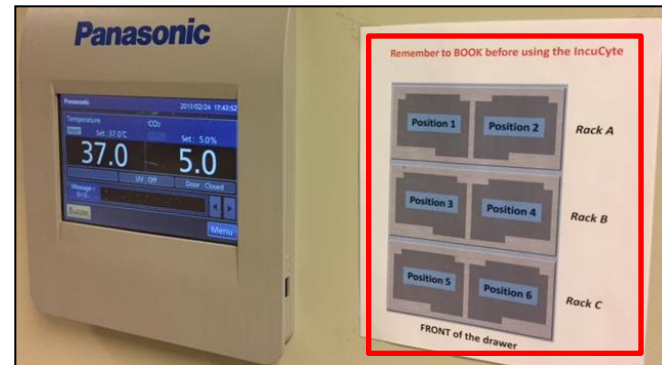
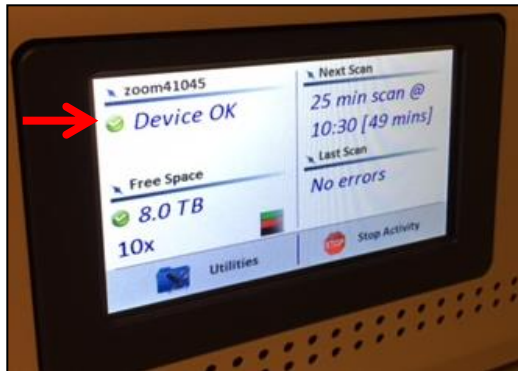


- General rules for using the instrument page 2
- Installing the software on your own pc page 3
- Setting up a timelapse page 4-11
- Creating a plate map page 7
- Setting up a “scan on demand” page 12
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- Deleting your raw data from the IncuCyte controller page 26
- Scratch wound maker page 27-28

General rules for operating the IncuCyte

- **Training is mandatory** for all users, please ask if you are not yet comfortable on the system (MIC or Halala). Once trained, you can get access to the Zoom software and connect remotely from your own pc.
- Every user will have his/her own **user account** (last name_first name initial). Log in using this account and remember to log out afterwards.
- The naming of files has to be in the following format: **yyyymmdd_scientist name_experiment name** (example: 20170209_Spriet_E_experiment name)
- As a general protocol, the IncuCyte will be set up to image **every 2 hours with the 10x objective** with sample holders for 6 plates.
- If you plan another interval, objective or sample holder than the general protocol, you need to talk to MIC personnel in advance and make a note about **special instructions** in the booking system. The objective can only be removed by MIC or Halala!
- **Booking in advance is compulsory**. Make sure you insert samples in the correct position.
- Make sure there are no **error messages** displayed on the control box after you have opened the incubator.

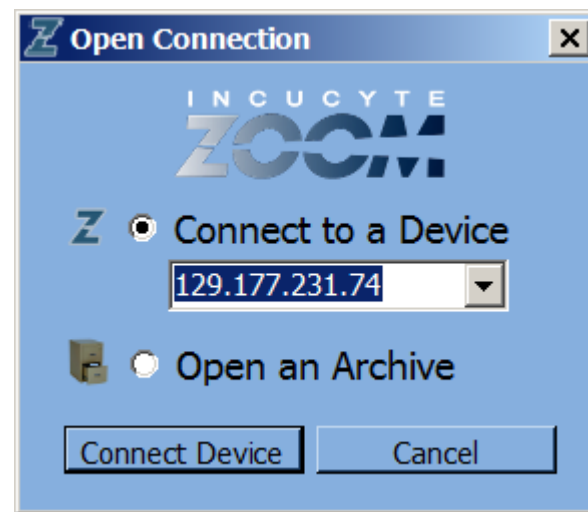


- You need special training for using the **wound-maker** as this involves special cell plates and cleaning procedure.
- It is mandatory to use **shoe protection** (blue plastic covers) and **gloves** when handling the IncuCyte. If you are working at the bench, then make sure you are wearing a **yellow lab coat** and follow general cell culture rules of trash and cleanliness.

Installing the IncuCyte ZOOM software

The IncuCyte ZOOM is unfortunately only compatible with windows (will work on mac if you have the windows interphase). The software is available on the Biomic server «free software» or by showing up directly in the MIC office with a usb-stick. Place the software folder directly onto «program files» and create a shortcut of the IncuCyte.exe file on your desktop. First time you connect to the instrument, put it the IP address: 129.177.231.74

Essen.IncuCyte.pdb	27.11.2016 02:20	PDB File	9 686 KB
Essen.IncuCyte.Win.dll	27.11.2016 02:20	Application extension	6 275 KB
Essen.IncuCyte.Win.pdb	27.11.2016 02:20	PDB File	5 172 KB
Essen.pdb	27.11.2016 02:20	PDB File	1 368 KB
Essen.Win.dll	27.11.2016 02:20	Application extension	517 KB
Essen.Win.pdb	27.11.2016 02:20	PDB File	816 KB
IncuCyte ZOOM User Manual.pdf	18.11.2016 18:22	Adobe Acrobat Doc...	3 848 KB
IncuCyte.exe	27.11.2016 02:20	Application	42 KB
IncuCyte.exe.config	26.01.2015 20:46	XML Configuration File	4 KB
IncuCyte.pdb	27.11.2016 02:20	PDB File	18 KB
IncuCyteArchive	11.2016 02:20	Application	99 KB
IncuCyteArchive	02.2014 17:03	XML Configuration File	1 KB
IncuCyteArchiveJobProcessor.pdb	27.11.2016 02:20	PDB File	18 KB
IncuCytePlateMapEditor.exe	27.11.2016 02:20	Application	30 KB
IncuCytePlateMapEditor.exe.config	27.02.2014 17:03	XML Configuration File	1 KB



The IncuCyte ZOOM software has also been installed on the workstations in the MIC PC room on the 6th floor.

Setting up a timelapse

Scans already in the drawer.
Make sure you select the position you've reserved through the booking system!

Time scale and indication of how long the schedules scans will actually scan. Blue line indicates cooling time. Do not delete by right-clicking here!

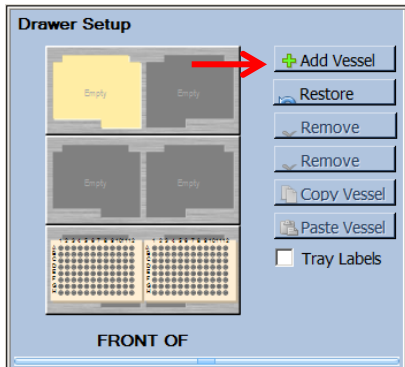
The screenshot shows the IncuCyte ZOOM software interface. The top status bar displays the IP address 129.177.231.74 (MIC) and the next scan time: 43 minutes (10:30). The main interface is divided into several sections:

- Task List (Left Panel):** Contains buttons for View Scans, Search, Schedule Scans (highlighted with a red arrow), Archives, and Administrator.
- Drawer Setup (Center-Left):** Shows a grid of 24 positions. A red box highlights the top two rows, which are currently empty. Below the grid is the label "FRONT OF".
- Scan Setup (Center-Right):** Includes sections for Channel Selection (Phase, Green, Red), Scan Mode (Sca, Wide Mod), Spectral Unmixing (0.00% removed), and Analysis Job Setup (Job Type, Processing Definition, Name, Job Version, Notes).
- Vessel Overview (Bottom-Left):** Displays "No Vessel Selected".
- Device Status (Bottom-Left):** Shows the current time as 09:47, next scan at 10:30, set by MIC, status as Ready To Scan, objective as Nikon 10x, filter as Dual Color Model 4459, free space as 8 TB (98%), and temperature as 37.3 °C.

