

## Course schedule and time-table

### Table of Contents

<b>Course schedule and time-table .....</b>	<b>1</b>
<b>Contact Details .....</b>	<b>1</b>
Presenters .....	1
Vendors .....	1
<b>Course Overview .....</b>	<b>2</b>
<b>Lab Schedule and Locations.....</b>	<b>3</b>
<b>Directions to Workshop Venues .....</b>	<b>4</b>
Directions to Seminars in HSC 1A4 .....	4
Directions to MacBiophotonics for Labs.....	4
Map of 4th Floor HSC for Facility.....	4
Map of MacBiophotonics Facility .....	4
<b>Details of Seminars .....</b>	<b>5</b>
The Anatomy of a Microscope: The components of a microscope and how to get the most from them - T. Collins ..	5
The New Test Tube of the Millennium, the Live Cell - R. Truant.....	5
Fluorescent Microscope Modalities: Picking the right microscope for your experiments - T. J. Collins.....	5
Automated Microscopy and High Content Screening - David W. Andrews .....	5
Practical FRET Measurement - R. Truant.....	6
Image Processing: Do's and Don'ts and Colocalisation Analysis - T. Collins.....	6

## Contact Details

### Presenters

David W. Andrews	McMaster University	andrewsd@mcmaster.ca
Tony J. Collins	McMaster University	tcollins@macbiophotonics.ca
Qiyin Fang	McMaster University	qiyin.fang@mcmaster.ca
Ray Truant	McMaster University	truantr@mcmaster.ca

### Vendors

Tony Bouquillon	Olympus Canada	Tony.Bouquillon@olympus.com
Jennifer Melo	Nikon Canada Instruments	jmelo@nikon.ca
Patrick Lapointe	Perkin Elmer	Patrick.Lapointe@perkinelmer.com
Egbert Verbrugge	PTI	EVerbrugge@pti-can.com
Vlad Zhukarev	Leica	vlad.zhukarev@leica-microsystems.com
Abdel Barraj	Zeiss	ABarraj@zeiss.com

## Course Overview

### Day 1: Monday July 20. HSC 1A4

Registration and coffee at 8 am. Coffee for registration is limited, please arrive in good time.

Please ensure you return to the lectures in good time. There is little spare seating and late attendees will be very disruptive. Thank you for your co-operation.

Start	End		Location
8:00	8:30	Coffee and Registration – Foyer outside HSC 1A4	HSC 1A4
8:30	9:00	Workshop introduction, notices and overview	
9:00	10:00	The Anatomy of a Microscope: The components of a microscope and how to get the most from them - T. Collins	
10:00	10:30	Coffee - Foyer outside HSC 1A4	
10:30	11:30	The New Test Tube of the Millennium, the Live Cell: Applications of Fluorescent Proteins - R. Truant	
11:30	12:30	Fluorescent Microscope Modalities: Picking the right microscope for your experiments - T. Collins	
12:30	13:30	Lunch – not provided	
13:30	14:30	Image Processing: The basics; the do's; the don'ts and colocalisation analysis done properly - T. Collins	
14:30	15:30	Practical FRET measurement - R. Truant	
15:30	16:00	Coffee - Foyer outside HSC 1A4	
16:00	17:00	Automated microscopy and High content Screening - D. Andrews	

### Day 2: Tuesday Jul 21

9.30	10.30	Labs 1	HSC 1H1-8 (ImageJ Lab in 4H22)
10.30	11.00	Coffee - Foyer outside HSC 1A4	
11.00	12.00	Labs 2	
12:00	13:00	Lunch (not provided)	
13.00	14.00	Labs 3	
14:00	14:30	Coffee - Foyer outside HSC 1A4	
14:30	15:30	Labs 4	

### Day 3: Wednesday Jul 22

9.30	10.30	Labs 5	HSC 1H1-8 (ImageJ Lab in 4H22)
10.30	11.00	Coffee - Foyer outside HSC 1A4	
11.00	12.00	Labs 6	
12:00	13:00	Lunch (not provided)	
13.00	14.00	Labs 7	

