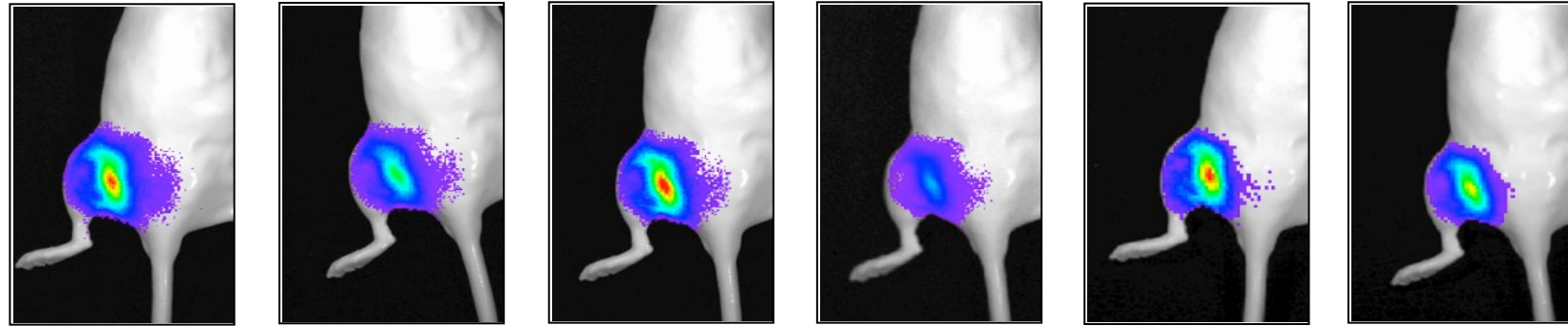


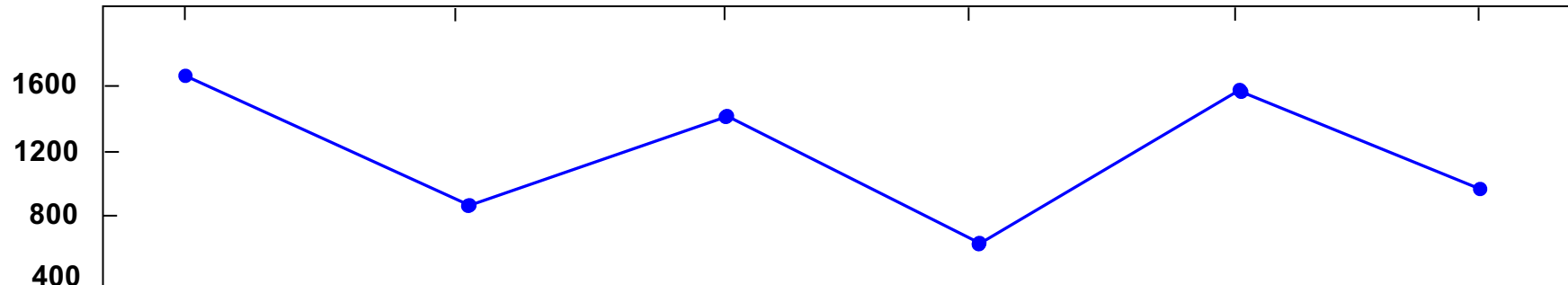
Raw Signal

Raw Signal
(Counts)



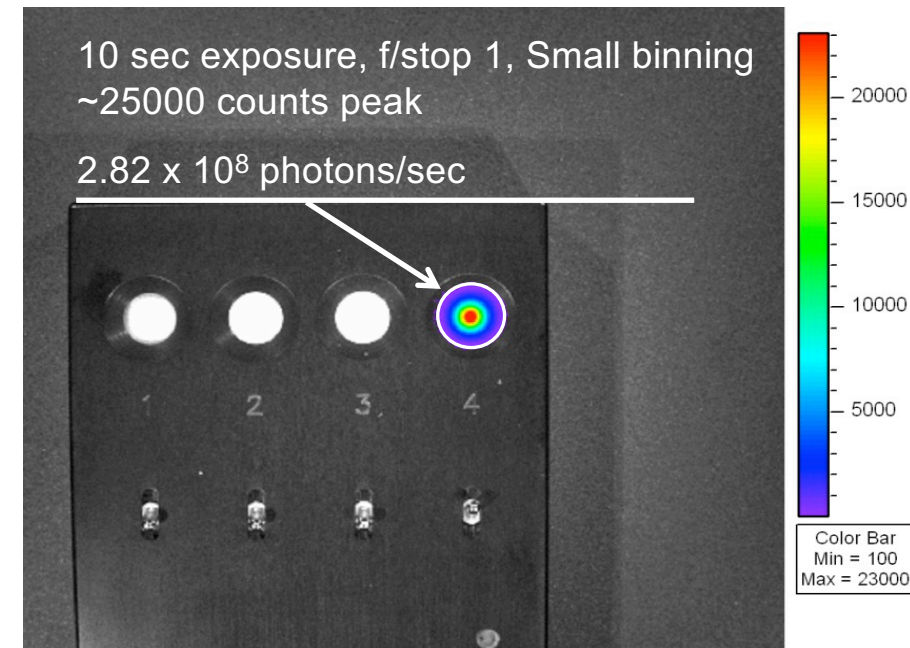
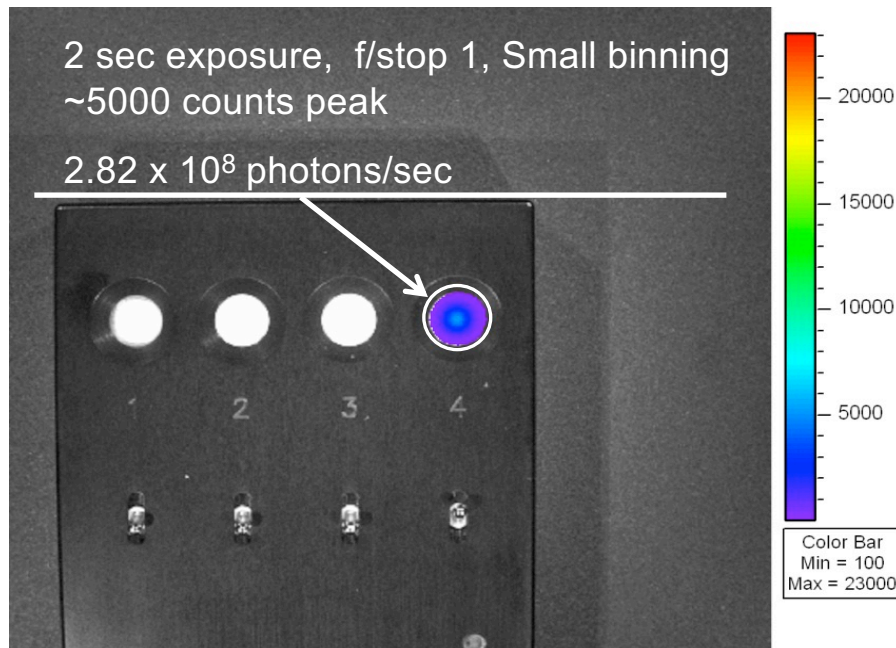
Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

Peak
Counts



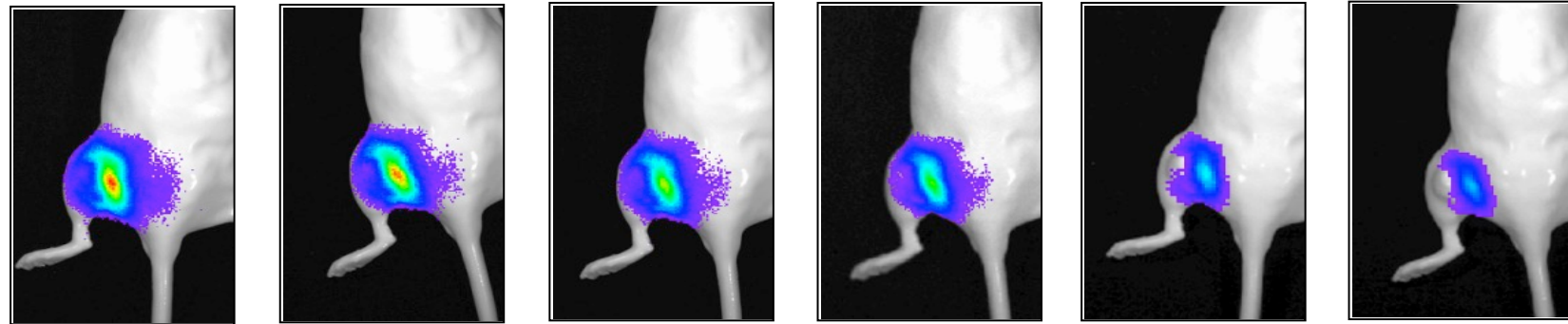
Calibrated Physical Units

- Living Image® automatically compensates for device settings: Exposure time, f /stop, binning and field of View.
- Calibrated units are Photons per Second, representing the flux radiating omni-directionally from a user-defined region



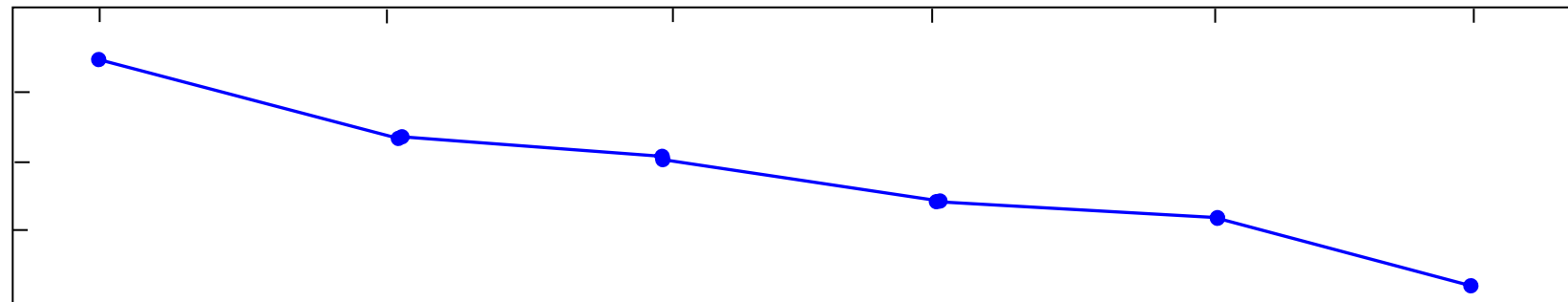
Calibrated Physical Units vs. Raw Signal

