Image Processing and Analysis

Time	Presentation	Speaker
1.00-1.10 pm	Welcome	Hong Yu/WIMR
1.10-1.45 pm	Things you need to know for publishing images	Hong Yu/WIMR
1.45-2.20 pm	A brief introduction to image analysis using ImageJ	Josh Studdert/CMRI
2.20-2.55 pm	An introduction to deconvolution with Huygens	Laurence Cantrill/KRI
2.55-3.15 pm	Afternoon tea	Hong Yu/WIMR
3.15-3.50 pm	Multi-dimensional image analysis using Imaris	Scott Page/CMRI
3.50-4.25 pm	Image analysis in Electron Microscopy	Emma Kettle WIMR/CMRI
4.25-4.30 pm	Wrap up + upcoming events	Hong Yu/WIMR

Finding cures. Saving lives. Giving hope.

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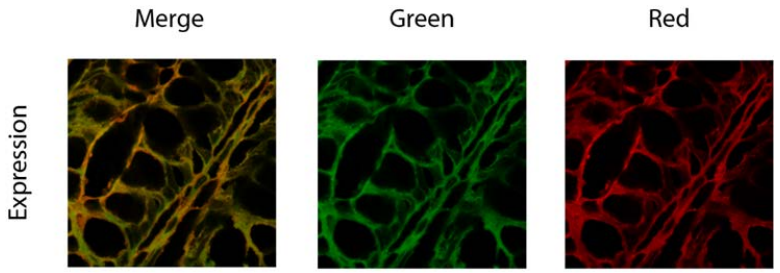
Things you need to know about figure-making for final publishing

Hong Yu, Cell Imaging Facility 24 May 2018

Introduction



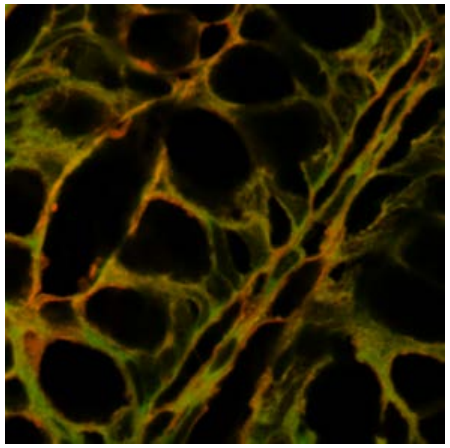
Figure

Raw images

Processing

Analysis

Publication



Fiji

Huygens Software
by Scientific Volume Imaging

Imaris
3D/4D Visualization and Analysis Software

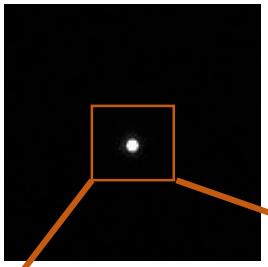
Digital Imaging Solutions
iTEM
Solutions for TEM Applications

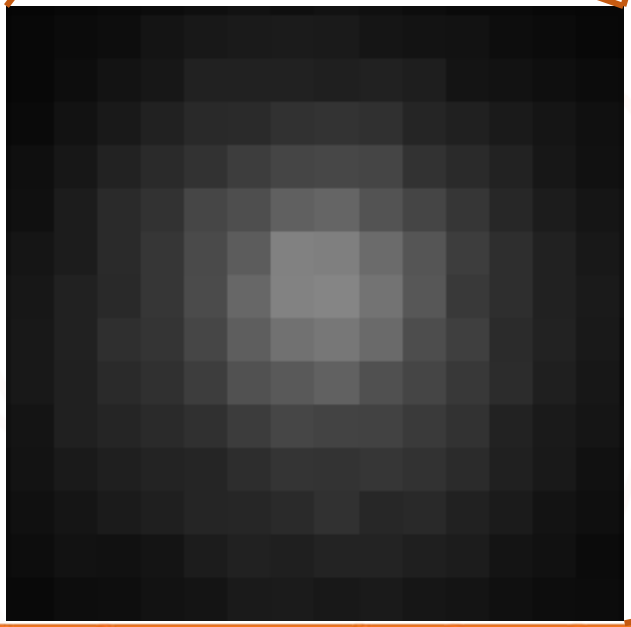
Images modified from https://fiji.sc/ ; https://svi.nl/HomePage; http://www.sciencemag.org/topic/optics ; https://science.institut-curie.org/platforms/cell-and-tissue-imaging/photonics/pict-lm-software/imaris/; https://www.behance.net/gallery/8956263/Nature-science-journal-cover
http://photobucket.com/gallery/http://s831.photobucket.com/user/hibari_sensei/media/cellcover_zps342b968d.jpg.html

Outlines

- Important concepts and terms
- What manipulations are “legal”?
- How to make figures for journals?

What is a digital image? A matrix of pixels





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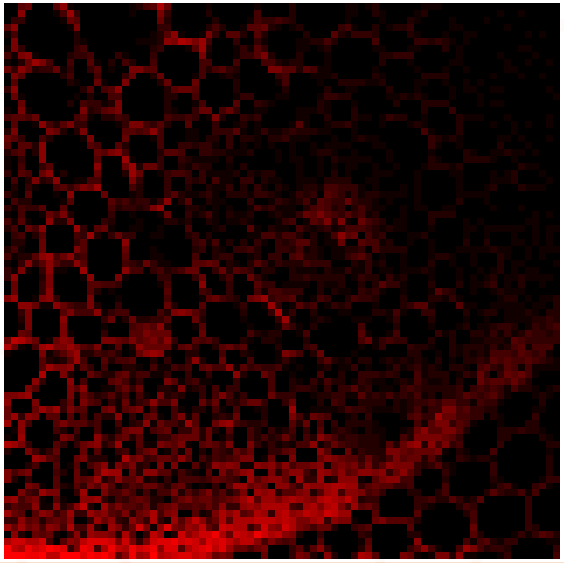
0	2	5	9	13	23	26	24	18	13	13	5	2	0
0	1	1	5	8	15	16	16	13	10	8	5	1	0
0	9	22	52	110	147	234	230	188	62	34	11	3	0
0	10	52	64	140	207	226	255	217	189	96	38	5	0
0	6	21	65	154	189	198	228	197	108	75	26	4	0
0	8	19	24	77	150	187	146	103	71	23	18	6	0
0	5	18	34	68	98	90	92	93	72	34	16	8	0
0	1	3	5	14	31	50	61	37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

