10 Solutions and tips for bioimage figures

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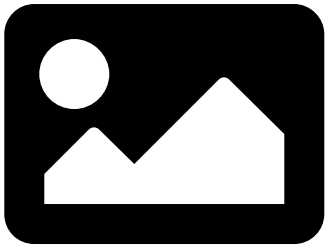
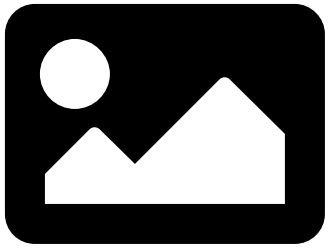
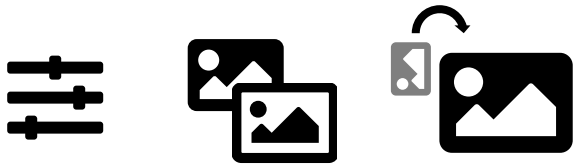
1

Make image copy





Work only on copy

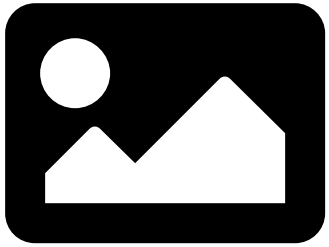


Save original microscope image

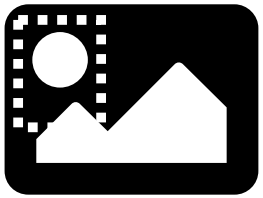
2

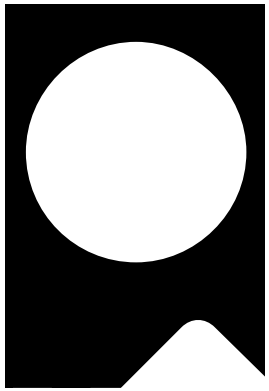
Magnification

What size on final page?



Crop, show zoom, always indicate position!

