23-30 October 2022

Lundberg Lab Symposium

Program

Stanford University

KTH VETENSKAP OCH KONST

Retreat Program

Sunday, 23 October 2022

15:00 - 18:00 Arrivals, Transportation to Best Western Plus Riviera
18:00 Optional informal dinners & gatherings in downtown Palo Alto

Monday, 24 October 2022

8:20 - 9:00 Walk to Shriram
9:00 - 11:30 Treasure-Hunt Tour of Stanford Campus by Trang
11:30 - 12:00 Tour of Shriram Center
12:00 - 13:30 LUNCH SESSION 1: “How to best use organoids in large-scale screenings to study cell states, heterogeneity, and cell organization.”

Catered by Rose International Market
Chair: Frida Björklund (PhD Student, Lundberg Lab)
Location: Shriram 262

SPEAKER: Dr. Calvin Kuo (Professor, Stanford University), 45 min
Title: TBD
LIGHTNING TALK, 5 min: Jan Hansen
Title: Shedding light on the function and mechanisms of cilia in cells and tissues
LIGHTNING TALK, 5 min: Frida Björklund
Title: Multiplexed imaging to study cell type organization in situ
LIGHTNING TALK, 5 min: Christian Gnann
Title: Studying metabolic cell states using the Human Protein Atlas workflow
BRAINSTORM SESSION, 30 min
13:30 - 16:30 Akoya visit, project meetings, or internal catch-up
16:30 Barbecue at Emma's (612 Alvarado Row)

Tuesday, 25 October 2022

7:30 - 8:20 Pick up cars at Enterprise (Emmie, Anthony, and a third driver)
8:20 - 9:00 Walk to Shriram
9:00 - 9:20 Coffee and Bagels with the Tony Wyss-Coray lab
9:20 - 11:00 SESSION 2: “How we can use spatial proteomics to understand cognitive decline”

Chair: Jan Hansen (Postdoctoral Scholar, Lundberg Lab)
Location: Shriram 262

INVITED LAB: Tony Wyss-Coray (Professor, Stanford University)
SHORT TALK, 10 min, 5 min Q/A: Sophia Shi (Wyss-Coray Lab)
Title: Vascular glycocalyx degeneration in the aging brain
SHORT TALK, 10 min, 5 min Q/A: Hamilton Se-Hwee Oh (Grad student, Wyss-Coray Lab)
Title: Organ-specific aging signatures in the plasma proteome track health and disease
SHORT TALK, 10 min, 5 min Q/A: Tal Iram (Wyss-Coray Lab)
Title: Novel bio-orthogonal mouse models for labeling of cell-specific proteomes in vivo
SHORT TALK, 5 min, 5 min Q/A: Jan Hansen (Postdoc, Lundberg Lab)
Title: Studying morphology, motility, distribution and interaction of microglia (and other cells) in 3D images
SHORT TALK (REMOTE), 10 min, 5 min Q/A: Mariya Mardamshina (Postdoc, Lundberg Lab)
Title: Building a foundation for large-scale Deep Visual Proteomics
SHORT TALK, 10 min, 5 min Q/A: Anthony Joseph Cesnik (Postdoc, Lundberg Lab)
Title: Deciphering hierarchical cell cycle controls by near-saturation phosphoproteomics
BRAINSTORM SESSION, 10 min

11:00 - 11:30 Break

11:30 - 14:00 SESSION 3: Micronuclei

Catered by Anthony Cesnik & Jeremy Rice (Lundberg Lab)
Chairs: Emmie Pohjanen (PhD Student, Lundberg Lab)
Location: Shriram 262

SPEAKER: Howard Chang (Professor, Stanford University), 45 min
SPEAKER: Paul Mischel (Professor, Stanford University), 45 min
LIGHTNING TALK, 10 min: Emmie Pohjanen
Title: Mapping the proteome of micronuclei reveals a diverse set of functions
LIGHTNING TALK, 5 min: Christian Gnann
Title: Enzyme spatiotemporal heterogeneity establishes functional cellular states
BRAINSTORM SESSION, 30 min
14:00 - 15:00 Project Meetings
15:00 - 17:00 Hike the Stanford Dish Trail
17:00 - 17:40 Transportation to dinner at AJITO Sushi in Cupertino
17:40 - 19:00 Dinner at AJITO Sushi
19:00 - 19:30 Transportation to Music Tunnel Karaoke
19:30 - 22:30 Evening activity, karaoke at Music Tunnel Karaoke

