User Guide
DNA STRANDING

Automatic DNA replication tract measurement to assess replication and repair dynamics at the single molecule level

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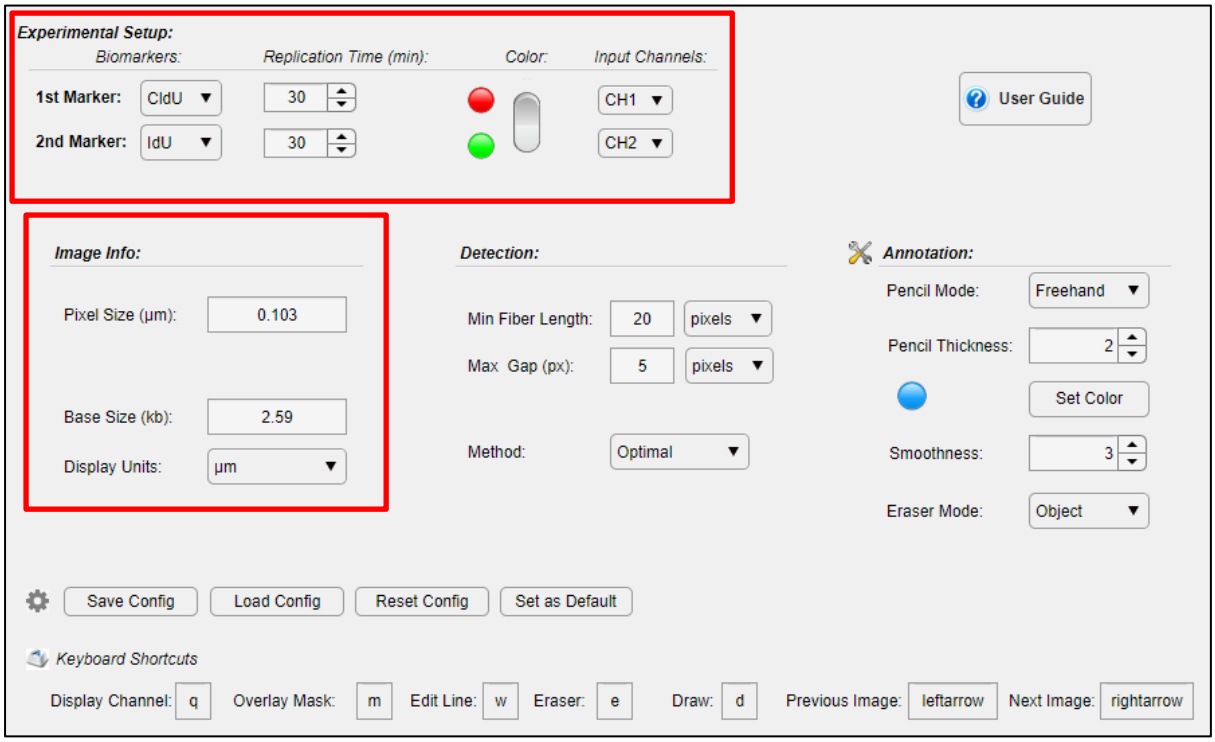
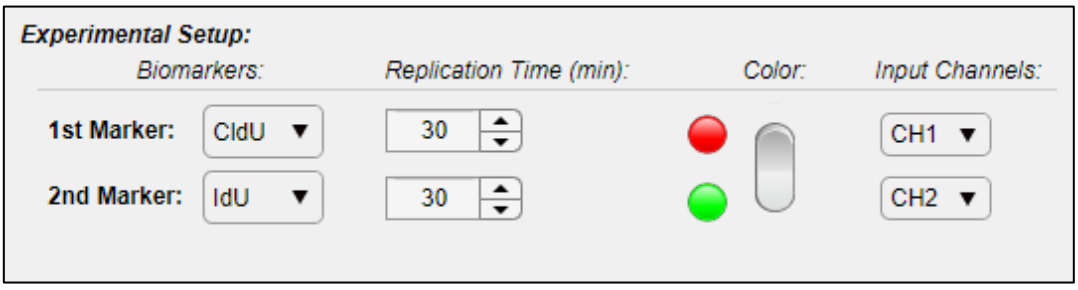
1. Image and analysis setting

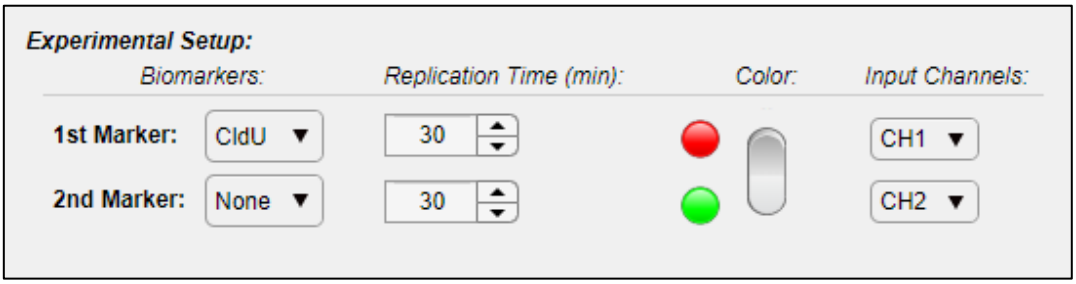


The experiment setup and other image configurations are recommended to be set before loading any image, any changes of configuration after you load images will only take effect when you reload them.

The experiment setup includes the sequence of the nucleotide analogues (currently only CldU and IdU are supported) and corresponding incubation time, color (currently only red and green are supported) and channel. The image below shows a typical setup: first put CldU for 30 minutes and then put IdU for 30 minutes; CldU is showed in red while IdU is showed in green; in scanner's output image, CldU and IdU signal are put into channel 1 and 2 respectively.

