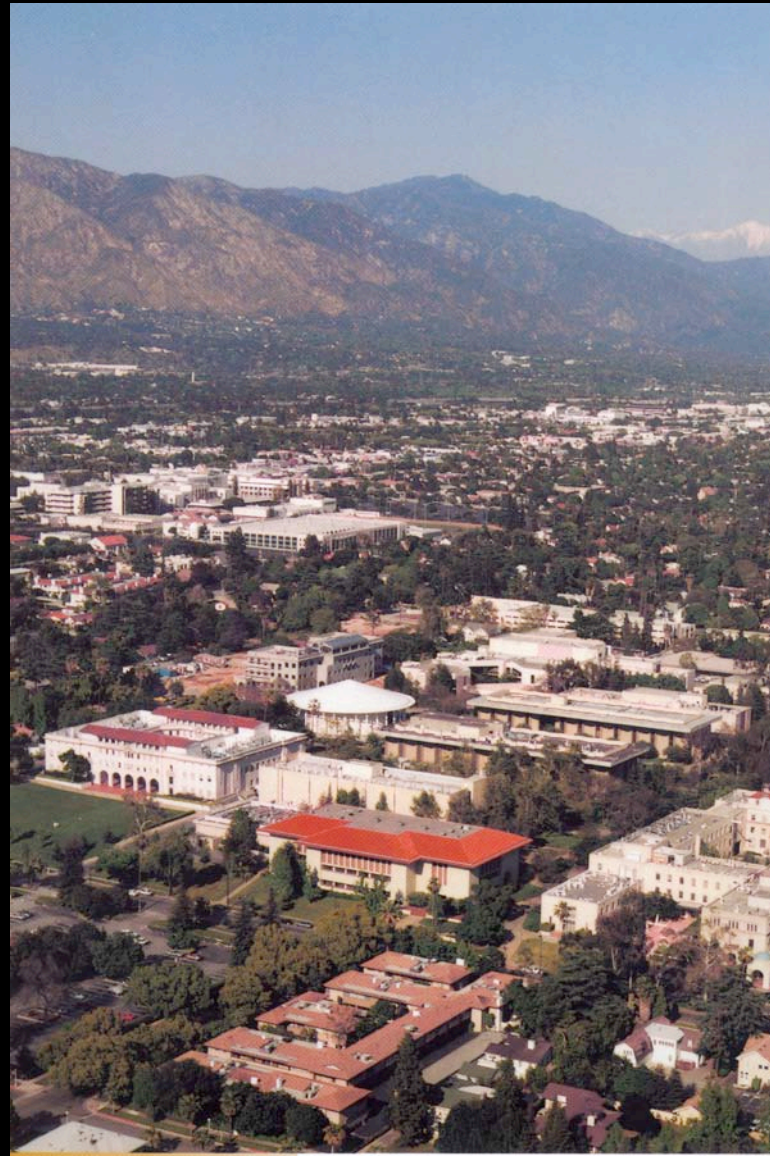


Dynamic Multi-Modal Imaging of Embryogenesis



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Improved Imaging

Embryo Survival

Visualization

Molecular Imaging

Tg Avians

Analysis

Fluorescent proteins



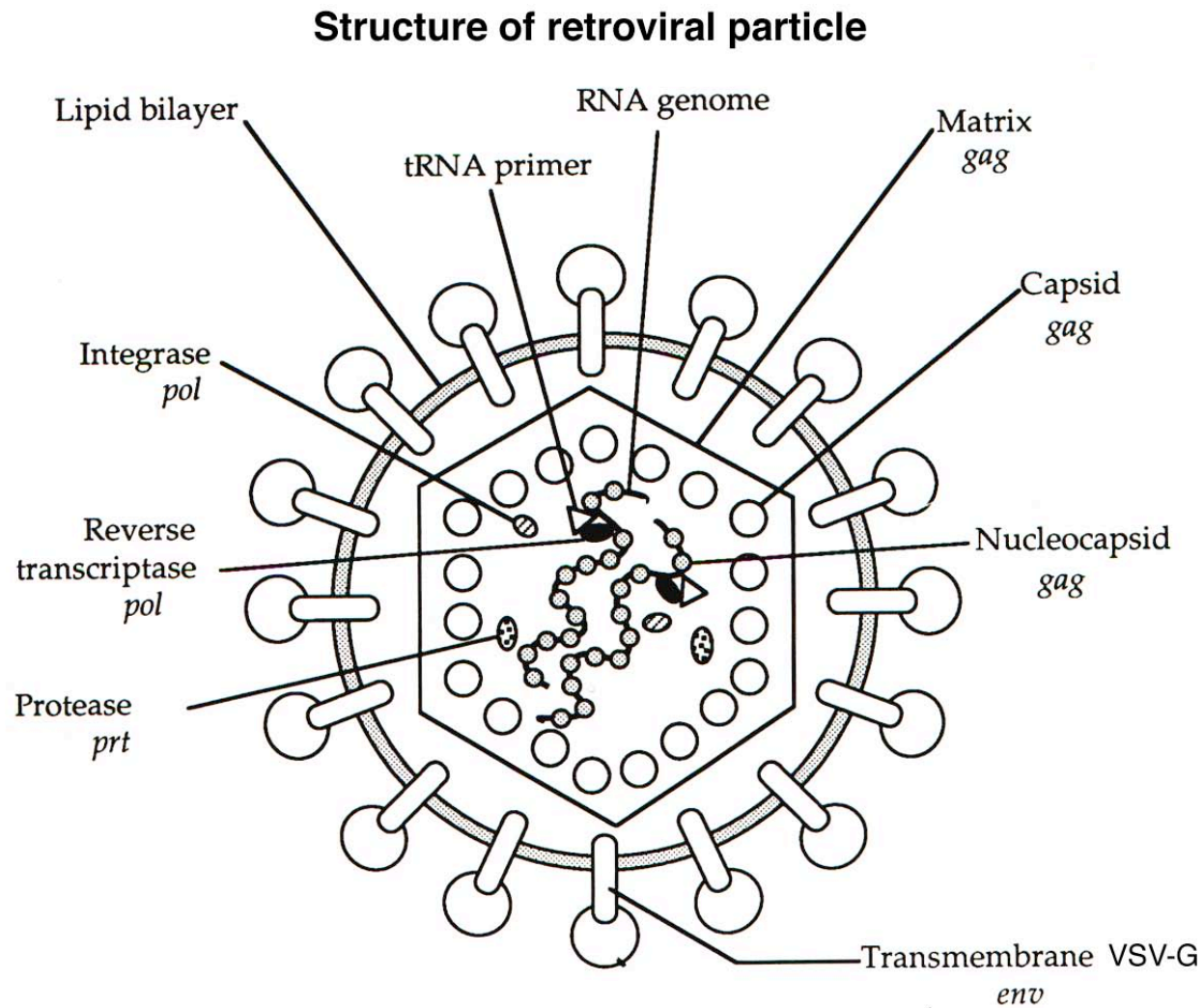
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GFP-expressing Retroviruses

- Ability to label most cells
- Fluorescent marker is not diluted by cell division
- Resistant to photo-bleaching

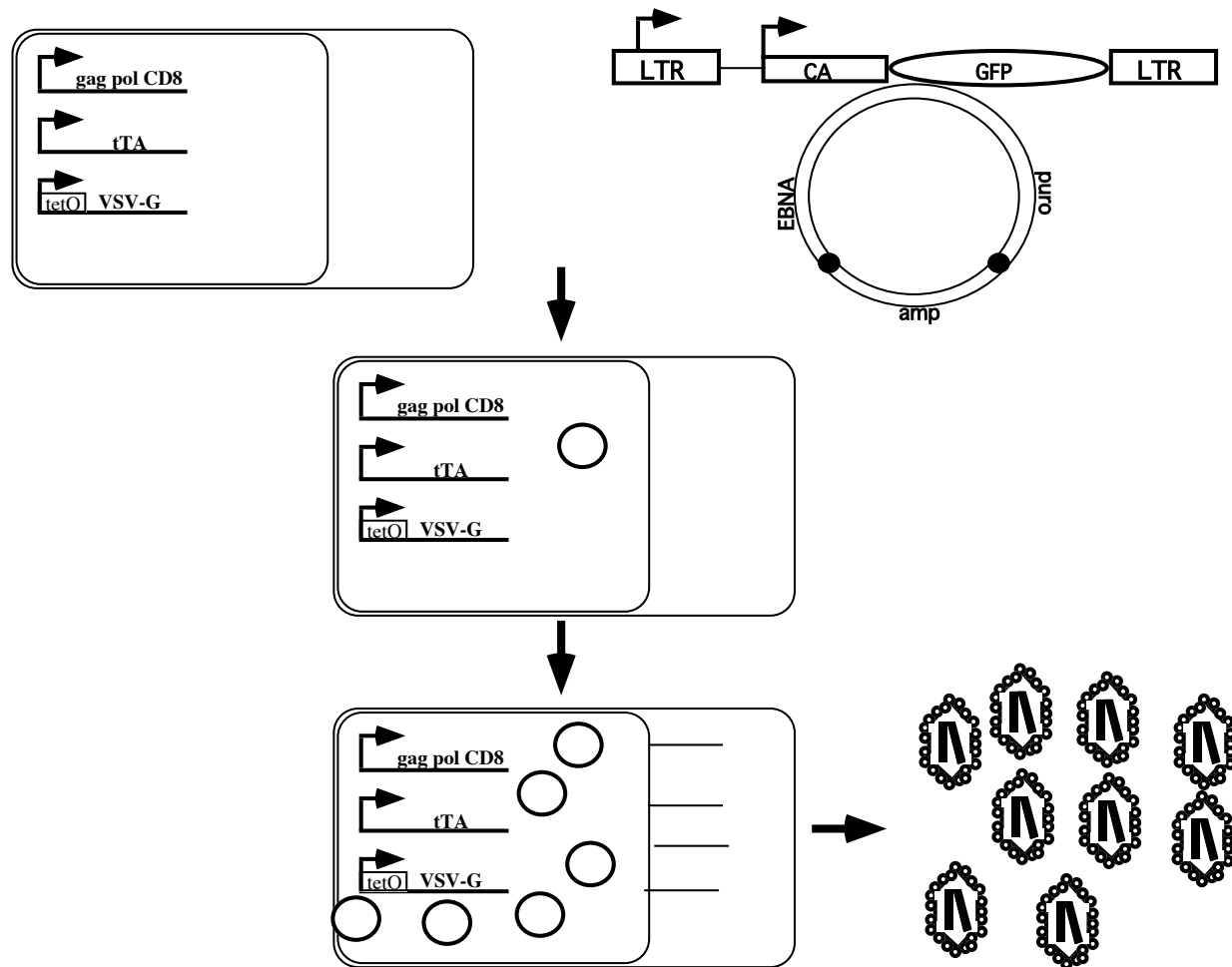
Structure of Retroviral Particle



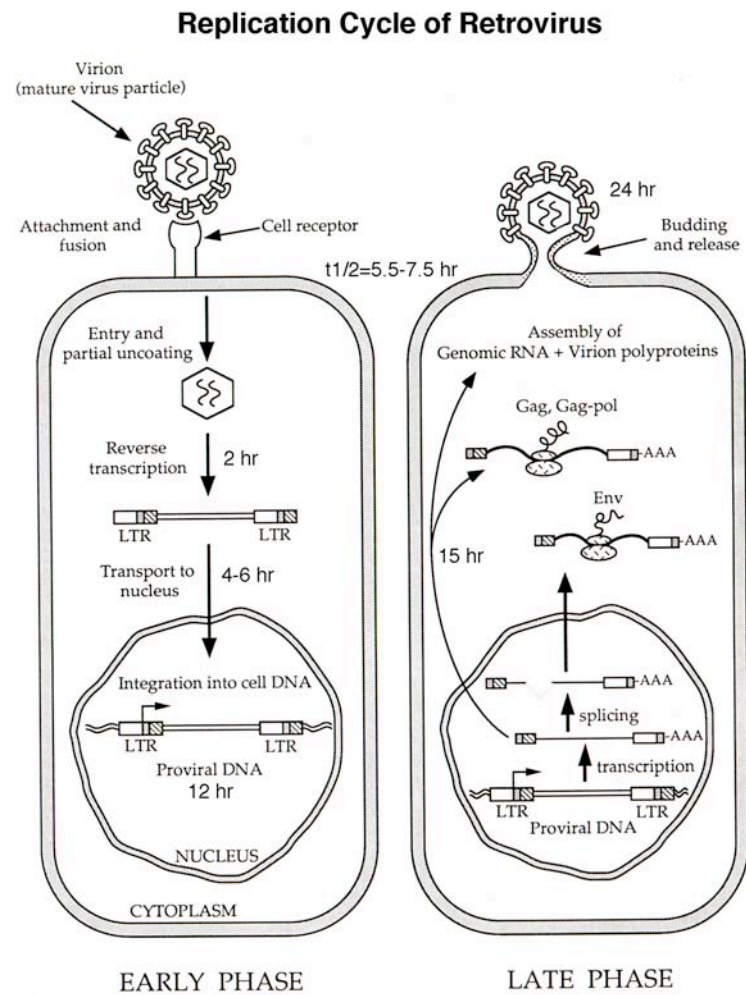
(copied from The Retroviridae, 1992)

