US Postal Stamp,
issued 2001

Non-invasive Imaging to Assess Transplanted Islets

Alvin C. Powers
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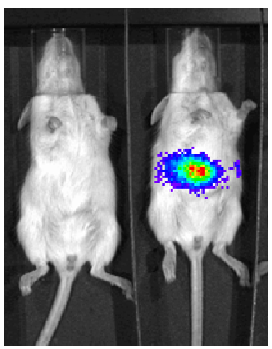
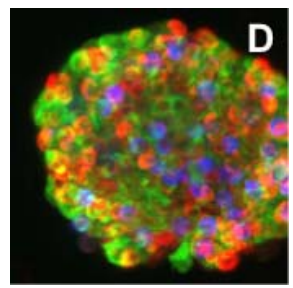
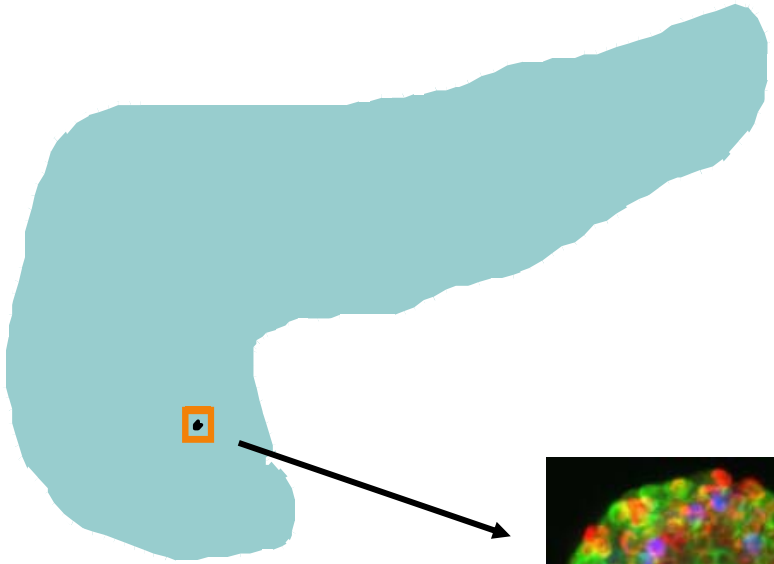




Today

- **Rationale and challenges for imaging pancreatic islets**
- **Overview of imaging modalities being used**
- **Bioluminescence to assess transplanted pancreatic islets**

Pancreatic Islets Are an Imaging Challenge



Pancreas

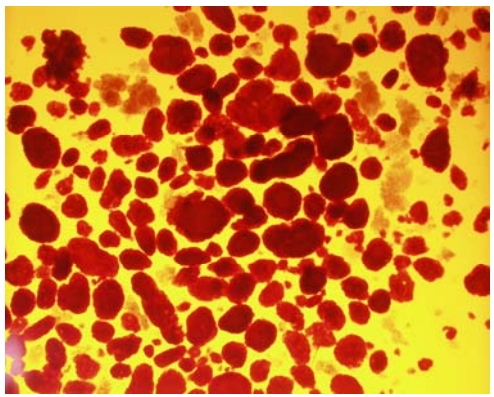
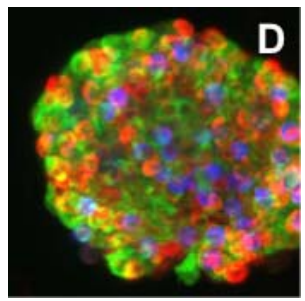


Photo courtesy of David Harlan

Purified Human Islets



Human Islet

α cells β cells δ cells

Islets size (≤ 250 μm) is less than resolution of imaging modalities (CT, MRI)

Non-Immune Barriers to Improving Islet Transplantation

- **Transplanted, intra-hepatic islets are relatively inaccessible**
- **Techniques to non-invasively assess or image islet mass are not available.**
- **Difficult to study islet survival following transplantation (must rely on islet function).**
- **Cannot assess interventions to sustain or improve islet survival after transplantation**

Features of Ideal Islet Imaging Modality

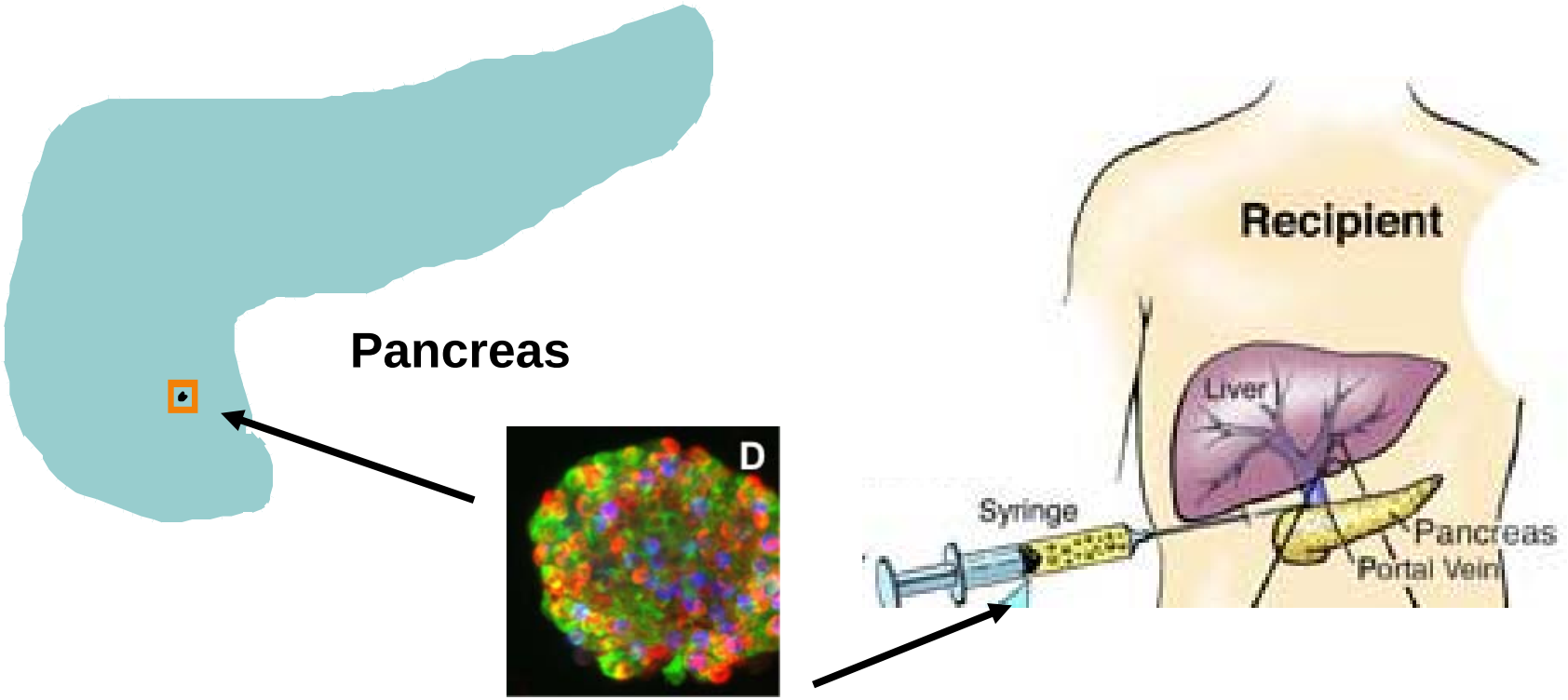
- Non-invasive and allow for serial, in vivo measurements in the same animal or person
- Non-toxic to islet cells
- Useful for study of islets in native pancreas and after transplantation
- Applicable to murine models
- Adaptable for human imaging

So what do you want to know from islet imaging?