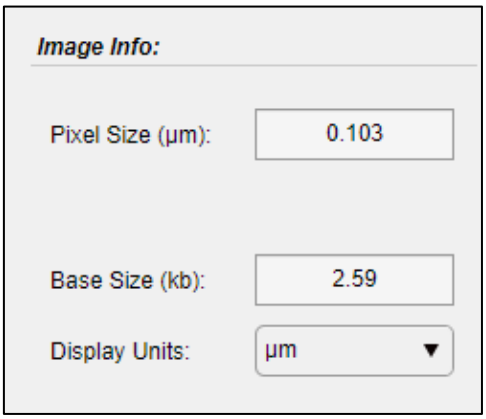


It is worth noting that if only one nucleotide analogues was used in the experiment (input image has only one channel), it should be set as the first marker, and the second marker should be set as "none".



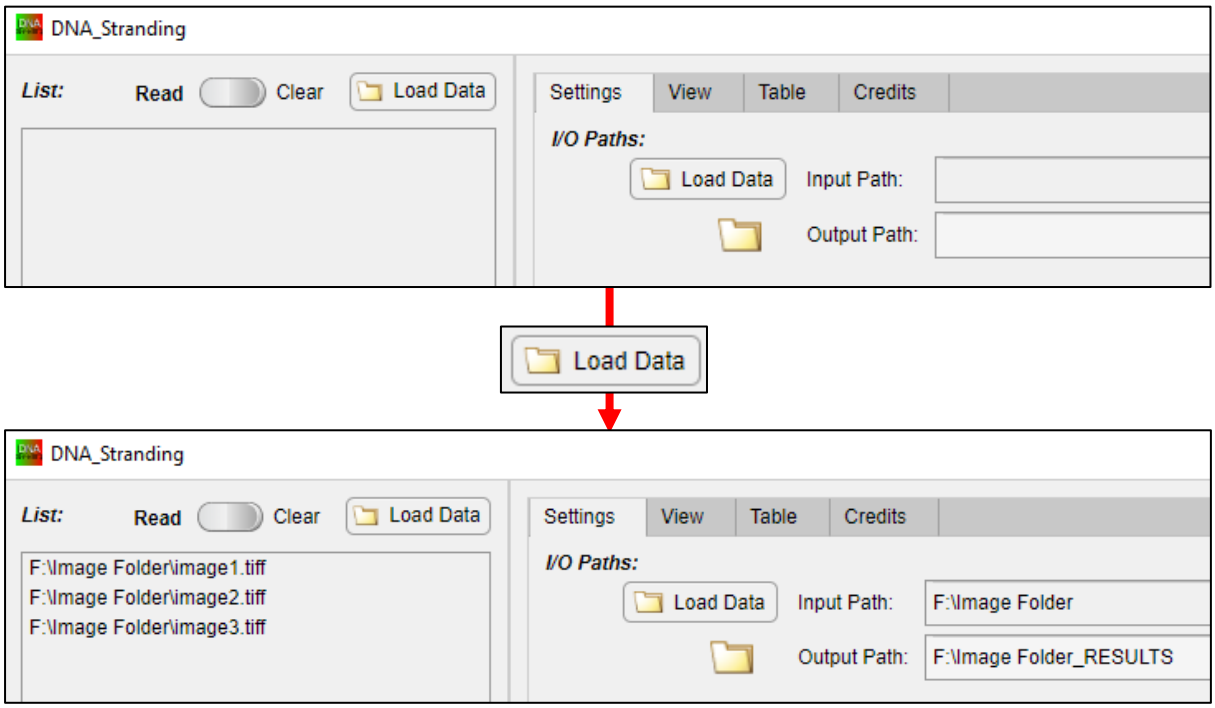
For the image that containing three channels, DNA_Stranding accepts its third channel for displaying only (not involved in any analysis). For the image that containing four or more channels, similarly, the first two channels will be used for analysis and the third one will be used for display, all other channels will be discarded.

The image info setup includes the pixel resolution (can be usually obtained by scanner metadata), base size and display unit. The setup in the image below is: pixel size 0.103um/pixel; base size 2.59 kb/um (which is a typical value); display unit micro meter.

