

## Instructions for Viewing Tracking Results

**1. Download FluoRender** from <https://github.com/SCIInstitute/fluorender/releases>

The recommended version for viewing the tracking results is 2.20. Make sure the computer hardware meets the requirements, which can be found in the user manual.

**2. Install FluoRender.**

**3. Extract the tracking results to their own directories.** Three tracking results are provided. `amat_result.zip` contains results from tracking *Drosophila* development using the tool by Amat et al. This sample data set was provided by Amat et al. `our_result.zip` contains results from tracking the same data set from Amat et al. but using our method. `fish_result.zip` contains results of tracking two time points of zebrafish eye development.

**4. Open FluoRender.**

**5. Enable tracking script.** In the “Record/Export” panel of FluoRender, click “4D Script”, and then check “Enable execution of a script on 4D data during playback”. From the list of built-in script files, choose “script\_4d\_selection\_tracking”.

**6. Load one sample data set.** Click “Open Volume” in the main toolbar of FluoRender. In the file open dialog, browse to one directory of extracted files, and then select the first time point file. Click “Open” to load this 4D sequence.

**7. Load tracking results.** Click “Tools->Tracking...” from the main menu to open the tracking dialog. Click “Load” from the “Track Map” tab to load a track file. Browse to the “\*.track” file in the same directory as the already loaded 4D data and open it.

**8. Select cells to view tracking results.** Hold the “Shift” key on keyboard and use the brush to select cells. Once cells are selected, go the “Selection” tab of the tracking dialog, and click “FullCompt” to load the selection into the tracking ID list. Alternatively, type “all” in the text control after “Selection tools:” and press “Enter” key to load all cells into the list. To view tracking results over time, click “Forward” or “Backward”.

**9.** To regenerate the tracking results, use the following parameters for track map generation. Times: 3, Size Thr.: 25, Contact F.: 0.6, Similarity: 0.85, Merge: check, Split: check.

**10.** Detailed operational instructions of FluoRender can be found in its manual. Also check the following YouTube video for a tutorial on cell tracking using FluoRender: <https://youtu.be/lyYPYHyWoic>.

## Source code for the uncertainty footprint

The implementation of the uncertainty footprint is integrated with the FluoRender system. Retrieve the source code of FluoRender from the following web link:

<https://github.com/SCIInstitute/fluorender>

Specifically, for the implementation of the uncertainty footprint for the EM algorithm, see this file:

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Cluster/exmax.cpp>

On line 62, the function GenUncertainty() calculates the uncertainty footprint.

For the implementation of the uncertainty footprint in graph matching, see these files:

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Tracking/Cell.h>

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Tracking/CellList.h>

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Tracking/TrackMap.cpp>

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Tracking/TrackMap.h>

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Tracking/Vertex.h>

<https://github.com/SCIInstitute/fluorender/blob/master/fluorender/FluoRender/Tracking/VertexList.h>

On line 48 and line 58 of the header file Vertex.h, there are definitions for the graph edge and vertex. There is a variable called count, which counts the changes. In the source code, search for the variable count to see how the uncertainty footprint is calculated.