Wednesday, 26 October 2022

8:00 - 8:45 Transportation by vans to CZ Biohub
8:45 - 9:00 Arrive and get settled at CZ Biohub, 2nd floor
9:00 - 12:00 SESSION 4: Mapping proteins in tissue and developmental contexts
Chair: Trang Le (PhD Student, Lundberg Lab)
CZ BIOHUB WELCOME, 10 min: by Shalin Mehta
SPEAKER, 25 min, 10 min Q/A: Dr. Shalin Mehta (Platform Leader, Imaging)
Title: [computational microscopy]
SHORT TALK, 10 min, 5 min Q/A: Trang Le (PhD Student, Stanford University)
Topic: Single cell localization classification & beyond
SPEAKER, 25 min, 10 min Q/A: Dr. Loïc Royer (Group Leader, Quantitative Cell Science, Organismal Architecture)
Title: Multimodal Atlas of Vertebrate Development
SHORT TALK, 10 min, 5 min Q/A: Sanem Sariyar (PhD Student, KTH Royal Inst. of Tech.)
Topic: High-parametric protein maps reveal the spatial organization in developing human lung
Break, 20 min
SHORT TALK, 10 min, 5 min Q/A: Dr. Jan Hansen (Postdoc, KTH Royal Inst of Tech)
Topic: Ciliary imaging and analysis
SPEAKER, 25 min, 10 min Q/A: Dr. Adrian Jacobo (Group Leader, Quantitative Cell Science)
Title: [tissue architecture]
GENERAL DISCUSSION & BRAINSTORMING, 15 min
12:00 - 13:00 Lunch at CZ Biohub, 4th floor
13:00 - 13:30 Tour of CZ Biohub imaging facilities
13:30 - 16:45 SESSION 5: Dissecting cell types, subcellular protein localizations, and infection biology
Chair: Christian Gnann (PhD Student, Lundberg Lab)
SPEAKER, 25 min, 10 min Q/A: Dr. Keir Balla (Group Leader, Infectious Disease)
Title: [zebrafish infection biology]
SHORT TALK, 10 min, 5 min Q/A: Dr. Charlotte Stadler
Topic: Spatial proteomics of Covid19 infected cells
SPEAKER, 25 min, 10 min Q/A: Dr. Manuel Leonetti (Group Leader, Quantitative Cell Science)
Title: [opencell]
SHORT TALK, 15 min, 5 min Q/A: Ulrika Axelsson
Topic: Current and future work of the Human Protein Atlas
Break, 20 min
SHORT TALK, 5 min, 5 min Q/A: Dr. Anna Martinez-Casals
Topic: Memento: web application for annotation and sharing images
SHORT TALK, 5 min, 5 min Q/A: Dr. Frederic Ballllosera Navarro
Topic: PIPEX: application for analysis of highly multiplexed image-data
SPEAKER, 25 min, 10 min Q/A: Dr. Angela Pisco (Associate Director, Data Science)
Title: Tools for Atlas
GENERAL DISCUSSION & BRAINSTORMING, 30 min

17:30 Walking tour through SF: Chinatown - North Beach - Coit Tower
Dinner in SF
Asynchronous transport back to Stanford

