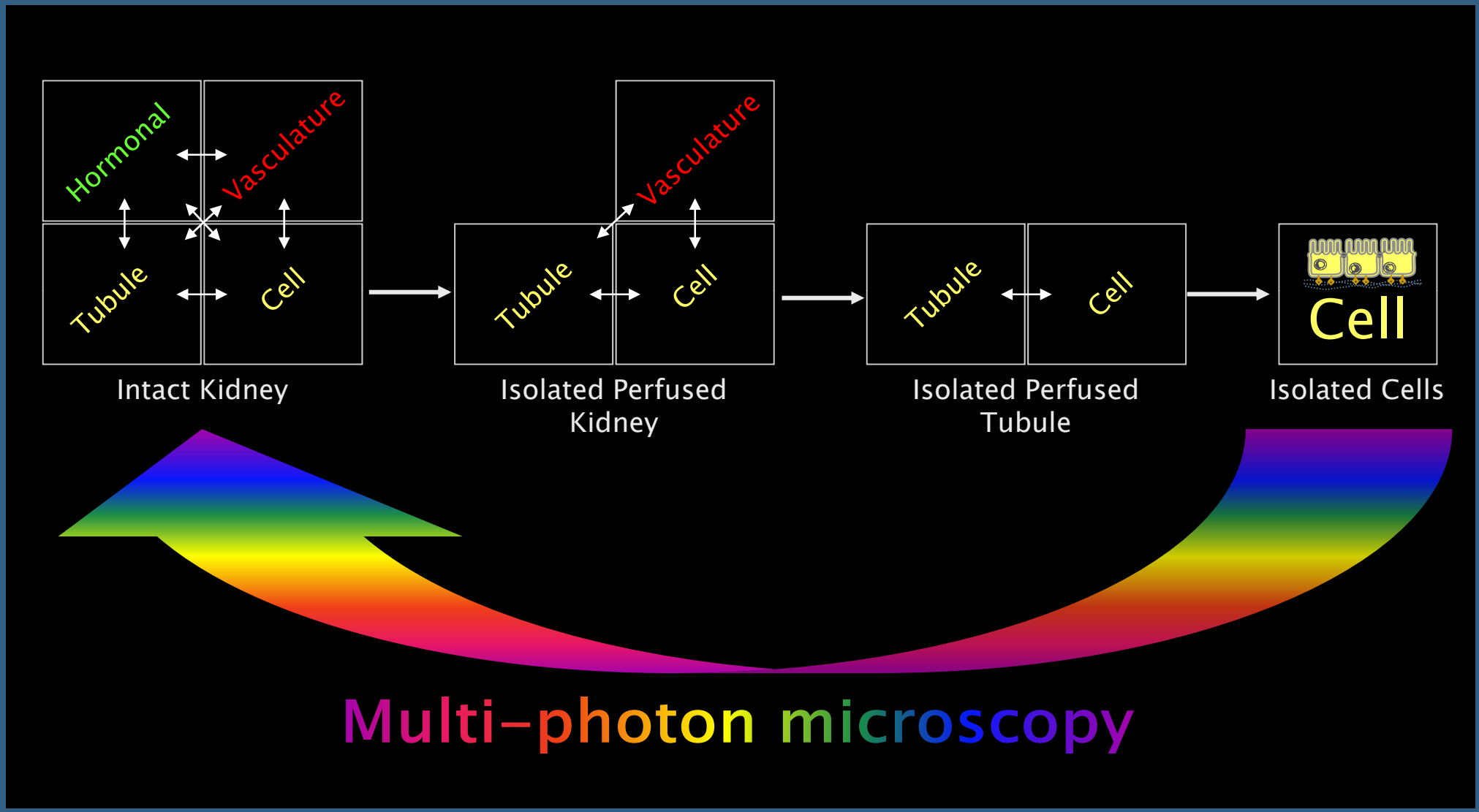
Visualization of Kidney Dynamics

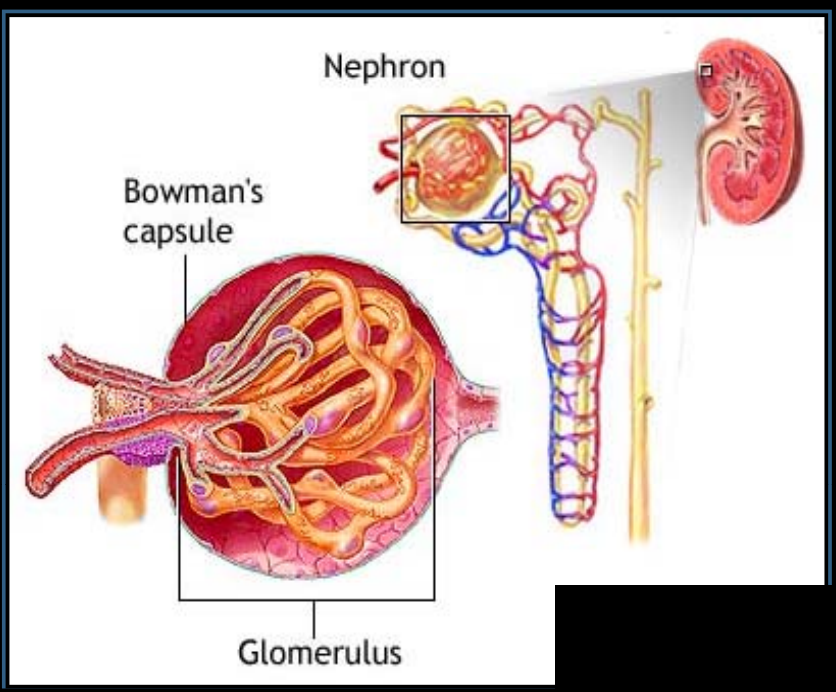
Bruce A. Molitoris
Department of Medicine
Indiana Center for Biological Microscopy
Indiana University School of Medicine

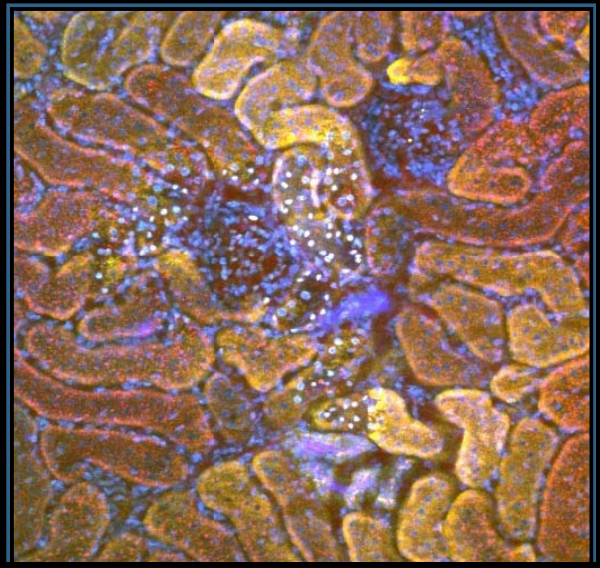
Functional and Therapeutic Studies *in Vivo*

