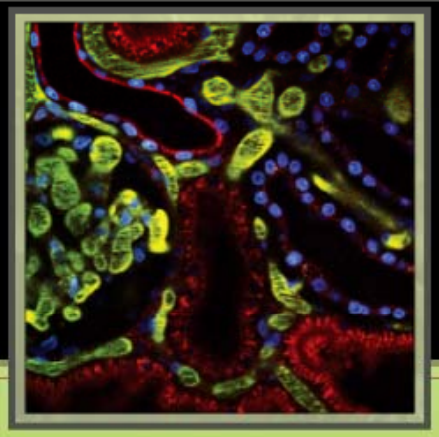
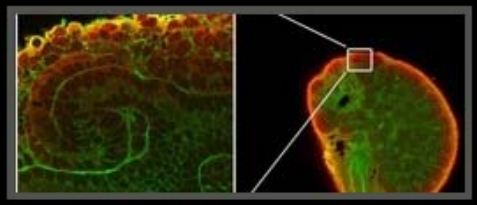
# George M. O'Brien Center

## for Advanced Renal Microscopy and Analysis

Download Application



*Hands-on training in state-of-the-art techniques in fluorescence microscopy, with an emphasis on intravital microscopy of the kidney.*



***2011 Workshop on Applied Microscopy in Kidney Research***

**April 26th - 29th 2011**

Indiana Center for Biological Microscopy
Indianapolis, Indiana

# Practical Intravital Imaging

# Outline

Preparation/ Surgery

Positioning

Landmarks: Unstained/ Stained
  Visual Indicators Determine Problems

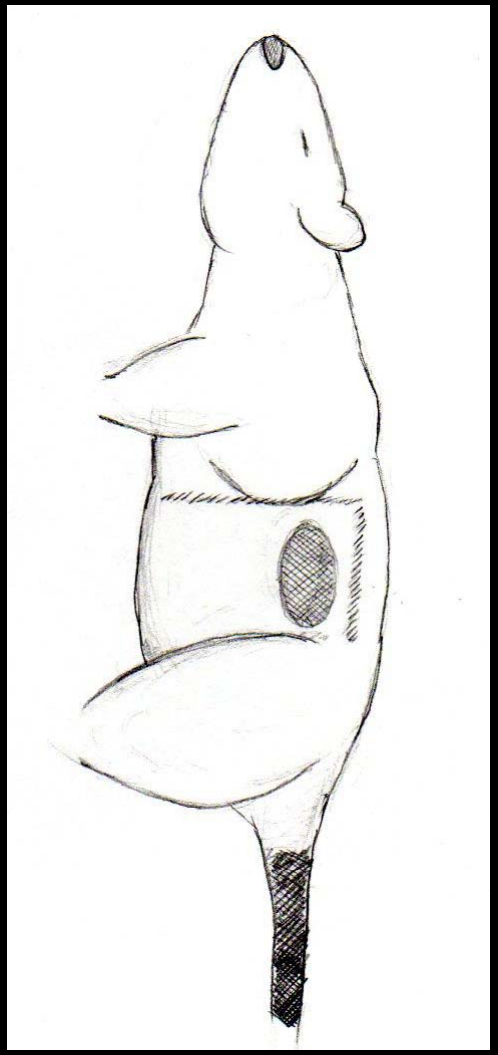
Fluorescent Markers: Dye Characterization

# Surgical Preparation

Short Term Imaging (less than 30')
Venous access:
Jugular- Infusion of probes
short line to minimize dead space
Rectal Temp Probe

Long Term Imaging (greater than 30-45')
Venous/Arterial access:
Femoral venous- continuous infusion of
0.9% Saline at ~1.5cc/Hr
Femoral arterial- BP/ HR monitoring, long line
Temp Probe in Saline dish, monitor fluid bath temp

During prep/ probe infusion: Receive ~ ¾ to 1.5 cc 0.9% Saline



# Surgical Preparation

Hemostat
Crushing outer skin and muscle layers to prevent bleeding

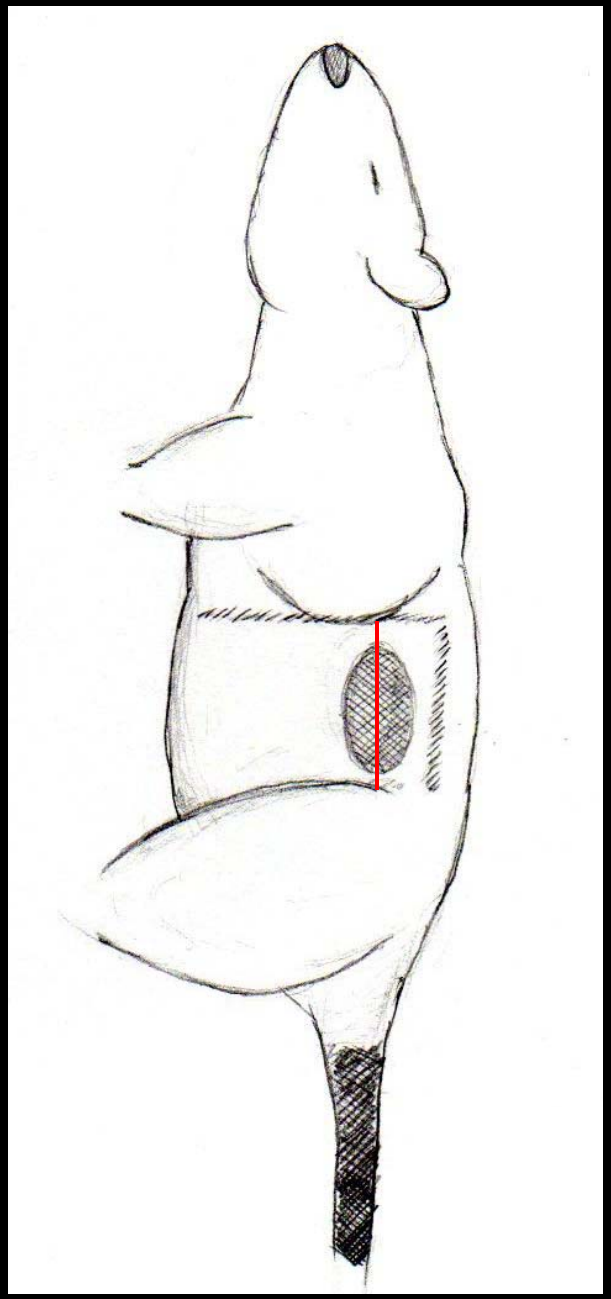
Long Forceps, w/ teeth
hold outer skin during cutting

Large Scissors
cutting outer skin

Small Scissors
cutting inner muscle layers

2 small forceps, blunt tipped w/ no Teeth to handle the fat/capsule surrounding the kidney

# Surgical Preparation



1). Lay rat perfectly on its side w/ left side facing you.

2). Palpate flank gently to find kidney, draw line down flank if necessary (make this line as large as necessary).

3). Lift skin w/ large forceps, crush tissue w/ hemostats, hold for 5-10 seconds.

4). Using large scissors, cut across crushed tissue line, there should be no bleeding.

