In vivo Imaging to boost pharmaceutical innovation and translational research:potential applications to dermo-cosmetology

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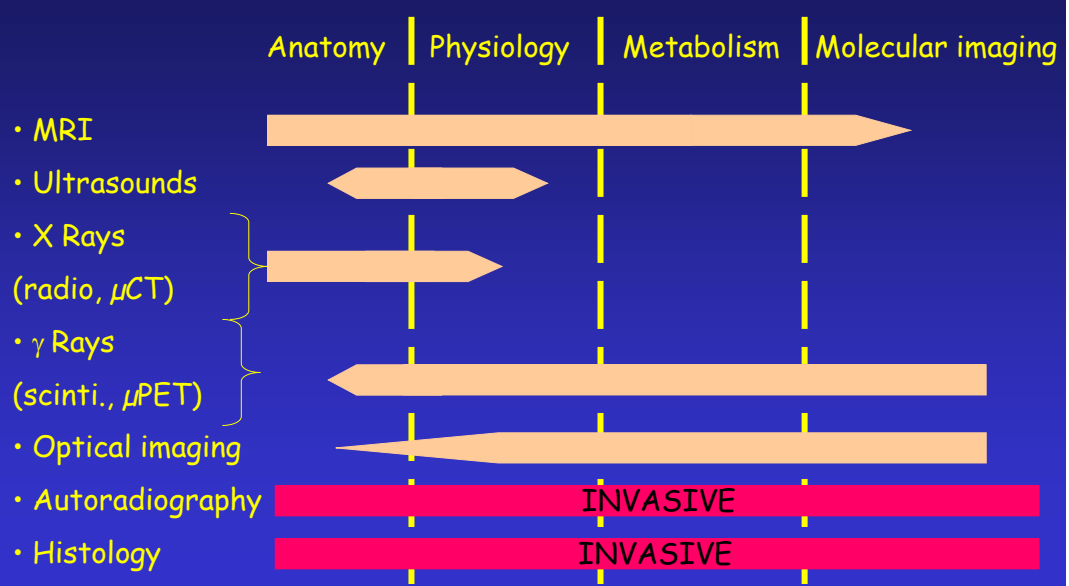
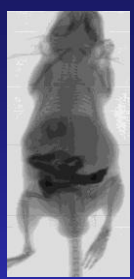
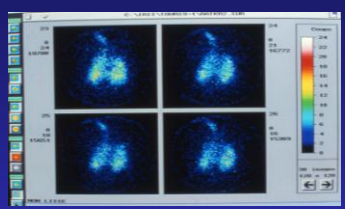
Imaging techniques available for small animal studies



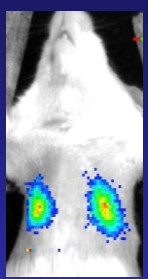
2D imaging with controled sanitary status and Quality Assurance (ISO 9001 certification since 2008) at CIPA



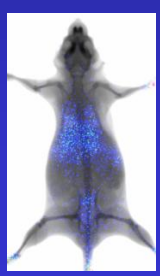
X ray



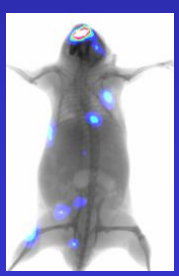
Gamma scintigraphy



Bioluminescence, fluorescence

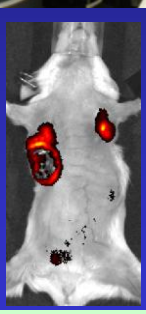


Scintigraphy + X

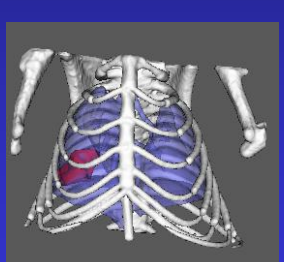


Bioluminescence + X

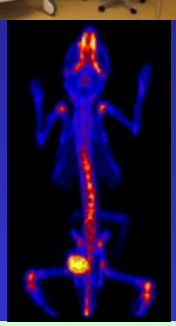
3D multimodality imaging at CIPA



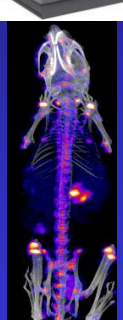
Bioluminescence
Infra red fluorescence



X Tomodensitometry



Positron Emission Tomography



SPECT/ X CT

in vivo imaging of biomarkers in preclinical drug discovery and development

Biomarkers: Parameters determined with validated accuracy and reproducibility to assess :

physiological or pathological processus and drug activity

Applications:

- -in vivo screening of candidate molecules
- -proof of concept
- -dose/response relationship and rationale for dose determination
- -safety assessment

→ prerequisite :

VALIDATED QUANTITATIVE IMAGING

Bioluminescence imaging

Firefly Luciferase

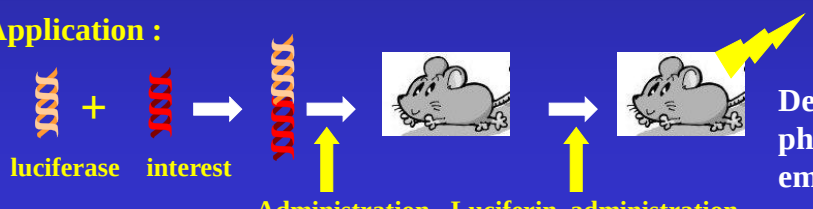
ATP O_2

Luciferin → Light emission

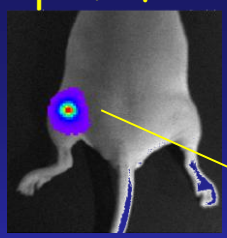
Gene expression + metabolism

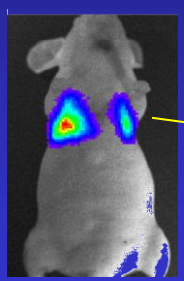
-gene vectorization, cell therapy
-oncology (luc tumor cells)
-infectiology (lux bacteria)
-toxicology (luc mice)

