Optical Imaging: Fluorescence and Bioluminescence

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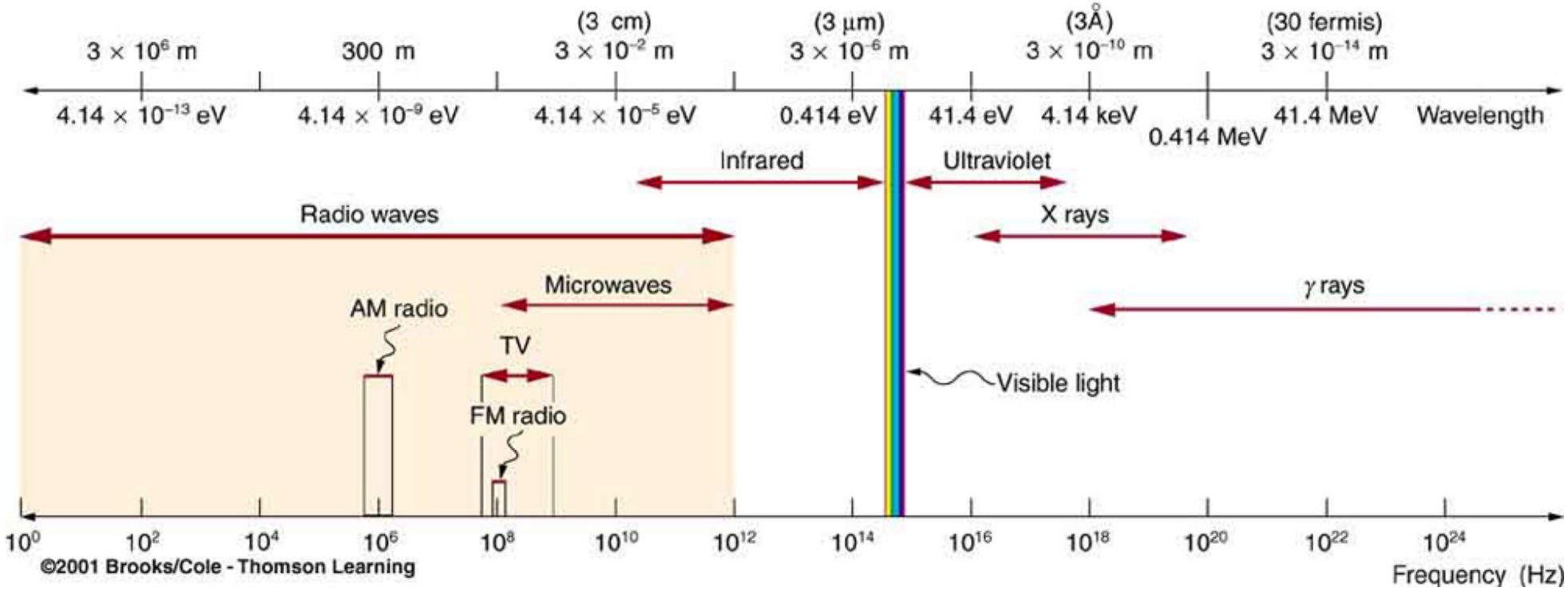
Why optical imaging?

Technique	Resolution	Depth	Time	Imaging agents	Target*	Cost‡	Primary small-animal use	Clinical use
MR	10–100 μm	No limit	Minutes–hours	Gadolinium, dysprosium, iron oxide particles	A, P, M	$$$	Versatile imaging modality with high soft-tissue contrast	Yes
CT	50 μm	No limit	Minutes	Iodine	A, P	$$	Lung and bone imaging	Yes
Ultrasound	50 μm	Millimetres	Minutes	Microbubbles	A, P	$$	Vascular and interventional imaging	Yes
PET	1–2 mm	No limit	Minutes	^{18}F, ^{11}C, ^{15}O	P, M	$$$	Versatile imaging modality with many different tracers	Yes
SPECT	1–2 mm	No limit	Minutes	^{99m}Tc, ^{111}In chelates	P, M	$$	Commonly used to image labelled antibodies, peptides and so on	Yes
FRI	2–3 mm	<1 cm	Seconds–minutes	Photoproteins (GFP), NIR fluorochromes	P, M	$	Rapid screening of molecular events in surface-based tumours	Development
FMT	1 mm	<10 cm	Seconds–minutes	NIR fluorochromes	P, M	$$	Quantitative imaging of targeted or 'smart' fluorochrome reporters in deep tumours	Development
BLI	Several millimetres	Centimetres	Minutes	Luciferins	M	$$	Gene expression, cell and bacterial tracking	No
Intravital microscopy (confocal, multiphoton)	1 μm	<400 μm	Seconds–minutes	Photoproteins (GFP), Fluorochromes	P, M	$$$	All of the above at higher resolutions but at limited depths and coverage	Limited development (skin)

Rudin and Weissleder, Nature Reviews in Drug Discovery, 2003

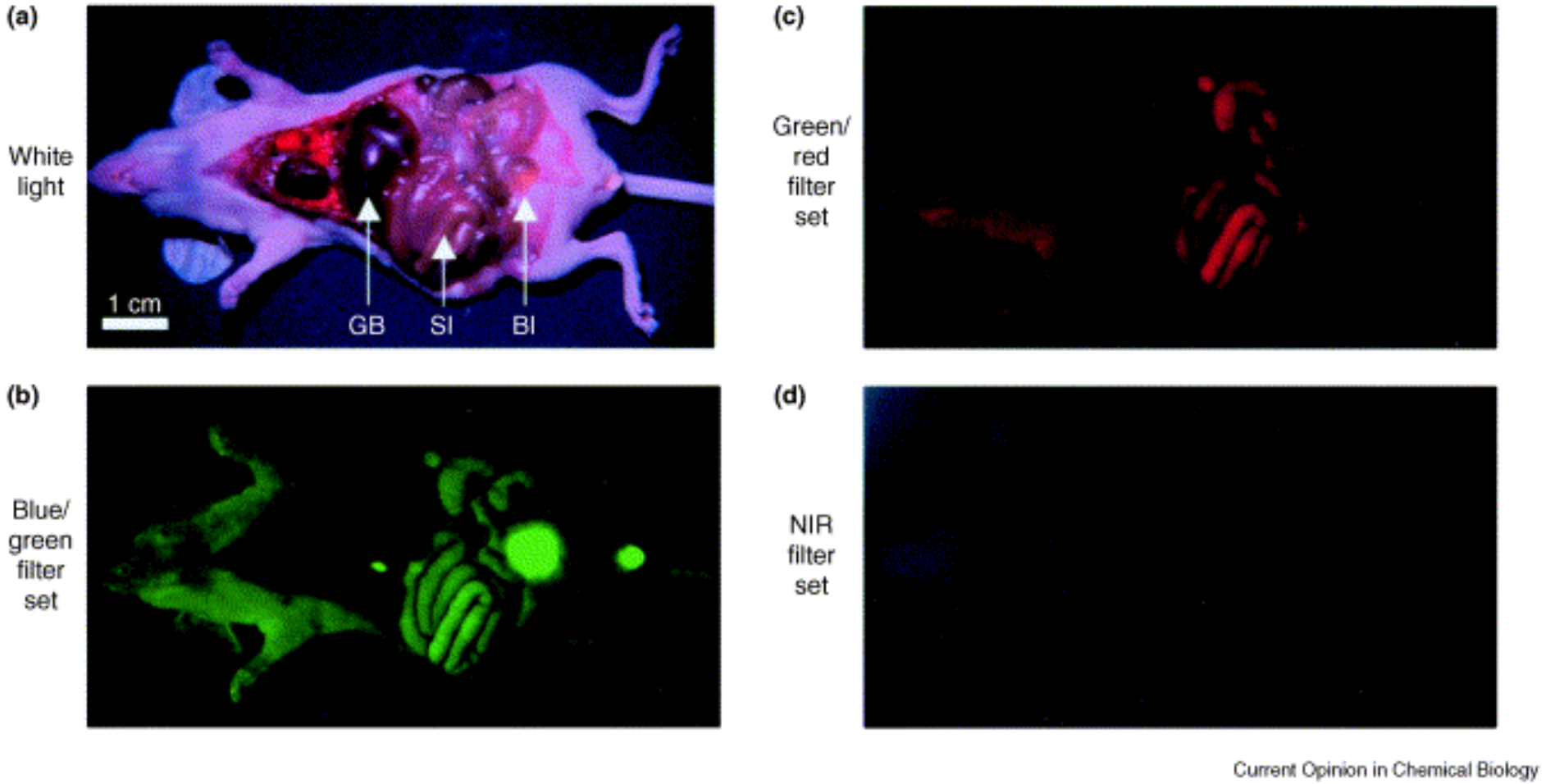
- Low cost of tracers and instrumentation

Why optical imaging?



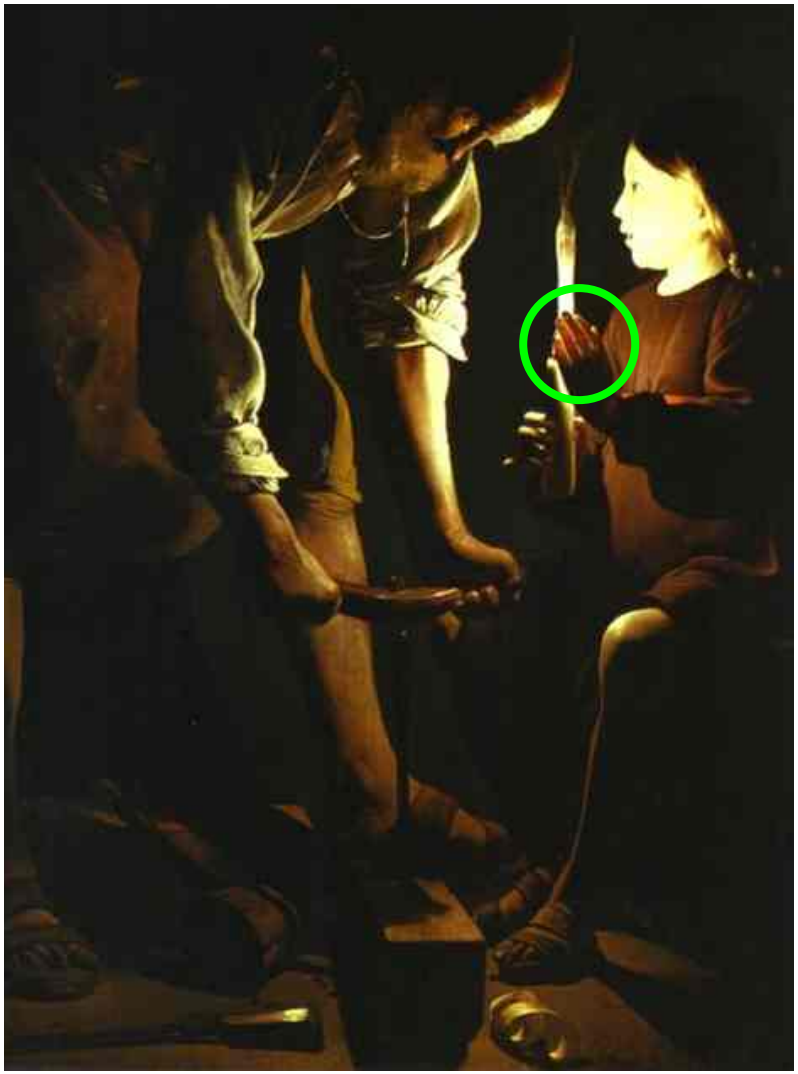
- Low-energy, non-ionizing radiation
- Tracers are stable (no radioactive decay), can be stored indefinitely
- Imaging of genetically encoded markers (fluorescent proteins, luciferases)
- Simultaneous detection of multiple tracers (filters)

Autofluorescence: wavelength-dependent

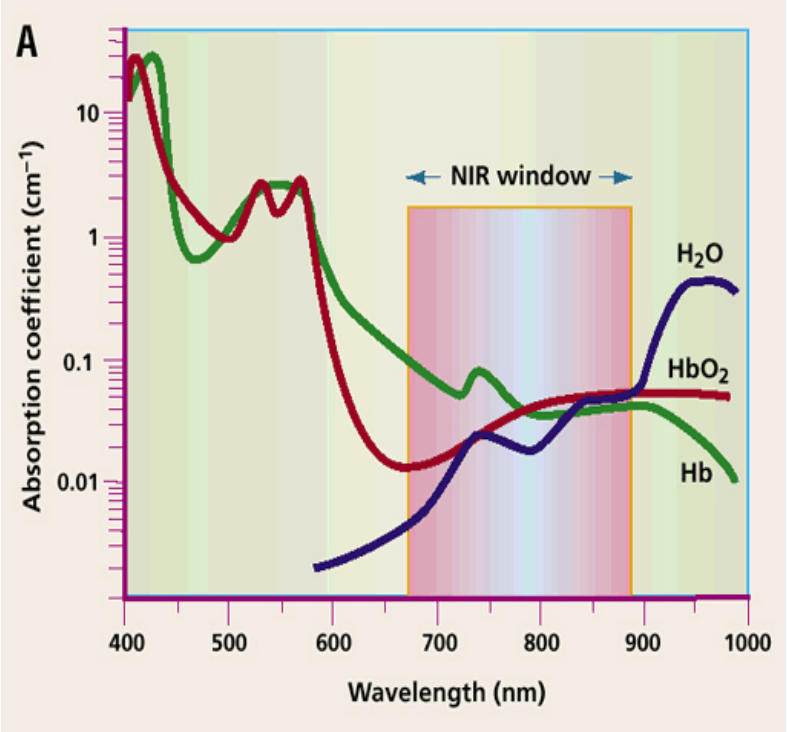


Frangioni-JV, *Curr Op Chem Biol*, 2003

Absorption in tissue: wavelength-dependent

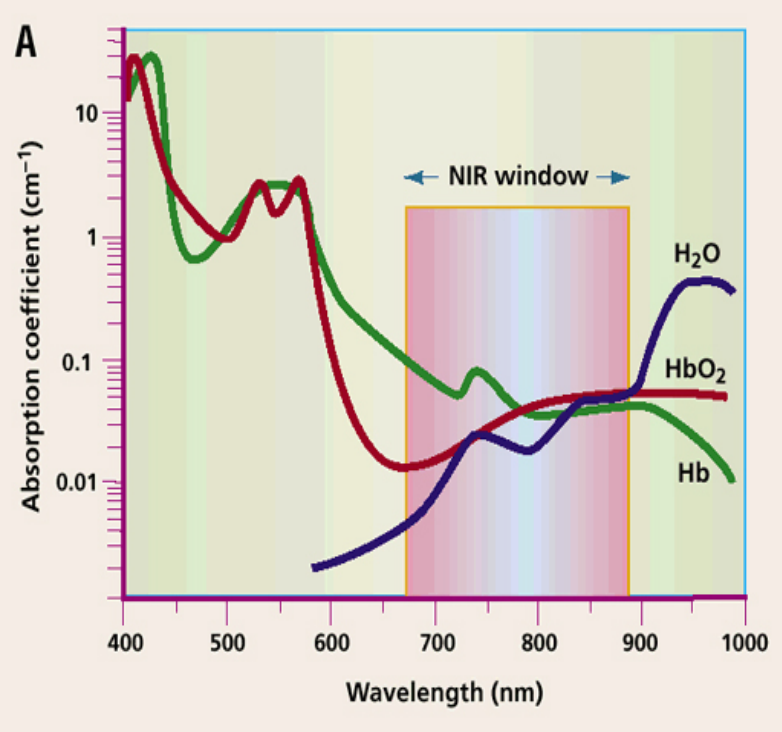


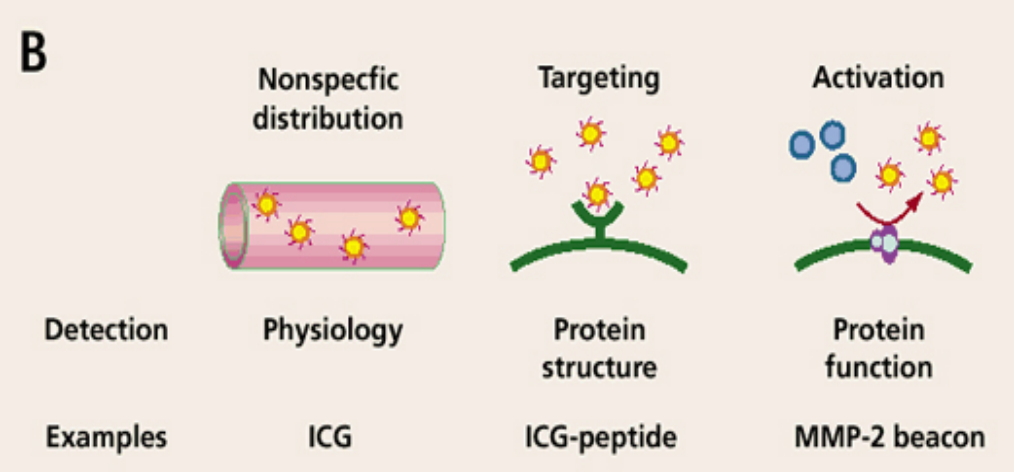
Georges de la Tour: *Saint Joseph charpentier*, 1643, Louvre