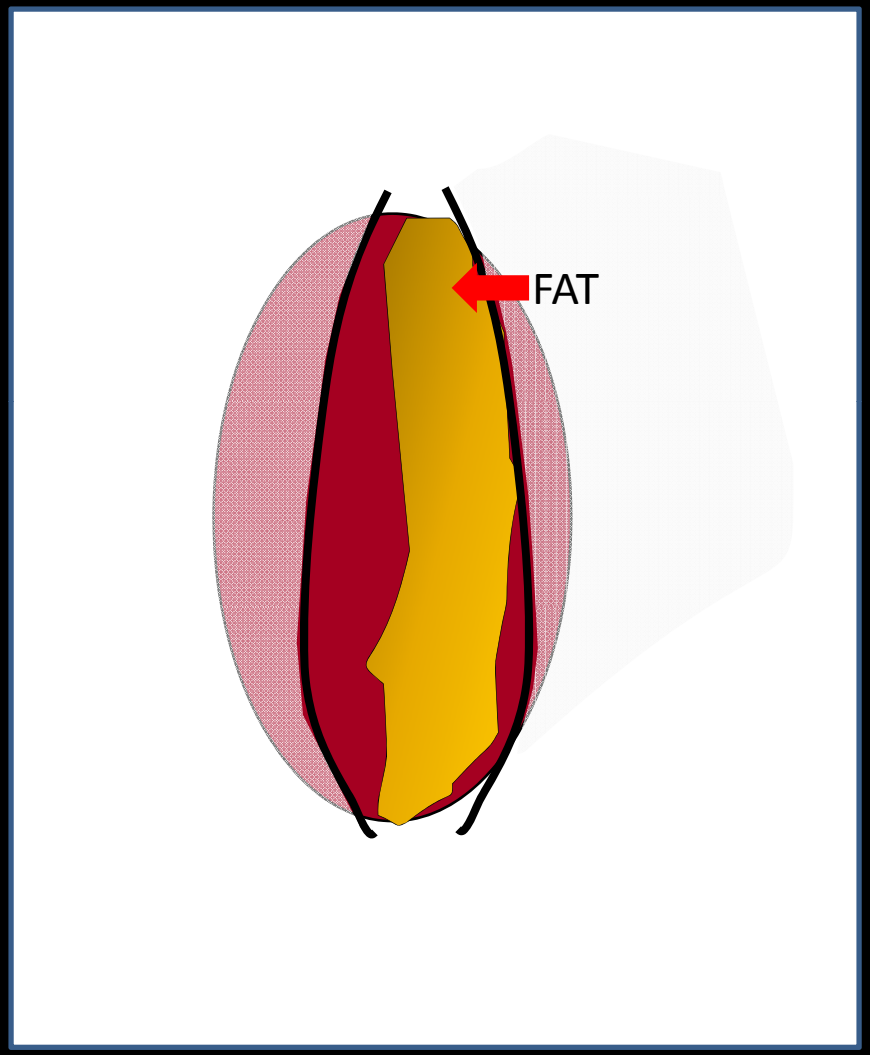
5). Repeat 3 & 4 w/ outer muscle layer, except use the small scissors

6). Crush the tissue on the 2nd muscle layer

7). Cut a small incision to visualize the kidney; a large incision will not hold the kidney out of the body.
It is easier to make another small cut than to stitch.

