CE Image Analysis

An Overview

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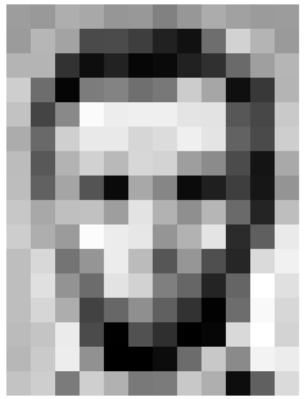
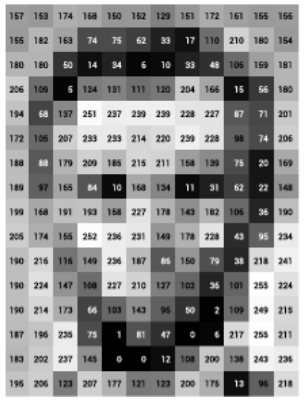
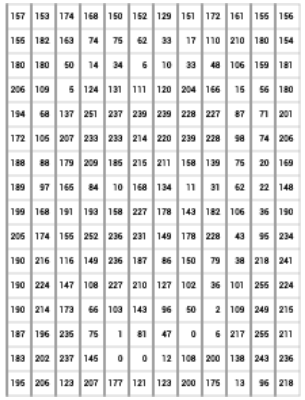
Conceptualizing Image Analysis

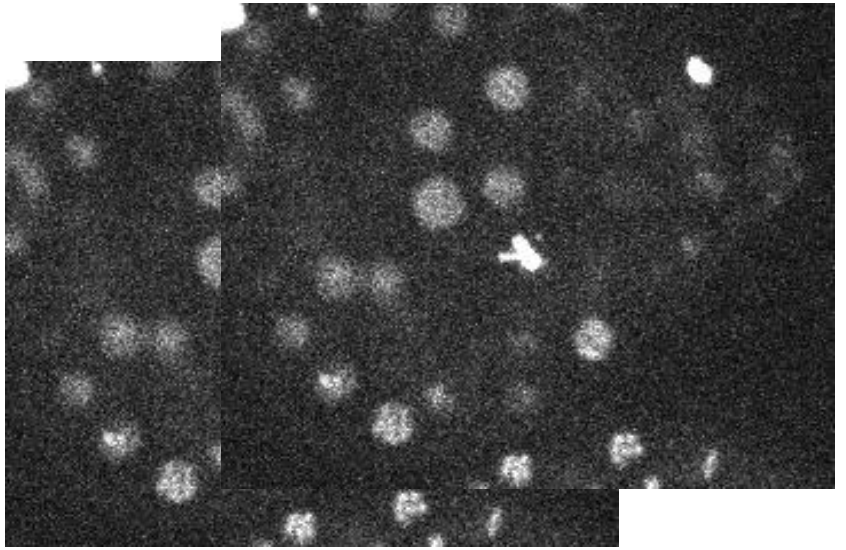
- Images
- Mapping from scientific problems
  - Validation
  - Computational tools
- Some Major Classes of Task
  - Object Segmentation and Measurement
  - Alignment
  - Tracking
- AI and Image Analysis
  - Introduction to deep learning
    - Architectures, Uses, Limitations
    - Advanced Examples Spatial Patterns

Images

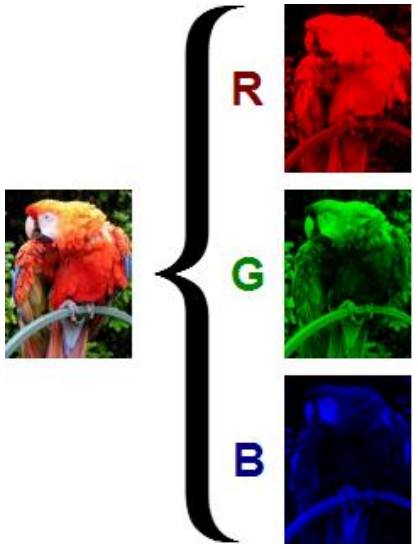


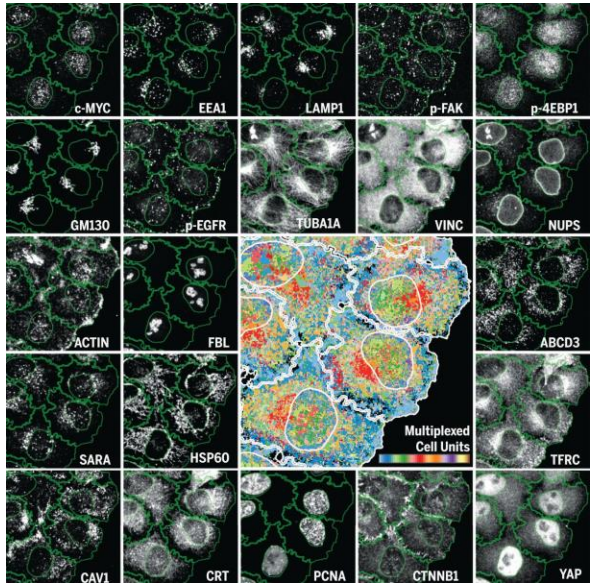


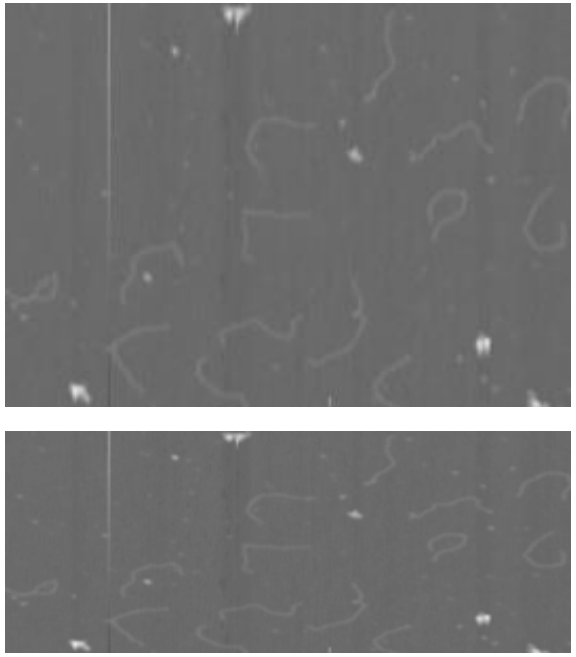




.25um x.25um x 1um pixel







Why might pixels not be square like these?

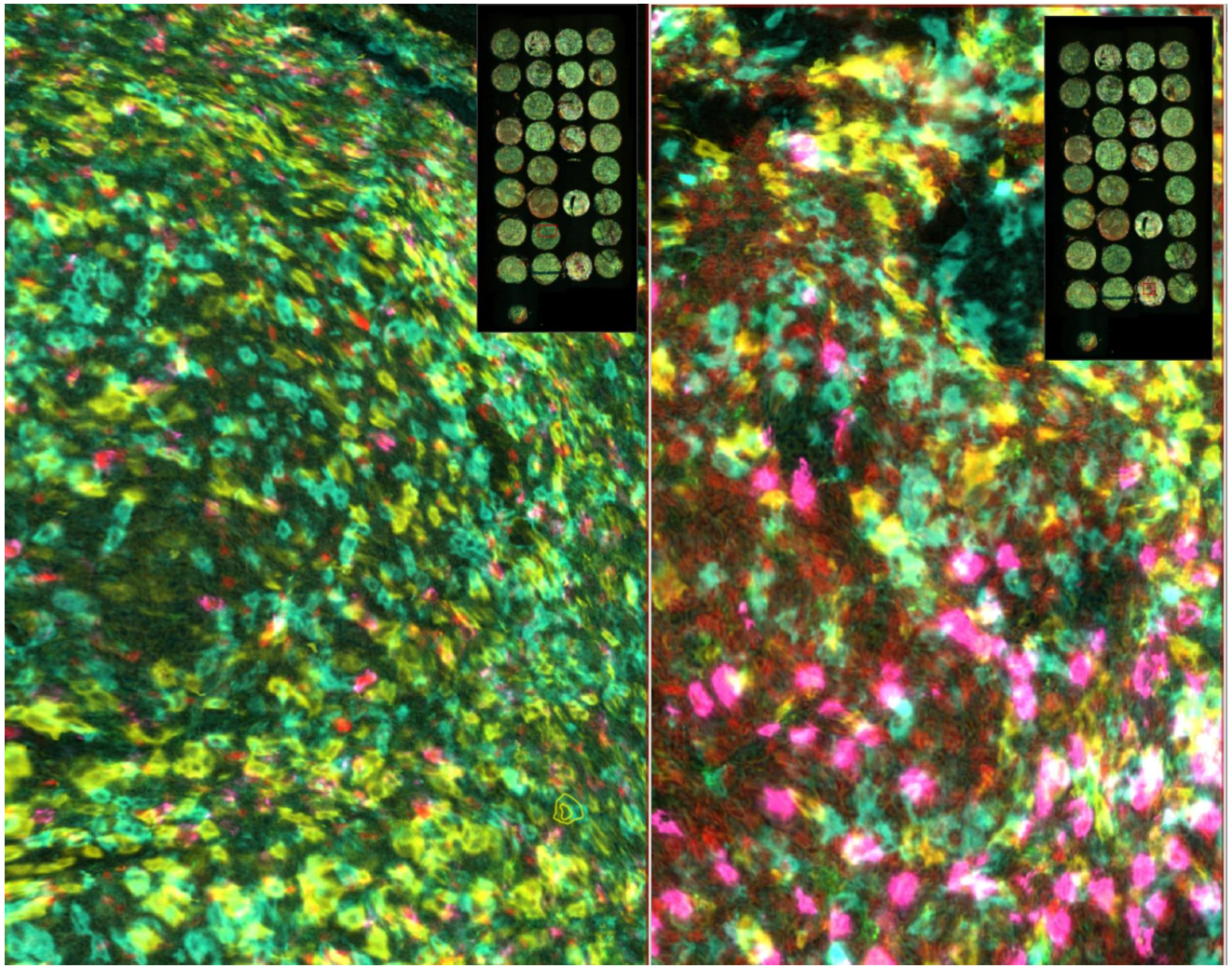
What is there? Objects, properties, relationships

- Mapping from a scientific problems
- Validation
- Computational tools

Mapping from a Scientific Problem

- What is the question?
  - Often (not always) prove condition x is different than condition y
- **What do I see?**
- What can I measure?

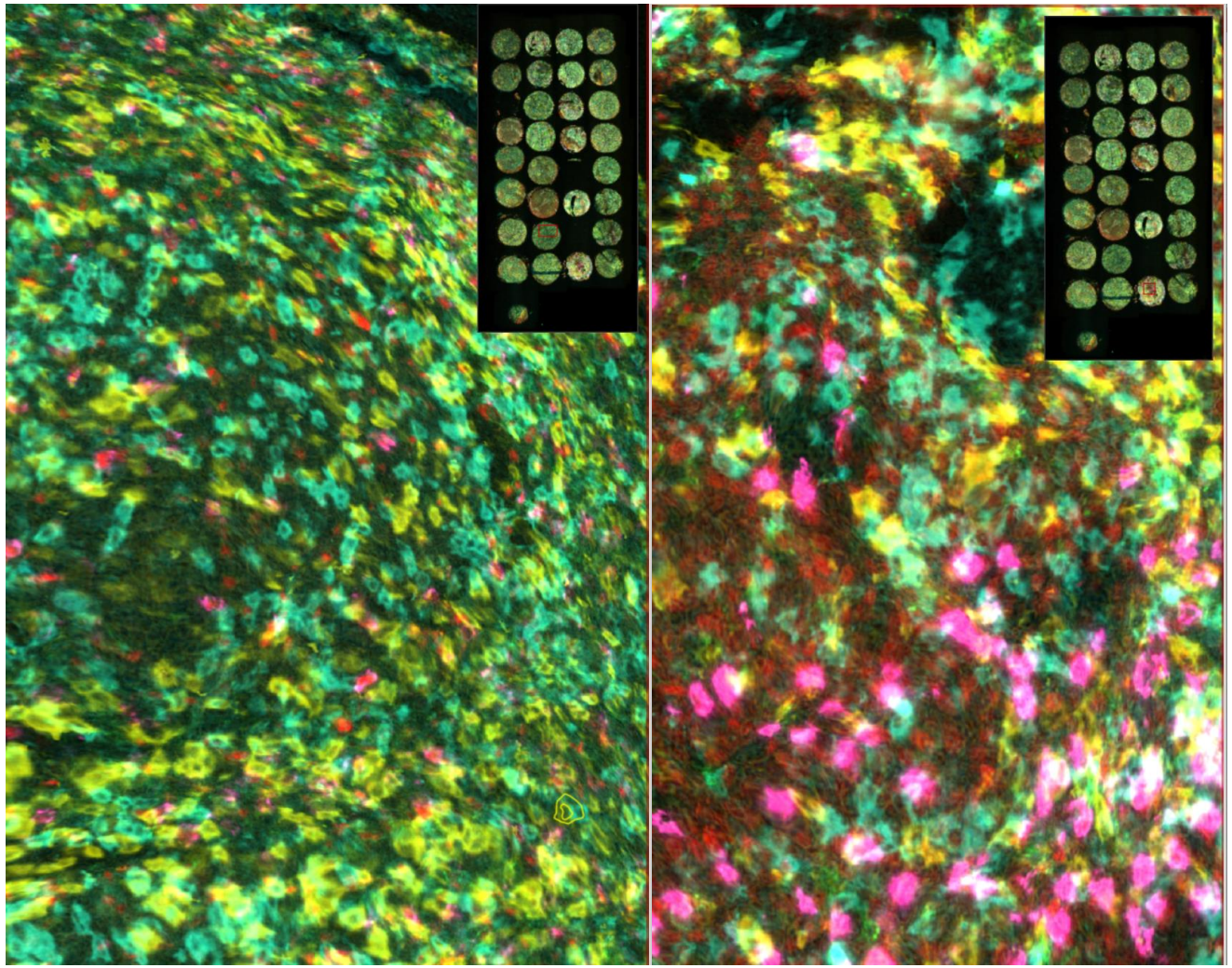
- What are some differences between these two cores?



Validation

- Spot checking vs formal evaluation
- **Chance of artificial correlation of measurement with experimental condition?**

- **How might this happen here?**



Computational Tools

- How easy is the workflow to
  - Create
  - Use
  - Replicate
- Layering on Flexible Products
  - Free: Cellprofiler, Ilastik, QuPath…
  - commercial: Imaris…
  - Libraries: napari, MATLAB…

Programs versus Software Products

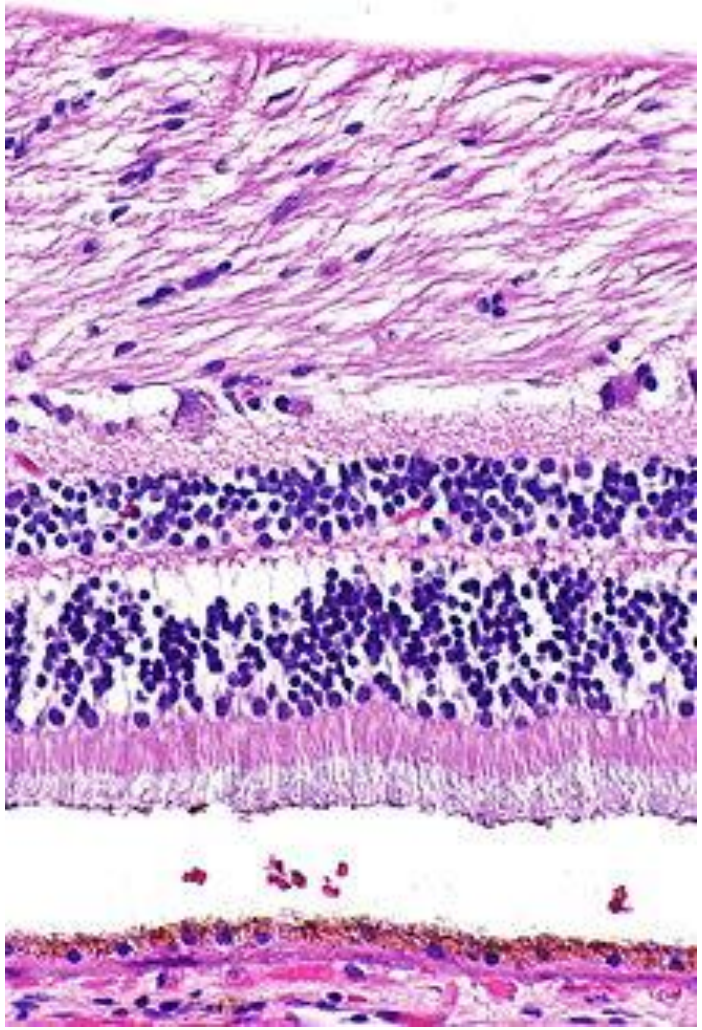
Usually small in size	Large
Author himself is sole user	Large number of users
Single developer	Team of developers
Lacks proper user interface	Well-designed interface
Lacks proper documentation	Well documented & user-manual prepared
Ad hoc development.	Systematic development