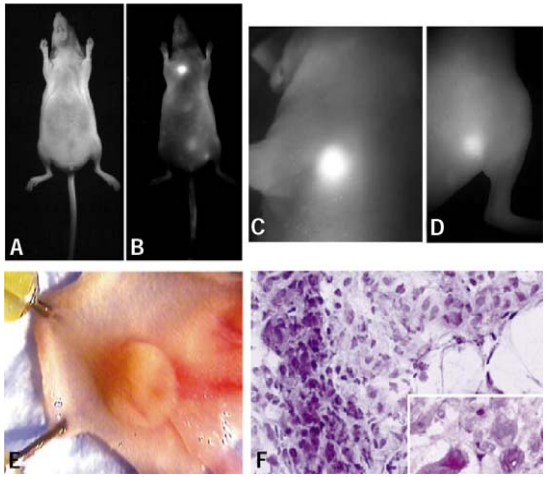
Weissleder et al., Nat Biotechnol, 2001

Advantages of near-infrared fluorescent (NIRF) detection





Weissleder, Nat Biotech 19:316 (2001)



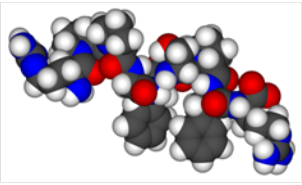
- high tissue penetration (mm-cm)
- low tissue autofluorescence
- many assay platforms & imagers
- translational: microscopy to human

Fluorescence

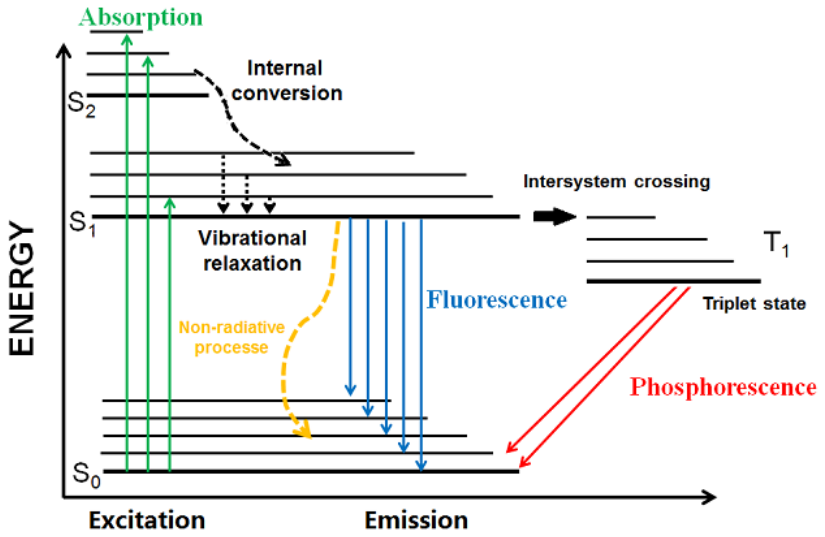
746 / 778

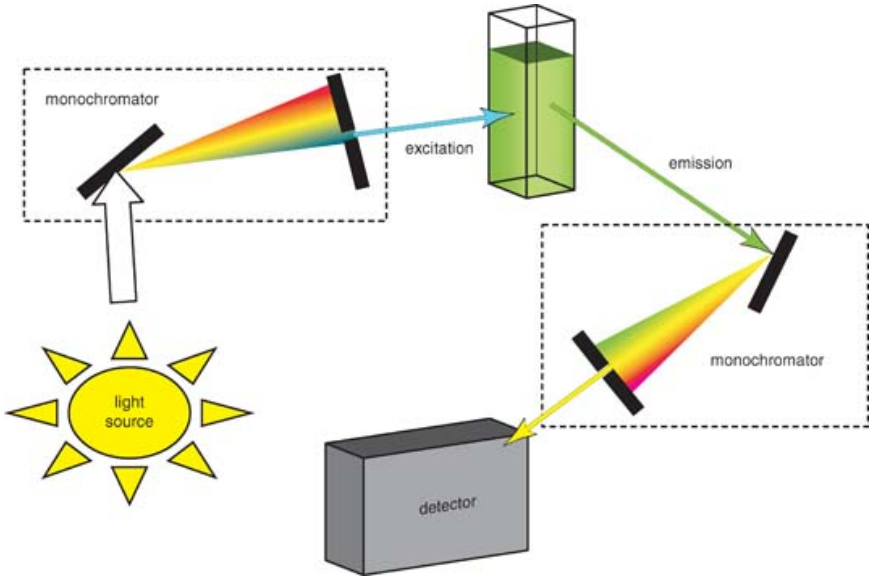
Indotricarbocyanin
ITCC

Linker

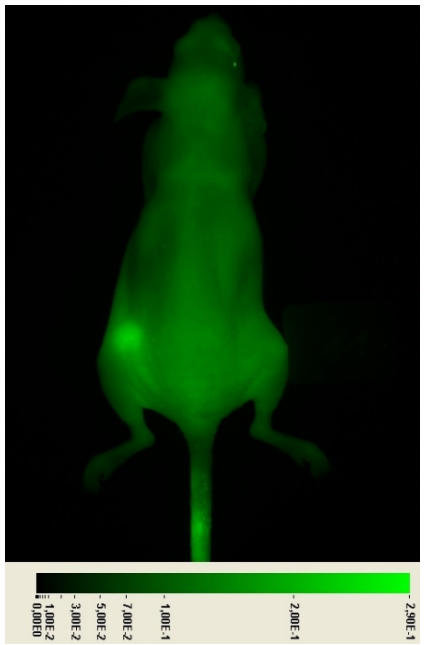


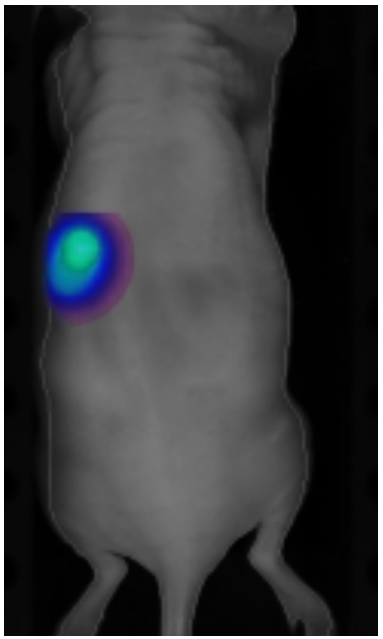
Peptid





Optical Imaging: near-infrared fluorescence





Fluorescence Reflectance Imaging (FRI)

➨ 2D detection of NIRF probes in subcutaneous tumor models

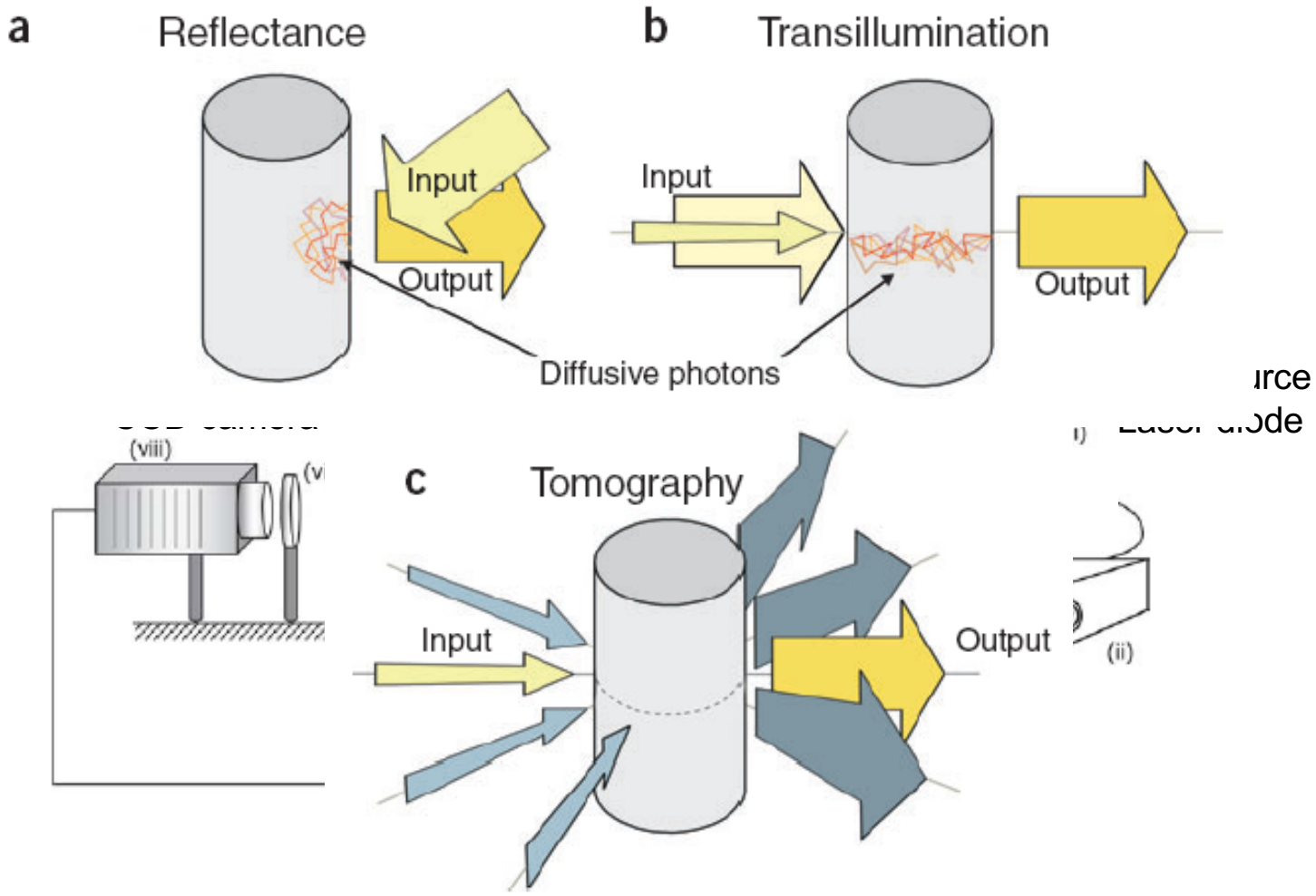
➨ fast, parallel detection of probes in two NIR channels (685, 785 nm)

Fluorescence Molecular Tomography (FMT)

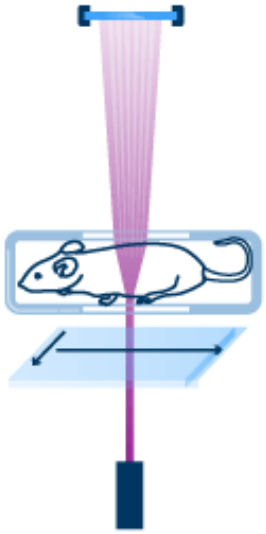
➨ 3D imaging of NIRF probes in orthotopic tumor models

➨ quantitation of NIRF probes in the animal

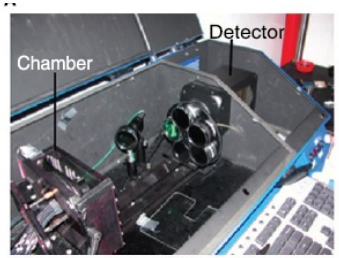
Optical Imaging: near-infrared fluorescence