Generating Pseudotyped Retrovirus

Making replication incompetent LZRS-based retroviral vectors
in Phoenix packaging lines

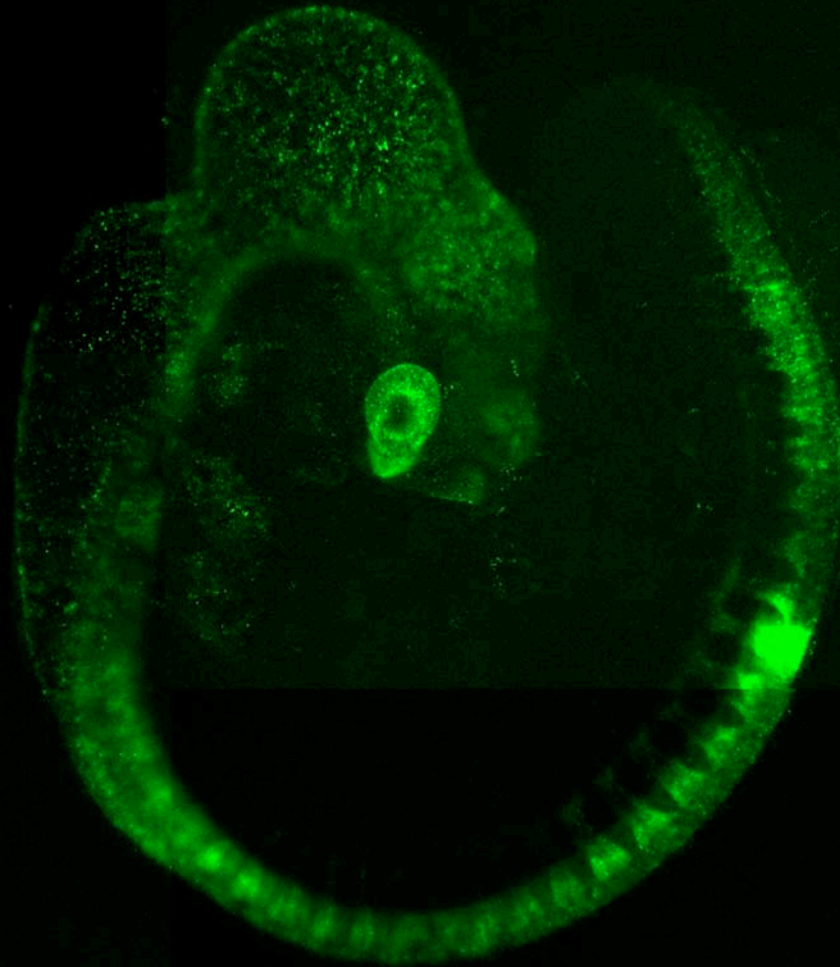


Replication Cycle of Retroviruses



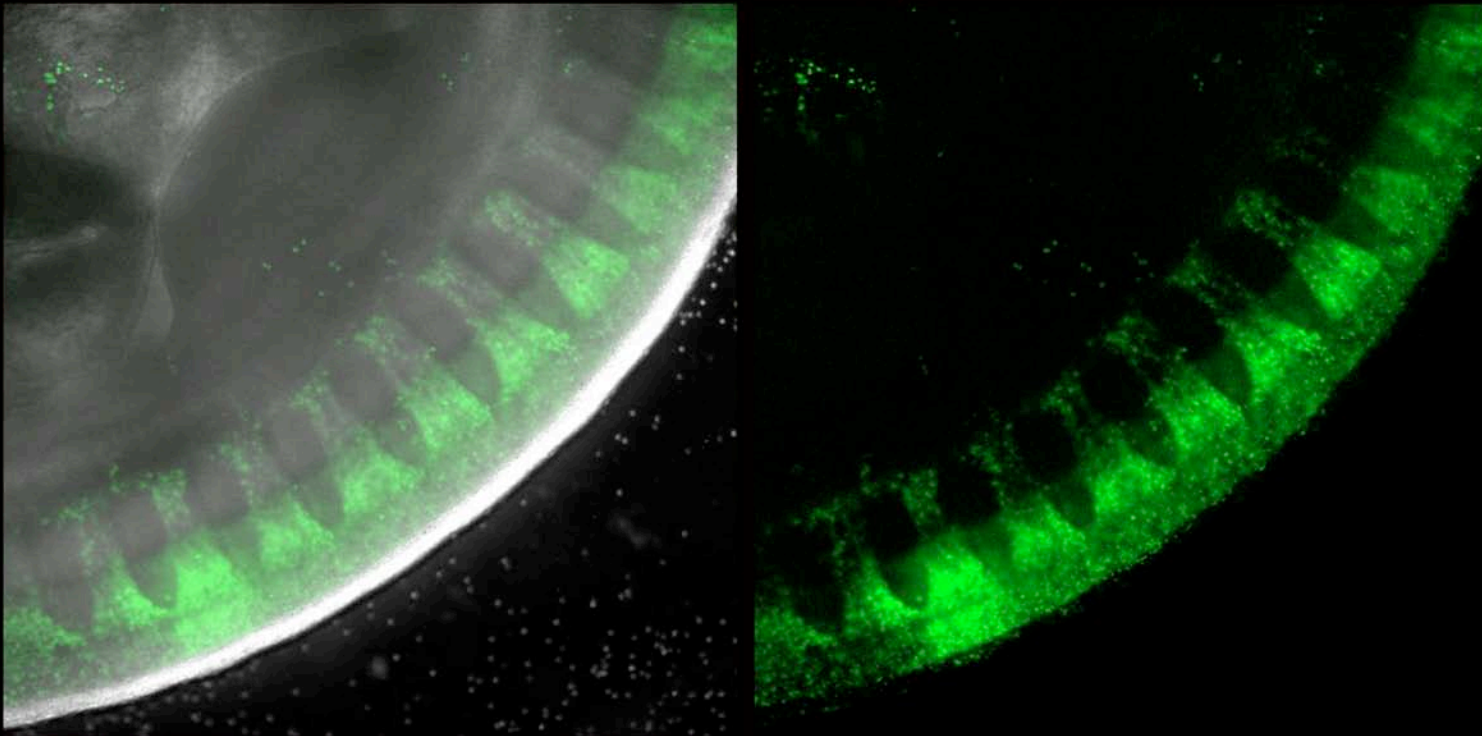
(adapted from The Retroviridae, 1992)