**Calibrated
Signal**
*(Photons per
second)*

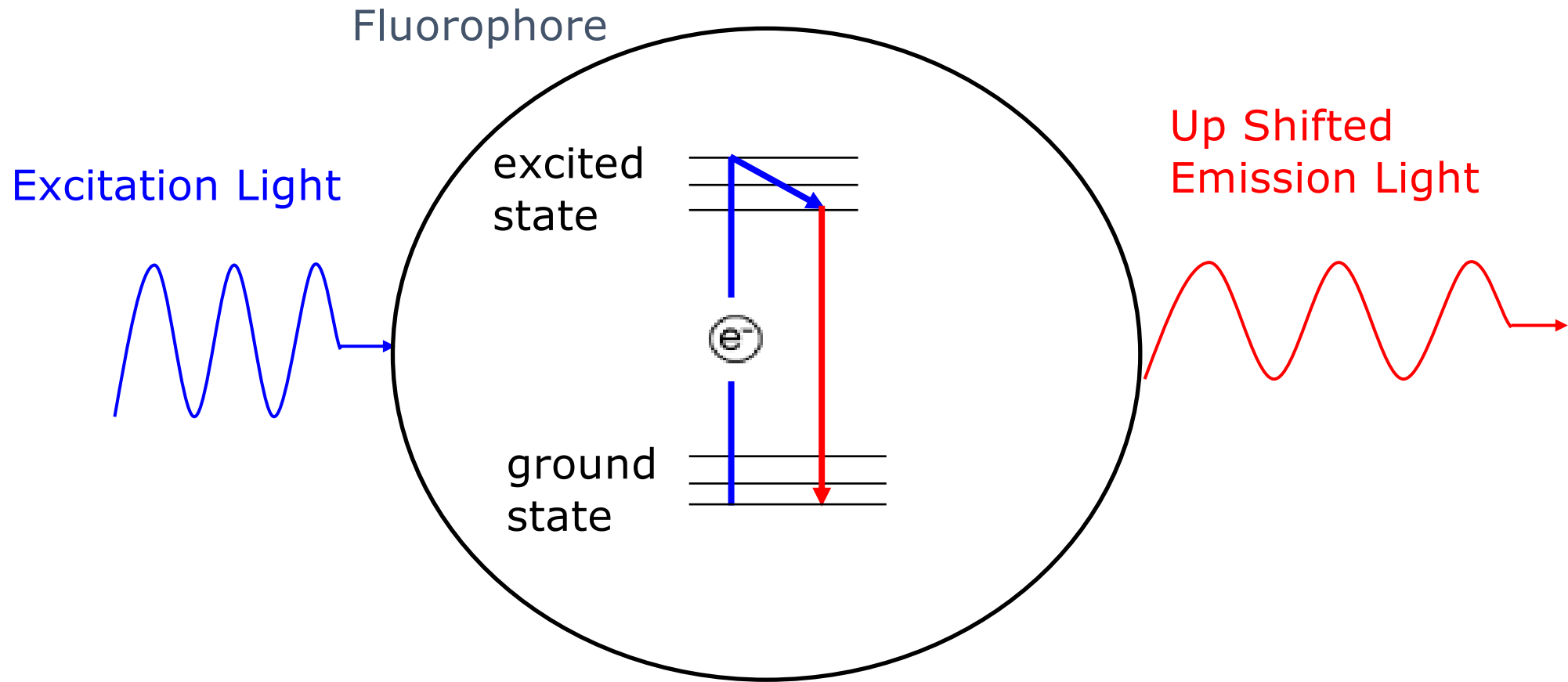


Exp time:	30 sec	30 sec	60 sec	60 sec	60 sec	60 sec
Binning:	small	small	small	small	medium	medium
Day:	1	2	3	4	5	6

Radiance:
*Photons per
second*



Fluorescence Process



Fluorescent Calibrated Units: Radiant Efficiency

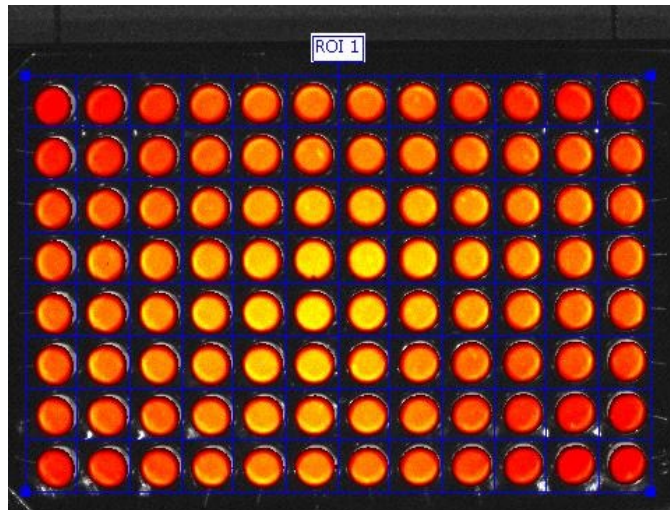
Excitation
Light Pattern



Units of 'Radiant Efficiency' compensates for non-uniform excitation light pattern

GFP Well Plate

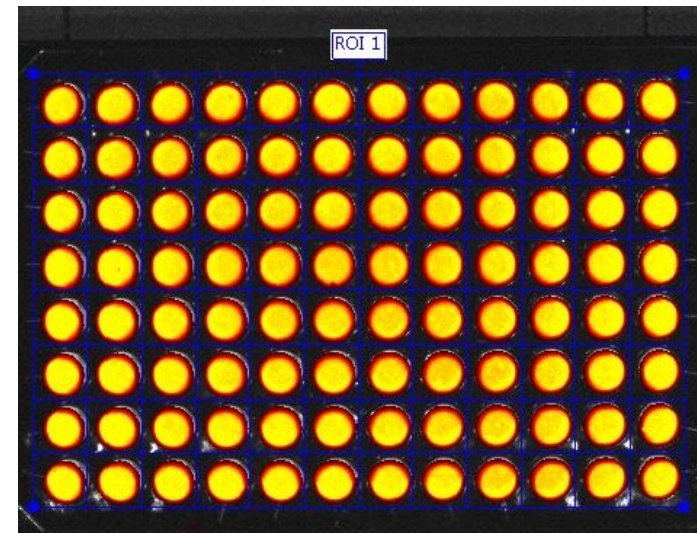
Counts



vs.

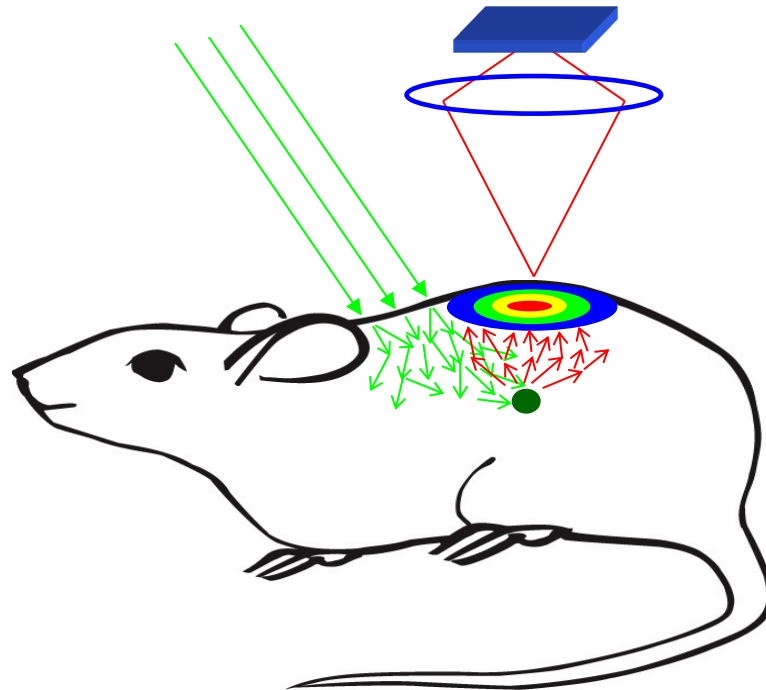
GFP Well Plate

Radiant
Efficiency



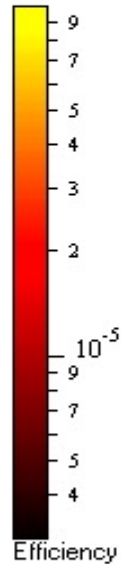
Epi-illumination Unit

$$\text{Radiant Efficiency} = \frac{\text{Emission Light (photons/sec/cm}^2\text{/str)}}{\text{Excitation Light (}\mu\text{W/cm}^2\text{)}}$$



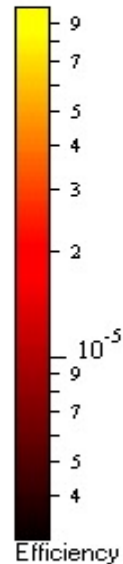
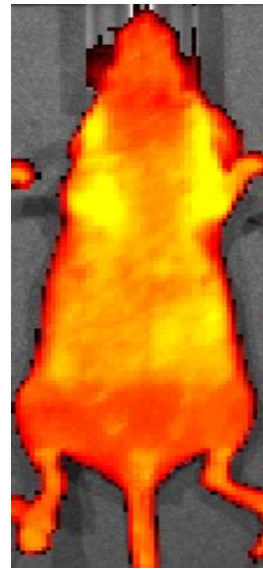
Autofluorescence of Control Mice

$\lambda = 550\text{nm}$



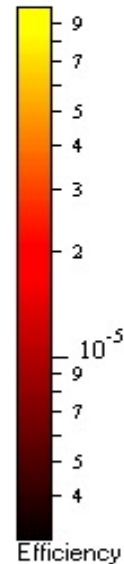
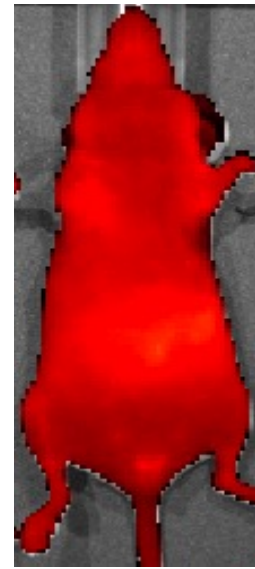
Color Bar
Min = 3.00e-6
Max = 1.00e-4

$\lambda = 610\text{nm}$



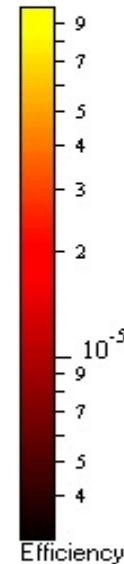
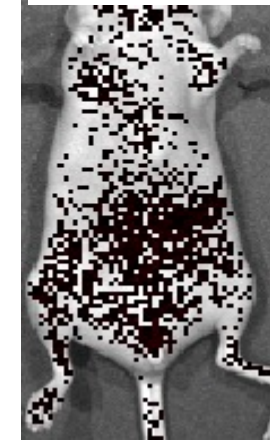
Color Bar
Min = 3.00e-6
Max = 1.00e-4

