Move "manipulation" and worm tracking using ImageJ version 0.9
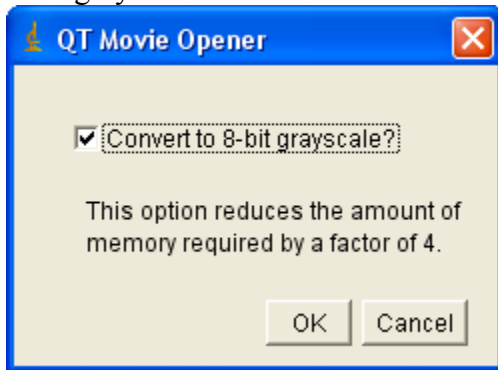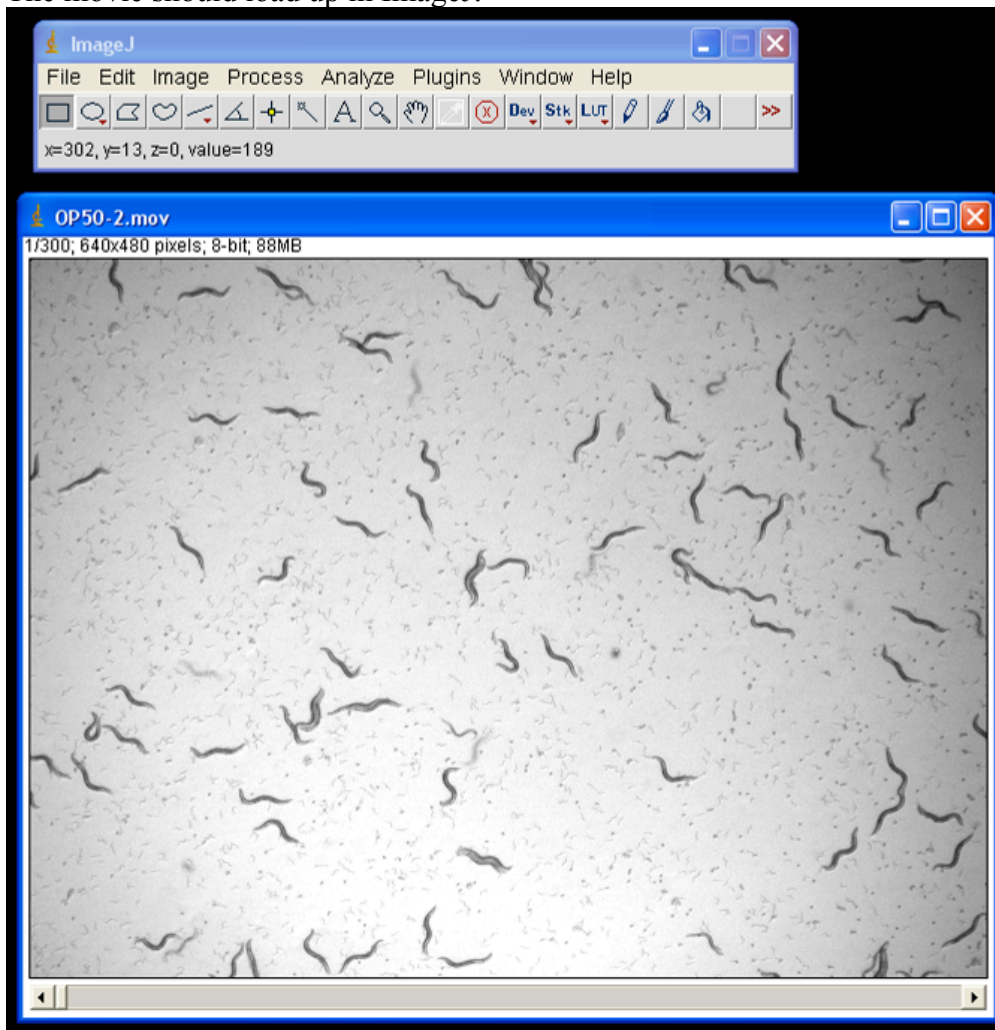Jesper S. Pedersen

How to use ImageJ for worm-tracking.

