Software Requirement:

Software:

MATLAB 2013a or newer is required. Some functions described in the user manual require MATLAB 2014 or newer and are noted. The MATLAB toolboxes of Statistics and Machine Learning, Image Processing, and Parallel Computing are also required. Additional downloads may be necessary including CUDA (Compute Unified Device Architecture) associated drivers for the desired OS. We also employed the FastSet toolbox developed by Lev Muchnik which can be found here: (http://www.levmuchnik.net/Content/ProgrammingTips/MatLab/FastSet/FastSet.html)

Hardware:

A CUDA compliant video card is required. This current Matlab package has been tested with GeForce GTX video cards with 2GB or 6GB of on-board memory.

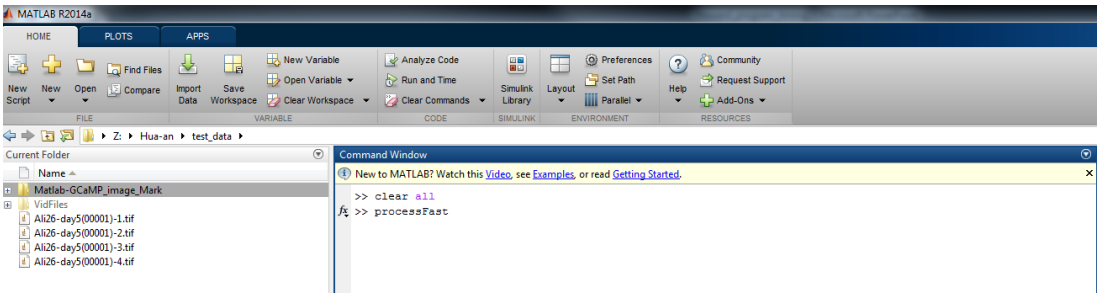
Software Use:

Prior to use, please be sure that the 3 associated MATLAB toolboxes have been installed and MATLAB 2013a or later is used. Download the MATLAB-Imaging Software folder and the demo video files (4) from the following url:
http://www.bu.edu/hanlab/resources/

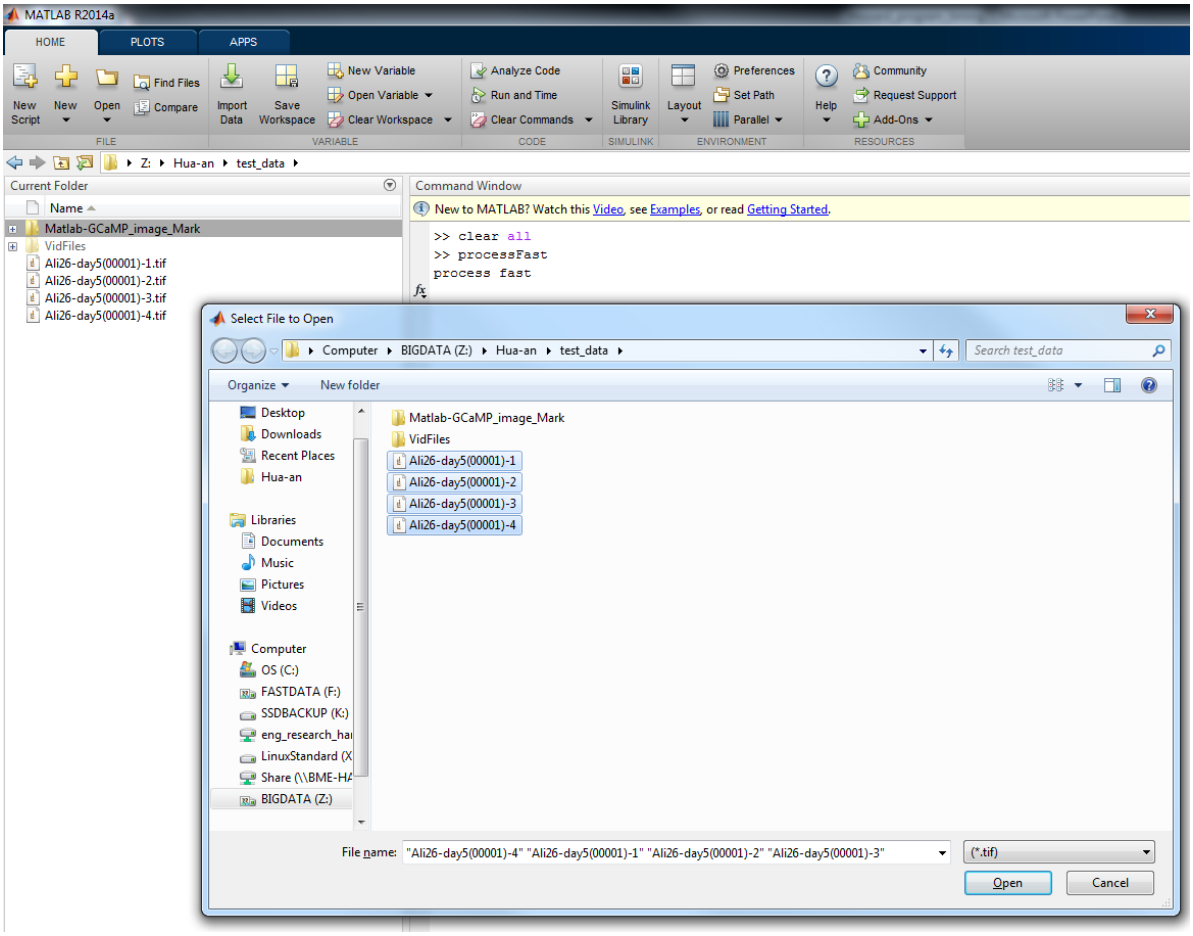
Tutorial:

First, ensure that the folder and associated files have been added to your MATLAB path. The primary function can be called by entering the following text at the command line:

```
>processFast
```

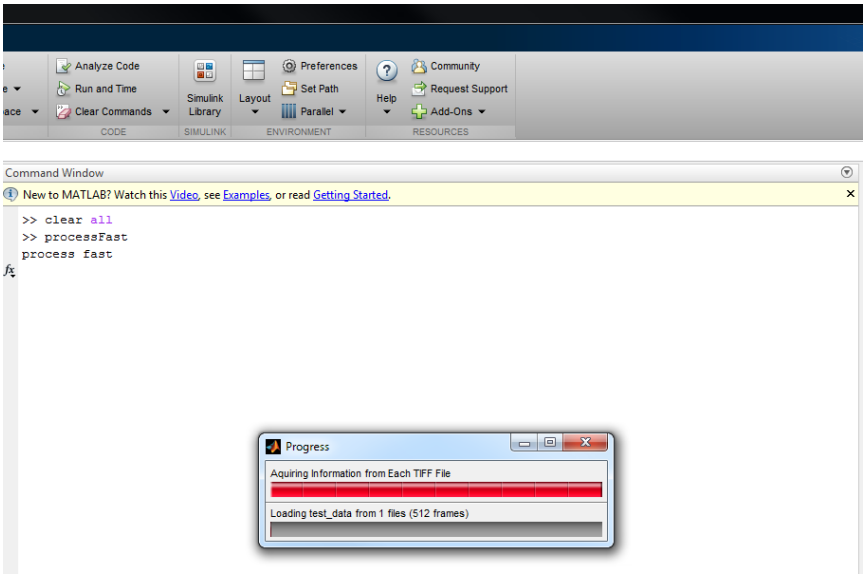


A dialogue box will open asking you to select the files you want to analyze. This program is designed to analyze mpTIFF formats. As mpTIFF's in standard format are limited to ~4 GB, longer recording sessions will include multiple sequential files. Files will be joined from top to bottom in the order listed, if more than one file is selected. Only files that represent continuous recording from a single imaging session should be selected, because ROIs will be identified and merged across all files selected in this step.



This step combines file loading, merging, filtering for contrast enhancement, image registration, and ROI generation as a single process, with a dialogue box providing instantaneous information of the current status information.

Loading:



Filtering:

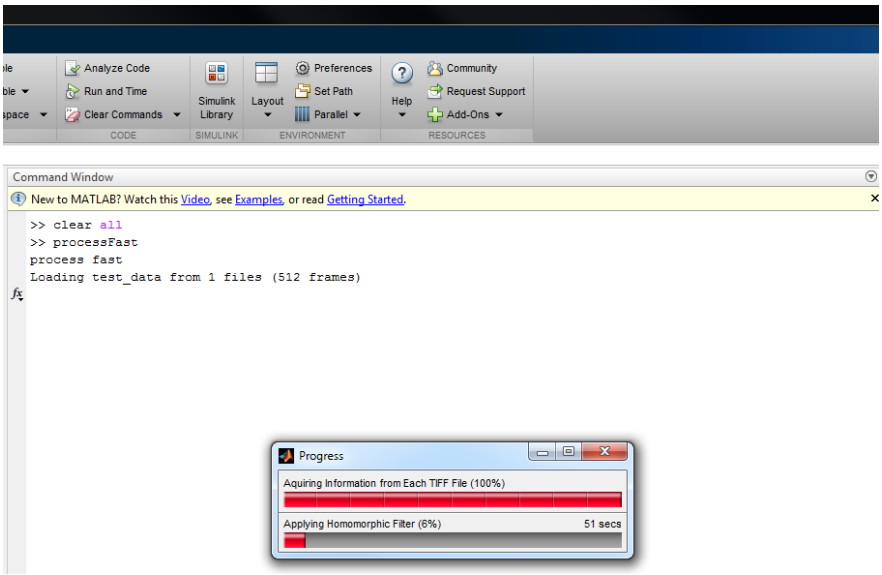
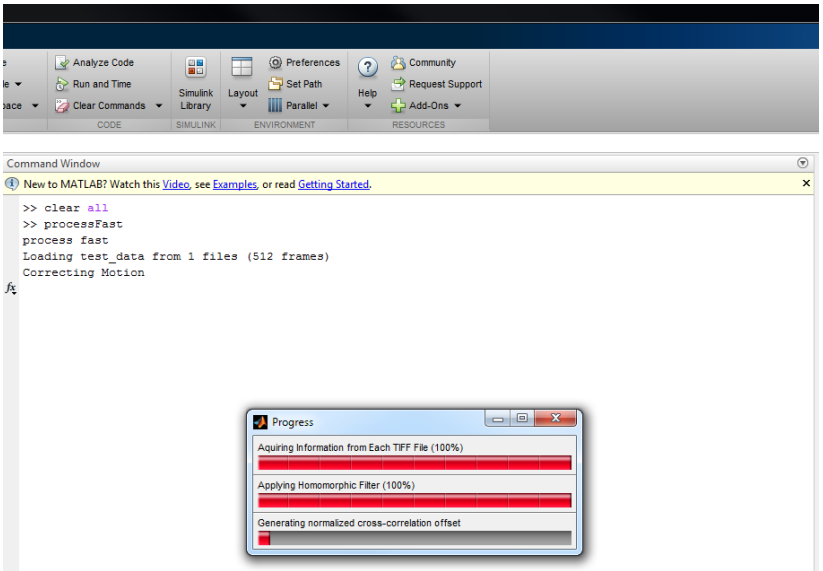
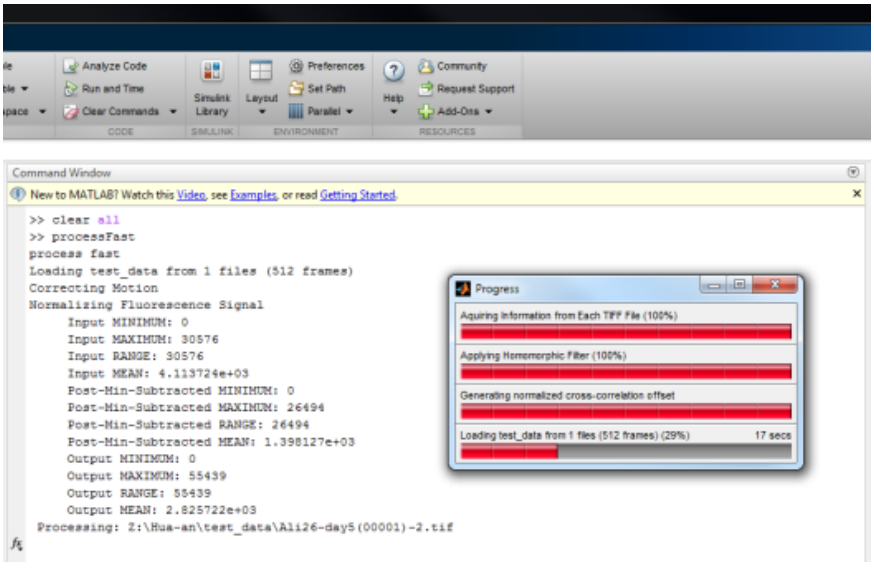


Image Registration:

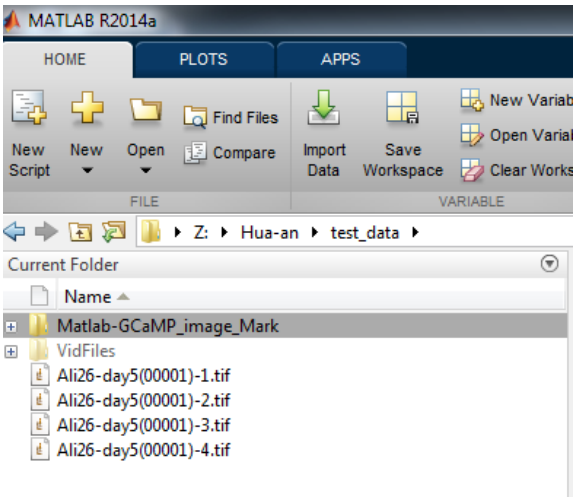


At the conclusion of this step, the second file will be automatically loaded and analyzed until all files have been completed.

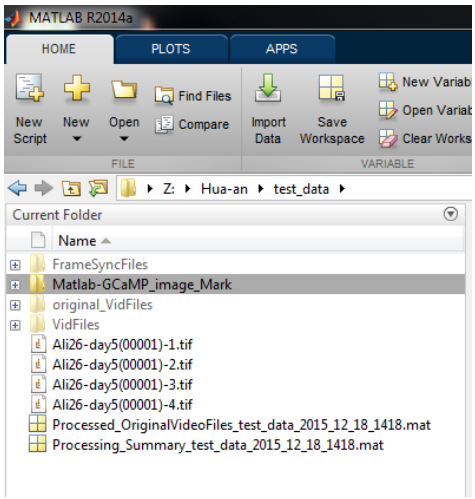


When the final file is finished processing, a new output file consisting of motion corrected and contrast enhanced image matrix will be automatically saved to your directory folder. Code to convert this array into a video format is included and is described later in this document.

