

SCALE AND MEASUREMENTS

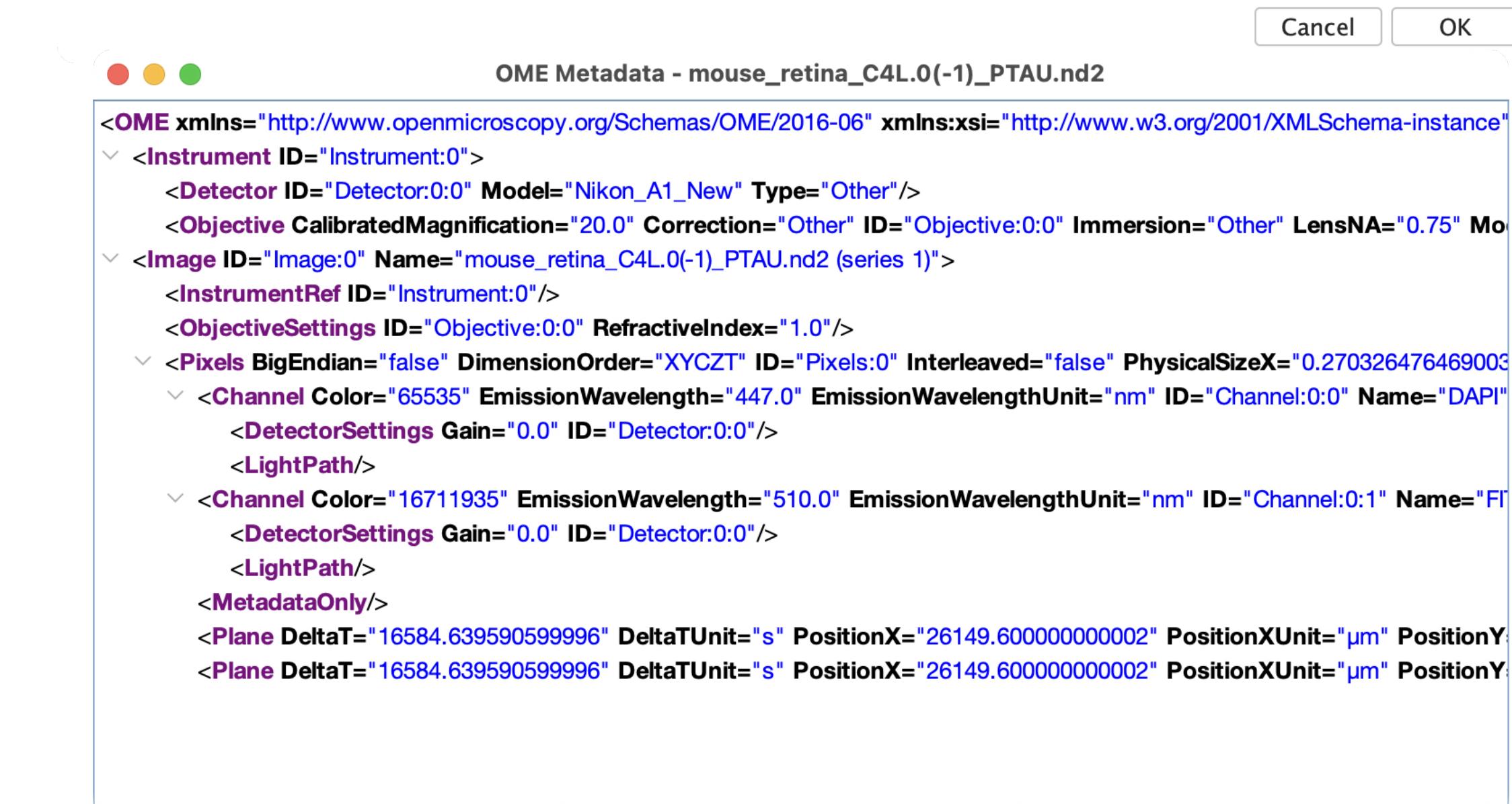
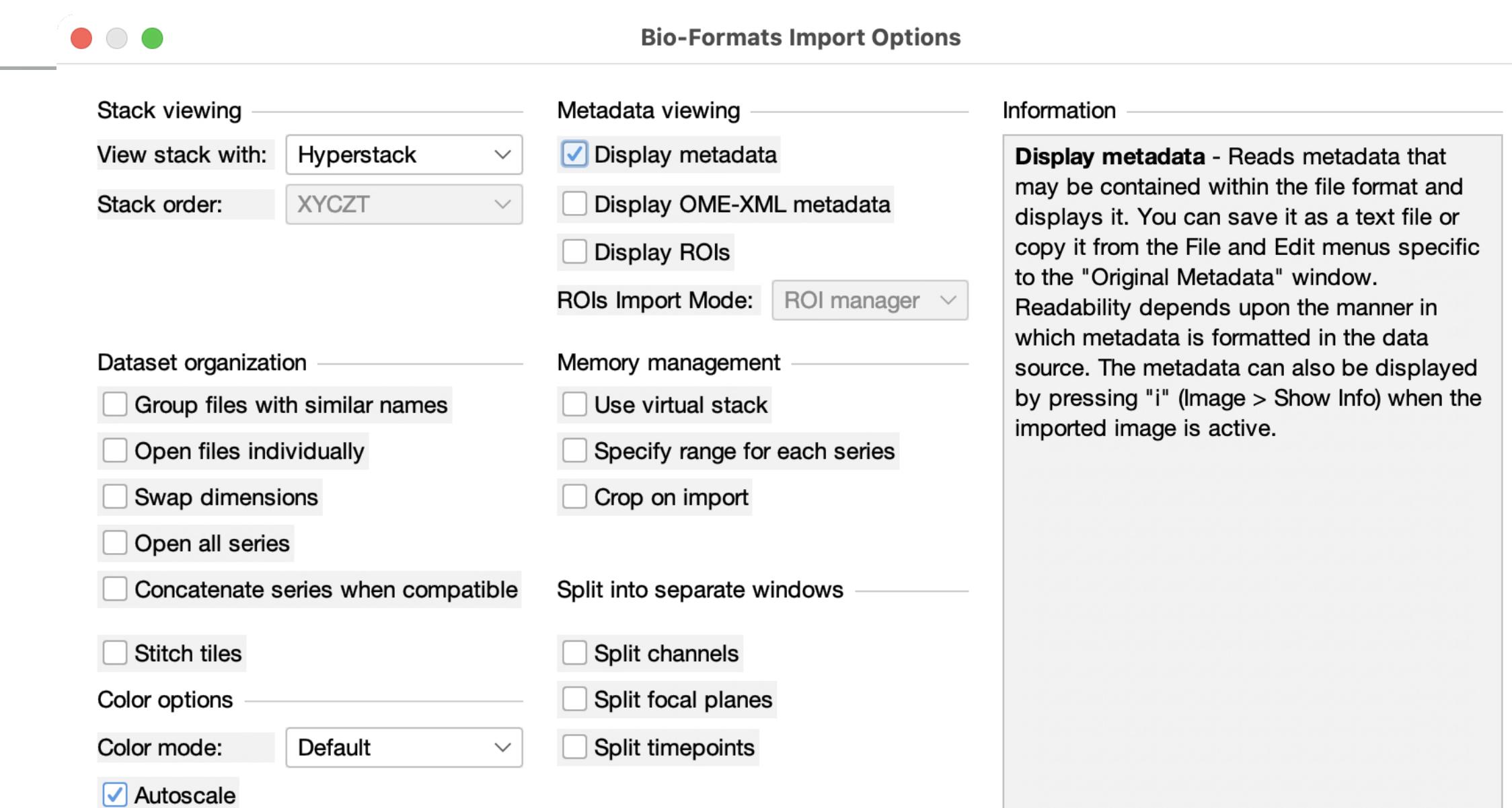
FJU

BIOFORMATS TOOLBOX

2 CHANNEL IMAGE

CONFOCAL IMAGE

- ▶ Open "mouse_retina_C4L.0(-1)_PTAU.nd2"
- ▶ Use the Indicated Settings in the Bio-Formats Import Options
- ▶ Review OME Metadata
- ▶ Review image in Hyperstack

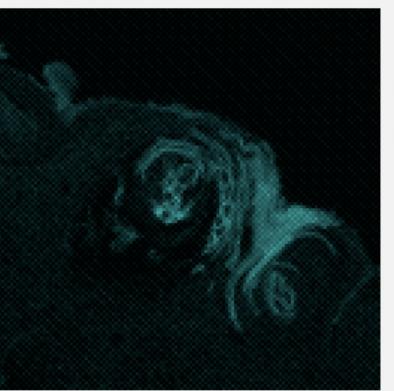


2 CHANNEL IMAGE

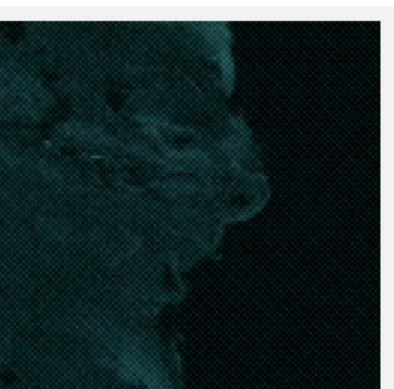
CONFOCAL IMAGE

- ▶ Open “confetti_mice.lif” using the BioFormats importer
- ▶ Now you get a series pop-up window
 - ▶ Notice Series 1 is called Series 1
 - ▶ Review OME Metadata
 - ▶ Review the image in Hyperstack

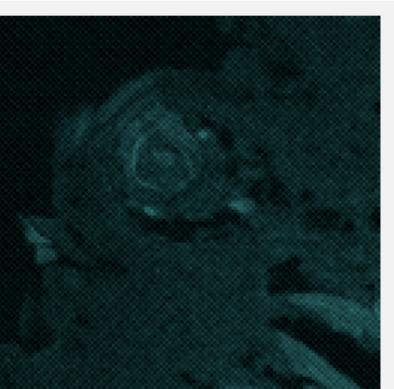
Series 1: Best overall: 512 x 512; 70 planes (5C x 14Z)



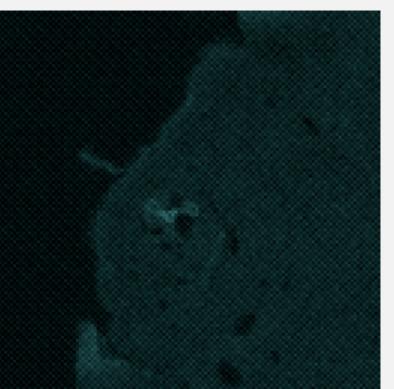
Series 2: easy to count cells 1: 512 x 512; 65 planes (5C x 13Z)



