

## Abstract Submission

All abstracts should be within 200 words and have a title, authors' names (presenting author's name underlined) and place of work.

The content of the abstract should be described under Introduction, Methodology, Results and Conclusions.

The authors should indicate in the registration form whether they would prefer to make a Poster or Oral presentation.

The last date for submission of abstracts is August 31, 2011.

Only registered delegates will be allowed to present papers.

The hard copy of the Registration Form complete in all respects along with the Abstract (if applicable) and the registration fee (as crossed bank draft/cheque payable at par) should be sent directly at the following address. A soft copy of the Abstract should also be sent to: [ilatimc@gmail.com](mailto:ilatimc@gmail.com) with cc to [spuri\\_1111@yahoo.com](mailto:spuri_1111@yahoo.com)

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### Workshop Coordinator

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**THE  
CYTOMETRY  
Society - INDIA**

## 4<sup>th</sup> ANNUAL MEETING OF The Cytometry Society

**Symposium on Applications of Flow  
Cytometry in Biomedical Research**

**&**

**12<sup>th</sup> Indo-US Workshop on Flow  
Cytometry in Clinical Research**

**8-12<sup>th</sup> October 2011**

*Under the aegis  
of  
The Cytometry Society (India)  
at*



**Panjab University, Chandigarh**  
*in collaboration with*



**Post Graduate Institute of Medical  
Education and Research, Chandigarh**

## THE CONCEPT

Flow cytometry is based on detection and rapid analysis of cellular markers and constituents stained with a fluorochrome and excited by a laser beam. Besides large cells (e.g., algae, bacteria, yeast, plant and mammalian cells) laser flow cytometry is used for study and sorting of human chromosomes and cells labeled with nanoparticles and beads.

The important value of laser flow cytometry is its ability to make measurements on a large number of single cells within a short period of time (millions of cells per minute) and generating multiparametric data. The heterogeneity of cell populations can be revealed and different subsets of cells identified and quantified. Selected cell populations can also be electronically sorted for further study.

During the symposium organized under the aegis of The Cytometry Society of India, flow cytometry experts from India and abroad will review use of this methodology in biomedical research and in monitoring of patients.



Gandhi Bhawan  
Panjab University

## Workshop: Flow Cytometry in Clinical Research

The wet lab workshop to be held at Panjab University and SIMER, Chandigarh will present lectures, tutorials and wet labs to review and demonstrate the various flow cytometric methods for clinical research.

The description of wet-labs being offered along with the time duration involved is given below. Each participant will get to attend either Two full-day wet labs or one full-day, two half-day wet labs or four half-day wet labs and is expected to give his/her preference of the wet labs as detailed in the workshop application form. Kindly submit a brief biodata alongwith a paragraph giving reason for the choice of wet lab module(s). An expert committee will select the candidates for participation and only selected candidates will be asked to send the full registration fee for the wet workshop.

S. No	Wet-Lab	Time duration
WL1	Basics of Flow cytometry	Half day
WL2.	Primary Immunodeficiency Disorders	Half day
WL3.	Paroxysmal Nocturnal Haemoglobinuria	Half day
WL4	Characterization of Mesenchymal Stem Cells (MSC)	Half day
WL5.	CD34 cell count: ISHAGE protocol	Half day
WL6.	Hematopoietic stem cell: side population analysis	Half day
WL7.	Cell Signaling	Half day
WL8.	Cell Sorting	Full day
WL9	DNA ploidy, proliferation and marker expression in human solid tumors and cells from body cavity fluids	Full day
WL10	MRD analysis in acute lymphoblastic leukemia	Full day
WL11	Multicolor Immunophenotyping	Full day
WL12	Monitoring of Phagocytosis, Free Radical Generation and Apoptosis in Neutrophils	Full day
WL13	Intracellular cytokine staining and analysis	Full day

We look forward to register about 50 participants for the wet-lab workshop.

## INVITED FACULTY

- Awtar Krishan, Miami
  - H. Krishnamurthy, Bengaluru
  - Mitali Chatterjee, Kolkata
  - William Telford, Bethesda
  - Sumeet Gujral, Mumbai
  - Geoffrey W. Osborne, St. Lucia
  - Manisha Madkaikar, Mumbai
- Brent Wood, Seattle
  - Amar Dasgupta, Mumbai
  - Vivek Tanavde, Singapore
  - Arvinder Singh, Gurgaon
  - Paresh Jain, Gurgaon
  - Madhu Dikshit, Lucknow
  - Martine Adelman, Nyon

