Image Processing in Medical Research

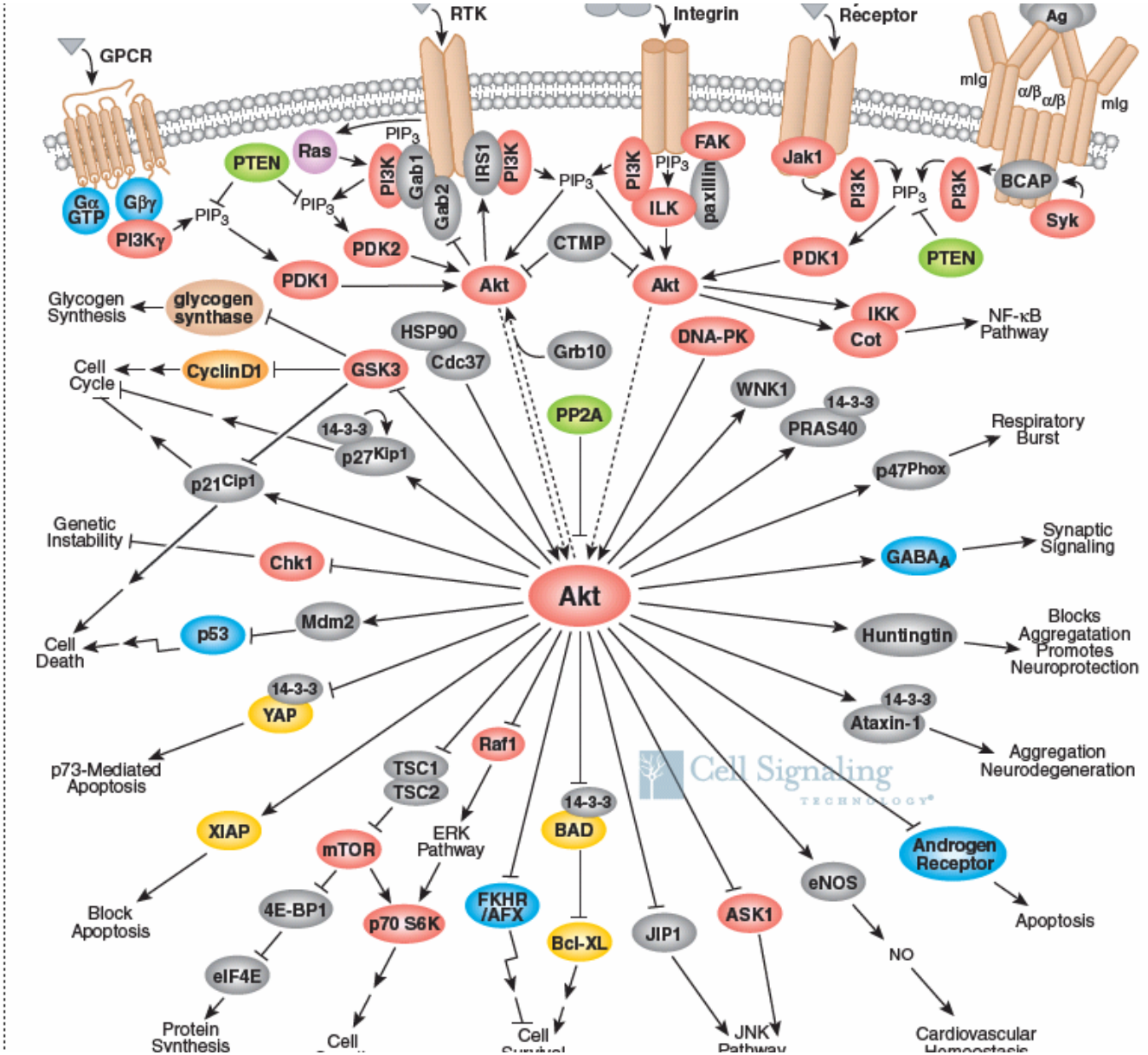
Catherine T. Nguyen
UTHSCH

Mills, Ph.D., M.D.
t of Molecular Therapeutics

- Our laboratory has made significant contributions to the understanding of ovarian tumorigenesis, including the identification and development of lysophosphatidic acid (LPA) as a possible marker for early-stage ovarian cancer and as a potential target for therapy. We have also played a major role in the understanding of the genetic aberrations in the phosphatidylinositol 3 kinase/PTEN/AKT pathway forwarding this cascade as a major target for therapy in multiple different cancers.
- Our research program is aimed at identifying the underlying genetic aberrations that occur in cancer (breast, ovarian, lung and prostate) and determining how they contribute to tumorigenesis. These observations should translate into novel indications of prognosis, response to therapy and outcome. Importantly this should lead to new therapies having a major impact on cancer.

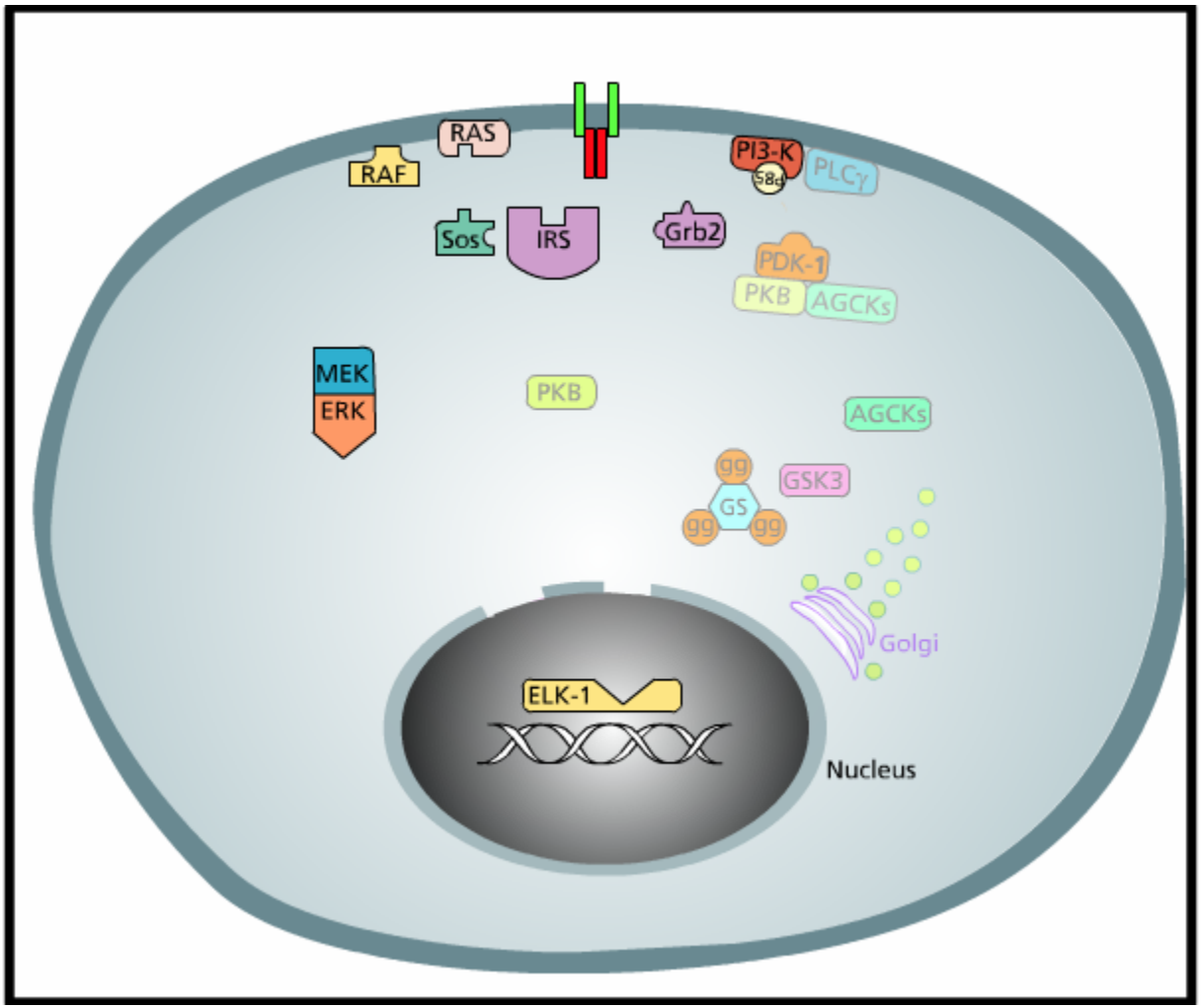
http://gsbs.uth.tmc.edu/tutorial/mills_g.html

PI3K and Cell Signalling



Translocation in signal transduction

...Interact-Modify-Translocate...



Redistribution® assays ‘target’ translocation

www.BioImage.com

Potential advantages of translocation modulators...

- Drugs are not directed at catalytic sites:
  - anticipate fewer side-effects through better targeting of action
  - approach well-validated targets with ‘difficult’ history
  - Can potentially target ANY signalling component where precise cellular location determines function
  - Not resticted to single Mode of Action per target.

- **Target Location**
  [anchoring, complex formation]
  Target Processes
  [nuclear import/export, transport, processing, half-life]
  Target Molecular Regulation
  [intermolecular and intramolecular interactions]

Types of microscopy

Brightfield
phase
Differential Interference Contrast (DIC)
darkfield
epifluorescence:
confocal
2-photon
digital decon

Fluorescence Microscopy

-The absorption and subsequent re-radiation of light by organic and inorganic specimens is typically the result of well-established physical phenomena described as **fluorescence.**

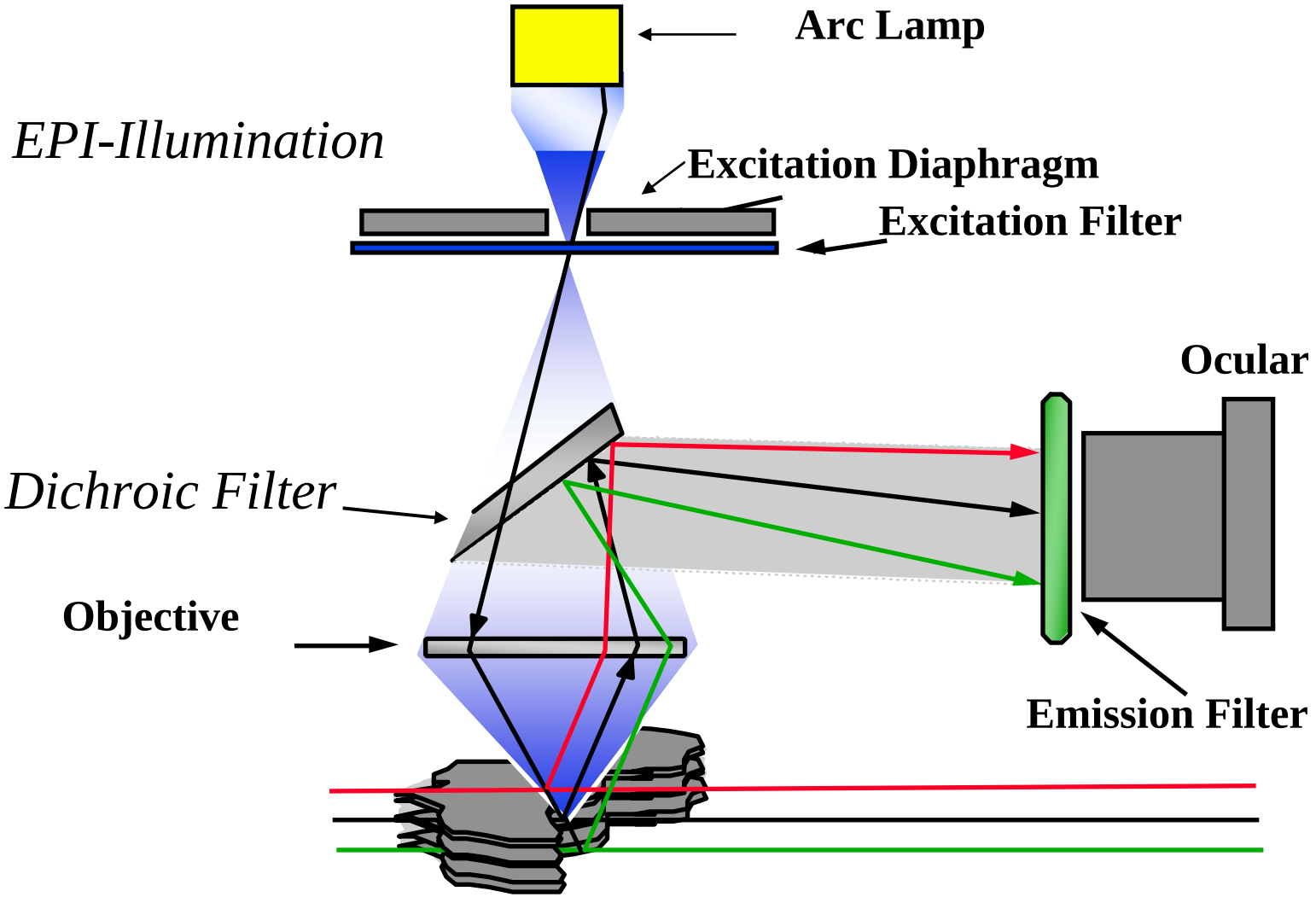
-The basic function of a fluorescence microscope is to irradiate the specimen with a desired and specific band of wavelengths, and then to separate the much weaker emitted fluorescence from the excitation light.

-In a properly configured microscope, only the emission light should reach the eye or detector so that the resulting fluorescent structures are superimposed with high contrast against a very dark (or black) background.

