# Things you need to know about figure-making for publication

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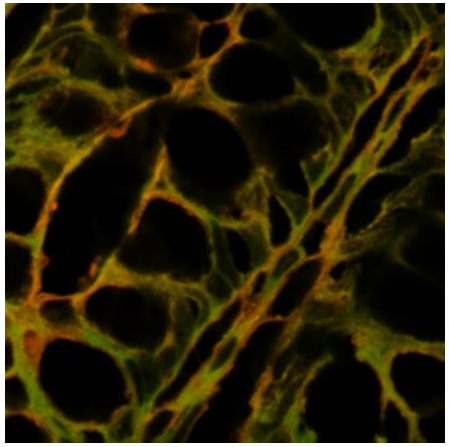
Collect data → Process → Analyse → Figure → Publish



Imaris
3D/4D Visualization and Analysis Software

Huygens Software
by Scientific Volume Imaging

Digital Imaging Solutions
iTEM
Solutions for TEM Applications


Hong Yu, Westmead Imaging Facility 11 Sept 2023

# Outlines

- Basic c
- What m
- How to
- How t

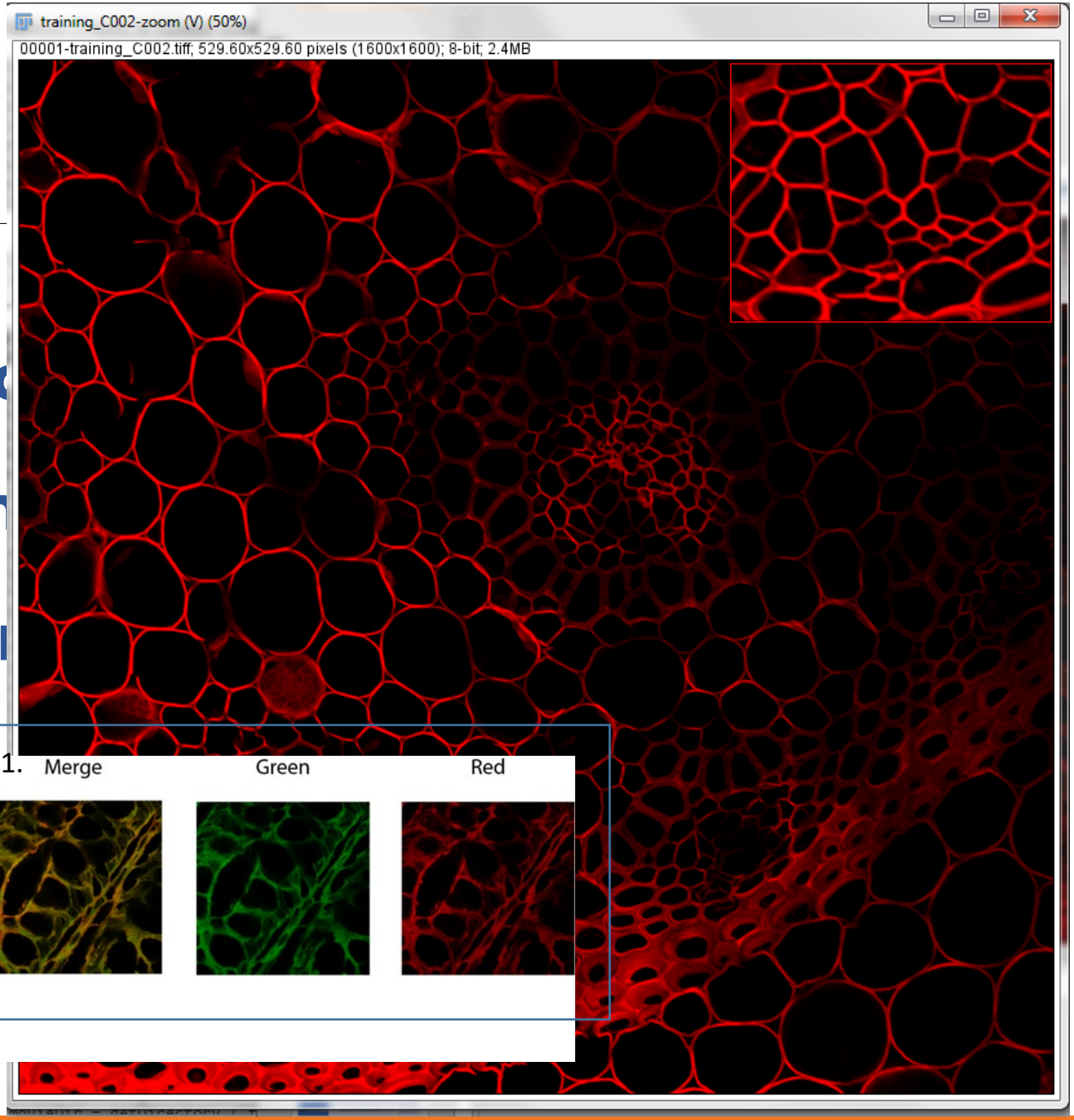



**Figure** 1.

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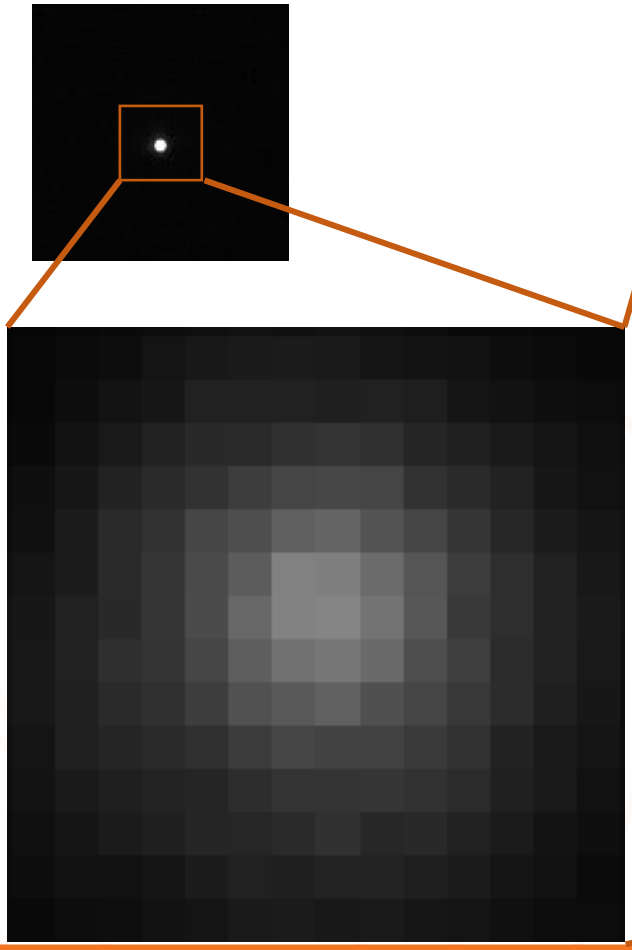
# What is a digital microscope image?