- **Should it measure islet cell mass or number? Should it reflect beta cell number of islet cell number?**
- **Should it reflect function or health of beta cells? The metabolic state and insulin secretory capacity of the beta cell will fluctuate in different physiologic and pathophysiologic conditions.**
- **Is spatial resolution of islets important?**

Islet mass ≠ Islet function

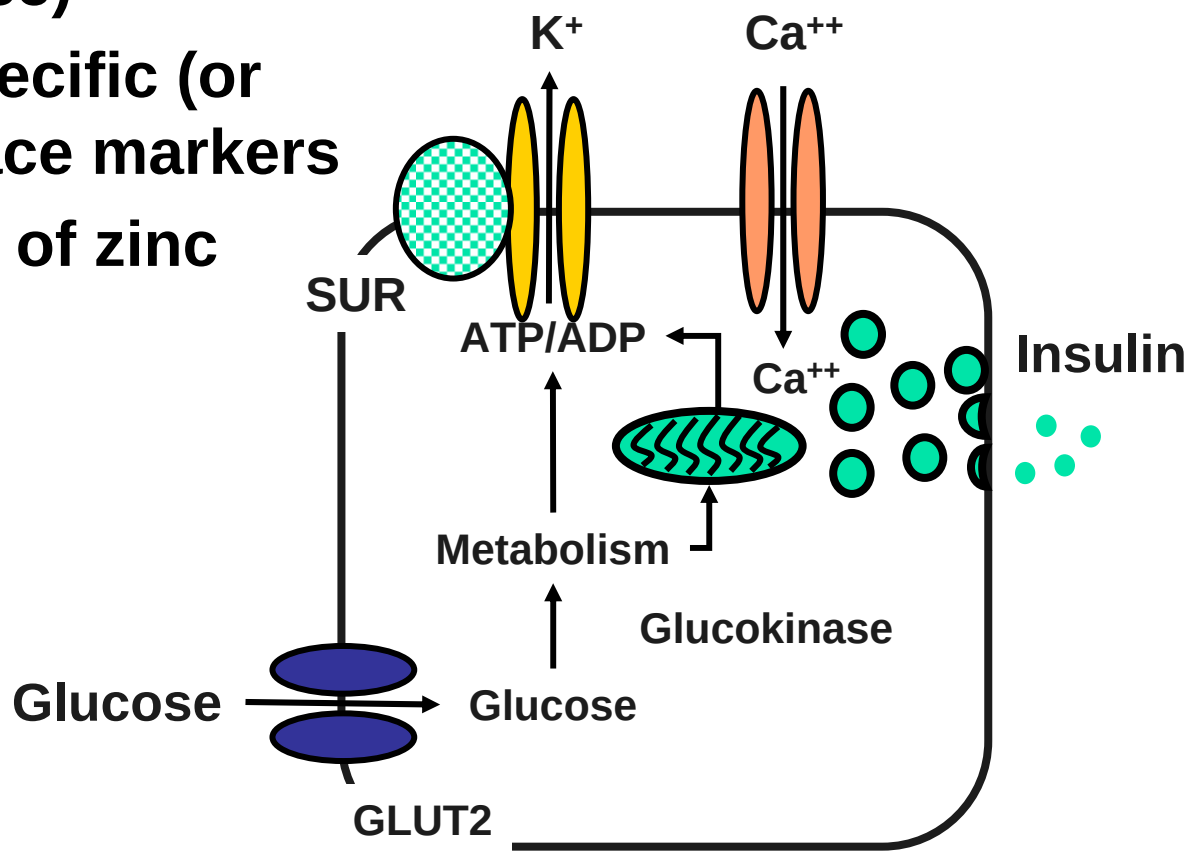
Approaches to Image Pancreatic Islets



- Utilize an islet-specific protein or process
- Introduce a reporter into islet cells

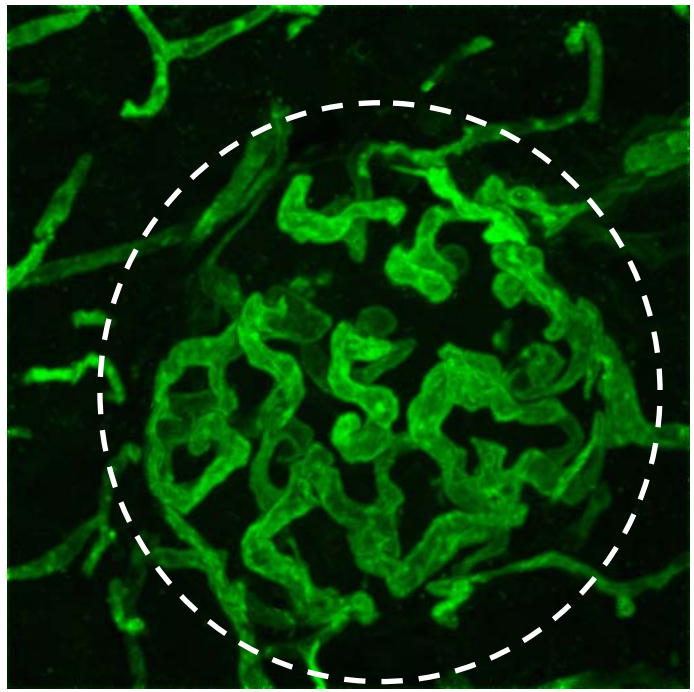
Features/Approaches That Could be Useful for Imaging

- Unique glucose metabolism (GLUT2, glucokinase)
- Islet or beta cell-specific (or enriched) cell surface markers
- High concentration of zinc



Features/Approaches That Could be Useful for Imaging

- Unique glucose metabolism (GLUT2, glucokinase)
- Islet or beta cell-specific (or enriched) cell surface markers
- High concentration of zinc
- In pancreas, islets are highly vascularized with a fenestrated endothelium
- MRI
- PET
- **Bioluminescence Imaging**



Islet in Mouse Pancreas

Non-invasive Assessment of Islets Using Bioluminescence

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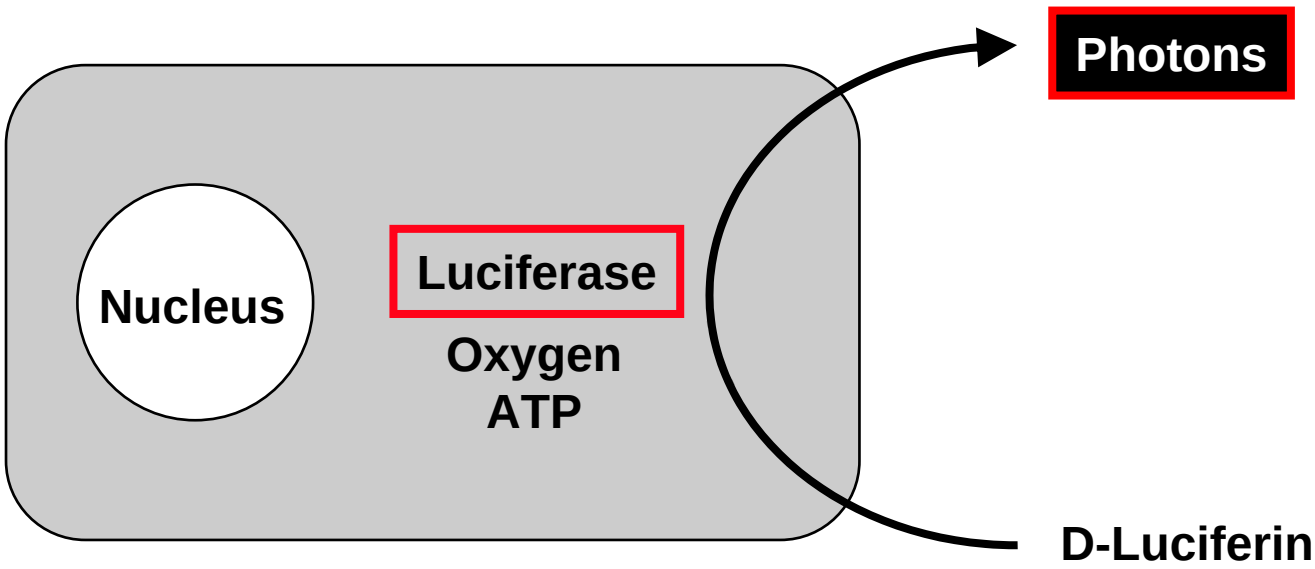
Bioluminescence Reaction

