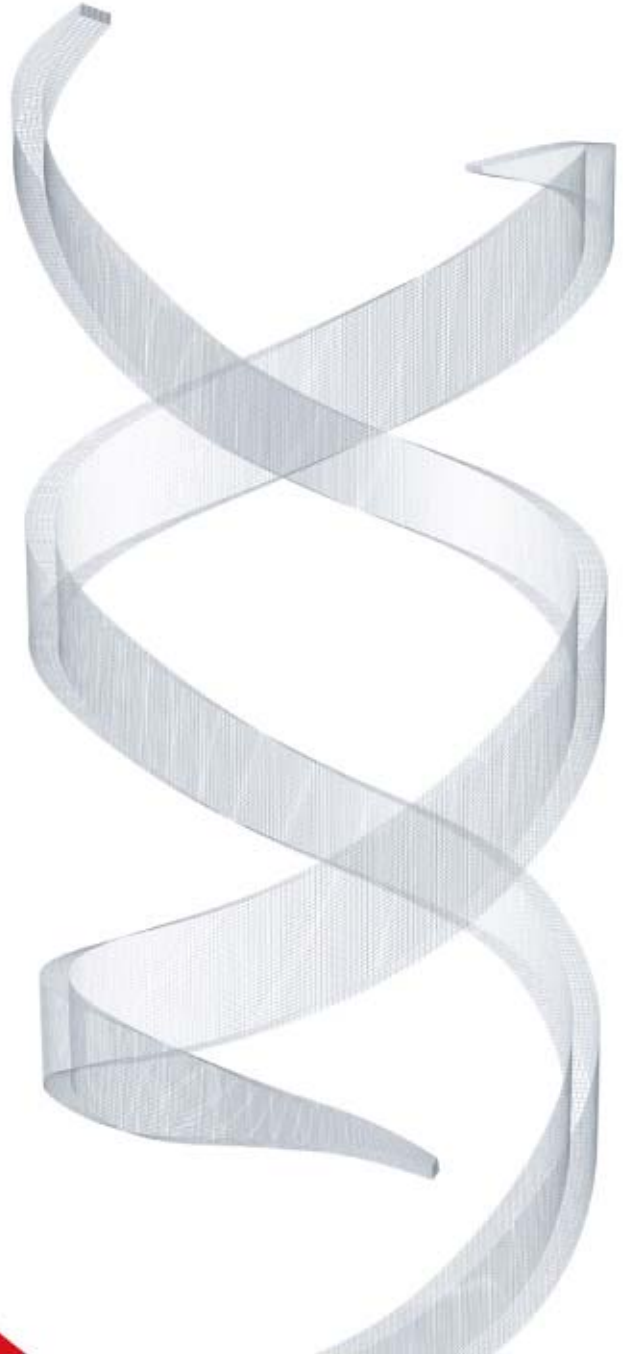
invitrogen™

Qdot® Conjugates: Sensitive, Multicolor, Stable Fluorescence

Patricia Whaley, Ph.D.

Molecular Probes Labeling and Detection Technologies

Invitrogen Corporation

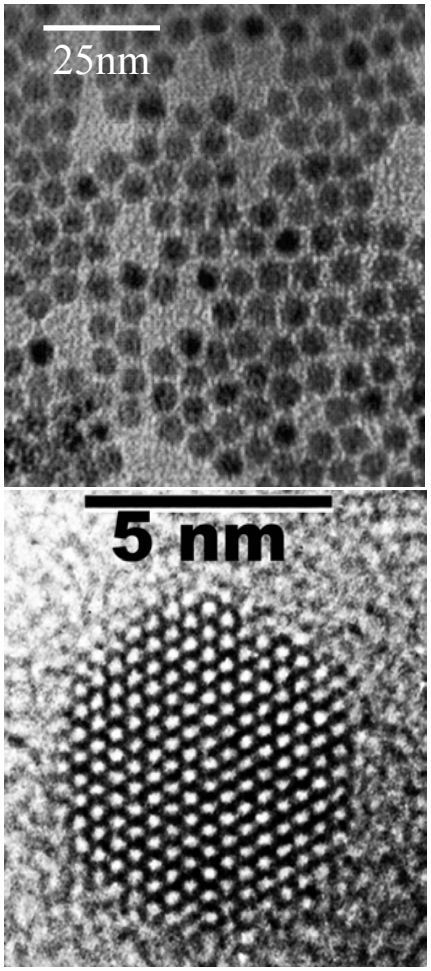


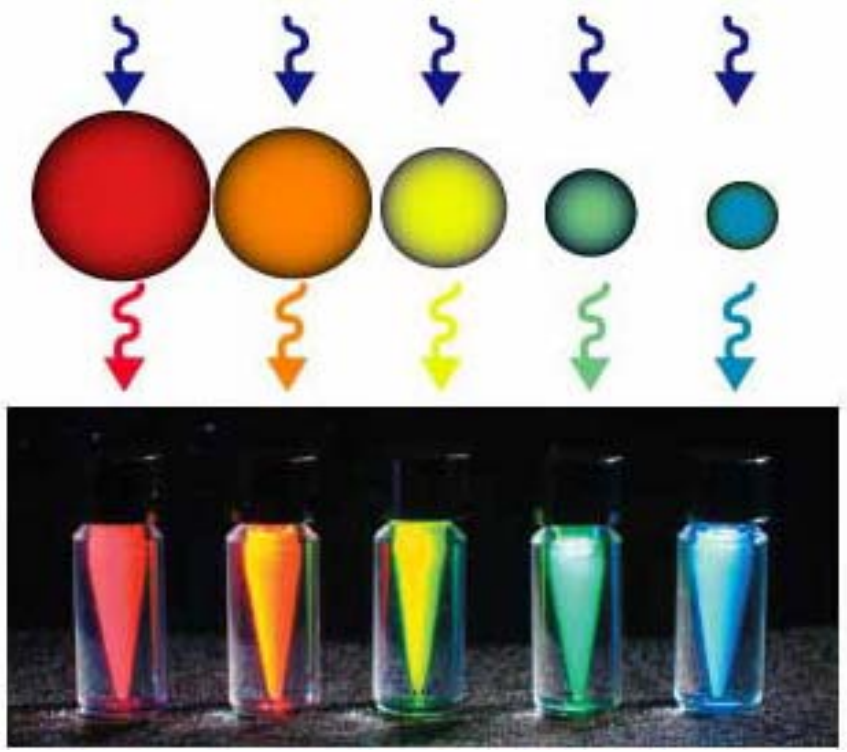
Outline

- Quantum Dot Basics
  - Materials
  - Spectral Properties
- Microscopy Applications
  - Immunofluorescent Cell Biology
  - Molecular Pathology
  - Cellular Assays
- Flow Cytometry
- In-vivo imaging
- Western Blotting

What Are Quantum Dots?

Highly fluorescent, nanometer-size, single crystals of semiconductor materials

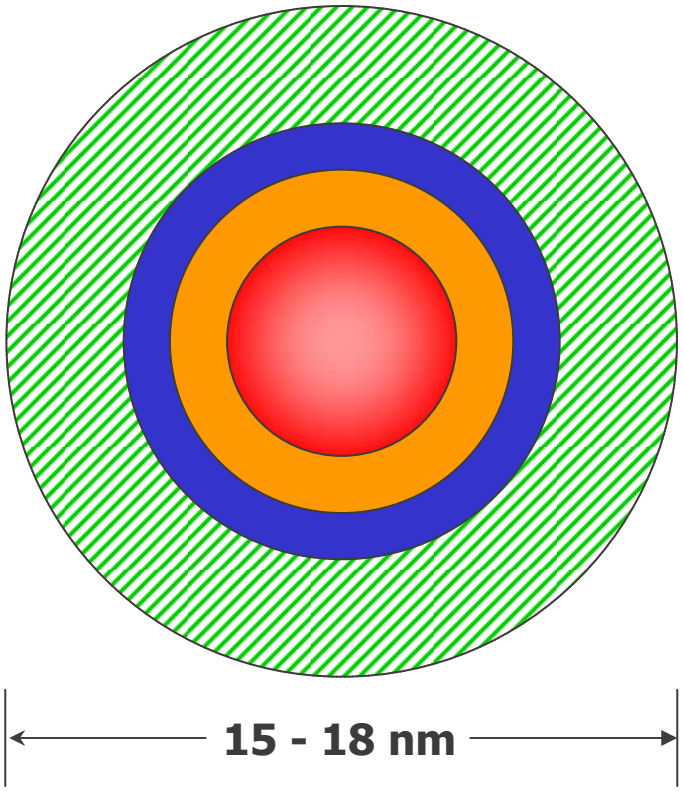




655 605 585 565 525 nm

Size of the nanocrystal determines the color
Size is tunable from ~2-10 nm (±3%)
Size distribution determines the spectral width

Qdot Conjugates are Engineered



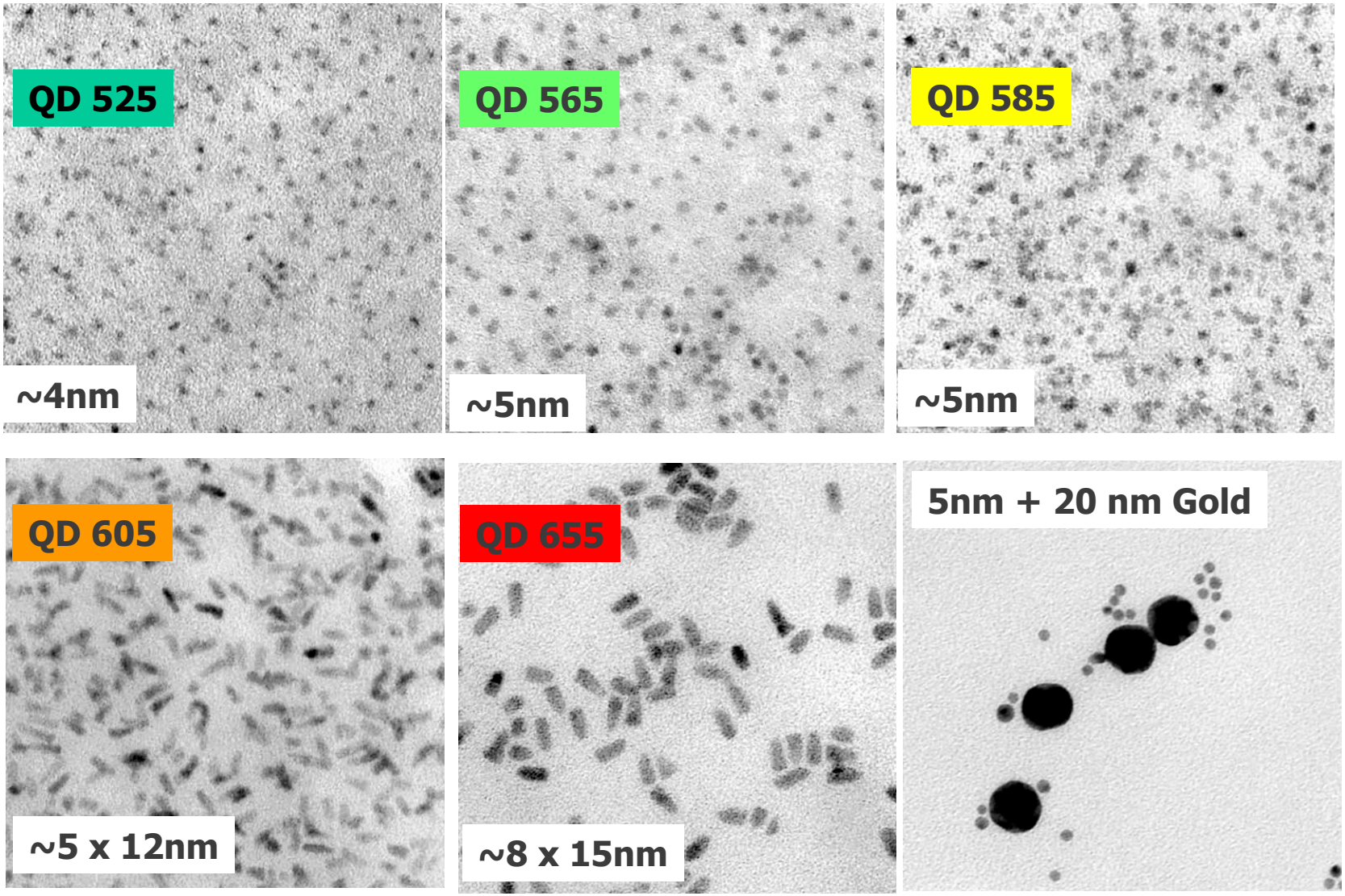
Core Nanocrystal (CdSe)
- Determines color

Inorganic Shell (ZnS)
– Improves brightness and stability

Organic Coating
- Provides water solubility and functional groups for conjugation

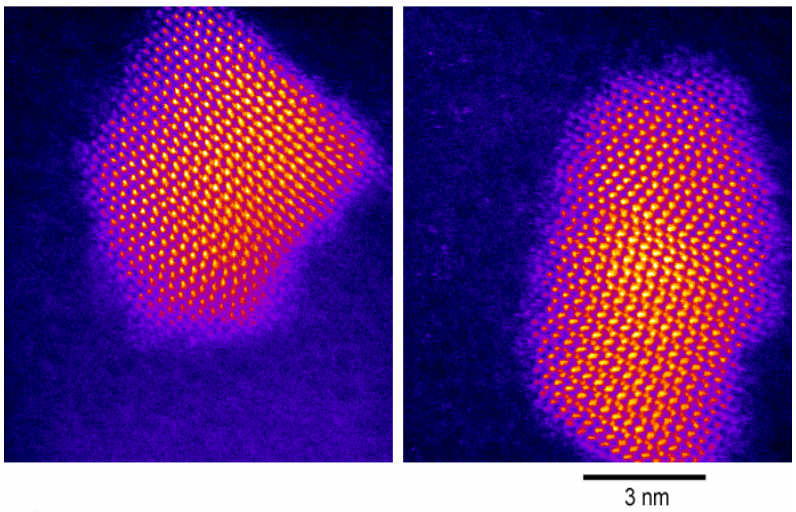
Biomolecule
-Covalently attached to polymer shell
- **Immuoglobulins**
- **Streptavidin, Protein A**
- **Receptor ligands**
- **Oligonucleotides**

Size of Qdot® Nanocrystals

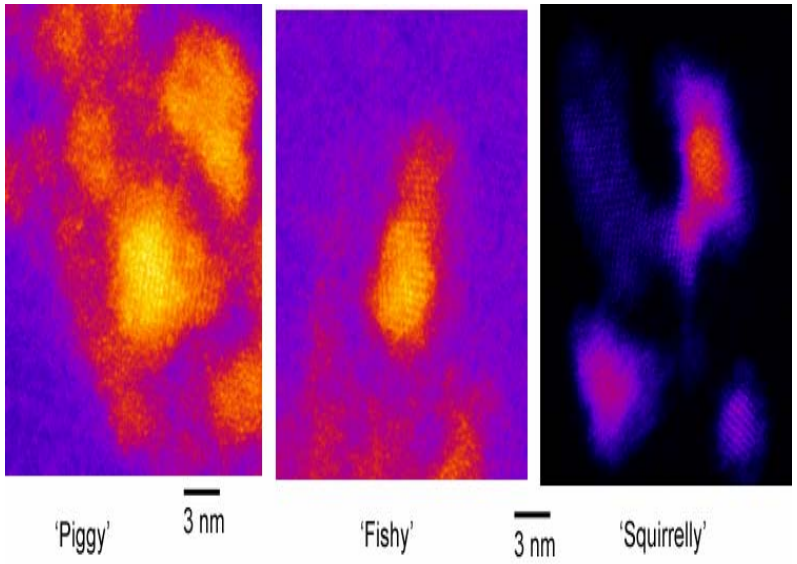


Images provided by Mark Ellisman, National Center for Microscopy and Imaging Research, UCSD, San Diego, CA

Quality Counts—ZStem Shows It



- Quantum Yield Above 90%
- Addition of Cd to shell material has improved shell coverage
- Contrast between core and shell has been reduced by the addition of Cd

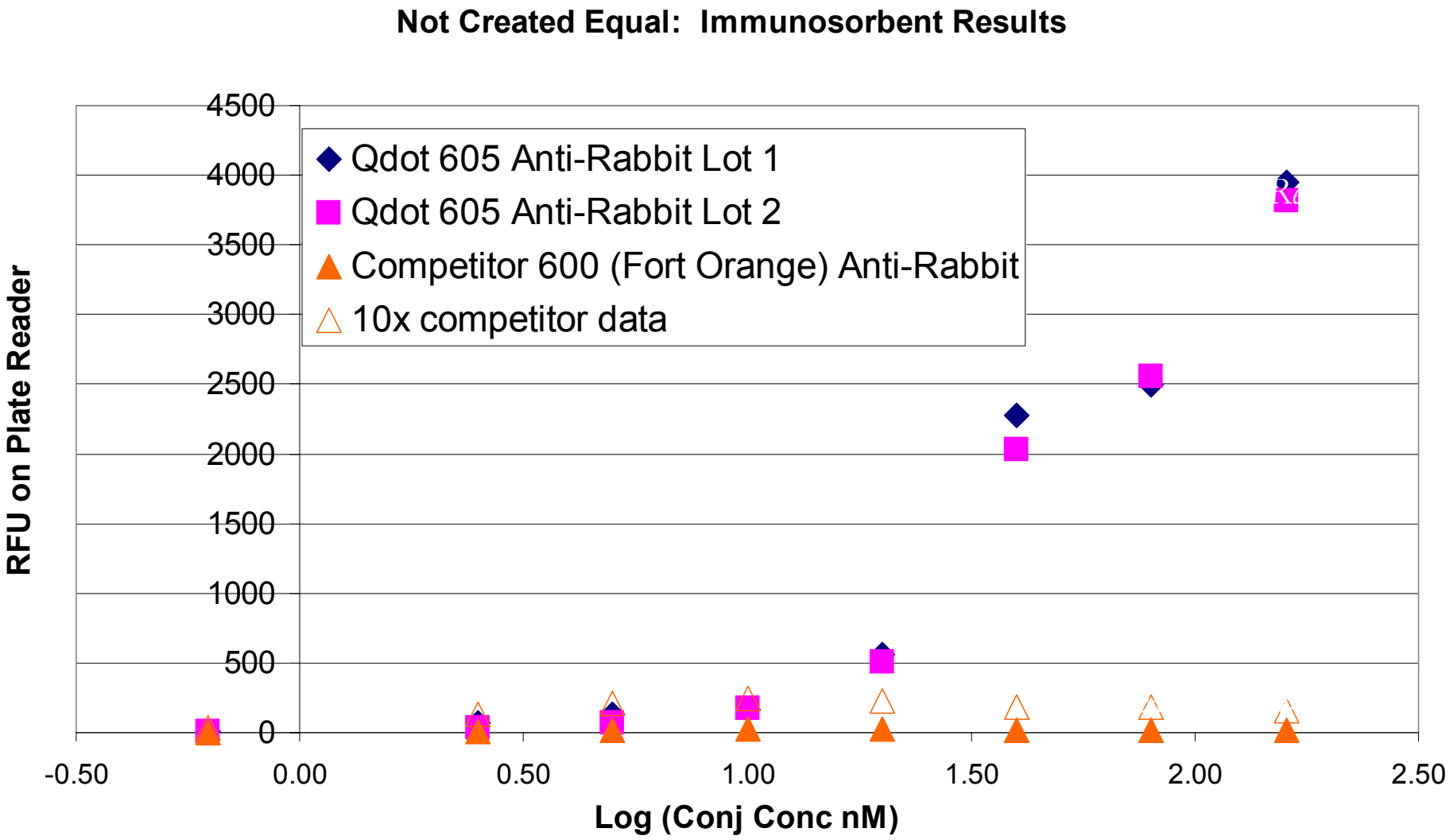


Quantum Dot Corp Materials

Literature Methods

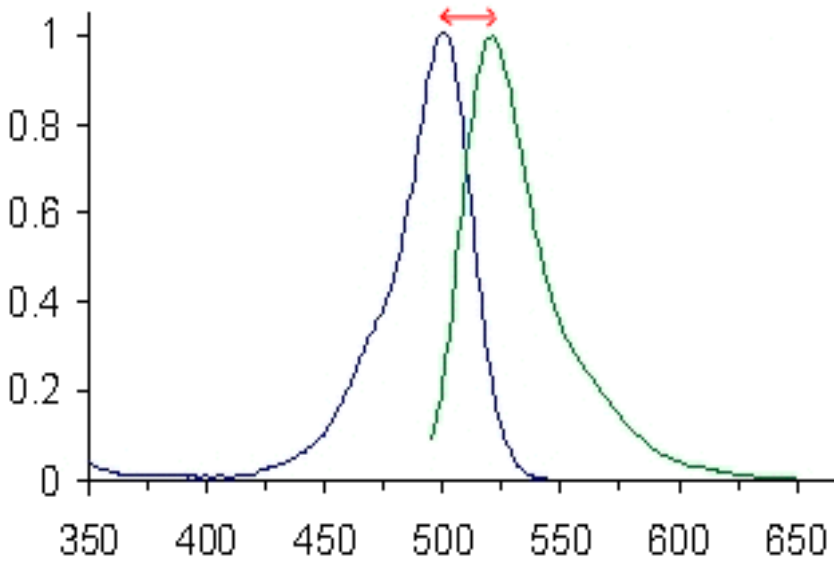
Data courtesy Steve Penneycook (ORNL), James McBride, and Sandy Rosenthal (Vanderbilt)

Qdot Conjugates vs. Competitors



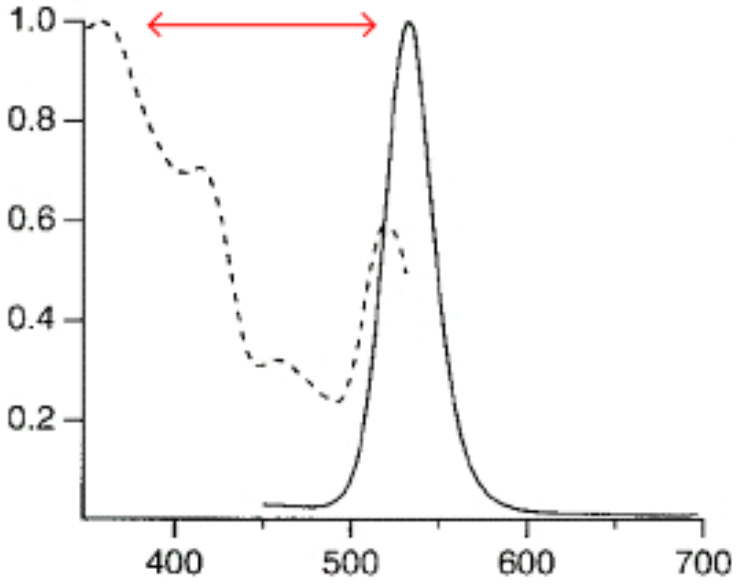
Spectral Properties

Organic dye (FITC)



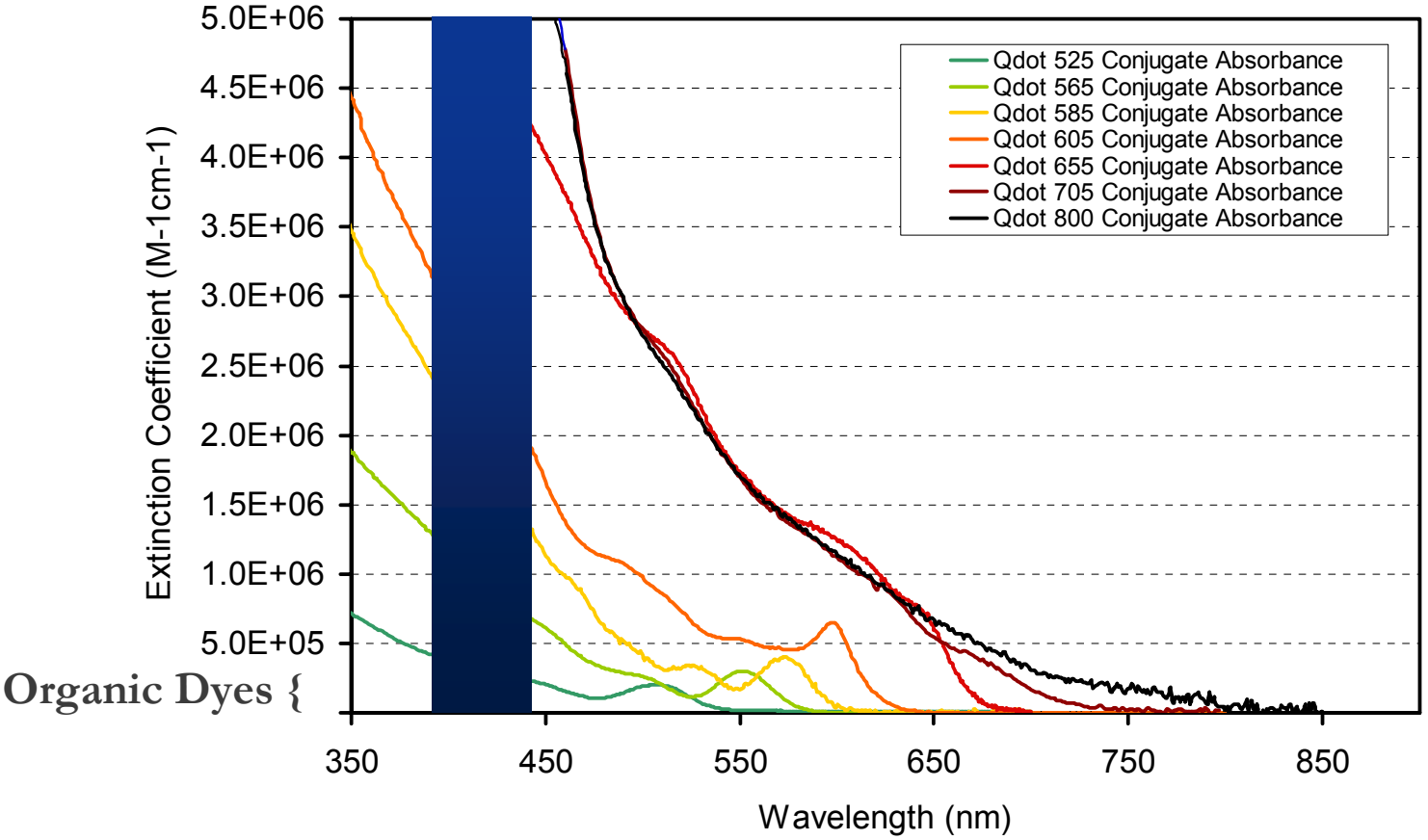
- Small Stokes shift
- Multiple source excitation req'd.
- Broad emission
- Poor photostability