Before



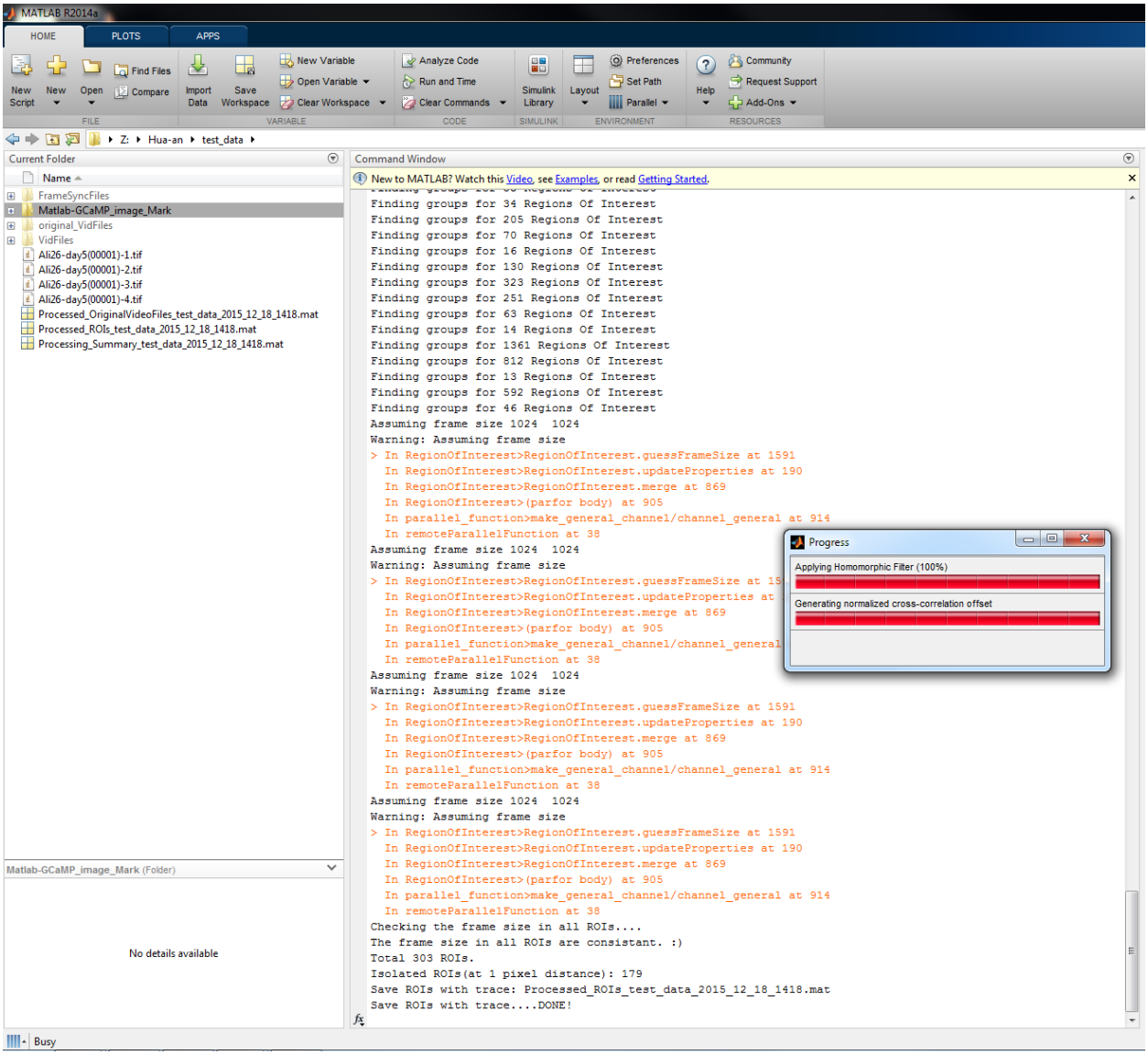
After



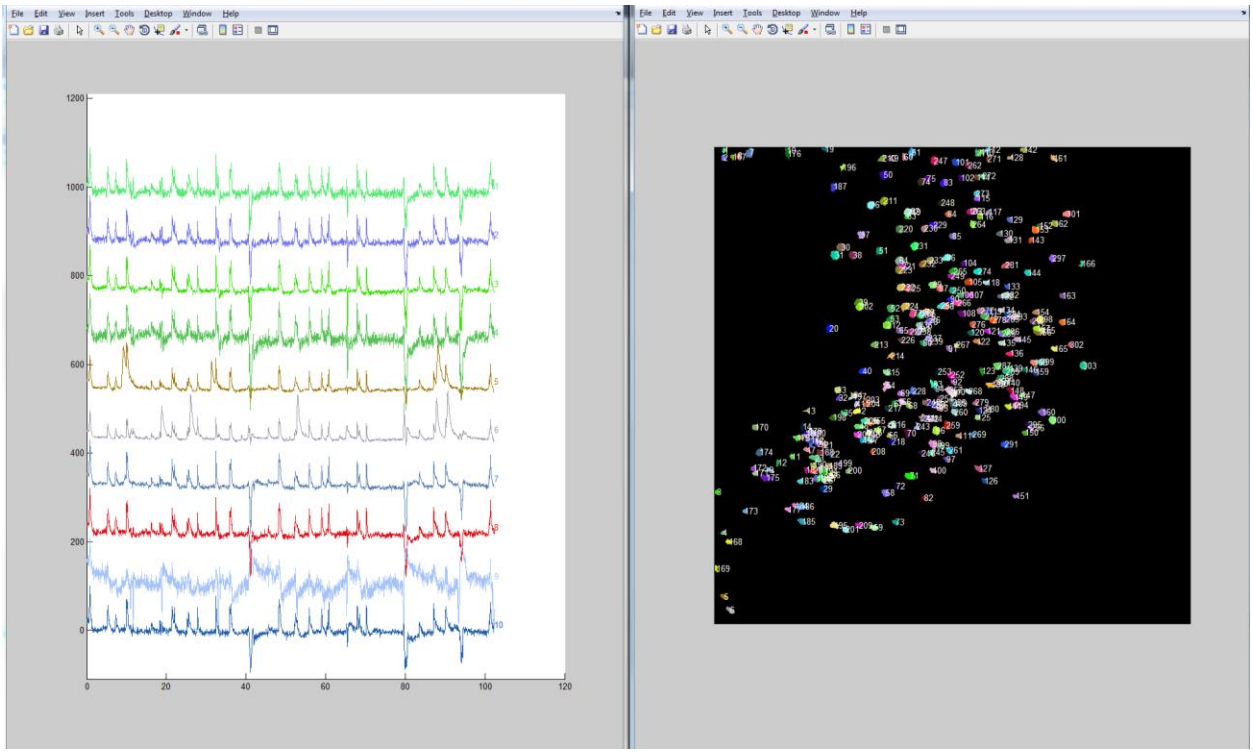
In the final step of software processing, single frame ROI's are generated for each image frame and then merged across all files. For a detailed description of this process please see the methods section of the accompanying paper. The accompanying functions have been commented to describe parameters that can be adjusted, allowing for user flexibility.

```
Loading test_data from 1 files (511 frames)
Correcting Motion
Normalizing Fluorescence Signal
     Input MINIMUM: 1
     Input MAXIMUM: 29814
