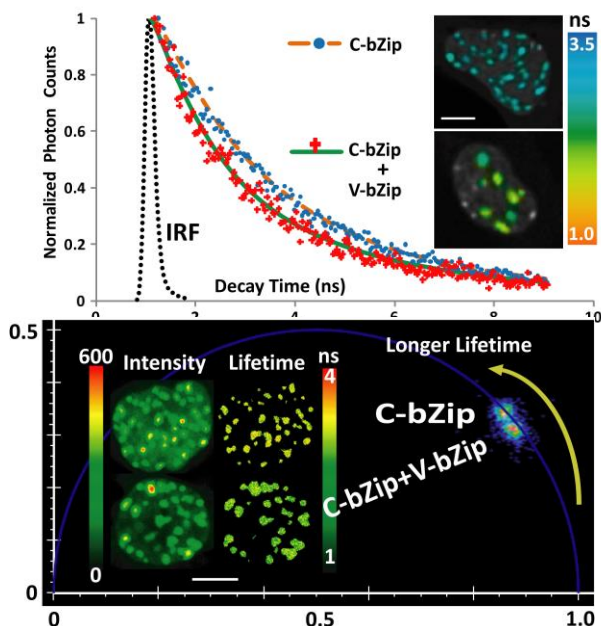


Metabolic response of segmented prostate cancer cells after treatment, based on FLIRR (Fluorescence Lifetime Redox Ratio) –NAD(P)H-a2%/FAD-a1%. Scientific Reports | (2018) 8:79, DOI:10.1038/s41598-017-18634-x



FLIM-FRET Microscopy to investigate proteins dimerizing in the nucleus. Top: Time correlated single photon counting (TCSPC) method (Becker & Hickl). Bottom: Frequency-domain technique (ISS). Nature Protocols 6 (9), 1324-1340, 2011

Systems and live model cell line - transfected with above proteins will be available at workshop for the practical.

### Faculty

- Dr. A. Periasamy, University of Virginia  
 Workshop Director, [ap3t@virginia.edu](mailto:ap3t@virginia.edu)  
 Dr. M. Barroso, Albany Medical College  
 Dr. M. Börsch, Jena University, Germany  
 Dr. J. N. Demas, Chemistry, UVA  
 Dr. M. Digman, Univ. of California-Irvine  
 Dr. A. Kenworthy, MPBP, UVA  
 Dr. A. Rück, Universität Ulm, Germany  
 Dr. S. Vogel, NIAA, NIH  
 Dr. A. Walsh, Texas A & M University

### Guest Lecturers

- Dr. J. Zhang, Univ. of California, San Diego  
 Dr. K. Siller, Research Computing, UVA  
 Dr. P. So, MIT  
 Dr. M. Skala, Univ. of Wisconsin, Madison  
 Dr. M. Stanley, Chroma Tech.

### For more info:

<https://kcci.virginia.edu/workshop/general-information>

### TUITION FEES:

\$2,500 non-profit organizations  
 \$2,900 for-profit organizations  
 (Includes lodging, breakfast, lunch, dinner, lecture materials)

### Contact:

**Prof. Ammasi Periasamy**  
[ap3t@virginia.edu](mailto:ap3t@virginia.edu)  
 434-243-7602

**Note: All participants must be fully Vaccinated and boosted.**

# 21<sup>st</sup> Annual Workshop on FLIM and FRET Microscopy and Label-free NAD(P)H/FAD Metabolic Imaging March 4-8, 2024

Hands-on instructions on microscopy systems

- \* Internationally recognized Faculty
- \* Best imaging and analysis solutions
- \* Personal attention for a maximum of 25 participants
- \* Individual problem solving



W.M. Keck Center for Cellular Imaging,  
 University of Virginia  
<https://kcci.virginia.edu/workshop-2024>

## AIM

The W.M. Keck Center for Cellular Imaging (KCCI), a university imaging center at the University of Virginia, is sponsoring an advanced practical course on (a) Förster (fluorescence) resonance energy transfer (FRET) for confocal and fluorescence lifetime imaging microscopy (FLIM-FRET); and (b) label-free FLIM microscopy of NAD(P)H and FAD to analyze the Redox metabolic states (FLIRR) in live cells before and after treatment.

Attendees are expected to be familiar with the basics of fluorescence microscopy. The curriculum, after a brief introduction to the principles of fluorescence, microscopy, fluorophores, FRET and FLIM, will concentrate on the practical aspects, hands-on individual instruction at the instruments followed by data analysis and interpretation.