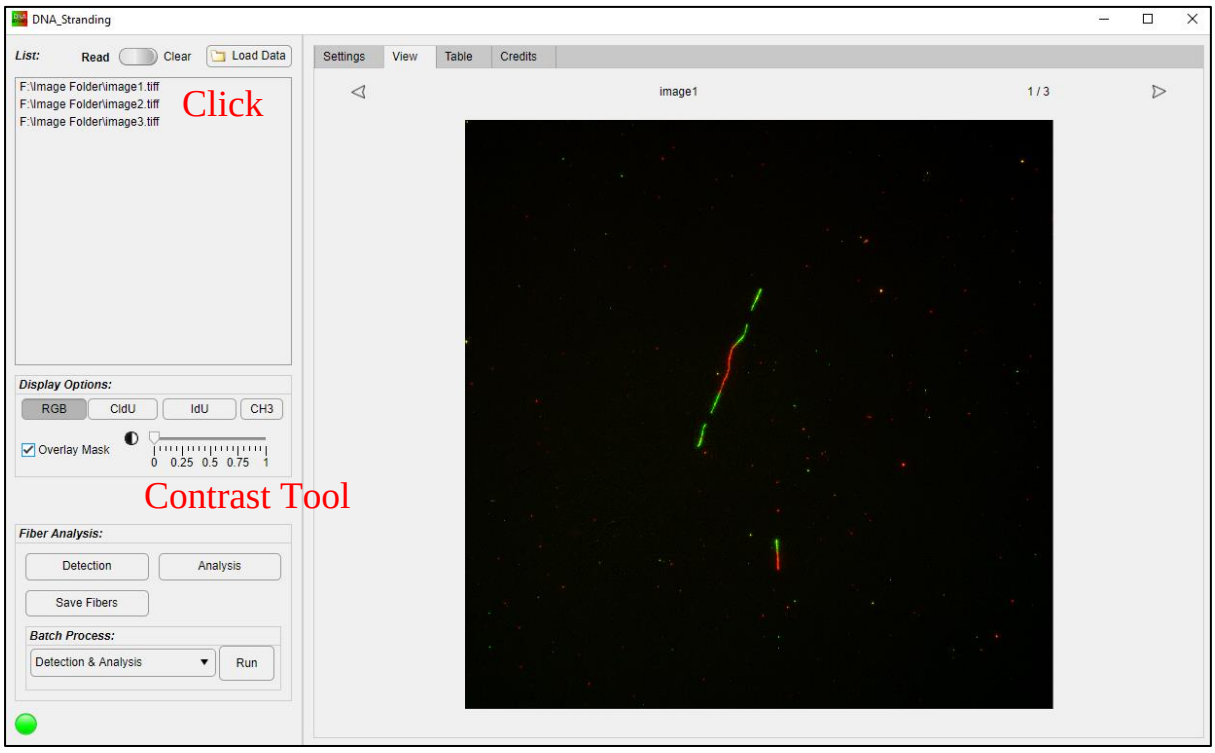


2. Load image

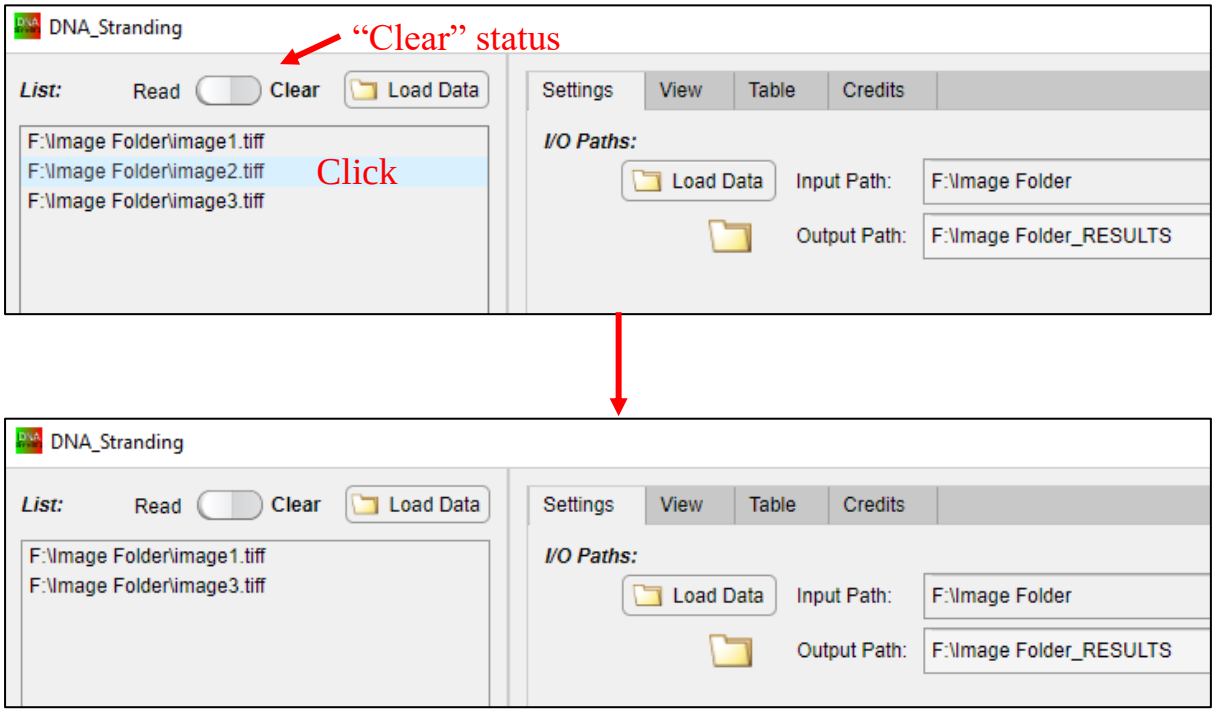
To load image, click “Load Data” button at top-left corner or Settings Panel and select the path that contains images. The software will load all the “.tif” and “.tiff” images in the specified folder (and subfolder).



Click a certain image in the Image List to visualize it (at View Panel). An extra contrast tool is provided for further contrast enhancement.

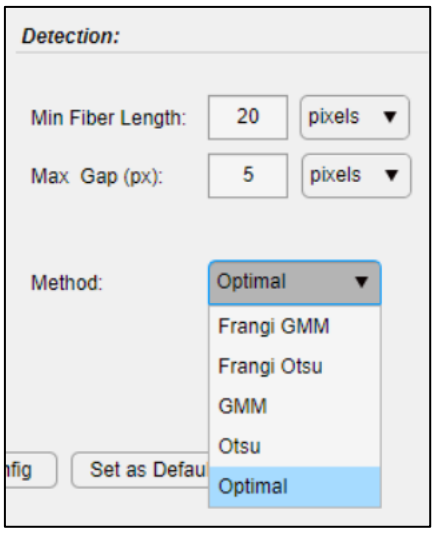


To remove any image from Image List, first click Read/Clear switch to “Clear” status and then click the image to be deleted.



