



















Fluorescence Tracker App User Guide

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References

- [Overview of MATLAB Apps](#)
- [App Building with MATLAB](#)
- [Feature Detection and Extraction with MATLAB](#)
- [Tracking and Motion Estimation with MATLAB](#)

Overview

The Fluorescence Tracker App uses computer vision point tracking to monitor changes in intravenous dye intensity of infrared videos used in fluorescence guided surgery.

Results obtained using this app can be found in "Digital Dynamic Discrimination of Primary Colorectal Cancer using Systemic Indocyanine Green with Near-infrared Endoscopy" by Jeffrey Dalli et al., UCD Centre for Precision Surgery, School of Medicine, University College Dublin, Ireland (2021).

Video Format

The Fluorescence Tracker App requires videos have the format specified **Figure 1**. In particular, the original video must have an aspect ratio of 16:9 with a column of three 4:3 inset frames along the left. The top left frame must contain the endoscopic white-light red-green-blue (RGB) image; and the middle left frame must contain the fluorescence intensity near infra-red (NIR) image of the same frame. Note, the cropping and timing of these two frames is assumed to be consistent.

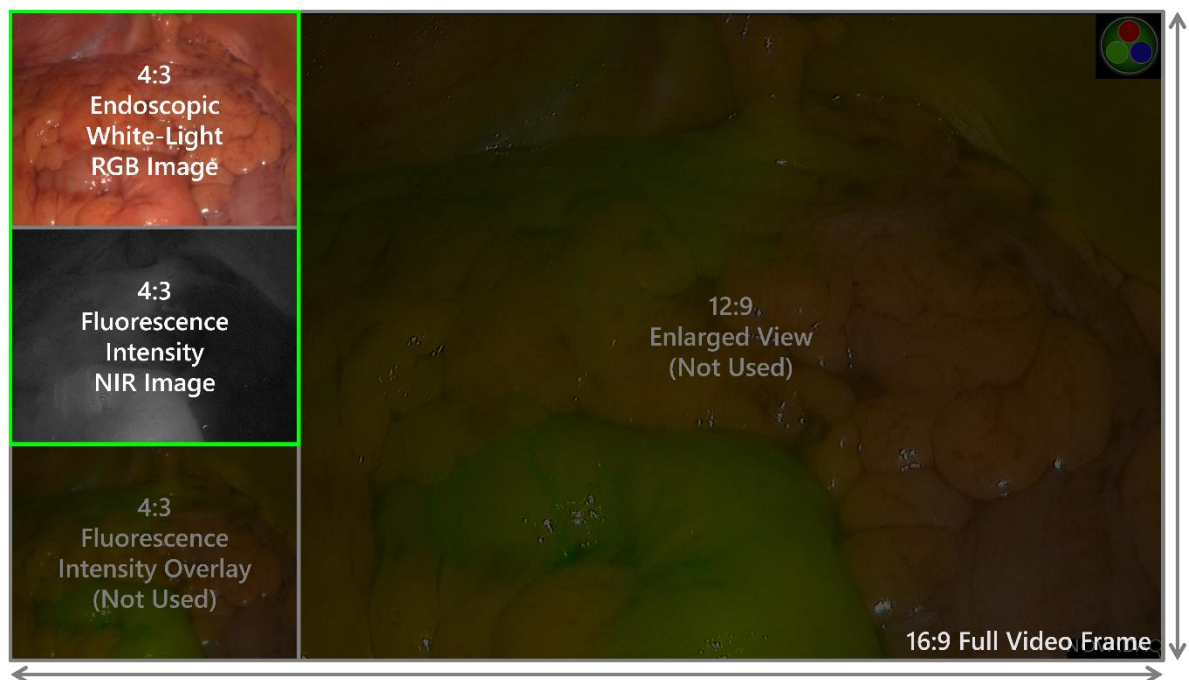


Figure 1: Required Video Format

The resulting 4:6 area containing these two frames has been outlined in green in **Figure 1**. This is the only portion of the video that is used for analysis. The remainder of the full video frame is ignored. *Note, the provided sample video has already been cropped and trimmed using the Trim and Save Video process described below.*

Setup Tab

Figure 2 illustrates the "Setup" tab after loading a video using the **Load Video** button. Other buttons become enabled as appropriate.

