Visualization of Kidney Dynamics

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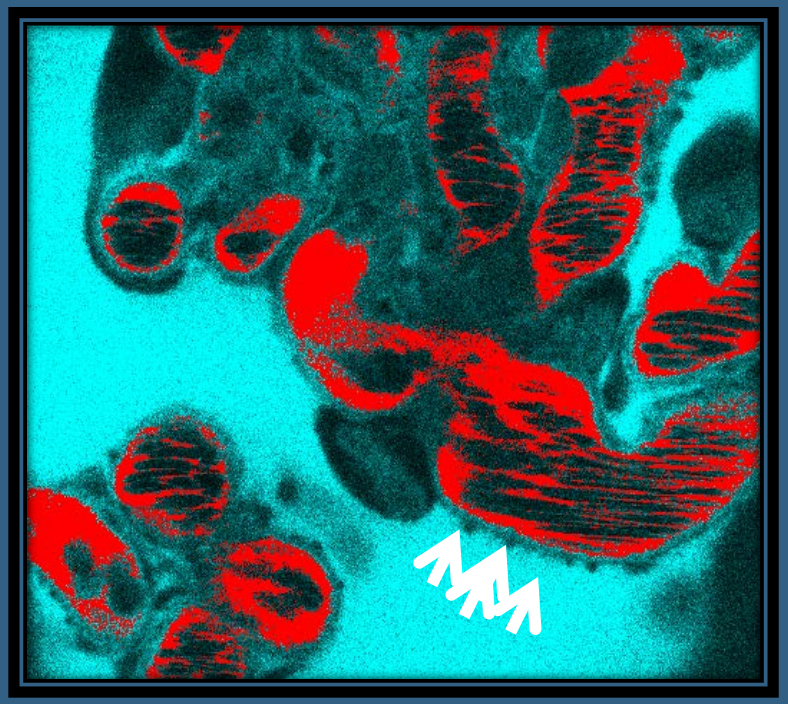
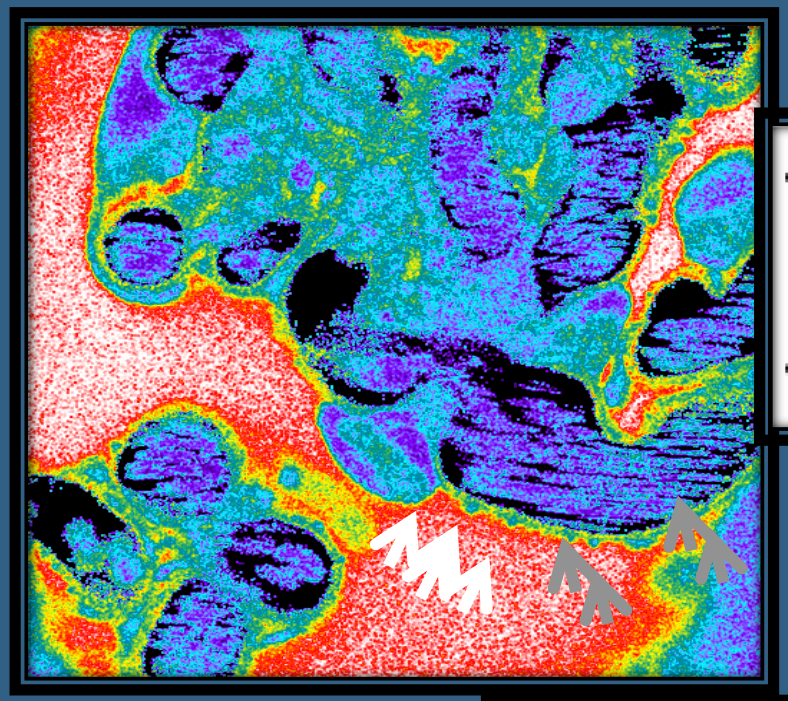
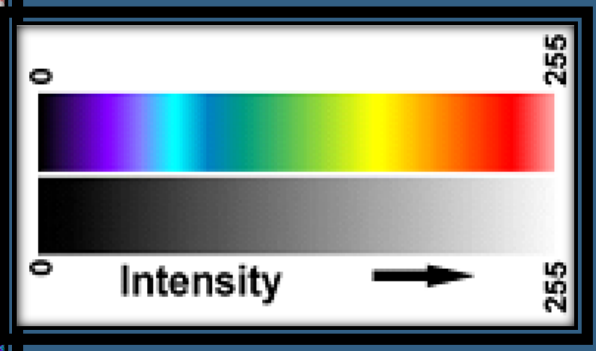
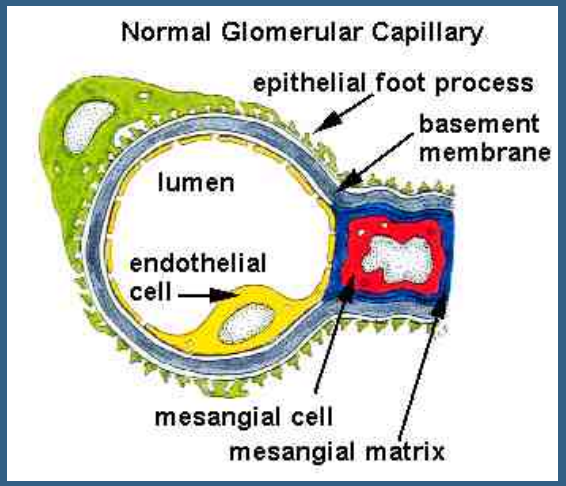
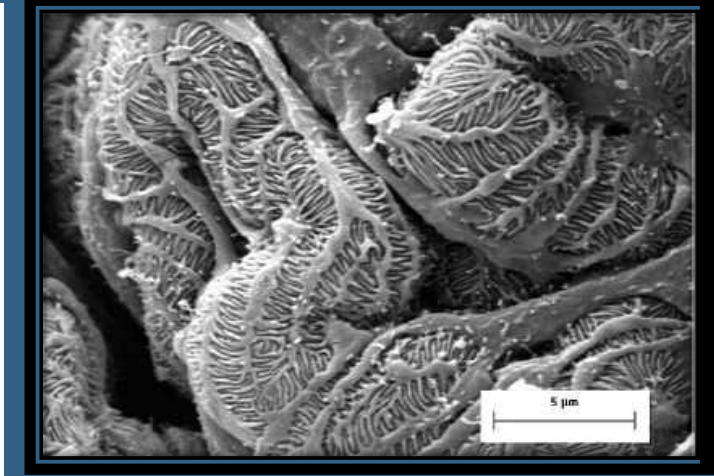
Visualizing Filtration the Basement Membrane & Podocyte

