International Conference on Nanotheranostics

ICoN 2013

Short Course

Nanotheranostics: all-in-one personalized medicine

28 September 2013

Golden Bay Beach Hotel
Larnaca, Cyprus

Short Course: Nanotheranostics: all-in-one personalized medicine

Session Chairs: George Potamitis, Chrysa Tziakouri-Shiakalli, Cyprus Medical Association

This short course will provide an overview of the major concepts behind the newly created field of nanotheranostics. Nanotheranostic agents have a number of significant advantages over current approaches: (i) Nanotheranostic agents can be customized to the disease and personalized to the patient. (ii) Active targeting and localization allows for better treatment with much less intense side effects compared to current regimens. (iii) The integration of therapy and monitoring provides real-time information on whether or not the specific treatment regimen is working for the specific patient. Given these attributes, it is not surprising that the field of nanotheranostics is considered the future of treatment of highly inhomogeneous and variable diseases such as cancer and chronic inflammatory disorders.

11.30-11.45 **Introduction**

Going to the lower limits: nanotechnology and nanomedicine

Theranostics: all-in-one personalized medicine

Andreani Odysseos, EFB, EPOS-Iasis R&D Ltd, Cyprus

11.45-12.05 **Theranostic Nanoparticles**

Rena Bizios, University of Texas at San Antonio, USA

12.05-12.25 **Clinical applicability of Optical Imaging**

Costas Pitris, University of Cyprus, Cyprus

12.25-12.45 **Nanotheranostics at the clinical fore**

Image-guided therapy: paving the way of nanotheranostic agents to the clinic

Monitoring therapy by imaging

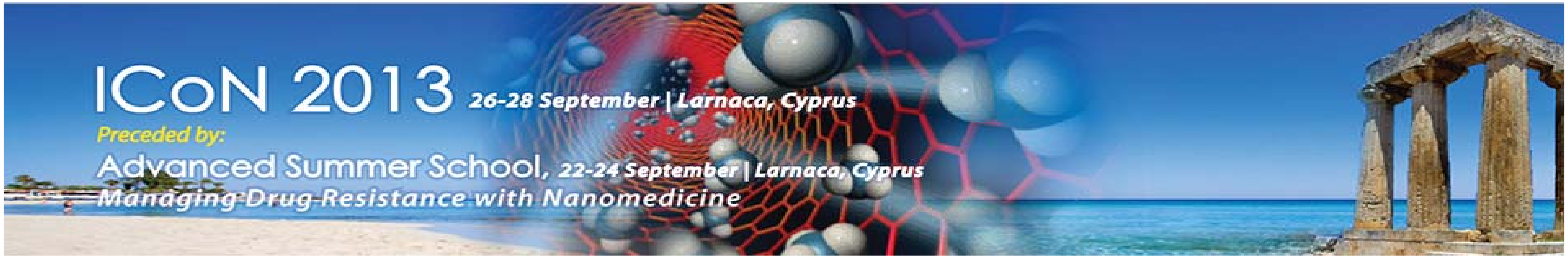
Andreani Odysseos, EFB, EPOS-Iasis R&D Ltd, Cyprus

12.45-13.00 **Image-guided quantification of drug delivery: a revolution in Radiology (Positron Emission Tomography (PET) and Magnetic Resonance Imaging (MRI))**

Radiation-based therapies

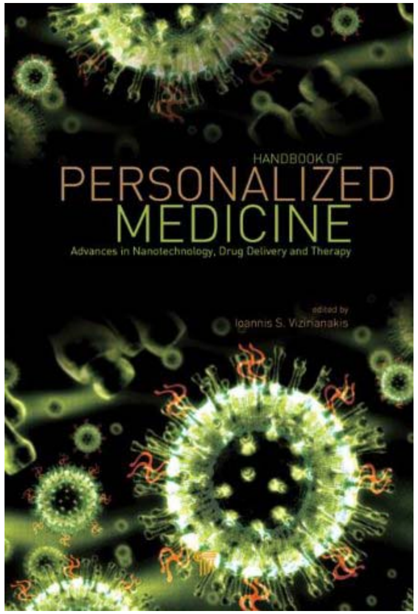
Costas Pitris, University of Cyprus, Cyprus

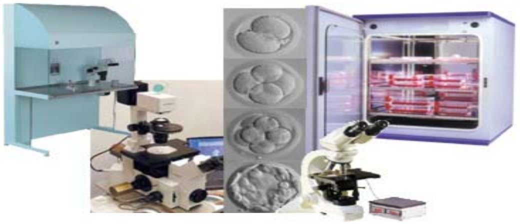
13.00-13.30 **Discussion of Clinical Challenges and Prospects**

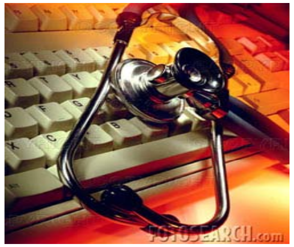


Special Short Course:

Nanotheranostics: all-in-one personalized medicine





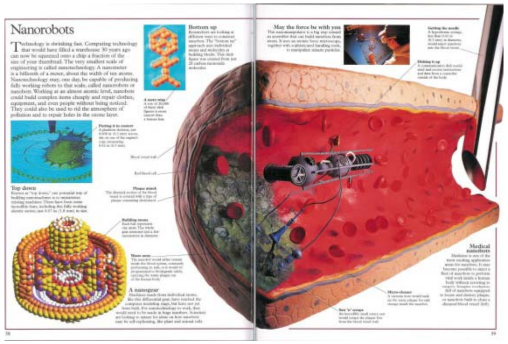


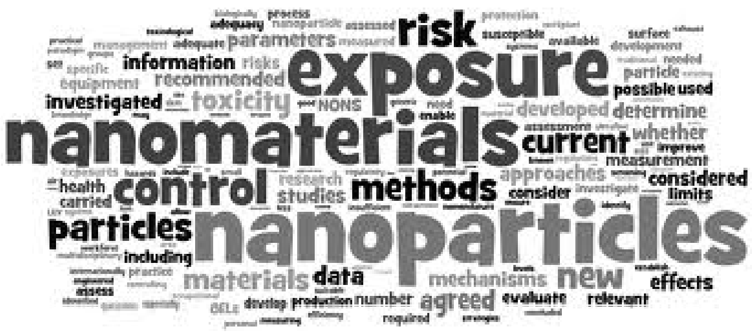
http://www.epos-iasis.com/IAPP/Education/

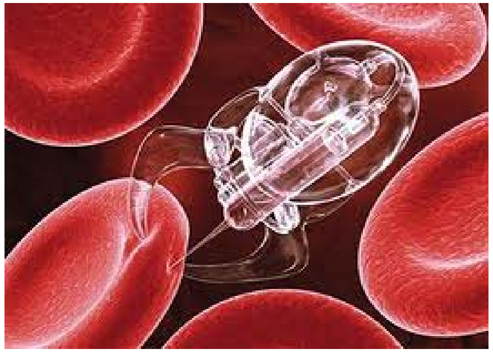
“ La médicine est un art fragil appuyé sur des sciences solides ”

Claude Bernard
French Physiologist (1813-1878)

Going to the lower limits: nanotechnology and nanomedicine







...An introduction to Nanotechnology...




...Big events happen in small worlds...

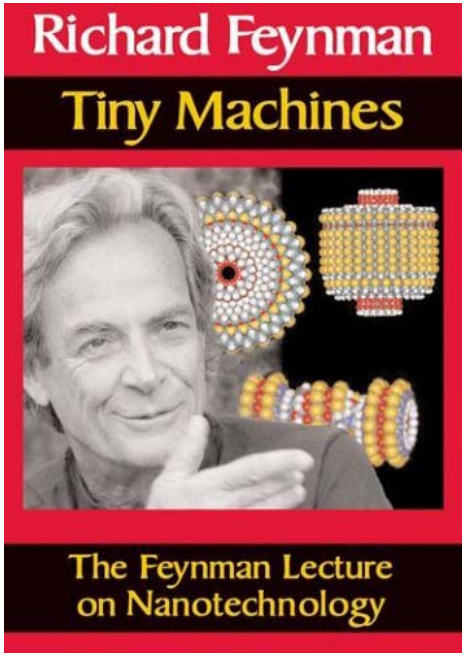
The Nano-world: a world of wonders, synthesis, complementarity , endurance and promise

Nanobioetechnology and NanoMedicine
The First Promise

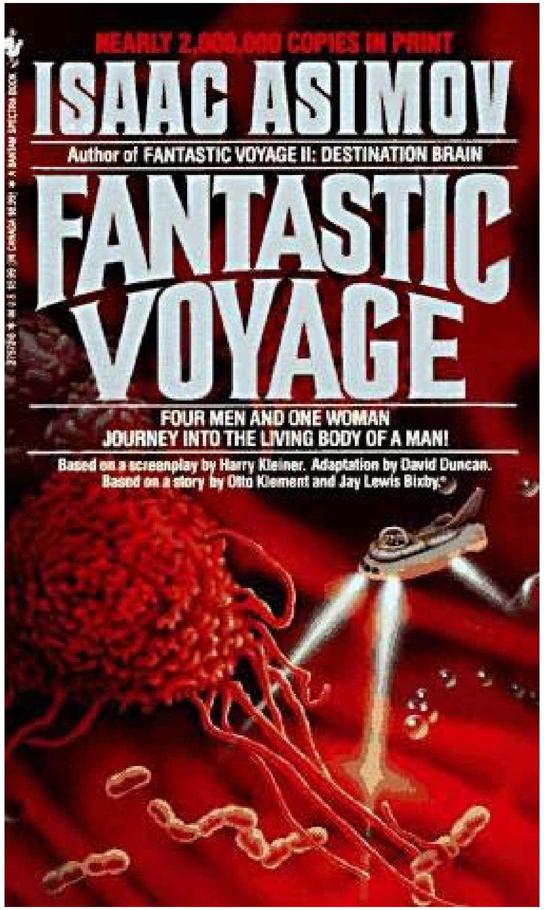
- Richard Feynman's lecture "*There's Plenty of Room at the Bottom*", a description of atomic scale machines: **the birth of Nanotechnology**
- Tiny machines, **self-assembling DNA**, molecular machines : **the conception of NanoBioTechnology**

"…I want to build a billion tiny factories, models of each other, which are manufacturing simultaneously. . . The principles of physics, as far as I can see, do not speak against the possibility of maneuvering things atom by atom. It is not an attempt to violate any laws; it is something, in principle, that can be done; ***but in practice, it has not been done because we are too big****".*

Richard Feynman, Nobel Prize winner in Physics, 1965



...An introduction to NanoBio technology and NanoMedicine...



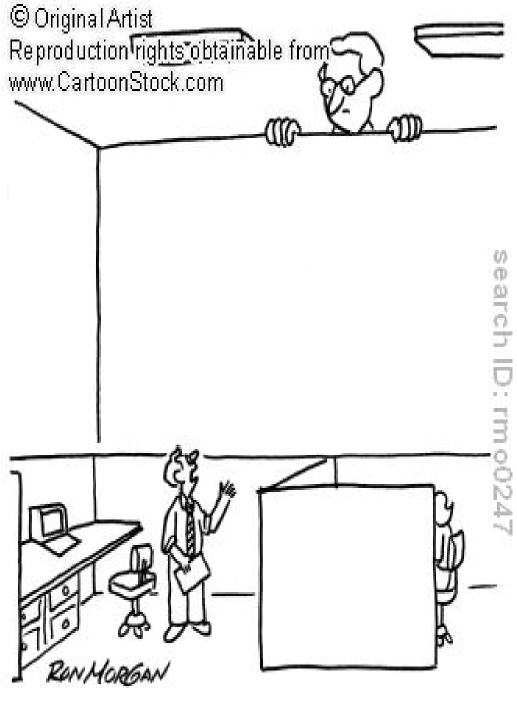
Fantastic Voyage:
A midget submarine swam in the human circulatory system to destroy a life-threatening clot

NanoBioTechnology: Nano, or, Mega Promises?

- **Nanobioechnology offers a promise to revolutionize the life sciences because it equips biologists with tools and materials that can interact directly with the biomolecules that they study on a daily basis**
  - **Biomolecule-material interaction , the *sine qua non* of nanobiotechnology**

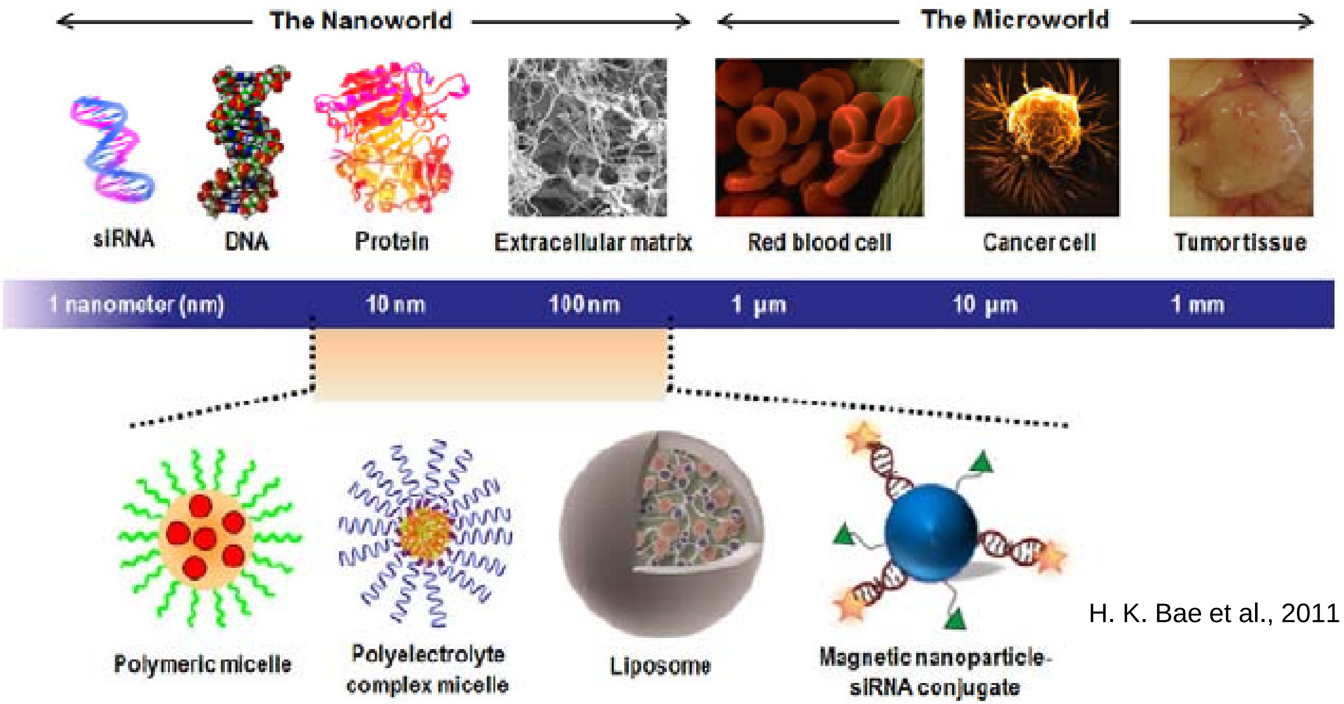
- **Both biotechnology and nanotechnology have matured to the point that their convergence offers opportunities for novel solutions to unmet needs in biology and medicine**

"If you increase the magnification another million times you can see the safety regulations."



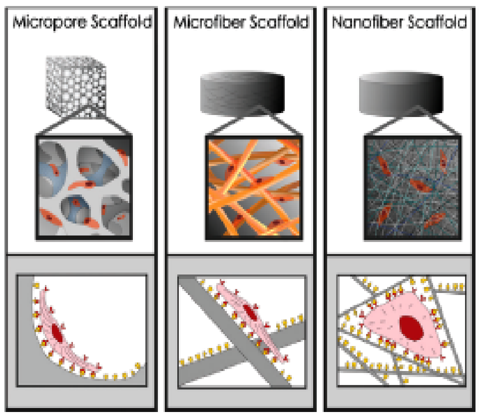
"It's simple: we're the nanotechnology department."

Why NanoBiotechnology?



H. K. Bae et al., 2011

All human tissues and extracellular matrix consist of nanofibrous forms



Of major significance in reconstructive Surgery!

