The following instructions are a best practice guide on how to generate images and videos in presentations and documents

If you have any questions, please direct them to cci@liverpool.ac.uk.

Table of Contents

Presentations with visual content – common problems

1. File size of the document is huge/unmanageable
    a. Sharing of documents is difficult
    b. Documents might not open on another PC
    c. Presenting documents on another PC might be difficult
2. Videos do not play
    a. Codecs missing → can be as bad as incompatibilities between different programs on same machine
    b. Incompatibility between operating systems

Keeping the document file size manageable

Initial considerations

What document should the file be included in?

- Word document (report, thesis, publication working copy)
- Presentation → Power Point, Keynote, Slides, Latex
- Poster

What is the reasonably largest number of pixels required for the item to display well?

- Video projector: HD: 1920 × 1080, 1280 × 720
- PC monitors: 1920 × 1080, 1280 × 960
- Single image on presentation slide:
    - Full frame: ≤ 1000 px
    - Half frame: ≤ 500 px
    - Third frame: ≤ 330 px
- Number of pixels required for word document might be even lower

Images

Software to generate figures

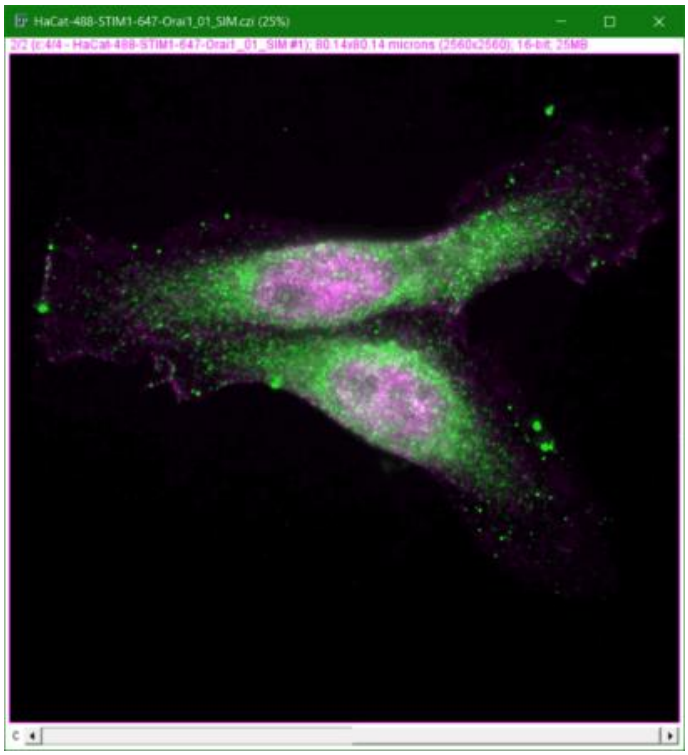
Imaris, Zen, Fiji (FigureJ), Omero.figure, Inkscape, Adobe Illustrator

Things to consider to reduce file size

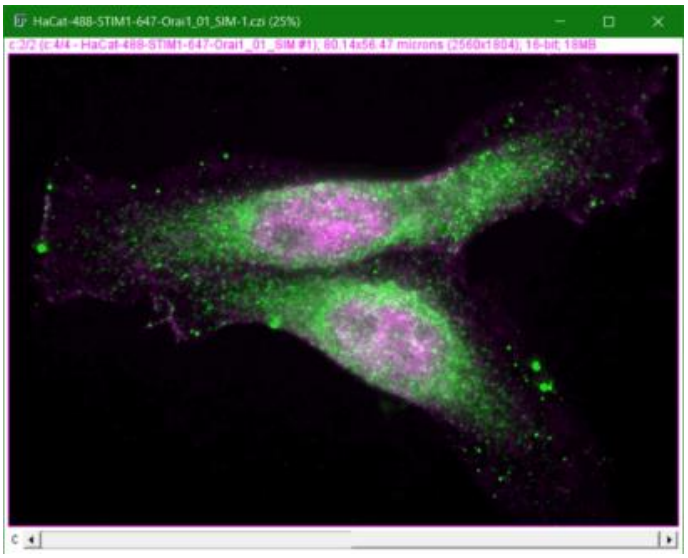
- Cropping
- Number of pixels
- Image type (bit depth, reduce to 8-bit grey scale or 8-bit Color)

