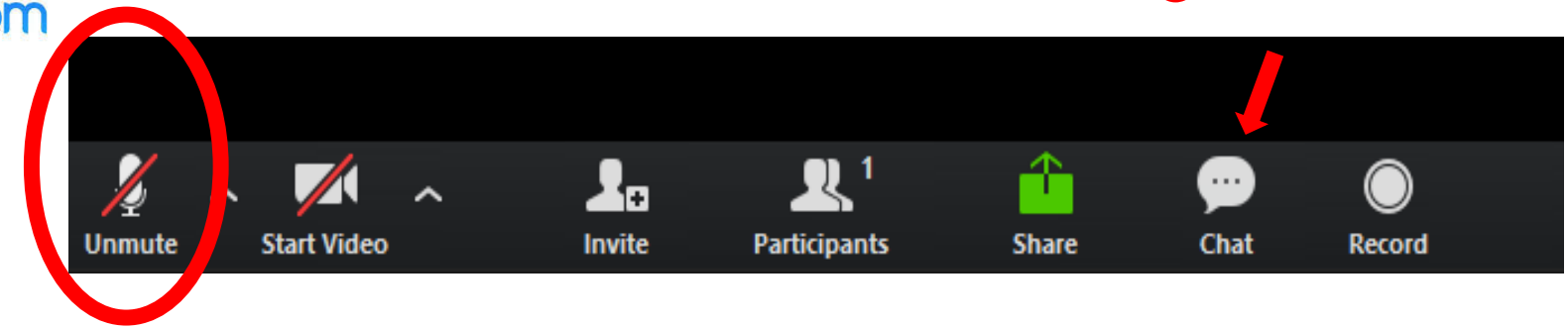
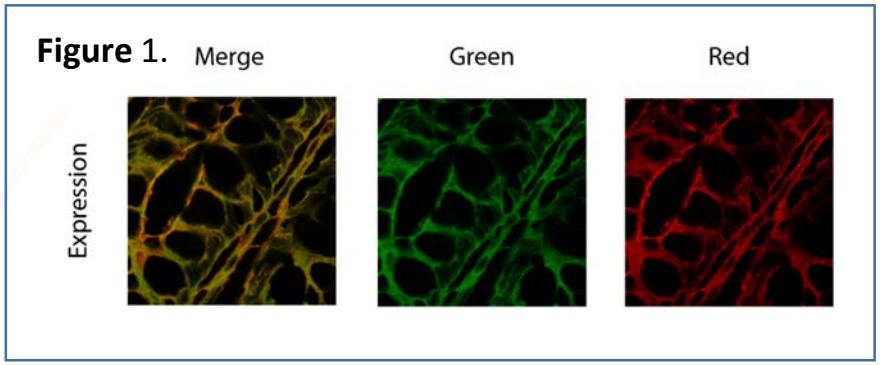
Questions



Things you need to know about figure-making for final publishing



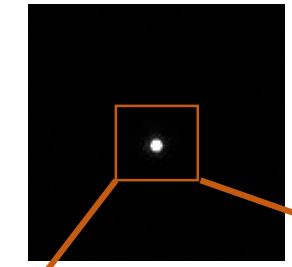
Hong Yu, Westmead Imaging Facility 18 Sept 2020

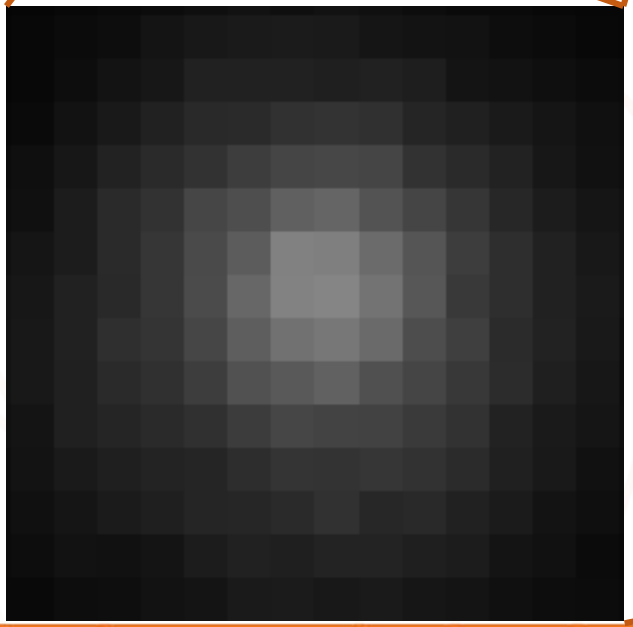
Outlines

- Basic concepts and terms
- What manipulations are “legal”?
- How to make figures for journals?
- How to create an inset within a picture?

What is a digital microscope image?

A matrix of pixels





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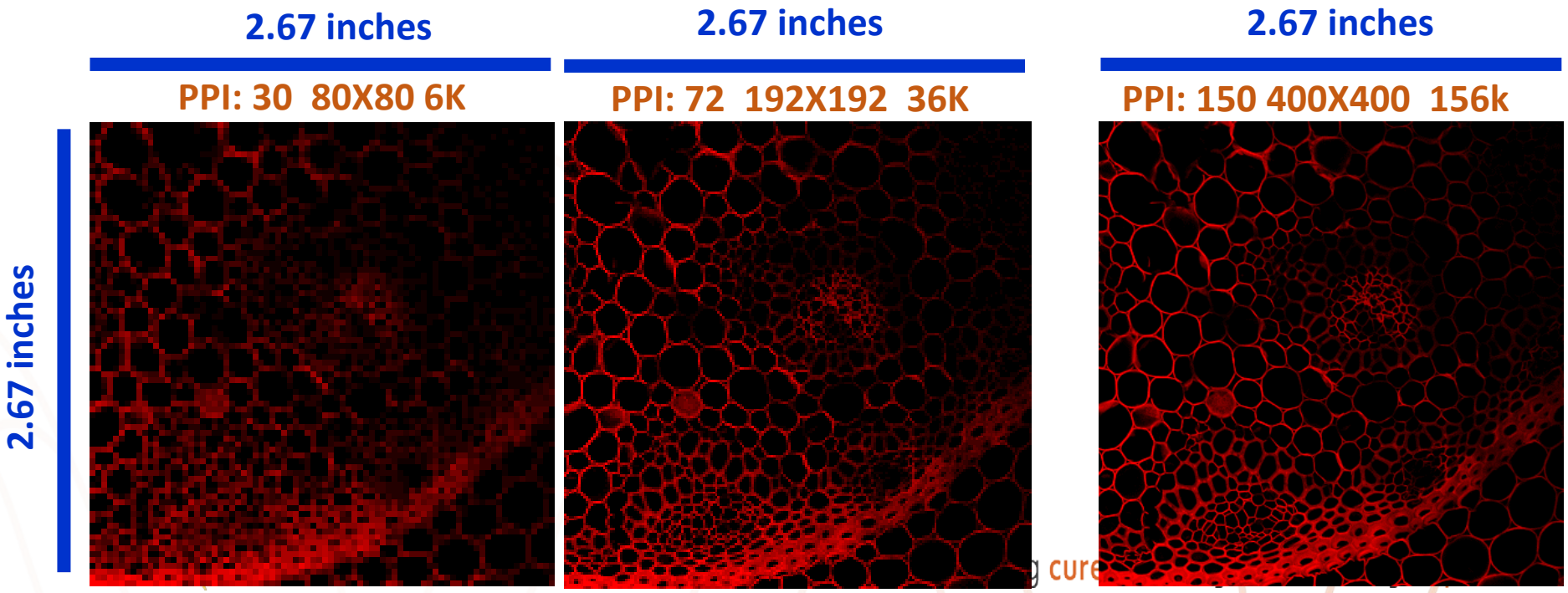
0	0	2	5	10	17	16	24	26	25	13	10	5	0
0	2	5	9	13	23	26	24	18	13	13	5	2	0
0	1	1	5	8	15	16	16	13	10	8	5	1	0
0	9	22	52	110	147	234	230	188	62	34	11	3	0
0	10	52	64	140	207	226	255	217	189	96	38	5	0
0	6	21	65	154	189	198	228	197	108	75	26	4	0
0	8	19	24	77	150	187	146	103	71	23	18	6	0
0	5	18	34	68	98	90	92	93	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 (dimensions): in pixels (512 X 512) or inches/ums (2.2 um X 2.2 um)
- Resolution (pixel density): in DPI/PPI, 50 nm/px (pixel size)