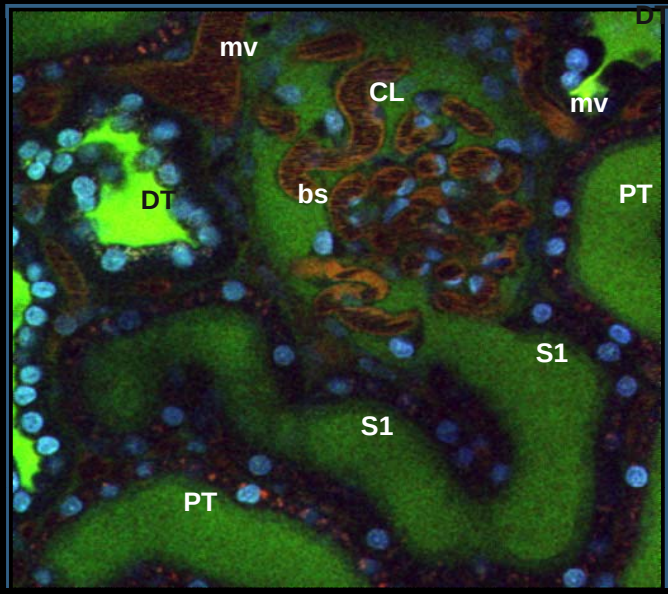


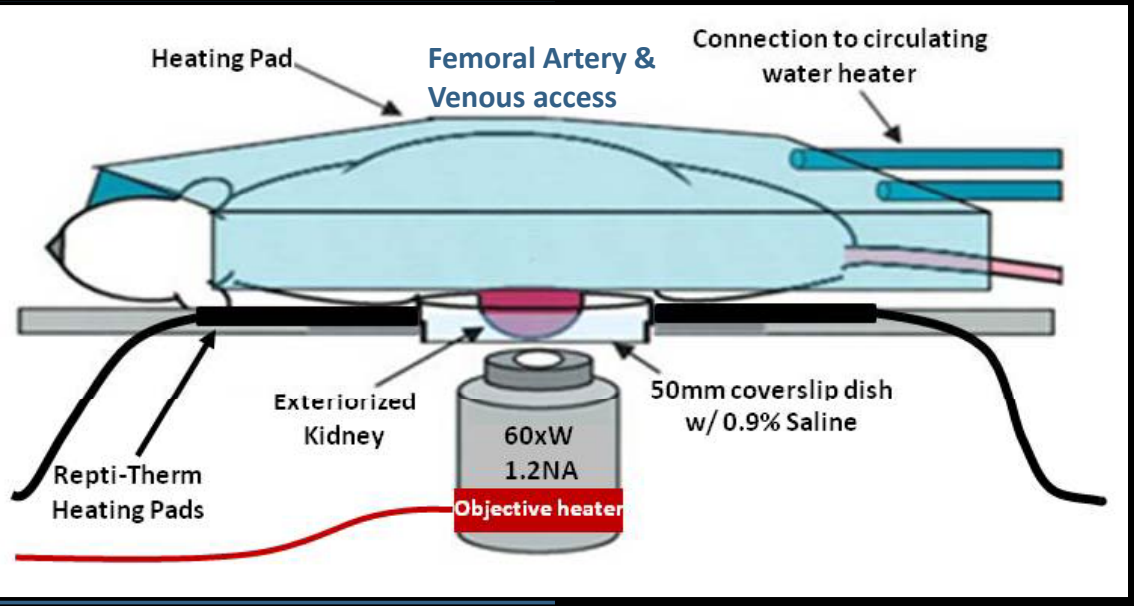
Molitoris and Sandoval AJP, 2005

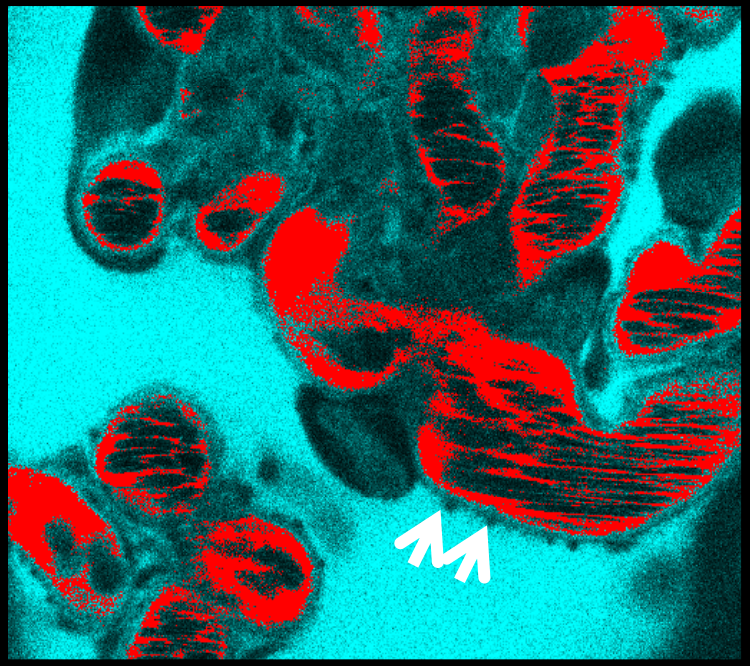
Visualizing Glomerular Function

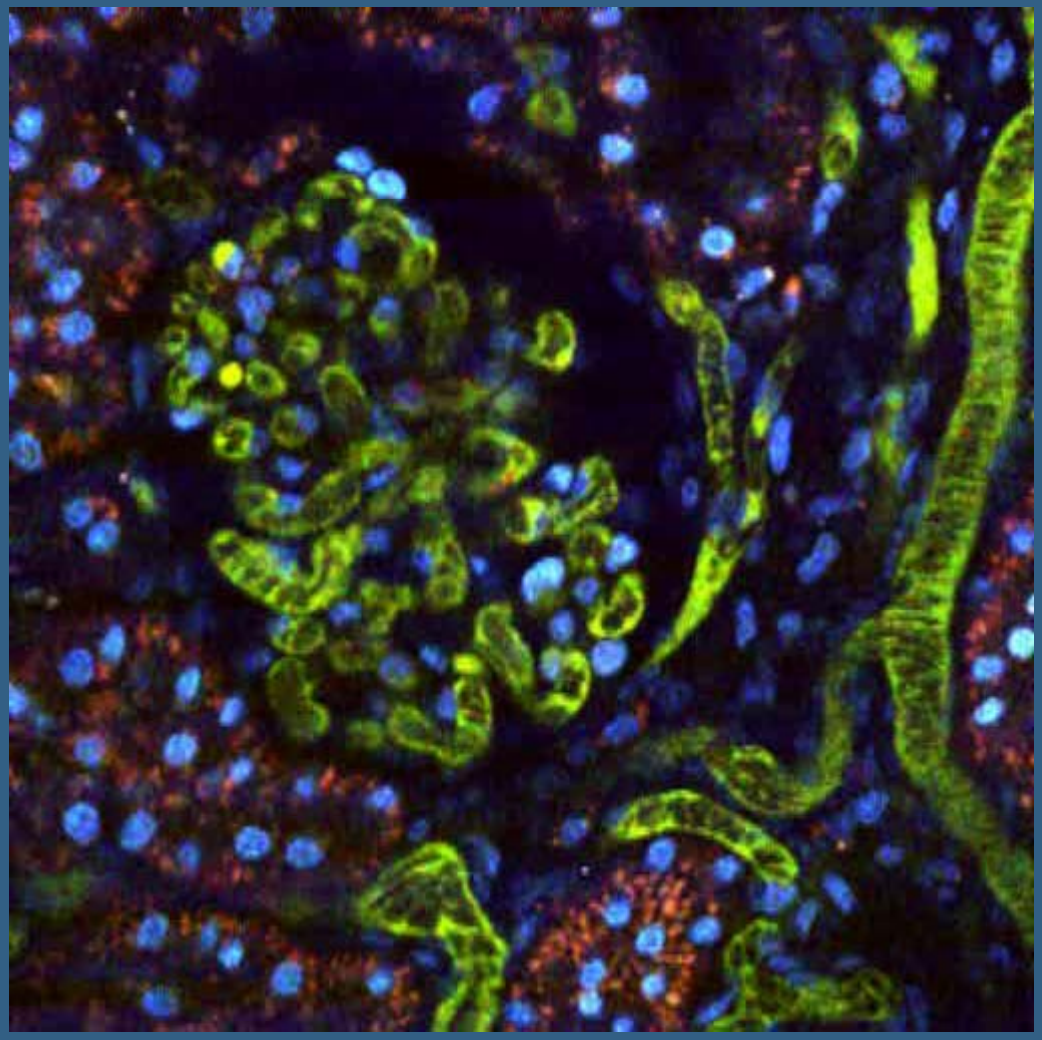








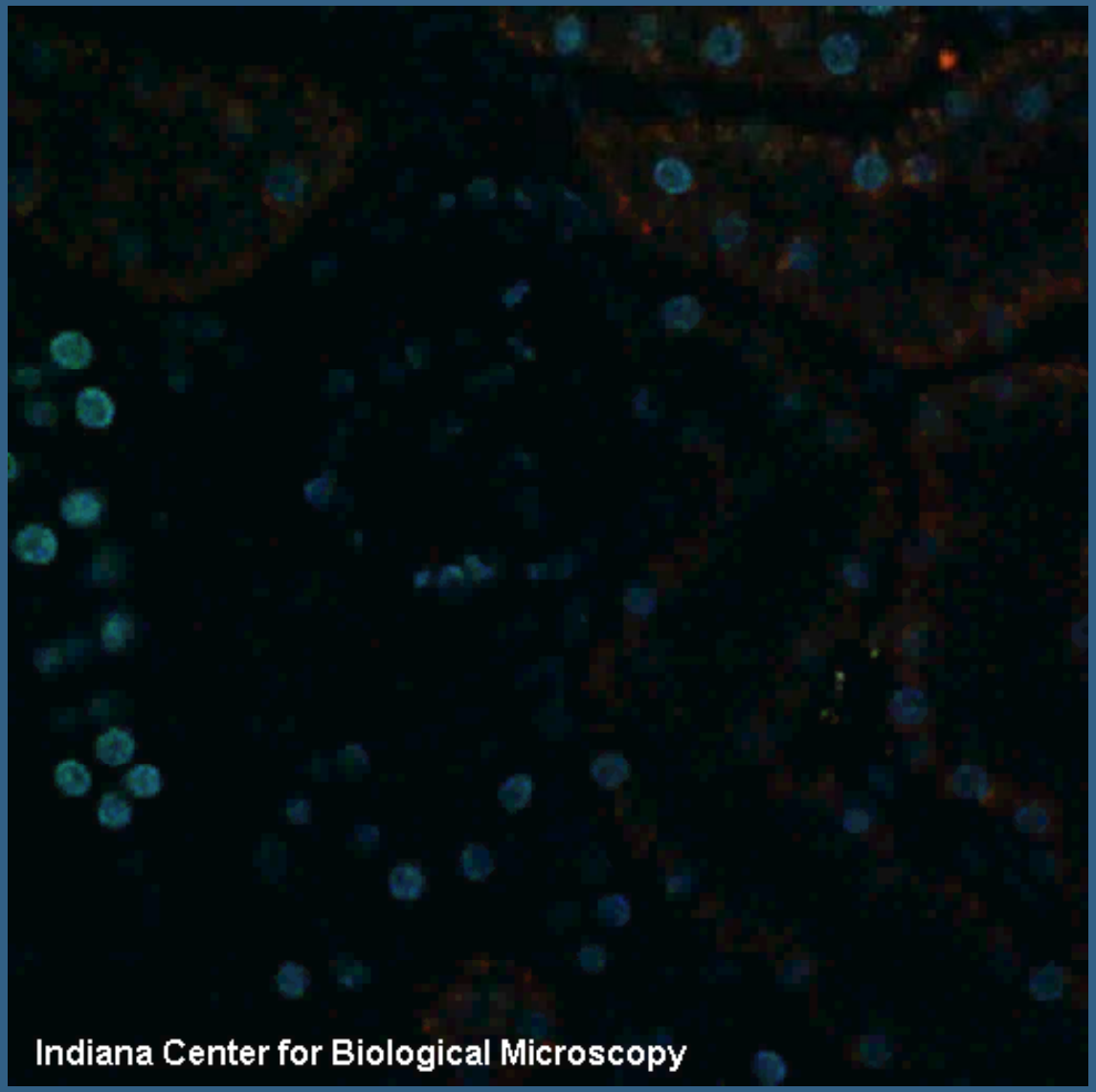




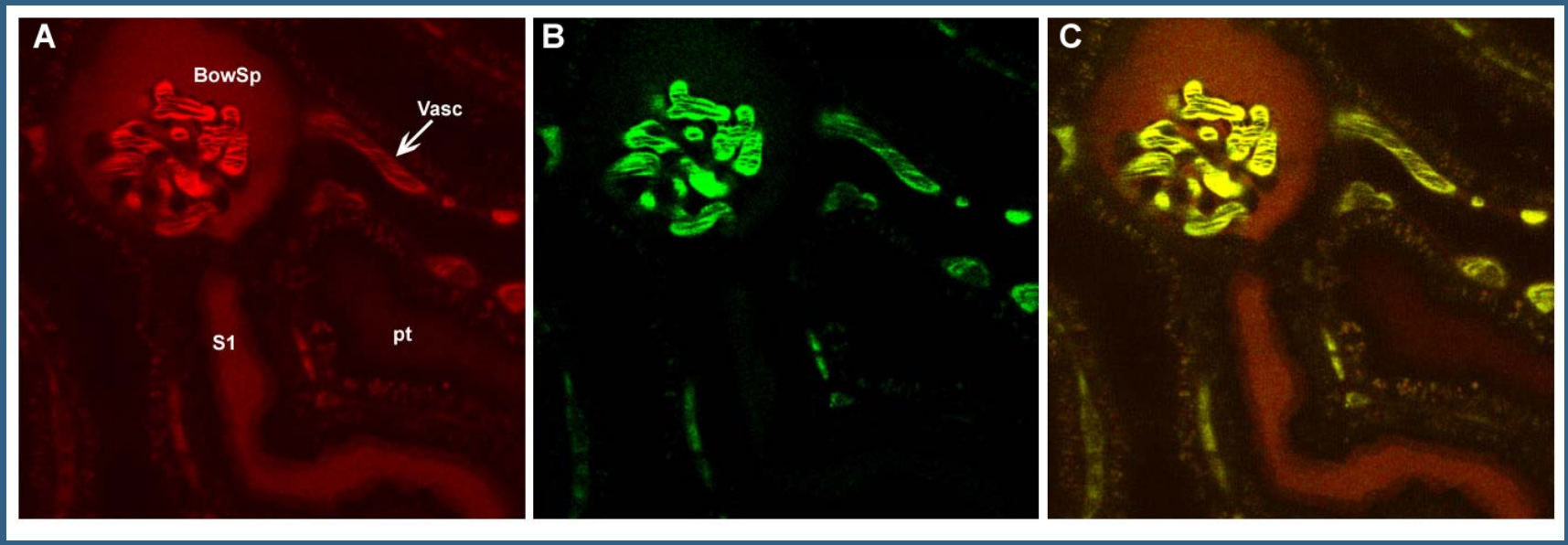
500K Mw FITC-Dextran (Green)

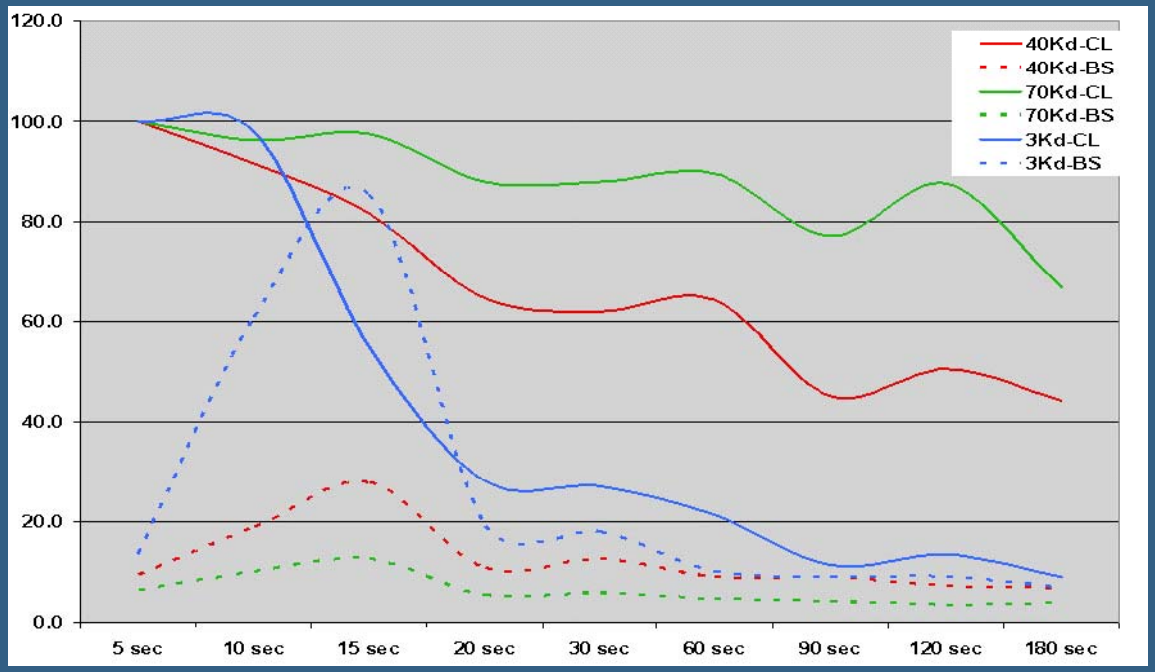
10K Mw Rhodamine Dextran (Red

Hoechst 44432 (Blue)


Indiana Center for Biological Microscopy

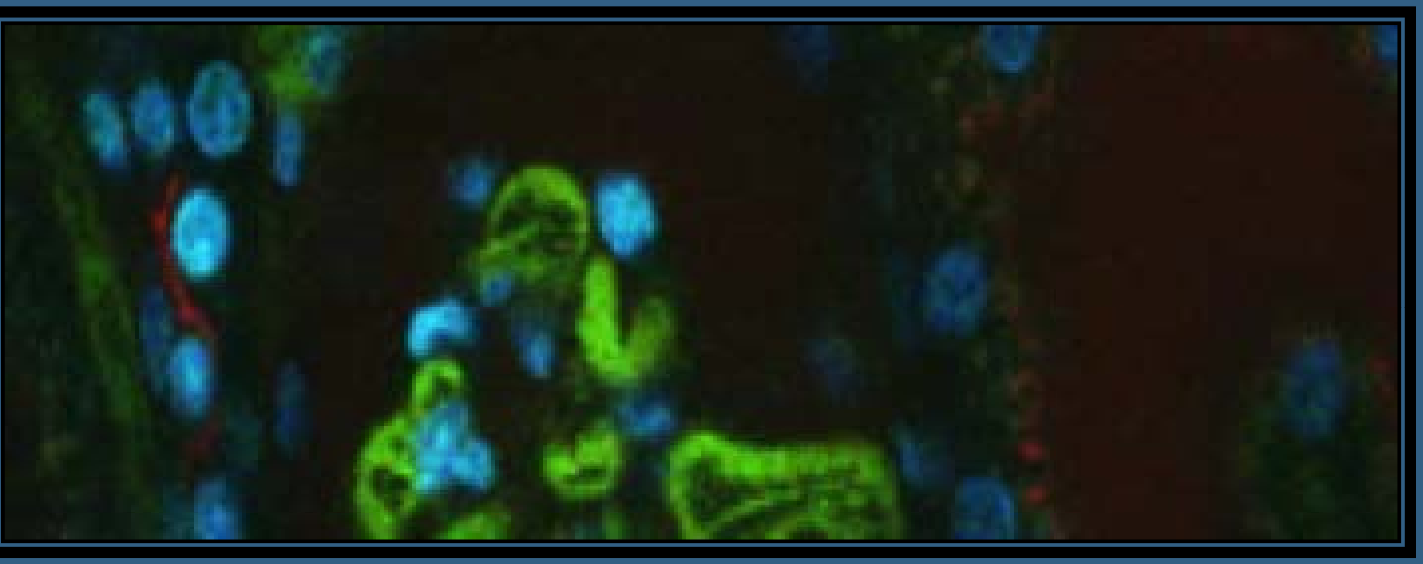
Glomerular Permeability and Vascular Clearance

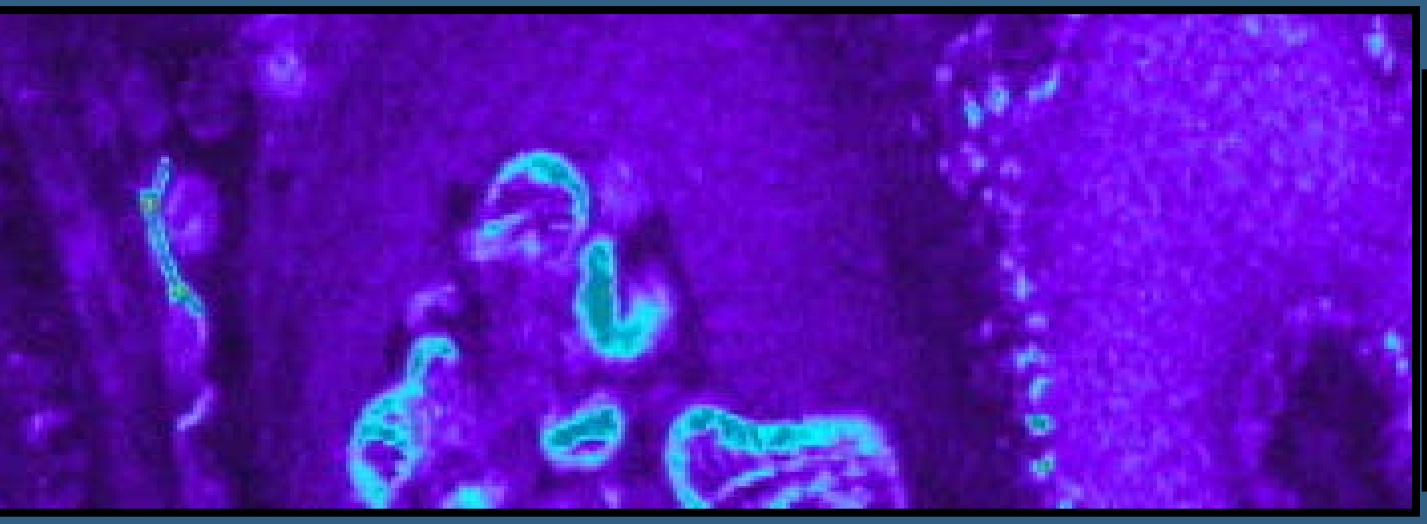


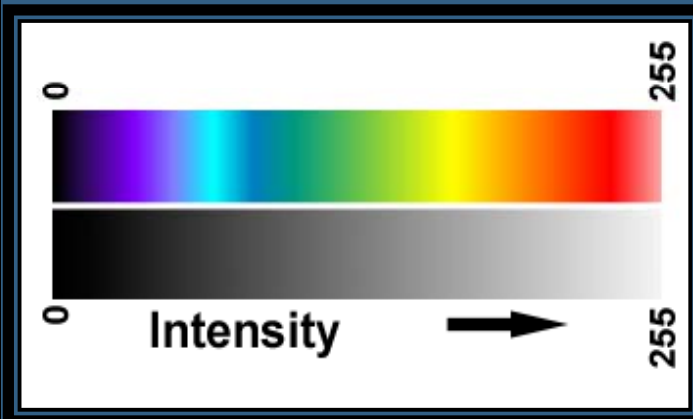


Reducing Scan Size

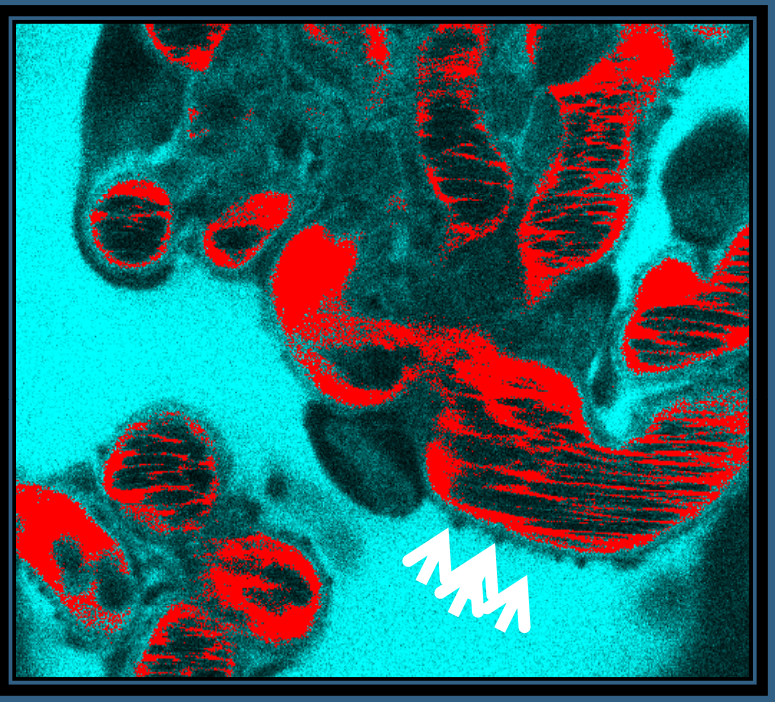
5 Frames/sec
500kDa FITC Dextran with 3kDa TR Dextran Injection

