

NIR Fluorescence Applications: From Basic to Translational Research

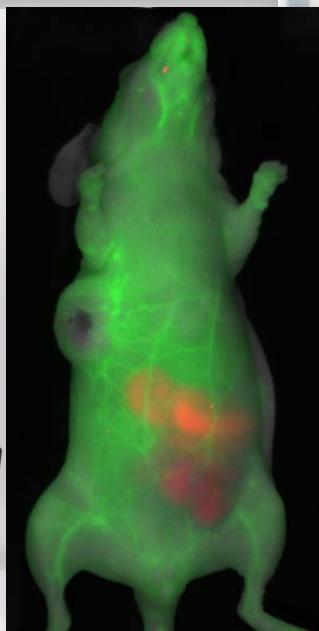
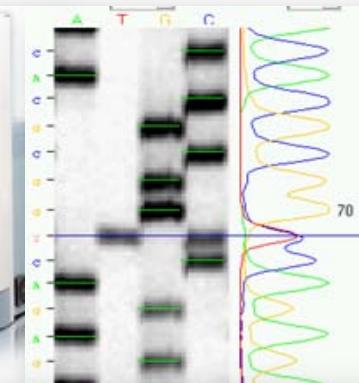
吳美萱
儀器部產品經理
騰達行企業股份有限公司

LI-COR® Biosciences



40 Years of Excellence

A History in NIR Detection



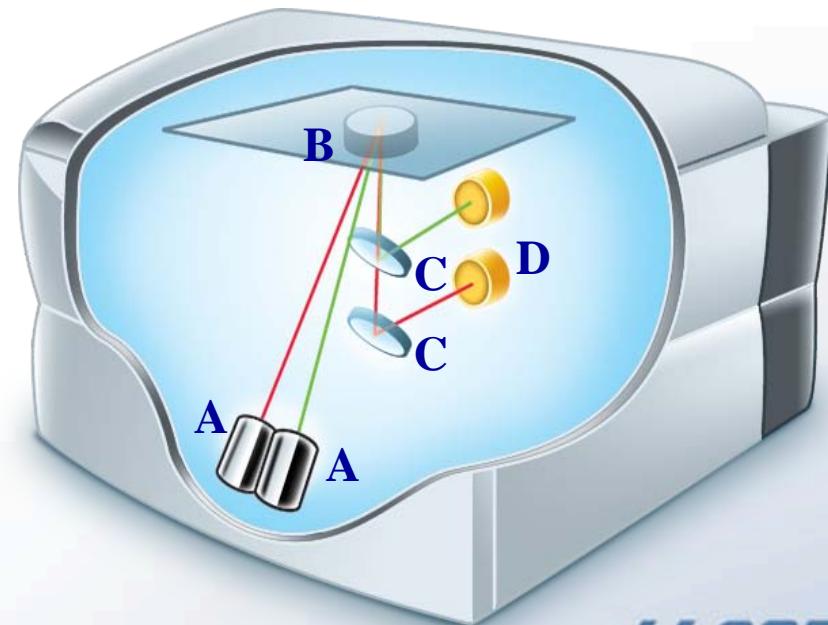
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UNIMED HEALTHCARE INC.

LI-COR
Biosciences



LI-COR Detection System

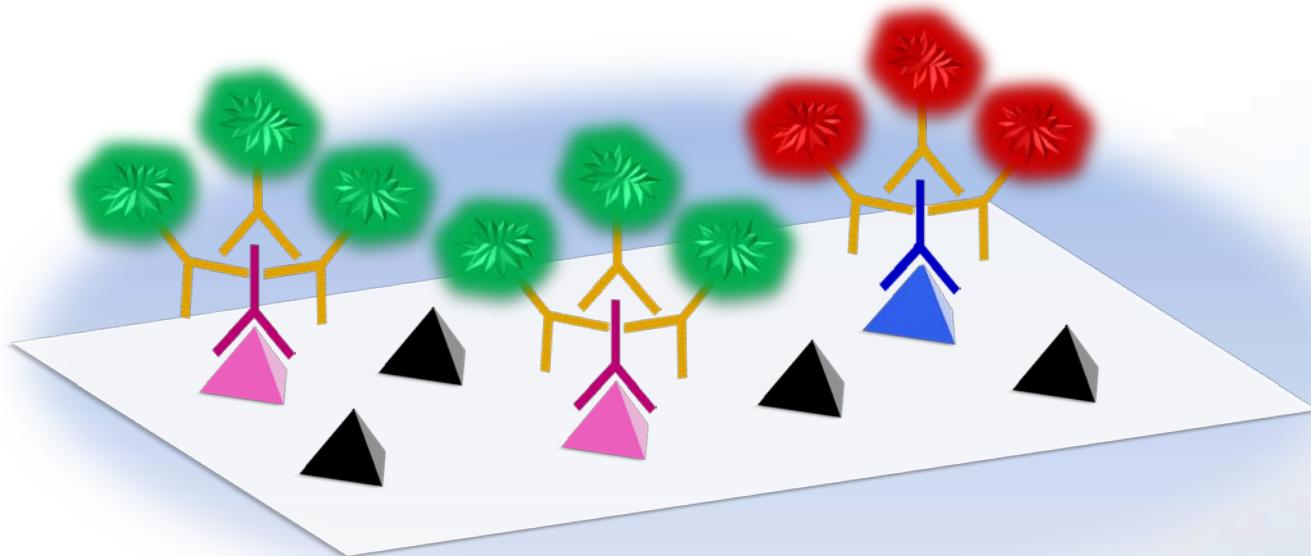
- 680 & 780 nm solid state laser diodes (A)
 - No spectral overlap
 - Low power consumption
 - Long life lasers (>40,000 hours)
- Silicon avalanche photodiode detectors (C)
- Resolution 21-337 microns
- Internal hard drive
- Secure network connectivity



Fluorescence Detection is Different

Enabling technology

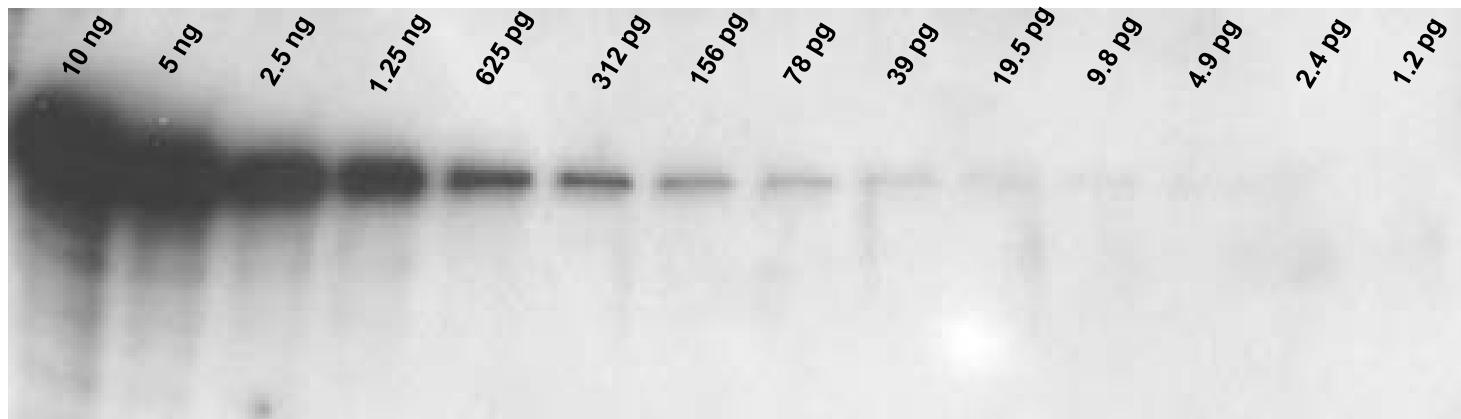
- Quantitative data
- Signal is directly proportional to amount of target





Chemiluminescence Detection

Tf protein:

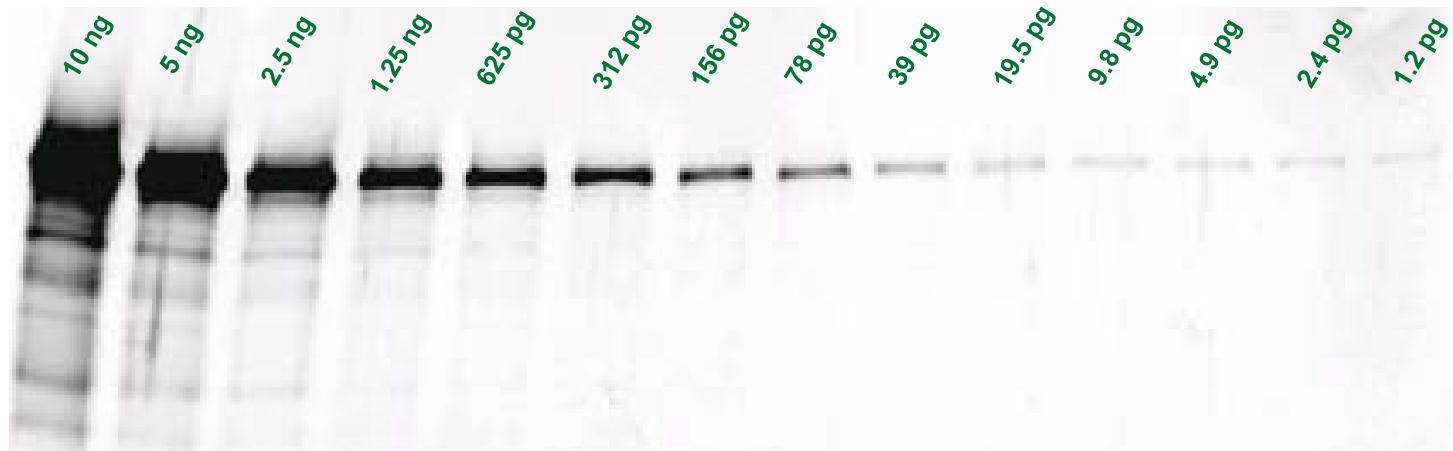


Dilutions of transferrin blotted on nitrocellulose, detected with rabbit anti-Tf primary and IRDye® secondary antibody



Direct Infrared Detection

Tf protein:



Dilutions of transferrin blotted on nitrocellulose, detected with rabbit anti-Tf primary and IRDye® secondary antibody

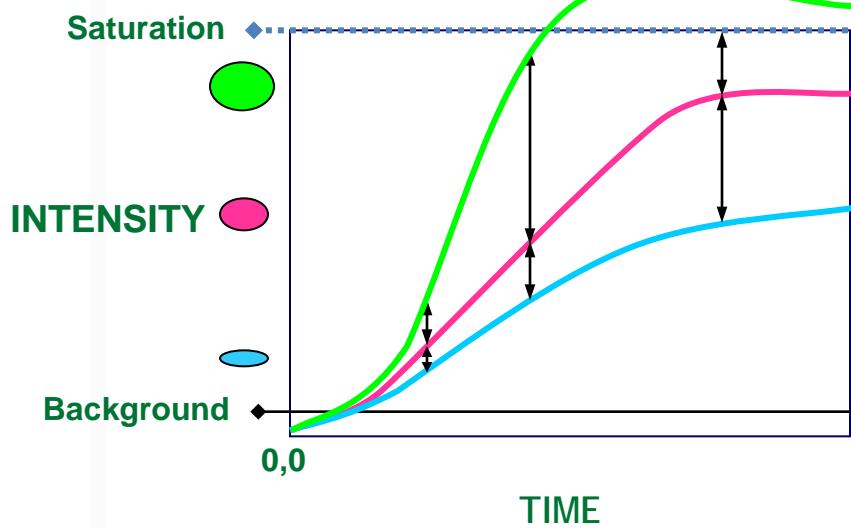
1. Signal is linear with amount of bound secondary antibody
2. No enzyme kinetics
3. No signal diffusion
4. Sensitivity equivalent *or better* than chemiluminescence



Benefits of Fluorescence Detection

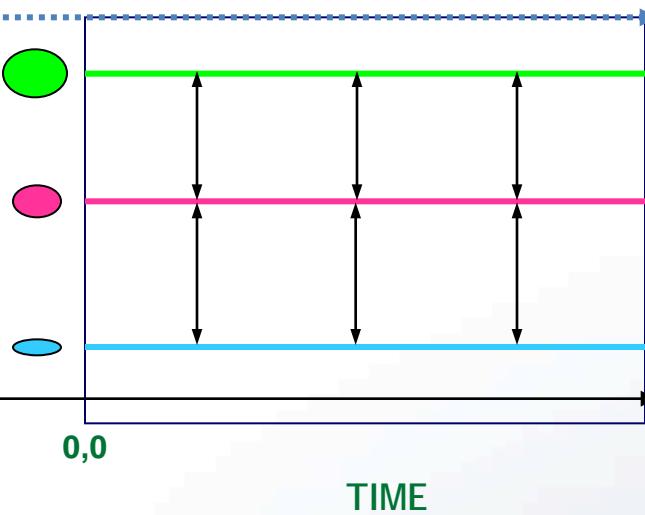
CHEMILUMINESCENCE

- Amplification of signal over time - *dynamic*
- Not proportional to target concentration



ODYSSEY

- No amplification of signal - *static*
- Signal proportional to target



High concentration /
signal



Medium concentration /
signal



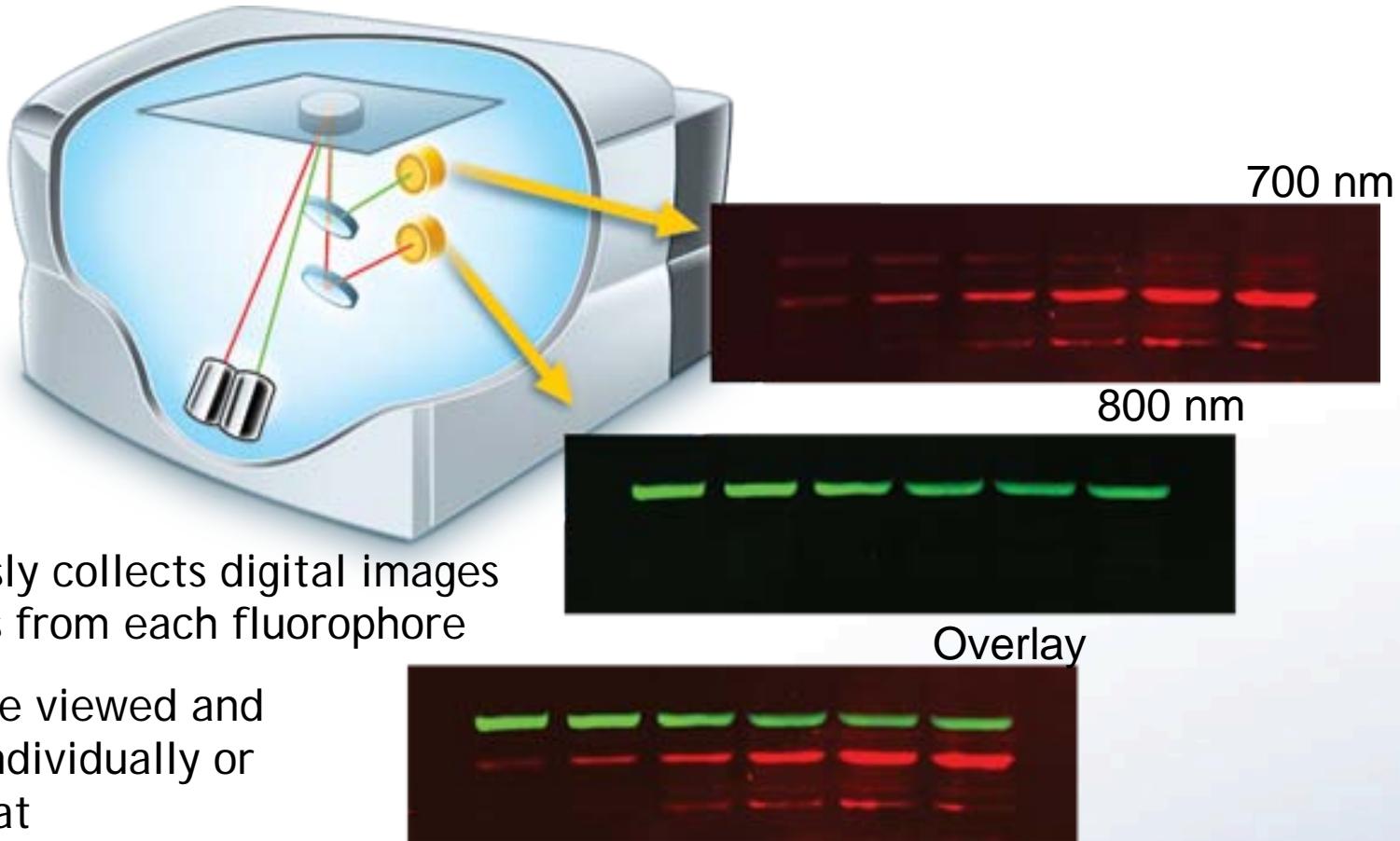
Low concentration /
signal



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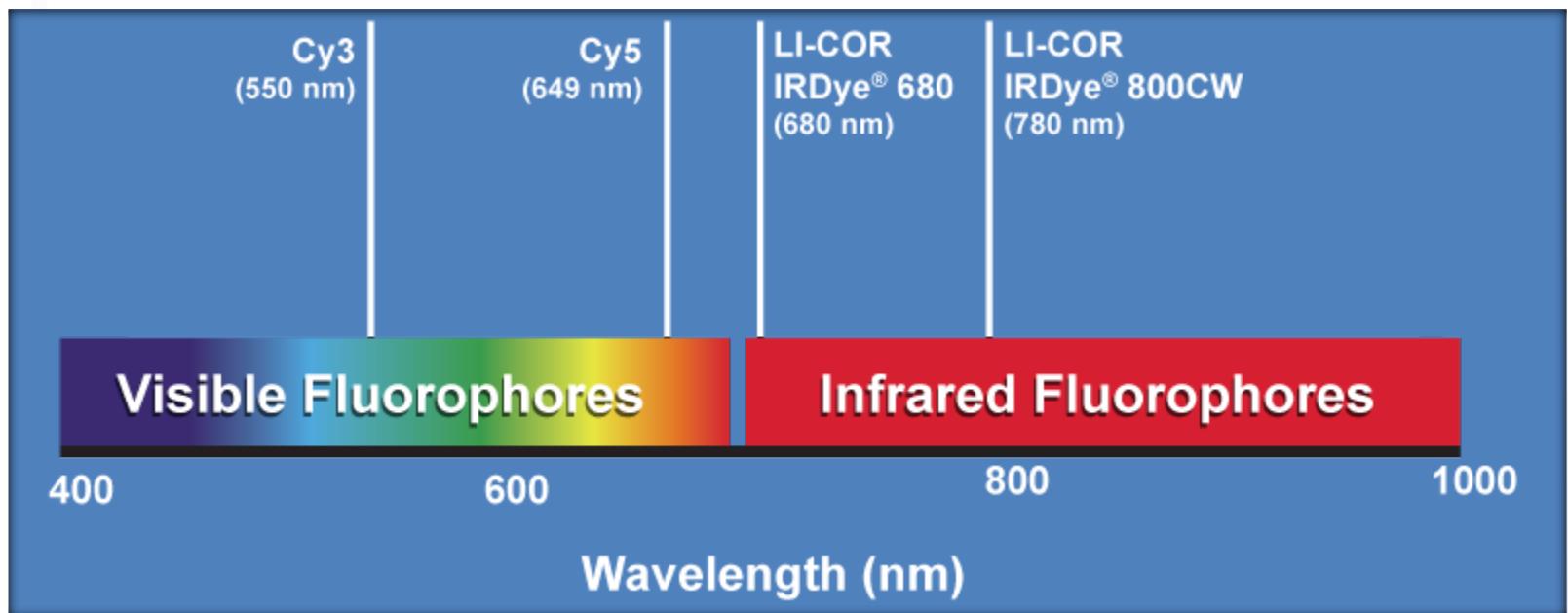
Multiplexed Quantitative Assay



