

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

Contents

1	Requirements and Comments	1
2	Tutorial	2
2.1	Initialization Window	2
2.2	Outline Window	4
2.3	Displacement Window	5
2.4	Parameter Window	6
3	Folder & Files Structure	9

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 `bf_gui_main.m`, and changing the working directory to the current folder. Figure (1) shows the main window of the analysis.

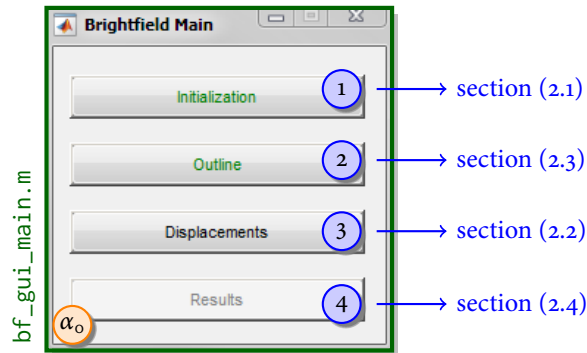


Figure 1: Main window; in blue, the relevant sections of the tutorial

- ① – ④ The analysis is split into four parts, each of them requiring user setup and review. The order of going through these 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 green.

2.1 Initialization Window

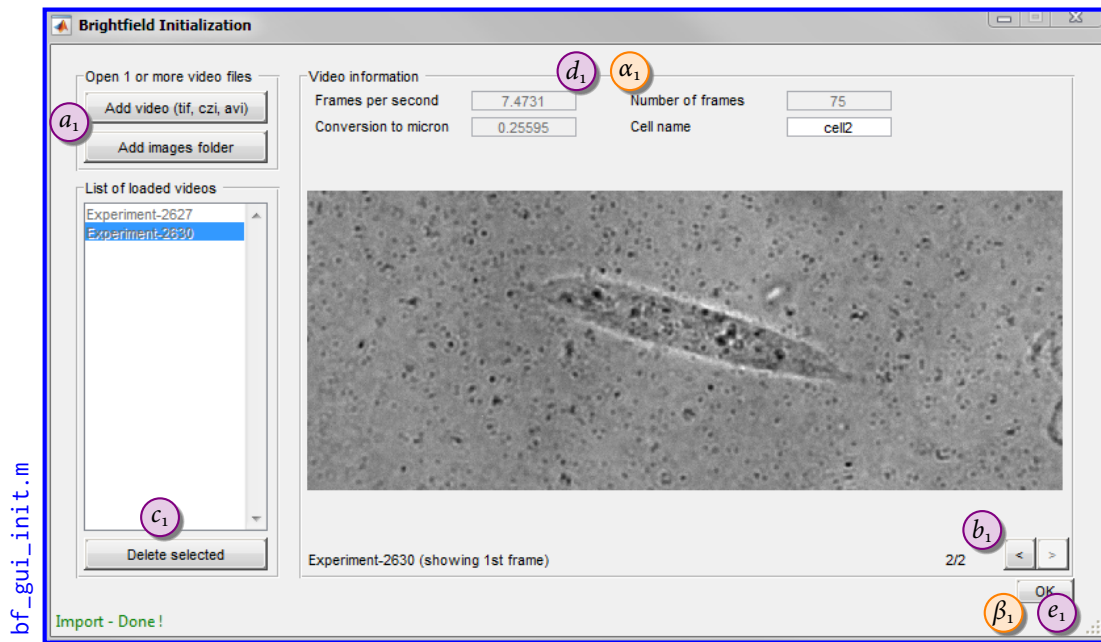


Figure 2: Initialization window

- 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 identification 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 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 Window