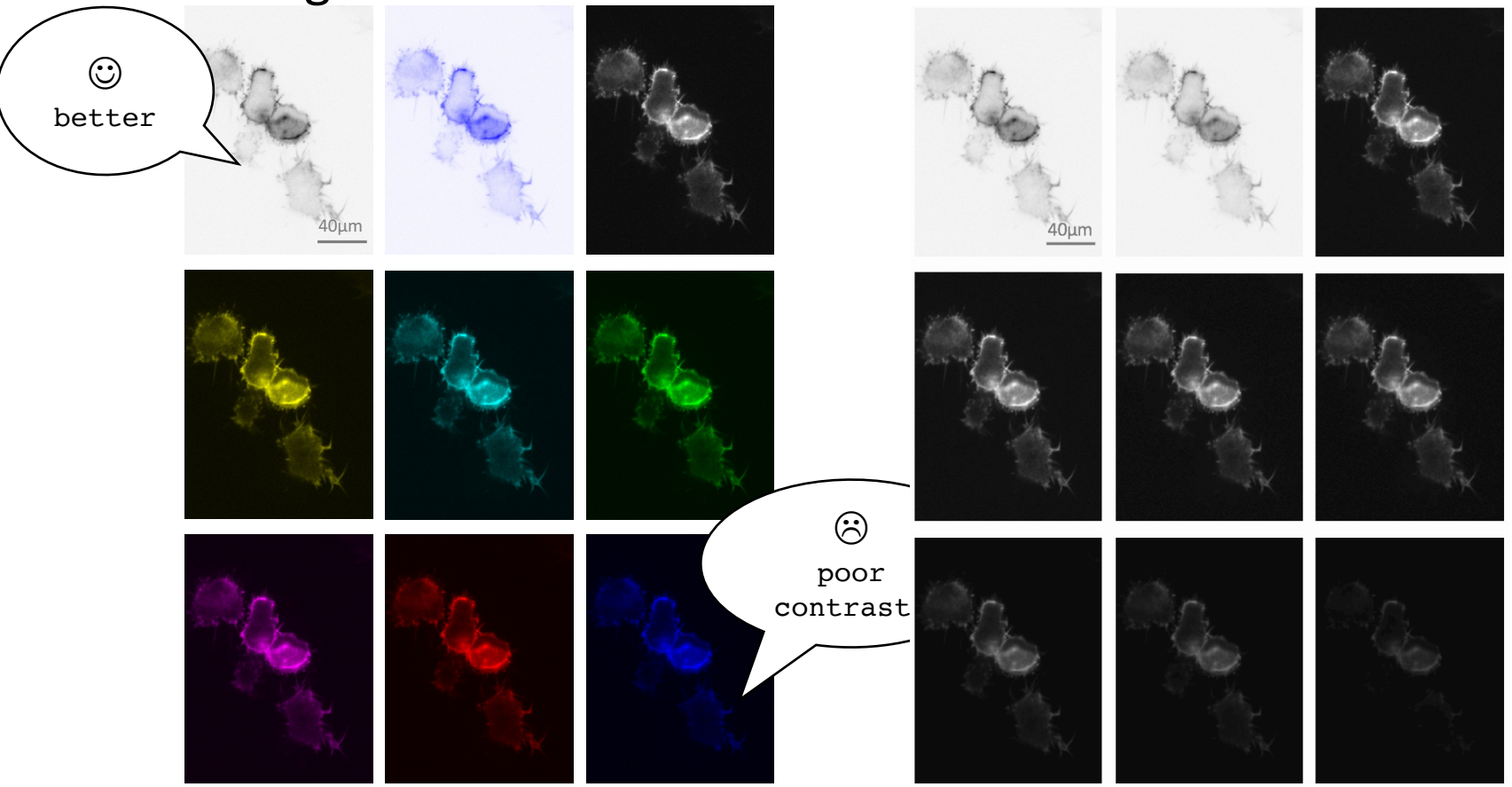




Colors: 1-color image

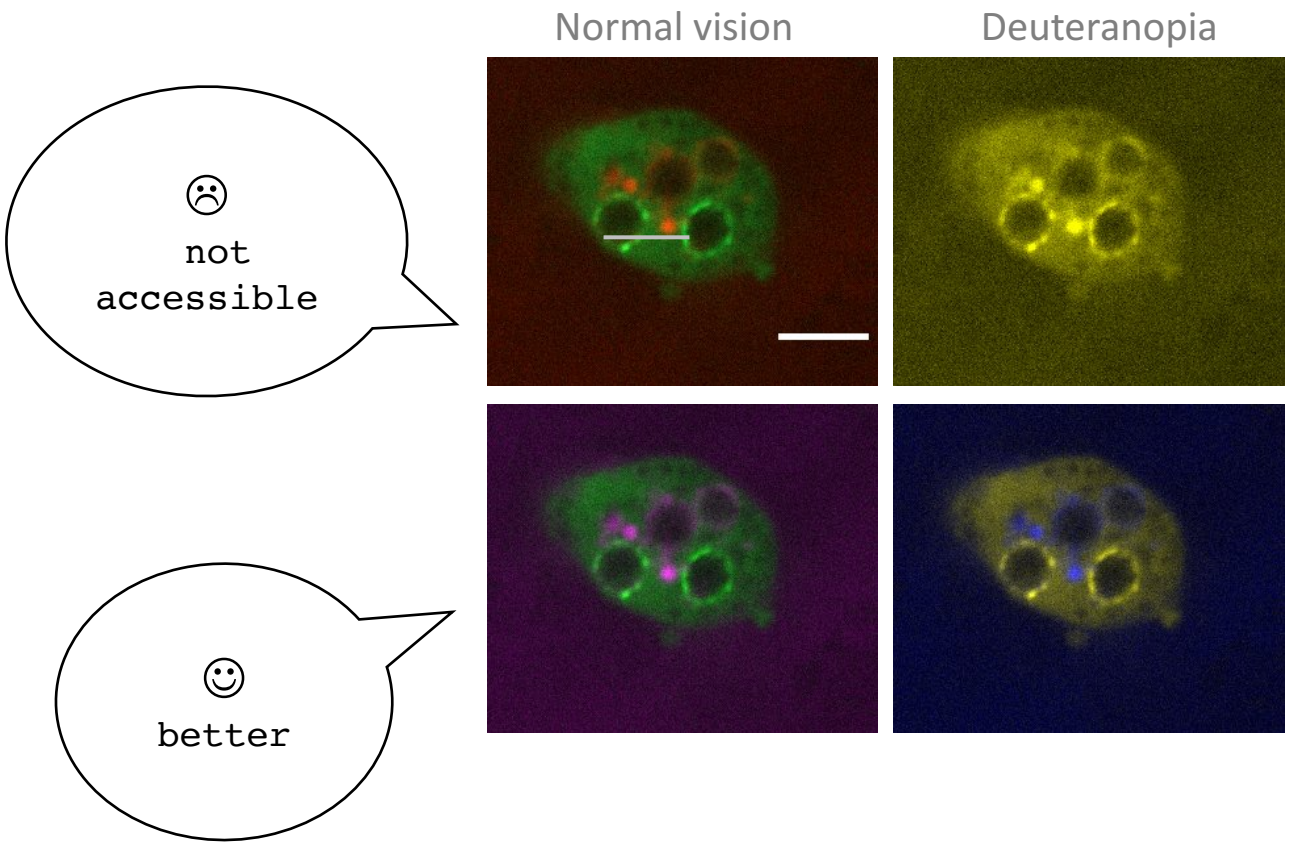
High contrast of signal on background color

Test visibility: greyscale



Colors: 2-color image

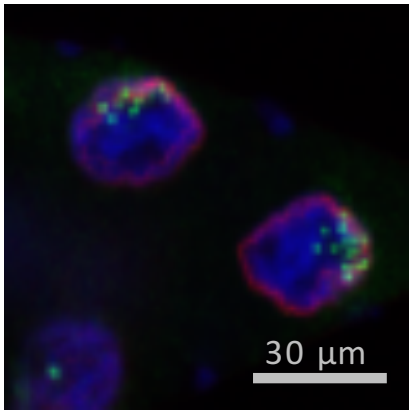
Be colorblind safe!



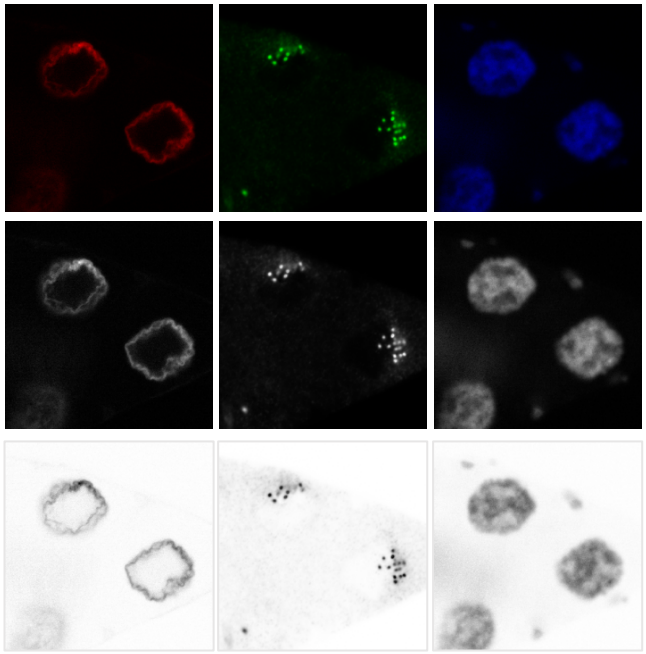
Colors: 3-color image

Be colorblind safe!

