

# Beginner's guide to calculating image scale ( $\mu\text{m}/\text{pixel}$ ) using the openEyecam and adding a scale bar with ImageJ/Fiji

## Introduction

Calculating the scale of an image helps ensure that measurements made from it are accurate and allows you to add scale bars, which are essential for the presentation and interpretation of microscopy images.

## How to calculate Image Scale

To calculate image scale you only need to know: (i) the **total magnification** used to image your sample; (ii) the **pixel size** of the camera used to capture your image.

If you have used the openEyecam to image, note that it is fitted with the Basler CMOS colour camera (part number 108275) (<https://www.digikey.co.uk/en/products/detail/basler-inc/108275/13245661>) with a **pixel size of 2  $\mu\text{m}$** .

**Total magnification** depends on the objective lens's magnification and any additional magnification in the optical path. Always record this!

Total magnification = Objective magnification  $\times$  0.5 (C-mount adapter has a 0.5 $\times$  magnification)

For example, if you have used the 20 $\times$  objective to capture your image:

Total magnification = 20 $\times$   $\times$  0.5 = 10

You are now ready to calculate image scale!

**The general formula is:**

$$\text{Pixel size in sample } (\mu\text{m}/\text{pixel}) = \frac{\text{camera pixel size } (\mu\text{m}/\text{pixel})}{\text{Total magnification}}$$

Following on with the example above, if you have used a 20X objective to capture an image on the openEyeCam:

$$\begin{aligned}\text{Pixel size in sample } \left( \frac{\mu\text{m}}{\text{pixel}} \right) &= \frac{2\mu\text{m}}{10} \\ &= 0.2 \mu\text{m}/\text{pixel}\end{aligned}$$

$$\underline{\underline{1 \text{ pixel} = 0.2 \mu\text{m}}}$$

You can use the image scale calculated above to generate a scale bar.

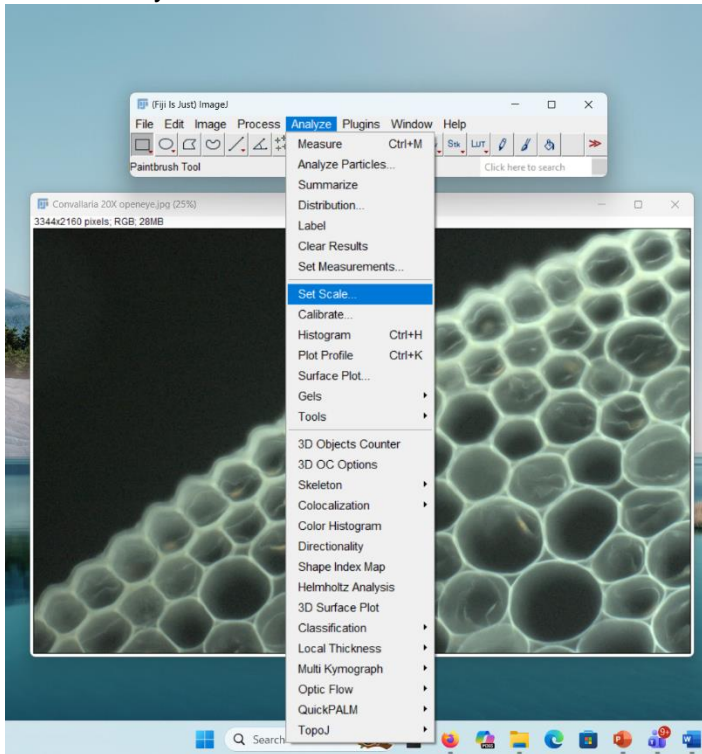
## How to set scale and add a scale bar in Fiji

### Step 1 – Open the Image

1. Launch Fiji.
2. Open the image of interest. Click File → Open. Select your image file. Alternatively, you can drag the saved image file into the grey ImageJ tool bar.

