Fluorescence Tracker App User Guide

Contents

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 in **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.

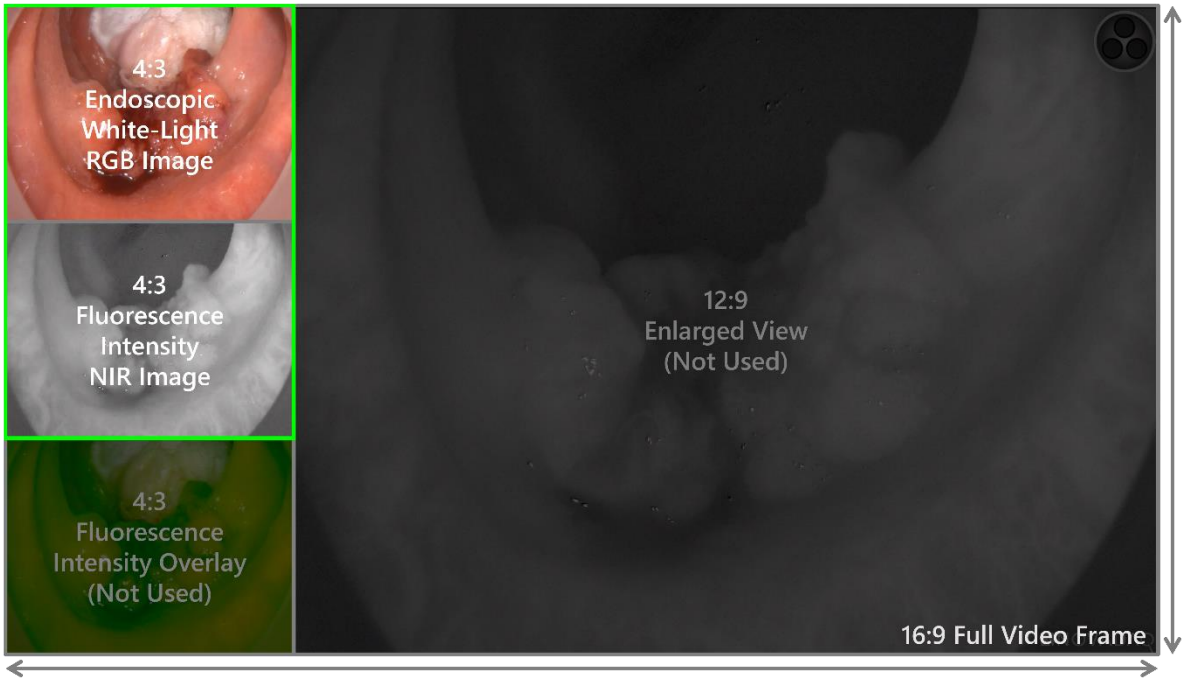


Figure 1: Required Video Format

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, to reduce its size the provided sample video has already been cropped and trimmed using the* **Trim and Save Video** *process described below. As noted, this step is optional.*

