RARE-EARTH DOPED NANOPARTICLES AS SHORTWAVE INFRARED REPORTERS

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Medical Imaging in Oncology

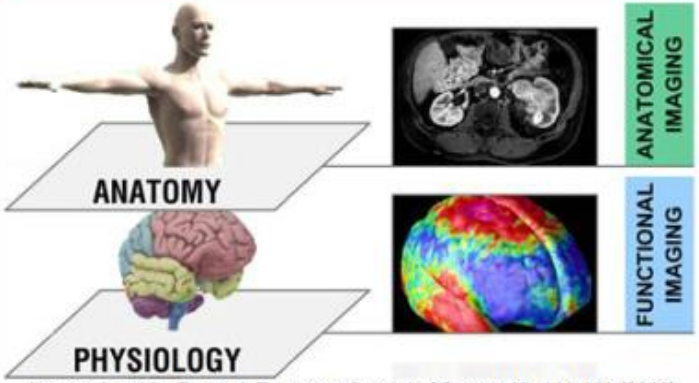


Image: Lambin, P., *et al. European Journal of Cancer* 48, 441-446 (2012).

- **Anatomical imaging** provides macroscopic biological information
  - Where and how large is a tumor?
  - Can treatment be initiated?
- **Functional imaging** detects changes in physiology
  - Has angiogenesis occurred?
  - What is the degree of hypoxia?
- Several modalities well-suited
  - Ultrasound
  - X-ray computed tomography (CT)
  - Magnetic resonance imaging (MRI)

Medical Imaging in Oncology

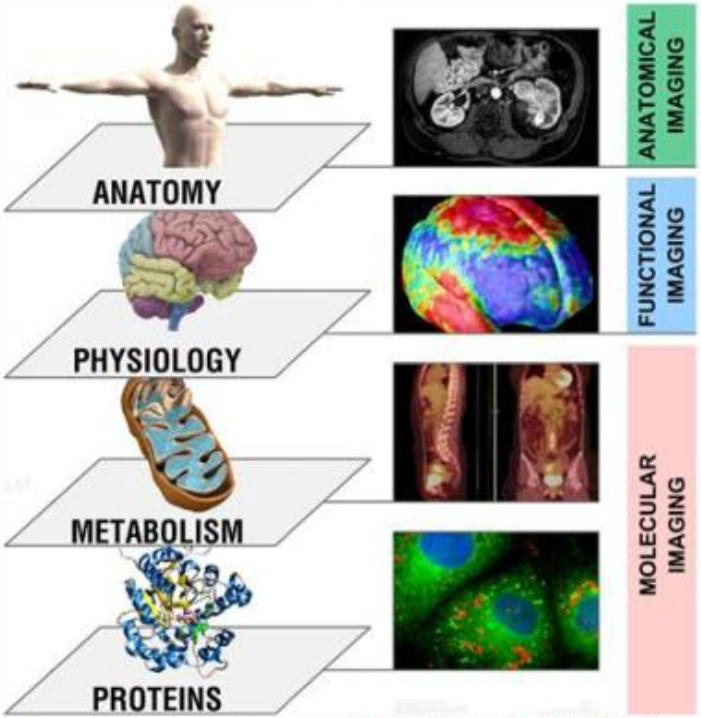
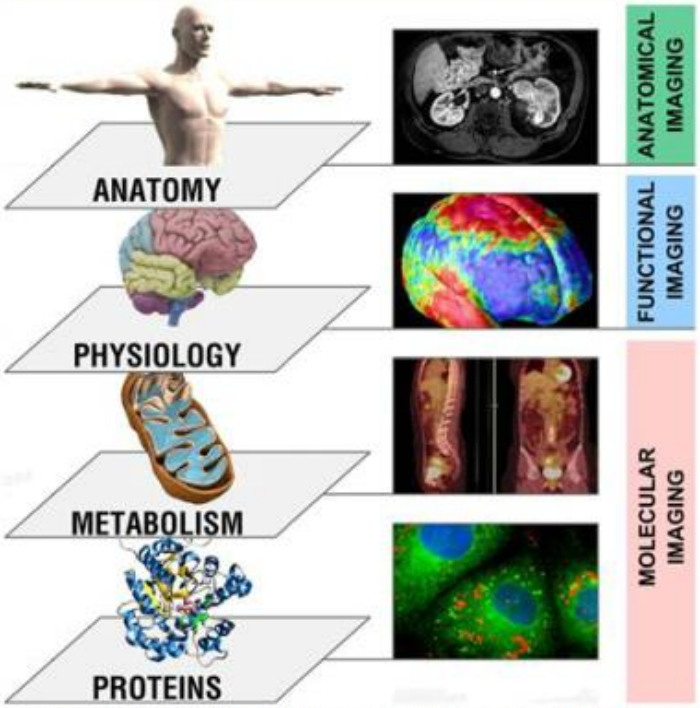


Image: Lambin, P., *et al. European Journal of Cancer* 48, 441-446 (2012).

- **Molecular imaging** visualizes processes at the microscopic level
  - How far has cancer progressed?
  - Has micro-metastasis occurred?
  - Is there response to treatment?
  - Can treatment be personalized?

- Capable modalities
  - MRI
  - Nuclear (PET/SPECT)
  - Optical imaging

Medical Imaging in Oncology



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  - How far has cancer progressed?
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- Capable modalities
  - MRI
  - Nuclear (PET/SPECT)
  - **Optical imaging**

Optical Imaging: An Emerging Field

- Non-invasive imaging technique
  - No harmful ionizing radiation
  - Portable and inexpensive
  - Easily translatable into the clinic

- Driven by the development of detectors and contrast agents[1]
  - Cameras (EMCCD, ICCD, InGaAs)
  - Exogenous contrast agents