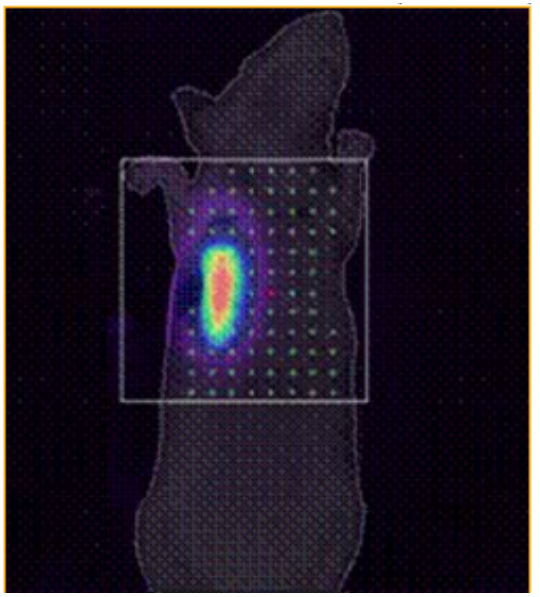


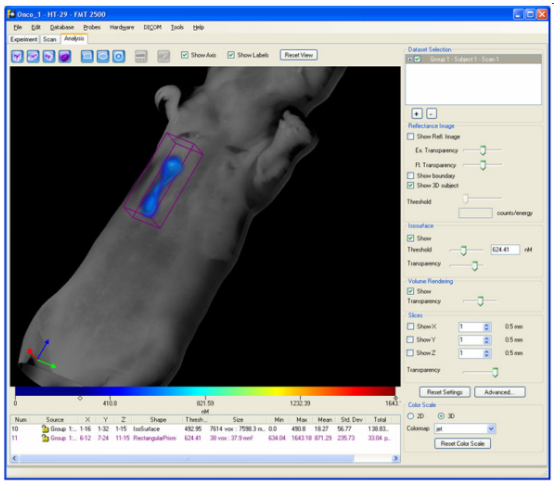
Optical Tomography: phantoms

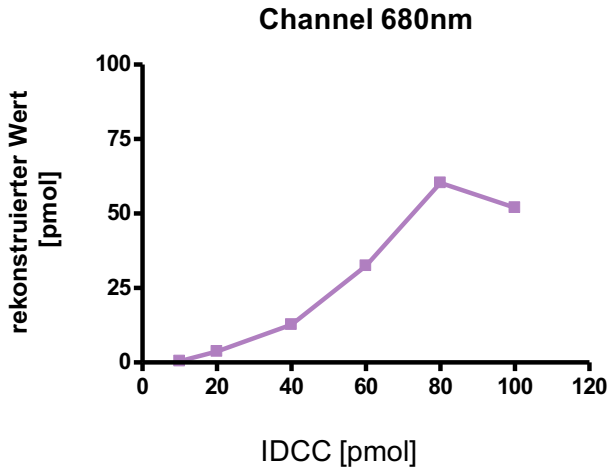


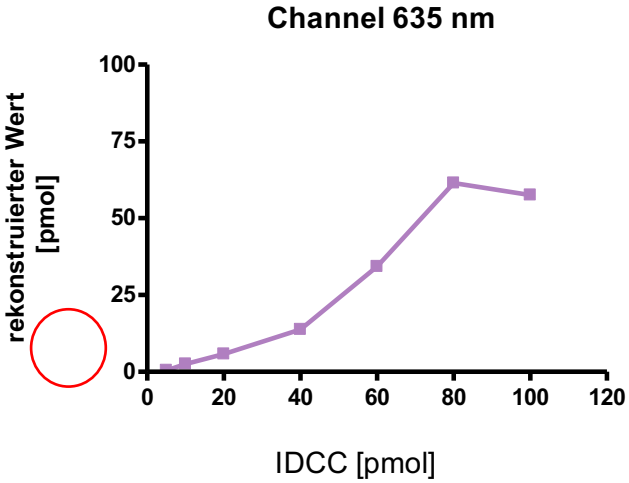
Laser	
Absorption	Emission
635nm	660nm
670nm	700nm
746nm	775nm
790nm	805nm





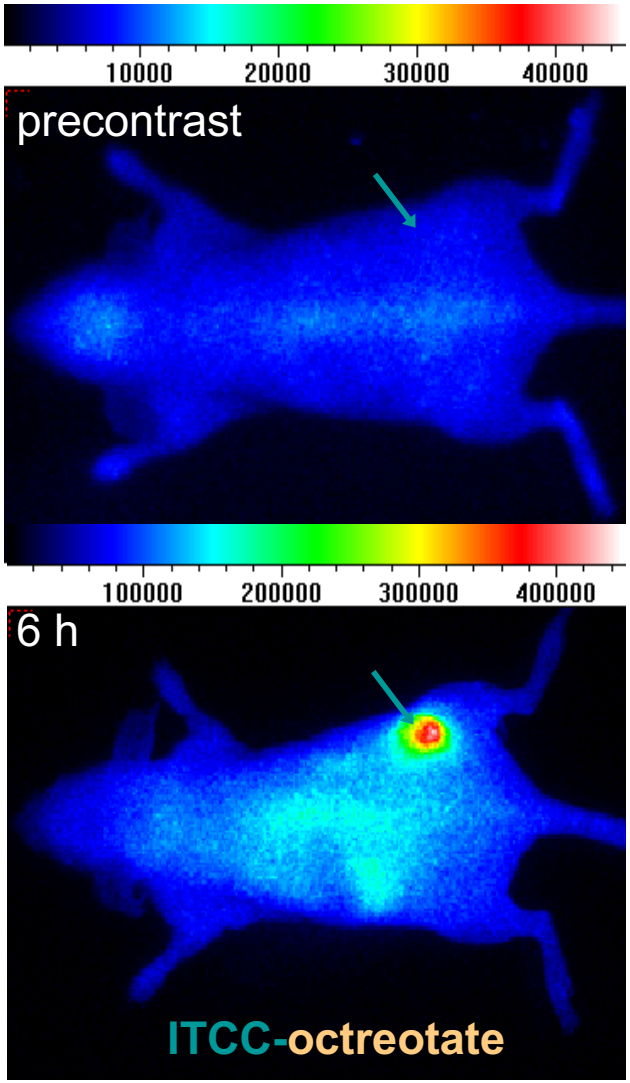


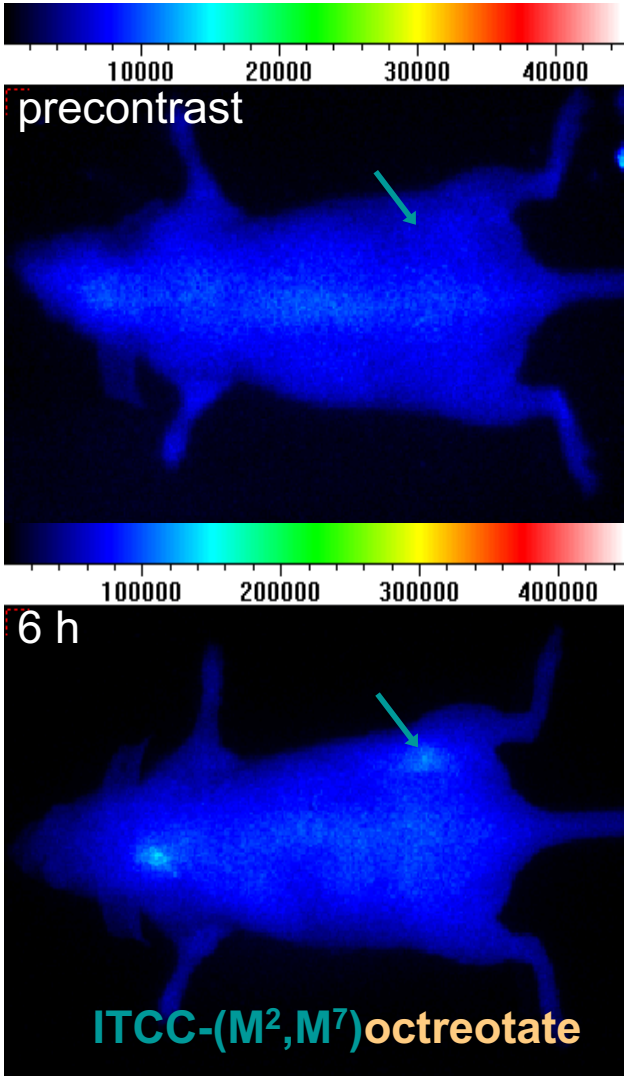


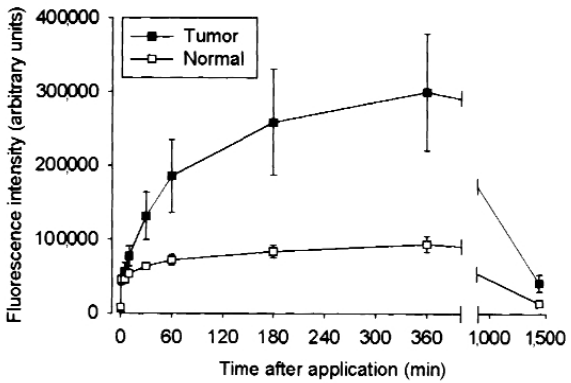


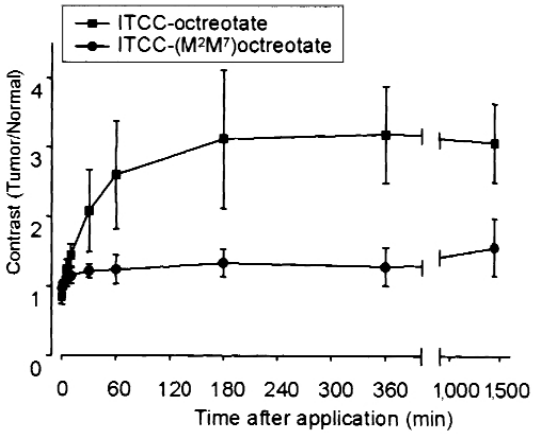
Preclinical near-infrared fluorescent tumor imaging

RIN38/SSTR2 tumor, dose: 0,02 µmol/kg i. v.





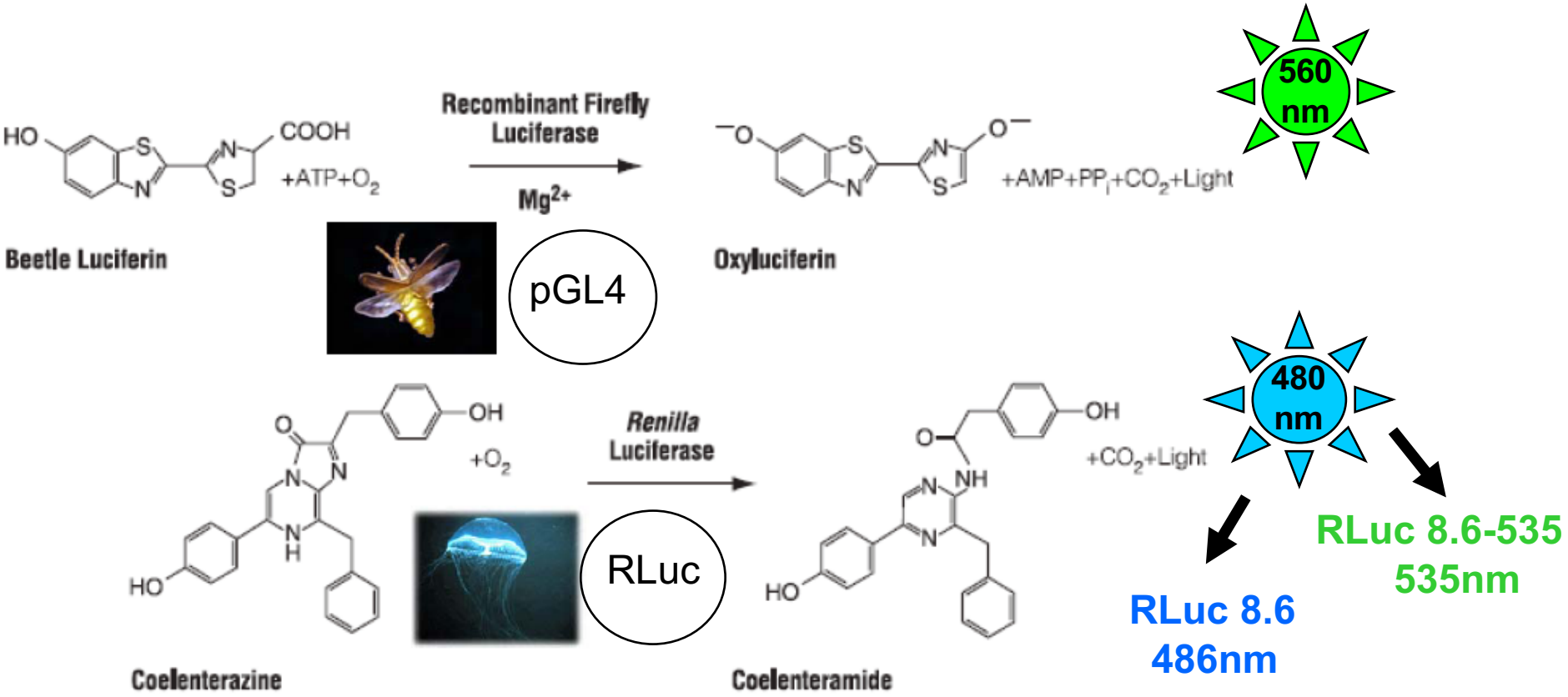




Becker, et al, Grötzinger; Nat Biotechn., 2001

Bioluminescence – a chemical reaction -

Production and emission of light in living organisms as a result of chemical reaction



Bioluminescence as a tool for molecular imaging of tumor models

- ✓ monitor tumor cells in preclinical studies
- ✓ Non-invasive
- ✓ detection of few cells and earlier
- ✓ No radiation
- ✓ High throughput
- ✓ Detection of micrometastases
- ✓ Monitor behavior right from the beginning of implantation
- ✓ functional analysis
- ✓ high sensitivity
- ✓ Monitor growth of orthotopic tumors non-invasively
- ✓ no tissue cut to fluorescence

Table 1. Current imaging modalities of interest in drug research and discovery

Technique	Spatial resolution and time scale	Clinical imaging	Application	Main characteristics
Ultrasound	50 μm; min	Yes	Anatomical, functional	Difficult to image through bone or lungs; microbubbles used for contrast enhancement
CT	50–100 μm; min	Yes	Anatomical, functional	Poor soft tissue contrast
MRI	80–100 μm; s to h	Yes	Anatomical, functional, molecular	High spatial resolution and soft tissue contrast
SPECT (low-energy γ-rays)	1–2 mm; min	Yes	Functional	Radioisotopes have longer half-lives than those used in PET; sensitivity 10 to 100 times smaller than PET
PET (high-energy γ-rays)	1–2 mm; min	Yes	Metabolic, functional, molecular	High sensitivity (picomolar concentrations); cyclotron needed
Bioluminescence	1–10 mm; s to min	No	Molecular	High sensitivity; transgene-based approach; light emission prone to attenuation with increased tissue depth
NIRF	1–3 mm; s to min	No	Molecular	Excitation and emission light prone to attenuation with increased tissue depth

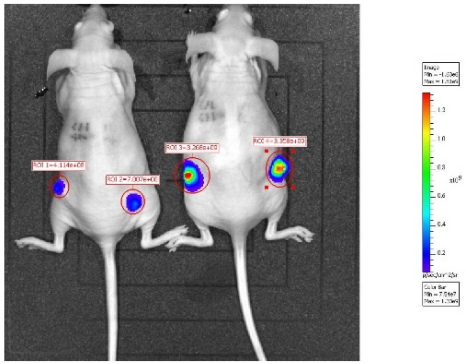
Applications

Oncology

Tumor growth and metastasis
Tumor related gene study

Functional genomics

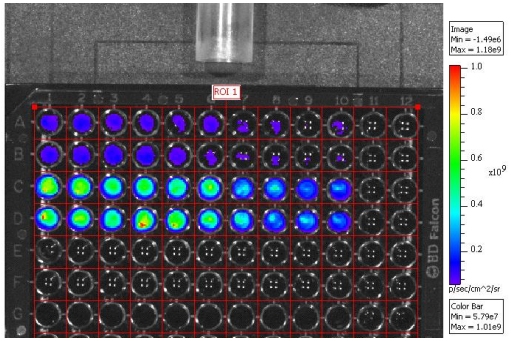
Expression profiles and
regulation studies,
protein-protein interactions,
apoptosis…



Stem cell research

Infectious disease

Infectious pathway
Molecular study



Pharmaceutical applications

Drug discovery (HTS)
Pharmacokinetics
absorption, distribution, metabolism

Gene delivery and therapy

Expression kinetics and localization

Toxicology research applications

Pharmaceutical kinetics and localization

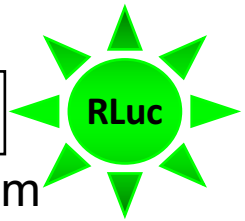
In vitro kinetics of luciferase expressing cell lines

