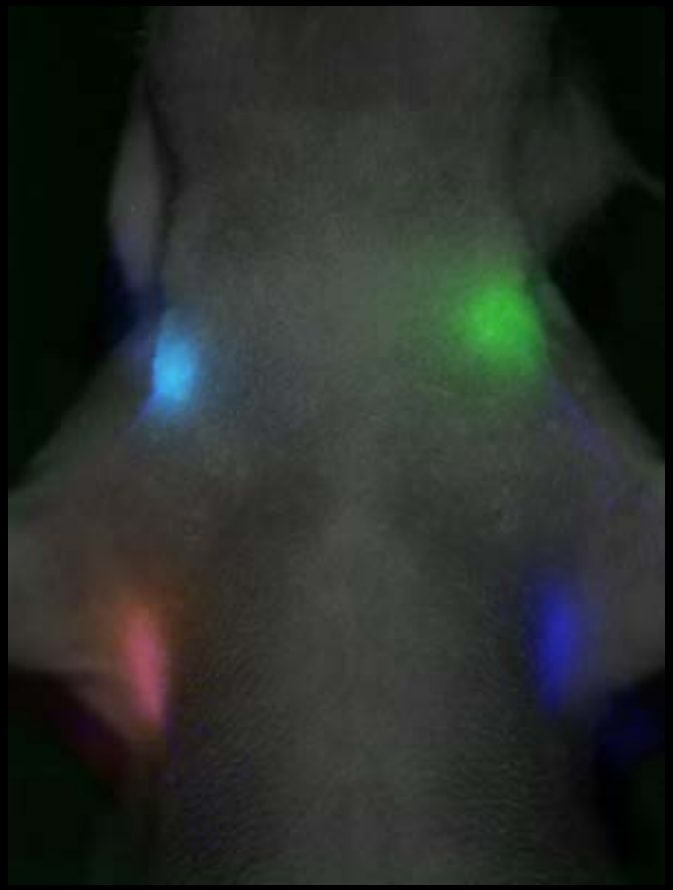
Mouse 101: *In vivo* Optical Imaging

Peter Choyke, M.D.
Molecular Imaging Program
National Cancer Institute

Optical Imaging: Why do it?

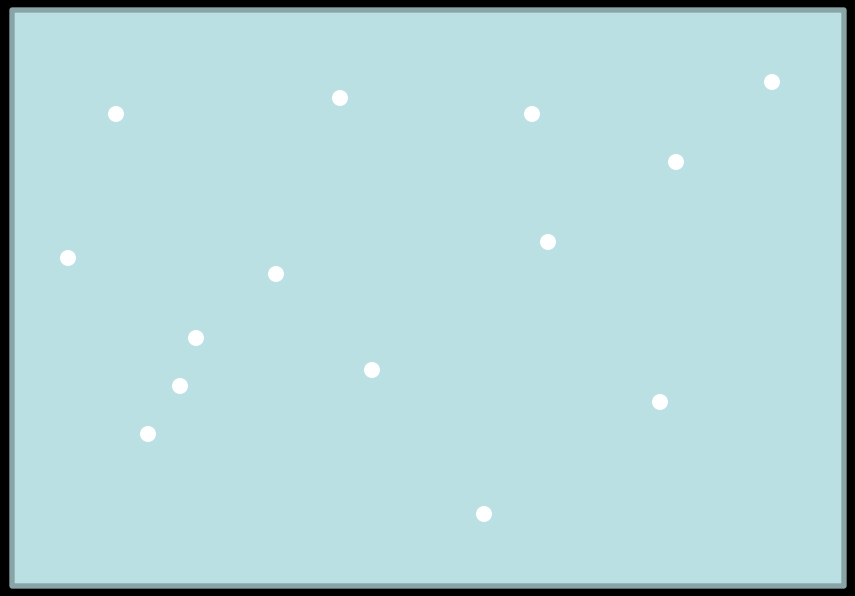
- Sensitivity
- Portability
- Low Cost
- Lack of Radiation
- Real time
- Multiplexing



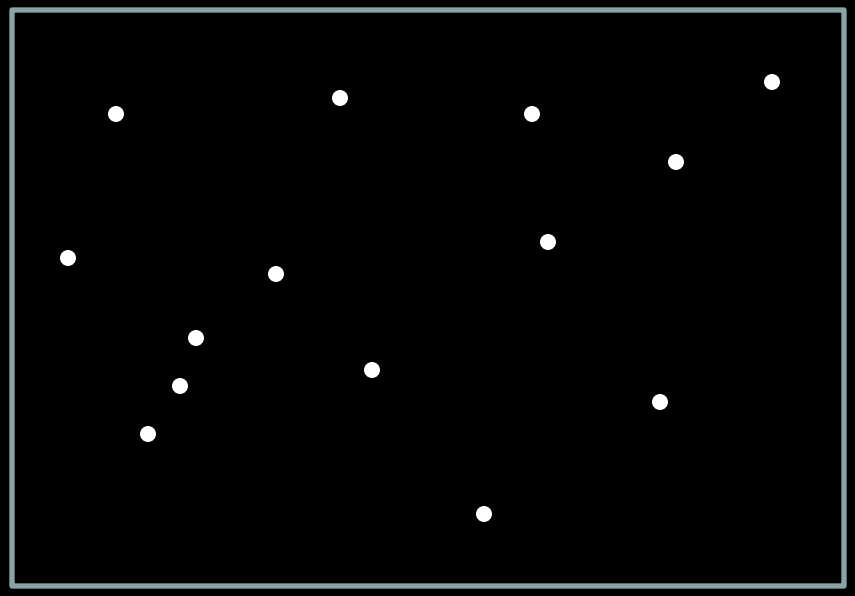
Optical Imaging: Why not?

- Poor penetration of light in tissue
  - Surface
  - Endoscopy
  - Surgical
- Autofluorescence

Target to Background



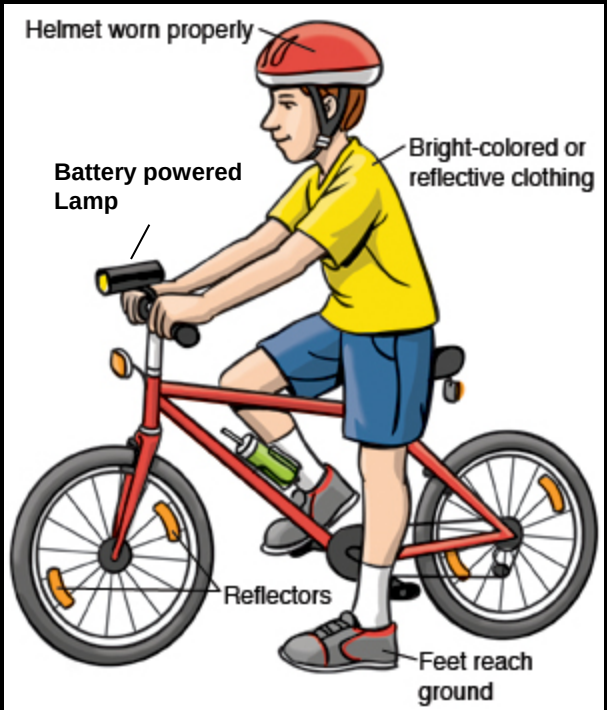
High Background: Low sensitivity



Low Background: High Sensitivity

Endogenous Optical Imaging

- Bioluminescence (BLI)
- Fluorescent Proteins (FPs)