(You will struggle…)



Split channels, better in greyscale



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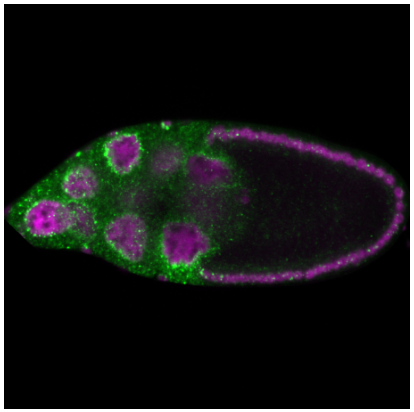
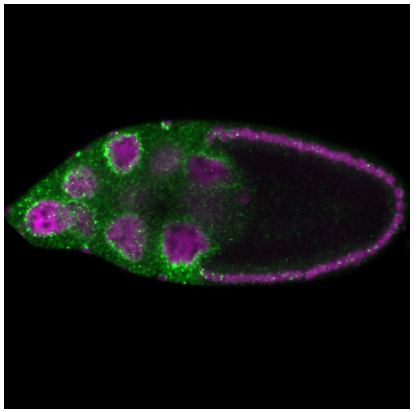
Annotate colors

Explain all colors used!





DNA, magenta
mRNA, green

Annotation also color blind safe! ☺

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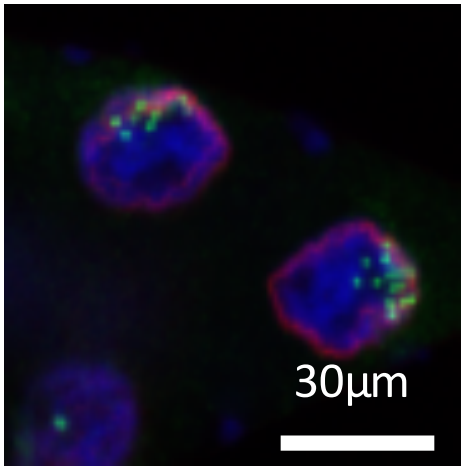
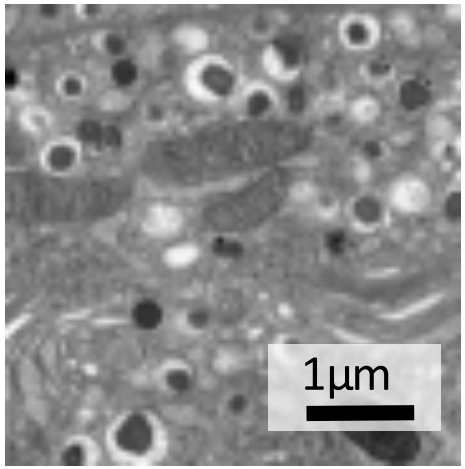
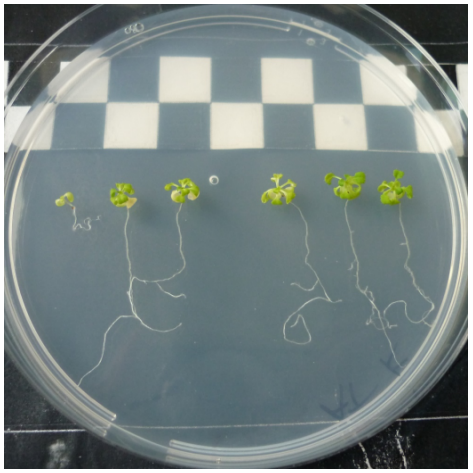
Annotate scale