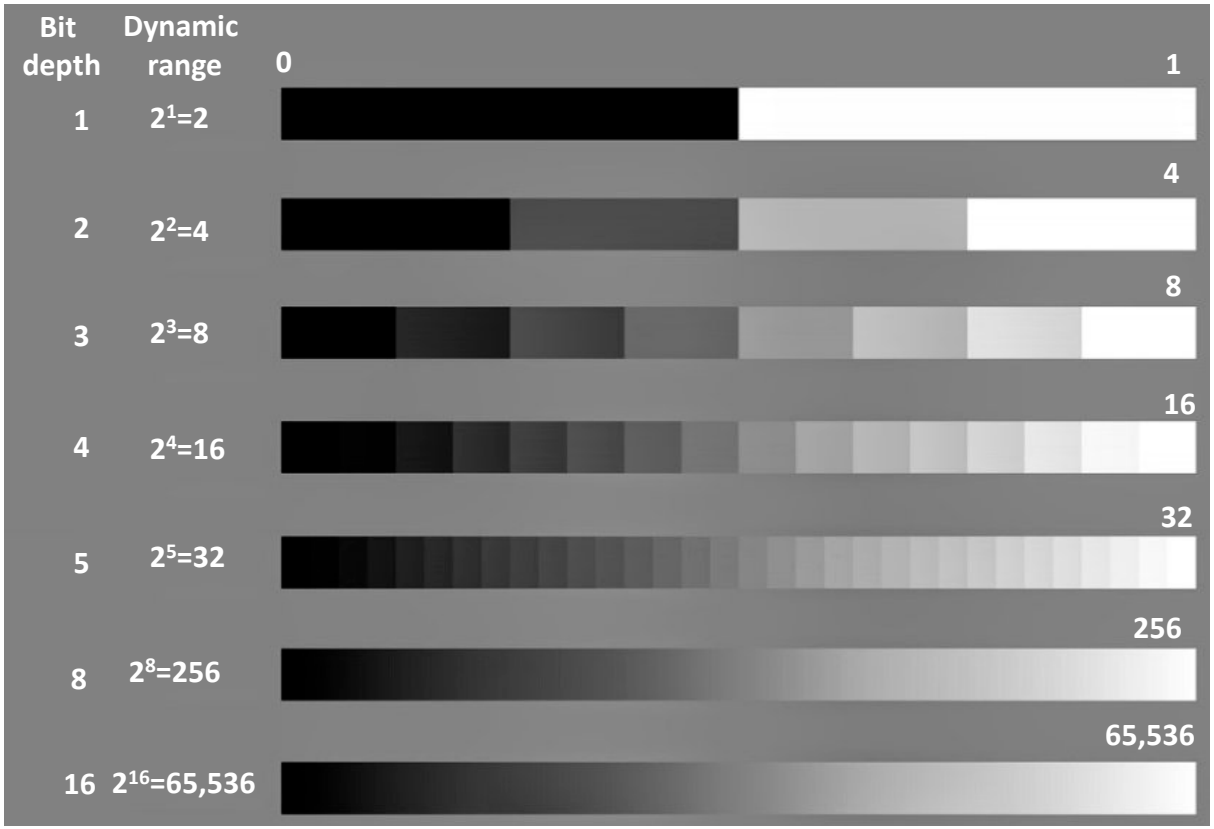


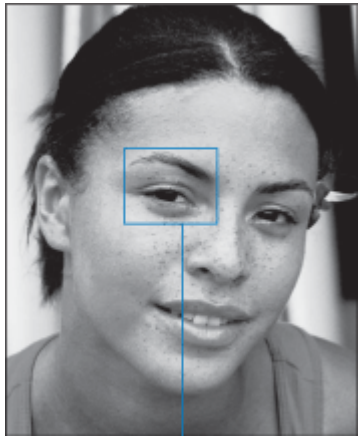
Bit depth & dynamic range

Bit (binary digit): smallest unit of data

Bit depth: the number of bits

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





1 bit

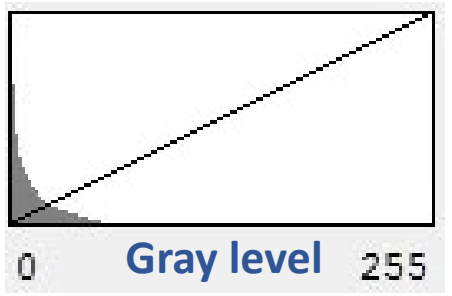
2 bits

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

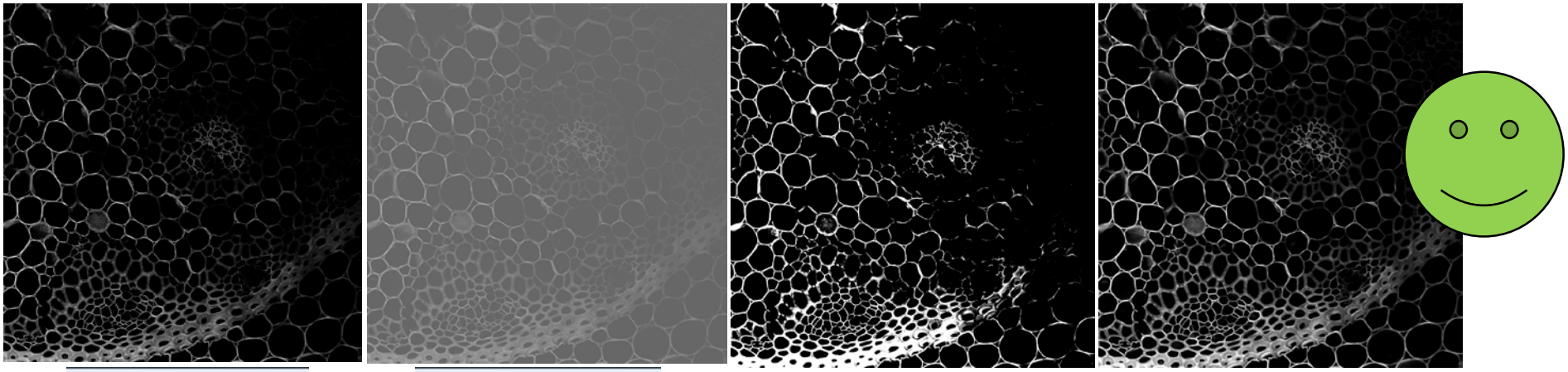