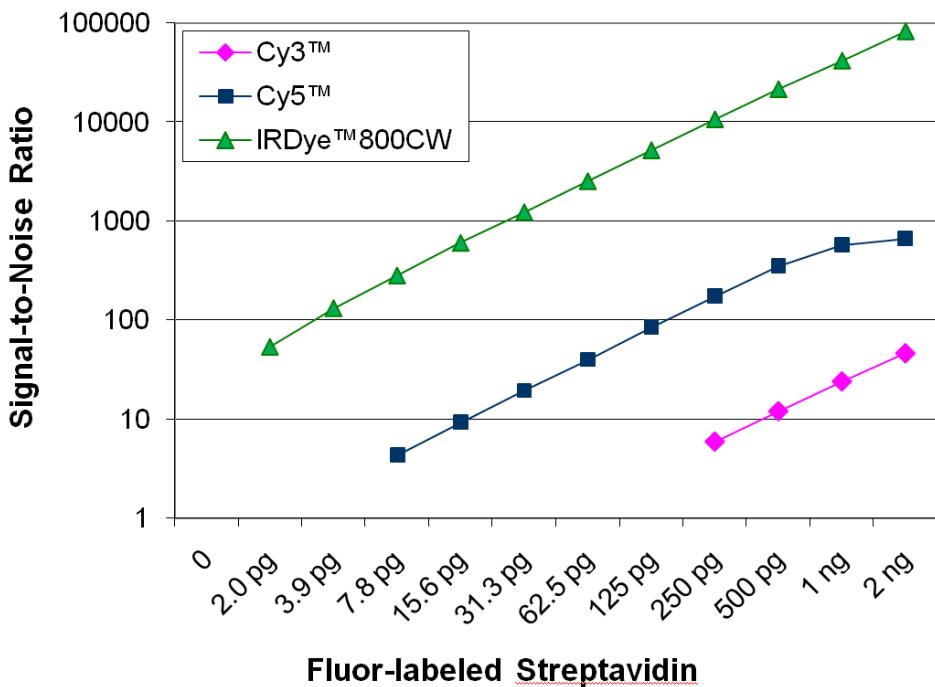


Why Use Infrared Fluorescence?

- Many natural and synthetic compounds autofluoresce in the visible region
 - **Nitrocellulose, PVDF, nylon, cellular content**
- Low background fluorescence in IR range translates to excellent sensitivity



Achieving High Sensitivity with NIR Fluorescence



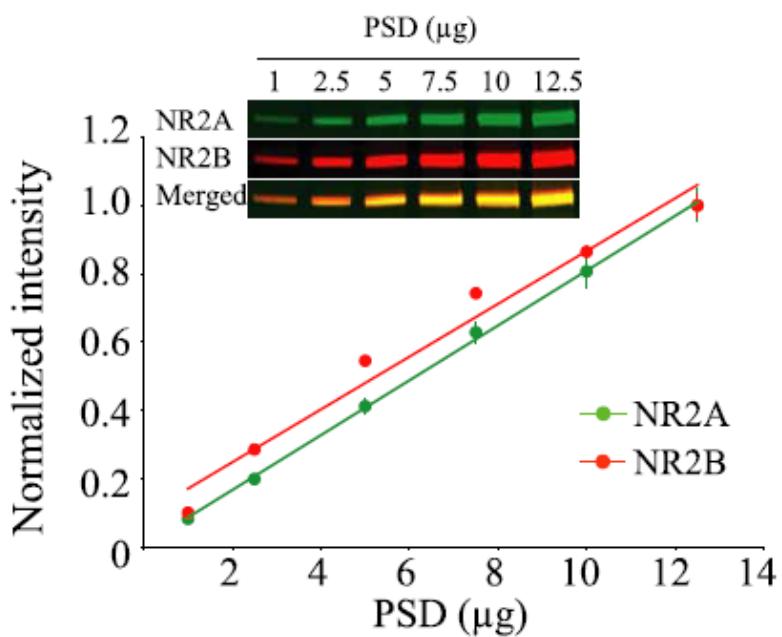
Comparison of visible and NIR dyes on nitrocellulose-coated slides.

Dilutions of streptavidin were spotted in 5 replicates on each of 3 slides, and imaged at the appropriate wavelengths.

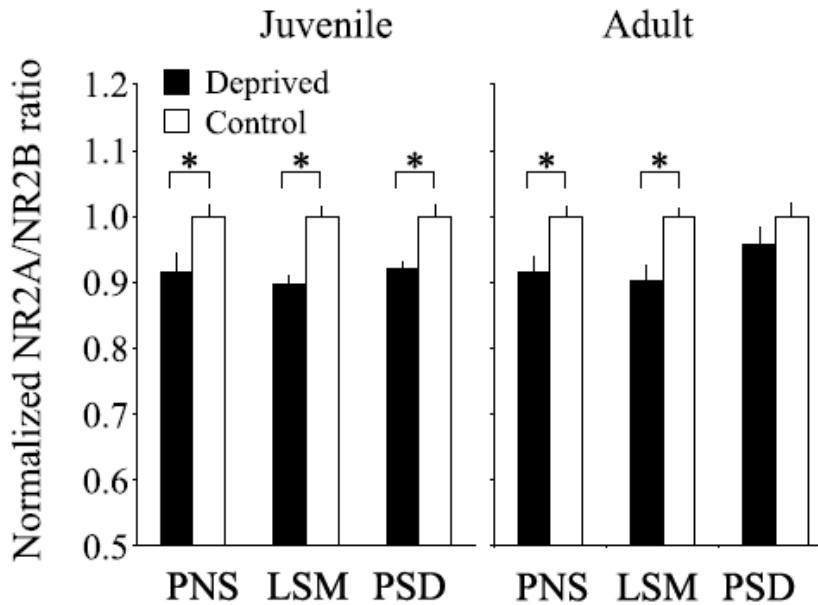
$\text{SNR} = (\text{fluorescence intensity} - \text{mean background}) / \text{std. dev. of background}$; SNR cutoff=3.

Reproducibility Uncovers Small Differences

B



C

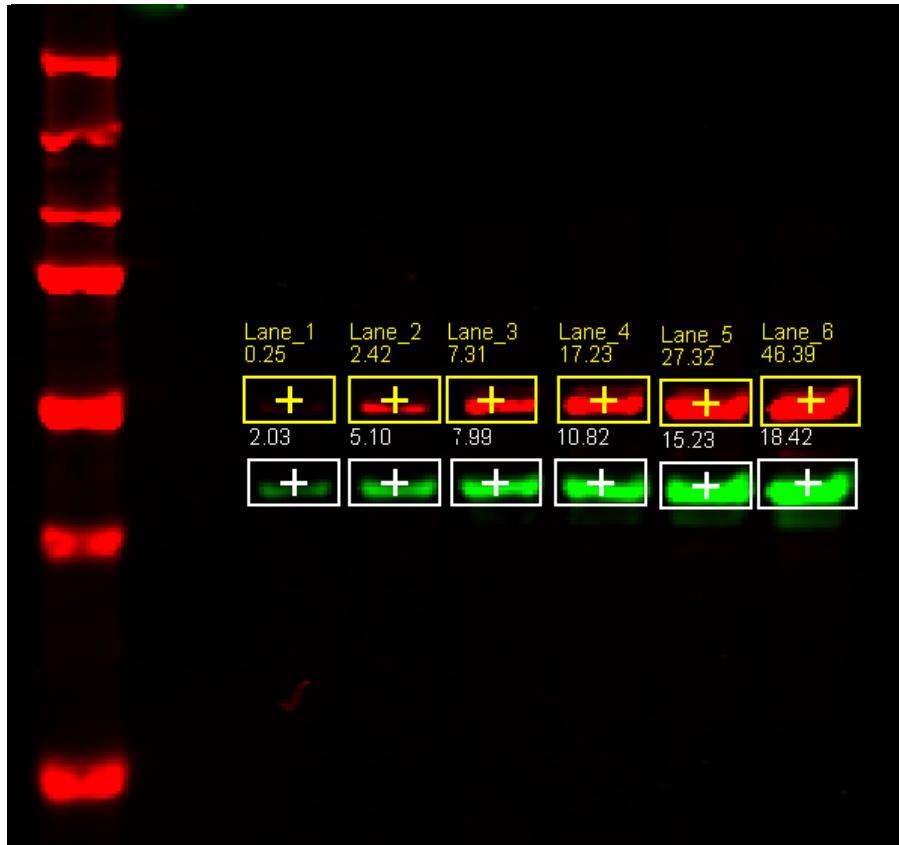


K. Yashiro et al. (2005) Neuroscience 25(50): 11684
University of North Carolina

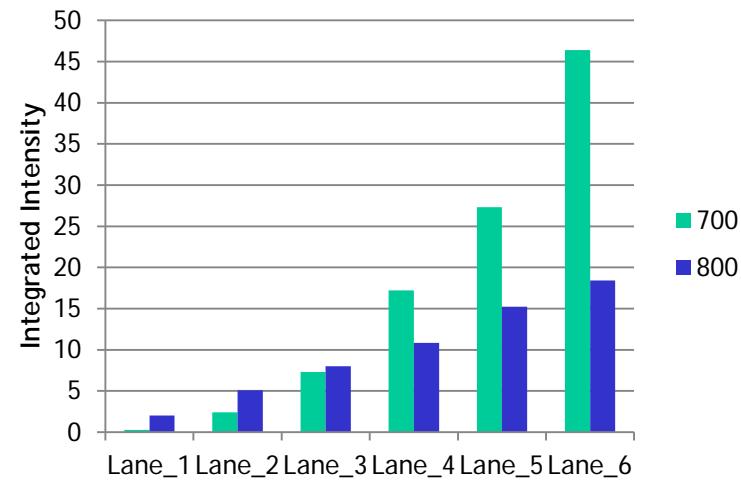
Applications



Two-color Simultaneous Detection

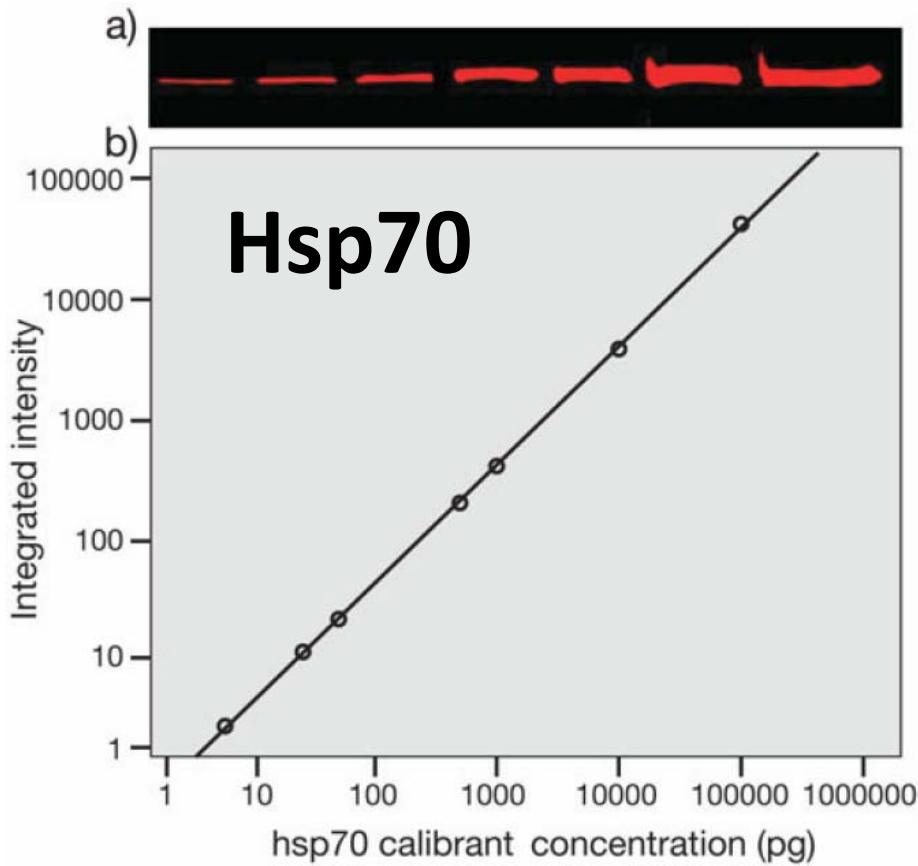


Name	700	800
Lane_1	0.25	2.03
Lane_2	2.42	5.1
Lane_3	7.31	7.99
Lane_4	17.23	10.82
Lane_5	27.32	15.23
Lane_6	46.39	18.42



* Two-color detection requires primary antibodies from different hosts

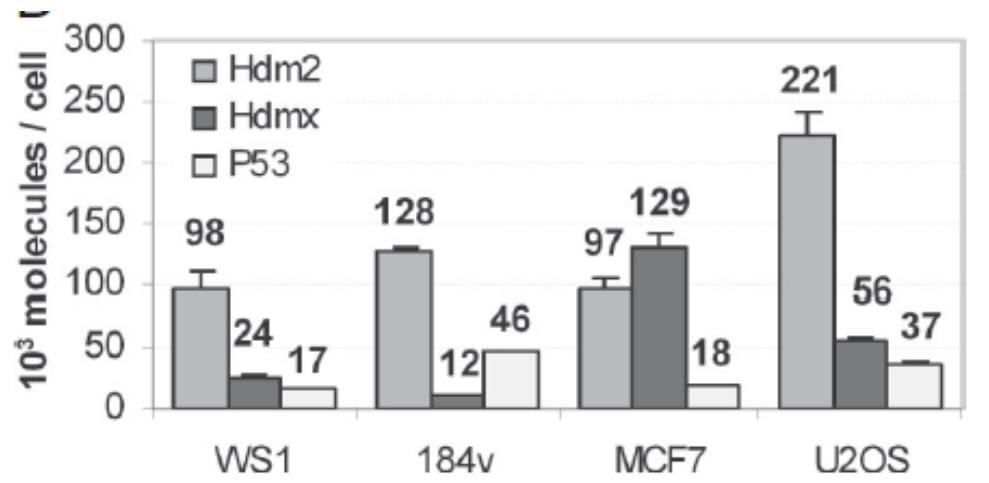
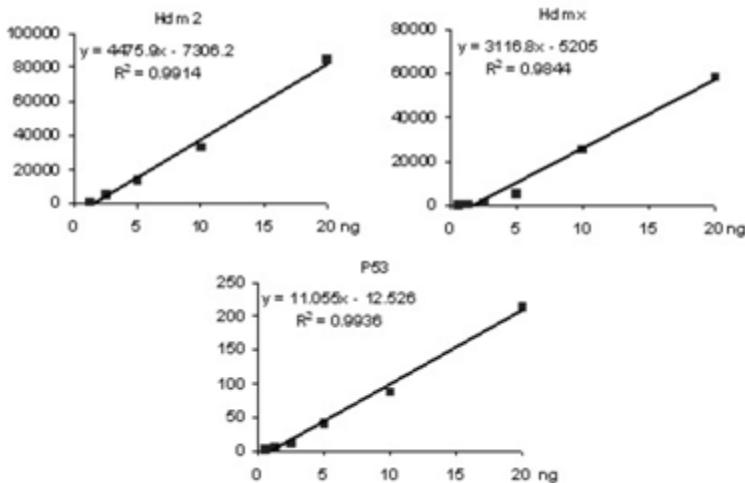
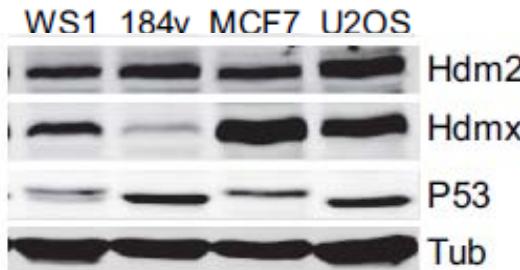
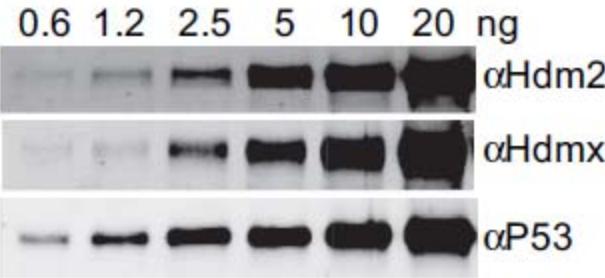
Absolute Quantification