Every scientific image need scale information



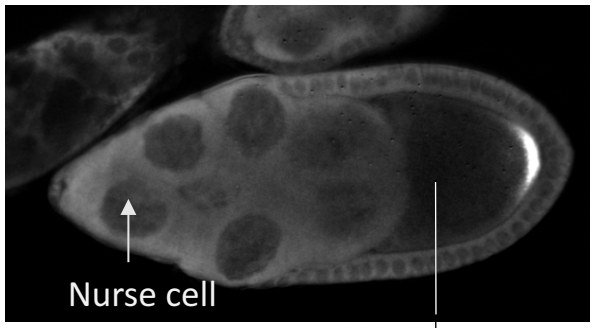


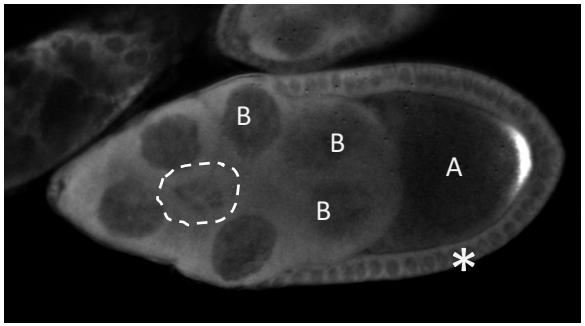


*** Magnification statement problematic

Annotate features

Lines, Arrows, Regions-of-interest, Symbols, Lettercode



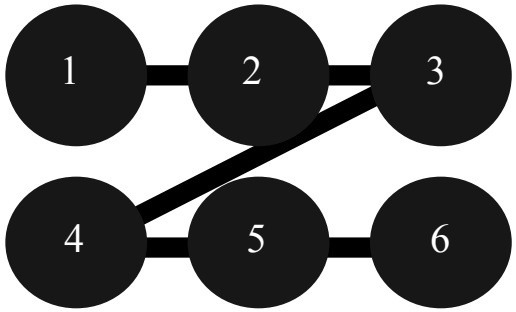


A Oocyte
B Nurse cells
* Somatic Cells

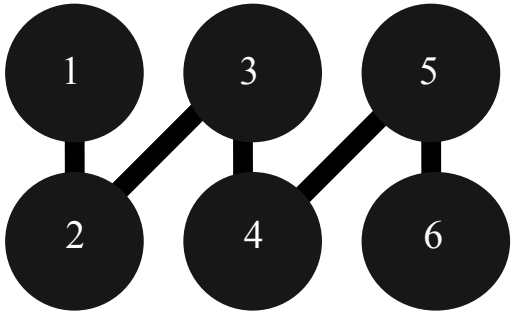
Layout

Audience understands reading direction of images

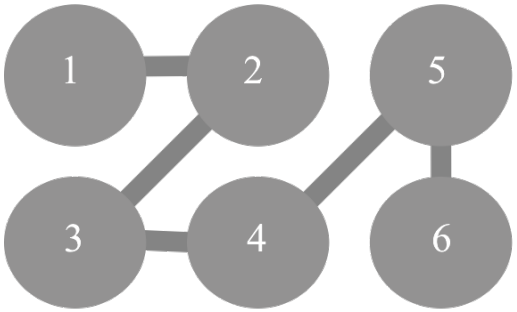
Images in rows



Images in columns



Don’t mix!



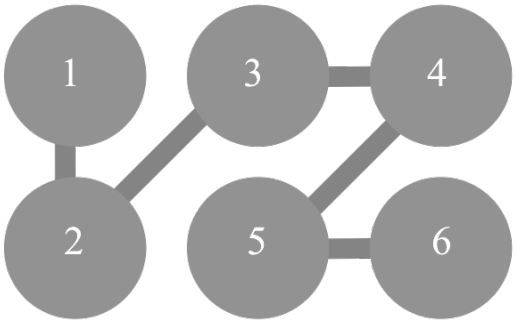


Figure legend

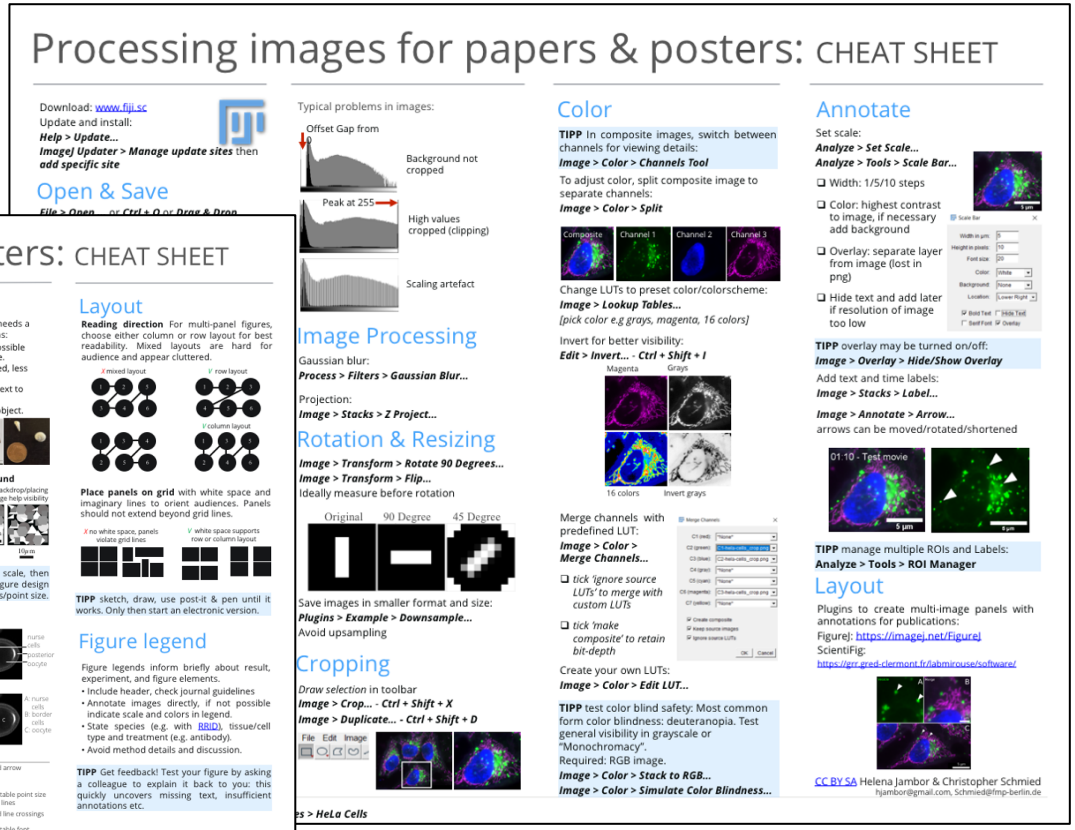
No image is self-explanatory

Blog Total Internal Reflection by Brooke:

https://totalinternalreflectionblog.com/2021/08/11/the-devil-in-the-details-a-short-guide-to-writing-figure-legends/

Help is near *Cheat sheets for novice users*

Christopher Schmied & Helena Jambor, F1000R, 2020



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Publishing images for papers & posters: CHEAT SHEET

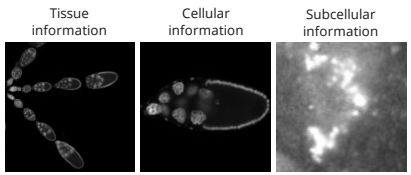
Image check list

Before publishing, ensure images are informative, truthful, and legible.