Open Quicktime or AVI movie directly in ImageJ[1]. It may ask if you want to convert to 8-bit grayscale. Since movies are actually recorded in grayscale check it to save memory.


The movie should load up in ImageJ:

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[1] ImageJ is free, Open-Source software available at: http://rsbweb.nih.gov/ij/

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Now select "Process" -> "Subtract Background..". A dialog like the one below will appear. You can adjust the "Rolling ball radius" and directly see what that does to your image by checking the "Create Background" and "Preview" options. (Remember to uncheck "Create Background" to actually process movie.)
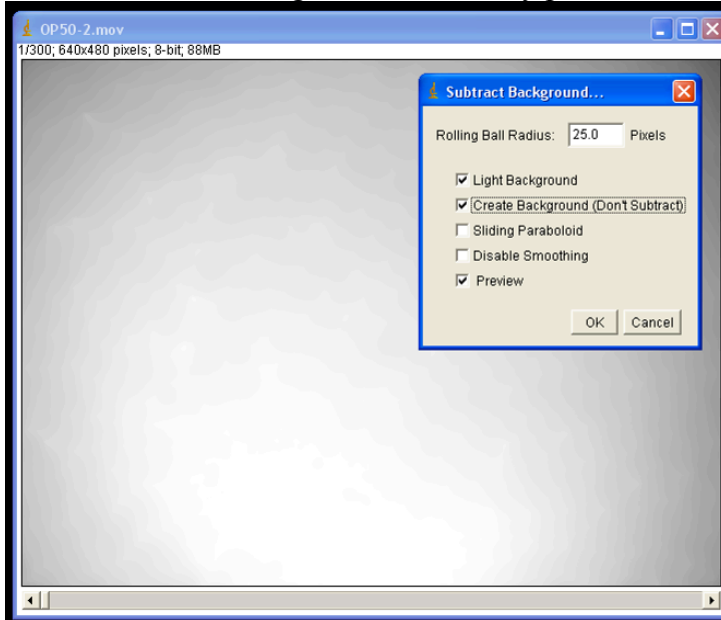

