The following document is a tutorial on our Lifeact analysis tool. It serves as an introductory getting-started guide.

Contents

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1 Requirements and Comments

- The code has been tested on all versions of Matlab between R2014a and R2016a. It has been tested for Windows (7, 8, 10), OS X (10.9) and Linux (Arch). The GUI looks best on Windows, with minor scaling issues on the other platforms.
- The code uses some third-party packages, that we include in our repository. This is the complete list:
  - **Ncorr**[1]: DIC algorithms.
  - **Bio-Formats toolbox**[2]: read image and video formats.
  - **xlwrite**[3]: write results to Excel files.
  - **peakdet**[4]: determine peaks on noisy curves.
  - **statusbar**[5] and **enableDisableFig**[6]: freeze the windows during calculations and estimate runtime.
  - **freezeColors**[7] and **COLORMAP and COLORBAR utilities**[8]: display overlays on figures.
  - **Exportfig**[9]: flexible plot exports.
- On recent versions of Matlab, the GUI produces a variety of warnings related to packages used for blocking the interface during calculation. These can safely be ignored.
- During execution, temporary files as well as result data is written to the disk. Thus it is advisable to have at least a few hundred MB of free disk space. Also, all the functions and the file structure has to be left unaltered, and put in a place that has writing permissions.
- Windows will generally freeze during intense calculation to prevent all user input, then resume post-computationally. Calculations can however be stopped using the usual ctrl + C key combination. It is mandatory to close all windows with there respective OK buttons, not using the OS window close button.

[1] http://ncorr.com/
[2] http://www.openmicroscopy.org/site/support/bio-formats5/users/matlab/
[3] http://www.mathworks.com/matlabcentral/fileexchange/38591-xlwrite--generate-xls-x--files-without-excel-on-mac-linux-win
[4] http://www.billauer.co.il/peakdet.html
[5] http://www.mathworks.com/matlabcentral/fileexchange/14773-statusbar
[6] http://www.mathworks.com/matlabcentral/fileexchange/15895-enable-disable-figure
[7] http://www.mathworks.com/matlabcentral/fileexchange/7943-freezecolors---unfreezecolors
[8] http://www.mathworks.com/matlabcentral/fileexchange/24371-colormap-and-colorbar-utilities--jul-2014-
[9] http://www.mathworks.com/matlabcentral/fileexchange/727-exportfig

2 Tutorial

The main window is opened by executing `lifeact_main.m`, and changing the working directory to the current folder. Figure 1 shows the main window of the analysis.

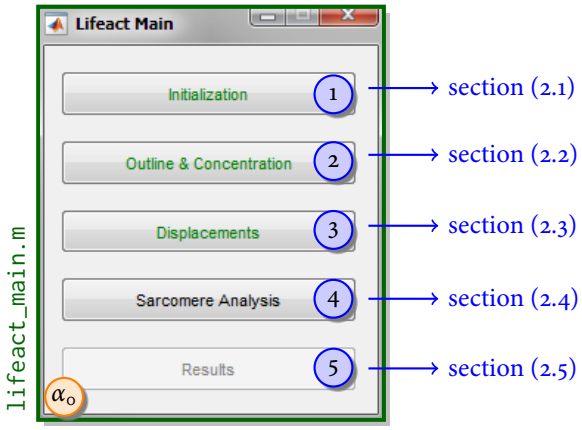


Figure 1: Main window; in blue, the relevant sections of the tutorial

1 – 5 The analysis is split into five parts, each of them requiring user setup and review. The order of progress is by increasing number.

α_0 Steps which are not accessible at user's current stage are greyed out, steps which have been completed show up in green typeset.