Qdot® Conjugate (525)



- Large "Stokes shift"
- Single-source excitation
- Narrow emission
- Excellent photostability

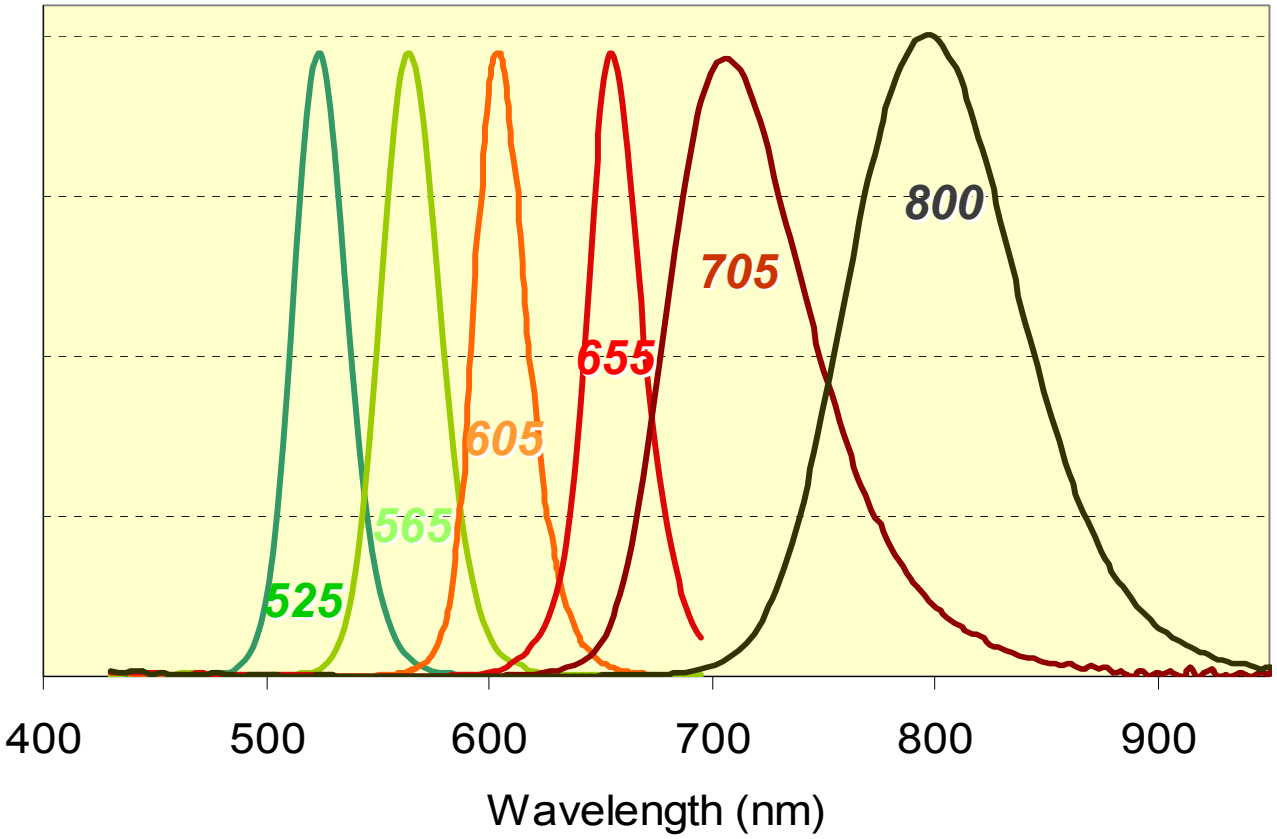
Excitation Spectra of Qdot Conjugates



High extinction >> High brightness

All colors can be excited at the same wavelength, 425DF45

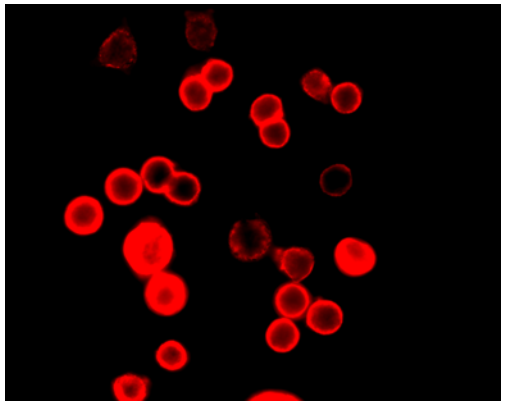
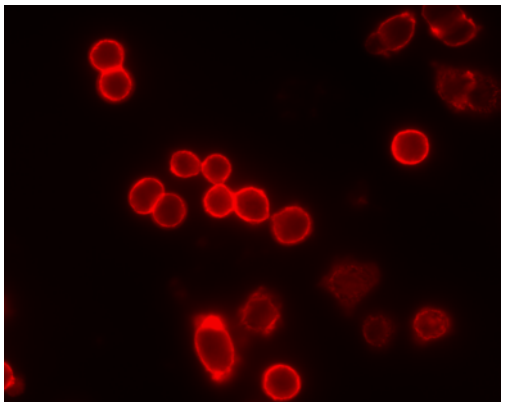
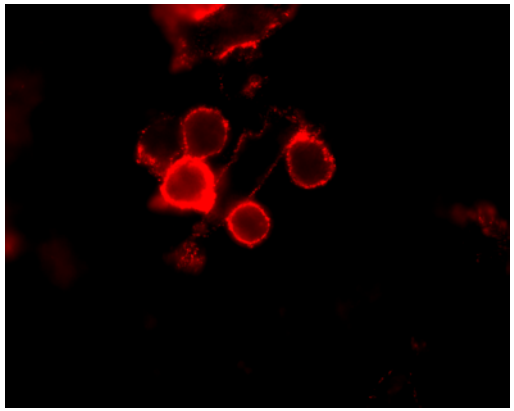
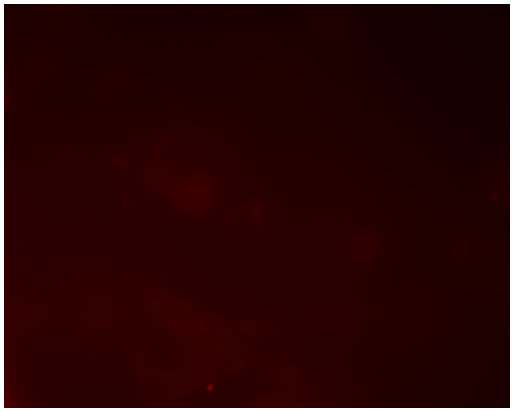
Emission Spectra of Qdot Conjugates



Minimal (<5%) cross-talk using 20nm bandpass filters

Simplified signal un-mixing >> simplified multiplex labeling

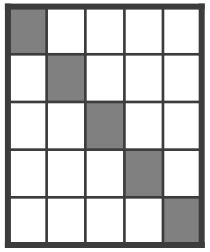
Excellent Brightness Provides High Sensitivity

Quantum dot	Organic dye
	
Exp. Time: 0.019 seconds	1.22 seconds
	
Exp. Time: 0.44 seconds	8.12 seconds

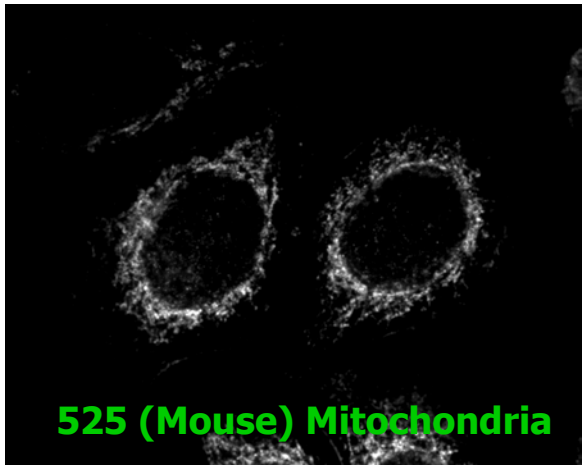
- High level Her 2/neu expression in SK-BR-3 Cells
- Quantum dots up to 50x brighter

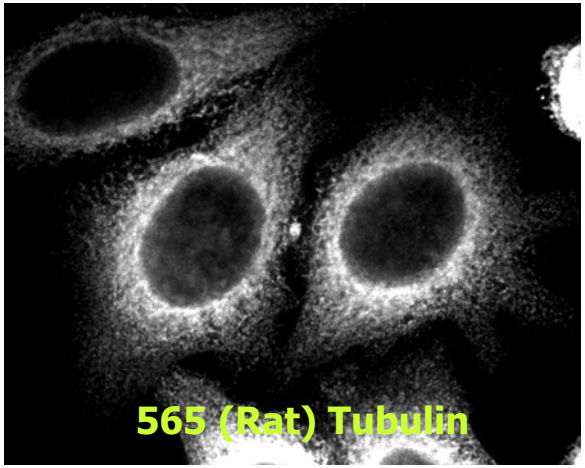
- Low level of Her 2/neu expression in MDA-MB-231 cells
- Quantum dots easy to detect but dye undetectable

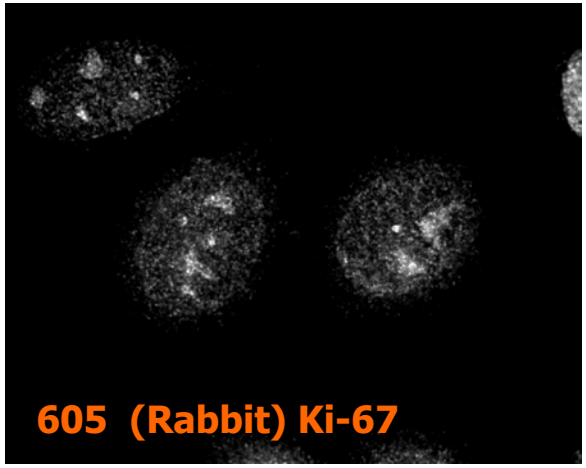
Detection of More Parameters in a Single Experiment

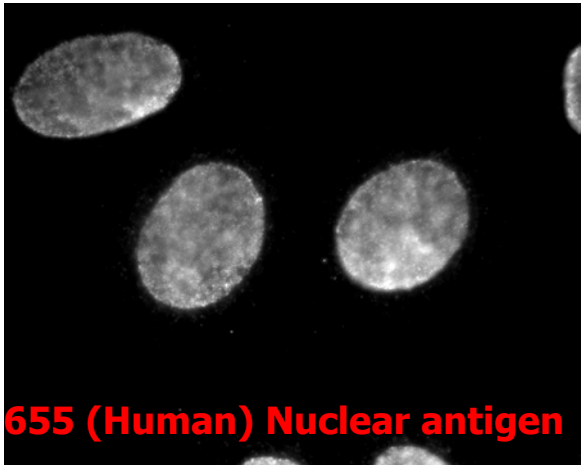


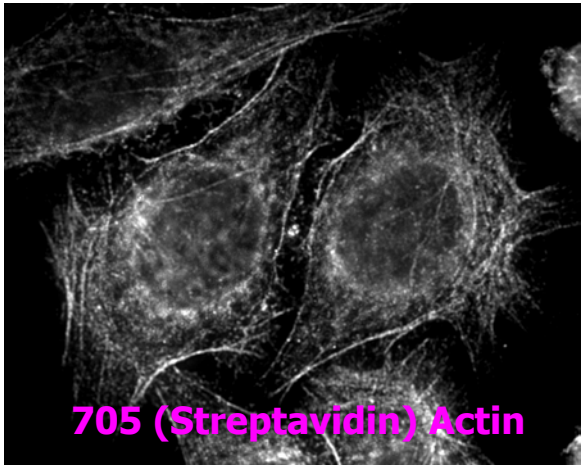
Partial information from single color experiments





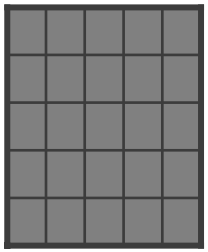




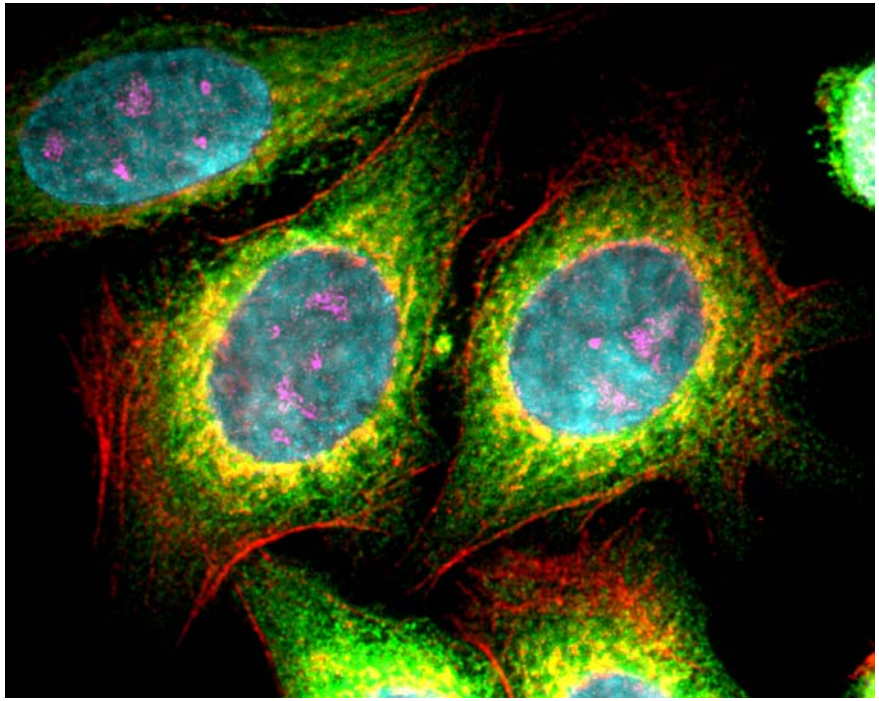


More information from every sample using Qdot Conjugates

Five Color Multiplexed Cell Labeling



Full information from multiplex experiments

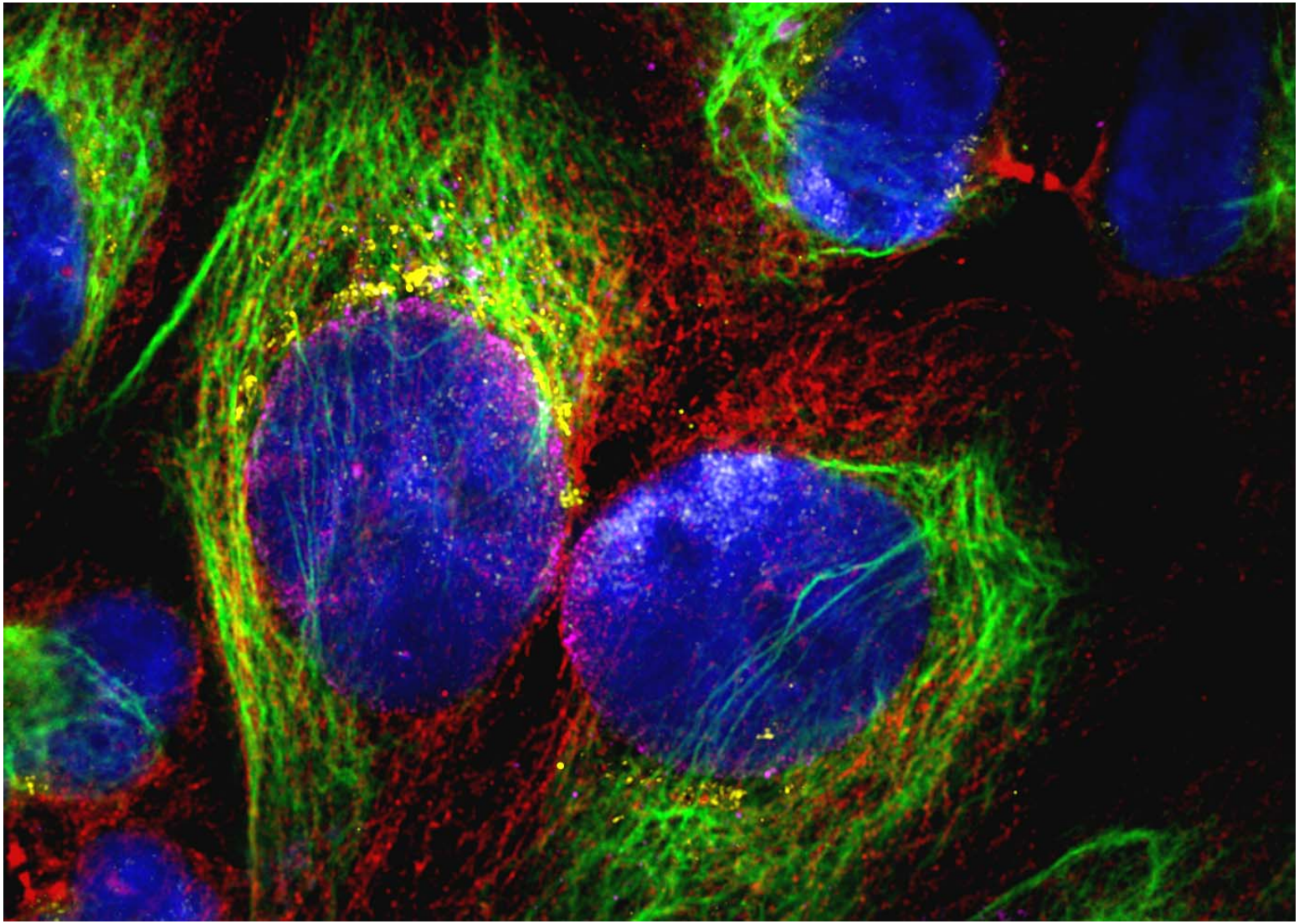


Overlayed Pseudocolor

Wu, X., et al., *Methods in Cell Biology, 75* **2004**.

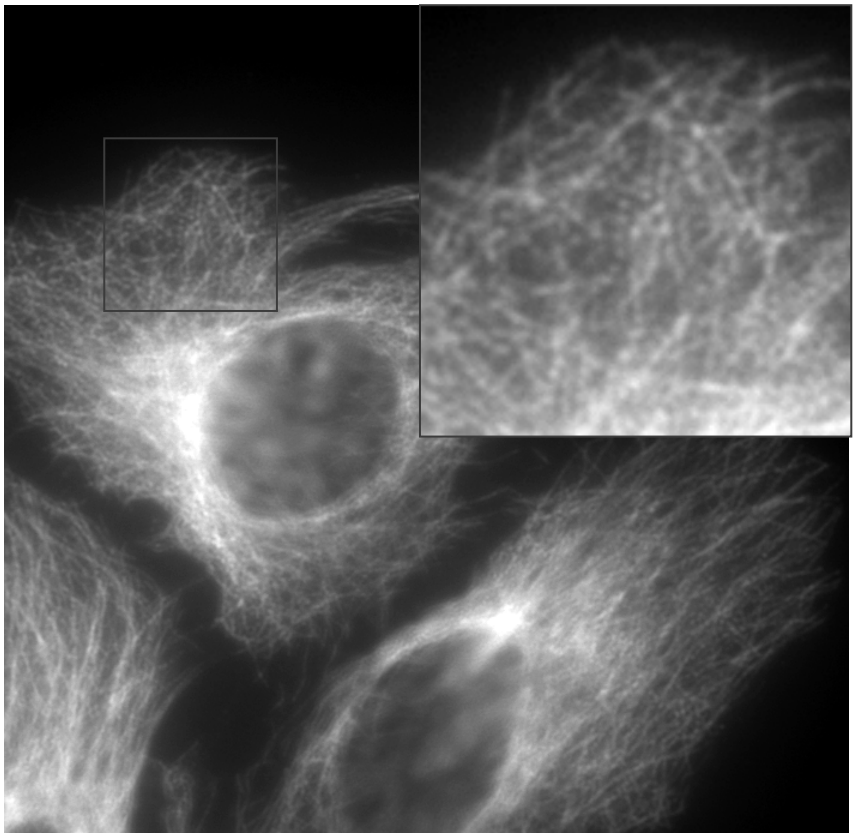
- 5-color labeling with dyes would be extremely difficult.
- Multiplexing gives more information from a single experiment.
- Multiplexing gives much more information than 5 single experiments.

Direct Conjugates Provide Ultimate Flexibility

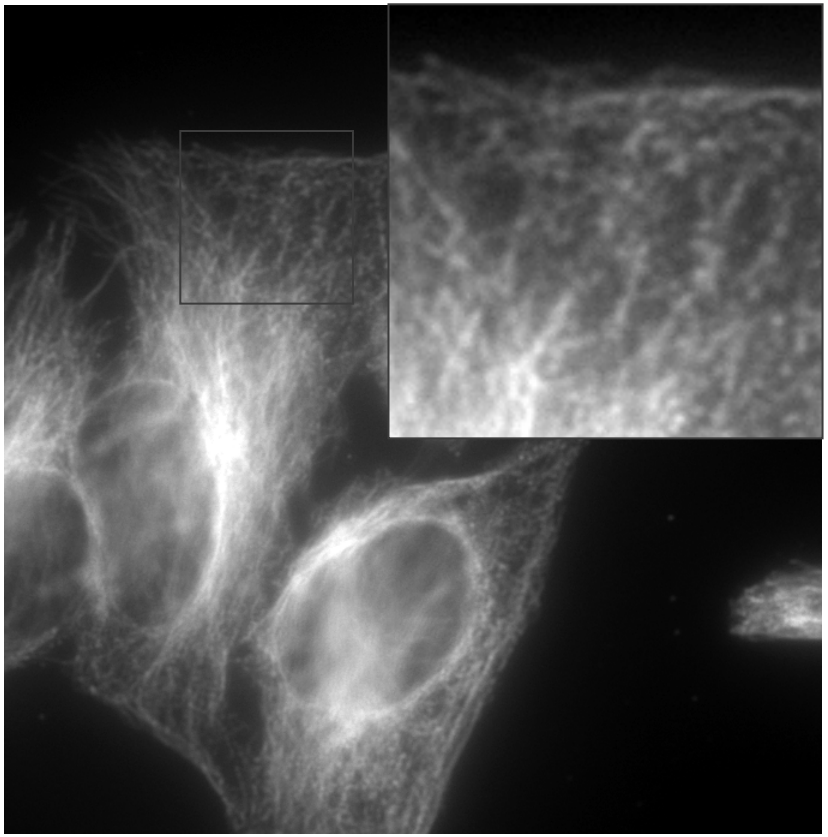


Qdot Conjugate Label Quality

HeLa cells fixed in paraformaldehyde and permeablized with Triton



Qdot 655 Conjugate



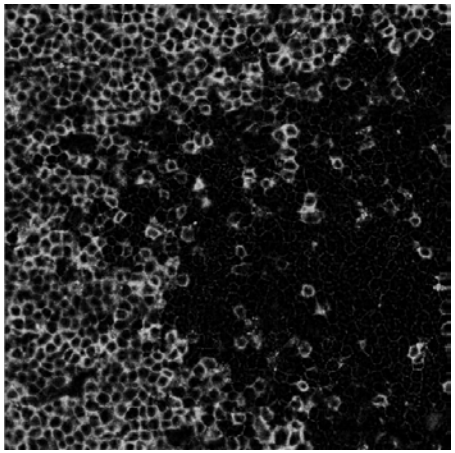
Alexa 594 conjugate

No significant differences in cytoskeletal protein labeling

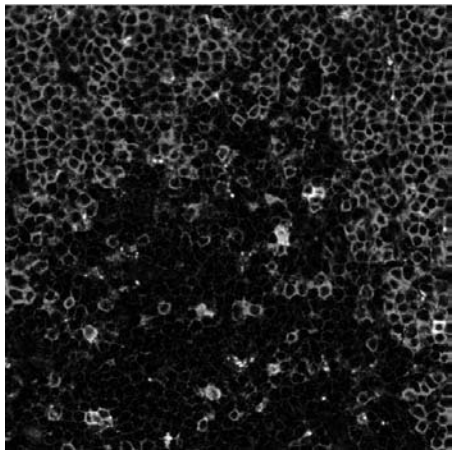
Qdot® Conjugates and Pathology

- Molecular Pathology
  - Patient diagnosis and prognosis
  - Patient stratification for "best" treatment regimen
  - Biomarkers for preclinical/clinical drug evaluation
- Traditional pathology loses all cellular/molecular correlations
  - Morphological correlation rather than molecular correlation
- Multiple markers are becoming the norm
  - Gene expression data → Protein analysis
- Qdot conjugate stability, brightness and multiplex capability are ideal.

