Image Processing
Part 2

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Types of Images

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!**

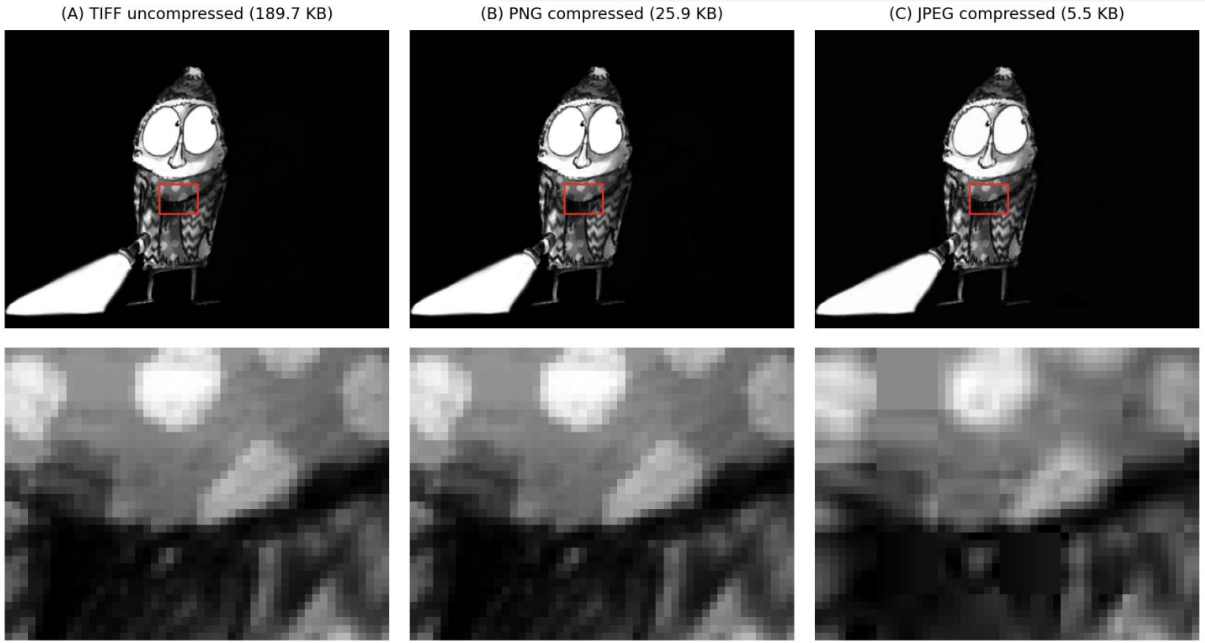


Fig. 53 Examples of images saved with (A) no compression, (B) lossless compression, and (C) lossy JPEG compression. The pixel values of (A) and (B) are identical. Image (C) looks similar, but zooming in on a detailed region reveals characteristic JPEG artefacts. #