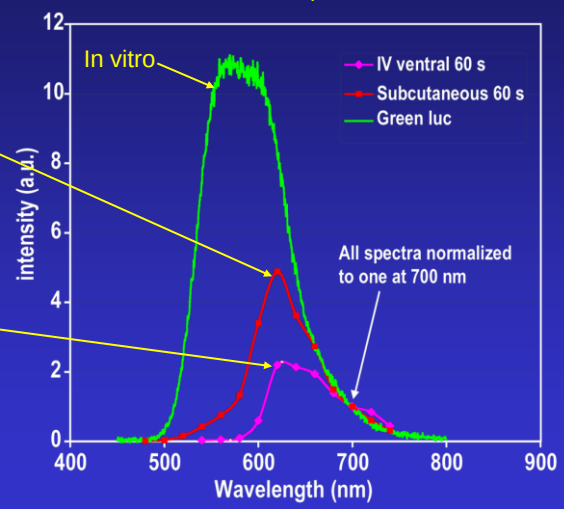
Application :



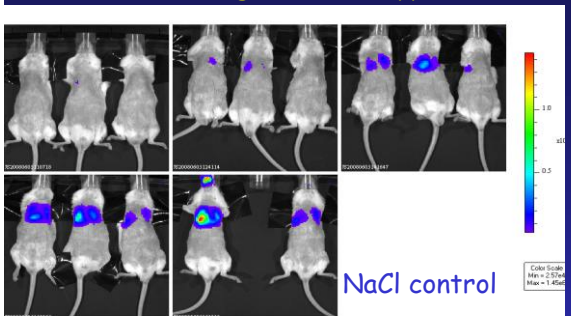
Spectral measurements provide information on depth of source and allows quantitative imaging



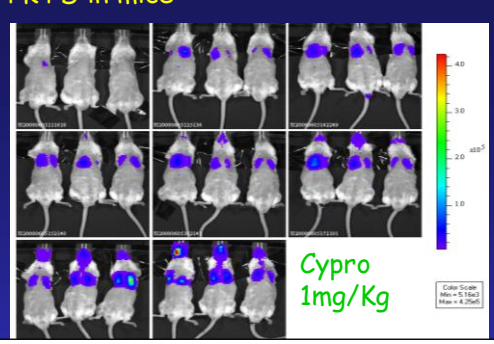




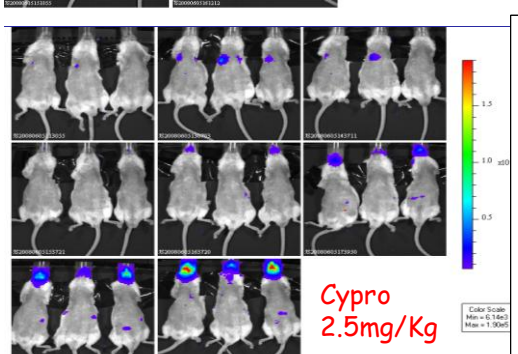
Pseudomonas aeruginosa luc+/cyprofloxacine PK PD in mice



