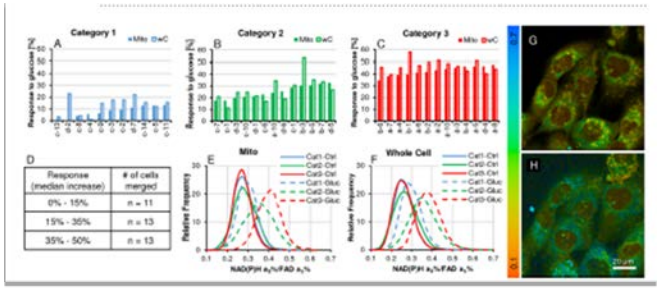
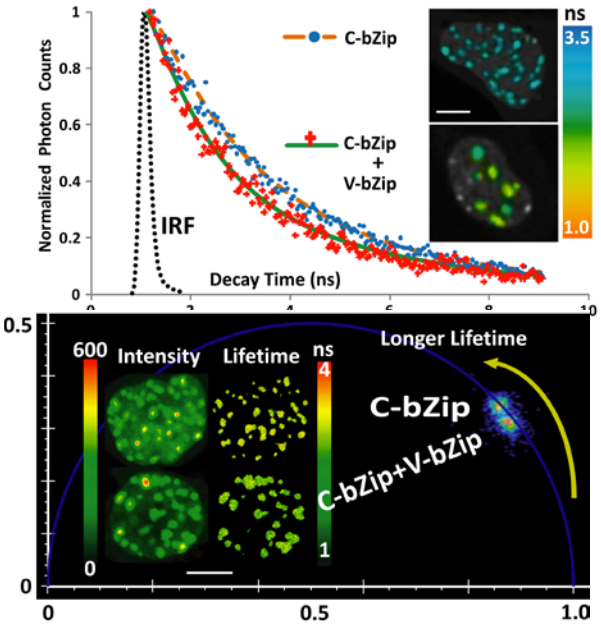


Metabolic response of segmented prostate cancer cells after treatment, based on FLIRR (Fluorescence Lifetime Redox Ratio) –NAD(P)H-a2%/FAD-a1%. Scientific Reports | *(2018) 8:79,* DOI:10.1038/s41598-017-18634-x



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. R. N. Day. Indiana University
Workshop Co-Director, rnday@iupui.edu

Dr. M. Barroso, Albany Medical College

Dr. M. Börsch, Jena University

Dr. J. N. Demas, University of Virginia

Dr. A. Kenworthy, University of Virginia

Dr. Angelika Rück, Universität Ulm, Germany

Dr. S. Vogel, NIAA, NIH

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

Guest Lecturers

Dr. M. Stanley, Chroma Tech.

Dr. P. So, MIT

For more info:

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

FEES: $2,200 non-profit organizations
$2,600 for-profit organizations
(Includes lodging, breakfast, lunch, dinner, lecture materials)

Contact:

Prof. Ammasi Periasamy
ap3t@virginia.edu
434-243-7602

All participants must be fully Vaccinated.

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

March 7-11, 2022

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
www.KCCI.virginia.edu/workshop/workshop2016

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 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 Leica, Olympus, Zeiss, Nikon, Chroma Tech, IdeaElan, Applied Precision, Lumen Dynamics, Becker & Hickl, Boston Electronics, ISS, Lambert.

Days 2-4 (8:30 AM – 12 PM)*

Short lectures and Q&A on the subjects:

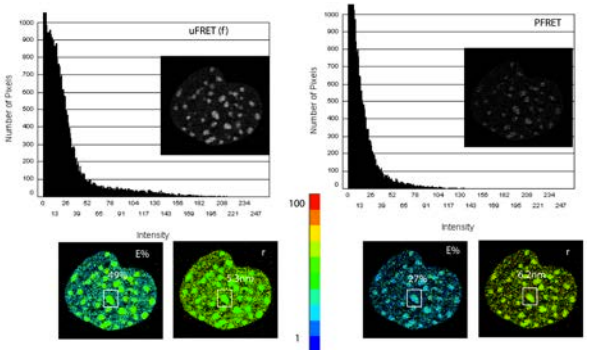
- Confocal/spectral FRET
- FLIM-FRET, NAD(P)H-TRP FRET
- Fluorophore pairs for FRET/FLIM-FRET
- FLIM-FRET,
- Redox states analyzed by NAD(P)H & FAD
- Metabolic Imaging
- Imaging live/fixed cells & tissue
- Spectroscopy FRET in suspensions
- Bacterial FRET

Days 2-4 (1 PM – 9 PM)*

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

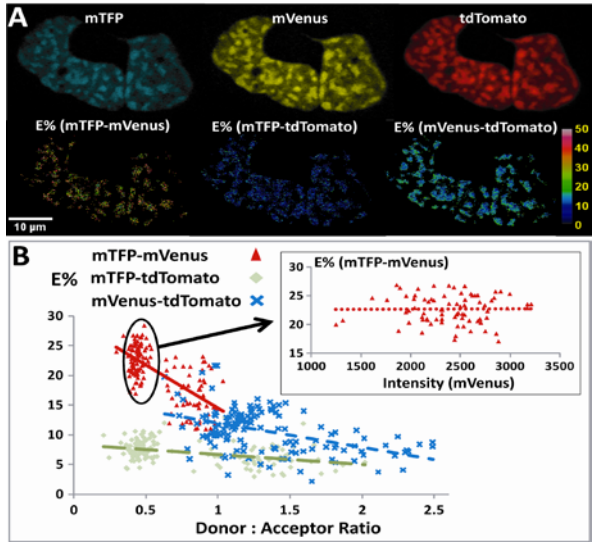
- Anisotropy / Homo-FRET
- NAD(P)H-TRP FRET
- FLIM analysis: Fitting and Phasor plots
- Single-molecule FRET
- Metabolic Imaging
- FRAP
- Working on your instrument of choice after formal curriculum ends

***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

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