

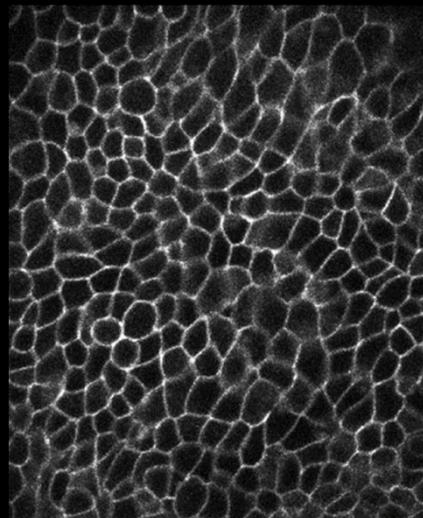
# Section 3: How to think like a Bio-image Analyst

Some slides and materials were adapted from:  
Lecture 12, 13, 14 of Bioimage Analysis 2020 by Robert Haase

In this section, we will...



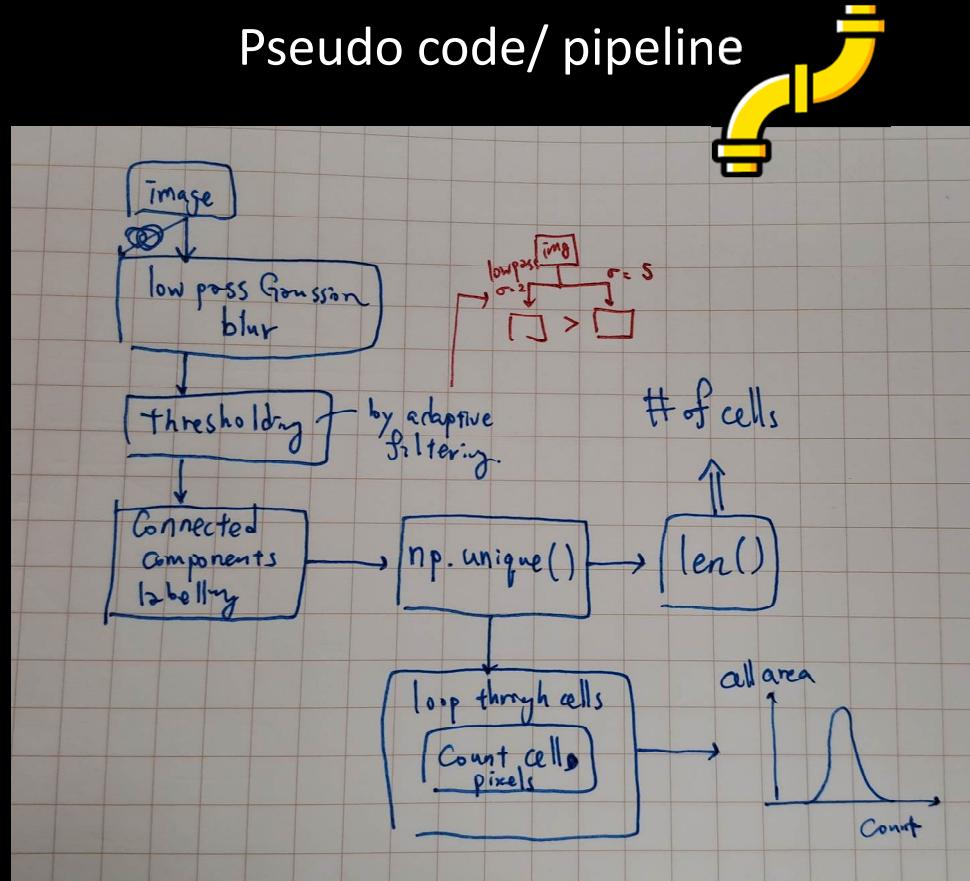
# For example...



