Fundamentals of Image Analysis and Visualization (the short version)

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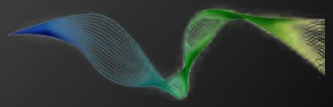
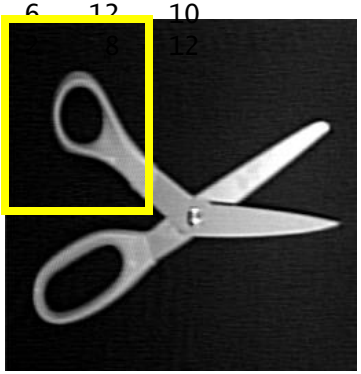


A digital image is a matrix of numbers (in this case bytes)

6	2	4	4	2	0	2	0	2	4	4	4	4	8	12	4	16	16	12	20
0	0	4	0	4	0	6	4	4	2	6	4	6	8	6	14	10	12	12	16
0	2	8	6	6	10	14	10	8	6	8	12	6	8	12	14	16	14	8	16
0	8	4	2	8	6	4	6	10	8	6	6	6	14	14	12	12	10	16	18
4	2	0	2	2	10	6	2	10	4	4	8	8	12	10	10	10	12	10	12
0	2	4	4	8	10	4	4	6	10	0	6	12	10	6	10	12	16	14	16
4	0	2	4	6	8	4	4	10	12	8	8	12	10	18	16	20	18	26	16
0	2	6	4	4	2	6	6	8	4	22	8	0	4	12	12	14	14	12	14
2	0	0	4	0	10	8	2	18	86	96	118	88	8	14	10	14	14	16	14
4	0	4	6	6	0	0	2	76	146	130	128	158	82	12	10	12	10	14	14
6	2	6	8	4	2	2	44	148	22	2	0	30	154	38	12	12	14	14	16
6	6	0	4	6	6	6	84	76	0	2	8	0	74	150	0	12	14	24	20
2	0	0	4	6	4	4	140	4	4	10	12	8	0	160	50	18	14	20	12
2	4	4	6	6	4	2	162	0	2	0	6	12	4	84	140	4	12	16	8
6	4	4	4	14	12	8	160	0	12	12	6	16	14	92	154	60	12	14	18
0	0	2	6	12	6	6	154	14	8	4	8	8	8	100	124	168	10	16	22
2	0	8	2	6	8	0	90	90	0	10	8	14	0	132	132	176	92	6	12
0	0	6	2	2	6	0	10	144	38	0	2	0	64	148	146	148	168	38	6
4	2	6	8	8	14	8	0	46	146	120	92	104	130	152	160	158	172	158	26
4	2	10	6	8	4	2	4	0	26	76	124	160	160	162	152	148	158	200	178
2	6	8	4	6	6	4	0	6	12	0	2	0	24	140	196	156	150	164	198
2	2	0	4	4	4	2	10	10	8	6	0	0	0	0	50	146	172	172	160
4	0	2	12	8	4	8	10	12	14	12	4	8	12	10	8	10	164	178	170
2	2	0	8	4	10	8	10	8	16	8	6	4	16	14	4	4	2	162	162
2	0	10	2	0	2	2	2	8	10	4	12	12	6	10	8	18	0	80	164
2	2	10	2	4	6	2	10	8	14	4	6	8	8	12	8	14	14	2	12
0	2	6	4	2	2	6	8	6	6	10	10	8	10	4	12	16	22	12	6
0	2	4	4	0	2	6	0	4	4	4	12	6	4	4	12	12	4	10	14
0	0	4	6	0	4	0	4	6	4	8	6	6	10	16	12	8	6	12	10
6	6	6	4	4	8	8	8	10	8	10	10	0	4	6	12	2	8	8	12

