

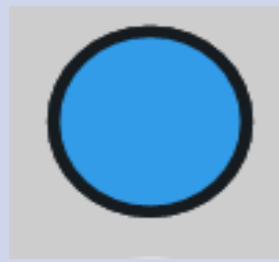
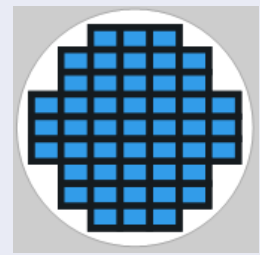
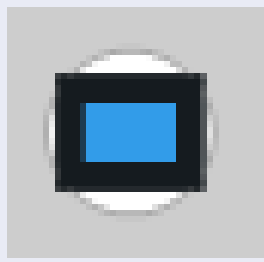
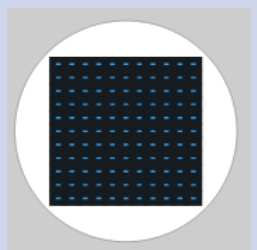
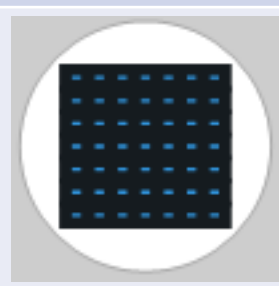
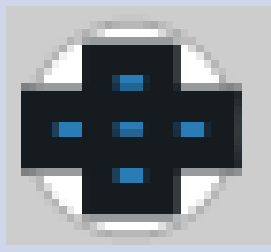
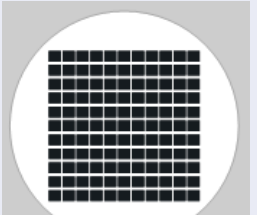
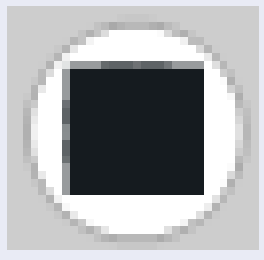
- Organoid and spheroid experiment should be run in Corning plates (you can buy these from us at MIC). If you run in a Nunc plate, then you will see the edges of the wells, especially on the left side of your plate. This might cause problems later when analyzing the data.

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

Trouble shooting – 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	

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

1. Contact MIC personnel to get access to the portal (mic@uib.no).
2. Put in the following link to access the portal.
3. Put in your uib user name and password (connecting can take minutes).
4. Open the IncuCyte 2021C software visible on the remote desktop.
5. Instead of the IP adress, put in ***incucyte.labit.intern***

https://portal.uib.no