Software engineering 101…

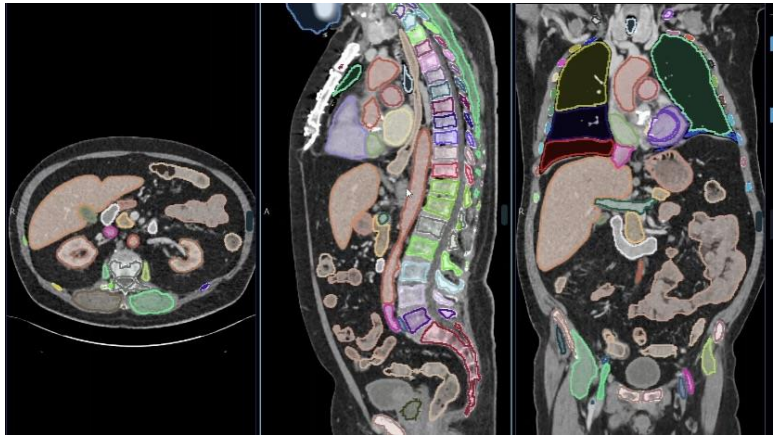
Major Classes of Task and Examples

- Object segmentation and measurement
- Alignment
- Tracking

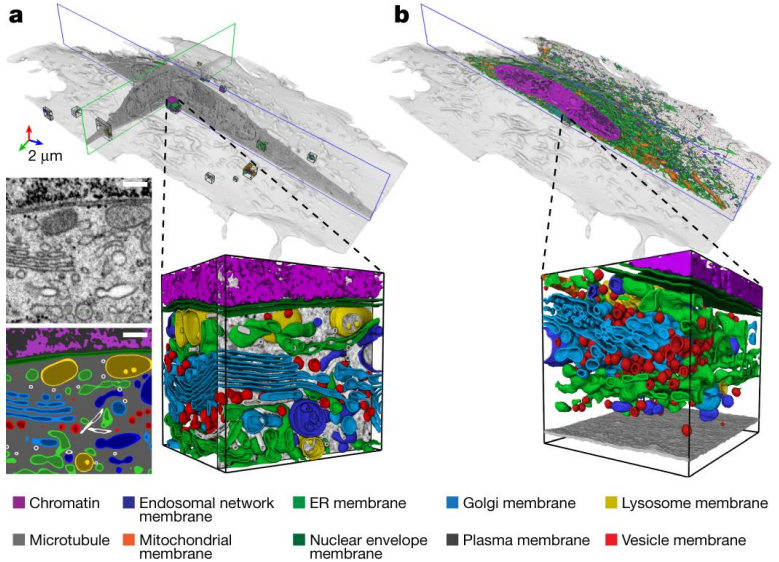
Object Segmentation and Measurement



wikipedia

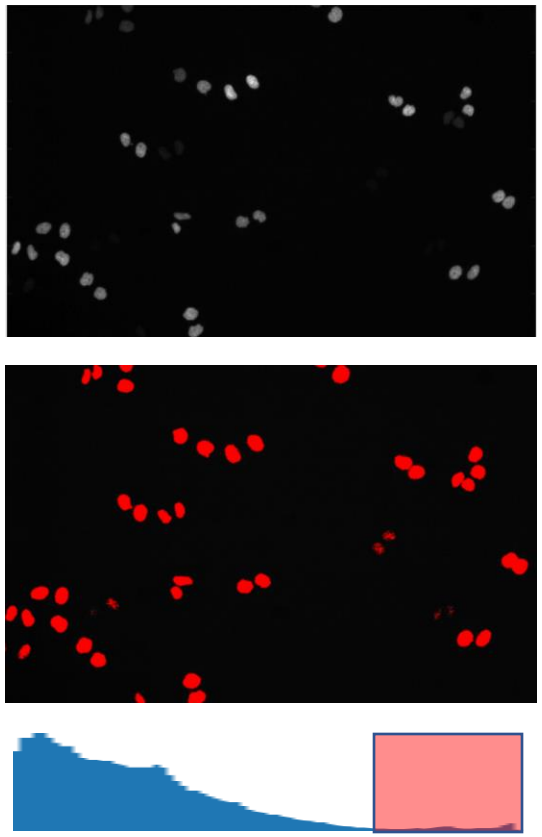


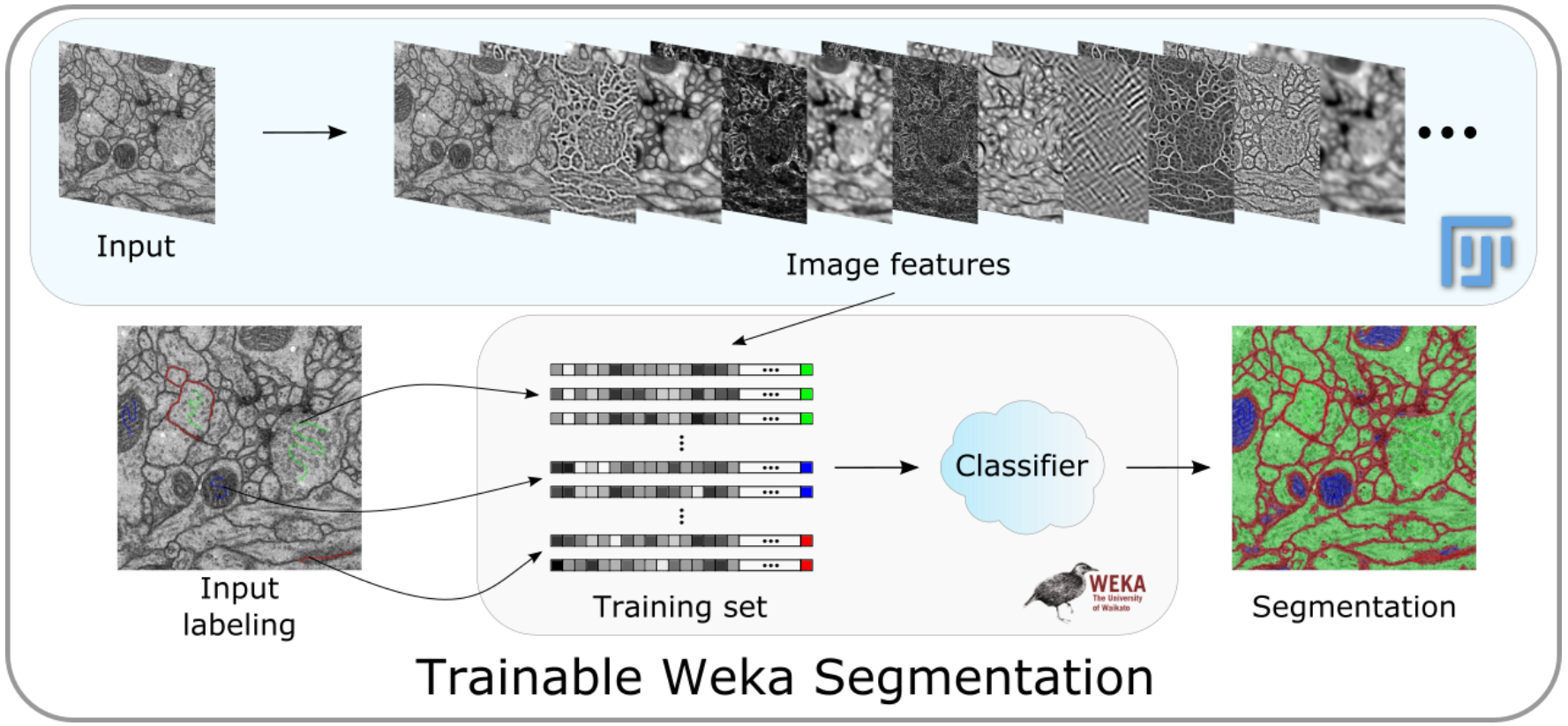
NVIDIA



Heinrich et al Nature, 2021

Object Segmentation

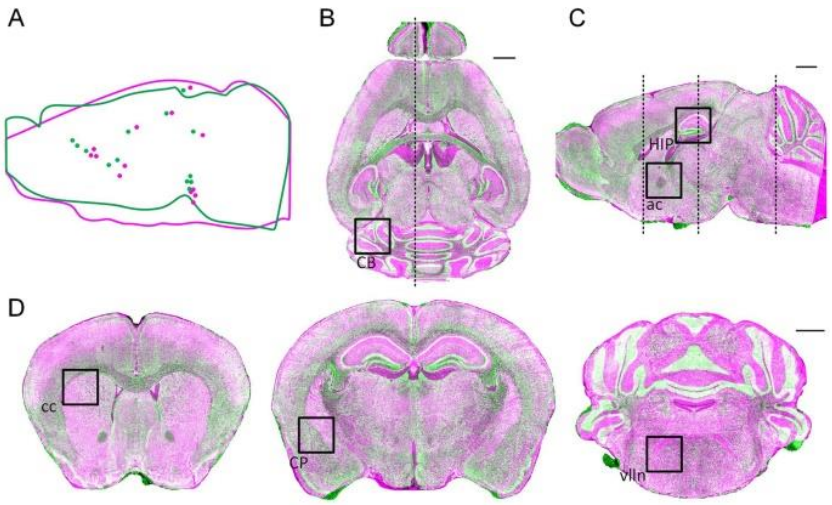




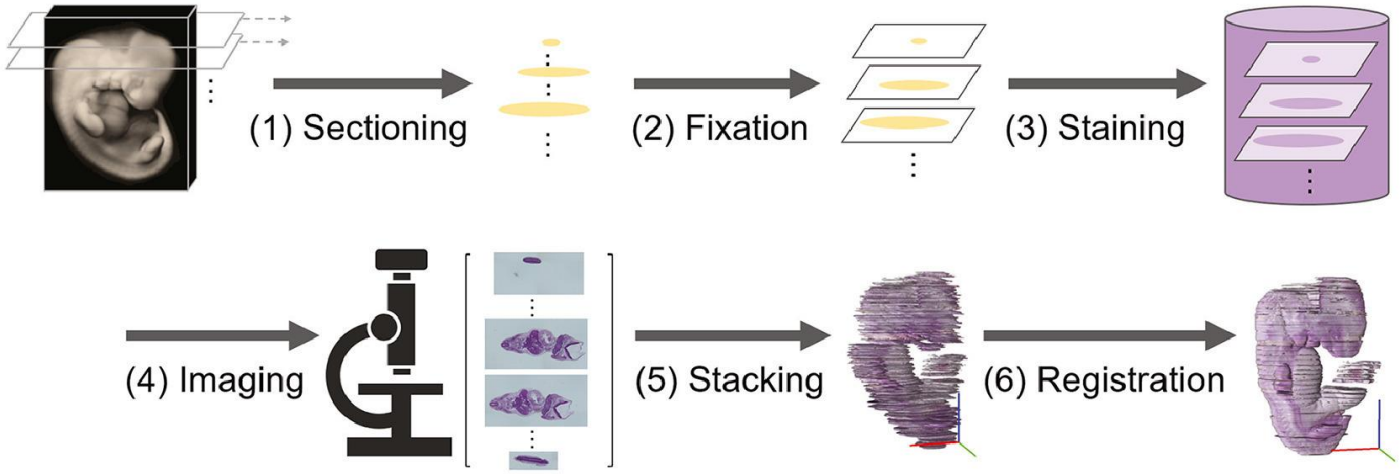
imagej.net/plugins/tws/

What are some likely advantages and disadvantages of these compared to deep learning approaches?

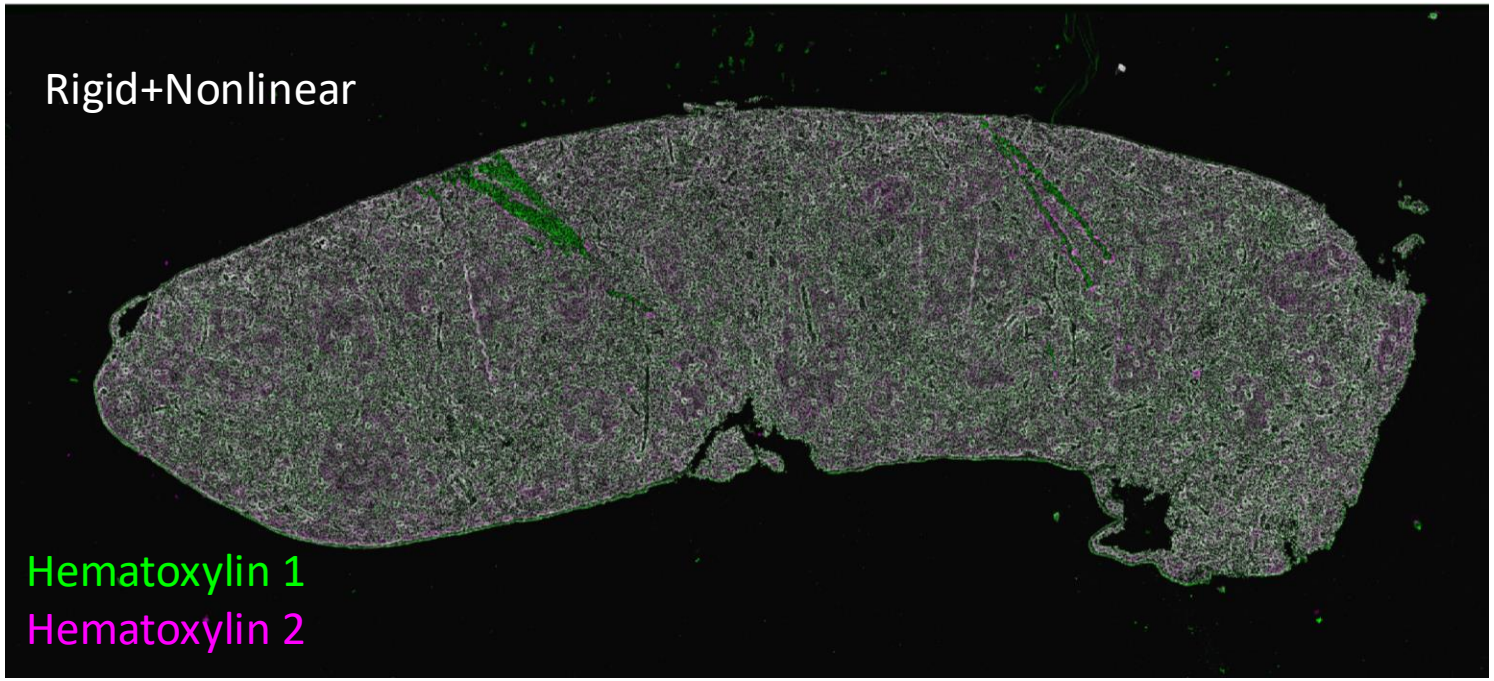
Alignment



Ni et al, Nature Scientific Reports, 2020



Kajihara et al, Pattern Recognition, 2019



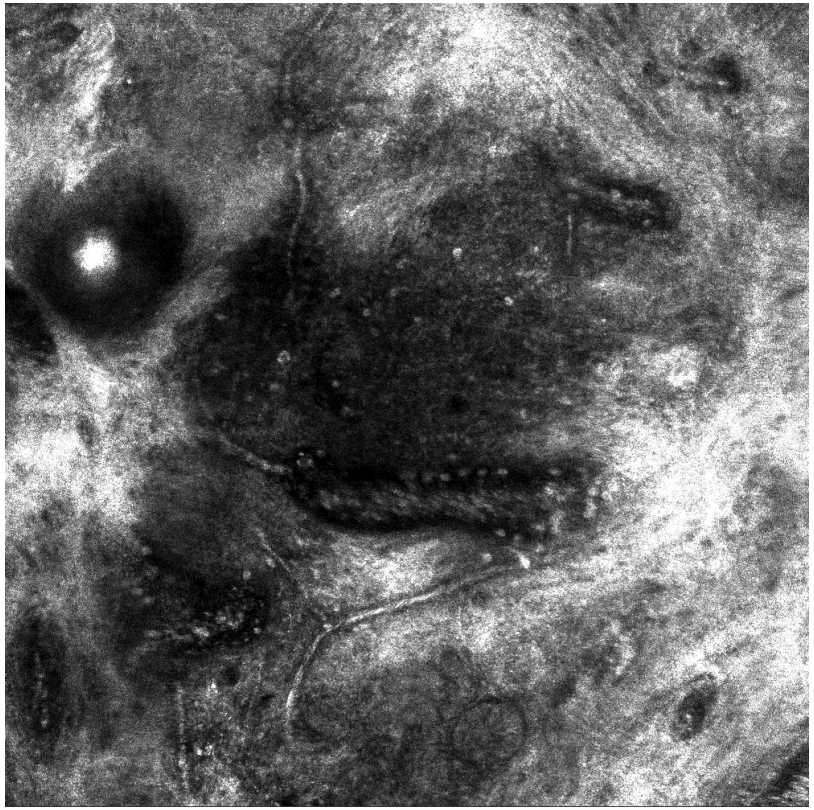
Alignment:Example

nature communications

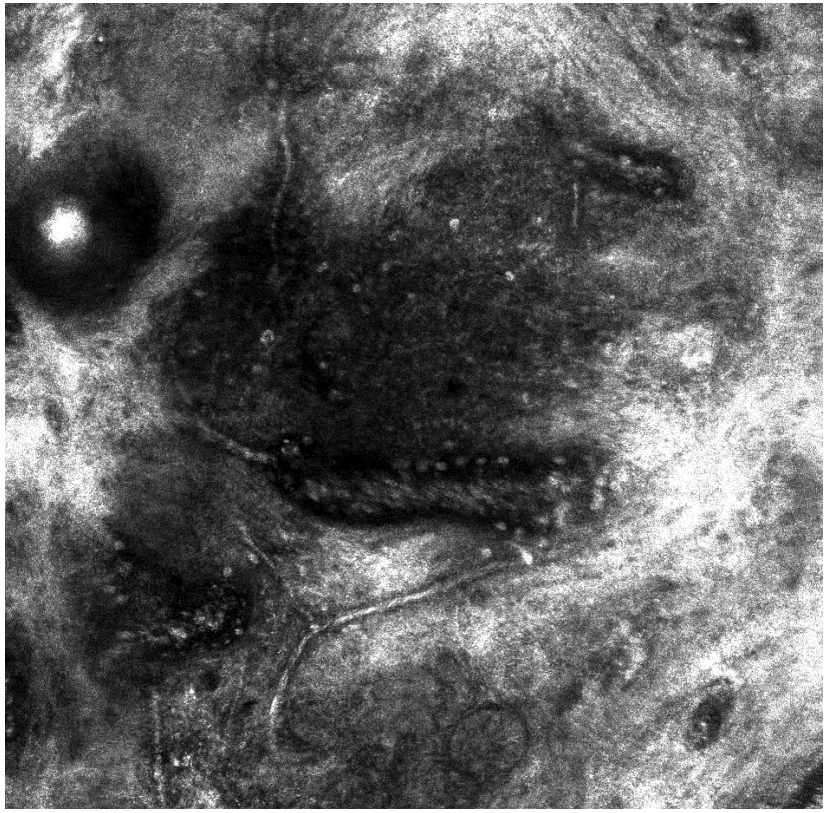
Article https://doi.org/10.1038/s41467-022-32738-7

In vivo tumor immune microenvironment phenotypes correlate with inflammation and vasculature to predict immunotherapy response

