

Preparation of Animals for Live Animal Imaging

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Ideal Condition of Rats during Experiments:

- adequate level of surgical anesthesia
- patent airway
 - supplemental oxygen
 - blood gases
- normal body temperature
- adequate cardiovascular function
 - fluid replacement
 - blood pressure monitoring
- normal renal function
 - measurement of GFR and renal blood flow
 - lissamine green passage time

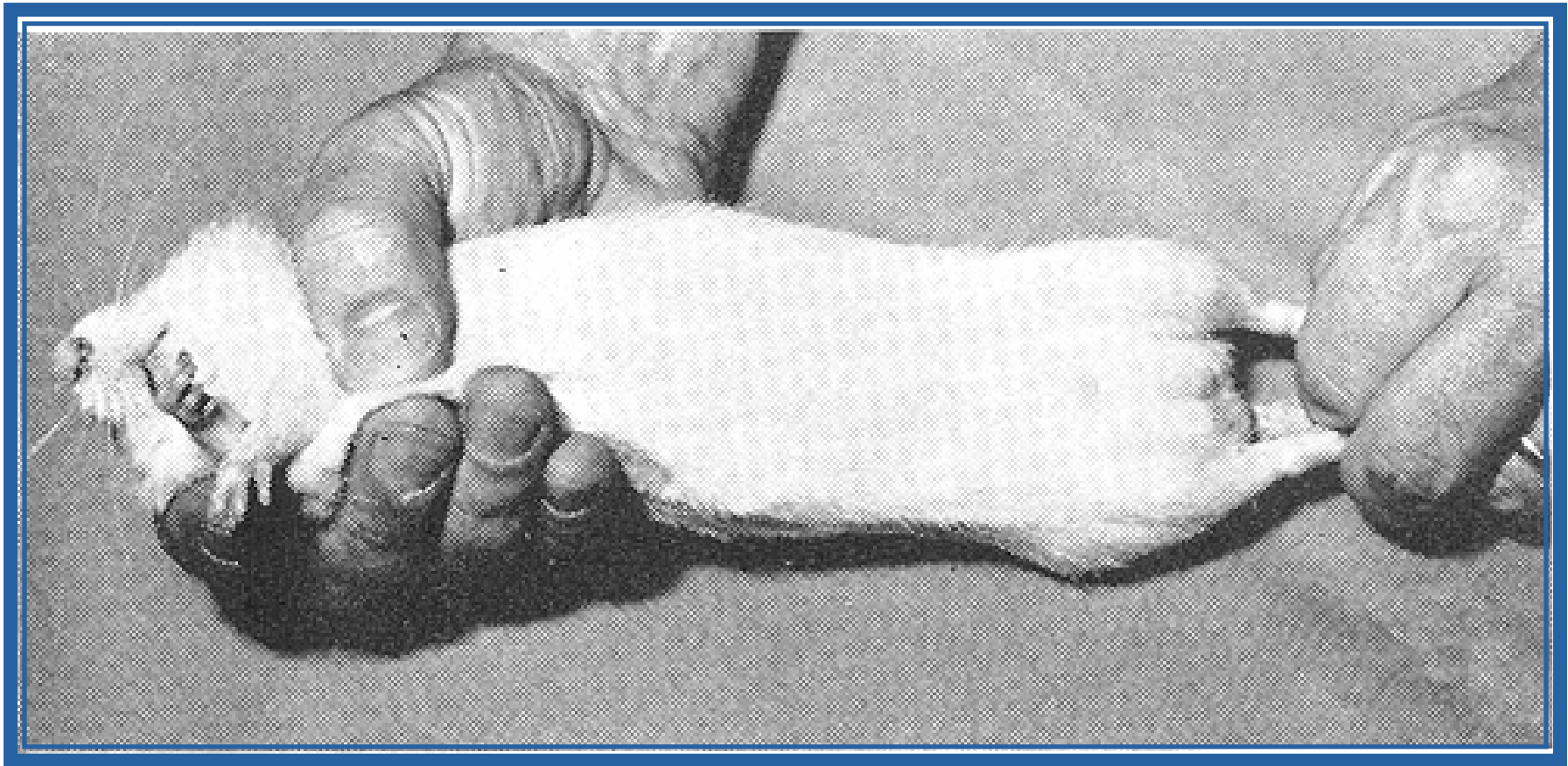
Anesthesia in the Rat:

1. It is best to fast the rat overnight, but allow ample water, prior to abdominal surgery.
2. The rat should be properly restrained (use gloves or towel wrap).
3. For recovery operations:
 - Halothane anesthesia (rapid recovery)
 - Pentobarbital, 40 mg/ml solution in isotonic saline, given i. p., 0.1 ml per 100 g body weight
4. For long-term operations:
 - Inactin, 100 mg/ml solution in isotonic saline, given i.p., 0.10 – 0.13 ml per 100 g body weight

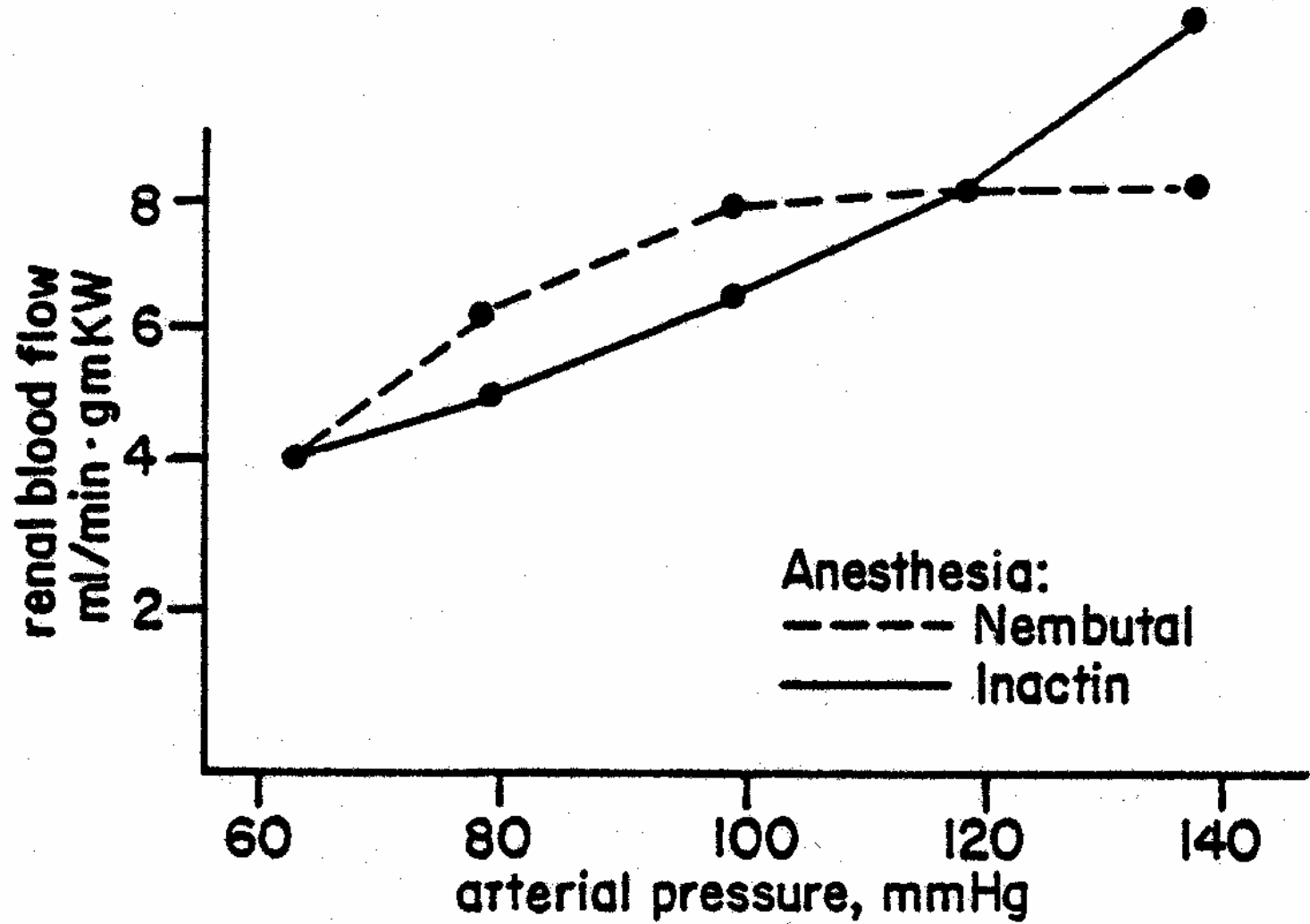
Anesthesia in the Rat (continued):

5. With surgical anesthesia level, respiration should be regular, ears and mucous membranes should be pink, no withdrawal of foot when the toes are pinched (painful reflexes extinguished), and the skeletal musculature should be flaccid. Rat will not blink when the eye or eyelid is touched.
6. Artificial respiration in the rat: Hold the forepaws with one hand and the hind paws with another, keeping the animal belly up, and swing it back and forth until the animal starts to breathe normally.
7. The particular anesthetic used may affect your results!

Ref.: Ben, Dixon, and Adamson. Anesthesia in the rat. Fed Proc 28:1522-1527, 1969; Taber and Irwin. Anesthesia in the mouse. Fed Proc 28:1528-1532, 1969







From: Conger and Burke. Am J Physiol 230:652-657, 1976

Rat Preparation:

1. Shave skin with fine clippers and remove hairs.
2. Place animal on heated board and monitor rectal temperature with a probe.
3. Normal rat rectal temperature is 37 – 38°C.
4. Rats become hypothermic rapidly when anesthetized.

