Optical imaging probes

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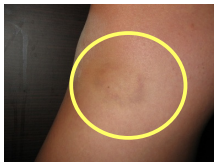
Optical Imaging

Q: - Why?

A: - It's the natural way
- We know how light travels through media
- It's easy (cheap) to see – detect light
- We can use probes to tag specific targets in model organisms
- Follow specific gene activities and pathways
- Study biological function, in vitro & in vivo
- Understand animal biology
- Translate to human level

Use:
- Absorption & Scattering (Light transport)
- Beacons – Fluorescent molecules



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Photonic Imaging

Methods

- Microscopy
- Photography
- Fluorescence reflectance imaging
- Diffuse optical tomography
  (contrast: Δabsorption, Δscattering)
- Fluorescence tomography
  (contrast: Δfluorescence)
- Bioluminescence
- Photoacoustic imaging
- Multimodal imaging

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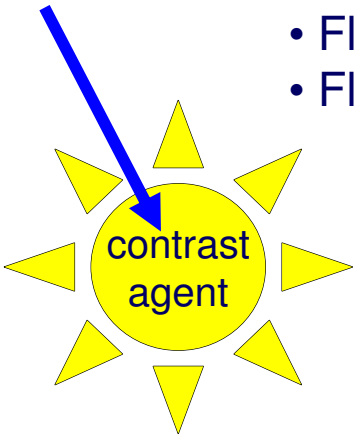
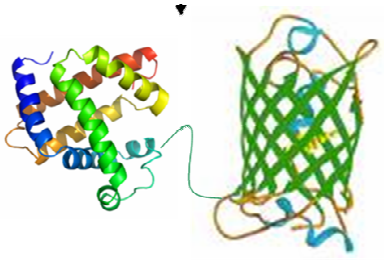
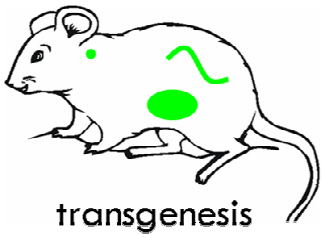
Photonic imaging

- Fluorescent dyes
- Fluorescent proteins



Labeling





targeted biological molecule

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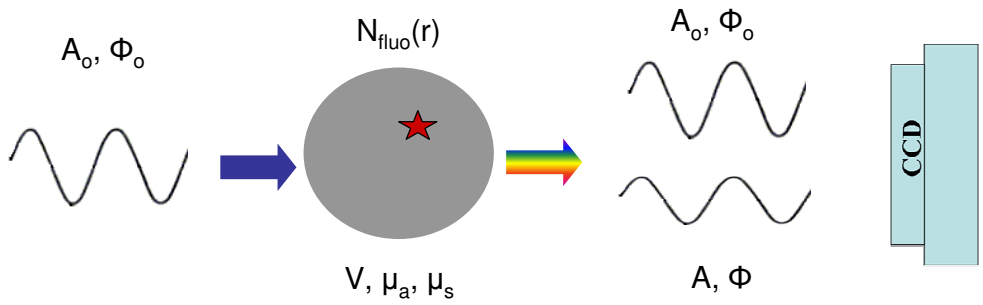
Photonic Imaging principle



A: Amplitude
Φ: Frequancy

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Imaging spectrum

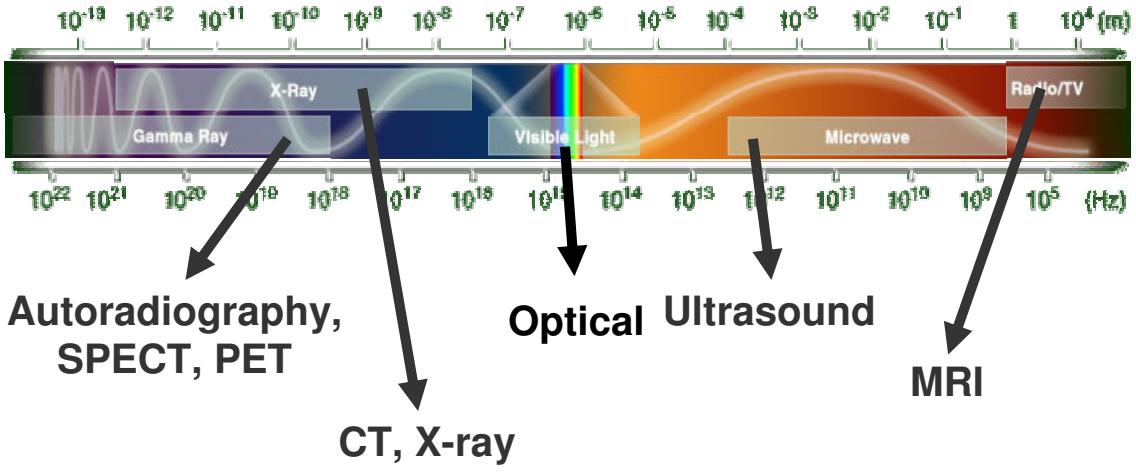

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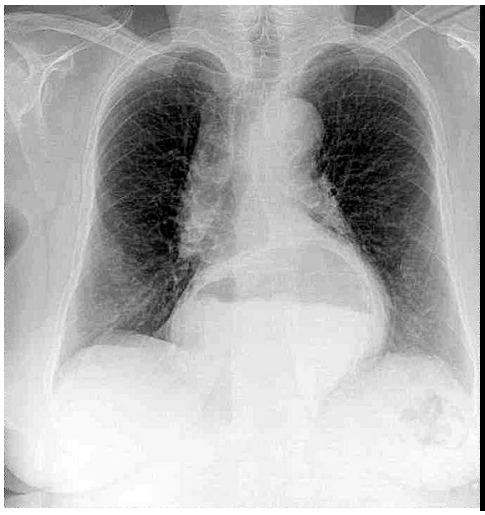
Image formation

Hardware based



Hardware & model based

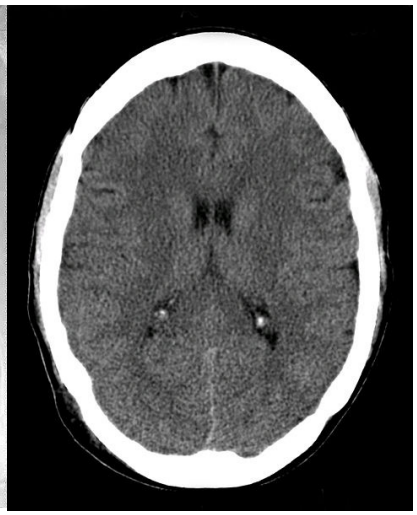


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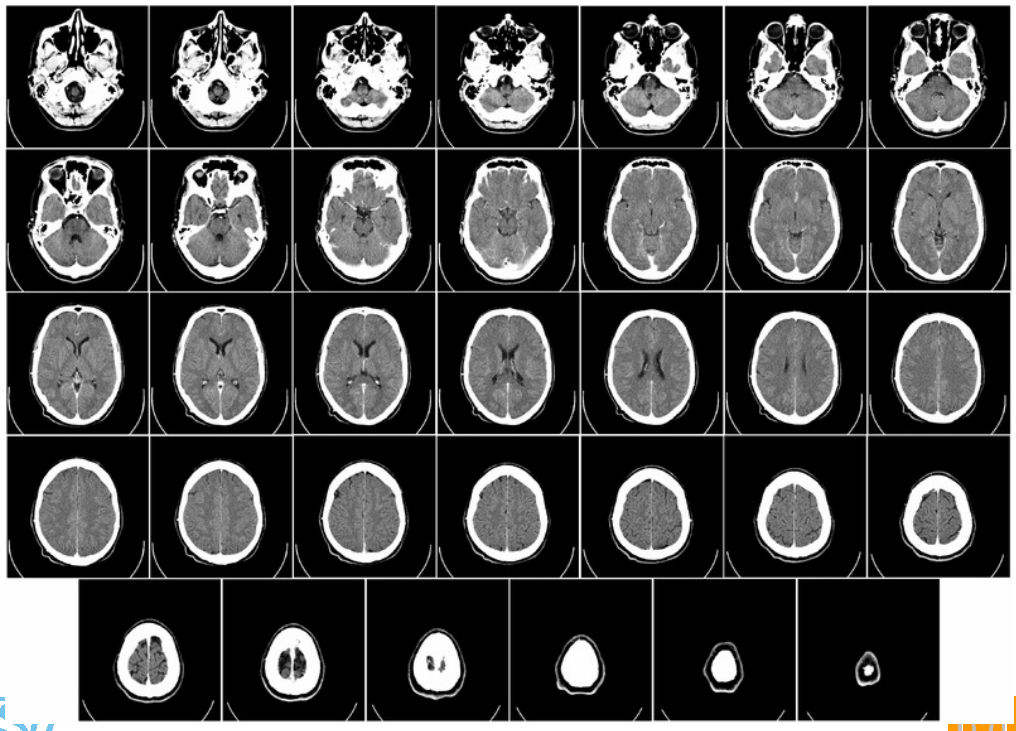
Image formation



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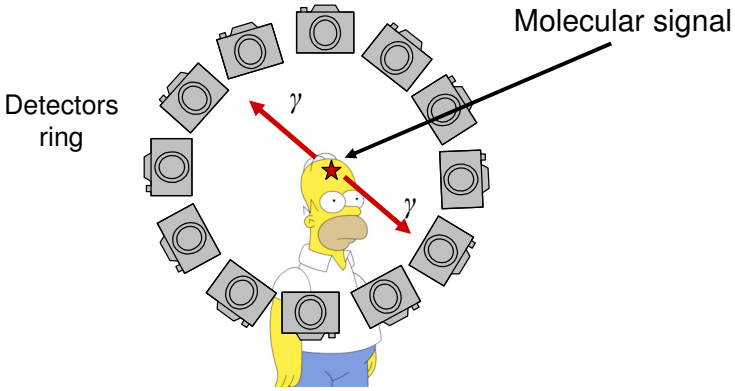
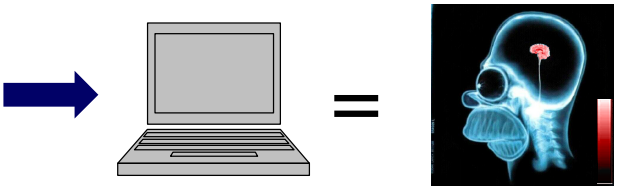
Imaging principle

1. **Hardware**



2. **Model**

$$P_c = P_1 P_2 = e^{-\int_0^a \mu(x)dx}$$



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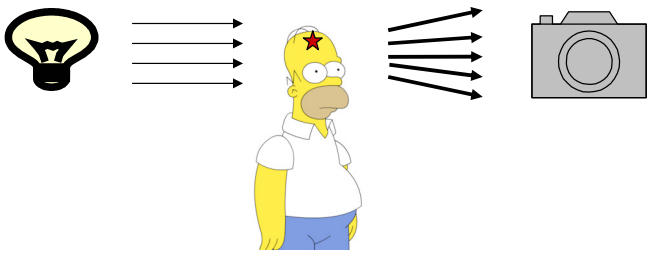
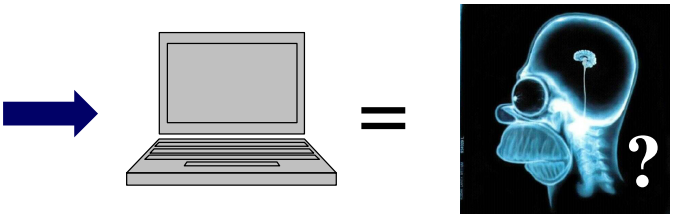
Tomographic imaging

1.