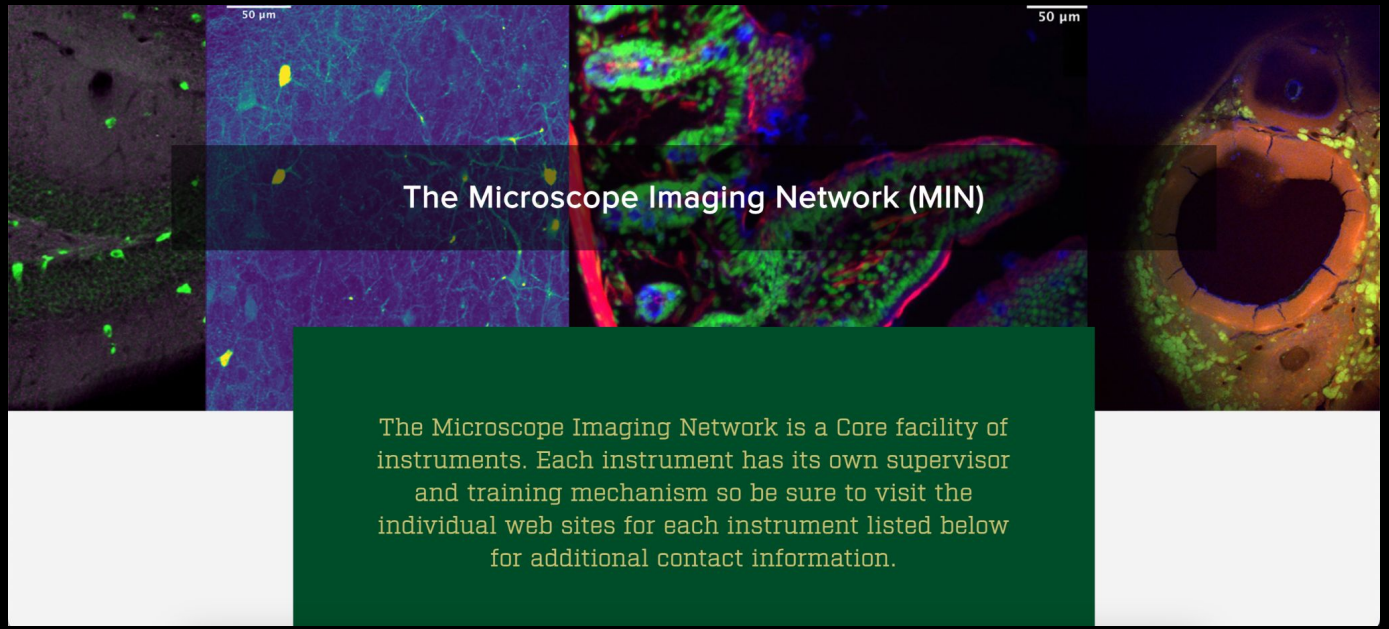
General Rule of Thumb

- **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.