$\lambda =$



Color Bar
Min = 3.00e-6
Max = 1.00e-4

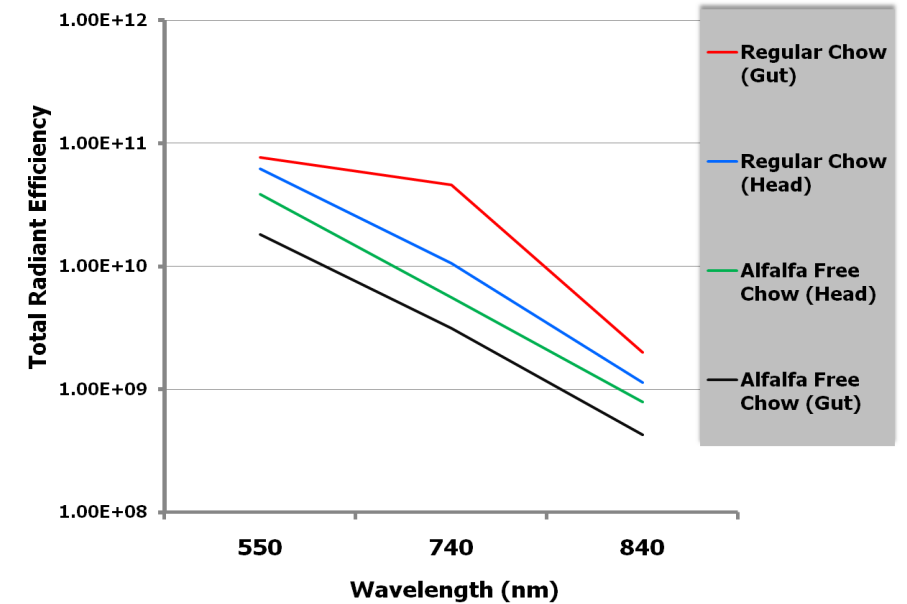
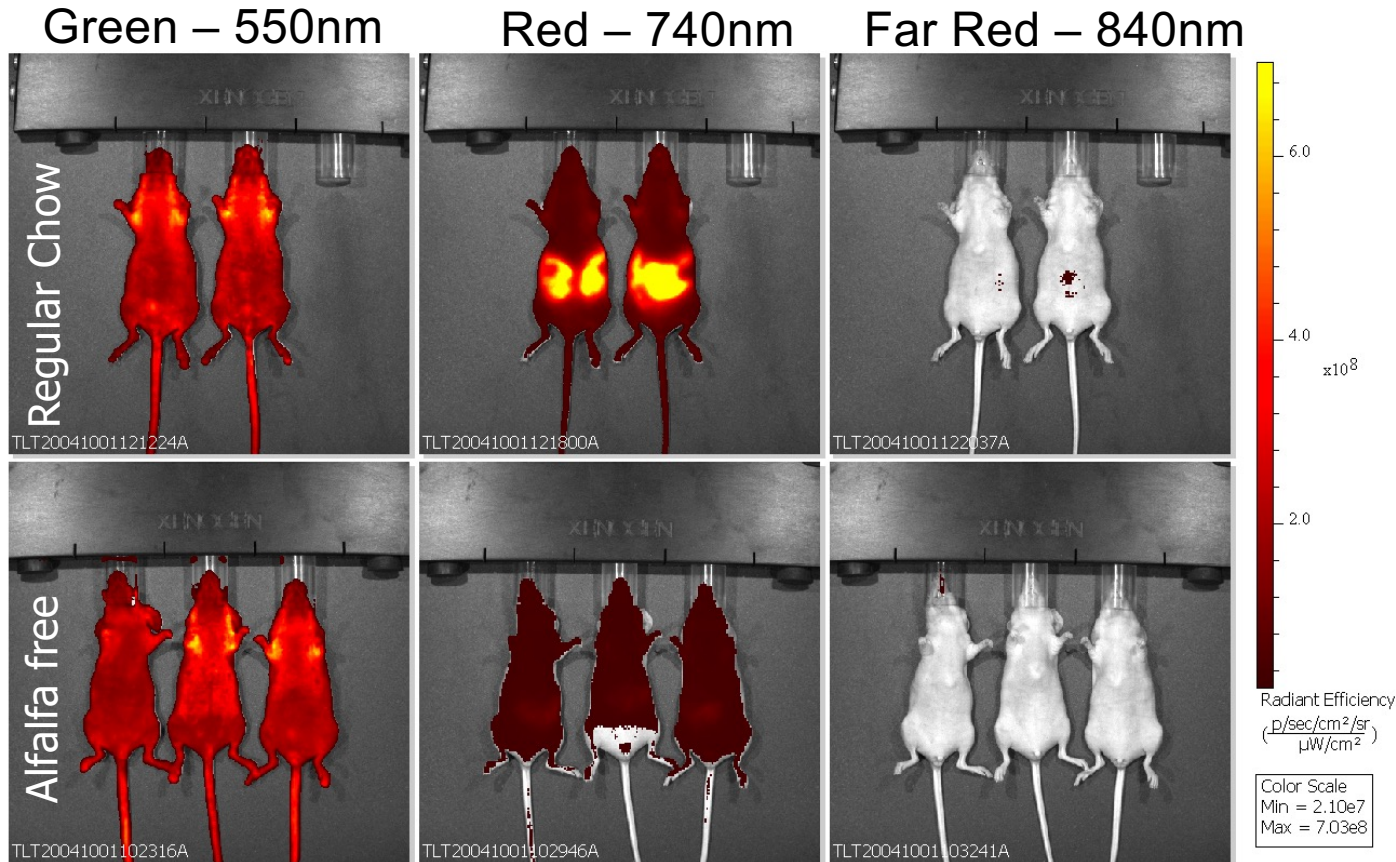
$\lambda =$
840n
m



Color Bar
Min = 3.00e-6
Max = 1.00e-4

Autofluorescence of Control Mice

- Unrefined chlorophyll-containing ingredients, particularly alfalfa, responsible for gut signal

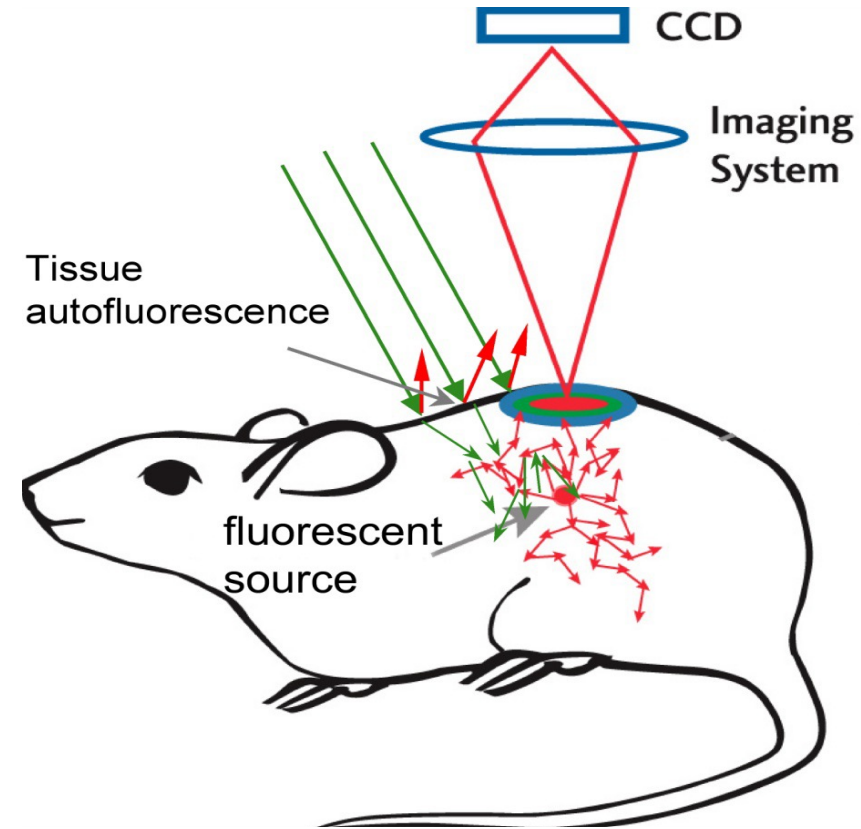


Research Diets
<http://www.researchdiets.com>

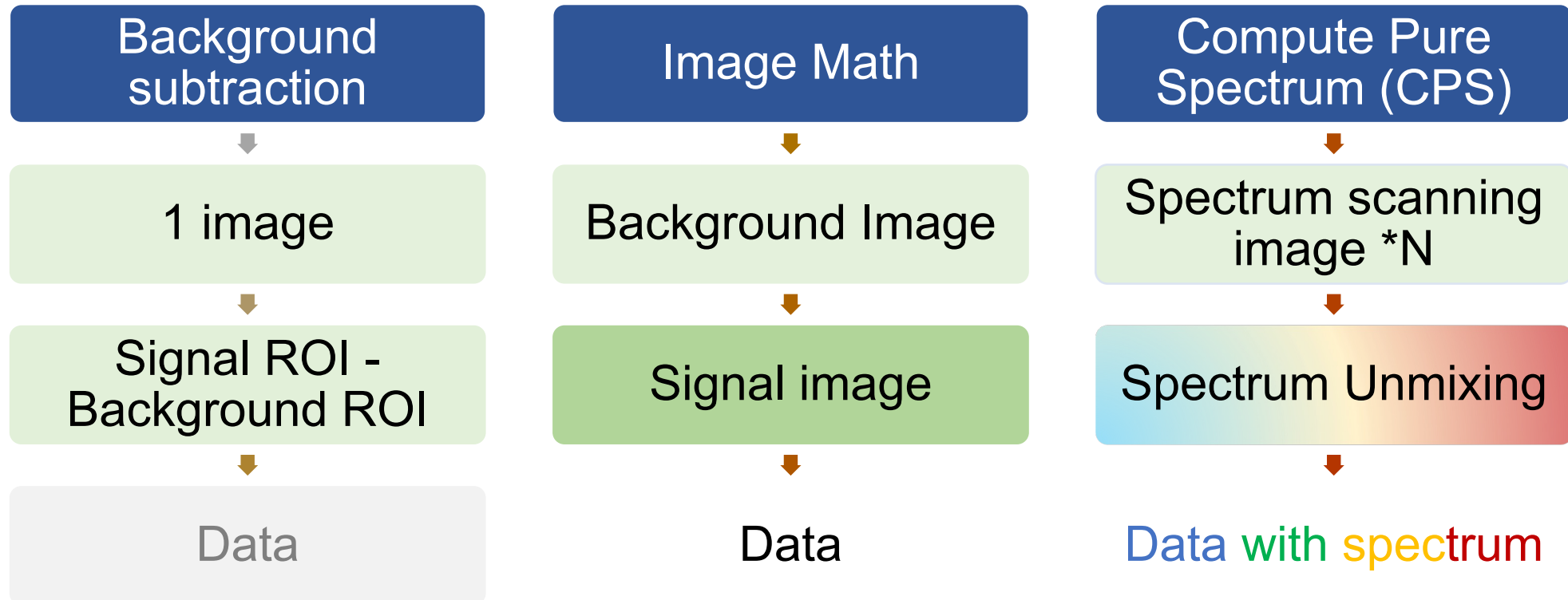
AIN-76A (D10001i) – alfalfa free

Challenges of *in vivo* fluorescent Imaging

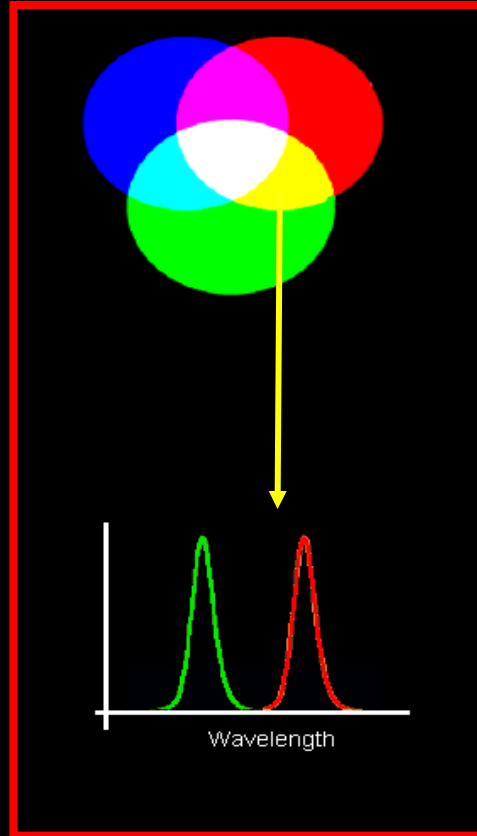
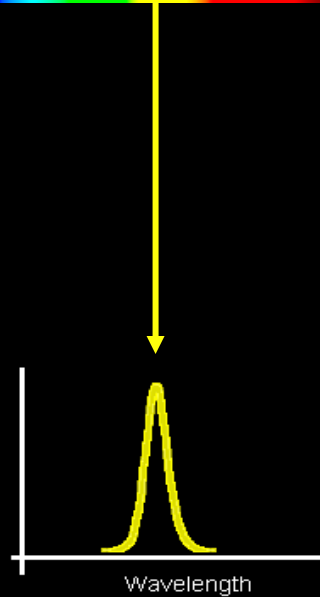
- Tissue Auto-fluorescence
 - RBC in all tissue
 - Background signal everywhere
 - The main restriction of sensitivity
- How to get better Signal to noise ratio?
 - Increase signal ?
 - Decrease background



PerkinElmer multiple solution - remove background



Compute Pure Spectrum (CPS) Most Useful in Animal!

