

# SIMPLE QUANTITATIVE METHOD OF NEURITE LENGTH MEASUREMENTS IN TRIANGULAR MICROFLUIDIC DEVICE

## APPLICABLE CHIPS REFERENCES

- NF\_2\_X\_TR
- NB6L2XTR
- NB24L2XTR

## PURPOSE

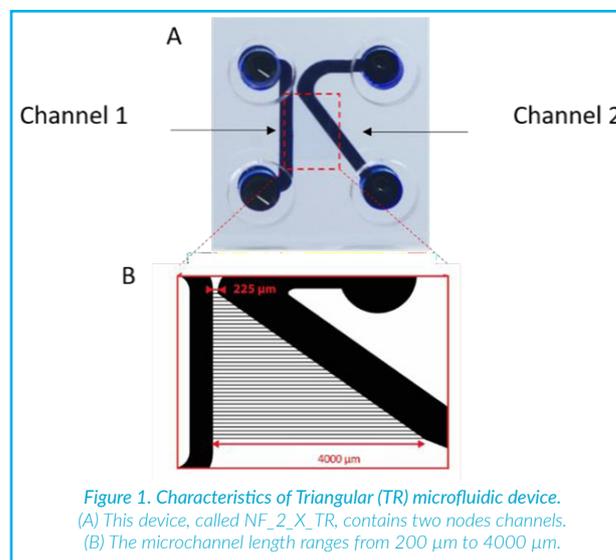
This application protocol describes a simple methodology to quantify the neurite length using the triangle-shaped microfluidic device from NETRI (Figure 1). Compartmentalized microfluidic devices enable segregation of the soma and growing neurites.

The measurement of neurites growth in conventional cell culture devices such as Petri dishes requires neurite tracer and/ or kymograph. It requires playing on cell density methods and are complicated to set up.

The triangular 2-nodes chip has specific characteristics to quantify the kinetic of neurite growth with a maximal neurite length up to 4000  $\mu\text{m}$  (Figure 1B).

This protocol can be used with animal/ human primary cells, cell lines or human induced pluripotent stem cells (iPSC).

Here, hippocampal primary neurons from embryonic rats are isolated using our previously published protocol<sup>1</sup>. Live cells are labelled using Vybrant® multicolor cell-labeling kit (ThermoFischer, V22889) in microfluidic system using our protocol described elsewhere<sup>3</sup>.



## MATERIALS

- Software Fiji with ImageJ core 1.53e2
- Graph Pad Prism 8
- Computer with Windows/Linux/MacOs compatible with Fiji

## PROCEDURE

### 1. Astall Fiji software

**Note:** Following Fiji user manual<sup>3</sup>.

### 2. Set up the scale

- 2.1. Open Fiji, keep it as a small window
- 2.2. Drag and drop the image of interest in the "Fiji" window
- 2.3. Click on "Analyze"
- 2.4. Select "Set scale"
- 2.5. Insert the parameters of your camera as provided by the suppliers (Figure 2)
- 2.6. Click on "OK"

**Note:** This first step is important to modify the size of your image from pixel to  $\mu\text{m}$ .

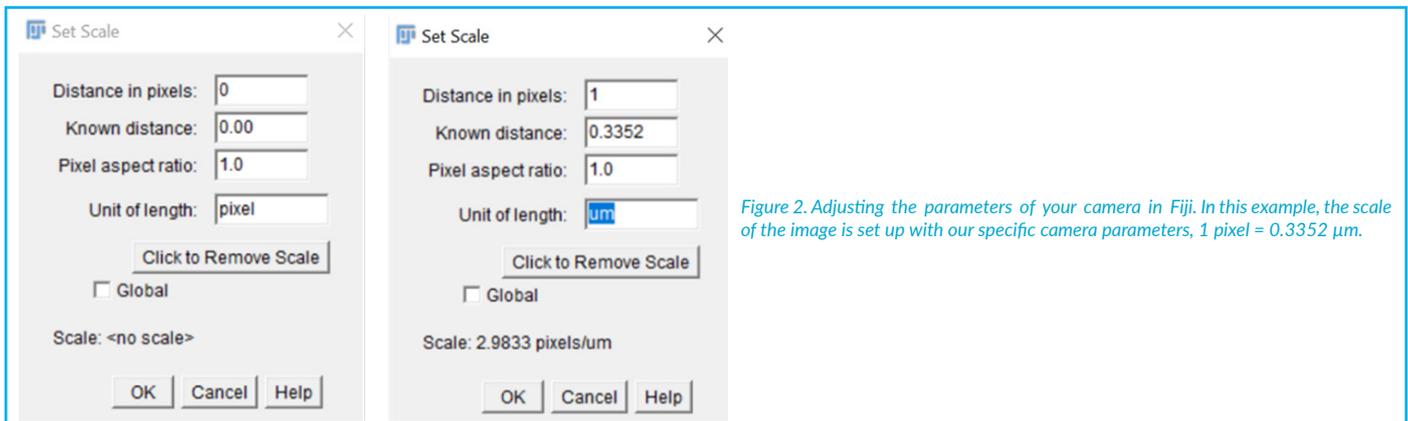


Figure 2. Adjusting the parameters of your camera in Fiji. In this example, the scale of the image is set up with our specific camera parameters, 1 pixel = 0.3352  $\mu\text{m}$ .