Stevens MM, George JH. Science 2005;310:1135–8

Current Applications – At a glance

Disease Diagnostics

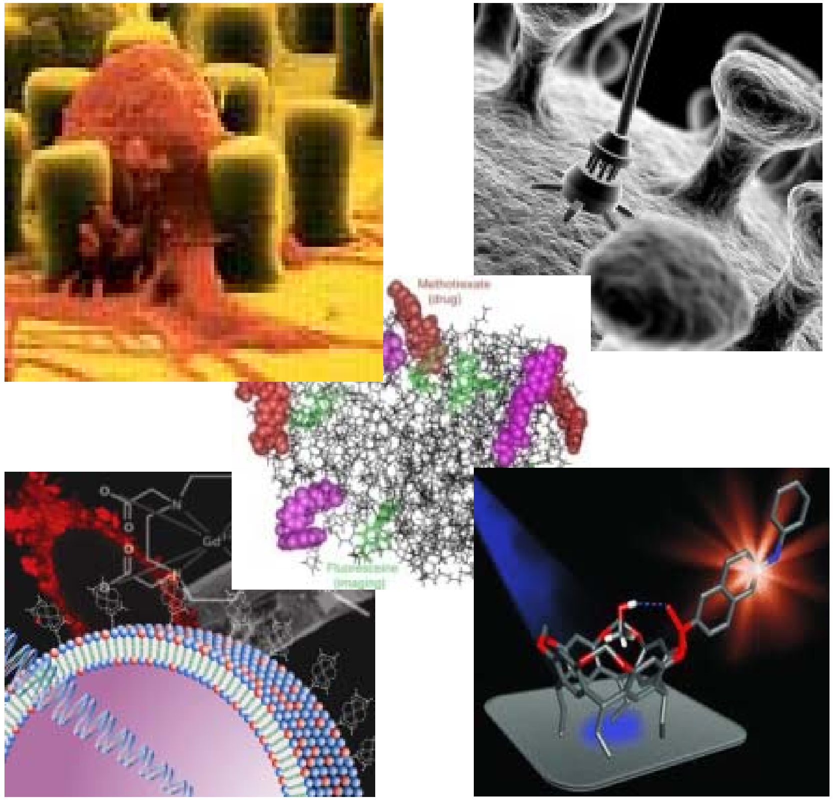
Ç **Advanced electrochemical Platforms based on switchable DNA architectures**

Ç **Advanced nanoparticles for *cell imaging* and *biological barrier crossing***

Ç **Integration of nanostructures and nanoparticles in *protein biomarker chips***

C **Nanotechnological toolkits for *diagnostics* and *treatment monitoring* of malignant and inflammatory processes**

Ç **Scaling of multiplex diagnostic test production using ultra-low liquid volume dispensing**

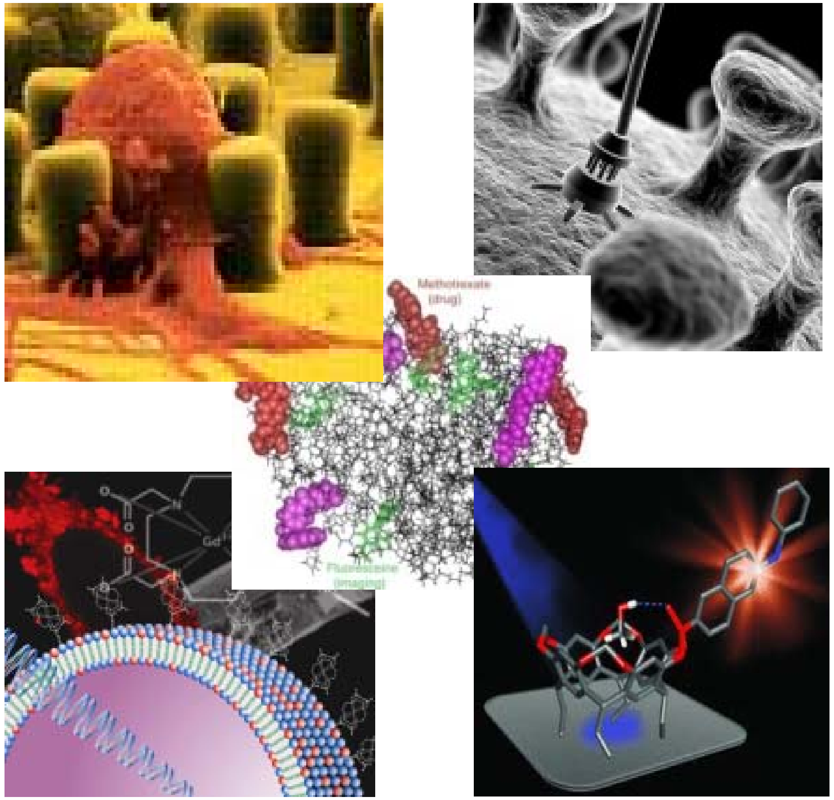


Current Applications – At a glance

Therapeutic Solutions

- **Nanoscale devices and novel technologies *targeting localized pathologies* with *controllable procedures***
- **Conventional pharmacy, Safety and Public Health**
- **Sophisticated *multifunctional drug carriers***
- **Drugability: virtual high throughput continuous *nanoformulation: the super-generics!***

- ***Sensorics***
  - **Efficient interaction of the large specific area of nanostructures**
  - **Improve the monitoring systems through better and smarter devices**
  - **Diverse read-out schemes in various health, security and safety applications**
  - **Automatic nanosensor systems for rapid food, pharmaceutical and biomedical analysis**



*Nano**medicine* : **natural extension of nanobiotechnology to medical interventions at a nano or molecular level**

ü *Imaging nanoparticles* guide contrast agents to specific tissues, making detection of diseases such as cancer feasible at ever earlier stages

- ü Drug delivery to specific tissues
- ü Targeting known markers of disease,
- ü More effective and less harmful to other organs

ü *Metal nanostructures* (gold nanoshells, nanorods, nanostars, etc,) -Hyperthermia-based therapy due to their ability to absorb radiation at certain wavelengths.

ü *Nanomaterial-based nanoscaffolds*, with embedded growth factors and other chemical signals, serve as guides for engineering artificial tissues and organs.

ü In the future, one can even envision a *surgical nanorobot*, either guided by a human surgeon or semi-autonomously

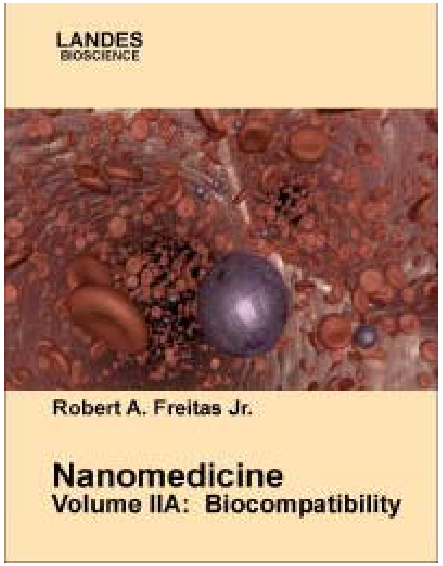
- ü Searching for disease
- ü Performing the diagnosis,
- ü Alleviating the problem by nanomanipulation of molecular structures and genes

Nanomedicine Bottlenecks

Biocompatibility:...being compatible with whatever defines life...
The realization of the "*Fantastic Voyage*"

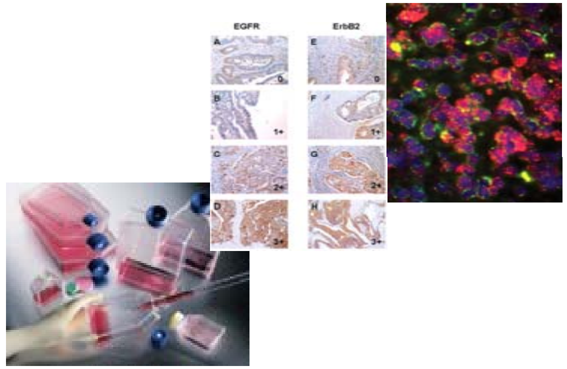
ü The safety, effectiveness, and utility of medical nanorobotic devices critically depend upon their **biocompatibilit**y with human organs, tissues, cells, and biochemical systems.

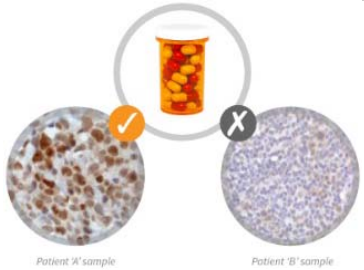
ü The definition of **nanomedical biocompatibility** is broadened to include all of the **mechanica**l, **physiological**, **immunologica**l, **cytological**, and **biochemical** responses of the human body to the introduction of artificial medical nanodevices, whether
  - ü "**particulate**" (large doses of independent micron-sized individual nanorobots) or,
  - ü "**bulk**" (nanorobotic organs assembled either as solid objects or built up from trillions of smaller artificial cells or docked nanorobots inside the body)

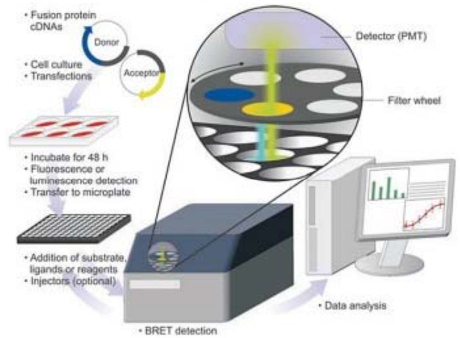


Nanotheranostics- Introducing the concept

- *Theranostics*: “it’s all Greek to me!”- Therapeutic + Diagnostics
- **A treatment strategy that packs a one-two punch**
  - A diagnostic test that identifies patients most likely to be helped or harmed by a new medication
    - Biomarker identification and validation
    - Patient stratification
    - “Predictive pharmacology”
  - **Targeted drug therapy based on the test**
    - Tests based on sophisticated technology involving genetics, molecular biology and testing platforms such as microchips
    - The test results are used to tailor treatment, usually with a drug that targets a particular gene or protein
    - “**Personalized medicine at the fore**”







The nano-theranostic process

ü The **nanotheranostic agent is injected** , it travels to the target site and accumulates there.

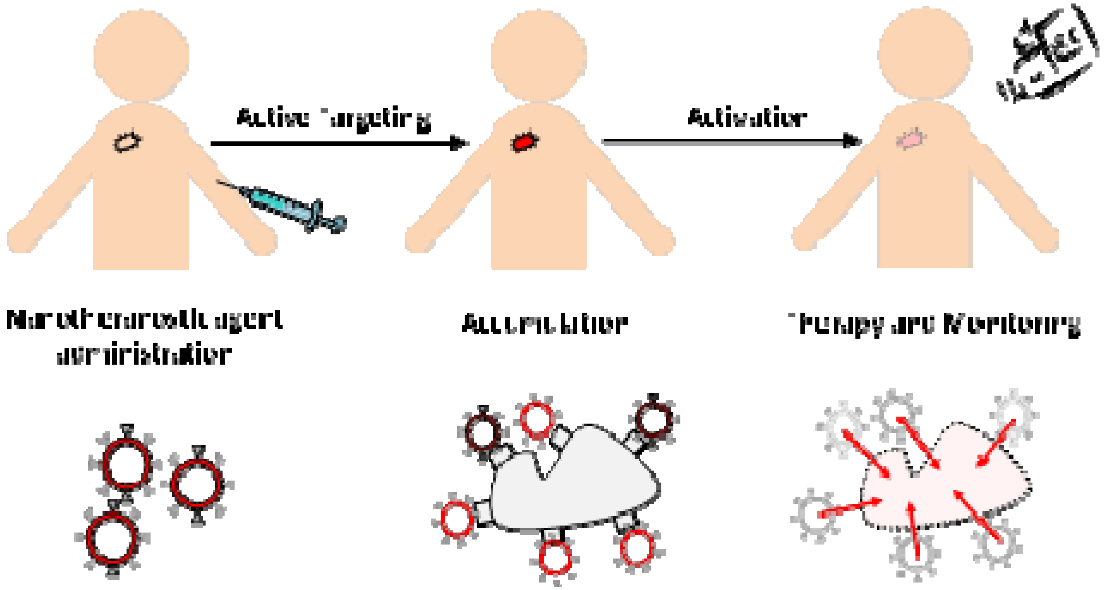
ü Moieties are **visible by means of imaging** (optical, endoscopic, MRI, PET, etc)

ü Process and final concentrations can be quantified and interpreted: More specific diagnosis.

ü Once at the target, nanostructures can be activated

  ü To heat and destroy the tissue (thermotherapy)

  ü To deliver drugs.



The nano-theranostic process

- **Drug delivery**: the particle is pre-loaded with a therapeutic agent and, once at the target, the outer shell disintegrates, releasing the medicine.
    - The medicine is released directly over the disease location at a high concentration resulting in a **more aggressive treatment.**
    - **Fewer** and much **less intense side effects** compared to current regimens, e.g. chemotherapy.
    - **This is probably the major reason why they are thought of as the future of cancer treatment!**

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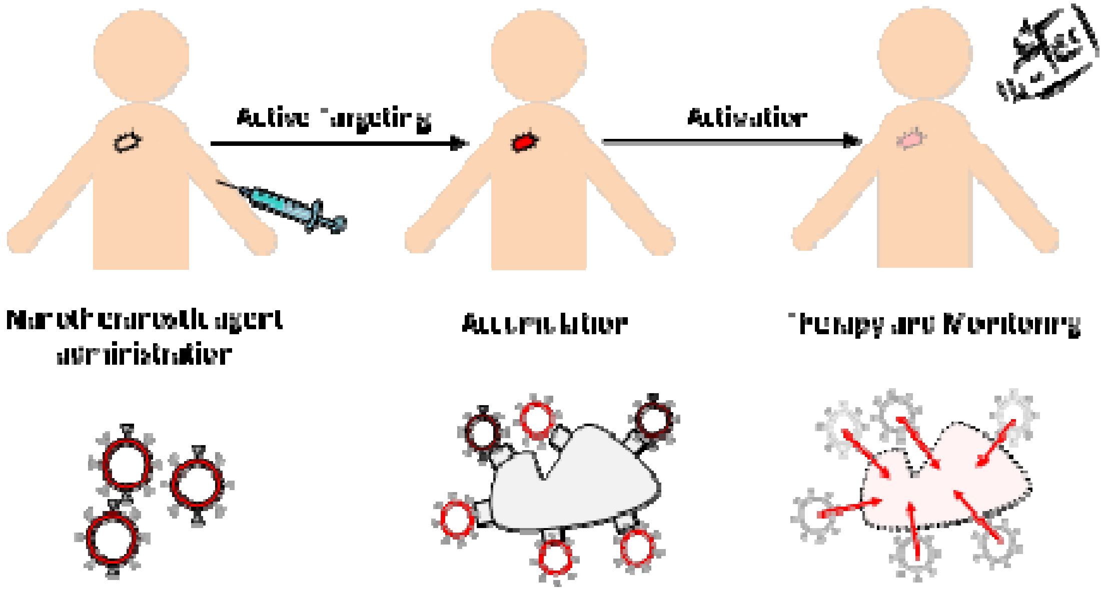


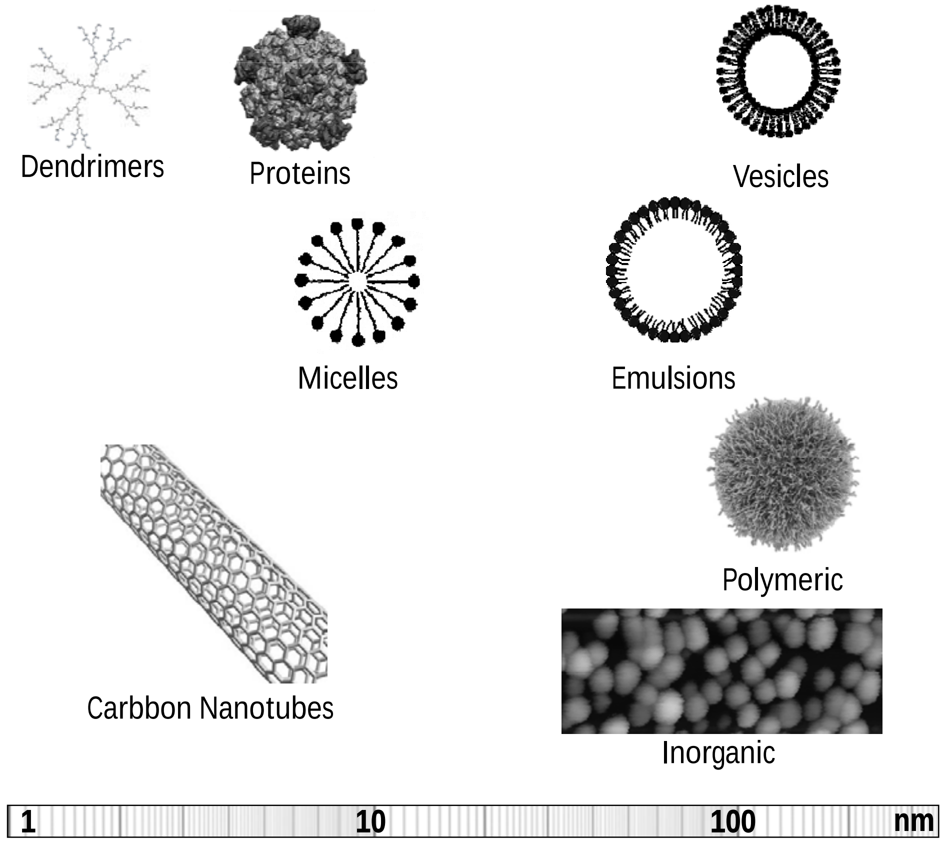
Theranostic nanoparticles provide real-time information on whether or not the specific treatment regimen is working for the specific patient. In addition, **theranostic nanoparticles can be tailored to the specific type of the disease,** leading to practical applications of personalized medicine.

Nano-theranostics: exploiting nanostructures with unique targeting and diagnostic capabilities

ü Many nanostructures are already imaging agents and can be readily "upgraded" to theranostic agents by mounting therapeutic functions on them

ü Developing biocompatible theranostic nanoparticles requires combining the disciplines of chemistry, biotechnology, physics, biology and medicine.

ü Initial trials of these nanoparticles have shown promising results which compare favorably to the current treatment options



Theranostic nanoparticles can be made into one of the following functionalized classes: drug conjugates; dendrimers; vesicles; micelles; metal based; microbubbles; carbon nanotubes

Clinical Applicability of Optical Imaging

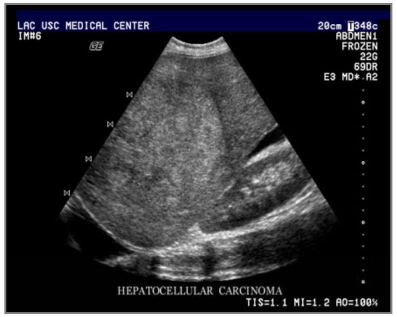
Constantinos Pitris, MD, PhD
KIOS Research Center
Department of Electrical and Computer Engineering
University of Cyprus

Medical Imaging Evolution

’80s Mainstream

X-Ray-based Anatomical Imaging: XR, CT

Ultrasound



Emerging

MR Anatomical Imaging (Soft-Tissue Visualization)

’90s Mainstream

CT, MR, US Anatomical Imaging

Digital Xray

Emerging

Functional Imaging MR, PET

MR Spectroscopy (Characterization)

‘00s Mainstream

Anatomical Registration of Functional Imaging: PET/CT, MR

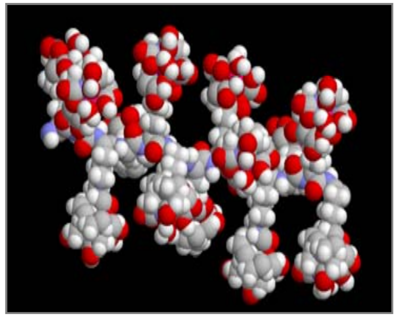
Emerging

Anatomical Registration of Molecular Imaging

Molecular Therapeutics, Diagnostics & DI

Next Mainstream

Molecular Imaging
+
Molecular Therapeutics
+
Molecular Diagnostics

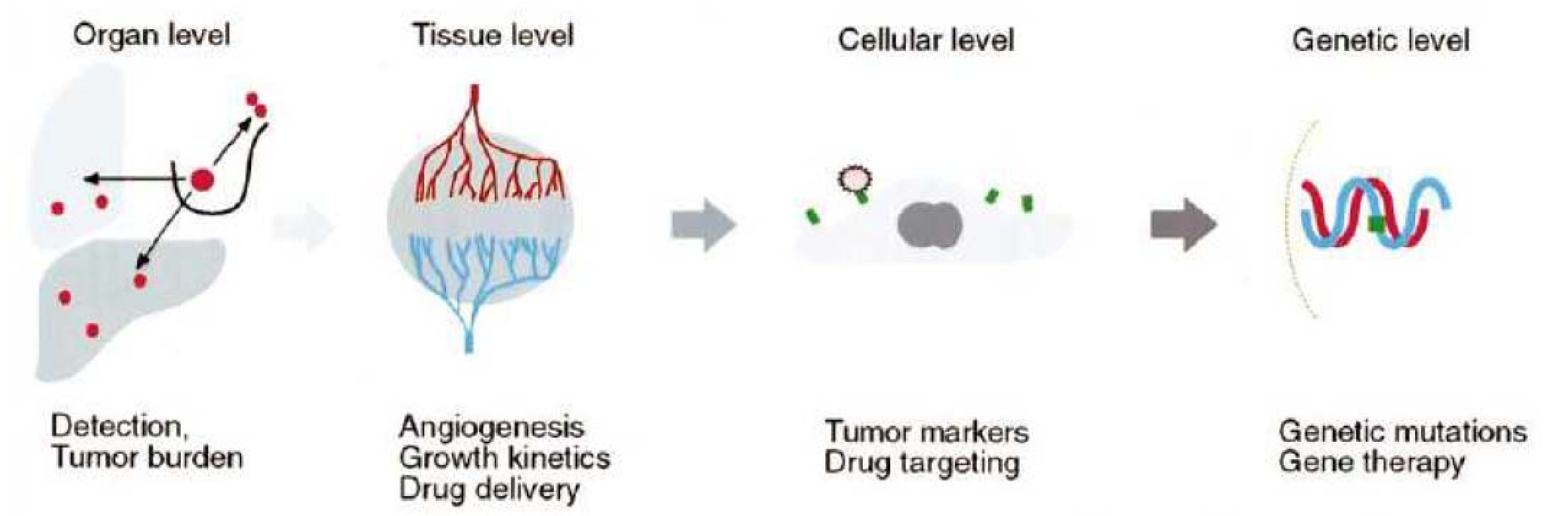


Emerging

Image-guided Gene Therapy

What is <u>Molecular</u> Imaging?