5 pg - 100 ng

20,000 fold

Absolute Quantification



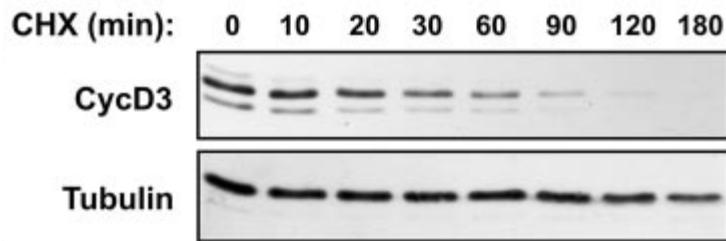
Wang et al., Salk Institute
PNAS 104: 12365–12370 (2007)

Relative Quantification

Time Course Analysis

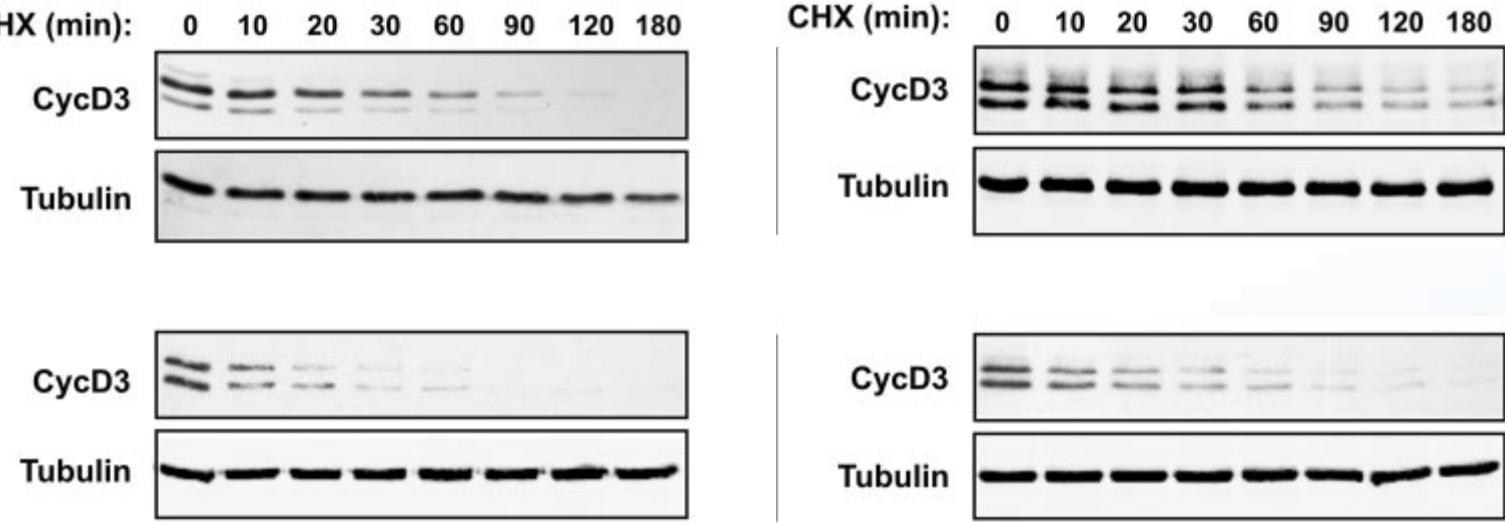
Rb^{+/+}

Proliferating



Rb^{-/-}

Differentiated





Pubs Citing “LI-COR Odyssey”



The Nobel Prize in Physiology or Medicine 2009

"for the discovery of how chromosomes are protected by telomeres and the enzyme telomerase"



Photo: U. Montan

Elizabeth H.
Blackburn



Photo: U. Montan

Carol W. Greider



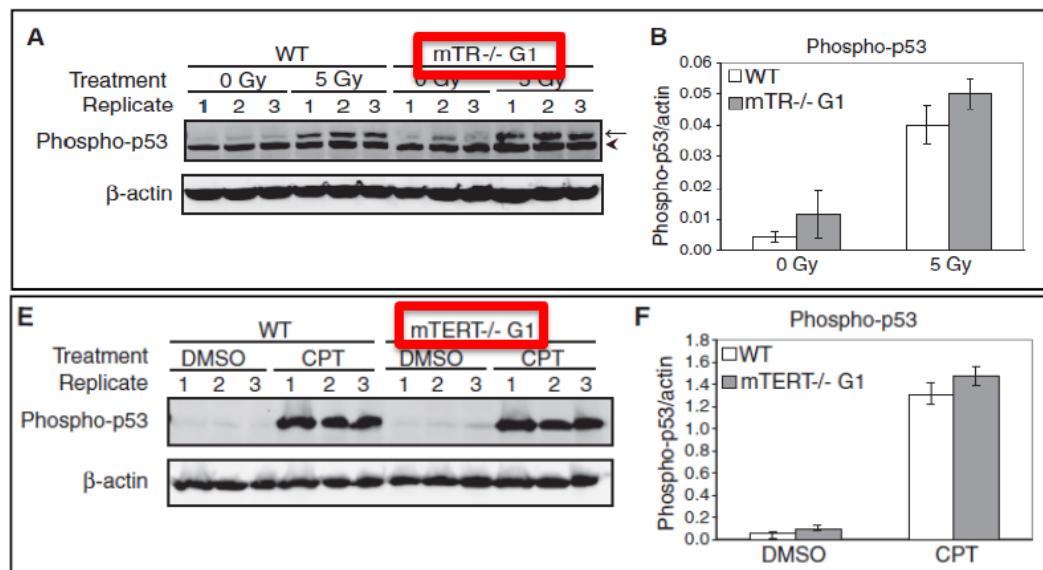
Photo: U. Montan

Jack W. Szostak

Comparing effects of mTR and mTERT deletion on gene expression and DNA damage response: a critical examination of telomere length maintenance-independent roles of telomerase

Sofia L. Vidal-Cardenas^{1,2} and Carol W. Greider^{1,*}

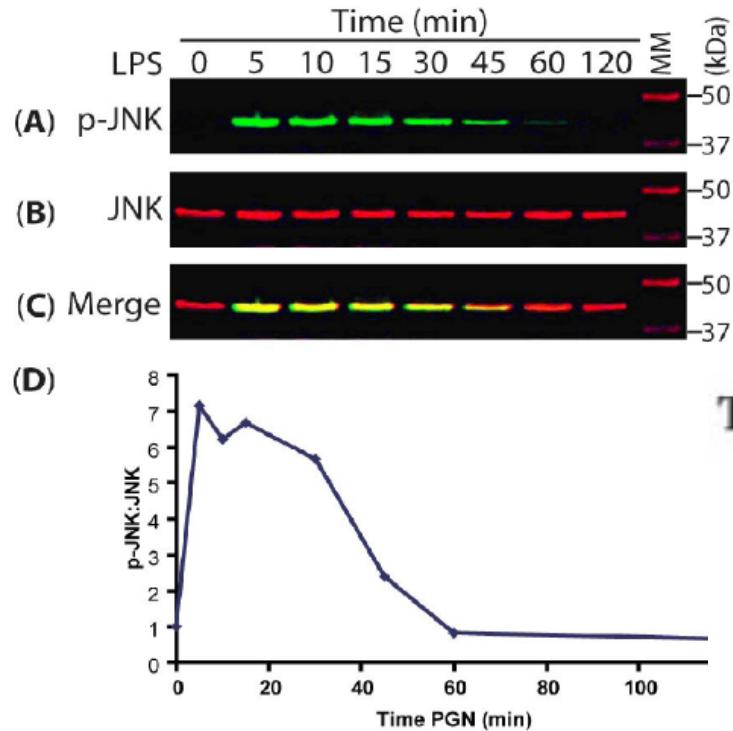
¹Department of Molecular Biology and Genetics and ²Graduate Program in Biochemistry, Cellular and Molecular Biology, The Johns Hopkins University School of Medicine, Baltimore, MD, USA



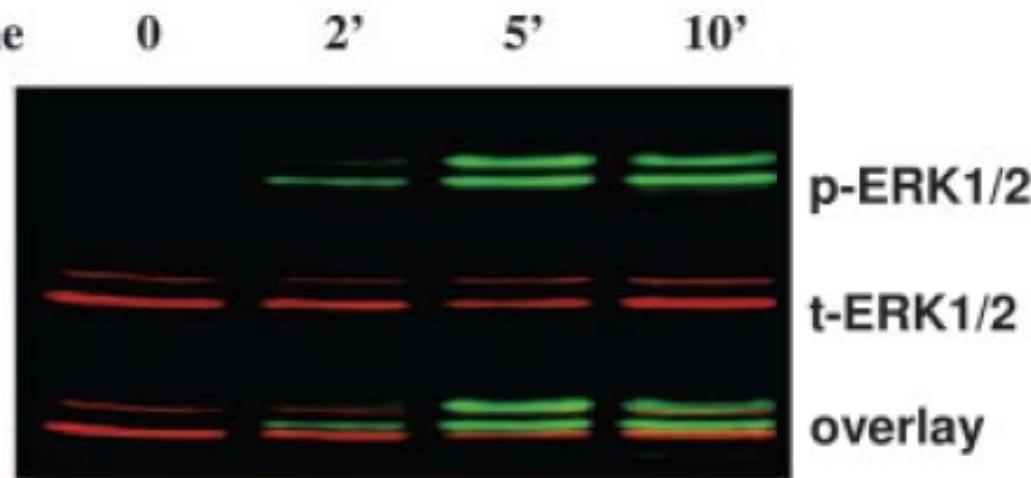
Nucleic Acids Research 38: 60–71 (2010) January

Phosphoprotein Analysis Signal Transduction

JNK Phosphorylation
in response to LPS treatment



Activation of MAPK by
Corticotropin Releasing Hormone

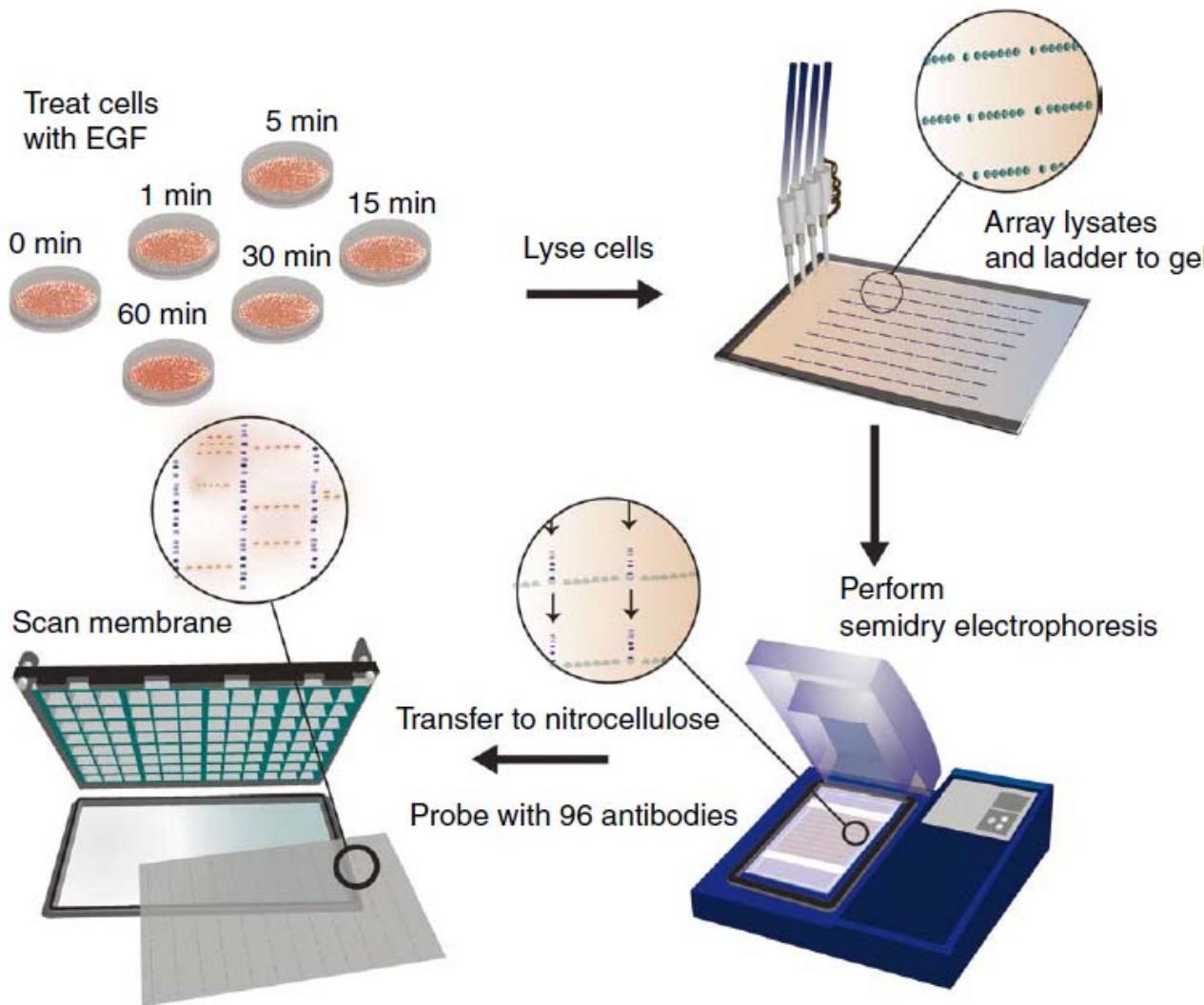


Biol. Proced. Online 2008; 10(1):20-28

Mol. Endocrinol. 20:3179-3195 (2006)



MicroWestern Array

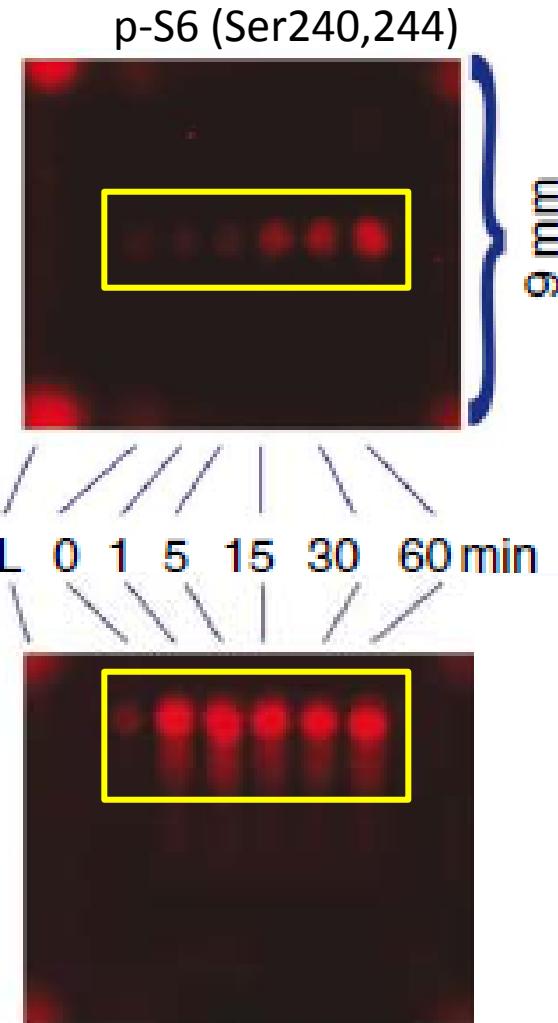
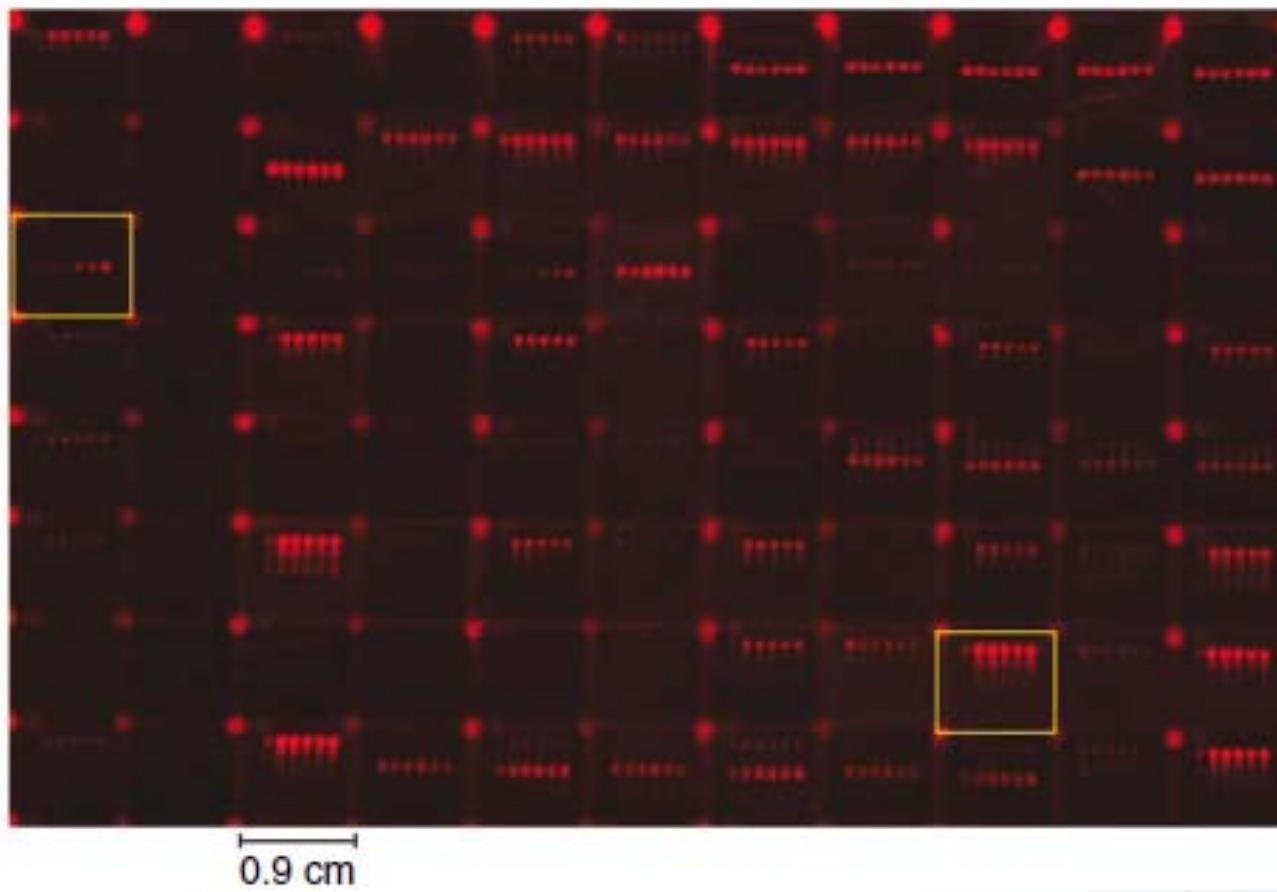


