

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

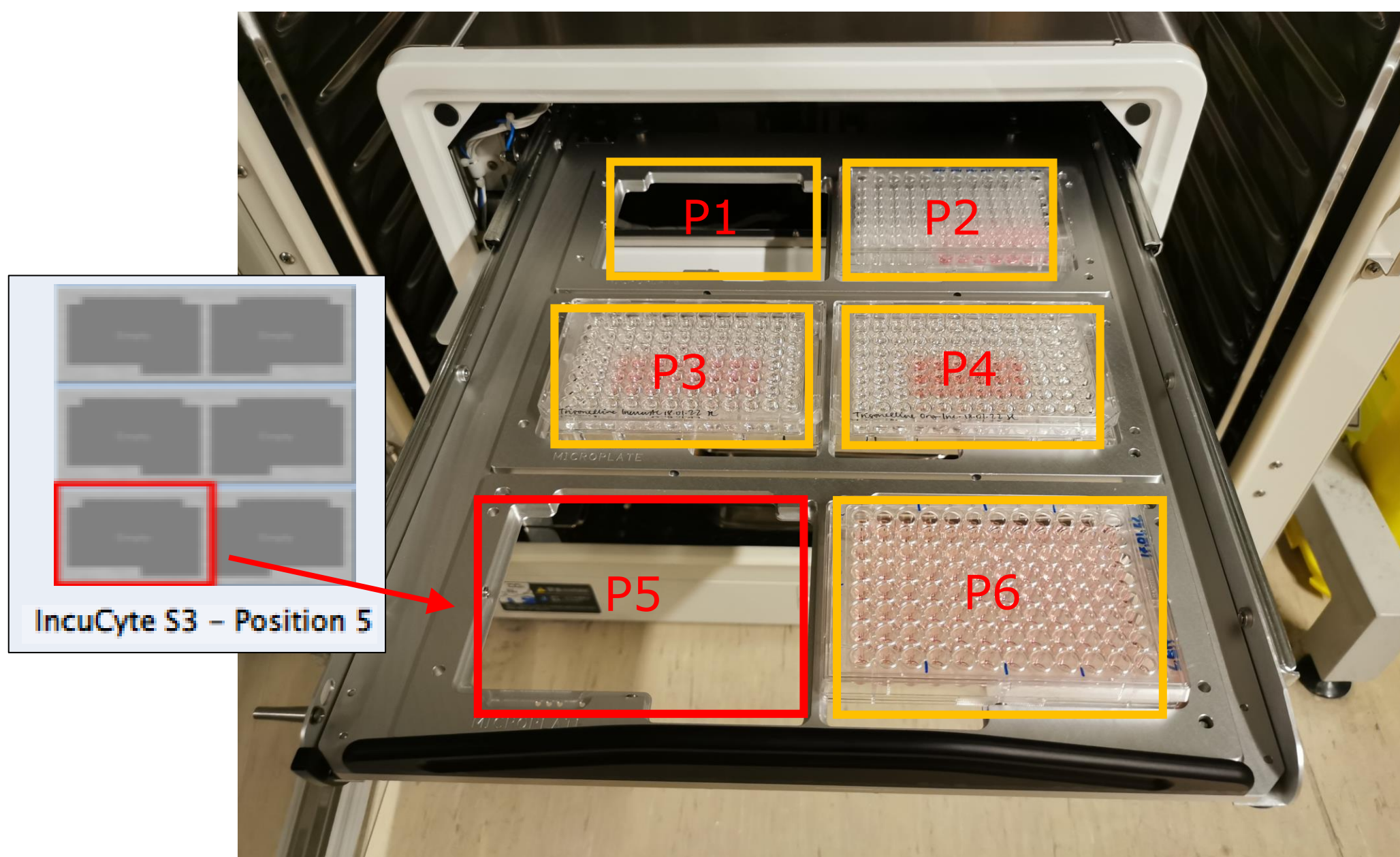
- The position you reserved is not free page 25
- Red and orange warnings when scan scheduling page 26
- I do not have the recommended plate page 27
- How much of the well area are we scanning page 28

Connecting remotely

page 29

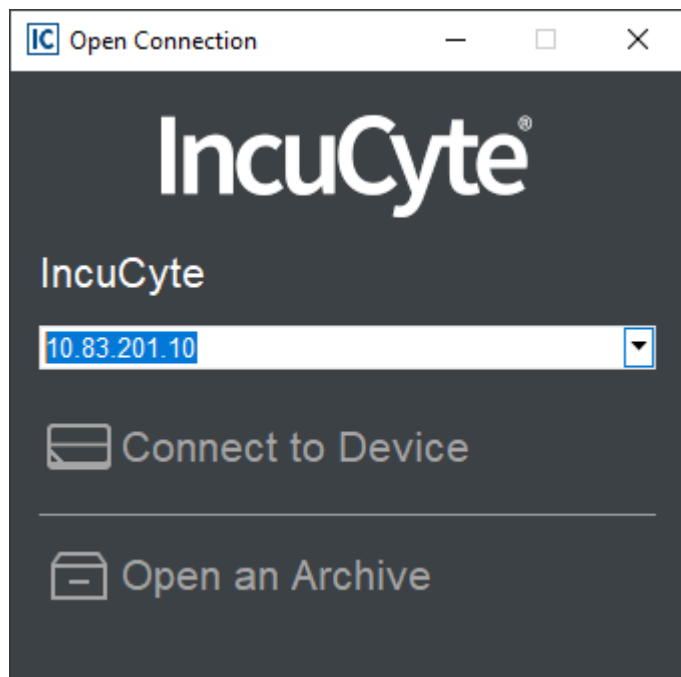
General rules for operating the IncuCyte

- 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

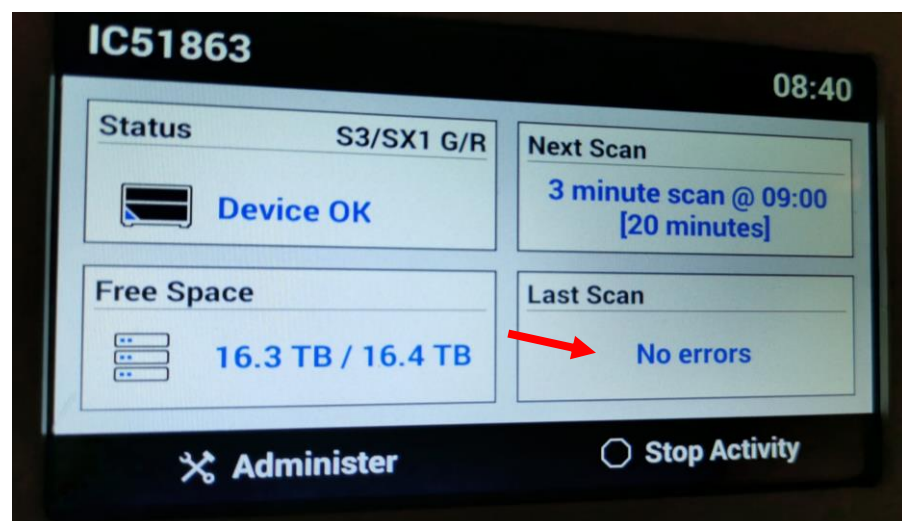
- Incucyte-2021C-GuiSetup
- Incucyte User Manual 2021C.pdf



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.



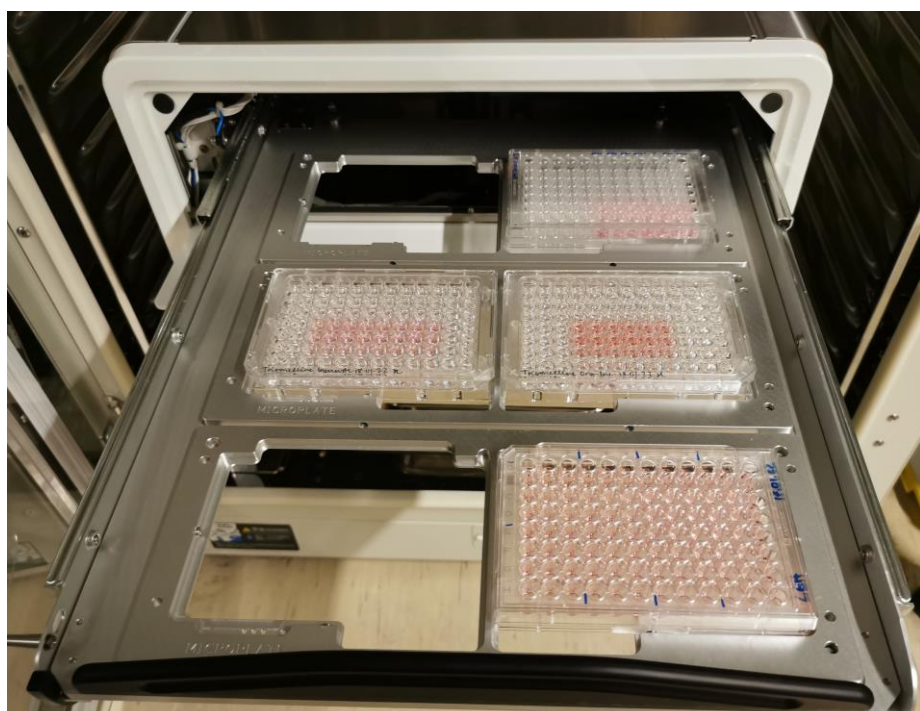
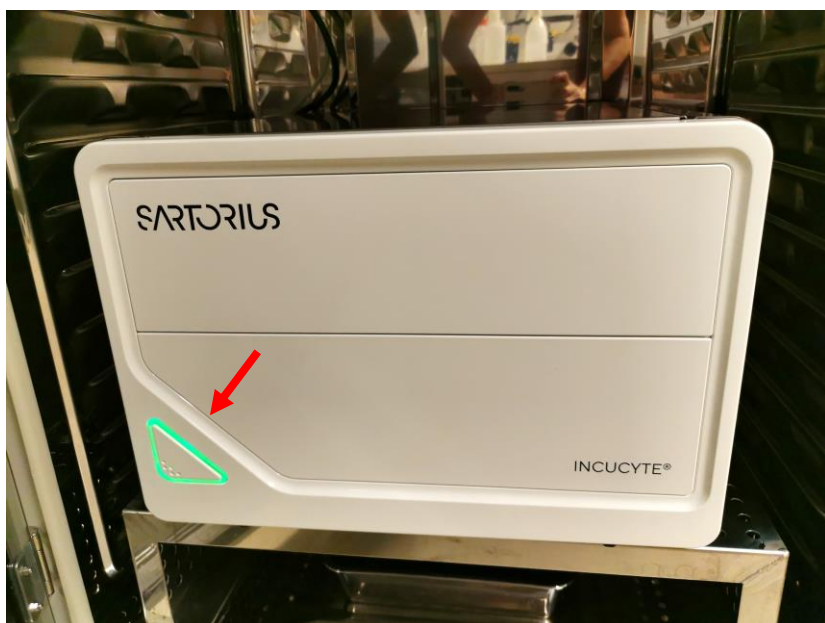
Log on to the controller with the IP address: 10.83.201.10

Put in your username and password. You can get this from Endy, Hege or Halala once you have done the training.

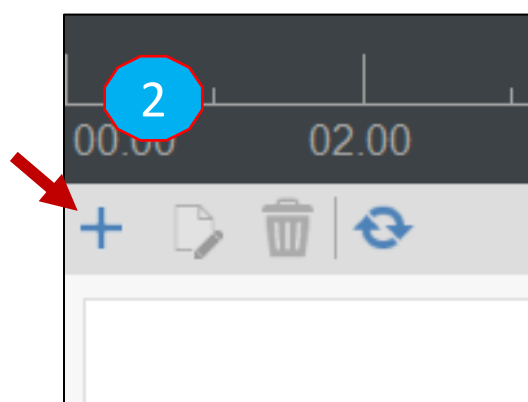
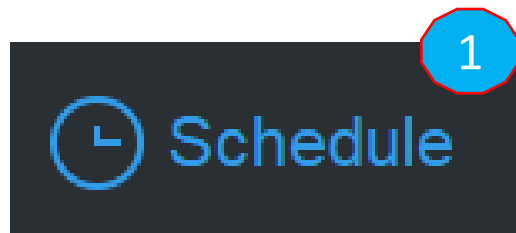
Make sure the system is not actively scanning (check the control panel) before opening the drawer (light will be green if you can open).

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

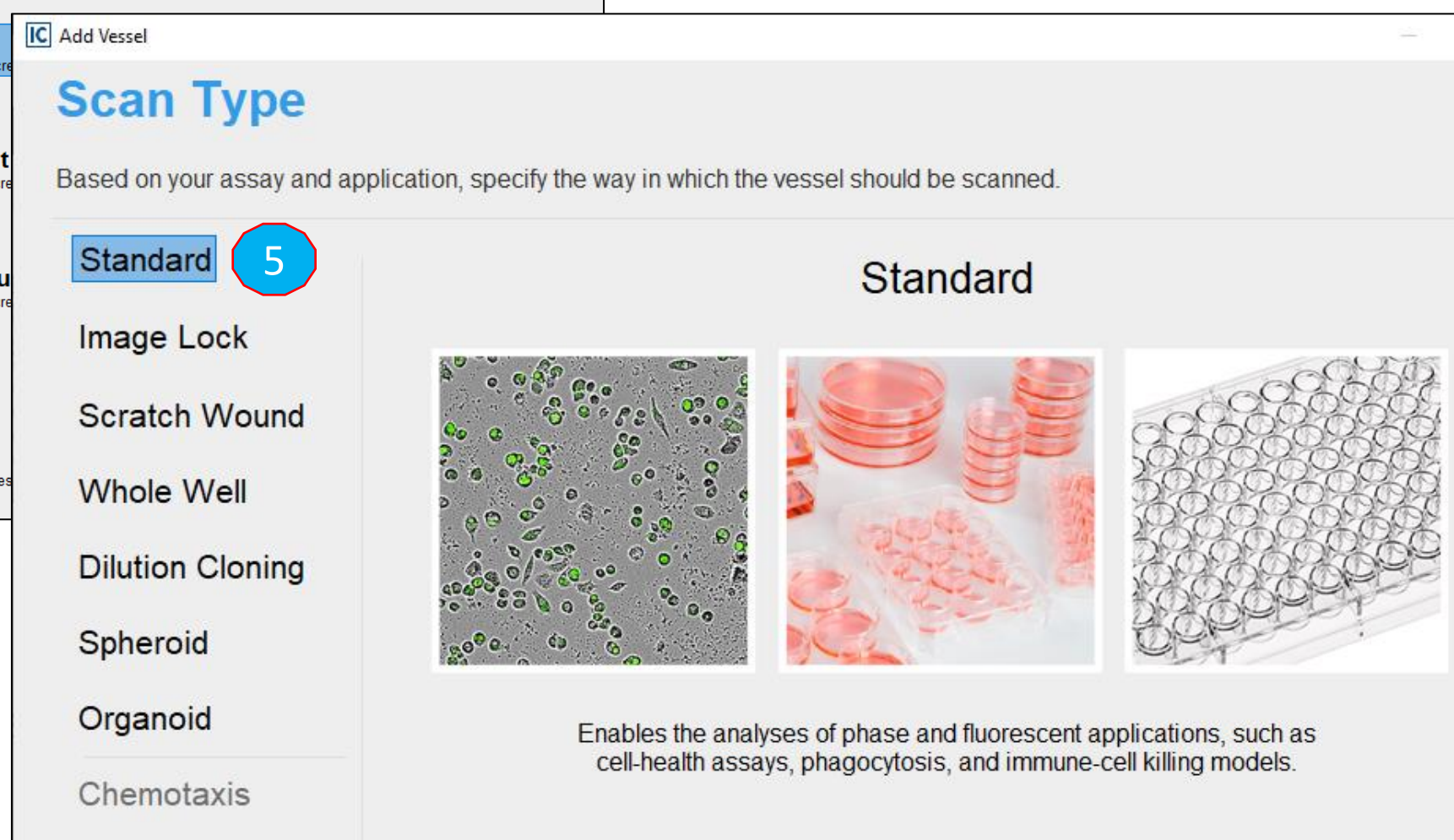
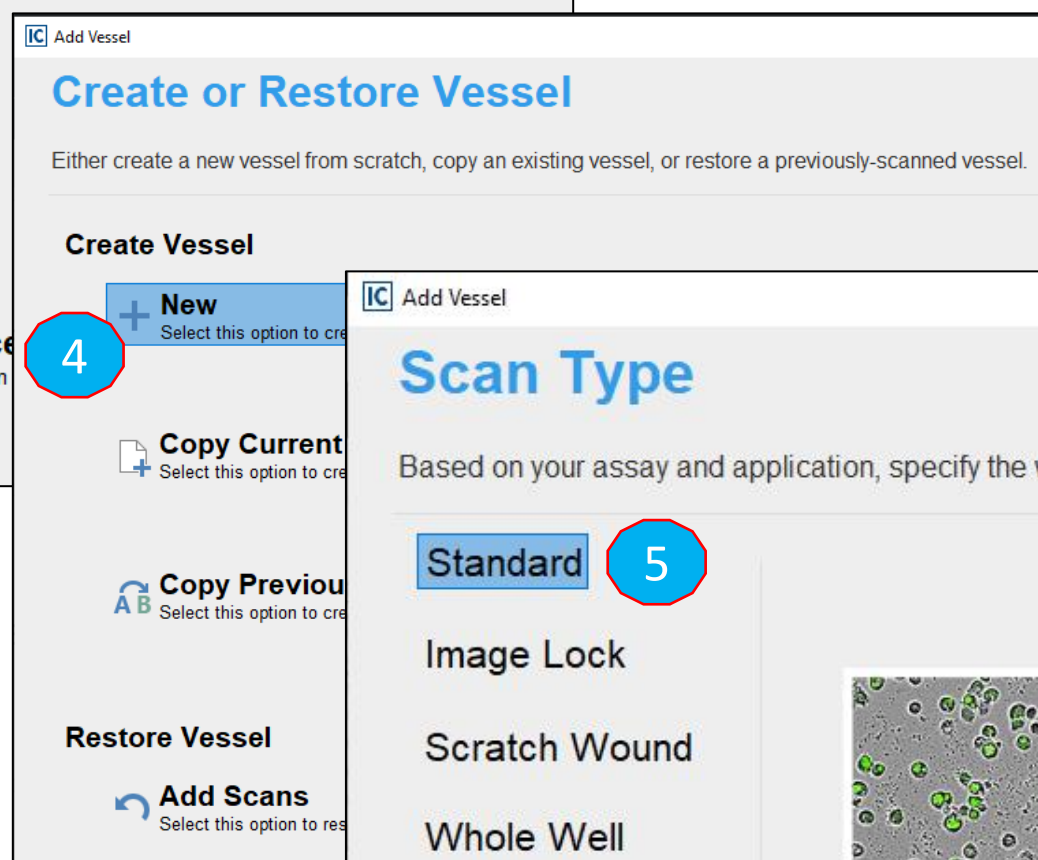
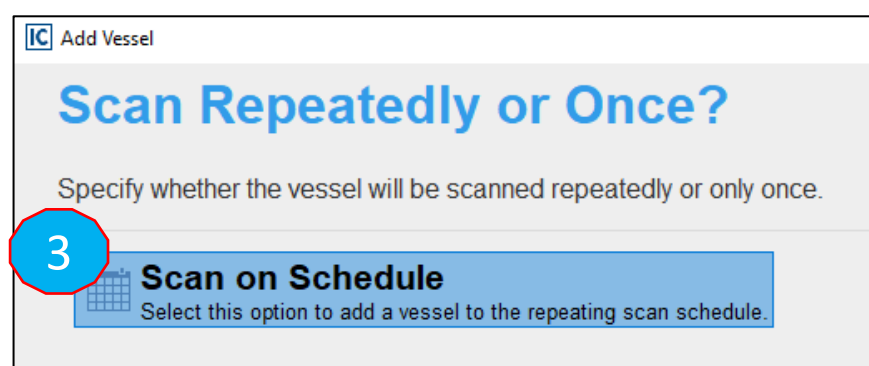
Make sure there are no error messages displayed on the control box after you have closed the incubator.