2.

$$U(\mathbf{r}) \propto \frac{e^{-\kappa r}}{r}$$



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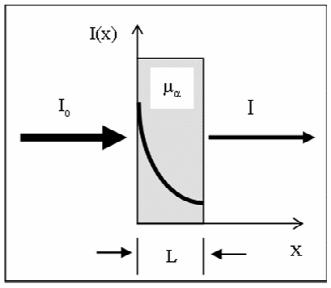
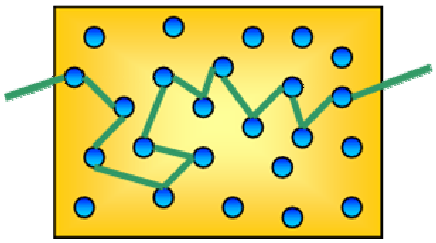
Light propagation

Low scattering



High scattering



Beer – Lambert law

$$I = I_0 e^{-\mu_t d}$$

Total attenuation coefficient $\mu_t = \mu_a + \mu_s$

Absorption coefficient μ_α

Scattering coefficient μ_s

Diffusion theory

$$\frac{1}{c}\frac{\partial U(\mathbf{r},t)}{\partial t} - D\nabla^2 U(\mathbf{r},t) + \mu_a U(\mathbf{r},t) = E(\mathbf{r},t)$$

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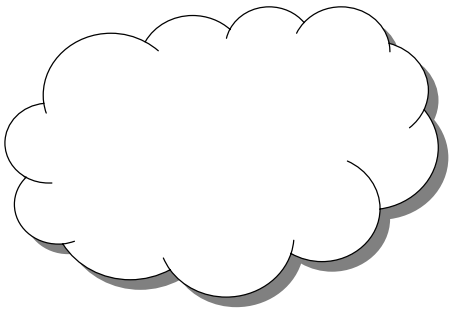
Scattering media



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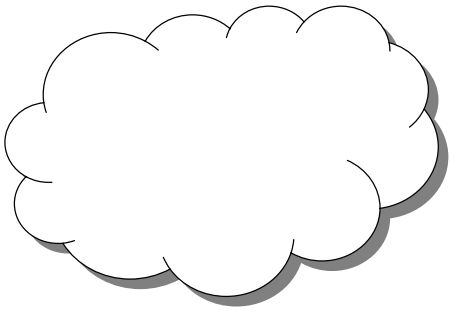
Scattering media



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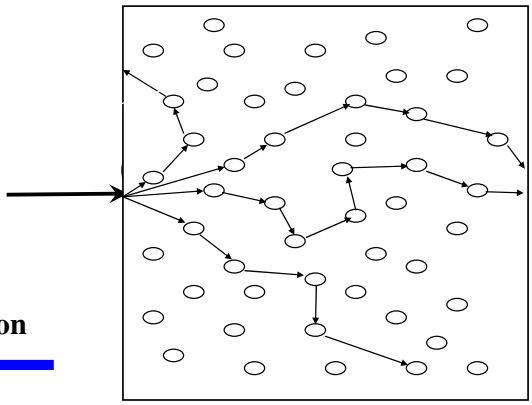
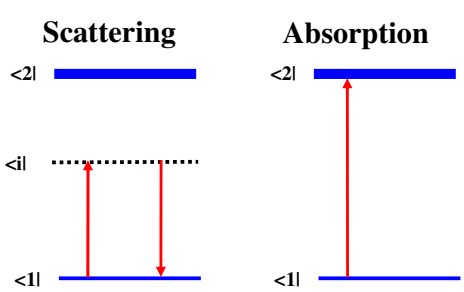
Scattering media





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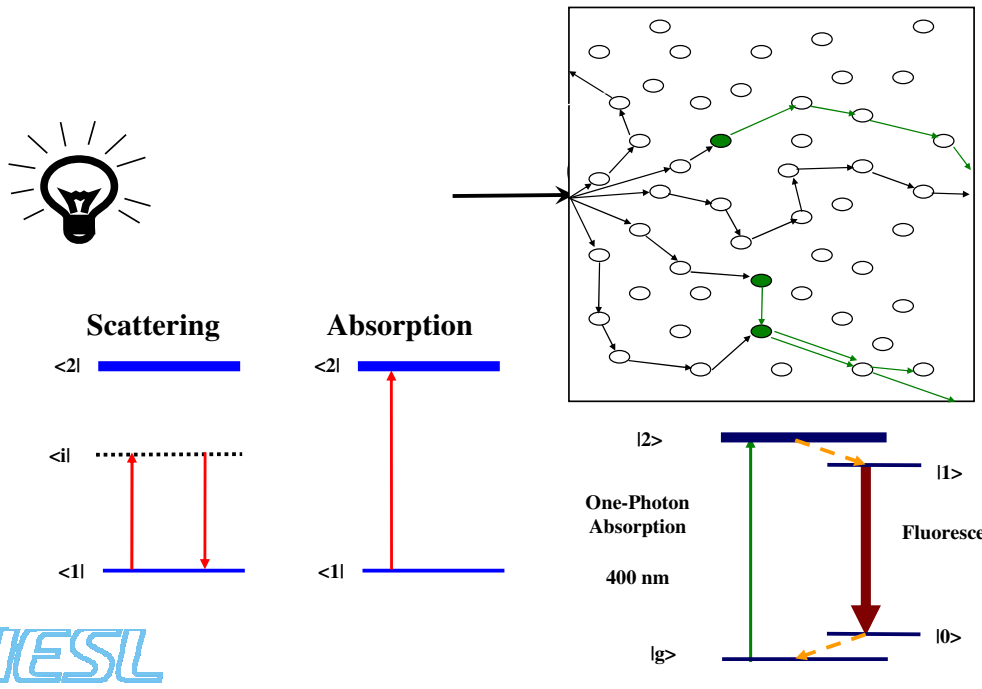
Scattering & fluorescing media



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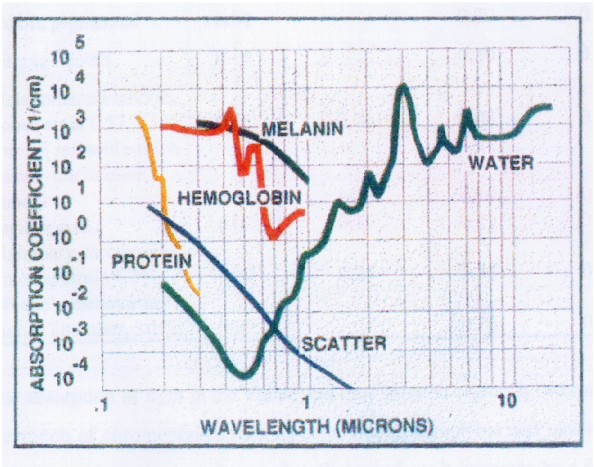
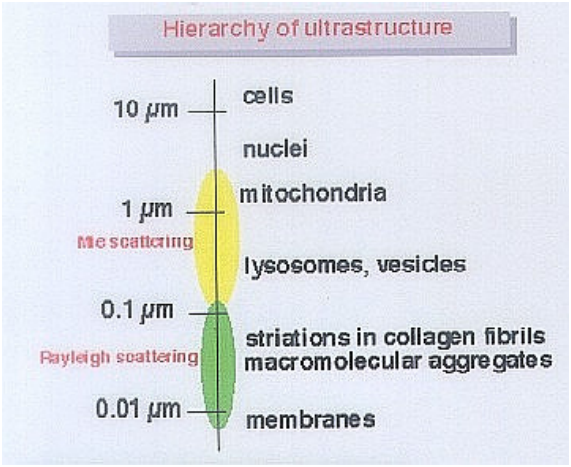
Biological tissue

Absorption



Scattering



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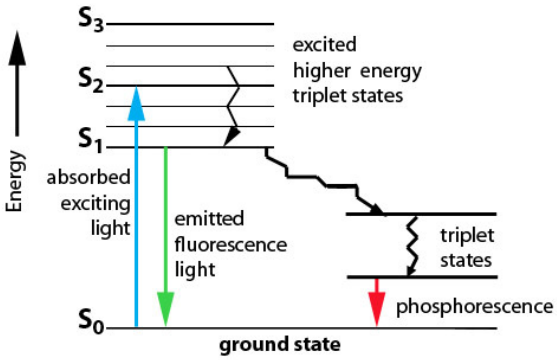
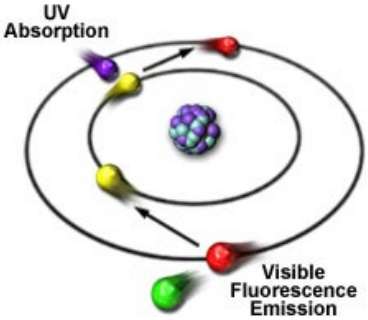
Fluorescence

Jablonski diagram





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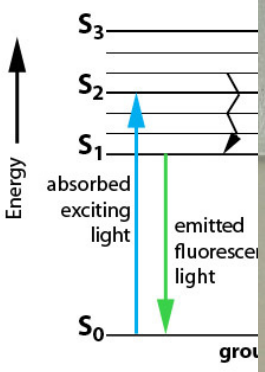
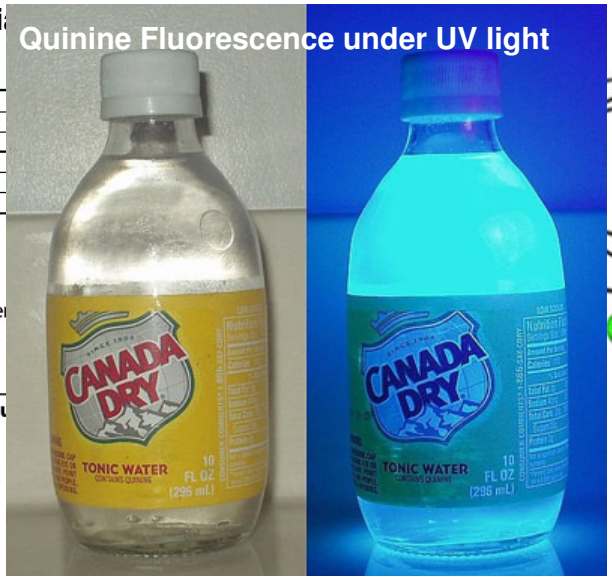
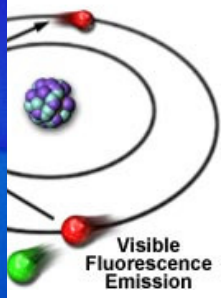
Fluorescence

Jablonski di







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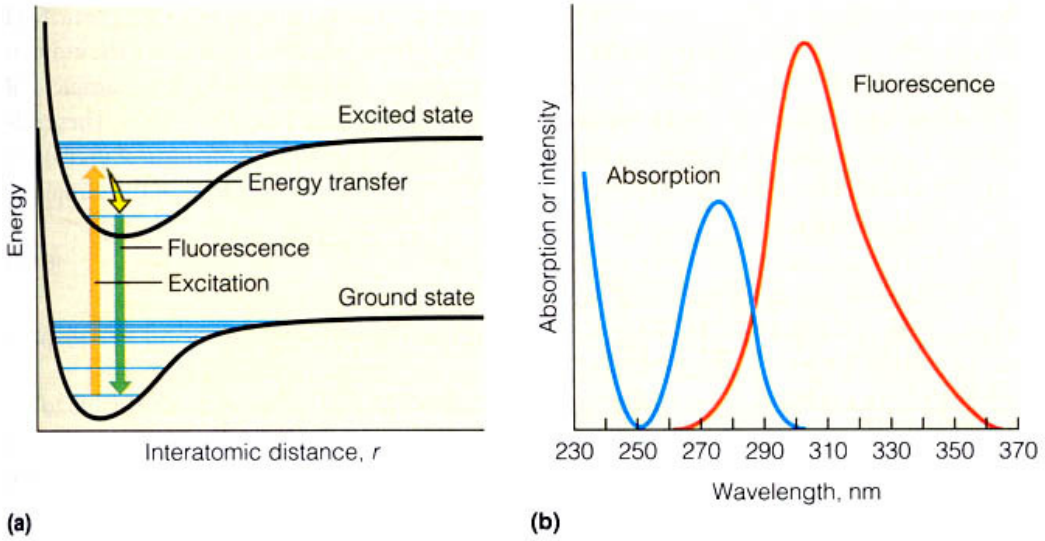
Fluorescence



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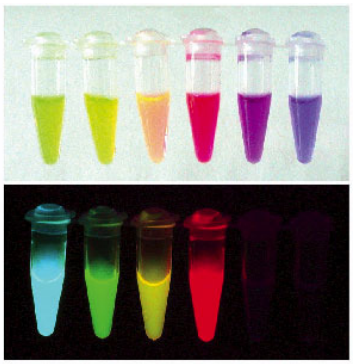
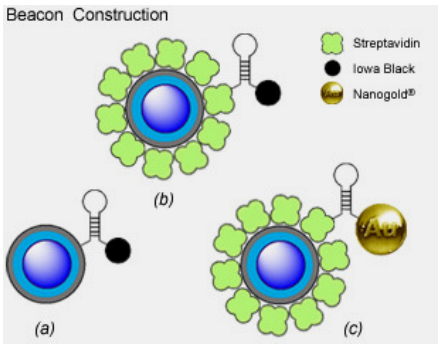
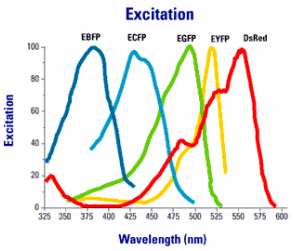
Contrast agents