Jones' laboratory

Ben May Institute
for Cancer Res.
Univ. of Chicago

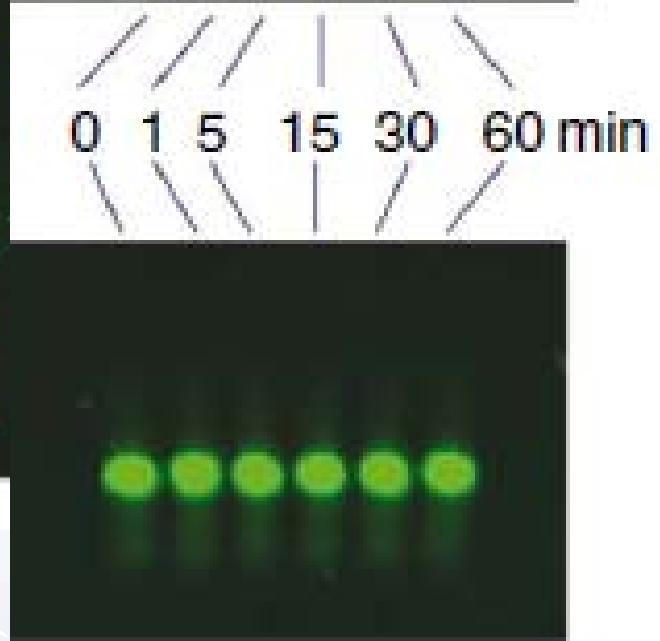
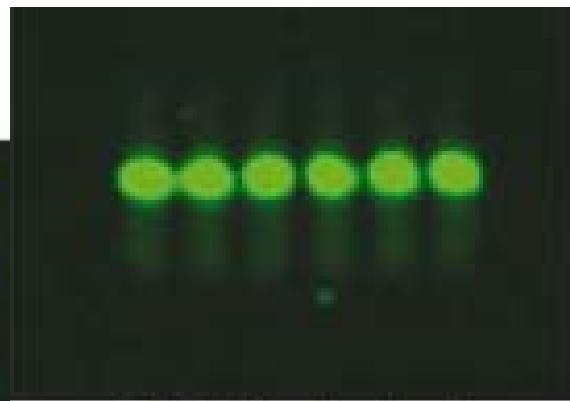


MicroWestern Array

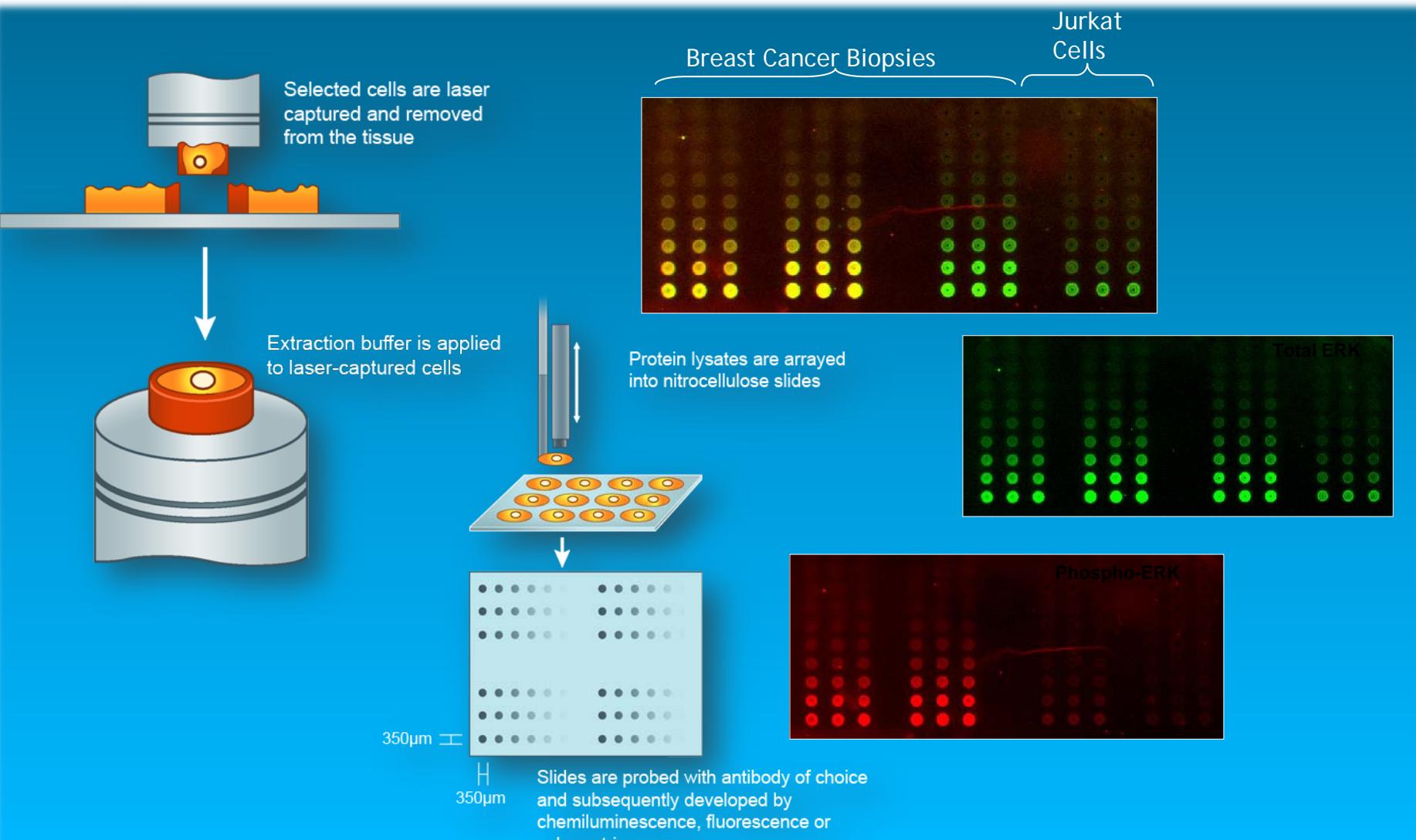




MicroWestern Array

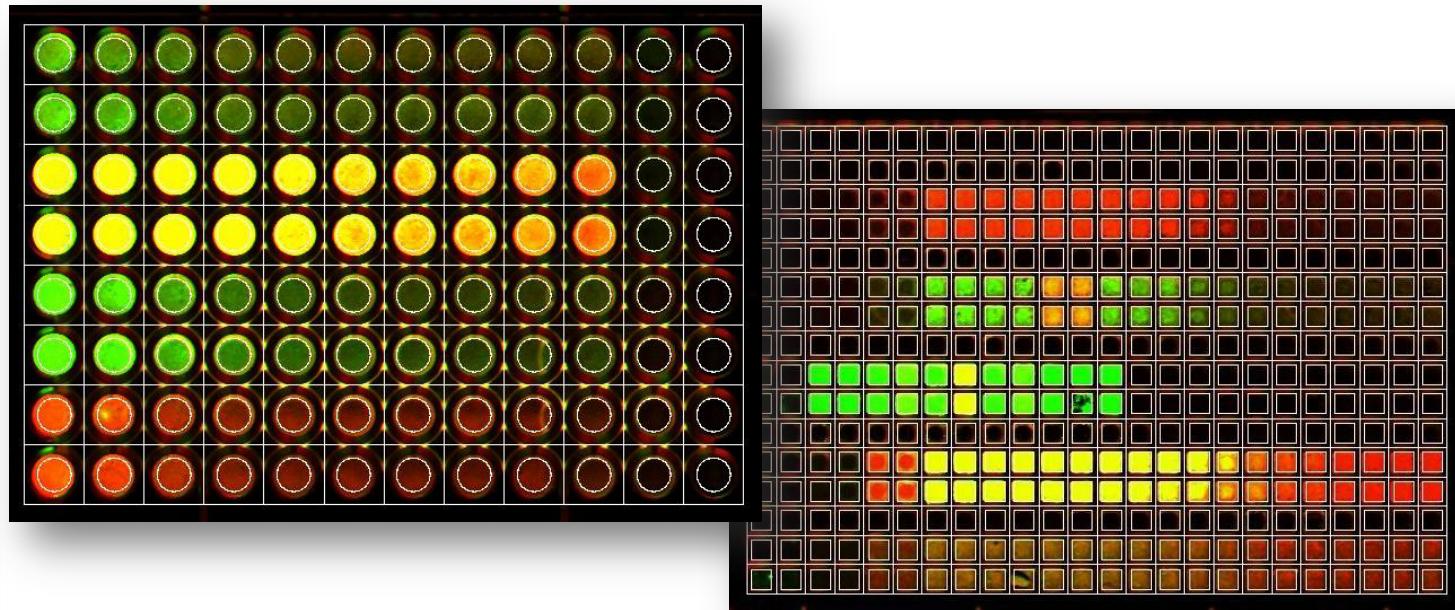


Reverse Phase Protein Arrays





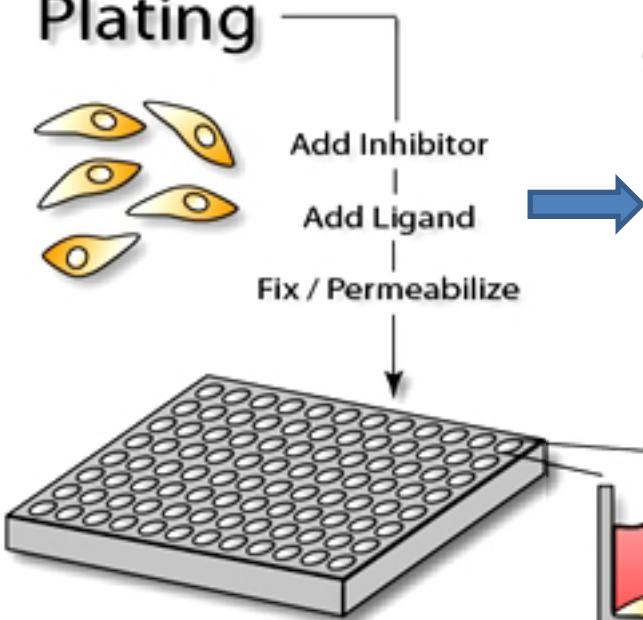
In-Cell Western™ Assay



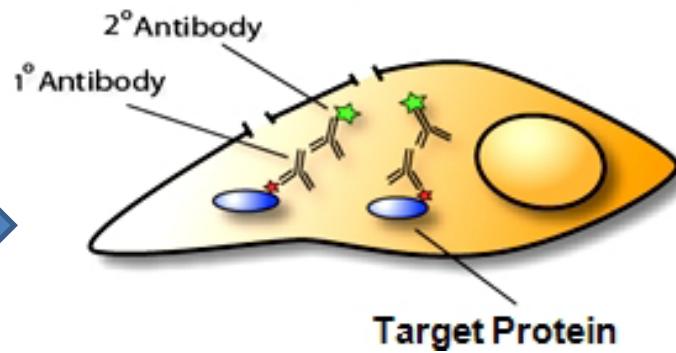


In-Cell Western™ Assay

Plating



Antibody Staining

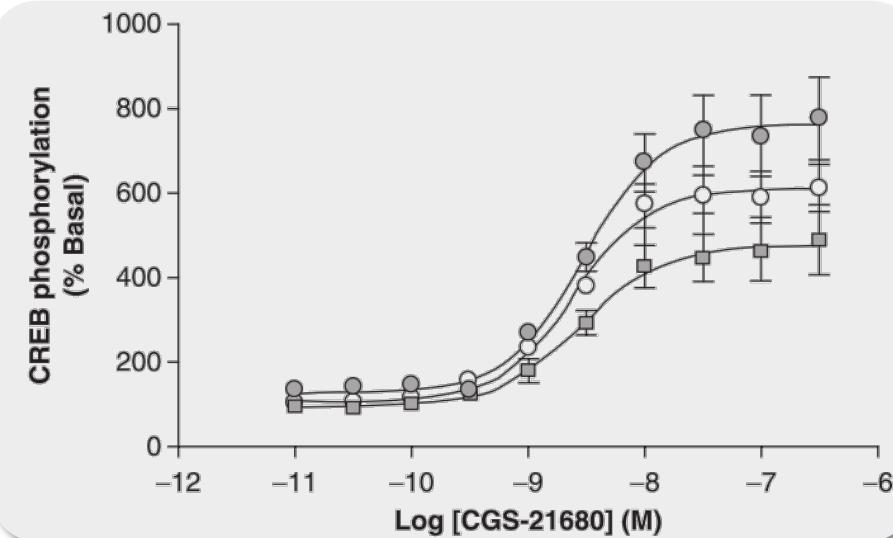
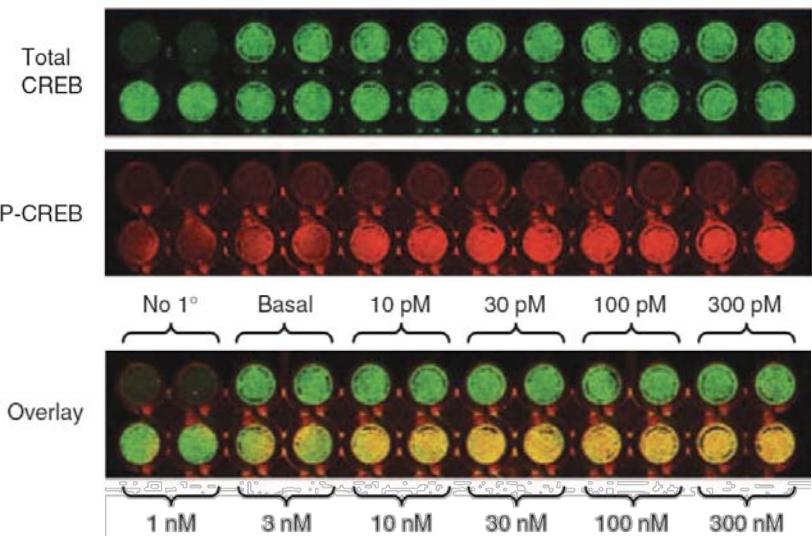