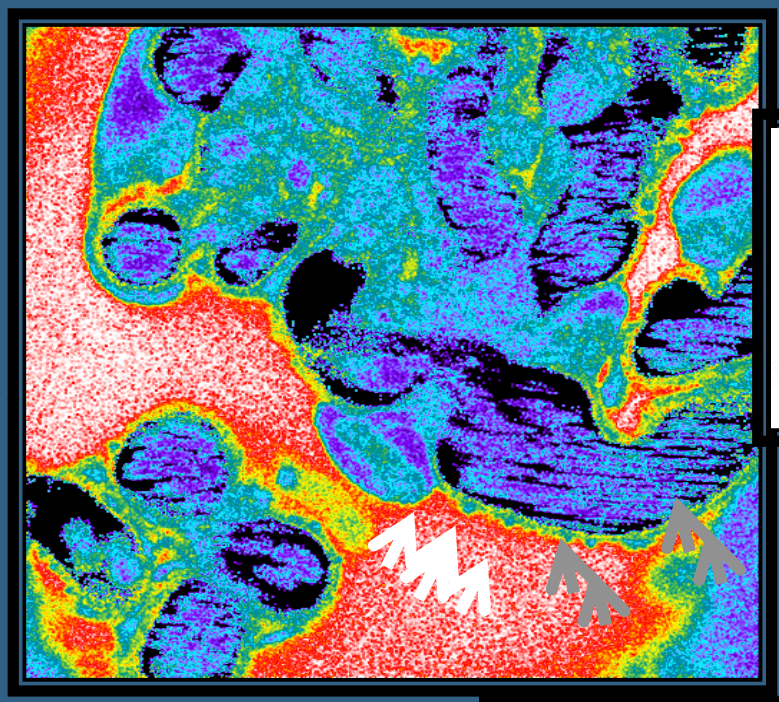


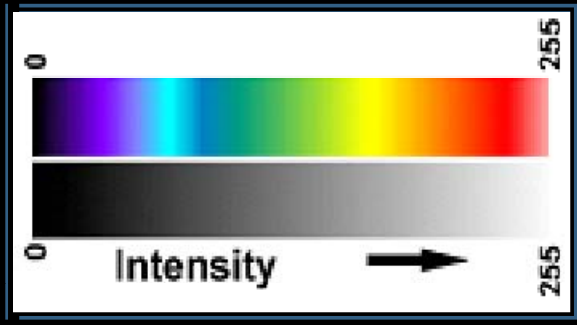


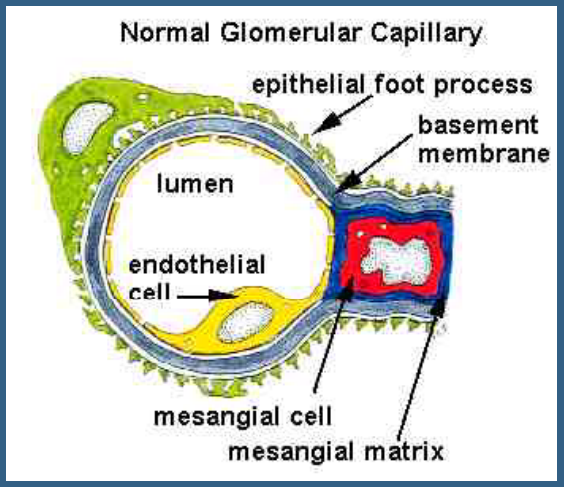


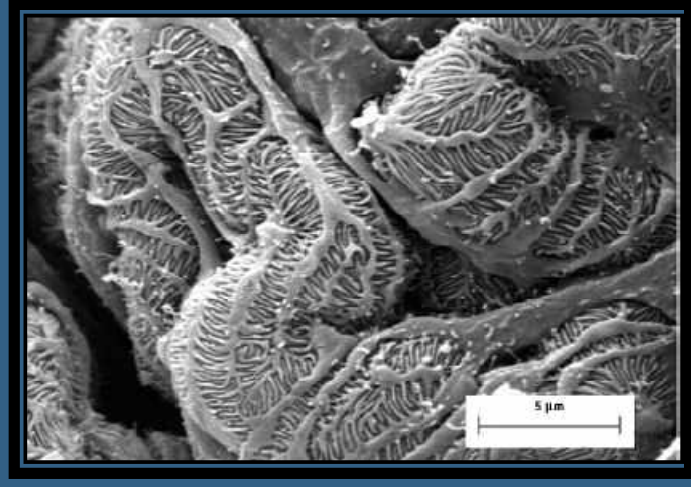
Visualizing Filtration the Basement Membrane & Podocyte

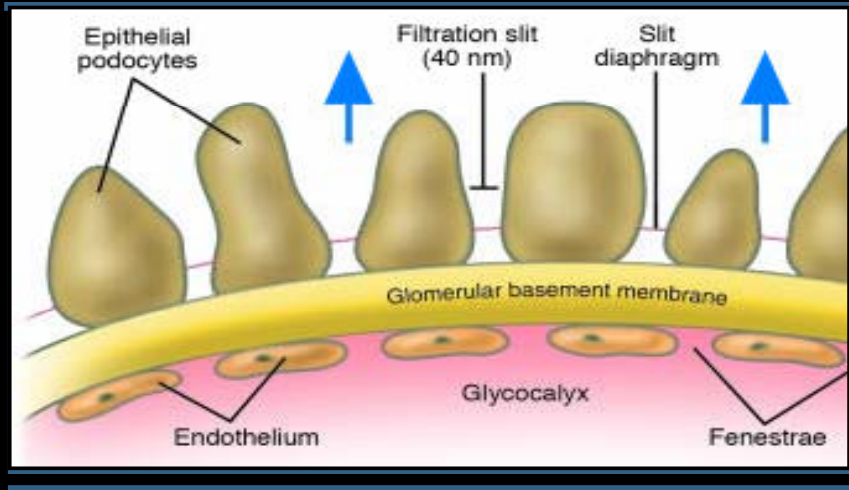












Question:

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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ISN
kidney
INTERNATIONAL
VOLUME 71 | ISSUE 6 | MARCH (2) 2007
http://www.kidney-international.org
Albumin filtration
Classification of lupus nephritis
Peritoneal dialysis solutions

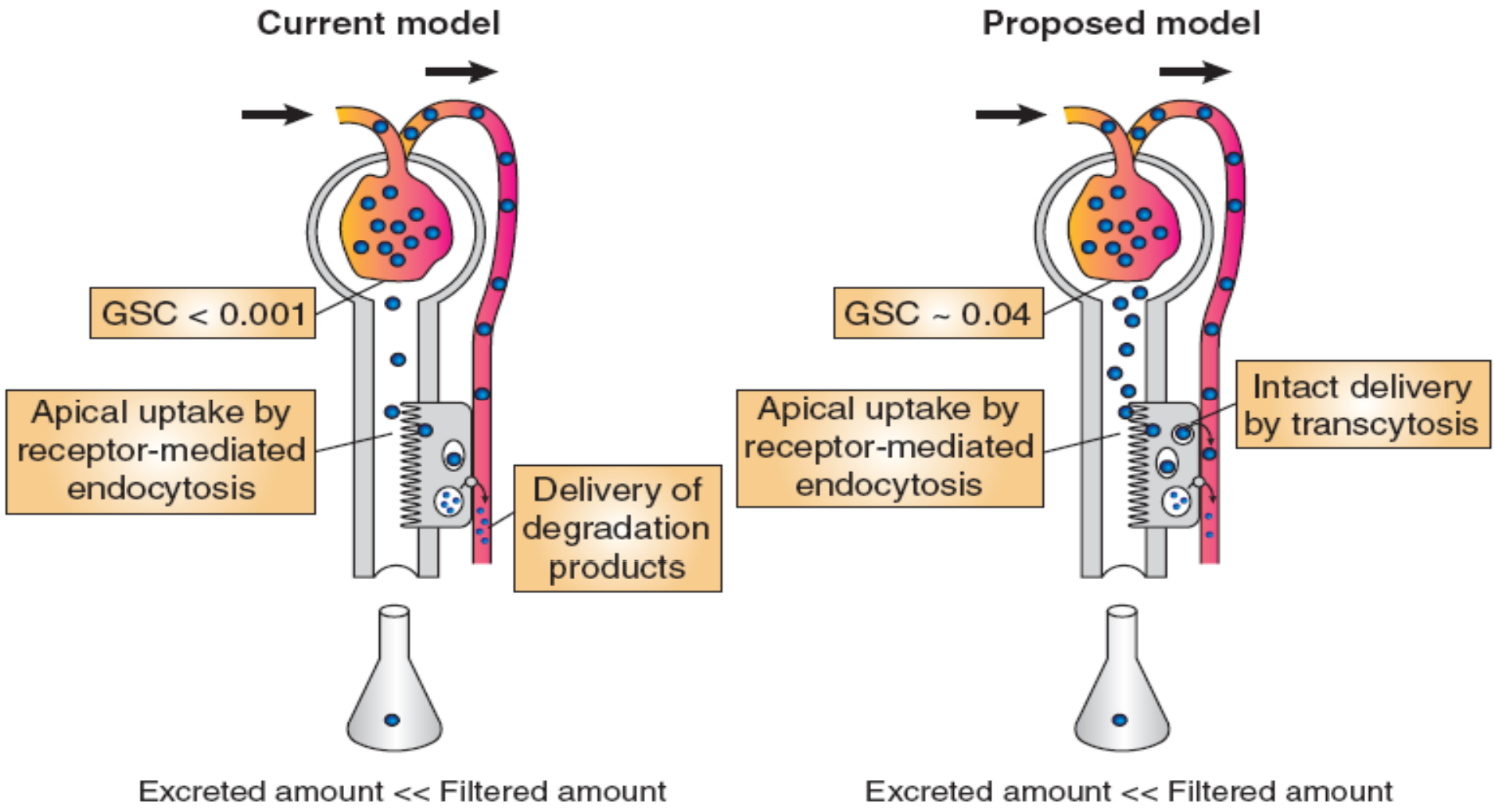
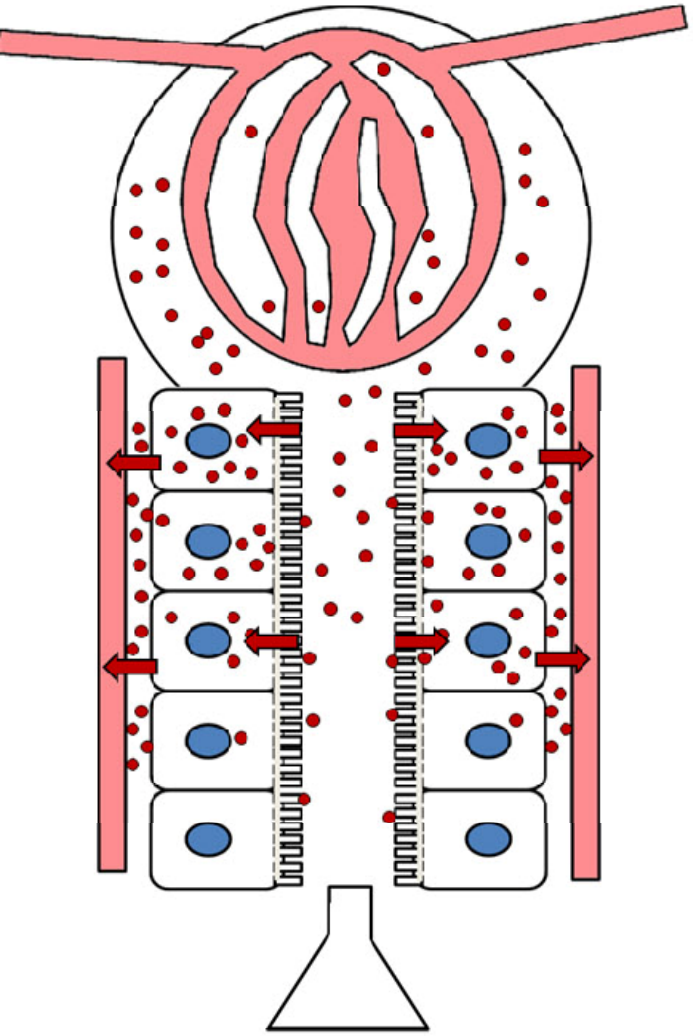
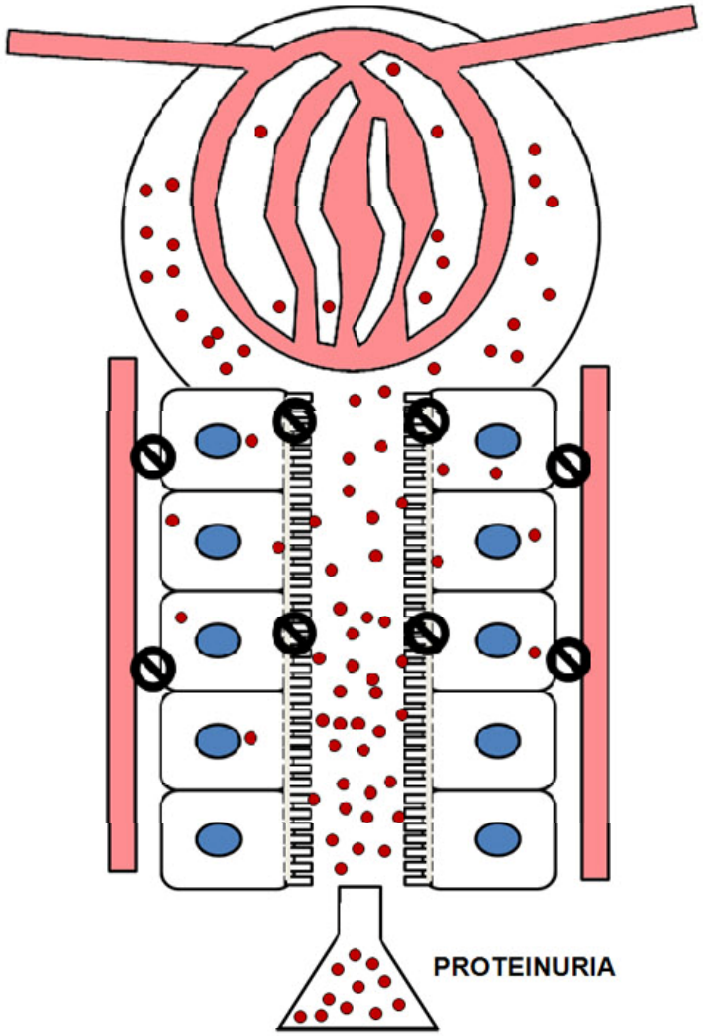
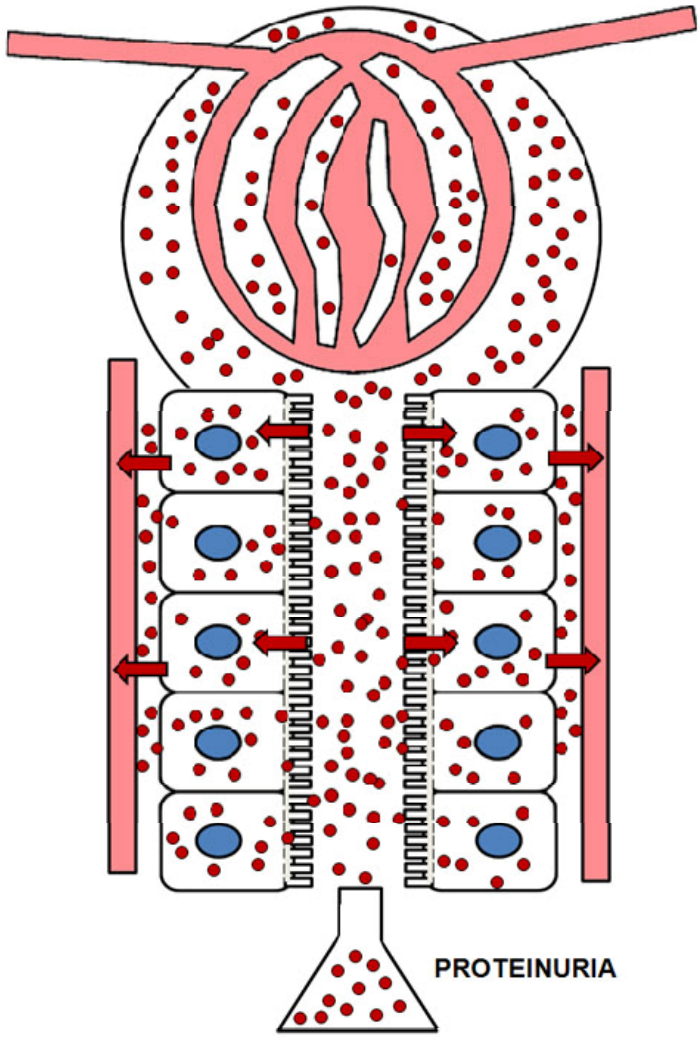


Figure 1| Comparison of the current model of renal albumin handling, as described in most of the publication, with the new model proposed by Russo *et al.* GSC, glomerular sieving coefficient.




