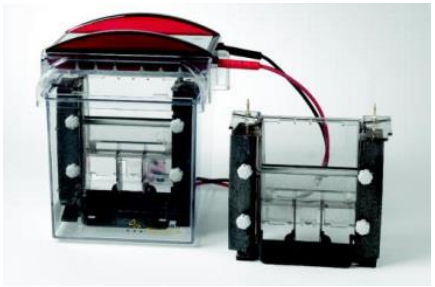
Plataforma de Proteómica

Dr. Carlos Río Bocos

Institut d'Investigació Sanitària de Palma (IdISPa)
Fundació d'Investigació Sanitària de les Illes Balears (FISIB)

19 Febrero, 2015

Equipos



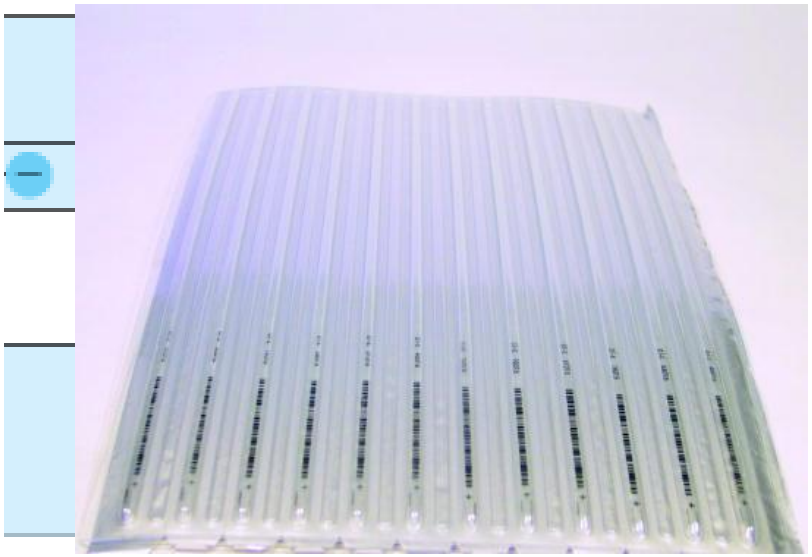
Protein Profiling

Medir la expresión de proteínas en dos, o más, muestras y compararlas – **"Comparative proteomics"**

- 1D gel electrophoresis (IEF - IPGphor3- or SDS PAGE - Ettan DALT*six* & MiniVE Vertical Electroforesis System -)
- 2D gel electrophoresis (IPGphor3 + Ettan DALT*six*)
- Fluorescence, DIfference Gel Electrophoresis (DIGE, Typhoon FLA 9500)…
- Fluorescence IR (Odyssey ® CLx)
- Gel & Membrane visualization (ImageQuant LAS 4000)
- Bioassays proprietary microsphere technology (MAGPIX® System - Luminex-)
- Plate, cuvette… reader (Synergy H1 Multi-Mode Reader - Bio-Tek -)
- LC-MS/MS using coded affinity tagging (ICAT, iTrac, SILAC..), ProteinChip Array (SELDI analysis), Antibody arrays

Electrophoresis (IEF)

Ettan IPGphor3 (GE Healthcare)

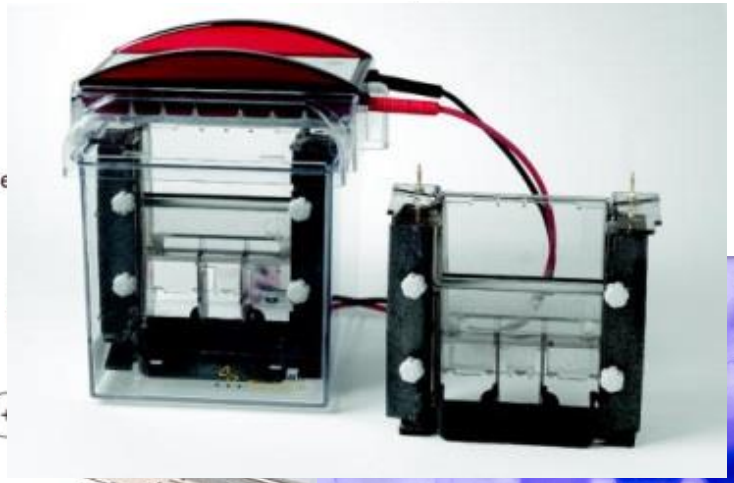


Immobiline DryStrip)

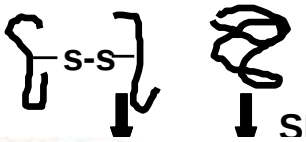
The Principle of Isoelectric Focusing. A pH gradient is established in a gel before loading the sample. (A) The sample is loaded and voltage is applied. The proteins will migrate to their isoelectric pH, the location at which they have no net charge. (B) The proteins form bands that can be excised and used for further experimentation.

Software: Ettan IPGphor3

Electrophoresis (SDS PAGE)



MiniVE Vertical Electroforesis System (GE Healthcare)



Ettan DALT*six* Large Vertical System (GE Healthcare)

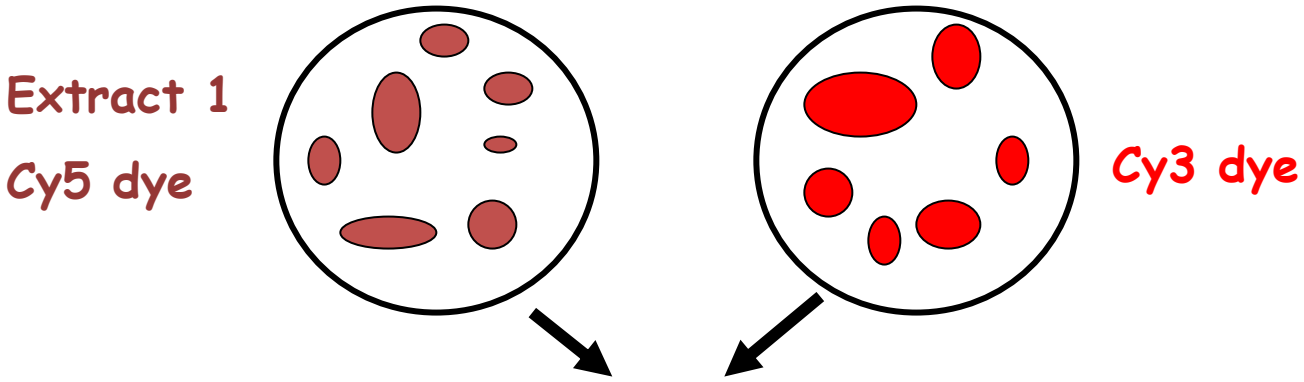
Trans-Blot® Turbo™ Transfer Starter System (Bio-Rad)

Separation by Molecular Weight

DIfference Gel Electrophoresis (DIGE)