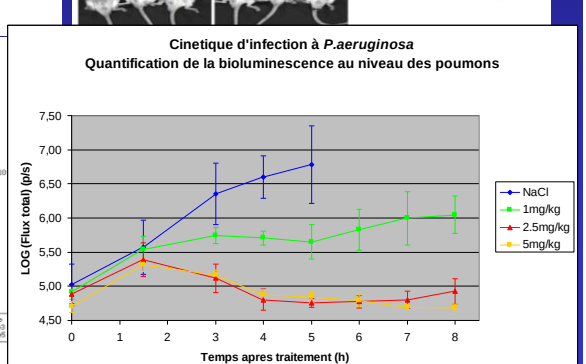
NaCl control

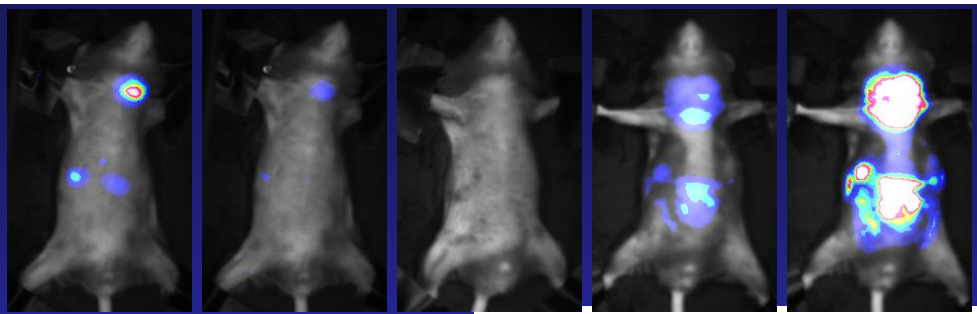


Cypro 1mg/Kg

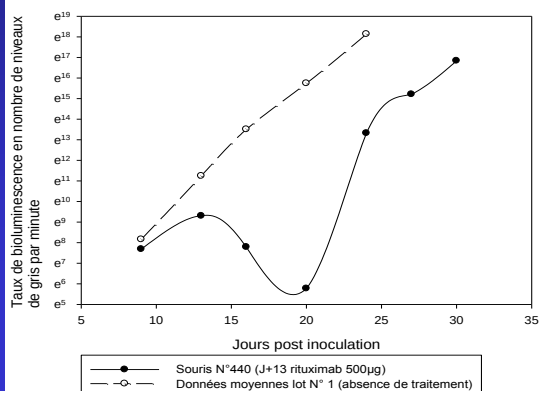


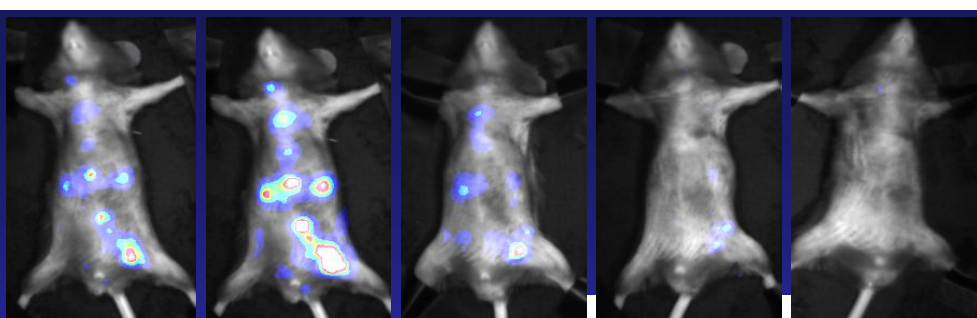
Cypro 2.5mg/Kg





Immunotherapy of a lymphoma model with CD20 luc EL4 cells with Mab:dose/efficacy and pharmacokinetics parameters

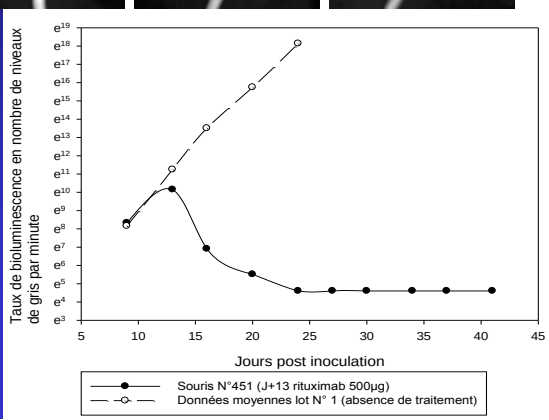




Immunotherapy of a lymphoma model with CD20 luc EL4 cells with Mab:dose/efficacy and pharmacokinetics parameters

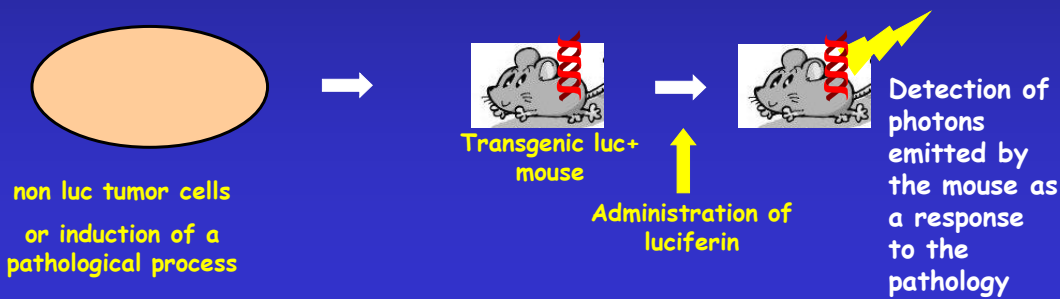
Daydé et al., Blood 2008

(Tumor burden influences exposure and response to rituximab : pharmacokinetic – pharmacodynamic modelling using a syngeneic bioluminescent murine model expressing human CD20)



Light Producing Transgenic Animals

Transgenic Animals expressing luciferase under the control of a specific promoter