Pixel values from the top left corner



Color information is commonly stored using an RGB format

Afghan Girl, National Geographic photographer Steve McCurry, 1984

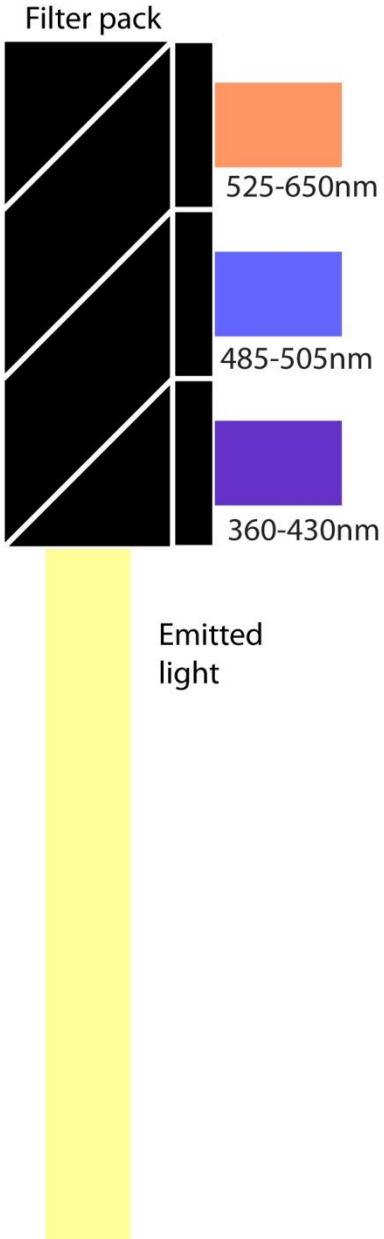
Red Channel

Green Channel

Blue Channel

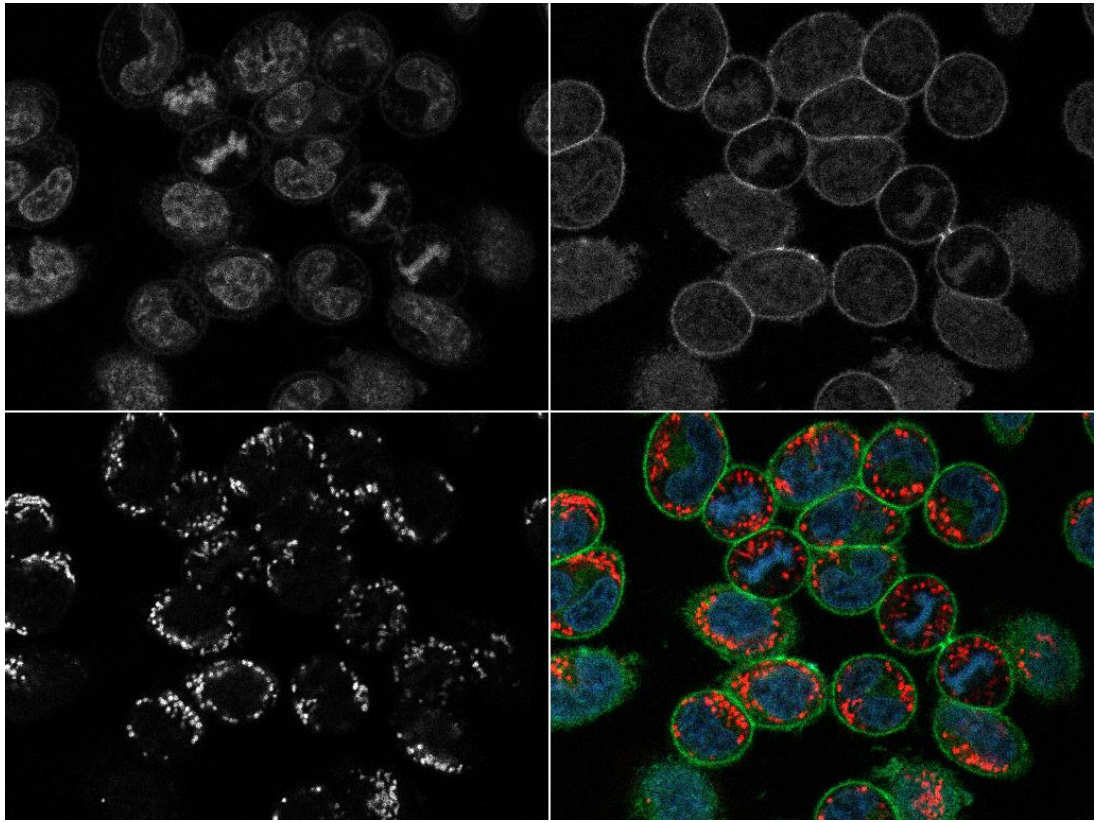
(CMYK, another color format, refers to the four inks used in color printing: cyan, magenta, yellow, and key (black). This format is often used for print documents).