After subtraction the movie will look something like this:
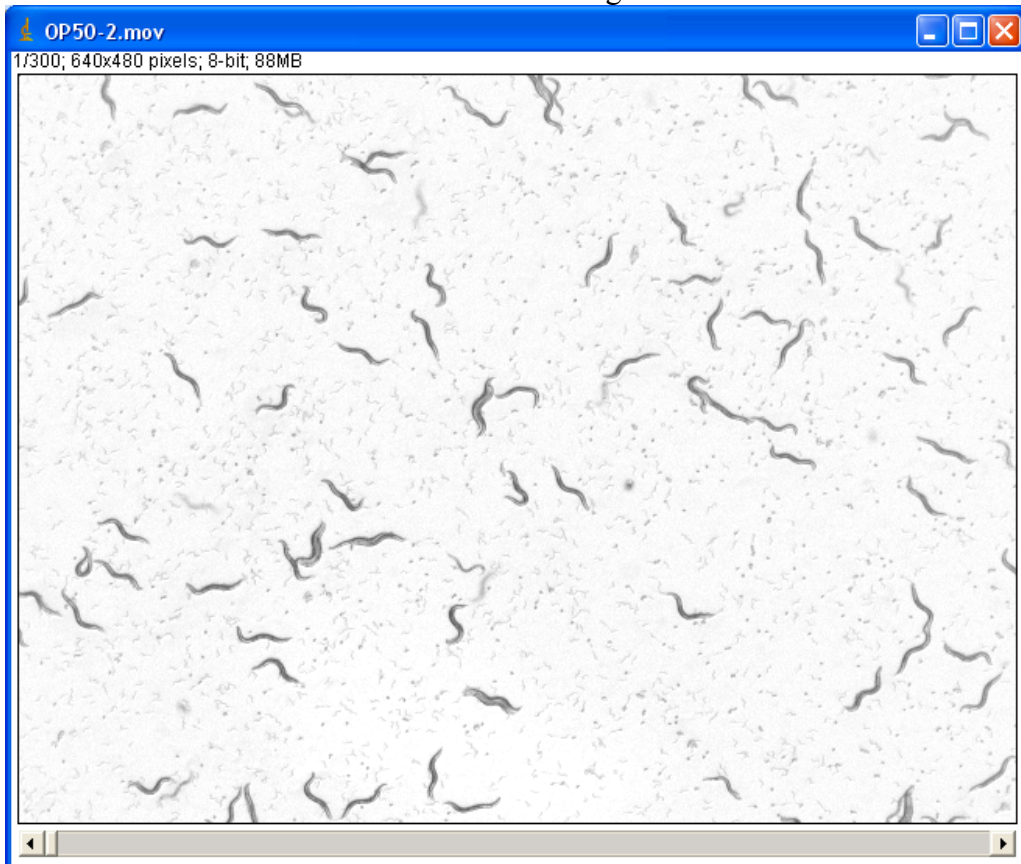
You're now ready to indicate what pixels are worms and what pixels are background by adjusting the threshold. Select "Edit" -> "Adjust->" -> "Threshold". Worms will appear as red. Adjust the sliders to find a proper level where worms are red and few spots appear on background. Once an appropriate level is found click "Apply".
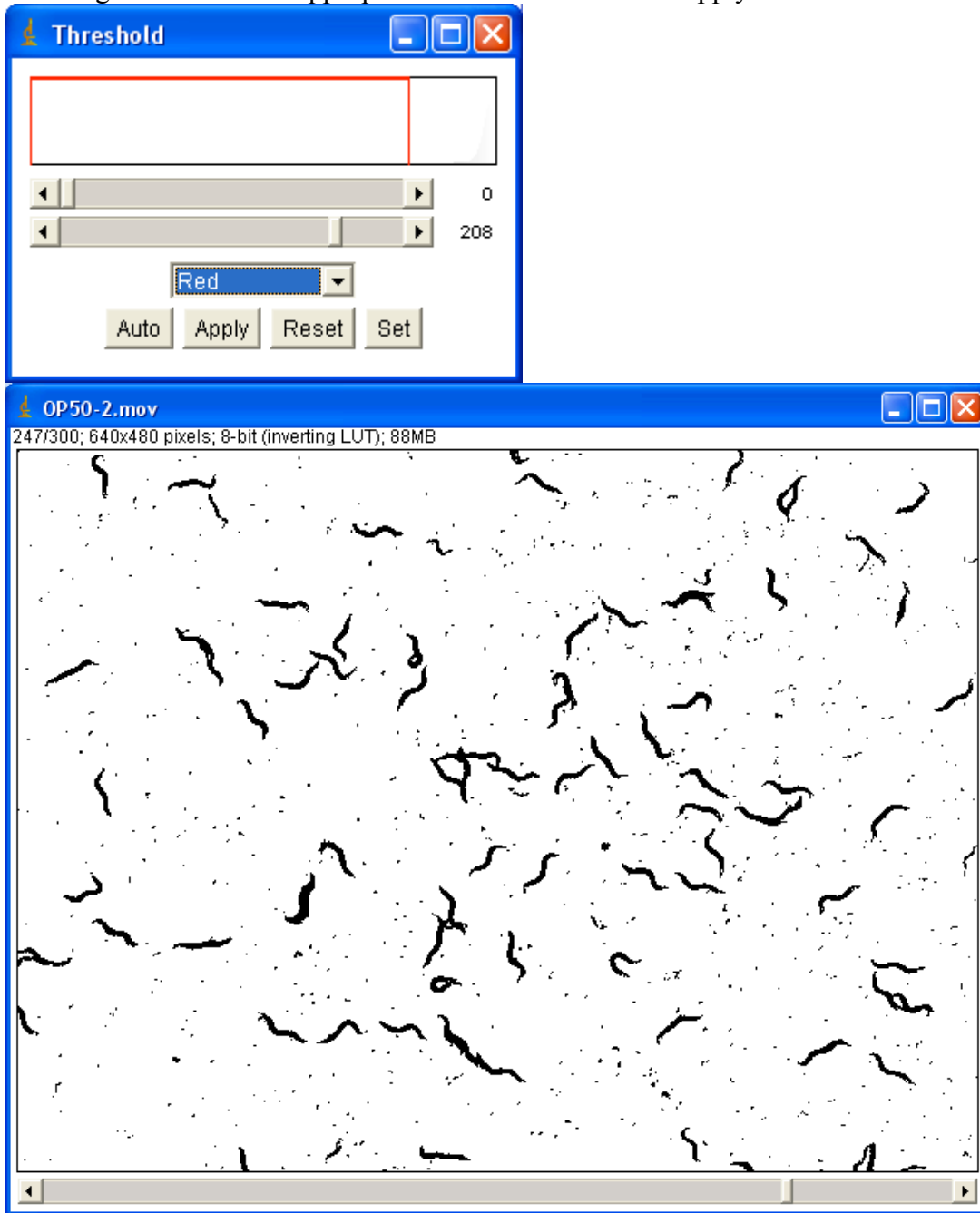