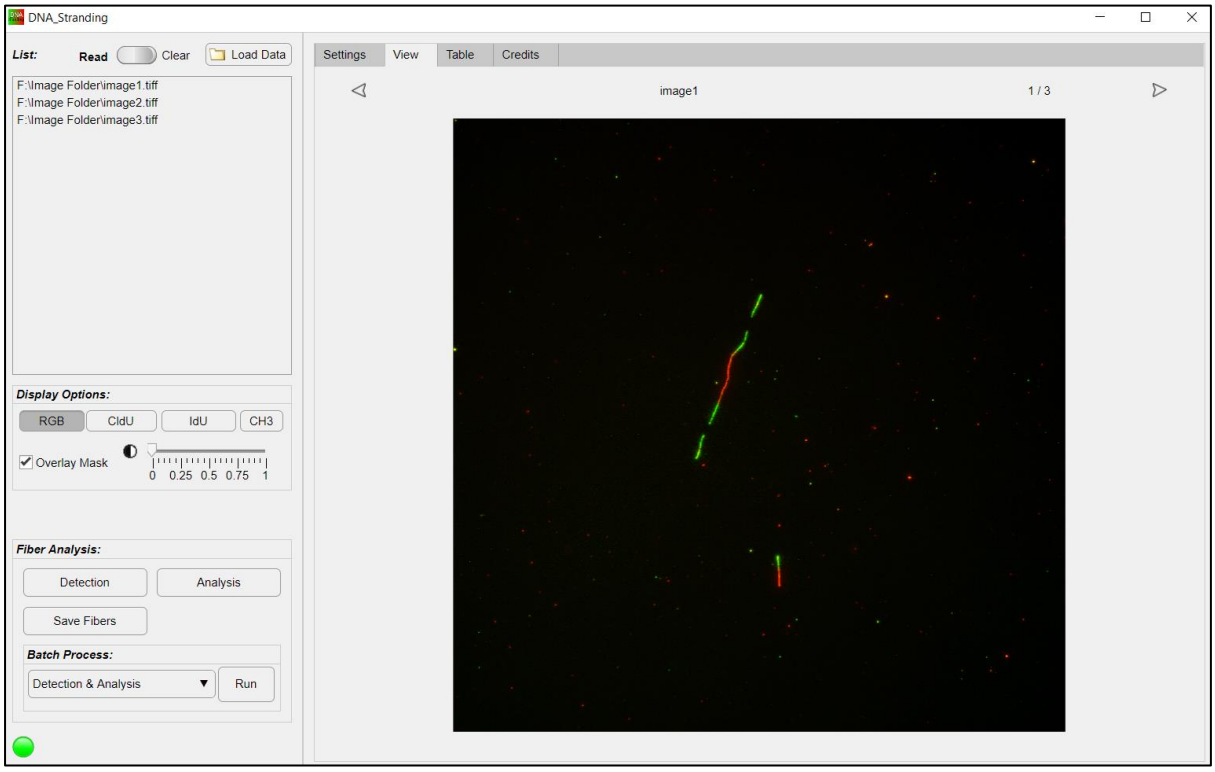
3. Automatic analysis pipeline

To start analysis process, the detection setup must be specified first. The recommended threshold value for minimum fiber length and maximum gap width are 20 pixels and 5 pixels respectively. The threshold can also be modified if necessary. There are five fiber detection methods supported by DNA_Stranding (see below), all five method have similar performances. In terms of speed, “Frangi Otsu” and “Otsu” are relatively faster while “Frangi GMM” and “GMM” are relatively slower, and the “Optimal” is the slowest one among all the methods. In general, “Frangi GMM” and “Optimal” are the most recommended methods.

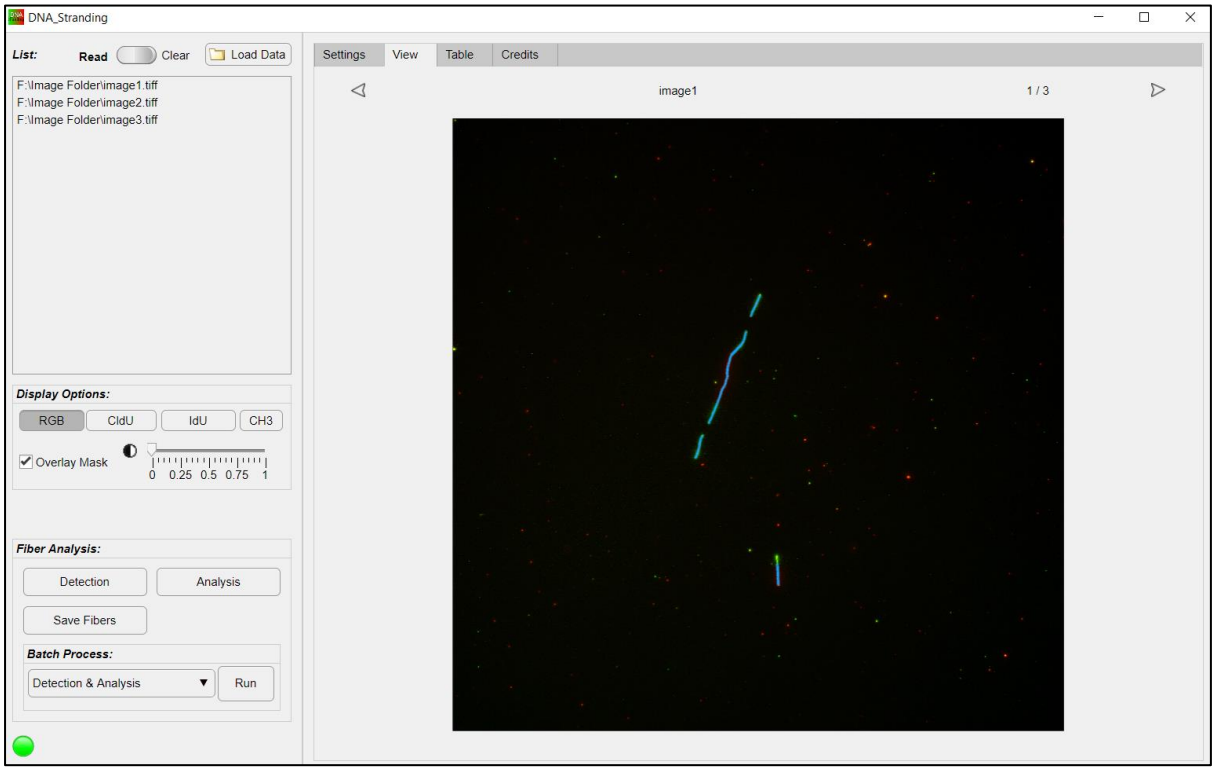


To perform automatic analysis, first select the image to be analysed, and click “Detection”, “Analysis”, and “Save Fibers” in sequence.