Intraoperative Use of Optical Imaging

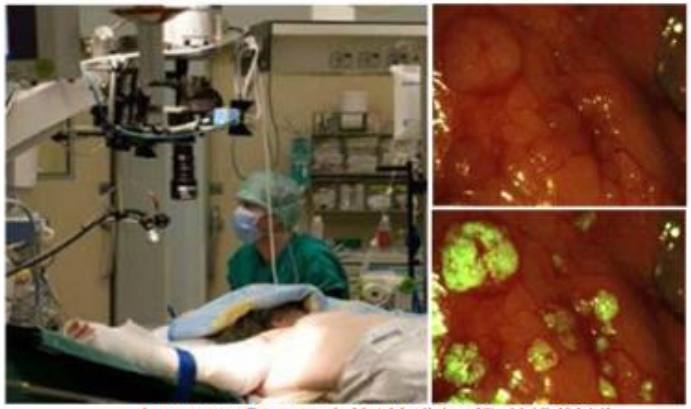
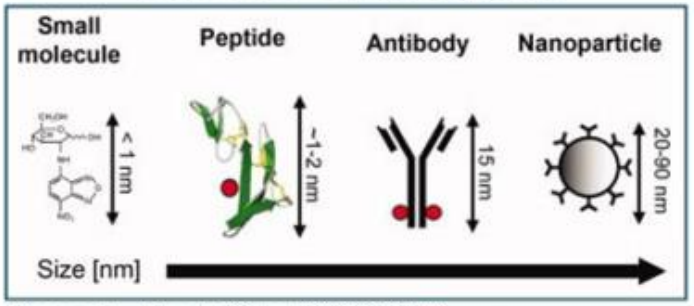


Image: van Dam, et al. *Nat Medicine* **17**, 1315 (2011)

Classes of Contrast Agents



[1]Pierce, et al., *Intern J of Cancer* 123,1979 (2008)

Overview of Research

Development of optical contrast agents for deep tissue cancer imaging, surgical guidance and molecular classification of disease at its earliest stages

- Contrast agent design requirements[1]
  - Biocompatible (non-toxic, biologically stable)
  - Sensitive and specific (detectable at low conc., targetable)
  - **Resolvable deep in living tissue**

[1] Weissleder, et al. *Nature* 452, 580 (2008)

Overview of Research

Development of optical contrast agents for deep tissue cancer imaging, surgical guidance and molecular classification of disease at its earliest stages

1. Principles of optical imaging
   - Interaction of light with tissue
   - Challenges of imaging using light

2. Recent advancements in biomedical optical imaging
   - Beyond the near infrared
   - Old probes with new potential

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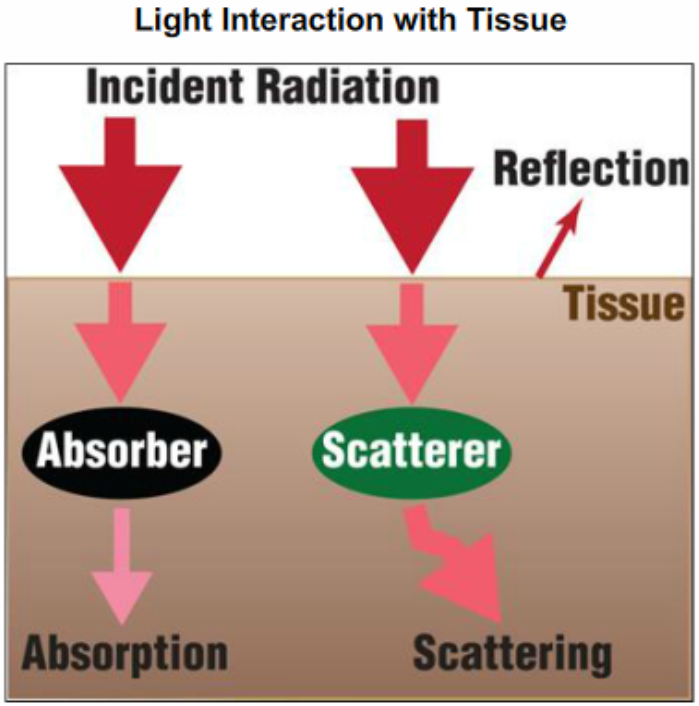
Photon Interactions in Tissue



- Optical imaging is governed by the interaction of light with tissue[1]
  - Absorption attenuates signal
  - Scattering diffuses signal
- Both absorption and scattering limit how deeply we can image[2]

[1] Bremer, et al. *European Radiology.* **(2003)** 13, 231
[2] Lim, Y.T. et al. *Mol Imaging.* **(2003)** 2, 50.

Photon Absorption in Tissue

- Tissue chromophores absorb light
  - Water
  - Hemoglobin/proteins
  - Melanin (in pigmented tissue)

- Near infrared (NIR, 700-1000 nm) is not absorbed by biological chromophores[1]
  - Low tissue auto-fluorescence
  - Improved penetration depth (cm)
  - "Biological window" for imaging

Tissue Auto-fluorescence

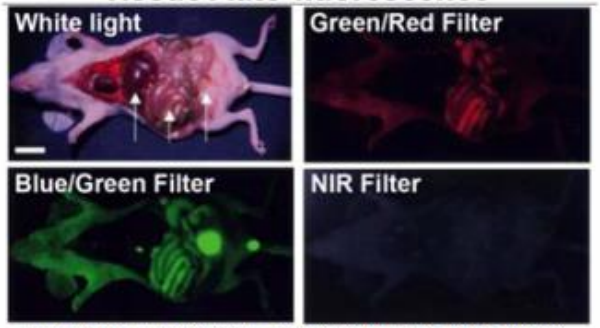
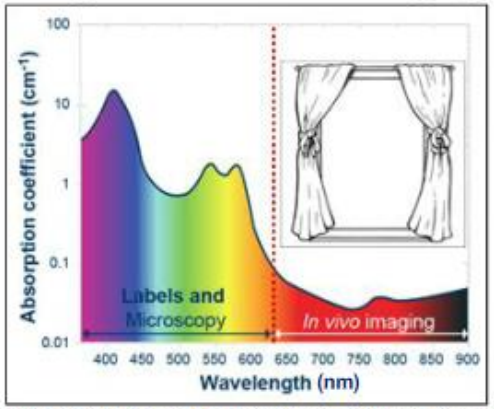


Image from Frangioni, *Cur. Opin. in Chem. Bio.* **2003**, 7, 626.