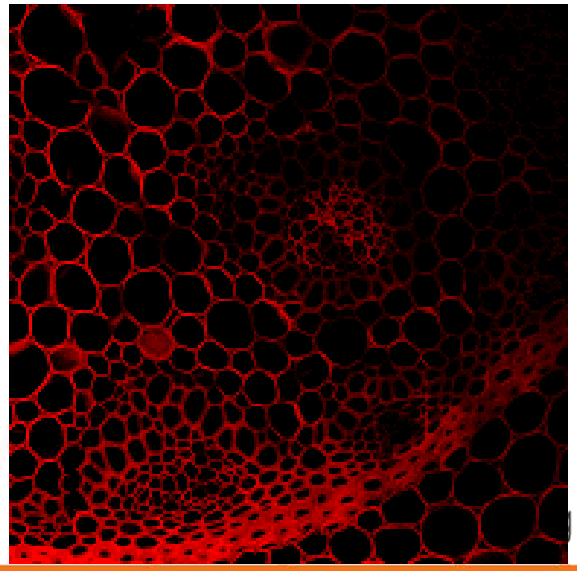
Image size and resolution

- Image size (pixel dimensions): 1024 X 1024
- Image resolution (pixel density):
  dpi (dots per inch) or ppi (pixels per inch)

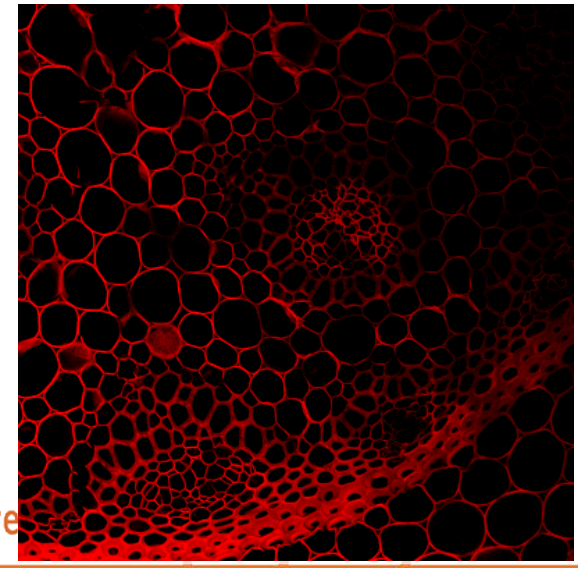
PPI: 30 80X80 6K



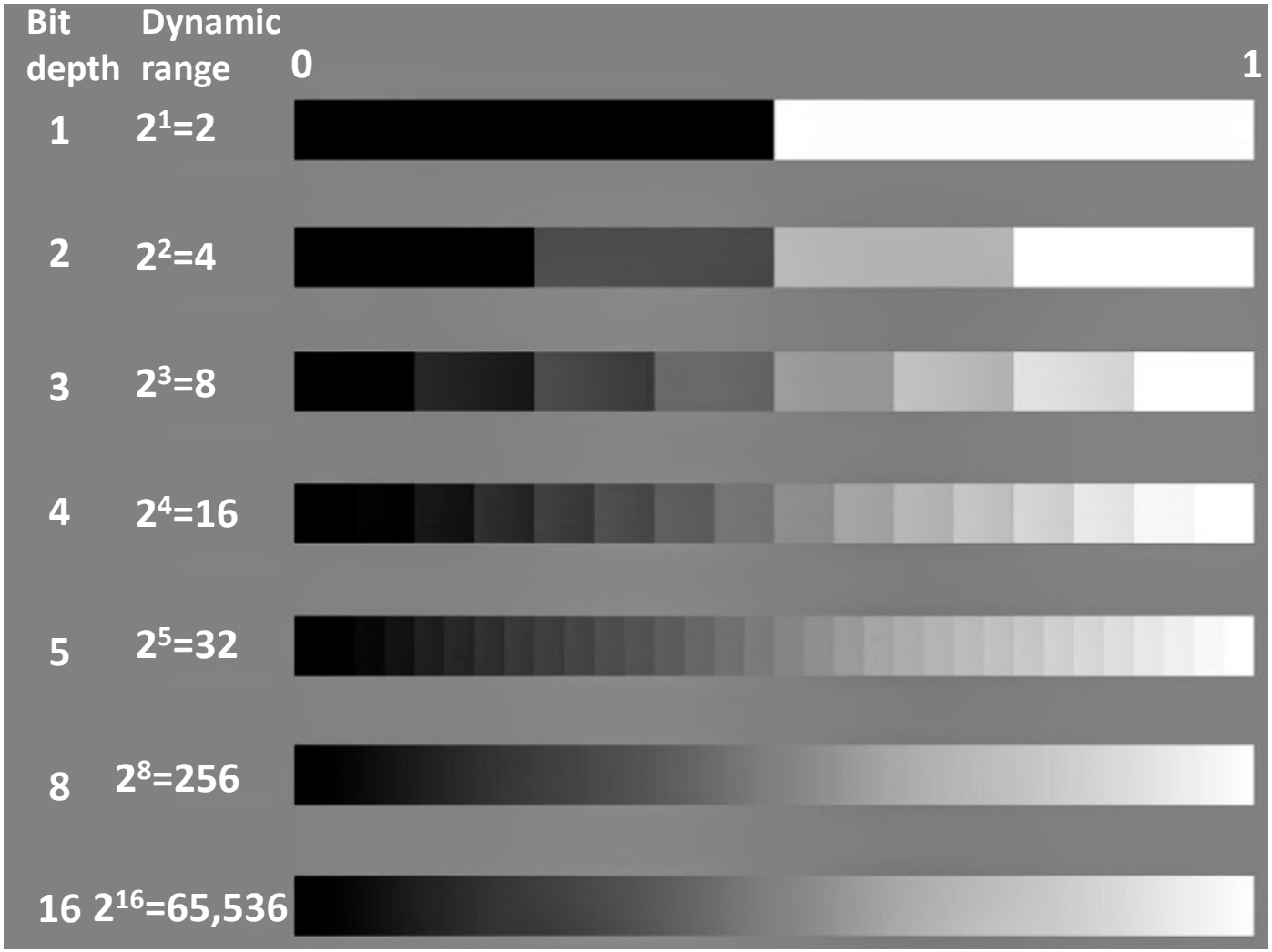
PPI: 72 192X192 36K

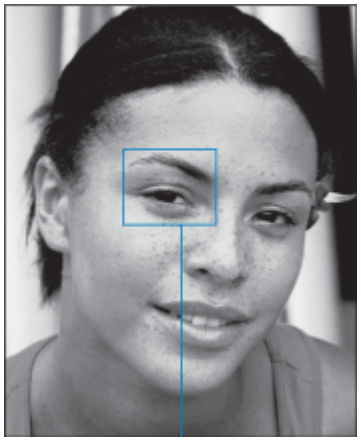


PPI: 150 400X400 156k



