In vivo Imaging in Rodents

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Considerations

- Human imaging ≠ rodent imaging
  - Mice are not just small humans with tails
    - Optical imaging is great in mice
    - CT is not so great in mice

- Cost
- Anesthesia time
- Throughput
Imaging Modalities

- **Human**
  - CT
  - Plain Films
  - Ultrasound
  - Magnetic Resonance
  - Radionuclide
  - PET
  - (Fluorescence)

- **Animal**
  - Bioluminescence
  - Fluorescence
  - Ultrasound
  - Plain film
  - Magnetic Resonance
  - Radionuclide
  - PET
  - CT
Hierarchy of Imaging

- PET
- SPECT
- CT
- Ultrasound
- Fluorescence
- Bioluminescence

Cost: PET > SPECT > CT > Ultrasound > Fluorescence > Bioluminescence

Time: PET < SPECT < CT < Ultrasound < Fluorescence < Bioluminescence
NCI-Frederick Small Animal Imaging Program

MRI
Philips 3.0T

PET
Siemens Inveon μPET
Xenogen/Perkin Elmer Spectrum

Optical
CRi Maestro

Ultrasound
Visual Sonics Vevo 770

γ-Well Counter
PerkinElmer 1480 Wizard

High Resolution
Fuji FLA-5100

Archival System
Siemens MIPORTAL

SPECT/CT
Siemens Inveon μSPECT/CT
Two Major Types of optical imaging

**Green Fluorescent Protein (GFP)**
Isolated from jellyfish, *Aequoria victoria*.

**Luciferase**
Isolated from firefly photocytes
What is Bioluminescence?

- Bioluminescence is a chemical reaction that produces light.
- Does not require excitation light
- Because the background tissue is non-light-producing there is high target to background.

CMV Luc
Reported Gene-Reporter Probe Paradigm

Massoud et al. Advances in Oncologic Imaging 2003
“Xenogen”

Xenogen
Perkin Elmer

Biospace
Bioluminescence

• Advantages
  – High sensitivity
  – High throughput
  – Relatively inexpensive
  – Highly specific

• Disadvantages
  – Requires transfection
  – Cells must be alive
  – No ex vivo BLI
  – Luciferin must be injected
  – Immune response
Rapid movement of pLNCX2-B16 into draining LNs
(Luciferase + B16 melanoma cells/ luciferine imaging)

Higher number of pLNCX2-B16 in the footpad was able to introduce rapid movement into draining popliteal LN.
PC-3 M-Luc-C6 Intraprostate

Day 7

Day 14

Day 21

Day 28

Average & Median PC-3M-Luc-C6 Intraprostate

Photons/sec

Average
Median

5.00E+09
4.50E+09
4.00E+09
3.50E+09
3.00E+09
2.50E+09
2.00E+09
1.50E+09
1.00E+09
5.00E+08
0.00E+00
Comparison of Luciferin Amounts

Luciferin was given IP at 150 mg/kg
Note loss of linearity at higher tumor mass – likely due to necrotic core.
Limitations

• Luciferin does not ‘disappear’ immediately after imaging
  – Minimum “rest” time required between imaging points
• Loss of luciferase activity by transfected cell lines
  – Immunologic reactions
• Loss of tumorigenicity with transfection
• Loss of linearity with increasing tumor volume
• Low signal/cell
What is Fluorescence Imaging?

- An excitation light produces an emission light (at higher wavelength)
- Requires an excitation light
- Background is high because of autofluorescence.
Fluorescent camera (Maestro)
Examples of Optical Imaging

Liver Metastatic Model: Day 9 after Intrasplenic Injection of $10^6$ MC38-GFP Tumor Cell Line in C57BL/6 Mice
Quantitative capabilities of GFP fluorescent imaging: Change in Signal Intensity over time
Luciferase versus GFP

**Luciferase**

_Earliest Detection = Day 1_

**GFP**

_Earliest Detection = Day 7_
GFP – Earliest Detection
Day 7

Skin is a major source of Autofluorescence

Post-thresholding of Autofluorescence
Tradeoffs-Fluorescent Proteins

- **Advantages**
  - No exogenous injection
  - Does not require live cells—use in histology
  - Wide variety of colors

- **Disadvantages**
  - Less sensitive
  - Requires transfection
  - Requires external light source
  - Autofluorescence

Neither BLI nor Fluorescence proteins are translatable to humans
Exogenous Fluorophores
Optical Imaging: The “Pros”

- Sensitivity
- Portability
- Expense
- Lack of Radiation
- Real time
- Multiplexing
- Activation
Optical Imaging: The “Cons”