# Surgical Preparation

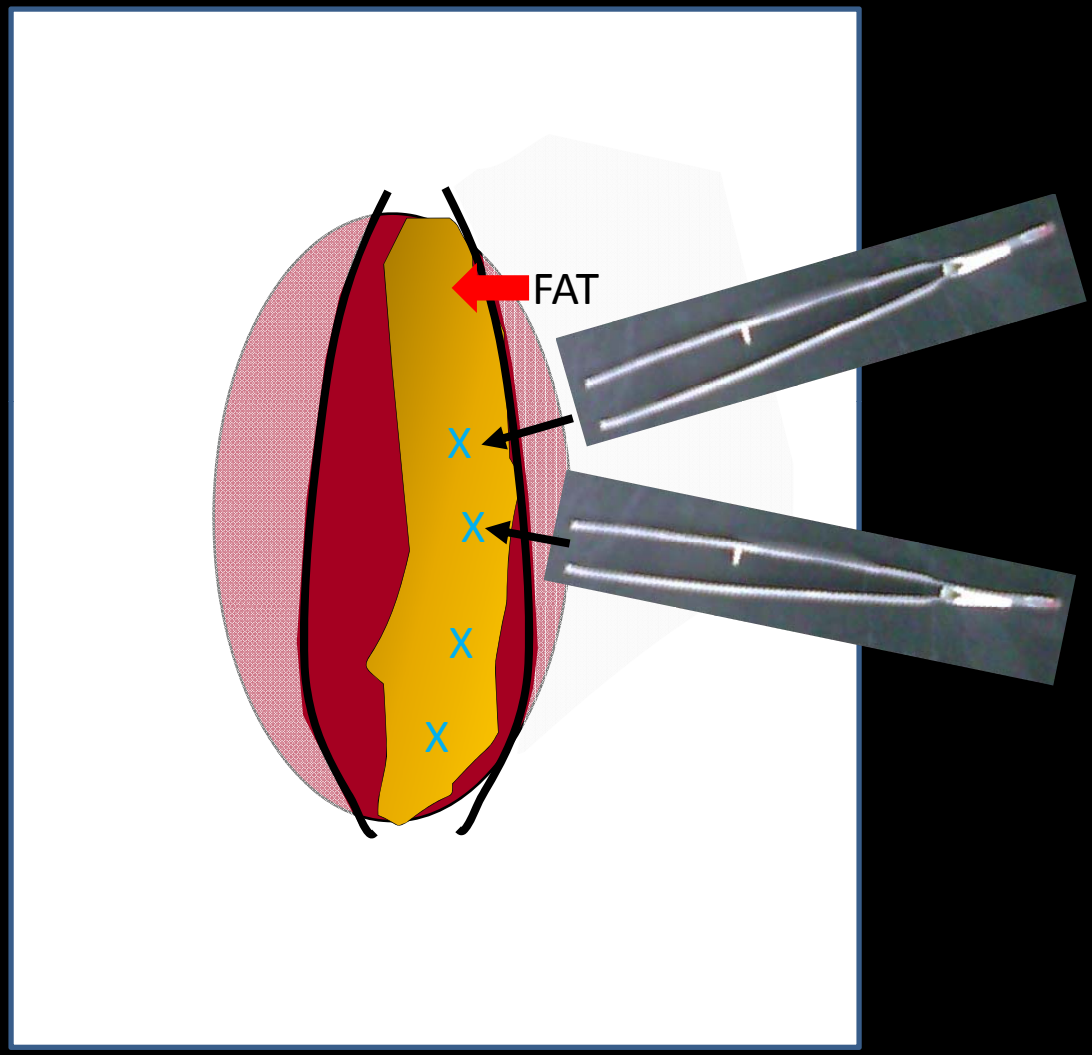
**Head**




**Tail**

# Surgical Preparation

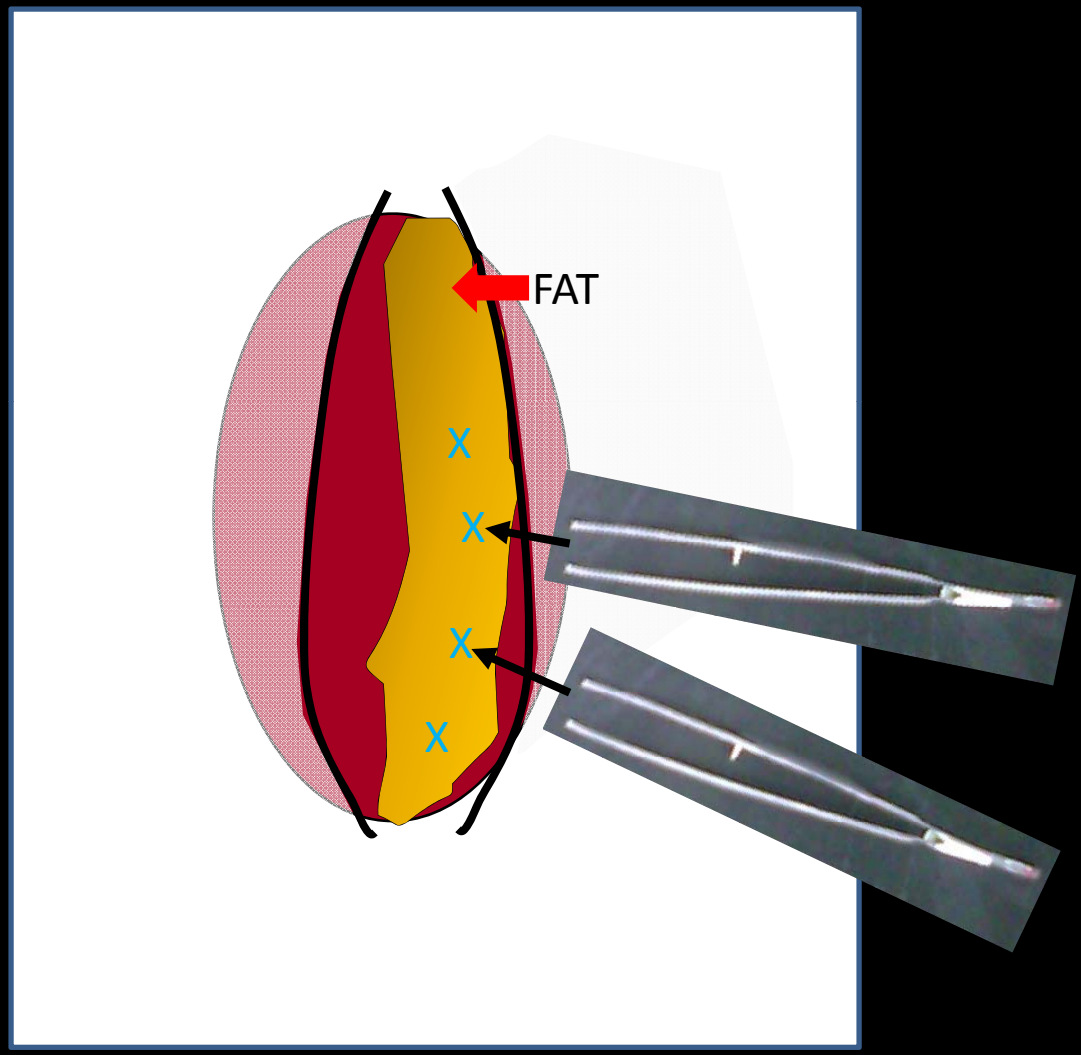