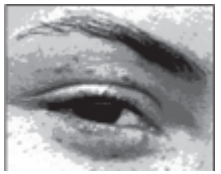
Bit depth and dynamic range



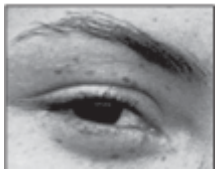


1 bit

2 bits



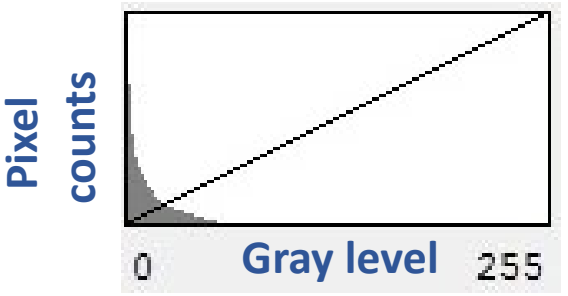
4 bits



8 bits

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

Manipulating image histogram

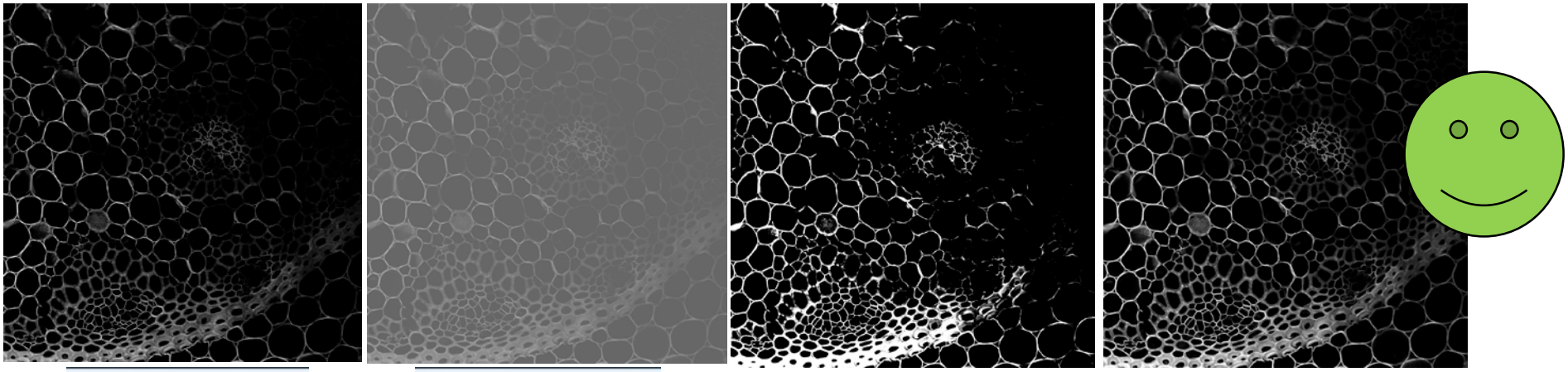


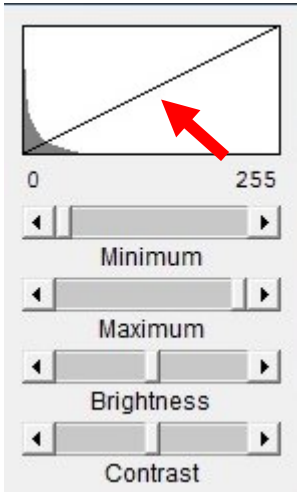
Unprocessed