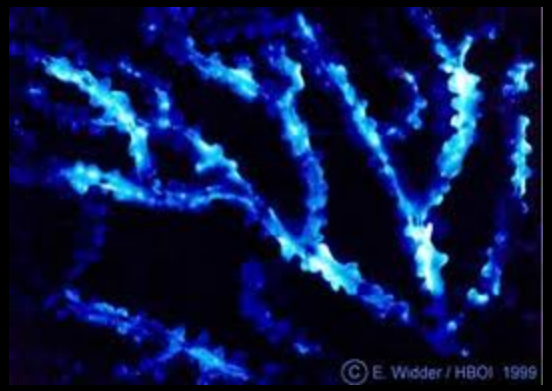


Bioluminescence

<u>Luciferase</u>

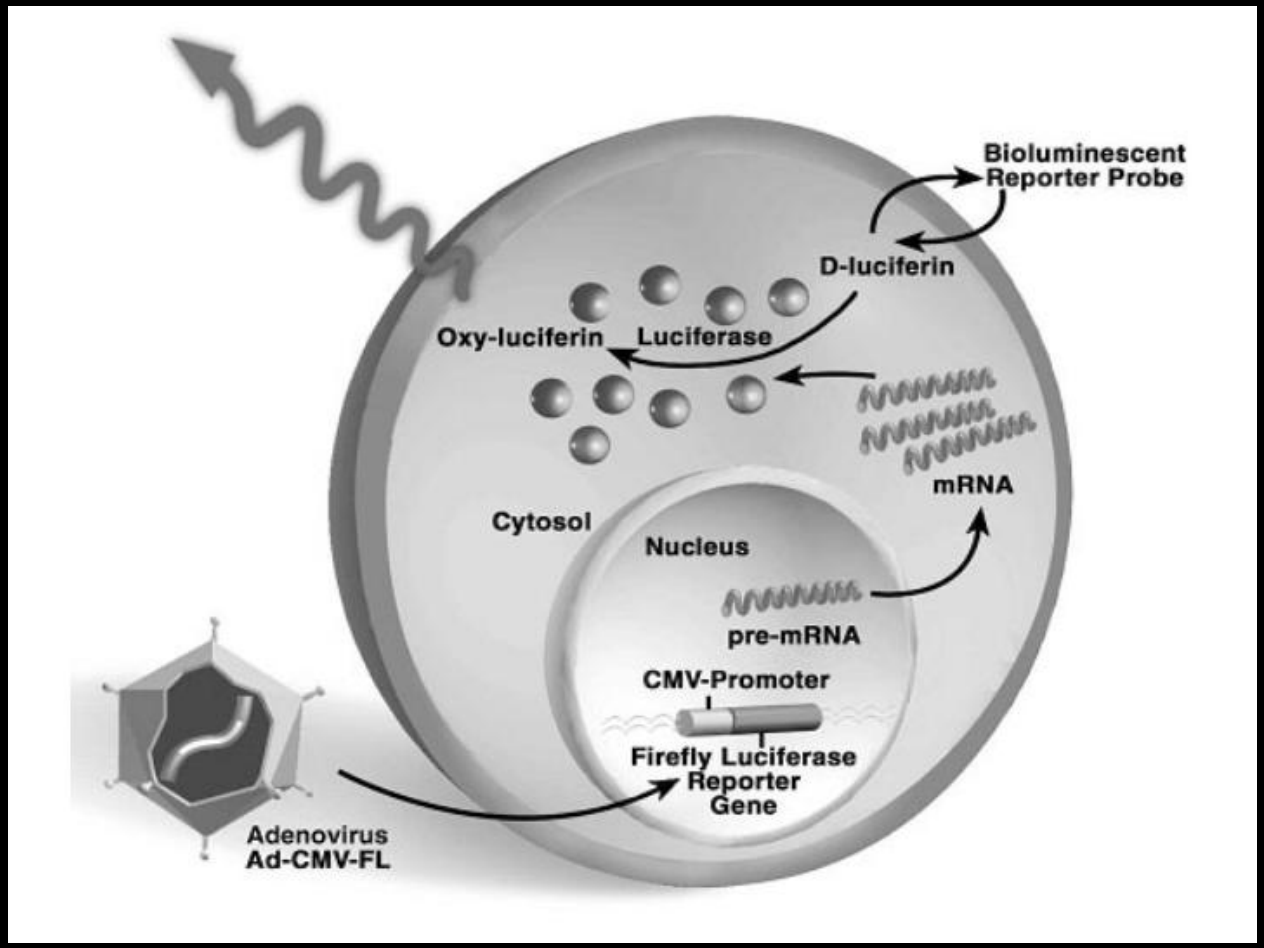
Isolated from firefly photocytes

Switchable



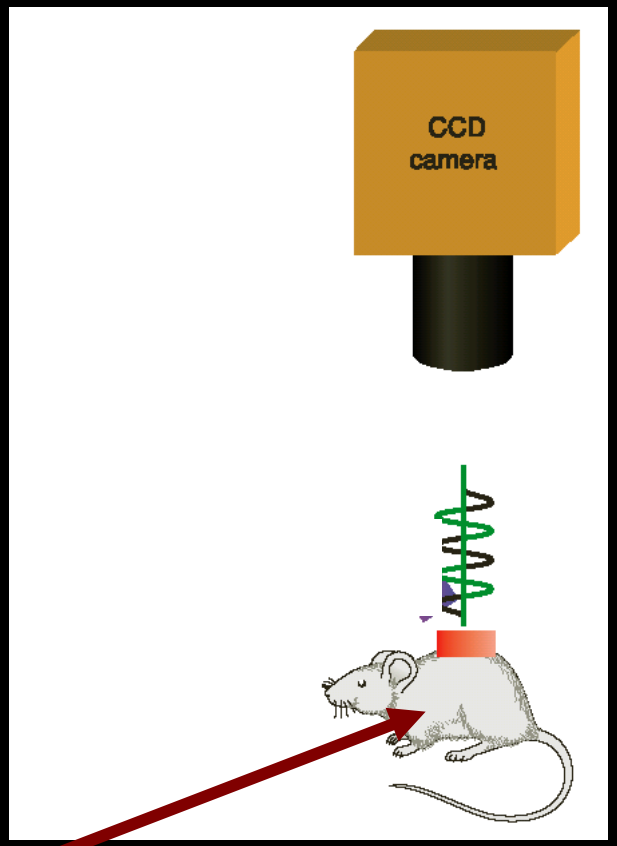
Reporter Gene-Reporter Probe Paradigm

Requires
Oxygen
And ATP



Massoud et al. Advances in Oncologic Imaging 2003

Bioluminescent Imaging: Luciferase



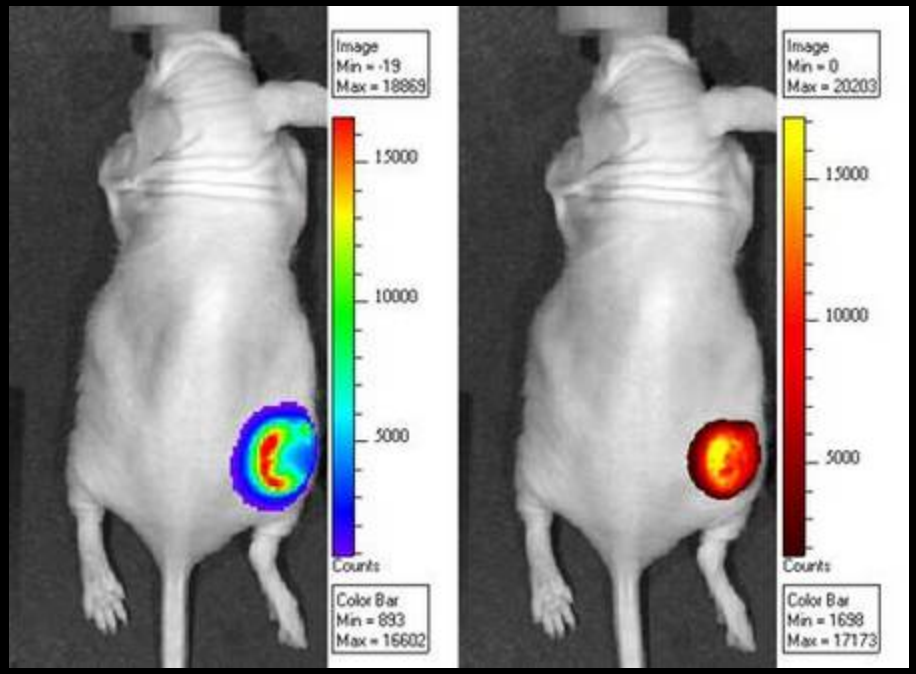
No Autofluorescence

Luciferin +
ATP +
O2

Luciferase

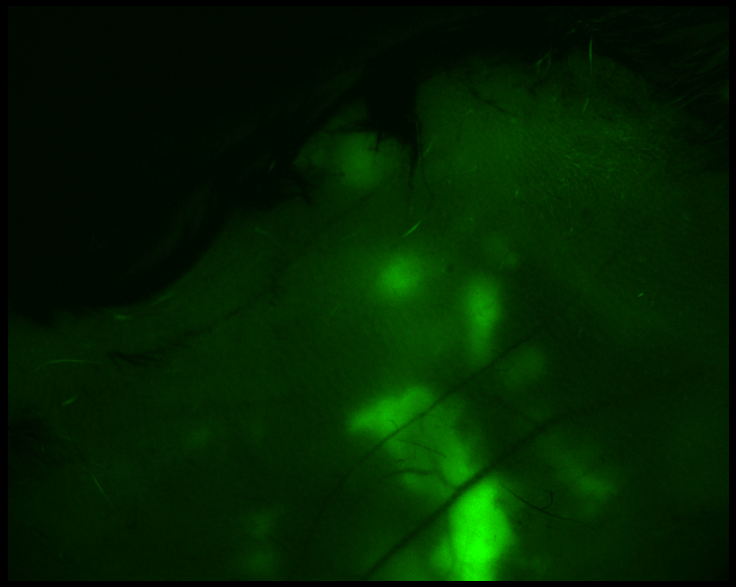
LIGHT

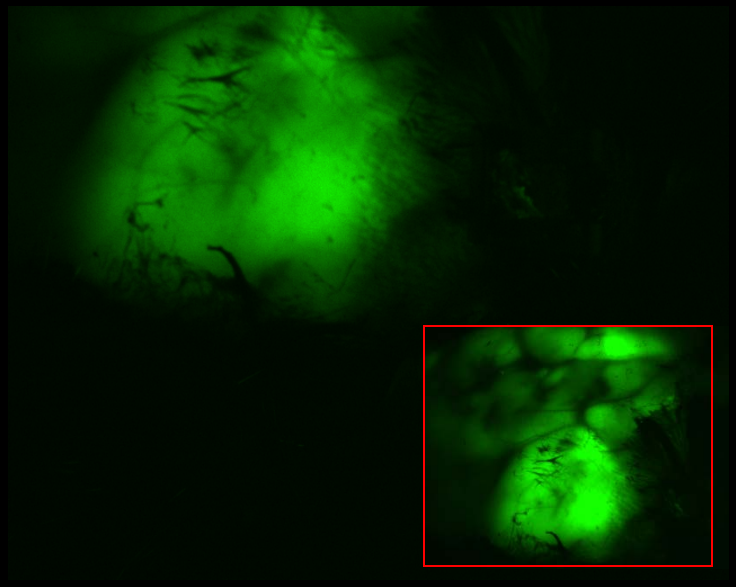
Xenogen Camera



Bioluminescence

Peritoneal Metastasis





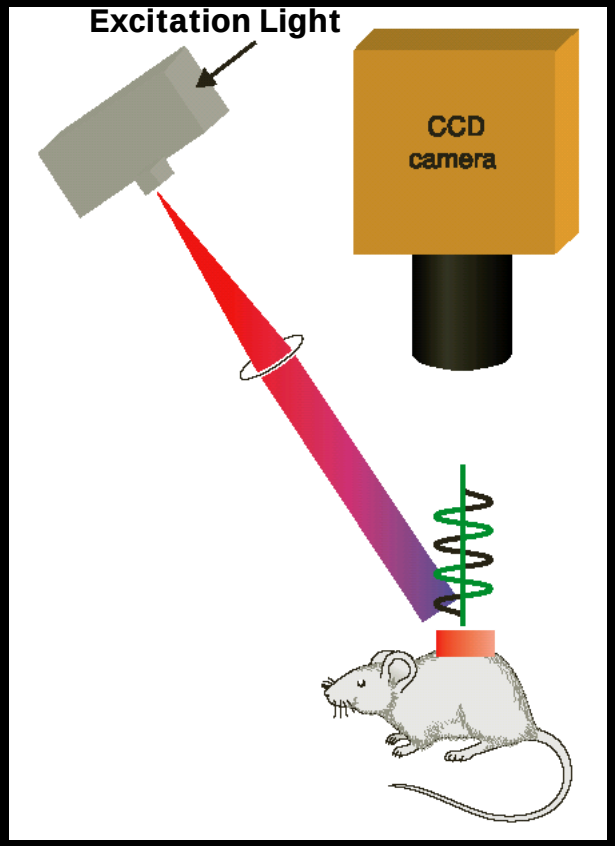
10 days post IP injection of 10^6 MC38-Luciferase Colon Carcinoma Tumor Cell Line in C57BL/6 Mice

Fluorescence Imaging: GFP

Green Fluorescent Protein (GFP)

Isolated from jellyfish, *Aequoria victoria.*

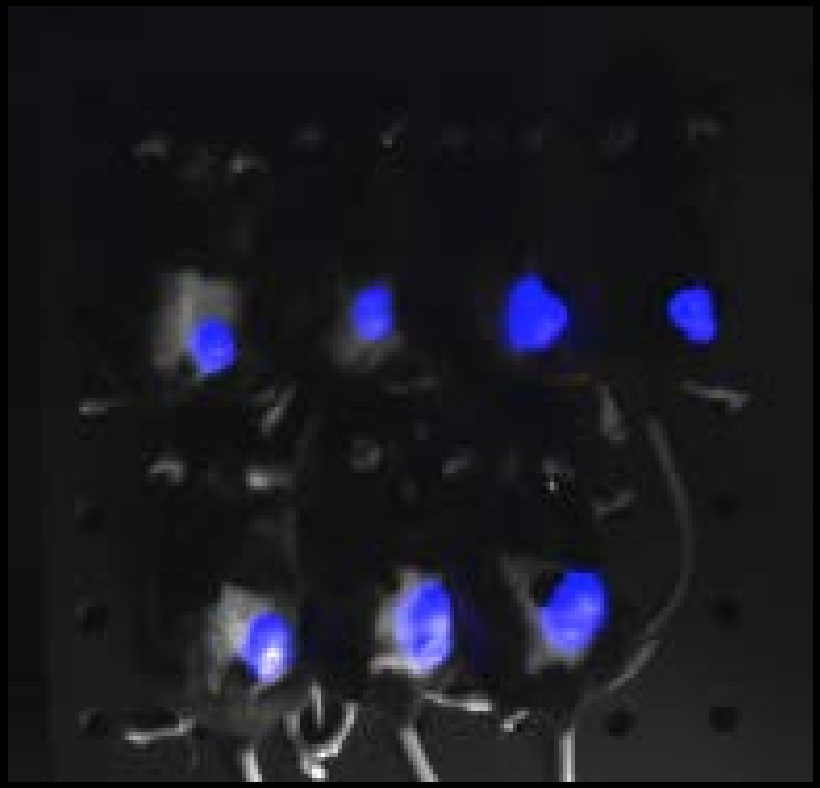
– *Always on, no energy requirements (except external light)*



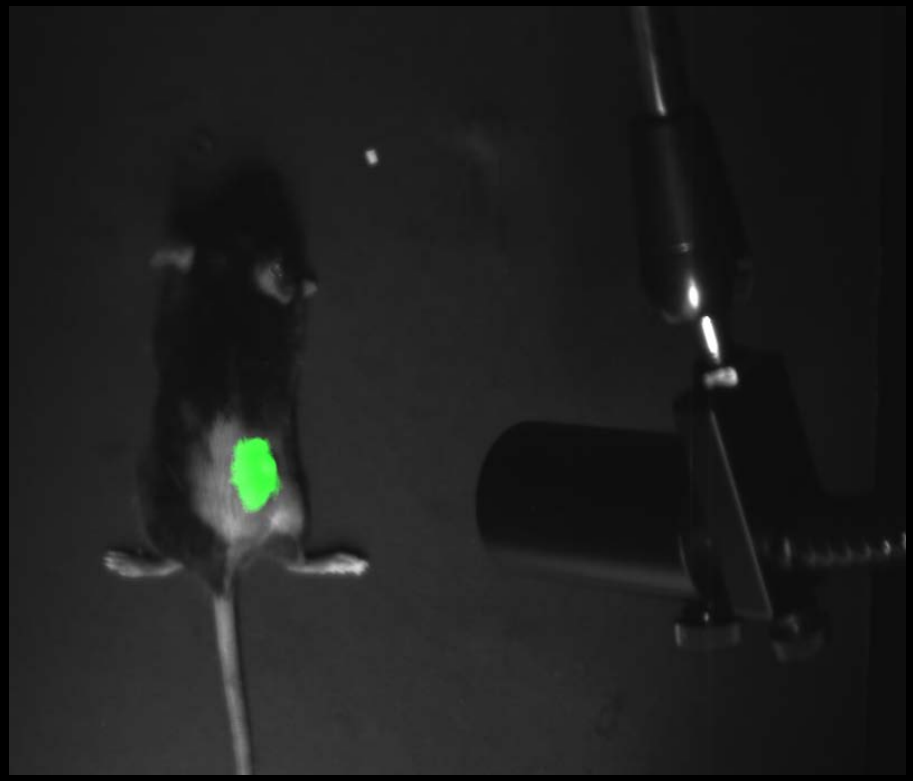
- Excitation Light source
- NO SUBSTRATE NEEDED

Sample Luciferase and GFP Images

Luciferase:



GFP:

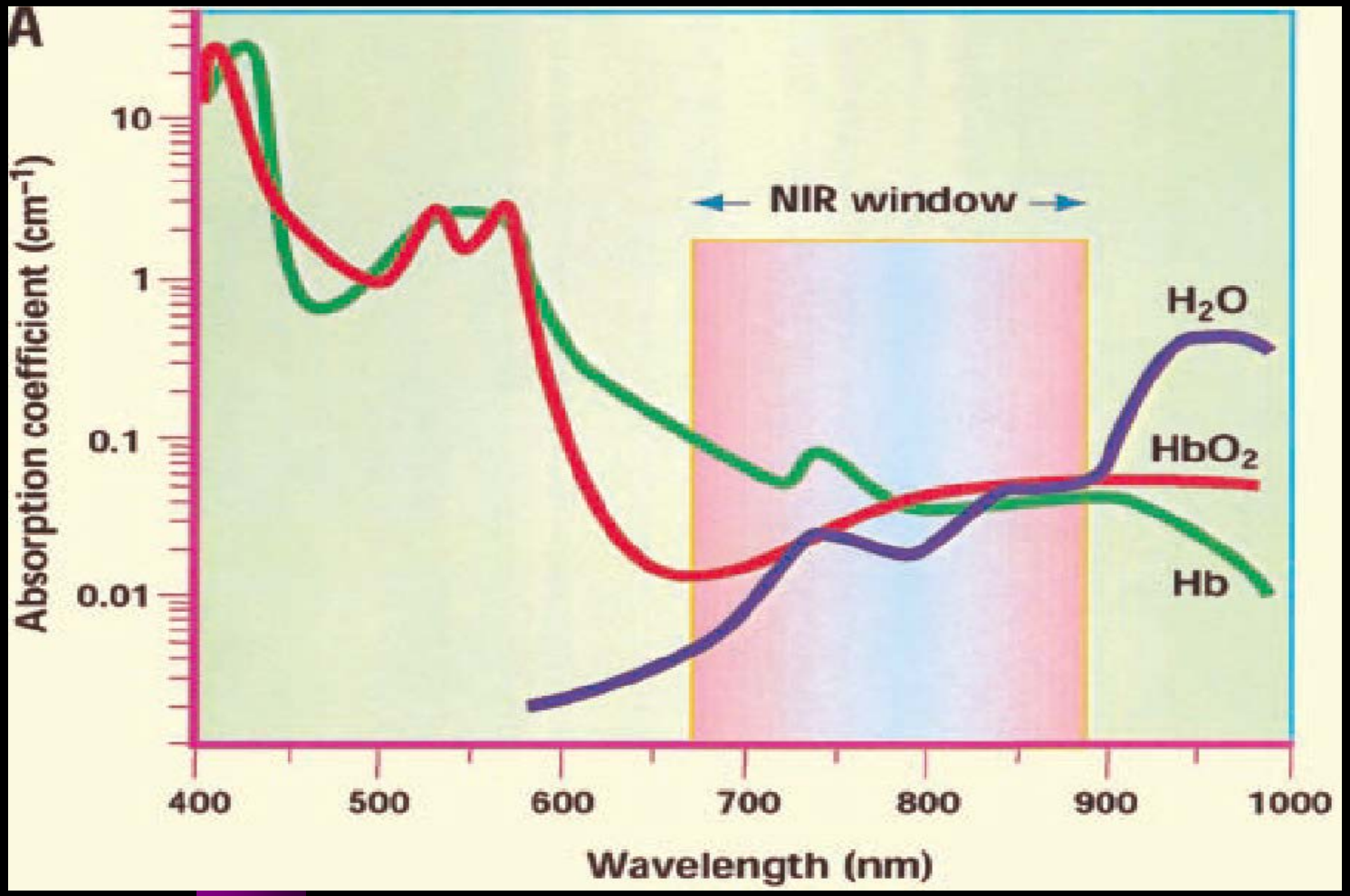


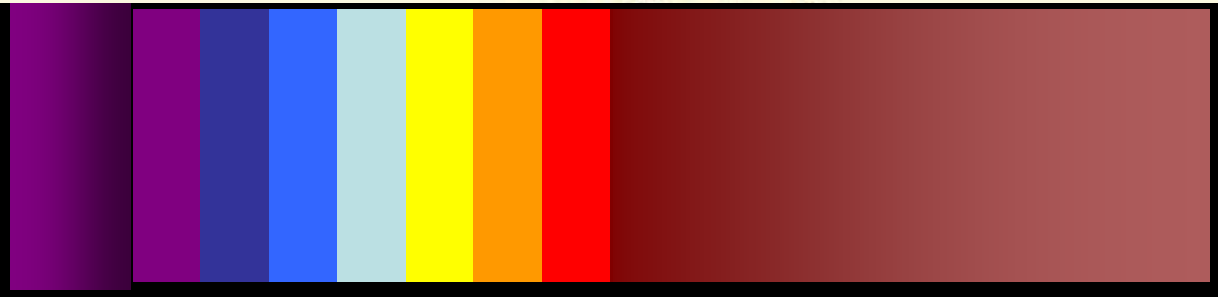
BLI vs. FP

Feature	Bioluminescence	Fluorescent Proteins
Mouse status:	Living only	Living and dead
Injection?	Yes, luciferin, i.p.	No
Sensitivity	Very high	Modest
Autofluorescence	Minimal	High (filter)
Cost	Moderate (luciferin)	Low
Camera	Xenogen	Many mfgs.
Overall Ease of use	V. Easy	Easy

Exogenous Fluorophores

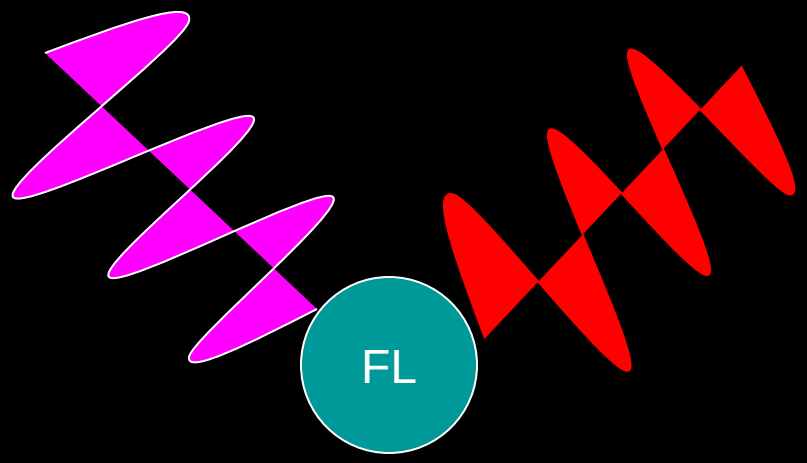
- Advantages:
  - No need to “engineer” mouse
  - More translatable to clinical application
  - More flexibility/choices
- Disadvantages
  - Requires more expertise
  - Chemistry
  - Requires injection of agent

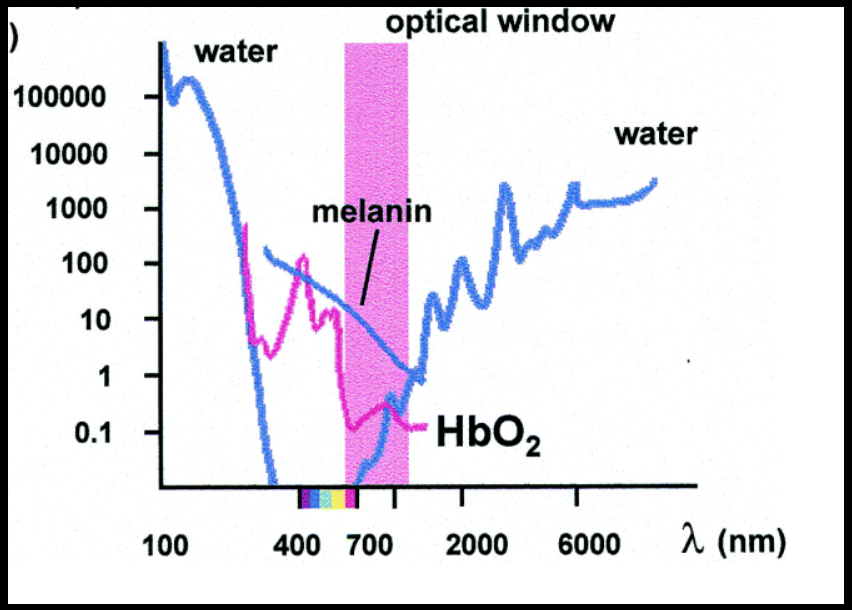




UV Visible NIR Infrared

Exogenous Fluorescence

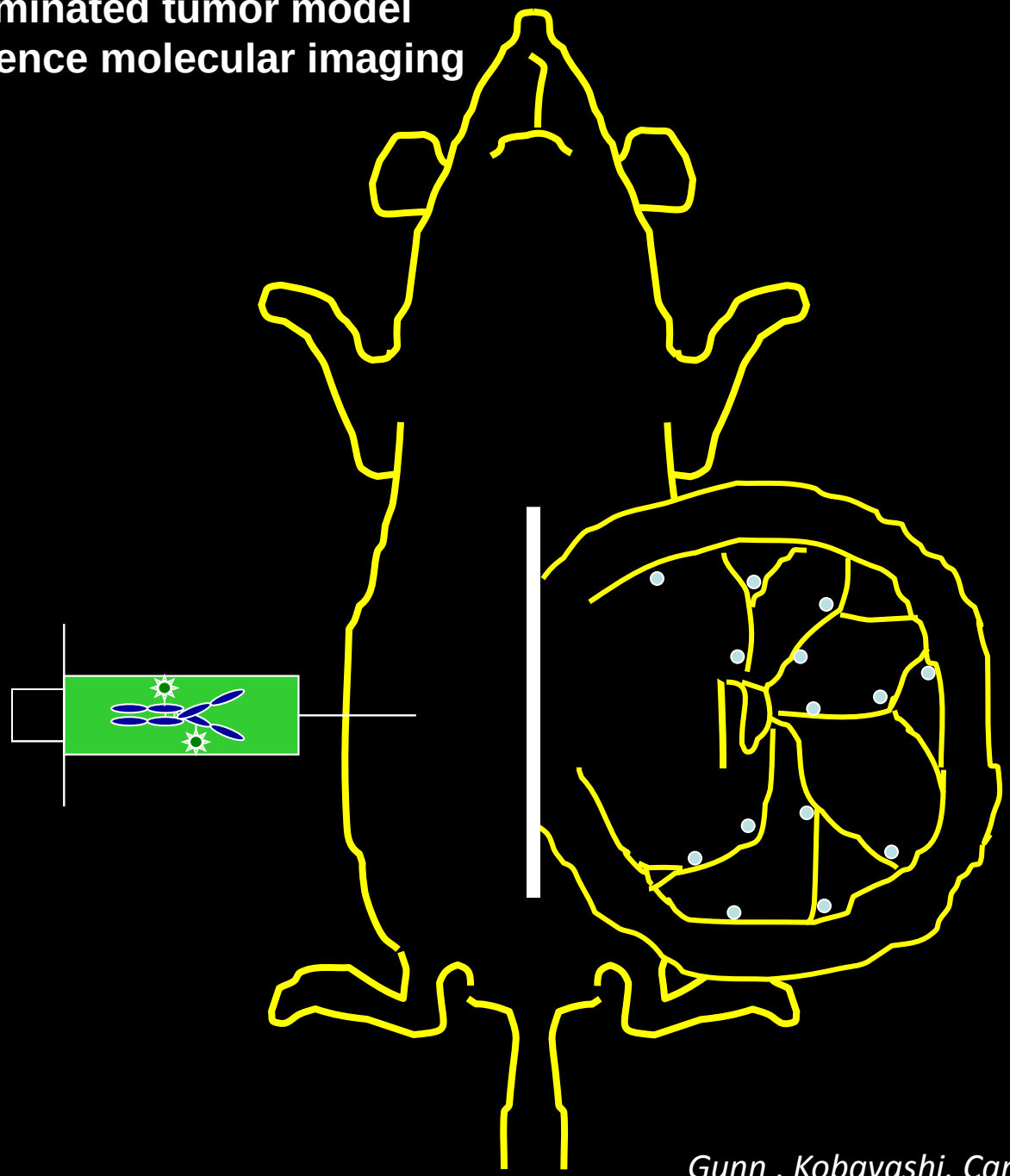


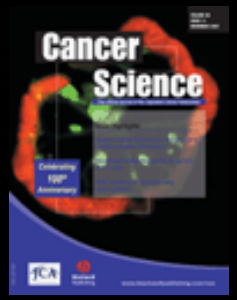


<u>Autofluorescence</u>
NADH
Collagen
Flavenoids

Mouse Chow

Peritoneally disseminated tumor model for target fluorescence molecular imaging

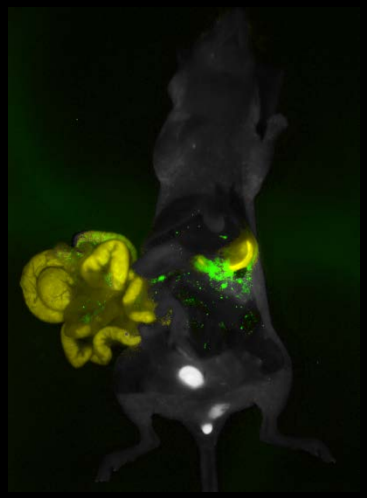


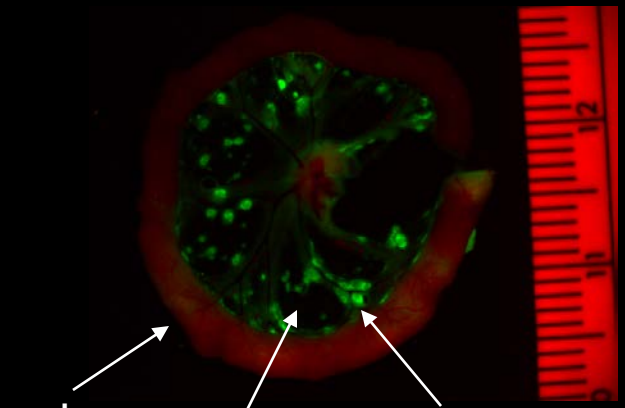


Gunn , Kobayashi, Cancer Sci, 2009/3

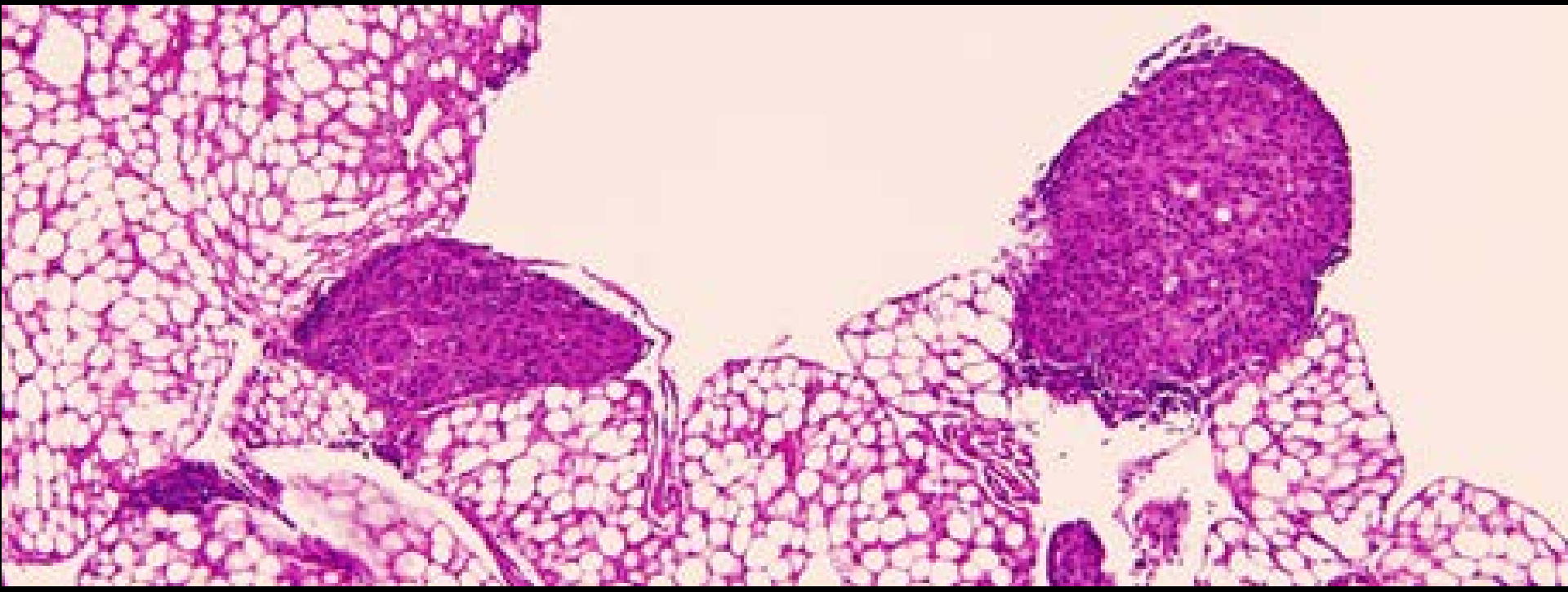
Animal Model

- SHIN3 Ovarian cancer model
- IP injection of tumor cells
- Incubate 10-20 days
- IP injection of Optical Probe
  - Maestro™ (CRi Inc)
  - Whole abdomen imaging
  - “Wagon wheel” mesenteric imaging