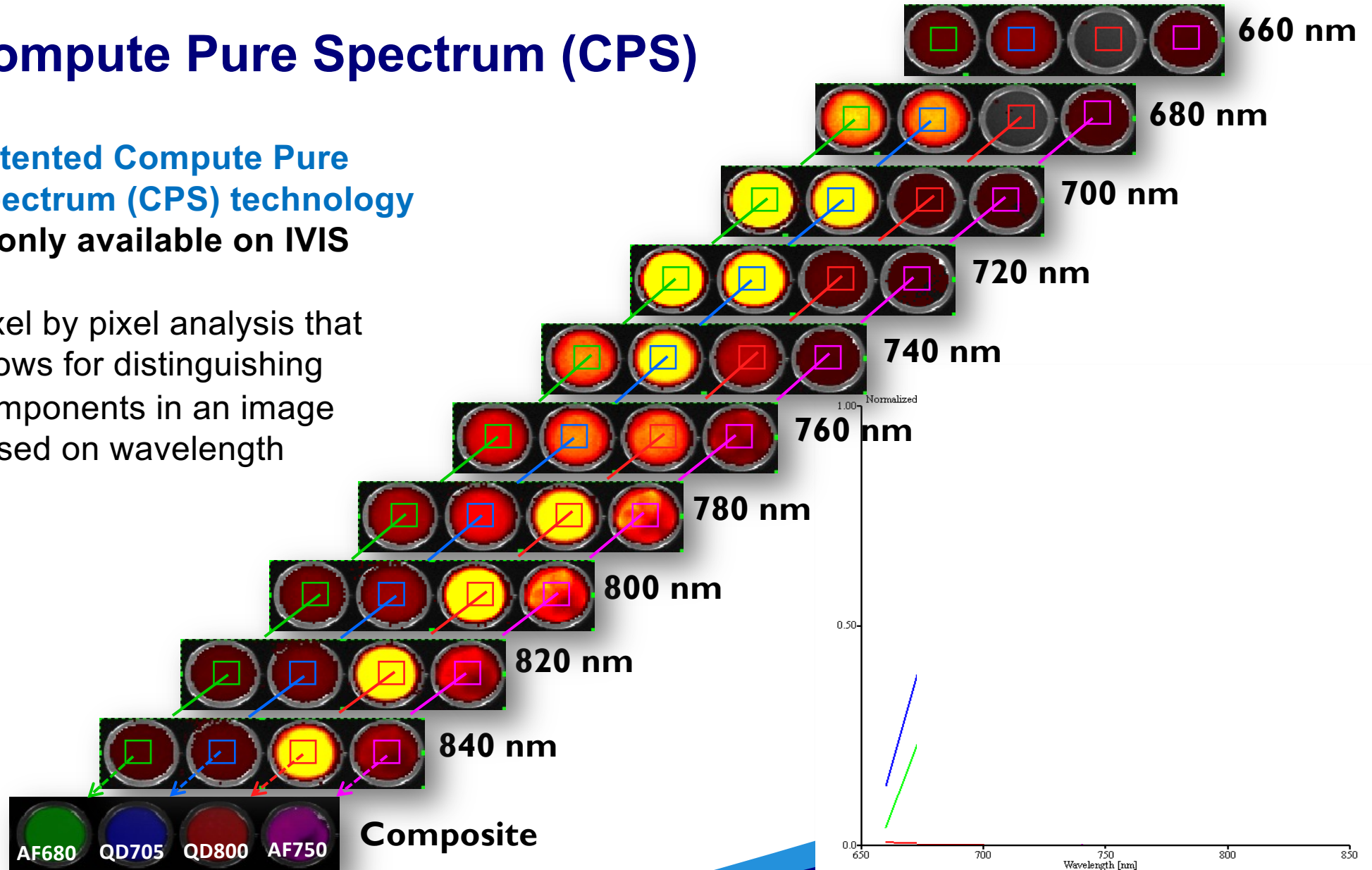


These two yellows
are *visually*
indistinguishable

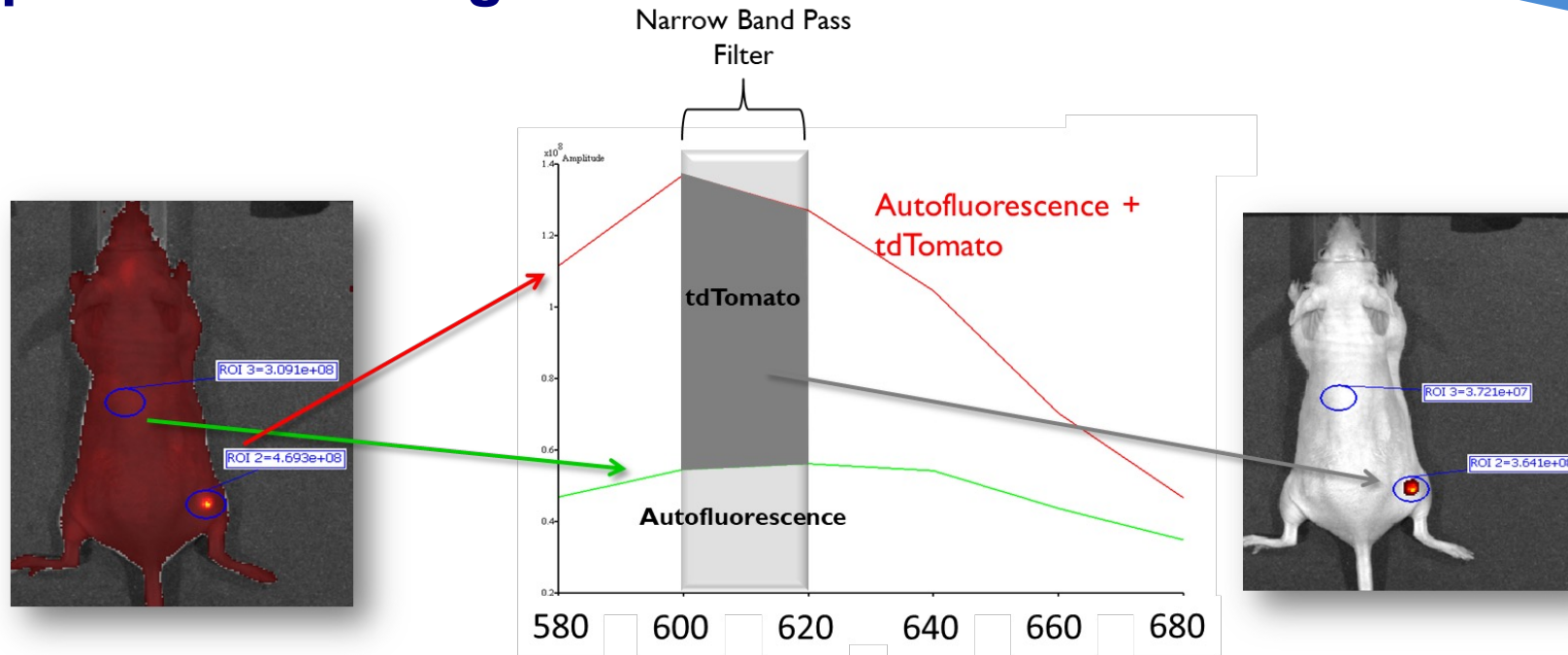
BUT multispectral
imaging can be used
to resolve their
spectral differences

Compute Pure Spectrum (CPS)

- Patented Compute Pure Spectrum (CPS) technology is only available on IVIS
- Pixel by pixel analysis that allows for distinguishing components in an image based on wavelength



Compute Pure Spectrum (CPS) - Spectral Unmixing



Background subtraction:

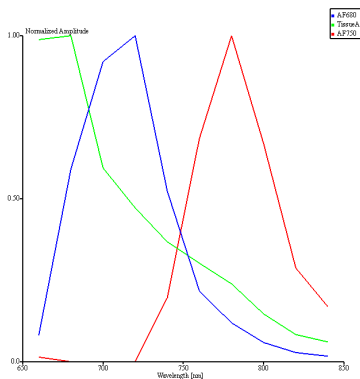
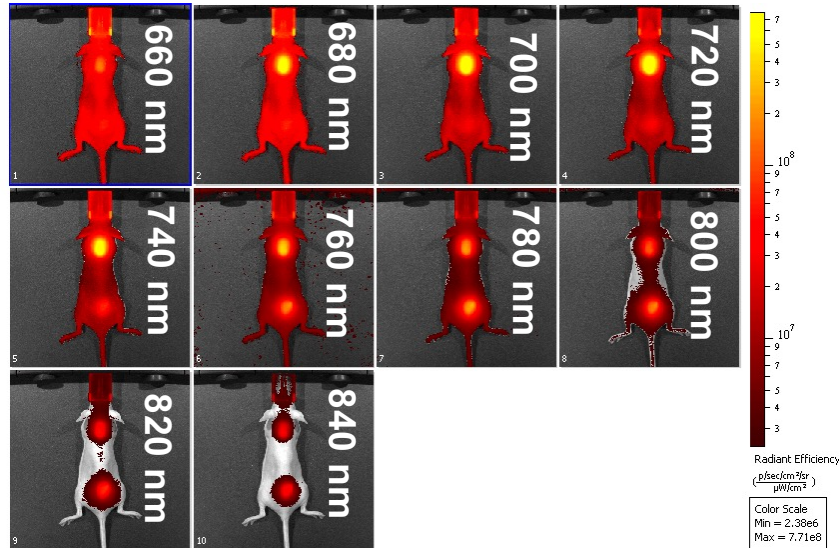
- Signal – Background = $ROI\ 2 = 1.602 \times 10^8$
- SN ratio = 1.5