**Head**




**Tail**

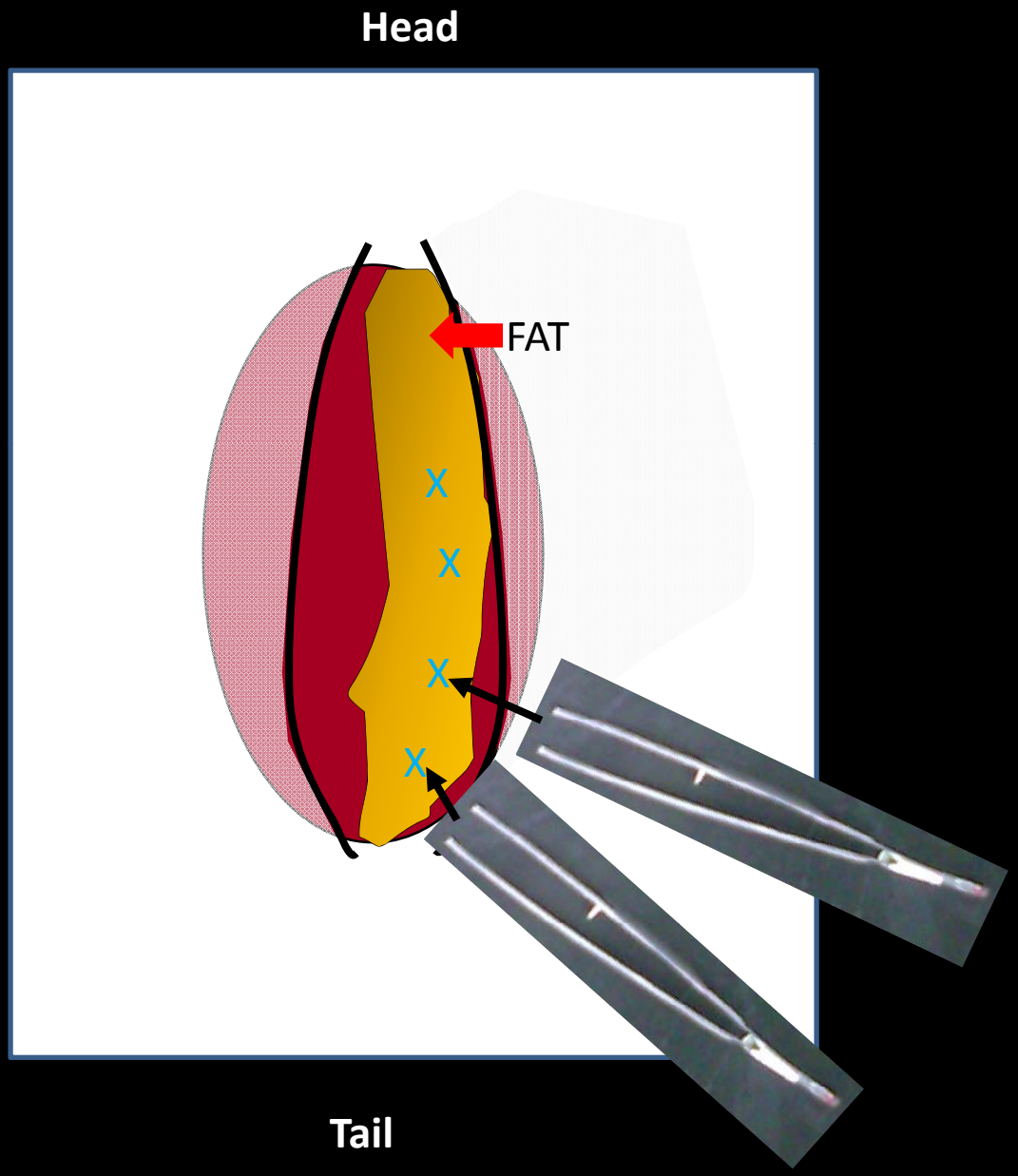
# Surgical Preparation

**Head**

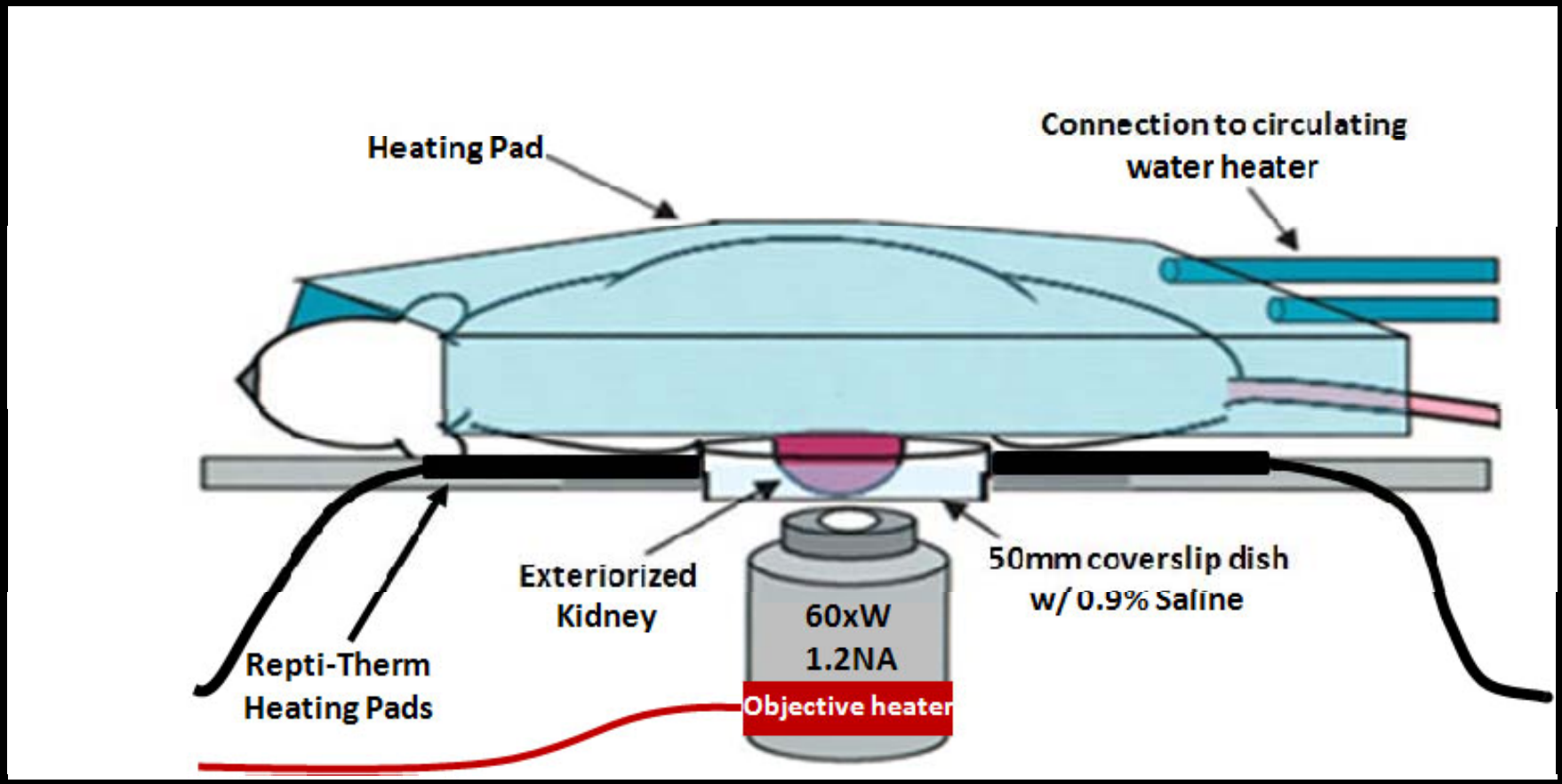



**Tail**

# Surgical Preparation



# Placement on Stage