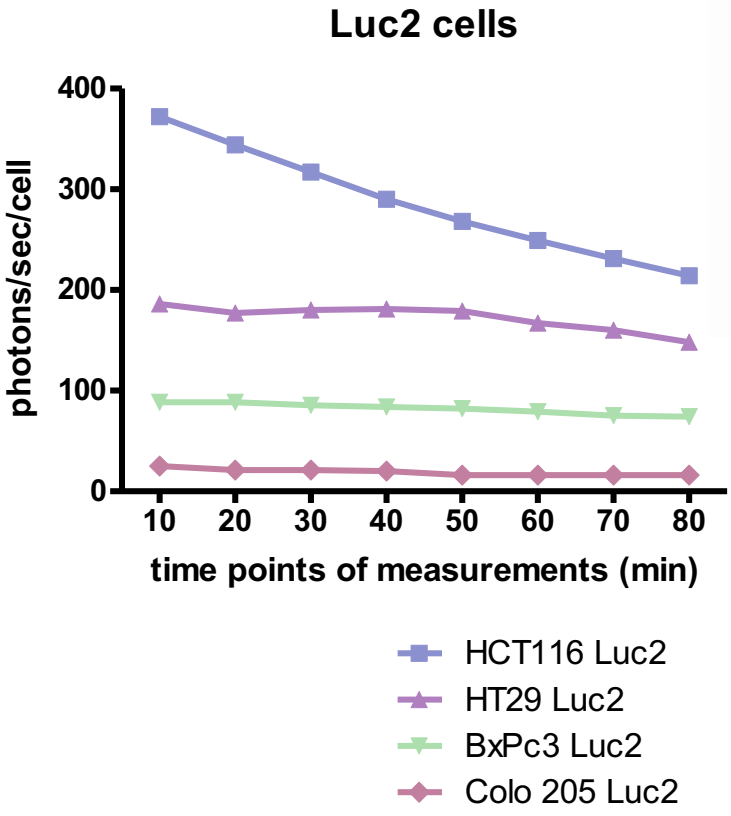
Firefly

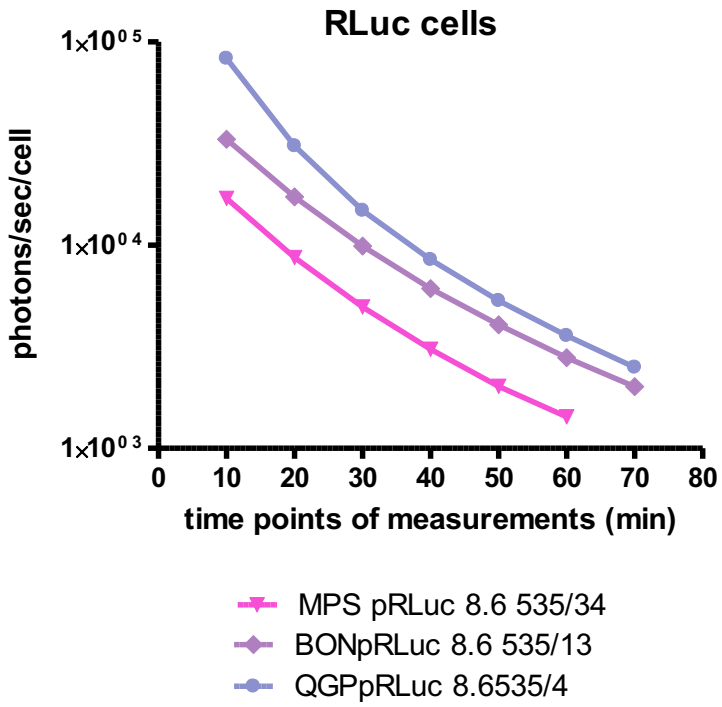
560nm

Renilla

535nm





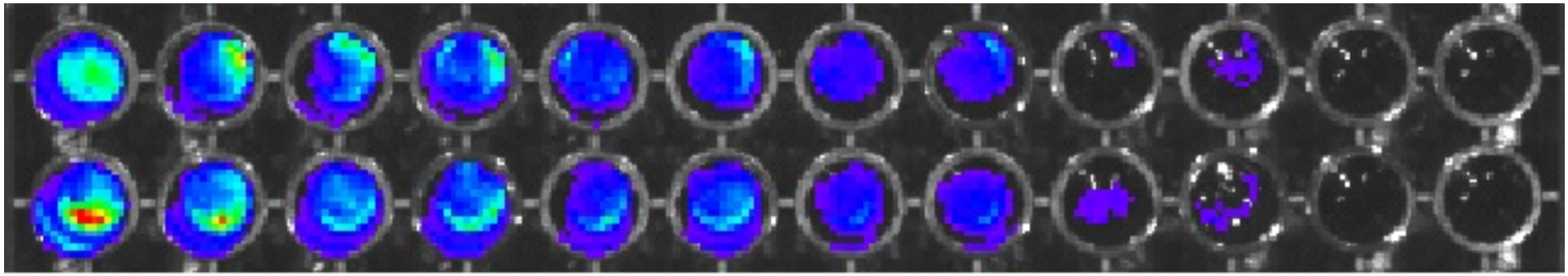


In vitro Bioluminescence

Bioluminescence kinetics

Black 96-well plate

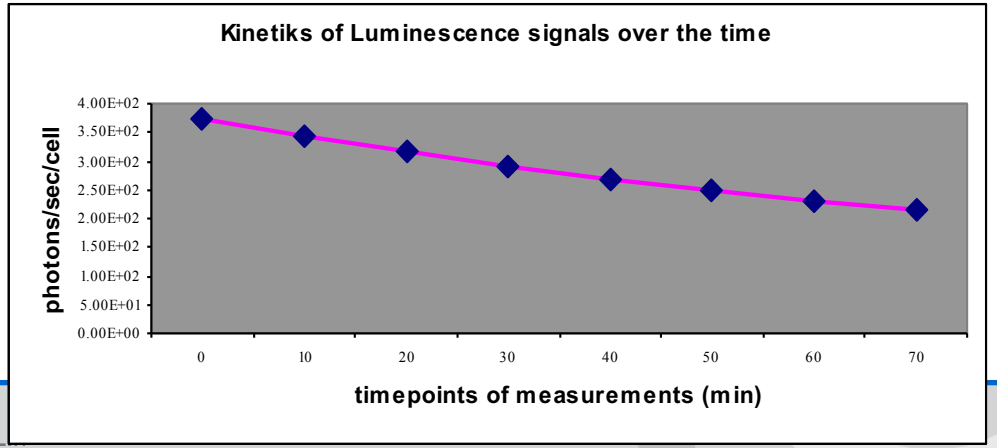
controls



- Seed cells according to scheme and incubate over night
- Add D-luciferin (150µg/ml) and measure photon emission every 10', during 1 hour.

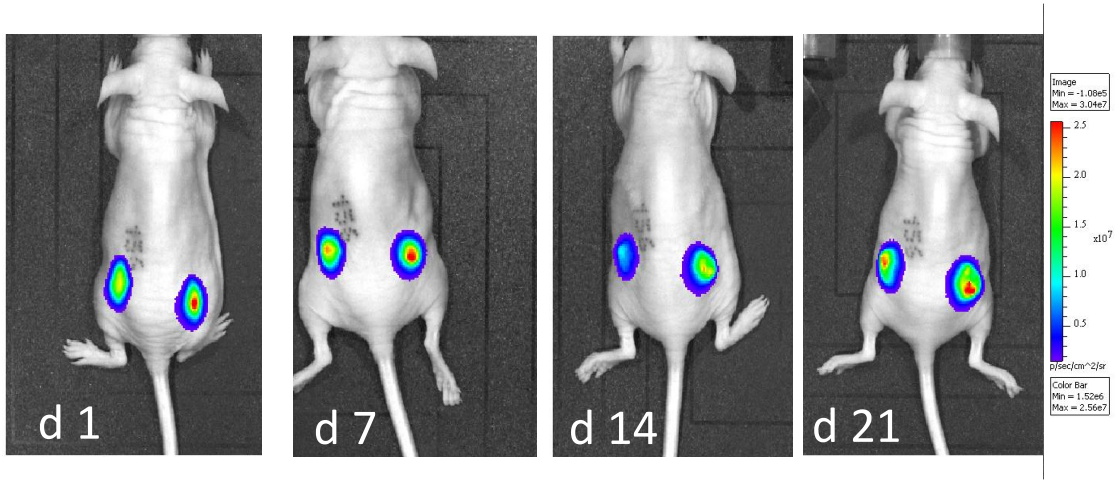
✓ Calculate photon/sec/cell

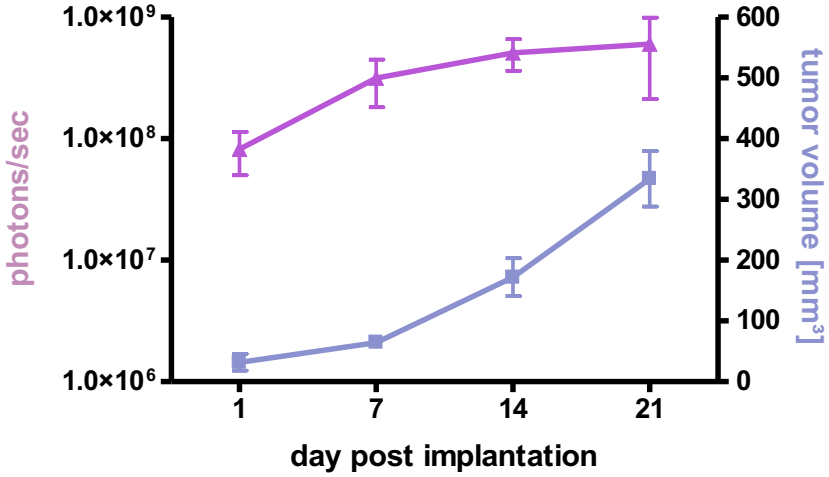
✓ Build kinetics curve

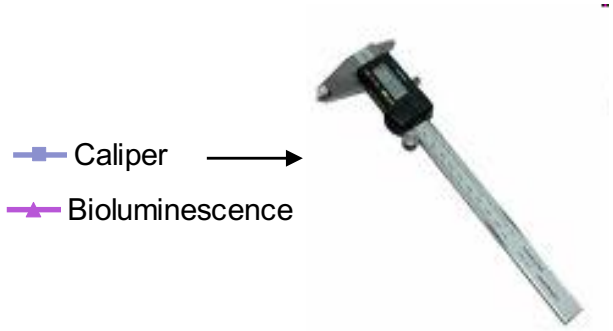


Bioluminescence

Subcutaneous implantation of pancreatic carcinoma cells in nude mice - BxPc3-Luc2 -



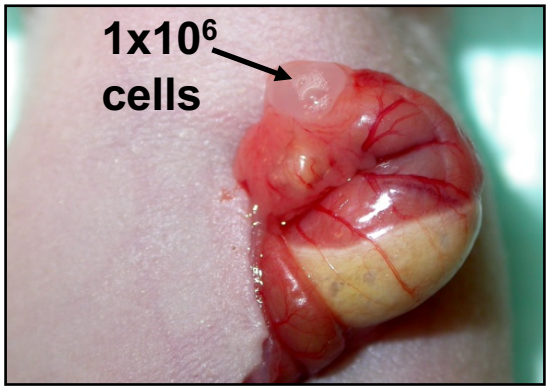


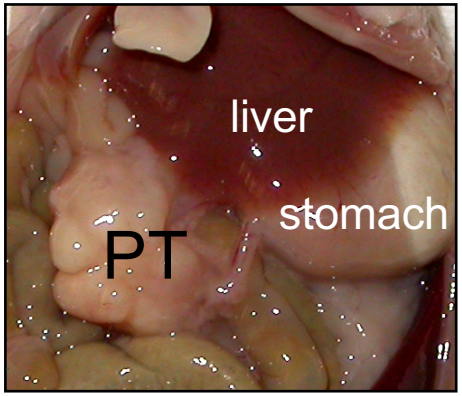


Advantages of orthotopic tumor models: pancreas

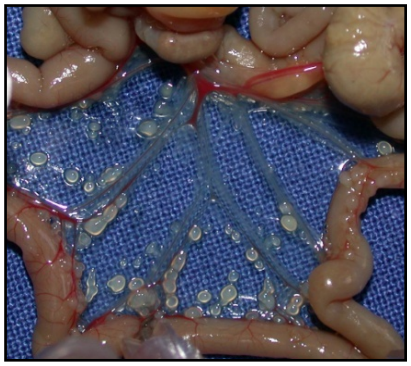
BON cells and other models

Inoculation





Primary tumor



Metastasis

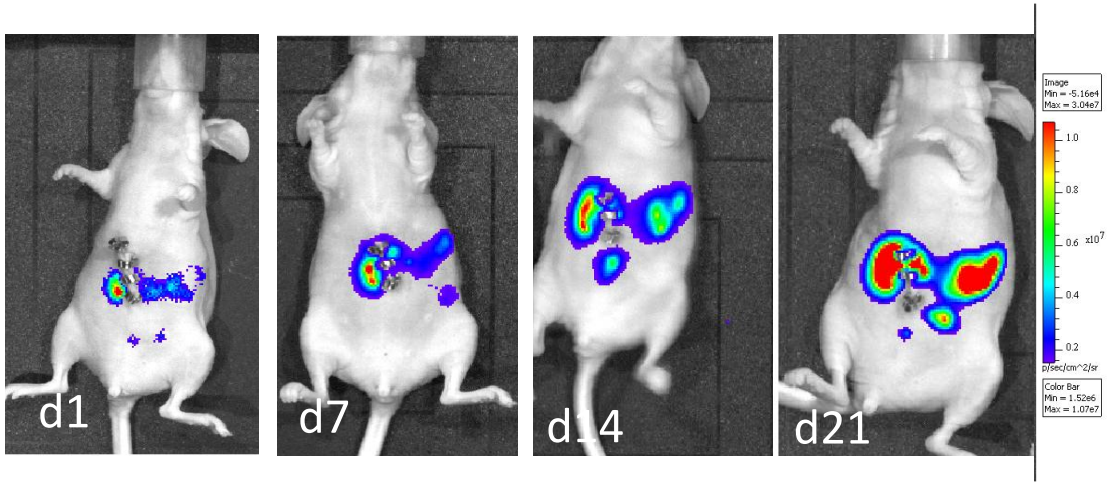
Ascites

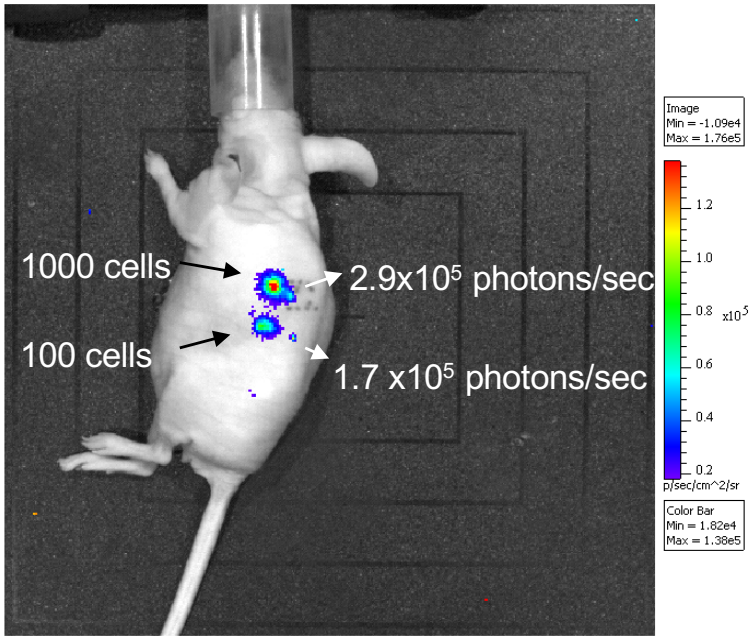
Icterus

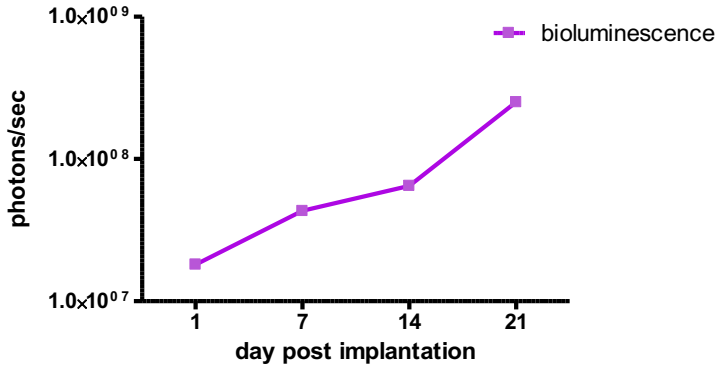
Bioluminescence

Orthotopic implantation of pancreatic carcinoma cells in nude mice - BxPc3-Luc2 -