### Day 4: Thursday Jul 23

9:00	10:00	Introduction to FLIM	HSC 1A4
10:00	11:00	FLIM-FRET case studies	HSC 1A4
11:00	11:30	Coffee break in 4H22	HSC 4H22
11.30	12:30	FLIM Lab1	MBF: HSC 4H14
12.30	13.30	Lunch (not provided)	
13.30	14.30	FLIM Lab2	
14.30	15.30	FLIM Lab3	
15.30	16.00	Coffee break in 4H22	
16.00	17.00	FLIM Lab4	

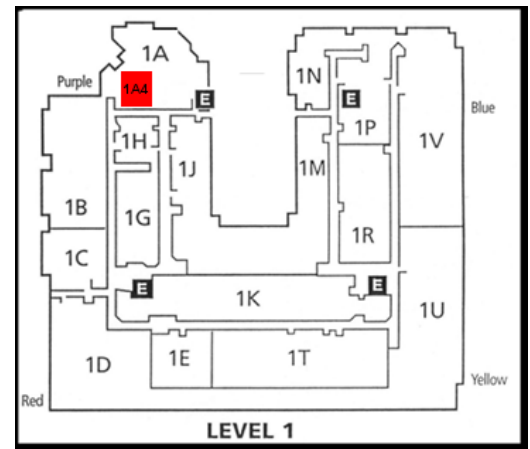
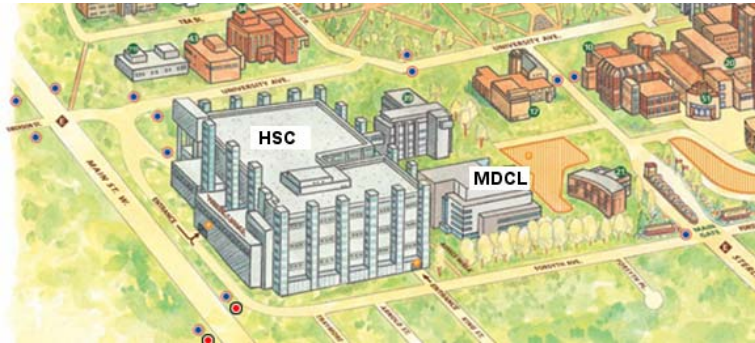
## Lab Schedule and Locations

<b>Introductory Lab groups</b>		<b>Tues. 21</b>				<b>Wed. 22</b>		
		Lab 1	Lab 2	Lab 3	Lab 4	Lab 5	Lab 6	Lab 7
Lab	System	9.30-10.30	11.00-12.00	13.00-14.00	14.30-15.30	9.30-10.30	11.00-12.00	13.00-14.00
FRET	Zeiss CellObserver	Group A	B	C	D	E	F	G
FRAP	Olympus FV1000	B	C	D	E	F	G	A
Ratio Imaging	PTI Easy Ratio Pro	C	D	E	F	G	A	B
Confocal	Leica White light SP5	D	E	F	G	A	B	C
Deconvolution	Nikon TEi	E	F	G	A	B	C	D
Live cell Confocal	Perkin Elmer Ultraview	F	G	A	B	C	D	E
ImageJ	Hosted by MBF	G	A	B	C	D	E	F

<b>FLIM Lab groups</b>			<b>Thurs. 23</b>			
			Lab 1	Lab 2	Lab 3	Lab 4
Lab	System	Location	11.30-12.30	13.30-14.30	14.30-15.30	16.00-17:00
TCSPC	Leica SP5	MBF: MP laser room	Group FL1	FL2	FL3	FL4
HCS-FLIM	PE FLIM-Opera	MBF: HCS room	FL2	F3	FL4	FL1
Widefield FLIM	LIFA	MBF: SDC room	FL3	FL4	FL1	FL2
Lifetime Fluorimetry	ISS Chronos	MBF: Fluorimetry area	FL4	FL1	FL2	FL3