Lectures and after dinner problem-solving discussions will address questions of fluorophore choices, the most suitable systems to achieve specific research objectives, qualitative vs. quantitative analysis and many more related subjects. Participants will also be introduced to a unique image processing and analysis software (PFRET) and Python code for FLIRR.

**10+** different and advanced microscopy systems will be available for a maximum of 25 students. With internationally recognized faculty in attendance, there is ample opportunity to interact with experts formally or informally.

Live-cell specimens are provided.

Participant's own specimens are welcome.

## PROGRAM SCHEDULE

### March 6 Advanced Microscopy Techniques

#### Lectures by leading scientists

#### March 4 – 8 Theory and Lab

(Monday 10-6pm theory; other days

Theory 8:30 AM – 12 PM)

- Introduction to workshop
- Basics of Fluorescence, FRET, FLIM, NADH, FAD, Trp, microscope choices
- Meet the experts from Abberior Case studies and Q&A on the subjects:
- Confocal/spectral FRET
- FLIM-FRET, NAD(P)H-TRP FRET
- Fluorophore pairs for FRET/FLIM-FRET
- FLIM-FRET,
- Redox states analyzed by NAD(P)H & FAD
- Metabolic Imaging
- Imaging live/fixed cells & tissue
- Spectroscopy FRET in suspensions
- Bacterial FRET

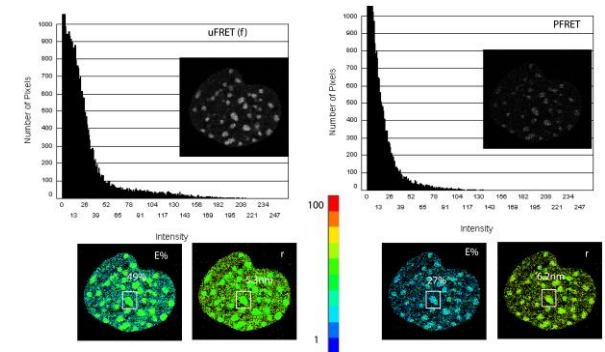
#### March 5-8 Lab (1 PM – 5 PM)\*

Hands-on practical instruction on various systems, data analysis, special demos, and general problem-solving discussions on

- Anisotropy / Homo-FRET
- NAD(P)H-TRP FRET
- FLIM analysis: Fitting and Phasor plots
- Single-molecule FRET
- Metabolic Imaging
- FRAP
- Working on your instrument of choice after formal curriculum ends

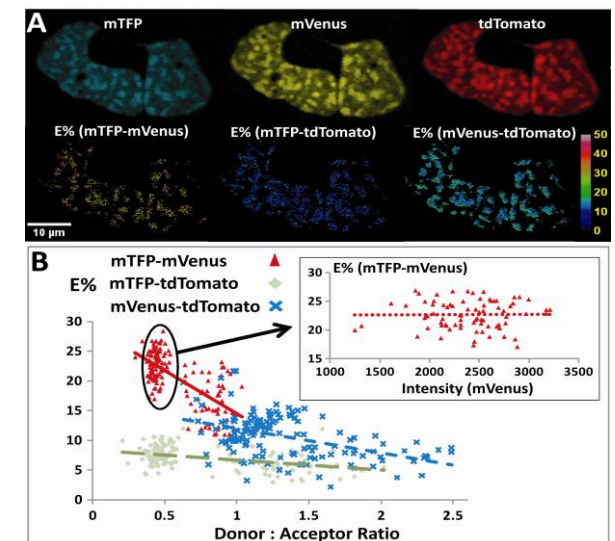
Instruments, Becker & Hickl, Boston Electronics, Carl Zeiss, Chroma Tech, ISS, Leica Microsystems, Olympus

**\*Including breakfast, breaks, lunch, and dinner.**



Comparing uFRET (uncorrected for spectral bleed-through - SBT) with PFRET (Processed FRET, corrected for SBT).

**This PFRET correction software will be available for workshop participants.**



An expanded PFRET software analyzes 3 FRET-interacting, labeled proteins simultaneously in live cells – a Keck Center for Cellular Imaging development. *Biophys. J.* 99, 1274-1283, 2010

### Participating Instrument Companies

Becker & Hickl, Boston Electronics, Carl Zeiss, Chroma Tech, ISS Inc., Leica Microsystems, Olympus.