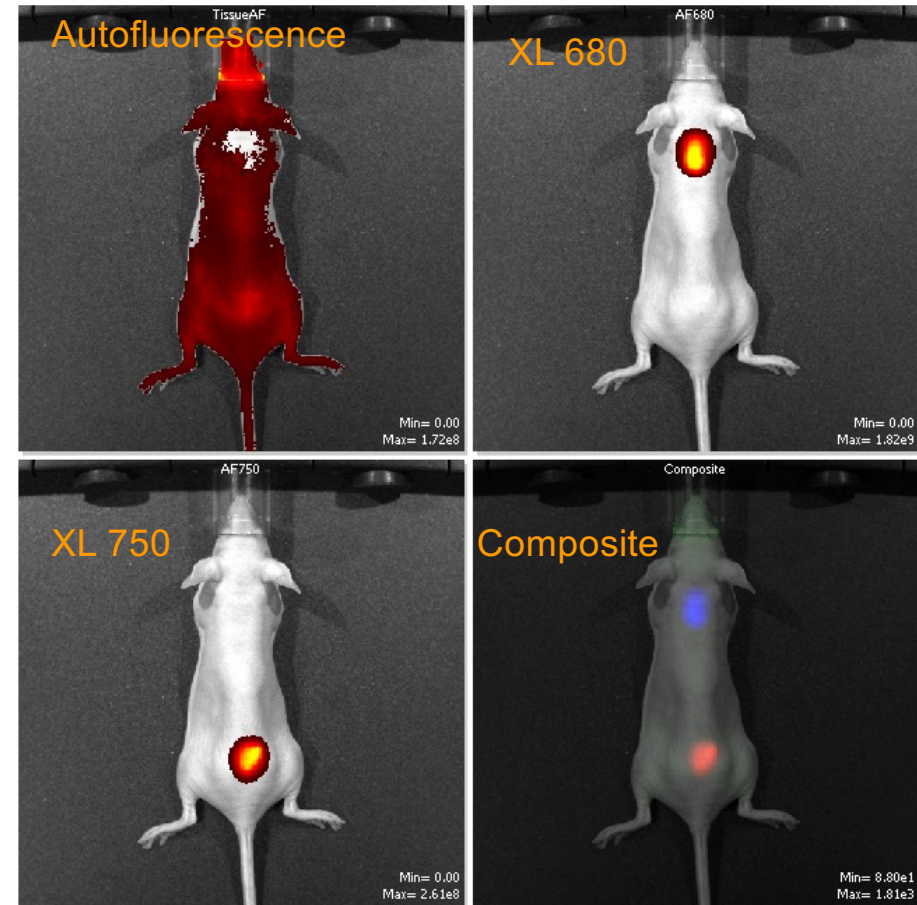
CPS spectrum unmixing

- Signal = $ROI\ 2 = 3.641 \times 10^8$
- Background = 0.372×10^8
- SN ratio = 9.7

XenoLight 680/750 in a Mouse

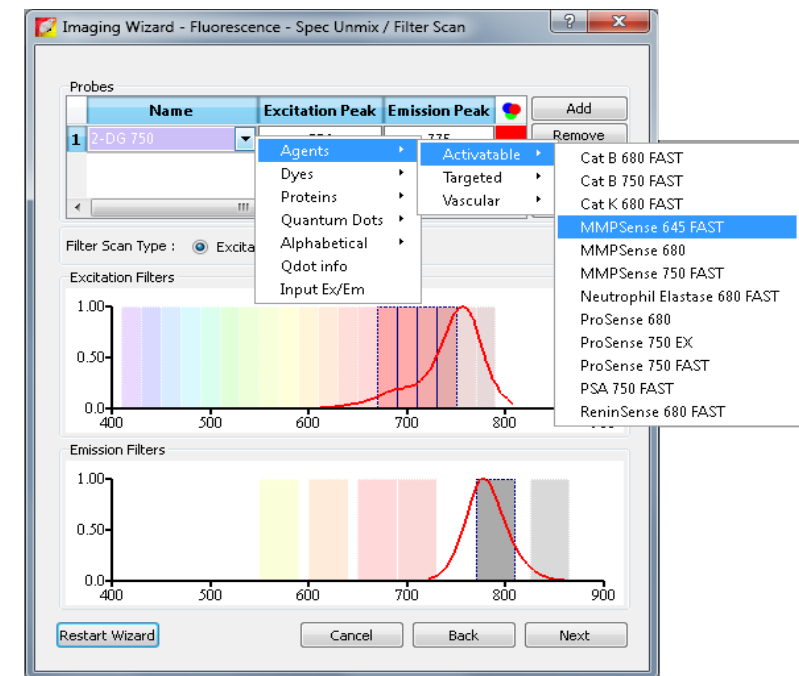


- ▶ Subcutaneous injections of 10^{14} molecules of XenoLight 680 (scruff)
- ▶ Subcutaneous injection of 10^{14} molecules of XenoLight 750 (lower dorsal region)
- ▶ 605nm excitation filter



A complete probe library for easier use

- Incorporated at least **98 probes** into Living Image software
- **Contains all the Perkin Elmer fluorescent Agent and Dyes**
- Contains commonly used Probes
 - Dyes
 - Alexafluor dyes
 - Cyanine dyes
 - VivoTag
 - Miscellaneous
 - Proteins
 - Quantum dots
- Data base can be expanded as needed
 - Input Ex/Em and Qdot info will allow user to input peaks if their probe isn't in database



Dual Cell tracking

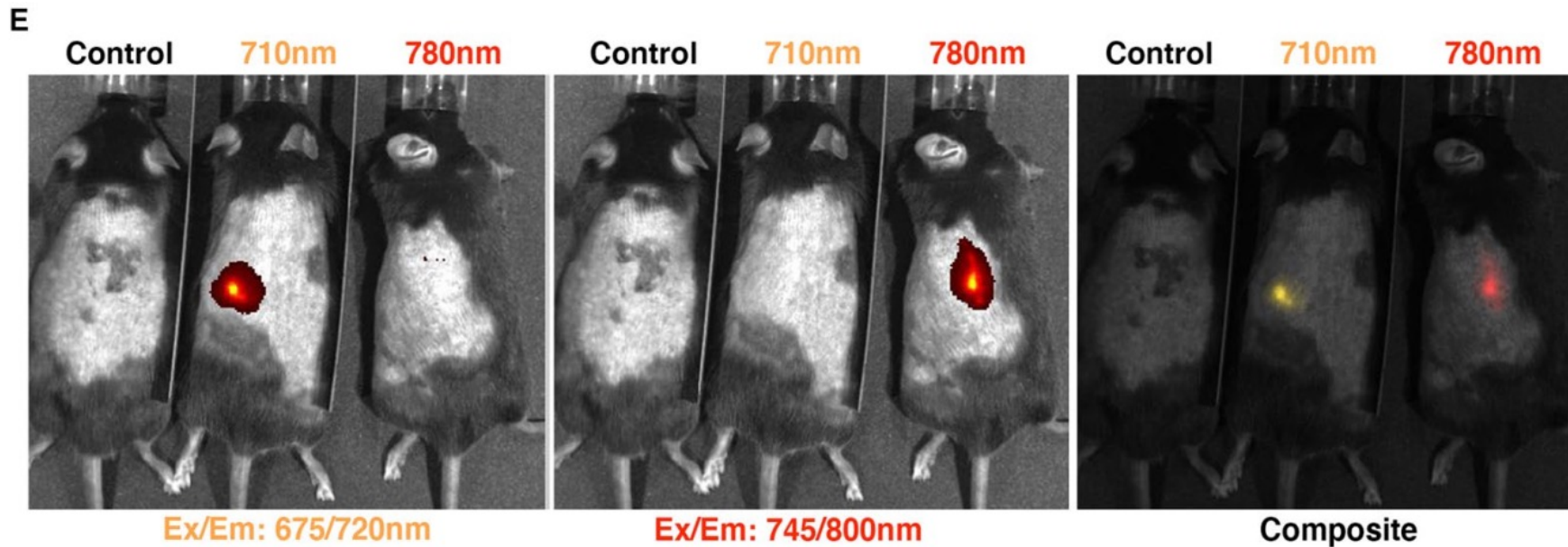
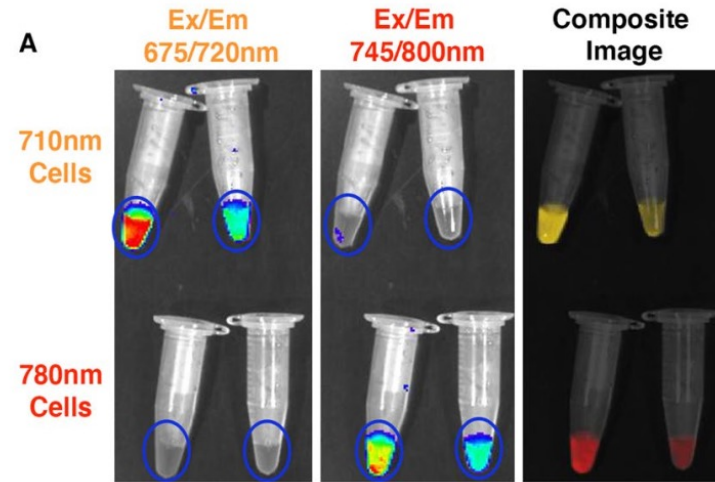
Article | [Open Access](#) | [Published: 14 January 2021](#)

Non-invasive synchronous monitoring of neutrophil migration using whole body near-infrared fluorescence-based imaging

[Jack Leslie](#), [Stuart M. Robinson](#), [Fiona Oakley](#) & [Saimir Luli](#) ✉

[Scientific Reports](#) **11**, Article number: 1415 (2021) | [Cite this article](#)

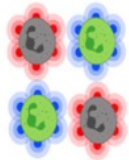
1306 Accesses | 2 Citations | 1 Altmetric | [Metrics](#)



Dual Cell tracking

A Neutrophil isolation & labelling

Group 1
WT Neutrophils 780nm
Rel^{-/-} Neutrophils 710nm



CCl₄ injured recipients



WT

Rel^{-/-}

IVIS imaging at 2 hr



WT Neutrophils

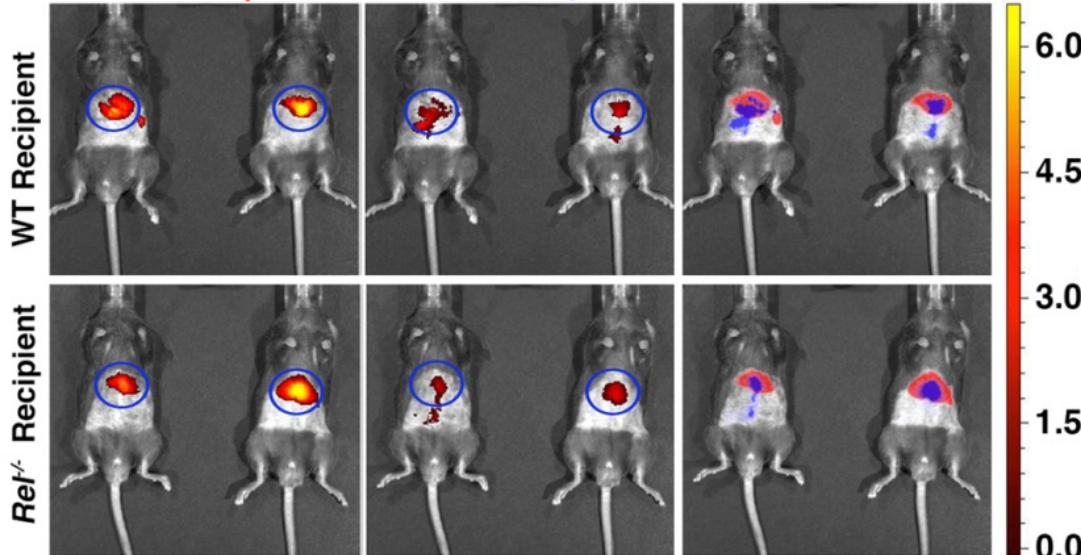
Rel^{-/-} Neutrophils

B

WT Neutrophils

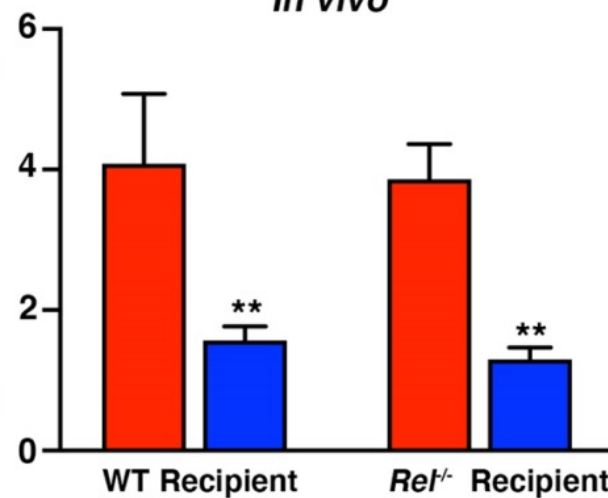
Rel^{-/-} Neutrophils

Composite Image



Average Radiant Efficiency x10⁷

In vivo



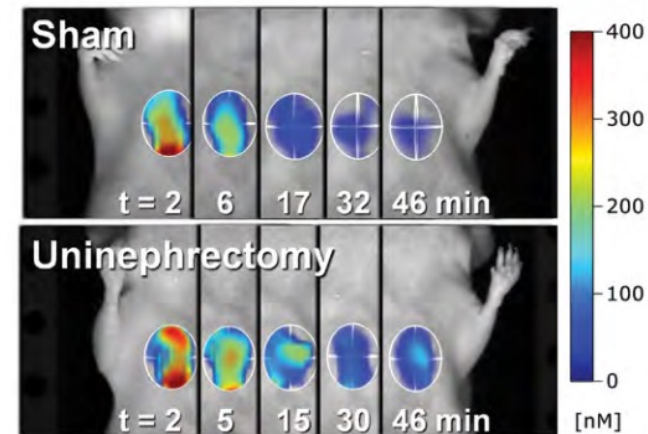
Vascular In Vivo Imaging Fluorescent Probes