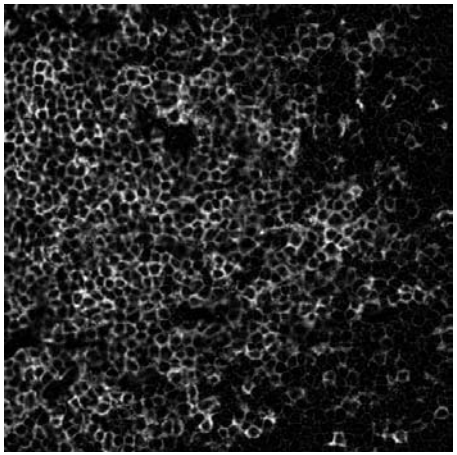
Obtain More Information From Each Specimen



"Slice 1"



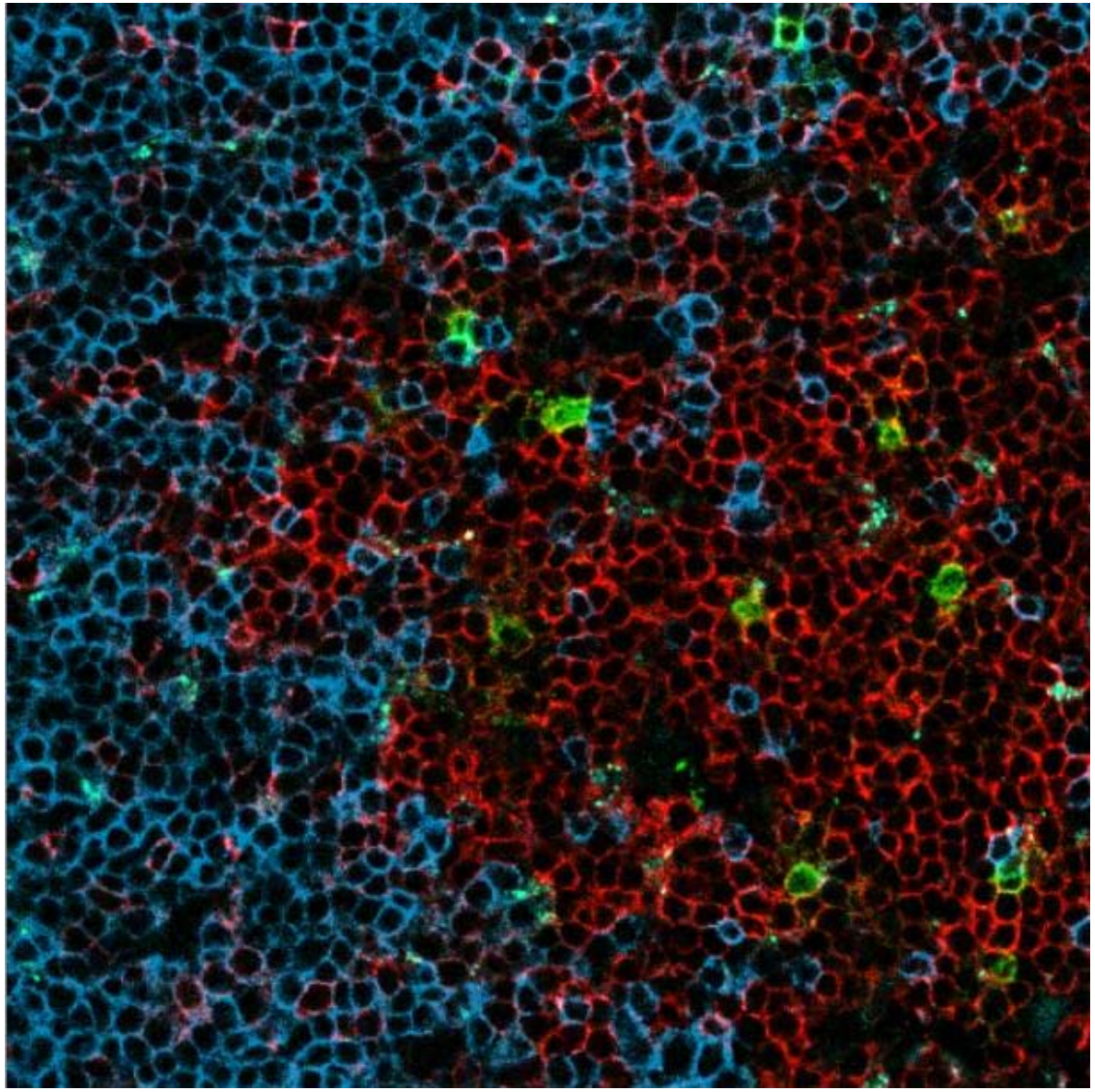
"Slice 2"



"Slice 3"

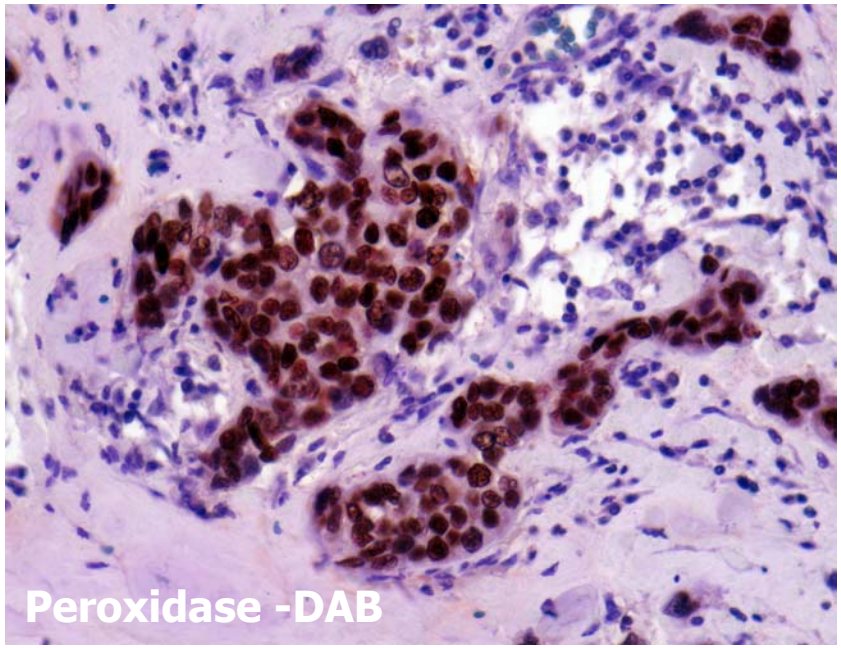
- Typical pathology: Single color, no relative measurements.
- Slices have no relative orientation.
- Marker information available is based on morphological correlations.

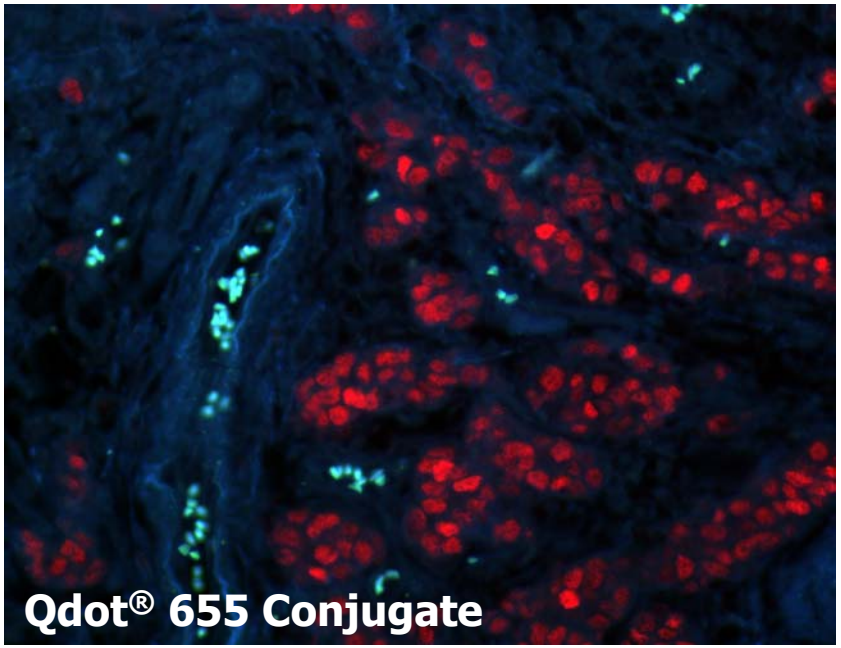
Pathology: See What You've Been Missing



Courtesy of
Jason Adams
UCSF

Estrogen Receptor: monoclonal Rb clone SP1 RM-9101-S



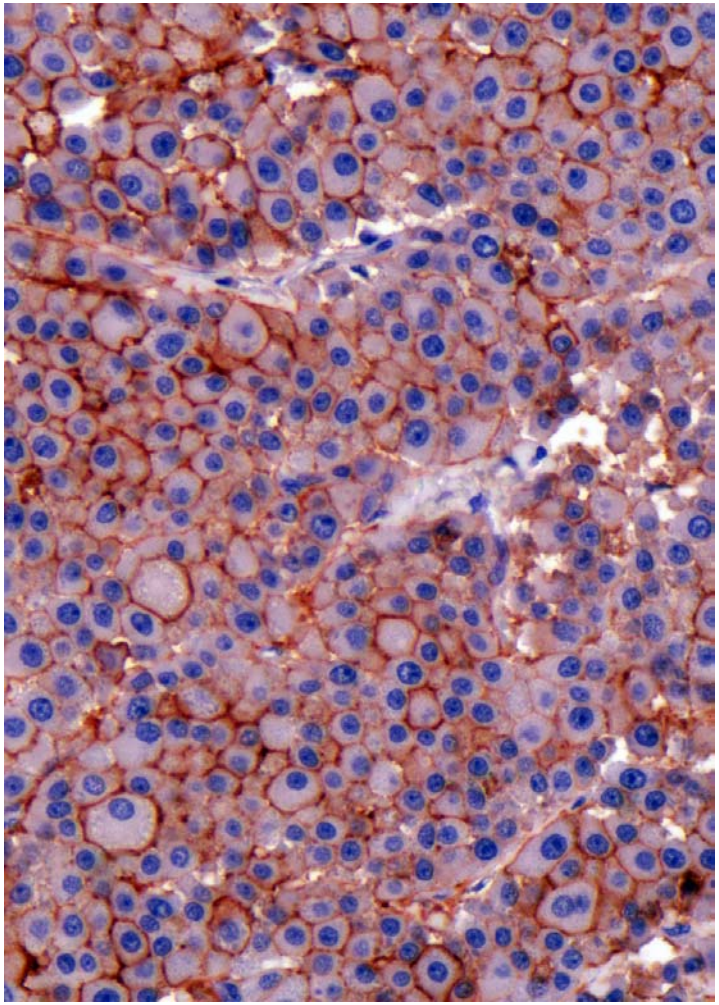


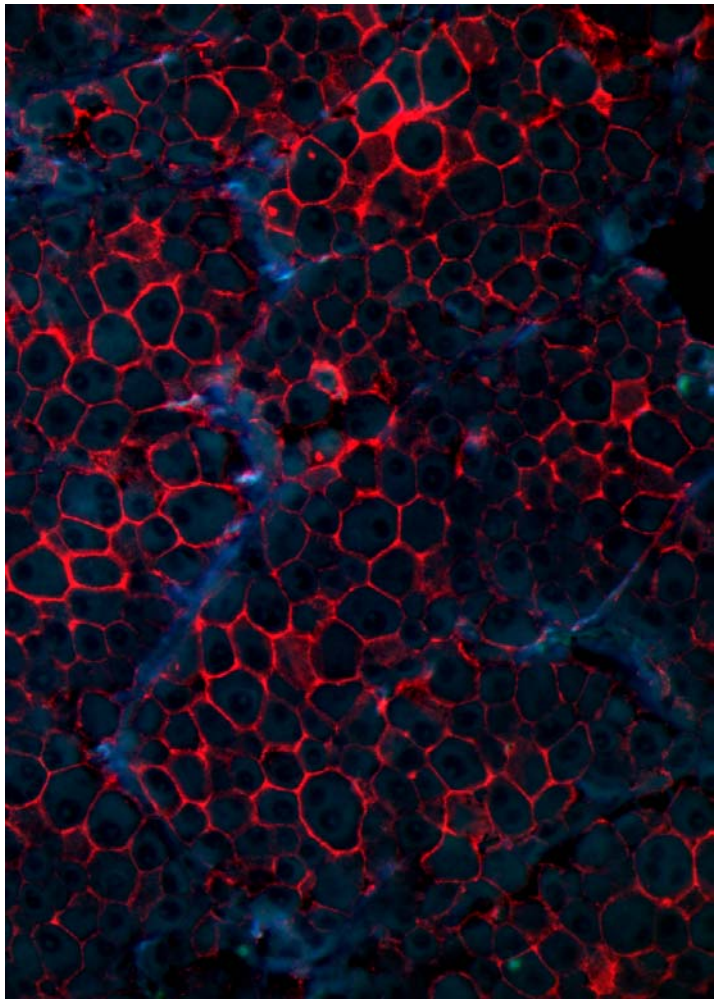
RGB images acquired a single gray scale images at 655/20, 565/20, and 450/58 nm and merged

Images courtesy of

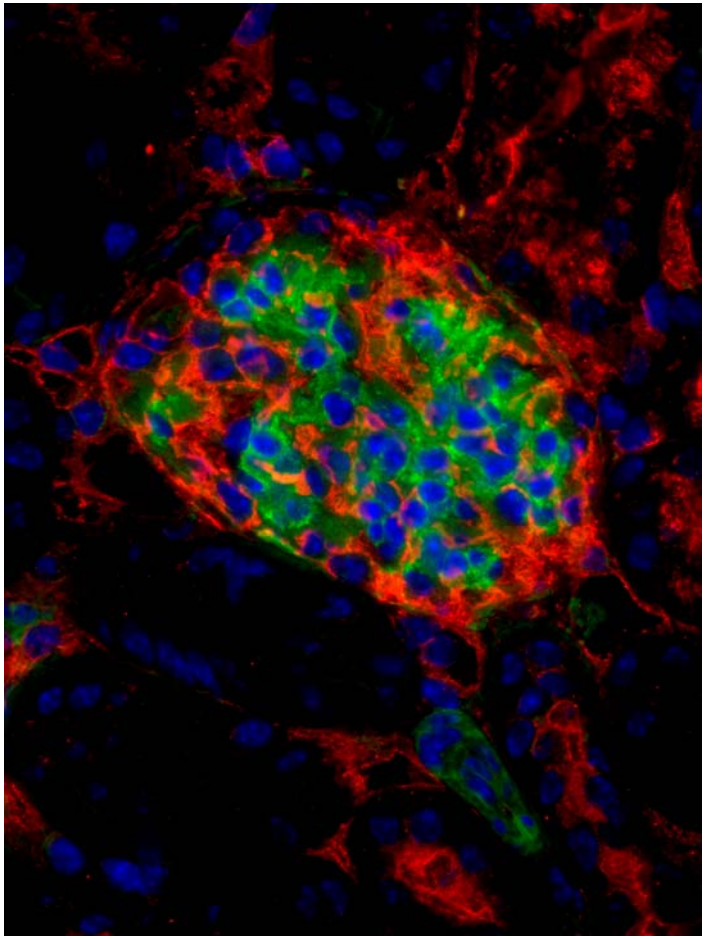
LAB VISION CORPORATION

Her-2 in Breast Cancer

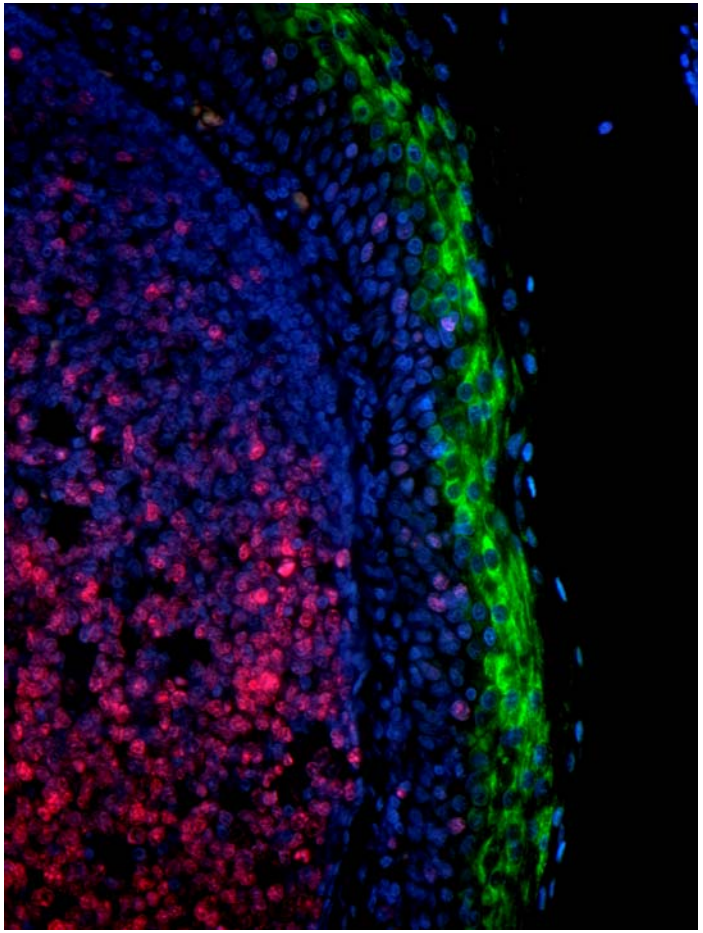




Qdot Primary Conjugates

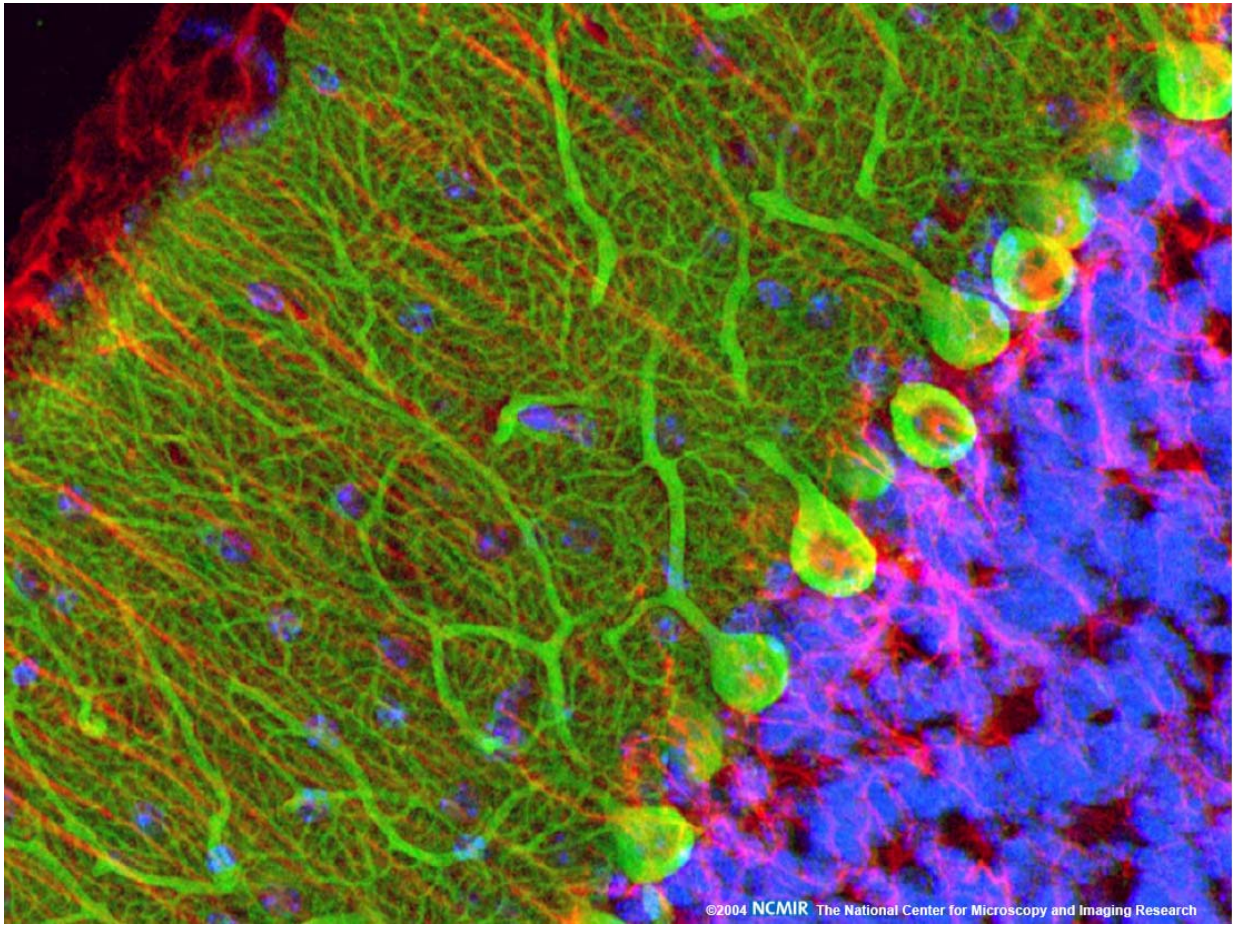


WGA Qdot 655 Conjugate
Phalloidin Qdot 525 Conjugate



Ki-67 Qdot 585 Conjugate
Vimentin Qdot 525 Conjugate (Clone V9)

Correlative Light and Electron Microscopy

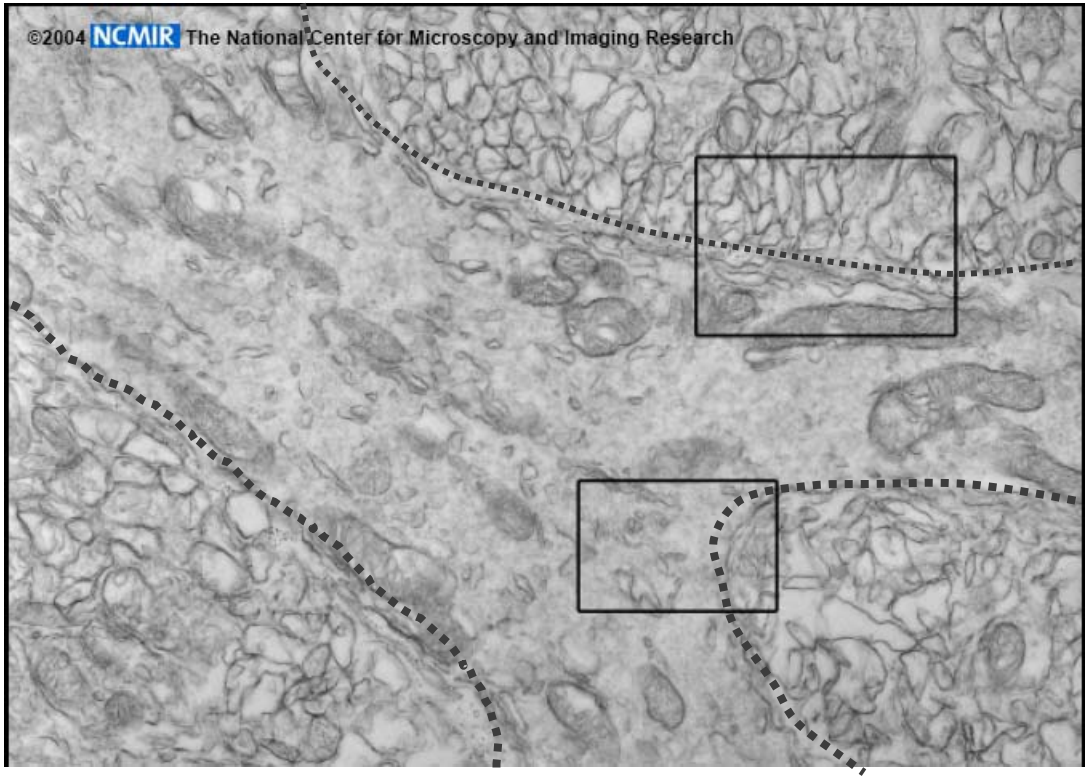


Giepmans, et al. Nature Methods 2(10), 2005.

