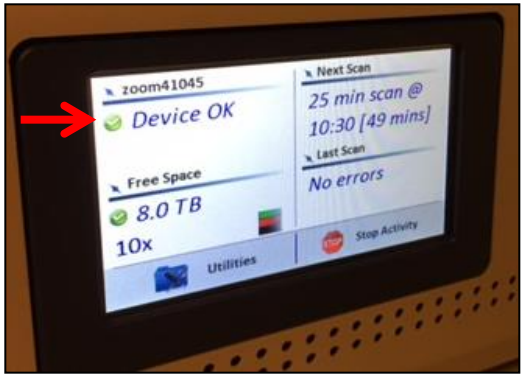
Quick user-guide to the IncuCyte

General rules for operating the IncuCyte

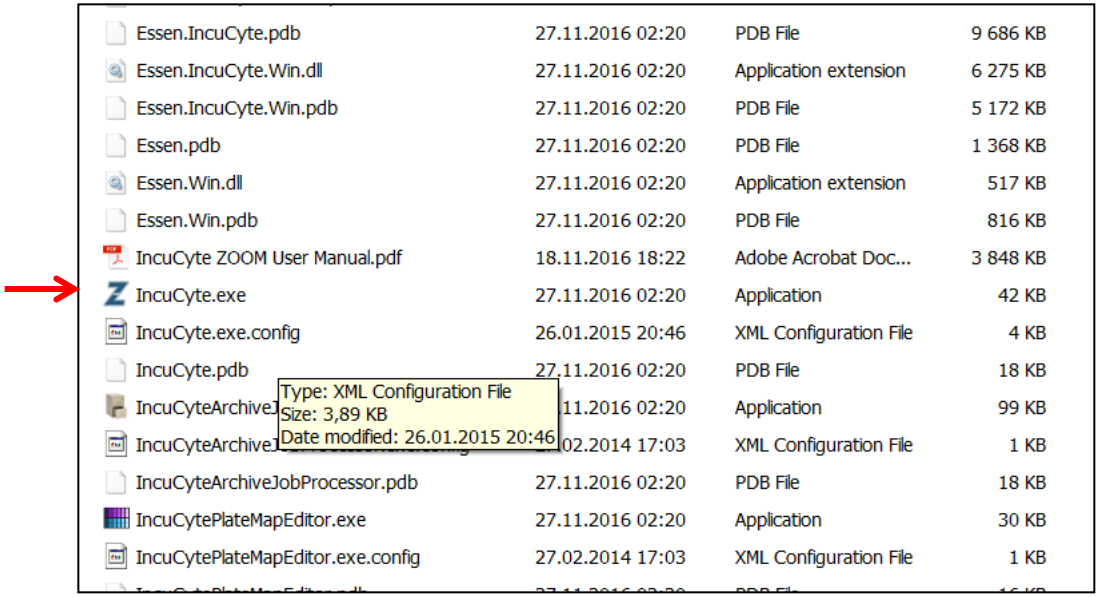
- **Training is mandatory** for all users, please ask if you are not yet comfortable on the system (MIC or Halala). Once trained, you can get access to the Zoom software and connect remotely from your own pc.
- Every user will have his/her own **user account** (last name_first name initial). Log in using 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

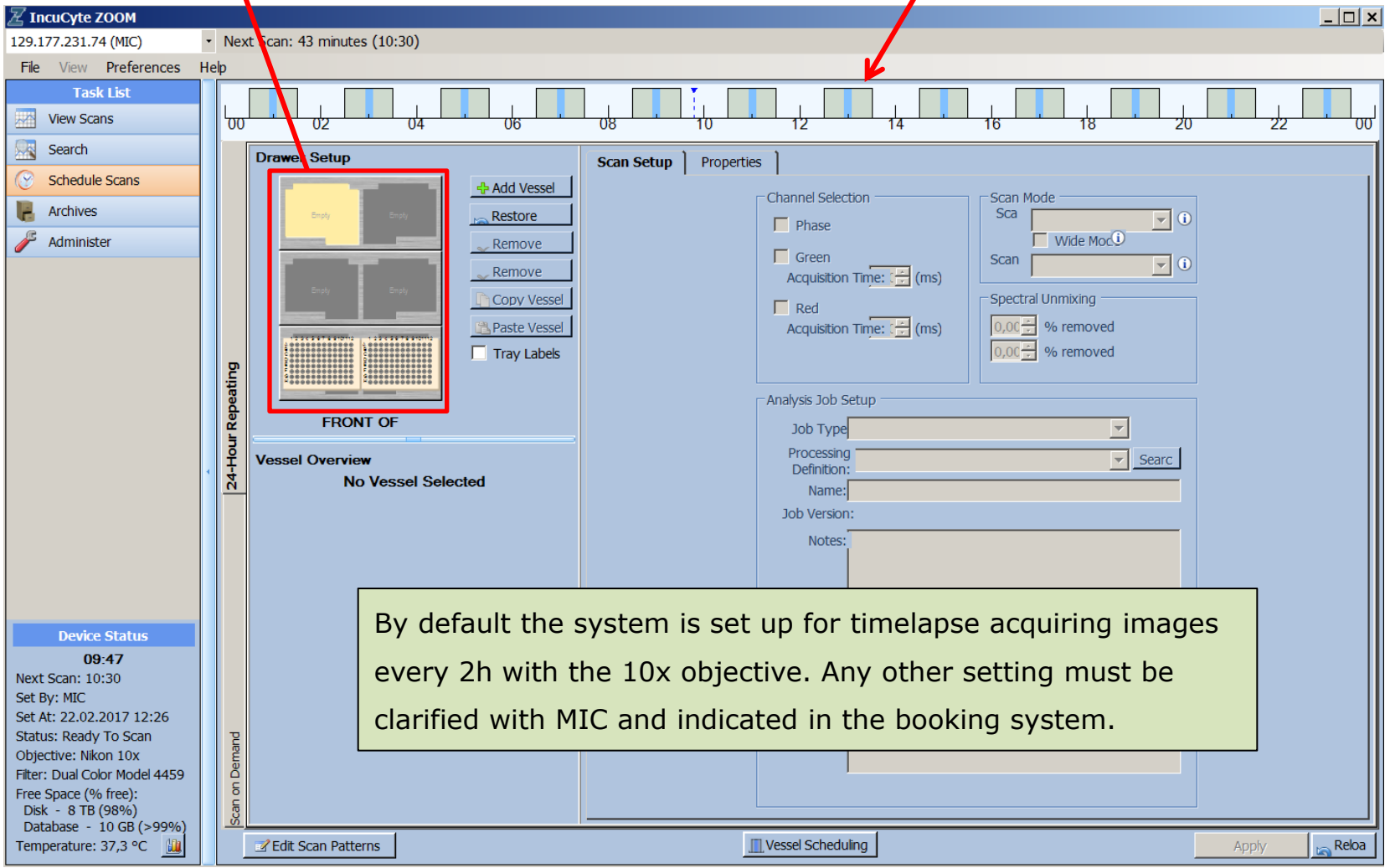


The IncuCyte ZOOM software has also been installed on the workstations in the MIC PC room on the 6th floor.

Setting up a timelapse

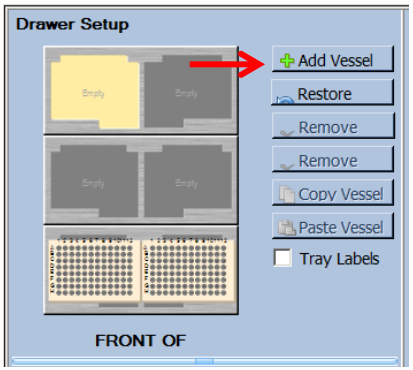
Scans already in the drawer. Make sure you select the position you've reserved through the booking system!

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!



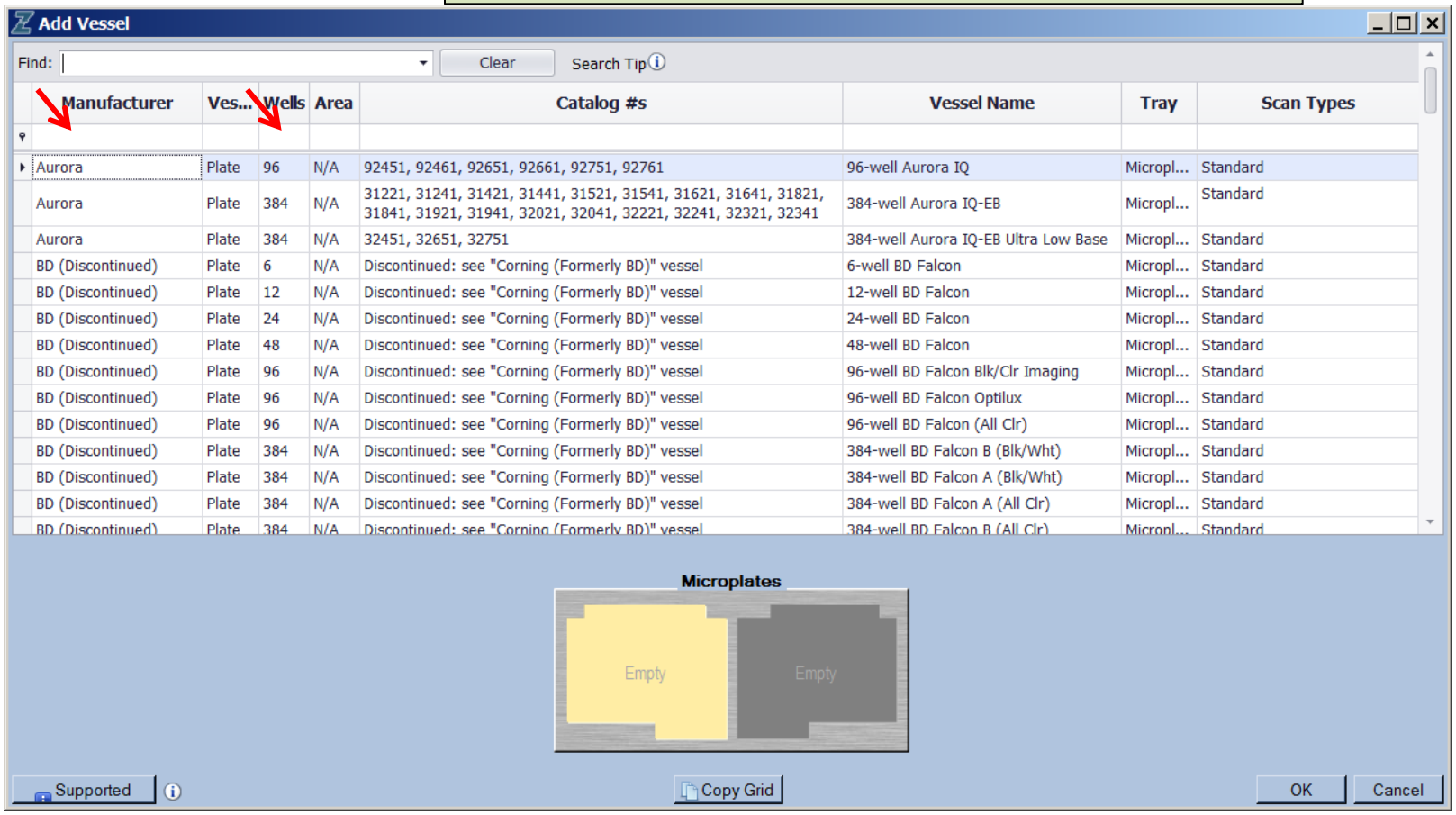
By default the system is set up for timelapse acquiring images every 2h with the 10x objective. Any other setting must be clarified with MIC and indicated in the booking system.

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.