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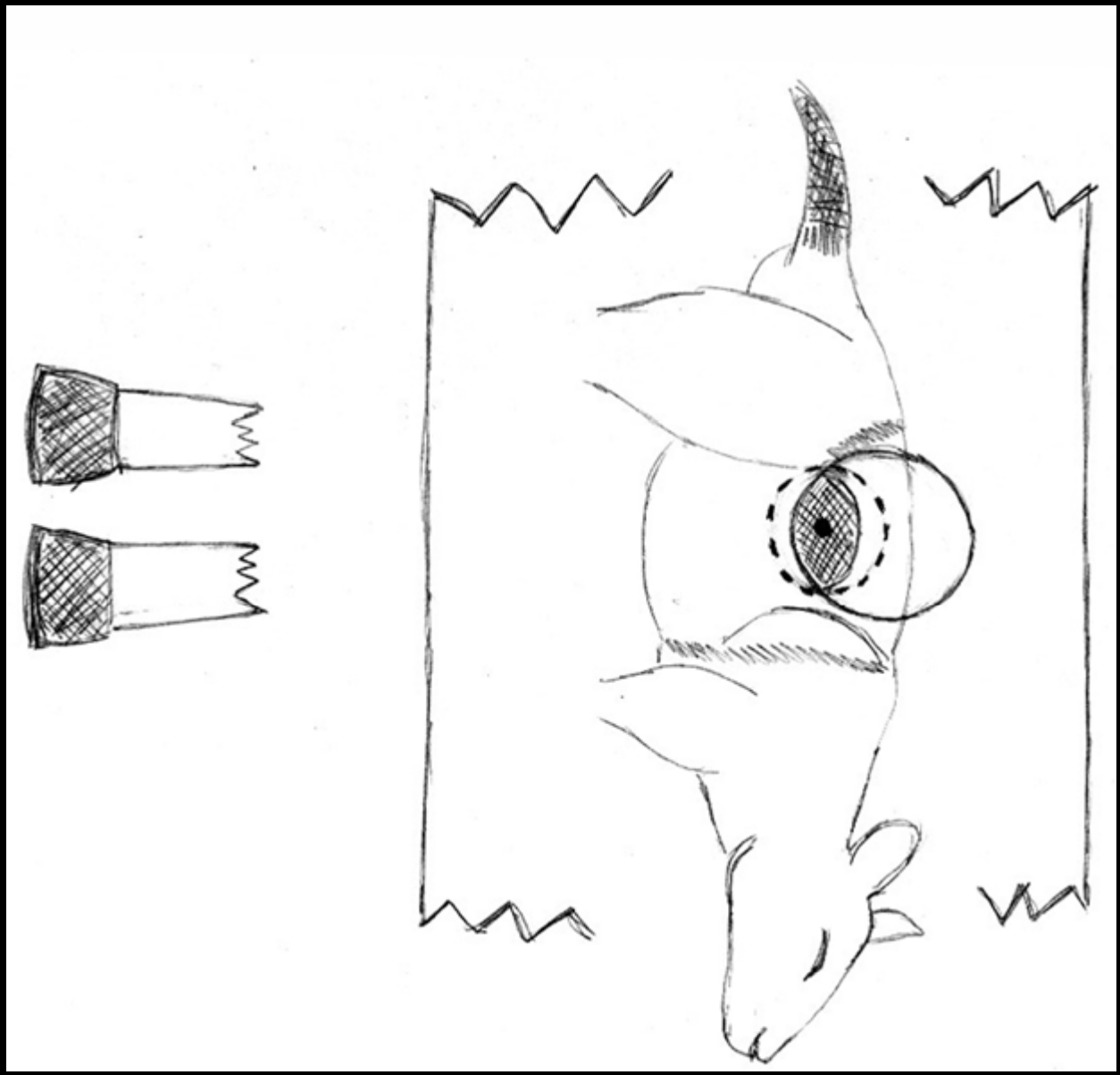
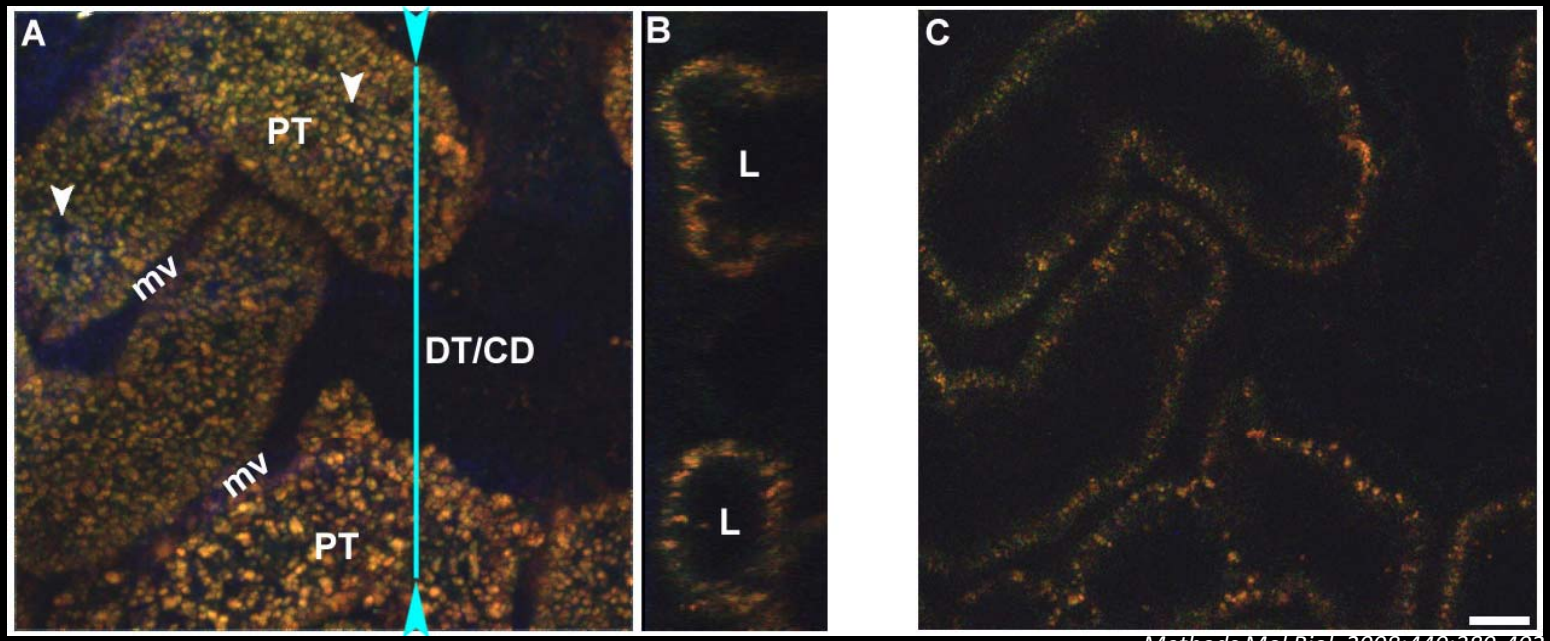
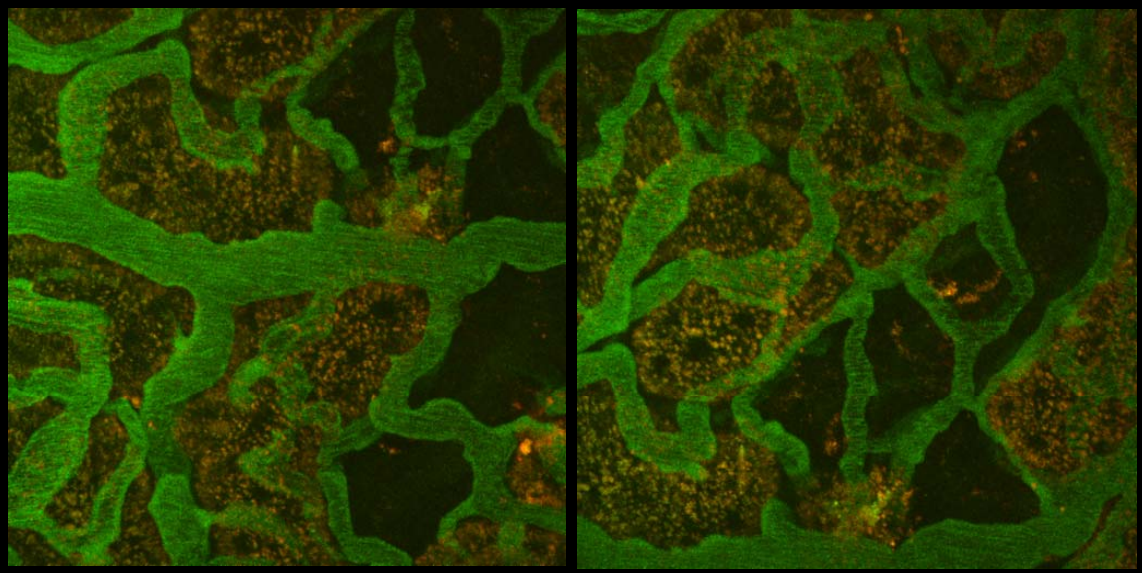


