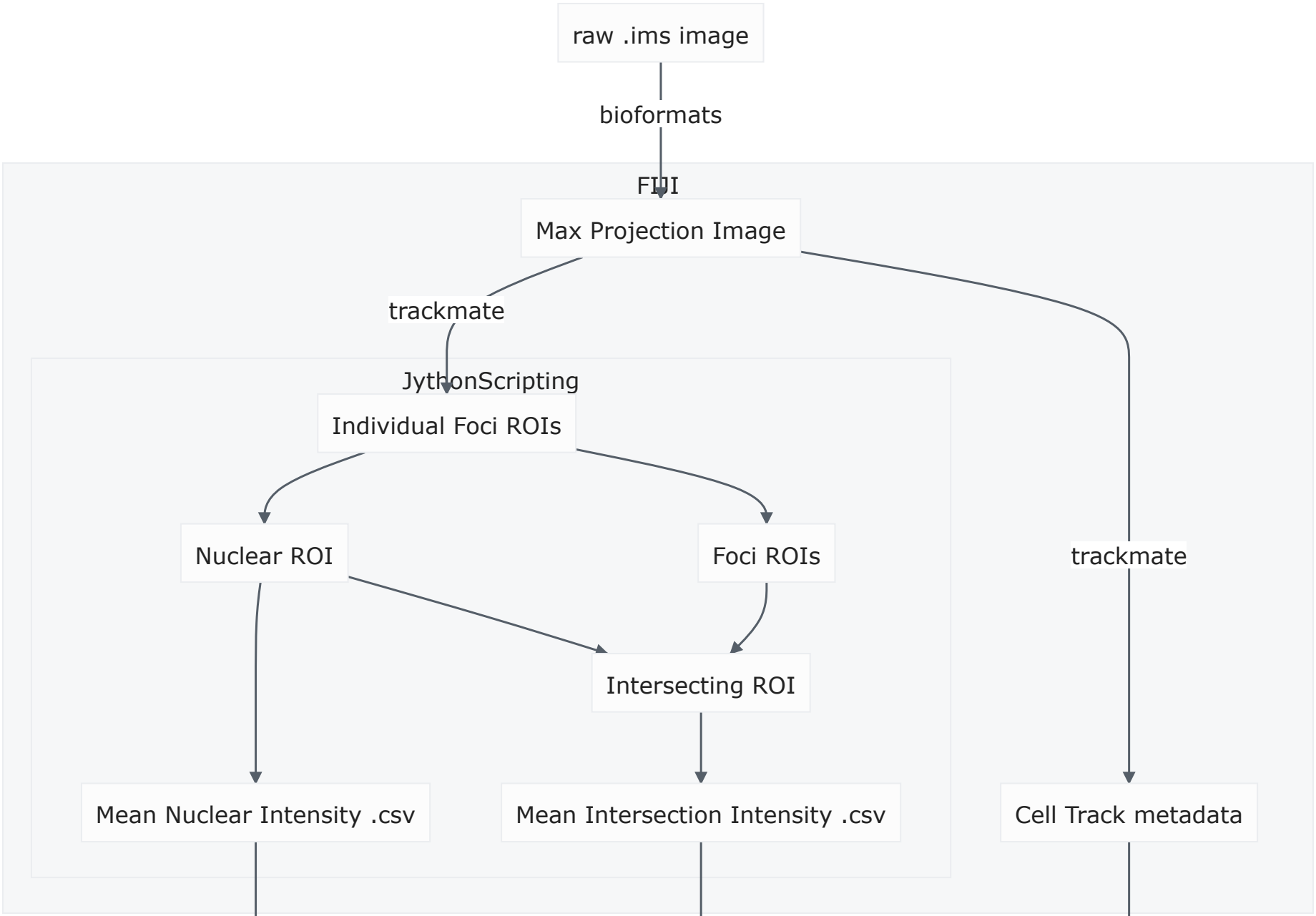
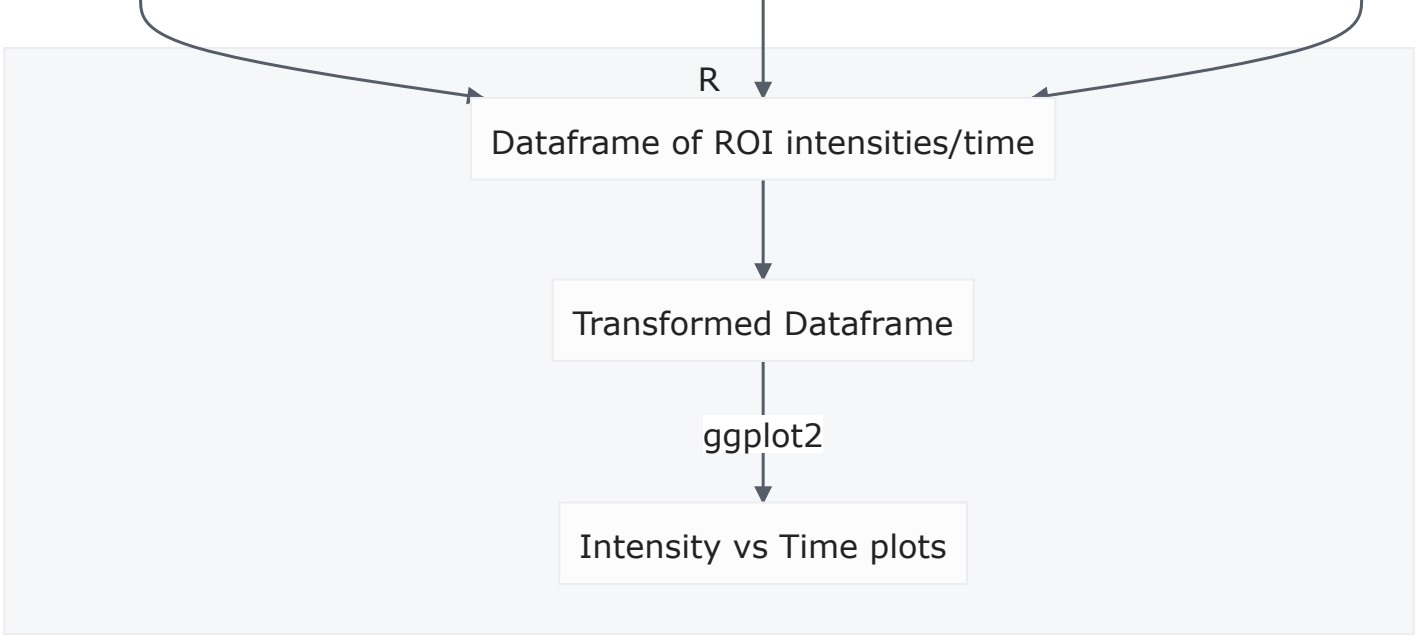