Infection of Chick Embryo with GFP-expressing Retroviruses

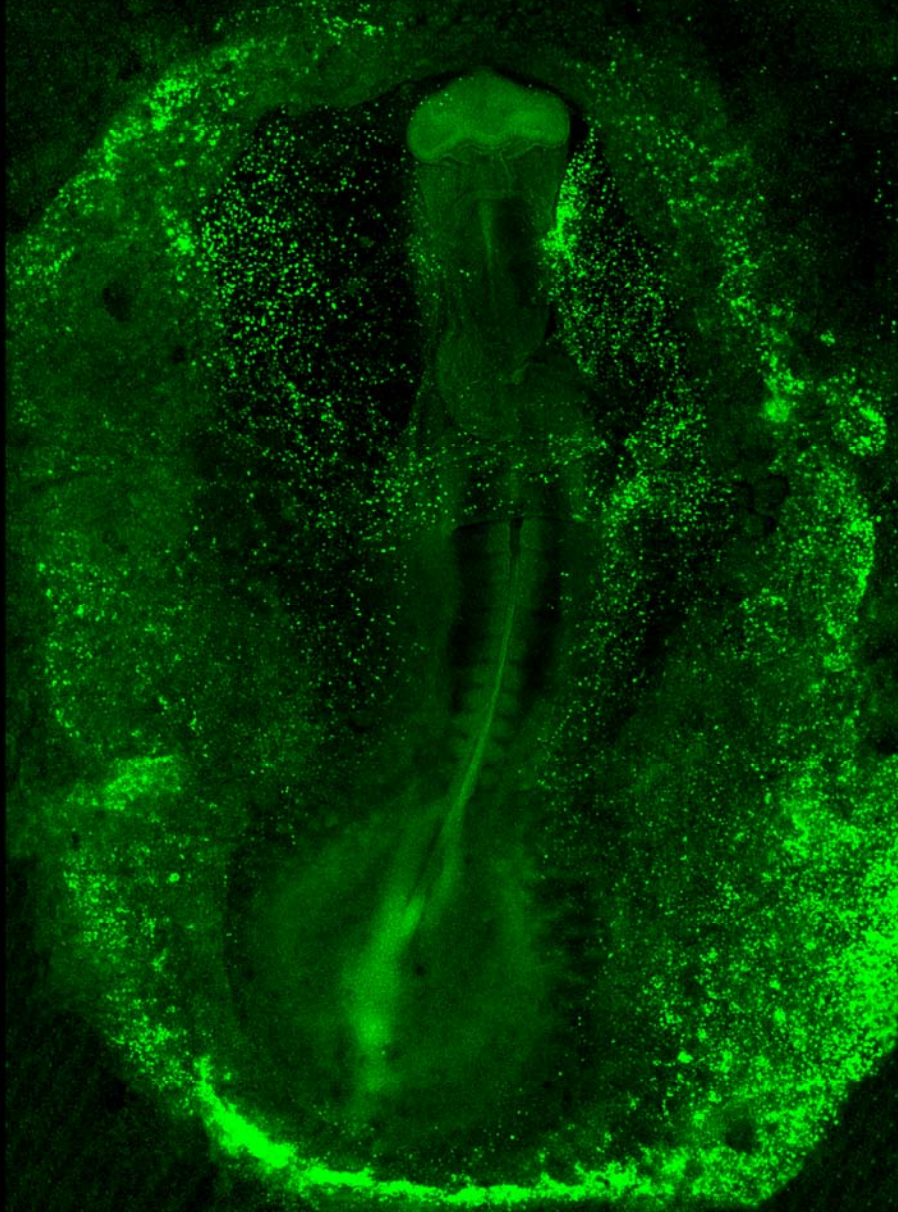


Chick infected with GFP expressing retrovirus
Imaged on Zeiss 410, 25X

Infected cells do not exhibit altered phenotypes

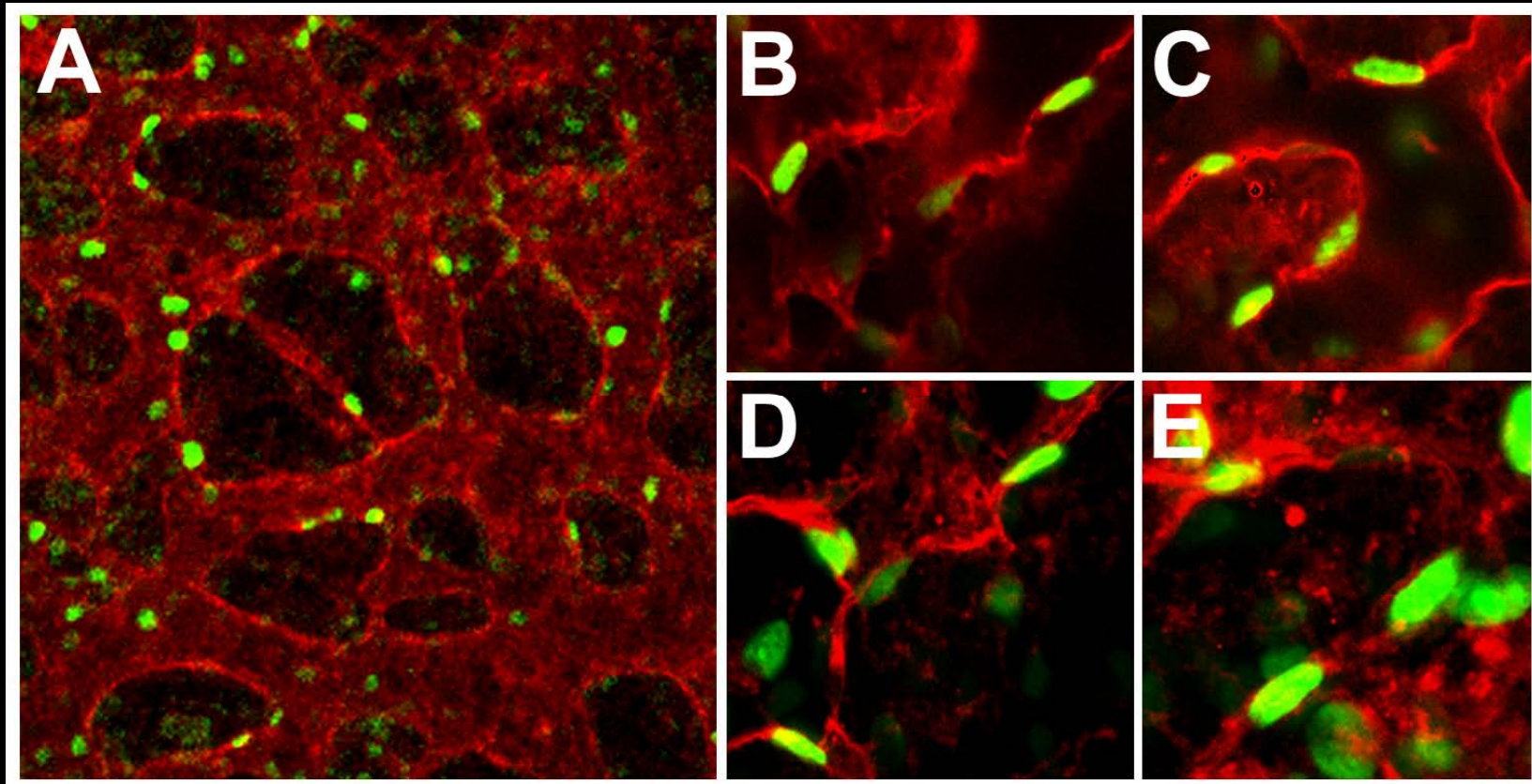


Chick NC cells infected with GFP expressing retrovirus

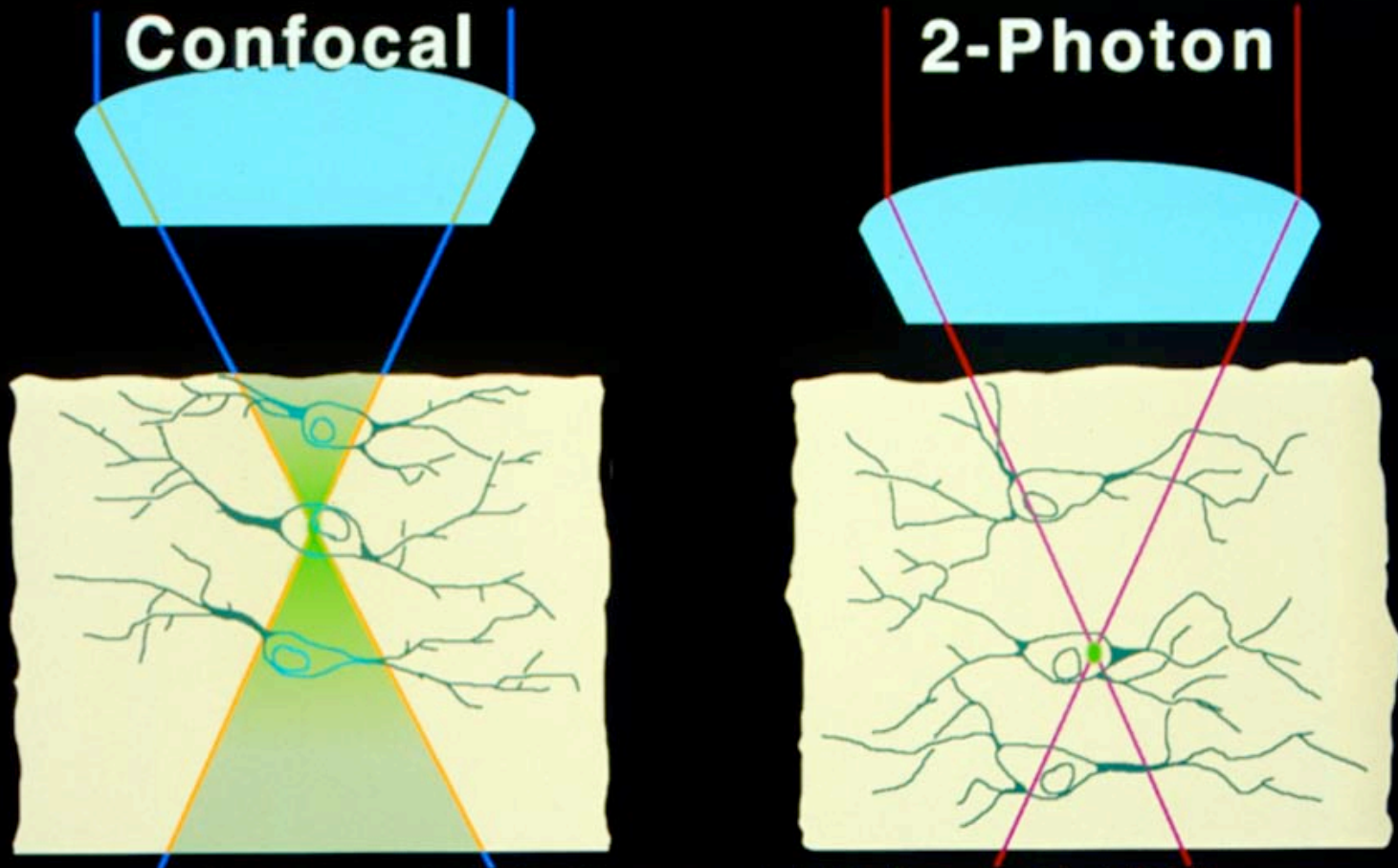


**Extensive YFP
expression is observed in
blood vessels 12hrs after
injection of H2B-YFP
into the blood islands of
a 4 somite quail embryo**

Blood island-derived cells infected with the H2B-YFP expressing retrovirus incorporate into quail intraembryonic vasculature. Panel A shows intraembryonic vessels of a 12 somite quail embryo 12 hours after injection of the blood islands with the H2B-YFP expressing retrovirus. Numerous green YFP⁺ cells are evident in the red, QH1 labeled blood vessels. Panels B-E are 40X magnification images of YFP⁺ incorporated into the vascular endothelium. Bar=50 μ m.