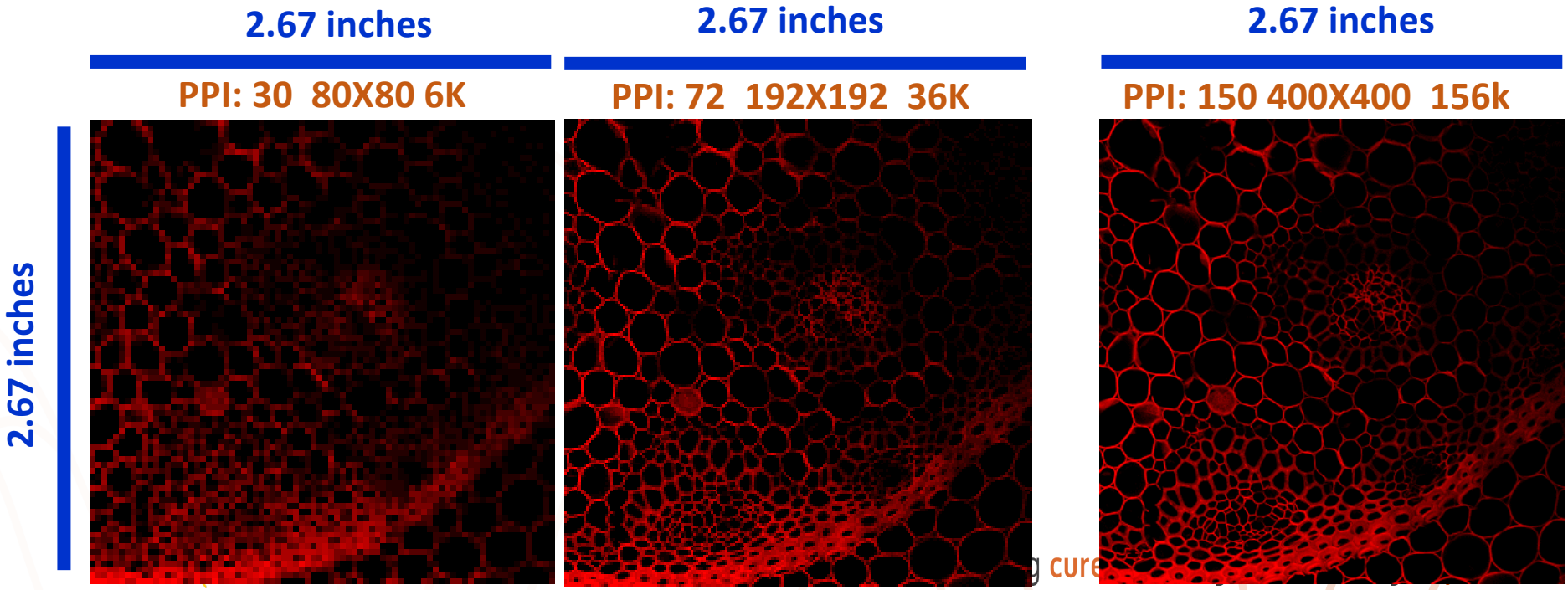
## A matrix of pixels



| | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 0 | 0 | 1 | 1 | 2 | 2 | 2 | 2 | 1 | 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 1 | 3 | 3 | 5 | 4 | 3 | 5 | 5 | 1 | 0 | 0 |
| 0 | 0 | 2 | 5 | 10 | 17 | 16 | 24 | 26 | 25 | 13 | 10 | 5 | 0 |
| 0 | 2 | 5 | 9 | 13 | 23 | 26 | 24 | 18 | 13 | 13 | 5 | 2 | 0 |
| 0 | 1 | 1 | 5 | 8 | 15 | 16 | 16 | 13 | 10 | 8 | 5 | 1 | 0 |
| 0 | 9 | 22 | 52 | 110 | 147 | 234 | 230 | 188 | 62 | 34 | 11 | 3 | 0 |
| 0 | 10 | 52 | 64 | 140 | 207 | 226 | 255 | 217 | 189 | 96 | 38 | 5 | 0 |
| 0 | 6 | 21 | 65 | 154 | 189 | 198 | 228 | 197 | 108 | 75 | 26 | 4 | 0 |
| 0 | 8 | 19 | 24 | 77 | 150 | 187 | 146 | 103 | 71 | 23 | 18 | 6 | 0 |
| 0 | 5 | 18 | 34 | 68 | 98 | 90 | 92 | 93 | 72 | 34 | 16 | 8 | 0 |
| 0 | 1 | 3 | 5 | 14 | 31 | 50 | 61 | 37 | 14 | 9 | 5 | 2 | 0 |
| 0 | 1 | 1 | 6 | 8 | 10 | 13 | 14 | 15 | 5 | 2 | 1 | 0 | 0 |
| 0 | 0 | 0 | 1 | 1 | 2 | 2 | 3 | 1 | 2 | 1 | 1 | 1 | 0 |
| 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 |

# Image size & image resolution

- Size (dimension)
  in pixels (i.e. 512 X 512)—pixel dimension or
  in inches/ums (i.e. 2.2 um X 2.2 um)-document dimension
- Resolution (pixel density): in DPI/PPI, pixel size (i.e. 50 nm/px )

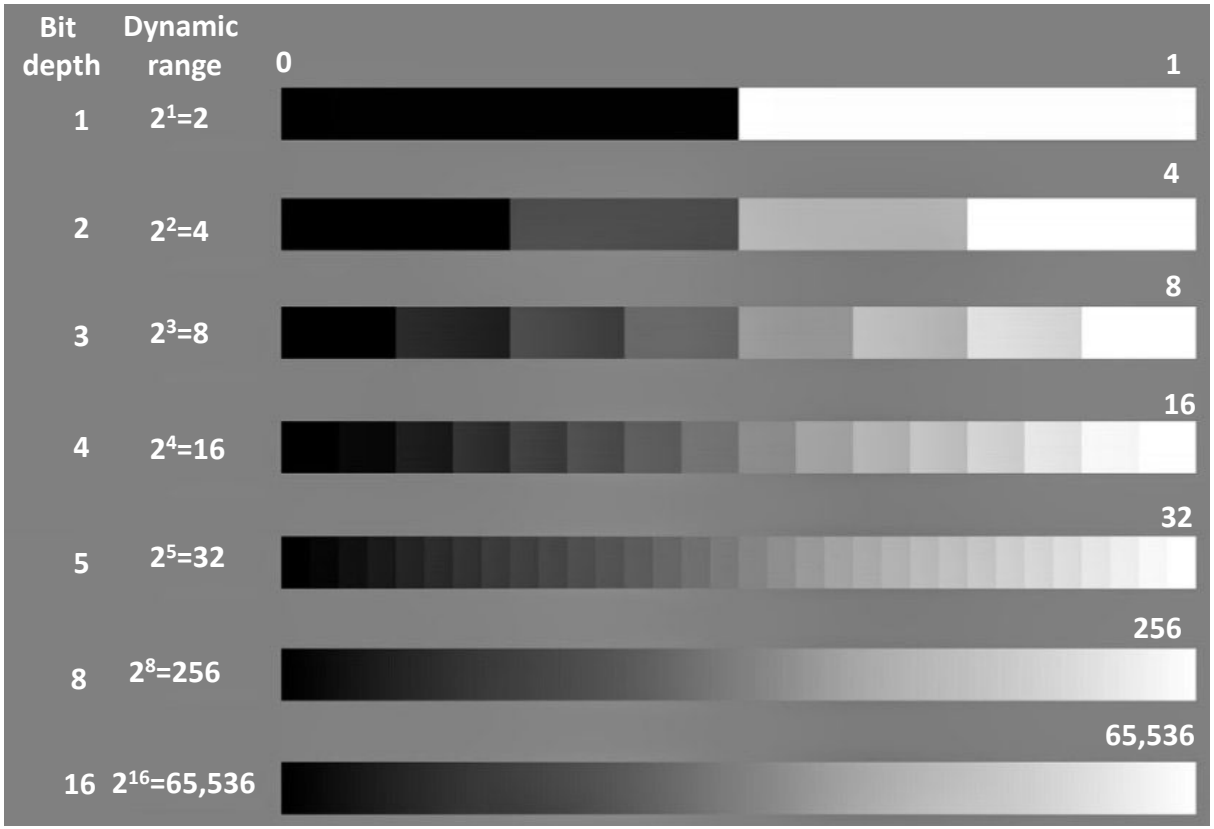


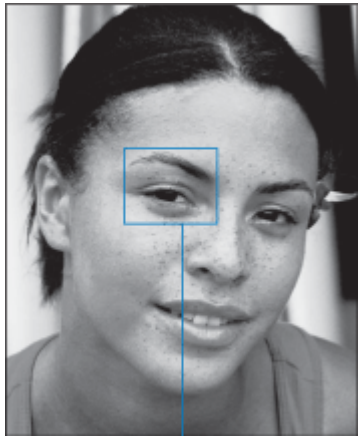
# Bit depth & dynamic range

**Bit**: short for binary digit 0 or 1, smallest unit of intensity data

**Bit depth**: the number of bits. i.e. 8-bit, 00000000-11111111

**Dynamic range:** possible grey shades, $=2^X$ (X: bit depth)



1 bit

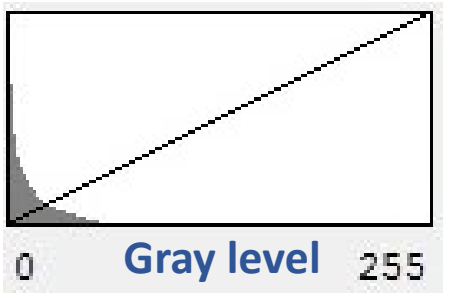
2 bits

4 bits

8 bits

Pictures modified from: https://www.azooptics.com/Article.aspx?ArticleID=1151 & https://jackinteractivemedia.files.wordpress.com/2014/10/bit-depth.gif