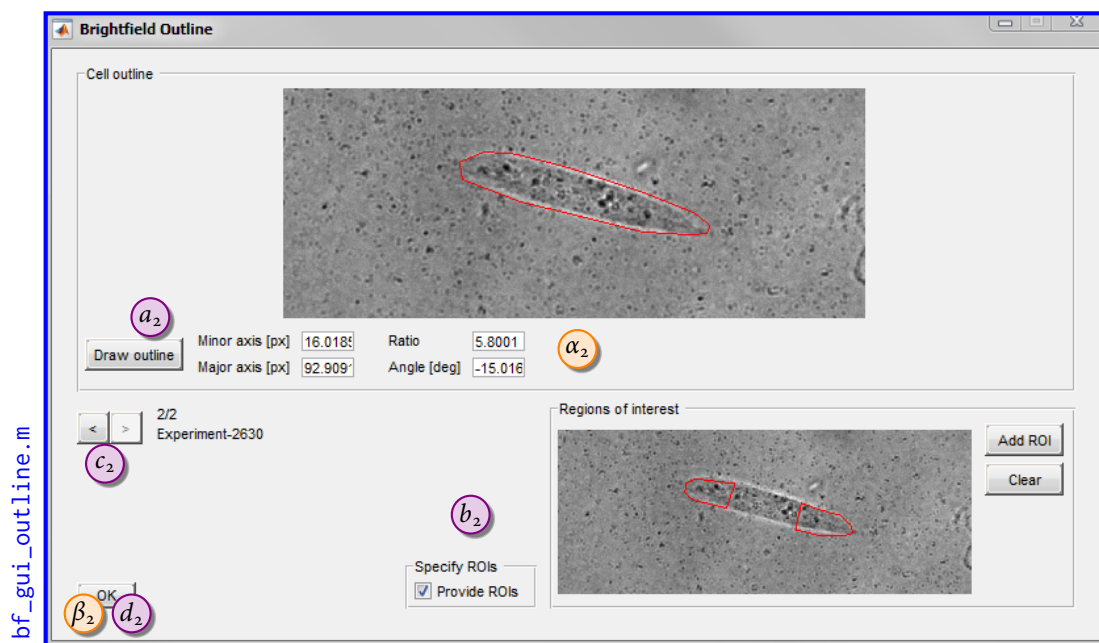


Figure 3: Outline window

- 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 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.
- c_2 These first two steps should be repeated for every video that has been loaded.
- d_2 Closes the window.
- α_2 Gives a preview of dimensions (in pixels), angle and ratio of the user-drawn outline. This is not used for any calculations, and is mainly given for information purposes.
- β_2 A warning message will ask the user to provide all necessary outline, in the case where some are forgotten.

2.3 Displacement Window

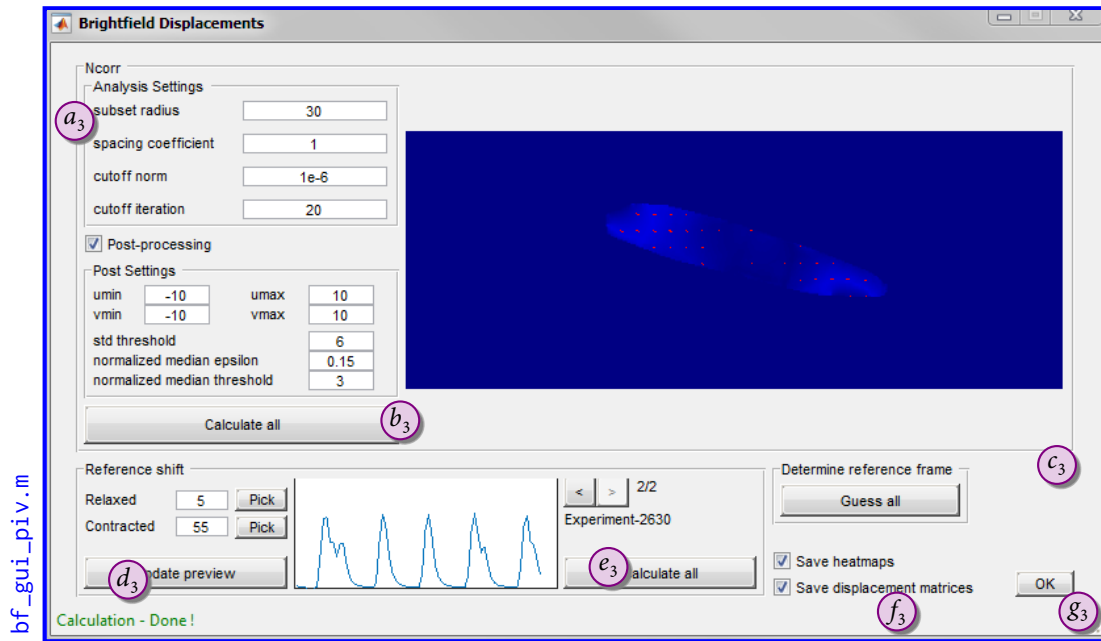


Figure 4: Displacement window

- a_3 Displacements are computed with the Ncorr DIC code. Brightfield 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.

