Optical -Omics and Single-Molecule Imaging

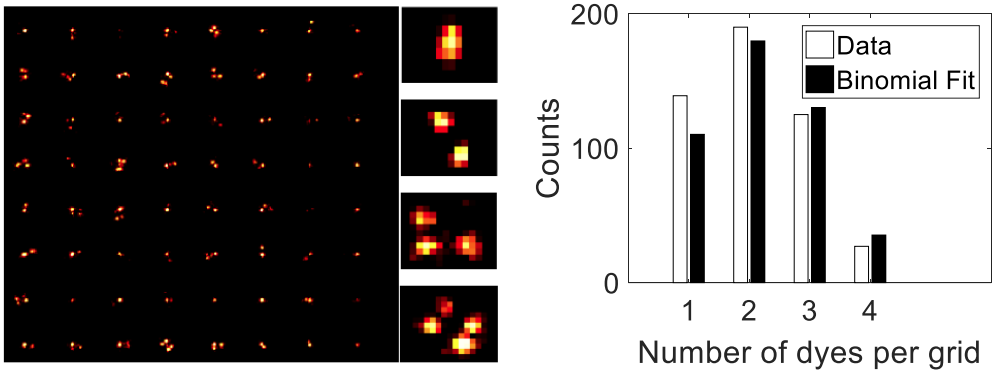
Milstein Lab USRA Project, Summer 2022

Overview:

We have a position available to a Physics student with a keen interest in Biological Physics. Over the years, we have had dozens of undergraduates work in the lab on a diverse array of projects. Many of them have wound up as authors on published manuscripts and have gone on to graduate school. This year, we have an opening for a Physics student to work on optical -omics and single-molecule imaging.

The use of advanced optical imaging techniques to quantify proteins or nucleic acids is termed 'optical proteomics' or 'optical genomics', respectively. Already, optical proteomics have been employed to probe complex protein networks involved in cancer formation. A single-molecule, fluorescence-based approach to molecular counting would be particularly powerful in single-cell, optical -omics applications where trace amounts of protein, DNA, or RNA, must be detected, and at spatial scales below the resolution limit of light microscopy. Single-molecule microscopy has the potential to significantly accelerate the optical -omics fields, leading to the development of new platforms for the high-throughput characterization and identification of proteins and nucleic acids at ultra-low concentrations *in vitro* or in single-cells. While currently confined to the research laboratory, as these techniques mature, we may see them have a significant impact on applications such as drug-screening and medical diagnostics.

The Milstein Lab has been working on applying statistical / machine learning based methods to extract accurate counts from single-molecule microscopy data in the hopes that the technique becomes the future gold standard for counting molecules.

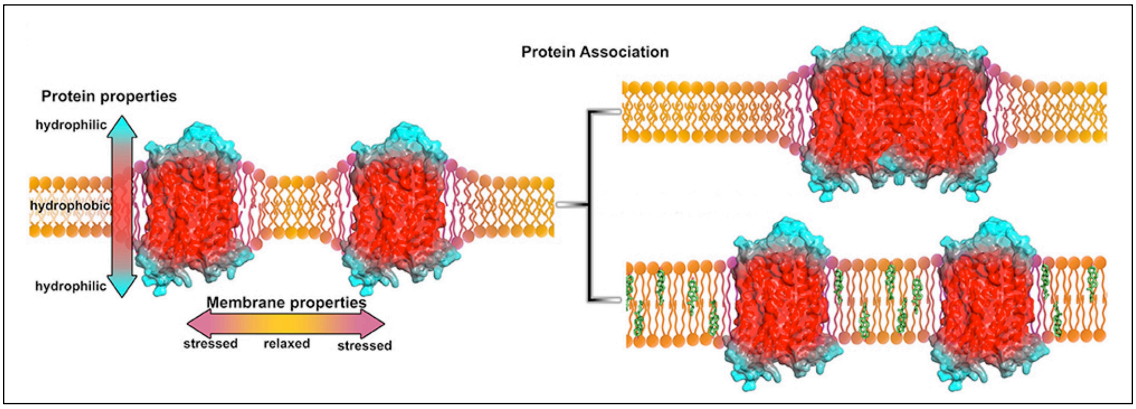


Left: Array of nanofabricated DNA origami grids displaying between 1-4 fluorescent labels. Arrays serve as a benchmark mimicking protein stoichiometry. *Right:* Histogram quantifying the distribution of labels.

Background Requirements:

No previous experience in Biology is necessary, but a solid foundation in physics is required. An ability to code in Python, or similar, is a plus. These projects are highly interdisciplinary, so expect to

work on a diverse array of challenges. All students will need to take the requisite biological and laser safety training.



Project Details:

We are currently applying quantitative single-molecule imaging to study an important class of membrane receptors known as G protein coupled receptors (GPCRs). GPCRs are an important drug target, >40% of all pharmaceuticals target GPCRs, and are associated with a range of maladies from heart disease to neurodegenerative disorders. GPCRs are signalling proteins that will often cluster into groups, and with other molecules. The purpose of this behavior is highly controversial in the field, and single-molecule imaging would provide a powerful new approach to understanding it.

Dependent upon the student, they may be involved in cell culturing and labeling, cell preparation for imaging, image acquisition, and optical alignment. They may also be involved in developing the data acquisition and image analytics pipeline, or in extending the statistical modeling that goes into extracting quantitative features from these images.

Note, the Milstein Lab is at the University of Toronto Mississauga campus. Student's will spend a good deal of time in the lab, so should either live nearby or on campus for the summer, or be willing to commute.

For more information about the Milstein Lab, please visit: https://www.utm.utoronto.ca/milsteinlab/

Recent Publications Involving Undergraduates (bold indicates undergrad):

An Expectation-Maximization Approach to Quantifying Protein Stoichiometry with Single-Molecule Imaging, **A. Boonkird**[1], D. F. Nino and J. N. Milstein (currently online at *Bioinformatics Advances*, 2021).

An Ultra-Stable and Dense Single-Molecule Click Platform for Sensing Protein-Deoxyribonucleic Acid Interactions, E. Visser, **J. Miladinovic**[2] and J. N. Milstein, *Small Methods* 5, 2001180, (2021).

FOCAL3D: A 3-dimensional clustering package for single-molecule localization microscopy, D. Nino, **D. Djayakarsana**[3] and J. N. Milstein, *PLoS Computational Biology* 16(12): e1008479 (2020).

[1]Current Student, University of Toronto Excellence Award (UTEA)
[2]Now at University of Saskatchewan, Medical School, UTEA
[3]Now at University of Toronto, Medical Biophysics Program, NSERC USRA