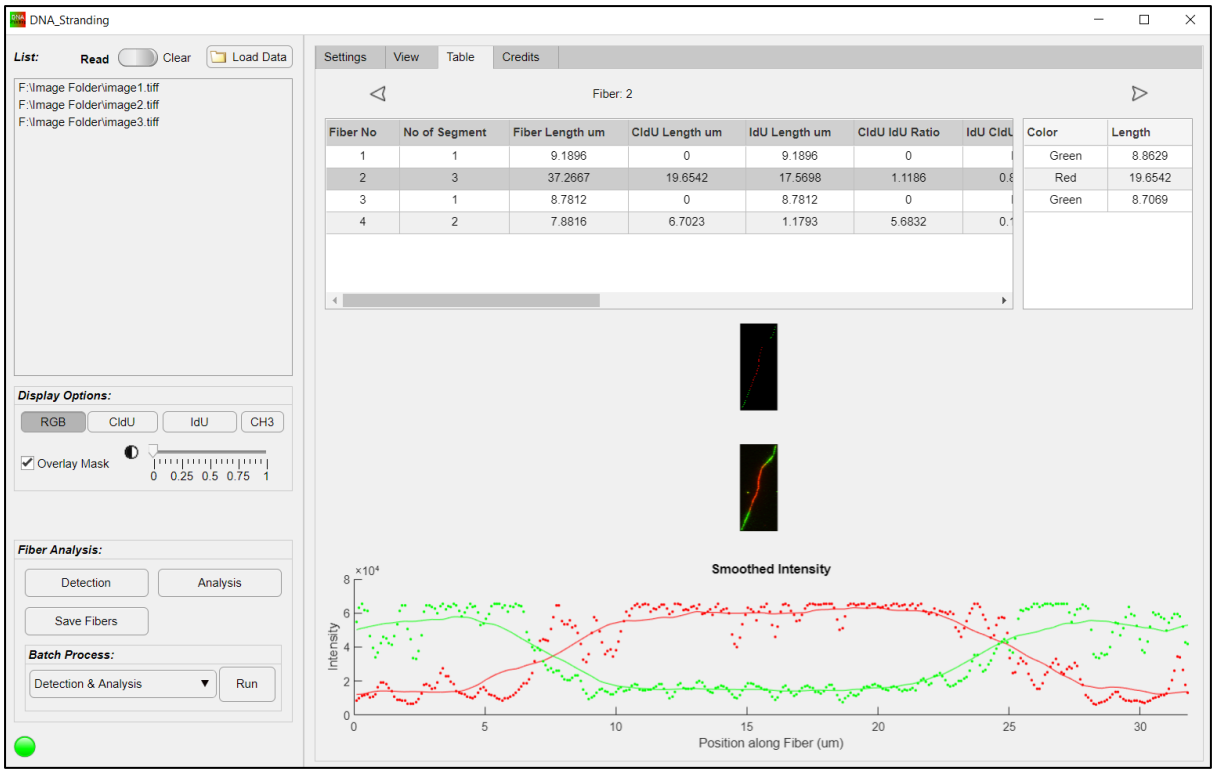
Select the image to be analysed (Image 1).



Click “Detection” to automatically detect fibers (the detected fibers are shown in light-blue).



Then click “Analysis” to measure fiber properties and view them in Table Panel.