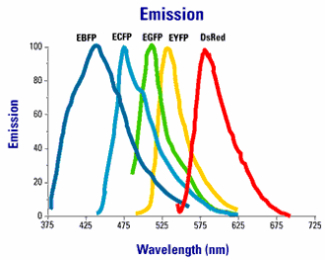
Fluorescing Proteins



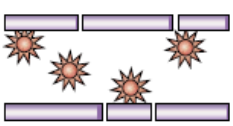
Nanoparticles





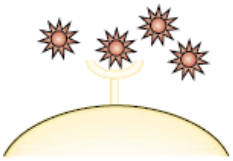


a Nonspecific probes



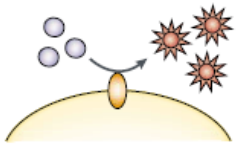
Detect physiology (blood volume, angiogenesis)

b Targeted probes



Used to localize proteins and determine structure

c Activatable 'smart' sensor probes



Used to localize enzymes and determine function

R. Weissleder, Nature Reviews, 2, 1-8 (2002)

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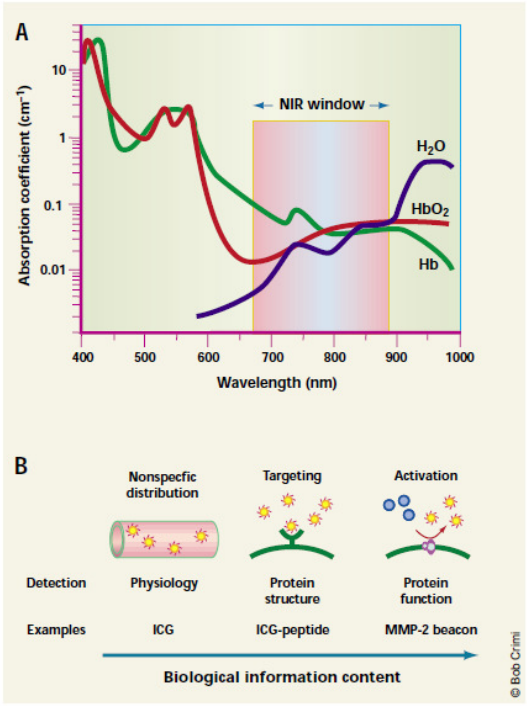
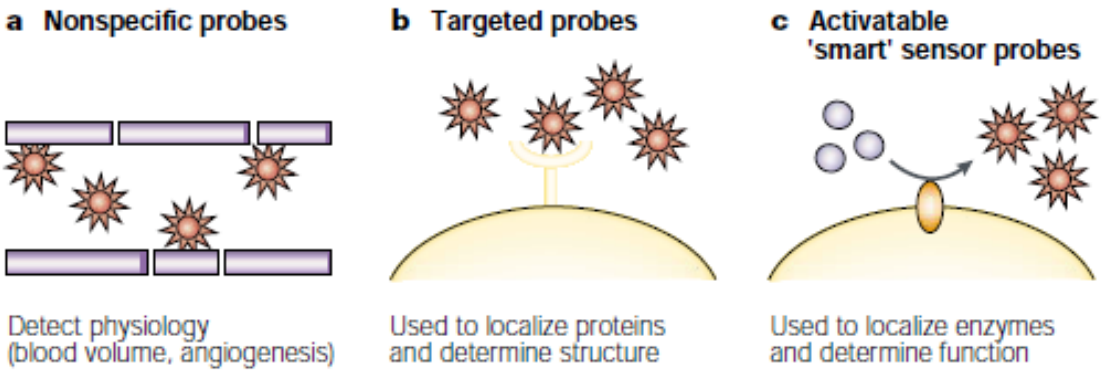
Contrast agents



R. Weissleder, Nature Biotech. 19, 316-317 (2001)

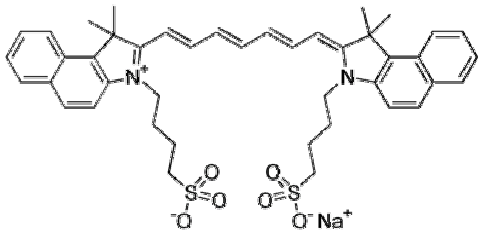
Contrast agents



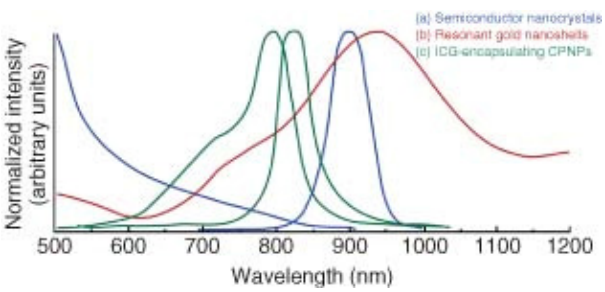
R. Weissleder, Nature Reviews, 2, 1-8 (2002)

Indocyanine Green (ICG)

Chemical structure



Spectra



- A tricarbocyanine dye with infrared absorption properties
- Absorption at 780nm and Emission at 820nm
- Very important because of the peak absorption and emission at the IR range
- Very little absorption in the visible range
- Approved for human use and has been used for decades

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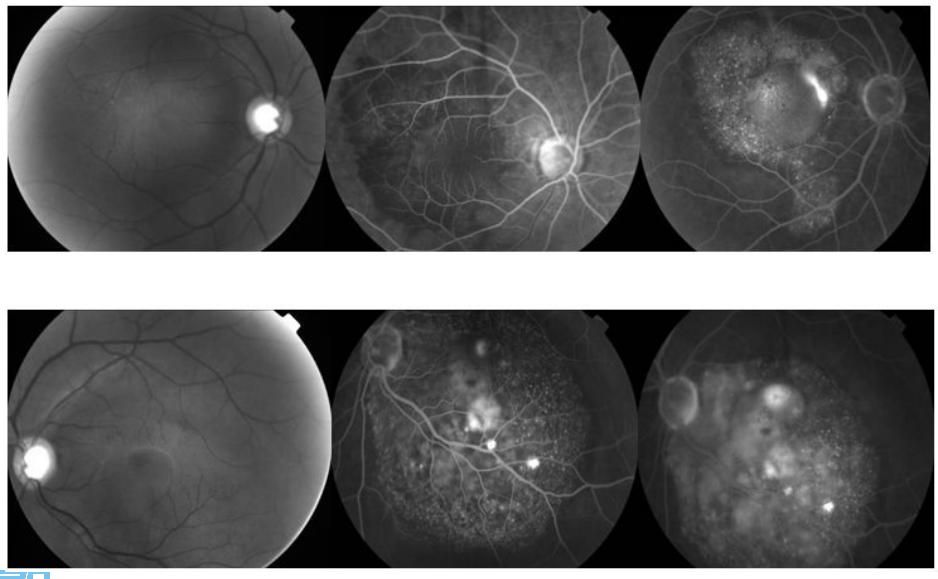
ICG clinical applications

Ophthalmic angiography



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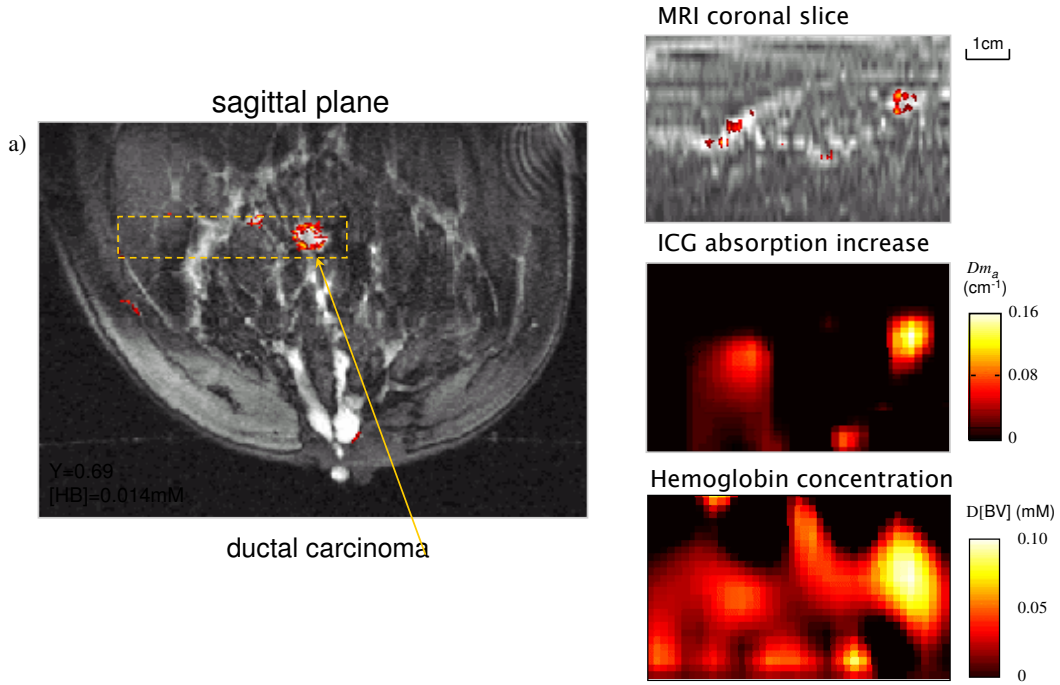
Mammography



Ntziachristos V., et. al. PNAS USA 97(6): 2767-2772 (2000).

MI luminescent probes

Table 2 Selected optical imaging probes

R. Weissleder et al, Nat. Biotech. 17, 375 (1999)

Reporter	Comment
Enzyme-activatable fluorochromes	
Cathepsin B	Cancer and inflammation marker
Cathepsin K	Osteoclasts
Cathepsin D	Cancer progression
Prostate-specific antigen (PSA)	Prostate cancer
Matrix metalloproteinases (MMP-2, -9, -13)	Cancer
Cytomegalovirus (CMV)	Infection
Human immunodeficiency virus (HIV) protease	Infection
Herpes simplex virus (HSV) protease	Infection
Thrombin	Thrombosis
Caspase-1	Apoptosis
Caspase-3	Apoptosis
Targeted fluorochromes	
Phosphatidylserine	Apoptosis
Somatostatin receptor	Cancer
Anti-tumor monoclonal antibody	Cancer
Hydroxyapatite (HA)	Calcification
Glucose transporter	Cancer
Folate receptor	Cancer
Fluorescent proteins	
Green fluorescence proteins (GFPs)	480–510 nm
DsRed (from *Discosoma*)	520–580 nm
HcRed (from *Heteractis crispa*)	600–650 nm
Bioluminescent proteins	
Firefly luciferase + benzothiazole luciferin	560–610 nm emission, high Q
Renilla reniformis luciferase + coelenterazine	460–490 nm, lower QY

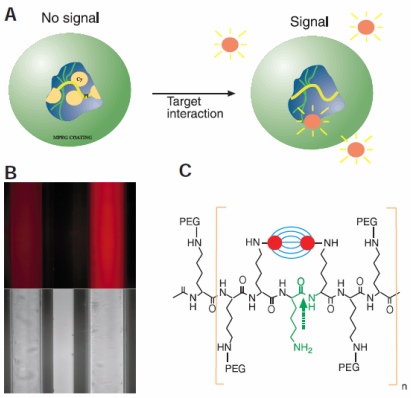


Figure 1. (A) Schematic diagram of probe activation. The initial proximity of the fluorochrome molecules to each other results in signal quenching (B) NIRF image (top) and bright light image (bottom) of nonactivated C-PGC (left) and activated probe (right). Fluorochrome concentration: 0.17 μM. Image acquisition time: 30 s. Excitation: 670 nm, emission: 700 nm. Note the difference in signal intensity between enzyme-activated and unactivated probe. (C) Chemical structure of repeating graft copolymer segment indicating quenching of Cy and enzymatic degradation site (green arrow).