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PROBE	PRODUCT DESCRIPTION	CAT. NUMBER
IVISense Vascular 680	Imaging of vascularity, perfusion, and vascular permeability. Remains localized in vasculature for 0-4 h; accumulates in tumors and arthritic joints at 24 h.	NEV10054EX
IVISense Vascular 750		NEV10011EX
IVISense Vascular NP 680	Fluorescent nanoparticles for imaging vascularity, perfusion and vascular permeability; long pharmacokinetic profile.	NEV10149
IVISense Vascular NP 750		NEV10150
IVISense Gastrointestinal 750	Imaging to monitor gastric emptying and the impact of various drugs on gastric motility; may also be used as an anatomical marker for the stomach.	NEV11121
IVISense Acute Vascular 680 (1 mg)	Fluorescent probes that enable imaging and quantitation of acute vascular permeability increase, bladder clearance via intravital ureter imaging, or incorporation into liposomes for drug delivery monitoring.	NEV10117
IVISense Acute Vascular 680 (5 mg)		NEV10130
IVISense Acute Vascular 750 (1 mg)		NEV10118
IVISense Acute Vascular 750 (5 mg)		NEV10177

PROBE	PRODUCT DESCRIPTION	CAT. NUMBER
IVISense GFR 680	NIR-fluorescent imaging agent to non-invasively determine glomerular filtration rate (GFR) <i>in vivo</i> in models of kidney disease, dysfunction, and drug toxicity.	NEV30000
IVISense Edema 680	Blood pooling fluorescent probe for imaging circulation, blood vessels, vasculature, vascular leak, including that associated with early oncologic and ophthalmologic lesions. This agent has a short pharmacokinetic profile with bladder clearance, and binds to albumin in blood for a modestly extended (30m-1h) circulation half-life.	NEV10116

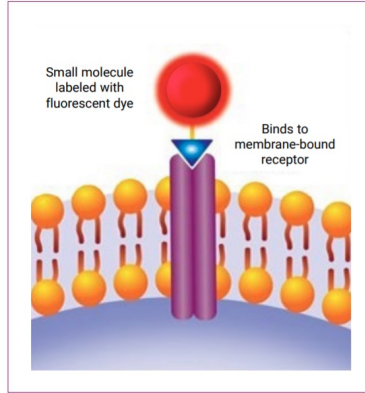


IVISense GFR 680 in SKH- 1E mice, FMT images were acquired at 1, 5, 15, 30, and 45 minutes post-injection GFR-Vivo 680

Targeted Fluorescent Agents

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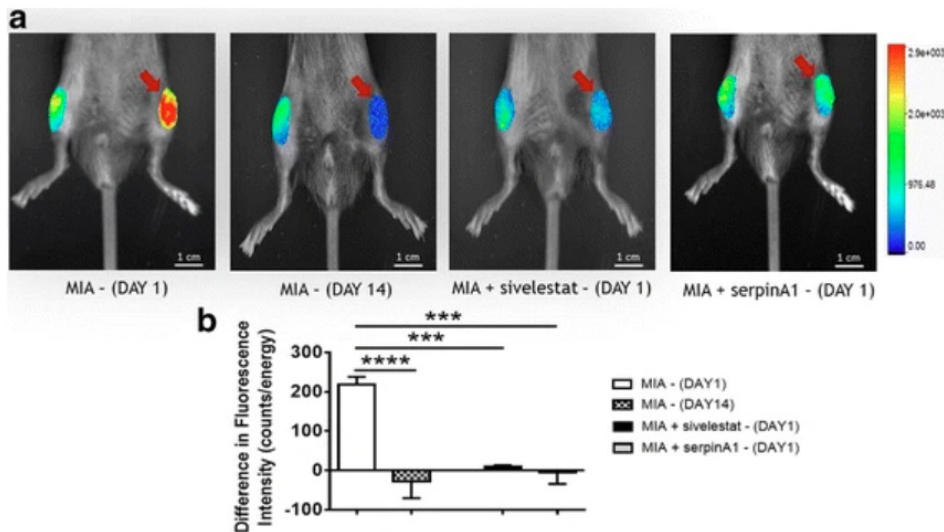
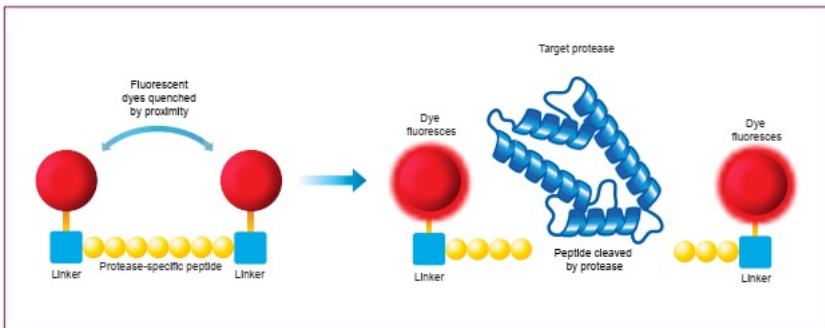
PROBE	PRODUCT DESCRIPTION	CAT. NUMBER
IVISense Annexin-V 750	<i>In vivo</i> targeting of membrane-bound phosphatidylserine exposed during the early stages of apoptosis.	NEV11053
IVISense Bombesin Receptor 680	Target and quantify up-regulation of bombesin receptors (BBR) <i>in vivo</i> associated with tumor proliferation. These receptors are also over-expressed in a variety of cancers.	NEV10090
IVISense Folate Receptor 680	Highly specific and sensitive in the detection of folate receptor proteins. Can be used to closely monitor and quantitate tumor growth and metabolism.	NEV10040
IVISense Hypoxia CA IX 680	Detects the tumor cell surface expression of carbonic anhydrase 9 (CA IX) protein, which increases in hypoxic regions within many tumors.	NEV11070
IVISense Integrin Receptor 645	Targets integrin $\alpha v\beta 3$ expressed in oncology, atherosclerosis, and angiogenesis disease models.	NEV10640
IVISense Integrin Receptor 680		NEV10645
IVISense Integrin Receptor 750		NEV10873

PROBE	PRODUCT DESCRIPTION	CAT. NUMBER
IVISense Osteo 680	Optimized imaging of bone turnover through binding of hydroxyapatite.	NEV10020EX
IVISense Osteo 750		NEV10053EX
IVISense Osteo 800		NEV11105
IVISense Tomato Lectin 680	NIR-labeled tomato lectin protein that has high binding affinity for glycoprotein N-acetylglucosamines on the surface of vascular endothelial cells. Use for vascular mapping <i>in vivo</i> .	NEV10060
IVISense Transferrin Receptor 750	NIR-labeled transferrin detects transferrin receptor upregulation associated with the increased cell metabolic need for iron in cancer and inflammatory cells. Also detects normal iron metabolism in the liver.	NEV10091
IVISense Bacterial 750 Probe in RediJect™ Solution (1 vial)	NIR-targeted probe for non-invasive detection of bacterial infections <i>in vivo</i> .	133397
IVISense Bacterial Probe 750 in RediJect Solution (4 vials)		133398
IVISense Bacterial 750 Control Dye in RediJect Solution (1 vial)	Non-reactive control dye for RediJect Bacterial Detection Probe.	133399
IVISense 2-DG 750 Probe in RediJect Solution (1 vial)	NIR-targeted probe for non-invasive imaging of glucose uptake <i>in vivo</i> .	760561
IVISense 2-DG 750 Probe in RediJect Solution (4 vials)		760562
IVISense 2-DG 750 Control Dye in RediJect Solution (1 vial)	Non-reactive control dye for RediJect 2-DG 750 probe.	760567

Activatable In Vivo Imaging Fluorescent Probes

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In vivo imaging of neutrophil elastase enzyme activity in MIA-injected knee joints

PROBE	PRODUCT DESCRIPTION	CAT. NUMBER
IVISense Cat B 680 FAST	Selective imaging of cathepsin B proteinases (Cat B). Optically silent in the inactivated state, becoming highly fluorescent when activated.	NEV11112
IVISense Cat B 750 FAST		NEV11098
IVISense Cat K 680 FAST	Imaging of cathepsin K (Cat K) activity in oncology applications involving metastasis to the bone as well as a broad range of bone applications, including bone loss, tumor-induced osteolysis, and bone changes following arthritis.	NEV11000
IVISense MMP 645 FAST	Imaging of metalloproteinase (MMP) activity is involved in many disease-related phenomena, including cancer propagation, invasion and metastasis, rheumatoid arthritis, and areas of cardiovascular disease.	NEV10100
IVISense MMP 680		NEV10126
IVISense MMP 750 FAST		NEV10168
IVISense Neutrophil Elastase 680 FAST	Fluorescent neutrophil elastase-activatable agent that is optically silent upon injection and produces a fluorescent signal after cleavage by elastase produced by neutrophil cells.	NEV11169
IVISense Pan Cathepsin 680 (formerly ProSense 680)	Versatile imaging of changes in cathepsin-based protease activity as seen in a number of pathological states and disease-related events, including rheumatoid arthritis, cancer, atherosclerosis, angiogenesis, and cardiovascular disease.	NEV10003
IVISense Pan Cathepsin 750		NEV10001EX
IVISense Pan Cathepsin 750 FAST	FAST version of ProSense, with faster kinetics and a broader imaging window.	NEV11171
IVISense Renin Receptor 680 FAST	Imaging of renin-angiotensin pathway associated with hypertension, and kidney and cardiovascular disease.	NEV11079

New dye - AIE dot

luminiCell

J&H

博克科技股份有限公司

J&H TECHNOLOGY CO., LTD.