At the bottom of the interface, there are buttons for "Edit Scan Patterns", "Vessel Scheduling", "Apply", and "Reloa".

By default the system is set up for timelapse acquiring images every 2h with the 10x objective. Any other setting must be clarified with MIC and indicated in the booking system.

Setting up a timelapse...continues



Click on «add vessel» and find the correct plate.
Different plates have different well location in xy, so find the correct one. Not all plates are compatible.

Sorting for manufacturer or wells is very useful.

The 'Add Vessel' dialog box features a search bar and a table of vessel options. Red arrows point to the 'Manufacturer' and 'Wells' columns. Below the table is a 'Microplates' section with a preview of a 96-well plate layout. At the bottom are buttons for 'Supported', 'Copy Grid', 'OK', and 'Cancel'.

Manufacturer	Ves...	Wells	Area	Catalog #s	Vessel Name	Tray	Scan Types
Aurora	Plate	96	N/A	92451, 92461, 92651, 92661, 92751, 92761	96-well Aurora IQ	Micropl...	Standard
Aurora	Plate	384	N/A	31221, 31241, 31421, 31441, 31521, 31541, 31621, 31641, 31821, 31841, 31921, 31941, 32021, 32041, 32221, 32241, 32321, 32341	384-well Aurora IQ-EB	Micropl...	Standard
Aurora	Plate	384	N/A	32451, 32651, 32751	384-well Aurora IQ-EB Ultra Low Base	Micropl...	Standard
BD (Discontinued)	Plate	6	N/A	Discontinued: see "Corning (Formerly BD)" vessel	6-well BD Falcon	Micropl...	Standard
BD (Discontinued)	Plate	12	N/A	Discontinued: see "Corning (Formerly BD)" vessel	12-well BD Falcon	Micropl...	Standard
BD (Discontinued)	Plate	24	N/A	Discontinued: see "Corning (Formerly BD)" vessel	24-well BD Falcon	Micropl...	Standard
BD (Discontinued)	Plate	48	N/A	Discontinued: see "Corning (Formerly BD)" vessel	48-well BD Falcon	Micropl...	Standard
BD (Discontinued)	Plate	96	N/A	Discontinued: see "Corning (Formerly BD)" vessel	96-well BD Falcon Blk/Clr Imaging	Micropl...	Standard
BD (Discontinued)	Plate	96	N/A	Discontinued: see "Corning (Formerly BD)" vessel	96-well BD Falcon Optilux	Micropl...	Standard
BD (Discontinued)	Plate	96	N/A	Discontinued: see "Corning (Formerly BD)" vessel	96-well BD Falcon (All Clr)	Micropl...	Standard
BD (Discontinued)	Plate	384	N/A	Discontinued: see "Corning (Formerly BD)" vessel	384-well BD Falcon B (Blk/Wht)	Micropl...	Standard
BD (Discontinued)	Plate	384	N/A	Discontinued: see "Corning (Formerly BD)" vessel	384-well BD Falcon A (Blk/Wht)	Micropl...	Standard
BD (Discontinued)	Plate	384	N/A	Discontinued: see "Corning (Formerly BD)" vessel	384-well BD Falcon A (All Clr)	Micropl...	Standard
BD (Discontinued)	Plate	384	N/A	Discontinued: see "Corning (Formerly BD)" vessel	384-well BD Falcon B (All Clr)	Micropl...	Standard

Setting up a timelapse

Open up Properties and give your experiment a label:
yyymmdd_full last name_first name initial_experiment
It is useful (for later sorting) to also fill in the cell line.

Task List

- View Scans
- Search
- Schedule Scans
- Archives
- Administer

Device Status

10:06
Next Scan: 10:30
Set By: MIC
Set At: 22.02.2017 12:26
Status: Ready To Scan
Objective: Nikon 10x
Filter: Dual Color Model 4459
Free Space (% free):
Disk - 8 TB (98%)
Database - 10 GB (>99%)
Temperature: 37,3 °C

24-Hour Repeating

Drawer Setup

- Add Vessel
- Restore
- Remove
- Remove
- Copy Vessel
- Paste Vessel
- Tray Labels

FRONT OF

Vessel Overview

96-well Essen ImageLock (13 min)

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

Scan Setup Properties

Label:

Cell:

Passag: 1

Plate Map:

Note:


Apply Relo

Creating a plate map

Scan Setup | **Properties**

Label:

Cell:

Passag: 1  Plate Map

Note:

The plate map can be designed before, during or after an experiment and is your custom-design map helping you in having the overview of the experiment. You can select compounds, cells types and growth conditions.

Plate Map Editor - IncuCyte Plate Map

- Click and drag to select areas.
- Click to toggle individual well selection.
- Use the tabs below to populate the selected wells.
- Click and drag to populate the selected wells.

Regions

Compounds | Cells | Growth Conditions

Compounds used in this plate:

- DMSO
- BGB324
- Warfarin
- Erlotinib

New Edit Delete

Select all wells that contain this

Add Compound to

Plate Map Notes

All	1	2	3	4	5	6	7	8	9	10	11	12
A			DMSO 0.2% ER3 (1) 20K cells / well		DMSO 0.2% ER10 (1) 20K cells / well							
B	DMSO 0.2% HCC827wt (1) 20K cells / well						DMSO 0.2% SUM149 (1) 20K cells / well EtOH	BGB324 1.6 µM SUM149 (1) 20K cells / well EtOH	BGB324 0.8 µM SUM149 (1) 20K cells / well EtOH	Warfarin 2 µM SUM149 (1) 20K cells / well EtOH	Warfarin 1 µM SUM149 (1) 20K cells / well EtOH	DMSO 0.2% SUM149 (1) 20K cells / well EtOH
C			Erlotinib 1 µM ER3 (1) 20K cells / well		Erlotinib 1 µM ER10 (1) 20K cells / well							
D												
E			BGB324 0.8 µM ER3 (1) 20K cells / well		BGB324 0.8 µM ER10 (1) 20K cells / well							
F	BGB324 0.8 µM HCC827wt (1) 20K cells / well						DMSO 0.2% SUM149 (1) 20K cells / well VitK	BGB324 1.6 µM SUM149 (1) 20K cells / well VitK	BGB324 0.8 µM SUM149 (1) 20K cells / well VitK	Warfarin 2 µM SUM149 (1) 20K cells / well VitK	Warfarin 1 µM SUM149 (1) 20K cells / well VitK	DMSO 0.2% SUM149 (1) 20K cells / well VitK
G			Erlotinib 1 µM BGB324 0.8 µM ER3 (1) 20K cells / well		Erlotinib 1 µM BGB324 0.8 µM ER10 (1) 20K cells / well							
H												

Export As Image

OK Cancel

Defining the scan pattern

The screenshot shows the 'Scan Pattern Manager for 129.177.231.74' window. On the left, there are configuration fields for Objective (10x), Tray (Microplates), Vessel (96-well Essen ImageLock), and Scan Type (Scratch Wound). Below these is a list of 'Available Patterns' including 'scratch_1'. A red arrow points to the 'Scratch Wound' dropdown menu. The main area displays a 96-well plate grid with columns 1-12 and rows A-H. A green callout box explains the 'Scratch Wound' scan type. At the bottom, there are buttons for 'Rename', 'New', and 'Delete', and a 'Pattern Editing' section with 'Images per well' set to 2, 'Save', 'Clear Pattern', and 'Cancel' buttons. A red arrow points to the 'New' button, and another points to the 'Save' button.

