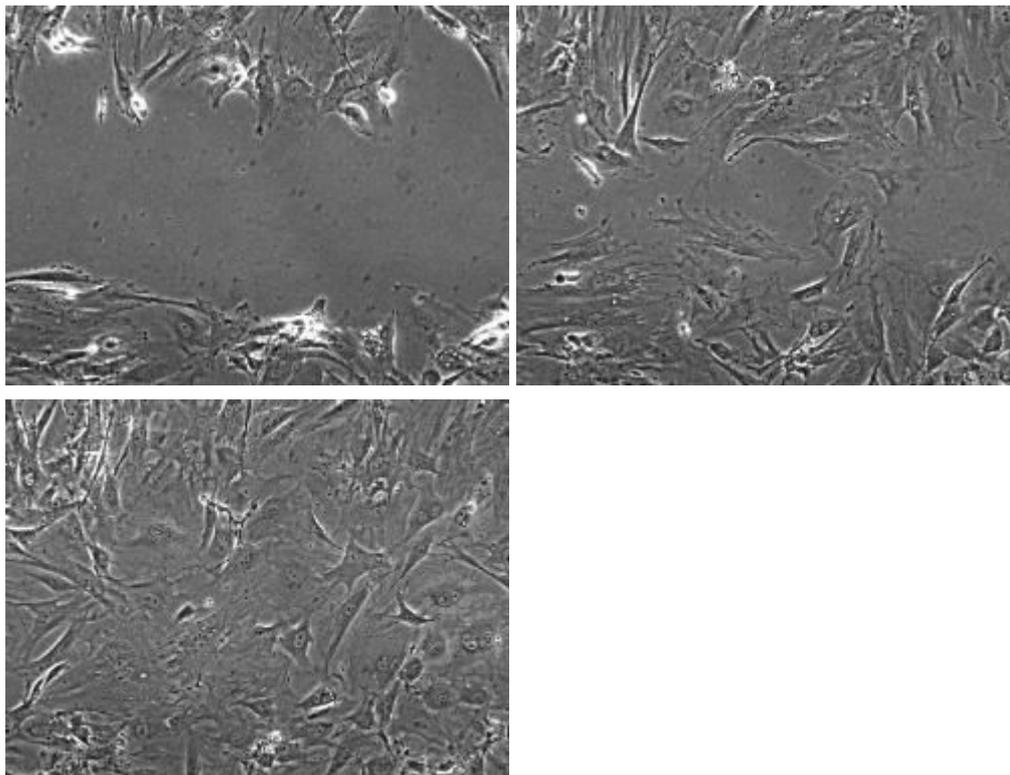


## Wound healing assay

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The wound healing assay allows the researcher to study cell migration and cell interactions. In some cases also single cell migration can be analyzed. This assay can be imaged using Nikon microscope 3 or the Olympus Cell<sup>R</sup>/Scan<sup>R</sup> system. Normally Nikon microscope 3 is used but if there is demand we can also write a protocol for the Olympus system. An automation is available for Nikon microscope 3 to image multiple positions in a multi well plate. At the moment we can image 60 positions. Normally we use a 10x Phase contrast objective, but recently we purchased a 4x objective that also can be used. CO<sub>2</sub> is available or you can add some HEPES to your medium.

Don't forget to pre-heat the microscope system for several hours or over night before you start the experiment.



Wound images after T=0h, T=12h and T=23h

## Protocol

1. Seed your cells in multi-well plates and culture until confluent. We normally use 6 well plates, but a plate with a larger number of wells is possible. If you use different treatments or different mutants you might have to adapt the seeding density. It is important that all the cultures are confluent at the start of the experiment.
2. Using a (yellow) pipette tip make a straight scratch, simulating a wound. Often we make a scratch keeping the pipette tip under an angle of around 30 degrees to keep the scratch width limited. This allows imaging of both wound edges using the 10x objective.
3. Check there is enough memory available on the HD; select Macintosh HD, go to 'File' ---> 'Get info'. The amount you need depends on the number of positions you image and the number of time points. For 18 positions and 144 time points 7.2 GB is enough.
4. Start the automation 'BF-timeseries-focusdrive'.
5. Double click 'Save image document' (next to yellow box) and select the subdirectory you want to save your files in.
6. Click 'run' to start running the automation. You will be asked a few questions about the number of files etc. The number of time points you can save in one go depends on the time intervals between time points and the total number of time points! Maximal number of positions on Nikon 3 is 60.
7. Select the positions you want to image. We often take 3 images per well. The first image you can focus using the focus knob on the microscope. After that **USE ONLY THE FOCUS KNOB ON THE JOYSTICK UNIT** to focus your image!!
8. When all positions are set the system will start imaging.