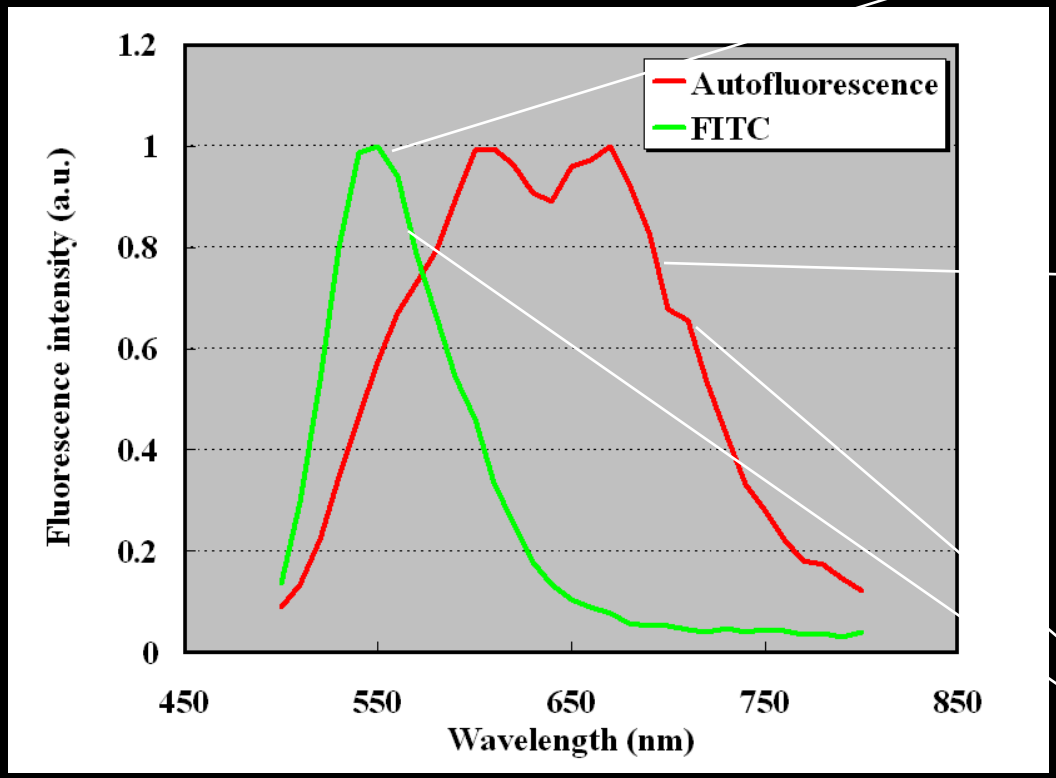


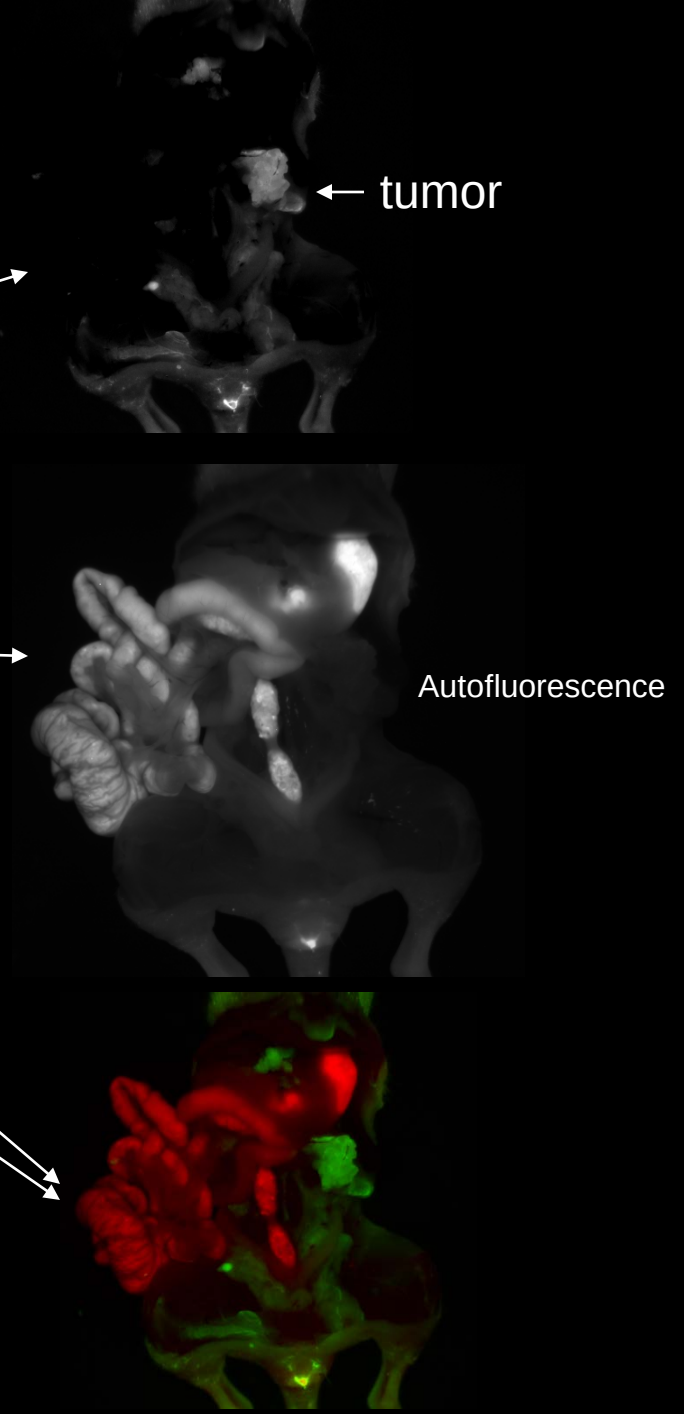
Microfoci (“studding”) of the Peritoneum



White light imaging

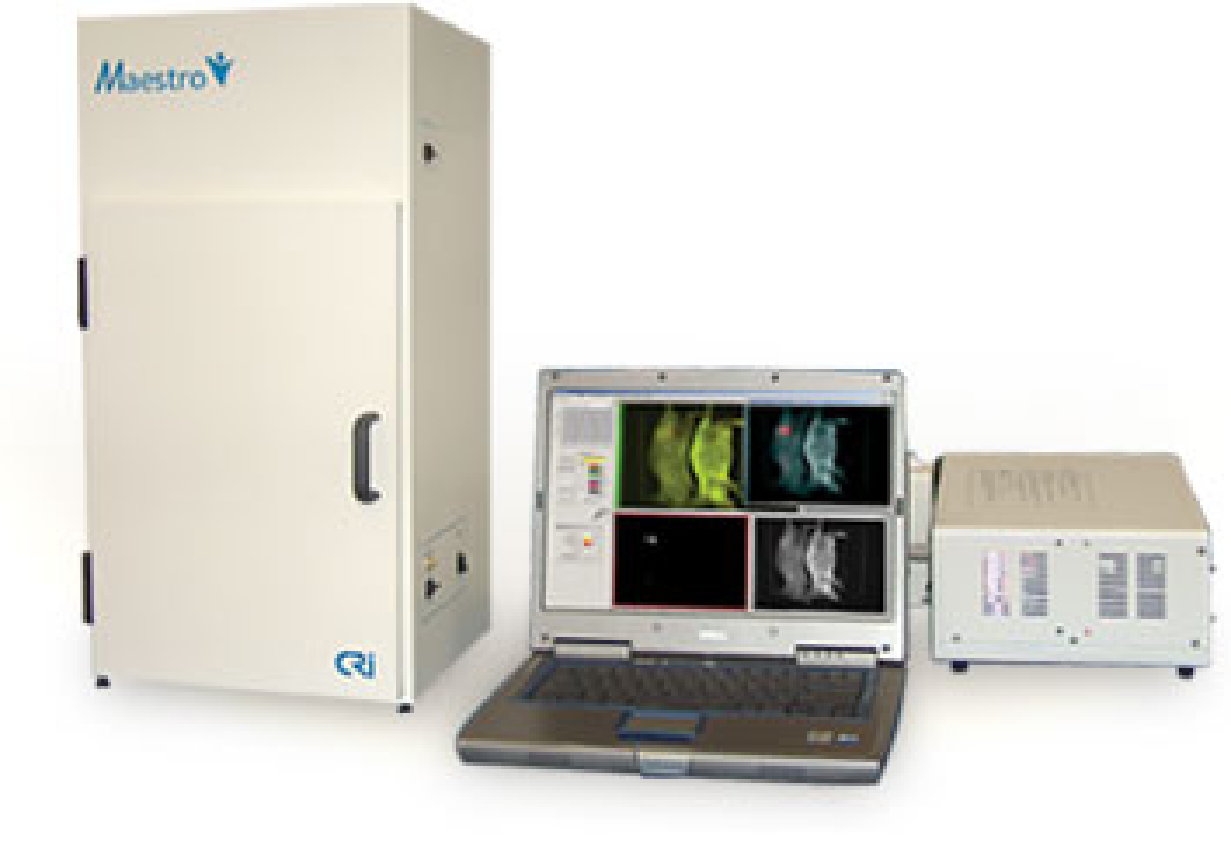
Spectral Imaging





Spectral images obtained at 5-10nm increments and then unmixing of the two spectra

In vivo spectral fluorescence imaging

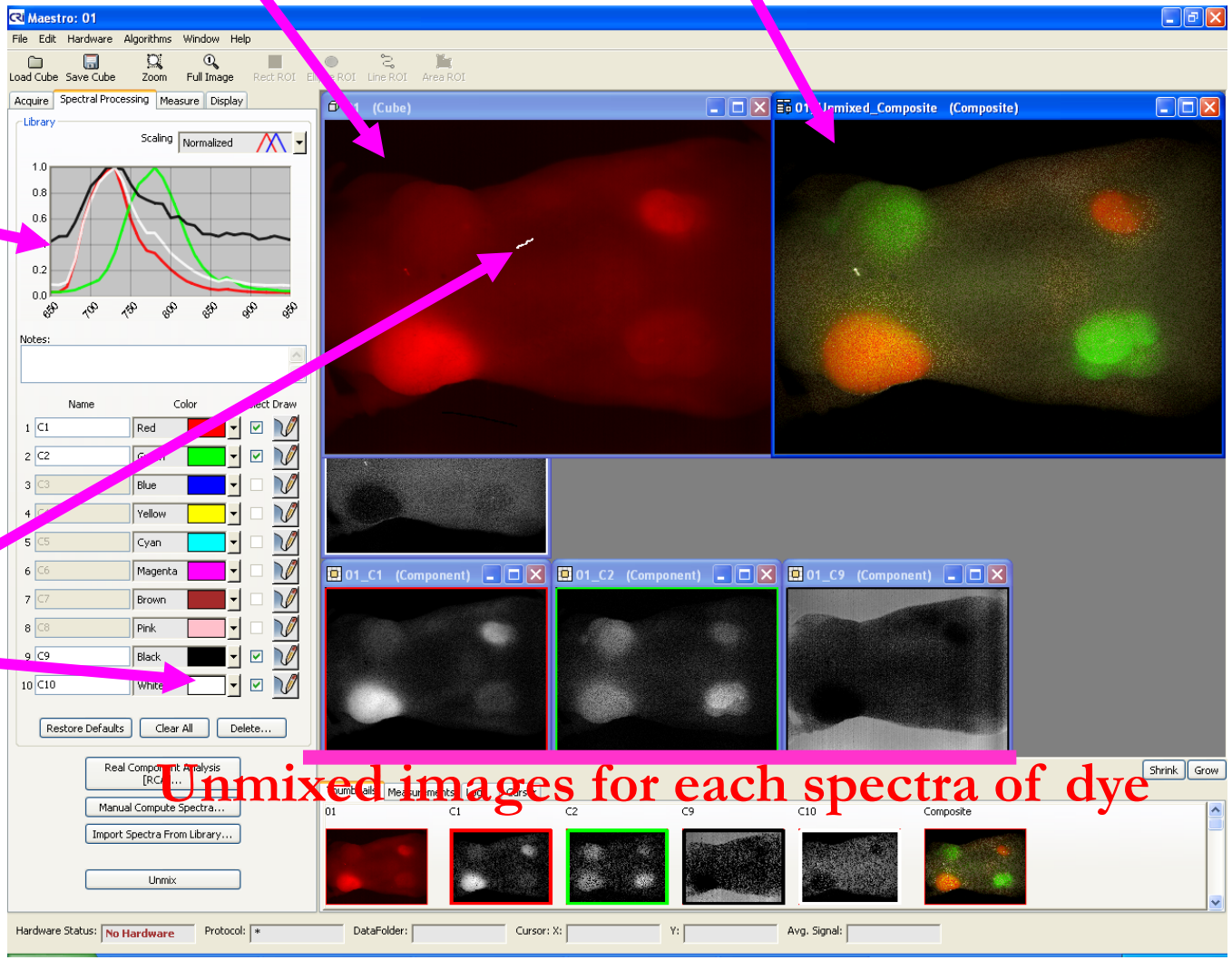


Imaging on Maestro™ In-Vivo Imaging System (CRi)