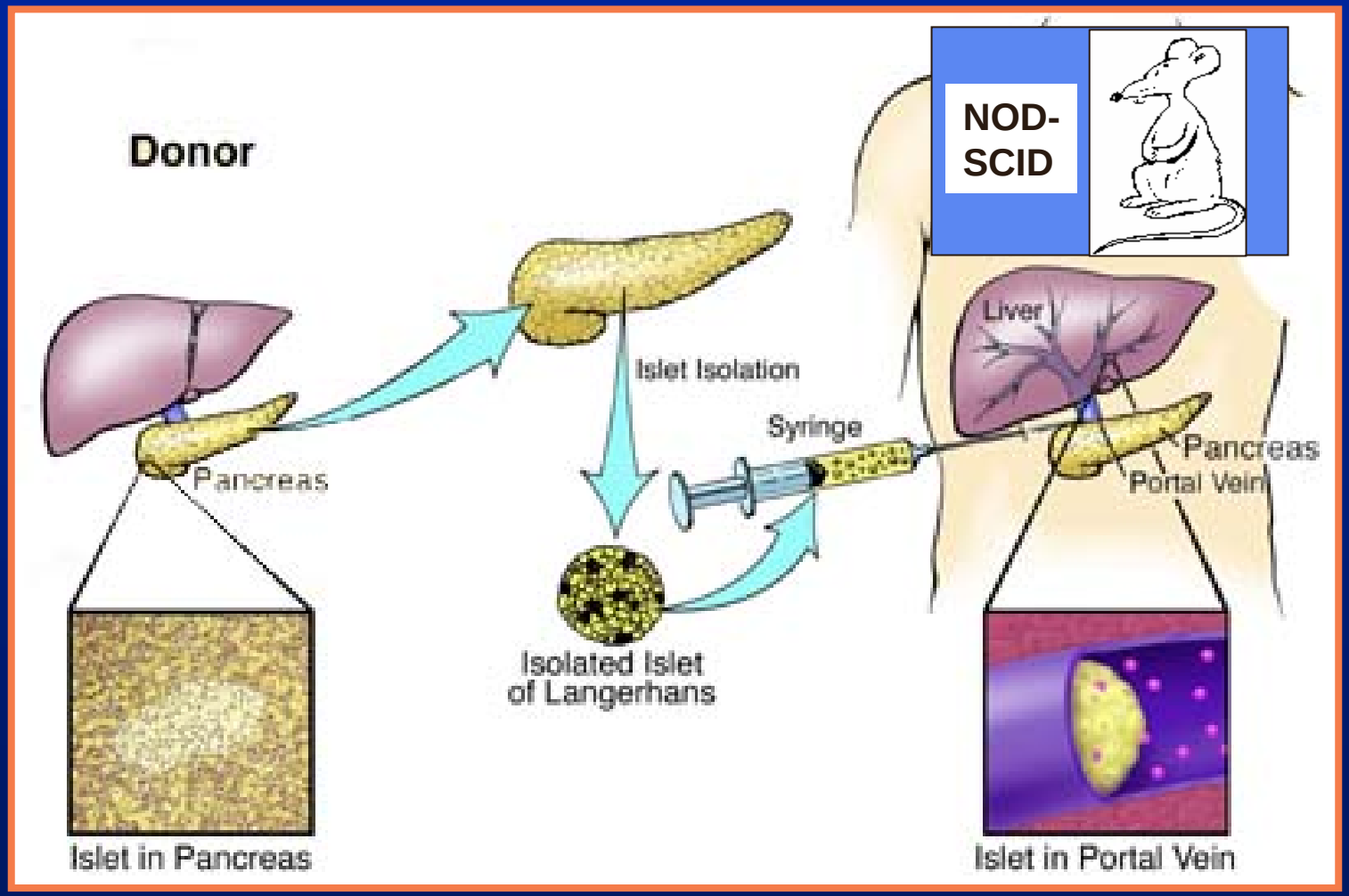
Luciferase + Luciferin + ATP + O_2 ⟶ Luciferase-Luciferin + AMP + PP_i

Luciferase-Luciferin + AMP + O_2 ⟶ Oxyluciferin* + CO_2 + AMP

Oxyluciferin* ⟶ Oxyluciferin + hν



Islet Transplantation Model

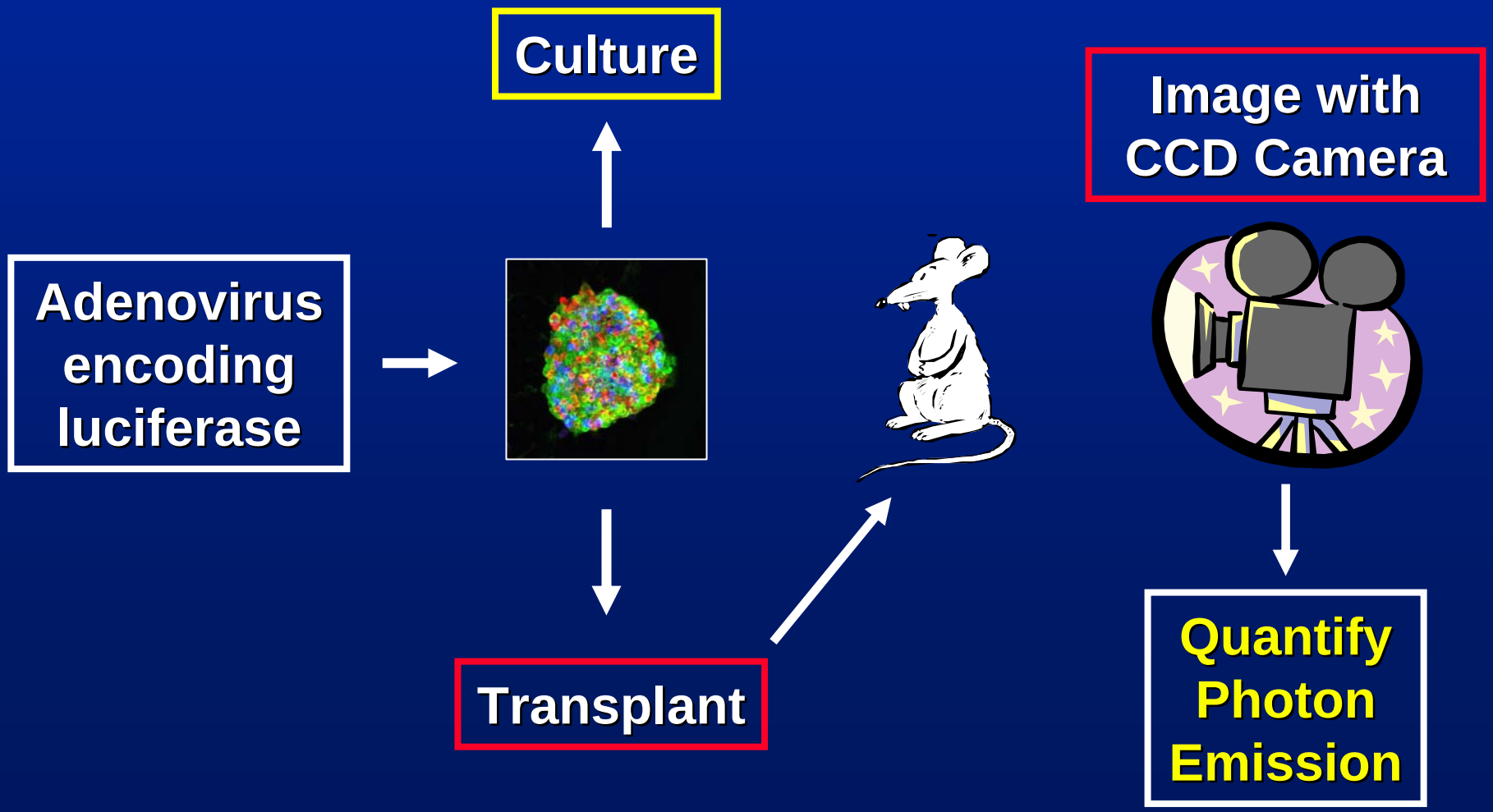


Adapted from JDRF figure

NOD-SCID Mouse Model

- Lack B- and T-Lymphocytes
- NOD background further reduces immunity because of NK cell deficiency
- Accept xenografts
- Do not develop insulitis or diabetes
- Allow long-term expression of adenoviral DNA
- Species-specific insulin assay to distinguish human insulin from endogenous mouse insulin