I want to quantify

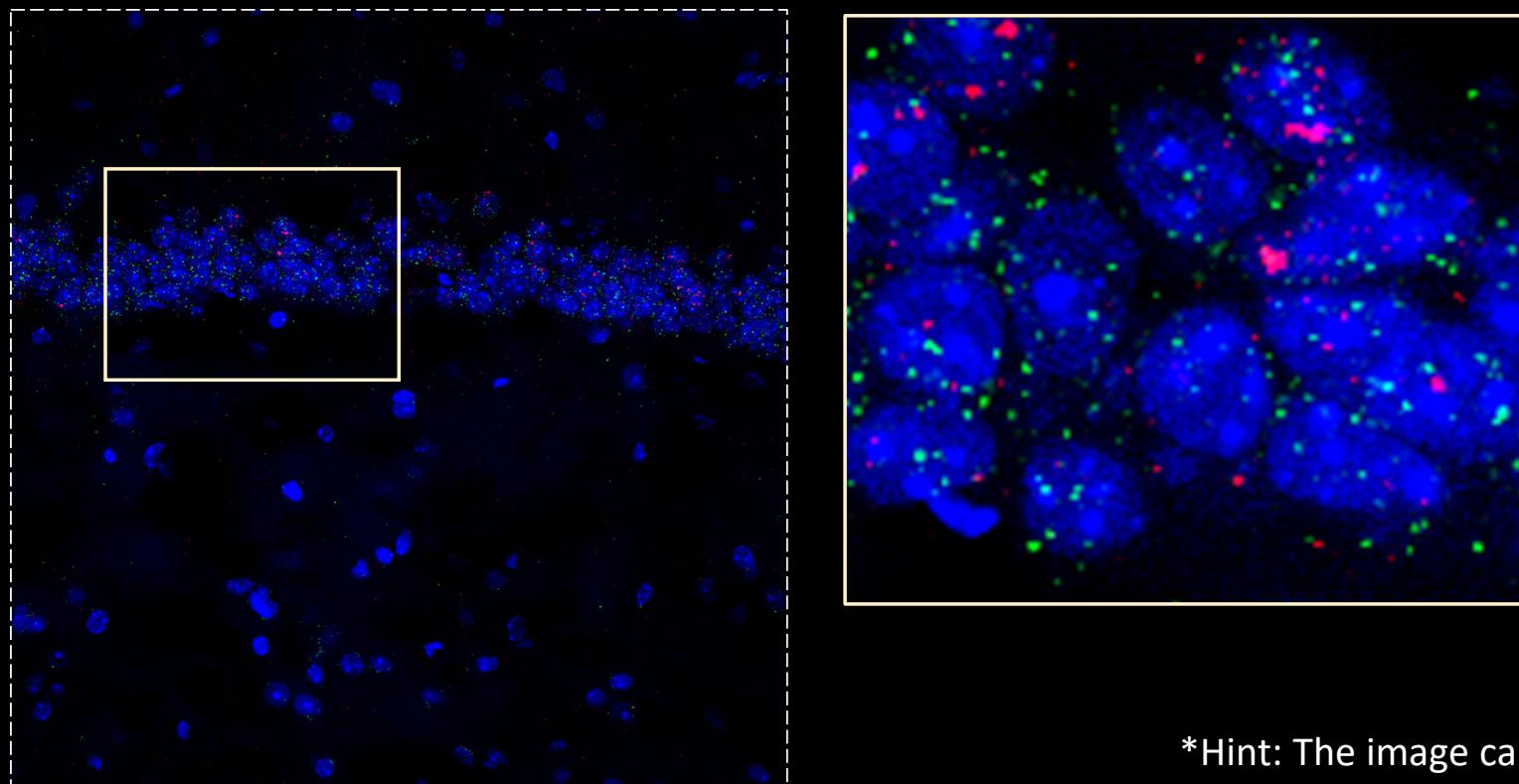
- Cell count
- Cell size distribution

## Pseudo code/ pipeline



Use what you learnt today!

# Case 1: Colocalization of neuro (FISH) images

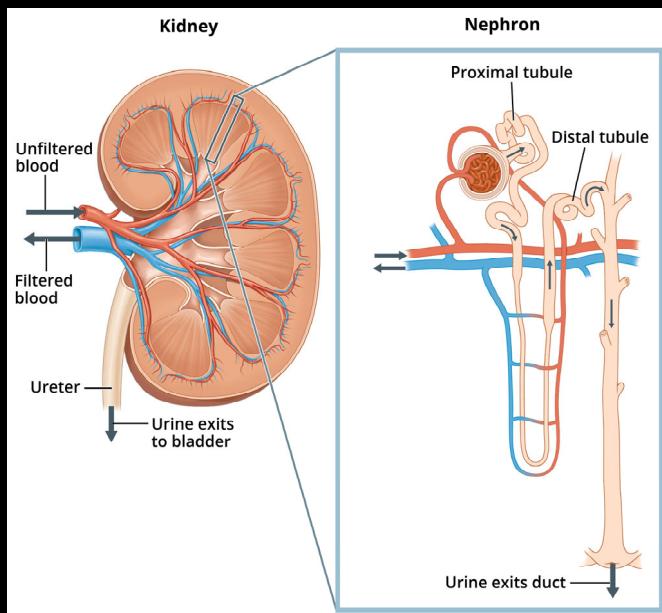


- I want to quantify:
- Number of cells
  - % of cells expressing R only, G only or both R&G
  - What else can you quantify?

\*Hint: The image can be split to three channels!