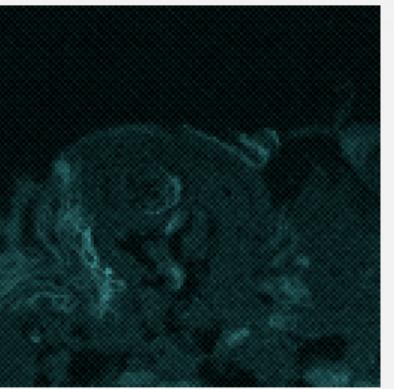
Series 3: hard to count cells 1: 512 x 512; 60 planes (5C x 12Z)



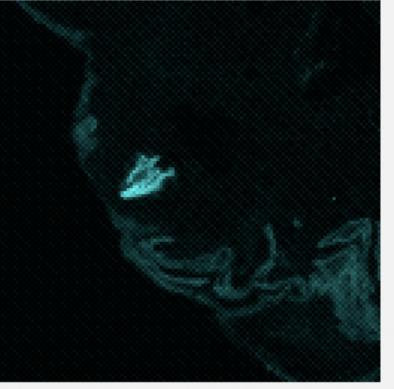
Series 4: hard to count 2: 512 x 512; 60 planes (5C x 12Z)



Series 5: worst overall: 512 x 512; 50 planes (5C x 10Z)



Series 6: easy to count 2: 512 x 512; 65 planes (5C x 13Z)



Cancel

OK

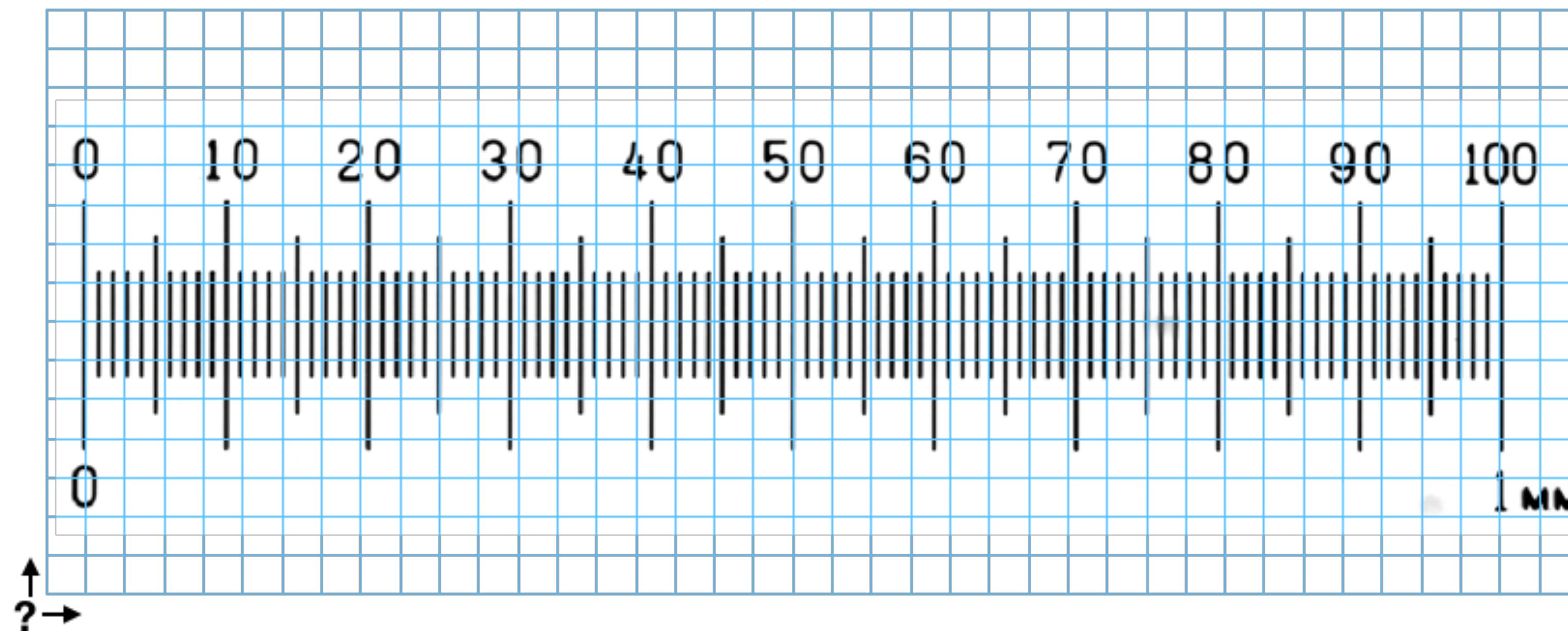
Select All

Deselect All

CALIBRATING A MICROSCOPE

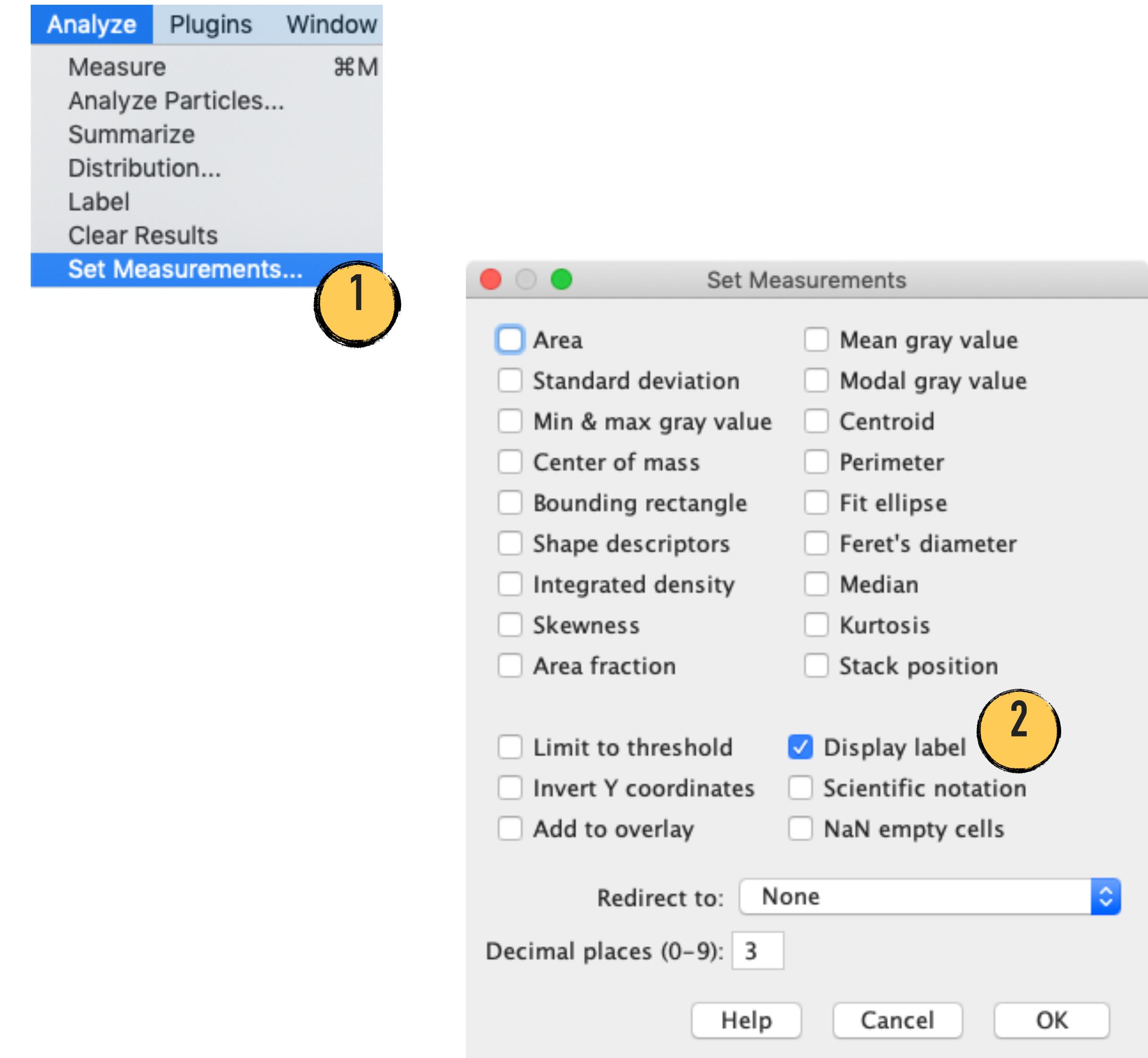
CALIBRATING MICROSCOPES

- ▶ In this section, we will learn how to calibrate a microscope by measuring a micrometer (tiny ruler)
- ▶ We will do this by measuring how many pixels cover the 1mm micrometer (1000 um) to come up with a um per pixel ratio that we can then use to measure objects in an image taken at the same magnification as the ruler
- ▶ For example, in this simplified illustration, the little blue squares represent the pixels in an image of a real micrometer. Note, the pixels depicted are not to scale. To calculate the size of one pixel in μm , we need to count the number of pixels that cover the 1000 μm in the micrometer.
- ▶ In this simplified example, roughly 36 pixels cover the ruler.
 - ▶ $1000/36 = \sim 26 \mu\text{m} \text{ per pixel}$



SET MEASUREMENTS

- ▶ Uncheck everything besides Display label
- ▶ The ImageJ website has [a description of all other measurements](#)



GETTING STARTED

OPEN AN IMAGE

1 File : Open...

▶ MATLAB Drive : Unit 2 : Data : Micrometers

- 2
- ▶ Captured 2xPlanApo.tif
 - ▶ Captured 4x Plan Flour.tif
 - ▶ Captured 10x Plan Fluor.tif
 - ▶ Captured 20x plan fluor.tif
 - ▶ Captured 40x Plan Fluor.tif



