Fluorescent Probes to Study Tubular Metabolism and Microvascular Function

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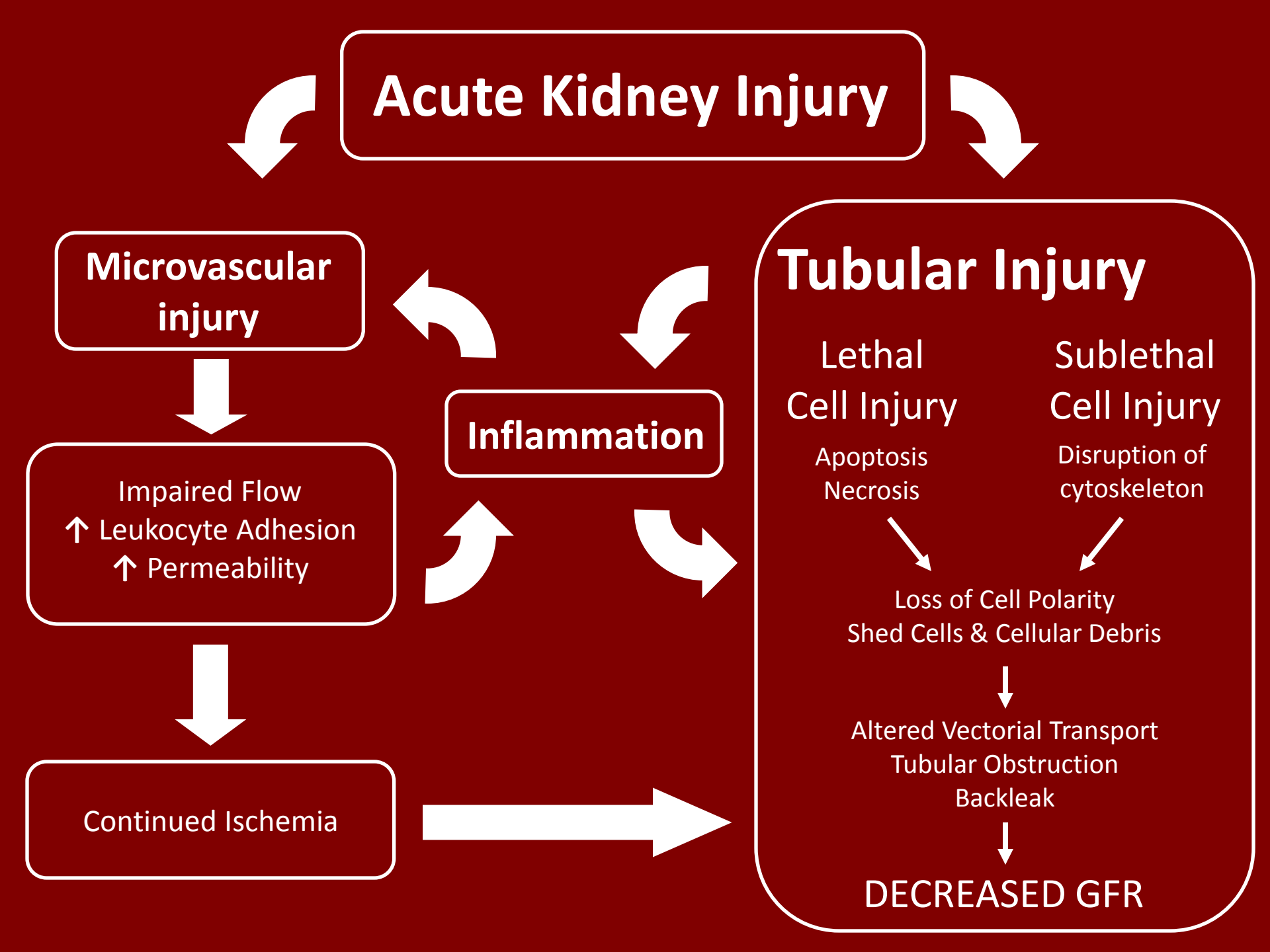
SCHOOL OF MEDICINE

INDIANA UNIVERSITY


Acute Kidney Injury
Microvascular injury
Impaired Flow
↑ Leukocyte Adhesion
↑ Permeability
Continued Ischemia
Inflammation
Tubular Injury
Lethal Cell Injury
Apoptosis
Necrosis
Sublethal Cell Injury
Disruption of cytoskeleton
Loss of Cell Polarity
Shed Cells & Cellular Debris
Altered Vectorial Transport
Tubular Obstruction
Backleak
DECREASED GFR

Outline

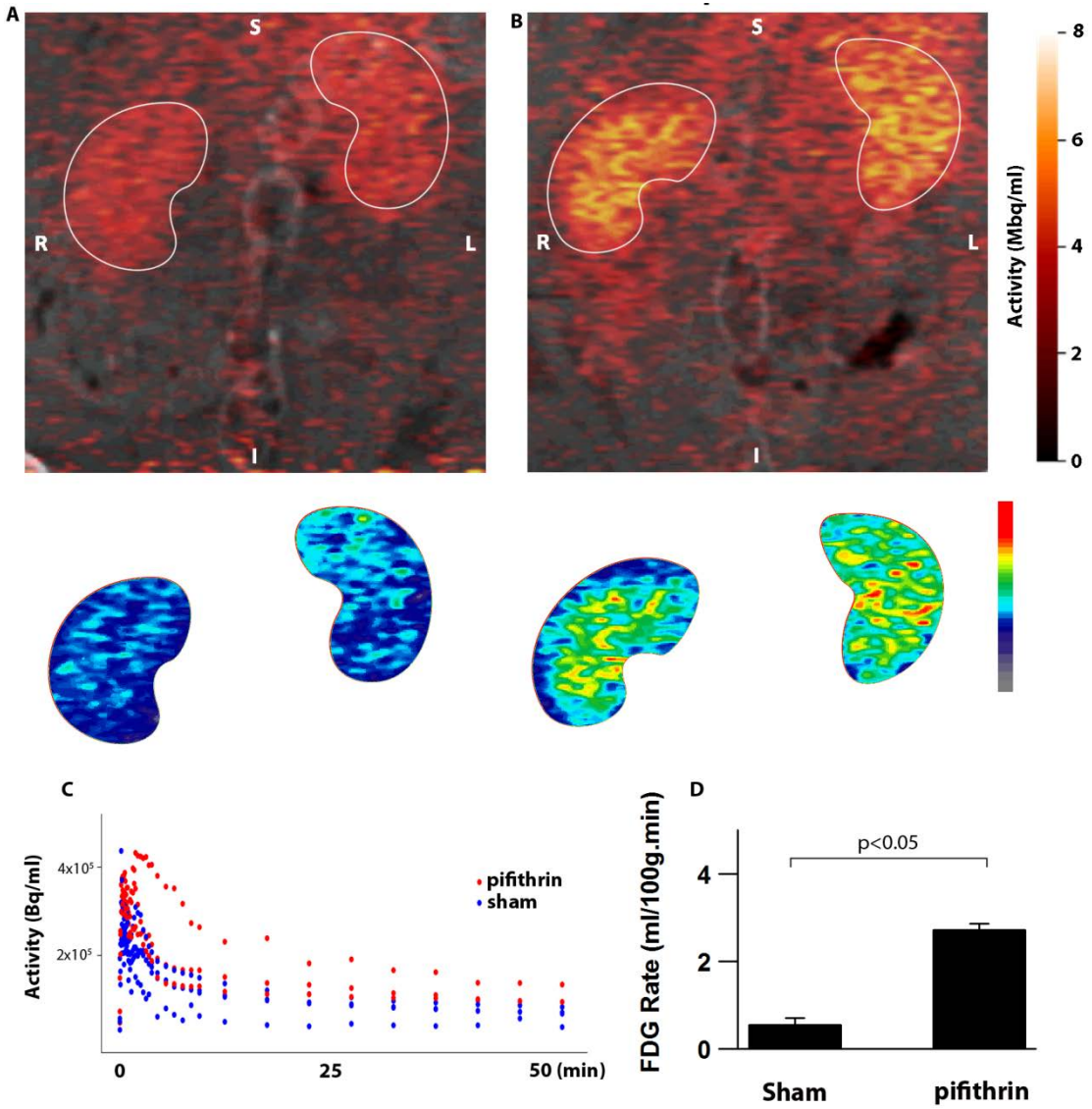
- Tubular metabolism
  - Glucose uptake
  - Oxidative phosphorylation
- Microvascular function
  - Permeability
  - Flow
  - Leukocyte trafficking
  - Thrombosis

Tubular Metabolism

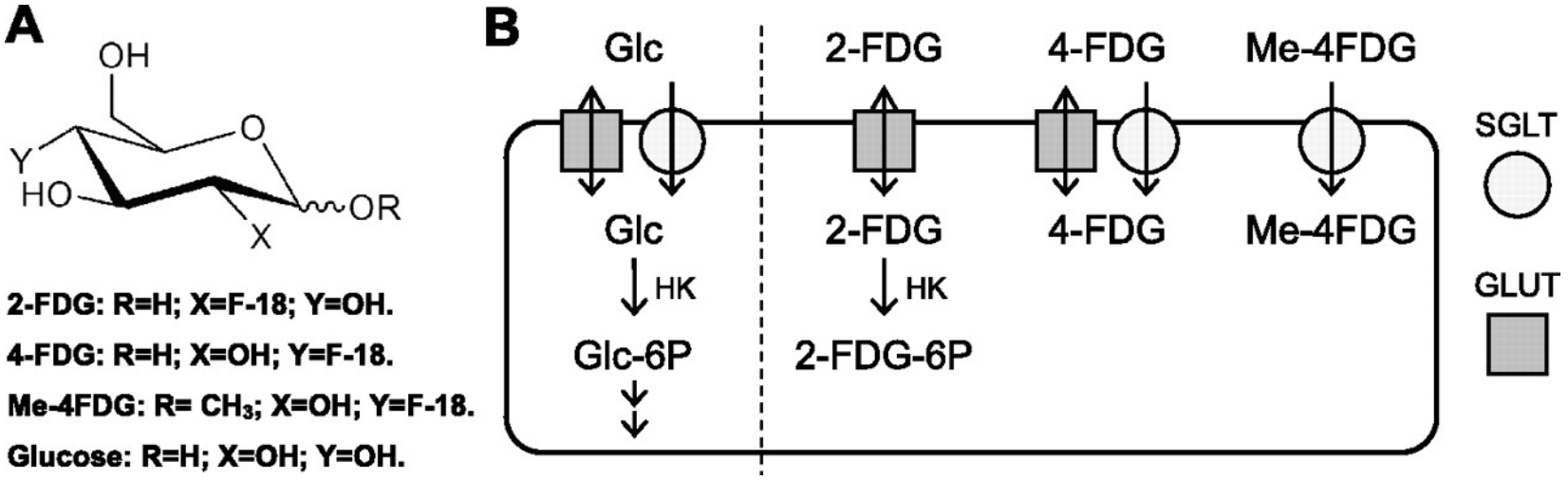
- ***Glucose uptake***

Positron Emission Tomography (PET)

- **Frequently used clinically to detect neoplastic tumors based on preferential uptake of glucose (Warburg effect)**
- **2-^{18}F deoxyglucose**
- **Application of PET to study of the kidney not widely utilized**
  - Dissection of tissue signal from filtered load
  - Relative affinity of 2-FDG for GLUT vs. SGLT

