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

For more info:
https://kcci.virginia.edu/workshop/general-information

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Note: All participants must be fully Vaccinated.
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 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.


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