CurveAlign Users Manual

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# Introduction

The GUI in CurveAlign is modular, so that the main user interface is in a separate window from the outputs. This allows for the user to resize the output windows to their preferred size. The main user interface window is shown below.

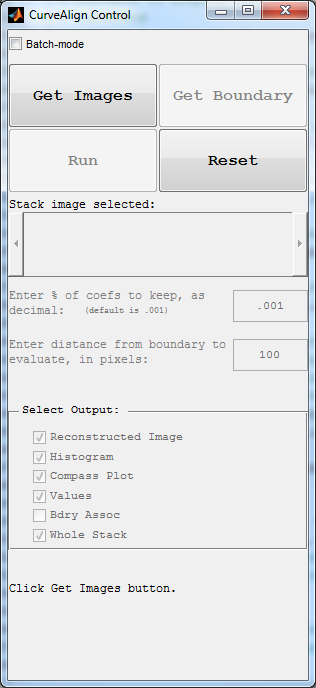


Figure . Main user interface for CurveAlign.

# QuickStart Instructions

1. Click “Get Images” and browse to an image. Images may be single or stacks. If you would like to process a directory of images use the “Batch-mode” checkbox (See batch mode section below).
2. If no boundary is required, go to step 3. To draw a boundary, hold down the Alt key and click points along the desired path on the image window. Release the Alt key when finished.
3. Enter the keep threshold level and select the desired outputs using the checkboxes on the bottom of the main window.
4. Click the run button.
5. You will be prompted to select an output directory where the output data will be placed.
6. If you have drawn a boundary, you will be prompted to give a file name and location for the boundary points to be saved. These will be saved as a \*.csv file. This file can be used again in the future by clicking the Get Boundary button.
7. The current function being performed by CurveAlign is listed in the Status label at the bottom of the main window.

# Output files

There are several files that are generated by the CurveAlign software for each image processed. These files and their descriptions are listed in the table below:

|  |  |
| --- | --- |
| **Filename** | **Description** |
| \*\_compass\_plot.csv | List of compass plot values |
| \*\_hist.csv | List of bin values and numbers of curvelets in each bin |
| \*\_values.csv | List of all curvelets, their angles (col 1), and their distances to the boundary in pixels (col 2 if a boundary is being used) |
| \*\_stats.csv | List of values containing:   * Mean angle * Median angle * Variance of angles * Std Dev of angles * Coef of Alignment (vector sum of angles) * Skewness of angle distribution * Kurtosis of angle distribution * Omni Test on angle distribution * red pixels in procmap image * yellow pixels in procmap image * green pixels in procmap image * evaluated pixels in boundary analysis |

# Output images

A few output images are saved in .tiff format in the selected output directory. These images are explained below.

## Overlay image

This image shows the position and angle of each curvelet that is being used in the analysis (green) and each curvelet that is ignored (red) as an overlay on the original image. If a boundary is selected, the boundary is also included in the overlay.

TACS-3a_overlay.tiff

Figure . Overlay image showing the curvelets that are within the specified distance from the boundary in green, the curvelets outside the specified boundary in red, and the boundary in yellow.

## Map Image

The map image is intended to help the user identify the spatial distribution of curvelet angles within the image.

The raw map (\_rawmap.tiff) file codes the angle of the curvelet into a grey scale value. The pixel in the image where the center of the curvelet is located is given a value between 0 and 255 that corresponds to 0 to 90 degrees when a boundary is selected and 0 to 180 degrees when a boundary is not selected. This file may be further processed in matlab or imagej according to the users preferences.

The processed map file (\_procmap.tiff) is a processed version of the raw map file overlayed on the original image. The output is intended to show regions of aligned structures that are perpendicular to the boundary, in the case of a boundary selection, or regions of generally aligned structures, in the case where no boundary is selected.