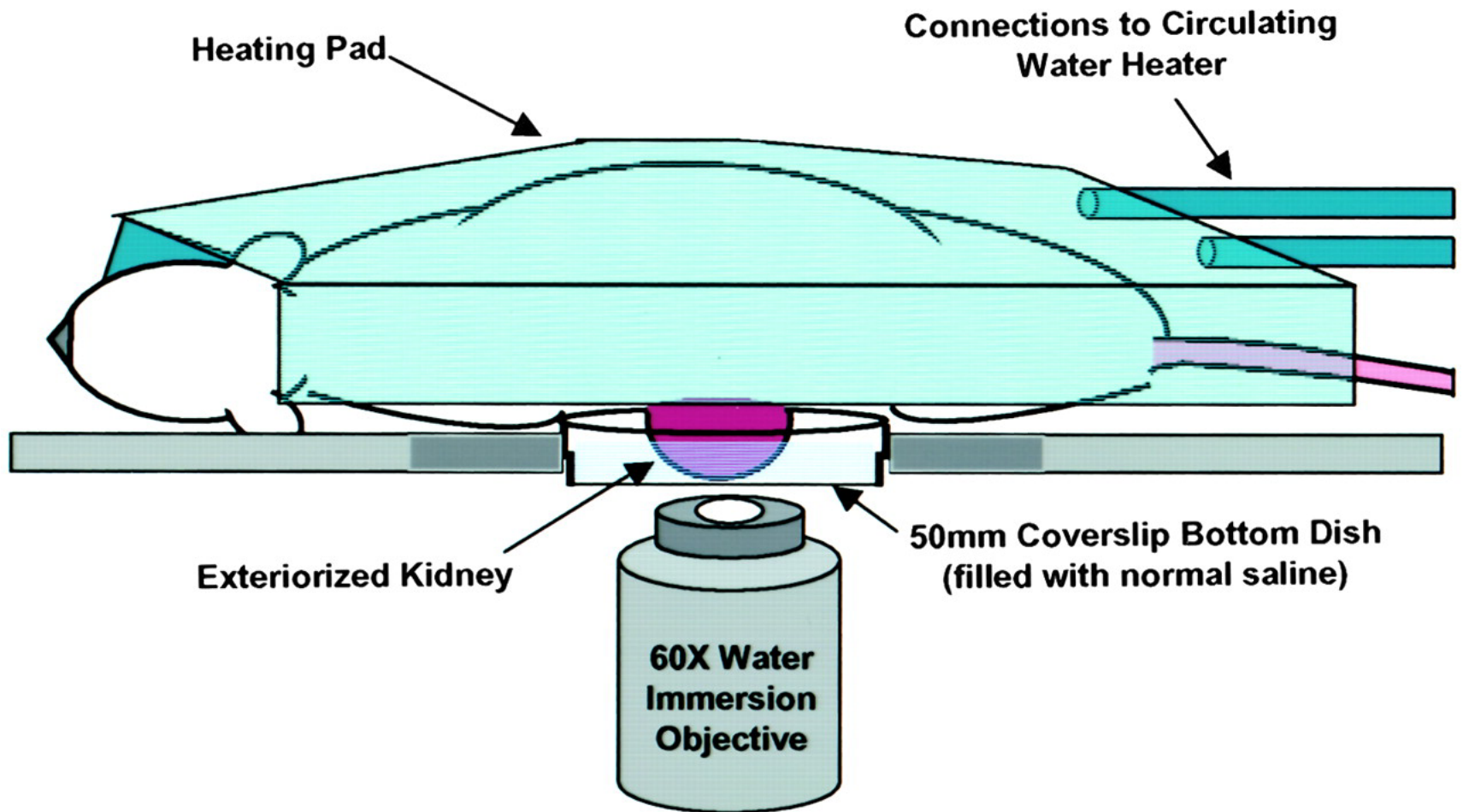


Anesthetized rats have a limited ability to regulate their body temperature, which will spontaneously decrease to 30–34°C if they are not warmed.

At a body temperature of 34°C, blood flow to the kidney cortex surface is half of normal.

Sensitive transport processes, such as PAH secretion in superficial proximal convoluted tubules, can come to a complete halt when body temperature falls.

Deetjen & Brechtelsbauer. Pfluegers Arch.
ges. Physiol. 281:26–27, 1964.



From Dunn et al., Am J Physiol Cell Physiol
283:C905-C916, 2002

Effect of Body Temperature on Response to Temporary Renal Ischemia:

Body temperature, °C	Plasma creatinine, mg/dl
33	0.56 ± 0.06 (5)
37	1.40 ± 0.25 (7)
39	3.20 ± 0.23 (9)

Values are means ± SEM (number of rats). Rats were subjected to 25 min of bilateral renal artery occlusion, and plasma creatinine was measured after 24 hr. Baseline plasma creatinine concentration was about 0.52 mg/dL in all groups. From: Zager & Altschuld. Am J Physiol 251:F87–F93, 1986.

Adequate Breathing:

- Maintain a patent airway

- Tracheostomy

Use 2 cm length of PE 240 for a 250 g rat.

- Give supplemental oxygen (moistened 35% oxygen, 65% nitrogen). Can also give 100% oxygen, but don't administer 95% O₂/5% CO₂ because you will induce a respiratory acidosis.

Acid-base Measurements in Inactin-Anesthetized Normal Rats:

Arterial pH	7.38 ± 0.03
Arterial P _{CO₂} , mm Hg	37 ± 4
Arterial plasma [HCO ₃ ⁻], mEq/L	22 ± 2.5
Arterial P _{O₂} , mm Hg	128 ± 21
Hemoglobin saturation with O ₂ , %	98 ± 0.6

Values are means ± SD (n = 14).

Reasons to Cannulate Femoral Artery and Vein:

- use for supplemental i.v. anesthetic doses (e.g., 0.03 ml 40 mg/kg body wt pentobarbital solution)
- maintain adequate hydration by constant i.v. infusion
- infuse substances i.v. to measure renal functions
- monitor arterial blood pressure
- collect arterial blood samples

The Hydration Issue:

1. Rats prepared for micropuncture may show a decreased blood volume and impaired circulatory function.
2. During preparation, I routinely give 1.0 ml 6% bovine serum albumin (fraction V) in isotonic saline i.v. to a 250 g rat.
3. Some authors remove blood from a donor rat, and infuse the plasma i.v. For example, Thomson et al. *Am J Physiol* 270:F461–F468, 1996 infused 1% body wt over 1 hr followed by continuous infusion at 0.15% body wt/hr.

The Hydration Issue (continued):

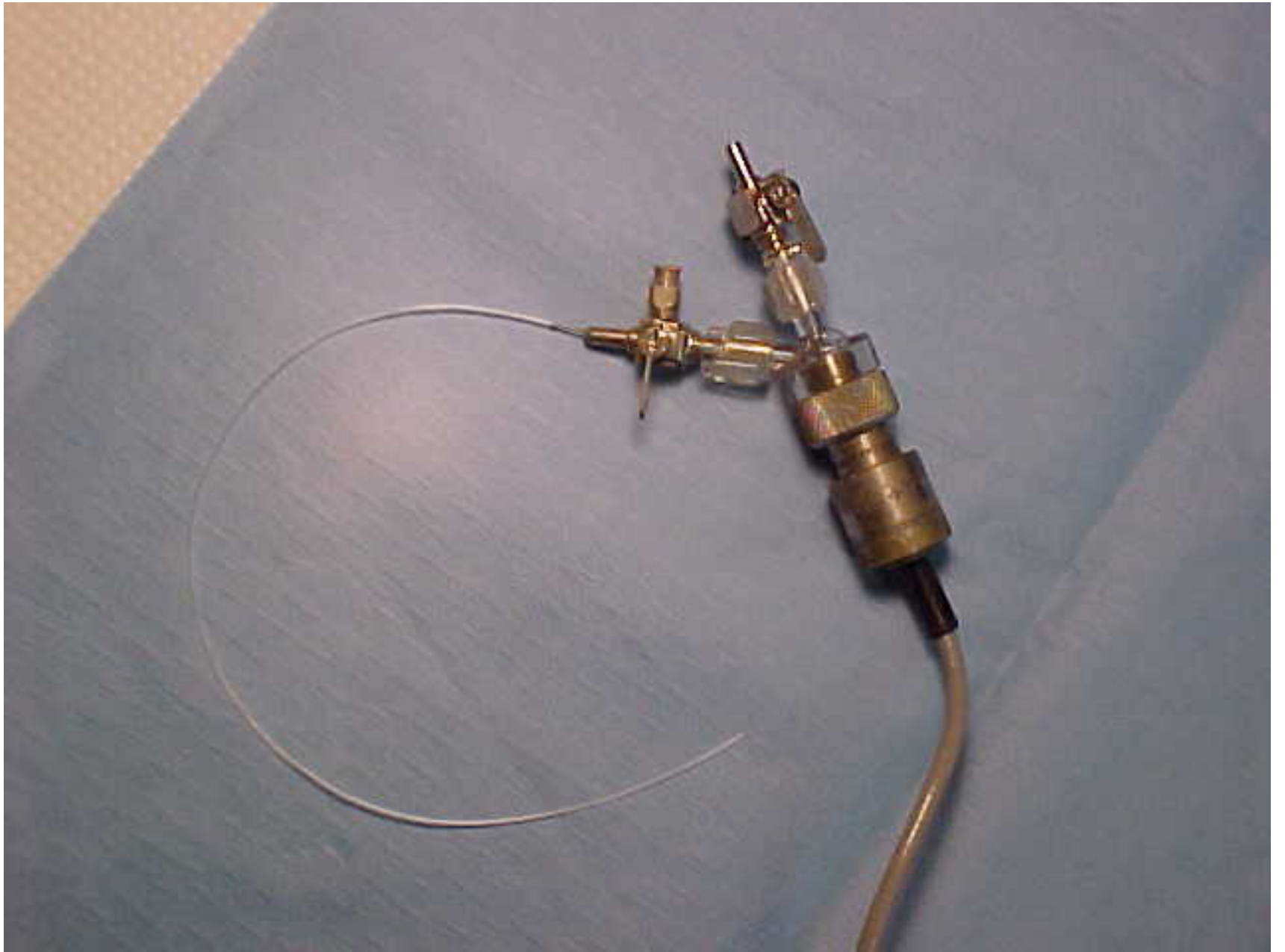
4. Start i.v. infusion as soon as possible. The infusion solution contains 3% polyfructosan in 0.9% saline and is delivered at a rate of 3 ml/hr.
5. Depending on the amount of blood sampling and other factors, the infusion solution may contain 2% BSA, PAH, mannitol, or 25 mM sodium bicarbonate/ 125 mM sodium chloride, instead of just 0.9% NaCl.

Blood Vessel Cannulation:

1. Cutting through the skin usually causes no significant blood loss.
2. Cut through the muscle layer by using hemostat to crush muscle, and then cut along track made by hemostat. Some people prefer a cautery.
3. Many investigators cannulate a carotid artery and the jugular vein, instead of the femoral vessels.
4. Use PE 50 tubing for cannulations. For small rats or mice, use PE 10.
5. The arterial cannula should be filled with heparinized saline (heparin concentration 20 units/ml).

Arterial Blood Pressure:

1. Arterial blood pressure is recorded using a pressure transducer connected to a chart recorder or computer. Check zero and calibrate. Keep transducer at level of heart.
2. Blood pressures are probably somewhat higher than normal in the anesthetized rat.
3. A typical mean arterial blood pressure is about 105 mm Hg in an anesthetized rat.
4. Many investigators will terminate an experiment if the mean arterial blood pressure falls below 90 mm Hg.
5. Don't trust the blood pressure reading unless the pressure oscillates (heart rate in rat is about 300–360 beats/minute).





Urine Collection:

1. Cannulate one or both ureters with PE 10 tubing, fitted into PE 50 tubing.
2. The urine is collected in weighed small vials, under water-equilibrated light mineral oil, to prevent evaporation.
3. Can collect urine from both kidneys together by catheterizing the urinary bladder with PE 50. Reduce the dead space of the bladder with ligatures.

Exposure of Kidney:

1. The left kidney is exposed by a small subcostal incision. The left kidney is somewhat farther from the diaphragm than the right, and its renal vessels are longer.
2. The kidney can be popped out. For the BioRad (inverted) microscope, the kidney lies under the animal in a dish of isotonic saline.
3. For the Zeiss (upright) two-photon microscope, the kidney should be placed in a kidney cup.