Image from Hsu Ching-Lung's Lab

# Case 2: Tracing tubule structures in the mouse embryo kidney

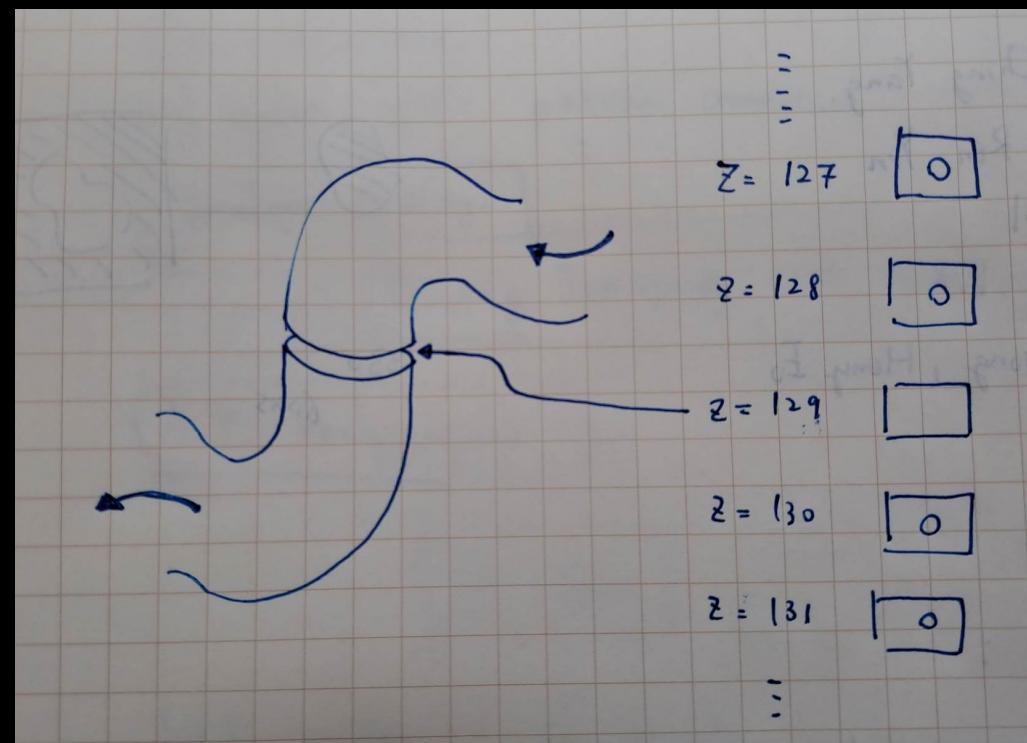
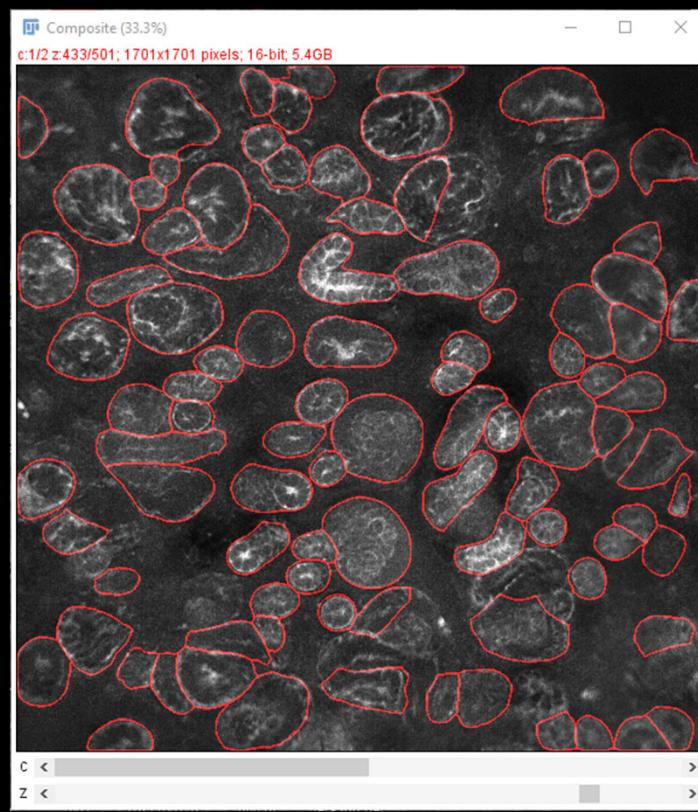


Question: How to get the final segmentation?  
Given the fact that Cellpose 3D works well with the data, just that

1. Cellpose cannot segment the entire tube. i.e. its fragmented
2. There are a few layers in the image where cellpose mis-identify (False Negative & False Positive)

Can you design a pipeline to overcome these problems?