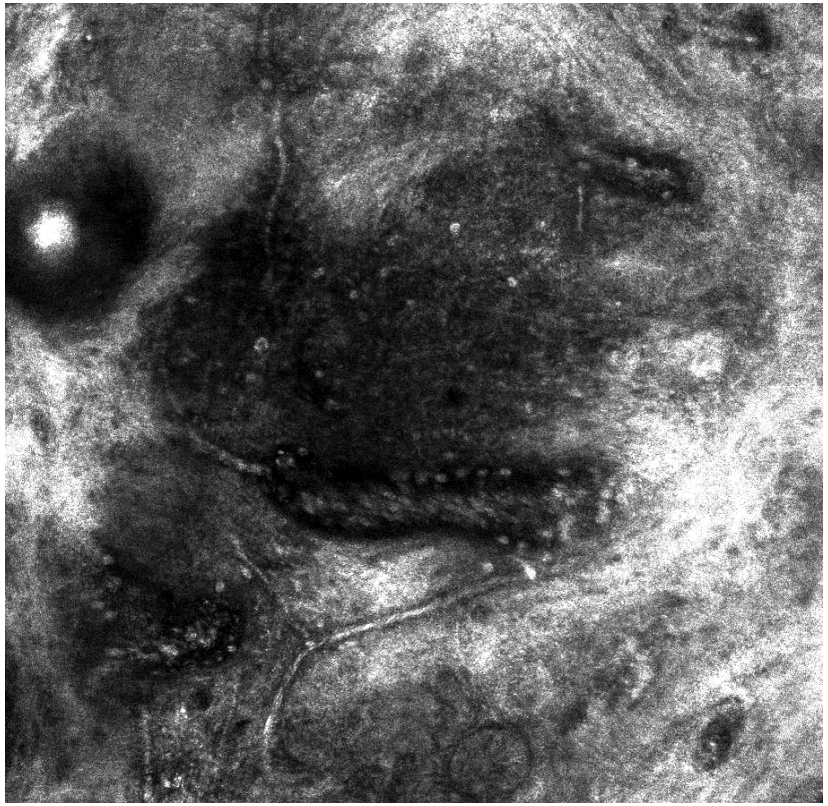
Sahu et al Nature Communications, 2022



Raw data



Fiji SIFT feature affine alignment

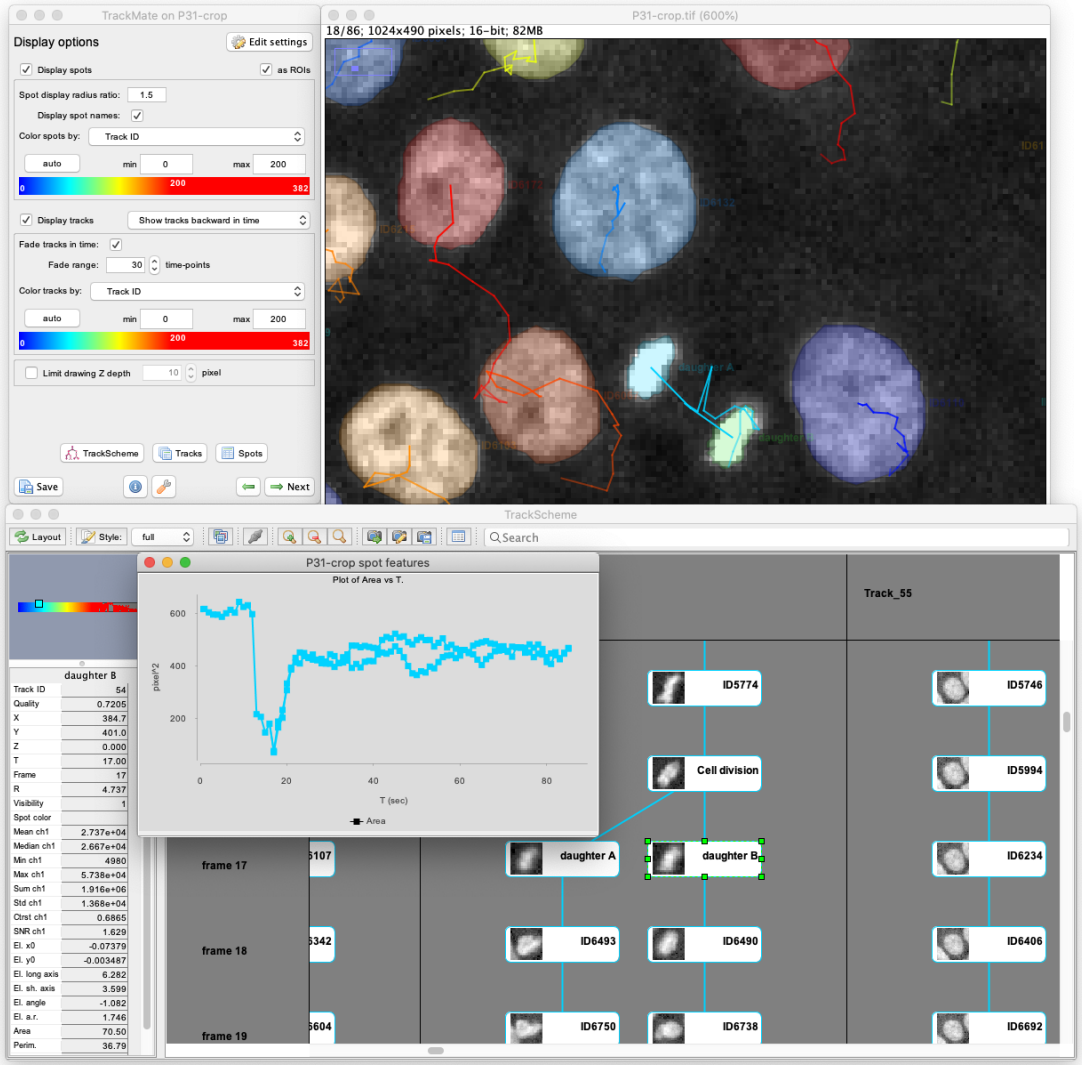


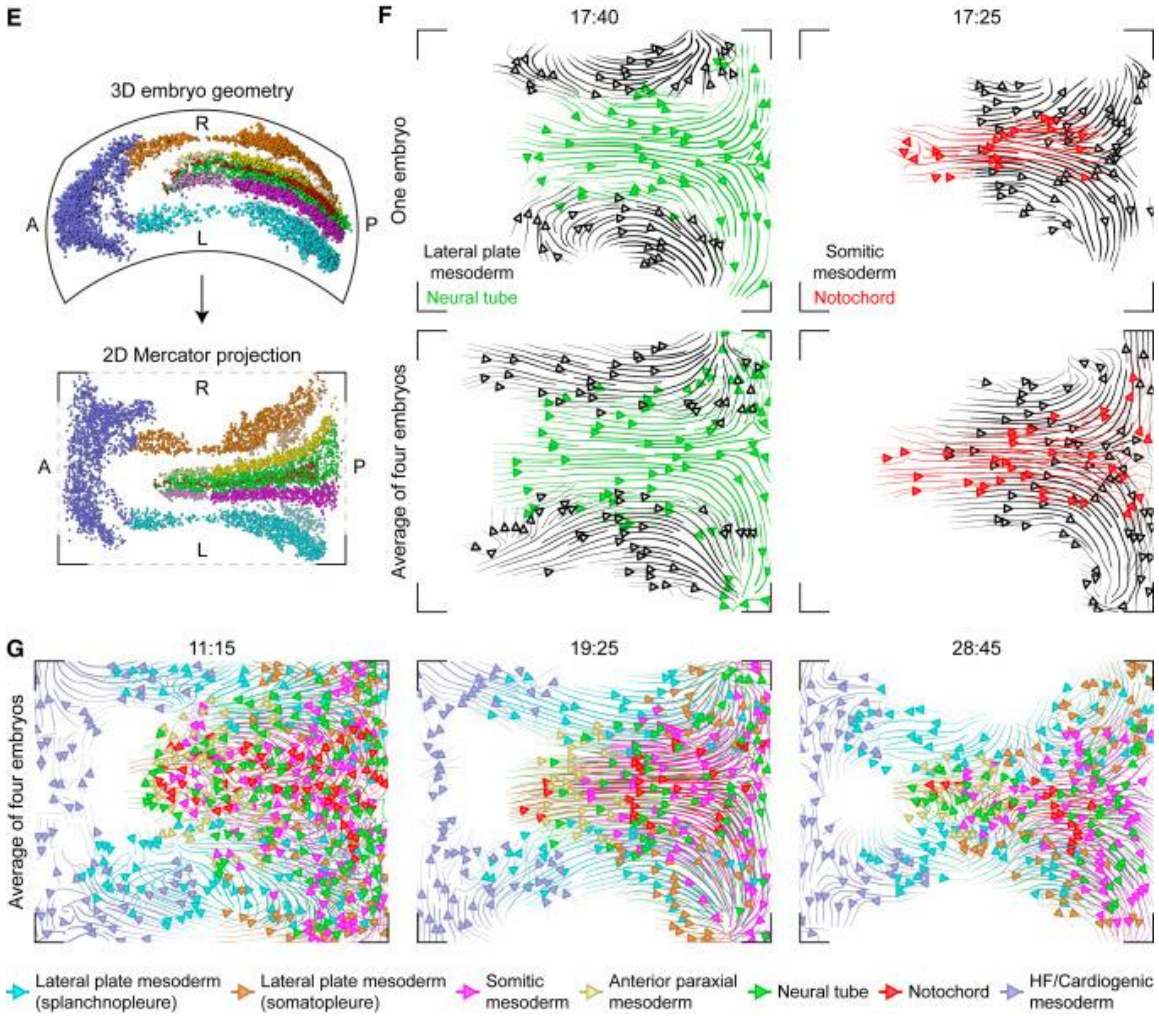
Plus MATLAB imregdemon nonlinear alignment

What might be some possible risks of this kind of nonlinear warping?

Tracking

Trackmate

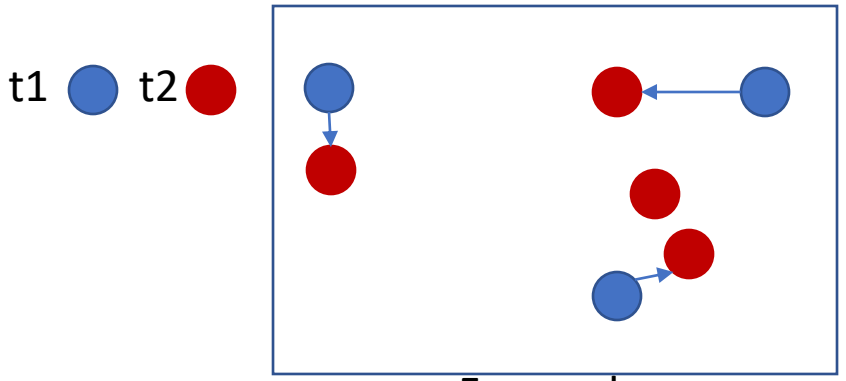




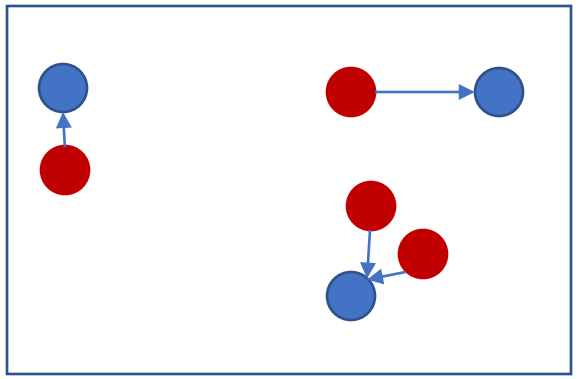
McDole, et al. Cell 2018

Tracking

- Detect and Link
  - Nearest neighbor
  - Linear Assignment

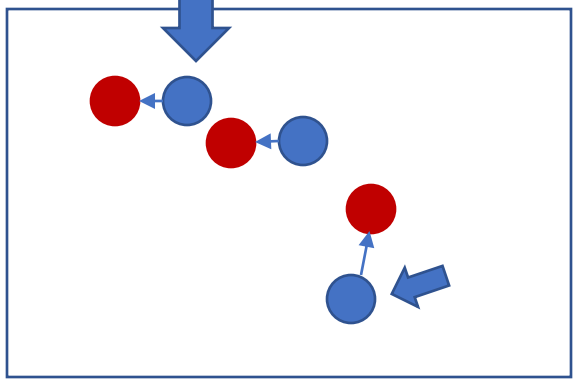


Forward

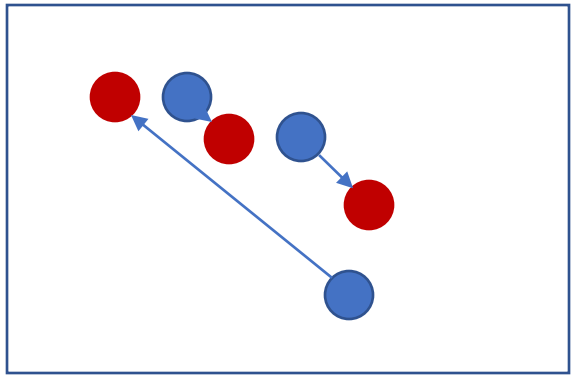


Backward

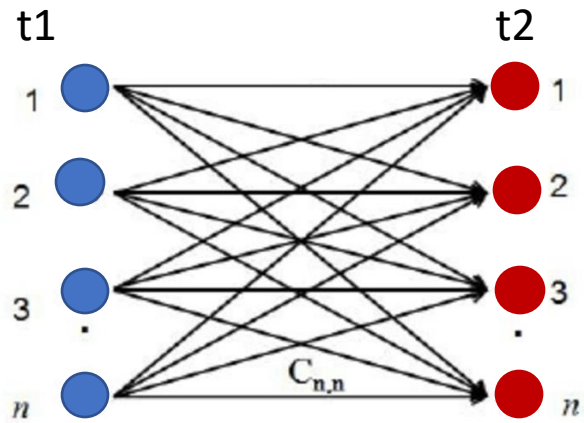
What result would we get if we started here?



Sensible match

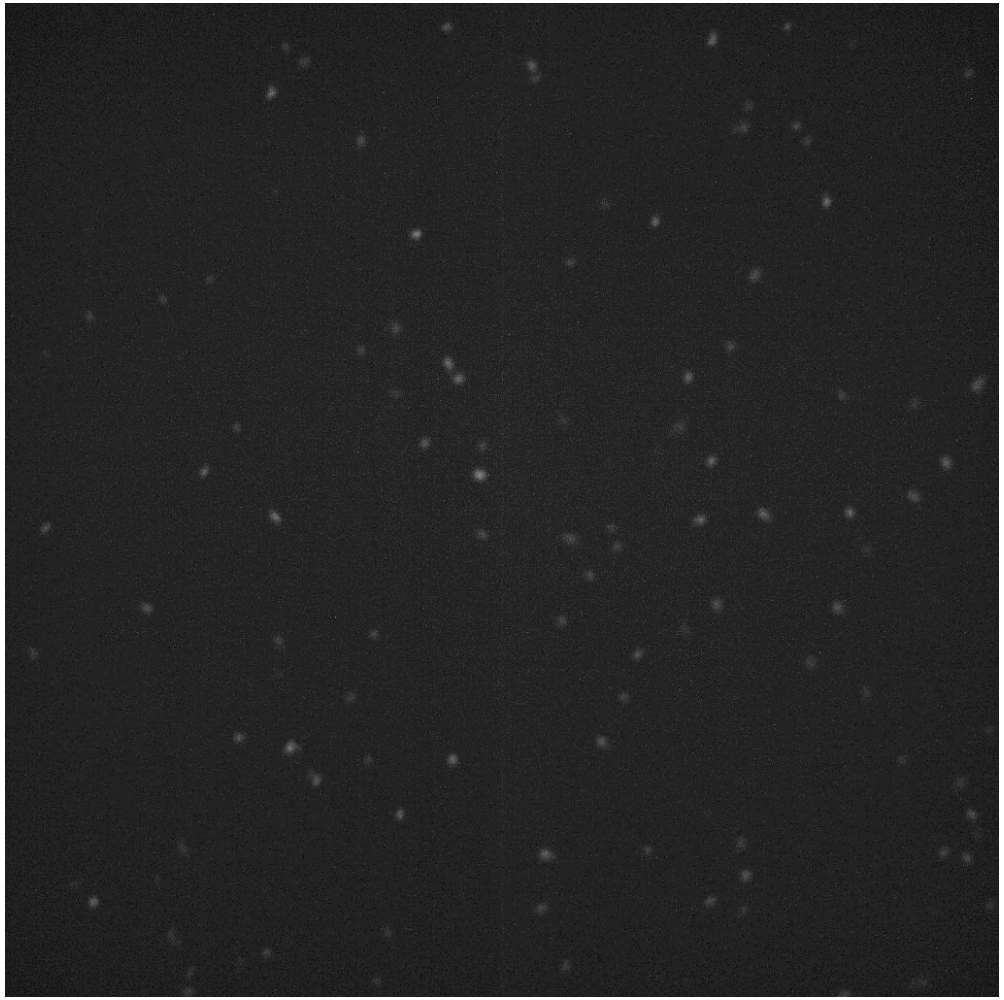


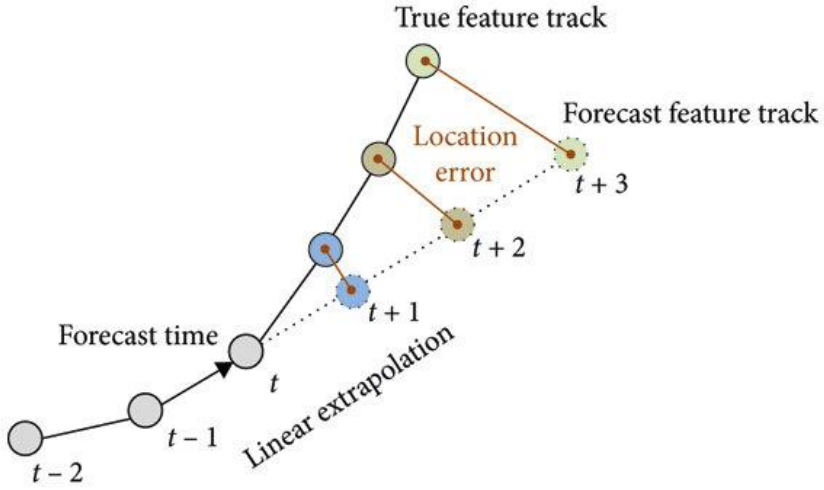
Not so sensible, but ‘correct’

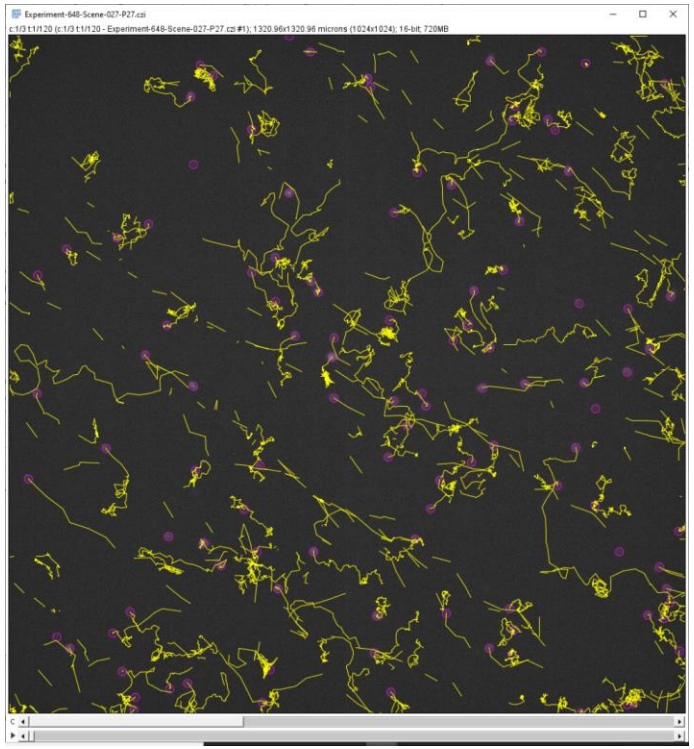


LAP represents the local cost of all possible matches

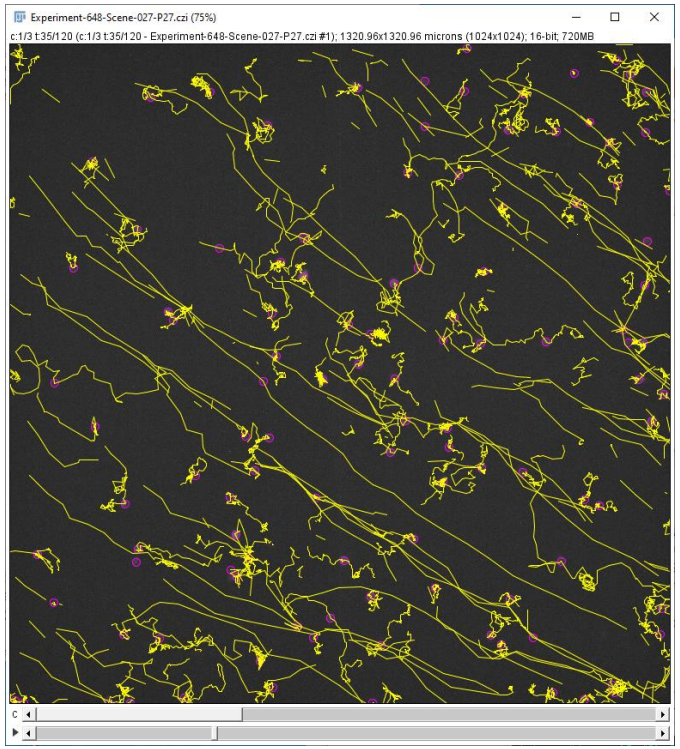
Motion Models







LAP threshold 40



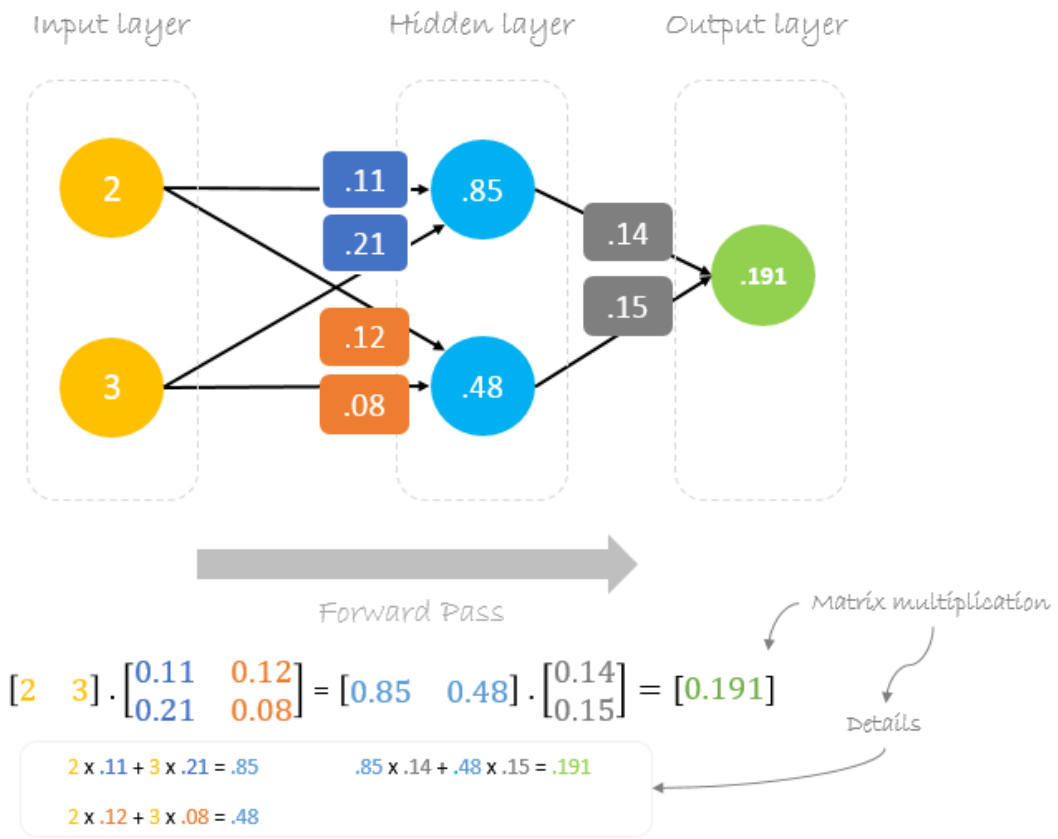
Linear Motion LAP threshold 40

AI and Image Analysis

- Introduction to deep learning
  - Overview of Uses
  - The pieces: Architecture, data, loss, postprocessing
  - Architectures
    - CNN
    - GAN
    - Vision Transformers
  - Limitations
    - Generalization
    - Opacity

AI is easy!