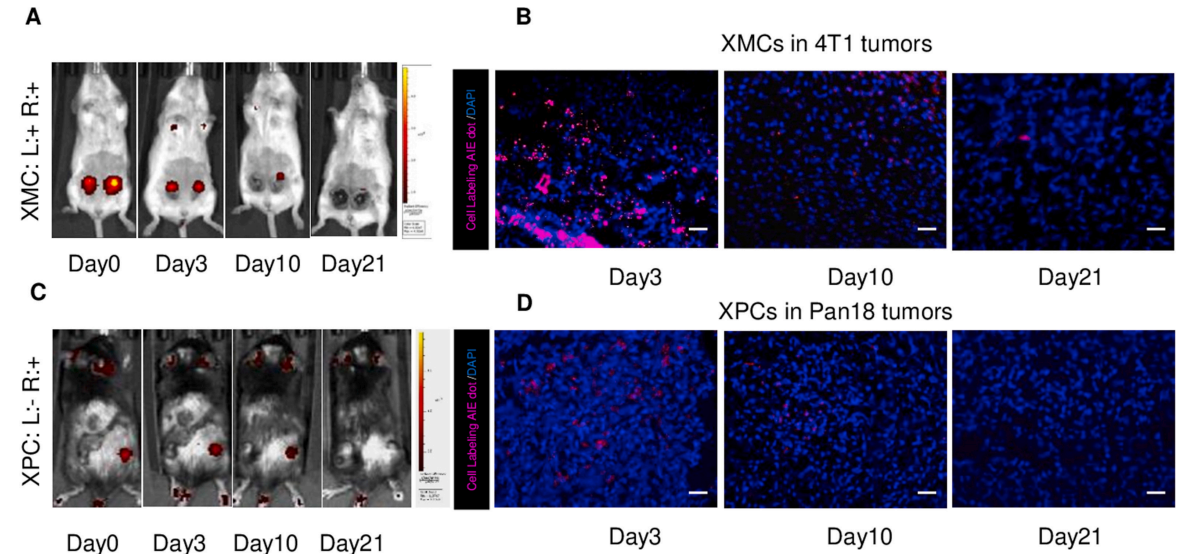
Intratumoral xenogeneic tissue-specific cell immunotherapy inhibits tumor growth by increasing antitumor immunity in murine triple negative breast and pancreatic tumor models

Chi-Ping Huang^a, Liang-Chih Liu^b, Chih-Chun Chang^c, Chun-Chie Wu^a, Chih-Rong Shyr^{c,*}

^a Department of Medicine, Department of Urology, College of Medicine, China Medical University and Hospital, Taichung, 404, Taiwan

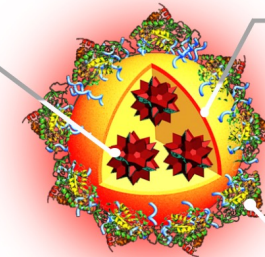
Xenogeneic tissue-specific cells were labelled with LuminiCell Trackers™, which are biocompatible organic fluorescent nanoparticles based on **aggregation induced emission (AIE dot) technology**.

AIE fluorogens are non-emissive in solutions but become highly fluorescent upon aggregate formation with long signal duration, low background auto-fluorescence and minimal signal quenching.



1. Unique AIE Fluorescence Dyes

Novel materials that have high brightness and unique spectral properties



2. Biocompatible encapsulation matrix

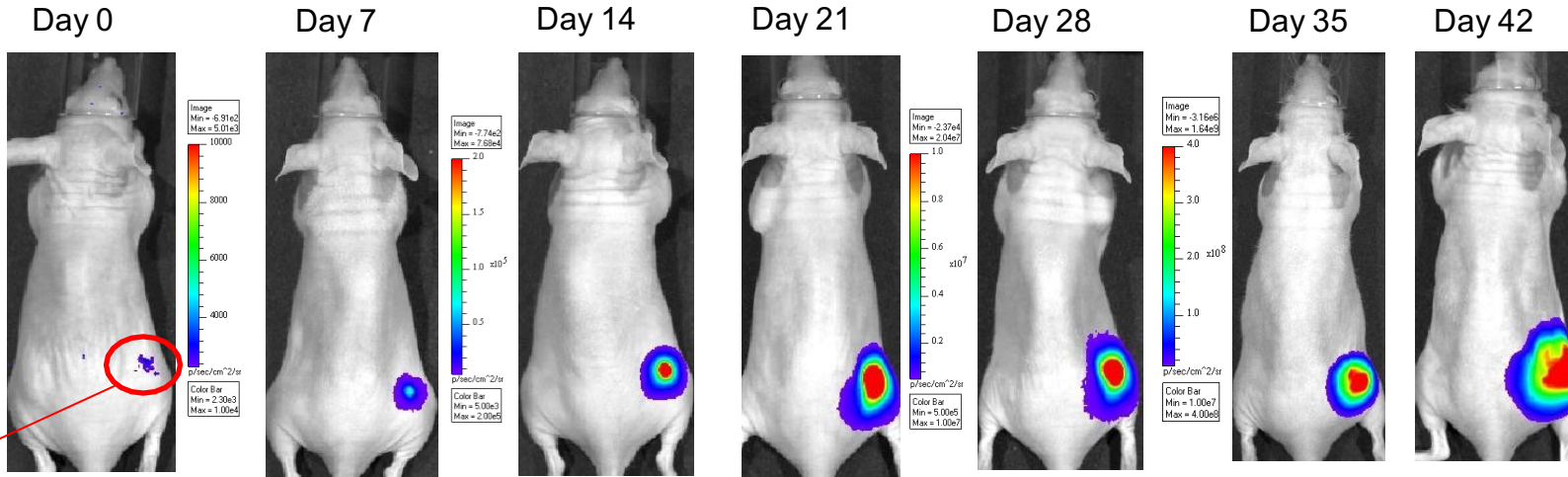
Biocompatible lipid polymer matrix that enables materials to be delivered and stable for both *in vitro* and *in vivo*

3. Tunable surface targeting groups

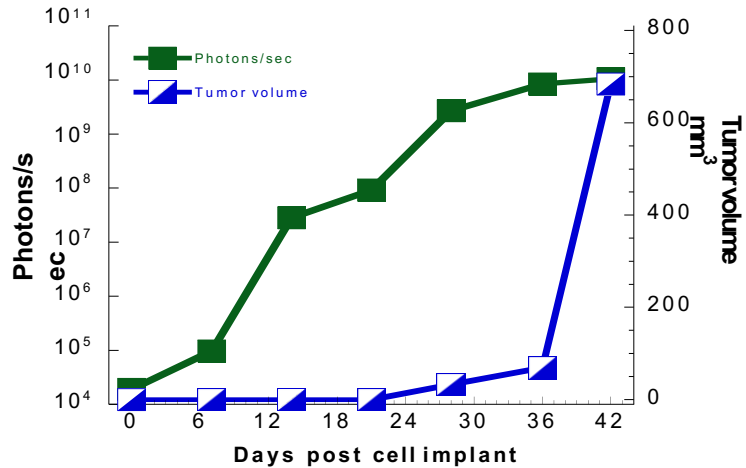
Proprietary surface moieties that enable high cellular uptake efficiency

Scheme 1. LuminiCell Tracker™ proprietary technology platform.

Longitudinal monitoring of tumor development

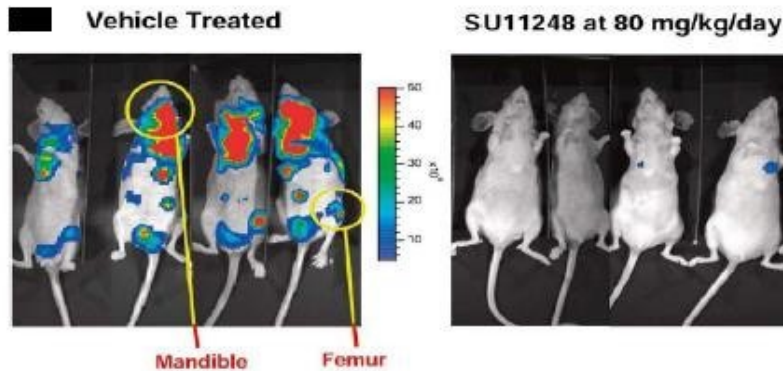
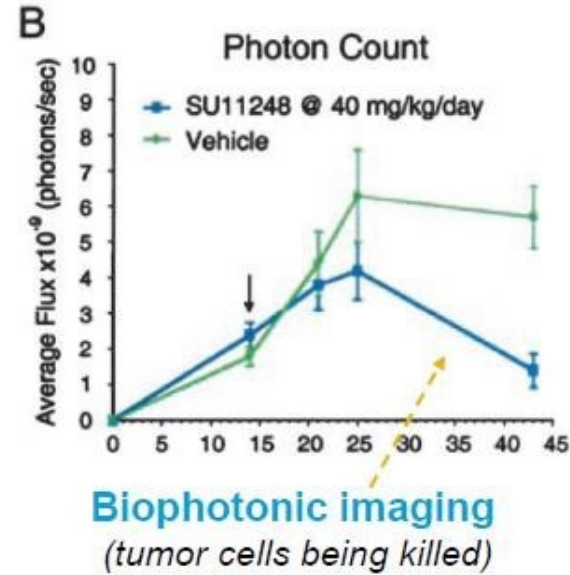
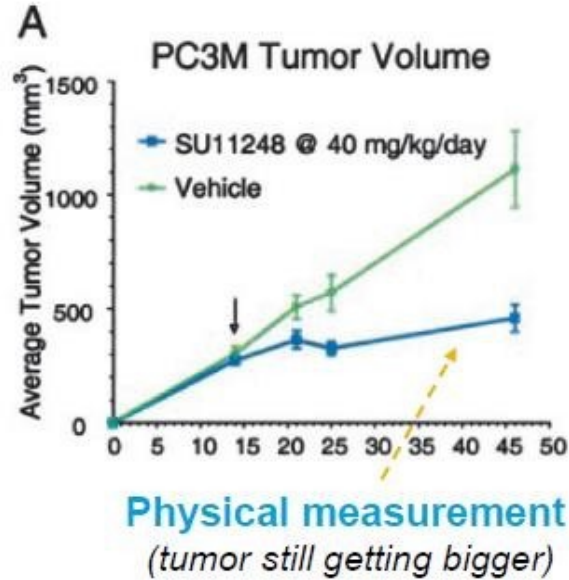


Bioware Ultra: 4T1-luc2
5 cells



With Bioware Ultra one can start collecting data from Day 0, while with caliper measurements one has to wait at least 28 days to see any tumor growth