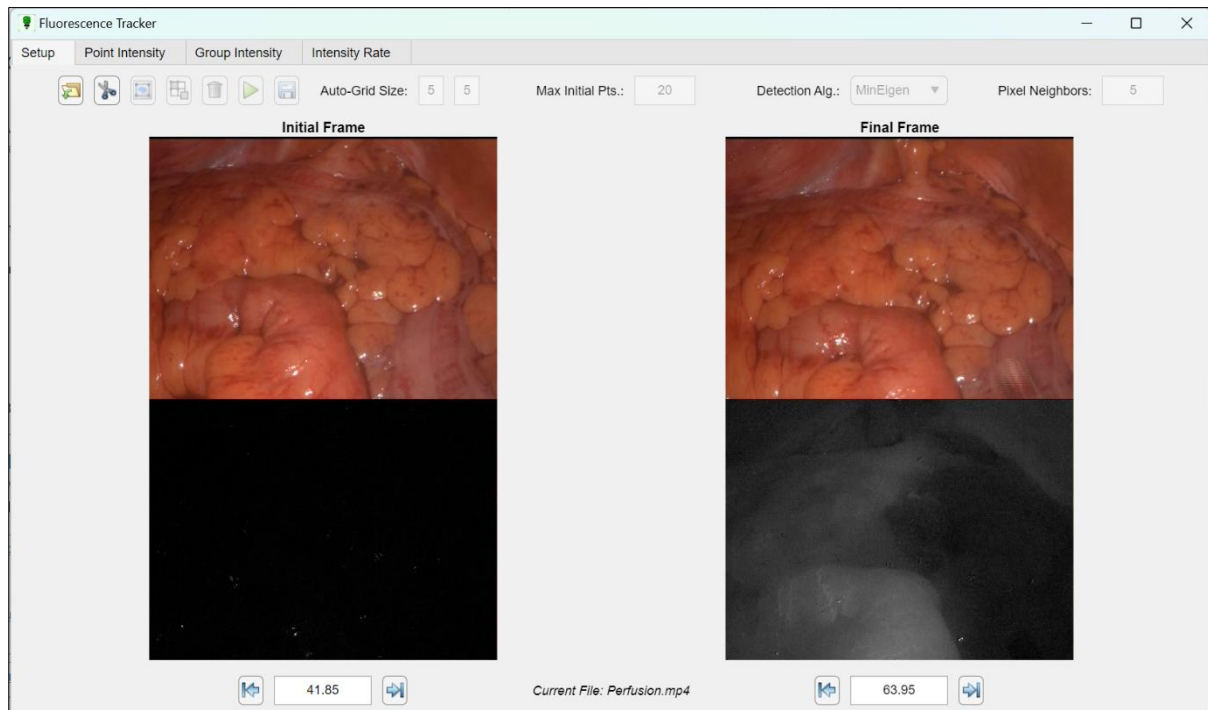


Figure 2: Setup Tab after Loading Video

Load Video

- Click the "Load Video" button to select a video file to load.
- This video must either meet the required **Video Format** specified above, or be an output of the **Trim and Save Video** process described below.

Trim and Save Video

- Click the "Trim and Save Video" button to create a new 4:6 video that contains only the two 4:3 frames specified in the **Video Format** section.
- *Before* doing so, you can also set the desired video time range using the **Initial / Final Time Fields** described below.
- Trimming and cropping the video speeds up later processing and only needs to be performed once. When complete, the trimmed/cropped video is automatically loaded, and can be loaded directly using the **Load Video** button in subsequent sessions.