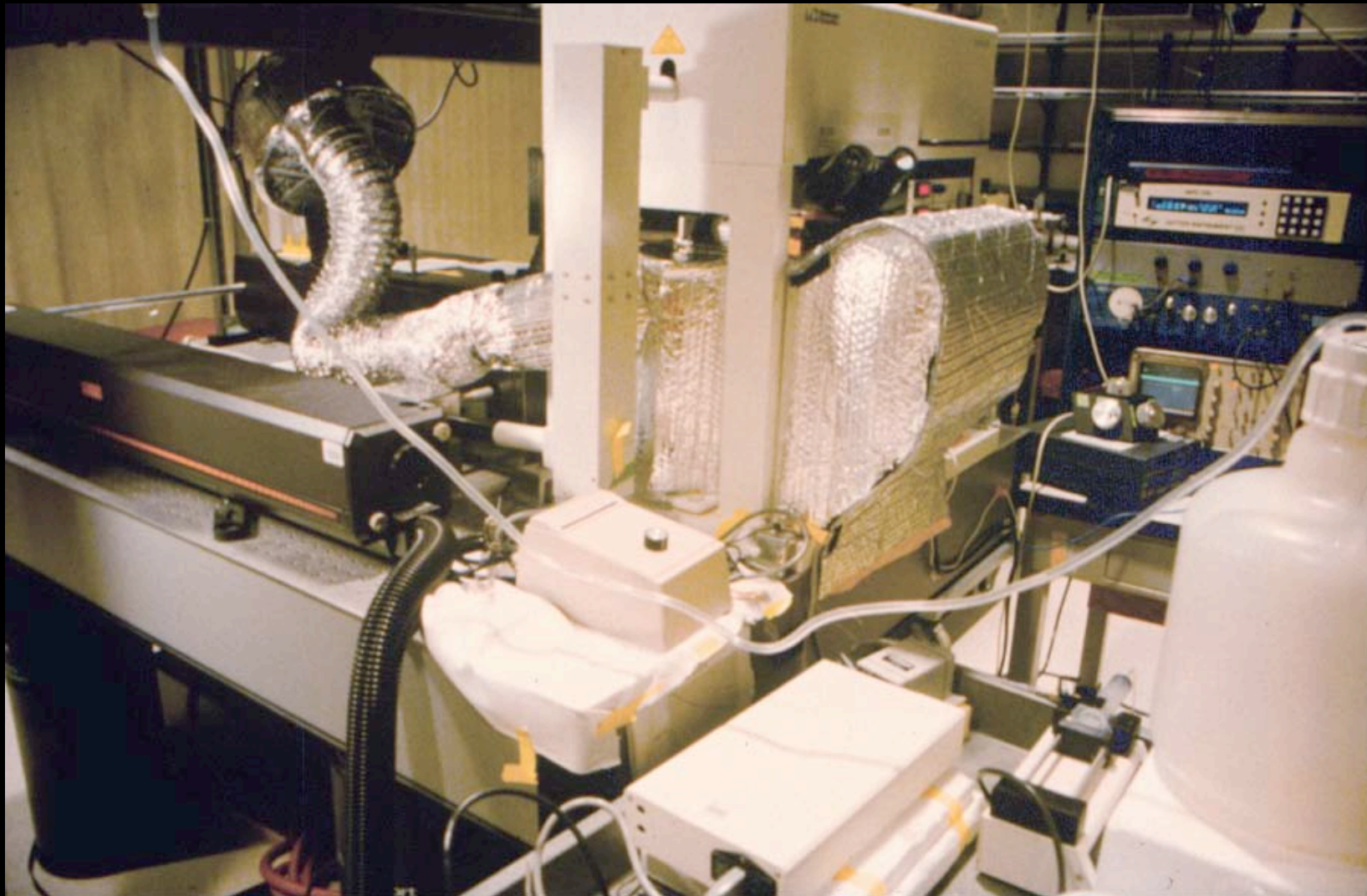


Laser Illumination Patterns



from **Potter, 1996 Current Biology 6:1595**

Classic TPLSM

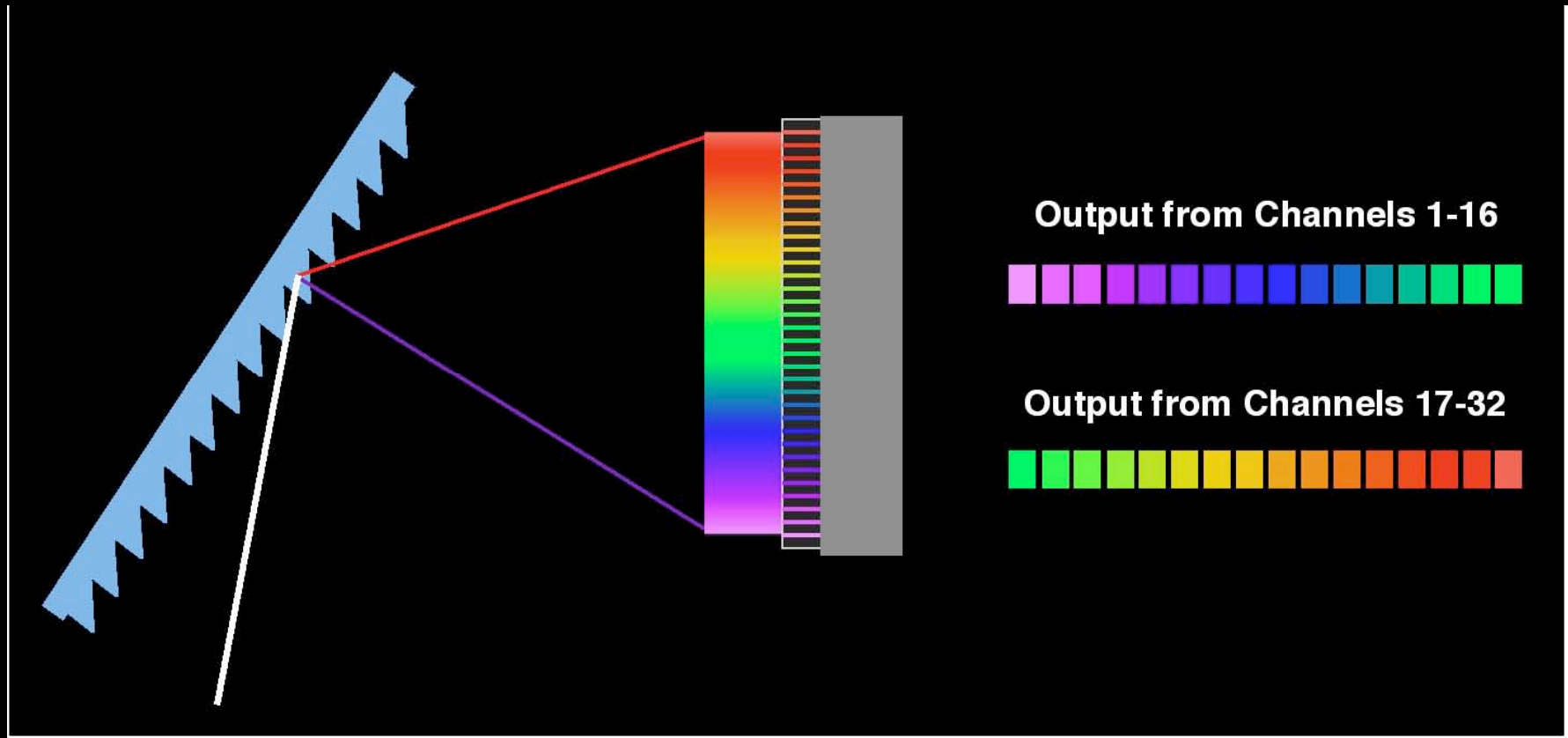


Spectral imaging using Zeiss 510 NLO fiber-coupled system

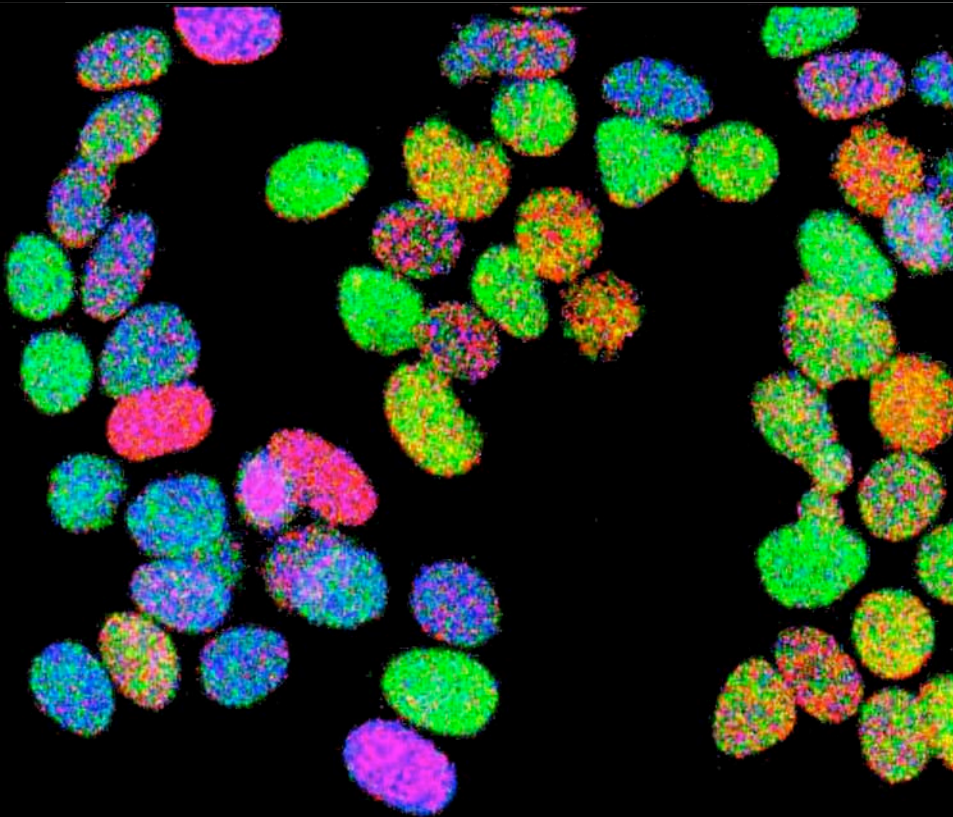


Using this system,
we have tested
the first prototype of
the spectral imager
(SPI) using both
single-photon and
multi-photon excitation
on a variety of
different samples.

Grating light dispersion



Linear unmixing algorithms can be used to identify overlapping CFP, GFP and YFP expression



CFP = blue
GFP = green
YFP = red

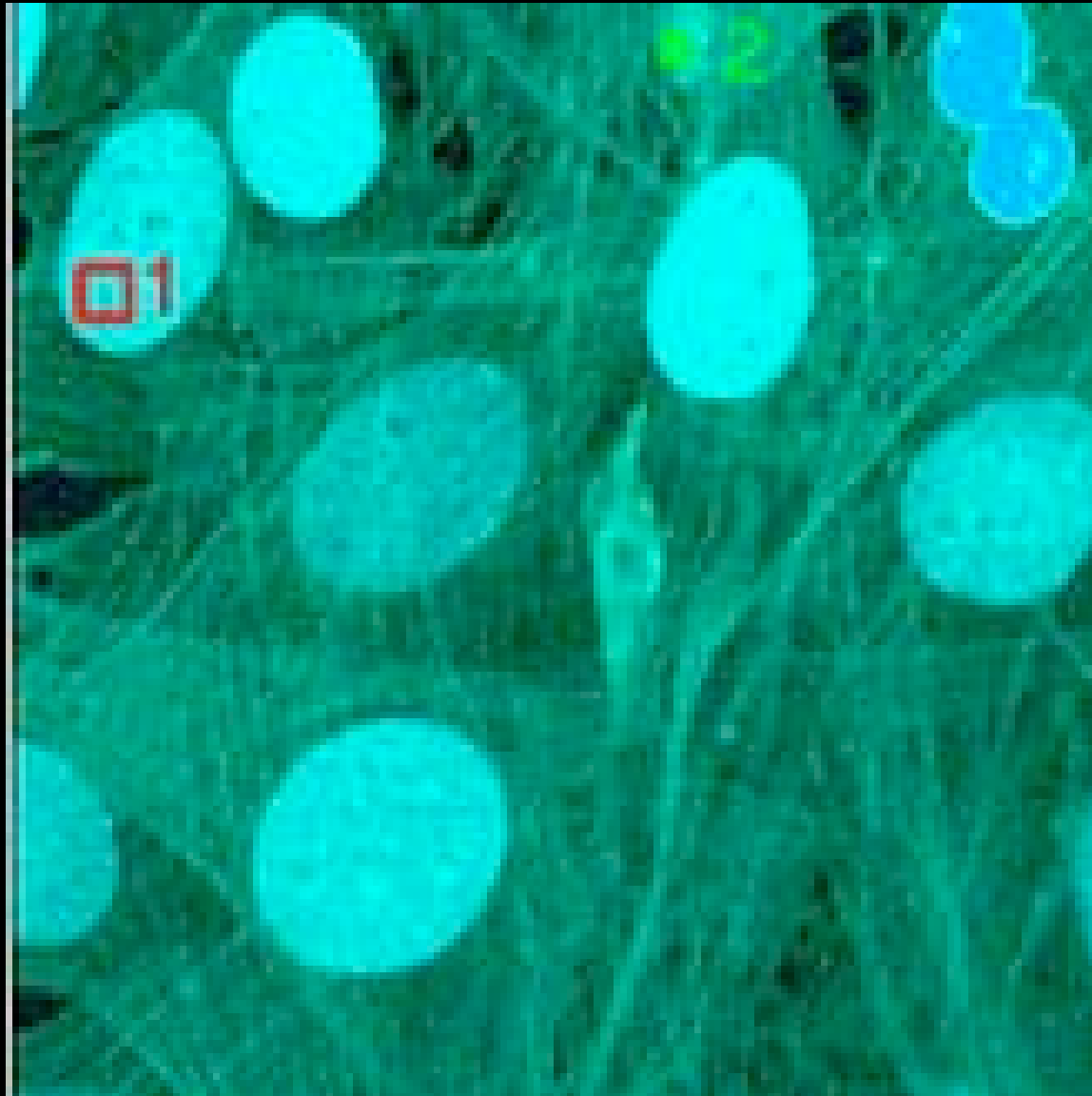
Nuclei of cells
infected with CFP, YFP
and GFP-retroviruses

(Lansford, Bearman and Fraser, (2001) JBO 6,311.)