2.1 Initialization Window

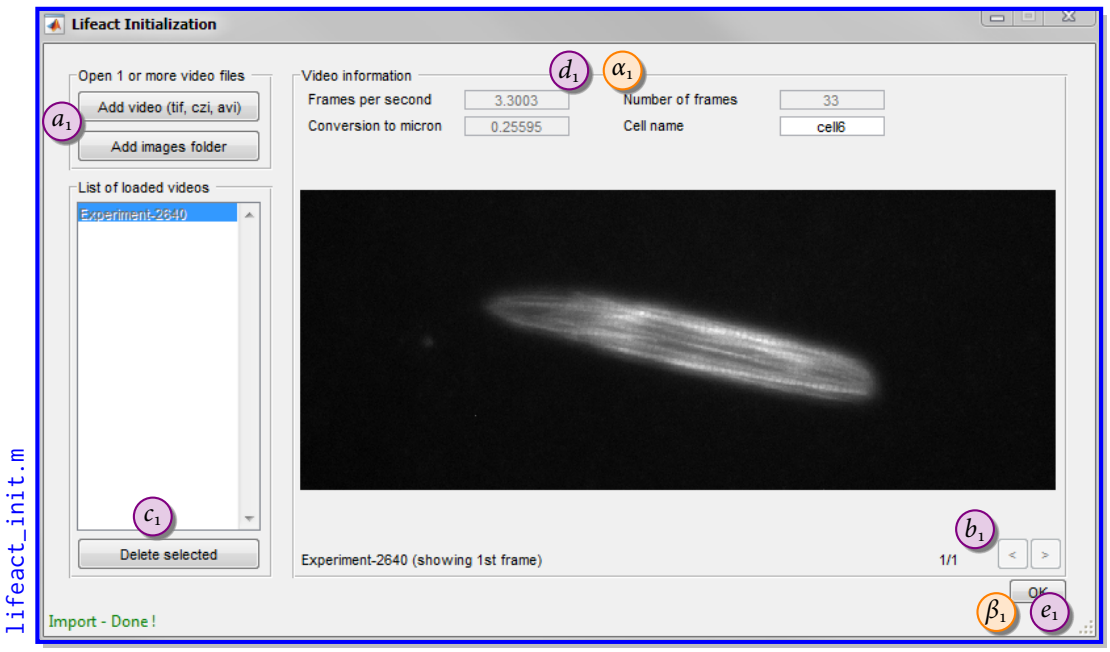


Figure 2: Initialization window

(a_1) One or more videos must be added to the analysis. Videos can be in the following formats:

- `*.czi` video files
- `*.avi` video files
- `*.tiff` image stacks

It is also possible to indicate a folder containing `*.png` images. Images are read in based on the order that they are listed in the folder, so images should be in the correct order, e.g. {`im_001.png`, `im_002.png`, `im_003.png`,...}. Be careful to pad numbers in file names with zeros, `im_11.png` generally list before `im_2.png`.

(b_1) Once multiple videos are loaded, it is possible to review and browse through them. The axes displays the first frame of the respective video, and the video information is chosen accordingly.

(c_1) Videos can be deleted from the analysis list, after they have been loaded. This may be useful if a whole list has been input, but one video is not suitable for analysis.

(d_1) Some information about the video is needed, in particular the framerate, the number of frames and the resolution. For indentification purposes in view of post-processing, cells should also be assigned a unique and characterizing name tag. No two videos are allowed the same cell name !

Depending on the format of the videos, the metadata is accessed automatically.

- For `*.czi` video files: whole metadata is available.
- For `*.avi` video files, `*.tiff` image stacks, image folders: user has to input framerate and resolution, whereas the number of frames is detected.

(e_1) The video information is saved in the cell-specific Excel sheet. For more information about this, see section 3 on folder and files organization.

(α_1) Based on video format, panels are enabled and disabled in a smart way. For instance for videos where the framerate is already available, no user input is required, which causes the respective input boxes to be greyed.

(β_1) In case that the OK button is activated, but important information is still missing, e.g. the conversion factor for one video has not been entered, a reminder message will pop up.

2.2 Outline & Concentration Window

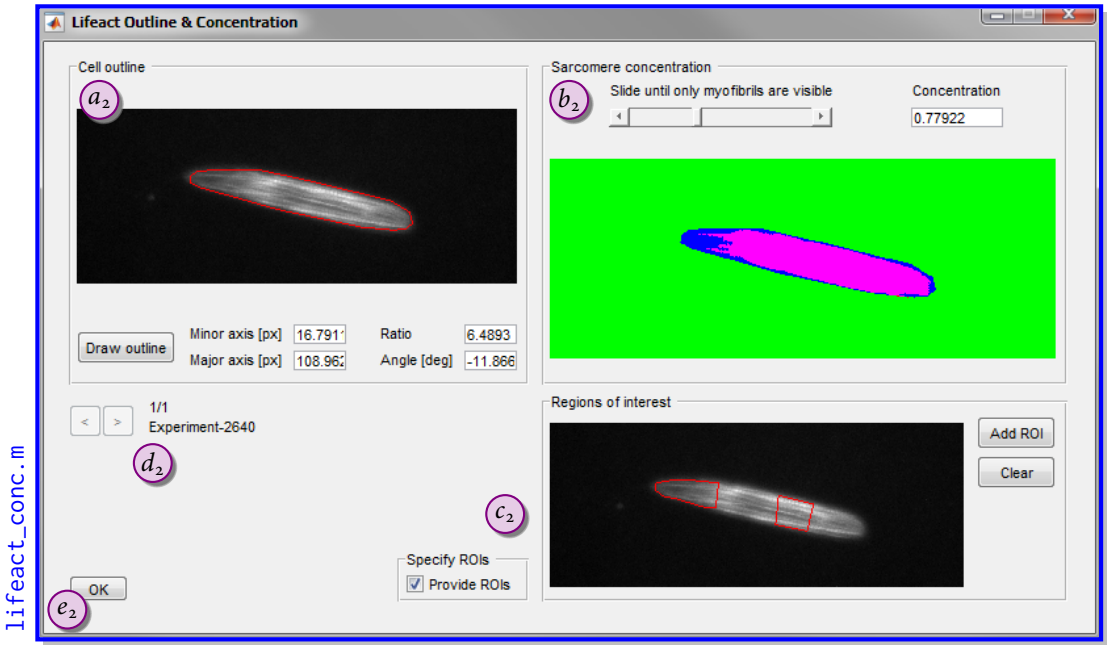


Figure 3: Outline & concentration window

a_2 The cell outline should be drawn. Then a preview of the cell ratio and angle is shown to verify the selection.