Initial / Final Time Fields

- If necessary, use the "Initial Time" and/or "Final Time" fields to trim the video to only include the desired time range to be analyzed.
- Change these values *before* using the **Trim and Save Video** button.

Previous / Next Frame

- If necessary, use the "Previous Frame" and/or "Next Frame" buttons to fine tune the desired video time range to be analyzed.
- The values in **Initial / Final Time Fields** will update accordingly.
- Use these buttons *before* using the **Trim and Save Video** button.

Select Region

- Click the "Select Region" button to specify a region of interest in the video.
- This will open a new figure window. Hover the cursor over the image and drag the resulting crosshairs to create a region of interest. (It may take a second for the crosshairs to appear.) The resulting rectangle can be moved and resized. Double-click inside the rectangle to accept the region.
- This can be done multiple times to add additional tracking points in the same or new regions.
- This will generate a number of tracking points less than or equal to the number specified in the **Maximum Initial Points Field** described below. If desired, change this value *before* using the "Select Region" button.
- Tracking points are detected using the method specified in the **Detection Algorithm Dropdown** menu described below. If desired, change this value *before* using the "Select Region" button.

Auto-Grid Regions

- Click the "Auto-Grid Regions" button to automatically create a grid of regions of the dimensions specified in the **Auto-Grid Size Fields** described below. If desired, change these values *before* using the "Auto-Grid Regions" button.
- Each region will then contain a number of tracking points less than or equal to the number specified in the **Maximum Initial Points Field** described below. If desired, change this value *before* using the "Auto-Grid Regions" button.
- Tracking points are detected using the method specified in the **Detection Algorithm Dropdown** menu described below. If desired, change this value *before* using the "Auto-Grid Regions" button.

Clear Regions

- Click the "Clear Regions" button to remove all points in the previously added region.
- This can be done multiple times until all previous regions have been removed.

Auto-Grid Size (Rows × Columns) Fields

- Use the "Auto-Grid Size" fields to specify the number of rows and columns of the gridded regions. The total number of regions is then the product of these two values. If desired, change these values *before* using the "Auto-Grid Regions" button.
- Note, excessive regions and/or points per region may increase the video processing time.

20 Maximum Initial Points Field

- Use the "Maximum Initial Points" field to specify the maximum number of initial tracking points per region.
- Set this value to `Inf` to find as many points as possible. Note, excessive points may increase the video processing time.

MinEigen Detection Algorithm Dropdown

- Use the "Detection Algorithm" dropdown menu to select the algorithm used to detect the initial tracking points.
- For more information, see the MATLAB documentation on "detect features" for each algorithm, for example: >> [doc detectMinEigenFeatures](#)

Figure 3 illustrates the "Setup" tab after the video has been trimmed and regions have been selected. In this case, three regions were selected with a maximum of 20 points each, detected using the "MinEigen" algorithm.