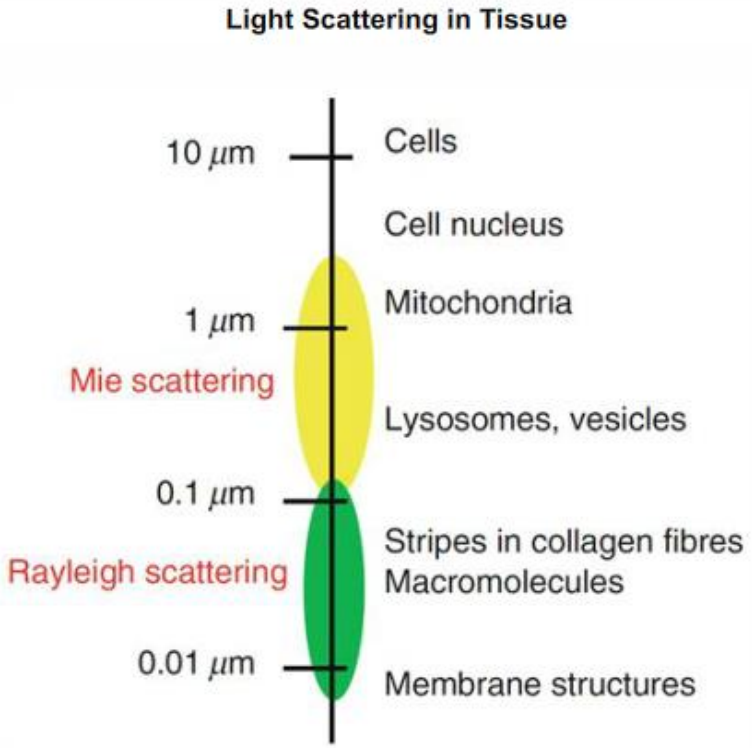
"Biological Window" for Imaging



[1]Adapted from Weissleder. *Nat Biotech.* **2001,** 19, *316 – 317.*

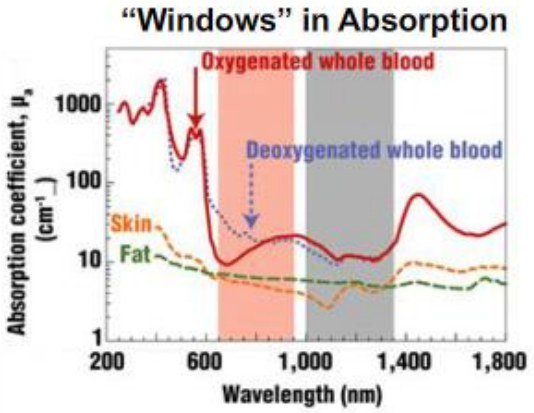
Photon Scattering in Tissue

- Scattering is the most dominate light-tissue interaction in NIR
- Tissue components and heterogeneity cause light scattering
  - Non-uniform distribution of refractive indices
  - Wavelength dependence tissue specific
- Contribution of absorption ***and*** scattering must be considered



New Window: Shortwave Infrared (SWIR)

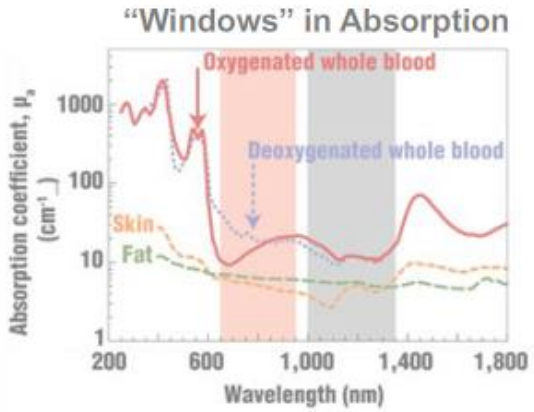
- "Second biological window"
  - Spanning 1,000 – 3,000 nm
  - Comparable absorption properties as NIR

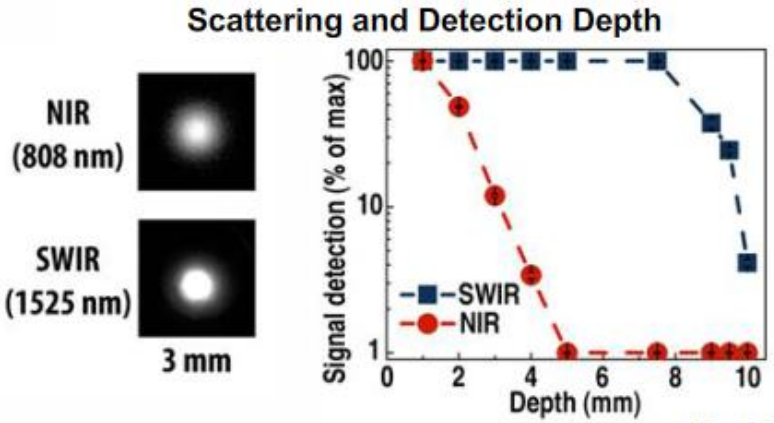


Top image adapted from Smith, et al. *Nat Nanotech.* **2009,** 4, *710.*

New Window: Shortwave Infrared (SWIR)

- "Second biological window"
  - Spanning 1,000 – 3,000 nm
  - Comparable absorption properties as NIR
- SWIR exhibits reduced scattering
- Improved resolution **and** depth
  - Simulated 100x improved S/N in tissue[1]