Thursday, 27 October 2022

8:20 - 9:00 Walk to Shriram
9:00 - 11:30 Free time for internal meetings
11:30 - 13:00
SESSION 7: “How to measure and build models of spatiotemporal interactomes?”
Catered by Bill’s Barbecue
Chair: Nicolai Dorka (PhD Student, Lundberg lab), Jan Hansen (Postdoc, Lundberg lab), Frida Björklund (PhD Student, Lundberg Lab)
Location: Y2E2 300
INVITED LAB: Alice Ting (Professor, Stanford University), 45 min
FLASH TALK, 6 min, 5 min Q/A
Title: "Mapping proteome trafficking in living cells via tandem proximity labeling", Wei Qin
FLASH TALK, 6 min, 5 min Q/A
Title: "Engineering reversible light regulation in TurboID for precise spatiotemporal control of proximity labeling", Joleen Shangyu Cheah
FLASH TALK, 6 min, 5 min Q/A
Title: “Laccase: a new enzyme for cell surface proximity labeling”, Song-Yi Lee
FLASH TALK, 6 min, 5 min Q/A: Nicolai Dorka
Title: “Combining Deep Visual proteomics with multiplexed IF to reveal interactomic insights”
FLASH TALK, 6 min, 5 min Q/A
Title: “Mapping cell structure across scales by fusing protein images and interactions”, Casper Winsnes
FLASH TALK, 6 min, 5 min Q/A
Title: “Studying the function and mechanisms of primary motile cilia - from cells to tissues and whole organisms”, Jan Hansen
BRAINSTORM SESSION, 30 min
13:00 - 13:15 Break / Clear Meeting Room
13:15 - 14:00 Transportation to Fogarty Winery
14:00 - 17:00 Wine tasting at Fogarty Winery