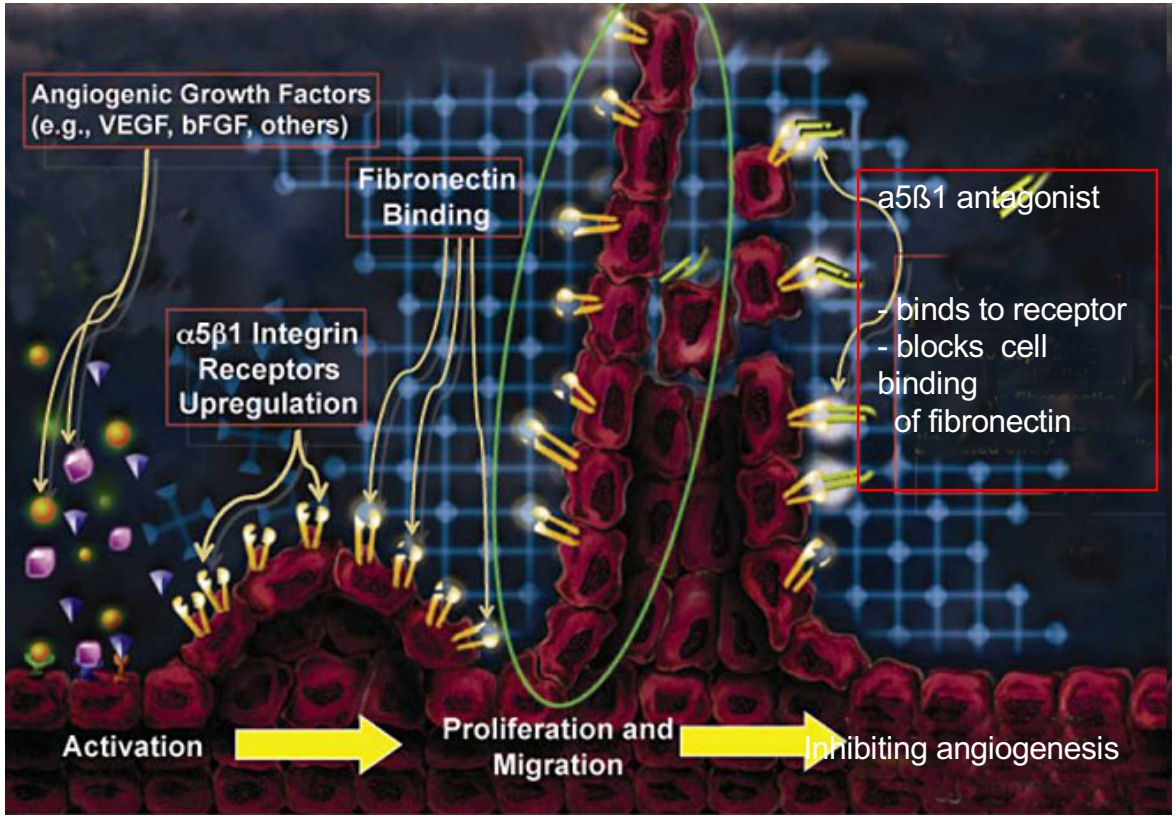
* Cell number and viability (necrosis!)
* Depth of localization (absorption, scatter)
* Type of luciferase, type of substrate







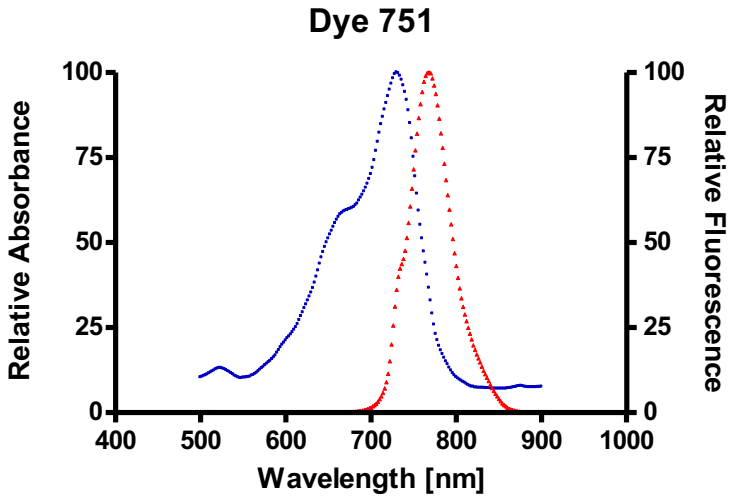
Anti-angiogenesis targets for imaging: integrin antagonists



Modifiziert nach (Quelle: Ophthotec.com)

- Inhibits neo-angiogenesis and lymphangiogenesis in various tissues (Umeda et al., 2006; Dietrich et al., 2007; Okazaki et al., 2009; …)
- Inhibits proliferation in a glioblastoma mouse model (Färber et al., 2008)

Photophysical & phtotochemical properties

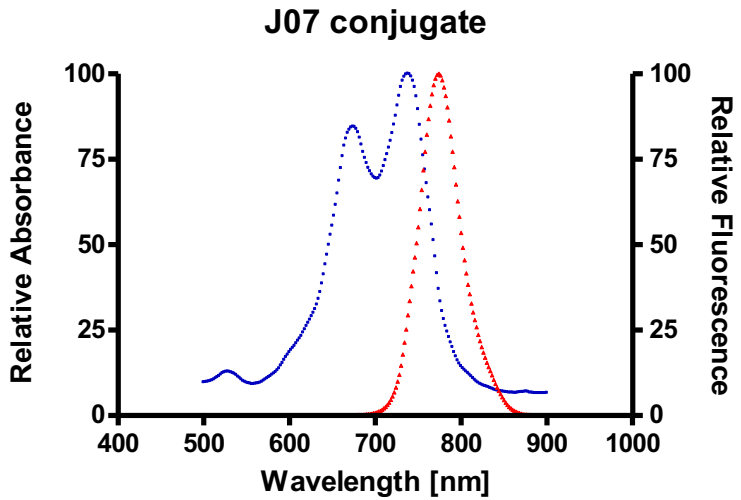


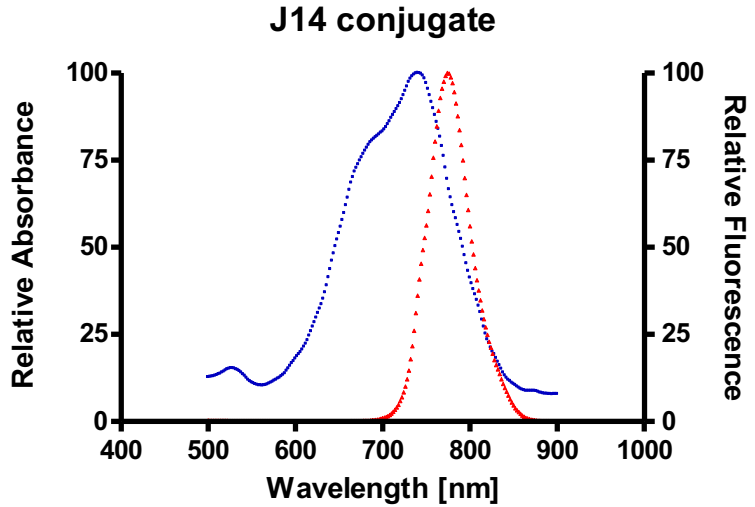
relative absorbance

relative Fluorescence

Abs_{max}	730 nm
Em_{max}	768 nm

Mol. Ext.coeff:	270000 m-1cm-1
Quantum yield	0,14





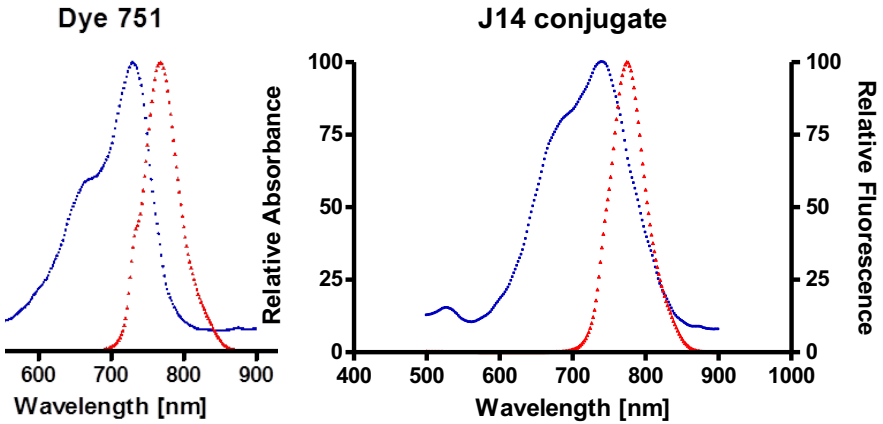
Abs_{max}	738 nm
Em_{max}	774 nm

Mol. Ext.coeff:	2550000 m-1cm-1
Quantum yield	0,12

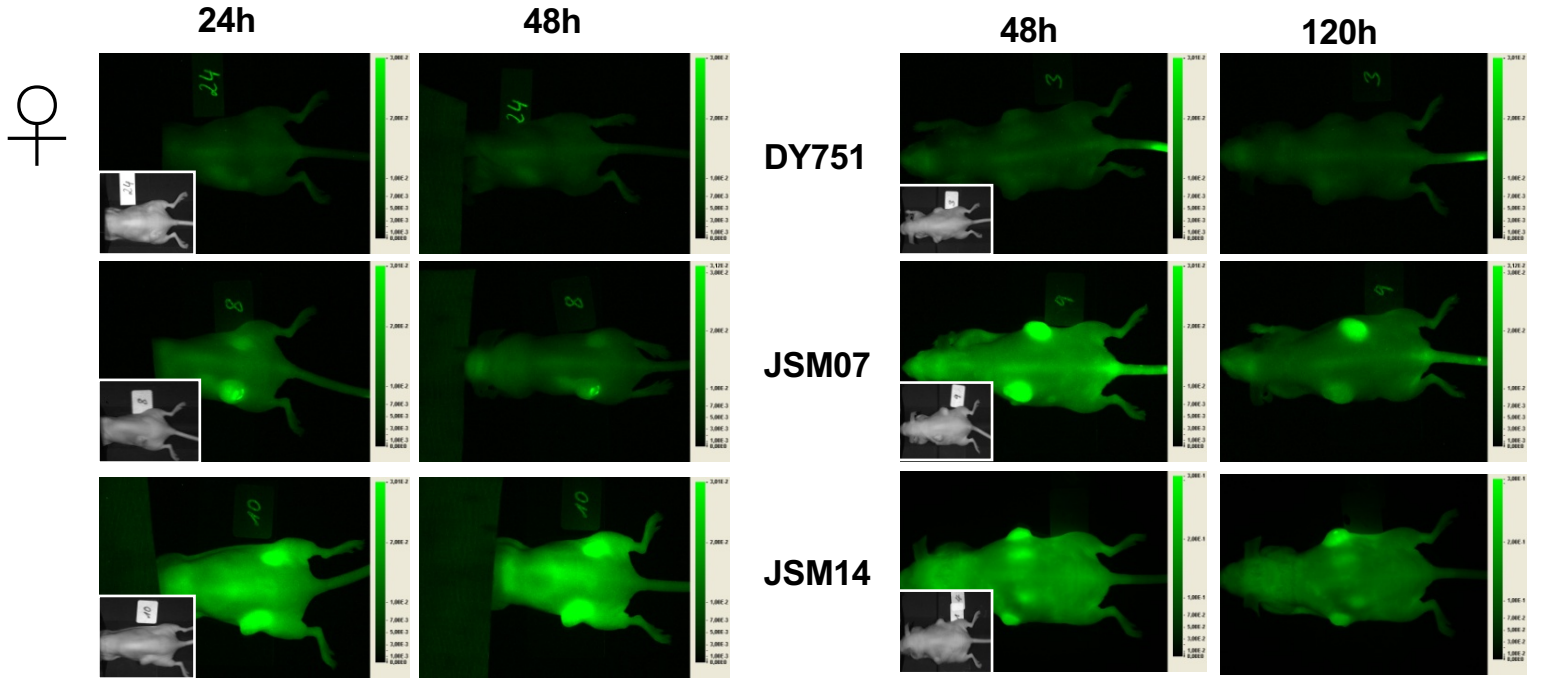
Abs_{max}	740 nm
Em_{max}	774 nm

Mol. Ext.coeff:	255000 m-1cm-1
Quantum yield	0,12

In vivo near-infrared fluorescent (NIRF) imaging of tumor-bearing mice using α5β1 integrin probes, A549 xenografts