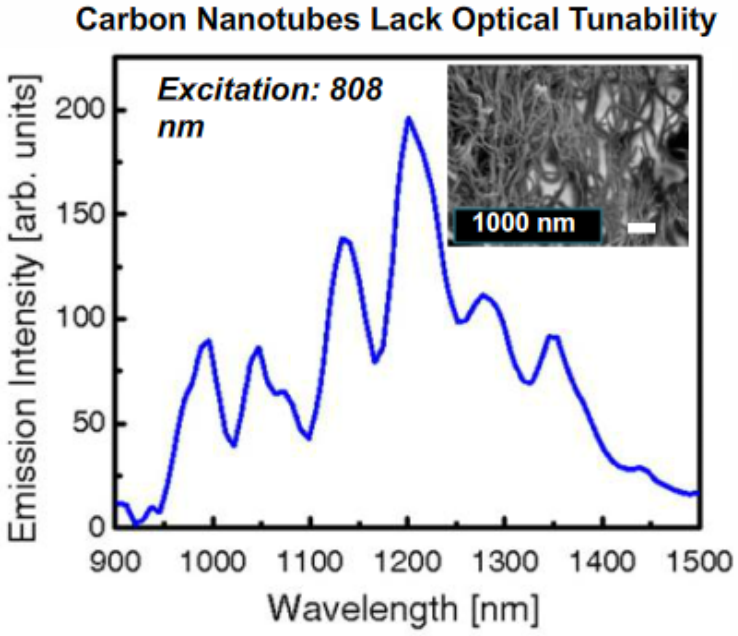


[1] Lim, Y.T. et al. *Mol Imaging.* **(2003)** 2, 50.

Top image adapted from Smith, et al. *Nat Nanotech.* **2009,** 4, *710.*

Challenges of SWIR-Based Imaging

- **Few** materials have biologically applicable SWIR contrast
  - Carbon nanotubes (SWNTs) have broad emission/sizes[1]
  - Quantum dots (QDs) exhibit contain toxic elements[2]
- **Lack** of commercially available imaging systems
  - Common systems have limited imaging capabilities beyond 850 nm



Images From Zhan, et al. *Nature Materials* **2**, 38 (2003) &
[1] Welsher K et al. *PNAS* **108**, 8943 (2011)
[2] Lim, et al. *Molecular Imaging* **2**, 50 (2003)

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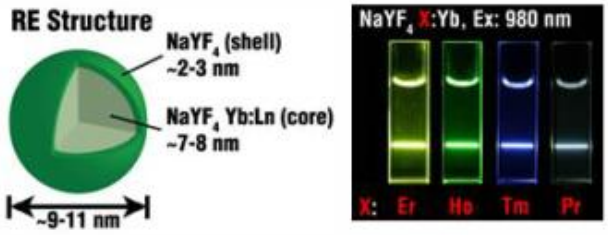
Rare-Earth Doped Nanoparticles (REs)

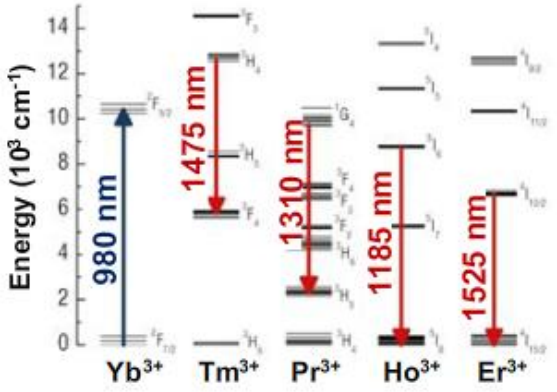
- Nanoparticles doped with Yb and rare earths
  - Doped "host" surrounded by an undoped "shell"
  - Numerous hosts available, $NaYF_4$ very efficient[1]

- Unique physical and optical characteristics
  - Low toxicity
  - Stable optical properties (non-bleaching)
  - Excited with NIR ("biological window")
  - Tunable emissions in visible (upconversion)
  - **Exhibit bright, tunable SWIR emissions[2]**

Structure & Visible Emission



Tunable SWIR Emission



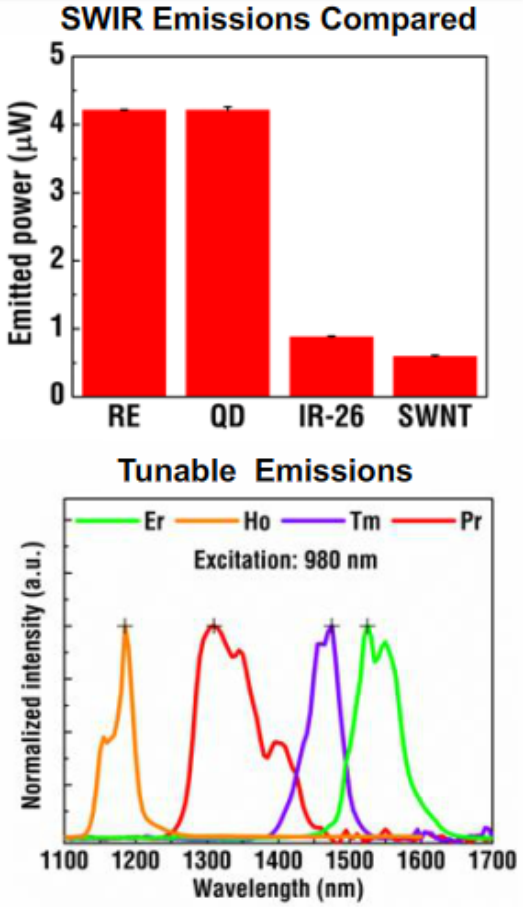
[1] Tan, MC, et. al *The Journal of Physical Chemistry C* (2011)
[2] Naczynski, D. J., et al. *Nature communications* (2013)

Developing REs for SWIR Imaging

- RE are **as efficient** as QDs and **significantly more efficient** as SWNTS at generating SWIR
  - Brighter than organic IR dyes (e.g. IR-26)

- Numerous benefits over SWNTs and QDs for biomedical SWIR imaging
  - Excitation in **first window (NIR)**, large Stokes shift
  - Excitation with **low power densities**
  - **Narrow and tunable** emissions, multiplex capability
  - Emissions are not size dependent

Naczynski, D. J., et al. *Nature communications* (2013)