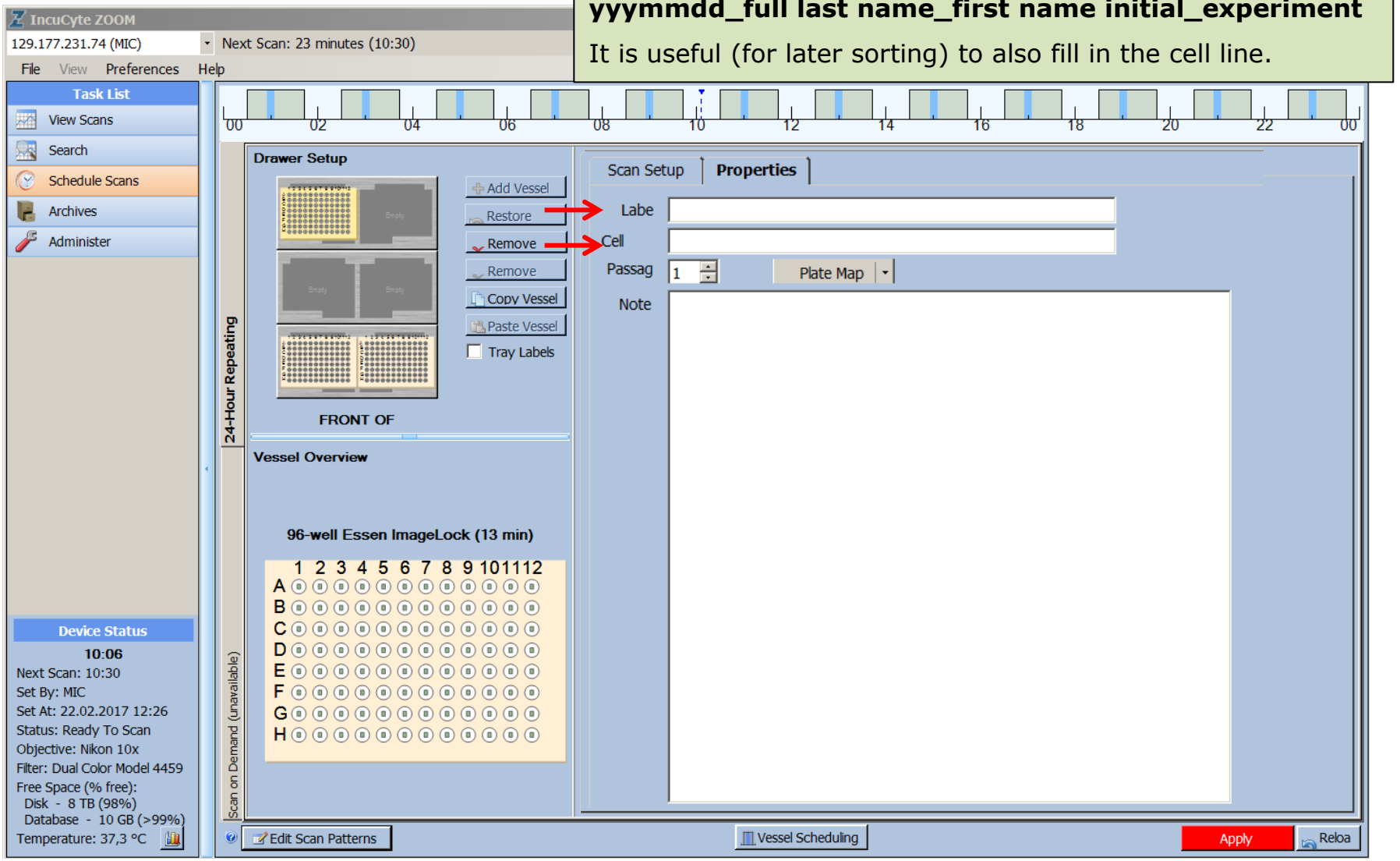


Setting up a timelapse

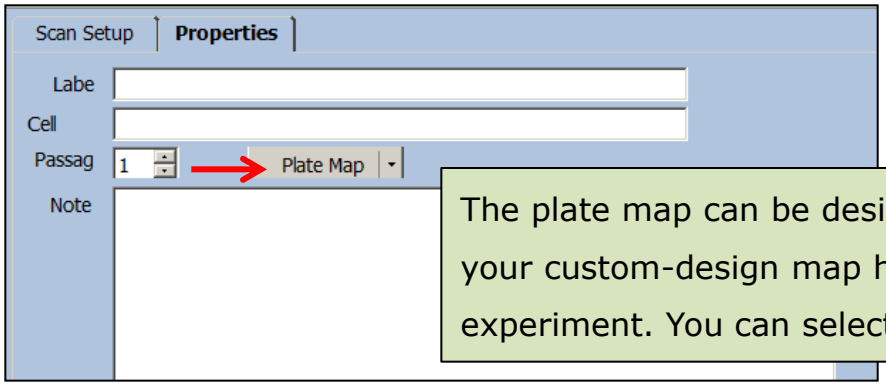
Open up Properties and give your experiment a label:

yyymmdd_full last name_first name initial_experiment

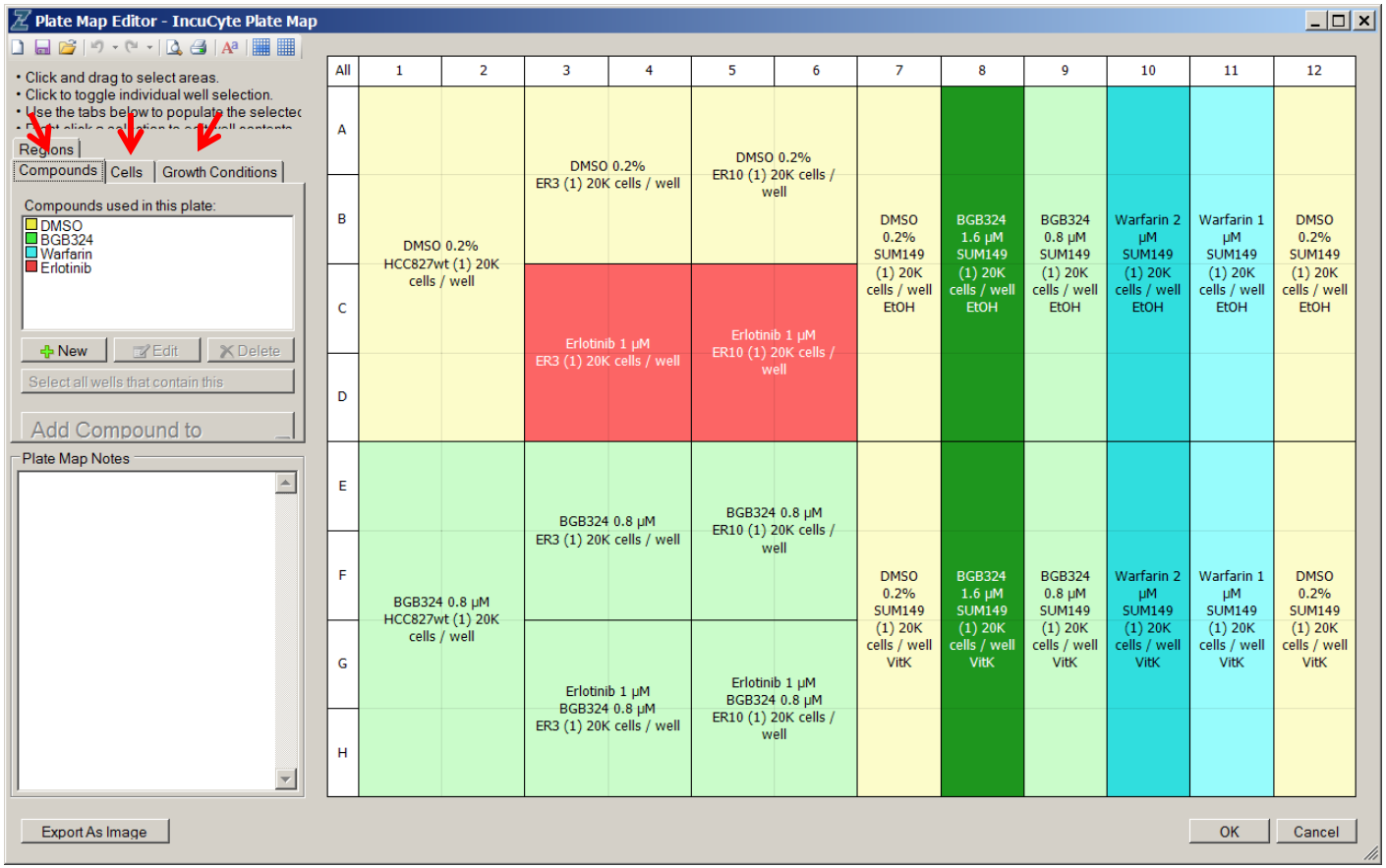
It is useful (for later sorting) to also fill in the cell line.



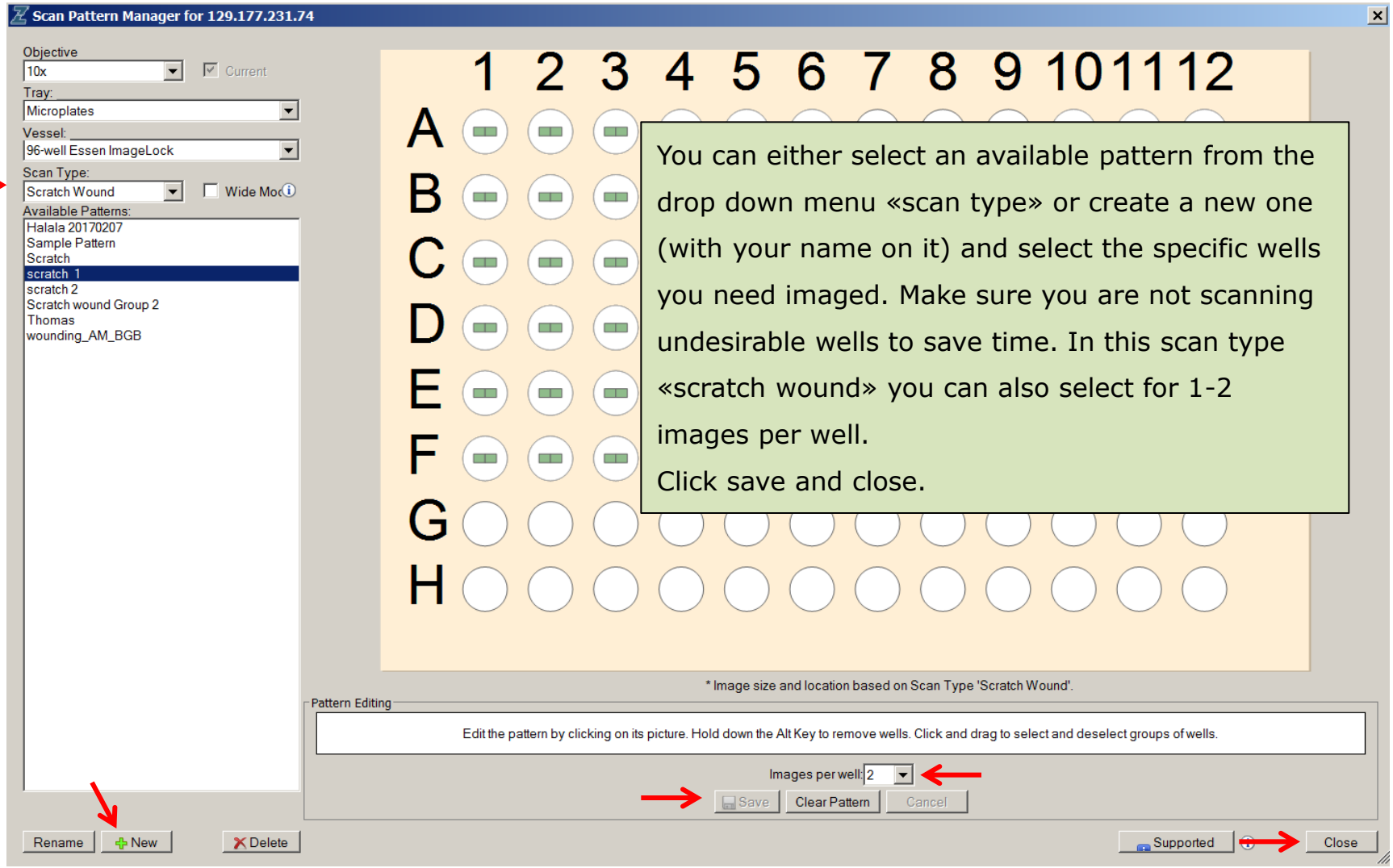
Creating a plate map



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.