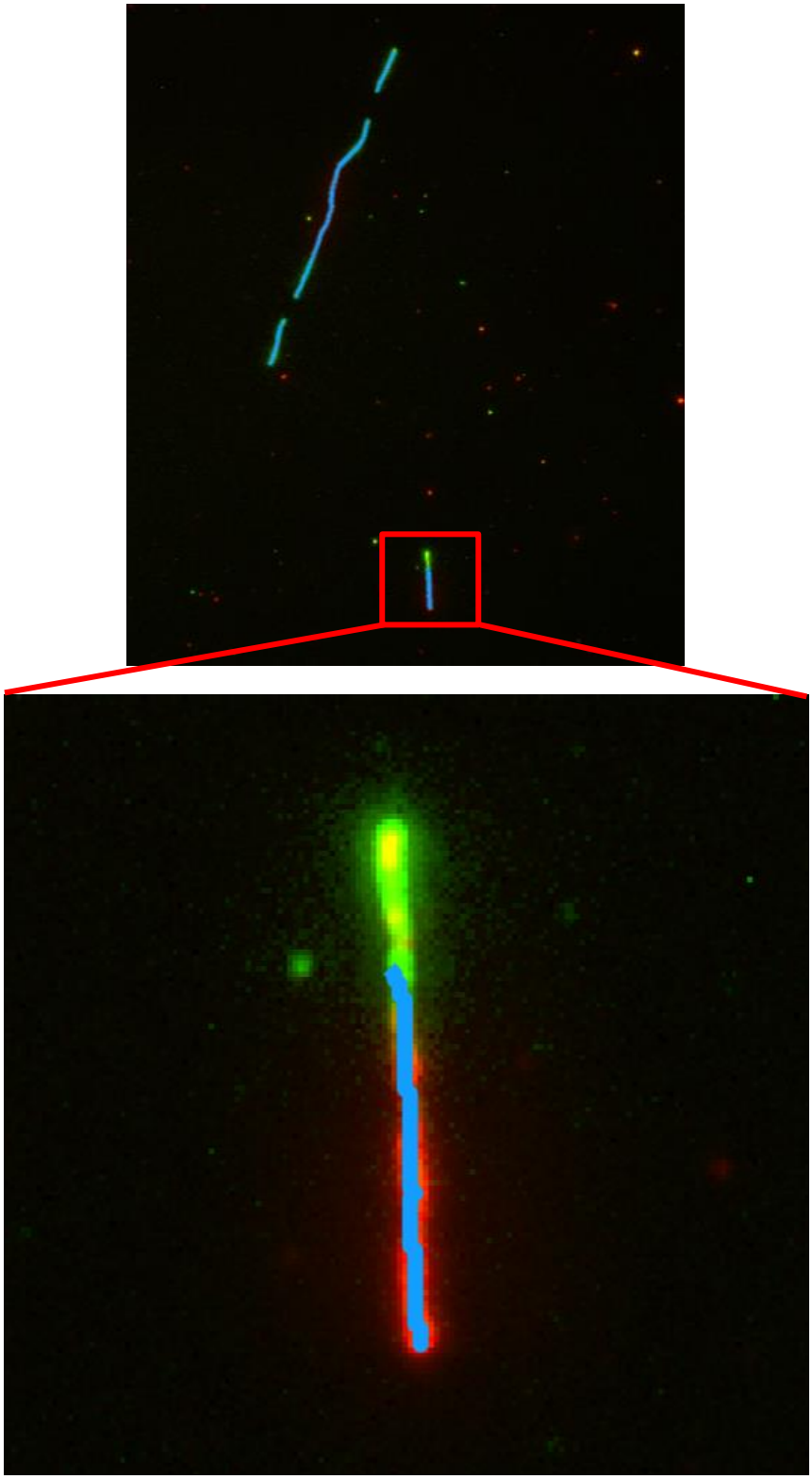


At last, click “Save Fibers” to save the detected fiber image and detailed fiber profiles to designated folder.

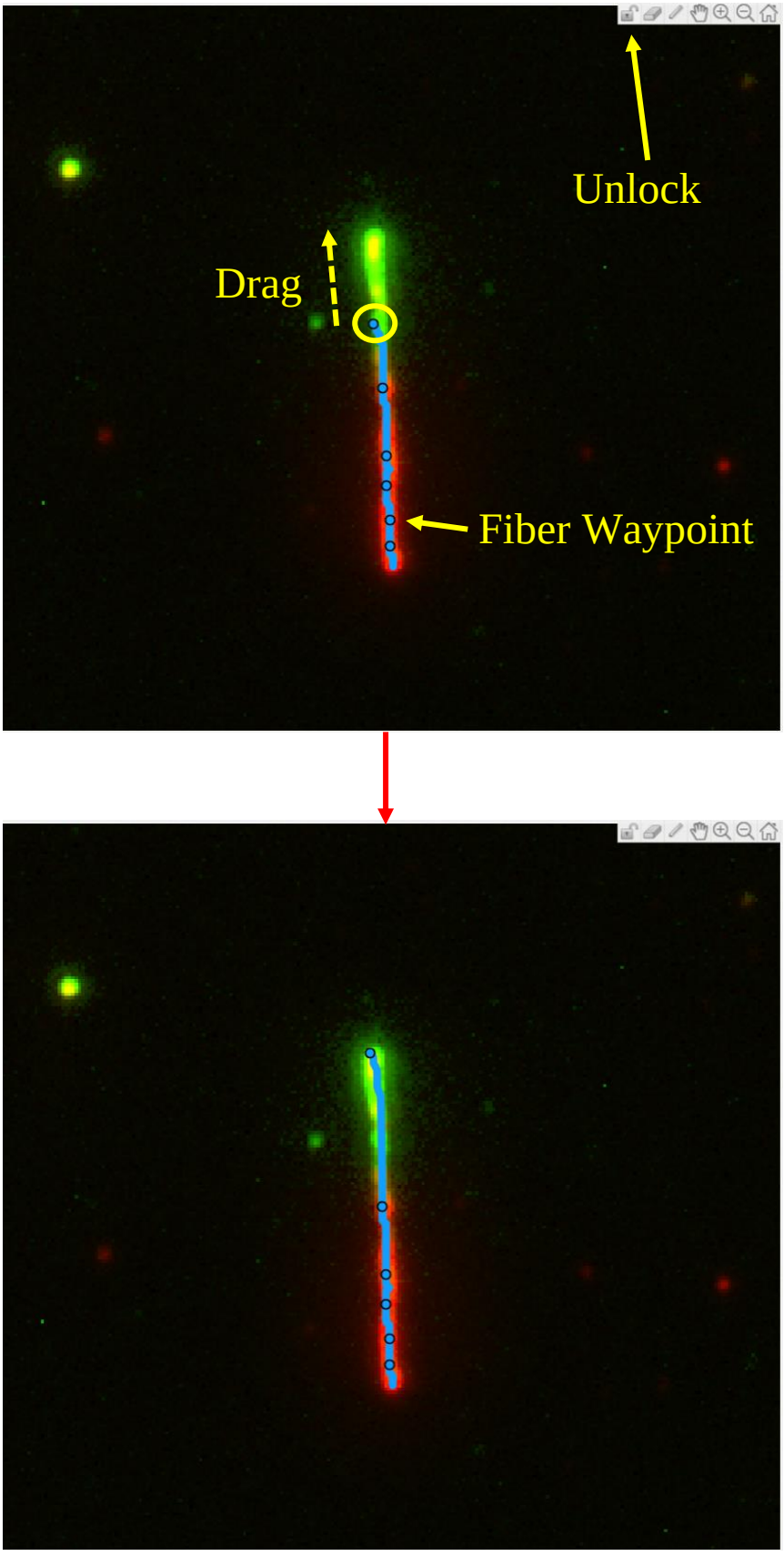
4. Semi-automatic and pure manual analysis pipeline

The highlight of this software is that it allows user to perform semi-automatic analysis to deal with the potential false positive and false negatives.

