Potential Biomedical Applications

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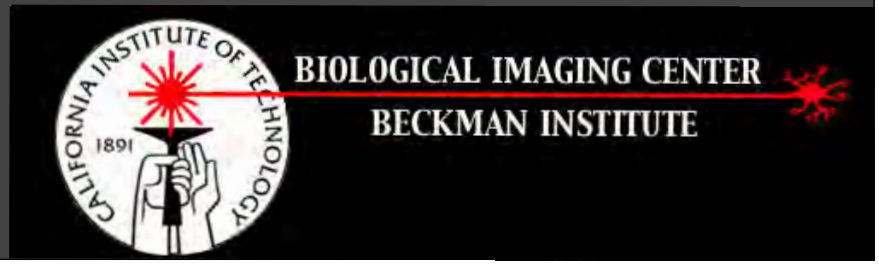
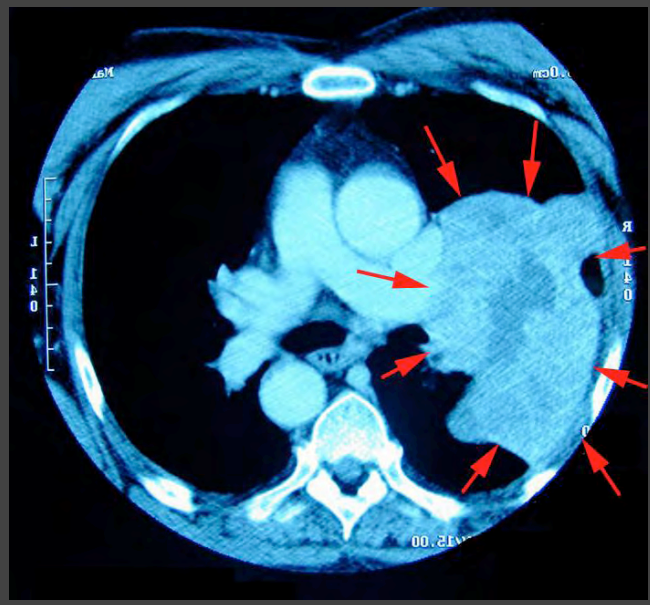


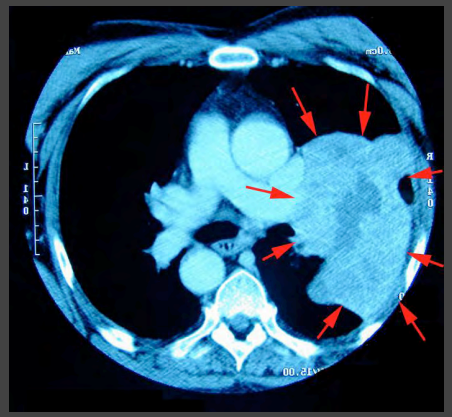
Potential Biomedical Applications

- Clinical imaging
  - Amazing advances
    - resolution
    - penetration
    - speed



Potential Biomedical Applications

Clinical imaging
- Amazing advances
  - resolution
  - penetration
  - speed
- May be reaching limits
  - moving mass
  - exposure
- CT: some studies suggest a significant increase in the incidence of cancer from a single scan series in brightest instruments



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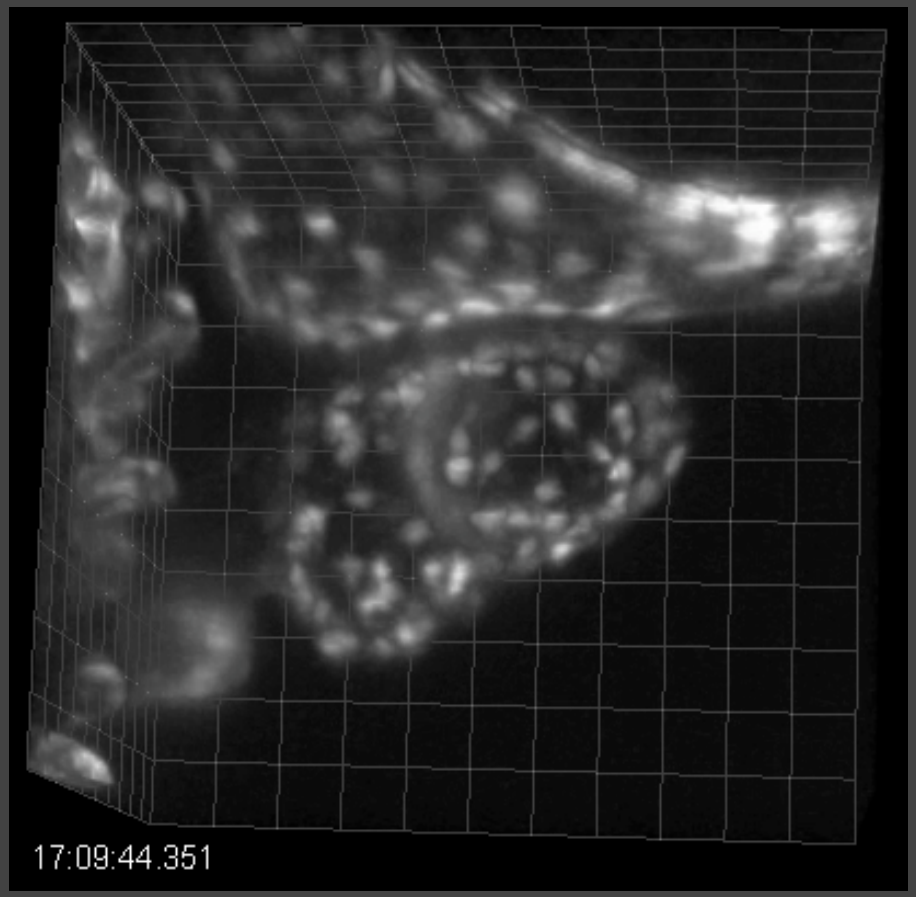
Potential Biomedical Applications

Research imaging
- Amazing advances
  - resolution
  - penetration
  - speed

- Based on fluorescence
  - (dyes, fluor. proteins)

- May be reaching limits
  - phototoxic byproducts
  - number of dyes



Beating heart in intact zebrafish
(collab with Gharib, Caltech; Leibling, UCSB)

Potential Biomedical Applications

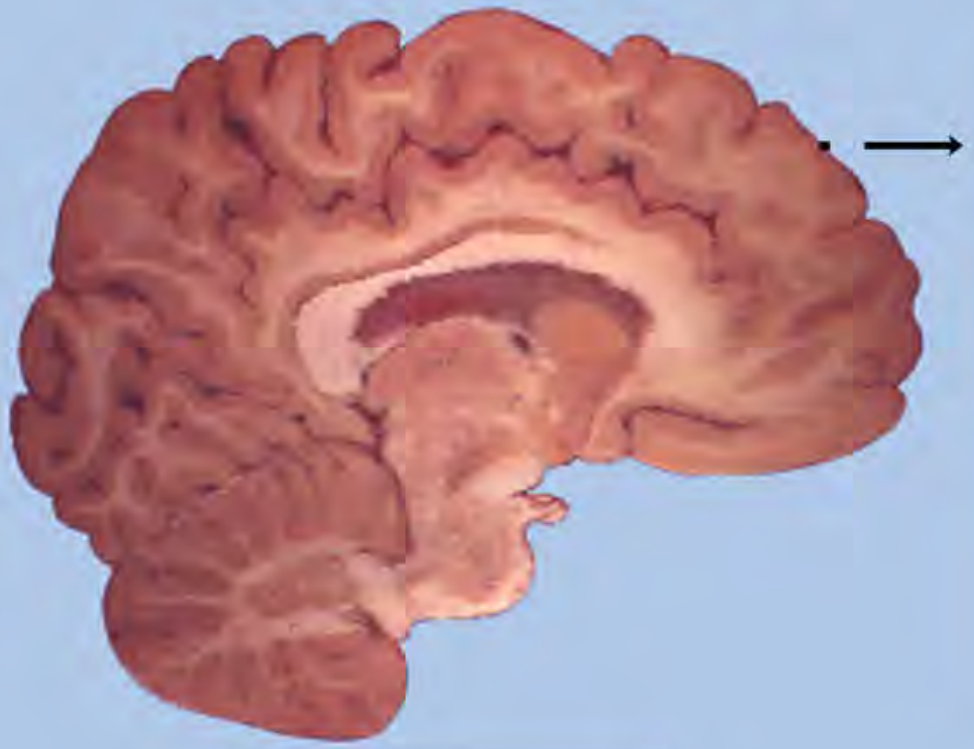
Illustrate challenge with two biological examples

- In vivo imaging of biological tissues
- “Next gen” DNA sequencing

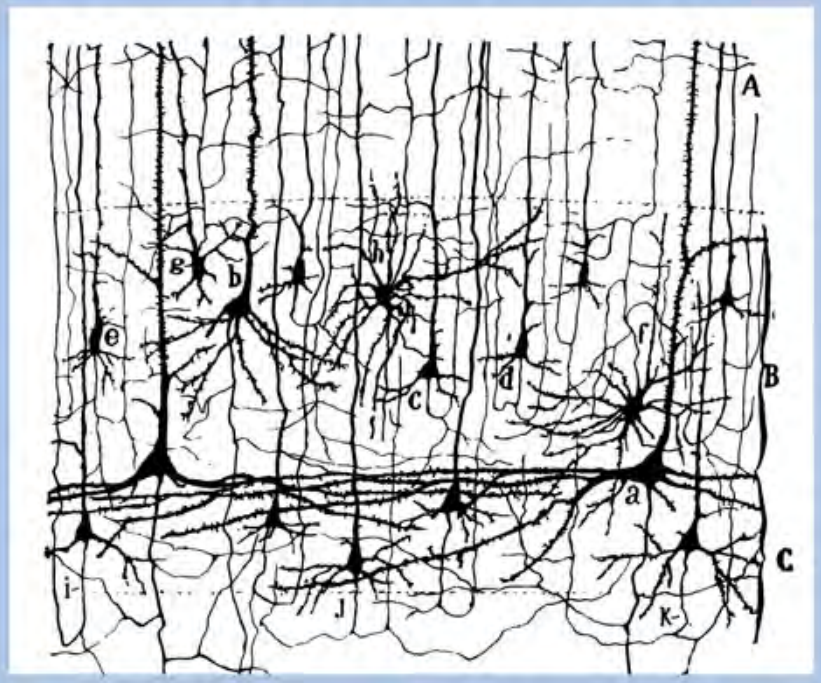
Both built on assumptions of large signals

Assumptions are starting to hamper progress

The challenge: understanding the macro- & micro-scale events of complex development



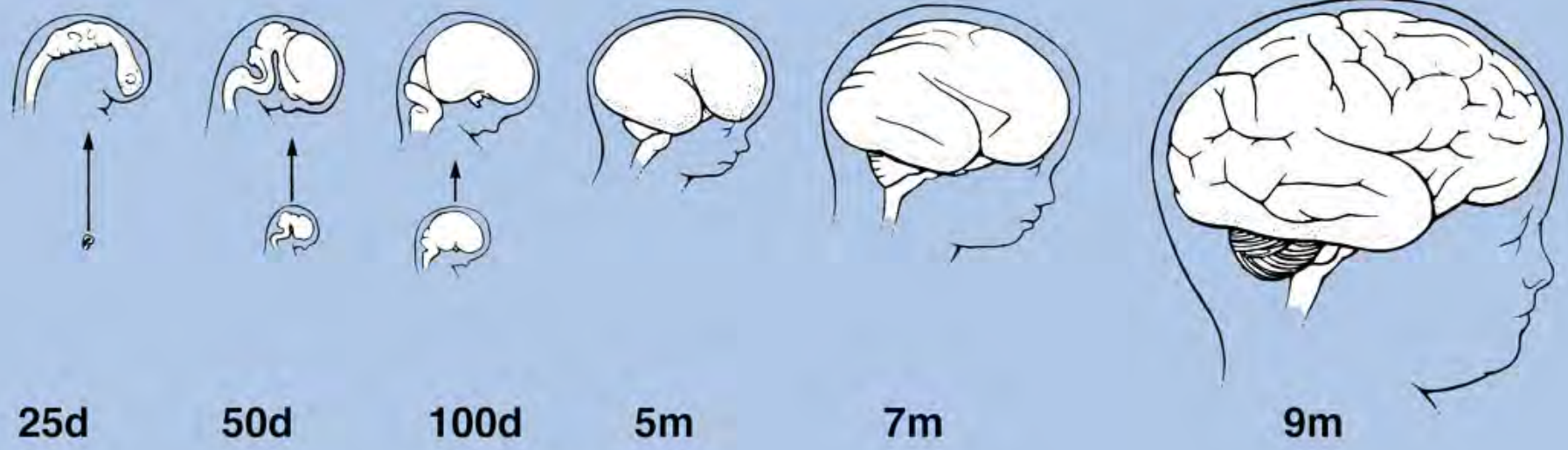
Human Brain:
100-1000 billion neurons



$(1mm)^2$ of cortex:

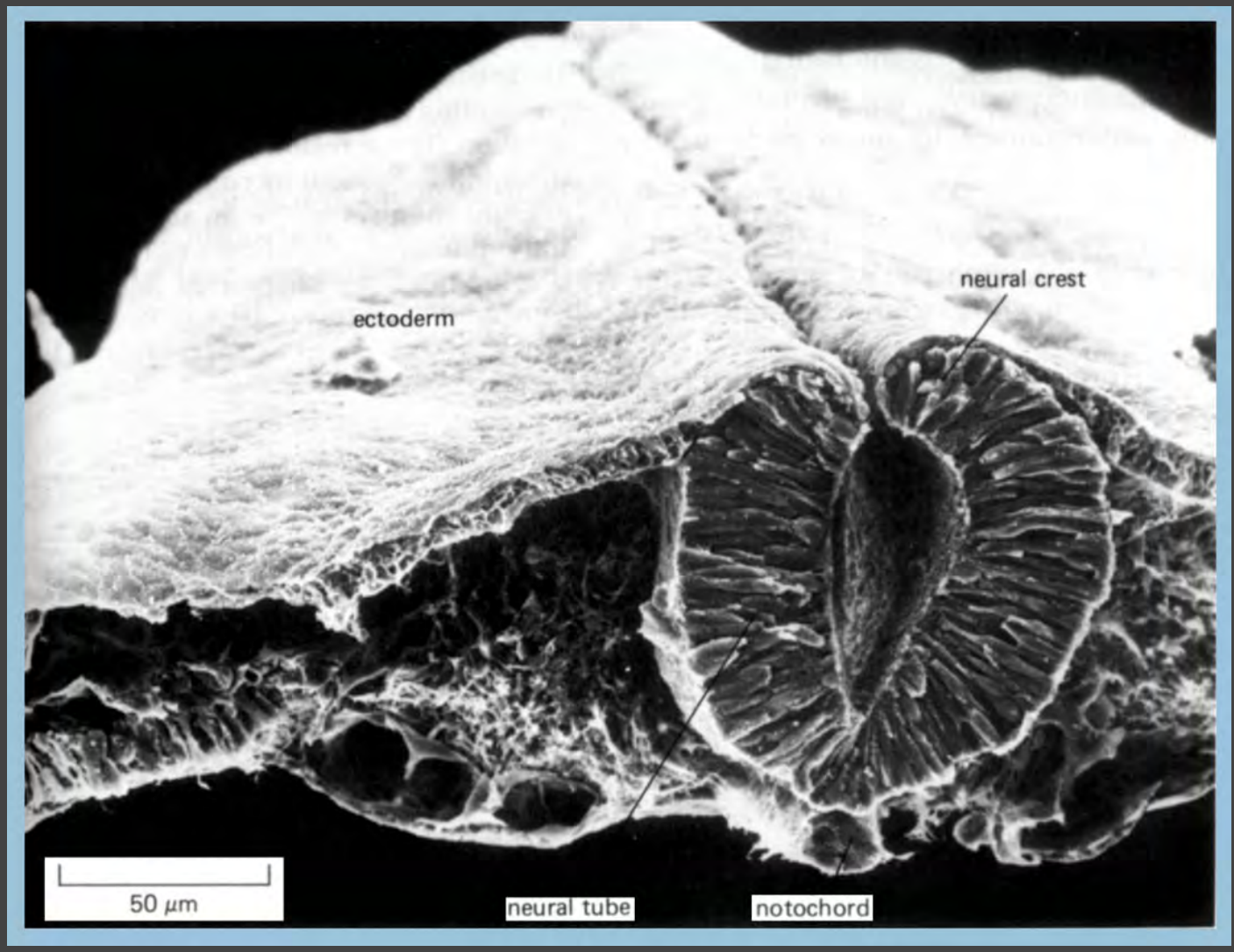
100,000 neurons
4 km of cabling
millions of synapses

The approach: Follow development in vivo



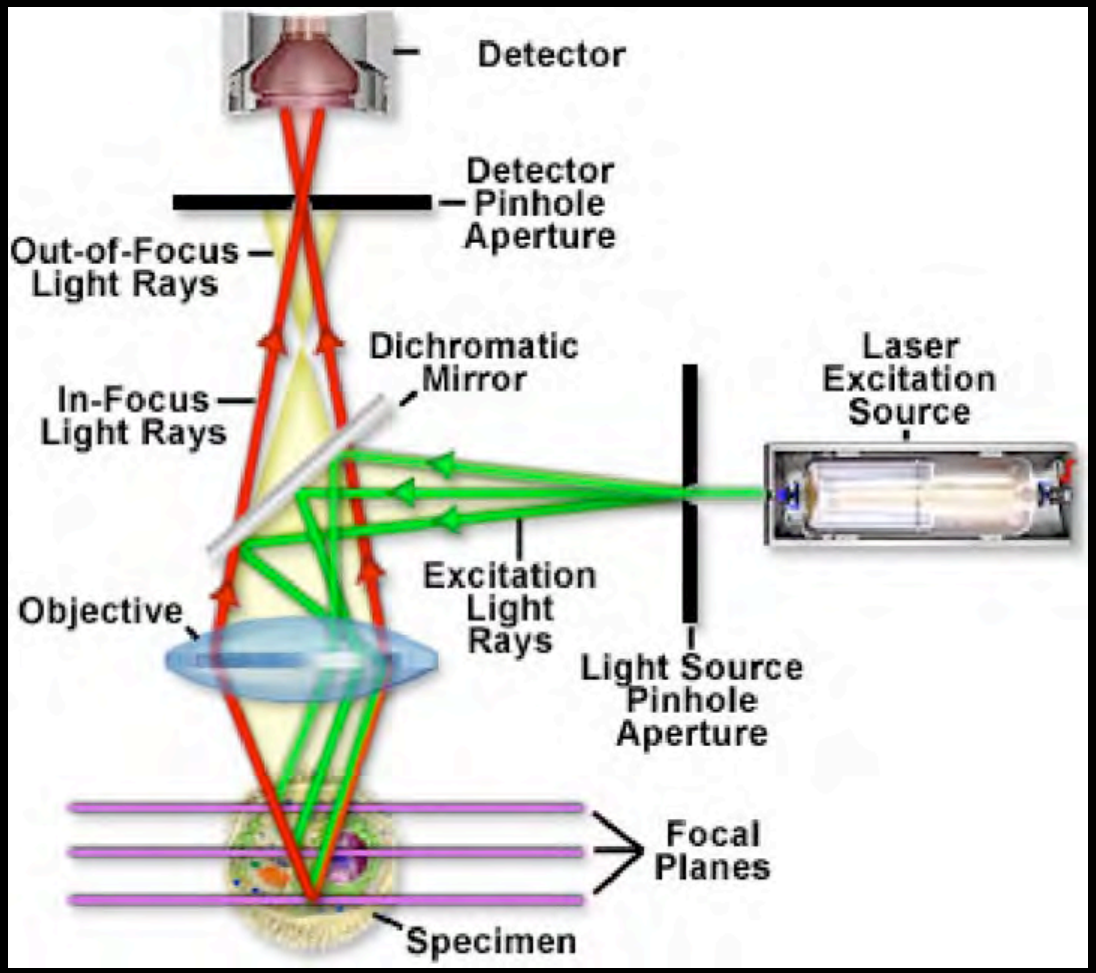
Following cells in living embryo offers two challenges:

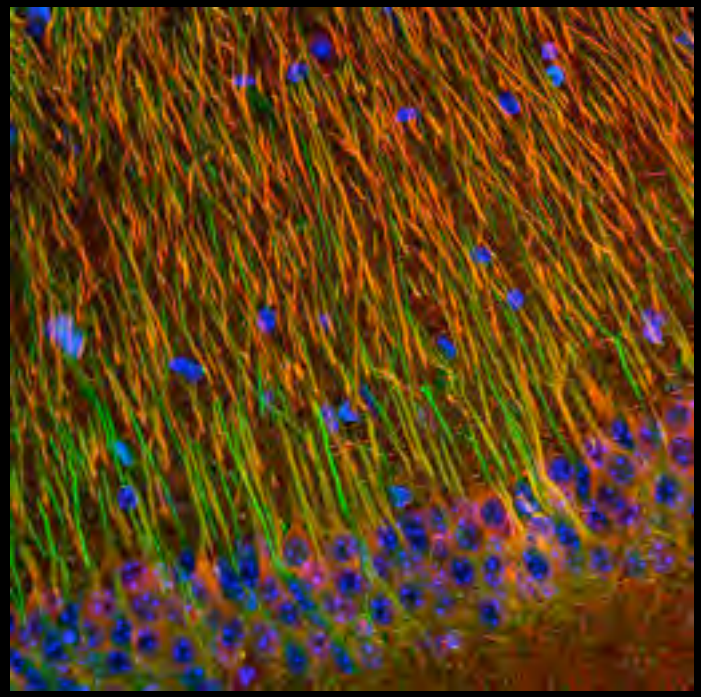
1. Many indistinguishable cells
2. Imaging living tissue



Biological intravital imaging

Confocal Microscope allows optical sectioning





Confocal image of fixed brain
(Rockefeller University)

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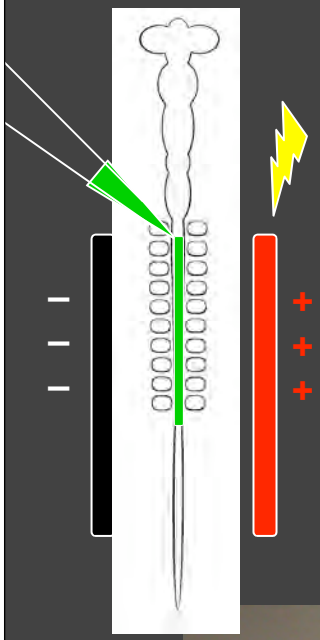
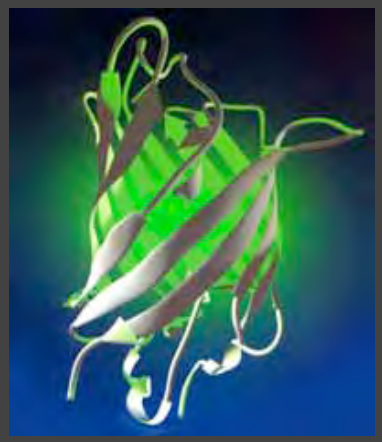
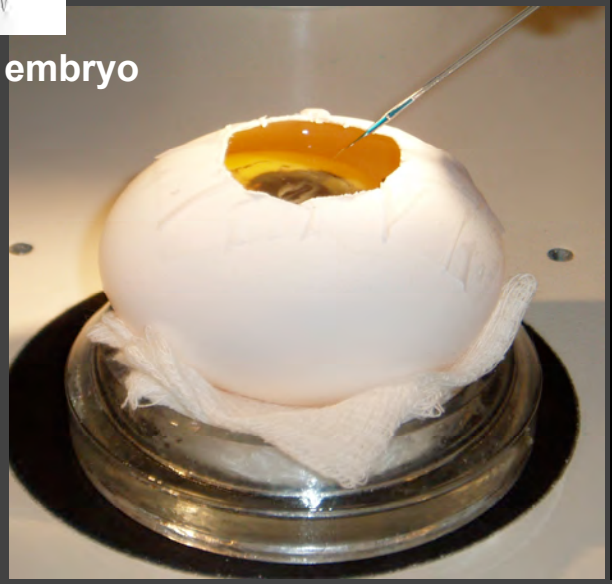
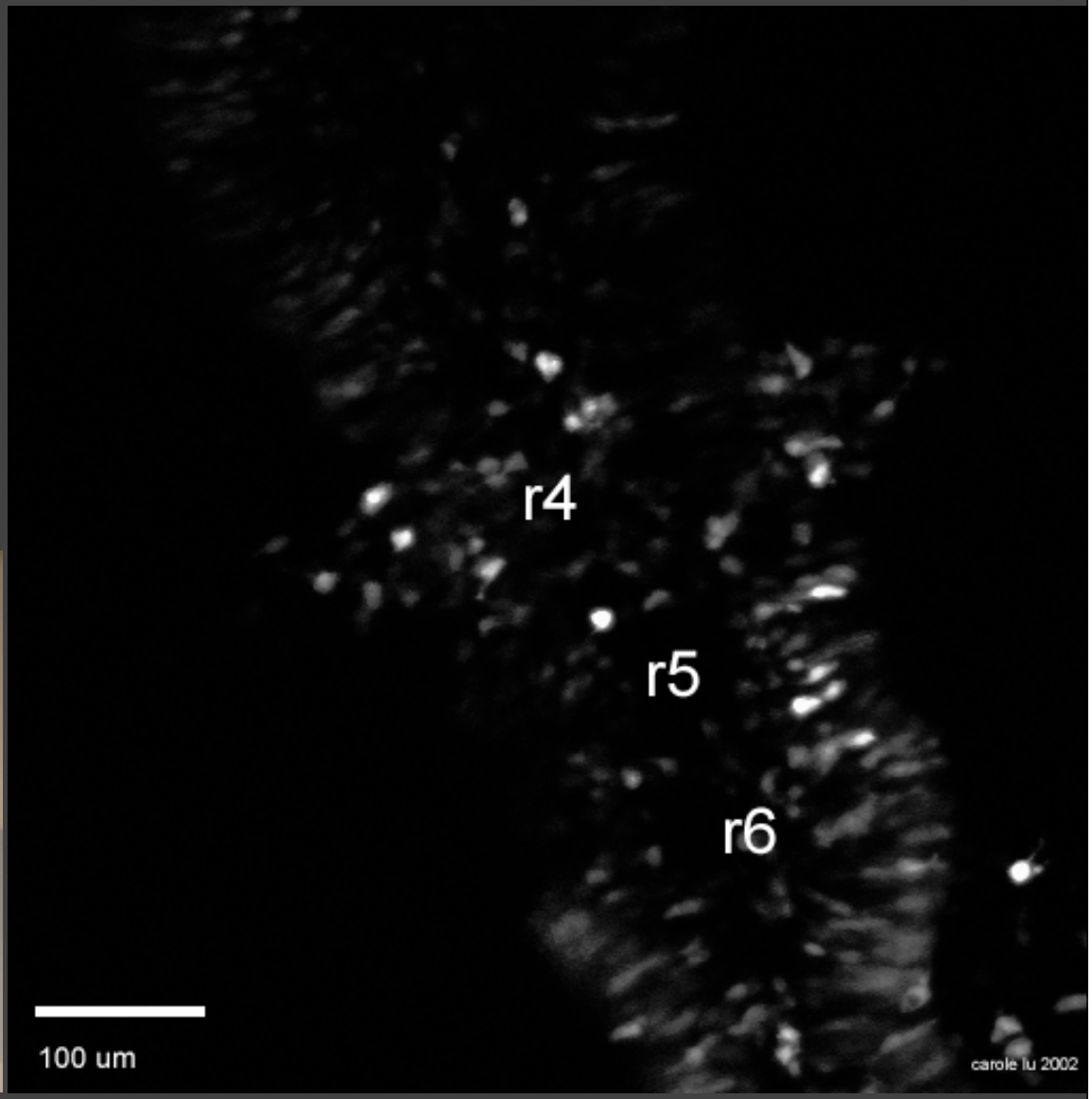
Fluorescent protein expression to visualize