# Histogram & manipulation

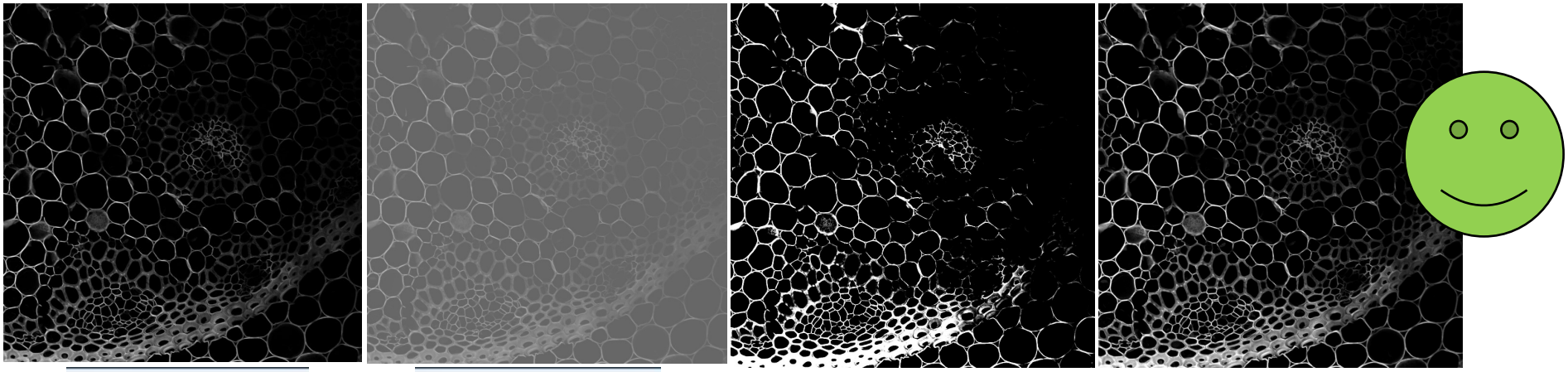


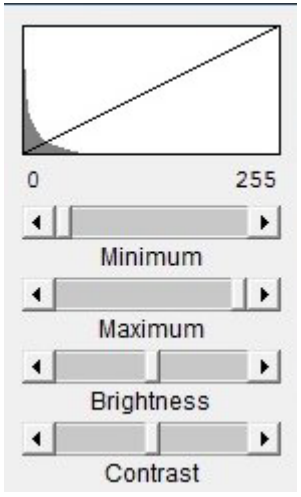

Unprocessed

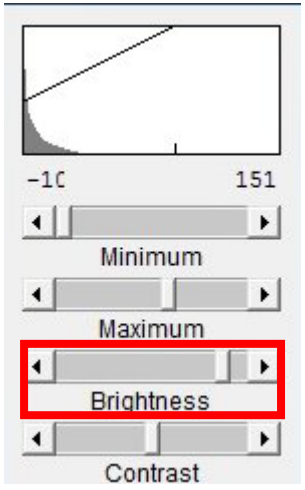
Brightness adjustment

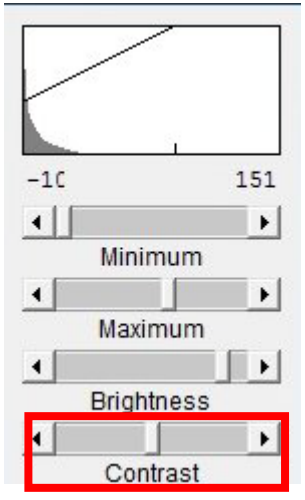
Contrast adjustment

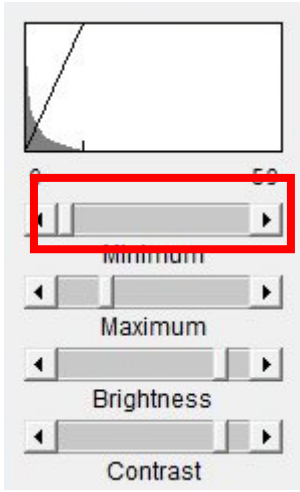
Contrast stretch

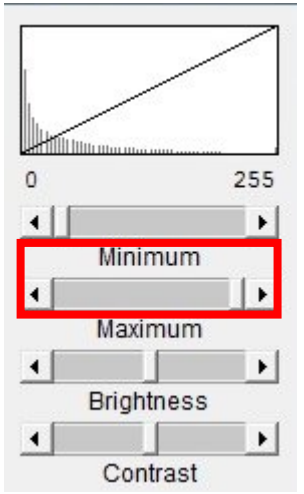




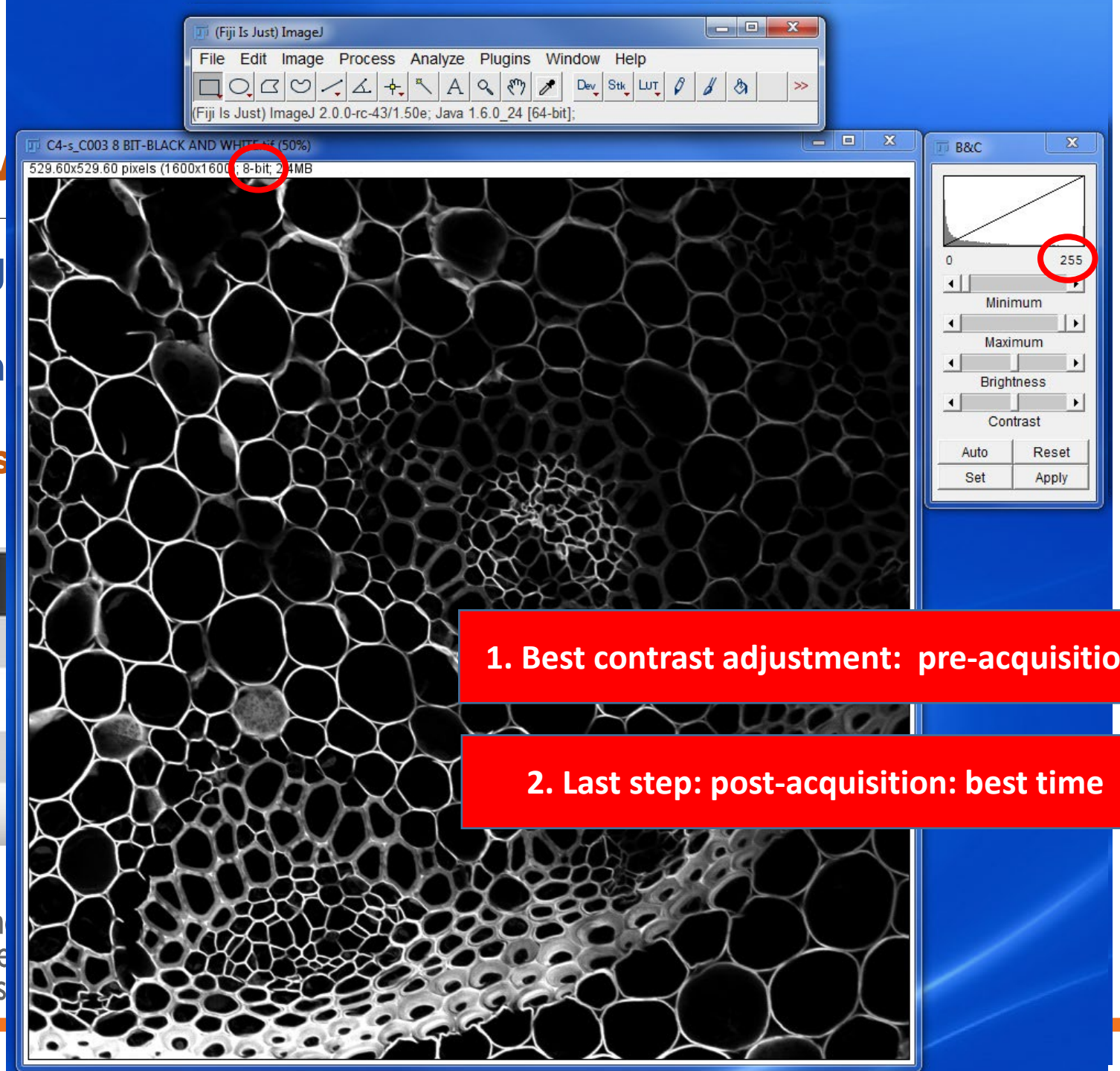



# Conv

- Imaging
- Low sign
- Journals

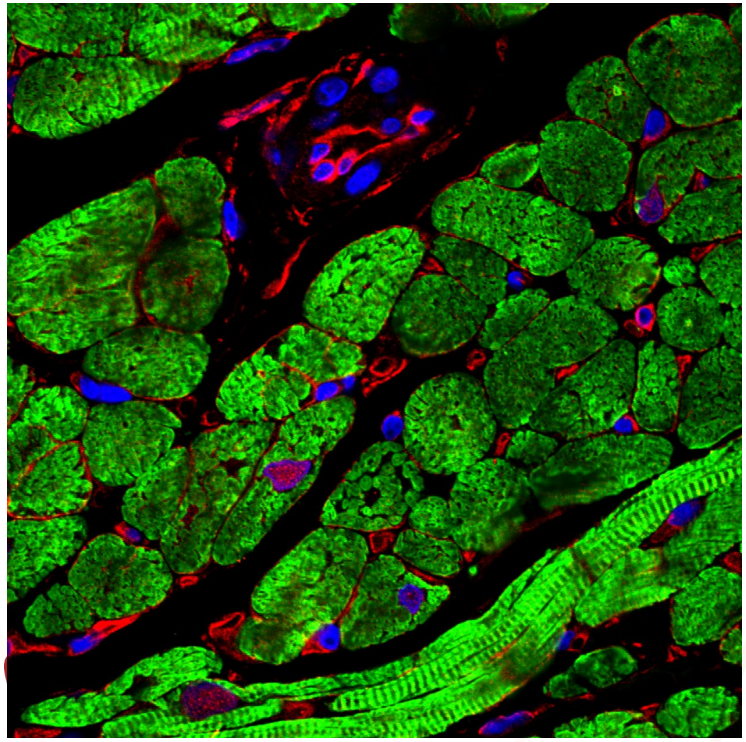



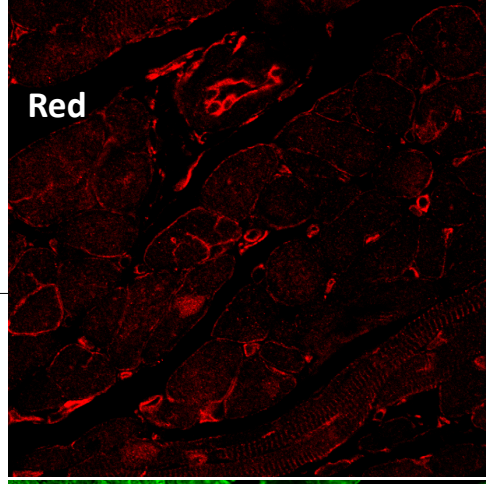

**1. Best contrast adjustment: pre-acquisition**

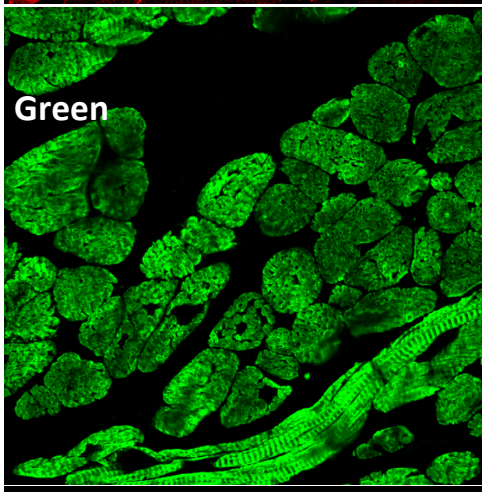
**2. Last step: post-acquisition: best time**

# Color images

- Simplest color representation is grayscale