On-Campus Resources

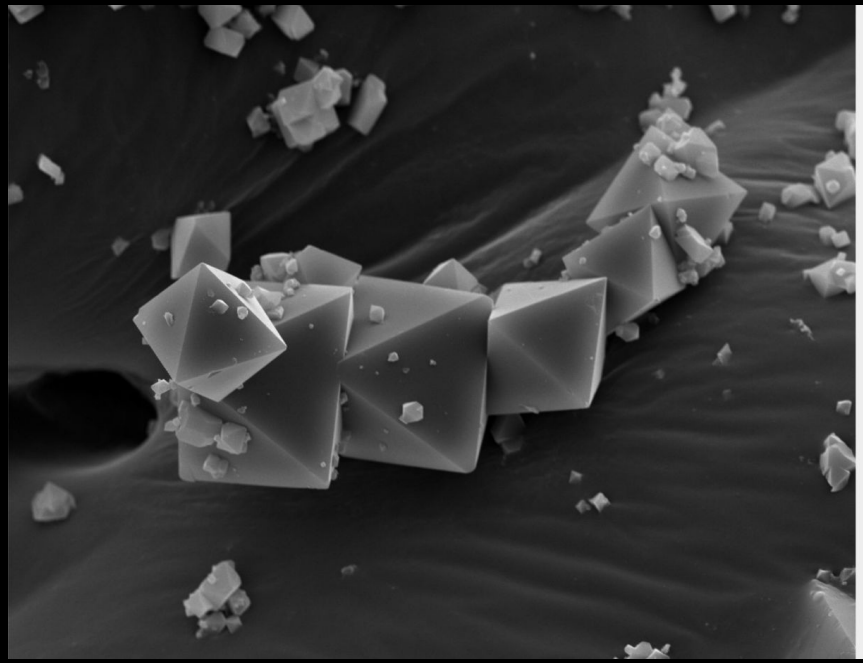
MIN Webpage

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

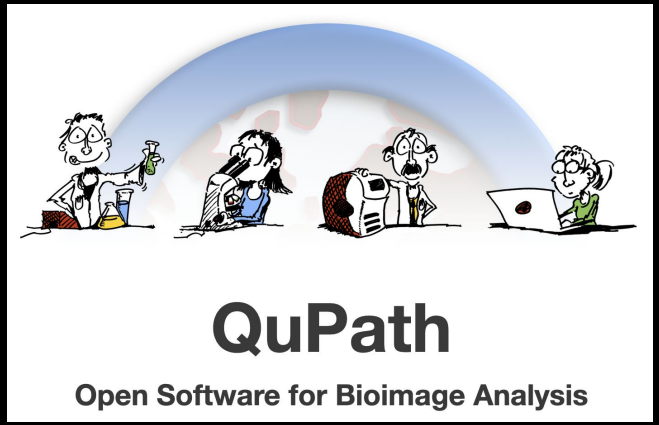
Coursework Offered via qCMB

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

Other Software for Image Analysis

QuPath Bioimage Analysis

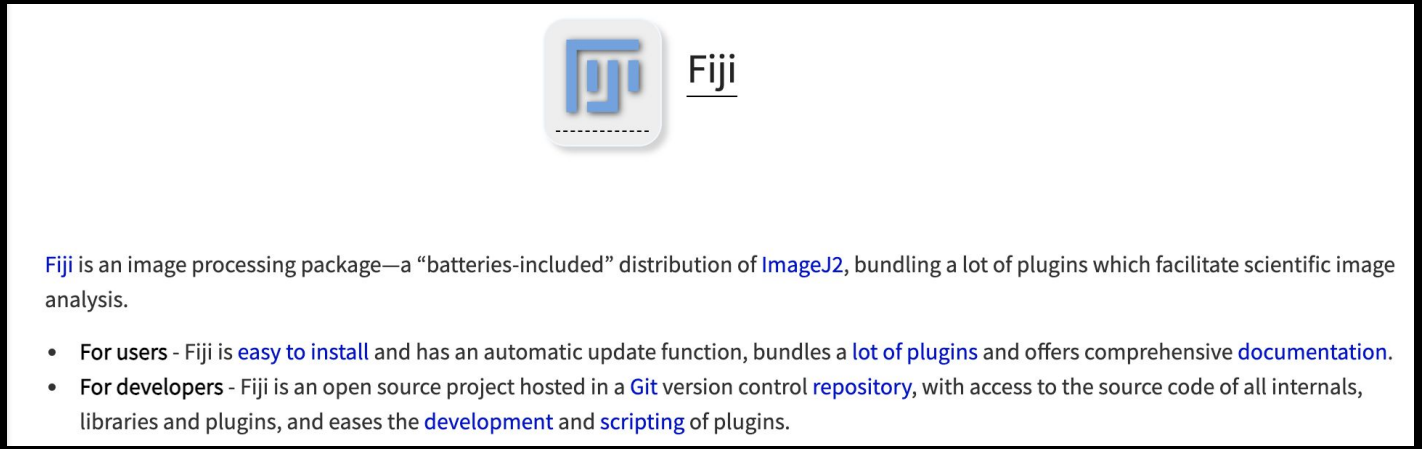
- https://qupath.github.io/
- Free Software
  - Mac OS
  - Windows



- 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

FIJI - an ImageJ associated software

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



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.