Enzyme activity

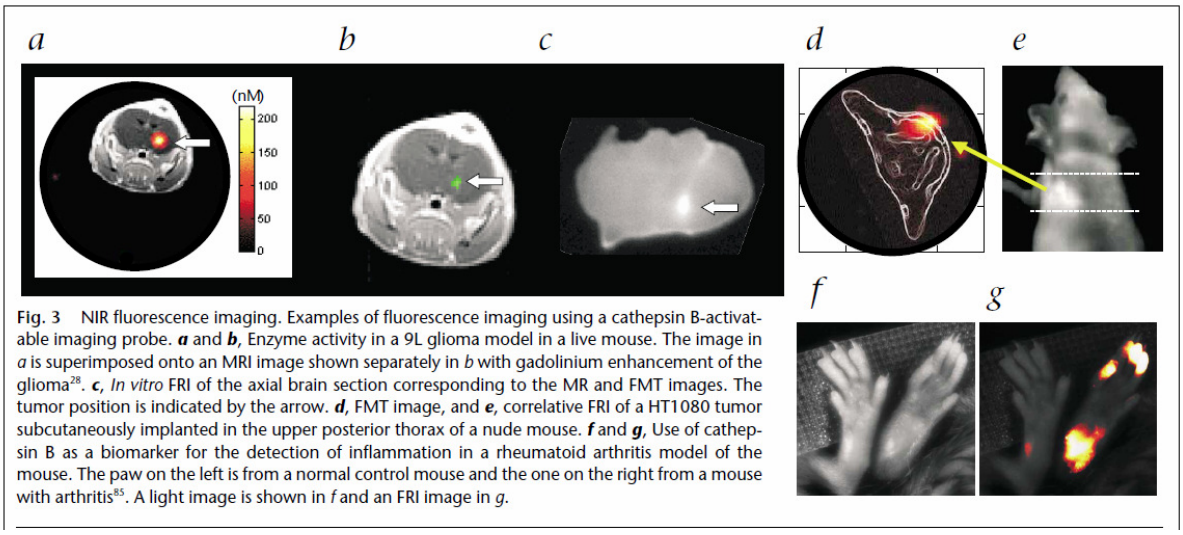
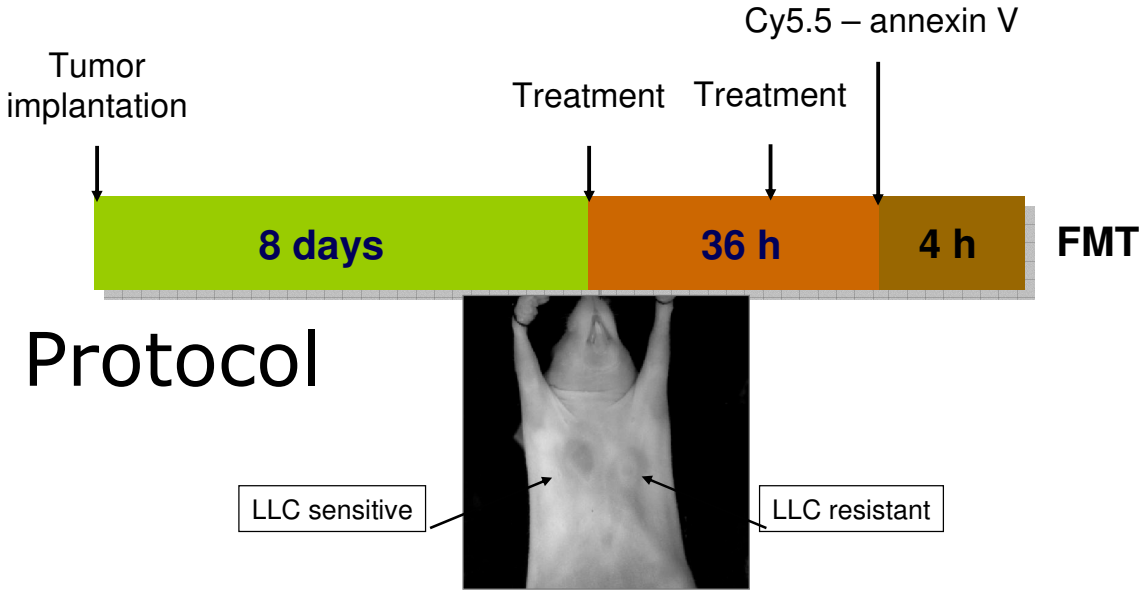


Fig. 3 NIR fluorescence imaging. Examples of fluorescence imaging using a cathepsin B-activatable imaging probe. ***a*** and ***b***, Enzyme activity in a 9L glioma model in a live mouse. The image in *a* is superimposed onto an MRI image shown separately in *b* with gadolinium enhancement of the glioma[28]. ***c***, *In vitro* FRI of the axial brain section corresponding to the MR and FMT images. The tumor position is indicated by the arrow. ***d***, FMT image, and ***e***, correlative FRI of a HT1080 tumor subcutaneously implanted in the upper posterior thorax of a nude mouse. ***f*** and ***g***, Use of cathepsin B as a biomarker for the detection of inflammation in a rheumatoid arthritis model of the mouse. The paw on the left is from a normal control mouse and the one on the right from a mouse with arthritis[85]. A light image is shown in *f* and an FRI image in *g*.

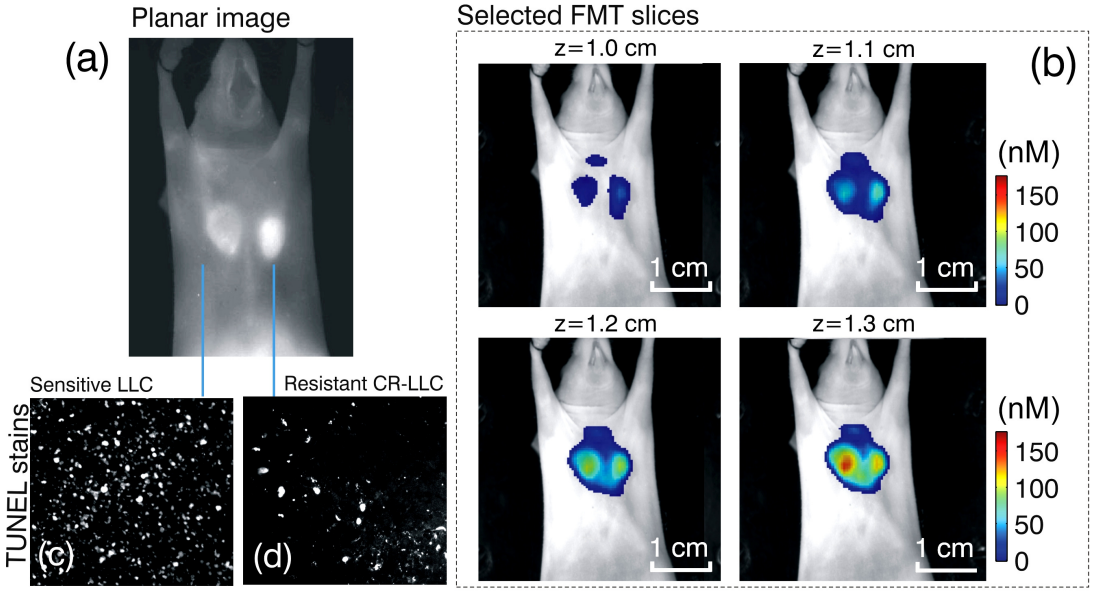
R. Weissleder and V. Ntziachristos, Nat. Med. 9, 123 (2003)

Treatment response



V. Ntziachristos et al, Proc. Natl. Acad Sci **101**(33) (2004).

Treatment response



V. Ntziachristos et al, Proc. Natl. Acad Sci **101,** 33 (2004).

Nanoparticles

Nanoparticles

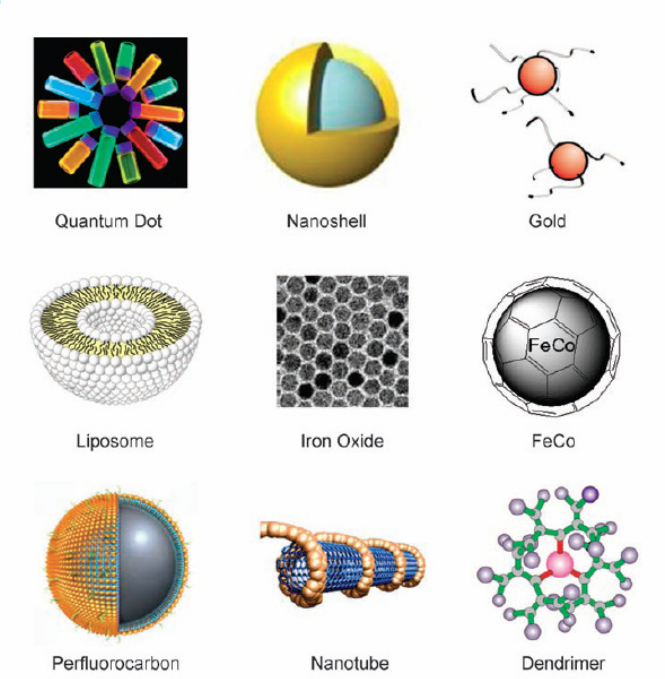
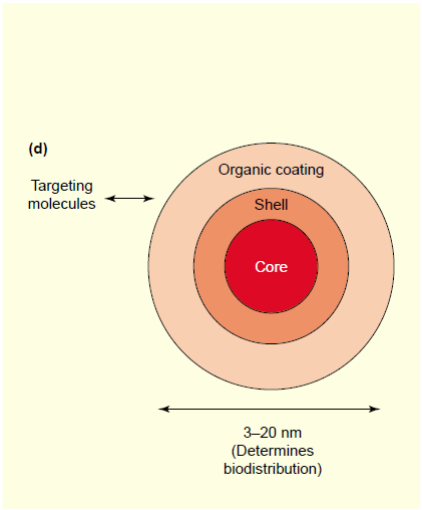
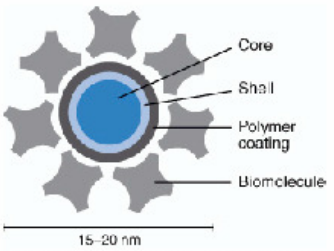


Figure 1. Representative nanoparticles that can serve as nanoplatforms for targeted molecular imaging in living subjects.

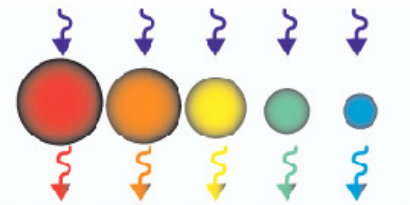


W. Cai and X. Chen, Small 3, 1840 (2007)

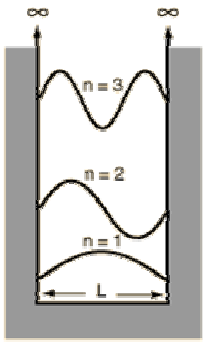
Quantum Dots



Quantum dot structure



Tuneability of quantum dot



- High quantum yield (90%)
- Tunable emission wavelength by varying the dimensions of the Qdot
- Resistant to photobleaching, very useful for 3D in vivo imaging
- Broad absorption spectrum compared to fluorophores
- **Photo-toxicity**

$$E_n = \frac{n^2 h^2}{8mL^2}$$

Quantum Dots

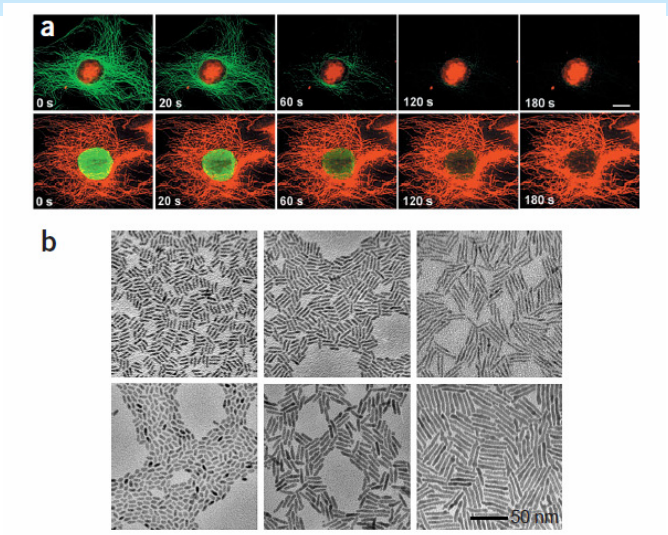
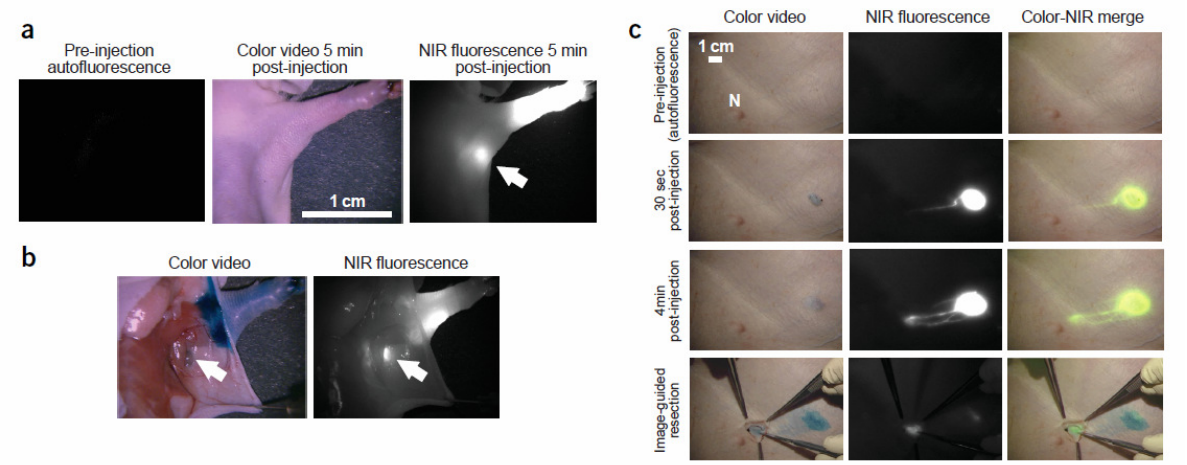