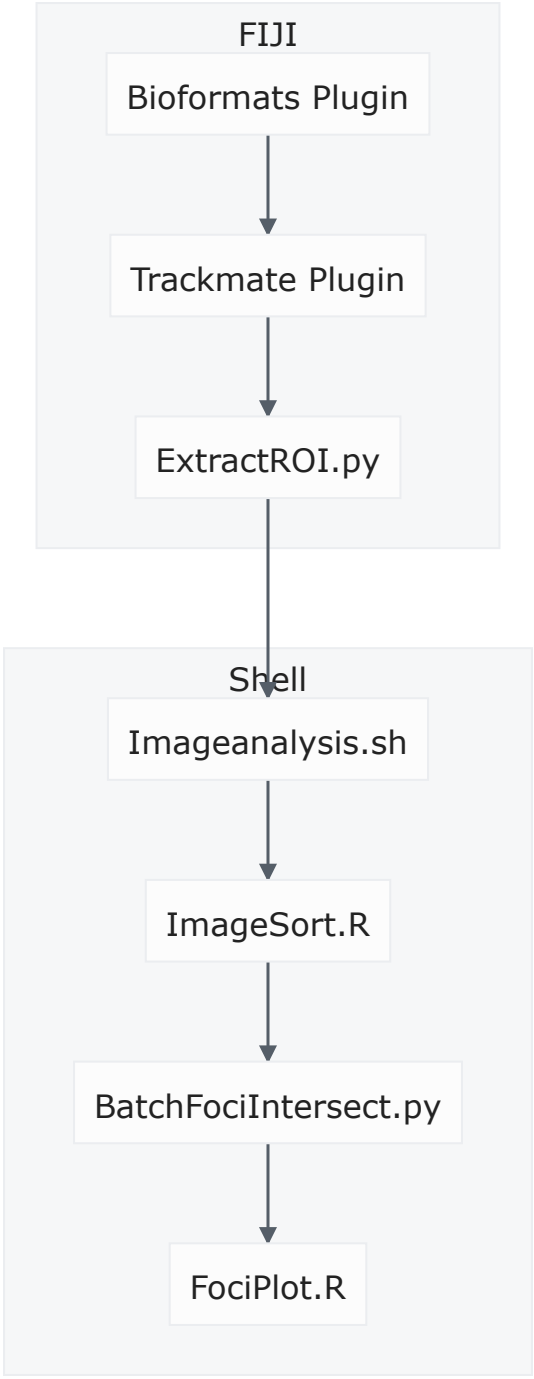
ImageAnalysis pipeline v2 (Timelapse)