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

IC Add Vessel

Scan Settings 6

Specify the image channels, microscope objective and other settings to use when scanning the vessel.

Cell-by-Cell Options ☒ None ☐ Adherent Cell-by-Cell ☐ Non-Adherent Cell-by-Cell

Image Channels ☒ Phase ☐ Green ☐ Red

Acquisition Time (ms) 300

Acquisition Time (ms) 400

Objective 10x

Ex: 440-480 nm
Em: 504-544 nm

Ex: 565-605 nm
Em: 625-705 nm

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.

IC Add Vessel

Vessel Selection 7

Select the type of vessel to scan.

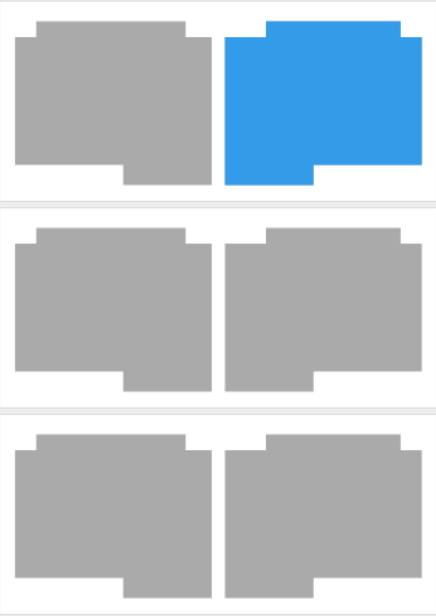
Enter text to search...

Manufacturer	Category	W...	Area	Catalog Numbers	Vessel Name	Tray Name
nunc		96				
Nunc	Plate	96	N/A	156545, 161093, 167008, 168055	96-well Nunc	Microplates
Nunc	Plate	96	N/A	167311, 167314, 267312, 267313	96-well Nunc Edge	Microplates
Nunc	Plate	96	N/A	152028, 152036, 152037, 152040, 165305, 165306	96-well Nunc opt bottom (Blk/Wht)	Microplates

IC Add Vessel

Vessel Location 8

Specify the location in the drawer for the vessel.



Front of Instrument

8. Specify the location in the incucyte for your plate.

9. Specify the scan pattern and number of images per well.

IC Add Vessel

Scan Pattern 9

Specify the scan pattern to use for image acquisition.

Select Wells

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

Images per Well 1

Scan Duration 2 min (estimated)

Use Sample Pattern

Scheduling a scan - continues

Add Vessel

Vessel Notebook

Provide information about the vessel.

Name

20220204_Spriet_Hela proliferation+drug

Cell Type

Passage

N/A ↕ ×

Plate Map

+ 📄 🗑️ 🔄 📁

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												
D												
E												
F												
G												
H												

No Plate Map Specified

Notes

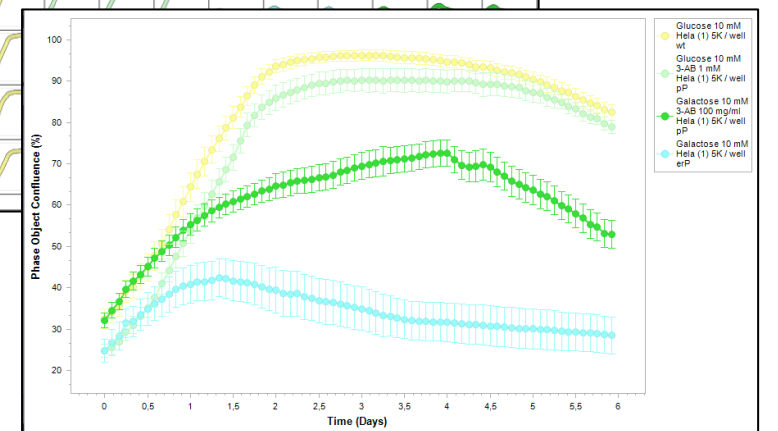
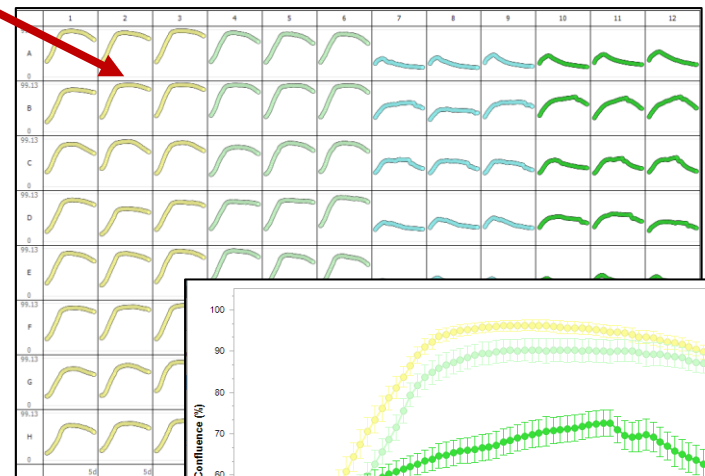
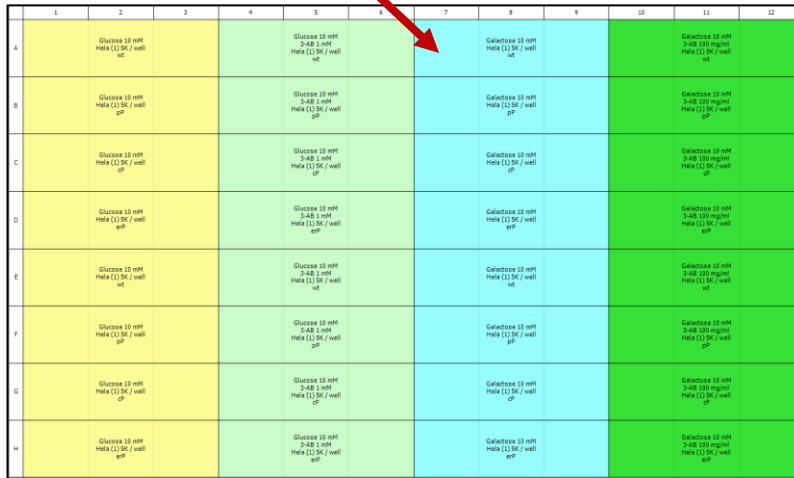
10. Save your plate information as following: **yyyymmdd_scientist name_experiment name**
11. Add a plate map. You can choose between compounds, cells and growth conditions.
12. The plate map can also be exported and imported for other plates later.

The screenshot displays the IncuCyte Plate Map Editor software. At the top, a blue header bar contains the text "IC IncuCyte Plate Map Editor - IncuCyte Plate Map*". Below the header is a toolbar with various icons for file operations and editing. A red circle with the number "11" is positioned in the top right corner. The main interface is divided into three sections: "Compounds", "Cells", and "Growth Conditions". The "Compounds" section is currently active, showing a list of compounds with a yellow background and the text "drug". A green callout box with a white border contains the text: "Compounds will give you concentration/dilution options." The "Cells" section is visible below, showing a list of cells with a red background and the text "Hela". A green callout box with a white border contains the text: "Cells will give you dilution type and directions of these." The "Growth Conditions" section is visible below that, showing a list of growth conditions with a green background and the text "Growth conditions will be simple without any concentration." The "Notes" section is at the bottom, showing a list of notes with a grey background and the text "Notes".

Add/edit

Add/edit to plate

A diagram of a toolbar with five icons: a blue square with a white plus sign, a blue square with a white minus sign, a red trash can, a blue square with a white grid, and a red plus sign. Red arrows point to the first four icons.



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

Add Vessel

13

Analysis Setup

Define an analysis to launch automatically after the vessel has been scanned. This is optional, but recommended.

Analysis Type

<Defer analysis until later>

Analysis Definition

Analysis Notes

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

Add Vessel

Scan Schedule

14

Define the scan schedule for this vessel.

Add Scans to Schedule

☒ Create new schedule with scans at intervals of: 3 Hours

☐ Add to existing schedule

☐ Create new schedule with advanced scheduling options

☐ Reserve tray location and defer scheduling until later

Total Duration of Experiment

☒ Scan indefinitely

☐ Stop scanning: 1 days, 0 hours after the first scan

☐ Stop scanning after: 15.01.2022 11.00

For advanced scheduling options, double click the timeline on the main schedule page.

Back Next

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 incubator when your experiment has ended.

How often should I scan?

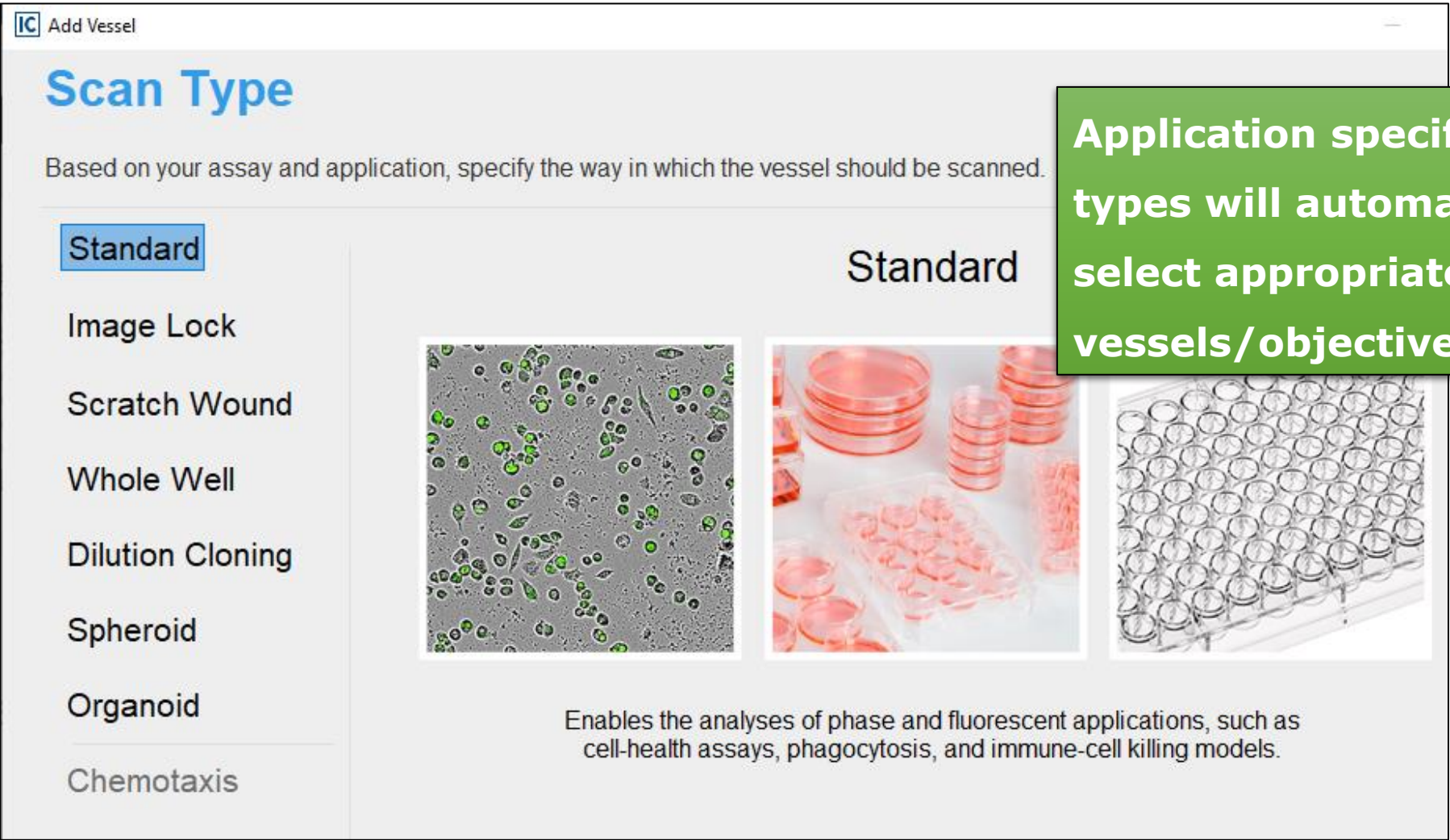
This will depend on the type of imaging you are doing.

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



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”.

IC Add Vessel

Scan Repeatedly or Once?

Specify whether the vessel will be scanned repeatedly or only once.

Calendar icon

Scan on Schedule

Select this

IC Add Vessel

Scan once now

Select this

Create or Restore Vessel

Either create a new vessel from scratch, copy an existing vessel, or restore a previously-scanned vessel.

Create Vessel

+ New

Select this option

Copy C

Select this option

Copy P

Select this option

Restore Vessel

Select this option

IC Add Vessel

Scan Type

Based on your assay and application, specify the way in which the vessel should be scanned.

Standard

Image Lock

Scratch Wound

Whole Well

Dilution Cloning

Spheroid

Organoid

Chemotaxis

IC Add Vessel

Scan Settings

Specify the image channels, microscope objective and other settings to use when scanning the vessel.

Cell-by-Cell Options

☐ None

☒ Adherent Cell-by-Cell

☐ Non-Adherent Cell-by-Cell

Image Channels

☒ Phase

☐ Green

☐ Red

Acquisition Time (ms)

300

Acquisition Time (ms)

400

Objective

10x

Adherent Cell-by-Cell acquisition requires Phase image channel and use of the 20x or 10x objective.

IC Add Vessel

Schedule Conflict Check

Verify scanning the vessel once immediately does not conflict with any daily scan times.

00.00 02.00 04.00 06.00 08.00 10.00 12.00 14.00 16.00 18.00 20.00 22.00 00.00

Yellow bar at 16:00

Vessel ID	Name	Owner	Location	Scan Duration h:mm
	test		Rear Tray, Left Position	0:02

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.

9

Viewing your data set

The screenshot shows the MICadmin - IncuCyte S3 interface. At the top, there's a navigation bar with icons for Schedule, View, Manage, Device, Archive, Security, and Status. Below this is a search bar. The main area displays a table of vessels. A red box highlights a specific vessel, and a red arrow points from its row in the table to a detailed view on the right.

Analyses	Vessel Name	Owner	Last Scan	Scan Type	Vessel ID
	Ritas_PARPconstructs_andCONTROLS	Rita-Guillot	26.01.2022 09.00	Standard	42
→	20220125_VelascoK_EA2	Kelly-Valesco	25.01.2022 14.06	Adherent Cell-by-Cell	40
→	20220125_VelascoK_EA1HEK	Kelly-Valesco	25.01.2022 13.58	Adherent Cell-by-Cell	38
→	Lepland_J_20220121_C11_Betaine_prolif_repeat	Johanna-Lepl...	25.01.2022 09.00	Standard	37
→	20220121_Guillot_proliferation-PARPconstructs	Rita-Guillot	24.01.2022 13.05	Adherent Cell-by-Cell	36
	Høyland_20012022_U2OS proliferation	Lena-Høyland	24.01.2022 11.52	Standard	35
	Høyland_180122_proliferation Hela	Lena-Høyland	24.01.2022 11.43	Standard	26
→	Lepland_J_20220119_C11_proliferation_propionylchol	Johanna-Lepl...	22.01.2022 21.05	Standard	32
→	Lepland_J_20220118_C11_trigonelline_proliferation	Johanna-Lepl...	21.01.2022 20.00	Standard	27
→	Lepland_J_20220119_C11_propionylchol_orgnorm	Johanna-Lepl...	20.01.2022 19.19	Standard	33
→	Lepland_J_20220118_C11_trigonelline_orgnorm	Johanna-Lepl...	19.01.2022 18.08	Standard	28
→	Lepland_J_20220114_C11_betaine_proliferation	Johanna-Lepl...	18.01.2022 19.00	Standard	18
→	Valesco_180122_confluency EA2	Kelly-Valesco	18.01.2022 09.57	Adherent Cell-by-Cell	24
→	Valesco_180122_confluency EA	Kelly-Valesco	18.01.2022 09.52	Adherent Cell-by-Cell	22
→	Lepland_J_20220114_C11_betaine_organoidnorm	Johanna-Lepl...	16.01.2022 12.00	Standard	19

The detailed view on the right shows a plate map (a grid of circles representing wells) and various parameters for the selected vessel (20220121_Guillot_proliferation-PARPconstructs):

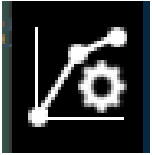
- Vessel Name: 20220121_Guillot_proliferation-PARPconstructs
- Scan Type: Adherent Cell-by-Cell
- Cell Type: HEK293
- Image Channels: ☒ Phase, ☒ Green
- Magnification: 10x
- Plate Map: [Yes](#)
- Vessel Type: 96-well Sarstedt
- Vessel ID: 36
- Vessel Notes:

- 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.