Image(s) from Bi-Chang Chen's lab



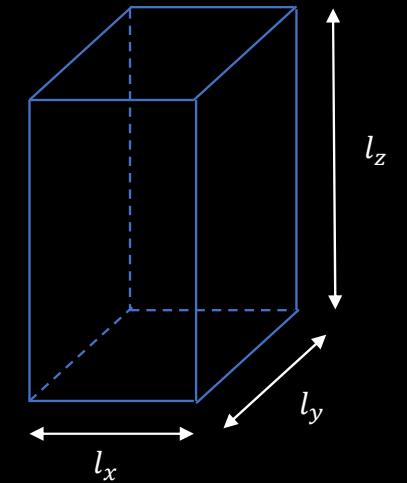
# Concept of Voxel



- 3D pixel; volume element
- Usually anisotropic: Not the same in all directions

$$l_x = l_y \neq l_z$$

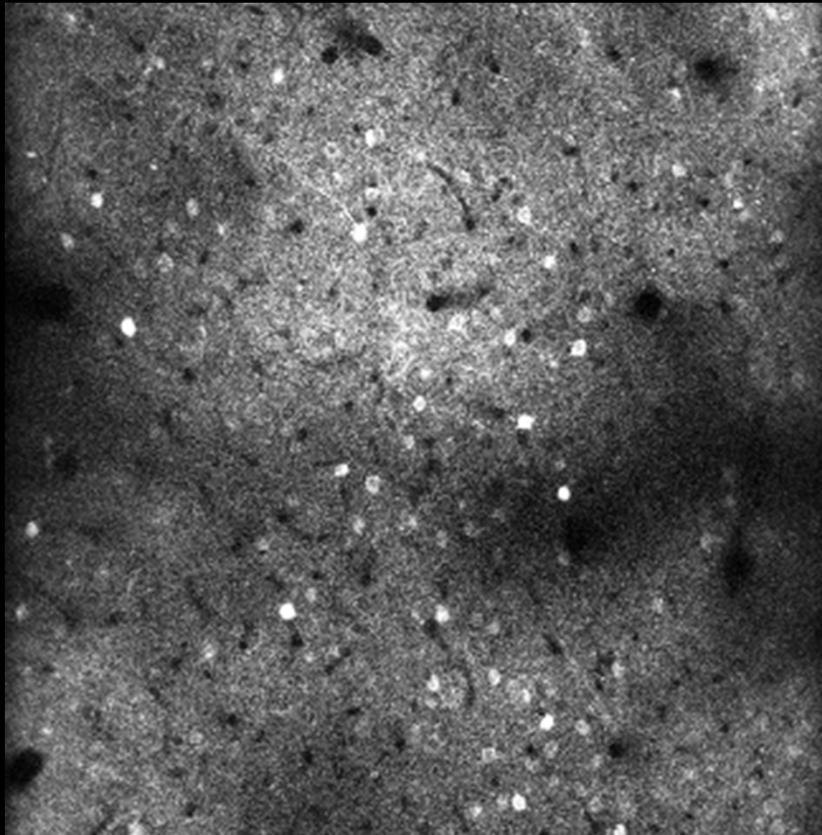
- Image analysts *love* to have isotropic voxels, but it's often not possible.