PROTEINURIA


PROTEINURIA

Fast Track

Rf-2 Gene Modulates Proteinuria and Albuminuria Independently of Changes in Glomerular Permeability in the Fawn-Hooded Hypertensive Rat

Artur Rangel-Filho,*† Mukut Sharma,‡ Yvonne H. Datta,†† Carol Moreno,*† Richard J. Roman,† Yoshiki Iwamoto,*‖ Abraham P. Provoost,** Jozef Lazar,*¶ Howard J. Jacob*†§

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J Am Soc Nephrol 16: 852–856, 2005. doi: 10.1681/ASN.2005010029

We report that *Rab38*, a gene within the *Rf-2* locus appears to influence the development of proteinuria (UPV) and albuminuria (UAV) in fawn-hooded hypertensive rats (FHH). Using congenic animals, we narrowed the region to eight genes; however, only one gene had a sequence variant. *Rab38* has a mutation in the start codon, resulting in a natural knockout in the FHH strain. Despite no differences in glomerular albumin permeability, congenic animals carrying the wild-type Brown Norway (BN) allele of *Rab38* on the FHH background exhibited, on average, 40% and 60% less UPV and UAV, respectively, than FHH. These findings suggest that *Rab38* may modulate the tubular processing of filtered proteins without affecting the glomerular filtration barrier. This is the first gene reported for an animal model of hypertension-associated renal failure. This gene resides on human chromosome 11, which has been linked to renal disease.

The genetic dissection of quantitative traits, such as renal failure, has proven a challenging task in humans because of their polygenic nature and interactions with the environment (19,27). One solution is to use animal models to study the genetic basis of ESRD (17,18). The first direct genetic evidence for hypertension-associated renal disease came from the FHH strain, in which five genomic regions or quantitative trait loci (QTL) (*Rf-1* through *Rf-5*) have been linked to the development of UPV, UAV, and focal glomerulosclerosis (1,2,31). Since then, several groups have found the homologous regions in humans to be also linked to renal failure (10,13,9,37). The *Rf-2* locus, located on rat chromosome 1, showed a recessive mode of inheritance with significant linkage to UPV (logarithm of the odds ratio score 5.39) and UAV (logarithm of the odds ratio score 6.50) (31). This locus accounts for approximately 30 to 40% of the urinary protein excretion (1). Studies in other rat models of renal failure have confirmed a role for the *Rf-2* region in the development of UPV and UAV (33,28,38,29). In addition, Winn *et al.* (37) have reported linkage to a familial form of focal segmental glomerulosclerosis (FGS) in a region of human chromosome 11 syntenic to *Rf-2* in rat. In this study, we report that a natural knockout of the *Rab38* gene is likely the *Rf-2* gene. We investigated the effects of restoring Rab38 protein expression on UPV, UAV, and the permeability of isolated glomeruli to albumin. Finally, through comparative genomics we constructed a map of the synteny between the rat and human QTLs at the gene level of resolution.

Materials and Methods

Generation of Congenic Animals and Sequencing of Candidate Genes

All experiments were performed in compliance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Congenic animals were developed by marker-assisted selective breeding of FHH and BN rats as reported previously (25). Sequencing of positional candidate genes was performed using genomic DNA and cDNA on an ABI3730 capillary sequencer according to the manufacturer's suggested protocol.

Urinary Protein/Albumin Excretion and Assessment of Glomerular Permeability

Urine from 12-wk-old animals, fed standard rat chow, was collected in two consecutive 24 h periods and analyzed for total protein by the Weichselbaum's Biuret method (36). Albumin excretion was measured using the AB580 assay (16). Results are reported as the average of the two collection days.

Glomerular permeability was determined using an *in vitro* functional assay as described previously (30).

Blood Pressure Measurement

BP was measured directly, in conscious rats, by cannulation of the right femoral artery as reported before (34).

Western Immunoblotting

Proteins from an SDS-polycrylamide gel electrophoresis of whole kidney homogenate of 12-wk-old FHH, BN, and FHH.BN-*Rab38* con-

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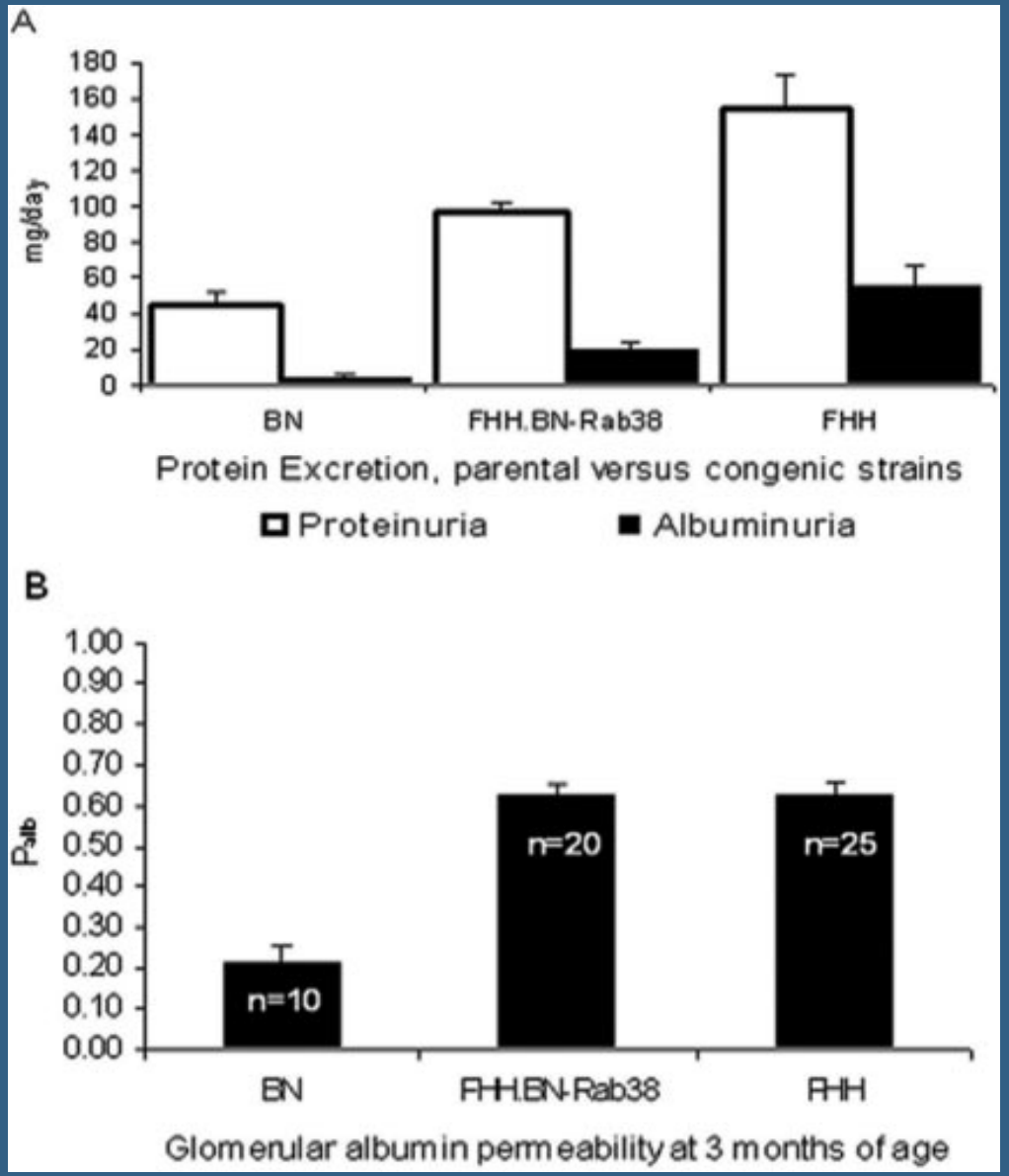


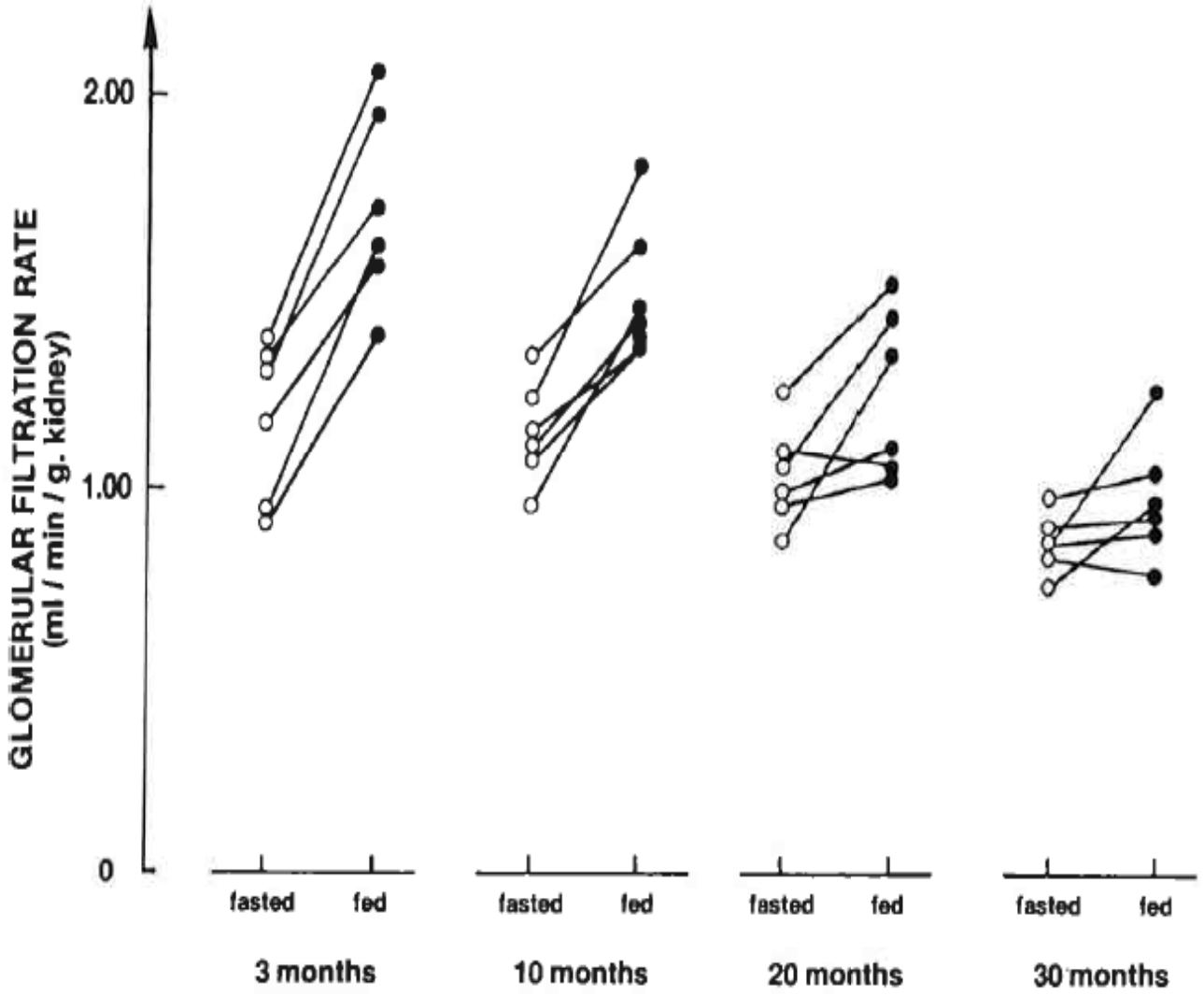


FIG. 2. Glomerular filtration rate in fed and fasted rats. Lines join data from same animals.