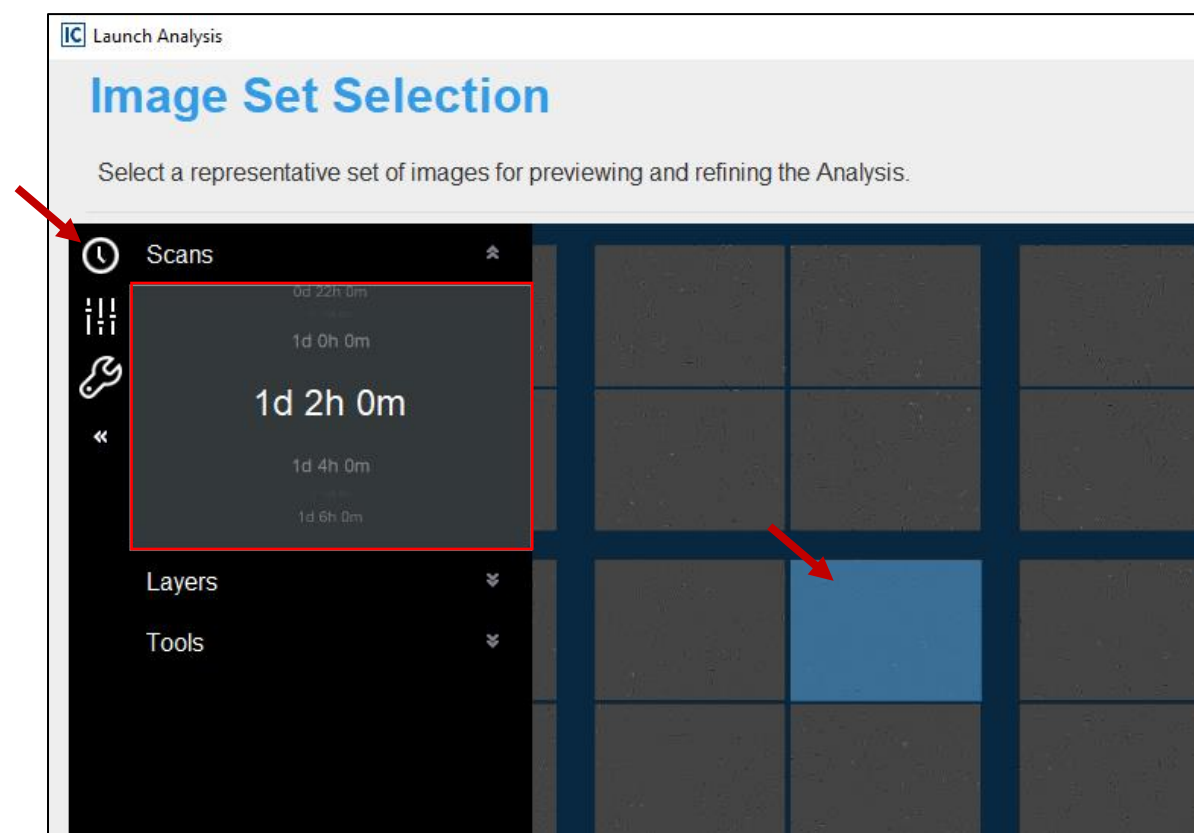
The screenshot shows a detailed view of a vessel (20220121_Guillot_proliferation-PARPconstructs) in the MICadmin - IncuCyte S3 interface. The interface includes a plate map (a grid of circles representing wells) and various toolbars. A red box highlights the toolbars, and a red arrow points from the toolbars to a list of functions.

- Opens vessel scan time.
- Image layers/channels. You can turn on/off channels and change display
- Tools
- Launch analysis
- Graph metrics
- Open vessel information
- Export images and movies

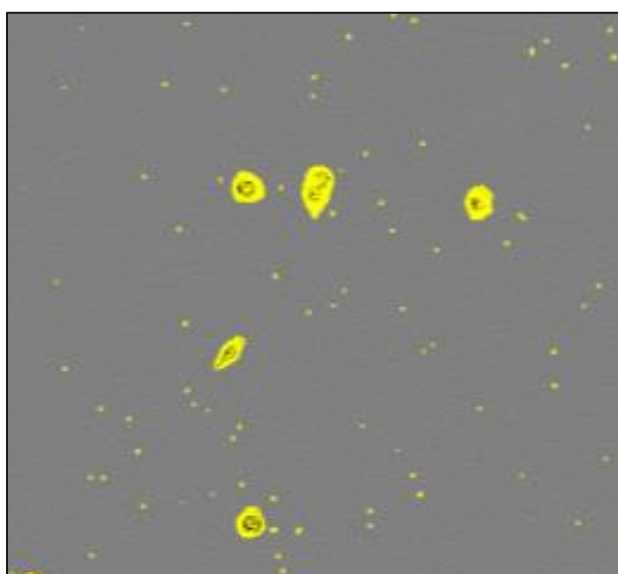
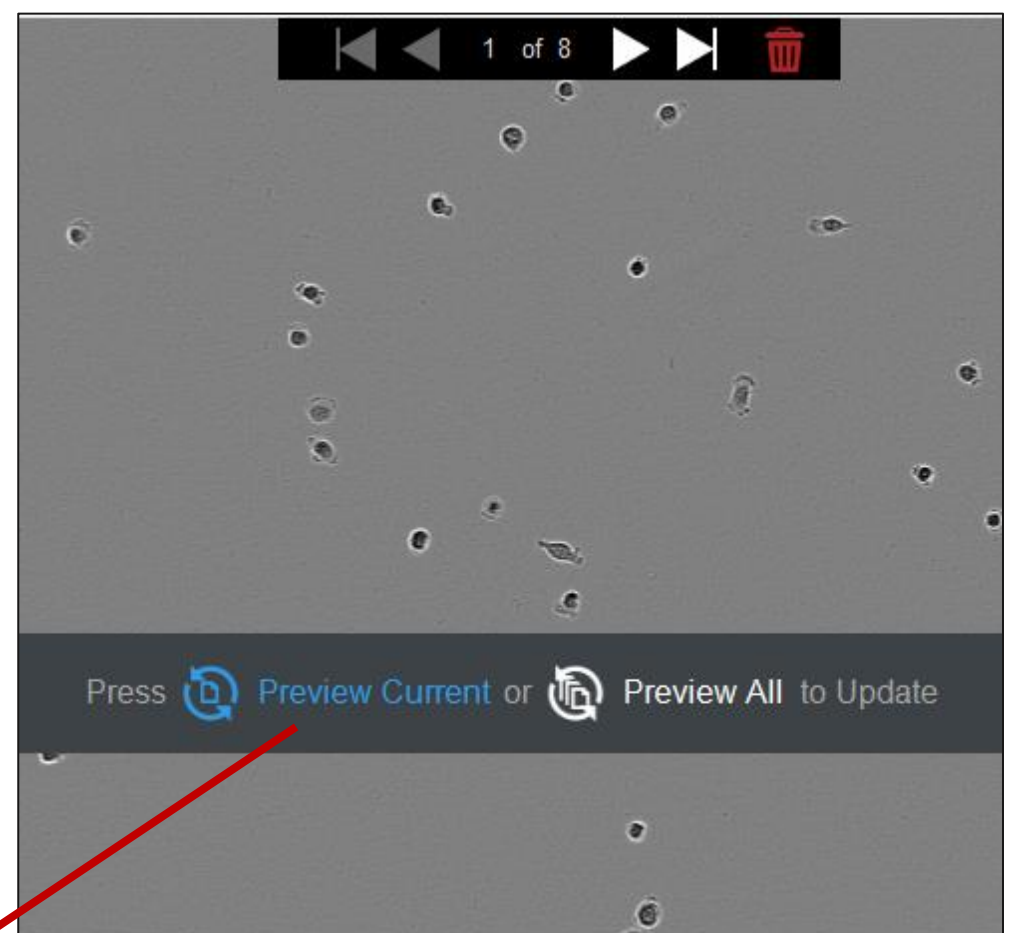
Analyzing a confluency experiment



- 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.

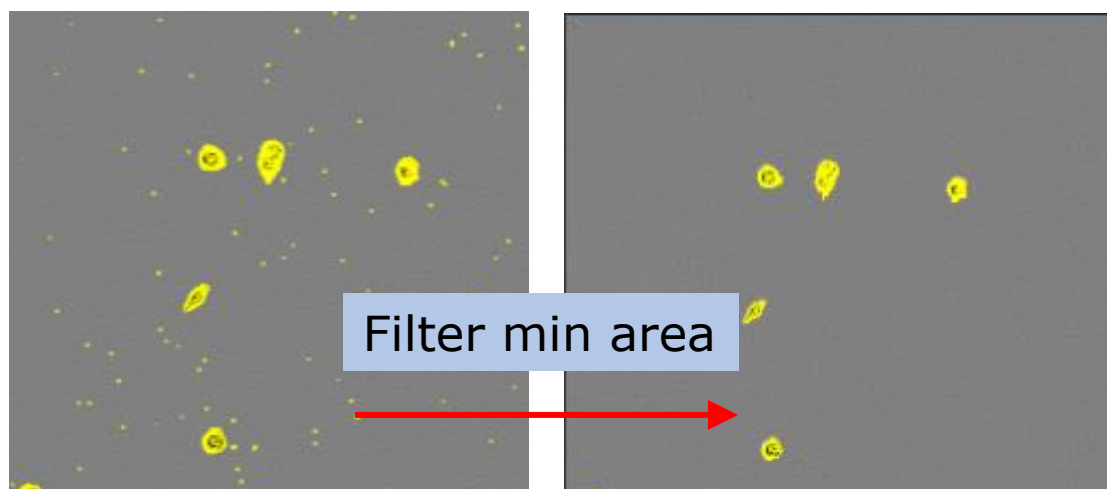
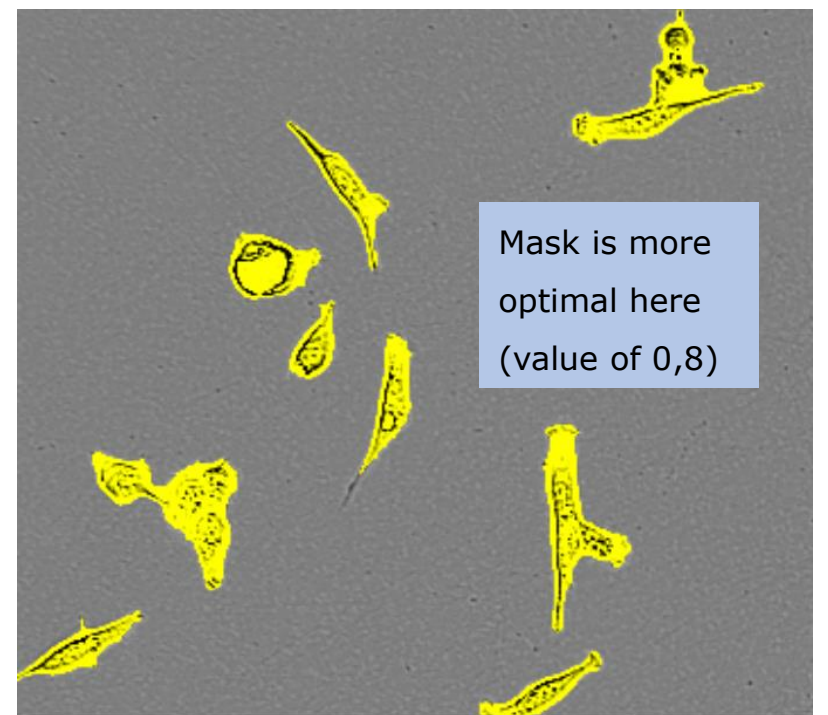
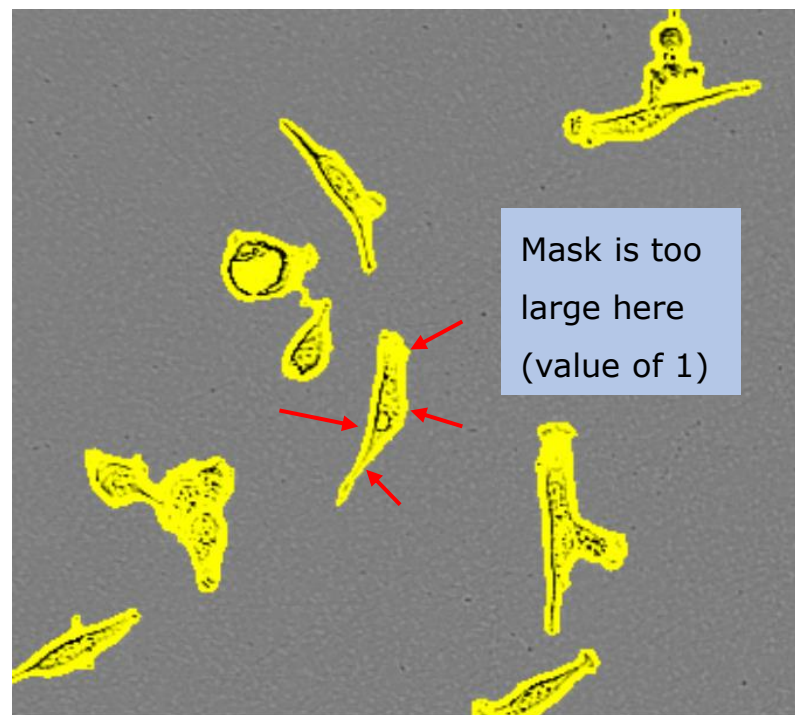
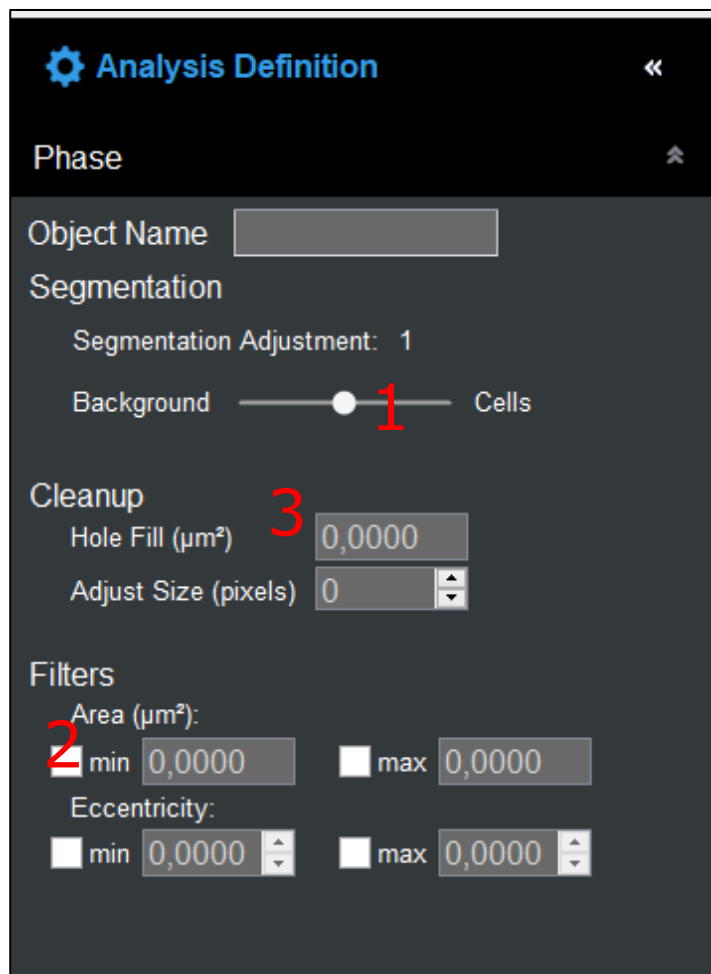


- The image selection will now be uploaded to your computer.
- To start the process of masking, you need to first press "preview current". A default masking will then appear on your image. On the next page you will see how we can improve the masking.

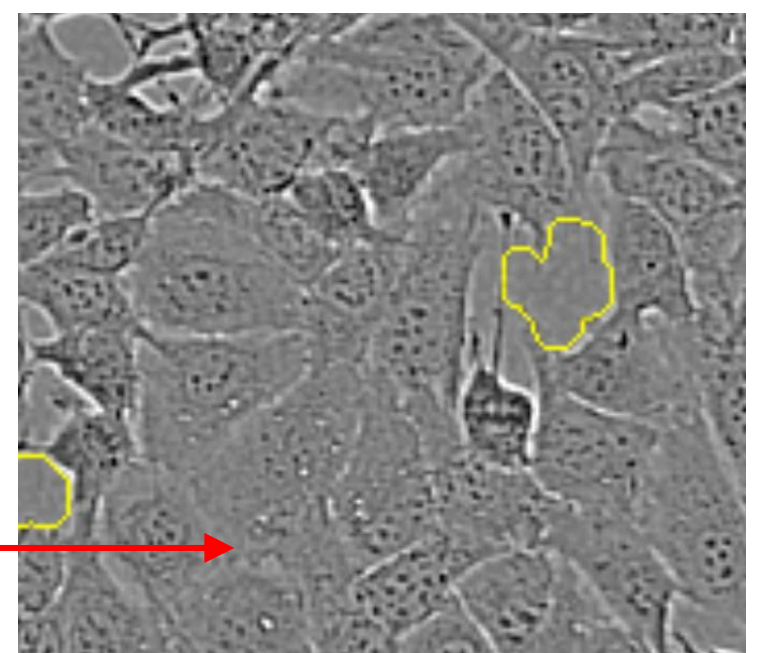
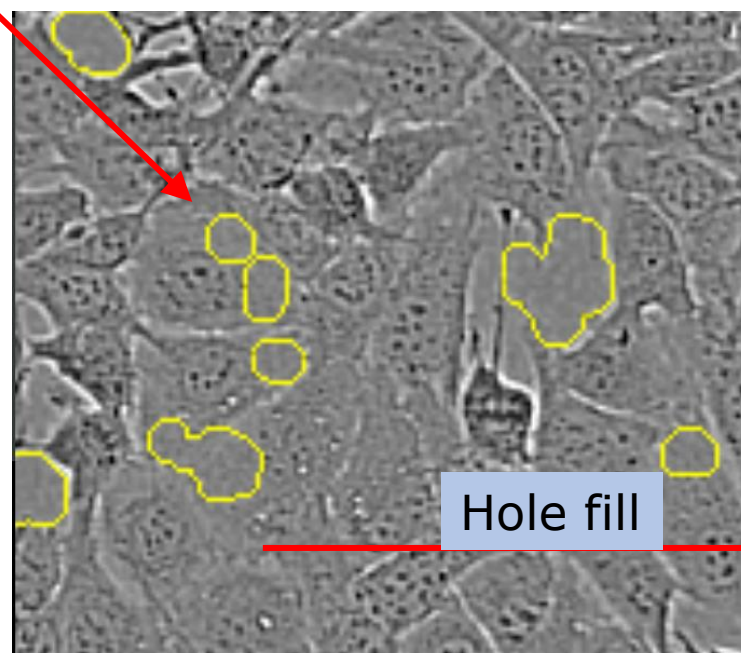
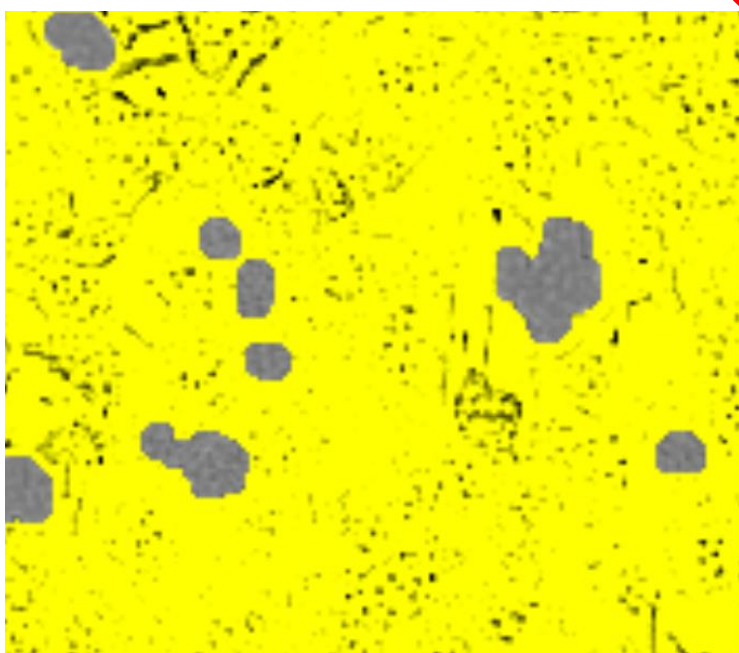
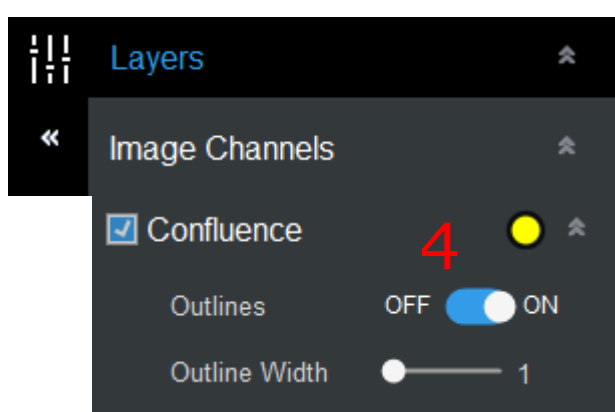


Analyzing a confluency experiment continues



- 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 $160 \mu\text{m}^2$.
- 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 $450 \mu\text{m}^2$ of Hole fill.