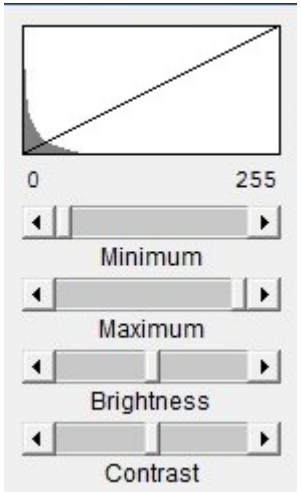
Unprocessed

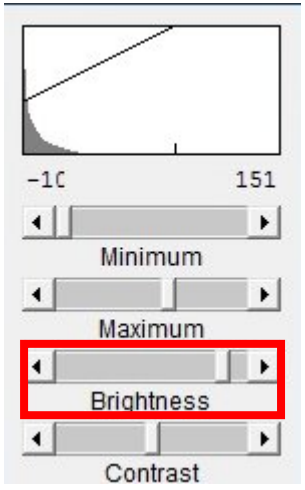
Brightness adjustment

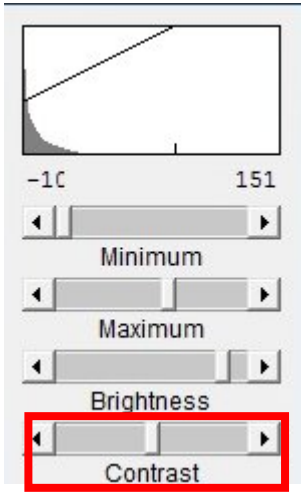
Contrast adjustment

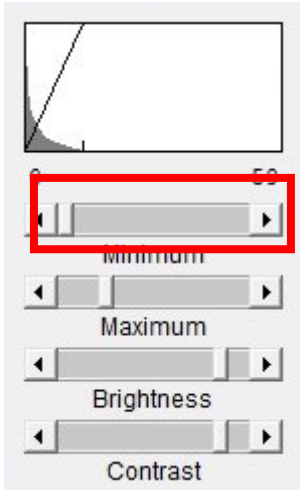
Contrast stretch

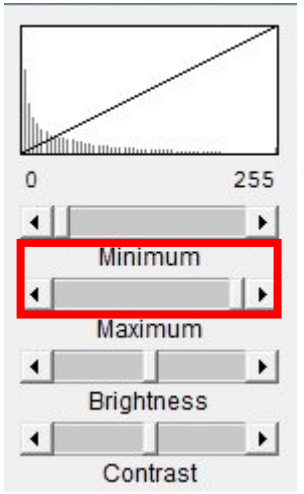












- Im
- Lo
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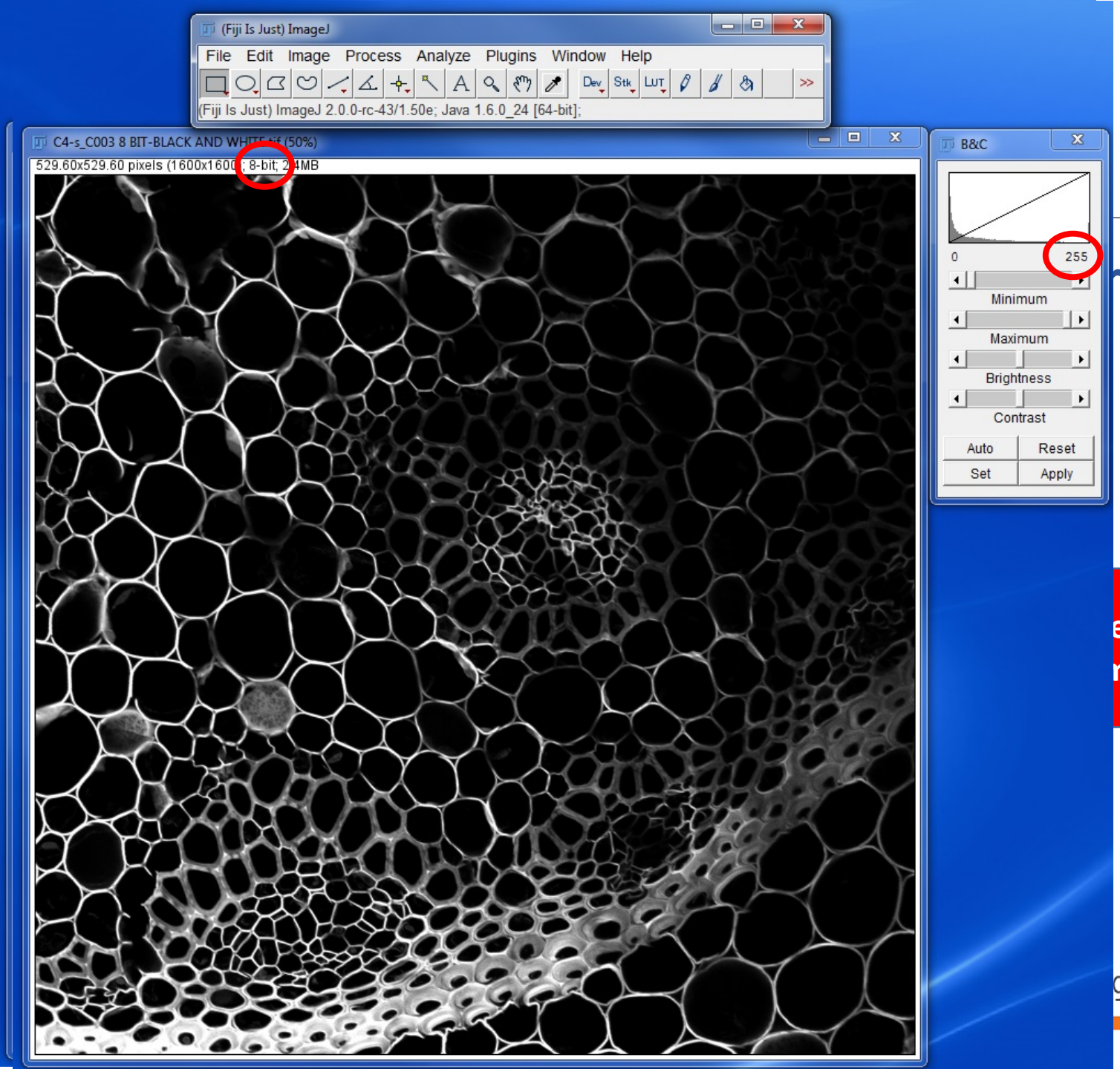
8-b

