Syllabus IMM203IH: Single Cell Resolution Assays in Immunology (Flow Cytometry)
Department of Immunology, University of Toronto

COURSE OUTLINE

Class Location: The course will be done in different rooms, please check below.

Class Time: January 12th – February 3rd, 2016

Professor/Coordinator: Dr. Juan Carlos Zuniga-Pflucker

Lecturer: Dr. Olga Rojas

Office Hours: Every day during the course**,** 12-1pm Room 7210, MSB.

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COURSE DESCRIPTION

The course aims at building basic knowledge in flow cytometry, from the experimental designs to data acquisition and analysis. The course plans to cover the various application of this technique and will include lectures, tutorials and practicum sessions.

The course will cover: instrumentation from basic design through performance evaluation to state-of-the-art innovations; data analysis from basic principles through advanced modeling methods; and applications in cell biology, biotechnology, immunology, cell signaling, fluorescent protein analysis, bead-based assays and cell sorting.

Laboratory sessions will address sample preparation, instrument features and operation, data collection and analysis in flow cytometry.

SPECIFIC LECTURE TOPICS

Lecture 1 - Introduction to Flow Cytometry - 2hrs:

- An overview of the history of flow cytometry (FC) and changes in the technology to the present time.
- How a flow cytometer works - Flow machines features and operation, fluidics, optical and electronic systems.
- Applications of FC in research, medicine, pharma, diagnostics (ie to convey the importance of the technology).
- Course policies, organization, grading, wet-lab sign-ups.

Lecture 2 - Multicolour Flow Cytometry - theory - 2hrs:

- When do you need multi-colour flow? - A case study in isolating specific cell populations based on iterative gating.
- Principals of light absorbance and emission for fluorochromes
- How to design a panel to interrogate specific cell populations including positive and negative controls.
- How to compensate.
- Data analysis boot camp

Lecture 3 - Other applications for Flow Cytometry 3hrs:

- Fixation choices.
- Fluorescence-based reporter systems

- Tetramers as reagents to identify antigen-specific lymphocytes.
- Transcription factors
- ICS
- Cell-sorting
- Bead-based assays
- Cell cycle/death analysis/proliferation.
- Measurement of mRNA by flow cytometry
- Phospho-flow

Lecture 4 - Other applications for Flow Cytometry: Mass Cytometry (CyTOF) 1:30hrs:

Cynthia Guidos

SPECIFIC PRACTICUMS

Practicum 1 - Introduction to Flow Cytometry

Pre-work:

- Online tutorials: BD Introduction to Flow Cytometry (to be completed prior to the first lecture): http://m.bdbiosciences.com/us/support/s/itf_launch (Check overview, fluidics, optics, electronics and optical measurements only, 1hr)

60 minute Tutorial section:

- Tutorial going through course expectations and outline as well as sign-ups for acquisition and analysis time.

Practicum 2 - Multicolour Flow Cytometry - theory

Pre-work:

- Invitrogen practical tutorials 1-4 (to be completed prior to the second lecture): http://www.thermofisher.com/ca/en/home/support/tutorials.html (40 mins).

Tutorial section:

- How to choose the right fluorochrome spectrum according to the FACS machine http://www.bdbiosciences.com/us/s/spectrumviewer
- Real-time panel design assisted by online tool: http://www.bdbiosciences.com/us/panelDesign

to be overseen by lecturer (ie, students bring their laptops).

Practicum 3 - Multicolour Flow Cytometry - surface and intracellular staining using reporter mice:

60 minute Tutorial section:

- Cell lineages in Lymph node will be briefly described (ie, lineage specific markers, concept of dump gates).
- How reporter mice are use by FACS will be explained
- Dissection of Lymph node (LN) will be explained.
- Preparation of single cell suspensions will be explained

- Staining procedure to be explained in detail.

Experimental section:

- Students will prepare LN single cell suspensions.
- Cells will be counted in order to tabulate absolute numbers.
- Cells will be stained to analyzed GL7, Fas, B220, CD138, live/dead plus expression of Blimp-1 YFP.

Practicum 4 - Multicolour Flow Cytometry - acquisition and analysis:

Pre-work:

- http://www.thermofisher.com/ca/en/home/support/tutorials.html (Tutorial 5 to be done prior to Practicum 4, 20 mins)

60 minute Tutorial section:

- Theory and practice of how to set up voltage parameters
- Walk them through an acquisition exercise.
- Talk about how to manage the machine while acquiring (things to watch out for).
- How to analyze and present data. Note they will prepare a lab report from this exercise, so it needs to be clear.

Experimental section:

- Acquire samples (pre-arranged time)
- Analyze samples (pre-arranged time)

Practicum 5 - Multicolour Flow Cytometry – sorting/Cell cycle and Proliferation Analysis

- *Pre-work:* Online tutorials: BD Introduction to Flow Cytometry (Analysis only to be completed prior to the first lecture):

  http://m.bdbiosciences.com/us/support/s/itf_launch (Check Sorting only, 20 mins)

Tutorial section: (we will use some case-based examples to develop in class)

- Why sort?
- How cells are prepared for cell sorting
- How the instrument works to sort highly purified cell populations
- Single cell sorting.
- How to QC final populations
- Methods of measuring cell cycle and different fixation parameters for each. Why choose one over the other
- Detailed overview of PI/7AAD staining protocol
- Principles of using proliferation protocols by FACS
- Overview of CFSE and KI67 protocols

EVALUATION

In Class Based Case Cytometry exercises:	20%
Lab report for Practicums 3 and 4	40%
Final Exam:	40%

ORGANIZATION OF PRACTICUMS

Each practicum will be preceded by a tutorial explaining the how the experiments are to be executed and some theory behind the choice of reagents/controls etc. The tutorials will be conducted by the course lecturer.