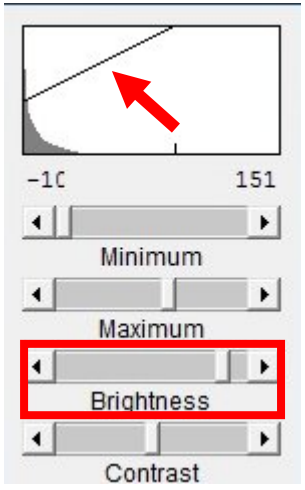
Brightness adjustment

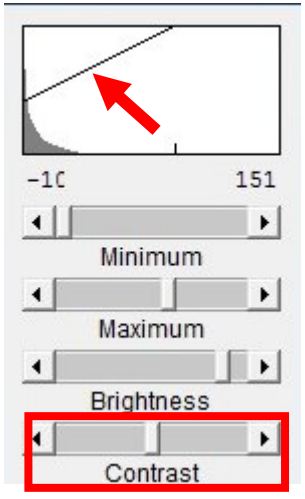
Contrast adjustment

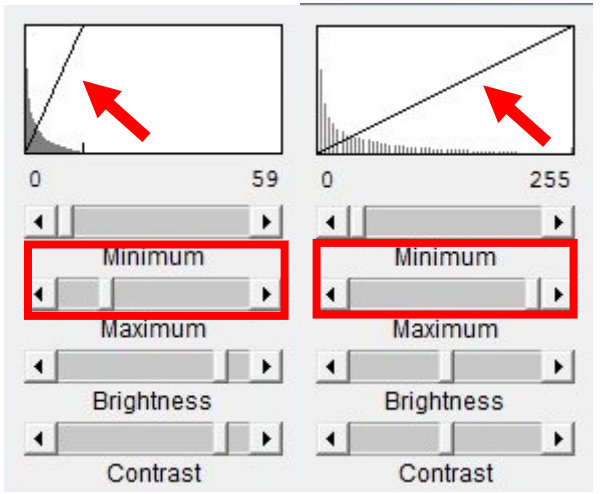
Contrast stretch









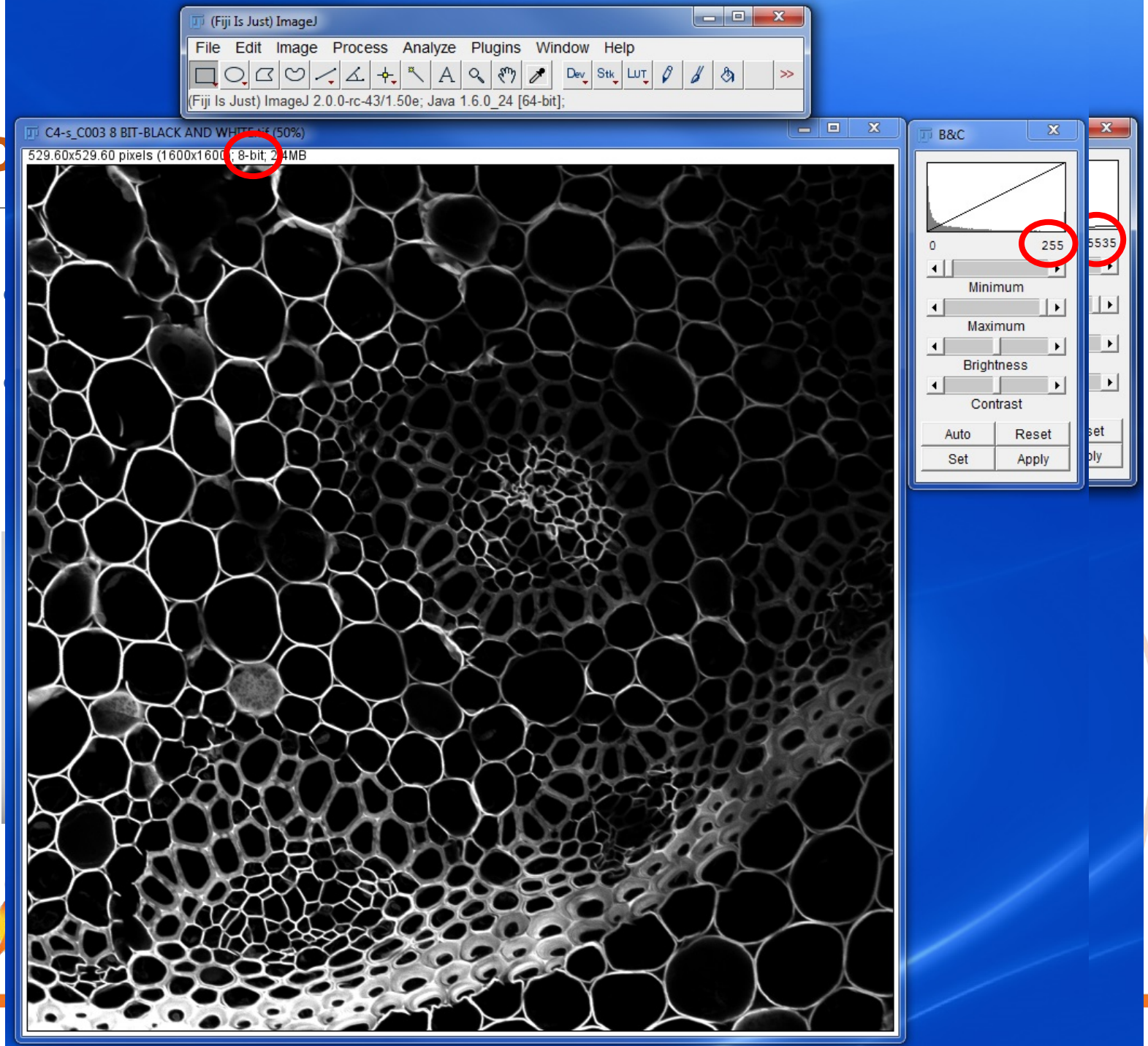


Co

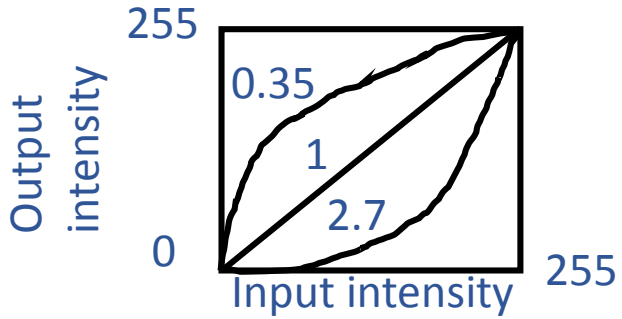
8-bit

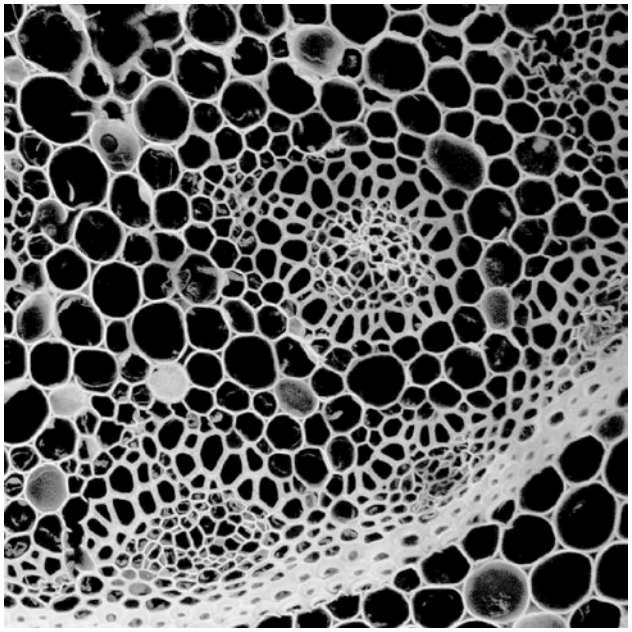
12-bit

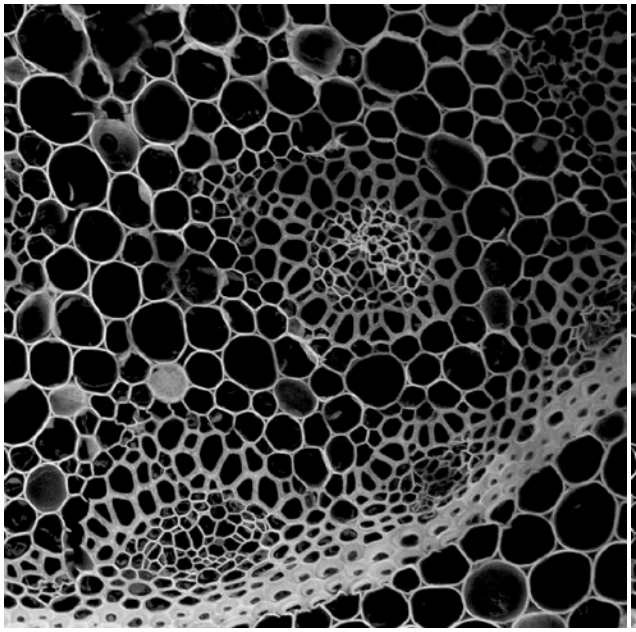
16-bit

