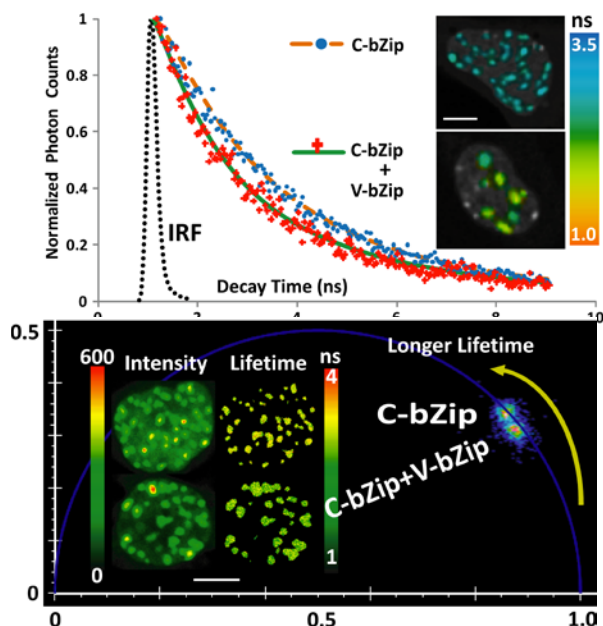


Metabolic response of segmented prostate cancer cells after treatment, based on FLIRR (Fluorescence Lifetime Redox Ratio) –NAD(P)H-a2%/FAD-a1%. Scientific Reports | (2018) 8:79, DOI:10.1038/s41598-017-18634-x



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

Dr. M. Barroso, Albany Medical College

Dr. M. Börsch, Jena University, Germany

Dr. J. N. Demas, Chemistry, UVA

Dr. A. Kenworthy, MPBP, UVA

Dr. A. Rück, Universität Ulm, Germany

Dr. M. Skala, Univ. of Wisconsin, Madison

Dr. S. Vogel, NIAA, NIH

Dr. A. Walsh, Texas A & M University

Dr. J. Zhang, Univ. of California, San Diego

Guest Lecturers

Dr. Karsten Siller, Research Computing, UVA

Dr. P. So, MIT

Dr. M. Digman, Univ. of California, Irvine

Dr. M. Stanley, Chroma Tech.

For more info:

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

TUITION FEES:

\$2,400 non-profit organizations

\$2,800 for-profit organizations

(Includes lodging, breakfast, lunch, dinner, lecture materials)

Contact:

Prof. Ammasi Periasamy

ap3t@virginia.edu

434-243-7602

Note: All participants must be fully Vaccinated and boosted.

20th Annual Workshop on FLIM and FRET Microscopy and Label-free NAD(P)H/FAD Metabolic Imaging March 6-11, 2023

Hands-on instructions on microscopy systems

- * Internationally recognized Faculty
- * Best imaging and analysis solutions
- * Personal attention for a maximum of 20 participants
- * Individual problem solving



W.M. Keck Center for Cellular Imaging,
University of Virginia

<https://kcci.virginia.edu/workshop-2022>

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

Day 1 (12 Noon – 9 PM)*

- Introduction to workshop
- Basics of Fluorescence, FRET, FLIM, NADH, FAD, Trp, microscope choices
- Meet the experts from Abberior Instruments, Becker & Hickl, Boston Electronics, Carl Zeiss, Chroma Tech, Excelitas Technologies Corp., ISS, Leica Microsystems, Olympus

Days 2-4 (8:30 AM – 12 PM)*

Case studies and Q&A on the subjects:

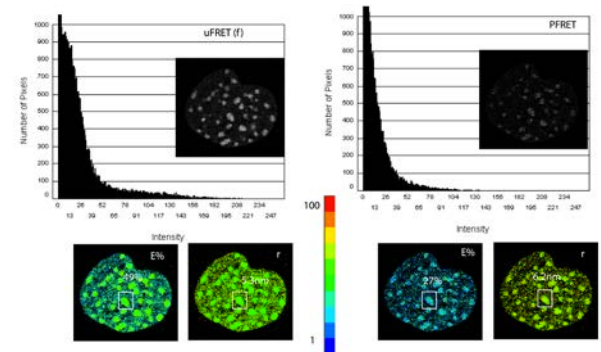
- Confocal/spectral FRET
- FLIM-FRET, NAD(P)H-TRP FRET
- Fluorophore pairs for FRET/FLIM-FRET
- FLIM-FRET,
- Redox states analyzed by NAD(P)H & FAD
- Metabolic Imaging
- Imaging live/fixed cells & tissue
- Spectroscopy FRET in suspensions
- Bacterial FRET

Days 2-4 (1 PM – 9 PM)*

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

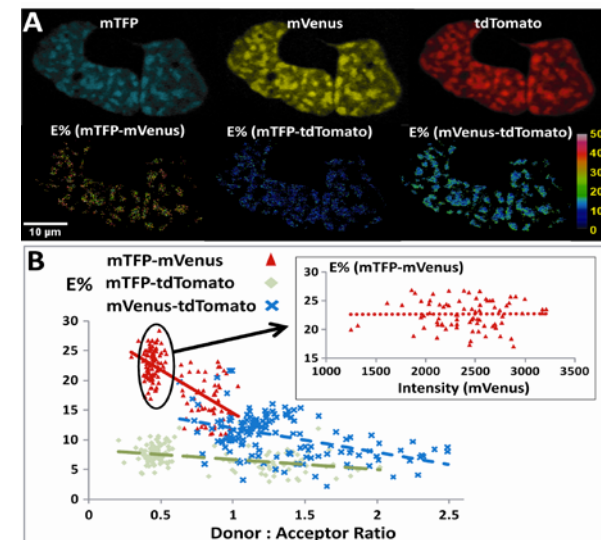
- Anisotropy / Homo-FRET
- NAD(P)H-TRP FRET
- FLIM analysis: Fitting and Phasor plots
- Single-molecule FRET
- Metabolic Imaging
- FRAP
- Working on your instrument of choice after formal curriculum ends

*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

Abberior Instruments, Becker & Hickl, Boston Electronics, Carl Zeiss, Chroma Tech, Excelitas Technologies Corp., ISS, Leica Microsystems, Olympus.