Forward Propagation

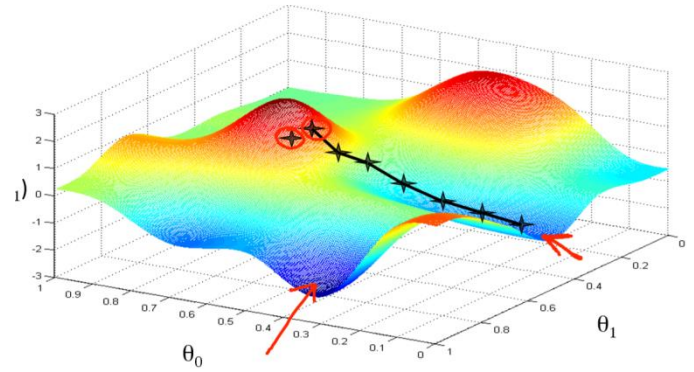


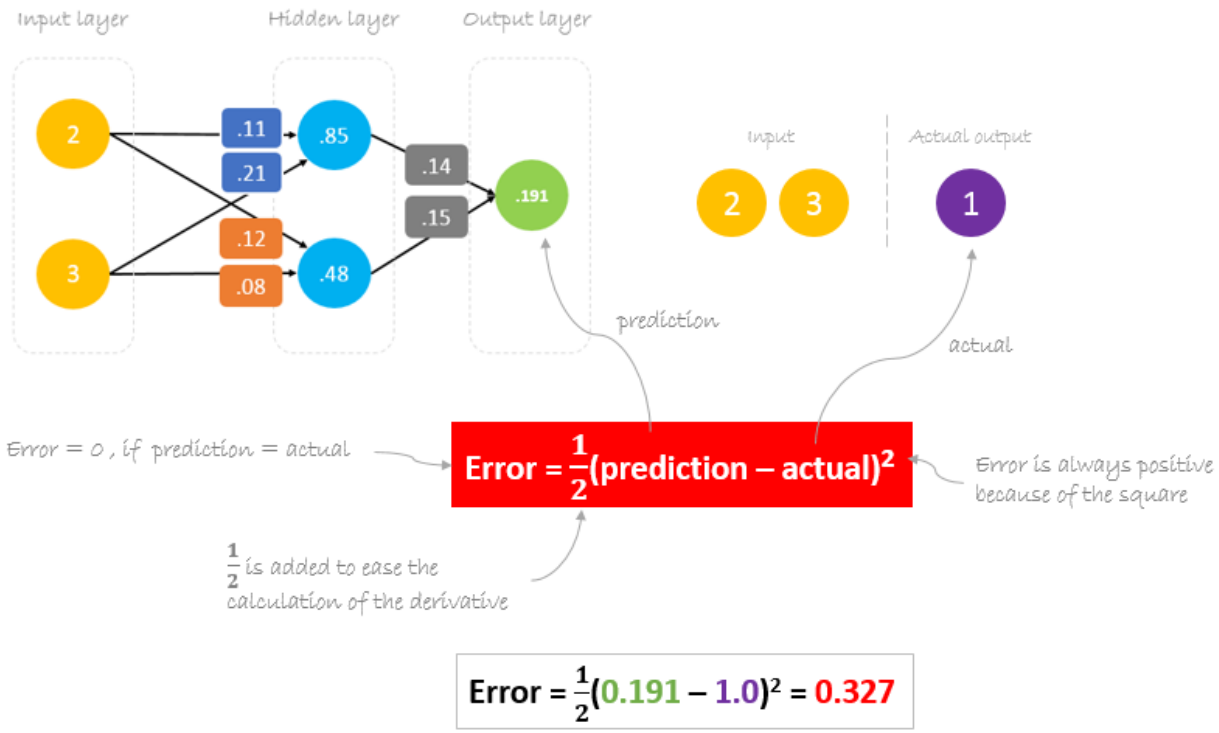
https://hmkcode.com/ai/backpropagation-step-by-step/

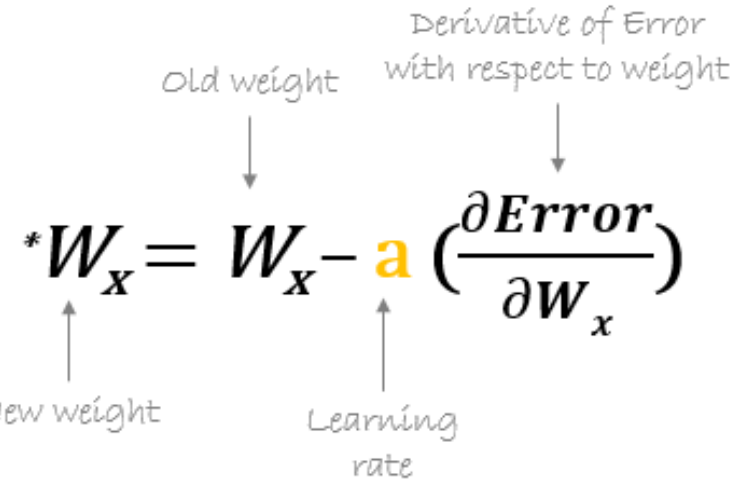
AI is hard! Backpropagation

What is a potential failure mode here?

Gradient descent





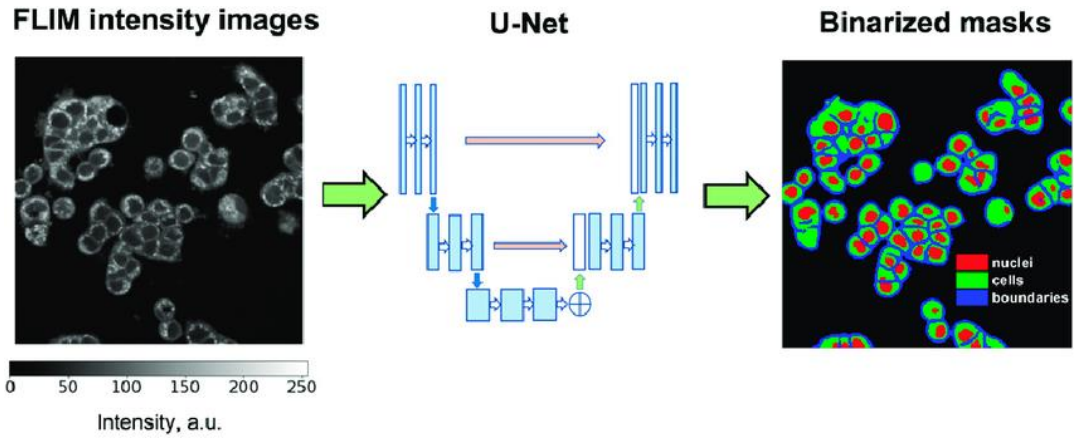


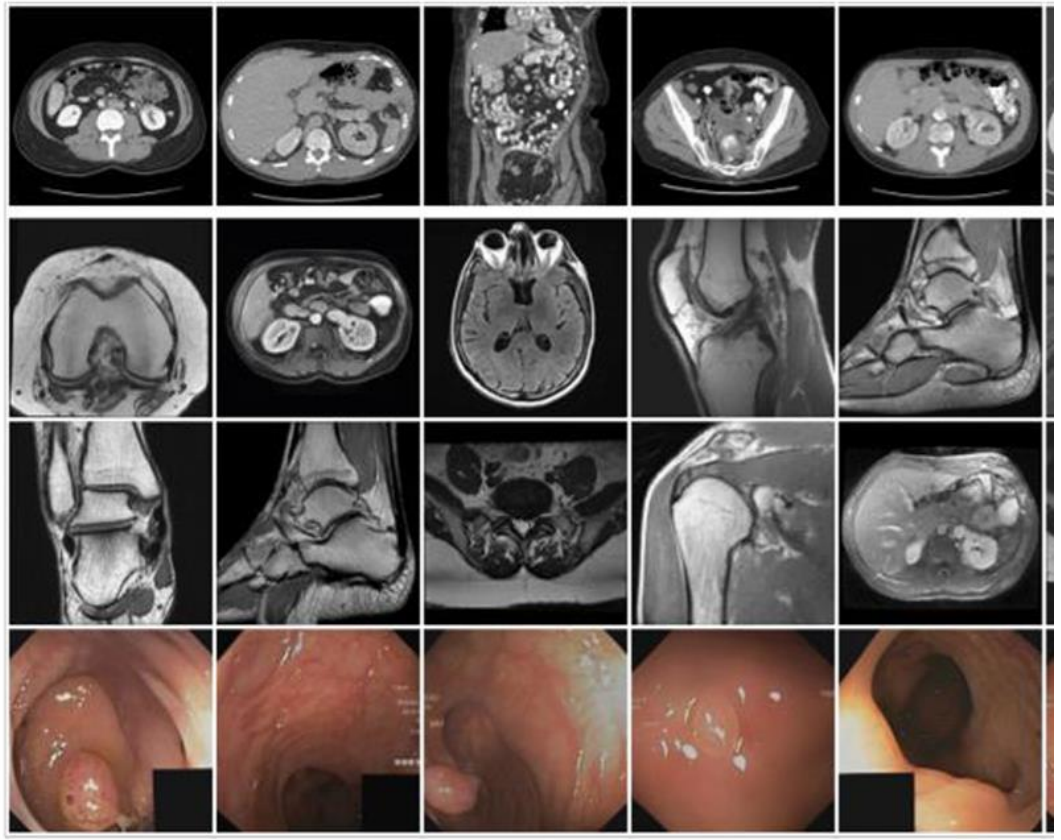
https://hmkcode.com/ai/backpropagation-step-by-step/

Uses

Regression to some Desired Output

E.G. Segmentation

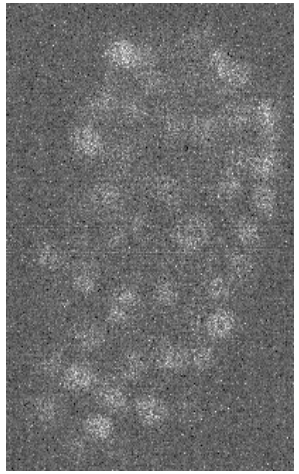




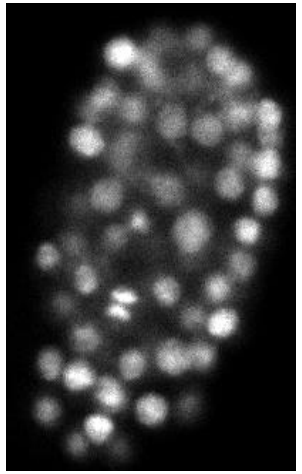
RadImageGAN Liu et al arXiv 2023

Generation of ‘Data’

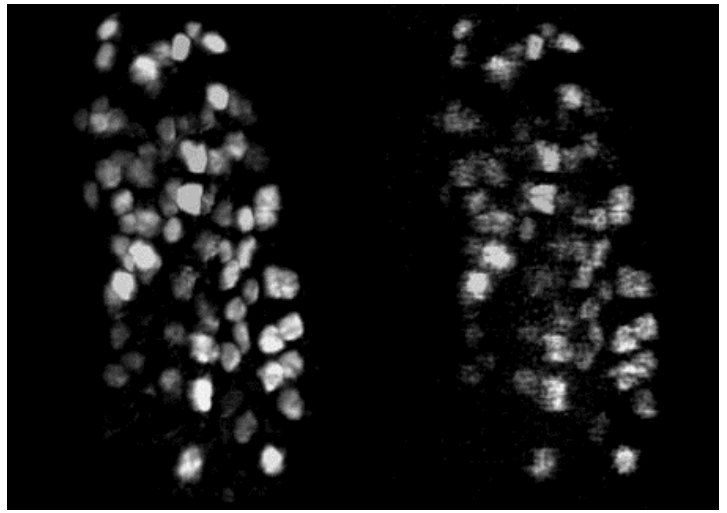
Components



Low contrast

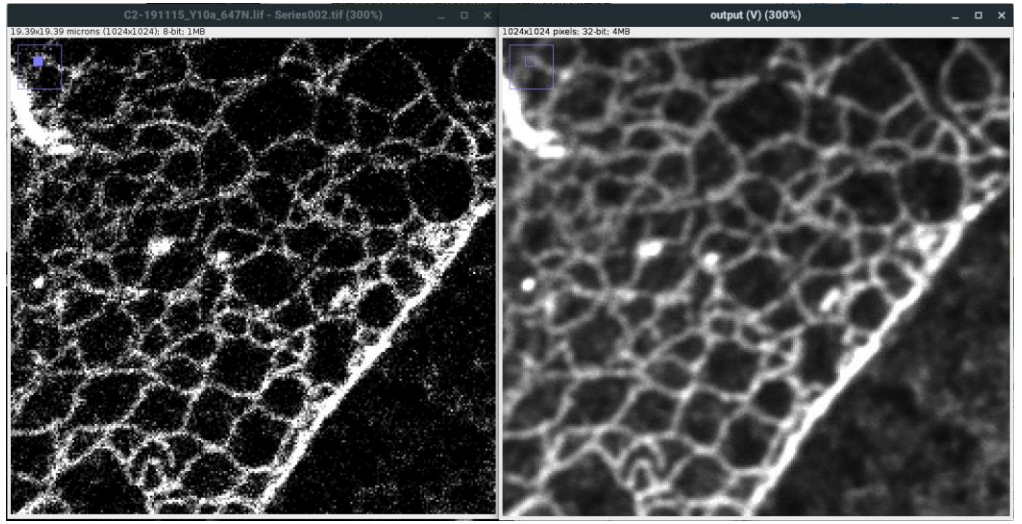


High contrast



CARE (CSBDEEP Unet) Weigert et al Nature Methods 2018

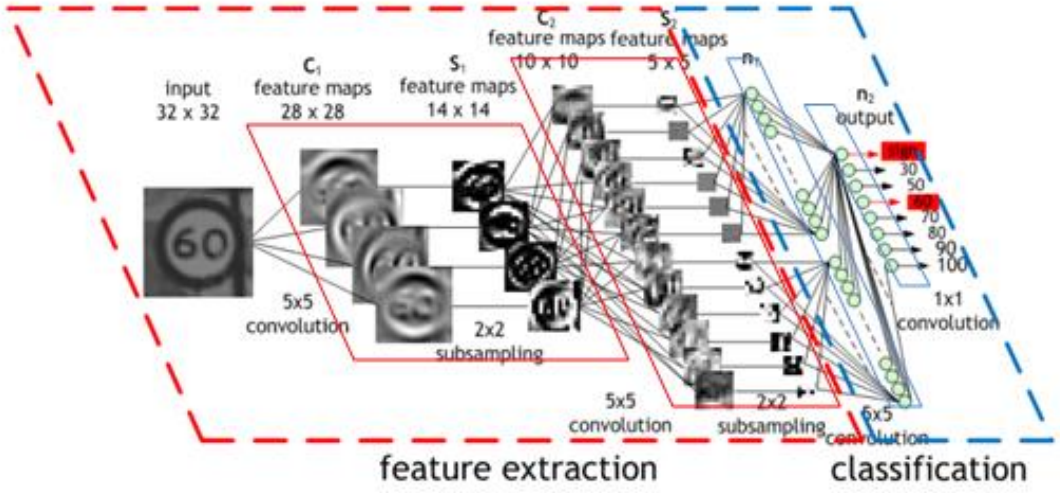
- Architecture –what can it do?
- Format, postprocessing –How to define task?
- Loss –will it learn to do task from this signal?
    - Error on?
        - The task you want to do?
        - An easier to supervise task?
            - Mask and predict
            - Discrimination
            - Relative position
- What might be some pros and cons of each approach?