Most simply, a *merged* image comes from 3 separate channels of data merged into an RGB image.



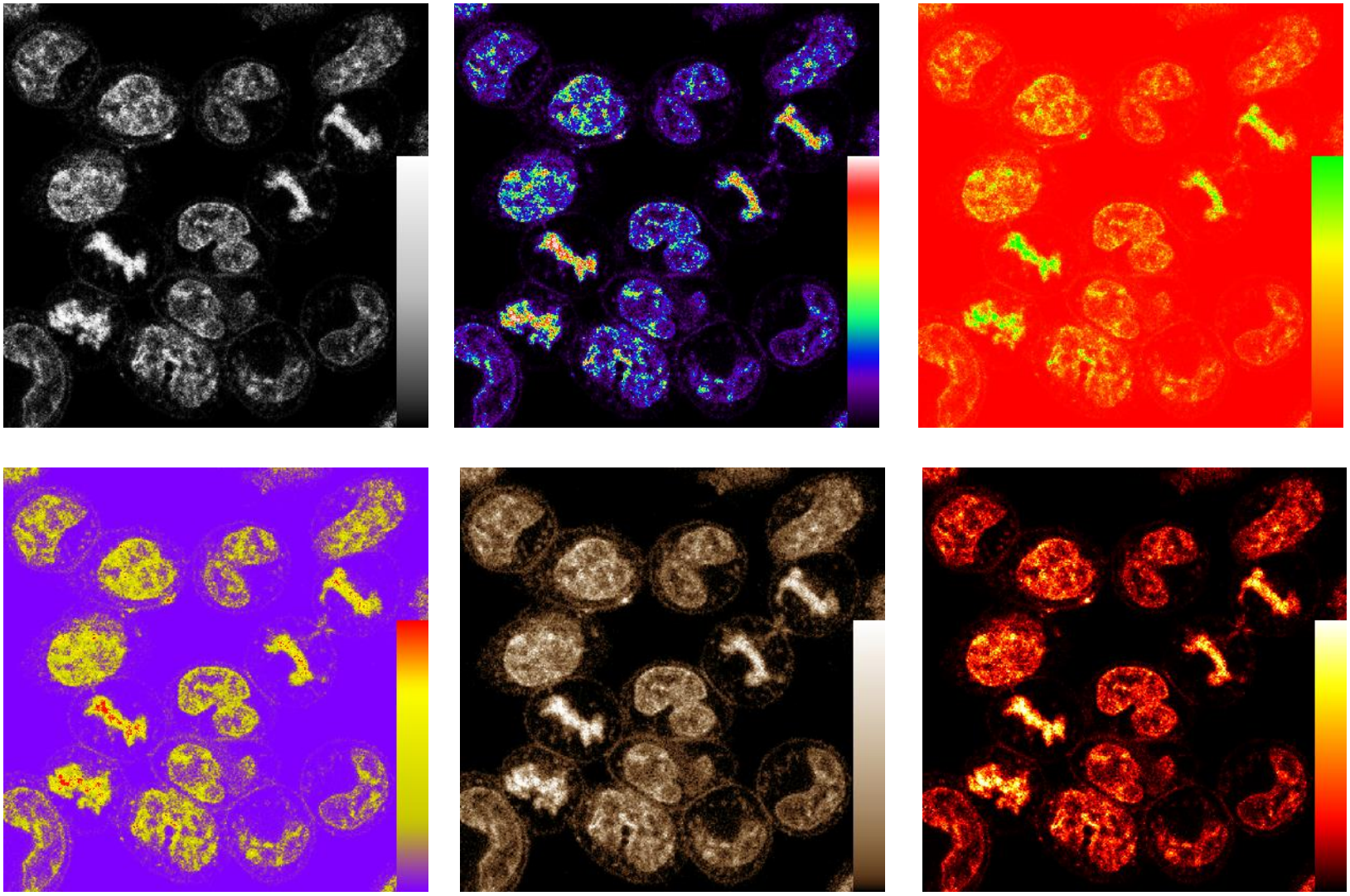
DAPI for DNA

PATMAN for plasma membrane



Rhodamine 123 for mitochondria

Look-up tables (LUTs) just map intensity to color for display. They have nothing to do with the actual imaging data



Bit Depth

Binary (1 bit) – 0 or 1 only

Unsigned 8 bit integers (BYTE) – 0 to 255 (2^8 gray levels)
11010111 (example pixel)

Unsigned 16 bit integers (WORD) – 0 to 65535 (2^{16} gray levels)
11001011 11101101 (example pixel)

Color – typically 24 bits (3 bytes) per pixel –
1 byte each for red, green and blue.
11111111 11111111 00000000 (example orange pixel)

512x512 pixel image has 262,144 pixels
A color byte image would be ~3*262 or 786 Kbytes

Image files have “headers” (and sometimes footers) that contain information about the image format.

Bit depth

16-bit
2^16 levels = 65,526 levels

8-bit
2^8 levels = 256 levels

6-bit
2^6 levels = 64 levels

4-bit
2^4 levels = 16 levels

2-bit
2^2 levels = 4 levels

1-bit (binary image)
2^1 levels = 2 levels

Common image formats:

GIF - Graphics Interchange Format. Developed by CompuServe. It was a widely used image format. Supports 8 bit pixel values which is OK for gray-level images and 'normal' color images, but is not suitable for very high quality color images.