12-b

16-b



16 bit

ep
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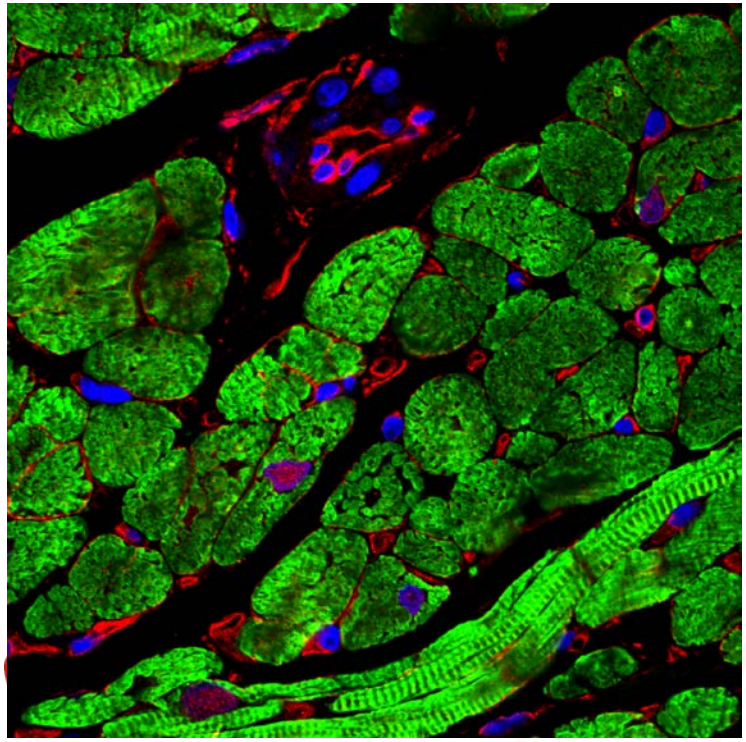
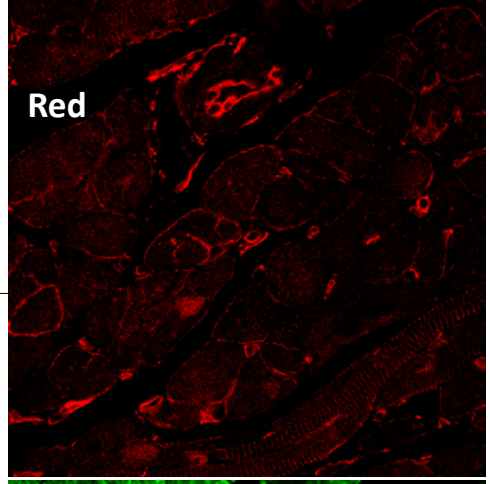
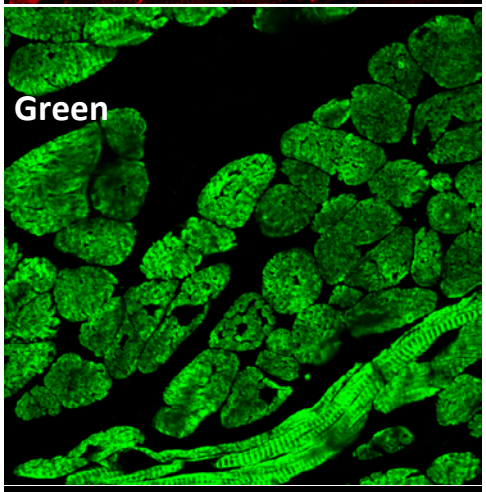
g hope.

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)