Setup Tab

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

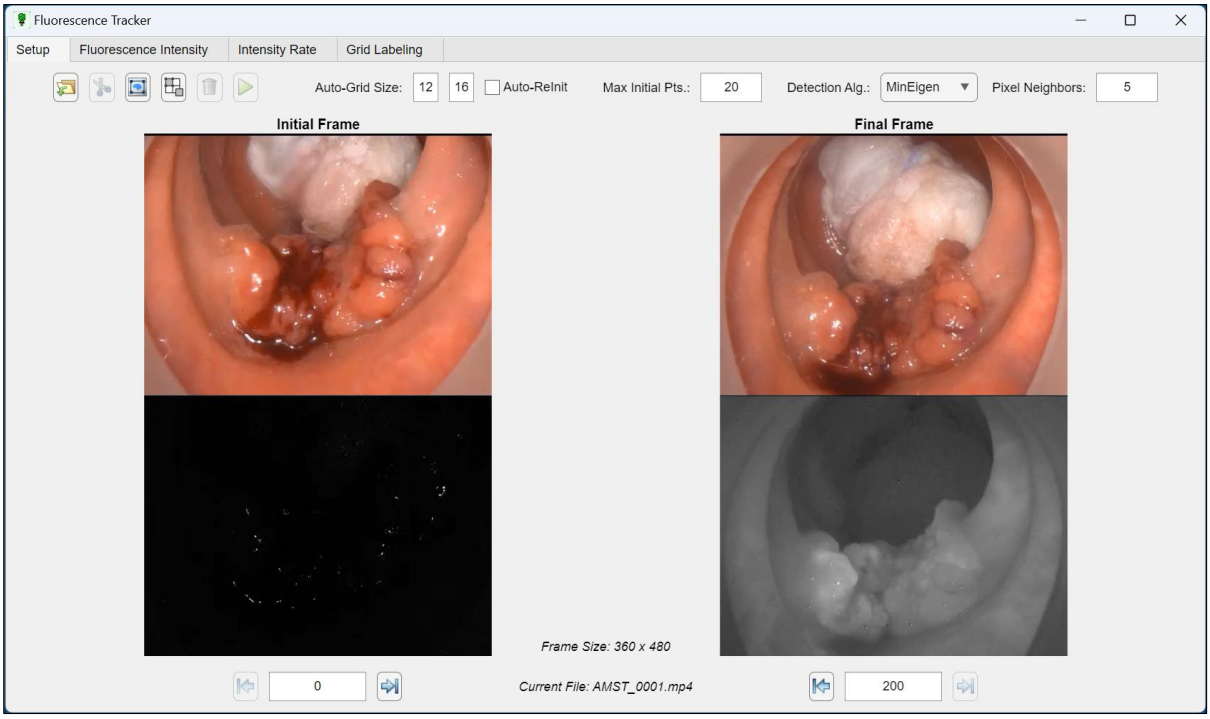


Figure 2: Setup Tab after Loading Video

Load Video

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

Trim and Save Video

- *This step is optional.* If desired, 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, set the desired time range using the **Initial / Final Time Fields**.
- When complete, the trimmed/cropped video is automatically loaded, and can be loaded directly using the **Load Video** button in subsequent sessions.

0 Initial / Final Time Fields

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

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** or **Run and Track Points** buttons.

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 new regions.
- This will generate a number of tracking points less than or equal to the number specified in the **Maximum Initial Points Field**. 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. If desired, change this value *before* using the "Select Region" button.
- In regions with limited features, the detection algorithm may not find the number of points specified in the **Maximum Initial Points Field**. If desired, this point deficit can be initialized with random points. To do so, tick the **Automatic Point Re-Initialization Checkbox** *before* using the "Select Region" button.
- To use the **Automatic Point Re-Initialization** feature only during tracking (without any initial random points), leave the checkbox unticked during region selection and only enable it *before* pressing the **Run and Track Points** button.

Auto-Grid Regions

- As an alternative to the manual **Select Region** process, click the "Auto-Grid Regions" button to automatically create a grid of regions of the dimensions specified in the **Auto-Grid Size Fields**. If desired, change these values *before* using the "Auto-Grid Regions" button.
- Tracking points are then uniformly dispersed across the grid, with the total number being less than or equal to the value specified in the **Maximum Initial Points Field**. 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. If desired, change this value *before* using the "Auto-Grid Regions" button.
- The tracked points are then used to compute the **affine transform** between the current frame and the initial frame. This transformation is used to perform **image registration**, which warps the current frame to keep features aligned with the initial frame. The mean intensity of each grid region is then computed using **distinct block processing** of the warped intensity frame.
- Edge regions may become padded with black due to warping. These regions are discarded since their mean intensity calculations will be corrupted with zero values.
- The **Automatic Point Re-Initialization Checkbox** is not applicable for "Auto-Grid Regions."

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.

12 16 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.

☑ Automatic Point Re-Initialization Checkbox

- Tick the "Auto-ReInit" checkbox to automatically re-initialize lost points during tracking and/or to add random points (as needed) during the **Select Region** process. If desired, change this value *before* using the **Select Region** or **Run and Track Points** buttons.
- Using the valid point region from the previous timestep, this feature attempts to reinitialize lost points using the method specified in the **Detection Algorithm Dropdown** menu. Any remaining point deficit is then initialized with random points in the region.
- Use caution when enabling "Auto-ReInit" with regions near the edge of the frame (or regions that become obstructed) as these regions may become invalid due to field of view changes.
- The "Auto-ReInit" checkbox is not applicable when using **Auto-Grid Regions**.

20 Maximum Initial Points Field

- Use the "Maximum Initial Points" field to specify the max number of initial tracking points. If desired, change this value *before* using the **Select Region** or **Auto-Grid Regions** buttons.
- 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 information on each algorithm, see the MATLAB documentation on **"Detect Features"**.

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. If desired, change this value *before* pressing the **Run and Track Points** button.
- 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.

Figure 3 illustrates the "Setup" tab after manually **Selecting Regions**. In this case, three regions were selected with a maximum of 10 points per region, detected using the "MinEigen" algorithm.