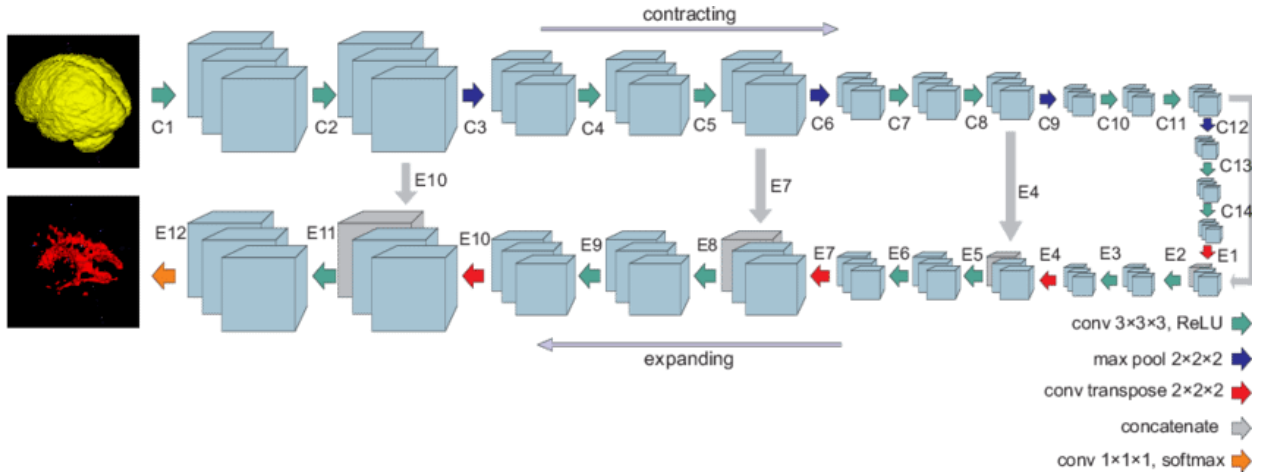


Noise2Void (CSBDEEP Unet) Krull et al CVPR 2019

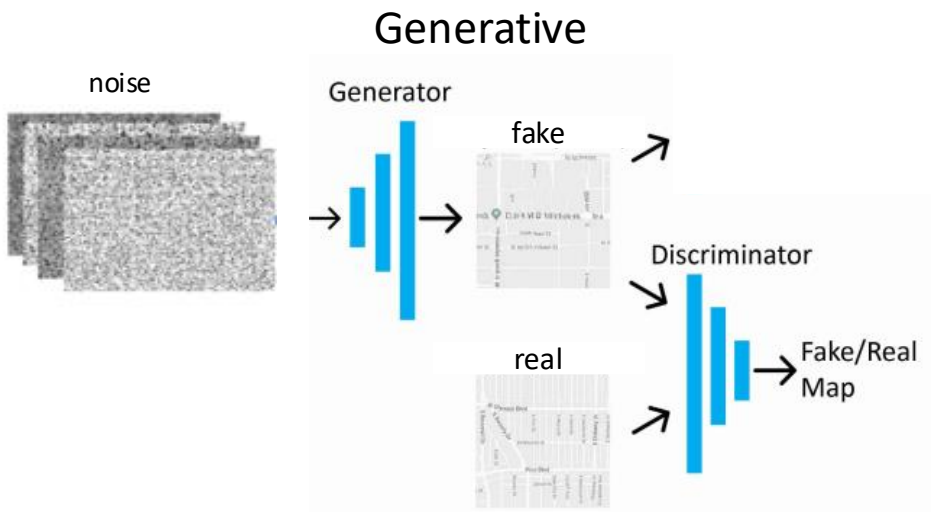
https://forum.image.sc/t/noise2void-for-fiji/34552/7

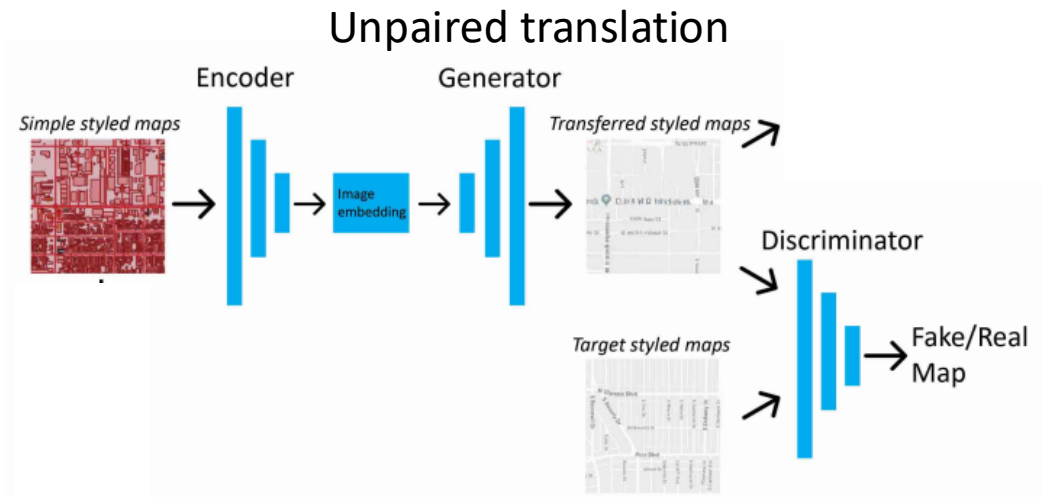
Architectures -CNN

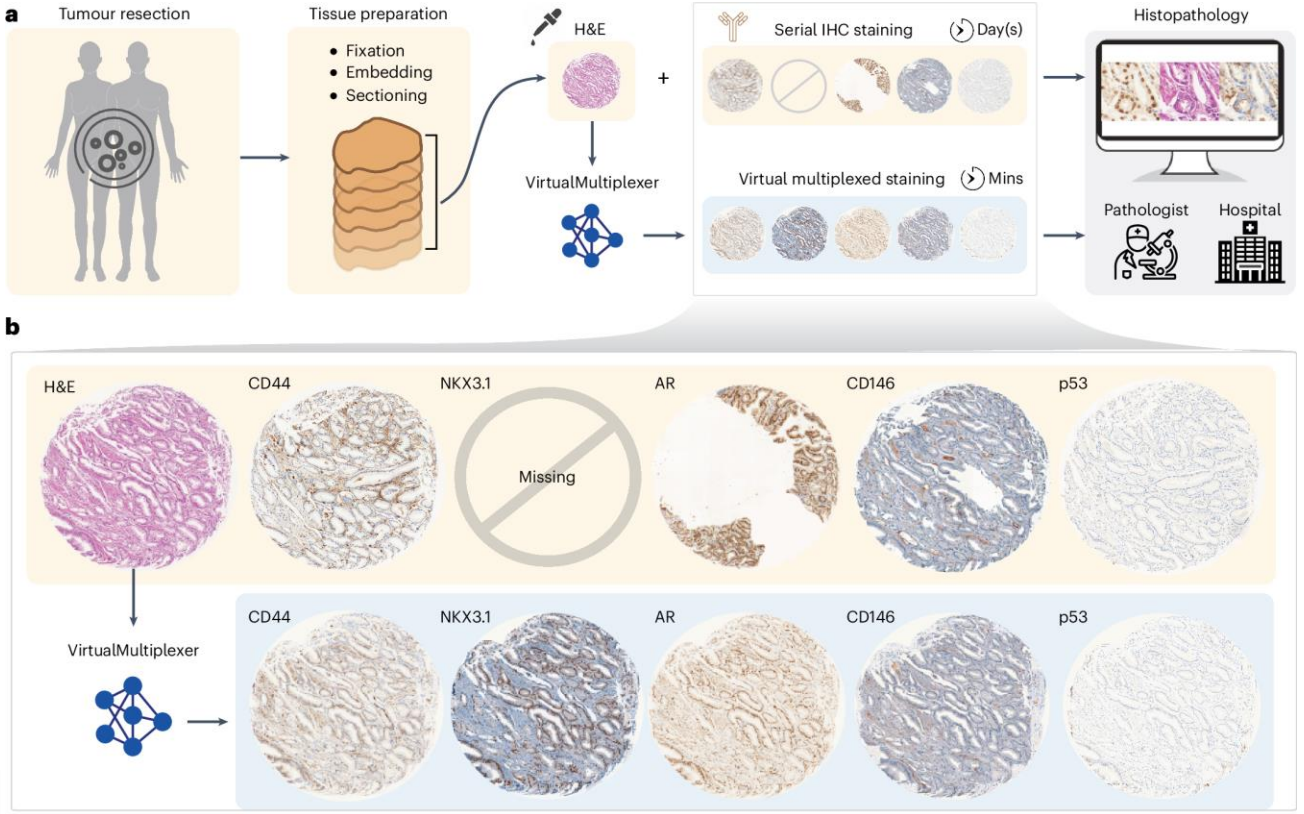




Architectures GAN

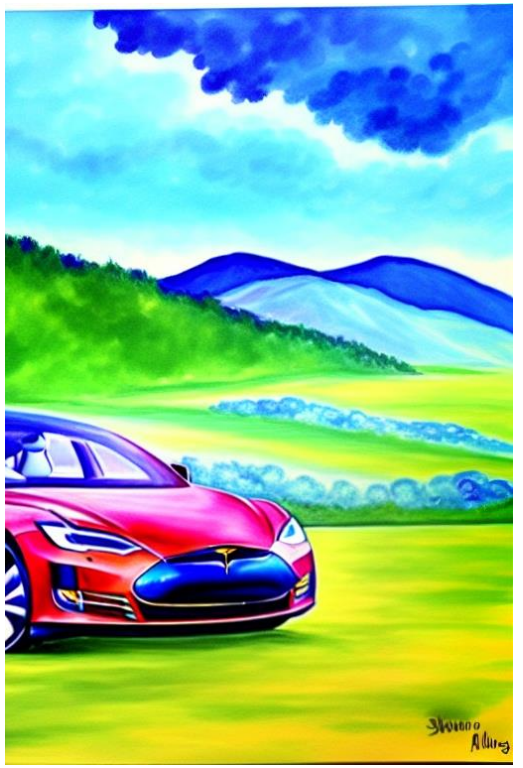






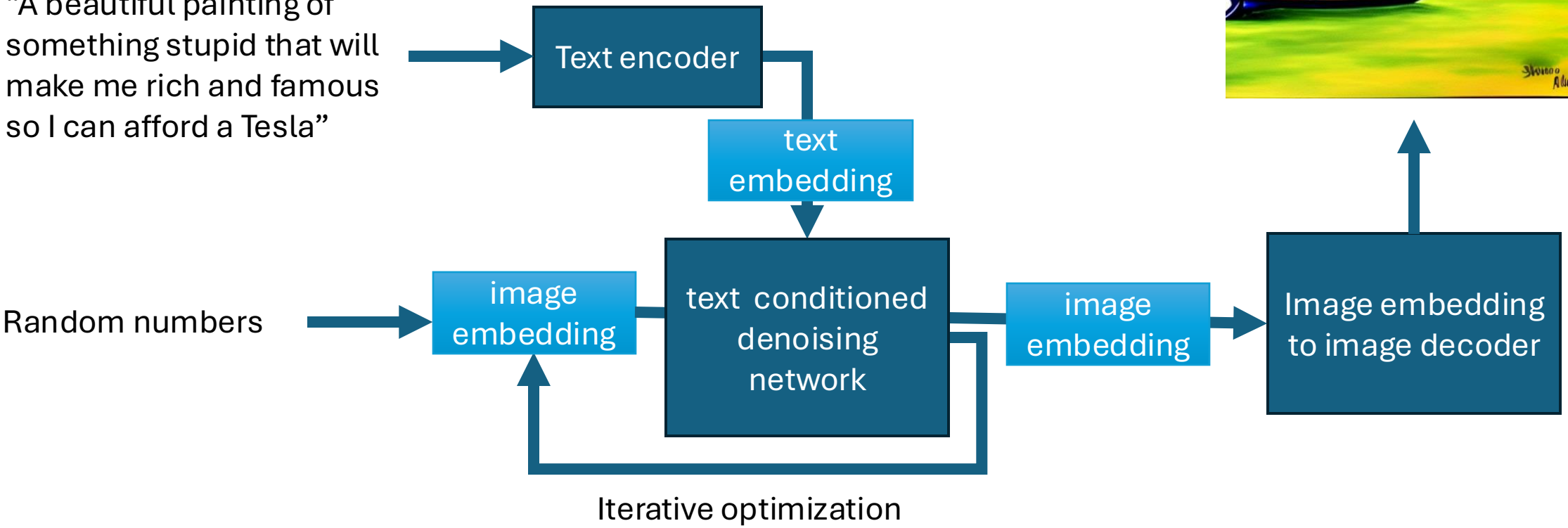
Pati et al Nature Machine Intelligence 2024

Image generation networks

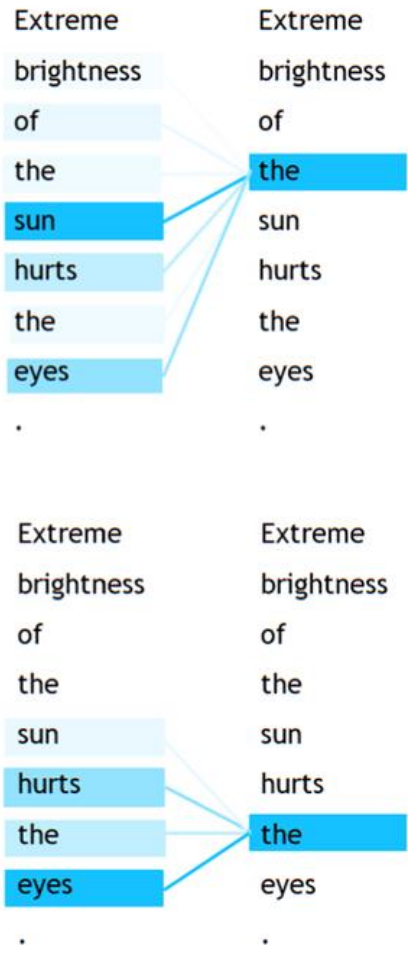


Prompt:

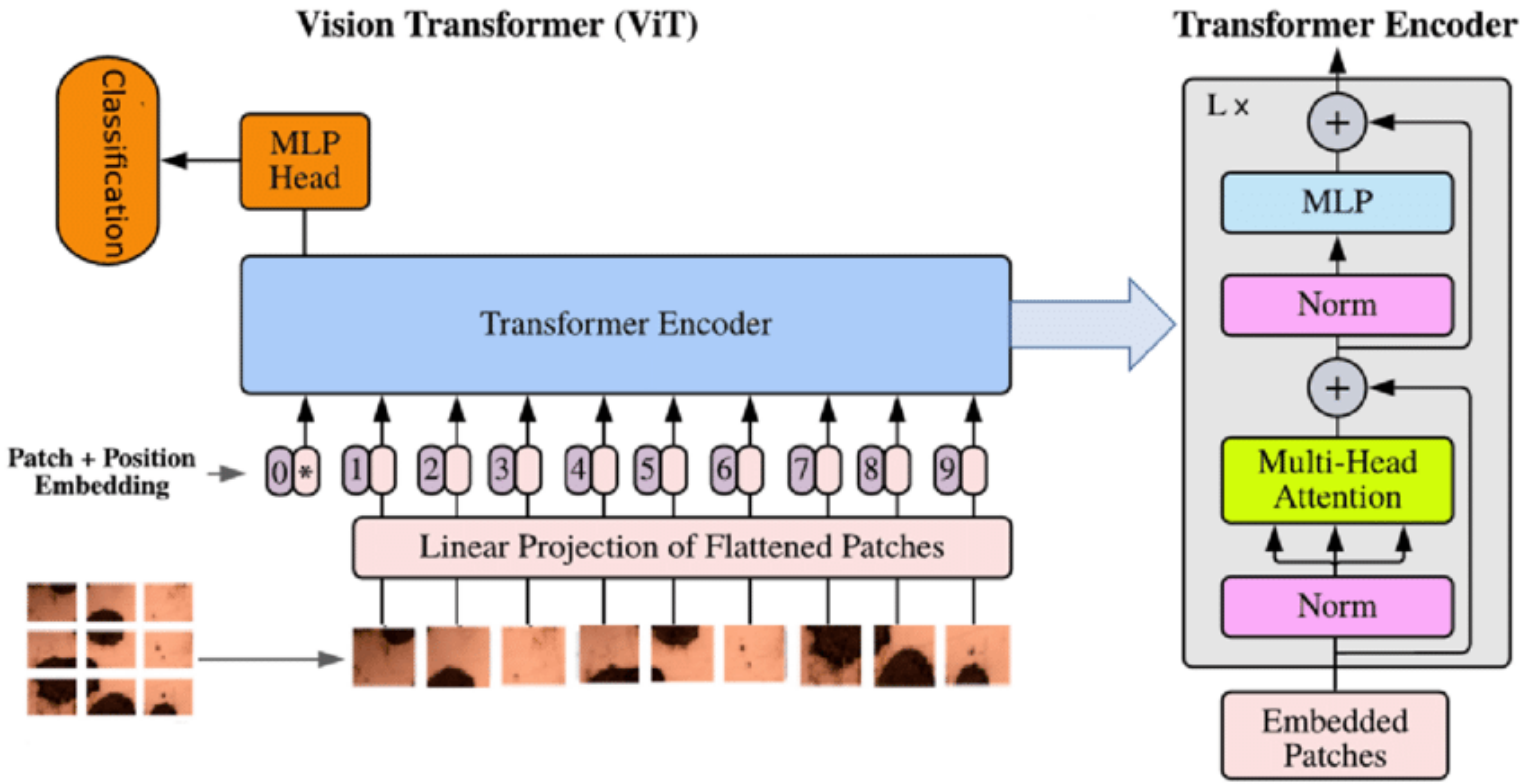
“A beautiful painting of something stupid that will make me rich and famous so I can afford a Tesla”



Architectures Vision Transformer



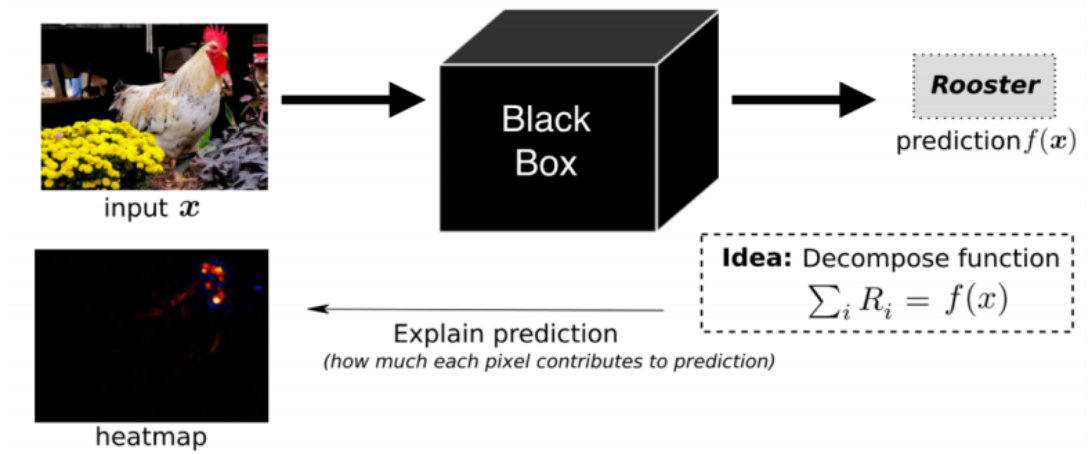
What are some potential tradeoffs for this flexibility?