Spectrally-resolved images on Maestro™

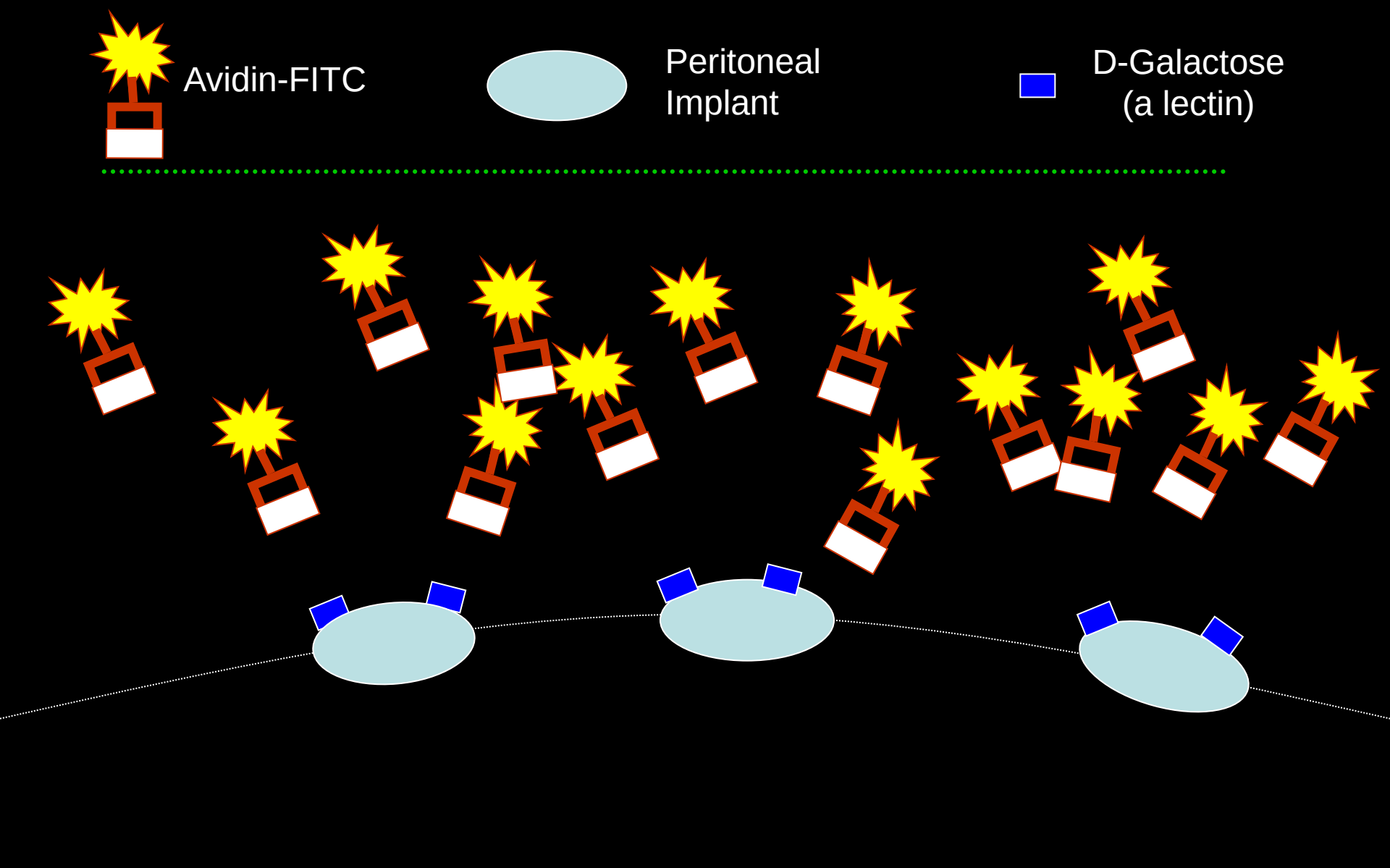
Spectral library

Correction for auto-fluorescence

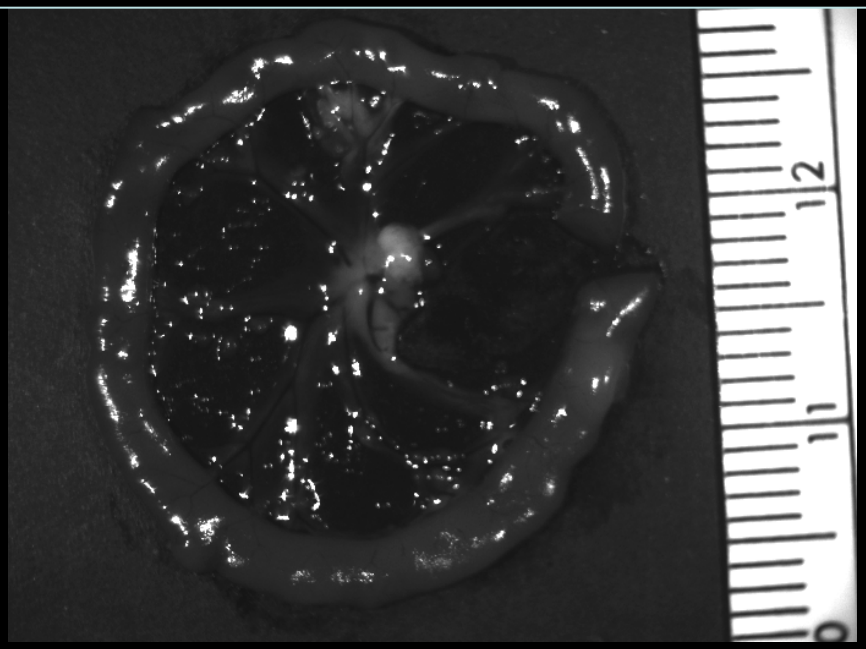


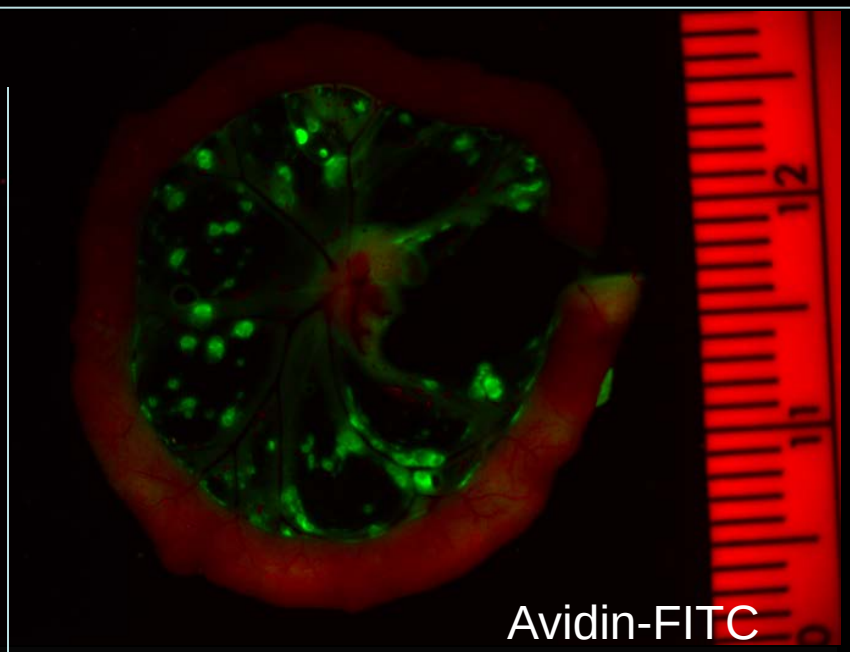
Unmixed images for each spectra of dye

Targeted Imaging of Peritoneum



Micro-cancer nodules of SHIN 3 ovarian cancer cells spread on the mesenteric membrane

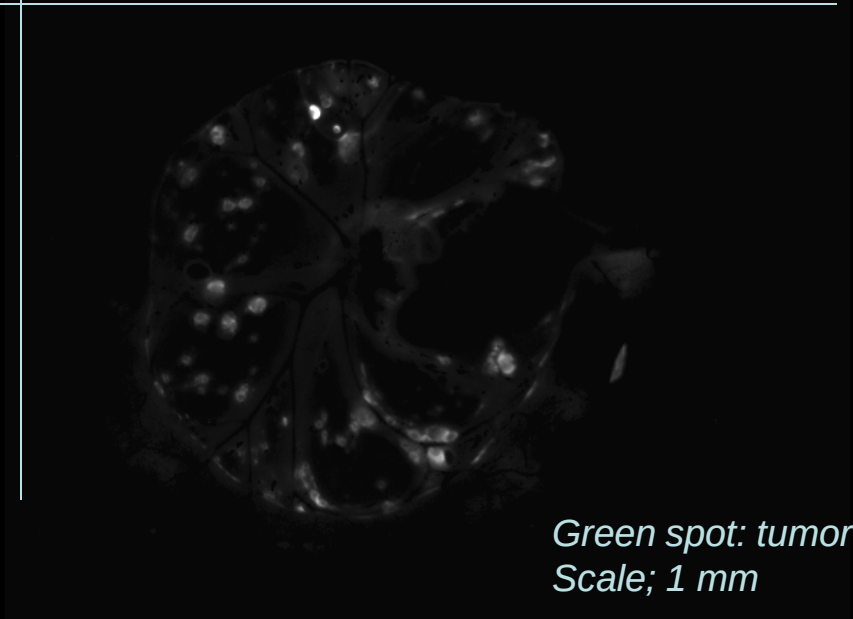




White light photo

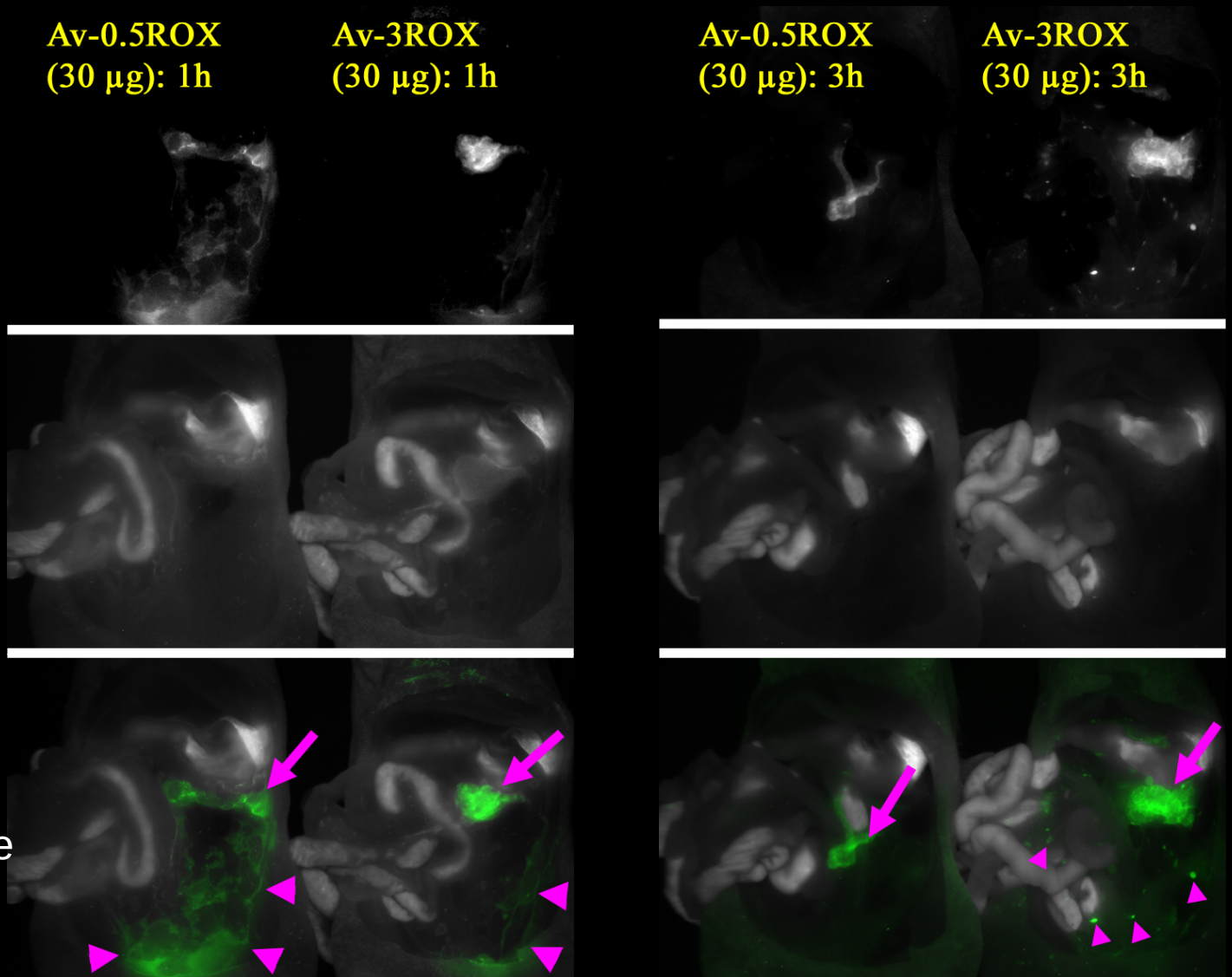
Green and red (auto-) fluorescence composite photo