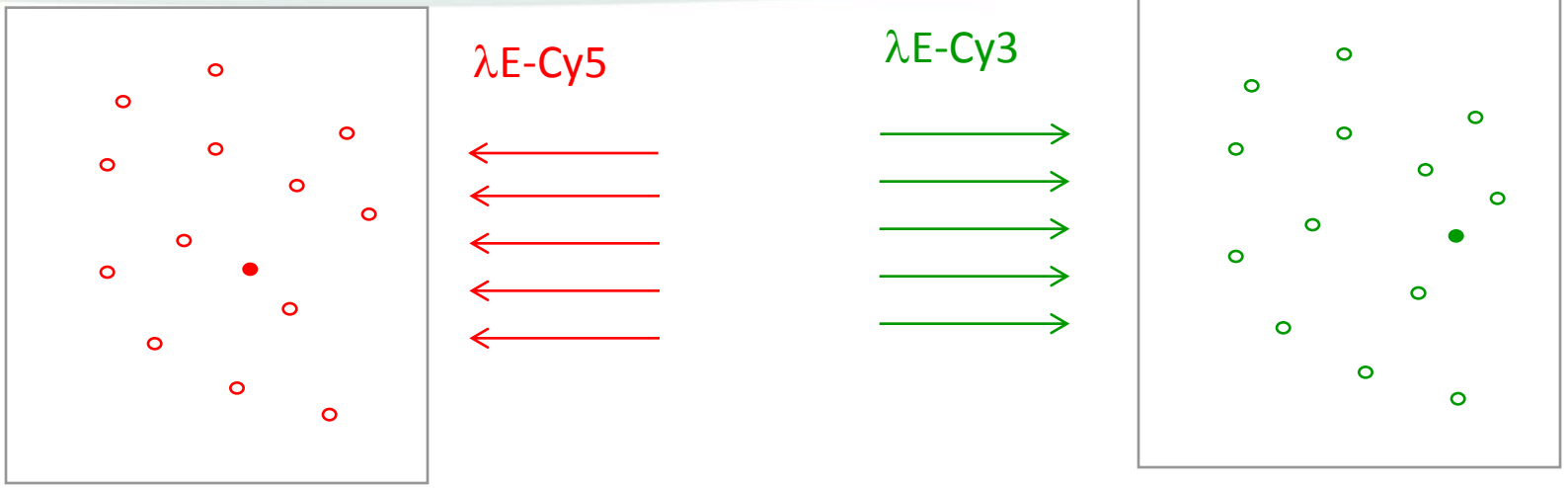
1) Proteins are extracted from the cells or tissues of interest.

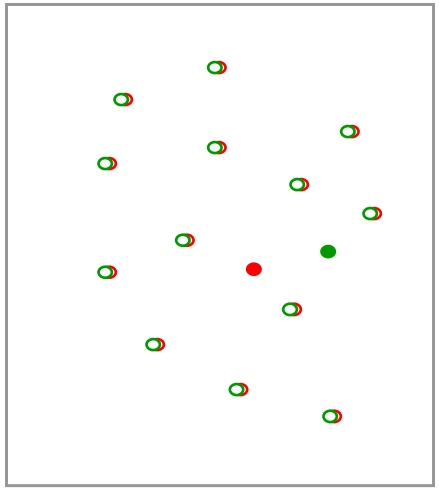
2) The protein extracts are labeled with different fluorescent dyes (Amersham CyDye DIGE Fluor Labeling Kit...):



3) The 2 extracts are mixed and then resolved by 2-D gel electrophoresis.

Dual Channel Imaging Technique (DIGE)





Excise spots; elute; digest, extract peptides; MS analyze, Protein identification

Typhoon FLA 9500

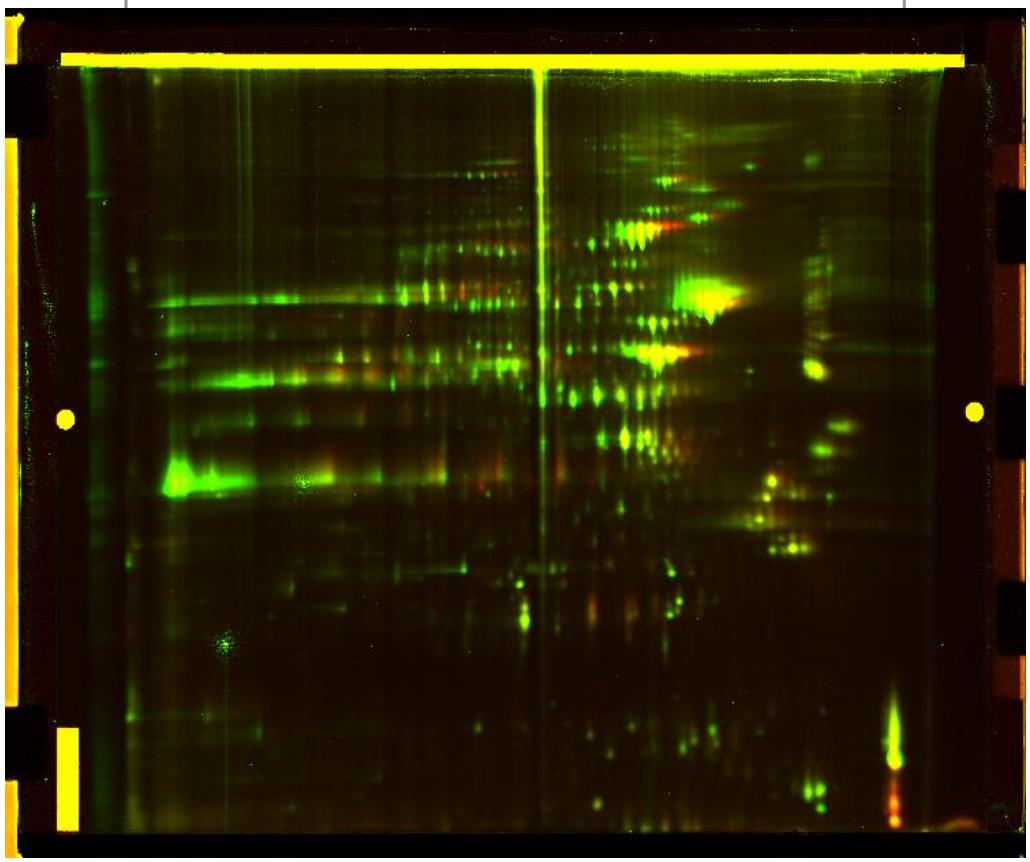
Excitation wavelengths:

473 nm (blue LD laser)

532 nm (green SHG laser)

635 nm (red LD laser)

Dual Channel Imaging Technique (DIGE)