PNG - Portable Network Graphics. This format was developed to replace GIF after CompuServe started to ask for royalties. This is a very good lossless image format that supports up to 16 bit gray scale images and 48 bit true color images.

TIFF - Tagged Image File Format. Developed by Aldus corporation and widely used. Allows up to 24 bit pixel values. Can save image stacks. Most important format for scientific imaging.

JPEG - Joint Photographics Experts Group. Incorporates data compression which ensures that files sizes are kept small, even when 24 bit pixel values are used. The JPEG compression algorithm is lossy - the reconstructed, uncompressed image will be slightly different from the original.

BMP - Microsoft Windows Bitmap. A relatively simple image format allowing up to 24 bit pixel values. Usually no data compression with BMP files

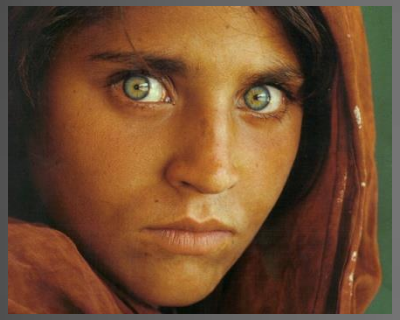
FITs – originally devised by NASA – can store image data, spectral data --- any kind of data. Has a well defined human readable header structure with predefined keywords and the ability to use user-defined keywords.

DICOM – digital imaging and communications in medicine – The standard for medical imaging.