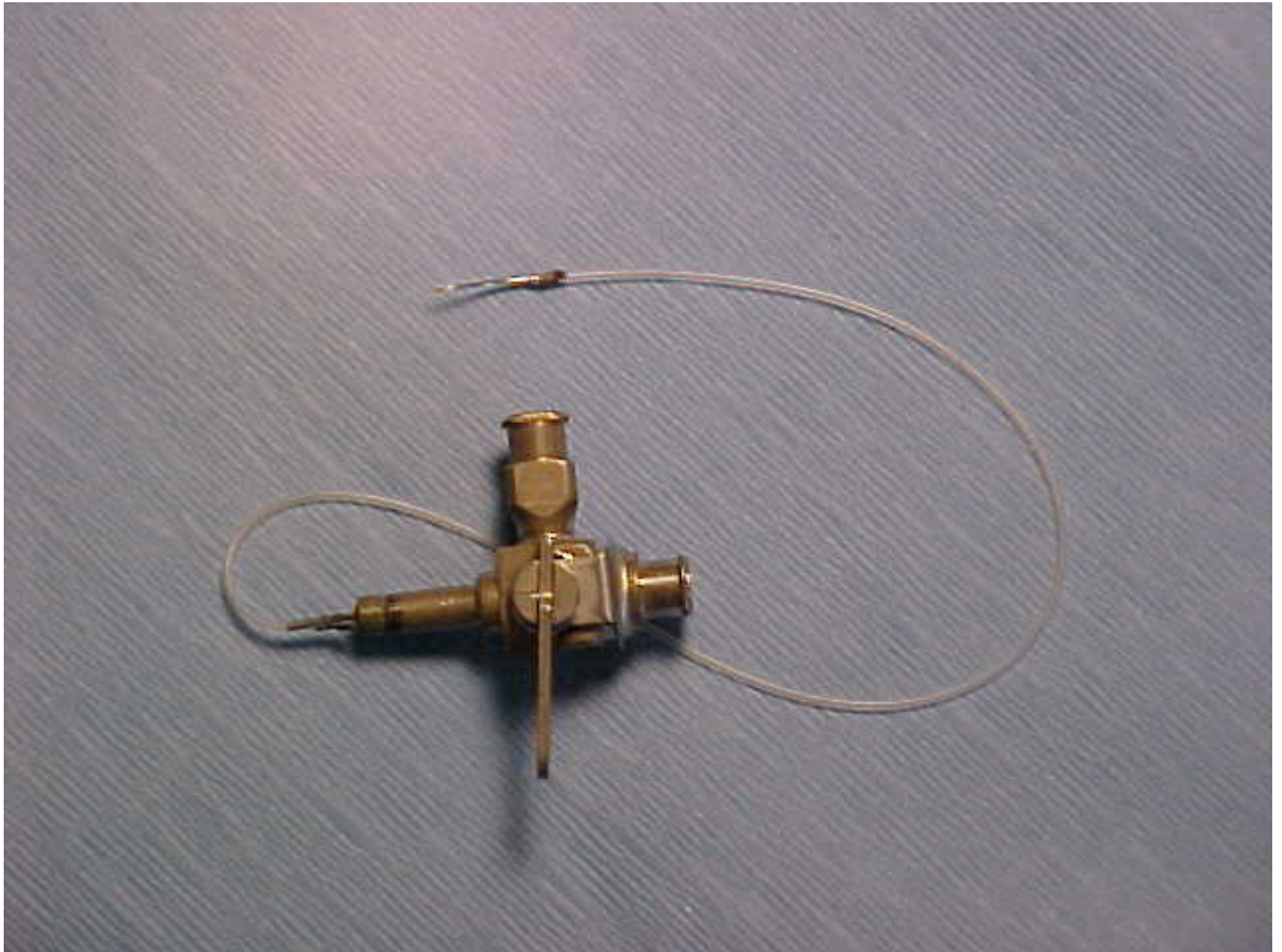


Measurement of renal function: GFR

1. Measure glomerular filtration rate (GFR) with inulin (polyfructosan).
2. Give a priming dose of 3% polyfructosan solution (0.2 ml/100 g body weight), followed by constant intravenous infusion at 3 ml/hr.
3. Wait 1 hr before starting urine collection periods.
4. Collect timed urine samples (20–30 min periods), with mid-period arterial blood collections (0.25 ml blood).
5. Analyze urine and plasma for inulin (anthrone method).
6. Calculate $GFR = C_{\text{inulin}} = U_{\text{inulin}} \times V/P_{\text{inulin}}$
7. A normal GFR in a rat is about 1 ml/min-100 g body weight.

Measurement of Renal Function: RBF

1. Renal blood flow (RBF) can be determined from the clearance and extraction of PAH, together with the blood hematocrit.
2. Give sodium PAH by constant intravenous infusion so as to establish a low (e.g., 2 – 8 mg/dL), steady plasma concentration.
3. RPF (renal plasma flow) = C_{PAH}/E_{PAH}
4. RBF (renal blood flow) = $RPF/(1 - \text{hematocrit})$



Measurements of Renal Function in Normal 6-Month-Old Sprague-Dawley Rats:

Body weight, g	464 ± 28
Mean arterial blood pressure, mm Hg	107 ± 6
Hematocrit, % cells	47 ± 1
GFR, $\mu\text{L}/\text{min}-100$ g body weight	392 ± 31
V, $\mu\text{L}/\text{min}-100$ g body weight	6.8 ± 1.7
PAH extraction ratio	0.88 ± 0.02
Renal blood flow, mL/min	18.5 ± 2.5

Values are means \pm SD for 14 rats. Kidney data are for the left kidney. From Tanner & Tanner. *Kidney Int* 58:1859–1869, 2000.

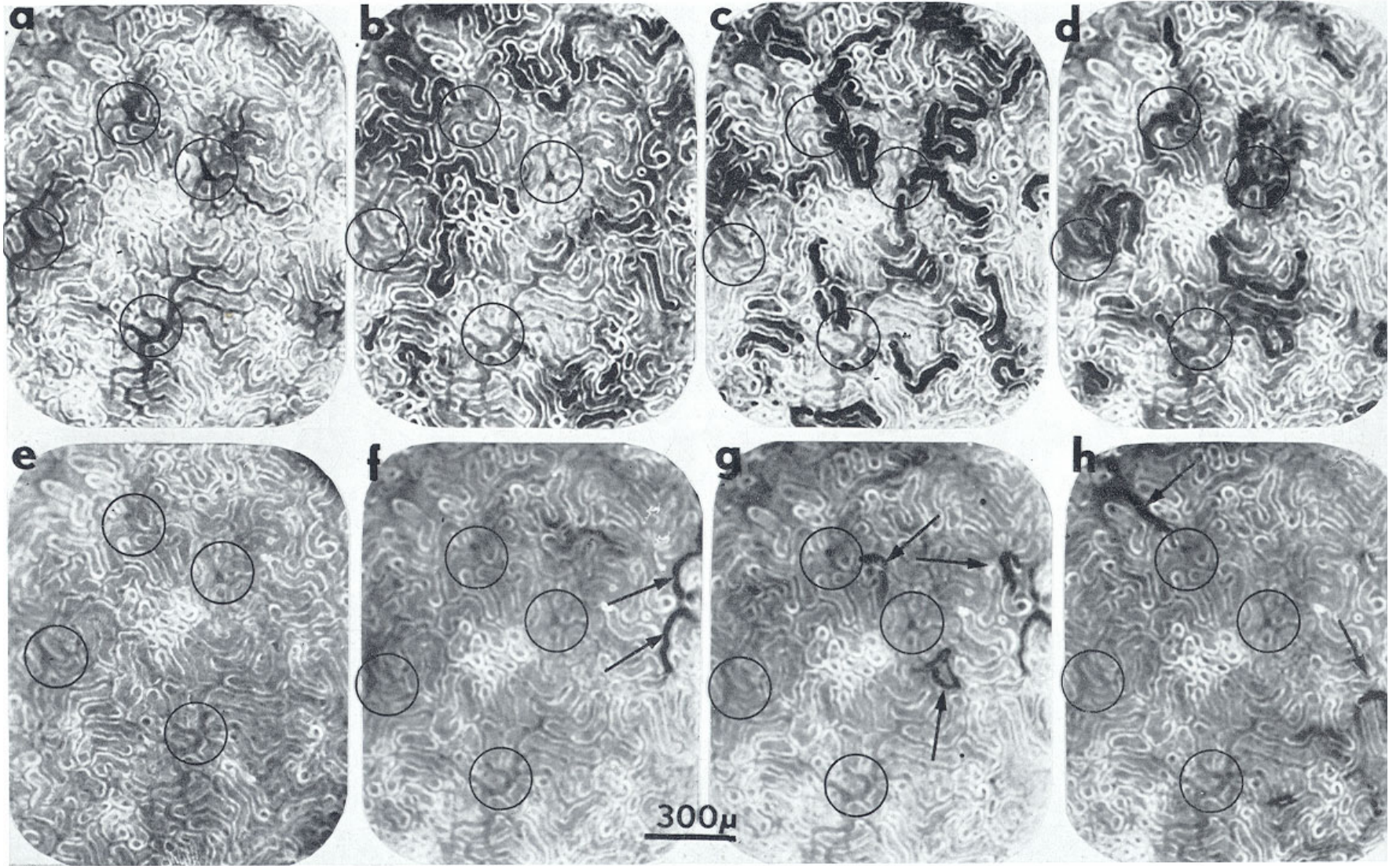
Measurement of Renal Function: Lissamine Green Passage Time

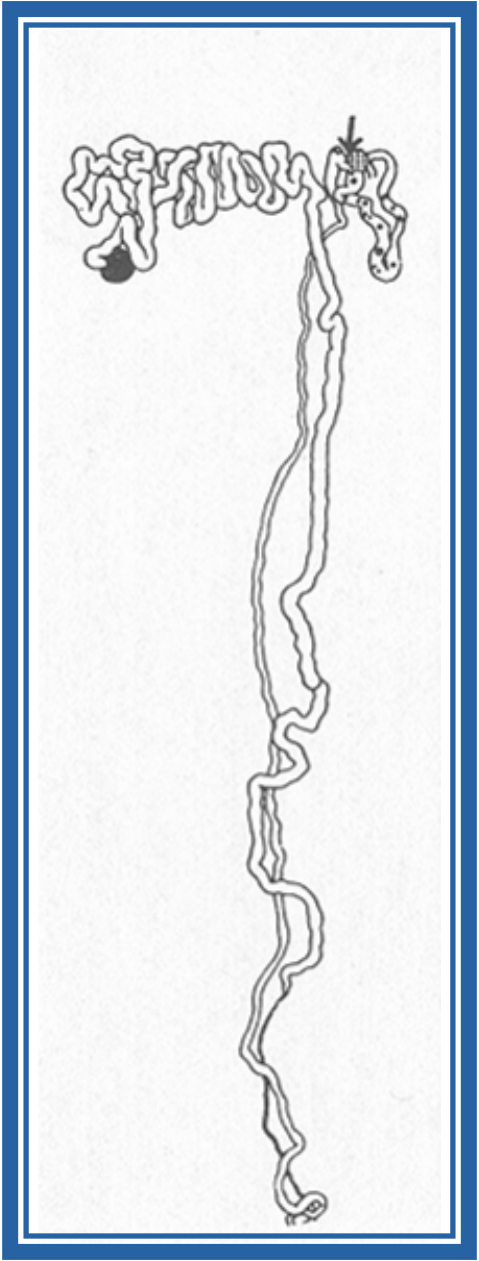
Rapidly inject a bolus of lissamine green (0.25 ml/kg body wt of 10% solution) into a jugular vein, and observe surface of kidney.