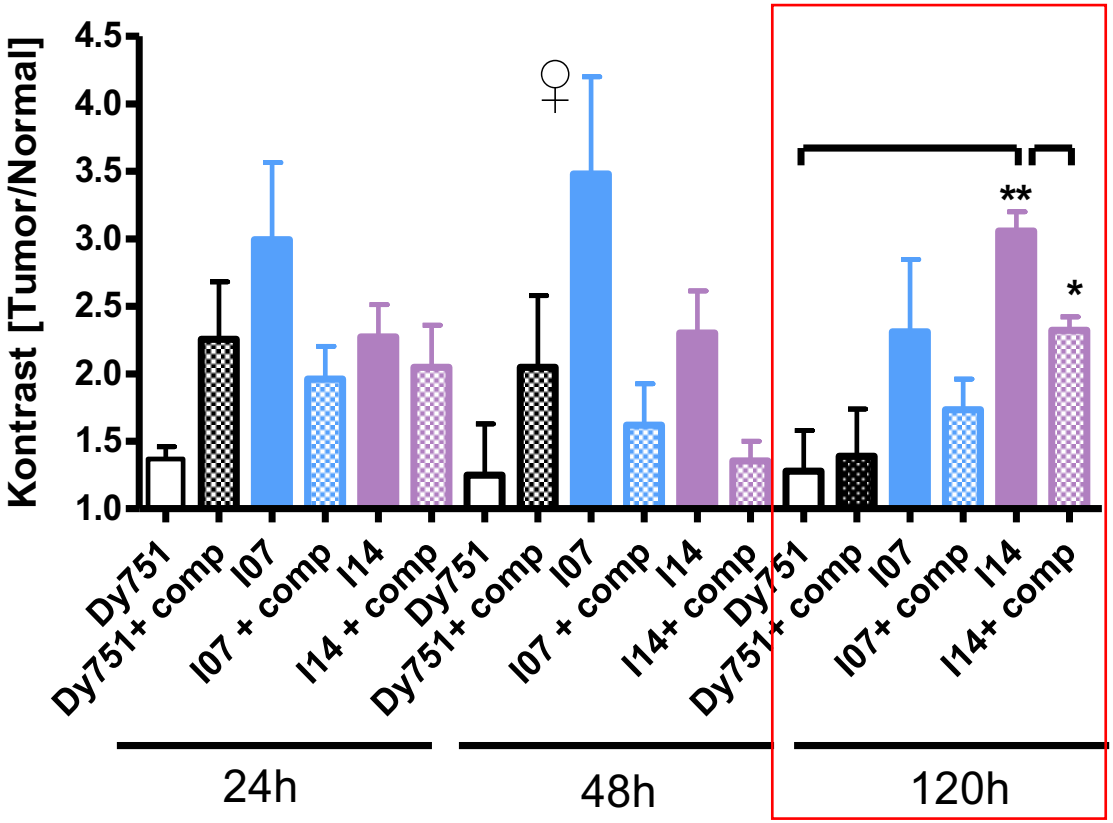
probe	Abs_{max} [nm]	Em_{max} [nm]	Φ	EC [L mol-1 cm-1]
DY751	731	775	≥ 19,9 %	116800
JSM07	736	771	≥ 21,5 %	85430
JSM14	737	770	≥ 20,9 %	98270

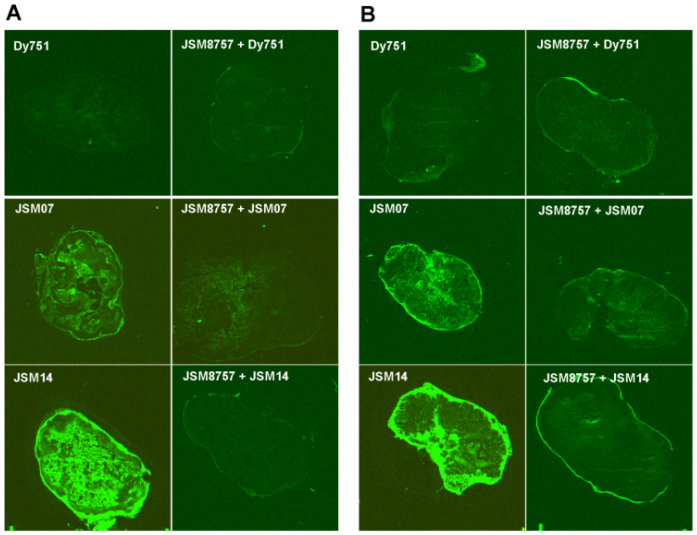


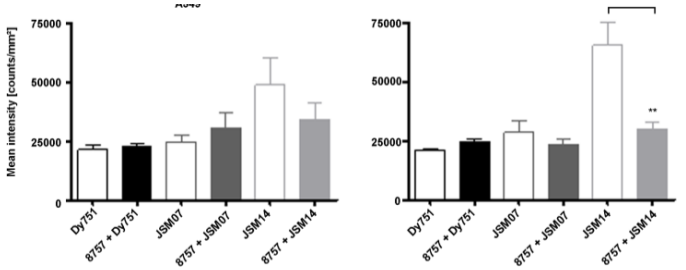
dosage per animal 0,6 nmol

Competition in A549 Tumors

➢ *Competition by injection of unlabelled integrin antagonist (1000 fold) 15 min before injection of fluorescent conjugates*

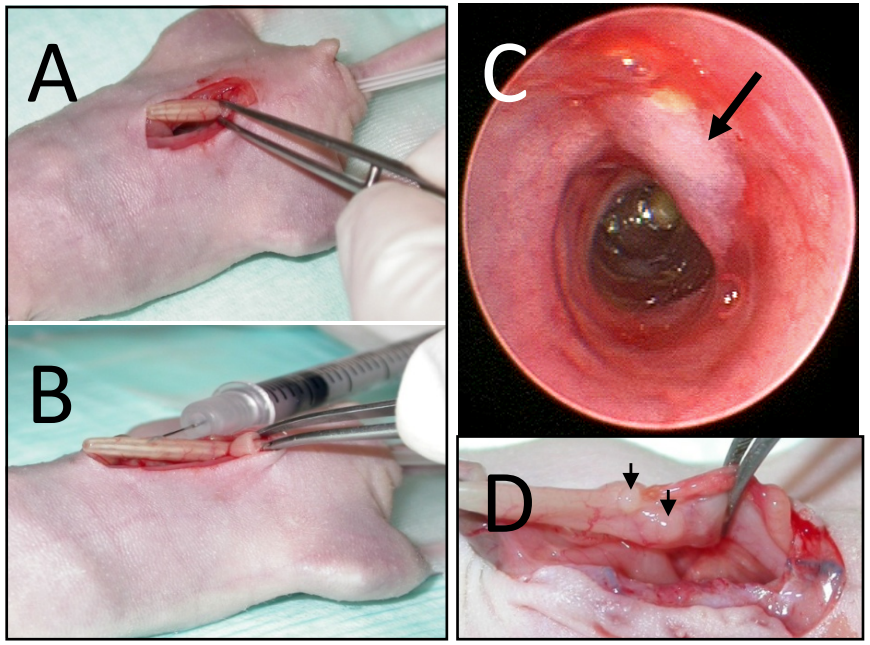


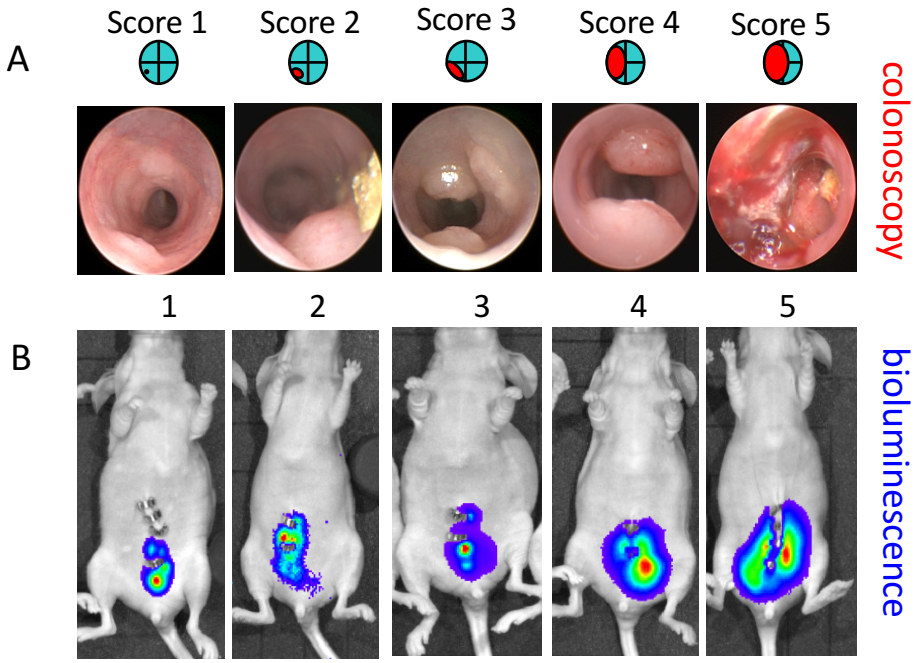


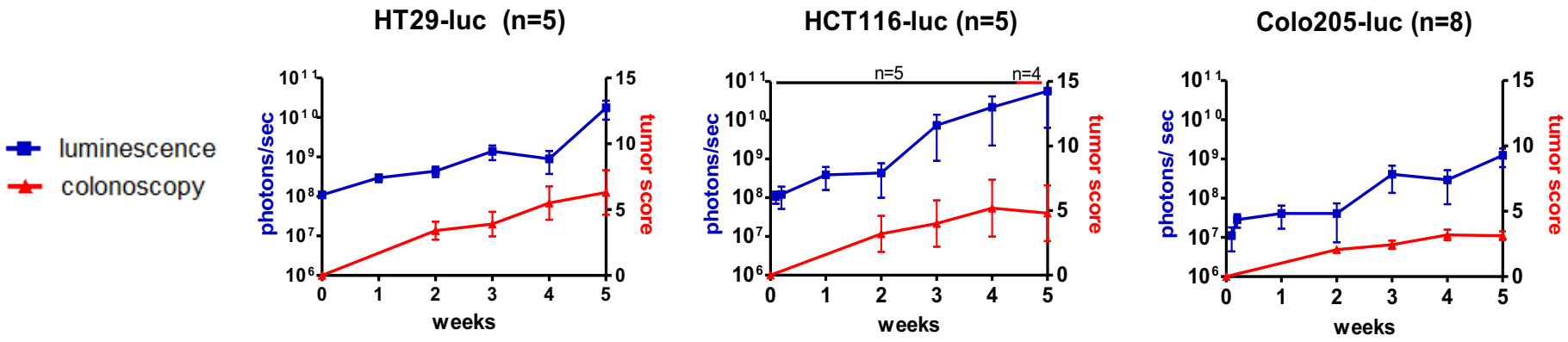


✓ Competition with unlabelled antagonist leads to inhibition of contrast enrichment of I07 and I14

Dual monitoring of a new orthotopic colorectal cancer mouse model

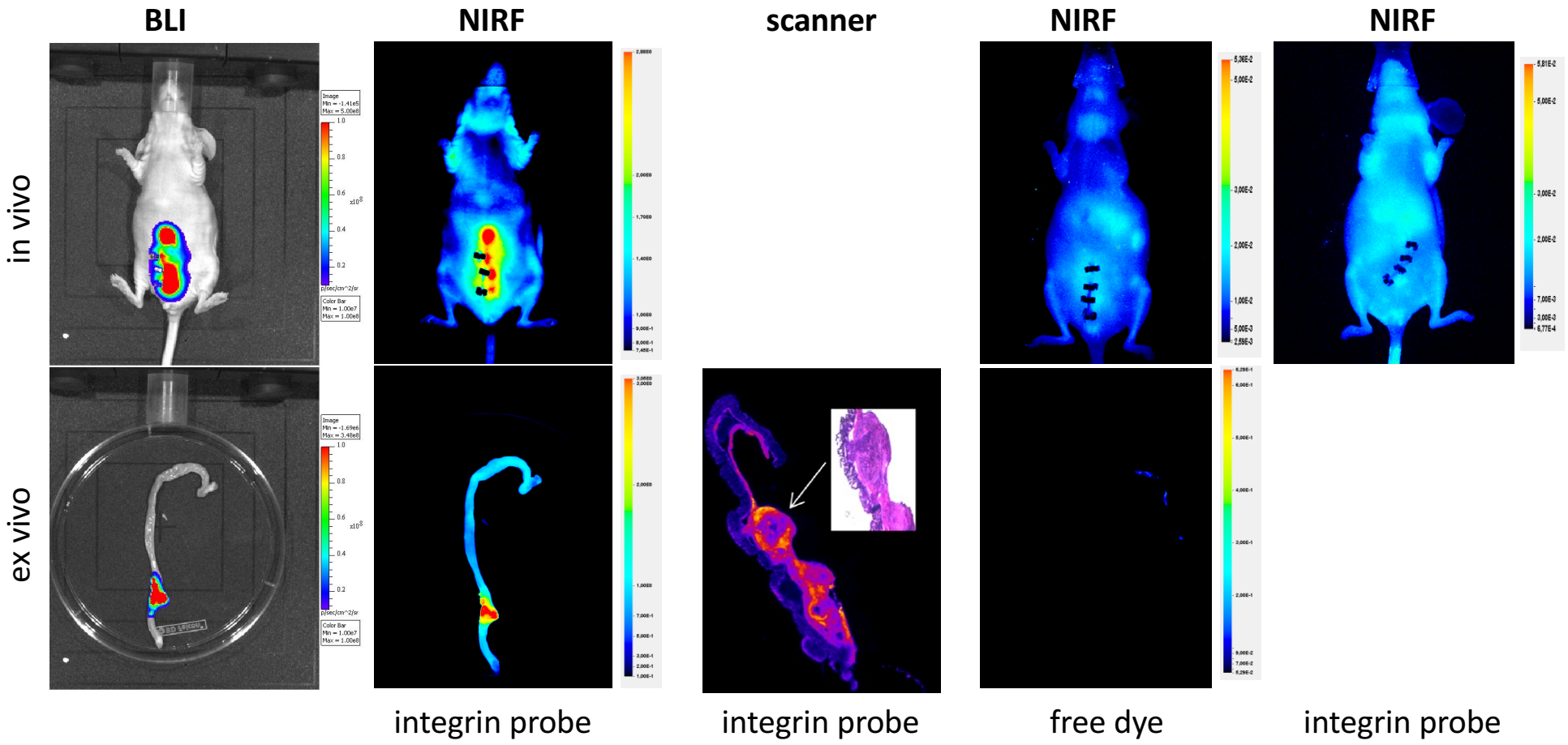






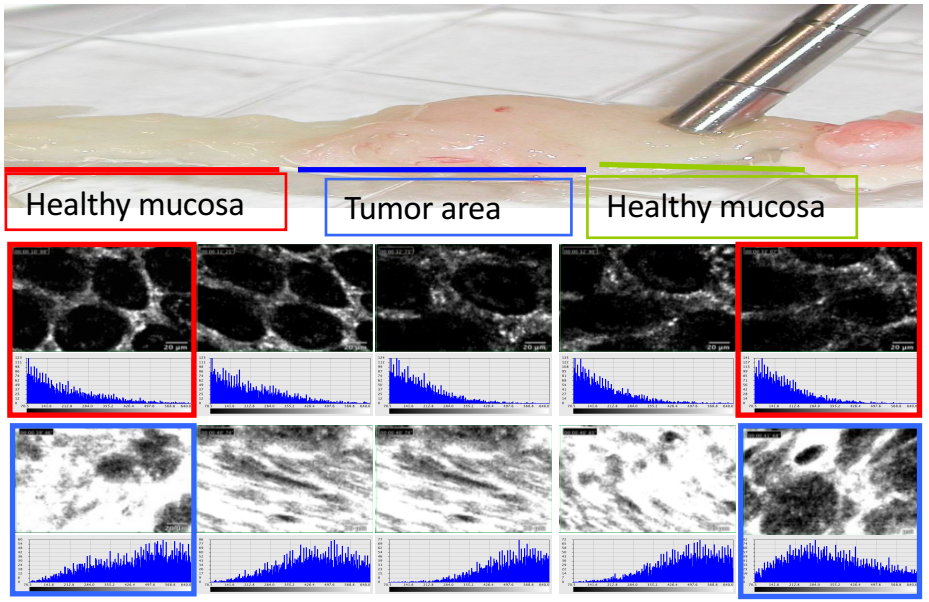
NIRF imaging of $\alpha_v\beta_3$ integrin in vivo/ex vivo