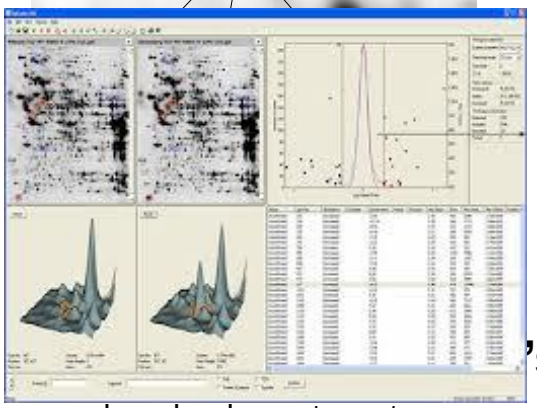
Human brain proteins

Differences in Expression Level in Thalamus

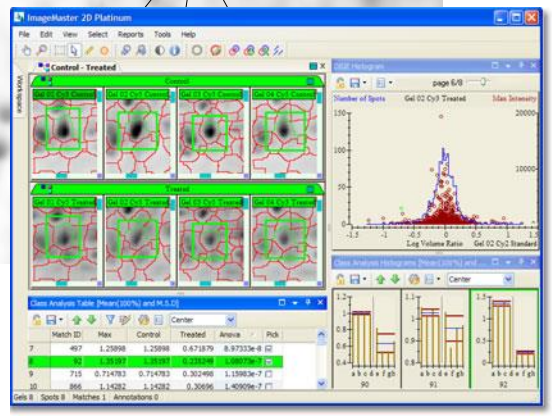
Control

Softwares: DeCyder 2D, Image Master 2D Platinum

phosphoglycerate mutase

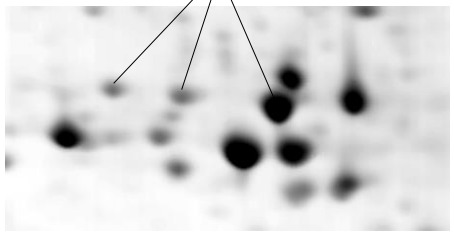


phosphoglycerate mutase

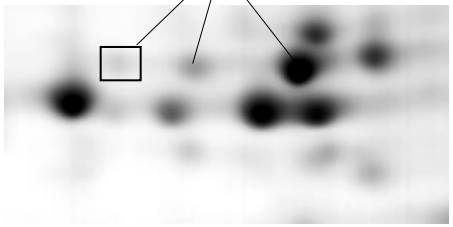


's

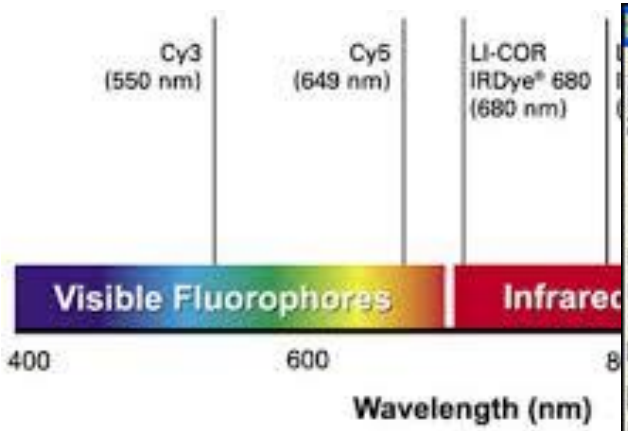
phosphoglycerate mutase

