
CONTRAX

STREAMLINED TFM GUI USER GUIDE

This document is a user guide for ContraX. It serves as reference and an introductory getting-started guide.

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REQUIREMENTS, INSTALLATION AND COMMENTS

1. REQUIREMENTS:

- ContraX should work without problem on computers equipped with a minimum of 8 GB RAM memory and 50GB disk space.
- ContraX has been tested for Windows 10, Windows Server 2018 and MacOS X 10.14.6.
- ContraX Streamlined TFM GUI code has been developed using Matlab R2018b.

2. INSTALLATION:

- Download Streamlined TFM GUI from our GitHub repository:
- Save source code on a disk that has enough free space to allow for analysis of videos. A disk with a fast read-write speed will perform best.

3. COMMENTS:

CODE DETAILS:

- Forward compatibility with Matlab and OS will depend on the changes made at that level. Updates will be provided on our GitHub to the best of our abilities.
- On recent versions of Matlab, the GUI produces a variety of warnings related to some of the packages. These can safely be ignored.
- The code uses some third-party packages, that are included in our repository release and do not require specific installation. Here is the complete list:
 - Ncorr1: DIC algorithms.
 - Bio-Formats for Matlab²: reads .czi image and video formats. This package is regularly updated to adapt to changes in proprietary formats. An update to a more recent version may be downloaded in the future.
 - xlwrite³: write results to Excel files.
 - peakdet⁴: determine peaks on noisy curves.
 - statusbar⁵ and enableDisableFig⁶: freeze the windows during calculations and estimate runtime.
 - freezeColors⁷ and COLORMAP and COLORBAR utilities⁸: display overlays on figures.
 - Exportfig⁹: flexible plot exports.¹
- It is important to leave the functions and the file structure unaltered. Matlab must have writing rights to the working repository.

USAGE DETAILS:

- Computing time is reduced with the use of parallel processing; hence, multicore CPU machines will perform better.
- Parallel processing is used in part of the code that loop over the stack of video to analyze and well as in some function that perform analysis at the frame level. Hence, some of the parallel processing benefits only become evident during the processing of multiple videos and/or long videos.

¹ <http://ncorr.com>

² <https://www.openmicroscopy.org/bio-formats/downloads/>

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-freezevalues---unfreezevalues>

⁸ <http://www.mathworks.com/matlabcentral/fileexchange/24371-colormap-and-colorbar-utilities--jul-2014->

⁹ <http://www.mathworks.com/matlabcentral/fileexchange/727-exportfig>

- When large batches of videos are analyzed, memory and disk space requirements will increase accordingly. During execution, a significant amount of temporary data as well as results files and variables are written to the disk or kept in RAM memory. Thus, it is advisable to have at least a few tenths GB of free disk space. In the PIV step, a tick mark option is available to relieve memory pressure for the processing of large batches of videos. In this case, some of the PIV output heatmap figures are not saved, but no data loss occurs. If out-of-memory error occurs, try to rerun the analysis with fewer videos at first.
- Calculations can be stopped at any time using the Matlab keyboard shortcut: ctrl + C key combination.

STEP-BY-STEP USER GUIDE

ContraX Streamlined TFM BUI is launched by executing tfm_gui_main.m.

The analysis is split into four sequential parts, each of them requiring user setup and review. Steps which are not accessible at a current stage are greyed out, steps which have been completed show up green.

We strongly recommend to close Matlab between subsequent executions of video batches, as some of the temporary data are saved as app data and may not be cleared without restarting Matlab.

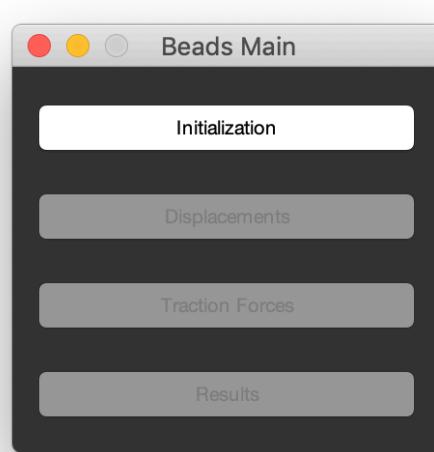


FIGURE 1 STREAMLINED TFM GUI MAIN CONTROL WINDOW.

1. INITIALIZATION PANEL:

The initialization panel is where the videos to analyze area loaded and image preprocessing is performed. Some image parameters also need to be provided.

1. In the main panel (Figure 1), launch the initialization panel by clicking on the *Initialization* button (Figure 2).

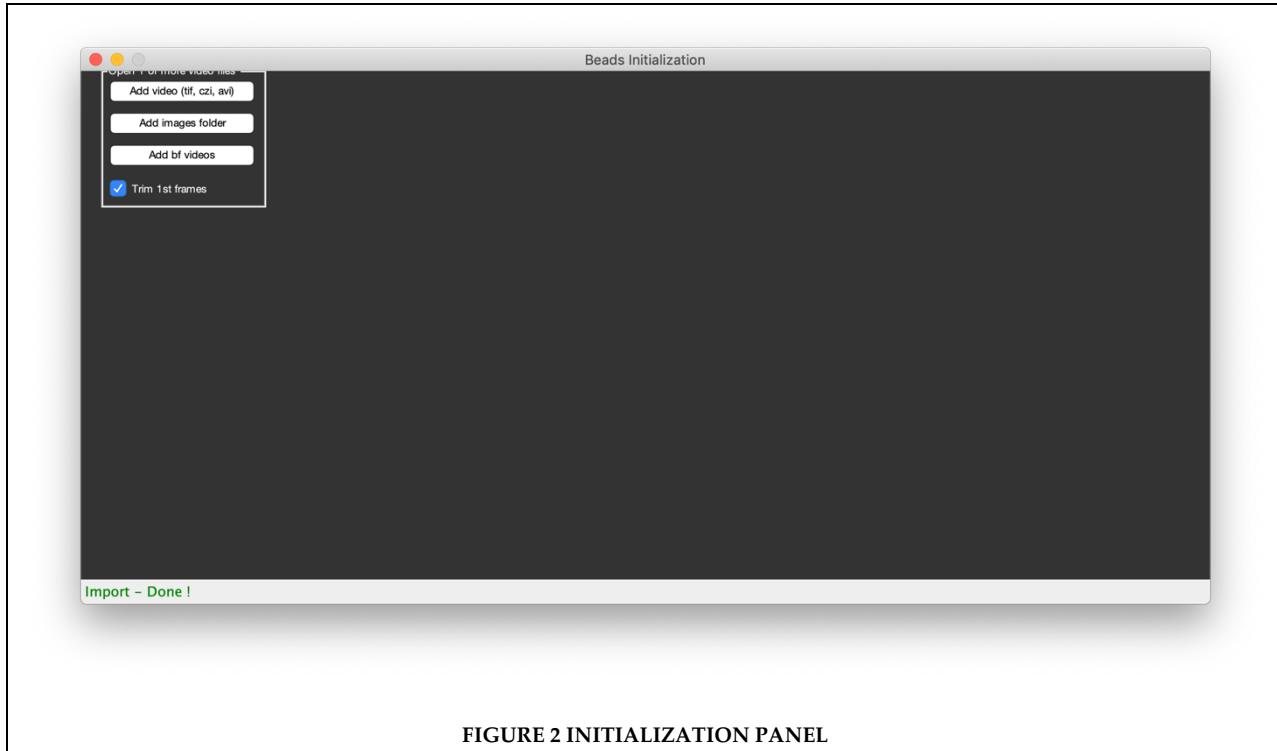


FIGURE 2 INITIALIZATION PANEL

2. Load videos of the fluorescent beads using Add video (tif, czi, avi) button. Use file explorer to locate the video of interest, toggling for the desired file extension type. Several videos can be selected and loaded at once, but they must ideally reside in the same folder.
 - Sometimes, we experienced that the first frame of the .czi video was not acquired correctly by our microscope camera, therefore we implemented an option to *trim the first frame* of the video. This tick mark must be selected prior to import the videos.

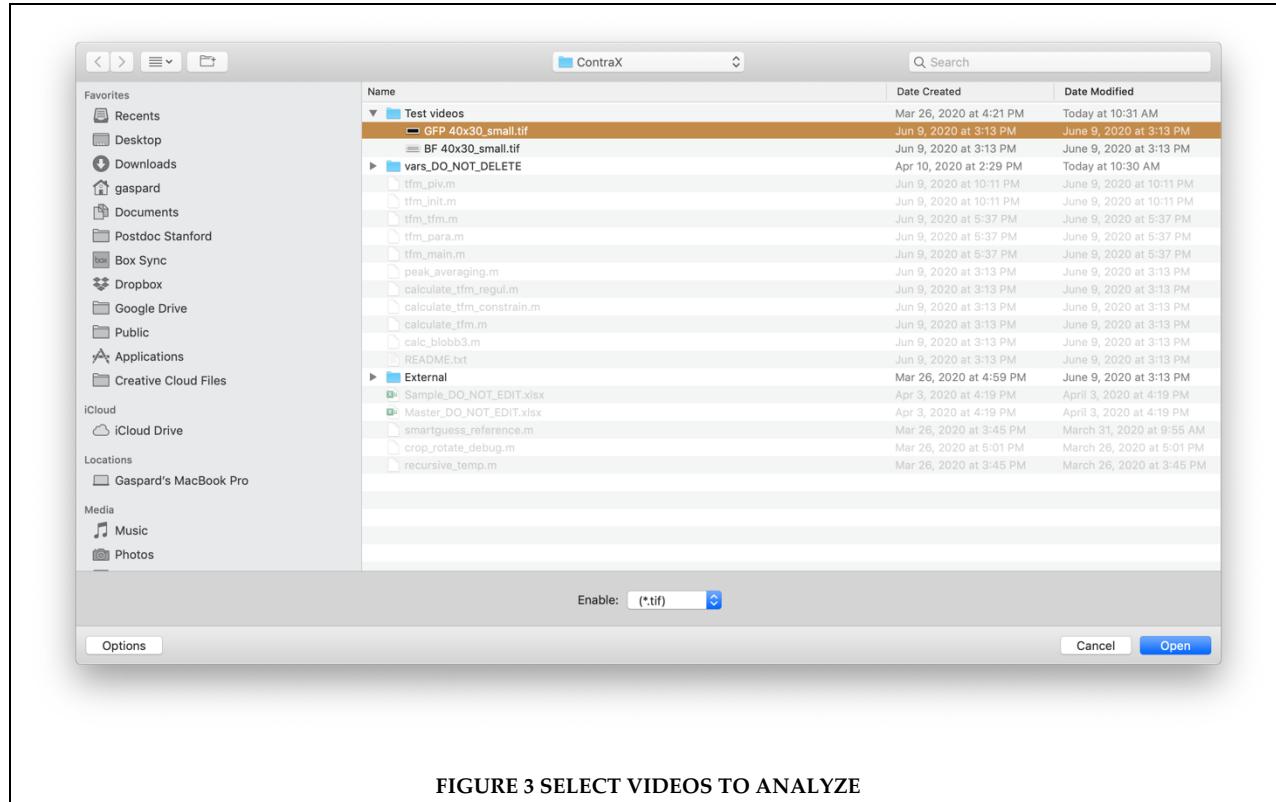


FIGURE 3 SELECT VIDEOS TO ANALYZE

- If cell outline masks were defined for some of the videos being loaded during a prior analysis, the following pop-up will appear asking if the user wants to reimport these masks automatically. This save precious time if a batch of cells needs to be analyzed a second time.

