

The Optical Imaging Facility (OIF) at the Universidad Central del Caribe, Bayamón, P.R., is organized around three major areas according to the distribution of the specialized equipment and complementary resources necessary for the effective utilization of the imaging technology.



These are

(1) the Immunocytochemistry Laboratory (ICCL),

(2) the Widefield Fluorescence Microscope Imaging Unit (WF) and

(3) the Laser Scanning Confocal Microscope Unit (LSCM).

A brief description of the equipment, capabilities and actual applications performed within each of the major components of the OIF is summarized in **Table I**.

Access to equipment is provided only upon completion of registration, acceptance of our established terms and conditions and instrument safety training. Upon user authorization all instrument access at the OIF is done in a first come basis. Users will be able to do on-line reservations through an electronic calendar provided at the OIF webpage. This step, in addition to expediting the process, helps OIF administrative assistant to tract instrument use for billing purposes. Any time on any piece of equipment must be logged in the appropriate record book. For live cell studies the interested user must discuss first with facility personnel. Users must always be alert for liquid spills on the stage, objective turret and microscope body.

TABLE 1

**UPRIGHT WIDEFIELD FLUOROESCEENCE
MICROSCOPY**

**EQUIPMENT &
RESOURCES**

- The system includes an **Olympus BH2 upright microscope** controlled by Metamorph (V. 6.3r7) software installed on a Windows 2000-based PC.
- The system is equipped for transmitted light (brightfield, phase contrast) and epi-fluorescence imaging. The microscope optics includes 10X, 20X, 40X oil and 100X oil objective lenses.
- Fluorescence imaging is achieved through automated ten position excitation and emission filter wheels containing appropriate filters for the fluorophores **FITC** (and **GFP**), **DAPI**, **Cy3** (and **Rhodamine**), **Cy.5** (and **Texas Red**).
- A focus motor allows the acquisition of series of images in the Z axis. Multi-channel, time lapses, Z-stack can be simultaneously programmed. Images are acquired with a 12 bit Photometrics Cool-Snap HQ cooled CCD-Camera (1365 x 1000 pixels).

Applications: Immunocytochemistry, *In situ*-hybridization, High-resolution single, double or triple fluorescence, Digital recording of the images from fixed tissue, Z-series of fixed specimens.

TABLE 1

**INVERTED WIDEFIELD FLUORESCENCE
MICROSCOPY**

**EQUIPMENT &
RESOURCES**

- The system includes an **Olympus IX70 inverted microscope** controlled by Metamorph / Metafluor (version 6.3r7) software installed on a Windows 2000-based PC.
- The system is equipped with optics for transmitted light (brightfield, phase contrast and differential interference contrast (DIC) and fluorescence imaging. This includes the 4x, 10x, 20x, 40x oil and 60x oil objective lenses.
- The system is equipped with appropriate filters for imaging the fluorophores **FITC** (and **GFP**), **DAPI**, **Cy3** (and **rhodamine**), **Cy5** (and **Texas Red**). The setup is also equipped with filters for ratiometric imaging of cytoplasmic Ca²⁺ with **Fura-2** and pH with **BCEF**.
- Images are acquired with high performance 12 bit cooled monochrome digital cameras: an Intensified Princeton Instruments Pentamax (512 x 512 pixels) or a 12 bit Hamamatsu ORCA-ER.

Applications: Live cell imaging: Single, double and triple fluorescence imaging, DIC imaging, Phase contrast imaging, Time lapses.

TABLE 1

**EQUIPMENT &
RESOURCES**

LASER SCANNING CONFOCAL MICROSCOPE

- The system includes an inverted **Olympus IX 81 microscope** with motorized focus module.
- Excitation is provided by
- the multi-line Argon Laser that allows excitation at 457 nm, 488 nm, 515 nm for dyes like **FITC, GFP**.
- HeNe-Green Laser exciting at (543 nm) for dyes like **Cy3, Rhodamine**.
- HeNe-Red Laser providing excitation line at 633 nm, for dyes like **Cy5**.
- A violet laser diode (405 nm) provides excitation of UV dyes (**DAPI, Hoechst**).
- Simultaneous or sequential detection of emission spectra in one, two, or three separate photomultipliers (PMTs) with 12 bit (4096 gray levels) resolution. There is a fourth channel to capture non-confocal transmitted light images.