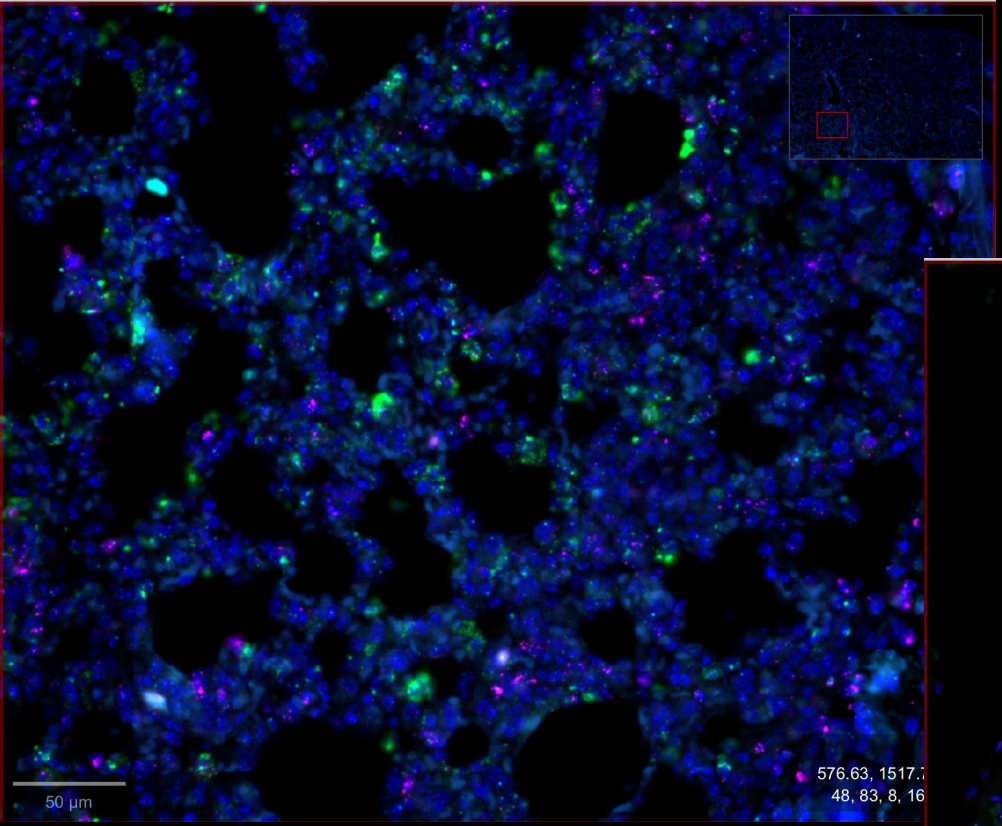
Ilastik: Free software that uses machine learning for interactive object recognition and segmentation.

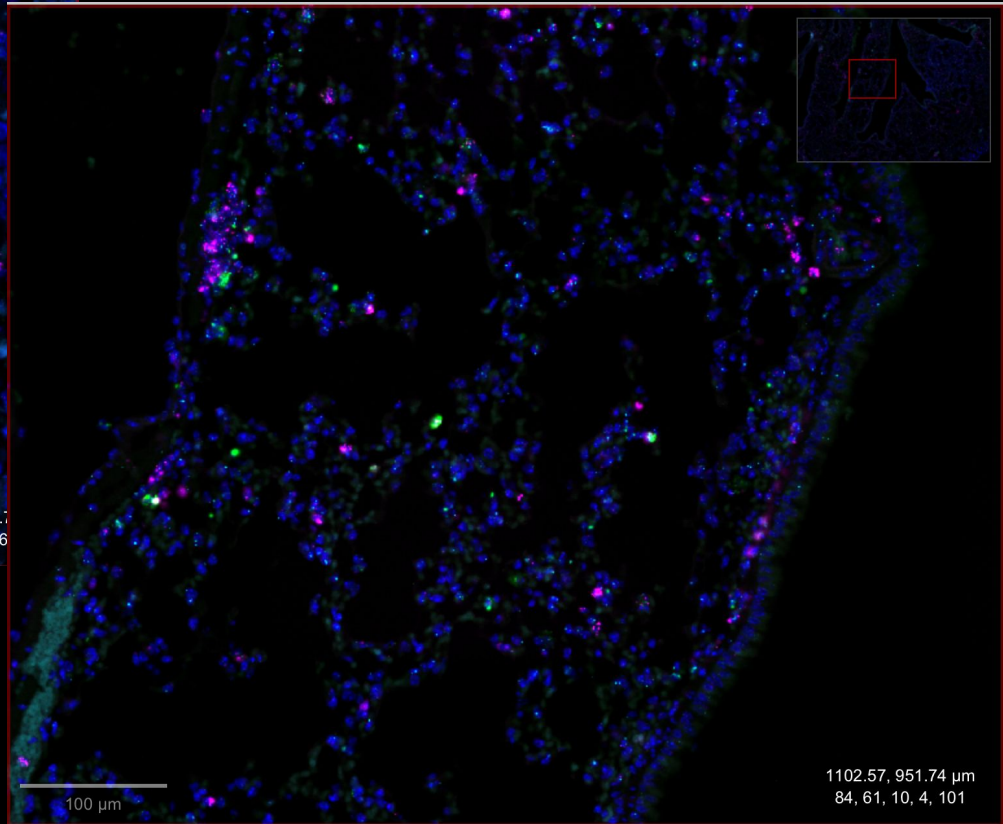
Imaris Viewer: A free version of Imaris with limited capabilities; useful for quickly viewing Imaris files or raw data.