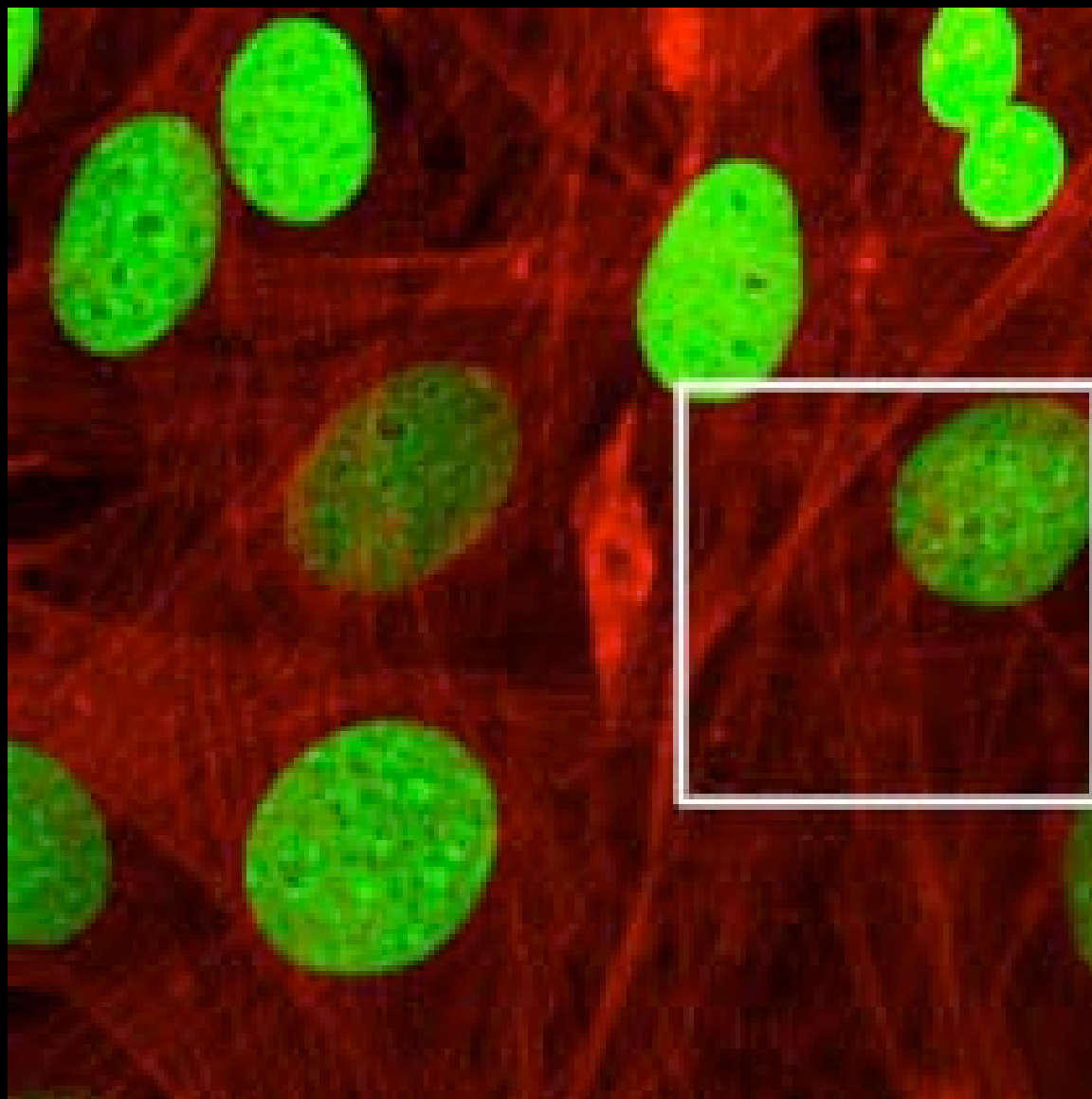


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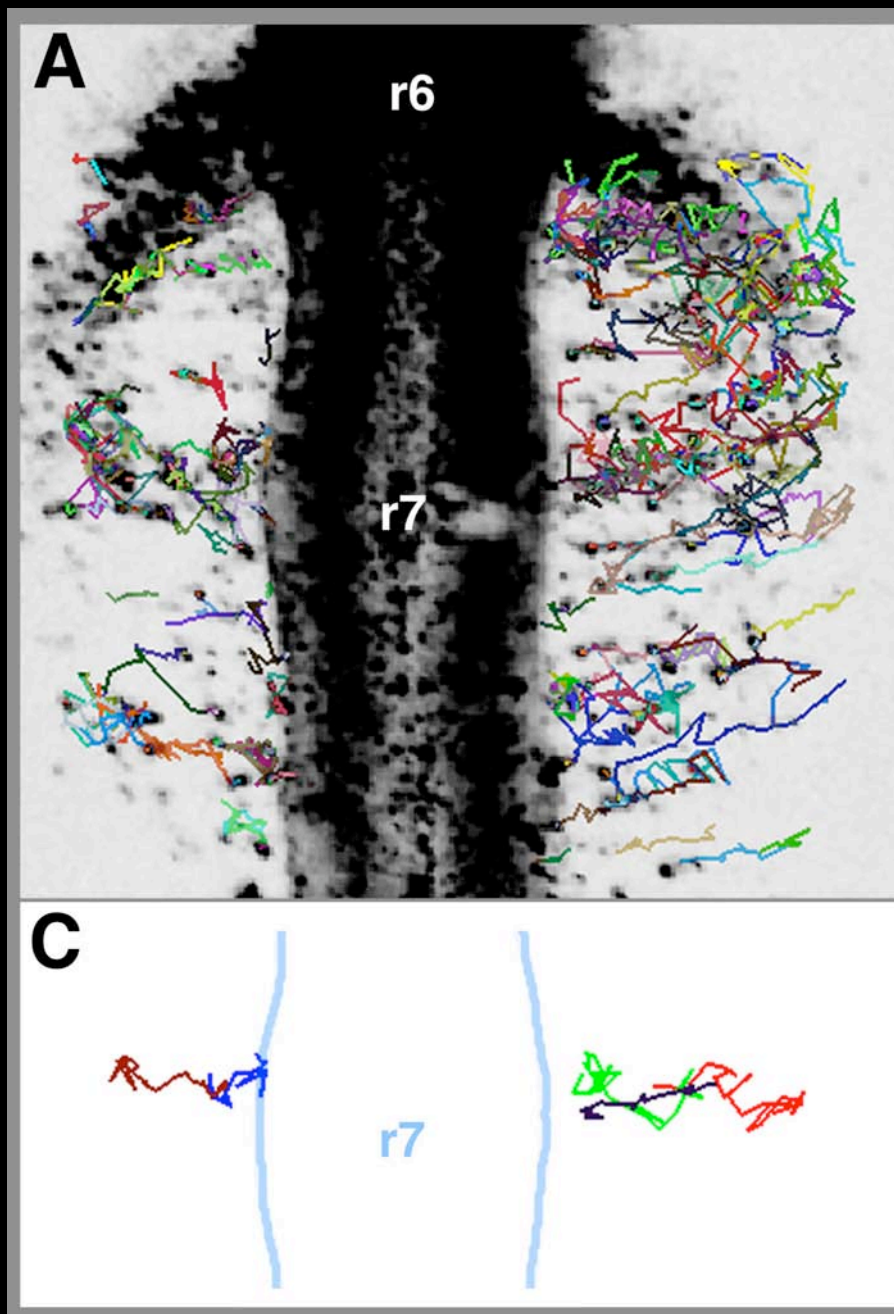
Unfiltered nuclear GFP and cytoplasmic fluorescein



Same image after linear unmixing!



XVTrack of hindbrain NC



Quail Developmental Atlas

- Anatomical atlas for quail
 - Computer and book access
- Integrate cell migration data and gene expression patterns into 3D embryo
- Develop Virtual Laboratory

Computational Biological Imaging

Establish a virtual environment for:

- collecting and storing images
- connecting images semantically
- visualizing correlations among anatomical and gene expression images
- determining the pattern similarities/dissimilarities between expressed gene
- the controlled sharing of images

BIC MRI



Biological Imaging Center Beckman Institute, Caltech

$B_0 = 11.7\text{T}$ (500MHz ^1H)

Magnetic field gradients $\sim 100\text{gauss/cm}$

low noise pre-amps

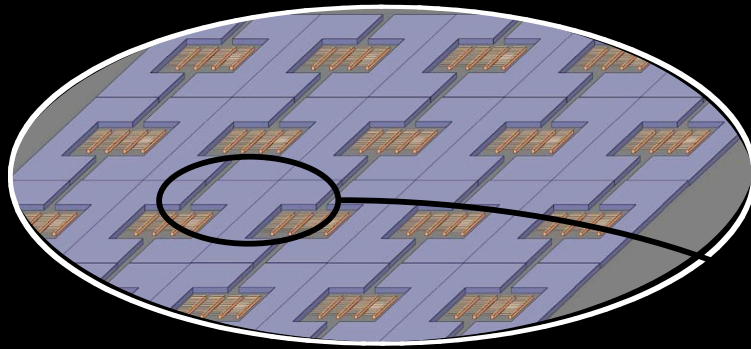
customize RF circuits for specimen of interest

specimen size $< 25\text{mm}$ (mice or less)

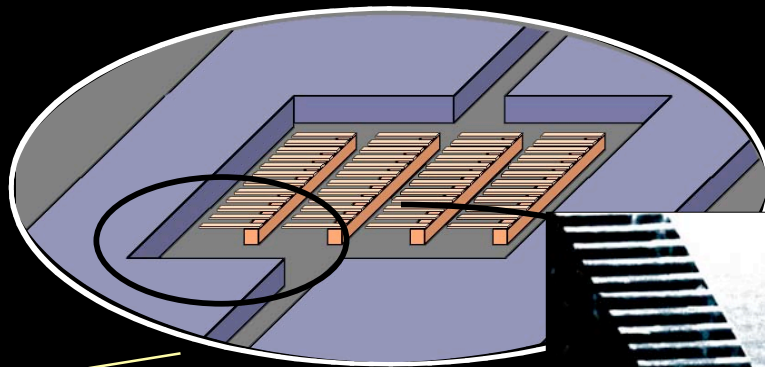
3D volume images at $\sim 25\mu\text{m}$

Five MRI movies deleted for
print version of presentation

Experimental Advances:

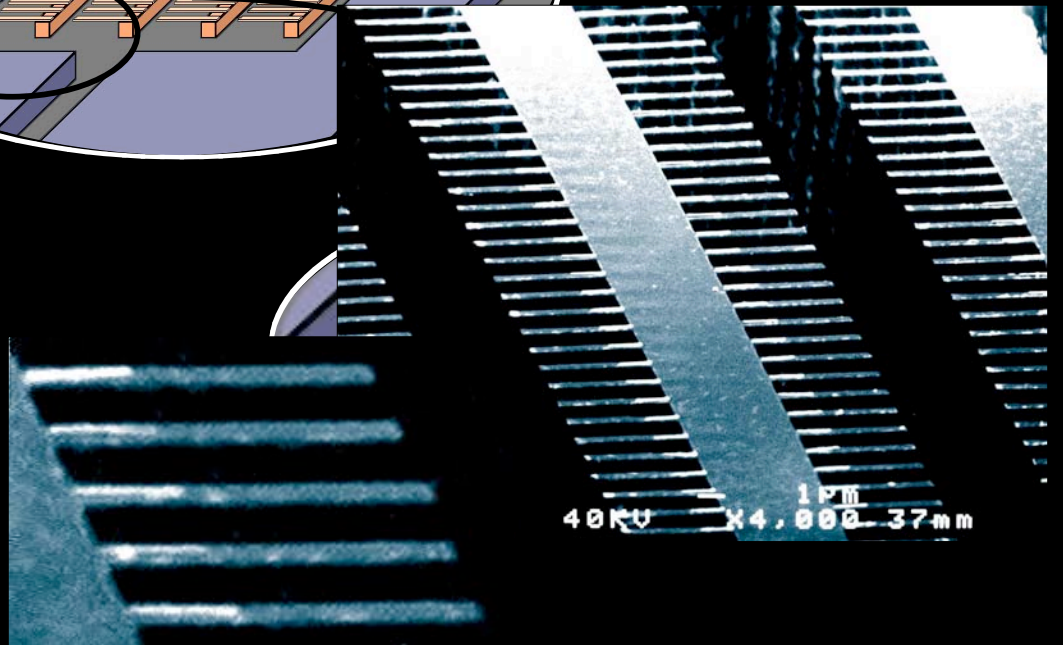


concept: NEMS bioarray with microfluidic analyte delivery



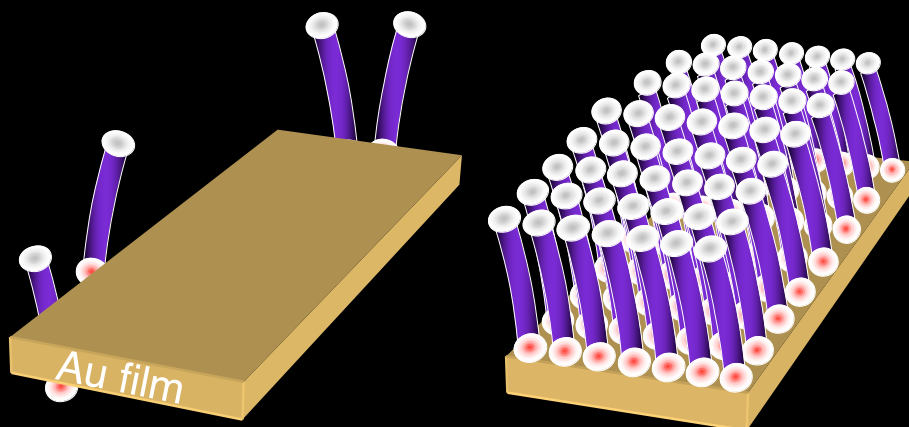
microfluidic flow channel

individual biofunctionalized NEMS element

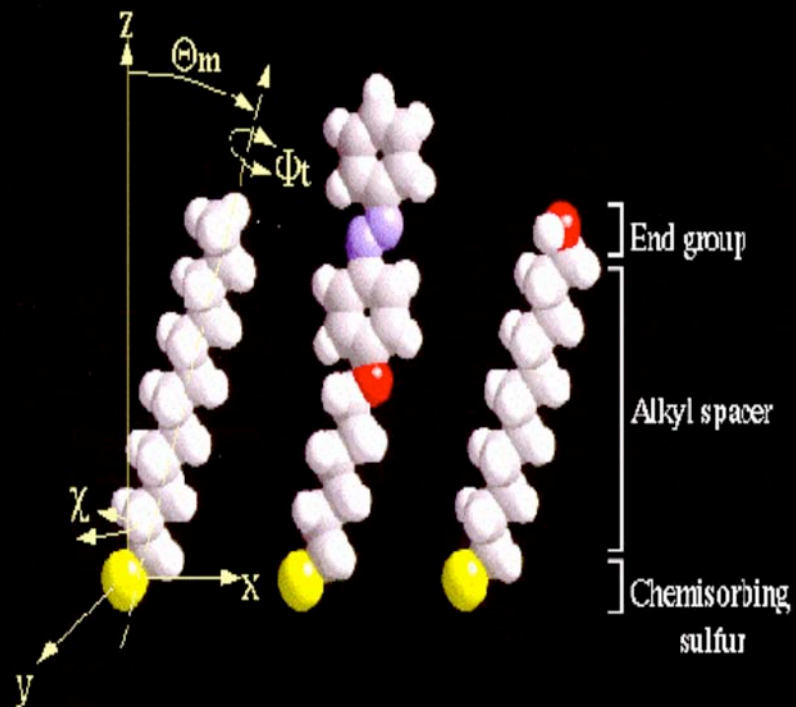


Experimental Advances:

Schematic for SAMs on Au

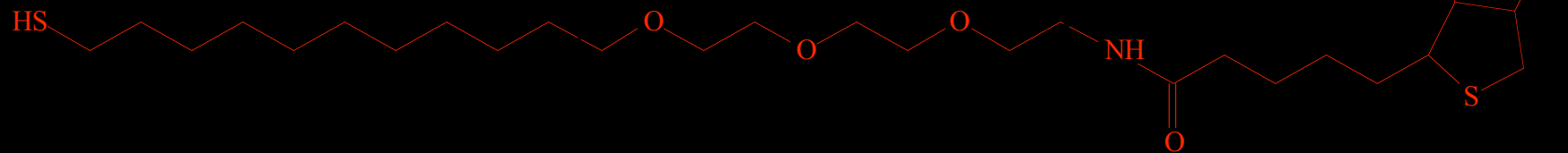
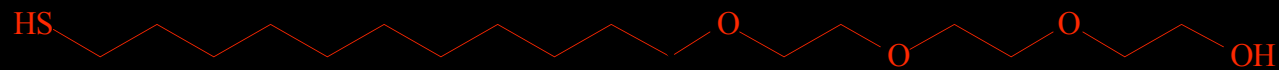
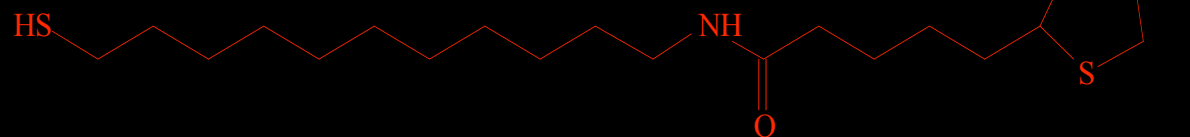
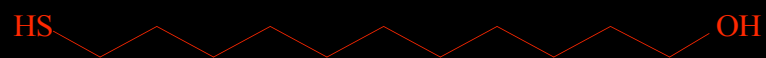


Our biofunctionalization effort centers upon use of SAMs for both uniform passivation (to obviate non-specific protein binding) and for local selective local functionalization for specific biological targets.



