Multimodal Preclinical Imaging for Translational Research

Joshua McHattan
Product Line Manager – Asia Cluster
Agenda

• Carestream Overview
• Introducing Molecular Imaging & Xtreme system
  • Multimodal Applications

• Development of a Novel Molecular Imaging Probe
  • Targeting Site of Infection
  • Tumor Imaging of Necrotic Core
  • Brain Trauma Inflammation & Cell Death Detection

• Conclusion
What We Do

A world leader in:

• Medical imaging

• Dental imaging and dental practice management software

• Molecular imaging
  – The most extensive portfolio on the market

• Healthcare information solutions (RIS & PACS)
  – More than 1,100 healthcare information management solutions currently installed worldwide (PACS, RIS, data management)

• Digital output solutions
  – More than 50,000 KODAK DRYVIEW laser imagers on the market worldwide

• All categories of Kodak film
Complete Portfolio for In-Vitro and In-Vivo Pre-clinical Imaging
How It All Ties Together

Genetic Influences
- Developmental
- Aging
- Diet
- Environmental
- Pharmaceutical

Genes
- DNA, RNA, Protein

Proteins
- Cells
- Tissue & Systems

Function
- Animal Models
- Patient

Health endpoints
- Osteoporosis
- Diabetes
- Cancer
- Etc.

Information and Image Capture, Storage and Analysis (RIS & PACS)
Why Whole Animal Molecular Imaging?

- Detect & Monitor Disease Models over time
- Monitor Drug Therapies Within the Same Model
- Biodistribution
- PK/PD
- Probe Development
- Fast, Efficient & Cost Reduction
- Proof of Principle Within a True Hostile Environment
Molecular Imaging in Disease Detection

- Injection of probes
- Red blood cell
- White blood cell
- Labeled probes
- Blood vessel

Circulation

Image capture

- Accumulate at disease site
- Imaged
- Disease site identified

Injection of probes

- Disease detection and diagnosis
- Disease response to therapy
- Guided surgery
An Unparalleled In-Vivo Product Offering for Small Animal Imaging

7 Powerful Imaging Modalities 2 Instruments

1. Fluorescence
2. Luminescence
3. Radioisotopic
4. Radiographic (X-Ray)
5. Single Photon Emission Computed Tomography (SPECT)
6. Positron Emission Tomography (PET)
7. Computed Tomography (CT)
Fused images your way with Multimodal Animal Transport System

Transport animals between Optical, PET, SPECT, CT and MRI imaging systems
Osteometabolic local activity

Osteosense750 Fluorescence with Animal Rotation

PET/CT with F18-Na

SPECT/CT with Tc-99 MDP
Fluorescence: Spectral Unmixing of dsRed
In vivo siRNA Delivery & Silencing in Tumors

MARS OsteoSense 750: Bone Remodeling
40min Dynamic Drug Delivery
Luminescent Prostate Cells

Visipaque Enhanced X-Ray  Prostate Signal  Co-registration of prostate Signal to below the bladder

Images Courtesy of Prof. Bob Handa, Arizona State Univ.
Three Day Time Lapse – Dual Modality

GFP
Pseudomonas

Luminescent
Salmonella

Morris, J.D.; Kamatkar, N. G.; Chapman, S. C.; Diener, J. M.; Courtney, A. J.; Leevy W. M.; Shrout, J. D. 
AEM, 2011, 77(23), 8310-8317.
In Vivo Radioisotopic Systems

$^{99m}\text{Tc}$ MAA uptake in lung

$^{125}\text{I}$ Uptake in Thyroid

$^{18}\text{F}$ FDG Uptake in heart and bladder

Radiolabeled Compounds Incorporating:

$^{18}\text{F}$, $^{111}\text{In}$, $^{99}\text{Tm}$, $^{125}\text{I}$, $^{64}\text{Cu}$, $^{32}\text{P}$

$^{111}\text{In}$ Biodistribution

$^{125}\text{I}$ Antibody Tumor Targeting
High Resolution Digital X-Ray

Anatomical Imaging

Bone Density Profiling

Dynamic X-ray Imaging
X-Ray Vascular Imaging Using MARS
• How Does it Work?
Inside In-Vivo Xtreme

X-ray Source
• High flux 500 uA X-ray head
• 45 kVp energy optimized for small animal imaging
• True microfocus X-ray head < 60 um spot size
• Geometric magnification stage
Inside In-Vivo Xtreme

Animal Management Zone

• Industry leading ultrathin, ultrauniform radiographic phosphor screen
• High sensitivity radio-isotopic screen
• Bottom up image imaging geometry prevents shadowing of excitation light from one animal onto the next, improves image quality by providing flat uniform focal plane
• Light tight ports
  – Catheter injection
  – Isoflurane anesthesia delivery
• MARS compatible
• Large 20 cm x 20 cm FOV
• Remote switching between imaging modalities, the animal never moves
Inside In-Vivo Xtreme

Camera and Optics

• Field serviceable and upgradeable camera
• Large fast f/1.1 lens coupled to CCD, largest sensor in class
• Choice of two cameras
  – High sensitivity, back-illuminated 4MP camera
  – High resolution, front-illuminated 16MP camera
• 6 patented wide angle emission filters standard
• 6 fixed FOVs with automated combination of vertical camera motion
• Cooled -60 degrees absolute
Inside In-Vivo Xtreme

**Excitation Source**

- Powerful 400W Xenon illuminator
- 28 excitation filters
- Image from the visible to the NIR
- Separate fluorophores using multiplex acquisition or excitation based spectral deconvolution
Easy to Use Standard User Interface

Select Modality…
Settings filtered by modality

Select Background Image

Single Click Protocol Execution

Click Expose…
Acquires functional image
Anatomical Background Image
Automatic Co-registration and Overlay

Image Station HT

File

Protocol: No Saved Protocols
Execute…

Modality: Fluorescence

Setting: Current Session
Save
Save As…

Annotation:

Background Image: Standard X-Ray

Camera Control

Exposure Time: 5.000 Sec.