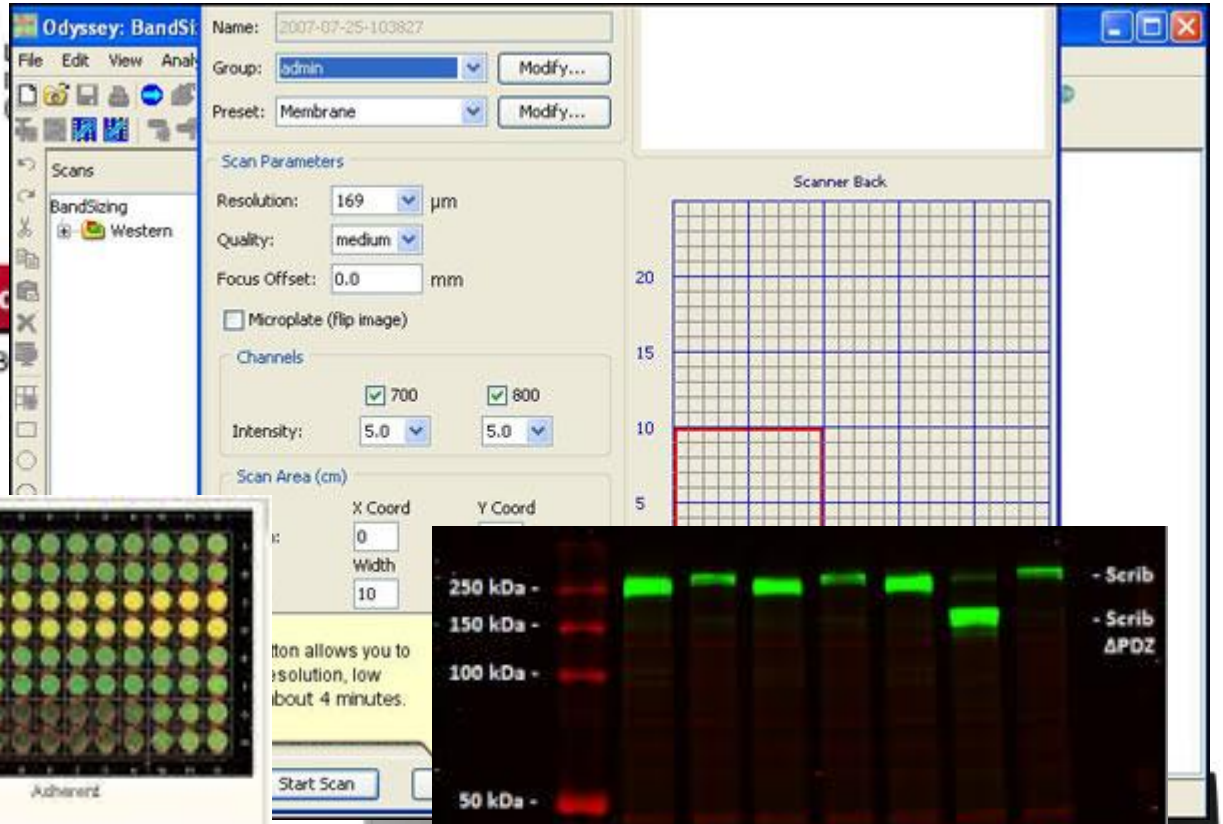


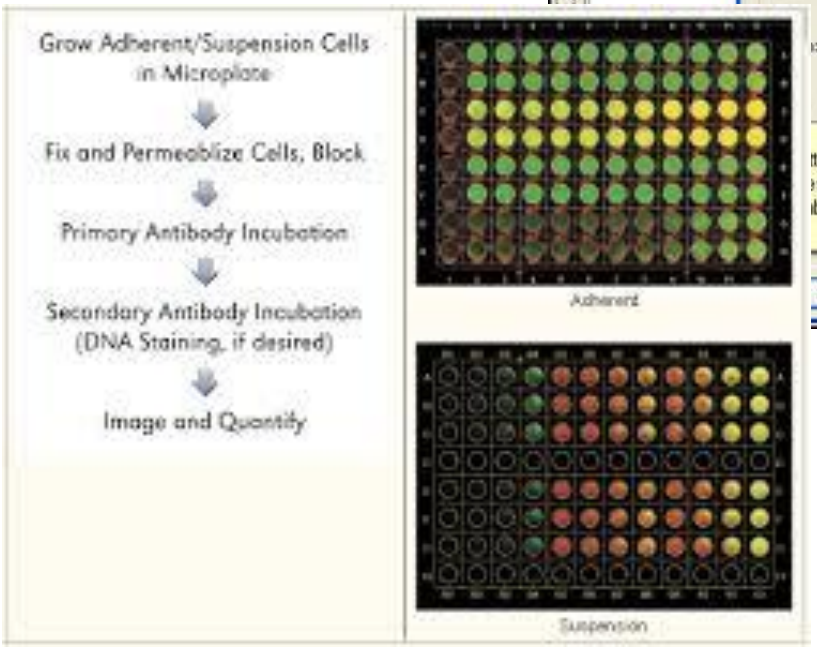
phosphoglycerate mutase

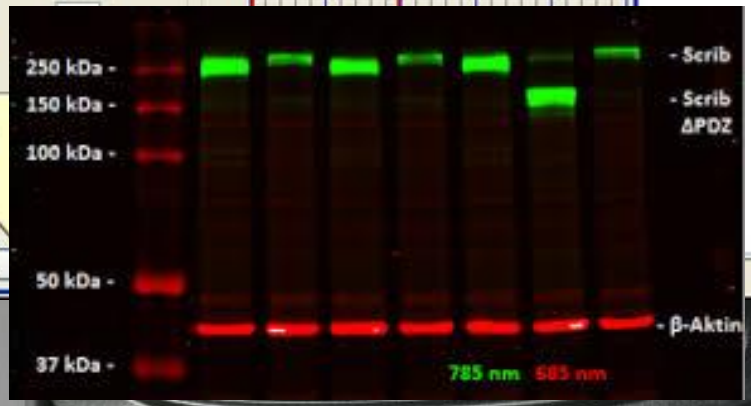


Odyssey ® CLx Infrared Imaging System - LI-COR Biosciences

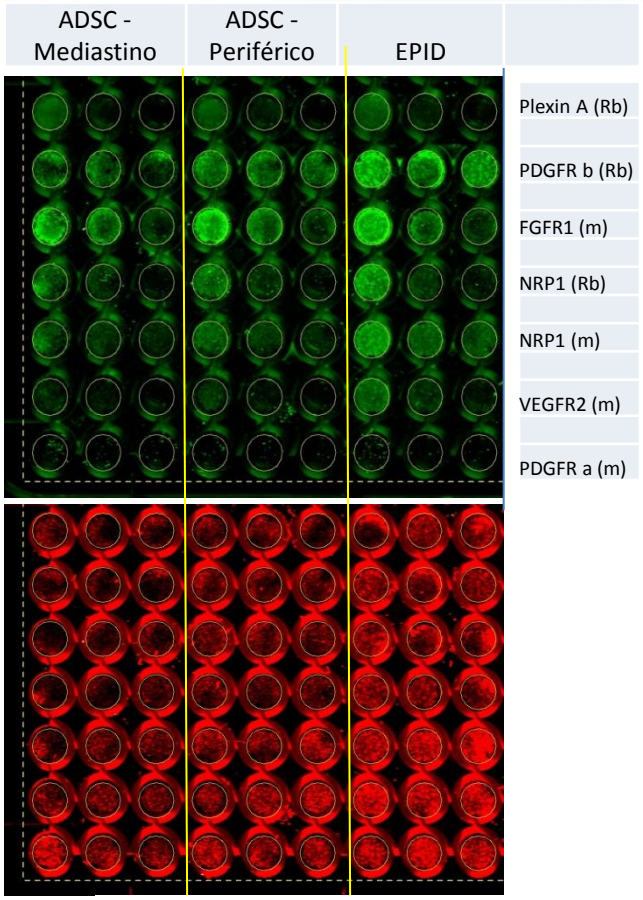


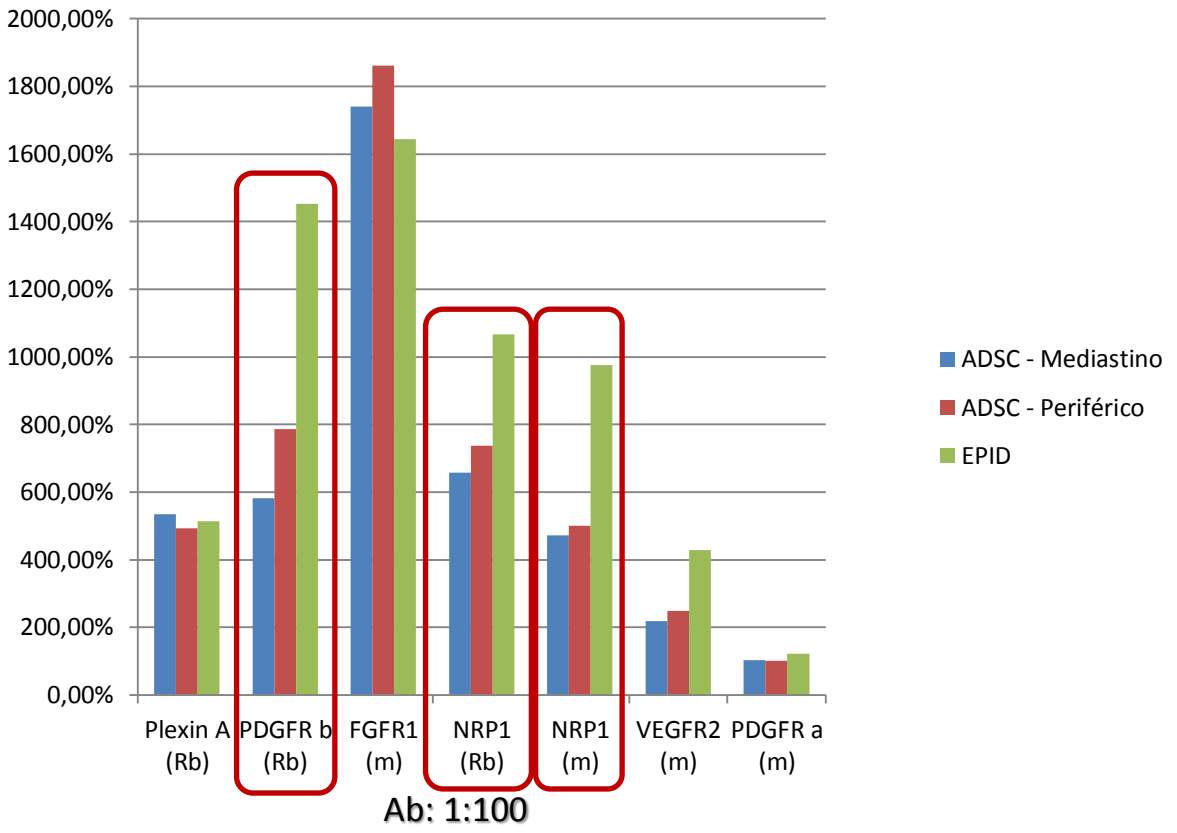






Receptor Expression (Odyssey)





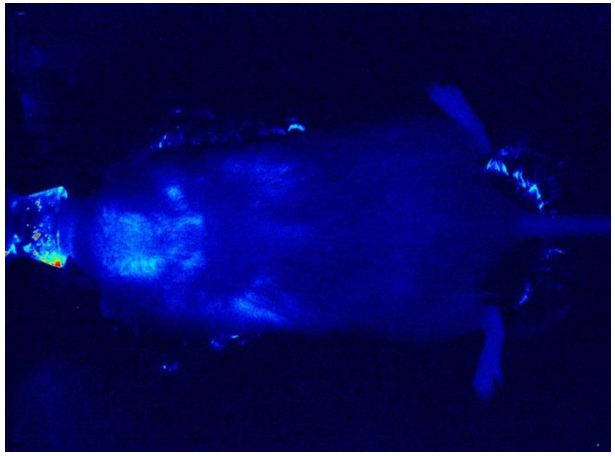
800 nm (verde) compensado * 700 nm — 100% ADSC-Periférico con PDFGR α 1:100