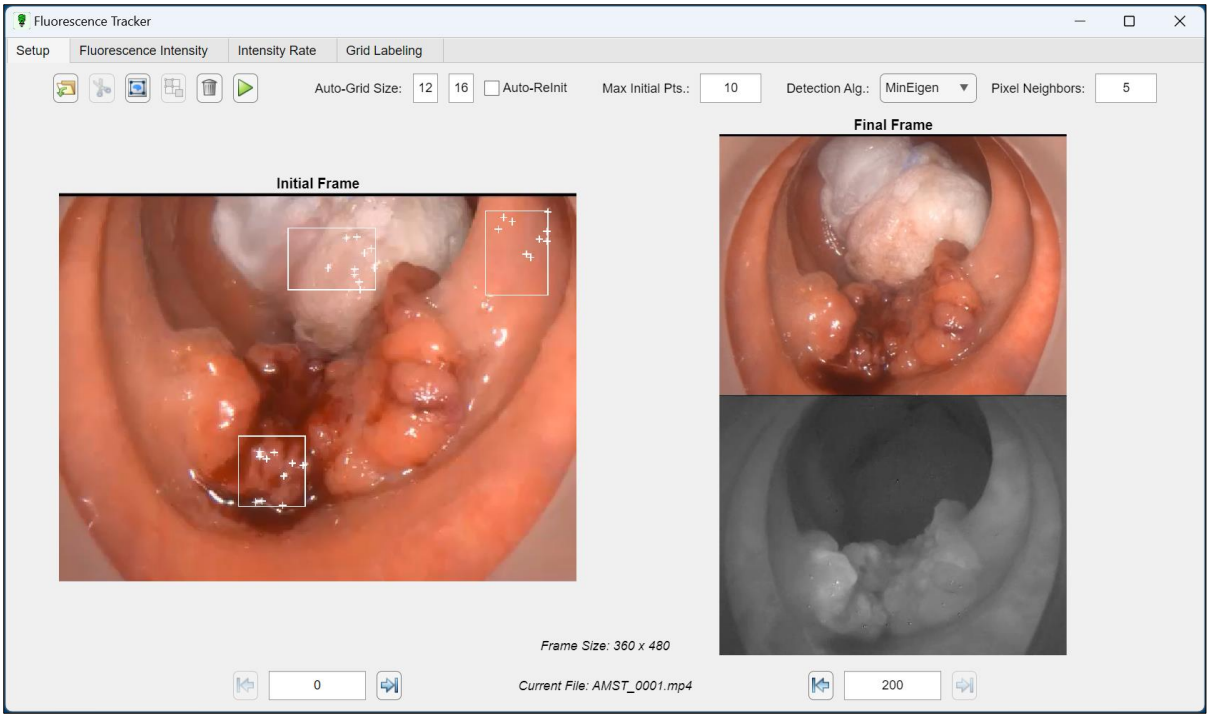


Figure 3: Setup Tab after Manually Selecting Regions

Figure 4 illustrates the "Setup" tab after using **Auto-Grid Regions**. In this case, 18 × 24 = 432 regions were auto selected with a maximum of 30 total points, detected using the "MinEigen" algorithm.