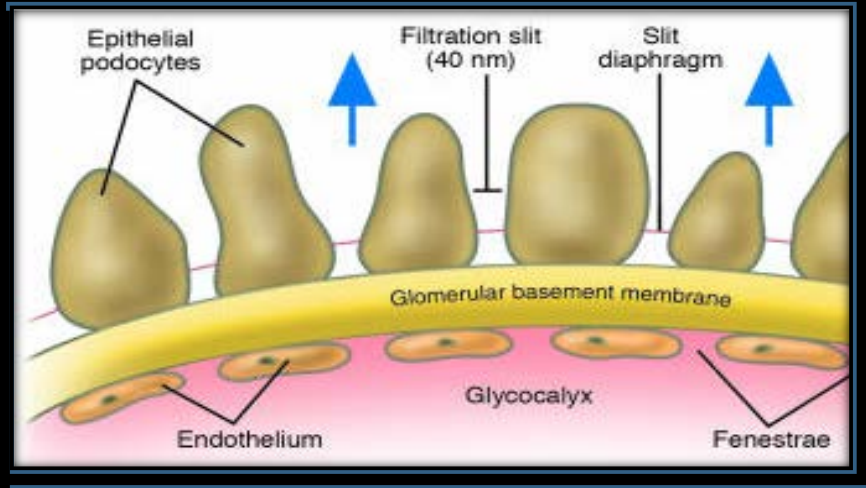




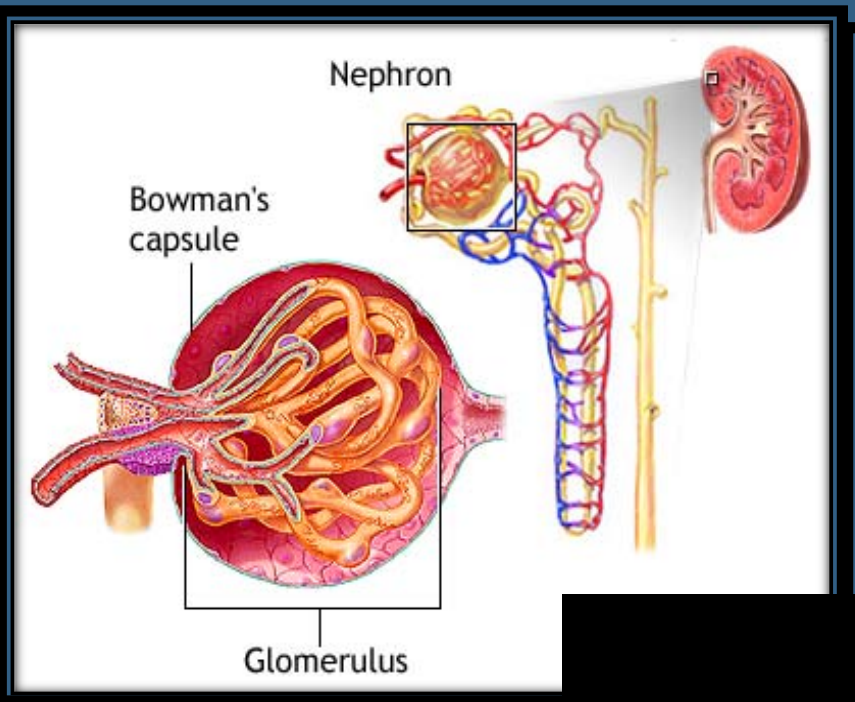


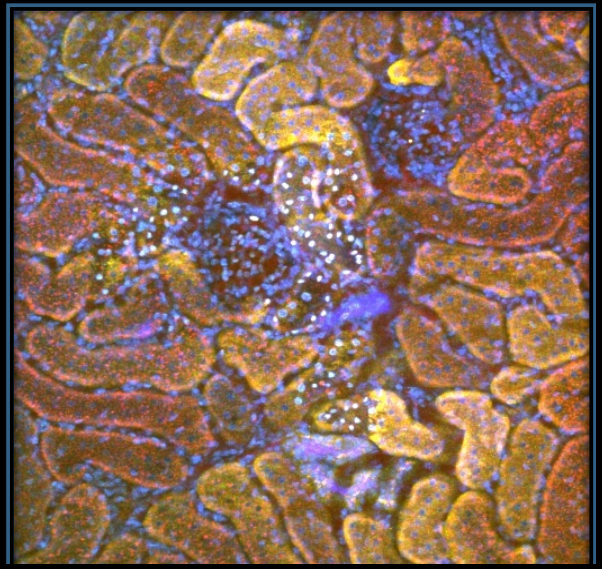


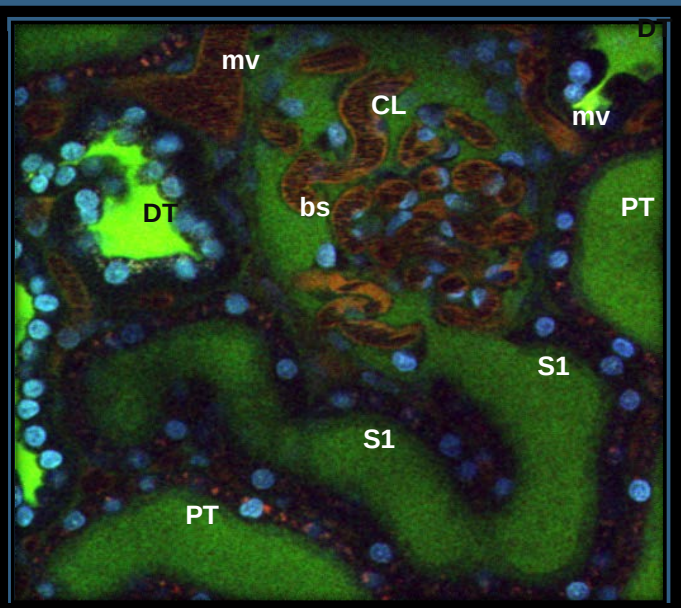


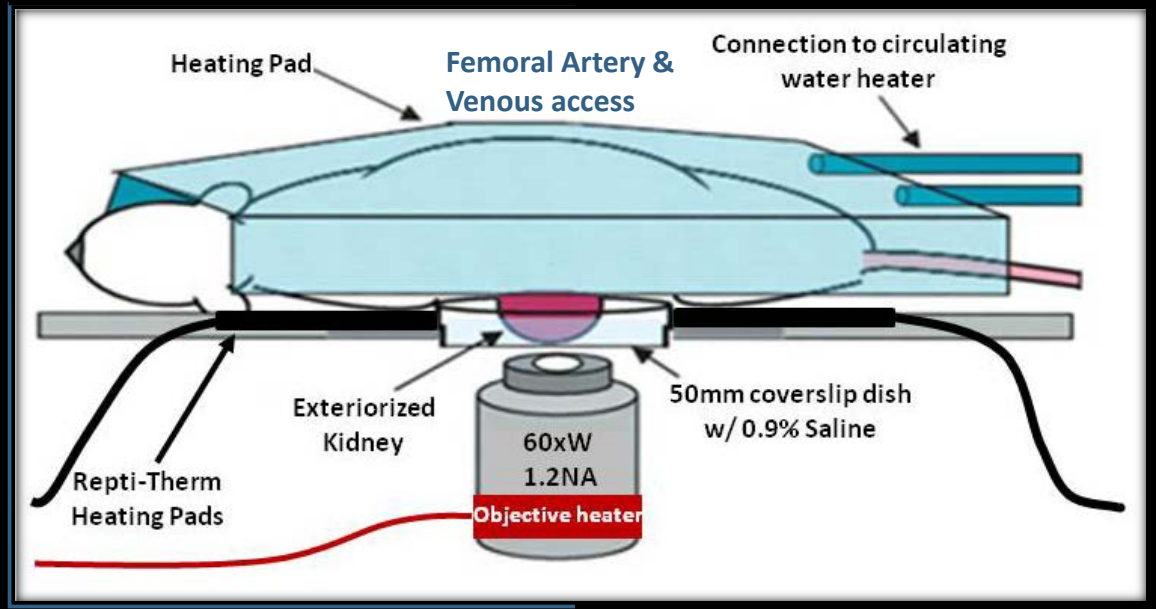


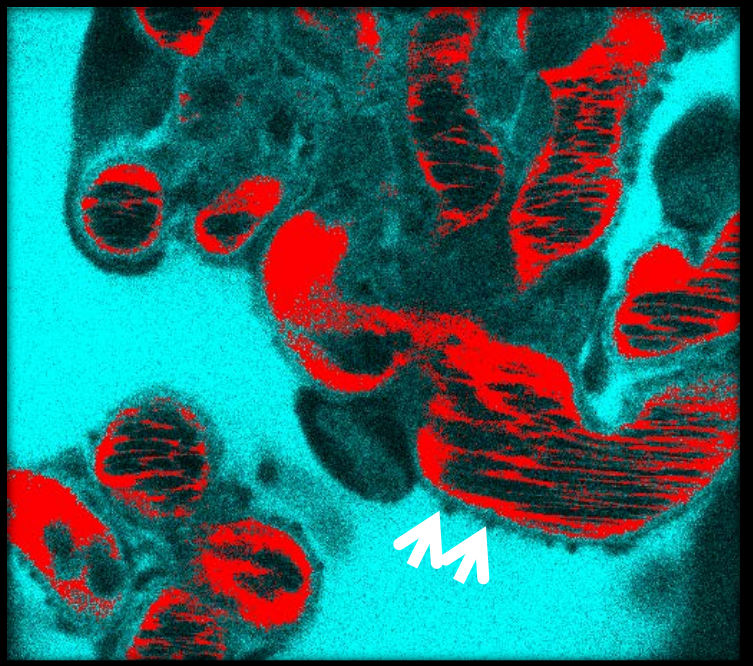
Visualizing Glomerular Function





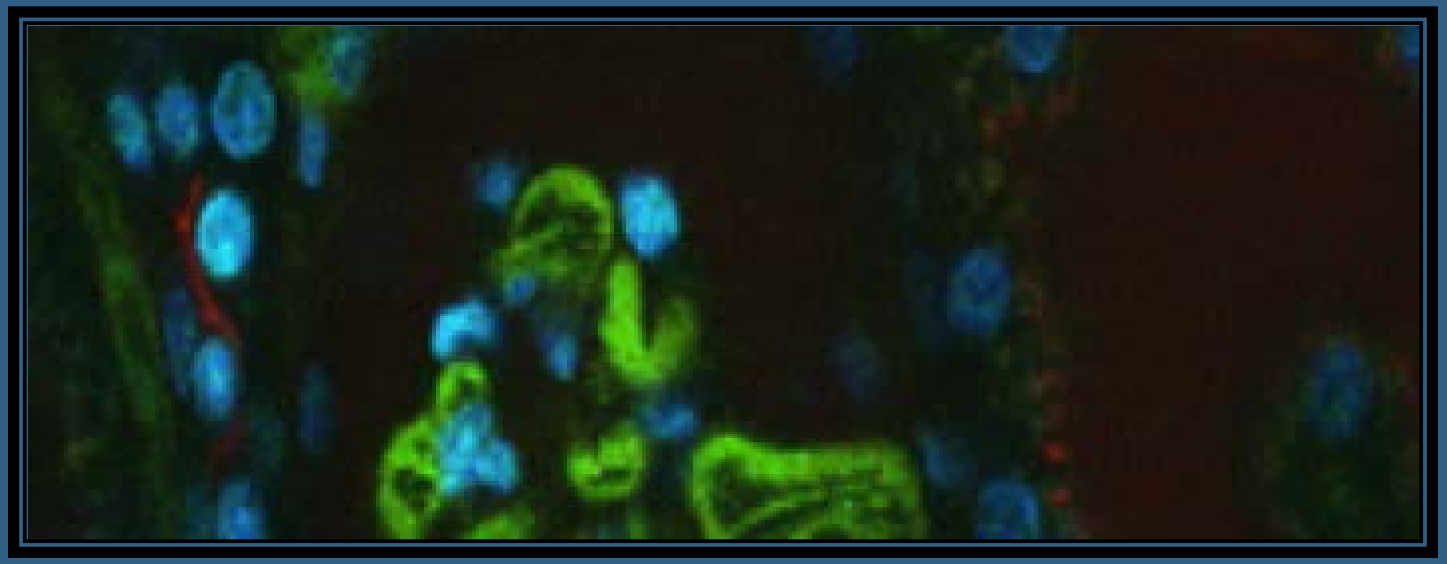


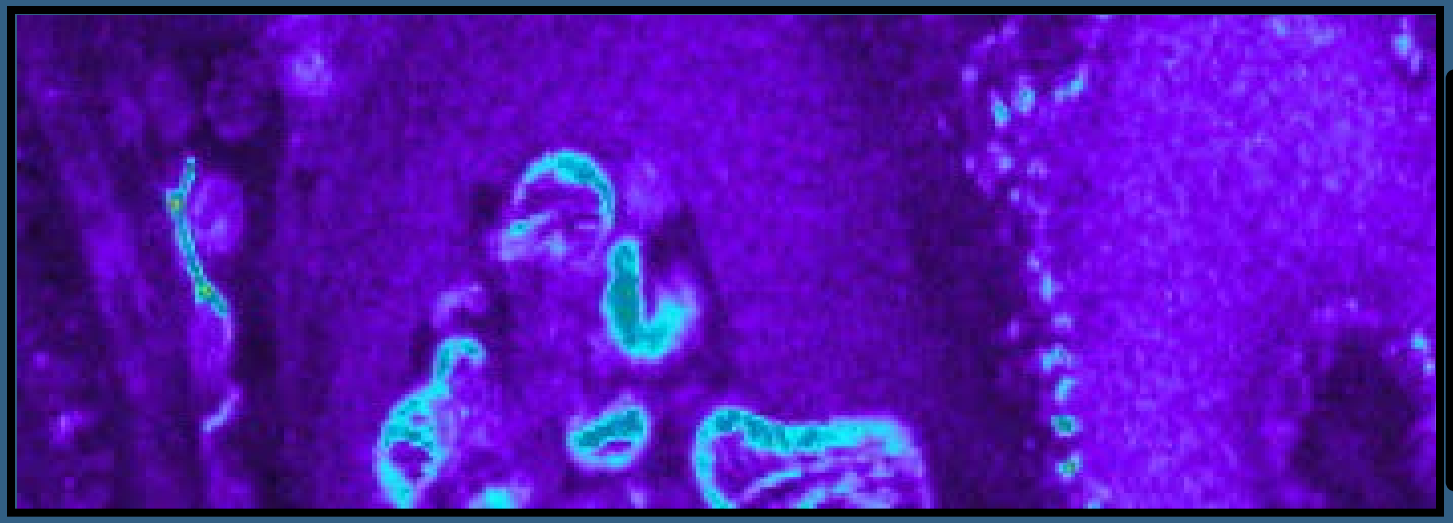


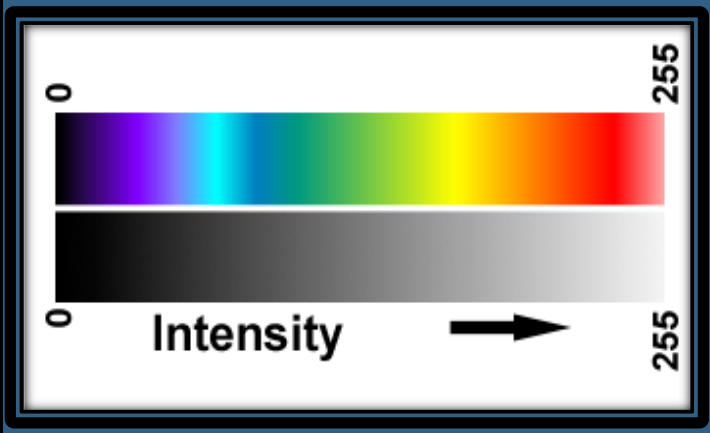


Reducing Scan Size

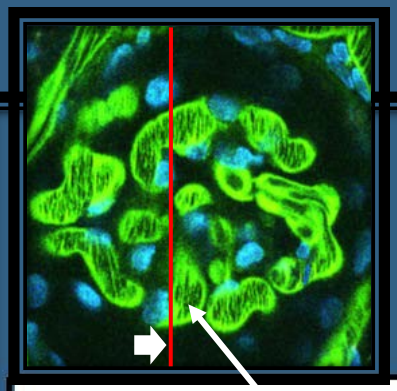
5 Frames/sec
500kDa FITC Dextran with 3kDa TR Dextran Injection

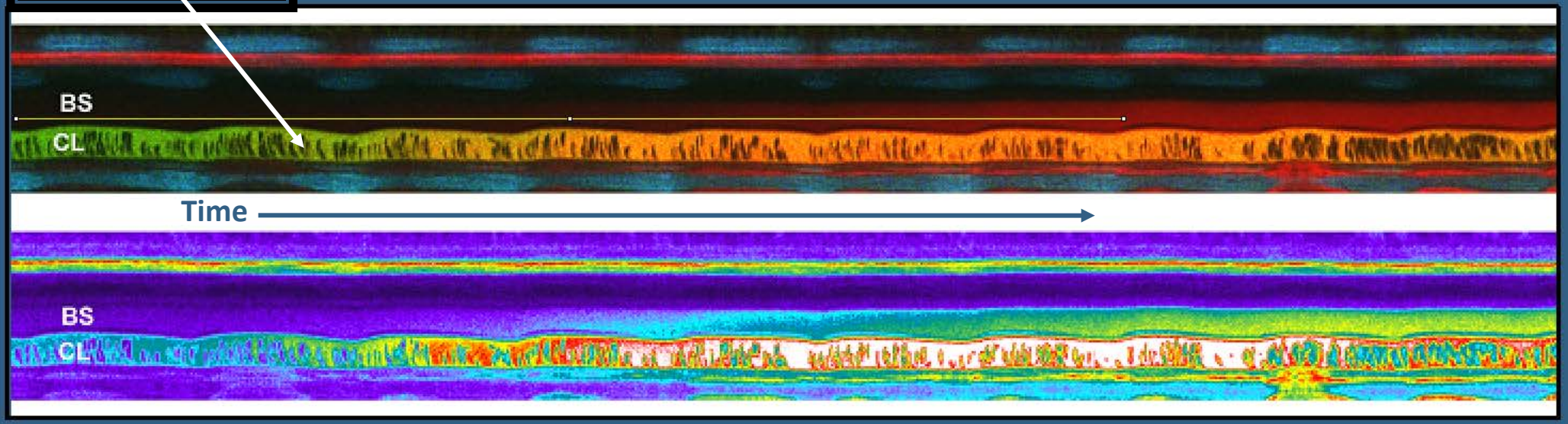


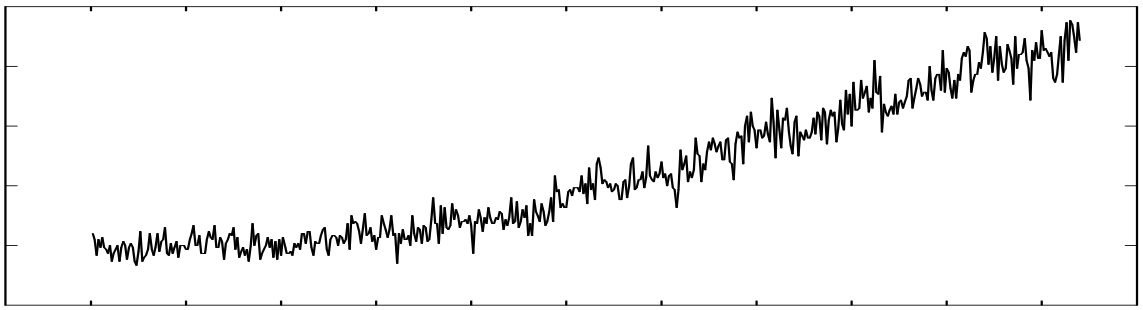


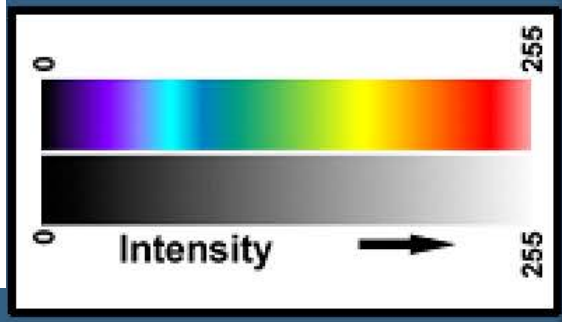


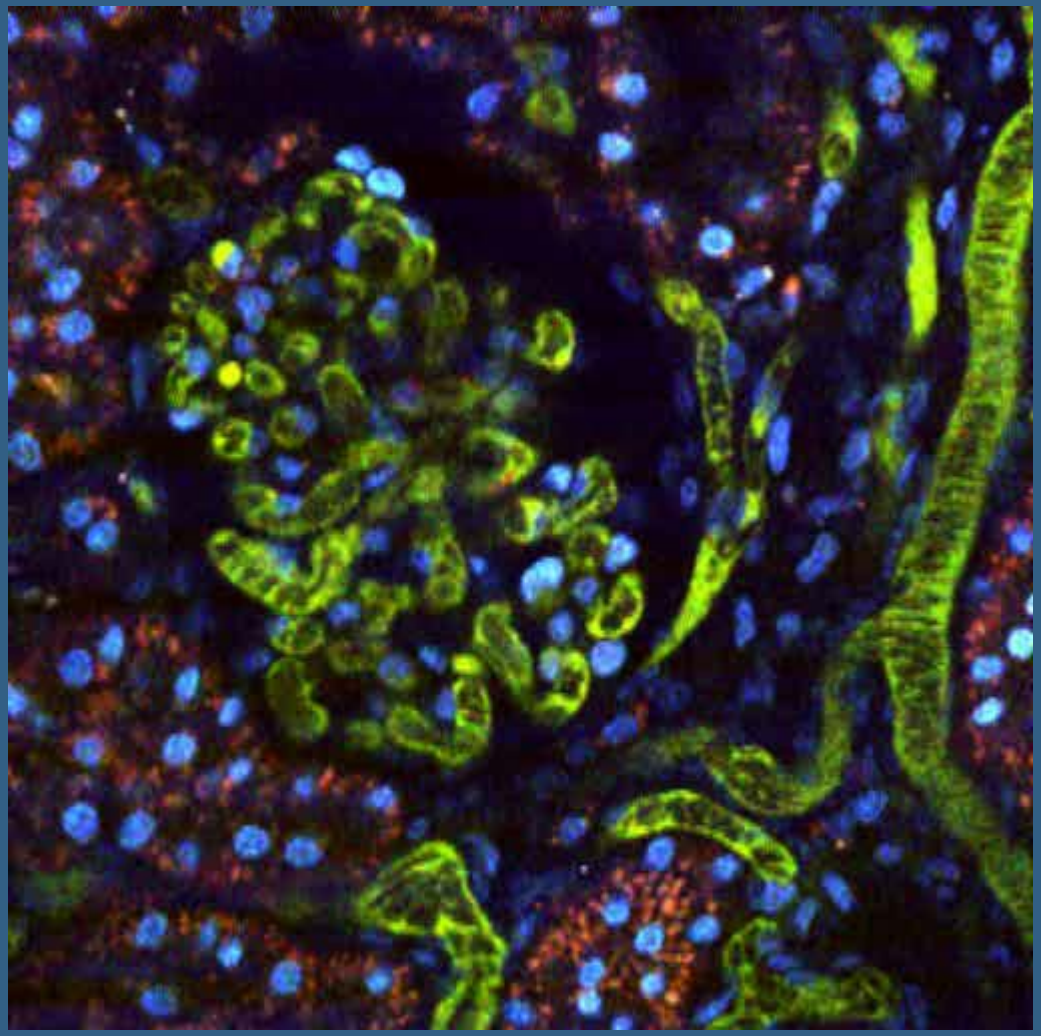
Line Scan Analysis of Glomerular Filtration







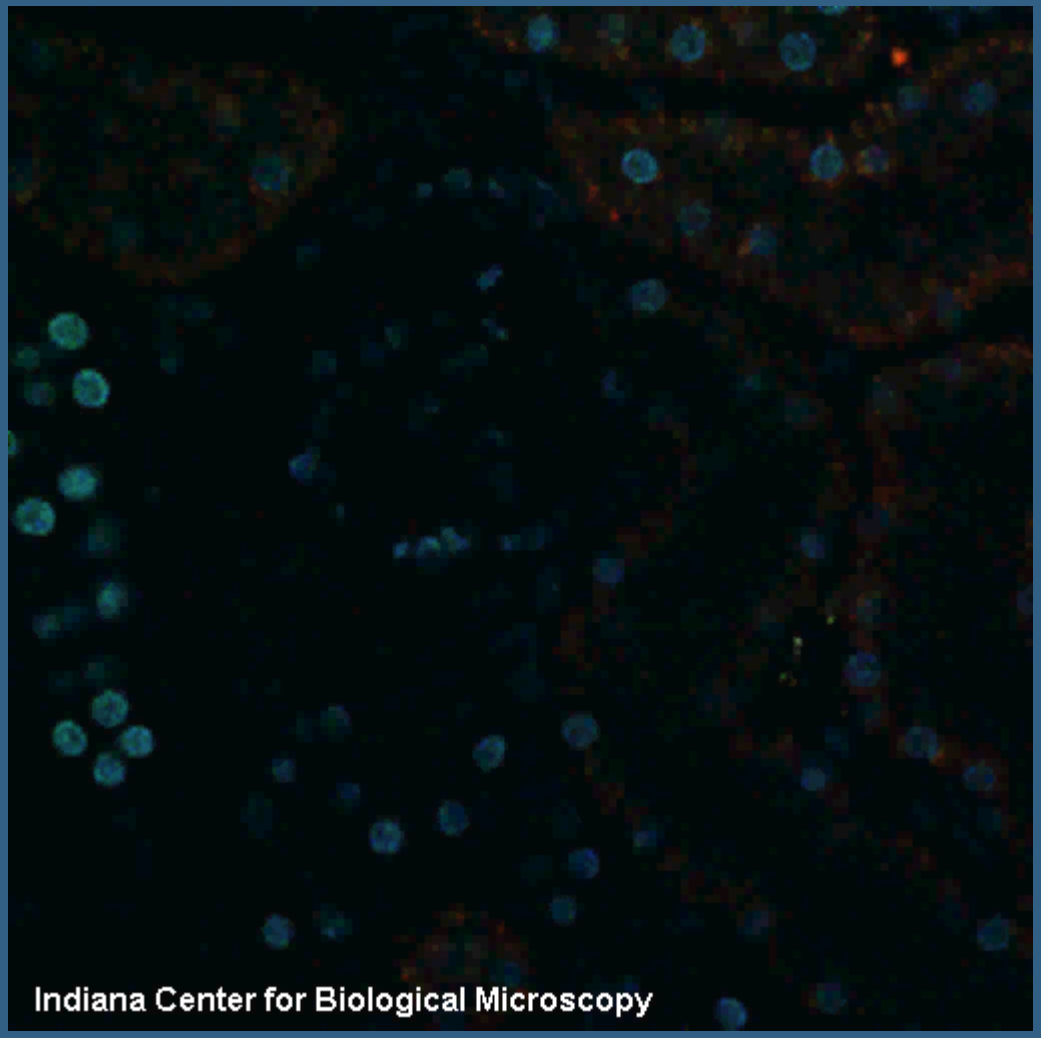




500K Mw FITC-Dextran (Green)

10K Mw Rhodamine Dextran (Red)

Hoechst 44432 (Blue)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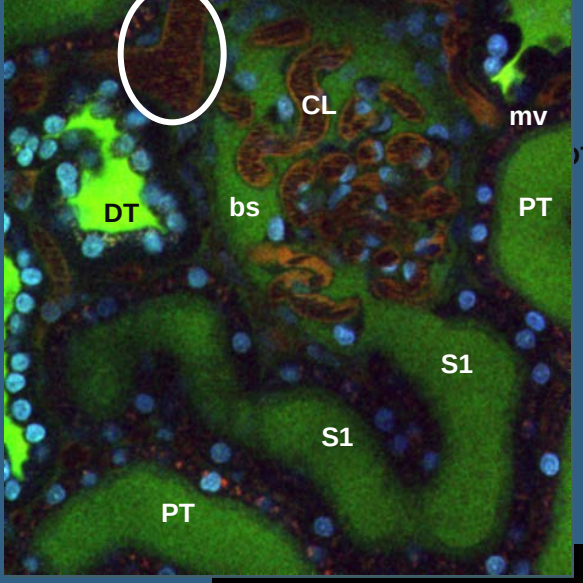
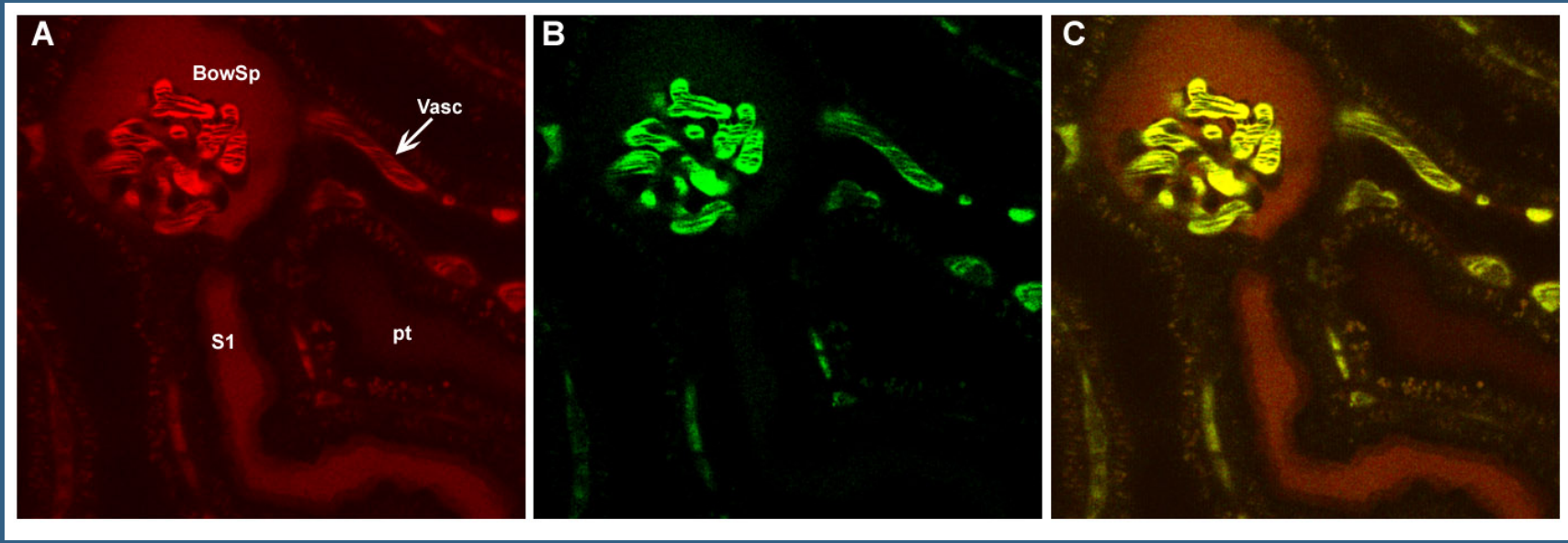
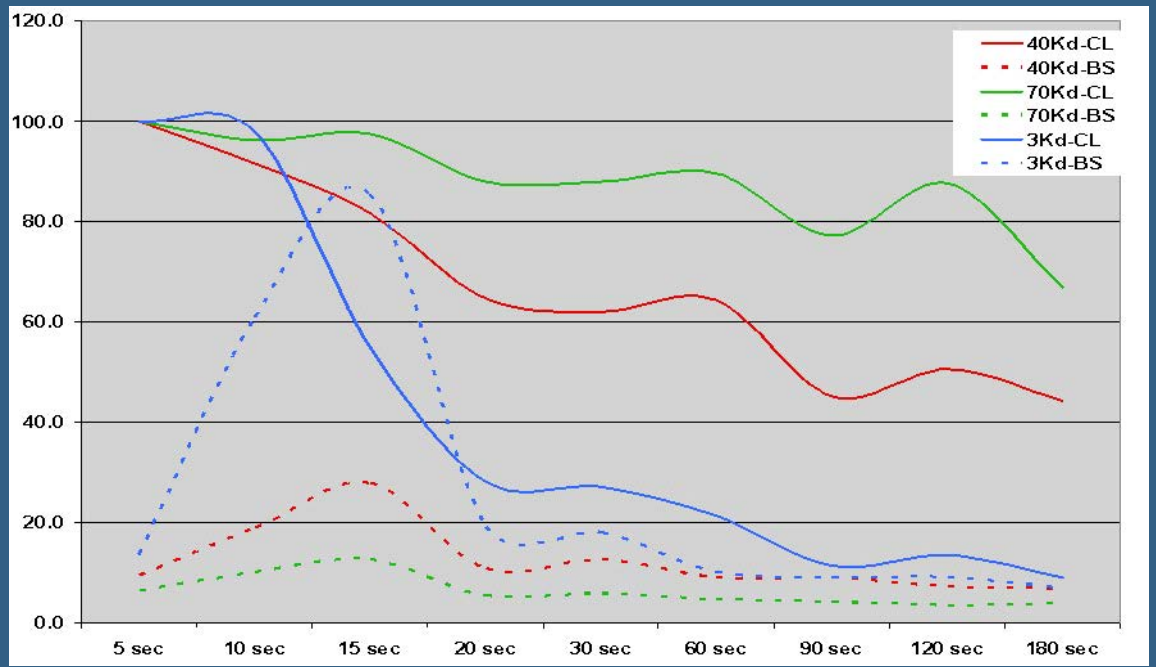
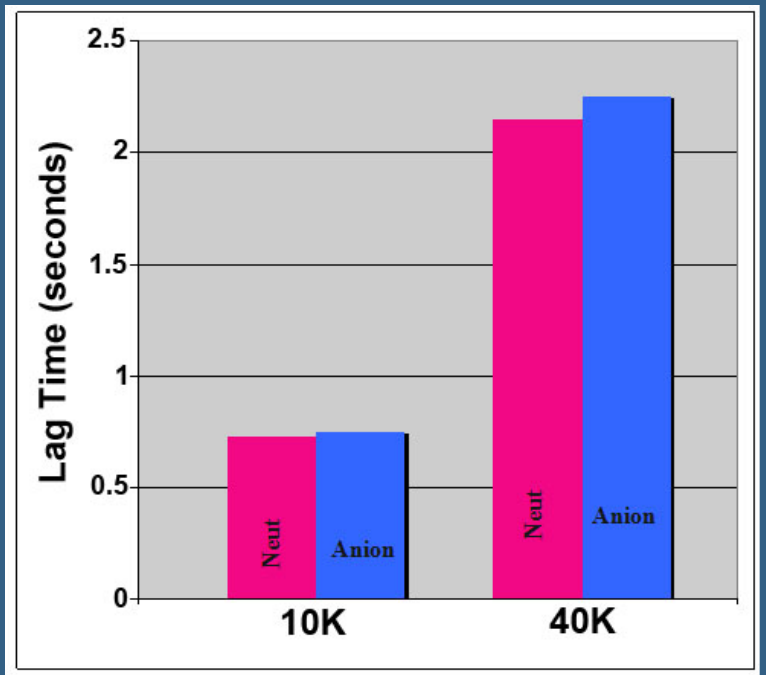


Quantifying Glomerular Filtration

Glomerular Permeability and Vascular Clearance







Question:

What are the Underlying Mechanisms of Proteinuria?