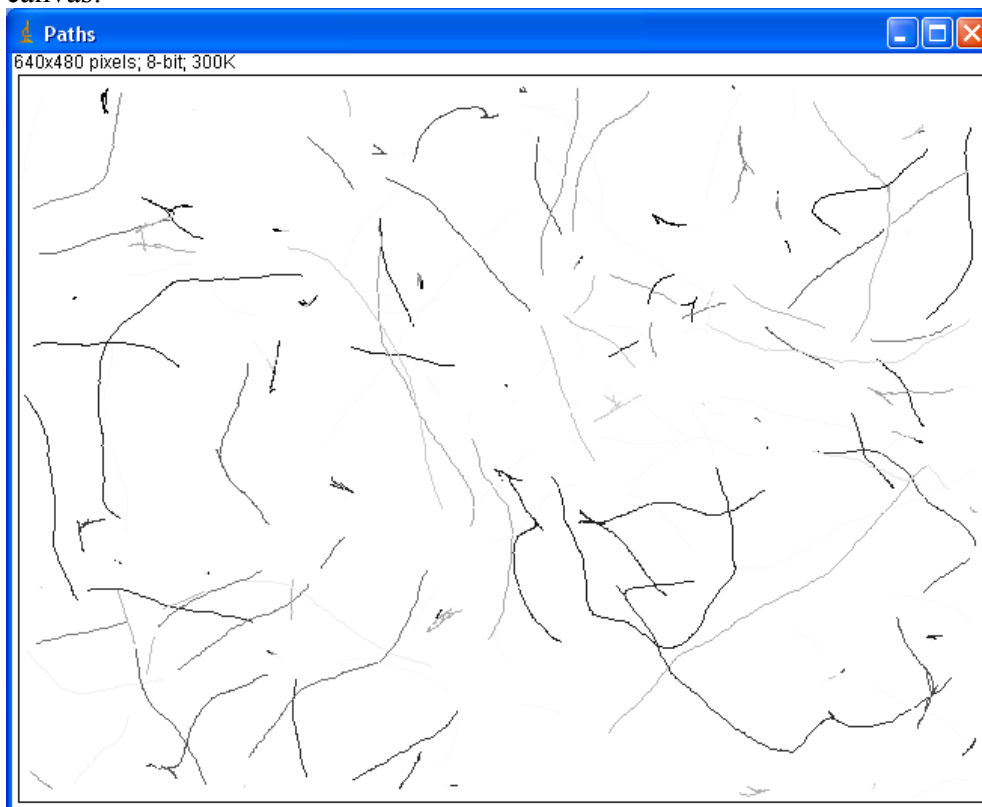Move "manipulation" and worm tracking using ImageJ version 0.9
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Now install the plugin "MTrack2"[2] or similar plugins[3] by selecting "Plugins" ->
"MTrack2" or use "Plugins" -> "Compile and Run…" and select the file
"MTrack2_.java". A dialog as the one below will appear:
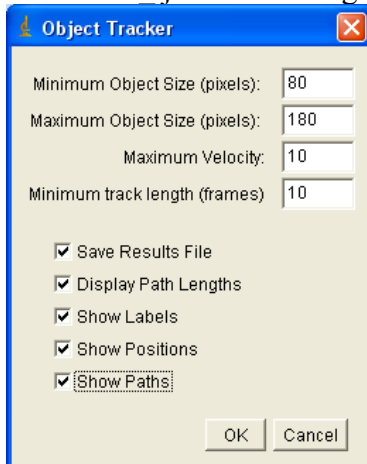
Indicate the minimum and maximum worm-size in pixels. The maximum velocity is the
number of pixels a worm can move in one frame.