	ADSC - Mediastino			ADSC - Periférico			EPID						
	1:100	1:200	1:400	1:100	1:200	1:400	1:100	1:200	1:400				
	01	02	03	04	05	06	07	08	09	10	11	12	
A	22,395	24,197	22,668	23,381	26,669	24,853	23,258	23,718	23,533	24,249	18,422	21,319	
B	535,34%	208,83%	135,62%	493,57%	195,09%	136,84%	513,82%	187,81%	122,93%	19,136	18,275	18,978	**Plexin A (Rb)**
C	582,35%	593,02%	400,13%	786,57%	680,74%	588,07%	1451,85%	981,03%	1008,45%	18,817	18,357	21,862	**PDGFR b (Rb)**
D	1740,04%	873,26%	313,13%	1861,30%	765,11%	343,20%	1643,59%	590,60%	262,10%	19,241	19,580	20,467	**FGFR1 (m)**
E	657,06%	317,29%	159,43%	737,21%	300,41%	167,72%	1066,75%	285,26%	167,78%	20,249	18,219	19,191	**NRP1 (Rb)**
F	471,58%	289,22%	217,23%	501,04%	290,67%	192,79%	975,30%	436,43%	291,18%	19,994	18,723	19,600	**NRP1 (m)**
G	218,56%	155,14%	132,48%	247,78%	143,49%	105,67%	428,42%	212,74%	130,45%	19,902	19,343	20,063	VEGFR2 (m)
H	103,59%	109,62%	114,02%	**100,00%**	96,67%	93,72%	121,78%	90,00%	88,39%	21,483	20,166	20,380	PDGFR a (m)

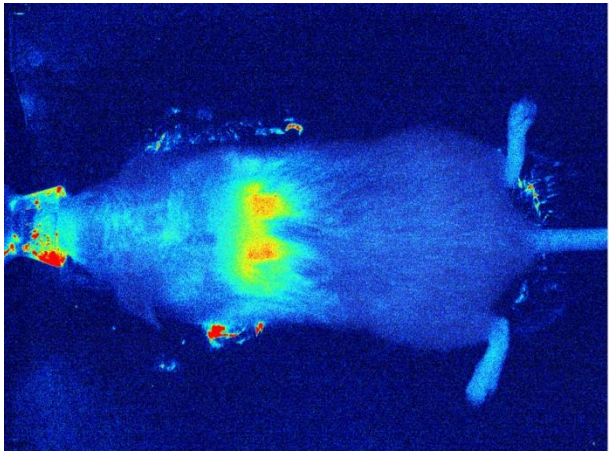
Pearl® Impulse (Li-Cor)

Estabulario UIB

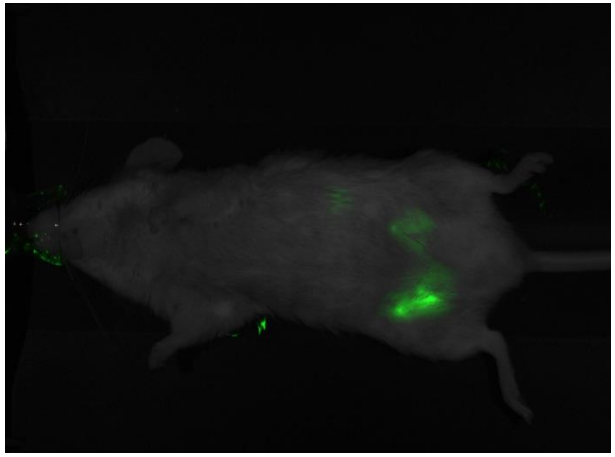
Mice ADSCs-CellVue® NIR815 with Pearl® Impulse



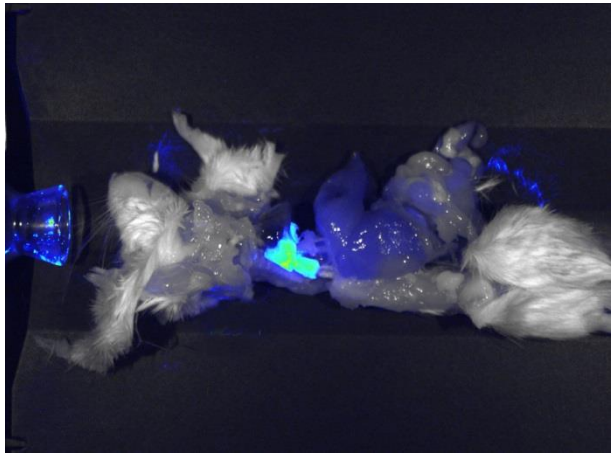
Control -12- (no tobacco, no ADSCs-CellVue®)



No. 6 i.v. 300 µl (tobacco, ADSCs-CellVue®)



Ventral - No. 8 i.p. 300 µl + i.p. 300 µl (tobacco, ADSCs-CellVue®)

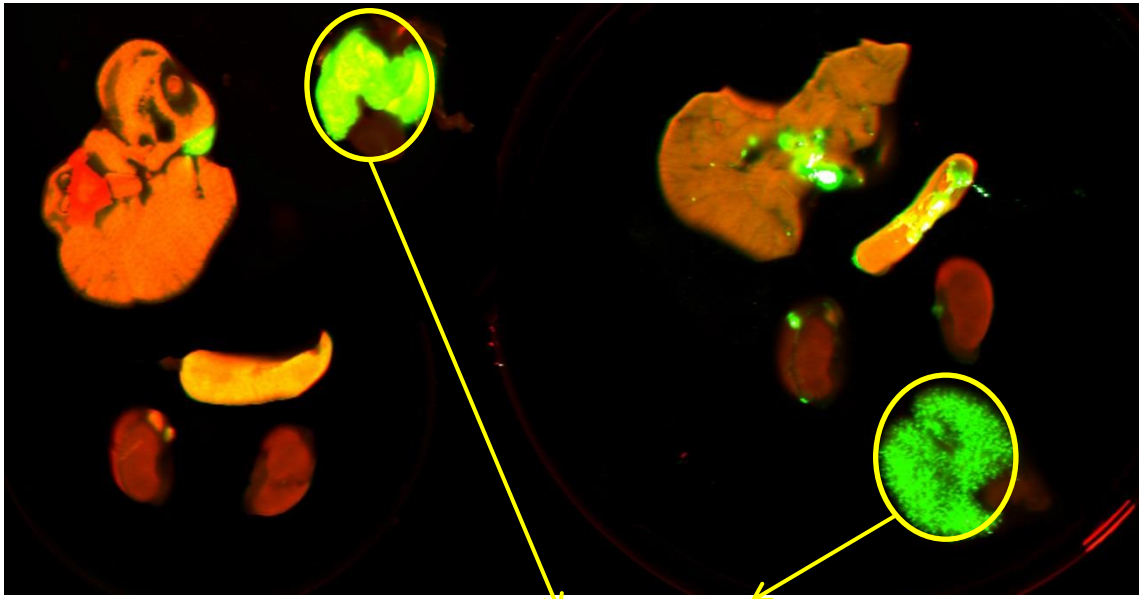


No. 10 i.v. 200 µl 1st + 300 µl 2nd (tobacco, ADSCs-CellVue®)