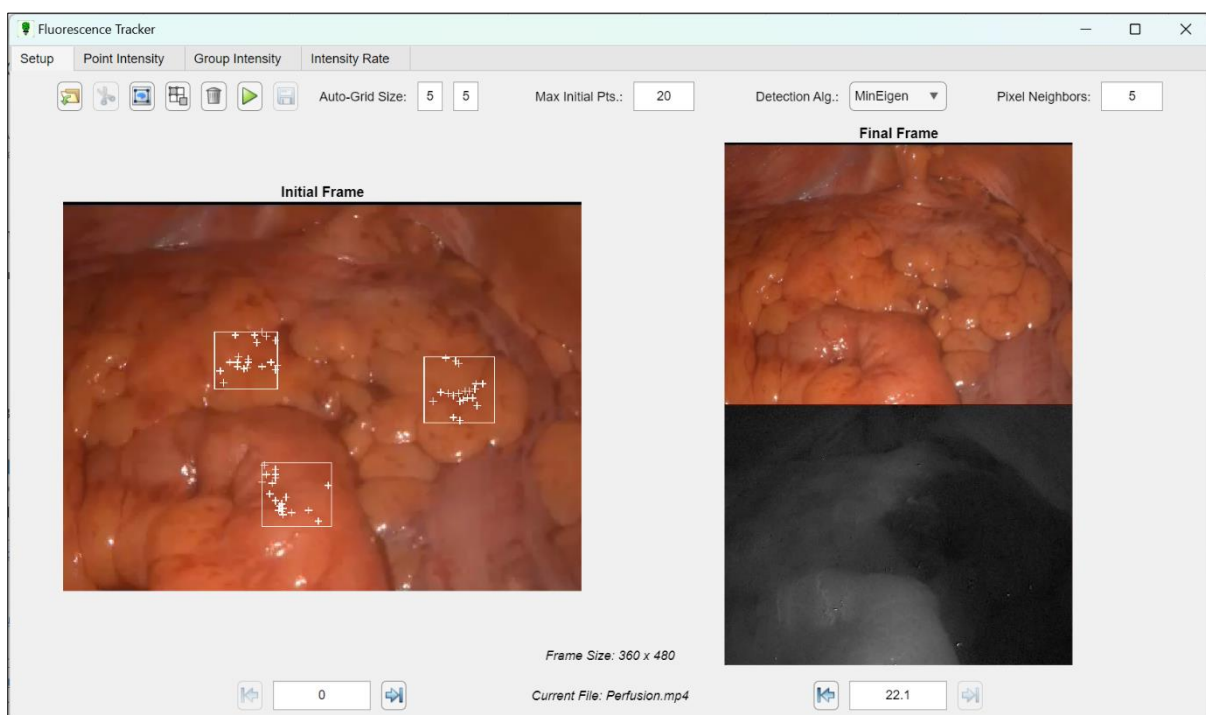


Figure 3: Setup Tab after Selecting Regions

▶ Run and Track Points

- Click the "Run and Track Points" button to begin processing the video.
- Processing will stop if all point tracks are lost. If so, try adding more initial points and make sure these points do not get obscured or go off frame over the video duration.
- If desired, change the **Pixel Neighbors Field** value *before* using the "Run and Track Points" button.

5 Pixel Neighbors Field

- Use the "Pixel Neighbors" field to specify the neighborhood about each tracking point to be used when computing the mean intensity value.
- A value of zero uses only the individual tracking point pixel; whereas, a value of 5 (for example) use a neighborhood of 11x11 pixels (5 pixels up/down and left/right of the tracking point) to compute a mean intensity value.
- This parameter can be used to decrease the noise associated with tracking and monitoring the intensity value of a single pixel.



Save Results

- Click the "Save Results" button to save variables to a file. If run from MATLAB, this will also automatically save variables to the MATLAB workspace (even if "Cancel" is selected).
- In the resulting save dialog window, use the "Save as type" drop-down menu to select a MAT file or an Excel spreadsheet.

Point Intensity Tab

Once video processing completes, the app will automatically switch to the "Point Intensity" tab to display the resulting time history of the (mean) intensity for each tracked point (neighborhood). Intensity values range from 0% (black) to 100% (white).



Toggle Legend On/Off

- Click the "Toggle Legend On/Off" button to display or remove the figure legend.
- The order of the points in the legend (from top to bottom) has been sorted to match the order of the final smoothed intensity values of the points (from high to low).
- These same colors are also used to label the points in the "Final Frame" figure on the right.

0.1

Smoothing Factor Field

- Use the "Smoothing Factor" field to set the desired smoothing of the intensity and intensity-rate curves.
- This value ranges from zero (no smoothing) to one. Note, excessive smoothing can result in an apparent delay in the smoothed signal.
- The light grey lines in the background display the results without smoothing.
- For more information, see the MATLAB documentation on "smoothing noisy data":
>> [doc smoothdata](#)

Group Intensity Tab

Figure 4 illustrates the "Group Intensity" tab, which displays the same light grey unsmoothed intensity curves as the **Point Intensity Tab**. However, these points are now divided into unique groups using [k-means clustering](#). The color curve displayed for each group is the mean of all the smoothed signals within that group.