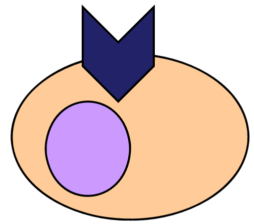
- " Molecular imaging is a growing research discipline aimed at developing and testing novel tools, reagents and methods to image specific molecular pathways in vivo, particularly those that are key targets in disease processes"
- **Weissleder et al, 2001**



What is Molecular Imaging?

1. Identify a Marker of disease

- **Acquired capabilities shared by most human cancers which collectively dictate malignant growth**
  - Self –sufficiency with respect to growth signals
  - Insensitivity to growth inhibitory signals
  - Evasion of programmed cell death
  - Limitless replicative potential
  - Sustained angiogenesis
  - Tissue invasion and metastasis

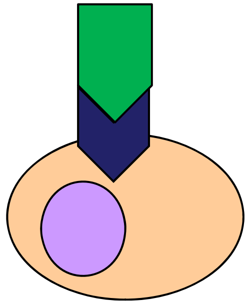


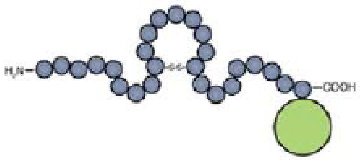
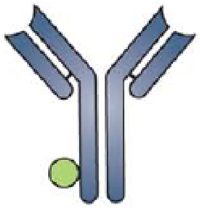
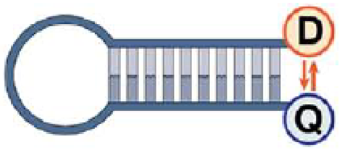
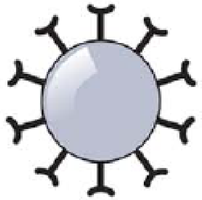
- **All of the above can be possible targets for molecular imaging**
  - Markers can be genes expressed or activated, cytoplasmic or free proteins, enzymes produced at the site of disease etc.
  - Important issues: epitope availability, specificity to disease and availability to suitable probe

What is Molecular Imaging?

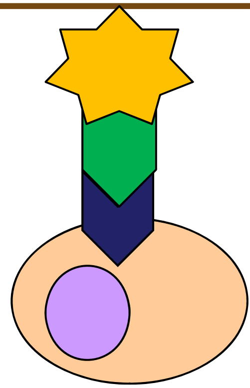
2. Identify a Probe for targeting the marker

- Non-specific (vascular flow)
- Targeted (Some molecule that preferentially binds to the target)
- Activatable (Activated by specific enzymes)



Type	Peptide	Antibody	Activatible probe	Nanoparticle
				
Advantages	• Easy delivery to target structure • Low immunogenicity • Low cost	• High specificity • Defined target • Defined and approved therapeutic ab may be labeled	• Specific activation • Optimized signal-to-noise ratio	• Loading with multiple proteins for multivalent targeting • Strong fluorescence
Disadvantages	• Variable affinity	• Potential immunogenicity	• Internalization frequently required for activation • Undefined safety profile	• Potential toxicity of non-biocompatible core • Renal clearance

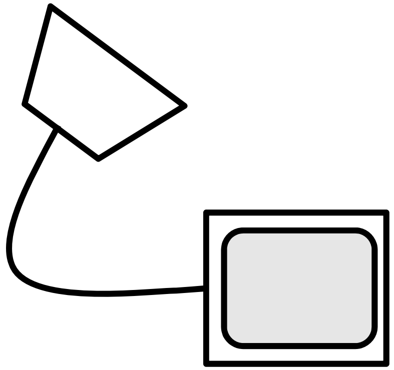
What is Molecular Imaging?



3. Attach an appropriate Beacon to the probe

- A molecule that can be detected non-invasively
  - Fluorescent or bioluminescent molecule (optical approach)
  - A metallic nanoparticle (MR approach)
  - Radioactive molecules (Nuclear approach)
  - An ultrasound air bubble (US approach),
  - Multimodal approaches

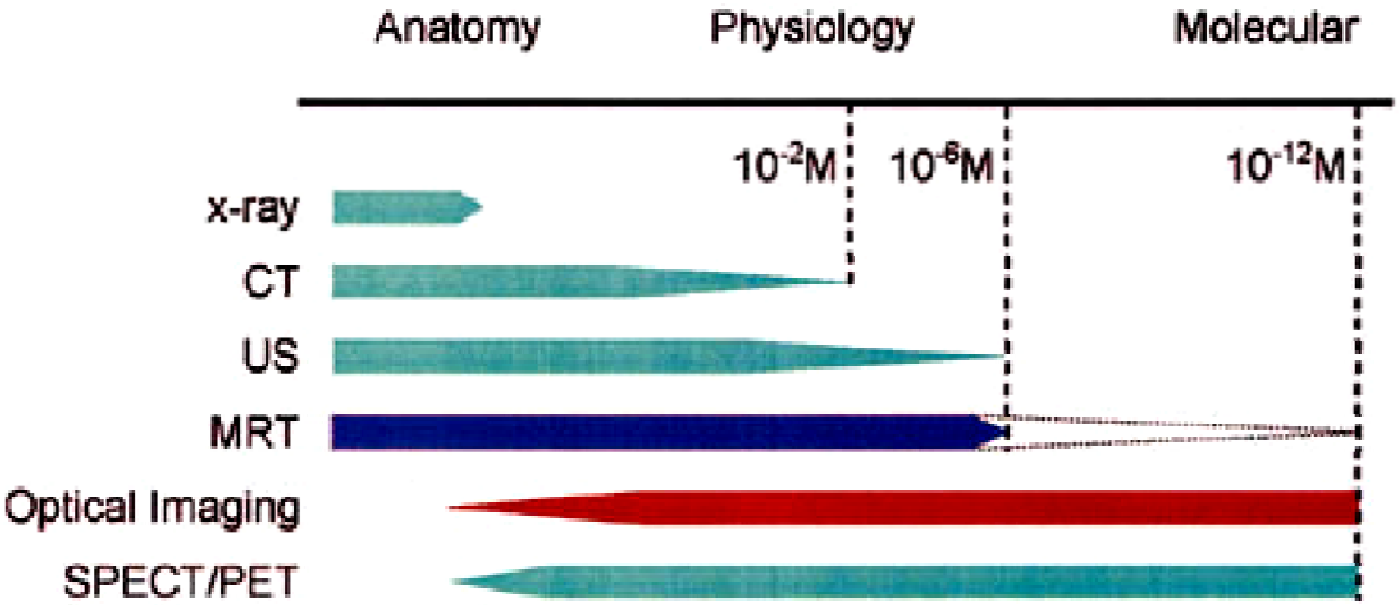
4. Detect the beacon



- The presence or absence of the marker can be detected indirectly
- Depending on the marker and its location, issues such as beacon uptake by the tissue and intracellular penetration may have to be considered.

Why Optical Molecular Imaging?

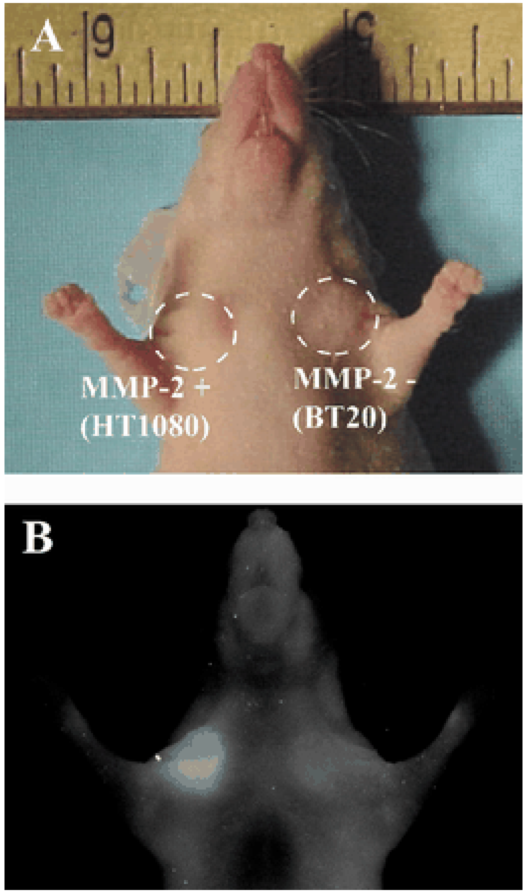
- **Exceptional resolution (μm)**
- **Exquisite sensitivity**
  - Optical probes enable imaging down to the nano-meter and nano-molar scales



(Weissleder R, 2001)

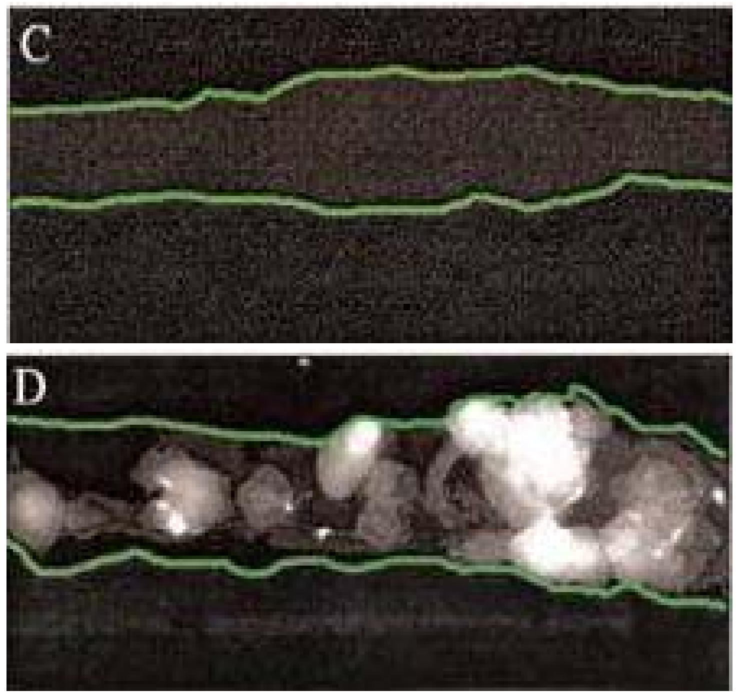
Why Optical Molecular Imaging?

- **Non-ionizing radiation**
  - Optical radiation is innocuous
  - Minimizing risks Ą Repeated imaging
  - Following a single animal over time to accurately monitor the effects of interventions progressively
    - Faster and less costly studies



Why <u>Optical</u> Molecular Imaging?

- **Multiplexing**
  - Multiple probes emitting at different wavelengths
  - Several targets imaged simultaneously
- **Little background**
  - (especially when imaging with Infrared (IR) excitation)
  - Autoflorescence (endogenous signal) of tissues is very low
  - Unlike, for example, proton contents in MRI, or scattering in US



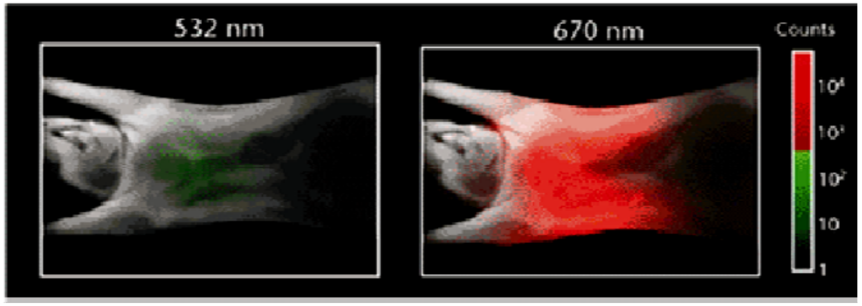
(Marten 2002)

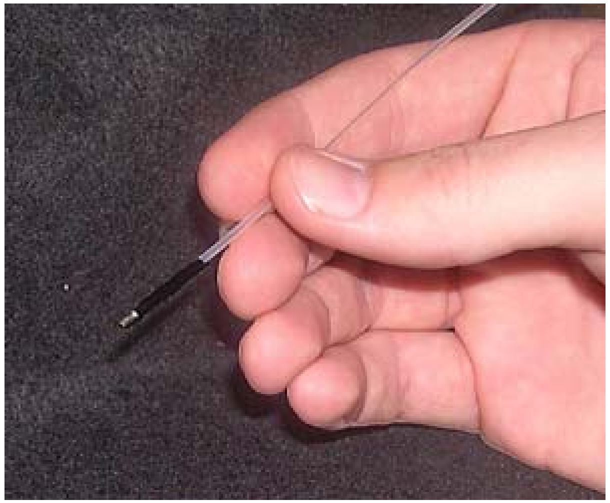
Why Optical Molecular Imaging?

- **Real-time imaging**
    - Time-dependent studies easily performed
- **Inexpensive and portable equipment**
    - Easily adapted to existing medical equipment (such as endoscopes)
- **Well-studied in biological systems**
    - E.g. fluorescent proteins or fluorescently labeled antibodies

What Optical Imaging Cannot Do?

- **Light can not penetrate deep into tissues**
  - A few mm to a few cm depending on the light's wavelength and the tissue
- **Whole-body optical imaging**
  - Can be performed in small animals
- **However, optical imaging in humans can be performed**
  - In the breast (around small external diameters)
  - Anywhere there is an 1-2 mm lumen
  - Through a fine needle



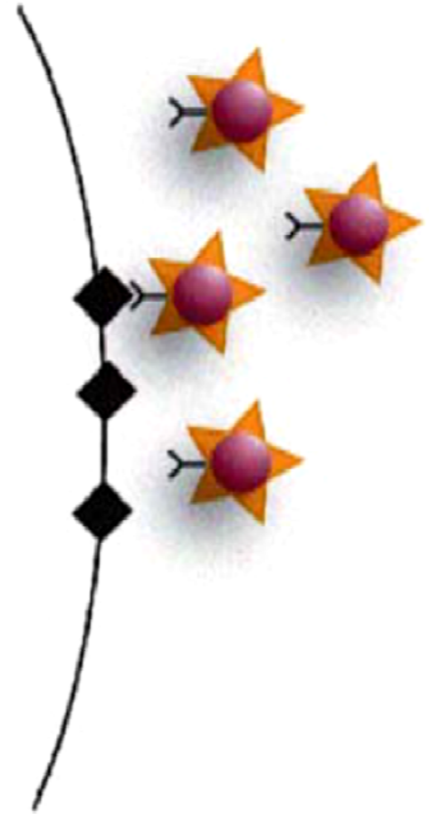


Optical Contrast Agents

- **Non-specific contrast agents**
  - Non-specific distribution pattern
  - An important tool for depicting tumor physiology
    - perfusion, vessel permeability, tissue blood volume
  - Contrasting mechanism based on
    - Angiogenesis Ą increased number of vessels
    - Permeability of tumor vessels Ą accumulation of fluorescent dyes in the tumor interstitium
  - E.g. Cyanine dyes (CD), clinically approved Indocyanine green (ICG, cardiogreen)

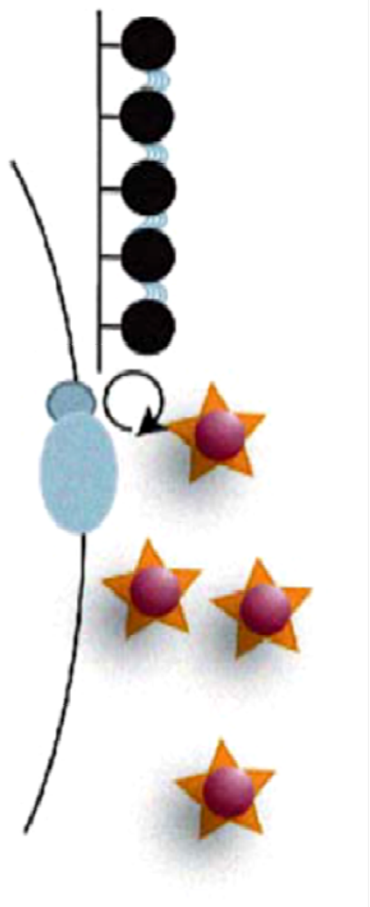
Optical Contrast Agents

- **Targeted / active contrast agents**
  - Combining efficient targeting strategies with sensitive detection techniques
    - Resolve molecular targets (e.g. tumor associated receptors) in the nM range in vivo
  - Possible forms
    - Large molecule (e.g antibodies or antibody fragments)
    - Small peptide derivatives
    - Affinity ligands (e.g. annexin V - apoptosis ; EGF for EGF Receptor-early carcinogenesis; biphosphonate derivatives-bone reconstruction)