### 3. Adjust the brightness and contrast

- 3.1. Click on "Image"
- 3.2. Select "Adjust"
- 3.3. Select "Brightness/Contrast"
- 3.4. Adjust the parameters to obtain the best visualization, start with Auto (Figure 3)

**Note:** This step is used for enhancing the neurite visualization in Channel 2.

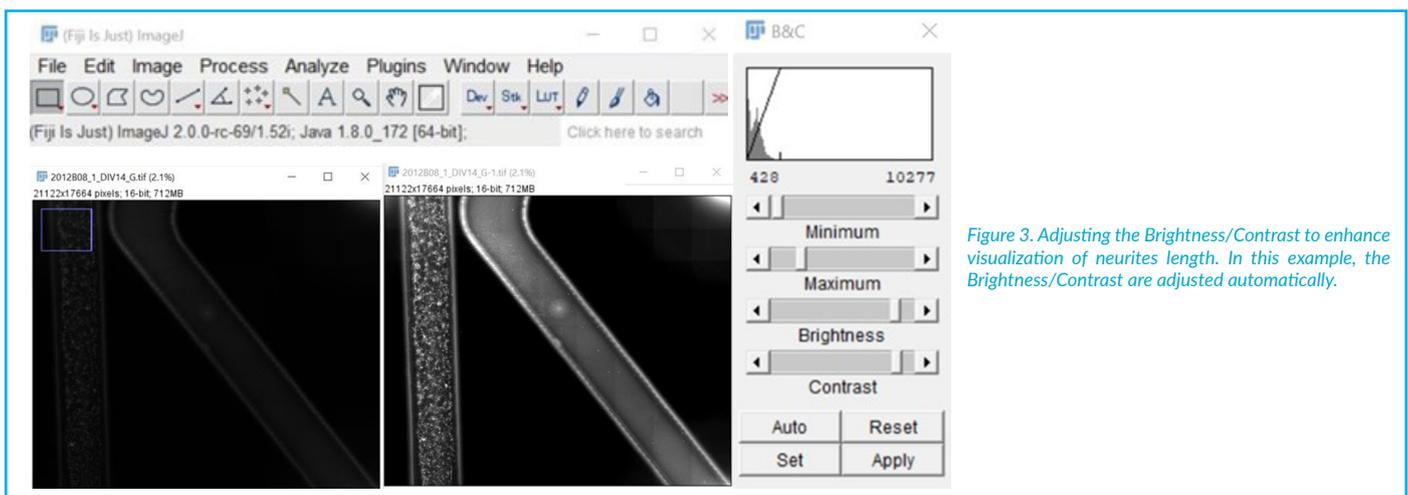


Figure 3. Adjusting the Brightness/Contrast to enhance visualization of neurites length. In this example, the Brightness/Contrast are adjusted automatically.



#### 4. Adjust the analysis parameters

- 4.1. Click on "Analyze"
- 4.2. Select "Set Measurement": a new window appears
- 4.3. Tick parameters to be measured
- 4.4. Click on "OK"

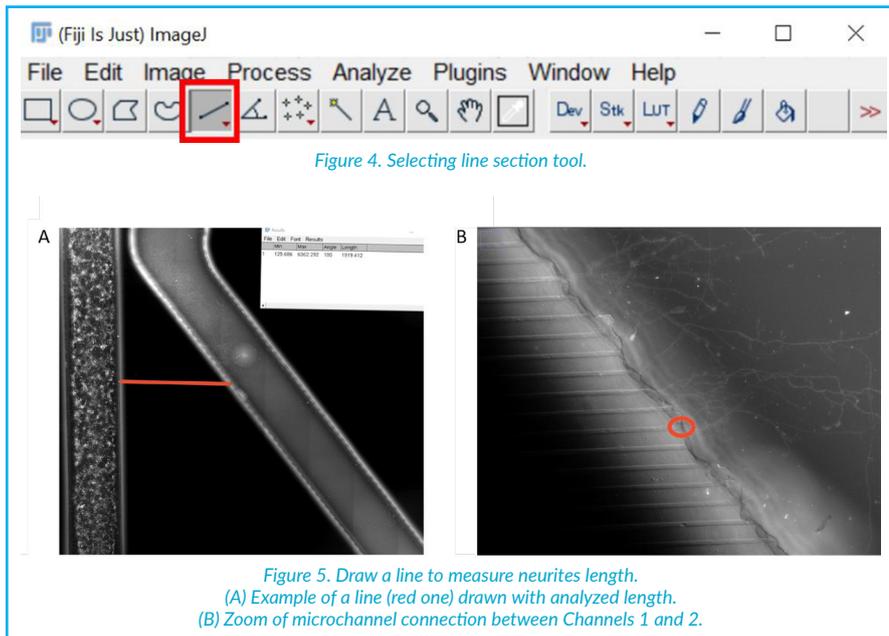
**Note:** By default, Fiji software analyzes the length and angle.