## REGISTRATION FEE

Registration and Tuition	Student	Faculty	Industry
Symposium only	₹ 1,500 <input type="checkbox"/>	₹ 3,000 <input type="checkbox"/>	₹ 5,000 <input type="checkbox"/>
Workshop only	₹ 10,000 <input type="checkbox"/>	₹ 10,000 <input type="checkbox"/>	₹ 15,000 <input type="checkbox"/>
Symposium & Workshop	₹ 10,500 <input type="checkbox"/>	₹ 12,000 <input type="checkbox"/>	₹ 18,000 <input type="checkbox"/>

## APPLICATION DEADLINE

For workshop : 31<sup>st</sup> August, 2011.  
Abstract submission : 31<sup>st</sup> August, 2011

## ACCOMMODATION

Panjab University Hostel : ₹ 1,500/day  
Hotel Accommodation : ₹ 3,000 onwards

An application form for the above can be downloaded from website, [www.cytometryworkshops.com](http://www.cytometryworkshops.com), and should be sent along with a two page biodata to the following

**Vice-Chancellor**  
Sector-14, Panjab University,  
Chandigarh-160014 INDIA

## REGISTRATION FORM

### FOR 4<sup>th</sup> ANNUAL MEETING OF TCS and Symposium on Application of Flow Cytometry in Biomedical Research

Panjab University, Chandigarh  
October 8-12, 2011

Full Name \_\_\_\_\_

Affiliation \_\_\_\_\_

City \_\_\_\_\_ Pincode \_\_\_\_\_

Email \_\_\_\_\_

Tel.(Office) \_\_\_\_\_ Mobile \_\_\_\_\_

Fax \_\_\_\_\_

Membership no. \_\_\_\_\_

Presenting paper: Yes\_\_\_/No\_\_\_;

If yes, Poster\_\_\_/Oral\_\_\_ presentation (tick one)

### Payment details

Cheque/D.D. No. \_\_\_\_\_

Date \_\_\_\_\_

Name of the bank & branch \_\_\_\_\_

Amount: \_\_\_\_\_

**APPLICATION FORM**  
**THE 12th INDO-US CYTOMETRY WORKSHOP**  
**10-12 October, 2011**  
**PGMIER, CHANDIGARH**

**Last Date of Submitting the Application: 31<sup>st</sup> August, 2011**

Please fill in all particulars and email the complete application form and enclosures to with copies to [skarora\\_in@yahoo.com](mailto:skarora_in@yahoo.com) and [spuri\\_1111@yahoo.com](mailto:spuri_1111@yahoo.com)

If selected, you will be informed by 10<sup>th</sup> September 2011 and will be asked to make the tuition remittance by 20<sup>th</sup> September 2011 to reserve your place.

**SURNAME:** \_\_\_\_\_ **FIRST NAME:** \_\_\_\_\_

Gender: \_\_\_\_\_ Highest Degree: \_\_\_\_\_

Place of Employment: \_\_\_\_\_

Mailing Address: \_\_\_\_\_

\_\_\_\_\_

Email: \_\_\_\_\_

Tel (Mobile): \_\_\_\_\_ Tel (Landline): \_\_\_\_\_

Accommodation Required: Yes / No

**Registration fee** Please tick the appropriate column

Registration and Tuition	Student	Faculty	Industry
Symposium only	₹ 1,500 <input type="checkbox"/>	₹ 3,000 <input type="checkbox"/>	₹ 5,000 <input type="checkbox"/>
Workshop only	₹ 10,000 <input type="checkbox"/>	₹ 10,000 <input type="checkbox"/>	₹ 15,000 <input type="checkbox"/>
Symposium & Workshop	₹ 10,500 <input type="checkbox"/>	₹ 12,000 <input type="checkbox"/>	₹ 18,000 <input type="checkbox"/>

**Enclosures** to be submitted along with the application form:

- Brief Bio-data (not more than two pages)
- A paragraph of about 150-300 words providing details of research interests and reasons for attending the workshop
- Wet Lab Preference Sheet

**Date:**  
**City:**

**Signature**

**WET LAB PREFERENCE SHEET**

(For any queries, please write to [skarora\\_in@yahoo.com](mailto:skarora_in@yahoo.com) and [spuri\\_1111@yahoo.com](mailto:spuri_1111@yahoo.com) )

An outline of the wet labs is enclosed in Appendix 1. Please select your preference on a scale of 1-10 for these wet labs.

Highest preference = Score 10; Lowest preference = Score 1

Each participant will get to attend either Two Full-day wet-labs OR One Full-Day, two-Half day wet labs OR Four Half-day wet labs.