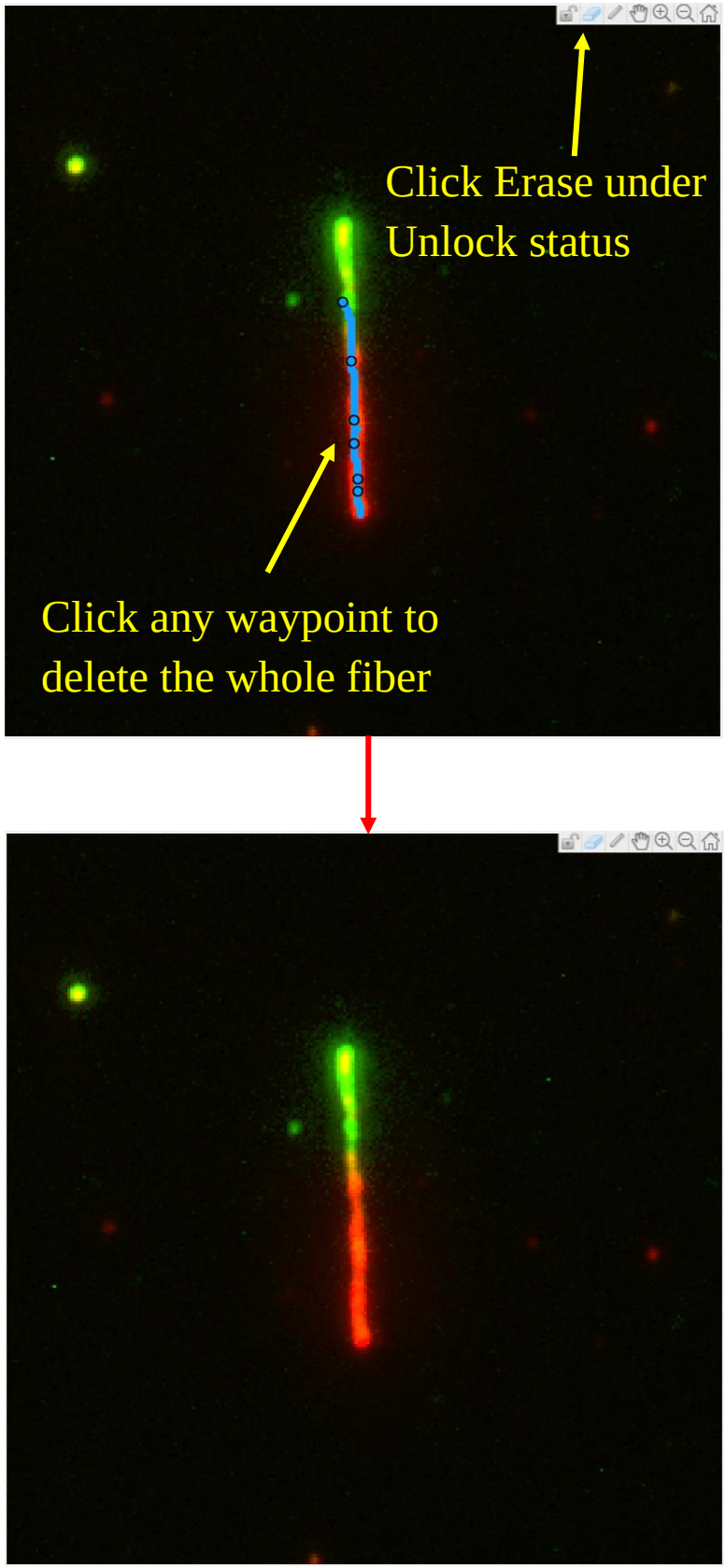
For semi-automatic pipeline, first click “Detection” to do automatic fiber detection. In Image 1, three out of four fibers are correctly identified by the algorithm and the fourth one is unsatisfying.



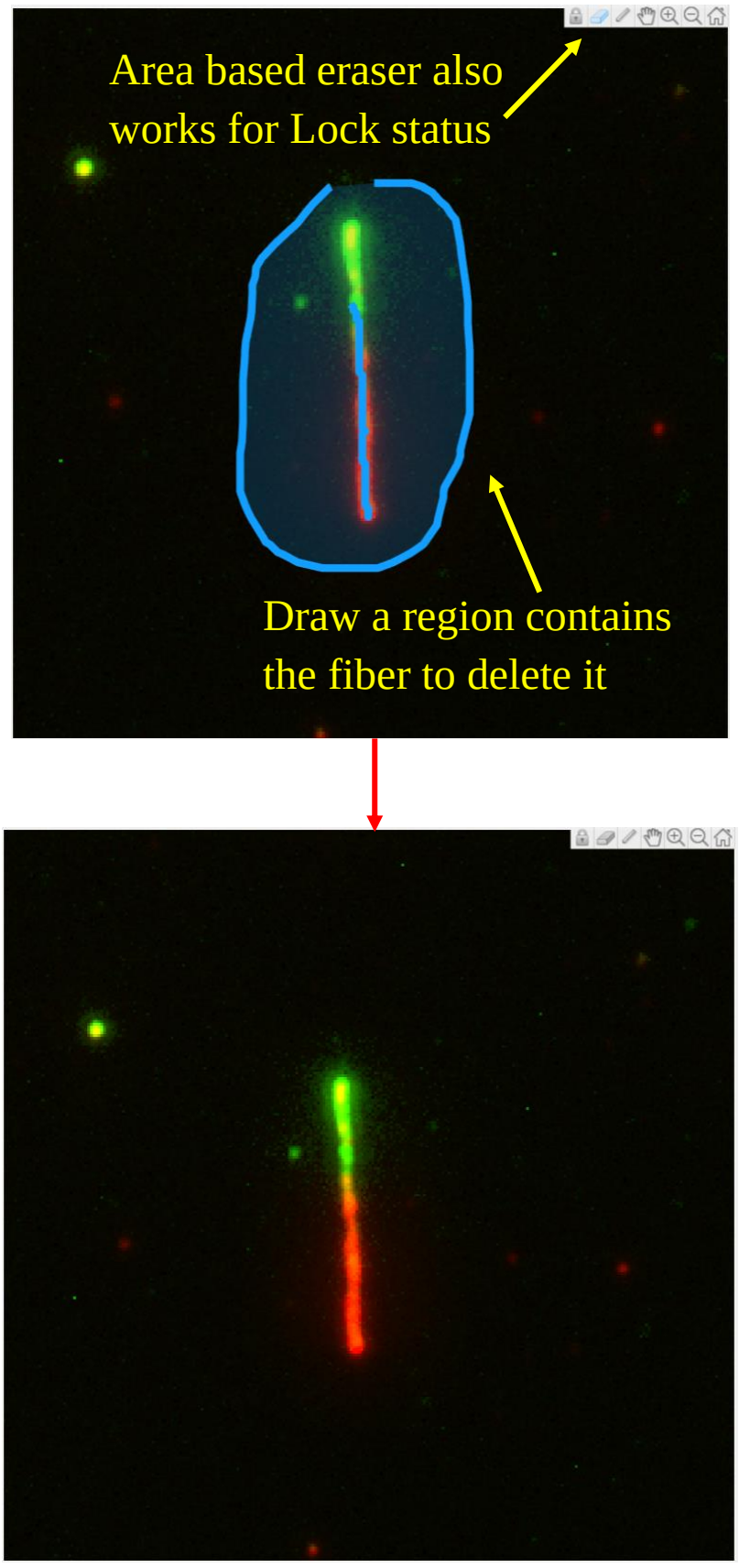
There are several ways to correct this fiber. The easiest method is to manipulate fiber's waypoints. First click lock button in image toolbar to enable fiber waypoint editing, then drag the top waypoint to proper location.