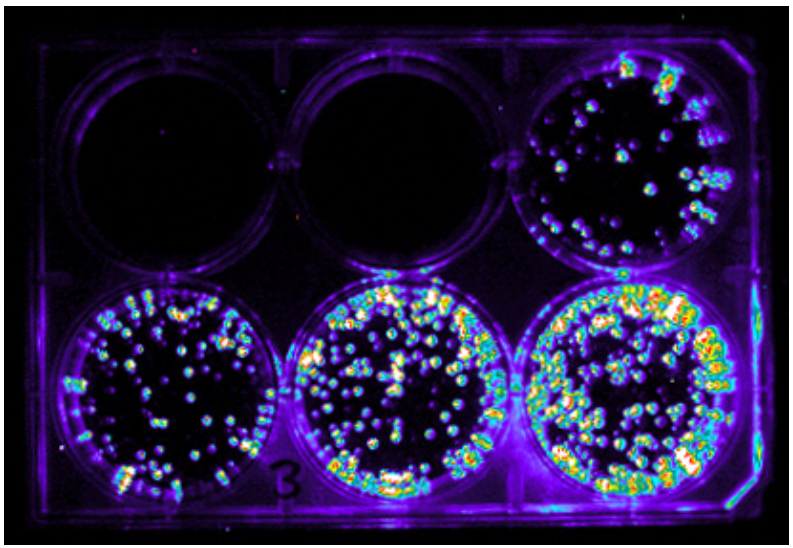
Bioluminescence of Transplanted Islets



In vitro Bioluminescence in Human islets

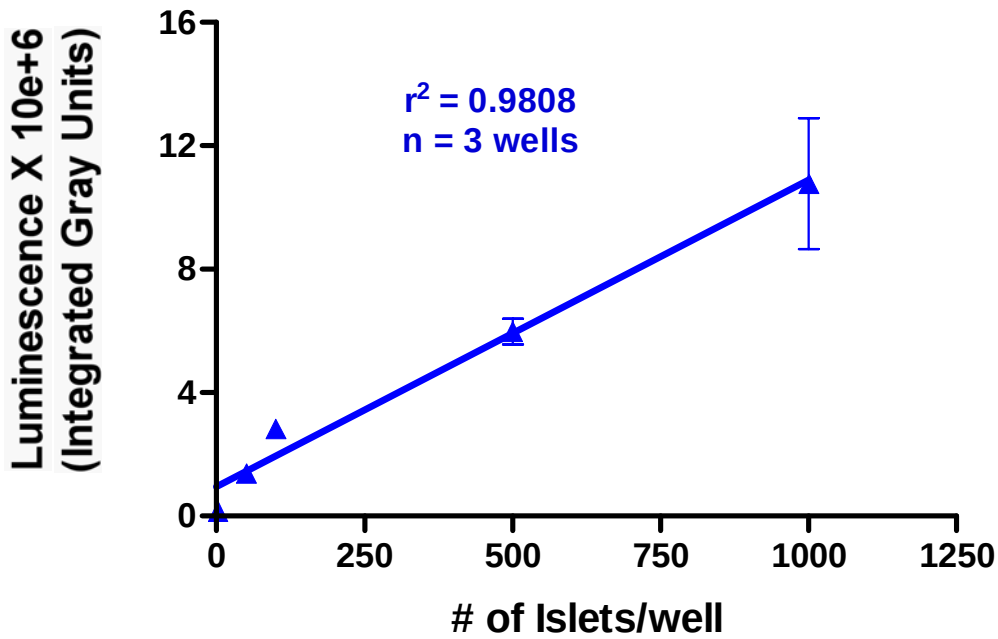
A. Islets (IEQ)/well

0 1000 50
(no virus)



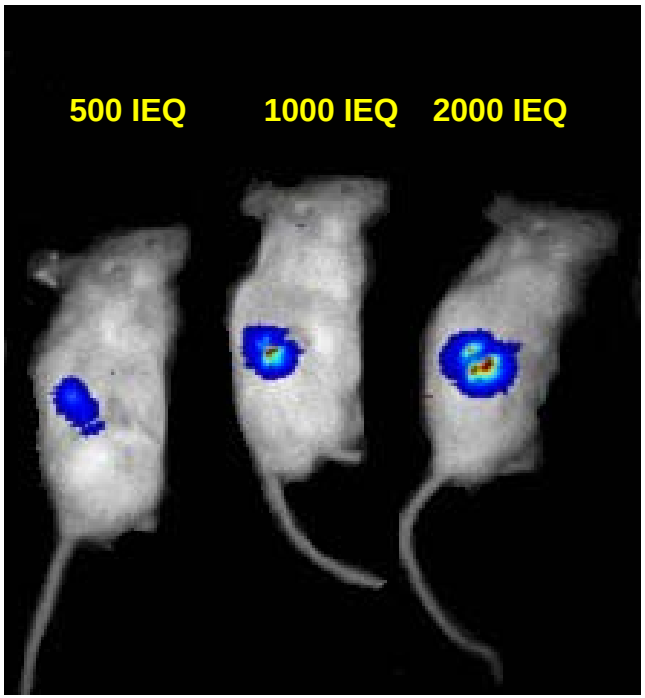
100 500 1000

B.

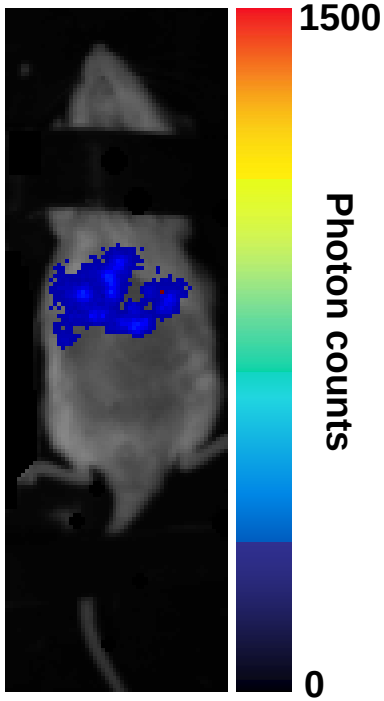


Imaging Transplanted Islets

Kidney Capsule (human islets)



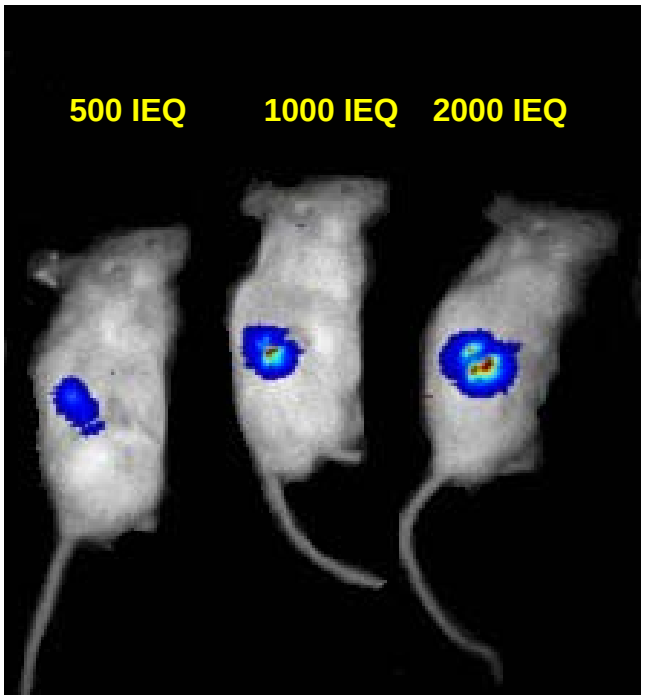
Liver (mouse islets)