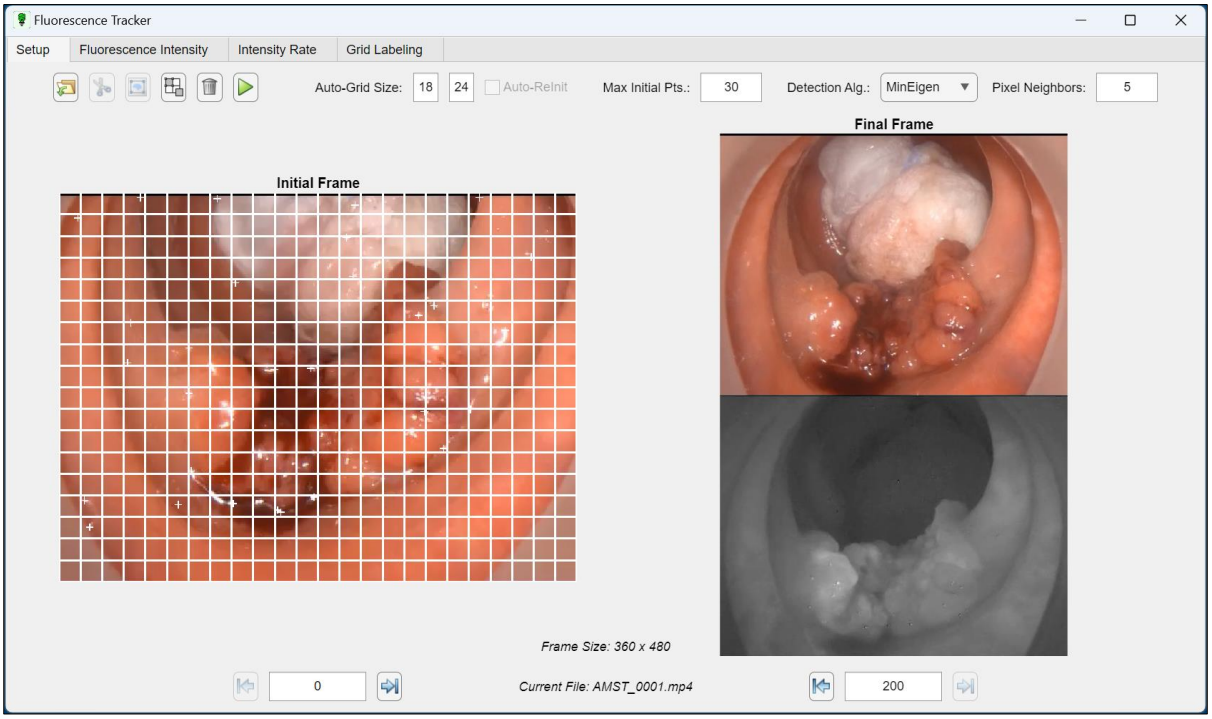


Figure 4: Setup Tab after Using Auto Grid Regions

Run and Track Points

- Click the "Run and Track Points" button to process the video with the specified selections.
- Before processing begins, a prompt will offer the option to save the resulting tracking video. To skip this option, press "Cancel" to process the video without saving the tracked video.
- Processing will stop if all point (or region) tracks are lost. If so, try adding more initial points (or regions) and make sure these points (or regions) do not get obscured or go off frame over the video duration. Or consider using **Automatic Point Re-Initialization** (if appropriate).
- If desired, change the **Automatic Point Re-Initialization Checkbox** or **Pixel Neighbors Field** values *before* using the "Run and Track Points" button.

Fluorescence Intensity Tab

As seen in **Figure 5** and **Figure 6** once video processing completes, the app will automatically switch to the "Fluorescence Intensity" tab to display the resulting intensity time histories for each tracked point. Intensity values range from 0 (black) to 255 (white). The light grey lines in the background display the individual point intensity results without smoothing. By default, the color curves displayed for each group represent the mean intensities across each of the originally selected regions. For comparison, **Figure 6** shows the **Auto-Grid Regions** results after changing the **Number of Groups Field** to 3.

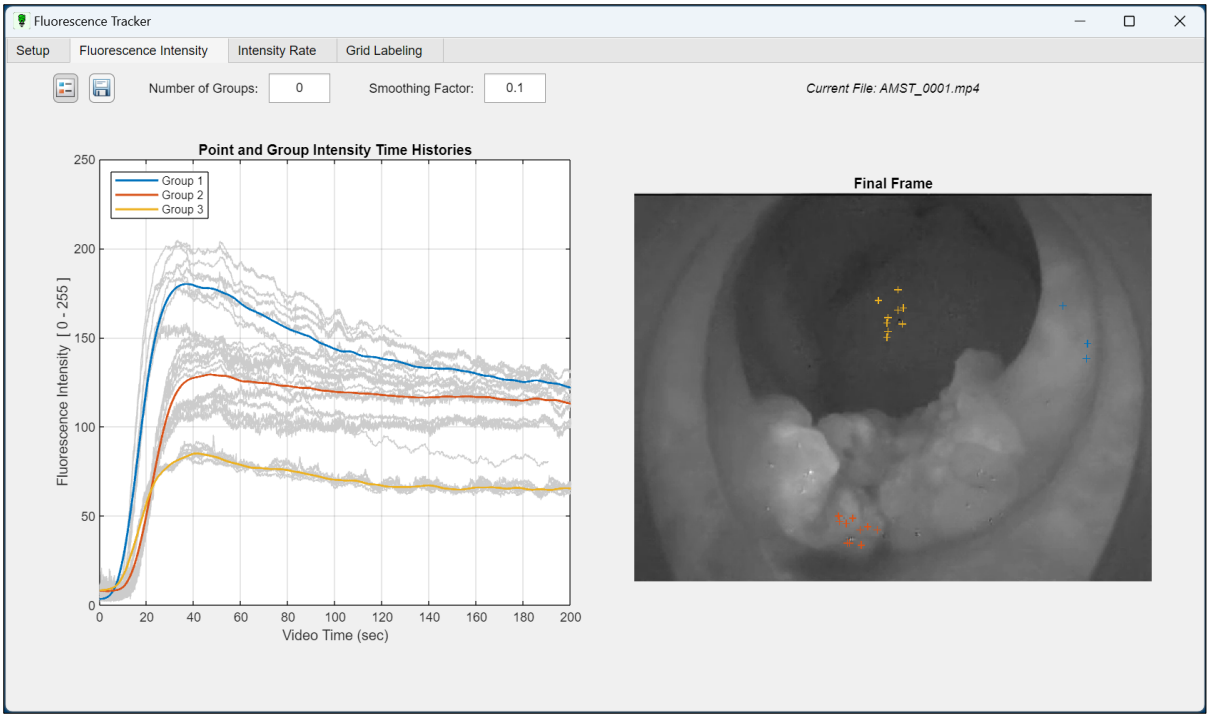


Figure 5: Fluorescence Intensity Tab (Manual Region Selection)