Cropping

In Imaris, adjust the size of the application window to reduce number of pixels and avoid "empty" pixels



25 MB

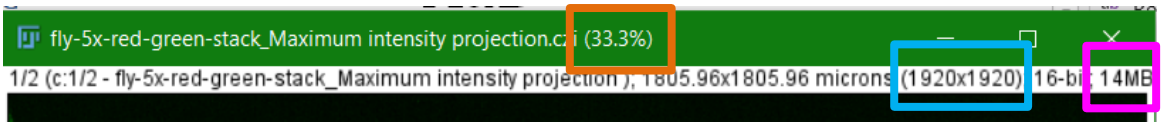


18 MB

Number of Pixels - Re-size/scale images in Fiji

Image status bar displays

- zoom at which the current image is displayed
- image dimension in pixels
- file size



Rule of thumb: zoom in/out to the desired image size on the screen, take note of the zoom and rescale accordingly via [Fiji> Image > Adjust >Size]:

For example for 33% zoom: (resized width) = (original width)/3, e.g. 640 = 1920 / 3

Resizing the image to 1/3rd in x and y, reduces the file size to 1/9th!

Image type

Greyscale

Imaging data should be 16-bit – 65536 grey levels

➔ down sample to 8-bit – 256 grey levels

Colour

RGB: 32-bit

- 8-bit red + 8-bit green + 8-bit blue + 8-bit Alpha (transparency)

➔ Down sample to 8-bit color: 256 colours

  - Caveat: Does not always result in a satisfactory image so skip if necessary

Practical Example

Input: 3 channel image: 2 fluorescence channels + TL

Output: montage 3 individual channels + merge

How to:

1. Open stack by dragging file into Fiji status bar or use [Fiji > File > Open…]
2. Re-order stack if required using stack sorter [Fiji > Image > Stacks > Tools > Stack Sorter]
3. Adjust LUTs as required [Fiji > Image > Lookup Tables]
4. Make composite [Fiji > Image > Color > Make Composite]
5. Adjust display parameters [Fiji > Image > Adjust > Brightness/Contrast…]

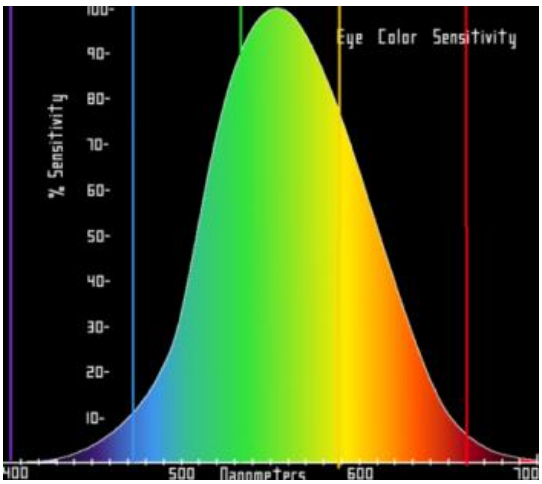
6. Crop [draw rectangle > right click > Duplicate] tick “Duplicate Stack”, close original stack
7. Re-size [Fiji > Image > Adjust > Size…]
8. Duplicate stack: [Right click in image > Duplicate…] tick “Duplicate Stack”
9. Add scale bar [Fiji > Analyze > Tools > Scale Bar …]
10. Flatten image to create RGB version of merged image [Fiji > Image > Overlay > Flatten]
11. Select cropped original stack, change all LUTs to greyscale [Fiji > Image > Lookup Tables]
12. Extract individual channels [Fiji > Image > Color > Split Channels]
13. Combine all images to one stack [Fiji > Image > Stacks > Images to Stack]
    a. Make sure no other single images are open in Fiji, otherwise they will be incorporated into the stack. Multidimensional stacks can be open and will be ignored during this operation.
    b. As the composite images is an RGB image, the other images are automatically converted to RGB images. The whole stack is of type RGB.
14. Re-order stack if required using stack sorter [Fiji > Image > Stacks > Tools > Stack Sorter]
15. Make montage [Fiji > Stacks > Make Montage]
    a. A scale factor can be applied at this stage but that might result in less clear scale bars so the re-sizing the single channel frame size is recommended in step 5
16. Convert RGB montage to 8-bit color [Fiji > Image > Type > 8-bit Color]
    a. Undo if resulting image is not satisfactory
17. Copy to system [Fiji > Edit > Copy to system] or on Windows: [Alt + c]
18. Paste into presentation/document