### How processed map files are created:

When a boundary is selected, the raw map file translates 0 to 90 degrees into 0 to 255 in gray scale. The center location of each curvelet is given a gray level corresponding to its angle with respect to the boundary. Then a square max filter is applied with a size of 12 pixels on a side, followed by a Gaussian disc filter with a sigma of 4 pixels. The color scale is then set to 0-20 degrees = black, 20-45 degrees = green, 45-60 degrees = yellow, and 60-90 degrees = red. The map image is overlayed on the original image with transparency set to 0.5. See Figure 3.

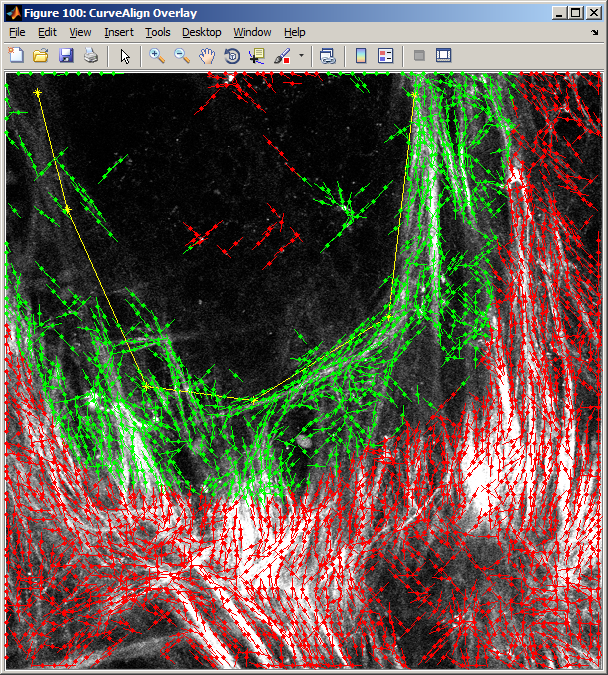
TACS-3a_procmap.tiff

Figure . Over lay (left) and map (right) images when a boundary is selected.

When a boundary is not selected, the raw map file translates 0 to 180 degrees into 0 to 255 in gray scale. The center location of each curvelet is given a gray level corresponding to its absolute angle in the image. Then a square, standard-deviation filter is applied with a size of 64 pixels on a side. The result is then subtracted from 127.5 (half of 255), followed by a 12 pixel max filter, followed by a Gaussian disc filter with sigma of 4 pixels. The color scale is then set to 0-80 black, 80-90 green, 90-100 yellow and 100-127.5 red. In this case, red indicates areas of highly aligned structures, while black and green indicate areas of more randomly aligned structures. The map image is overlayed on the original image with transparency set to 0.5. See Figure 4.

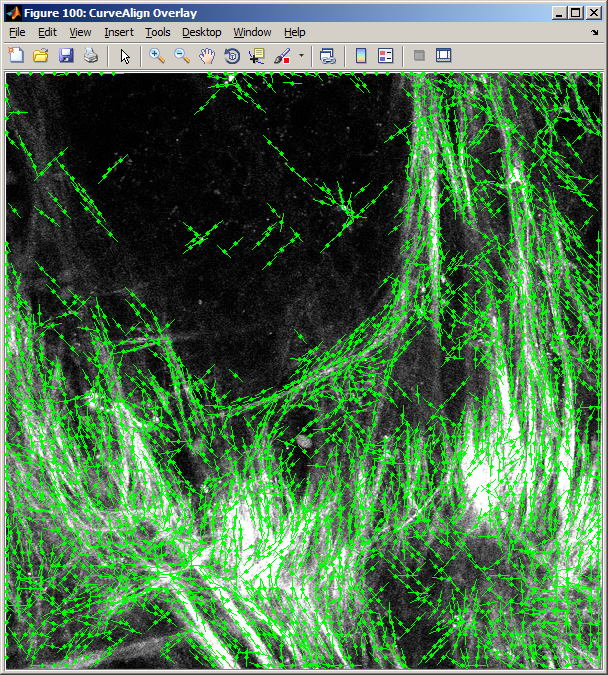
TACS-3a_procmap.tiff

Figure . Overlay and map images when no boundary is selected.