Analyzing a confluency experiment - continues

Press  Preview Current or  Preview All to Update

- 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”

Launch Analysis

Scan Times and Wells

Select the scan times and images to analyze along with the option to analyze future scans.

Select Scans

☐ Analyze Future Scans

0d 0h 0m

0d 2h 0m

0d 4h 0m

0d 6h 0m

0d 8h 0m

0d 10h 0m

0d 12h 0m

0d 14h 0m

0d 16h 0m

0d 18h 0m

0d 20h 0m

0d 22h 0m

1d 0h 0m

1d 2h 0m

1d 4h 0m

1d 6h 0m

1d 8h 0m

1d 10h 0m

1d 12h 0m

1d 14h 0m

1d 16h 0m

1d 18h 0m

1d 20h 0m

1d 22h 0m

2d 0h 0m

2d 2h 0m

2d 4h 0m

2d 6h 0m

2d 8h 0m

2d 10h 0m

2d 12h 0m

Select Wells

123456789101112

A

B

C

D

E

F

G

H

- 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.

10.83.201.10 - MICadmin - IncuCyte S3

Schedule

View

Manage

Device

Archive

Security

St

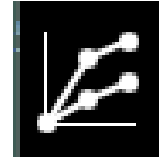
Enter text to search...

Analyses	Vessel Name	Owner	Last Scan	Scan Type	Vessel ID
<div><div></div><div></div></div>					
<div><div></div><div></div></div>	Lepland_J_20220114_C11_betaine_proliferation	Johanna-Lep...	17.01.2022 15.00	Standard	18
Analysis Definition Name	Analysis Type	Creator	Date Completed	Analysis ID	Analysis Notes
Spriet_170122_test	Basic Analyzer	MICadmin		3	

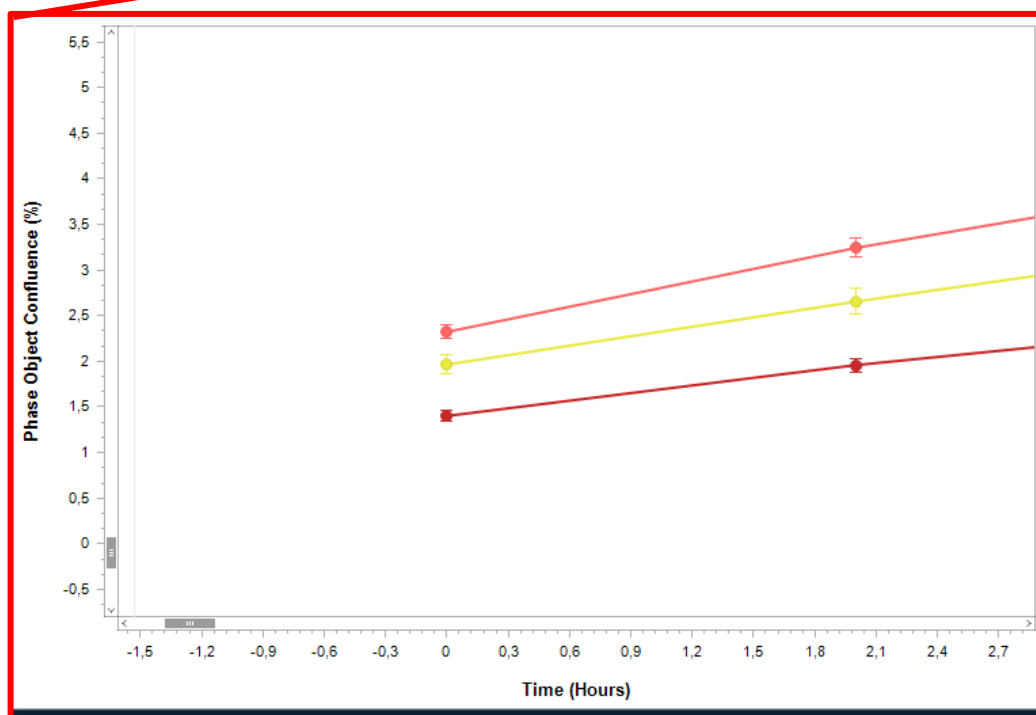
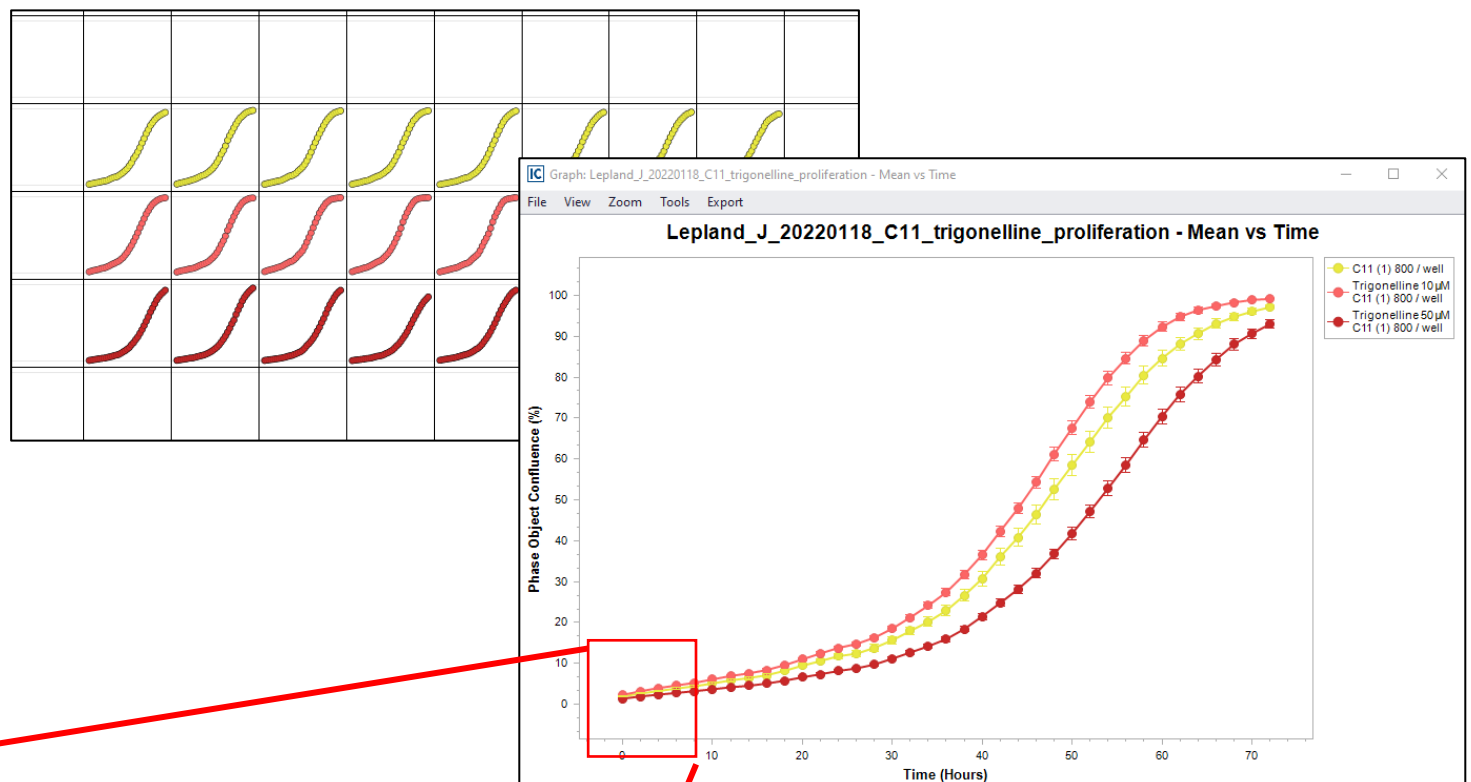
Analyzing a confluency experiment continues

Lepland_J_20220118_C11_trigonelline_proliferation	
Analysis Definition Name	Analysis Type
Johanna_C11_proliferation A	Basic Analyzer

B



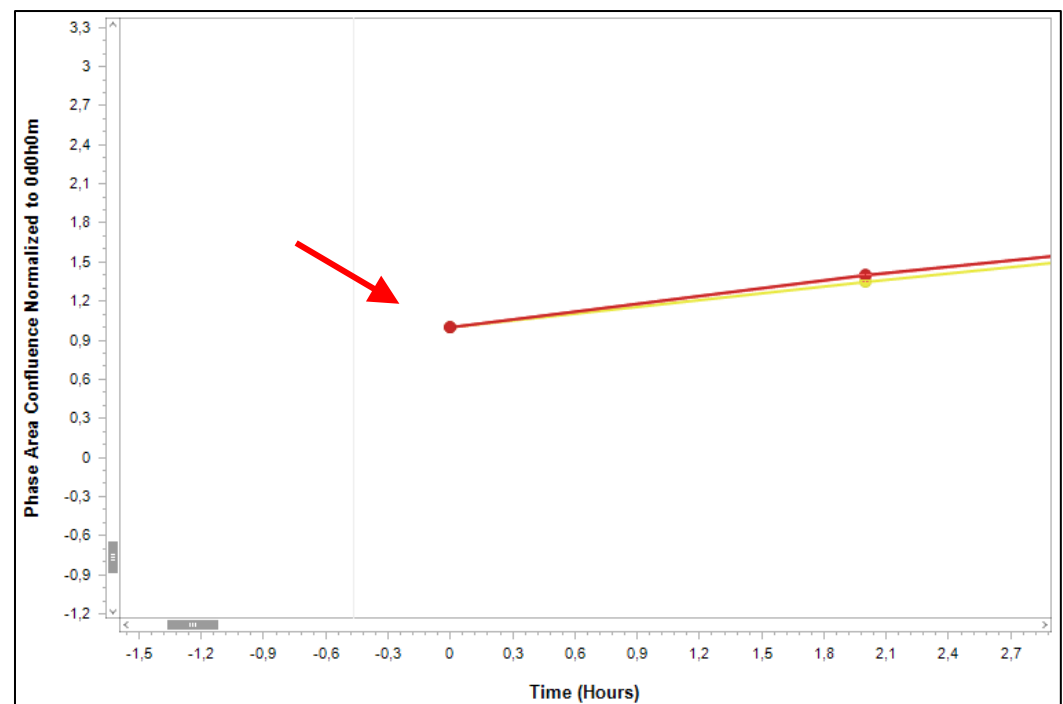
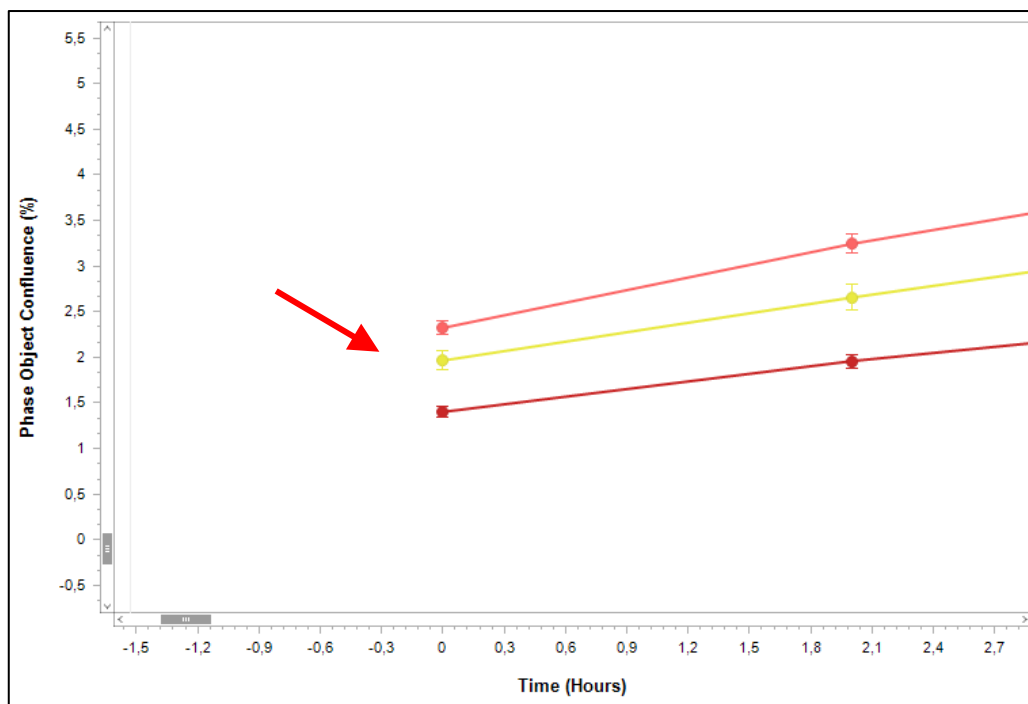
- Open the final analysis by either clicking on the blue arrow next to your experiment under “view” and open the analysis definition (A) or simply open your experiment click on the “graph metrics” icon (B).
- Check the microplate graph to have a quick overview over all the wells.
- Create a graph and “**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.



Select Metric

→ + [icon] [icon] [icon]

- ▼ User Defined Metrics
Click the + button above to add additional metrics and normalizations.
- ▼ Phase
 - Confluence (%)
- ▶ Scan Metrics

Select Metric

+ [icon] [icon] [icon]

- ▼ User Defined Metrics
Phase Area Confluence Normalized to 0d0h0m
- ▼ Phase
 - Confluence (%)
- ▶ Scan Metrics

Create Metric

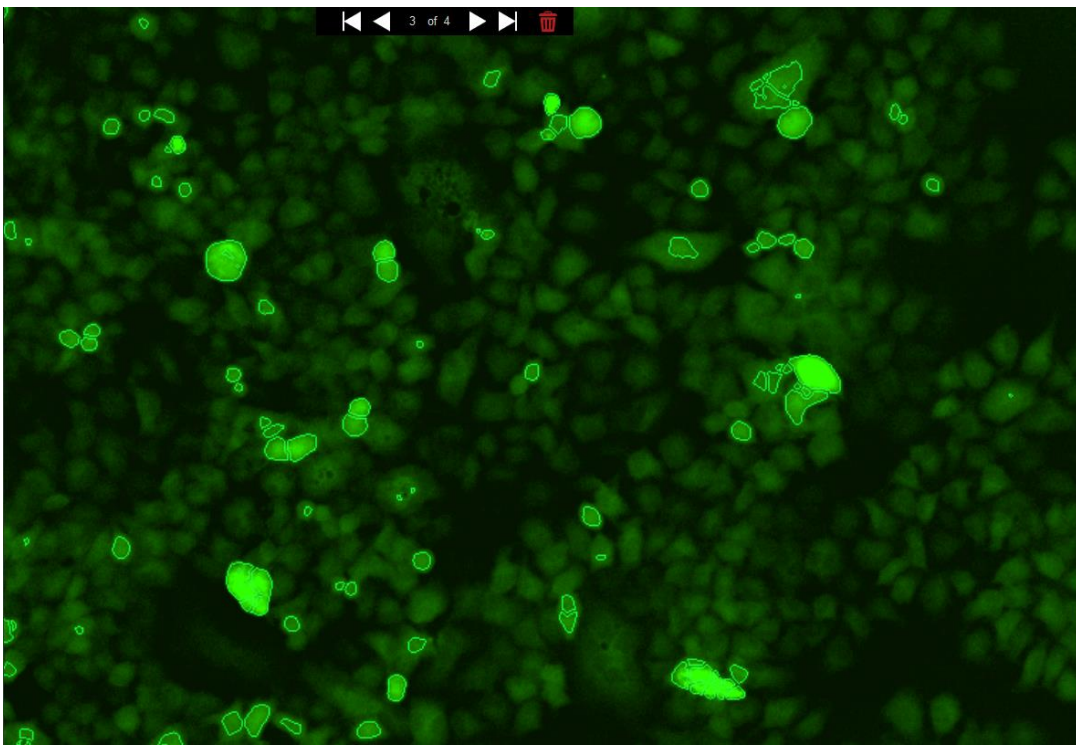
	Channel	Metric	Value
Metric	Phase	Area	Confluence
Normalized to (by division)	None		
Normalize to Scan (by division)	0d 0h 0m		
Display as	Ratio		
Default Name	Phase Area Confluence Normalized to 0d0h0m		
Name Override			

OK

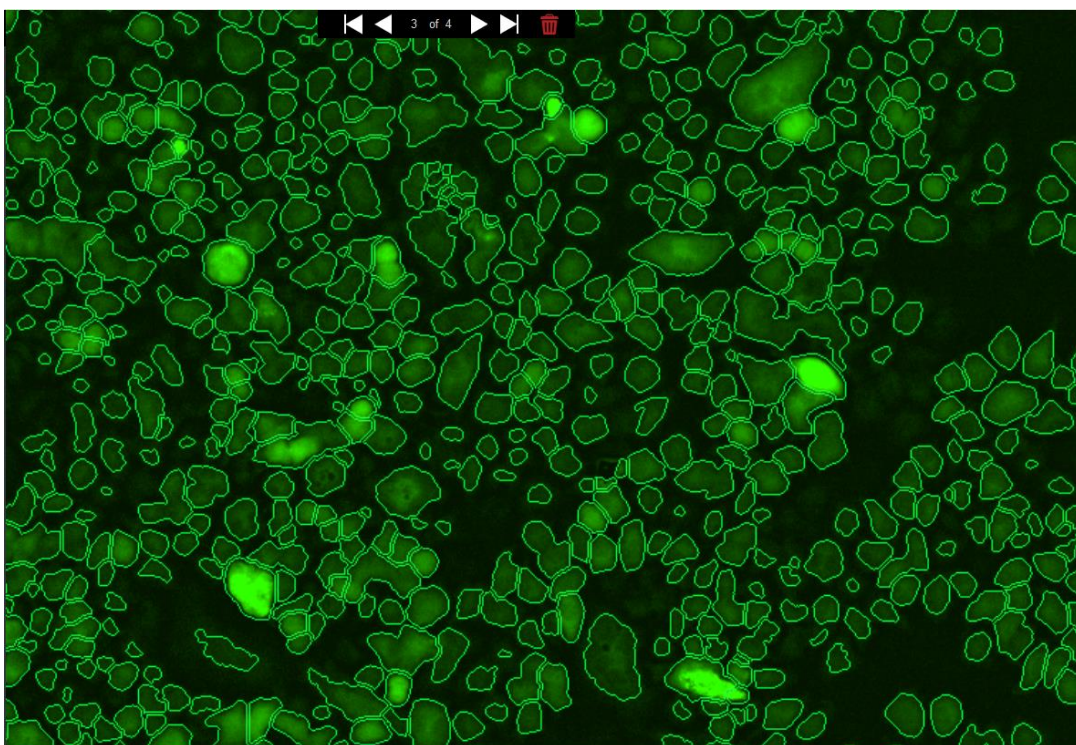
- Now, run the new user defined metrics and see the results in the graph. You can also export your normalized data to excel. Timepoint will then be 1 and the rest of the timepoints show up in relation to this.

