Image Processing
Part 2

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Examples of Image Types

Format	Extensions	Main use	Compression	Comment
TIFF	.tif, .tiff	Analysis, display (print)	None, lossless, lossy	Very general image format
OME-TIFF	.ome.tif, .ome.tiff	Analysis, Display (print)	None, lossless, lossy	TIFF, with standardized metadata for microscopy
Zarr	.zarr	Analysis	None, lossless, lossy	Emerging format, great for big datasets – but limited support currently
PNG	.png	Display (web, print)	Lossless	Small(ish) file sizes without compression artefacts
JPEG	.jpg, .jpeg	Display (web)	Lossy (usually)	Small file sizes, but visible artefacts

Loss of Information

> **Warning**
>
> **Lossy compression is bad for analysis!**

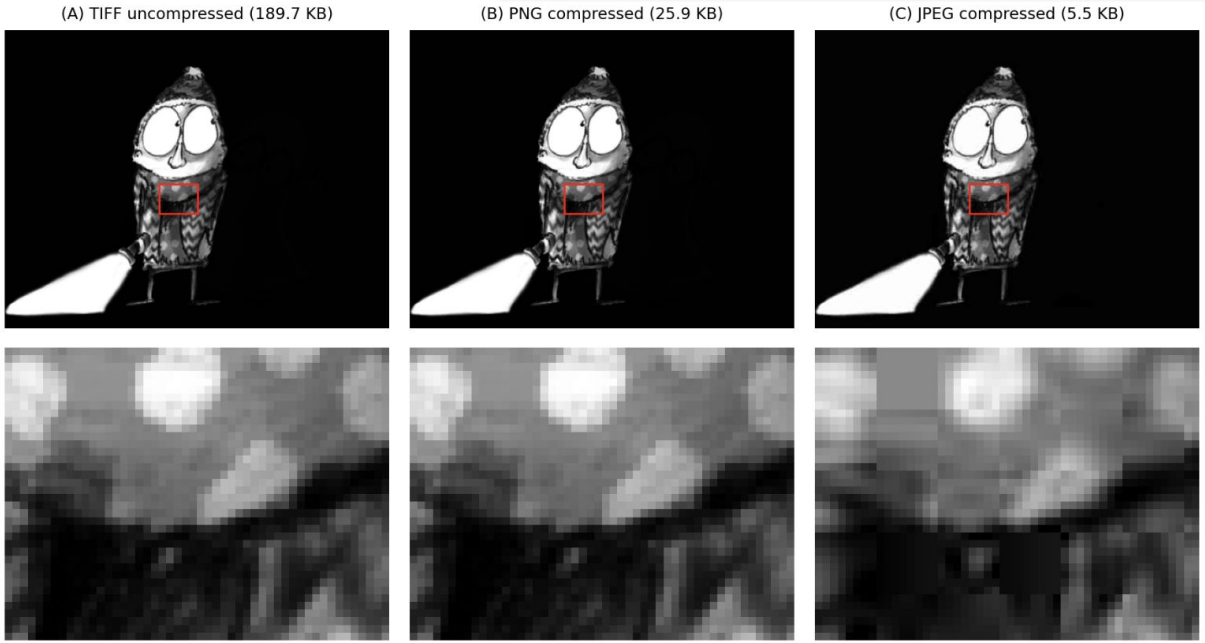


Fig. 53 Examples of images saved with (A) no compression, (B) lossless compression, and (C) lossy JPEG compression. The pixel values of (A) and (B) are identical. Image (C) looks similar, but zooming in on a detailed region reveals characteristic JPEG artefacts. #

General Rule of Thumb

- **Journal figure: TIFF.**
  Often the journal requests this anyway. Even if I'm not convinced it always makes sense.
- **Presentation: PNG.**
  File size is not usually a problem, and PNG provides some compression without introducing artefacts.
- **Website: JPEG or PNG**,
  JPEG (usually) because smaller file sizes mean the website can load quicker (and eat less data). But PNG for images that contain few colors, including most 'artificial' images such as drawings, dialog boxes or logos. JPEG artifacts can look especially ugly in such cases, while PNG can compress them very well.

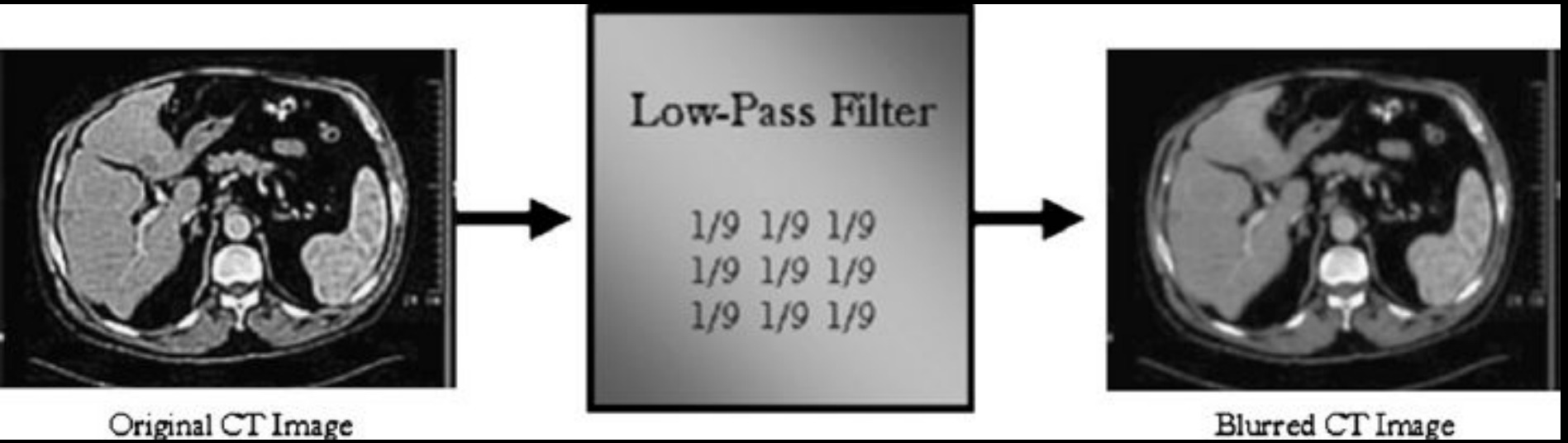
Filters

Original	*Gaussian Blur*	*Sharpen*	*Edge Detection*
$\begin{bmatrix} 0 & 0 & 0 \\ 0 & 1 & 0 \\ 0 & 0 & 0 \end{bmatrix}$	$\frac{1}{16}\begin{bmatrix} 1 & 2 & 1 \\ 2 & 4 & 2 \\ 1 & 2 & 1 \end{bmatrix}$	$\begin{bmatrix} 0 & -1 & 0 \\ -1 & 5 & -1 \\ 0 & -1 & 0 \end{bmatrix}$	$\begin{bmatrix} -1 & -1 & -1 \\ -1 & 8 & -1 \\ -1 & -1 & -1 \end{bmatrix}$
			