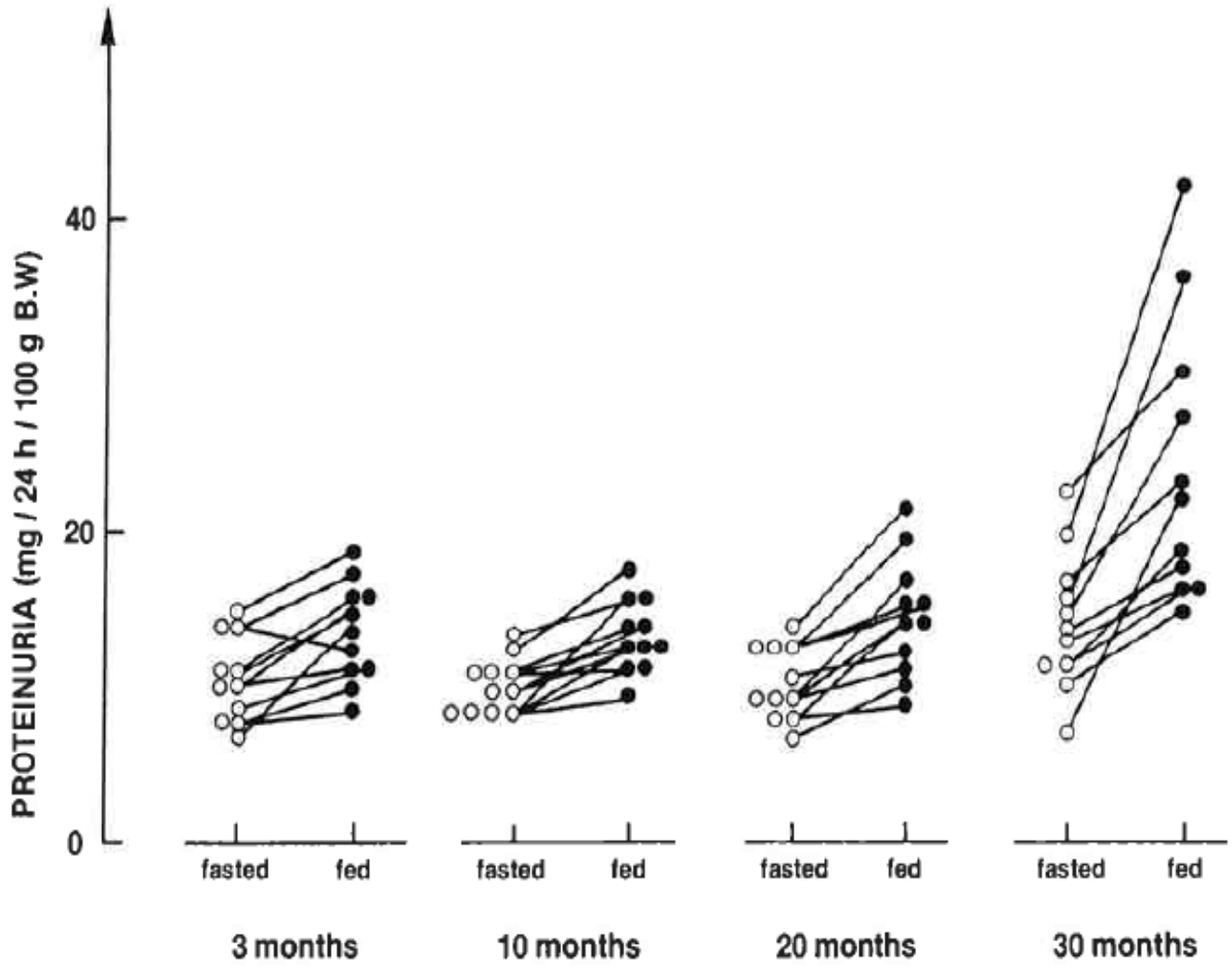
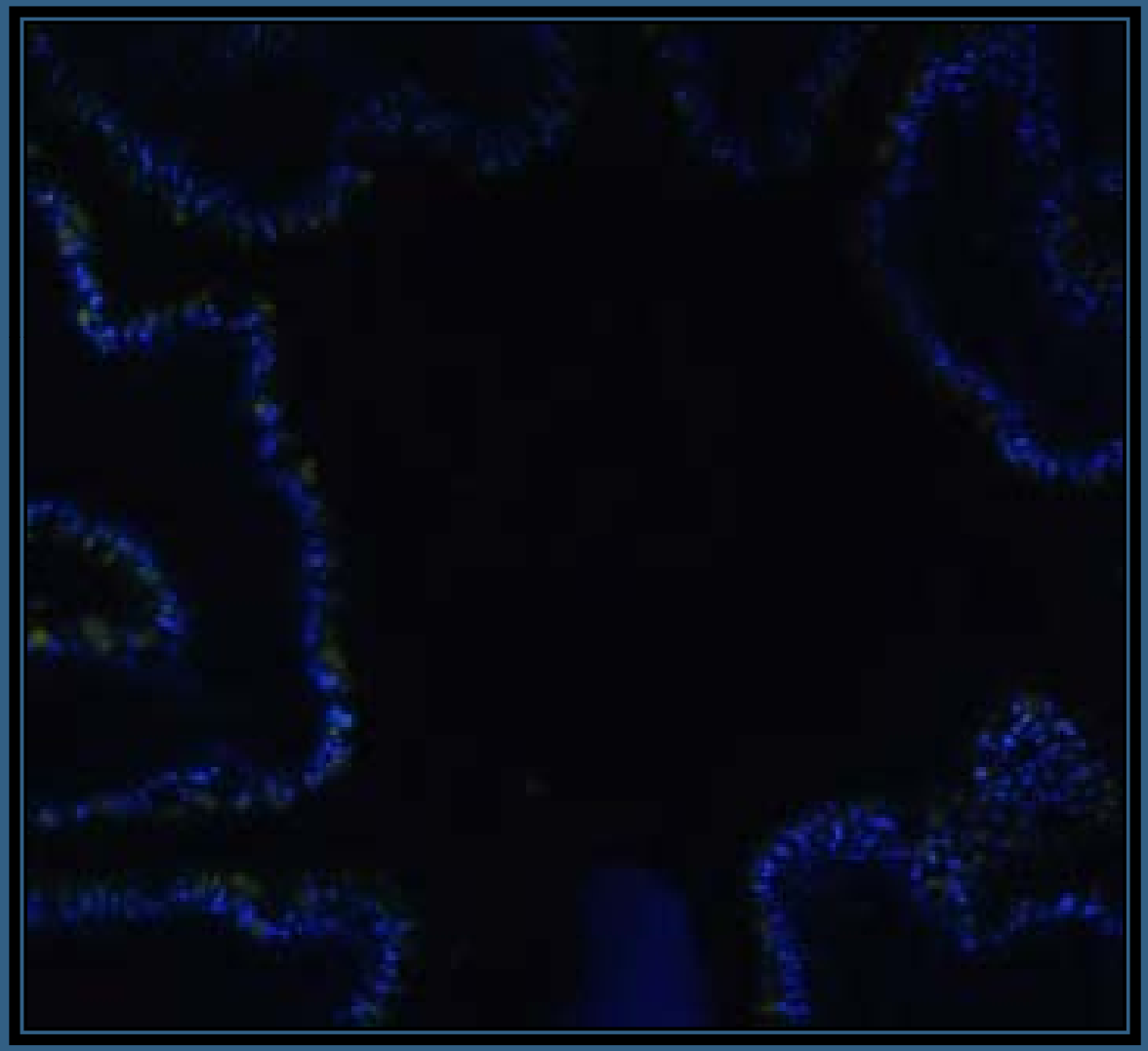
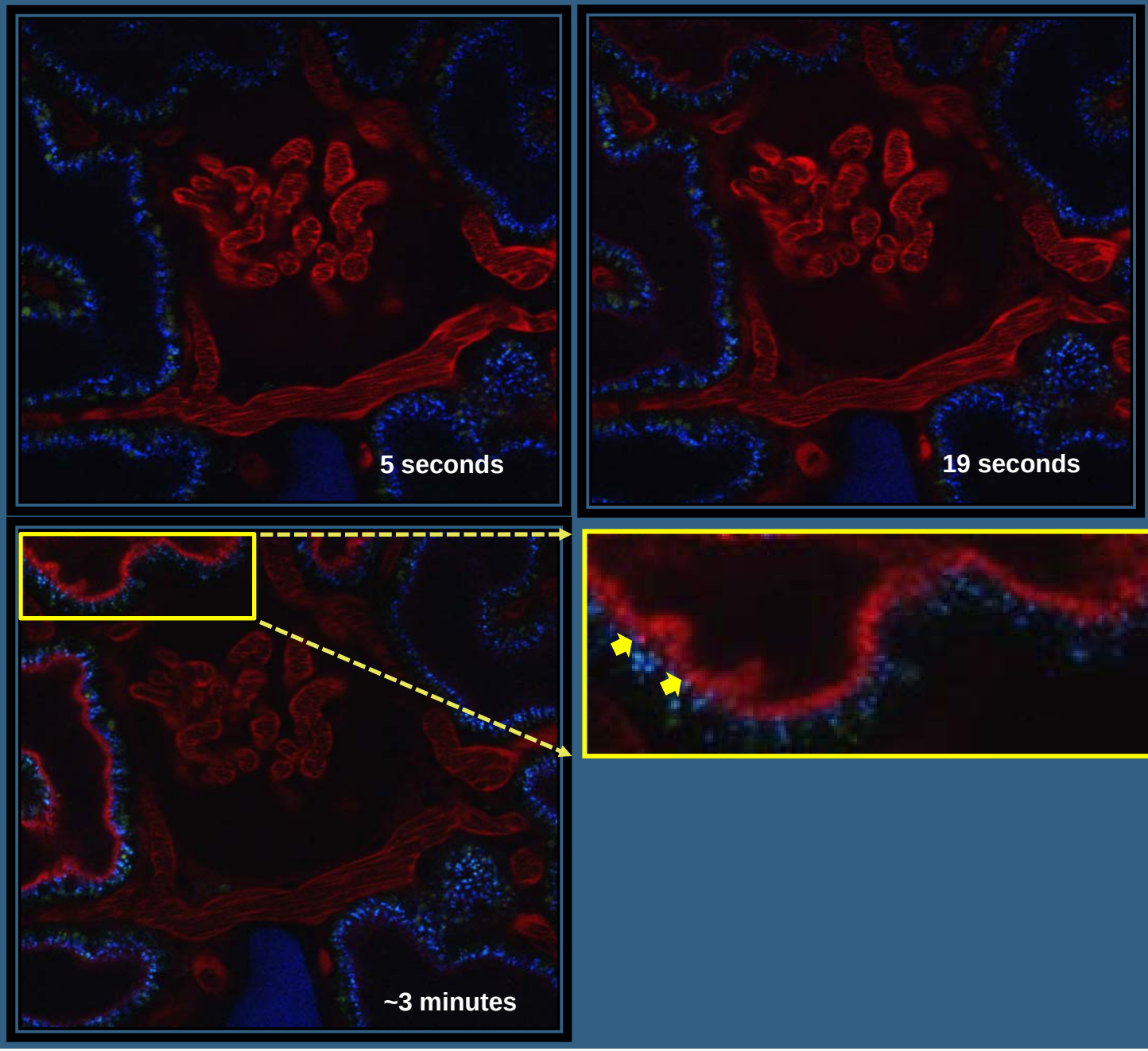


FIG. 1. Daily proteinuria in fed and fasted rats. Lines join data from same animals.

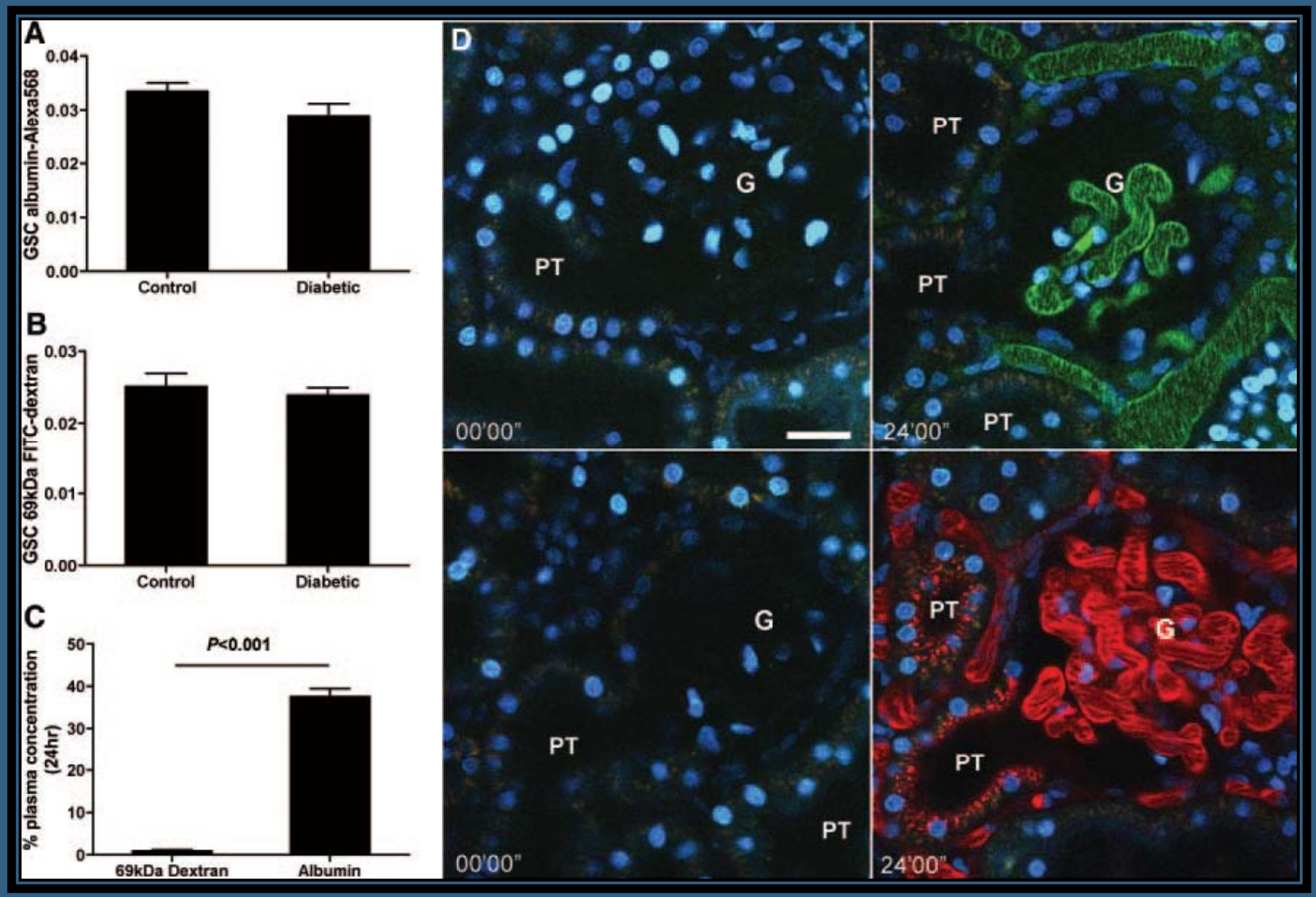
Albumin Filtration and Reabsorption in the Rat