### Step 2 – Set the Image Scale

1. Select Analyze → Set scale



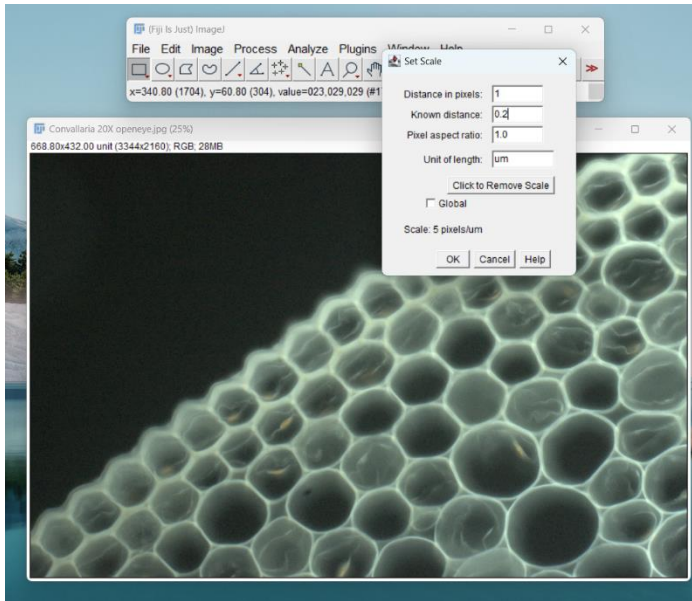
2. Enter the scale you calculated above. In this example we used a 20× objective on the openEyecam, so 1 pixel is equal to 0.2  $\mu\text{m}$  (this will depend on the objective you used to generate the image as described above).

Distance in pixels = 1

Known distance = your calculated value (e.g., 0.2)

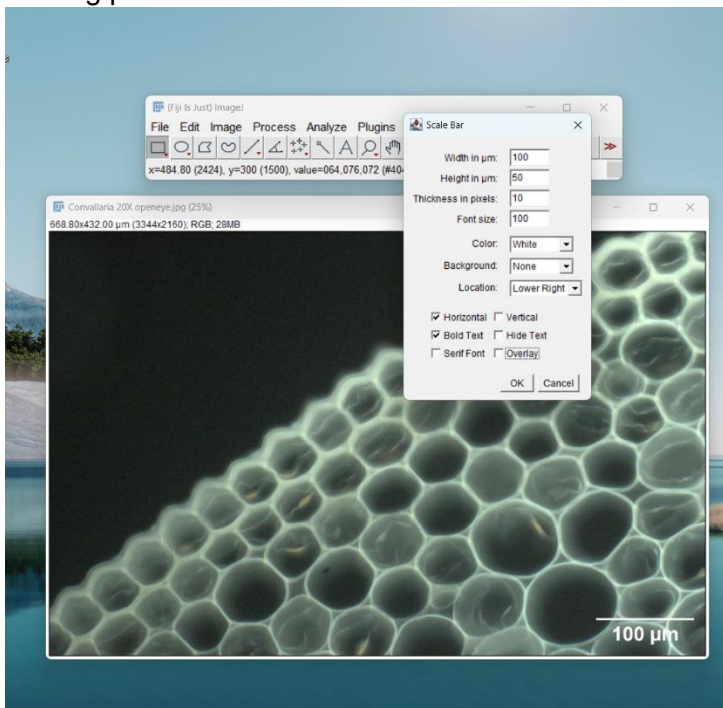
Unit =  $\mu\text{m}$

Click OK.



### Step 3 – Add the scale bar

1. Select Analyze → Tools → Scale Bar
2. Choose appropriate scale bar settings for your image, see example below as a starting point.



The choice of scale bar settings depends on the image dimensions and the size of key features within the sample. The following settings can be used as general guidance:

- Width in  $\mu\text{m}$  (between 2 and 200)
- Width in  $\mu\text{m}$  (50)
- Thickness in pixels (10)
- Font size (100)

It is recommended to experiment with different combinations to determine the most appropriate scale bar for your specific sample and imaging conditions.

#### **Step 4 – Save your image**

1. **Select File** → Save As → remember to save as an appropriate file type, generally TIFF (for downstream analysis) data or PNG (for inclusion in presentations or reports).