OME – open microscopy environment – Developed at Madison for better communication and transport of research images.

Example Image Compression

Afghan girl



Detail

Uncompressed TIFF, 608Kb (500x400 pixels *3 byte / pixel = 600Kb)

Compressed JPEG, 22Kb

Examples of manufacturer specific formats
(when in doubt, try ImageJ)

LSM – Zeiss confocal Laser Scanning Microscope

LIF – Leica confocal

OIB, OIF – Olympus FluoView 1000 confocal

NEF – Nikon Electronic Format

SIF – Andor camera

SLD – Slidebook

ZVI – Zeiss AxioVision

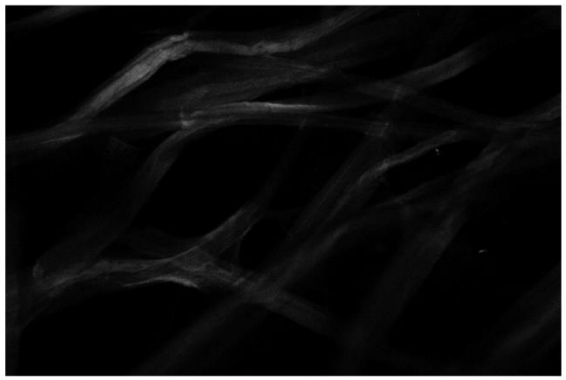
IMS - Imaris

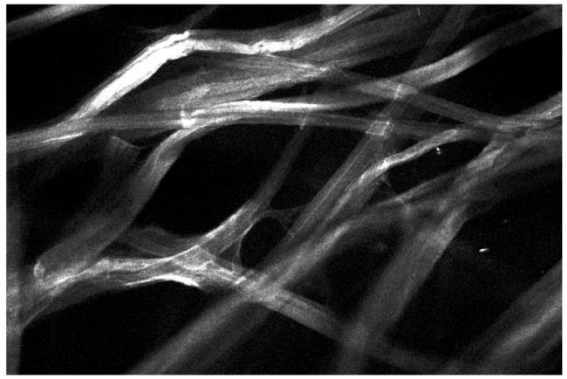
#1 rule of image analysis

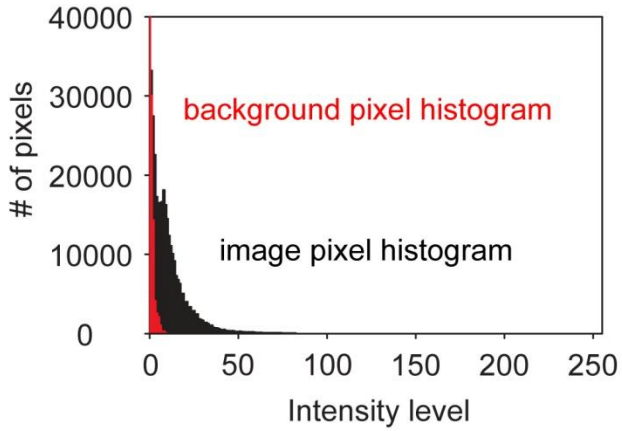
Take quantitative images.