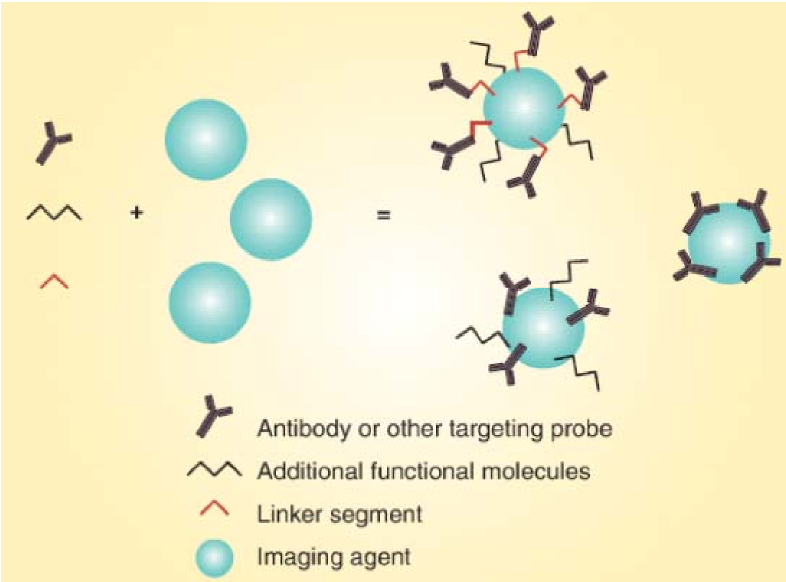
Optical Contrast Agents

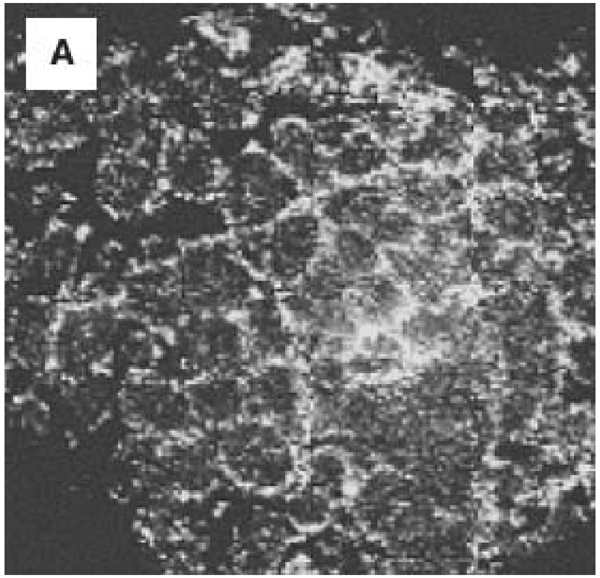
- **Smart/ activatable contrast agents**
  - Alter signal characteristics upon interaction with the specific target
  - Optical smart probes
    - Very little signal in the native stage
    - Brightly fluorescent after enzymatic cleavage
  - Provide the highest SNR
  - More complex probe design and synthesis
  - They have been shown to image various proteases
    - Tumor-associated lysosomal proteinases, intracellular proteinases and matrix metalloproteinases



Nano-Optical Beacons

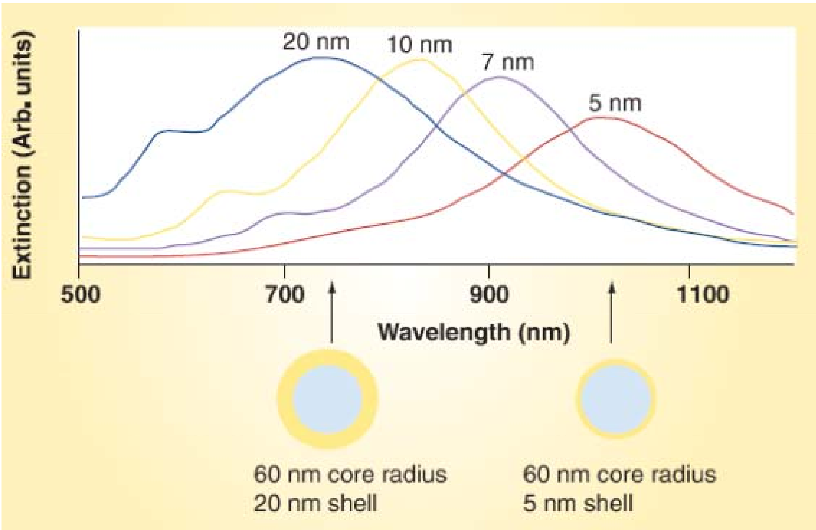
- **Metal Nanoparticles**
  - Provide optical contrast by exploiting surface plasmon resonance
  - Contrast in live cells and cervical biopsies using gold nanoparticles targeted for detection of EGFR, a hallmark for many epithelial cancers (Sokolov et al)

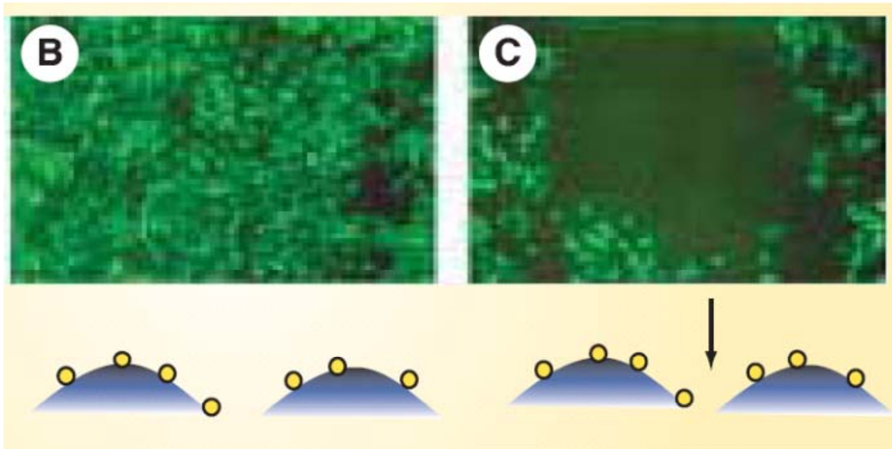




Nano-Optical Beacons

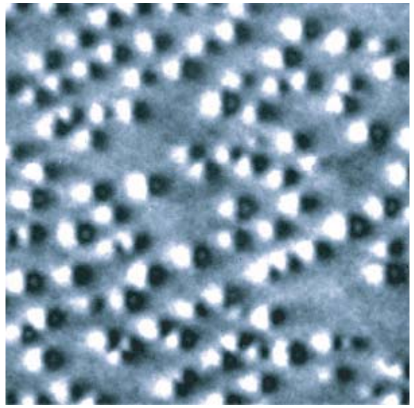
- **Nanoshells**
  - Metal shell with a dielectric core
  - Optical properties can be tailored to the desired wavelength
  - Can be used for nanoparticle-assisted photothermal therapy
  - Dual imaging/therapy is possible with nano-shells that simultaneously exhibit scattering and absorption a specific wavelengths

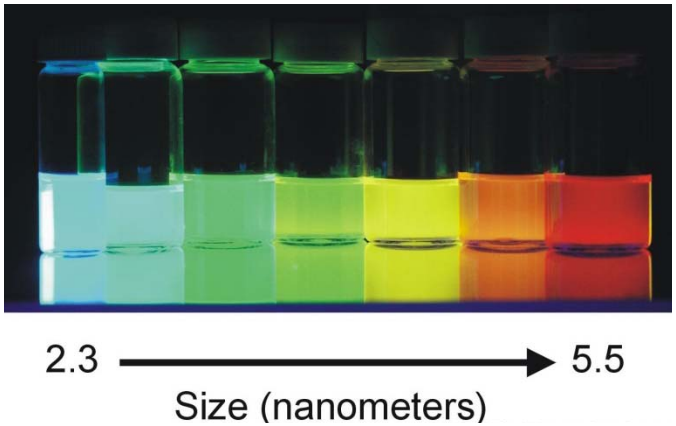




Nano-Optical Beacons

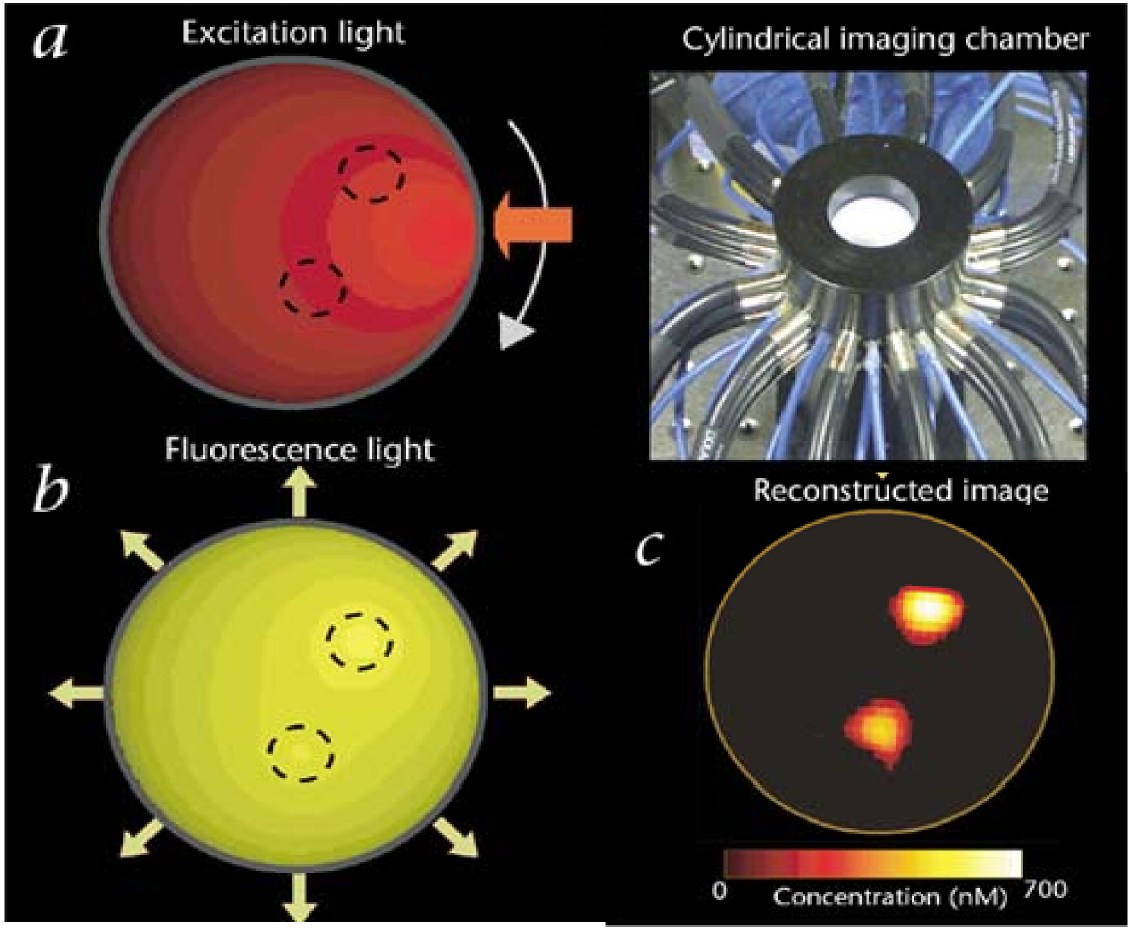
- **Quantum Dots**
  - 2-10 nm semiconductor nano-crystals that fluoresce
  - Improved brightness and long luminescence lifetimes
  - Broad absorption and narrow emission spectra
    - Multicolor labeling
  - Resistance to photobleaching
  - Have been used successfully to target human prostate cancers grafted in mice as well as in guiding the real-time, in vivo resection of sentinel lymph nodes





Applications

- **Fluorescence Molecular Tomography (FMT).**
  - Three-dimensional, quantitative detection of probe distribution in vivo



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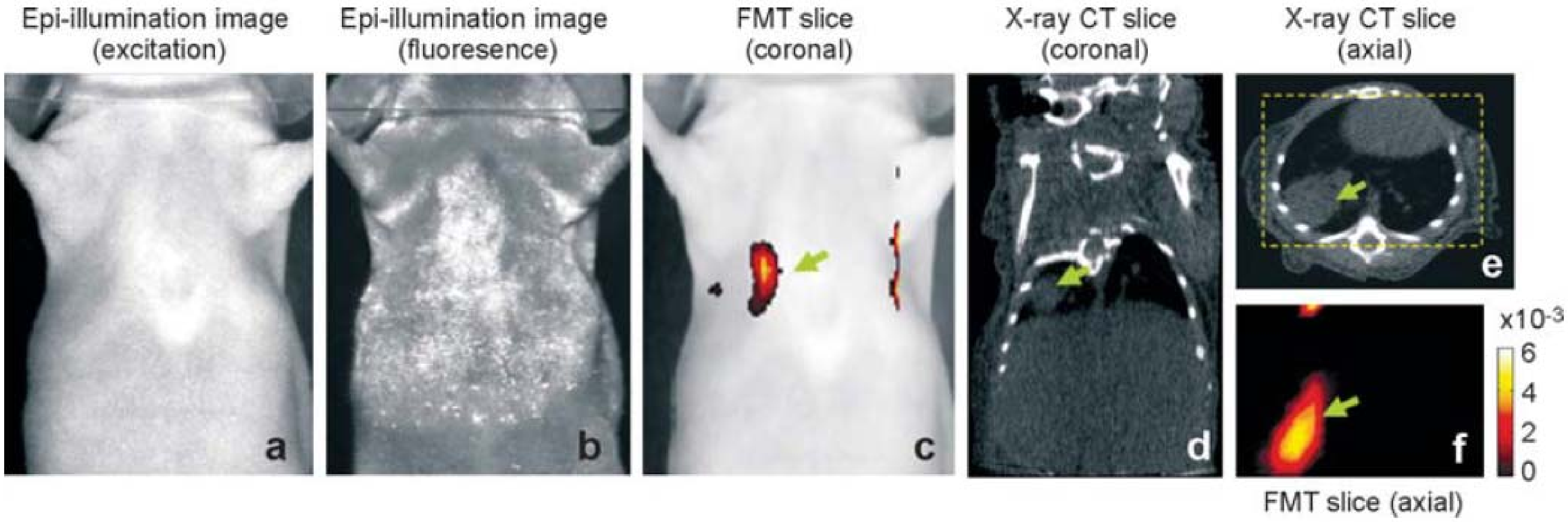
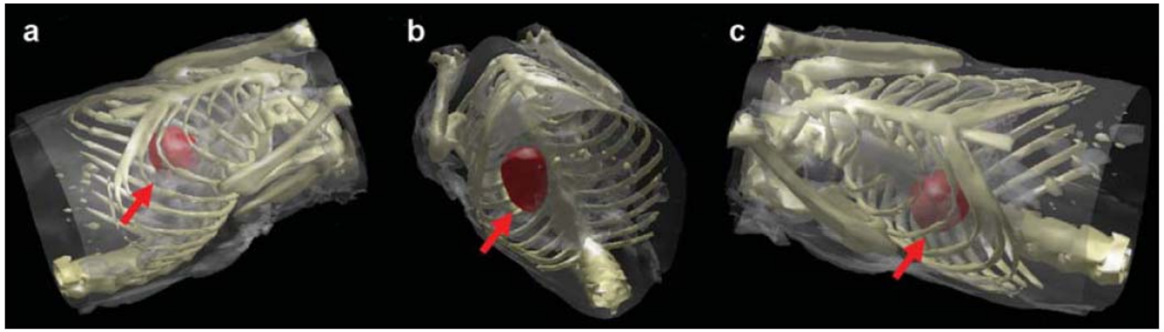
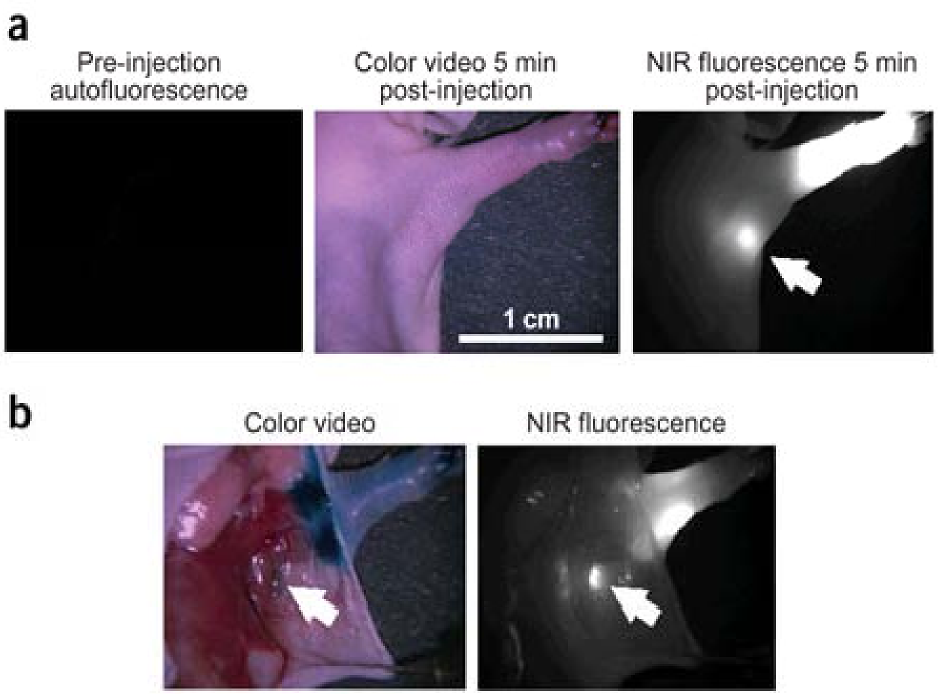


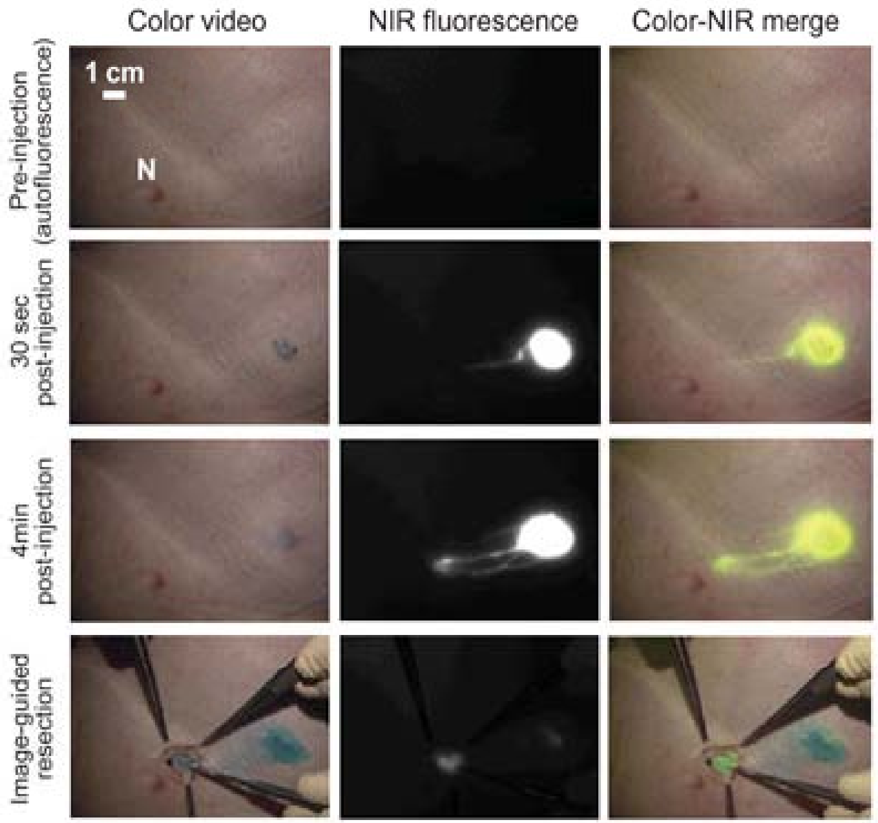


(Ntziachristos, Annu. Rev. Biomed. Eng. 2006.8:1-33.)