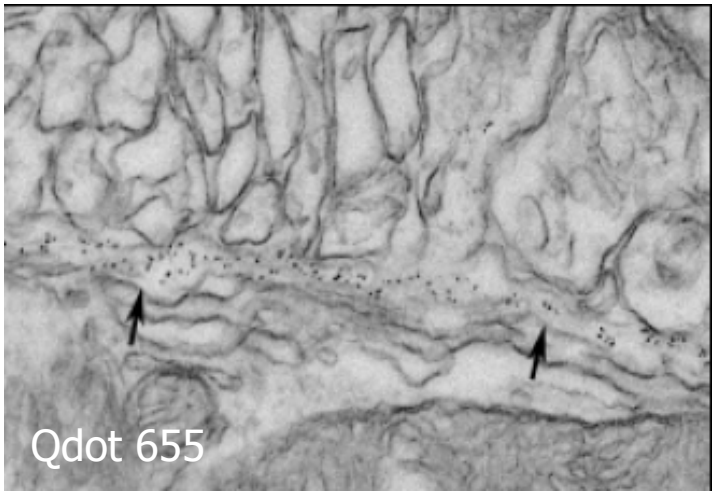
Images provided by Mark Ellisman, National Center for Microscopy and Imaging Research, UCSD, San Diego, CA

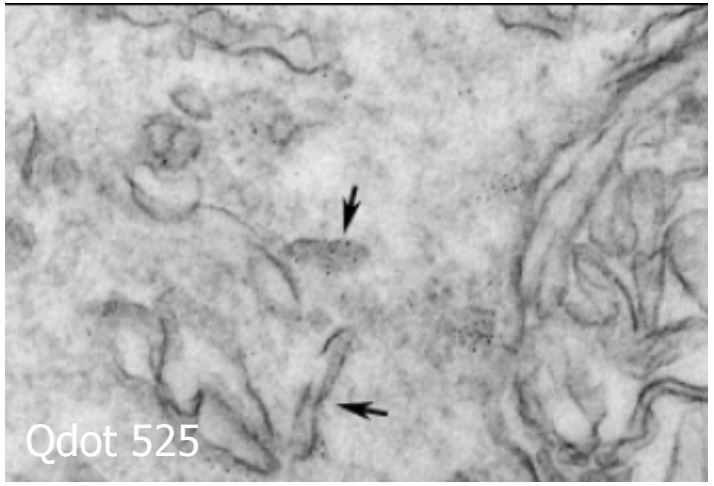
Glial fibrillar acidic protein - Qdot 655
Inositol triphosphate Receptor – Qdot 525

Better EM labels than colloidal gold because of superior penetration

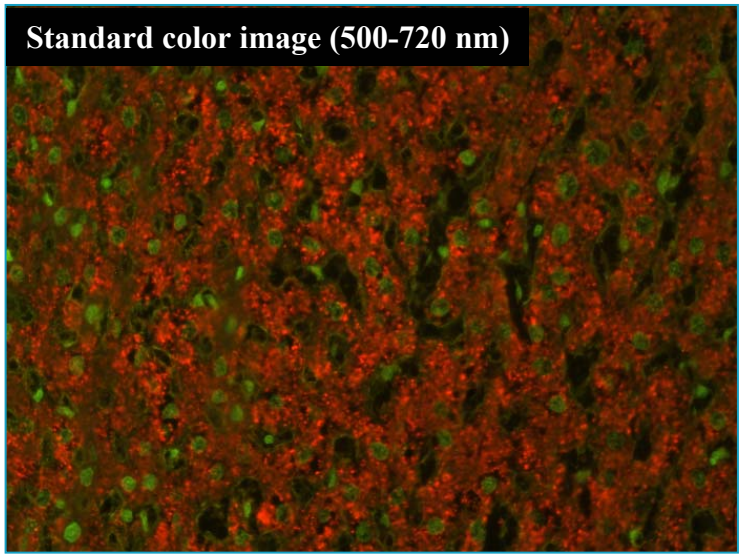


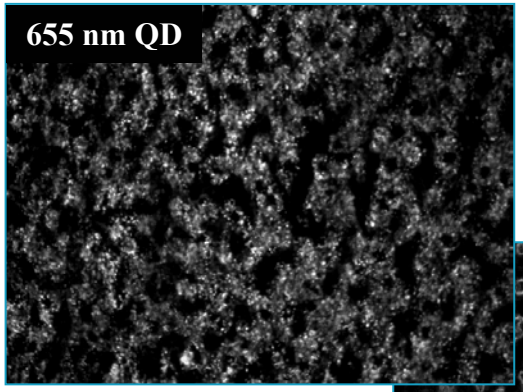
Glial fibrillar acidic protein - Qdot 655
Inositol triphosphate Receptor – Qdot 525

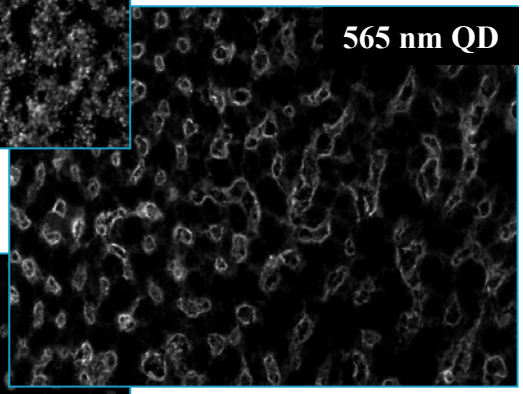


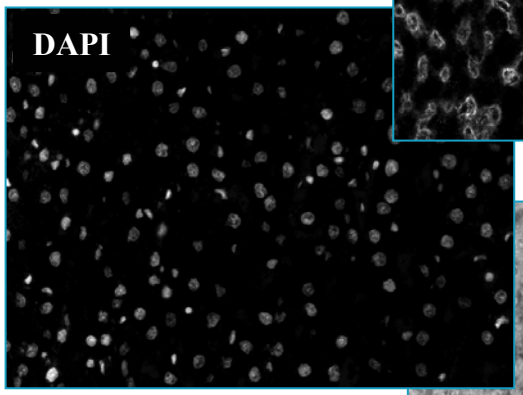


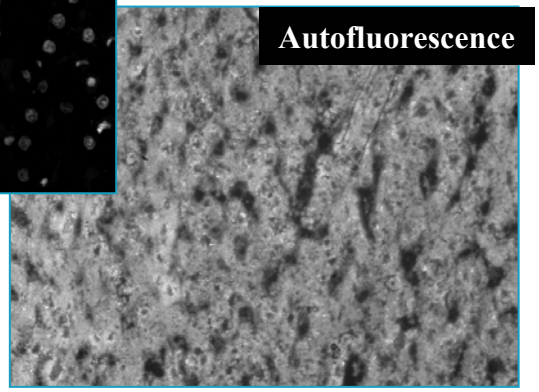
Qdot Conjugates and Spectral Imaging

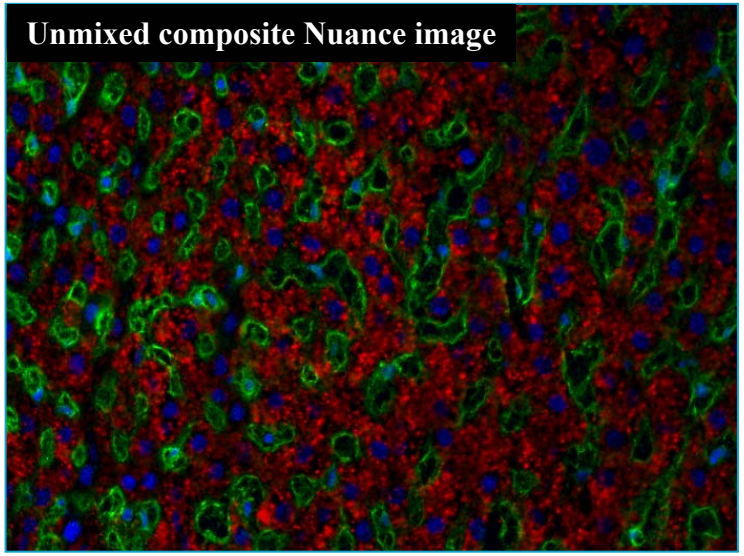








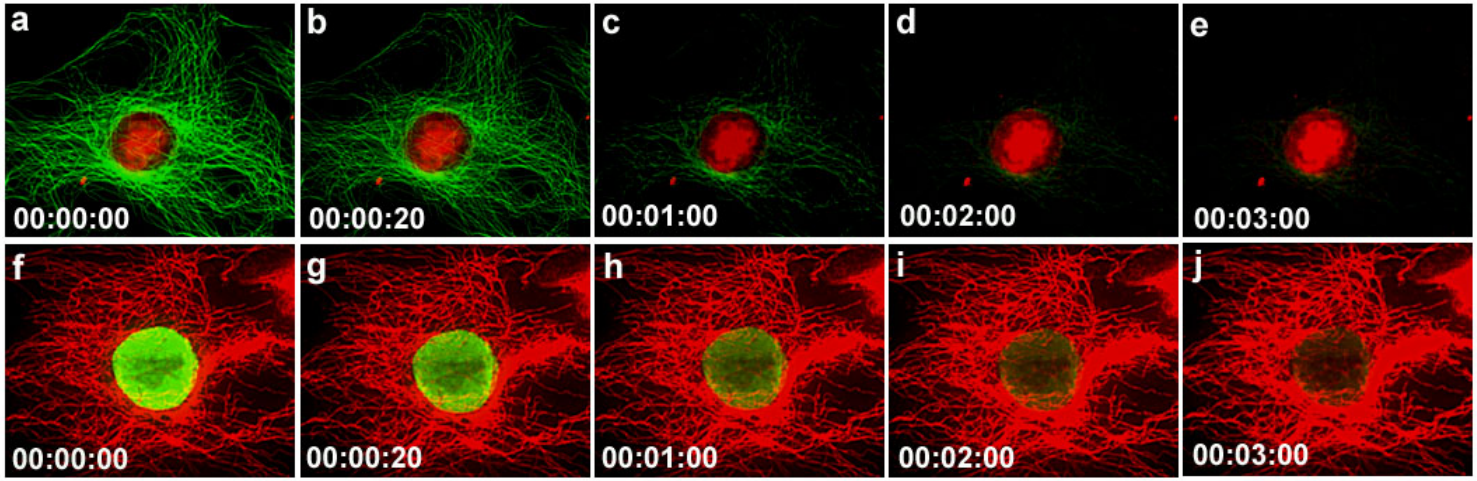


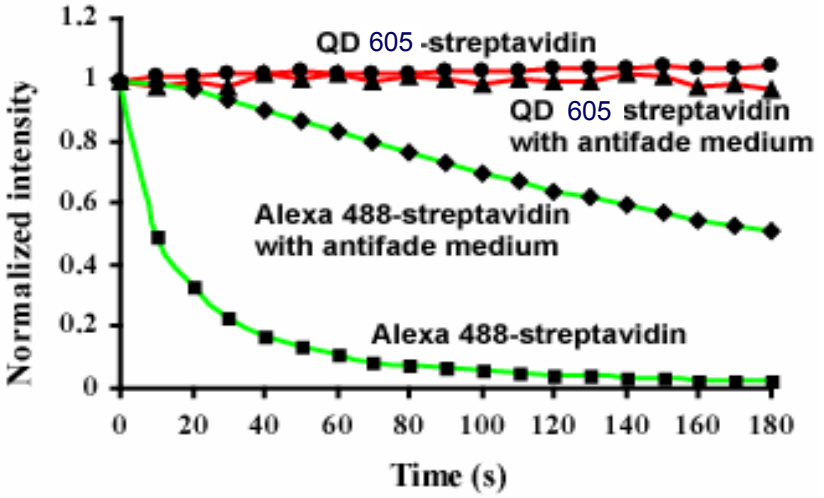


Data Courtesy of CRI-Inc on a Nuance Camera System

You can see clearly now.

Photostability of Qdot® Conjugates





Wu, X., et al. *Nature Biotechnology, 21(1)* **2003**.

Top panel (a-e): Nucleus labeled with Qdot conjugates and microtubules labeled with Alexa Fluor 488

Bottom panel (f-j): Nucleus labeled with Alexa Fluor 488 and microtubules labeled with Qdot conjugates

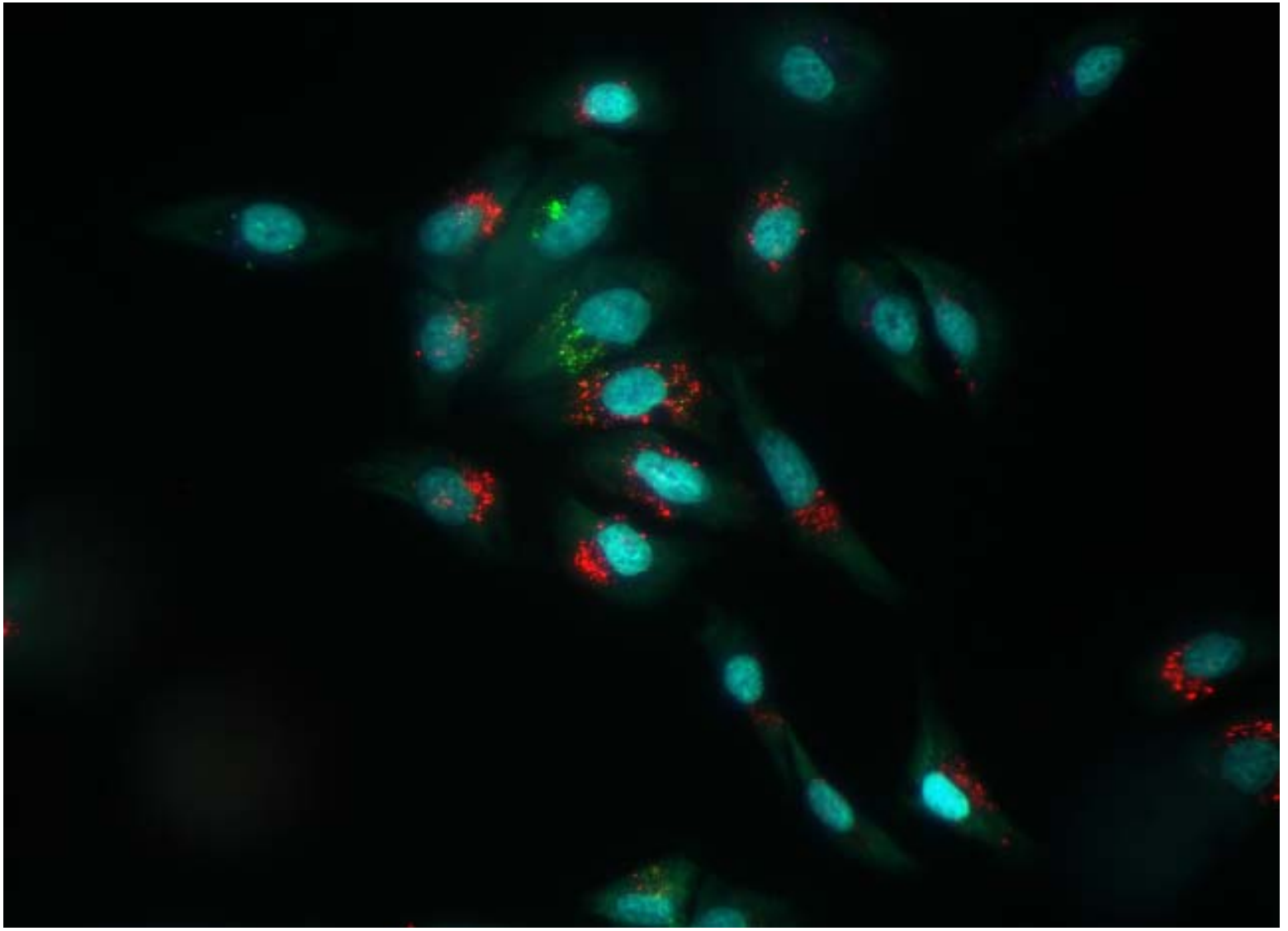
Left: quantitative data showing effect of anti-fade medium

Qdot® Conjugates for Fluorescent Labeling

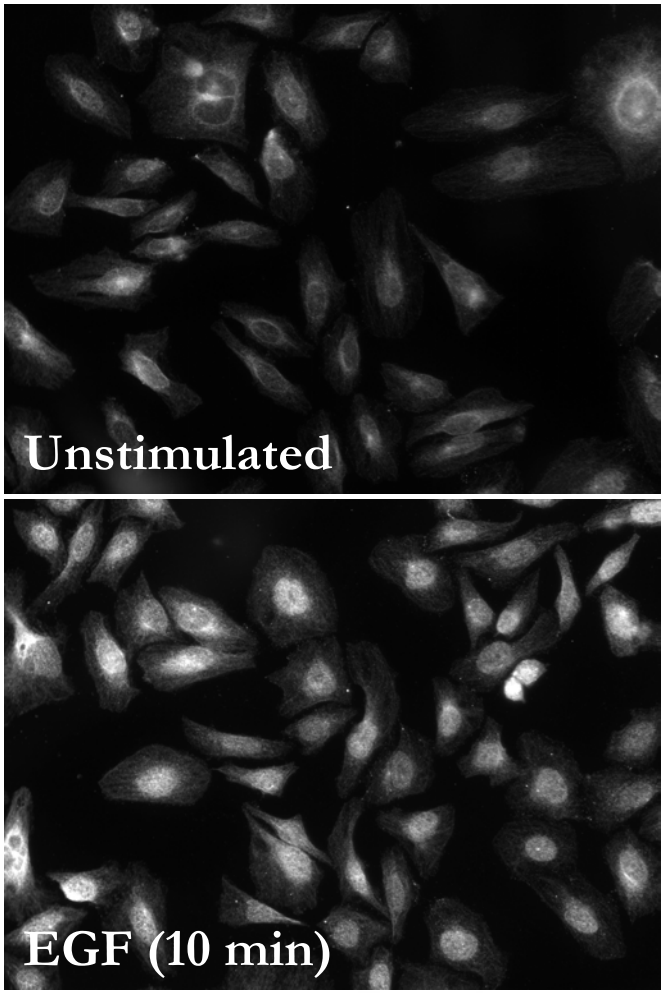
- Extremely bright for sensitive detection
- High photostability provides:
  - Ability to monitor signal over long periods of time
  - Ease of use (time for focusing and image collection)
  - Enables pathology and live cell imaging applications
- Narrow emission peaks for simple multiplexing
- Easily illuminated by many excitation sources
- Easily conjugated to a variety of biomolecules

- Key issues: Fixation Protocol, Filter Selection, PAP Pen Quenching, Photobrightening

Cellular Assays



Nuclear Translocation Assay



Nuclear expression of phosphorylated Erk in starved HeLa cells following EGF stimulation

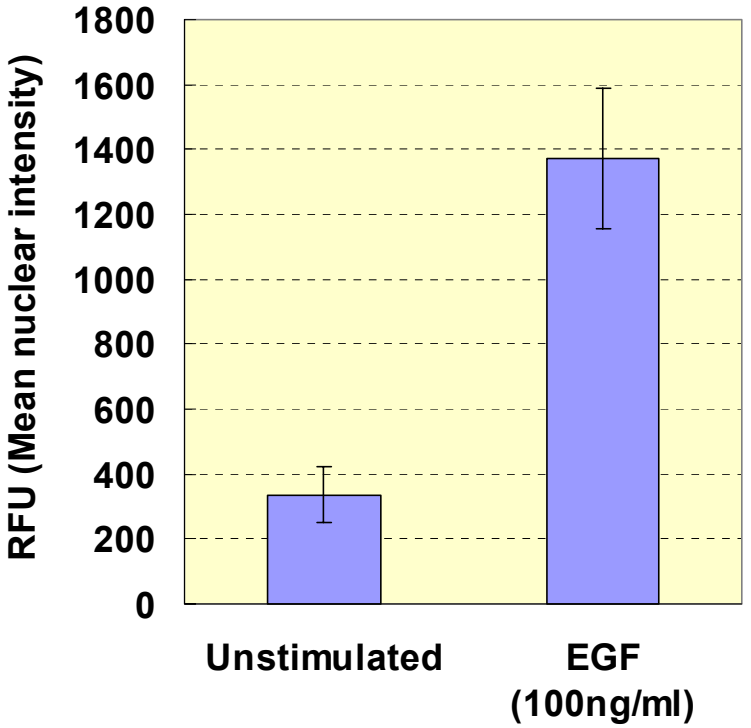
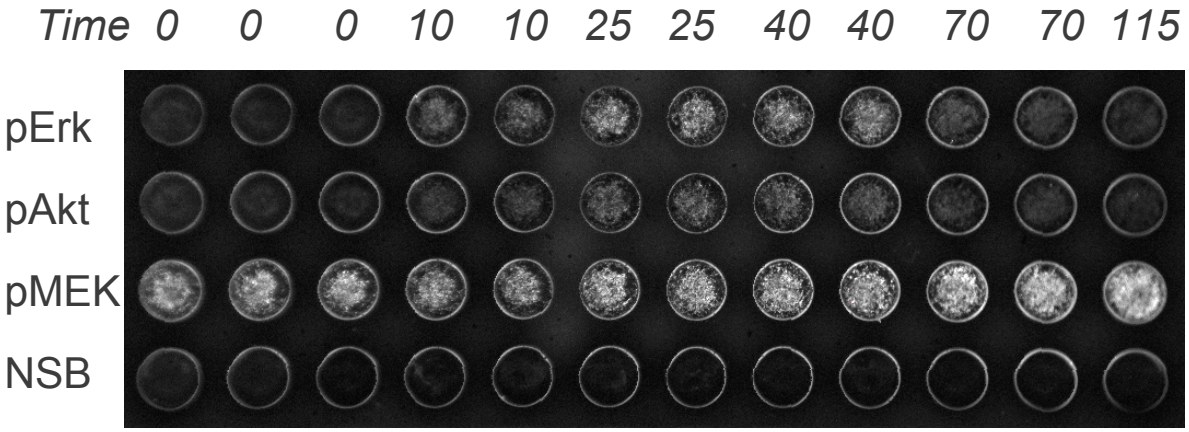
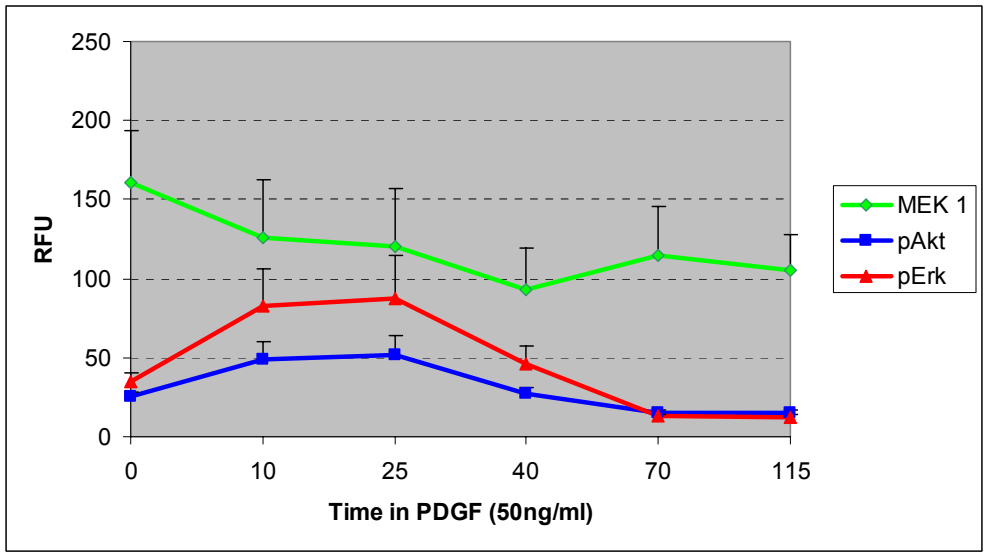


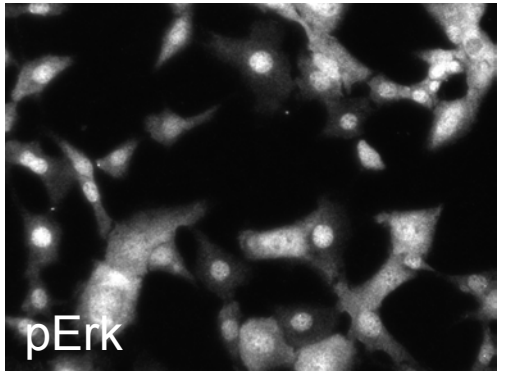
Plate-based Cellular Immunoassays Benefits

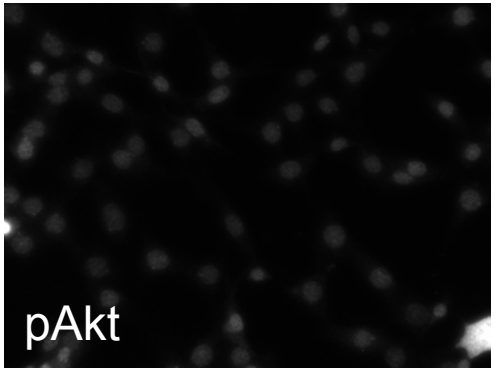
- High information content in high throughput
- Lower cost and simpler instrumentation requirements
  - Plate readers are readily available in all target labs
- More valid biology
  - More flexibility in assay configuration
  - More controls or reference markers may be included
- Lower level of expertise required
- Instant data reduction
  - Image analysis and storage not required
- Label reagents can be transferred to tissue characterization in preclinical models

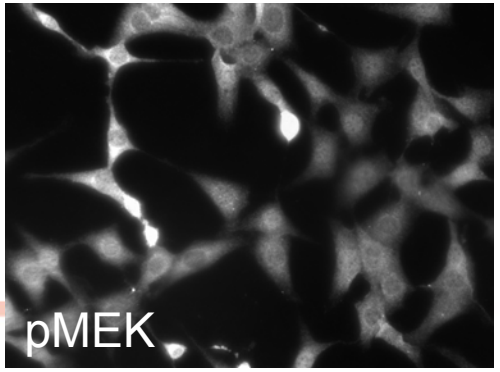
Qdot Conjugate Labeling of MAP Kinase Pathway in 3T3 cells





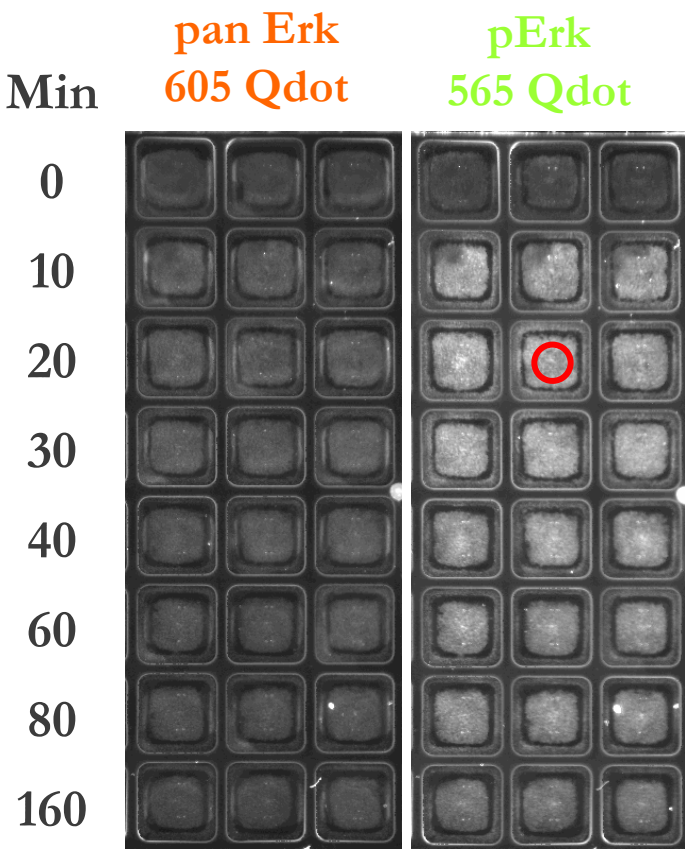




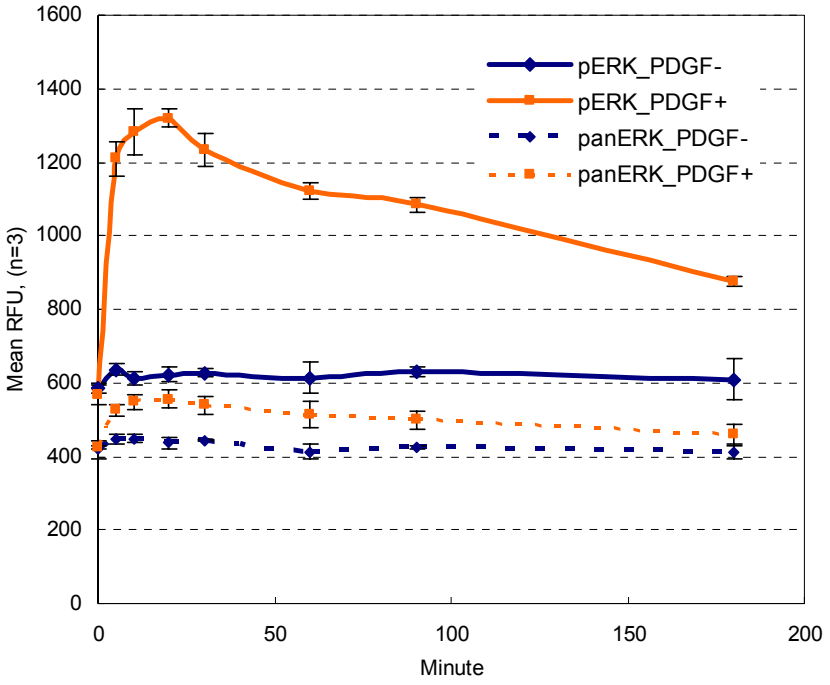


Serum starved cells were stimulated with 50 ng/ml PDGF for the times indicated. Cell (left) were stimulated for 25 min for fixation.