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-Hat**” 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 μm^2 .
- Launch the analysis.

Segmentation

Top-Hat

Radius (μm) 30,000

Threshold (GCU) 0,5300

Edge Split On

Edge Sensitivity -20

Cleanup

Hole Fill (μm^2) 50,000

Adjust Size (pixels) 1

Filters

Area (μm^2):

☒ min 60,000 ☐ max 60,000

Eccentricity:

☐ min 0,0000 ☐ max 0,0000

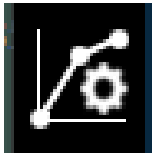
Mean Intensity:

☐ min 0,0000 ☐ max 0,0000

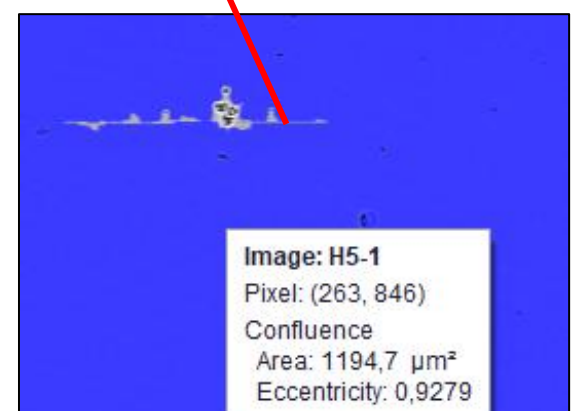
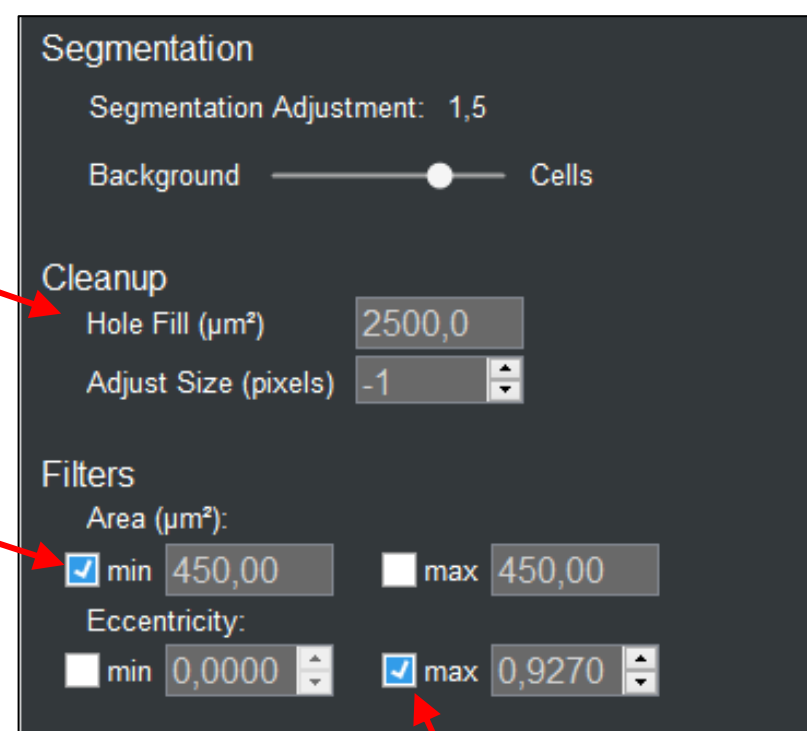
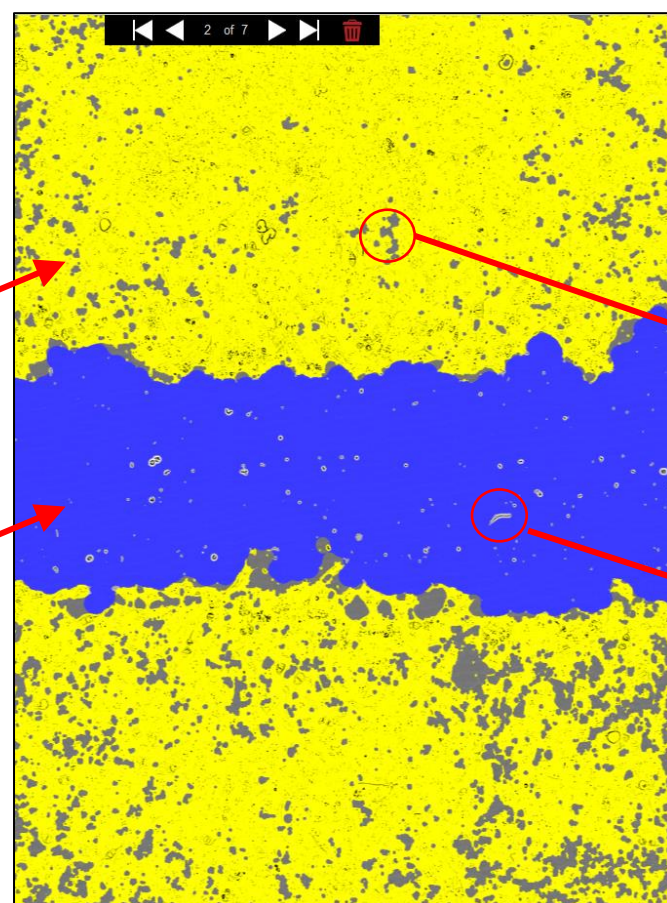
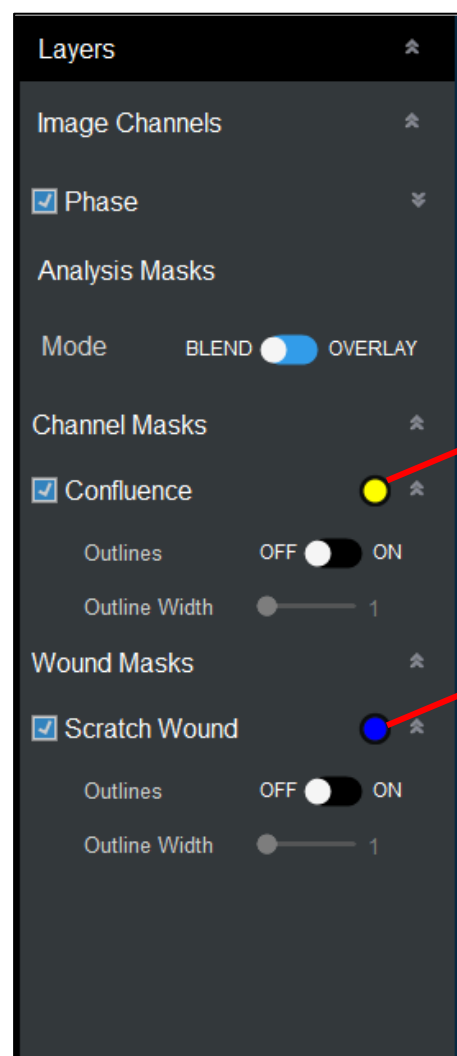
Integrated Intensity:

☐ min 0,0000 ☐ max 0,0000

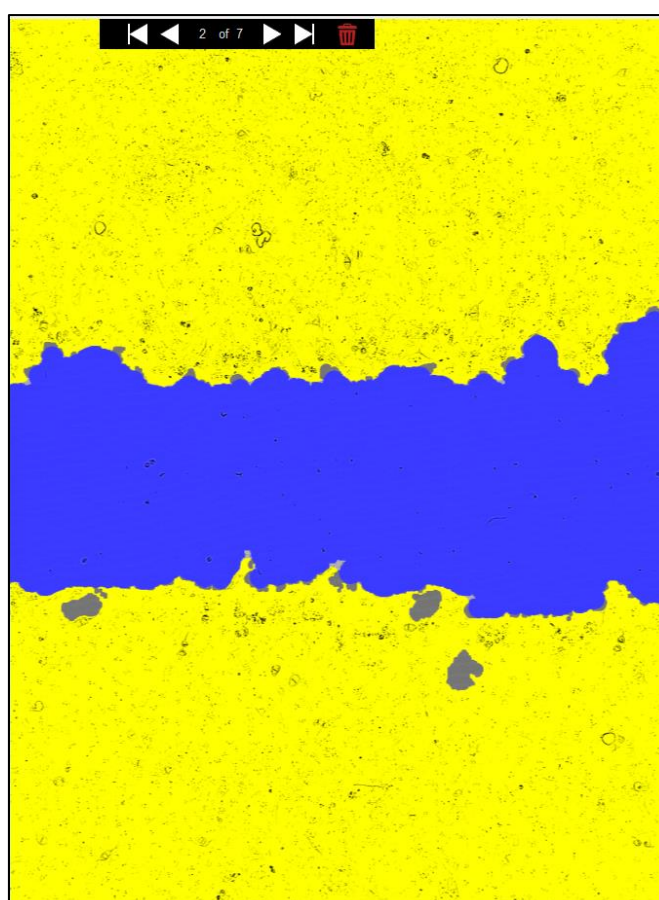
Analyzing a scratch wound



- 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.

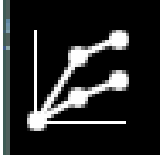


- In this experiment segmentation of 1,5 towards cells with a -1pixel adjustment seem to pick up cells accurately.
- We choose to filter away particles and free cells in the wound.
- We also used “eccentricity” to remove some minor scratches in one of the wells.

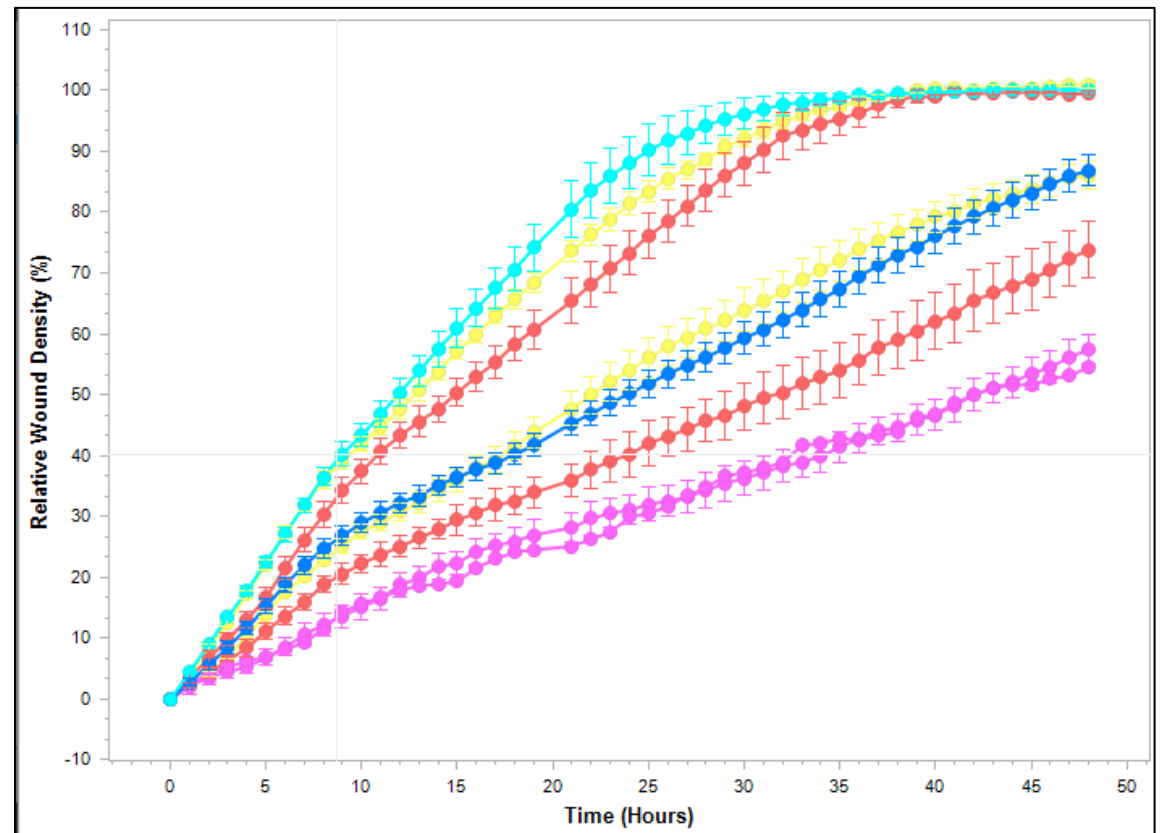
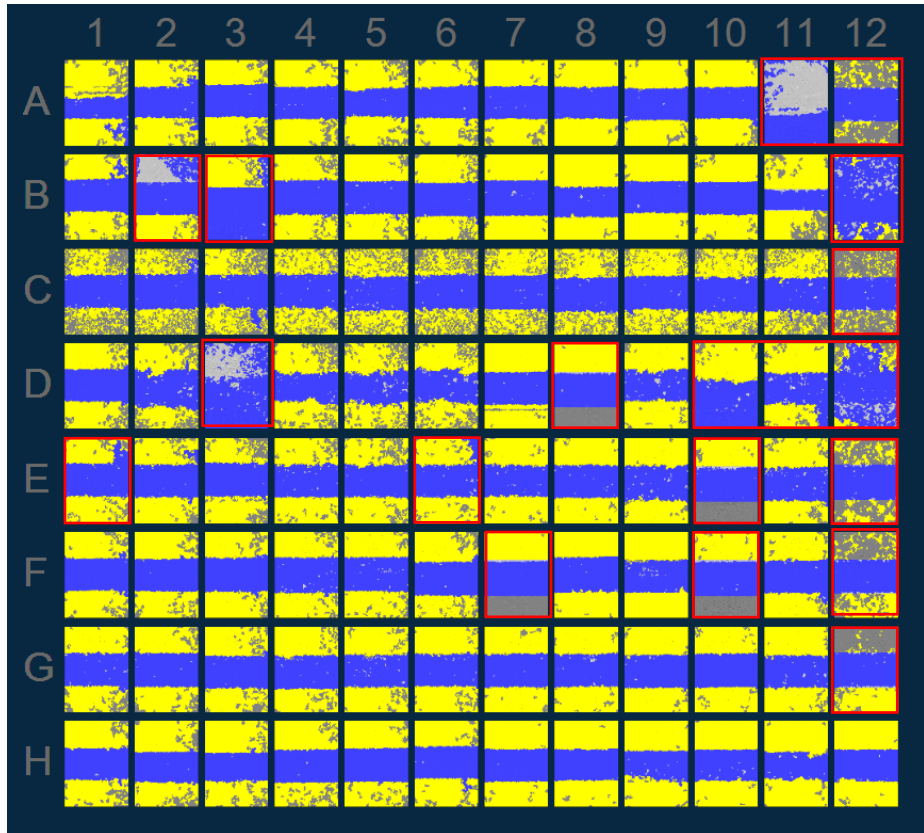


- Continue to “launch analysis”. Select wells and time points to be analyzed.