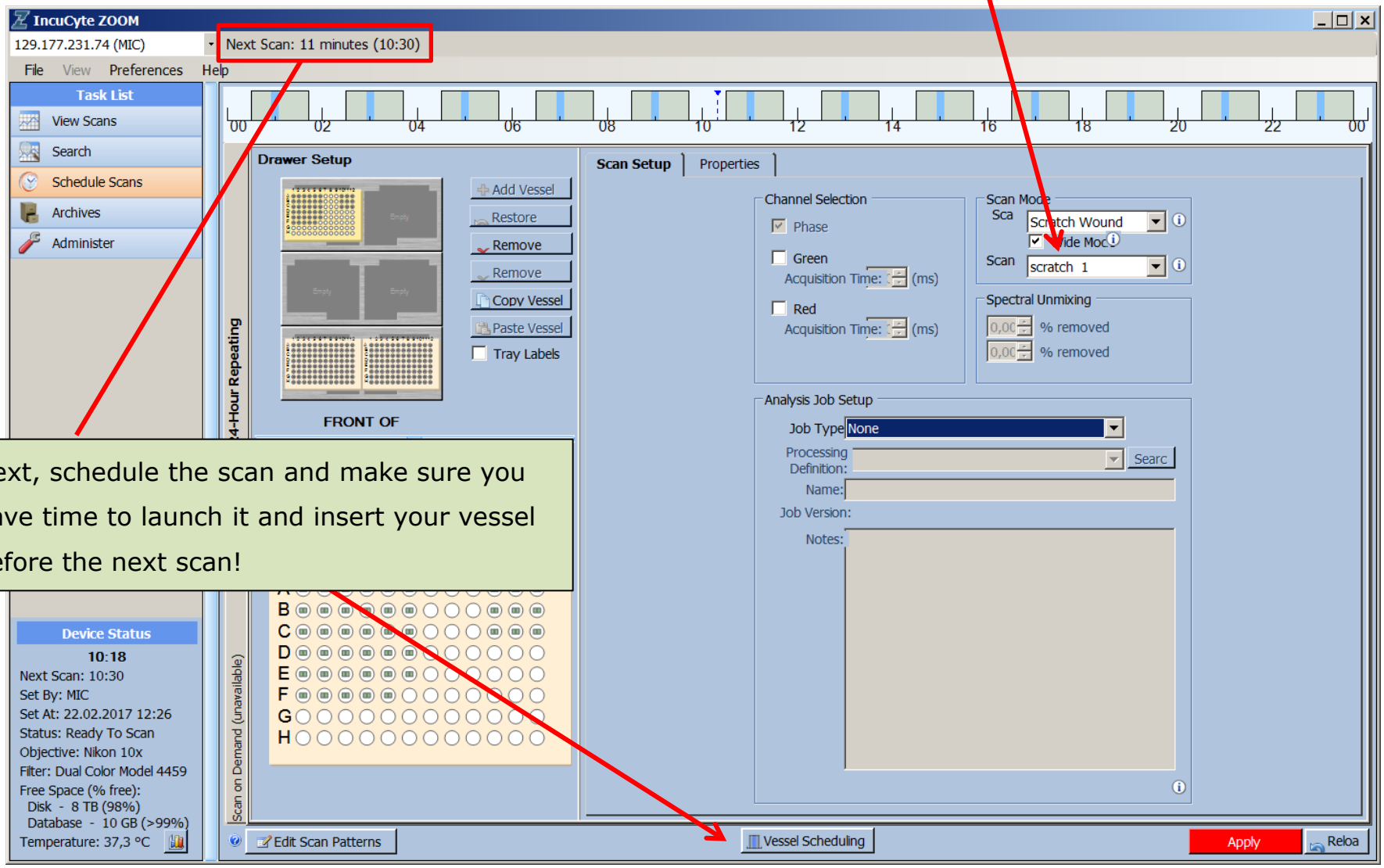


Defining the scan pattern



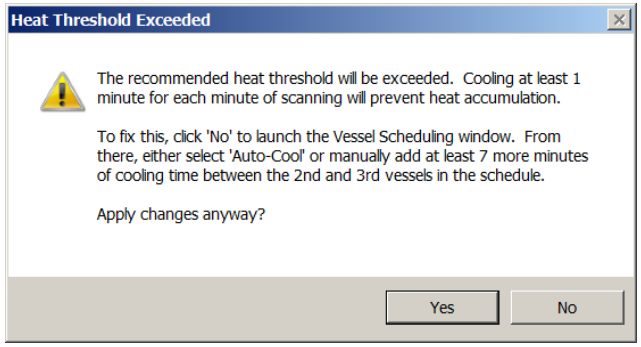
Setting up a timelapse

Retrieve your scan pattern from the scan list

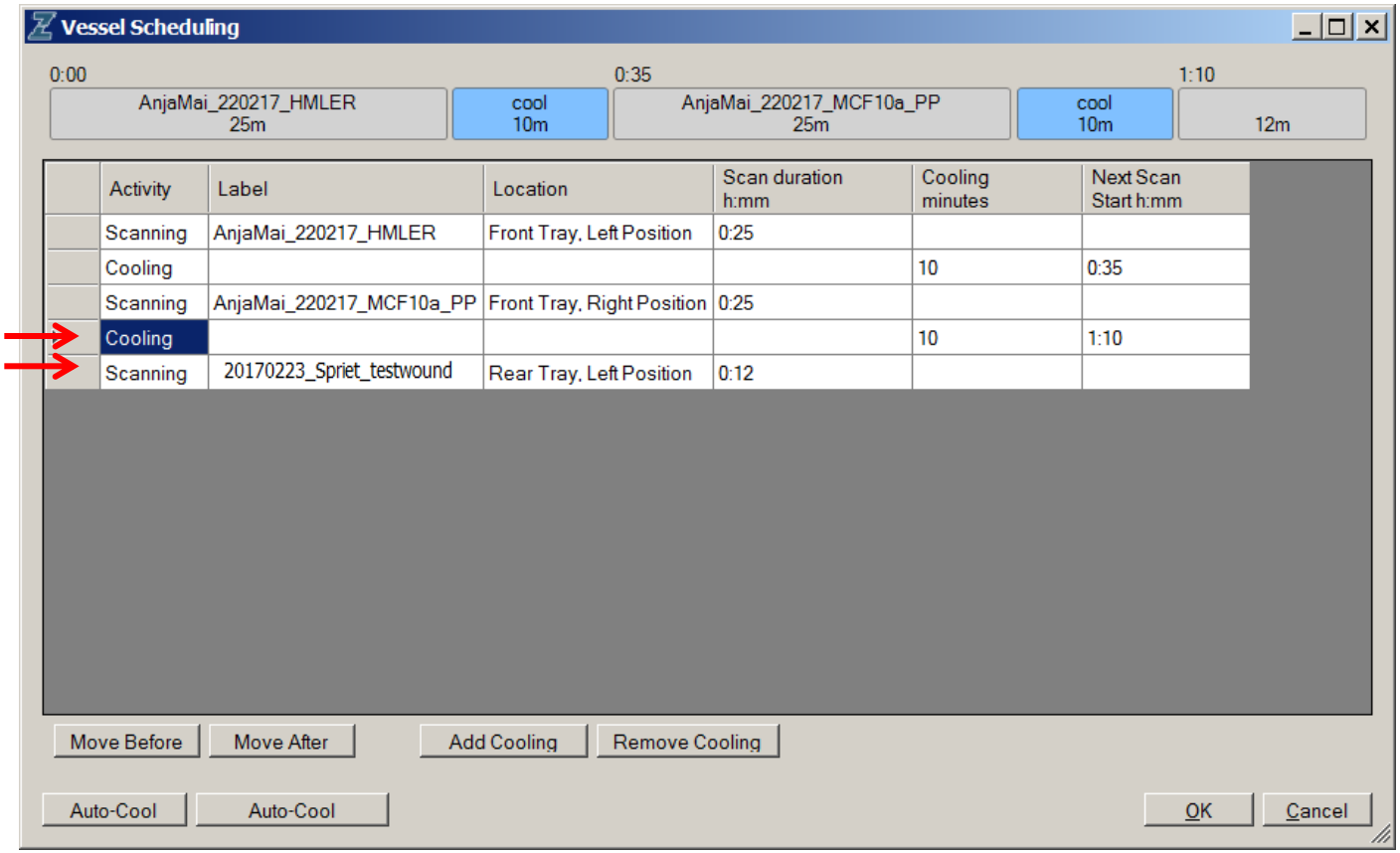


Next, schedule the scan and make sure you have time to launch it and insert your vessel before the next scan!

Setting up a timelapse – vessel scheduling

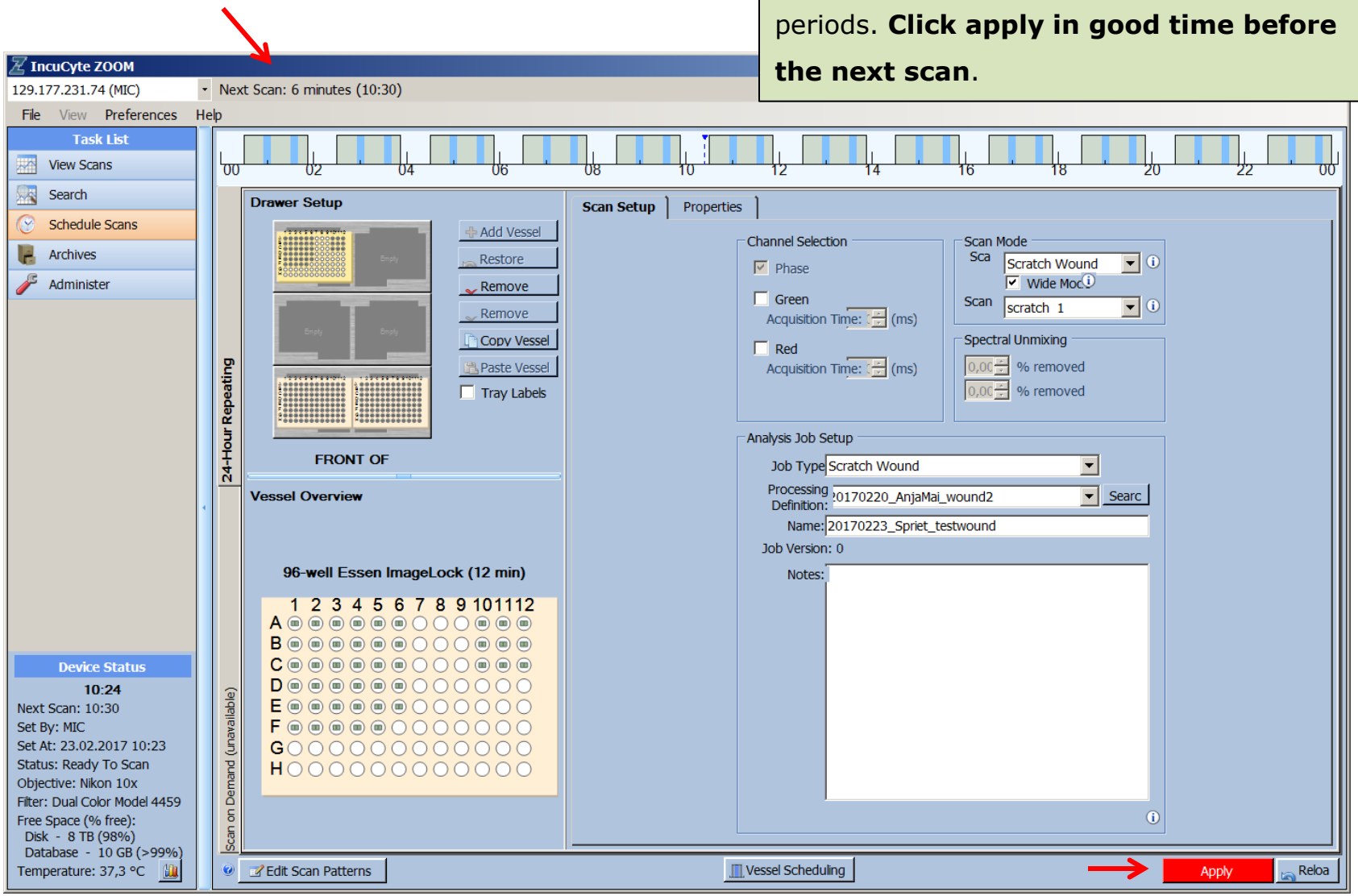


This message indicates that you need to add cooling time into the schedule and in order not to shift everyones scanning, **put your scan last and add the cooling time right before**.