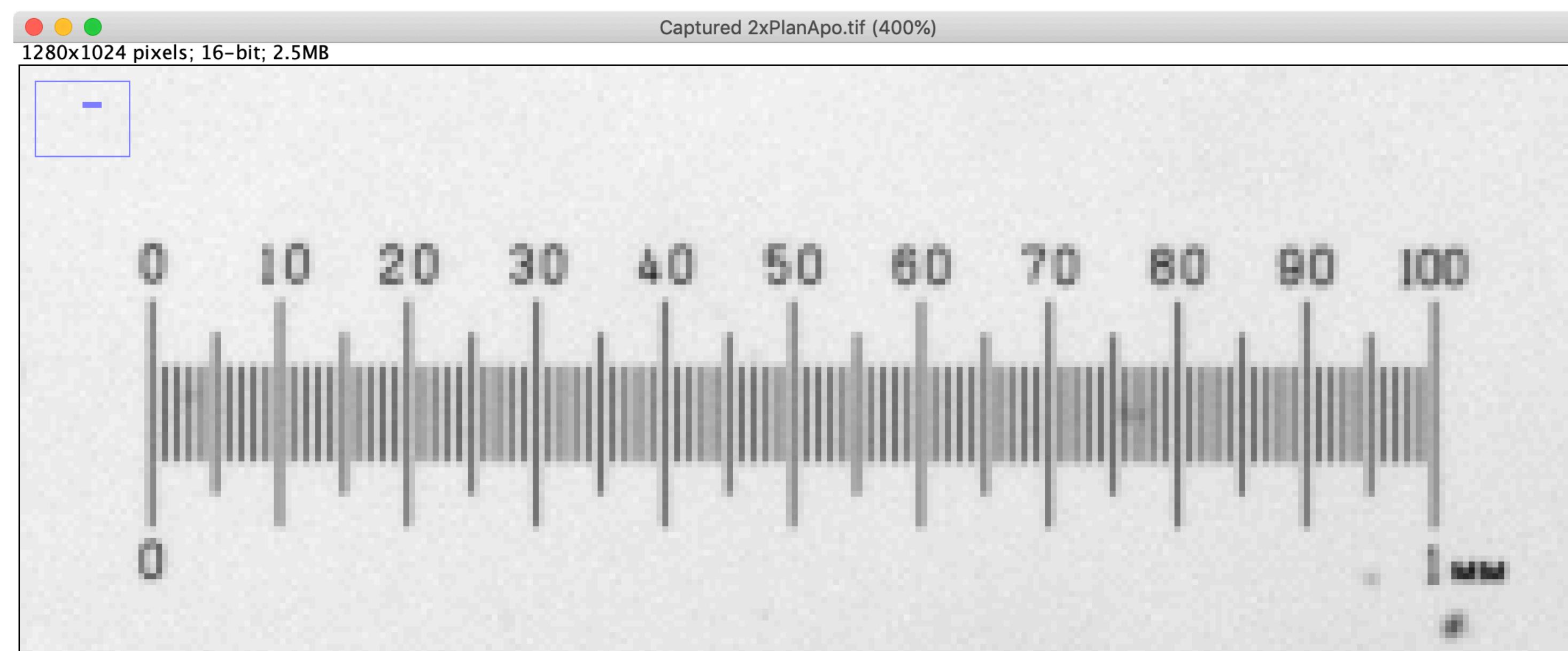
- 3
- ▶ Ruler = 1 mm
 - ▶ 1000 μ m
 - ▶ 1 tick = 10 μ m
 - ▶ Multiply each number by 10 to get the number in μ m

GETTING STARTED

ZOOM IN



- ▶ **1.** Select Magnifying glass
- ▶ **2.** Click on image to zoom in. Right-click to zoom out. (or type '+' or '-'
- ▶ **3.** Click on the hand icon () to pan the image around (so the ruler is centered)



GETTING STARTED

MEASURE

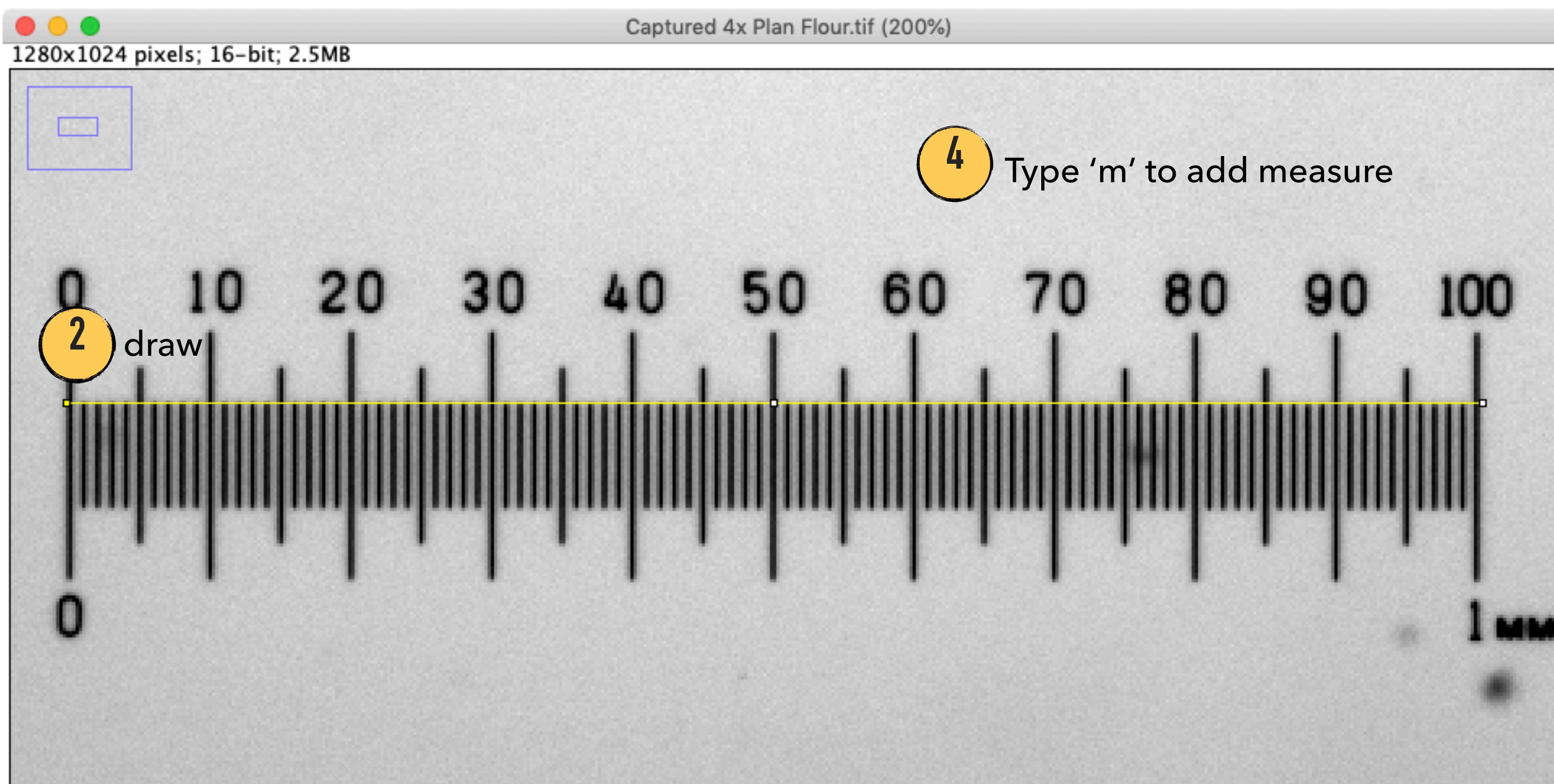
1

Select Line tool



3

Ensure an angle of 0.00



GETTING STARTED

MANAGE RESULTS

1

Fiji File Edit Font Results

Click on the Results window. Notice that the Fiji Menu changes

	Label	Angle	Length
1	Captured 2xPlanApo.tif	0	217.500
2	Captured 4x Plan Flour.tif	0	433.500
3	Captured 10x Plan Fluor.tif	0	438.000
4	Captured 20x plan fluor.tif	0	435.500
5	Captured 40x Plan Fluor.tif	0	865.000

2

FILE

- Save As... ⌘S
- Rename...
- Duplicate...
- Rename...
- Duplicate...

save as results.csv

RESULTS

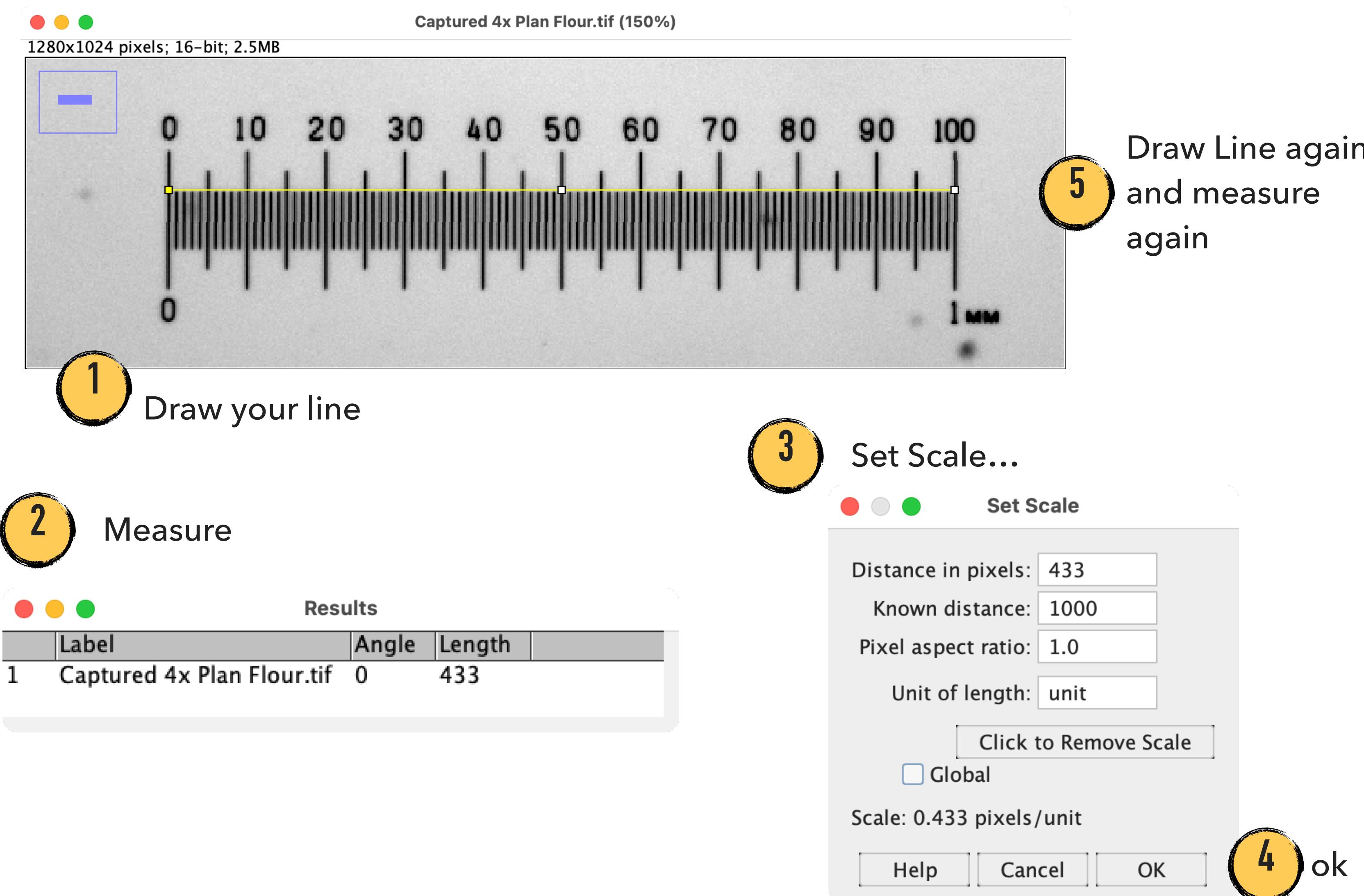
- Clear Results
- Summarize
- Distribution...
- Set Measurements...
- Sort...
- Plot...
- Options...