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

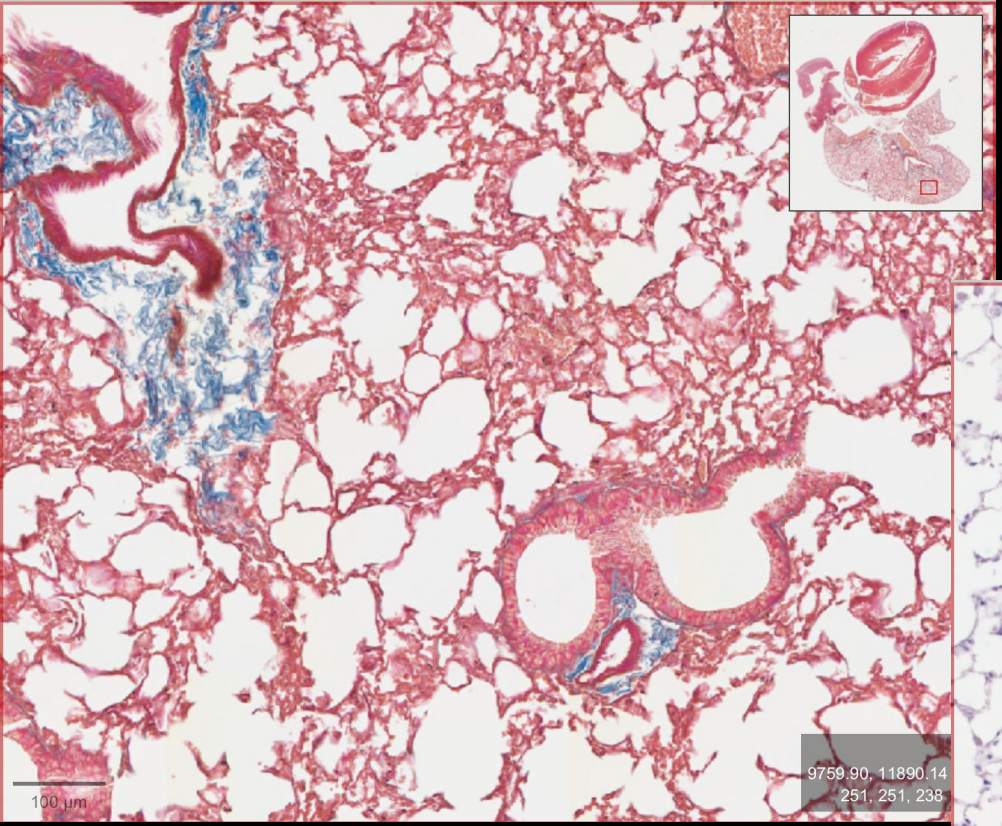
LAS X Core: Free software from Leica to open images acquired on the Leica SP8 confocal or any microscope controlled by LAS X. Scroll down the linked page to find the version appropriate to your operating system. Windows only.