24h post injection, 2 nmol

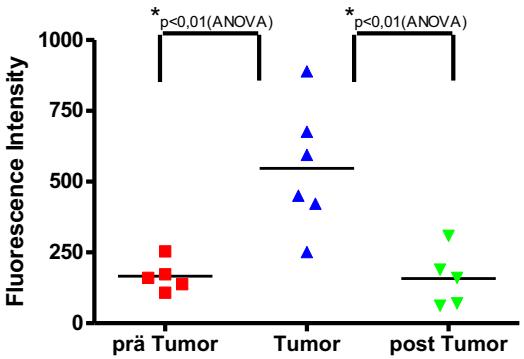


Endomicroscopic ex-vivo analysis of $\alpha_v\beta_3$ integrin targeting

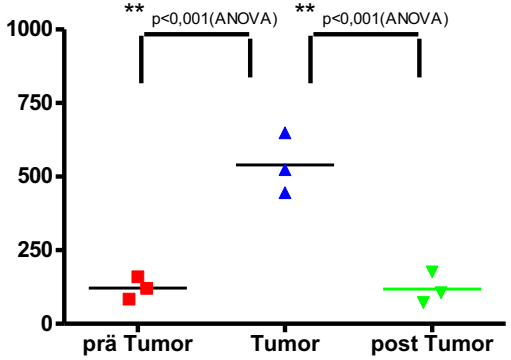
Confocal fluorescence endomicroscopic fiber probe
Exc 660 nm, Em >675 nm
(MaunaKea)



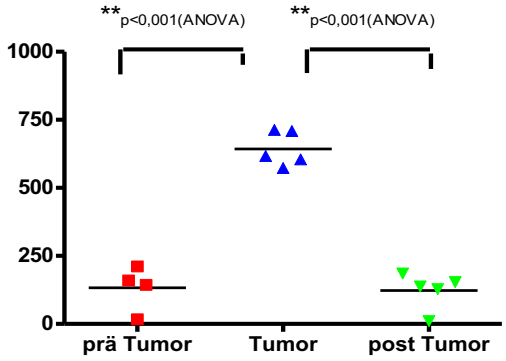
HT29 -Luc (n=5)



HCT116 (n=3)



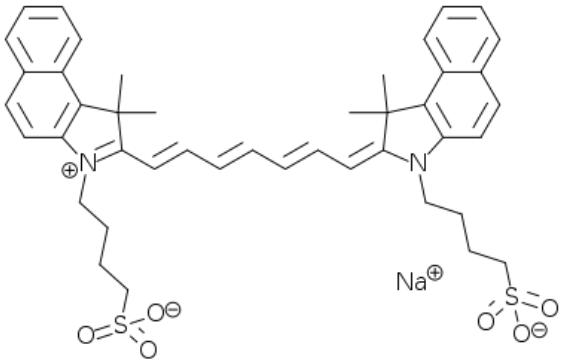
Colo 205 Luc (n=5)

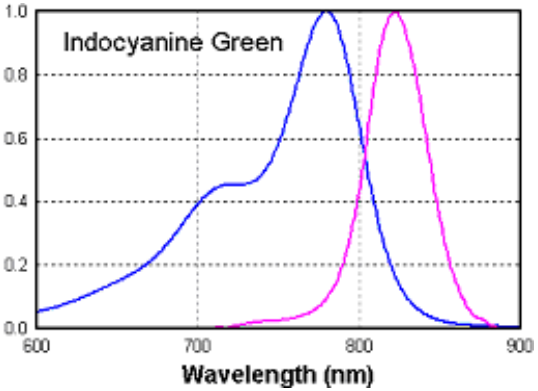


Schulz et al Mol Imaging Biol 2015

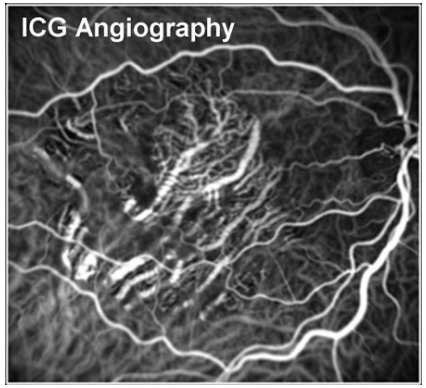
Broggini et al Eur Spine J 2015

Contrast agent: ICG

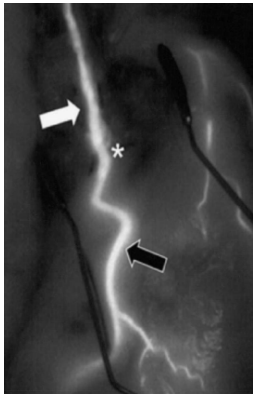




ophtalmology

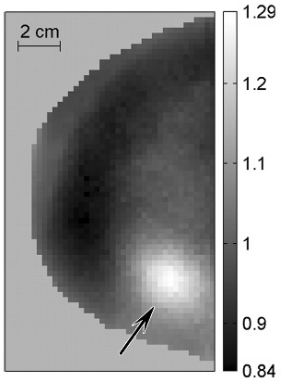


cardiology

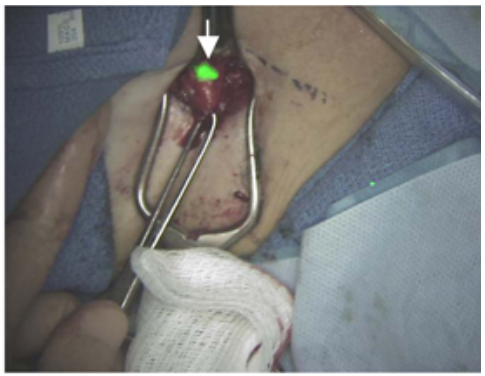


Desai et al, J Am Coll Cardiol 2005

mammography



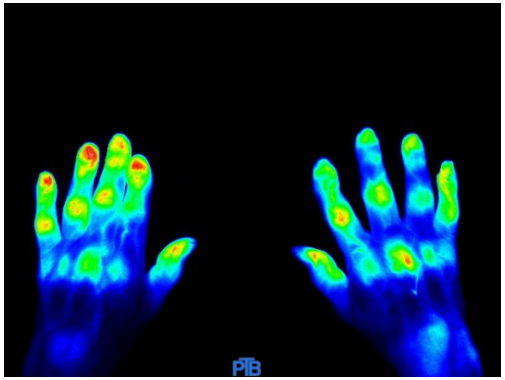
sentinel LN detection



Poellinger et al, Radiology, 2011
Troyan et al, Ann Surg Oncol 2009

control

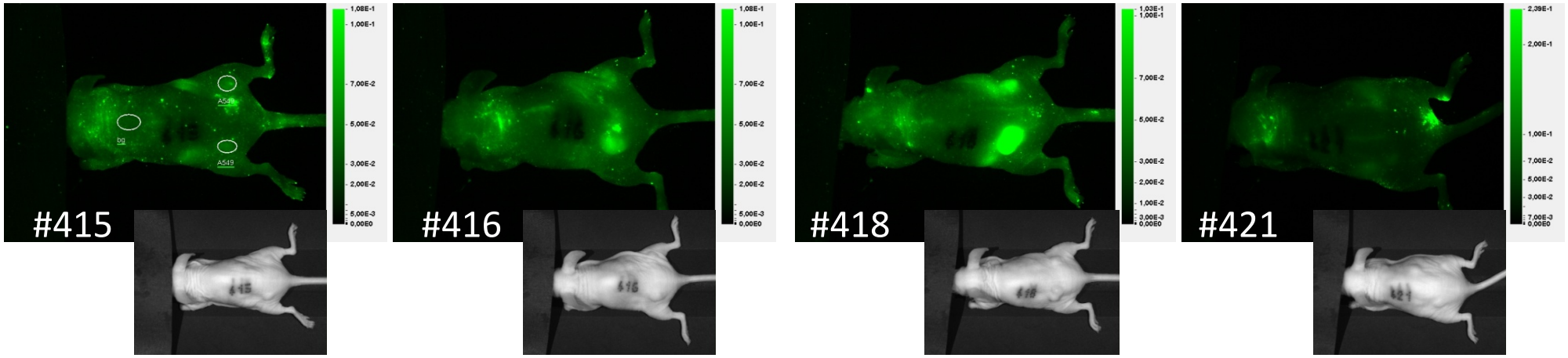
rheumatoid arthritis



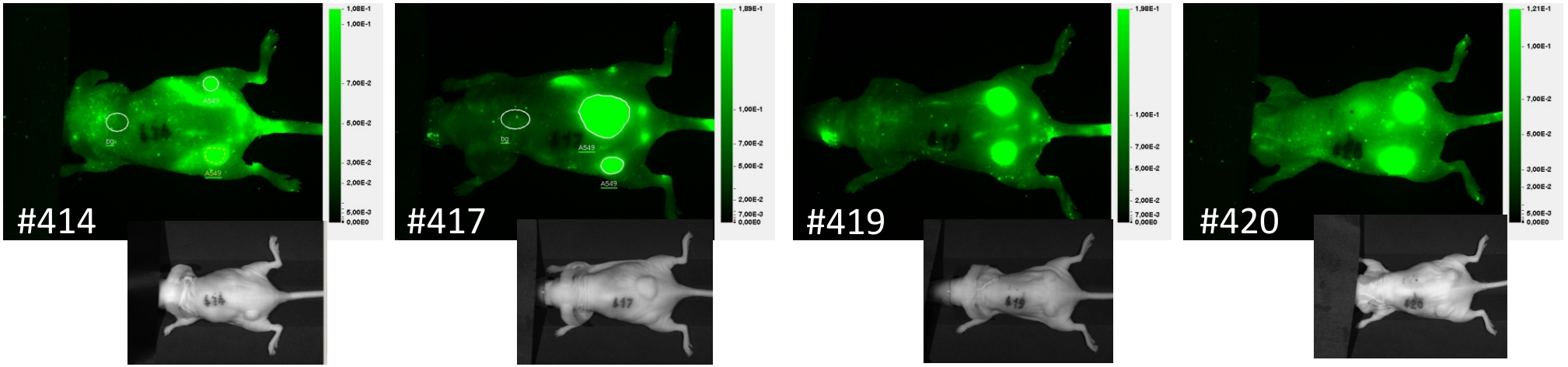
Bernd Ebert (Physikalisch-Technische Bundesanstalt)
Kai Licha, Michael Schirner (mivenion)

Comparison of nanoICG with ICG, 24 h post injection, 800nm channel, A459 lung tumor xenografts