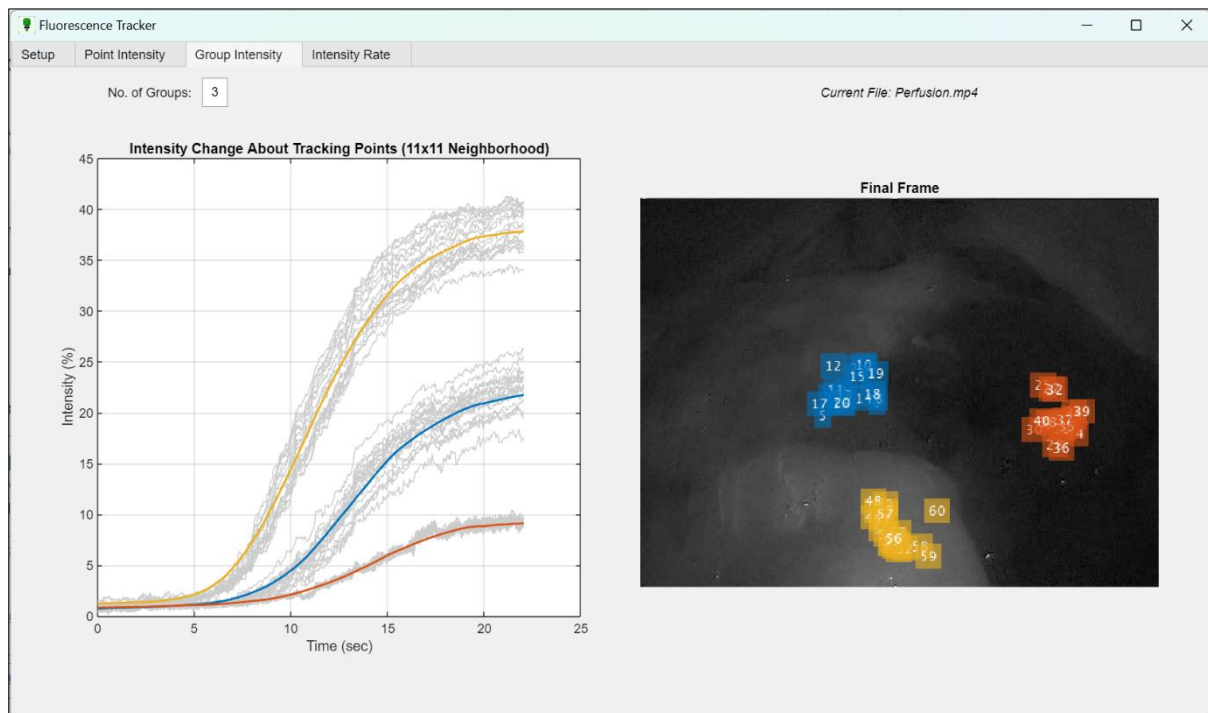


Figure 4: Group Intensity Tab

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Number of Groups Field

- Use the "Number of Groups" field to change the default value. The default value is set based on differences in the final intensity values.
- The resulting point groups are color-coded and the same colors are used to label the points in the "Final Frame" figure on the right.
- This value can also be set to "0" (zero) to reset the groups to correspond to the originally selected regions of interest. If these regions were selected based on a known diagnosis (e.g. cancer or benign), this then allows for easy labelling of all the exported intensity signals using the group ID.

Rate Intensity Tab

The "Rate Intensity" tab displays the intensity rate of change over time. The intensity rate of change is computed using a simple back-difference between video frames (the change in smoothed or grouped intensities divided by the change in time). The resulting rate is then smoothed using the value specified in the **Smoothing Factor Field**. The resulting figure uses the same color-coding as described in the **Group Intensity Tab**. There are no user options available in the "Rate Intensity" tab.