Mice ADSCs-CellVue® NIR815 with Odyssey® CLx

(Organs ~1 week after 2nd injection)

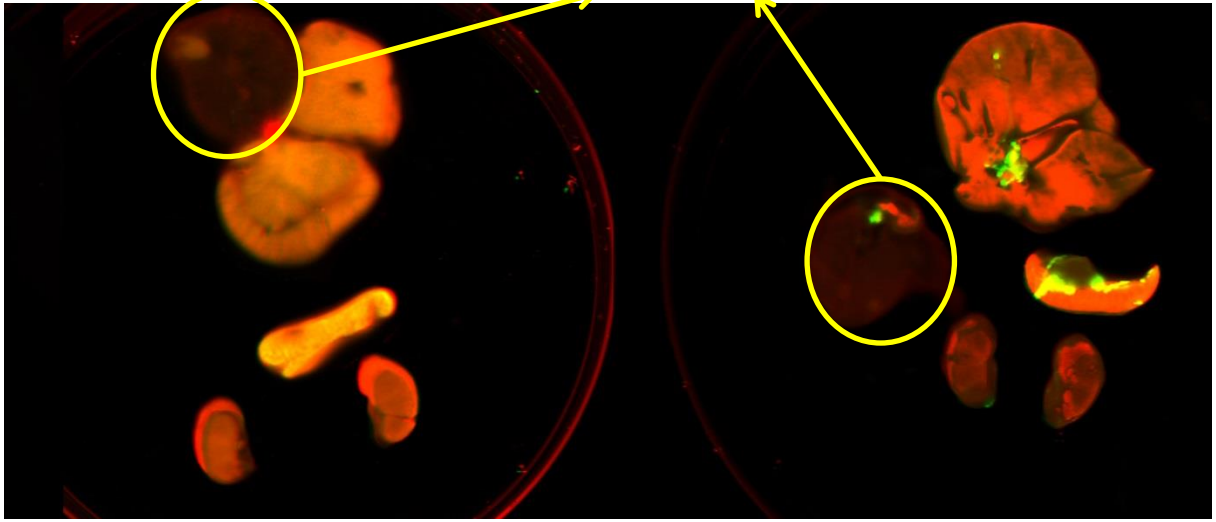
No. 6
1st: 300 µl i.v.
2nd: 50 µl i.v. + 250 µl s.c.



No. 2
1st: 50 µl i.v. + 200 µl i.p.
2nd: 300 µl i.p.

Lungs

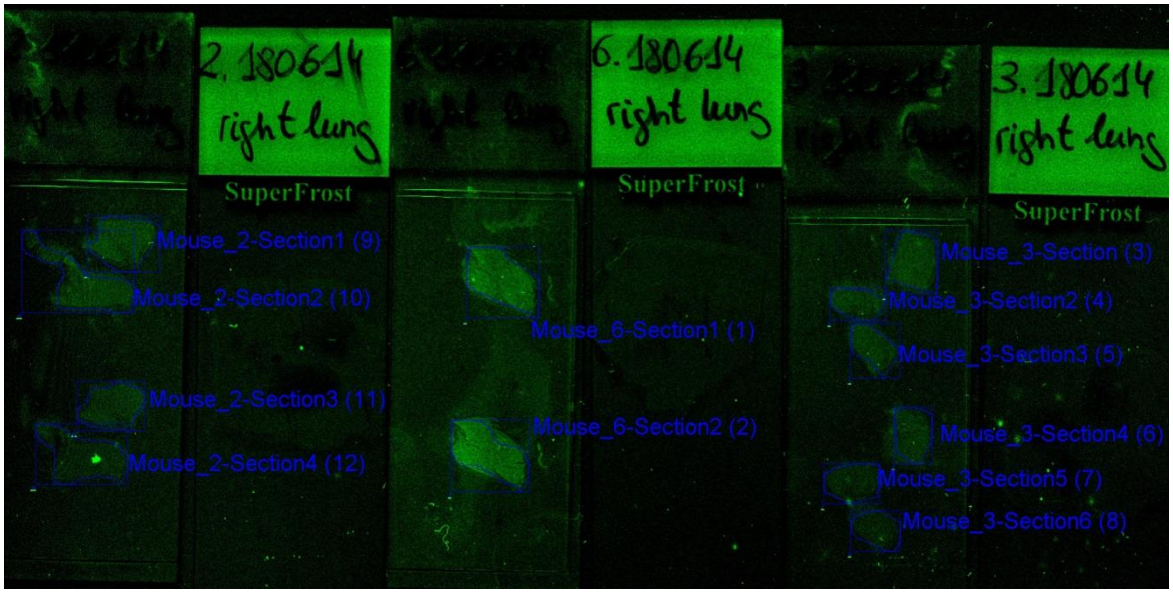
No. 14
Control

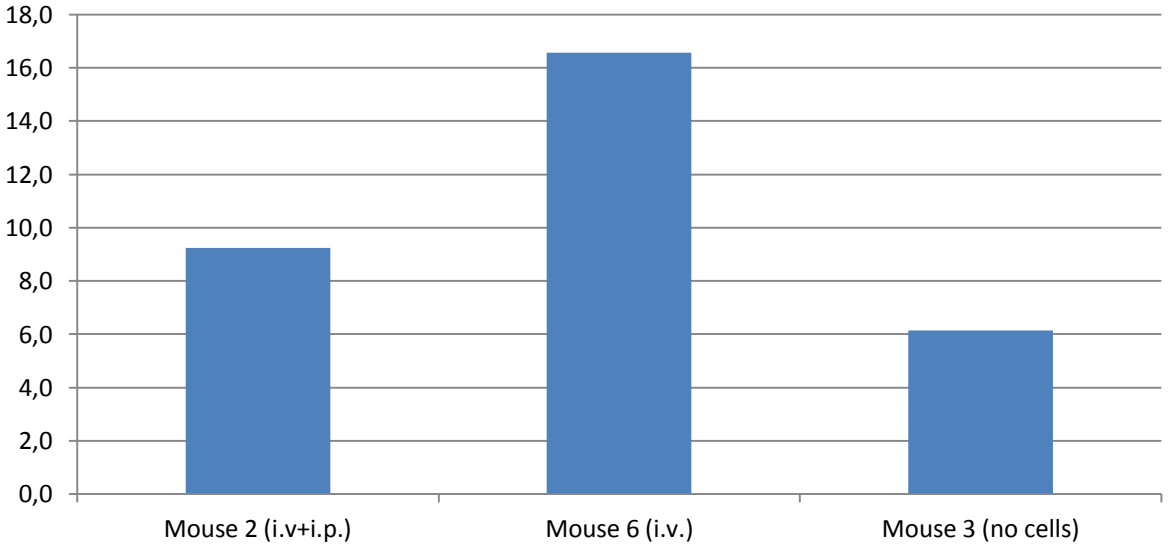


No. 8
1st: 300 µl i.p.
2nd: 300 µl i.p.