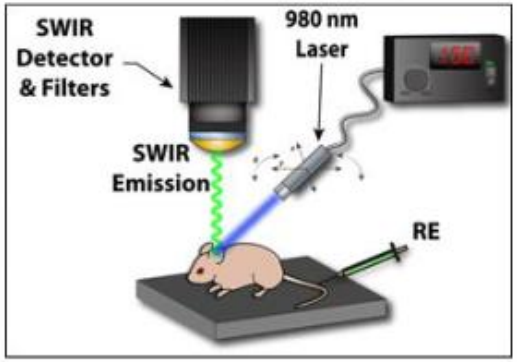
Designing a SWIR Imaging Prototype

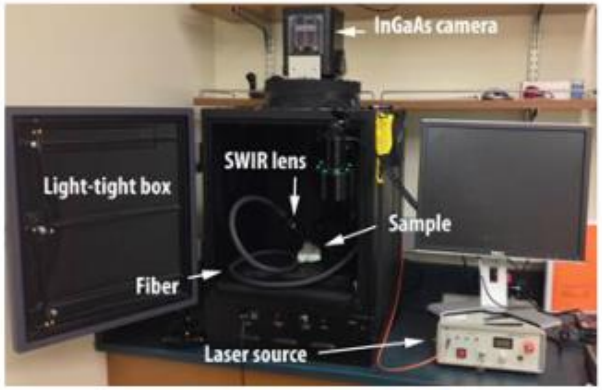
- Preclinical translation requires the development of an imaging platform

- Developed of a low-cost (<$100k) small animal system
  - InGaAs camera
  - NIR laser
  - Interchangeable emission filters
  - SWIR lens/objective
  - Anesthesia unit/heating system

- Operating features
  - Fast exposure, video rate imaging (20-50 ms)
  - Low power excitation (>100 mW/cm^2)
  - Excellent detection sensitivity (3 nM for REs)

SWIR Imaging Prototype

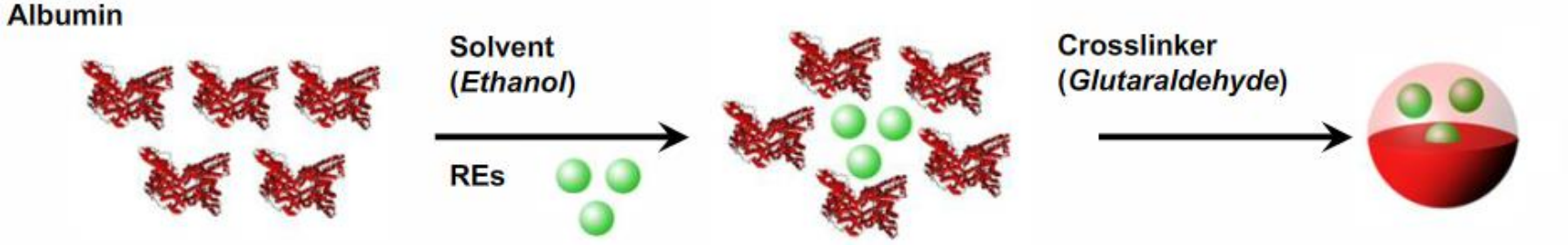




Naczynski, D. J., et al. *Nature communications* (2013)

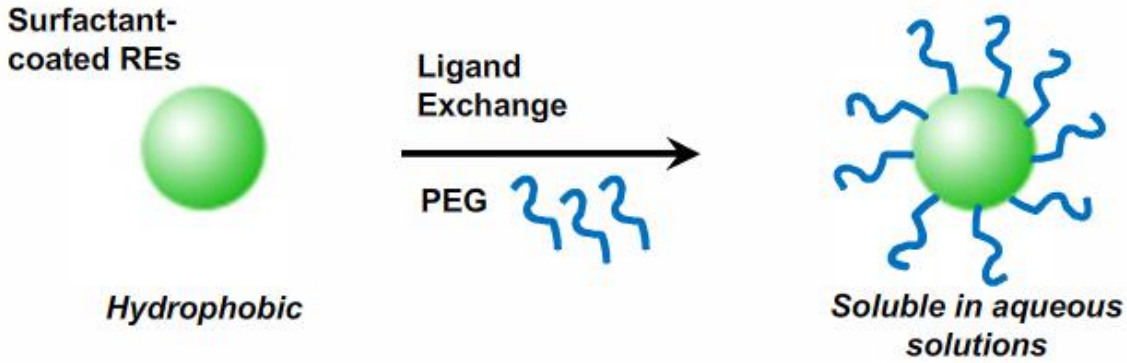
Developing REs for Biological Use

- **REs** encapsulated in **human serum albumin**[1]



[1] D. J. Naczynski, et al. *Small* (2010)

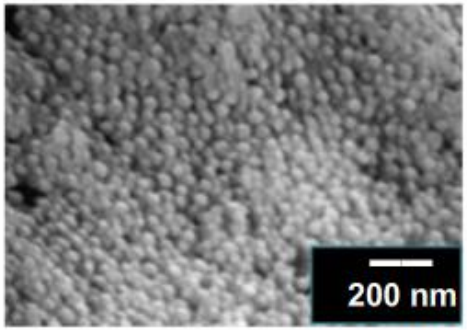
- **REs** stabilized using **PEG [poly(ethylene glycol)]**



Developing REs for Biological Use

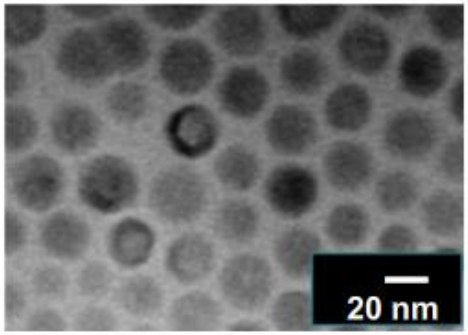
Albumin encapsulation

- Tunable sizes (75-300 nm)
- Increased biocompatibility
- Drug binding regions[1]
- Clinical precedent (Abraxane)