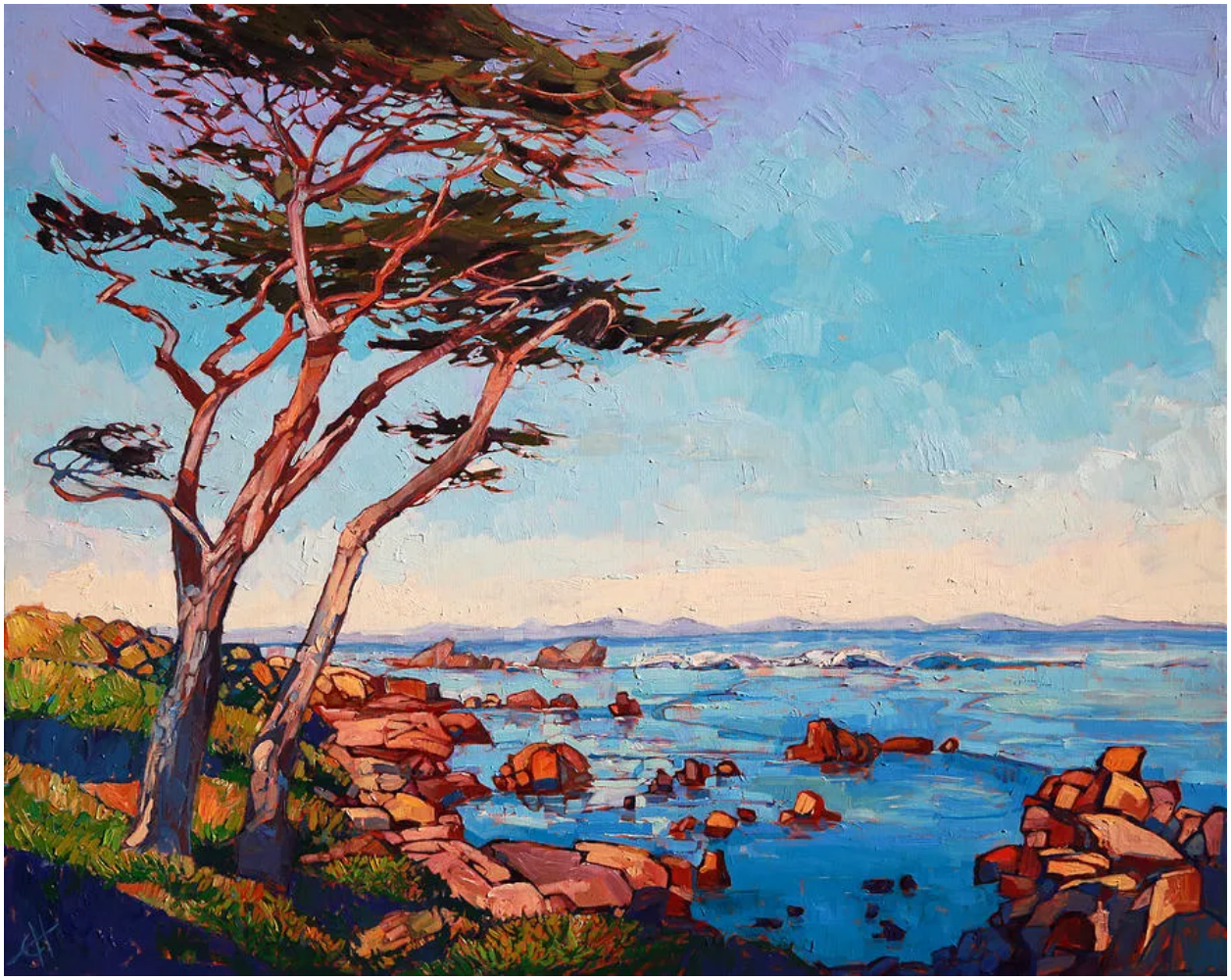

STANFORD NEUROSCIENCES BUILDING

Friday, 28 October 2022

7:30 - 9:00 Transportation to Monterey
9:00 - 9:30 Arrive and drop off luggage at Monterey Tides
9:30 - 12:00 SESSION 8: Research update, live from Stockholm
Virtual Chair: Sanem Sariyar
Titles & Abstracts: Please see "Lundberg Lab Research Presentations"
LAB VISION & WELCOME, 10 min, 5 min discussion: Emma Lundberg
9:45 (EU 18:45) VIRTUAL TALK, 15 min, 5 min Q/A: Burcu Ayoglu
10:05 (EU 19:05) VIRTUAL TALK, 15 min, 5 min Q/A: Sanem Sariyar
10:25 (EU 19:25) VIRTUAL TALK, 15 min, 5 min Q/A: Kalliopi Tzavlaki
10:45 (EU 19:45) *Break, 15 min*
Virtual Chair: Mariya Mardamshina
11:00 (EU 20:00) RECORDED TALK, 15 min, 5 min Q/A: Eleanor O'Brien & Pranauti Panshikar
11:20 (EU 20:20) RECORDED TALK, 15 min, 5 min Q/A: Carolina Oses & Tony Ullman
11:40 (EU 20:40) VIRTUAL TALK, 15 min, 5 min Q/A: Mariya Mardamshina
12:00 - 13:30 Lunch at Cannery Row
13:30 - 15:30 Monterey Bay Aquarium
16:30 - 17:00 Check in to rooms at Monterey Tides
18:00 - 19:30 SESSION 9: Multiplexing, microdissecting, and microscopic structures
Chair: Anna Martinez Casals
Titles & Abstracts: Please see "Lundberg Lab Research Presentations"
SHORT TALK, 15 min, 10 min Q/A: Jan Hansen
SHORT TALK, 15 min, 10 min Q/A: Christian Gnann
SHORT TALK, 15 min, 10 min Q/A: Nicolai Dorka
19:30 - 20:15 *Dinner at Monterey Tides*
20:15 Beach campfire

Saturday, 29 October 2022

7:00 - 9:00 Breakfast available at Monterey Tides
9:00 - 10:50 SESSION 10: Visions, instruments, and software for spatial proteomics
Chair: Charlotte Stadler
Titles & Abstracts: Please see "Lundberg Lab Research Presentations"
SHORT TALK, 15 min, 10 min Q/A: Charlotte Stadler
SHORT TALK, 15 min, 10 min Q/A: Anna Bäckstöm
SHORT TALK, 15 min, 10 min Q/A: Ulrika Axelsson
SHORT TALK, 15 min, 10 min Q/A: Frederic Ballllosera Navarro
Break, 20 min
11:10 - 12:50 LUNCH SESSION 11: Deciphering heterogeneity in pancreas, micronuclei, and the cell cycle
Lunch delivery: Taqueria Super Pollo
Chair: Anthony Cesnik
Titles & Abstracts: Please see "Lundberg Lab Research Presentations"
SHORT TALK, 15 min, 10 min Q/A: Anna Martinez-Casals
SHORT TALK, 15 min, 10 min Q/A: Emmie Pohjanen
SHORT TALK, 15 min, 10 min Q/A: Anthony Cesnik
SHORT TALK, 15 min, 10 min Q/A: Frida Björklund
12:50 - 15:00 Beach activities
15:00 - 17:10 SESSION 12: Cell shape, image analysis, and whole-cell modeling
Chair: Wei Ouyang
Titles & Abstracts: Please see "Lundberg Lab Research Presentations"
SHORT TALK, 15 min, 10 min Q/A: Casper Winsnes
SHORT TALK, 15 min, 10 min Q/A: Wei Ouyang
SHORT TALK, 15 min, 10 min Q/A: Ishan Gaur
SHORT TALK, 15 min, 10 min Q/A: Trang Le
17:30 - 19:00 *Dinner delivery (Osteria Al Mare) & sunset views at Monterey Tides*
19:00 - 21:00 Escape room 831, 599 Lighthouse Ave, Monterey, CA
21:00 Pumpkin carving & beach campfire