Albumin Filtration and Reabsorption in the Rat

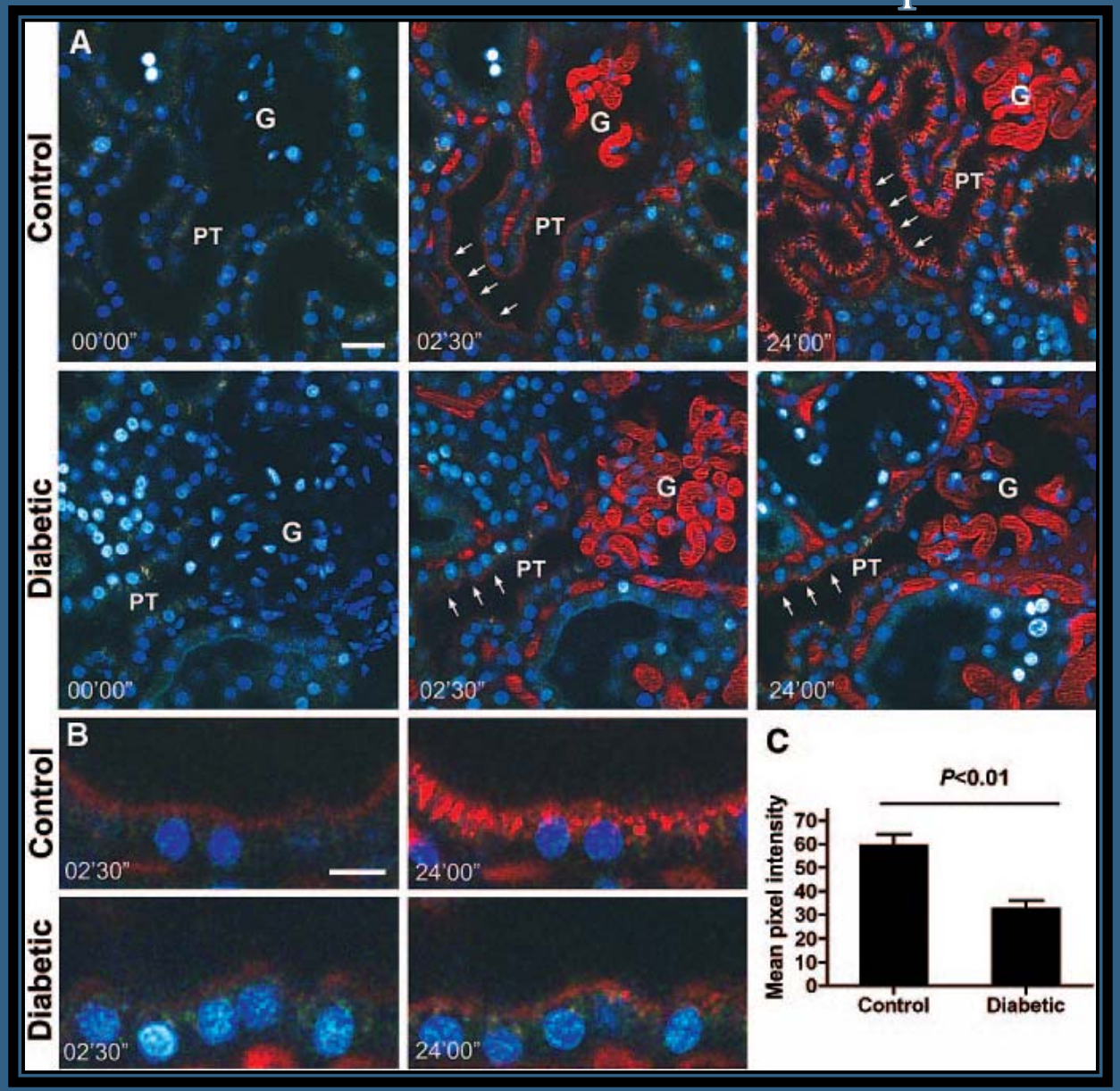


Effect of Early Diabetes in the Rat on Albumin Filtration and Reabsorption



Russo et.al. JASN 2009

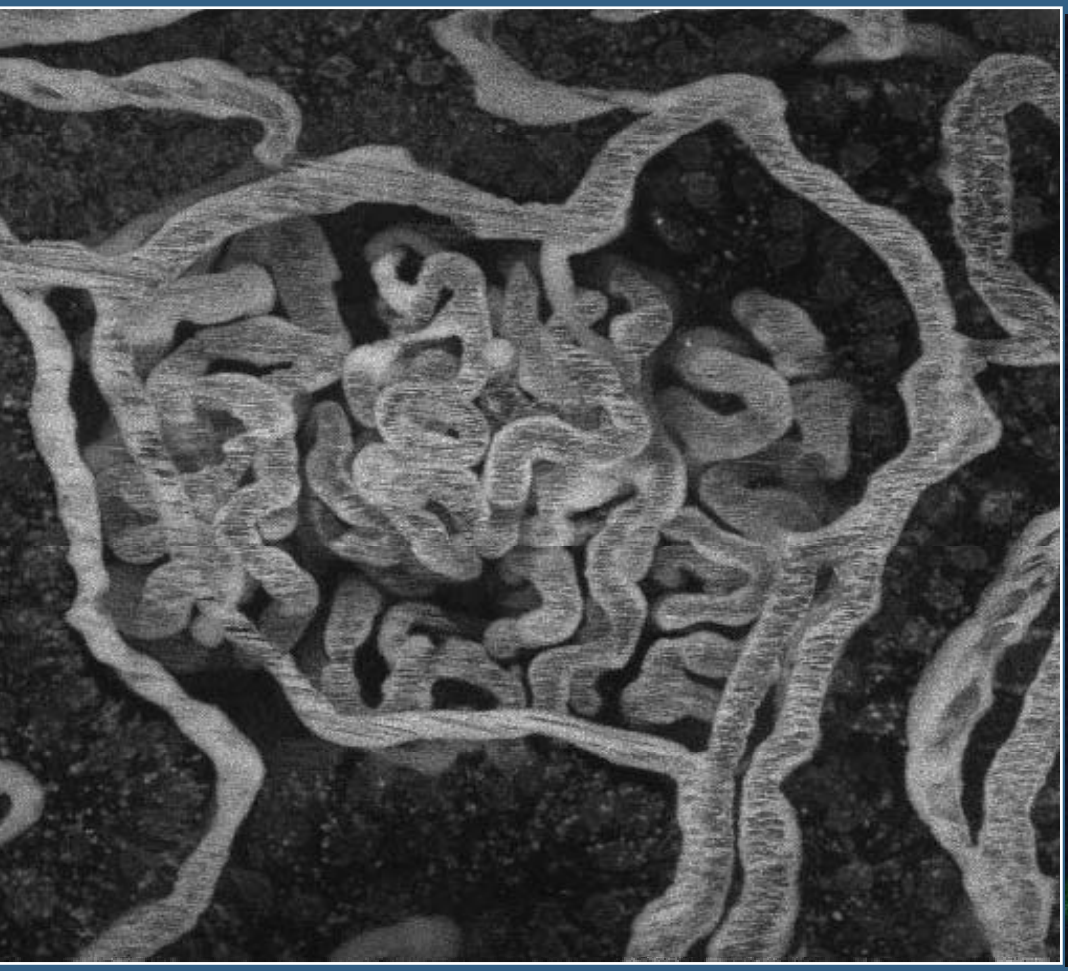
Effect of Early Diabetes in the Rat on Albumin Filtration and Reabsorption

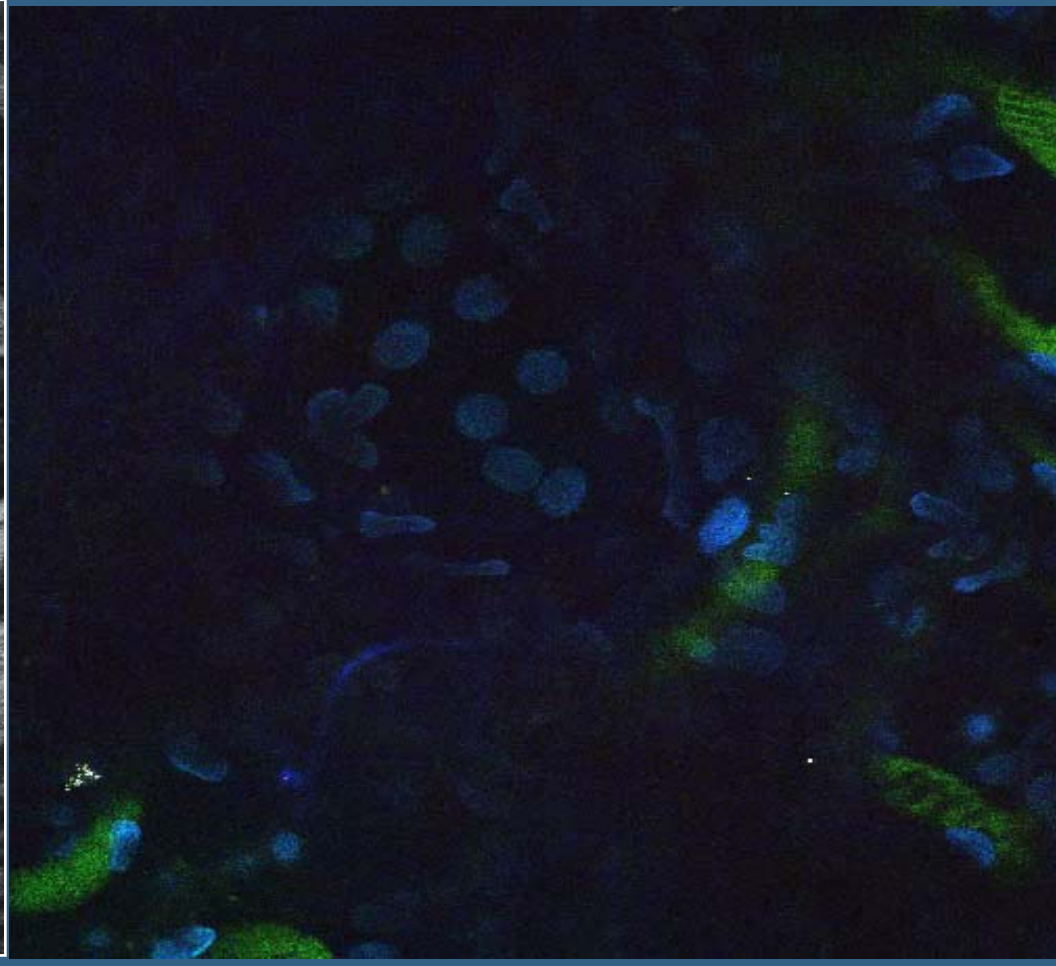


Russo et.al. JASN 2009

Challenges

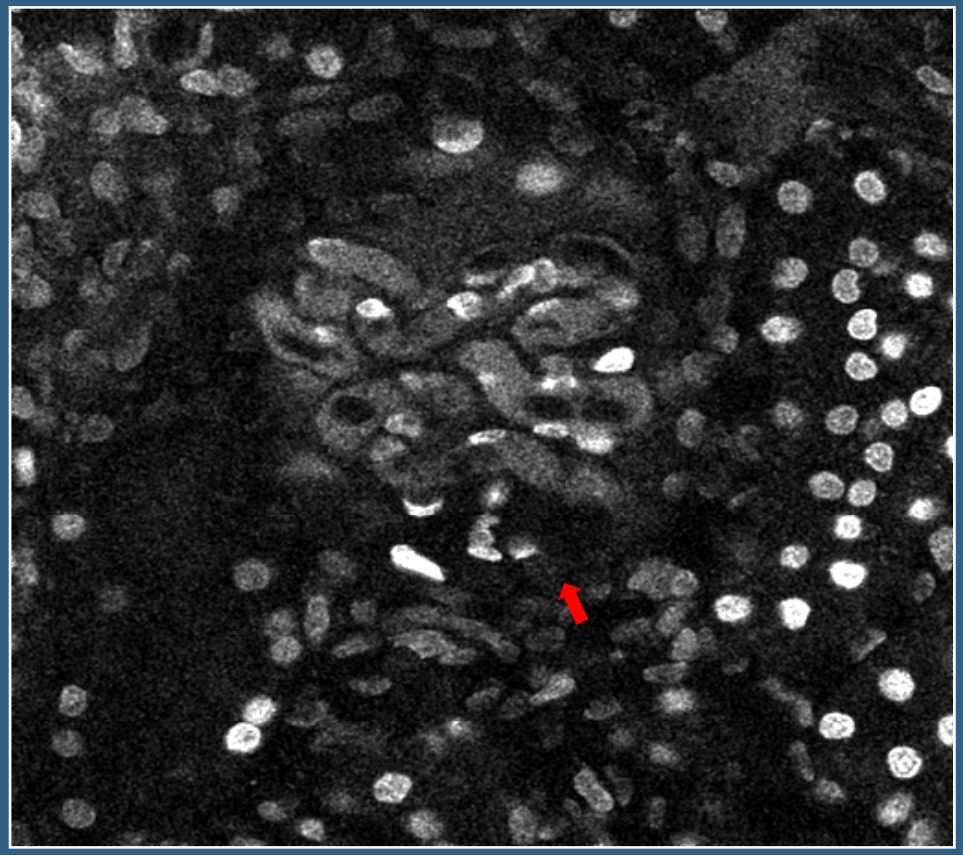
- 1. Dogma, Assumptions, Biology, Reagents, Sensitivity
- 2. Quantitative Analysis without Gold Standards
- 3. You See What you are Looking For
- 4. Correcting for Depth of Field
- 5. Going Deeper
- 6. Out of Focus Fluorescence
- 7. Physiologic state of the rat



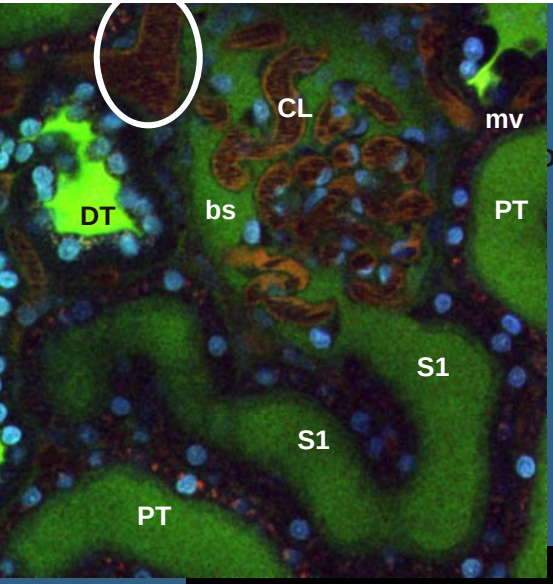


24 Hr CLP Glomerular Flow Heterogeneity

Large 150 kDa dextran



Small 3 kDa dextran



Quantifying Glomerular Filtration

GFR as a Marker of Kidney Function in AKI

1. Historical Marker of Global Kidney Function
2. Multiple Techniques but either Lack Accuracy or Speed of Determination
3. No Clinically Usable Technique for AKI
4. In AKI would have Diagnostic and Severity of Injury Capabilities
5. An Accurate GFR would allow for Earlier Initiation and Termination of RRT
6. May have a Role in Surveillance Technology

Ideal Characteristics of GFR Technique

1. Safe, Inexpensive , Repeatable and Accurate
2. Rapid at Bedside Readout
3. Display Data for Interpretation and Evaluation of Test
4. Minimally Invasive or Noninvasive
5. Administered by Nursing Personnel
6. Independent of Vascular Permeability

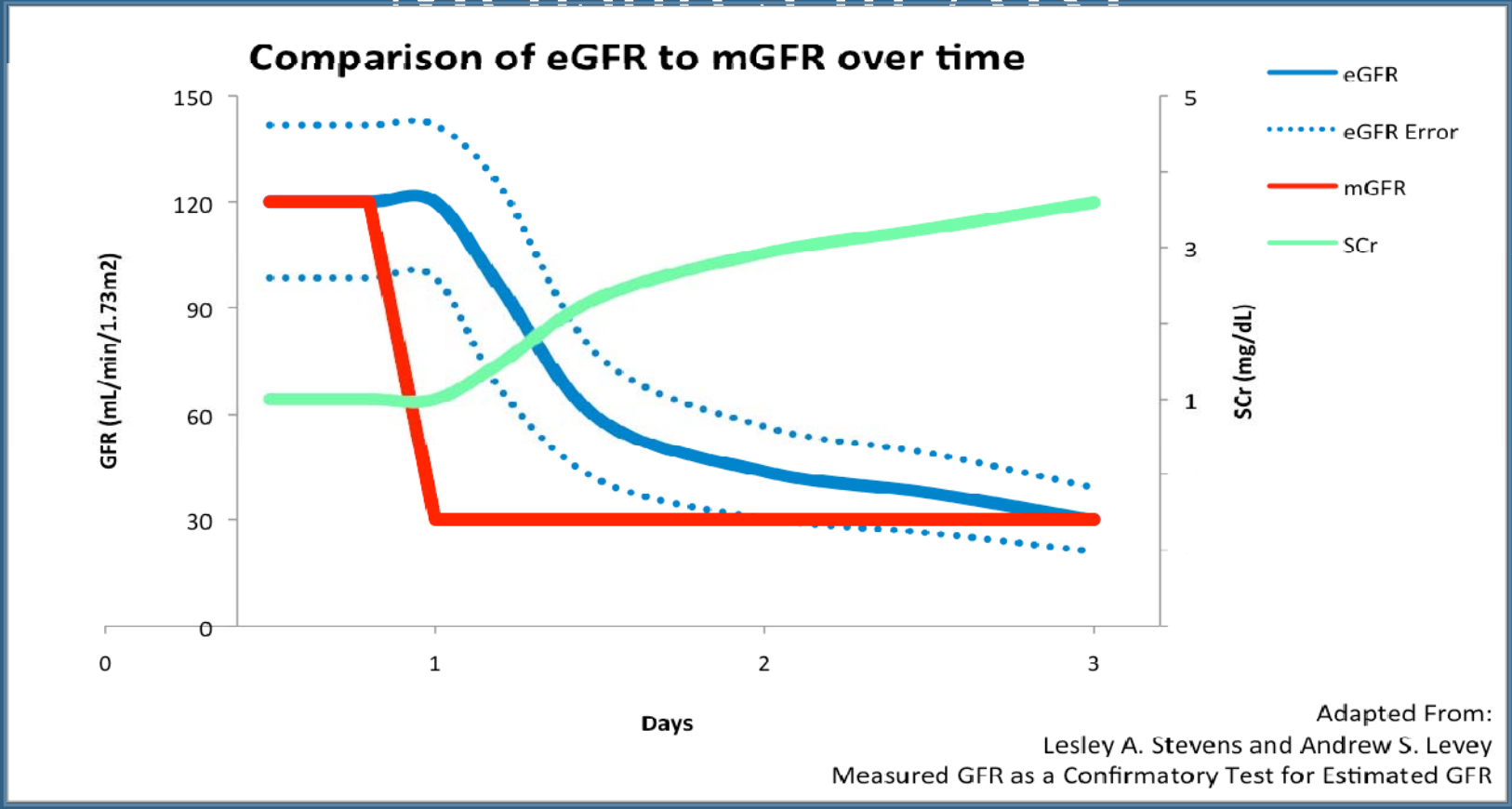
Why is GFR not Determined in AKI Now?