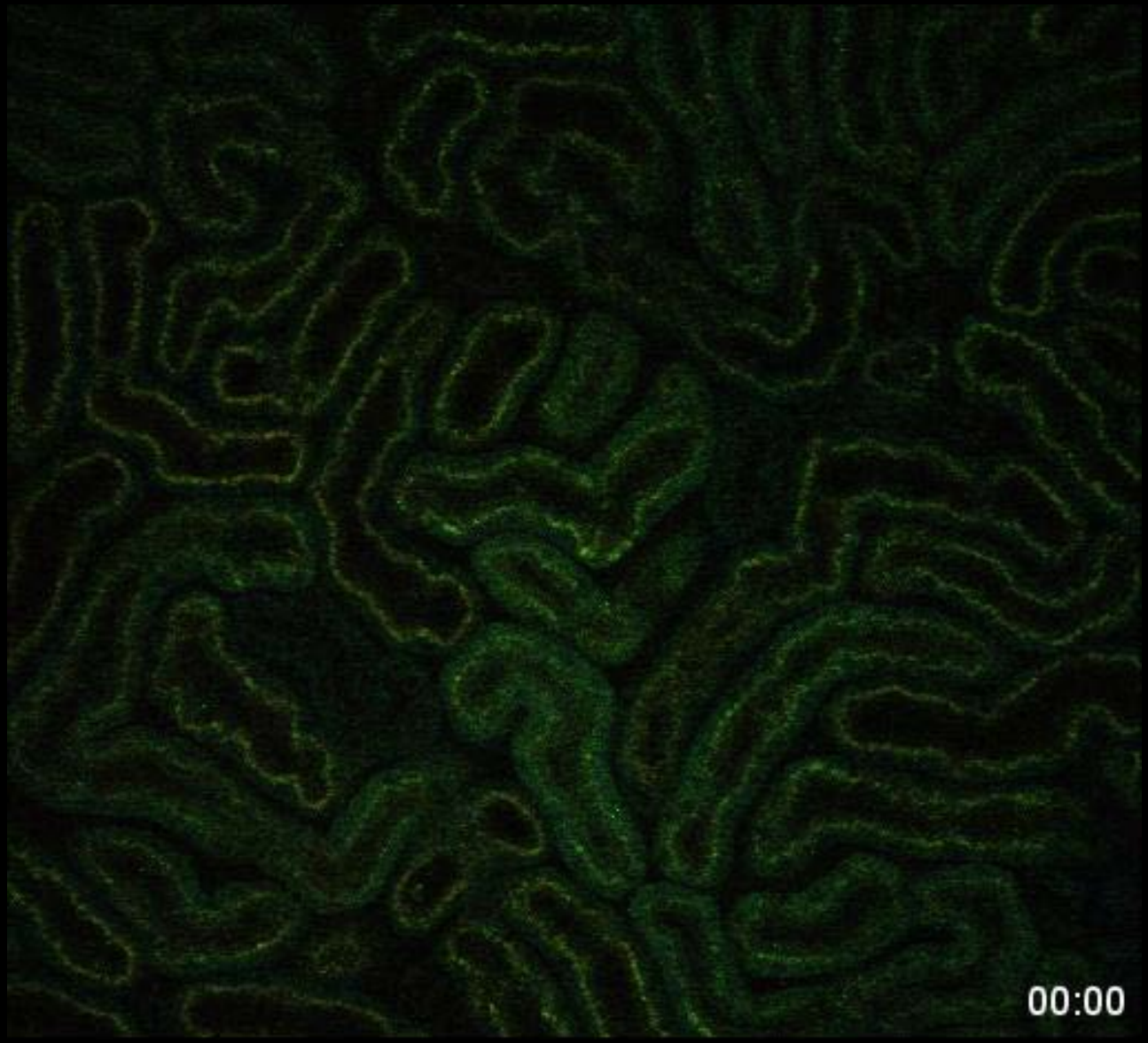


Glucose transporter imaging probes and their transport cascades into cells



Yu A S et al. Am J Physiol Cell Physiol 2010

2-[*N*-(7-nitrobenz-2-oxa-1,3-diazol-4-yl)amino]-2-deoxy-D-glucose
excitation/emission maxima of ~465/540 nm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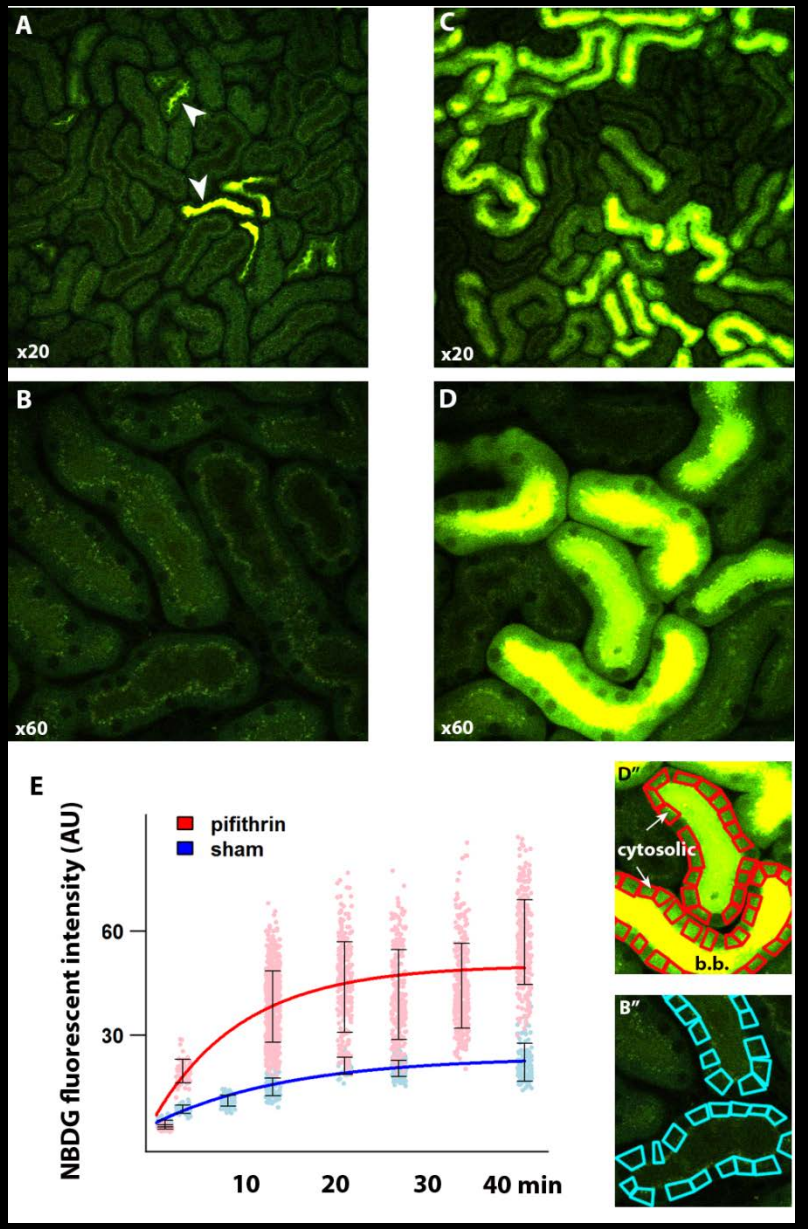
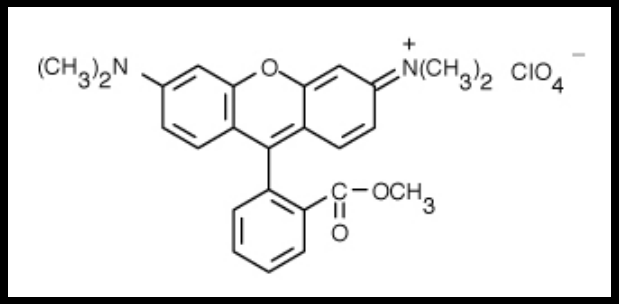
Quantitation of NBD-glucose uptake



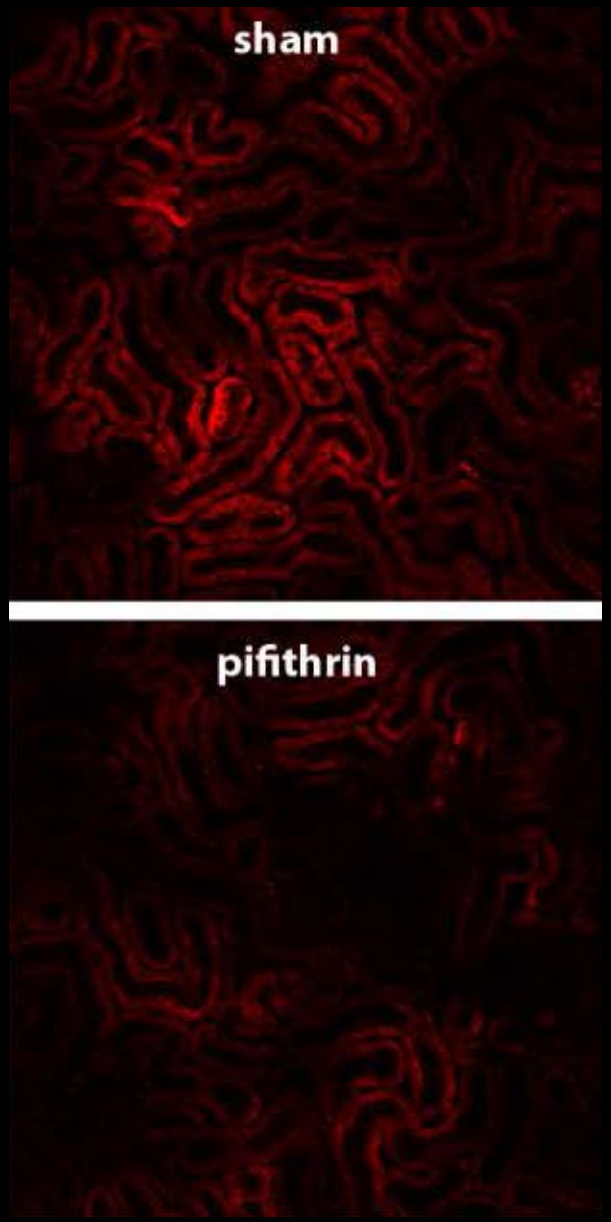
Tubular Metabolism

- Glucose uptake
- ***Oxidative phosphorylation***

Tetramethyl rhodamine methyl ester



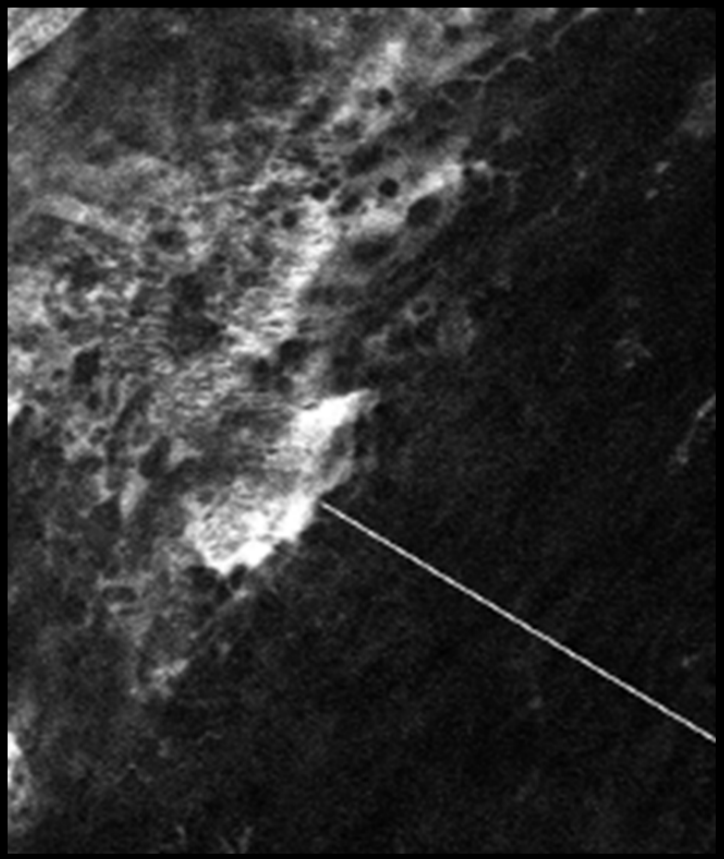
- Lipophilic cationic fluorophore
- Rapidly and reversibly taken up by live cells
- Accumulation in mitochondria driven by mitochondrial membrane potential ($\Delta\Psi_m$)