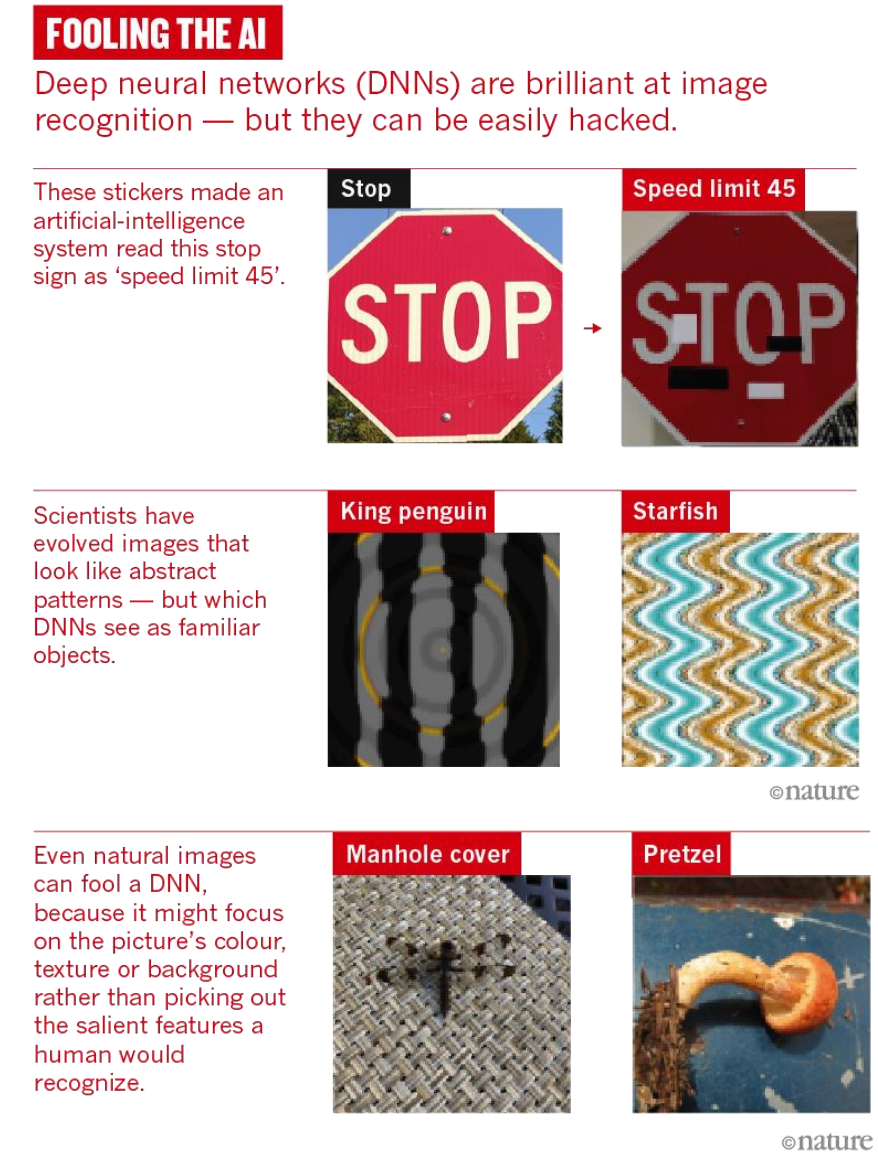
ViT, Dosovitskiy et al ICLR 2021

Limitations

- Wrong and Confident
  - sensitivity to out of sample testing whether hostile or chance
- Largely Opaque

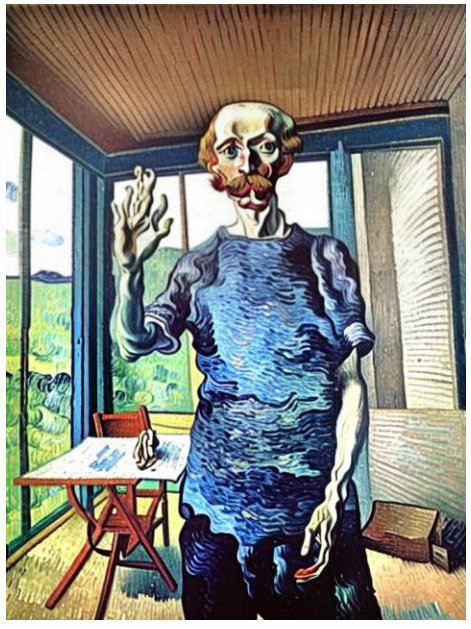


Piccinotti et al Reports on progress in physics, 2020

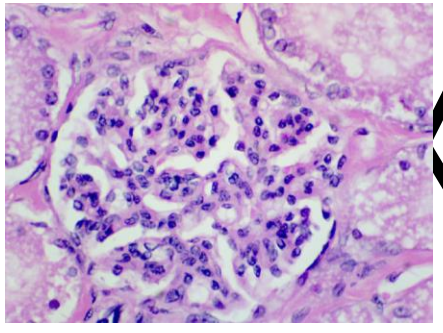


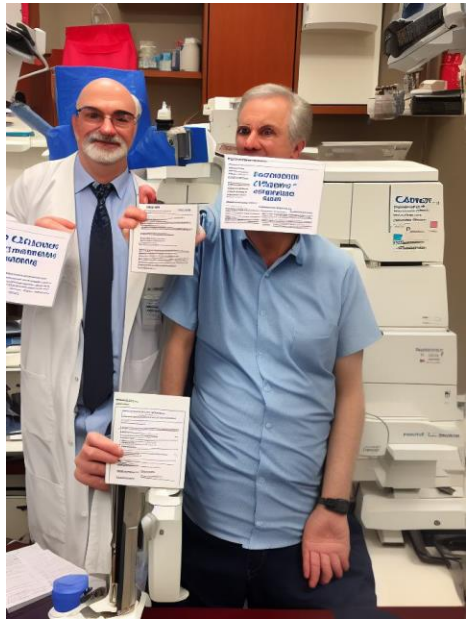
Why deep-learning AIs are so easy to fool, Nature Oct 2019

Out of Sample:
You will always get a result, you may not like it
img2img

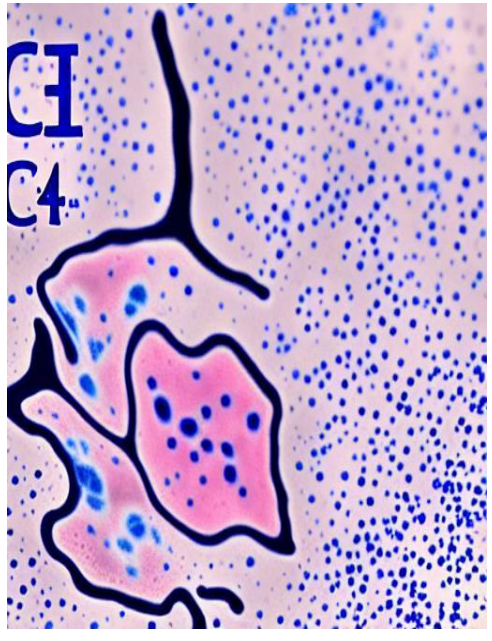


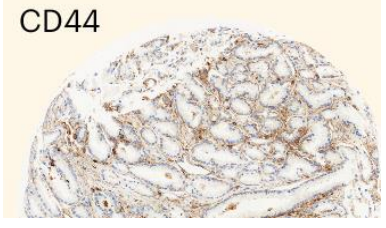
An oil painting of a man waving by Vincent Van Gough





CD44 IHC special stain cancer diagnosis science

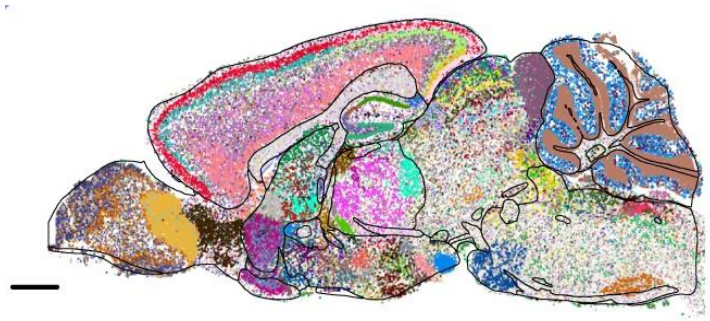


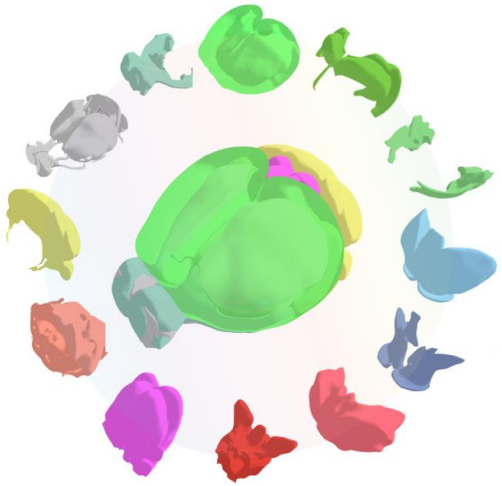


AI in Depth Examples

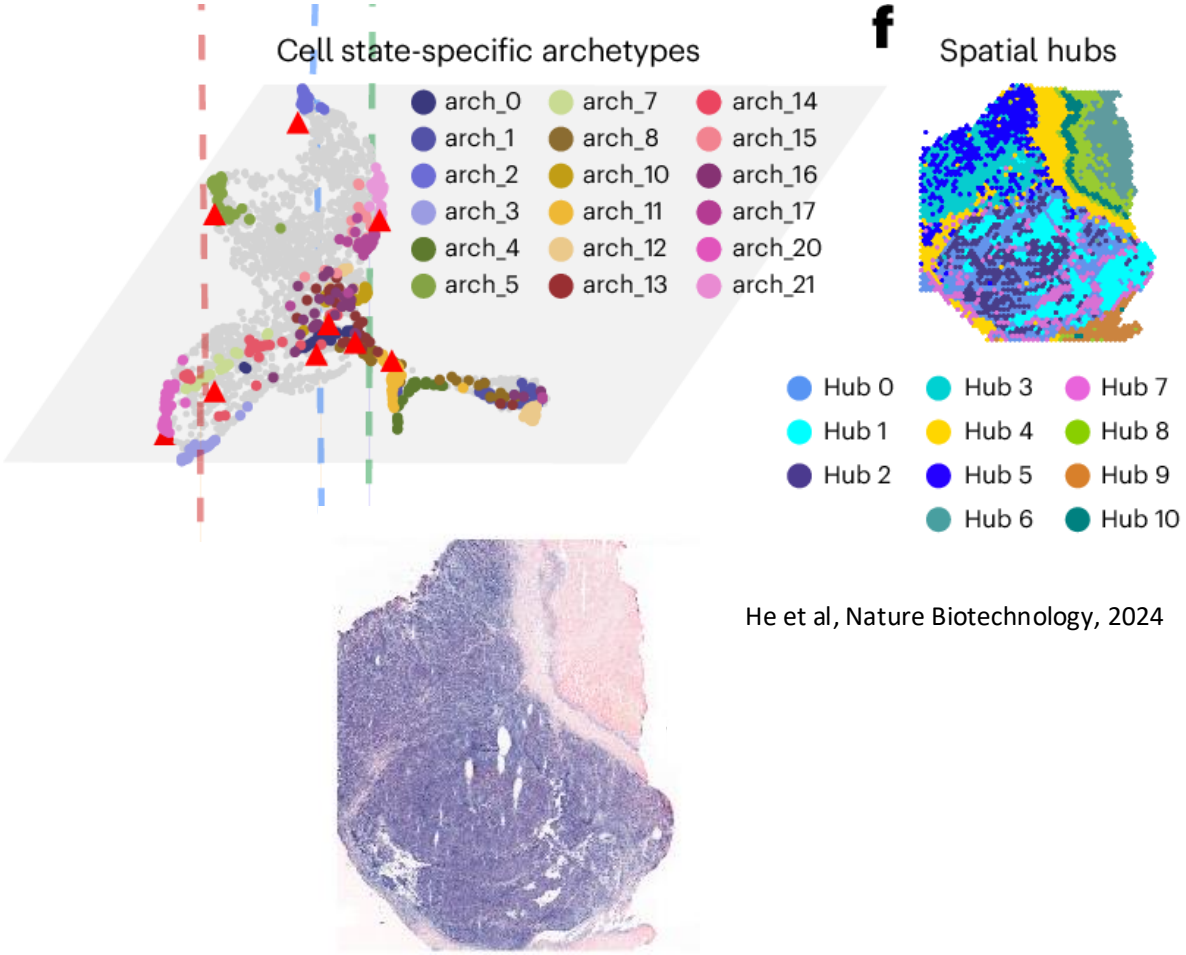
- Spatial Temporal Pattern Quantification
  - Development
  - Behavior

Spatial Molecular Patterns





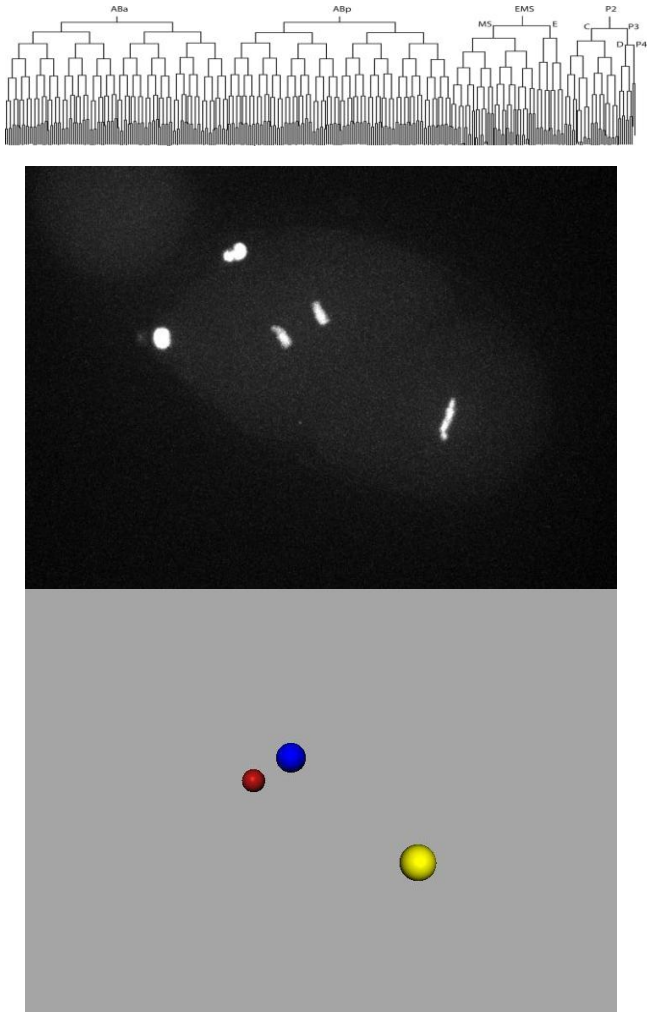
Zhang et al, Nature, 2023



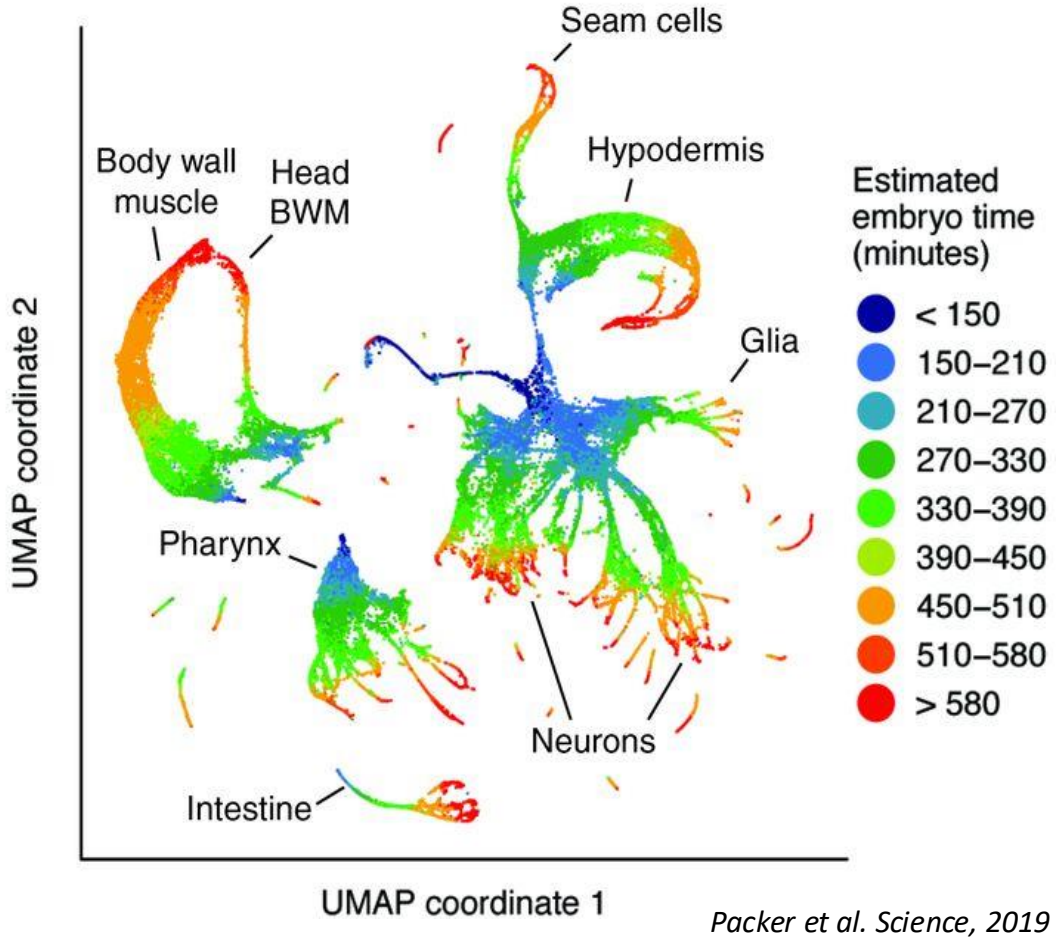
He et al, Nature Biotechnology, 2024

Space, Time and Gene Expression in *C. elegans* embryogenesis

Combining morphogenesis and molecular manifolds



Cell Positions Over Development

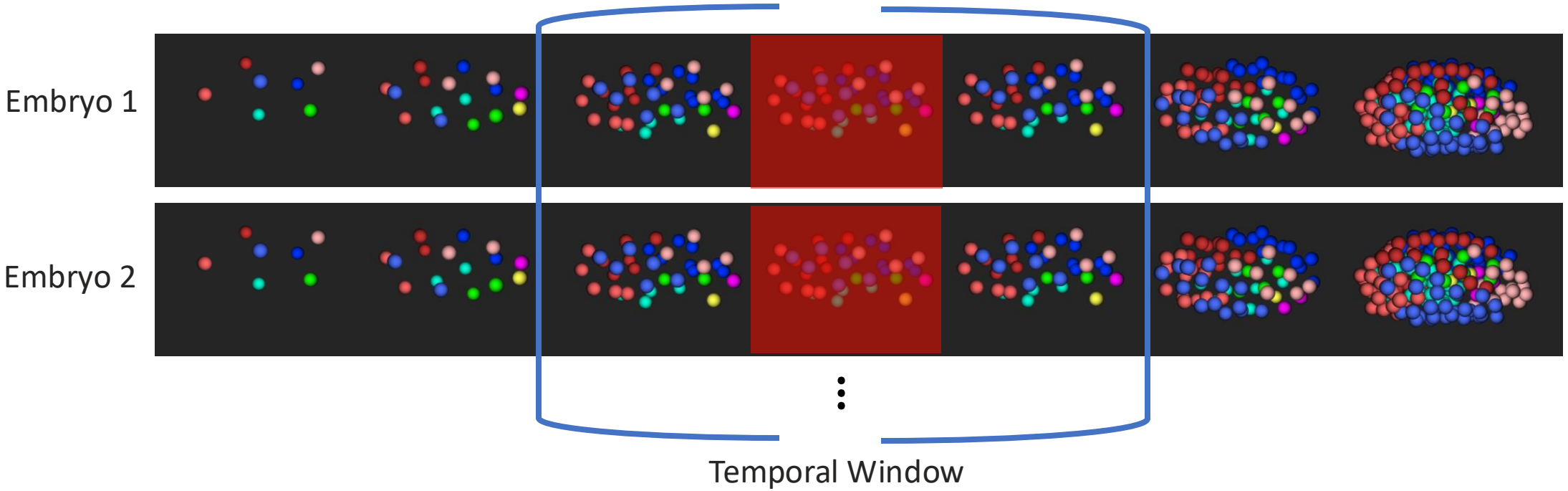


Packer et al. Science, 2019

Molecular manifold

Efficient Joint Spatial Temporal Learning

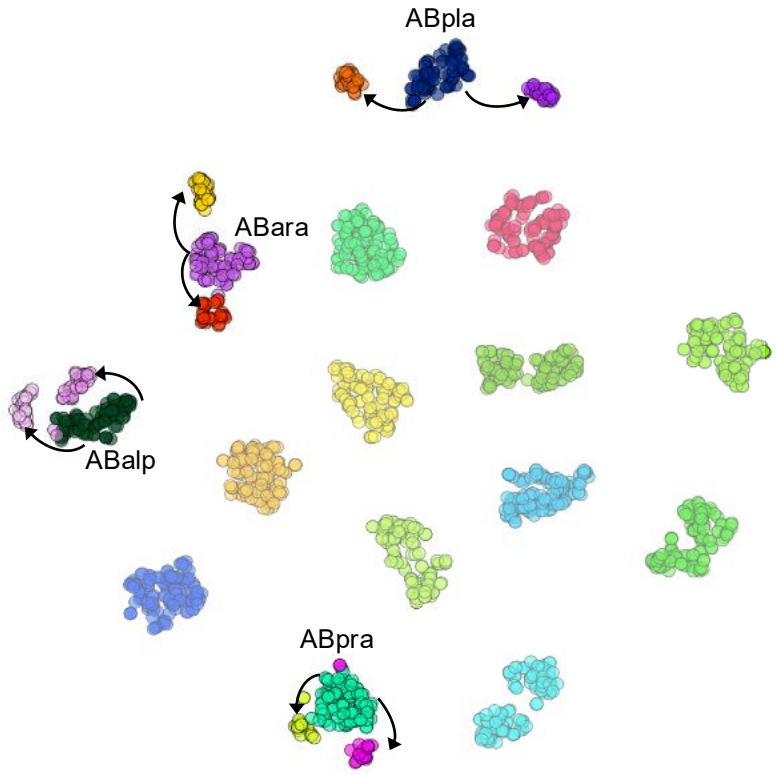
Transformer Based Spatial Representation