Applications:

High-resolution fluorescence and digital recording of the images from thick specimens (20-200 μm).

TABLE 1

EQUIPMENT & RESOURCES

METAMORPH / METAFUOR SOFTWARE-

(Three online and two offline licenses). The software allows the integration of a variety of hardware devices into a single, intuitive acquisition interface. It also provides image display and processing tools that are crucial for performing accurate image analysis. MetaMorph offers analysis tools to handle from simple intensity logging to advanced morphometry analysis, colocalization, FRET, 3D measurements. Moreover, MetaMorph also enable users to further automate acquisition, processing, and common analysis routines.

APPLICATIONS:

Brightness measurements, cell counting, cell cycle, cell migration, cell proliferation, cell viability, colocalization cytotoxicity and apoptosis, morphometry, motion analysis and particle tracking.

OTHER RESOURCES:

The facility also provides access to a tissue culture hood, freezer, refrigerators and a water-jacketed, 37° C, CO₂ incubators.

SERVICES

IMMUNOCYTOCHEMISTRY LABORATORY:

- Assistance in the development of protocols for new antibodies.
- Titration/optimization of antibody.
- Identification of potential sources of problems.

WIDEFIELD AND CONFOCAL MICROSCOPES:

- Image acquisition and image analysis for registered users by staff members.
- Training on the correct use of equipment and software for image acquisition and analysis at the OIF.

Widefield Fluorescence
Microscope Unit (WF)

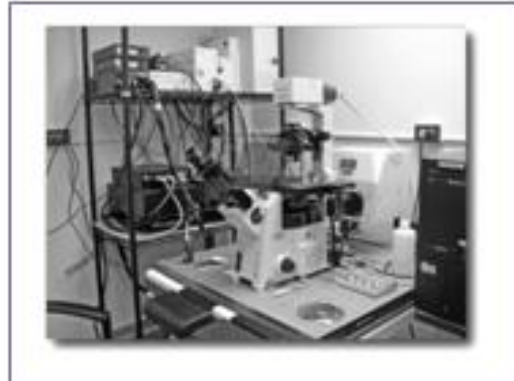


Olympus BH2 upright microscope



Olympus IX70 inverted microscope

Laser Scanning Confocal
Microscope Unit
(LSCM)



Olympus IX 81 microscope

Immunocytochemistry
Laboratory (ICCL)



REQUEST FORM FOR SERVICES OPTICAL IMAGING FACILITY

Universidad Central del Caribe, Bayamón, Puerto Rico

FIRST NAME _____

LAST NAME _____

Affiliation: University _____

Department _____

Position:

- _____ Faculty
- _____ Post-Doctoral student
- _____ Doctoral student
- _____ Master student
- _____ Medical student
- _____ Research Associate
- _____ Research specialist
- _____ Undergraduate student.

Phone _____ Ext _____

e-mai _____

Please select all of those that apply

SPECIMEN PROCESSING

- ___ Optimization of Primary and Secondary Antibodies
- ___ Optimization of tissue fixation
- ___ Cryostat in CIAS (basement)
- ___ Cryostat in CIAS (second floor)
- ___ Vibratome

CONSULTATION ON APPLICATIONS Yes ___ No ___

INSTRUMENT TRAINING Yes ___ No ___

INSTRUMENTATION USAGE Yes ___ No ___

- ___ Upright Widefield Fluorescence Microscope
- ___ Inverted Widefield Fluorescence Microscope
- ___ Laser Confocal Microscope

INSTRUMENTATION SERVICE

- ___ Upright Widefield Fluorescence Microscope
- ___ Inverted Widefield Fluorescence Microscope
- ___ Laser Confocal Microscope

IMAGE PROCESSING AND ANALYSIS MetaMmorph, ___ MetaFluor

Briefly explain the specific objective that you will expect to achieve with an imaging technology.