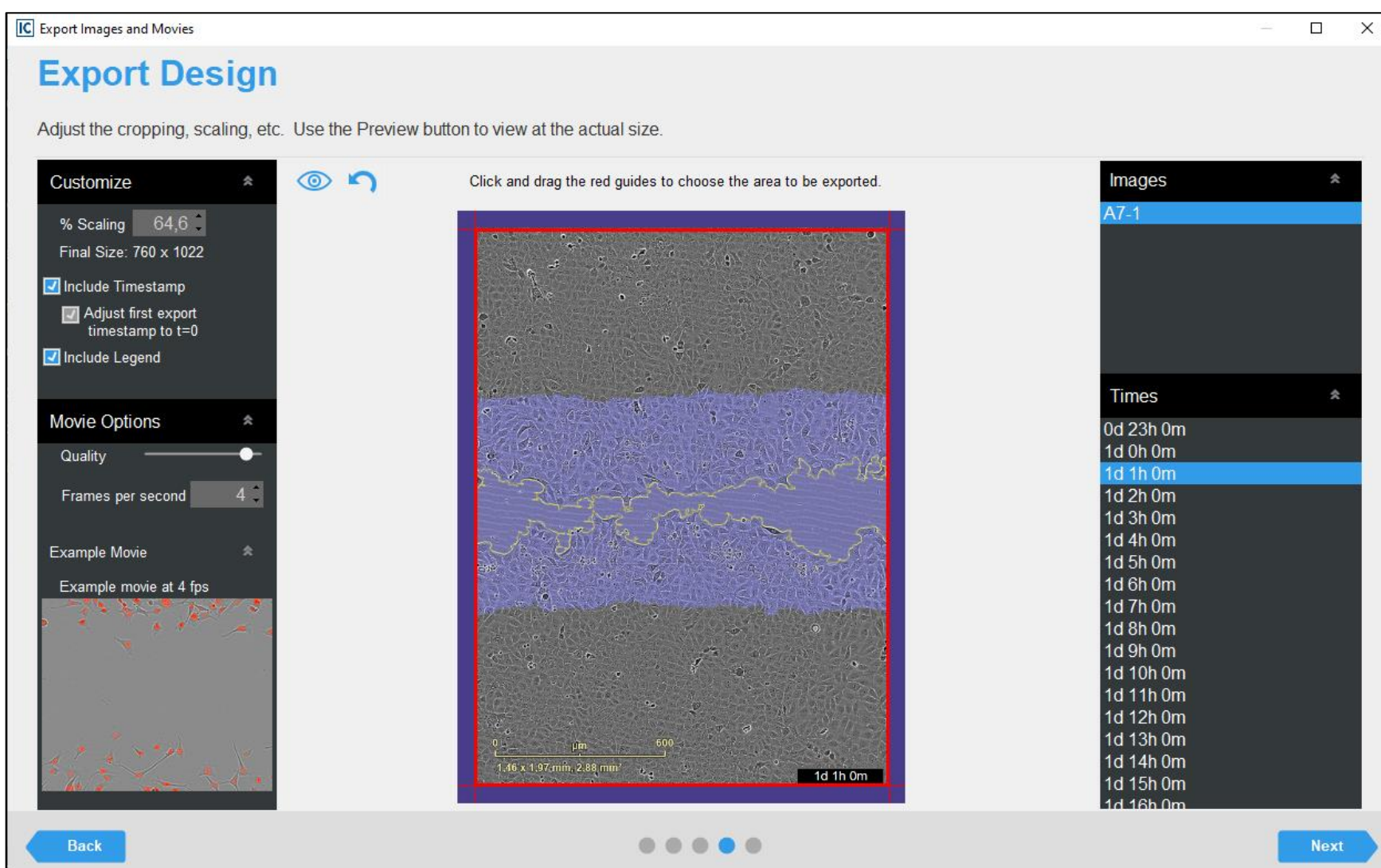
Analyzing a scratch wound continues



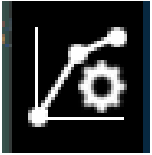
- Open your experiment and click on “**graphics metrics**”.
- Check the relative wound density/wound confluency of the microplate graph to get an idea of how good the masking was. Remove wells which are problematic or consider running a new analysis definition.



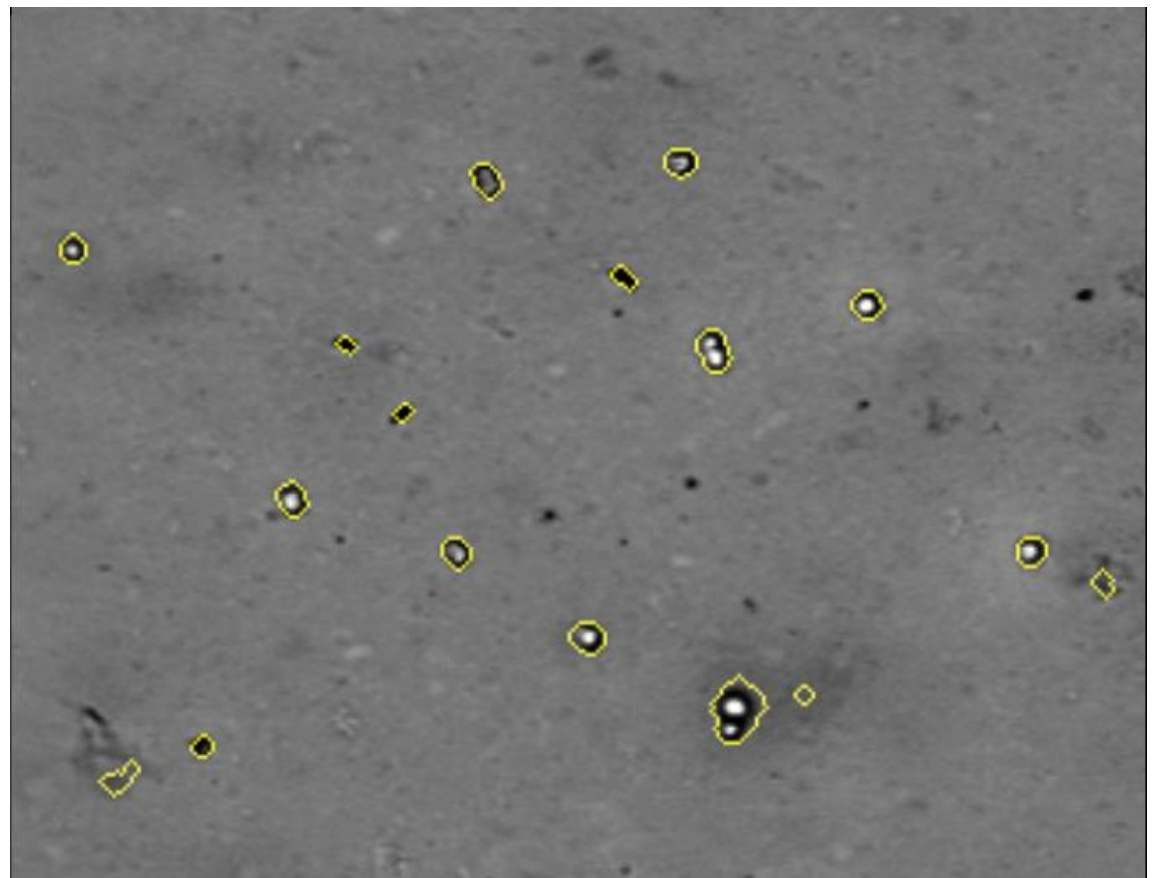
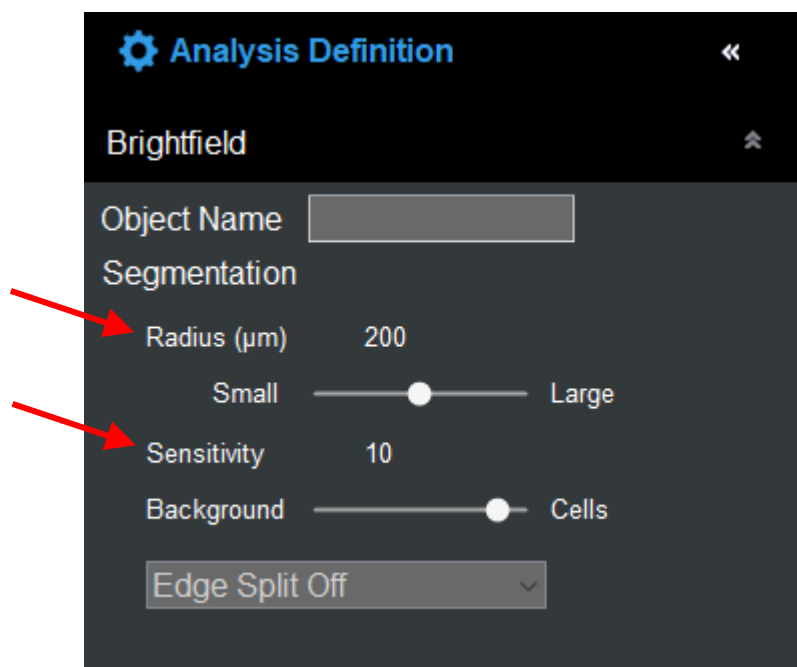
- You can for example export a movie from a representative well and leave the “confluency” and “initial scratch wound” masks on.



Analyzing a spheroid experiment

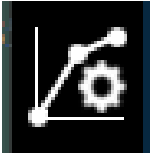


- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select “**create new analysis definition**” and select “**spheroid**”.
- Select the Image channels you want to analyze.
- Select a few representative images in the “image set selection” by clicking 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.

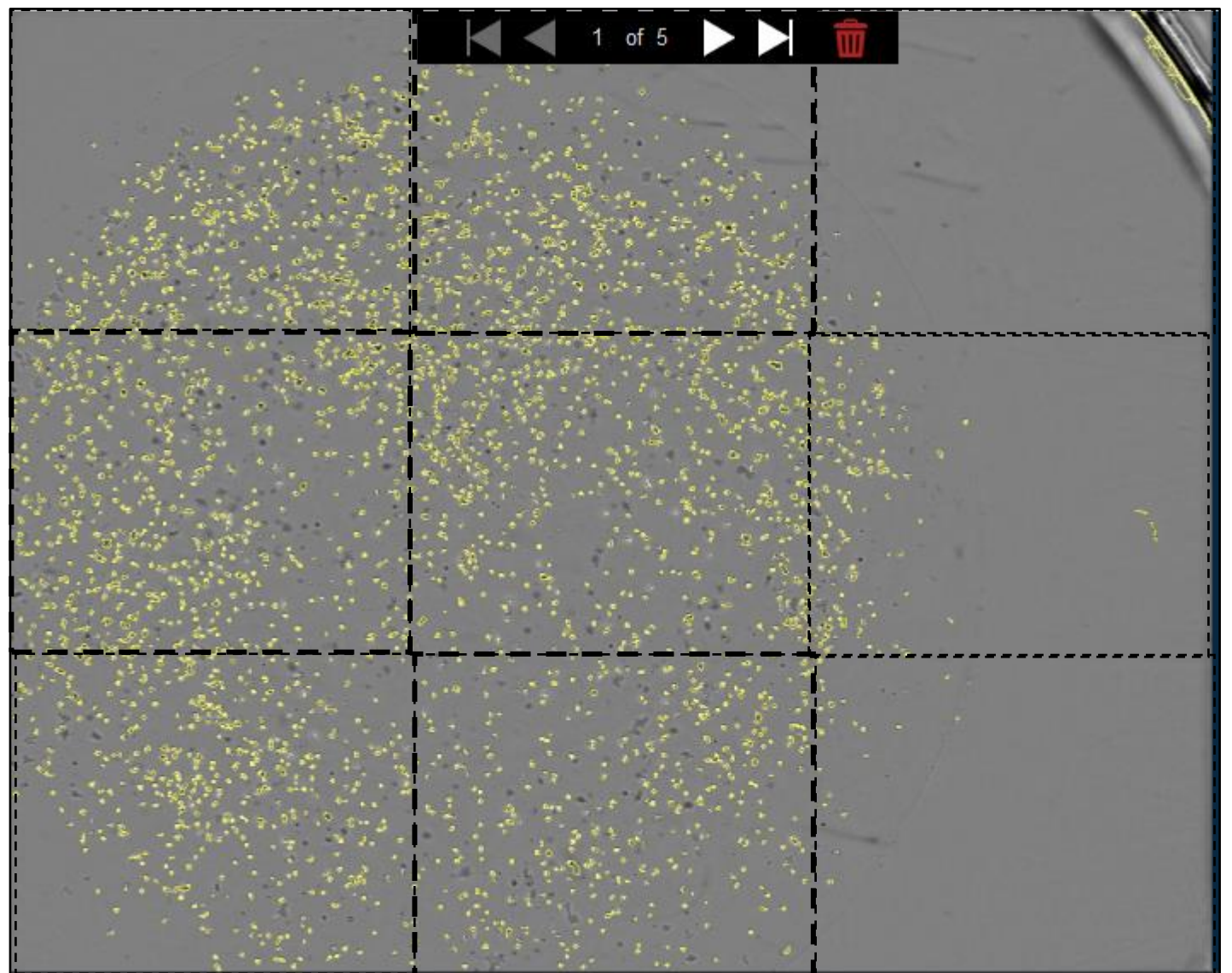
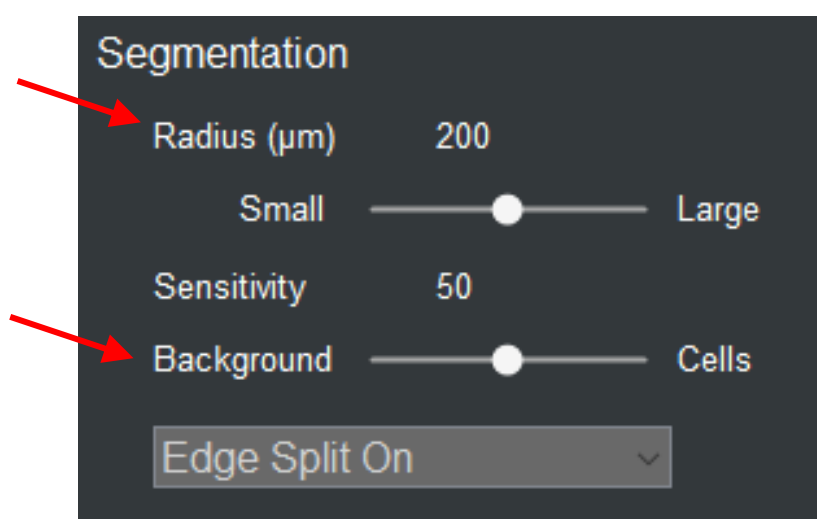


- **Radius** = background remover. Move slider towards “large” to remove more from the background.
- **Sensitivity** = spheroid brightness relative to the background. Move towards “cells” if you want to pick up spheroids which have lower contrast.

Analyzing an organoid experiment



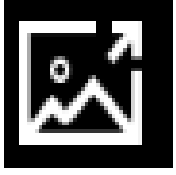
- Open your experiment and launch the analysis. Go through the guided assistant wizard step by step.
- Select “**create new analysis definition**” and select “**organoid**”.
- Select the Image channels you want to analyze.
- Select a few representative images in the “image set selection” by clicking 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).



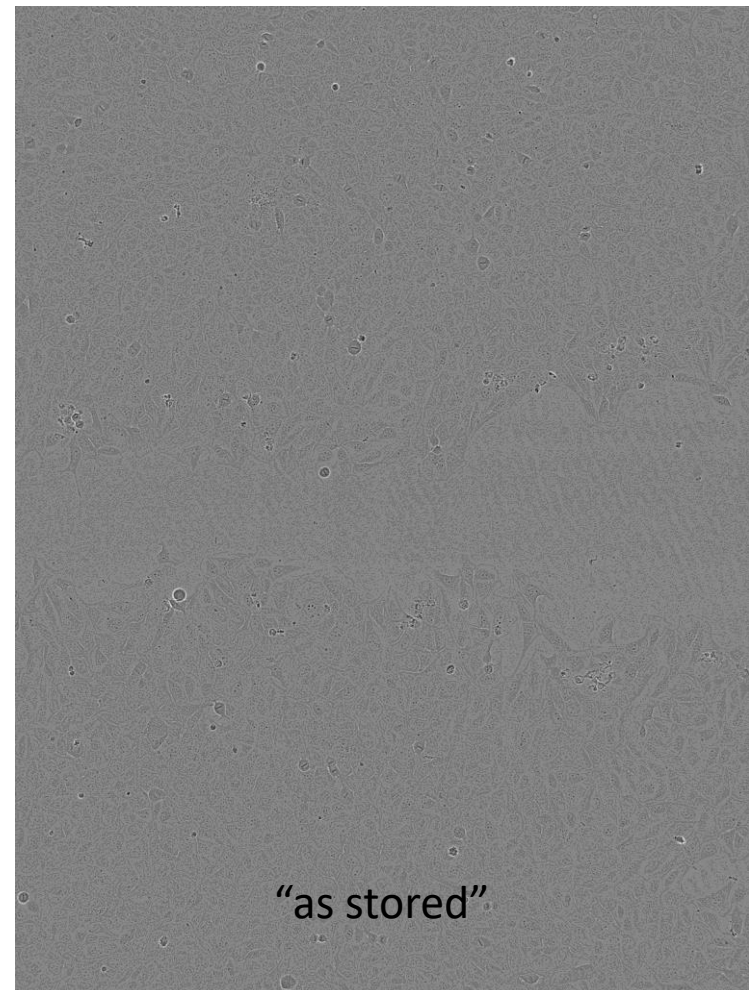
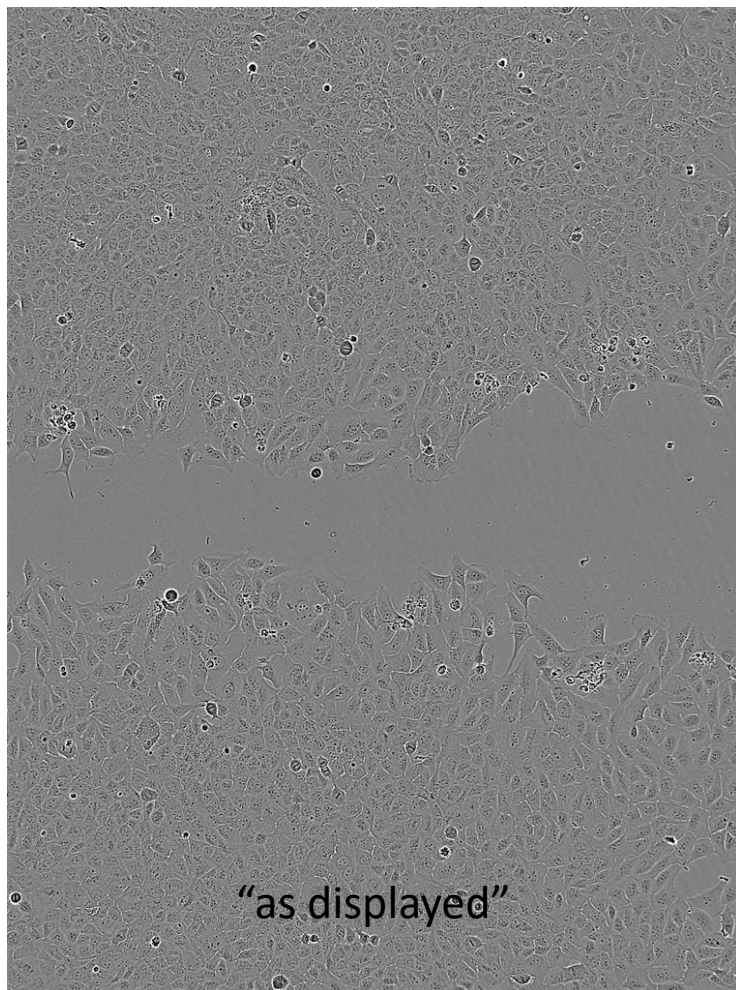
- Making changes to the mask and refreshing the image takes a longer time because your image consists of 9 images (if using 12 well plate). Be patient!