## Reconstructed Image

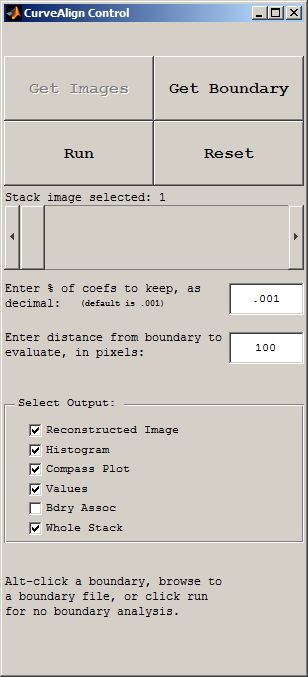
This is an image reconstructed from the thresholded curvelets. It shows all of the edges in the image that were measured.

TACS-3a_reconstructed.tiff

Figure . Reconstructed image.

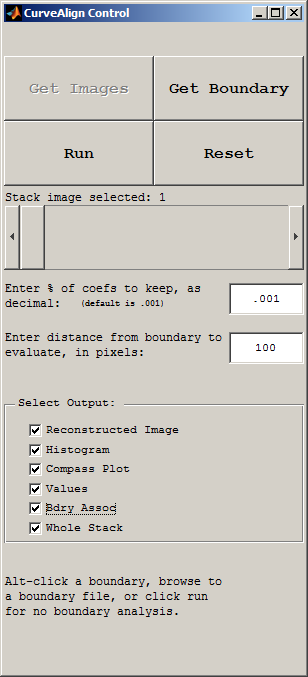
# Boundary Analysis

The user is able to analyze the curvelets that fall only within a certain distance from a boundary. To enter this distance, first a boundary must be drawn or opened from file. Then the distance in pixels should be entered into the edit box.



Boundaries are created by alt-clicking on the original image that is opened in the CurveAlign GUI. When alt is released, the boundary is ended and no additional points may be added to the boundary. Boundary files can also be opened by browsing to a saved boundary file using the Get Boundary button.

The position on the boundary that is being compared to each curvelet may be visualized as well. This allows the user to see where the angle comparisons are being made. Blue lines are drawn on the overlay output image from each curvelet to the point on the boundary that the curvelet is associated with.



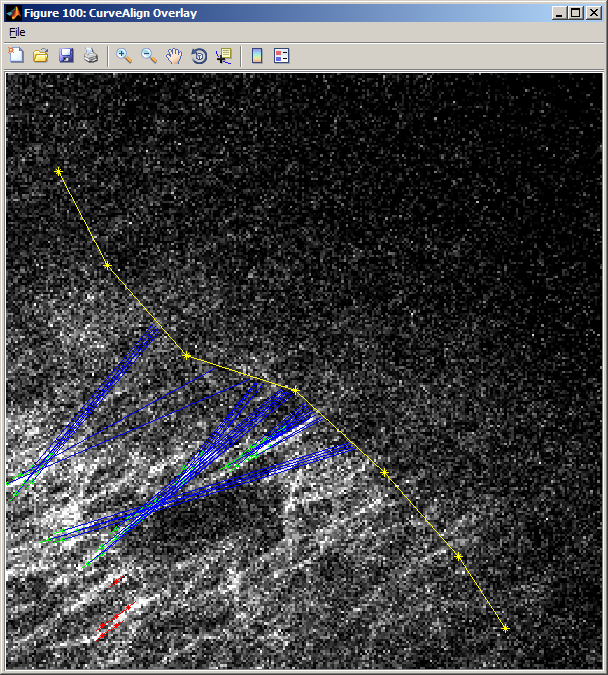
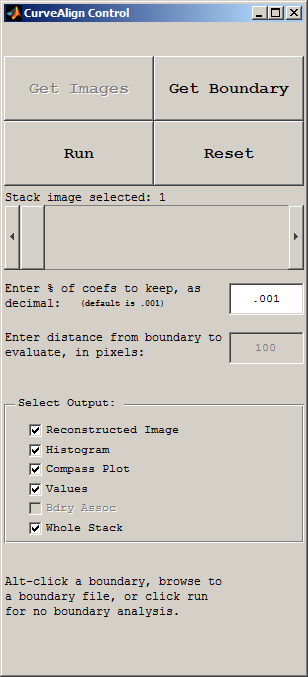