Figure 1 Quantum confinement in semiconductors and new biological labels. (**a**) Cell labeling with quantum dots and illustration of quantum dot photostability, compared with the dye Alexa 488. In the upper panels, the nucleus is stained red with quantum dots and the actin fibers are stained green with the dye. In the lower panel, the labeling is reversed. (Reprinted from ref. 19.) (**b**) Transmission electron micrographs of quantum rods—a new nanostructure that may have uses as a biological label with polarized emission, reduced blinking and faster radiative rates than dots. The time

P. Alivisatos, Nat. Biotech 22, 47 (2004)

Quantum dots

Sentinel lymph node mapping in mice



J. V. Frangioni et al, Nat. Biotech. 22, 93 (2004)

Quantum dots

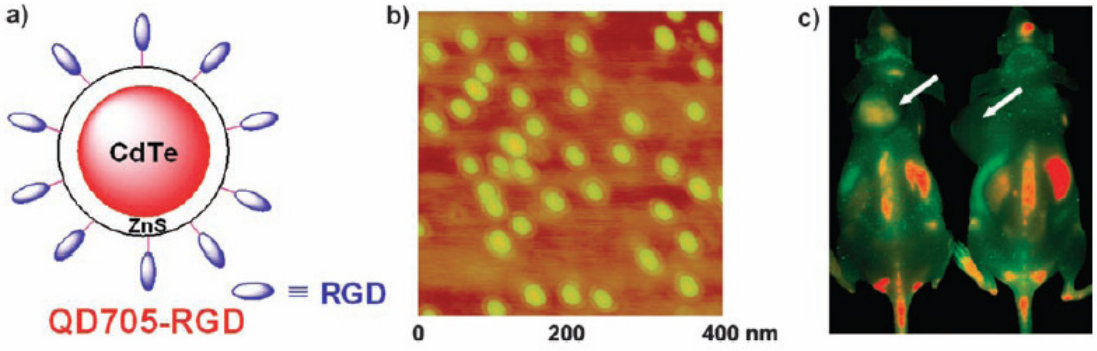
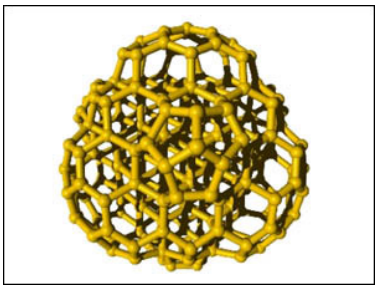
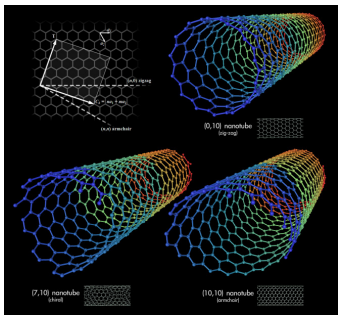


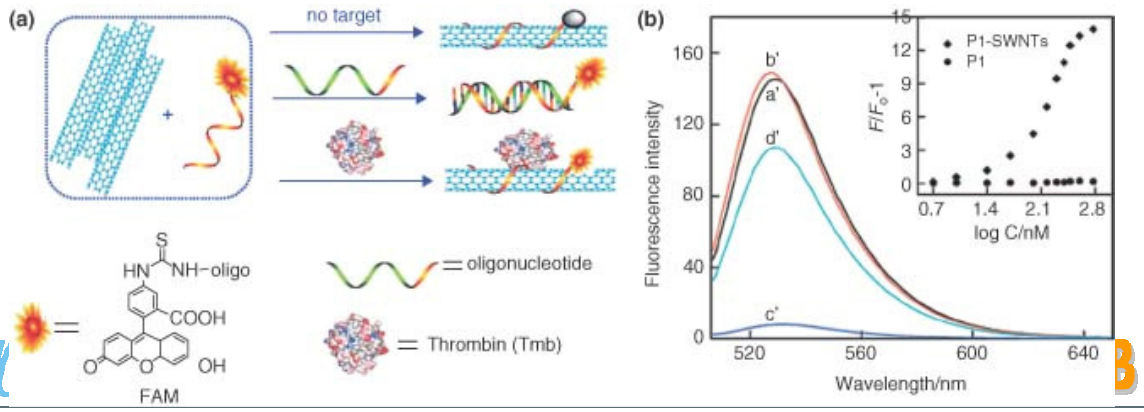
Figure 2. RGD peptide-conjugated QD705 for NIRF imaging of tumor vasculature. a) A schematic illustration of the probe QD705-RGD. b) An atomic force microscopy image of QD705-RGD deposited on a silicon wafer. c) In vivo NIRF imaging of tumor vasculature in U87MG human glioblastoma-tumor-bearing mice. The mouse on the left was injected with QD705-RGD and the mouse on the right was injected with QD705. Arrows indicate tumors (adapted from Ref. [48]).

W. Cai and X. Chen, Small 3, 1840 (2007)

Carbon dots and wires







Nanoparticles

Table 1. Nanoplatforms composed of different materials have been reported for biomedical applications using various molecular imaging modalities. Note that some nanoparticles do not possess intrinsic imaging signal, thus the modality depends on the label used in a particular study.

Nanoplatform	Composition	Imaging modality	References
Quantum dot	CdSe, CdTe	optical fluorescence	[47, 48, 53, 55]
Nanoshell, nanocage	gold	OCT	[62–64]
Iodinated nanoparticle	iodine	CT	[70–75]
Bi_2S_3 nanoparticle	Bi_2S_3	CT	[77]
PFC nanoparticle	perfluorocarbon	depends on the label	[89–91, 122–126, 138]
SPIO, CLIO	iron oxide	MRI	[97–117, 146-152]
FeCo nanoparticle	Fe, Co	MRI	[118]
MnMEIO	Mn, iron oxide	MRI	[132,133]
SWNT	carbon	depends on the label	[144]
Liposome	phospholipid	depends on the label	[121, 145, 156]

W. Cai and X. Chen, Small 3, 1840 (2007)

Nanoparticles

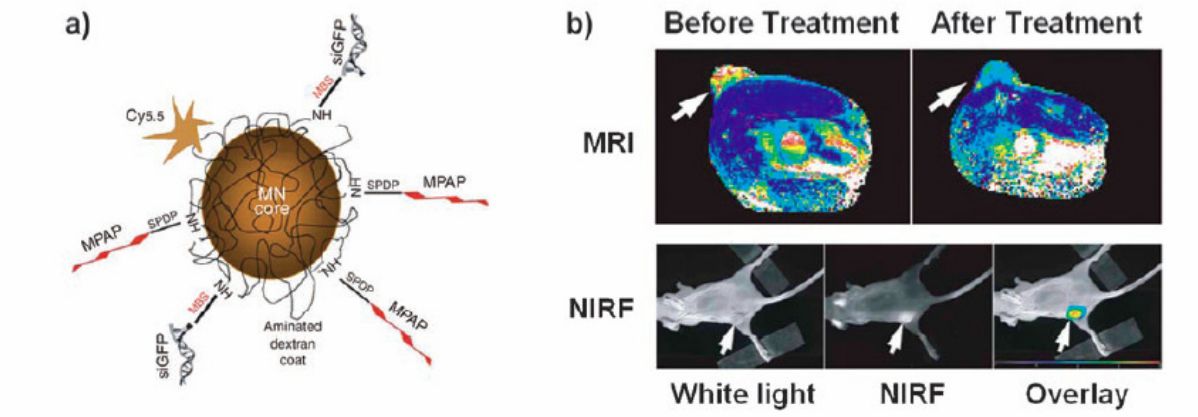


Figure 9. A multifunctional probe for in vivo dual-modality imaging and therapy. a) Schematic illustration of the multifunctional probe consisting of a magnetic nanoparticle labeled with a near-infrared dye Cy5.5, membrane translocation peptides (MPAP), and siRNA molecules targeting green fluorescent protein (siGFP). b) In vivo MRI of mice bearing subcutaneous LS174T human colorectal adenocarcinoma (arrows) before and after treatment. A high-intensity NIRF signal in the tumor confirmed the delivery of the nanoparticle (adapted from Ref. [157]).

W. Cai and X. Chen, Small 3, 1840 (2007)

FLUORESCING PROTEINS

Timeline

1955: Green fluorescent substance in jellyfish first described.

1962: GFP identified as protein, extracted from 10,000 jellyfish

1969: “Green protein” named green fluorescent protein.

1974: Intermolecular energy transfer between aequorin and GFP in jellyfish

1979: Shimomura characterized structure of chromophore.

1985: Prasher clones and expresses aequorin.

1992: GFP cloned.

1994: GFP expressed in *E. coli* and *C. elegans*. Mechanism for chromophore formation. First new color (blue).

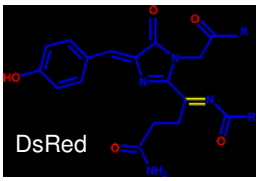
1996: Tsien designs a mutant based on crystal structure of GFP. It is yellow fluorescent.

1997: FRET between two fluorescent proteins used as a Ca2+ sensor named Cameleon

Timeline

1999: Red fluorescent proteins (DsRed) discovered in anthozoan corals. Leads to discovery of many new fluorescent proteins and chromoproteins.

2000: The biggest difference between green fluorescent protein and it's red analog, DsRed, is that the chromophore of DsRed has an extra double bond which extends the chromophores conjugation and causes the red-shift.



2003: Kindling protein (KFP) can undergo irreversible photo-conversion from non-fluorescent to stable red fluorescent form upon photo-activation.

2004: New “fruit” FP’s generated by in vitro and in vivo directed evolution.

2007: mKate, bright far-red FP.

2008: The first mutant of the *Aequorea victoria* GFP that forms a red chromophore reported.

