Supplemental Text 1: Directions on using the application

***Opening the Application***

If using the stand-alone app, you can simply call the .exe or by double clicking a desktop shortcut. However, if you wish to run the application through the source code within MATLAB, you will need MATLAB 2018b or later and must do so within the appdesigner app within MATLAB by calling “appdesigner” in the command window. Once appdesigner is open, you can go into open and select the application .mlapp file. While no licenses are needed for using the stand-alone application, you must have a license for MATLAB and for multiple sub licenses that are used within the code if running on MATLAB, including the image processing toolbox and, if you wish to use parallel computing, the parallel computing toolbox. Once complete, you will have the main application open (Fig 1).

***Selecting Data***

Once the application is open, the first step is to select the data in which you wish to process. To do so, you hit the “Select Main Folder” button (Fig 2a). A select folder prompt will open. Here, you select the folder in which all the data you which to process is stored, selecting only architectural or proliferation staining at a given time. The application will automatically go through the folder structure and find the deepest subfolders throughout and list them in the “List of Data Sets” text box (Fig 2c). You will then be able to select items of the list and can delete the items that you do not want to process (Fig 2f). The app will also bring up the staining drop down list, in which you can select if you are processing raw, architectural, or proliferation staining (Fig 2d). The second drop down will give functions that can be completed based on the selected data type (Fig 2e).

***Dealing with Raw Data***

*Convert to individual channels*

Typically, raw data will come directly from the microscope and must be translated into a different form in order to process. Here, our first step is to convert a microscope file into individual channel 3d images. To do so, you select raw for the staining and convert to individual channels as the function (Fig 2d-e). Another drop down will show which will allow for you to choose if what you are converting is proliferation or architectural staining (Fig 3a). You then can select to run only highlighted items from the list of data sets or to run all data sets (which is suggested, Fig 3b) and hit run (Fig 3c) to open a secondary app. Within this app, you can choose the staining order (Fig 3d), the file type you are converting from (Fig 3e), and then run (Fig 3f). If each individual image does not have its own folder, new folders will be created automatically, where raw images are grouped with their corresponding individual channel images (C1 – C4). Once complete, you should reselect the main file if your folder structure was altered in order to regather the list of data sets.

*Visualize and Cut*

It is typical, when utilizing z-stack imaging, that a few frames at the start and at the end of the images are of poor quality and should be removed. To do so, we need to visualize the images, determine which frames of the images are unusable, and then remove those parts from the images. To do so, you select **Raw -> Visualize and cut ->** hit the **select** button **->** **cut and remove from list** (suggested) or **cut and keep in list** **-> run**. This will open a secondary app that will allow for you to look through an individual image, find the frames you want to be first and last, and cut the images at those positions (Fig 4). The cut and remove will remove the item you visualize from the list of data sets once complete. You can regather all data sets by reselecting the main folder. To navigate, you can change channel and frame (Fig 4a-b). , If the image is up and selected (last item clicked on), these can be changed by hitting left and right to move through channels or up and down to move through the z stack. To cut the image, you put your desired first and last frame into their respective bins (Fig 4c-d) and hit cut (Fig 4e). This will exit this secondary app. If you exit out of the image by accident, you can reopen it by hitting the visualize button (Fig 4f).

***Architecture and Proliferation Image Processing***

Once the data is cleaned up, the image processing is very straight forward. You select the type of staining and what function you wish to complete (Fig 2d-e). The functions will be to segment, gather, collect, and visualize. Segmenting finds the voxels belonging to each component within the images, gathering finds the parameters for the segmentation for each individual image set, and collecting consolidates individual image set parameters, with details about the individual image sets, together. You will want to go through the process in the order of segmenting -> gathering -> collecting for both staining protocols. The visualize function will allow you to visualize the segmentation in 2d after the images have been segmented. Once you select the function, an options drop down list will appear. It will give you the ability to do all the function at one time or break into individual components. It is recommended to select the all options. For the segmentation, you can check the run-in parallel check box to run in parallel (Fig 2g). The app will automatically determine the number of cores and free ram to optimize the number of workers the parallel computing is broken into and show these values in text boxes in which the values can be lowered by the user. You then hit run, and a progress bar will appear and work to estimate the remaining time of the process.