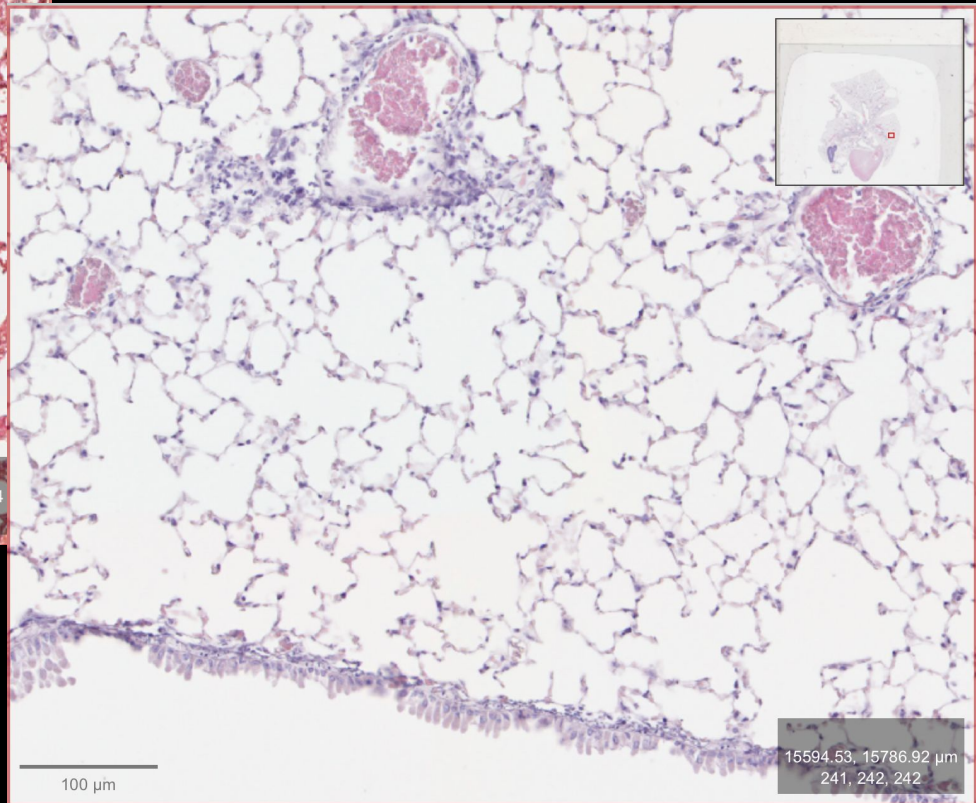
Images from Our Research



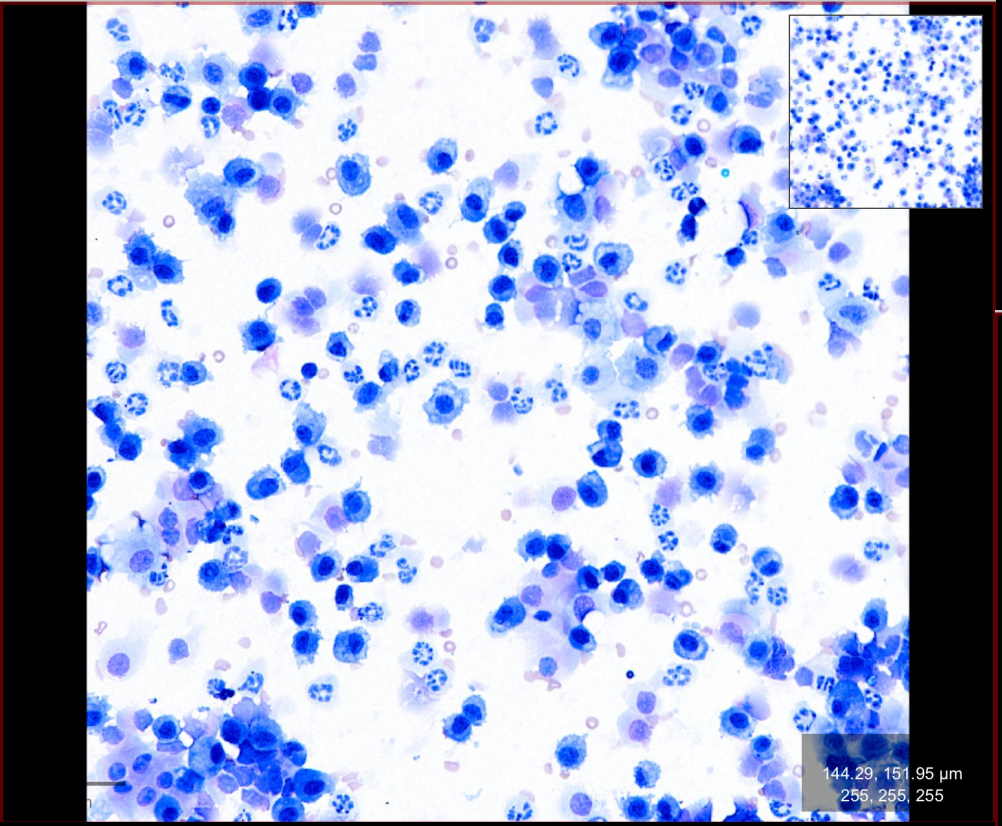


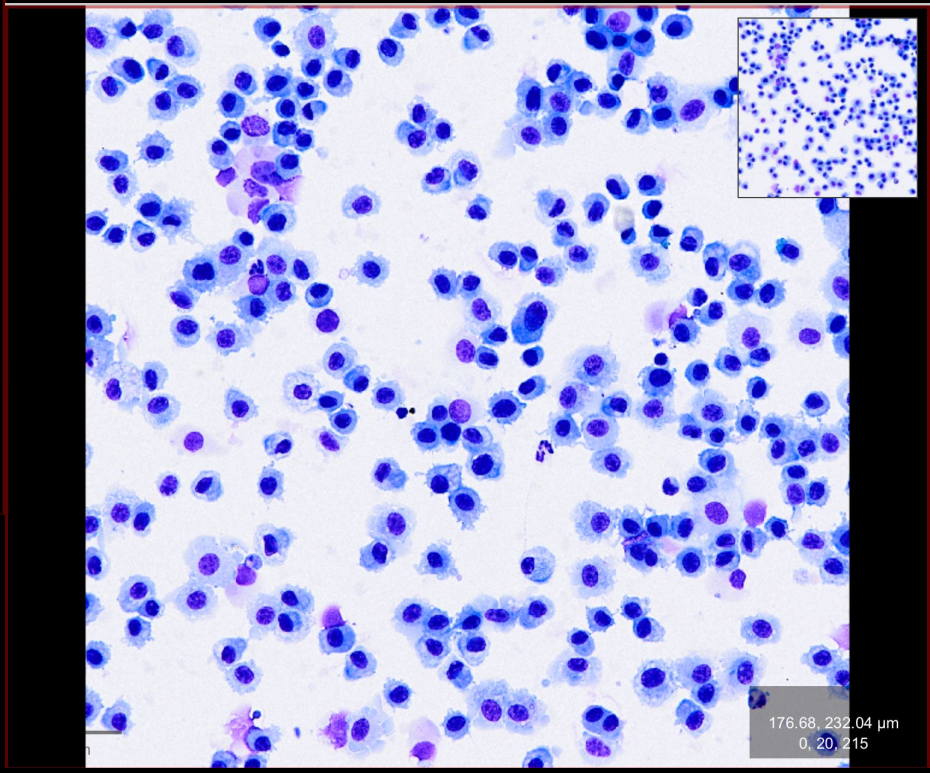
RNAScope Images - Puncta quantification



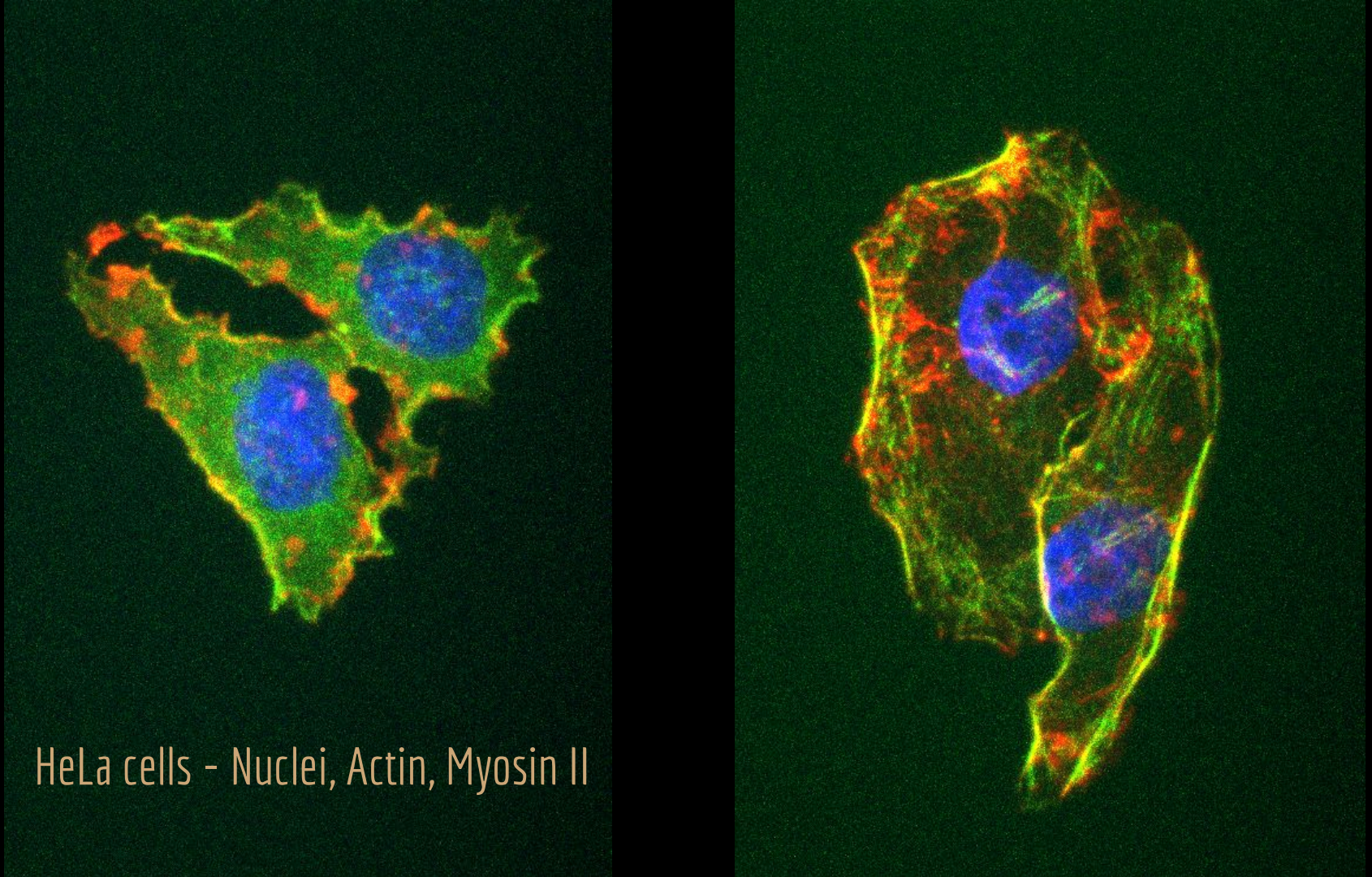


Histology and Pathology Scoring





Cytospins - Immune cell counts



HeLa cells - Nuclei, Actin, Myosin II

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 4 questions in 600 words or less. (20 points)

Turn in: images, .cpproj and .csv files (your pipeline and output) and the Homework Questions (word or pdf)

Due date: Sunday April 7th, 2024 by midnight.

Grades: based on the written worksheet 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

Must haves: 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 of the forum for help

Use ImageJ to pre-processes images if needed

Work together

*Use all tools 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? Are there specific techniques and/or types of data you hope to collect from your 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? Include references.
4. Describe an image analysis platform we haven't discussed in class. What types of images can be analyzed and what types of analysis are possible? Include references.