Students will be divided into groups of 2 for all lab work, however lab reports will be written individually. Students will be required to sign up for acquisition and analysis time for their flow cytometry experiments in the Faculty of Medicine flow cytometry facility. Practicums will be overseen by the lecturer, who will be available for technical assistance as necessary.

Although it would be ideal to have each of the flow cytometry reagents titrated as a separate experiment in the context of valid positive and negative controls, this is not practical within the course time frame and the recommended dilutions will be provided. In addition, the lecturer will be responsible for euthanizing the experimental animals to be used for the necropsies.

PREPARATION OF LAB REPORT

Lab reports must consist of:

- An introduction explaining the concept that will be tested with references/citations where appropriate
- A faithful narrative of the methods applied with all relevant details.
- A results section describing the data that were produced
- A discussion section describing interpretation of results and any unexpected findings/technical problems.

Lab reports will be graded with the following rubric:

Clarity and organization of report:	25%
Quality of data:	30%
Interpretation of data:	30%
Grammar/Writing quality:	15%

FINAL EXAM

The final exam will test the student's theoretical and practical knowledge of the course in short answer and multiple choice format as well as one long answer question.

GENERAL SCHEDULE

Session	Topic	Activities	Time
1	**Introduction to Flow Cytometry**	*Lecture 1:* 2hrs, 10am-12pm, MSB3290 *Practicum 1:* 2hrs, 1pm-3pm, MSB3290 • 60 minute tutorial by the lecturer on course design and scheduling of acquisition and analysis time, to be handled during this period. • http://www.bdbiosciences.com/ca/services/training/itf_launch.jsp (to be completed prior to the first lecture on one's own, 1hr)	**Week 1, Day 1;** **4 hours** **Tues, Jan 12**
2	**Multicolour Flow Cytometry - theory**	*Lecture 2:* 2hrs, 10am-12pm, MSB 3290 *Practicum 2: 1-5* 4hrs, 1pm-5pm, MSB 3290 • http://www.thermofisher.com/ca/en/home/support/tutorials.html (Tutorials 1-4 to be done prior to Practicum 2, 40 mins) • How to choose the right fluorochrome spectrum according to the FACS machine http://www.bdbiosciences.com/us/s/spectrumviewer • Real-time panel design assisted by online tool: http://www.bdbiosciences.com/us/panelDesign to be overseen by lecturer (ie, students bring their laptops).	**Week 1, Day 2;** **6 hours** **Wed, Jan 13**
3	**Multicolour Flow Cytometry - surface and intracellular staining using reporter mice**	*Practicum 3:* 7hrs, 10am-5pm, Flow Cytometry Facility MSB7226 • 60 minute tutorial on cell preparation • Cell preparation (dissection, preparing single cell suspensions) • Staining and counting	**Week 2 - Day1;** **8 hours** **Tuesday, Jan 19**
4	**Multicolour Flow Cytometry - intracellular cytokine staining**	*Practicum 4:* 1hr, 9am-10am, MSB 3290 2hrs, 10am-12pm, Flow Cytometry Facility MSB7226 3hrs, 1pm-4pm, MSB 3290 • http://www.thermofisher.com/ca/en/home/support/tutorials.html (Tutorial 5 to be done prior to Practicum 4) • 60 minute tutorial on optimizing voltages • Acquisition and analysis	**Week 2 - Day2;** **6 hours** **Wed, Jan 20**

5	**Multicolour Flow Cytometry – sorting/ Cell cycle and proliferation analysis**	*Practicum 5:* 4hrs, 1pm-5pm, MSB 2290 • *Pre-work:* Online tutorials: BD Introduction to Flow Cytometry (Analysis only to be completed prior to the first lecture): http://m.bdbiosciences.com/us/support/s/itf_launch (Check Sorting only, 20 mins) *Tutorial section:* • Why sort? • How cells are prepared for cell sorting • How the instrument works to sort highly purified cell populations • Single cell sorting. • How to QC final populations • Methods of measuring cell cycle and different fixation parameters for each. Why choose one over the other • Detailed overview of PI/7AAD staining protocol • Principles of using proliferation protocols by FACS • Overview of CFSE and KI67 protocols	**Week 3 - Day 1;** **4 hours** **Tues, Jan 26** **1-5 Tutorial comp rm**
7	**Other applications for Flow Cytometry**	*Lecture 3:* 3hrs, 1pm-4pm, MSB 7231	**Week 3, Day 2;** **3 hours** **Wed, Jan 27** **1-4**
8	**CyTOF**	*Lecture 4:* 1:30hrs, 10:30am – 12pm, MSB 7231	**Thursday 28** **10:30-12**
8	**Final Exam**	Administered and graded by lecturer. Room : TBD	**Week 4;** **2 hours.** **Wed, Feb 3**