Microvascular Function

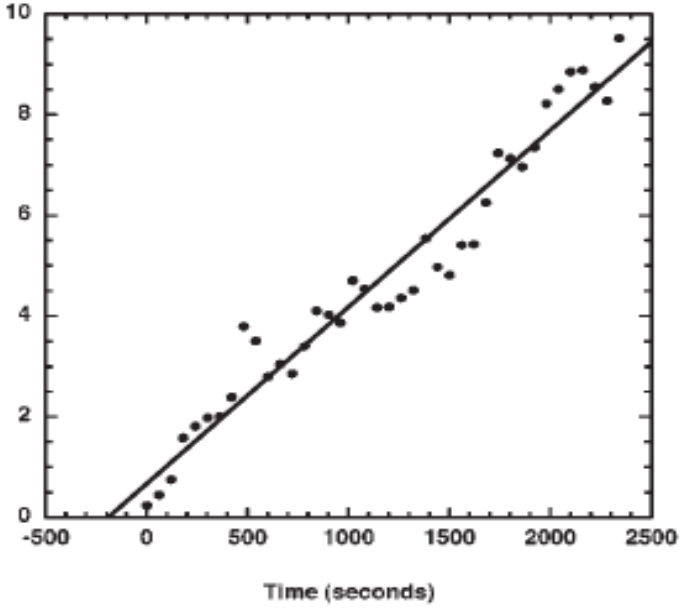
- ***Permeability***

Permeability of Tumor Vessels

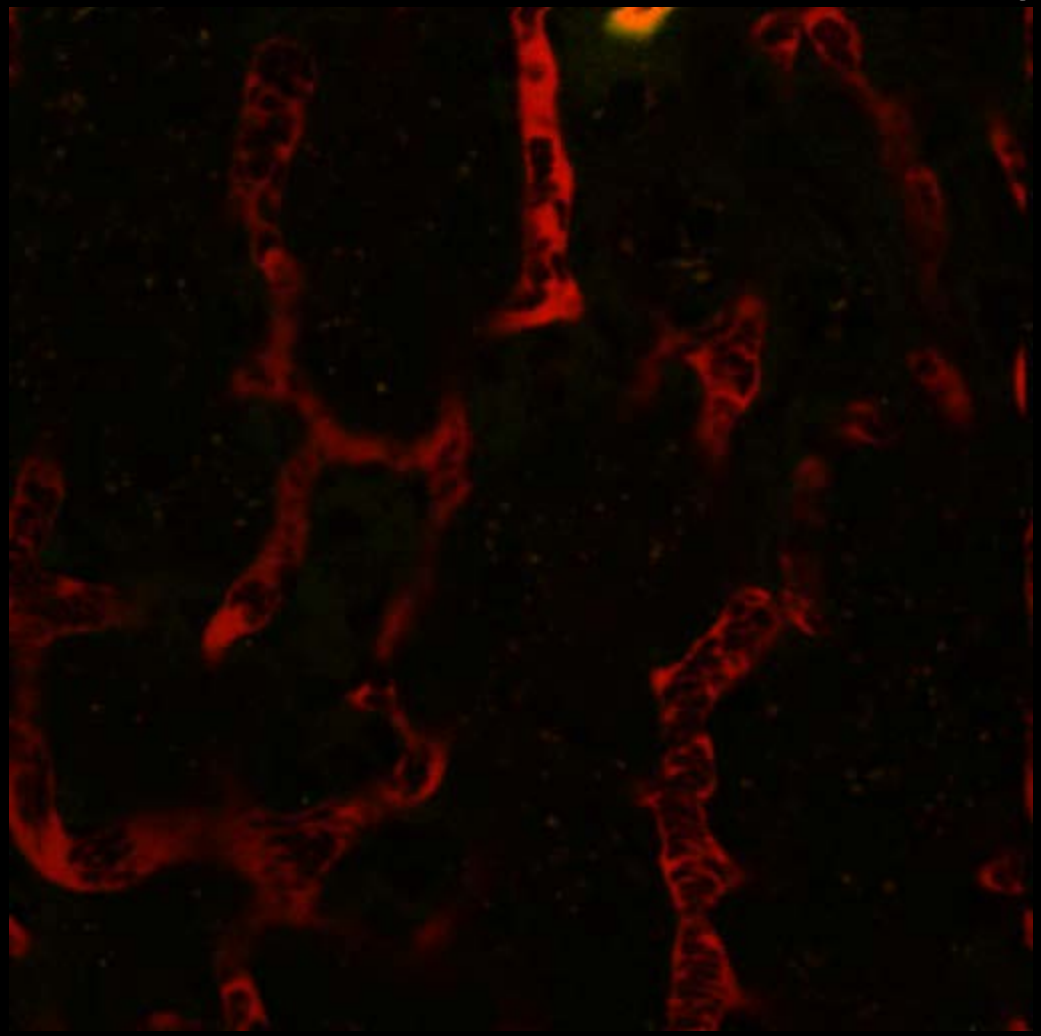


Brown E. et. al. Nature 2001; 7: 864-868

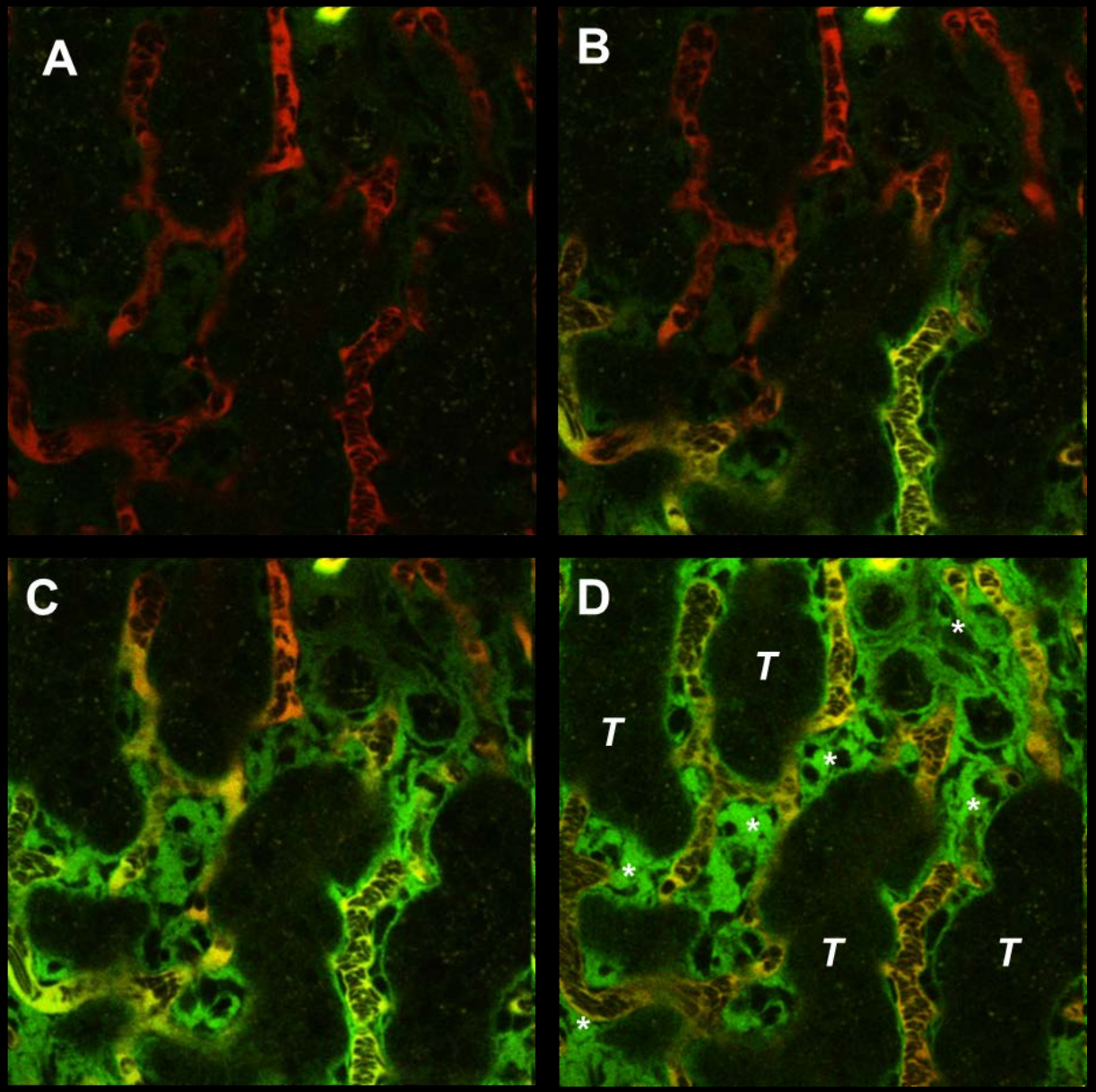
$$P = \lim_{t \to 0} \frac{\delta}{\delta t} \frac{\int_{r=R}^{\infty} F(r) r dr}{(F_v - F_i) R}$$