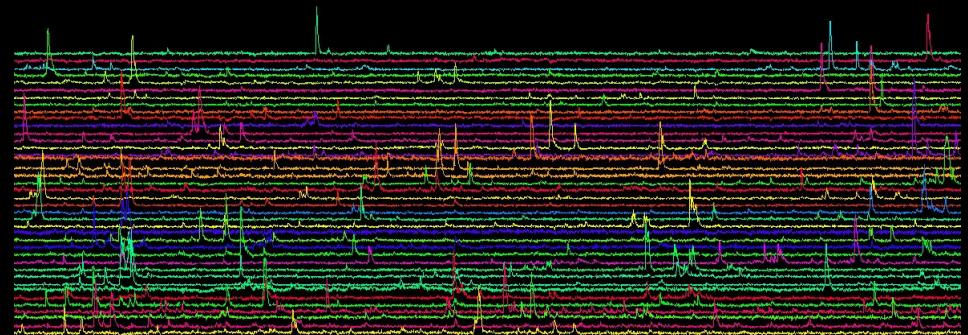
# Dimensional Reduction

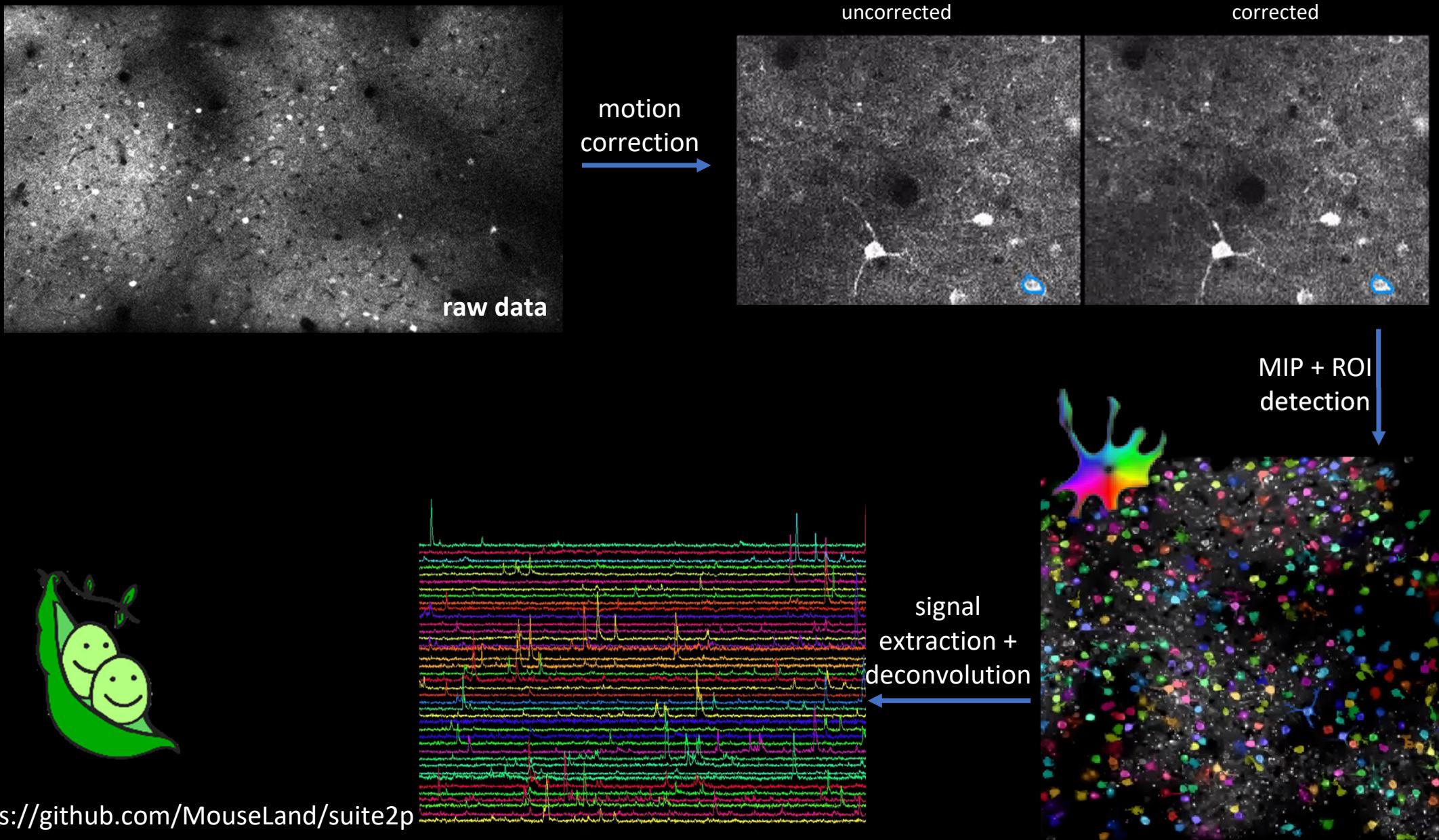
- Cropping
- MIP

# Case 3: 2-photon Calcium imaging



How to go from the video recording from the left to the raster plot below?





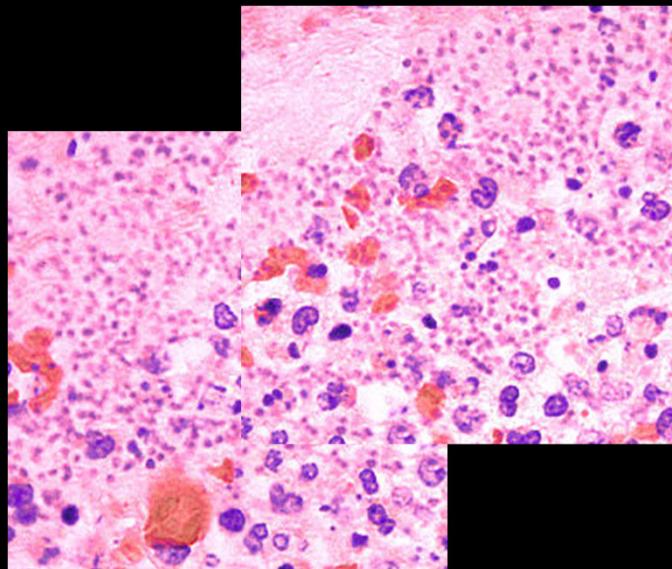
<https://github.com/MouseLand/suite2p>

# Very brief chat about image registration

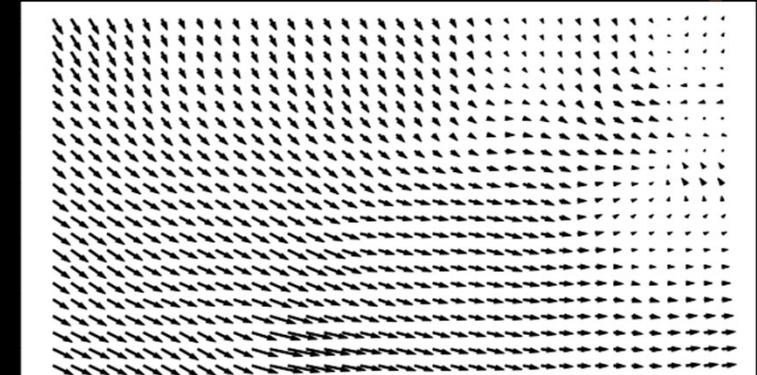
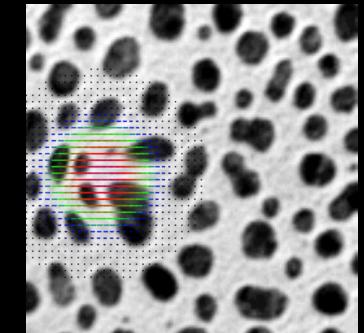
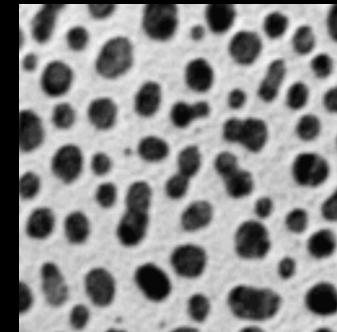
Used in motion correction, image stitching, object tracking

## Rigid registration

Translation, rotation...