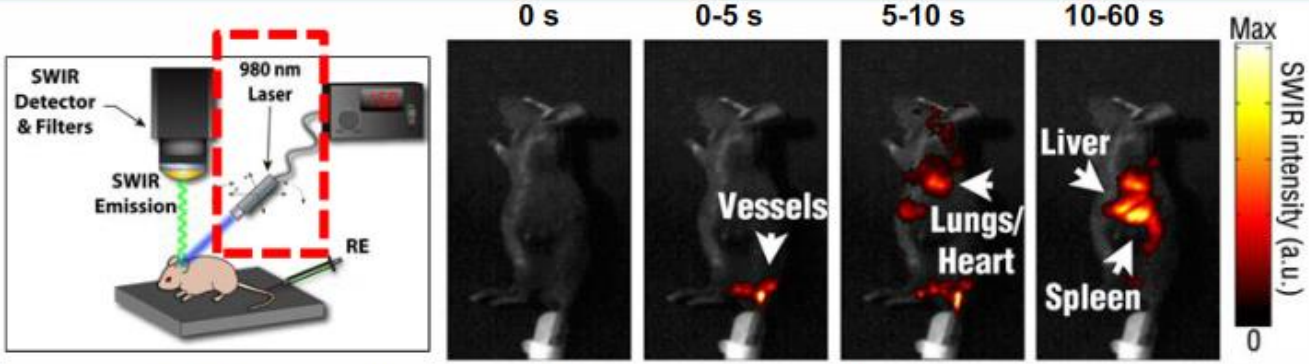
PEGylation

- Simple procedure
- Small size achievable (30-50 nm)
- Increased biocompatibility
- Improved serum half-time



[1] Cui, Mingjie, et al. Advanced healthcare materials (2013)

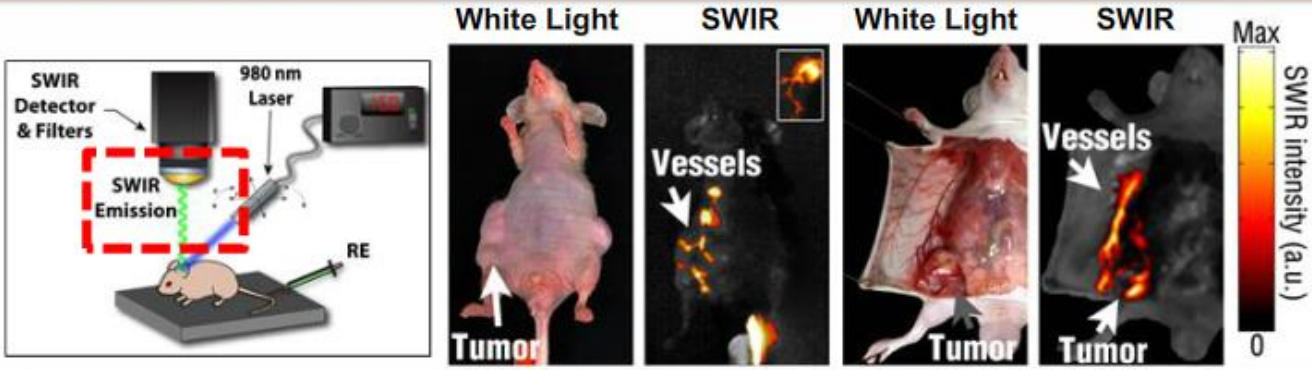
Real-Time Tissue SWIR Imaging



- REs injected via tail vein catheter in healthy mouse
  - Video-rate image acquisition over 60 s
- Circulation of REs could be visualized in real time
  - Transport to heart and lungs
  - SWIR emissions could distinguish individual organ structures
  - Tissue distribution was rapidly assessed non-invasively

Naczynski, D. J., et al. *Nature communications* (2013)

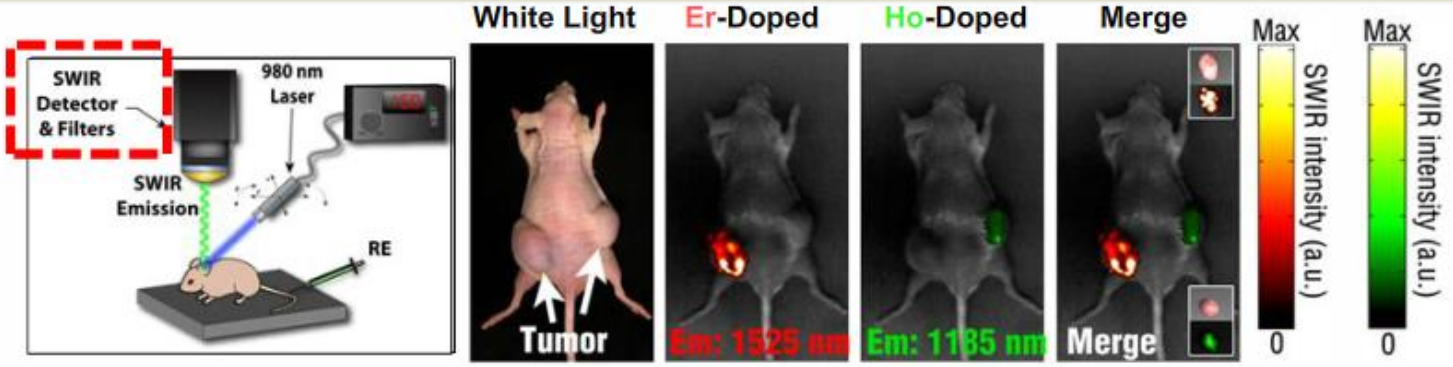
Vascular SWIR Imaging



- REs injected into mice bearing melanoma xenografts
  - Tumor regions surgically exposed following injection
- SWIR reveals vessel patterns in tumor xenograft model
  - Vasculature architecture visualized
  - Irregular patterns near tumor
  - Individual vessels observed after dissection

Naczynski, D. J., et al. *Nature communications* (2013)

Multispectral SWIR Imaging



- Two "color" multispectral SWIR imaging after intra-tumoral injection
  - Injected REs doped with **Er (em: 1525 nm)** and **Ho (em: 1185 nm)**
- **First** demonstration of multispectral *in vivo* SWIR imaging
  - Single source excitation identified both signals with no crosstalk
  - Tunable SWIR emissions can be use to probe multiple disease markers

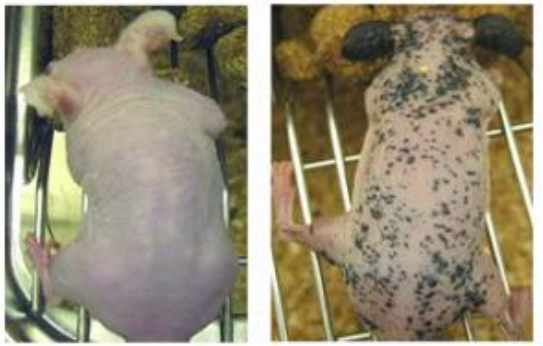
Naczynski, D. J., et al. *Nature communications* (2013)