- **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.

Sequence Type and Scans

Select the type of sequence to export (a single movie or a series of individual images) and the set of scans.

Select Sequence Type

- ☐ Single Movie of Images as Displayed
- ☒ Series of Images as Displayed

Select Scans

0d 3h 0m
0d 4h 0m
0d 5h 0m
0d 6h 0m
0d 7h 0m

Customize

% Scaling64,6Final Size: 760 x 1022

☒ Include Timestamp

☒ Adjust first export timestamp to t=0

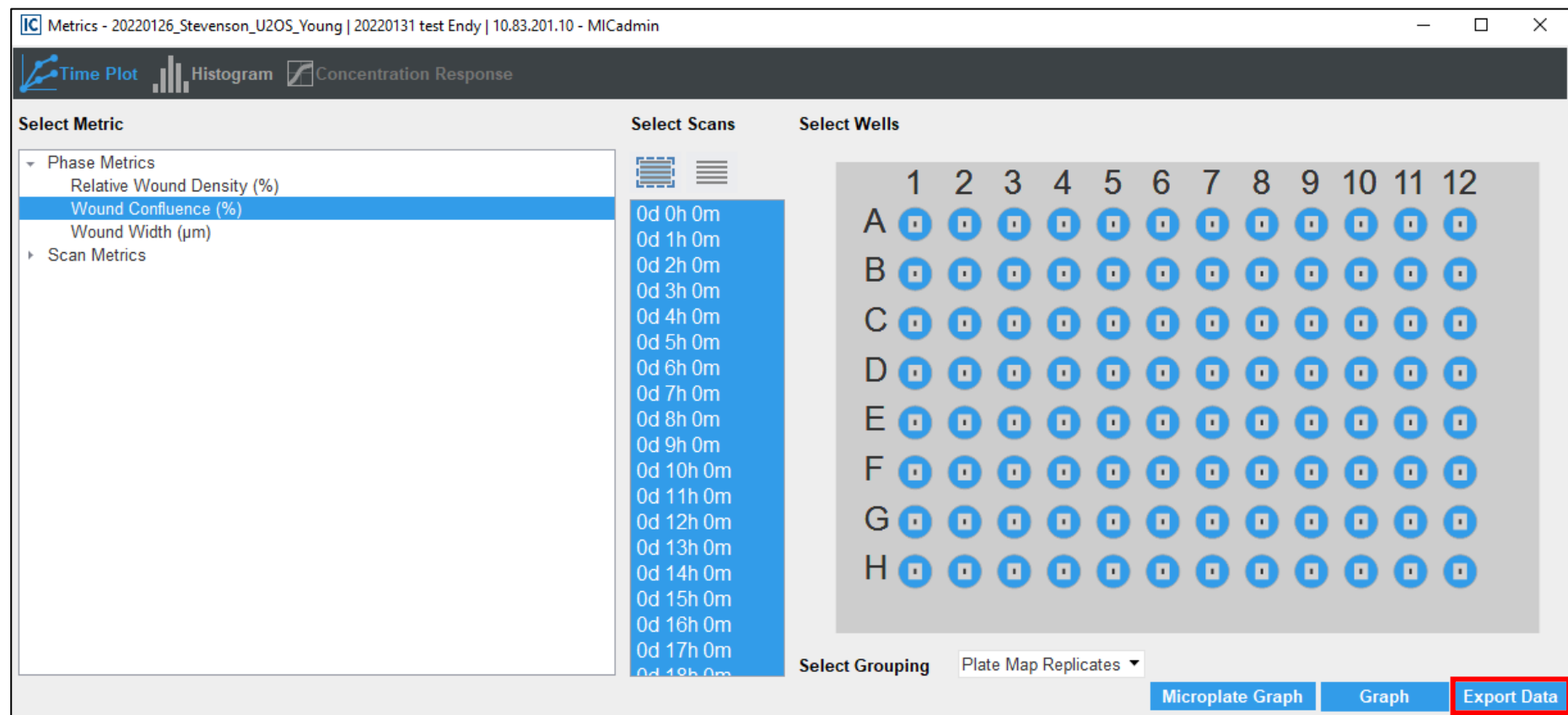
☒ Include Legend

Movie Options

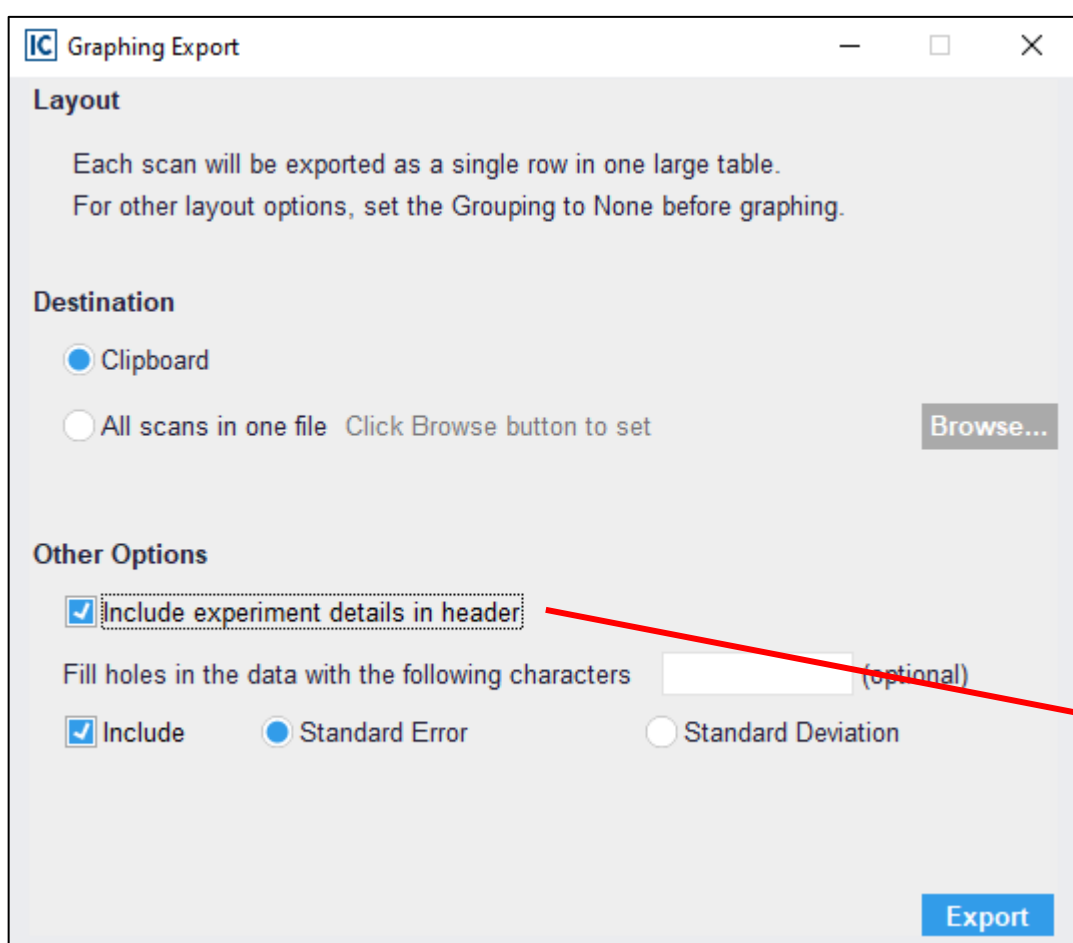
Quality

Frames per second6

How to export data sets



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

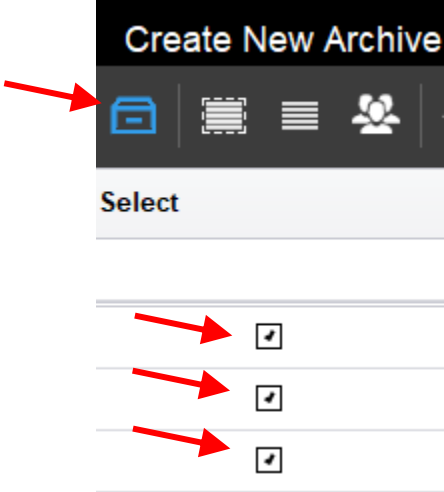


The screenshot shows an Excel spreadsheet with the exported data. The data is organized into columns for 'Date Time Elapsed', 'Cond Med', and various 'SA' (SA100, SA200, SA400) values. The first few rows contain header information like 'Metric: Wound Confluence (%)', 'Cell Type: U2OS WT, U2OS KO', 'Passage: 1', 'Notes', and 'Analysis: 20220131 test Endy'. The main data rows start from row 8, showing time points from 0 to 30 hours and corresponding confluence values for different conditions.

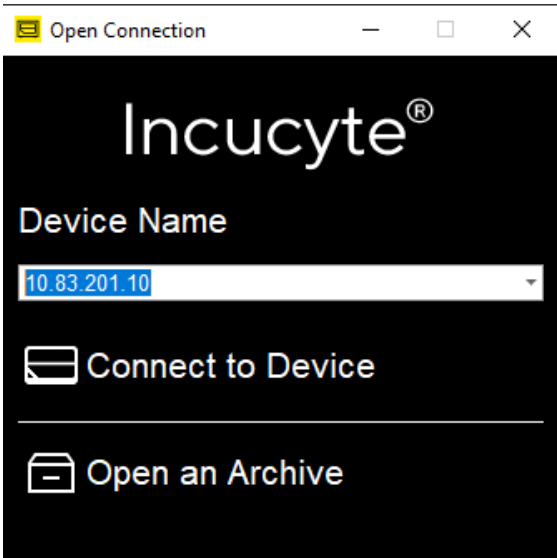
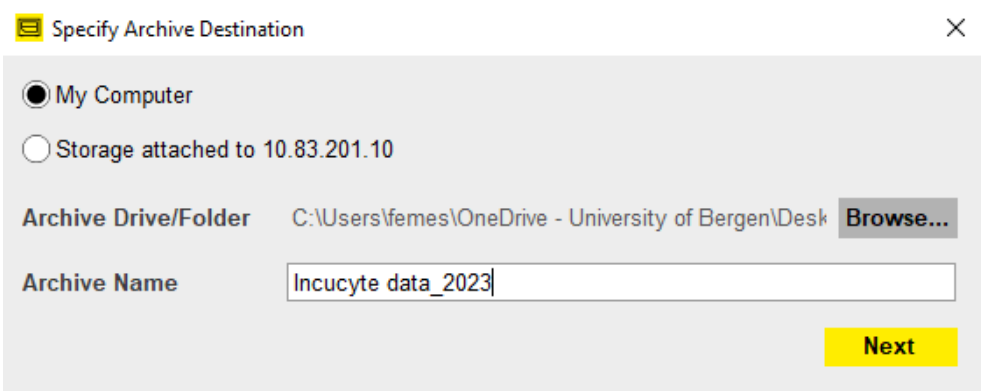
	A	B	C	D	E	F	G	H	I	J
2	Metric: Wound Confluence (%)									
3	Cell Type: U2OS WT, U2OS KO									
4	Passage: 1									
5	Notes:									
6	Analysis: 20220131 test Endy									
8	Date Time Elapsed	Cond Med	Cond Med	SA100 100	SA200 200	SA100 100	SA200 200	SA400 400	SA800 800	
9	26.01.2022	0	0,744569	9,683495	9,415191	0,416214	0,674415	5,473887	0,923702	1,41
10	26.01.2022	1	2,611088	12,66226	9,926576	1,035027	1,675147	5,578084	1,714178	1,9
11	26.01.2022	2	4,385651	15,26606	9,702394	1,070064	2,887306	6,090958	1,706893	2,2
12	26.01.2022	3	6,560804	18,42259	10,09437	1,10397	4,094858	6,337874	1,563869	1,9
13	26.01.2022	4	9,06391	20,94021	10,33558	1,182343	5,670241	7,025234	1,414145	2,21
14	26.01.2022	5	12,02381	28,68268	10,12134	1,296091	8,020673	6,867832	1,618007	2,49
15	26.01.2022	6	15,18148	32,45089	10,40388	1,241659	11,19519	6,766445	1,751505	2,40
16	26.01.2022	7	17,40484	35,40007	11,11364	1,264002	14,51873	6,767357	1,573975	2,15
17	27.01.2022	8	19,678	38,2556	10,65496	1,364955	17,64026	6,785025	1,695219	2,48
18	27.01.2022	9	22,21572	40,88298	11,04378	1,34126	20,59283	6,810476	1,72402	2,12
19	27.01.2022	10	23,81079	43,04874	11,0844	1,447548	23,41864	6,824713	1,960214	2,5
20	27.01.2022	11	25,50501	45,368	10,92521	1,459309	25,78172	7,020018	1,712007	2,60
21	27.01.2022	12	27,65988	47,72744	11,35059	1,431675	28,05044	7,179265	1,759711	2,27
22	27.01.2022	13	28,59215	50,03247	11,59708	1,360775	30,13824	7,085369	1,721251	2,48
23	27.01.2022	14	30,75535	52,31842	11,73691	1,456727	31,8427	7,073599	1,851563	2,51
24	27.01.2022	15	32,51548	54,70159	11,83946	1,335149	33,89228	6,971819	1,648048	2,51
25	27.01.2022	16	34,10647	56,88034	12,42117	1,549744	35,17454	7,012428	1,858084	2,57
26	27.01.2022	17	36,51551	58,7829	12,61277	1,485133	36,68457	7,124371	1,604978	2,3
27	27.01.2022	18	38,46995	60,49199	12,92551	1,477174	38,27282	7,028399	1,687469	2,53
28	27.01.2022	19	40,8326	62,56483	12,94764	1,504114	39,54512	7,33329	1,525325	2,57
29	27.01.2022	21	45,06596	66,23177	18,13304	1,603454	41,36158	7,00377	1,482061	2,22
30	27.01.2022	22	47,35208	67,99209	13,31064	1,544623	42,5323	7,21234	1,64325	2,17
31	27.01.2022	23	49,79553	69,77773	18,42854	1,642643	43,38449	7,116712	1,715378	2,43
32	27.01.2022	24	52,08223	71,60505	18,03847	1,399312	43,9727	7,124641	1,711363	2,38
33	27.01.2022	25	54,00624	73,06618	18,3476	1,504652	45,27998	7,316431	1,813927	2,63
34	27.01.2022	26	55,77993	74,59618	18,75575	1,59144	46,19467	7,210802	1,620966	2,42
35	27.01.2022	27	57,34894	75,93295	18,50669	1,480189	47,20059	6,927742	1,686951	2,30
36	27.01.2022	28	58,79927	77,27345	17,58855	1,525595	48,17598	7,112288	1,68816	2,62
37	27.01.2022	29	60,45702	78,70217	17,62126	1,401702	48,85669	7,332609	1,611813	2,59
38	27.01.2022	30	62,12133	79,6878	13,55154	1,56442	49,60636	6,906094	1,436688	2,44

- 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.

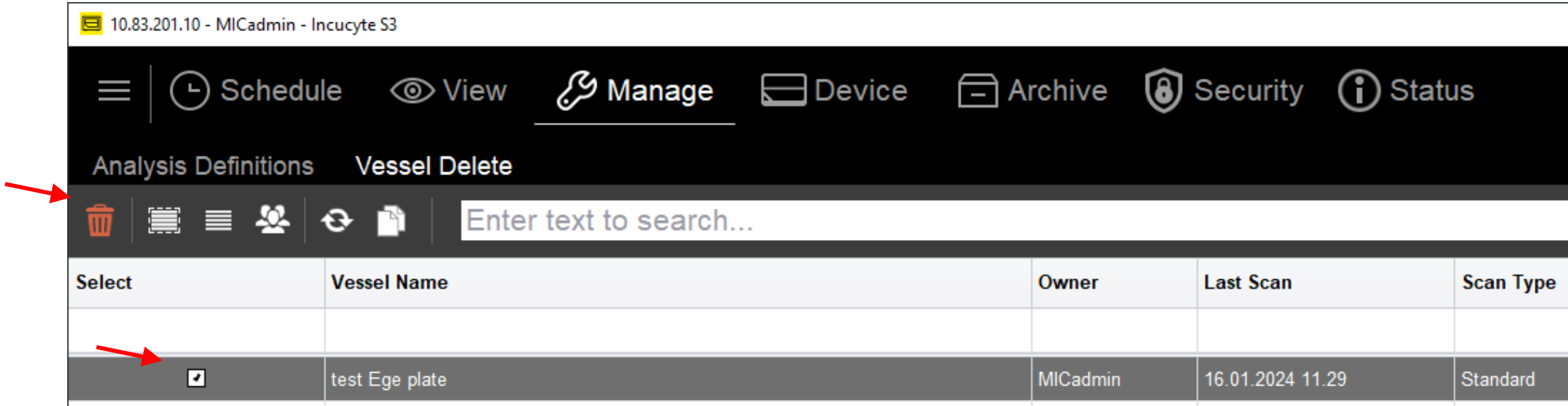
How to archive and delete you data from the remote controller



- 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.

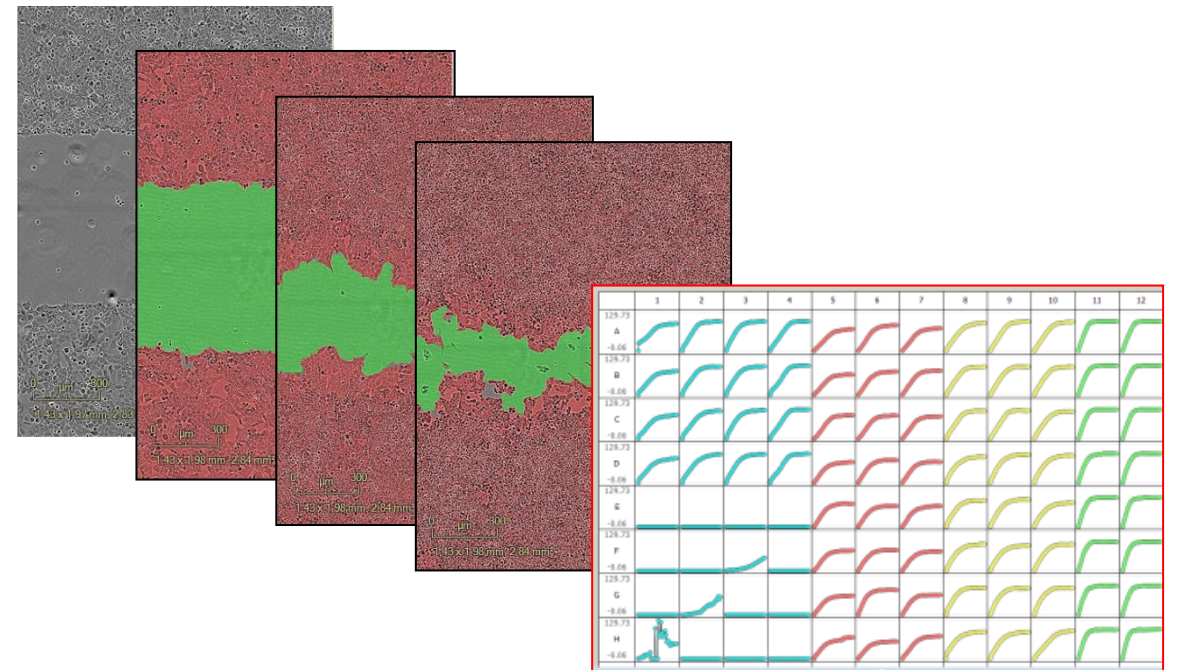


- To delete your data from the remote controller, simple go to “Manage”, filter to find your data, check the box in front of each data set and click on the trash icon.