3

explore Result menu

HOW TO SET THE SCALE FOR THE 4X IMAGE

SET SCALE...



MEASURING STUFF

MEASUREMENTS

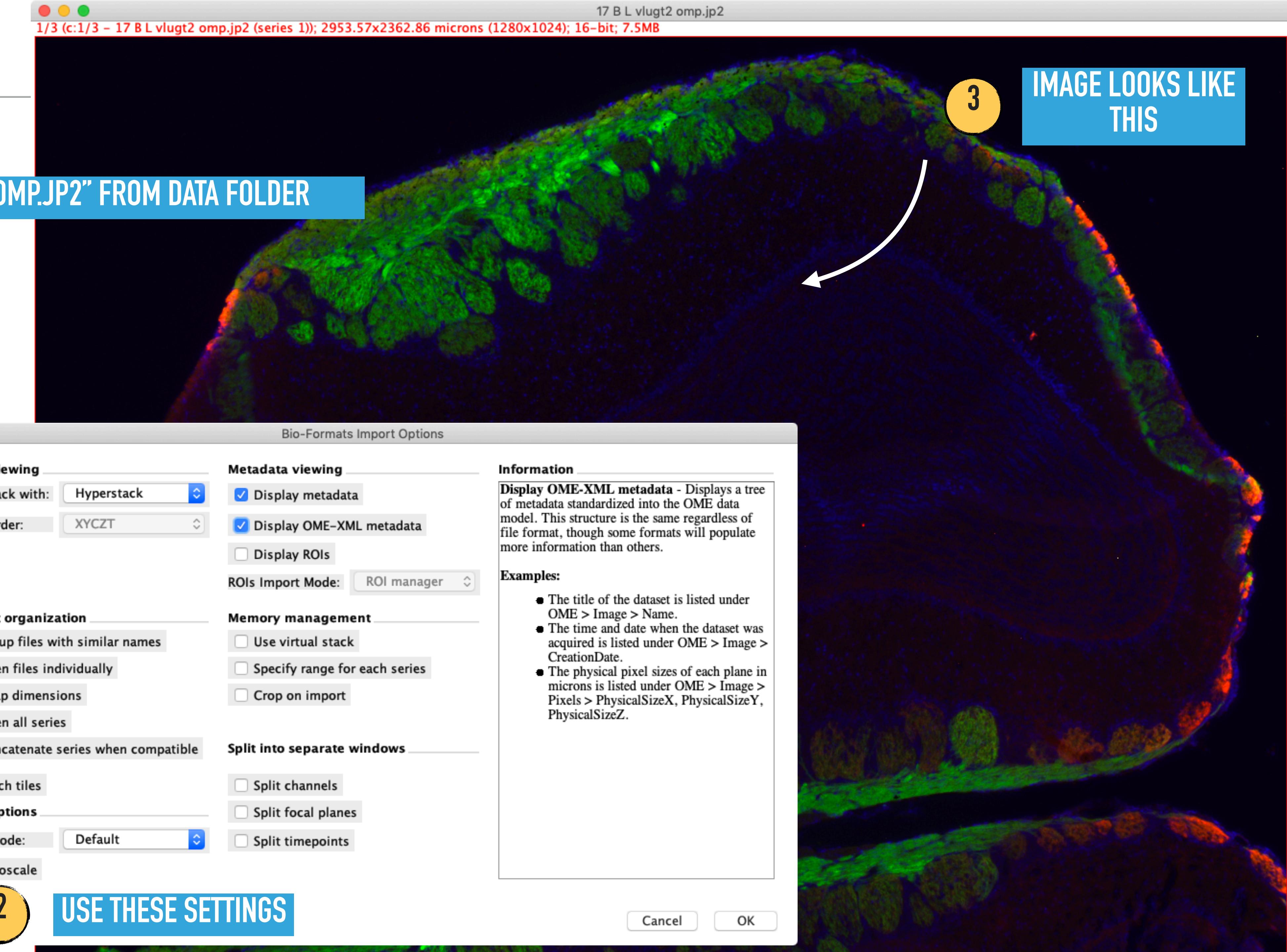
OPEN IMAGE

1

OPEN "17 B L VLUGT2 OMP.JP2" FROM DATA FOLDER

MATLAB Drive : Unit 2 : Data : Micrometers

- Captured 2xPlanApo.tif
- Captured 4x Plan Fluor.tif
- Captured 10x Plan Fluor.tif
- Captured 20x plan fluor.tif
- Captured 40x Plan Fluor.tif



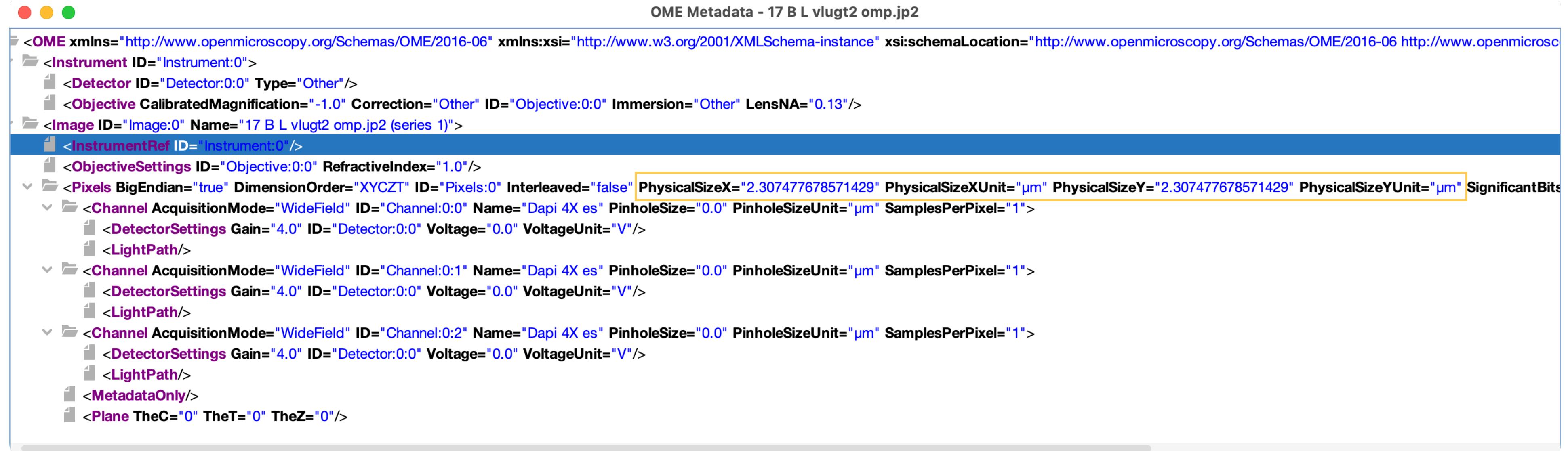
2

USE THESE SETTINGS

IMAGE LOOKS LIKE THIS

INSPECT METADATA

- ▶ Fiji recognizes the pixel dimensions
 - ▶ 2.3 μm x 2.3μm (4X image)



The screenshot shows the 'OME Metadata' viewer in Fiji. The title bar reads 'OME Metadata - 17 B L vlugt2 omp.jp2'. The XML code is displayed in a tree structure:

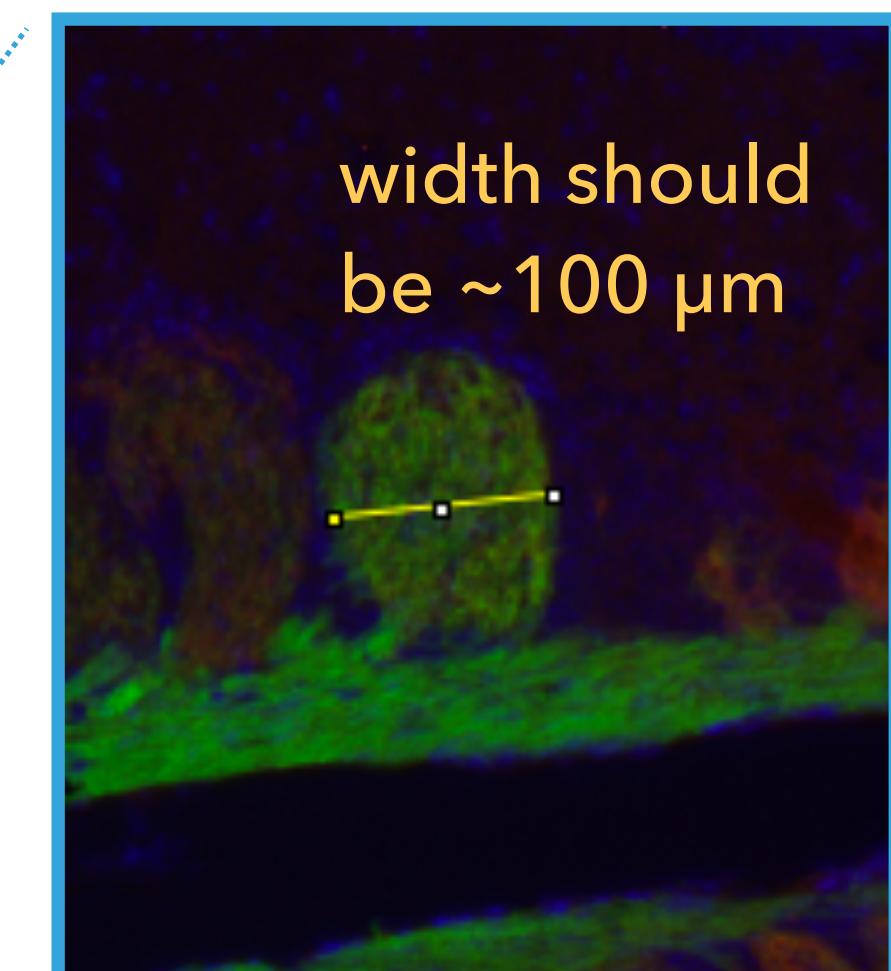
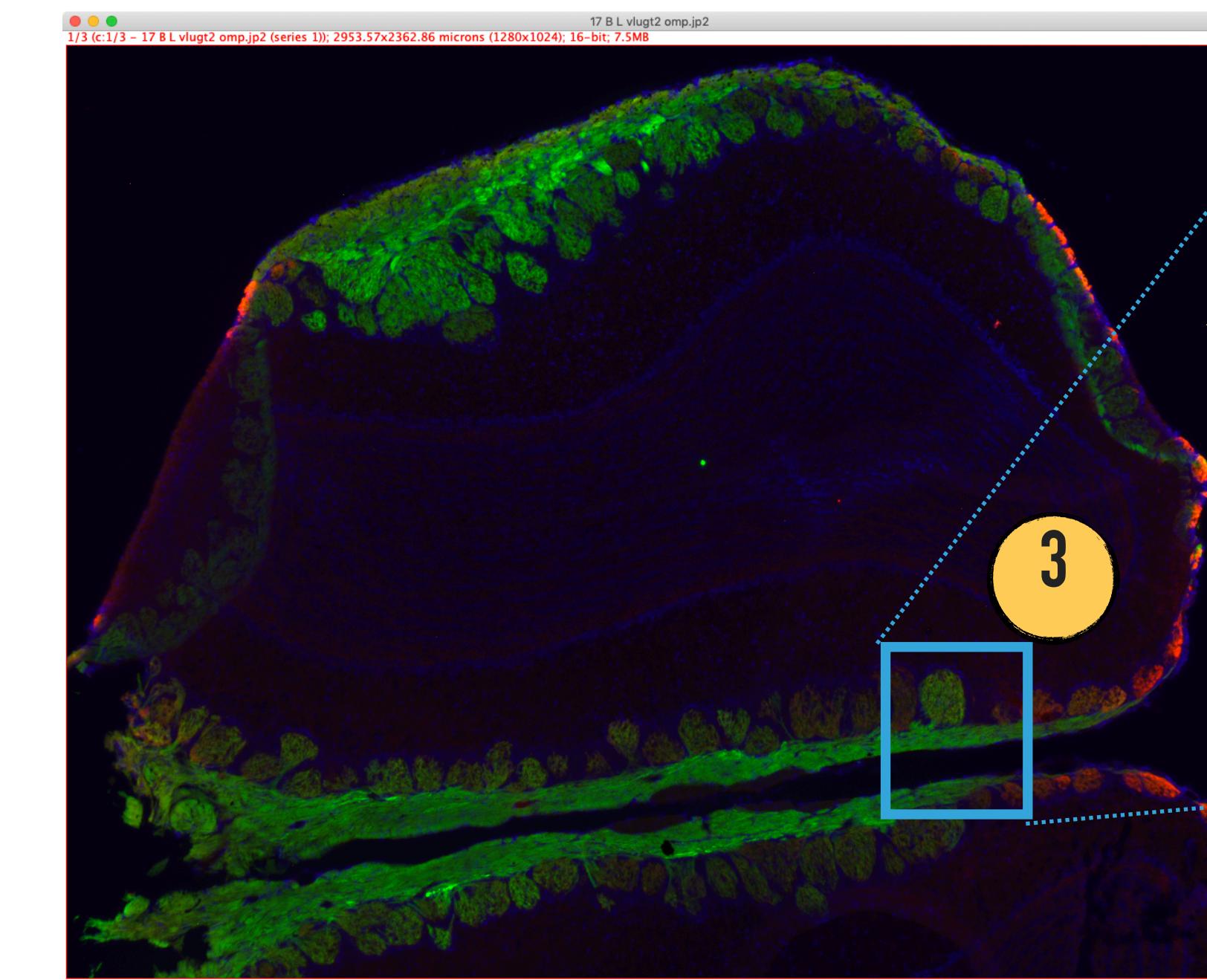
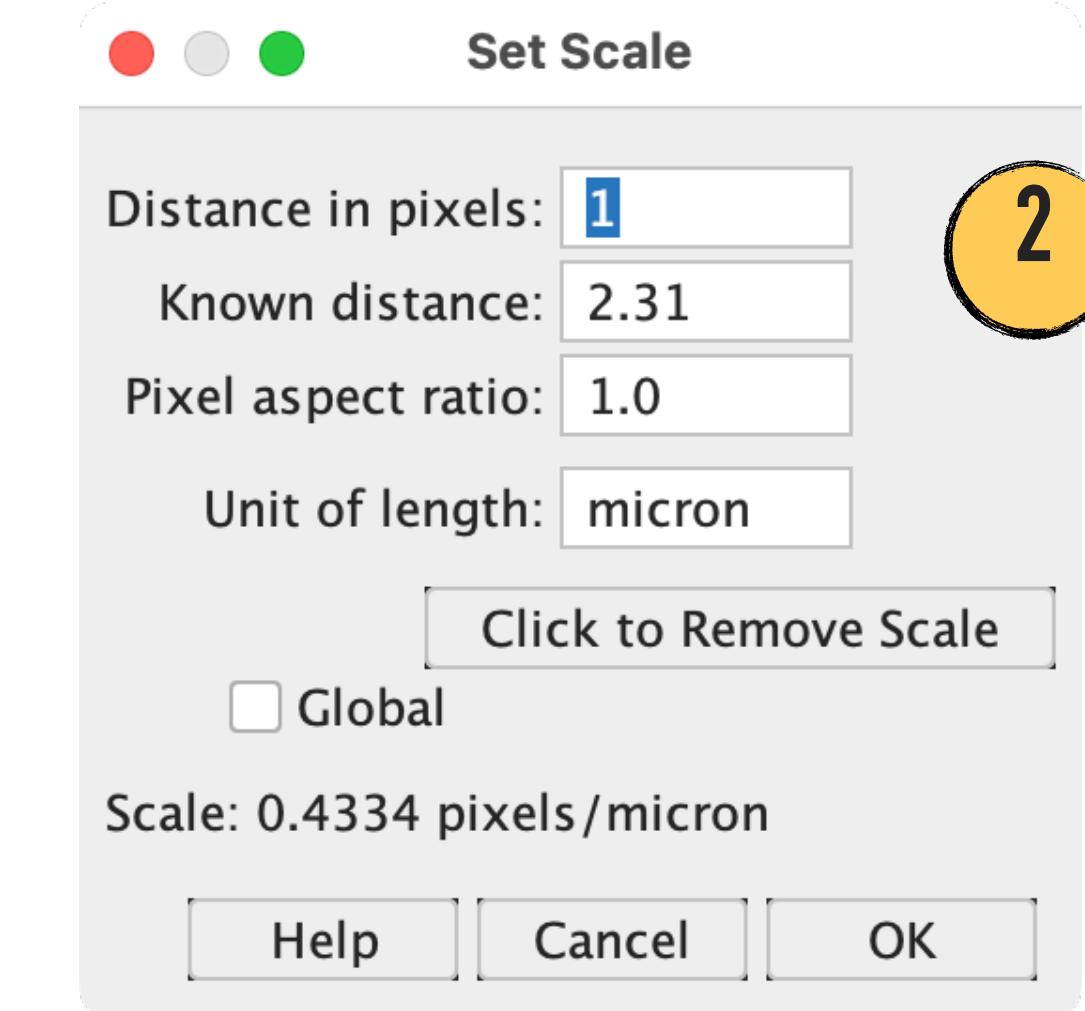
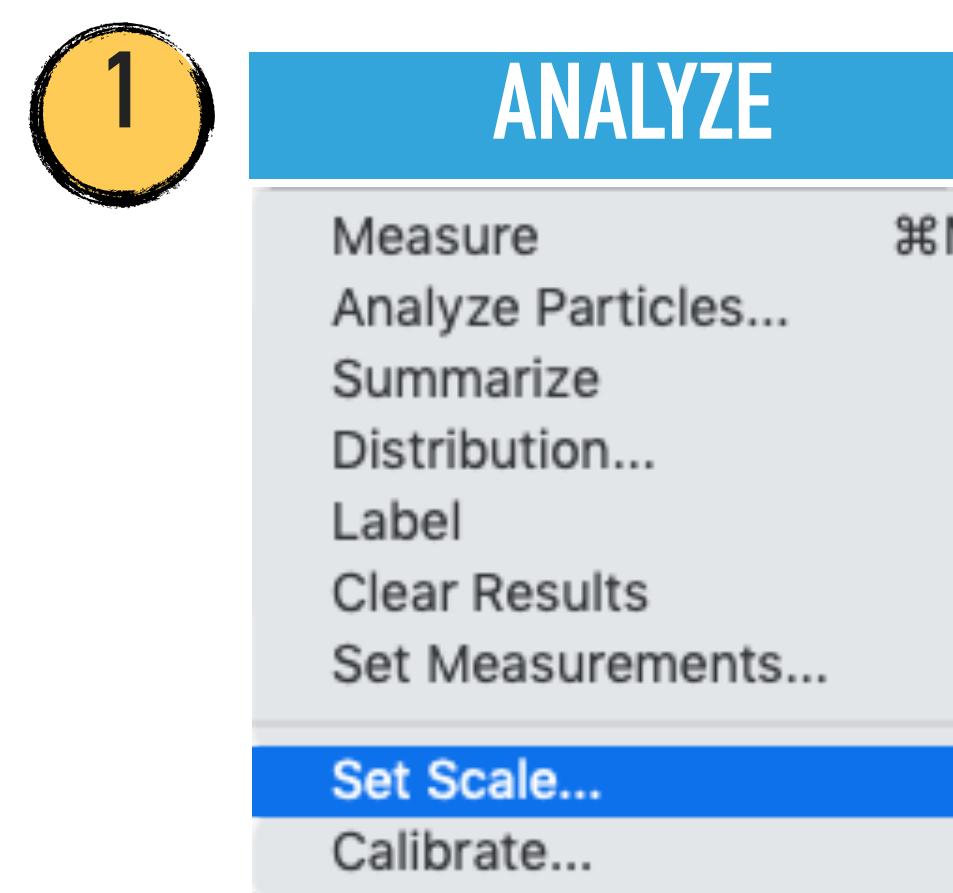
```
<OME xmlns="http://www.openmicroscopy.org/Schemas/OME/2016-06" xmlns:xsi="http://www.w3.org/2001/XMLSchema-instance" xsi:schemaLocation="http://www.openmicroscopy.org/Schemas/OME/2016-06 http://www.openmicroscopy.org/Schemas/OME/2016-06.xsd">
  <Instrument ID="Instrument:0">
    <Detector ID="Detector:0:0" Type="Other"/>
    <Objective CalibratedMagnification="-1.0" Correction="Other" ID="Objective:0:0" Immersion="Other" LensNA="0.13"/>
  <Image ID="Image:0" Name="17 B L vlugt2 omp.jp2 (series 1)">
    <InstrumentRef ID="Instrument:0"/>
    <ObjectiveSettings ID="Objective:0:0" RefractiveIndex="1.0"/>
    <Pixels BigEndian="true" DimensionOrder="XYCZT" ID="Pixels:0" Interleaved="false" PhysicalSizeX="2.307477678571429" PhysicalSizeXUnit="μm" PhysicalSizeY="2.307477678571429" PhysicalSizeYUnit="μm" SignificantBits="16" SamplesPerPixel="1" Z="1">
      <Channel AcquisitionMode="WideField" ID="Channel:0:0" Name="Dapi 4X es" PinholeSize="0.0" PinholeSizeUnit="μm" SamplesPerPixel="1">
        <DetectorSettings Gain="4.0" ID="Detector:0:0" Voltage="0.0" VoltageUnit="V"/>
        <LightPath/>
      <Channel AcquisitionMode="WideField" ID="Channel:0:1" Name="Dapi 4X es" PinholeSize="0.0" PinholeSizeUnit="μm" SamplesPerPixel="1">
        <DetectorSettings Gain="4.0" ID="Detector:0:0" Voltage="0.0" VoltageUnit="V"/>
        <LightPath/>
      <Channel AcquisitionMode="WideField" ID="Channel:0:2" Name="Dapi 4X es" PinholeSize="0.0" PinholeSizeUnit="μm" SamplesPerPixel="1">
        <DetectorSettings Gain="4.0" ID="Detector:0:0" Voltage="0.0" VoltageUnit="V"/>
        <LightPath/>
      <MetadataOnly/>
      <Plane TheC="0" TheT="0" TheZ="0"/>
    </Pixels>
  </Image>
</Instrument>
</OME>
```

