Focus tracking in TrackMate

Intro

TrackMate[1] is preinstalled in Fiji

## Open movie

1. Open fluorescence movie in Fiji (ImageJ)

## Start TrackMate

1. Start TrackMate through Plugins > Tracking > TrackMate

## Selecting spots

1. Change nothing in Calibration and Crop settings; check that time dimension is listed under T, not Z in crop settings
2. Select LoG Detector
3. Zoom in to a spot on the first (brightest frame) to estimate blob diameter (around 7 px)
4. Check ‘Use median filter’ and ‘Do sub-pixel localization’
5. Try out a few Threshold values and click ‘preview’, judge by eye if spot selection is reasonable
6. Click ‘next’ to start detection
7. Select all spots by dragging the right bounds of the ‘quality variable’ to the left

## Linking spots

1. At ‘Select a view’ select ‘HyperStack Displayer’
2. At ‘Select a tracker’ select ‘LAP Tracker’
3. Set ‘LAP Tracker’ settings to the following (convert to px using resolution where appropriate)
   1. 1 μm