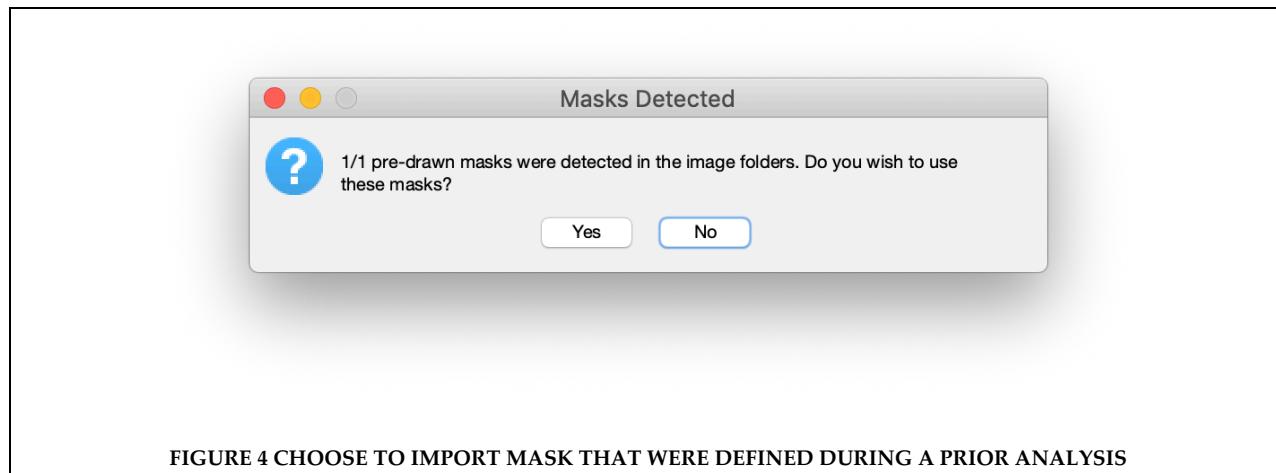


FIGURE 4 CHOOSE TO IMPORT MASK THAT WERE DEFINED DURING A PRIOR ANALYSIS

- Multichannel .czi video can be loaded and a pop-up window will ask to assign each channel to the corresponding TFM or brightfield/fluorescence image for cell outlining (Figure 5).



FIGURE 5 MULTICHANNEL .CZI FILES CAN BE IMPORTED AND EACH CHANNEL MUST BE ASSIGNED TO TTFM OR BRIGHTFIELD/FLUORESCENCE FOR CELL OUTLINING

3. After loading, the list of loaded videos and a preview frame of the fluorescent beads video are displayed. If masks are loaded from a prior analysis, the cell outline and encompassing analysis ellipse are displayed in overlay in red and blue, respectively. If no mask was loaded, the preview frame does not display any outline.

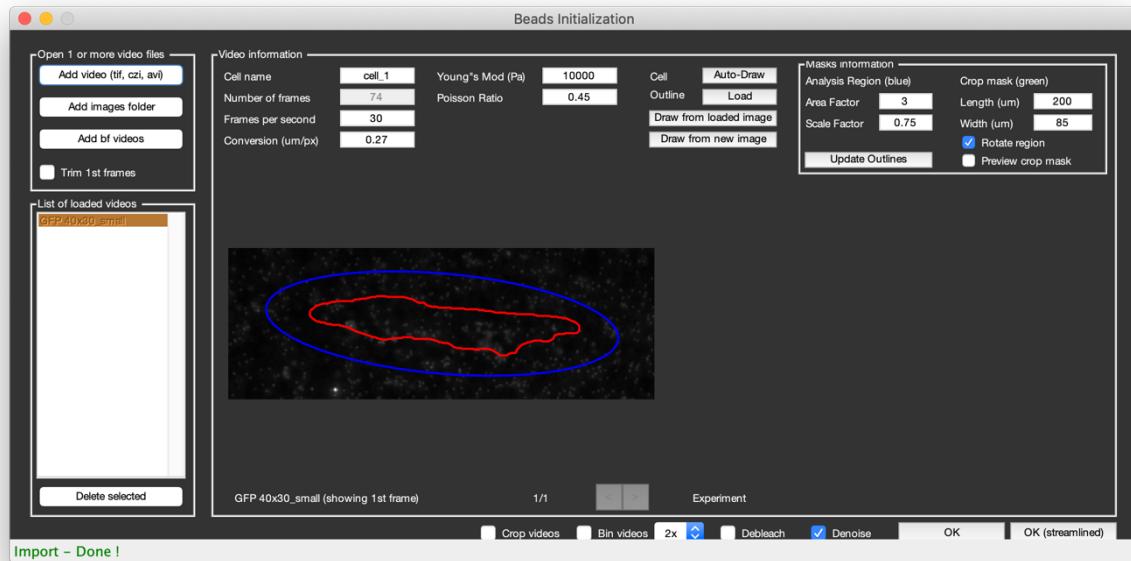


FIGURE 6 ONE VIDEO WAS LOADED WITH A PREVIOUSLY DEFINED MASK AND A PREVIEW IS SHOWN

4. Load brightfield/fluorescent image or video to define the cell outline by clicking on the *Add bf video* button.

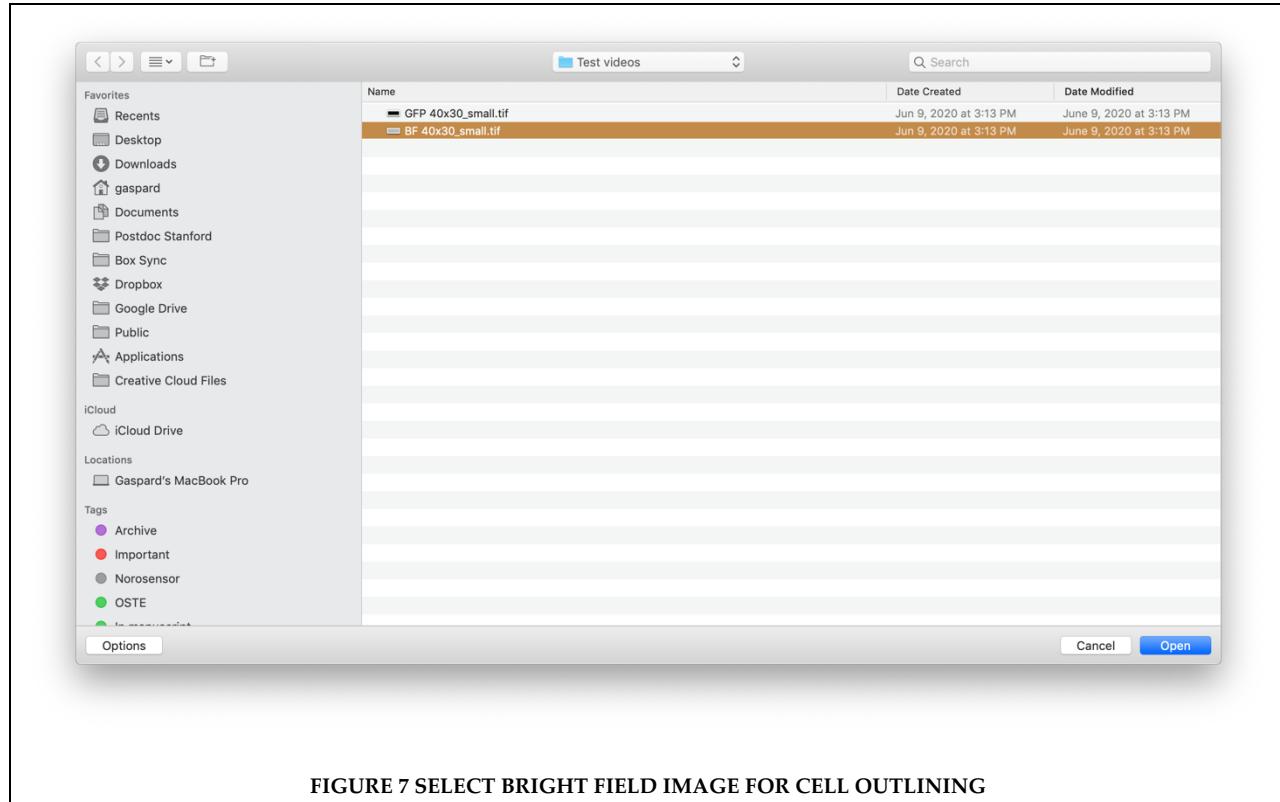


FIGURE 7 SELECT BRIGHT FIELD IMAGE FOR CELL OUTLINING

5. Draw a cell outline for each of the loaded videos: 4 options are available and must be performed for each video:
 - a. *Auto-draw*: The software uses a built-in algorithm to automatically detect cell objects and outline in the brightfield/fluorescent image for each video sequentially. The user just needs to select in each black and white mask image which object to outline (Figure 8). Once the stack of videos is processed, the user can refine the outlines manually using the option below. The process can also be programmatically fully automated (from within the code), but, as currently implemented in the code, will only be able to select the one object detected in the center of the image frame.

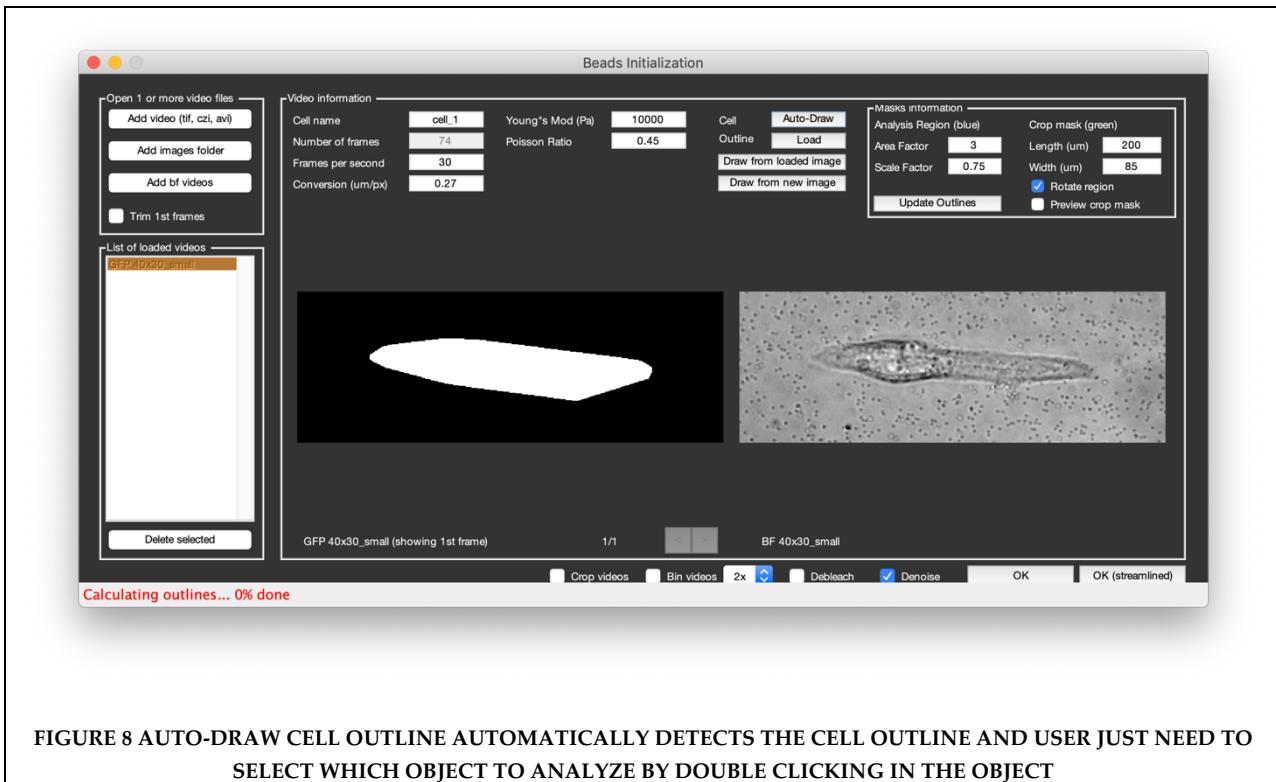


FIGURE 8 AUTO-DRAW CELL OUTLINE AUTOMATICALLY DETECTS THE CELL OUTLINE AND USER JUST NEED TO SELECT WHICH OBJECT TO ANALYZE BY DOUBLE CLICKING IN THE OBJECT