1. Multiple “Gold Standard” Techniques have been developed

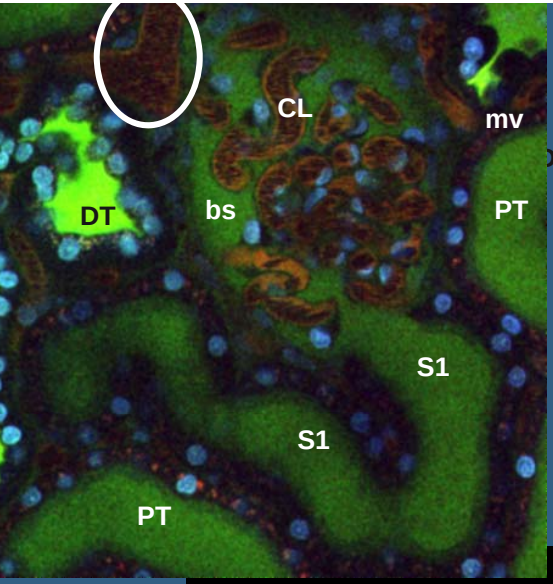
2. Sampling methods such as inulin, iohexol, or iothalimate clearance:

   - All require 6+ hours to administer test - multiple blood draws
   - Require samples to be sent for outside lab analysis, requiring days
   - Possible radiation exposure from injected marker
   - Require moving the patient
   - Too expensive, time consuming and cumbersome to be practical

SCr and eGFR: Inadequate Measures in AKI

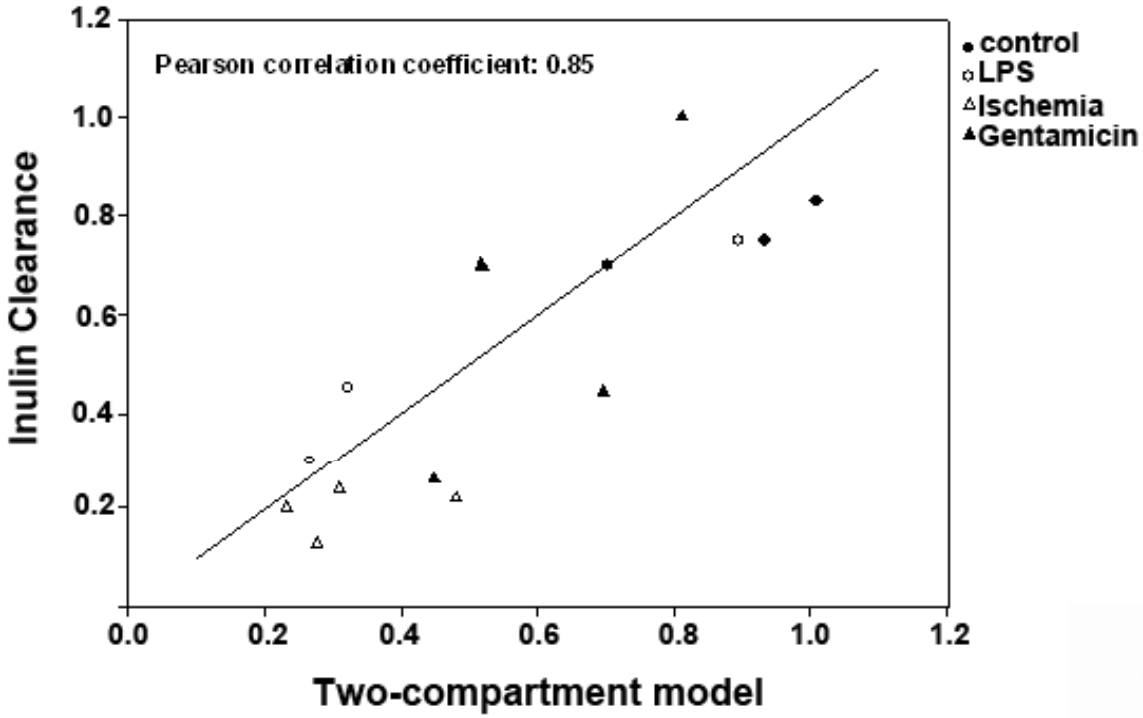


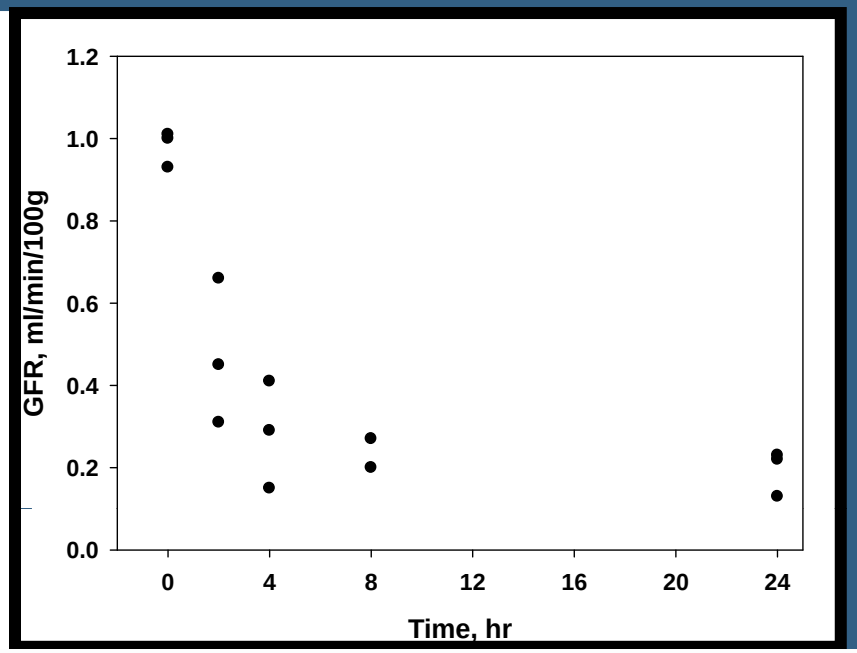
- By the time SCr rises above normal, 50% of kidney function has been lost