Hypothesis:

Glomerular Filtration as well as Proxmal Tubule Reabsorption are Critical Determinants of Proteinuria

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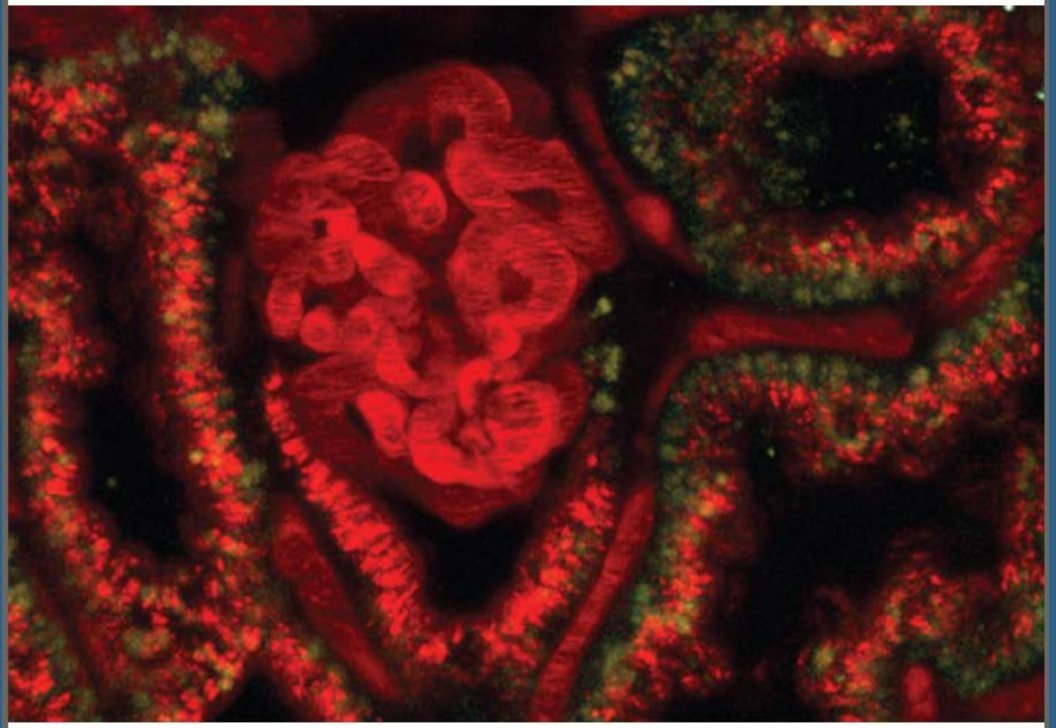
kidney INTERNATIONAL



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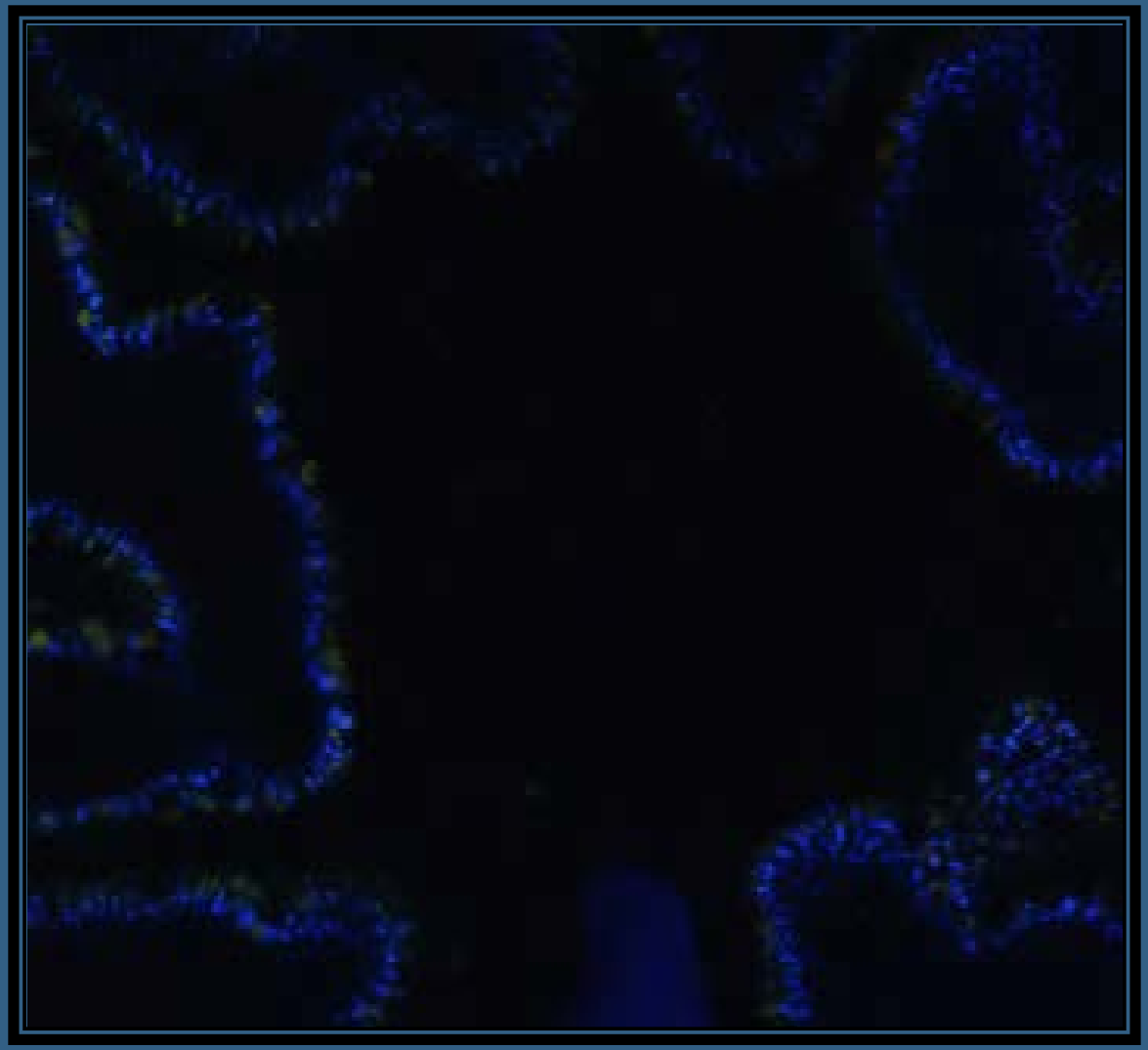
http://www.kidney-international.org

Albumin filtration

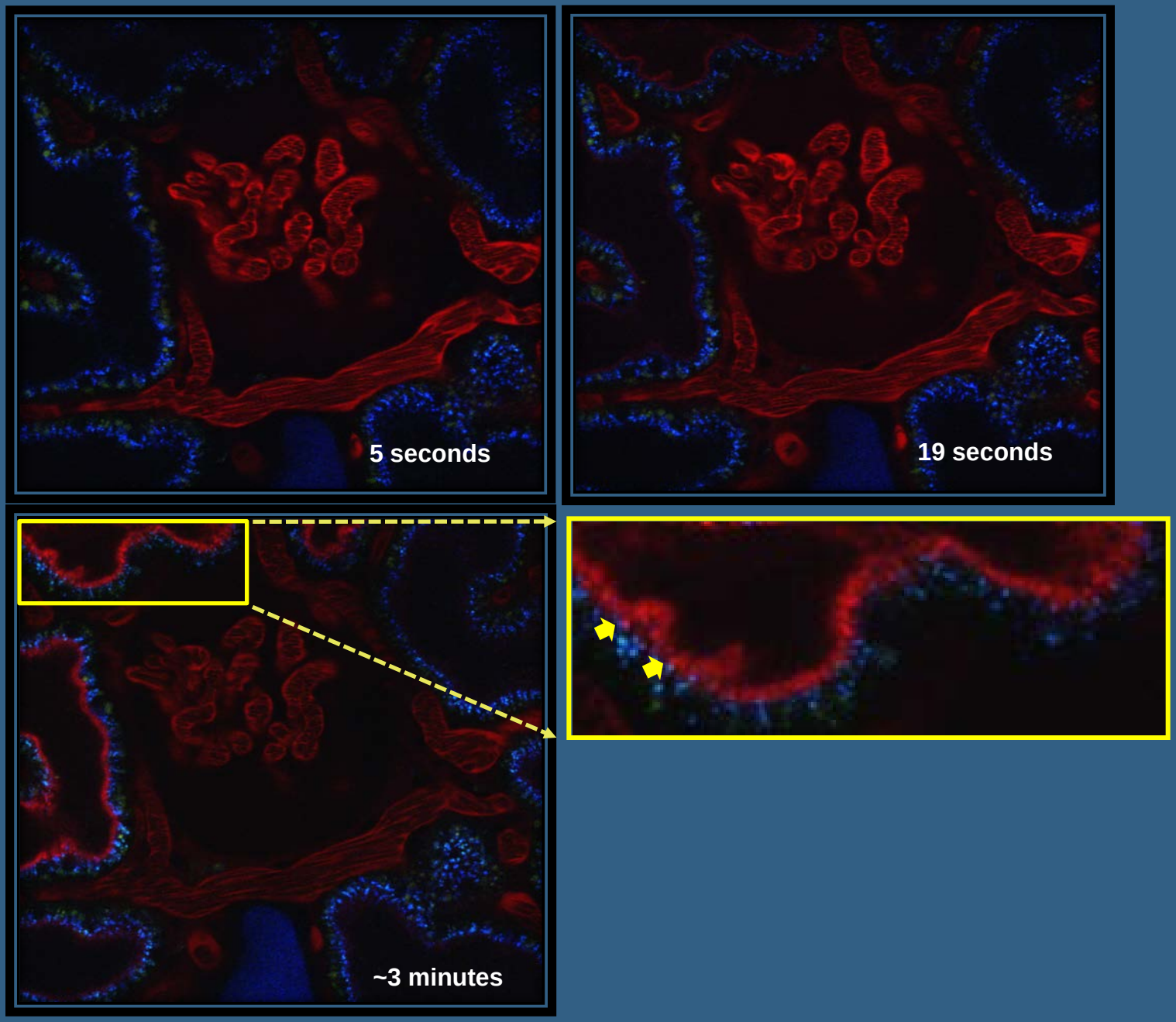
Classification of lupus nephritis

Peritoneal dialysis solutions

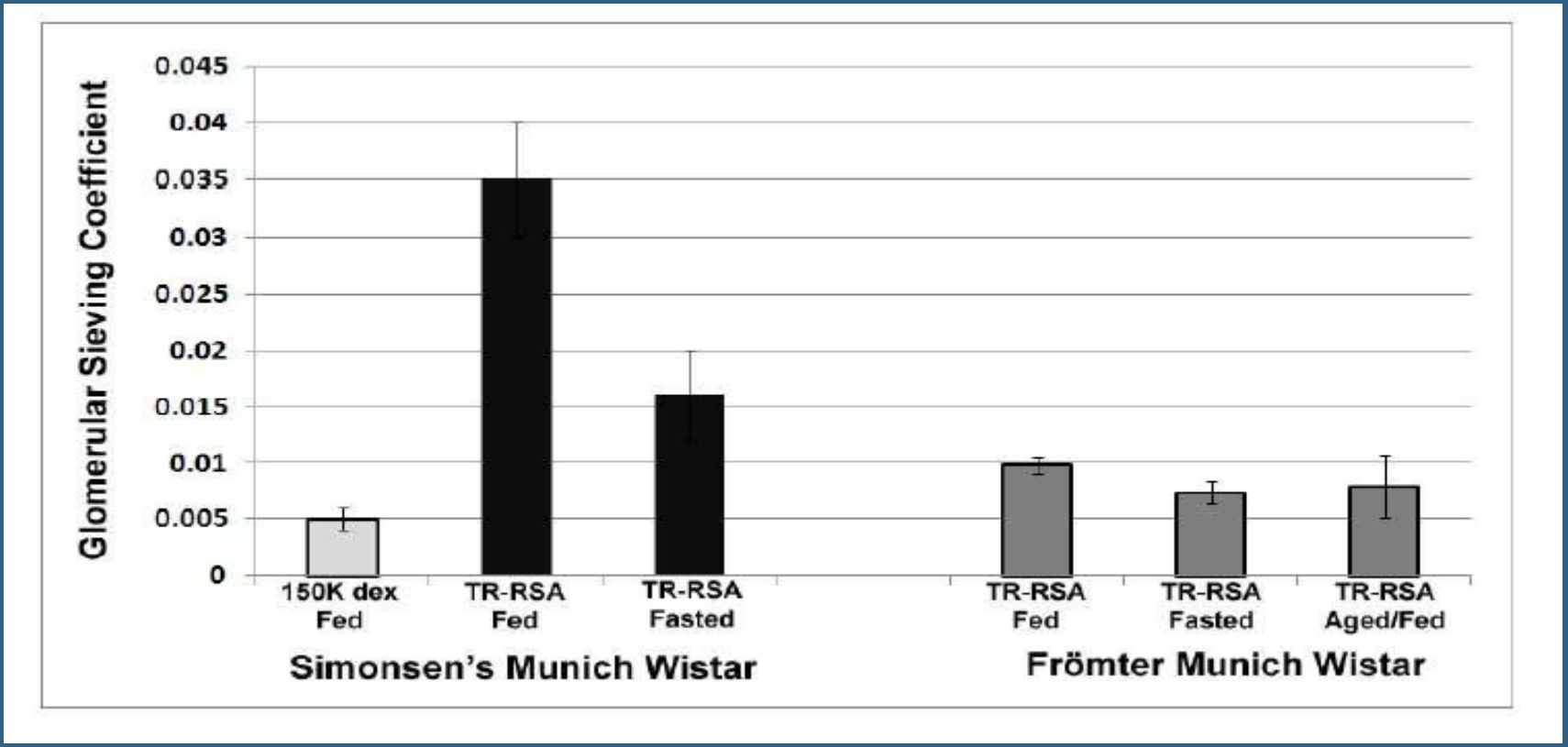
Albumin Filtration and Reabsorption in the Rat



Albumin Filtration and Reabsorption in the Rat



Feeding and Genetics influence the Glomerular Sieving Coefficients



Modified from Sandoval et al. JASN 2012,

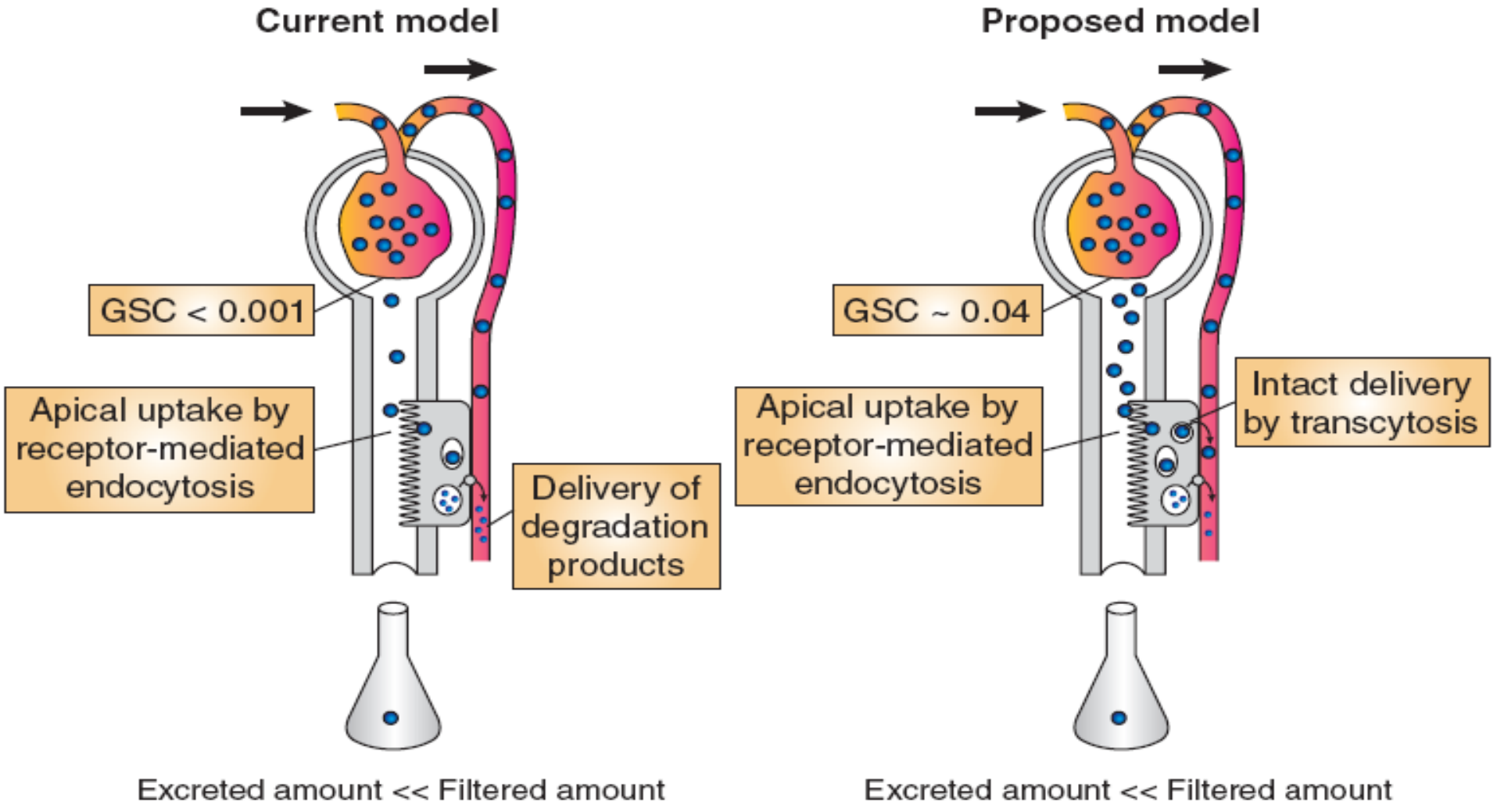


Figure 1| Comparison of the current model of renal albumin handling, as described in most of the publication, with the new model proposed by Russo *et al.* GSC, glomerular sieving coefficient.