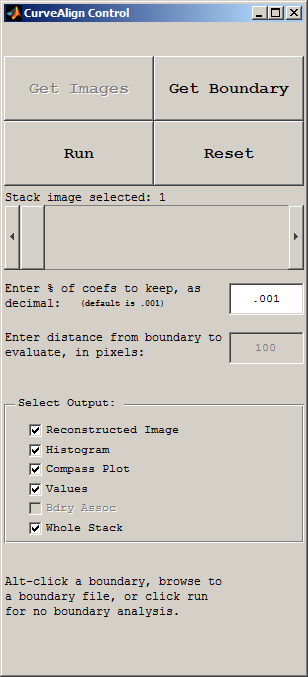
Figure . An example of an overlay images where the associations between curvelets and the boundary are indicated by blue lines.

# Stacks

CurveAlignV2.0 allows for the user to select stacks of images. When a stack is opened, the slider bar is enabled, allowing the user to choose which image in the stack should be displayed.



There is a checkbox at the bottom of the main window that allows for the user to select if the whole stack should be processed, or only the current image should be processed.



When a stack is being analyzed, then the output files that are images will also be in stack format. To open these stacks in ImageJ, the LOCI bioformats importer must be used. For some reason, these tiff stacks cannot be drag and dropped into ImageJ, this will be investigated further in the next release of CurveAlign.

The other output files, for example the histogram file and the curvelet angle spreadsheet, are produced such that each image in the stack creates a new output file. For example a stack with 4 images will generate 4 histogram files named stack\_1\_hist.csv, stack\_2\_hist.csv, stack\_3\_hist.csv, etc.

## Stacks with Boundaries

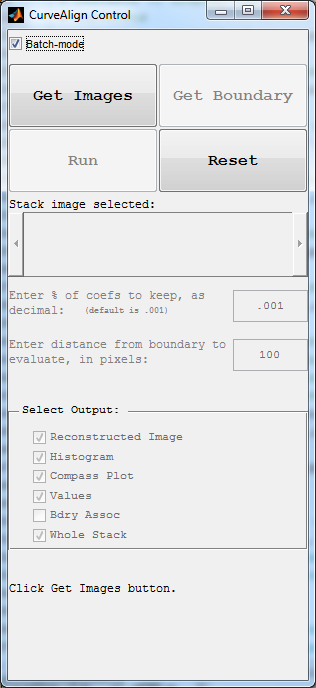
If a stack is being processed, then only a single boundary can be used for the entire stack. Future versions of this tool will allow for a different boundary in each image plane in a stack.

## Stacks without Boundaries

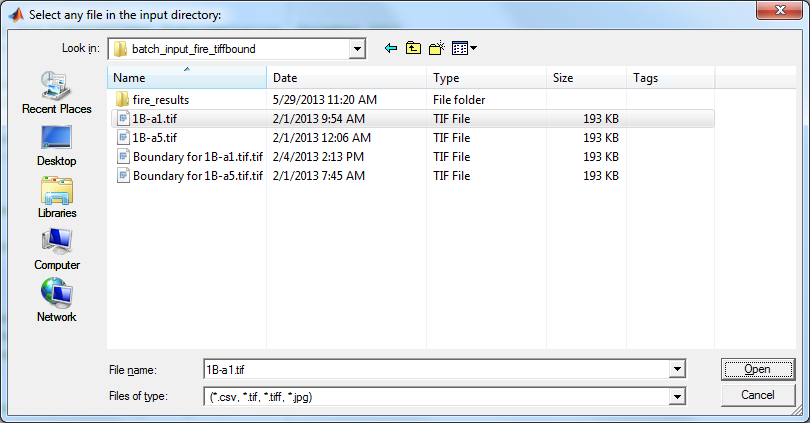
When no boundary file is selected, then each image in the stack is analyzed as described in the section above about image analysis without a boundary.