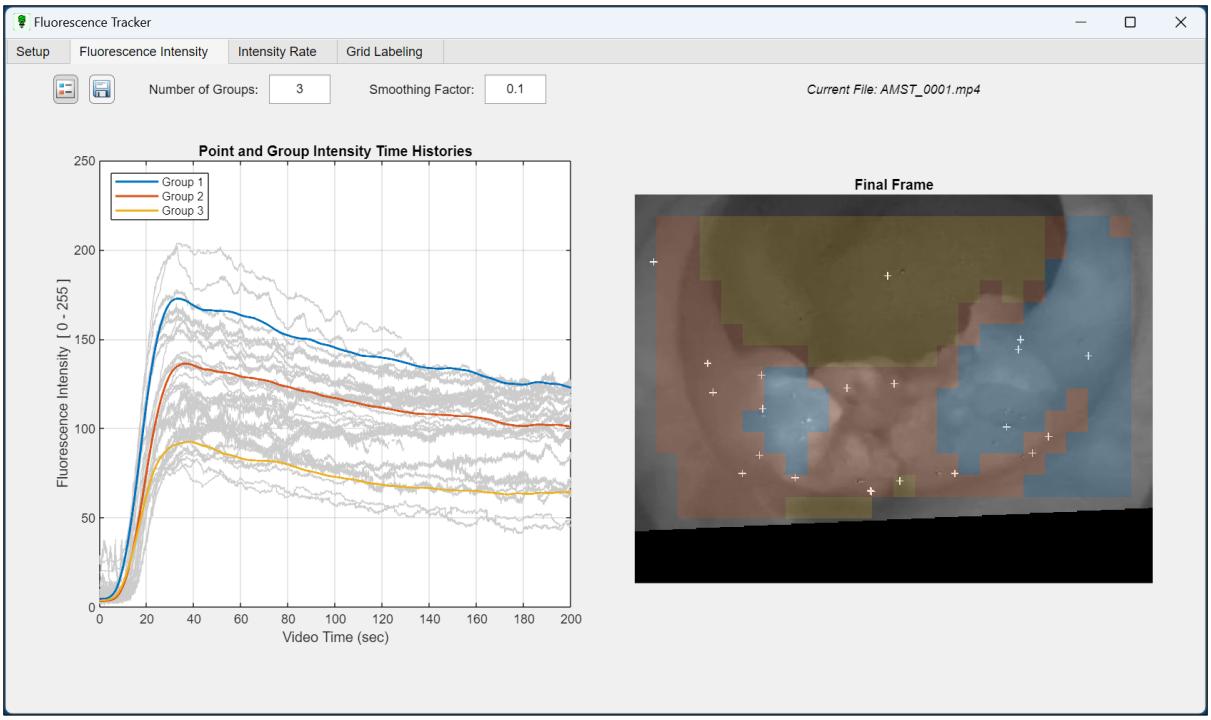


Figure 6: Fluorescence Intensity Tab (Automatic Grid Regions with "3" Groups)

Toggle Legend On/Off

- Click the "Toggle Legend On/Off" button to display or remove the figure legend.
- Initially the groups are labelled and displayed based on the order the regions were selected.
- If the **Number of Groups Field** is set to a nonzero value, the order of the groups in the legend (from top to bottom) will be sorted to match the order of the final smoothed mean intensity values of the groups (from high to low).
- These same colors are then used to label the corresponding points (or regions) in the "Final Frame" figure on the right.

Save Results

- Click the "Save Results" button to save all variables (including labels, if any) 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.

0 Number of Groups Field

- Use the "Number of Groups" field to perform ***k*-means clustering** to partition the tracked intensity time histories into distinct groups ($k > 0$).
- The default value of 0 (zero) indicates the groups 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.
- The smoothed mean intensity of each group is then color-coded, and the same colors are used to label the corresponding points (or regions) 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 group intensity and group intensity-rate time histories.
- This value ranges from zero (no smoothing) to one. Note, excessive smoothing can result in an apparent delay in the smoothed signal.

Intensity Rate Tab

As seen in **Figure 7**, the "Intensity Rate" 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 the smoothed grouped intensities divided by the change in time.

The light grey lines in the background display the result for each individual tracked point. The color curves displayed for each group are then the smoothed version of the grouped rate signals, based on the values specified in the **Number of Groups Field** and **Smoothing Factor Field**. The resulting figure uses the same color-coding as described in the **Fluorescence Intensity Tab**. There are no user options available in the "Intensity Rate" tab.