     Input RANGE: 29813
     Input MEAN: 4.045571e+03
     Post-Min-Subtracted MINIMUM: 0
     Post-Min-Subtracted MAXIMUM: 25890
     Post-Min-Subtracted RANGE: 25890
     Post-Min-Subtracted MEAN: 1.329995e+03
     Output MINIMUM: 0
     Output MAXIMUM: 54026
     Output RANGE: 54026
     Output MEAN: 2.822854e+03
Start merging single frame ROIs....
```

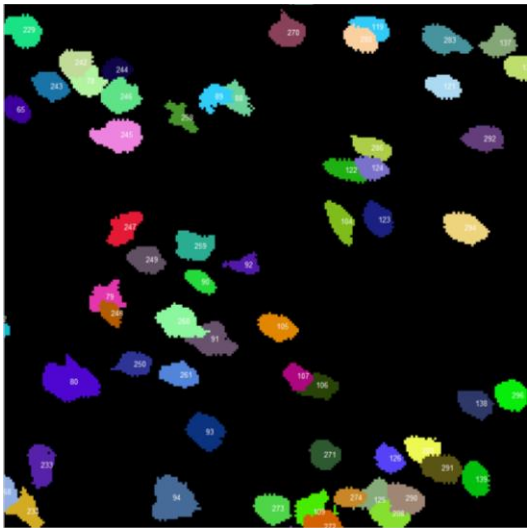
When the software finishes, it will return the number of ROIs found, descriptive statistics for each ROI, and image maps that allow the user to view the data.



Automated MATLAB images for quick visualization of data:



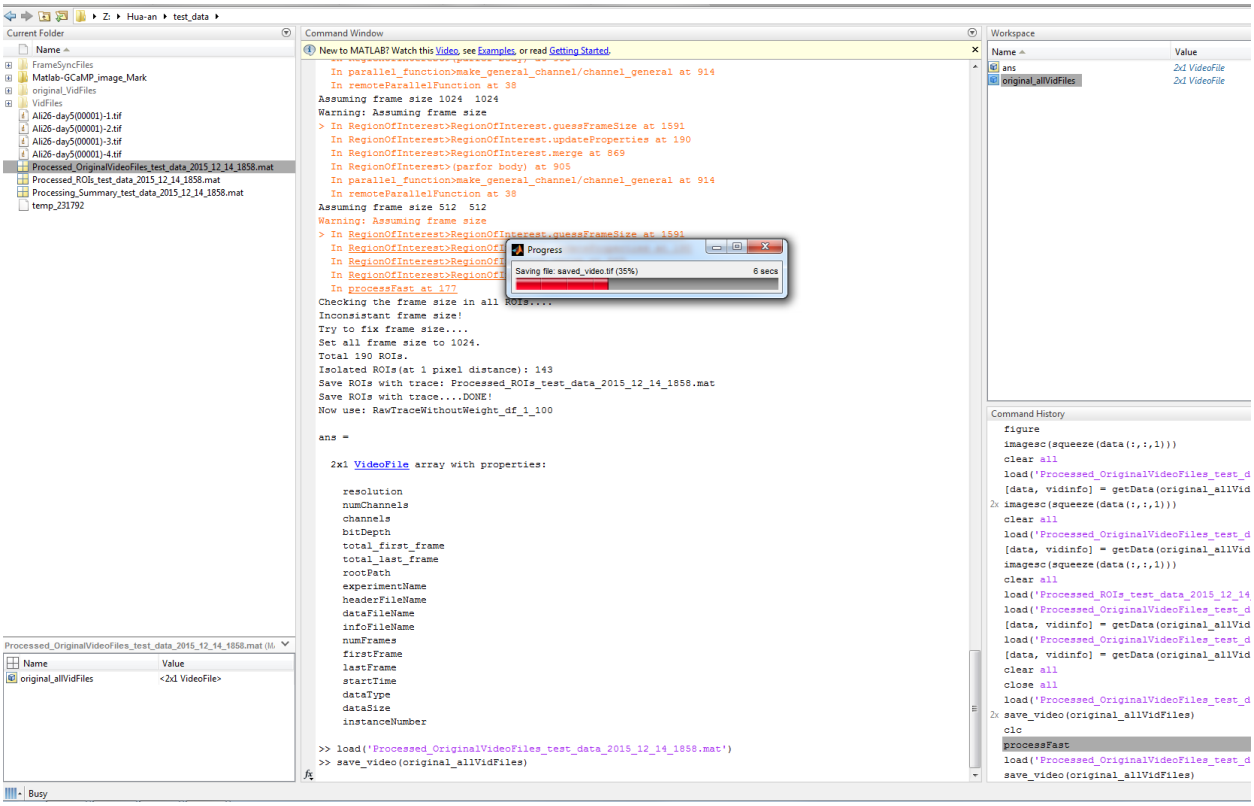
The accompanying figure includes ROI identifiers and can be zoomed into to look at individual ROI morphology and location.



Manipulating and processing imaging data:

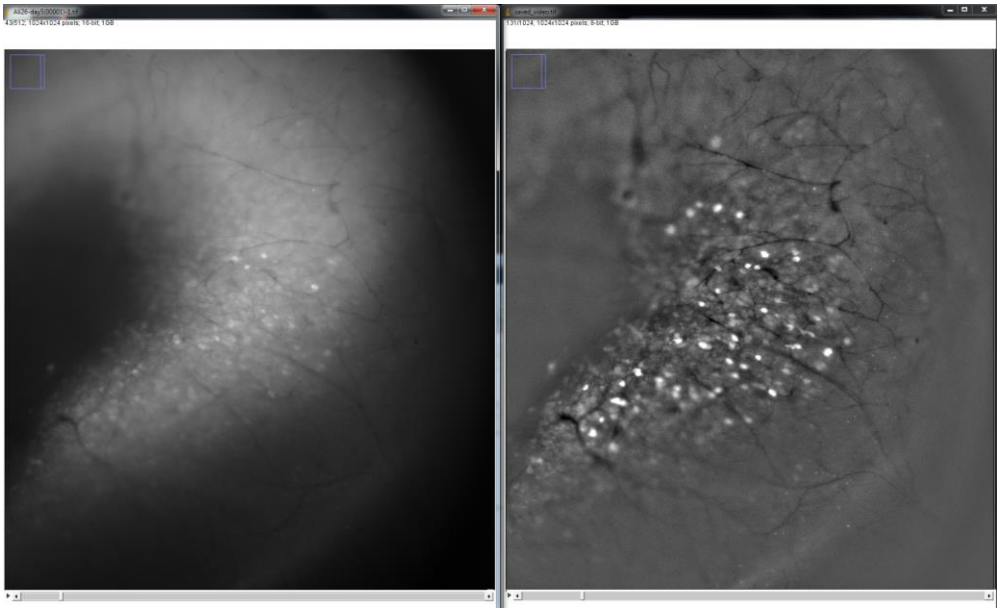
As described above, a matrix containing both the processed video and information regarding individual ROIs are now saved to the working directory folder.

To create new video, users can load the processed video files into their active workspace by clicking on the video.mat file, then run the save_video function at the command line to produce an mpTIFF file of the motion corrected video as shown below.