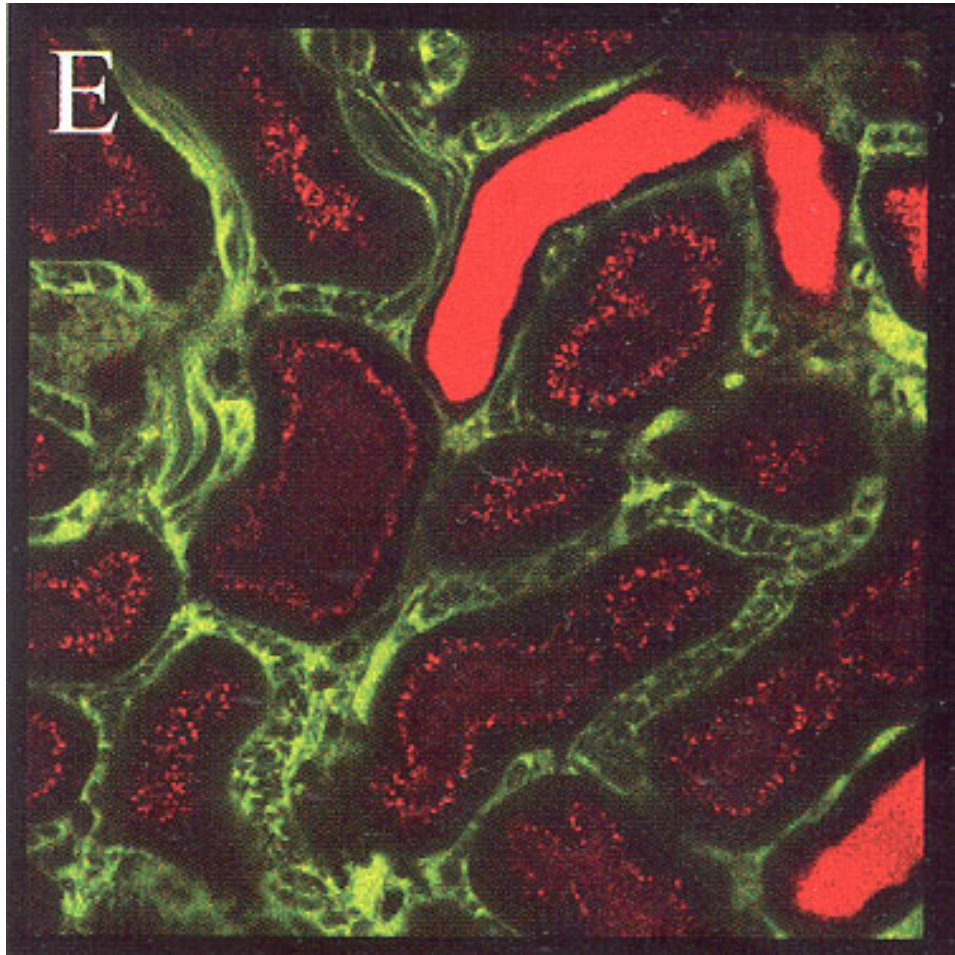
Can use this technique to identify early and late proximal tubule segments and surface distal convoluted tubules.

Prolonged proximal passage time (>10 seconds) suggests impaired kidney function.

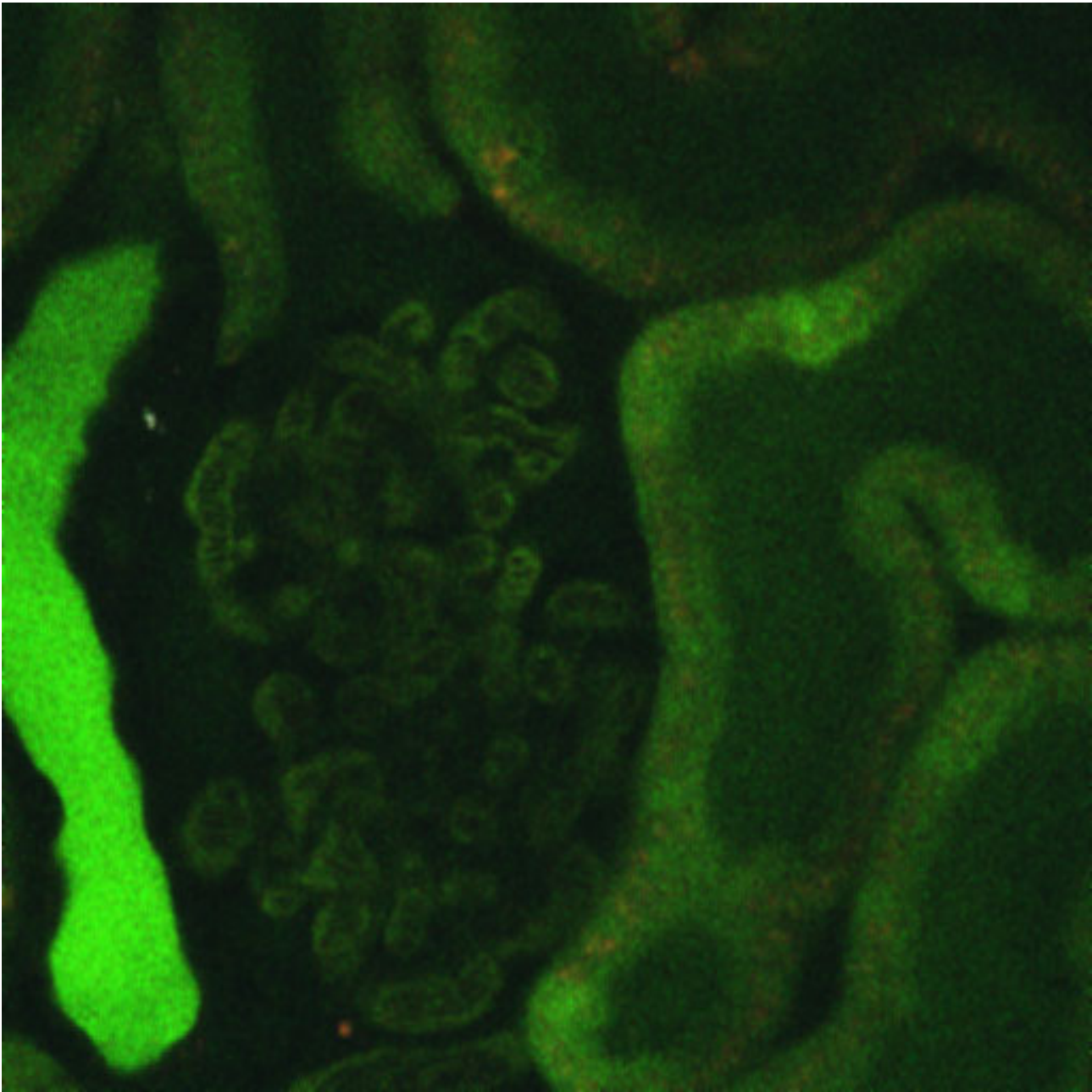
Ref.: Steinhausen. Pfluegers Arch ges Physiol 277:23–35, 1963.