Applications

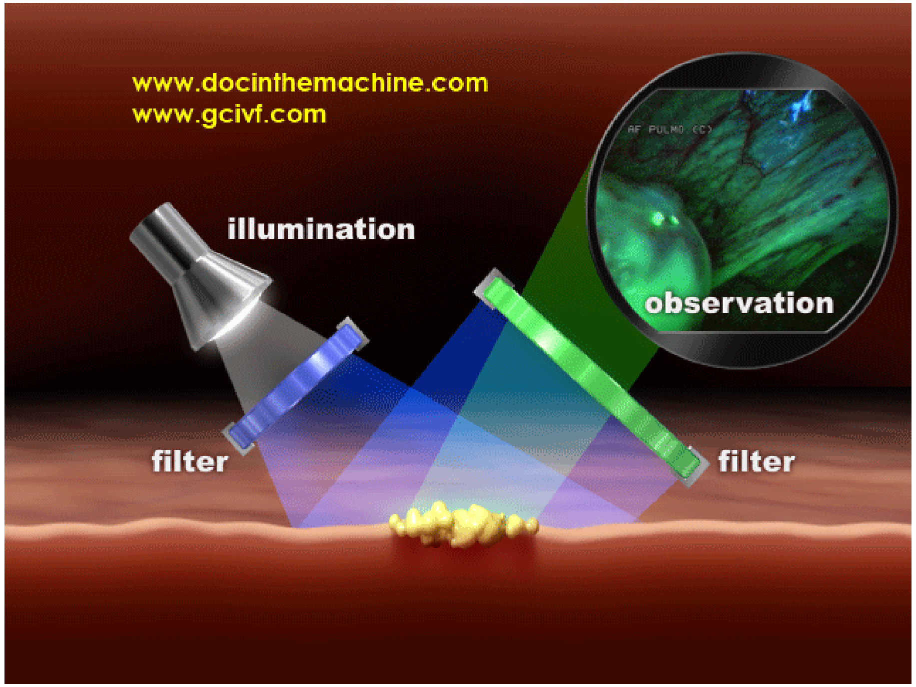
- **Inorganic Quantum Dots (CsSe) in surgery of sentinel node**





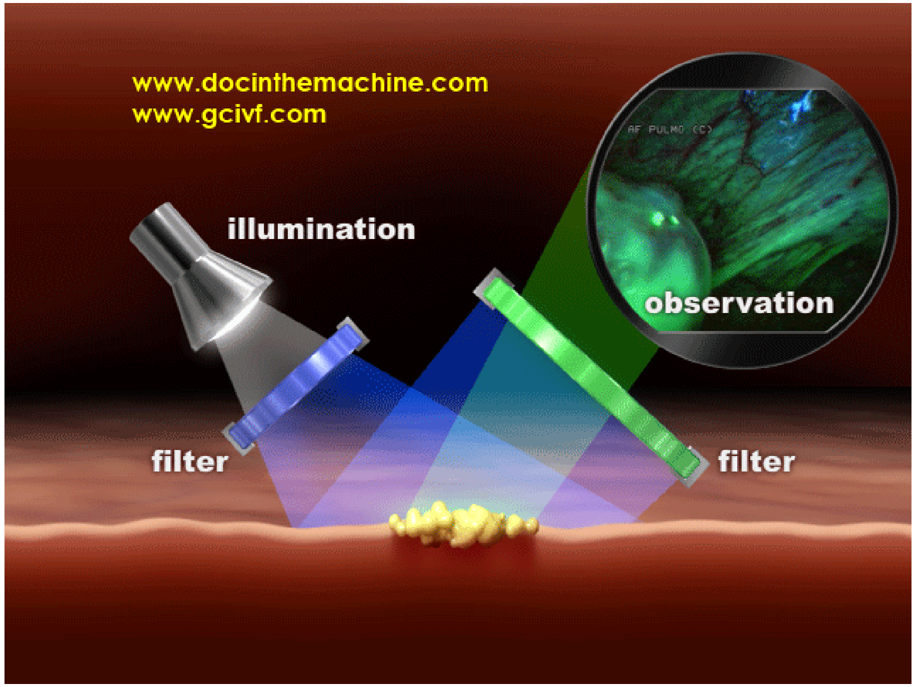
Fluorescence Endoscopy

- **Autofluorescence imaging**
  - No exogenous contrast agent
  - Provides information about biochemical composition and metabolic rate of tissue
  - Major Fluorophores:
    - Structural proteins (collagen, elastin) NAD/NADH, Flavins, Aromatic amino acids e.g. tyrosin, Porphyrins
    - Lipopigments e.g. ceroids, lipofuscin

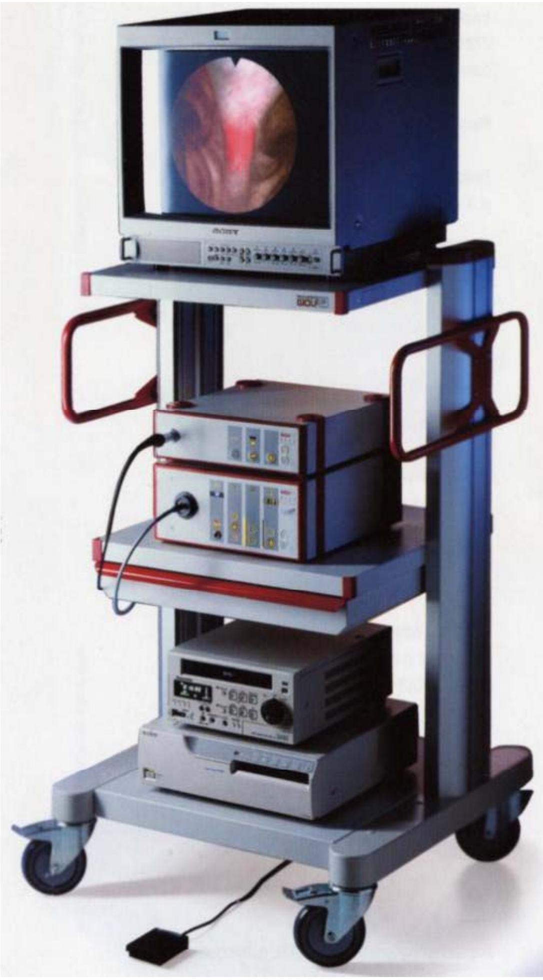


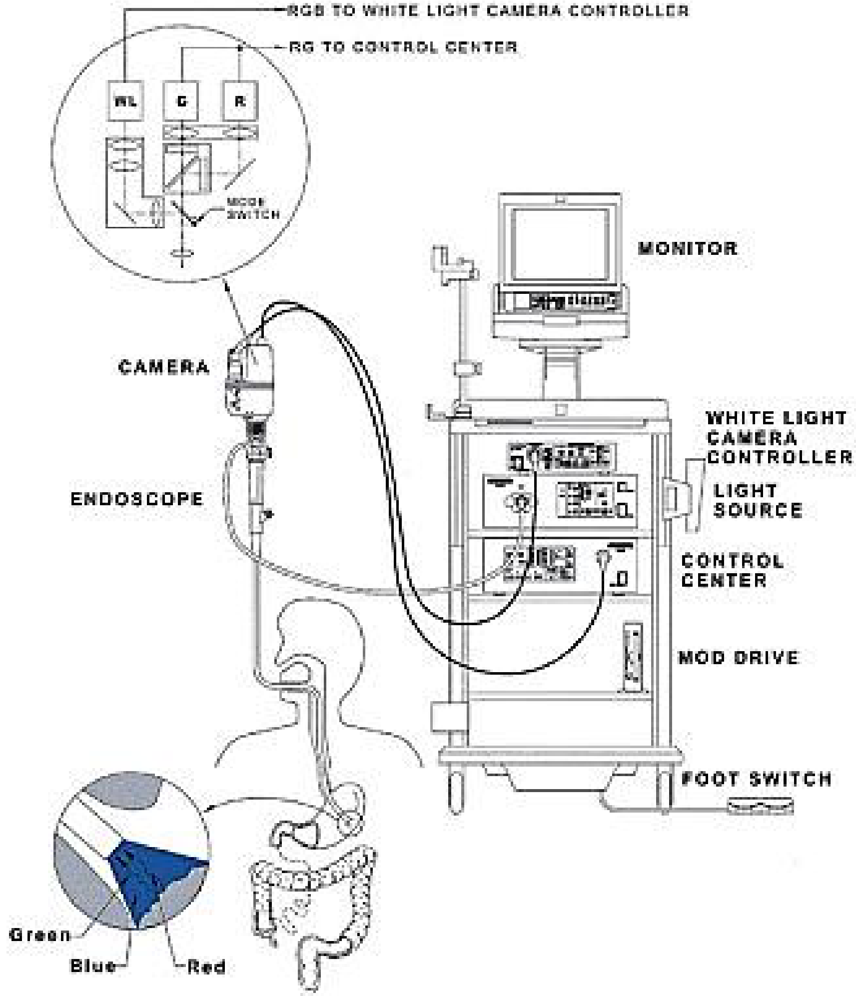
Fluorescence Endoscopy

- **Clinical Applications of Autofluorescence Imaging**
  - Detection Of Neoplasia In High-risk Patients
  - Endoscopic Therapy With Curative Intent



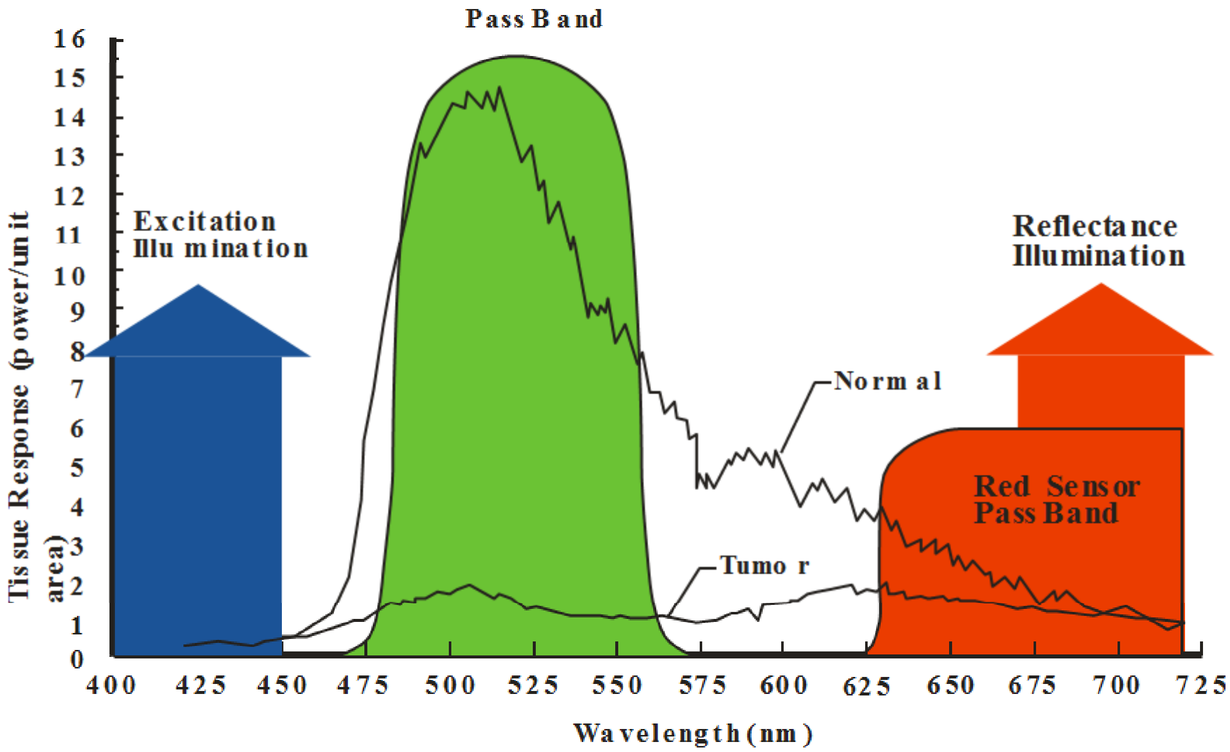
Fluorescence Endoscopy

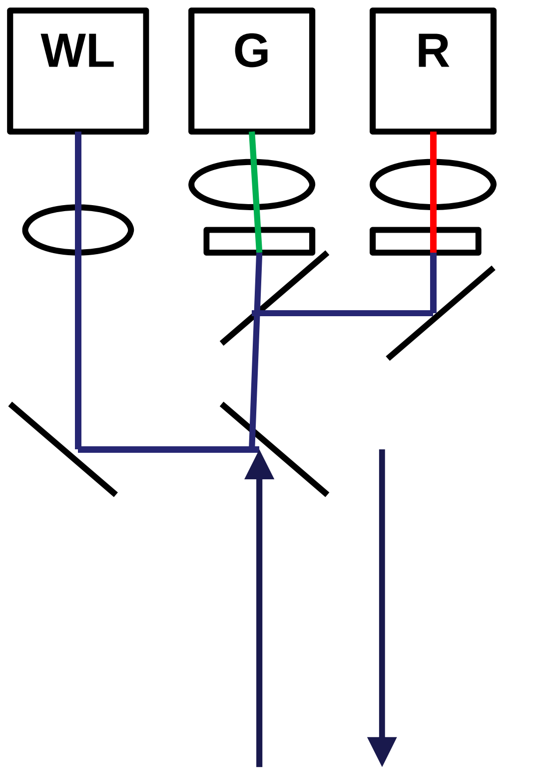




Fluorescence Endoscopy

Onco-LIFE Combined Fluorescence-Reflectance Imaging

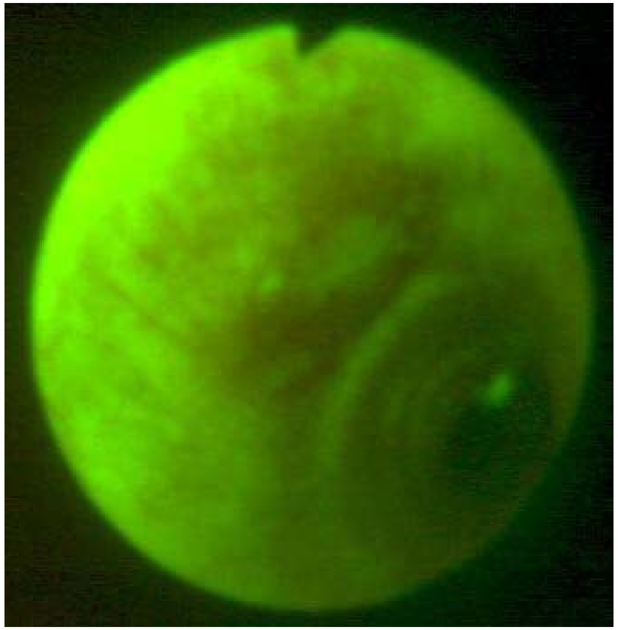




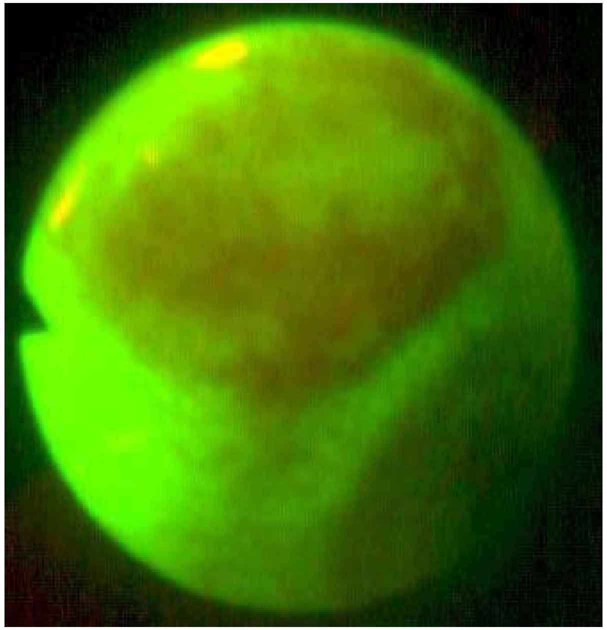
Fluorescence Endoscopy

Tracheal Carcinoma in-situ

White Light



Fluorescence



Fluorescence-Reflectance

Xillix Onco-LIFE Combined Fluorescence-Reflectance Imaging

Fluorescence Endoscopy

Bronchial Carcinoma in-situ

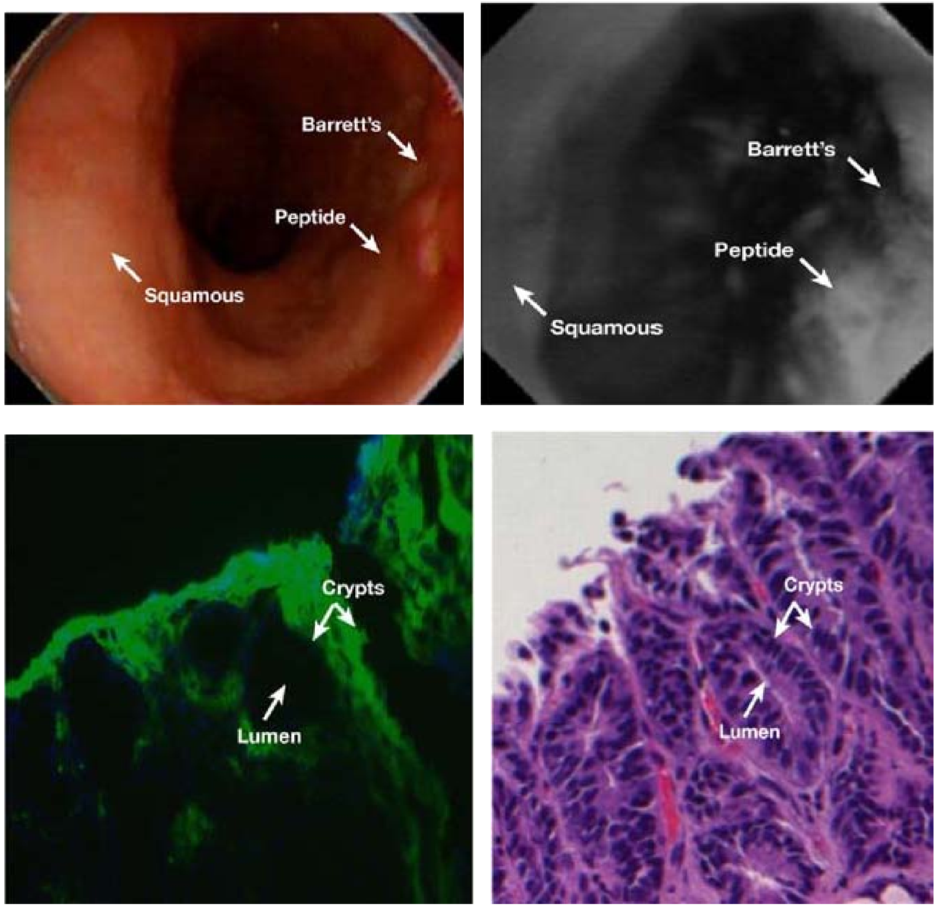
DAFE II(Diagnostic AutoFluorescence Endoscopy) commercialized by Richard WOLF GmbH

Fluorescence Endoscopy

- **Limitations of Autofluorecscence**
  - Sensitive but not specific
  - Many false positives
  - Generates many biopsies
  - Without validated screening technique
    - who should have AFE???
- **Endoscopic Molecular Imaging**
  - High sensitivity and specificity
  - Can be integrated with therapy (theranostics)

Applications

- **Targeted detection of high-grade dysplasia in Barrett's esophagus with fluorescence imaging.**
  - Molecular image
    - Topical administration of labeled peptides
    - Increased fluorescence intensity at sites of high-grade dysplasia.
  - Binding of the peptide to the outer surface of the dysplastic crypts.