After the analysis the worm-tracks will appear as lines of different shades of gray on a
canvas:

[2] Available at http://valelab.ucsf.edu/~nico/IJplugins/MTrack2.html
[3] i.e: CASA http://rsbweb.nih.gov/ij/plugins/casa.html

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Moreover, you can also see a movie where each worm is indicated by a number and x-coordinate.
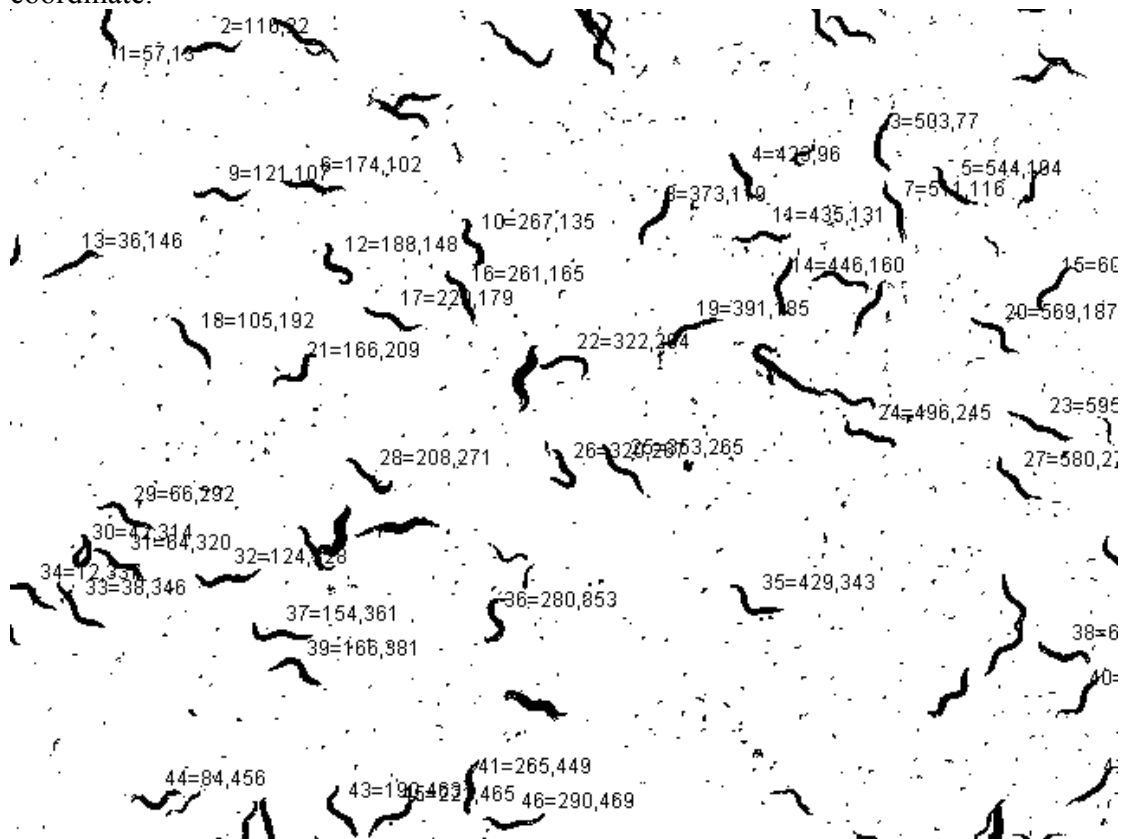
The text-file produced will contain the tracks of all worms. At the bottom of the text file there will a list of all the tracks showing the length of the track (sum of all movements) and distance traveled (distance from start to finish).