- ❑ **Colors visible?** Grayscale and dark colors on light background have highest contrast. Test visibility in color blind mode e.g. with colororacle.org
- ❑ **Annotations complete?** Explain annotations and abbreviations in figure legend. Hint: best avoid abbreviations.
- ❑ **Image and text size on page:** Test print figures: are image features visible is text legible?
- ❑ **Image resolution:** Be careful with image compression when saving, avoid pixelated images.
- ❑ **No manipulation** No individual image feature may be enhanced, obscured, moved, removed, or introduced.

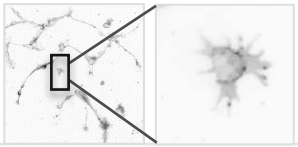
Magnification

Evidences in images must be visible to be convincing. Scientists should decide on the necessary detail and fill the frame to maximize the information/pixel.



Zoom, Insets

Insets or zoomed regions-of-interest are used when two magnifications are needed. Indicate inset position in original image, do not obstruct key image features.



Color

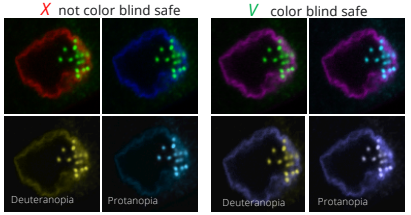
Color or not?

- Photo of natural appearance, color helps.
- Micrograph of a colored stain (e.g. histology), color helps.
- Micrographs of fluorescent stain, imaged in grayscale, consider grayscale.
- Micrographs of 2-3 fluorescent stain color, use color-blind safe combination.
- Electron micrographs, grayscale by default, use grayscale.

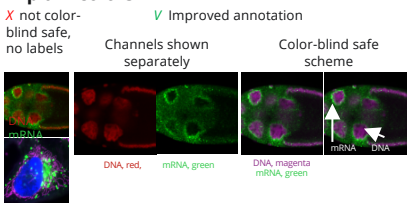
Which color? Visibility depends on color lightness and background color.

To a color blind, some colors are not distinguishable, often red and green (right).

Pick color blind safe combination:

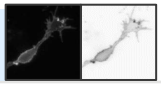


Explain colors



Resources for color Channel colors are adjustable in ImageJ/FIJI, referred to as lookup tables (LUT). Colors can also be inverted and new LUTs defined.

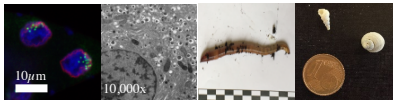
TIPP Highest contrast: dark objects on light background, and in grayscale.



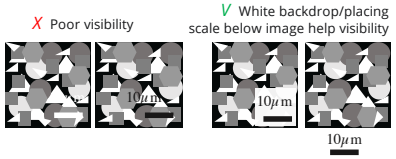
Annotate

Scale information Every image needs a scale to relate it to reality. Options:

- Scale bar of known length, if possible dimensions annotated in image.
- Magnification of set-up indicated, less intuitive and error-prone.
- Tape measure/ruler included next to object in photo.
- Every day item shown next to object.

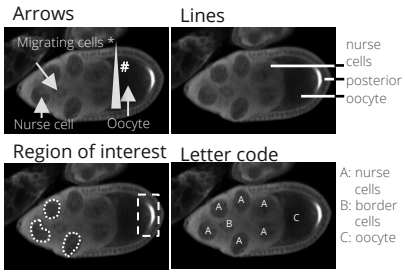


Annotation on a busy background



TIPP in FIJI to insert a very thin scale, then add rectangle as scale bar with figure design software to better adjust thickness/point size.

Point out details

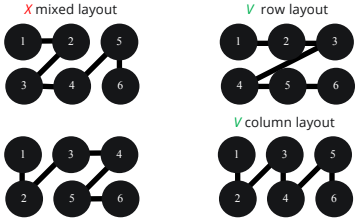


	Use	Tips
Arrows	Point to structure, show direction/ movement (*)	Align, avoid arrow crossings
ROI	Point to entire structure	Choose suitable point size for dashed lines
Lines	Label at line end	Align, avoid line crossings
Letter	Label many features	Choose suitable font (sans serif)

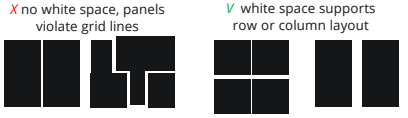
TIPP Avoid overlapping annotations and obstruction of key image features. Annotation colors should be color blind safe and aligned.

Layout

Reading direction For multi-panel figures, choose either column or row layout for best readability. Mixed layouts are hard for audience and appear cluttered.



Place panels on grid with white space and imaginary lines to orient audiences. Panels should not extend beyond grid lines.



TIPP sketch, draw, use post-it & pen until it works. Only then start an electronic version.

Figure legend

Figure legends inform briefly about result, experiment, and figure elements.

