Fluorescence Bioimaging in Translational Research

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Topics

• Overview of fluorescence in translational research
  ➢ Challenges
  ➢ Options
  ➢ Opportunities
• Images
• What lies ahead?
Fluorescence in Bioimaging

• Advantages
  - Multiplexing
  - Chemistry
  - Resolution
  - Speed

• Disadvantages
  - Chemistry
  - Translatability
Bioimaging in Translational Research

- Fluorescence microscopy
- Flow cytometry
- High throughput screening
- High content screening
- Animal models
Fluorescence In Vivo

- Introduced fluorescence
  - Dyes and probes
  - Transformed cells
  - Labeled cells
- Inherent fluorescence
  - Food origin
  - Autofluorescence
Dyes and Probes

• Adapted from microscopy
  ➢ FITC, Alexa Fluors, ICG

• Newer technologies
  ➢ Nanocrystals, Q dots

• Functional probes
  ➢ FRET pairs
  ➢ Protein complementation
  ➢ “Smart” probes
Challenges of Imaging Fluorescence In Vivo

• Autofluorescence
  ➢ Gut and fur
    ➢ Solutions: Alfalfa-free chow, shave and use depilatory, spectral unmixing, wavelength

ex 460-490nm  
em 510-550nm

ex 595-635nm  
em 650-700nm
Challenges of Imaging Fluorescence In Vivo

- Near Infrared (NIR)
  - Less scattering through tissue
  - Avoids absorption due to tissue Hb
  - Avoids autofluorescence

Weissleder, Nature Biotechnology(2001) 19,316
Challenges of Imaging Fluorescence In Vivo

(a) White light

(b) ex 460-500 em 505-560

(c) ex 525-555 em 590-650

(d) ex 725-775 em 790-830

Challenges of Imaging Fluorescence In Vivo

- Autofluorescence
- Physics of light
- Invasive or Non-invasive
Challenges of Imaging Fluorescence In Vivo

dorsal skin fold

or skin flap
Olympus In Vivo Imaging Systems

- **IV100**
  - minimally invasive intravital fluorescence microscopy
  - laser scanning with tilting scan head
  - 3 PMTs with filter detection
  - MicroProbe objective lenses

- **OV110**
  - single cell to whole animal fluorescence – Macro to micro
  - arc lamp light source
  - CCD and bandpass filter detection
  - 4 lenses for 114-fold range of magnification
Olympus IV100
Olympus OV110
Mouse Models

- Mimic human disease states
- Highly characterized
- Specificity conferred to genetic strain, molecule expression, and pathway function
  - Fluorescent fusion proteins
- Advances in knock-out, knock-in and transgenic approaches
- Relative low cost of maintenance
- Rapid reproduction (~9 weeks)
IV100: Vascular leakage in reperfusion injury

MicroProbe lens inserted through incision in jejunum, apposed to the mucosal surface of colonic villus labeled with Rhodamine 6 (green), blood pool with Angiosense 680 (red)

Courtesy of R. Weissleder, MGH/Harvard
IV100: CNS Injury

GFP expressed in axon (green)

Tissue macrophages autofluorescence (red)
OV110: Bone Remodeling
OV110: Cardiology
IV100: Biomarker “typing” of colon adenoma

Green: Mucosa
Red: Protease activity
Blue: Blood pool

Courtesy of R. Weissleder, MGH/Harvard
IV100: Biomarker "typing" of colon adenoma
IV100: Tumor vasculature
OV110: Cardiology
OV110: Vascular imaging
OV110: Immunology

FITC-labeled T-cells tracking in lymph node

Courtesy of R. Germaine, NIAID
OV110: Tumor cells in circulation

Courtesy of AntiCancer, Inc.
OV110: Tumor cell extravasation

A

B

C

D

E

F

15hr

27hr

Courtesy of AntiCancer, Inc.
OV110: Protease activity in tumor

Cathepsin B
IV100: Inflammation

Tissue: Kidney
Green: Endothelial cells
Red: Blood pool

Courtesy of R. Weissleder, MGH/Harvard
What lies ahead?

- Imaging drug efficacy
- Diagnostics
- Drug delivery/disposition
- Photo therapy
- Nano-surgery
Thank you!
SHOW TIME !