ImageJ advantages compared to Senchu:

- Not as "picky" about movie quality as Senchu.
- Many parameters are adjustable.
- Runs on both OS X and Windows (linux too).
- Completely freeware (Senchu requires a commercial math plugin)
- Open-Source - everyone who knows a bit of java can enhance the software.

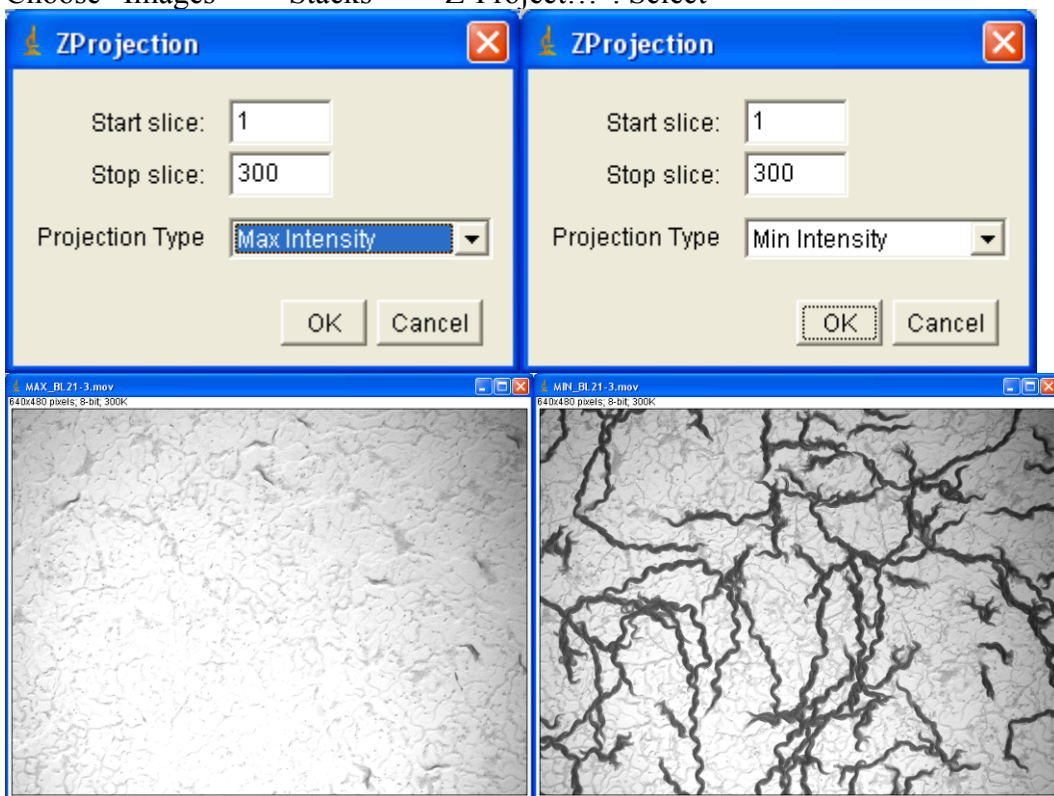## ImageJ disadvantages compared to Senchu:

- Not high-throughput (analyzes one file at a time).
- Slow analysis (may be due to java).