Experimental Advances:

Target Alkanethiols for SAMs



Experimental Advances: Biofunctionalization

Initial "Microfunctionalization" Results with SAMs



Biofunctionalization of Au with self-assembled monolayers

Au:Si pads were reacted with Neutra-avidin-Cy3

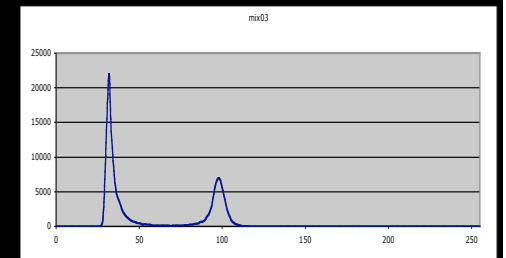
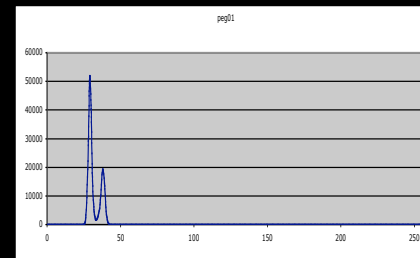
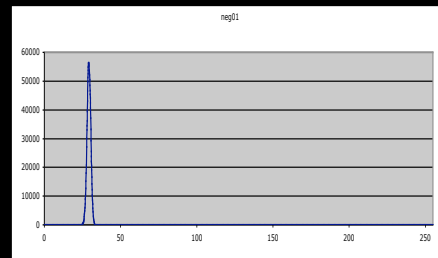
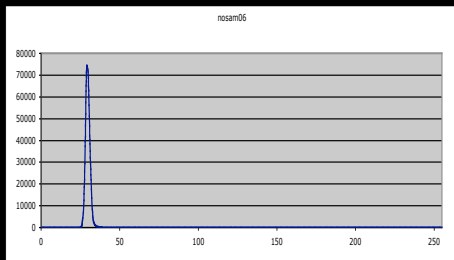
Three images of SAMs on Au--1. MeOH only negative control, 2. HS-C11-OH negative control, 3. HS-C11-OH and HS-C11-biotin mixed monolayers showing binding specificity to Au and not Si. Neutraavidin-Cy3 used for binding study.

Note that you can faintly see CIT in the two negative controls.

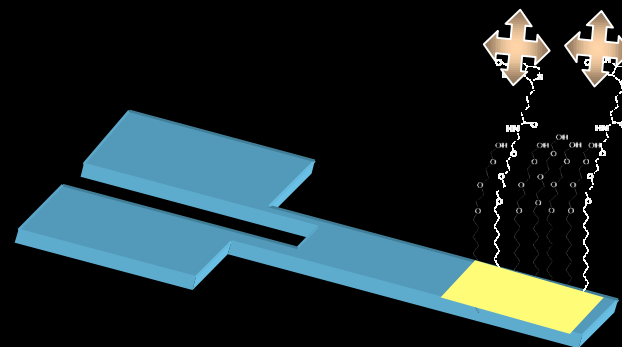
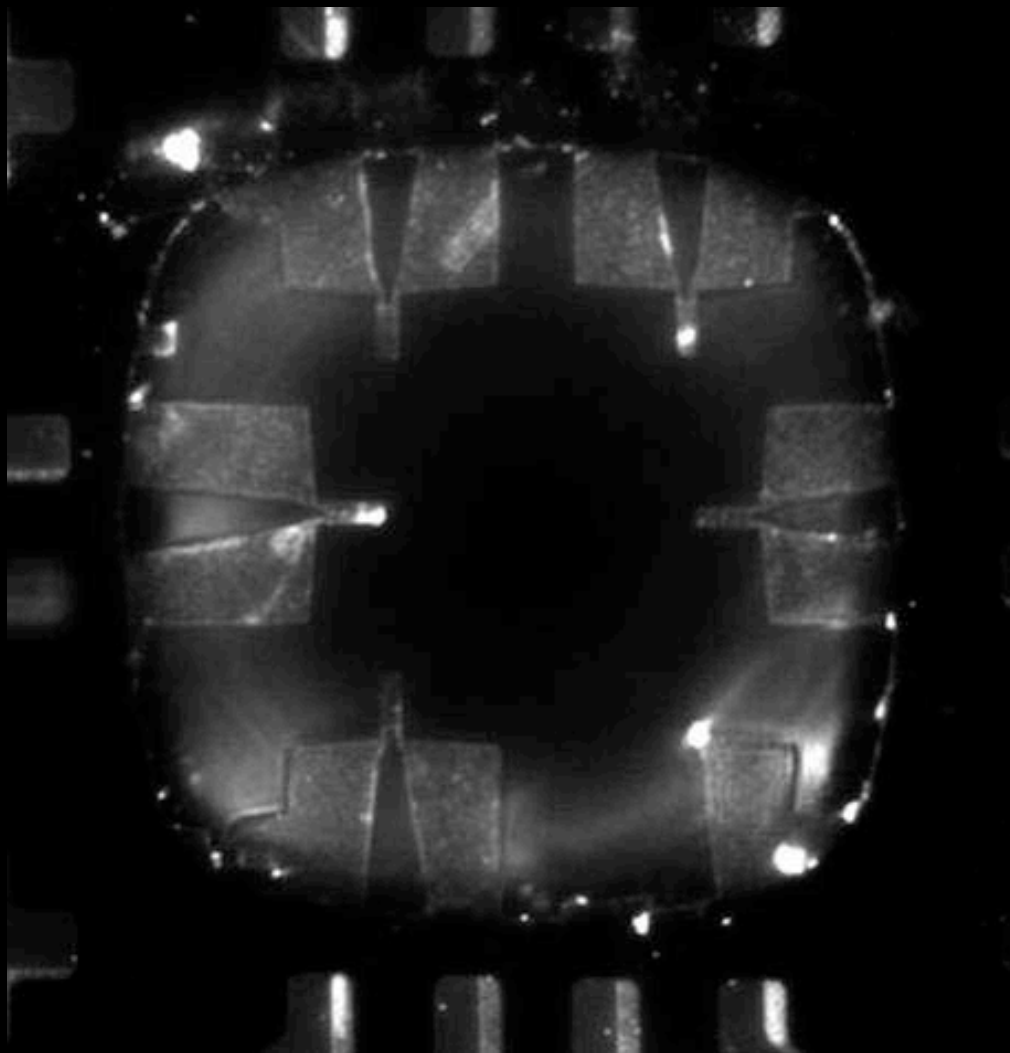
Functionalized Au

Incubated with QD-streptavidin

(-) (-) C11-PEG C11-biotin



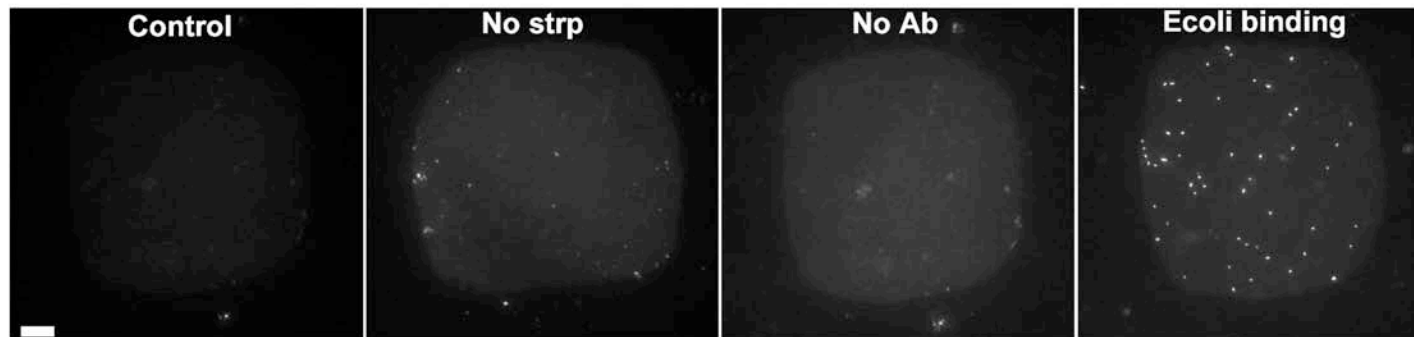
Successful biofunctionalization on chip: fluorescence characterization



Cy3-coated Streptavidin
fluorescence reveals
presence of Biotin on Au pad

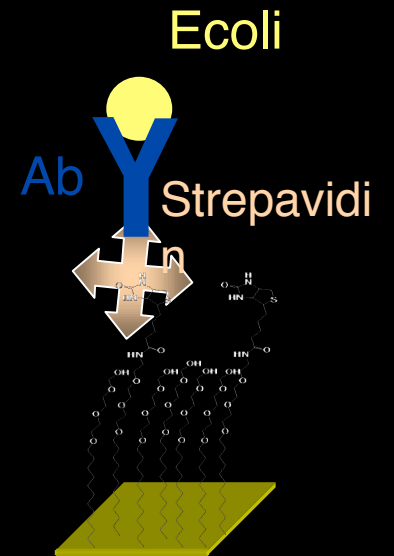
Specific binding with bacteria antibody

Specific binding w/ anti-*E.coli* Antibody onto Biotinylated SAMS



1. C11-PEG/C11-PEG-bt on Au
2. Attach strp layer/wash
3. Separately bind anti-*E.coli* to *E.coli*/wash
4. Inc #3 w #2/wash; stain w Syto11/ image x400 on Axio2.