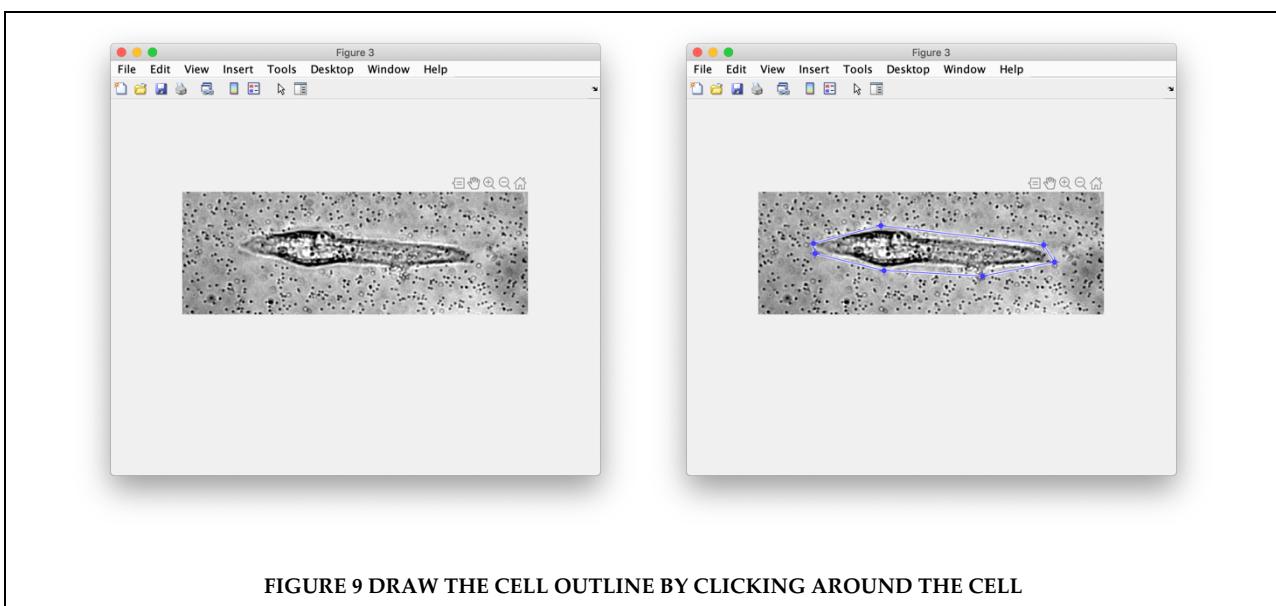


FIGURE 9 DRAW THE CELL OUTLINE BY CLICKING AROUND THE CELL

- d. *Draw from new image:* Let the user load another brightfield / fluorescence image to draw a cell outline on, in a similar way as c). This will replace the brightfield / fluorescence image previously loaded for a corresponding fluorescent beads video.
6. The cell outline is displayed in red and the analysis area in blue (Figure 10). The size of the analysis area can be changed, see step 9.

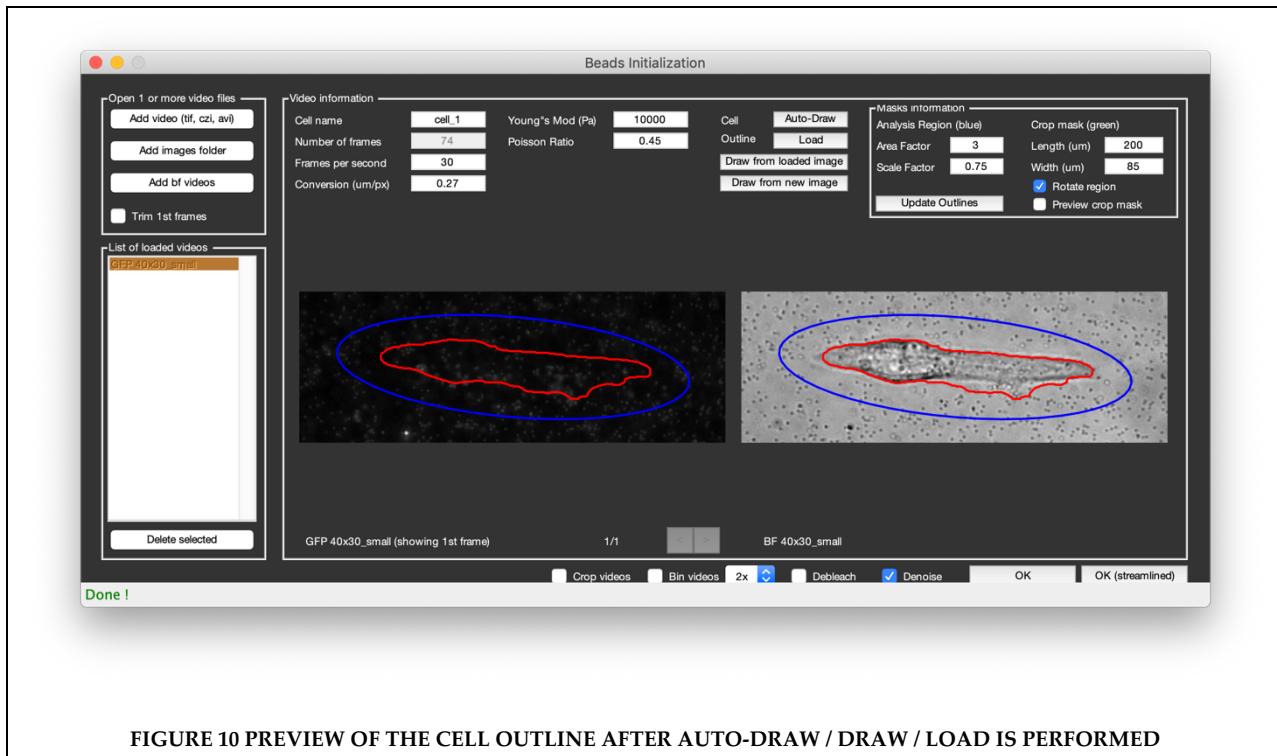


FIGURE 10 PREVIEW OF THE CELL OUTLINE AFTER AUTO-DRAW / DRAW / LOAD IS PERFORMED

7. Make sure that all cells have been outlined, that the video parameters, *i.e.*, *Frame per second*, *Conversion (um/px)*, *Young's Modulus (Pa)*, and *Poisson ratio* are correct for each video. Note: the default values can be edited in the code prior to start.
8. Make sure to use the appropriate *Analysis region (blue)* parameters for each video. The *Area Factor* controls the area of the blue ellipse to be x time larger than the ellipse that best fit the cell outline. The *Scale Factor* enables to control the aspect ratio of the ellipse, *i.e.*, a scale factor < 1 makes the ellipse less elongated, which allows to avoid having the ellipse going much out of frame in the case an edge of the video frame is too close to the cell. In some cases, that could result in analysis bias or unwanted noise from edge effect in the PIV step.
9. Select the size of the cropping mask if cropping of the video is desired. Note that enough space between the cell and the video frame edges is necessary to capture gel deformation that can propagate far from the cells in the case of a soft hydrogel formulation. Select if the cropping region should also rotate the image while cropping. Click on the *Update Outline* button to refresh the outlines preview (Figure 11).

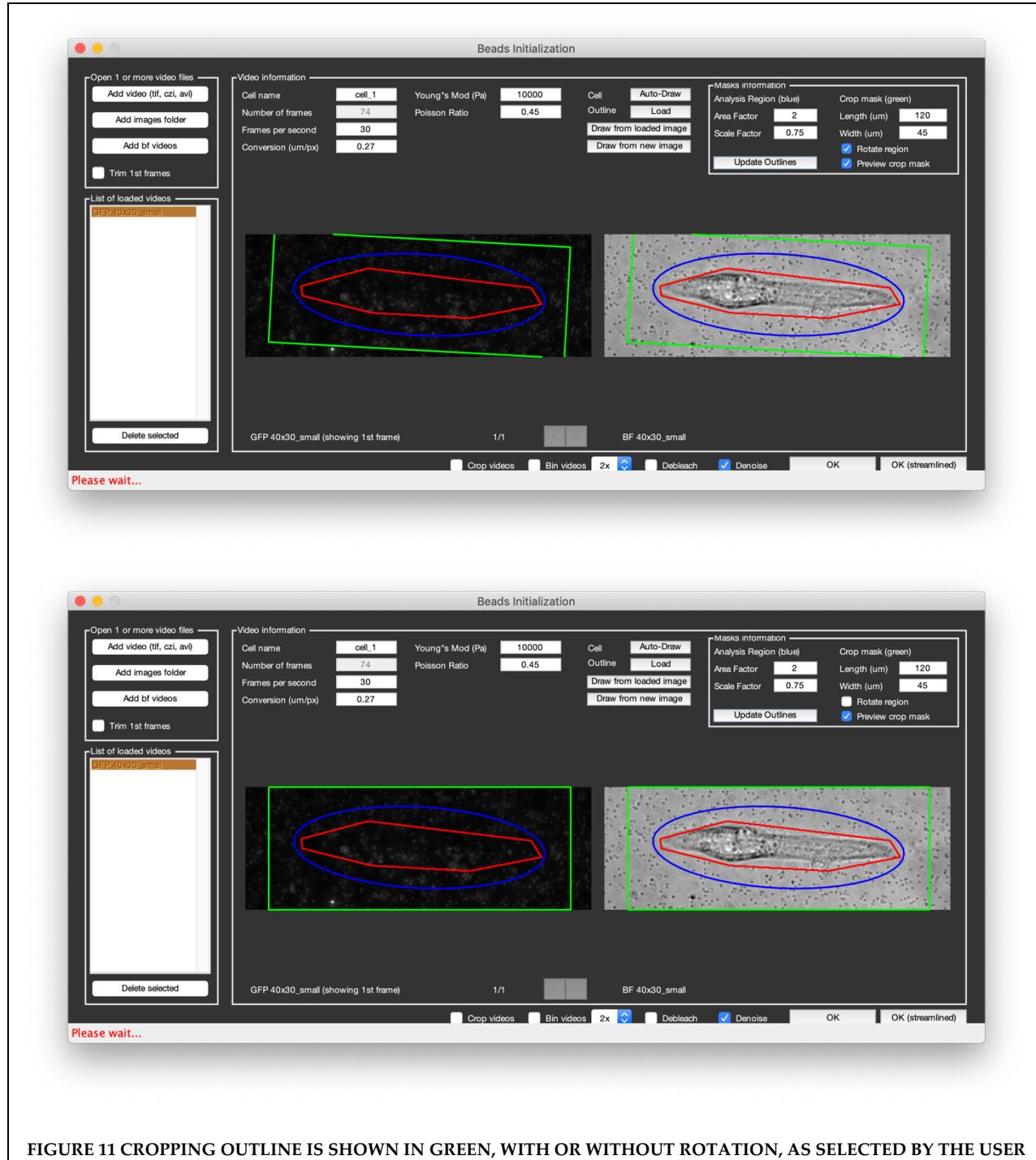


FIGURE 11 CROPPING OUTLINE IS SHOWN IN GREEN, WITH OR WITHOUT ROTATION, AS SELECTED BY THE USER

10. Before to finalize the initialization, verify the parameters a last time and decide whether to *Crop*, *Bin*, *Debleach* or *Denoise* the videos (Figure 12).
11. Finally, decide to proceed with the analysis step-by-step, which allows for editing the defaults analysis parameter in subsequent steps, by clicking on the *OK* button, or in streamlined mode, which will proceed with the analysis using the default parameters (editable in the code), by clicking on *OK (streamlined)*.