- Made up of 3 gray scale channels (RGB)
- Can be 8 or 16 bits per channel (255/65536)





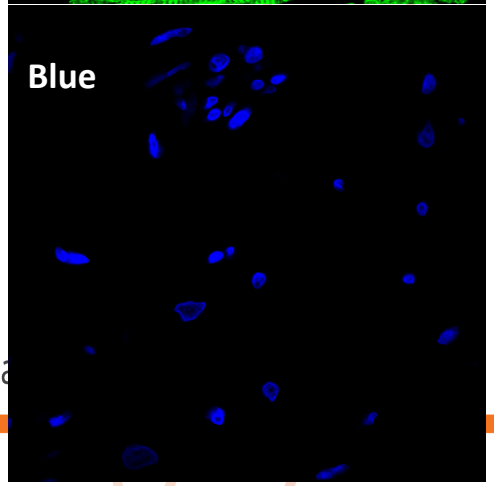






Image courtesy: Kyi from Cardiology

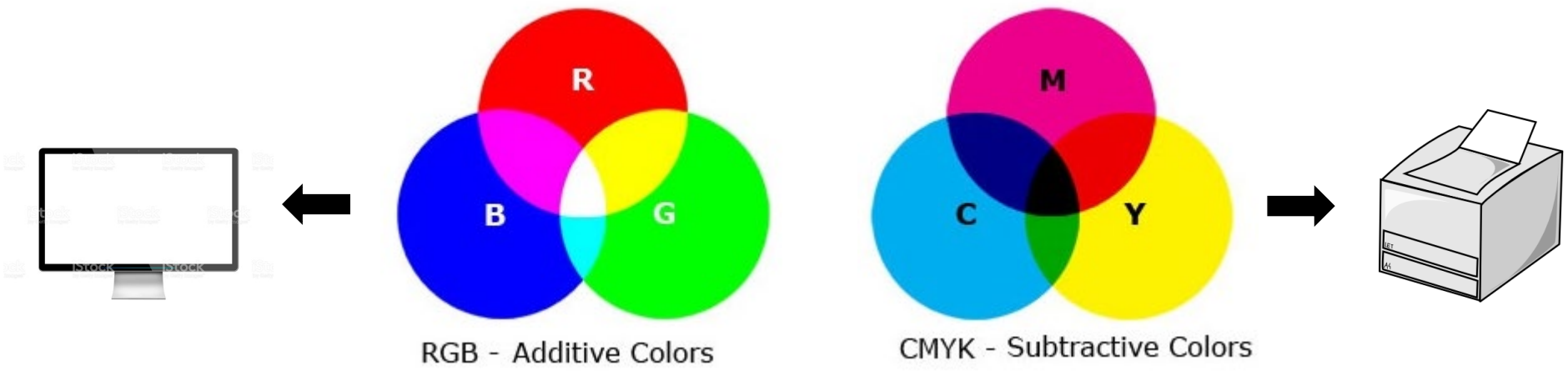
# Color models: RGB & CMYK

## RGB

Red Green Blue

## CMYK

Cyan, Magenta, Yellow, Black




RGB - Additive Colors

CMYK - Subtractive Colors

Images modified from: http://inkmonstr.com/2018/02/23/cmyk-vs-rgb/

# Image formats

## The contents of an image file

- Image data: pixel values (numbers, only numbers)
- Metadata: data about data (image type, bit depth, pixel size, microscope settings etc)