- Poor penetration of light in tissue
  - Surface
  - Endoscopy
  - Surgical
- Autofluorescence
Spectral Imaging

Spectral images obtained at 5-10nm increments and then unmixing of the two spectra.
Spectrally-resolved images on Maestro™

- Spectral library
- Correction for auto-fluorescence
- Unmixed images for each spectra of dye
Non-Targeted Imaging of Peritoneum

FITC

Peritoneal Implant

D-Galactose (a lectin)
Targeted Imaging of Peritoneum

Avidin-FITC

Peritoneal Implant

D-Galactose (a lectin)
Micro-cancer nodules of SHIN 3 ovarian cancer cells spread on the mesenteric membrane.

White light photo

Green and red (auto-) fluorescence composite photo

Green fluorescence photo

Avidin-FITC

Green spot: tumor nodules
Scale: 1 mm
PeT based activatable fluorophore
Exogenous dyes

- No radiation
- Low cost equipment
- Translatable

**Problems**
- Requires chemistry usually
- Autofluorescence
- Depth of imaging is limited (but good for surface tumors)
Ultrasound

• Rapid method
• No radiation
• Minimal sedation
• Limited access
  – No bone
  – No air
  – Example: pancreas
• Requires considerable user skill
US basics

Imaging dependent on the speed of sound in tissue
3D Volume: 1,231.4 mm³

Modality: Ultrasound
Transducer: 40 MHz
Axial Resolution: 40 µm

Tumor: Pancreatic ductal adenocarcinoma
Animal Model: KPC
Model Courtesy of Dr. S. Kozlov (CAPR)
Interventional procedure

MRI

• Advantages
  – Resolution
  – Coverage
  – Specificity

• Disadvantages
  – Slow
  – Expensive
  – Training
Excellent detail in abdomen
Contrast Injection (Gadolinium)
Imaging angiogenesis
Modality: MRI
Sequence: T2
Axial Resolution: 170 \( \mu m \)
Animal Model: NSG-PDX
Courtesy of Drs. J. Doroshow, P. Jacobs, J. Tatum
**Pre-Contrast Sequence: T2**
Modality: MRI
Axial Resolution: 170 µm
Animal Model: TFE3-fusion mouse model
Courtesy of Drs. W. Marston Linehan (CCR) and L. Schmidt (FNCLR)

**Post-Contrast Sequence: T1**
Contrast: Gadoterate meglumine (Dotarem)

LASP/FNLCR
Single Photon Emission Computed Tomography - SPECT

- Single Photon Emission
- Computed Tomography
Single Photon Emission CT (SPECT)
Collimation = Inefficiency

Collimation reduces the sensitivity and resolution of SPECT by rejecting the majority of events.
Uptake of $[^{99m}\text{Tc}]\text{Met}$ after 60 min in MKN-45 mouse xenografts

Tumor $\sim 2.5\% \text{ ID/g}$

Kidneys $\sim 7\% \text{ ID/g}$
Positron Emission Tomography

- Positron emission

- Electron (e-)

- 511 keV photon

- 180 degrees apart

- 511 keV photon

- Positron (e+)
PET: Advantages and Disadvantages

- Highly sensitive
- Metabolic information
- Better spatial resolution than SPECT

Disadvantages
- Expense
- Radiochemistry
- Regulatory
- Short half life
F-18 Deoxyglucose

Otto Warburg

Lou Sokoloff
FDG Metabolic PET/CT imaging
Sodium Fluoride PET
Zr-89 MetMAb in MKN-45 xenografts
Tracking of Zr89-labeled DCs transferred to a mouse by PET

(4.5 uCi/5 million cells injected i.v.)
Computed Tomography

- The workhorse of human imaging is not scaled well to mice
- Technology is improving
- MRI is generally better than CT in mice
- Problems:
  - Cannot distinguish most organs
  - High radiation dose
  - Time consuming
  - Expensive
Mouse with lung metastasis (10 weeks post MDA-MB-231 cell injection)

Healthy mouse
Summary I

- Bioluminescence is the workhorse imaging technique for rodents.
- Endogenous fluorescent proteins (e.g., GFP) are less sensitive but more stable.
- Ultrasound is an excellent technique for identifying and monitoring tumors but requires a high degree of skill.
Summary II

- MRI is the best cross sectional imaging agent.
  - Contrast agents can be used for perfusion studies
- SPECT and PET are specialized but expensive modalities reserved for particular targeted imaging probes.
- CT in rodents does not perform as well as in humans. Dose is high, Resolution low
Imaging Resources at NCI

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