Phospho-Erk Assay Results



Induction of phosphorylated Erk by PDGF in NIH3T3 cells



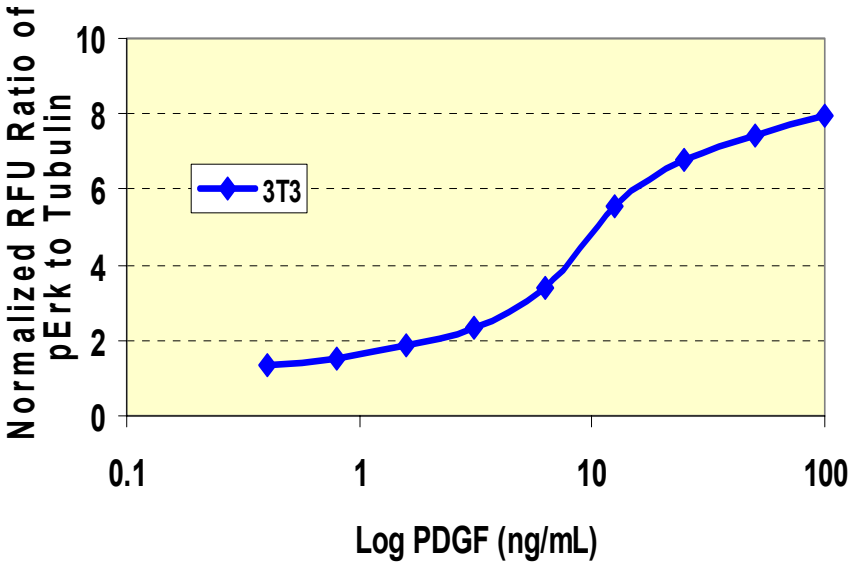
At left: Images of the same set of wells acquired at two colors assaying pan Erk and phospho Erk. The red circle represents the area used for measuring mean intensity in each well.

At right: Results plotted showing time course of PDGF induced phosphorylation of Erk

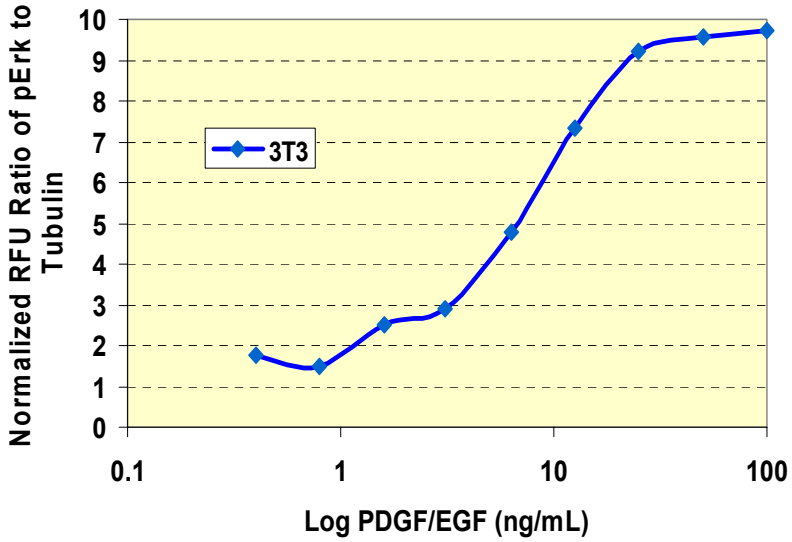
Z' values at 20 through 80 minutes are 0.63-0.77

More Controls Leads to More Robust Data

Kodak MM2000 gel imager

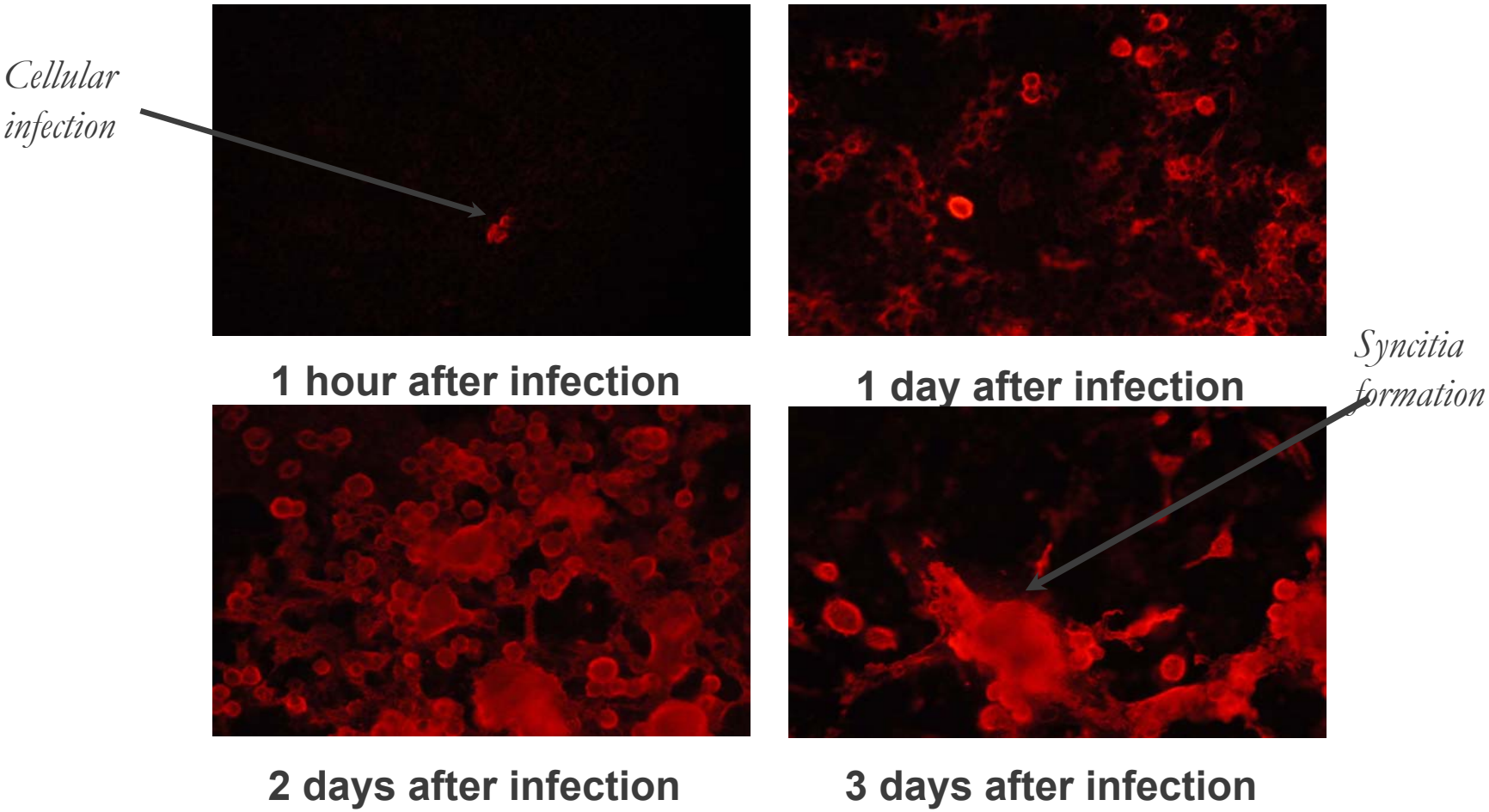


BMG PolarStar Plate Reader



- p42/44 MAPK phosphorylation was indexed to tubulin but can be normalized to pan protein expression or wheat germ agglutinin (Qdot WGA Conjugate) expression.
- Multiplexing with Qdot Conjugates allows extensive information and referencing in a single well readout.

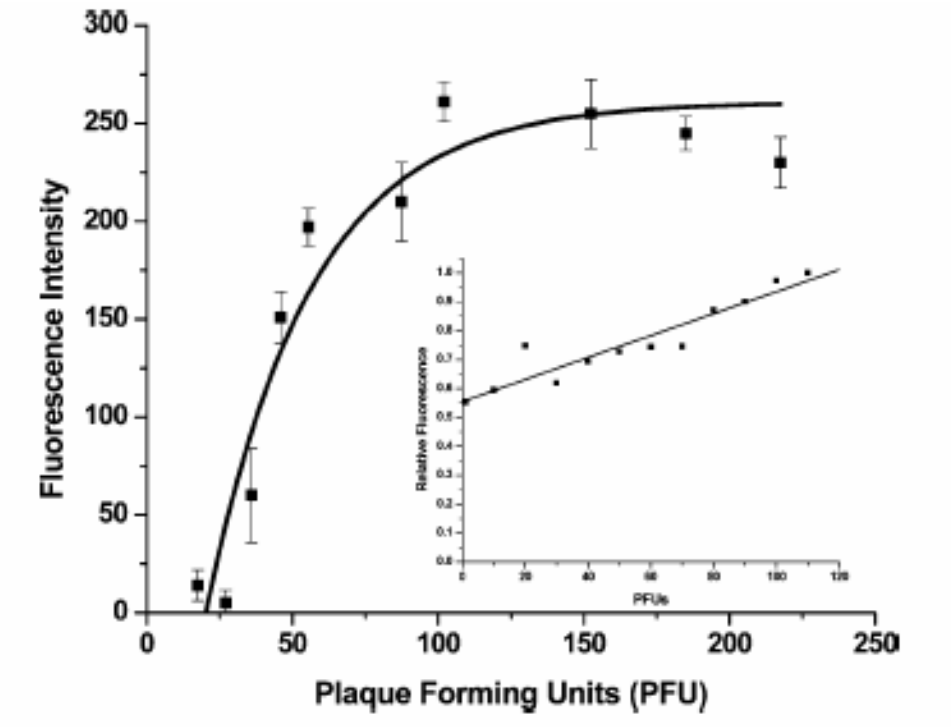
Respiratory Syncitial Virus Progression



E. Bentzen, *Nano Letters, 5* **2005**.

Dose-Response for Viral Infection in Culture

Whole well F-Protein cell intensity vs initial infection load



Data collected on a Bio-Tek Synergy HT

Ex:250 nm
Em:598+/-18 nm

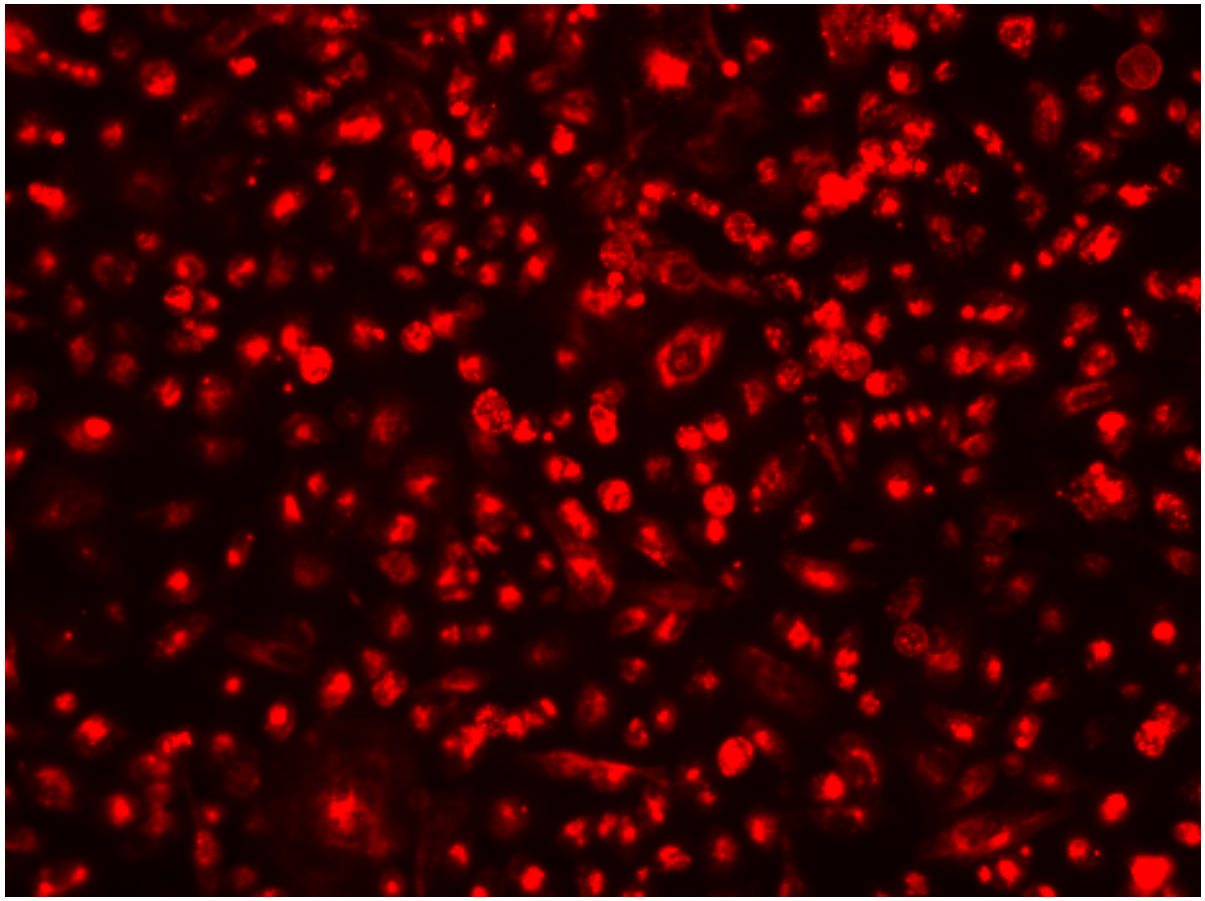
Qdot 605 Streptavidin Conjugate
Biotinylated anti-RSV F-Protein

Detection limit of 35-50 PFU in 24 hours

*Progression can be monitored by image analysis **or** by quantitative analysis of the cell population.*

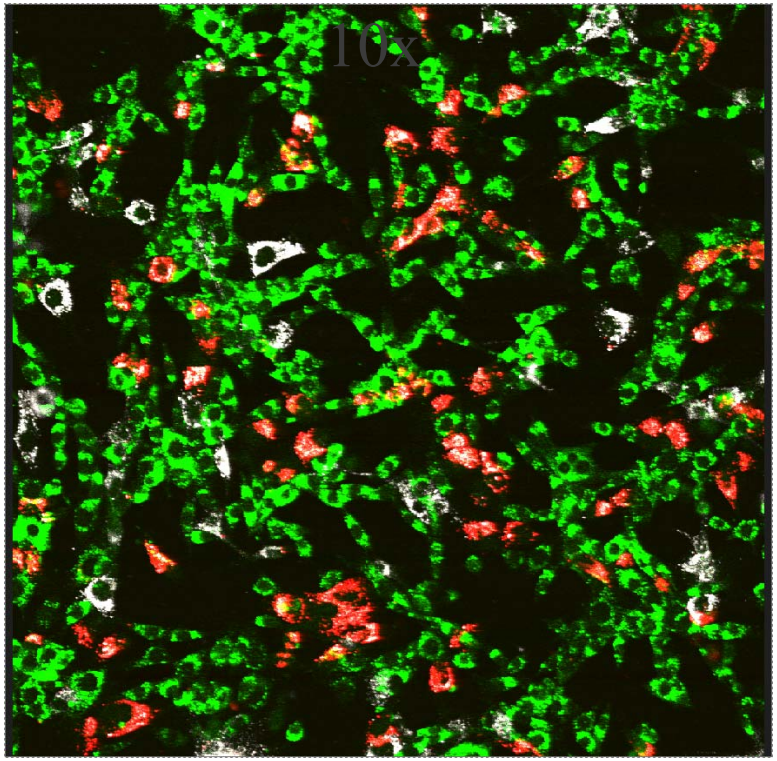
Faster and easier to look at the whole population.

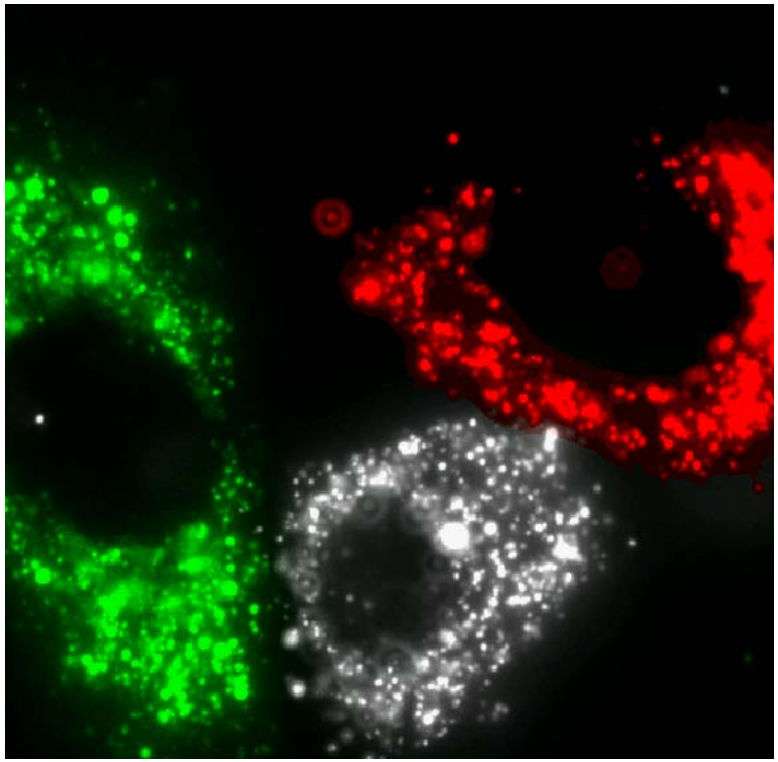
Qtracker Cell Labeling Kits



Lagerholm, B.C., et al., Nano Letters, 4(10) **2004**.

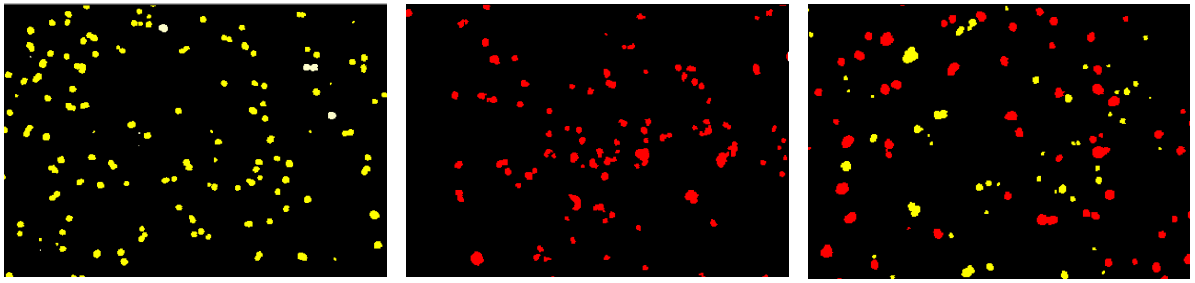
Three Color Qtracker® Cell Labeling





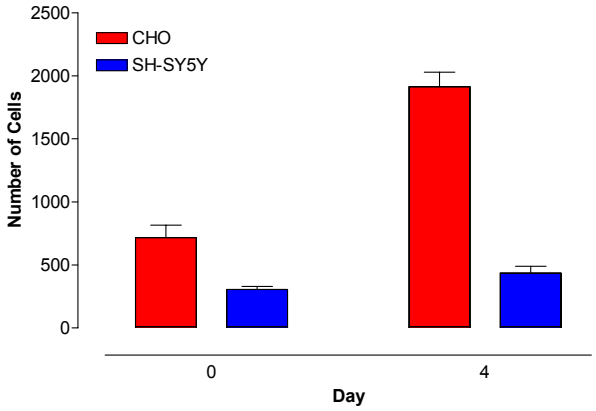
- 3T3(green), HeLa(red), and U188(white) cells labeled with Qtracker 565, 655, and 705 respectively.
- Co-cultured in 8-well chambers for 24 hrs. Images captured with a Leica Confocal microscope (ex. = 488nm).

Multiplexed Cellular Assays

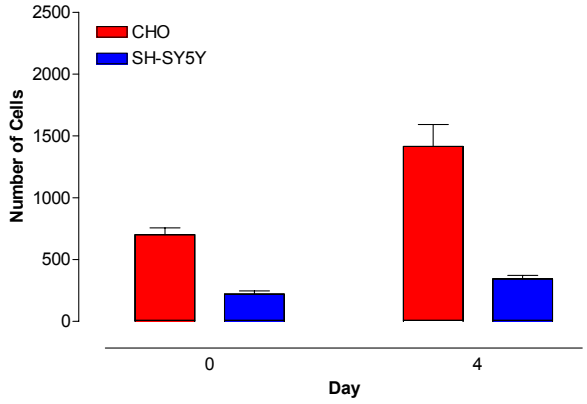


CHO-655 **SH-SY5Y-705** **Mixed**

Single cell proliferation over 4 days

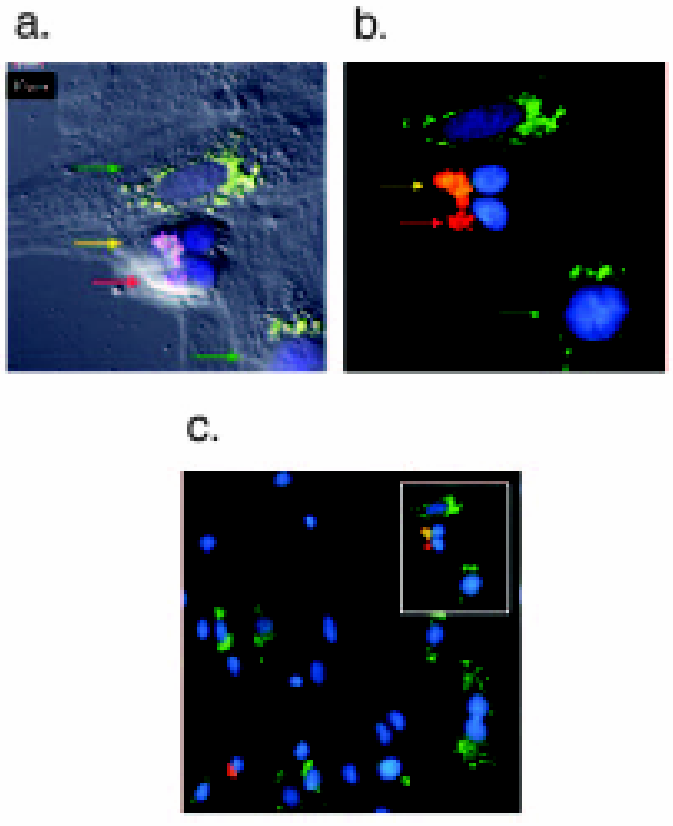


Mixed cell proliferation over 4 days



- TTP Acumen® Explorer for single-excitation, multiplexed cellular analysis.
- Real-time proliferation readouts from multiple cell-lines within a single well.
- Other multiplexed cellular readouts possible (Internalization, Calcium, etc.)