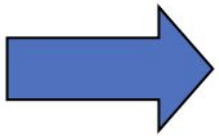
Smoothing Filters (low-pass)

Leaves (passes) low spatial frequencies untouched

High frequencies are attenuated, almost absent



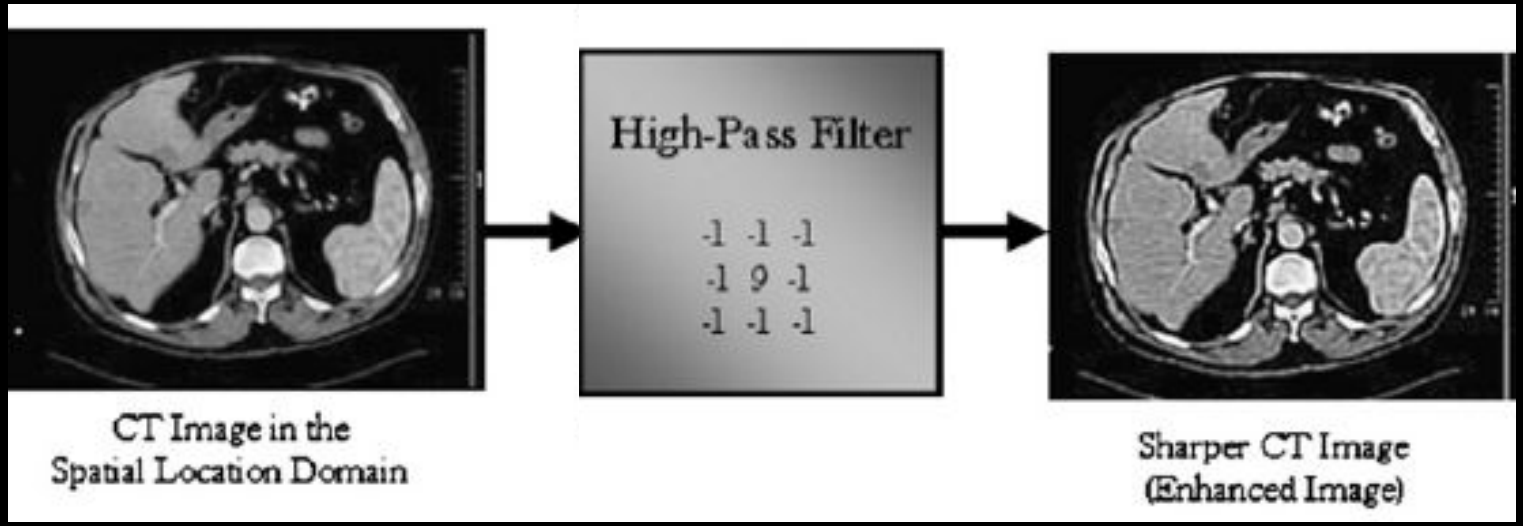
Low pass filter - smoothing



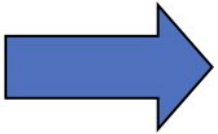
Sharpening (High-Pass Filter)

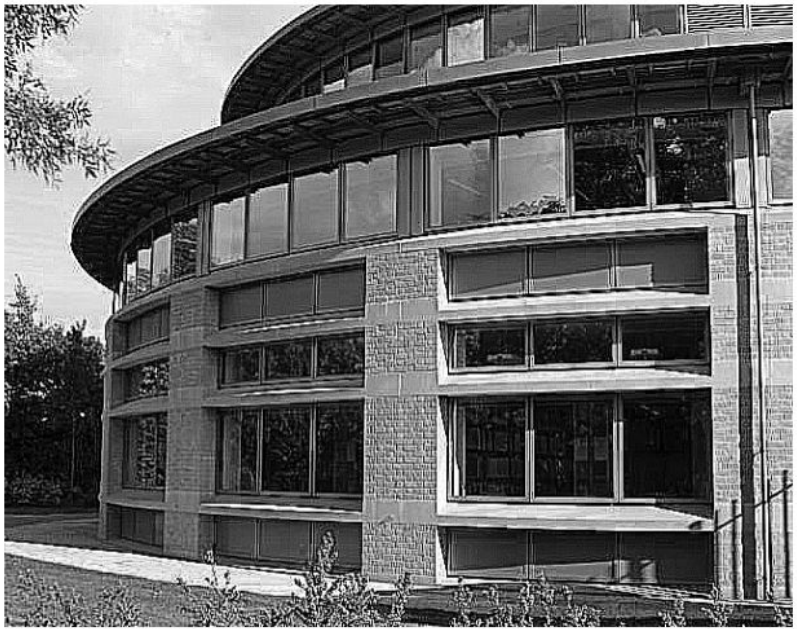
Passes up the high spatial frequency pixels,