- Warning is shown before to proceed with Streamlined analysis. Close it to proceed (Figure 13).

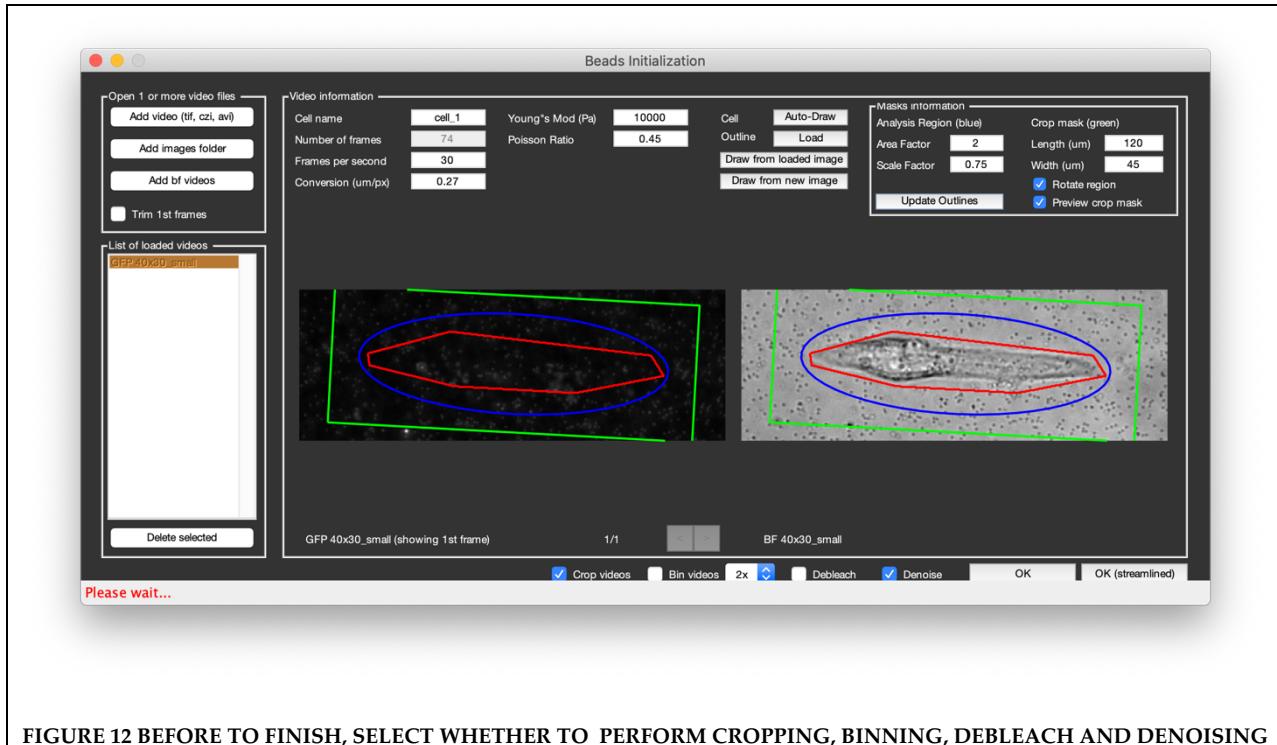
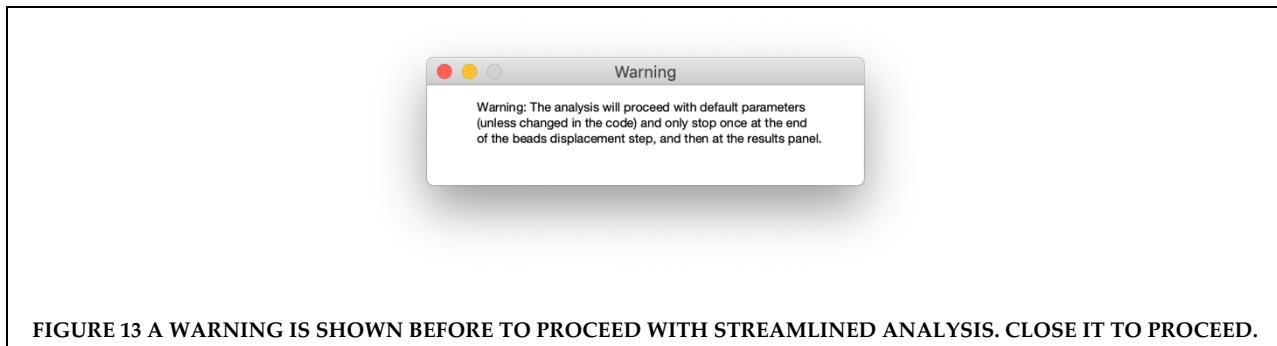


FIGURE 12 BEFORE TO FINISH, SELECT WHETHER TO PERFORM CROPPING, BINNING, DEBLEACH AND DENOISING



2. DISPLACEMENT PANEL:

The displacement panel is where the PIV analysis of the gel deformation is performed by calculating the displacement of the fluorescent beads using the Ncorr algorithm. This step uses parallel processing over the videos stack and video frames.

1. In the main panel (Figure 14), launch the initialization panel by clicking on the *Initialization* button (Figure 15).

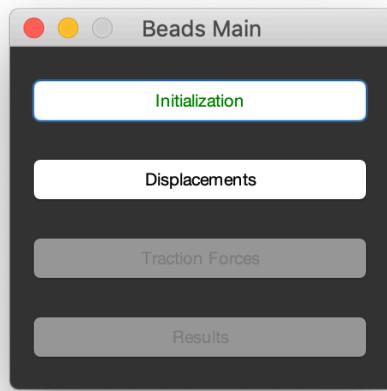


FIGURE 14 AFTER INITIALIZATION IS COMPLETED, THE DISPLACEMENT STEP IS ENABLED

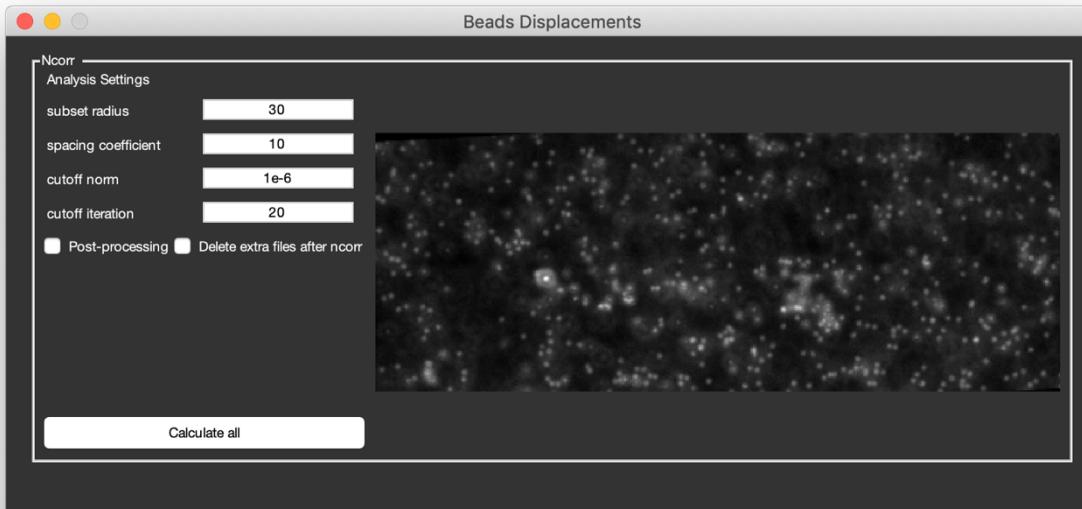


FIGURE 15 DISPLACEMENT PANEL

2. The first step calculates the beads displacement with the Ncorr PIV algorithm and requires choosing values for the processing parameters:
 - *Subset radius*: controls the size (in pixels) of the image windows whose position is tracked using the beads. The adequate value depends on the experimental conditions, such as the beads density, depth of field of the objective used for imaging, image noise, etc.
 - *Spacing coefficient*: controls the spacing (in pixels) between each image windows analyzed. A small value will result in a measurement of the displacement at many spatial locations while a large value will spatially down sample data and underestimate the hydrogel deformation.
 - i. Note: Both values will strongly influence the computing time, and it is important to always use the same values for all the data analysis to obtain comparable results.
 - *Cutoff norm* and *cutoff iteration* control the accuracy of analysis.
3. Select whether post-processing is desired, in which case additional parameters must be chosen to filter data during a post-processing step. Such post-processing must be used with caution and with rigorously the same parameter values for experimental consistency (Figure 16).
4. Select whether to delete extra files after the Ncorr PIV calculation step to preserve memory. In this case, it is not possible to save the .png images and .mat displacement field data of the PIV analysis results. It can be a necessary choice depending on the hardware used and the number of videos processed in the batch. If selected, it is not possible to rerun this step without going through the *Initialization* panel again to reload some of the data.
5. Once parameters are chosen, launch the PIV calculation by clicking on the *Calculate all* button.

