

**21st Annual
Workshop on FRET, FLIM, and FLIRR (Metabolism) Microscopy
W. M. Keck Center for Cellular Imaging (KCCI)
University of Virginia, Charlottesville**

March 4, 2024

Monday

[Gilmer Room #390](#)

9:30-10am Registration (Gilmer Hall 390; 485 McCormick Rd.)

- 10:00 – 10:30 **Introduction to the workshop, the staff, and the participants**
Dr. Ammasi Periasamy, Workshop Director
Center Director, Keck Center for Cellular Imaging
Prof. of Biology and Biomedical Engineering
- 10:30 – 11:10 **Basics of Fluorescence**
Dr. James N. Demas, Emeritus Prof of Chemistry, UVA
- 11:10 – 11:40 **Basics of Light Microscopy**
Dr. Ammasi Periasamy
- 11:40 – 12:10 **New developments in mirror and filter design for FRET and FLIM**
Dr. Michael Stanley, Chroma Tech, Vermont.

12:10 – 1:00 Lunch (Gilmer 490)

- 1:00 – 1:40 **Visible Fluorescence Proteins (Zoom)**
Dr. Michael Boersch, Abbe Center for Photonics, Single Molecule
Spectroscopy, University of Jena, Germany
- 1:40 – 2:10 **Basics of FRET Microscopy**
Dr. Ammasi Periasamy
- 2:10 – 2:50 **Two-color FRET-The Algorithm (Wide-field, Confocal, & Spectral
FRET)**
Dr. Ammasi Periasamy
- 2:50 – 3:10 **Probing of Kidney Cancer metabolism using steady state
fluorescence spectroscopy.**
Dr. Santhosh Chidangil, Professor of Atomic and Molecular Physics;
Centre of Excellence for Biophotonics, Manipal Academy of Higher
Education, Manipal, Karnataka, India.

3:10 – 3:30 Coffee break

- 3:30 – 4:00 **Quantitative FRET data Analysis: Case study**
Mr. Horst Wallrabe, Keck Center for Cellular Imaging, UVA
- 4:00 – 4:30 **Basics of FLIM Microscopy**
Dr. Ammasi Periasamy
- 4:30 – 5:00 **FLIRR Microscopy: measurement of metabolism in Prostate cancer cells**
Dr. Shagufta Alam, Keck Center for Cellular Imaging, UVA.
- 5:00 – 5:40 **FRET, FLIM, and FLIRR Data Acquisition and Issues (Confocal, 1p & 2p FLIM)**
Dr. Ammasi Periasamy
- 5:40 – 6:10 **Software tools for biological image analysis**
Dr. Karsten Siller, Research Computing, UVA
- 6:30- 7:30 Dinner (Gilmer 490)**

21st Annual Workshop on FRET, FLIM, & FLIRR Microscopy

March 5, 2024
Tuesday
Gilmer Room #390

- 8:30 – 8:45 Revision (Questions about previous day's activities)
- 8:45 – 9:20 **Time-Domain FLIM / FRET data analysis by Maximum Likelihood Estimation.**
Dr. Wolfgang Becker, CEO and Founder of Becker & Hickl, Germany
- 9:20 – 10:00 **Metabolic FLIM and oxygen PLIM/dFLIM: New Techniques, algorithms, and applications (Zoom)**
Dr. Angelika Rueck, Senior Scientist, Leader of the microscopy core facility, University of Ulm, Germany.
- 10:00 – 10:30 Coffee (Room# 390)**
- 10:30 – 11:00 **Single molecule FRET and its applications (Zoom)**
Dr. Michael Boersch
- 11:00 – 11:30 **The Orientation of Light and Time-resolved Fluorescence Anisotropy**

Dr. Steven Vogel, National Institute on Alcohol Abuse & Alcoholism
National Institutes of Health (NIH)

11:30 – 11:50 Dr. **Anastasiia Aleksandrova**
Advanced Workflow Specialist - Confocal (Mid-Atlantic US)
Leica Microsystems, Inc

11:50 – 12:10 **Dr. Alma Arnold**
3D Product and Application Support Specialist
ZEISS Research Microscopy Solutions

12:10 – 1:00 Lunch Gilmer 490

1:00 – 5:00 Hands-on Training - Data Acquisition, Analysis
(see the Lab Schedule)

3:00 – 3:30 Coffee break

5:30–6:30 Room 490 - Discussion on “Selection of Microscopes, Detectors, Filters & Techniques for FRET, FLIM & FLIRR” – Questions and answers or any other issues

6:30- 7:00 Dinner (Gilmer 490)

7:00 – 8:00 Room PLSB 030
Fluorescence Recovery after Photobleaching (FRAP) (demo)
Dr. Anne Kenworthy, Prof of Molecular Physiology and Biological Physics;
Associate Director, Center for Cell and Membrane Physiology, University
of Virginia School of Medicine.