Sunday, 30 October 2022

9:00	Breakfast at hotel, including CLOSING REMARKS, 10 min: Emma Lundberg
11:00	Check out of Monterey Tides
12:00	Hike at Año Nuevo State Park
16:00	Transportation to SFO

Symposium Host

Emma Lundberg

Associate Professor of Bioengineering & Pathology, Stanford University
Professor of Protein Science, KTH Royal Institute of Technology

Invited Speakers & Groups

Russ Altman

Professor, Stanford University

Keir Balla

Group Leader, Infectious Disease

Howard Chang

Professor, Stanford University

Tony Wyss-Coray

Professor, Stanford University

Adrian Jacobo

Group Leader, Quantitative Cell Science

Calvin Kuo

Professor, Stanford University

Manuel Leonetti

Group Leader, Quantitative Cell Science

Shalin Mehta

Platform Leader, Imaging, Chan-Zuckerberg Biohub

Paul Mischel

Professor, Stanford University

Angela Pisco

Associate Director, Data Science

Loïc Royer

Group Leader, Quantitative Cell Science, Organismal Architecture, Chan-Zuckerberg Biohub

Alice Ting

Professor, Stanford University

Lundberg Lab Research Presentations

Ulrika Axelsson

Researcher, SciLifeLab & KTH Royal Institute of Technology

A guide to LIMS states and scores

One of the most common questions that I get, or at least one of the most common topics of discussions that I have with people in our research group, is about the different states and reliability scores that exist in our LIMS. For this session, I will do my best at guiding you through the somewhat overwhelming stages of the pipeline as well as the scoring for antibodies in the subcellular section. We have sent more than 40 000 antibodies on this winding journey, and now it is your turn to follow. The goal is to provide some insight to the meaning of different stages and scores in LIMS, and to help you understand which ones you need to pay attention to when working with the huge ICC-IF data set in your research projects.

Burcu Ayoglu

Researcher, SciLifeLab & KTH Royal Institute of Technology

Protein mapping studies aiming to shed light on human diseases and early human life

Our understanding of how different organs and systems in the human body develop is relatively limited on a molecular and cellular level. This early phase of human life is not only interesting to explore from a basic science perspective, but it also has tremendous implications for several adult-onset diseases. In this talk, I will mainly introduce some on-going and planned studies where we generate protein-based knowledge on the dynamics of immune adaptations taking place in mother's body over the course of early pregnancy; when and in which fetal organs human immune system cells and other cell types reside before birth; and how early life environmental exposures such as systemic infections might affect normal organ development and thus predispose for adult-onset diseases. In addition to these projects exploring early human life, I will also introduce a few new projects starting in 2023, where we leverage our spatial protein mapping power to validate potential biomarkers e.g. in neurodegenerative and fibrotic disorders.

Frederic Ballllosera Navarro

Research Engineer, Stanford University

A general image repository infrastructure for the group

The current lab capabilities to store, share and re-utilize the images of the different researchers are not satisfactory. After trying different incomplete solutions over the years, mainly focused in limited personal storage space, we need a more robust, scalable and generalistic approach. The new image vault project (preliminarily code-named "Bifröst") shall also outgrow the basic concept of file manager by offering a tag-based search engine and enhance the sharing possibilities of the data generated by the group. Designed to be interoperable through different entry points, Bifröst ambition is to grant image access to present (memento, PIPEX) and future platforms (HPA-like projects), becoming the gateway to the lab knowledge riches.