## Directions to Workshop Venues

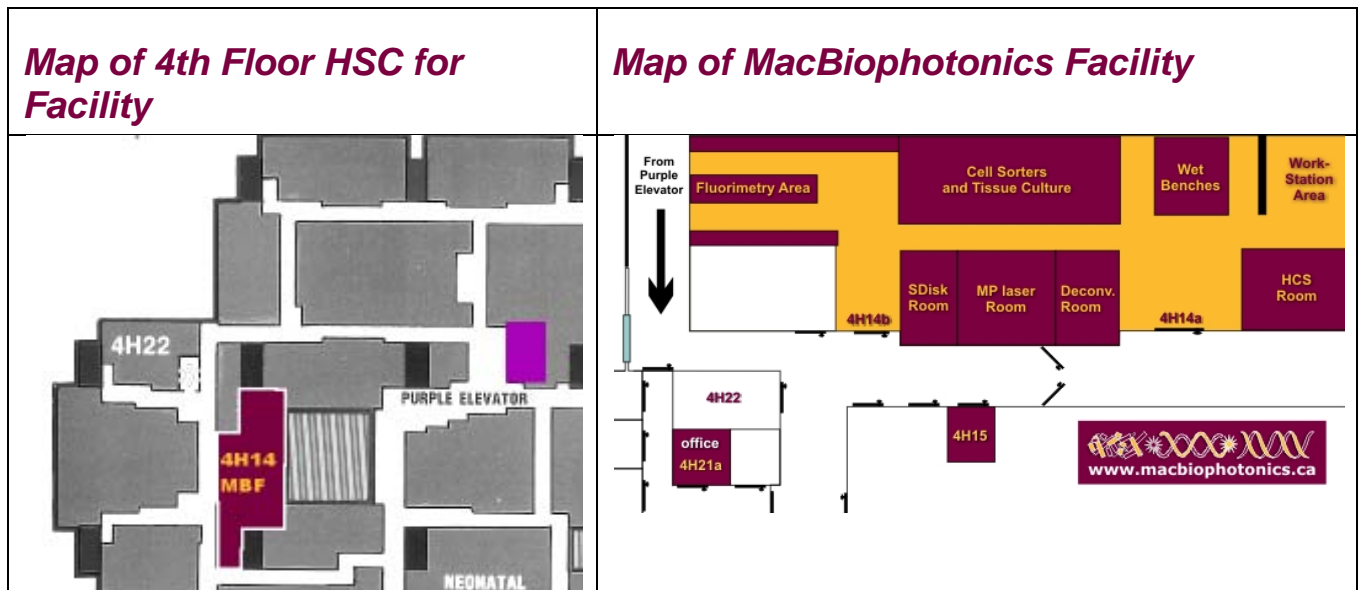
### Directions to Seminars in HSC 1A4



**HSC = Health Sciences Centre** The Health Sciences Centre is the largest building on campus, located towards the south of campus between University Ave., Main St., and Forsyth Ave.. Enter HSC from University Avenue, not Main Street. The workshop lectures and labs take place in the purple section (North West Wing) of the building. Lectures are located in room HSC 1A4 on the first floor. Some labs are in room HSC 1H1-8 located in the 1H area on the first floor.

### Directions to MacBiophotonics for Labs

Please reference map above to locate the Health Sciences Centre. The facility is located in rooms 4H14 and 4H15 on the 4th floor in the North West Wing (purple section) of the Health Sciences Centre (HSC). Enter HSC from University Avenue, not Main Street. Take the Purple elevators to the 4<sup>th</sup> floor then exit to the right of the 'Dormant Elevators.' 'Dormant Elevators' are non-functional. Continue to the end of the corridor then turn left. Do not take a sharp right towards the Biochemistry Department sign.



## **Details of Seminars**

### ***The Anatomy of a Microscope: The components of a microscope and how to get the most from them - T. Collins***

A proper understanding of the components of the microscope tools is necessary to ensure correct acquisition and interpretation of microscope data.

The different optical components will be introduced from light source to detector. The most important part of the microscope is the objective. Containing multiple lenses, this complex piece of optics determines the performance of the microscope. This presentation covers the anatomy of the objective and how this affects the way in which your data can be interpreted. The roles of magnification, the numerical aperture, corrections and contrast techniques will be covered.