Objective: 10x Current

Tray: Microplates

Vessel: 96-well Essen ImageLock

Scan Type: Scratch Wound Wide Mod

Available Patterns:

- Halala 20170207
- Sample Pattern
- Scratch
- scratch_1**
- scratch 2
- Scratch wound Group 2
- Thomas
- wounding_AM_BGB

You can either select an available pattern from the drop down menu «scan type» or create a new one (with your name on it) and select the specific wells you need imaged. Make sure you are not scanning undesirable wells to save time. In this scan type «scratch wound» you can also select for 1-2 images per well. Click save and close.

* Image size and location based on Scan Type 'Scratch Wound'.

Pattern Editing

Edit the pattern by clicking on its picture. Hold down the Alt Key to remove wells. Click and drag to select and deselect groups of wells.

Images per well: 2

Save Clear Pattern Cancel

Rename **New** Delete

Supported Close

Setting up a timelapse

Retrieve your scan pattern from the scan list

The screenshot displays the IncuCyte ZOOM software interface. At the top, a status bar shows the IP address 129.177.231.74 (MIC) and a notification for the next scan: "Next Scan: 11 minutes (10:30)". A red box highlights this notification, with a red arrow pointing to a text box above it that says "Retrieve your scan pattern from the scan list".

The main interface is divided into several sections:

- Task List:** A sidebar on the left with options: View Scans, Search, Schedule Scans (highlighted), Archives, and Administrator.
- Drawer Setup:** A central area showing a grid of wells. A red arrow points from the "Next Scan" notification to this area. Below the grid, it says "FRONT OF".
- Scan Setup | Properties:** A panel on the right with the following settings:
 - Channel Selection:** Phase, Green, Red. Acquisition Time: (ms).
 - Scan Mode:** Sca: Scratch Wound, Wide Moc. Scan: scratch 1.
 - Spectral Unmixing:** 0,00 % removed (twice).
 - Analysis Job Setup:** Job Type: None, Processing: (dropdown), Definition: (dropdown), Name: (text field), Job Version: (text field), Notes: (text area).
- Device Status:** A bottom-left panel showing: 10:18, Next Scan: 10:30, Set By: MIC, Set At: 22.02.2017 12:26, Status: Ready To Scan, Objective: Nikon 10x, Filter: Dual Color Model 4459, Free Space (% free): Disk - 8 TB (98%), Database - 10 GB (>99%), Temperature: 37,3 °C.
- Bottom Panel:** Includes "Edit Scan Patterns", "Vessel Scheduling" (highlighted with a red arrow), "Apply", and "Reloa" buttons.

Next, schedule the scan and make sure you have time to launch it and insert your vessel before the next scan!

Setting up a timelapse – vessel scheduling

The schedule looks fine with two cooling periods. **Click apply in good time before the next scan.**

The screenshot displays the IncuCyte ZOOM software interface. At the top, the status bar shows the IP address 129.177.231.74 (MIC) and the next scan time: 6 minutes (10:30). A red arrow points to this status bar. The main interface is divided into several sections:

- Task List:** Includes View Scans, Search, Schedule Scans (highlighted), Archives, and Administer.
- Drawer Setup:** Shows a grid of 96 wells with buttons for Add Vessel, Restore, Remove, Copy Vessel, Paste Vessel, and Tray Labels. A red arrow points to the 'Remove' button.
- Scan Setup | Properties:** Contains settings for Channel Selection (Phase, Green, Red), Scan Mode (Scratch Wound, Wide Mod), Scan (scratch 1), Spectral Unmixing (0.00% removed), and Analysis Job Setup (Job Type: Scratch Wound, Processing Definition: 20170220_AnjaMal_wound2, Name: 20170223_Spriet_testwound).
- Vessel Overview:** Shows a 96-well Essen ImageLock (12 min) grid with columns 1-12 and rows A-H.
- Device Status:** Shows the current time as 10:24, next scan at 10:30, and various system metrics like free space and temperature.

At the bottom right, a red arrow points to the 'Apply' button. The interface also features a '24-Hour Repeating' vertical label and a 'Scan on Demand (unavailable)' label.

Removing plates: click on your plate and select «remove» and click on APPLY.
Open the drawer in between scans and remove your plate.

Scan on demand

You can run a one-time scan over your plan through the "scan on demand".

The screenshot displays the IncuCyte ZOOM software interface. The main window shows a 24-hour repeating scan schedule on a timeline from 00 to 00. A red arrow points to a yellow bar on the timeline at approximately 13:00. A red box highlights the configuration window for the 'Scan on Demand' scan, which includes the following settings:

- Tray Type: Microplates
- Tray Position: Front
- Cutout Position: Left
- Vessel Type: 96-well Nunc
- Scan Type: Standard
- Scan Pattern: Sissel
- Unique ID: 2017y6m26d12h39

Below these settings, the 'Channel Selection' section is visible, with red arrows pointing to the 'Phase' and 'Green' checkboxes, both of which are checked. The 'Acquisition Time' for 'Green' is set to 400 (ms) and for 'Red' to 800 (ms). The 'Label' is 'test Sissel' and the 'Passage' is '1'. A red box highlights the 'Scan on Demand' button in the bottom left corner of the configuration window. A red arrow points to the 'Apply' button in the bottom right corner of the main software window.

Carefully select the correct position and vessel type. Retrieve the scan pattern and define a name to the scan. Make sure the correct channels are selected and press apply. The IncuCyte will start a one-time scan immediately and between regular 24-hour repeating scans.

Analysing scratch wound assay

Inside «archives» you will see all the data acquired on the IncuCyte. Search for your sample using your surname under «label». Double-click to open the image set.

The screenshot shows the IncuCyte ZOOM software interface. The top bar displays the IP address 129.177.231.74 (essenbio) and the status Scanning (4%). The left sidebar contains navigation options: Task List, View Scans, Search, Schedule Scans, Archives (highlighted with a red arrow), and Administer. The main window shows a table of scan data with columns: Select, Start, User, Label, Cell Type, Scan Type, Color1, Color2, Phase, and Objective. The table lists various scans, including a highlighted row for a scratch wound assay performed by 'lie_m' on 19.05.2017. The bottom status bar shows the current time as 15:02 and provides details about the next scan, including the set by user (H-Aksnes), set at time (08.06.2017 14:56), objective (Nikon 10x), filter (Dual Color Model 4459), and free space information (Disk - 7,3 TB (89%), Database - 9,8 GB (98%), Temperature: 37,1 °C). A 'Device Status' section is also visible in the bottom left corner.