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.

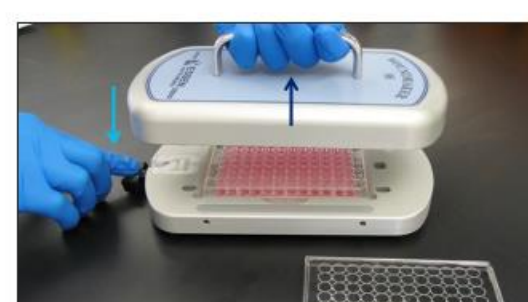


Users must remember the following:

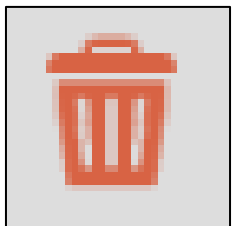
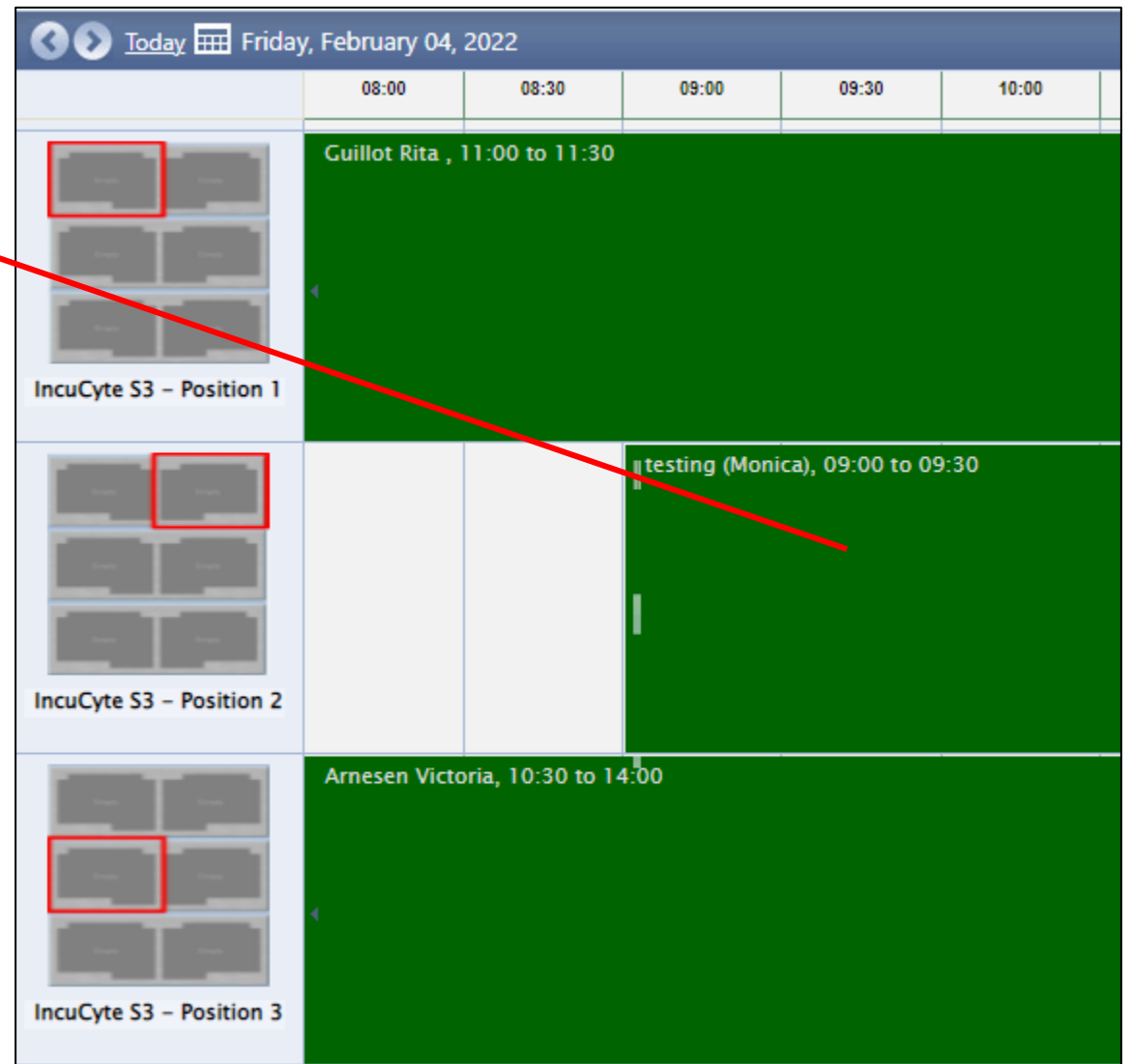
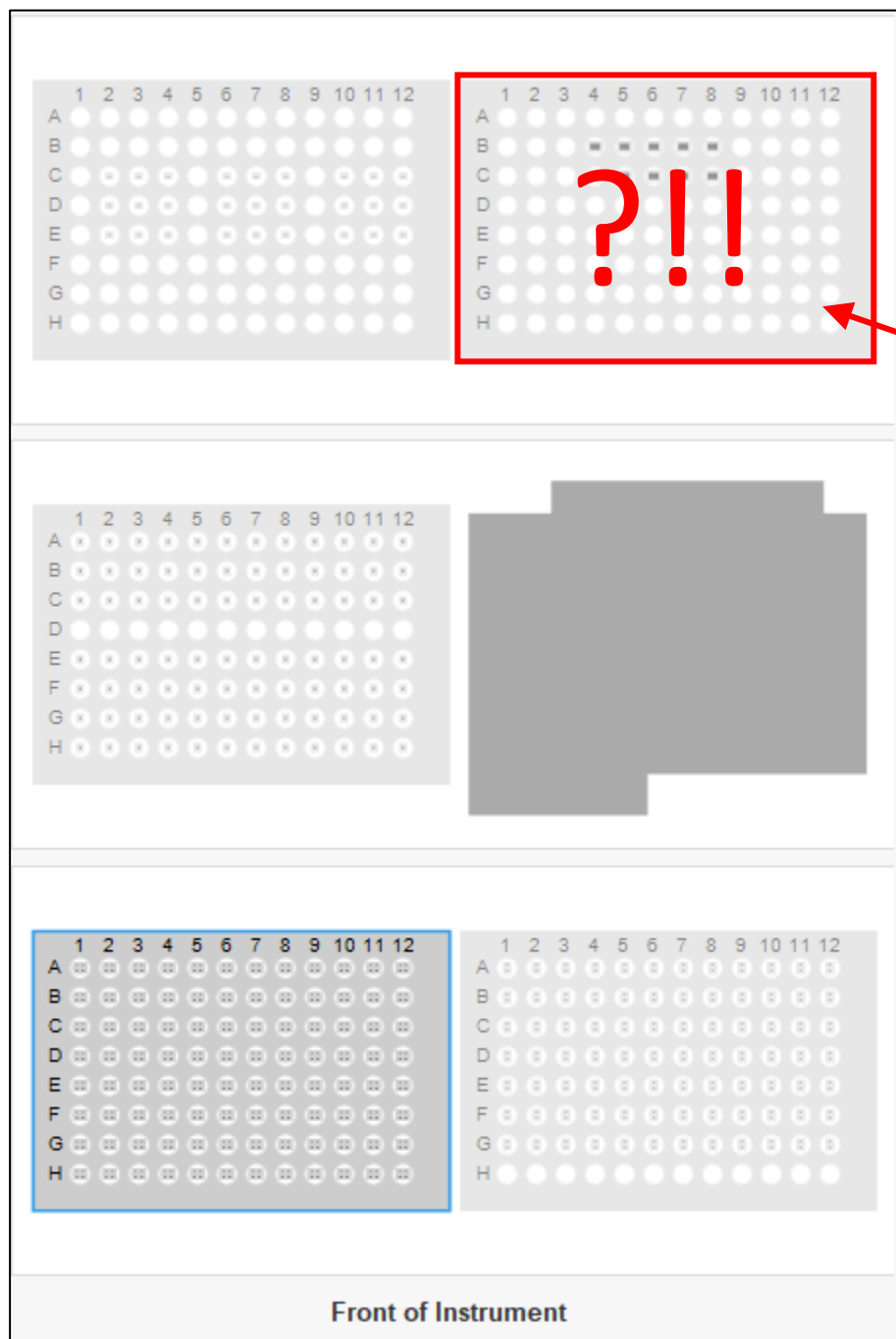
- Preferably user **ImageLock plates** from (Essenbio) from MIC (booking system-supplies).
- Seed cells overnight or 24h in advance to a **90-95% confluency (10-40.000 cells/well)**. If cells are left growing longer there will be a lot of matrix left on the bottom surface disrupting the wound healing. If cells do not attach well, coat the bottom with collagen or poly L-lysine.
- 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 **100 μl** 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 matrix (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

Scan Schedule

Define the scan schedule for this vessel.





00.00 02.00 04.00 06.00 08.00 10.00 12.00 14.00 16.00 18.00

Add Scans

☒ Create

Scan Schedule

Define the scan schedule for this vessel.

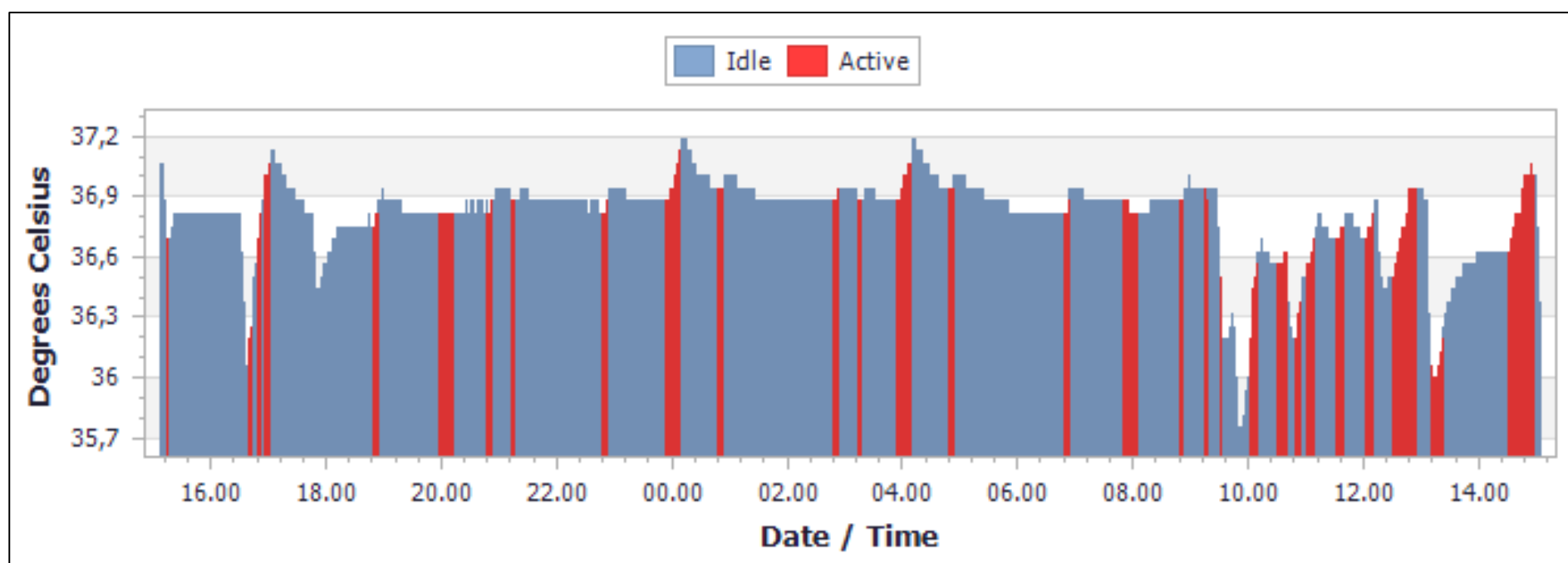


00.00 02.00 04.00 06.00 08.00 10.00 12.00 14.00 16.00 18.00

Add Scans to Schedule

☒ Create new schedule with scans at intervals of

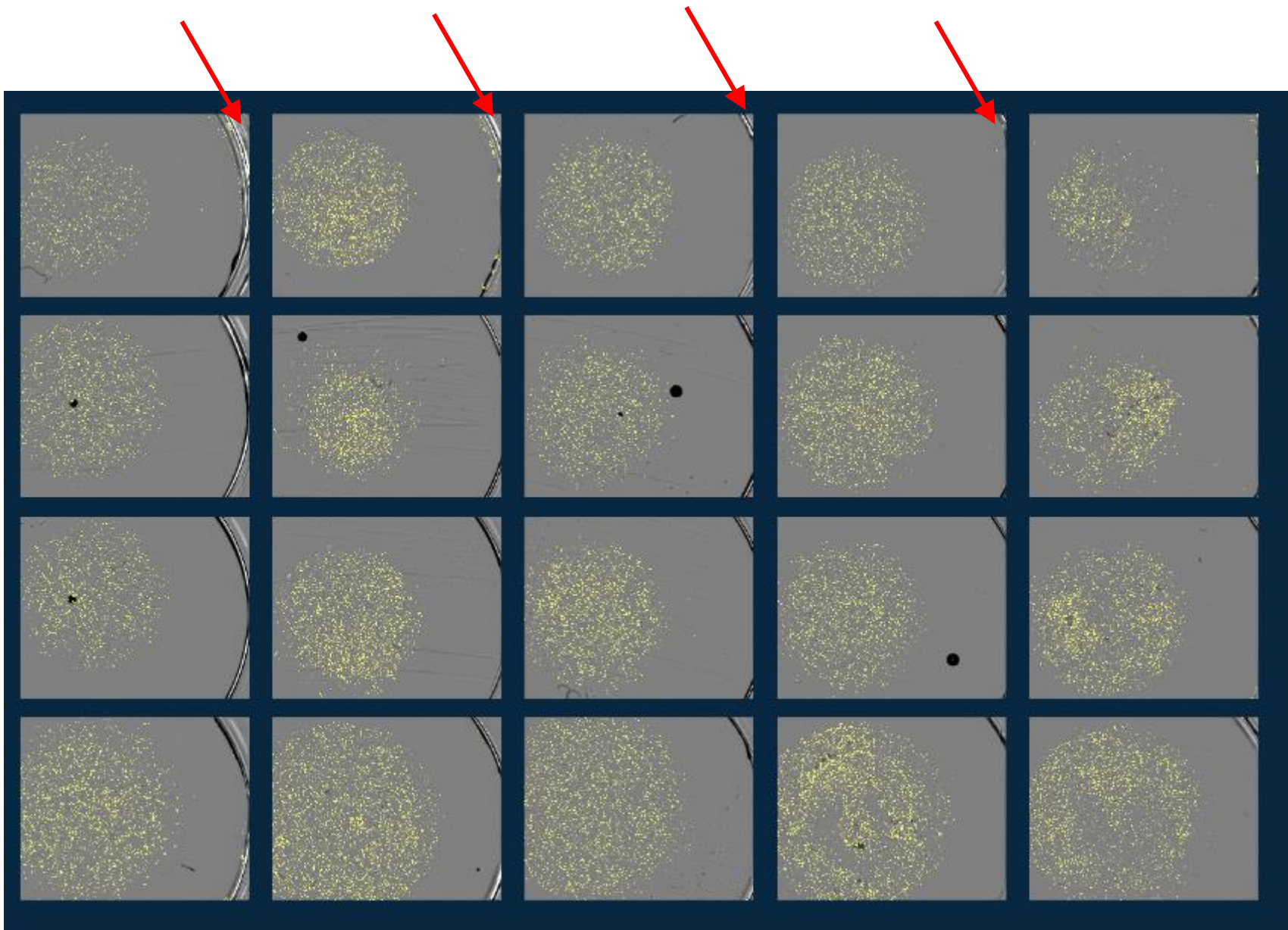
- If there are conflicts in the time schedule, you will not be able to click next and launch the job.
- Use the mouse arrow and drag the schedule till it is not showing conflict anymore.
- Keep in mind that it is optimal to keep some cooling time in between scans in order for the cell environment to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



Trouble shooting – I do not have the recommended plate


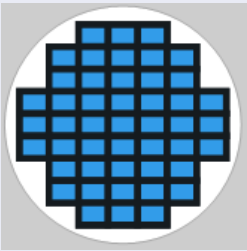

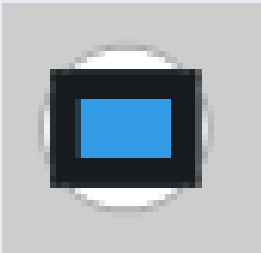
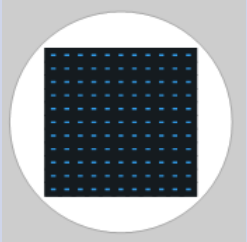

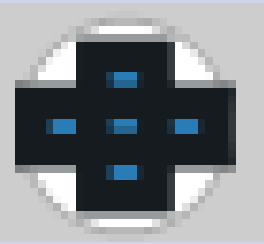


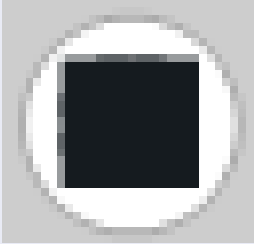
- It is recommended that you seed your cells/spheroids and organoids in the recommended vessels. This is because different brands have different xyz positions for their wells. In some cases we have been able to run an experiment in a “wrong” brand.
- Wound healing plates have special crosses marked on 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>