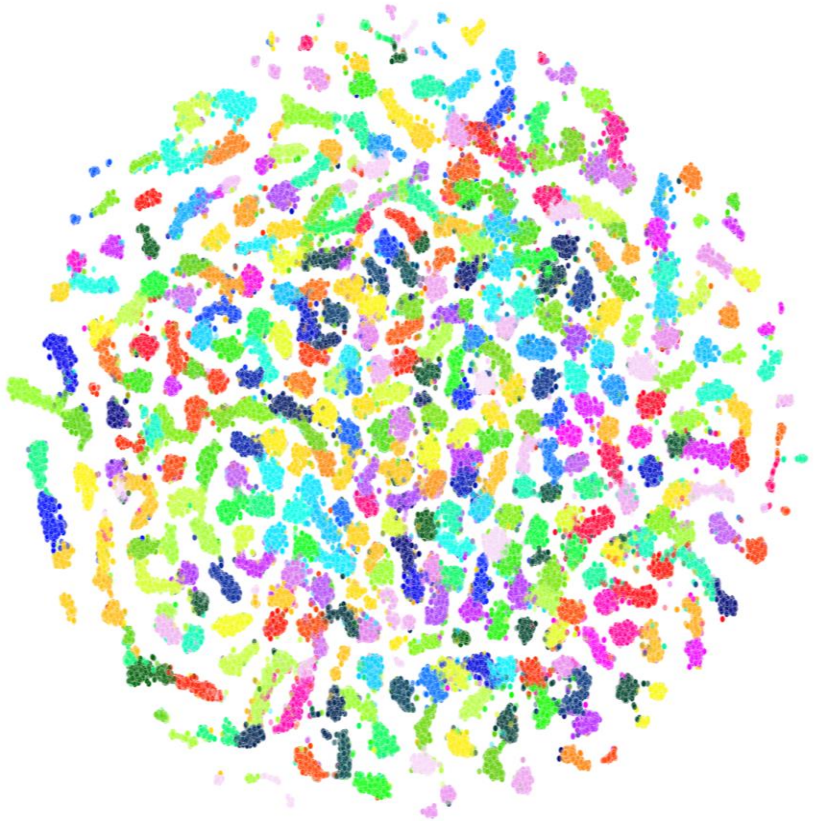


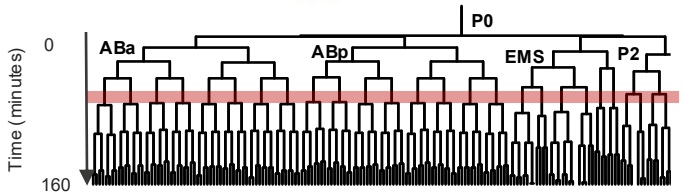
1. Transformer Encoder
2. Twin Attention: Training to match cells in similar pairs
3. Sliding Window

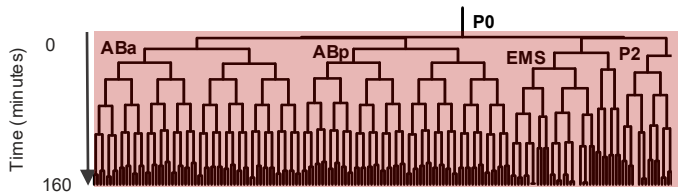
Properties of Learned Embeddings

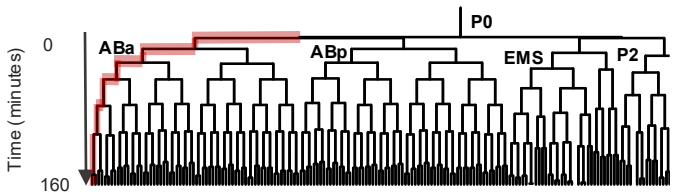
Learned Manifold of Development





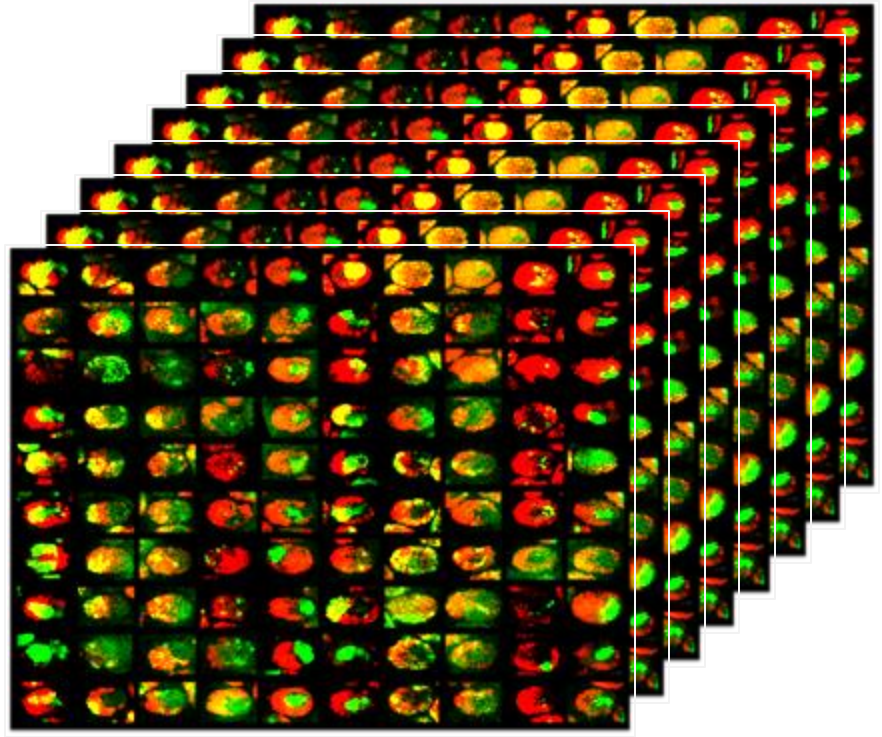




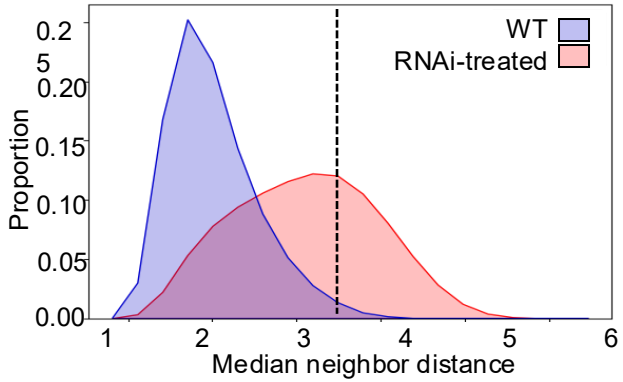


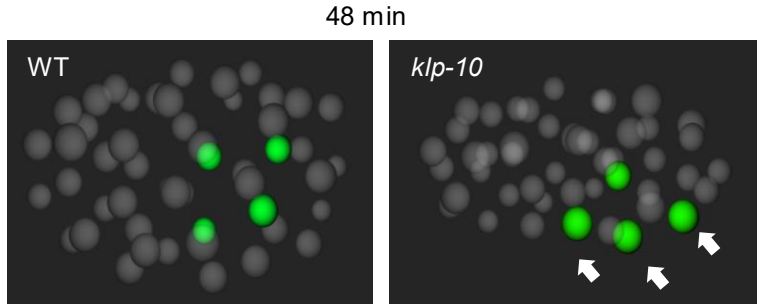
Applications

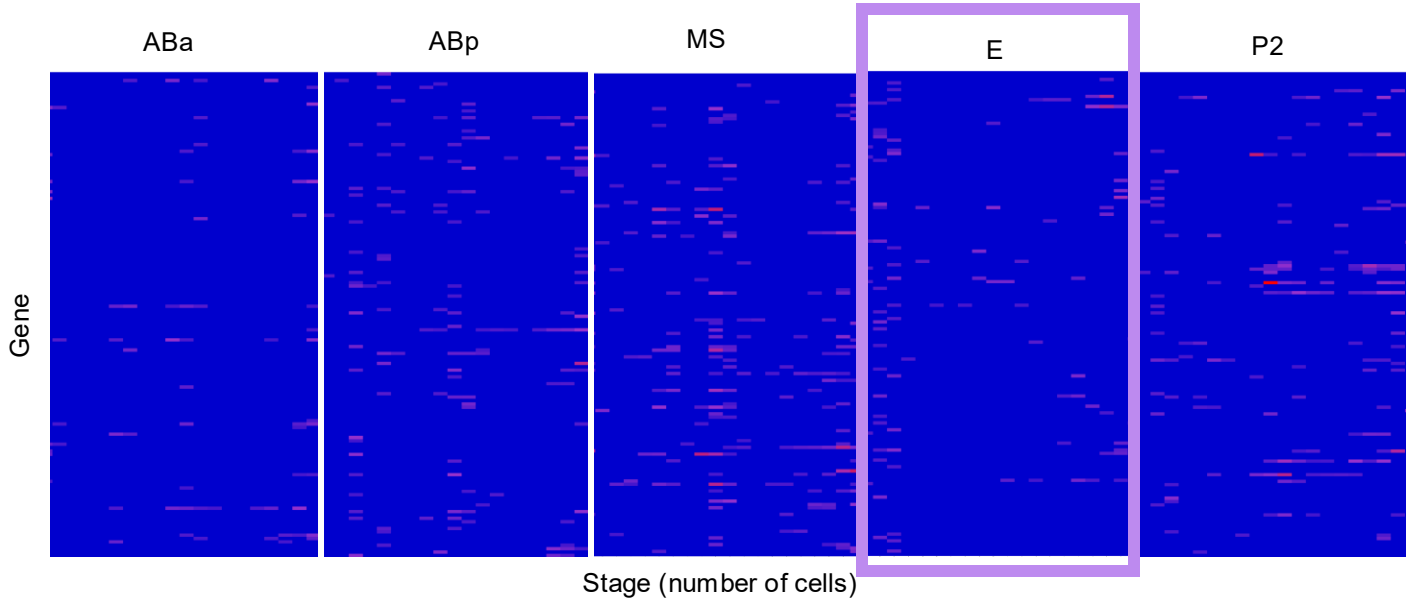
Transient, Correctable Defects



Du et al Cell, 2014







Dataset:

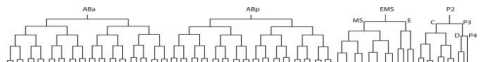
embryos that survived RNAi treatment for 144 embryonic lethal genes

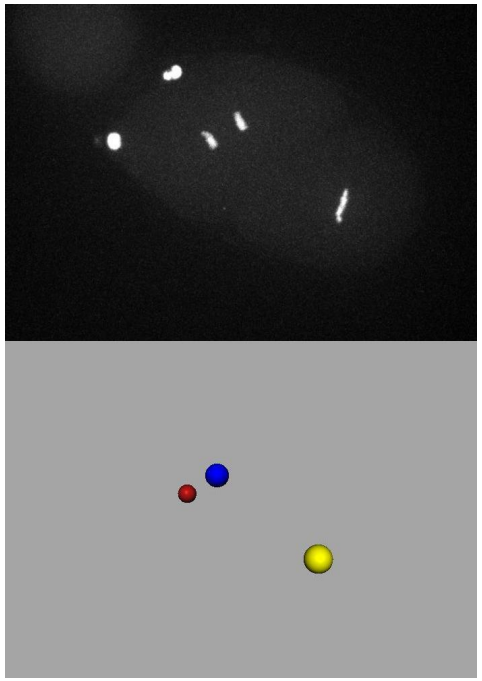
MSK Confidential — do not distribute

Spatial Molecular Correlation

Combining morphogenesis and molecular manifolds

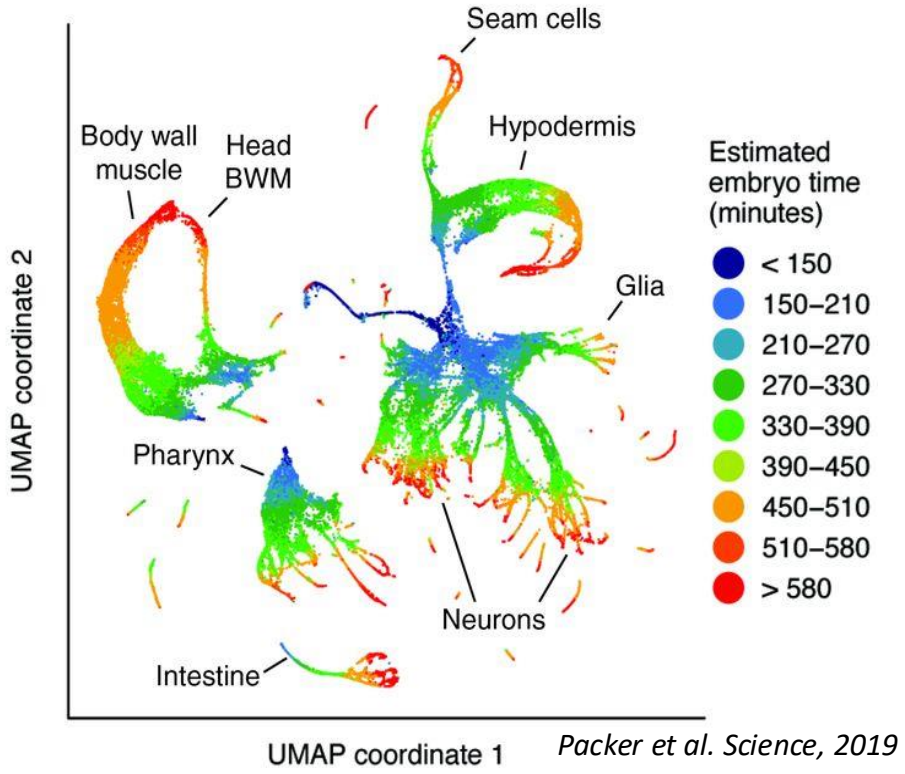
Lineage





How could we use these two manifolds now that we have them and can map between them?