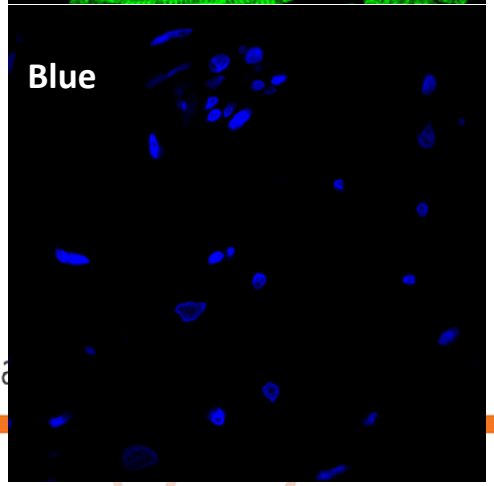


Image courtesy: Kyi from Cardiology

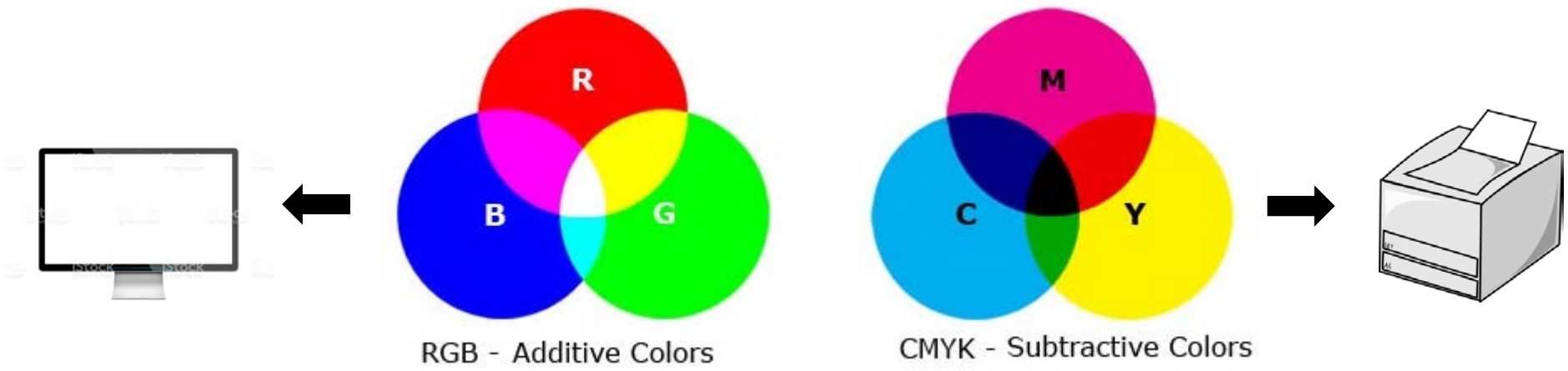
Color models: RGB & CMYK

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/

Bitmap (raster) & vector images

https://www.sciencemag.org/authors/instructions-preparing-revised-manuscript

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

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 preserving the metadata

Display: general formats

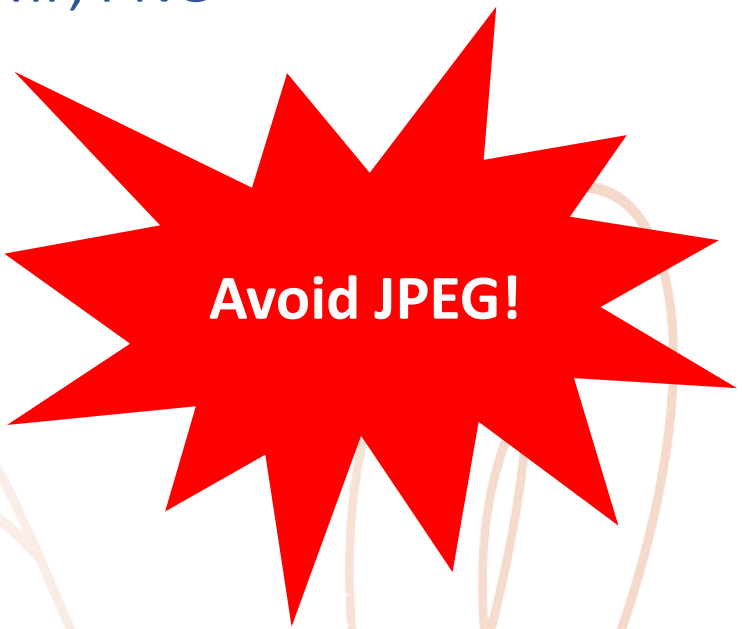
Always keep your original data!

Commonly-used general formats

Recommended (lossless): **Tiff**

Generally good (lossless): JPEG2000, BMP, PNG

Generally bad (lossy): JPEG, JPG, GIP

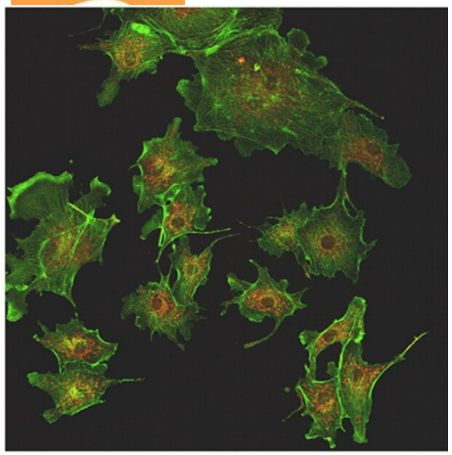


What manipulations are “legal”?

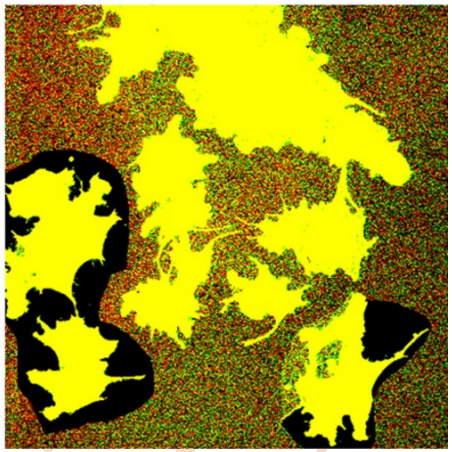
- Linear adjustment of brightness, contrast, color balance in moderation
- Background subtraction
- Cropping
- Reduce image resolution

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

Manipulated image



Manipulation revealed by contrast adjustment



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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 process or adjustment
- 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!

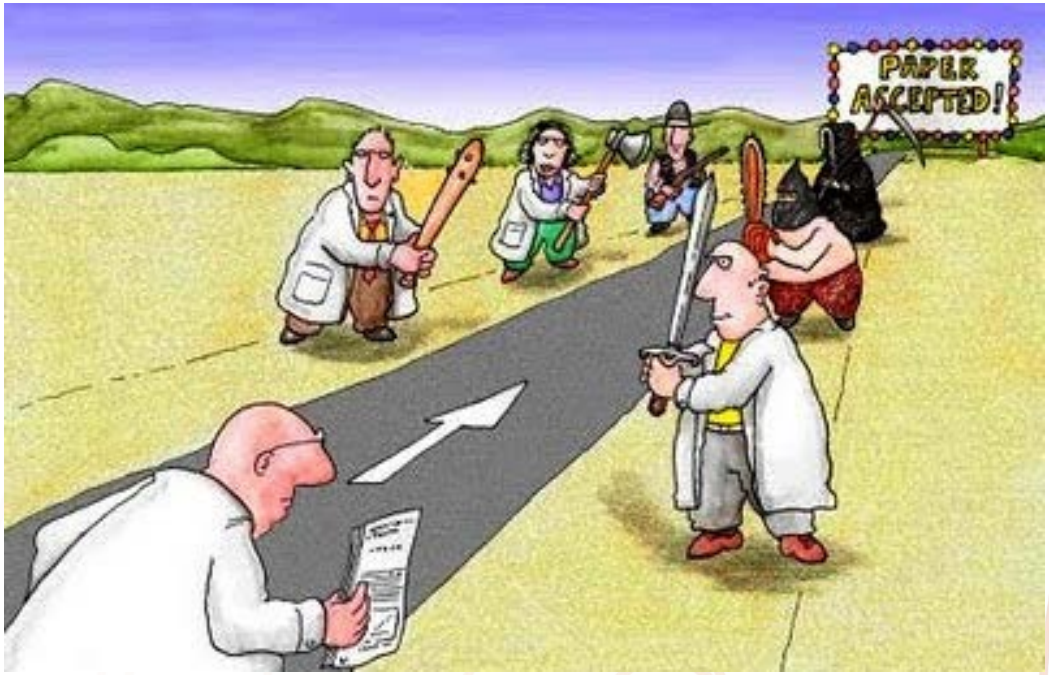


Image courtesy: http://tripleed.com/lindberg-rantatalo-hallgren-has-article-accepted-on-making-sense-of-paradoxes/

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

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

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

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

A figure-making example

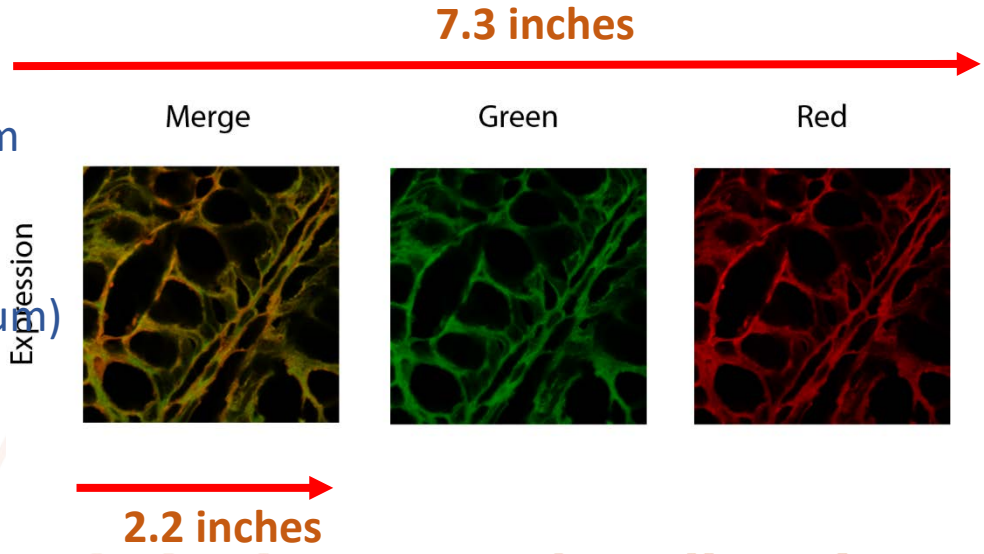
Journal requirements

- Double column figure: 7.3 inches wide
- Output: 8 bit RGB, 300PPI, Tiff

Task: assemble 3 fluo images
Plan:
each image width 7.3/3=2.4 inches
let's do 2.2 inch 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)