Green fluorescence photo

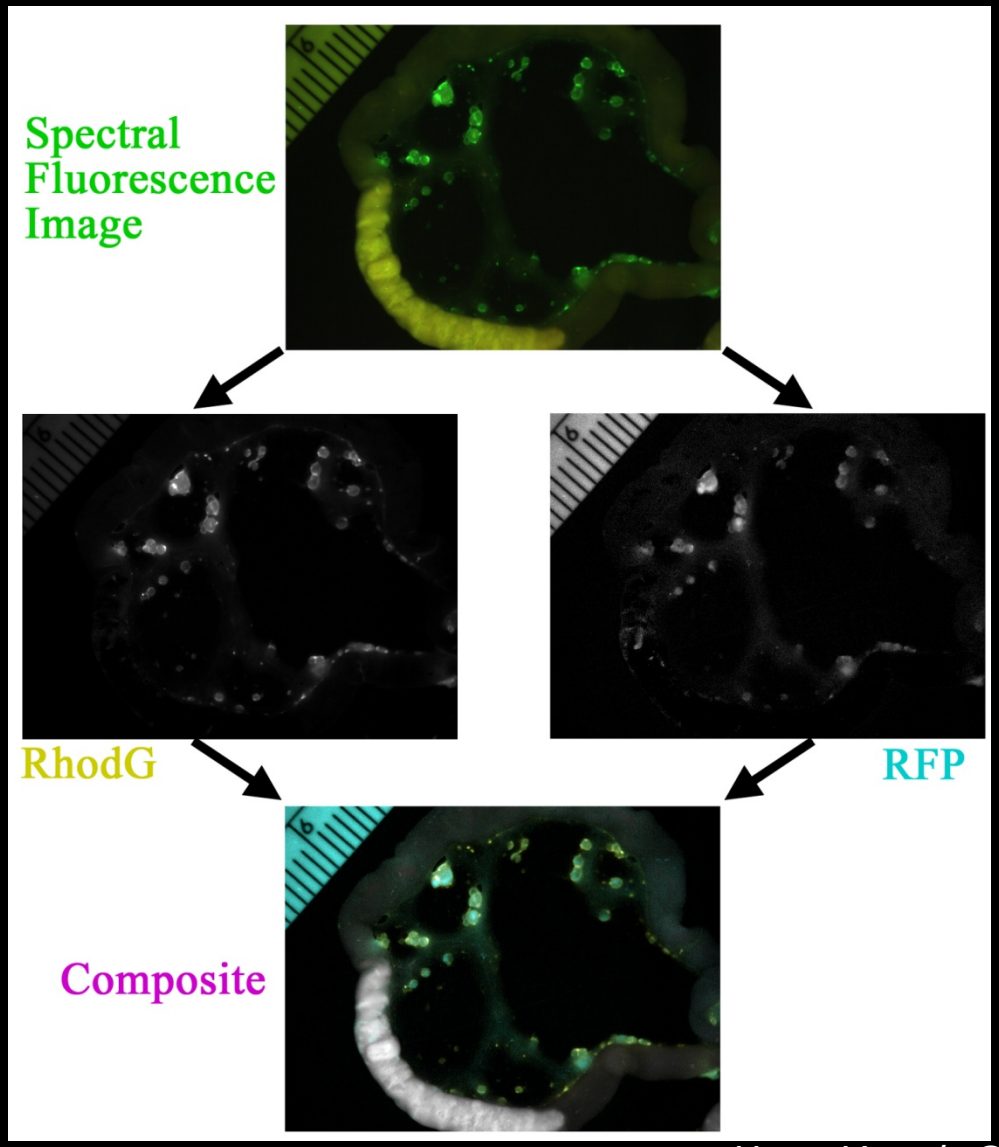


Green spot: tumor nodules
Scale; 1 mm

Self Quenching-Dequenching Mechanism of Enhancement



Validation with dysRed(RFP)



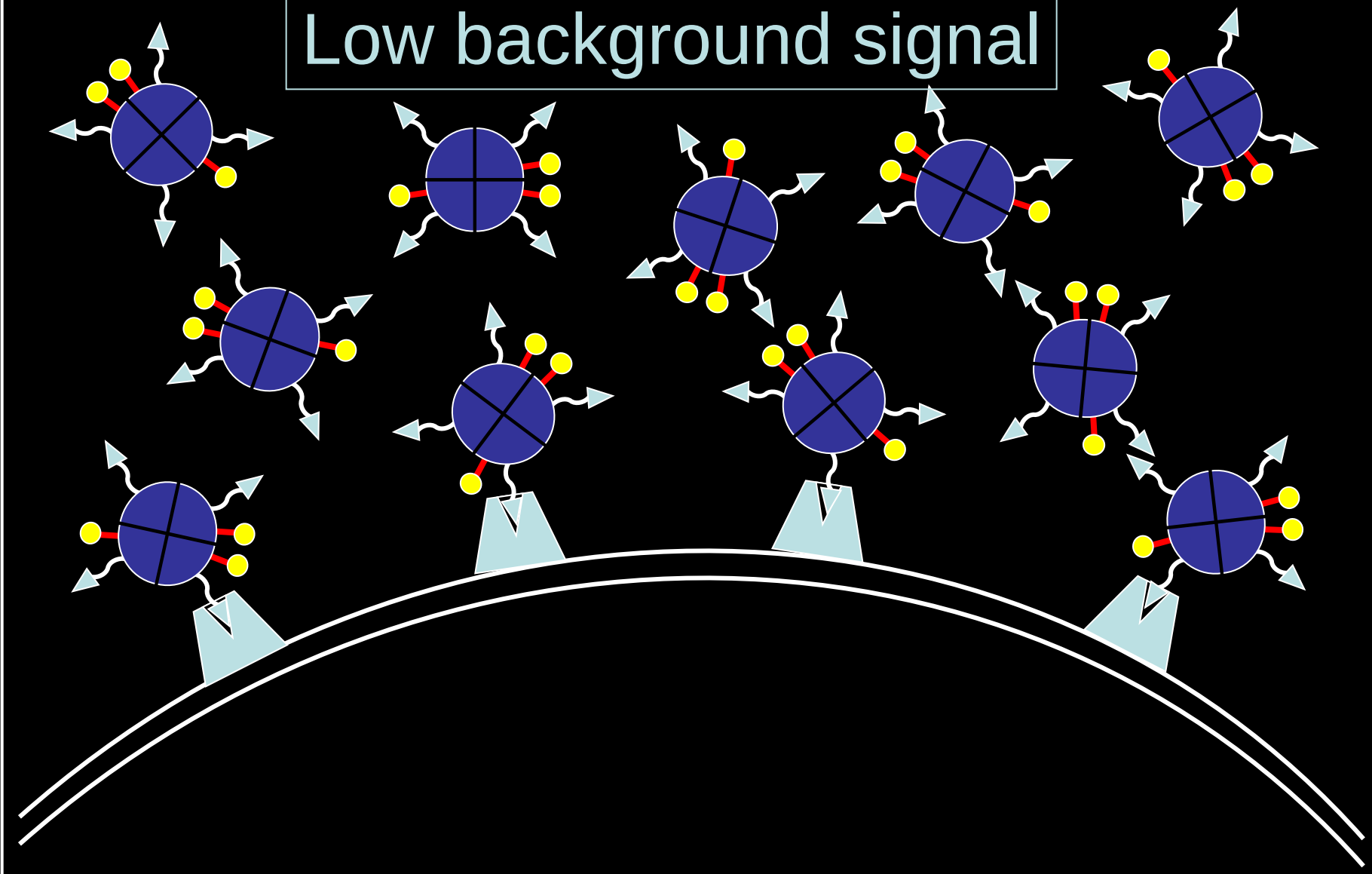
Hama Y et al. Soc. Molec Imaging 2006

Optical imaging

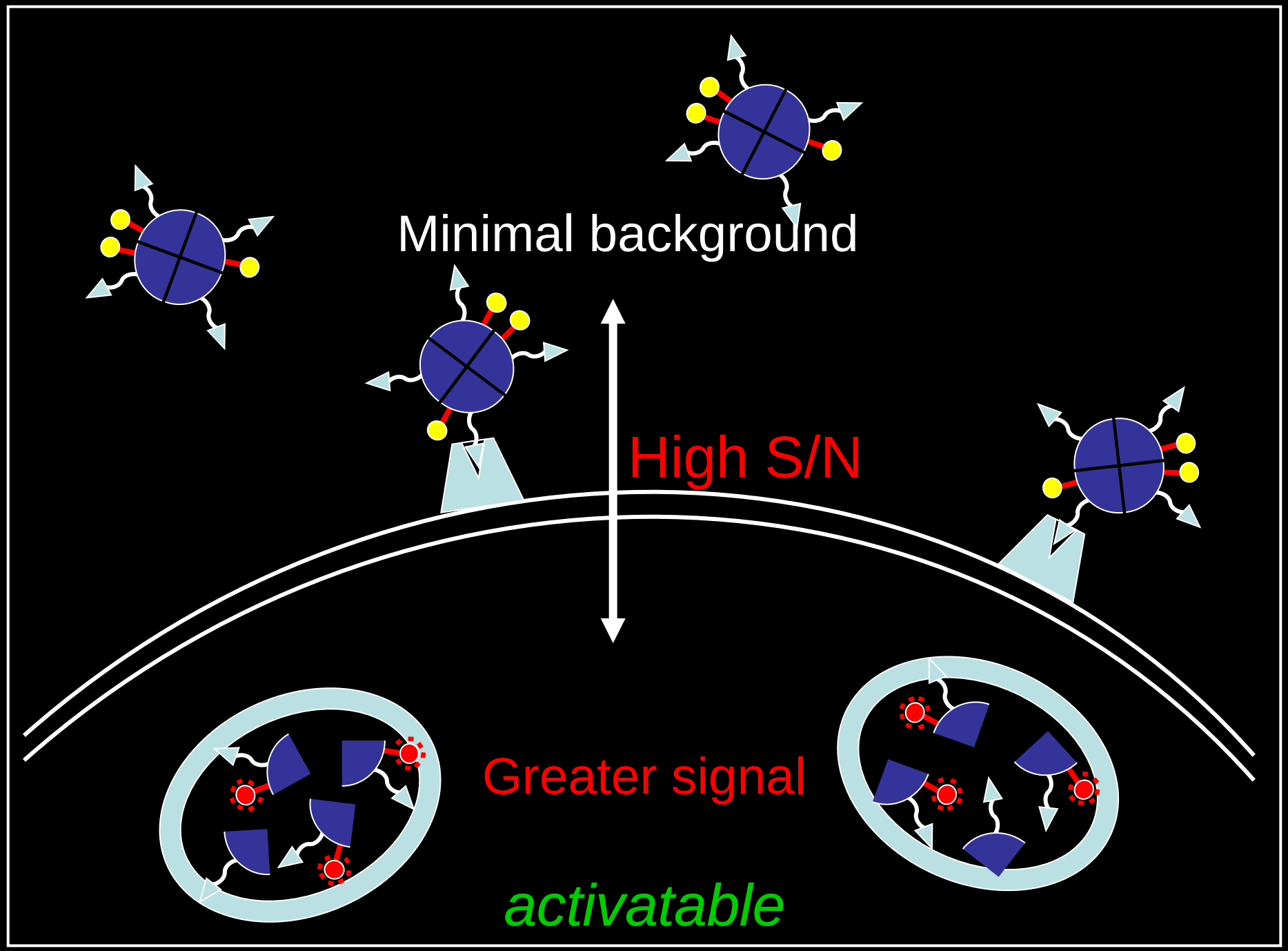
Switches

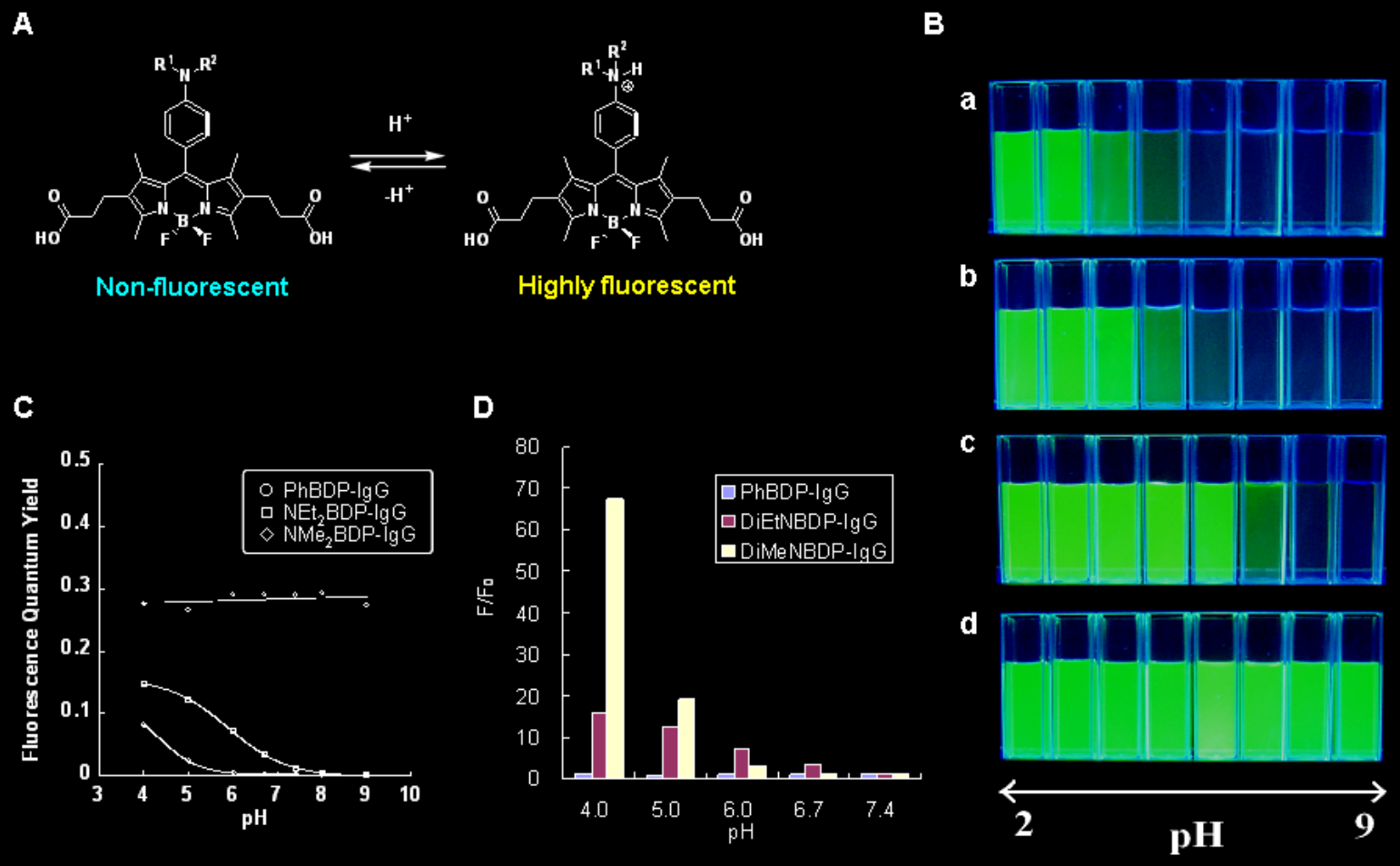
- Enzyme
  - Caspase, MMP (MGH group)
  - Molecular interaction with two hybrid system (Washington U. group)
  - β-galactosidase (reporter gene)
- pH (proton concentration)
- Oxidation
- Reactive oxygen species