Multispectral imaging with the LSM 510 Meta: 6 labels at a time in living cells (32 anode PMT)

Product of a Caltech/JPL collaboration

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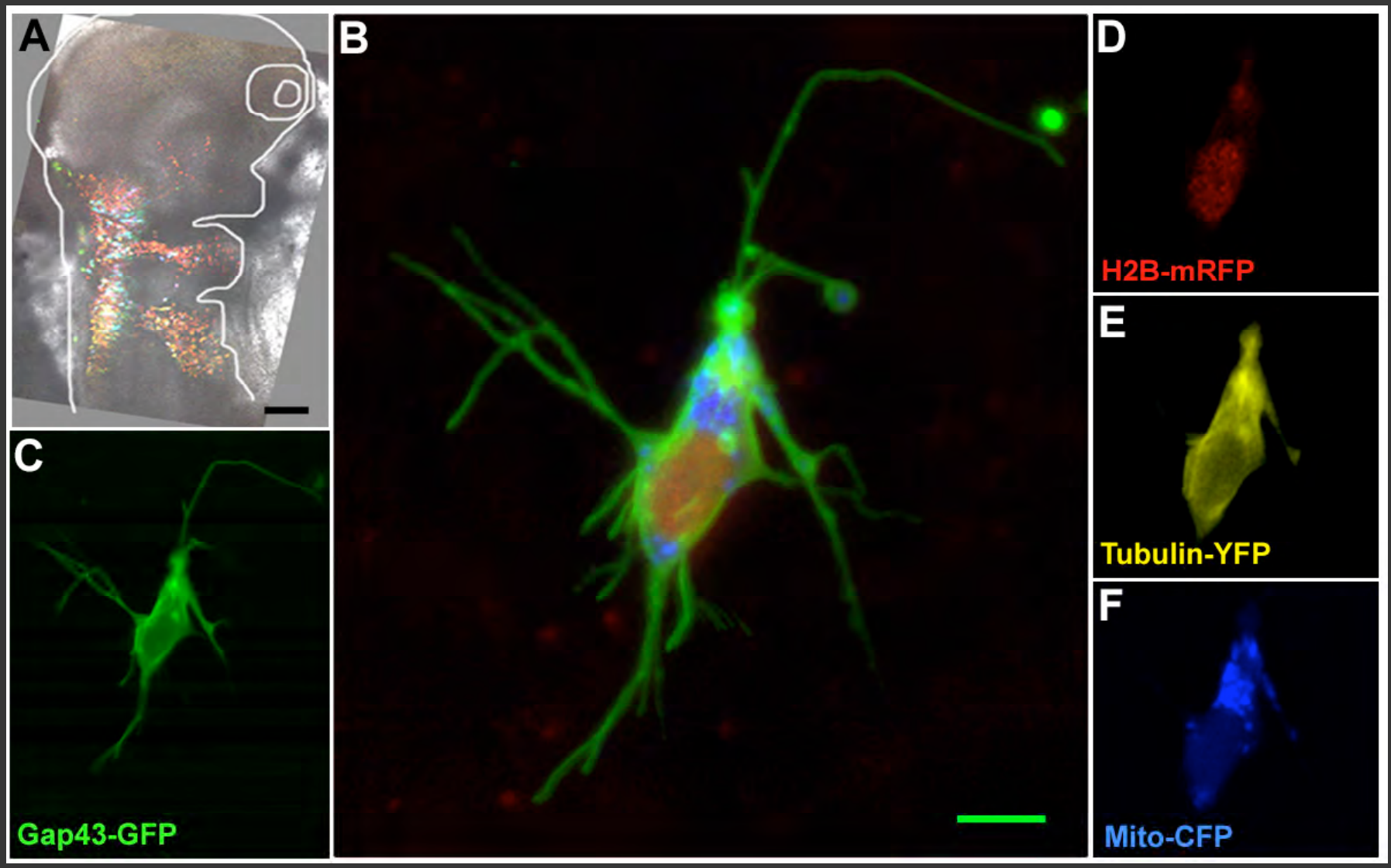
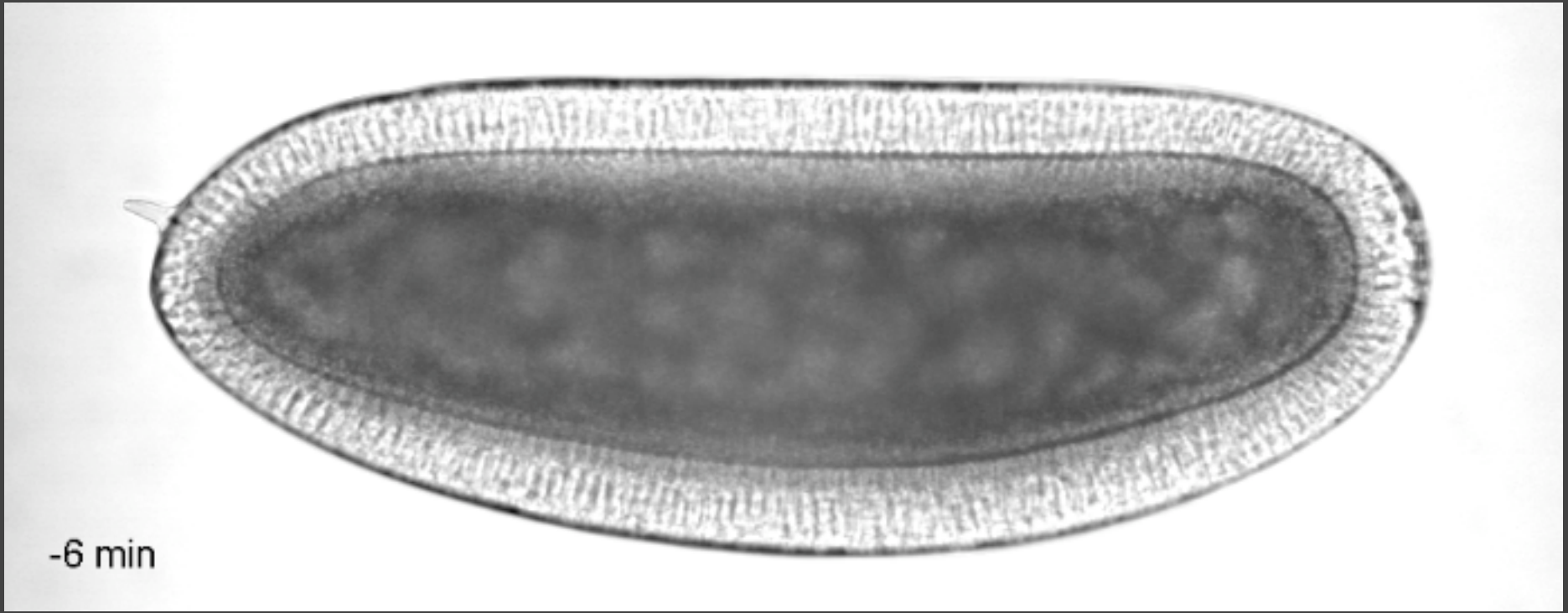
Multi-spectral CLSM of neural crest