# Batch mode

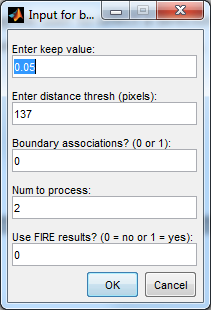
Batch mode is used to process entire directories of images and potentially their associated boundaries. Check the “Batch-mode” checkbox and click Get Images to start batch mode.



A file selection GUI will then open allowing the user to select a directory where a collection of images are stored. Browse to the desired directory and select any file within that directory and click Open.



Once the directory has been selected, a simple batch mode GUI starts that allows the user to enter input parameters related to the batch analysis.



The keep value, distance thresh, and boundary associations are explained elsewhere in this document.

The “num to process” input field allows the user to select how many files to process in the directory. For example, if there are 1000 files in a directory, and the user only wants to process the first 2, then the user should enter the number 2 in this edit box. The order of processing depends on the operating system of the computer CurveAlign is running on. By default, the number that is in that box is the total number of images found in the selected directory.

The “Use FIRE results?” input field allows the user to use the output of the CT-FIRE software as input to CurveAlign. If this is selected, then the CurveAlign program does not perform a curvelet transform. Instead, the fiber database that was generated by the CT-FIRE software is used to gather angle information about the fibers in the image.

CurveAlign will first search for boundary files in the chosen directory. If there are boundary files, then CurveAlign will process all images that are found to be associated with the located boundary files. If no boundary files are found, then CurveAlign will process all images in the directory and produce absolute angle information. Images may be a mixture of both individual images and stacks. If the image is a stack, then the entire stack will be processed.

The batch mode output files will be stored in a directory named CA\_Out and will include all of the outputs available from the CurveAlign software.

## Batch mode with CSV boundaries

If boundaries are drawn with the CurveAlign program, they should be saved in the same directory as the images. The boundary files should be named like the following:

Image file name = TACS-3a.jpg

Boundary file name = boundary for TACS-3a.jpg.csv

## Batch mode without boundaries

To process a directory of images without boundaries, then just place the images in a directory by themselves (without any boundary files), then run CurveAlign with batch mode checked and select any image that directory. In this case, the distance from the boundary and boundary association edit boxes will be ignored.

## Batch mode with Tiff boundaries

In batch mode, boundaries may also be imported as tiff files. Tiff boundary files must be 8bit binary mask images where the boundary has a value of 255 and everything else must be 0. Boundary tiff files must have the same number of pixels (length and width) as the original image and may be produced by hand or by segmentation in ImageJ/FIJI. Tiff boundary files should be named similar to csv boundary files.

Image file name = 1B-a5.tif

Boundary file name = boundary for 1B-a5.tif.tif

## Batch mode with CT-FIRE input

To compare the results of the CT-FIRE software to boundaries (either TIFF or csv), then enter 1 into the “Use FIRE results?” edit box in the batch mode GUI. The fibers in the database will be treated as fiber segments and their angles will be measured relative to a boundary if a boundary file is present in the same directory as the original images. If “Use FIRE results?” is selected, then the user is prompted to select the directory where CurveAlign can find the CT-FIRE results. The following naming convention should be used:

Image file name = 1B-a5.tif

Boundary file name = boundary for 1B-a5.tif.tif

CT-FIRE results file = FIREout\_1B-a5.tif.mat

# Status Label

To allow the user to keep track of what is happening in the program, there is a status label on the bottom of the main window. This label gives hints about what the user should do next or shows the current task that the program is working on.

