Potency test

Fractional beta-cell Viability
Cellular composition assessment

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Why is difficult to assess islet potency?

Current methodologies for the evaluation of islet cell viability are largely based on tests that rely on DNA-binding dyes.
While these tests identify cells that have lost selective membrane permeability, they do not allow us to recognize apoptotic cells, which do not yet stain with DNA-binding dyes.

Current methods of analysis do not discriminate between cell subsets in the preparation and they do not allow us to selectively define beta-cell viability.

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Why is difficult to assess islet potency?

1. In clinical islet transplantation, 30-90% pure islets assessed by DTZ can be transplanted.
   We transplant not only islet cells, but also acinar and ductal cells.
   Average purity is 50-60% in Miami

Beta	25%
Alpha	15%
Acinar	30%
Ductal	15%

2. Each preparation has different cellular composition, different viability, and sensitivity to noxious stimuli.

3. It is not impossible to evaluate islet viability and cellular composition without dissociation. However, it is very difficult to assess the viability of each cell subset individually in a short time before transplantation.

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Assessment of Human Islet Cell Composition and Viability at the University of Miami

We have developed methods for the specific assessment of **beta-cell content** and **viability** in human islets based on:

- cellular composition analysis (**Laser Scanning Cytometry**; LSC)

and

- identification of beta cell-specific apoptosis at the mitochondrial level (**Flow Cytometry**)

We have validated our methods using *in vivo* assessment of islet potency (transplantation into **immunodeficient mice**).

H. Ichii et al. Am J Transplant 2005; 5: 1635-45

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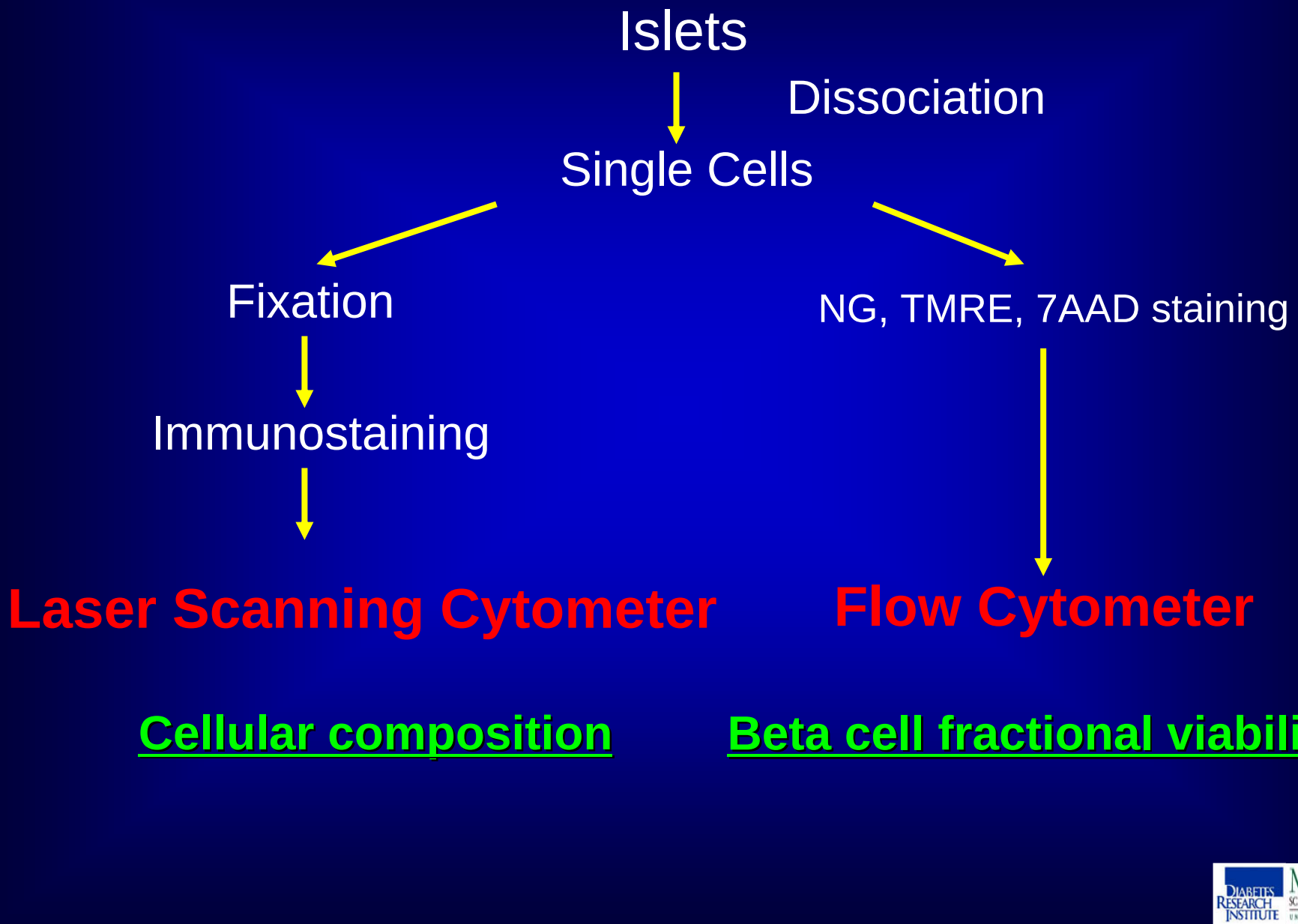
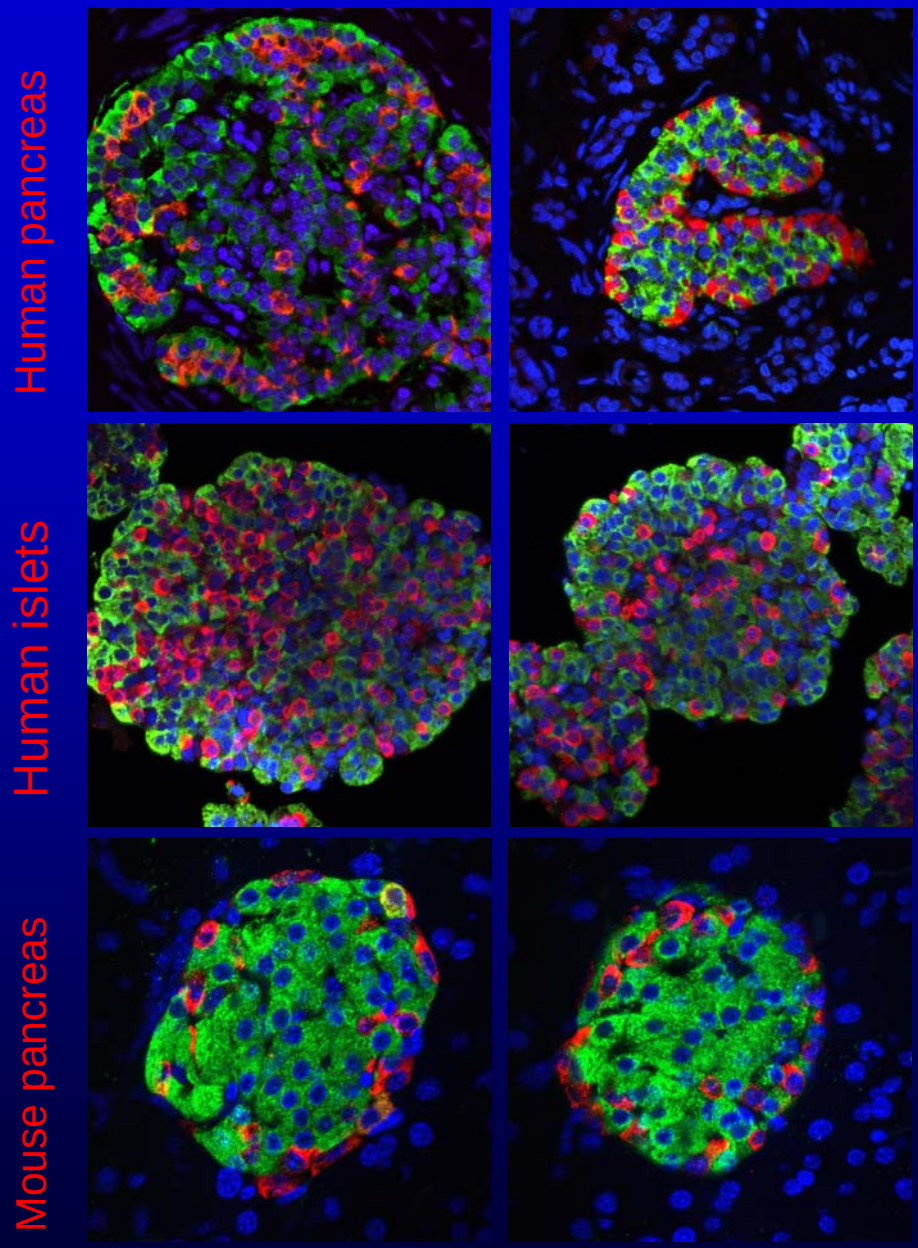
Experimental procedure