# Landmarks




*Methods Mol Biol. 2008;440:389-402*



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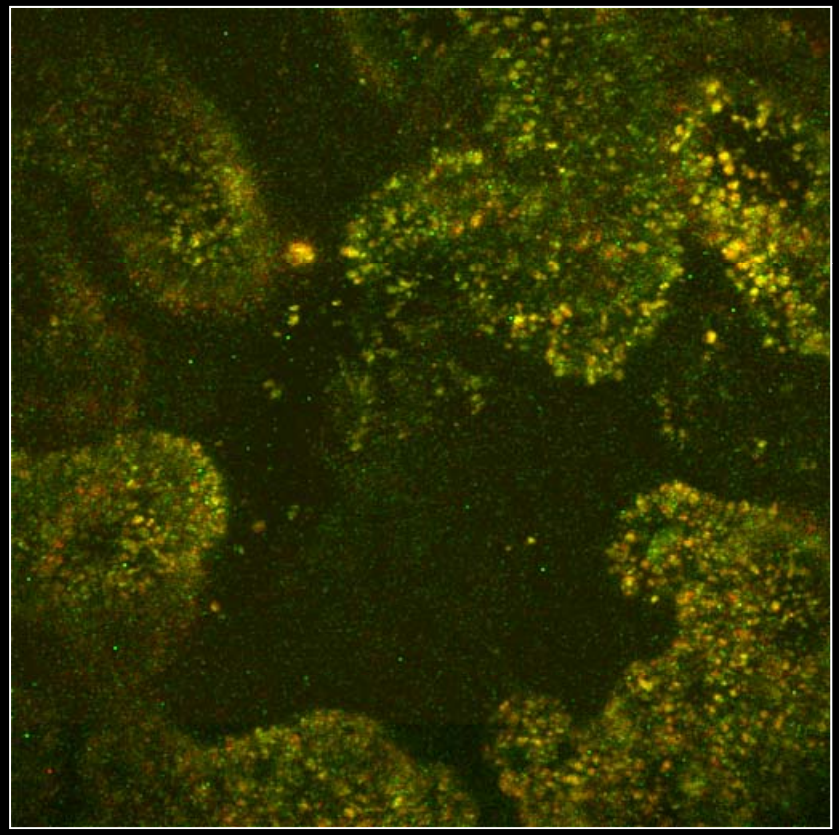
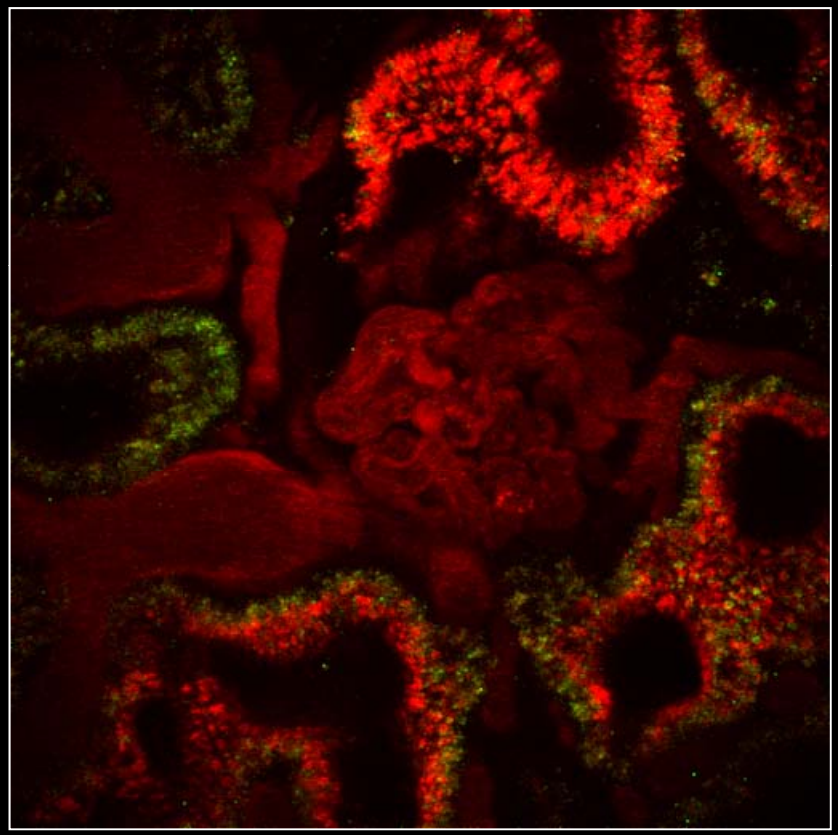
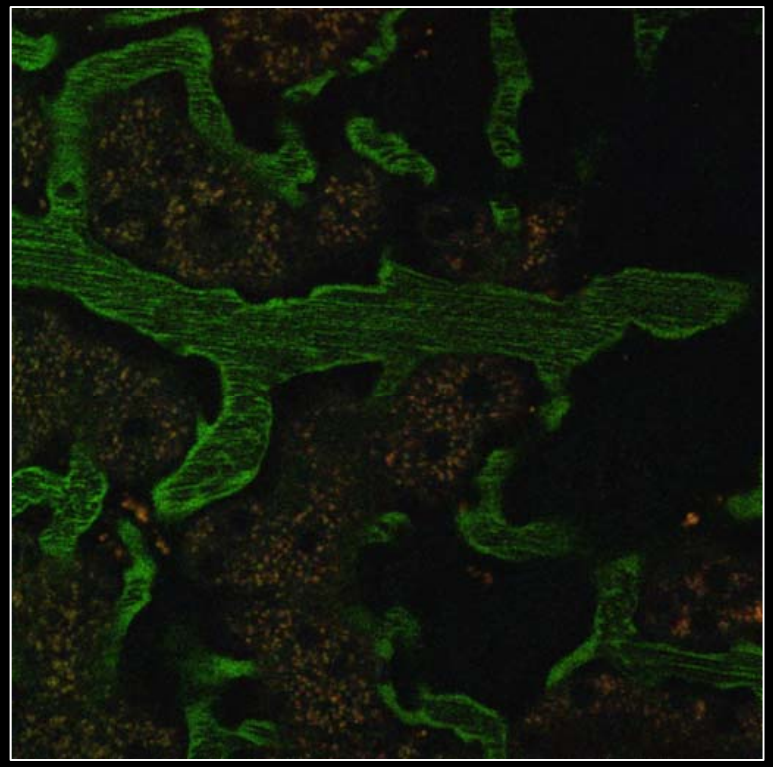


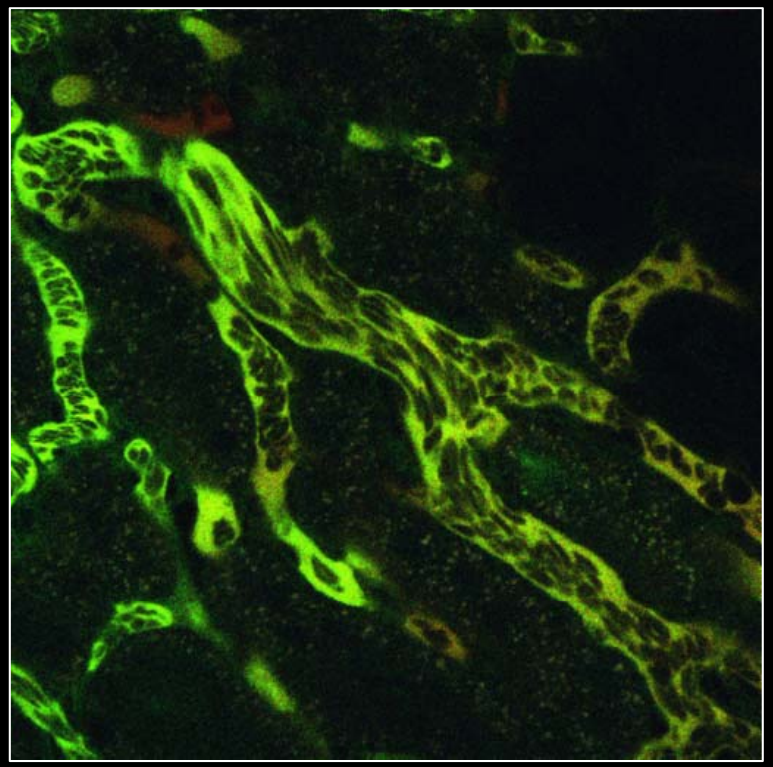


# Landmarks/ Anomalies

Normal Infusion:
1). Should see dye in bloodstream within 5-7 seconds
2). Appearance in field is fairly uniform
3). RBC's should appear as streaks across straight blood vessels

Alterations:
1). Appearance of dye exceeds 10 seconds
2). Appearance within field is staggered among vessels
3). Shape of RBC's is discernible, abundant plasma in vessels