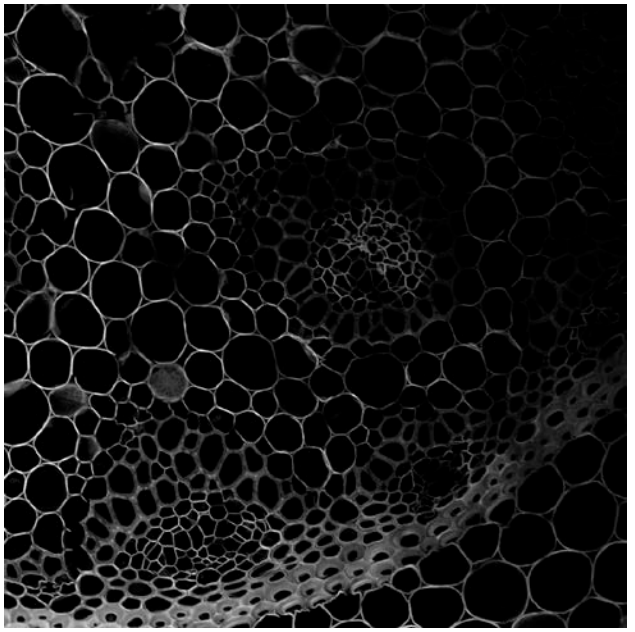


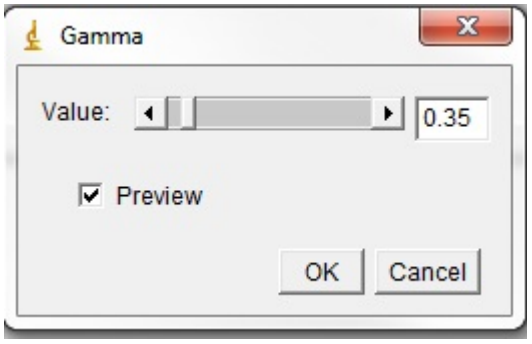
Gamma adjustment

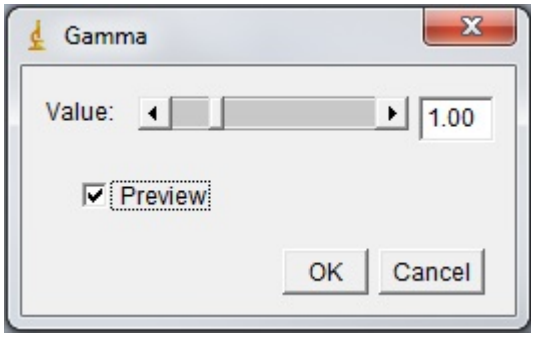


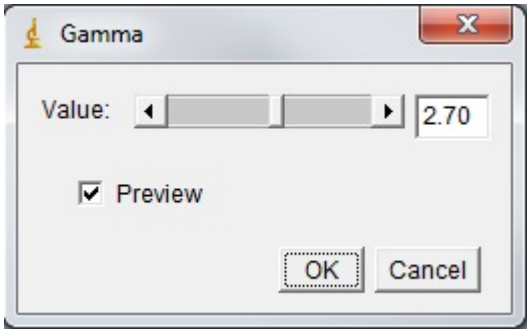






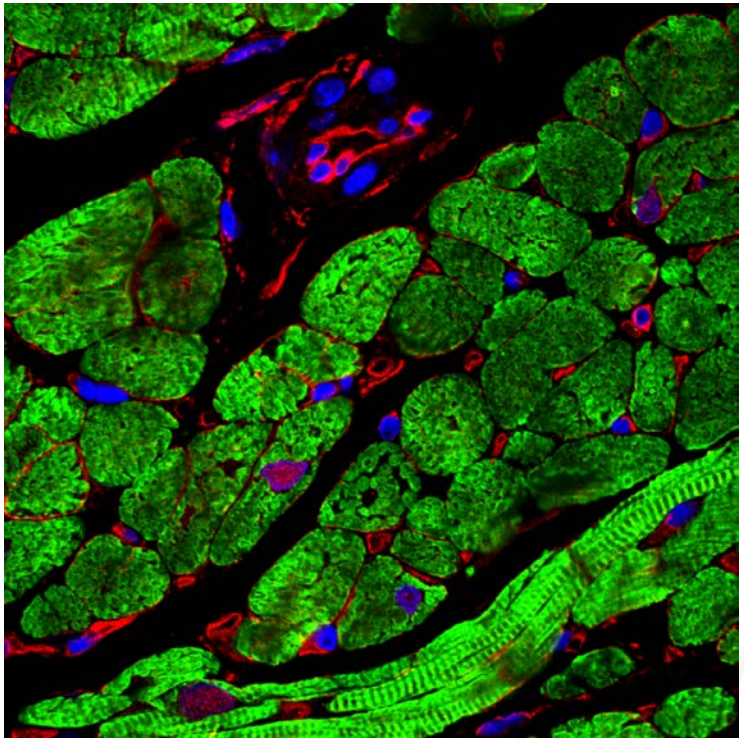




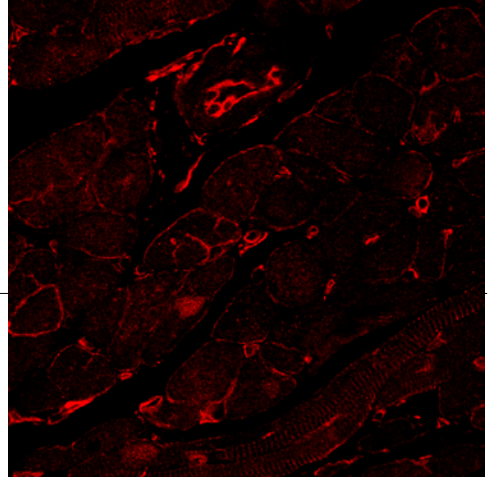


Color images

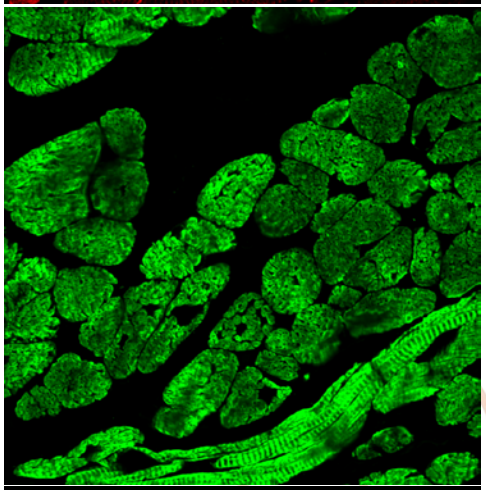
- Made up of 3 gray scale images
- Can be 8 or 16 bits per channel