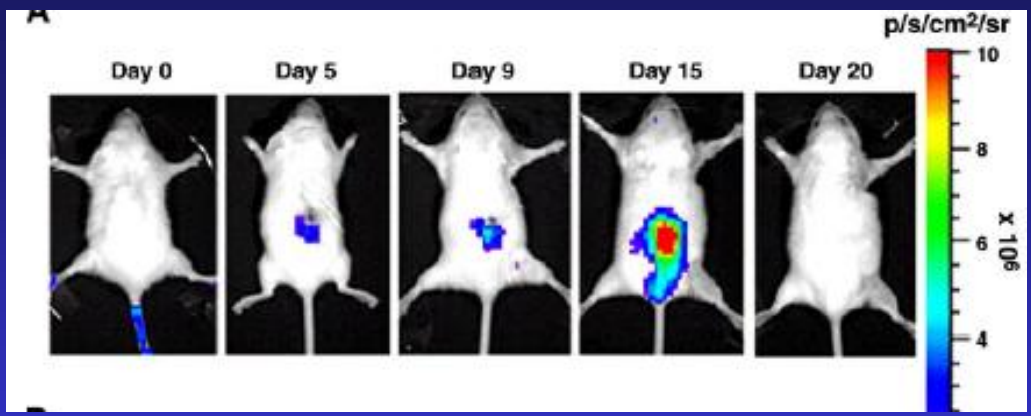
Light Producing Transgenic Animals

Inflammation

Animal Model	Background Strain	Applications
Gadd45b (growth arrest & DNA damage inducible 45-Beta)	CD-1	Cancer–apoptosis; MAP kinase- and NF-κB-mediated signaling pathways; inflammation
iNos or Nos2 (macrophage nitric oxide synthase)	FVB/N	Inflammation; sepsis
Epx* or Epo (eosinophil peroxidase)	FVB/N	Eosinophila–parasitism or asthma; bone marrow transplantation
Saa1 (serum amyloid A-1)	BALB/C	Arthritis, amyloidosis, sepsis
IL-2 (interleukin-2)	CD1	Inflammation, cancer
Cox2 or Ptgs2 (cyclooxygenase-2)	BALB/C	Inflammation, pain
TNFα (tumor necrosis factor-alpha)	BALB/C	Inflammation–arthritis or inflammatory bowel disease; cancer–apoptosis; sepsis
NFκB-RE (NFκB response elements)	BALB/C	Inflammation–arthritis or inflammatory bowel disease; cancer–apoptosis
NFκB-RE (Oslo) (NFκB response elements)	BALB/C & DBA/1	Inflammation–arthritis or inflammatory bowel disease; cancer–apoptosis
IκBα ([inhibitor of NFκB]α)	BALB/C	Inflammation–arthritis or inflammatory bowel disease; cancer–apoptosis; sepsis

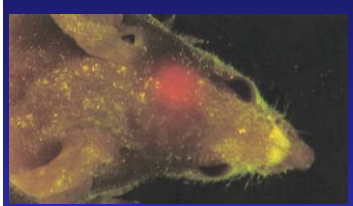
Oncology/Angiogenesis

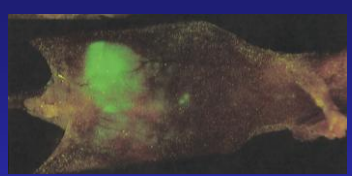
Animal Model	Background Strain	Applications
Kdr or Vegfr2* (vascular endothelial growth factor receptor-2)	FVB/N	Inflammation; angiogenesis processes incl. embryonic or post-natal development, wound healing, cancer
Kdr or Vegfr2* (in NUDE [Nu/Nu] background)	FVB/N	Tumor-induced angiogenesis
Vegf * (vascular endothelial growth factor)	FVB/N	General angiogenesis reporter
PSA (prostate specific antigen)	FVB/N	Tracking prostate tumor cells growth/metastasis

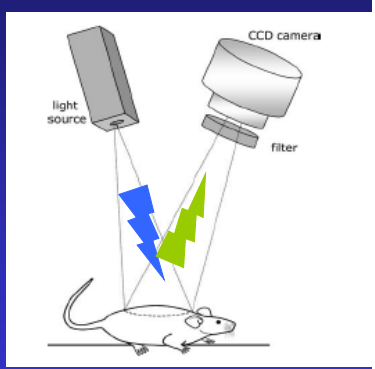


Evolution of bioluminescence signal after induction of a skin lesion in a VEGFr luc mouse

In vivo fluorescence imaging
GFP or RFP expression in tumor cells

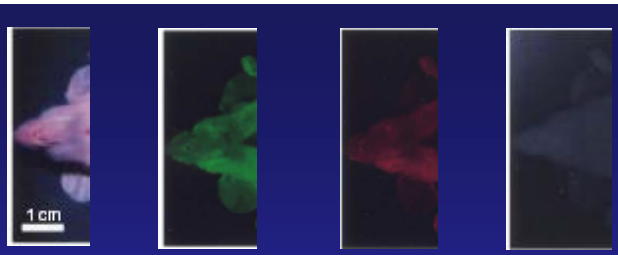




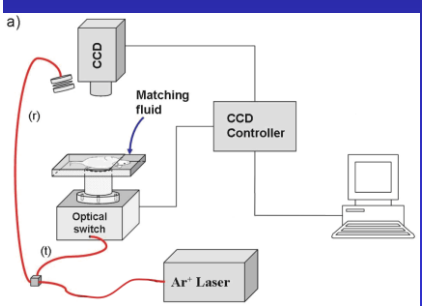


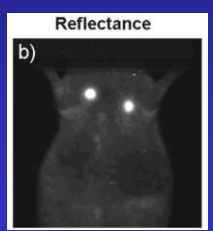
limitations for quantitation and deep foci due to absorption of excitation and fluo photons

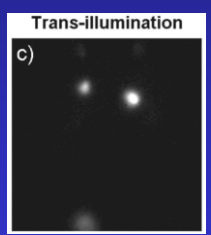
Autofluorescence from skin when observed as visible conventional fluo green 1) , red 2) and in the Near Infra Red 3)



1) 2) 3)





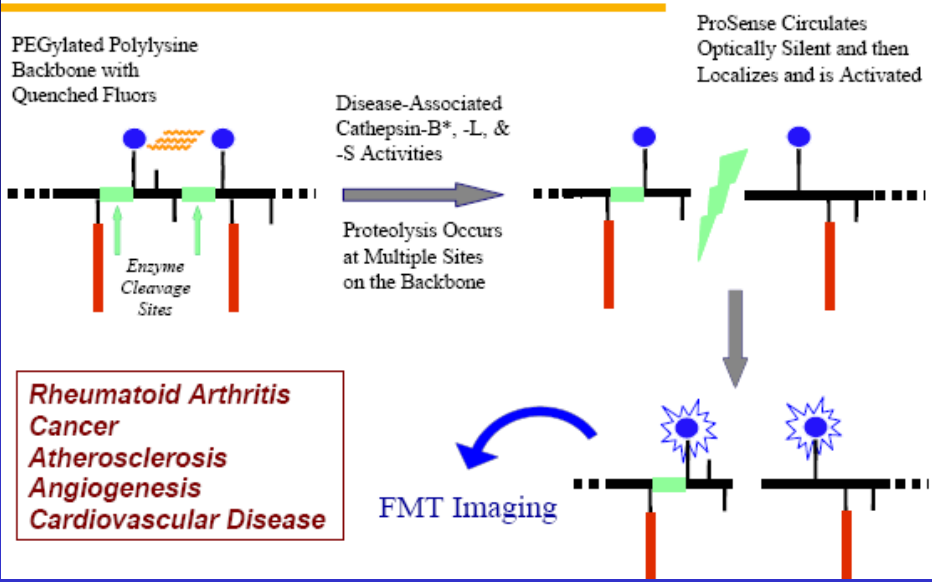


2D fluorescence by reflectance or trans-illumination
3D by trans-illumination, FMT and Time Domain Fluorescence