Assessment of Islet Preparation

Alpha (Glucagon)
Beta (insulin)
Nuclei (DAPI)



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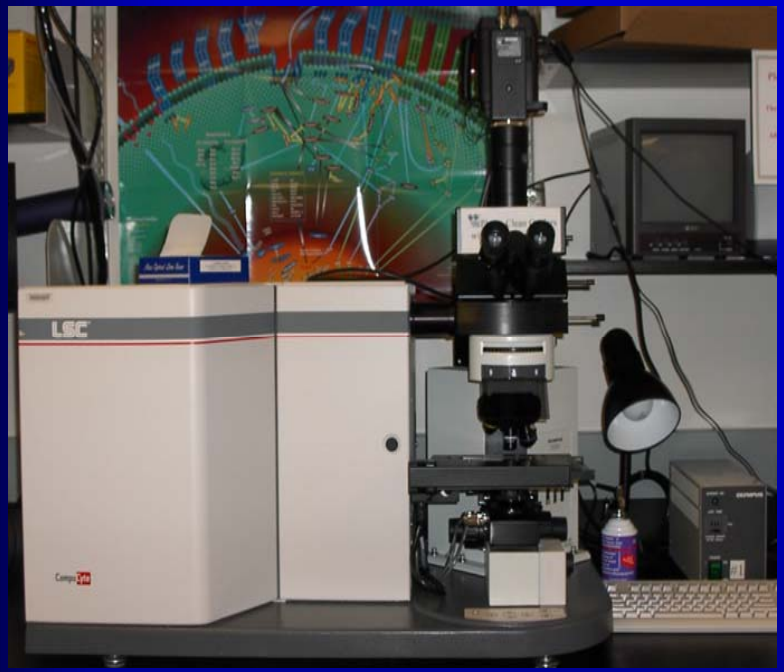
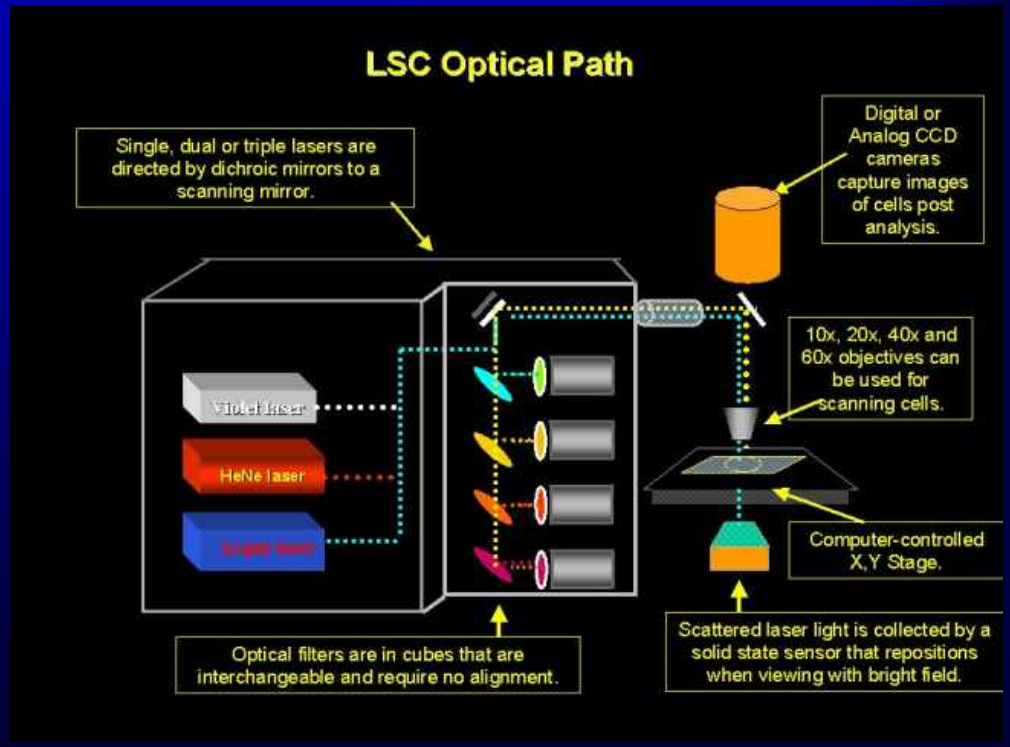
Laser Scanning Cytometer