Cell Fusion Assays and Stem Cells



EPC fusion / total human cells :

(mean +/- standard deviation)

0.50 +/- 0.23 %

Endothelial Progenitor Cells
Qtracker 565

H9C2 Cells (Cardiac Lineage)
Qtracker 655

Cell Fusion Rate:
Both colors, one cell
0.50 +/- 0.23%

EPC committed to Cardiac Lineage
30-50% Transdifferentiation

Conclusion: Cell Fusion cannot account for differentiation behavior

Murasawa, et al. Arterioscler Thromb Vasc Bio, 25(7) 2005, 1388-1394

Multiplexed Cellular Assays

- Qtracker Cell Labeling Kits are non-toxic
  - Analysis of phenotype, metabolism, proliferation, differentiation
- Qtracker Reagents do not transfer between cells
- Qtracker Reagents are passed to daughter cells for 6-8 generations typically

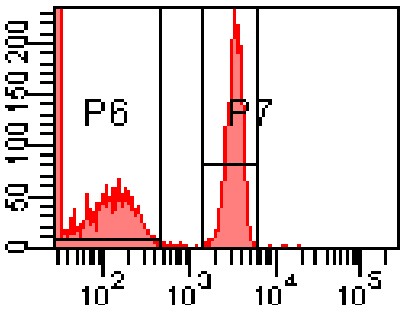
- Ideal tools for studying cell-cell interactions
- Ideal tools for tracking cell fate in living systems

Qdot Conjugates in Cytometry

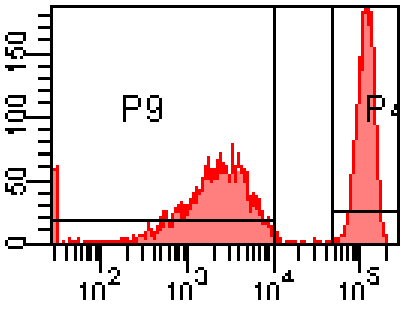
- Flexible excitation
  - Usable with all common platforms
  - Perfect for single laser, multicolor systems
- High brightness
  - Comparable to or better than best dye molecules
- Very low cross-talk
  - No or minimal compensation required from single laser.
- Improving platform
  - QDC R&D producing ongoing improvements to brightness, width, and NSB.
- Unlimited colors available
  - 6 colors from 525-800 nm with < 5% cross-talk
- Photostability allows for imaging and resorting

Flow Cytometry: Qdot Streptavidin Sampler Kit

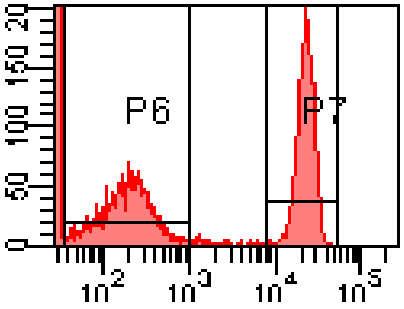
CD4-biotin + Streptavidin Sampler Kit on human PBMCs, 405 nm excitation



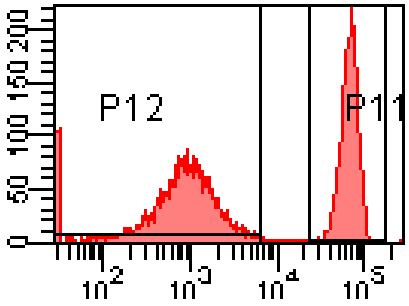
Qdot 525 streptavidin
525/20



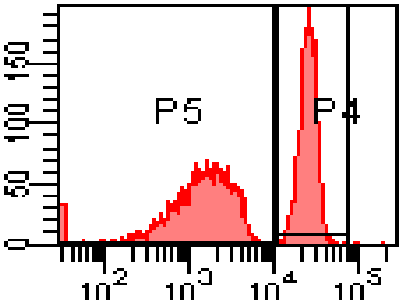
Qdot 605 streptavidin
605/20



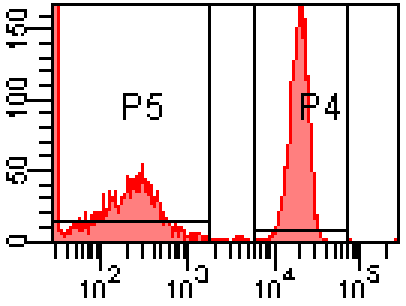
Qdot 565 streptavidin
565/20



Qdot 655 streptavidin
655/20

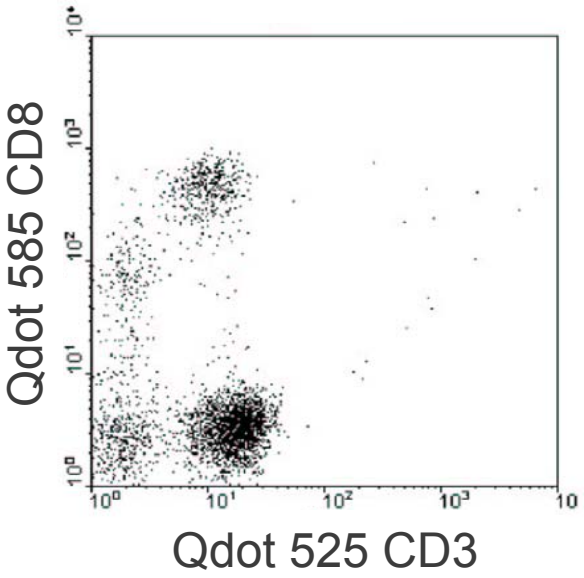


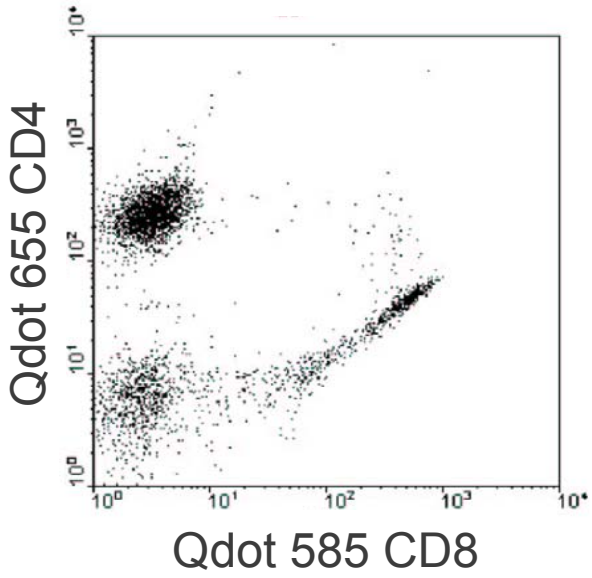
Qdot 585 streptavidin
585/20



Qdot 705 streptavidin
695/40

Single Laser, Multicolor Flow Without Compensation





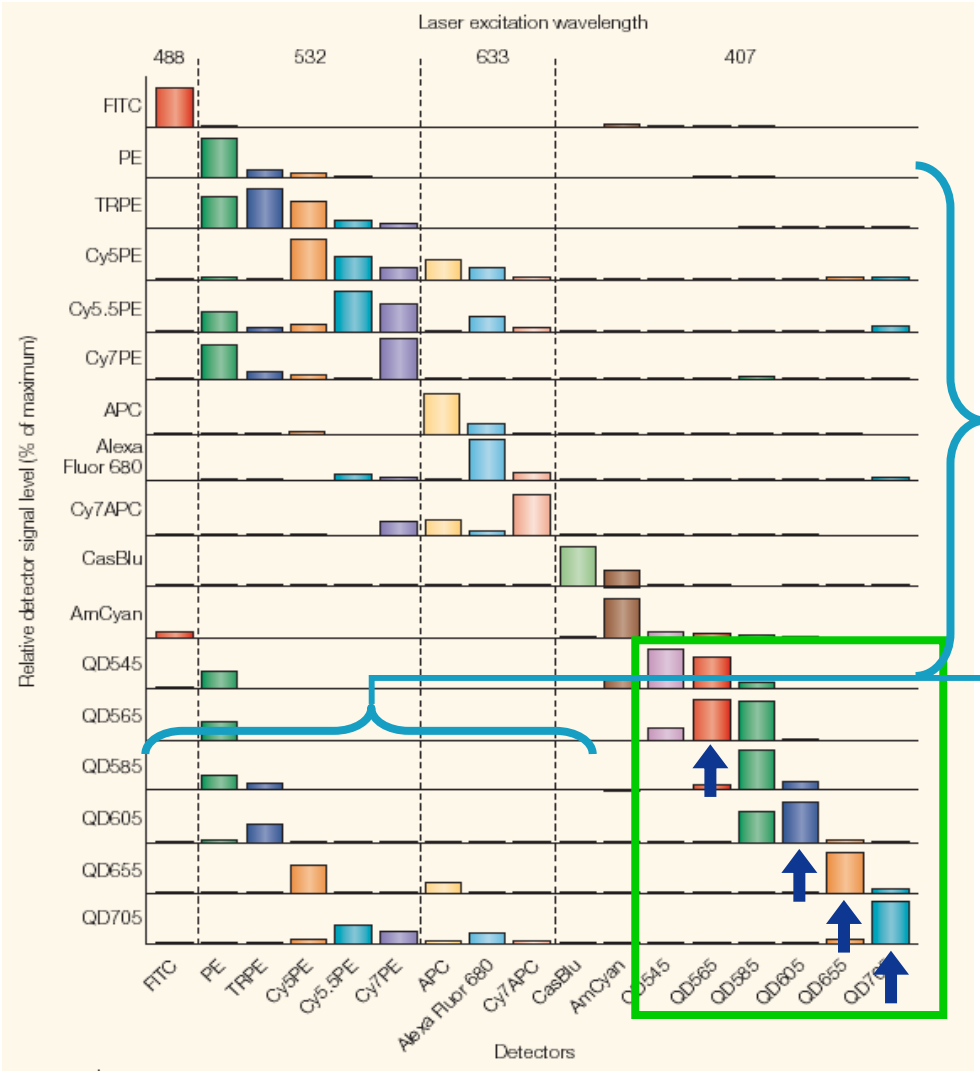
PBMCs + direct conjugate cocktail

Modified BD FACScan:
- FL1: 530/30
- FL2: 585/42
- FL3: 620 LP

Run without compensation

Reagent	Intensity off 488 nm	Spectral Overlap
Qdot 525 crystal	~ Fluorescein	2% into FL2
Qdot 585 crystal	~ 50% RPE	11% into FL3 (>620 nm)
Qdot 655	~ RPE/Cy5	0.2% into FL2

Clean Spectral Signals from Qdot Conjugates



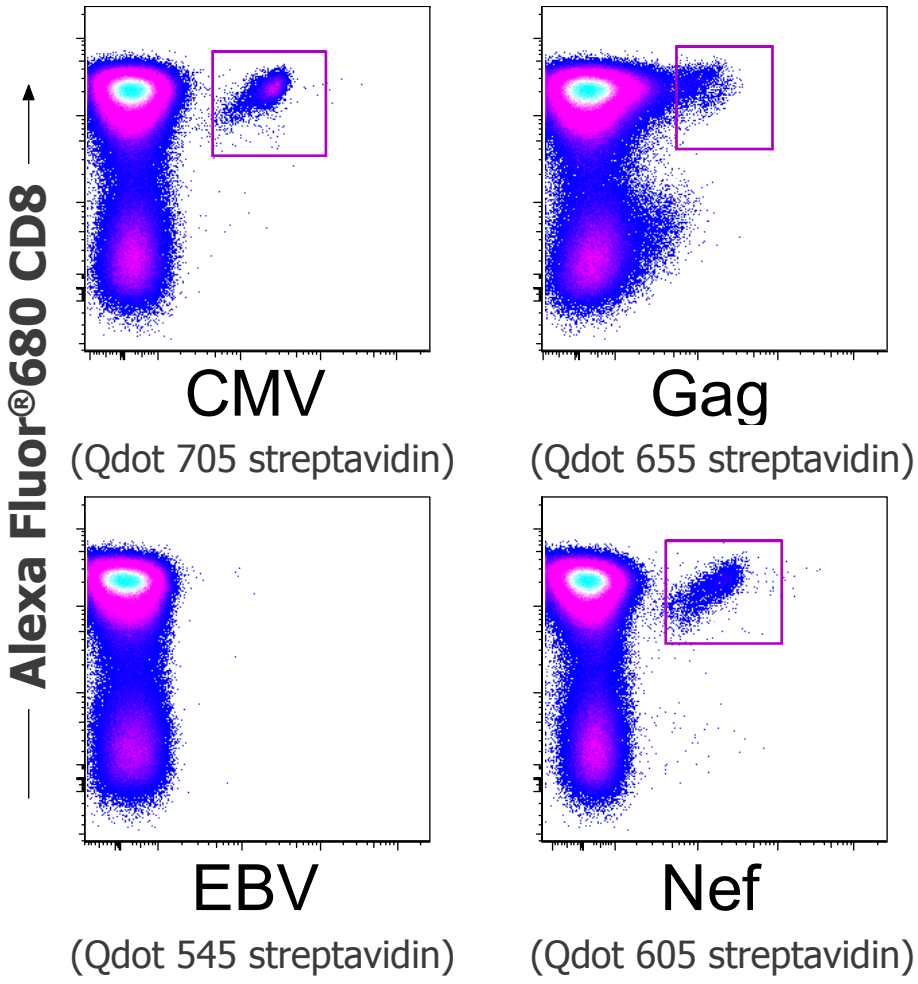
17 colors with manageable compensation

Other dyes don't cross-over into Qdot Conjugate channels

Qdot Conjugates don't cross-over into other dye channels substantially (in spite of broad excitation)

Perfetto, S.P., Chattopadhyay, P. K., Roederer, M.; Nature Reviews Immunology; V.4 (2004); 648-655.

4 Qdot Tetramer Reagents



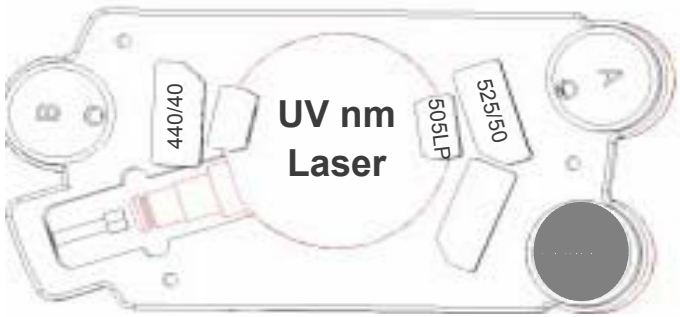
More information per sample possible

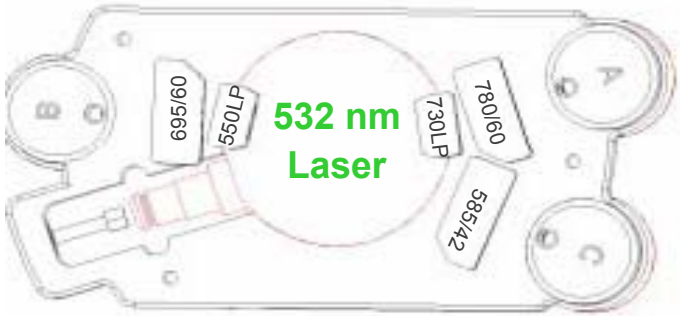
Phenotyping & Activation in a single sample context

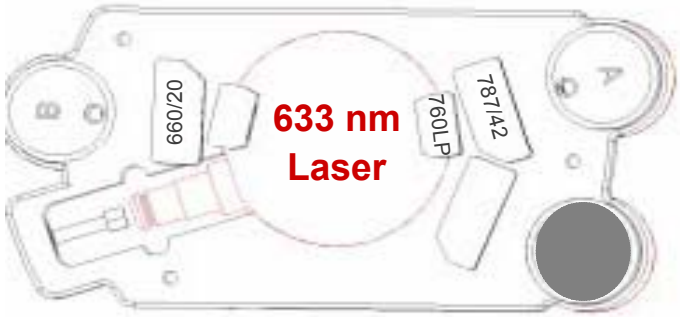
Streptavidin Conjugates used with Biotin-MHC-Peptide complexes to elucidate T-cell specificity

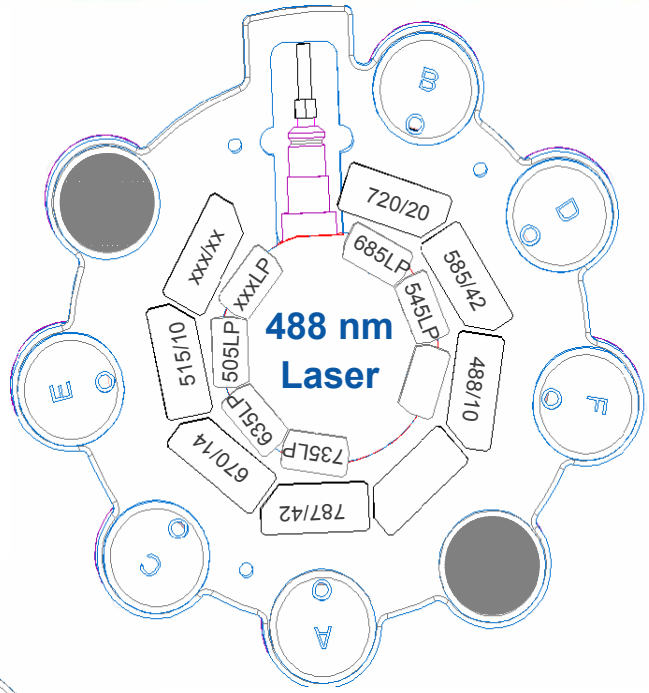
Data courtesy Stephen DeRosa (FHCRC) and Mario Roederer (NIH-VRC)

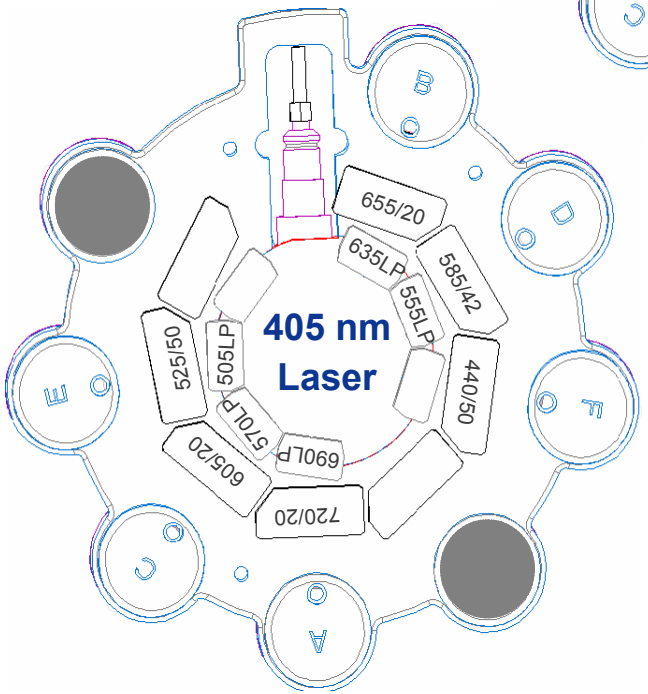
LSR II: Trigon / Octagon Configurations



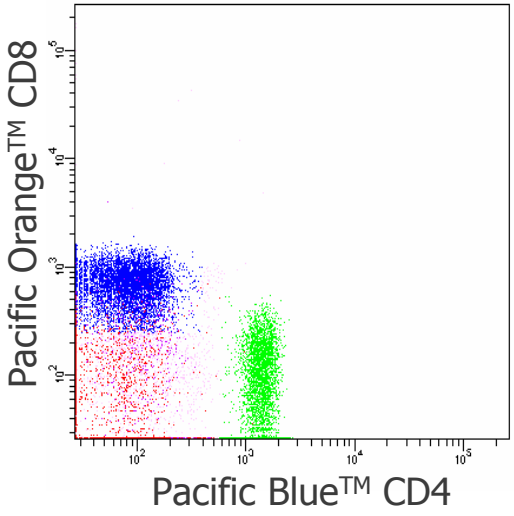


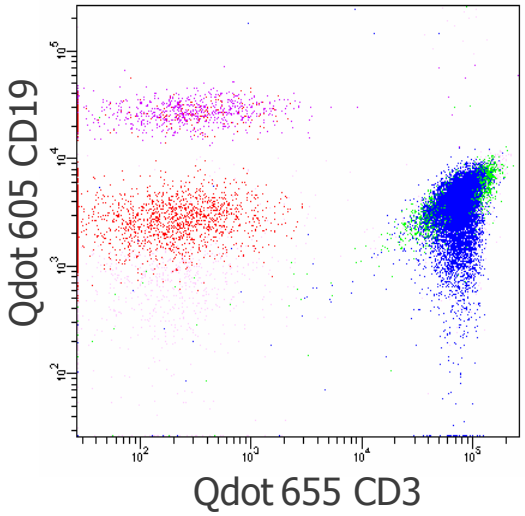






5-Color Stain: Violet Excitation



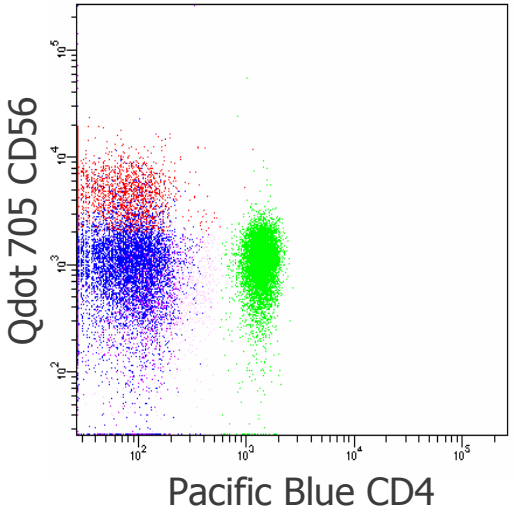


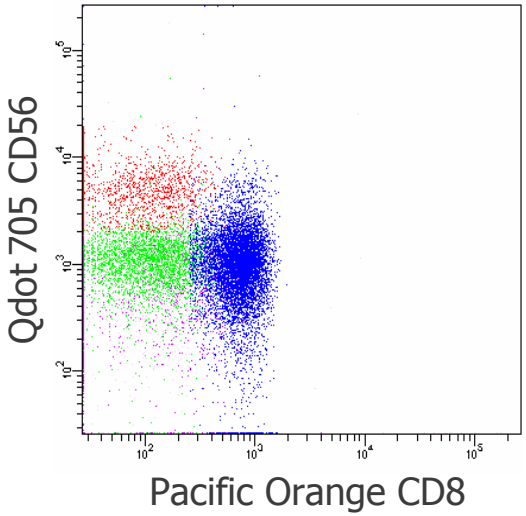
CD3-b + Qdot 655 SA

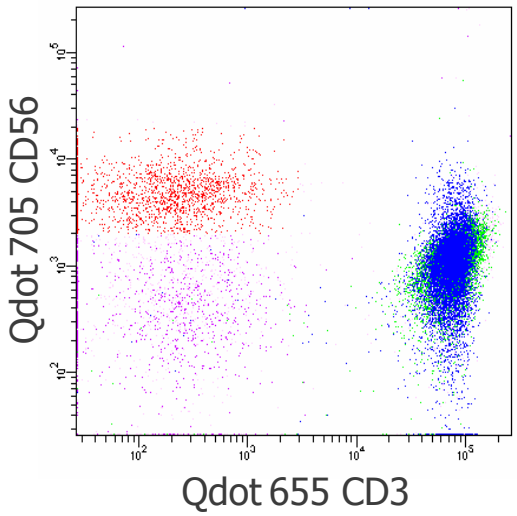
CD56-b + Qdot 705 SA

CD19-b + Qdot 605 SA

Pacific Orange CD8 / Pacific Blue CD4



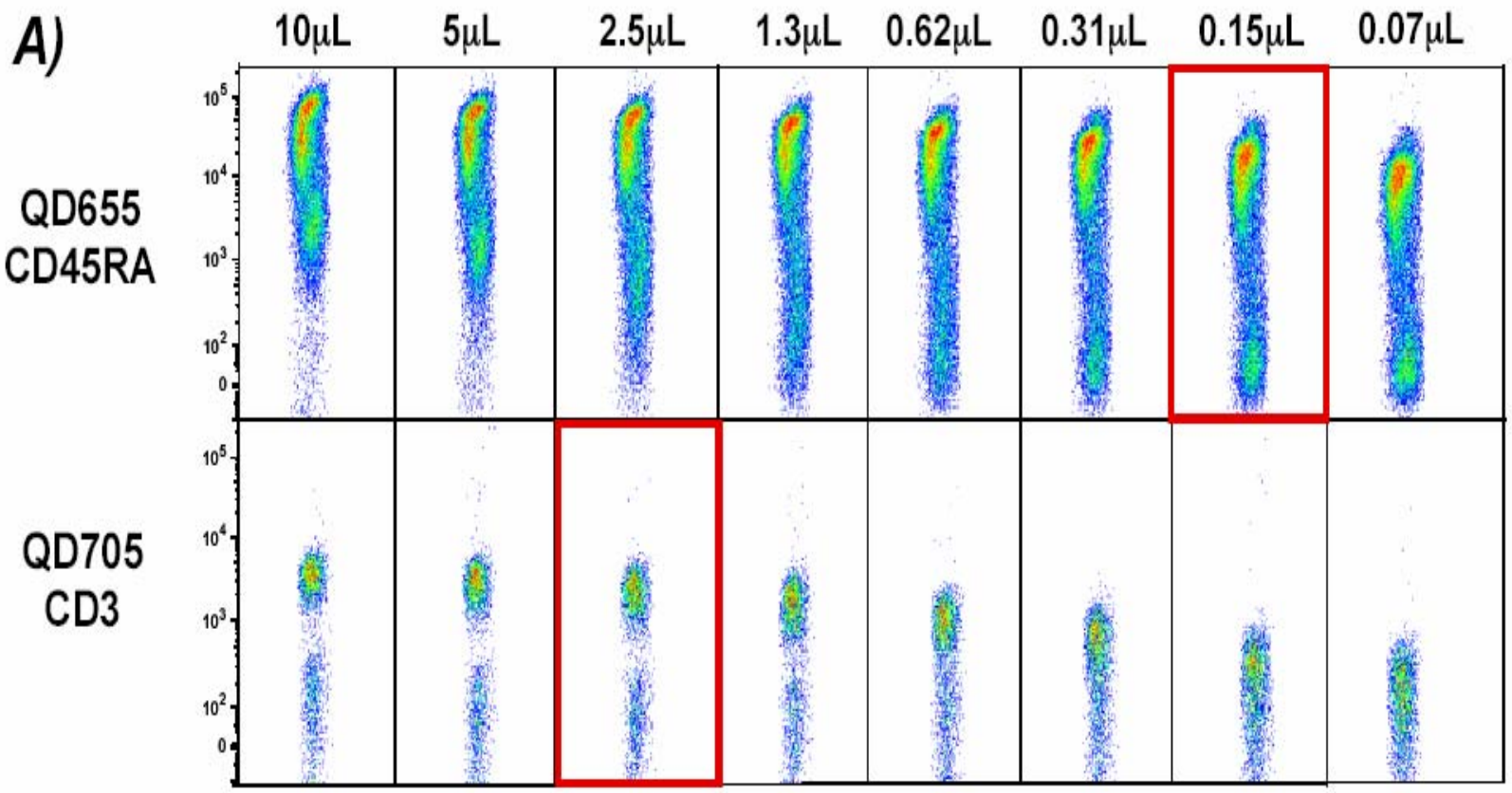




Summary

- Current Qdot nanocrystals are comparable to best organic dyes in intensity.
- Narrow emission spectra of the Qdot nanocrystals result in very low cross-talk.
- Selected Qdot reagents exhibit minimal spectral overlap
- Qdot reagents can be multiplexed for multicolor analysis in single- and multi-laser systems
- Current solutions available:
  - Streptavidin and anti-immunoglobulin conjugates
  - Conjugation kits
  - Reactive Qdot nanocrystals
  - Custom conjugation services

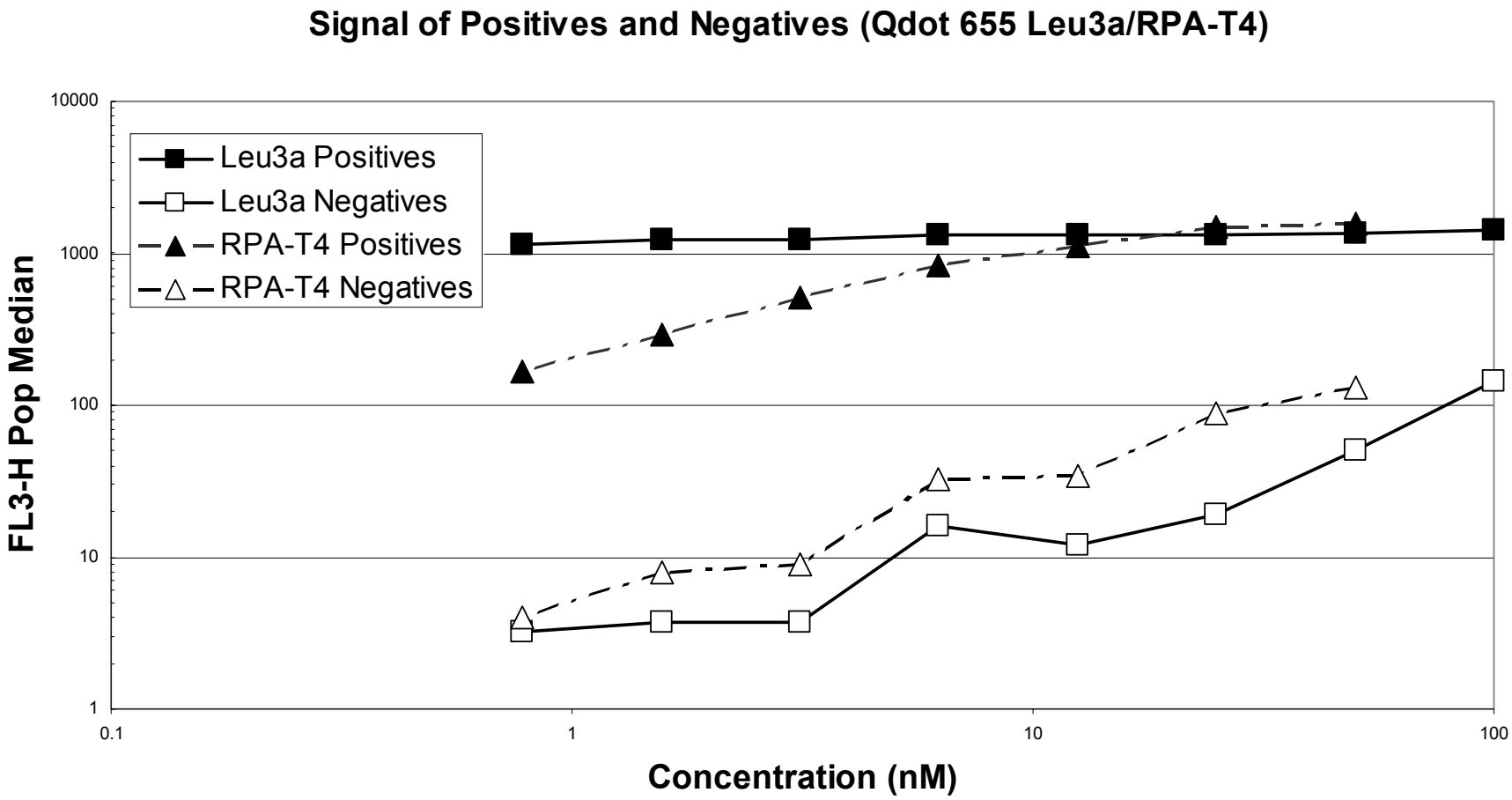
Direct Conjugates Give Excellent Results



Chattopadyhay, et al. Methods in Molecular Biology (in press)

At the right titration level…

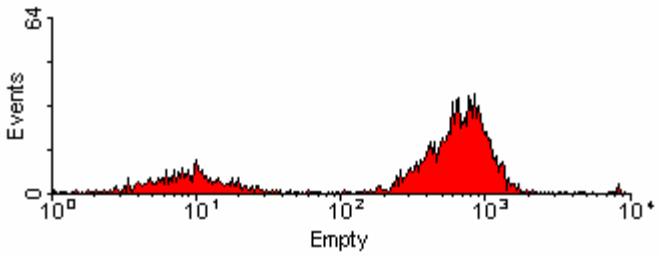
Importance of Optimal Titration and Clone



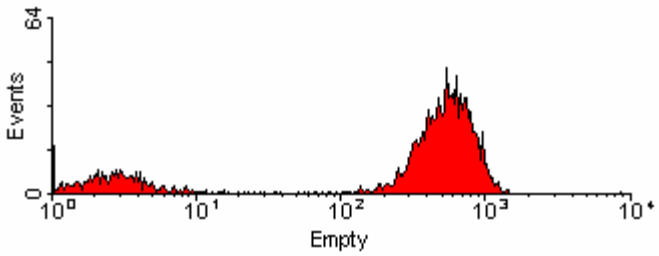
PBMC stained with Qdot 655 Leu3a/RPA-T4 Conjugate
Washed 2x with HBSS. 100 ul staining with 10^6 cells.
High affinity clones give high quality products.