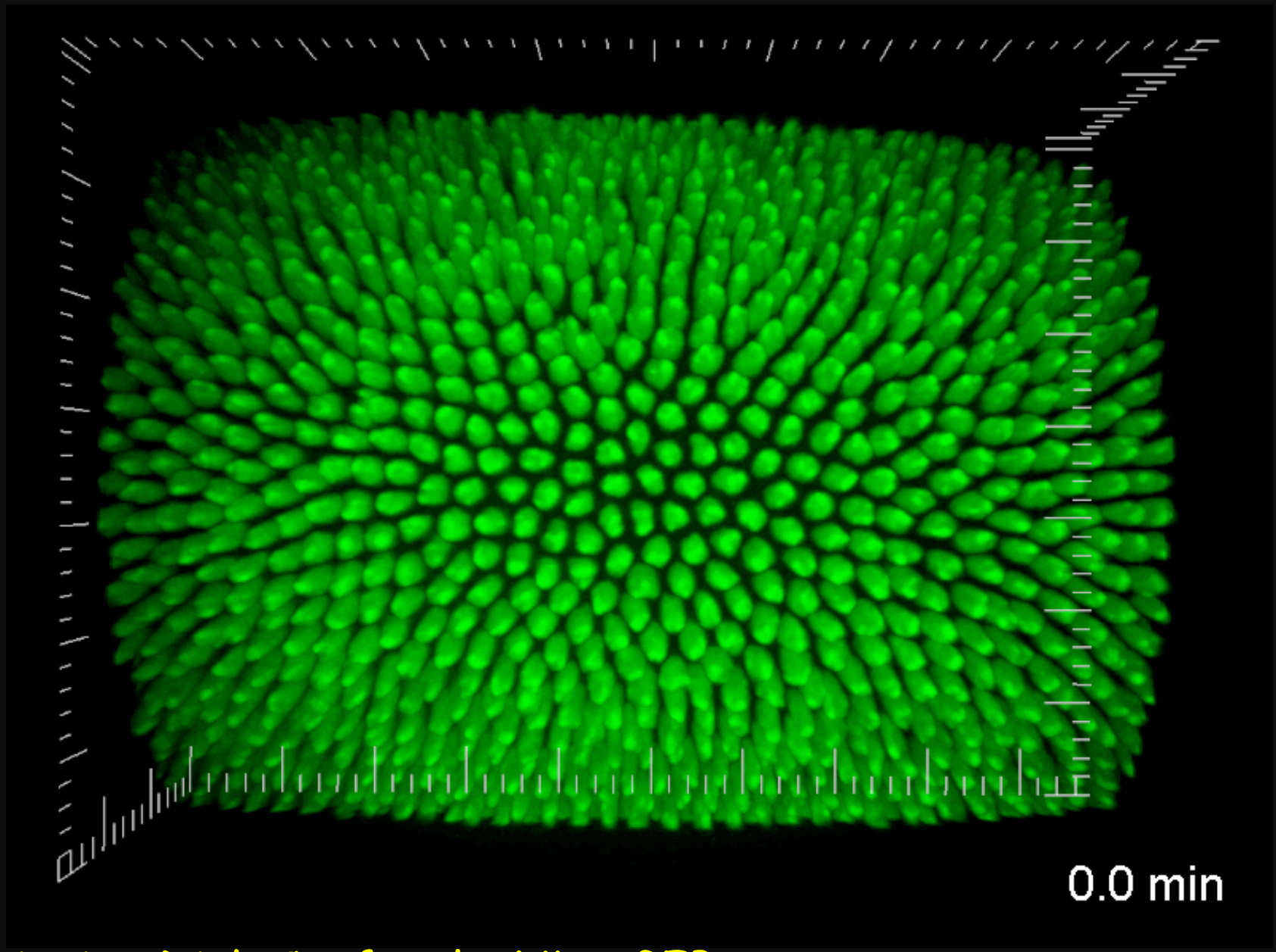
Kulesa and Lansford; LSM-510 META

Example 1 -
Drosophila gastrulation & germ-band elongation



Transmitted light movie of fly embryo development

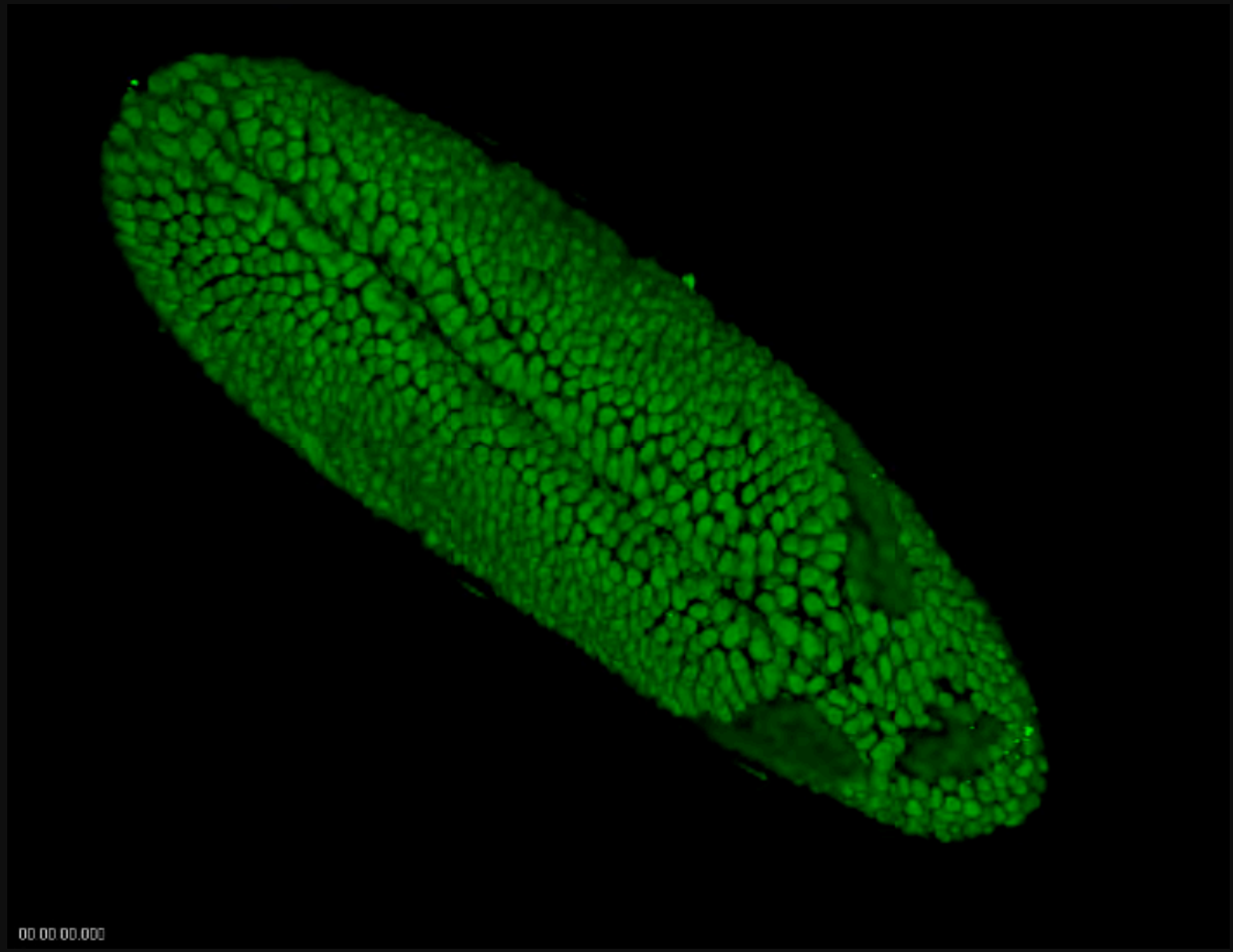
Dynamic Imaging of ventral furrow formation



Spinning Disk Confocal - NLS:GFP

W Supatto

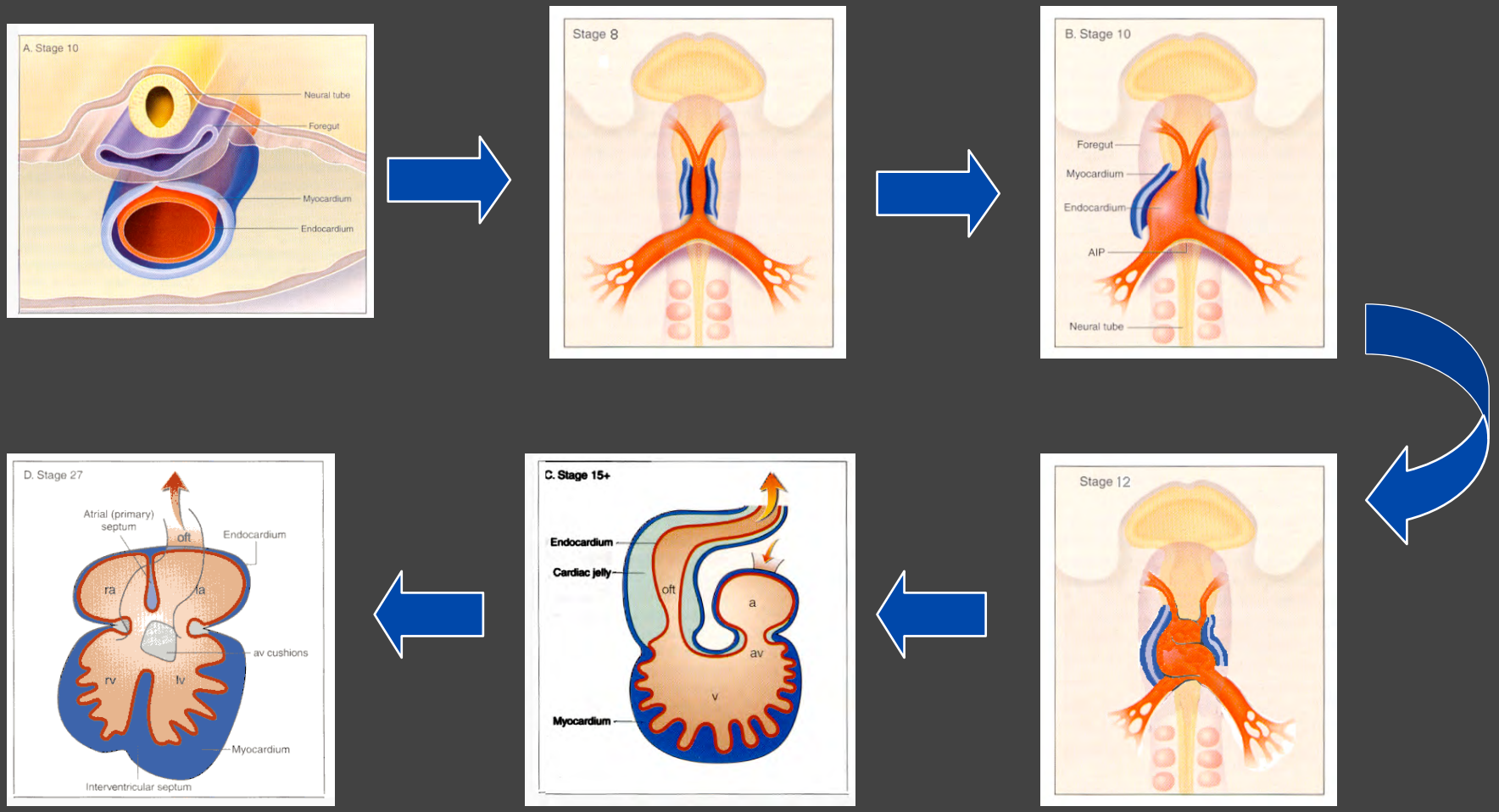
Greater penetration from two-photon imaging



TPLSM - NLS:GFP

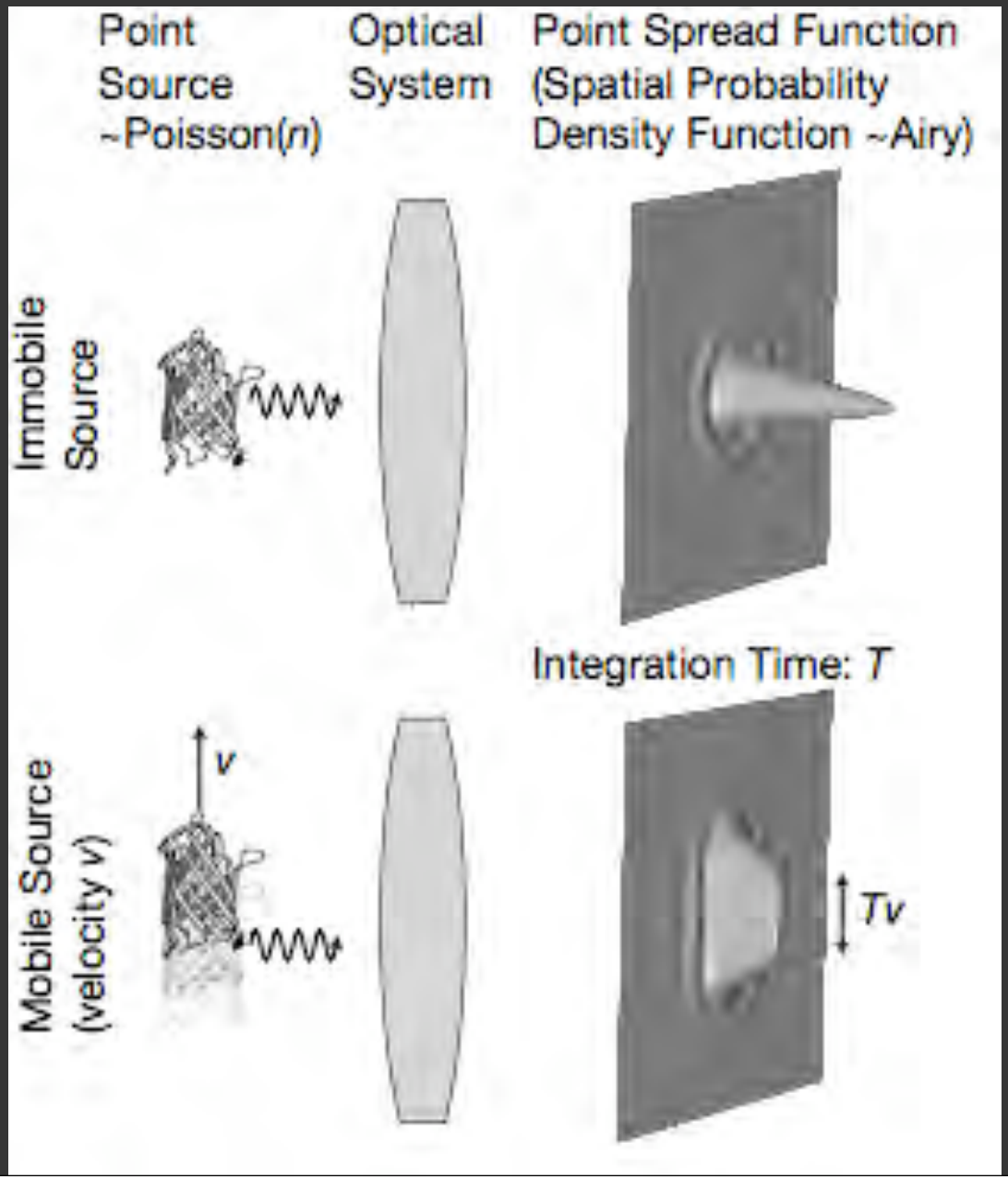
W Supatto

Example 2:
Role for flow in vertebrate cardiogenesis?
(morphogenesis as it pumps)



Fishman & Chien, 1997

Poisson meets Airy meets motion



PSF width:

Δx

New PSF width:

$\Delta x' = T\,v + \Delta x$

T: integration time

v: velocity

Example:

f_s=25 Hz,

$T=1/f_s$=0.04 s

v=1 mm/s

Tv= 40μm

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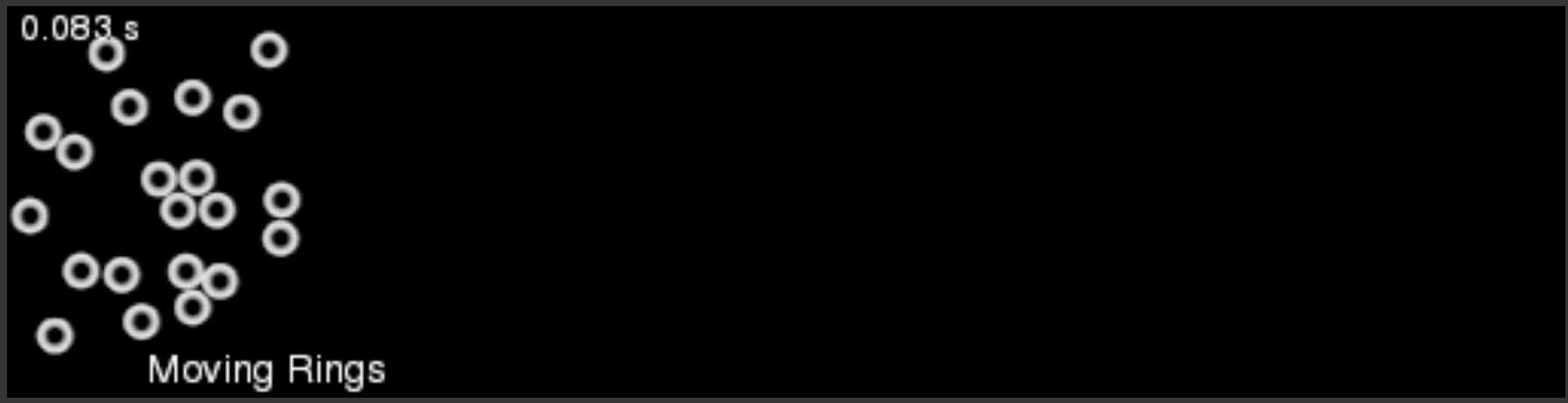
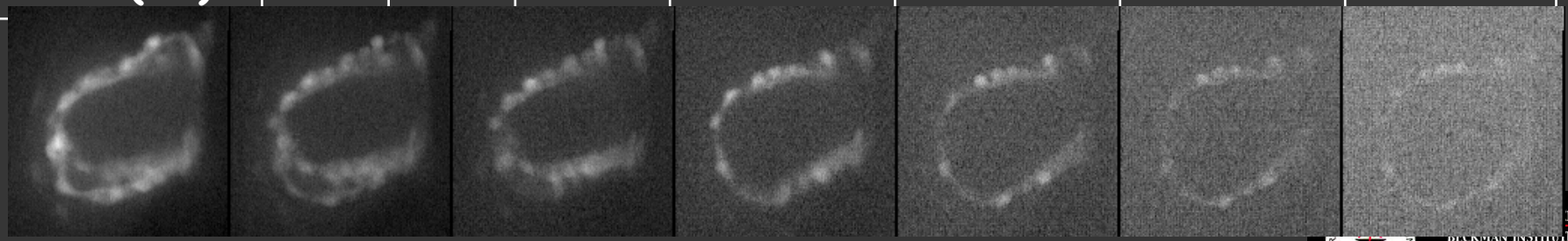
Motion Blur and Integration Time



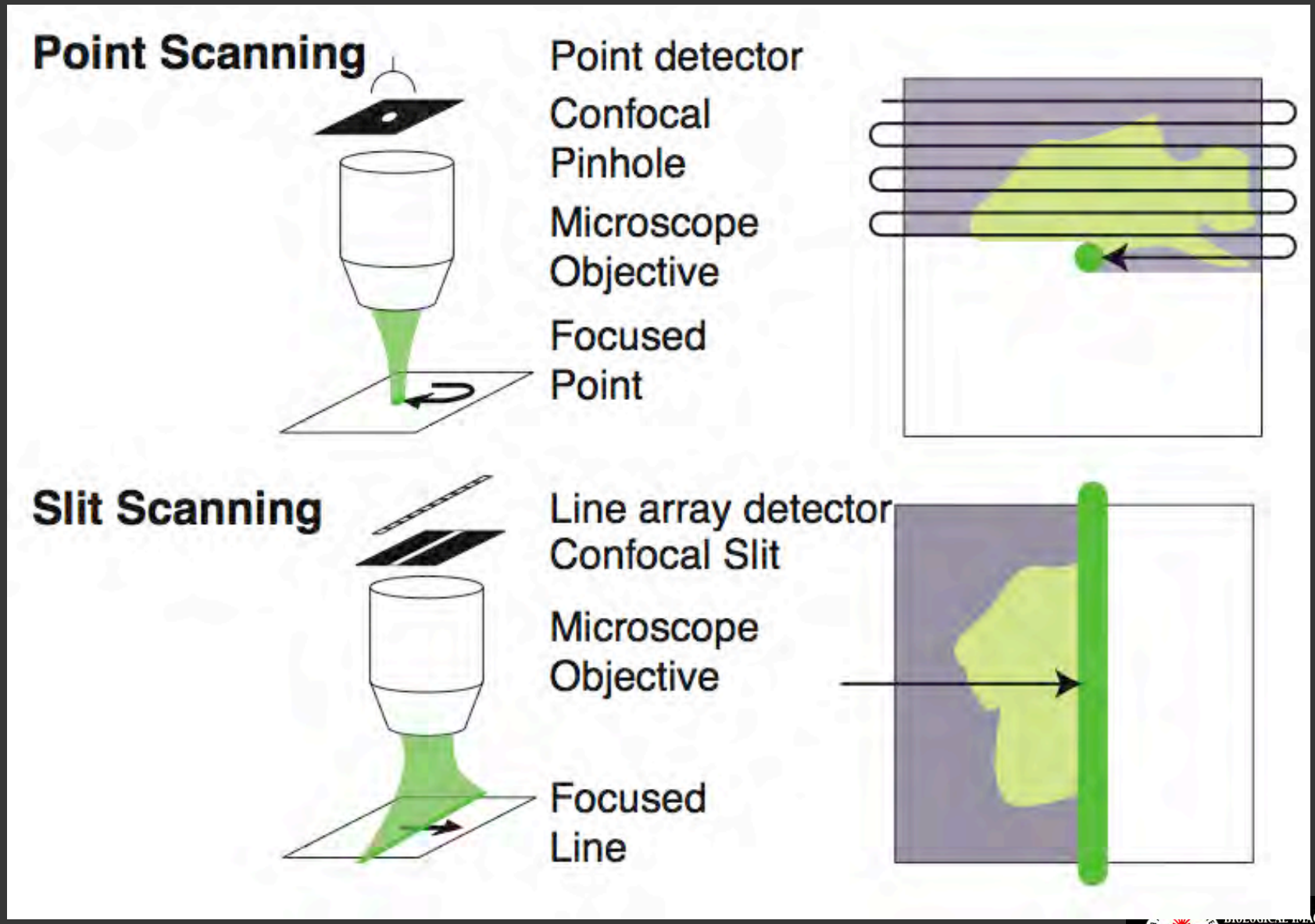
Best integration time

Compromise between motion blur and insufficient photon count

Framerate (fps)	2.5	5	10	20	35	68	104
Integration Time (ms)	400	200	100	50	25	12	6

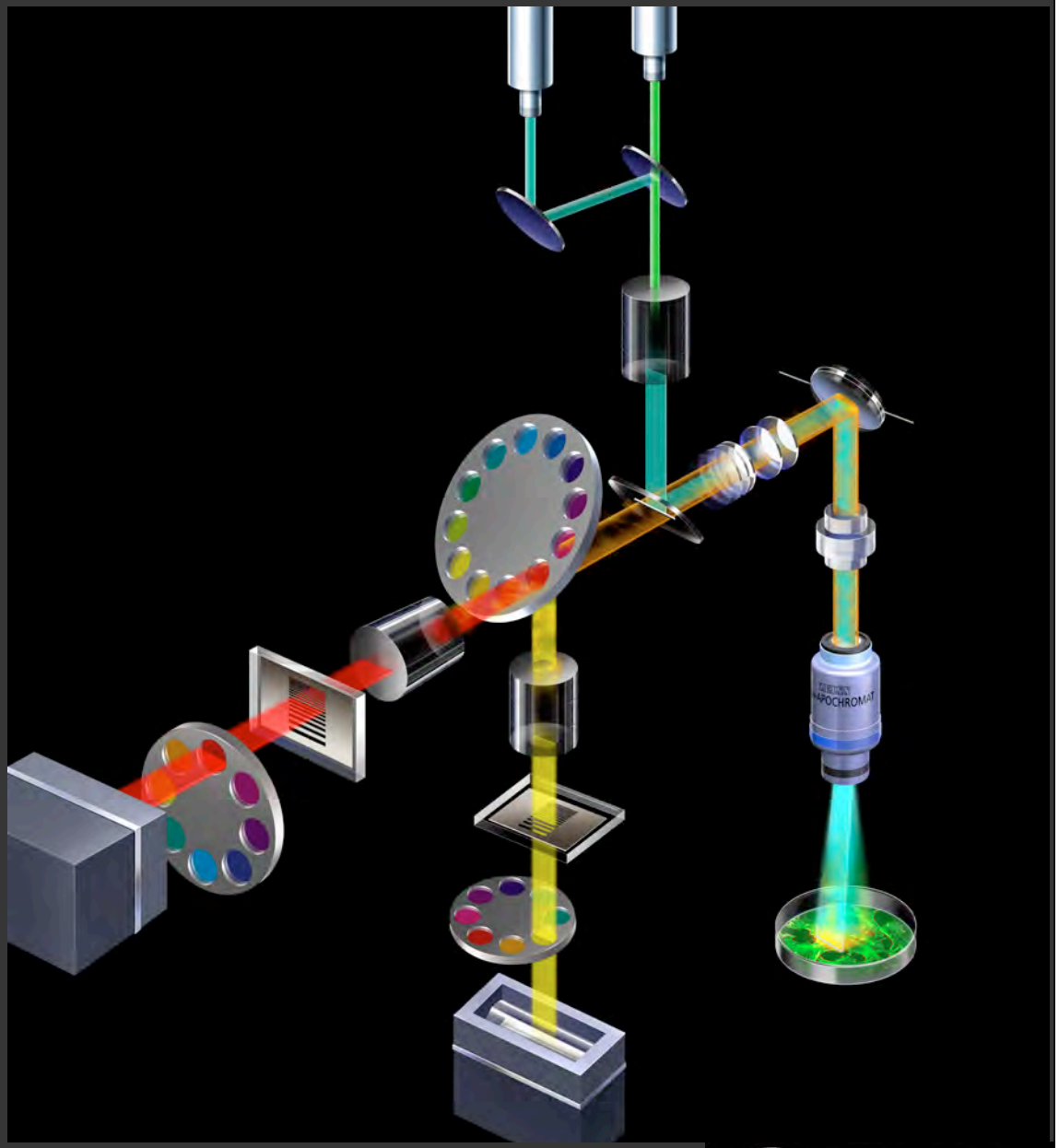


Making a Fast Confocal Microscope



LSM 5 LIVE

- **Parallel illumination / detection**
- Up to **120 frames/sec** at 512x512
- **AchroGate** color-independent high-efficiency main beamsplitter