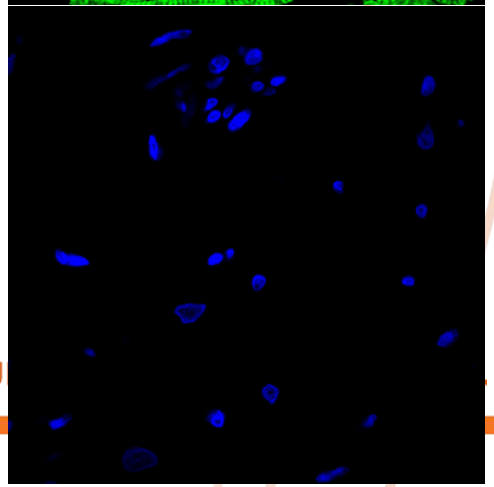
Red



Green



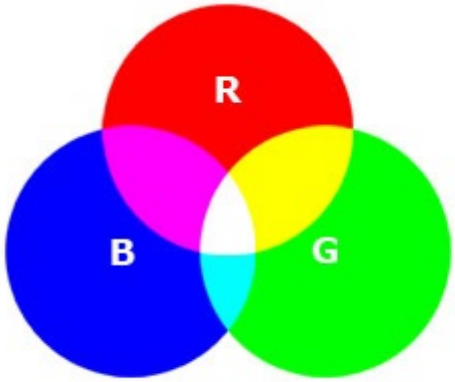
Blue



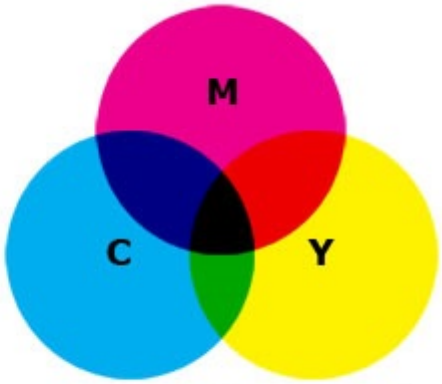
Finding cu

Image courtesy: Kyi from Cardiology

Color models: RGB & CMYK



RGB - Additive Colors



CMYK - Subtractive Colors

Red
Green
Blue

Cyan
Magenta
Yellow
Black

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 preserving the metadata

Display: general formats

Always keep your original data!

Image formats: compression & general formats

Compression

- Lossy: JPEG, GIP, etc
- Lossless: Tiff, JPEG2000

Commonly used formats

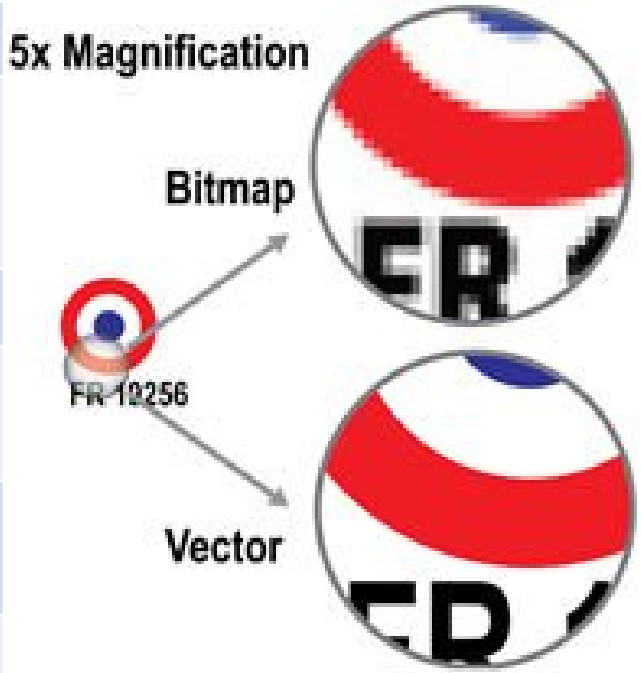
Recommended: **Tiff**

Generally good: **Tiff**, OME-Tiff, JPEG2000, BMP, PNG

Generally bad: JPEG, JPG, GIP

Bitmap & Vector images

	Bitmap/Raster	Vector
Made up of	pixels	paths
Produced by	digital image capture devices (true images)	drawing softwares: illustrator, ppt (math formula)
Example formats	jpg, tiff, bmp	Ai, cdr, dwg
Good used for	color images, mic images	Logos, texts
When enlarged	may appear pixelated	no resolution impact
Resolution	PPI/DPI. Res-dependant	res-independent. Res of output device
Image size	large	small

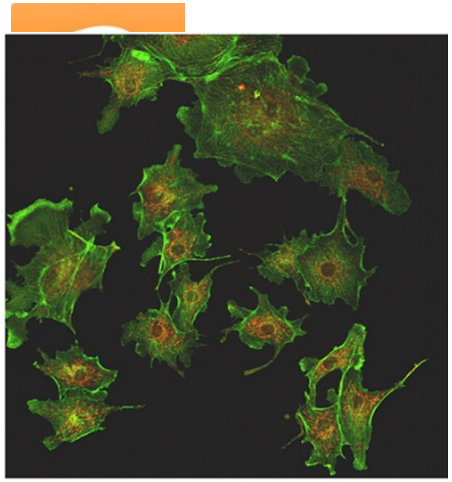


What manipulations are “legal”?

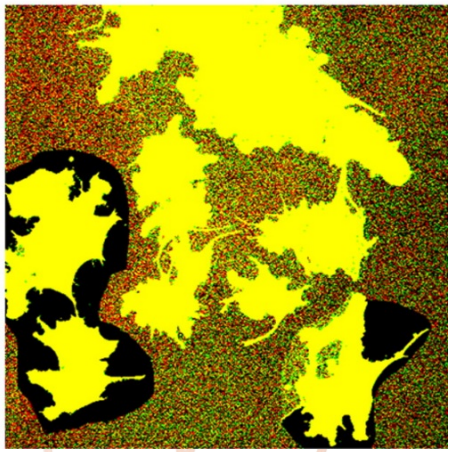
- Linear adjustment of brightness, contrast, color balance in moderation
- Background subtraction
- Cropping
- Reduce image resolution

- 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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Suggestions on image manipulations

- Safe-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 softwares 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/

Why building figures?

- Increase clarity of data
- Each figure should be submitted as a single file
- Meet journal formatting requirements