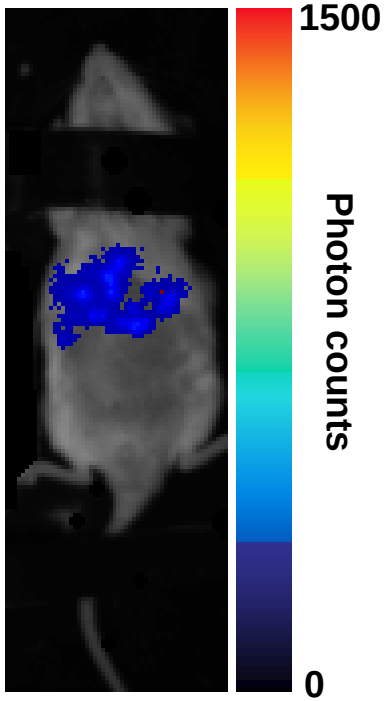
Luminescent Rod

Imaging Transplanted Islets

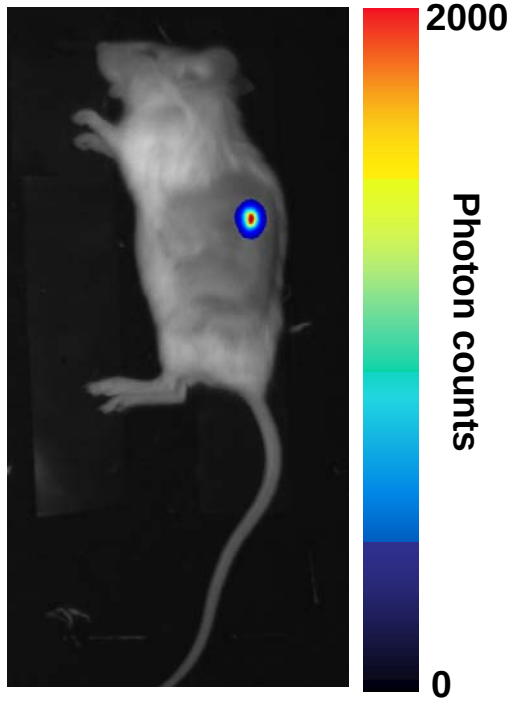
Kidney Capsule (human islets)



Liver (mouse islets)

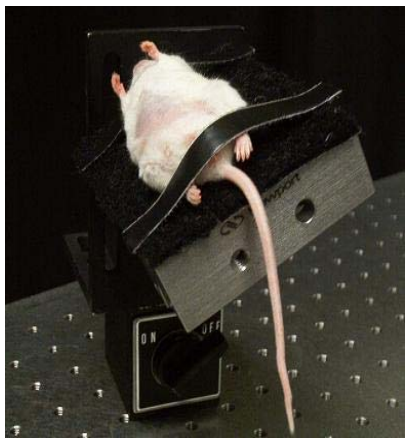


Kidney Capsule (luminescent rod)



Standardization of Imaging Using Luminescent Bead

A



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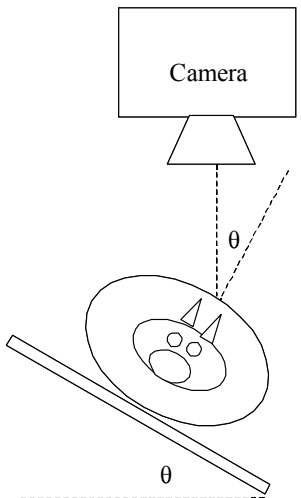

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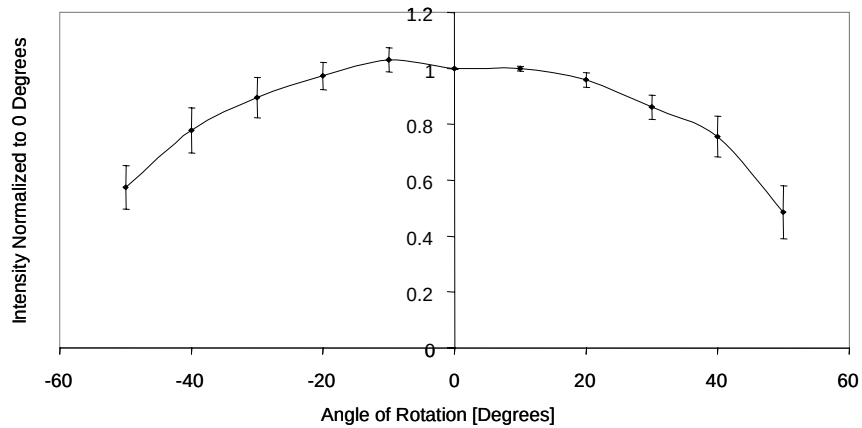

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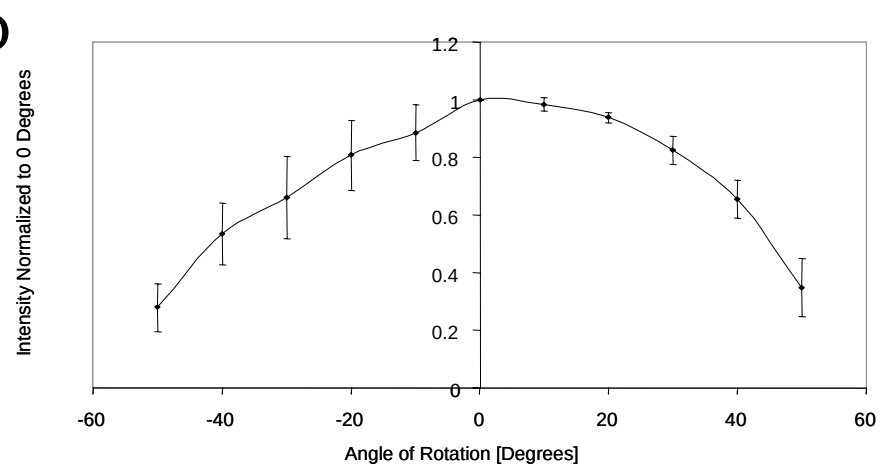
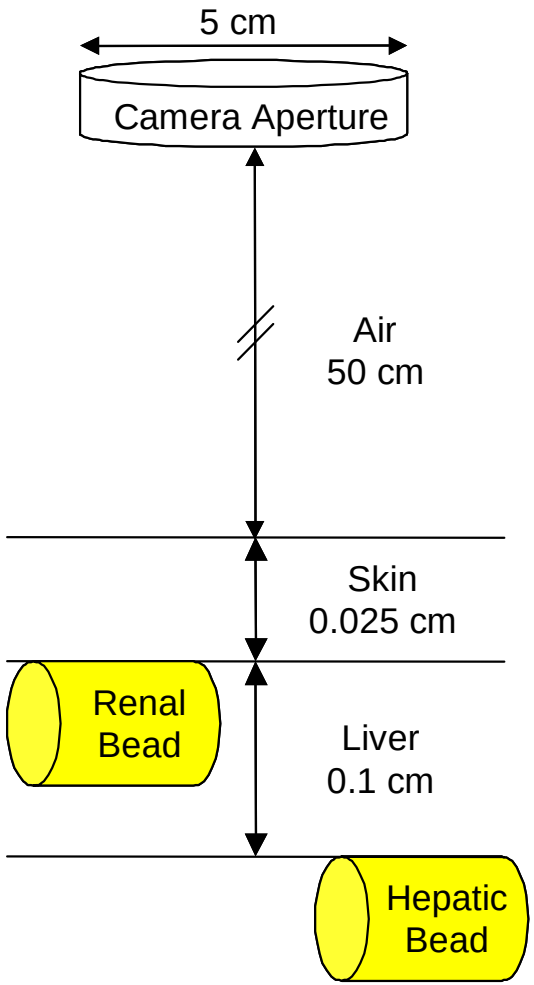


Absorption of Photons by Surrounding Tissues



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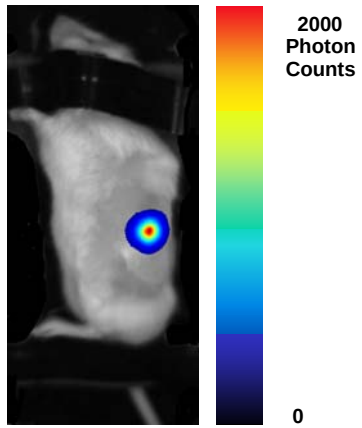

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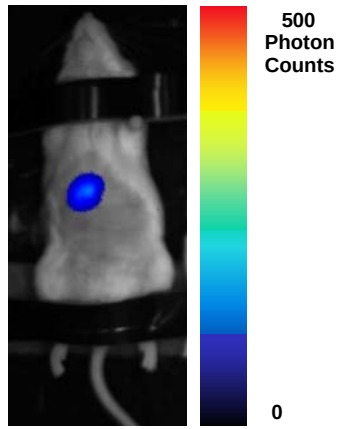