Setting up a timelapse – vessel scheduling

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

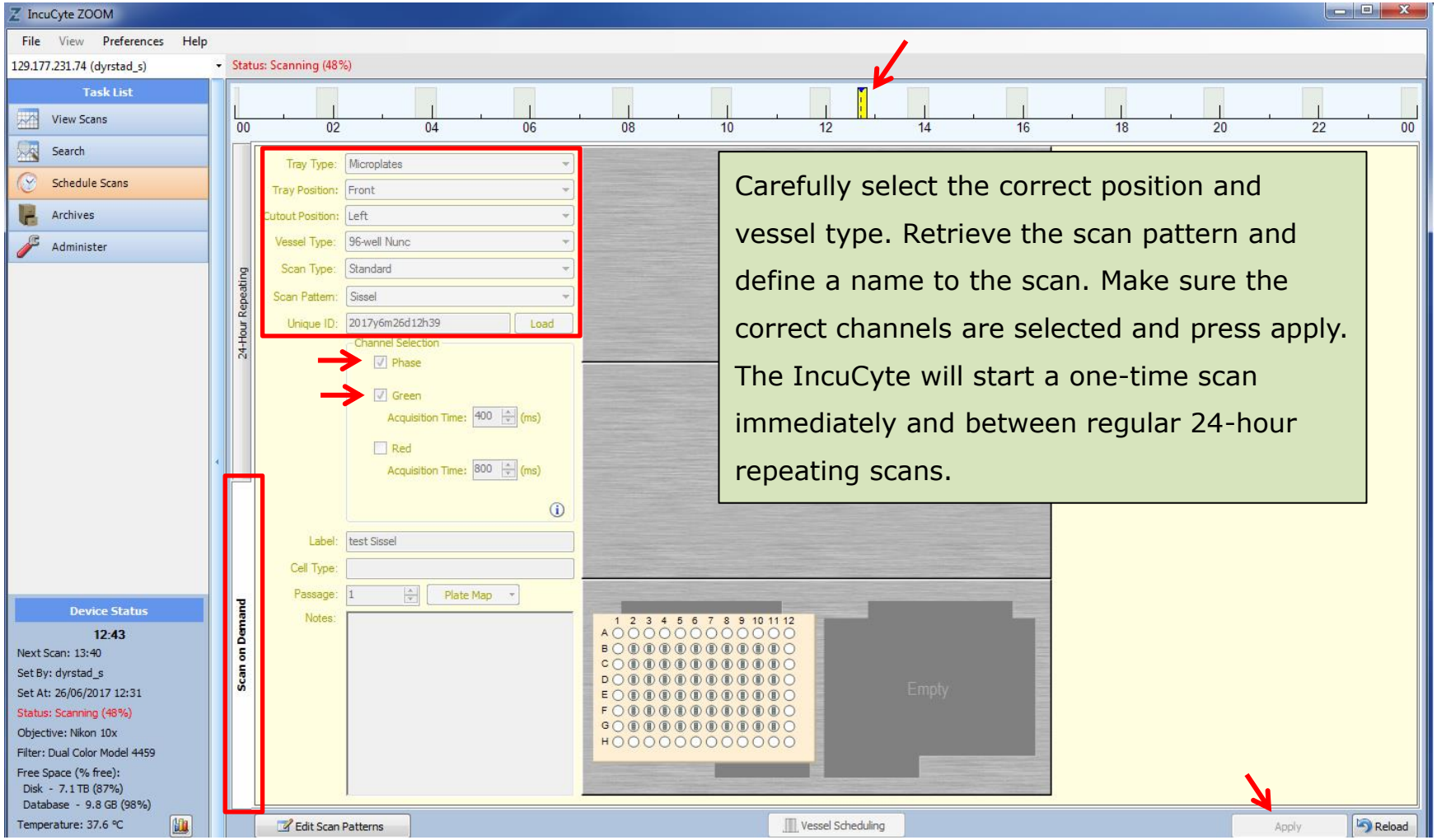


Removing plates: click on your plate and select «remove» and click on APPLY.

Open the drawer in between scans and remove your plate.

Scan on demand

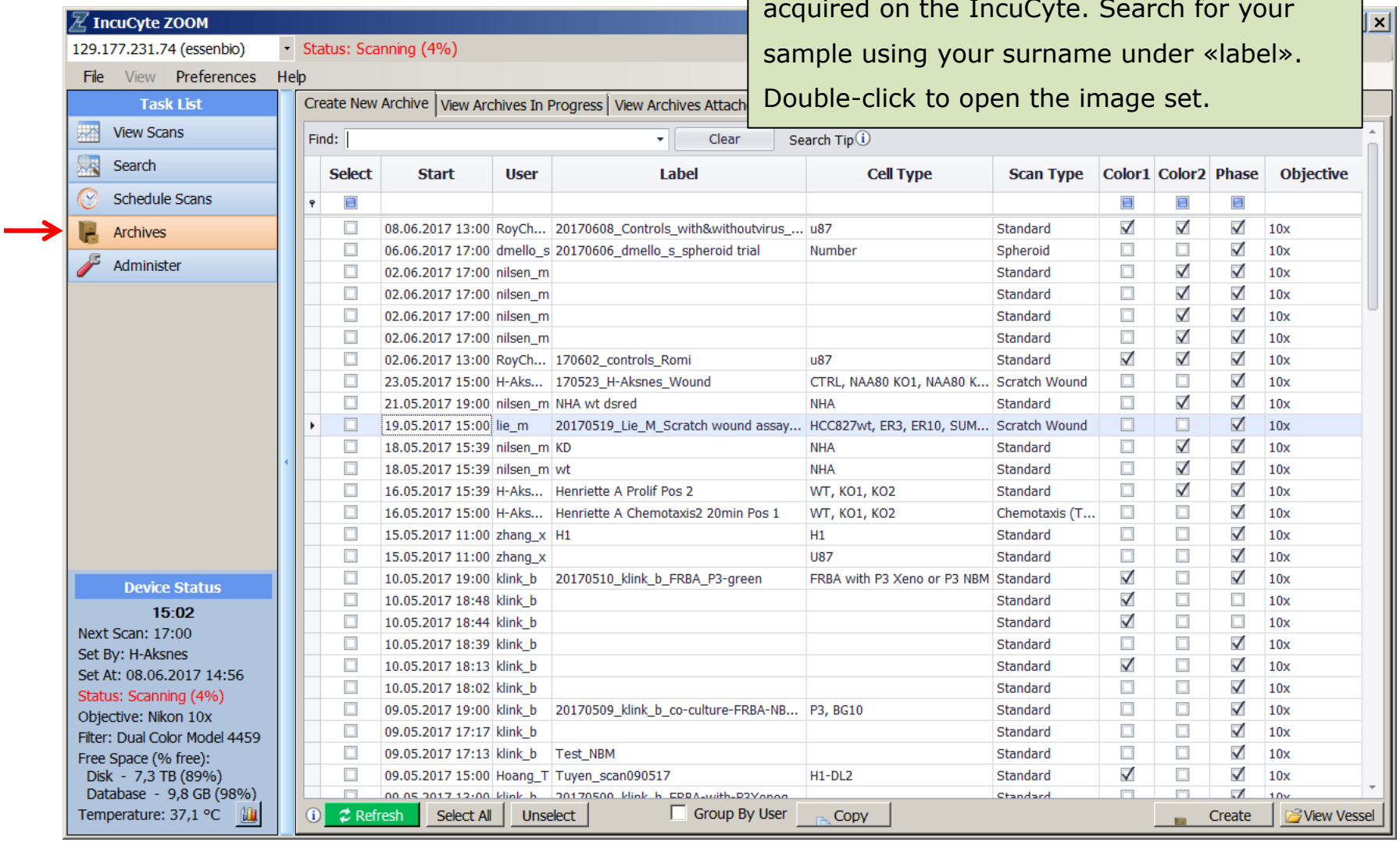
You can run a one-time scan over your plan through the "scan on demand".



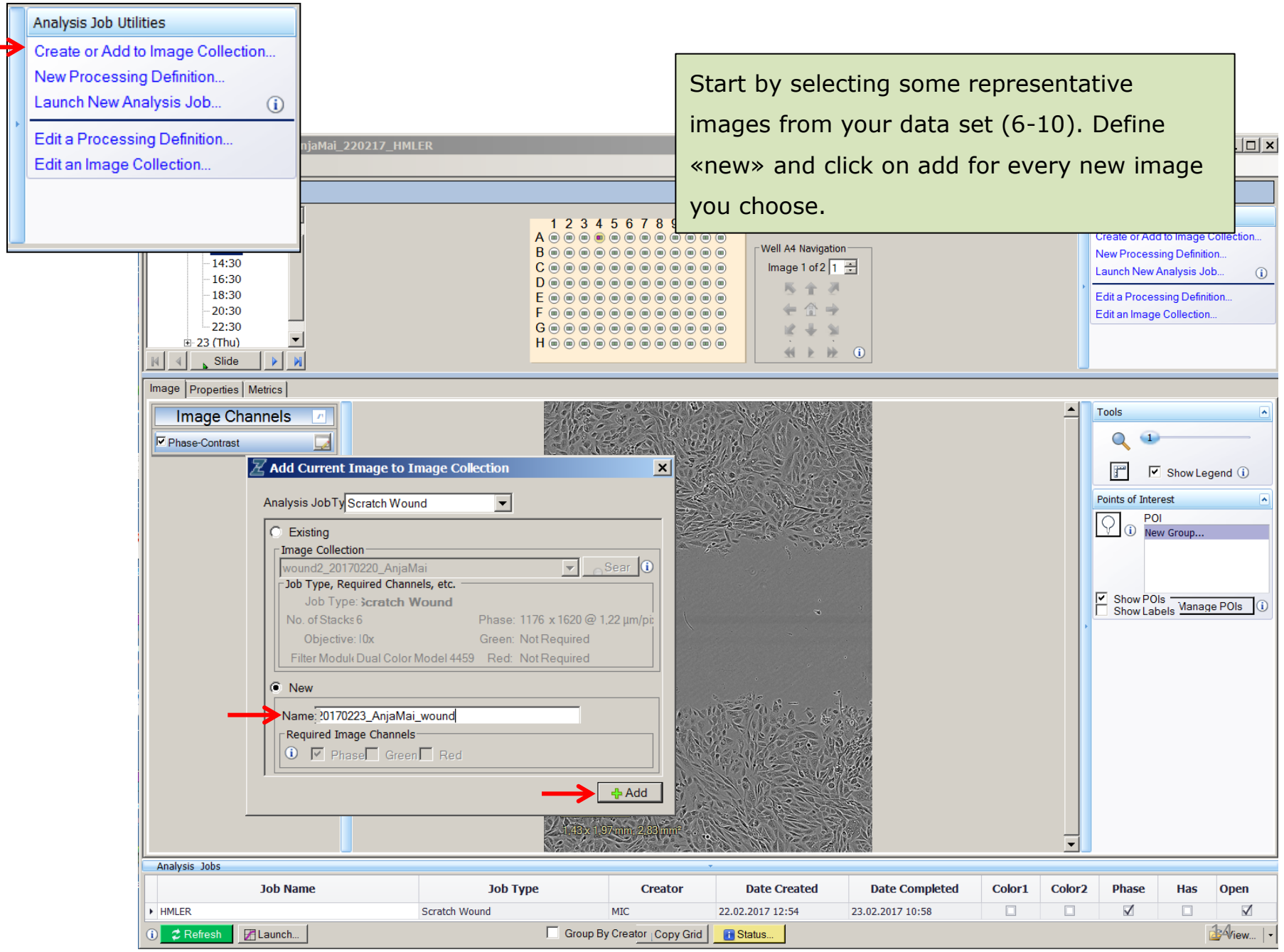
Carefully select the correct position and vessel type. Retrieve the scan pattern and define a name to the scan. Make sure the correct channels are selected and press apply. The IncuCyte will start a one-time scan immediately and between regular 24-hour repeating scans.

Analysing scratch wound assay

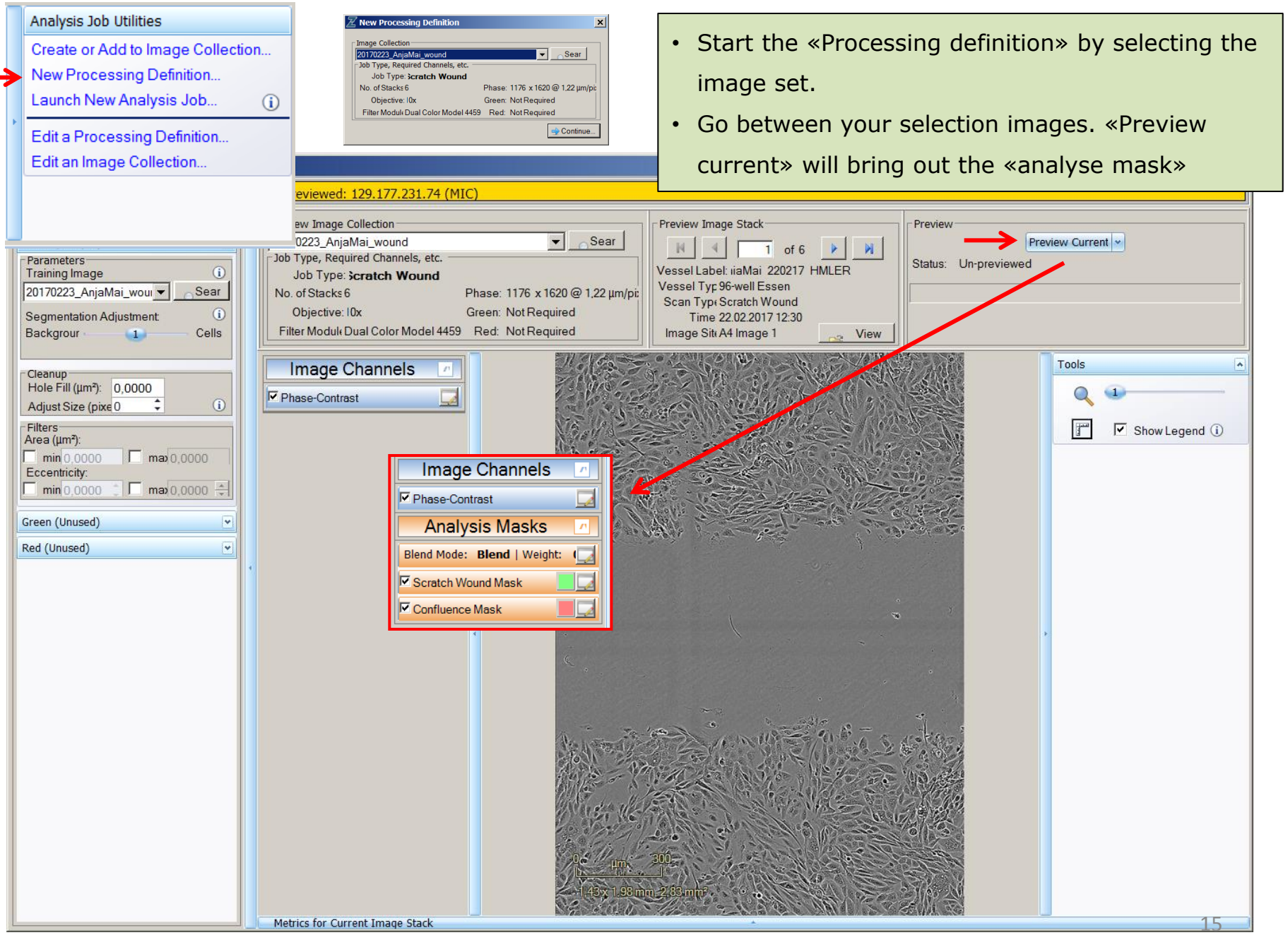
Inside «archives» you will see all the data acquired on the IncuCyte. Search for your sample using your surname under «label». Double-click to open the image set.