-The limits of detection are generally governed by the darkness of the background, and the excitation light is typically several hundred thousand to a million times brighter than the emitted fluorescence.

http://www.microscopyu.com/articles/fluorescence/fluorescenceintro.html

Fluorescent Microscope



Ray Grill, PhD

Light Sources - Lasers

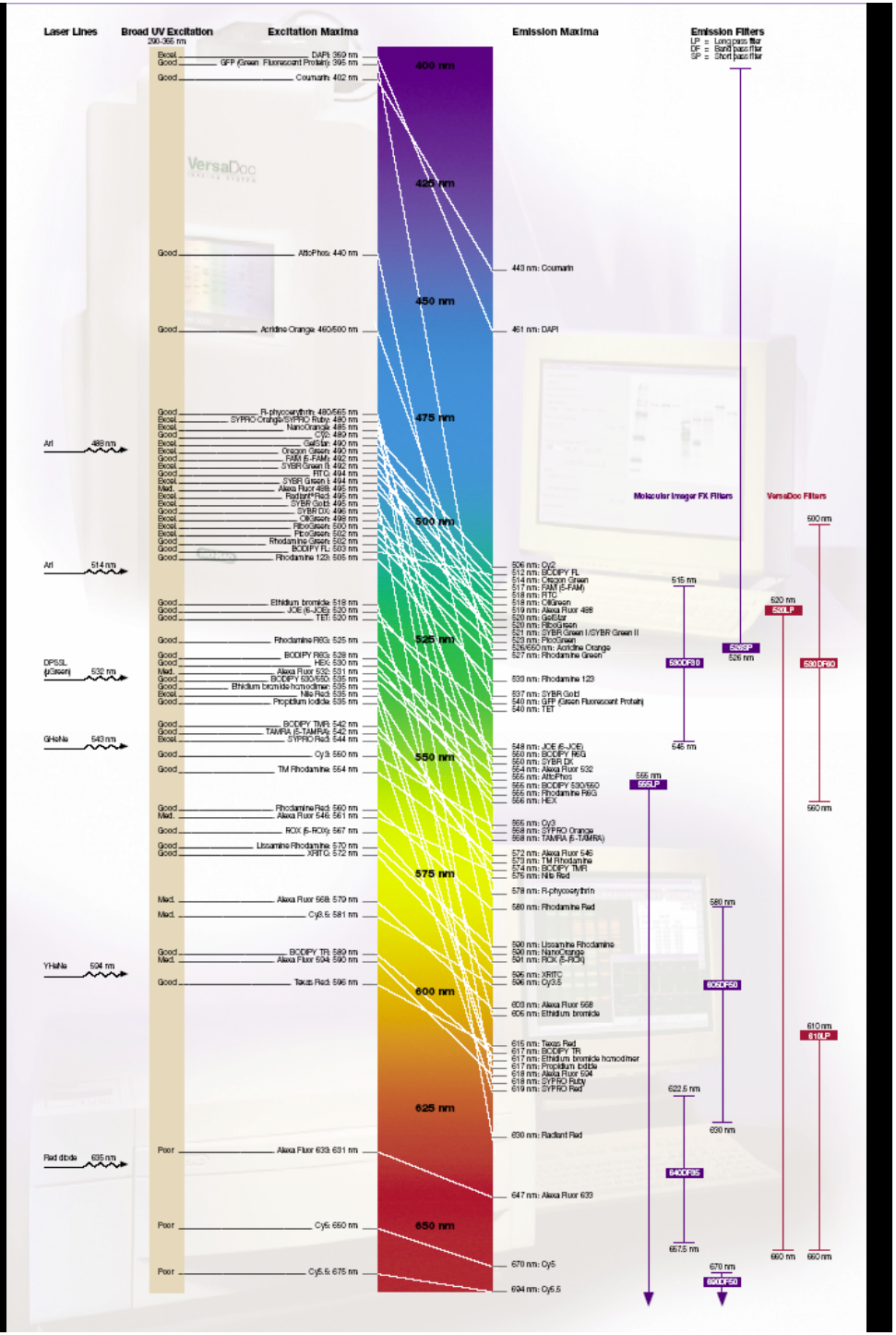
Laser	Abbrev.	Excitation Lines
• Argon	Ar	353-361, 488, 514 nm
• Violet Diode		405 nm
• Krypton-Ar	Kr-Ar	488, 568, 647 nm
• Helium-Neon	He-Ne	543 nm, 633 nm
• He-Cadmium	He-Cd	325 - 441 nm
• Red Diode		647 nm

(He-Cd light difficult to get 325 nm band through some optical systems)

Fluorophores:

-There is a wide range of fluorophore molecules available for fluorescence and confocal microscopy

-with a 3-laser line confocal system, one can label up to 3 different targets within a tissue source



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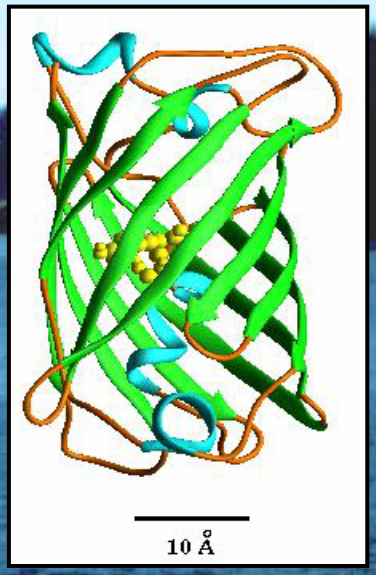
GFP



- Green Fluorescent Protein (GFP) isolated from *Aequorea victoria*

- **GFPs fused to signalling components to track translocations in cells**

www.BioImage.com

Translocation Assay

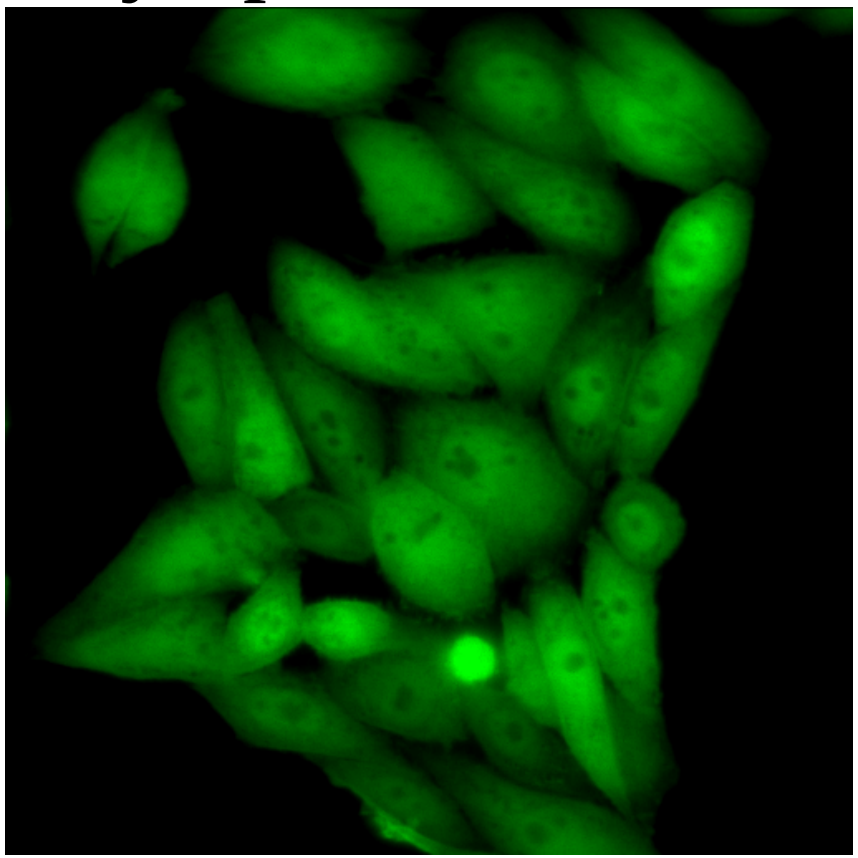
- Protein translocations are vital to information flow in signal transduction

- **Assays to identify and screen protein-protein interactions in context, in mammalian cells**

- monitor protein translocations, real-time in live cells

- find potentially useful therapeutic compounds which target intracellular signal transduction

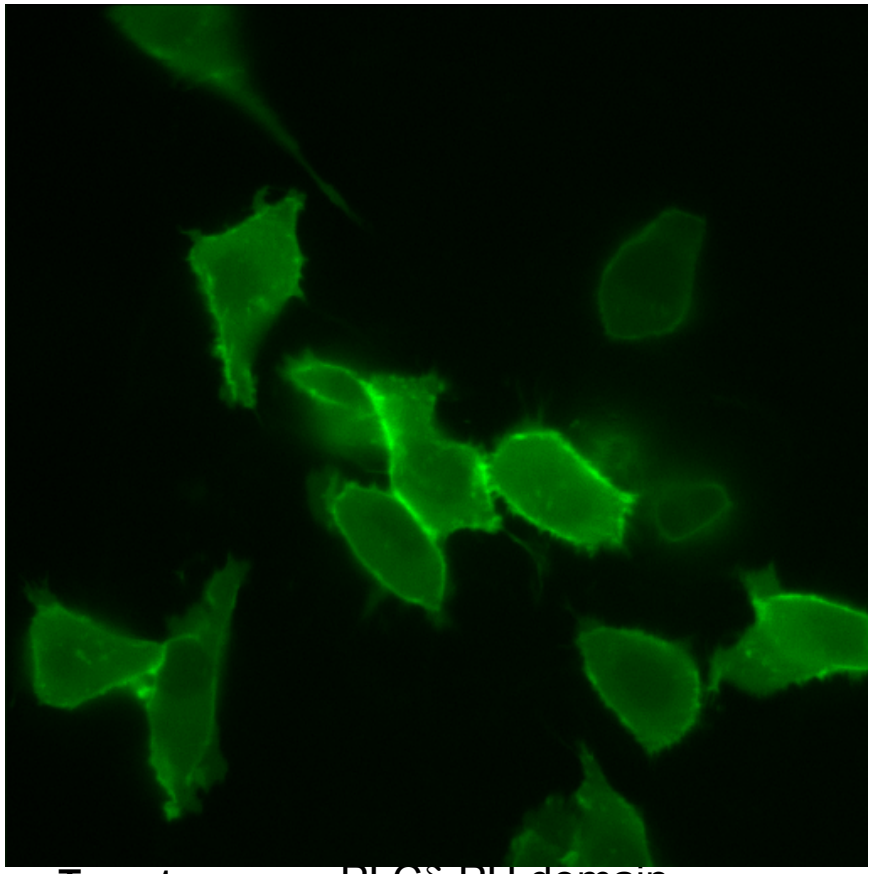
Cytoplasm to Membrane Translocation



Example	Therapeutic area
PKCα	Cancer
PKCβ	Diabetic complications
PKCε	Cardiovascular
Akt	Cancer & Diabetes
Arno	Many
β-Arrestin	Many

Target: Akt1
Host cell type: CHO-hIR
Stimulation: IGF-1 / insulin
Timescale: ~5 min

Membrane to Cytoplasm Translocation



Target: PLCδ-PH domain
Host cell type: CHO-hIR
Stimulation: ATP
Timescale: ~30 sec

Example	Therapeutic area
PLCδ	Many
MARCKS	Cancer, Diabetes
Membrane Receptors	Many

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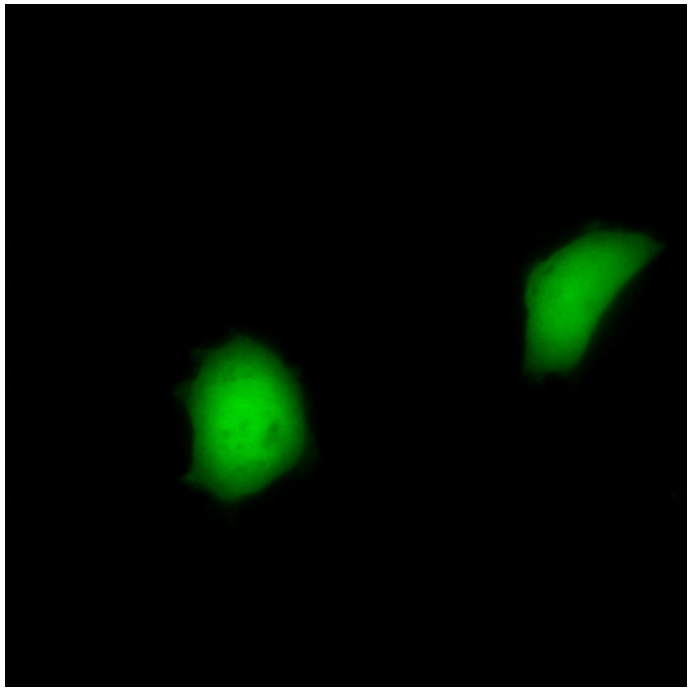
Cytoplasm to Nucleus Translocation

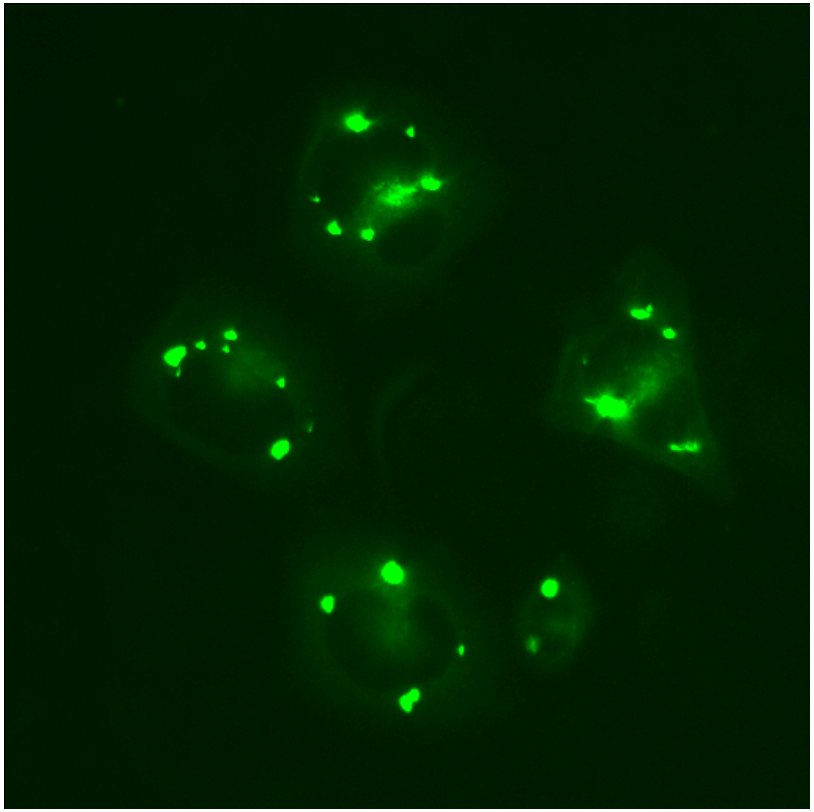


Example	Therapeutic area
p65 NFκB	Inflammation
ERK1	Cancer
p38	Inflammation
JNK	Cancer
β-catenin	Cancer
TFs	Many