Frida Björklund

PhD Student, SciLifeLab & KTH Royal Institute of Technology

Mapping spatiotemporal protein variations in human pancreatic islets

Pancreatic islets are composed of specialized endocrine cells that secrete hormones that are essential for blood-glucose homeostasis. Emerging evidence suggests that islet cells are heterogeneous in both their gene expression and functionality. However, it is not yet understood how the transcriptional, proteomic, and functional heterogeneity correlates to normal islet cell function and dysfunction in diabetes pathogenesis. Characterization of heterogeneity in islet cells is being aided by the integration of several different technologies, one of which is patch-seq which combines electrophysiological measurements of exocytosis and channel activity with single-cell RNA sequencing. This study resulted in the identification of a subset of genes correlated with functional activity and heterogeneity in human nondiabetic and diabetic islet cells. To investigate what proteomic markers define islet cell heterogeneity and its relation to transcriptomic profiles, multiplex antibody-based approaches are being used to map islet cell heterogeneity and the islet microenvironment. In this presentation, I will discuss some preliminary results from annotated IHC images of nondiabetic patients from the Human Protein Tissue Atlas, relating proteomic and transcriptomic profiles.

Anna Bäckström

Research engineer, Spatial Proteomics Facility, SciLifeLab & KTH Royal Institute of Technology

The COMET instrument in general and a 10-plex antibody panel on a Swiss roll in particular

“Rapid hyperplex made easy” is how Lunaphore sums up the Comet instrument. It is an all-in-one staining and imaging platform for hyper-plex immunofluorescence, where the key component is a microfluidic chip. Thanks to the microfluidic technology of the chip the molecule deposition on the sample depends on convection rather than solely on diffusion. This in turn allows rapid delivery to the sample and short and efficient incubations of the reagents and buffers. The Comet instrument operates in cycles of staining, image acquisition and elution. Two primary antibodies of different species can be added in each cycle and there are 20 spaces for primary antibodies i.e. possible to run a 40-plex protocol.

In the Spatial Proteomics Facility we have just finished a project with a user who is studying estrogen receptor beta (ERβ) in the colon. The user hypothesizes that intestinal ERβ affects the immune cell landscape during inflammation and carcinogenesis and that it works to impede the initiation and progression of colorectal cancer. Together with the user we set up a Comet experiment including a 10-plex panel of primary antibodies on FFPE (Formalin-Fixed Paraffin-Embedded) mouse colon, shaped as a Swiss roll. The abundances and compositions of infiltrating immune cell populations will be analysed and compared between untreated mice and mice with induced colonic inflammation. The results are not yet evaluated but in my presentation I will show some examples of healthy and inflamed samples.

Anthony Cesnik

Postdoctoral Scholar, Stanford University

Deciphering hierarchical cell cycle controls by near-saturation phosphoproteomics

The cycle of cellular division is a tightly coordinated process that involves the cyclical expression of thousands of genes, proteins, and post-translational modifications (PTMs). Recently, we identified hundreds of proteins with newly identified associations to the cell cycle by interrogating the expression of these proteins on the single-cell level. We showed that a large majority of these novel proteins were not regulated at the level of transcript expression, but instead found indications that they are regulated post-translationally, such as by phosphorylation. In this study, we perform a deep phosphoproteomic study of the interphase between cellular divisions that achieves near saturation. This additional depth of phosphoproteomic analysis is achieved by a method to boost the signal for most phosphopeptides and that also allows a rough estimation of the occupancy of phosphosites. These rough estimates importantly allow the prioritization of sites with high estimated occupancy for follow-up experiments. These results have allowed us to show an expanded network of cell cycle regulation beyond that of transcript and protein expression.

Phosphoproteomics often does not reach saturation, due to there being many low-occupancy phosphorylation sites. In this study, we achieve the deepest phosphorylation study to date by quantifying over 77,000 phosphosites, which even exceeds a recent metaanalysis of 17 phosphoproteomic experiments for HeLa cells (around 60,000 phosphosites), including the deepest analysis to date, which alone had over 50,000 phosphosites. In a single experiment, we observed nearly the same number of phosphosites as the around 90,000 observed for many human sample types over 575 days of instrument time in that metaanalysis.

Nicolai Dorka

PhD Student, SciLifeLab & KTH Royal Institute of Technology
January 2023: PhD Student, Stanford University

Deep proteomic profiling of healthy human pancreatic islet cells by combining multiplexed imaging with Deep Visual Proteomics (DVP)

Mapping the proteomic profile of human pancreatic islet cells on a single cell level facilitates a deeper understanding of spatial proteomic islet composition and their phenotypic functionality. Here we integrate CODEX ® (CO-Detection by indEXing) by Akoya Bioscience, enabling highly multiplexed imaging, into Deep Visual Proteomics (DVP).