Targeted SWIR Imaging of Melanoma

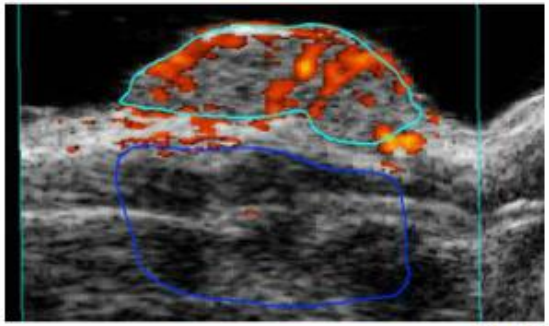
- Transgenic murine melanoma model (**TGS**)[1]
  - Spontaneous tumor development
  - Mimics human cutaneous melanoma
  - Highly metastatic
  - Pigmented lesions

- Angiogenesis in larger lesions
  - Relevant to other aggressive cancers

SKH (*left*) & TGS Mice (*right*)

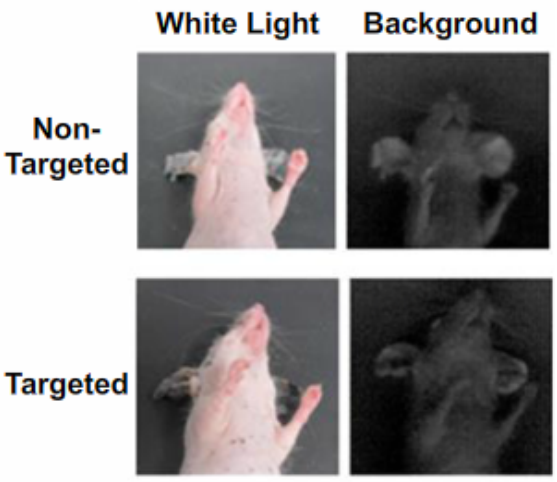


Angiogenesis in TGS Tumor



[1] Chen, S. et al. *Nature Genetics* **34**, 108-112 (2003).

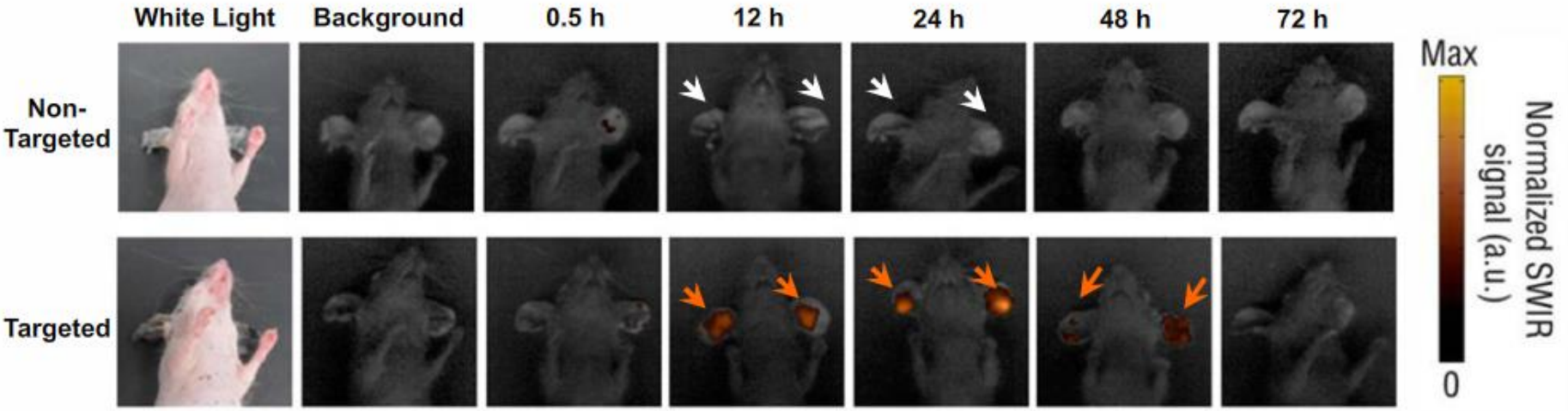
Targeted SWIR Imaging of Melanoma



- Very low autofluorescence observed before imaging
  - Backlit images resolved with incandescent lighting

Naczynski, D. J., et al. *Nature communications* (2013)