Analysing scratch wound assay

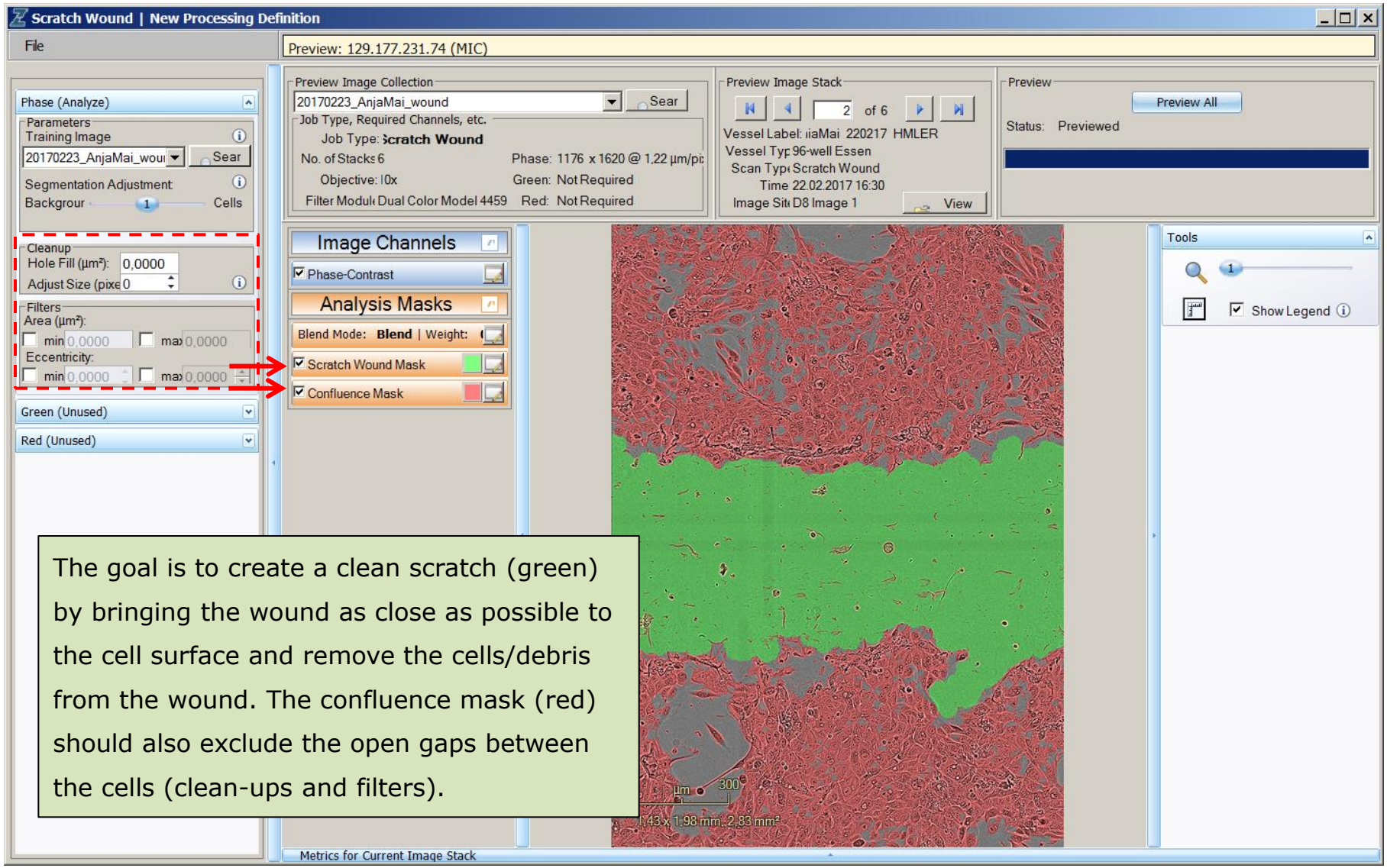


Analysing scratch wound assay



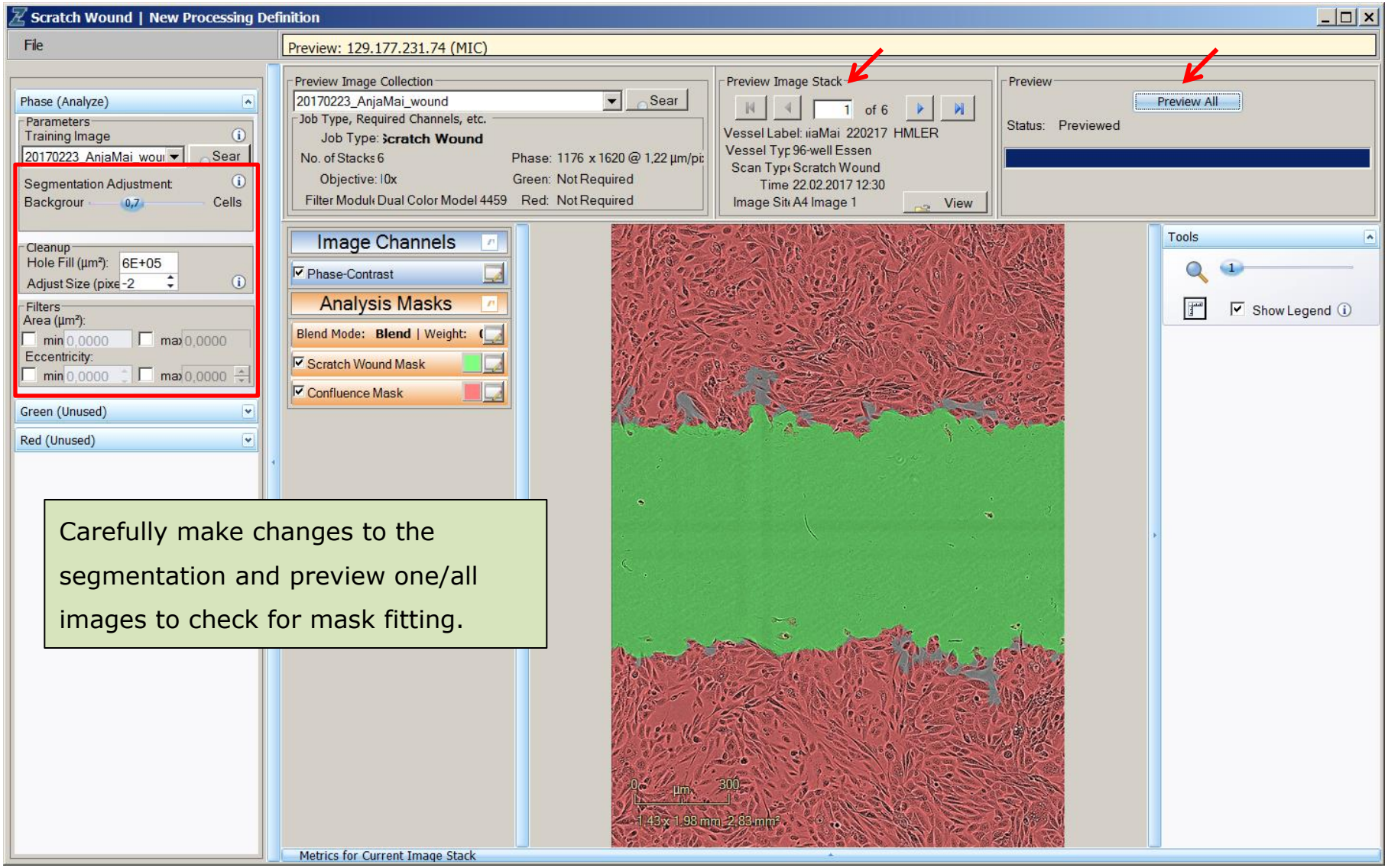
- Start the «Processing definition» by selecting the image set.
- Go between your selection images. «Preview current» will bring out the «analyse mask»

Analysing scratch wound assay



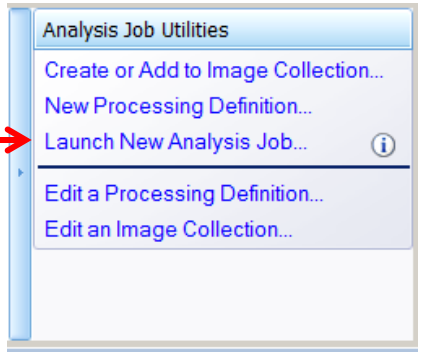
The goal is to create a clean scratch (green) by bringing the wound as close as possible to the cell surface and remove the cells/debris from the wound. The confluence mask (red) should also exclude the open gaps between the cells (clean-ups and filters).

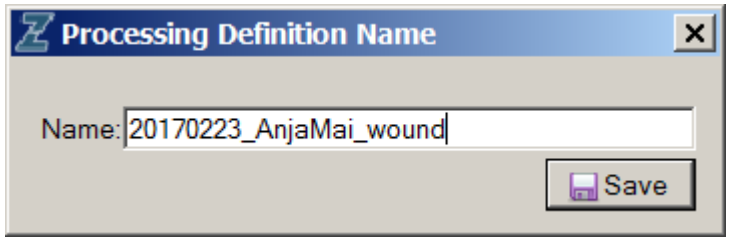
Analysing scratch wound assay



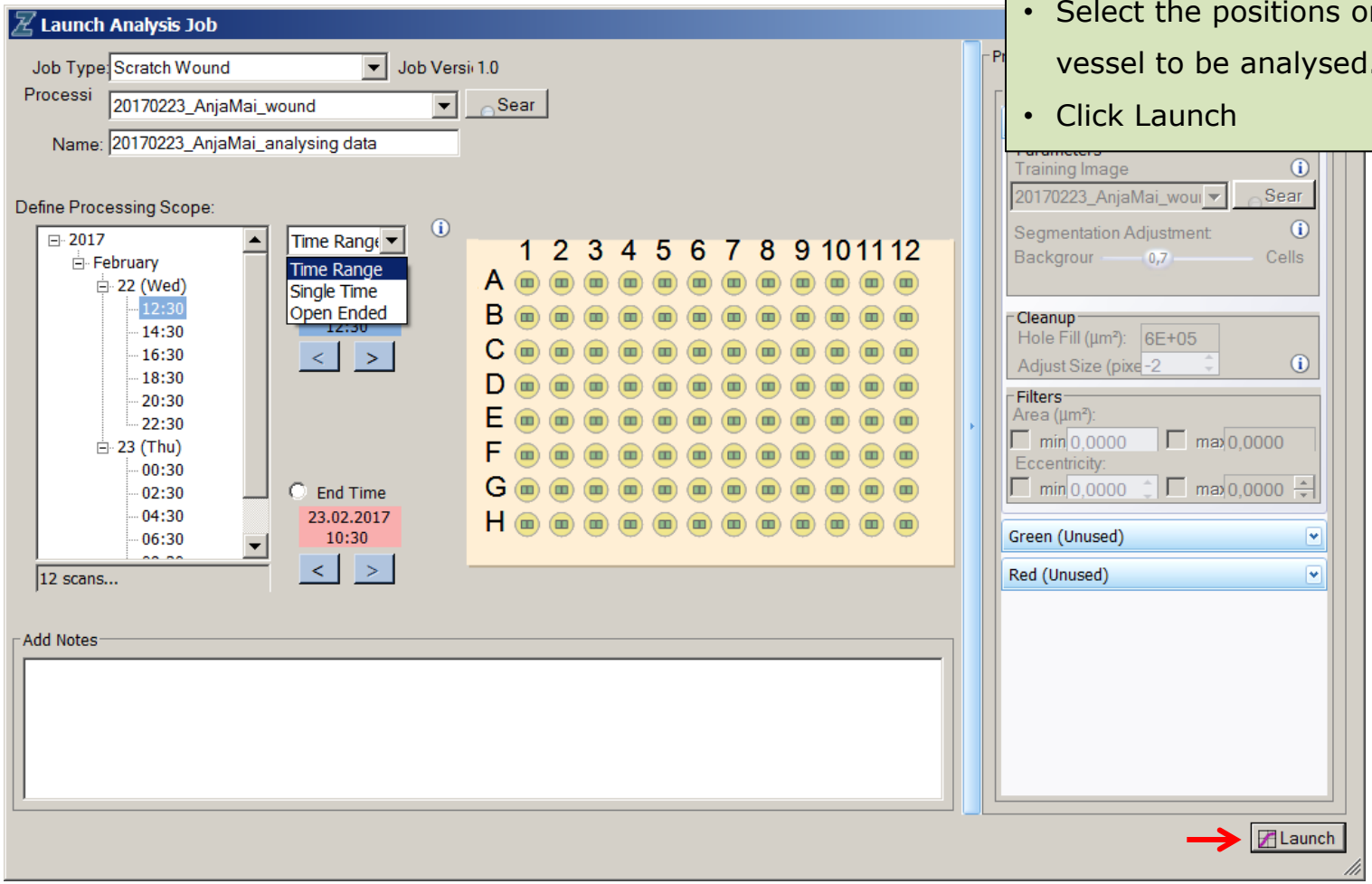
Carefully make changes to the segmentation and preview one/all images to check for mask fitting.