Detection



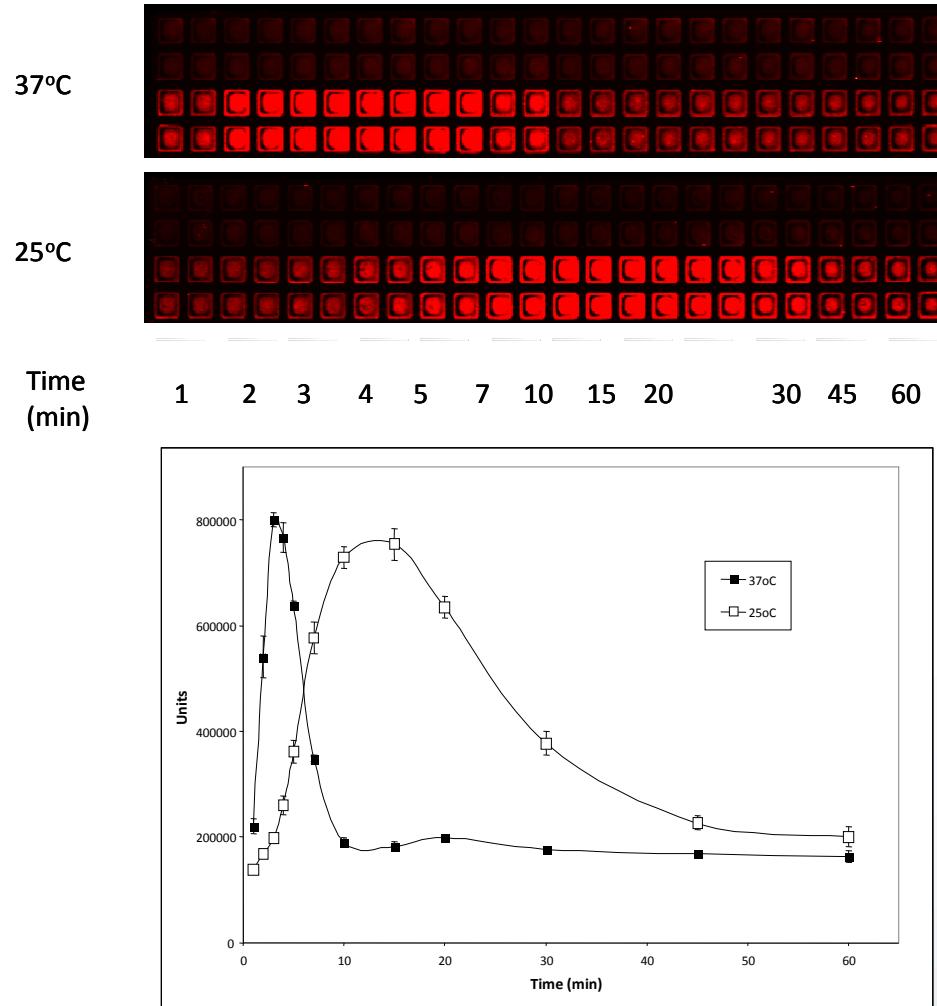
Odyssey
Imager

Dose-Response Analysis



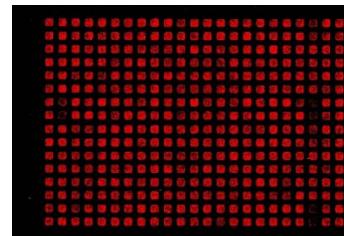
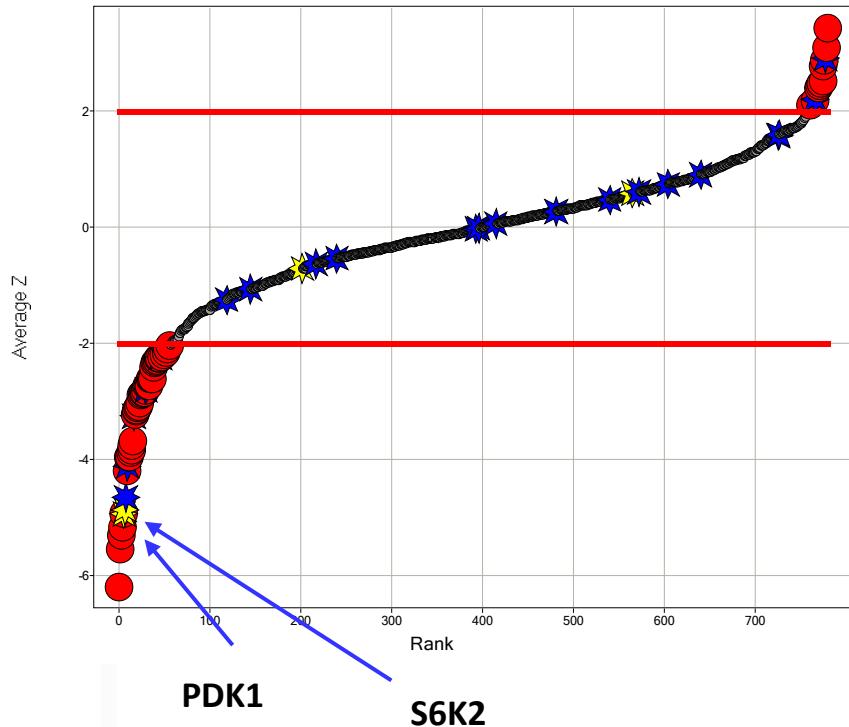


Sygnaling Dynamics

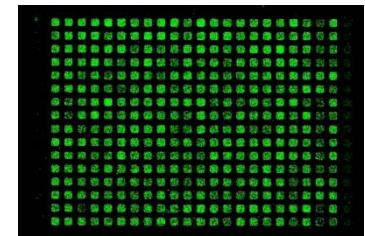




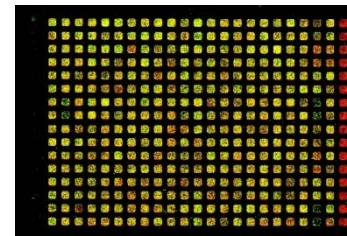
siRNA Screen



700 Channel: Alexa-680
(cell # Normalization)



800 Channel: phospho-S6



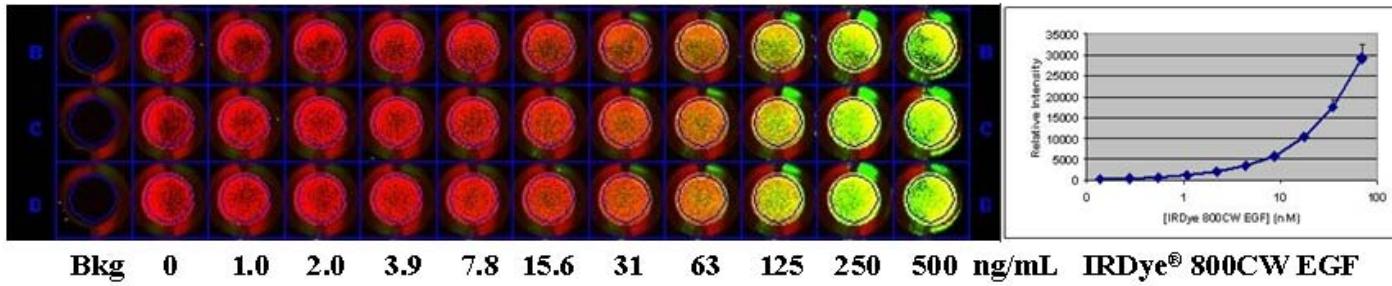
Overlay

Human kinome screen in asynchronously growing HeLa Cells



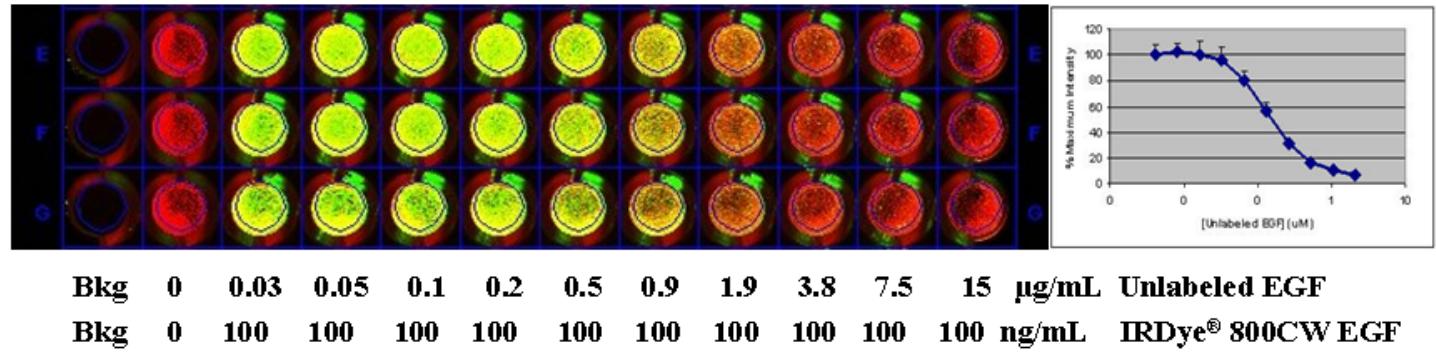
On-Cell Western™ Assay

Analysis of Cell Surface Proteins



Optical Agent Binding Assay:

- A dose response was noted when A431 cells were incubated with increasing concentrations of IRDye 800CW EGF (green). Cells were normalized to TO-PRO-3 staining (red).



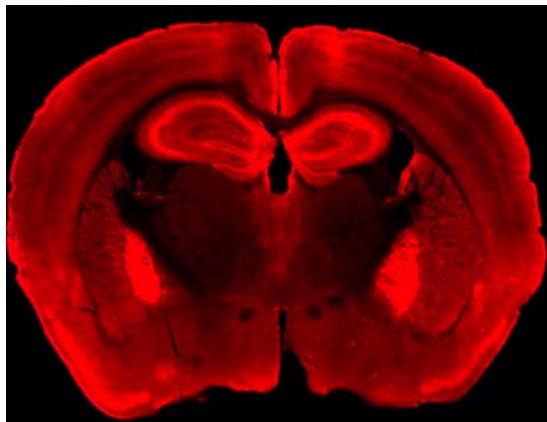
Optical Agent Competitive Challenge:

- Increasing concentrations of unlabeled EGF successfully competed for binding sites with IRDye 800CW EGF (green). Cells were normalized to TO-PRO-3 staining (red).

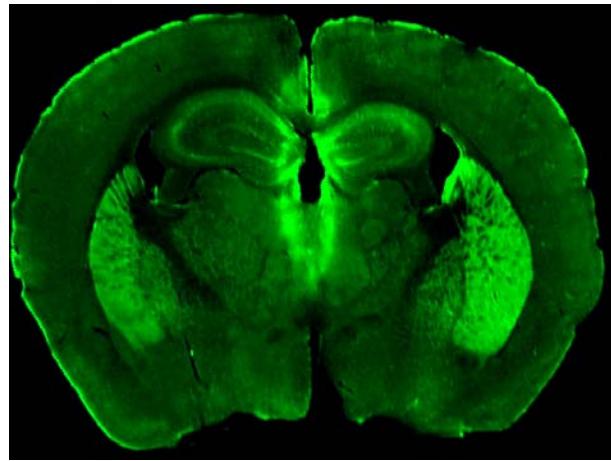


Immunohistochemistry Imaging

CB1 and Dopamine D2 receptors



CB1



Rabbit α D2



Merged



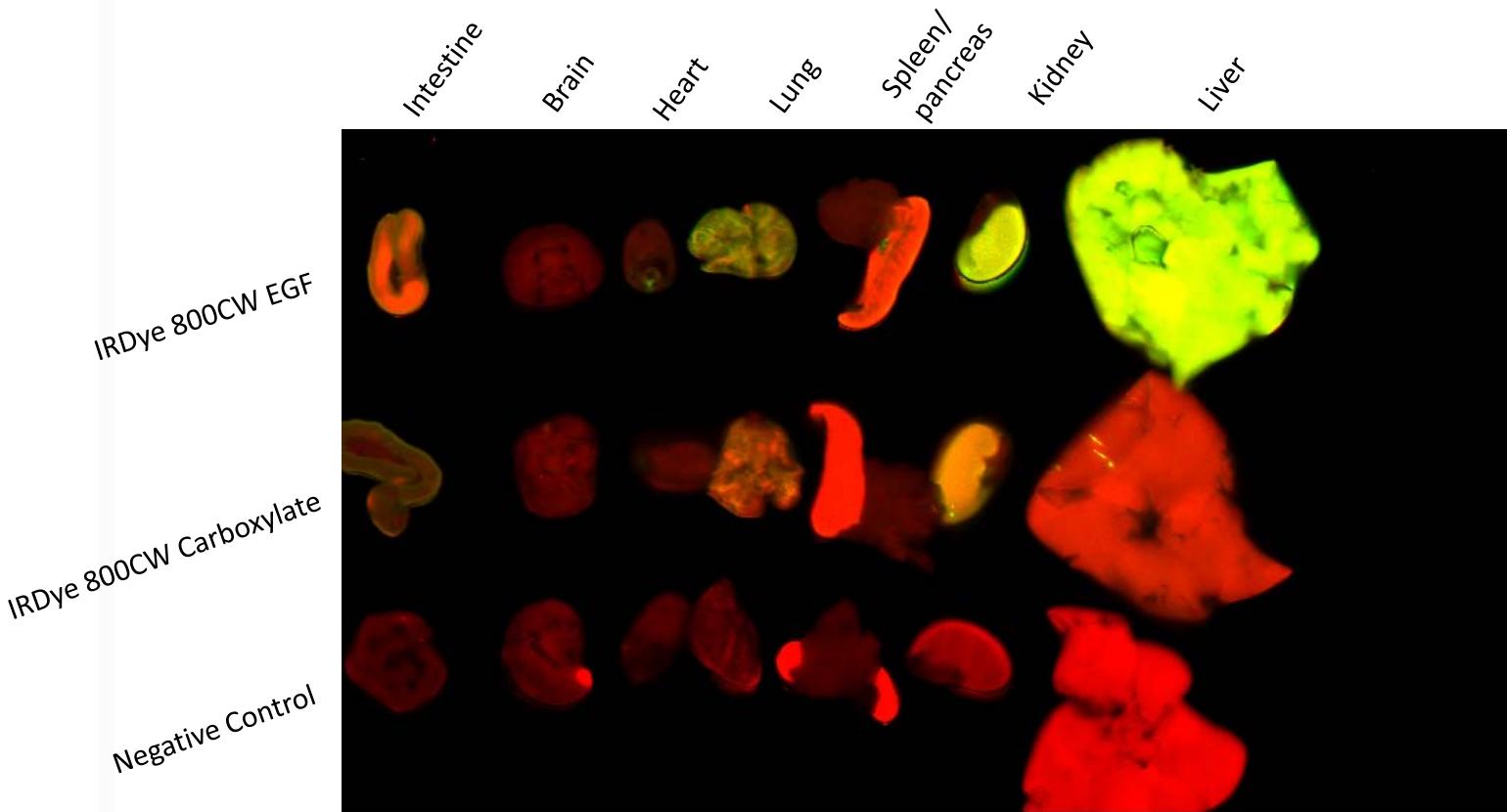
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Data Courtesy of Dr. Chris Kearn

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Molecular Imaging – Organs

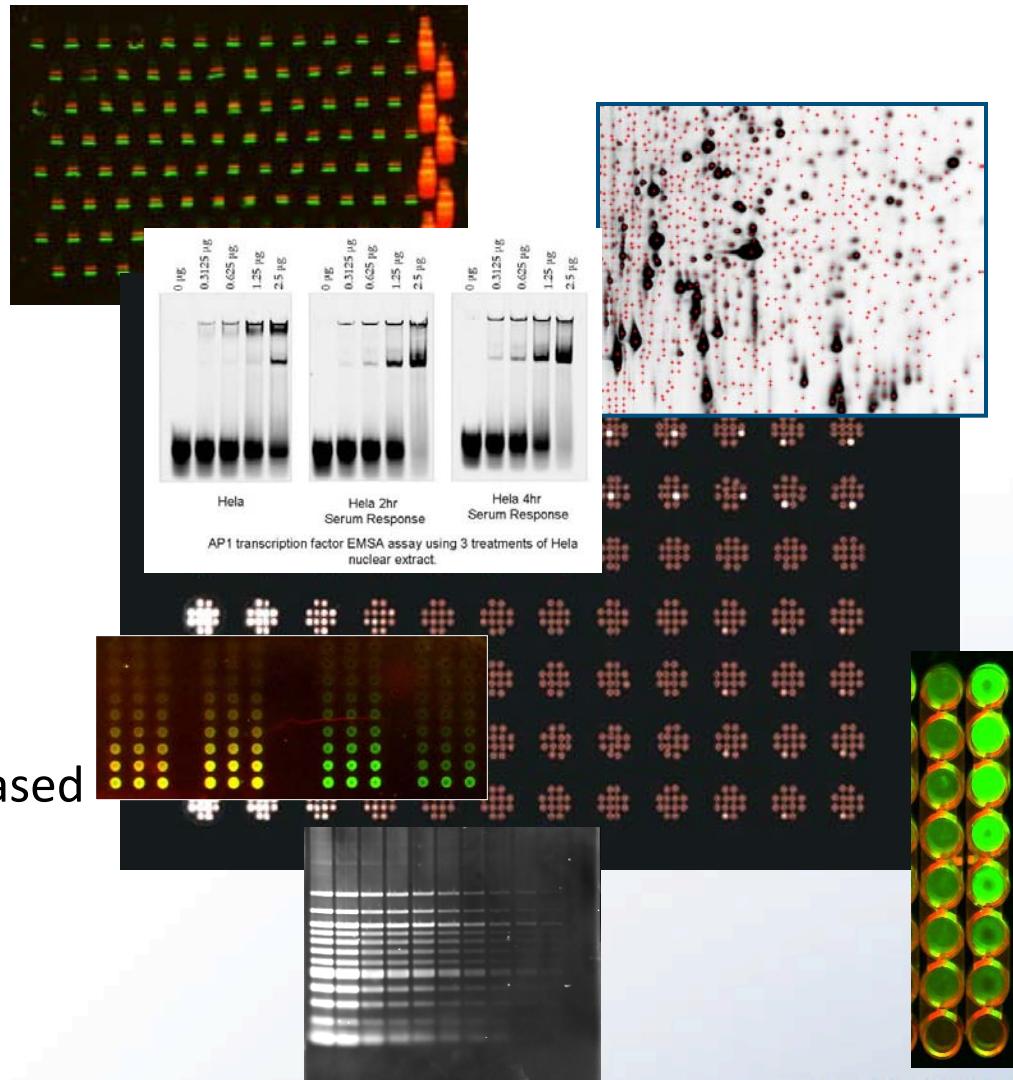


Mice were imaged post injection of 1X PBS, IRDye 800CW carboxylate, or IRDye 800CW EGF. Animals were sacrificed and organs excised for imaging on Odyssey. 800 nm channel is presented in green; 700 nm presented in red.