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Alternative ways to remove background from movies:

Choose "Images" -> "Stacks" -> "Z-Project…". Select
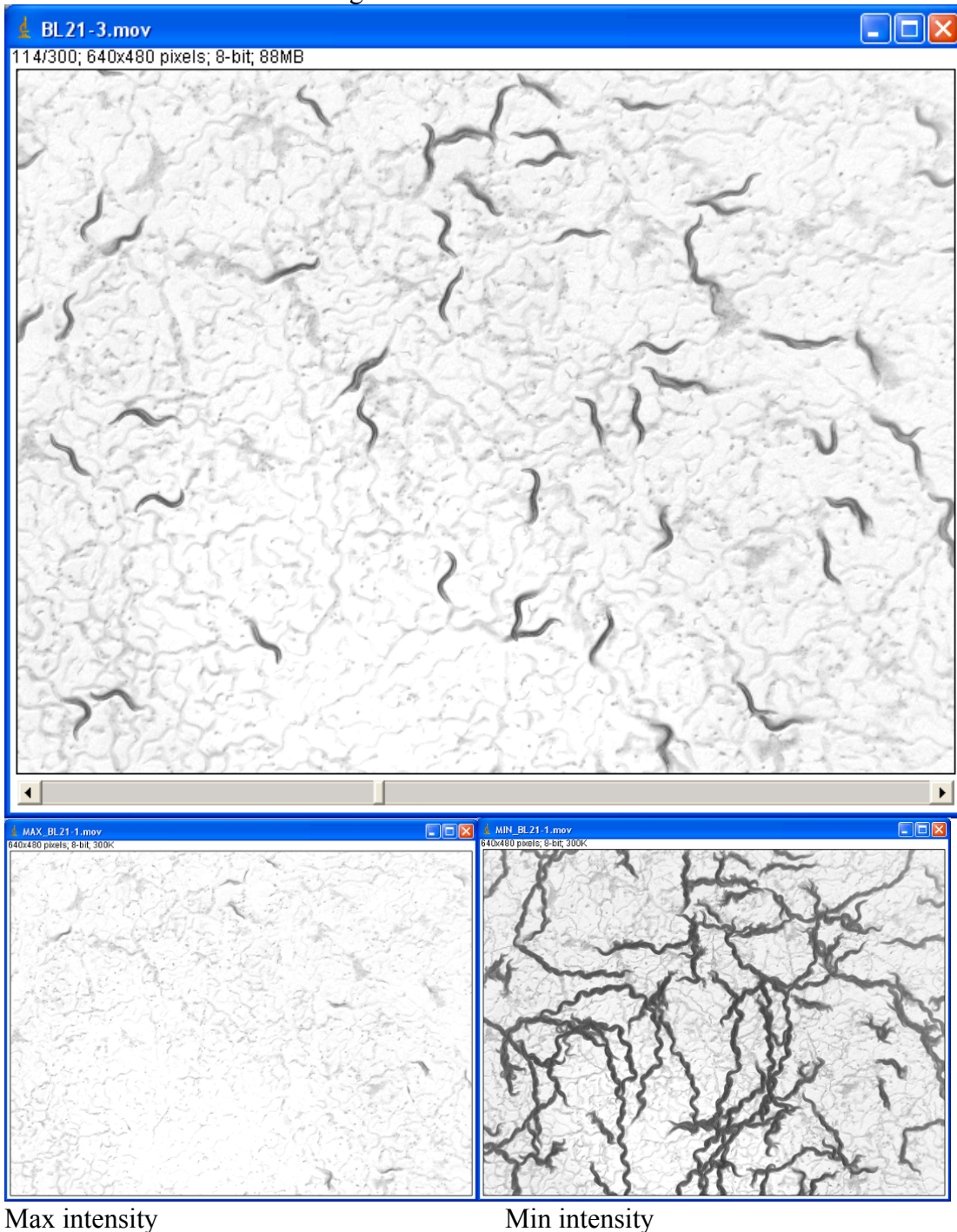