Mice ADSCs-CellVue® NIR815 with Odyssey® CLx

(Paraffin sections with +/- H&E)





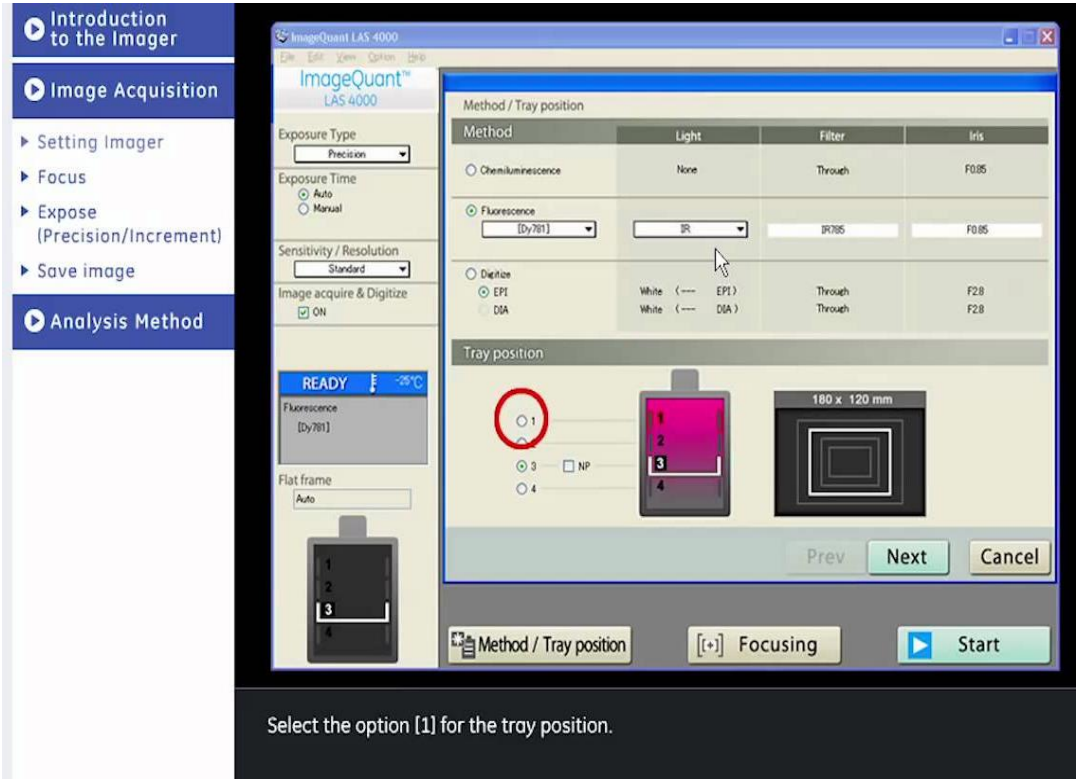
ImageQuant LAS 4000 (GE Healthcare Life Sciences)

Digital imaging of protein and nucleotides in gels and membranes:

- Dark sample cabinet
- Camera system
- Filter wheel
- Light sources

ImageQuant LAS 4000 (GE Healthcare Life Sciences)

Software: Image Quant LAS 4000 v1.2



Light sources:

Chemiluminescence (ECL)

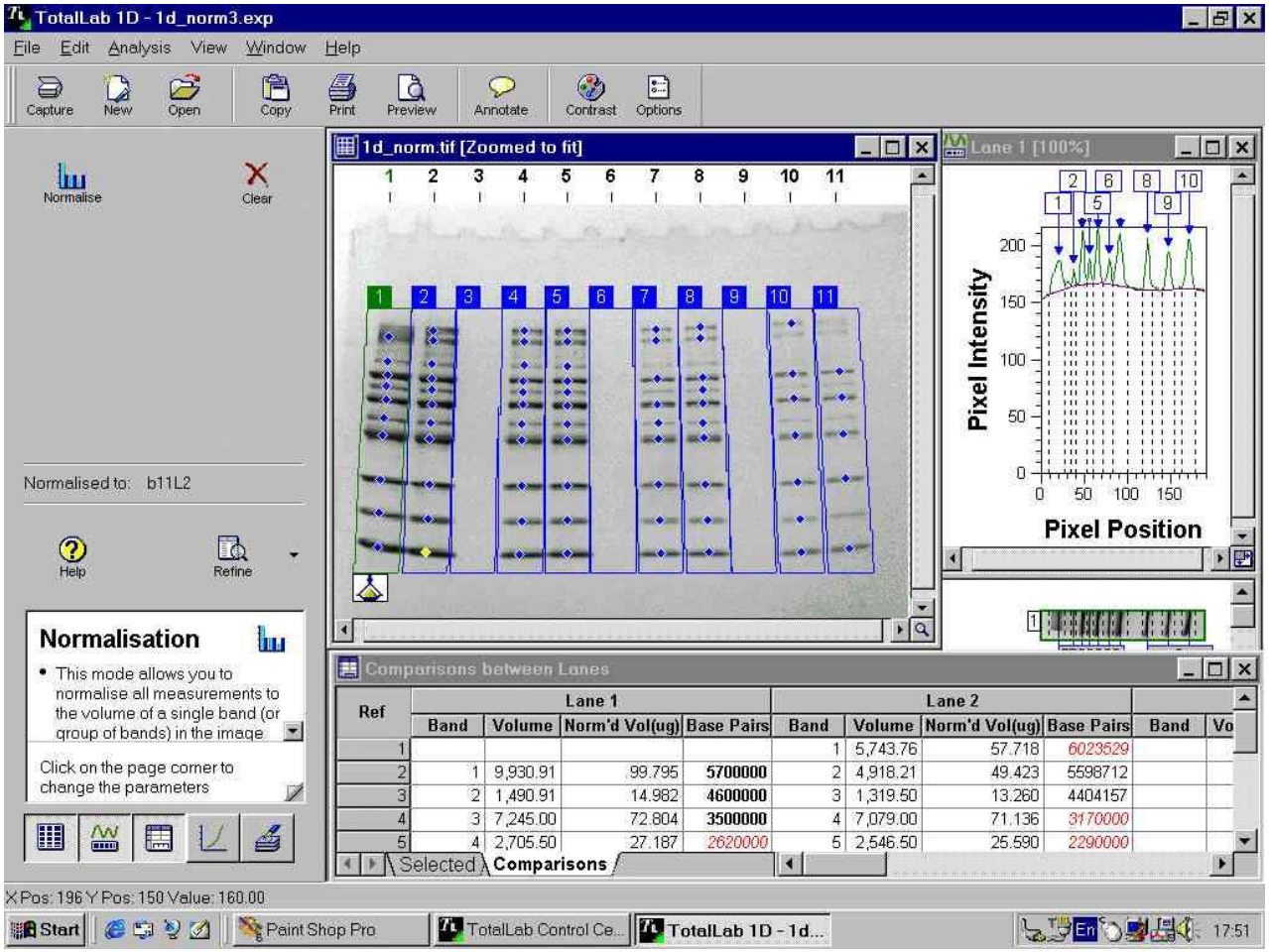
Fluorescence (Epi / Trans)