Attenuates low frequencies...this accentuates high frequencies

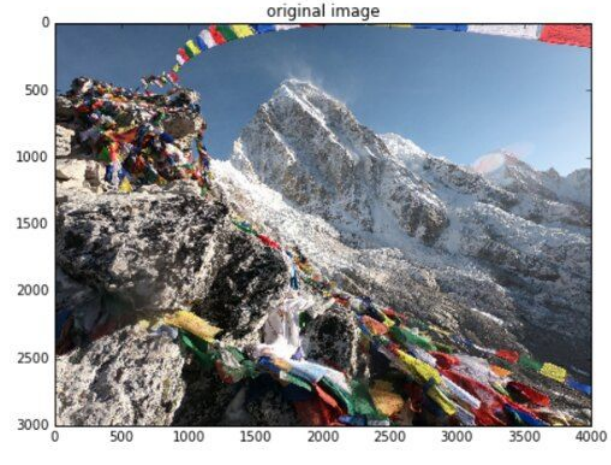


High-pass filter - sharpening





Comparison


original image
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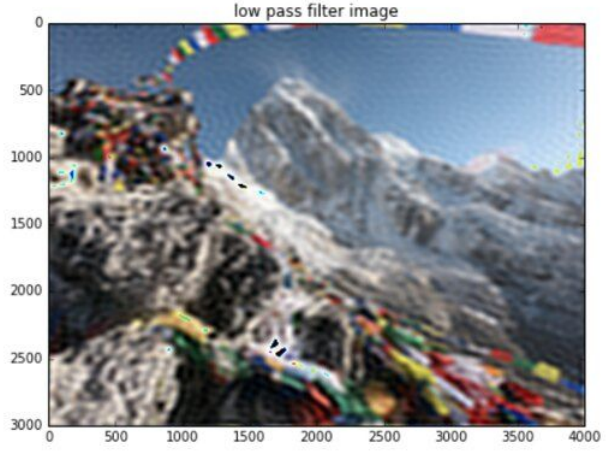
low pass filter image
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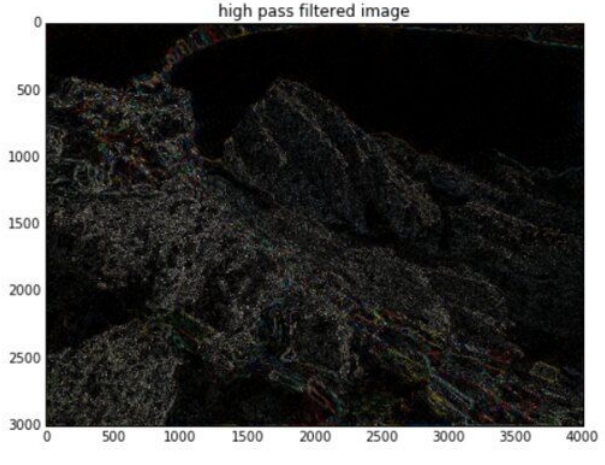
high pass filtered image
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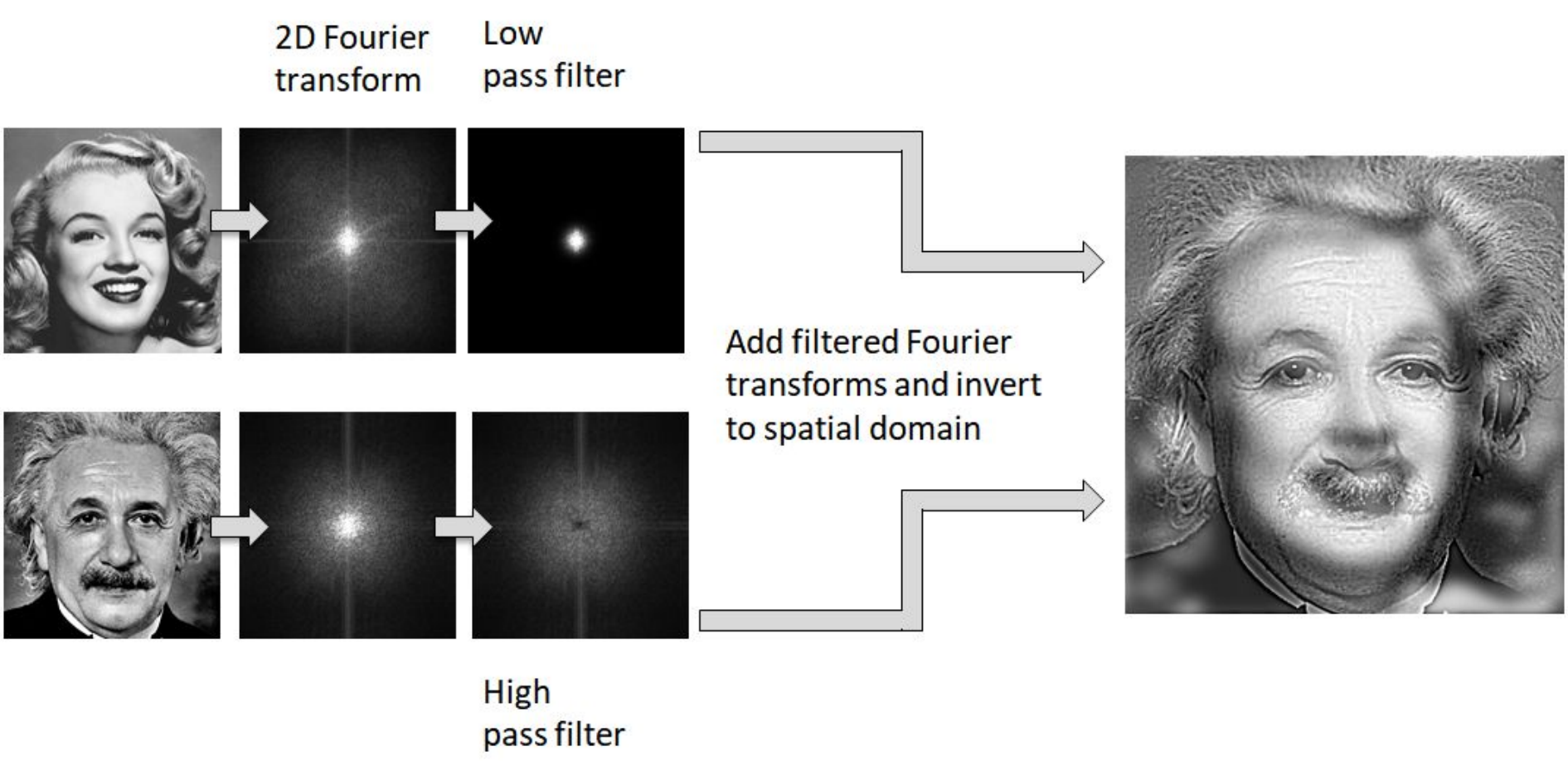
Who is it?

Now squint...


2D Fourier transform
Low pass filter
Add filtered Fourier transforms and invert to spatial domain
High pass filter