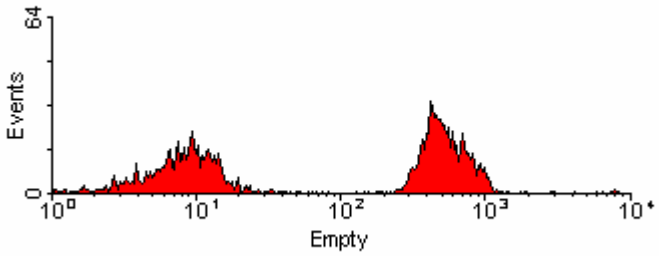
Direct Antibody Conjugate Comparison

Qdot 655 vs PE-Cy5 Antibody Conjugates

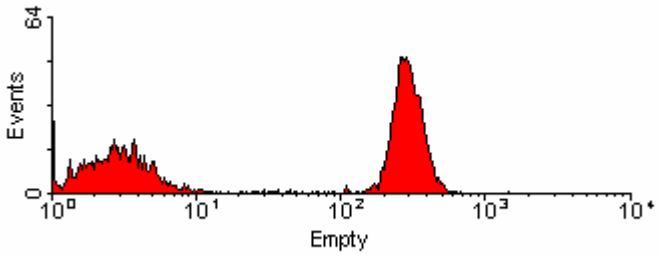


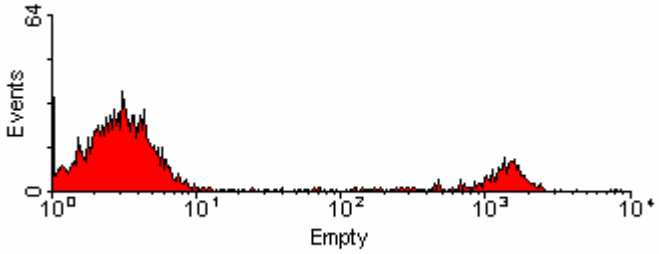
CD3



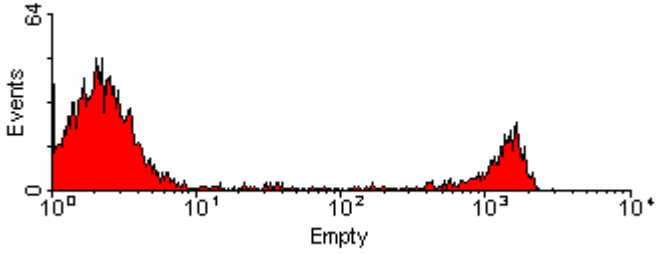


CD4





CD8



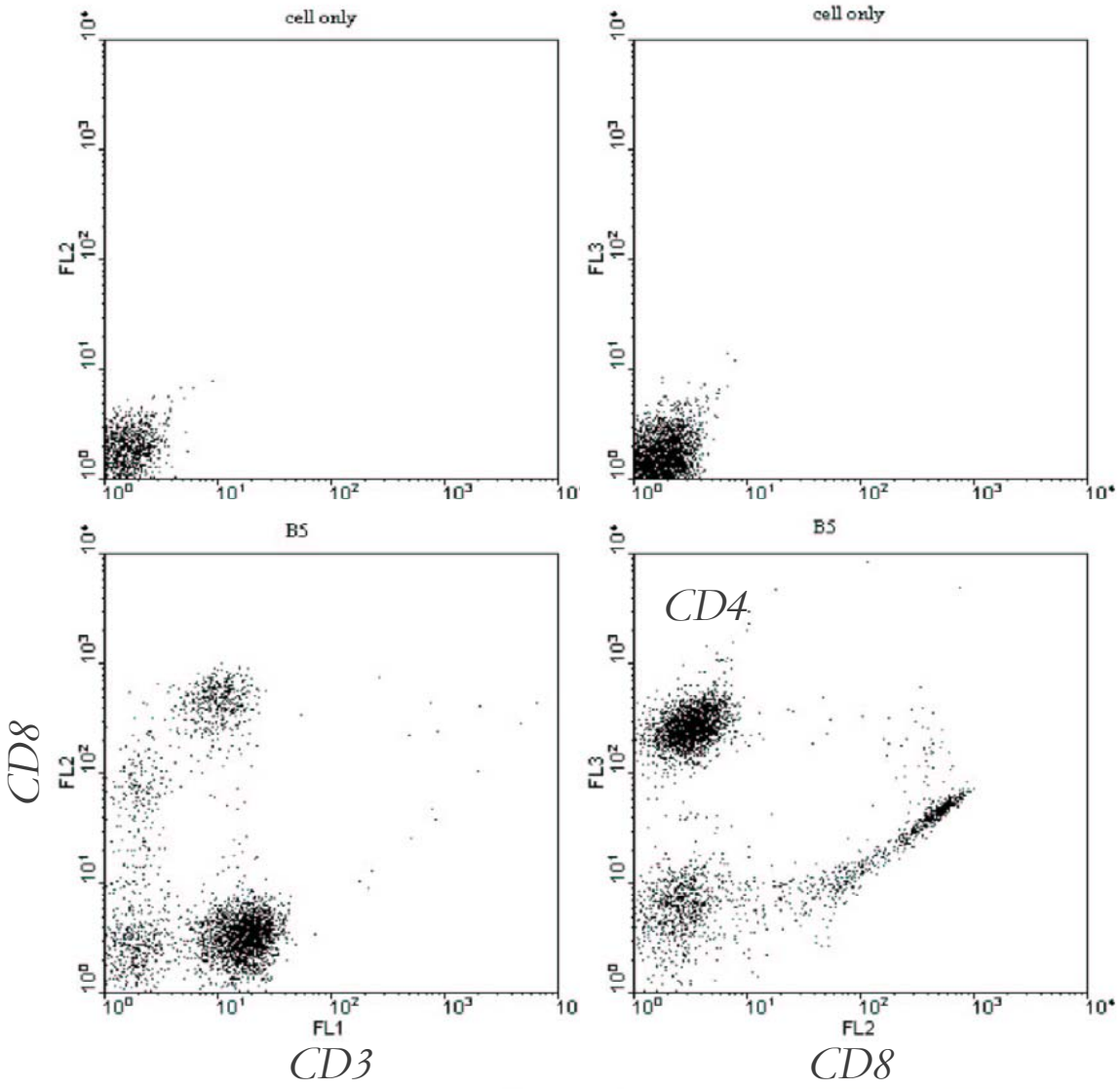
Qdot 655 Antibody Conjugate

PE-Cy5 Antibody Conjugate

Current (488 based) and Future Performance

	Intensity	*Crosstalk*	*Potential Crosstalk*
655	Equal to PE-Cy5	0.2% into FL2 *1% PE-Cy5*	<1%
585	~1/2 PE signal	11 % into FL3 (620 LP) 52% PE	<1 % (on standard 650 LP FL3)
525	Equal to FITC	2% into FL2 14% A488 23% FITC	<1%

Single Laser, Multicolor Flow Without Compensation



Buffy coat stained
Direct Qdot Conjugate reagent cocktail
Lysed/Washed 2x

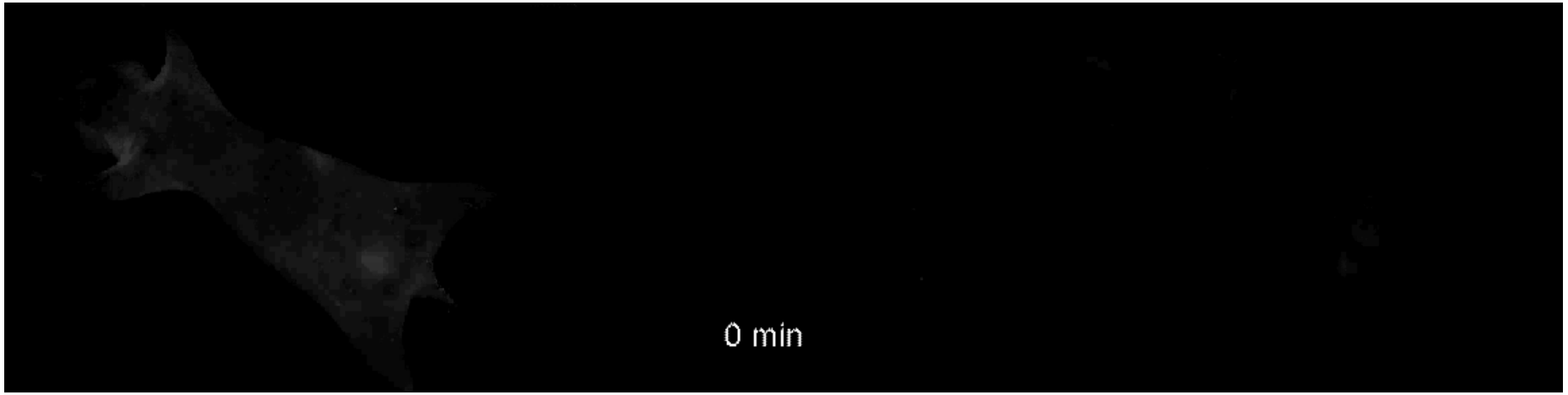
488 ex FACScan
530/30 FL1
585/42 FL2
620 LP FL3
UNCOMPENSATED

Stock 650 LP would have substantially lower FL2/3 cross-talk

Flow Summary

- Current Qdot materials are comparable to best dyes in intensity.
- The narrow emission spectra of the Qdot materials result in very low cross-talk.
- The Qdot channels off the violet laser are free of compensation.
- There are significant benefits to using Qdot materials in multicolor single-laser analysis.
- Current solutions available:
  - Conjugation Kits
  - Streptavidin Conjugates
  - Reactive Qdot Nanocrystals
  - Custom Conjugation Services

In vivo *Imaging*



Ballou, B. et al., *Bioconjugate Chemistry, 15(1)* **2004**.

Qdot nanocrystal materials can be imaged at scales from centimeters to nanometers.

Real-time Receptor Ligand Tracking

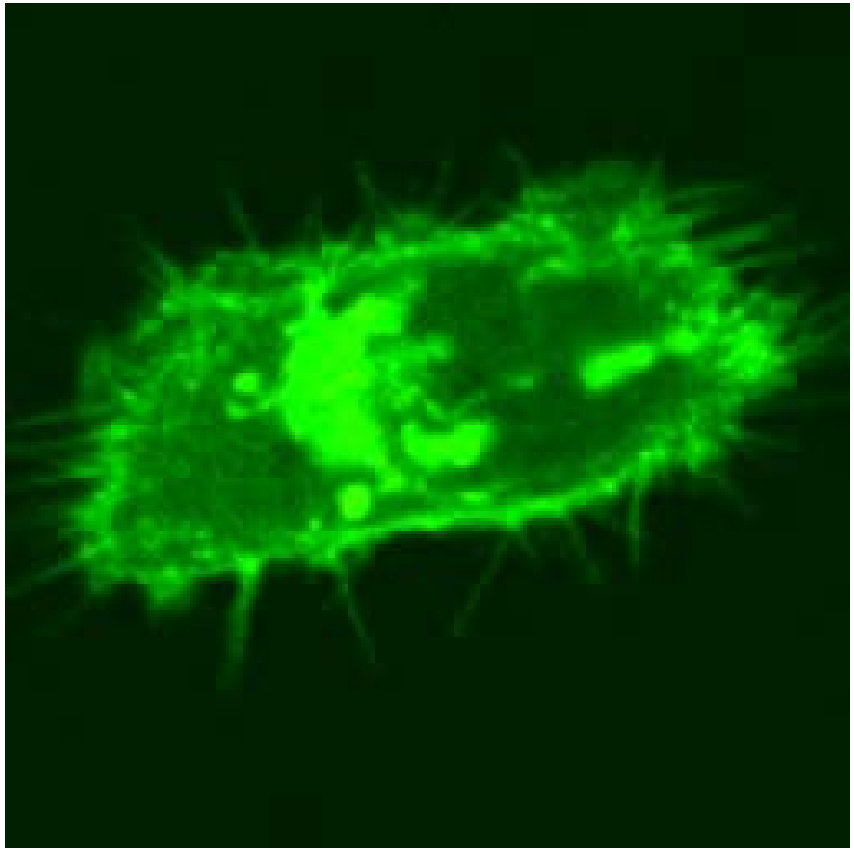
EGF Receptor Internalization

Qdot 605-EGF conjugate (erbB1)
erbB3-Citrine

- EGF-Qdot Conjugate co-internalizes with ErbB2
- Novel retrograde transport mechanism via filopodia
- EGFR homodimerizes with erbB2 but erbB3

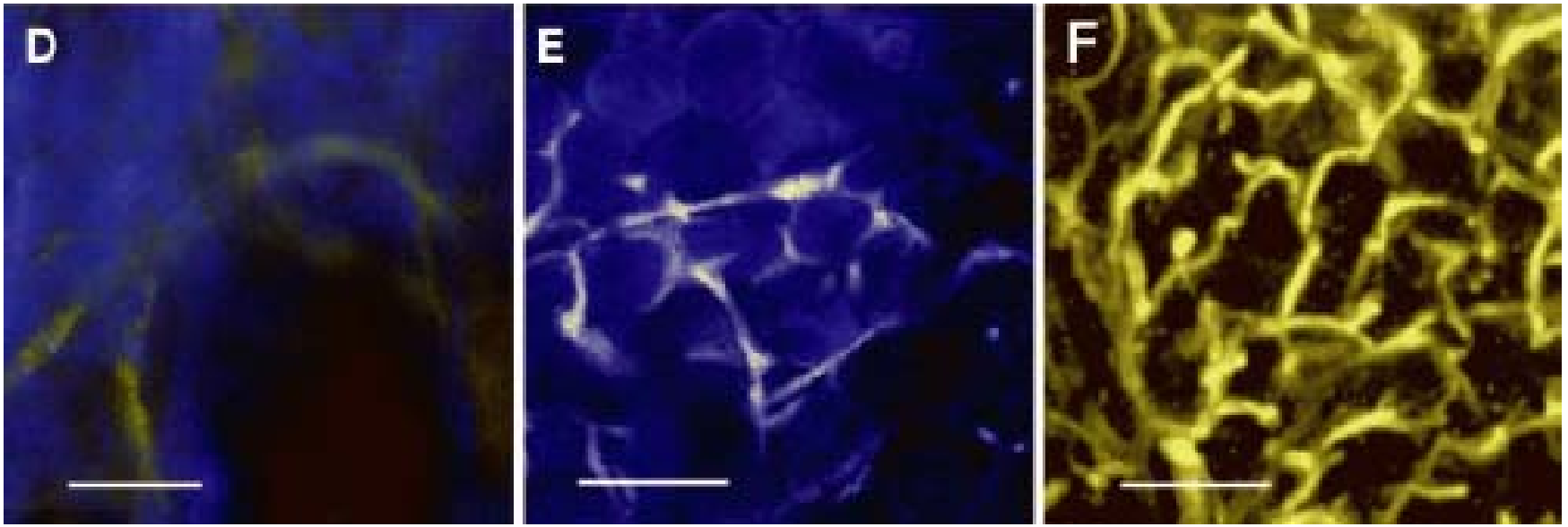
Photostability allows unprecedented real-time continuous monitoring of receptor-molecule (ligand, drug) dynamics

Lidke, D. e*t. al.* Nature Biotechnology 22 (20), 2004



4.5 sec/frame; 100 frames
Sequential confocal scans

Multiphoton Imaging –Vascular Imaging in Ovary



FITC - Dextran

Qdot ITK Carboxyl QD's
Single MP slice

Qdot ITK Carboxyl QD's
250 µm image stack

Larson, et.al., Science 300(5624) 2003.

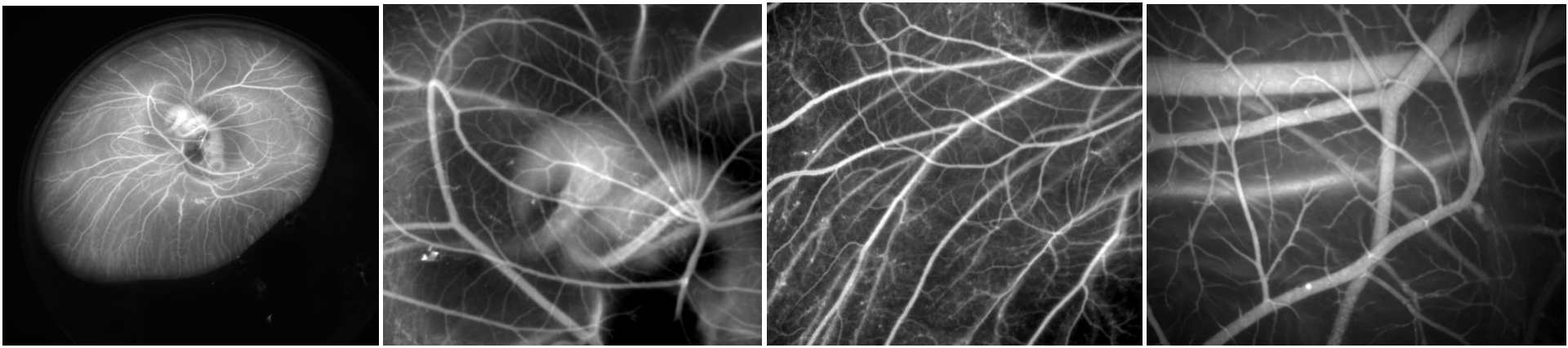
Bright
- Very high S/B
- Excellent discrimination from auto-fluorescence
- Fine structural and dynamic information can be obtained

Noninvasive
- Imaging through skin
- High contrast angiography

Stable
- No toxicity observed after venous injection

In-vivo Vascular Imaging

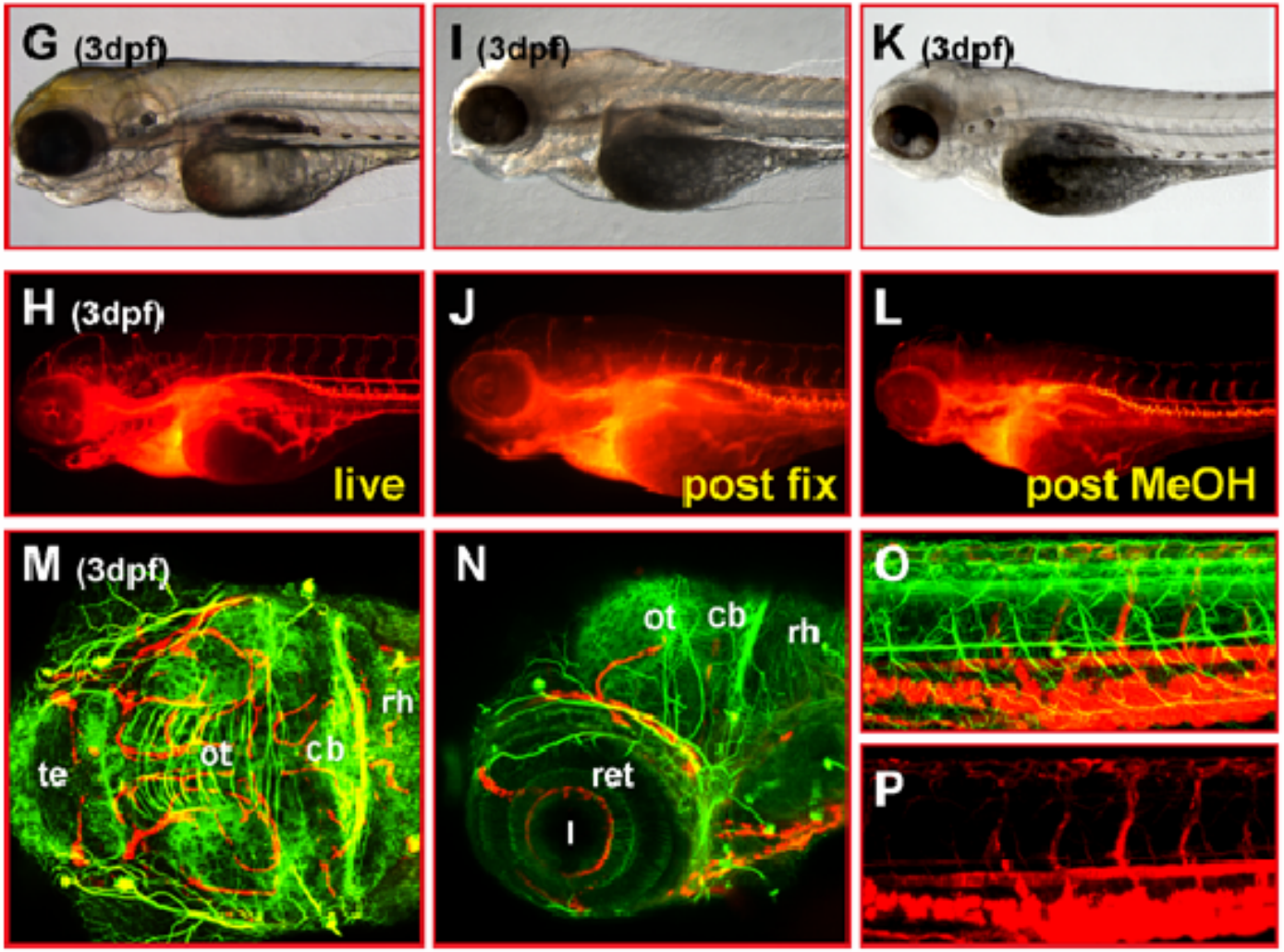
- Chick embryo venous injection at increasing resolution
- Bright signal allows highly detailed vascular analysis
- Red colors allow deeper, higher resolution imaging than dyes



Courtesy of Greg Fisher, Byron Ballou and Alan Waggoner, Carnegie Mellon University

- Long circulation time allows detailed vascular imaging.
- Also useful for marking vascular structures in tissue sections.