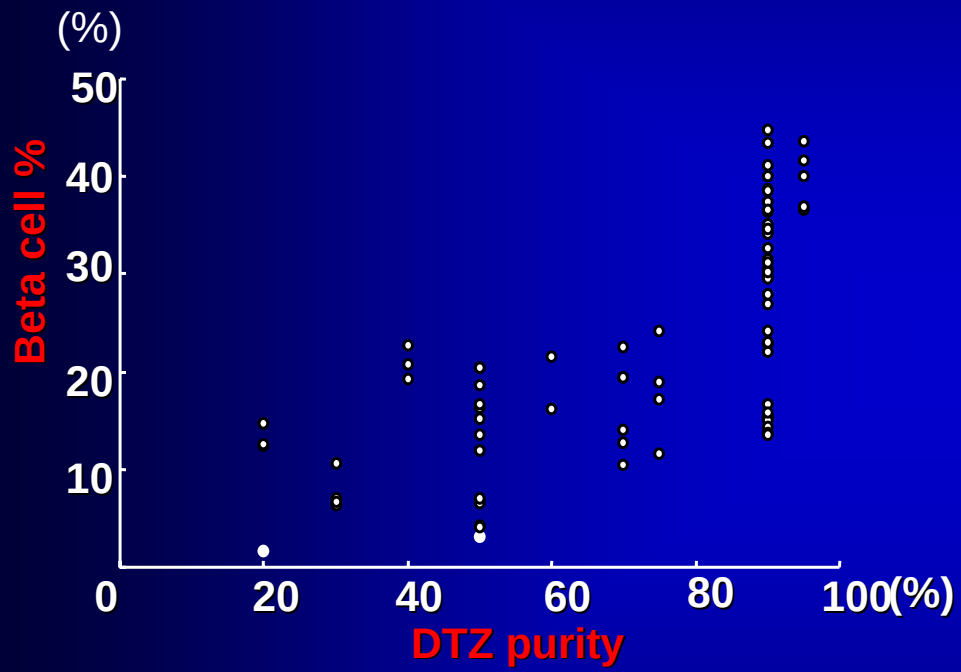


The LSC is an instrument designed to enable fluoresence-based quantitative measurements on tissue sections or other cellular preparations at single-cell level.

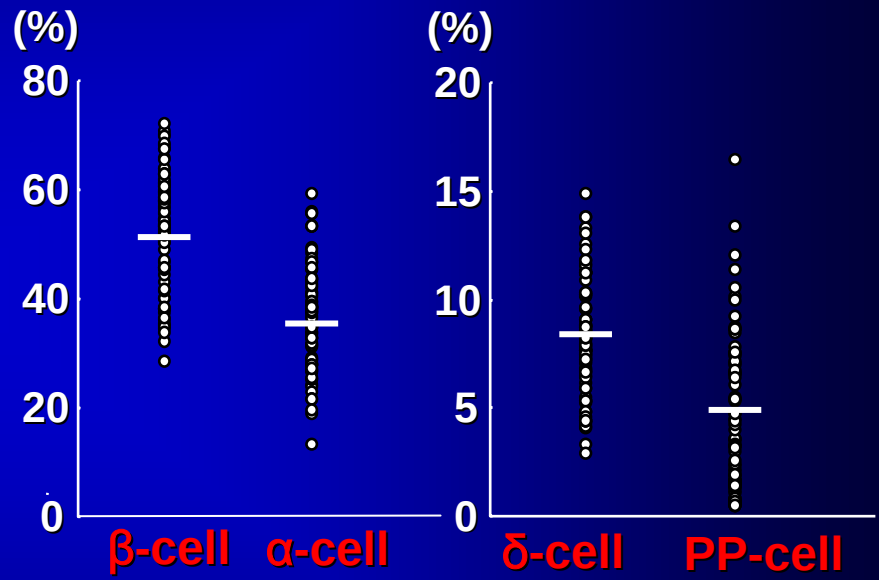
The instrument consists of a base unit containing fluorescent microscope, optics/electronics unit coupled to an argon, HeNe and Violet laser, a computer-controlled motorized stage.



