International Advanced Microscopy Course

Confocal Events 2003

Washington
in cooperation with the
National Institute of Health
December 2nd - 4th, 2003

Final
Course Program

Speakers:

G. Burger (LEICA, Mannheim, Germany)
A. Kenworthy (Vanderbilt University)
W. Knebel (LEICA, Mannheim, Germany)
J. Lippincott-Schwartz (NIH, Bethesda)
D. Malide (NIH, Bethesda)
J. McNally (NIH, Bethesda)
G. Patterson (NIH, Bethesda)
L. Peachy (University of Pennsylvania)
R. Pepperkok (EMBL, Heidelberg, Germany)
M. Wachsmuth (LEICA, Mannheim, Germany)

For detailed information, please, refer to:
www.confocal-microscopy.com

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Course Program

Advanced Microscopy Course

Date:	December 2nd – 4th , 2003
Location:	Washington DC (USA)
Course Fee:	Lectures free of charge Practical sessions: US$ 1,000.00 (limited to 18 participants)
Hotel accommodation	Holiday-Inn Bethesda 8120 Wisconsin Ave Bethesda, MD 20814 Phone +1 – 877 – 888 – 3001 Rate: US$ 129.00 (Leica special rate)

Tuesday, December 2nd

Lectures

9:00 – 9:15	Introduction	G. Burger
9:15 – 9:45	Basics and principles in confocal microscopy	W. Knebel
9:45 – 10:30	Insights into secretory trafficking and sorting using FRAP	J. Lippincott-Schwartz
10:30 – 11:00	Coffee break	
11:00 – 11:45	FRAP: An essential tool for understanding transcription	J. McNally
11:45 – 12:30	Development and use of a photoactivatable green fluorescent protein	G. Patterson
12:30 – 14:00	Lunch break	
14:00 – 14:45	FRET applications	R. Pepperkok
14:45 – 15:30	FRET in membranes: special considerations	A. Kenworthy
15:30 – 15:45	Coffee break	
15:45 – 16:30	FCS and FLIM basics and principles	M. Wachsmuth

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Wednesday December, 3rd

Practical Session

9:00 – 9:30	Introduction	all
9:30 – 10:15	Multicolor Imaging	L. Peachy
10:30 – 11:00	Tips and Tricks in Confocal Imaging	W. Knebel
11:00 – 11:15	Coffee break	
11:15 – 11:45	Tips in Live Cell Imaging	D. Malide
11:45 – 12:30	FRET methods	G. Burger
12:30 – 14:00	Lunch break	
14:00 – 17:00	Practical sessions 1	
	FRET	Group 1
	Photoactivation, FRAP and FLIP	Group 2
	FCS	Group 3

Thursday December, 4th

Practical Session

9:00 – 12:30	Practical session 2	
	Photoactivation, FRAP and FLIP	Group 1
	FCS	Group 2
	FRET	Group 3
12:30 – 14:00	Lunch break	
14:00 – 17:00	Practical sessions 3	
	FCS	Group 2
	FRET	Group 3
	Photoactivation, FRAP and FLIP	Group 1

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- Abstract 1 -

Frontiers in fluorescent protein imaging of living cells

Jennifer Lippincott-Schwartz,

Cell Biology and Metabolism Branch,
NICHD,
NIH,
Bethesda, MD

The development of fluorescent proteins as molecular tags over the past decade has spurred a revolution by allowing complex biochemical processes to be correlated with the functioning of proteins in living cells. Fluorescent proteins such as green fluorescent protein (GFP) from the jellyfish Aequorea victoria and its variants can be fused to virtually any protein of interest to analyze protein geography, movement and chemistry in living cells.

As such, they have provided an important new tool for understanding protein function, filling an urgent need now that the genome sequence of many organisms is complete. The modified GFPs have been used as markers to track and quantify individual or multiple protein species, as probes to monitor protein-protein interactions, and as photochemically modulatable proteins to highlight and follow the fate of specific protein populations within a cell. The focus of this talk will be on the kinetic microscopy methods of photobleaching and photoactivation that are being used to monitor the appearance, location, movement and degradation of GFP fusion proteins in living cells. Results from these applications are providing profound new insights into protein function and cellular processes in the complex environment of the cell.