- 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 be 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 Parameter Window

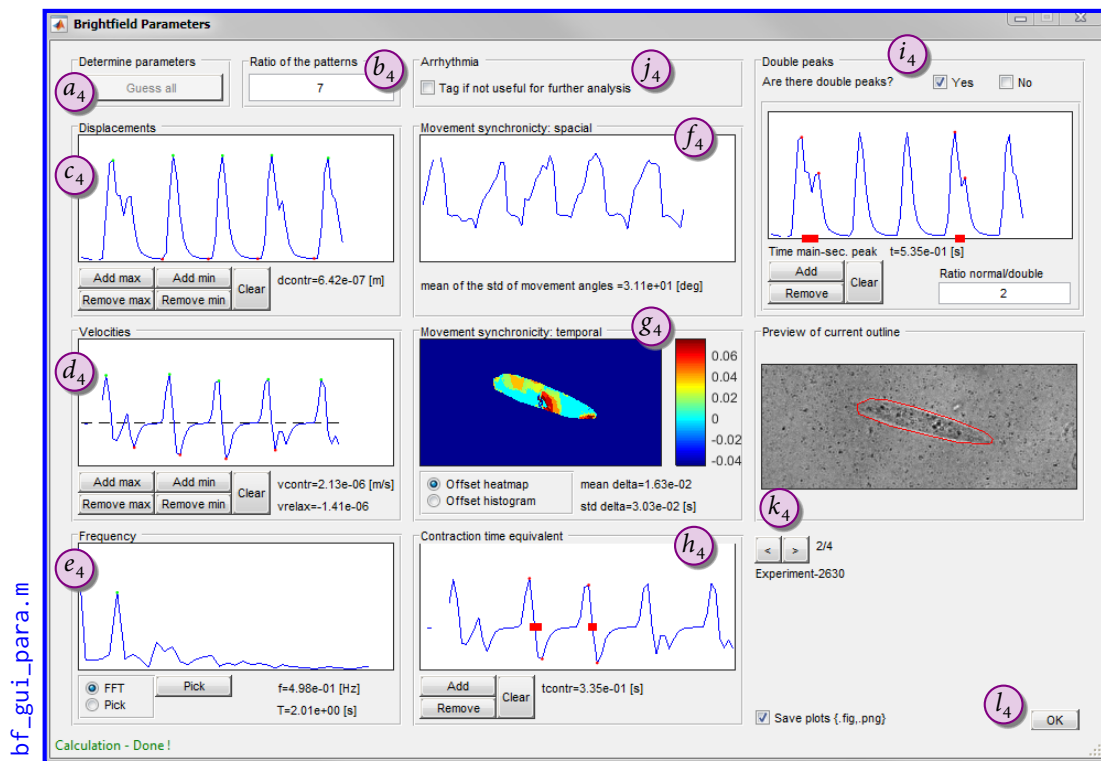


Figure 5: Parameter window

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_4 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_4 Input the aspect ratio of patterns.

- c_4 Average displacement defined for each timepoint (i.e. frame) as the mean of all displacements inside cell blob,

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

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,

$$d_{\text{contr}} = \frac{1}{m} \sum_i^m \max(d(t_k)) - \frac{1}{n} \sum_j^n \min(d(t_k)) . \quad (2)$$

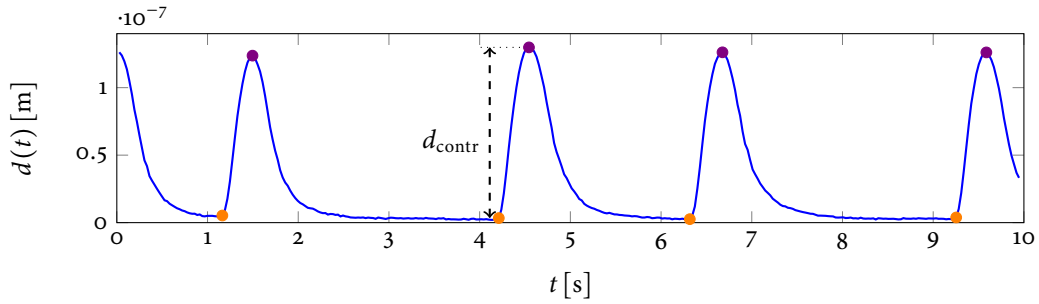


Figure 6: Displacement plot

- d_4 Average velocity defined for each timepoint (i.e. frame) using the mean displacements $d(t_k)$,