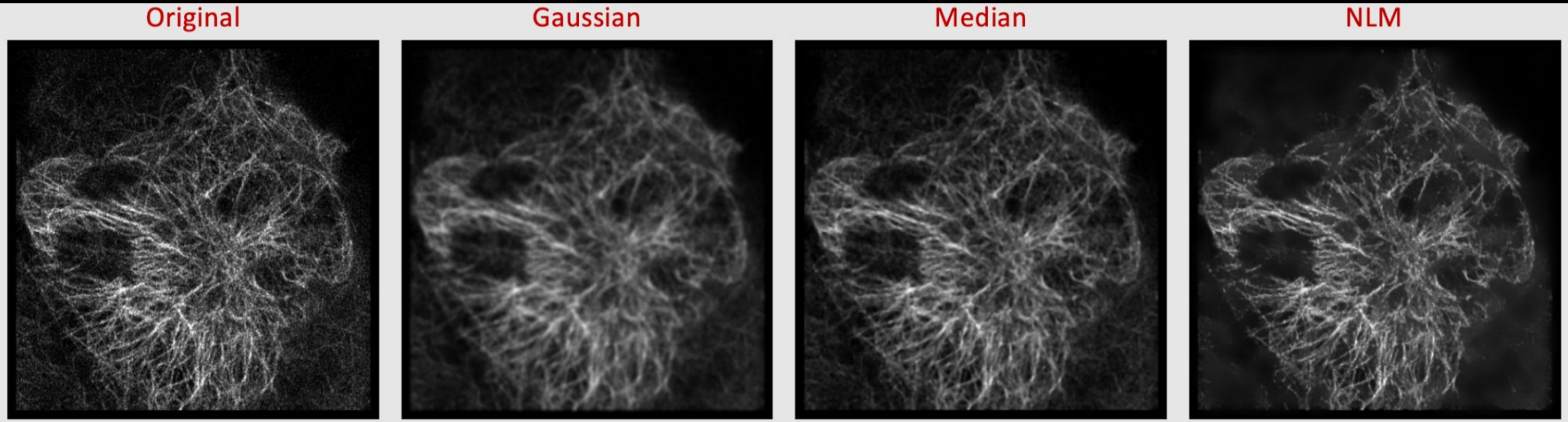
Denoising

Neighborhood methods

- Uses surrounding pixel values
- Mean, median, gaussian
- Performs poorly on noisy images

Non-Local methods

- Whole image is scanned by kernels
- Averages similar pixels to reduce noise
- Considers similarities of pixel values across whole image



Gaussian Blur Filter

Kernel is bell shaped (gaussian curve)

Kernel = 2 * sigma + 1
(you set the sigma)

Center is heavily weighted

Sigma = 2 (5X5)

1	1	2	1	1
1	2	4	2	1
2	4	8	4	2
1	2	4	2	1
1	1	2	1	1

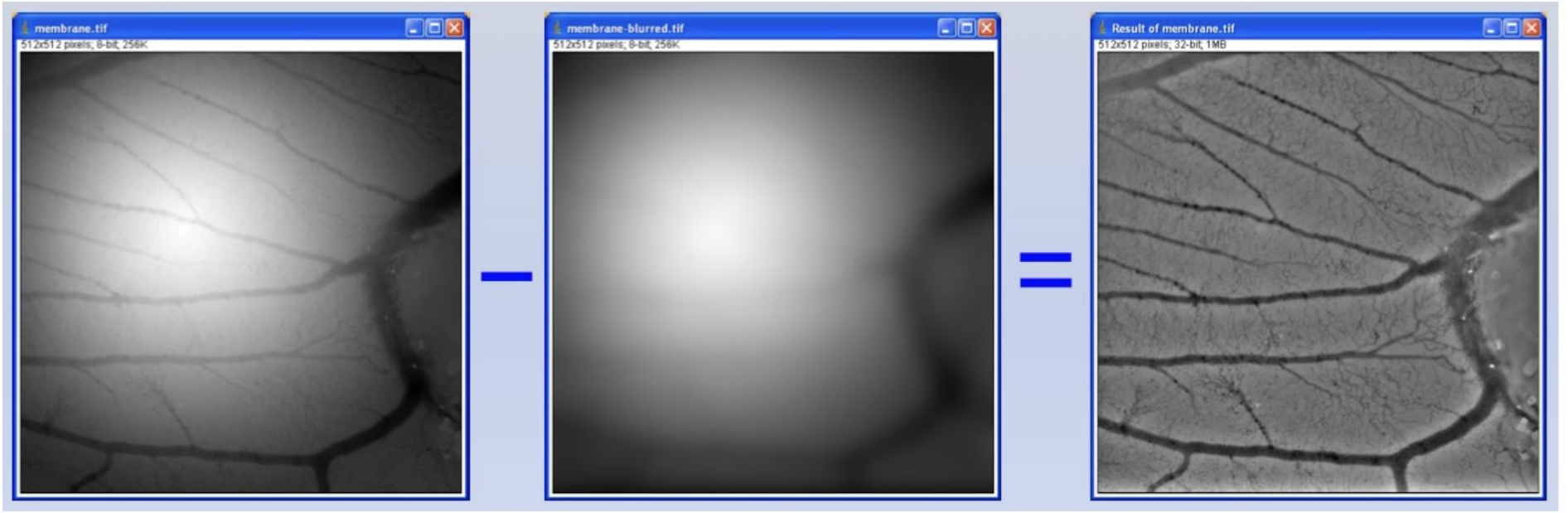
Sigma = 3 (7X7)

1	1	1	2	1	1	1
1	2	2	4	2	2	1
2	2	4	8	4	2	2
2	4	8	16	8	4	2
2	2	4	8	4	2	2
1	2	2	4	2	2	1
1	1	1	2	1	1	1

Sigma = 7 (15X15)

2	2	3	4	5	5	6	6	6	5	5	4	3	2	2
2	3	4	5	7	7	8	8	8	7	7	5	4	3	2
3	4	6	7	9	10	10	11	10	10	9	7	6	4	3
4	5	7	9	10	12	13	13	13	12	10	9	7	5	4
5	7	9	11	13	14	15	16	15	14	13	11	9	7	5
5	7	10	12	14	16	17	18	17	16	14	12	10	7	5
6	8	10	13	15	17	19	19	19	17	15	13	10	8	6
6	8	11	13	16	18	19	20	19	18	16	13	11	8	6
6	8	10	13	15	17	19	19	19	17	15	13	10	8	6
5	7	10	12	14	16	17	18	17	16	14	12	10	7	5
5	7	9	11	13	14	15	16	15	14	13	11	9	7	5
4	5	7	9	10	12	13	13	13	12	10	9	7	5	4
3	4	6	7	9	10	10	11	10	10	9	7	6	4	3
2	3	4	5	7	7	8	8	8	7	7	5	4	3	2
2	2	3	4	5	5	6	6	6	5	5	4	3	2	2

Why would you BLUR!!



Non-Linear Filters

Uses a nonlinear function to modify pixels → uneven modifications (be careful!)

Don’t Think Ahead

One puzzle at a time, be creative!

Let’s try together…

Open CellProfiler

Drag in Brain_input.jpg

Reporting your processes

Aaron et al., 2021 A Guide to reporting in digital image processing - can anyone reproduce your quantitative analysis? https://www.aicjanelia.org/_files/ugd/835c50_cc34305735124c7092f2216e03b9bd5d.pdf

Heddleston et al., 2021, A guide to reporting in digital image acquisition - can anyone replicate your microscopy data? https://www.aicjanelia.org/_files/ugd/e43f7a_021d414cb2b14e24bbe65285d5938887.pdf

Microscopy Reporting

“ I used 60X objective.”