Title of Project _____

Funding support (Source and #) : _____

Terms and conditions:

- First time users will have to receive training only by OIF staff before attempting unassisted operation of OIF equipment.
- Equipment and service reservation is done on a first come first served basis.
- Sign-up for the instrumentation and services will be accessible through on-line scheduler. In the event of internet connection problems you may alternatively contact Katiria Colon, and Natalia Skachkova (OIF technicians) at 787-798-3001 x 2061, 2054 and 2101. All cancellations must be notified at least 24 hrs in advance in order to avoid charges.
- Experimental data on any OIF computer hard drives will not be backup and it is the user responsibility to remove it promptly after its acquisition.
- Each user is required to have his/her own data storage system and should include the transfer time to perform this activity in its scheduled equipment usage.
- Data acquisition must be saved in the identified User Files directory. Any data left on the equipment computer's desktop will be deleted without warning.
- No radioactive material or pathogens should be used in the facilities.
- Equipment should be cleaned and left in standard (default) configuration after each usage.

I agree to abide with the established terms and conditions to obtain access and services at the OIF.

I agree to include an acknowledgement to the RCMI supported OIF in presentations and publications that incorporate images and data acquired at this facility and will provide an electronic version of the article to the OIF Oadministration.

PRINTED NAME _____ **SIGNATURE** _____ **DATE** _____

OIF COST RECOVERY SYSTEM					
Activity	Training (per hour)	Equipment Use	Hourly Rate*	Semester Fee*	Annual Fee*
Live Cell Time Lapse	\$30.00	Assisted	\$35.00	\$1,200.00	\$1,800.00
		Unattended	\$25.00	\$780.00	\$1,200.00
Image Analysis	\$25.00	Assisted	\$15.00	\$500.00	\$600.00
		Unattended	\$10.00	\$350.00	\$350.00
Image Acquisition/ Processing	\$50.00	Assisted	\$55.00	\$1,800.00	\$2,800.00
		Unattended	\$40.00	\$1,000.00	\$1,500.00
Antibody signal optimization	\$30.00	Assisted	\$20.00	\$500.00	\$800.00

The funds would be used to cover expenses related to:

- Equipment maintenance (microscopes, CO₂ incubator, fluorometer, imaging computer, digital cameras, excitation devices, excitation and emission filter wheels)
- Replacement of supplies (excitation lamp, coverslides, culture slides, incubation chambers, chamber tubing, gas tanks, fluorescence filters, immersion oil, lens paper),
- Imaging software updates.
- Common reagents (calcium indicators, ionophores, ion solutions, alcohol)



UNIVERSIDAD CENTRAL DEL CARIBE, INC.

Fiscal Year 2010__

ACCOUNT SETUP FORM

OPTICAL IMAGING FACILITY (OIF)

TITLE OF ACCOUNT:

Explain the need for this account (supplemental information acceptable):

The OIF is designed to offer training, technical support and maintenance of a group of equipment needed to run applications that required microscope-based systems.

The revenue obtained from these services is intended to cover for replacement of supplies and equipment maintenance and service repairs.

Responsible Person:

Priscila Sanabria

Email:

psanabrir@gmail.com

Department:

Physiology

Phone:

797-798-3001 x 2053

Is there a limitation on type of expenses (please check)?

Yes

No

If yes, describe:

Duration:

Limited Term (less than 1 year)

Continuing (more than 1 year)

X

Estimated Total Revenue Budget \$

5,000

Total Expense Budget \$

5,000

Source of Funds (Must be Completed)

Training and
equipment use _____

***If the new account is institutionally funded please indicate which account will be funding it.**

***If the new account is non-institutionally funded please attach a budget detailing where revenue and expenditures should be allocated.**

Request Prepared by:

*Priscila
Sanabria*

Date:

October, 5, 2010

Responsible Person's Signature:

Date:

October, 11, 2010

Dean's Signature:

Date:

Approved by:

Dean of Administration Signature

Date:

TO BE COMPLETED BY FISCAL SERVICES:

SL Account Code: _____

GL Map Code: _____

Function: _____

Fiscal Services Approval: _____

Date: _____