Some applications

- Cell labeling for imaging domiciliation
- Biodistribution of molecules
- Systemic/lymphatic absorption from skin /intradermal admin.
- Apoptosis : annexine V-Cy5.5 and caspase 1 with quenched substrate
- Specific function: angiogenesis (molecular and /or functional)
- Antibody-antigen or ligand-receptor interaction
- Enzymatic activities using activatable quenched probes

Activatable Probe: ProSense 680/750

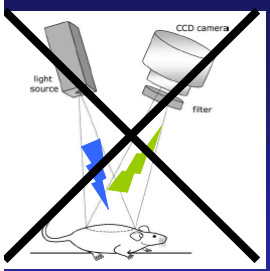


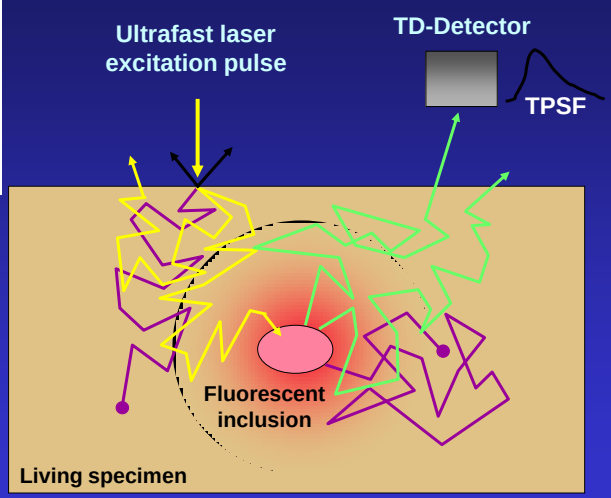
Rheumatoid Arthritis
Cancer
Atherosclerosis
Angiogenesis
Cardiovascular Disease

Some NIRF probes from Visen medical as biomarkers

Probe	Type	MW	Clearance $t_{1/2}$	Description/Uses	Ch1 ~680nm	Ch2 ~750nm
MMPSense™	Activatable	~450,000	4-8d (tissue)	Activated by *MMP2, 3, 9 & 13* protease activities/Inflammation, Arthritis, Oncology	✓	--
ProSense®		~450,000	24h (blood) 4-8d (tissue)	Activated by Cathepsin B (primarily), L, and S/ Inflammation, Arthritis, Oncology	✓	✓
OsteoSense®	Targeted	~1,000-1,500	17.5d (arthritic joint)	Binds to sites of Bone Remodeling/Osteoarthritis, Bone Formation/Resorption, Calcification	✓	✓
AngioSense®-IVM	Passive Distribution	~250,000	~2h	Images Vasculature/Intravital Microscopy (IVM)	✓	✓
AngioSense®			5h (blood) 5d (arthr. joint)	Images Vasculature and Blood Vessels/IVM; Blood Pooling in Tumors and Inflammation/FMT	✓	✓
Superhance™		~1,500	1.5h-2h (blood)	Binds to Albumin and Images Vasculature & Inflammation/IVM	✓	--
Genhance™		~800-1,200	Minutes	IntraVital Microscopy	✓	✓
AminoSPARK™	Nanoparticle (Targetable)	5-10nm(core) 20-40nm >10^6	Varies	Fluorescent Nanoparticle Tag for variety of Amine Reactive Molecules	✓	✓
AngioSPARK™			14h-20h (blood) Weeks (tissue)	Fluorescent Nanoparticle/ Long Term Imaging of Blood Pooling in Tumors and Inflammation	✓	✓
VivoTag™-S	NIR Fluorochrome (Targetable)	~900-1,200	Varies	Fluorescent Tag for NHS Ester Conjugation to Peptides, Small Molecules, Proteins, Fab Antibodies, etc.	✓	✓
VivoTag™		~1,250	Varies		✓	--

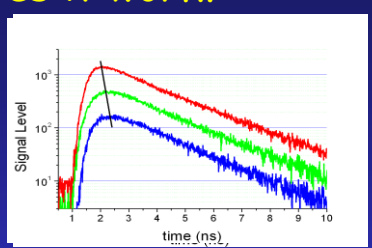
Fluorescent time-domain measurements



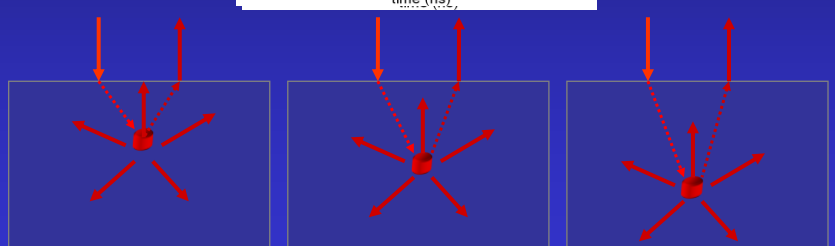


Time Domain Imaging

How does it work?