Select	Start	User	Label	Cell Type	Scan Type	Color1	Color2	Phase	Objective
<input type="checkbox"/>	08.06.2017 13:00	RoyCh...	20170608_Controls_with&withoutvirus_...	u87	Standard	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	06.06.2017 17:00	dmello_s	20170606_dmello_s_spheroid trial	Number	Spheroid	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	02.06.2017 17:00	nilsen_m			Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	02.06.2017 17:00	nilsen_m			Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	02.06.2017 17:00	nilsen_m			Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	02.06.2017 17:00	nilsen_m			Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	02.06.2017 13:00	RoyCh...	170602_controls_Romi	u87	Standard	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	23.05.2017 15:00	H-Aks...	170523_H-Aksnes_Wound	CTRL, NAA80 KO1, NAA80 K...	Scratch Wound	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	21.05.2017 19:00	nilsen_m	NHA wt dsred	NHA	Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input checked="" type="checkbox"/>	19.05.2017 15:00	lie_m	20170519_Lie_M_Scratch wound assay...	HCC827wt, ER3, ER10, SUM...	Scratch Wound	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	18.05.2017 15:39	nilsen_m	KD	NHA	Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	18.05.2017 15:39	nilsen_m	wt	NHA	Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	16.05.2017 15:39	H-Aks...	Henriette A Prolif Pos 2	WT, KO1, KO2	Standard	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	16.05.2017 15:00	H-Aks...	Henriette A Chemotaxis2 20min Pos 1	WT, KO1, KO2	Chemotaxis (T...	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	15.05.2017 11:00	zhang_x	H1	H1	Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	15.05.2017 11:00	zhang_x		U87	Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	10.05.2017 19:00	klink_b	20170510_klink_b_FRBA_P3-green	FRBA with P3 Xeno or P3 NBM	Standard	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	10.05.2017 18:48	klink_b			Standard	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	10x
<input type="checkbox"/>	10.05.2017 18:44	klink_b			Standard	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	10x
<input type="checkbox"/>	10.05.2017 18:39	klink_b			Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	10.05.2017 18:13	klink_b			Standard	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	10.05.2017 18:02	klink_b			Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	09.05.2017 19:00	klink_b	20170509_klink_b_co-culture-FRBA-NB...	P3, BG10	Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	09.05.2017 17:17	klink_b			Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	09.05.2017 17:13	klink_b	Test_NBM		Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	09.05.2017 15:00	Hoang_T	Tuyen_scan090517	H1-DL2	Standard	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	09.05.2017 12:00	klink_b	20170509_klink_b_FRBA-with-P3Xeno...		Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x

Analysing scratch wound assay

Analysis Job Utilities

- Create or Add to Image Collection...
- New Processing Definition...
- Launch New Analysis Job... (i)
- Edit a Processing Definition...
- Edit an Image Collection...

Start by selecting some representative images from your data set (6-10). Define «new» and click on add for every new image you choose.

Add Current Image to Image Collection

Analysis Job Type: Scratch Wound

Existing

Image Collection: wound2_20170220_AnjaMai Search

Job Type, Required Channels, etc.

Job Type: Scratch Wound

No. of Stacks: 6 Phase: 1176 x 1620 @ 1,22 µm/px

Objective: 10x Green: Not Required

Filter: Modulr Dual Color Model 4459 Red: Not Required

New

Name: 20170223_AnjaMai_wound

Required Image Channels

Phase Green Red

+ Add

Job Name	Job Type	Creator	Date Created	Date Completed	Color1	Color2	Phase	Has	Open
HMLER	Scratch Wound	MIC	22.02.2017 12:54	23.02.2017 10:58	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

Analysing scratch wound assay

- Start the «Processing definition» by selecting the image set.
- Go between your selection images. «Preview current» will bring out the «analyse mask»

The screenshot displays a software interface for image analysis. On the left, a vertical menu titled "Analysis Job Utilities" contains options: "Create or Add to Image Collection...", "New Processing Definition..." (highlighted with a red arrow), "Launch New Analysis Job...", "Edit a Processing Definition...", and "Edit an Image Collection...". Below this is a "Parameters" panel with settings for "Training Image" (20170223_AnjaMai_wound), "Segmentation Adjustment" (Backgroun: 1), "Cleanup" (Hole Fill: 0.0000, Adjust Size: 0), "Filters" (Area: min 0.0000, max 0.0000; Eccentricity: min 0.0000, max 0.0000), and "Green (Unused)" and "Red (Unused)" channels.

The main workspace is divided into several sections. At the top, a "New Processing Definition" dialog box is open, showing "Image Collection" (20170223_AnjaMai_wound), "Job Type" (scratch Wound), "No. of Stacks" (6), "Objective" (10x), and "Filter Moduli" (Dual Color Model 4459). Below this, a "Preview Image Stack" panel shows "Preview" (1 of 6) and "Status: Un-processed". A red arrow points to the "Preview Current" button in this panel.

The central area displays "Image Channels" with "Phase-Contrast" checked. Below it, an "Analysis Masks" panel (highlighted with a red box) shows "Blend Mode: Blend | Weight:" and three checked masks: "Scratch Wound Mask" (green) and "Confluence Mask" (red). The main image shows a scratch wound assay with a scale bar (0 to 300 μm) and dimensions (1.43 x 1.98 mm, 2.83 mm²).

At the bottom, a "Metrics for Current Image Stack" panel is visible. On the right, a "Tools" panel includes a magnifying glass (set to 1) and a "Show Legend" checkbox.

Analysing scratch wound assay

Scratch Wound | New Processing Definition

File Preview: 129.177.231.74 (MIC)

Phase (Analyze)

Parameters

Training Image: 20170223_AnjaMai_wound

Segmentation Adjustment

Backgrou: 1 Cells

Cleanup

Hole Fill (μm^2): 0,0000

Adjust Size (pixel)

Filters

Area (μm^2):

min: 0,0000 max: 0,0000

Eccentricity:

min: 0,0000 max: 0,0000

Image Channels

Phase-Contrast

Analysis Masks

Blend Mode: Blend | Weight:

Scratch Wound Mask

Confluence Mask

Preview Image Collection

20170223_AnjaMai_wound

Job Type: Required Channels, etc.

Job Type: **Scratch Wound**

No. of Stacks: 6 Phase: 1176 x 1620 @ 1.22 $\mu\text{m}/\text{pix}$

Objective: 10x Green: Not Required

Filter Modul: Dual Color Model 4459 Red: Not Required

Preview Image Stack

2 of 6

Vessel Label: iiaMai 220217 HMLER

Vessel Typ: 96-well Essen

Scan Typ: Scratch Wound

Time: 22.02.2017 16:30

Image Sit: D8 Image 1

Preview

Status: Previewed

Tools

Show Legend

Metrics for Current Image Stack

300 μm

1.43 x 1.98 mm, 2.83 mm²

The goal is to create a clean scratch (green) by bringing the wound as close as possible to the cell surface and remove the cells/debris from the wound. The confluence mask (red) should also exclude the open gaps between the cells (clean-ups and filters).

Analysing scratch wound assay

Scratch Wound | New Processing Definition

File Preview: 129.177.231.74 (MIC)

Phase (Analyze)

Parameters
Training Image
20170223_AnjaMai_wound

Segmentation Adjustment
Background: 0.7 Cells

Cleanup
Hole Fill (μm^2): 6E+05
Adjust Size (pixels): -2

Filters
Area (μm^2):
 min 0.0000 max 0.0000
Eccentricity:
 min 0.0000 max 0.0000

Green (Unused)
Red (Unused)

Preview Image Collection
20170223_AnjaMai_wound

Job Type, Required Channels, etc.
Job Type: **Scratch Wound**
No. of Stacks: 6 Phase: 1176 x 1620 @ 1.22 $\mu\text{m}/\text{pix}$
Objective: 10x Green: Not Required
Filter Module: Dual Color Model 4459 Red: Not Required

Preview Image Stack
1 of 6

Vessel Label: iiaMai_220217_HMLER
Vessel Type: 96-well Essen
Scan Type: Scratch Wound
Time: 22.02.2017 12:30
Image Site: A4 Image 1

Preview
Status: Previewed
Preview All

Image Channels
 Phase-Contrast

Analysis Masks
Blend Mode: Blend | Weight: 1
 Scratch Wound Mask
 Confluence Mask

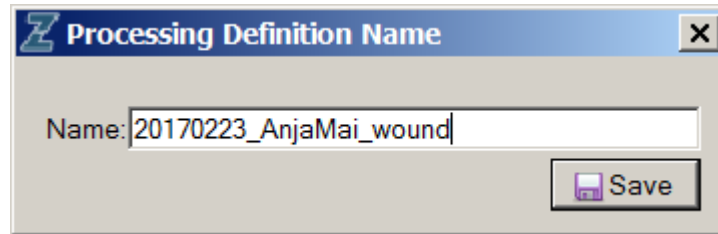
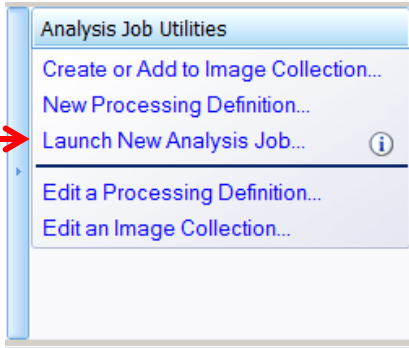
Tools
1
 Show Legend

Carefully make changes to the segmentation and preview one/all images to check for mask fitting.