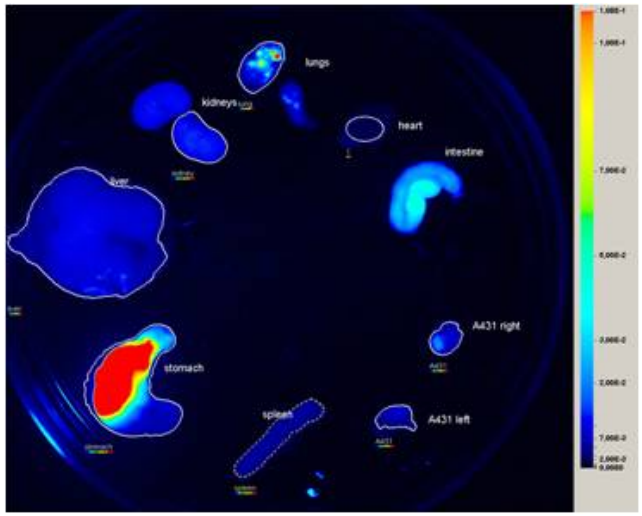
24h p.i. **ICG (0,2 mg/kg)**

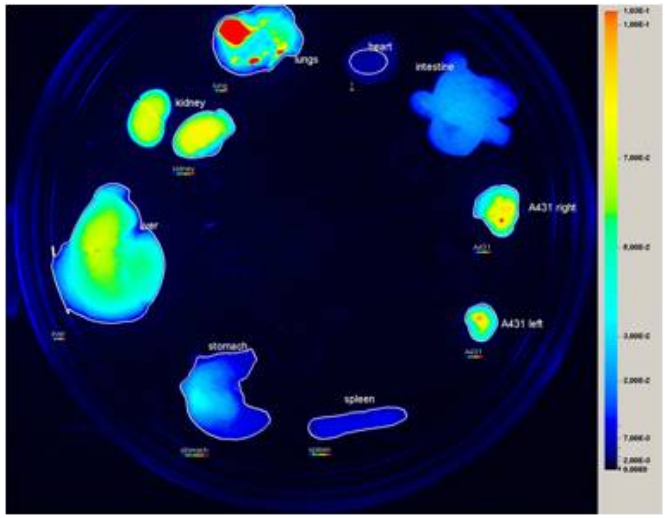


24h p.i. **nanoICG (0,2 mg/kg)**

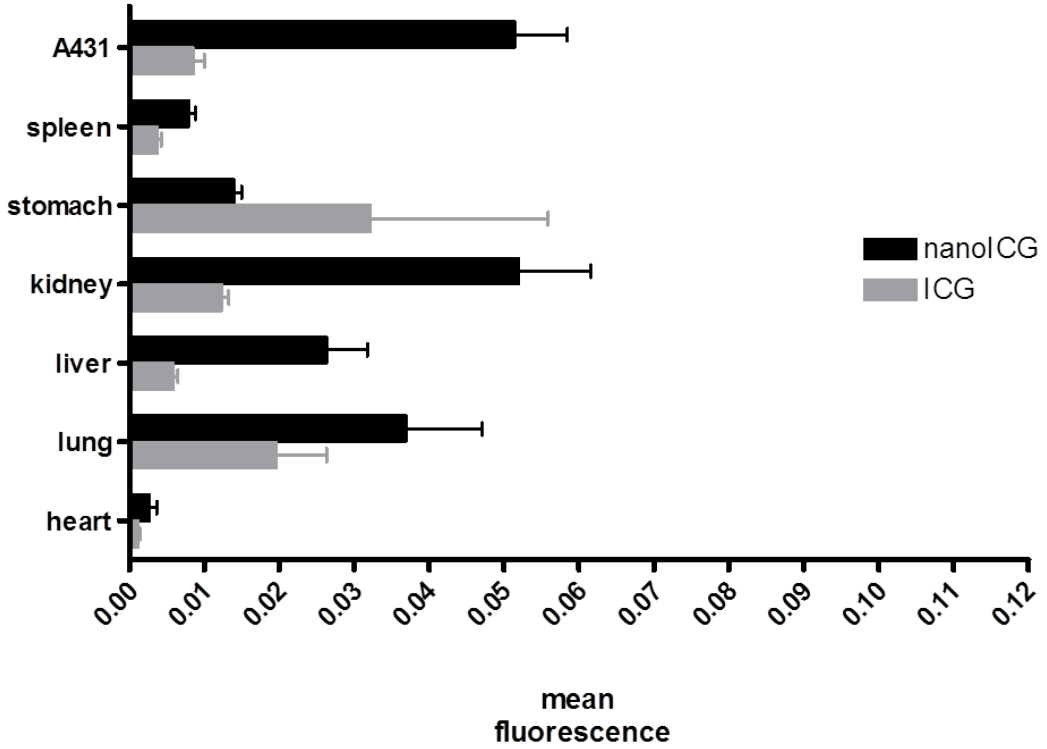


Ex vivo imaging nanoICG, 800 nm, A459 tumors



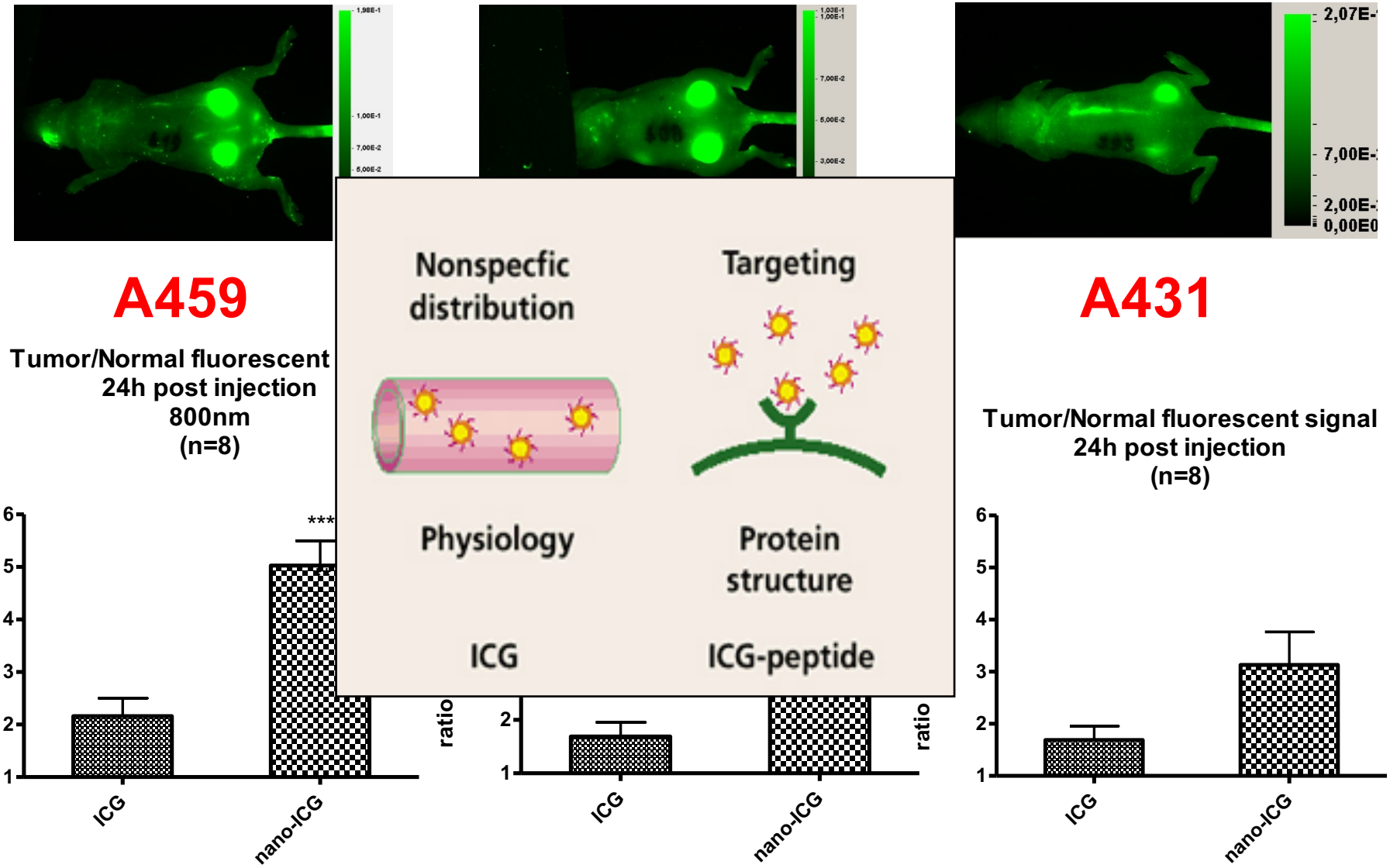


24h p.i. **ICG (0,2 mg/kg)**



24h p.i. **nanoICG (0,2 mg/kg)**

Semi-quantitative analysis from dorsal view, 24 h post injection, 800 nm channel



TECHNICAL REPORTS

nature medicine

Detection of colonic dysplasia *in vivo* using a targeted heptapeptide and confocal microendoscopy

Pei-Lin Hsiung[1], Jonathan Hardy[1], Shai Friedland[2,3], Roy Soetikno[2,3], Christine B Du[1], Amy P Wu[1], Peyman Sahbaie[2], James M Crawford[4], Anson W Lowe[3], Christopher H Contag[1] & Thomas D Wang[2,3]

VOLUME 14 | NUMBER 4 | APRIL 2008 NATURE MEDICINE

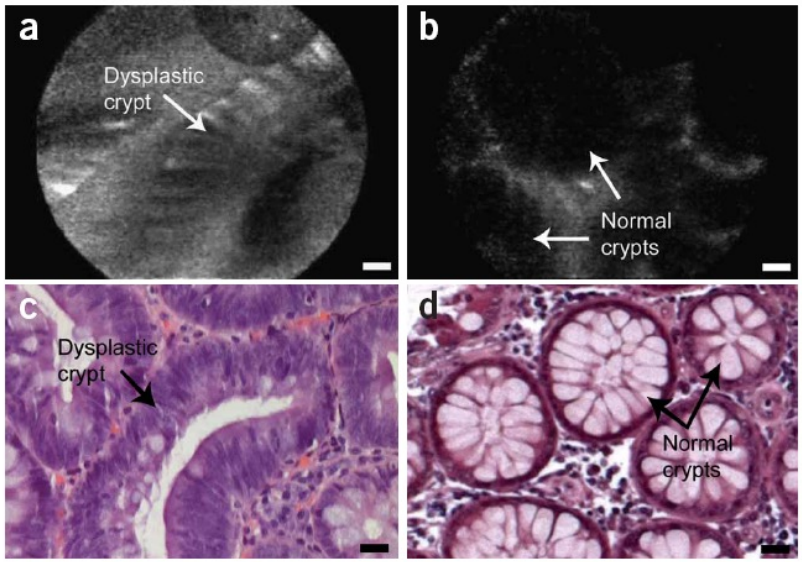


Figure 2 *In vivo* confocal fluorescence images of peptide binding. (**a**) Binding to dysplastic colon polyp. (**b**) Binding to adjacent normal mucosa. (**c**,**d**) Histology of dysplastic colon polyp (**c**) and normal mucosa (**d**) stained with H&E. Scale bars, 20 μm.

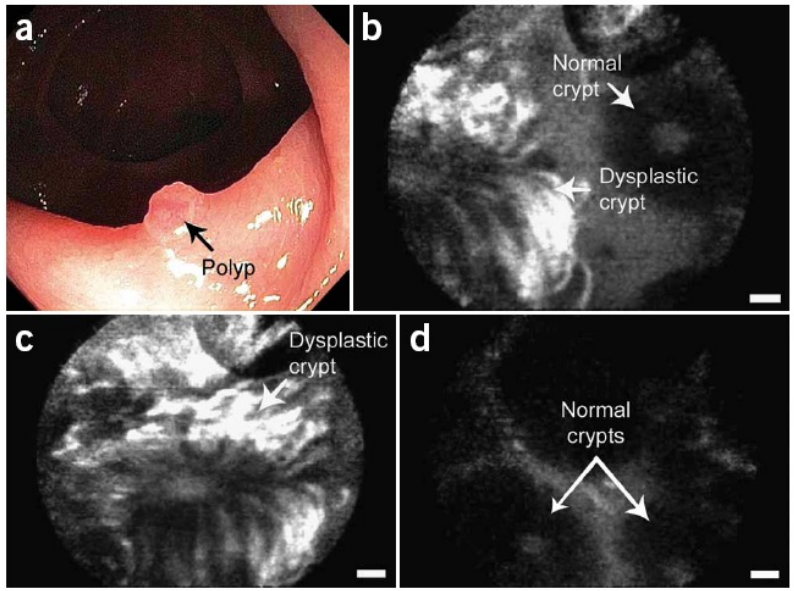
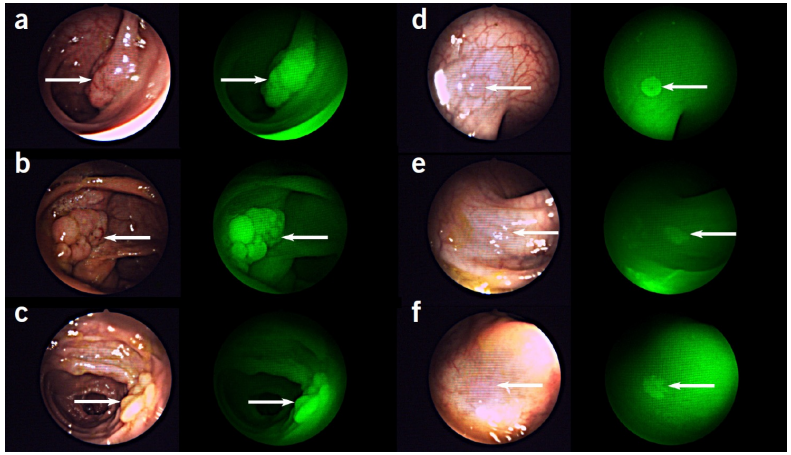


Figure 3 *In vivo* confocal fluorescence images of the border between colonic adenoma and normal mucosa, showing peptide binding to dysplastic colonocytes. (**a**) Endoscopic view. (**b**) Border. (**c**) Dysplastic crypt. (**d**) Adjacent mucosa. Scale bars, 20 μm.

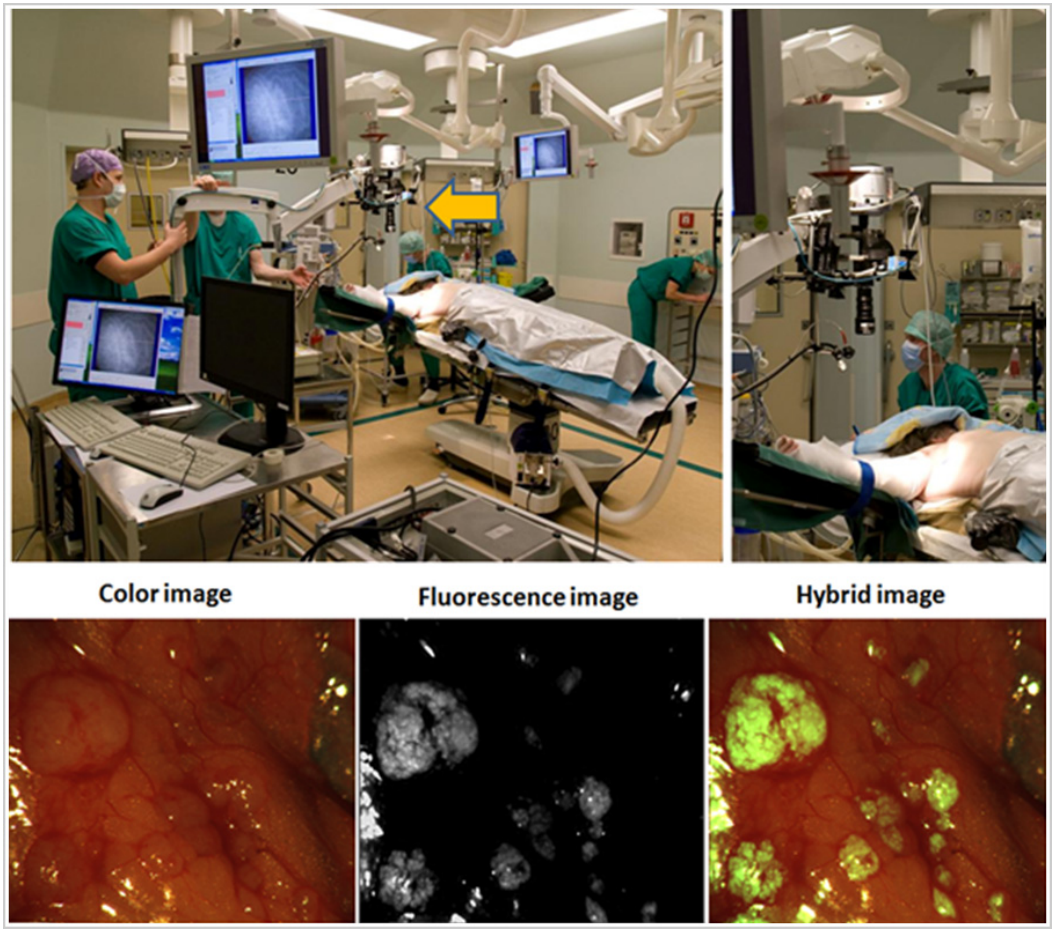
Human study. IRB approval was granted by Stanford University Medical Center and the VA Palo Alto Health Care Systems. We recruited individuals undergoing routine screening colonoscopy and obtained informed consent from all individuals. Polyps that were identified endoscopically during routine colonoscopy were washed with water for ~5 s to remove excess mucus. Approximately 3–6 ml of peptide at a concentration of 100 μM was then administered topically to 1–2 cm^2 of the surface of the colon using a standard endoscopic spray catheter. Excess peptide was removed by gently rinsing the region with water. Imaging was performed within ~5 min of peptide administration using the Cellvizio-GI confocal fluorescence imaging system (Mauna Kea Technologies). The fibered confocal microscope was passed through the instrument channel of a standard colonoscope (Olympus CFQ-160). Imaging of the polyp and adjacent endoscopically normal-appearing mucosa was performed before and after peptide administration. After imaging, the polyp was removed according to standard protocol, submitted for routine histology, and analyzed by staff pathologists at the Palo Alto VA Hospital.

Clinical application of NIRF probes

Endoscopy

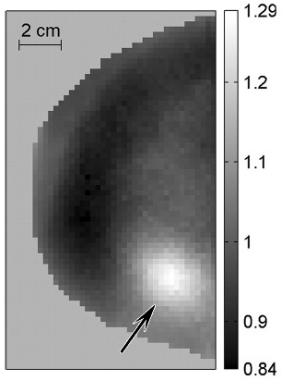


Intraoperative imaging

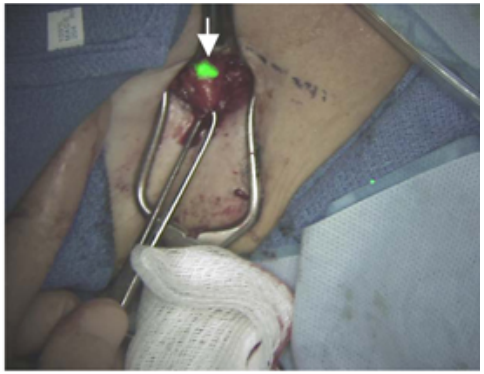


Van Dam et al, Nat Med 2011

Mammography



Sentinel LN surveillance



Burggraf et al, Nat Med 2015
Poellinger et al, Radiology, 2011
Troyan et al, Ann Surg Oncol 2009

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