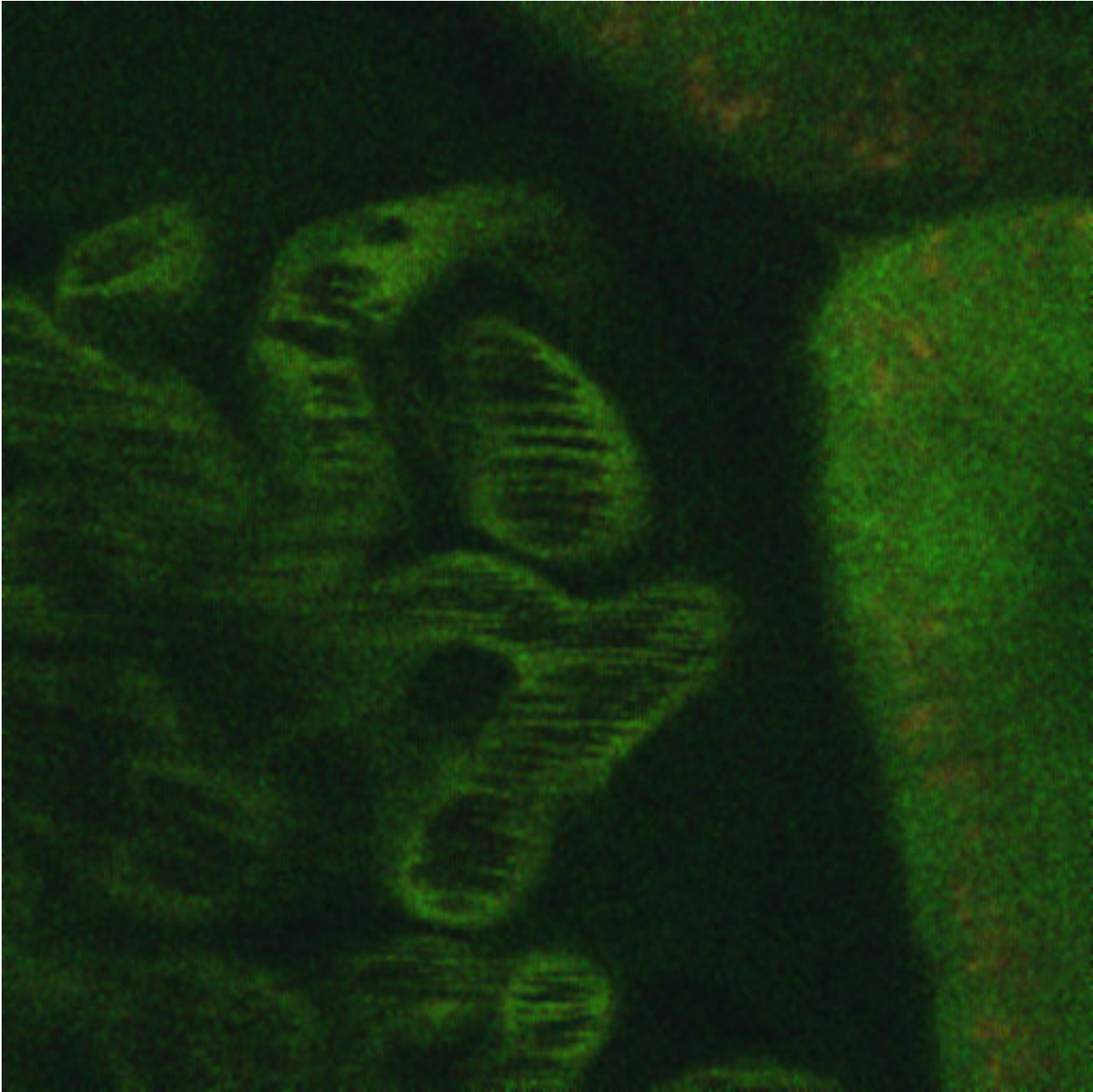


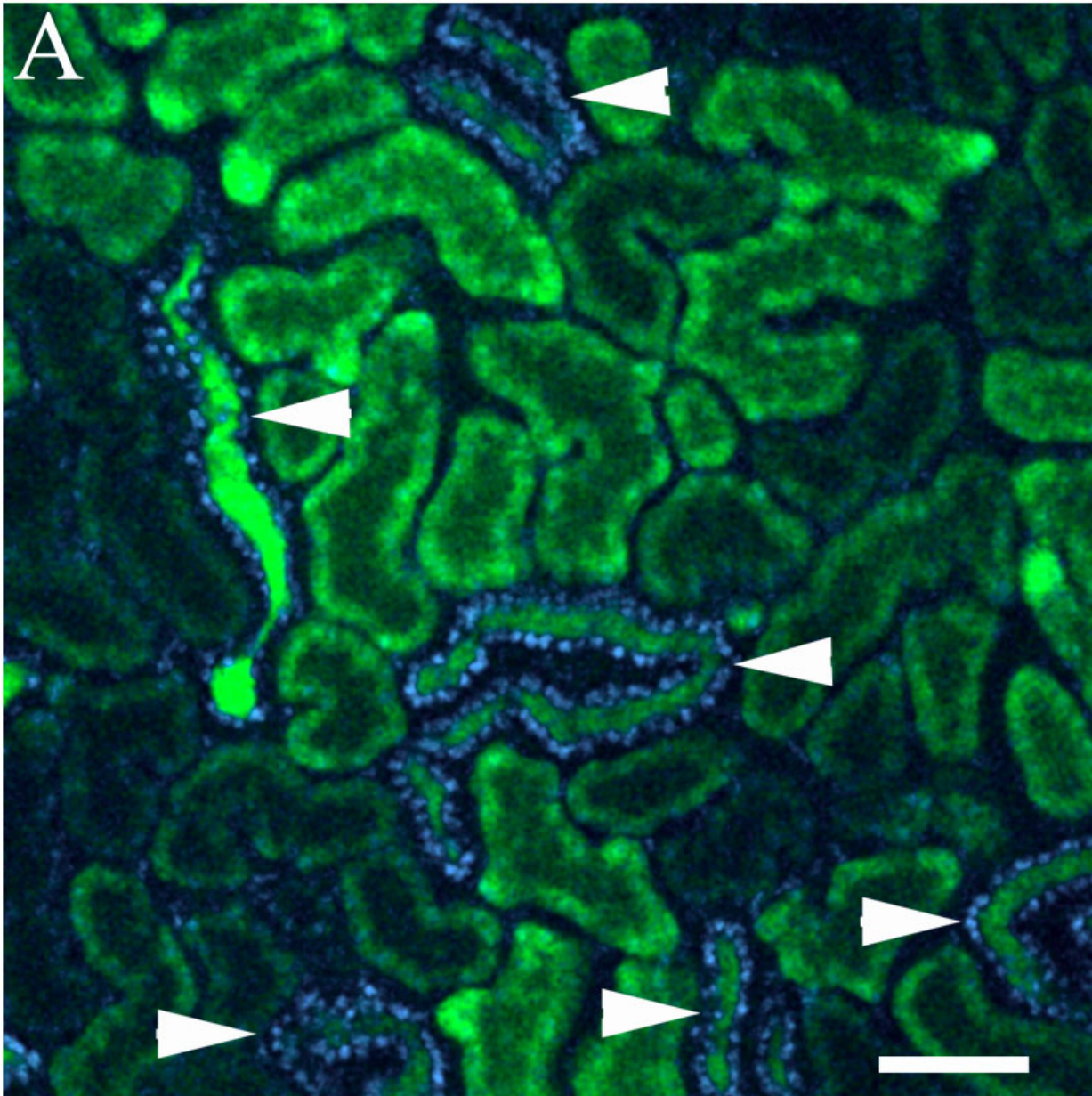


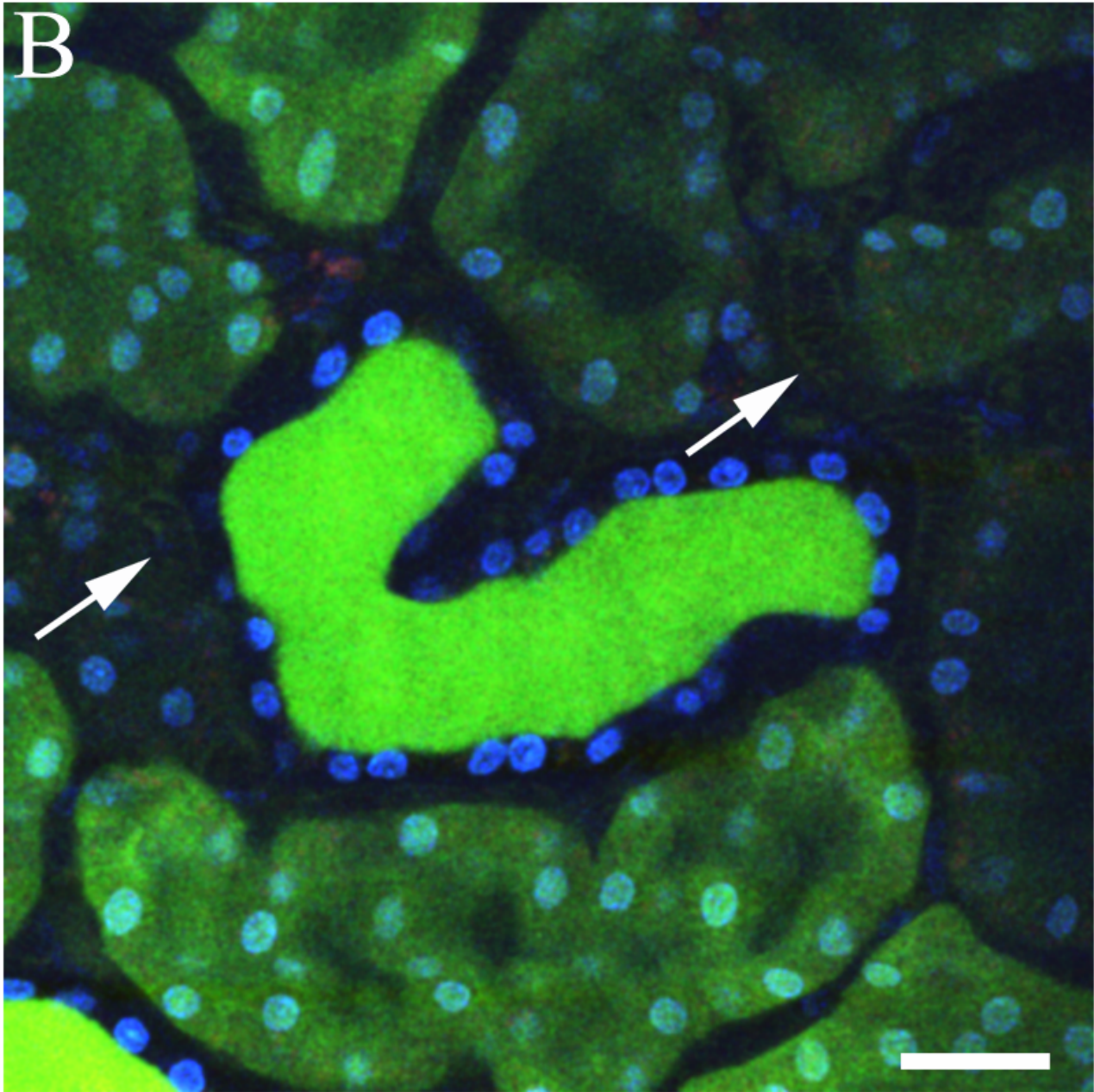


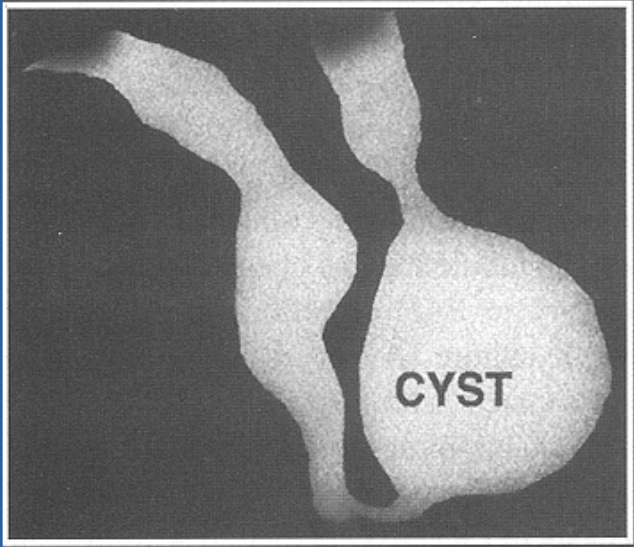
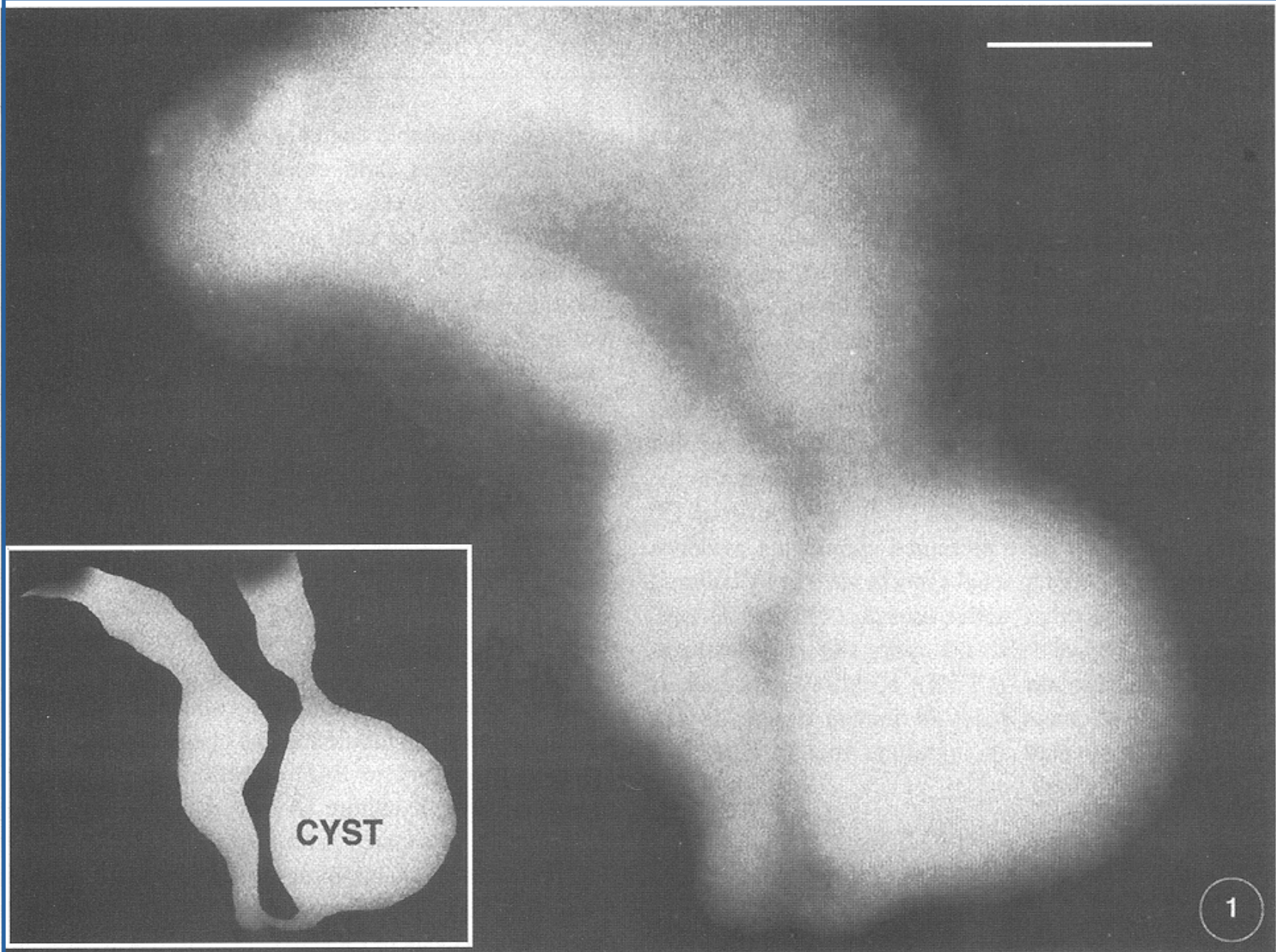