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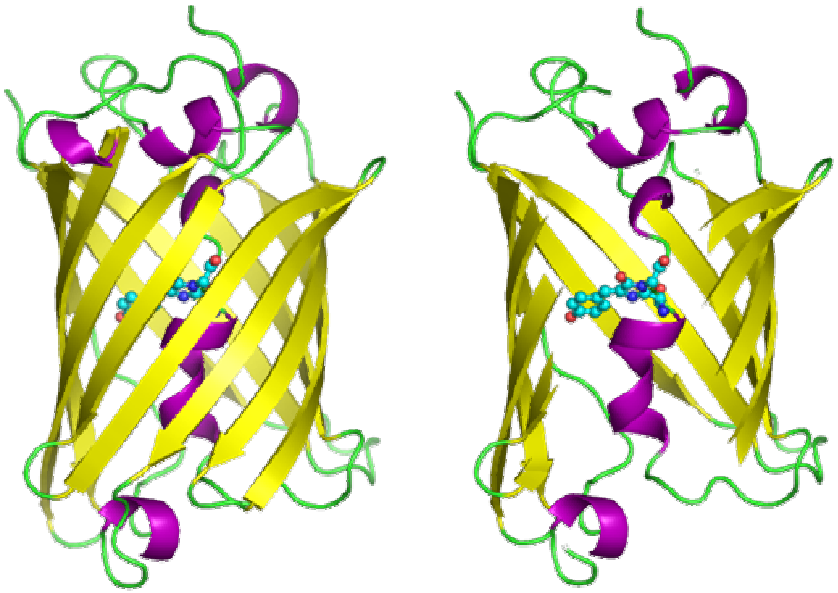
GFP Structure



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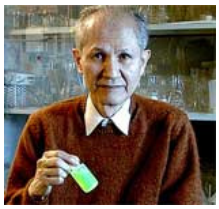
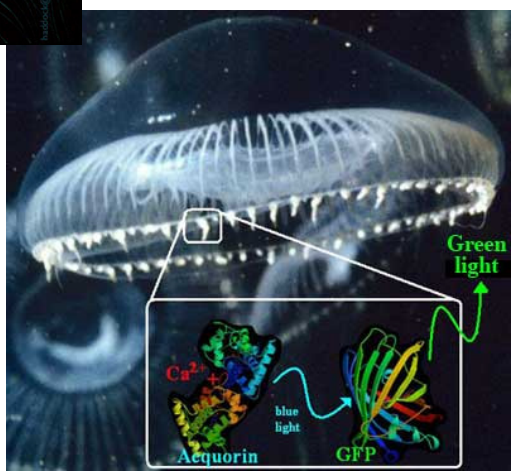
Osamu Shimomura



Osamu Shimomura was the first person to isolate GFP and to find out which part of GFP was responsible for its fluorescence. His meticulous research laid the solid foundations on which the GFP revolution was built. In 1960, shortly after he arrived in Princeton from Japan, Shimomura started studying the bioluminescence of the crystal jellyfish, *Aequorea victoria.*



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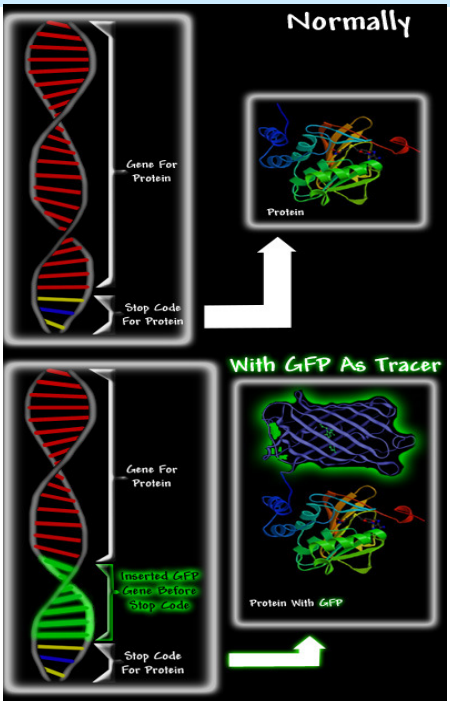
Douglas Prasher

Douglas Prasher was the first person to realize the potential of GFP as a tracer molecule. In 1987 he got the idea that sparked the GFP revolution. He thought that GFP from a jellyfish could be used to report when a protein was being made in a cell. Proteins are extremely small and cannot be seen, even under an electron microscope. However if one could somehow link GFP to a specific protein, for example hemoglobin, one would be able to see the green fluorescence of the GFP that is attached to the hemoglobin. It would be a bit like attaching a light bulb to the hemoglobin molecule.



Gene 111, 229-233, (1992)

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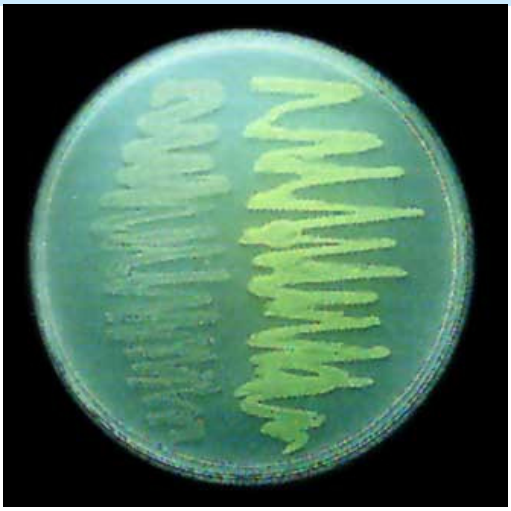
Marty Chalfie



Science 263, 802 (1994)

Chalfie wanted to use GFP as a marker that could be attached to a promoter. The promoter is a region of DNA located in front a gene, when the cell needs to make a specific protein, it binds to the promoter for that gene, which in turn activates the gene. By attaching GFP to a promoter, Chalfie was hoping that GFP would be produced whenever the promoter it was attached to was activated; in this way GFP fluorescence could be used to signal activation of the GFP-tagged promoter.

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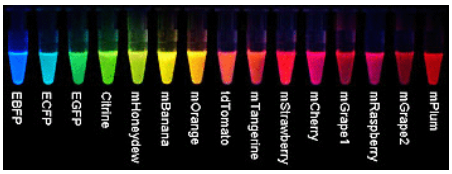
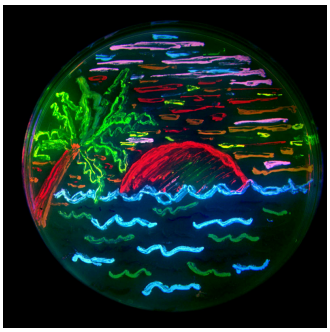
Roger Tsien





While Shimomura, Prasher and Chalfie were all instrumental in taking GFP from the jellyfish and showing that it can be used as a tracer molecule, it is Roger Tsien who is responsible for much of our understanding of how GFP works and for developing new techniques and mutants of GFP. His group has developed mutants that start fluorescing faster than wild type GFP, that are brighter and have different colors.

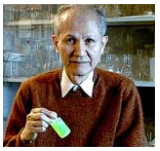
IESL IMBB

2008 Nobel Prize in Chemistry

Press Release

8 October 2008

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry for 2008 jointly to:



Osamu Shimomura, Marine Biological Laboratory (MBL), Woods Hole, MA, USA and Boston University Medical School, MA, USA,

Martin Chalfie, Columbia University, New York, NY, USA
and

Roger Y. Tsien, University of California, San Diego, La Jolla, CA, USA

"for the discovery and development of the green fluorescent protein, GFP".

A MOLECULAR MICROSCOPE

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Spectra

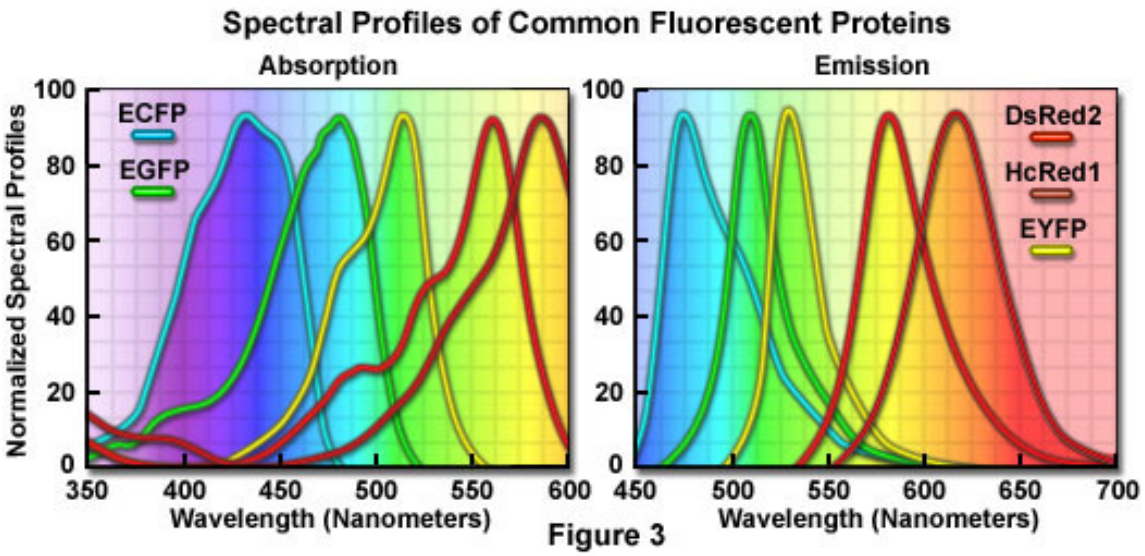
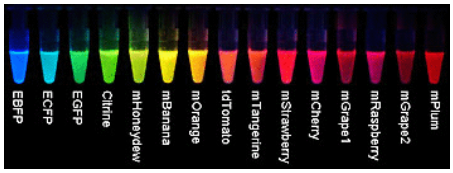


Figure 3



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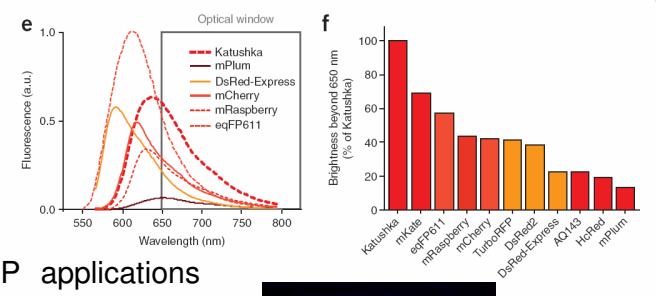
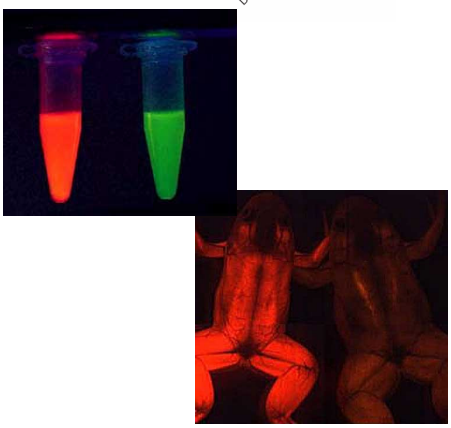
Red coral proteins



A major breakthrough in GFP applications came when Sergey Lukyanov found some GFP-like proteins in corals. No one before Lukyanov had tried looking for GFP-like proteins in corals because corals do not bioluminesce (that is give off light using chemical energy like fireflies do). Lukyanov also found a red fluorescent protein (called DsRed). His findings resulted in the discovery of many new GFP-like proteins in non-bioluminescent and sometimes even non-fluorescent marine organisms.



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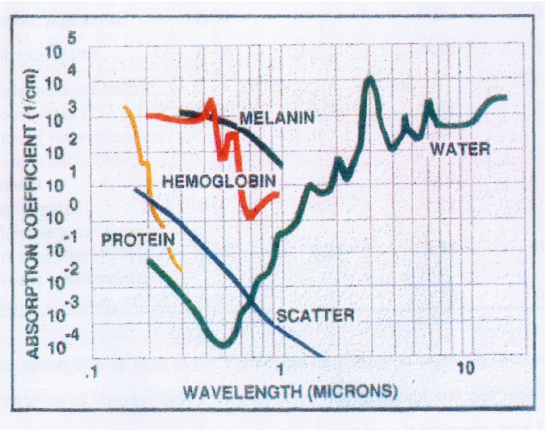
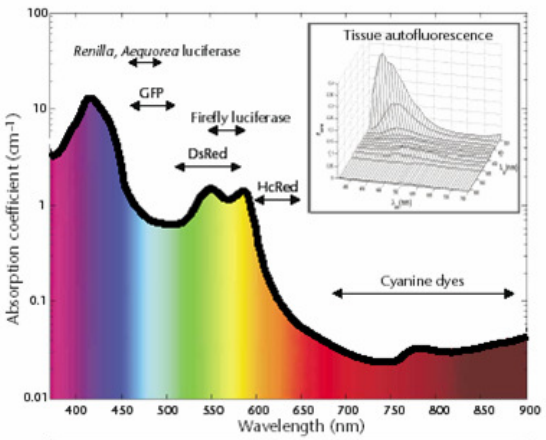
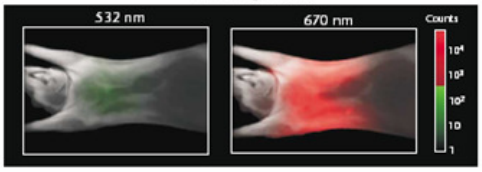
Why Near Infra Red?