The XML code describes the metadata for a 4X image, including instrument settings, objective, and pixel dimensions. The pixel dimensions are highlighted with a yellow box.

MEASUREMENTS

SET SCALE....

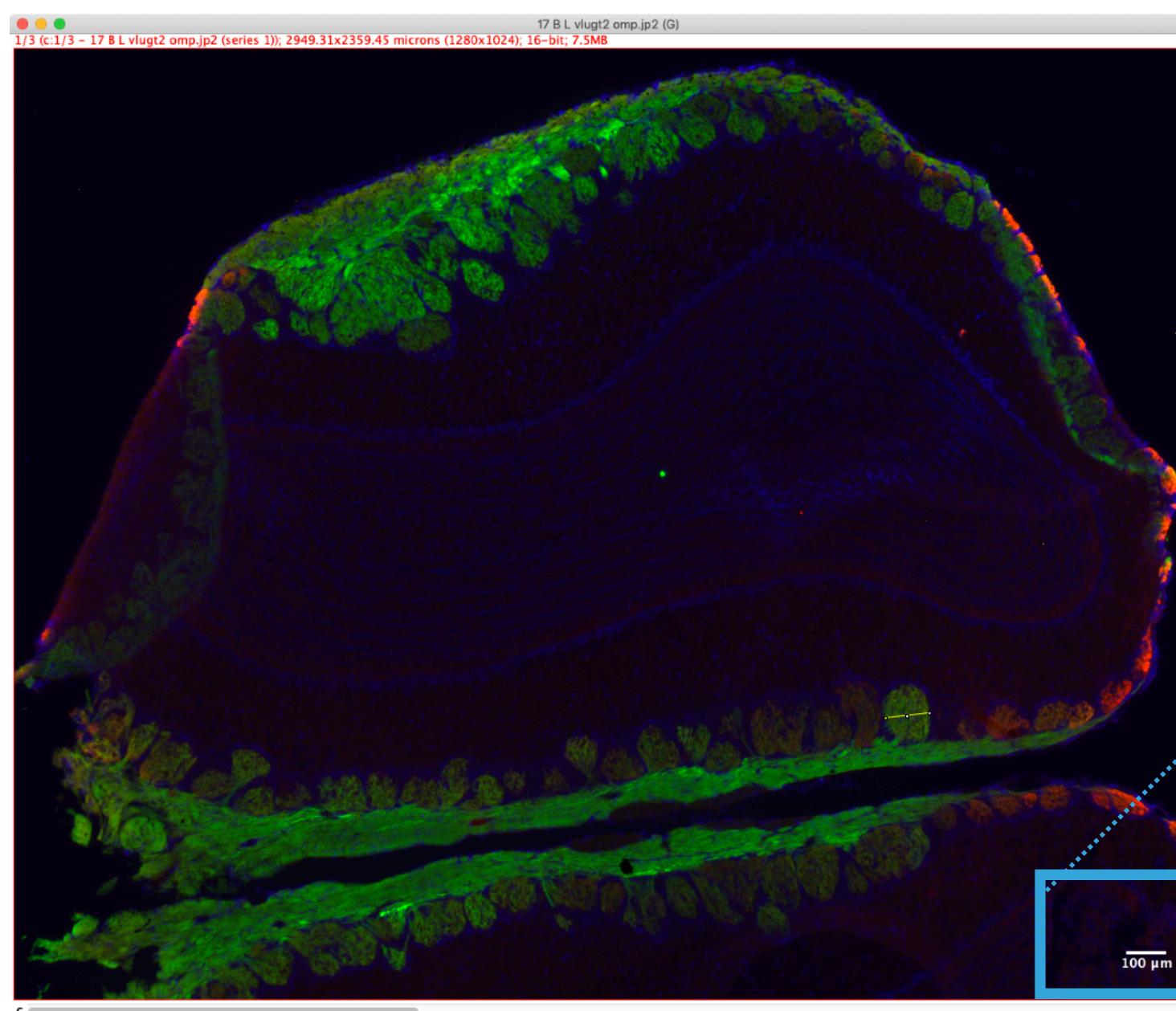
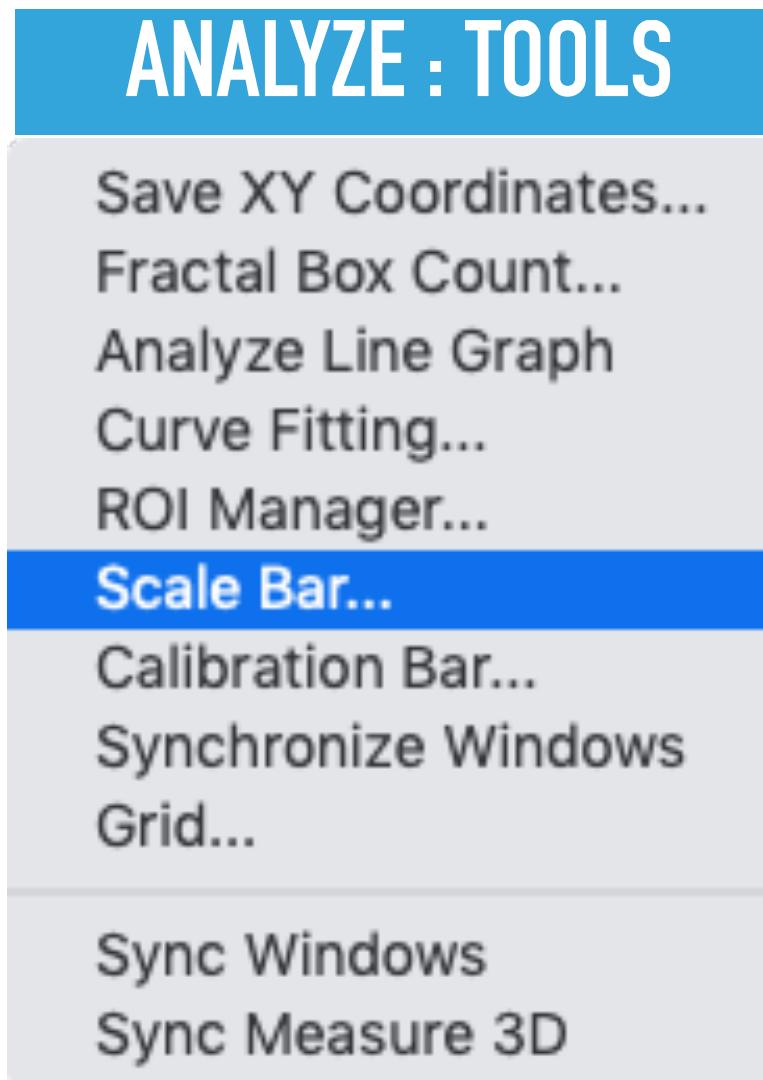
- ▶ The scale is already set
- ▶ Notice that Fiji reports pixels / micron, which is just the inverse of $\mu\text{m}/\text{px}$
- ▶ $1 / 2.31 = 0.43$