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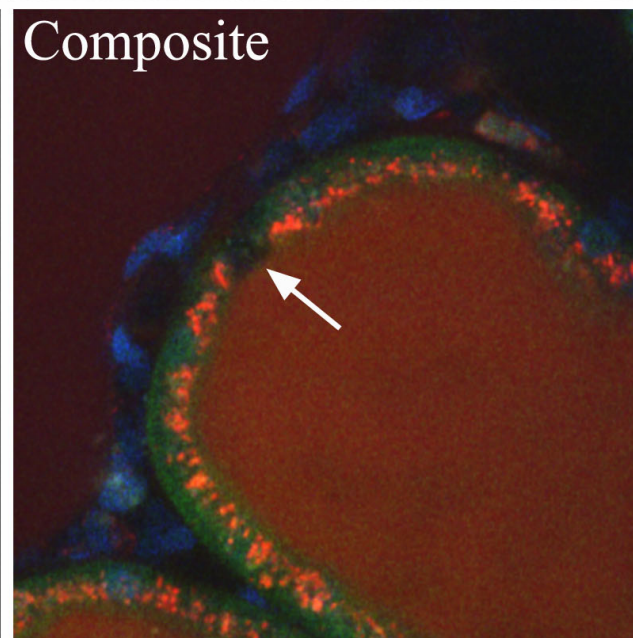
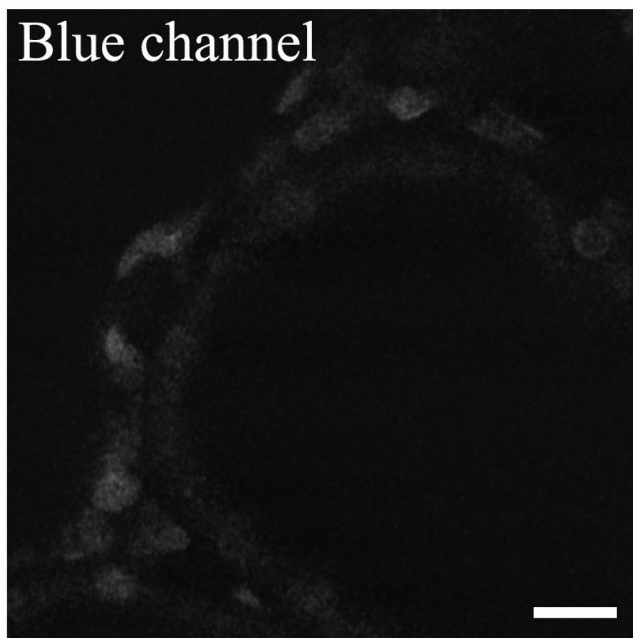
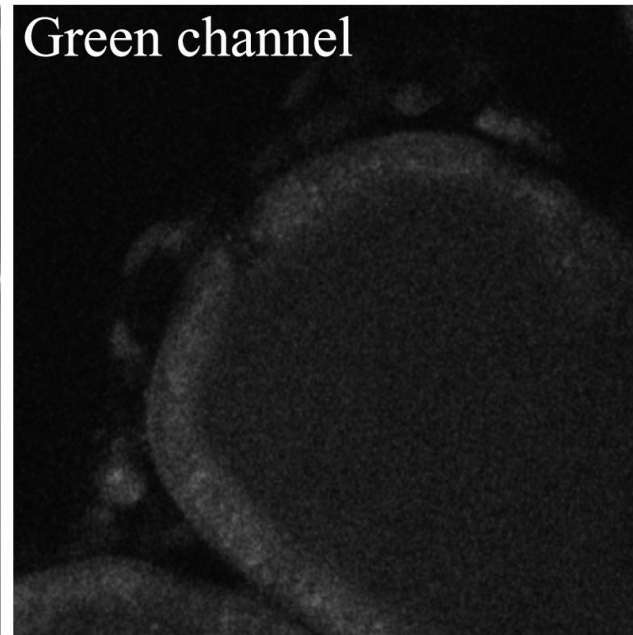
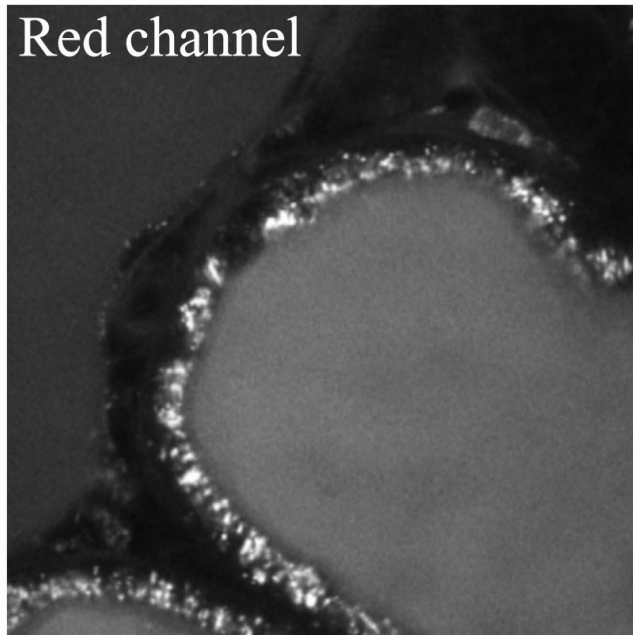