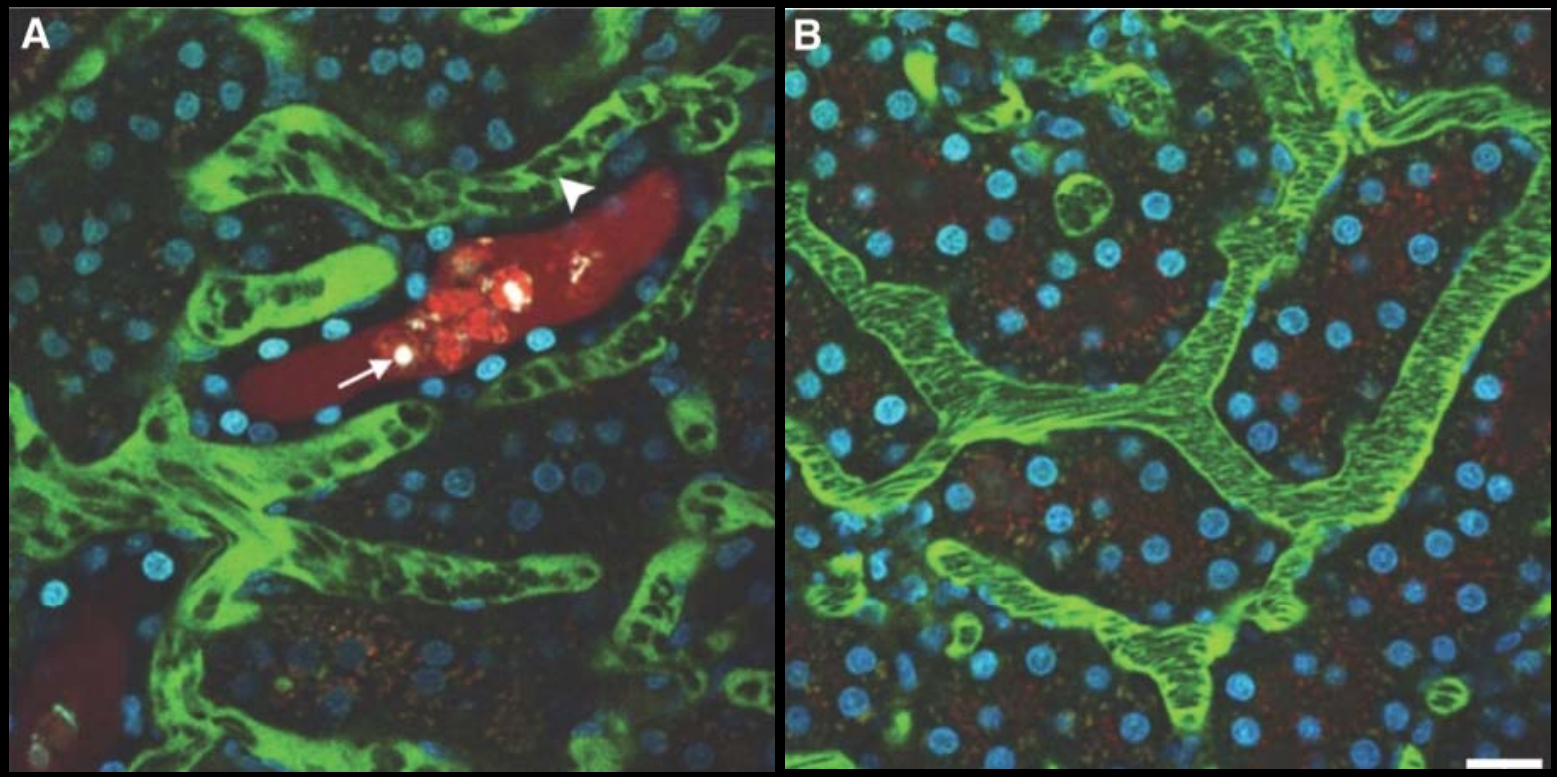


# Landmarks/ Anomalies

Sclerotic Glomerulus


Damaged Glomerulus

# Landmarks/ Anomalies



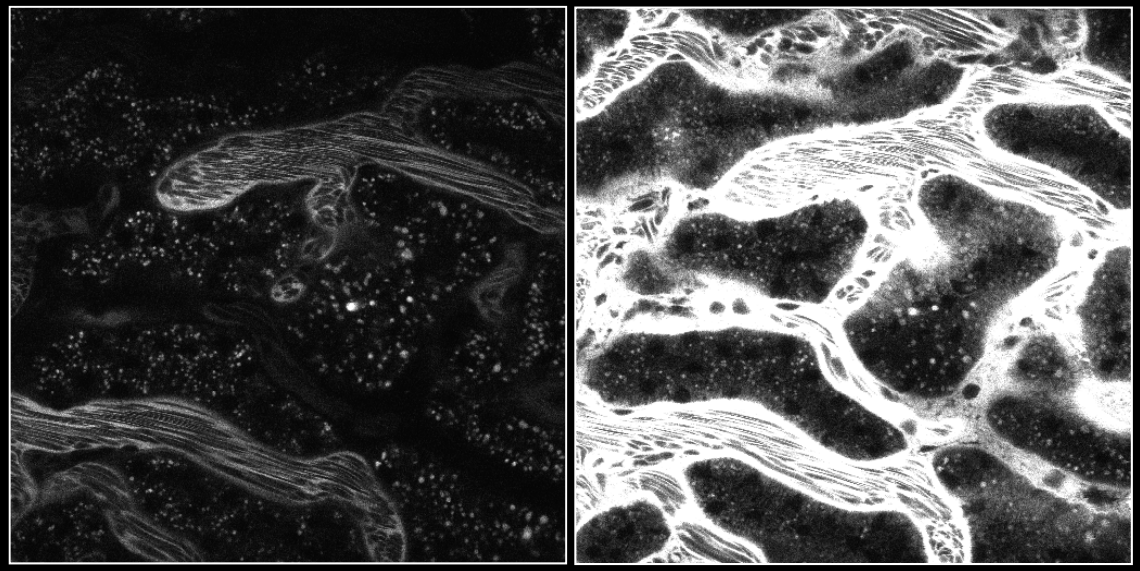

Soluble thrombomodulin protects ischemic kidneys. Sharfuddin AA et al, *J Am Soc Nephrol*. 2009 Mar;20(3):524-34.

Flow Anomolies
Proximal Tubule anomalies
Cast material

# Fluorescent Markers: *in vitro* calibration

Important when setting up experiment to collect quantitative data.

Involving collection of images that will later be used to determine and subtract background values.