Script to Autmatically downsample and generate multichannel montage

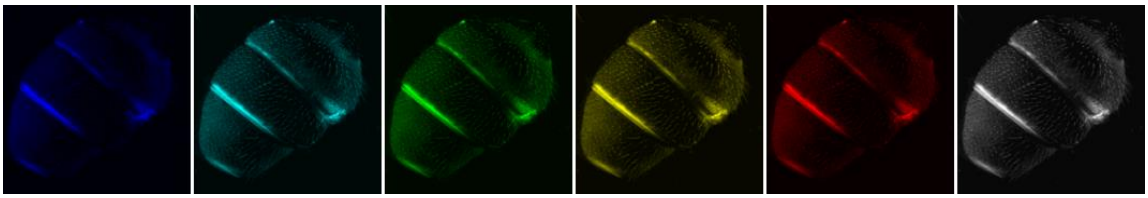
For easier and faster creation of downsampled multichannel montages, download the `multi_channel_montage.ijm` Fiji script from this Github repository. Click on the green “Code” button and download the repository as a zip file. Unzip and open the .ijm file in Fiji [Fiji > Open]. The script editor opens automatically. Open the stack that you want to convert into a montage. Adjust the channel LUTs to the final colours as you require for the composite image and re-order the stack if required. Delete any channels that should be excluded from the montage. [Fiji Script Editor > Run].

Pro tips

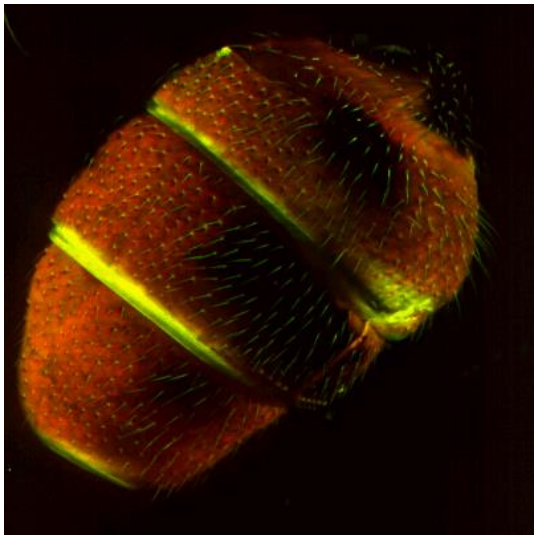
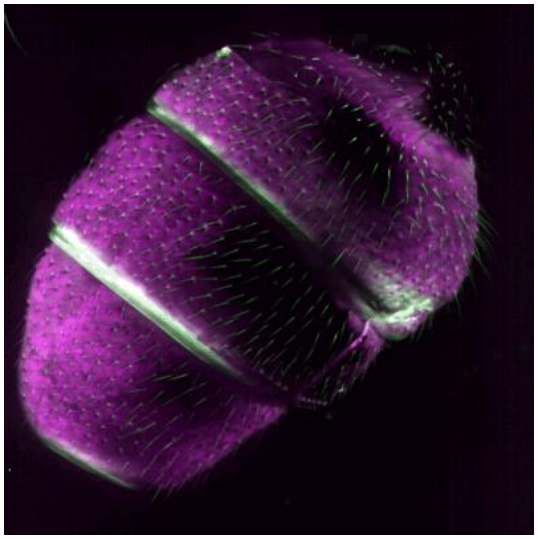
- Display single channel data as grey scale:
  - sensitivity of the human eye varies with wavelength/colour ↓ (image source)