ß-cell percentage and purity

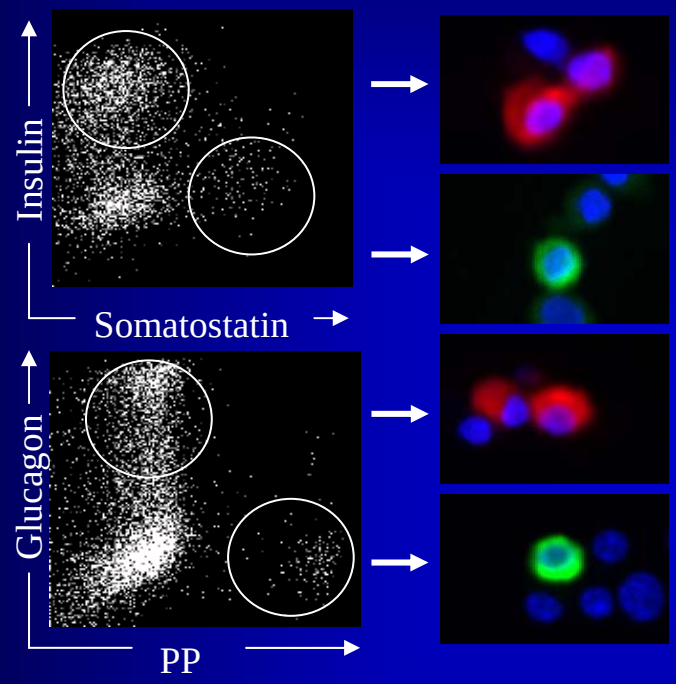


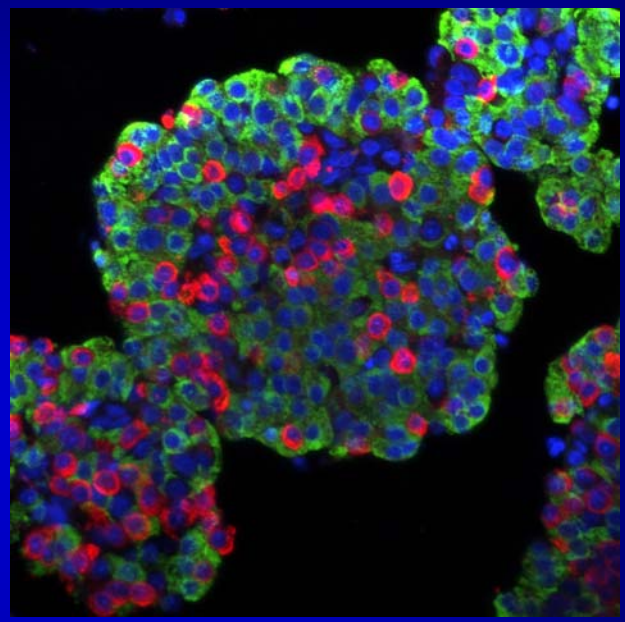
Cellular Composition in Islet



$$\frac{\beta\text{-cell (\%) or } \alpha, \delta, \text{PP}}{\beta+\alpha+\delta+\text{ PP-cell (\%)}}$$

Comparison of cellular composition assessment between dissociated and non-dissociated islet cells





Alpha (Glucagon)
Beta- (insulin)
Nuclei (DAPI)

	β -cells	α -cells	δ -cells
Non-dissociated islet	57.4±19.6%	32.8±16.3%	9.8±2.9%
Dissociated islet cells	54.4±11.4%	34.7±12.5%	10.9±4.6%

No statistically significant differences are observed.

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Human Islet Cellular Composition

Whole Islet

	#	β (%)	α (%)	δ (%)
Miami	5	57	33	10
Nashville	32	54	34	10

Dissociated Islet

	#	β (%)	α (%)	δ (%)
Miami	63	54	35	11
Edmonton	69	57	23	10

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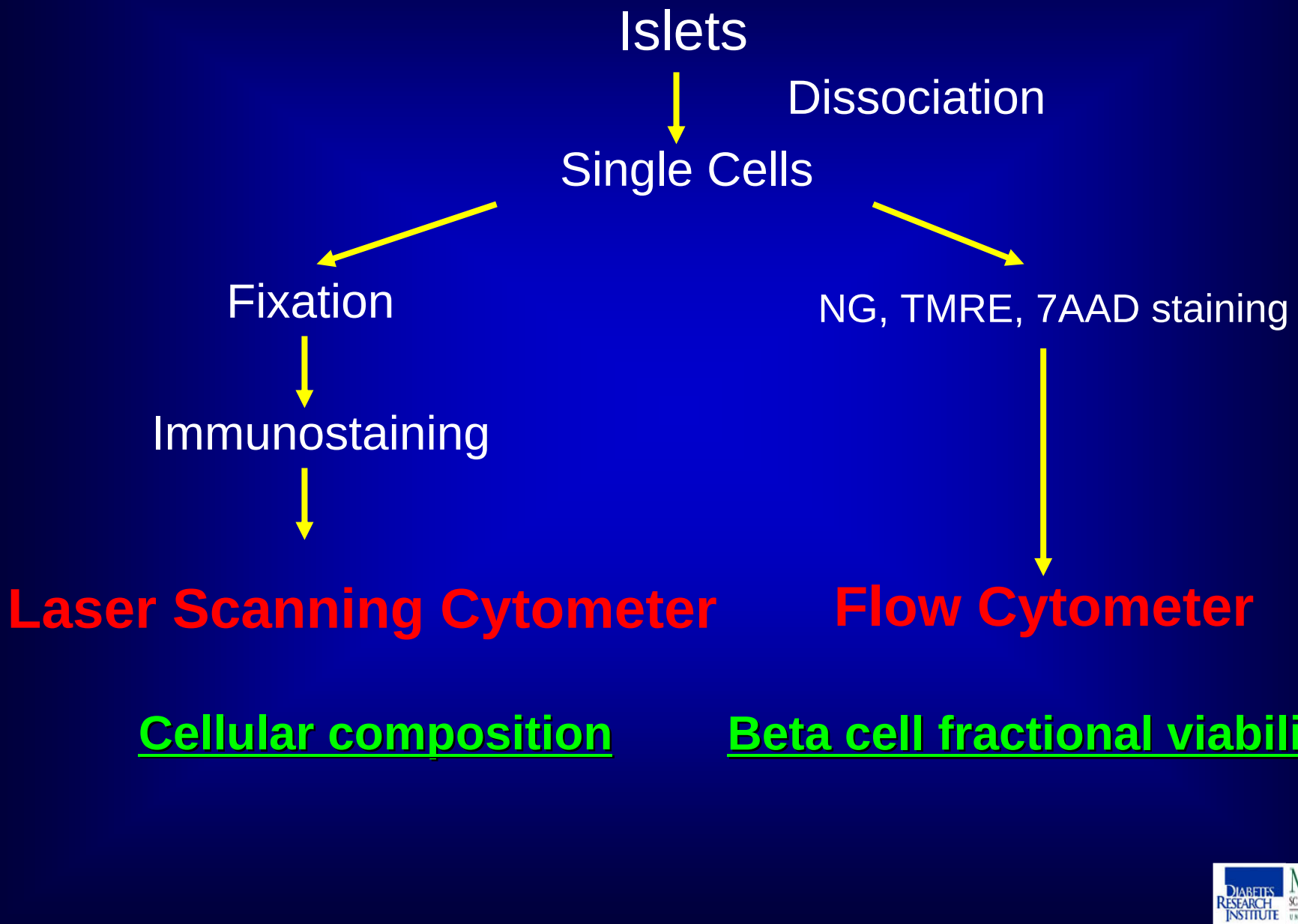
Experimental procedure