Microvascular Permeability

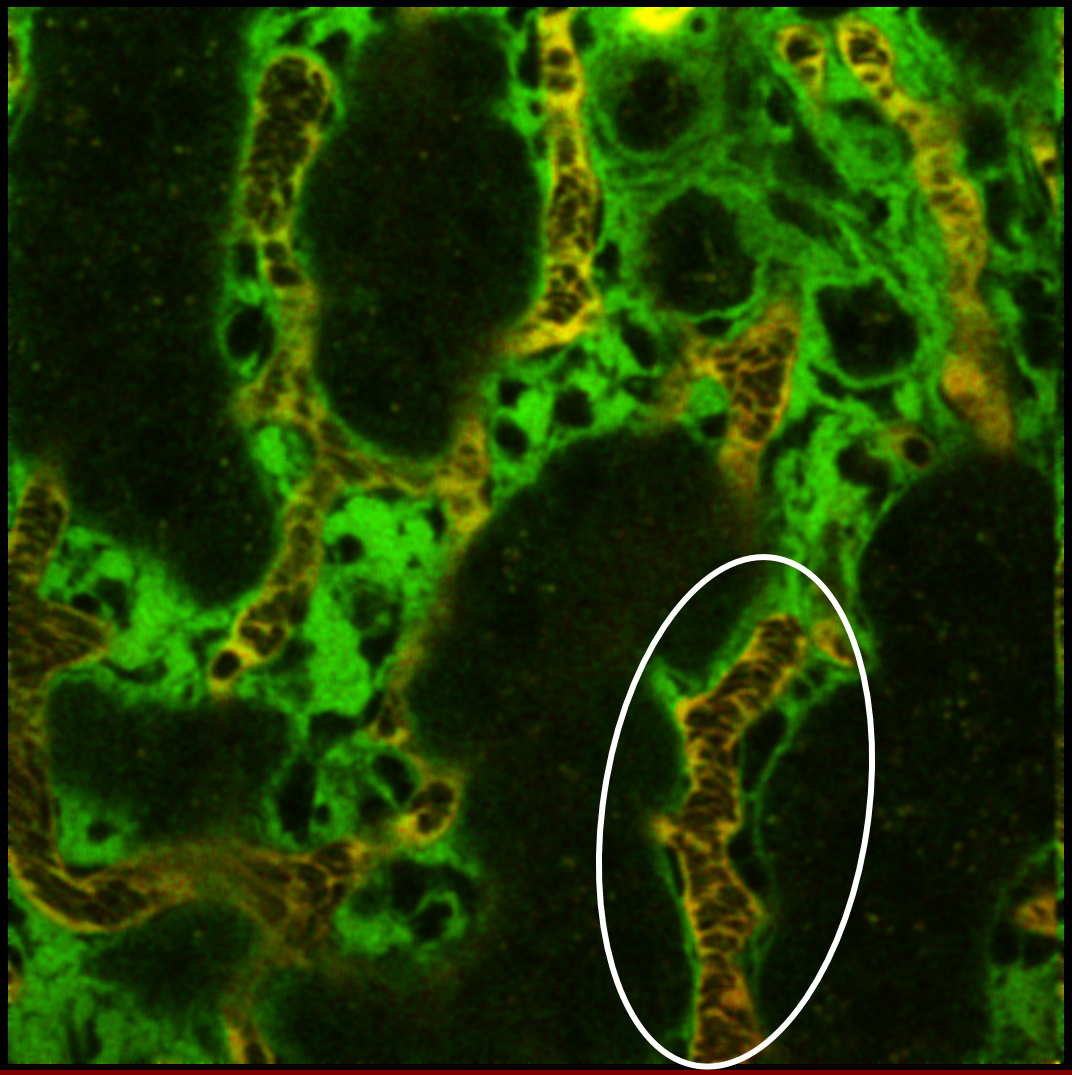


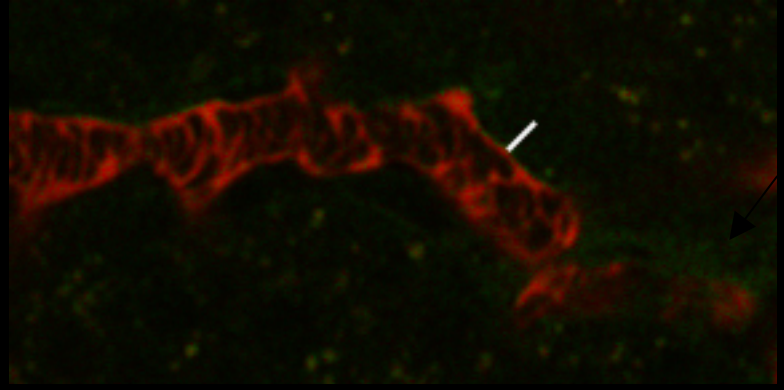
150K dextran
3K dextran

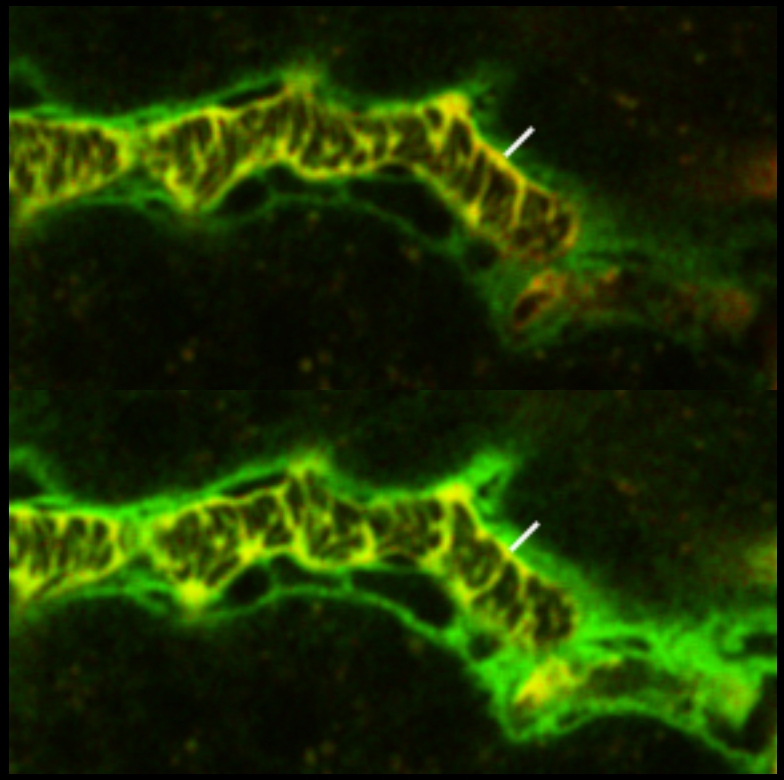


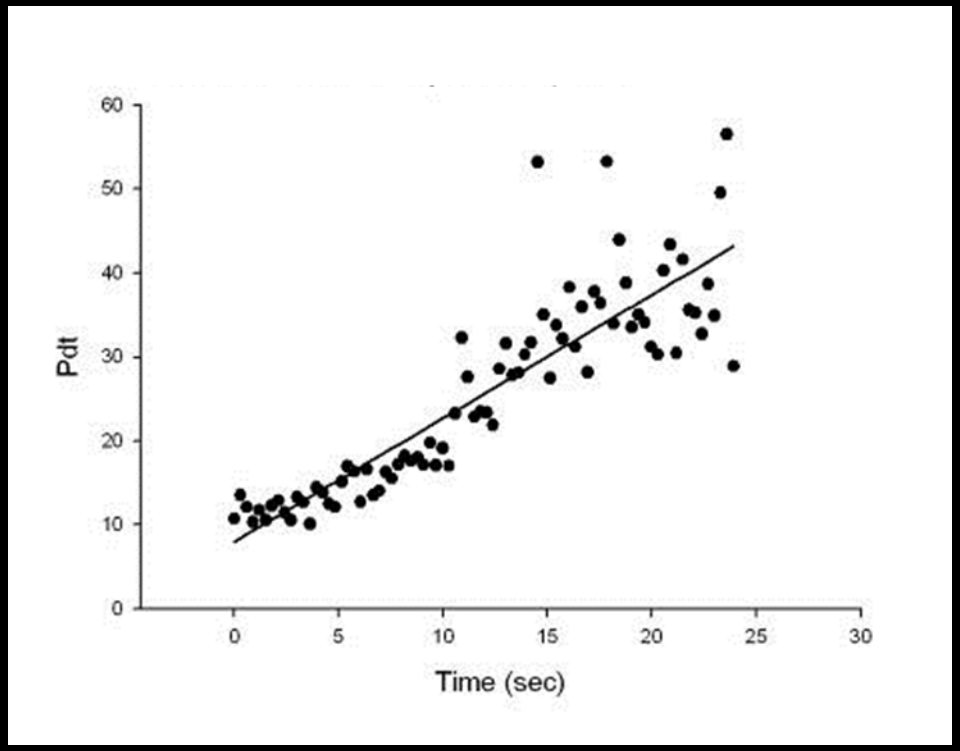
150K dextran
3K dextran

Microvascular Permeability

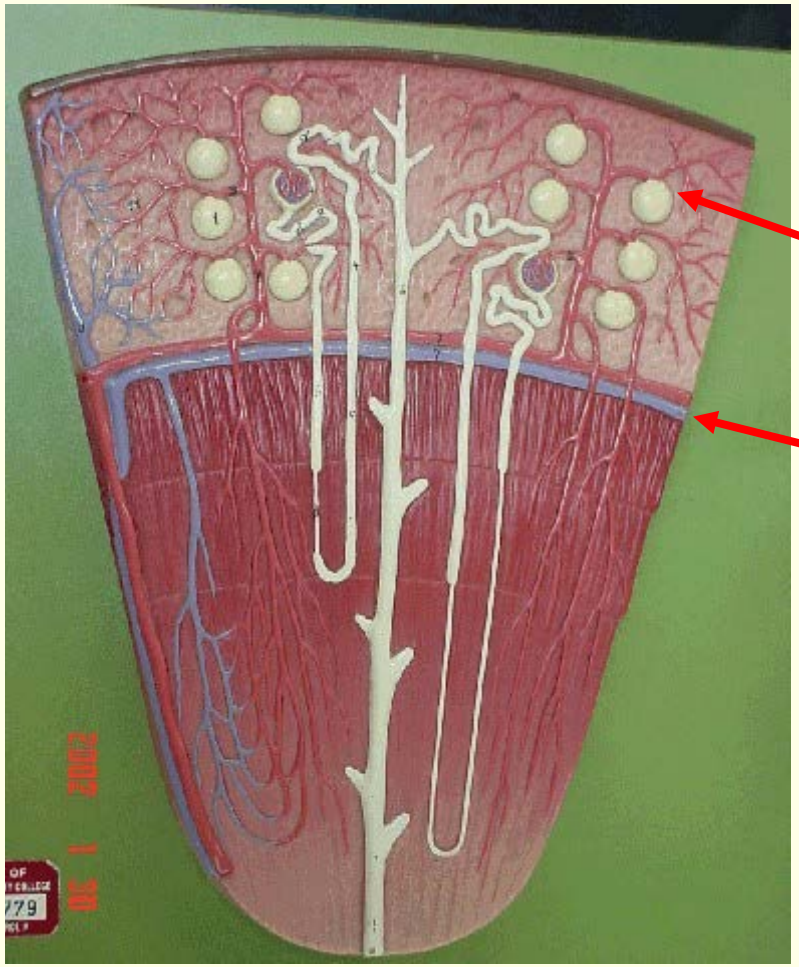







Pdt
60
50
40
30
20
10
0
0
5
10
15
20
25
30
Time (sec)

Considerations



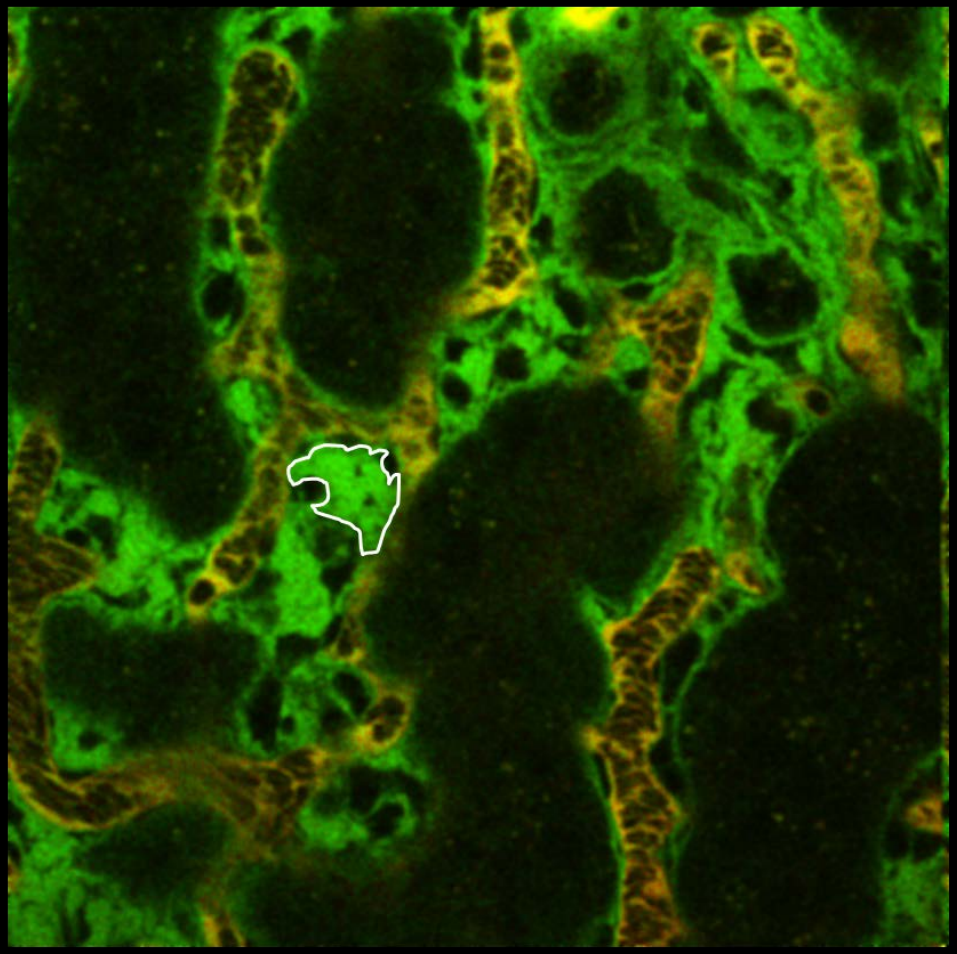
Glomeruli of most rats, mice
400 microns deep