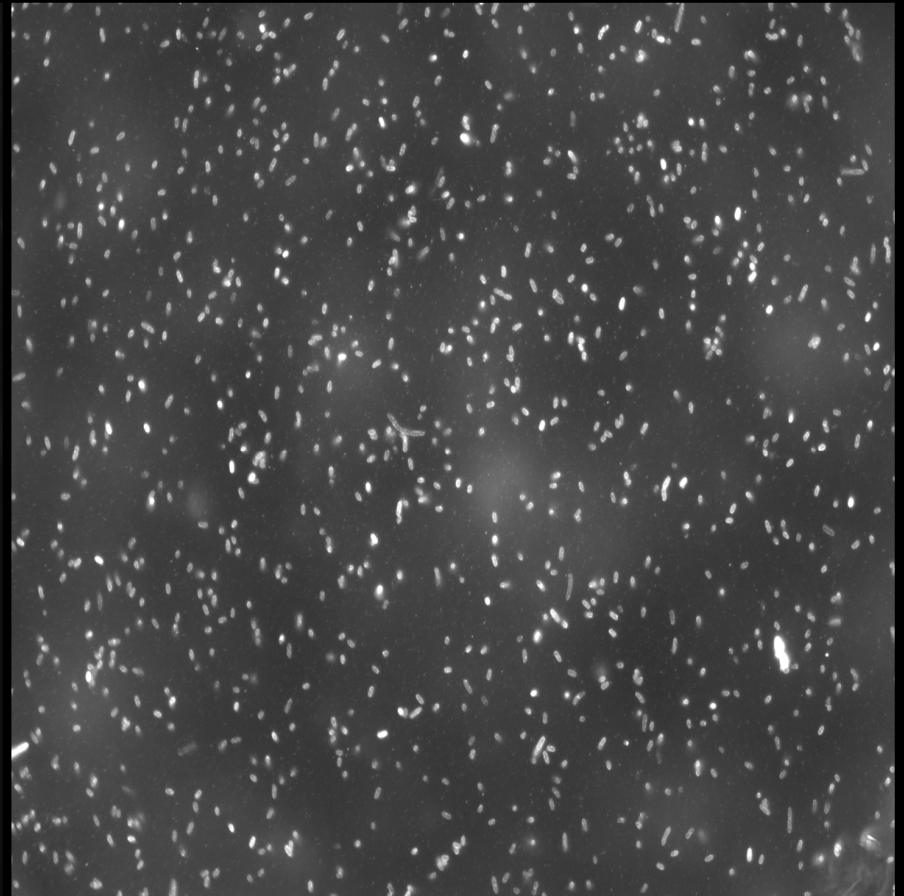
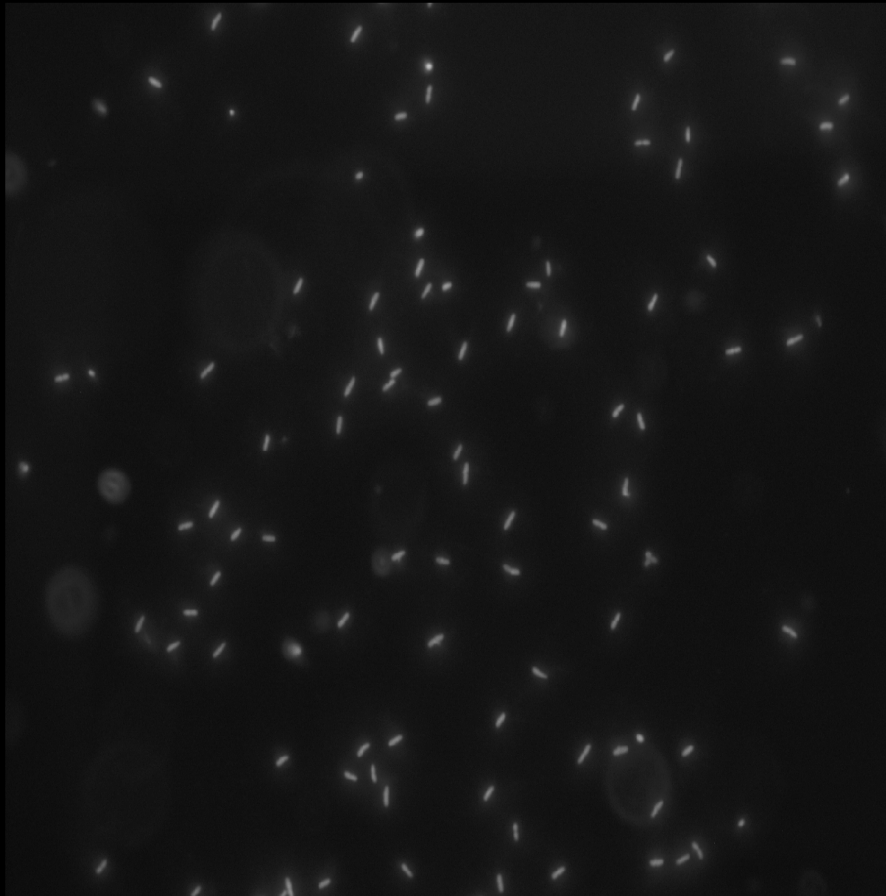
Scale 20 um