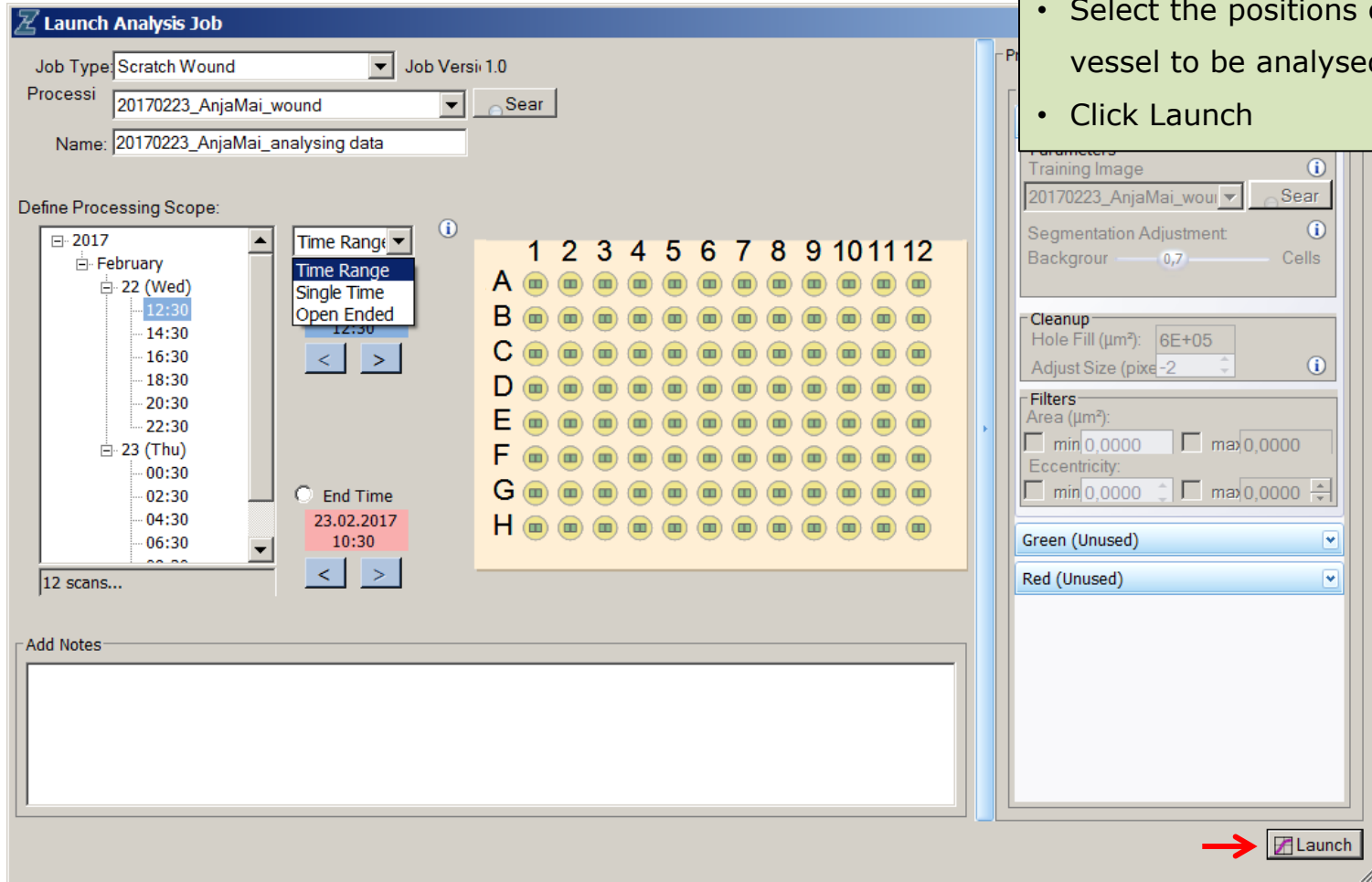
0 300 μm
1.43 x 1.98 mm, 2.83 mm²

Metrics for Current Image Stack

Analysing scratch wound assay




- Give the processing job a name.
- Select the time range (select open ended if your acquisition is not finished yet).
- Select the positions on your vessel to be analysed.
- Click Launch