b_2 Sarcomere concentration, i.e. ratio of the cell that is filled with myofibrils, can be determined by changing the slider value until the desired picture is achieved. It is impossible to a have a concentration bigger than 1, therefore everything outside the cell that was selected with the slider threshold is neglected. Uneven illumination can be an issue here.

c_2 For each cell, in addition to the cell as a whole, which is a mandatory step, the user can opt to additionally specify one or more regions of interest, for which parameters should also be determined.

d_2 These first three steps should be repeated for every video that has been loaded.

e_2 Closes the window.

2.3 Displacement Window

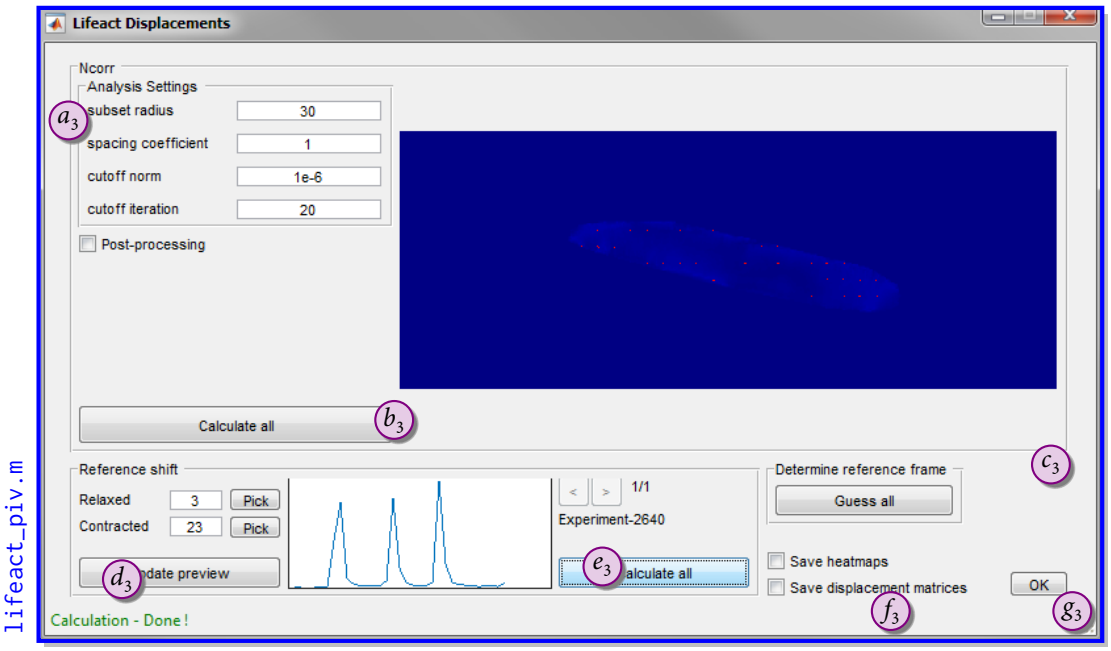


Figure 4: Displacement window

(a_3) Displacements are computed with the Ncorr DIC code. Lifeact videos are sparse on markers for exact displacement calculation. It is absolutely essential that the computed displacements are as accurate as possible.

For DIC, the important parameters are subset size and radius. The size parameter is a tradeoff between accuracy and time-efficiency. Ideally it would be set very low (ideally 0) to get very high resolution, whereas large values produce an excessive smoothening effect. The radius is pretty tricky to set. It should ideally be as small as possible without getting noise effects.

To improve performance, only areas inside the cell outline are considered for displacements.

Important Note: Also it is possible to change these parameters, it is not recommended. The optimal settings are a very small spacing value (0 or 1 ideally), as well as no post-processing.

(b_3) All videos are processed with identical analysis settings. The preview gives a heatmap preview of the current frame, with a fixed pre-set axis scale and vector length. For some videos, the preview thus might not look amazing, however usually this is a scaling issue.

(c_3) Displacements are calculated with an arbitrary reference frame, the first frame of the video. For display and interpretation purposes, it is generally favourable to have a relaxed cell state as reference. This step smart-guesses the optimal reference frame.

(d_3) The reference auto-guessing usually is very reliable. However there are cases, where the prediction is not accurate, e.g. when the videos are really small, or the cell movement is particularly unsynchronized, coupled with excessive image noise. A new reference value can be selected by picking a point as reference or inputting a frame number.