- Include header, check journal guidelines
- Annotate images directly, if not possible indicate scale and colors in legend.
- State species (e.g. with RRID), tissue/cell type and treatment (e.g. antibody).
- Avoid method details and discussion.

TIPP Get feedback! Test your figure by asking a colleague to explain it back to you: this quickly uncovers missing text, insufficient annotations etc.

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hjambor@gmail.com, Schmied@fmp-berlin.de
Images © Helena Jambor, Andreas Müller (EM)
Icons © Font Awesome, Template Rstudio.

Processing images for papers & posters: CHEAT SHEET

Download: www.fiji.sc
Update and install:
Help > Update...
ImageJ Updater > Manage update sites
then ***add specific site***

Open & Save

File > Open ... or ***Ctrl + O*** or ***Drag & Drop***
Special Formats:
Plugins > Bio-Formats > Bio-Formats Importer

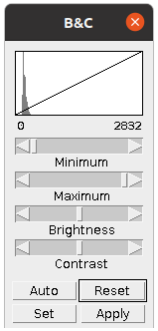
Review existing metadata:
Image > Properties... - *Ctrl + Shift + P*

File > Save As ...
TIFF: analysis, quantifications
PNG: presentations, figures
AVI: movies, animations, GIFs

TIPP duplicate image before processing:
Image > Duplicate... - Ctrl + Shift + D

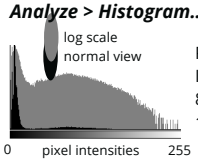
Brightness & Contrast

Image > Adjust > Brightness/Contrast...
Ctrl + Shift + C



- ❑ Auto: saturates the image by 0.35%
- ❑ Reset: to min & max or 0-255 for 8-bit
- ❑ Set: fixed values - use for comparisons
- ❑ Apply: histogram stretch using set min & max. **Use with caution!**

Check for problems with intensity sampling:
Analyze > Histogram... - ***Crtl + H***



Pixel count
Intensity range:
8-bit image [0-255]
16-bit image [0-65535]

TIPP during acquisition use ~75% of the intensity range histogram to avoid saturation or too low signal capture.

Typical problems in images:

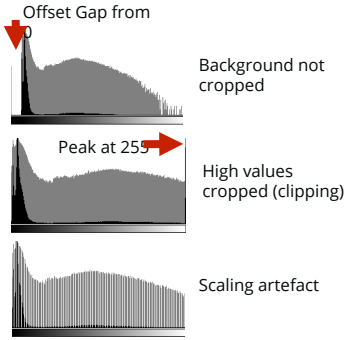


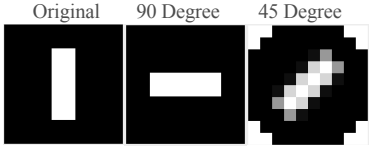
Image Processing

Gaussian blur:
Process > Filters > Gaussian Blur...

Projection:
Image > Stacks > Z Project...

Rotation & Resizing

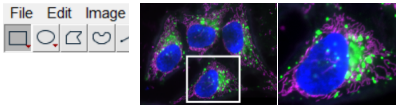
Image > Transform > Rotate 90 Degrees...
Image > Transform > Flip...
Ideally measure before rotation



Save images in smaller format and size:
Plugins > Example > Downsample...
Avoid upsampling

Cropping

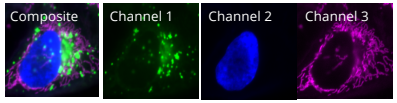
Draw selection in toolbar
Image > Crop... - ***Ctrl + Shift + X***
Image > Duplicate... - ***Ctrl + Shift + D***



Color

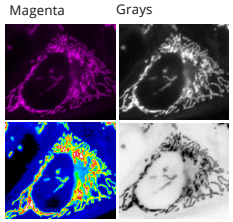
TIPP In composite images, switch between channels for viewing details:
Image > Color > Channels Tool

To adjust color, split composite image to separate channels:
Image > Color > Split

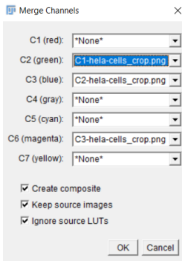


Change LUTs to preset color/colorscheme:
Image > Lookup Tables...
[pick color e.g grays, magenta, 16 colors]

Invert for better visibility:
Edit > Invert... - ***Ctrl + Shift + I***



Merge channels with predefined LUT:
Image > Color > Merge Channels...



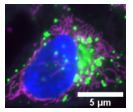
- ❑ *tick 'ignore source LUTs' to merge with custom LUTs*
- ❑ *tick 'make composite' to retain bit-depth*

Create your own LUTs:
Image > Color > Edit LUT...

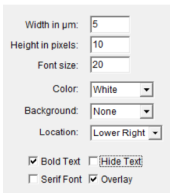
TIPP test color blind safety: Most common form color blindness: deuteranopia. Test general visibility in grayscale or "Monochromacy".
Required: RGB image.
Image > Color > Stack to RGB...
Image > Color > Simulate Color Blindness...

Annotate

Set scale:
Analyze > Set Scale...
Analyze > Tools > Scale Bar...



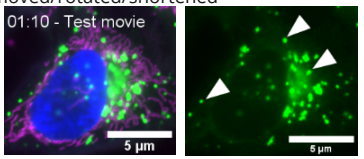
- ❑ Width: 1/5/10 steps
- ❑ Color: highest contrast to image, if necessary add background
- ❑ Overlay: separate layer from image (lost in png)
- ❑ Hide text and add later if resolution of image too low



TIPP overlay may be turned on/off:
Image > Overlay > Hide/Show Overlay

Add text and time labels:
Image > Stacks > Label...