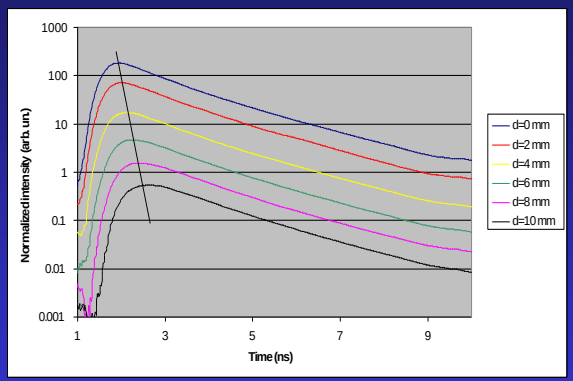


Analogy with Sonar system



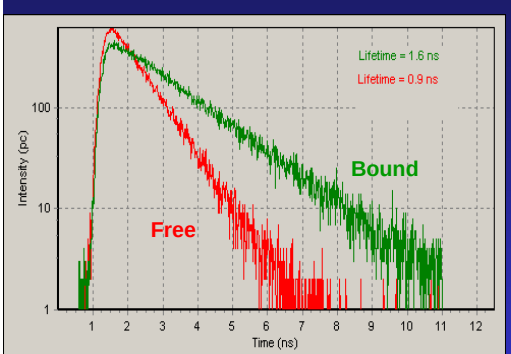
Depth and Concentration Probing

Different depths
100 nM Cy5.5 concentration



- **Peak position shifts to the right as depth increases**
- **Intensity decreases with depth due to tissue absorption**
- **Correction from absorption allows determination of actual concentrations**

Relationship between fluorescence lifetimes and free/bound ratio of a drug in a given territory

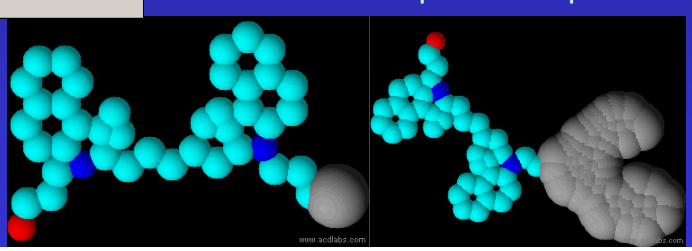


- Lifetime (τ) affects slope of decay curve:

  Slope = -1/τ

- The shorter the lifetime, the steeper the slope

Emission wavelength = 700nm



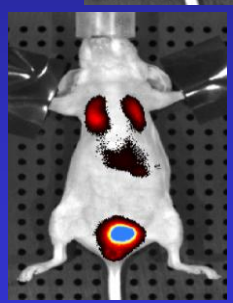
Limitations of infra red fluorescence imaging

-molecular weight of fluorochroms : 700 to 1200 and possible effects upon properties of labeled molecules

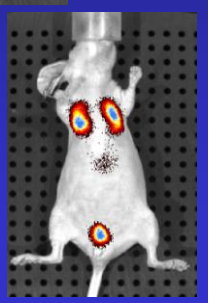
-excitation light provided by lasers and not by filtered white light: fluorochroms must fit with lasers

-assessment of free-bound ratio is not possible for high molecular weight drugs (mAb)