Analysing scratch wound assay

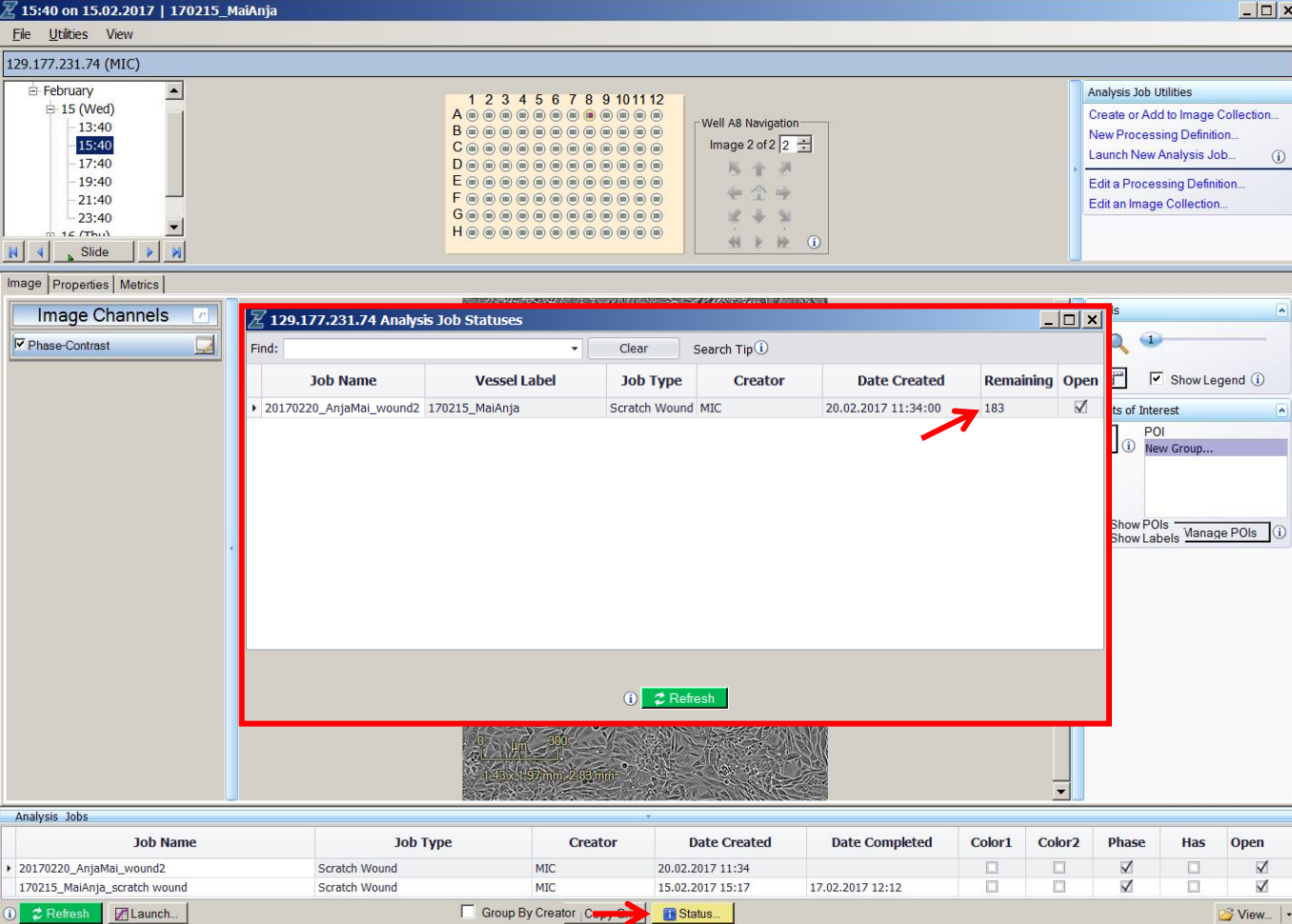
Analysis Job Created

 Your analysis job has been created.

- The controller (129.177.231.74) will process this job as soon as possible.
- There is currently no time estimate, but progress is available via the Status button at the bottom of the Vessel View.

Do not show this message again OK

Once you launch the analysis, the controller will launch the job in between scanning jobs. You can at any time see the time remaining by pressing «status» at the bottom.



The screenshot shows the software interface with a window titled "129.177.231.74 Analysis Job Statuses" highlighted in red. This window contains a table with the following data:

Job Name	Vessel Label	Job Type	Creator	Date Created	Remaining	Open
20170220_AnjaMai_wound2	170215_MaiAnja	Scratch Wound MIC		20.02.2017 11:34:00	183	<input checked="" type="checkbox"/>

A red arrow points to the "Remaining" column value of 183. Below the table is a "Refresh" button.

At the bottom of the main interface, there is another table with the following data:

Job Name	Job Type	Creator	Date Created	Date Completed	Color1	Color2	Phase	Has	Open
20170220_AnjaMai_wound2	Scratch Wound	MIC	20.02.2017 11:34		<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>
170215_MaiAnja_scratch wound	Scratch Wound		15.02.2017 15:17	17.02.2017 12:12	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>

At the bottom of the interface, there is a "Status..." button with a red arrow pointing to it.

Viewing analysed data

Open your data set (double-click) under «archives». Your image set will open up. On the bottom you will see the «analysis job». To check mask, simply double click on the analyse job.

The screenshot shows a software interface for image analysis. At the top, there is a status bar with the time '14:40 on 09.02.2017' and the file name '170209_Aksnes_wound'. Below this is a menu bar with 'File', 'Utilities', and 'View'. A file browser on the left shows a directory structure for 'February' and '09 (Thu)', with '14:40' selected. A grid of buttons labeled A through H is visible. The main image area shows a grayscale image of a wound with a red and green mask overlaid. The 'Image Channels' panel on the left shows 'Phase-Contrast' checked and 'Green' unchecked. The 'Analysis Jobs' table at the bottom lists the analysis job 'Aksnes_wound170213'. A red arrow points to the first row of the table.

Job Name	Job Type	Creator	Date Created	Date Completed	Color1	Color2	Phase	Has	Open
Aksnes_wound170213	Scratch Wound	H-Aksnes	13.02.2017 15:05	13.02.2017 16:41	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Viewing analysed data – microplate graph

Go to Metrics and select the time range and positions you want to investigate (or define regions) and click on «microplate graph».

«Microplate graph» gives a quick overview of the dataset.

Phase Metrics

	Mean	Std. Dev.	Min	Max
Vessel	75,378	32,418	0,0000	99,979
A1	92,325	-	-	-

Metric Graph/Export

Time Plot

Phase Metrics

- Relative Wound Density (Percent)
- Wound Confluence (Percent)
- Wound Width (µm)

Scan Metrics

Statistic: Mean

Histogram

Phase Metrics

Time Range: Start Time 09.02.2017 2:40, End Time 13.02.2017 11:40

Region: All Wells

Group: All

48 scans

Include Vessel Label in trace names

Buttons: Graph, Microplate Graph, Data Export...

170209_Aksnes_wound - All Wells Mean vs Time

Relative Wound Density (Percent) over 3 days

Time	1	2	3	4	5	6	7	8
09:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
09:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
10:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
11:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
12:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
13:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
14:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
15:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
16:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
17:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
18:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
19:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
20:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
21:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
22:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
23:30	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
24:00	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

Metrics for Current Image Stack

Image Channel	Wound Confluence (Percent)	Wound Width (µm)	Relative Wound Density (Percent)
Phase	68,558	255,80	77,164

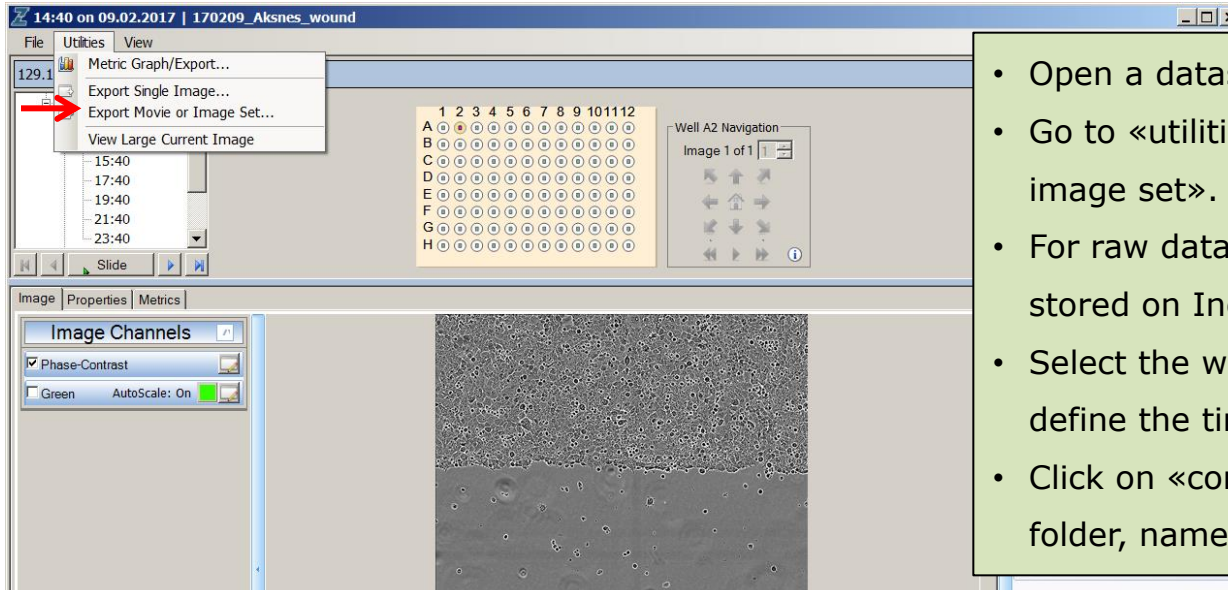
Exporting analysed data

The screenshot displays the 'Metric Graph/Export' software interface and a Microsoft Excel spreadsheet. The software interface includes a 'Time Plot' section with a list of scan times, a 'Time Range' selector, and a 'Phase Metrics' list. The 'Phase Metrics' list includes 'Phase Object Confluence (Percent)', 'Phase Object Count (1/Image)', 'Phase Object Count (1/mm²)', 'Phase Object Count (1/Well)', 'Avg Phase Object Area (µm²)', and 'Avg Phase Object Eccentricity'. The 'Scan Metrics' section is also visible. The 'Statistic' dropdown is set to 'Mean'. The 'Histogram' section is also present. The 'Region' dropdown is set to 'All Wells'. The 'Time Range' is set to 'Start Time' from '16.05.2017 3:39' to 'End Time' '19.05.2017 1:39'. The 'Microplate Graph' shows a grid of wells with yellow circles indicating data points. The Excel spreadsheet shows the following data:

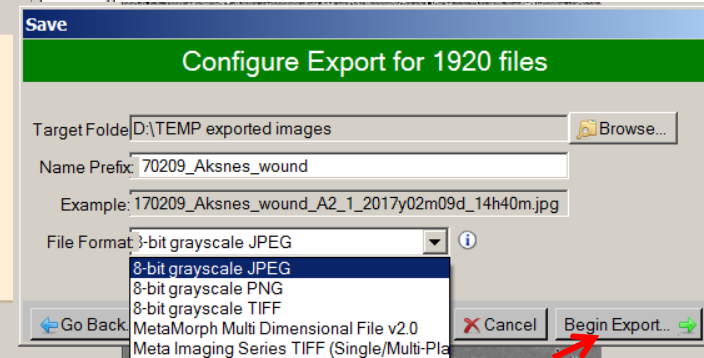
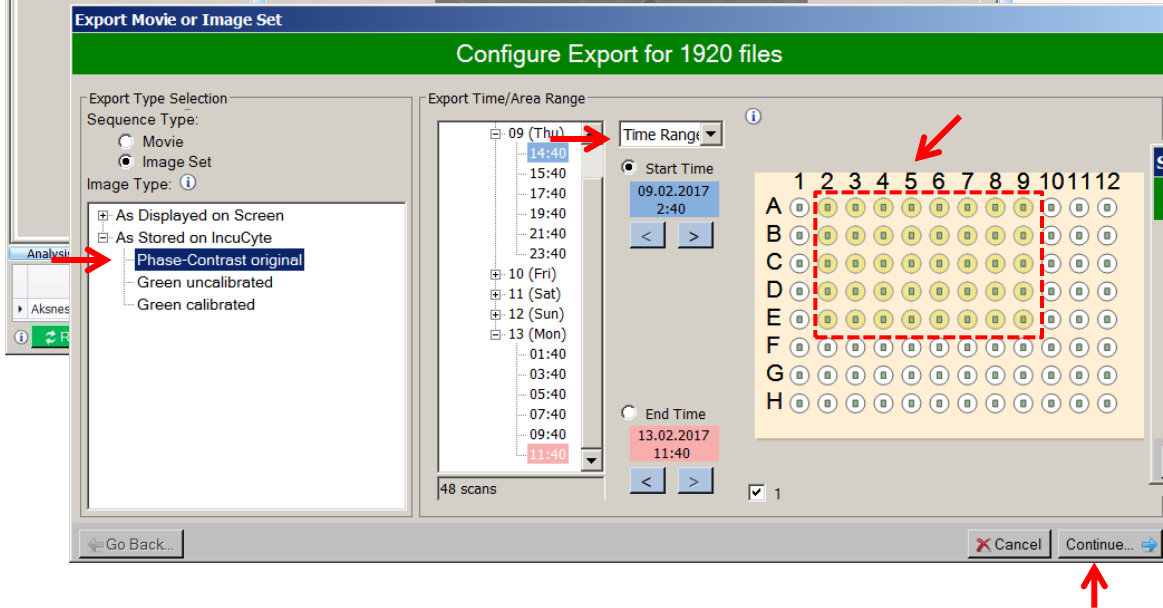
Date Time	Elapsed	Henriette A Prolif Pos 2: All
16.05.2017 15:39	0	272,7168
16.05.2017 17:39	2	255,2746
16.05.2017 19:39	4	271,9302
16.05.2017 21:39	6	303,8762
16.05.2017 23:39	8	321,2609
17.05.2017 01:39	10	340,1018
17.05.2017 03:39	12	350,9247
17.05.2017 05:39	14	380,7954
17.05.2017 07:39	16	400,0266
17.05.2017 09:39	18	415,1024
17.05.2017 11:39	20	443,612
17.05.2017 13:39	22	465,1187
17.05.2017 15:39	24	505,0276
17.05.2017 17:39	26	542,2175
17.05.2017 19:39	28	614,3526
17.05.2017 21:39	30	621,5753
17.05.2017 23:39	32	710,7784
18.05.2017 01:39	34	787,9478
18.05.2017 03:39	36	865,5345
18.05.2017 05:39	38	936,542
18.05.2017 07:39	40	1023,012
18.05.2017 09:39	42	1170,149
18.05.2017 11:39	44	1300,123

Select the metrics of interest and click «data export». Paste the data into excel. It will require a few exports to get all the metrics values over into the table.