Numerical Aperture (NA)

Higher NA→higher resolution

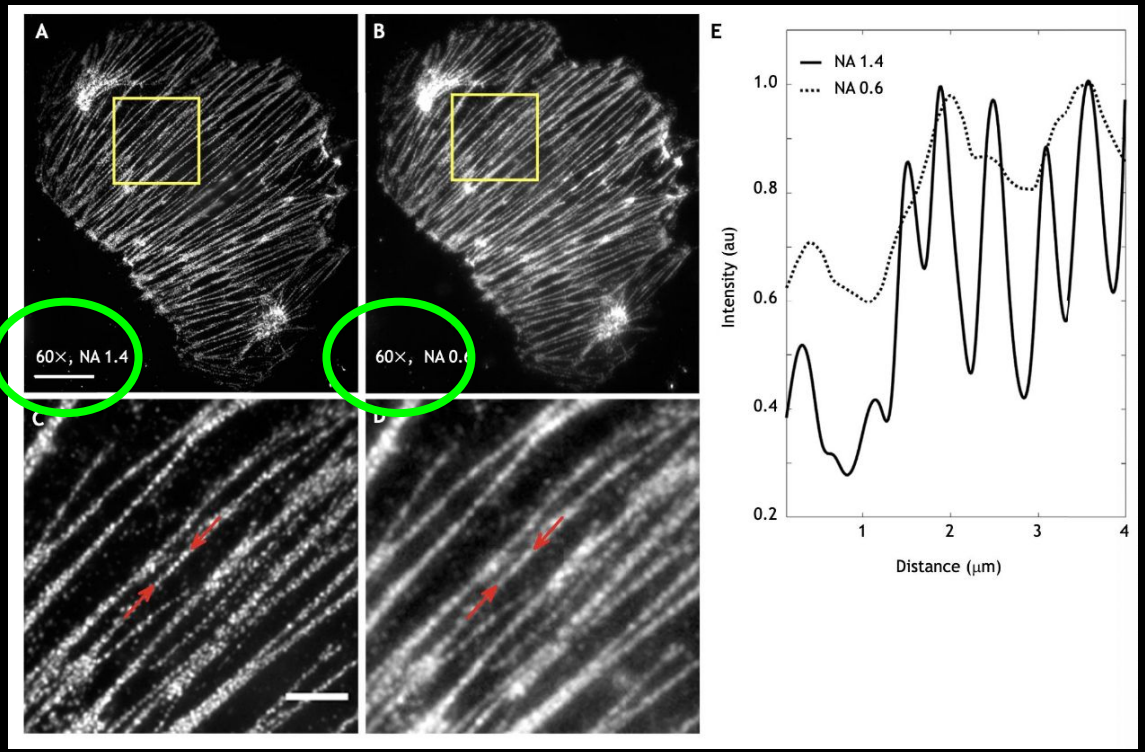
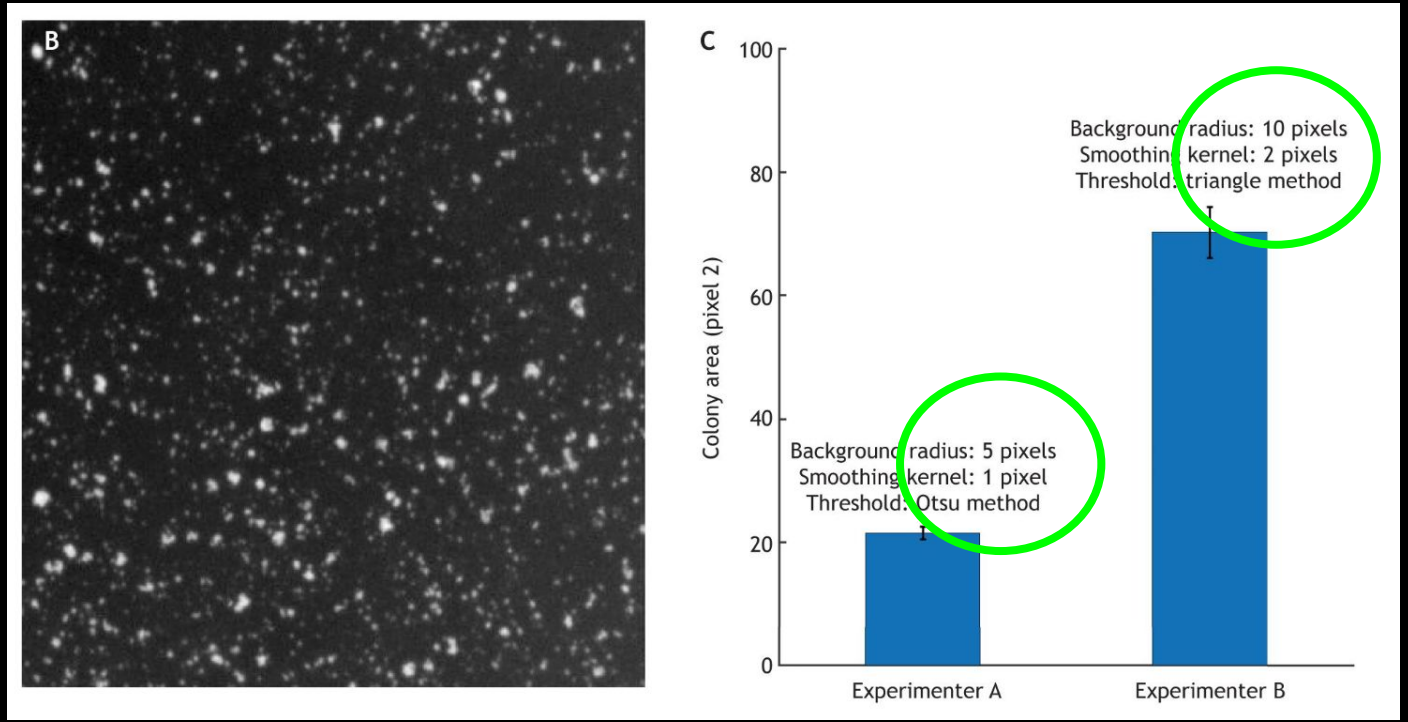