## File saving

For analysis: formats best preserving data

Display: general formats

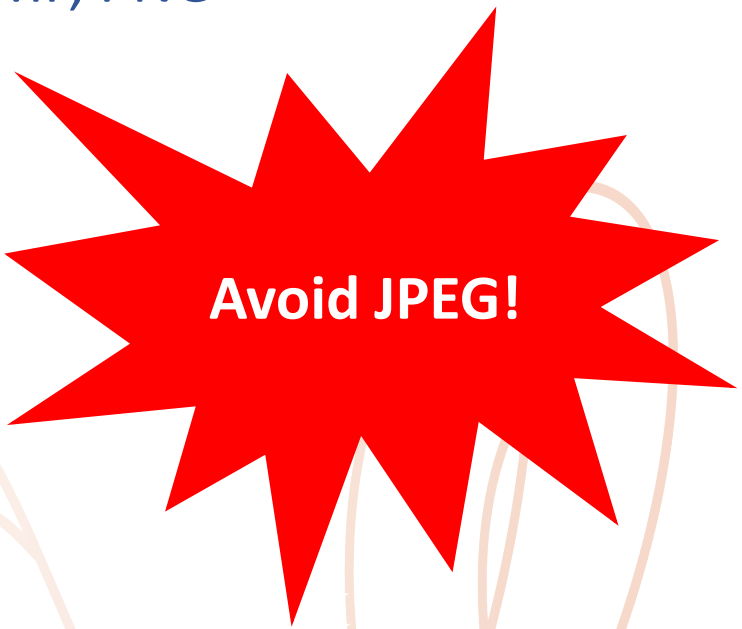
**Always keep your original data!**

# Commonly-used general formats

Recommended (lossless): **Tiff**

Generally good (lossless): JPEG2000, BMP, PNG

Generally bad (lossy): JPEG, JPG, GIP



# Bitmap (raster) & vector images

sciencemag.org/authors/instructions-preparing-revised-manuscript

**Format**

Figure files at the revision stage must be in one of the following formats (in preferred order):

*Vector illustrations and diagrams (preferred)*: Adobe Portable Document Format (PDF) Encapsulated PostScript (EPS), or Adobe Illustrator (AI).

*Raster illustrations and diagrams*: Tagged Image File Format (TIFF)(minimum 300 dpi).

*Vector and raster combinations for photographs or microscopy images*: Adobe Portable Document Format (PDF) or Encapsulated PostScript (EPS)

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Please keep an archive of all original images used in figures as *Science* may request delivery of these images for production purposes. Save these at the highest resolution possible, preferably as the original file in its native format.

*At this stage in the process, we cannot accept files in formats other than those specified above; in particular, we **cannot** accept:*

- Figures embedded in Microsoft Word files.
- Microsoft PowerPoint files.



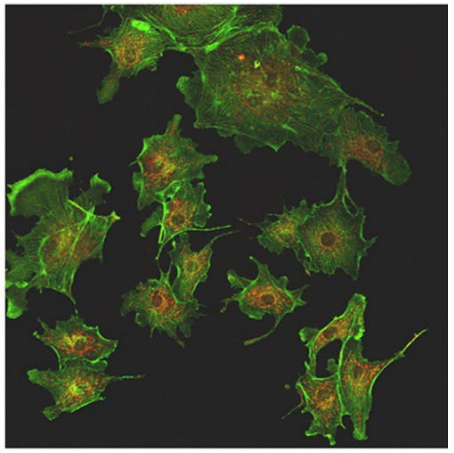
Image courtesy: http://www.differencebetween.info/difference-between-bitmap-and-vector

# What manipulations are “legal”?

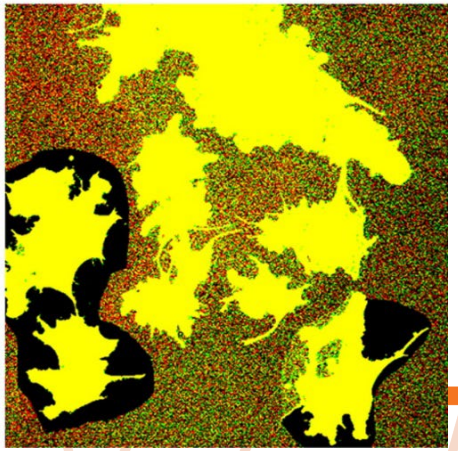
- Linear adjustment of brightness, contrast, color balance in moderation
- Background subtraction
- Cropping
- Reduce image resolution

- Increasing image resolution
- Adjusting only a specific part of an image or erasing spots
- No cutting/pasting into a single picture
- Control and experiment are not treated identically

Manipulated image



Manipulation revealed by contrast adjustment



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Findir

# Suggestions on image manipulations

- Keep original data as it was acquired
- Perform adjustments on a copy of the unprocessed image
- Save processed images separately with important processes or adjustments
- Disclose handling software and specific processing
- Do not increase the resolution of an image when exporting
- Ethical guidelines http://jcb.rupress.org/content/166/1/11, https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4114110/

# **Figures:** increase clarity of data

## Meet Journal formatting requirements!

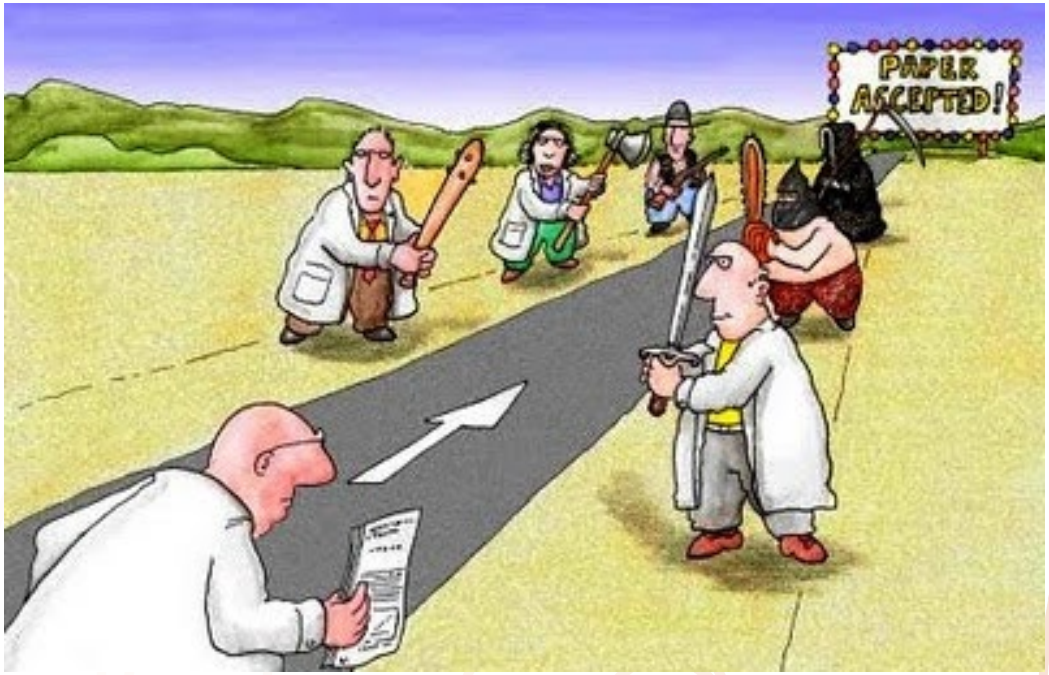


Image courtesy: http://tripleed.com/lindberg-rantatalo-hallgren-has-article-accepted-on-making-sense-of-paradoxes/

# Figure-making rules

- Read the journal instructions **first**:

  *Image type: raster/vector, 8 bit, RGB/CMYK*
  *Image size (dimensions): 1 (3.5 inch/9cm) or 2 column (7.3 inch/18.5 cm)*
  *Image resolution—input>>> output: 300 or 600 or 1200 dpi/ppi?*
  *File size (< 5Mb)*
  *Format (Tiff, PDF, etc)*

- Be mindful of **acquisition** resolution > 300 dpi
- Don't manipulate images excessively
- Avoid the use of lossy compression (use recommended format)
- Each figure should be submitted as a single file

# Figure-making software tools

## We need proper software to

- arrange, lay out, and annotate your images;
- bring in raster images;
- make/draw vector graphics;
- export the final figure.