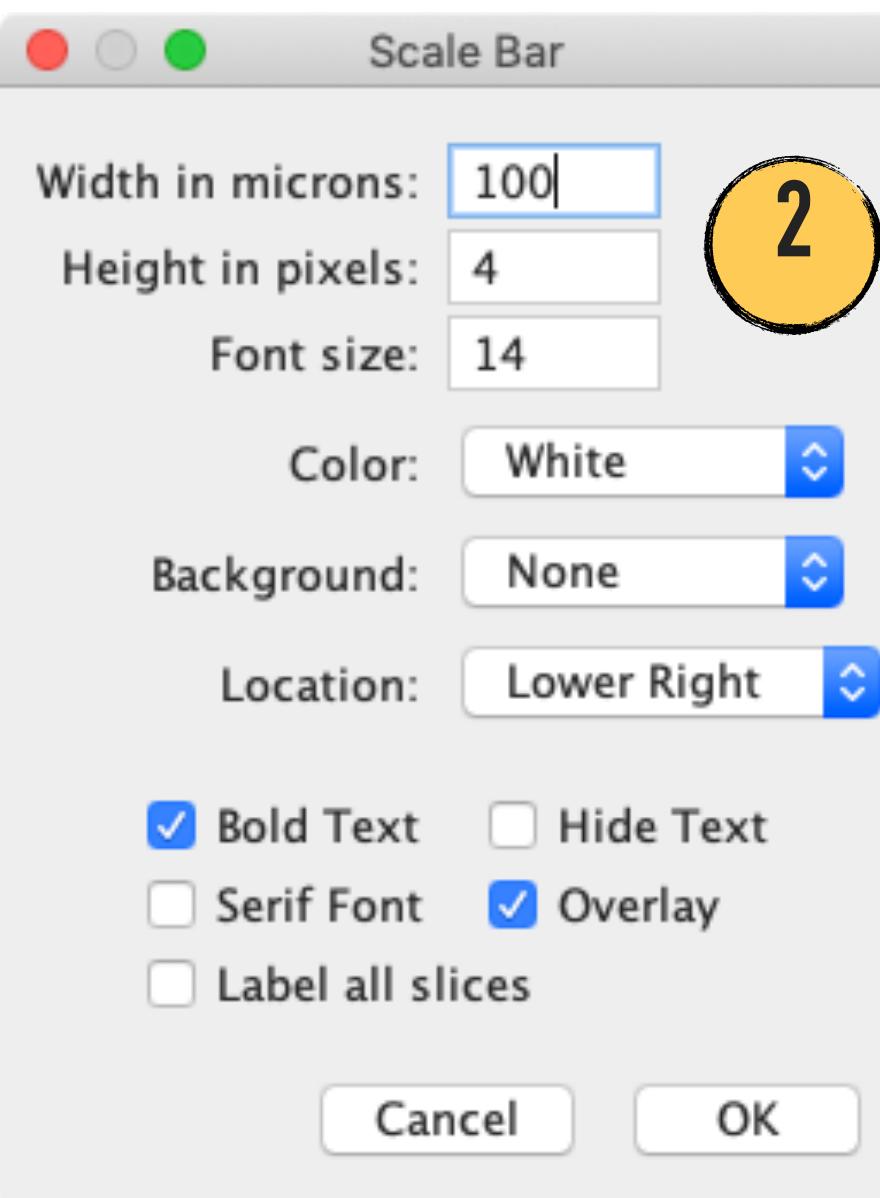
MEASUREMENTS

SCALE BAR . . .

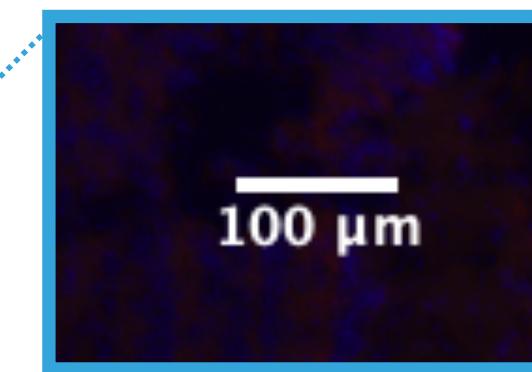
1



2



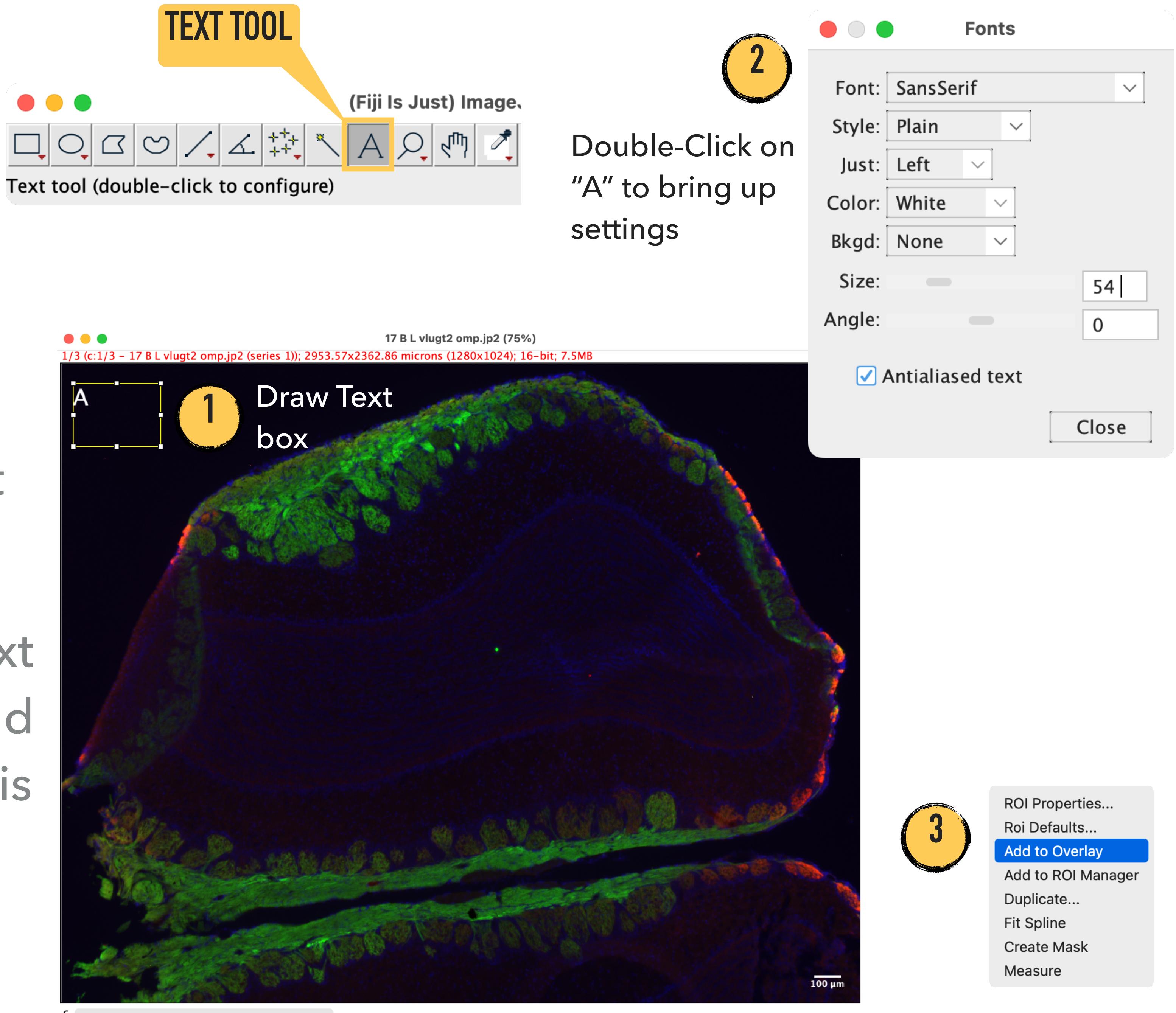
3



► Enjoy
scale bar

TEXT TOOL ADDS TEXT

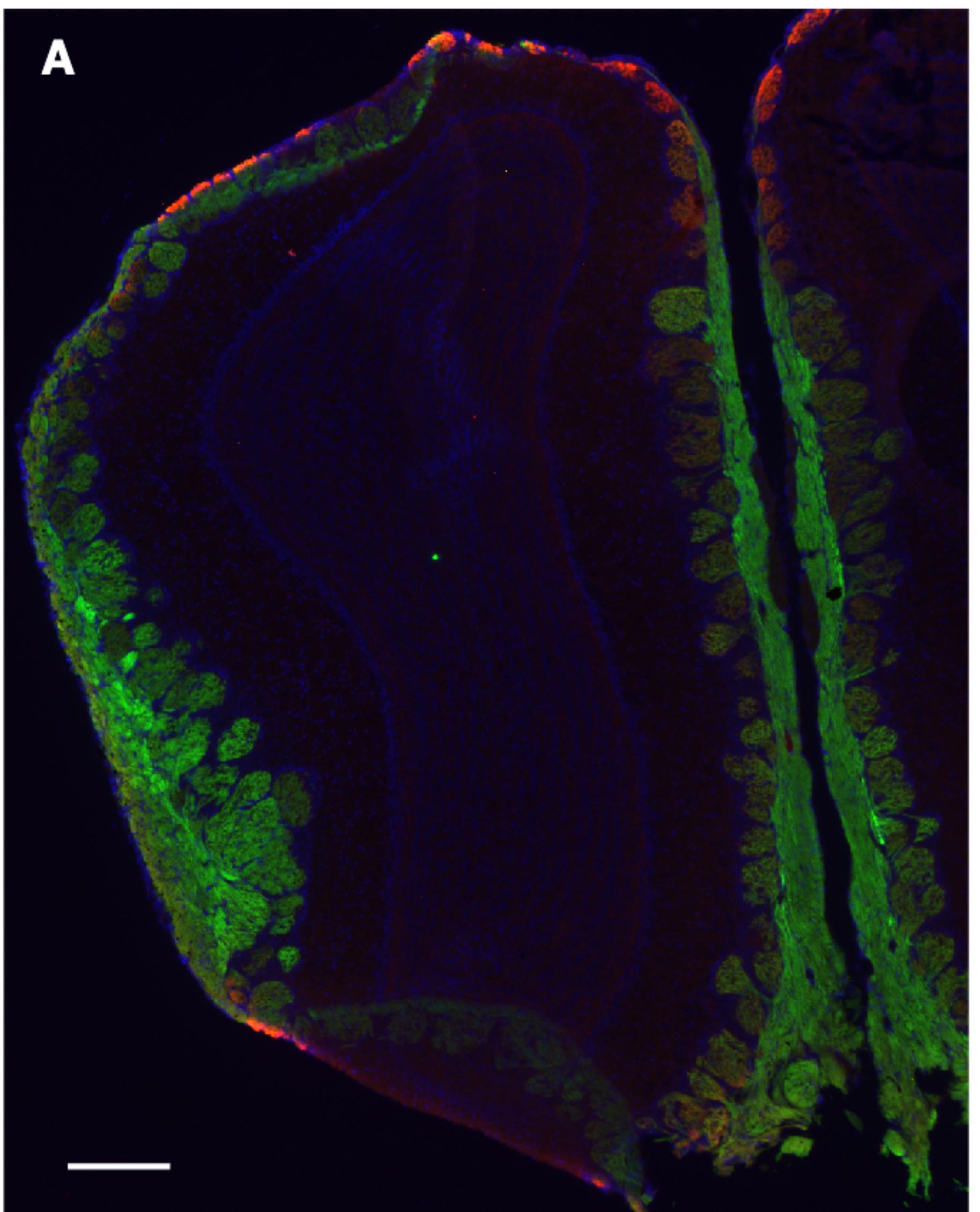
- ▶ After adding text, you can move the text box around.
- ▶ Be careful, if you click anywhere else besides directly on the text, the text will disappear.
- ▶ Once you like where the text is, right-click on the text and select "Add to Overlay". This will keep the text in the image.