Target: Erk1
Host cell type: CHO-hIR
Stimulation: FCS / hEGF
Timescale: ~10 min

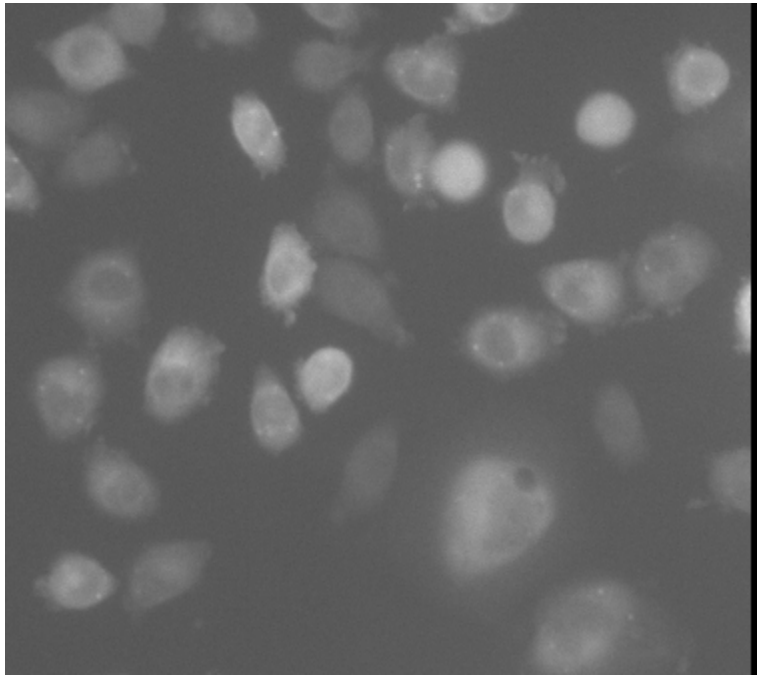
www.BioImage.com

Granules to Cytoplasm Translocation



Target: PKAcat
Host cell type: CHO-hIR
Stimulation: Forskolin, then G_i stimuln
Timescale: ~40 min total

Example	Therapeutic area
PKAcat	Gs/Gi GPCRs, many...
PDE4As	Inflammation, many



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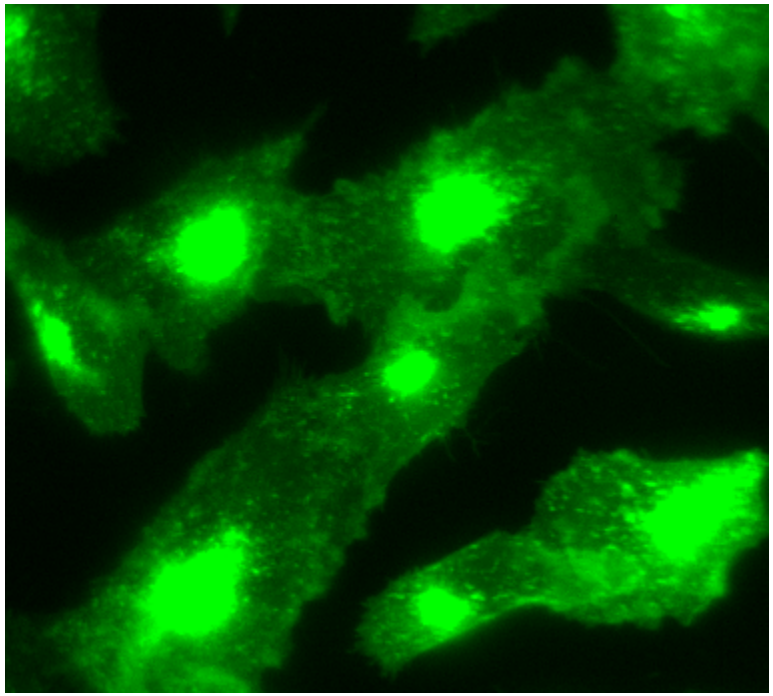
Vesicle to Plasma Membrane Translocation

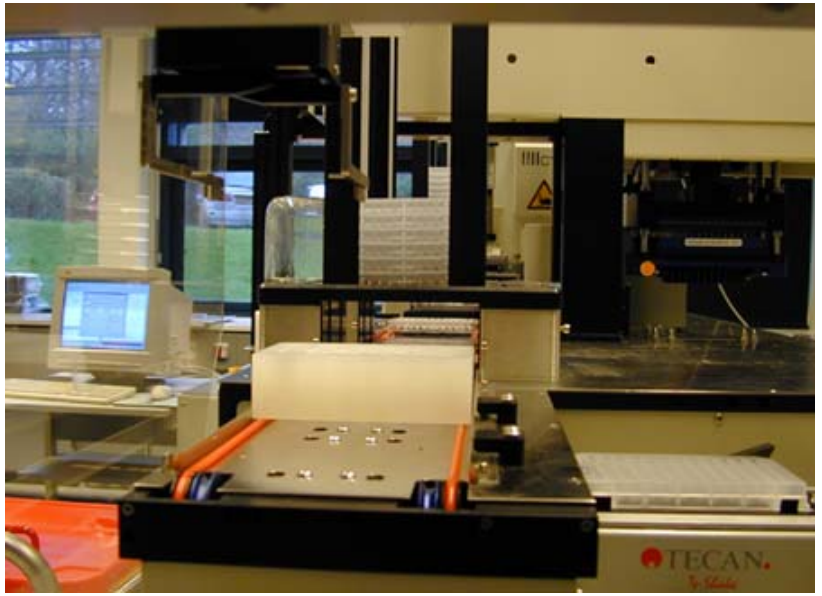


Example	Therapeutic area
GLUT4	Diabetes
Receptor & channel recycling	Many

Target: GLUT4
Host cell type: CHO-hIR
Stimulation: Insulin
Timescale: ~20 min

www.BioImage.com

High Throughput Imaging

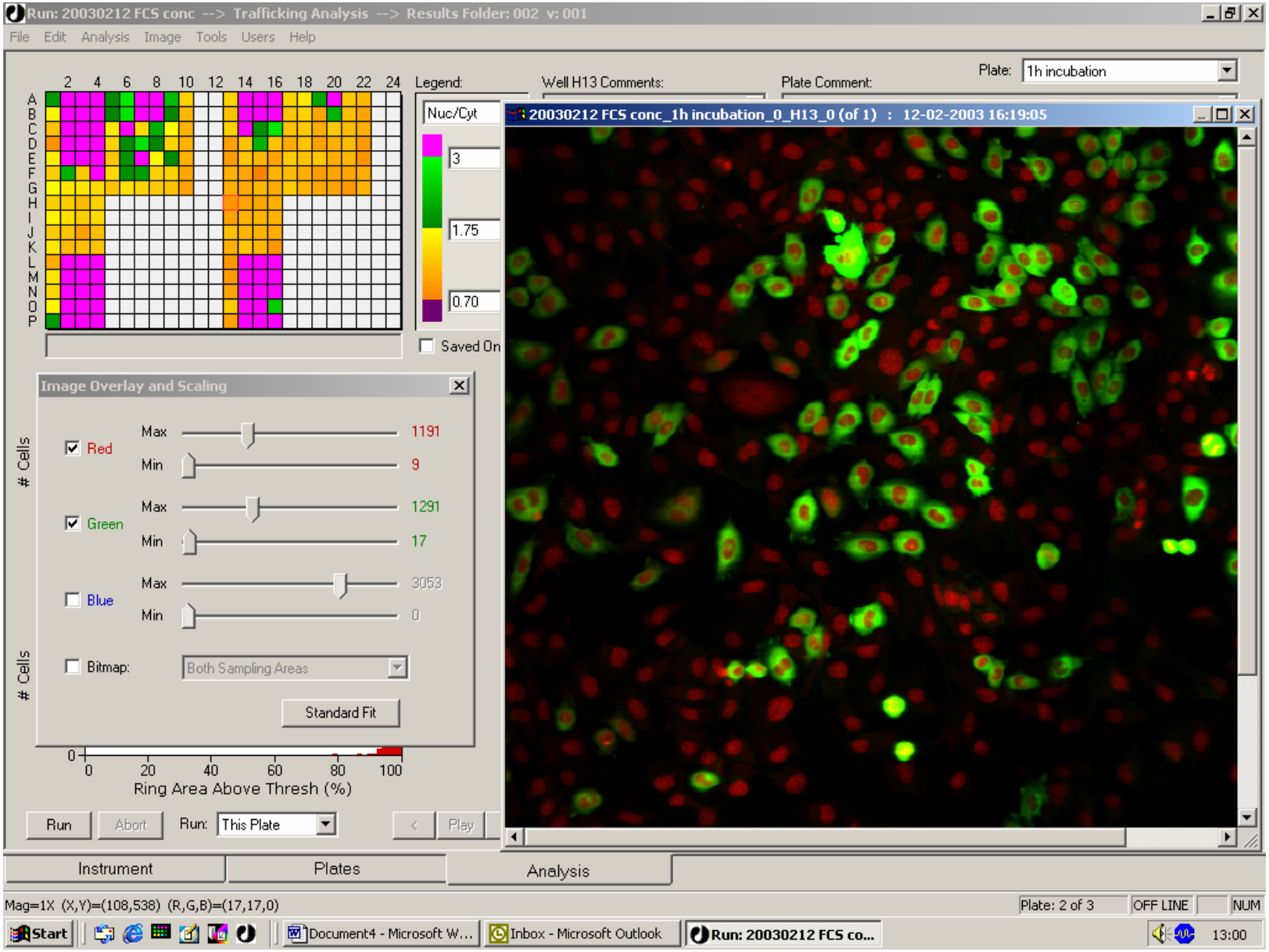


260,000 compounds
Selected for diversity

INCell Throughputs:
20,000 /day/machine

www.BioImage.com

Redistribution Assays in HTS – IN Cell - 1



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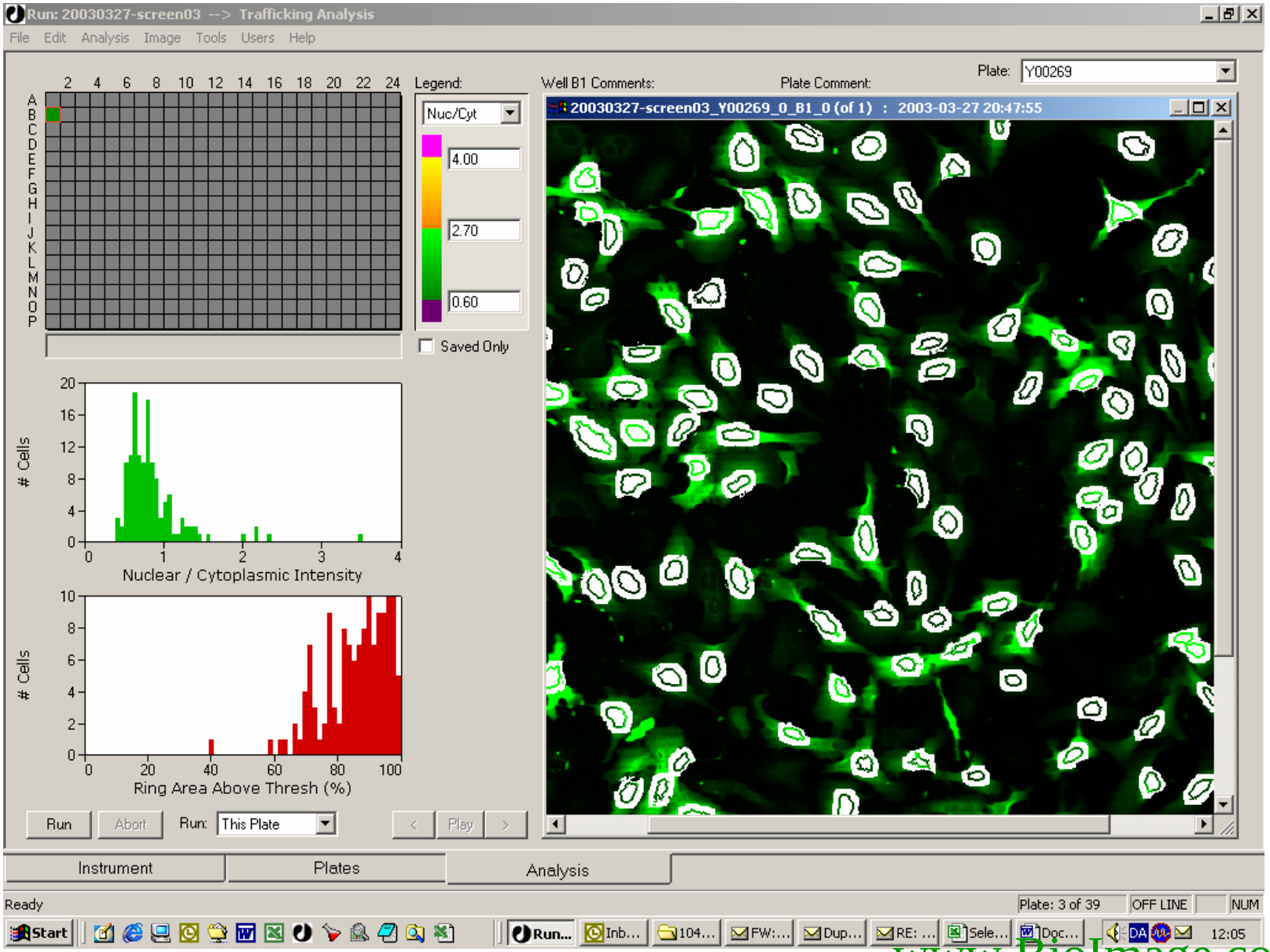
Redistribution Assays in HTS – IN Cell - 2