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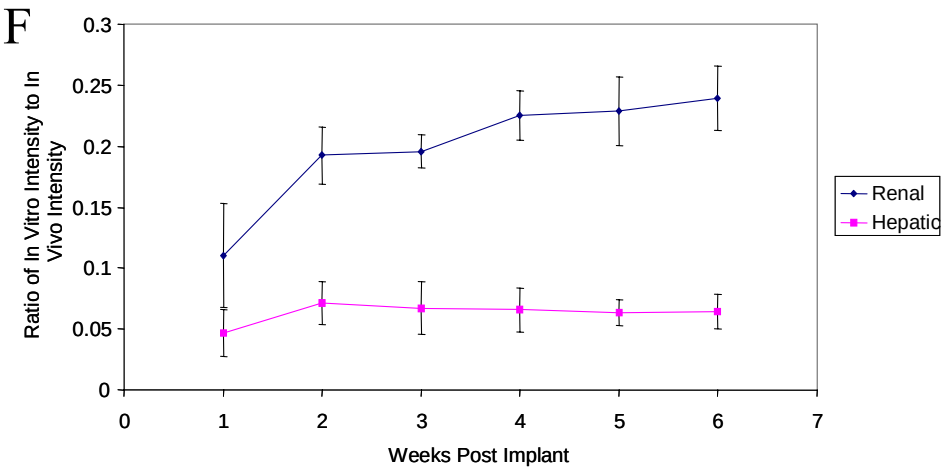
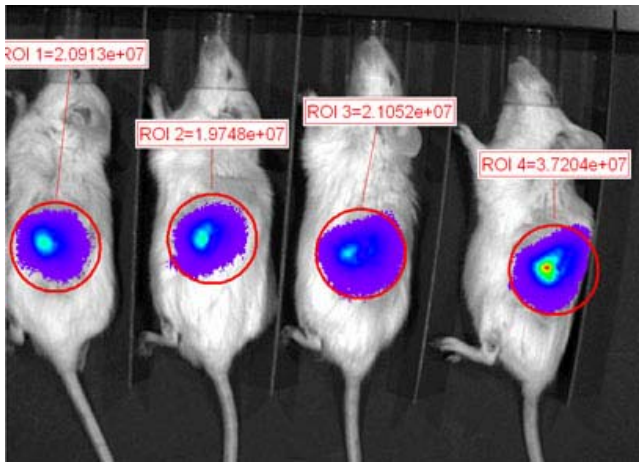
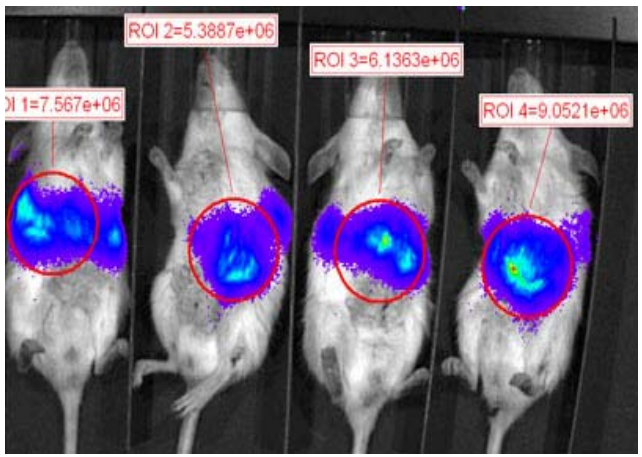


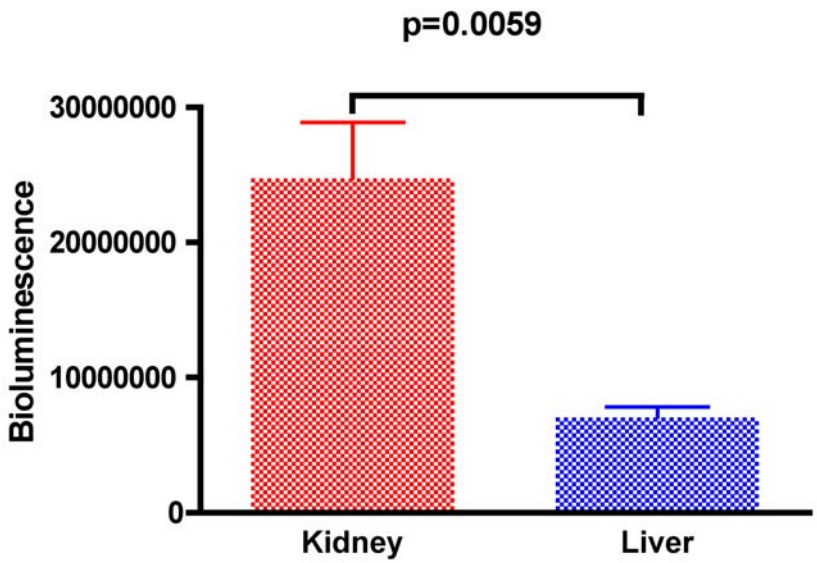
Bioluminescence of Islets in Kidney or Liver