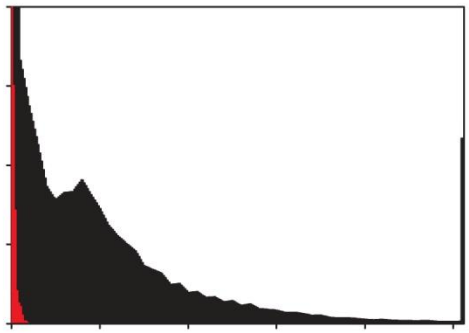
Raw data

Scaled display









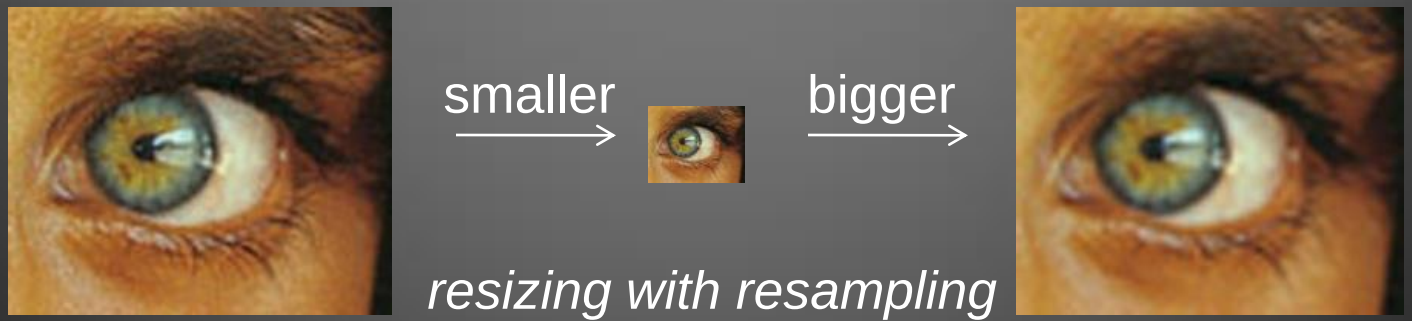
Best practices for scientific data

Never change original “raw” data.

Keep a tag to the original data with any processed image.

Keep records of steps in any image analysis procedure

In preparing images for presentation, resample as little as possible. To size images, use pixels/inch rather than resampling!



resizing with resampling

Image presentation, analysis and visualization software (not comprehensive)

Paint – Presentation tool available as an accessory on Windows systems

Adobe Photoshop and Illustrator – Presentation and some analysis

ImageJ/FIJI – Imports and exports many different image formats and is capable of a wide variety of analysis and visualization processes (Originally NIH Image, there are many free pluggins and macros are available on the Web. Support is minimal and many pluggins are somewhat glitchy.)

Metamorph (BRC license on any platform) -- Good at 4D visualization and 2D analysis.

Volocity – Visualization, Classification, Tracking and Deconvolution, BRC site license for download anywhere at Cornell ($10/hour) – tailored for confocal data.

Osirix/Microview – Free visualization tools tailored to CT data.

Avizo/Amira (BRC license on any platform) – Powerful analysis, segmentation and visualization with a somewhat steep learning curve.

IDL, Matlab and Mathematica – extremely powerful for analyzing matrix data with custom needs, but require a significant learning curve.

ImageJ History

Wayne Rasband (right) at the 1st ImageJ Conference 2006 (picture courtesy of Marc Seil, CRP Henri Tudor, Luxembourg).

Date	***Package***	***Creator***	***Comments***
1987	NIH Image	Wayne Rasband, NIH	Pascal language --For Mac II computer (At this time PC's had limited graphics functionality). Pluggin functionality added in 1993
Early 1990's	Scion Image	Scion Corporation (frame grabbers)	C language -- For PC platform, but users found it buggy and this version died out.
1997	 ImageJ (ImageJ1)	Wayne Rasband, NIH	Java -- All platforms with Pluggin capability
2005	MBF_ImageJ	Tony Collins of **MacBiophotonics** (then of WCIF)	**O**nline manual and version of ImageJ bundled with useful plugins. Last update = 2009.
2008	 FIJI ("Fiji Is Just ImageJ—batteries included").	Albert Cardona of the **Institute of Neuroinformatics** at **ETH Zurich** and Johannes Schindelin of the **Max Planck Institute of Molecular Cell Biology and Genetics** (MPI-CBG)	An automatically updating version of ImageJ with many useful pluggins and a development environment for scripting languages.
2009	ImageJ2	ImageJDev project, NIH (instigated by Grant Harris's Google group ImageJX)	Complete rewrite of ImageJ with backward compatibility to ImageJ1