Data Implicating PTC in Albuminuria	Reference
D-Serine induced PTC injury	Carone & Ganote, 1975
Megalin-Cubilin complex	Birn et al., 2000; Christensen & Birn, 2001; Wang et al., 2005
CLC-5 knockout	Piwon et al., 2000; Norden et al., 2002; Christensen et al., 2003
Total body irradiation	Yammani et al., 2002
NHE-3 knockout	Gekle et al., 2004
Statins	Sidaway et al., 2004; Verhulst, D'Haese, & De Broe, 2004; Atthobari et al., 2006
Rab 38	Rangel-Filho et al., 2005; Williams, Burke, Lazar, Jacob, & Roman, 2011
Neonatal Fc receptor	Kim et al., 2006; Sarav et al., 2009
Increased glomerular sieving coefficients	Russo et al., 2007
Transcytosis	Russo et al., 2007, Sandoval et al., 2012
Carbon nanotubes	Ruggiero et al., 2010
Bardoxolone	Reisman et al., 2012
Diptheria toxin-induced PTC injury	Grgic et al., 2012; Sekine et al., 2012; Zhang et al., 2012

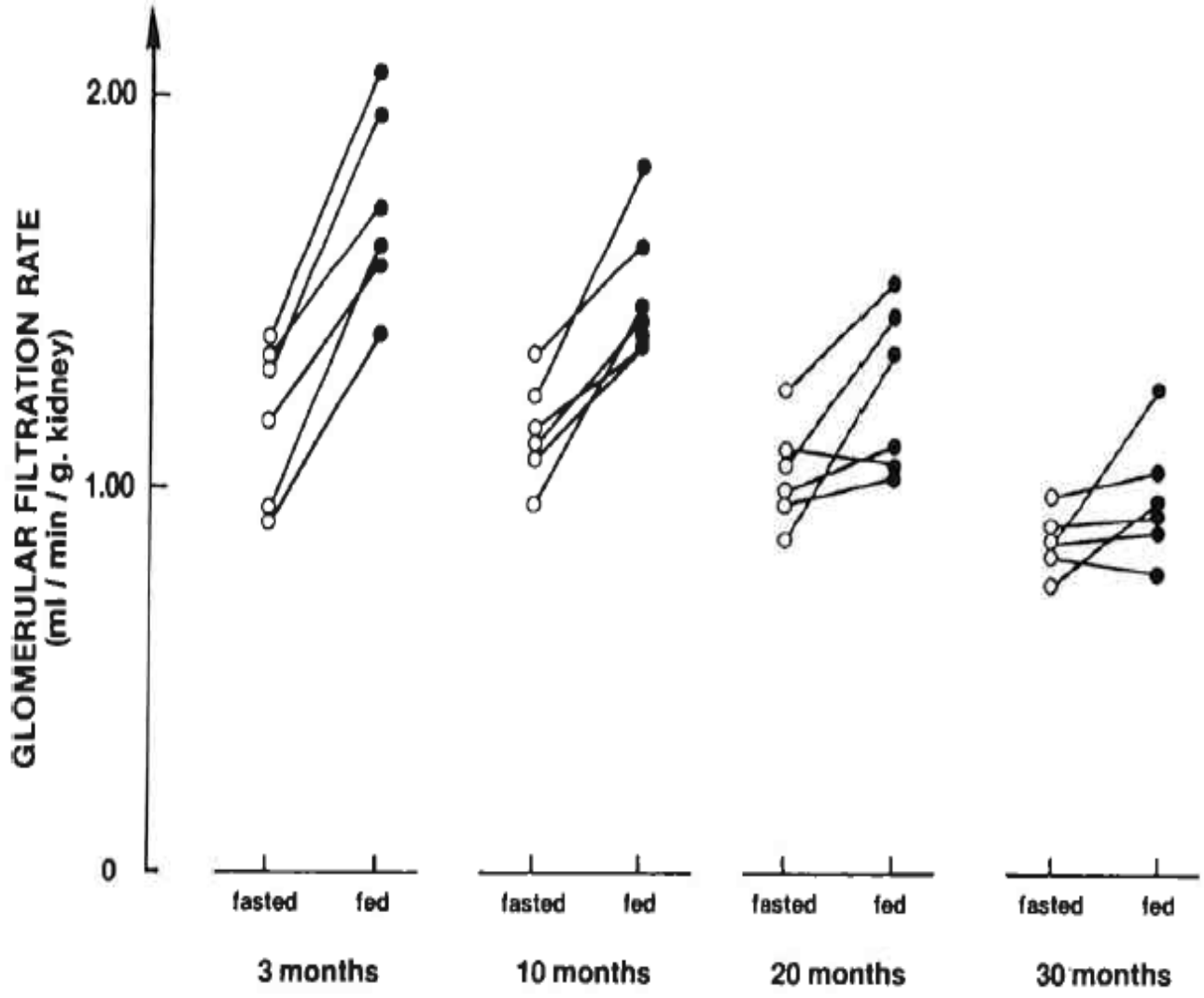


FIG. 2. Glomerular filtration rate in fed and fasted rats. Lines join data from same animals.

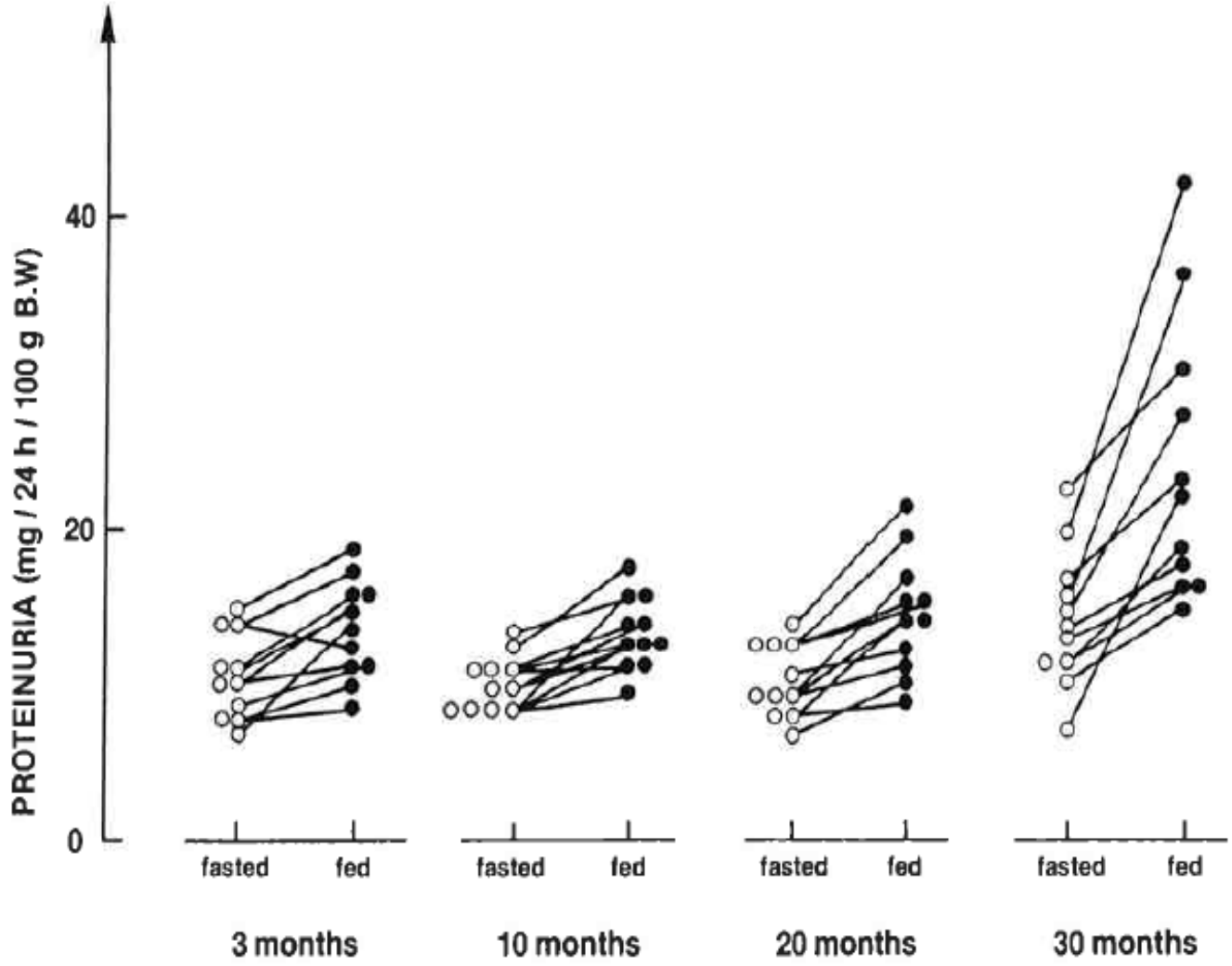
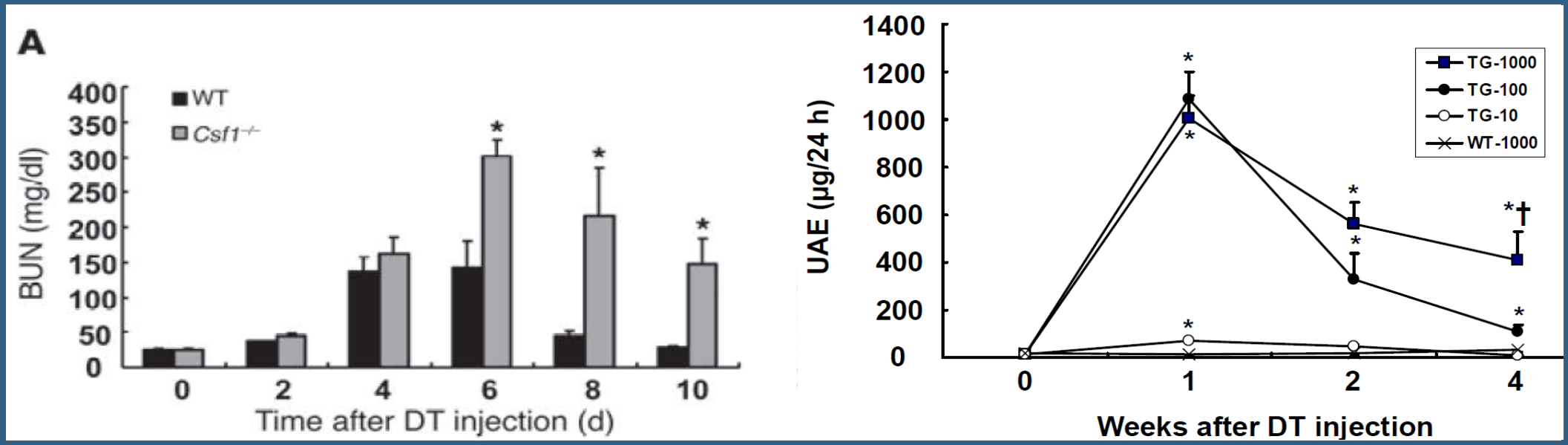


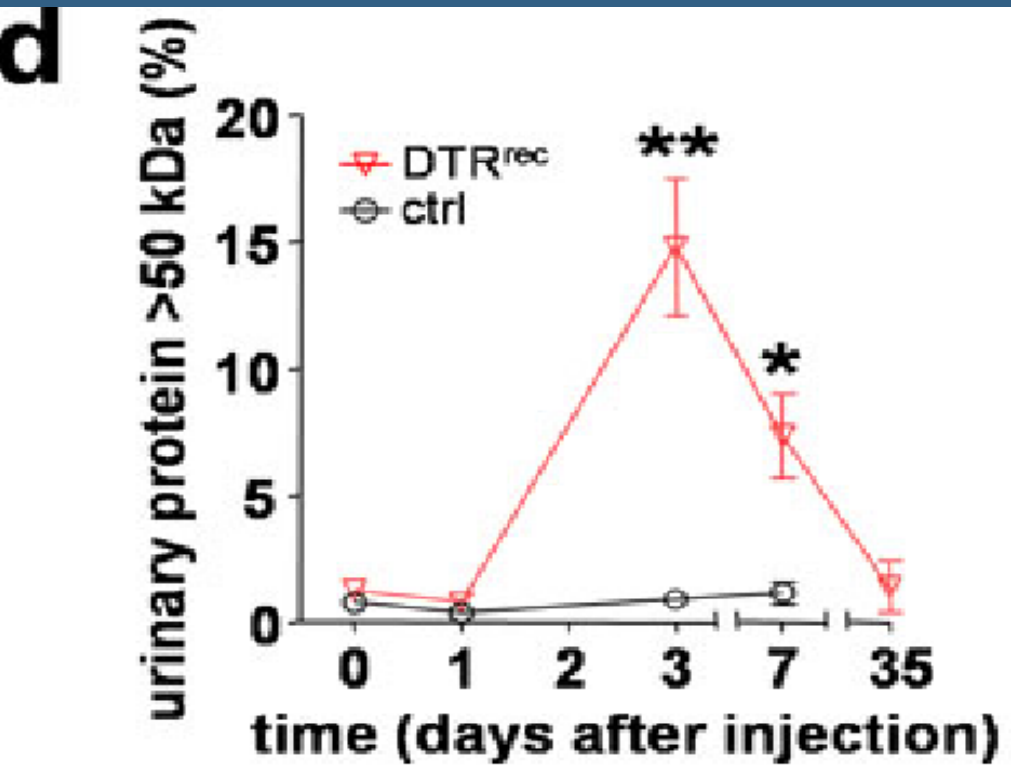
FIG. 1. Daily proteinuria in fed and fasted rats. Lines join data from same animals.

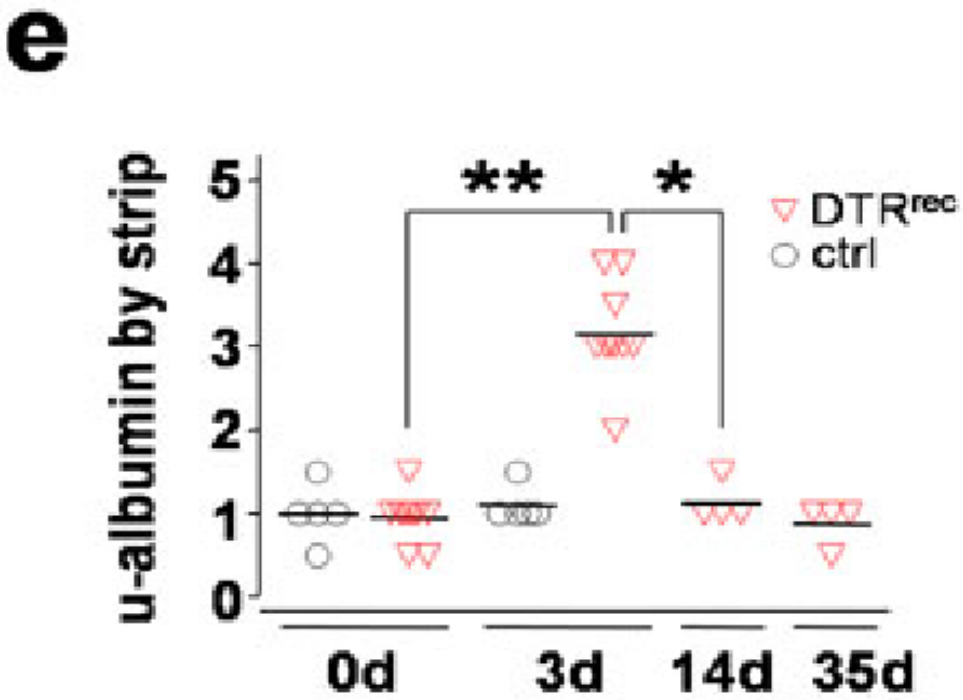
DT-Induced AKI and PT Proteinuria



Zhang MZ, et al. CSF-1 signaling mediates recovery from acute kidney injury. J Clin Invest, 122(12), 4519-4532, 2012.

Increased Urinary Albuminuria Post DT Injection





Grgic I, et al. Targeted proximal tubule injury triggers interstitial fibrosis and glomerulosclerosis. KI, 82, 172-183, 2012.