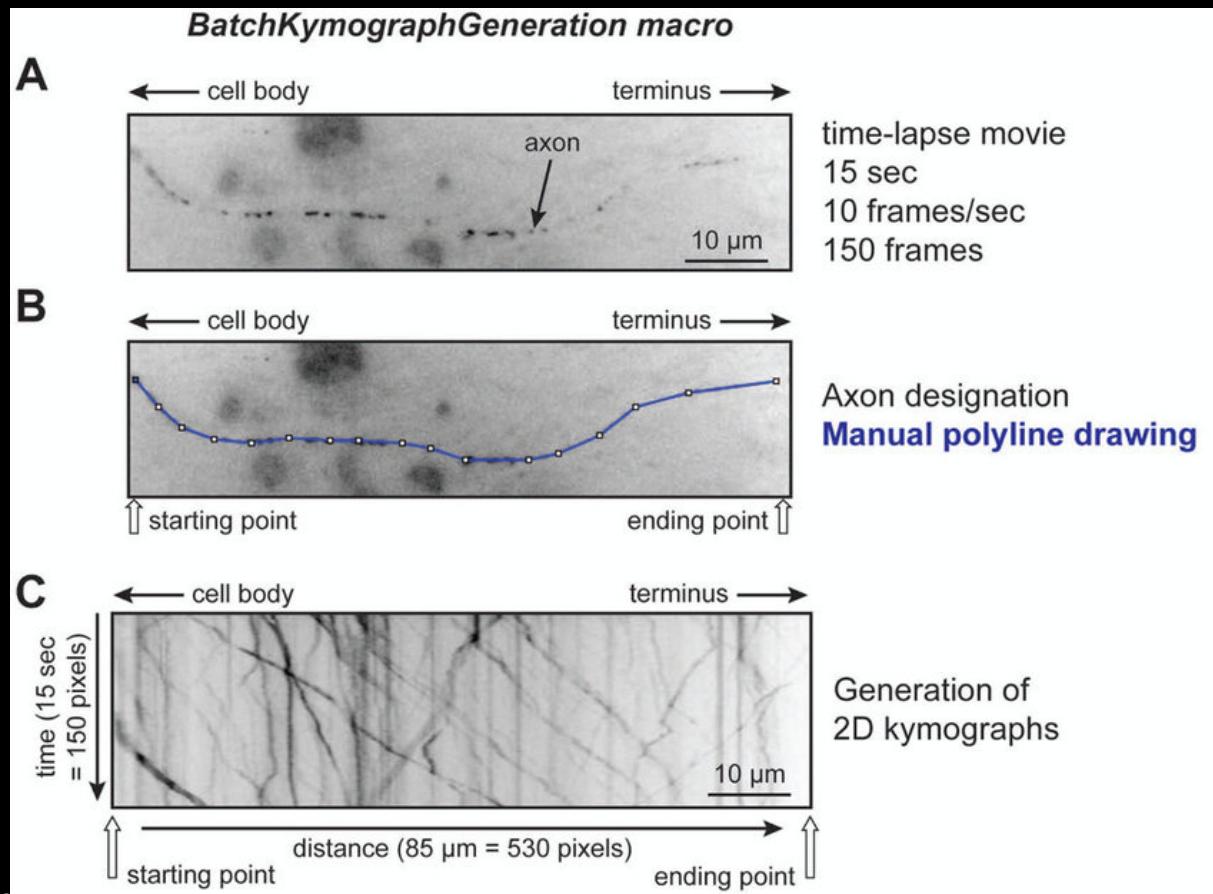


## Non-rigid registration



# Introduction to time series imaging

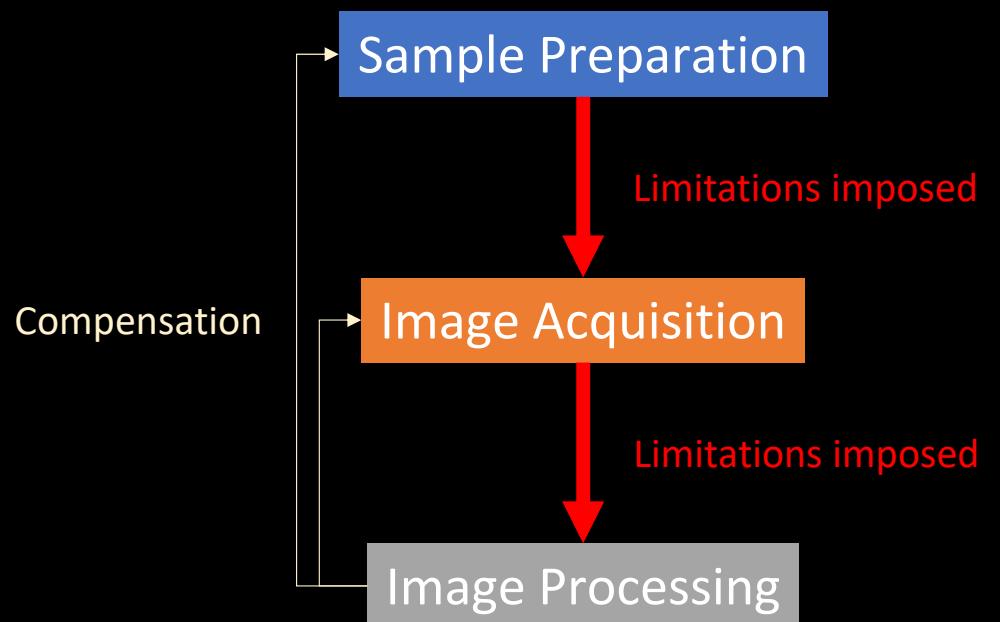
- idea of kymograph



Sequential images of PrPC-EGFP vesicles moving in a mouse hippocampal axon

# Recognizing the problems

- Sometime you cannot quantify your image very well. Think about the source of the problem. Upstream limitation
- Is it the problem of image processing? Image acquisition? Sample preparation?
- My criteria: if you can't see the features by eyes, it's the problem of the upstream
- Though there are recent effort to compensate the limitation in the first two steps via image processing methods
- For example, computational noise reduction, motion correction and so on
- E.g. emergence of computational microscopy. Compensation for photon budgets



## Further reading & learning...

- Due to the limitation of time and ignorance of mine, here are some topics that are not covered in this workshop.
- Image deblurring, image registration, deconvolution

# Useful Resources



# Image.sc

If you have a question  
you want to ask about  
image analysis:

<https://forum.image.sc/>

The screenshot shows the homepage of the Image.sc forum. At the top, there is a navigation bar with links for "Zulip chat", "Related Forums", "Sign Up", "Log In", a search icon, and a menu icon. Below the navigation bar, there is a section titled "Community Partners" with a "Latest" tab selected. There are also tabs for "all categories", "all tags", "all", "Top", and "Categories". A "Topic" filter is applied. The main content area displays several forum posts:

- Related Communities**  
There are several other community forums whose topics overlap with this one: Microforum's focus is hardware, acquisition, and specimen-related aspects of scientific imaging, particularly (but not limited to) theory ... [read more](#)
- Welcome to the Image.sc Forum!**  
Announcements  
Welcome to the Scientific Community Image Forum at [forum.image.sc!](https://forum.image.sc/) This forum's focus is software-oriented aspects of scientific imaging, particularly (but not limited to) image analysis, processing, acquisition, storag... [read more](#)
- Reading 2D images as a multi channel 3D stack in python**  
Image Analysis python  
A post by user C with 4 replies, 40 views, and 2 hours ago.
- Qupath ImageCombiner alignment apply the affine transform to the image**  
