## Prepare directories

Transfer “.lif” (microscope) files to “Data/lif\_files”

The directory “Data/wound\_outlines” will contain all outline files. The directory should be organized as follows:

**Experiment\_name** 🡪 **Condition\_name** 🡪 **well\_number** 🡪 **position\_number** 🡪 outline files

Things in bold should be folder names. All outline files should be “.txt” files.

## Outline cells

### Load a video

Open Fiji

Click: Plugins 🡪 Bio-Formats 🡪 Bio-Formats Importer

Select the appropriate lif file from the “Data/lif\_files” directory.

Click “Open”.

A menu will pop up. These options should be selected:

* Stack viewing 🡪 View stack with: 🡪 Hyperstack
* Color options 🡪 Color mode: 🡪 Default

Make sure that “Memory management 🡪 Use virtual stack” is unselected.

Click “OK”.

A menu will pop up.

Click “Deselect All”.

Check the box next to the video that should be imported. The line should end with something that looks like “(##T)”. If it ends with “(##C)” then it is a multichannel still image, not a video.

Click “OK”. The stack will load.

It is possible that a second menu labeled “Console” will pop up during the previous step. Close the “Console” pop up.

### Rotate the video

Click: Image 🡪 Transform 🡪 Rotate…

A menu will pop up.

Check “preview”.

Change the value for “Angle (degrees)” until the wound is vertically oriented with the cell sheet located on the left side of the image. The value for “Grid Lines” should be 1 and the value for “Interpolation” should be “Bilinear”.

Click “OK”.

A menu will pop up and ask if you want to process all images in the stack. Click “Yes”.

### Correct for stage shift

Click the square selection (the first shape in the main Fiji menu). Outline a temporally invariant region with which to perform stage shift correction.

Click: Plugins 🡪 Template Matching 🡪 Align slices in stack…

A menu will pop up.

Select:

* Matching method 🡪 Normalized correlation coefficient
* Search area (pixels around ROI) 🡪 10 (this choice depends on the amount of shift. A higher value may be needed if there is substantial shift.)
* Leave “Subpixel registration?” unchecked.
* Uncheck “show align coordinates in results table?”.

A menu will pop up.

Click “OK”.

Two menus will pop up. Close all of them and if prompted to save the output select “No”.

Check that the stage shift has been corrected. If it has not, then repeat the above steps with different parameter values.

### Set the scale

Click: Analyze 🡪 Set Scale…

A menu will pop up.

Click “Click to Remove Scale”.

Check “Global”.

Click “OK”.

### Outline the sheet

Click the freehand selection tool (the fourth shape in the main Fiji menu).

Starting on the wound front, outline the wound. Keep the mouse flush against the image edge when not tracing the wound front. Outline the side of the image that contains the cell sheet.

Click: File 🡪 Save As 🡪 XY Coordinates

Change the file name so that it terminates with the suffix “\_##m.txt”, where ## is the time point in minutes (i.e. 0, 60, 120). Save the image under the appropriate directory in “Data/wound\_outlines”. If the file name for any file is incorrect, then the analysis will fail. If any file in a different format ends in “\_##m.txt” then the analysis will fail.

In file explorer, navigate to the file that was just saved. Open the file. The following should be true:

* There should be two columns.
* Both columns should be composed solely of integers. If there are decimal values (or either of the next two bullets are not true) then you failed to perform “Set Scale” above.
* No value in the first column should exceed the number of pixels wide the image is.
* No value in the second column should exceed the number of pixels tall the image is.

The image dimensions are given in the upper-left of the image window. Example: “1/97 (t:1/97 – Mark\_and\_Find\_002/MPSA w2-l1-01009); **1392x1040** pixels; 8-bit; 134MB”. Here the x-dimension is 1392. The y-dimension is 1040.

Outline the next time point and save it as described above.

When done saving the outlines for all time points, close the video. When prompted to save the changes, select “Yes”. Save the “.tif” file to the appropriate “Data/wound\_outlines” directory.

### Analyze the data

Open the program “R Studio”.

Click: File 🡪 Open File…

Select the file “Main.R” in the analysis directory.

Click: Session 🡪 Set working directory 🡪 To source file location

Click: Code 🡪 Source

When a new line with only the symbol “>” appears in the console, analysis has completed.

Occasionally, red text will appear that says something along the lines of “There were 16 warnings (use warnings() to see them)”. If this appears type “warnings()” into the console and hit enter. If all of the errors say something along the lines of

1: In .Internal(isNamespaceEnv(ns)) :

closing unused connection 18 (<-JonesLabENVY:11597)

then there is no problem. R is simply warning that the socket connections to parallel R threads generated during analysis are being automatically closed.

Navigate to the “Results” directory to retrieve the analysis results.