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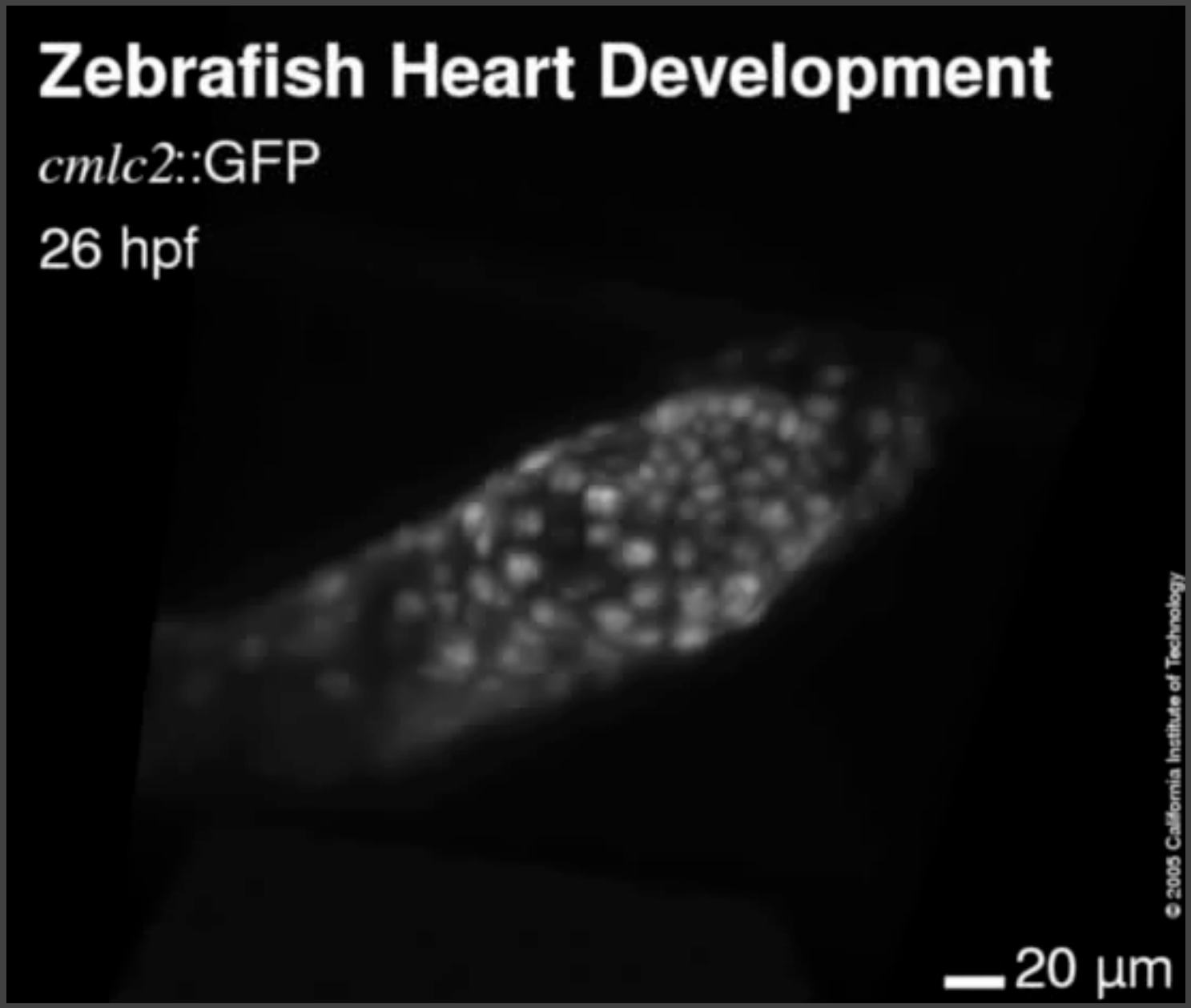
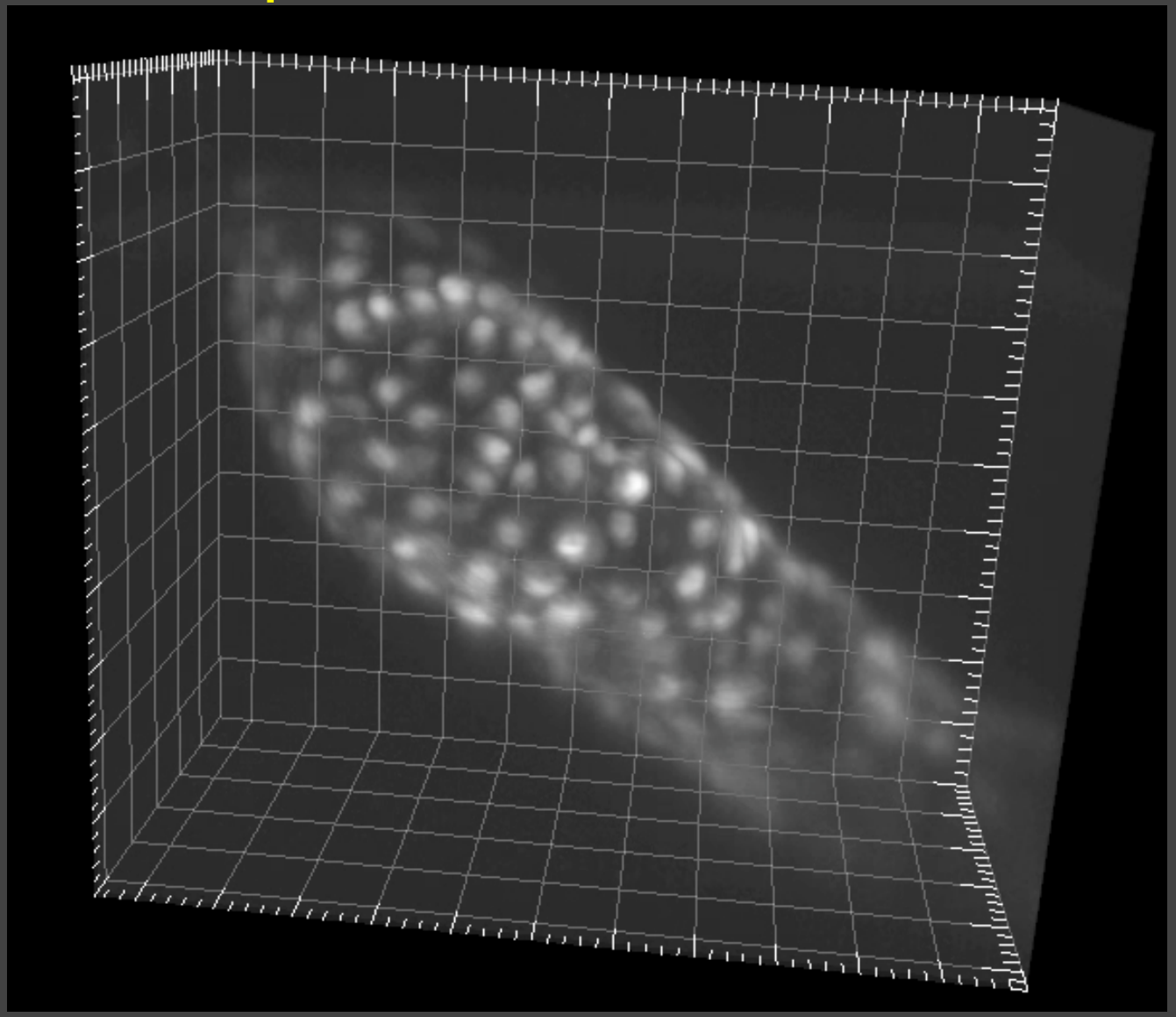


Forouhar, Liebling

Heart development followed with cmlc2::GFP



Grid = 10 um

Forouhar, Liebling

Challenge for biological imaging

Based on fluorescence of dye or protein
- 30% - 80% of excitations result in an emitted photon
- 1% - 5% of excitations result in interstate conversion
  - Interstate conversion creates free radicals

Lifetime of a dye: 1000-100000 photons
- Optics collect a minority

Imaging with increased excitation can't help
- Saturation of dye
- Dose of radicals to the specimen

Potential Biomedical Applications

Illustrate challenge with two biological examples

- In vivo imaging of biological tissues
- “Next gen” DNA sequencing

Both built on assumptions of large signals

Assumptions are starting to hamper progress

Predictive, Preventive, Personalized Medicine

- Predictive:
  - Probabilistic health history--DNA sequence
  - Biannual multi-parameter blood measurements
  - Diagnostic measurements to stage disease
- Preventive:
  - Design of therapeutic and preventive drugs via systems approaches
- Personalized:
  - Unique individual human genetic variation mandates individual treatment

(Lee Hood & Nanosystems Alliance)

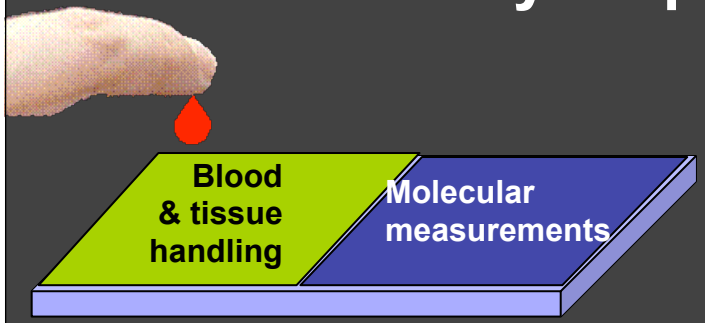
Next Generation Molecular Diagnostics

Blood Test:
1000-2000 proteins
Dozens of genes and variants

- **identify personal health risks;**
- **identify drug incompatibilities;**

- **stratify disease;**
- **progression of disease;**

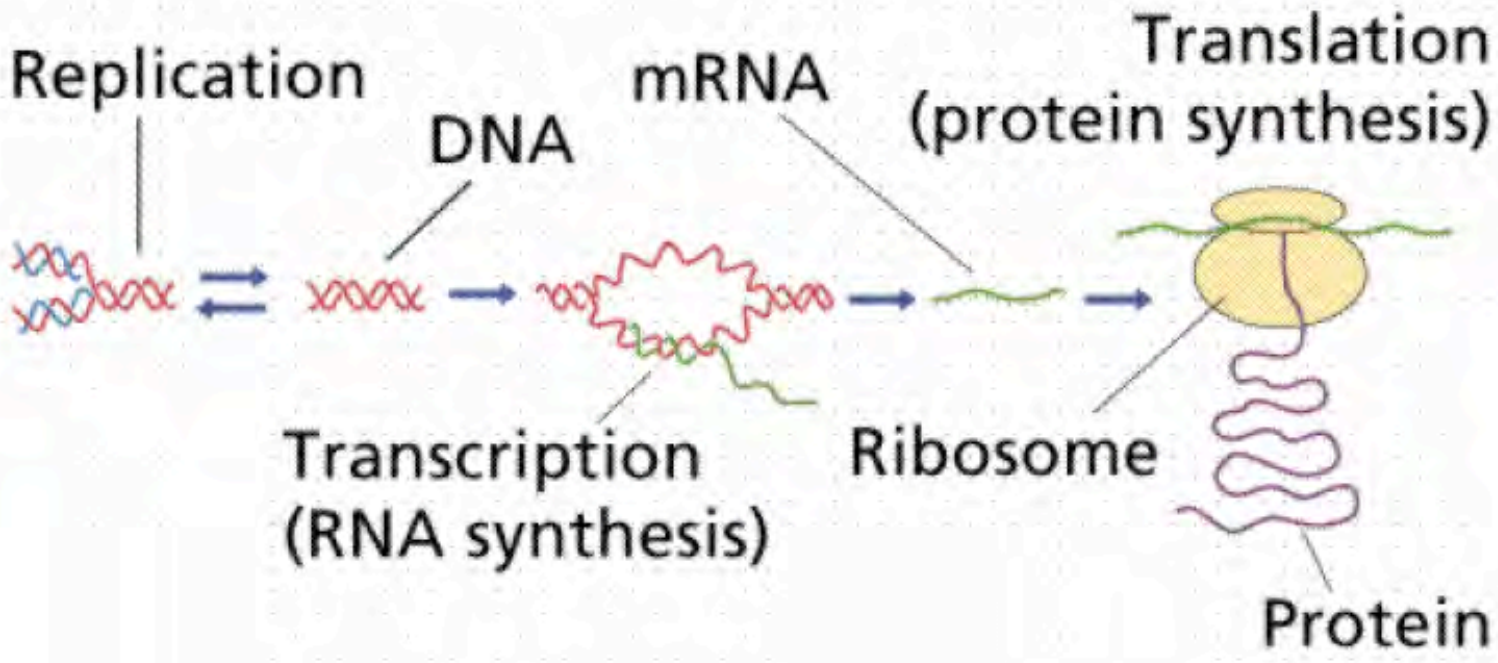
- **choose therapeutic strategy;**
- **assay response of disease to therapy**



Simple (finger prick sampling)
Point of care
Fast read-out

The Central Dogma of Molecular Biology
(Crick)

- **DNA** DNA → DNA (Replication)
- **RNA** DNA → RNA (Transcription / Gene Expressio
- **Protein** RNA → Protein (Translation)



Before Hood: DNA sequencing used gel electrophoresis

To sequence template:

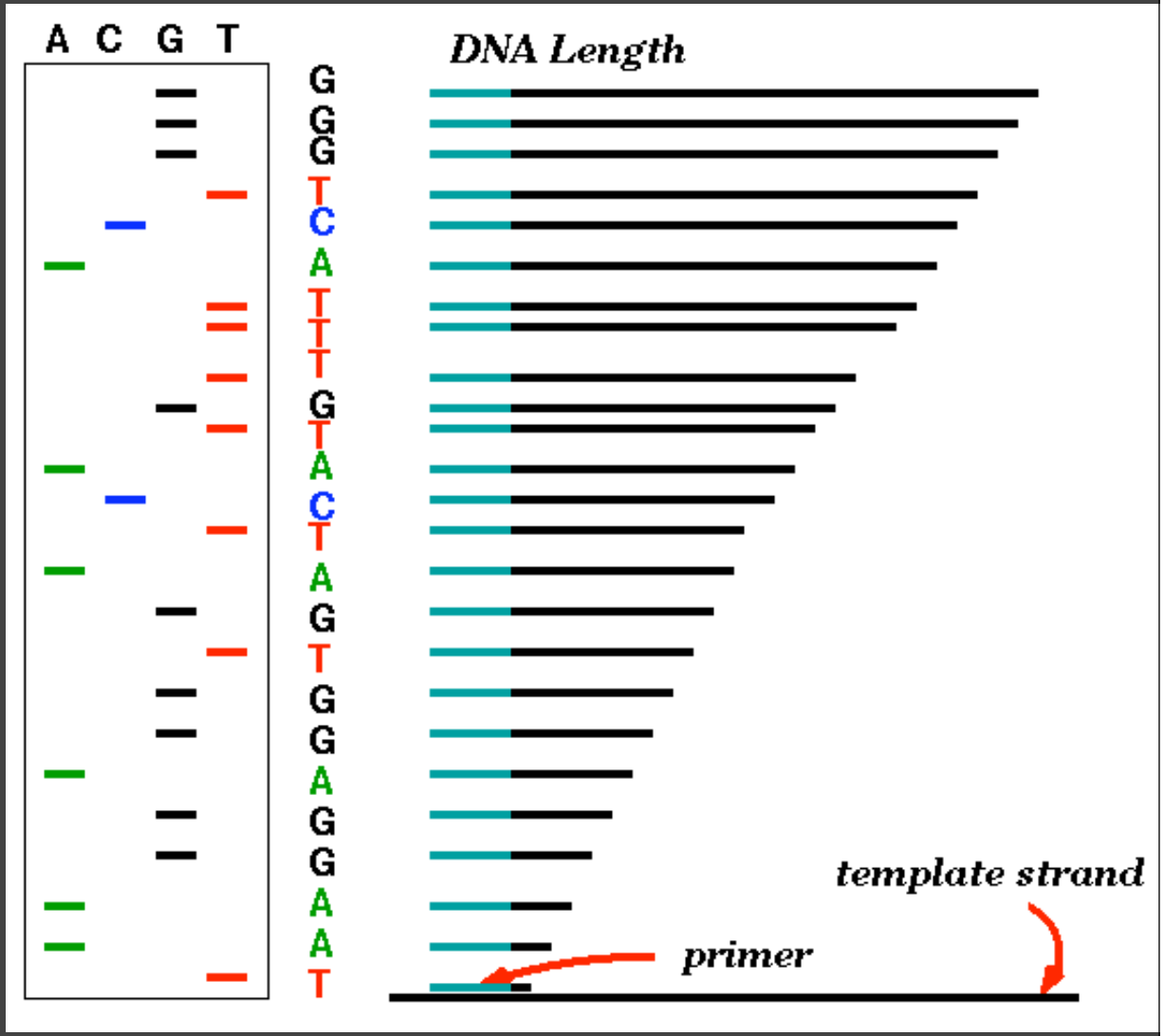
Start at primer

Grow DNA chain

Include “poison” bases (modified a, c, g, t)