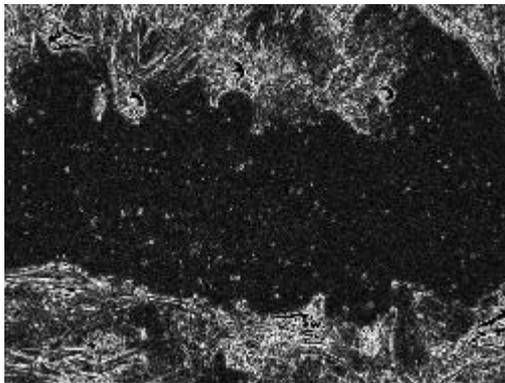
## Analyze your data

Separate the different positions stored in single files in one file per position using the automation 'separate-timeseries' and save as \*.tif files. Don't forget to select the directory your files are stored and to select a directory to save the new files as well as to change the file extension of the new files from \*.lif to \*.tif.

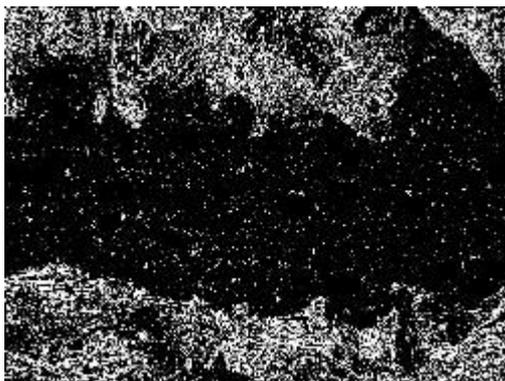
Open a \*.tif file in ImageJ

Follow the next steps:

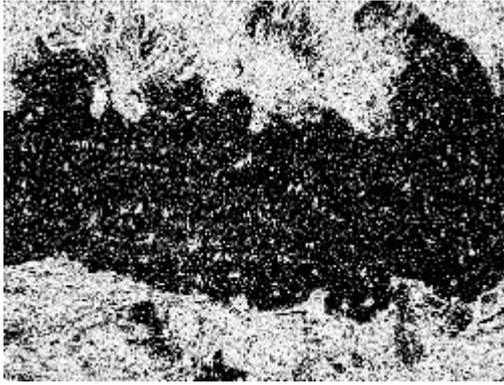
- Process ----> Find Edges
- Process ----> Sharpen



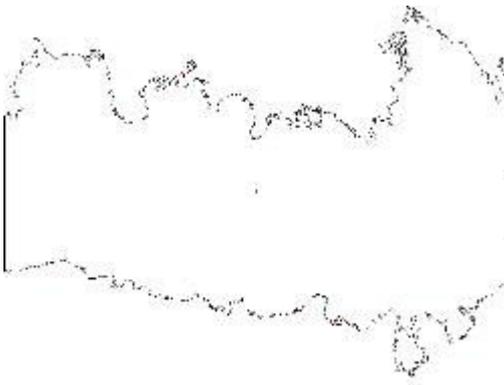
- Image ----> Adjust ----> Threshold; Select Black/White. Upper slider to 0 (left). Set lower slider in such a way that it is clear where the cells are. You might want to check a time point where the wound is partially closed to see if the difference between cells and wound are still visible.



- Process ----> Find Edges
- Image ----> Lookup Tables ----> Invert LUT

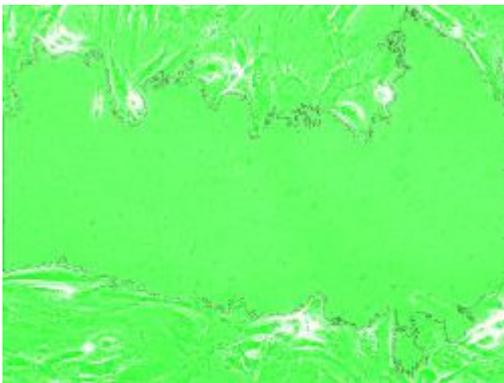


- Analyze Particles. Size: select a number that make sense if you look at a time point where the wound is partially closed (often a value between 100000 and 50000000; dependent on image size). Circularity: 0.00-1.00. Show: Outlines. Flag: Summarize.
- Save both the Summarized data file and the file with outlines. The data file will show the percentage of wound area in your image on every time point.



Outline of the wound

- The data file can be further analyzed using Excel.
- The outline file can be used to analyze if the wound area found corresponds with the real wound by merging this file with the original \*.tif file using Image ----> Color ----> RGB Merge (or using the Plugin 'RGB Gray Merge'). If necessary repeat the analysis with a different setting at the 'Threshold' or 'Analyze Particles'.



Merged image to check accuracy of the analysis.