Corticomedullary boundary
2 mm deep

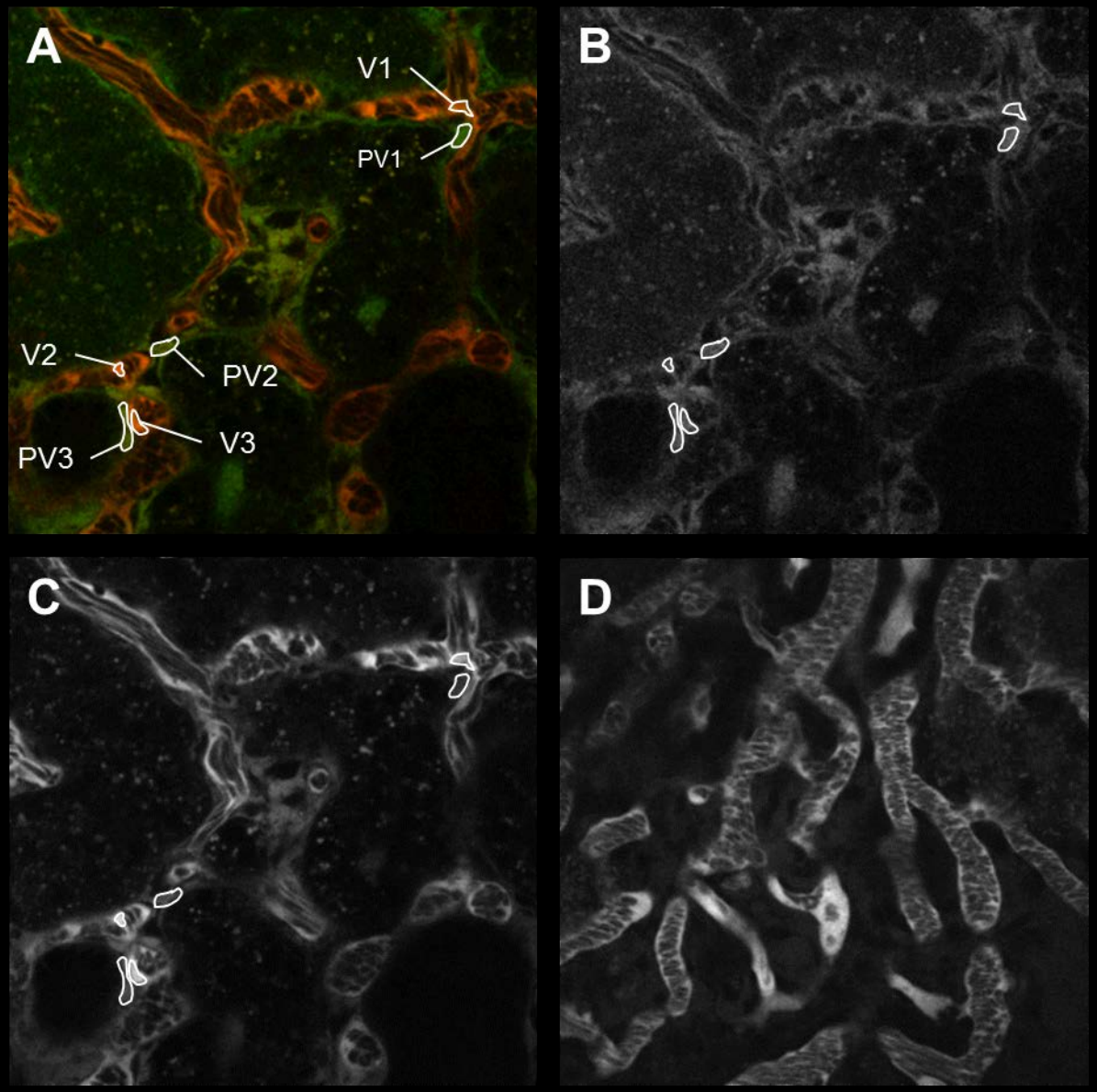
Considerations

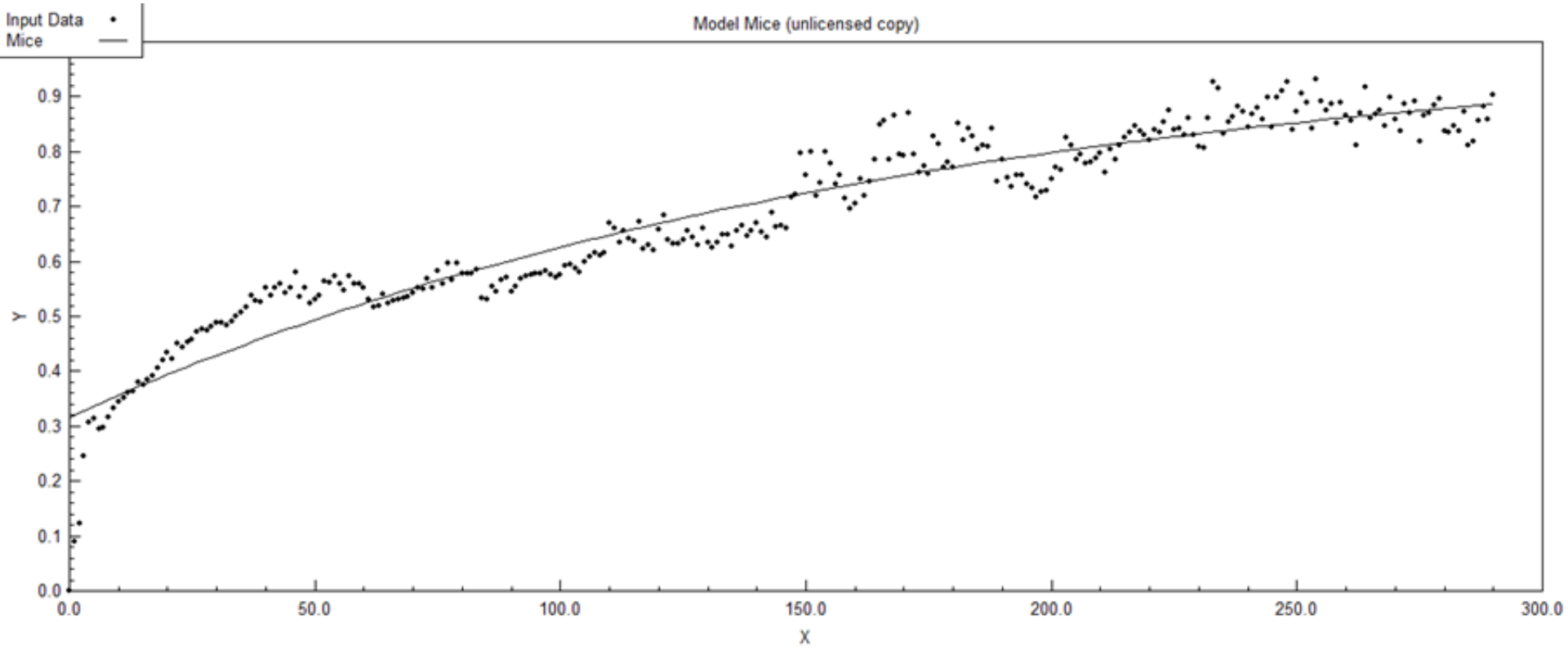
- General
  - Stochastic nature of permeability for larger molecular weight probes
  - Picking the correct MW probe for discerning the altered permeability to be studied
- Defining vessel wall limit
- Prep stability
- Limited number of measurements

Region Analysis



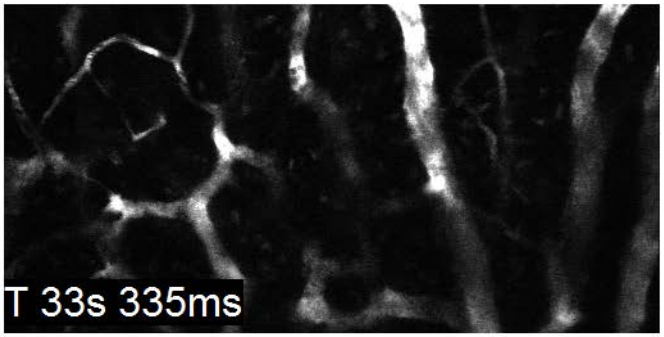
- Record average intensity inside region over time
- Ratio with average intensity of blood vessels over time

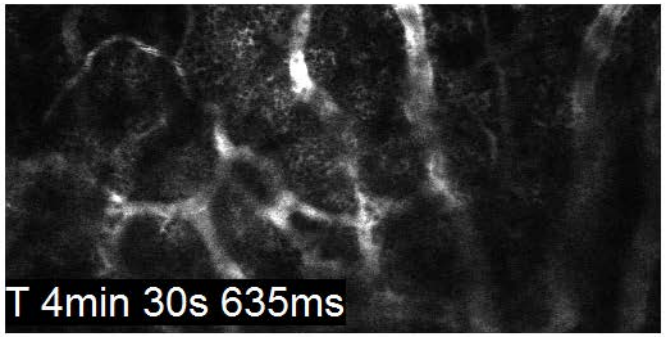

A
V1
PV1
V2
PV2
PV3
V3
B
C
D

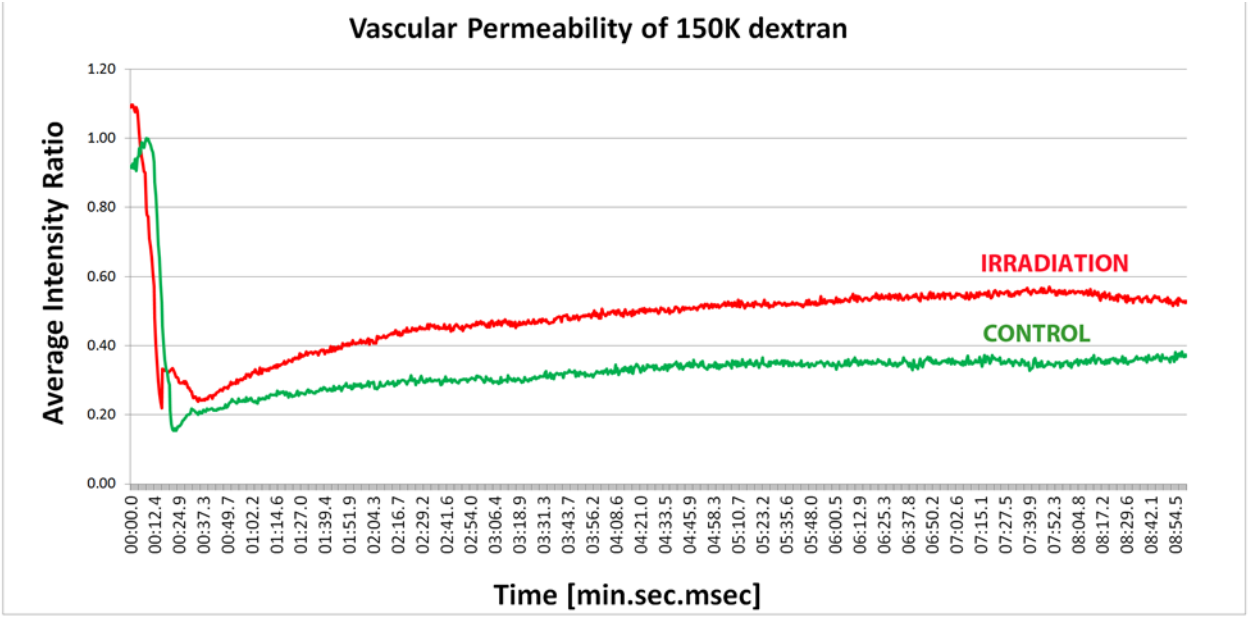


$$I_{avg} = a\left(1 - e^{-bt}\right) + c$$

$$\text{b} = 0.0058917453665142 3$$





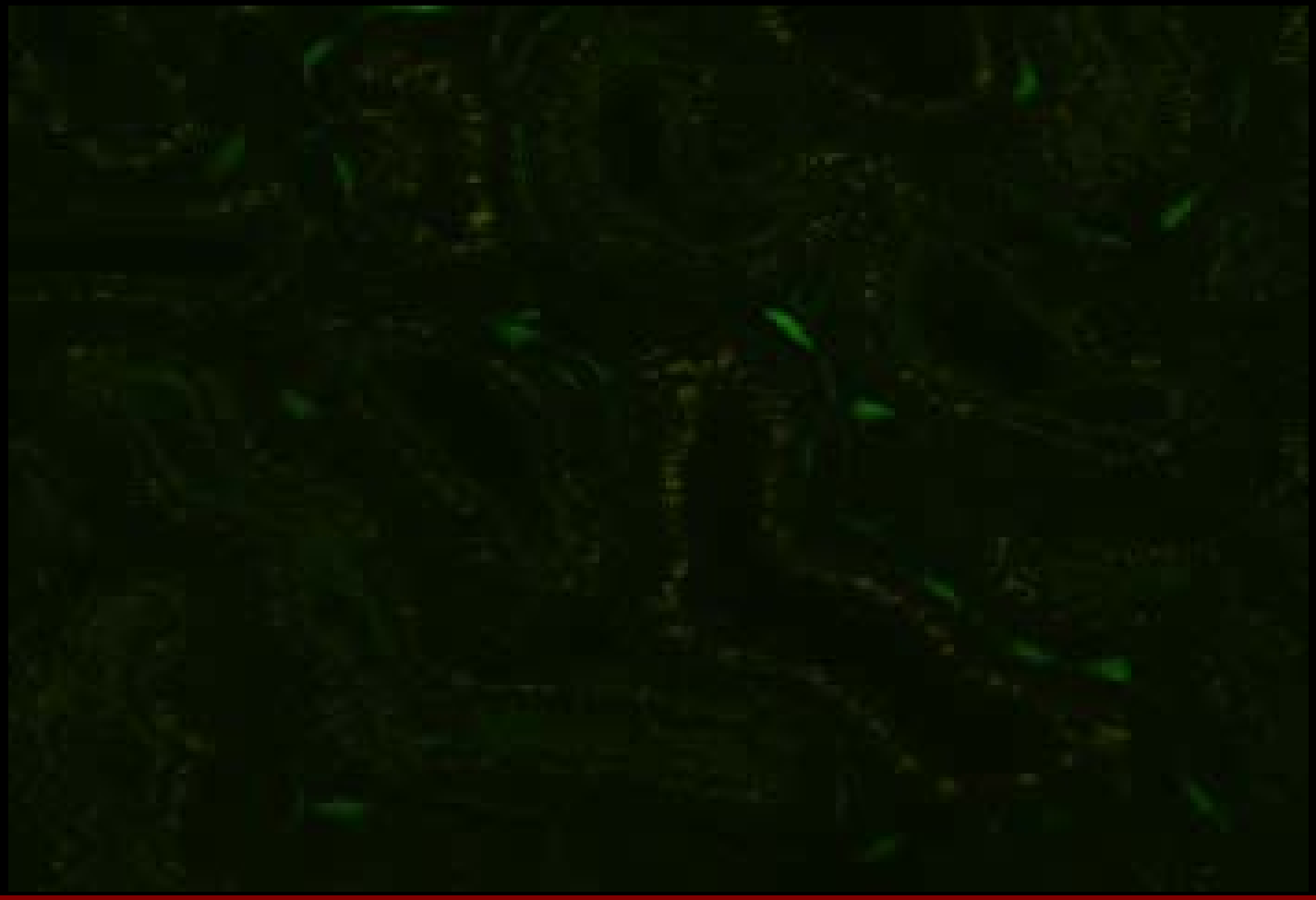


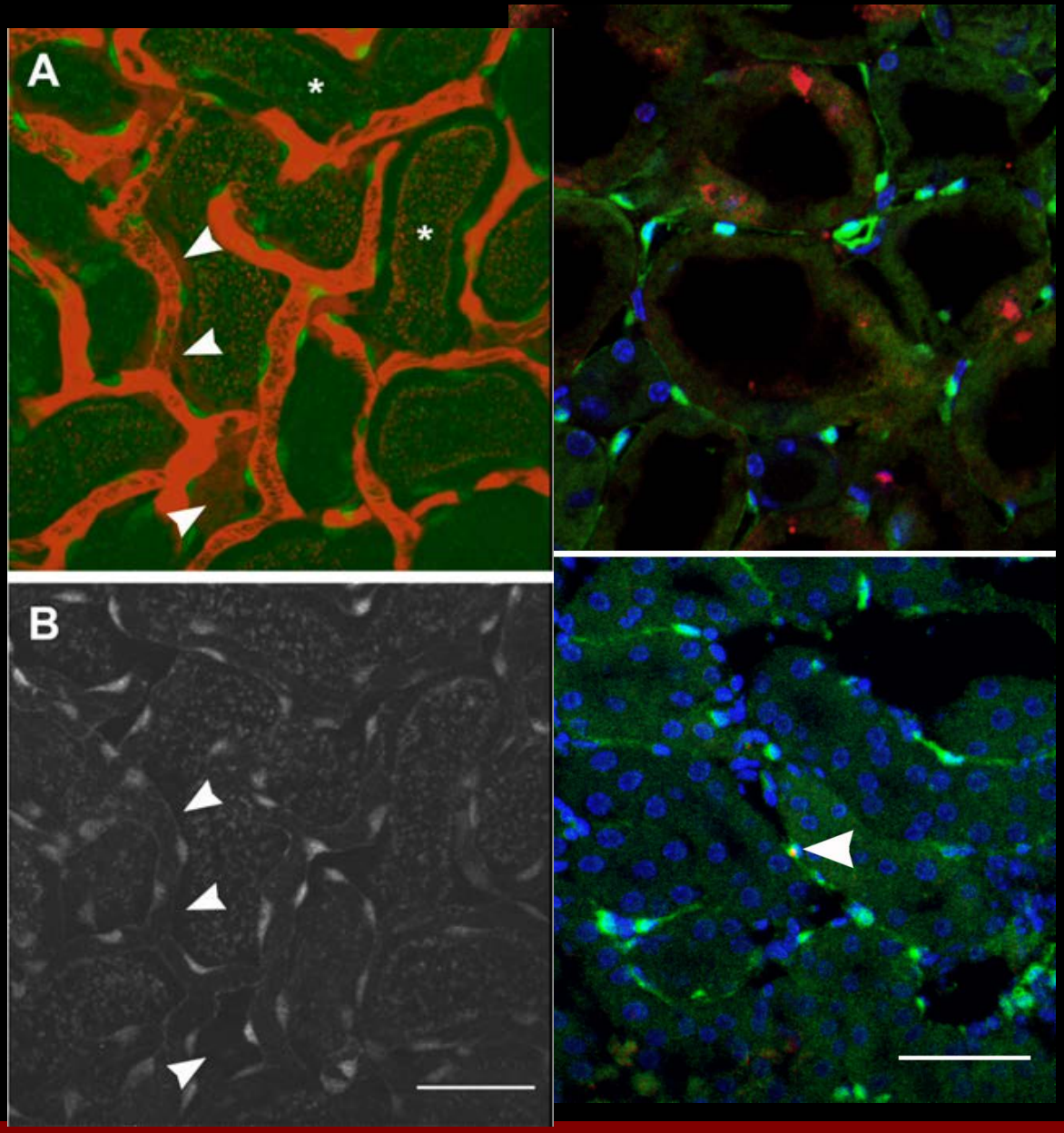
Courtesy of Gosia Kamocka

Additional Considerations

- Limited perivascular space in kidney
- Balance between intravascular and extravascular signal