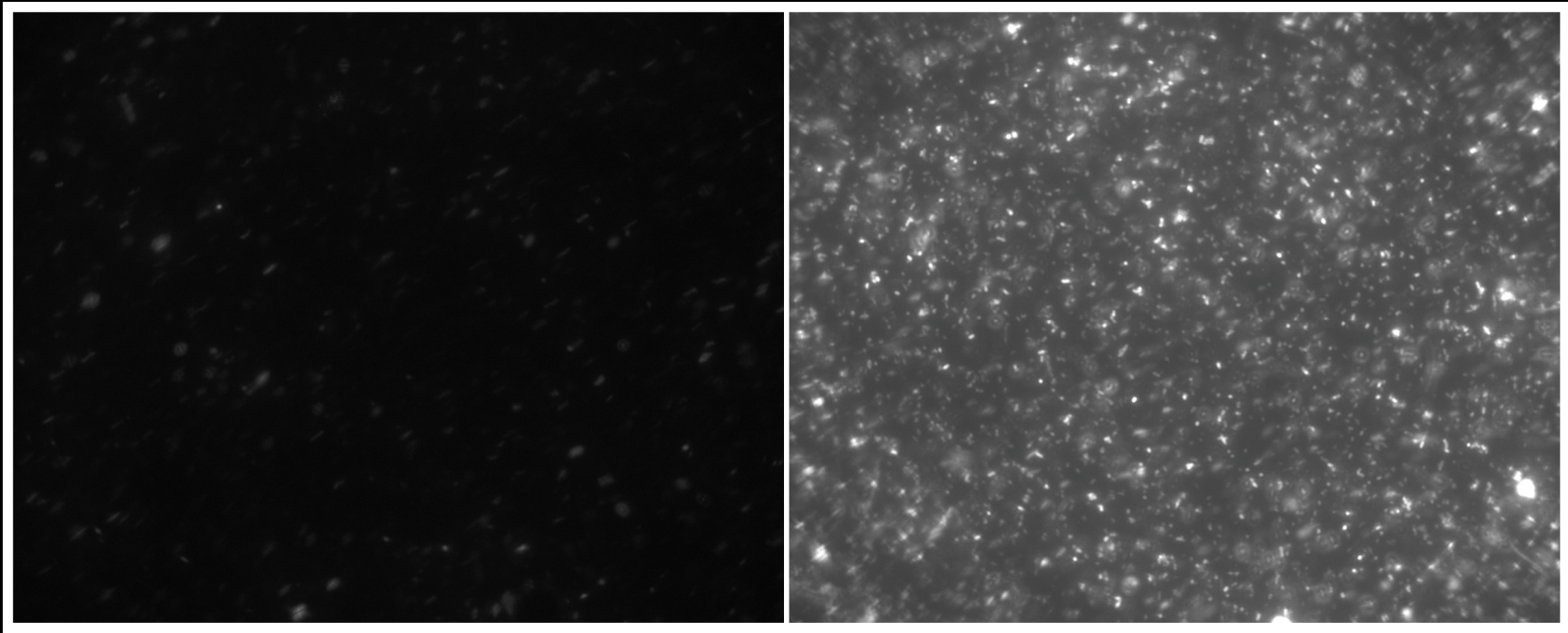
E.coli

EtBr

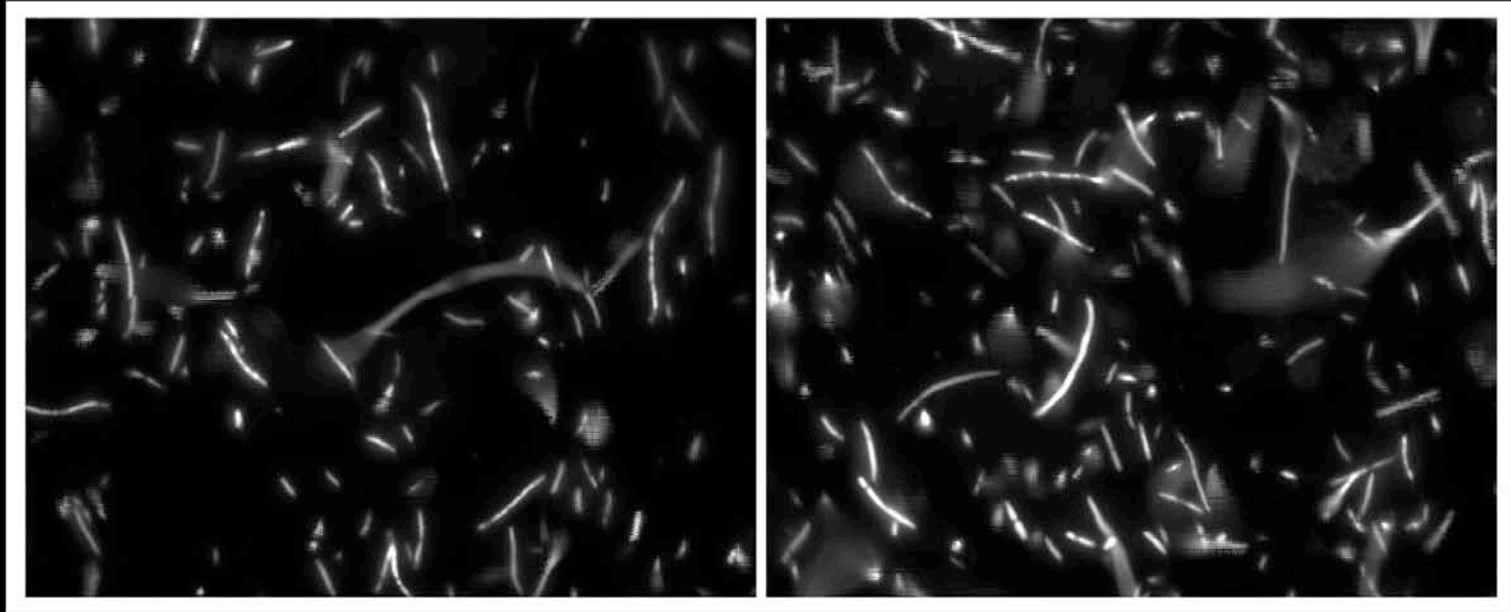
OD605



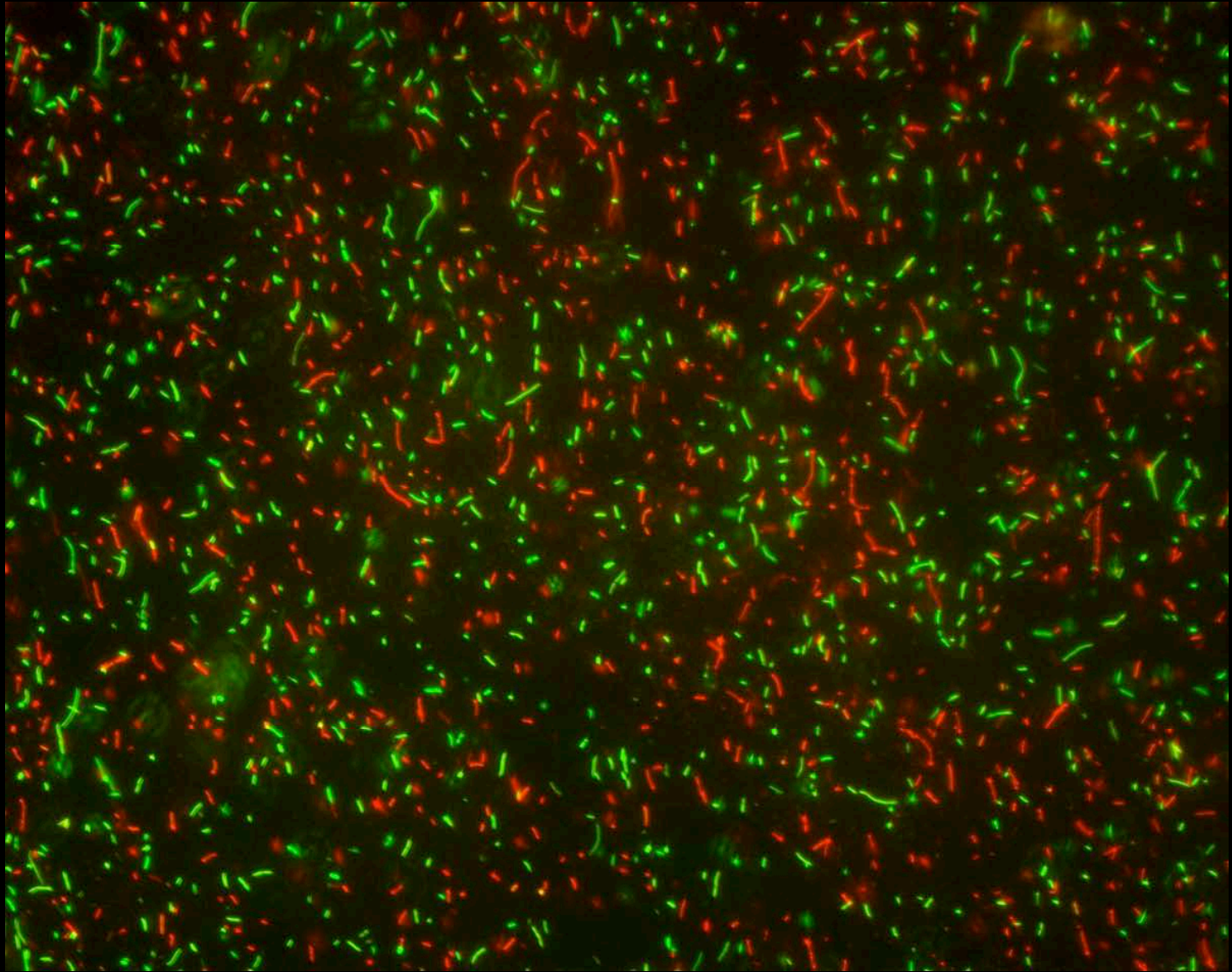
E.coli+SE.biotin+Stp-Cy3

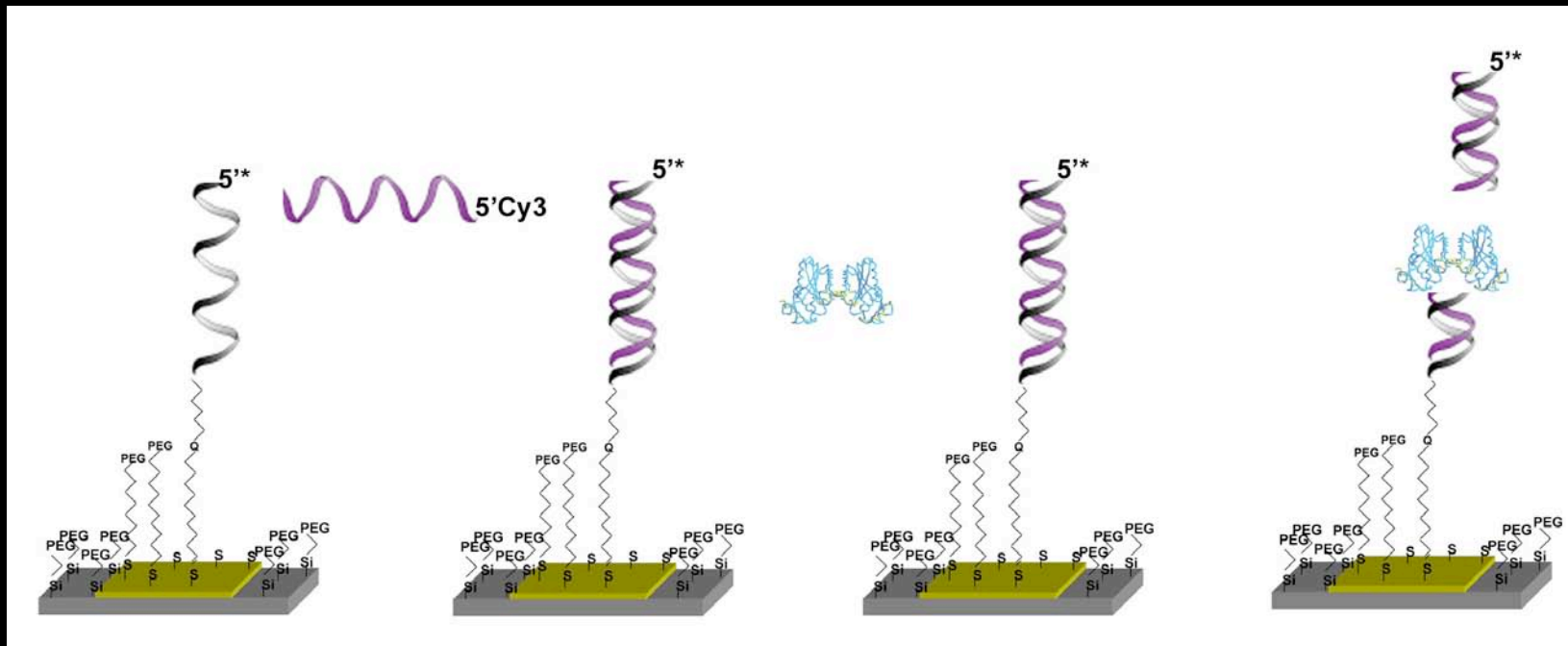


E.coli labeled with Syto 11 (x1000)



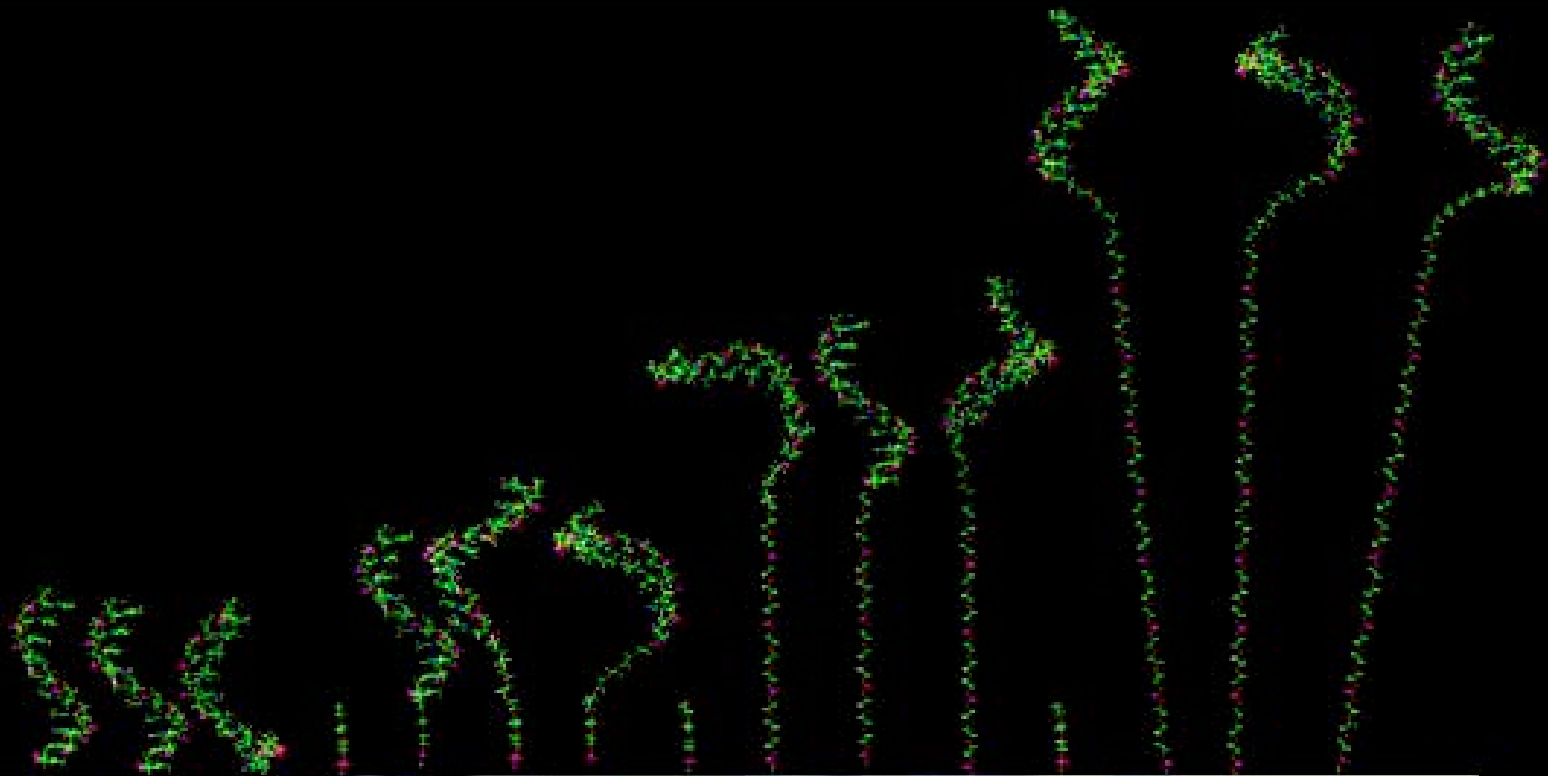
081303

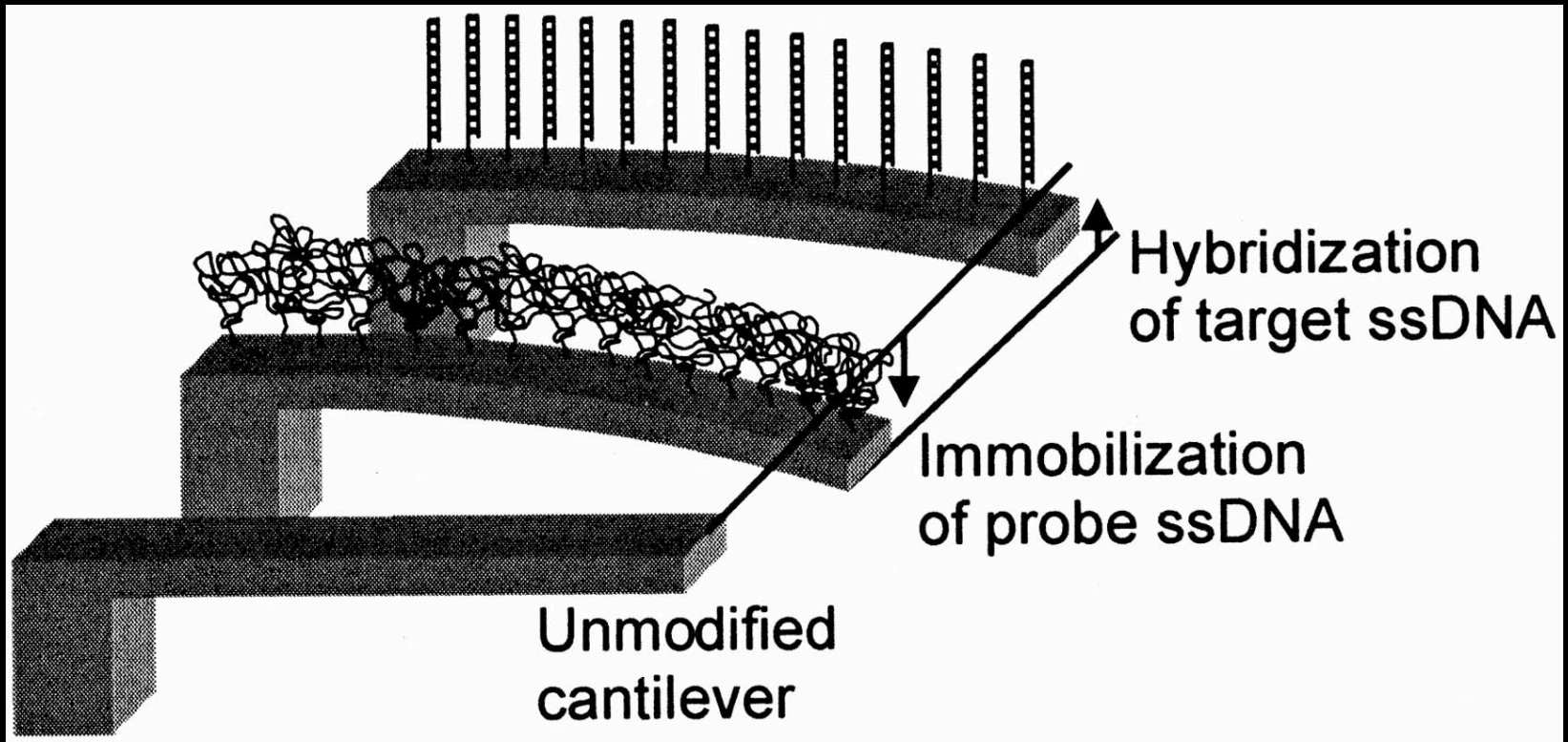




The density of oligonucleotides on the surface is approximately 10 pmol per mm² on aminated polypropylene, approximately 0.1 pmol per mm² on glass after ammonia deprotection—equivalent to one molecule per 39 square angstroms.

(Southern et al)





Co-conspirators

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Zeiss Jena

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Transgenic Quail

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Janet Baer-CIT

Retrovirus

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BioNEMS

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