Low background signal

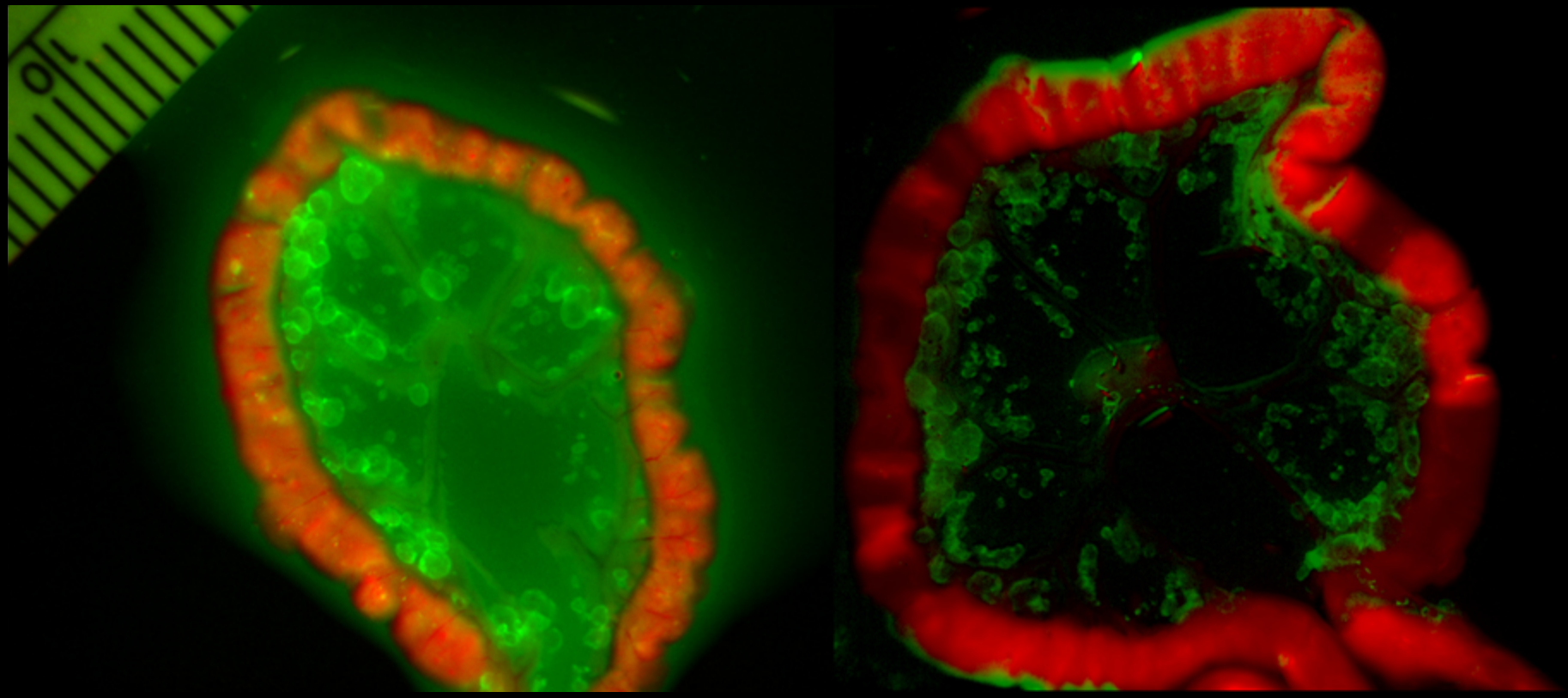


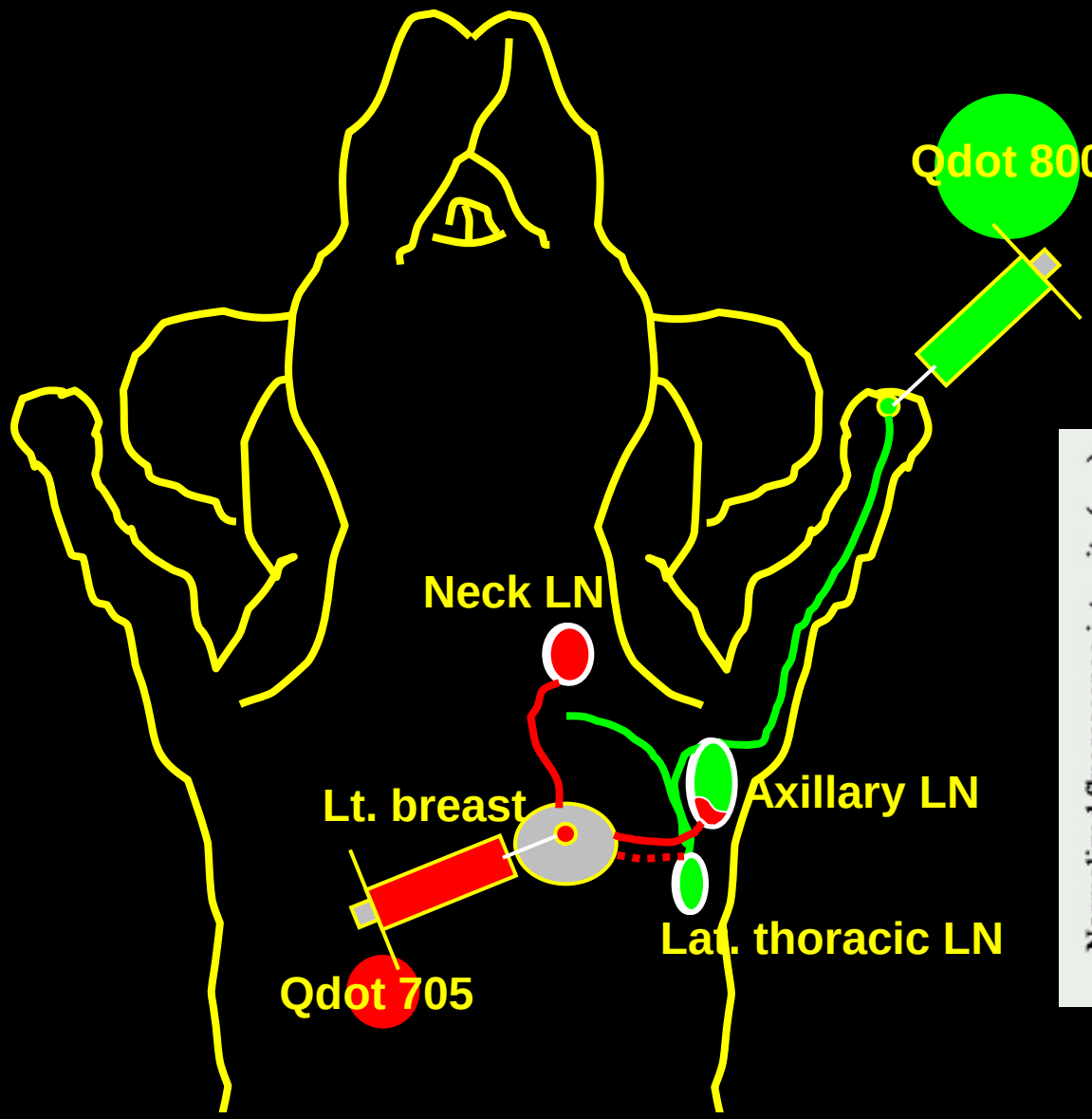
activatable

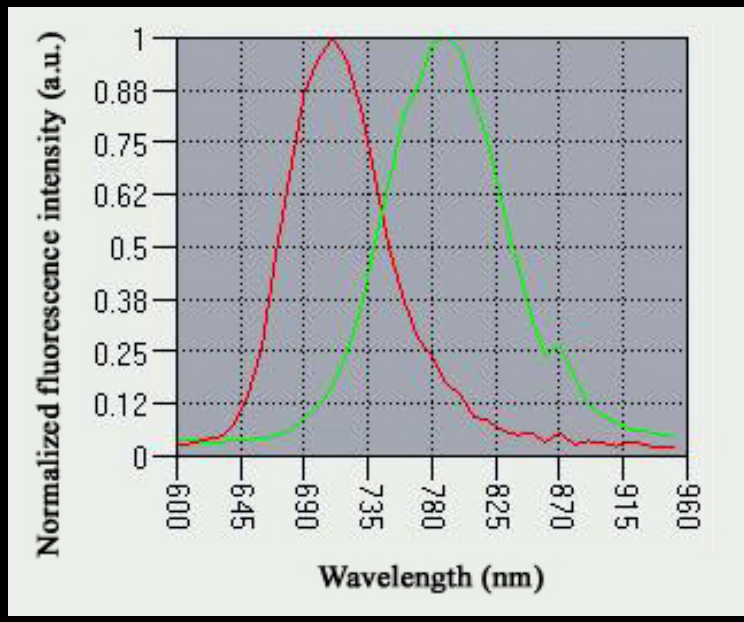

Minimal background
High S/N
Greater signal
activatable


A
H+
-H+
Non-fluorescent
Highly fluorescent
B
a
b
c
d
2
pH
9
C
Fluorescence Quantum Yield
pH
PhBDP-IgG
NEt2BDP-IgG
NMe2BDP-IgG
D
F/F0
pH
PhBDP-IgG
DiEtNBDP-IgG
DiMeNBDP-IgG