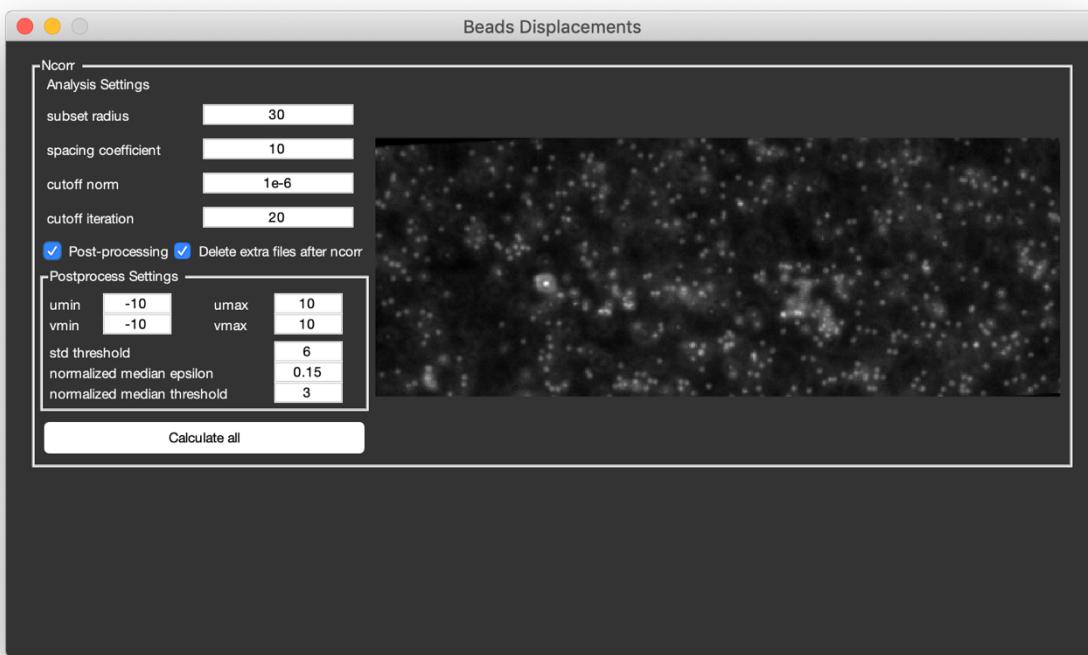
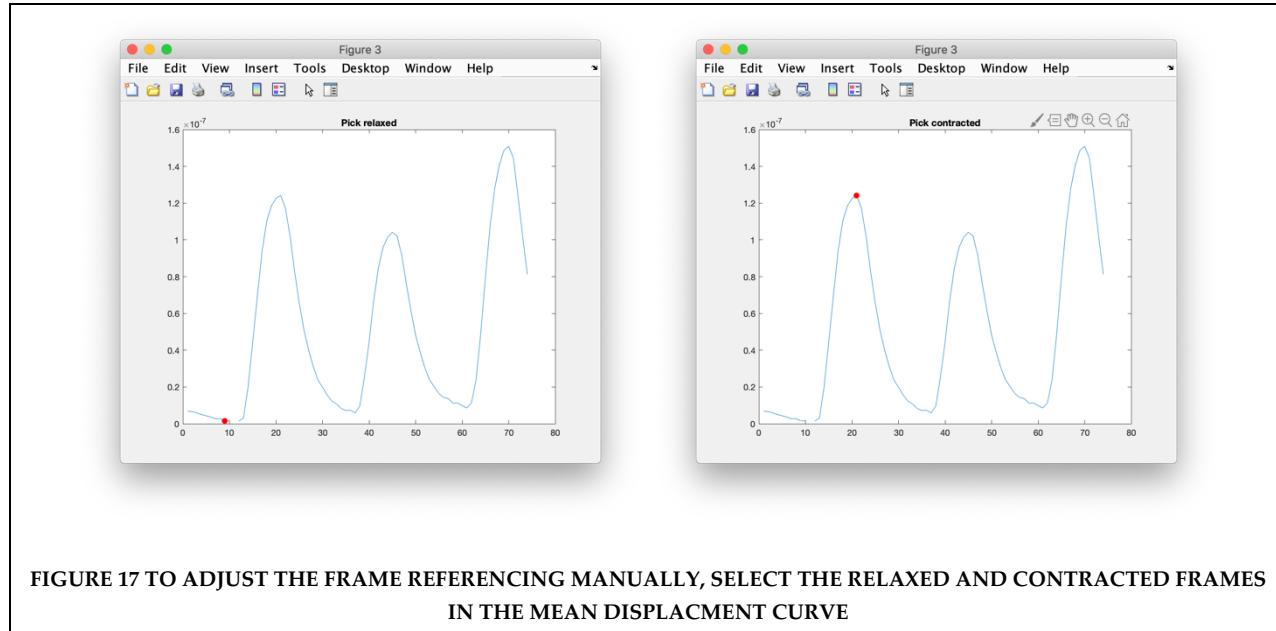
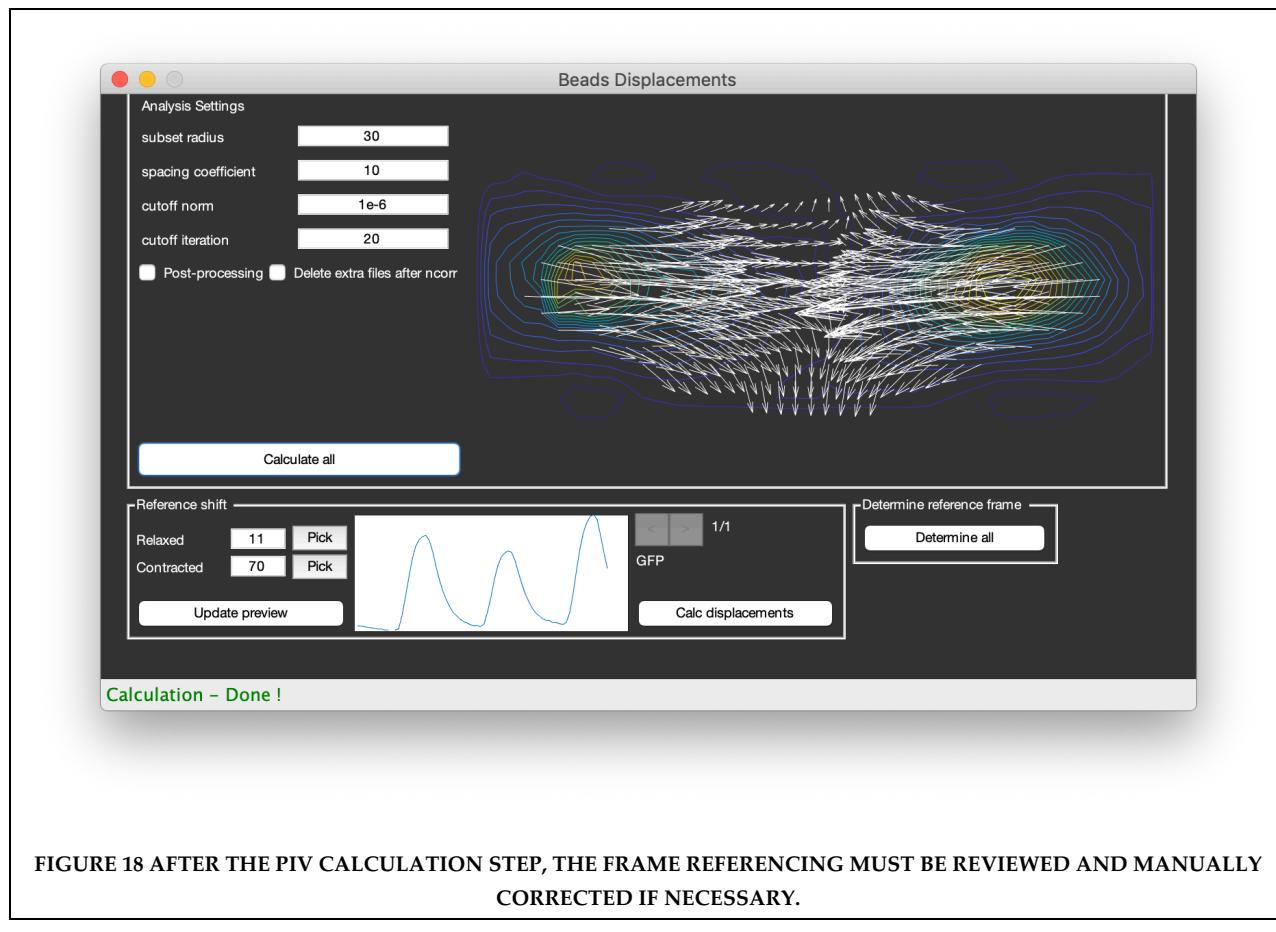


FIGURE 16 POST-PROCESSING CAN BE PERFORMED IN POST-PROCESSING. MUST ONLY BE USE AFTER TESTING AND WITH CONSISTENCY.

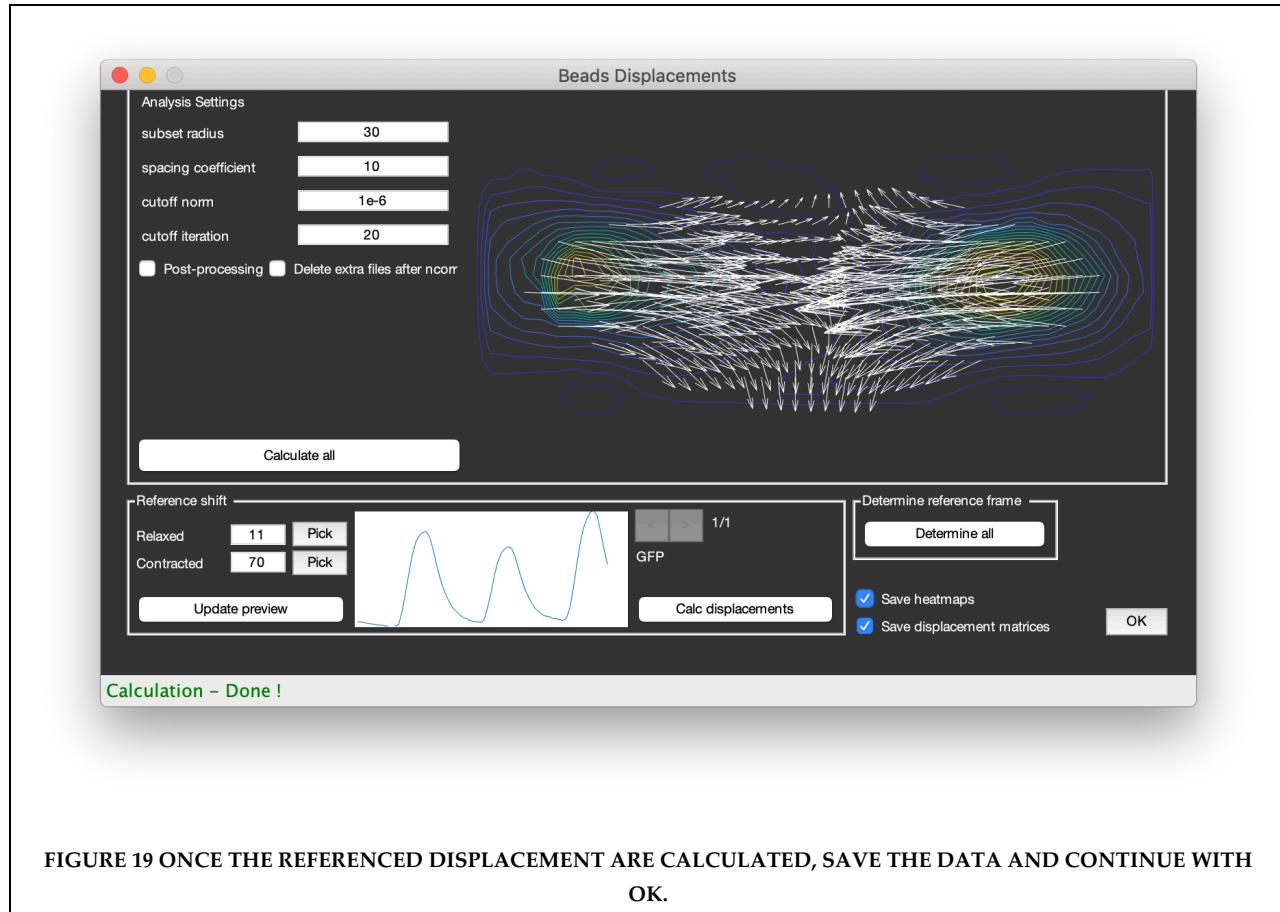
6. After the PIV step is performed, the displacement is automatically referenced to the most relaxed image frame. The average displacement trace is displayed for each video for review. (Figure 18) It must be verified for each video and eventually manually corrected. It can happen that the algorithm does not adequately identify the relaxed frame. In some instance, a contracting frame is mistaken for the relaxed frame, and the curve in up-side-down, the arrow directions in the image preview helps verify that the displacement points inwards towards the center of the cell. In some instance, *i.e.*, weak contraction and/or noisy data, the frame identified by the algorithm is not ideal and a manual identification can sometimes improve the data. If unsure, proceed with the analysis and decide whether to exclude or reprocess the data at the results step. To adjust the frame referencing, either enter the frame numbers for a (the most) relaxed and contracted frames or click on the respective *Pick* button. The latter option opens a pop-up with the average displacement curve where the reference point can be picked by clicking on the curve (Figure 17).