Kidney



Liver



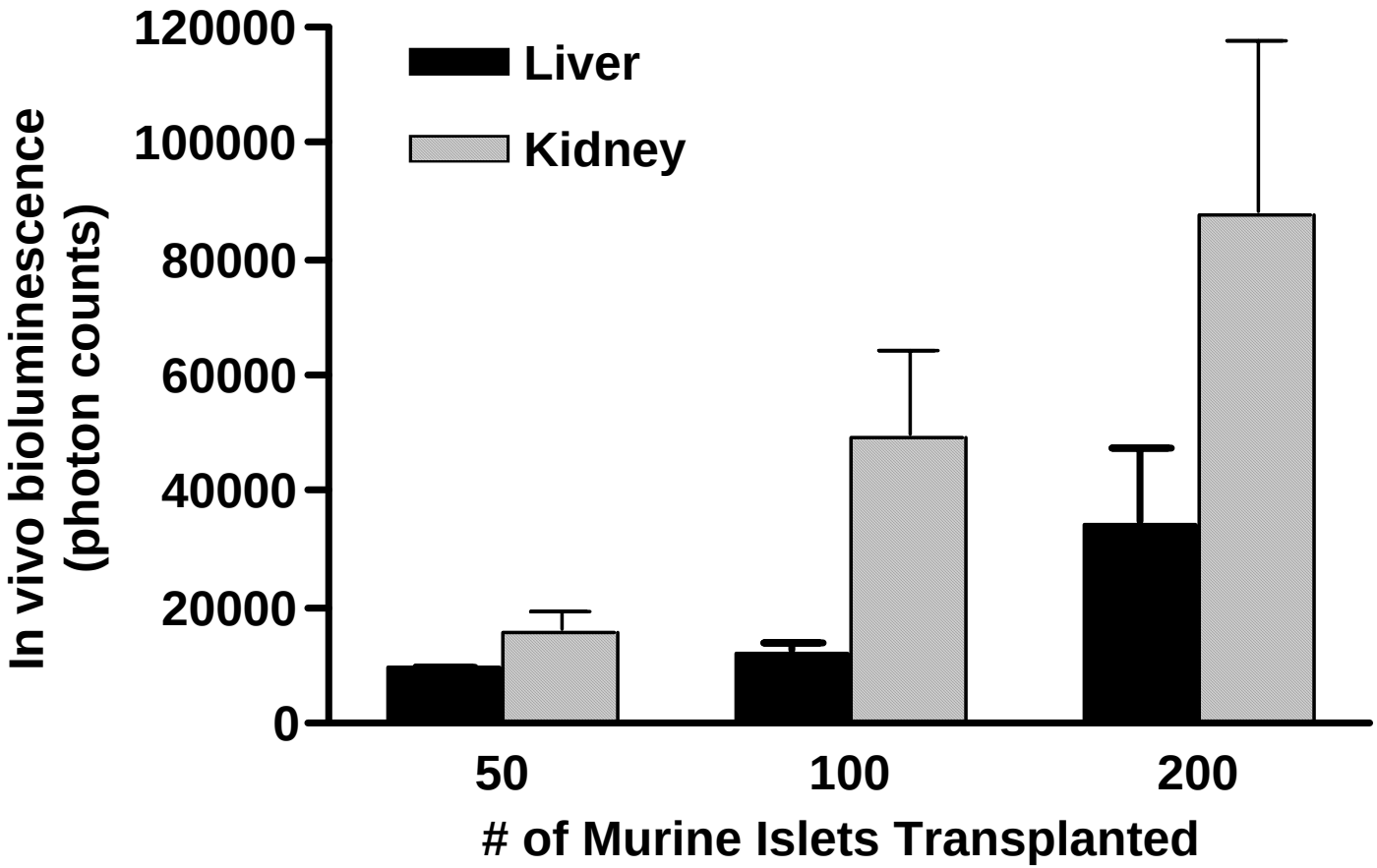


- **200 mouse islets**
- **Imaged two weeks post-transplant**

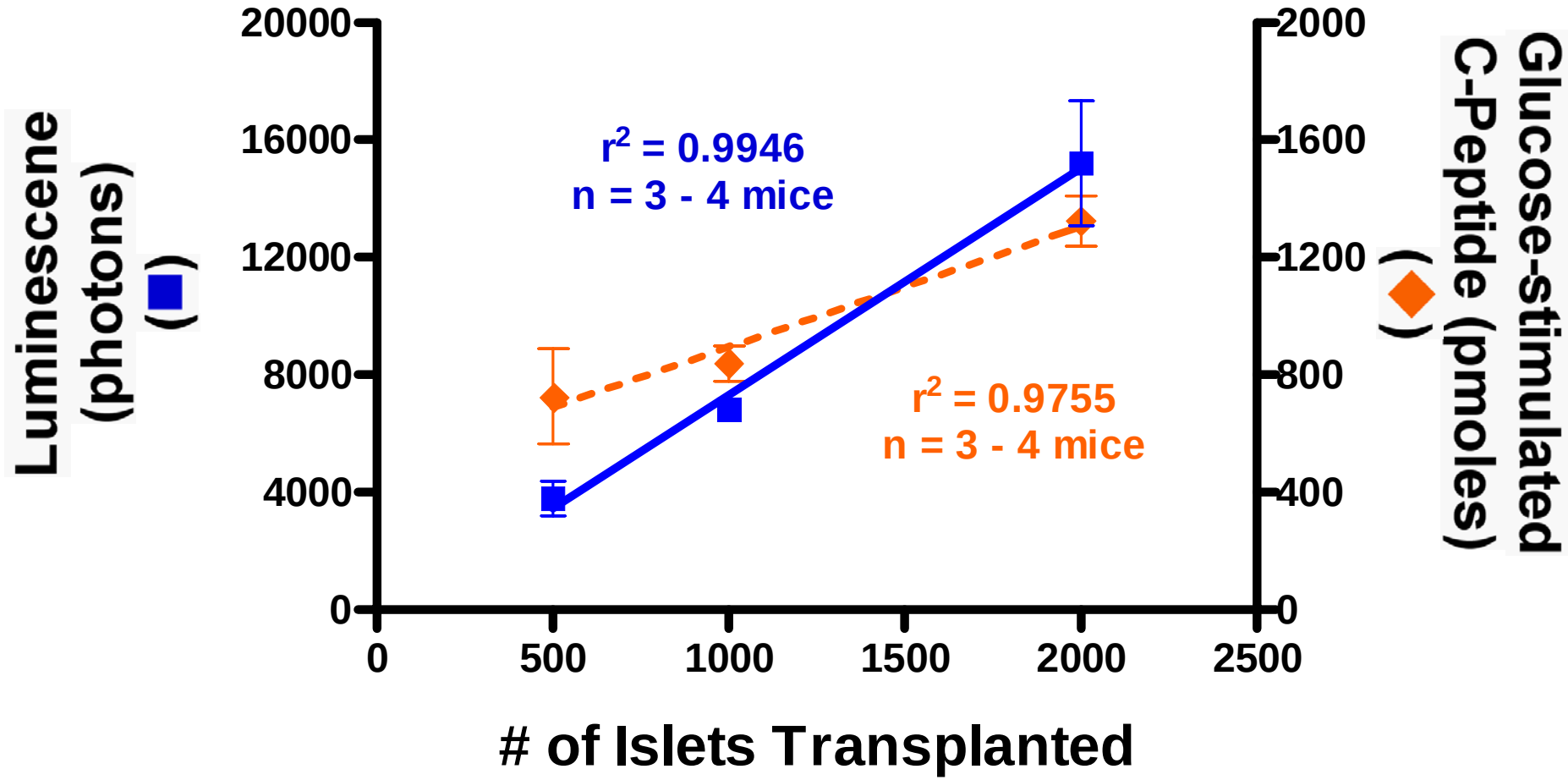
Bioluminescence is Influenced by Site of Transplantation

	Renal Intensity (in vitro/in vivo)	Hepatic Intensity (in vitro/in vivo)
Bead	0.2384 ± 0.0261	0.0645 ± 0.0140
100 islets	0.0476	0.0116
200 islets	0.0284	0.0112

Bioluminescence of Transplanted Murine Islets



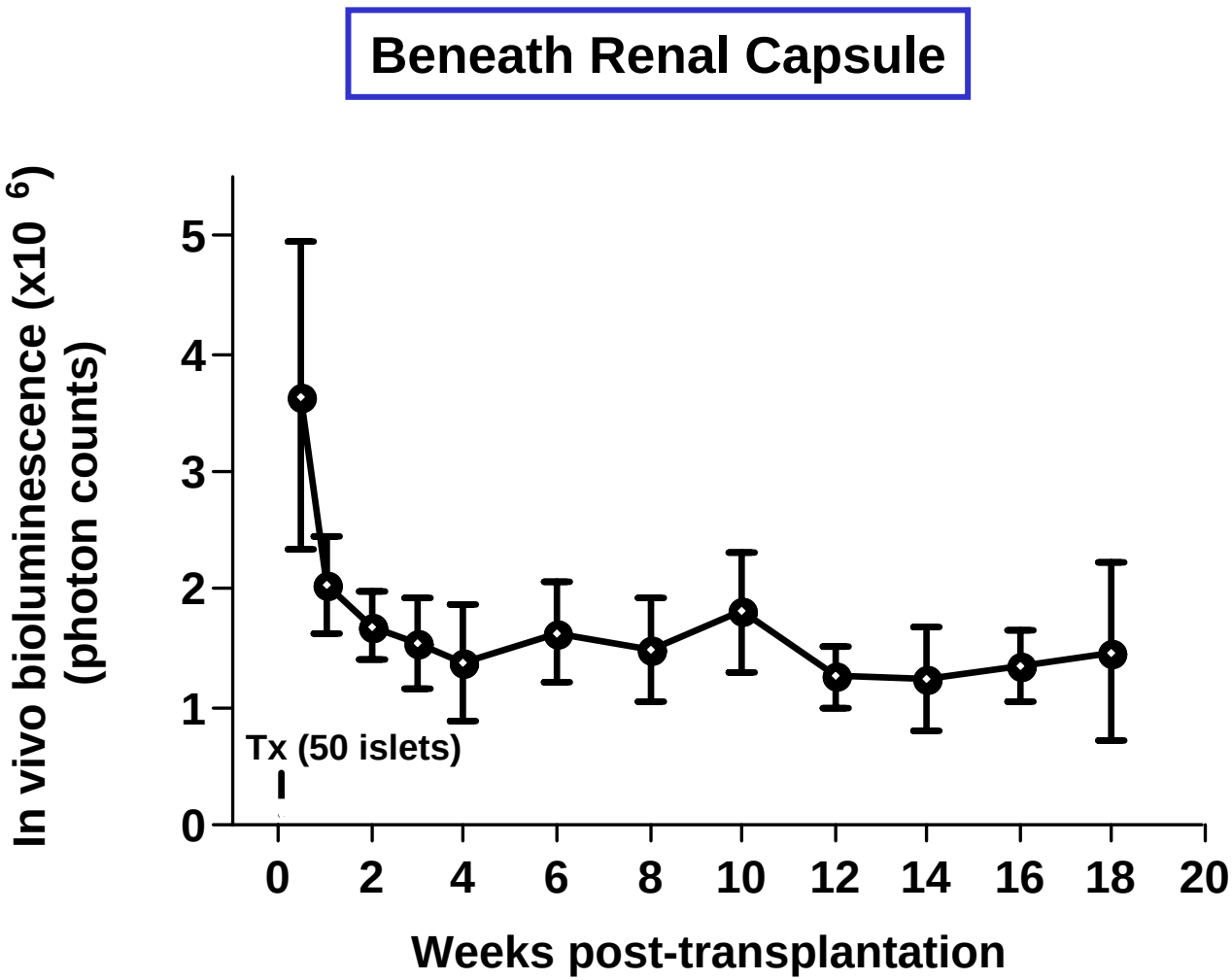
Transplanted Human Islet Number and Luminescence