MEASURING STUFF IN MATLAB

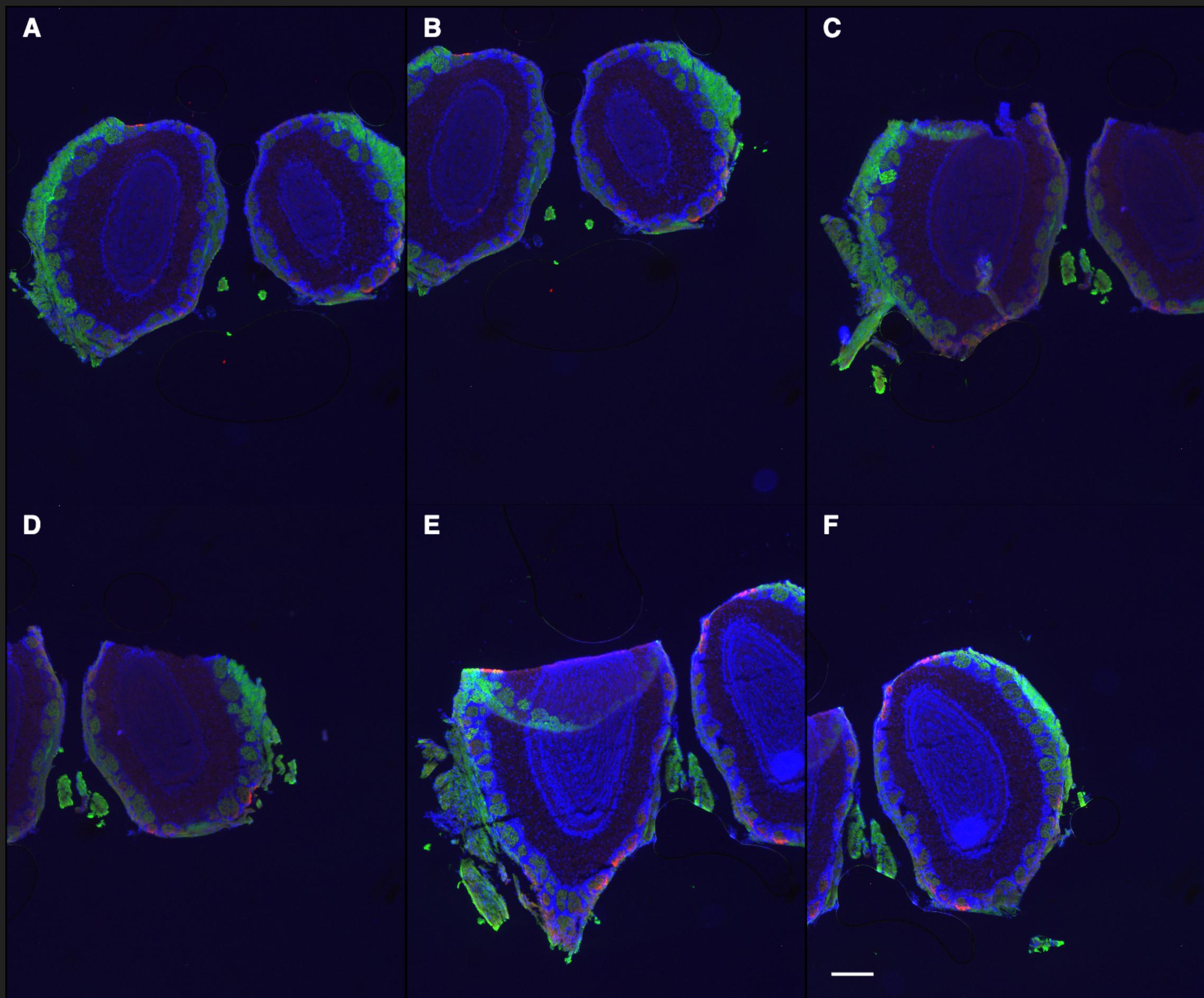
MATLAB

- ▶ Open "17 B L vlugt2 omp.jp2"
- ▶ Correct bit-depth to display the image properly
- ▶ Rotate Image 90° using imrotate
- ▶ Display the image
- ▶ Add a 250 µm scalebar using the mmAddScaleBar function
 - ▶ `mmAddScaleBar(gca,250,2.3,'w')`
 - ▶ Drag the blue line to the bottom left-corner (without resizing the line)
 - ▶ Double-click on line when done
- ▶ Add a Letter to the top left corner



CHALLENGE

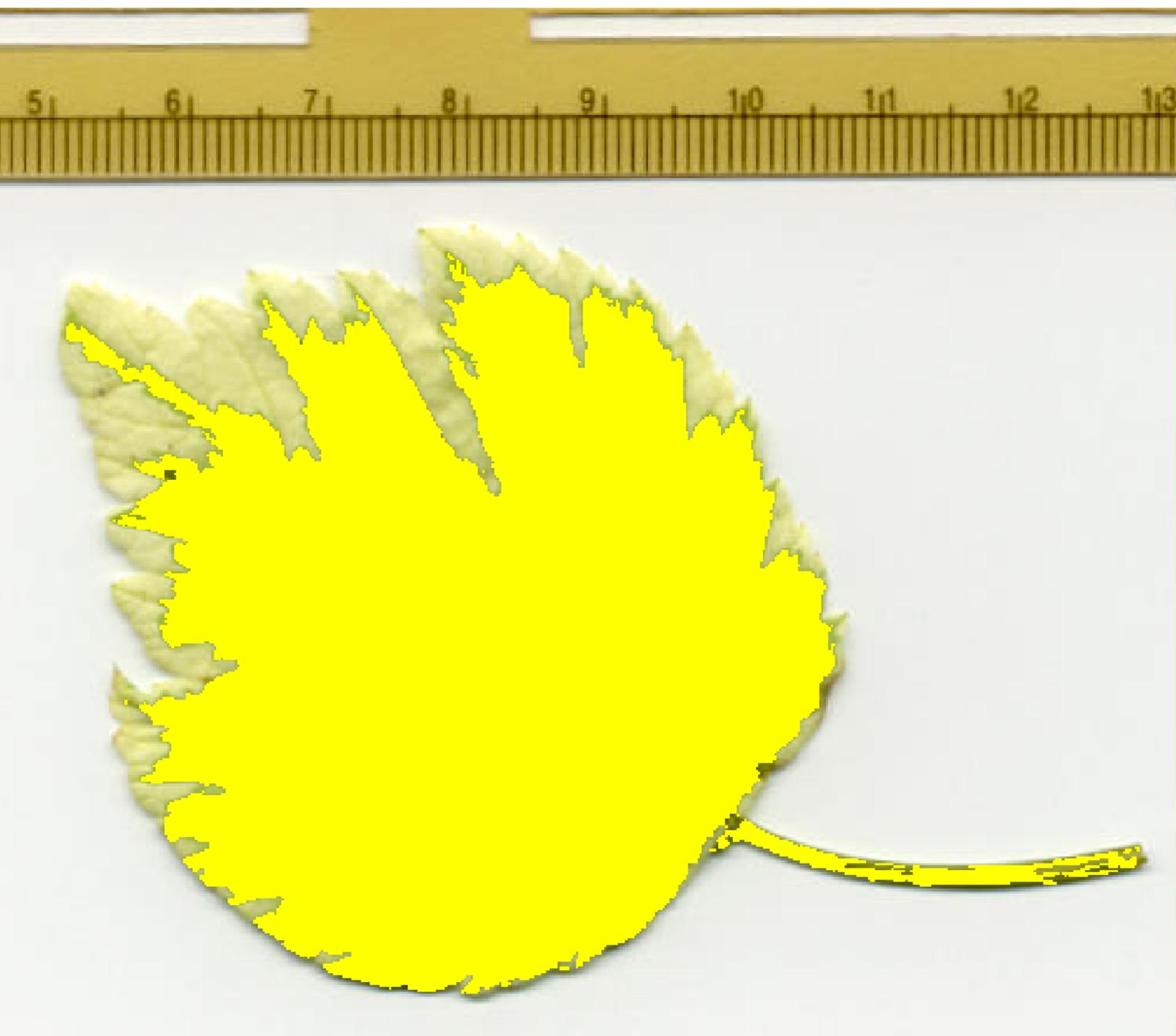
- ▶ How would you plot the first 6 images from the MOB_VGLUT2 folder
- ▶ Notes
 - ▶ Letters in each corner of the image
 - ▶ Scale bar only added to last image



COLOR SEGMENTATION

SEGMENT THE LEAF

- ▶ Open “leaf.png” in MtMdata unit2 folder
- ▶ Segment the Green Part of the Leaf
- ▶ Segment the Whole Leaf
- ▶ Calculate the size of a pixel (in/pixel) - use imageViewer
- ▶ Calculate the area of the green in in^2



COLOR SEGMENTATION

SEGMENT THE WHOLE LEAF

- ▶ Segment all the leaf
- ▶ Calculate the ratio of Green to Whole Leaf