Goetz, Gastroenterology, Vol. 138, No. 3, p. 831

Nanotheranostics at the clinical fore

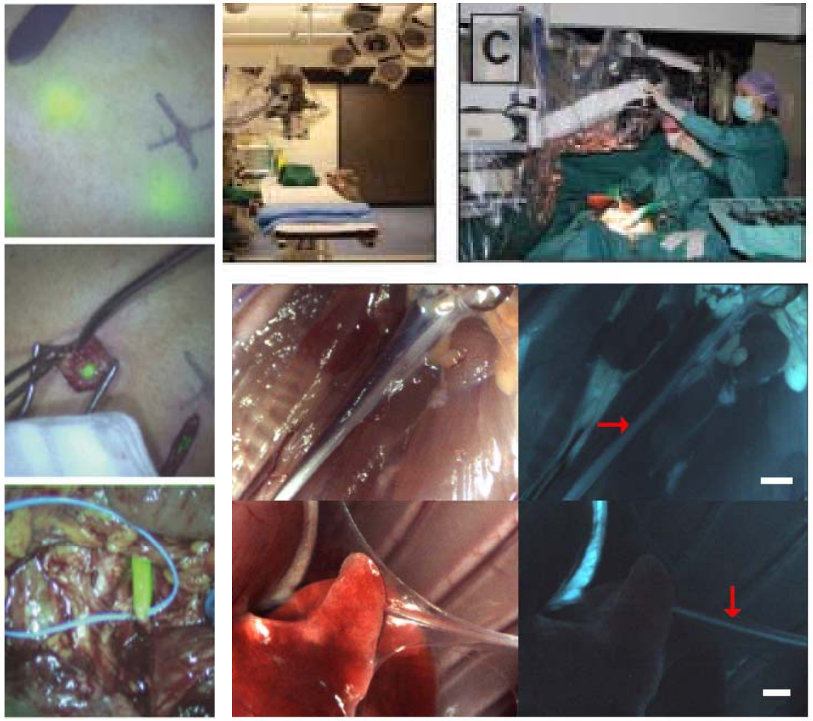
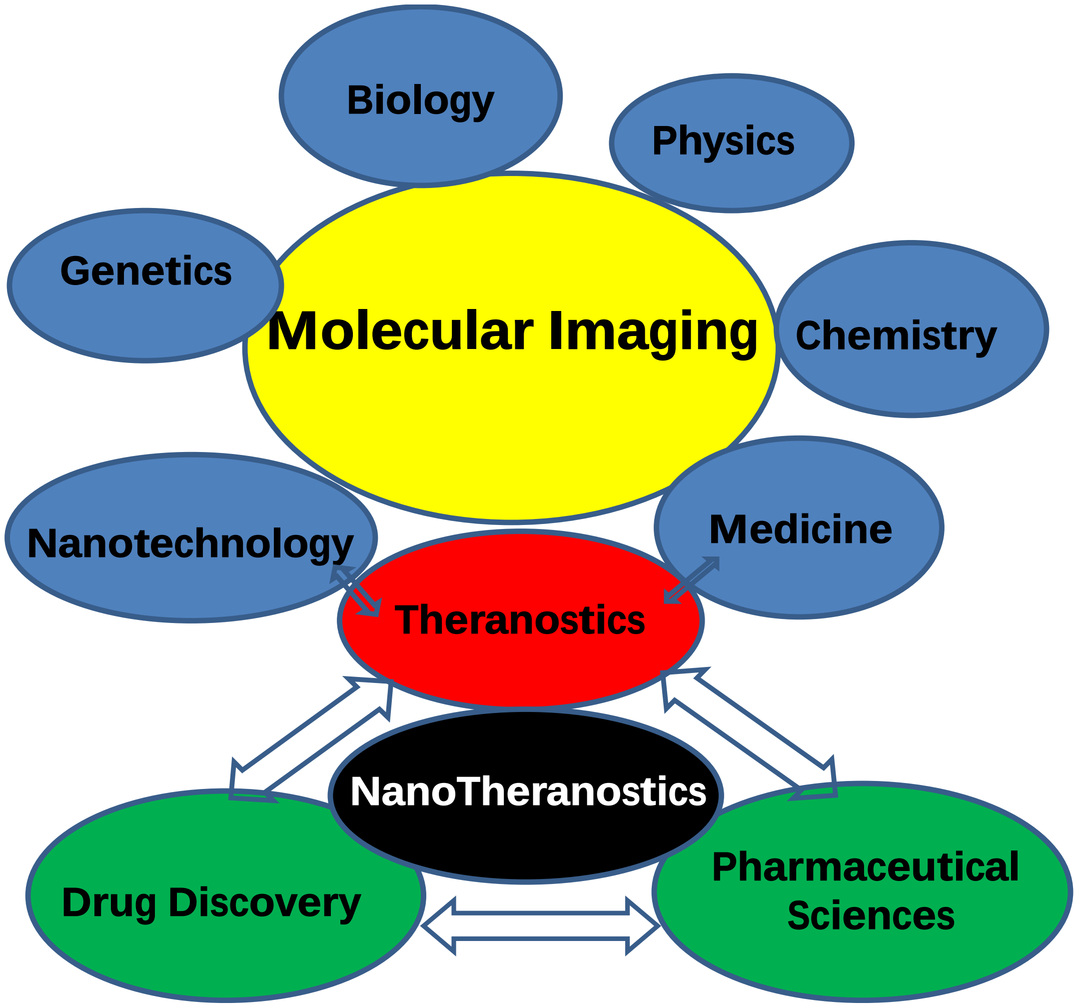


Image-guided therapy: paving the way of nanotheranostic agents to the clinic

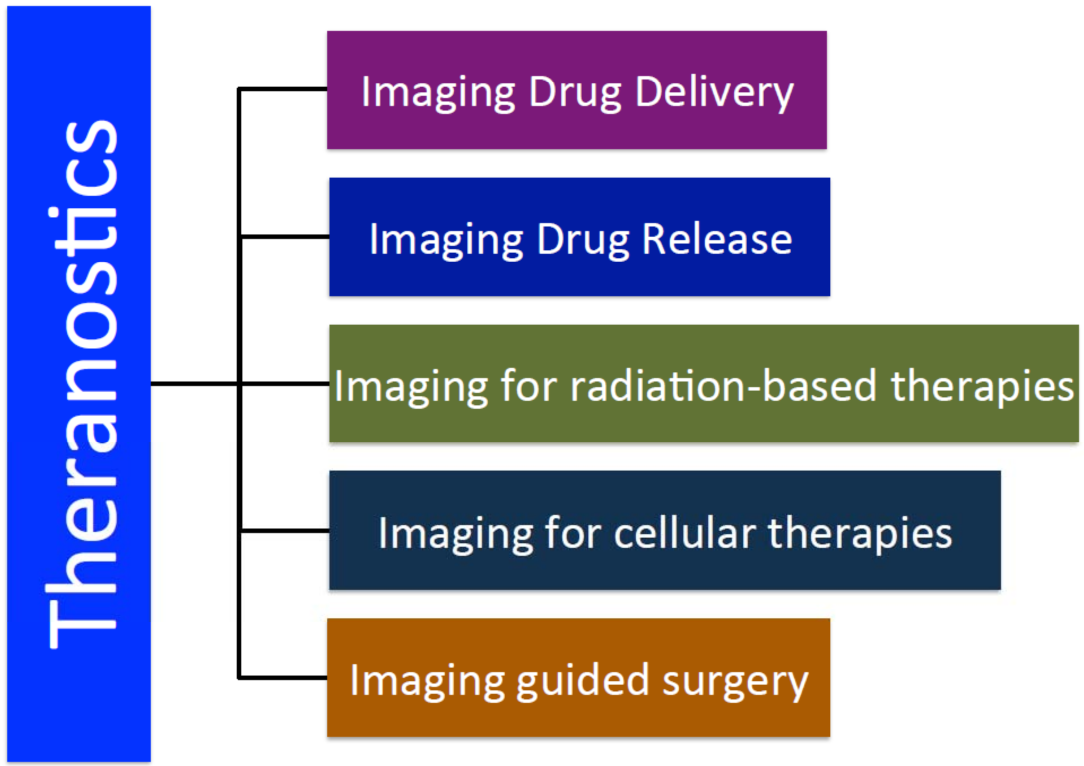
Theranostics in the clinic: where pharmaceutical sciences meet *In vivo* molecular imaging



The epitome of personalized approaches

Nanotheranostics in interventional procedures

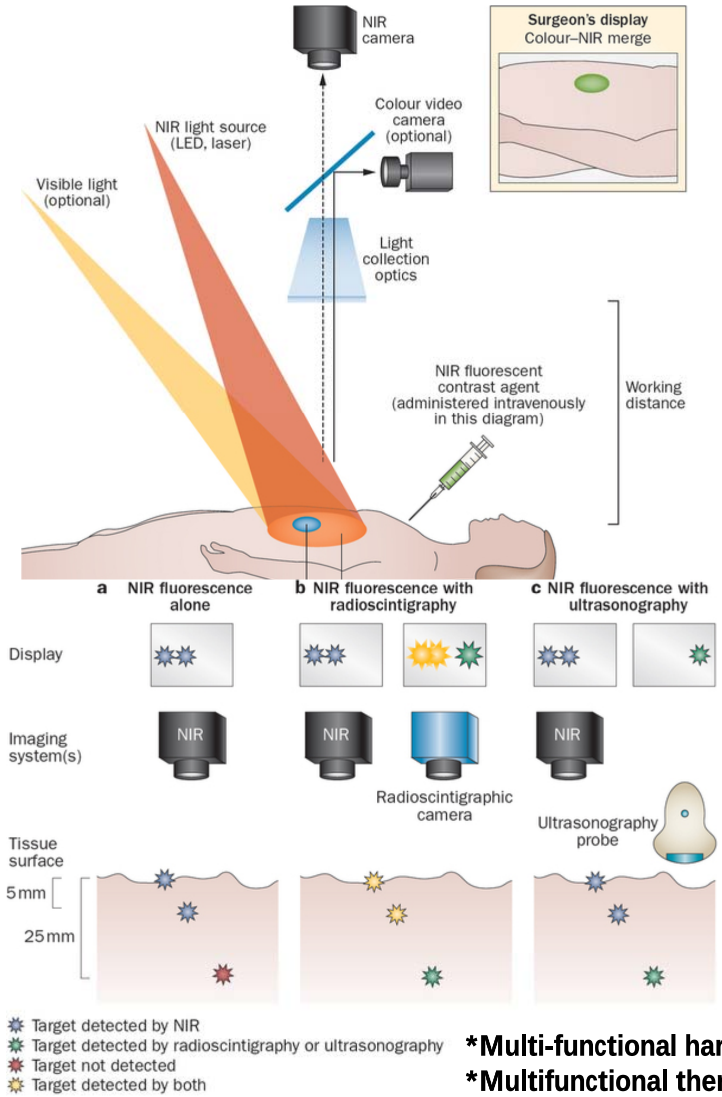
- A robust platform for **personalized, minimally invasive**, **in vivo drug delivery** with **on-demand release** and therapy, while enabling **real-time treatment monitoring**.
- Novel strategies enabling **integration of diagnosis and therapy** across many major specialties and sub-disciplines of clinical practice.
- **Highly interdisciplinary** research integration which is the outcome of the **synergism** between **molecular imaging**, **therapy**, and **nanomedicine**.



Imaging in interventional procedures: at present, appears to be one of the applications that is much closer to clinical translation

Fluorescent imaging in interventional procedures

The value of optical imaging in (nano)theranostics

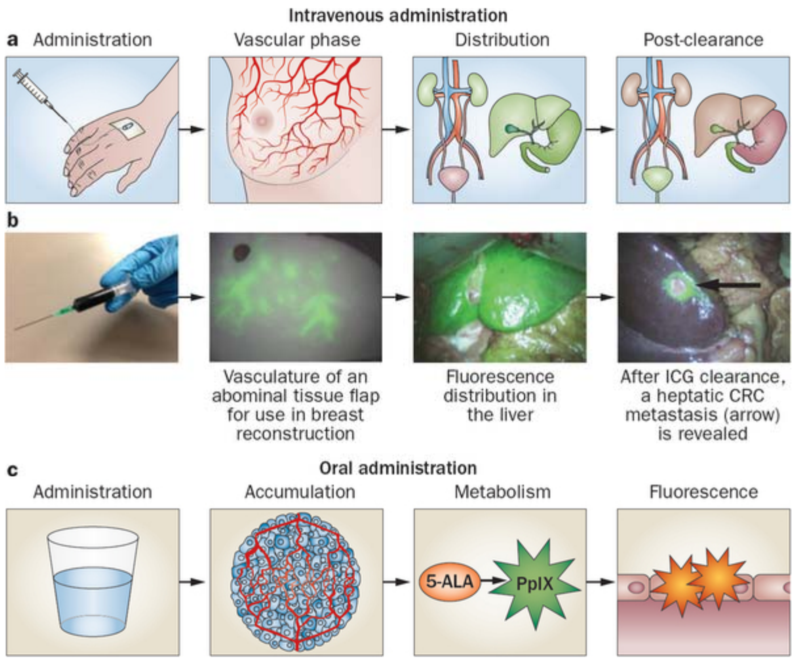


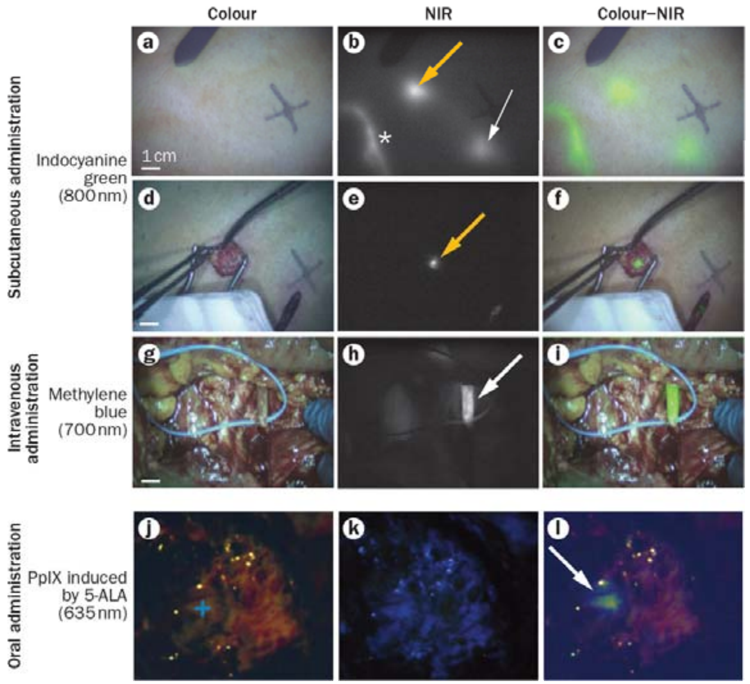
***Multi-functional hardware**
***Multifunctional theranostic probes**

- Image-guided surgery.
  - Promising, highly anticipated applications, of theranostics and nanotheranostics
- It involves:
  - Guided debulking
  - surgical margin specification with the ultimate goal of optimal organ preservation.
- Most of the clinical interventional procedures currently depend to a large extend on human visual perception and, to a lesser extent, on intra-operative diagnostic procedures.
- The detection of the cellular or molecular signature of disease, at which level complete cure could be achieved, is currently unavailable in the operating room.
  - A compelling need to incorporate advanced optical and opto-acoustical methods, in order to complement the critical decision making process, during clinical interventions

Image-guided cancer surgery using NIR Fluorescence

(Vahrmeijer AL et al)





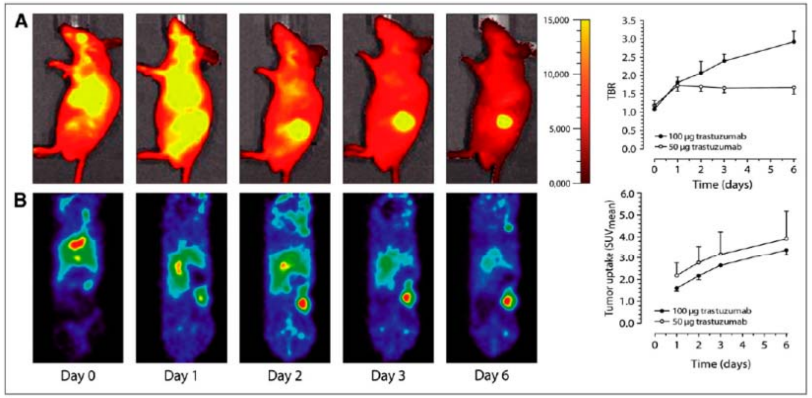
ü Optical-based techniques of intra-operative guidance have emerged with the potential to enable surgeons to **accurately assess the tumor margins.**

ü The evolution and transfer of these optical devices to the operating room has been made possible by significant technological advances in the field of **medical optics**

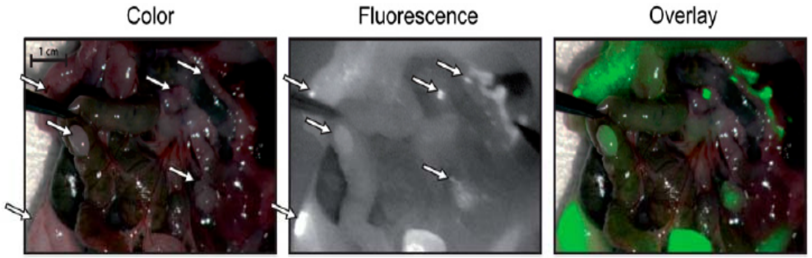
ü Paradigm shifts in surgery arise when surgeons are empowered to perform surgery **faster, better and less expensively than current standards.**

ü Optical imaging that exploits invisible near-infrared (NIR) fluorescent light (700–900 nm) has the potential to **improve cancer surgery outcomes**, **minimize the time patients are under anaesthesia and lower health-care costs** largely by way of its improved contrast and depth of tissue penetration relative to visible light.