7. Once reviewed, select *Calc. Displacement* to calculate the referenced displacement.



8. Once reviewed, select *Calc. Displacement* to calculate the referenced displacement (Figure 18).
 - Note: If streamlined analysis is selected in the initialization panel, the computation proceeds automatically up to this point. The referenced displacement is calculated but we recommend verifying the referencing (step 6) and recalculating the referencing if any of the reference frame is changed (step 7). If no change is made, proceed with saving the data directly.
9. Select what data to save and click on *OK* to proceed (Figure 19).



3. TRACTION FORCE PANEL

1. In the main panel (Figure 20), launch the initialization panel by clicking on the *Initialization* button (Figure 21).

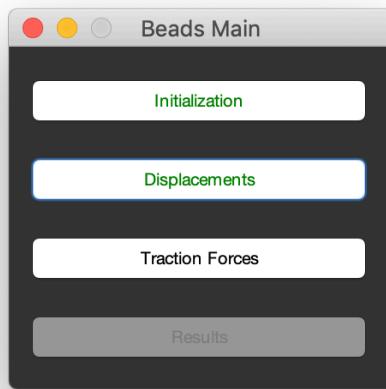


FIGURE 20 AFTER THE DISPLACEMENT STEP IS COMPLETED, THE TRACTION FORCE STEP IS ENABLED

2. When the *Traction Force* panel is launched, the calculation of the estimation of the regularization parameter is performed automatically.
3. Update the material parameters for each video if necessary (that is normally already set and verified in the *Initialization* panel).
4. Choose whether to proceed with *unconstrained analysis* or *constrained analysis* of the traction stress. *Unconstrained analysis* computes the traction stress for the whole frame, while *Constrained analysis* will assume zero traction stress outside of the analysis area (blue ellipse in the *Initialization* panel). The choice is left to the user to be made and more information can be found in Sabass et al. (2008), but this choice should be maintained the same for all videos analyzed and dataset to be compared.
5. Proceed with the computation of the traction stress by clicking on the *Calculate all* button.

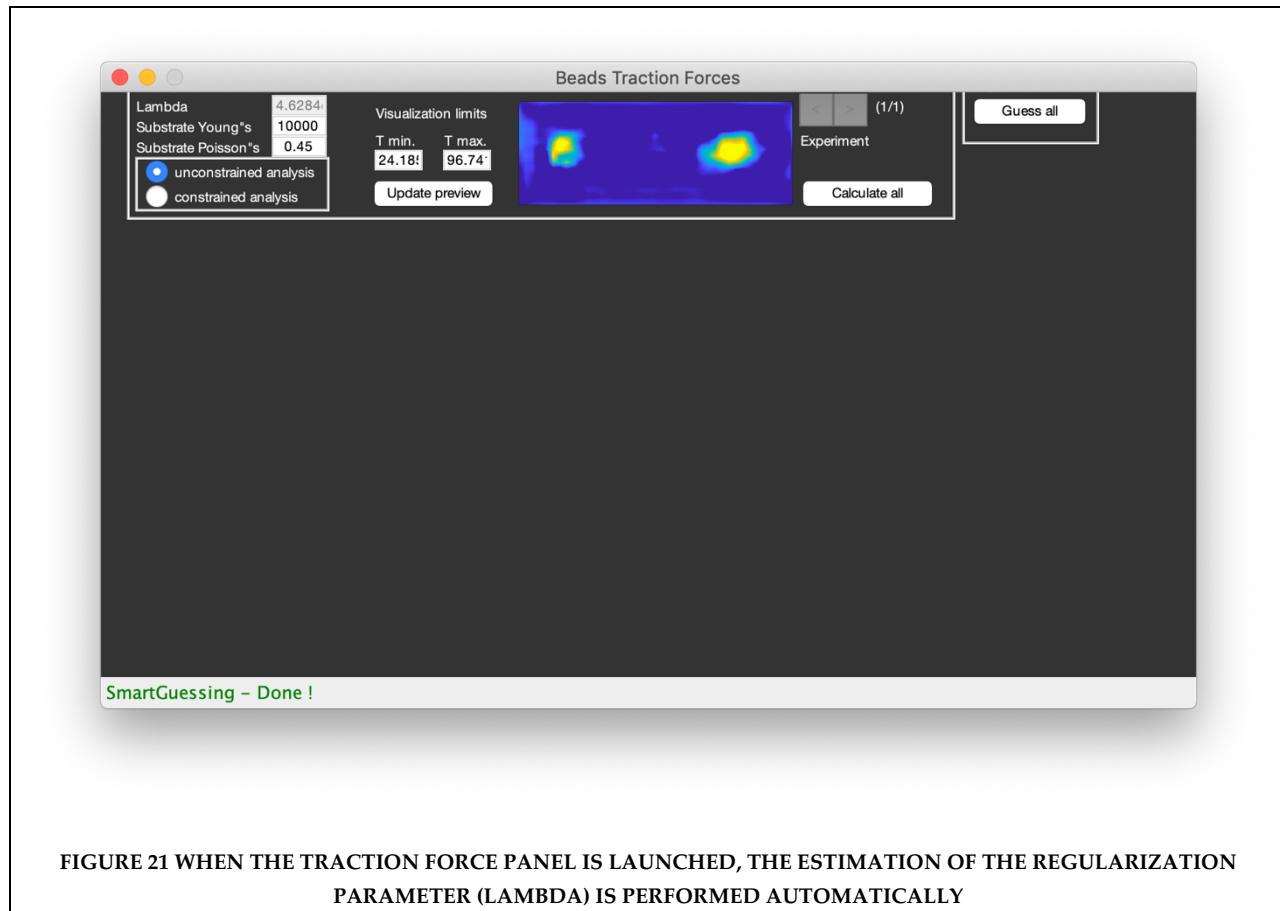


FIGURE 21 WHEN THE TRACTION FORCE PANEL IS LAUNCHED, THE ESTIMATION OF THE REGULARIZATION PARAMETER (LAMBDA) IS PERFORMED AUTOMATICALLY

6. Select what data to save and click on OK to proceed (Figure 22).

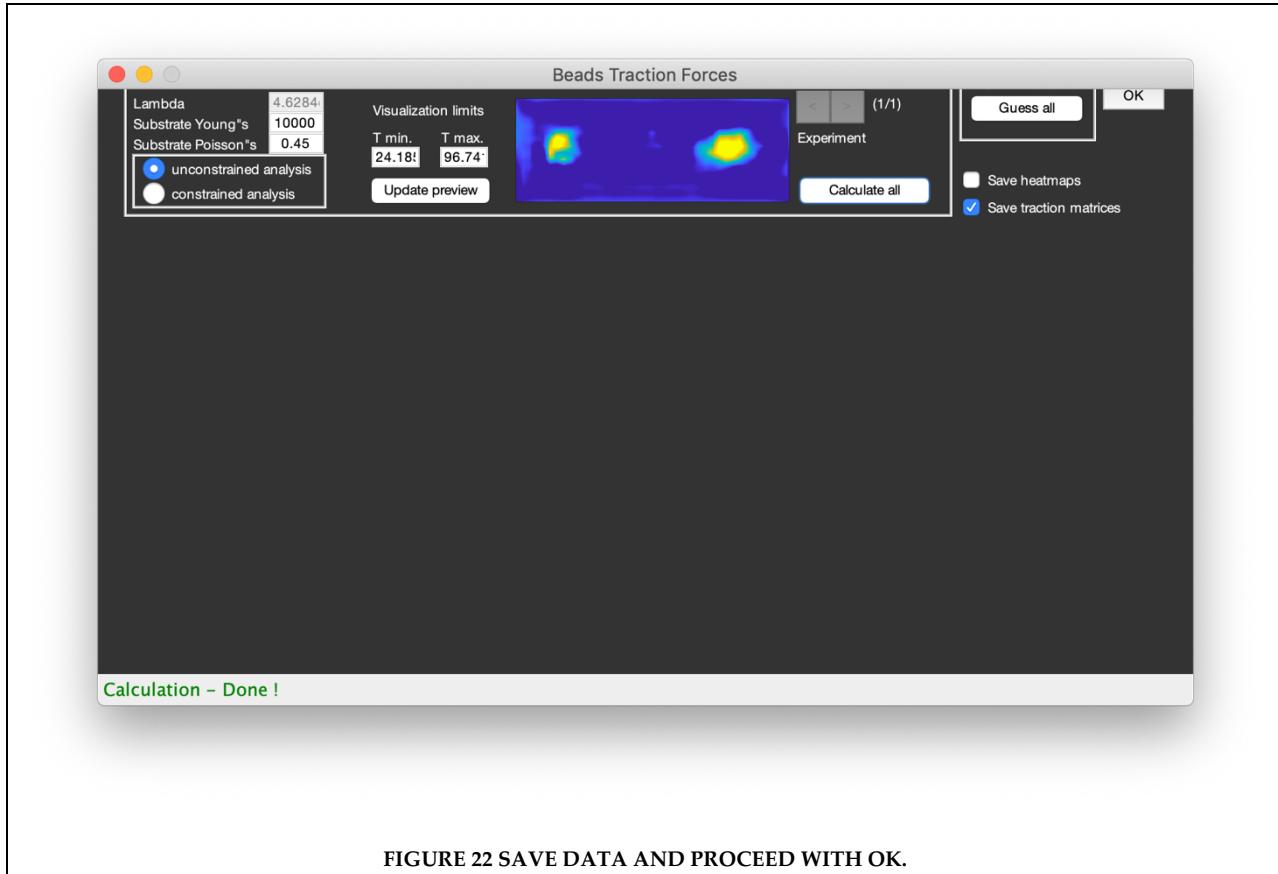


FIGURE 22 SAVE DATA AND PROCEED WITH OK.

4. RESULTS PANEL

1. In the main panel (Figure 23), launch the initialization panel by clicking on the *Initialization* button (Figure 24).

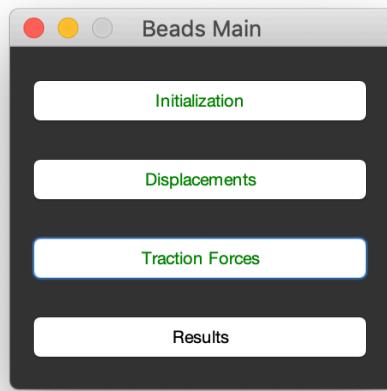


FIGURE 23 AFTER THE TRACTION FORCE STEP IS COMPLETED, THE RESULTS PANEL IS ENABLED.