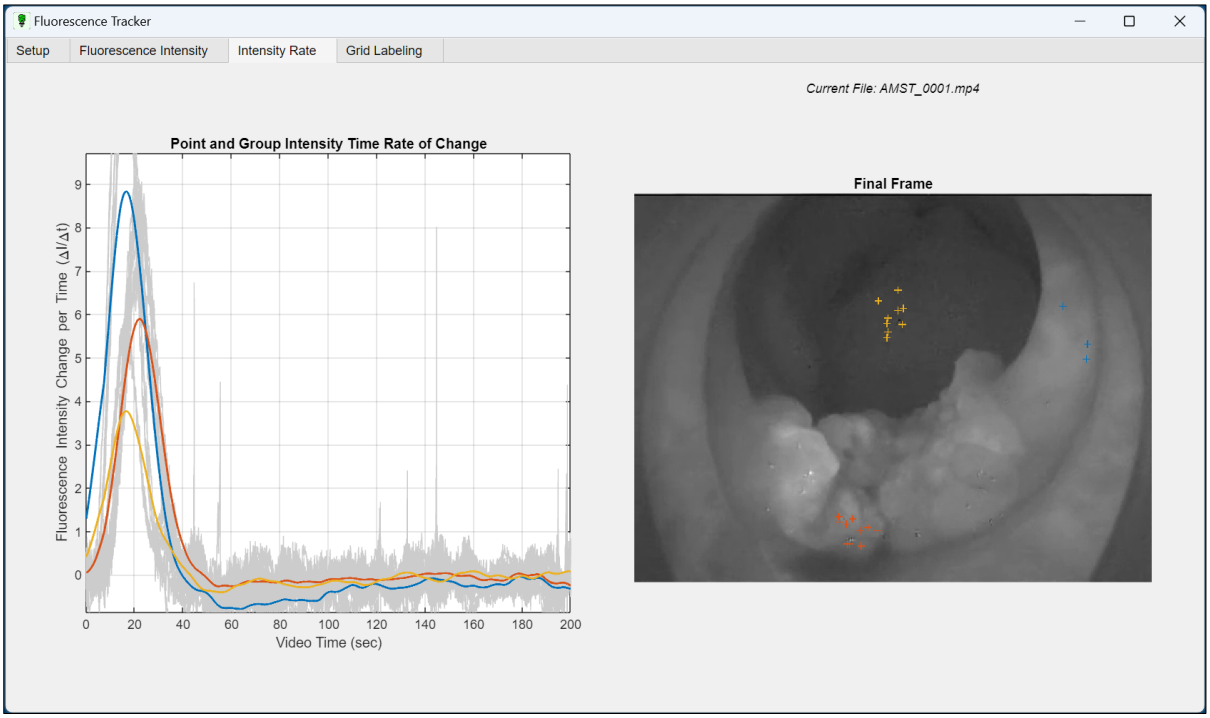


Figure 7: Intensity Rate Tab (Manual Region Selection)

Grid Labeling Tab

As previously described, when set to a value greater than zero, the **Number of Groups Field** can be used to cluster the intensity time histories into distinct groups. While these **"unsupervised"** clusters may be insightful, they do not explicitly diagnosis what each group represents. Diagnosis predictions can be performed using **"supervised"** machine learning. To train a machine learning model in this way requires a labeled dataset. For example, by processing multiple videos and labelling regions with a known diagnosis, the intensity data from the labeled regions can be used to train a machine learning model to distinguish between the specified diagnoses. Once sufficiently trained, this model could then use intensity data from undiagnosed regions in new videos to predict the corresponding diagnosis for each region. Note, this tab is only applicable when using **Auto-Grid Regions**.

Label Region

- Click the "Label Region" button to specify a region to be labeled.
- This will open a new figure window. Hover the cursor over the image and drag the resulting crosshairs to label the corresponding grid regions. (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 labels to other grid regions.
- Grid regions are then labeled with the text specified in the **Label Edit Field**. If desired, change this value *before* using the "Label Region" button.

Clear Label

- Click the "Clear Label" button to remove the previously added label.
- This can be done multiple times until all previous labels have been removed.

Refresh Plots

- Click the "Refresh Plots" button to apply the labeled grouped. The app will automatically change the **Number of Groups Field** to "0" and switch to the **Fluorescence Intensity Tab**.
- To export intensity data for labeled groups, use the "Refresh Plots" button *before* using the **Save Results** button on the **Fluorescence Intensity Tab**.

Save Labels

- Click the "Save Labels" button to save *only* the label table 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.

Label Edit Field

- Use the "Label Edit Field" field to specify a region label. If desired, change this value *before* using the **Label Region** button.

Figure 8 shows the **Fluorescence Intensity Tab** after processing the video with **Auto-Grid Regions** enabled (and **Number of Groups Field** set to "0"), but before labeling. As such, the color curves displayed for each group represent the mean intensities across each of the valid auto-grid regions.