The contracted frame is not used for further calculations, so it is not necessary to change this, too. The reason the guess for the contracted frame is given is that for the unlikely case that the auto-guess failed, usually the issue can be fixed by exchanging relaxed and contracted guesses.

e_3 The previews should be visually inspected for all videos. Afterwards, displacement data has to transformed to the new reference. In addition, the synchronicity calculation is performed at this stage.

f_3 A lot of data is generated during the DIC analysis, which can optionally be stored for further processing. The options here are: saving the full displacement field in matrix form, as they are used during the analysis, and saving the preview heatmaps.

Important Note: Saving takes some time and should only be enabled if necessary.

g_3 The window is closed and the selected saving options are written to the disk. All Excel result files are updated with reference and contracted frame.

2.4 Sarcomere Analysis Window

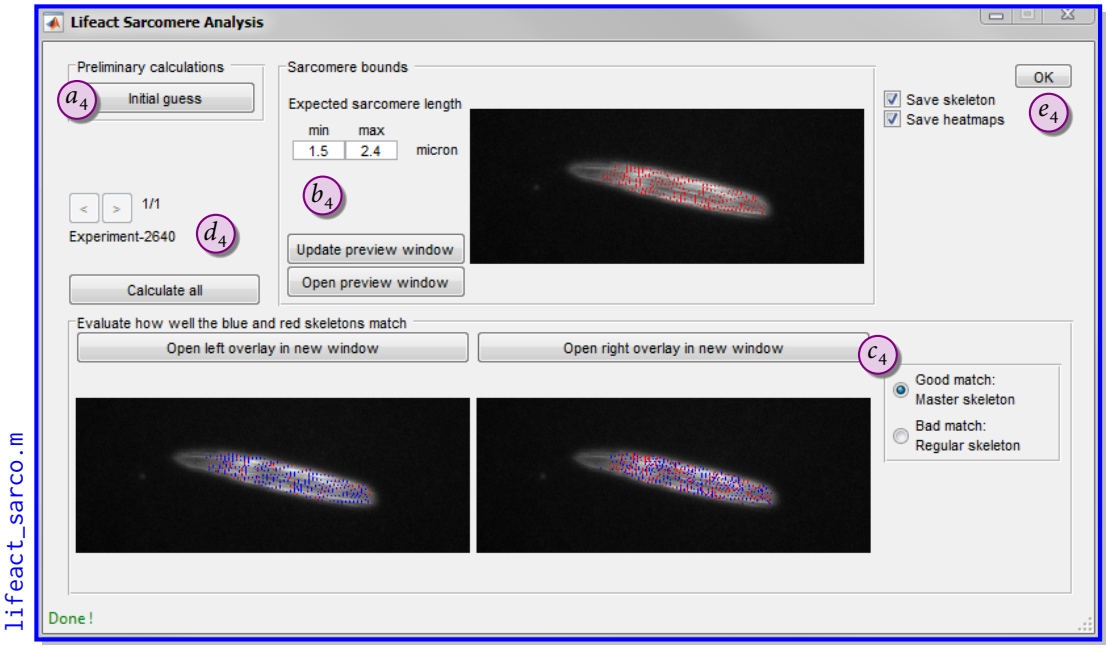


Figure 5: Sarcomere Analysis window

a_4 Using an initial guess for sarcomere ranges, the skeleton for the first video frame is previewed. In addition, both the relaxed and contracted state are translated back to the first frame, for user evaluation if the displacement information is suitable for master skeleton analysis.

b_4 The skeletonization needs guesses for the sarcomere ranges, in order to generate valid z-lines. Special care has to be taken to restrict this to a useful range. For instance if sarcomere means are expected to lie around 1.3 micron, the maximum should be lower than 2.6, and the minimum larger than 0.65, in order to avoid double or half periods.

c_4 It is preferable for both accuracy and comparability to generate a single master skeleton for each cell. However, in order for this to work, displacement calculations have to be really precise. The user should review both the left and right image, and evaluate how well red and blue skeletons correspond.

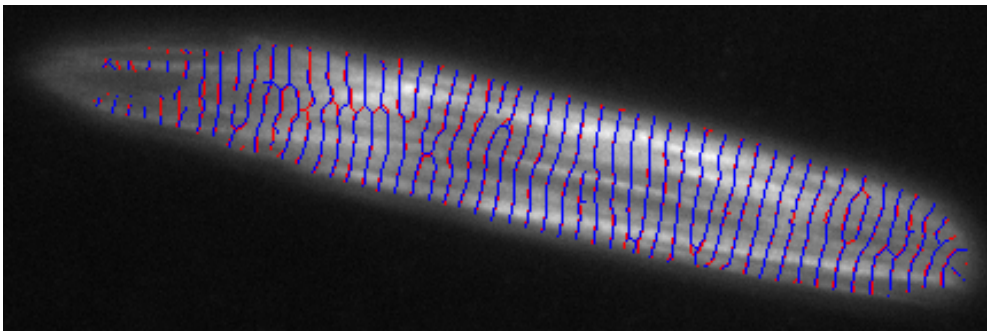


Figure 6: Example of a good match

Then a choice is made whether the regular or the master analysis shall be performed.

d_4 After the choice has been made for all videos in the stack, the calculation can begin. Skeleton images as well as distance heatmaps are saved to the disk (c.f. section 3). If unsure, the user should review these after calculation is done to make sure the sarcomere ranges were chosen correctly.

e_4 If the heatmaps and the skeleton images should be kept, the user can opt to do so. This also closes the window.