**Note: Kindly list your preference score for all of the following wet labs**

S. No	Wet-Lab	Time duration
WL1	Basics of Flow cytometry	Half day
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WL13	Intracellular cytokine staining and analysis	Full day

Previous experience in flow cytometry (Please tick as appropriate)

- New user (Less than 6 months)
- 6-12 months
- 1-3 years
- More than 3 years

Have you attended a basic course in flow cytometry before? (Please tick as appropriate)

- No
- Yes (If yes, where \_\_\_\_\_)

**Date:**  
**City:**

**Signature**

## APPENDIX 1: OUTLINE OF THE WET LABS

### **Wet Lab 1: Basics of Flow Cytometry (Half day)**

**Faculty: H Krishnamurthy and Navya Jain**

To drive an automobile one need not know how to design an alternator but should understand if it's operating correctly and optimally. Likewise with a flow cytometer or cell sorter; to properly operate a cytometer and interpret data, one must understand its basic components and their operation in order to obtain valid and optimized data. The goal of this lecture is to familiarize those new to the field of flow cytometry with its basic components and their correct usage. The key components including fluidics, lasers, optics, electronic detectors, analog to digital converters and pulse processors will be described in sufficient detail to give the new operator/user a basic understanding. Likewise, the integration of all these components into a functional unit will be described and characterized.

### **Wet Lab 2: Primary Immunodeficiency Disorders (Half-day)**

**Faculty: Manisha Madkaikar and Biman Saikia**

Primary immunodeficiency disorders (PID) are the inherited disorders of immune system characterized by increased susceptibility to various infections, autoimmunity, allergies and malignancies. Flow cytometry offers a rapid, sensitive and reproducible tool for evaluation of different components of immune system and diagnosis of various PID. We will discuss laboratory approach and application of flow cytometry based assays for diagnosis of PID. The wet labs will include T, B and NK cell enumeration and their interpretation and measurement of neutrophil oxidative burst activity using Dihydrorhodamine (DHR) test.

### **Wet Lab 3: Paroxysmal Nocturnal Haemoglobinuria (Half-day)**

**Faculty: Manisha Madkaikar and Jasmina Ahluwalia**

Paroxysmal nocturnal haemoglobinuria (PNH) is a rare hematopoietic stem cell disorder characterized by a somatic mutation in the PIGA gene, leading to a deficiency of proteins linked to the cell membrane via glycosylphosphatidylinositol (GPI) anchors. Flow cytometry is the method of choice for identifying cells deficient in GPI-linked proteins and is, therefore, necessary for the diagnosis of PNH. In this half day workshop, we will discuss important issues like panel selection, sample processing, gating strategies and data interpretation for diagnosis of PNH. In the wet lab detection of PNH clone will be demonstrated on red blood cells using CD59/CD55 and on neutrophils using FLAER and CD66b respectively.

### **Wet Lab 4: Characterisation of Mesenchymal Stromal Cells (Half-day)**

**Faculty: Vivek Tanavde, Vivek Jha**

Mesenchymal Stromal Cells (MSC) have the potential to differentiate into a wide variety of tissues including bone, cartilage and fat. There are a variety of markers

available for enumerating MSC in culture. The International Society for Cellular Therapy (ISCT) developed a consensus definition for identifying cultured MSC. This definition has proven useful to define MSC used in cellular therapy protocols. Since the ISCT criteria for defining MSC measure the expression of multiple cell surface markers on single cells, multi parameter flow cytometry is a useful tool to identify MSC. MiltenyiBiotec has developed a flow cytometry based MSC phenotyping kit that identifies MSC fulfilling ISCT criteria on a 2 laser/4 color instrument. This wet lab will focus on identifying cultured MSC fulfilling the ISCT criteria using multi parameter flow cytometry.

### **Wet Lab 5: CD34+ cell enumeration: ISHAGE protocol (Half-day)**

**Faculty: Vivek Tanavde and Neelam Marwaha**

CD34 enumeration is a useful tool to enumerate the numbers of hematopoietic stem cells in bone marrow, cord blood or mobilized peripheral blood grafts used for transplantation. This lab will introduce the general principles involved in rare cell analysis, their application to CD34 enumeration and discuss the ISHAGE protocol which enumerates the numbers of viable CD34+ cells using single platform flow cytometry. Critical issues in this assay as outlined by Sutherland & Keeney will also be discussed.

### **Wet Lab 6: Hematopoetic Stem Cells: Side population assay (Half-day)**

**Faculty: William Telford and Neelam Marwaha**

Flow cytometry plays a critical role in the identification and characterization of mammalian and human stem cells. We will review recent advances in stem cell analysis by flow cytometry, including both traditional and novel cell surface phenotyping approaches; physiological characteristics such as ABC transporter activity using pump-specific efflux probes and pump-specific antibodies; and flow cytometric measurement of other stem cell-specific physiological characteristics, including aldehyde dehydrogenase activity. Modern application to stem cells and recent technological innovations will be emphasized. We will do either a Hoechst 33342 or DCV side population labeling of mouse bone marrow, combined with cell surface labeling with three to six markers, allowing identification of SP activity in both early hematopoetic stem cells (HSCs) and more committed common lymphoid progenitors (CLPs). We will use the resulting polycolor samples to demonstrate the acquisition and analysis of complex multicolor samples, particular detector setup and both real-time and post-acquisition compensation setup using both the BD DiVa software and third-party packages like FlowJo.