Redistribution Assays in HTS – IN Cell - 3

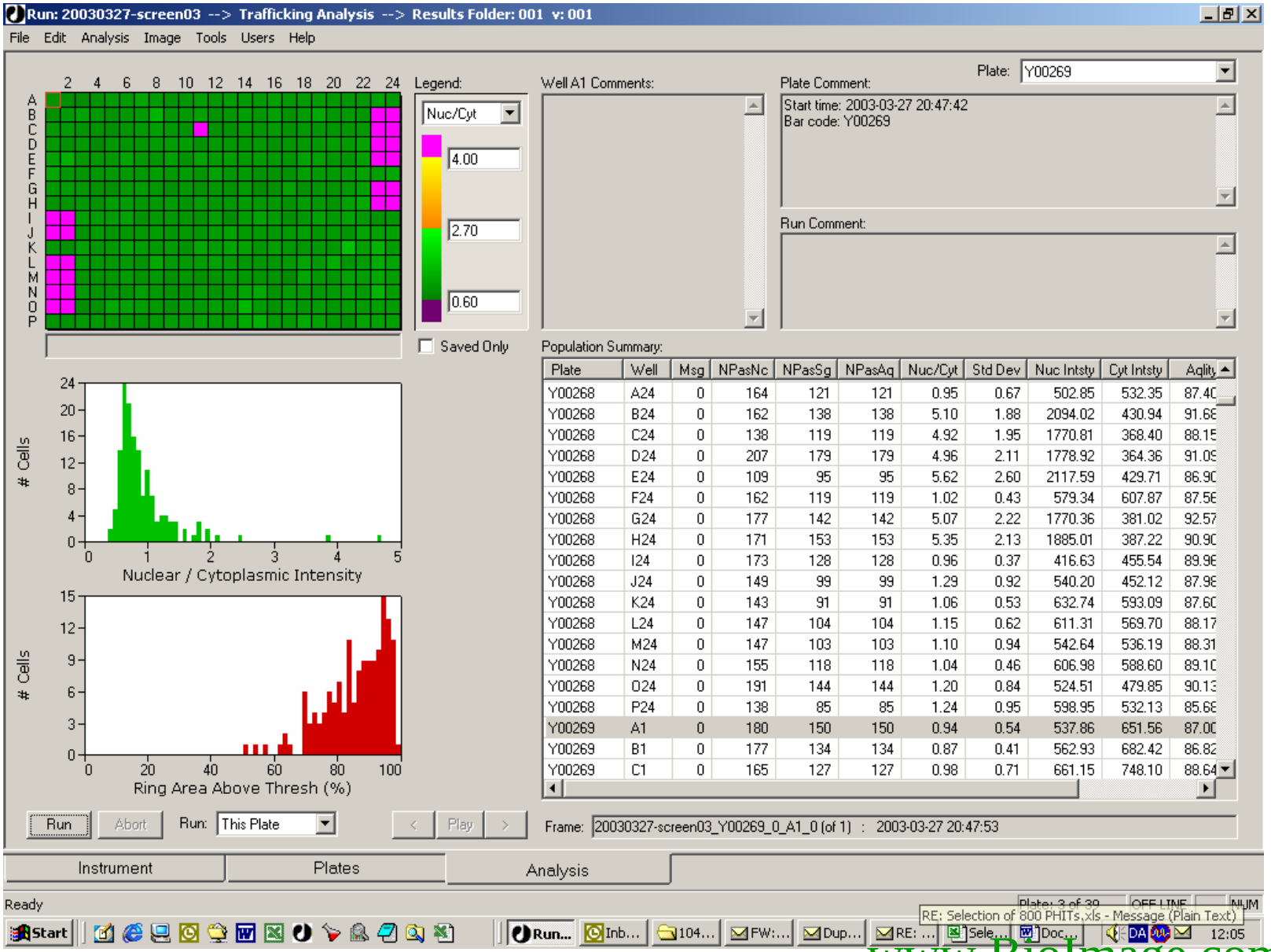
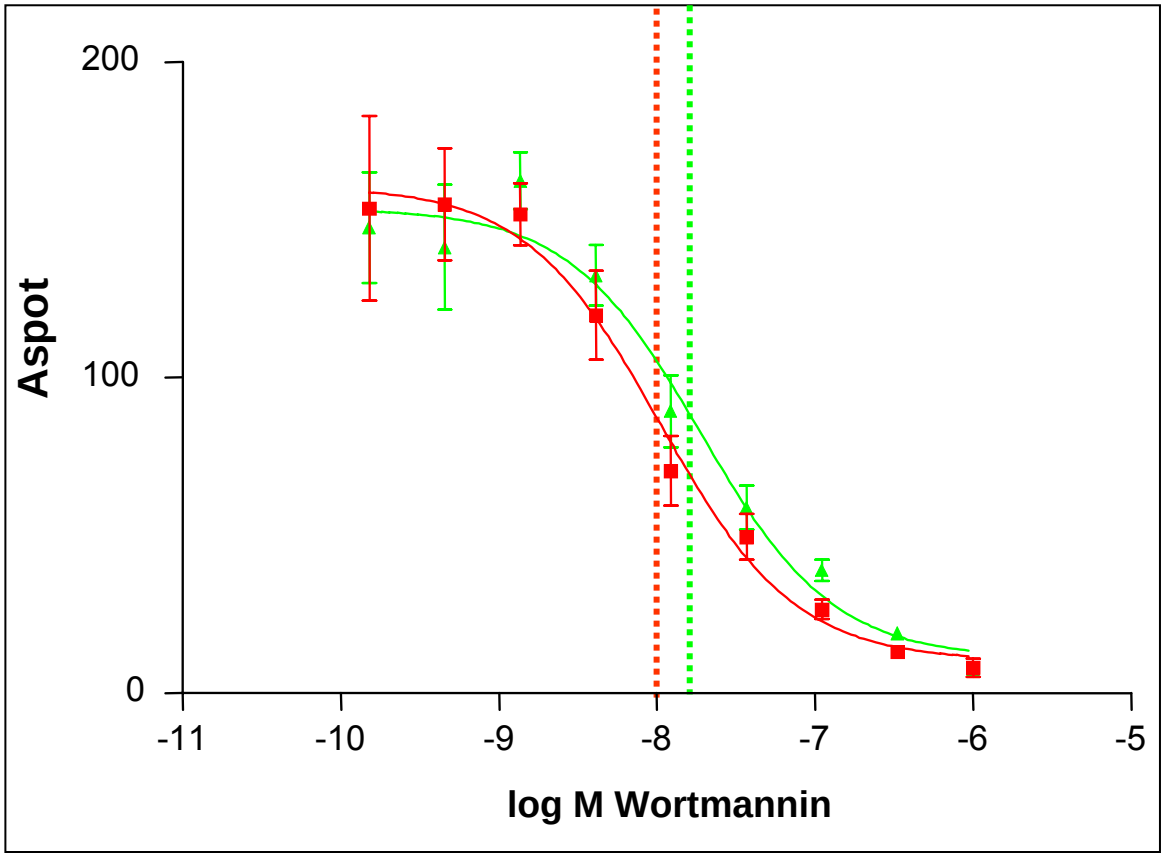


Plate	Well	Msg	NPasNc	NPasSg	NPasAg	Nuc/Cyt	Std Dev	Nuc Intsty	Cyt Intsty	Aglity
Y00268	A24	0	164	121	121	0.95	0.67	502.85	532.35	87.4
Y00268	B24	0	162	138	138	5.10	1.88	2094.02	430.94	91.6
Y00268	C24	0	138	119	119	4.92	1.95	1770.81	368.40	88.1
Y00268	D24	0	207	179	179	4.96	2.11	1778.92	364.36	91.0
Y00268	E24	0	109	95	95	5.62	2.60	2117.59	429.71	86.9
Y00268	F24	0	162	119	119	1.02	0.43	579.34	607.87	87.5
Y00268	G24	0	177	142	142	5.07	2.22	1770.36	381.02	92.57
Y00268	H24	0	171	153	153	5.35	2.13	1885.01	387.22	90.9
Y00268	I24	0	173	128	128	0.96	0.37	416.63	455.54	89.9
Y00268	J24	0	149	99	99	1.29	0.92	540.20	452.12	87.9
Y00268	K24	0	143	91	91	1.06	0.53	632.74	593.09	87.6
Y00268	L24	0	147	104	104	1.15	0.62	611.31	569.70	88.1
Y00268	M24	0	147	103	103	1.10	0.94	542.64	536.19	88.3
Y00268	N24	0	155	118	118	1.04	0.46	606.98	588.60	89.1
Y00268	O24	0	191	144	144	1.20	0.84	524.51	479.85	90.1
Y00268	P24	0	138	85	85	1.24	0.95	598.95	532.13	85.6
Y00269	A1	0	180	150	150	0.94	0.54	537.86	651.56	87.0
Y00269	B1	0	177	134	134	0.87	0.41	562.93	682.42	86.8
Y00269	C1	0	165	127	127	0.98	0.71	661.15	748.10	88.6

PKB redistribution™ run on IN Cell Analyser

Full-length PKBα-GFP in CHO: cyt → PM, fixed after 5 mins stimulation



Stimulation: 1 μg/mL IGF-1

Wortmannin IC_{50} = 10 - 20 nM

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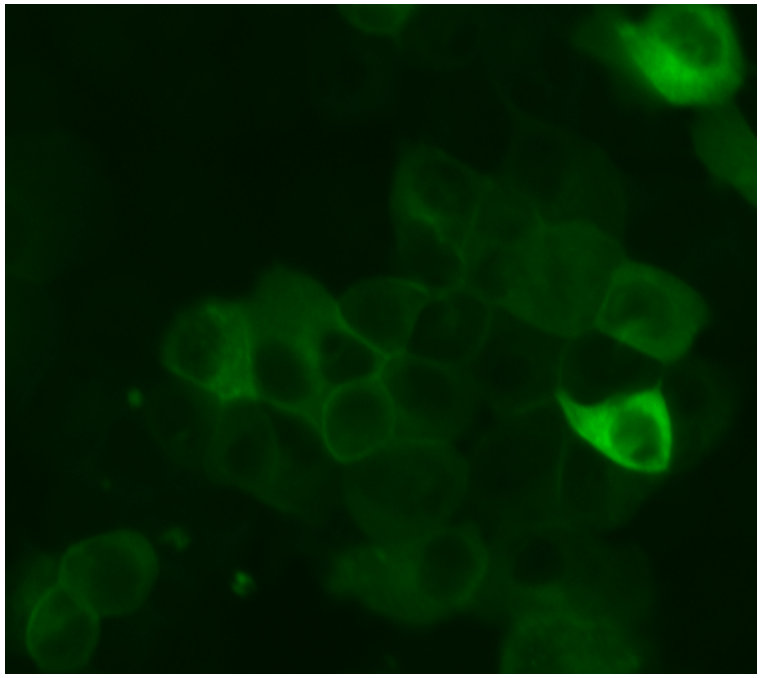
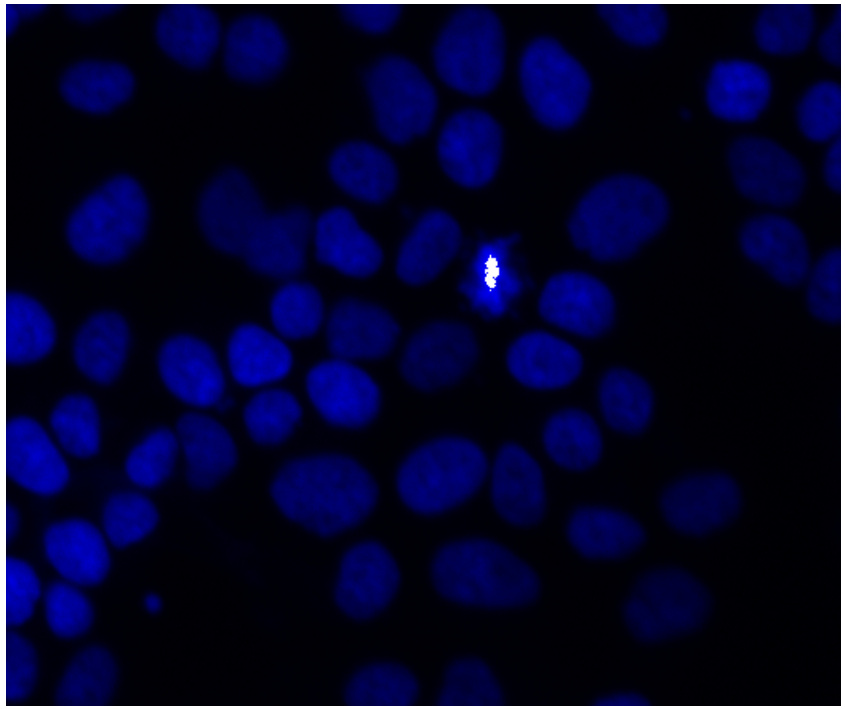
C5_FLD1_GREEN_YILING2_08_05 COLLECTED 4_19_05
InCell
PDK1-EGF