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

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

$$v_{\text{max,contr}} = \frac{1}{m} \sum_i^m \max(v(t_k)) \quad ; \quad v_{\text{max,relax}} = \frac{1}{n} \sum_j^n \min(v(t_k)) . \quad (4)$$

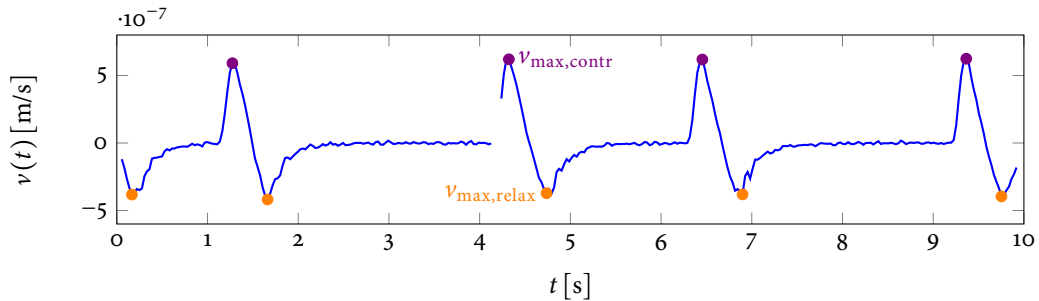


Figure 7: Velocity plot

- e_4 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.

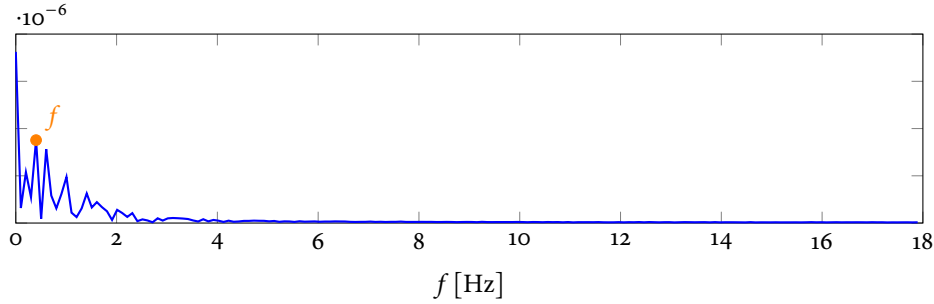


Figure 8: Fourier transformed signal

- (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,

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

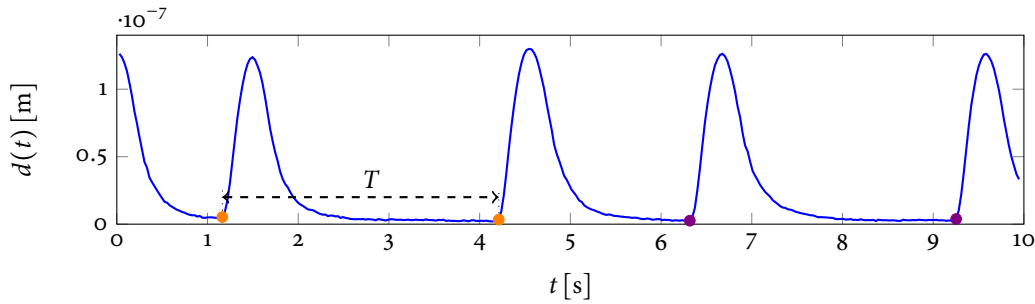
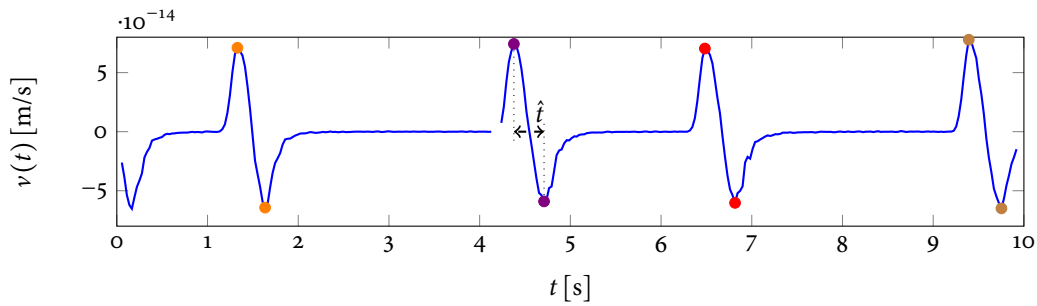


Figure 9: Displacement plot with time period

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.