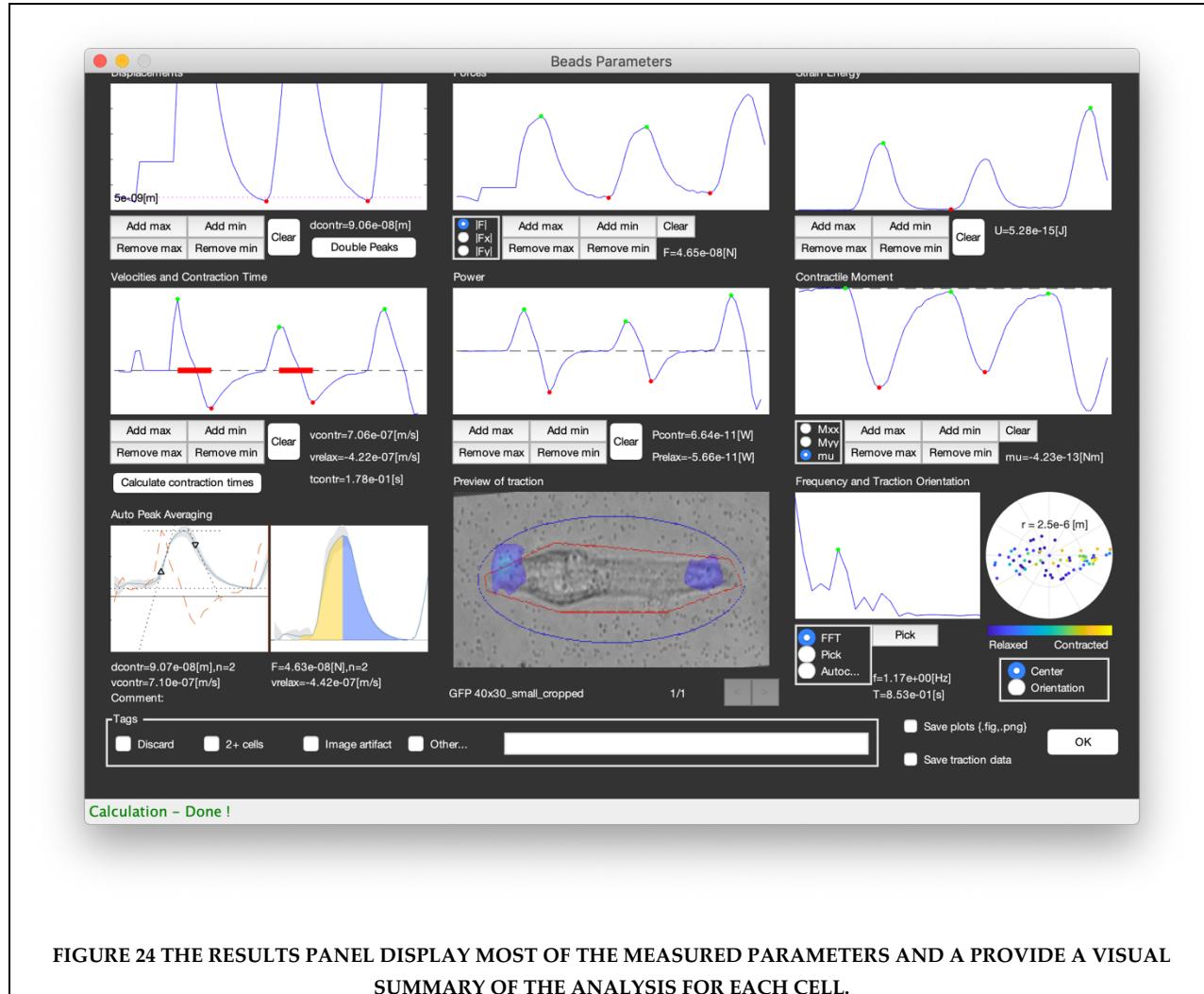


FIGURE 24 THE RESULTS PANEL DISPLAY MOST OF THE MEASURED PARAMETERS AND PROVIDE A VISUAL SUMMARY OF THE ANALYSIS FOR EACH CELL.

- When the results panel is launched, the software calculates all the contractile parameters for each cell. The results panel displays a summary of the analysis for each cell, with top 6 panels left-to-right row-by-row, the time traces of the:
 - Average bead displacement* (i.e., the average value of the displacement amplitude within the analysis area (blue ellipse)),
 - Total force* (i.e., the surface integration of the traction stress amplitude (or along and perpendicularly to the cell main axis) within the analysis area (blue ellipse)),
 - Strain energy* (i.e., measured within the analysis area (blue ellipse)),
 - Velocities and Contraction time* (i.e., the maximum and minimum velocities of contraction calculated as the derivative of the average bead displacement, and the duration of contraction measured as the duration between maximum and minimum contraction velocities),
 - Contraction Power* (i.e., the product of the contraction force and velocity),
 - Contractile moment* (i.e., the measure of the strength of the force dipole created by the cell).

- For each of these parameters, the peaks of interested are automatically selected and can be manually added or remove by the user.
 - In addition, if users are interested to capture addition data regarding the presence of double peaks, due to contraction twitch or cell doublet, this can be done by selecting the *Double peak* button and adding the relevant parameter in the pop-up panel.
3. The bottom row figure shows left to right:
- The results of the automated peak detection and averaging for the *Average displacement* and Contraction velocities, and of *Total force* with the area integrated for the computation of the *Contraction impulse*. Here, the peak detection is entirely automated, and the other parameters are also calculated in a similar way in the background. The user has no direct access to the data which remove the risk for user bias in the peak selection. The number of peaks that are detected and used for the averaging is mentioned below the figure with the valued of the measured amplitude.
 - A .gif video showing the contraction stress superimposed onto the brightfield/fluorescent preview image for each cell. This provide the user with rapid way to further assess the qualitative value of a measurement and potentially flag a cell using the predefined flags tick boxes and comment field.
 - A radio-button-switchable figure showing:
 - *FFT*: the FFT transformation of the *Average displacement* used to measure the *Contraction frequency*, enabling the user to manually refine the peak selection.
 - *Pick*: the user can measure the contraction frequency by picking peaks manually in the *Average displacement* curve, as well.
 - *Autocorrelation*: the autocorrelation of the *Average displacement* trace used to automatically identify and average the contraction peaks, which provide an additional way to qualitatively assess the quality of the automated analysis.
 - A radar plot showing the:
 - *Position* of the center of pression with respect to the center of the cell and provides a measure of the contraction coordination and homogeneity within the cell.
 - *Orientation* of the contraction dipole with respect to the cell morphology main axis, which provides a measure of the alignment of the contraction dipole with the cell geometry. In patterned elongated cell, this angle should remain minimal, while in non-patterned cells, the angle may vary in time.
4. *Tags*: The user can select between four predefined tags to mark cell videos that may not match the user required data quality and a comment field. The flag value for each tag and the comments left by the users are saved in the results file and enable easy data curation post-analysis (Figure 25).
5. Once the results are verified for each cell, the user select what additional data to save and proceed with *OK* to finalize the analysis. Finally, the main panel can be closed, and the analysis is finished (Figure 26).

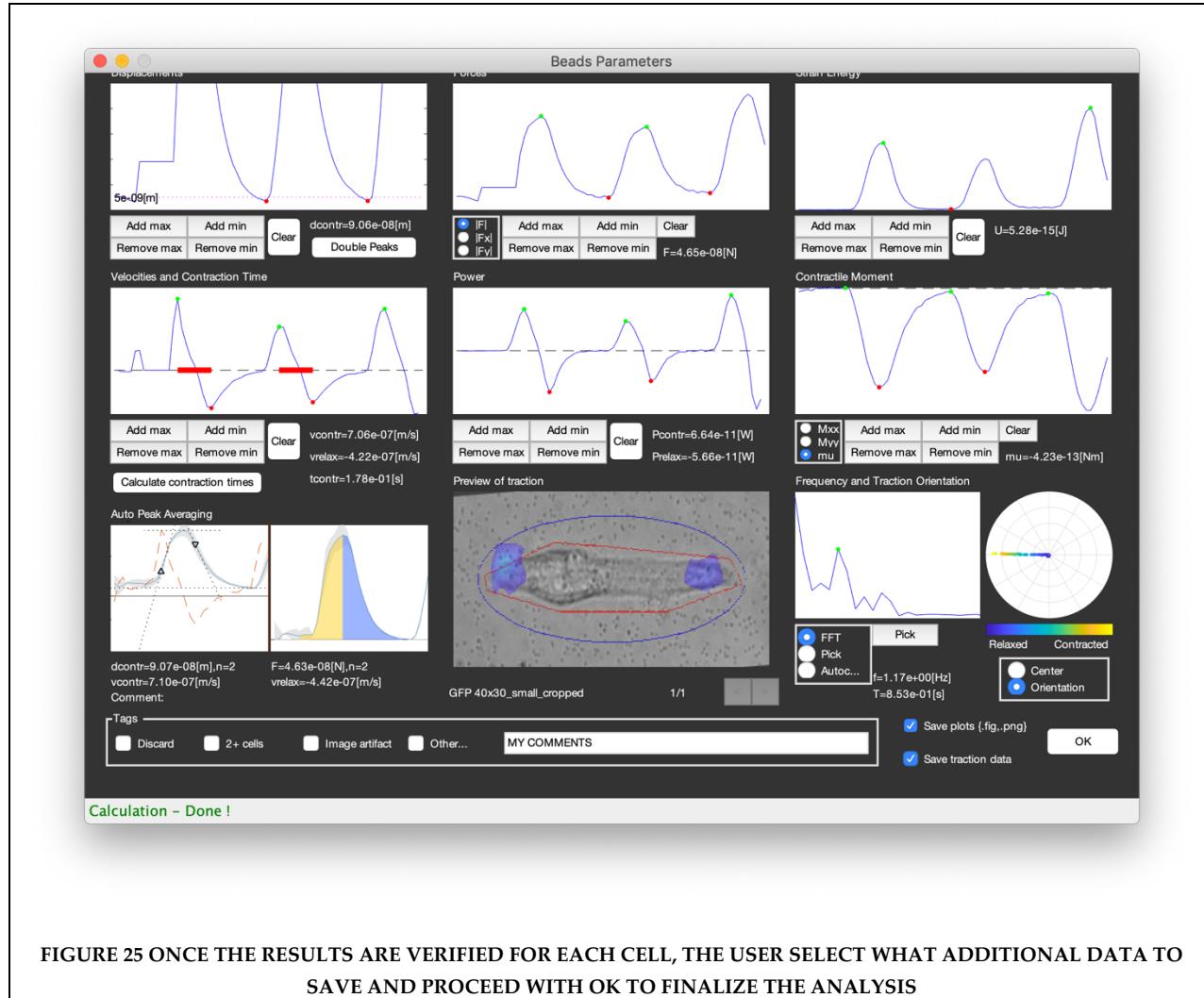


FIGURE 25 ONCE THE RESULTS ARE VERIFIED FOR EACH CELL, THE USER SELECT WHAT ADDITIONAL DATA TO SAVE AND PROCEED WITH OK TO FINALIZE THE ANALYSIS

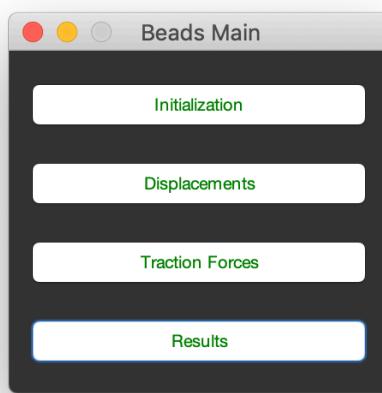


FIGURE 26 ONCE ALL THE STEP ARE COMPLETED, THE MAIN PANEL CAN BE CLOSE AND THE ANALYSIS IS FINISHED

OUTPUT OF COMPUTATION

The analysis produces a large amount of data and many output files and enough disk space is required.

The output file structure is as follow (Figure 27):

- If the videos are cropped and/or binned, this new version of the video is saved, and the original version is preserved on disk.
- A *Batch_results.xlsx* file is generated at the end of the results step, which combine the measured parameters for each of the videos analyzed during a batch analysis. The data for the manually selected peaks and for the automated peak averaging analysis are saved on separate tabs. This allows for rapid analysis and interpretation of data.
- A folder is created for each video loaded. A separate folder is created for the brightfield/fluorescent video/image if it has a different name than that of the corresponding beads video. For multichannel .czi, only one folder is created.
 - In the folder for the brightfield/fluorescent video/image, the cell outline mask only is saved (Figure 28).
 - In the folder for the beads video, the substructure shown in Figure 29 is generated:
 - *Plots:*
 - *Curve* plots: contains .png and .fig files of the results panel figures for easy and rapid review for each cell.