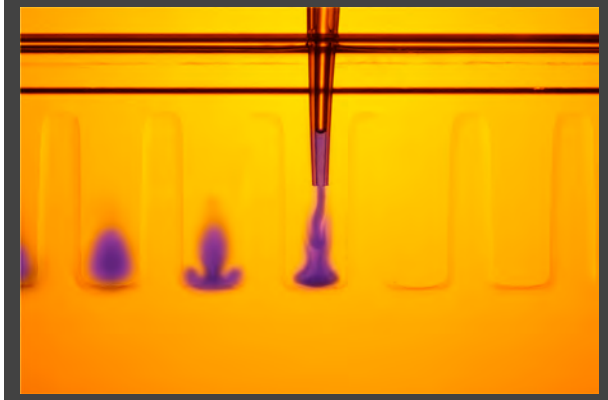
Stops reaction at all possible points

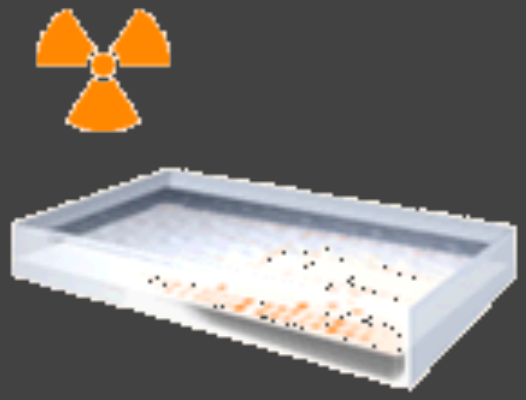
Separate products with length, using gel electrophoresis



DNA sequencing before 1986 was read out by hand

electrophoresis box

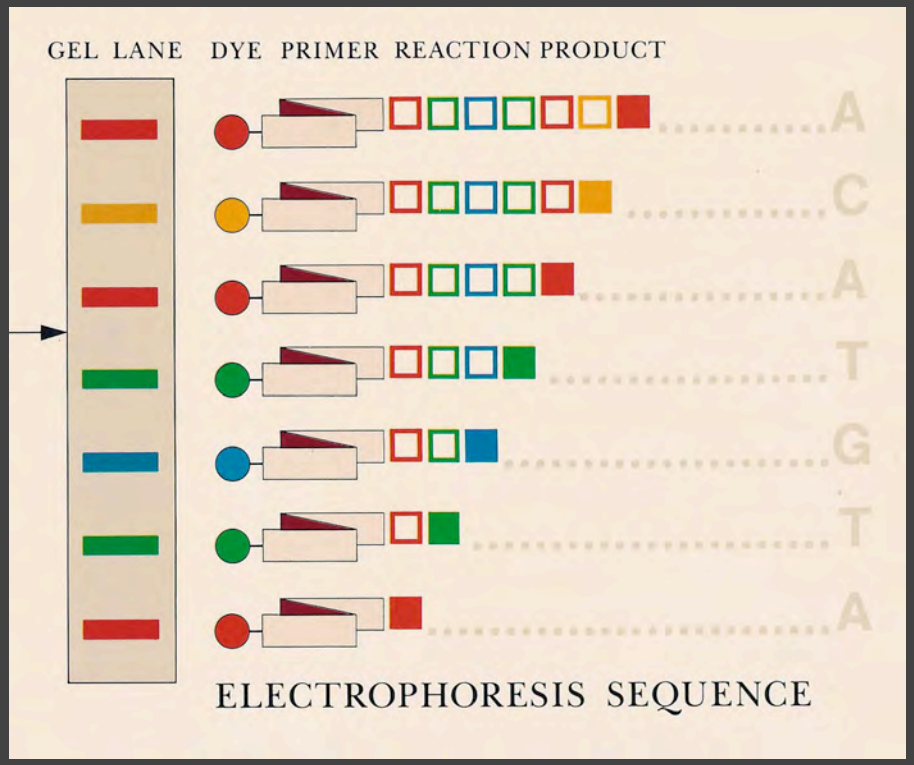




gel on x-ray film

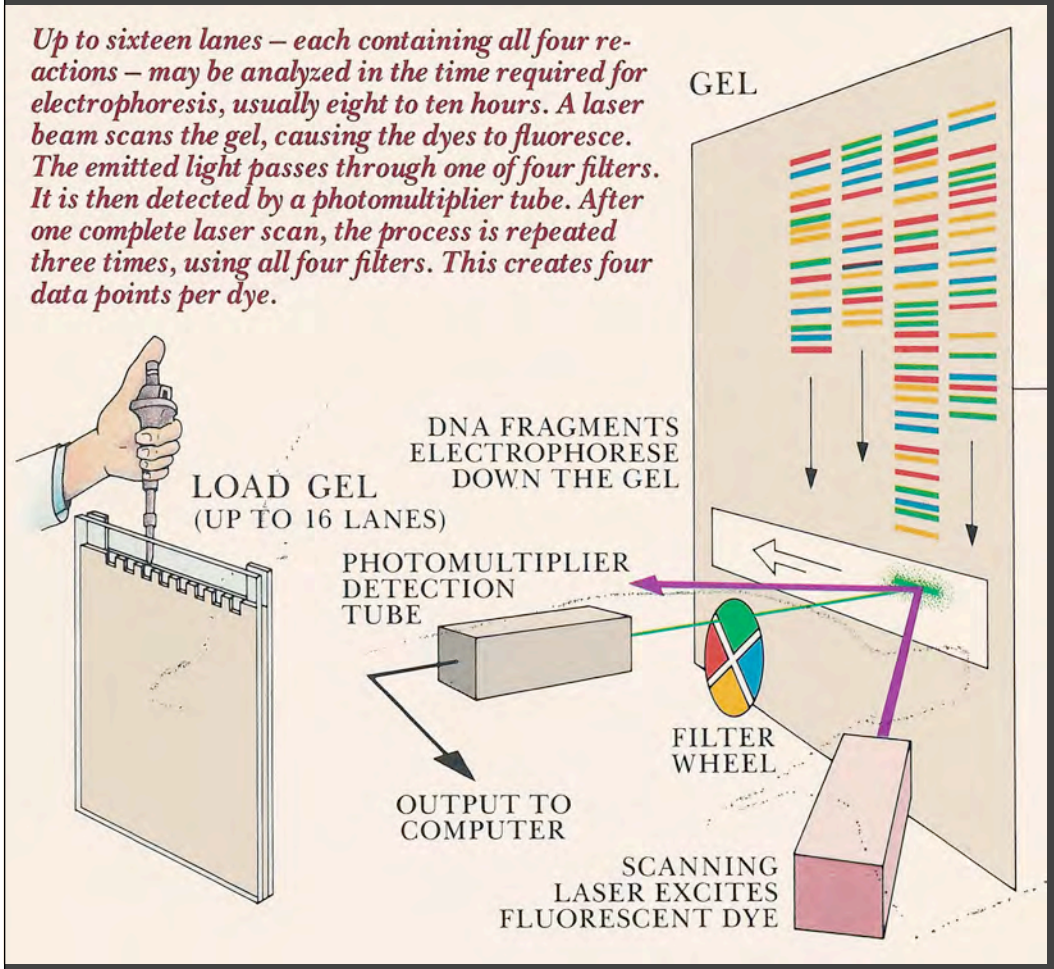
reading out the bands

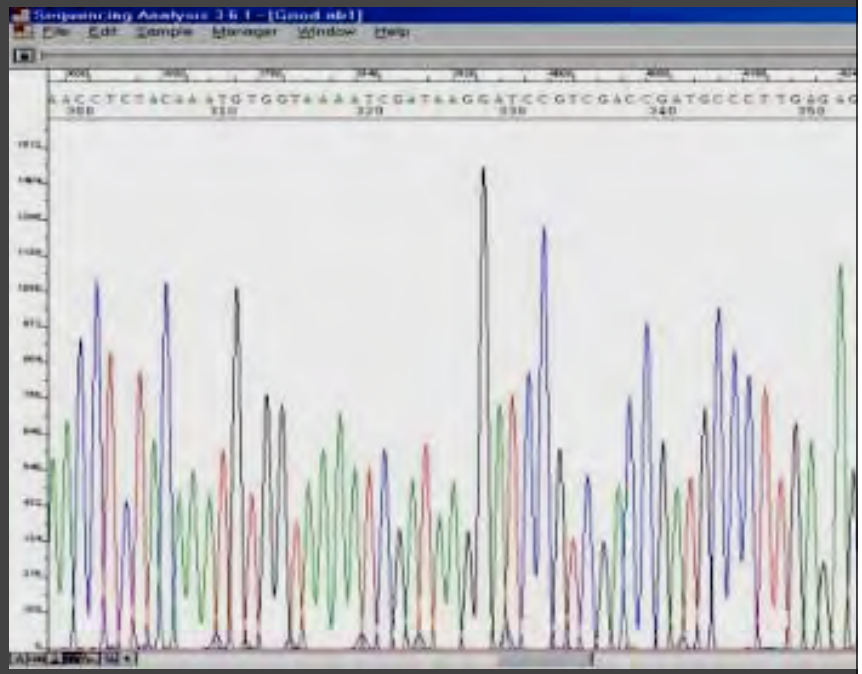
Fluorescent primers avoided radioactivity and permitted automated sequencing