## Commonly used programs:

- **Word**: bad choice
- **Photoshop**: not recommended
- **Powerpoint**: try to avoid
- **Illustrator**: recommended
- Others: Inkscape, InDesign etc


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http://www.sciencemag.org/site/feature/contribinfo/prep/figguide.pdf

# 4-step figure-making workflow: recommended

**2 software tools are involved: Fiji ImageJ & Illustrator**

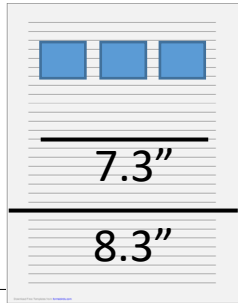

Step 1: Planning: journal requirements, raw data

Step 2: Getting individual images ready: **FIJI ImageJ** (better than Photoshop) size, res, bit depth, format, etc

Step 3. Assembling components: **Illustrator**
raster & vector images, texts & annotation (vector), etc

Step 4. Export file: **Illustrator**
resolution (300 DPI), RGB/CMYK, format (PDF/Tiff), etc

# A figure-making example




## Journal requirements

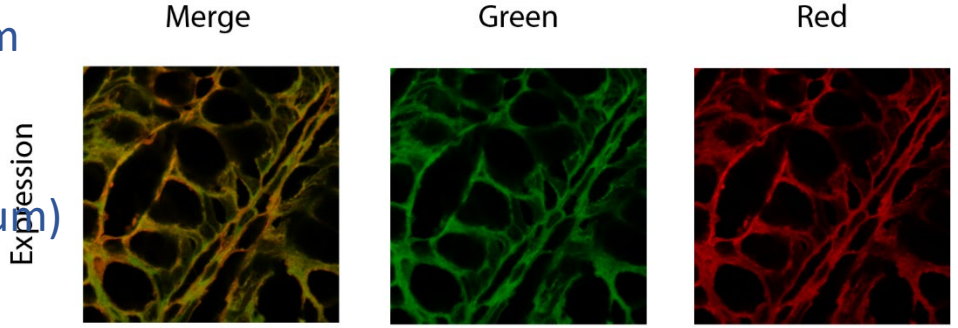
- Single column figure
- Output: 8 bit RGB, 300PPI, Tiff

**Task**: make a figure embedded with 3 fluo images
**Plan**: A4: 8.3” x 11.7”
single column: 8.3” → → *7.3*”
individual channel image: 7.3”/3=*2.4*”
let’s do **2.2**” width!

## Raw images

- Size: 1600X1600 Pixel, 70.356x70.356 um
- Conversion: 1 um = 0.00003937 inch
- Resolution:1600 pixel/70.356 um (pixel/um)
  1600/70.356/0.00003937=**577,635** PPI
- Format: oif →→ Tiff (lossless)

**7.3 inches**




**2.2 inches**

**Reduce to 300 PPI**

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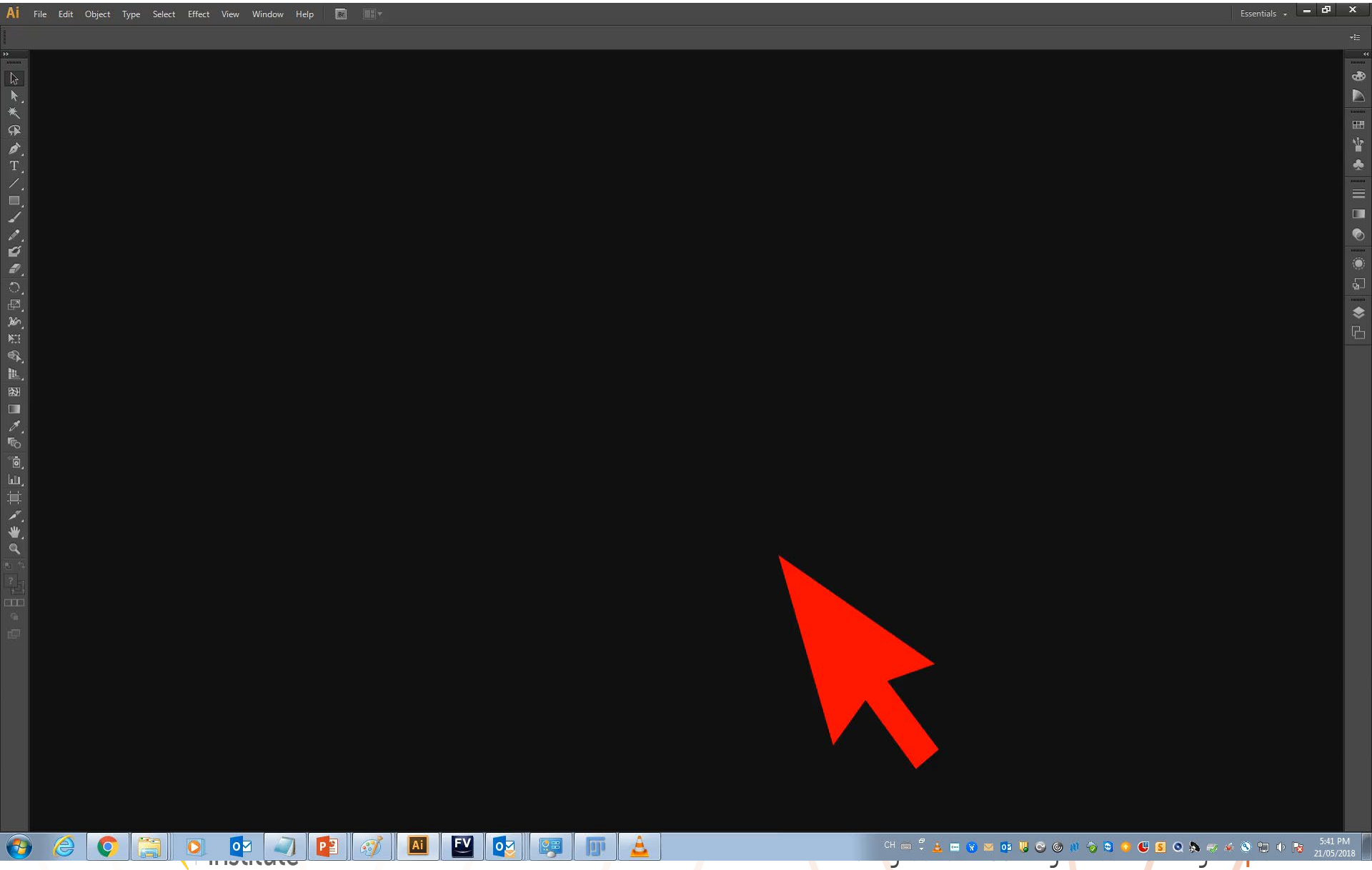
File
Edit
Object
Type
Select
Effect
View
Window
Help
Essentials
5:41 PM
21/05/2018