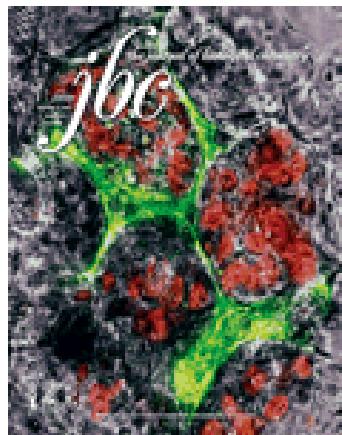


Additional Applications

- Protein Detection
 - 1-D / 2-D Coomassie®
 - In-Gel Westerns
- Nucleic Acid Detection
 - EMSA / Gel Shift Assays
 - DNA Staining
- Microwell Assays
 - ELISA/FLISA
 - Cell Proliferation
 - Protease Assays
 - Cell Mobility and Other Cell-based Assays
- Protein arrays
 - RPPA, Direct Phase arrays



Gel Shift / Quantitative EMSA



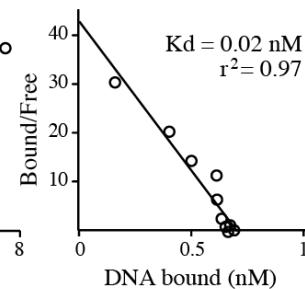
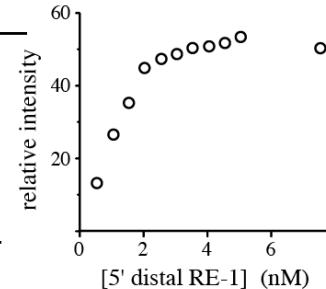
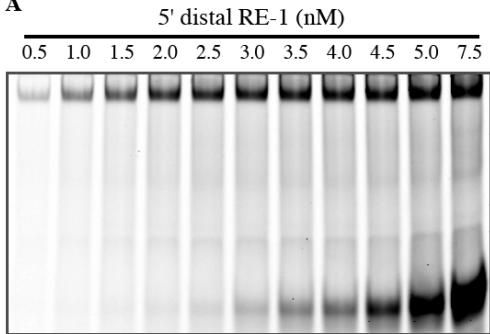
J. Biol. Chem., Vol. 281, Issue 6, 3190-3197, February 10, 2006

Characterization of Distinct Stat5b Binding Sites That Mediate Growth Hormone-stimulated *IGF-I* Gene Transcription*

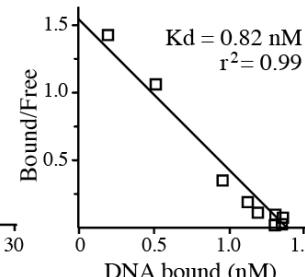
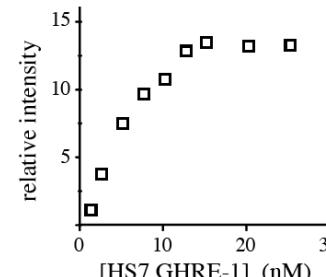
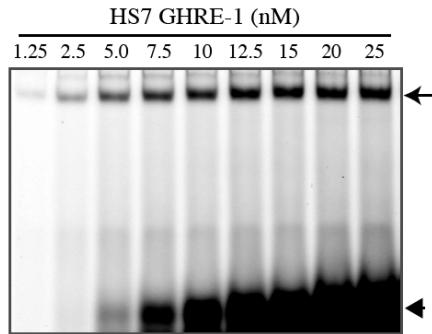
Dennis J. Chia[†], Mitsuru Ono[†], Joachim Woelfle[†], Mylynda Schlesinger-Massart[†], Honglin Jiang[§], and Peter Rotwein^{†1}

From the [†]Department of Biochemistry and Molecular Biology, Oregon Health & Science University, Portland, Oregon 97239 and the [§]Department of Animal and Poultry Sciences, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061

A

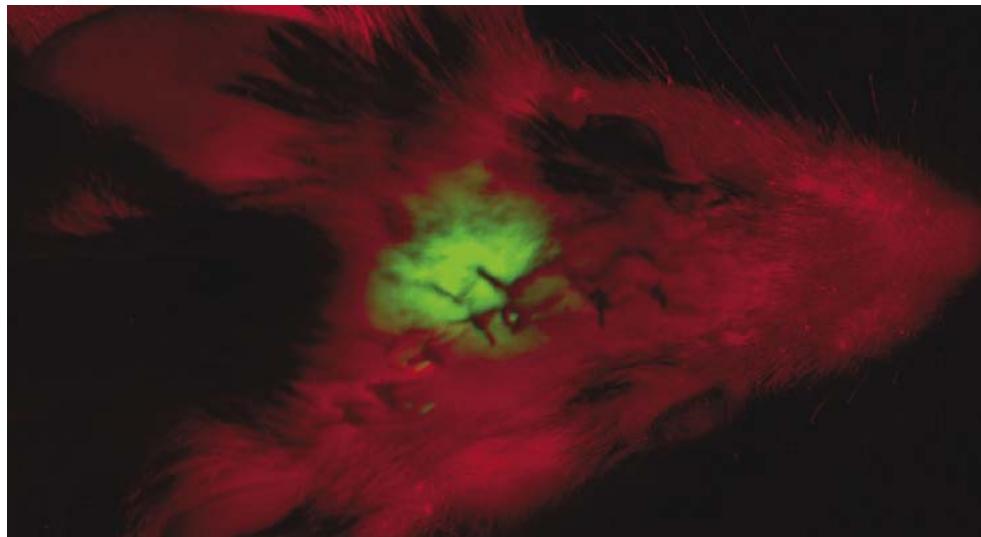


B



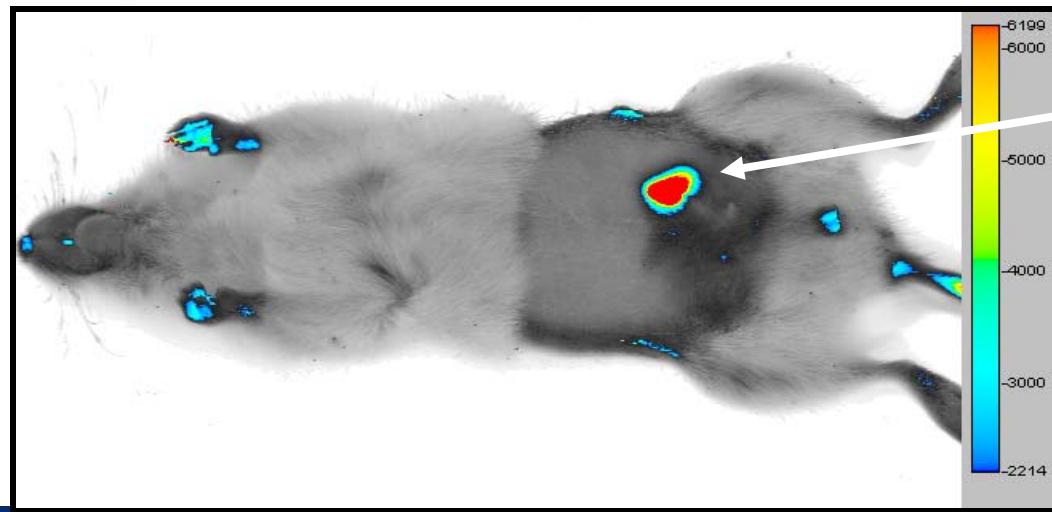


In vivo Imaging with Odyssey®



Intracranial anti- β -amyloid antibody injection.

Skoch & Backskai, Mass. Gen. Hosp.



Orthotopic prostate tumor detected with EGF - IRDye® 800CW

* Imagen tomada 4 días post-inyección



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Odyssey® MousePOD™

- Mouse imaging accessory
- Can be connected to gas anesthesia (3 animals)
- Controlled temperature
- Doesn't require any modifications to the instrument

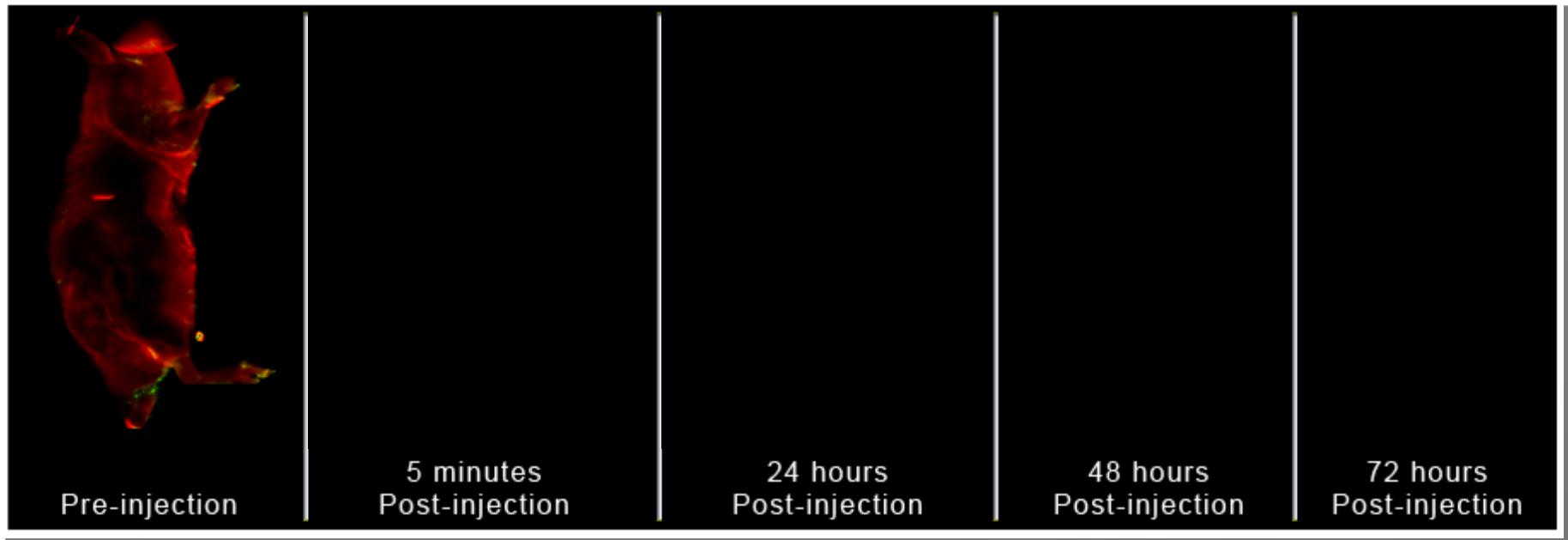


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Imaging Timeframe



- IRDye® 800CW EGF localization and clearance in subcutaneous tumor

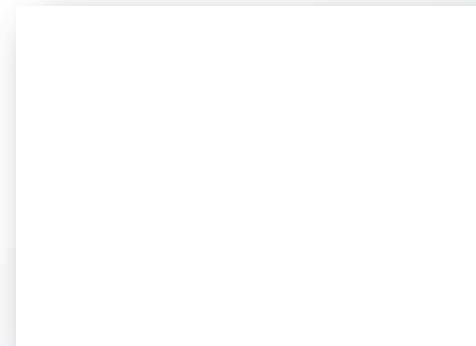
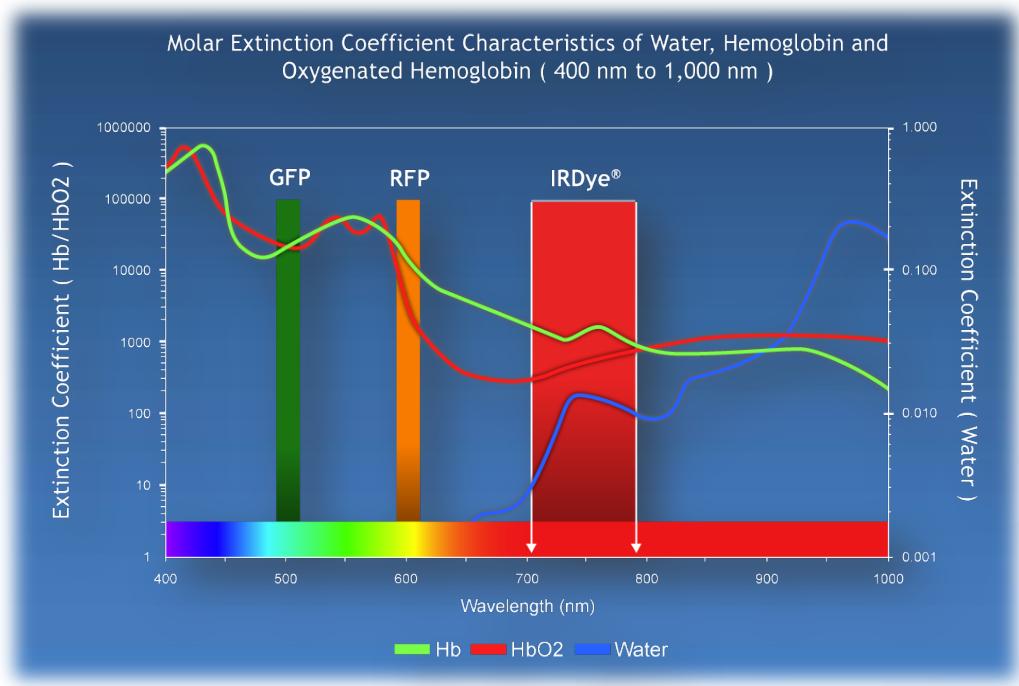
The NIR Advantage

- NIR: Lower absorbance and fluorescence from tissues and cells
- Lower scattering at higher wavelengths



Lower background

Higher signal : noise



Courtesy Dr. Mark Whitney, UCHSC

Fixed NIH3T3cells
Exposure Times:
430 nm: 5592 msec
488 nm: 4977 msec
532 nm: 6007 msec
784 nm: 17,578 msec

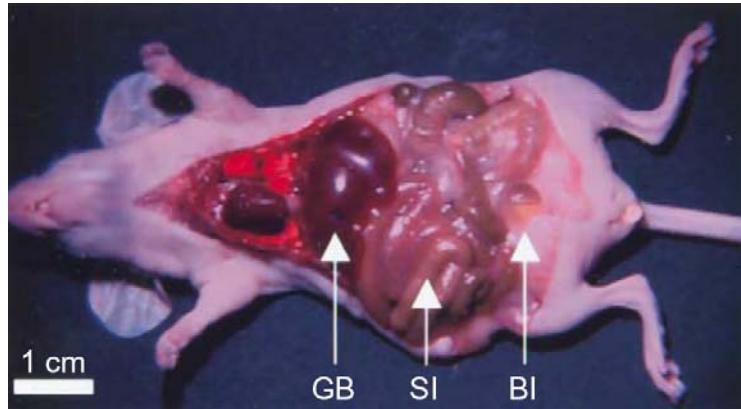


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The NIR Advantage

white light



green/red



blue/green

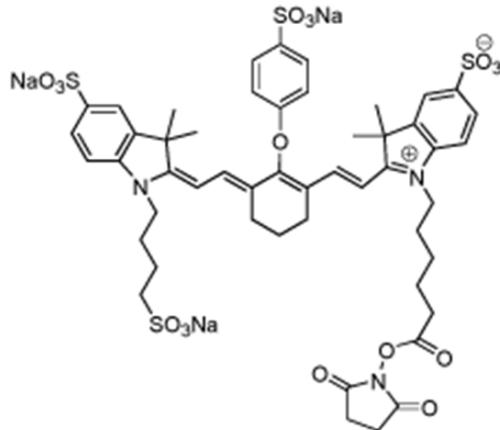


NIR

IRDye Contrast Agents

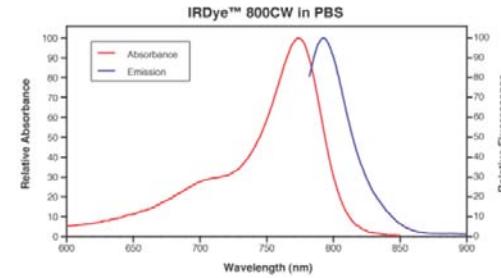
- NIR spectrum -> High Signal/Noise
- High Extinction coeff. -> Bright
- High solubility -> Good clearance
- Low Toxicity

- IRDye800 CW – NHS ester
- IRDye800 CW - maleimide
- IRDye800CW Carboxylate
- IRDye680 – NHS ester
- BrightSite™ Optical probes
- QC-1 Quencher



IRDye® 800CW NHS Ester

C₅₀H₅₄N₃Na₃O₁₇S₄
Exact Mass: 1165.20291
Mol. Wt.: 1166.20297



Absorbance Spectrum | Emission Spectrum | Combination Graph

Solvent	Extinction Coefficient M ⁻¹ cm ⁻¹	Abs Max nm	Em Max nm	Stokes Shift nm	MW g/mole
Methanol	300,000	778	794	16	1166
Water	240,000	774	789	15	1166
1X PBS	240,000	774	789	15	1166
PBS/Methanol	270,000	777	791	14	1166



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IRDye® 800CW Toxicity Study Published

LI-COR Reports on Toxicity Study of IRDye® 800CW Carboxylate

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RESEARCH ARTICLE

Single-Dose Intravenous Toxicity Study of IRDye® 800CW in Sprague-Dawley Rats

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Abstract

Objective: Fluorophore-labeled contrast imaging agents are moving toward clinical use for a number of applications. The near-infrared dye IRDye 800CW is frequently used in its N-hydroxysuccinimide (NHS) ester form for labeling these agents. Following conjugation or breakdown of a labile ligand, excess NHS ester is converted to the carboxylate form. To prepare for clinical use as a near-infrared fluorophore, a toxicity study was conducted on IRDye 800CW carboxylate.

Methods: Male and female Sprague-Dawley rats were given a single intravenous or intradermal administration of IRDye 800CW carboxylate. Indamine Green was used as a comparative control. Survival was monitored for 14 days. At the end of the study, rats were euthanized and observed for up to 14 days. Clinical chemistry, hematology, and pharmacokinetic analyses were performed on subgroups of animals. Organs were analyzed for content of the test article. Tissues were analyzed microscopically for pathological changes.

Results: Based on hematology, clinical chemistry, and histopathologic evaluation, single doses of IRDye 800CW carboxylate at dose levels of 1, 5, and 20 mg/kg or 20 mg/kg intradermally produced no pathological evidence of toxicity.

Conclusion: A dose of 20 mg/kg was identified as the no observed adverse effect level following IV or ID routes of administration of IRDye 800CW.

Key words: IRDye 800CW, Toxicity study, Pharmacokinetics, Optical imaging

Introduction

With recent advances in optical imaging technology and instrumentation, targeted fluorophore-labeled contrast agents are moving toward translation to human clinical diagnostic use. The primary applications for clinical optical imaging are envisioned to be in real-time intraoperative surgical resection of tumor and nodal metastasis, endoscopy, and lymphovascular imaging. Near-infrared (NIR) fluorescent

dyes (Ex>750 with red-shifted emission) are the preferred signal-generating molecules for labeling targeted ligands owing to the significant tissue penetration of emission light and the clear distinction from the lack of autofluorescence owing to endogenous fluorophores [1, 2].

Indocyanine green (ICG) has been used for decades as an angiographic contrast agent and has been recently translated to the clinic for noninvasive lymph mapping [3–6]. More recently, ICG has been used for fluorescence molecular tomography [7, 8] bodies well for NIR fluorescence imaging as a clinically viable molecular imaging modality. Unfortunately, the clinically approved form of ICG does not have a ready functional group and cannot be used to label targeting agents. There has been considerable recent effort to develop general-purpose NIR fluorophores that can be conjugated to

❖ In 2009, LI-COR reported on a Toxicity Study of IRDye® 800CW Carboxylate.