Usage & Issues qupath, alignment, qupath-extension, imagecombiner  
A post by user C with 4 replies, 48 views, and 2 hours ago.
- DLC 2.3.0 fails to start on macOS 12.6.2 - TensorFlow or Qt issue? How to resolve?**  
A post by user C with 0 replies, 0 views, and 2 hours ago.

# Applied Bio-Image Analysis Lecture 2020

If you want to have a good overview on current trend in bioimage analysis:

<https://www.youtube.com/watch?v=p4vYlybltQM&t=2584s>

The image shows a YouTube video player interface. At the top, there are logos for BIOTEC, CENTER FOR SYSTEMS BIOLOGY DRESDEN, and CBG. The video title is "Applied Bio-Image Analysis A Summary" by Robert Haase, Myers lab, MPI CBG. The video was uploaded in April-July 2020 and has 982 views from 2 years ago. The video duration is 1:34:32. The player shows a thumbnail of the speaker, Robert Haase, and various control buttons like play, volume, and settings.

Applied Bio-Image Analysis  
A Summary

Robert Haase, Myers lab, MPI CBG

April-July 2020

14c Applied Bio-Image Analysis Lecture 2020 - A Summary

haesleinhuepf 2.3K subscribers Subscribed 21 Share ...

982 views 2 years ago  
A summary about what we learned in the 2020 lecture about Applied Bio-Image Analysis at BioTec TU Dresden

0:01:30 An Introduction to Applied Bio-Image Analysis Show more

# 100DaysCode

If you want to become more competent in python programming

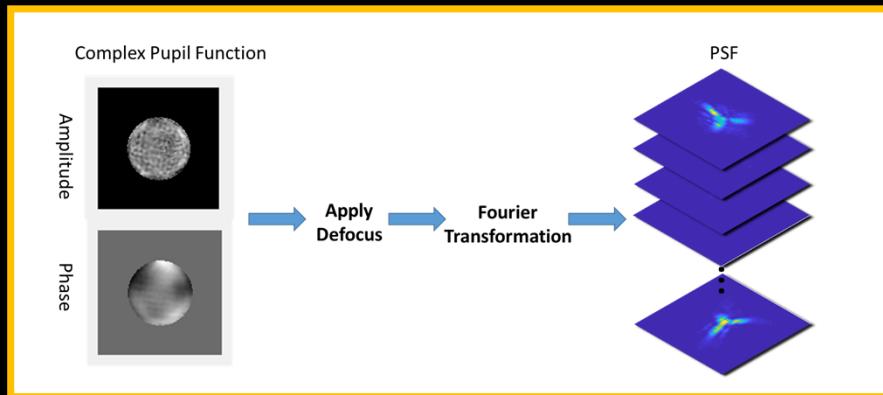
<https://www.udemy.com/course/100-days-of-code/>

The screenshot shows the Udemy website interface. At the top, there's a navigation bar with the Udemy logo, a search bar, and user account links. Below the header, the breadcrumb navigation shows 'Development > Programming Languages > Python'. The main title of the course is '100 Days of Code: The Complete Python Pro Bootcamp for 2023'. A brief description follows: 'Master Python by building 100 projects in 100 days. Learn data science, automation, build websites, games and apps!'. Key metrics are displayed: 'Bestseller', '4.7 ★★★★★ (163,693 ratings)', and '729,990 students'. The course is created by 'Dr. Angela Yu'. It was last updated in August 2022 and is available in English, Arabic [Auto], and 15 more languages. To the right, there's a preview video thumbnail featuring a woman, a 'Preview this course' button, and a note indicating the user purchased it on Aug. 29, 2022. A large 'Go to course' button is prominently displayed at the bottom.