Quantifying Glomerular Filtration

Quantifying Glomerular Filtration in Rats

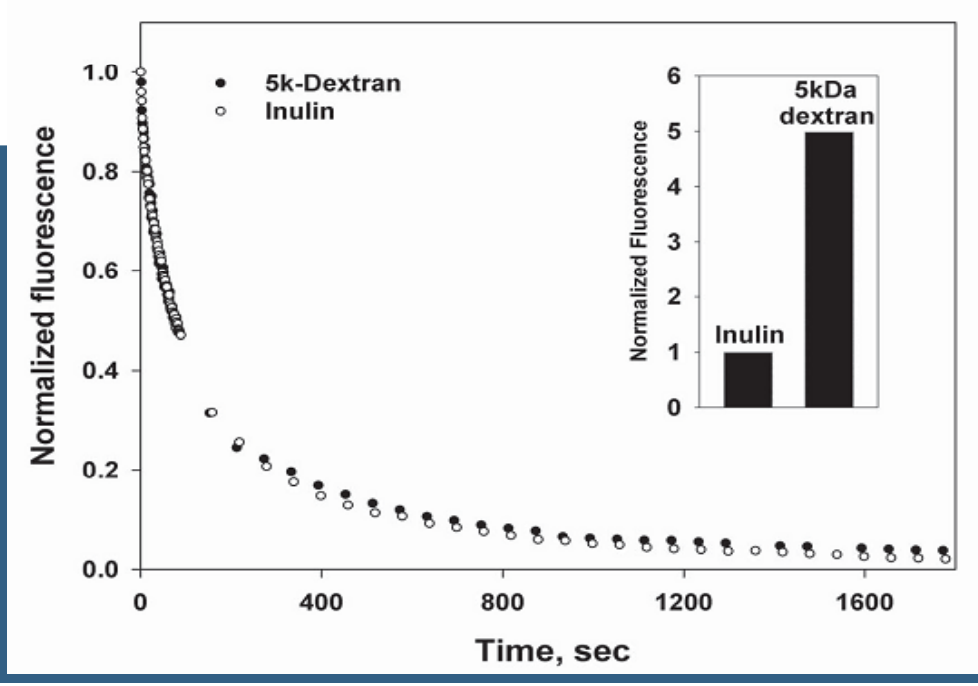




Data Collection Only Required 10-15 minutes per GFR Determination Post Injection

Advantages of Dextrans

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



Wang. Am J Physiol Renal Physiol, 2010

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

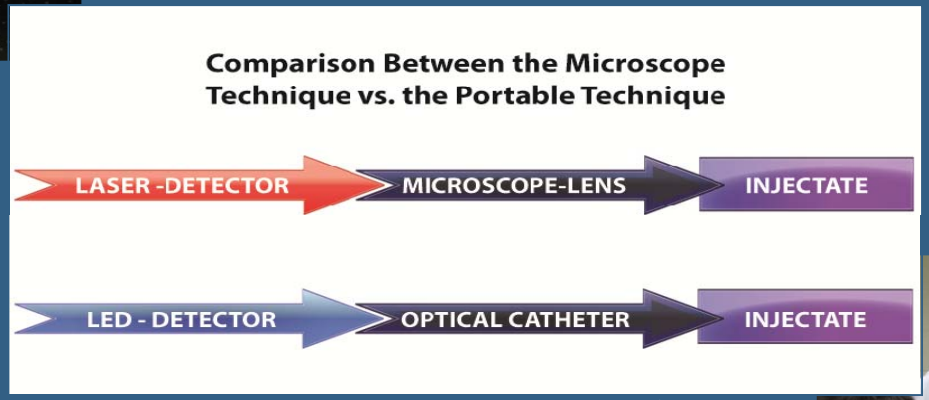
4. Data Storage and Software Analysis

5. Raising MONEY

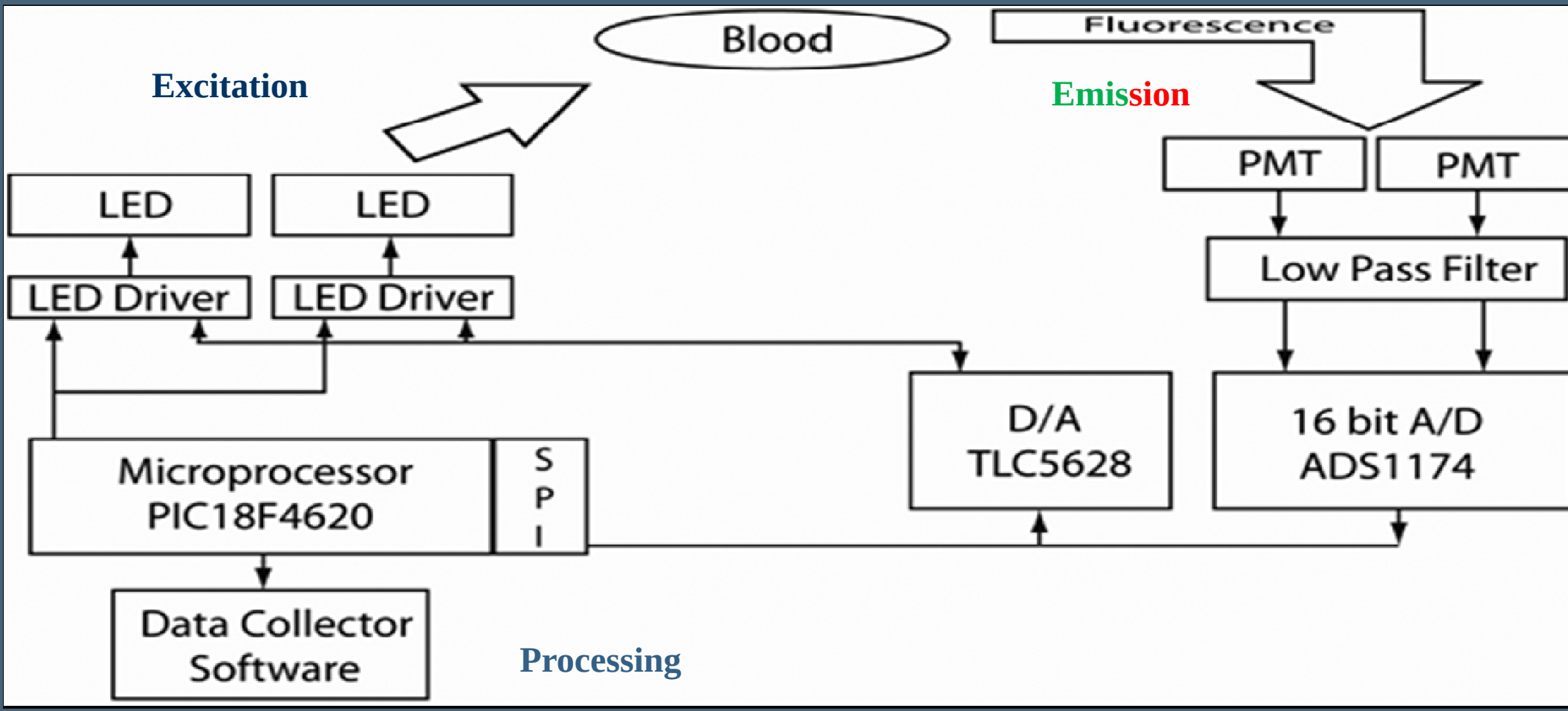
"Advancing" to the Dark Side

Commercialization: Bench to Bedside

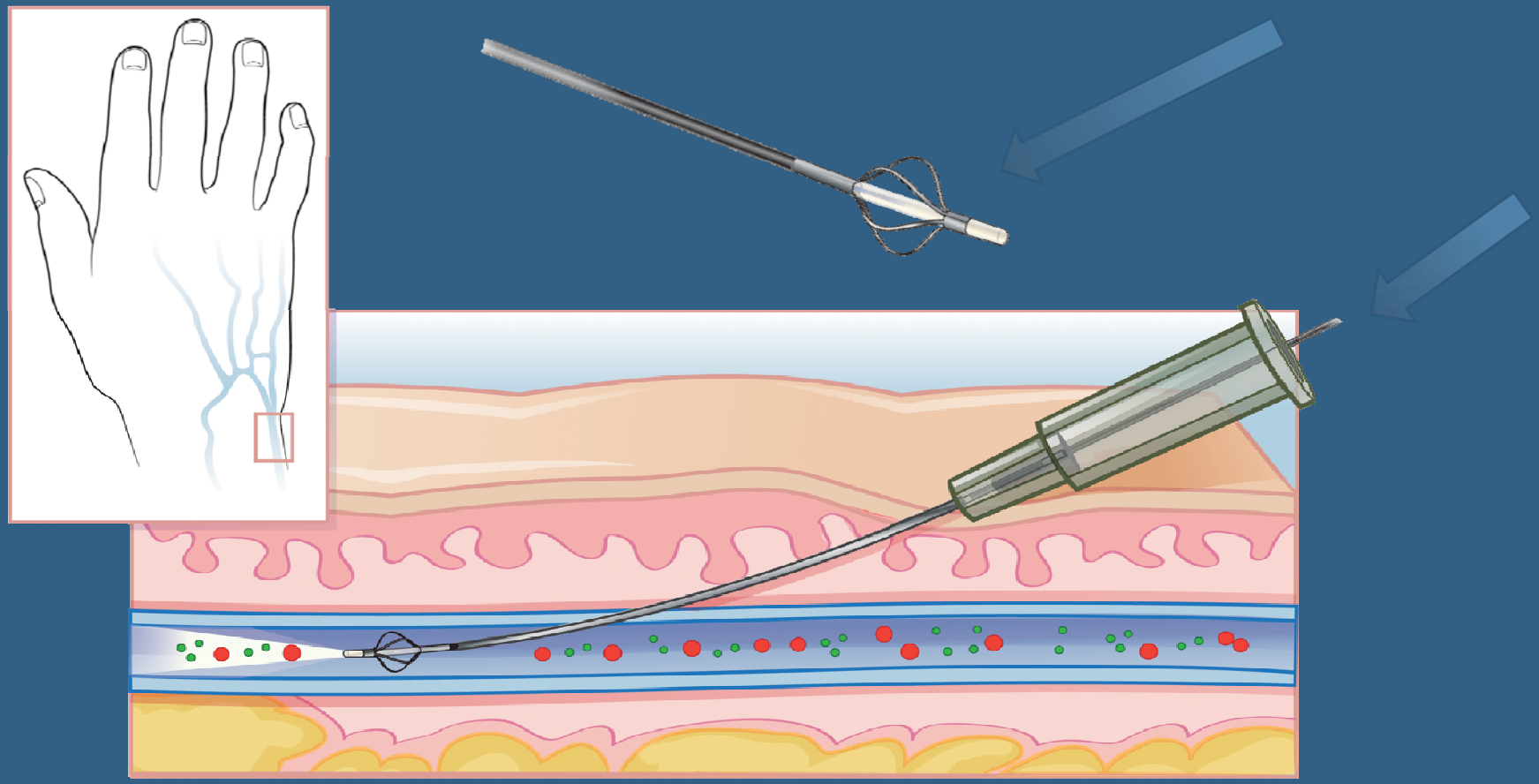
Discovery using expensive 2-photon laser requiring surgery to visualize exteriorized kidney



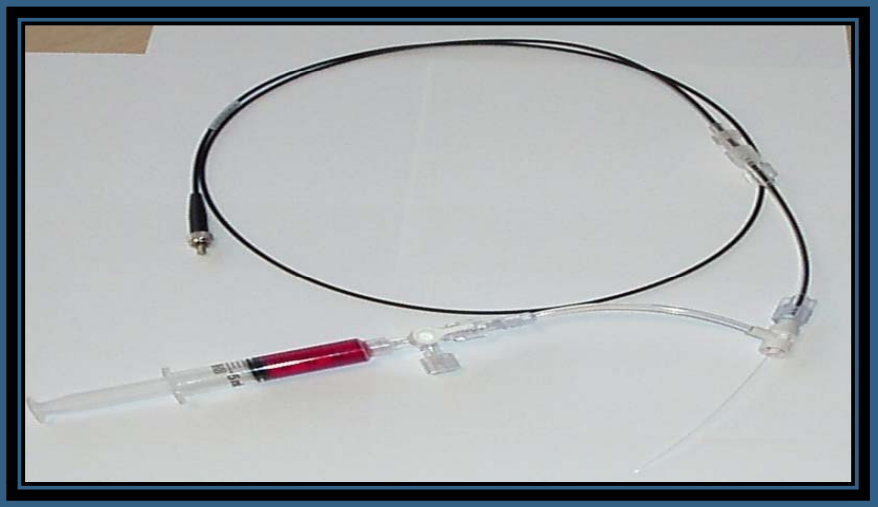
Inexpensive, portable LED-based device using fiber optic introduced through standard 18g catheter to read markers

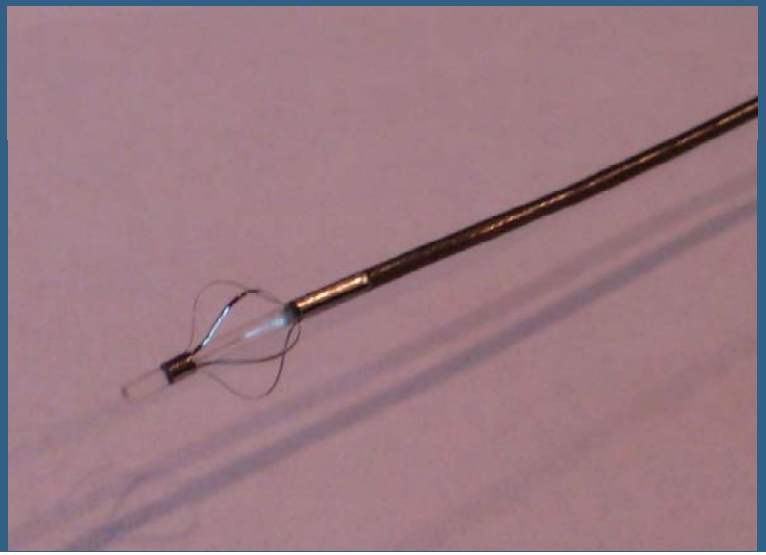

Blood
Fluorescence
Excitation
Emission
PMT
PMT
LED
LED
Low Pass Filter
LED Driver
LED Driver
D/A
TLC5628
16 bit A/D
ADS1174
Microprocessor
PIC18F4620
S
P
I
Data Collector
Software
Processing

Measuring Fluorescence Through 18g Catheter

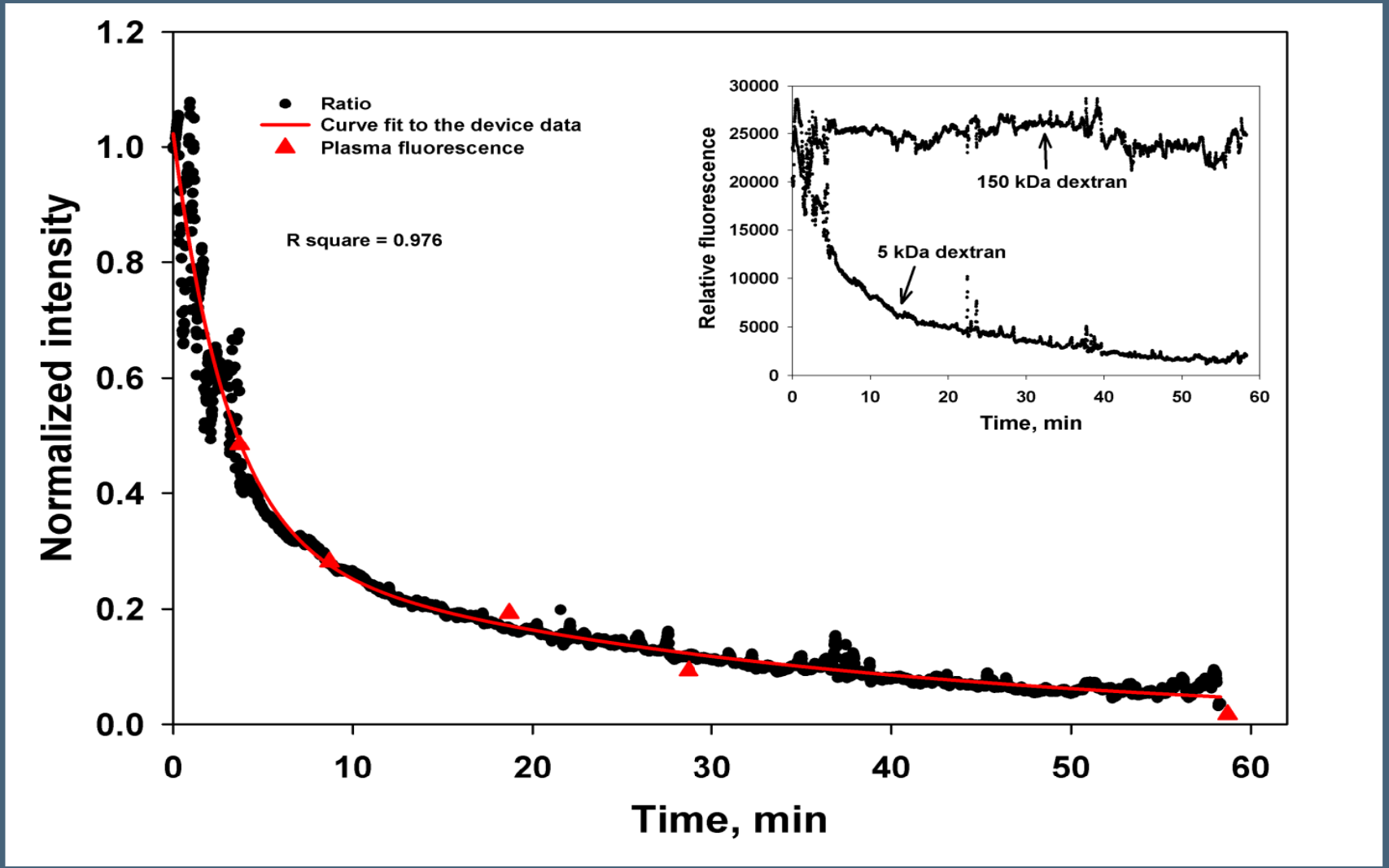


FAST Clinical GFR Technique





GFR Determination in Dogs Via A Peripheral Vein



New Technology In Development for Rapid GFR

1. Repeatable, accurate, rapid GFR determination at bedside
2. Minimally invasive optical device
3. Rich data for interpretation and evaluation of test
4. Able to be administered by nursing personnel
5. Independent of vascular permeability

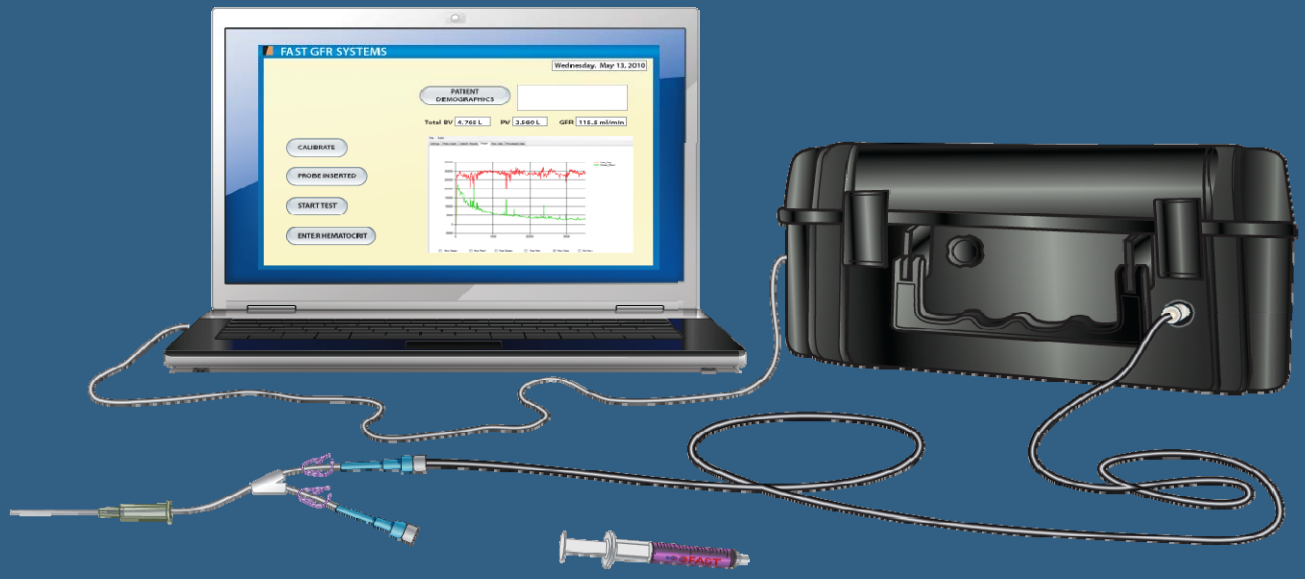
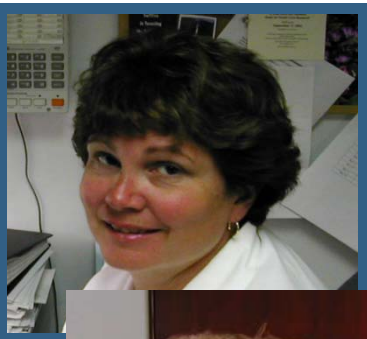


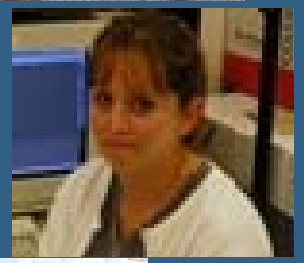
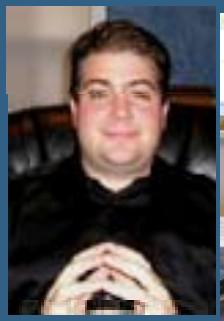
Table 1. Investigational uses for multi-photon microscopy

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



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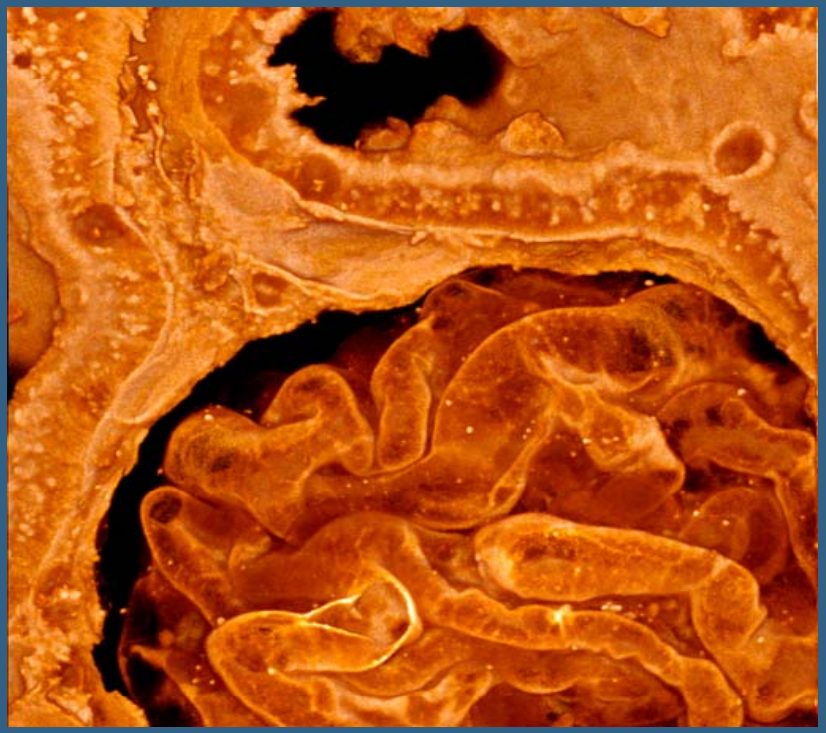
NIH O'Brien Center for Advanced Renal Microscopic Analysis



- Develop new optical methodologies for renal investigators.

- Assist investigators in implementing these new techniques in their laboratories, or in the facilities of the Center.

Investigators interested in using the facilities of the Center can find details and application information at:

www.nephrology.iupui.edu/obrien