# Why Illustrator not Powerpoint?

sciencemag.org/authors/instructions-preparing-revised-manuscript

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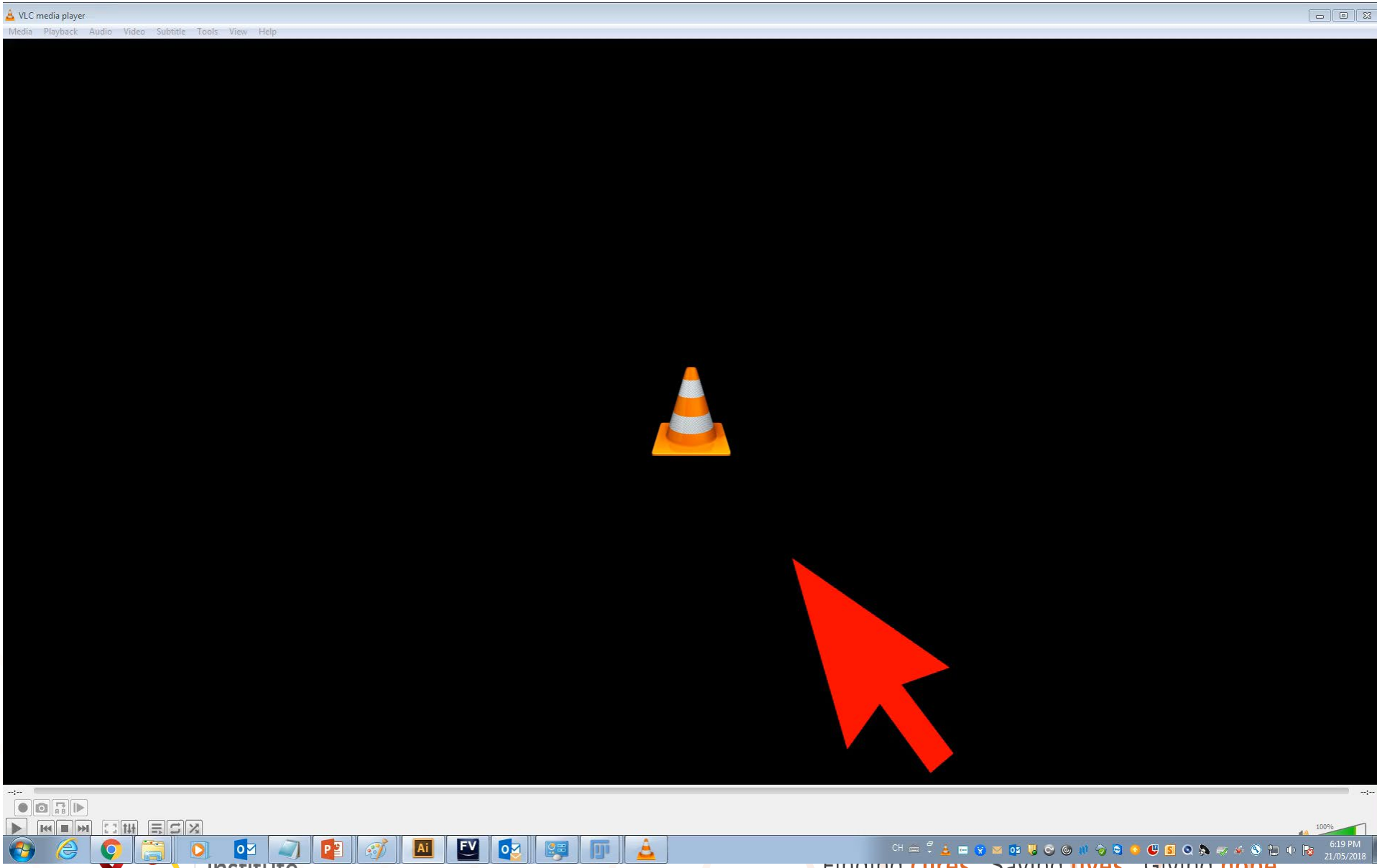
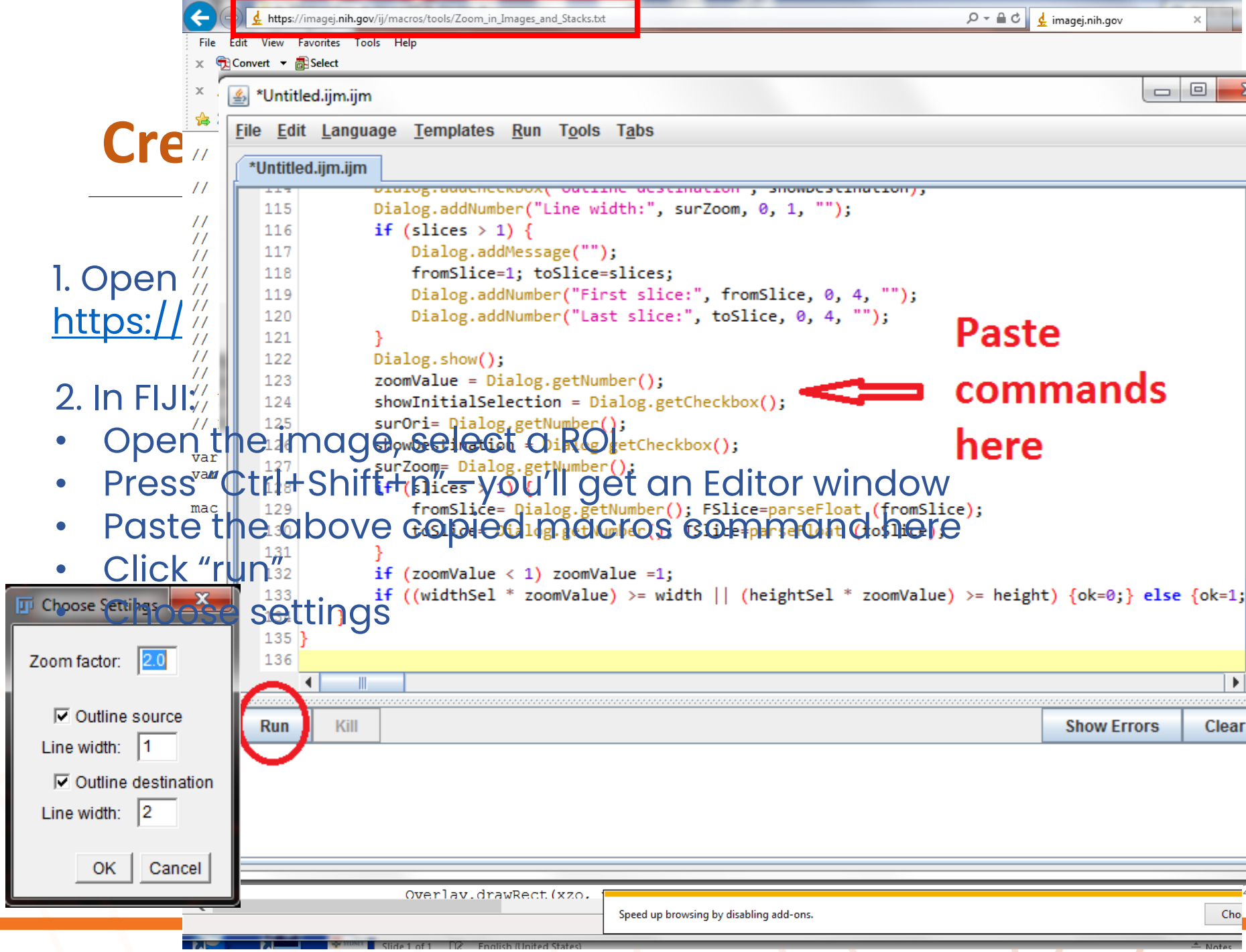