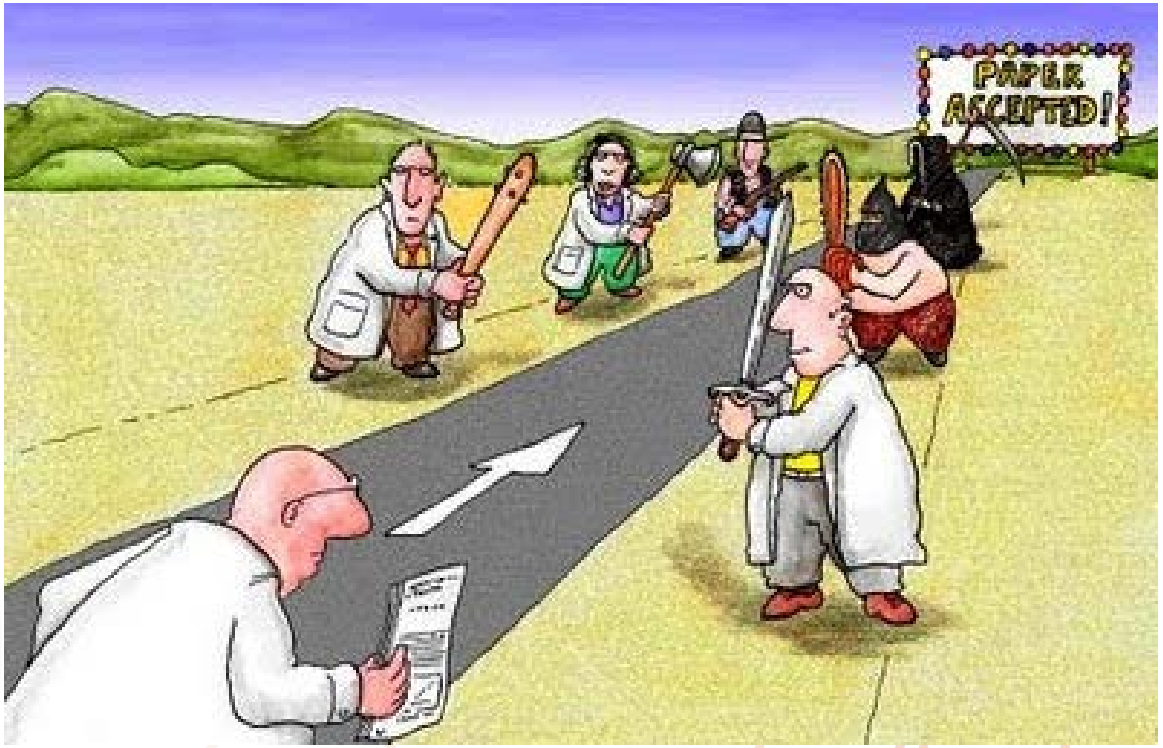


Image courtesy: http://tripleed.com/lindberg-rantatalo-hallgren-has-article-accepted-on-making-sense-of-paradoxes/

Figure making: rules

- Read the journal instructions first*:*

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

- Be mindful of acquisition resolution > 300 dpi
- Only manipulate images using image manipulation software
- Don’t manipulate images excessively
- Avoid the use of lossy compression

Figure making: software tools

We need a 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

Figure making workflow---3 steps

After you process and analyse your original images…

1. ImageJ (better than Photoshop): size, res, bit depth

2. Illustrator: assemble vector and raster components

3. Illustrator: export the final figure file:
   RGB/CMYK, vector/raster, format, resolution etc

Figure making: an example

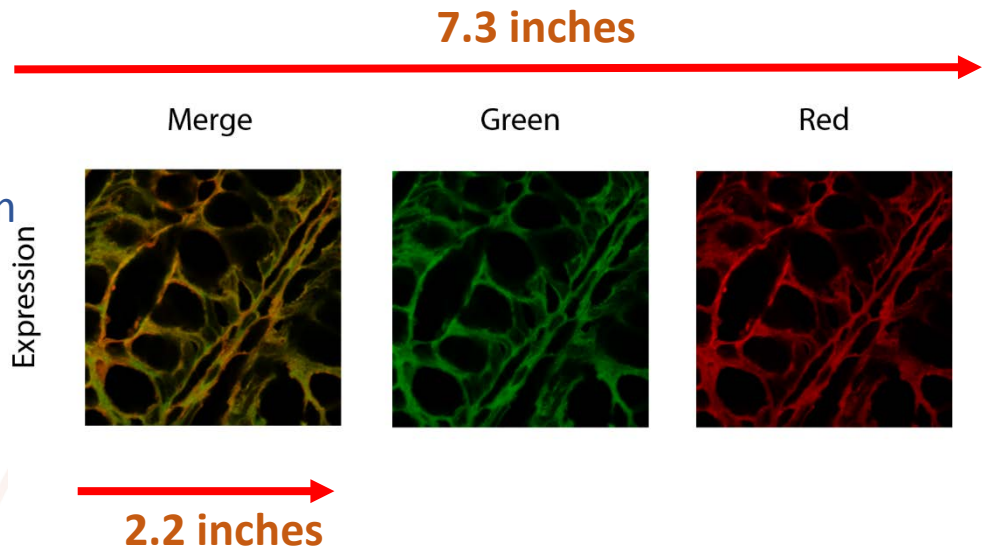
Journal requirements

- Double column figure: 7.3 inches/18.5 cm wide
- Output: 8 bit RGB, 300PPI, Tiff

3 fluo images & texts
each image width: 7.3/3=2.4
let's do 2.2 inch width

To be assembled images

- Size: 1600X1600 Pixel, 70.356x70.356 um
- Conversion: 1 um = 0.02 inch
- Acquisition resolution
  1600/70.356/0.02=**1137** PPI



Why Illustrator not Powerpoint?

