Workshop structure

Part 1 **Introduction**	What are filopodia and how have they been studied
Part 2 **Intro to Filopodyan workflow (Fiji)**	A guided walk through the plugin for segmentation & tracking using a simple demo file
Part 3 **Intro to Filopodyan workflow (R)**	Phenotype comparison; correlations between properties; filopodium initiation; tip elongation
Part 4 – Workflow reproduction	
a. **Analysis: Phenotype comparison**	Batch processing (Fiji) and downstream analysis
b. **Analysis: Fluorescence & tip movement**	Tip fitting, direction-corrected tip movement, cross-correlation analysis
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Part 5: **Deconstruction**	

Workflow reproduction

Test run of Filopodyan in Fiji:

- Sample growth cone (short single-channel time series)

A: Phenotype comparison between datasets

- 1) Analysis results with batch automation vs manually curated dataset (ctrl)
- 2) Phenotype in control vs ENA overexpression dataset

B: Tip Fluorescence quantification

Test run / Interactive demonstration

Sample file: growth-cone-test-file.tif

Filopodyan user guide: https://github.com/gurdon-institute/Filopodyan/blob/master/Filopodyan%20User%20Guide.pdf

1. Start Fiji

2. Open sample file

3. Launch Filopodyan

4. Segment and track filopodia:
   (segmentation) set suitable segmentation parameters (with the help of Preview)
   (filtering) set filtering parameters (hit Apply to preview)
   (manual editing) explore possibilities to delete tracks or timepoints

5. Inspect output:
   - image with ROI overlays
   - data tables

Workflow reproduction

Test run of Filopodyan in Fiji:

- Sample growth cone (short single-channel time series)

A: Phenotype comparison between datasets

- Analysis results with batch automation vs manually curated dataset (ctrl)

B: Tip Fluorescence quantification

Workflow reproduction (4a)

Image data: 4a_Big-ctrl-dataset
Data tables from manually curated analysis: 4a_Manualy-curated-tables
Data tables from batch automated analysis: [generate in 4a_RESULTS] (also in batch_preprocessed)

1. Open Filopodyan in batch mode
(Fiji > Plugins > Filopodyan, without any images open)

2. Run analysis on big ctrl dataset using the same parameter settings as used in the curated dataset (→), save resulting tables in [new folder] as .txt files (this may take a while!)

Parameter settings:

```
Map C: 1
Measure C: 2
Threshold: RenyiEntropy
Adaptive thresholding: false
Fit tip to measure C: false
Join fragments: false
ED Iterations: 4
Base back frames: 0
LoG sigma: 2.6

Filter settings-
Min start frame: 1
Min frames: 3
Min max length: 1.8
Min length change: 0.1
Min max DCTM: 0.0
Min max DCBM: 0.0
Max mean waviness: 0.35
```

R script analysis: MASTERSCRIPT

1. On top of the script, adjust file paths for:
   - `folder.names`, (data tables: 4a_RESULTS)
   - `Loc.save`, (output of R analysis), and
   - `Loc.Modules` (R scripts)

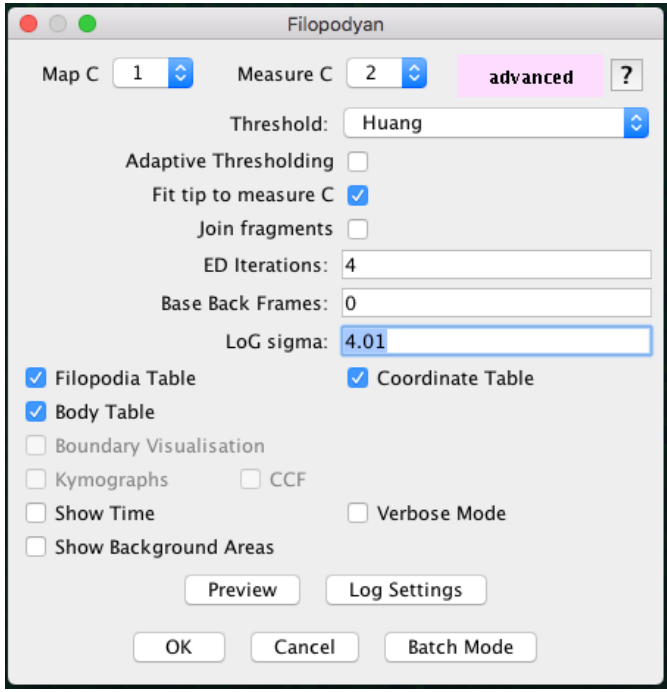
2. Test all filepaths (`setwd()`). Check that the folder defined as Loc.Modules contains all required scripts (Modules 1, 2 and 3, GraphingTemplates and ColourSchemes)

3. Run Masterscript

Workflow reproduction (4b)

Image data: 4b_TipFluorescence
Record of manual curations: ”Track Edits” .txt files (in 4b_TipFluorescence)
Data tables: [generate & move to 4b_RESULTS] (also in TipF_preprocessed)

1. Open an image from the 4b dataset in Filopodyan
2. Run analysis using these parameter settings →



3. In Edit Tracks window: find reconstructed tracks in need of manual curation, eliminate falsely reconstructed timepoints (or tracks). Finally, try applying the corresponding track edits file.

FilopodyanR MASTERSCRIPT.R:

- set folder.names[1] to filepath of the TipF dataset, [2] to “ ”
- set Loc.save to filepath 4b_RESULTS folder
- set compare.phenotypes to FALSE
- set dataset.names[1] to “ENA”, [2] to “ “
- workspace saving (bottom of script), e.g. as LastWorkspace_TipF.Rdata
- Execute Masterscript

FilopodyanR CCF.R:

- set loading filepath (line 126) (e.g. LastWorkspace_TipF.Rdata, with full filepath)
- set saving filepath for .Rdata file (end of script)

FilopodyanR CCF subcluster.R:

- set loading filepath (line 43) and guide.size (line 187), run sections 1-7