Multi-photon microscopy of the kidneys of living animals

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Vital Imaging by Multiphoton Microscopy

• The extended depth provided by multi-photon microscopy permits high-resolution imaging of the cells of living animals.

• The ability to image multiple fluorophores supports correlations of multiple probes and physiological processes.
Major Advantages of Vital Imaging by Multiphoton Microscopy

• Dynamic nature of events

• Three dimensional aspects of cellular interactions

• Repeat evaluations of same area over time

• Structure-Function relationships

• See is believing
Novel Utilization of Vital Imaging Using Multiphoton Microscopy

• Investigative

• Diagnostic applications

• Therapeutic applications
Major Challenges of Vital Imaging by Multiphoton Microscopy

- Anesthesia/Ventilation to eliminate movement
- Water immersion lens 60X
- Probe delivery-vascular barrier
- Quantitative aspects
- Acquisition rate
Human Renal Schema

A  Control

B  Ischemic
Ischemia induces alterations of the apical F-actin structure

FITC - F-actin
Texas Red - G-actin
Ischemia induces ADF localization to vesicles in the proximal tubule lumen
Urinary Vesicles Following Ischemia Contain ADF and Actin

**A**

**B**

**Phase Contrast**

**ADF: FITC secondary**

**F-actin: Texas Red-phalloidin**
Kidney proximal tubule cells expressing XAC(wt)-GFP
Propidium iodide used to identify necrotic cells *in situ* remains in nuclei after fixation –
Identification of apoptotic and necrotic nuclei in ischemic renal tissues

1. TUNEL-positive apoptotic nucleus
2. TUNEL-positive apoptotic nuclei, secondary necrosis – weak TUNEL label –
3. TUNEL-negative, necrotic nuclei

Pierre Dagher and Katrina Kelly
Hoechst Nuclear Stain

Rhodamine 6G
(Mitochondrial Stain)
500K Mw FITC-Dextran (Green)
10K Mw Rhodamine Dextran (Red)
Hoechst 44432 (Blue)
Parenchymal Window
Clamp during the cutting (30’ Ischemia total) Imaged 5hrs post
6-5-2002
DAY 2

4min Post dextran injection

3,000 Rhodamine Dextran (RED)
500,000 FITC Dextran (GREEN)
2nd Rat Pre-Folate Incubation, 3D Data

Red Channel (TR Folate)

Green Channel (No Dye)

Blue Channel (Hochst Nuclear Dye)

Color Overlay
Green = FITC Folate
Red = Rhodamine Albumin
Blue = Hoechst 33342

Munic Wistar, Normal Diet
2nd Rat, Time 0, 16th Frame of Movie

Red Channel (TR Folate)
Green Channel (No Dye)
Blue Channel (Hochst Nuclear Dye)
Color Overlay
1st Rat Time 40 min

Red Channel (TR Folate)

Green Channel (No Dye)

Blue Channel (Hochst Nuclear Dye)

Color Overlay
TRANSCYTOSIS?
2nd Rat, Time 52 min, 3D Data

Red Channel (TR Folate)

Green Channel (No Dye)

Blue Channel (Hochst Nuclear Dye)

Color Overlay