Endothelial Structure-Function

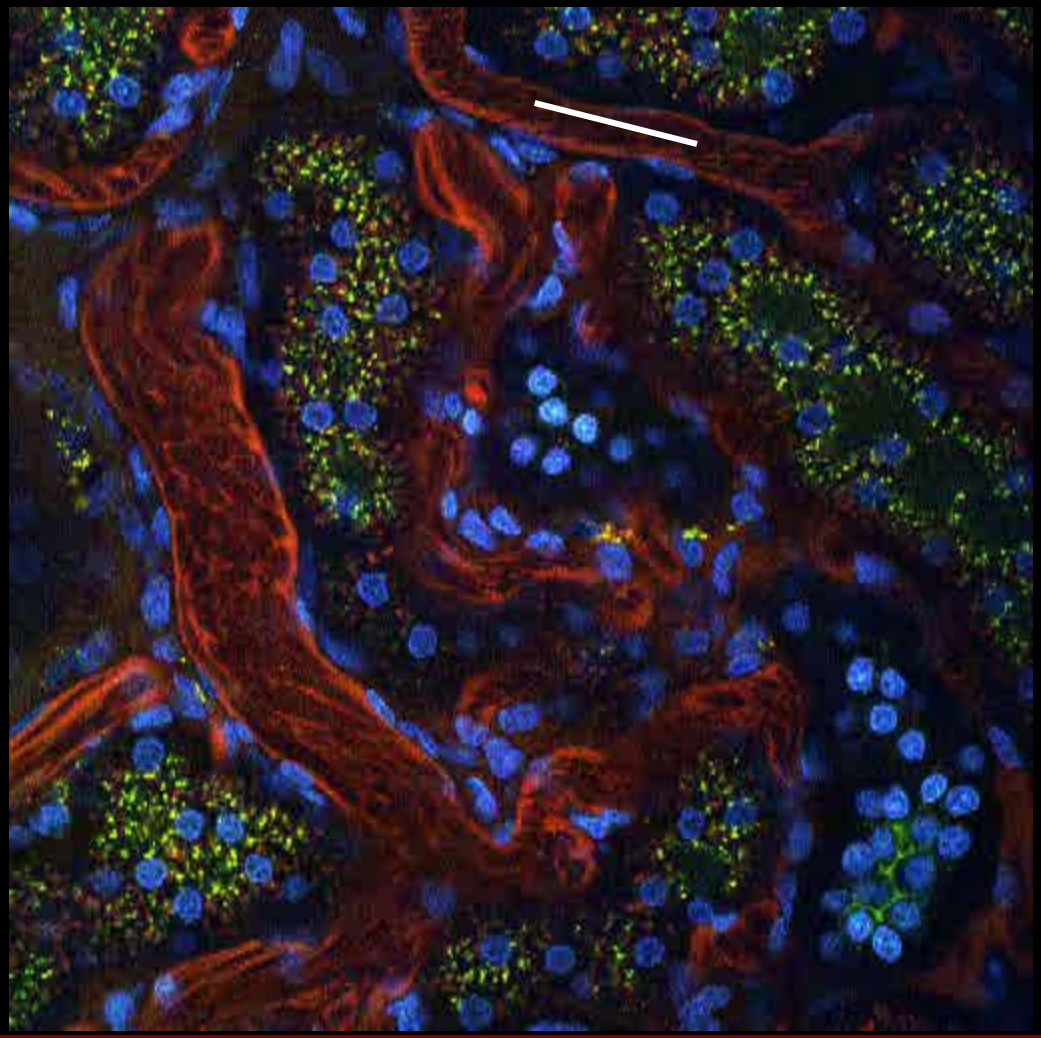



A
*
*
B

Microvascular Function

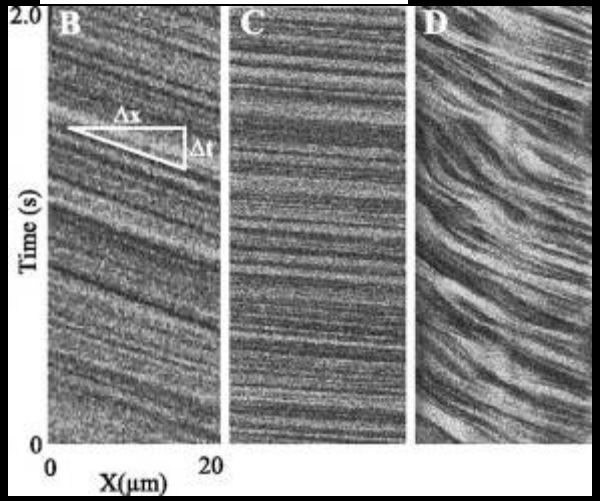
- Permeability
- ***Flow***

Microvascular Flow



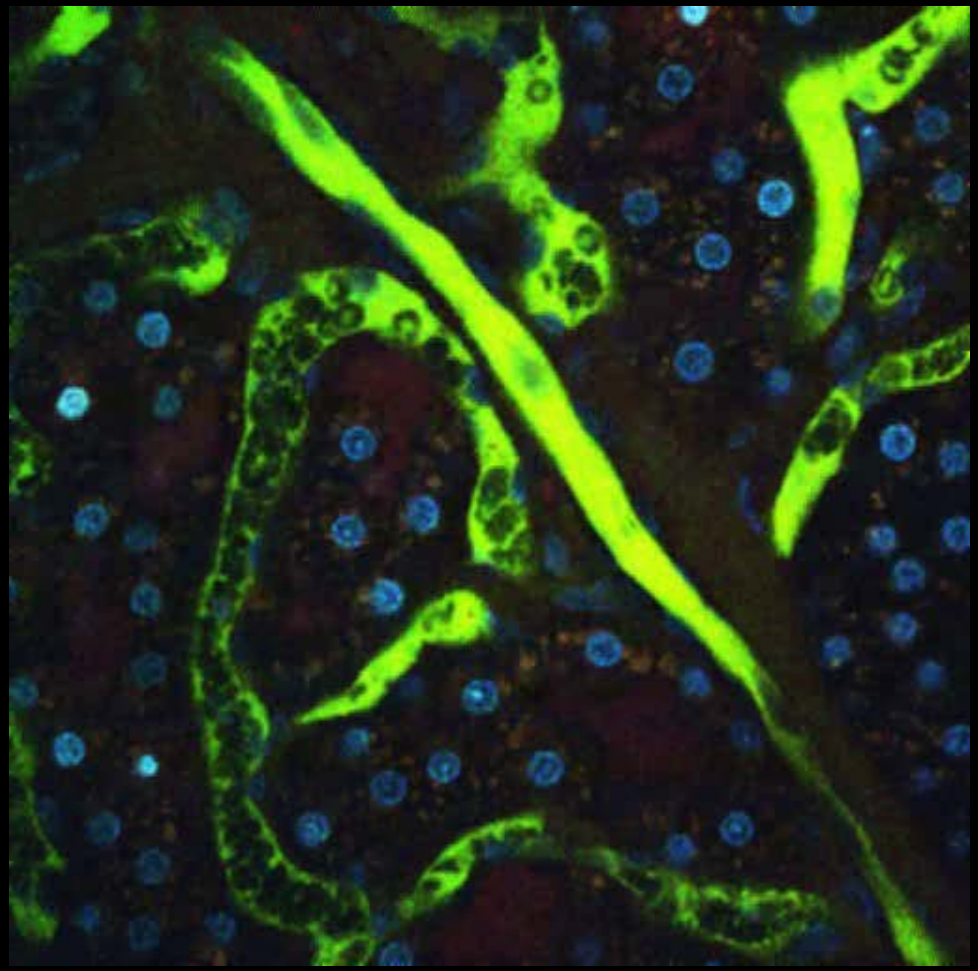
Mean Flow (mm/sec)