Analysing scratch wound assay

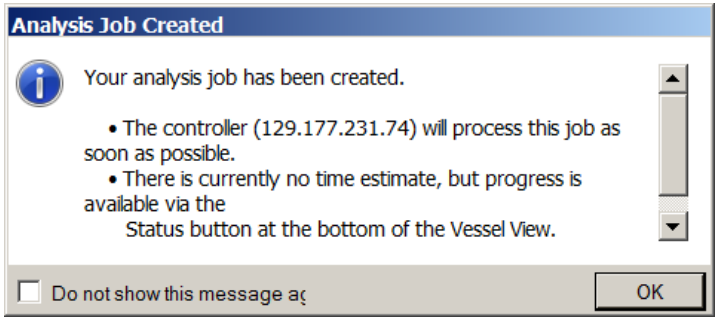




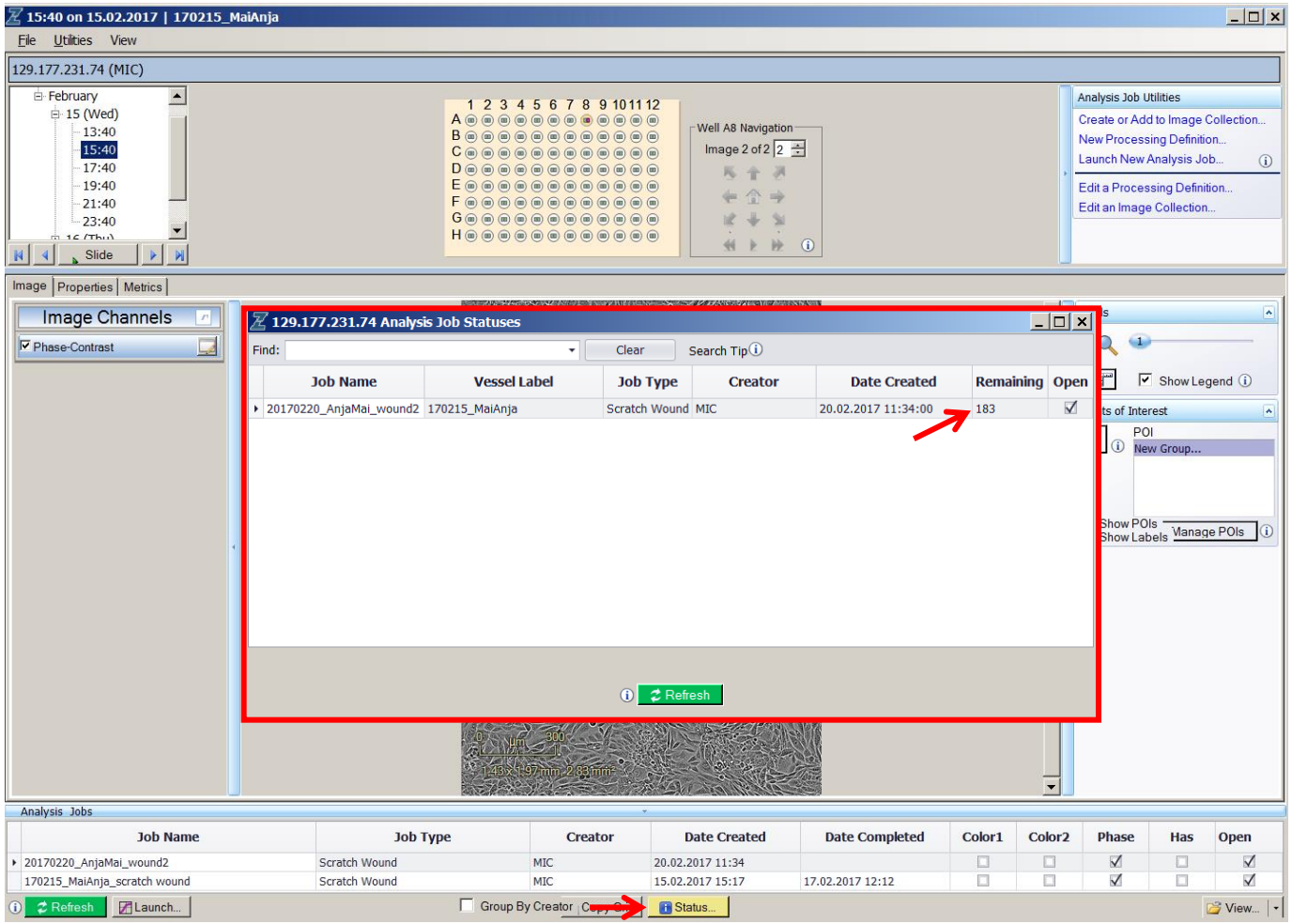
- Give the processing job a name.
- Select the time range (select open ended if your acquisition is not finished yet).
- Select the positions on your vessel to be analysed.
- Click Launch



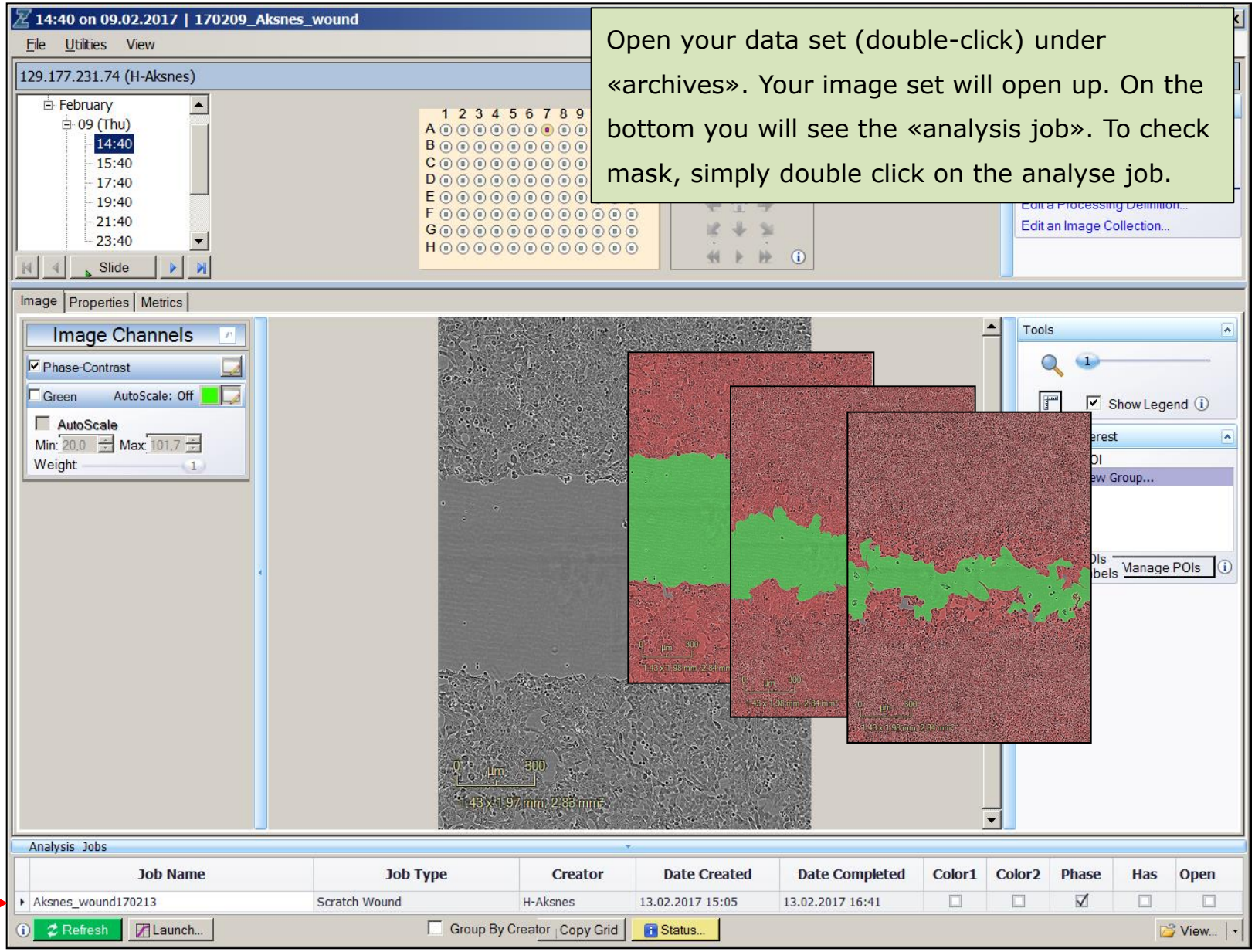
Analysing scratch wound assay



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.