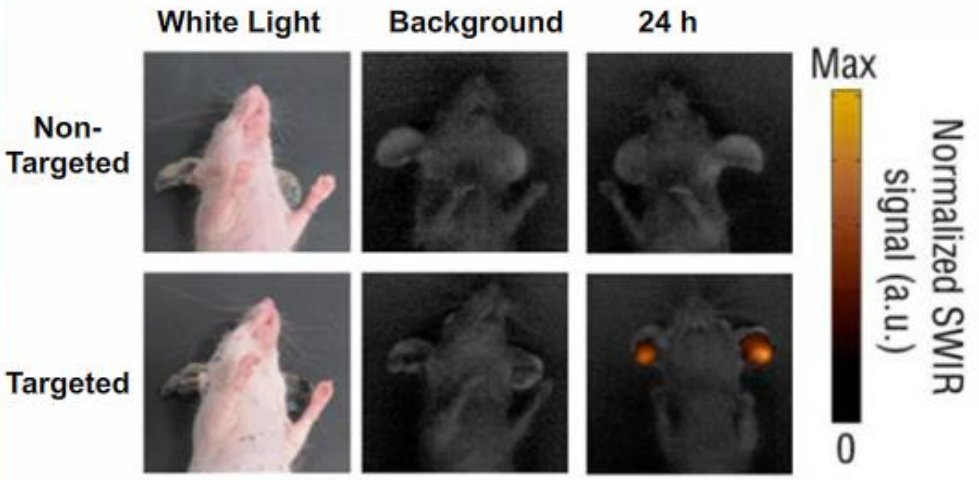
Targeted SWIR Imaging of Melanoma



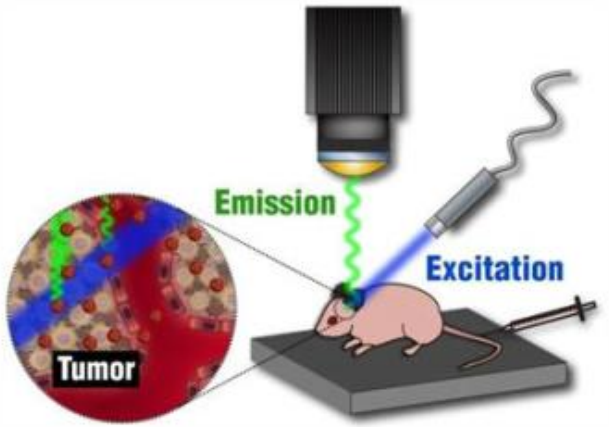
- Tumors imaged over time after injection of RE formulations
- Negligible fluorescence observed in non-targeted formulations

Naczynski, D. J., et al. *Nature communications* (2013)

Targeted SWIR Imaging of Melanoma



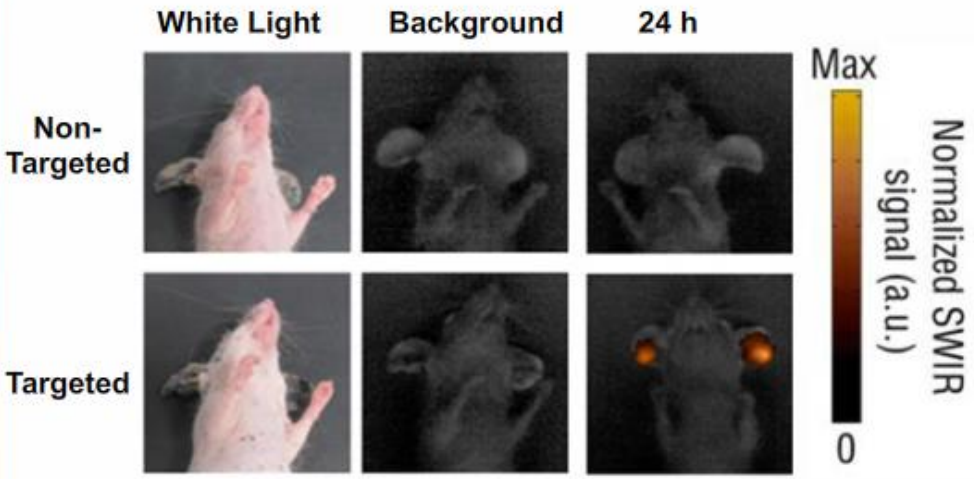
Proposed Mechanism of Targeting

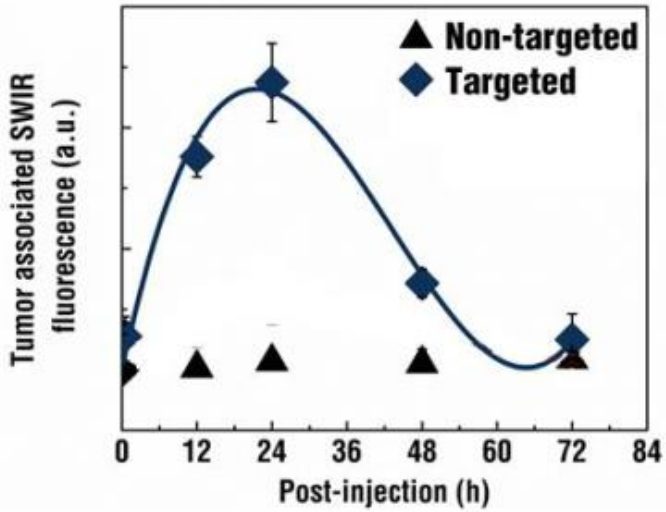


- Enhanced SWIR signal at tumor sites with targeted REs
- Only SWIR emissions observed
  - Easily detectable through dense, pigmented tumors

Naczynski, D. J., et al. *Nature communications* (2013)

Targeted SWIR Imaging of Melanoma





- Peak emission observed and validated at 24 h
  - Over a **10-fold increase** in tumor accumulation over non-targeted

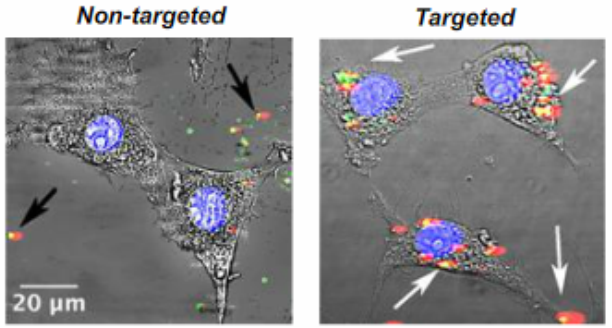
Naczynski, D. J., et al. *Nature communications* (2013)

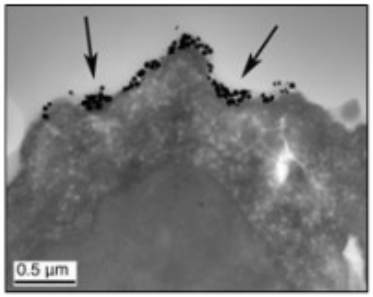
Future Directions

- Biomarker imaging using **multiplexed SWIR**
  - Monitoring disease response after treatment
  - Visualizing the interplay between multiple cancer indicators

- Extension of SWIR imaging toward **deeper tissue** and **intraoperative imaging**
  - Fluorescence endoscopy
  - Lymph node mapping/biopsy guidance
  - *In situ* histopathology

Molecular Targeting Disease Markers





D. J. Naczynski, et al. *Small* (2010)

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- Dr. Richard Riman (Materials Science)
- Dr. Suzie Chen (Cancer Biology)
- Dr. Charles Roth (Chemical Eng.)

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