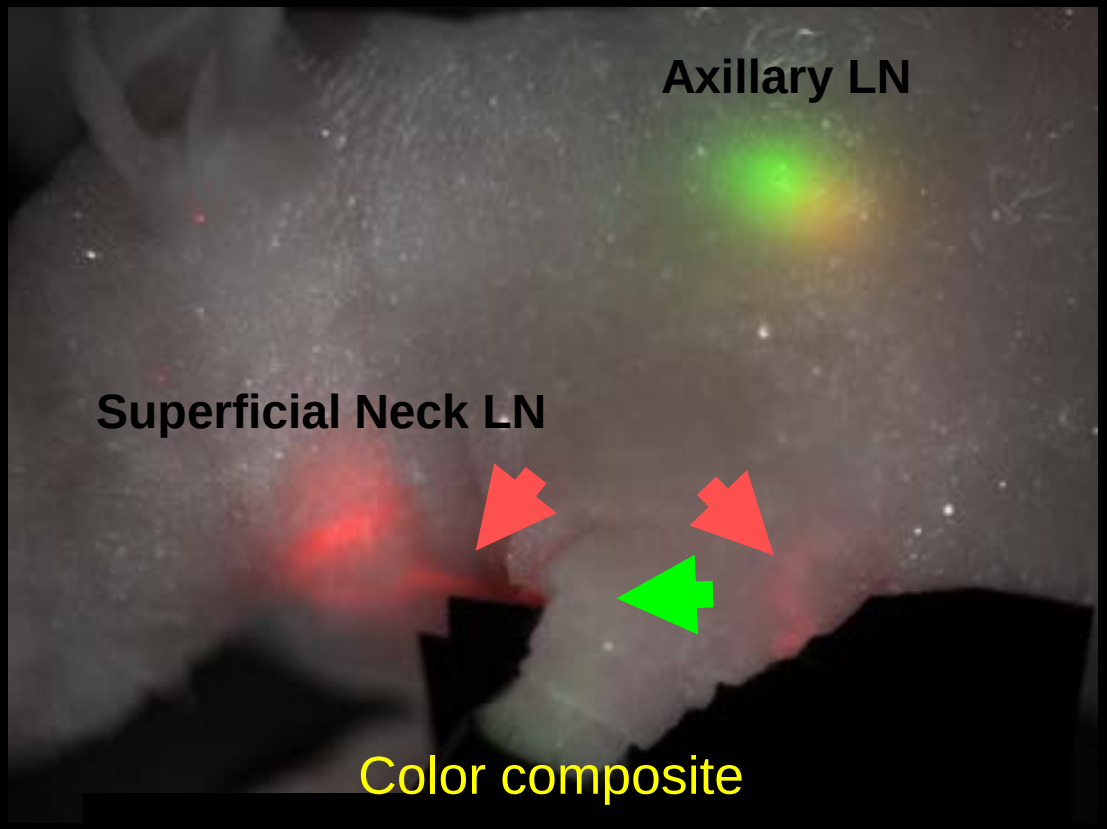
PeT based activatable fluorophore



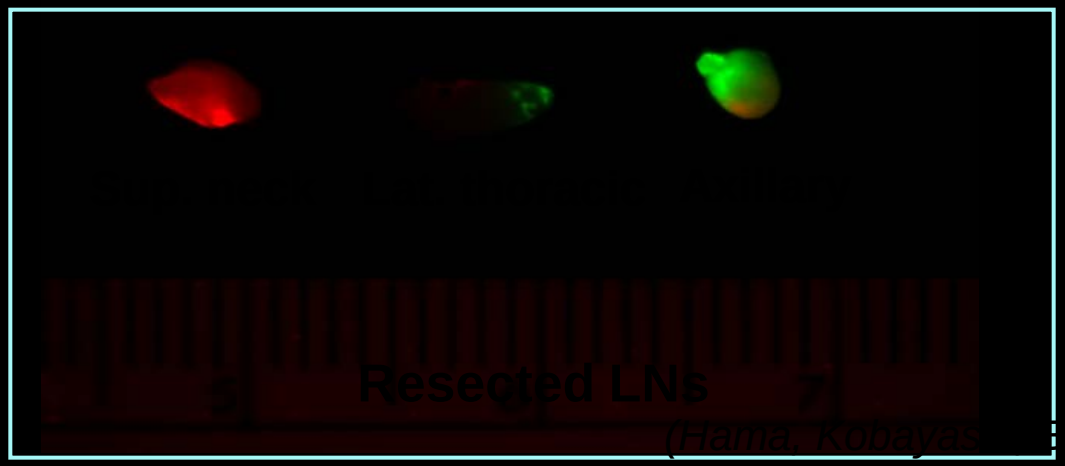




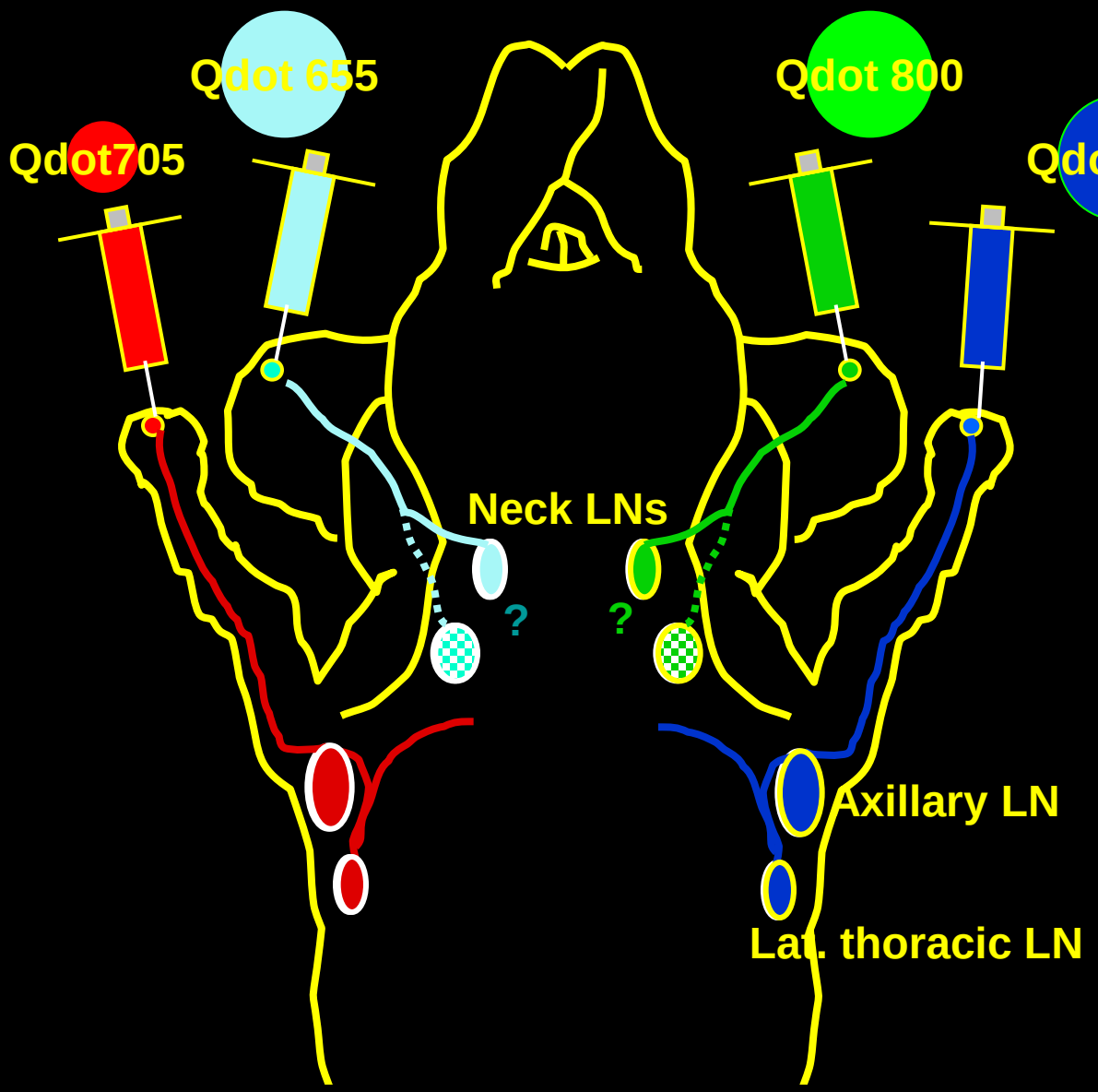
(Hama, Kobayashi; Breast Cancer Res Treat, 2006 in press)

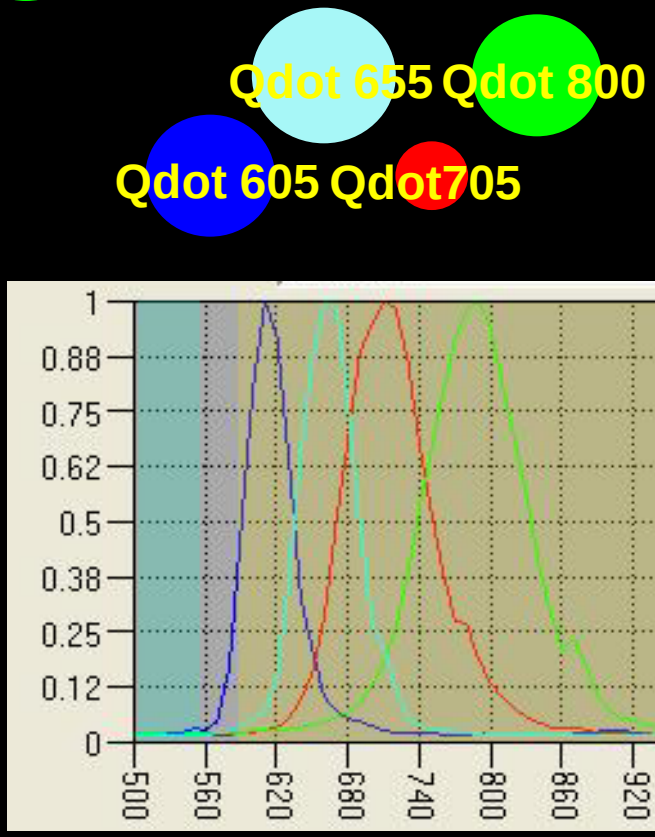


Qdot 705 from the breast (red)

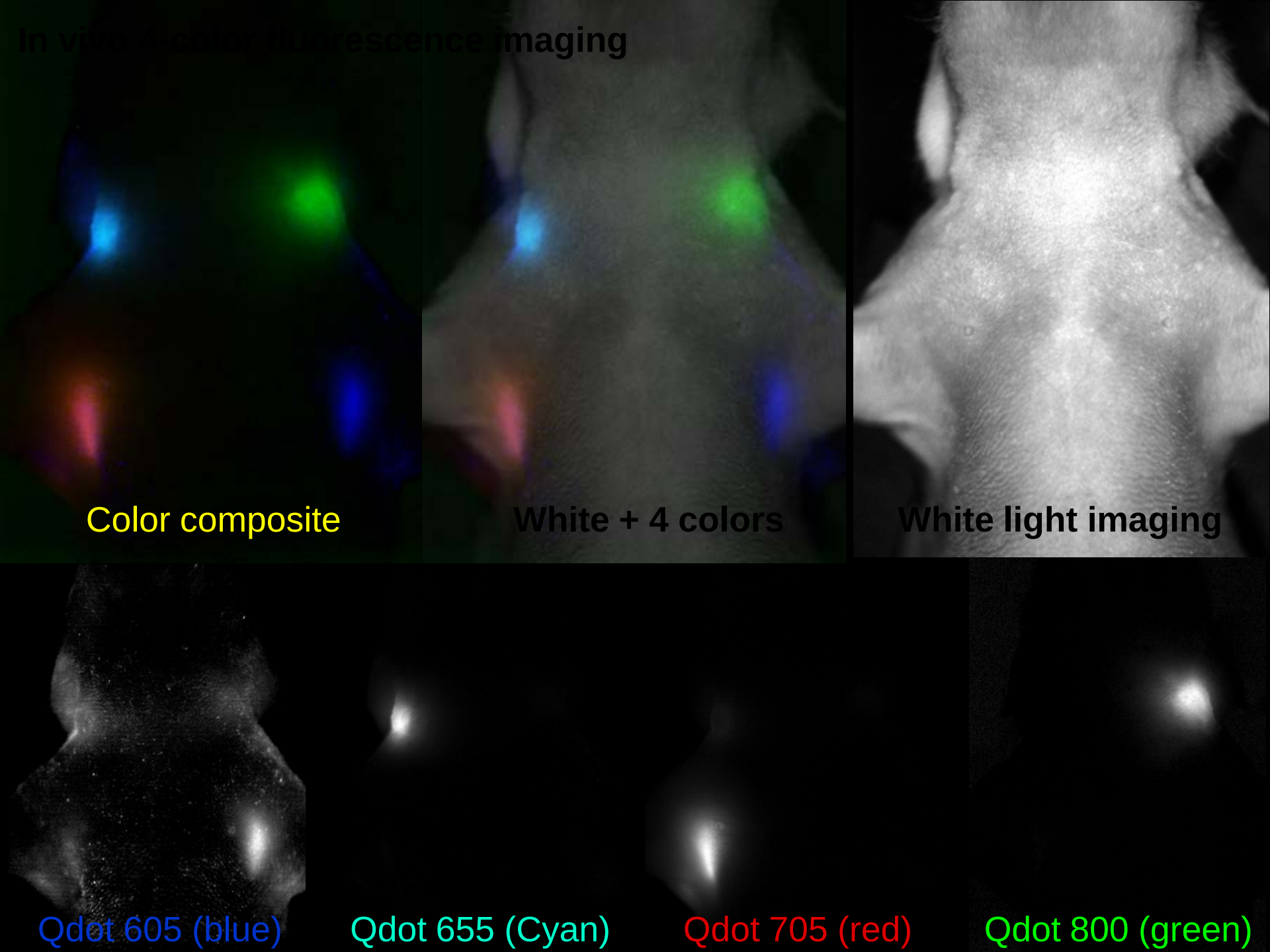


Qdot 800 from the upper Extremity (green)


Qdot705
Qdot 655
Qdot 800
Qdot 605
Neck LNs
?
?
Axillary LN
Lat. thoracic LN

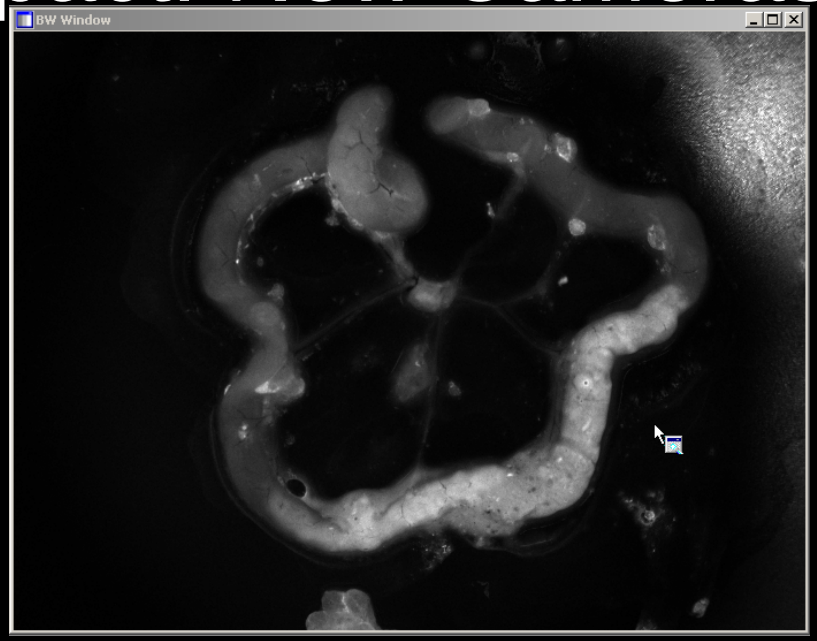

Qdot 655
Qdot 800
Qdot 605
Qdot705
1
0.88
0.75
0.62
0.5
0.38
0.25
0.12
0
500
560
620
680
740
800
860
920
980

In vivo 4-color fluorescence imaging

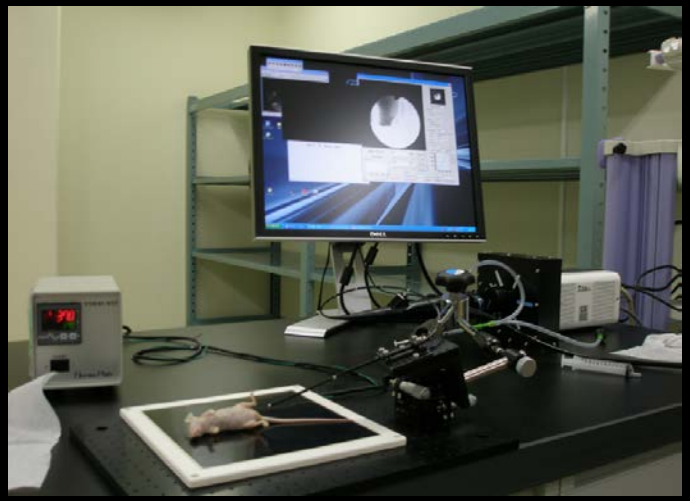


Towards Clinical Impact: New Cameras

Collaboration with Paul Smith, NIBIB



Gunn et al Cancer Sci 2007
Longmire et al. Top in Qntm Elect 2008



Collaboration with Yasuteru Urano, Univ. of Tokyo

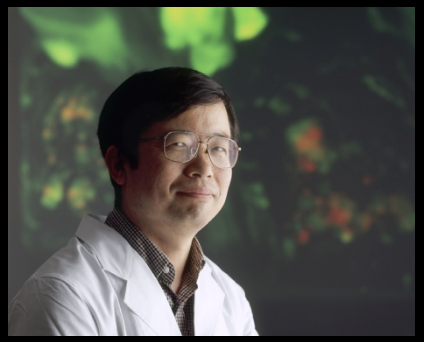
Summary

- Two most common in vivo imaging methods are Bioluminescence and Fluorescent Proteins
  - Convenient and straightforward to do
- Using exogenous contrast agents is more challenging but also potentially translatable clinically

Summary

- A variety of optical mechanisms can be used:
  - Always on agents
  - Intravenous activated agents
  - Intradermal/body cavity agents
- Requires knowledge of spectral processing and optimizing cameras

Acknowledgements



- Hisataka Kobayashi MD PhD
- Mikako Ogawa
- Nobu Kosaka
- Y. Hama (U. Tokyo)
- Marcelino Bernardo
- Yasuteru Urano (U. Tokyo)
- John Elkas, MD (INOVA Fairfax Hospital)
- AJ Gunn
- Raphael Alford
- Michelle Longmire