Challenges

- 1. Dogma, Assumptions, Biology, Reagents, Sensitivity
- 2. Quantitative Analysis without Gold Standards
- 3. You See What you are Looking For
- 4. Correcting for Depth of Field
- 5. Going Deeper
- 6. Out of Focus Fluorescence
- 7. Physiologic state of the rat
- 8. Quantifying what you cannot see with the eye and believing it

Tissue Depth Fluorescence Detection is Fluorophore Dependent

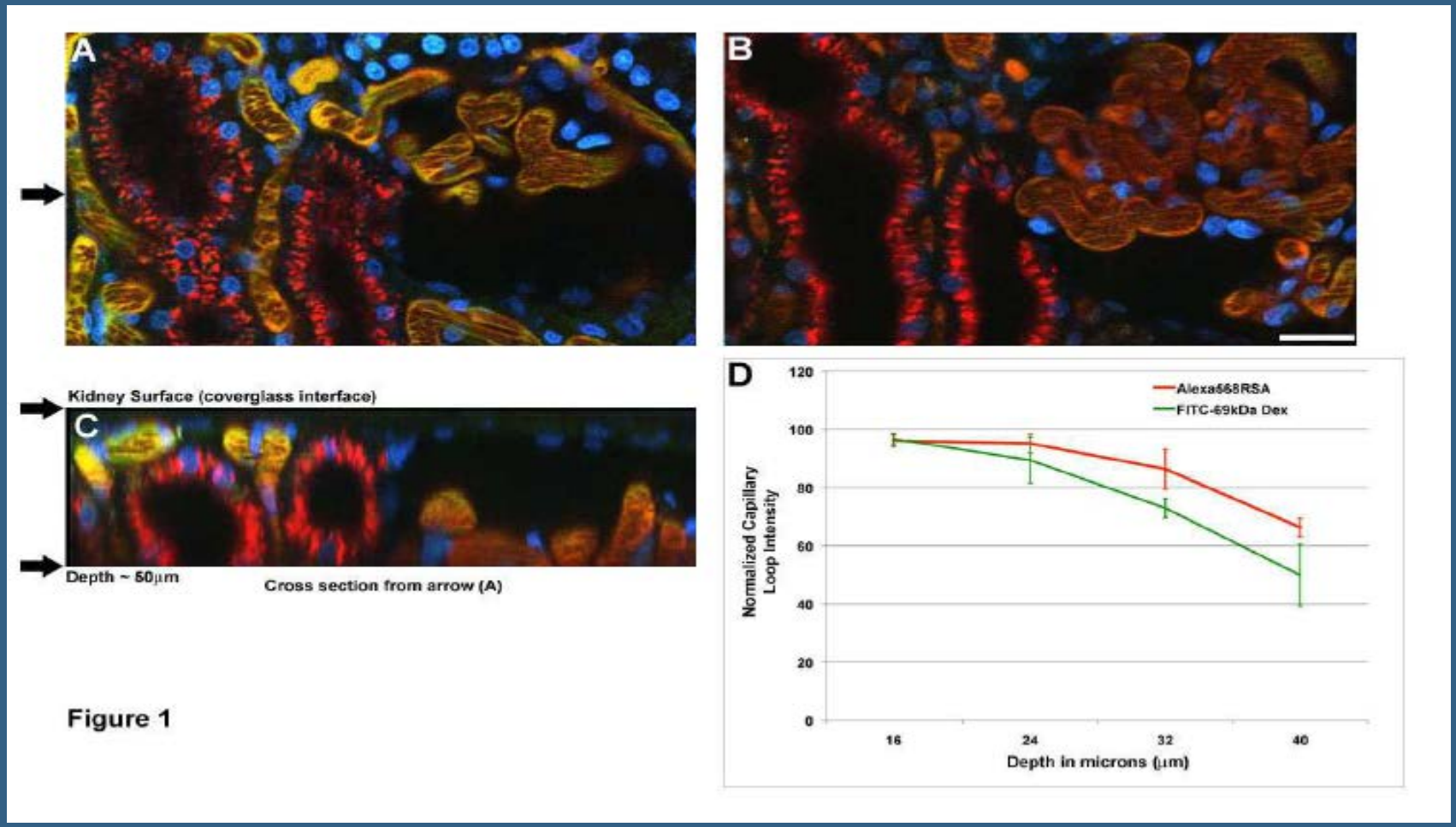


Figure 1

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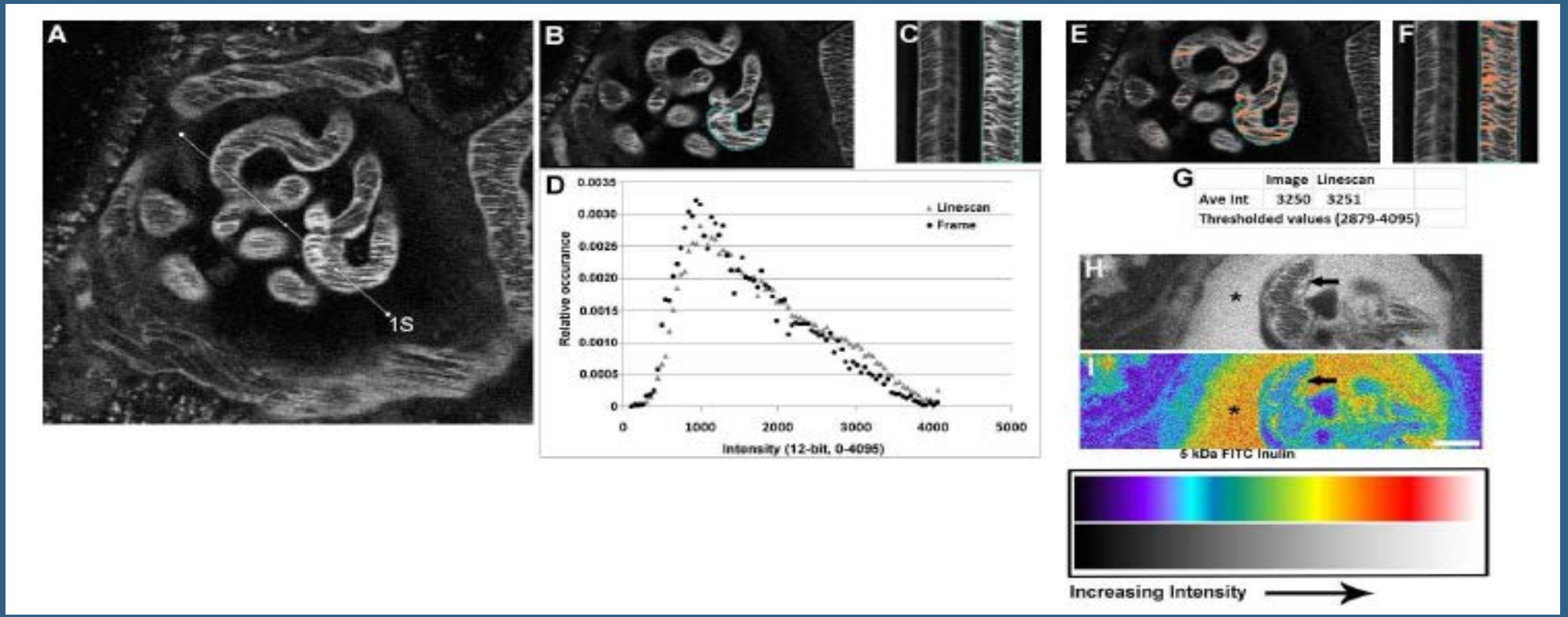
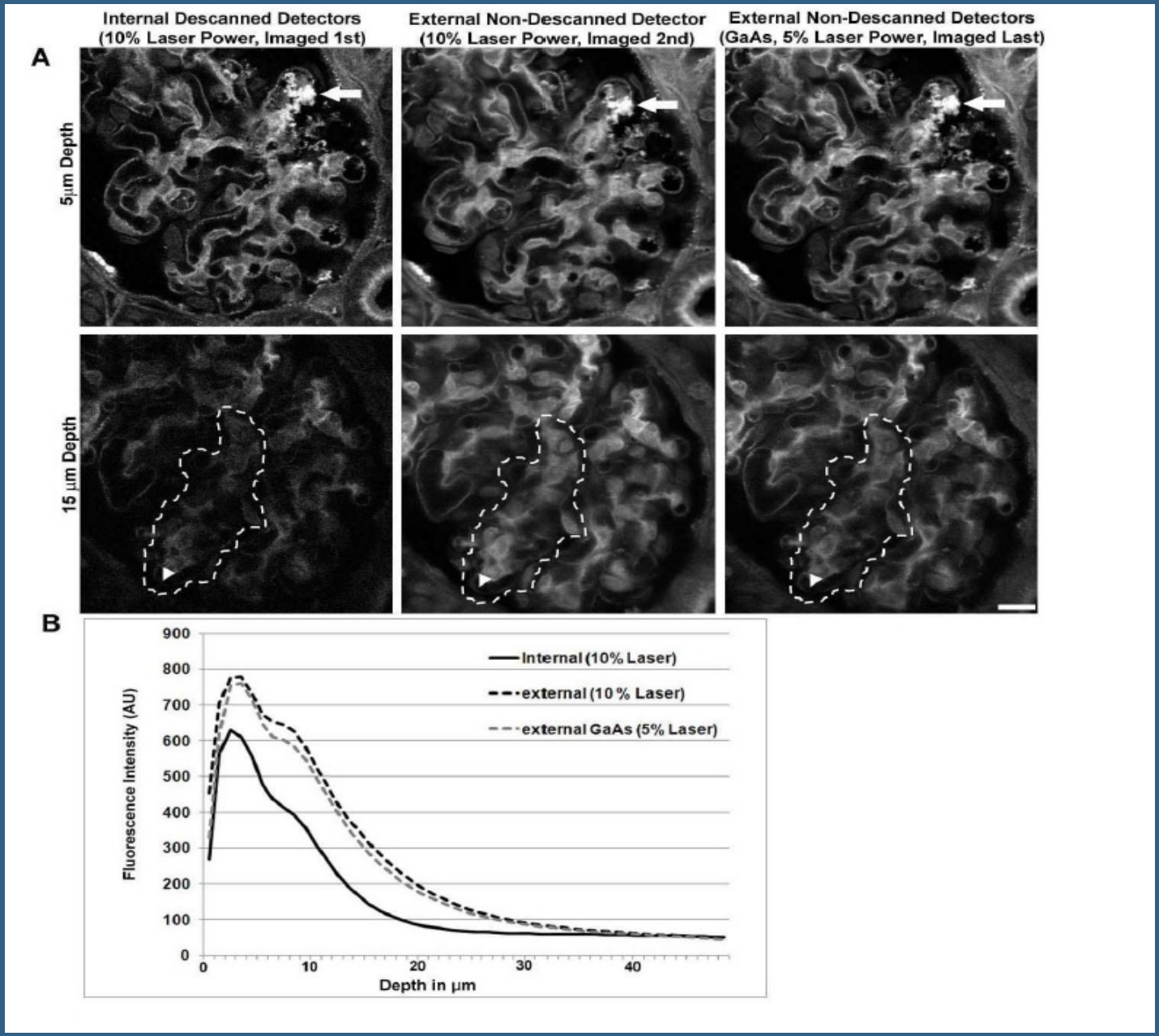
Capillary Fluorescence by Two Different Techniques are Equivalent



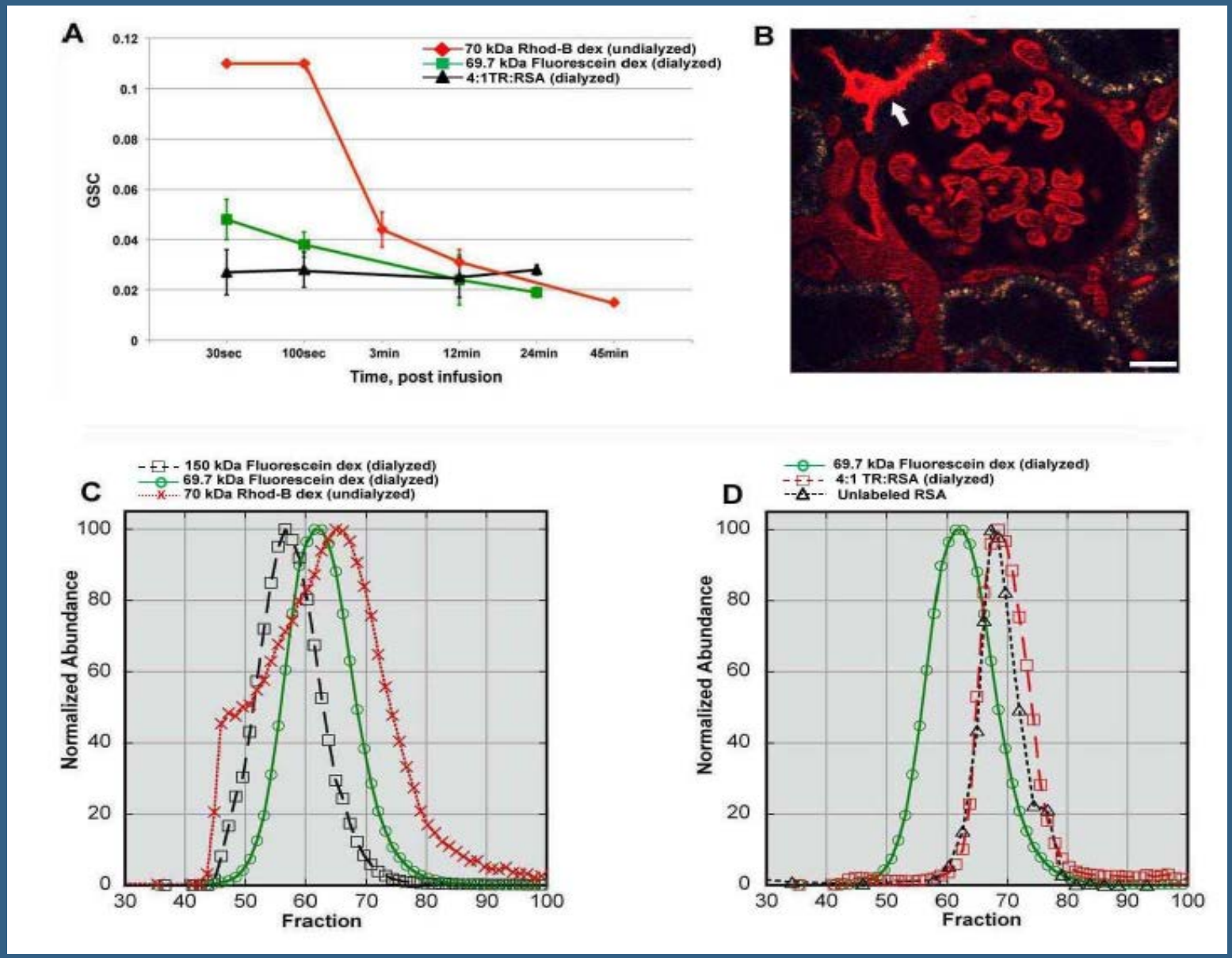
Sandoval et.al. JASN 2012

Laser Detection Devices Vary Greatly in Sensitivity and Resolution



Sandoval et.al. JASN 2012

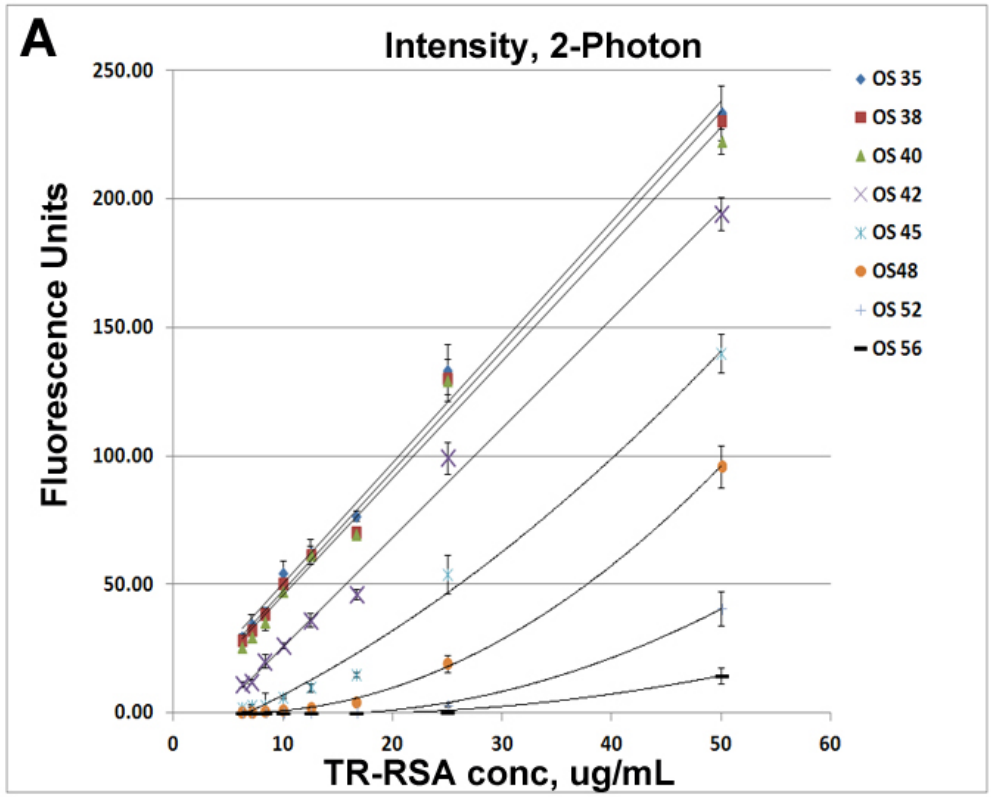
Dextrans can Lead to Misinterpretation of GSCs