### ***The New Test Tube of the Millennium, the Live Cell - R. Truant***

This section of the course is focused on the use of Aequorea Fluorescent Proteins (AFPs) in live cell imaging. The current palette of AFPs ranges in numbers of 18-20, and in colors from 440 to 650 nm, but the properties of these fluorophores mean that some are more practical than others for live cell imaging. The properties of many of these proteins will be summarized and discussed, as well as methods used to derive new fluorescent proteins with desired properties. A brief overview of fluorescent microscope design and setup to optimize for AFP visualization and live cell imaging will be discussed, as well as practical conditions of cell growth and maintenance for live cell imaging over short and long time periods. Paired fluorophores will be discussed for techniques such as co-localization of two proteins and Förster resonant energy transfer (FRET), and lifetime imaging. Location and dynamics of proteins in live cells will be discussed with an overview of qualitative fluorescent recovery after photo bleaching (FRAP) and fluorescence loss induced by photo bleaching (FLIP) with multiple fluorophores. Newer AFPs and their use in fluorescent photo activation will also be summarized.

A second revolution from the use of AFPs is in the design and use of AFPs as biosensors, either as individual protein markers responding to local nano environments, as location sensors to detect enzymatic activity, or as conformational switching sensors to detect a conformational change in a protein in response to a protein-protein or protein-factor interaction. Finally, we will conclude with an overview of detecting protein complex interactions in vivo by the use of bimolecular fluorescent complementation, or BiFC, with newly developed vectors.

### ***Fluorescent Microscope Modalities: Picking the right microscope for your experiments - T. J. Collins***

Fluorescence Microscopy has undergone a revolution in recent years with the development of cheaper, more powerful light sources; the increased processing power of personal computers and the development of novel fluorescent reagents. Along with these developments, new microscope modalities have emerged. The traditional wide-field microscope has been joined by single-point scanning confocal and multi-point scanning confocal microscopes. This seminar will compare these microscopy modalities, how they work, their advantages and disadvantages and how to best use each one.

### ***Automated Microscopy and High Content Screening - David W. Andrews***

Fluorescent proteins are most often used as tags to identify the subcellular localization of proteins in live cells. They can also be used as organelle specific markers and as reporters for both protein:protein interactions and cellular processes such as organelle assembly. When fluorescent proteins are used as reporters, assays do not have to 'endpoint'. The advantages to maintaining cell viability throughout screening includes facilitating functional cloning and time course experiments that can extend over prolonged periods. We are exploring the use of fluorescent proteins for image based small molecule and genetic screens in yeast and human cells. I will highlight our recent progress using FRET-FLIM to study transcription factor interactions for a planned chemical screen. I will also describe the use of high resolution automated fluorescent imaging and the yeast specific gene deletion library to

examine the effect of 5100 non-essential genes on the assembly of proteins into the endoplasmic reticulum. Automated analysis of colocalization, texture and morphology features was carried out for > 75,000 images. Multidimensional clustering of the images and support vector machines were used to classify the images of the different strains of yeast. Some surprising effects on the regulation of the expression of exogenous genes in cells that create difficulties for screening were discovered. An array of phenotypes including changes in ER morphology as well as other processes required to maintain the unique protein and lipid composition of the organelle were revealed. Our preliminary data suggest that a surprising diversity of information can be recorded in image based screens using live cells and fluorescent proteins.

### ***Practical FRET Measurement - R. Truant***

This section will involve the theory and practical application of the use of Forster Resonant Energy Transfer, or FRET, to detect direct molecular interactions in vivo, or in use as biosensors. We will discuss the theory behind FRET, and the detection of FRET by multiple methods including stimulated emission, acceptor photobleaching and FRET detection by fluorescence lifetime imaging, or FLIM. Advantages and disadvantages of this method will be discussed, as well as some well characterized uses of FRET as biosensors for calcium measurement and protein conformation, and the use of FRET for protein-nuclei acid or protein-lipid interactions in vivo. Some typical fluorescent protein donor-acceptor pairs will be discussed, as well as typical fluorescent protein-fluorescent dye donor-acceptor pairs. Focus will be on two molecule FRET detection.

### ***Image Processing: Do's and Don'ts and Colocalisation Analysis - T. Collins***

Digital manipulation and complex processing techniques are now available at the click of a mouse button. This powerful technology can be used, and misused, by anybody. This lecture will cover the issues relating to image manipulation and some of the limitations as to what is and is not allowed when enhancing your image data. The second part of the seminar will cover the much misunderstood method of colocalisation analysis.