- UV (EtBr)
- Blue
- Green
- Red
- IR

Digitization (Epi / Trans)

ImageQuant LAS 4000 (GE Healthcare Life Sciences)

Software: Image Quant TL



MAGPIX® System (Luminex)

MAGPIX® System (Luminex)

Based on the principles of fluorescence imaging. Lasers and Photo Multiplying Tubes (PMTs) are replaced with Light Emitting Diodes (LEDs) and a CCD camera to deliver a cost effective, compact, and reliable multiplexing platform.

xMAP Technology

xPONENT® software:

Synergy H1 Multi-Mode Reader - (Bio-Tek)

Absorbance/ Fluorescence / Bioluminescence

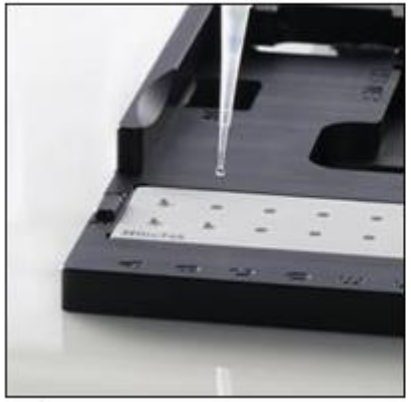
Synergy H1 Multi-Mode Reader - (Bio-Tek)

Patented Hybrid Technology™ combines flexible monochromator detection (**230 - 999** nm, 1 nm increment) with high performance dichroic-based detection.

Compatible with **Take3™ Micro-Volume Plate**: samples down to 2 µL volume can be measured. Especially useful when working with precious samples, for fast and accurate DNA/RNA quantification at 260 nm

Dichroic-based filter optics, for best performance and advanced detection technologies such as fluorescence polarization and time resolved fluorescence.

Compatible with the Gas Controller for control and monitoring of CO2 and O2.



A

B

Synergy H1 Multi-Mode Reader - (Bio-Tek)

http://www.biotek.com/resources/index.html

Gen5 Sample Files

- Absorbance (33)
- AlphaScreen / AlphaLISA / SureFire (4)
- Fluorescence - Time Resolved / TR-FRET (9)
- Fluorescence Intensity (47)
- Fluorescence Polarization (6)
- Imaging (4)
- Luminescence (19)
- Sample Experiments with Data (2)
- System Files - Cytation Imaging Cubes (16)
- System Files - Cytation Imaging Objectives (8)
- System Files - Plate Types (10)
- System Files - Synergy Neo Filter Cubes (47)

Synergy H1 Multi-Mode Reader - (Bio-Tek)

absorbance/ fluorescence / bioluminescence

Flexible monochromator-based multi-mode microplate reader

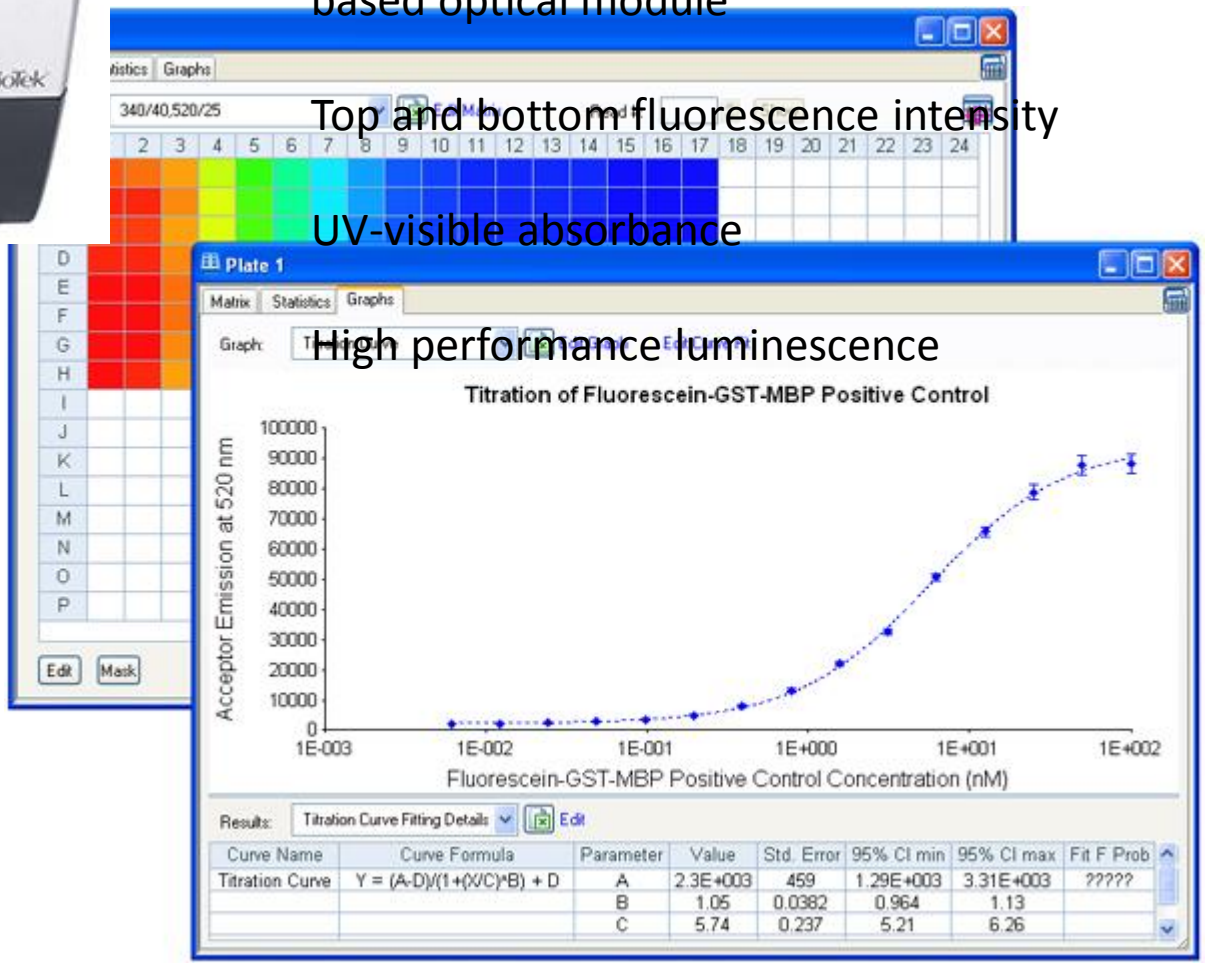
Gen5 software: reader control, advanced data analysis and flexible Excel export in one software package.

Hybrid system with the addition of a filter-based optical module

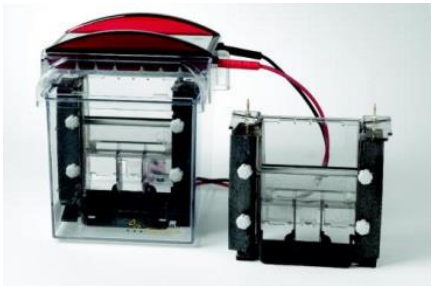
Top and bottom fluorescence intensity

UV-visible absorbance

High performance luminescence



Equipos



¡GRACIAS!