Image Processing Reporting

Setting details matter



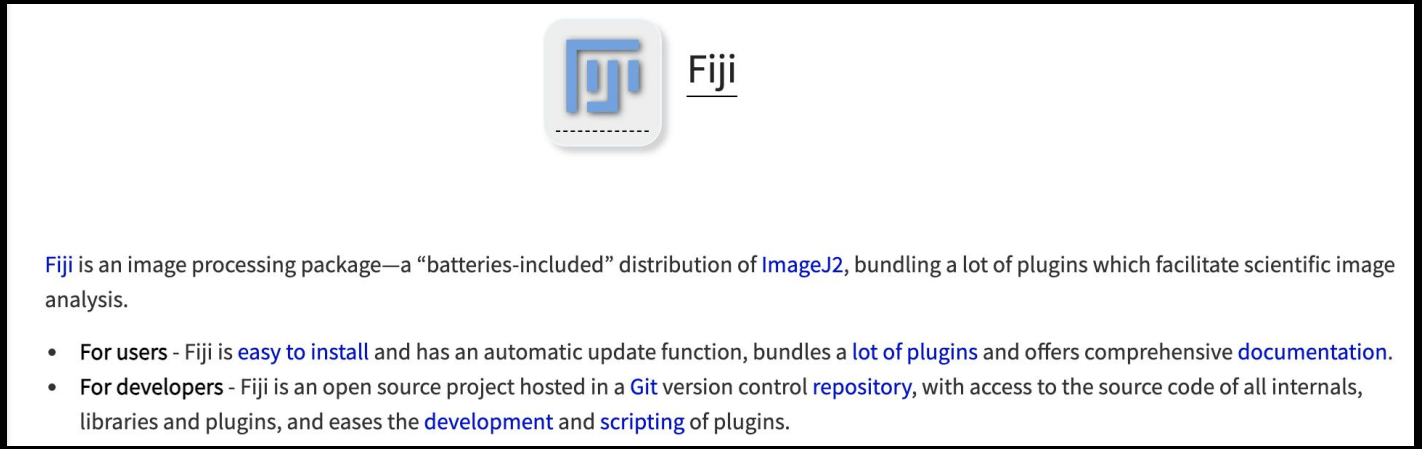
PROCESSING TASK	COMMON ALGORITHMS	PARAMETER(S) TO REPORT
Background Subtraction	Rolling Ball/Rolling Minimum	Kernel size and shape
	Gaussian Smoothing and Subtraction	
	FFT high pass filter	Frequency cut-off values
Denoising	Gaussian Smoothing	Kernel size and shape
	Median Filtering	
	Non-Local Means	Noise sigma and smoothing value
Deconvolution	Weiner Deconvolution	PSF and Wiener factor (1/SNR)
	Richardson-Lucy	PSF and number of iterations
	Blind	Estimated PSF (or none)
Intensity Threshold	Manual	Intensity value or percentile
	Automatic	None
	Local	Varies
Segmentation	Pixel Connectivity	4 or 8 (2D images) 6, 18, or 26 (3D images)
	Morphometric Filtering	Shape/size parameter(s) with upper and lower bounds
	Binary Operations	Operations type (e.g. erosion, dilation), number and order of implementations

MICROSCOPE COMPONENT	PARAMETERS TO REPORT
Light source	Source type, make and model
	Wavelength (if laser)
	Power/Power Density at Sample
Excitation/Emission Optics	Dichroic mirror manufacturer and wavelength characteristics
	Excitation and Emission Filters manufacturer, and wavelength characteristics
	Instrument-Specific Components (e.g. confocal pinhole size)
Sample and Acquisition Parameters	Sample Preparation and Mounting Protocol
	Z-interval spacing
	Time-lapse Interval
Objective Lens	Manufacturer
	Magnification
	Numerical Aperture
	Optical Aberration Corrections
Detector	Detector Type and Manufacturer
	Exposure/Dwell Time
	Gain
	Offset
	Binning (if used)

Other Software for Image Analysis

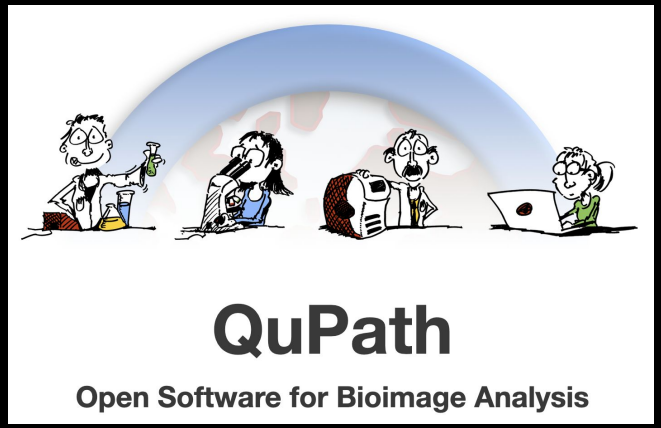
FIJI - an ImageJ associated software

- https://imagej.net/software/fiji/
- Free
  - Mac OS
  - Windows



QuPath Bioimage Analysis

- https://qupath.github.io/
- Free Software
  - Mac OS
  - Windows



- Getting started
  - First steps
  - Viewing images
  - Annotating images
  - Manual counting
  - Two essential tips
  - Getting help
- Tutorials
  - Projects
  - Separating stains
  - Detecting tissue
  - Measuring areas
  - Cell detection
  - Cell classification
  - Density maps
  - Exporting measurements
  - Multiplexed analysis
  - Pixel classification
  - Superpixels

Free software for analyzing and processing image data

Fiji: ImageJ plus a large bundle of plugins, all packaged together in one easy-to-download application. Runs on Mac OS, Windows, and Linux. Very handy for new users who don't want to download individual plugins. Includes utilities for 3D viewing, video editing, measuring colocalization among others. **Recommended for all CDB Micro Core users.**

QuPath: Free, open-source software designed primarily for annotation and analysis of large-format whole-slide images (color brightfield and multi-channel fluorescence)

CellProfiler: Free, open-source software from the Broad Institute for segmentation and quantitative analysis of microscope image data.

Ilastik: Free software that uses machine learning for interactive object recognition and segmentation.

Imaris Viewer: A free version of Imaris with limited capabilities; useful for quickly viewing Imaris files or raw data.