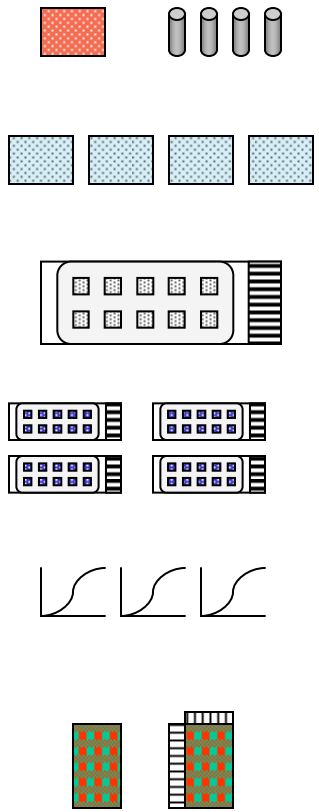




Reverse-Phase Protein Array

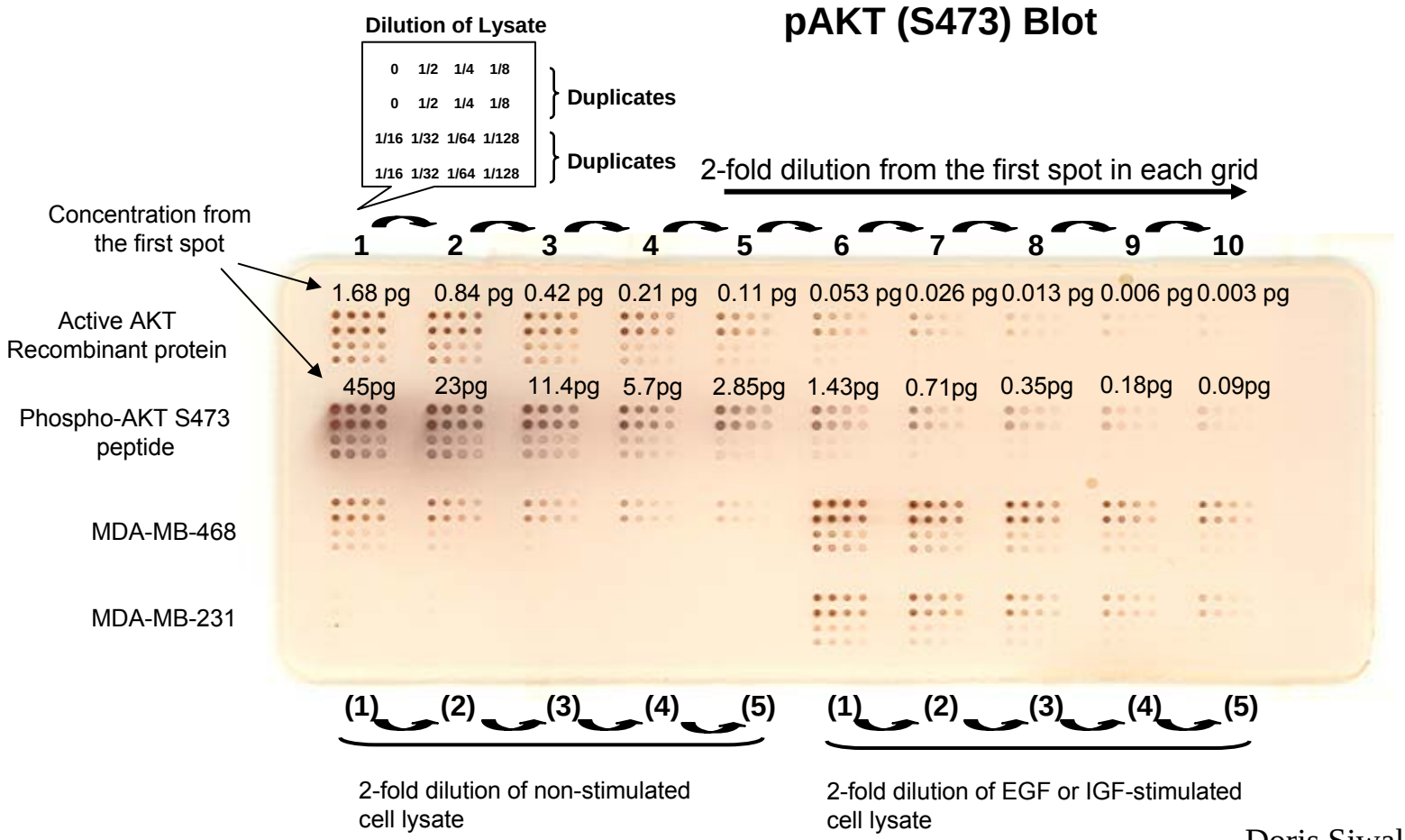
- Able to use denatured lysates
  - Antigen retrieval is not a problem (cf. tissue arrays)
- Able to use non-denatured lysates
  - Can detect protein-protein, protein-DNA or protein-RNA complexes
- Each sample is arrayed in a miniature dilution curve
  - Enables full quantitation of samples
- Does not require direct tagging of the protein of interest
  - Allows marked improvement in the reproducibility, sensitivity, and robustness of the assay

Basic Steps Involved in Protein Lysate Arrays



- Lyse cells/tissues
- Perform multiple serial dilutions on samples
- Print array of diluted samples on slides
- Stain slides to detect proteins of interest
- Construct dilution curves for each sample, determine sample intensity
- Compare sample/protein (antibody) results with heatmaps, cluster analyses

Sample Reverse-Phase Protein Lysate Array



Doris Siwak, PhD

Quantitation of Protein Lysate Arrays

Ln(Y0)	Ln(Ymin)	Ln(Ymax)	x0	slope	cv_pct	r2	linear_pct
8.679	8.646	8.715	4.604	0.7058	0.9762	0.9871	-47
8.213	8.175	8.245	4.605	0.8299	0.8498	0.9874	-33
8.638	8.599	8.664	3.383	0.6819	0.7514	0.9939	64
7.961	7.917	8.003	4.328	0.5544	1.021	0.9514	31
8.167	8.125	8.199	4.057	0.642	1.12	0.984	53
8.685	8.658	8.722	2.931	0.6424	0.8435	0.9934	78
7.853	7.806	7.887	4.605	0.7429	1.109	0.9715	-31
8.09	8.06	8.129	4.605	0.6336	0.9867	0.982	-41
8.584	8.565	8.629	2.734	0.7213	0.7829	0.9951	78
8.163	8.125	8.198	3.825	0.5149	0.7325	0.9794	42
7.834	7.79	7.881	3.527	0.4925	1.35	0.9558	53
7.722	7.698	7.75	4.605	0.1425	0.69	0.9455	-63
8.96	8.93	9.015	-0.246	0.626	1.881	0.9702	84
8.41	8.379	8.438	4.605	0.6786	0.7774	0.9934	-54
7.83	7.789	7.869	3.648	0.5229	1.047	0.9732	49
8.305	8.26	8.335	3.379	0.7721	0.8575	0.9918	56
7.953	7.909	7.98	4.29	0.7034	0.9234	0.9867	42
8.638	8.598	8.697	3.878	0.6068	2.12	0.9499	65
8.687	8.662	8.724	2.996	0.681	0.8037	0.9949	80
8.997	8.98	9.025	2.492	0.6241	0.3943	0.9984	83
8.7	8.658	8.718	4.585	0.5797	0.9519	0.9925	-100
8.524	8.489	8.539	3.135	0.5795	0.6088	0.9968	91
8.244	8.214	8.275	4.605	0.7497	0.73	0.9923	-41
9.225	9.19	9.254	3.877	0.4451	0.8269	0.984	67
8.387	8.358	8.42	3.737	0.6756	0.8428	0.9936	66
8.402	8.389	8.44	3.776	0.6676	0.5112	0.9971	60
8.451	8.425	8.471	4.605	0.6962	0.4747	0.9973	-50
8.33	8.313	8.368	4.605	0.7507	0.6957	0.9955	-53
9.182	9.145	9.201	-0.223	0.5452	0.8014	0.9932	95
8.472	8.44	8.503	3.06	0.6543	0.8922	0.9942	85
9.074	9.049	9.105	3.333	0.4422	0.7375	0.9906	85
8.779	8.749	8.818	3.823	0.5553	1.162	0.986	86
9.148	9.113	9.166	1.552	0.5135	0.7031	0.9949	100
8.823	8.777	8.855	2.987	0.5683	1.499	0.9813	100
8.926	8.897	8.954	3.082	0.5473	0.7633	0.9941	94
8.563	8.538	8.604	3.057	0.6009	0.9683	0.991	80
8.552	8.514	8.584	3.237	0.6782	0.7857	0.9924	62
8.807	8.784	8.832	4.605	0.5816	0.5503	0.9963	-67
8.75	8.727	8.801	3.184	0.6806	1.049	0.9896	71
8.853	8.824	8.91	3.012	0.6136	1.689	0.9761	91

- Analyze scanned .tif file images in MicroVigene 2.100
  - Protocol to be placed in the server
- Ln(Y0) of sample intensities determined by MicroVigene are in .dxt files, which are compiled in Excel

Doris Siwak, PhD

Gelovani, M.D., Ph.D.
nderson Cancer Center
tal Diagnostic Imaging

- Molecular imaging combines new molecular agents with traditional imaging tools to create more targeted therapies with the objective to simultaneously find, diagnose and treat disease. Our research is focused on developing new approaches to molecular imaging that would help detect cancer at an earlier stage and enable care teams to assess the extent of the disease sooner – making a significant impact on the diagnosis, therapy and management of cancer.

Gelovani Lab objectives:

- **develop and translate novel non-invasive molecular imaging approaches into clinical practice to improve cancer detection and non-invasive molecular-genetic profiling, selection of novel anti-cancer therapies and monitoring their efficacy.**

- **development of a premiere research program aimed at the discovery of novel and more specific chemical, genetic, and cellular tracers for imaging different tumor-specific targets.**

- **A variety of PET, NMR and optical imaging tracers will be developed in my research laboratory that would allow for the translation of current molecular-biological assay systems into clinical imaging applications.**

- **implement recent advances in molecular probe designs and novel methods of molecular biology in the process of development of these novel-imaging tracers.**

http://gsbs.uth.tmc.edu/tutorial/gelovani.html

Molecular imaging of temporal dynamics and spatial heterogeneity of hypoxia-inducible factor-1 signal transduction activity in tumors in living mice.

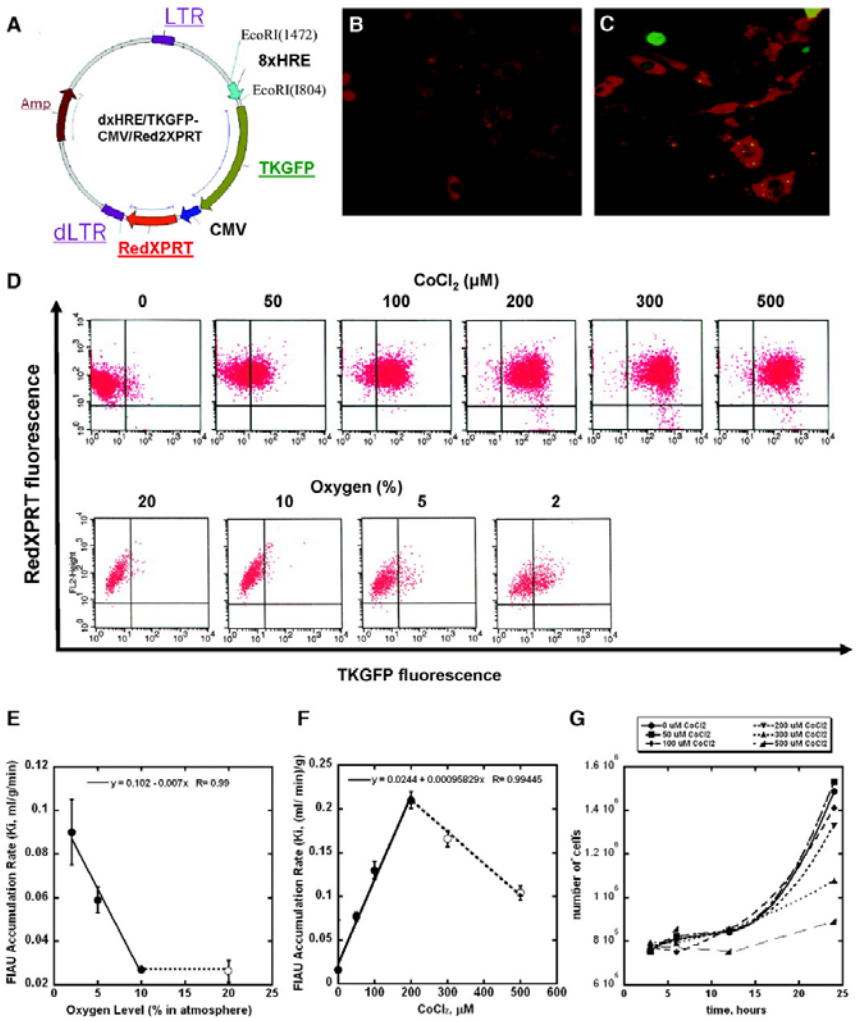
HIF-1a-hypoxia inducible factor

- Hif-1a transcriptional activity plays a major role in neoangiongenesis during tumor cell growth
- Increased oxygen demands on tissues due to rapid growth at the tumor sites
- HIF1a is a protein that is stabilized under hypoxic conditions
- Under normoxia, it is rapidly degraded in the cell

HIF-1a dual reporter

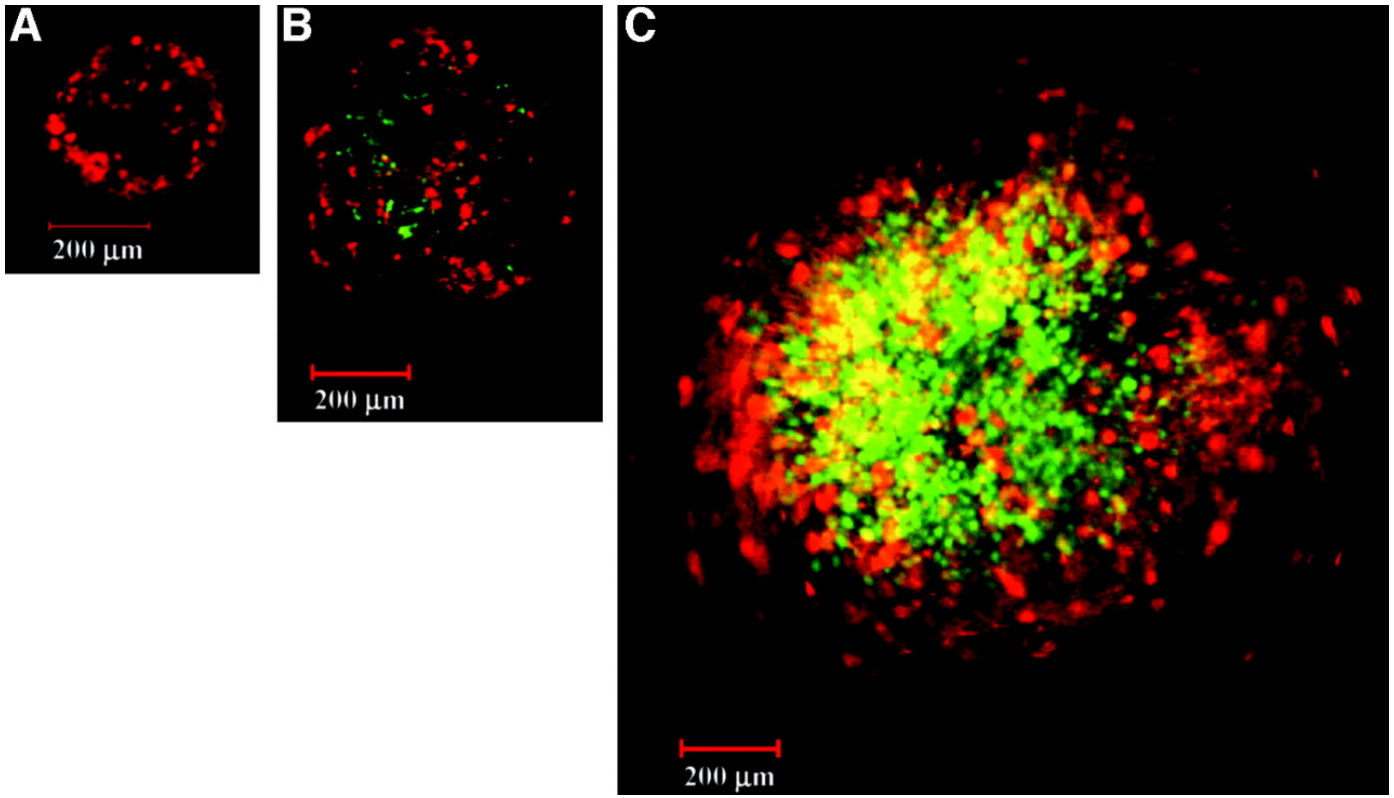
- Reporter probe selectively converts radiolabeled substrate which is trapped in the cells and can be detected using different diagnostic modalities. (using ^{18}F-FEAU) (2'-fluoro-2'-deoxy-1B-D-arabionofuransoly-5-ethyl-uracil)
- Non-invasive imaging of transcriptional regulation of endogenous genes
- HIF-1a inducible sensor reporter gene (GFP)
- Constitutively expressed beacon reporter gene (DsRed)