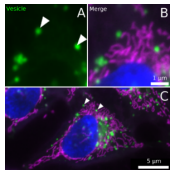
Image > Annotate > Arrow...
arrows can be moved/rotated/shortened



TIPP manage multiple ROIs and Labels:
Analyze > Tools > ROI Manager

Layout

Plugins to create multi-image panels with annotations for publications:
FigureJ: https://imagej.net/FigureJ
ScientiFig:
https://grr.gred-clermont.fr/labmirouse/software/



CC BY SA Helena Jambor & Christopher Schmied
hjambor@gmail.com, Schmied@fmp-berlin.de

Example image used: HeLa Cells - ***File > Open Samples > HeLa Cells***

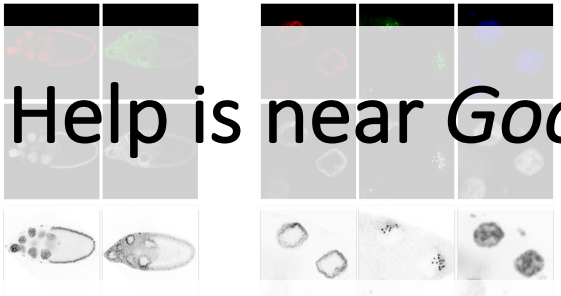
Help is near *Good/Bad Examples*

Jambor, Antonietti, Weissgerber et al PLOS Bio 2021

2. Split channels: **colorblind safe**, some colors have low contrast (see Fig. 6)

3. Show split channels in grayscale: **colorblind safe, high contrast**

4. Show split channels in inverted grayscale: **colorblind safe, highest contrast**



Example

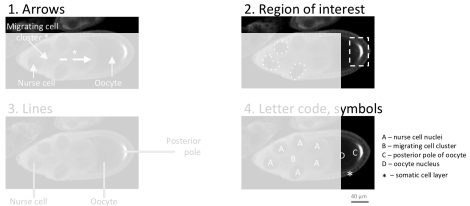
1. Colored annotation, not colorblind safe
2. Colored annotation, colorblind safe
3. Grayscale annotation

Visibility test

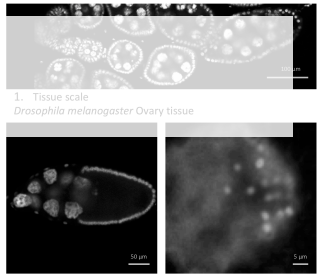
Colorblind vision render

Annotation strategies

1. Arrows
2. Region of interest
3. Lines
4. Letter code, symbols



A Magnification/zoom must match message

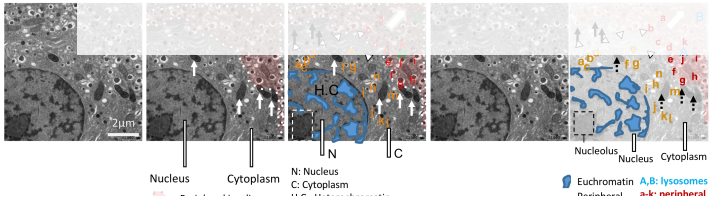


1. Tissue scale
Drosophila melanogaster Ovary tissue

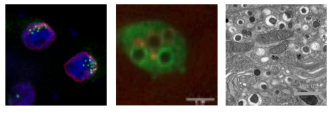
2. Cellular scale
Drosophila melanogaster
Egg chamber with oocyte

3. Subcellular scale
Drosophila melanogaster,
RNA granules in epithelial cell

1. No anno

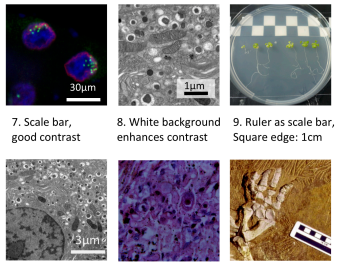


Poor scale bar examples



1. No scale bar
2. Scale bar illegible, poor compression
3. Scale bar blends into the background
4. Scale bar in color
5. Scale bar too small
6. Scale bar blends into the background

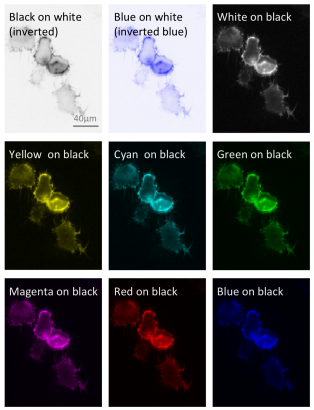
Clear scale bar examples



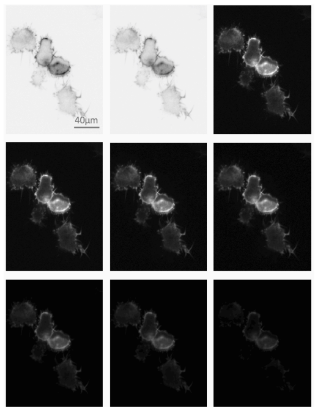
7. Scale bar, good contrast
8. White background enhances contrast
9. Ruler as scale bar, Square edge: 1cm
10. Scale bar, good contrast
11. Scale bar below image
12. Ruler as scale bar, Square edge: 1cm

Situation	Example	Visibility tests: Colorblind simulation	Visibility tests: Grayscale simulation
Color photo e.g. photograph, tissue staining with dyes.			
Microscope image, 1 color			
Microscope image, 2 colors			
Microscope image, 3 colors			
Electron microscope image			