4.7 ± 0.2 16.7 ± 0.4



Kang, J. J. et al. Am J Physiol Renal Physiol 291, 2006

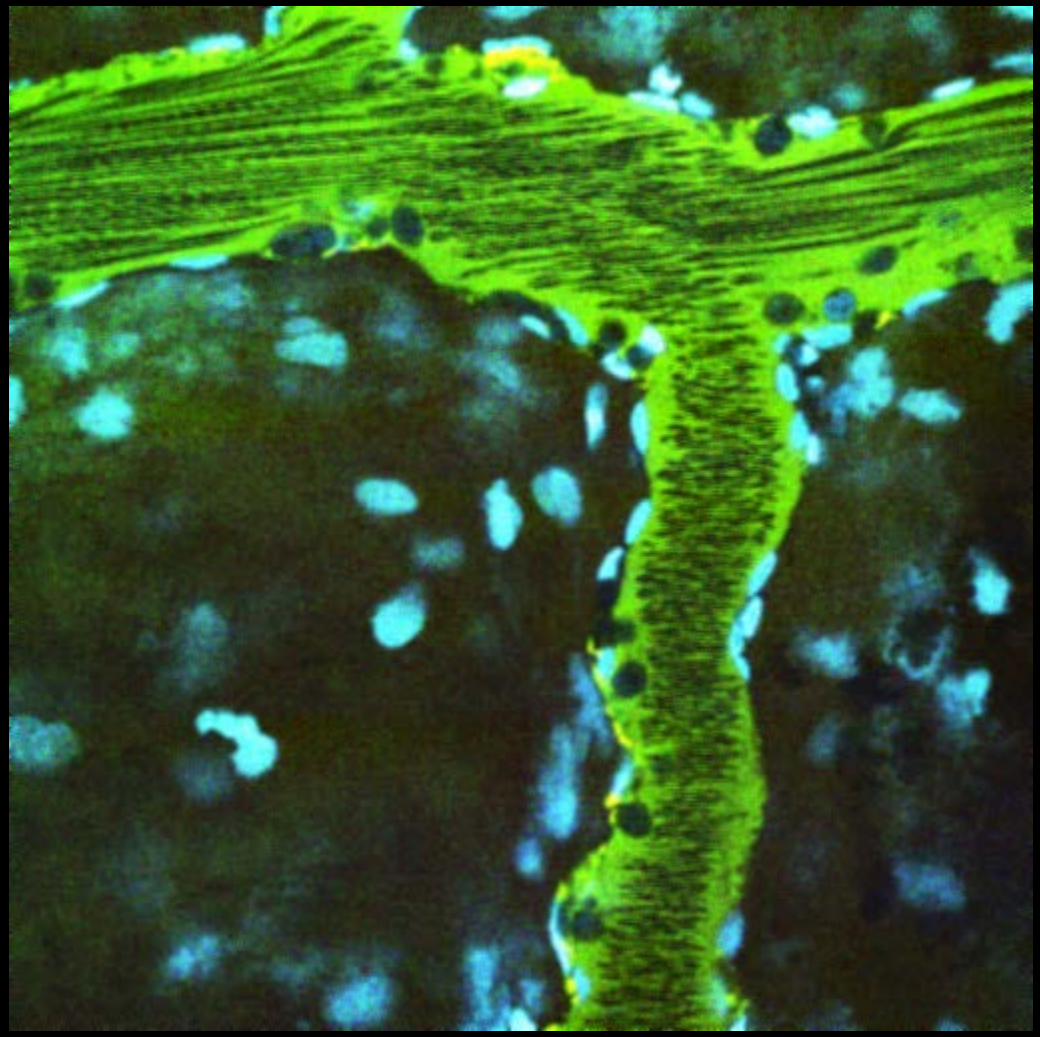
Considerations



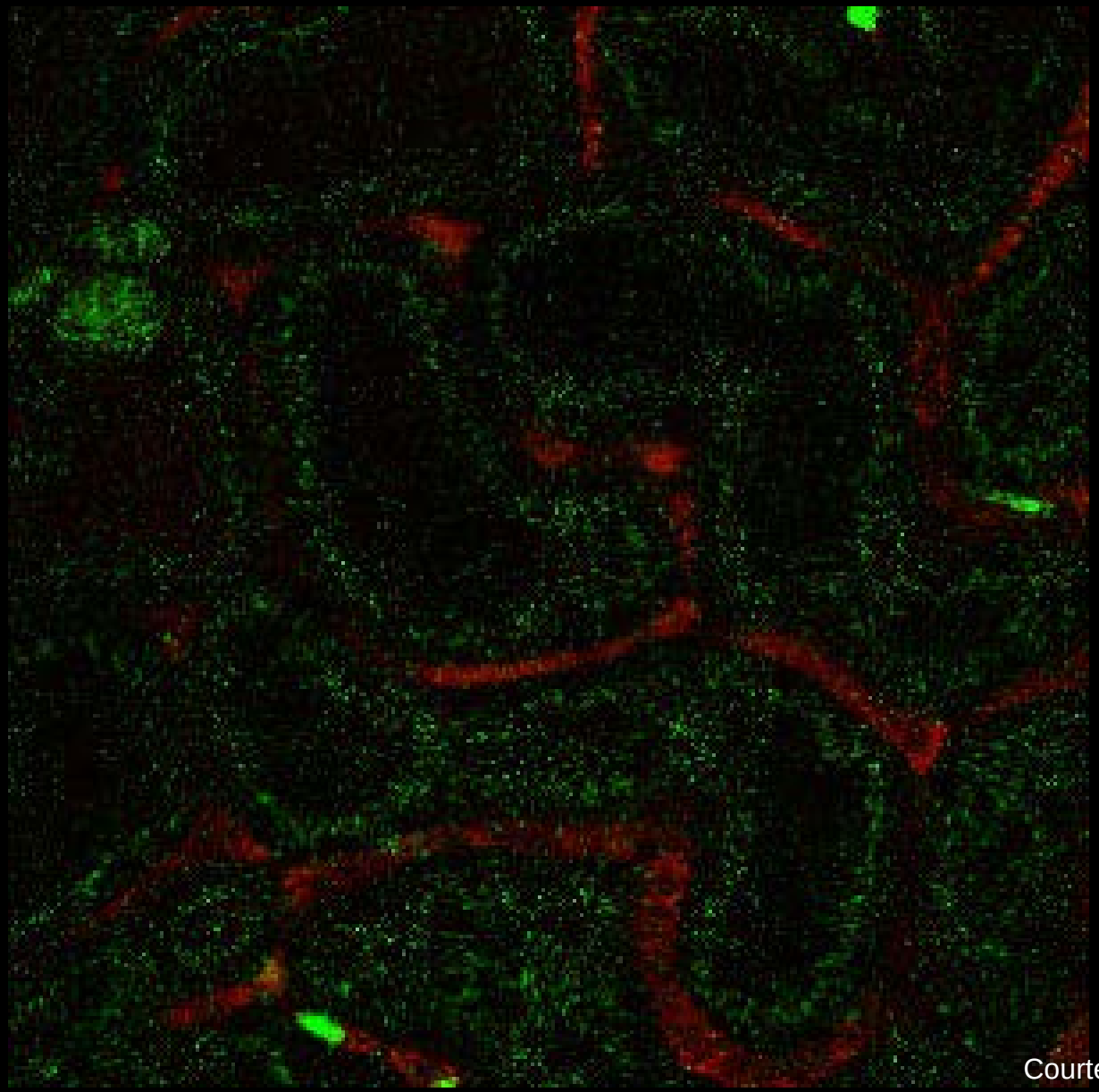
Microvascular Function

- Permeability
- Flow
- ***Leukocyte trafficking***

Leukocyte Trafficking

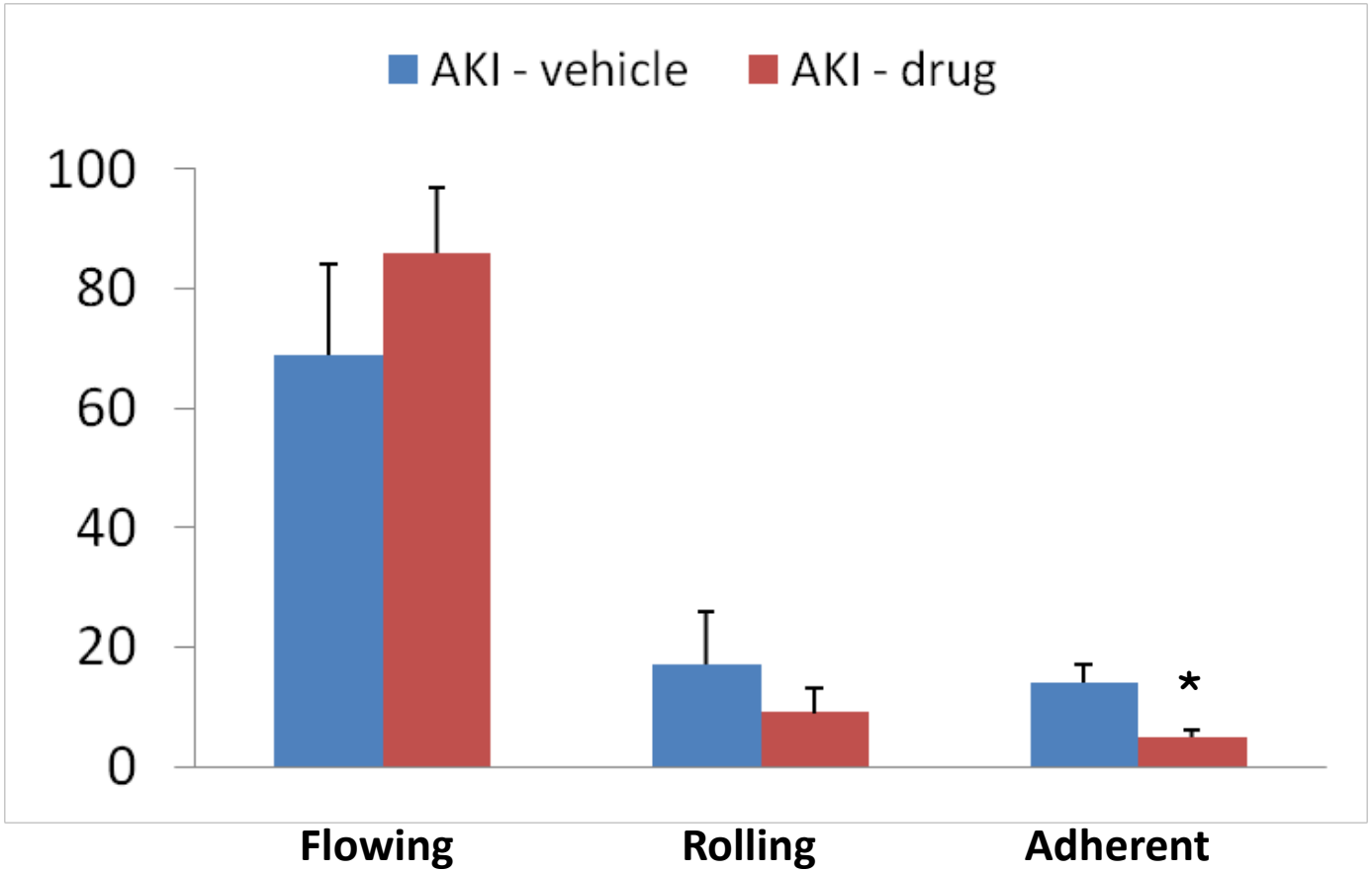


Courtesy of Ruben Sandoval

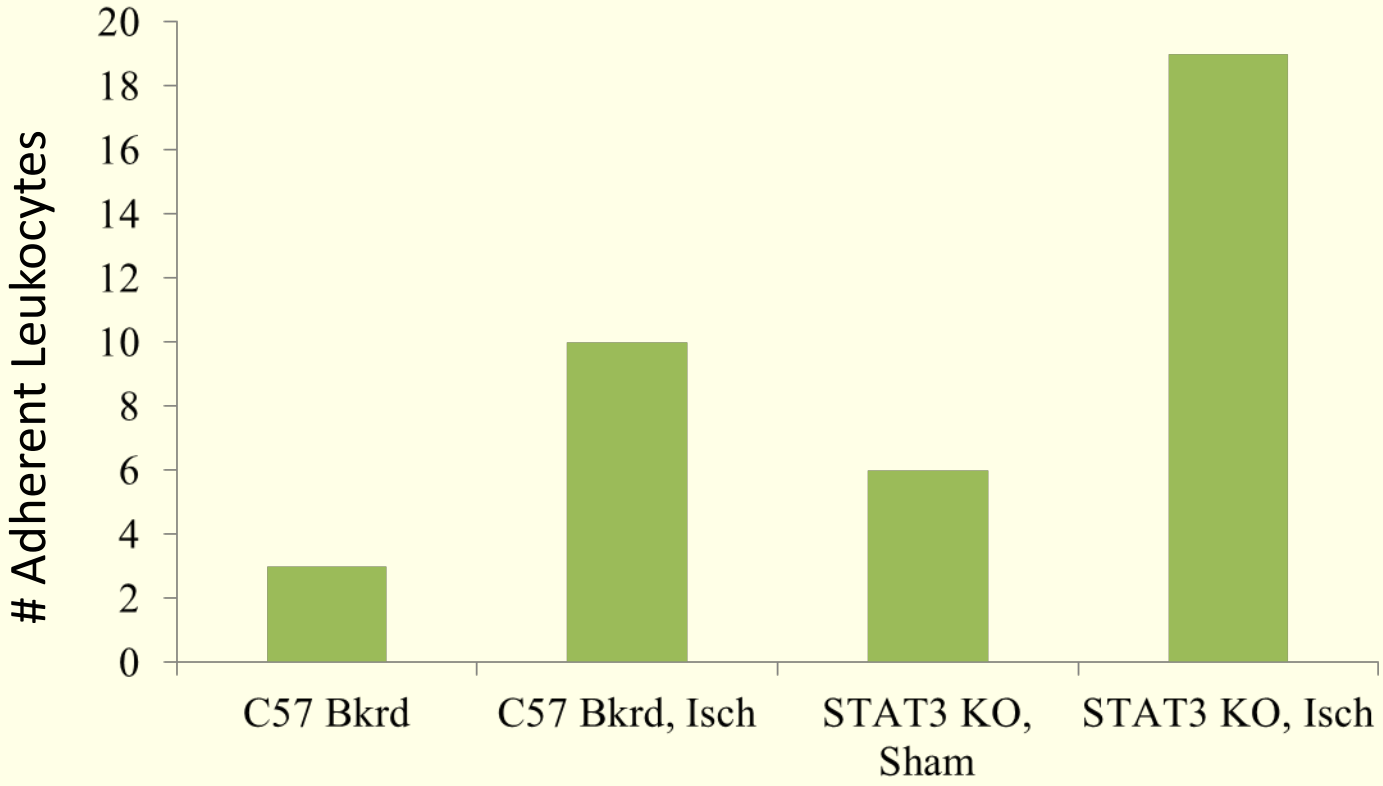


Courtesy of Simon Atkinson

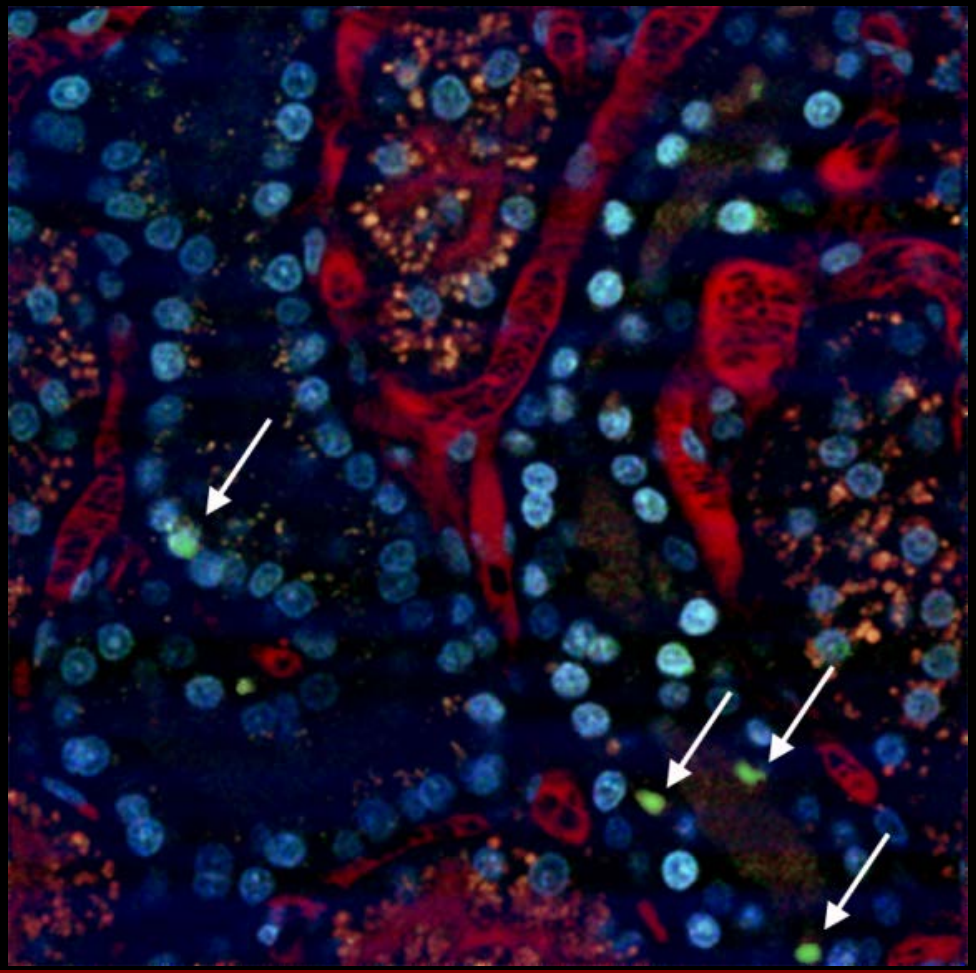
Anti-inflammatory Effect of Drug on Leukocyte Trafficking Following Ischemia