- *Traction heatmaps*: contains .png images of the traction heatmap for each frame and enables to generate a movie of the traction stress.
- *Dataset*:
 - *Traction Data*: contains the full resolution interpolated x and y component of traction stress as .mat files.
 - *Traction Forces*: contains the raw traction stress data at the point of analysis, *i.e.*, at the position of each PIV window tracked at the PIV step.
 - *Displacements*: contains the raw data of the displacement at the point of analysis, *i.e.*, at the position of each PIV window tracked at the PIV step.
 - A .gif file of the overlay of the traction stress over the cell image (same as shown in the *Results panel*)
 - *Mask*: contains the mask of the cell outline as .mat file.
 - *Results*: contains a .xlsx file with the complete results for each cell, including all the parameters save in the *Batch_results.xlsx* file and the time trace for all the parameters and the averages peaks curve.

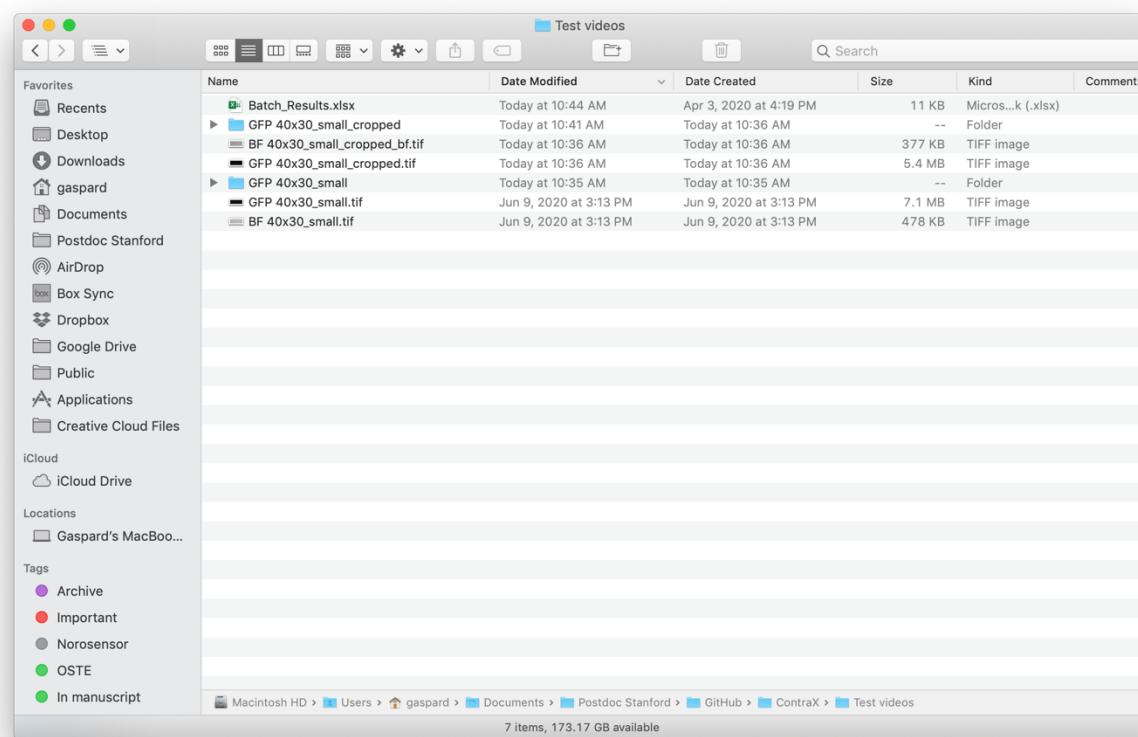


FIGURE 27 FILE STRUCTURE CREATED FOR EACH OF THE ANALYSED VIDEOS IN THE ROOT FOLDER

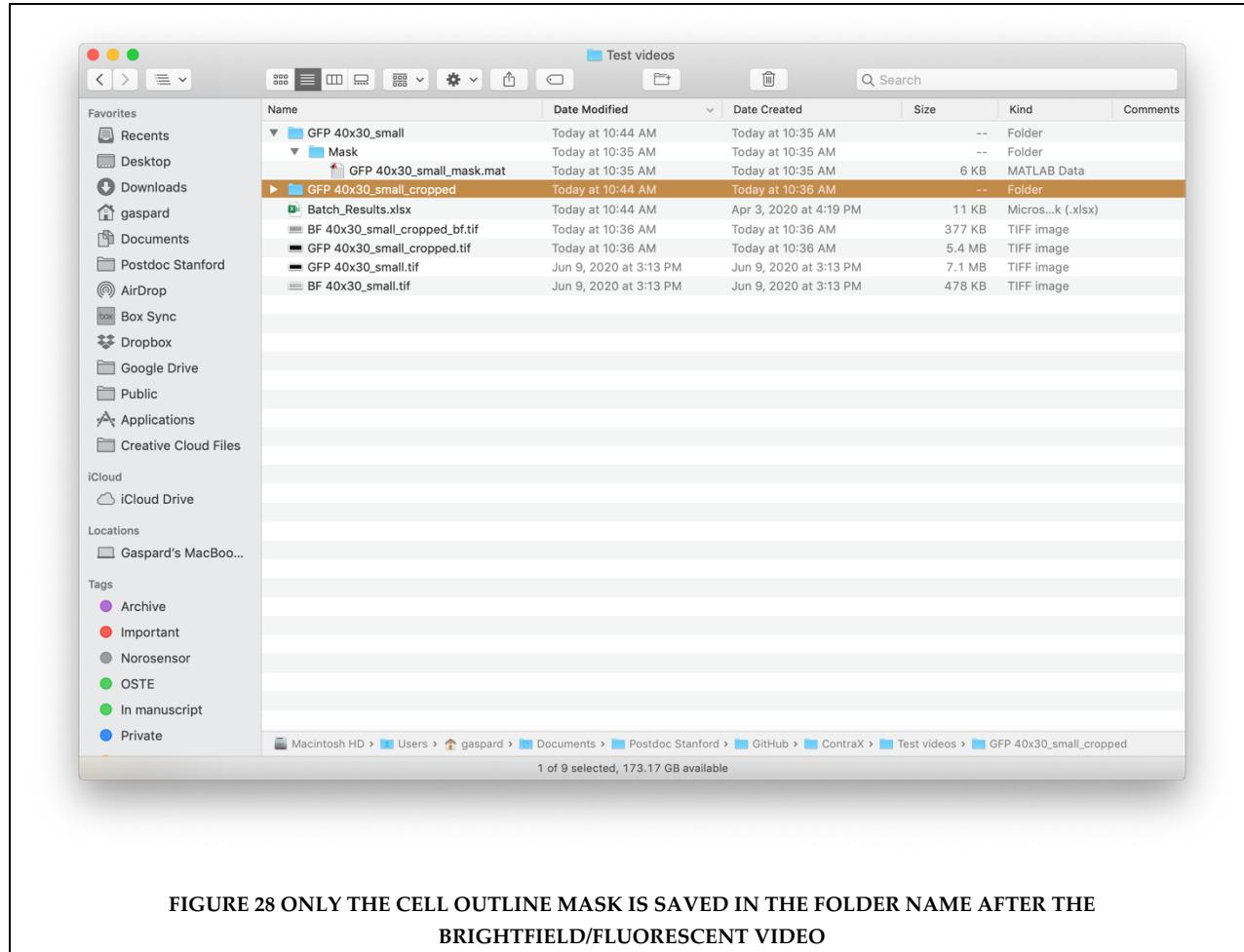


FIGURE 28 ONLY THE CELL OUTLINE MASK IS SAVED IN THE FOLDER NAME AFTER THE BRIGHTFIELD/FLUORESCENT VIDEO

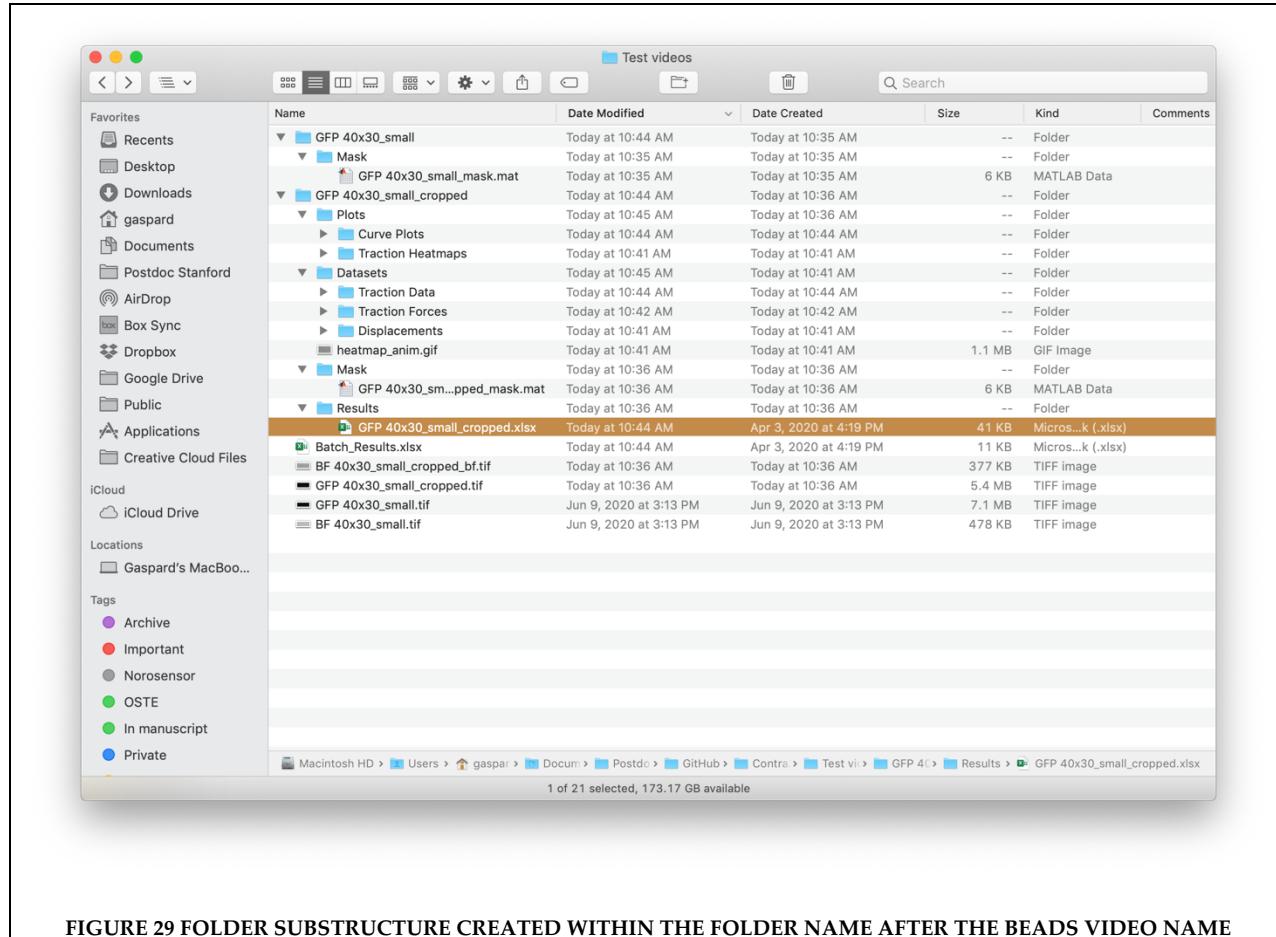


FIGURE 29 FOLDER SUBSTRUCTURE CREATED WITHIN THE BEADS VIDEO NAME