VLC media player
Media Playback Audio Video Subtitle Tools View Help

# Cre

1. Open https://

2. In FIJI:

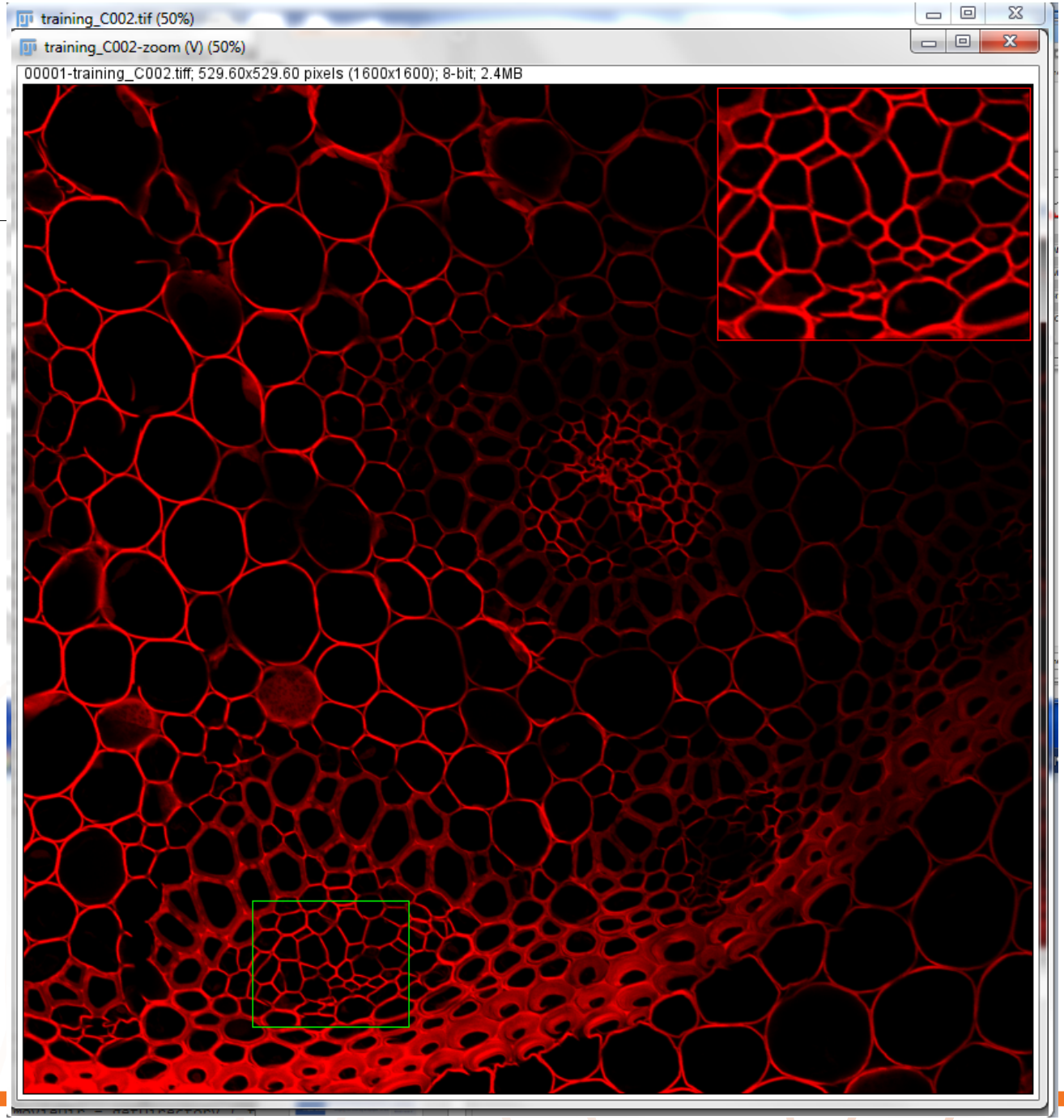
- Open the image, select a ROI
- Press "Ctrl+Shift+n"—you'll get an Editor window
- Paste the above copied macros command here
- Click "run"
- Choose settings



# Creating an

4. Now you decide where to place your inset (red square)

5. Finally click on the red square place the inset

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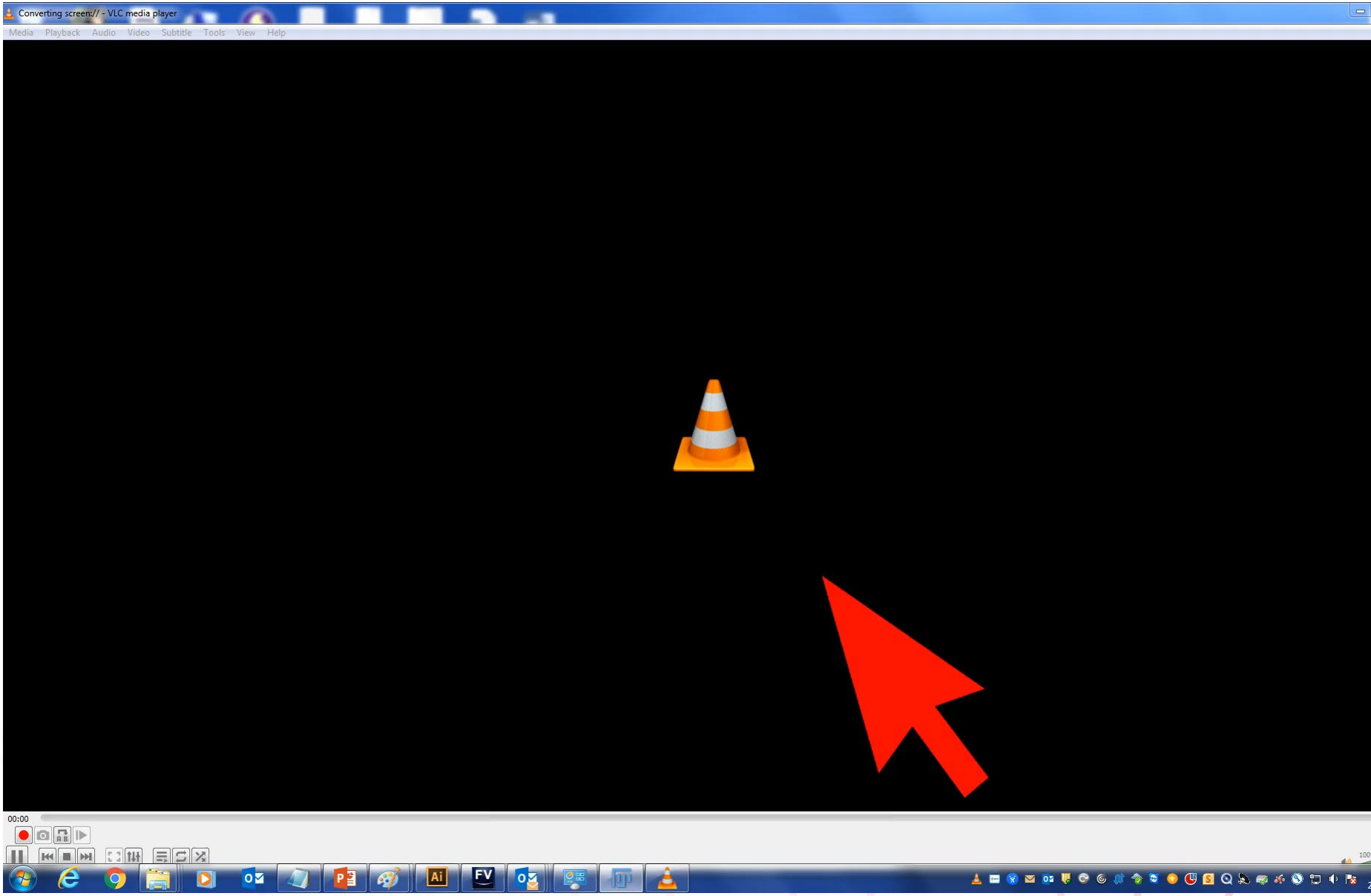
Converting screen:// - VLC media player
Media Playback Audio Video Subtitle Tools View Help
00:00

# Summary & take-home messages

Say

# Available image processing & analysis software

1. Fiji ImageJ (general, open source)

2. Aivia: general (analysis, DPL)

3. Desktop 200: general (analysis, DPL)

4. Others: ASW (Olympus VS 120), LASX (Leica), Zen (Zeiss), NDP view2 (NZ), Imagescope (Leica)

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# Thank you!