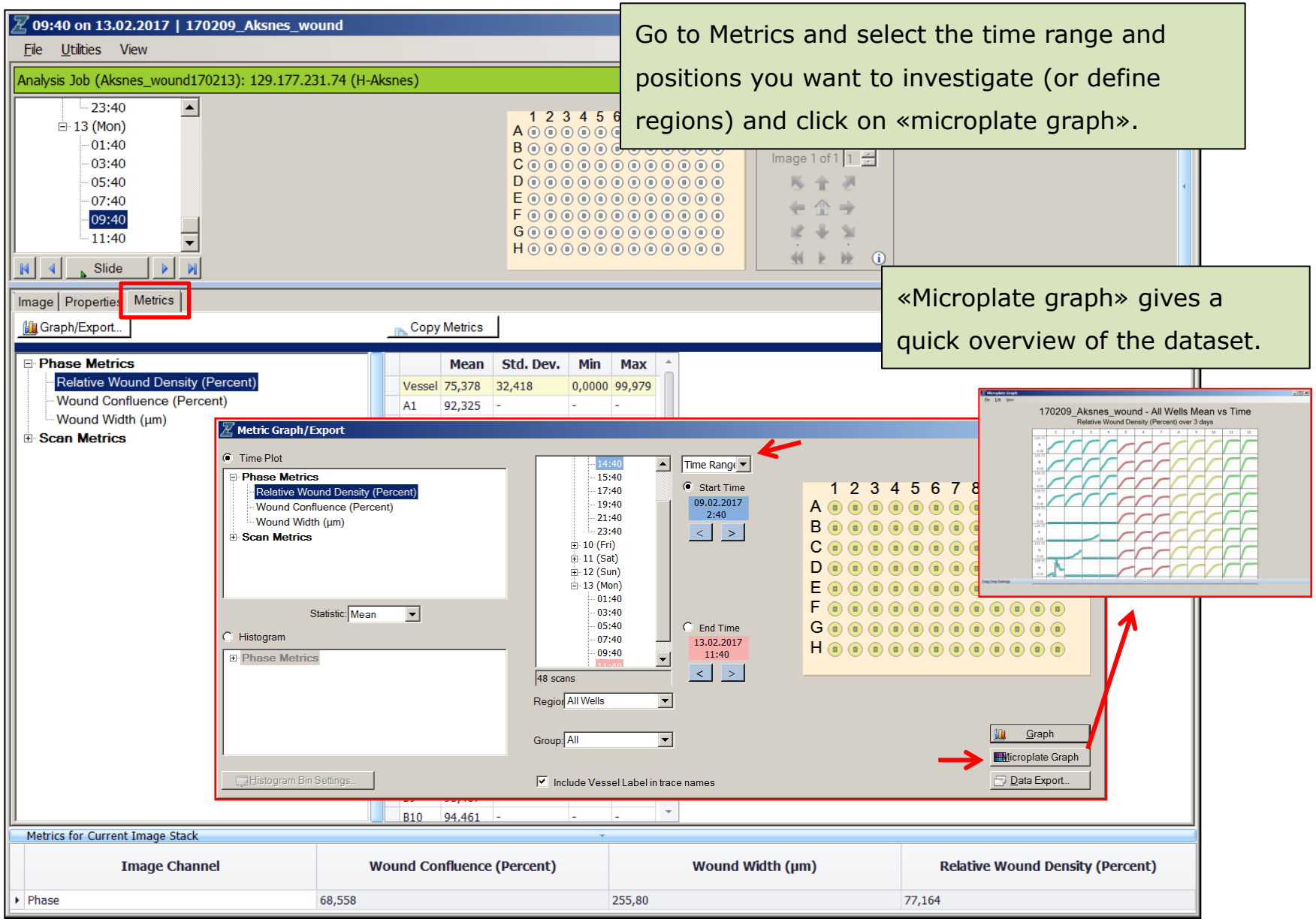


Viewing analysed data

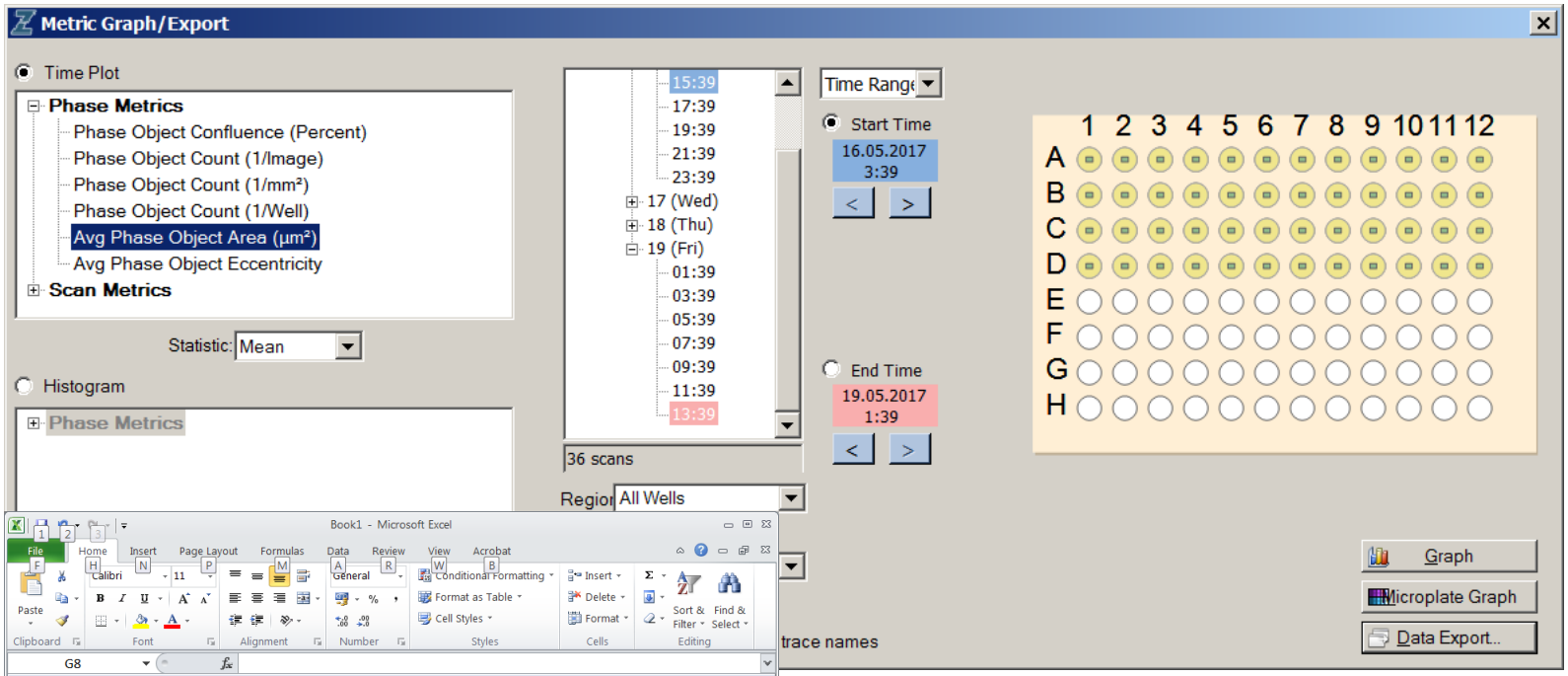


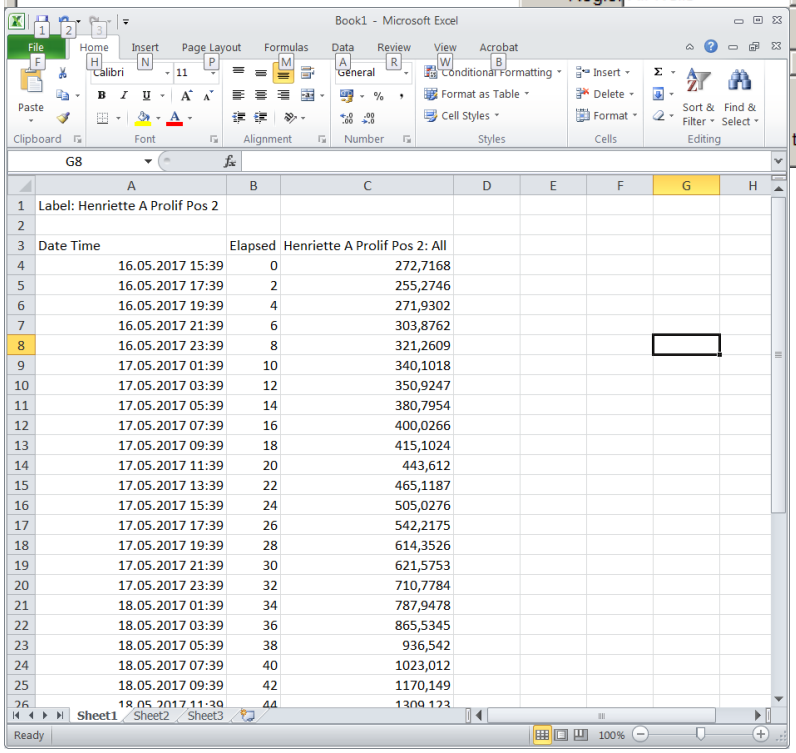
Open your data set (double-click) under «archives». Your image set will open up. On the bottom you will see the «analysis job». To check mask, simply double click on the analyse job.

Viewing analysed data – microplate graph



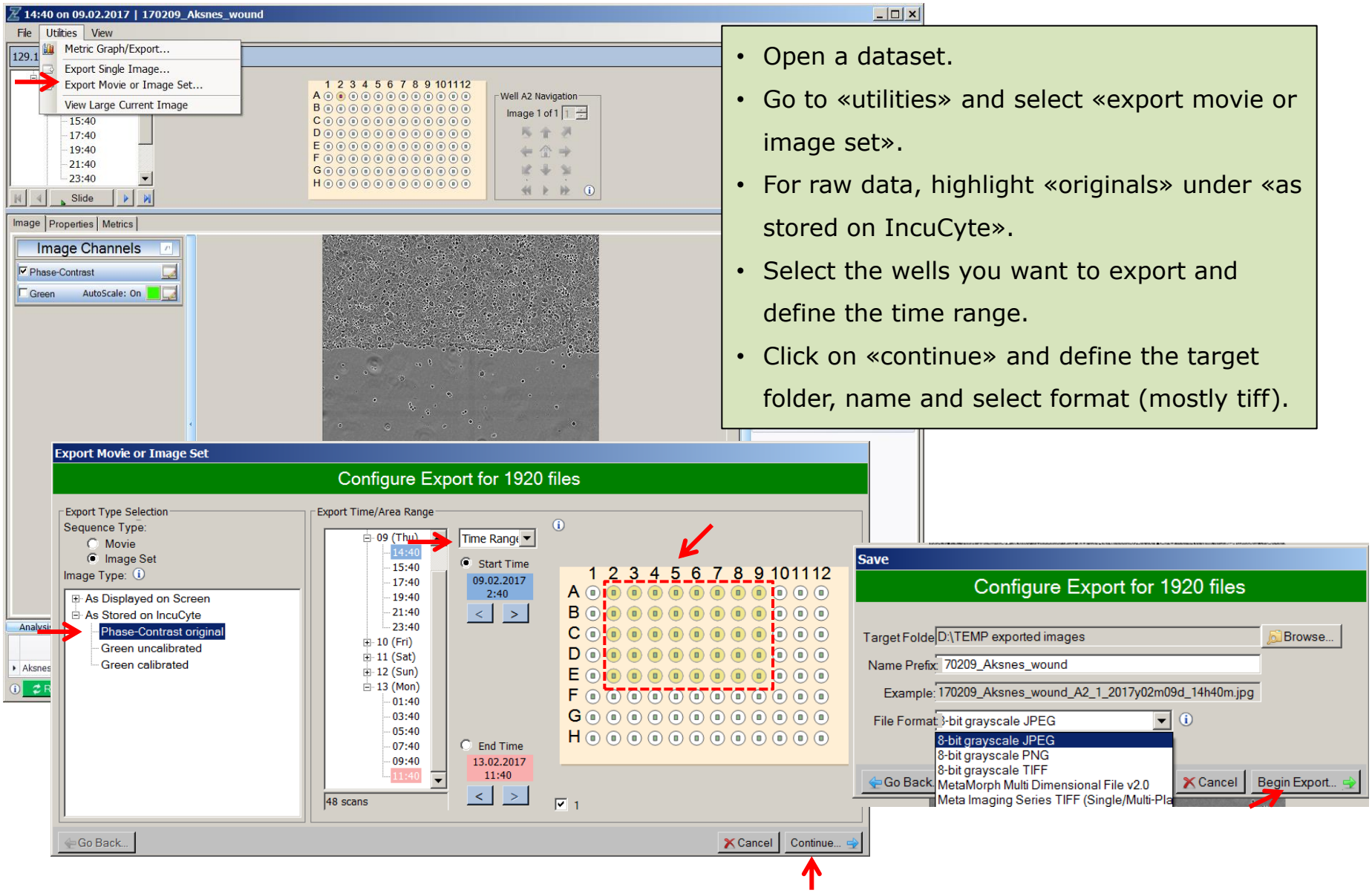
Exporting analysed data





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.