Zebrafish with Qdot Conjugates



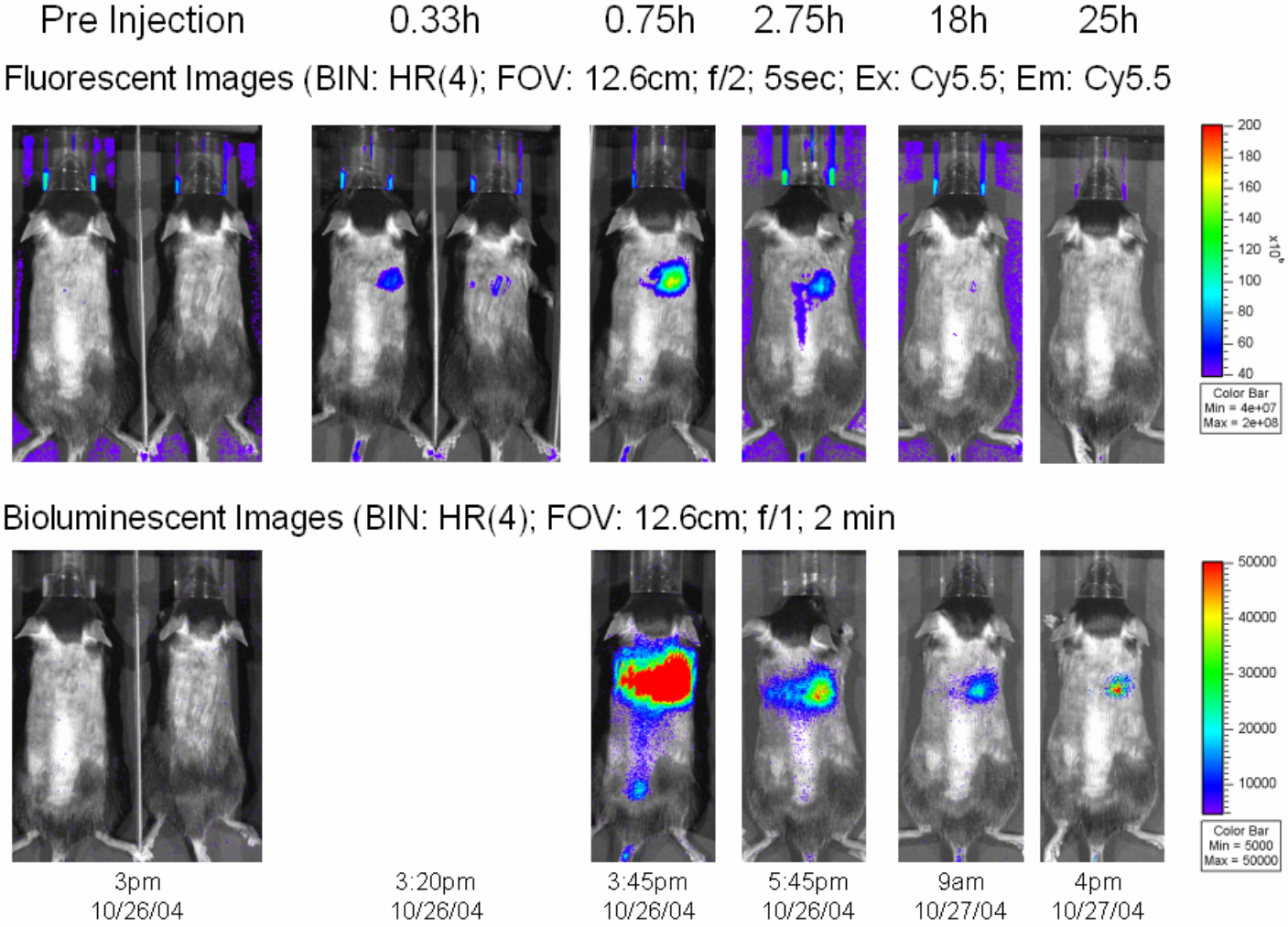
Whole Mount
IHC from in-vivo

Acetylated Tubulin
axons
Qdot 605 SAv Conj.
vascular

Rieger, et al. Dev Dyn 18, 2005.

Tracking cells by fluorescence and luminescence

Dorsal Images



C57B1/6 Mice

B16F10 luc cells Loaded with Qtracker 705 Cell Labeling Kit

$2x10^6$ cells injected via tail vein

Cell fate monitored for 24 hours.

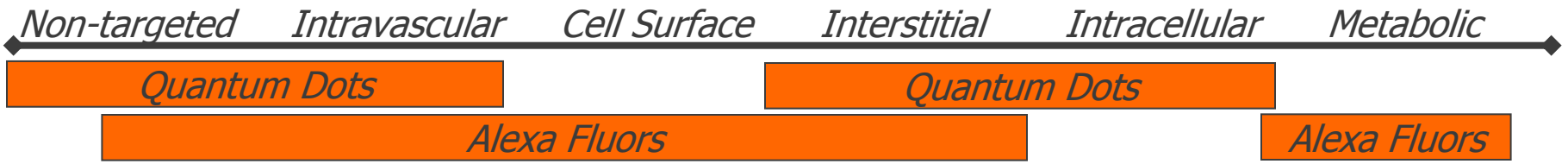
Fluorescence tracks bioluminescence results until colony formation.

Xenogen IVIS Expt Courtesy Steve Smith

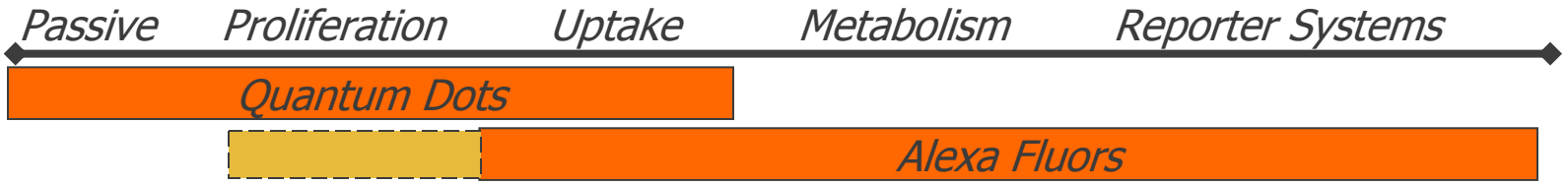
Results like bioluminescence without transformed cells…

In-vivo imaging across the continuum

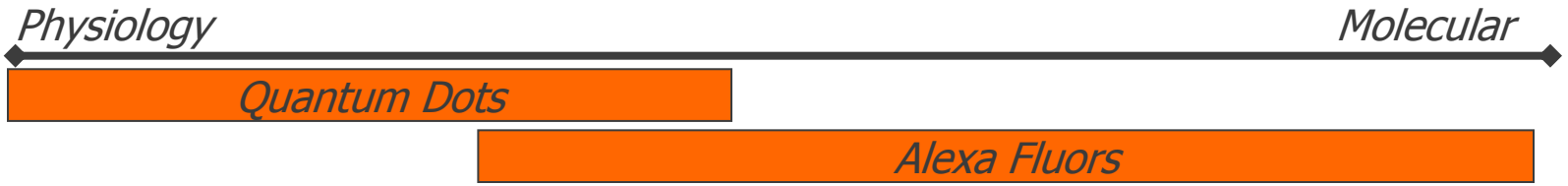
Molecular Targeting



Cellular Tracking



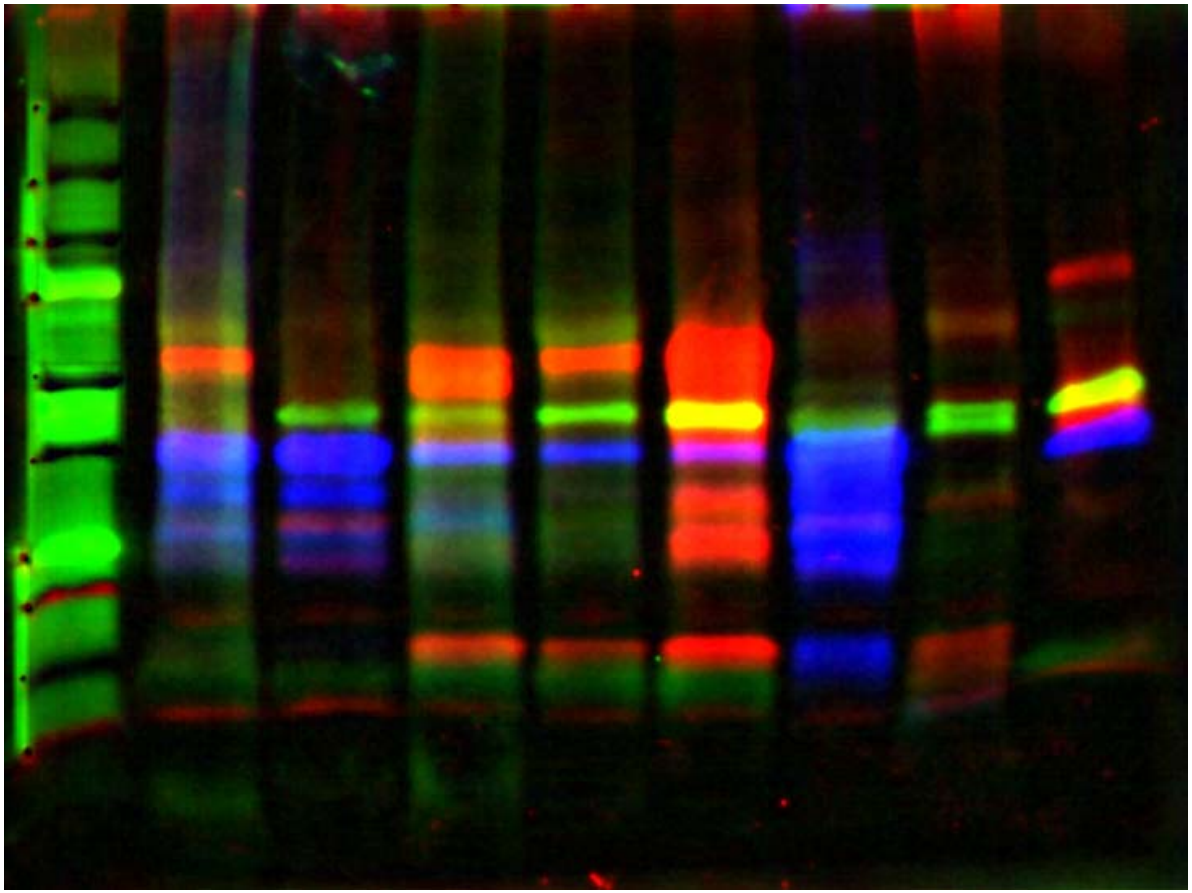
Post-mortem analysis



Live Cell and Animal Imaging

- Image noninvasively, then follow up at higher resolution
- Compatible with GFP imaging
- Imaging at greater depth and resolution
- Infrared materials can be imaged through skin and other tissues effectively
- Repeated imaging without repeated dosing.
- Longitudinal imaging from in-vivo to intravital to post-mortem to electron microscopy.

Qdot Western Blotting products



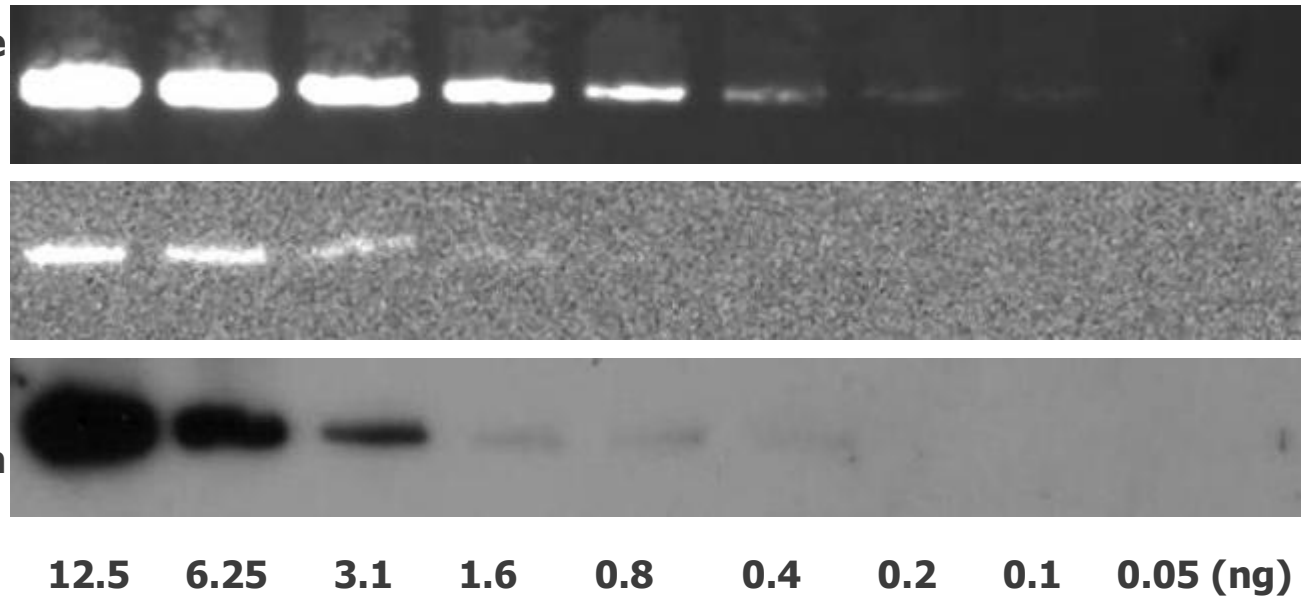
Actin
Vimentin
GAPDH

Rat Tissue
Lysate Blot

Western Analysis with Qdot Conjugates

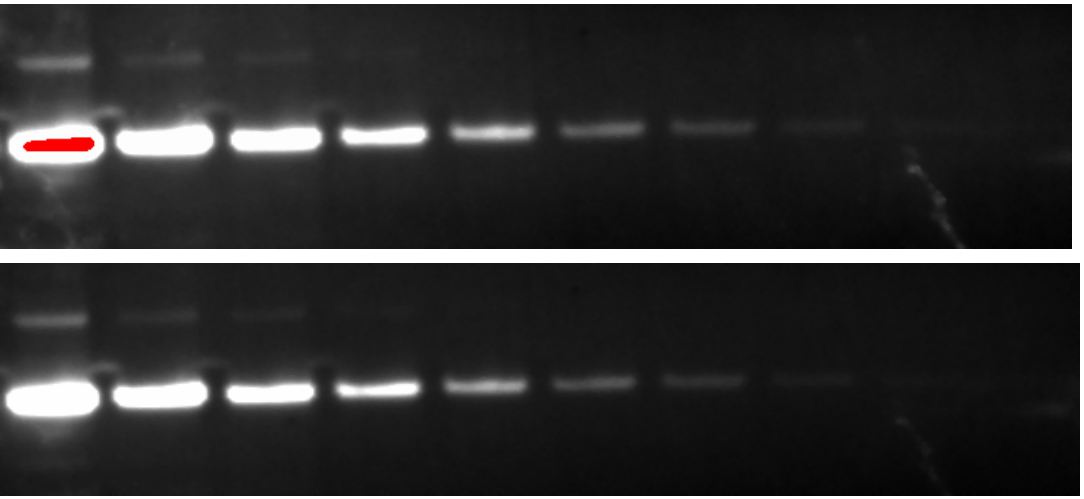
- Sensitivities comparable or better than reported chemiluminescence levels.
- NO FILM, NO DARKROOM, NO RUSH.
- Simple multicolor detection without dedicated instrument
  - Chemiluminescence imaging systems
  - Gel Documentation systems
  - Trans-illuminator and color camera with anti-haze filter
- No stripping and reprobing required
  - Faster experiments
  - More reliable data
- More reliable quantitative analysis
  - 2-3 orders of magnitude linear dynamic range with single exposure
  - Extended exposures extend dynamic range to 4-5 orders of magnitude

More Sensitive than the "Gold Standard" ECL



- Purified protein dilution series with identical antibodies
- Qdot Conjugates deliver sensitivity dramatically higher than ECL Reagents even under the optimal film-based detection.
- Combination with Millipore Immobilon-FL Transfer Membrane provides highest sensitivity.

Qdot Western Blots Can be Stored for Months



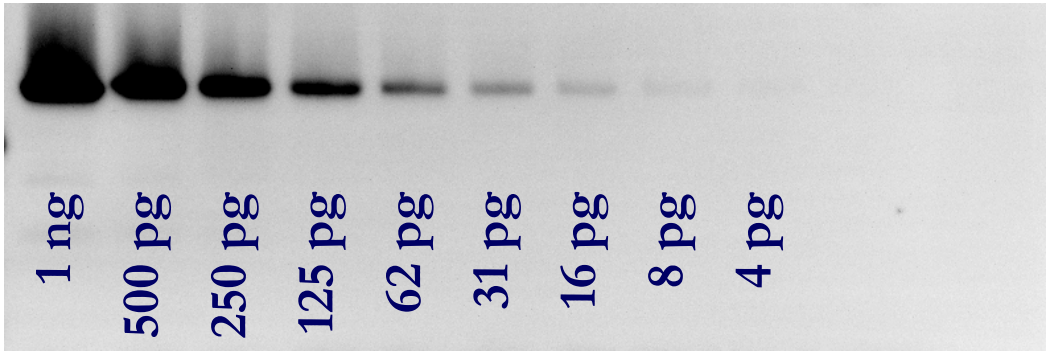
Day 2: 40 pg detection

3 months later: 150 pg detection
Stored in TBS

- Stored in TBS, the Qdot Western Blots retain signal for months.
- Stored dry, they may retain signal even longer.
- Plenty of time to get imaging conditions optimized for publication.

Sensitive Quantitative Western Analysis

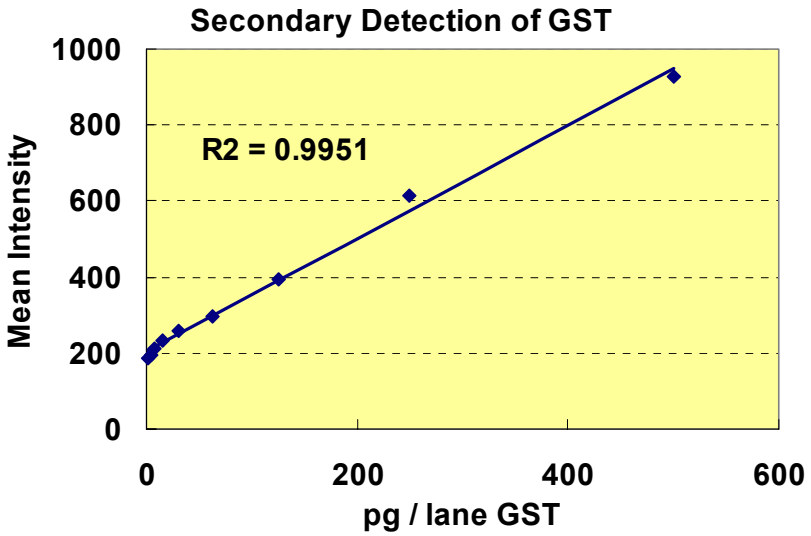
Pure GST—Goat anti-GST—Qdot® 655 anti-Goat Conjugate



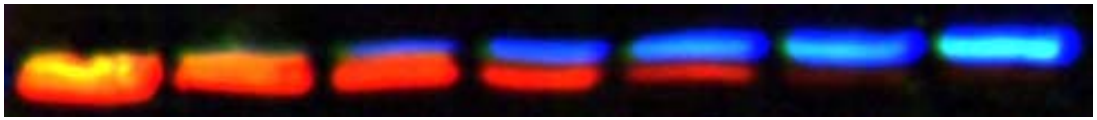
- Detection limit 4-8 pg GST
- Fluorescence detection with chemiluminescent sensitivity
- Stability for repeated analysis

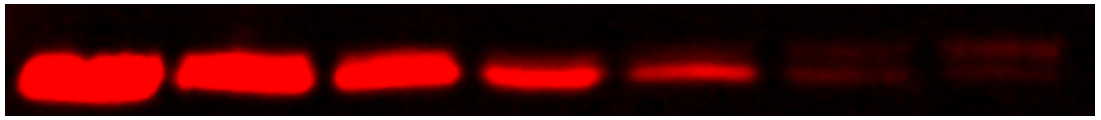
Images acquired with a KODAK Image Station 2000MM Multimodal Imaging System

- Broad linear range
- Progressive exposures extend range
- 100 fold linear range for each exposure

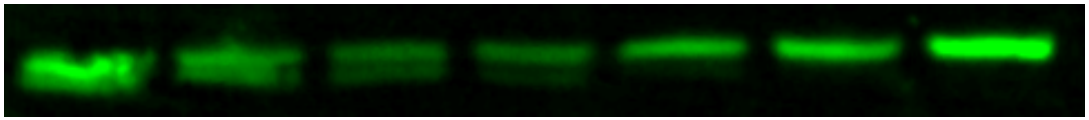


Useful in Protein Expression and Purification

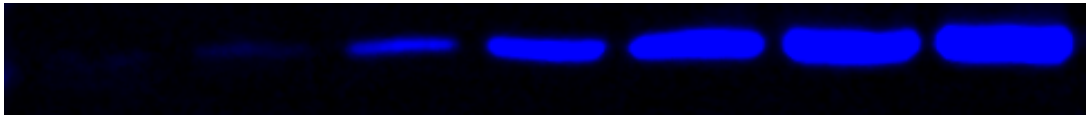




HA detected with a primary antibody followed with Qdot® 605 anti-Rabbit conjugate.



GST detected with Qdot® 565 anti-GST conjugate



c-Myc detected with a primary antibody followed with Qdot® 705 anti-Mouse conjugate.

- Multicolor analysis allows simultaneous measurement of protein and fusion domains.
- Single blot analysis eliminates questions of band alignment between multiple blots.
- Qdot conjugates allow 3 or 4 color analysis of overlapping bands with simple filters.

Simple, Fast and Economical

ECL on Film

Transfer (1 hr)
|
Block (1 hr)
|
Primary (1 hr)
|
Rinse (0.25 hr)
|
Secondary (1 hr)
|
Rinse (0.25 hr)
|
Substrate, Film, Develop (1 hr)
|
Substrate, Film, Develop (1 hr)
|
Substrate, Film, Develop (1 hr)

Qdot Conjugate

Transfer (1 hr)
|
Block (1 hr)
|
Primary (1 hr)
|
Rinse (0.25 hr)
|
Secondary (1 hr)
|
Rinse and Image 3x
(0.25 hr)

Save hours in the darkroom with Qdot Western Blotting detection

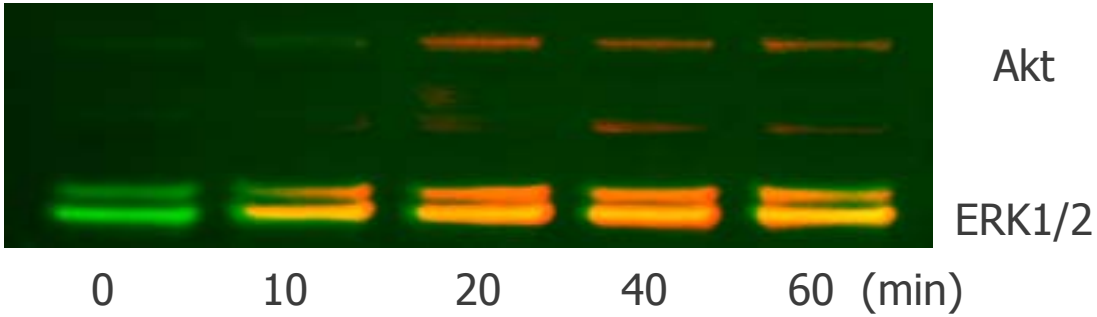
Simple, Fast and Cost Effective

Detection Method	Product Number	Antibody Dilution	Cost per blot
ECL Advance	RPN2138 (GE-Amersham)	1:25000	$35.45
ECL Plus	RPN2132 (GE-Amersham)	1:5000	$37.05
ECL	RPN2108 (GE-Amersham)	1:5000	$31.05
Qdot Conjugate	1100-2	1:1000	$28.03
Qdot Conjugate	1100-2	1:2000	$19.28

Phosphoryation of Akt and Erk in A431 Cells Stimulated by EGF (25ng/mL)
This is a typical cell-signalling model system.

phospho Akt
phospho ERK 1/2 } **Qdot 705**

pan Akt
pan ERK 1/2 } **Qdot 605**



Ornberg, et al., Nature Methods 2(1) 2005, 79-81

- Color value indicates ratio of modified to total protein
- This is a typical class of experiment in cell-signalling research.
- Analysis of multiple bands allows single experiment with rich content.
- The alternative experiment would take several days with standard methods.

Qdot Western Blotting Benefits

- Simple
  - Fluorescence imaged directly, no substrate addition
  - No dependence on time (CL) or photostability issues (dyes)
  - Robust, stable signals allow repeated imaging
  - Imaging can be done on gel imagers, CCD imagers, and laser scanners—very flexible
- Quantitative
  - Linear quantitative range over 2.5 orders of magnitude
  - Stable signal ensures reliable measurements
  - Sensitivity as good as best reported chemiluminescence methods
- Multiplexed
  - No stripping and reprobing to detect multiple bands
  - Single source excitation with emission filters ensures reliable signal ratios

Conclusions

- Qdot® Conjugates provide substantial benefits in detection
  - Ultimate in photostability
  - Sensitivity rivals or exceeds the best methods
  - Multiplexing capability dramatically simplified
  - Wide variety of available products ensures application needs are met

- Distinct product lines appropriate for many applications
  - Microscopy
  - Flow Cytometry
  - Live cell/live animal imaging
  - Western Blotting
  - Immunoassays

- Reactive Qdot nanocrystal materials available for your chemistry (Innovator's Tool Kit Quantum Dots)
  - Organic
  - Carboxyl
  - Amino(PEG)

Independent Accolades

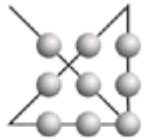
Science magazine's Top 10 Scientific Breakthroughs of 2003.
"[Quantum dot bio-imaging is]…the most exciting new technique to emerge from the collaboration of physicists and biologists."

Science

Forbes/Wolfe Nanotech Report's Top 5 Breakthroughs of 2003. Number 1: In vivo labeling with quantum dots

Forbes/Wolfe Nanotech Report

LARTA Nano Republic Conference 2003. "Most promising innovation" award.



Larta

Small Times magazine's 2003 Researcher of the Year. Quantum Dot founder Paul Alivisatos.

smalltimes
BIG NEWS IN SMALL TECH.

2004 Fortune Cool Companies winner.

2004 FORTUNE® cool companies

Publications—Invitrogen Materials Are the Standard

- ~230 peer reviewed publications (Biological Apps since 1998)
  - 92 used QDC Materials (2003-2005)
    - Pathology—Fluorescence/EM/FISH
    - Live Cell Microscopy (dynamics)
    - Single Molecule Analysis
    - FRET
    - Arrays
    - Microfluidics/Patterning
    - Pathogen Detection
    - And many more
  - 2 used competitor materials (reporting quenching)
  - Others used self-fabricated materials (great ideas)
  - Large number of review articles

- Quantum Dot—Invitrogen Nanocrystal Technologies materials ensure consistency, support, and optimal performance every time.

Collaborators

• Mark Ellisman	NCMIR-UCSD
• Alan Waggoner	Carnegie Mellon University
• Paul Wylie	TTP Labtech
• Watt Webb	Cornell University
• Mario Roederer	NIH-Vaccine Research Center