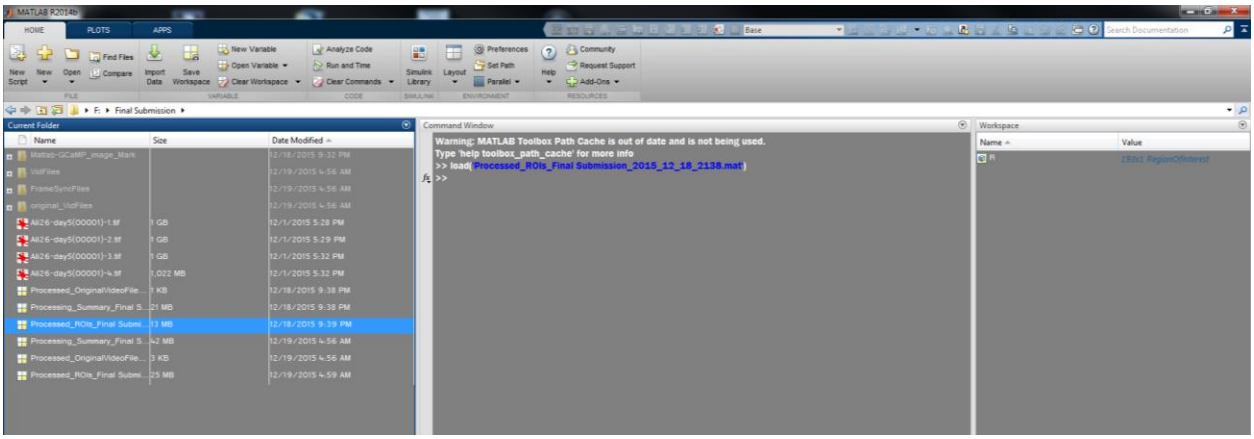
The video can now be opened in viewed in any video player that supports mpTIFF formats. Here we show the demo data side by side with the original video using the open source program Image J.

Original Corrected

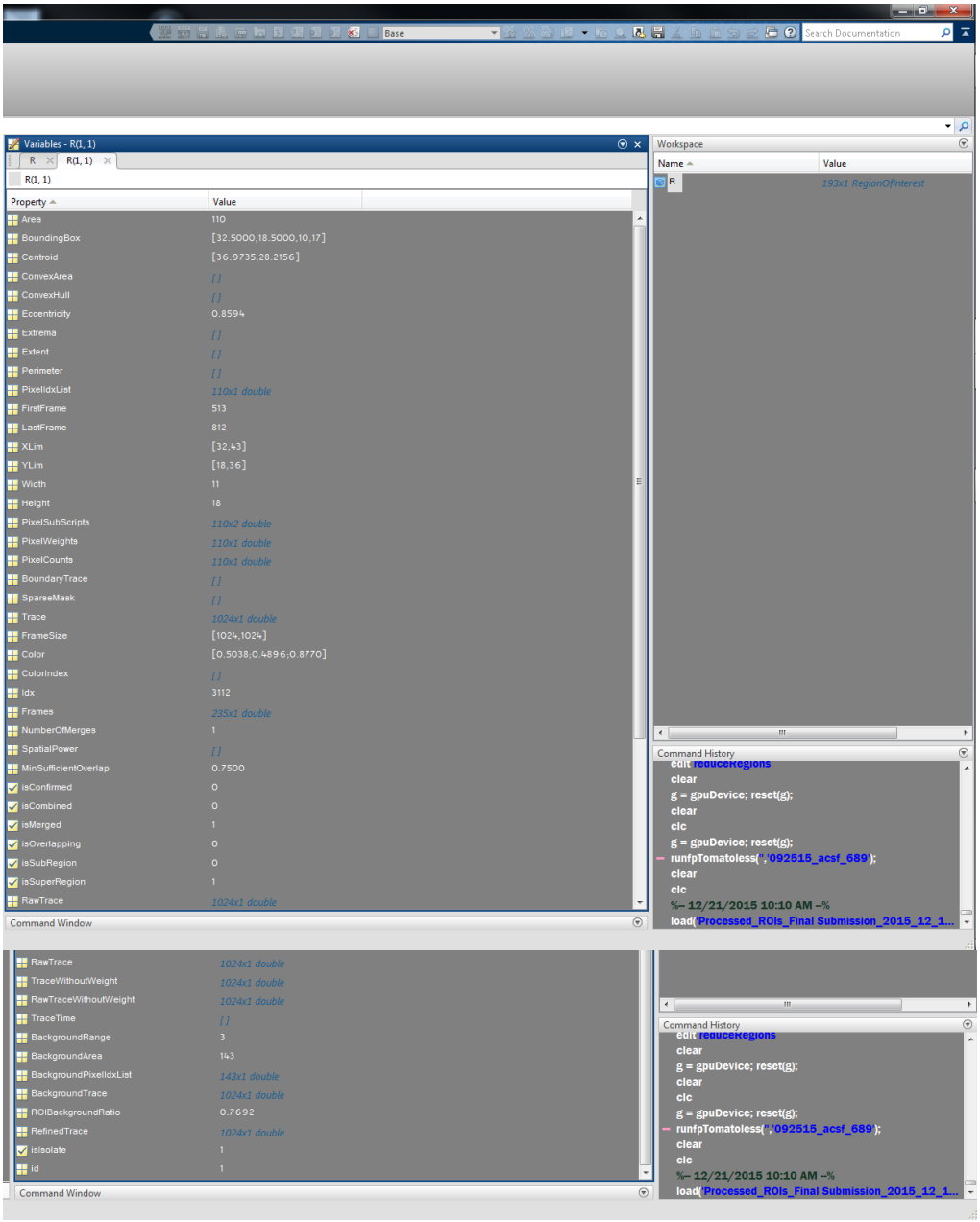


Visualizing identified ROIs:

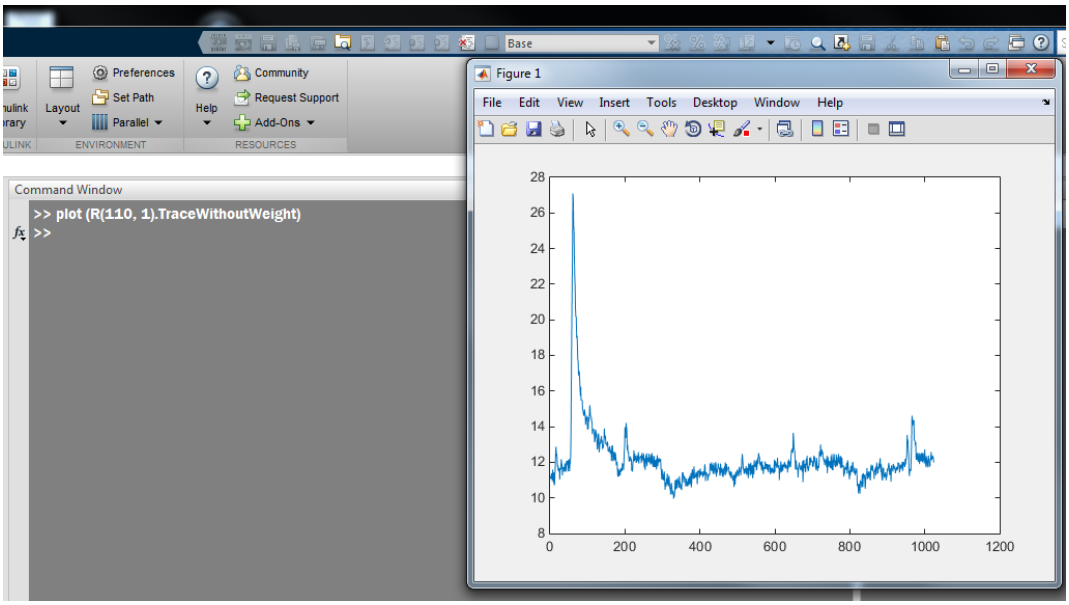
All ROI information, including descriptive statistics can be accessed by loading the ProcessedROI.mat file into the active workspace.



This returns the object R. By clicking on R, users can access all statistics returned by the processFast function related to each identified ROI.

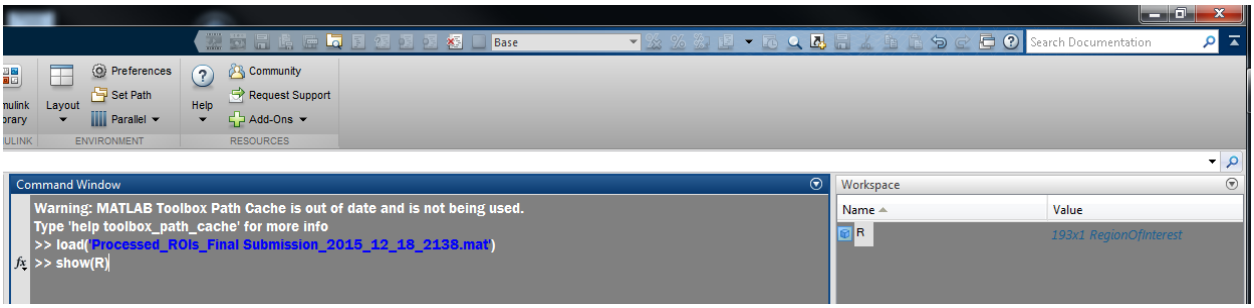


Plotting individual ROIs can be done from the command line using traditional MATLAB commands:

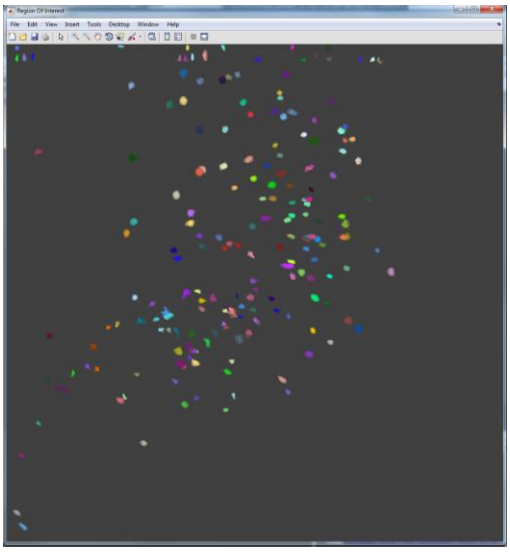


For users running MATLAB versions compatible with hGversion2 graphics (2014 and later), additional functions allows for an interactive plotting tool to highlight or compare identified ROIs. To take advantage of this tool, users with compatible versions of MATLAB can use the show function. The function requires that the ProcessedROI.mat file is in the workspace and can be called by entering the following text at the command line:

>show(R)



This will produce an interactive figure allowing the user to highlight and compare individual ROIs.



Users then can click on individual ROIs to get the time series plot for any ROI in the imaging window. Left clicks produce a single trace and clear all current traces. A right click adds traces to the current time series allowing for multiple ROIs to be shown in the same figure.