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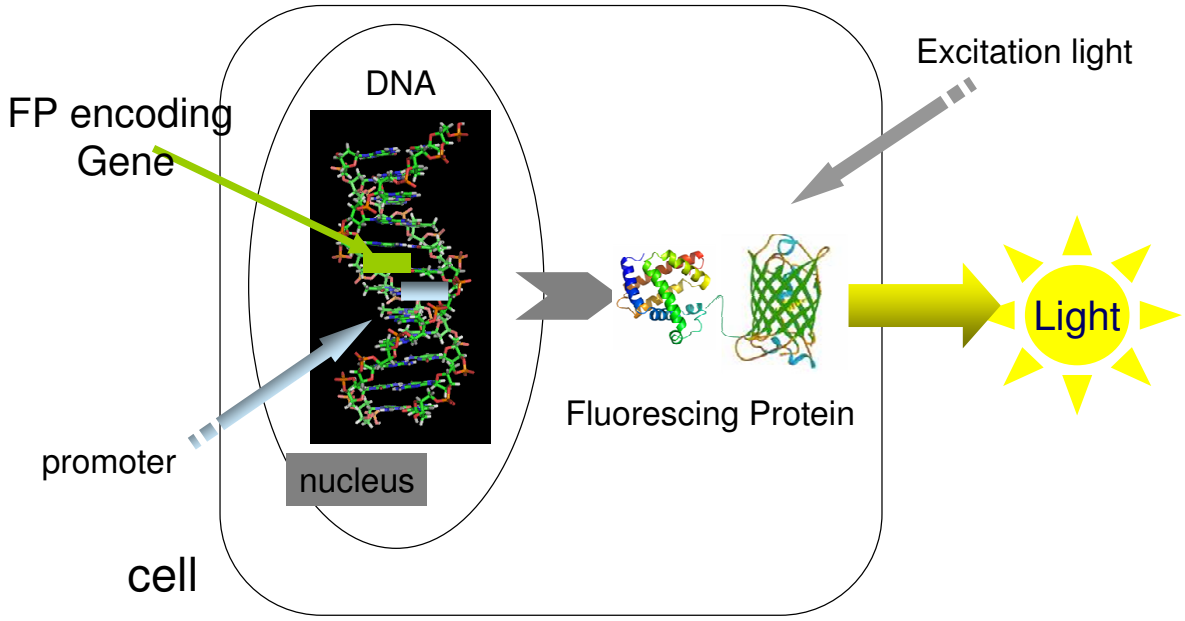
Fluorescence protein labeling



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Significance

- **FPs are visual markers**
- **Study of biological processes (example: synthesis of proteins)**
- **Localization and regulation of gene expression**
- **Cell movement**
- **Cell fate during development**
- **Formation of different organs**
- **Study disease progression and therapy efficiency**
- **Etc…**

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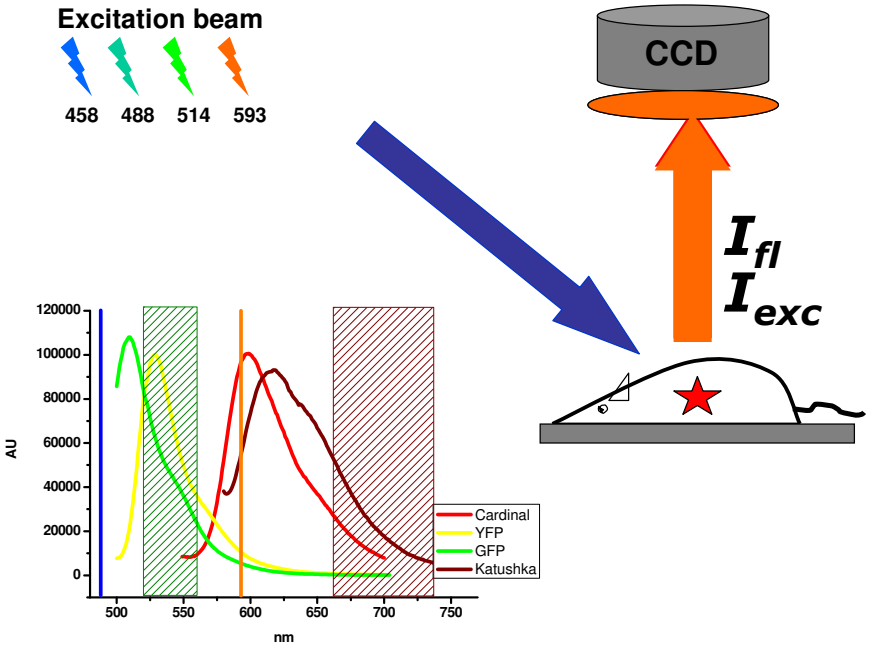
Detection



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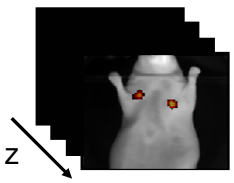
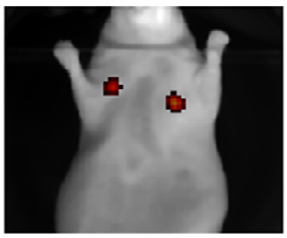
GFP subcutaneous tumors

9L-GFP Human Glioma cells
Injected under the mammary fat pad
Allowed to grow for 21 days

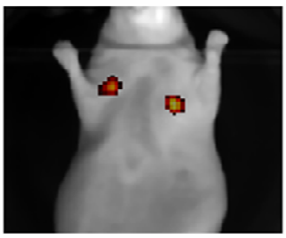
Tomographic slices



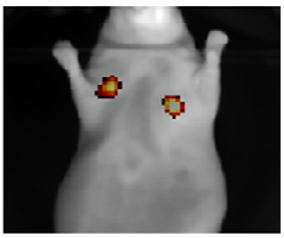
z = 0.89 cm

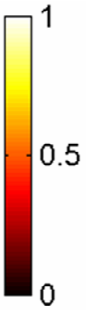


z = 0.96 cm



z = 1.03 cm





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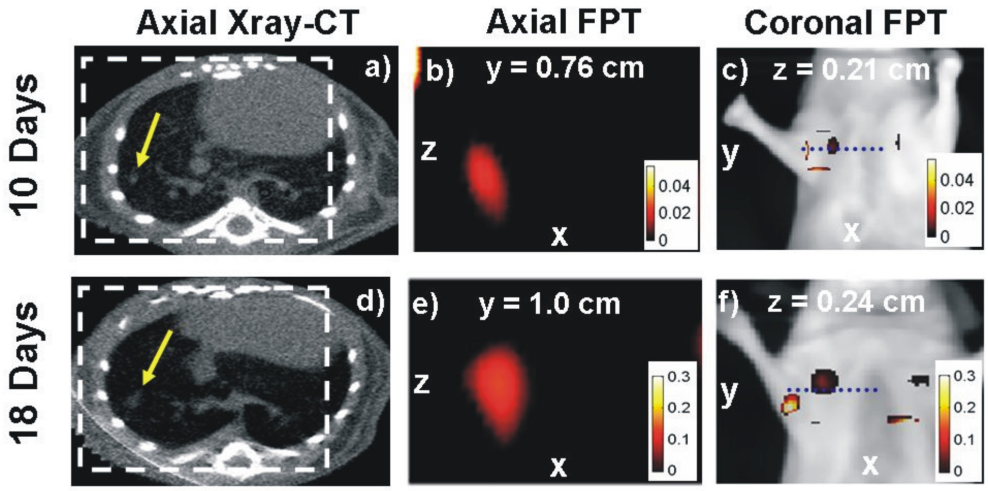
Lung tumor model

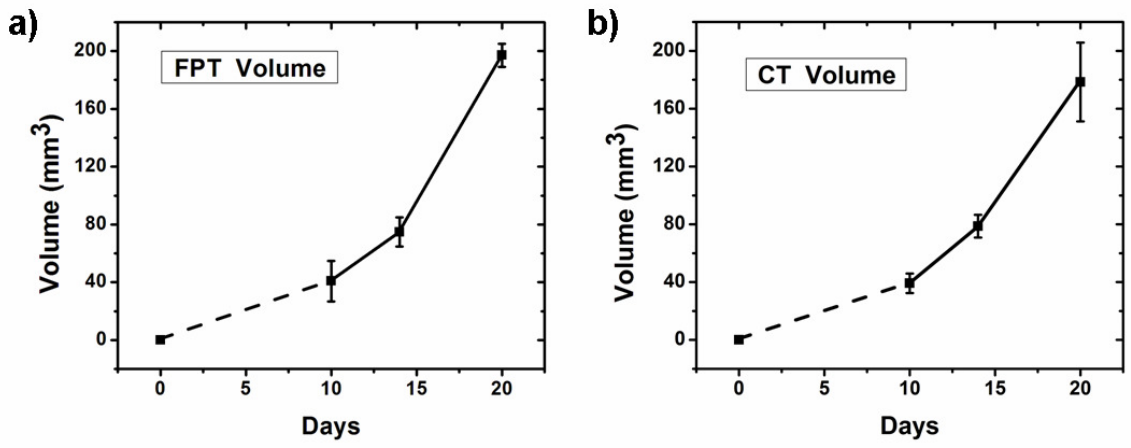
9L – GFP human Glioma cells
Injected into the right lung

Imaged at Day 10, 18 and 20
With FPT & X-ray CT



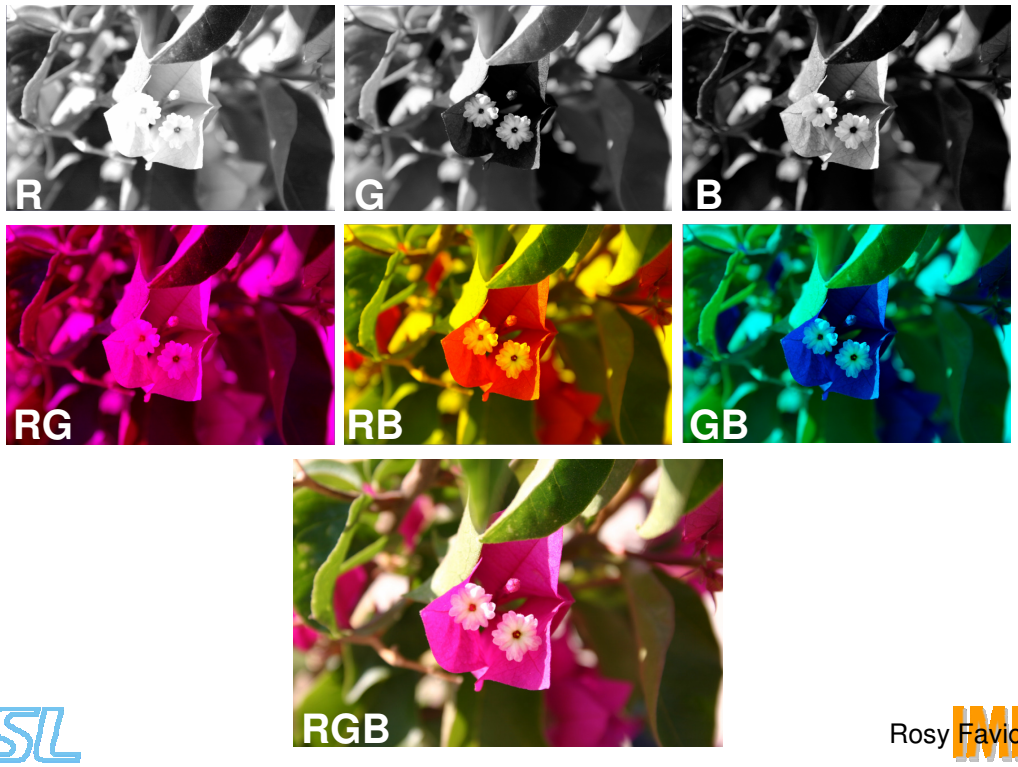
G. Zacharakis et al., PNAS **102**, 18252-18257 (2005)

Lung tumor growth



G. Zacharakis et al., PNAS **102**, 18252-18257 (2005)

Multicolour imaging



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Rosy Favicchio IMBB

Multi-color imaging

Tomographic calculations

Fluorescence reconstruction

$$U^{nB} = \frac{U_{fluo}}{U_{inc}} = W_{fluo} \cdot fluo(\vec{r})$$

$$fluo_{\lambda} = s_G C_G + s_R C_R$$

$$\begin{vmatrix} fluo_1 \\ fluo_2 \end{vmatrix} = \begin{vmatrix} s_{G_1} & s_{R_1} \\ s_{G_2} & s_{R_2} \end{vmatrix} \begin{vmatrix} C_G \\ C_R \end{vmatrix}$$

Absorption reconstruction

$$U^{mua} = \frac{U_{exc} - U_{inc}}{U_{inc}} = W_{abs} \cdot \delta mua(\vec{r})$$

$$mua_{\lambda} = \varepsilon_{Hb} \cdot C_{Hb} + \varepsilon_{HbO} \cdot C_{HbO}$$

$$\begin{bmatrix} mua_1 \\ mua_2 \end{bmatrix} = \begin{bmatrix} \varepsilon_{Hb1} & \varepsilon_{HbO1} \\ \varepsilon_{Hb2} & \varepsilon_{HbO2} \end{bmatrix} \begin{bmatrix} C_{Hb} \\ C_{HbO} \end{bmatrix}$$