### **Wet Lab 7: Cell signaling (Half-day)**

**Faculty: Martin Aldelmann and Shipra Thukral**

This wet lab will describe the basic protocol of staining of intracellular phosphorylated molecules along with surface staining for characterization of particular subset of cells where the signalling is being studied. Routine fixation and permeabilization of tissue culture cells (anchorage independent cell lines) is

accomplished using formaldehyde fixation followed by permeabilization of cytoplasmic and nuclear membranes using absolute methanol. Various other possibilities of fixation and permeabilization will also be discussed.

### **Wet Lab 8: Cell Sorting (Full day)**

**Faculty: Geoff Osborne, TA Nagarjuna and Meenakshi**

This wet lab aims at introducing course participants to the theory and practice of electrostatic cell sorting, where one group or population of cells is physically separated from others for further downstream analysis or processing. We will take you through:

1. The concepts that are critical to understanding how the technology actually works.
2. Move to a “hands on” tour of the hardware (the “which bit does what” approach).
3. Apply what we have learned to this point to sort both beads and biological samples and verify the outcomes of these sorts.
4. Learn the basics of problem recognition and what steps we can take to address the most common problems involved in cell sorting.

### **Wet Lab 9: Solid tumors: DNA ploidy and Cell markers (Full day)**

**Faculty: Awtar Krishan, S Radhika, Rajendra Kumar**

Flow cytometry is a rapid method for monitoring of cellular DNA content, cell cycle distribution and cells in proliferation. In this lab module, we will prepare cells from tissue culture, tumors and paraffin embedded blocks for flow cytometric determination of DNA content and cell cycle phase distribution.

### **Wet Lab 10: MRD analysis in acute lymphoblastic leukemia (Full Day)**

**Faculty: Brent Wood, Sumeet Gujral, Neelam Varma**

Minimal residual disease (MRD) analysis by flow cytometry provides important prognostic information following chemotherapy in patients suffering from acute lymphoblastic leukemia. The flow-MRD assay is useful for post-therapy risk stratification and has potential to identify patients who may benefit from treatment intensification protocols. In this wet lab we will cover laboratory aspects of MRD analysis in ALL by flow cytometry including panel design, sample preparation, instrument setup and data analysis and reporting.

### **Wet Lab 11: Multicolor Immunophenotyping (Full Day)**

**Faculty: Paresh Jain and Amitav Mohanty**

Designing and performing a multicolor immunophenotyping experiment requires a sound understanding of instrument, reagent, sample processing, and data interpretation related variables. In this full-day laboratory, we will illustrate the importance of these variables through a mix of wet labs and tutorials. While the tutorials will cover grammar and vocabulary of building a multicolor panel and

experiment, the practical session will focus on instrument setup, compensation and FMO controls using a 8-color experiment designed to characterize circulating naïve, memory and regulatory CD4 T-cells in human peripheral blood.

### **Wet Lab 12: Monitoring of Phagocytosis, Free Radical Generation and Apoptosis in Neutrophils (Full Day)**

**Faculty: Madhu Dikshit and Sachin Kumar**

Neutrophils popularly known as polymorphonuclear leukocytes (PMNs/ Neutrophils) orchestrate the innate immunity and perform important roles in host defense. Circulating neutrophils recognize and kill the invading pathogens by chemotaxis, phagocytosis, and by generation of highly reactive oxygen species and release of microbicidal proteases. Neutrophils ultimately die by inevitable constitutive apoptotic process and subsequently get engulfed by macrophages to resolve the inflammatory response. Neutrophils thus need to be studied within a short period of collection and all the neutrophil activities listed above can be studied by laser flow cytometry and the use of fluorescent tags or probes. In this wet-lab we demonstrate the protocols that can be used for neutrophil phagocytosis, free radical generation and apoptosis.

### **Wet-Lab 13: Intracellular Cytokine staining (Full Day)**

**Faculty: Sunil K Arora and Jaideep S. Toor**

Estimating the profile of cytokines being expressed by a population of cells is a very important question being asked by researchers working in the field of immunology or clinical medicine. Staining and detection of cells based on phenotypic characteristics along with the cytokine being produced intracellular is technically demanding and requires dual staining of surface markers as well as making the cell membrane permeable to reagents for staining of particular cytokine inside the cells. In this one full- day workshop we envisage to demonstrate the various steps involved in isolation of cells, activation protocol, surface staining, permeabilisation and then intracellular staining followed by analysis and interpretation of results after acquisition in a flowcytometer. The tutorial and wet-lab will also be followed by a session on troubleshooting and problem solving.