2.5 Parameter Window

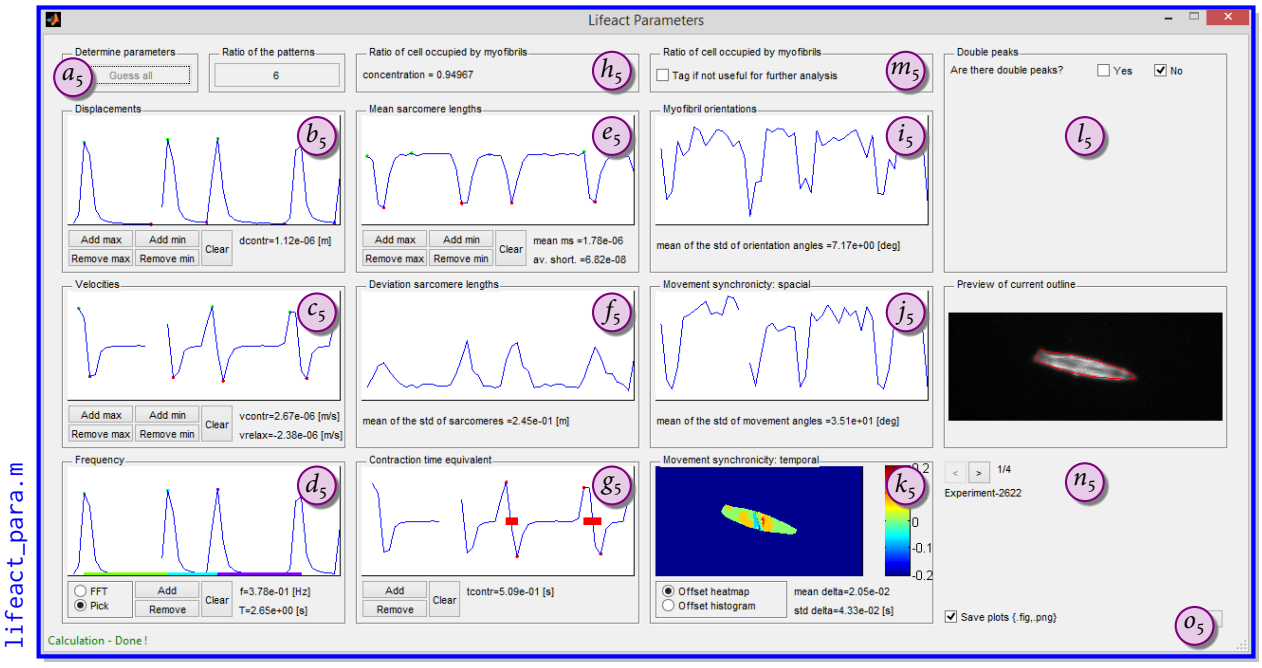


Figure 7: Sarcomere Analysis window

Note: all of the following parameters can be left empty. If for instance one curve does not look good, and it is not possible to extract reliable information, just leave it out. The associated parameter will appear as NaN, which is left empty in Excel later.

a_5 The maxima and minima of the displacement and velocity curves can be estimated in an automated way. This is not always accurate, and should be reviewed and corrected.

b_5 Average displacement defined for each timepoint (i.e. frame) as the mean of all displacements inside cell blob,

$$d(t_k) = \frac{1}{N} \sum_{k}^{N} \sqrt{u_{k,x}^2 + u_{k,y}^2} \,. \tag{1}$$

The contraction displacement then is the total distance between relaxed and contracted state, or in other words, the distance between minima and maxima on the $d(t)$ curve,

$$d_{\text{contr}} = \frac{1}{m} \sum_{i}^{m} \max_i \left(d(t_k)\right) - \frac{1}{n} \sum_{j}^{n} \min_j \left(d(t_k)\right) \,. \tag{2}$$