Max intensity   Minimum intensity

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The trick also works after background subtraction:
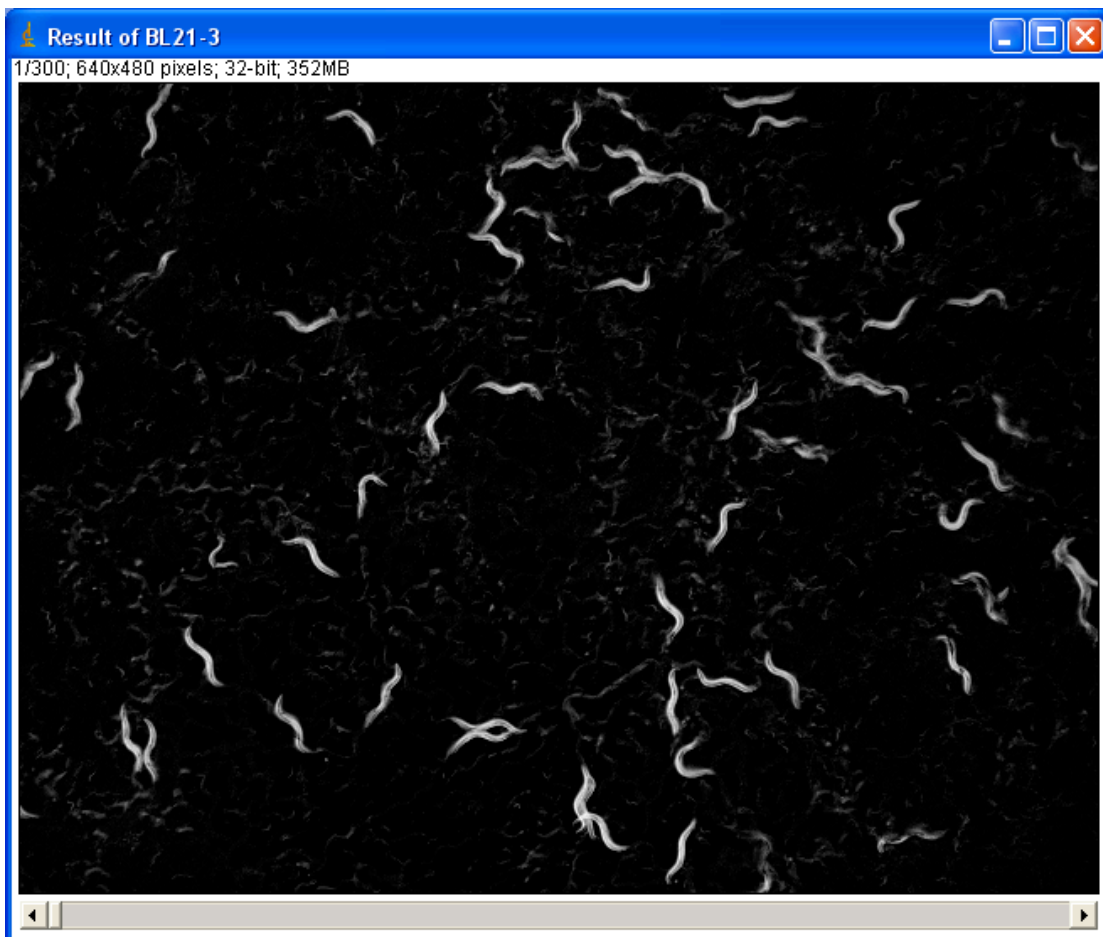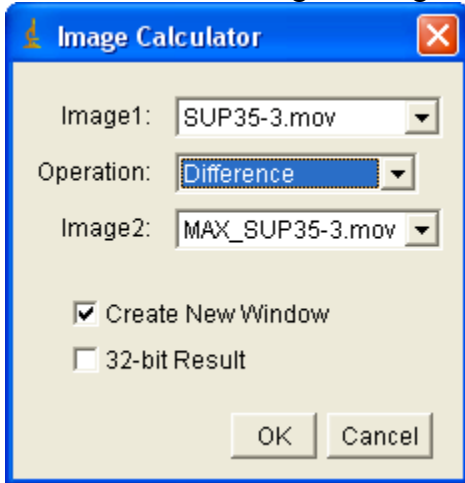

Max intensity   Min intensity

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Select "Process" -> "Image Calculator". Select difference and have your movie as image1 and the maximum image as image2.

Disadvantage is that immobile worms will be removed from the resulting movie.