Another way is to erase the unsatisfying fiber and redraw a new one. There are two eraser modes supported by this software, object mode and area mode (user can choose eraser mode in Settings Panel). To erase any fiber using object based eraser, first unlock fiber editing, then click erase button to activate eraser and delete the unsatisfying fiber.

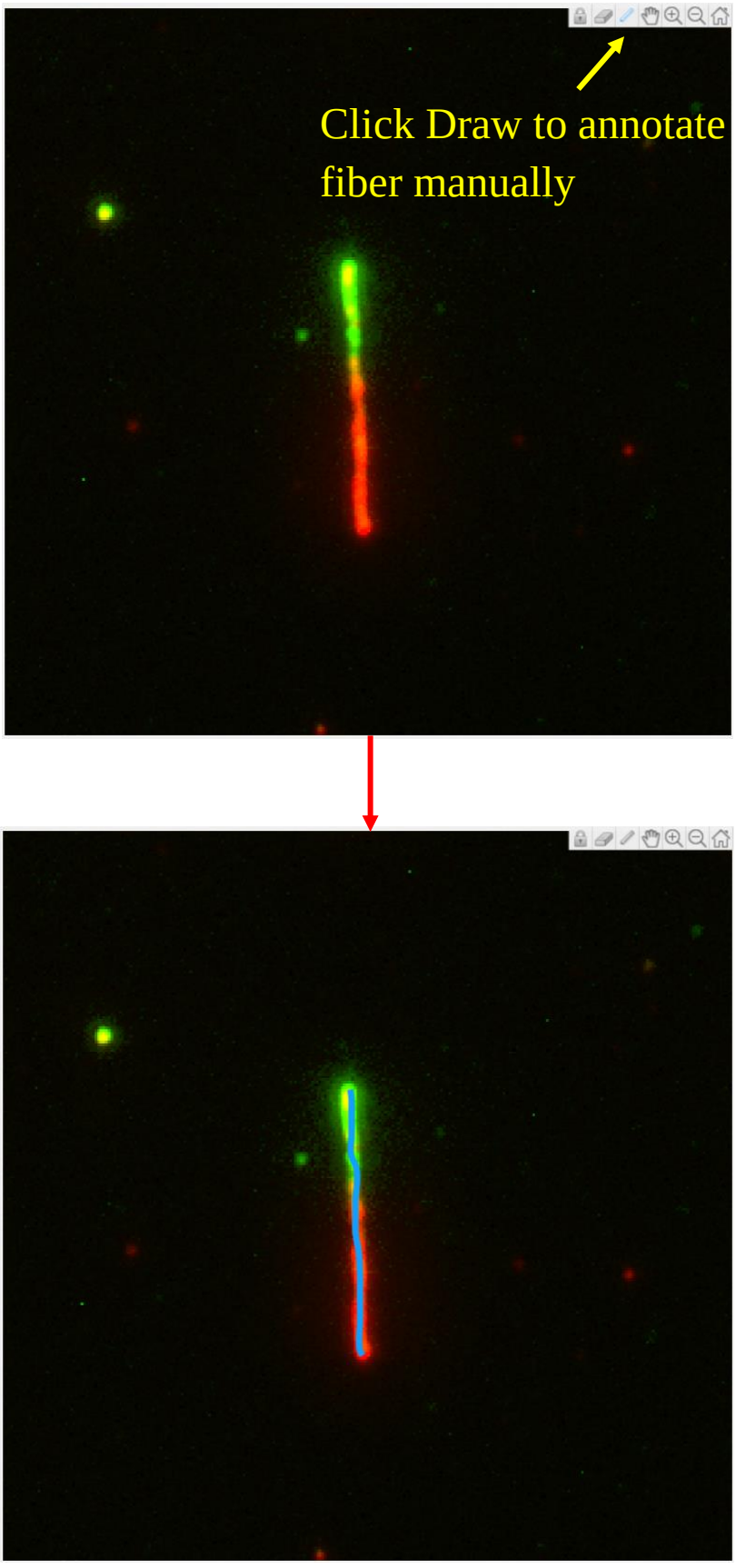


The use of area based eraser does not require Unlock status. To erase any fiber using object based eraser, simply click erase button to activate eraser and draw a region that contains the unsatisfying fiber to delete it.



The area based eraser can also delete multiple fibers at the same time, as shown below.

After deleting the unsatisfying fiber, user can manually annotate a new fiber use drawing tool, as shown below. A key feature of drawing tool is that it allows user to draw multiple fibers which crossed with each other (not shown here).



After the elimination of all the false positive and false negatives, similar to automatic analysis pipeline, click “Analysis” and “Save Fibers” button in sequence to quantify and save fiber profiles.

Pure manual analysis pipeline is also supported in the software. As the name implies, user can annotate all the fibers manually without any automatic fiber detection and then let software do the analysis and quantification of fibers.