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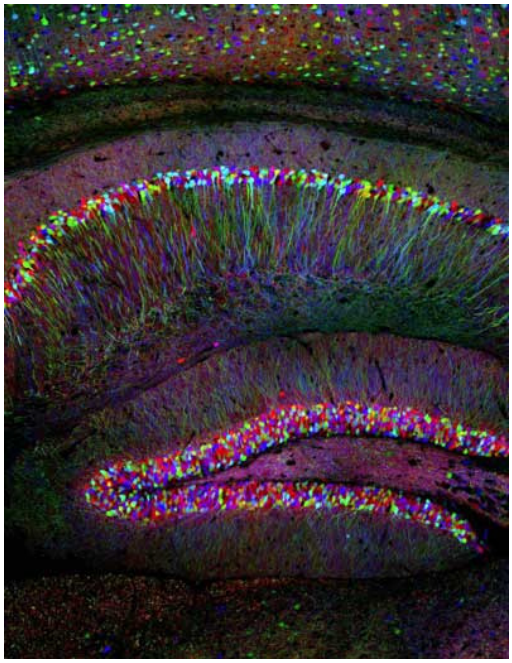
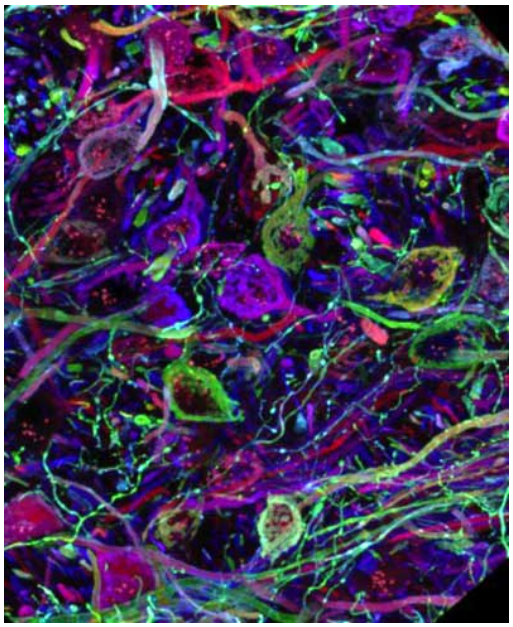
Brainbow





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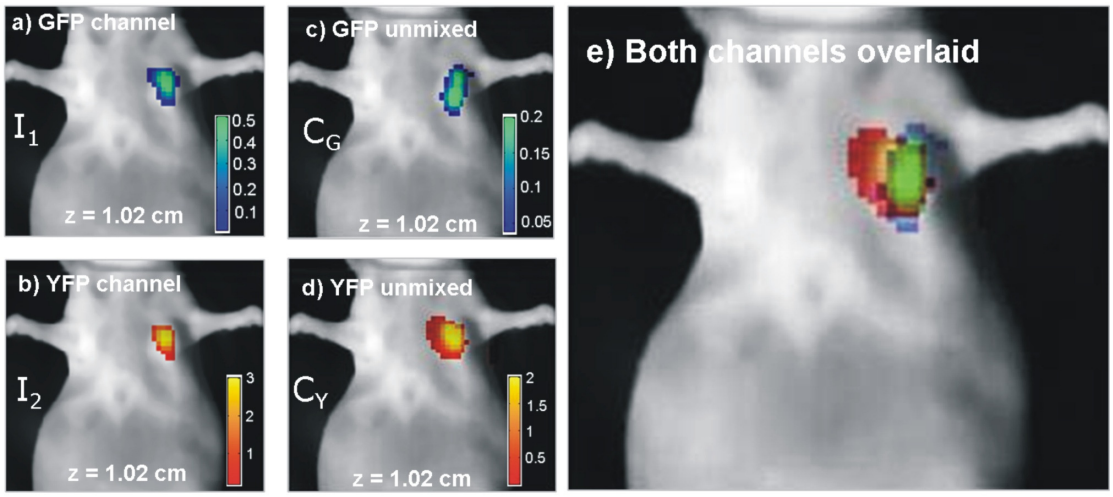
Viral gene delivery

First:

- Image @ 510nm ± 5nm and 570nm ± 5nm

Then:

- Apply the algorithm ⇒ Deconvolved images of YFP-tumor & GFP-virus



IESL MCH 1811

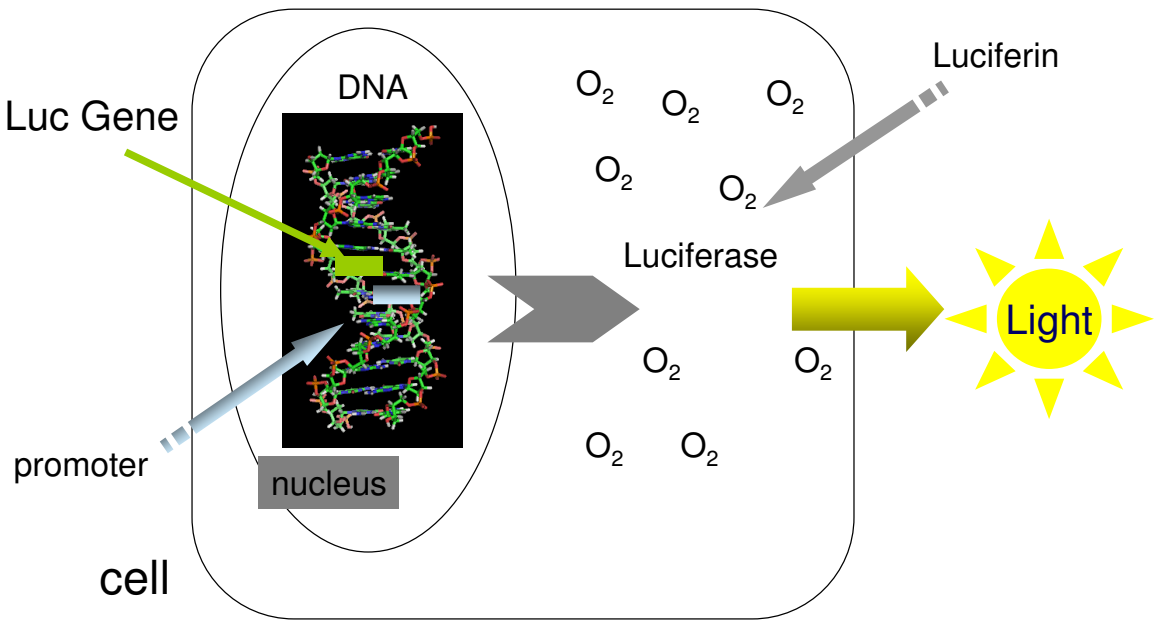
G. Zacharakis et al., PNAS ***102****, 18252-18257 (2005)*

IMBB

BIOLUMINESCENCE

IESL IMBB

Bioluminescence



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Bioluminescence

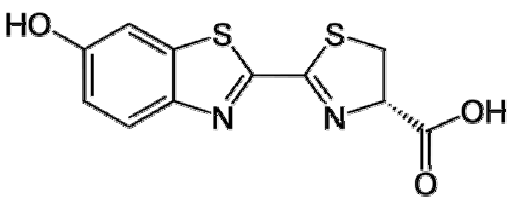
Chemical Reaction

Luciferin
+
O_2

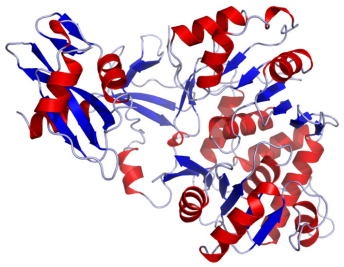
→ (Luciferase)

Oxy-luciferin
+
Light

Luciferin



Luciferase



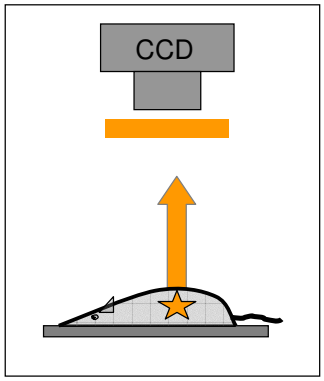
IESL

IMBB

Bioluminescence

BLI setup

Sensitive detector
Filters

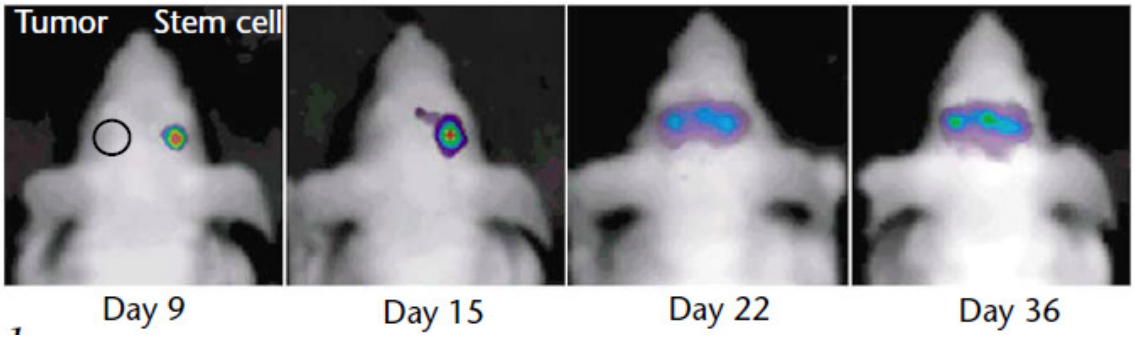


IES

IMBB

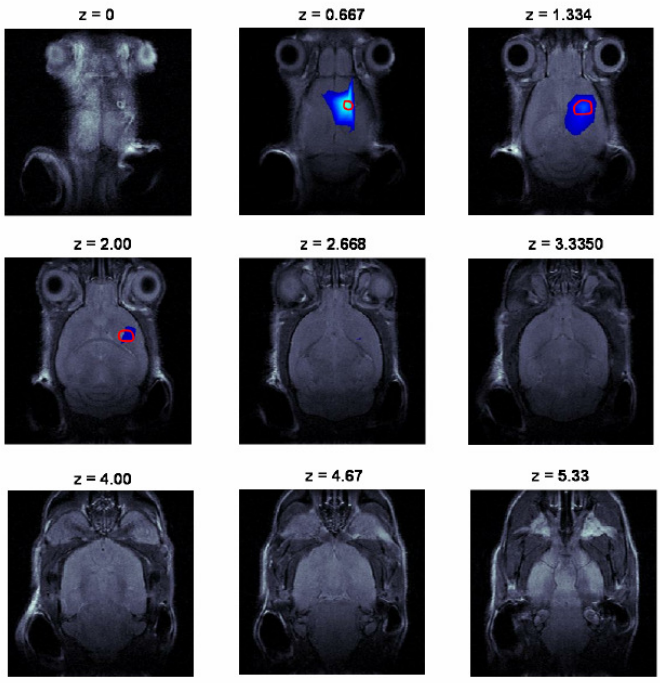
Bioluminescence

Migration of luc-labeled neural progenitor cells across the brain midline attracted by a contralaterally implanted glioma



R. Weissleder, Nat. Med. 9, 123 (2003)

Bioluminescence 3D imaging



A. Chaudhari et al. Phys. Med. Biol. 50 5421 (2005)

The search for Red Proteins

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Table 1 Optical *in vivo* imaging systems[a]

Technique	Contrast[b]	Depth	Commonly used wavelength	Clinical potential
Microscopic resolution				
Epi	A, Fl	20 μm	Visible	Experimental
Confocal	Fl	500 μm	Visible	Experimental
Two-photon	Fl	800 μm	Visible	Yes
Mesoscopic resolution				
Optical projection tomography	A, Fl	15 mm[c]	Visible	No
Optical coherence tomography	S	2 mm	Visible, NIR	Yes
Laser speckle imaging	S	1 mm	Visible, NIR	Yes
Macroscopic resolution, intrinsic contrast				
Hyperspectral imaging	A, S, Fl	<5 mm	Visible	Yes
Endoscopy	A, S, Fl	<5 mm	Visible	Yes
Polarization imaging	A, S	<1.5 cm	Visible, NIR	Yes
Fluorescence reflectance imaging (FRI)	A, Fl	<7 mm	NIR	Yes
Diffuse optical tomography (DOT)	A, Fl	<20 cm	NIR	Yes
Macroscopic resolution, molecular contrast				
Fluorescence resonance imaging (FRI)	A, Fl	<7 mm	NIR	Yes
Fluorescence molecular tomography (FMT)	Fl	<20 cm	NIR	Yes
Bioluminescence imaging (BLI)	E	<3 cm	500–600 nm	No

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