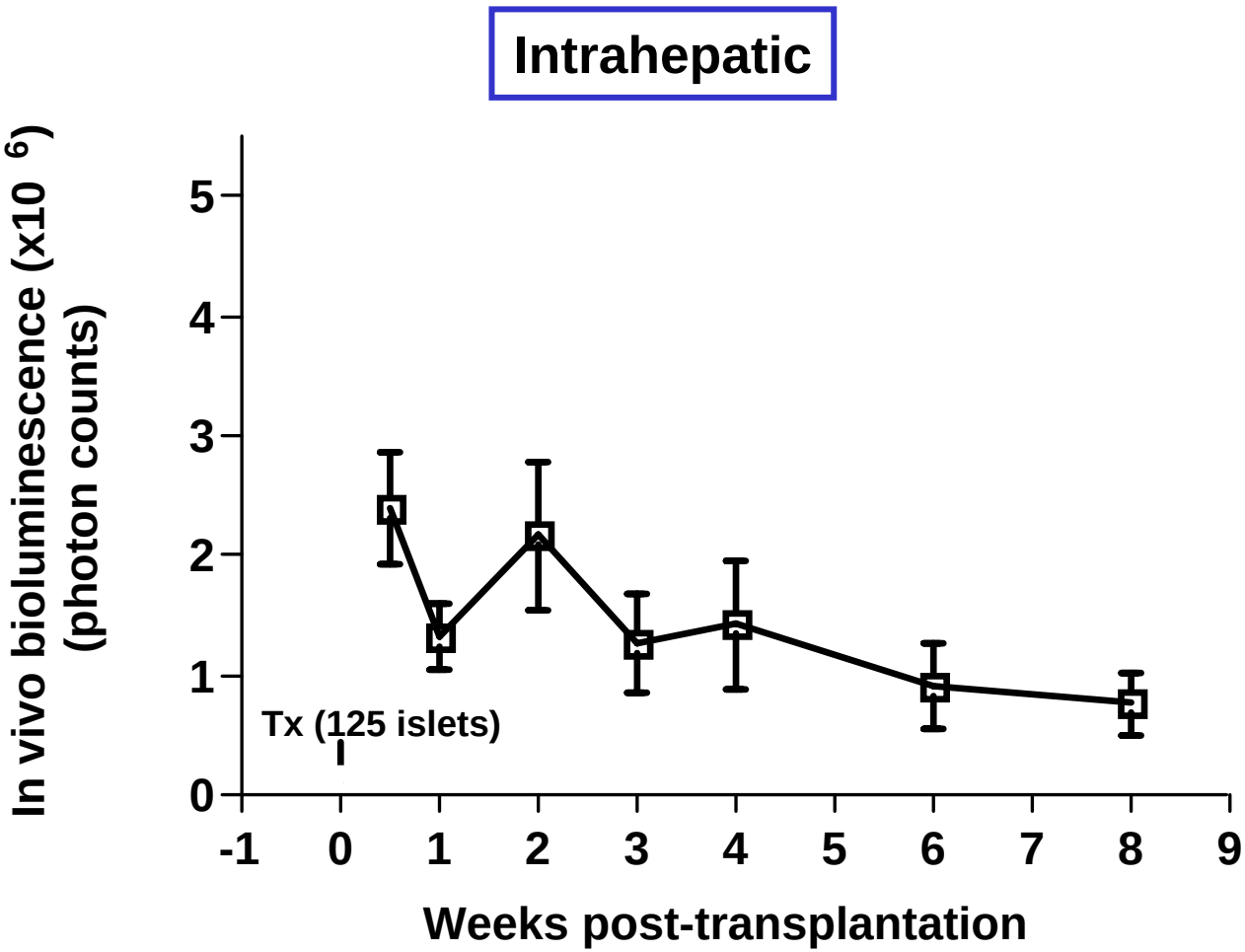
Bioluminescence of Transplanted Islets

- **Dependent of level of luciferase expression within mouse or human islets; (requires ATP and oxygen and viable islets)**
- **Optical scattering properties of tissue in which luciferase-expressing islet reside and tissues through which emitted light must exit the animal influence bioluminescence.**
- **If these are considered in calculations, bioluminescence reflects transplanted islet # and function.**

Bioluminescence of Transplanted Murine Islets



Bioluminescence of Transplanted Murine Islets



Decline in Bioluminescence of Transplanted Islets

- Large decline (> 60%) suggesting islet loss in first week after transplantation
- Beginning 2 weeks post-transplant, greater loss from intra-hepatic islets of all types
  - Liver is unfriendly site?
  - Destroyed by immune attack against luciferase or adenoviral/primate proteins?
- Islets no longer express luciferase
  - Cell division and progeny cells no longer express luciferase

Alternative Approaches

- **Lentivirus (D. Kaufman, UCLA)**
- **AAV virus**
- **Transgenic expression of luciferase**

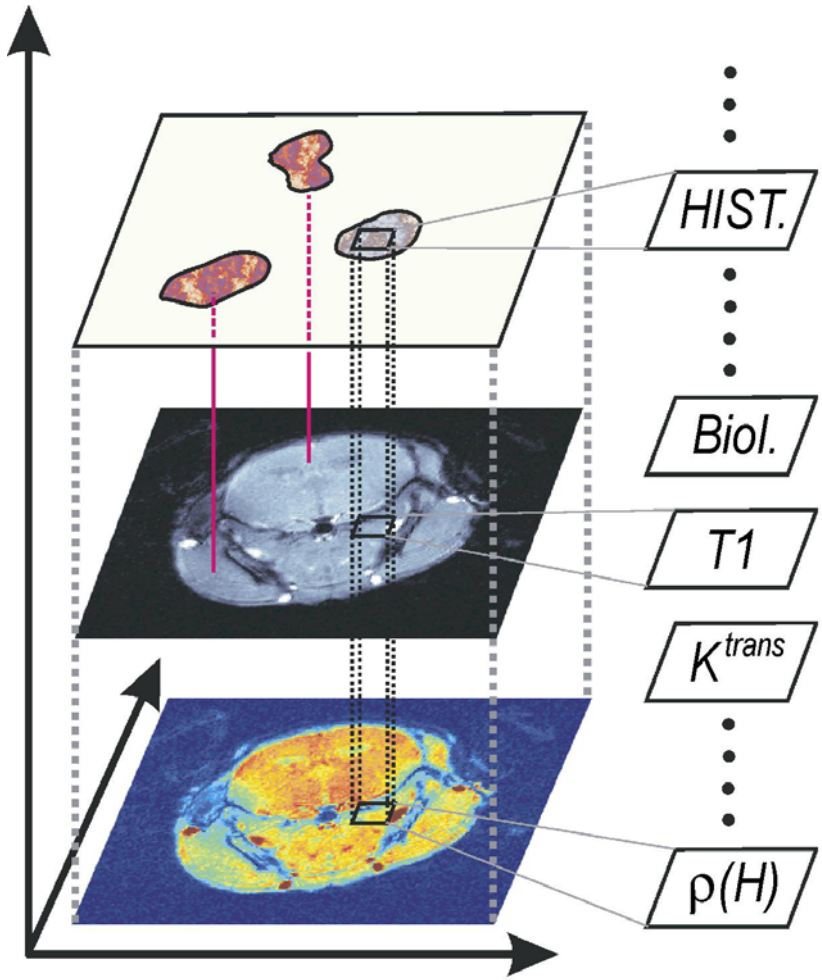
Bioluminescence to Assess Transplanted Islets

- Non-invasive
- Sensitive (detect 25-50 transplanted mouse islets)
- Photon generation likely reflects islet cell number (and maybe islet function)
- Poor spatial resolution
- Allows for serial measurements of intra-hepatic islets
- Allows testing of interventions to increase or sustain transplanted islet mass

Bioluminescence as Islet Imaging Modality

Non-invasive and allow for serial, in vivo measurements in the same animal	**Yes**
Non-toxic to islet cells	**Yes**
Useful for study of islets in native pancreas and after transplantation (in kidney and liver)	**Probably**
Applicable to murine models	**Yes**
Adaptable for human imaging	**No**

Co-Registration of Multiple Imaging Modalities and Biologic Information



Martin Lepage, John Gore, Vanderbilt Imaging Institute

- All imaging modalities will have limitations.
- No single modality will answer all questions about islets in pancreas or transplanted islets.
- Understanding islet survival and function will require integration of complementary imaging modalities and physiology in animal models and in humans.

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