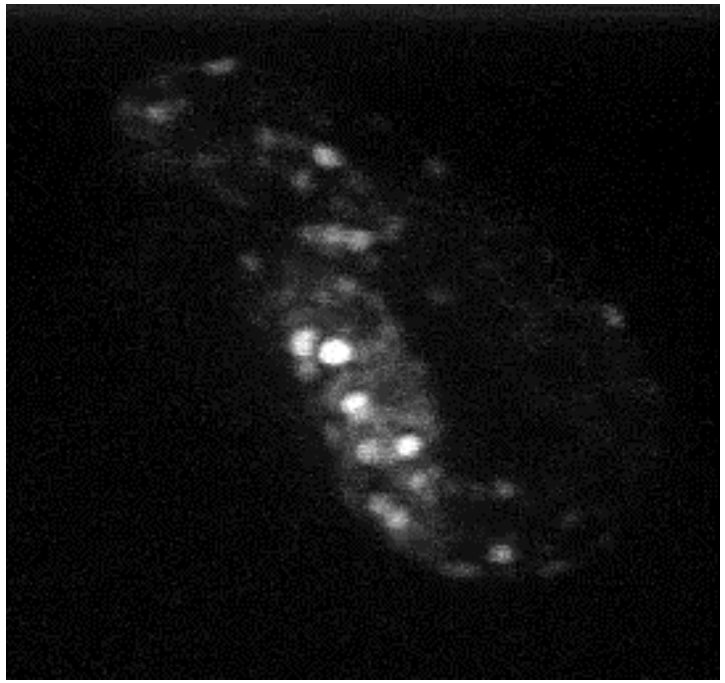
Tissue development manifold

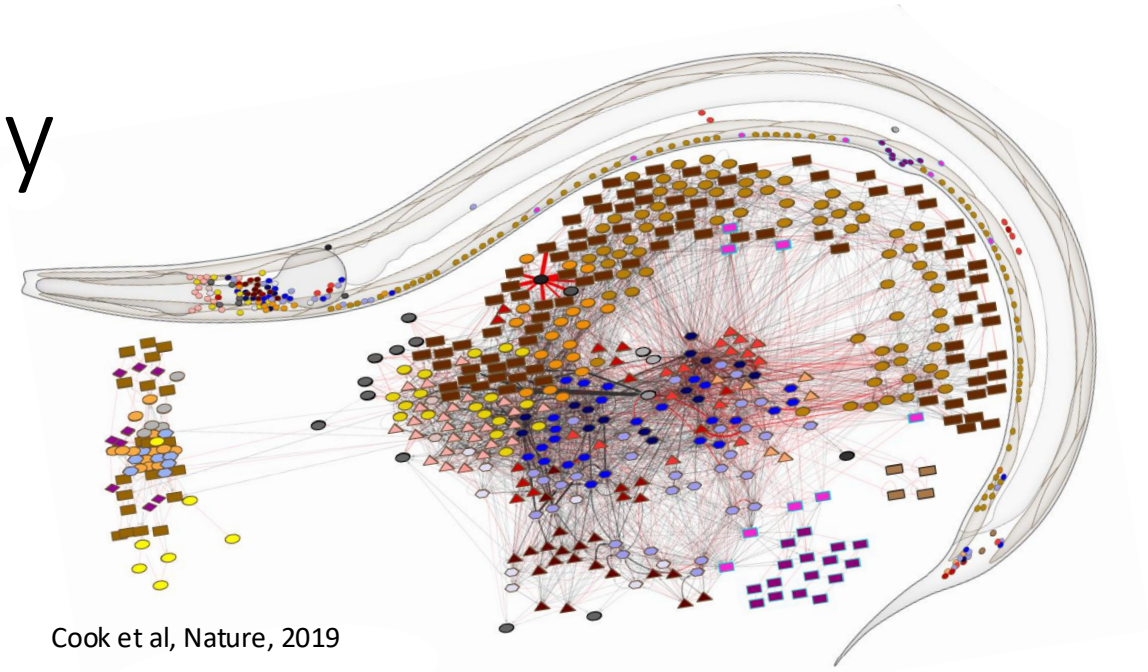


Packer et al. Science, 2019

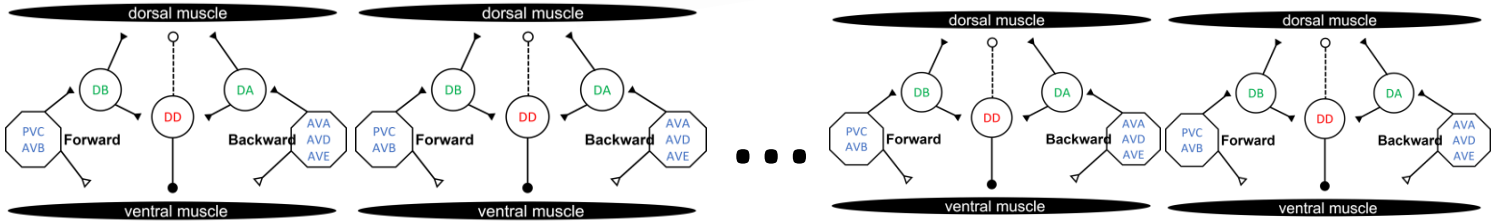
Molecular manifold

Motion and Neural Activity



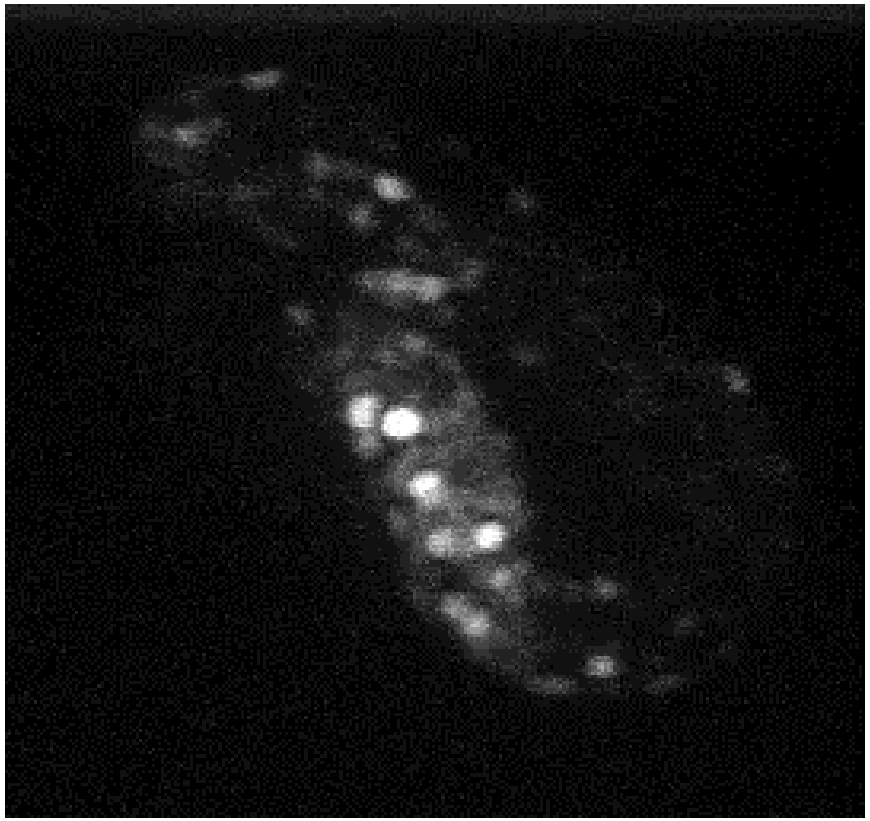


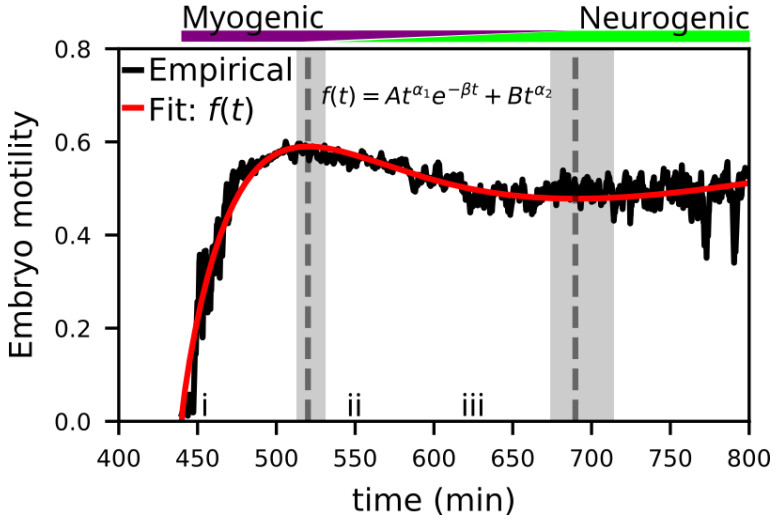
Cook et al, Nature, 2019

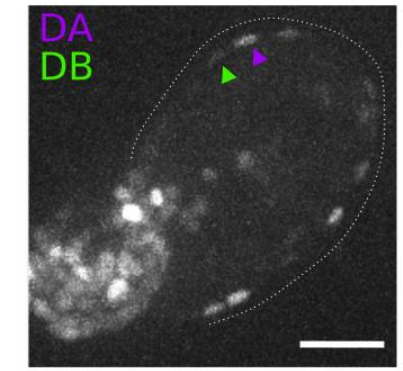


Question: How does circuit activity pattern emerge over development?

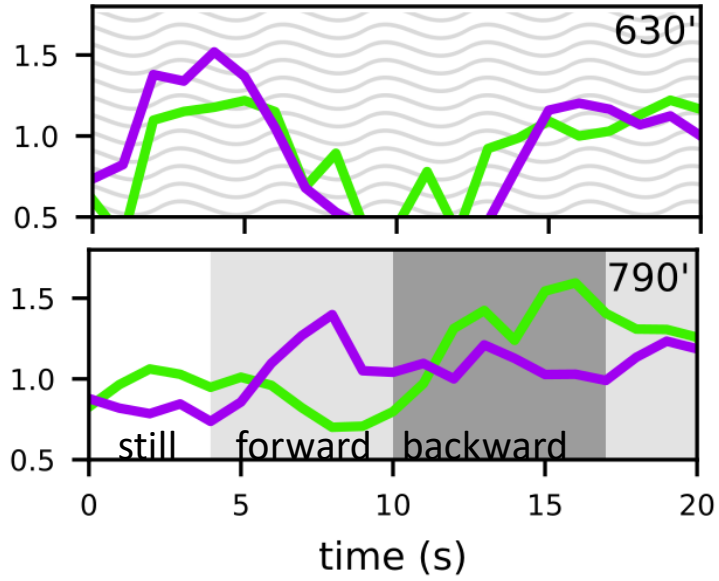
Motion and Neural Activity



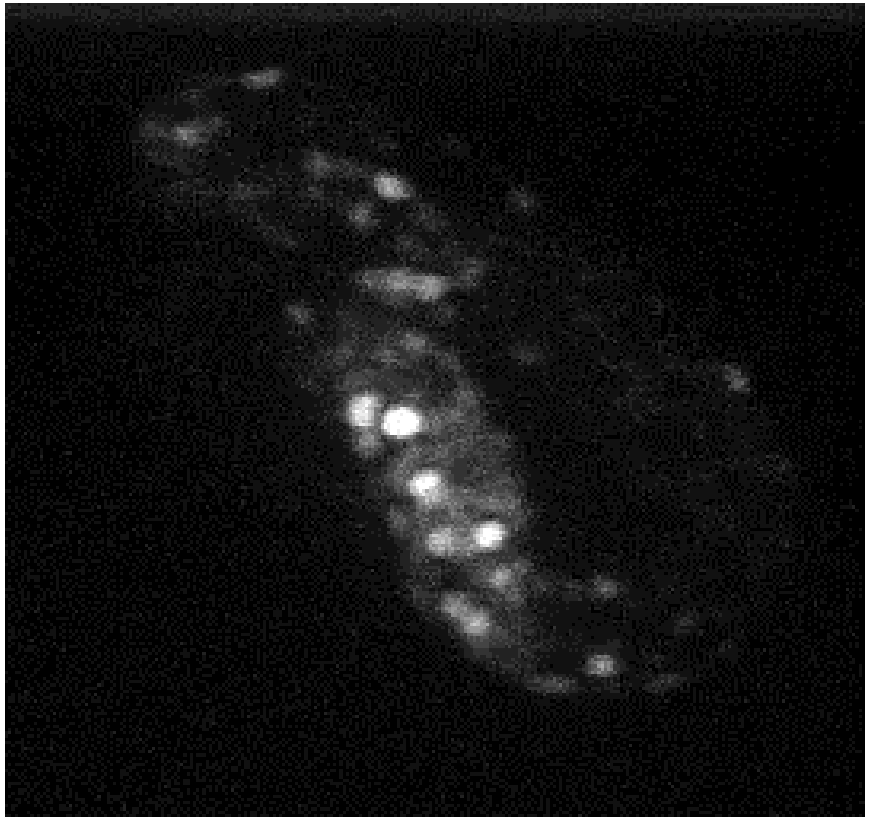




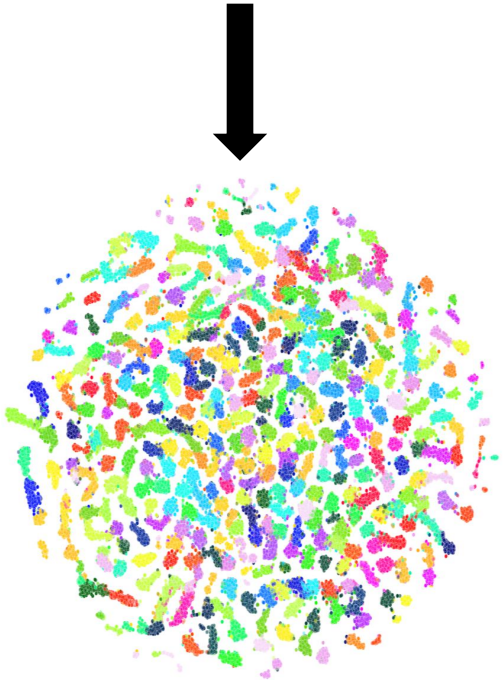
Pan-neuronal NLS::GaMP7F

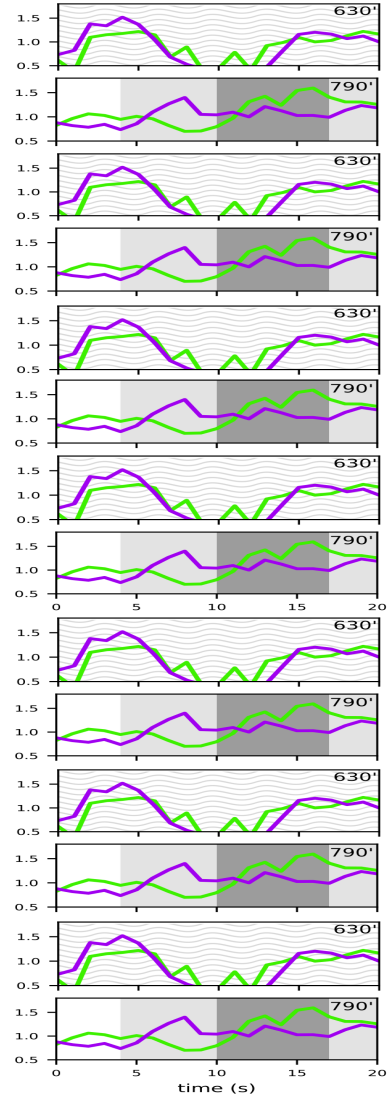


Engineering Challenge
Tracking and Identification

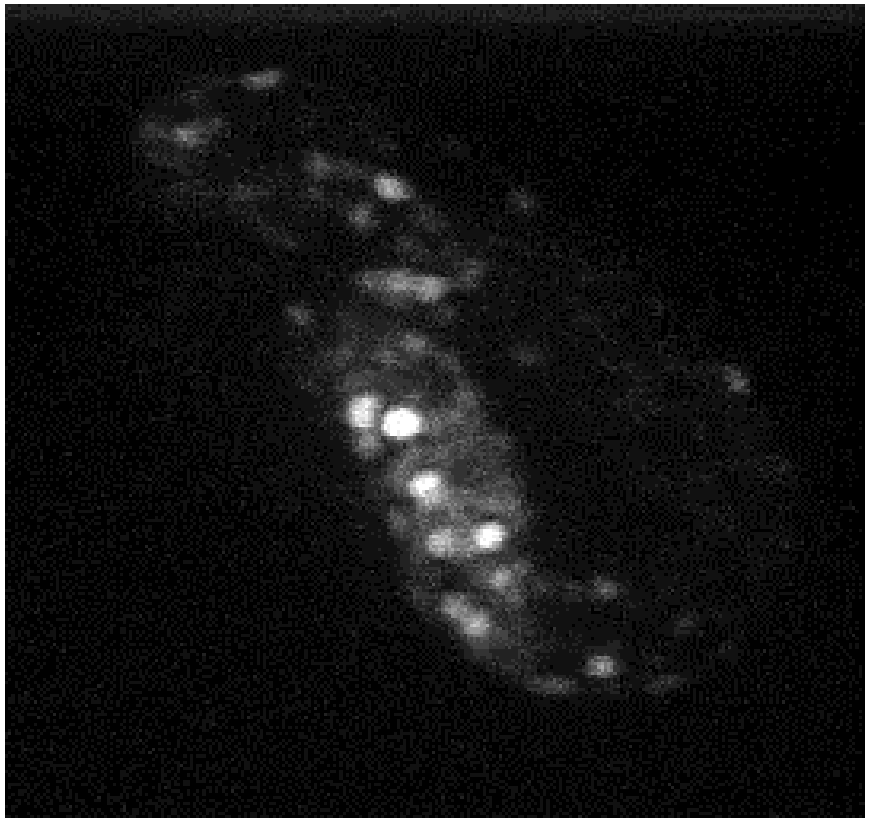


Twin Attention Encoder

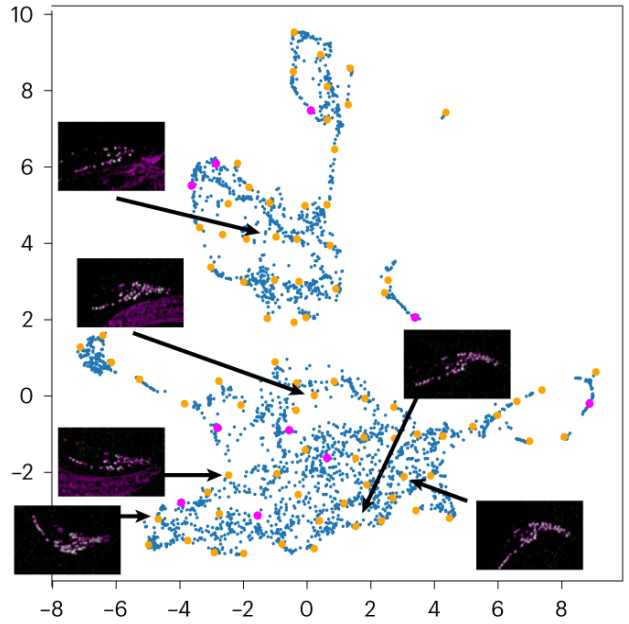




Conceptual Challenge
Posture-Activity Correlation

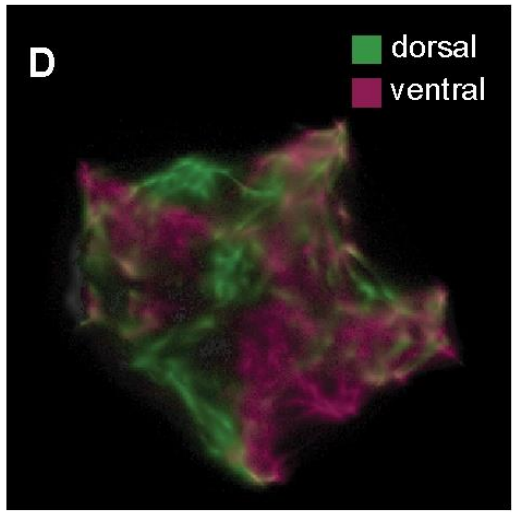


Posture Space



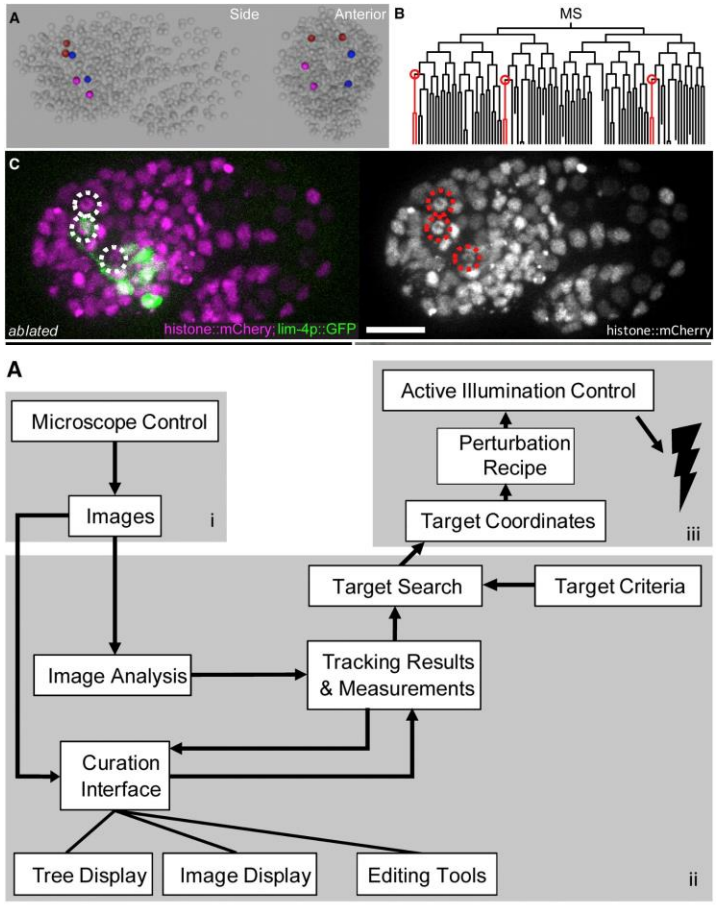
Park et al, Nature Methods 2024

Activity Space



Atanas et al, Cell 2023

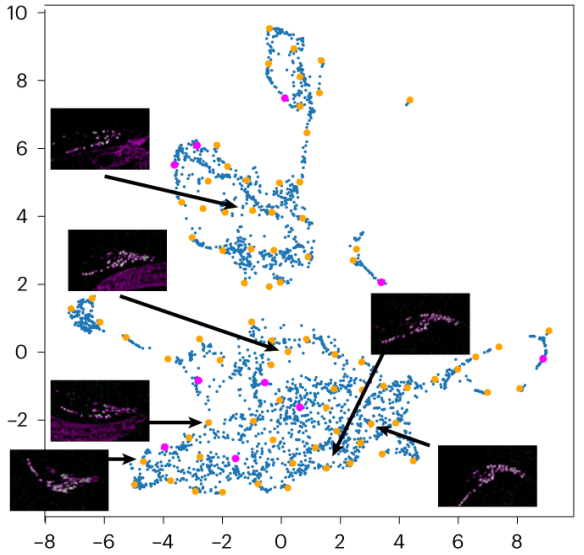
Perturbation



Shah et al, Developmental Cell, 2017

ShootingStar: Identity guided ablation, photoconversion, photoactivation

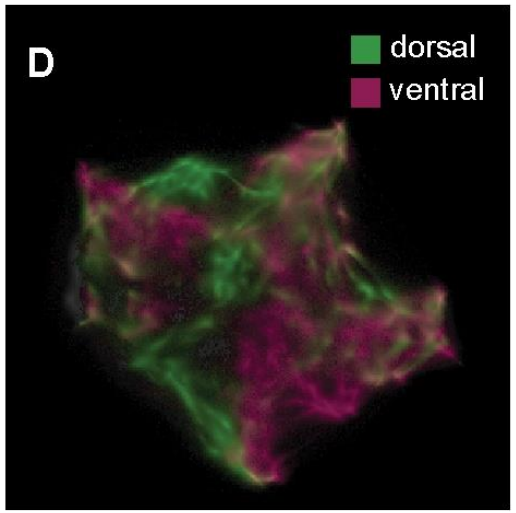
Posture Space



Park et al, Nature Methods 2024

Engineering challenges of photoactivation targeted to both *specific cells* and *specific states*

Activity Space



Atanas et al, Cell 2023

All together now

- The fundamental problem underlying image analysis:
  - turn a biological question into a measurement question
- If the question is is answerable depends on the interplay of
  - imaging
  - analysis methods
  - tool ergonomics
- This often requires a team, but knowing what is possible informs experimental choices

Questions?