This workflow combines the spatial dimension with the resolution of ultra-high sensitivity mass spectrometry using deep learning driven image processing and single cell laser dissection. By applying multiplexed immunostaining on healthy human pancreas, we aim for advanced clustering of complex phenotypes that reveal deeper insights into the cell type specific proteomic signatures of pancreatic islets.

Trang Le

PhD Student, Bioengineering Department, Stanford University

Mapping of subcellular structures into cell shape

Studying protein expression, localization and interaction is fundamental to achieving a mechanistic understanding of a functioning cell at the systems level. Previously, we were able to predict protein locations by neural network models that can classify protein patterns at the single-cell level. The next step is viewing the proteins in the context of a cell. All human cells have a specific shape and organization which allow for their defined function. In fact, precise control of the specific protein's distribution is essential for many biological processes. An example of which are the cell cycle markers, which we know can be predicted (in both intensity and locations) by DNN trained on cell morphology alone (unpublished work). Therefore, the cell shapes should play a vital role in protein distribution, or vice versa. However, the relationship between cell shapes and protein patterns are surprisingly not well characterized. With signal processing and dimensionality reduction techniques, we aim to create a mean cell organization with the natural range of cell-to-cell variation to study the relationships of proteins and organelles in relation to cell's shape. The pilot study is planned in U-2 OS cell lines, in which more than 12,000 proteins are mapped in HPA images and cover most of the cellular structures.

HuBMAP + HPA - Hacking the Human Body

https://www.kaggle.com/competitions/hubmap-organ-segmentation

Christian Gnann

PhD Student, SciLifeLab & KTH Royal Institute of Technology

Metabolic moonlighting and heterogeneity create phenotypic diversity in human cells

Non-genetic cellular heterogeneity in the spatial expression of metabolic enzymes has been implicated in a variety of cellular processes such as drug resistance, metastasis, differentiation or immune cell activation. In this study, we provide insight into the extent of metabolic complexity by analysing the expression of metabolic enzymes with subcellular resolution using antibody-based imaging data from the Human Protein Atlas (HPA) project. We show that metabolic enzymes exhibit higher degrees of expression variability compared to the human proteome. Interestingly, the observed heterogeneity is mainly established on the proteomic level and is conserved in situ. Furthermore, it correlates strongly between related metabolic pathways, indicating that cellular states are manifested through intra- and intercellular fluctuations of the respective enzymes. The spatiotemporal complexity of the metabolic proteome is further extended as over 50% of all enzymes localize to multiple cellular compartments, suggesting potential multifunctional properties. By integrating public affinity purification-based protein-protein interaction data with subcellular location data from the HPA, we identified several enzymes with potentially novel non-canonical functions. Altogether, we believe that the spatial complexity and single-cell heterogeneity of metabolic enzymes can establish dynamic metabolic states which can provide a readout for cell and tissue phenotypes ultimately allowing for targeted studies of their impacts on cell function in normal and pathological cell systems.

Ishan Gaur

Masters Student, Stanford University

Whole cell modeling using diffusion models

Jan Hansen

Postdoctoral Scholar, SciLifeLab & KTH Royal Institute of Technology
From Summer 2023: Postdoctoral Scholar, Stanford University

Shedding light on ciliary signaling and function with spatial proteomics techniques

The primary cilium is a filamentous membrane protrusion emanating from the surface of almost any vertebrate cell. Most of what we know about the function of primary cilia stems from hereditary diseases that are caused by primary cilia dysfunction, so-called ciliopathies. Ciliopathy patients present with a broad spectrum of phenotypes, including polydactyly, cognitive impairments, obesity, kidney defects, or blindness, underlining that the primary cilium fulfills pleiotropic and tissue-specific functions. However, the molecular mechanisms underlying primary cilia function are largely unknown. The picture has emerged that the primary cilium functions as a cellular antenna: it senses extracellular signals and locally transduces the information into the cell body. Yet only few signaling pathways have been mechanistically attributed to the cilium. This is mainly due to the lack of tools to reveal the protein composition of individual cilia.