Reduce to 300 PPI

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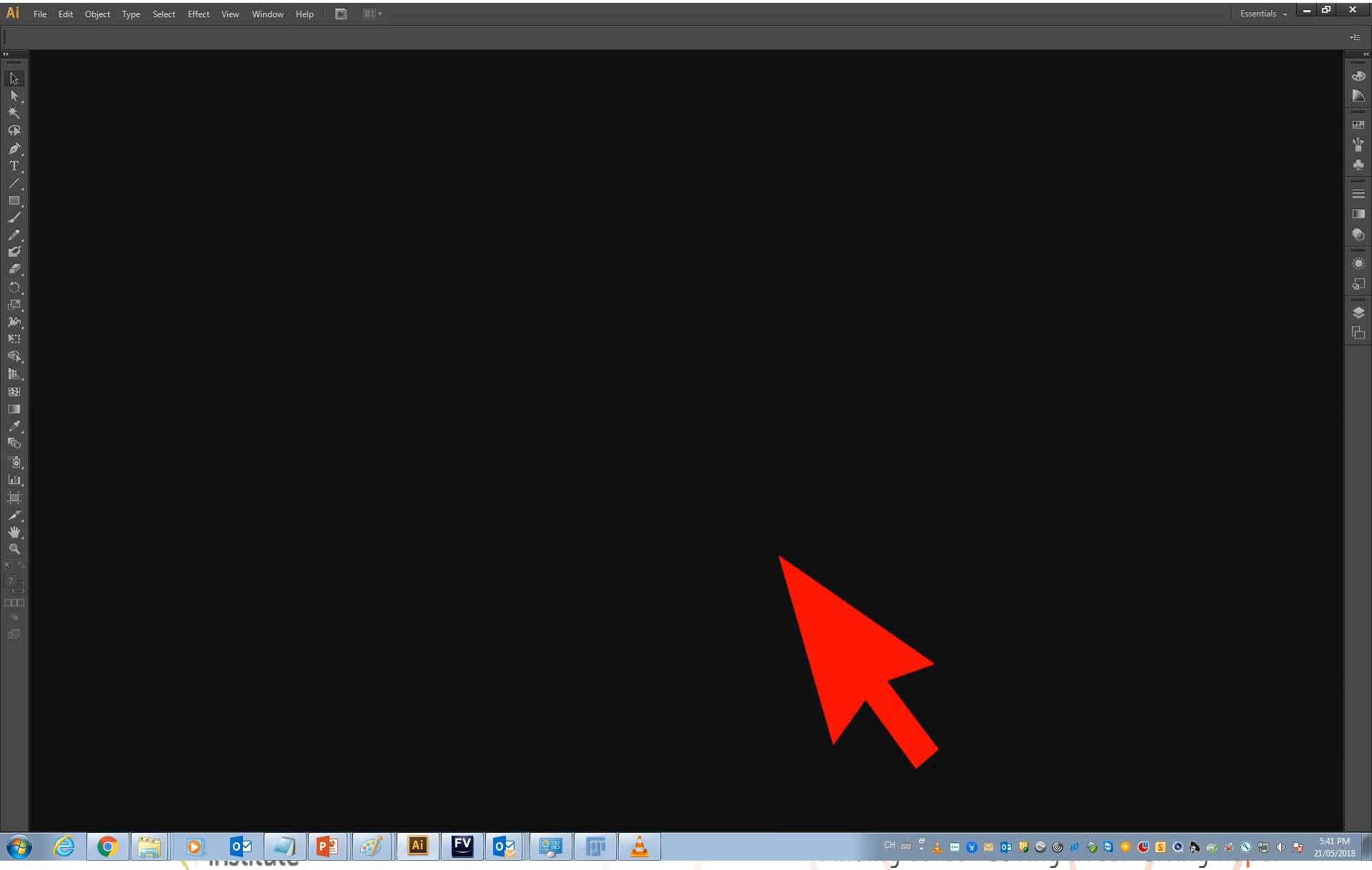
File Edit Object Type Select Effect View Window Help
Essentials
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21/05/2018

Why Illustrator not Powerpoint?