Plasmid Construct of Reporters



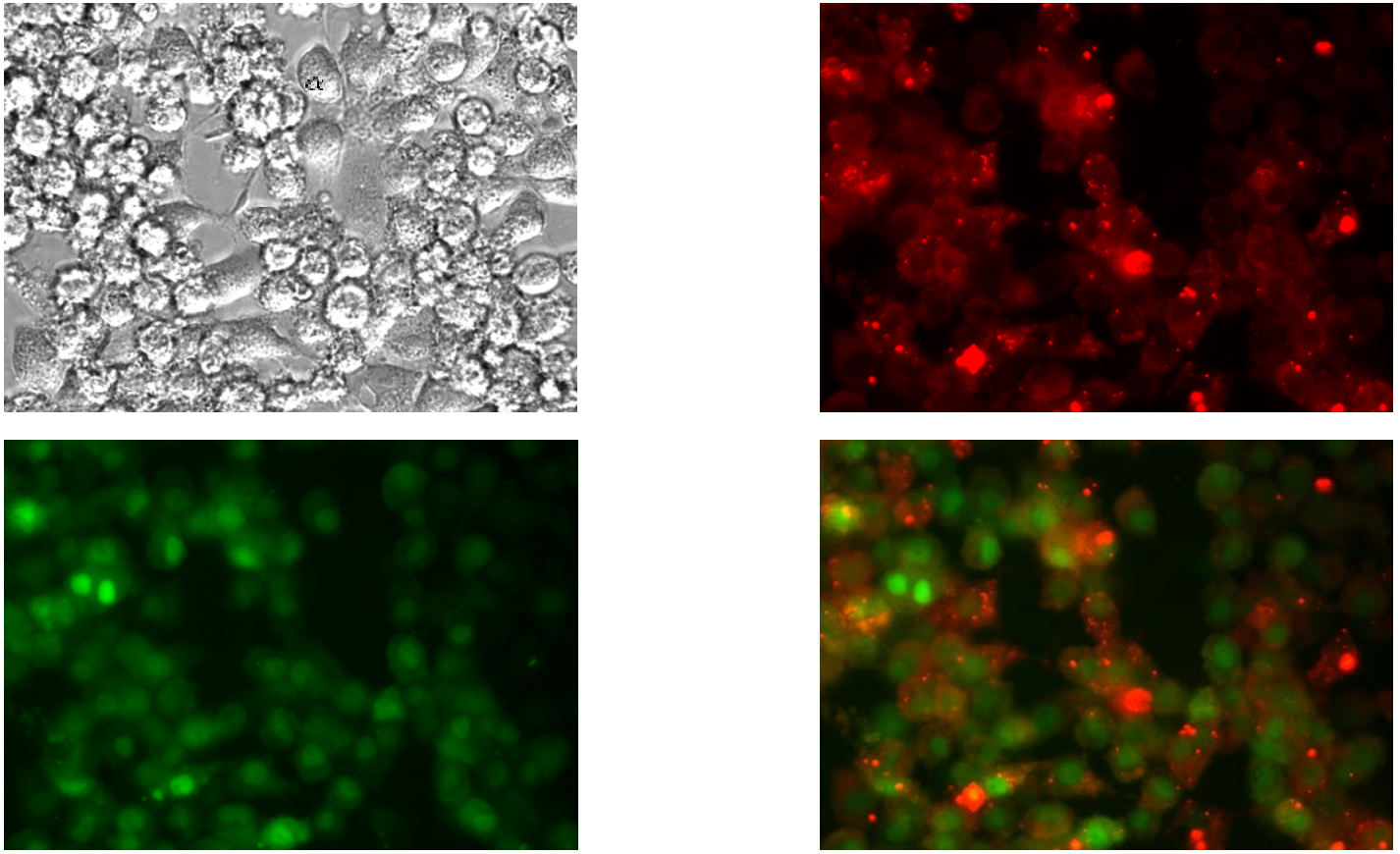
Serganova, I. et al. Cancer Res 2004;64:6101-6108

Experimental tumor model-using HIF-1a



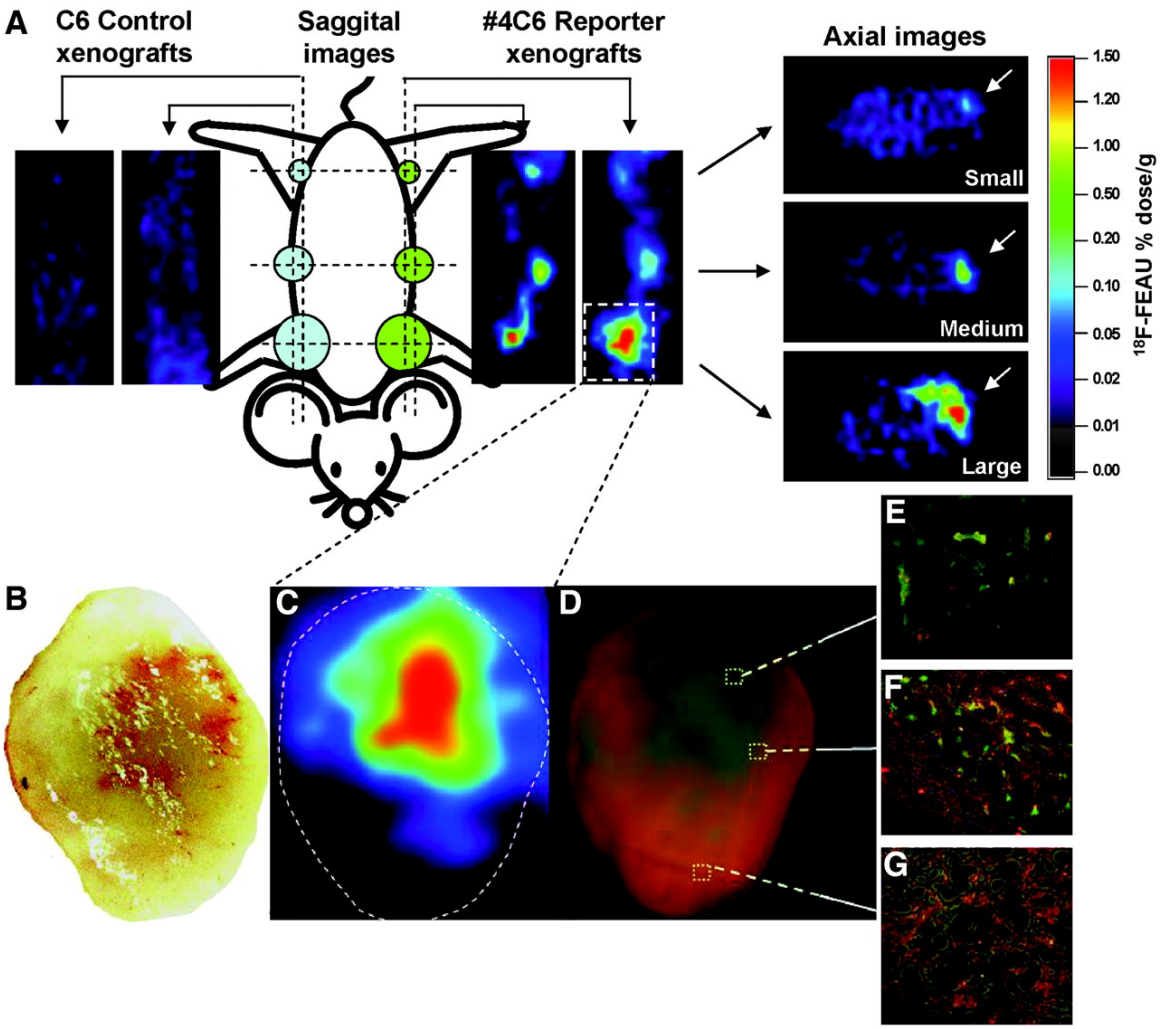
Serganova, I. et al. Cancer Res 2004;64:6101-6108

4c6 cells induced with 400uM $CoCl_2$



Under hypoxic conditions, HIF-1 level is increased and it forms a complex with HIF-1ß and the CBP/p300 coactivator (4) and translocates into the nucleus, where it binds to the core DNA sequence 5'-RCGTG-3'

Fig. 5



Serganova, I. et al. Cancer Res 2004;64:6101-6108

Cancer Research

A preclinical model for noninvasive imaging of hypoxia-induced gene expression; comparison with an exogenous marker of tumor hypoxia

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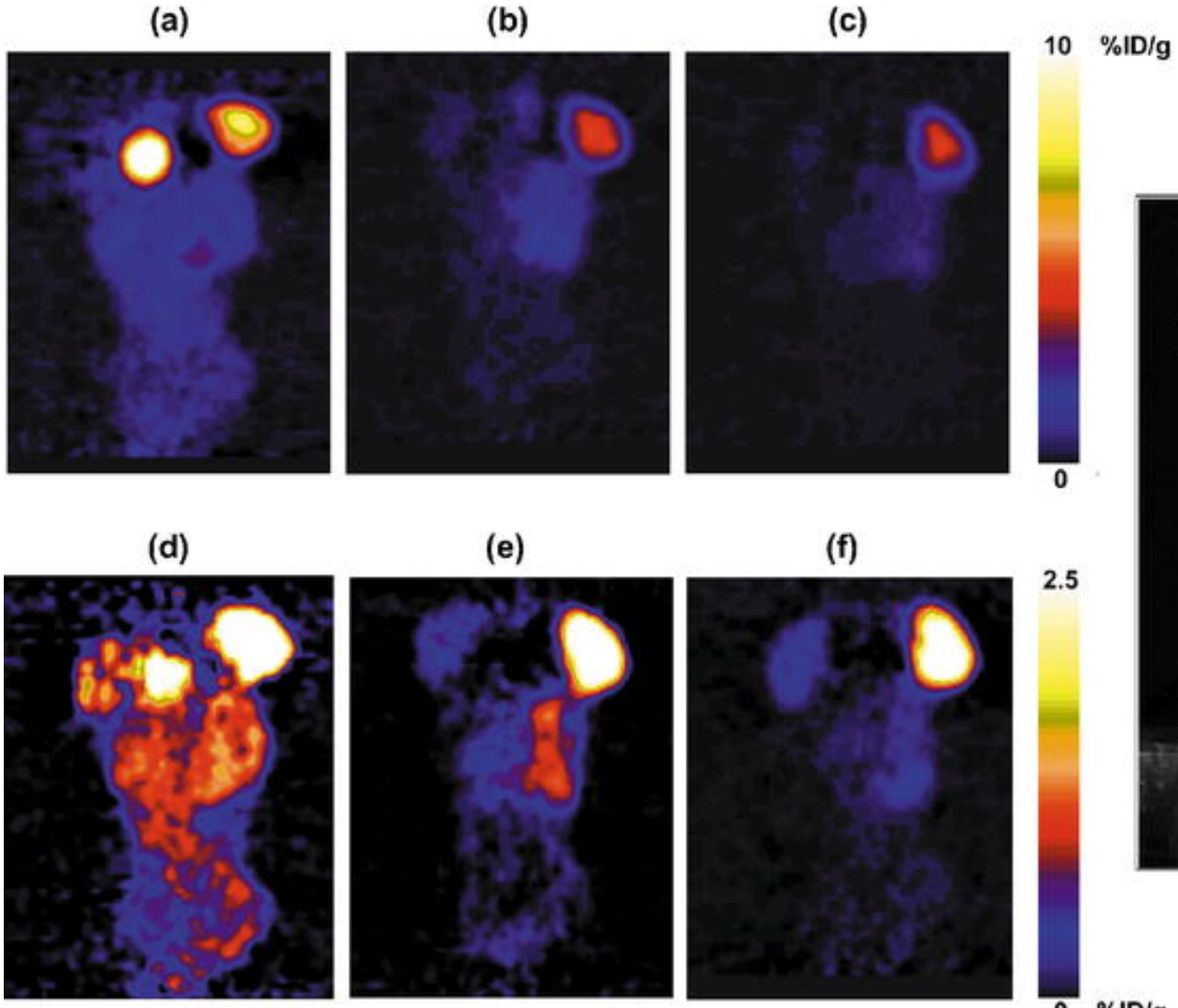

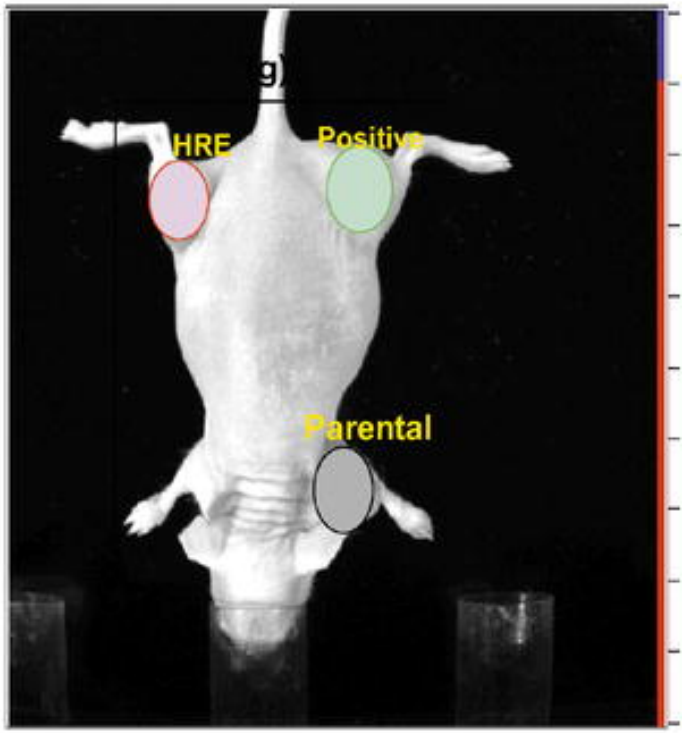

Eur J Nucl Med Mol Imaging. 2004 Nov;31(11):1530-8. Epub 2004 Sep 16.