Fractional beta cell viability assessment

DNA-binding dye

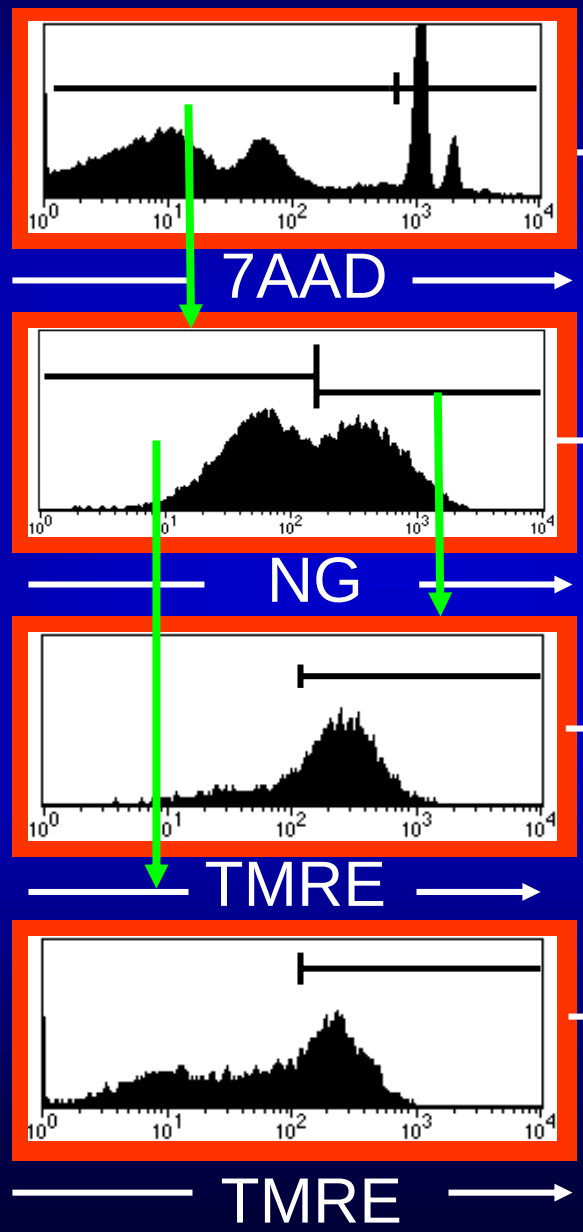
7-AAD

Zinc-binding dye

Newport green

Mitochondorial Membrane Potential indicating dye

TMRE



dead cells (%)

Beta cell content (%)

Beta cell viability (%)

Other cell viability (%)

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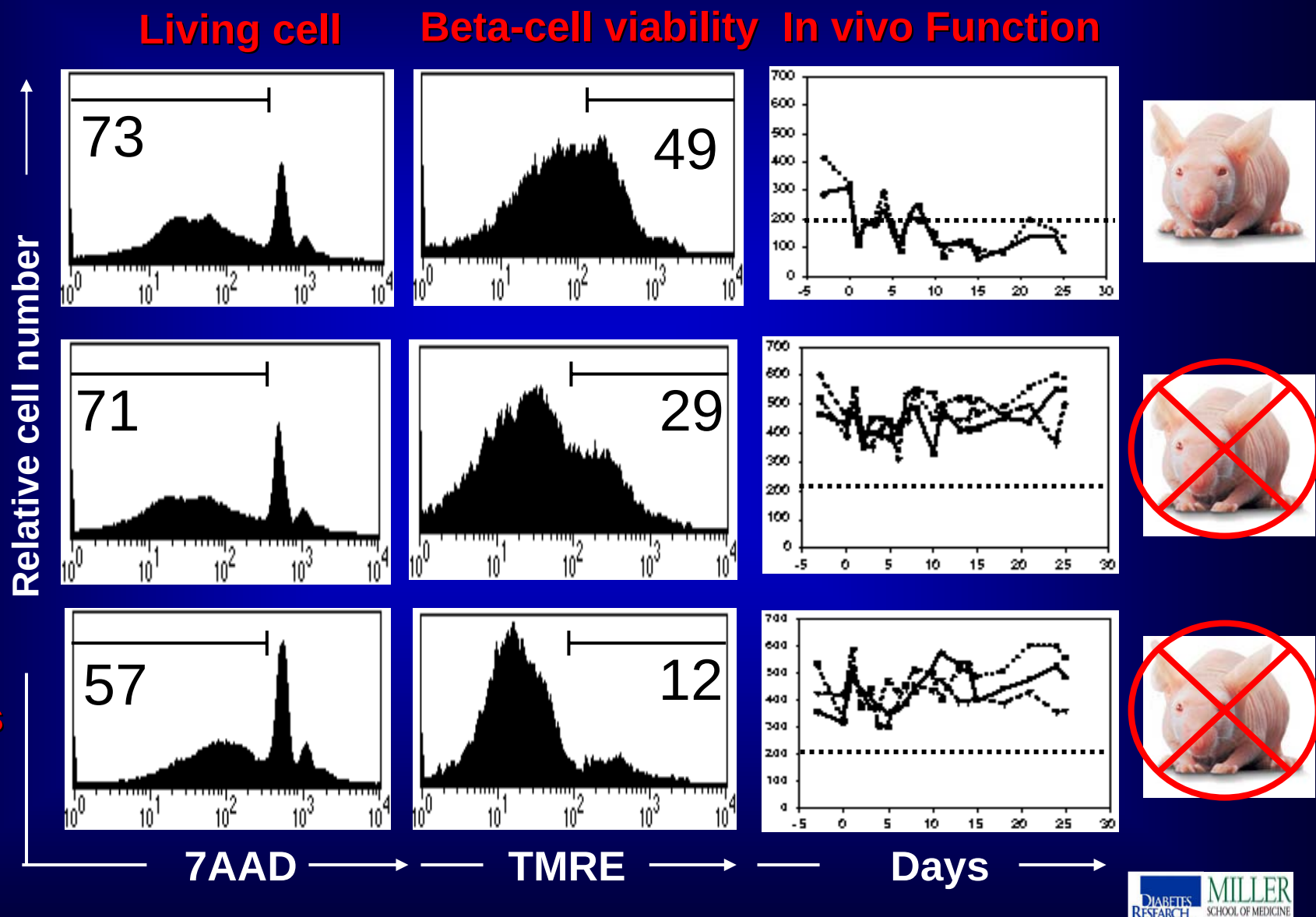
Comparative analysis of cell viability, β-cell apoptosis and *in vivo* islet function.