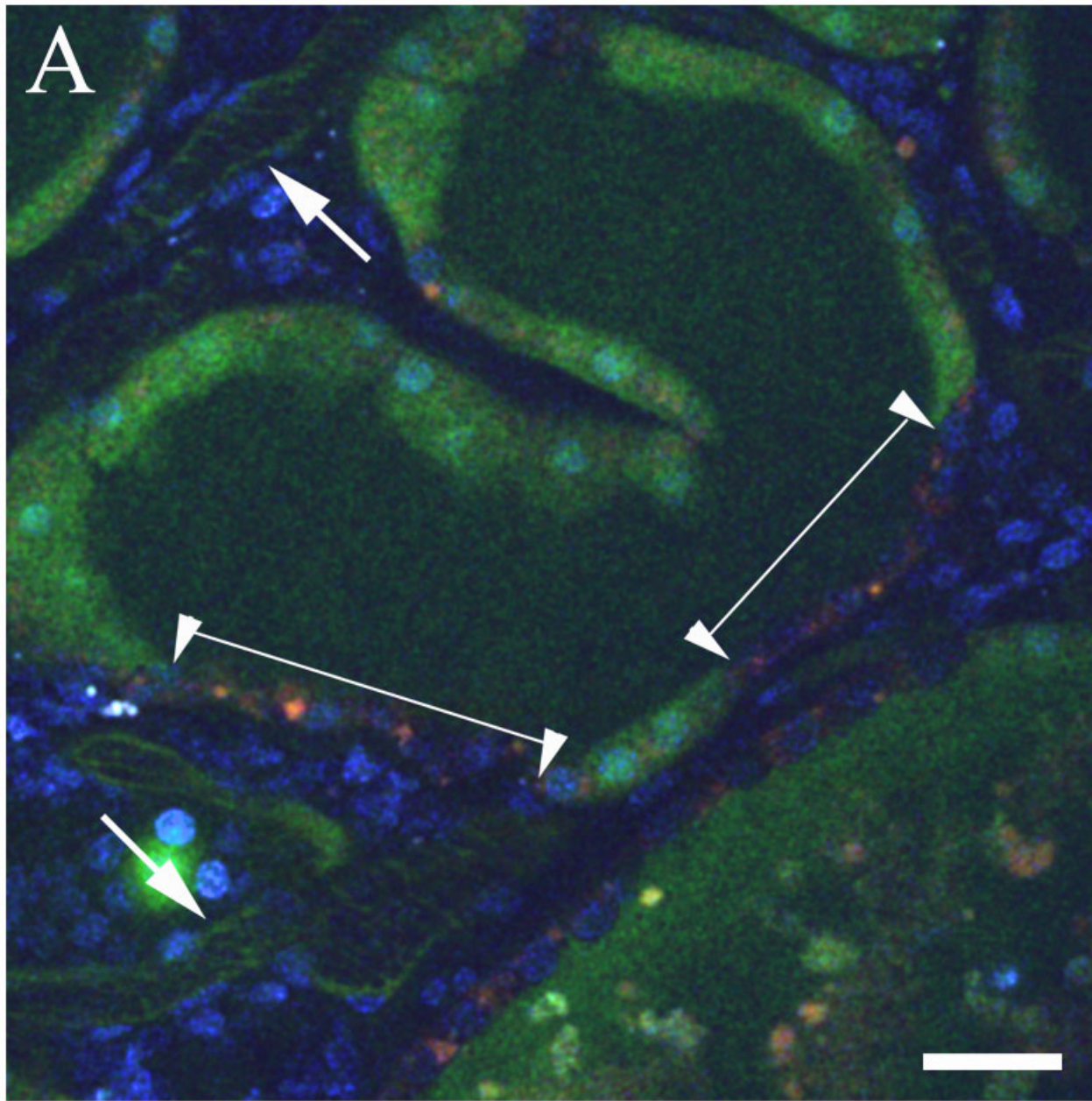


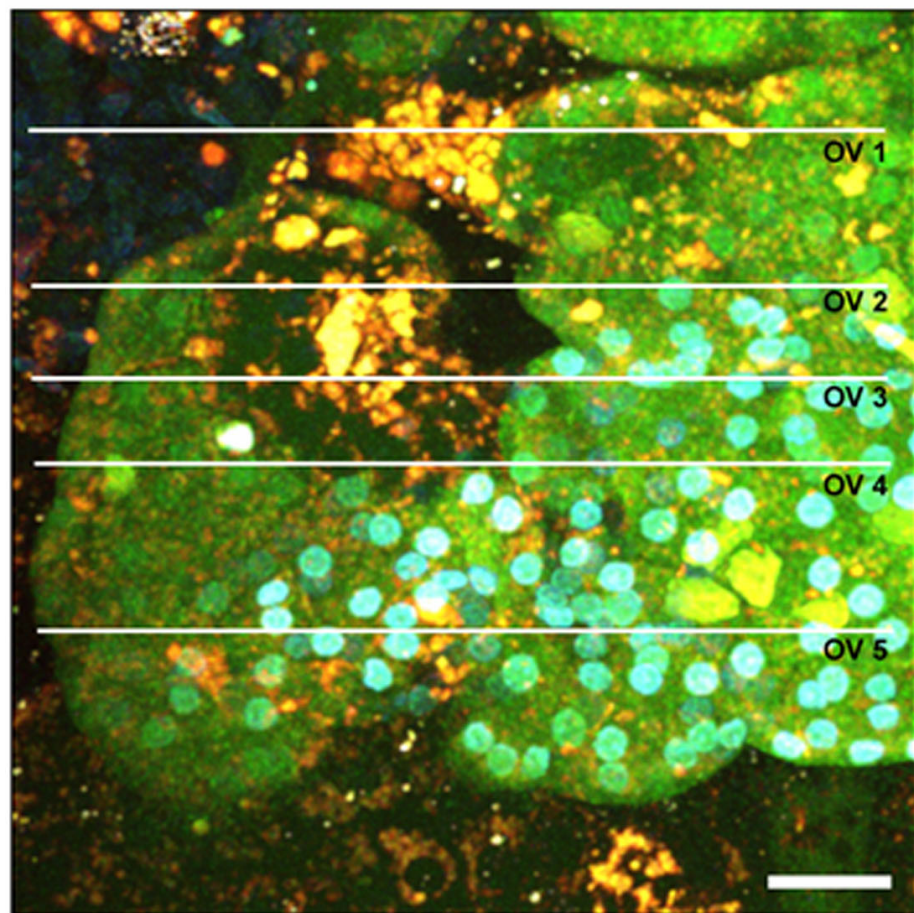




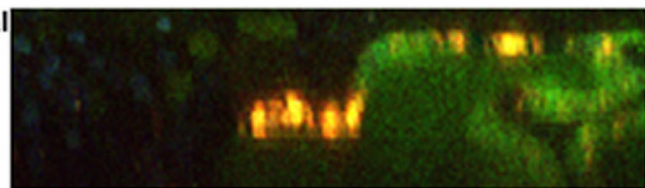




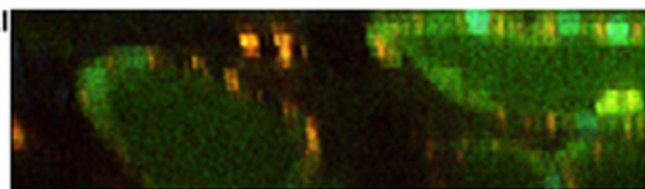




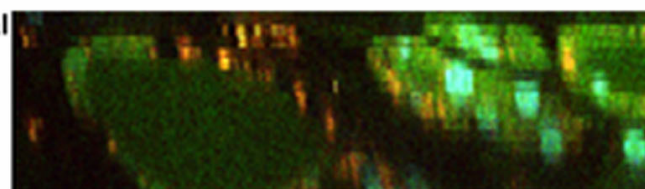
Orthogonal View 1



Orthogonal View 2



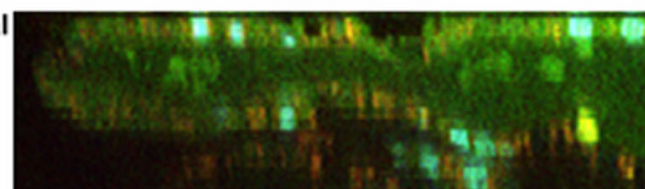
Orthogonal View 3



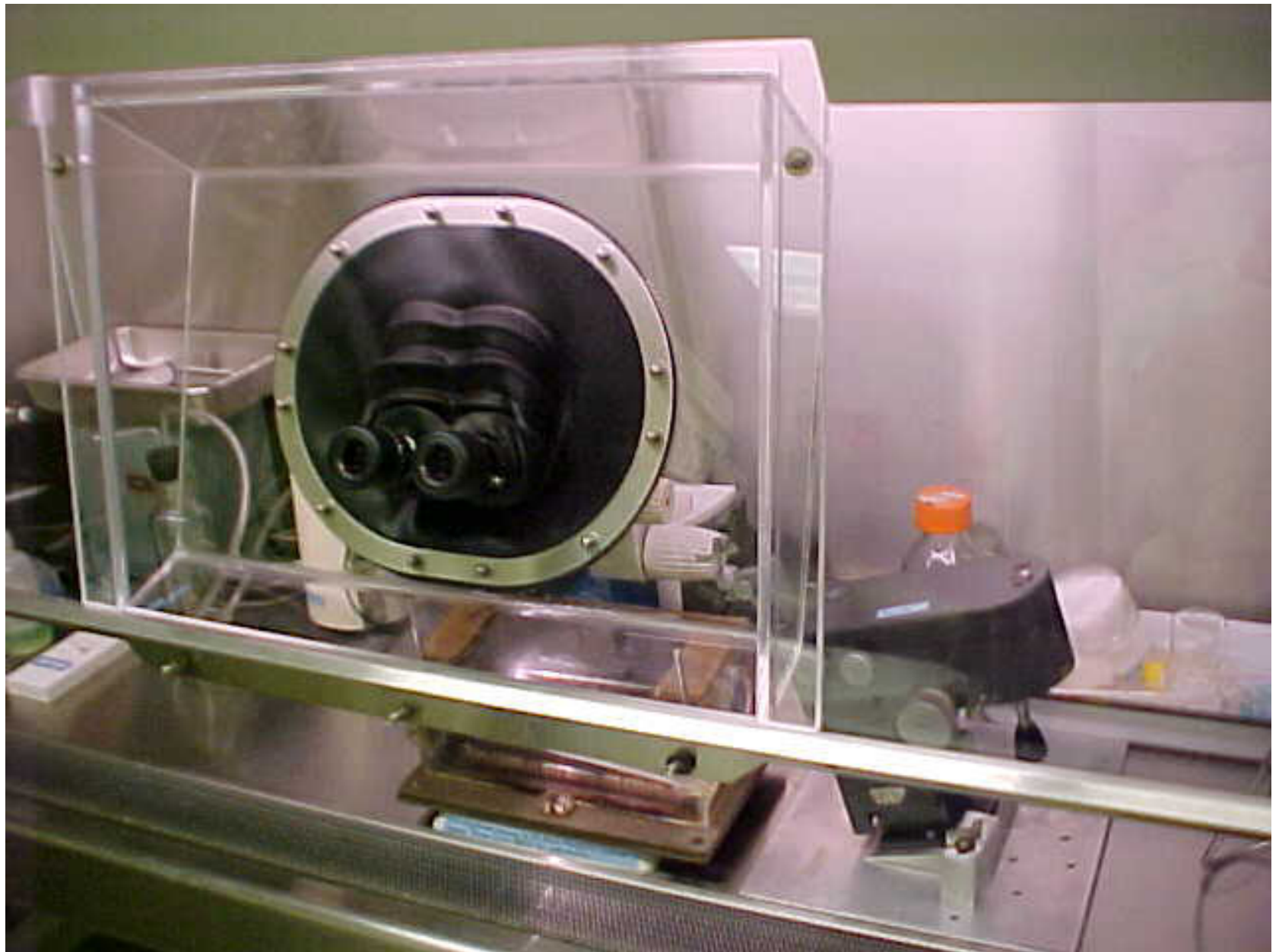
Orthogonal View 4

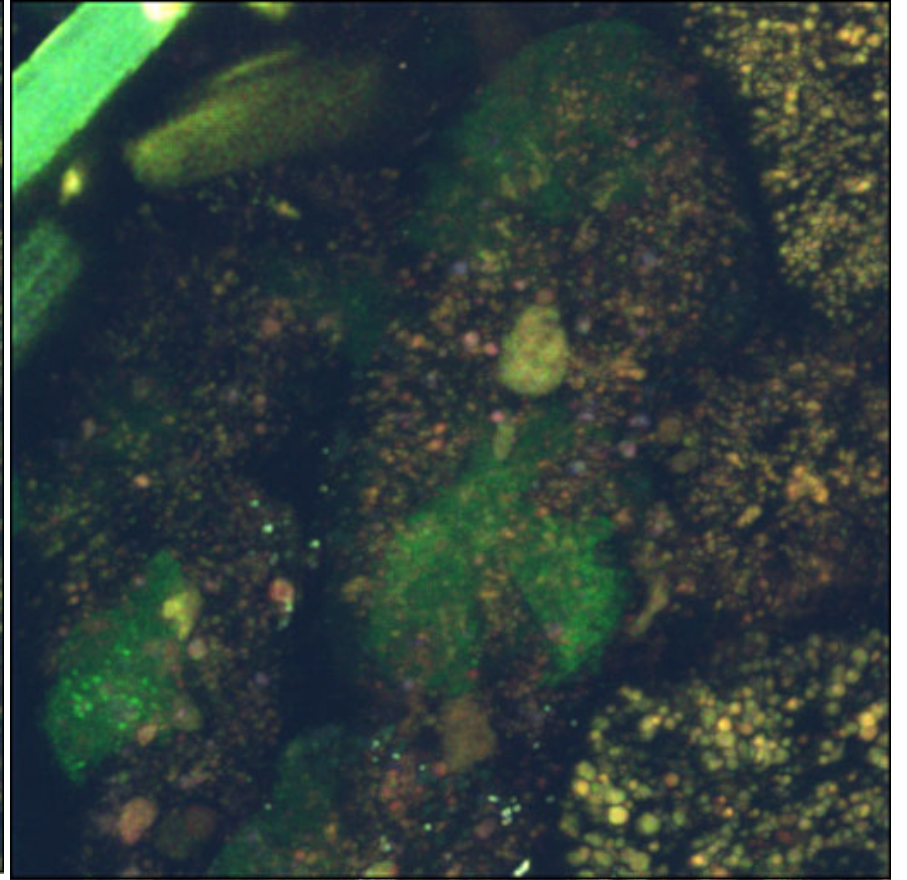
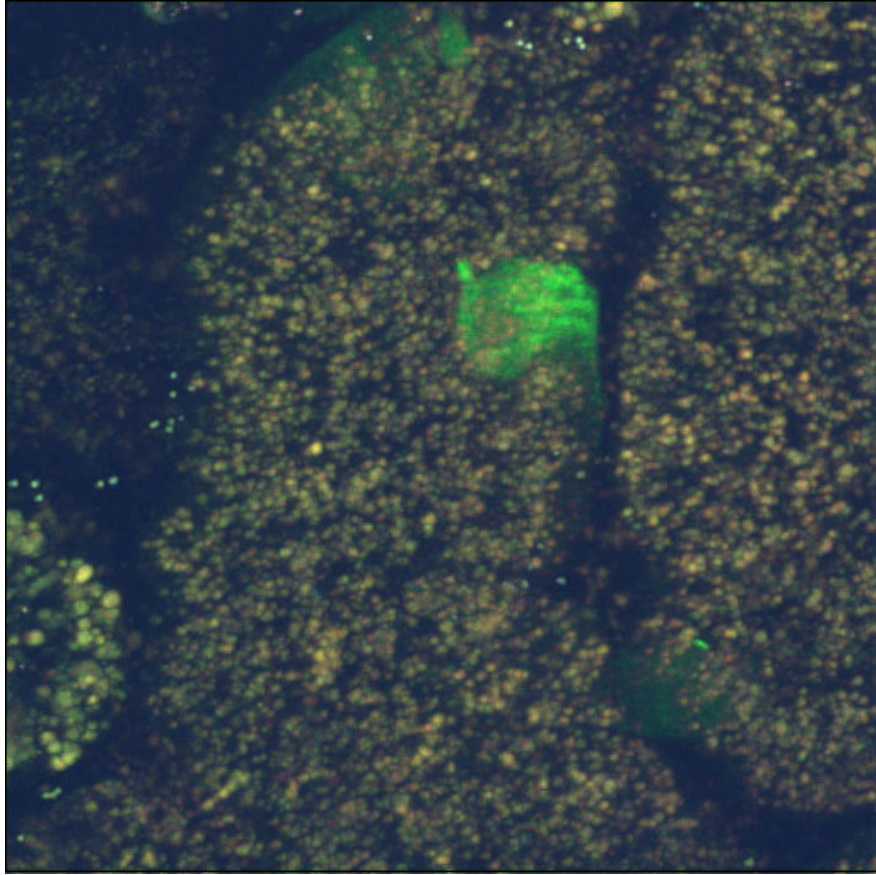