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❖ LI-COR is preparing to submit a Drug Master File on IRDye 800CW to the U.S. Food and Drug administration

❖ LI-COR is currently producing IRDye800CW using a cGMP process available for clinical studies

QUESTIONS? Contact LI-COR Business Development at busdev@licor.com

IRDye 800CW has not been reviewed or approved by the FDA and is for investigational use only



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BrightSite™ Optical Agents

Developed systematically for *in vivo* research

Optimized specificity

IRDye 800CW EGF Optical Probe

Optimized binding capacity

IRDye 800CW 2-DG

Optimized clearance

IRDye 800CW RGD

IRDye 800CW HA

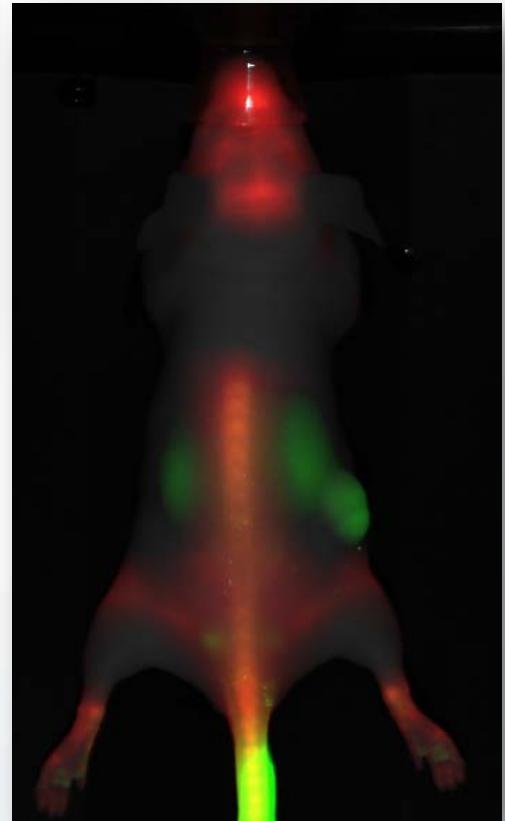
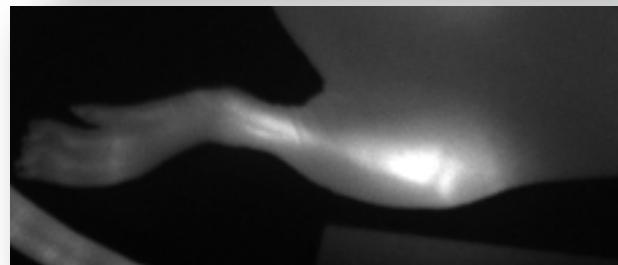
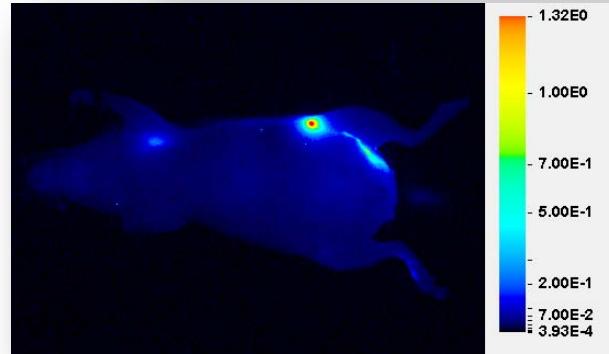
IRDye 800CW PEG

IRDye 800CW or 680 BoneTag™

CellVue® NIR 815 / Burgundy

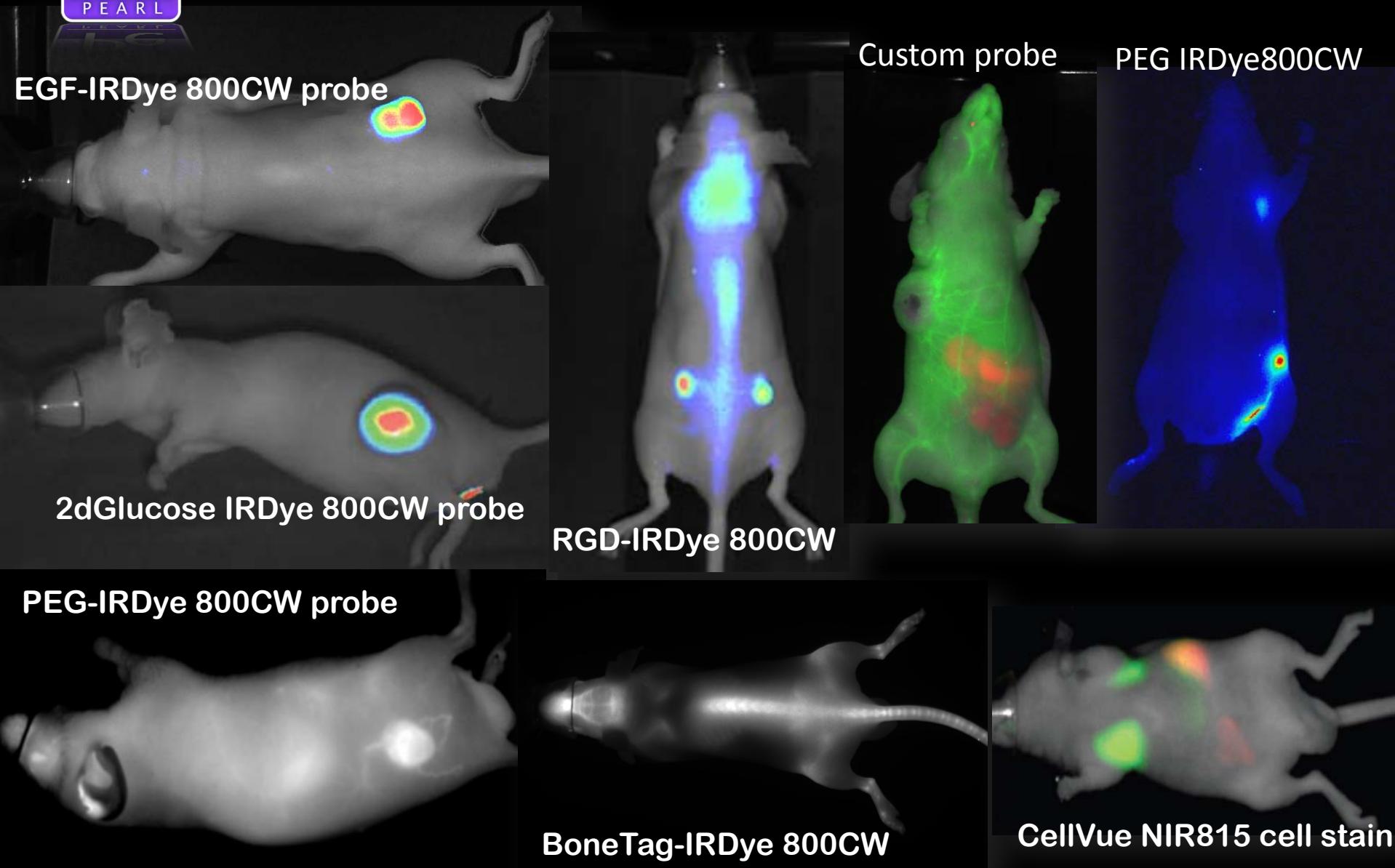


Pearl Imager: The NIR Fluorescence *in vivo* Imager



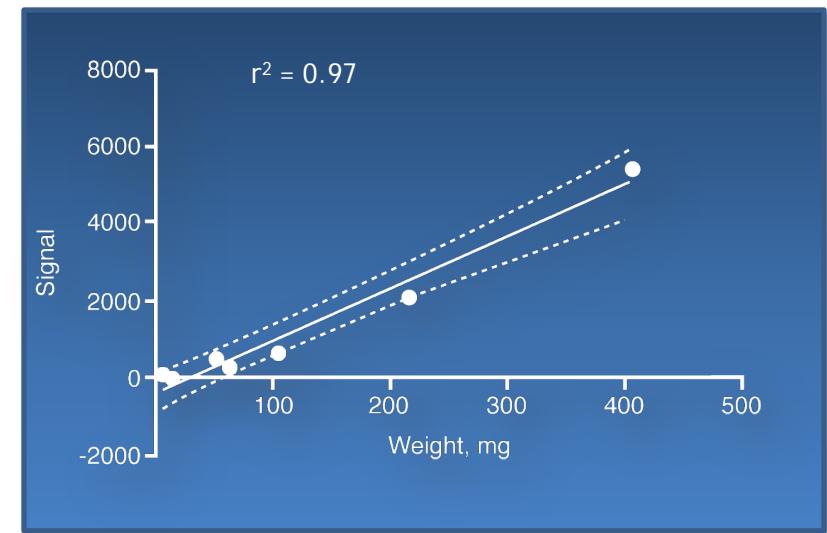
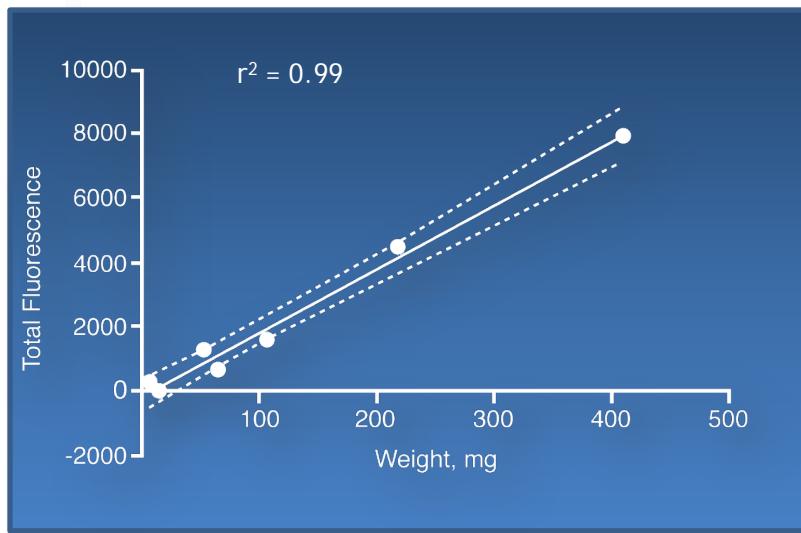
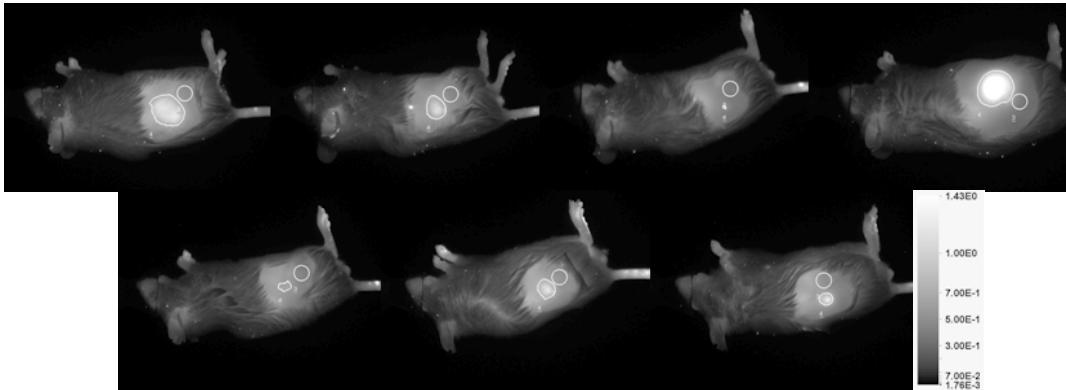


Animal Imaging Applications





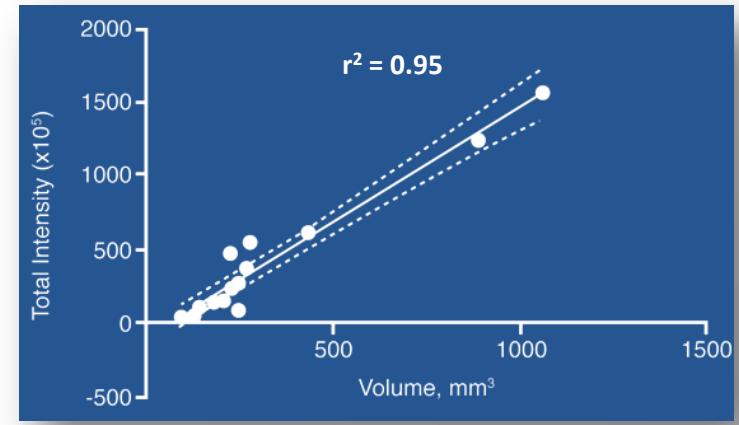
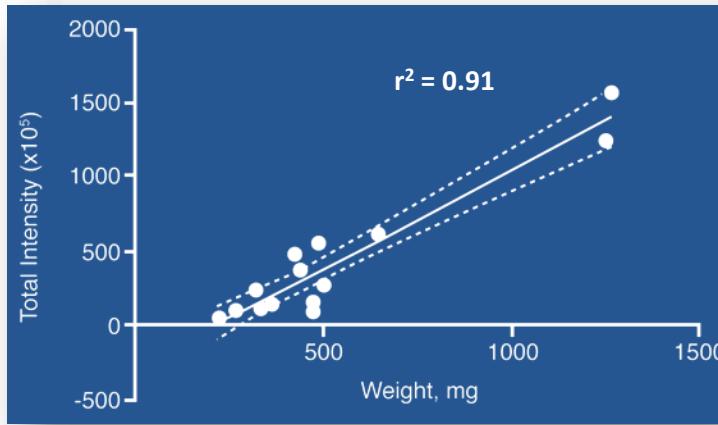
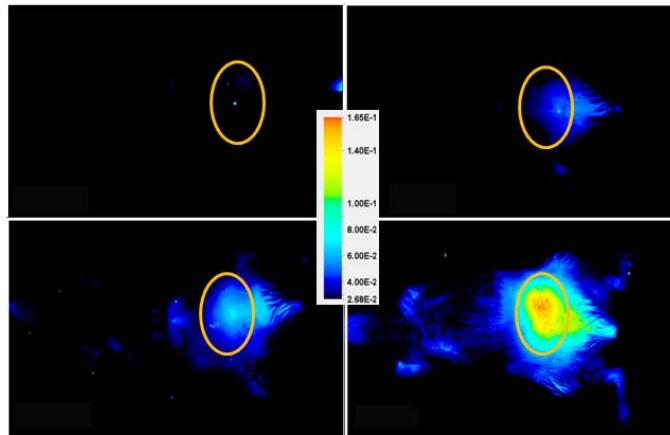
Correlation of Tumor size to Signal Subcutaneous Implantation



The data show that 99% and 97% of the variance in weight can be explained by the variation in Total fluorescence and Signal ($P = 0.05$)



Correlation of Tumor size to Signal Orthotopic Implantation

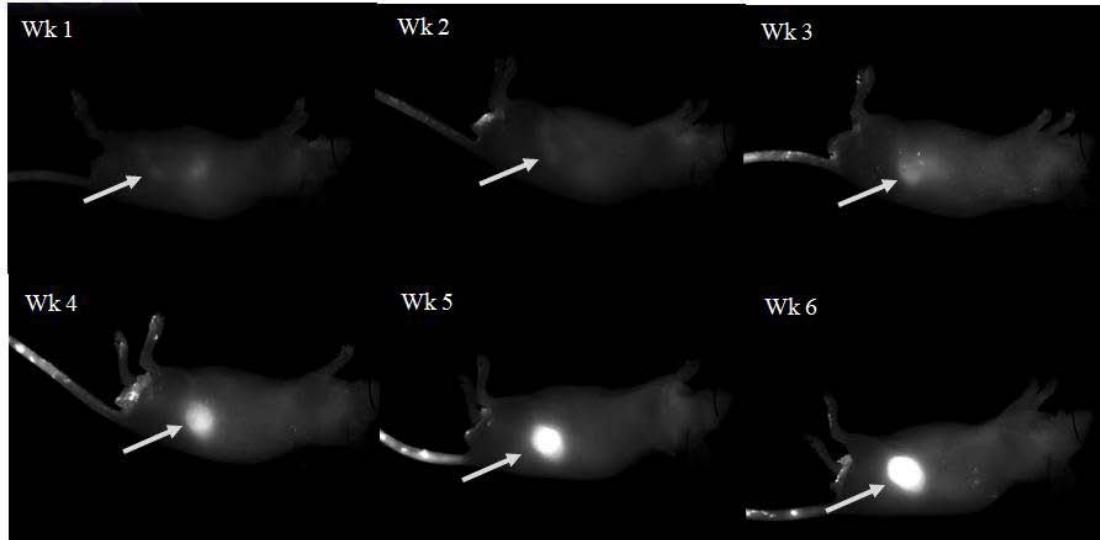


For this orthotopic tumor model and optical agent combination,
>91% of the variability in Total intensity can be explained by tumor weight.