Jennifer Lippincott-Schwartz
Cell Biology and Metabolism Branch
Nat. Inst. of Child Health & Human Development
National Institutes of Health
Bethesda MD 20892
mailto: jlippin@helix.nih.gov

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- Abstract 2 -

Development of a Photoactivatable Fluorescent Protein from *Aequorea victoria* GFP

George Patterson and Jennifer Lippincott-Schwartz

Cell Biology and Metabolism Branch,
NICHD,
NIH,
Bethesda, MD

The green fluorescent protein (GFP) provides a specific and non-invasive fluorescent marker that can be used in a variety of imaging techniques to monitor protein dynamics within living cells. We describe the development of a photoactivatable fluorescent protein based on a T203H mutant of the *Aequorea victoria* GFP (referred to as PA-GFP). After photoactivation with light of ~400 nm, PA-GFP exhibits a new absorbance peak at ~505 nm that results in a 60-100-fold fluorescence increase over background under 488 nm excitation. The photoactivation occurs in purified protein samples as well as when the proteins are expressed in living cells under aerobic conditions. We have demonstrated the utility of PA-GFP expressed as a free protein to measure protein diffusion across the nuclear envelope and as a chimera with a lysosomal membrane protein (lgp120) to demonstrate rapid inter-lysosomal membrane exchange. Photoactivation of PA-GFP provides a good complement to the assortment of photobleaching methods currently employed to highlight selectively a pool of fluorescent proteins in a cell. Because only photoactivated molecules exhibit noticeable fluorescence under 488 nm excitation, PA-GFP has the advantage that newly synthesized molecules will not become fluorescent and complicate experimental results. Therefore, these data indicate that PA-GFP will be an excellent tool for biological studies monitoring protein, organelle, and cell dynamics.

Dr. George H. Patterson
Cell Biology and Metabolism Branch
Nat. Inst. of Child Health & Human Development
National Institutes of Health
Bethesda MD 20892
mailto: geopa@helix.nih.gov

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- Abstract 3 -

Dr. James McNally

Laboratory of Receptor Biology and Gene Expression,
NCI,
NIH,
Bethesda, MD

We are using fluorescence recovery after photobleaching (FRAP) to study the dynamics of transcription-factor binding at a promoter. We have shown with FRAP that a steroid-hormone transcription factor (the glucocorticoid receptor) remains bound for only ~1 min with its target promoter and then is replaced by another glucocorticoid receptor. This rapid exchange is surprising given that transcription from the promoter continues for at least one hour. We are now using FRAP to analyze the function and mechanism of rapid exchange. Our recent studies suggest that rapid exchange is coupled to the transcription process itself and that it is regulated in part by chaperones and proteasomes.

Dr. James McNally
Laboratory of Receptor Biology and Gene Expression
National Cancer Institute
National Institutes of Health
Building 41, Room B602
Bethesda MD 20892
mailto: mcnallyj@dce41.nci.nih.gov

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- Abstract 4 -

FRET in membranes: special considerations

Anne Kenworthy, Ph.D.,

Department of Molecular Physiology and Biophysics,
Vanderbilt University School of Medicine,
Nashville, TN

FRET offers great potential to study events such as receptor dimerization in living cells. However, FRET measurements between membrane proteins or lipids require careful interpretation, because molecules confined to membranes are much more crowded than in solution. In a membrane, even randomly distributed donors and acceptors can give rise to substantial FRET. I will outline the theoretical background of two-dimensional FRET and its implications for the design of FRET measurements in cell membranes. I will then discuss how FRET can be used to study membrane protein oligomerization, obtain structural information, and probe the organization of membrane microdomains such as lipid rafts.

Dr. Anne K. Kenworthy
Molecular Physiology and Biophysics Dept.
Vanderbilt University School of Medicine
718 Light Hall
2215 Garland Ave.
Nashville TN 37232-0615
mailto: anne.kenworthy@vanderbilt.edu