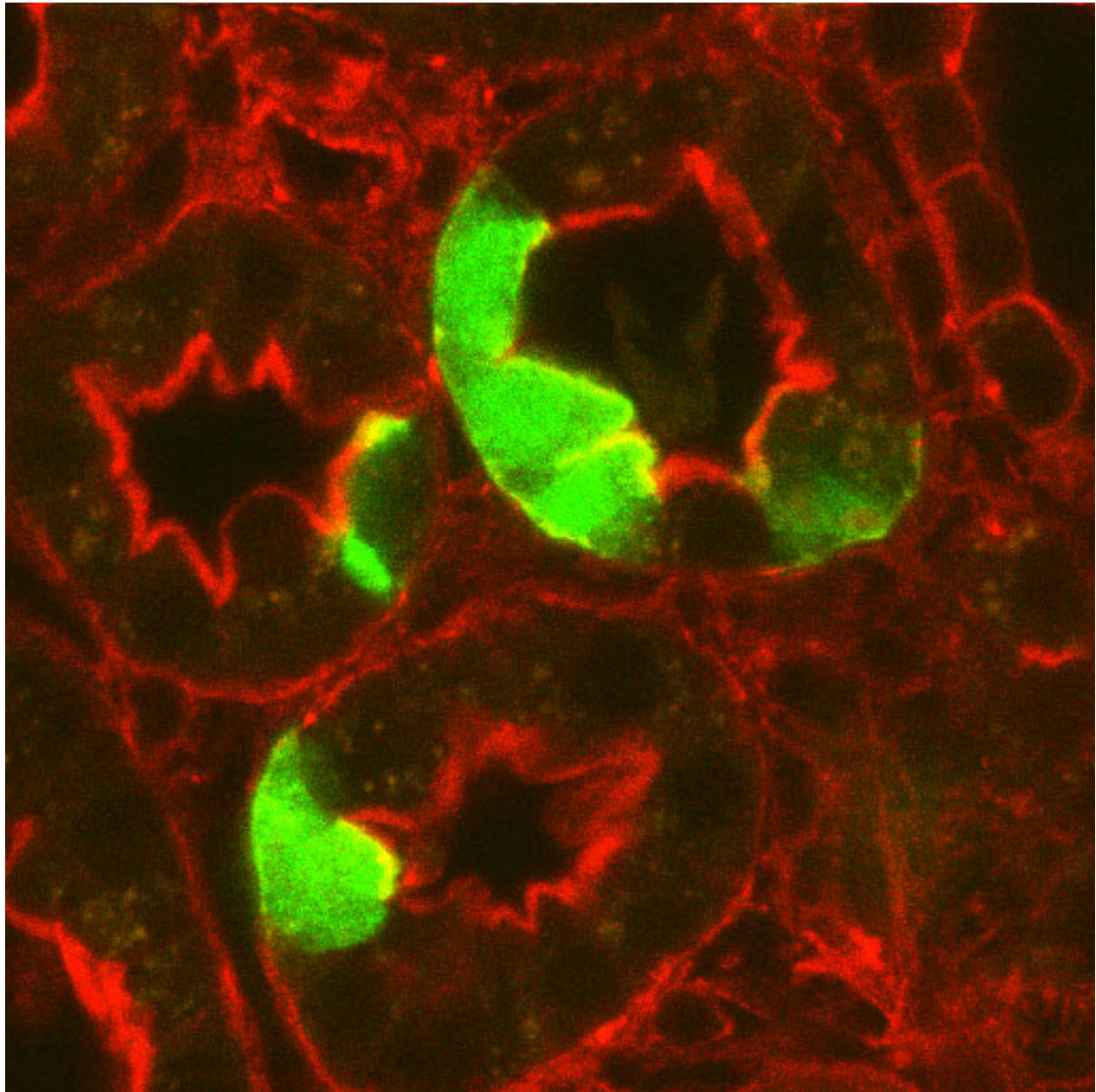
Orthogonal View 5











Fluorescent Markers (see Dunn et al. Am
Physiol Cell Physiol 283:C905–C916, 2002):

Rhodamine-dextran

Hoechst 33342 dye

Fluoresceinated compounds

Mitochondrial dyes

Need to be aware of possible toxicity (good reason to monitor functions).

Advisable to dialyze dextrans or labeled proteins before injection.

Mice Studies (25–30 g body weight):

Anesthesia: ketamine i.m. (50 µg/g) + Inactin i.p. (100 µg/g)

Maintain rectal temperature at 37–38°C

Tracheostomy (PE 90); 100% oxygen

Cannulate carotid artery, jugular vein, or femoral vessels
with PE 10

Infuse 2.25 g BSA/100 ml 0.9% NaCl at 0.5 ml/hr

Urine bladder catheterized with PE 50

Refs.: Gross et al. J Am Soc Nephrol 9:346–354, 1998

Traynor et al. Am J Physiol 275:F922–F927, 1998

Schnermann. J Am Soc Nephrol 10:2614–2619, 1999

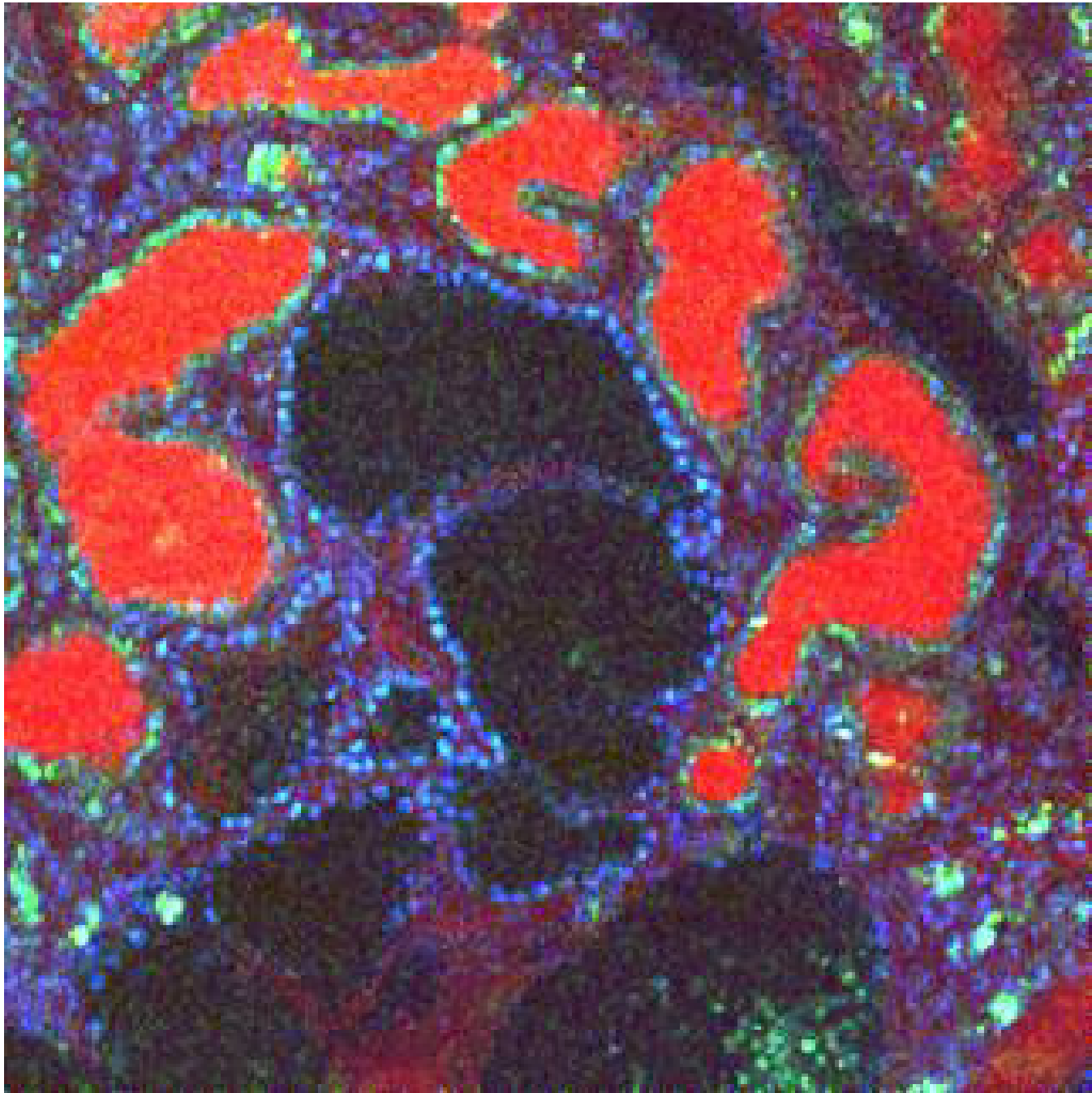
Conclusions:

- Two-photon in vivo microscopy is a powerful way to analyze kidney function.
- We need to pay close attention to the physiological condition of the animals we study.

“The history of renal physiology has been in too large measure a history of traumatic procedures which have in the end only misled investigation.”

Homer W. Smith

Harvey Lectures. 35:166–222, 1939-1940



Thanks to the Following:

Dr. Bruce Molitoris

Ruben Sandoval

Dr. Ken Dunn

Dr. Exing Wang

Dr. Sharon Ashworth

and other members of the
Indiana Center for Biological Microscopy