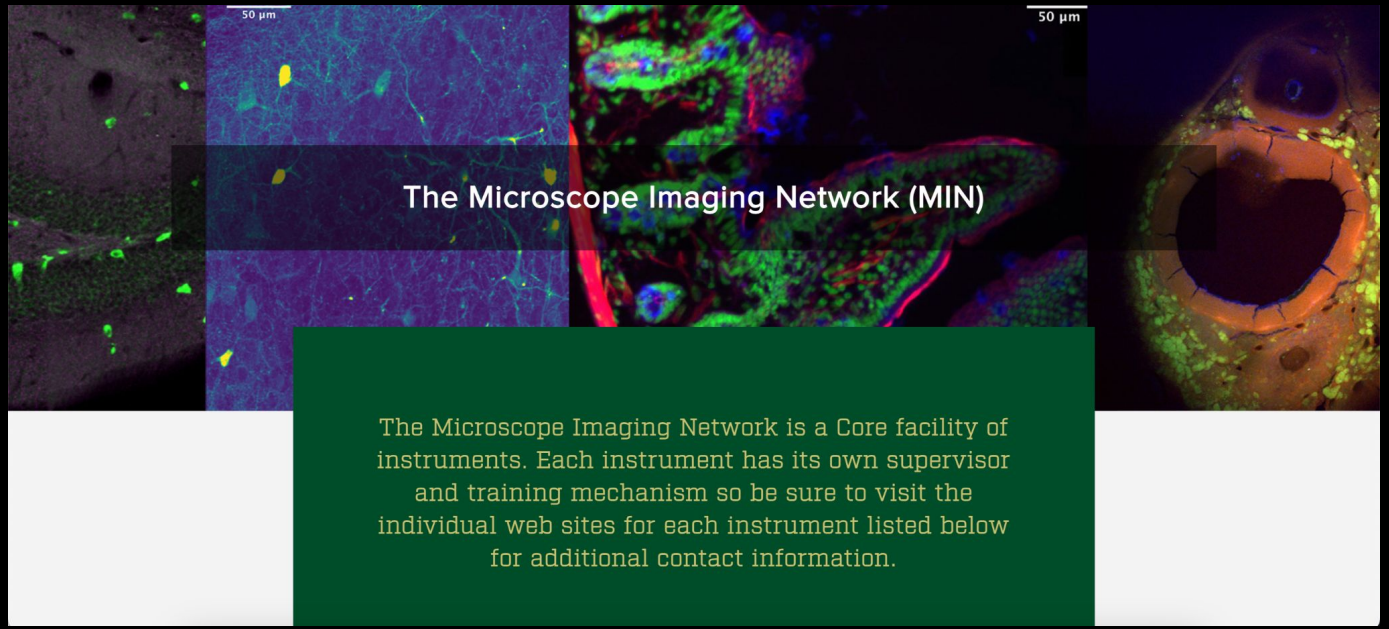
ZEN Lite : Free software from Zeiss to open .czi files. Windows only.

LAS X Core: Free software from Leica to open images acquired on the Leica SP8 confocal or any microscope controlled by LAS X. Scroll down the linked page to find the version appropriate to your operating system. Windows only.

On-Campus Resources

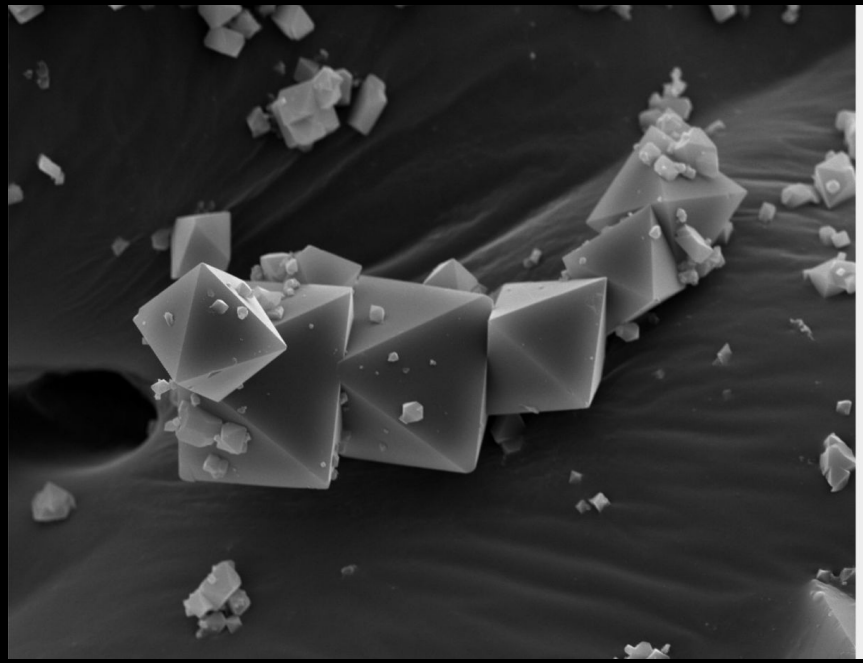
MIN Webpage

https://www.research.colostate.edu/min/



ARC-ISS Webpage

https://www.research.colostate.edu/iss/



Center for Imaging and Surface Science

The ISS Center enables research and development programs by providing expertise and access to state-of-the-art equipment for electron microscopy, spectroscopy, and other surface characterization measurements.

CuBTC metal organic frameworks. Dr. Jon Thai, Reynolds group, Chemistry Department, CSU

Coursework Offered via qCMB

NSCI 677	Microscopic Image Collection & Processing	2
BC 665A	Advanced Topics in Cell Regulation: Microscopic Methods	2
CS 510	Image Computation	4

Other Learning and Communities

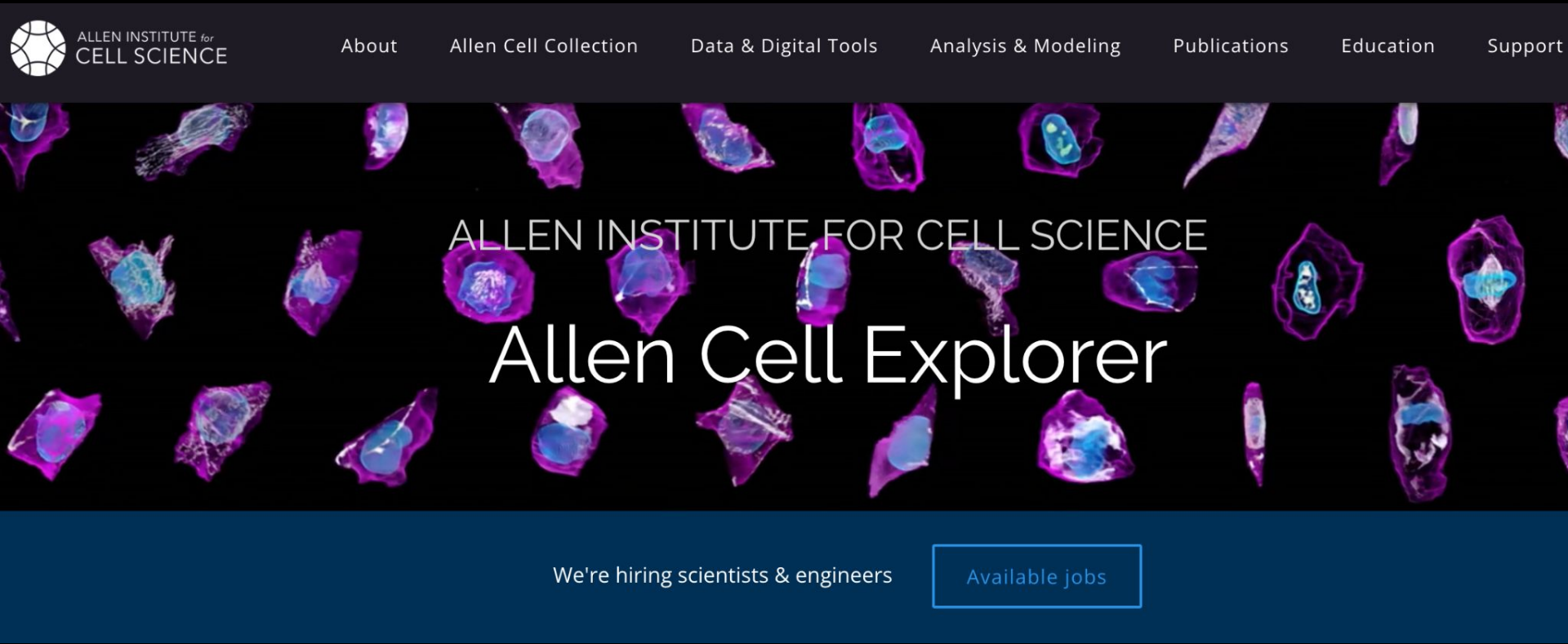
MDI Biolabs spring Workshop - Quantitative Fluorescence Microscopy: https://mdibl.org/course/qfm-2025/