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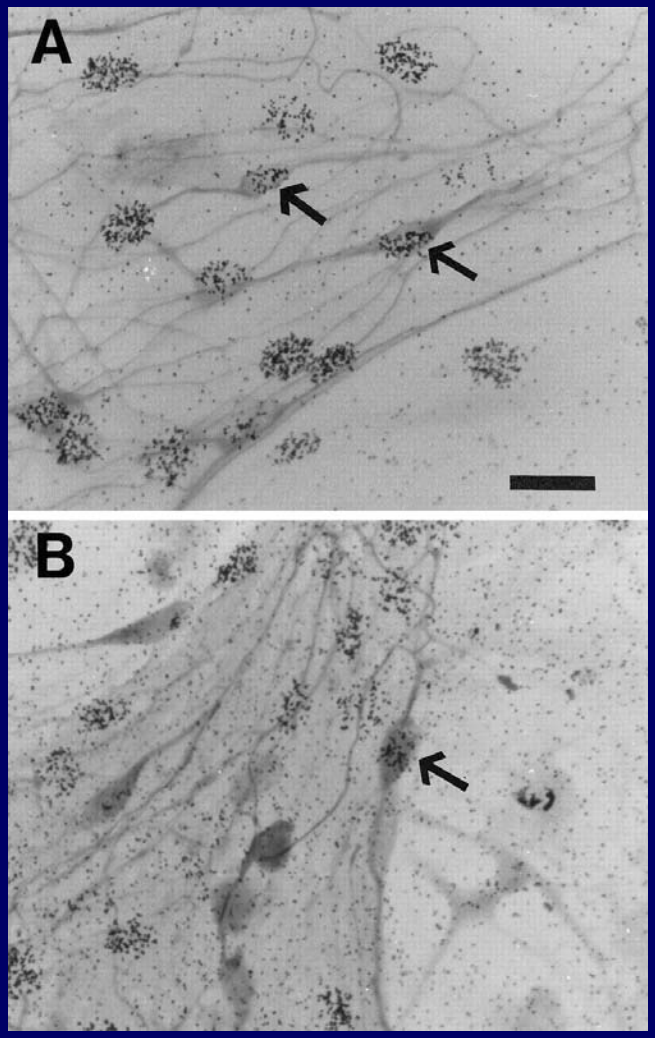
The main focus of the lab is the generation of a multi-level approach to treating spinal cord injury. This involves minimizing functional loss in the acute phase of injury by decreasing inflammation and repairing damaged blood vessels. In the chronic phase of injury, attention is focused on stimulating axonal regeneration through the application of target-derived growth factors and the manipulation of the spinal extracellular matrix to provide a positive growth environment

In Vitro Generation of Adult Rat Olfactory Sensory Neurons and Regulation of Maturation by Coculture with CNS Tissue
Grill, R. J. et al. J. Neurosci. 1997;17:3120-3127

- **Olfactory sensory neurons are continually generated throughout life in mammals**
- **This neurogenesis process is unique and understanding its mechanism will be useful as potential therapy clinically in:**
  - **as use of stem cells in diseases/ injury of the CNS**

Study carried out in different growth media and cell environment to determine which cells and factors functions as trophic/maturation factors.

Newborn rat nasal cells in vitro-



cells are labelled with [^{3}H] thymidine

Neuron specific tubulin labelling and olfactory marker protein shows both immature olfactory sensory neurons and mature olfactory neurons

Grill, R. J. et al. J. Neurosci. 1997;17:3120-3127

SOCIETY FOR NEUROSCIENCE The Journal of Neuroscience

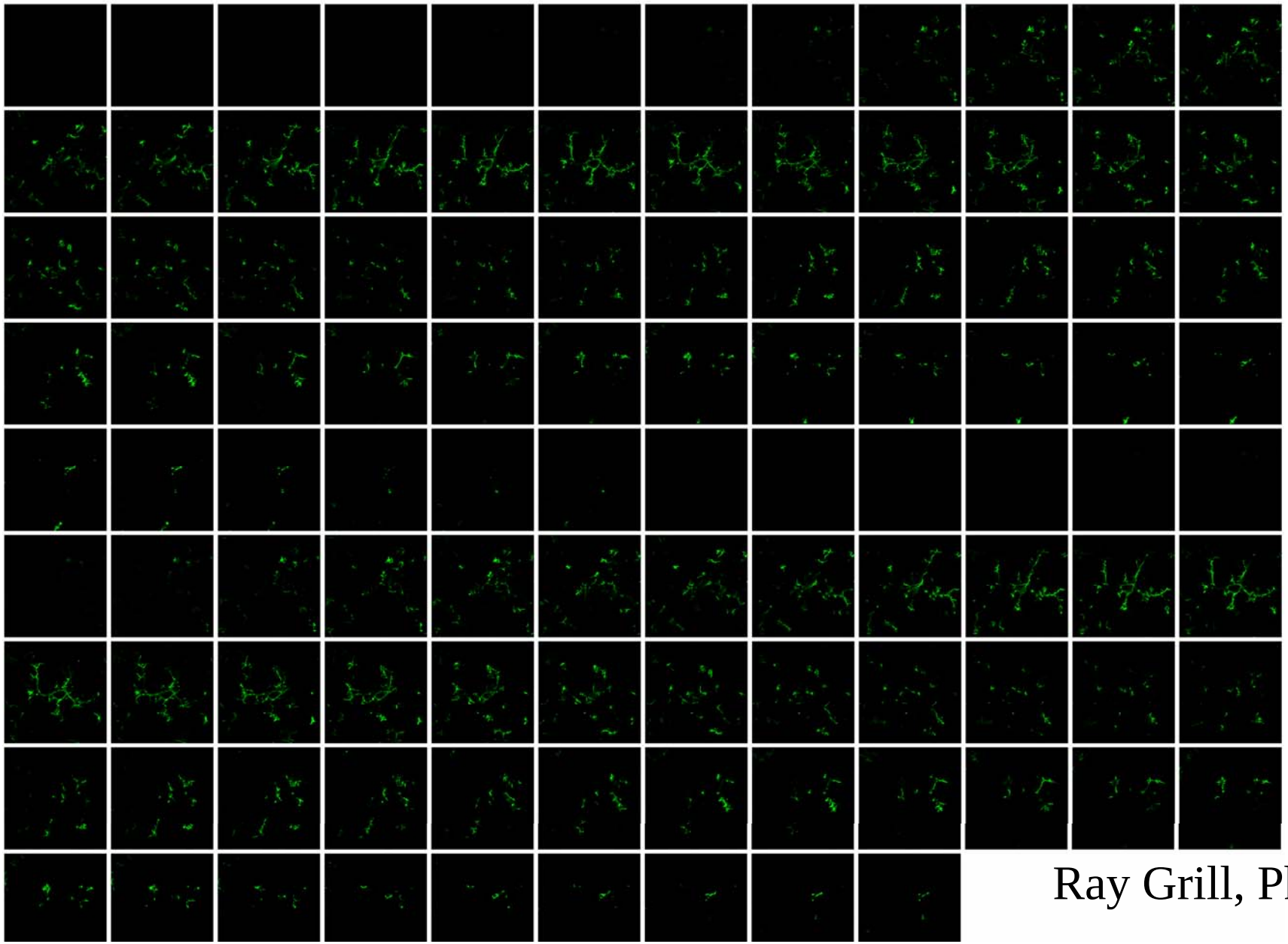
Multi-Color Confocal Imaging

-confocal microscopy provides the ability to visualize multiple targets at the same time

-This provides the user with a wide-range of approaches to study: 1) cell-cell interactions, 2) selective expression of antigens across a range of cell types, etc.

Ray Grill, PhD

Confocal Imaging: Generation of a stack of 105 images of microglial cells labelled with calcium binding protein Ab



Ray Grill, PhD

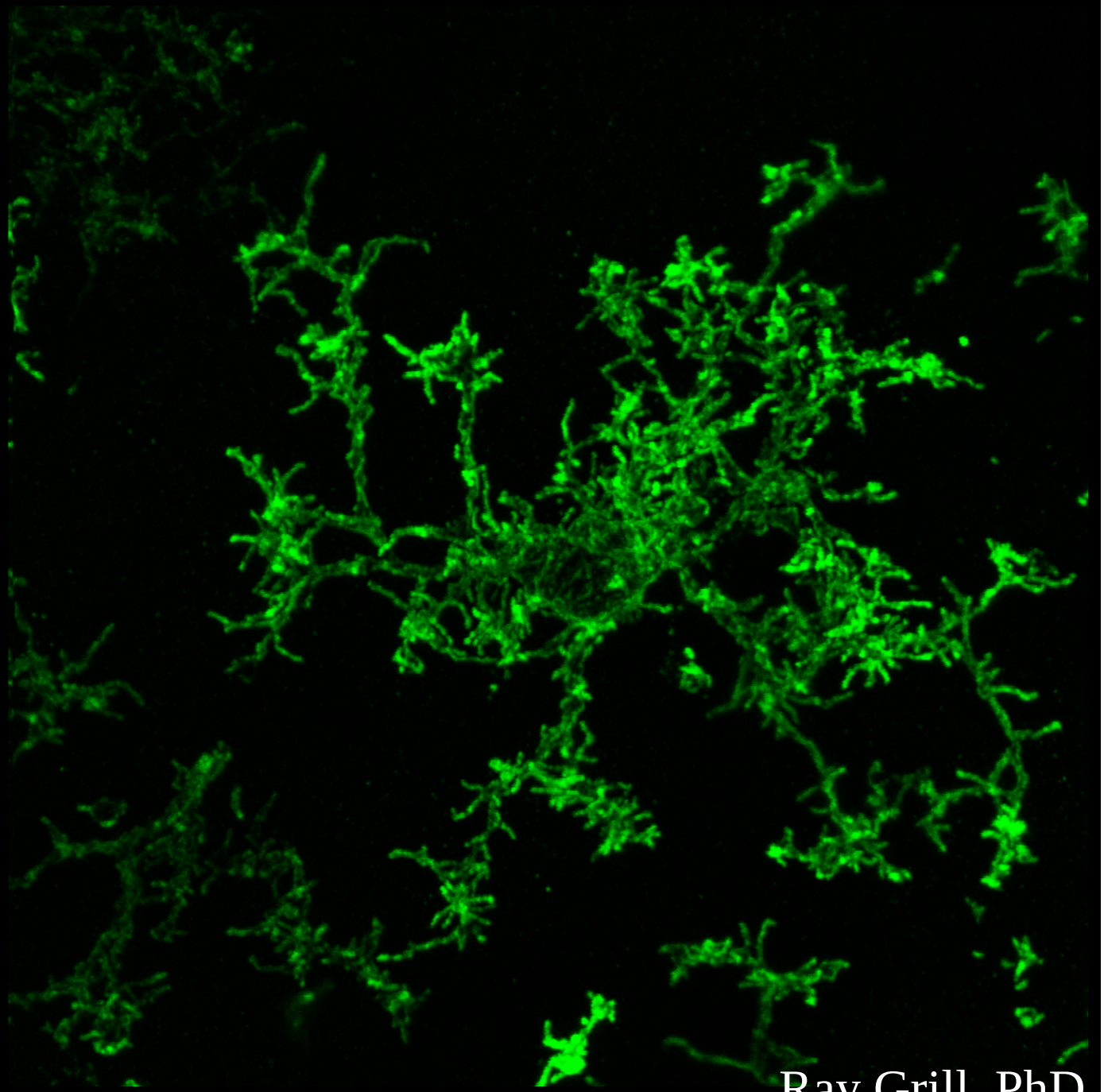
Confocal Imaging:

Conversion of a stack of images into a single projected image

This is an image of a microglial cell taken at 100x magnification

Cell labeled with an antibody against a microglial-specific calcium-binding protein

visualized using a fluorophore excited at the 488 nm wavelength

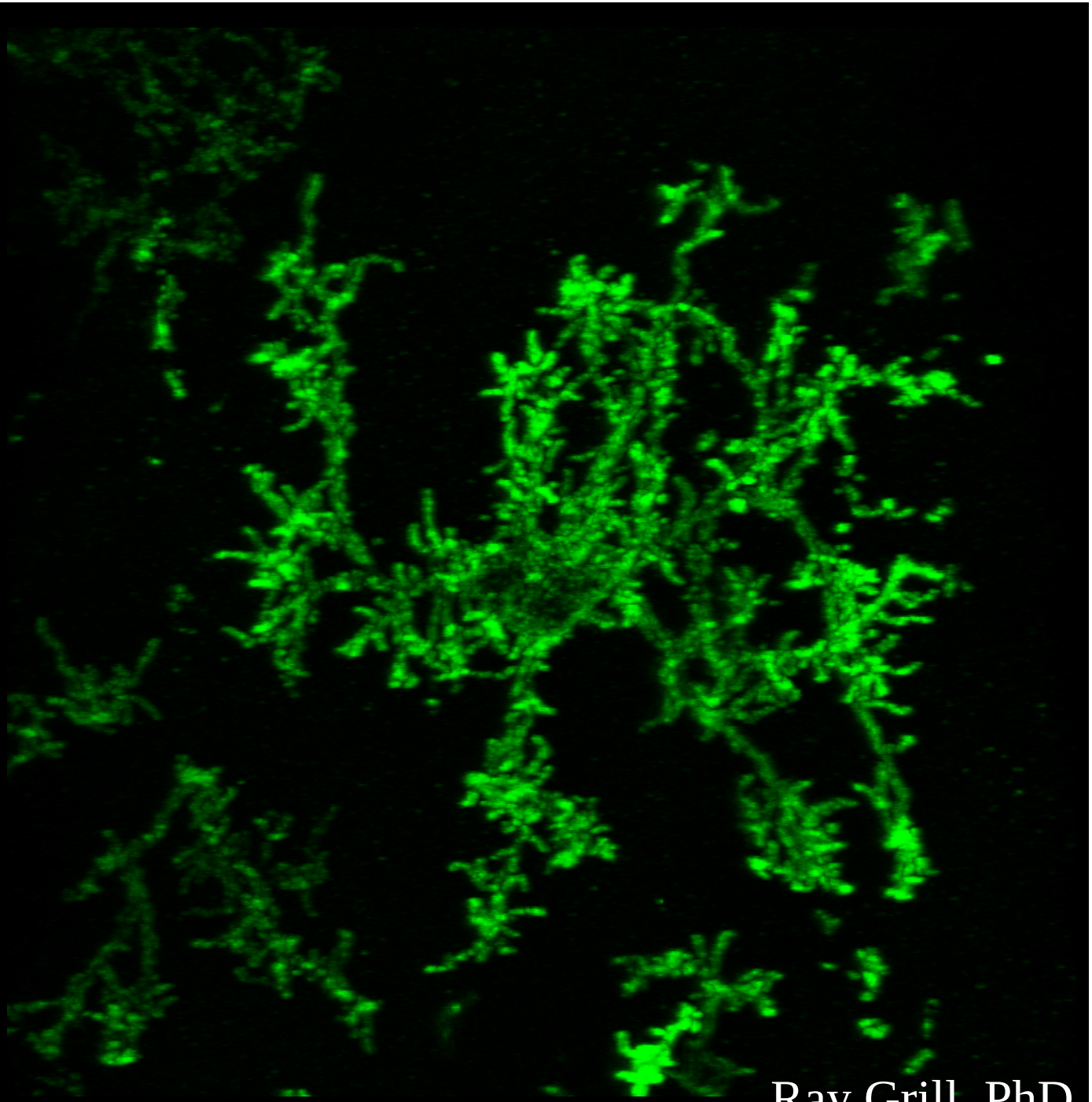


Ray Grill, PhD

Confocal Imaging:

Same stack of images converted into a 3D rotation

Allows one to study the target of interest from virtually any angle



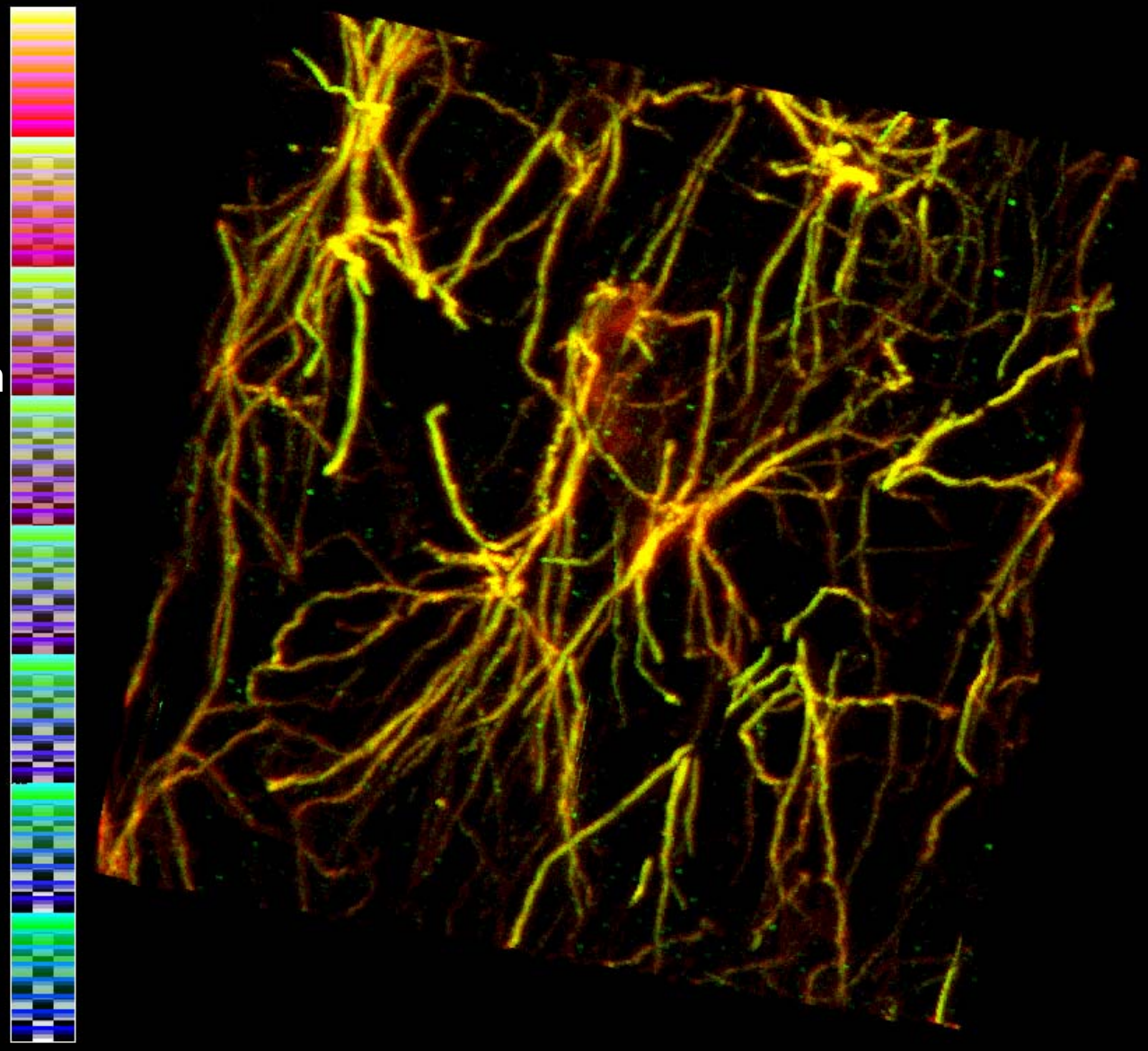
Confocal:

allows one to distinguish between co-association vs co-expression

GFAP: green

VEGF: red

overlap: yellow



Volume: 512 x 512 x 75 x 8 bits = 19200 Kbytes

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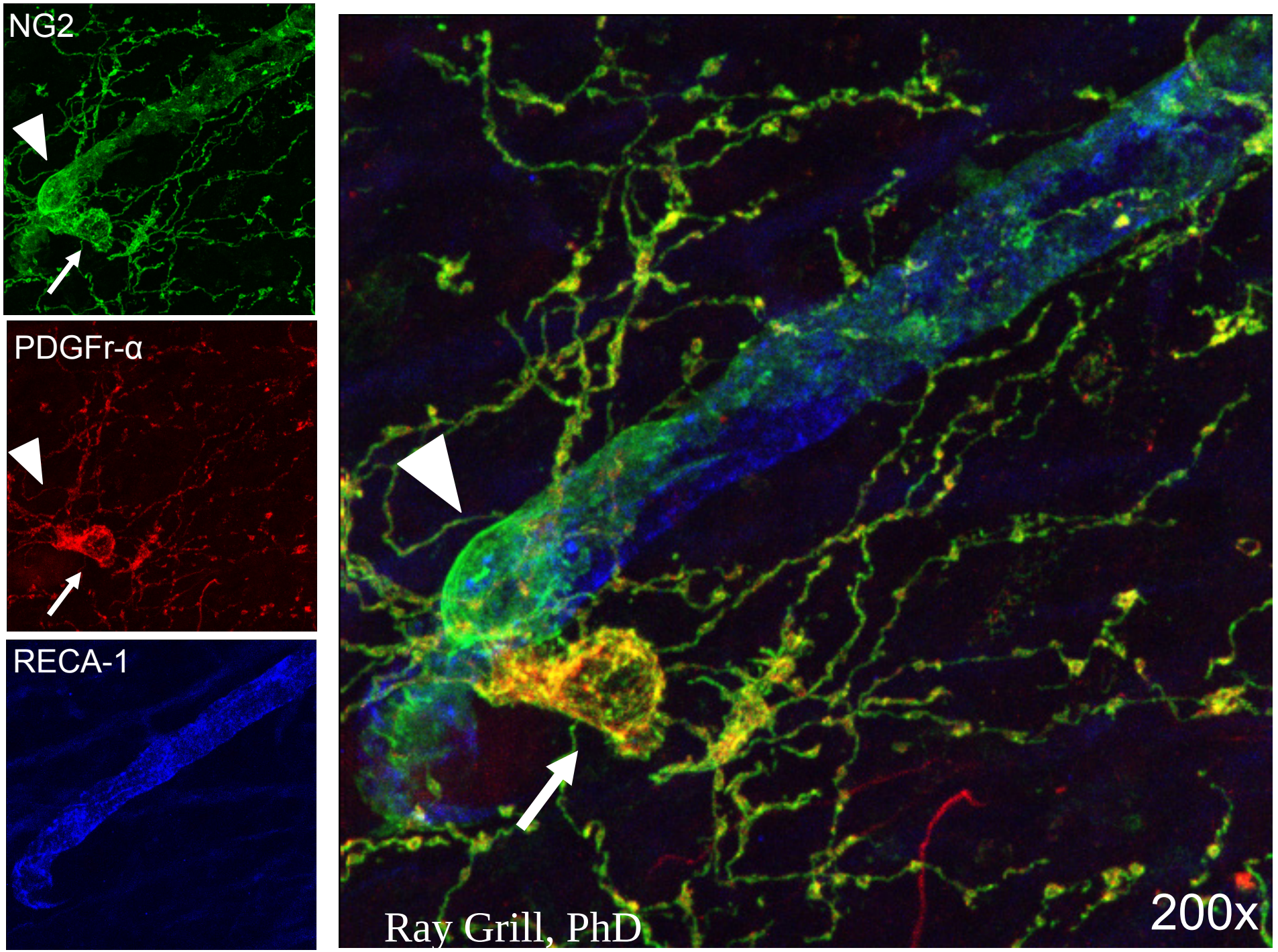
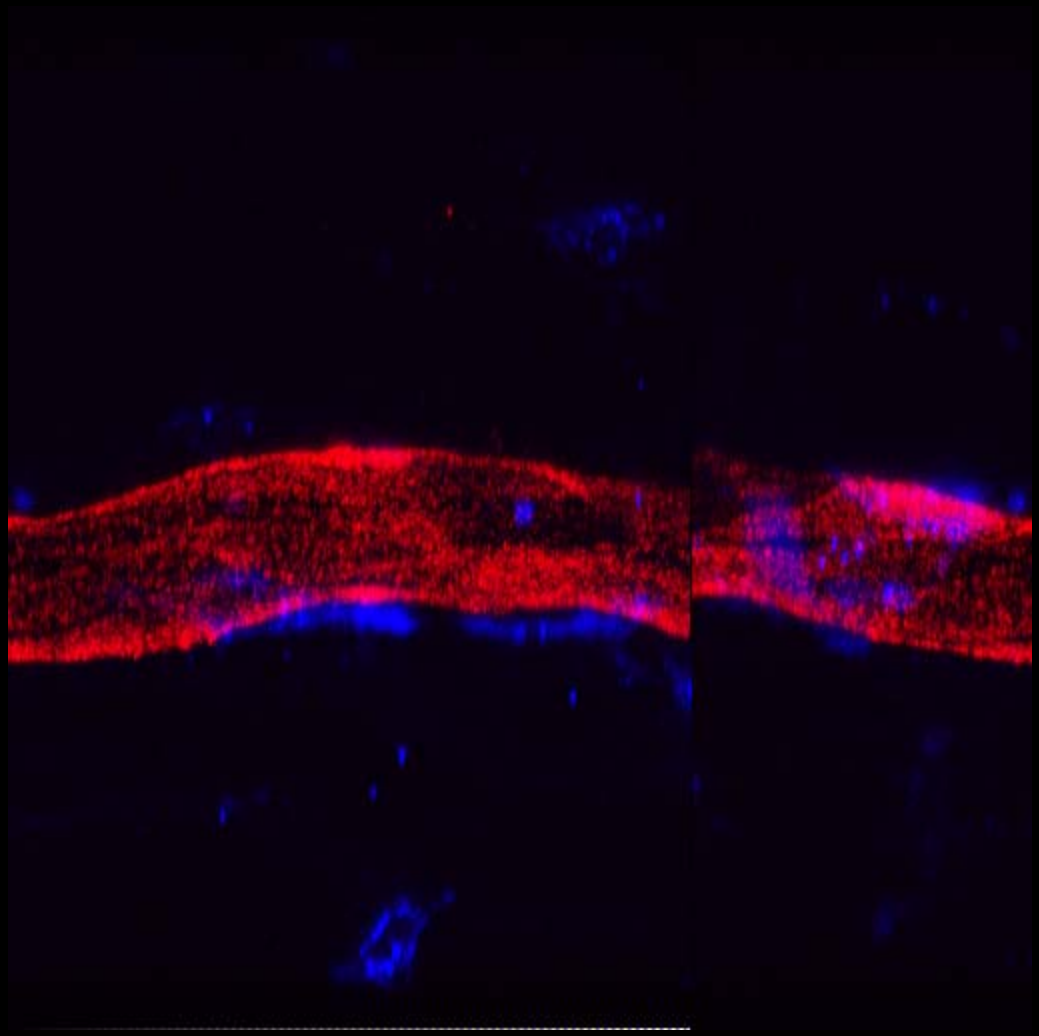

NG2
PDGFr-α
RECA-1
Ray Grill, PhD
200x

Confocal Microscopy allows the scientist to study cell-cell interactions

blood vessel within the injured adult rat spinal cord immuno-labeled with antibodies recognizing endothelial cells (green) and horse-radish peroxidase (HRP), a vascular tracer normally excluded from the intact nervous system



Capillary:
green

HRP:
red

Ray Grill, PhD