#### 5. Measure the length of the neurites

- 5.1. Click on "Line section tool" (Figure 4)
- 5.2. Draw a line on the microchannel between Channels 1 and 2 to measure the length
- 5.3. Click on "Analyze"
- 5.4. Select "Measure": the "Results" window appears

**Note 1:** To verify that the line was drawn correctly, angle measure parameter seen in "Results" window should show 0.

**Note 2:** If you want to compare neurites length between pictures, be careful to always draw your line at the intersection between microchannel and channel (Figure 5B).



#### 6. Copy raw data to save the measurement

- 6.1. On the "Fiji" window, click on "File"
- 6.2. Select "Save as".
- 6.3. Choose the picture format: Tiff/PNG/Jpeg
- 6.4. Select folder and name of the merged picture.

#### 7. Save the pictures

- 7.1. On the "Fiji" window, click on "File"
- 7.2. Select "Save as".

## Expected Results

Slice	Count	Total Area	Average Size	%Area	Mean	Perin
Picture of live cells	1287	171195.85404	133.01931	4.63917	255	44.2
Picture of dead cells	2672	71441.05337	26.73692	1.93596	255	18.7

Figure 7. Quantitative results of cells counting on live cells and dead cells pictures.

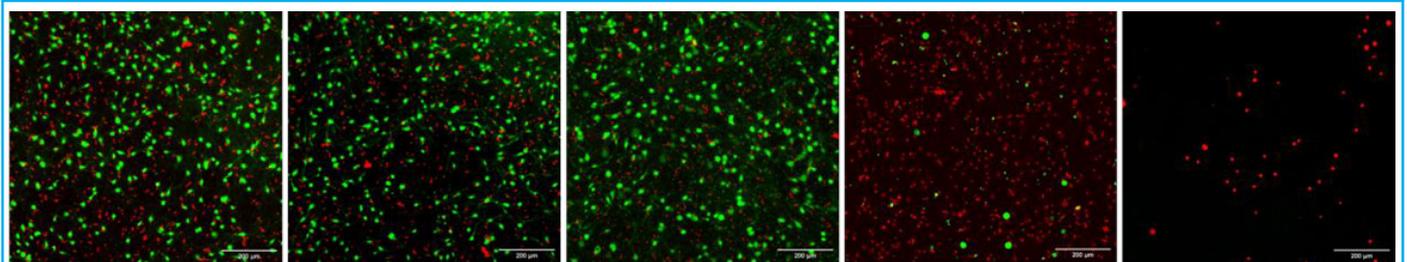


Figure 8. Fluorescent pictures of LDA assays according to different concentration of AβO exposure from 0 μM to 100 μM. Green fluorescent correspond to live cells and red fluorescence correspond to dead cells.

## TROUBLESHOOTING

Troubles	Solutions
Incoherence in length measurements of neurites OR measurements out of the expected range	<ul style="list-style-type: none"> <li>• Verify the parameters set up in the "Set up scale" step.</li> <li>• Update the distance in pixel with your camera acquisition parameters.</li> </ul>
Difficulty to visualize neurite in Channel 2	<ul style="list-style-type: none"> <li>• Crop a section and adjust the Brightness/Contrast.</li> </ul>
Difficulty to draw a straight line, as close as possible to microchannel	<ul style="list-style-type: none"> <li>• Draw your line with "line section tool" holding the shift key simultaneously.</li> </ul>
Difficulty to keep your angle at 0	<ul style="list-style-type: none"> <li>• Draw your line with "line section tool" holding the shift key simultaneously.</li> </ul>

Table 1. List of potential issues that can happen and proposed solutions.

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1. Maisonneuve, B. G. C., Vieira, J., Larramendy, F. & Honegger, T. Microchannel patterning strategies for in vitro structural connectivity modulation of neural networks. doi: <https://doi.org/10.1101/2021.03.05.434080>
2. Schindelin, J. et al. Fiji: An open-source platform for biological-image analysis. Nature Methods vol. 9 676–682 (2012).
3. Maisonneuve, B. et al., Neurite growth kinetics regulation through hydrostatic pressure in a novel triangle-shaped neurofluidic system. doi: <https://doi.org/10.1101/2021.03.23.436675>

## CONTACT



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