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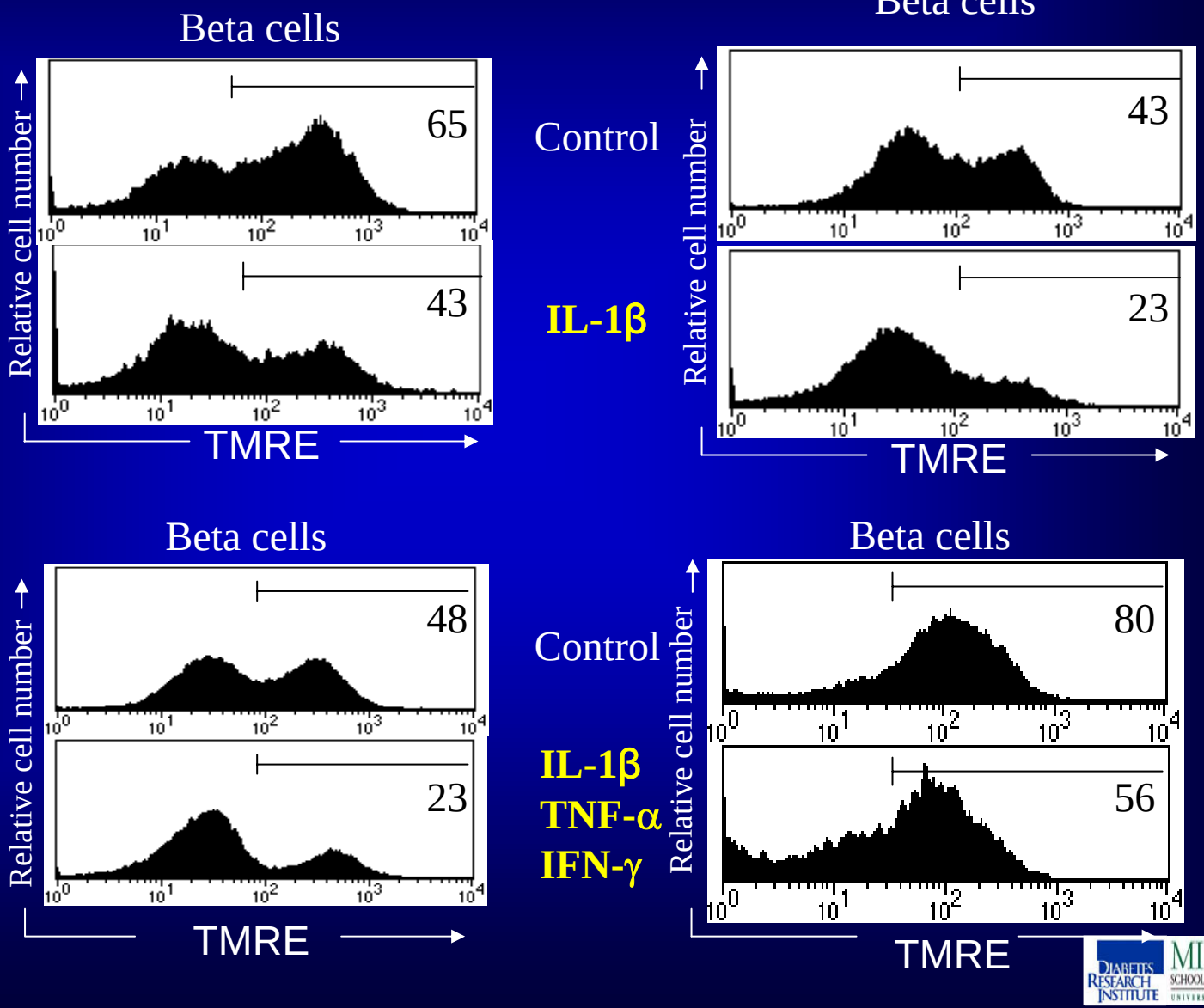
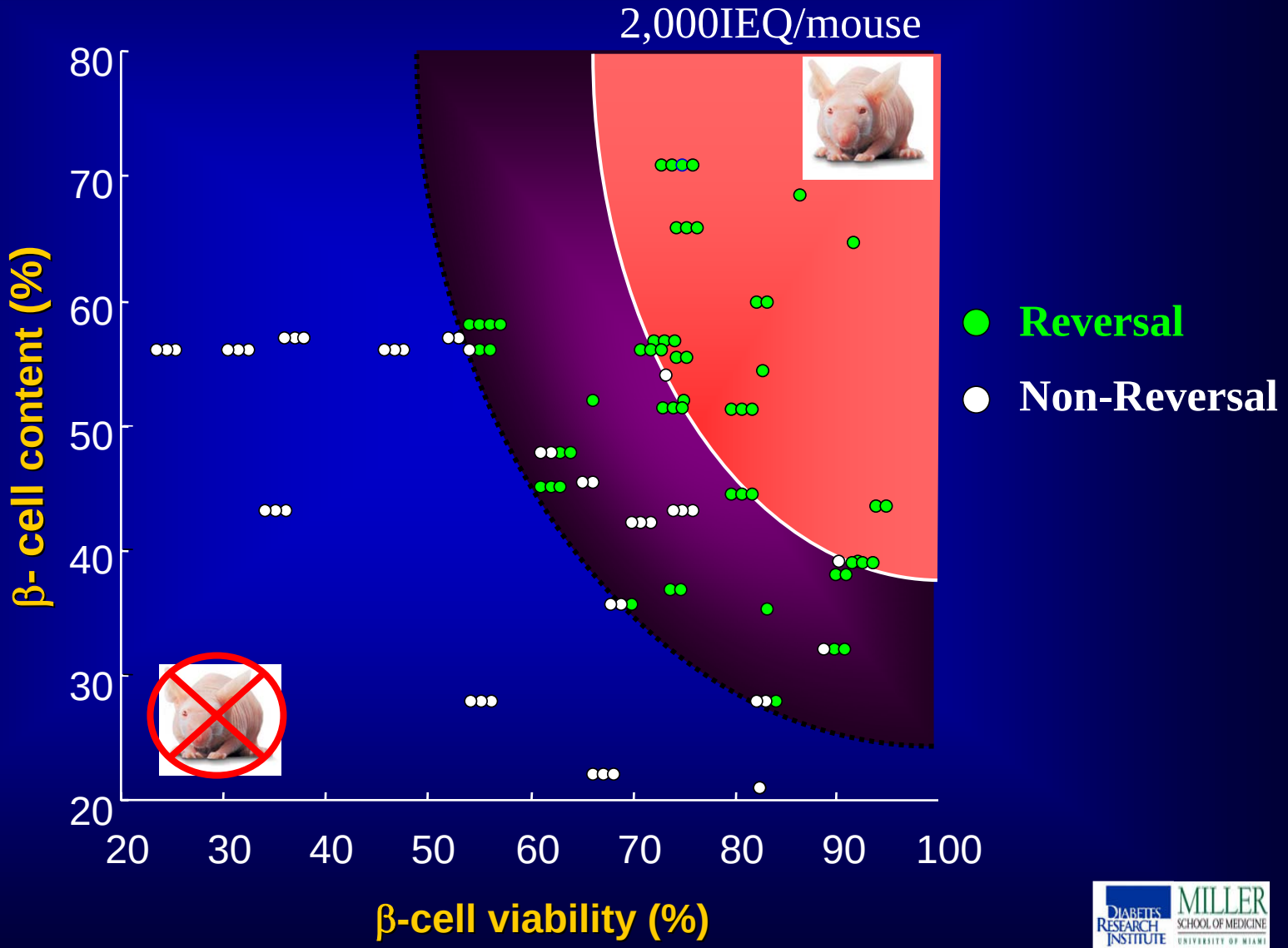
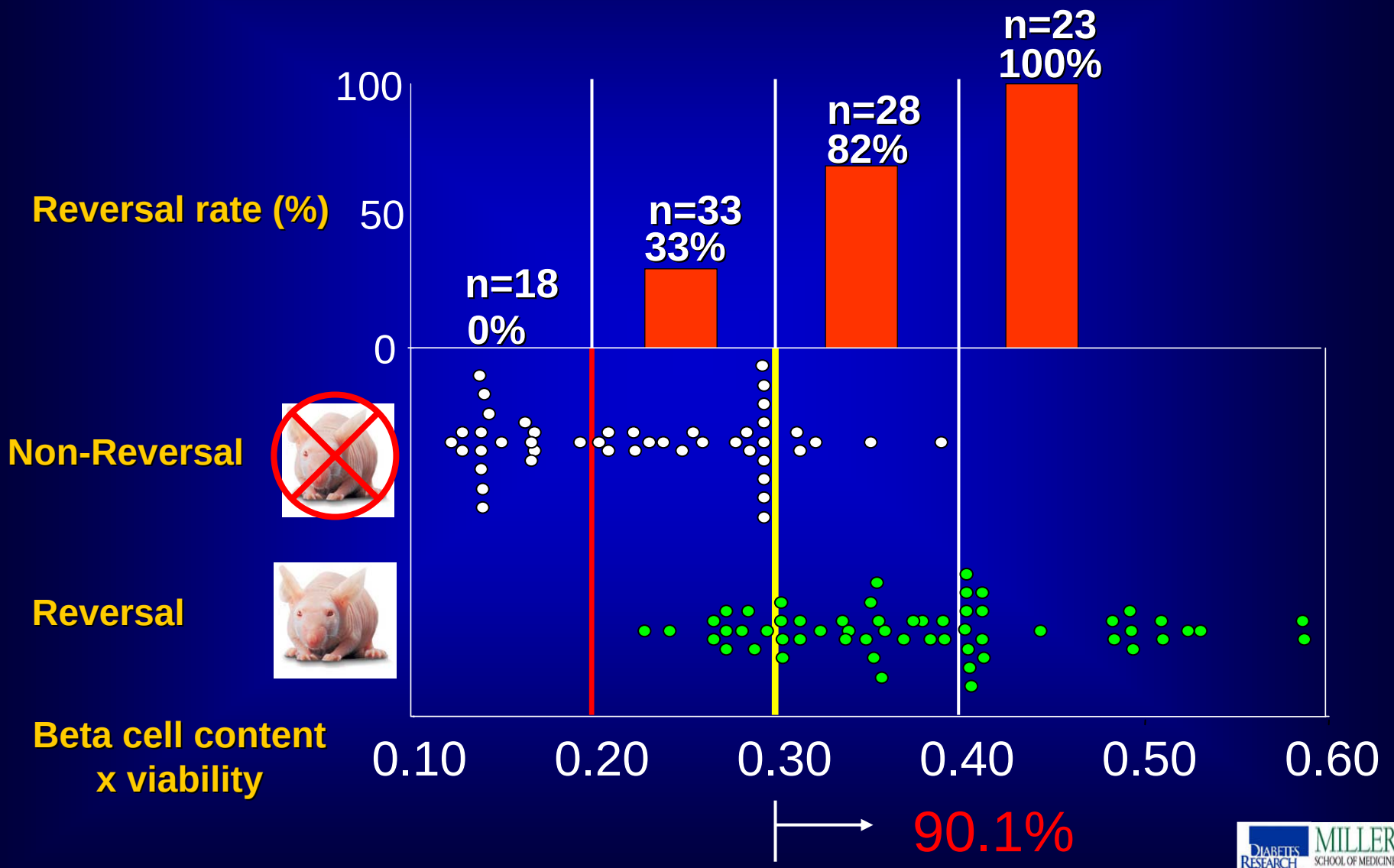
Analysis of β-cell Fractional Viability After Noxious Stimuli *in vitro*