FOV: 6.6 10 12 15 18 20

fStop: 1.1 1.4 2.0 2.8 4.0 5.6 8.0 11 16

Bin: 1x1 Pixels

Excitation: 620

Emission: 535

Advanced…
Preview…
Expose…

TCP: Auto Select
Serial No: 0
CCD Temp:
Automatic Co-registration
Easy Quantification Including Ratiometric Analysis
Easy Quantification including Ratiometric Analysis

19X background in less than 5 sec in NIR!
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Results from Notre Dame Integrated Imaging Facility (NDIIF) in collaboration with Prof. Bradley Smith, Department of Chemistry and Biochemistry, University of Notre Dame, Indiana, USA
Probes for Molecular Imaging

Reporter

Linker

Affinity Group

DPA = dipicolylamine

DPA-Cy7

Affinity for anionic lipids

DPA = dipicolylamine
Absorbance Properties of Tissue

\[
\varepsilon \text{ (mM}^{-1}\text{cm}^{-1})
\]

\[
\alpha \text{ (cm}^{-1})
\]

\[\begin{align*}
\lambda \text{ (nm)}
\end{align*}\]
Microscopy: *S. aureus* with DPA-Cy7

*S. aureus*
Experimental Setup: *In Vivo* Targeting

IM injection of *S. aureus*
Experimental Setup

Intravenous injection of DPA-Cy7
In Vivo Imaging of Bacterial Infection

This montage was prepared using ImageJ (free imaging software from NIH)

Excitation 755 nm, Emission 830 nm, No binning, 60 s acq, Fstop = 2.4

Region of Interest (ROI) Analysis

21 h

“Target” (T)

“Liver” (L)

“Non-Target” (NT)

Whole Mouse
Fluorescence Quantitation: T/NT and T/L Ratios

T/NT = Target to Non-Target Ratio
T/L = Target to Liver Ratio
Probe Pharmacokinetics

![Graph showing pharmacokinetic data for different tissues over time.](image)
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<th>3</th>
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<td>Heart/Lungs</td>
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<td>Left/Right Leg</td>
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Ex Vivo Quantitation

![Bar graph showing mean fluorescence (a.u.) for different organs in both control and infected and treated conditions.](image-url)
Multi-Modal Labeling

Probe is now translatable into PET/SPECT imaging – Future goal to move into a clinical environment
Evaluation of Novel Imaging Agent

• PET infectious model left leg of a mouse

• 95 uCi $^{64}$Cu labeled imaging agent

• Imaged 3 hrs post-injection

• 10 min PET scan
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Targeting Tumor Necrotic Core Post Taxol Treatment

Collaboration with Prof S. Achilefu at Washington University in St Louis, School of Medicine

Using a PS Sensor to Detect Necrotic Tissue Within Tumors

PSS-794 is now commercially available from MTTI
Histological Analysis of Tumor Sections
Imaging Cell Death Using Labeled Annexin V

- Living Cell
  - PS actively confined to inner membrane surface
  - Annexin A5
  - Tracer
- Apoptosis
  - Auto phagy
  - Early Necrosis
  - Mitotic Catastrophe
- Cell Death
- Nuclear imaging
- MRI imaging
- Optical imaging
- Ultrasound imaging

Final stage of apoptosis

Cancer Research Reviews
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Brain (Frontal Cortex) Cryolesion Protocol

- Frontal Lobe cryolesion induced
- 1 hour later, PSVue 794 targeted probe injected IV
- 4 hours after injection, mice received 5 mg luminol injected IP
- X-ray, Near-infrared fluorescence, luminescence and RGB reflectance (not shown) images taken
- Imaging repeated at 20 hours post cryolesion induction
Brain (Frontal Cortex) Cryolesion - Xtreme

X-ray

Luminescence (Luminol)

Fluorescence PSVue 794

Control  Cryo 1  Cryo 2  Control  Cryo 1  Cryo 2  Control  Cryo 1  Cryo 2

5 hr post injury
Brain (Frontal Cortex) Cryolesion – 5 Hour Time Point
Brain (Frontal Cortex) Cryolesion

X-ray

Luminescence (Luminol)

Fluorescence PSVue 794

Control Cryo 1 Cryo 2

Control Cryo 1 Cryo 2

Control Cryo 1 Cryo 2

20 hr post injury
Brain Cryolesion/ Co-registration
Traumatic Brain Injury Model

Treatment at 24 hr post TBI challenge:
→ Test rat: 3 mg/kg PSVue 794 IV
→ Neg. control rat: saline IV

Images courtesy of Dr. Nae Dun, Temple University
At 24 hr post NIR probe IV injection (48 hr post TBI challenge):

Peak NIR probe observed in TBI-region, as well as in several major organs: kidneys, lung, liver, pancreas, and intestinal track.

Images courtesy of Dr. Nae Dun, Temple University
Conclusion

• Whole animal imaging is a very useful tool for research development

• The In Vivo Xtreme system provides a powerful multimodal solution for molecular imaging
  – Fluorescence
  – Luminescence
  – Radioisotopic
  – High-resolution digital x-ray

• With up to 7 imaging modalities you have the freedom to combine many different molecular and anatomical imaging experiments

• Novel probes to detect disease can be easily assessed for effectiveness
Thank you!

Although the Carestream In-Vivo Xtreme can be used for *in vivo* and *in vitro* molecular imaging of materials, researchers should be aware that the methods of preparing and viewing the materials for molecular imaging may be subject to various patent rights.

Contacts:

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