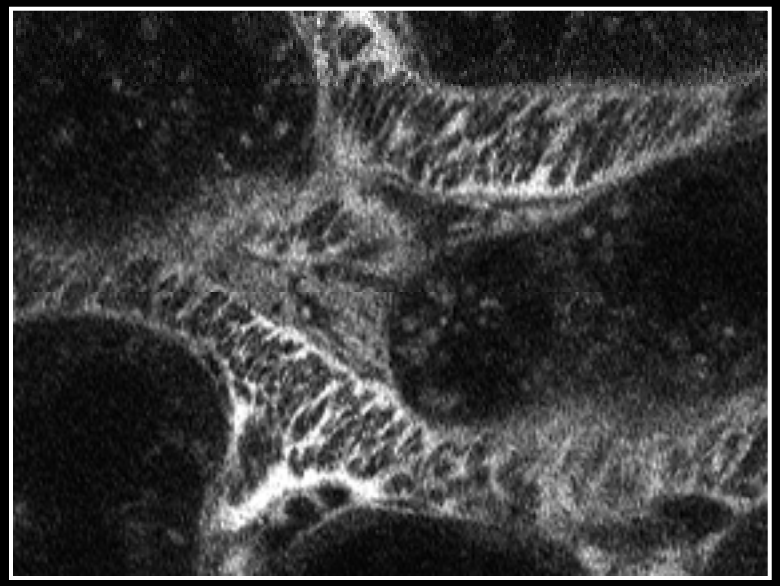


# Fluorescent Markers: *in vitro* calibration
# Prior use in cell culture

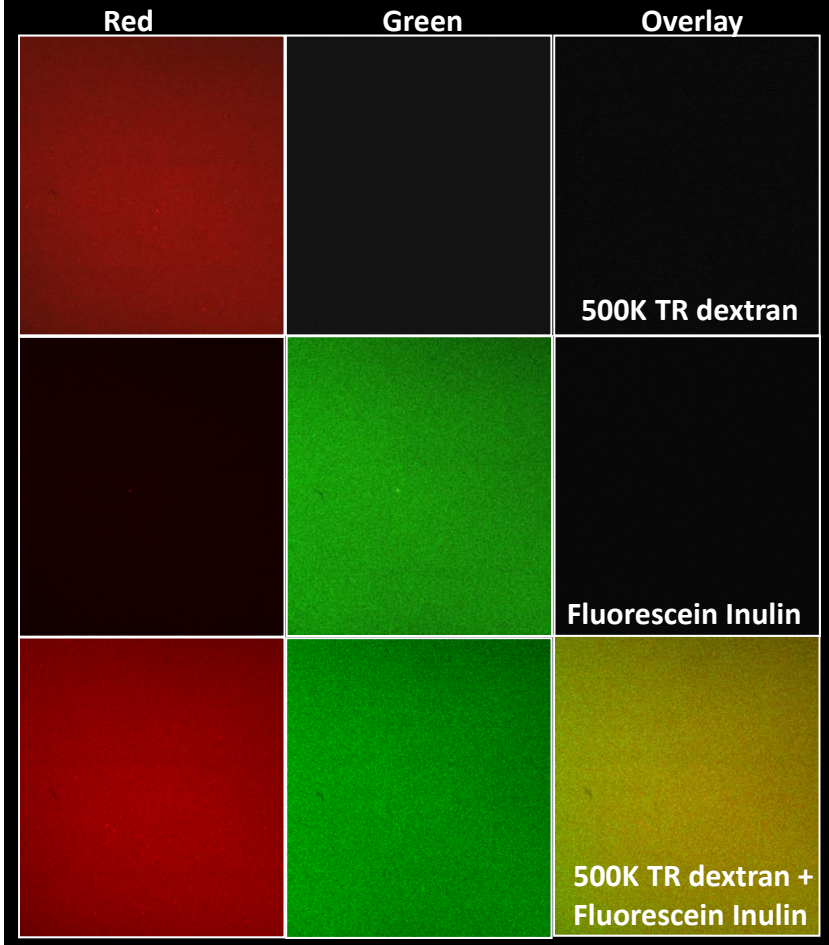
1.) Determine Cell Culture Concentrations ( 500ug/mL)

2.) Determine Plasma Volume of Rat (Typically ~3.5%; dyes do not cross into RBC's)
250g rat = 8.75 mL of plasma
Dose = 4.3mg; Add ~20%, final Dose= 5.16mg

3). Load syringe w/ 2-3 doses, carefully monitor fluorescence during infusion



# Fluorescent Markers: *in vitro* calibration




1). Carry out a dilution series of your stockmarker in PBS

2). Calculate a dilution factor of the stock solution (1:150, etc) that best uses the dynamic range of the microscope.

3). Calculate approximate plasma volume of rat bases on body weight (250g)= 8.75mL

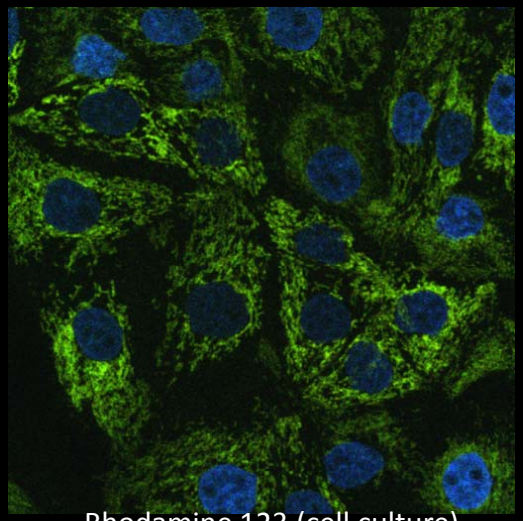
4). Divide Plasma Volume (in uL)/ Dilution Factor) 8750/150= ~58.333uL stock

5). Add at least 20% extra (light scatter through tissue, dead space in venous line)

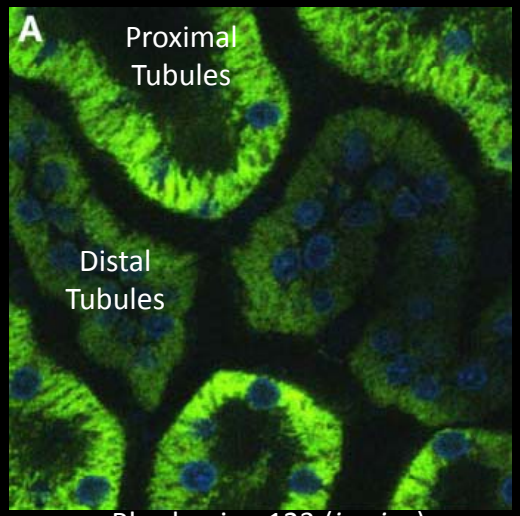
6). Dilute into at least 500-750uL of Normal Saline, watch monitor as you infuse to assure you do not saturate your specimen.

*Crucial probes retained in the blood.

# Fluorescent Markers: *in vitro* characterization

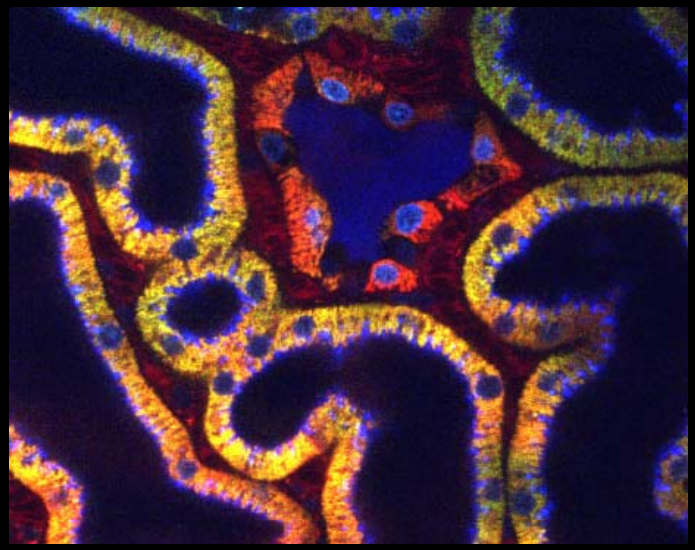


Rhodamine 123 (cell culture)

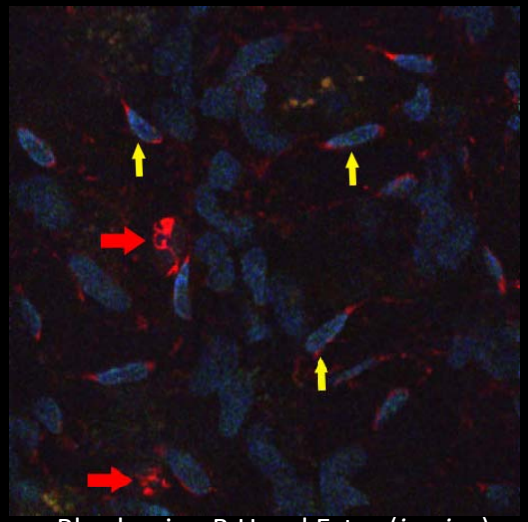



Rhodamine 123 (*in vivo*)

*Adv Drug Deliv Rev. 2006 Sep 15;58(7):809-23 2006 Aug 15. Review*



Rhodamine 123 (green)/TMRM (red)



Rhodamine B Hexyl Ester (*in vivo*)
White Blood Cell (red arrows)
Endothelial Cell (yellow arrows)

*Adv Drug Deliv Rev. 2006 Sep 15;58(7):809-23 2006 Aug 15. Review*

# Acknowledgements

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