Sutent – Fast Tracked FDA Approval



Murray et al 2003

Sutent (舒癌特)是一種口服的小分子多靶點受體酪氨酸激酶抑制劑

2006年1月26日被FDA批准用於治療對標準療法

胃腸道基質腫瘤和轉移性腎細胞癌。

舒尼替尼是第一種被批准用於同時治療兩種類型癌症的藥物。

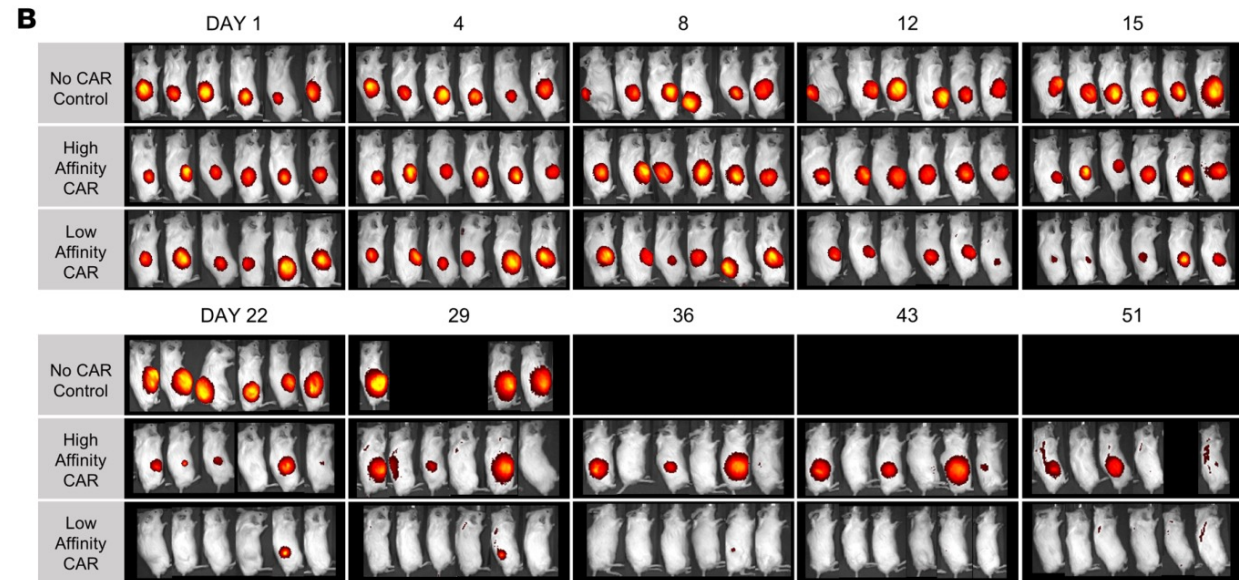
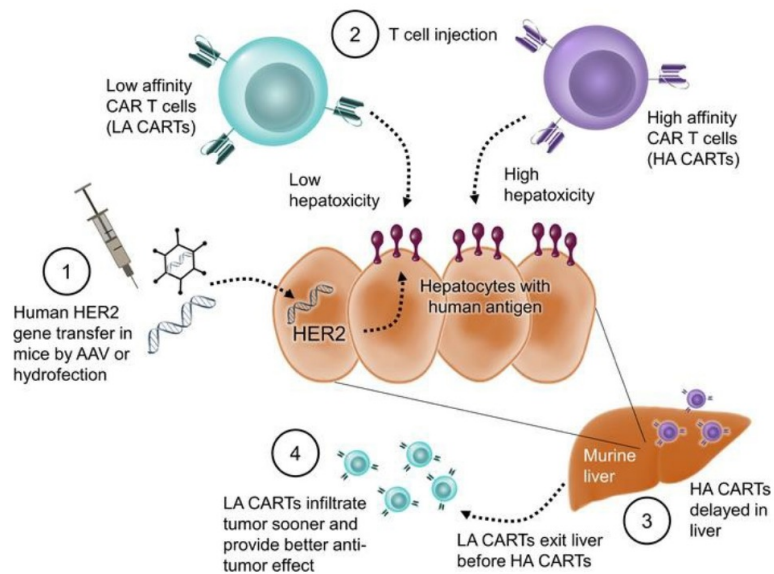
JCI insight

A rational mouse model to detect on-target, off-tumor CAR T cell toxicity

Mauro Castellarin, ... , Yangbing Zhao, Carl H. June

JCI Insight. 2020;5(14):e136012. <https://doi.org/10.1172/jci.insight.136012>.

Graphical abstract



(B) The Her2+ tumor cells, SKOV3, were genetically modified to express the fluorescent reporter, IRFP720, for in vivo imaging. Tumor xenograft fluorescence is shown in a yellow-to-red spectrum.

JCI insight

A rational mouse model to detect on-target, off-tumor CAR T cell toxicity

Mauro Castellarin, ... , Yangbing Zhao, Carl H. June

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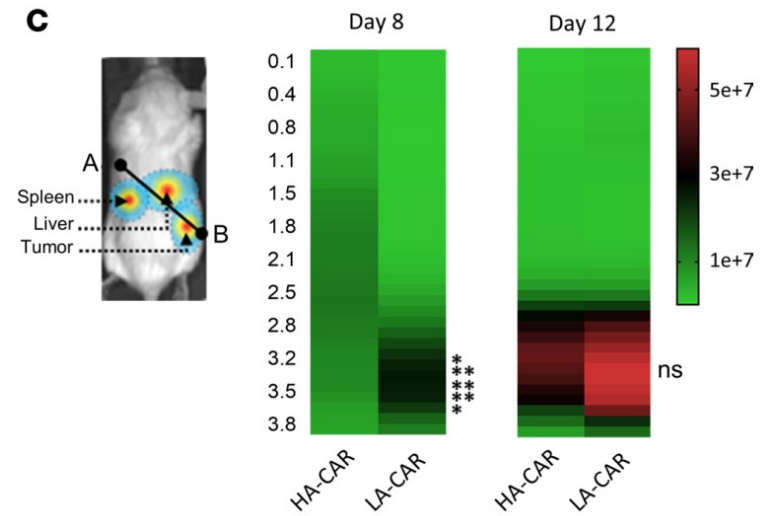
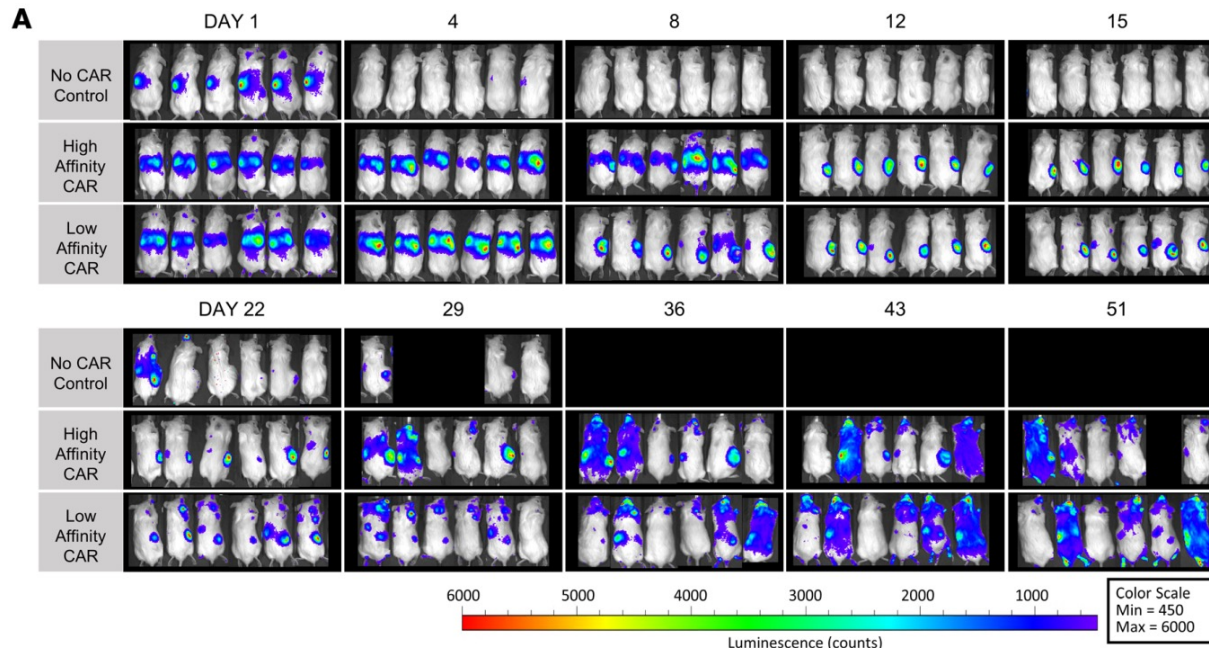
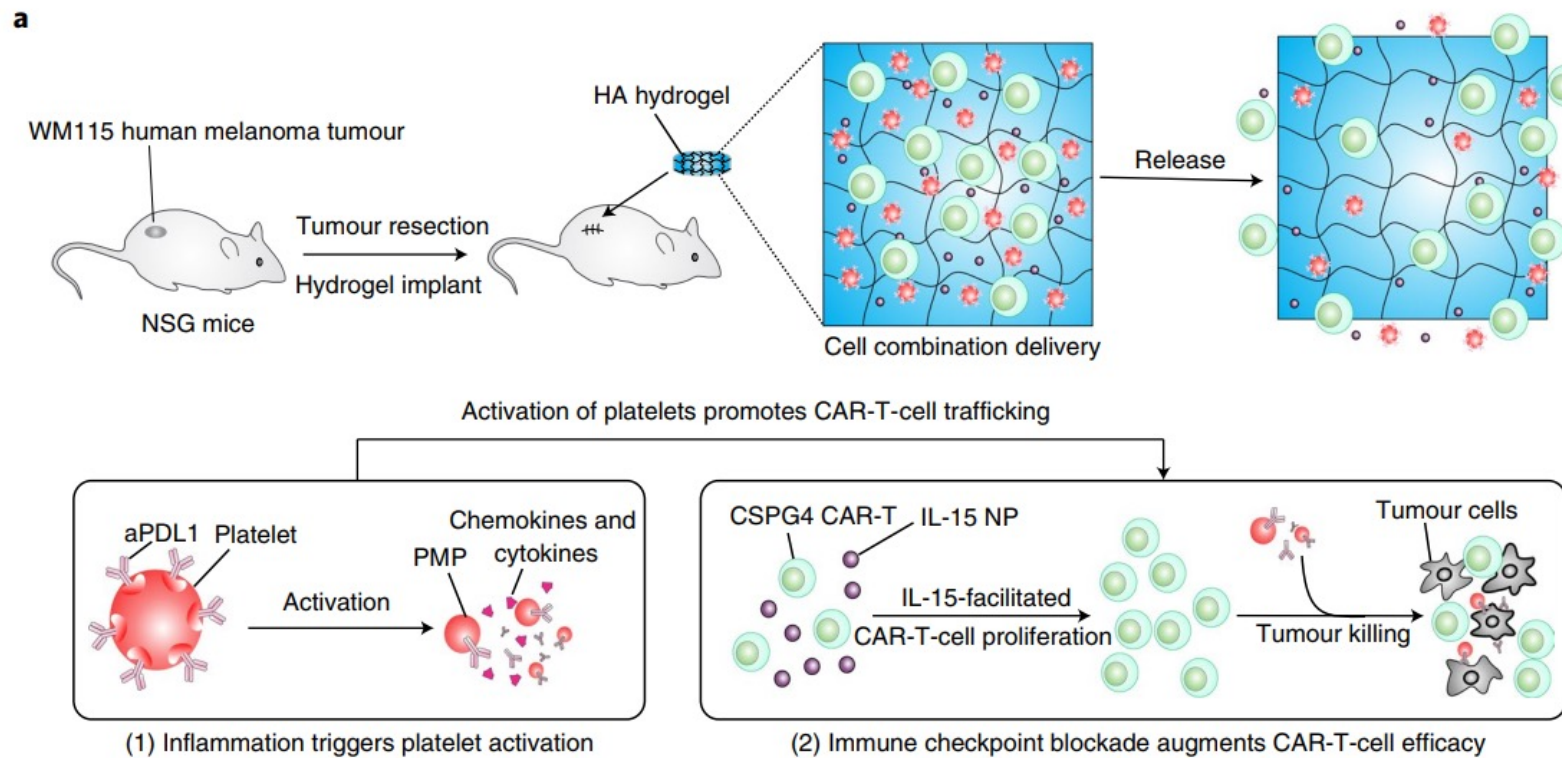


Figure 6. Low-affinity CARTs spend less time off-tumor than high-affinity CARTs

Inhibition of post-surgery tumour recurrence via a hydrogel releasing CAR-T cells and anti-PDL1-conjugated platelets



Inhibition of post-surgery tumour recurrence via a hydrogel releasing CAR-T cells and anti-PDL1-conjugated platelets