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

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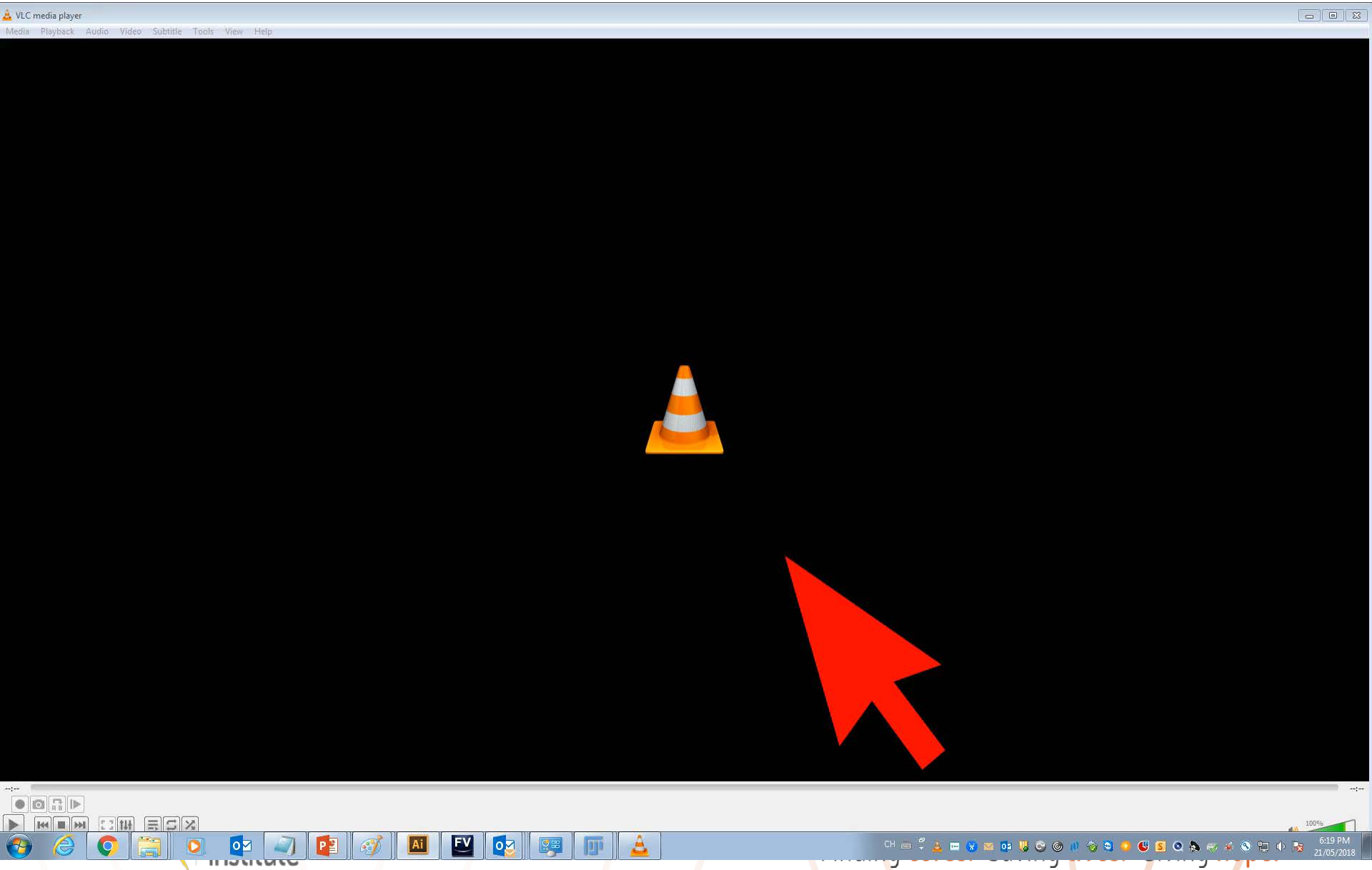
VLC media player
Media Playback Audio Video Subtitle Tools View Help
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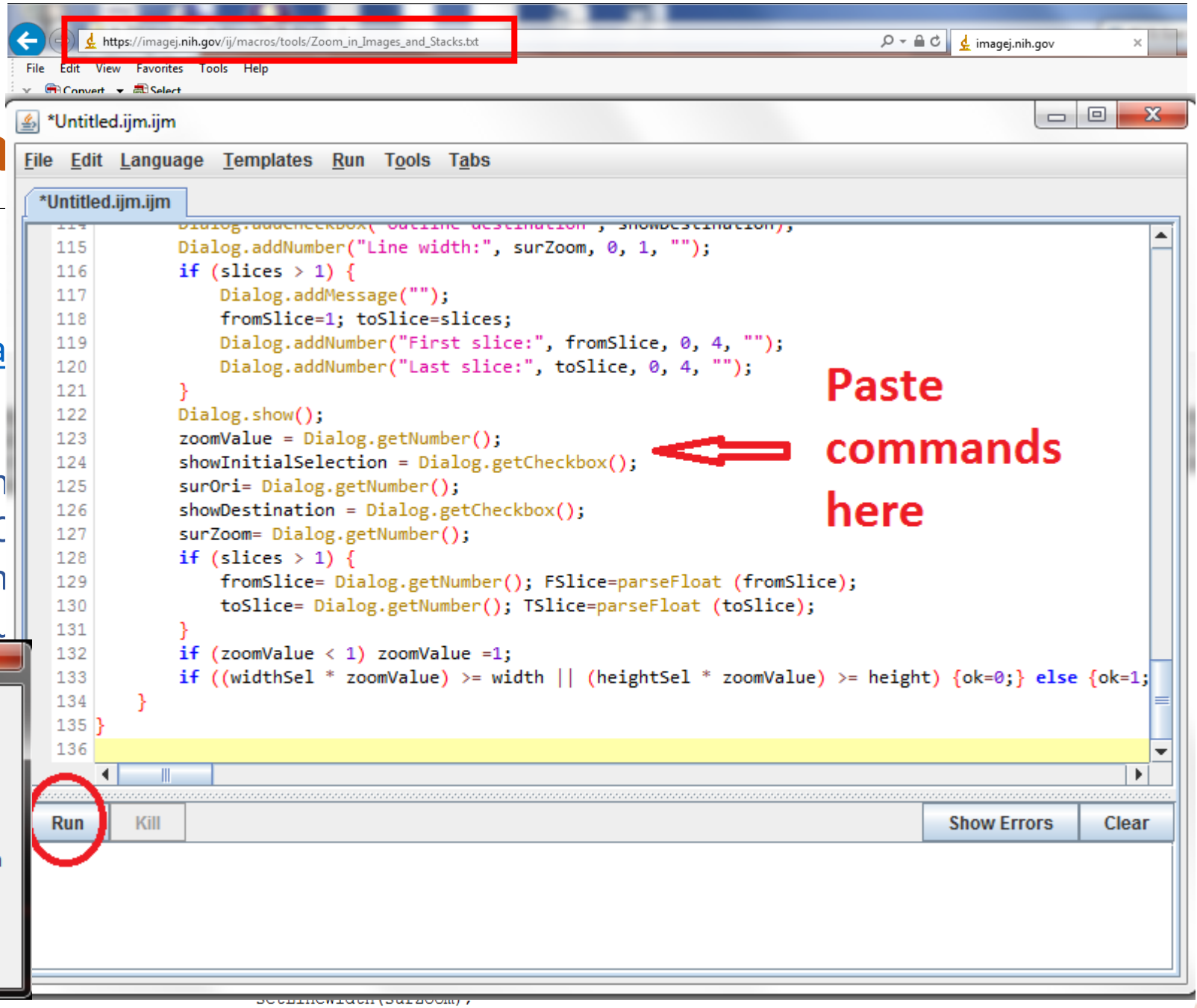
21/05/2018