Leukocyte Adhesion

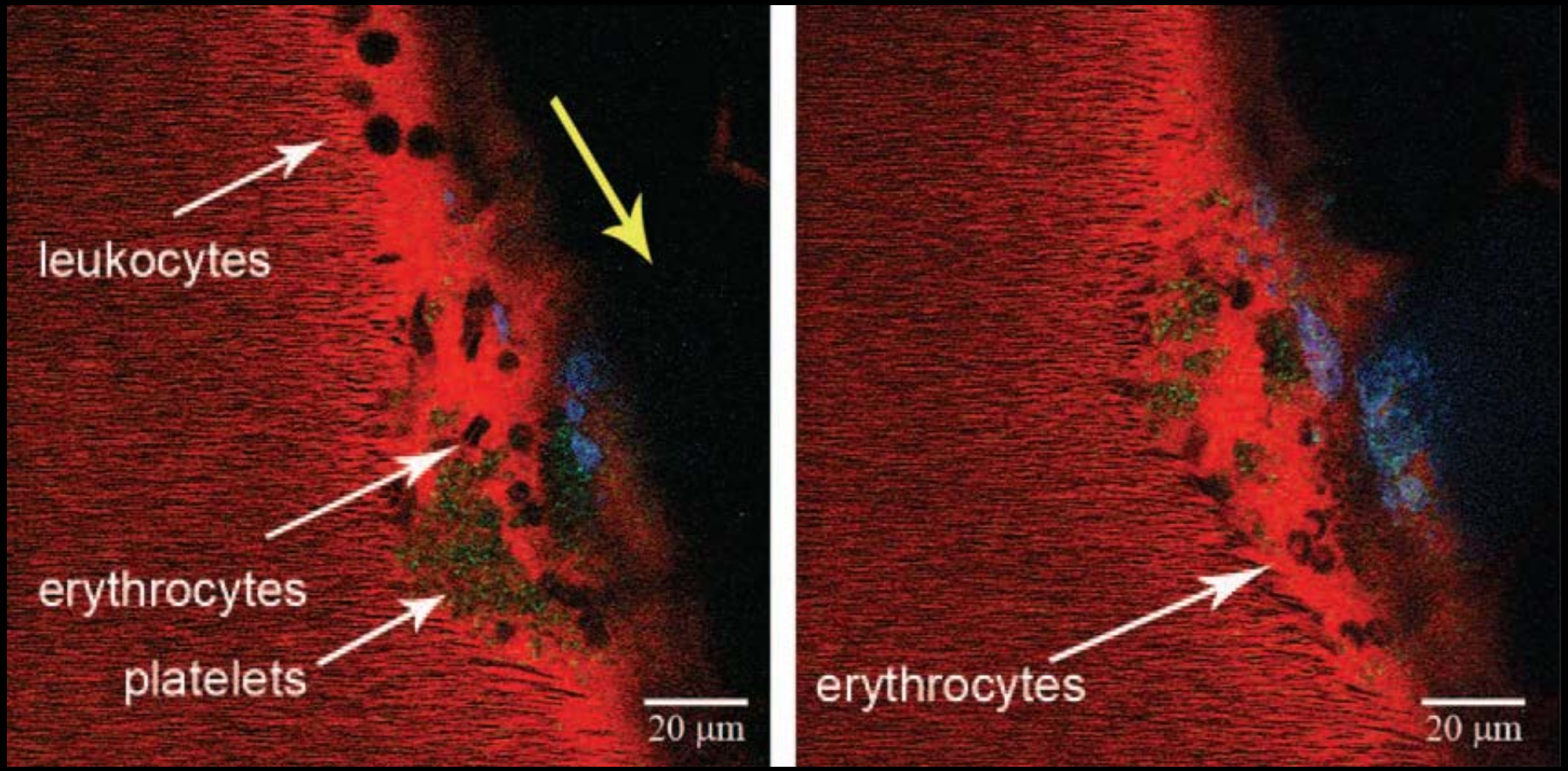


“Structure”-Function



Microvascular Function

- Permeability
- Flow
- Leukocyte trafficking
- ***Thrombosis***

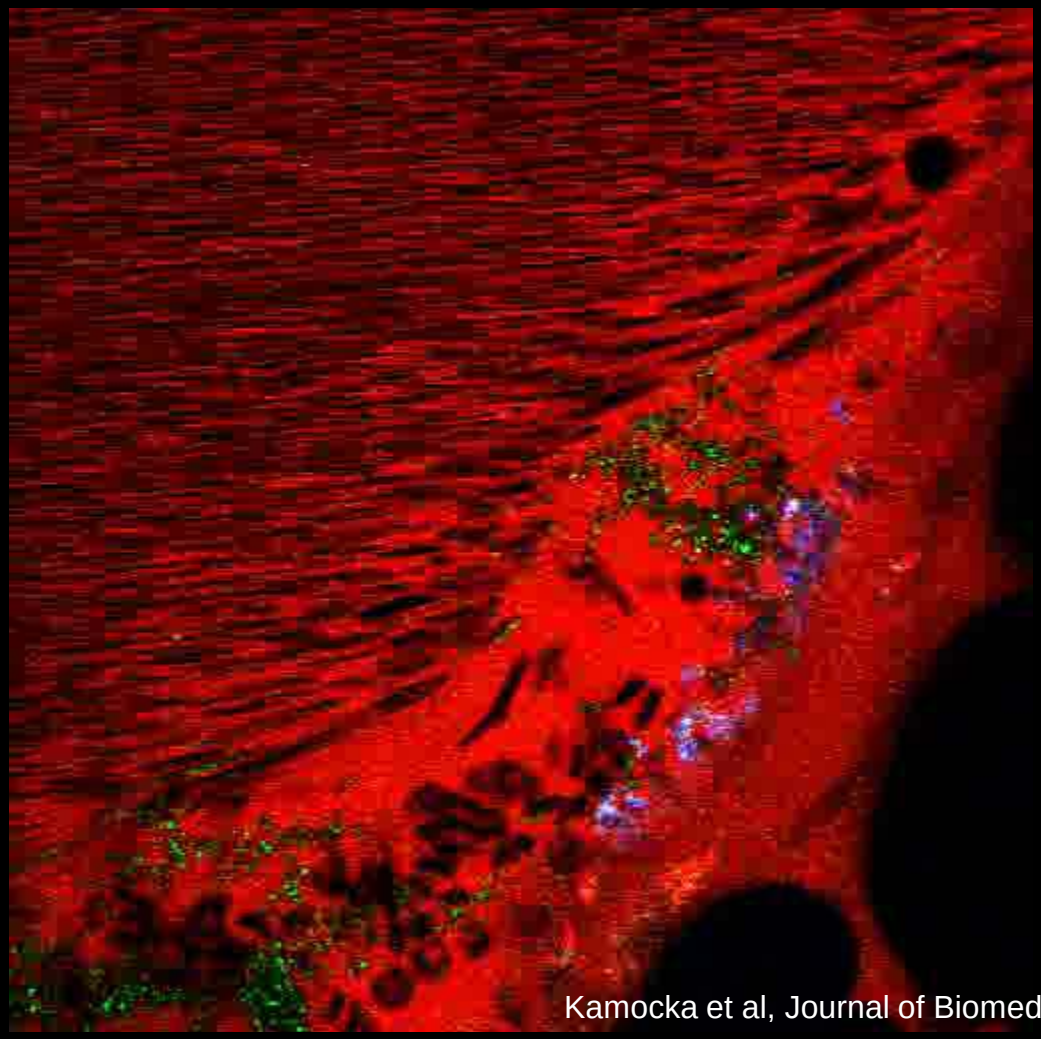


Platelets Fibrin Dextran

Alexa Fluor488 Alexa Fluor 388

Kamocka et al, Journal of Biomedical Optics 15:016020, 2010

Thrombus Formation



Platelets
Fibrin
Dextran

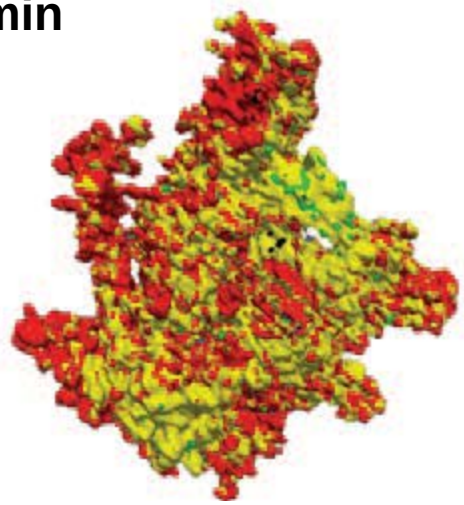
Kamocka et al, Journal of Biomedical Optics 15:016020, 2010

Platelets

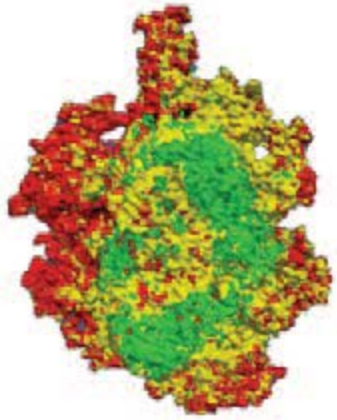
Fibrin–bound platelets

Fibrin

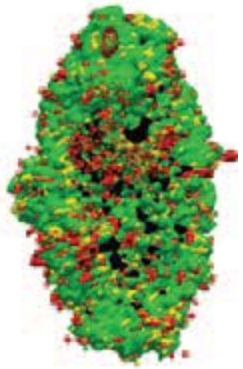
1 min



2 min

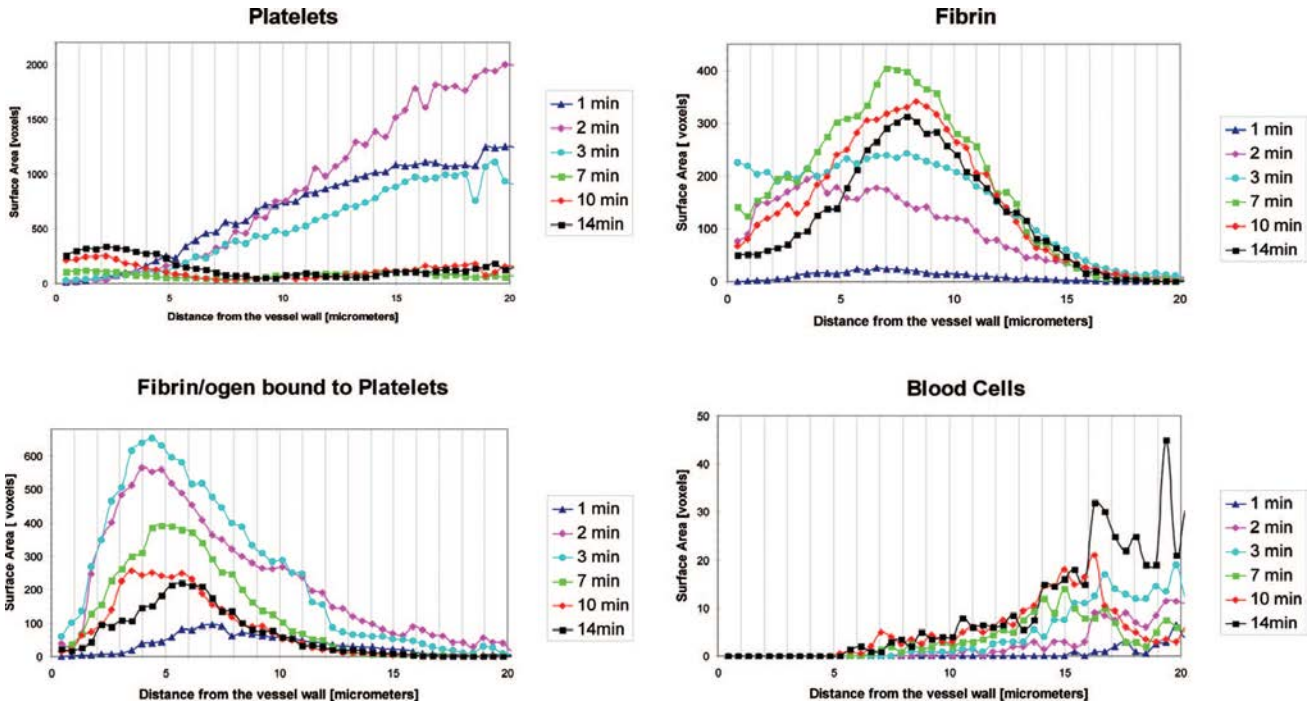


6 min



Kamocka et al, Journal of Biomedical Optics 15:016020, 2010

Thrombus analysis



Kamocka et al, Journal of Biomedical Optics 15:016020, 2010

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