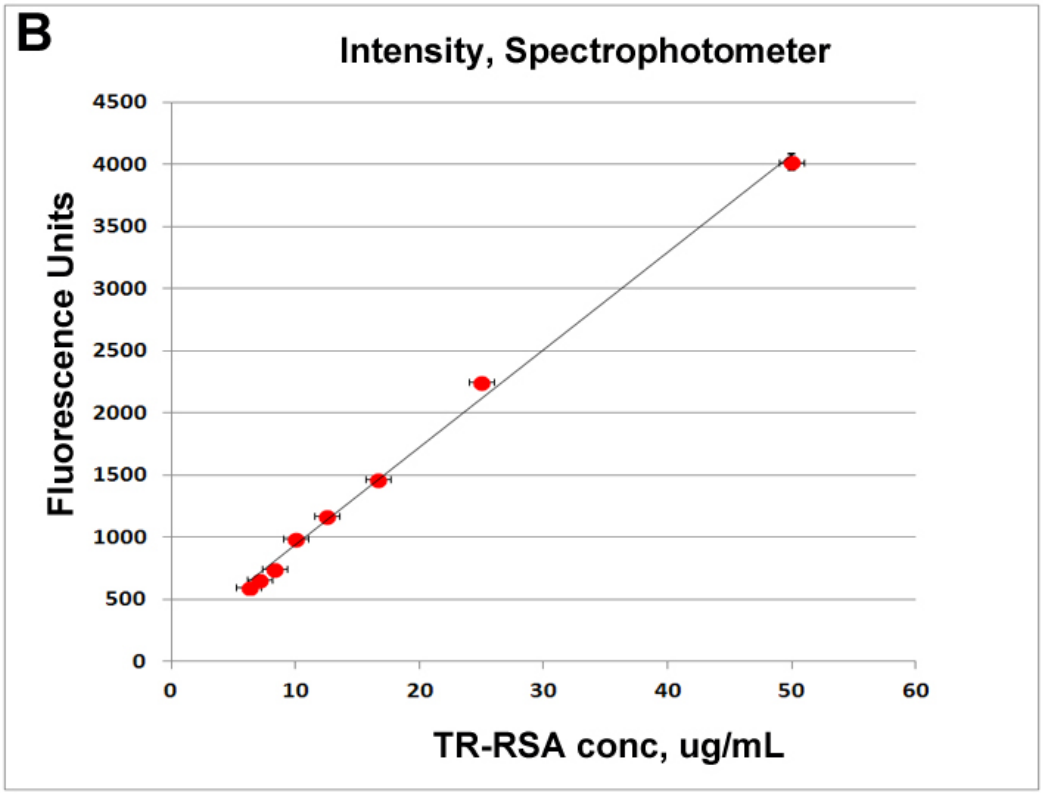


Sandoval et.al. JASN 2012

Setting Background Level Determines Sensitivity

Figure 2





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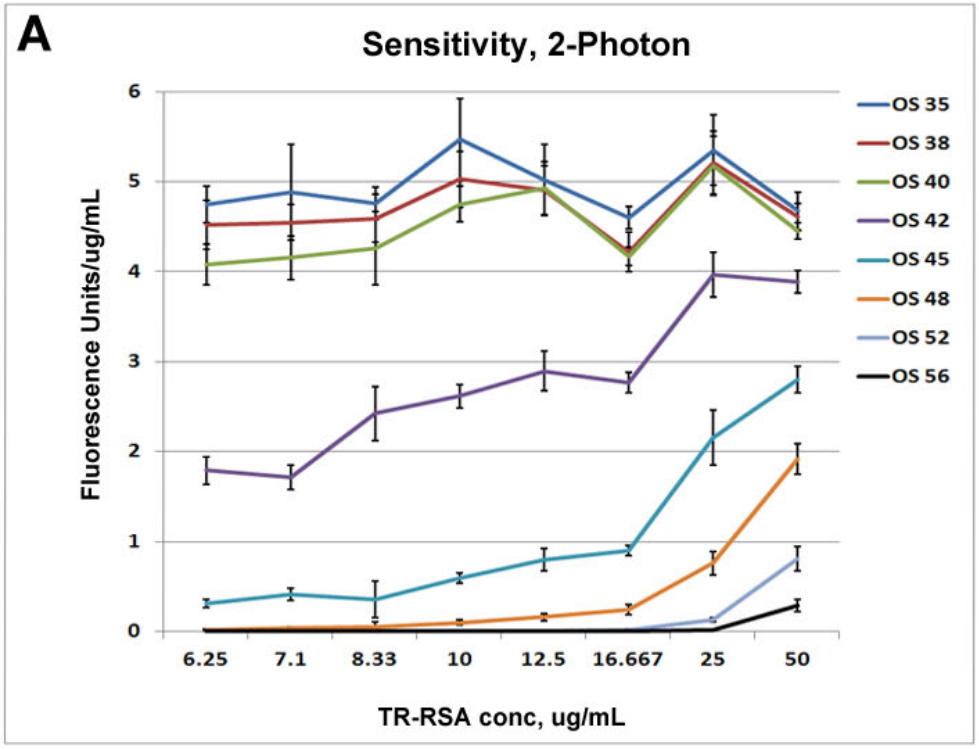
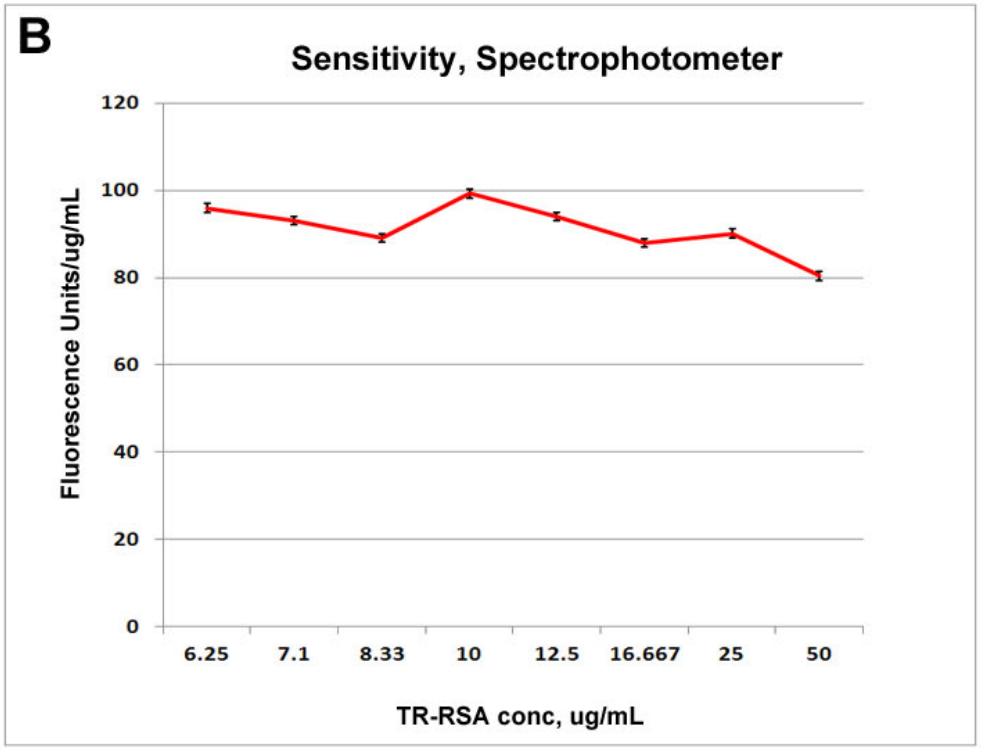
In vitro analysis of effect of changing the background setting

Setting Background Level Determines Sensitivity

Figure 4





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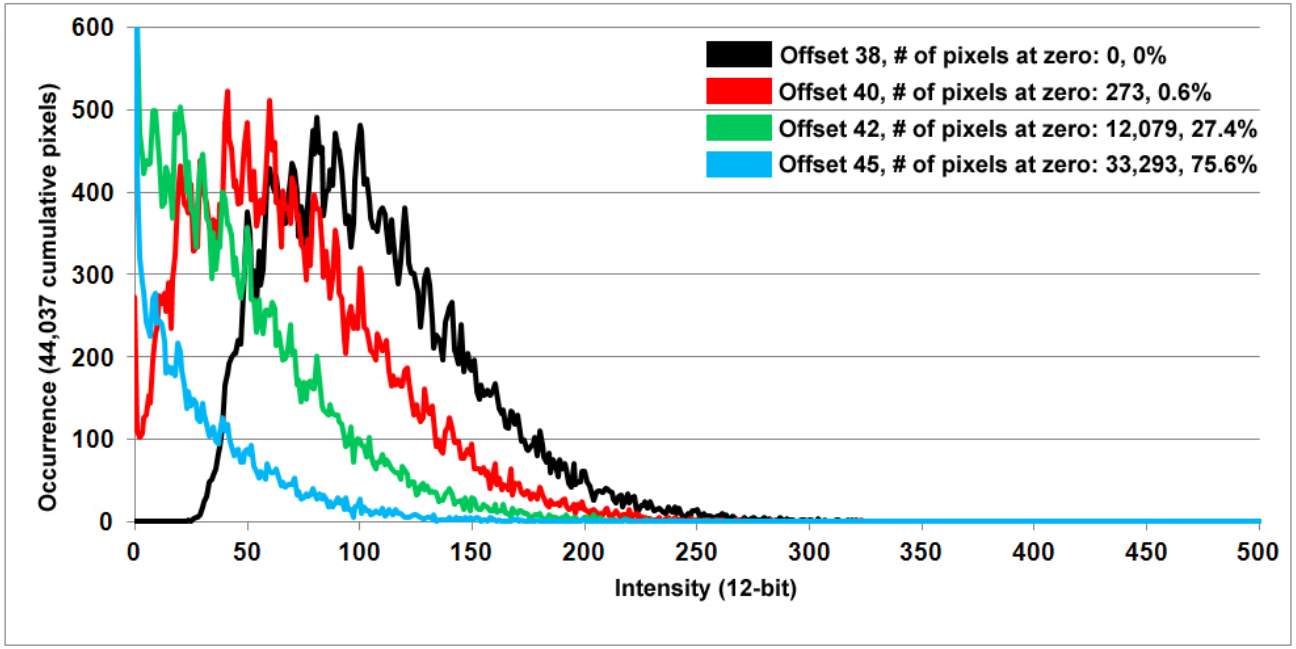
In vitro **analysis of fluorescence per unit TR-RSA at different background levels**

Setting Background Level Determines Sensitivity

Figure 3

A

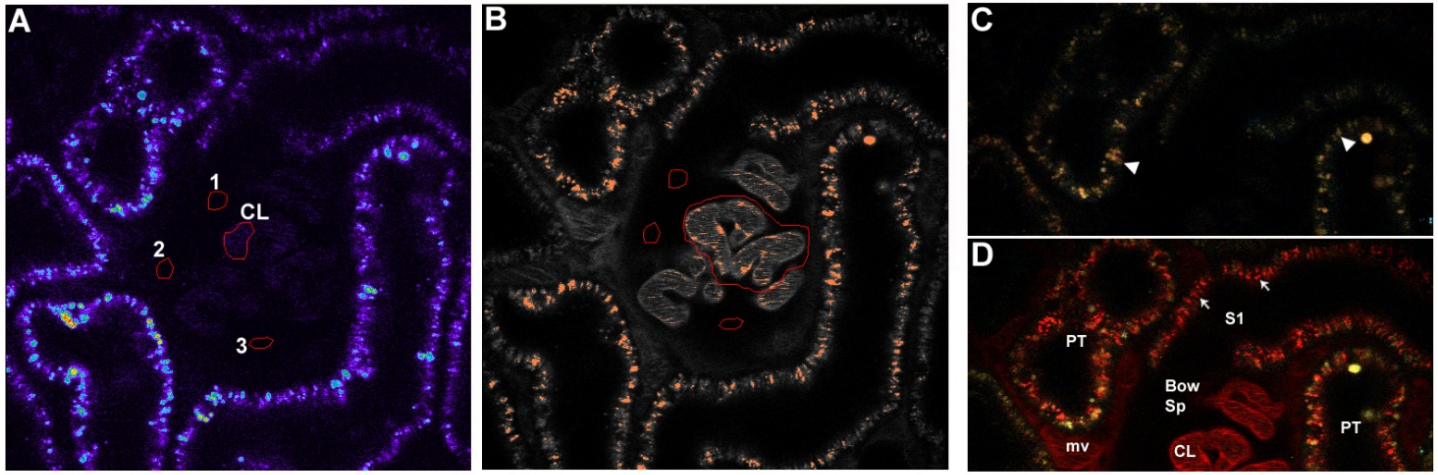


B

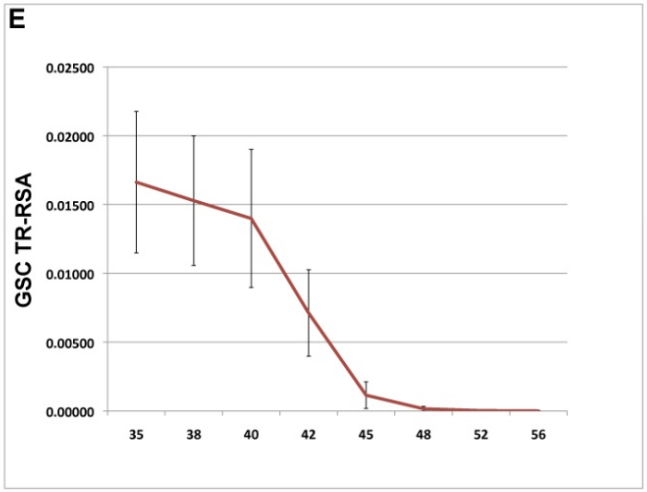
TR Albumin Conc	35	38	40	42	45	48	52	56
50 ug/mL	225.2	220.3	217.3	186.9	135.0	84.7	31.6	10.6
25 ug/mL	120.4	117.3	118.5	90.7	43.2	15.2	2.3	0.3
16.7 ug/mL	73.7	71.8	70.0	44.9	14.7	3.2	0.2	0.1
12.5 ug/mL	57.2	56.5	56.0	32.9	8.2	1.4	0.0	0.0
10 ug/mL	50.4	47.8	46.5	25.7	5.5	0.8	0.0	0.0
8.3 ug/mL	37.9	37.5	36.8	17.4	2.7	0.4	0.0	0.0
7.1 ug/mL	30.4	30.3	29.0	12.9	2.2	0.2	0.0	0.0
6.3 ug/mL	30.9	30.5	27.5	12.4	1.8	0.1	0.0	0.0

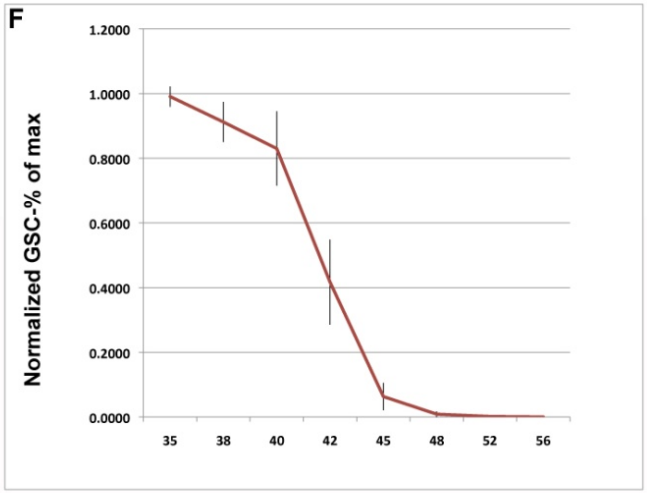
Setting Background Level Determines Sensitivity In vivo

Figure 6



$$GSC = \frac{\text{BowSp Int (Raw ave R1, R2, R3) - (Bkg ave R1, R2, R3)}}{\text{CapLoop Int (Thresholded Plasma Int Post Infusion) - (CapLoop Int Background)}}$$





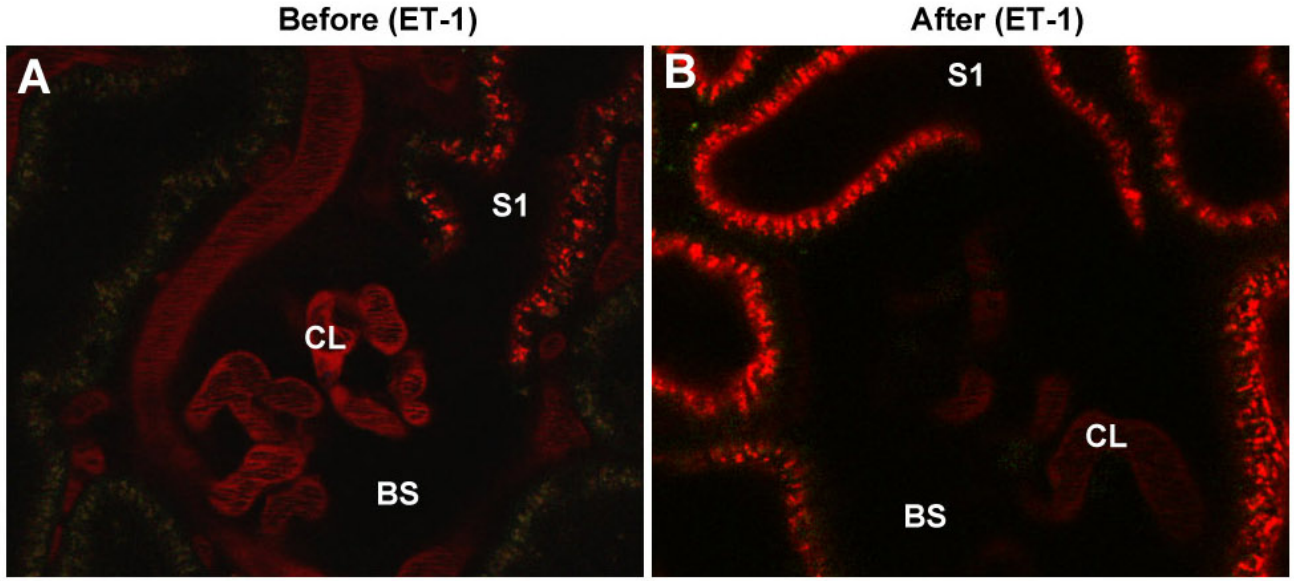
Sandoval et.al. Intravital 2013

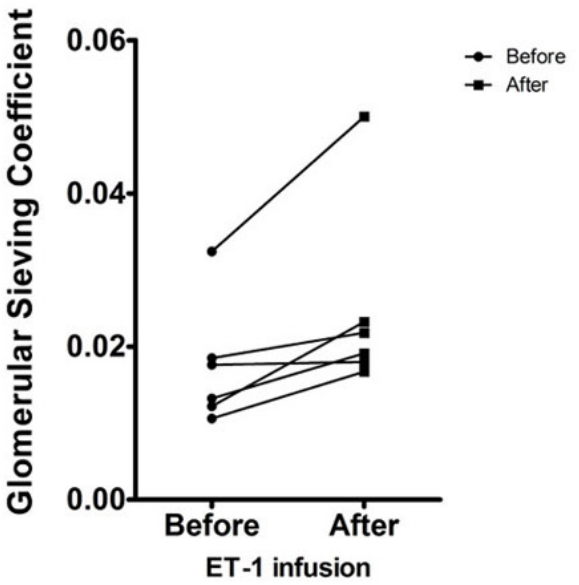
Working Hypothesis

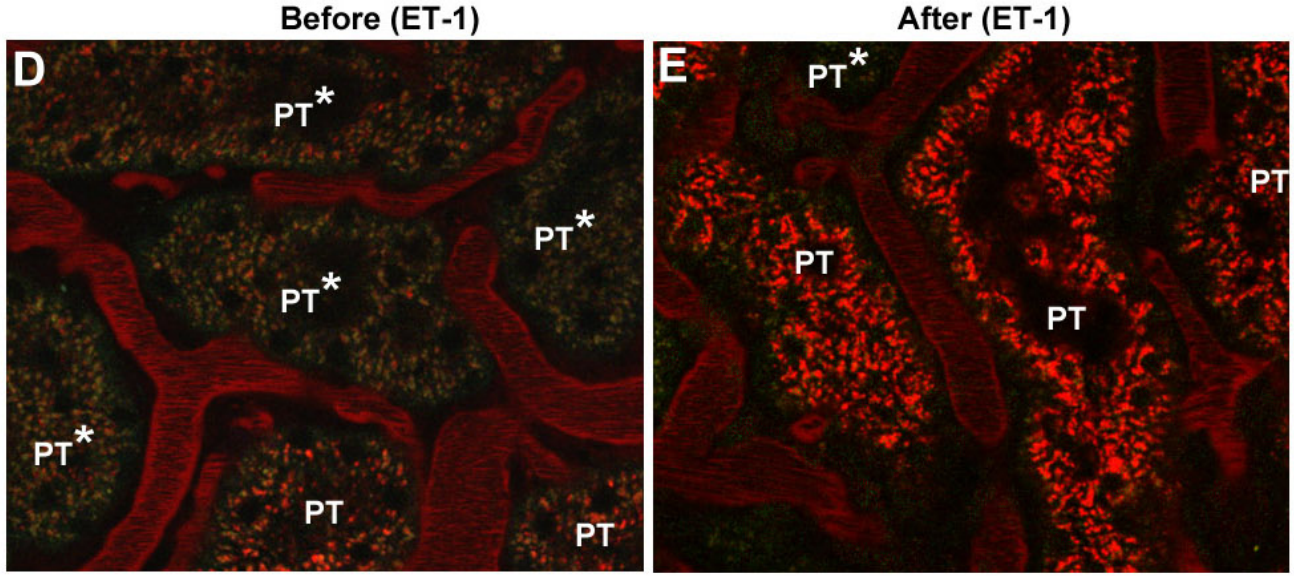
Both glomerular permeability and proximal tubule cell reabsorption play fundamental, physiologic, synergistic, interactive and inducible roles to try and maintain the physiological state and minimize albuminuria.

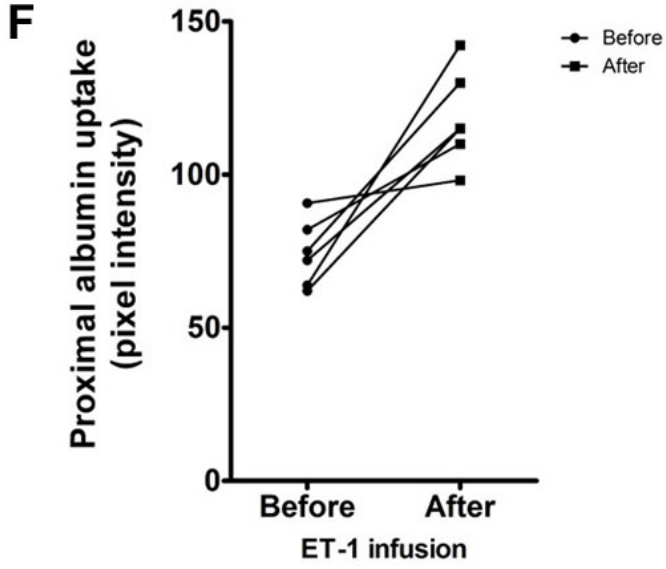
We further hypothesize that acute or chronic alterations in glomerular albumin permeability or in proximal tubule albumin reclamation can directly affect albuminuria.