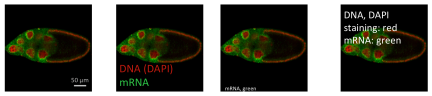
Color images



Grayscale test for visibility

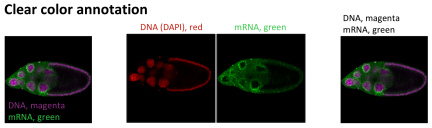


Poor color annotation



1. No color annotation
2. Color annotation not colorblind safe
3. Illegible and/or incomplete annotation
4. Annotation covers image content

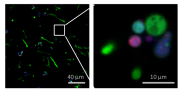
Clear color annotation



5. Colorblind safe annotation
6. Colorblind image and annotation (split color channels). Note: red has low contrast on black background, see Fig. 6.
7. Name colors when text must be in grayscale

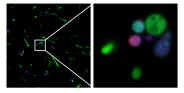
B Insets allow readers to see more than one scale

Poor inset examples

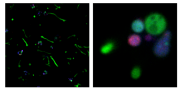


1. Wrongly placed inset (no cells in marked region)
2. No inset marked, inset obstructs data

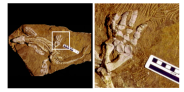
Clear examples



5. Inset indicated

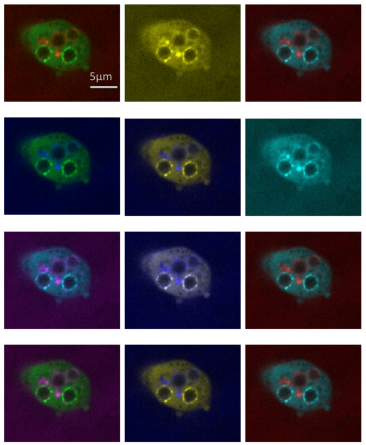


3. Inset origin not marked
4. No inset marked,

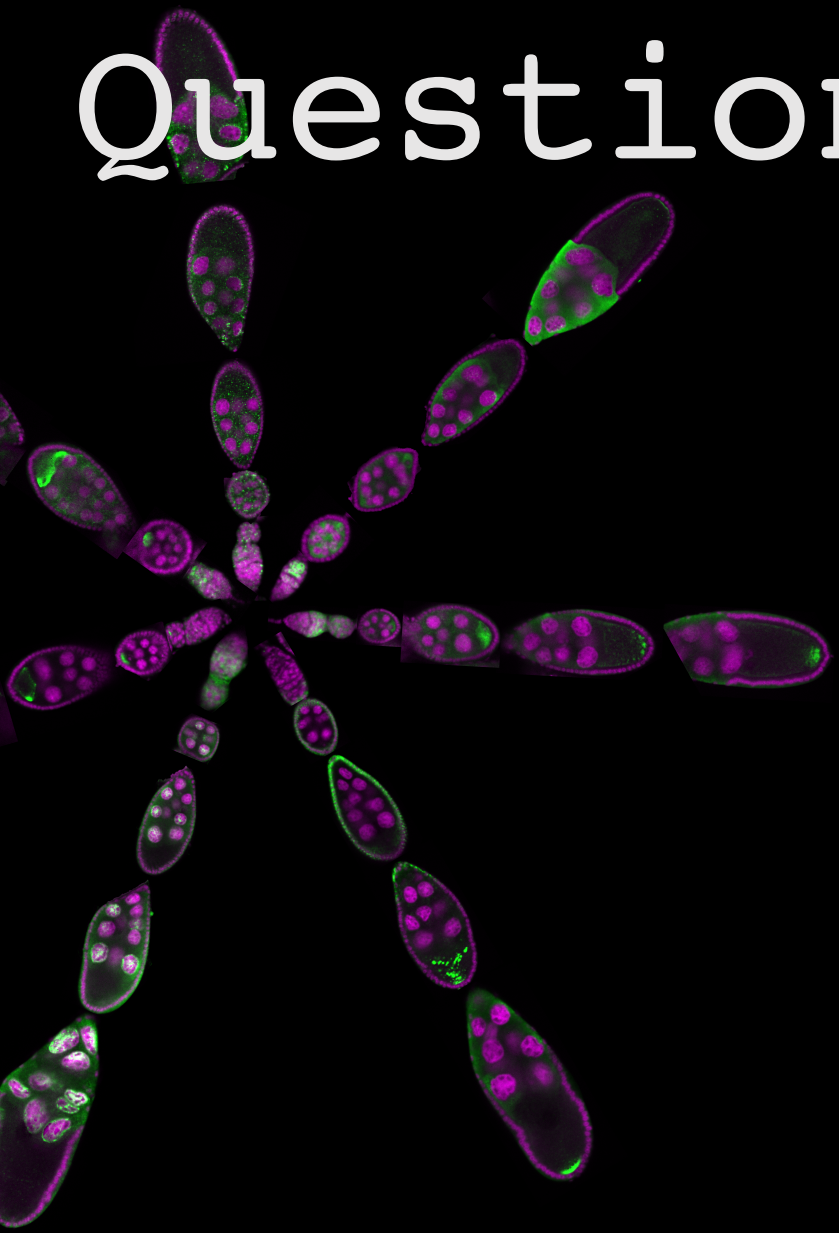


6. Inset indicated

Selected hue	Normal vision	Deuteranopia	Tritanopia
Green & red			
Green & blue			
Cyan & magenta			
Green & magenta			



Question time!



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