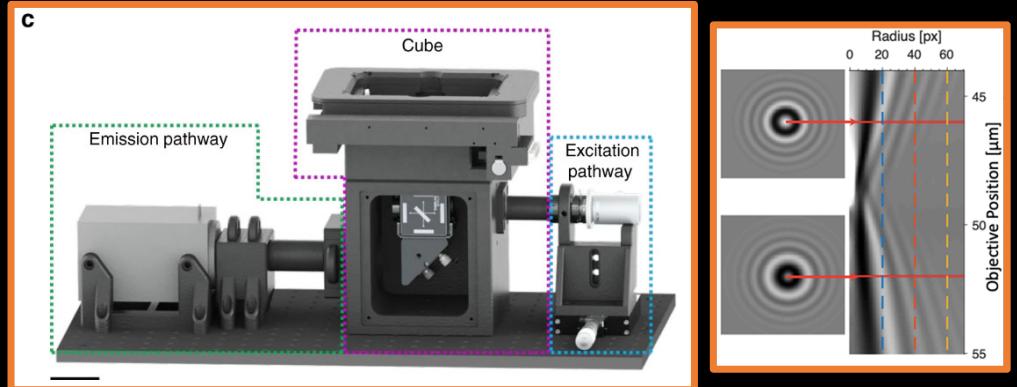
... and I



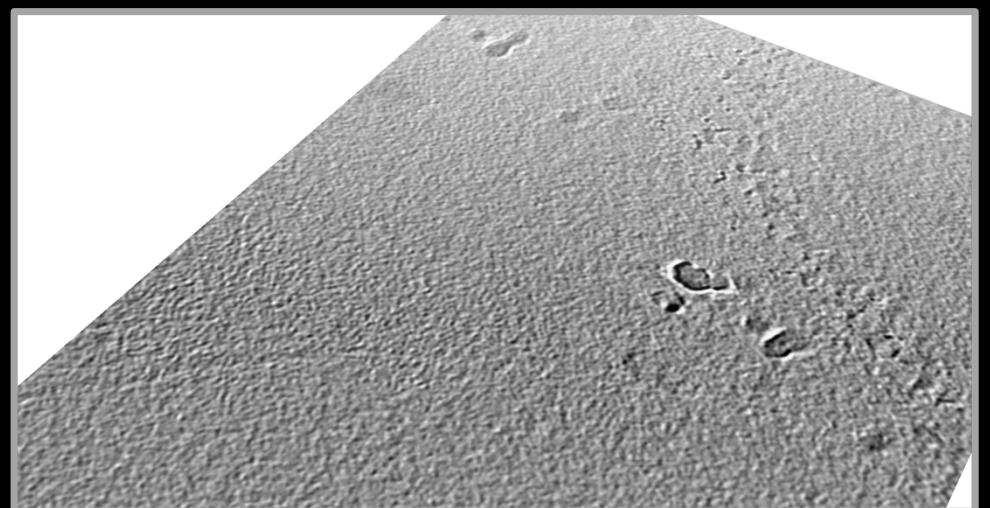
### Light-sheet image processing



A little bit of Theoretical Optics



(Super-resolution) Microscopy Hardware/ Control



Cryo-EM Tomography and sub-tomogram averaging

# Other useful resources

- To learn about microscopes: Nikon's MicroscopyU  
<https://www.microscopyu.com/>

# Acknowledgement

My Teaching Materials are mostly based on the following resources:

- *Bioimage Book* by *Peter Bankhead*
- *Lecture Bioimage Analysis 2020* by *Robert Haase*
- *Bioimage Analysis in Python Jupyter Notebook* by *Jonas Hartmann & Toby Hodges*
- *Google Colab Notebook* by *Marius Pachitariu*

# Helps and Advice

## Inside Sinica:

Hsu Ching-Lung 徐經倫老師 (NPAS) and his lab (Esp. Hsuan-Pei Huang 黃宣霈)

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Jung-Kun Wen 温榮崑學長 (IBC ImageCORE)

Wei-Chen Chu 朱韋臣學長 (ICOB ImageCORE)

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You-Hsuan Liu 劉又萱 (IOP)

My parents (Koukounut Family)...



## Outside Sinica

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Jonas Hartmann (UCL London)

Chien-Cheng Michael Shih (**Novartis US**)

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- Ricardo Henriques's (LMCB UCL London)
- Steven Lee's (Chemistry Cambridge)



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- Imaging Core Facilities, ICOB, AS