MBL fall workshop: https://www.mbl.edu/education/advanced-research-training-courses/course-offerings/optical-microscopy-imaging-biomedical-sciences

Cold Spring Harbor Lab spring workshop: https://meetings.cshl.edu/courses

BINA community: https://www.bioimagingnorthamerica.org/join/

Public Image Databases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Data & Digital Tools
Analysis & Modeling
Publications
Education
Support
ALLEN INSTITUTE FOR CELL SCIENCE
Allen Cell Explorer
We're hiring scientists & engineers
Available jobs

IMAGESCIENCE.ORG

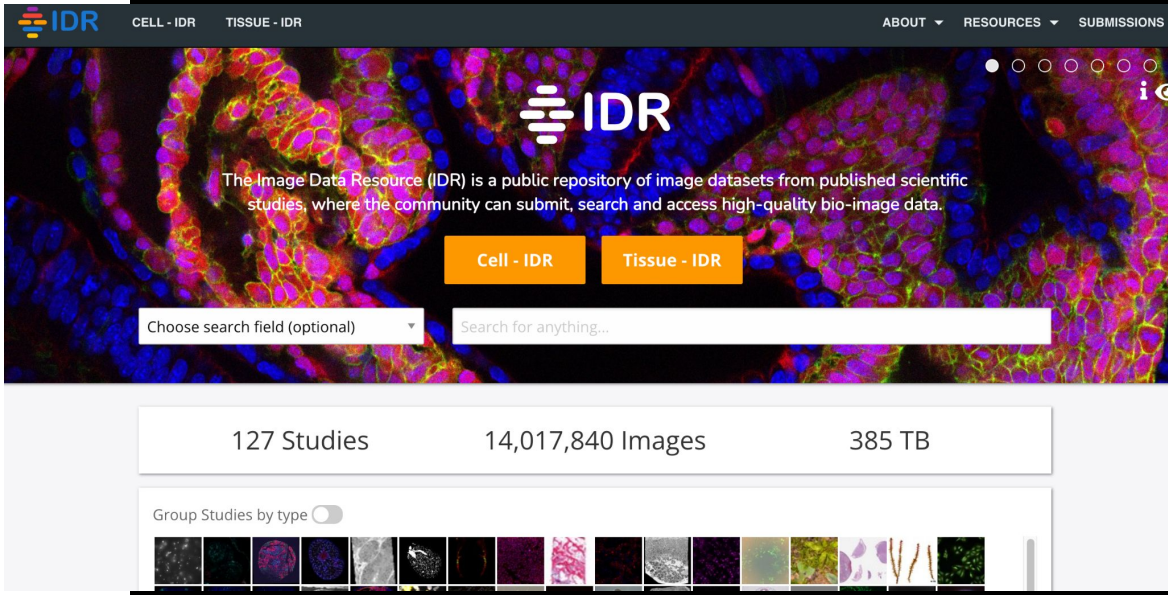
Images

Biological Images

- Acute Lymphoblastic Leukemia Image Database
- Allen Brain Atlas
- American Society of Hematology Image Bank
- Blood Cell Images Dataset
- Broad Bioimage Benchmark Collection
- Cell Image Library
- Euro-BioImaging FAIR-Sharing Collection
- Malaria Cell Images Dataset
- Microscope Cell Nuclei Images Dataset
- Molecular Expressions Photo Gallery
- Multimodal Videos of Human Spermatozoa

Challenge Images

- BigNeuron Gold166 Benchmarking Images
- DIADEM Neuron Reconstruction Challenge
- Fingerprint Verification Competition
- Grand Challenges in Biomedical Image Analysis
- ImageNet Large Scale Visual Recognition Competition
- PASCAL Visual Object Classes Challenge
- Retrospective Image Registration Evaluation



Homework: Two parts (outlined in following two slides)

1) Image Processing: Use your own images (or borrowed) to construct a new pipeline (5 points)
2) Questions: Answer the 3 questions in 600 words or less. (20 points)

Turn in: 1) a screenshot of your pipeline

2) Homework Questions (word or pdf)

Due date: Sunday May 4th, 2025 by midnight (almost two weeks from today)

Grades: based on answers to the questions and attempt at pipeline (this does not have to be fully functional)

Homework: Image processing

Process your own images (5 points):

- Use 2-3 of your or a colleagues images (or our images uploaded to this module on canvas)
- Build a pipeline to:
  - Save a modified image for each input image
  - Extract data: at least 3 measurements of your choosing to save to .csv file

Include: Meaningful notes, attempted multiple modules

Suggestions:

Use .tif files

Be careful with metadata and Names and Types (this is usually where problems happen)

Don't be ambitious - only use a few images at most and limit the number of modules used.

Post on the forum for help

Use ImageJ to pre-processes images if needed

Work together

*Utilize all resources at your disposal

Homework: Questions

Answer the following questions in less than 600 words. Include references. (20 points)

1. Where do you see image analysis fitting into your future project? What data you hope to collect from your images (if you use images)?
2. What was the most challenging part of working through the example in CellProfiler? Why was it challenging?
3. Find an image from a recent paper you read. What type of data did they collect from the image and what technique/software did they use to analyze it? Provide the reference.