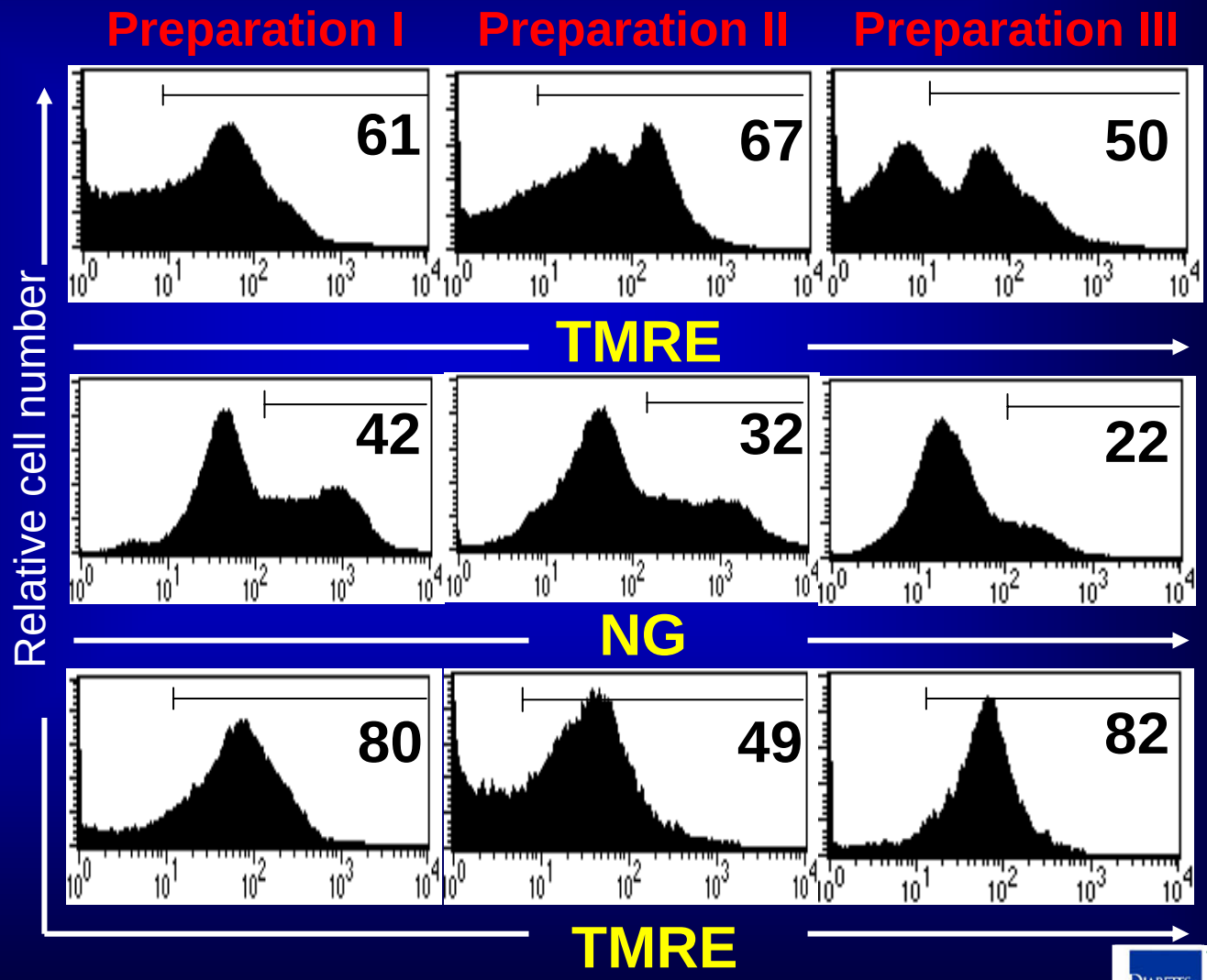
Correlation of β-cell content and viability with *in vivo* Islet Function