Script workflow





Running enviroment



#==========

Running steps

1. Import/open image in FIJI

2. Select ROI boundary (*will ensure that all identified ROIs will not be straddling the boundary of the image*)```

```
//setTool("rectangle");
makeRectangle(50, 50, 924, 924);
```

3. Run Trackmate

Trackmate Params:
##

```
1. . Image parameters
        1. X (0, 1023)
        2. Y (0, 1023)
        3. Z (0, 0)
        4. T (0, 59)
2. LoG detector
        1. Detect in channel: 3 (lacO)
        2. Estimated object diameter: 0.5
        3. Quality Threshold: 50(Confocal) - 200 (TIRF)
3. Select all for initial thresholding
4. For filters
        1. Contrast Ch3 (place the lower limit of the gate at the beginning of the plateau)
5. LAP Tracker
        1. frame-to-frame linking
                1. max distance: 1.0 micron
        2. Segment closing gap:
                1. max distance: 1.0
                2. max frame gap: 3
6. Filters on tracks:
        1. TrackDisplacement: ~0-1.8
        2. TrackStart: 0 *Must be visible and measurable at time = 0, to synchronize measurements*
        3. TrackStop: ~45-50
```

```
7. Open *Tracks* tab, select all tracks, press *Export to csv*
8. In trackmate menu, select *Export spots to IJ ROIs
```

##

4. Open ExtractROI.py in FIJI (*Do not touch FIJI as this script opens, writes, and closes continuously which may lead to closing the wrong image*)
    1. Select output directory for individual images
5. Copy ImageAnalysis.sh into the working directory
    1. Place the following sub-scripts somewhere on the system (make a note of the path). Edit the corresponding paths in the Imageanalysis.sh script

```
ImageAnalysis.sh
        -imageSort.R
        -BatchFociIntersect_v4.py
        -FociPlot_v2.R
```

6. Open terminal and run ImageAnalysis.sh from the working directory

```
bash ImageAnalysis.sh
```

7. Select output target output directory, Run script.

8. Done

Output

the Resulting directory should have the following folder structure:

```
Experiment Folder/
                        Export.csv
                        ImageAnalysis.sh
                        Original_maxProj.tif
                        ROIs/
                                N_cellID/
                                        N_cellID_stack.tif
                                        N_cellID_IntensityTrace.png
                        Outputs/
                                AvgCell_intensity.csv
                                log.txt
                                PerCell_intensity.csv
                                ROI_values.csv
                                Figures/
                                        FirstSlope.png
                                        FociInt_Ch1.png
                                        FociInt_Ch2.png
                                        MaxIntensityPlot.png
                                        Rplots.pdf
                                        econdSlope.png
```