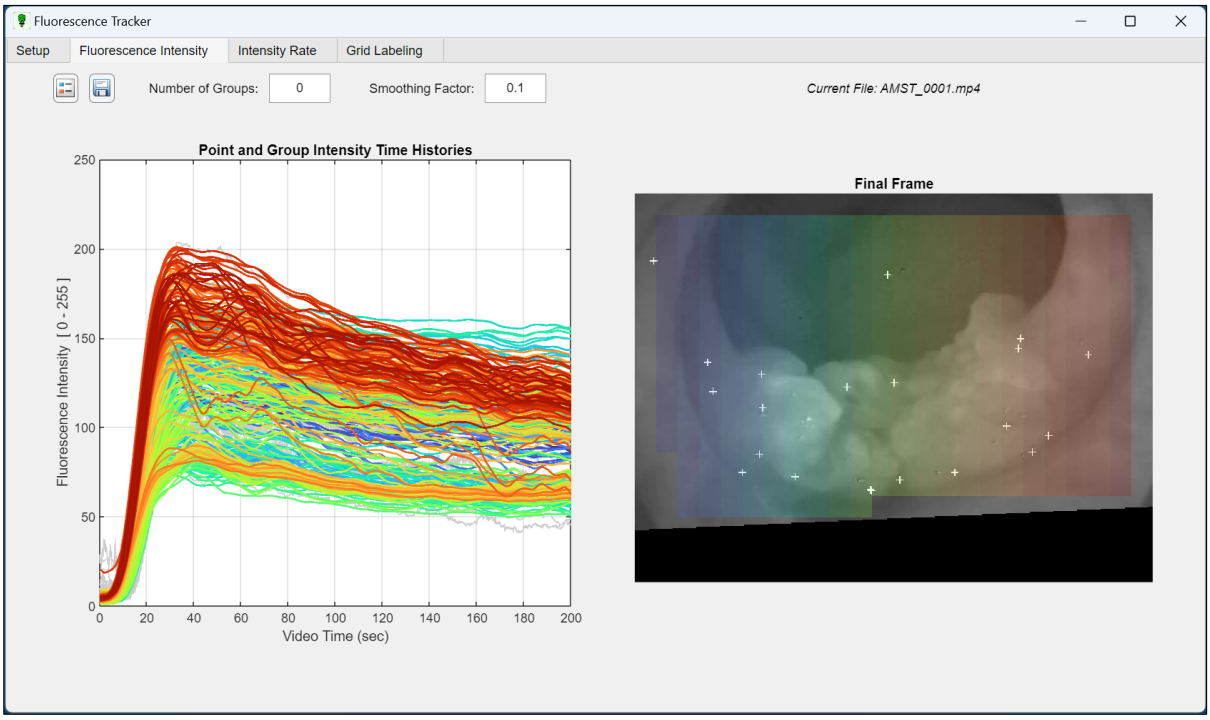
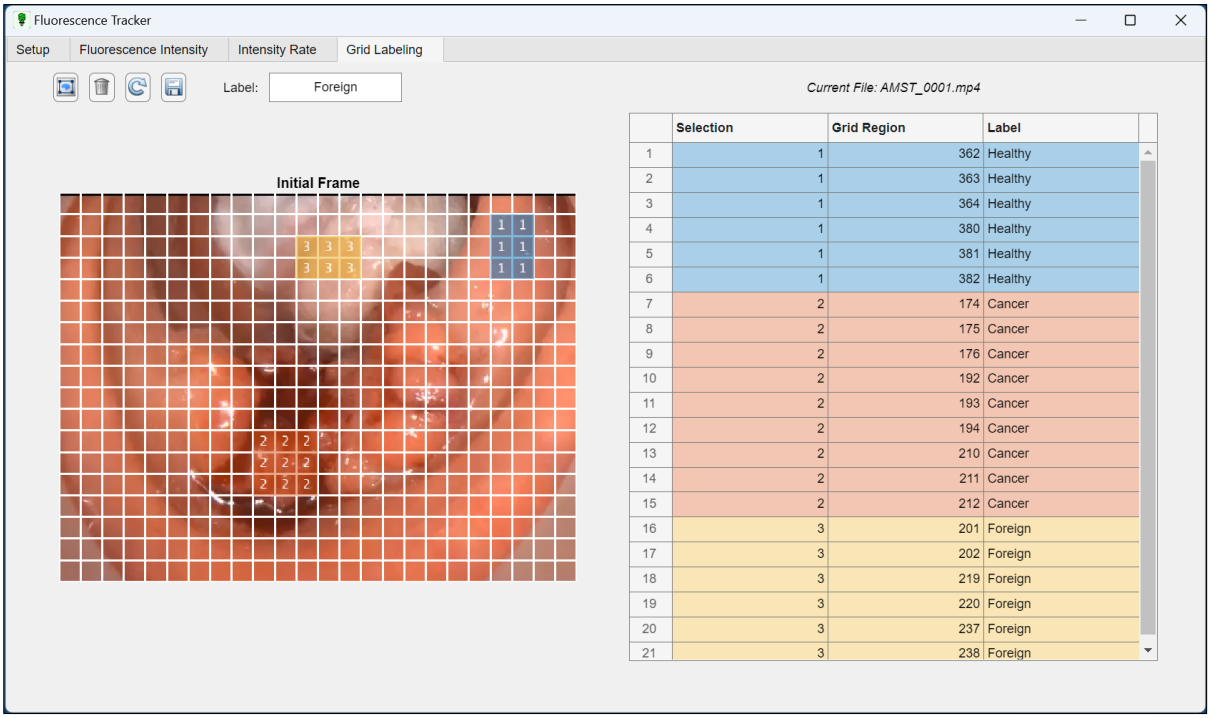