Chronic ET-1 Increases GSC for Albumin without Proteinuria

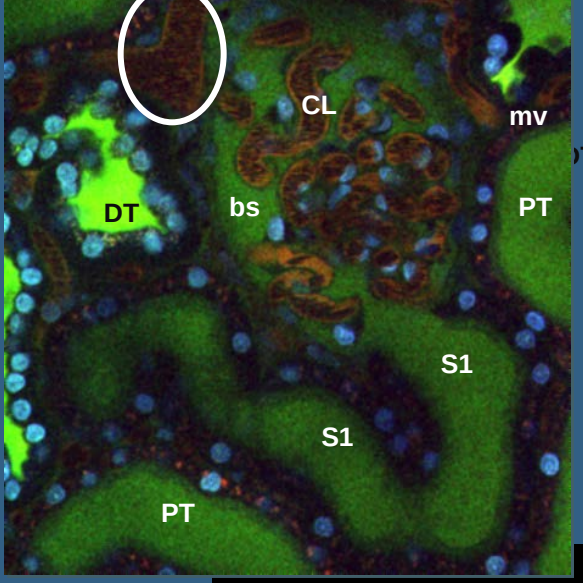






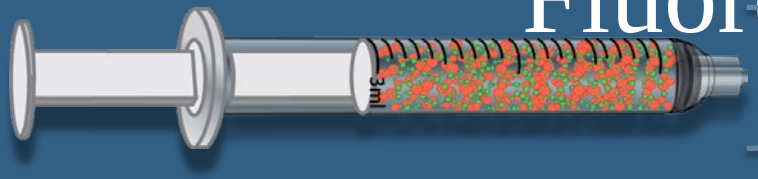


M. Saleh, D. Pollock, et al., Life Sci 2012



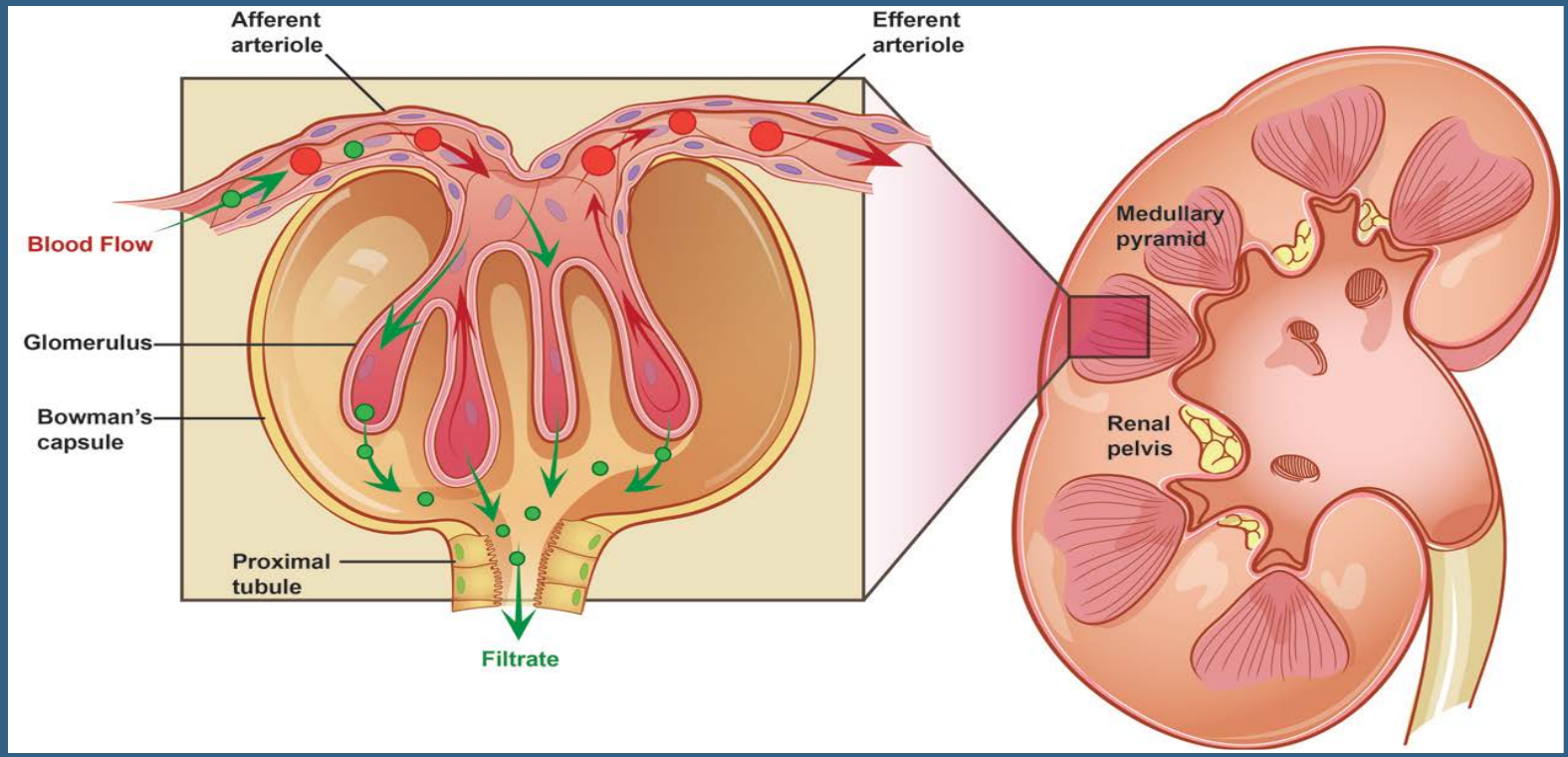
Quantifying Glomerular Filtration

Clearance of Intravenous Fluorescent Injectate

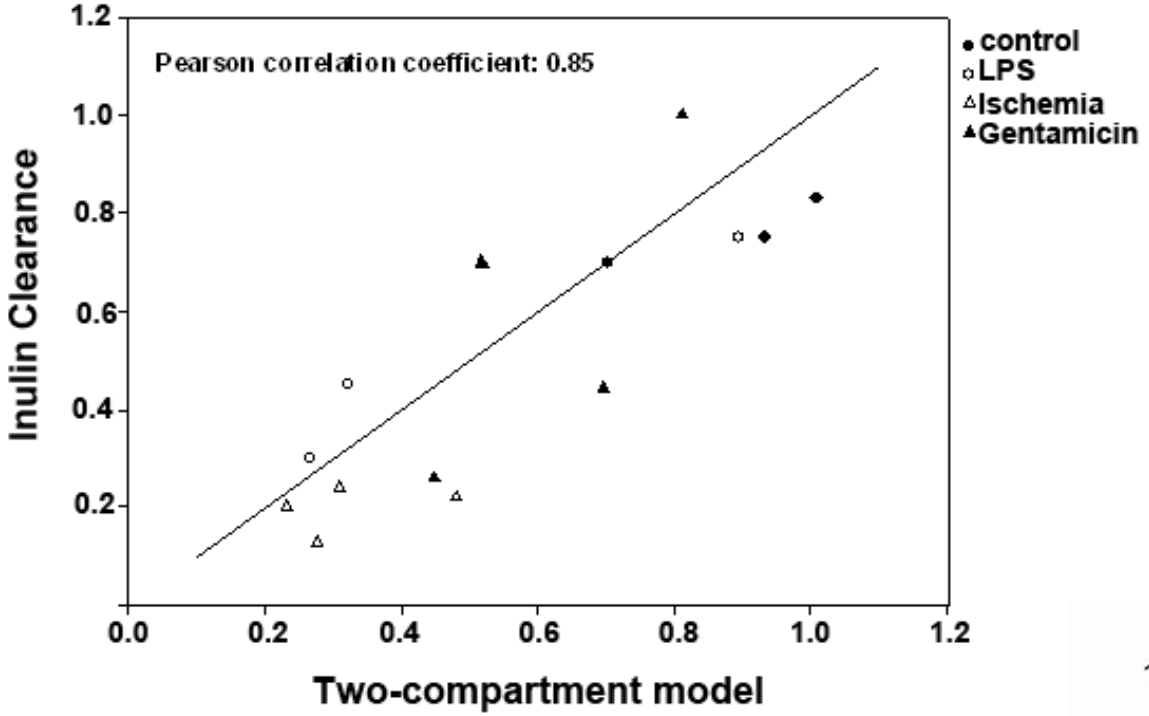


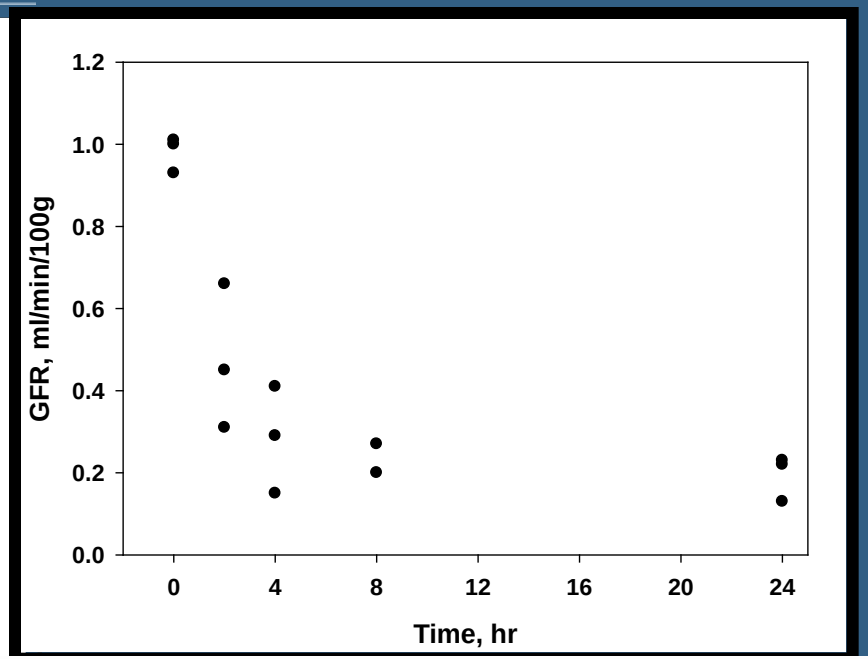
3ml IV injection of large & small fluorescent marker

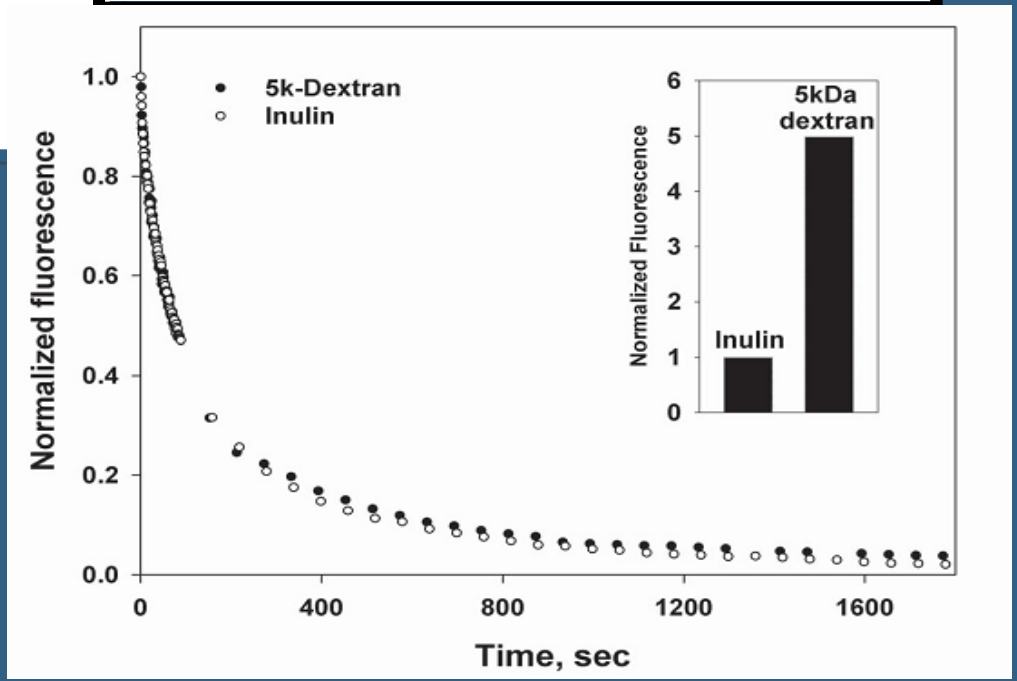
Small marker filtered across glomerulus, large marker retained in vascular space



Quantifying Glomerular Filtration in Rats







Data Collection Only Required 10-15 minutes per GFR Determination Post Injection

Advantages of Dextrans

- **Solubility**
- **None Immunogenic**
- **High labeling effeciency**

Wang. Am J Physiol Renal Physiol, 2010

"Advancing" to the Dark Side

Converting from a Microscope to Portable Technique

1. Fluorescent Markers, No Change form Microscopic Technique
   A. Large Dextran for Vascular Volume Measurement
   B. Small Free Filterable Dextran for Rate Determination

2. Excitation Generation and Emission Detection Device Needed

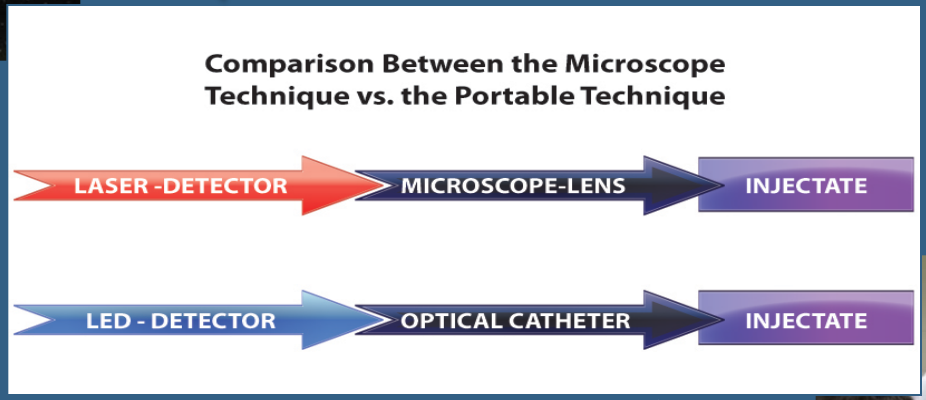
3. Optical Probe for Delivery of Excitation Pulse and Recovery of Emission Signal

4. Data Storage and Software Analysis

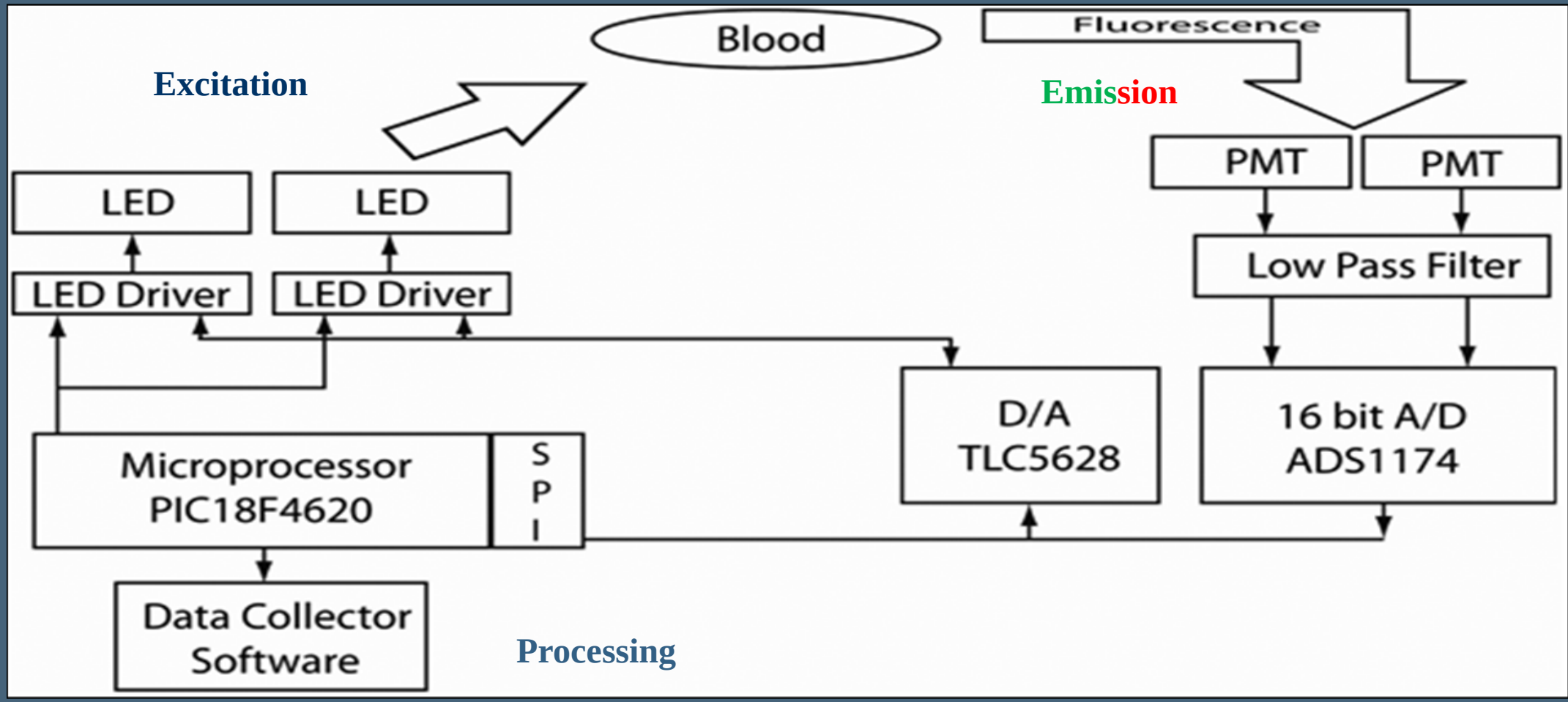
5. Raising MONEY

Commercialization: Bench to Bedside

Discovery using expensive 2-photon laser requiring surgery to visualize exteriorized kidney

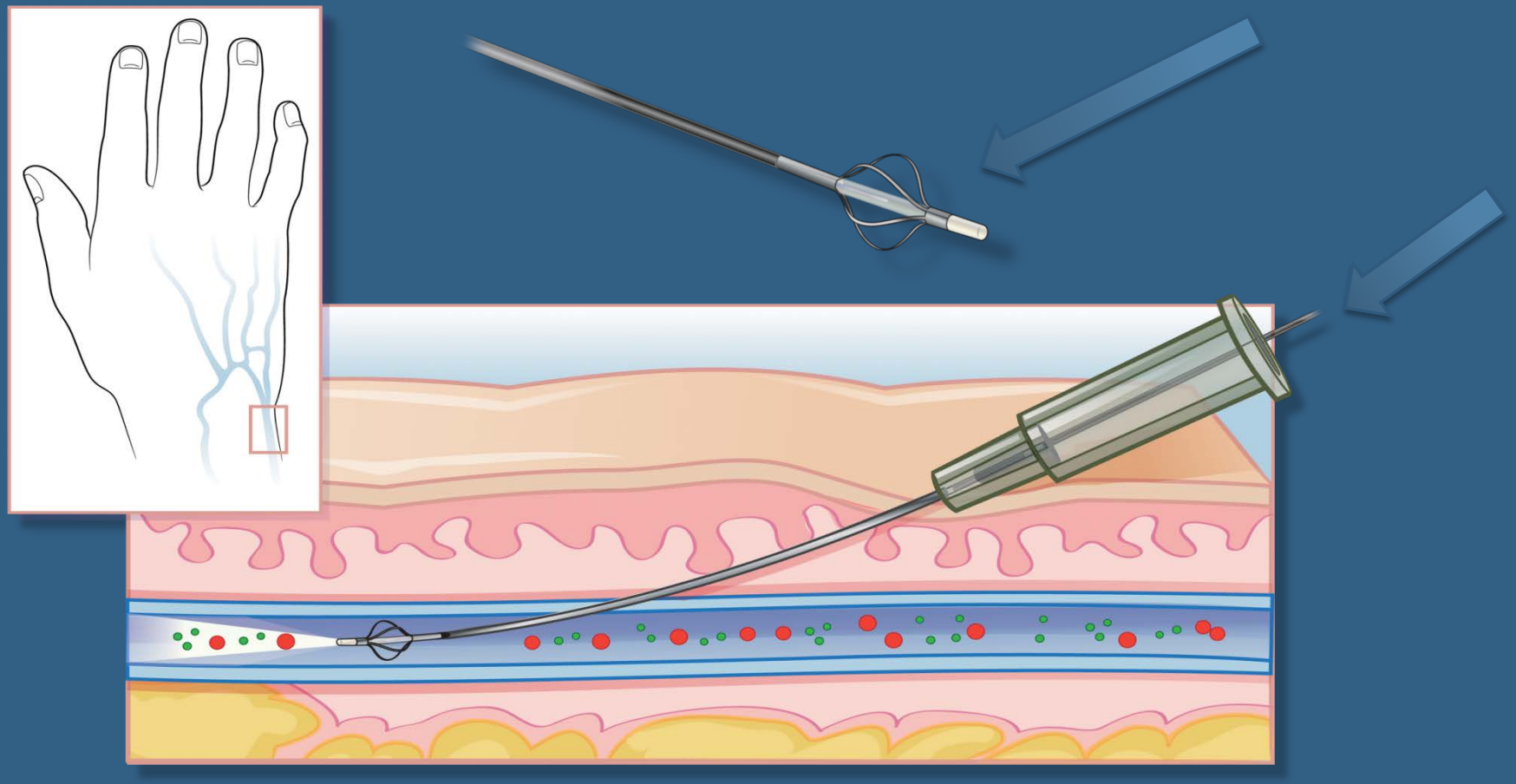


Inexpensive, portable LED-based device using fiber optic introduced through standard 18g catheter to read markers