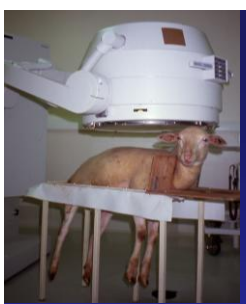
24h

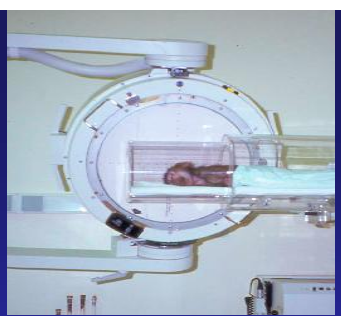


48h

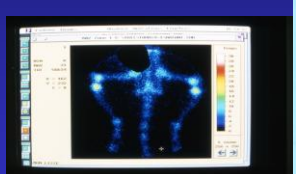


72h

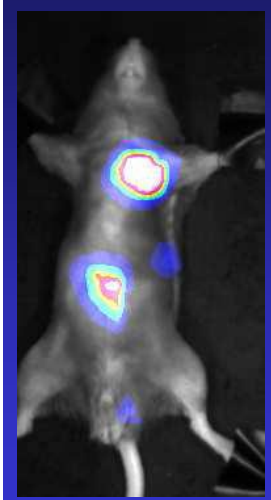


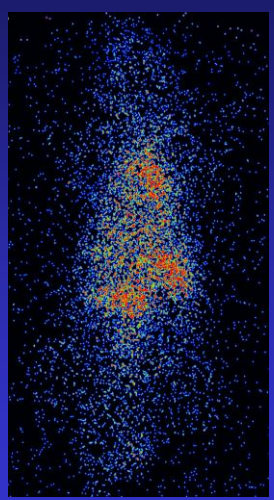


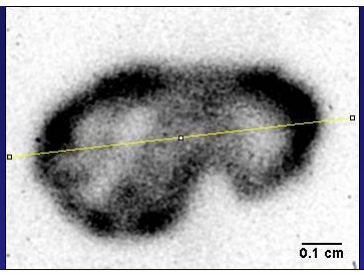
Imaging with gamma isotopes

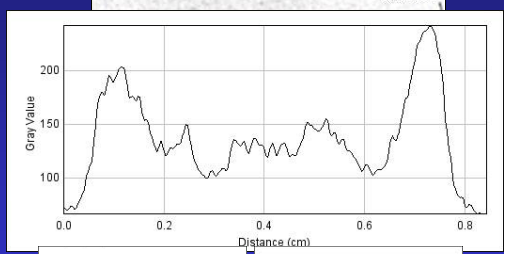


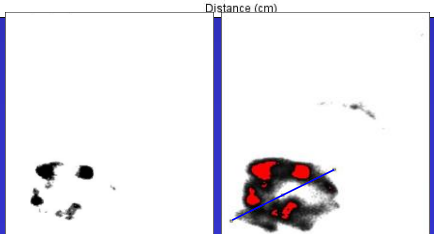
S1464 biolum versus anti CD20 Mab ^{111}In scintigraphy and distribution inside tumor



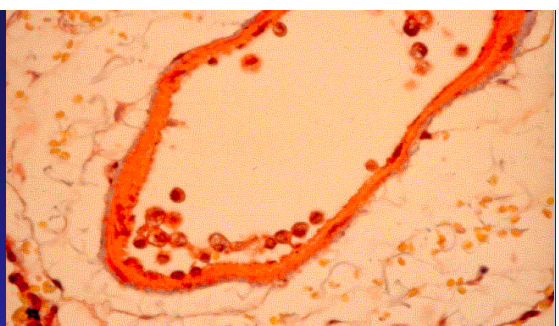


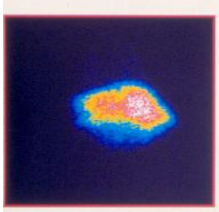


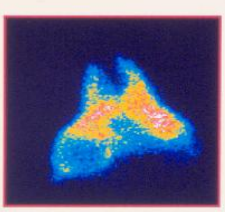


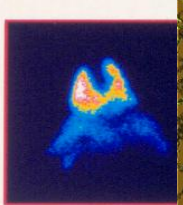


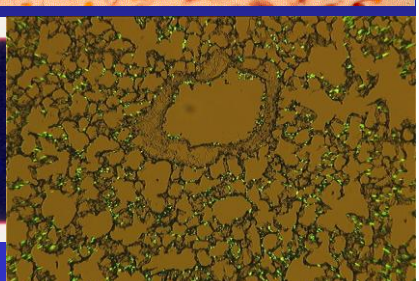
PIMs phagocytosis in vivo imaging by bimodal 99mTc - fluorescent colloids











Sham	Patho. (d21) :	Patho. (d35)
Liver : 90%	Liver : 83%	Liver : 27%
Lungs<2%	Lungs : 13%	Lungs : 65%

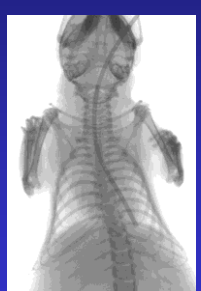
Examples of multimodal strategy for translational research

Rationale approach:

-biophotonics i.e. bioluminescence and fluorescence from in vitro to in vivo screening, proofs of concept and selection of leads

-in vivo modalities derived from medical imaging to assess efficacy, PK, improve drug targeting or vectorization then move to patients

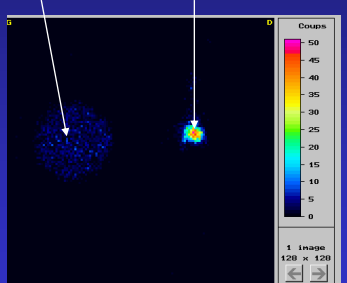
Interventional Imaging to optimize induction of bronchial carcinoma for aerosolized chemotherapy development



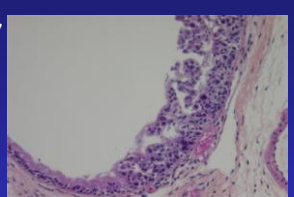
X ray guided bronchial catheter

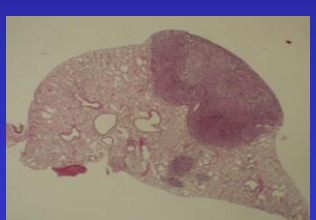
Standard 99mTc activity

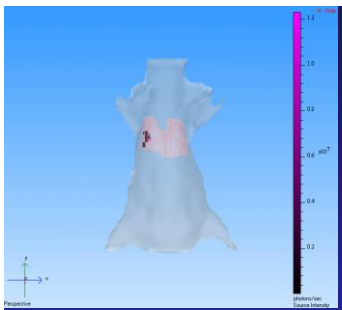
Number of cells actually deposited



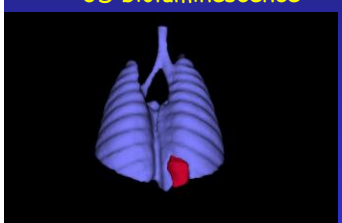
Scintigraphic quality control of tumor cells inoculum