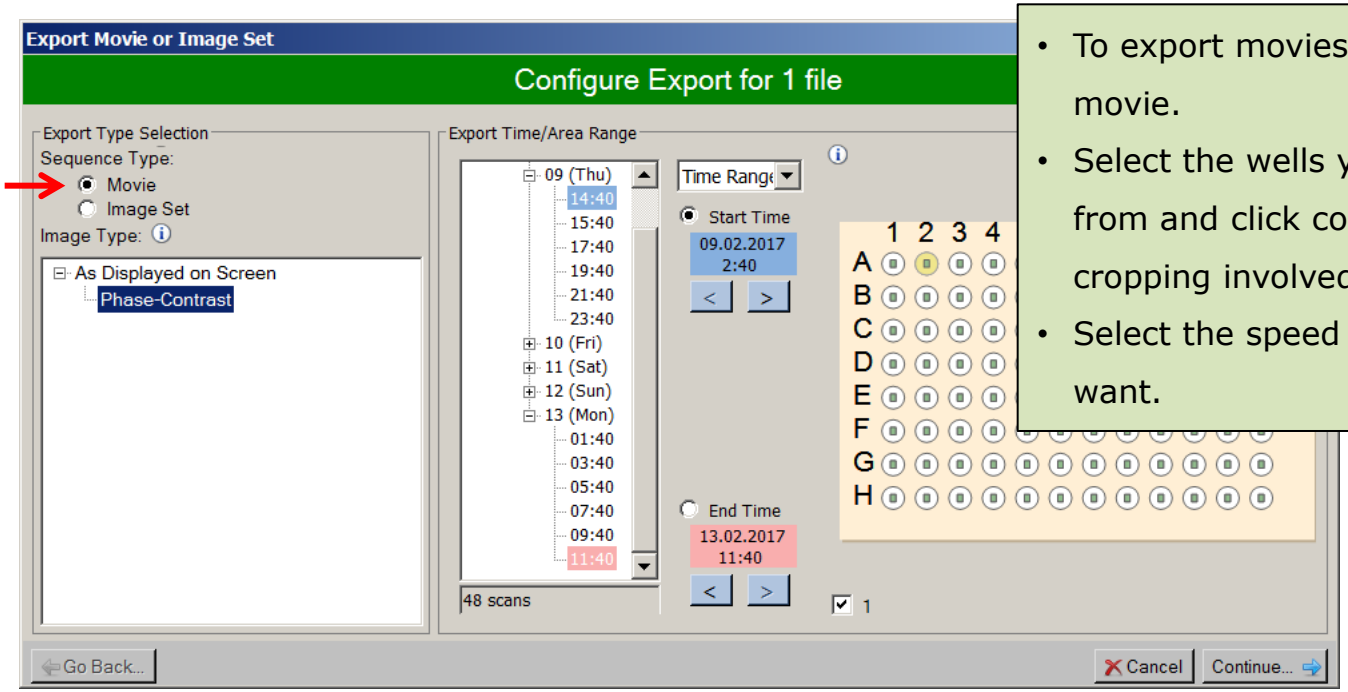
How to export image sets

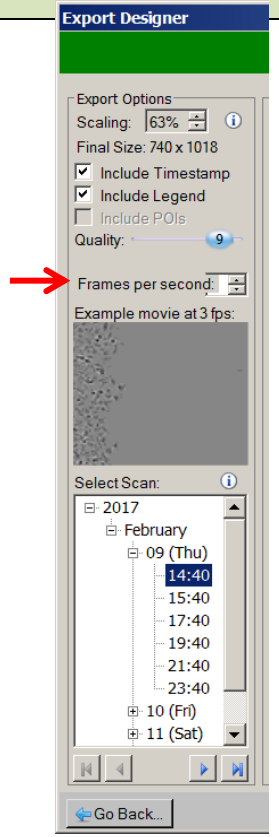


- Open a dataset.
- Go to «utilities» and select «export movie or image set».
- For raw data, highlight «originals» under «as stored on IncuCyte».
- Select the wells you want to export and define the time range.
- Click on «continue» and define the target folder, name and select format (mostly tiff).

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.



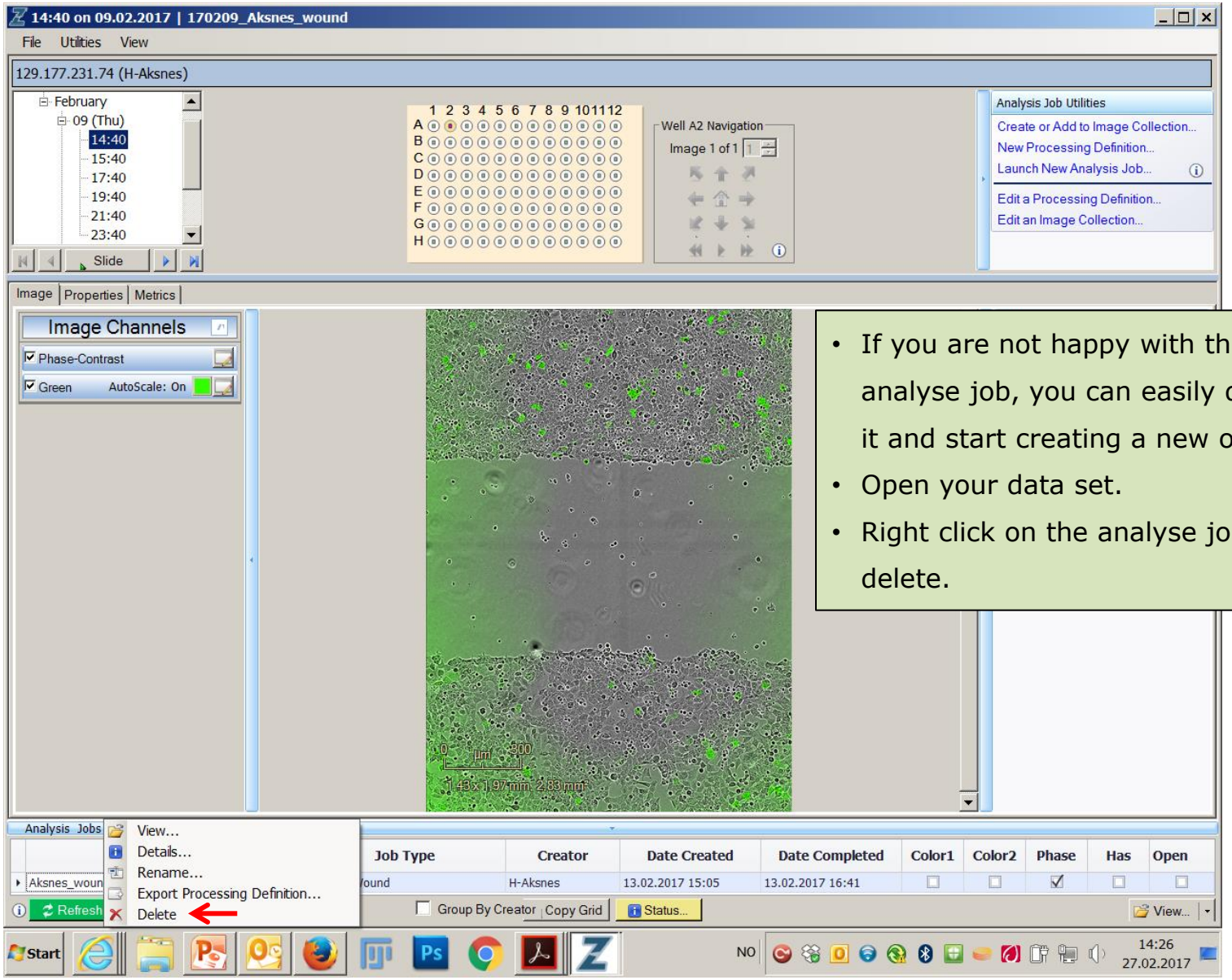


- You can either export the movie as raw data (phase contrast + fluorescent channel) or the data set with the masks.
- For the latter click on analyse (at the bottom) and check scratch and confluence mask before going to utilities-export movie.

170209_Aksnes_wound_A2_1.mp4

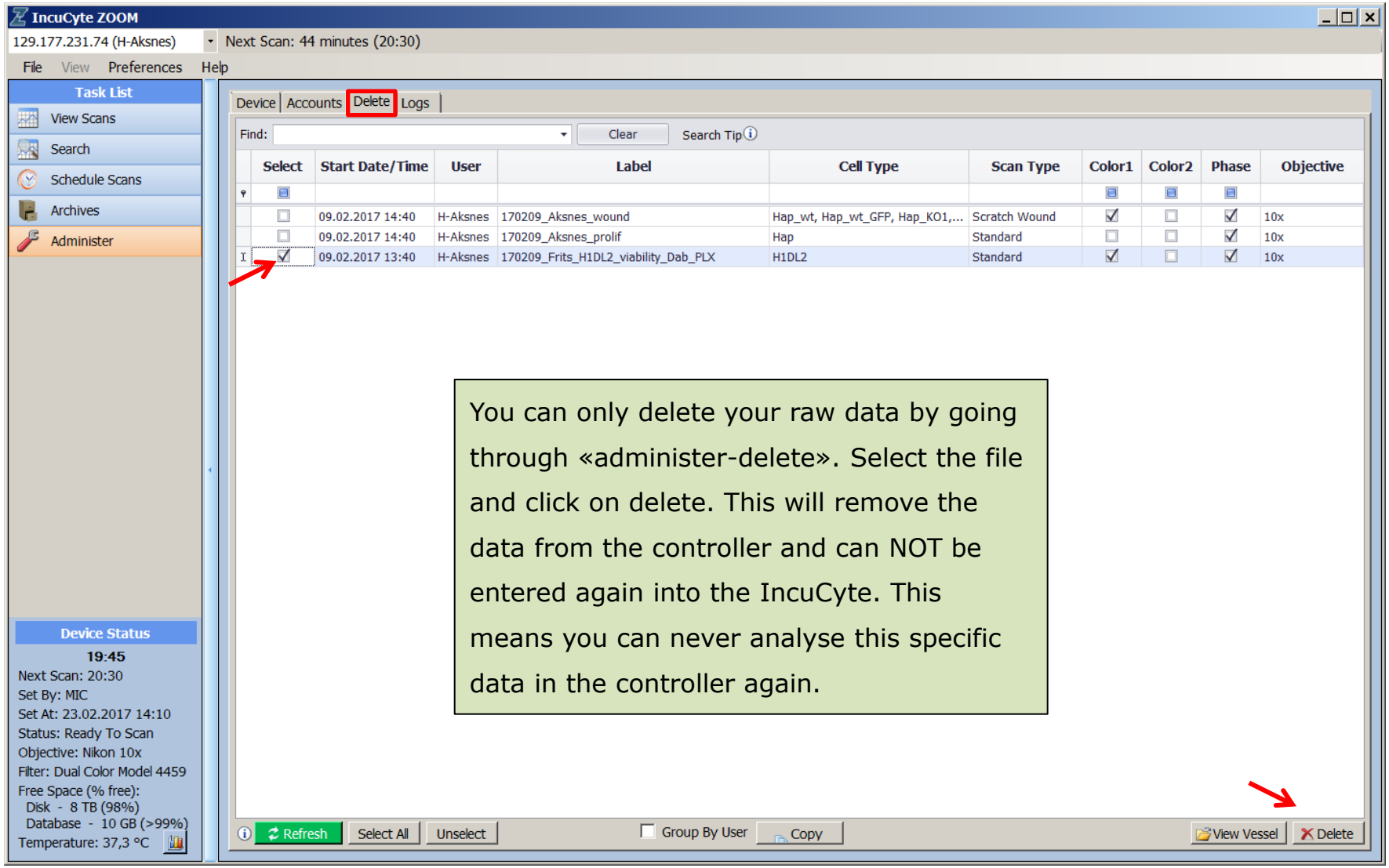
170209_Aksnes_wound2_A2_1.mp4

Deleting analysis jobs



- 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



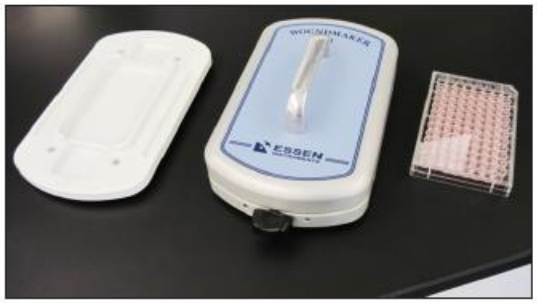
You can only delete your raw data by going through «administer-delete». Select the file and click on delete. This will remove the data from the controller and can NOT be entered again into the IncuCyte. This means you can never analyse this specific data in the controller again.

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

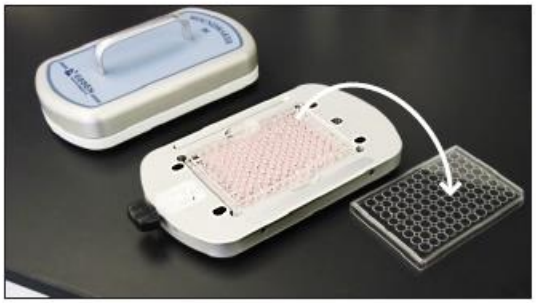
- Creates 96 homogeneous, 700-800µm wide wounds.
- Woundmaker needs to be cleaned before and after every use.
- Cleaning solutions must be **45 ml,** not more not less.
- Never place woundmaker directly on **bench** and never turn woundmaker **upside down**!
- Never leave any wells **dry**, this will damage pins.
- Never use woundkmaker with more than **100µl** 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



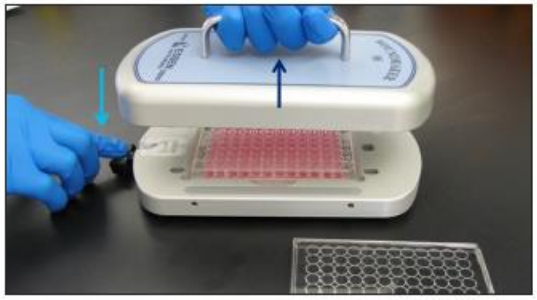
Step 1
- Remove top of WoundMaker™.
- Set top in empty wash boat.



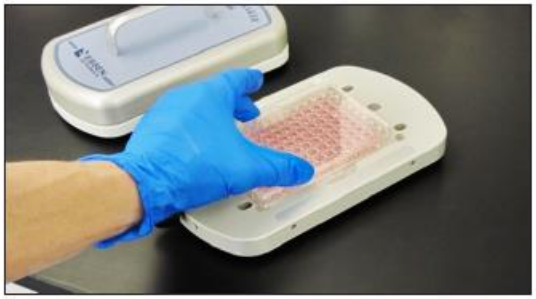
Step 2
- Insert plate (containing cells & media) into base plate holder.
- **Remove plate cover.**

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.