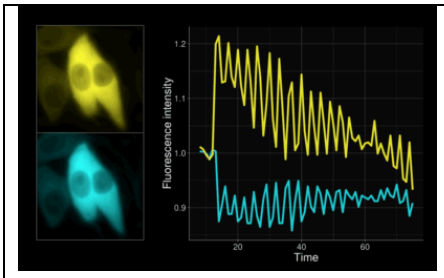
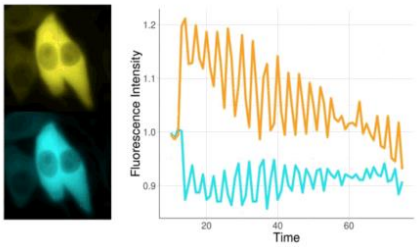
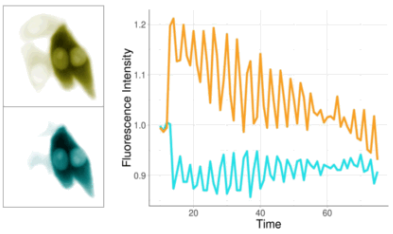
  - unaffected by colour blindness
  - any Look-Up-Table (LUT) on top of grey scale image data makes the displayed pixels seem darker:



- Multi-channel images: choose colour blind friendly combinations of pseudo colours
  - Red-green colour blindness is most common followed by blue-yellow
    - Opt for magenta-green
  - Use [Fiji > Color > Simulate Color blindness]

DON'T ☹	DO ☺
	

- Display fluorescence images on black slide background
    - Graphs on same slide should have black background as well

		
☺ High contrast between image details and black background Source	☹ High contrast between image details and white graph background → distracting	☺ High contrast between image details (inverted LUT) and white background Trick on inverting LUTs

Further reading

1. Effective image visualization for publications – a workflow using open access tools and concepts
2. Reproducible image handling and analysis

Videos

Video formats

Imaris:

- mp4 (H.264) – frequently used codec, playable on a lot of devices but not universal
- avi (uncompressed) – many possible codecs
- TIFF stack – the world is your Oyster
    - Create videos in Fiji/another software
    - Create animated GIFs in Fiji

Fiji

- Ships with save as “avi” or “animated gif”
    - Save any image stack as movie
- ~~Plugin: Save as movie (Last update 2015)~~
- ~~Mac users plugin: QuickTime Movie Writer (Last update 2010)~~

Why animated gif?

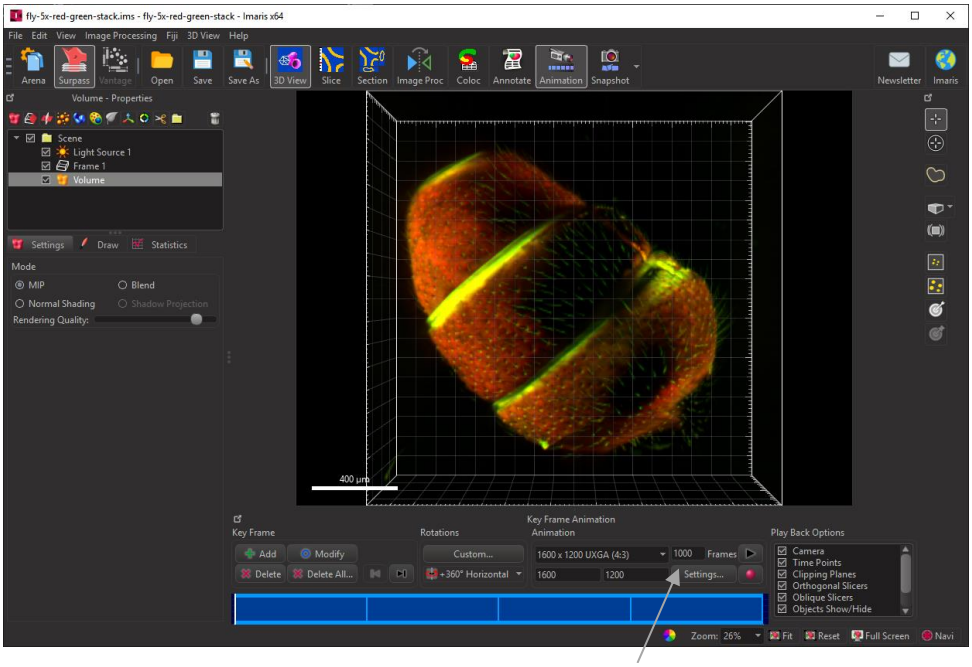
Pros	Cons
Small file size	Limited colours (256 colour depth)
Opens in any browser on most operating systems (without special plugins) – no dedicated player required	Few more steps to include as video in ppt
Create in Fiji	
Embeds fully in ppt presentation	