- f_4 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.
- g_4 Displays temporal synchronicity: heatplot/histogram of the synchronicity δ .
- h_4 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,

$$\hat{t} = \frac{1}{m} \sum_i^m \left\| \left[t_k \mid \max_i v(t_k) \right] - \left[t_k \mid \min_i v(t_k) \right] \right\|. \quad (6)$$

Figure 10: Velocity plot with \hat{t}

- i_4 Tag if there are double peaks.
- j_4 Tag if cell does not look useful for further analysis.
- k_4 Go through all the videos and input parameters. Also gives a preview of the current cell outline or ROI.
- l_4 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 Brightfield GUI:

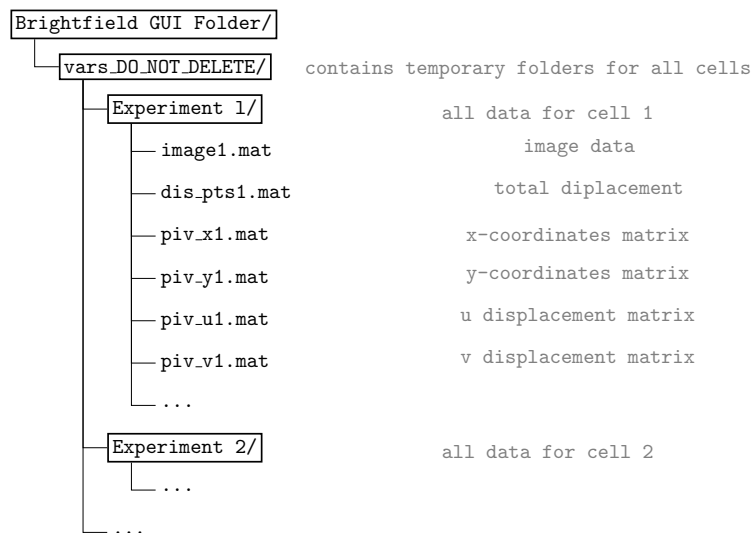


Figure 11: Folder tree for temporary data

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

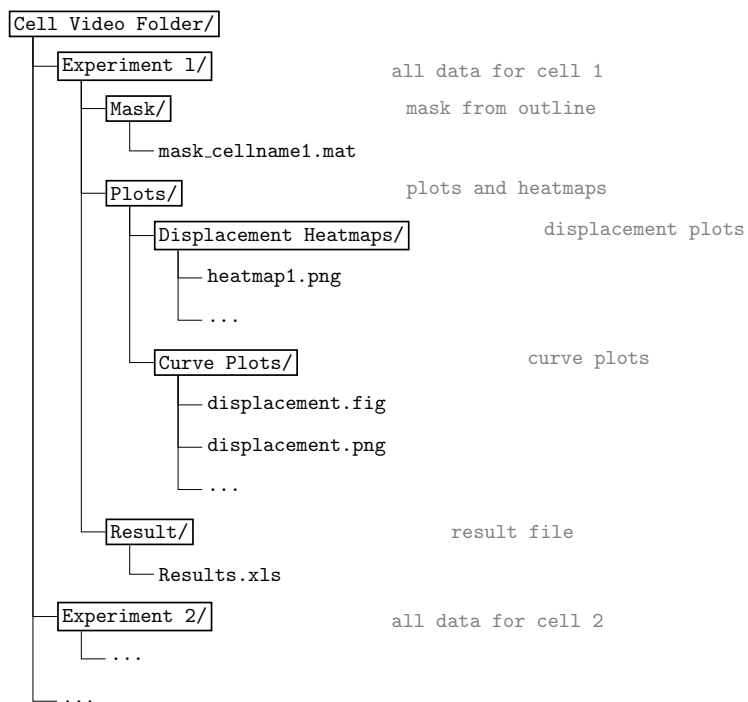


Figure 12: Folder tree for result data

- 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.