Crea

1. Open up https://ima

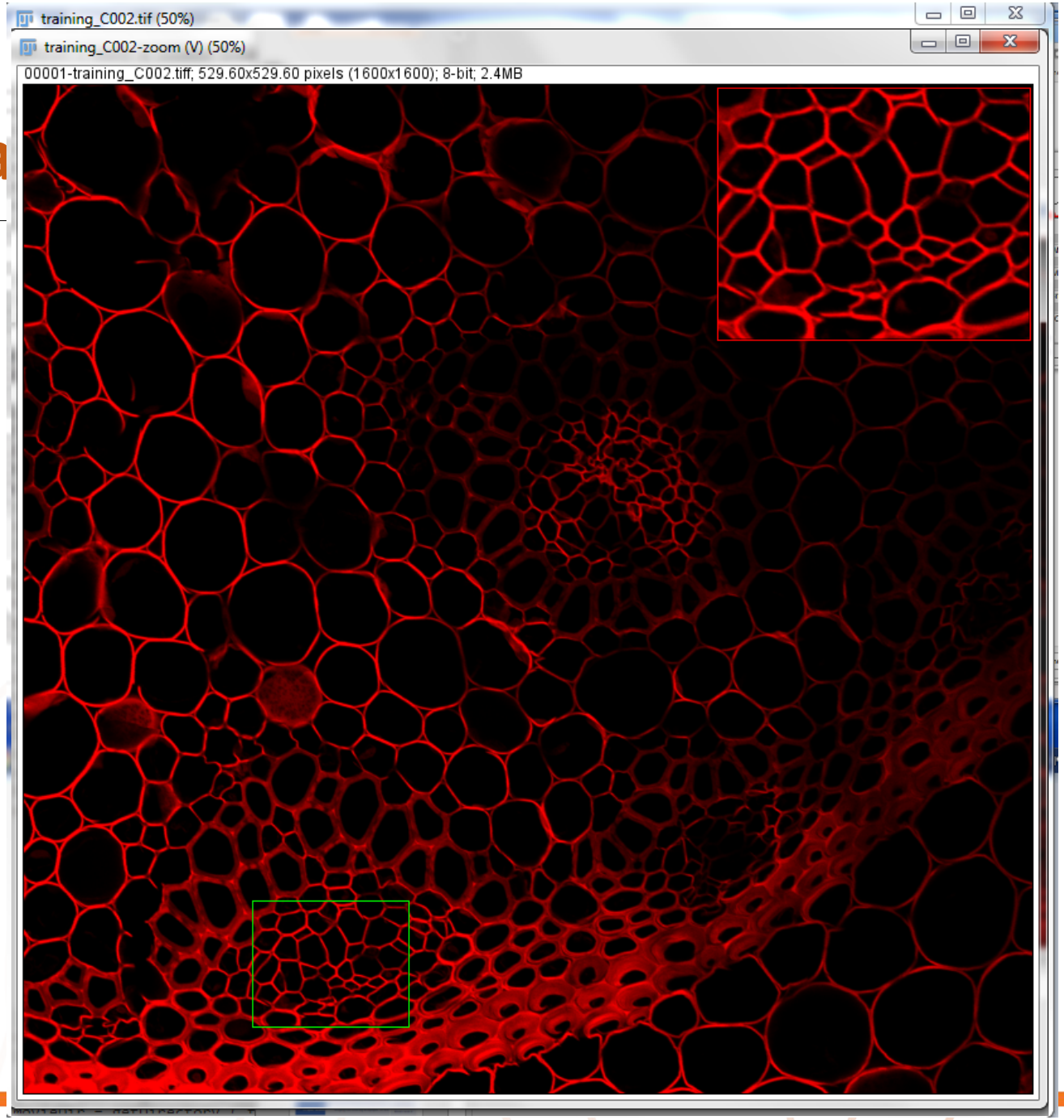
2. In FIJI:
   - Open th
   - Press "C
   - Paste th
   - Click "ru



Creating a

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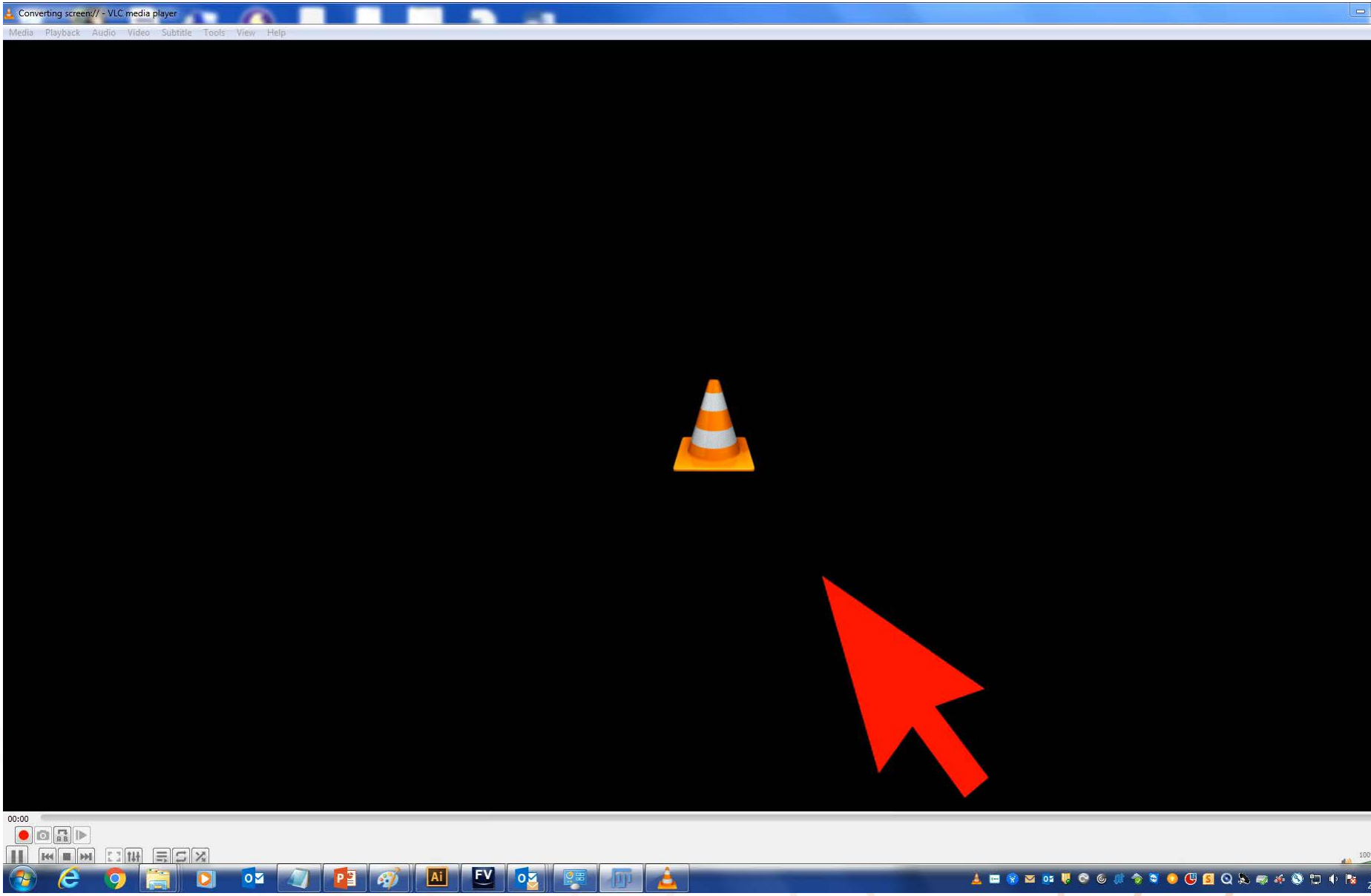
Converting screen:// - VLC media player
Media Playback Audio Video Subtitle Tools View Help
00:00

Summary & take-home messages

Its hard to say…

Visit Our website to find this presentation…

https://sydneyuni.atlassian.net/wiki/spaces/WIF/pages/765397549/Tips+Tricks

https://wimr.sharepoint.com/sites/ScientificPlatform2/SitePages/Imaging%20Facility.aspx

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Thank you!