21st Annual Workshop on FRET, FLIM, & FLIRR Microscopy

March 6, 2024
Wednesday

[Gilmer Room #390](#)

- 8:30 – 8:45 Revision (Questions about previous day's activities)
- 8:45 – 9:15 **FRET & FLIM in biological and Clinical applications using Phasor plot.**
Dr. Michelle Digman, Associate Prof. of Biomedical Engineering, University of California at Irvine.
- 9:15 – 9:45 **Fluorescent proteins and FRET sensors**
Huiwang Ai, Prof of Molecular Physiology and Biological Physics
- 9:45 – 10:15 **In vivo quantitative FRET small animal imaging: intensity versus lifetime-based FRET.**
Dr. Margarida Barroso, Prof. and Director of Imaging Core Facility, Dept. of Molecular and Cellular Physiology, Albany Medical College, Albany, NY
- 10:15 – 10:40 **Coffee (Room #390)**
- 10:40 -11:10 **FRET in Membranes: Special Considerations**
Dr. Anne Kenworthy
- 11:10 – 11:40 **Binary-FRET: A new tool for studying activity dependent CaMKII - NR2B binding, adaptation, and translocation.**
Dr. Steven Vogel
- 11:40 – 12:00 **Dr. Yuansheng Sun, ISS, Inc**
- 12:00 – 12:20 **Dr. Bo Faust**
Research Imaging Specialist, Confocal and Multiphoton Evident/Olympus
- 12:20 – 1:00 **Lunch (Gilmer 490)**
- 1:00 – 5:00 **Hands-on Training - Data Acquisition, Analysis & Demo**
- 3:00 – 3:30 **Coffee**

5:30–6:30 Room 490 - Discussion on “Selection of Microscopes, Detectors, Filters & Techniques for FRET, FLIM & FLIRR” – Questions and answers or any other issues

6:30- 7:00 Dinner (Gilmer 490)

7:00 – 8:00 Phasor plot demonstration, Dr. Yuansheng Sun, ISS, Inc., Keck Center, room 043

21st Annual Workshop on FLIM, FRET and FLIRR Microscopy

March 7, 2024

Thursday

Gilmer Room #390 (lectures)

8:30 – 8:45 Revision (Questions about previous day’s activities)

8:45 – 9:15 **Molecular imaging by TCSPC FLIM- Application to metabolic and FRET imaging.**
Dr. Wolfgang Becker

9:15 – 9:45 **Quantitative analysis of label-free FLIM images of single cells measurement of metabolism**
Dr. Alexandra (Alex) Walsh, Assistant Prof of Biomedical Engineering, Texas A & M University, College Station, Texas.

9:45 – 10:00 **Biolmaging North America (BINA) – a network organization for imaging scientists.**
Dr. Nikki Bialy, BINA Program Coordinator, (Kevin Eliceiri group), Morgridge Institute for Research, Madison, WI.

10:00 – 10:30 Coffee break

10:30 – 11:00 **Autofluorescence imaging to study neutrophil metabolism.**
Dr. Rupsa Datta, Associate Scientist, (Melissa Skala group), Morgridge Institute for Research, Madison, WI.

11:00-11:30 **Phasor approach to FLIM: Metabolism in lung carcinoma cells**
Dr. Michelle Digman

- 11:30 – 11:50 **Voltage measurements with FLIM.**
Dr. Holly Aaron, Director, CRL Molecular Imaging Center,
University of California Berkeley.
- 11:50 – 12:05 **Software tools to administrate the Microscopy Facility**
Mr. Masilamani Elangovan, CEO and Founder of “Idea Elan”.

12:05 -12:30 **Group Picture**

12:30 – 1:00 **Lunch break (490)**

1:00 – 5:00 **Hands-on Training - Data Acquisition, Analysis & Demo**

3:00 – 3:30 **Coffee**

5:30 pm Travel to Michie Tavern

6:00 – 8:30 **Workshop Dinner at Michie Tavern**

683 Thomas Jefferson Pkwy, Charlottesville, VA 22902

Historic tavern with a period restaurant serving Southern fare, plus a gift shop.

<https://www.michietavern.com/>

21st Annual Workshop on FRET, FLIM, & FLIRR: Microscopy

March 8, 2024
Friday
Gilmer Room #390

8:00 – 12:00 Lab
Hands-on Training - Data Acquisition, Analysis & Demo

12:00 – 1:00 **Lunch (Gilmer 390)**

1:00 – 1:40 **Macroscopy fluorescence lifetime FRET monitors tumor drug-target engagement in vivo.**
Dr. Margarida Barroso

- 1:40 – 2:20** **Towards High Throughput, High Resolution, Deep, Two-Photon FLIM**
Dr. Peter So; Professor of Mechanical Engineering and Biological Engineering, MIT, Boston, MA
- 2:20 – 3: 00** **Certificate distributions**

The workshop ends at 3pm.