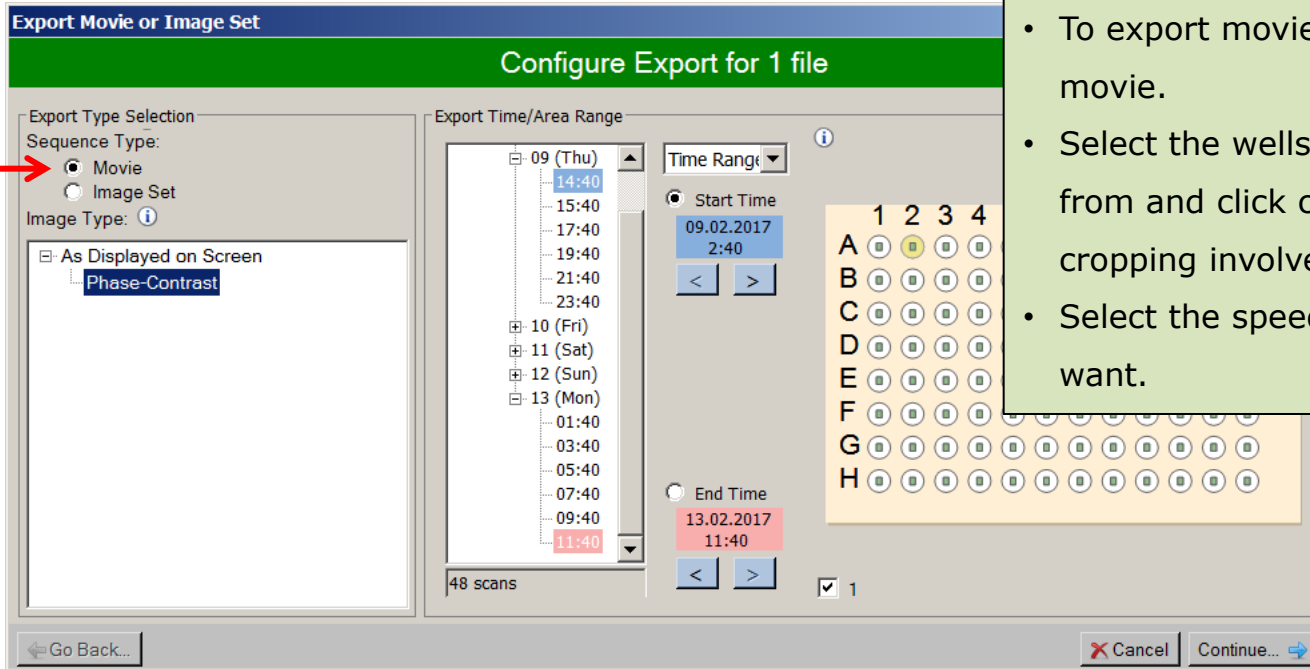
How to export image sets



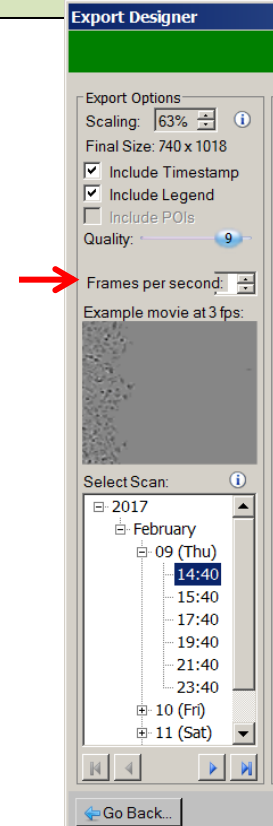
- Open a dataset.
- Go to «utilities» and select «export movie or image set».
- For raw data, highlight «originals» under «as stored on IncuCyte».
- Select the wells you want to export and define the time range.
- Click on «continue» and define the target folder, name and select format (mostly tiff).



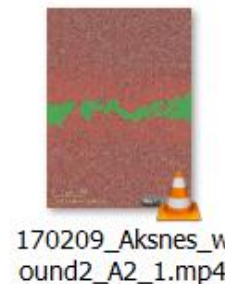
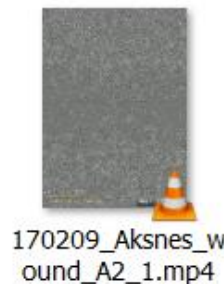
How to export movies



- To export movies, select sequence type: movie.
- Select the wells you want to create movies from and click continue. There will be some cropping involved due to size.
- Select the speed (frames per second) you want.



- You can either export the movie as raw data (phase contrast + fluorescent channel) or the data set with the masks.
- For the latter click on analyse (at the bottom) and check scratch and confluence mask before going to utilities-export movie.



Deleting analysis jobs

14:40 on 09.02.2017 | 170209_Aksnes_wound

File Utilities View

129.177.231.74 (H-Aksnes)

February

09 (Thu)

14:40

15:40

17:40

19:40

21:40

23:40

Slide

Well A2 Navigation

Image 1 of 1

Analysis Job Utilities

- Create or Add to Image Collection...
- New Processing Definition...
- Launch New Analysis Job...
- Edit a Processing Definition...
- Edit an Image Collection...

Image Channels

- Phase-Contrast
- Green AutoScale: On

Analysis Jobs

- View...
- Details...
- Rename...
- Export Processing Definition...
- Refresh
- Delete

Job Type	Creator	Date Created	Date Completed	Color1	Color2	Phase	Has	Open
wound	H-Aksnes	13.02.2017 15:05	13.02.2017 16:41	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Group By Creator Copy Grid Status...

Start

14:26 27.02.2017

NO

- If you are not happy with the analyse job, you can easily delete it and start creating a new one.
- Open your data set.
- Right click on the analyse job and delete.

Deleting your raw data from the IncuCyte controller

The screenshot shows the IncuCyte ZOOM software interface. The top bar displays the IP address 129.177.231.74 (H-Aksnes) and the next scan time: 44 minutes (20:30). The menu includes File, View, Preferences, and Help. On the left, a Task List sidebar contains View Scans, Search, Schedule Scans, Archives, and Administrator (highlighted). The main window has tabs for Device, Accounts, Delete (highlighted with a red box), and Logs. Below the tabs is a search bar with a 'Find:' field, a 'Clear' button, and a 'Search Tip' icon. A table with the following columns is displayed: Select, Start Date/Time, User, Label, Cell Type, Scan Type, Color1, Color2, Phase, and Objective. The table contains three rows of data. The third row is selected, and a red arrow points to the 'Delete' button in the bottom right corner of the interface.

Select	Start Date/Time	User	Label	Cell Type	Scan Type	Color1	Color2	Phase	Objective
<input type="checkbox"/>	09.02.2017 14:40	H-Aksnes	170209_Aksnes_wound	Hap_wt, Hap_wt_GFP, Hap_KO1,...	Scratch Wound	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input type="checkbox"/>	09.02.2017 14:40	H-Aksnes	170209_Aksnes_prolif	Hap	Standard	<input type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x
<input checked="" type="checkbox"/>	09.02.2017 13:40	H-Aksnes	170209_Frits_H1DL2_viability_Dab_PLX	H1DL2	Standard	<input checked="" type="checkbox"/>	<input type="checkbox"/>	<input checked="" type="checkbox"/>	10x

Device Status
19:45
Next Scan: 20:30
Set By: MIC
Set At: 23.02.2017 14:10
Status: Ready To Scan
Objective: Nikon 10x
Filter: Dual Color Model 4459
Free Space (% free):
Disk - 8 TB (98%)
Database - 10 GB (>99%)
Temperature: 37,3 °C

Refresh Select All Unselect Group By User Copy View Vessel Delete

You can only delete your raw data by going through «administer-delete». Select the file and click on delete. This will remove the data from the controller and can NOT be entered again into the IncuCyte. This means you can never analyse this specific data in the controller again.

Scratch woundmaker – useful and VERY expensive toy (only for specially trained users)

- Creates 96 homogeneous, 700-800 μ m wide wounds.
- Woundmaker needs to be cleaned before and after every use.
- Cleaning solutions must be **45 ml**, not more not less.
- Never place woundmaker directly on **bench** and never turn woundmaker **upside down!**
- Never leave any wells **dry**, this will damage pins.
- Never use woundmaker with more than **100 μ l** of media per well.
- Wear gloves, cell culture coat and shoe protection when entering the cell lab.
- Get your ImageLock Essen microplate from MIC (booking system-supplies).



- **Before usage**, soak pins in sterile water for 5 min and then 70% ethanol, and let air dry.
- Perform the wound following the 6 step procedure (next page). If you are wounding multiple plates of the same cell line, simply soak pins in 45ml sterile distilled water between wounding.
- **After the last experiment:** 45ml of 0.5% Alconox for 5min, 45ml of 1% Virkon S. for 5 min, 45ml of sterile distilled water for 5min, twice with 45ml 70% ethanol for 5 min.

Scratch woundmaker 6 steps to create wound



Step 1

- Remove top of WoundMaker™.
- Set top in empty wash boat.



Step 2

- Insert plate (containing cells & media) into base plate holder.
- **Remove plate cover.**



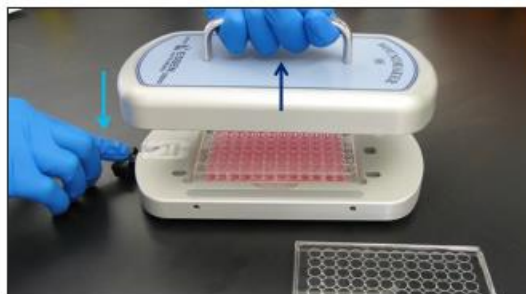
Step 3

- Replace pin block by guiding the rear dowels of pin block into the rear holes of the base plate.
- **Do not push down.**



Step 4

- Push and hold the black lever.



Step 5

- Lift pin block while continuing to hold the black lever down.



Step 6

- Replace plate cover.
- Wash wells (up to two washes).
- Add treatment conditions.
- Put into the IncuCyte™ and start acquiring data and images.