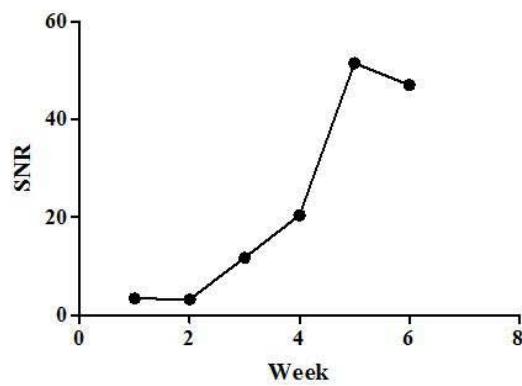


2-DG Longitudinal Study

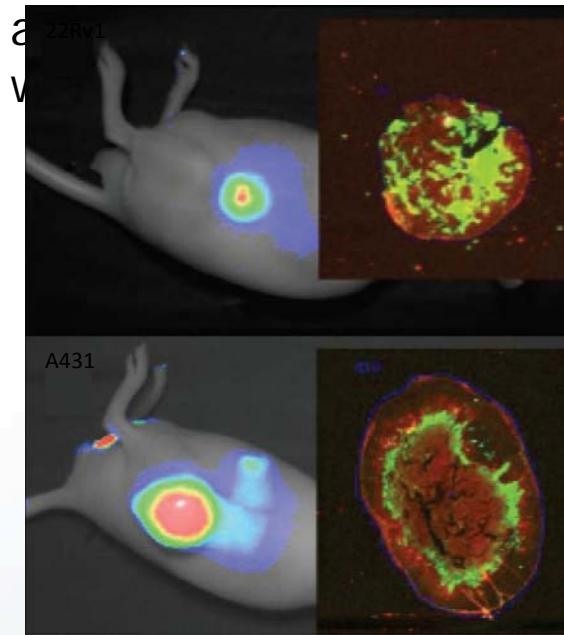
A



B



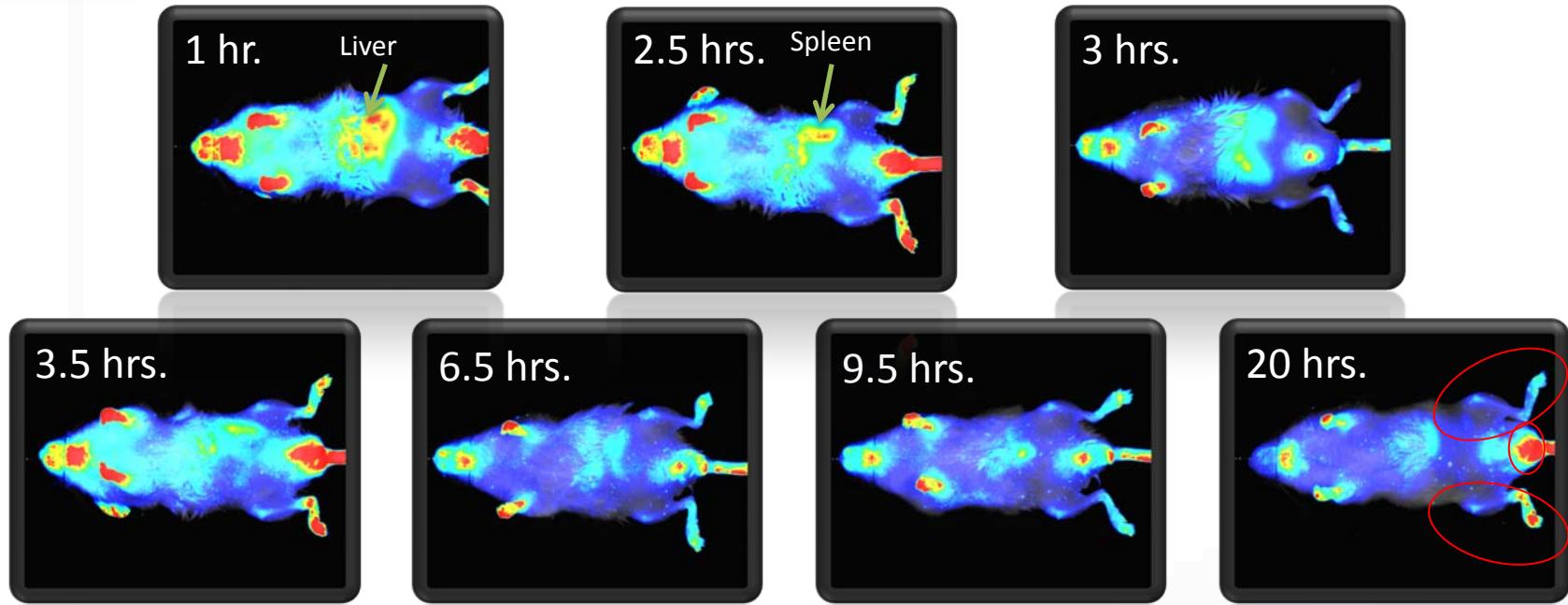
- Subcutaneous prostate tumor
- Imaged weekly 24 hrs.



Kovar et al. LI-COR Biosciences
Anal. Biochem. (2008) in press



Imaging of Leukemia Cells with CellVue® NIR815

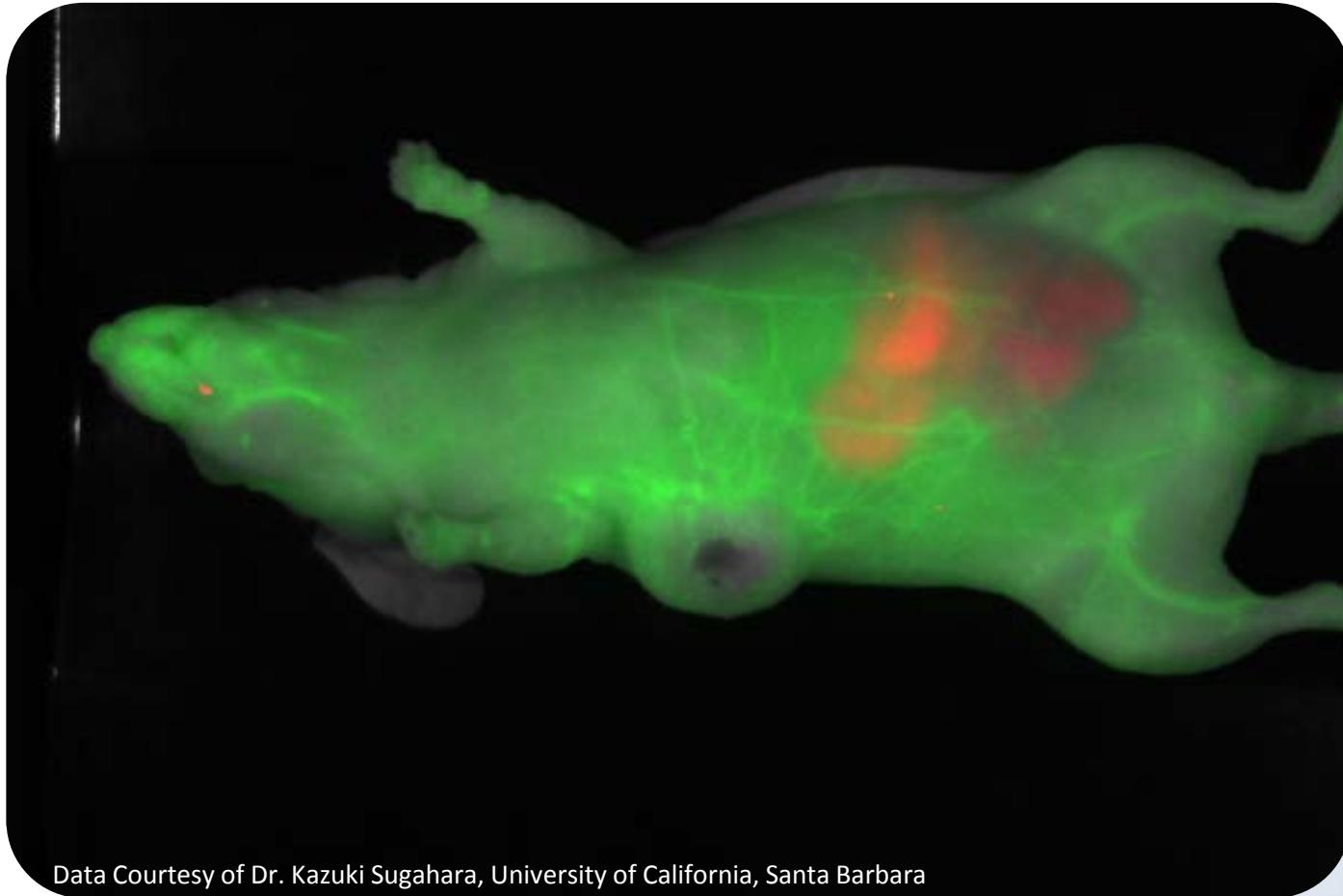


- 5×10^6 Nalm 6 cells were labeled with CellVue® NIR815 and injected i.v. into two animals
- Images were taken post injection at the indicated times

Data Courtesy of Dr. Linda J. Bendall and Dr. Carole Ford Westmead Millennium Institute University of Sydney, Australia



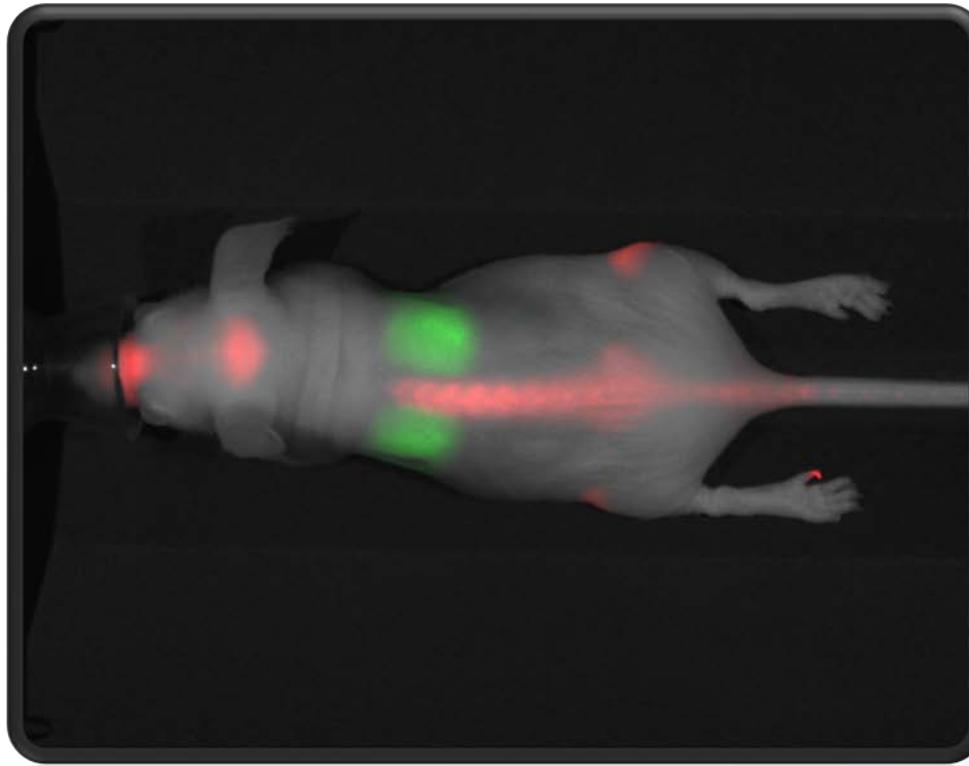
Arterial Wall Imaging



Data Courtesy of Dr. Kazuki Sugahara, University of California, Santa Barbara



Imaging Lung Engraftment

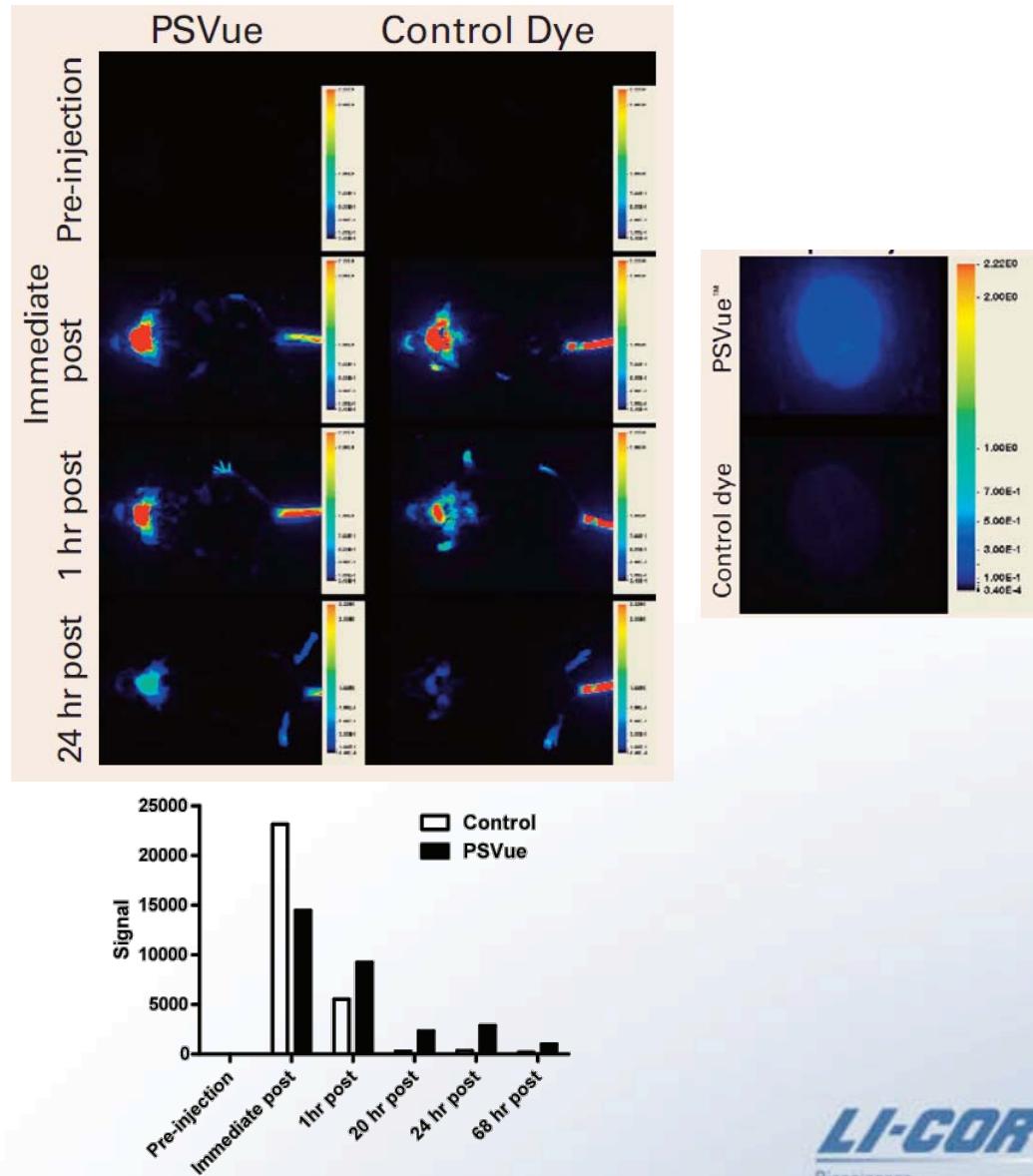


Dorsal view of a nude mouse injected with MDA-231 Luc breast cancer cells (IC injection 3 weeks prior to imaging) which demonstrated engraftment to the lungs (dorsal view). IRDye® 680 BoneTag was injected 7 days prior to imaging (Red). IRDye® 800CW EGF was injected 4h prior to imaging (Green).



Fluorescence Imaging in Brain

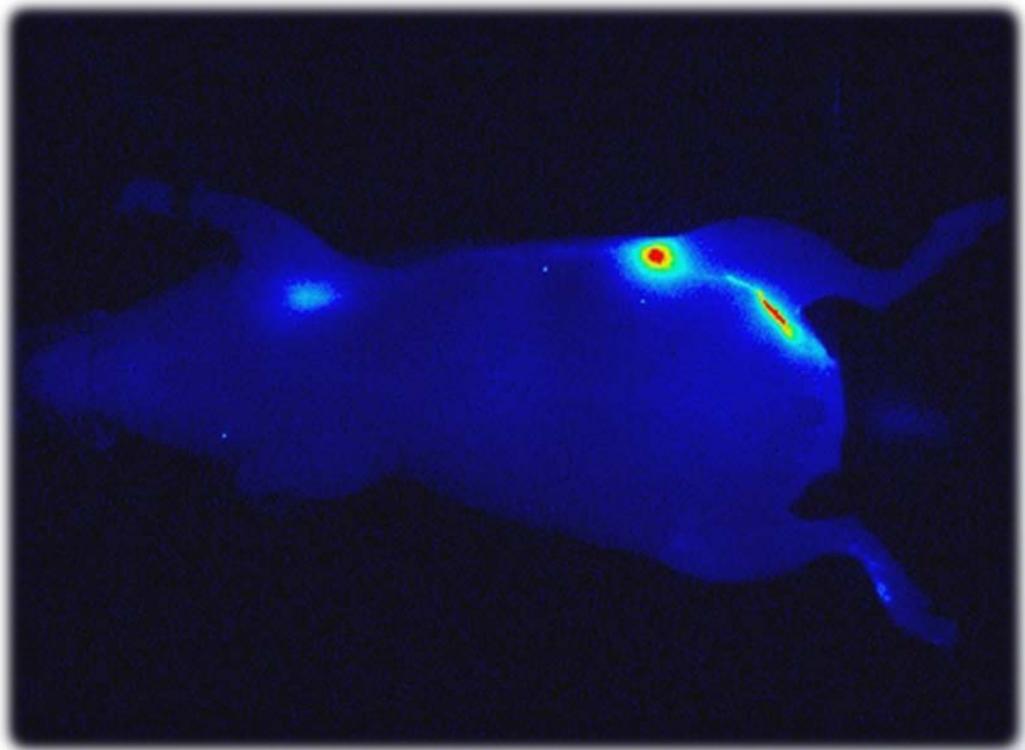
- Damage to dopaminergic neurons was induced with MPTP
- Mice were injected i.v. with PSVue® or a control dye
- Whole brain was imaged at 48 hrs post injection





Real-Time Imaging

- Intradermal injection (0.1 nmole) of IRDye® 800CW PEG
- Agent seen as it distributes through the lymphatic system and pools in the lymph node



Pearl®
impulse

NIR Fluorescence Advantages

Summary

- Fluorescence allows for quantification in Westerns
- Long wavelengths ensure good signal:noise ratio and sensitivity
- Better image quality
- Wide dynamic range
- Easy transition from other methods
- Versatility of applications
- Translatable to clinical applications
- Low cost of operation



Questions?



THANK YOU!!!