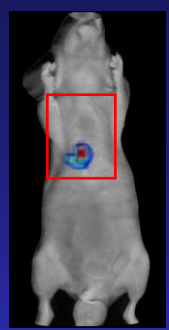


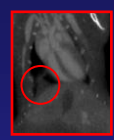
3D bioluminescence



X CT/Respiratory gating

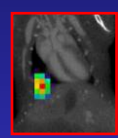
FMT2500 Reconstruction





Lung Tumor

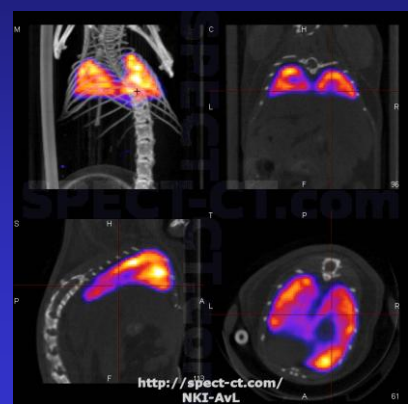
micro-CT / FMT Fusion



MMPs 7,9,12,13
Polylysine backbone with peptide sequence CPLGVRGKC to link IR fluo for specific activation

Cathepsines B,L,S and plasmine

Aerosolized chemotherapy



Aerosol lung distribution

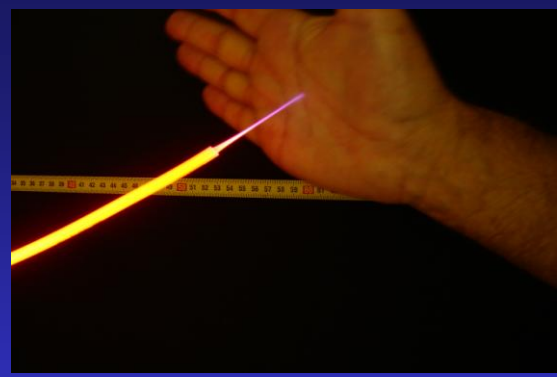
Aerosolized gemcitabine in the treatment of NSCLC

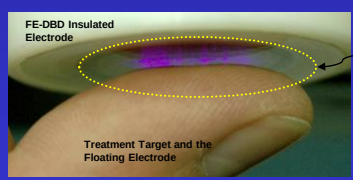
First clinical trial, 2008
11 patients, 9 weekly administrations
Escalating dose from 0.5mg/kg
Maximum Tolerated Dose
1.5mg/kg
Cmax= 10 to37micromol/l
AUC= O.O33 to O.137mg/L/h

Phase 2 study at 1.5mg/kg, 2009

GREMI, UMR 6606
CNRS-University of Orleans

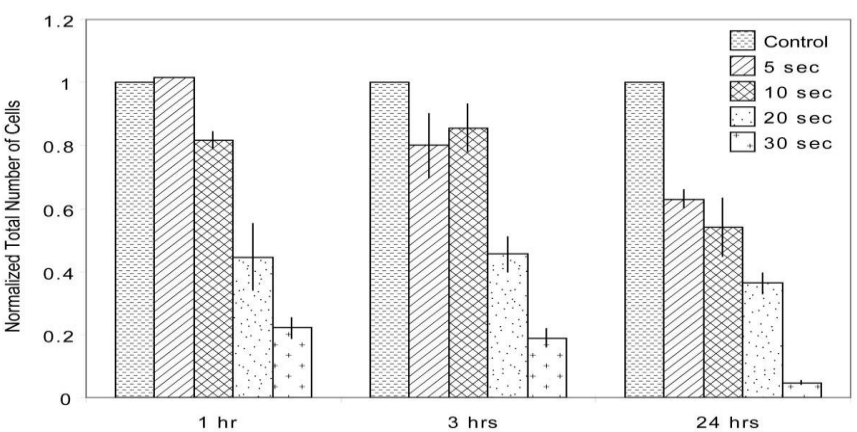
Development of cold atmospheric plasmas for therapeutical applications



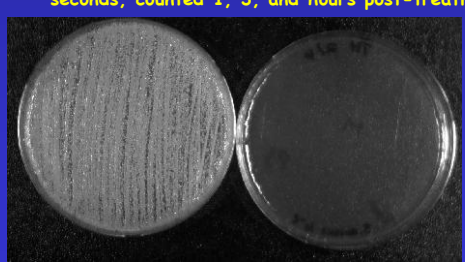


Dielectric Barrier Discharge Plasma in Air

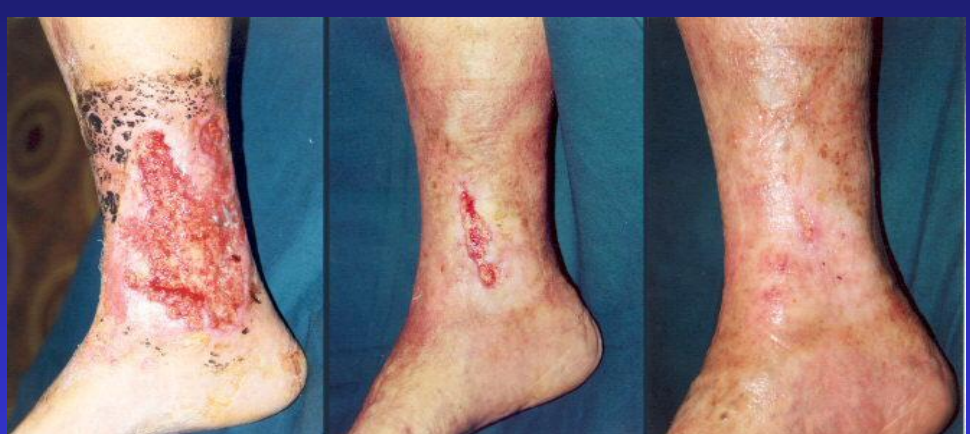
Floating potential DBD – Inset on the right shows filamentary nature of the discharge



24 FE-DBD treatment of Melanoma cancer cells: Control, 5, 10, 20, and 30 seconds, counted 1, 3, and hours post-treatment.



Staphilococcus aureus 5 minutes exposure



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