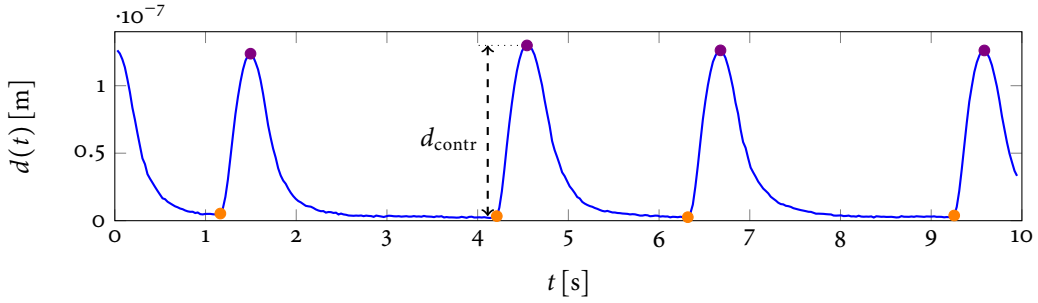


Figure 8: Displacement plot

c_5 Average velocity defined for each timepoint (i.e. frame) using the mean displacements $d(t_k)$,

$$v(t_k) = \frac{\Delta d}{\Delta t} = \frac{d_{k+1} - d_{k-1}}{t_{k+1} - t_{k-1}} \tag{3}$$

The maximal contraction and relaxation velocities then correspond to maxima and minima of the $v(t_k)$ curve,

$$v_{\text{max,contr}} = \frac{1}{m} \sum_{i}^{m} \max_i \left(v(t_k)\right) \quad ; \quad v_{\text{max,relax}} = \frac{1}{n} \sum_{j}^{n} \min_j \left(v(t_k)\right) \,. \tag{4}$$

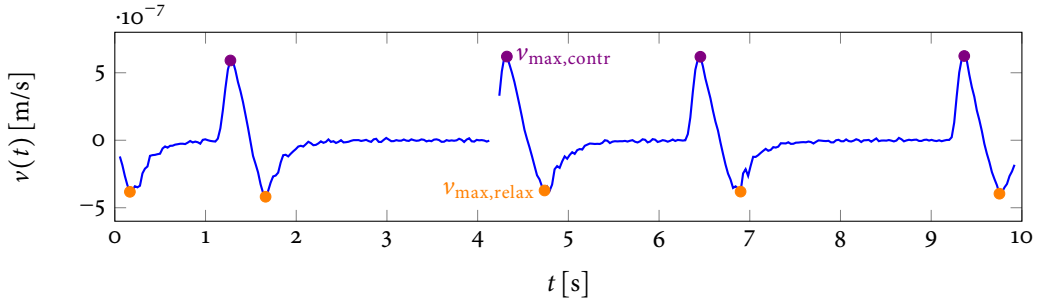


Figure 9: Velocity plot

d_5 To determine frequency of contraction, there are two possibilities.

(i) The signal can be Fourier transformed. Then the peaks correspond to main frequencies of the signal.

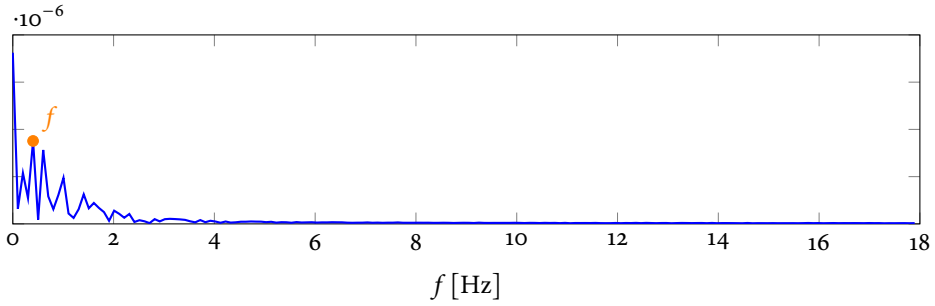


Figure 10: Fourier transformed signal

(ii) Use the definition of frequency as inverse of the period $T = \frac{1}{f}$. Using the displacement signal $d(t_k)$, it is easy to draw periods on the curve. For multiple contractions, the mean can then be computed,

$$f = \left(\frac{1}{m} \sum_i^m T_i \right)^{-1} . \tag{5}$$

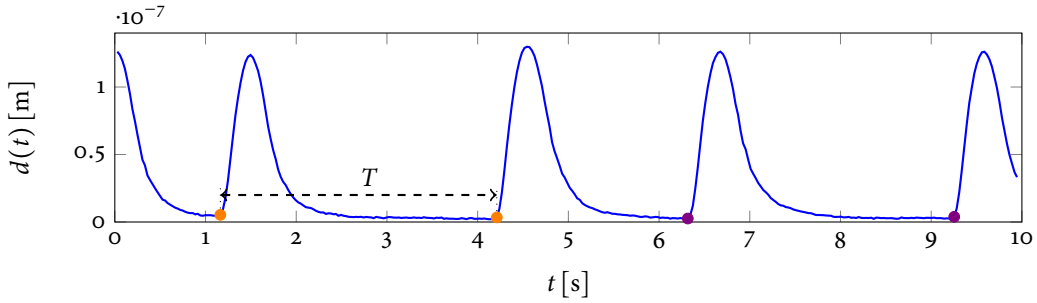


Figure 11: Displacement plot with time period

Method (ii) is probably the safer choice, as it is quite clear what is selected, especially in cases where the curve is not really periodic.