Things to consider to reduce file size

- Cropping - (same as in image section)
- Number of frames
- Number of pixels - (same as in image section)
- Image type (bit depth, reduce to 8-bit grey scale or 8-bit Color) - (same as in image section)

Number of frames

- How many frames are required so that the animation does not appear “stutter”?
- Z-stack – image analysis requires much larger number of slices than an animation
- Time series – image analysis requires a larger number of slices than an animation although the difference is not a great as for a z-stack



Number of frames between “key frames”
Aim for just about smooth
2.78 frames/degree → way too many

How to create an animated gif in Fiji with a small file size?

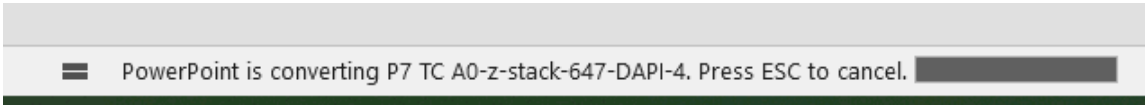
1. Open image file by dragging file into Fiji status bar or use [Fiji > File > Open…] or image sequence via [Fiji > File > Import > Image Sequence…]. Open as virtual stack if file is too large for PC RAM either as BioFormats Option ticked or [Fiji > File > Import > TIF Virtual Stack]
2. Adjust LUTs as required [Fiji > Image > Lookup Tables]
3. Adjust display parameters [Fiji > Image > Adjust > Brightness/Contrast…]
4. If multichannel data, make composite [Fiji > Image > Color > Make Composite]
5. Subset in z/time [Fiji > Image > Stacks > Tools > Make Substack…]
6. Re-size [Fiji > Image > Adjust > Size…]
7. Crop [draw rectangle > right click > Duplicate] tick “Duplicate Stack”, close original stack
    a. Use Maximum intensity projection as guide [Fiji > Stacks > Z Project… > Max Intensity]
    b. Draw rectangle in Max. Intensity Projection, add to regions of interest (ROI) manager [t]
    c. Select stack, click on region of interest in ROI manager, click on [Show all]
8. Duplicate stack: [Right click within ROI in image > Duplicate…] tick “Duplicate Stack”

9. Add scale bar [Fiji > Analyze > Tools > Scale Bar …]
10. Flatten image to create RGB version of merged image [Fiji > Image > Overlay > Flatten] → Images now RGB
11. Convert RGB montage to 8-bit color [Fiji > Image > Type > 8-bit Color]
12. Save as animated GIF [Fiji > File > Save as > Animated GIF…]
    a. Set global lookup table options: "Load from Current image"
    b. Set delay in milliseconds: default: 500ms → 2 fps
    c. Number of plays: 0 for looping continuously

Embedding an animated gif animation in ppt

1. Open Power point
2. [Insert > Video > Video on My PC…]
3. Navigate to folder containing animation file, set video format to "All files (*.*)"

4. Wait for file to be converted – status bar at the bottom of the ppt window



5. The file is now embedded and recognised as a video and video options are available in the top ribbon/menu
    a. No need to keep the .gif file in the same folder as the ppt presentation as it is fully embedded

Sharing of large files

1. WeTransfer for file size up to 2 GB on free account
2. Smash unlimited file size without account but files larger than 2 GB have lower priority over paid plan accounts and might have to "queue"

Bonus: Power Point presentations as animated gif

If you are using Microsoft office 365 Business [File > Export > Create an Animated GIF]

Microsoft 2019

1. Power point presentation [File > Export > Create PDF document]

2. Open pdf in Fiji [Fiji > File > Import > PDF]
3. Resize and convert to 8-bit color 😉
4. Save as animated gif