Figure 8: Fluorescence Tab (Automatic Grid Regions before Labeling)

Figure 9 shows how the "Grid Labeling" tab can be used to label regions with known diagnoses and extract their corresponding grid indices. In this way, the exported intensity data can easily be labeled and prepared for training a machine learning model.



	Selection	Grid Region	Label
1	1	362	Healthy
2	1	363	Healthy
3	1	364	Healthy
4	1	380	Healthy
5	1	381	Healthy
6	1	382	Healthy
7	2	174	Cancer
8	2	175	Cancer
9	2	176	Cancer
10	2	192	Cancer
11	2	193	Cancer
12	2	194	Cancer
13	2	210	Cancer
14	2	211	Cancer
15	2	212	Cancer
16	3	201	Foreign
17	3	202	Foreign
18	3	219	Foreign
19	3	220	Foreign
20	3	237	Foreign
21	3	238	Foreign

Figure 9: Grid Labeling Tab

Figure 10 shows the **Fluorescence Intensity Tab** after using the "Grid Labeling" tab and pressing the **Refresh Plots** button. As such, the color curves displayed for each group now represent the mean intensities for each labeled region.

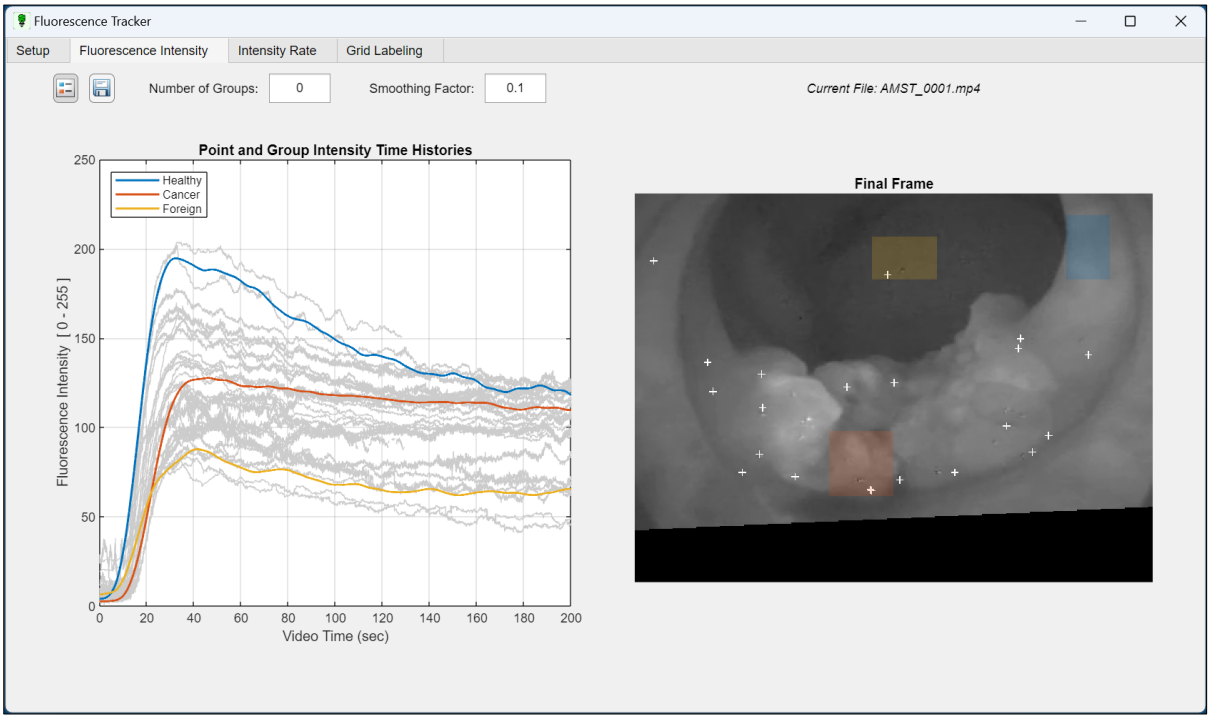


Figure 10: Fluorescence Tab (Automatic Grid Regions after Labeling and "Refresh Plots")

References

- *Overview of MATLAB Apps*
- *App Building with MATLAB*
- *Feature Detection and Extraction with MATLAB*
- *Tracking and Motion Estimation with MATLAB*
- *Image Registration with MATLAB*
- *MATLAB for Machine Learning*