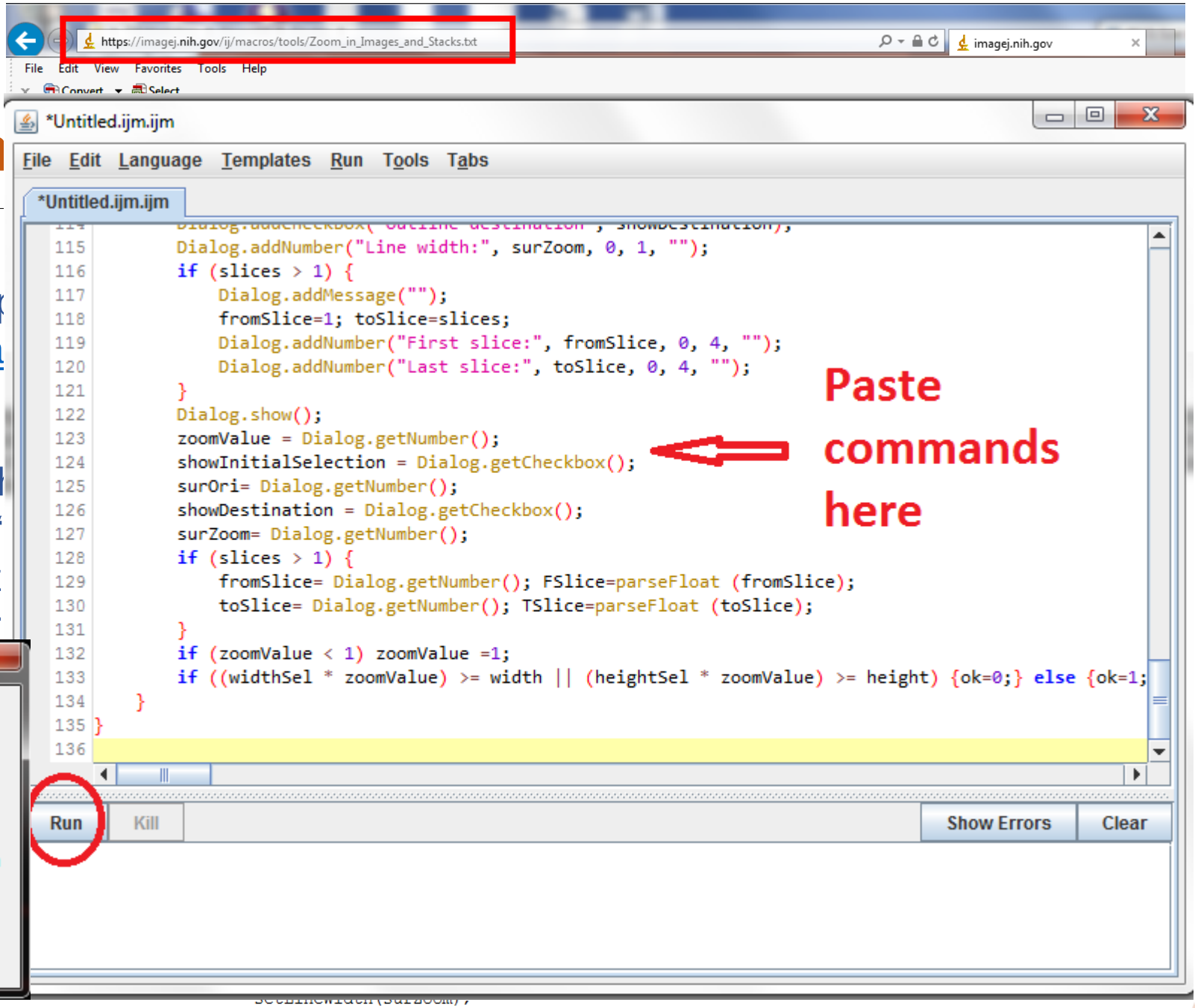
	Powerpoint	Illustrator
Image resolution (new file)	No control	Controllable
Image resolution (export)	Default: 72 DPI < 300 DPI	Controllable
White margin	No control	Auto adjust
Meet journals requirements	May not*	Yes
Good for	Presentation	Printing

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

Crea

1. Open up
https://ima

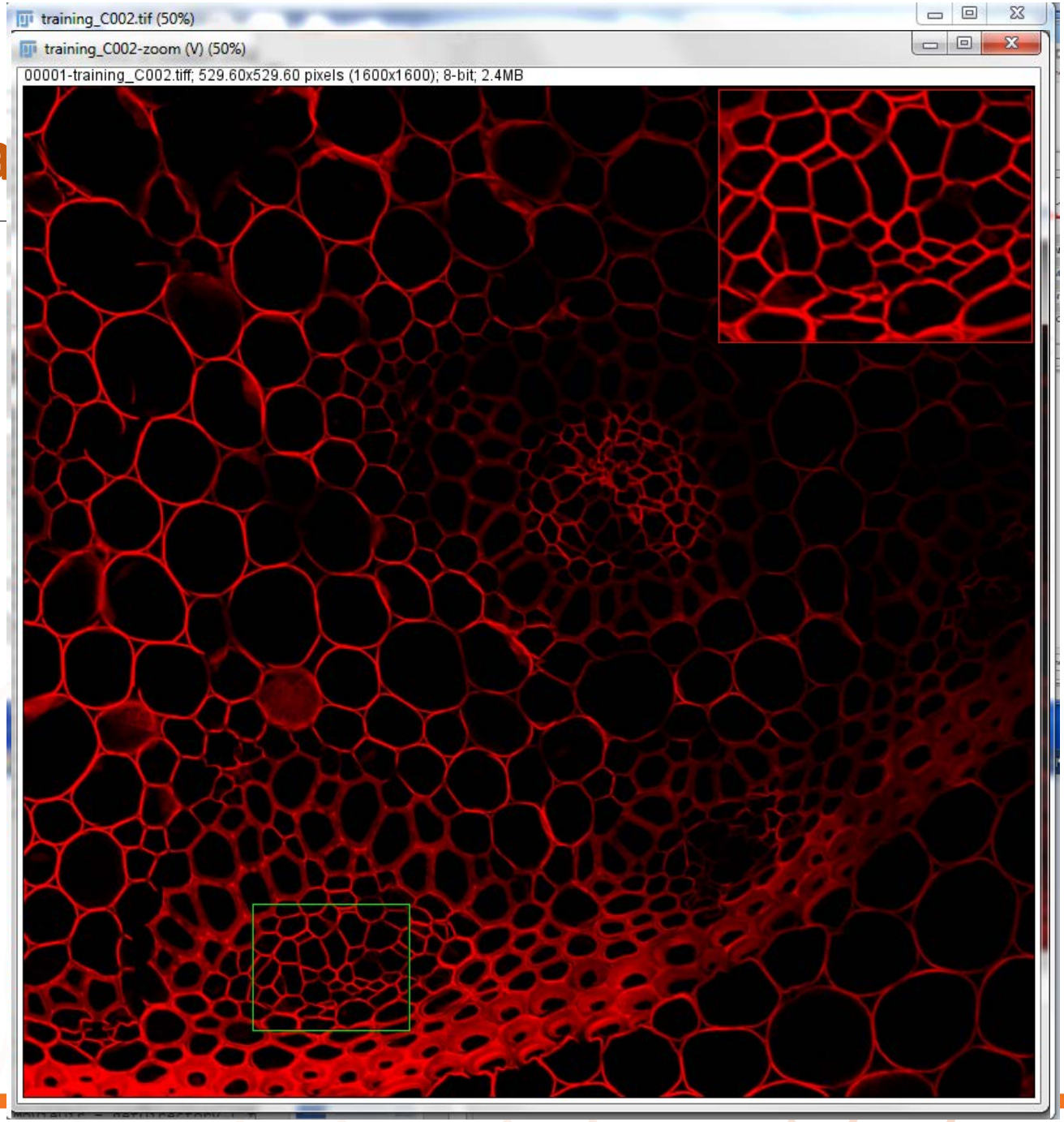
2. In FIJI:
- Open th
- Press "
- Paste t
- Click "r



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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Thank you!

Upcoming Imaging Events

When	What	Where
2:30-4:00 pm June 13	Westmead Imaging Community: New Products from Leica and Introduction to Cell Profiler	CMRI Seminar Room 1
11-5pm 14 June	User meeting: QC for acquisition & analysis Workshop on Olympus VS 120 image analysis	WIMR Conference Room Level 2
April-July ?	Art in Science	TBC
1-4.30pm 4 July	Seeing is believing	WIMR Conference Room Level 2
11-1pm 22 Nov?	Live cell imaging (TBC)	WIMR Conference Room Level 2