e_5 Displays the mean sarcomere length in the cell body over time. The user has to select maxima and minima, in order to calculate the average sarcomere shortening,

$$\text{shortening} = \frac{1}{m} \sum_i^m \max_i (\bar{s}(t_k)) - \frac{1}{n} \sum_j^n \min_j (\bar{s}(t_k)) . \tag{6}$$

The mean over average sarcomere lengths in time is also shown.

f_5 Plot of the deviation of sarcomeres inside the cell as a function of time. No user input required.

g_5 It is difficult to evaluate the contraction time of one contraction, as it is hard to determine when it exactly starts and ends. However one can take a value that scales proportionally to contraction time: we take the

time between a velocity maximum and minimum of a contraction cycle,

$$\hat{t} = \frac{1}{m} \sum_{i}^{m} \left\| \left[t_k \middle| \max_i v(t_k) \right] - \left[t_k \middle| \min_i v(t_k) \right] \right\| . \tag{7}$$

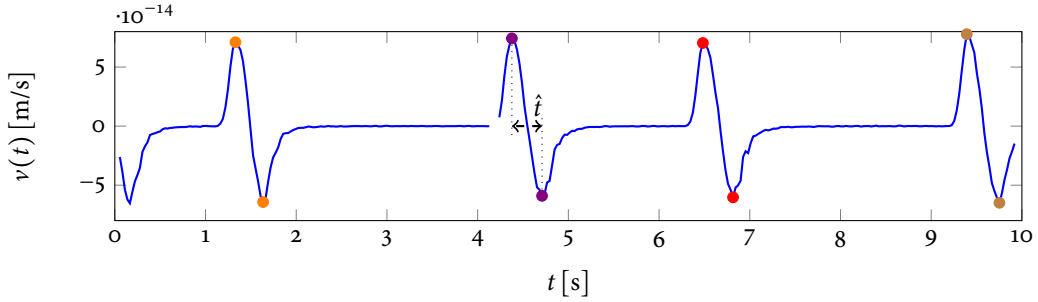


Figure 12: Velocity plot with $\hat{t}$

(h_5) Ratio of the cell that is taken by sarcomeres,

$$c = \frac{A_{\text{cell}} \cap A_{\text{sarco}}}{A_{\text{cell}}} \tag{8}$$

(i_5) Deviation of myofibril orientation angle, as a measure of how organized the myofibrils are. No user input required.

(j_5) Displays the evolution of the mean of the standard deviation of the direction angles of displacements inside the cell (measure of spacial synchronicity). No user input required.

(k_5) Displays temporal synchronicty: heatplot/histogram of the synchronicity δ.

(l_5) Tag if there are double peaks.

(m_5) Tag if cell does not look useful for further analysis.

(n_5) Go through all the videos and input parameters. Also gives a preview of the current cell outline or ROI.

(o_5) Curve data and generated parameters are saved in the result file. The user can optionally select to also save curve plots. This then closes the window and concludes the analysis.

3 Folder & Files Structure

- Creation of a temporary folder for each video in the path of the Lifeact GUI:

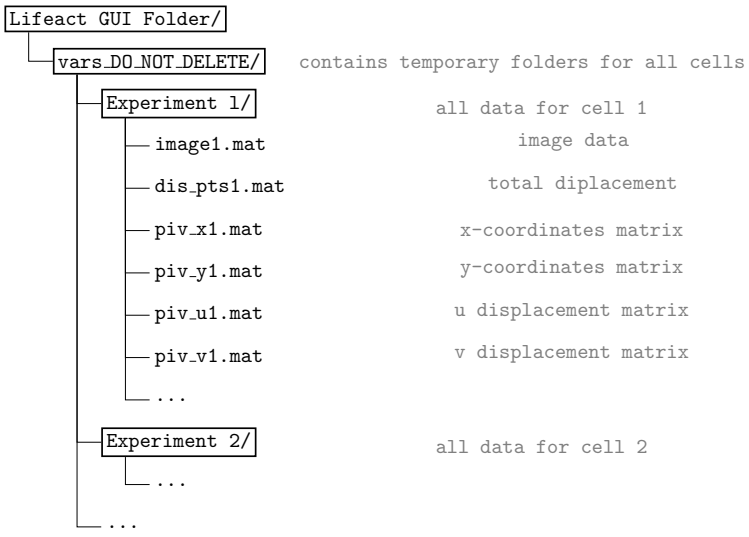


Figure 13: Folder tree for temporary data

- Creation of a result folder for each video in the path of the video file:

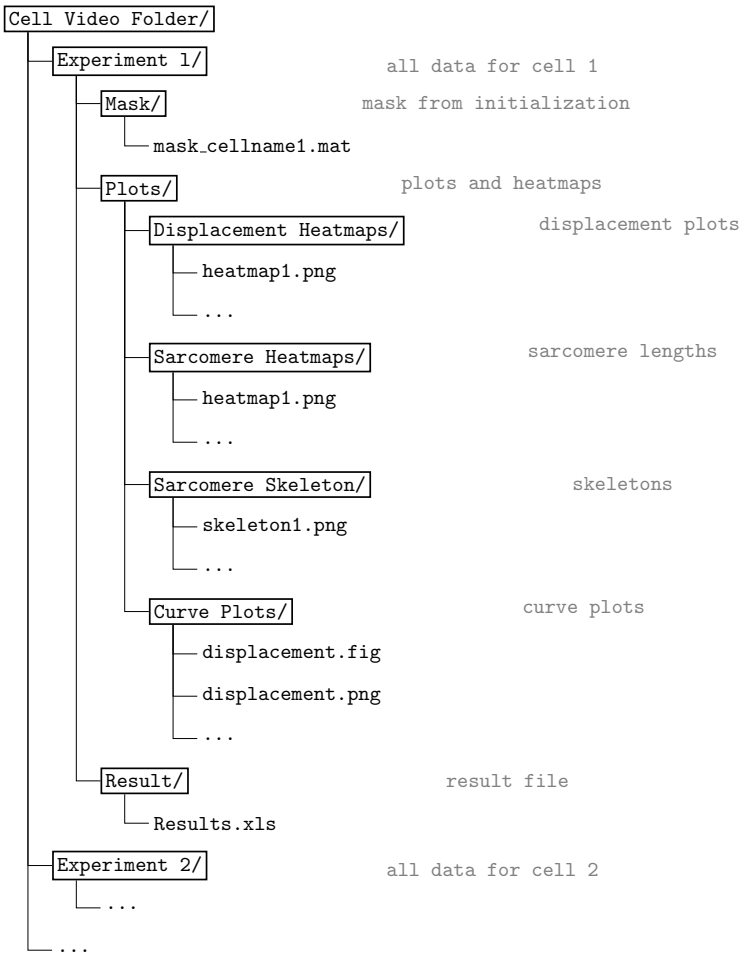
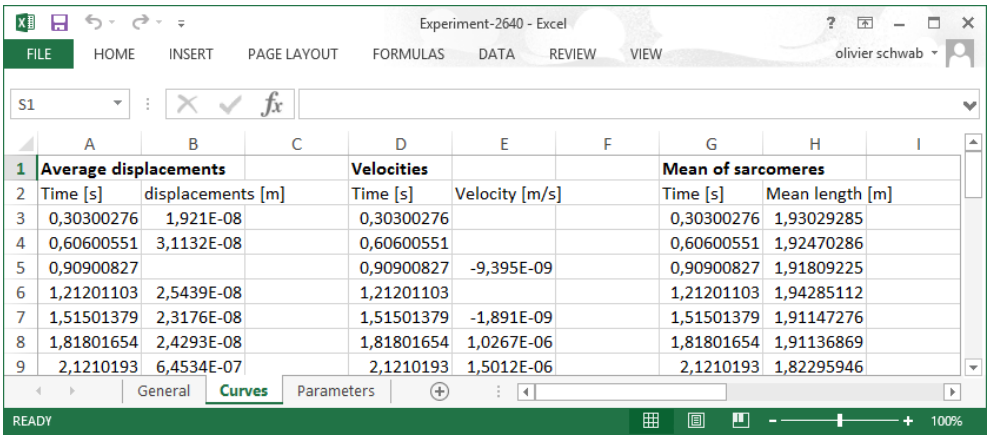


Figure 14: Folder tree for result data

- For each cell video, a main Excel result file is created, which contains the microscope data, the curve data as well as the generated parameters. The file is organized into separate worksheets.



	A	B	C	D	E	F	G	H	I
1	**Average displacements**			**Velocities**			**Mean of sarcomeres**		
2	Time [s]	displacements [m]		Time [s]	Velocity [m/s]		Time [s]	Mean length [m]	
3	0,30300276	1,921E-08		0,30300276			0,30300276	1,93029285	
4	0,60600551	3,1132E-08		0,60600551			0,60600551	1,92470286	
5	0,90900827			0,90900827	-9,395E-09		0,90900827	1,91809225	
6	1,21201103	2,5439E-08		1,21201103			1,21201103	1,94285112	
7	1,51501379	2,3176E-08		1,51501379	-1,891E-09		1,51501379	1,91147276	
8	1,81801654	2,4293E-08		1,81801654	1,0267E-06		1,81801654	1,91136869	
9	2,1210193	6,4534E-07		2,1210193	1,5012E-06		2,1210193	1,82295946	

Figure 15: Excel result file