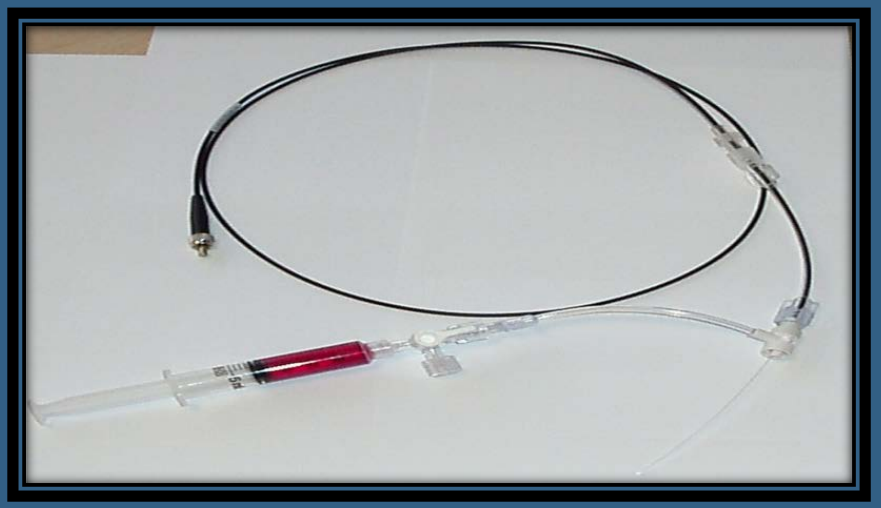

Blood
Fluorescence
Excitation
Emission
PMT
PMT
LED
LED
Low Pass Filter
LED Driver
LED Driver
D/A
TLC5628
16 bit A/D
ADS1174
Microprocessor
PIC18F4620
S
P
I
Data Collector
Software
Processing

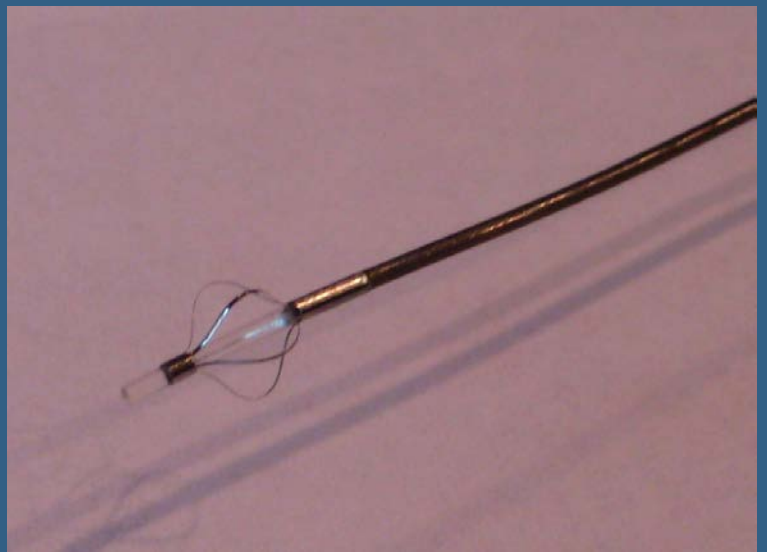
Measuring Fluorescence Through 18g Catheter

Self Centering Fiber Optic Inserted Through 18g Peripheral Catheter

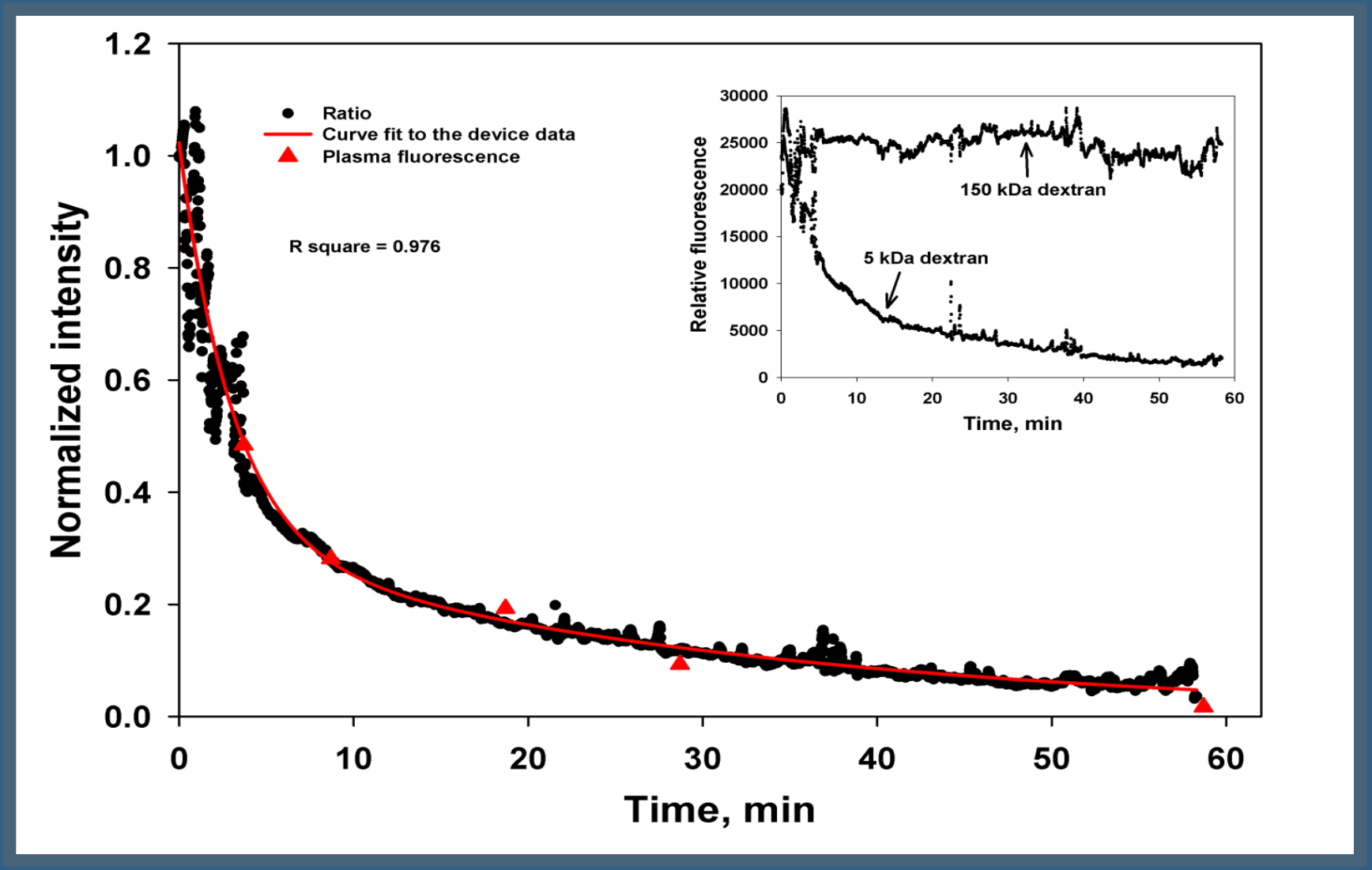


FAST Clinical GFR Technique





GFR Determination in Dogs Via A Peripheral Vein

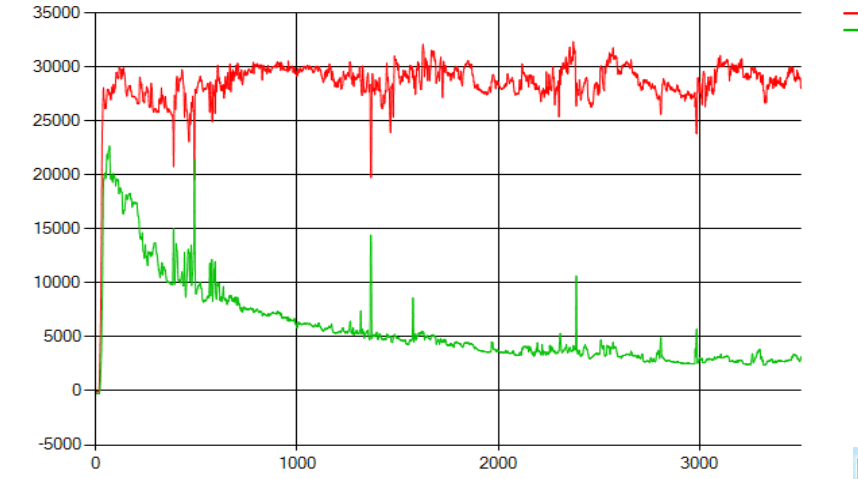


Wang et.al. Kidney Int 2012

Fast Version 1.5

File Edit

Settings | Ratio Graph | DataFit Results | Graph | Raw Data | Processed Data



Slow Green | Slow Red | Fast Green | Fast Red | Raw Data | Std Dev

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File Edit

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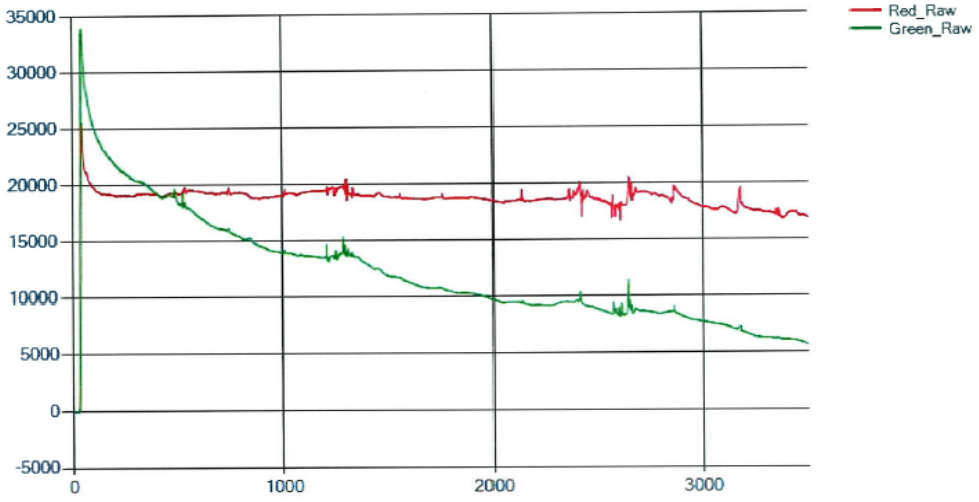

Slow Green | Slow Red | Fast Green | Fast Red | Raw Data | Std Dev

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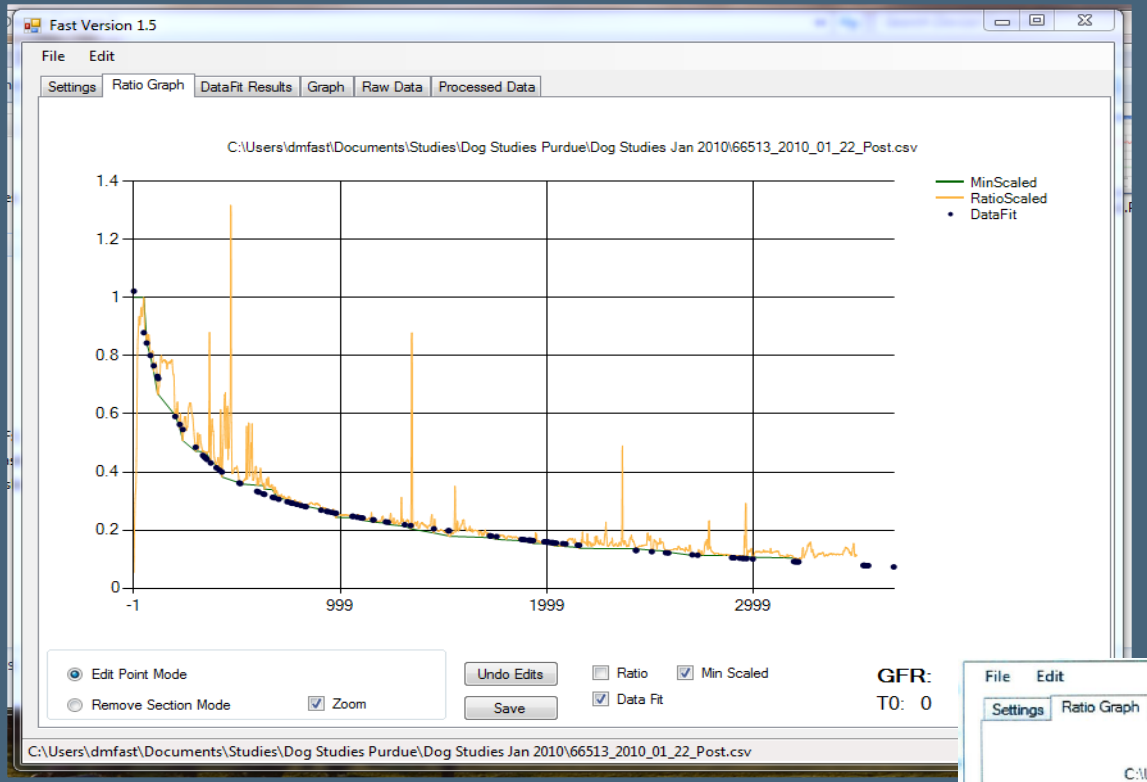
Fast Version 1.5
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RatioScaled
DataFit
1.4
1.2
1
0.8
0.6
0.4
0.2
0
-1
999
1999
2999
Edit Point Mode
Remove Section Mode
Zoom
Undo Edits
Save
Ratio
Min Scaled
Data Fit
GFR:
T0: 0
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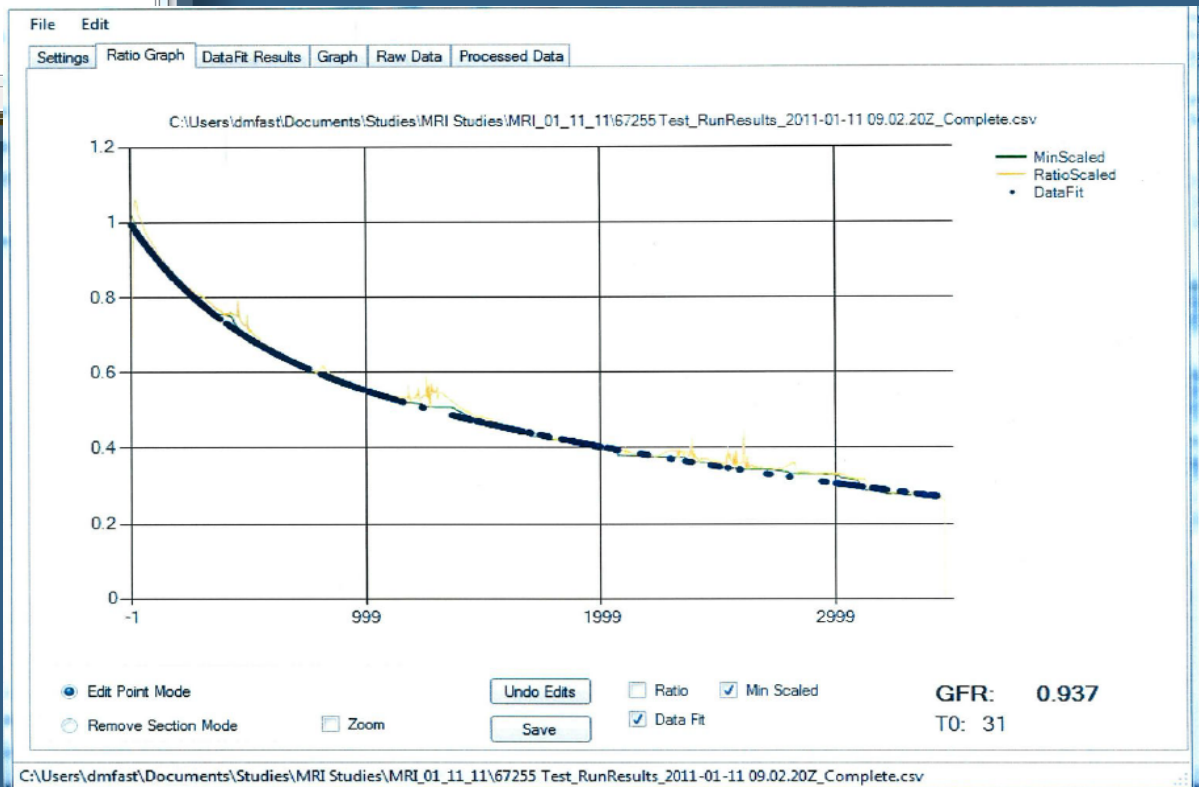
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Graph
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MinScaled
RatioScaled
DataFit
1.2
1
0.8
0.6
0.4
0.2
0
-1
999
1999
2999
Edit Point Mode
Remove Section Mode
Zoom
Undo Edits
Save
Ratio
Min Scaled
Data Fit
GFR: 0.937
T0: 31
C:\Users\dmfast\Documents\Studies\MRI Studies\MRI_01_11_11\67255 Test_RunResults_2011-01-11 09.02.20Z_Complete.csv

Table 1. Investigational uses for multi-photon microscopy

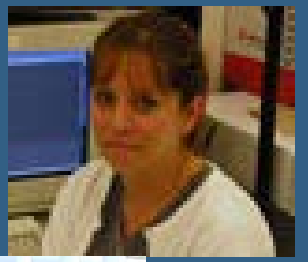
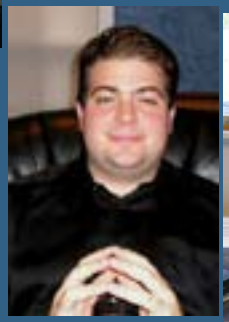
Glomerular
Size/volume
Permeability/filtration
Fibrosis/sclerosis
Microvasculature
RBC flow rate
Endothelial permeability
WBC adherence/rolling
Vascular diameter
Cellular uptake
Cell type-specific uptake
Site – apical vs. basolateral membrane
Mechanism – endocytosis vs. carrier/transporter mediated
Cellular trafficking
Intracellular organelle distribution
Cytosol localization
Cellular metabolism
Fluorescence decay over time
Cell toxicity
Cell injury in necrosis, apoptosis
Surface membrane/blebbing
Mitochondrial function
Glomerular filtration rate determination










IUSM Cellular & Integrative Physiology
Mouhamad Alloosh
Michael S Sturek