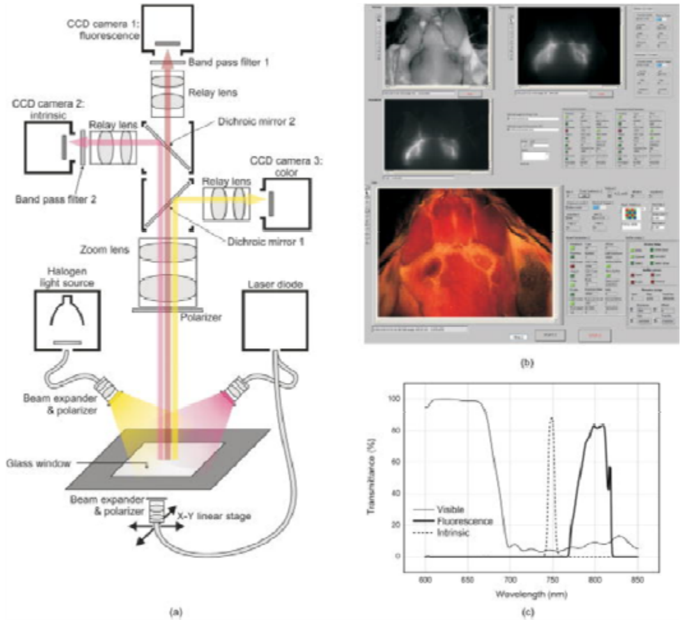
Use of theranostic agents for Intraoperative guidance I: Searching for HER2-expressing tumors



Dual modality of imaging HER2 in subcutaneous xenograft model of SK-BR3 (HER2+++ breast cancer)
A: representative two-dimensional fluorescence images of trastuzumab-800CW in a time course and tumor relative uptake
B: Correlated PET images after89 Zr –trastuzumab and uptake as determined by micro-PET

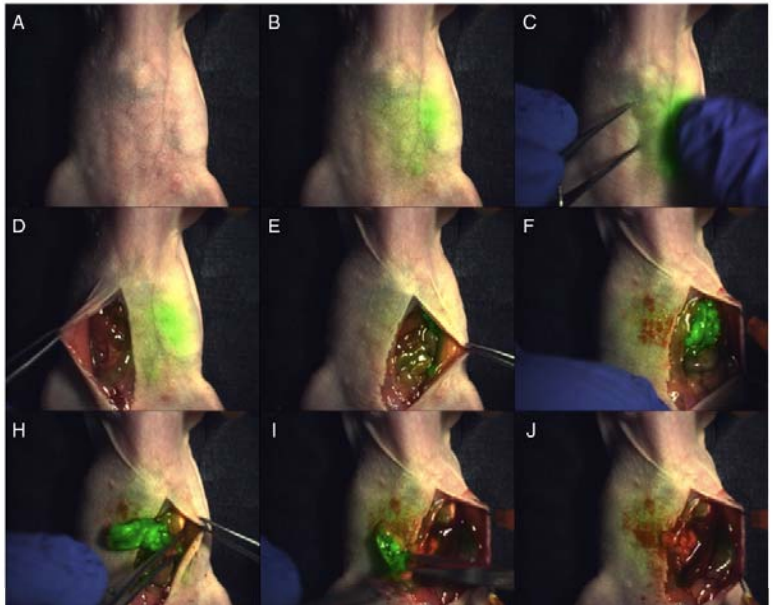


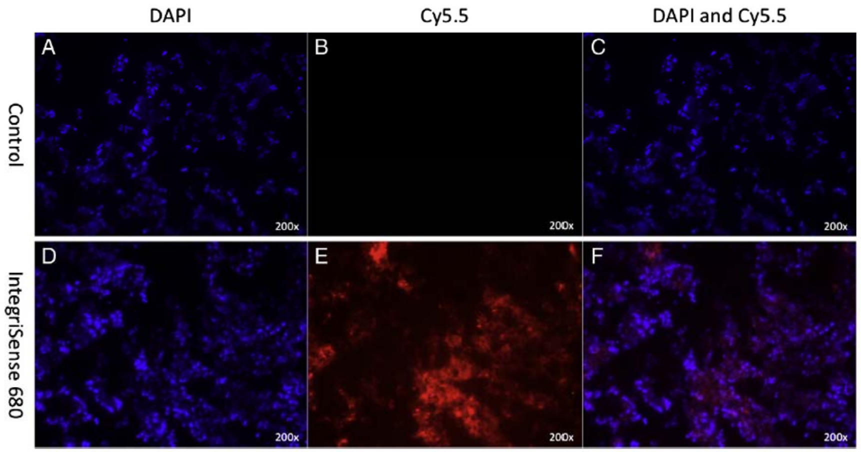
Intraoperative images of trastuzumab-800CW in human gastric cancer KATO-III (HER+++) intraperitoneal tumor model



Real-time intraoperative fluorescence imaging system using light-absorption correction. (Themelis G, et al. J. Biomed. Opt. 2009;14(6):064012-064012-9.

Use of (nano)theranostic agents for Intraoperative guidance II: Real-time monitoring of NIR fluorescent targets

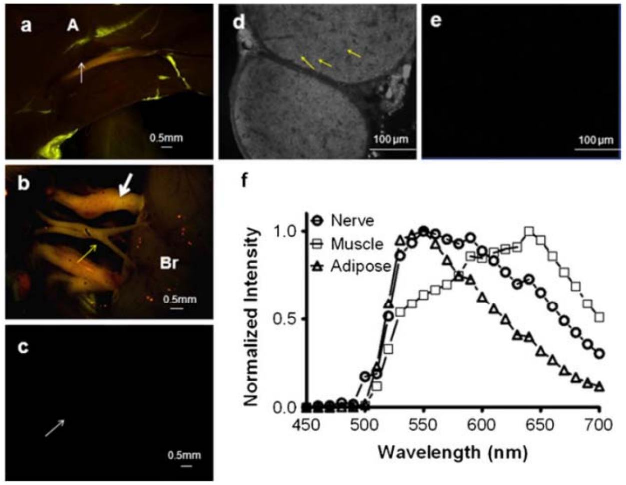


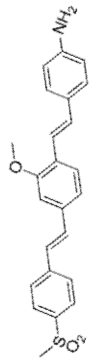


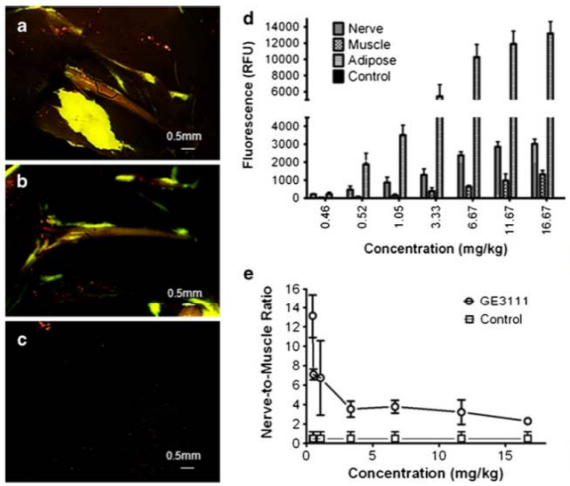
Fluorescence microscopy of cryo-resected tissues

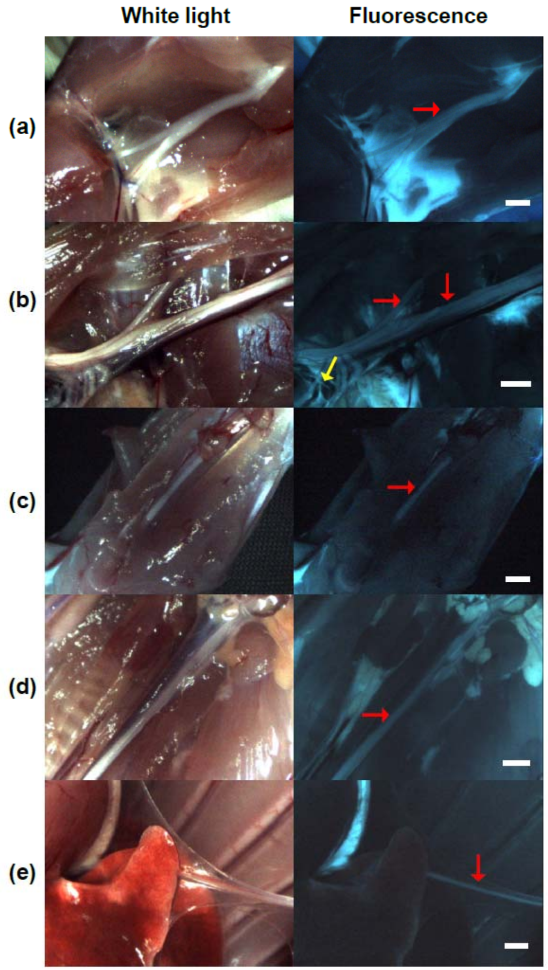
- ü *NIRF-theranostic agent –guided tumor debulking of ovarian cancer*
- ü **Using laser for excitation in combination with real-time intraoperative fluorescence imaging system and light-absorption correction, allowing for the simultaneous imaging of color and corrected NIR fluorescence**
- ü **Nine steps in the image-guided resection after injection with IntegriSense (non-protein antagonist targeting avβ3 integrin**
- ü **(A-C)When the laser light is turned-on, the signal is visible through the skin and enables palpation**
    - ü **(D-F) After opening and inspection of the abdomen the tumor lights u in pseudocolor green**
    - ü **(H-J)After resection of the tumor tissue, no apparent fluorescent signal is left (J)**

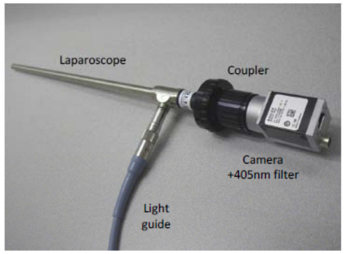
Use of (nano)theranostic agents for laparoscopic surgery



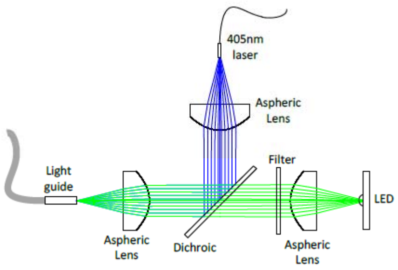








(a)



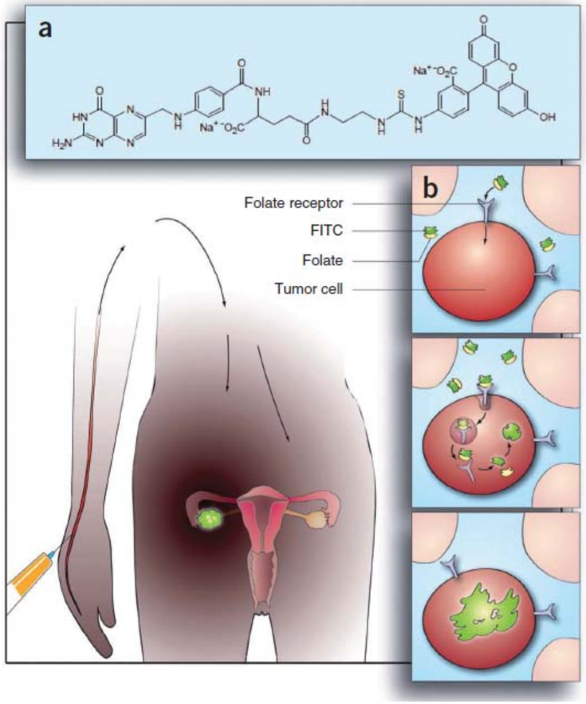
(b)

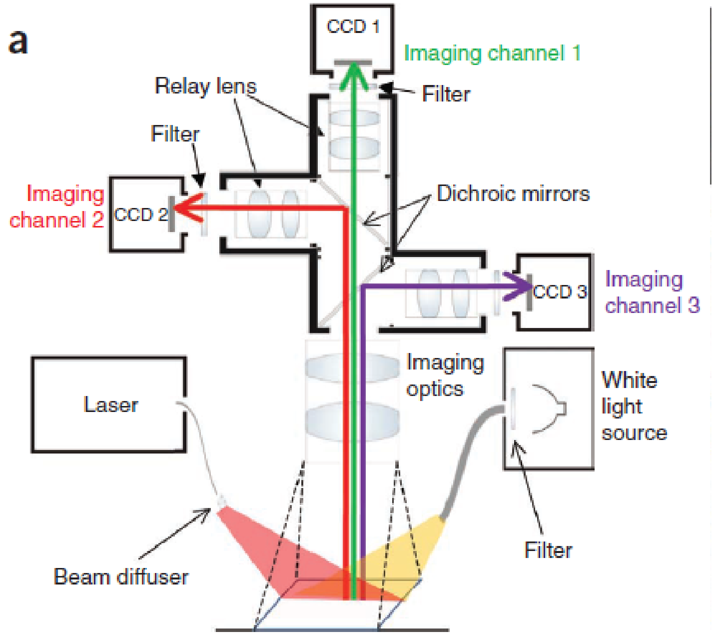
Single camera, dual-mode laparoscopic instrument

Laparoscopic surgery of fluorescently labeled nerves in rodents.
Red arrows: Primary nerves -Sciatic, (b)Brachial, (c)Median, (d) Vagus, (e) Phrenic
Yellow arrow: Branch of phrenic; branches of radial, ulnar, medium in close proximity

Image-guided surgery: Clinical Translation

Disseminated Ovarian Cancer: the proof-of-principle for (nano)theranostics in the Operating Room

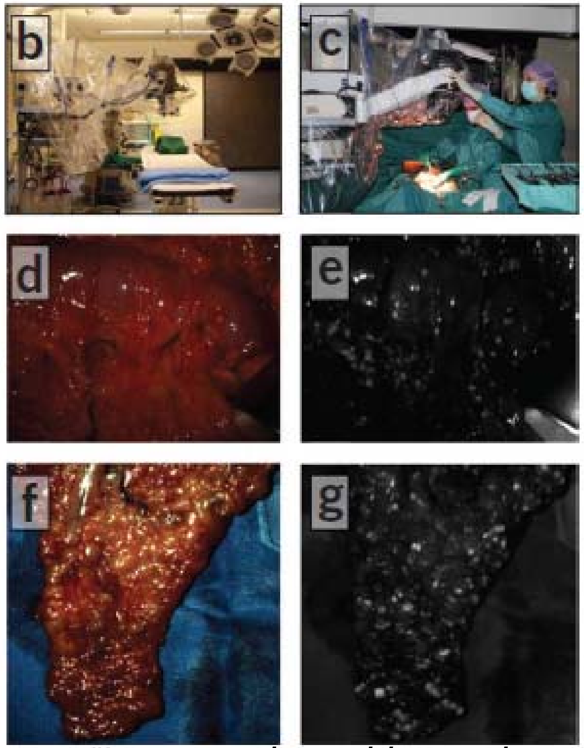




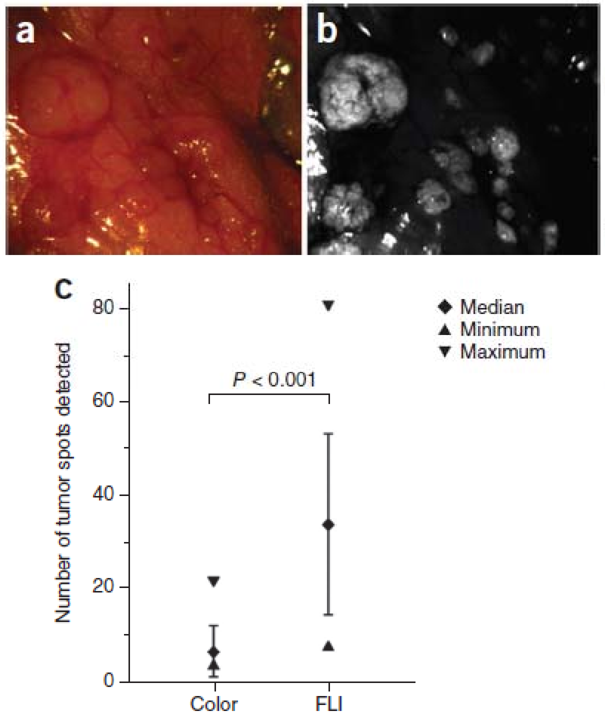
- **Both the fluorescent probe and the hardware are validated and in place**
  - Folate receptor-α has been established as a biomarker of epithelial Ovarian Cancer
  - Fluorescein Isothyocyanate (FITC) is readily available as a biocompatible stain
  - Feasible chemistry for Folate-FITC conjugation
  - Extensively studied cellular biology of FR-α after Folate binding

- **Real-time intraoperative fluorescence imaging system using light-absorption correction has been extensively used and optimized in pre-clinical studies**

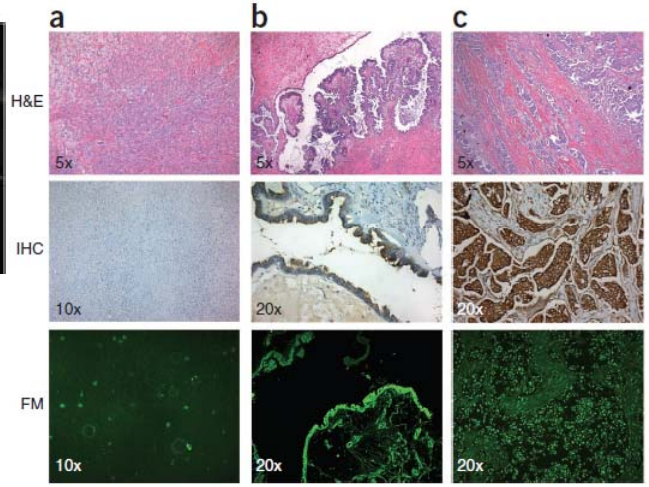
Ovarian Cancer Surgery in the 2010s



- *"Intraoperative multispectral imaging system"*
- **Patient with high-grade, disseminated OC, stage III, FR-α++**
- **Intraoperative snap-shots of simultaneously detected images: (d,f) in colour; (e,g) corresponding fluorescence**