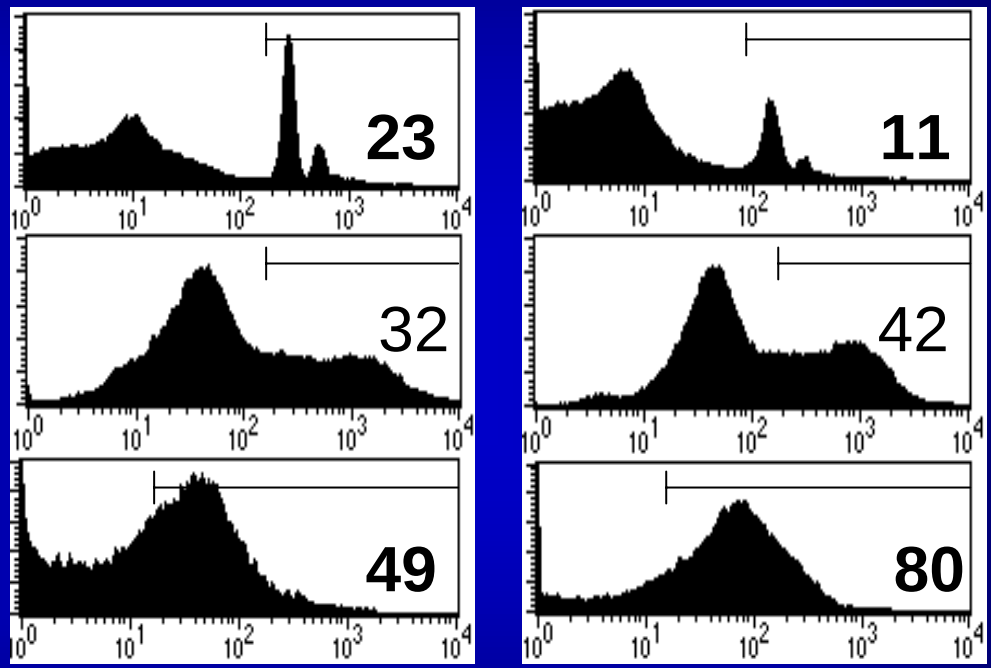
Predictive Value of β-cell Content/Viability on *in vivo* Islet Function.



β-cell-specific analysis of viability/apoptosis in human islet preparations



	Preparation 1	Preparation 2
Pre-culture	450,000 IEQ	380,000 IEQ



Post-culture	170,000 IEQ	380,000 IEQ
	Not Transplanted	Transplanted

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Islet Dissociation

- **Does it affect the cellular composition?**

  NO.

  The cellular composition of dissociated islets is comparable to that of whole islets by IHC.

  If cell loss occurs, this is not selective for any specific cell subset and the overall proportion is maintained.

- **Does it affect cell viability?**

  MAYBE.
  However, it may not matter!
  Good correlation between *Viable Beta Index* and *in vivo* function suggests that this approach can PREDICT the potency of isolated islets and that it is REPRESENTATIVE of the quality of the preparation

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Ongoing Studies

- Evaluation of the correlation between Viable Beta-Index and islet engraftment in the clinical setting

- Develop a method to assess the viability of multiple islet cell subsets (i.e., ductal, alpha…)

- Identify more sensitive marker than MMP for islet cell potency assessment

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