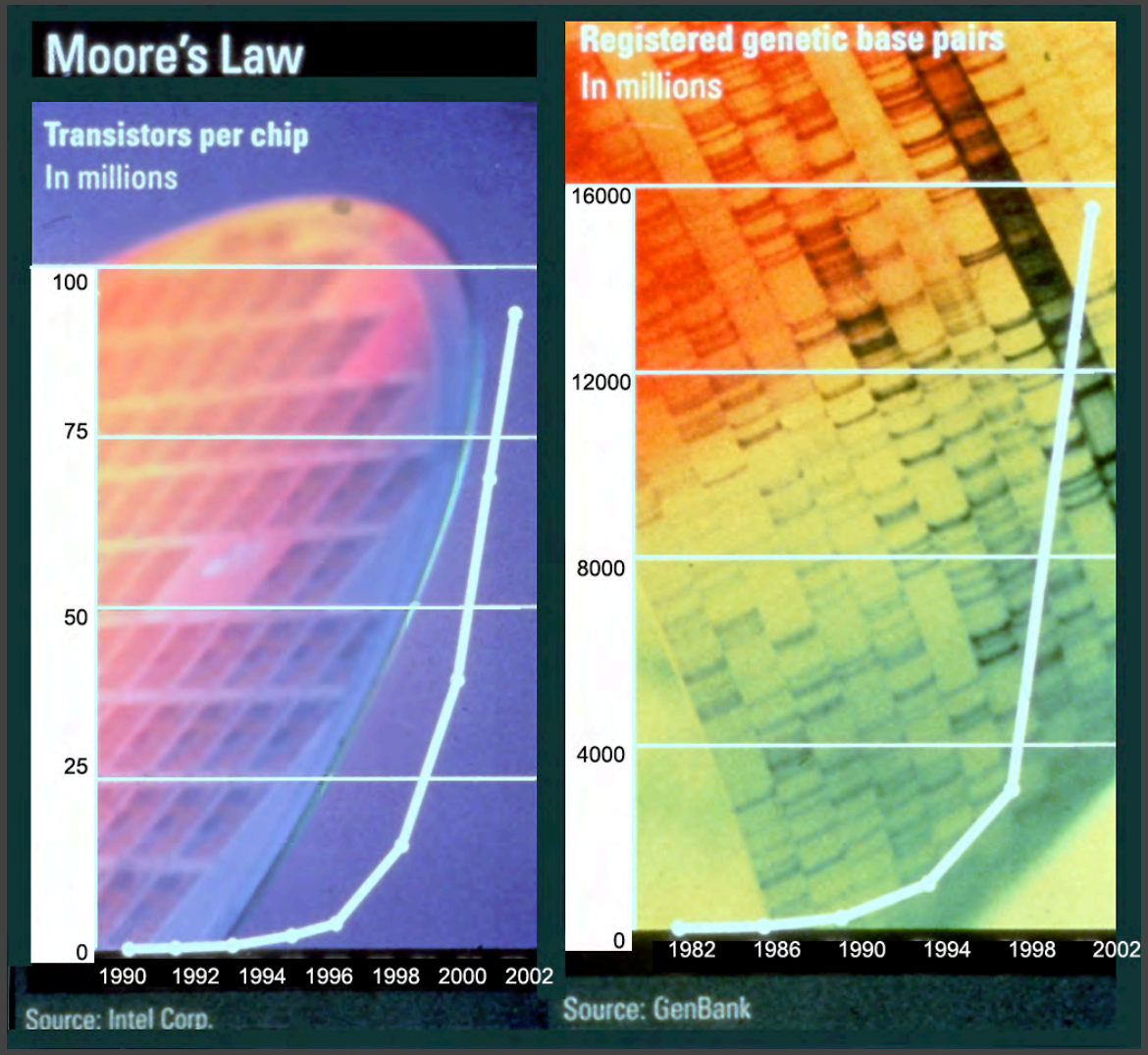
Automated DNA sequencer & output

Up to sixteen lanes – each containing all four reactions – may be analyzed in the time required for electrophoresis, usually eight to ten hours. A laser beam scans the gel, causing the dyes to fluoresce. The emitted light passes through one of four filters. It is then detected by a photomultiplier tube. After one complete laser scan, the process is repeated three times, using all four filters. This creates four data points per dye.





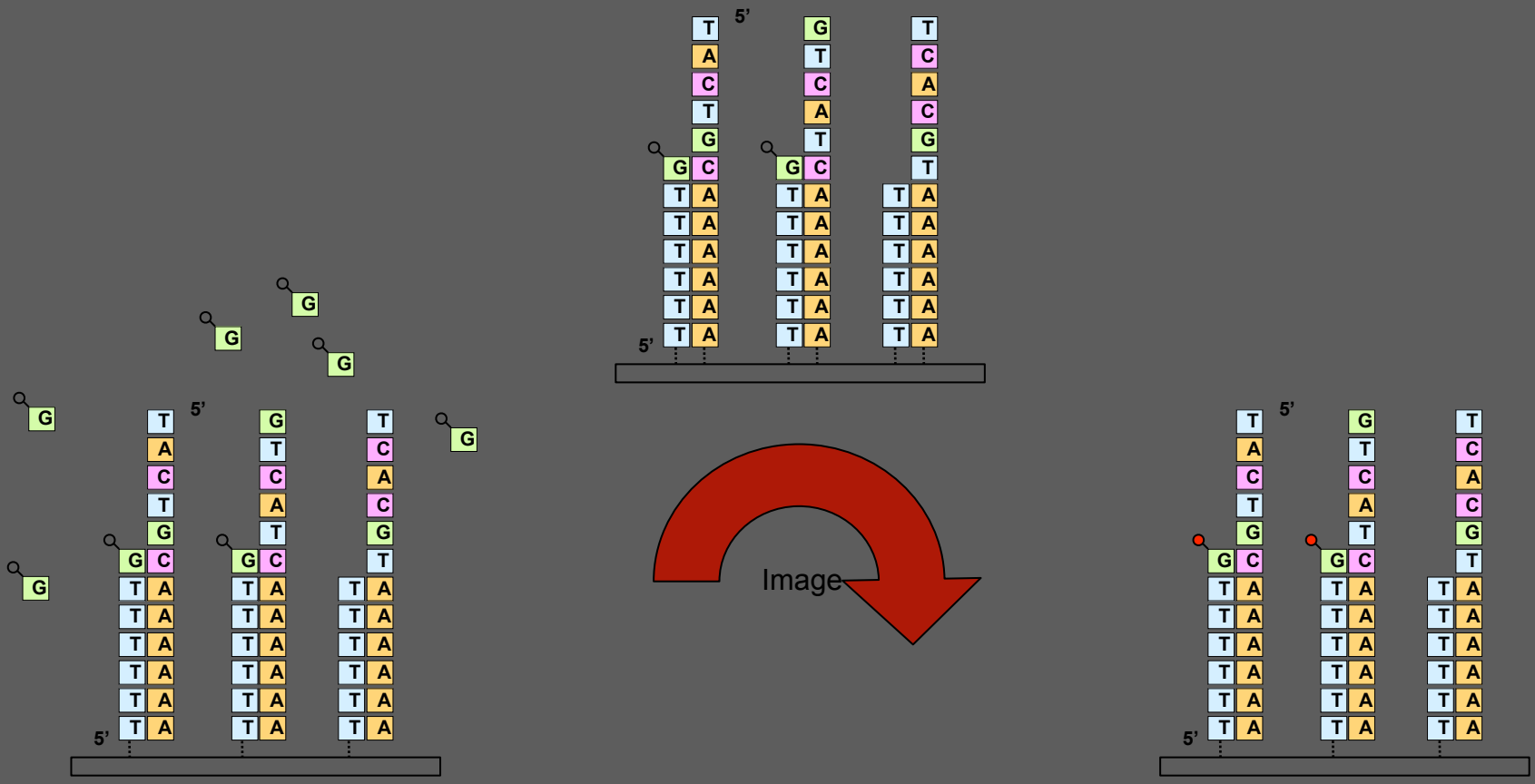
“Moore’s law” of chips and bases



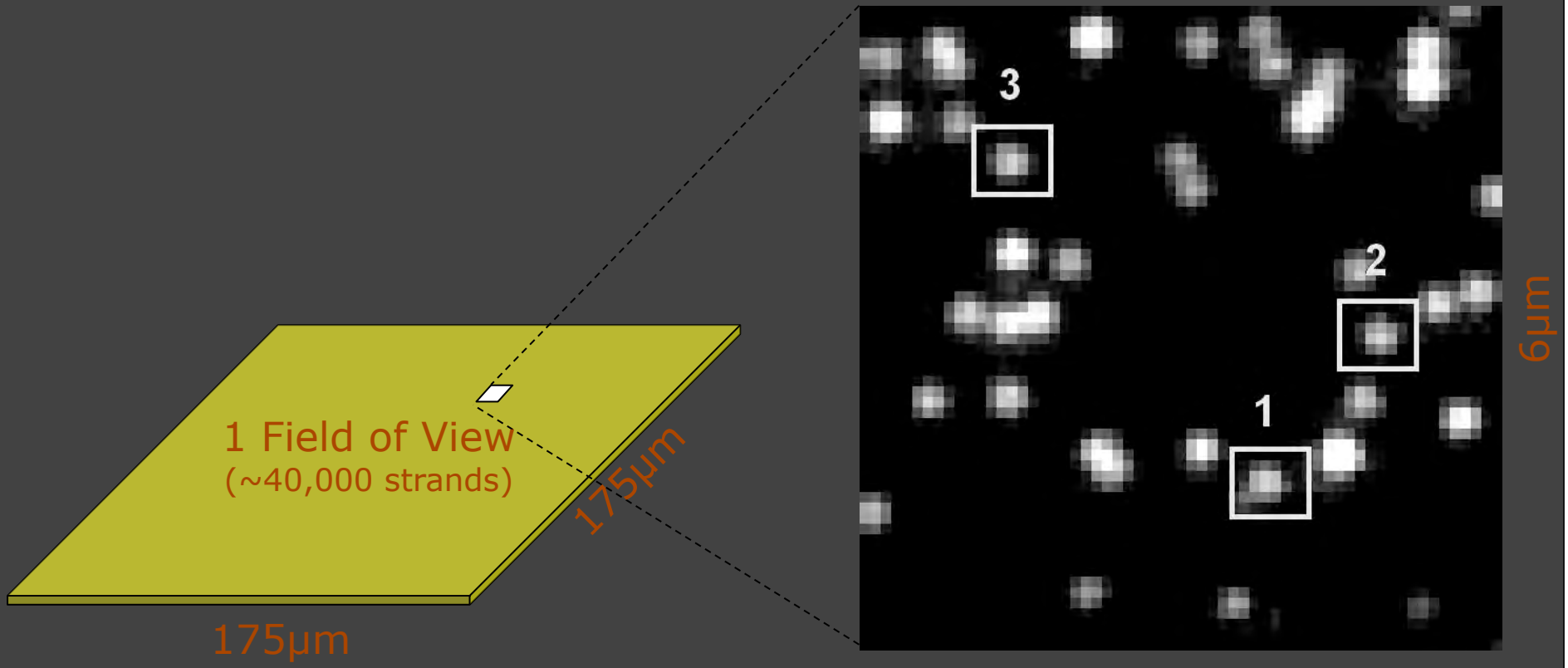
Update: Sequencing now growing at 10x Moore’s law

Next gen sequencing

Sequence by synthesis



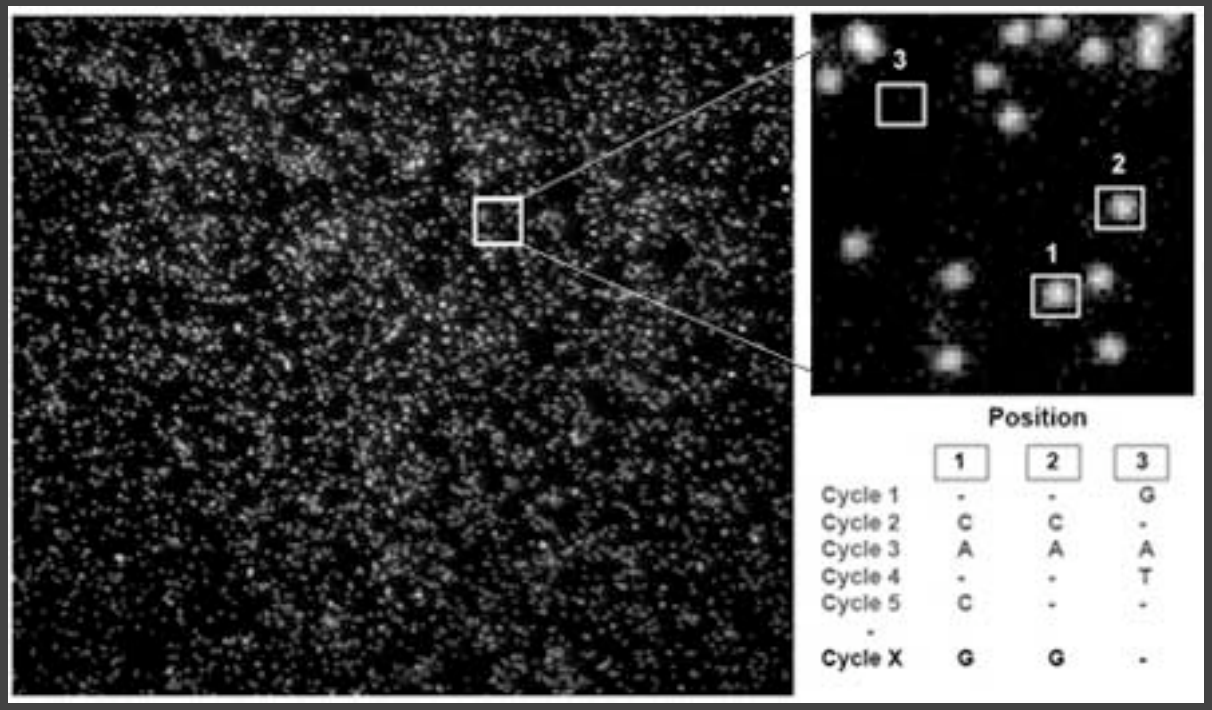
Next gen sequencing



an actual
tSMS image

Next gen sequencing

Imaging of spots before they disappear is key
(and fluorescence labels make that a challenge)



Challenge for sequencing

Based on fluorescence of dye
- 30% - 80% of excitations result in an emitted photon
- 1% - 5% of excitations result in interstate conversion
  - Interstate conversion creates free radicals

Lifetime of a dye: 1000-100000 photons
- Optics collect a minority

Imaging with increased excitation can't help
- Saturation of dye
- Dose of radicals to the specimen

Potential Biomedical Applications

Must move to a new photon budget

"Every emitted photon is sacred"*

So must have better detectors and collection optics

The challenges:

- intrascene dynamic range (1-10000 photons/pixel)
- at least 1usec pixel rate (prefer 10nsec)
- light not collimated (need large area sensor)
  - scattering medium before optics
  - 2cm exit pupil (±30 degree divergence)
  - (need non-imaging collection optics?)
- low dark counts
- low cost, low running costs, low maintenance