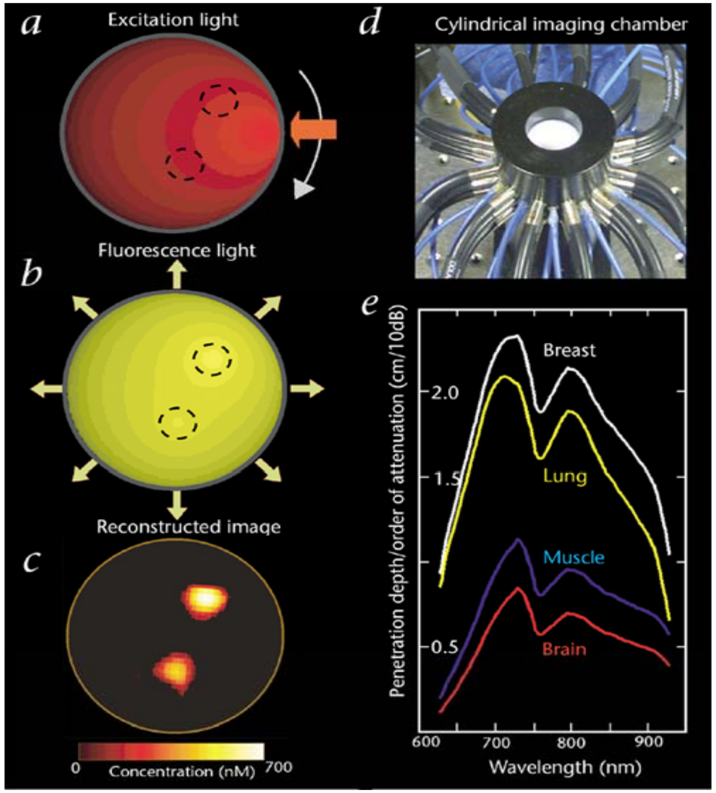
- **FITC-conjugate Folate Acid was administered IV (a,b)**
- **Color image (a) with the corresponding tumor-specific fluorescence image (b) of a representative area in the abdominal cavity.**
- **Scoring(c) was based on three different color images and their corresponding fluorescence images (FLI); P<0.001 by five independent surgeons.**

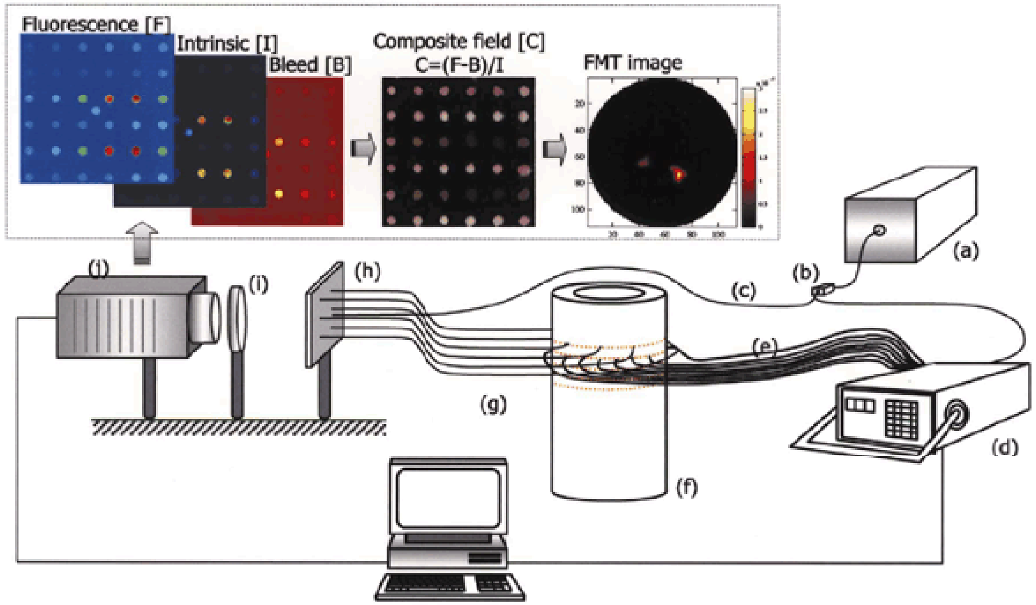


- **Immunohistologic Verification: the golden standard**
  - **Fibrothecoma; (b) borderline serous tumor; (c) High-grade serous carcinoma**
- **H+E vs Immunohistochemistry vs Fluorescence microscopy of frozen sections after FITC-FR-α administration.**
- **Data verify the assessment of visible lesions intraoperatively**

- **Functionalization of the probe with a potential therapeutic target is expected to introduce theranostics) within the same session Ą HIF; VEGR, ERBB, HSP90; CXCL; MMPs**
- **Odysseos A, Pitris C et al: U.S Pr. Patent-61,382,875:** Multimodal and Multifunctional Theranostic Agents for In vivo Detection, Monitoring and Targeted Therapy of TKR-mediated Disorders

Fluorescent Molecular Tomorgraphy: answer to the hardware challenge
In vivo molecular imaging meets with drug computerized tomography

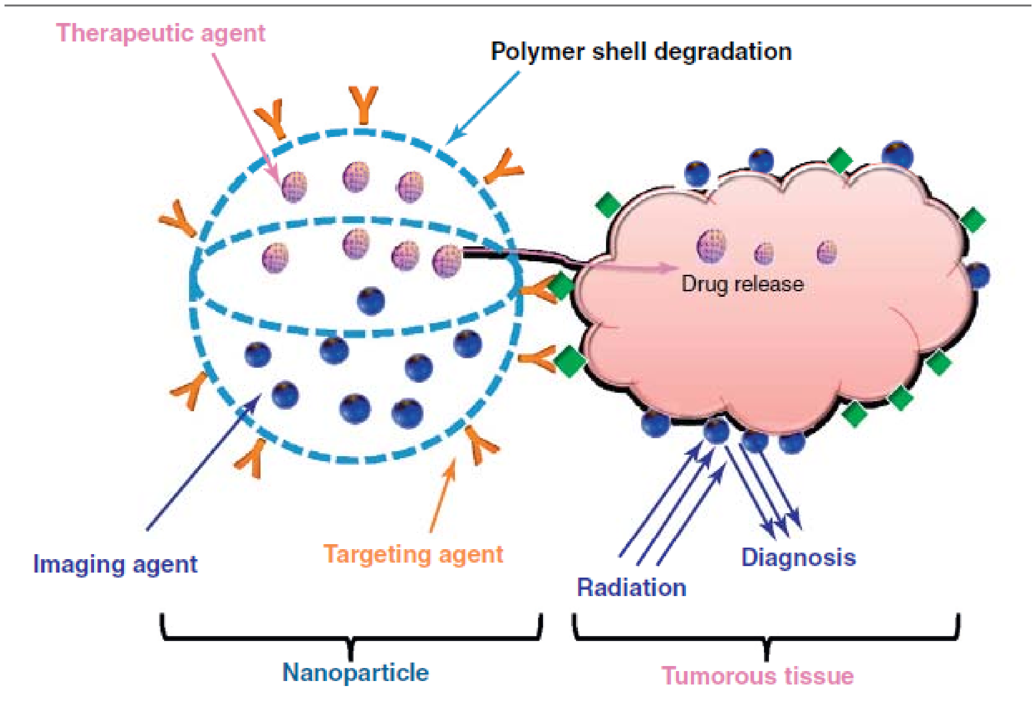




Based on (a) the use of target-specific molecular fluorescent reporters (probes) and (b) volumetric reconstruction of light emitted from the probe

a. A single light source illuminates into tissue –excitation of fluorochromes in tissues
b. In each position the fluorochromes act as secondary sources at a higher wavelength
   -Both collected from multiple points using appropriate filters
c. Measurements are tomographically combined to yield 3D fluorochrome distribution
d. A cylindrical FMT imaging system: blue-excitation, black-collection fibers
e. Modelling of the distance that NIR light can propagate into different tissues (before attenuation by an order of magnitude)

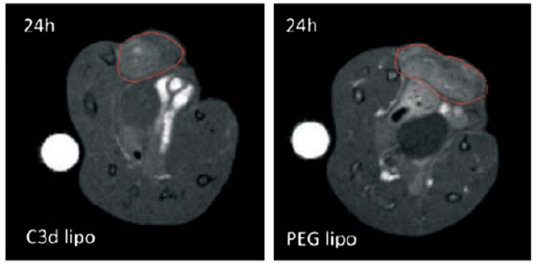
Monitoring Therapy by Imaging

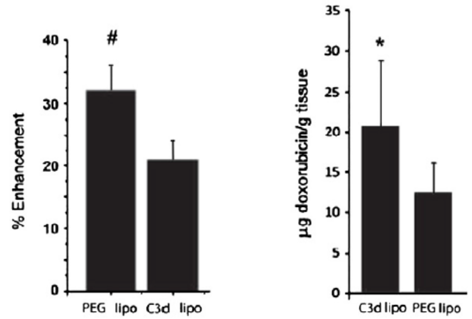


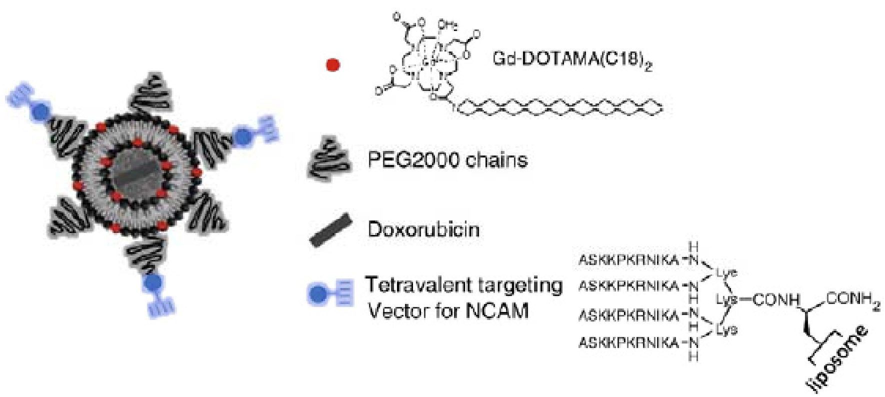
(Drug Discovery Today. V17,17/18, Sept 2012)

Imaging-guided Drug Release

- ü **MRI** has outstanding spatial resolution (down to tens of µm), it is minimally invasive, and has superb potential for longitudinal studies
- ü These advantages are opposed, however, by the relatively low sensitivity of MRI contrast agents, especially when the administered probe is intended to report on biological processes at the cellular level.
- ü Several nanomedical approaches have been developed to address these limitations.
  - ü Paramagnetic agents (e.g. Gd(III) or Mn(II) compexes), tightly bound to the structural components of the nanocarriers, result in an elongation of the rotational tumbling of the metal complex leading to a remarkable positive effect on the enhancement of MR contrast, even at clinically relevant field strengths.







- V Lipid-based nanoparticles, are the nanotheranotic agents closest to clinical translation.
- V Stealth liposomes, encapsulating Doxorubicin, tagged with a Gd-based agent and exposing a tetravalent petide targeting the neoplastic-epithelium-expressed receptor NCAM (Neural Cell Adhesion Molecule)
- V MRI imaging and targeted therapy of solid tumors such as Kaposi sarcomas
- V An excellent correlation between the MRI response (longitudinal water protons relaxation enhancement) and the amount of Doxorubicin uptaken by tumor cells was also observed in in vitro studies.

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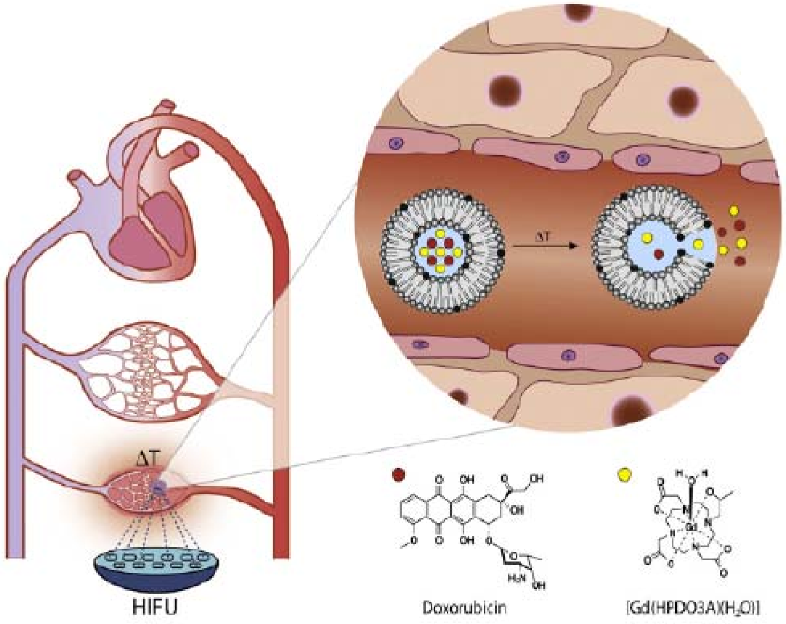
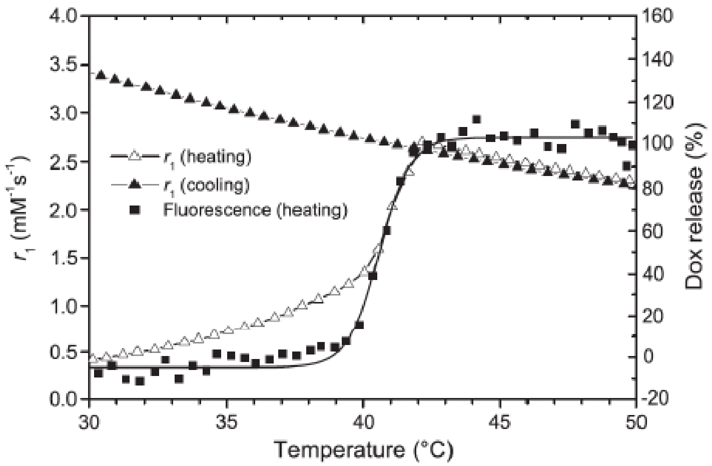
Hyperthermia - Imaging Drug Release



Temperature-induced release from a Temperature Sensitive Liposome with the application of Hyperthermia-Inducing Fluorescence Unit (HIFU).

- **MR images of KS tumor-bearing rats**
- **1 control; 2 treated**
- **T1 maps of the tumor and leg overlaid**
- **Differential response between the two treated animals**
- **Drug release over time is verified by Dox levels**



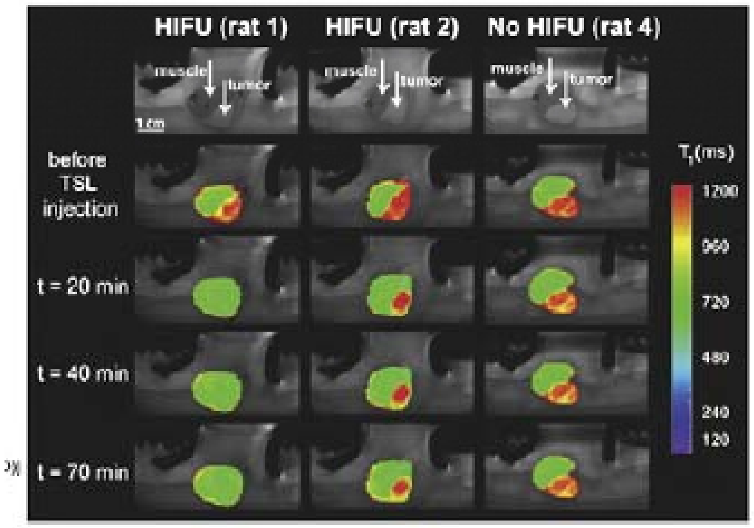
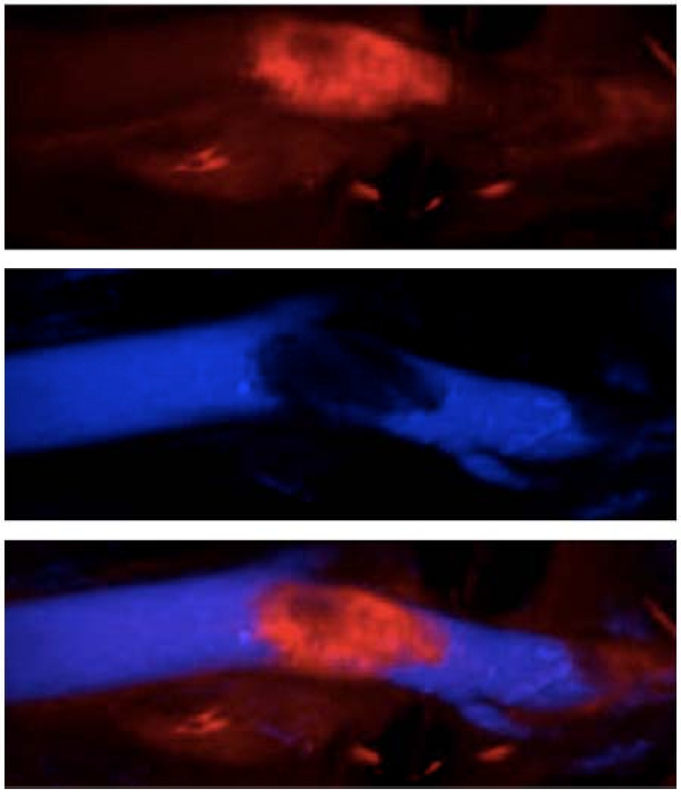


Photo-toxic therapy- Activating Therapy with Light Imaging Drug Release in Cardiology

- **A multimodal, multifunctional theranostic nanoagent**
  - Magneto-fluoroscent imaging attributes (SPIO+NIR fluorophore)
  - Light-activated therapeutic chlorin (CLIO): localization and phototoxic activation
  - Phagocytosis by activated macrophages
  - In vivo validation with uptake by macrophage-rich atherosclerotic lesions
  - Imaging with intravital microscopy
  - Therapeutic effect: exposure to light photons led to stabilization of the lesion due to eradication of inflammatory macrophages



- **In vivo intravital fluorescence microscopy of the theranostic nanoagent to carotid atheroma**
- **NIR emission demonstrates particle uptake**
- **Fluorescence angiogram with standard fluorescein-dextran for vasculature outlining**
- **Merged pictures**