One reason why we know very little about primary cilia is that their analysis is technologically challenging because they are so tiny: Their volume is more than 1000 times smaller than the volume of the cell to which they attach. Thus it is difficult to reveal the protein composition of cilia with common laboratory techniques.

I aim to overcome technological challenges and establish “antibody-based spatial proteomics” technologies for primary cilia. Using the antibody library from the human protein atlas (https://www.proteinatlas.org/), we map the ciliary protein repertoire in cell lines from different tissue origins. We will reveal the protein composition of primary cilia and generate maps of ciliary proteins in human cells. By establishing new image analysis pipelines we plan to leverage these maps for revealing new functional signaling networks in cilia, how these engage with cellular functions, and how dynamic this relationship is on the single cell level, within a cell population of the same cell type and across cell types. This will provide a new understanding of the functions and mechanisms of primary cilia.

In follow-up studies we plan to translate these studies to tissues and motile cilia. I am to establish multiplexed imaging techniques for characterizing ciliary signaling states in tissues. Profiling cilia in tissues will open up new avenues for understanding the role of this tiny organel not only on the subcellular but also on the tissue, organ, and whole body level.

Mariya Mardamshina

Postdoctoral Scholar, SciLifeLab & KTH Royal Institute of Technology
January 2023: Postdoctoral Scholar, Stanford University

Deciphering proteomic breast cancer heterogeneity using Target-guided Deep Visual Proteomics: challenges of establishing pipeline for single-cell resolution

Many solid tumors are typically composed by several cellular clones with different properties and phenotypes, embedded in varying microenvironmental conditions. This heterogeneous tumor nature might define rates of growth, progression, and treatment responses. The pattern of treatment response in many cancer types became more stable with introduction of new therapeutic regimens, however, the success rate still did not reach 100% for majority of tumors with only 5% of cancer patients found to benefit from genome-targeted therapies. Therefore, better assessment of tumor heterogeneity is essential for the development of more effective treatment modalities.

In this study we aim to acquire a comprehensive portrait of breast cancer heterogeneity and establish pipeline for systematic spatially resolved proteomics. Highly-multiplexed staining enables classification of cell types and in-depth spatial mapping of coexisting cell populations and microenvironmental niches. Using the same tissue section for automated single-cell microdissection based on unbiased AI-driven selection allows to analyze diverse cellular phenotypes and associate it with stages of progression and metastatic niches in distant organs. To acquire in-depth proteomic profiling of the different isolated cell populations with single-cell resolution, we will implement a recently developed state-of-the-art mass spectrometry-based proteomic workflow.

Anna Martinez Casals

Basic Life Research Scientist, Stanford University

Human Cell Atlas of the Pancreas

The human pancreas is a physiologically unique organ involved in the secretion of several hormones as well as the secretion of enzymes for the digestive system. Several human diseases are associated with the pancreas: a very common chronic disease is Diabetes mellitus that involves an inadequate control of blood levels of glucose. In the context of generating a first version of the Human Cell Atlas of the Pancreas, we contributed to the Expression and Spatial analysis Pancreas Atlas Consortium Europe (ESPACE) project aiming at defining the cellular composition and heterogeneity of the pancreas, using spatial proteomics approaches at single cell level. To increase the number of multiplexed proteins, we utilized the iterative method named co-detection by indexing (CODEX): since designing a marker panel is a complex task, first we performed a large IHC screening of 144 HPA antibodies on non-disease formalin-fixed paraffin-embedded tissue microarrays sections to enable selection of the targets showing higher heterogeneous expression. Making use of Memento, an in-house developed web application, 12558 single tissue cores were manually annotated from which we concluded that 70% of the 144 screened proteins were expressing inter- and/or intradonor heterogeneity. Based on the screening results, we created a 24-plex antibody panel which was applied to 184 tissue cores derived from different parts of pancreas of 17 non-disease donors (ND) and 15 additional tissue cores from type-2 diabetes donors (T2D). The produced images were subjected to an in-house computational pipeline, PIPEX, for cell segmentation and to obtain cell data matrices (including cell id, size, cell spatial coordinates XY, antibodies intensities). The results allowed us to analyze almost 2 millions of cells showing until now clear heterogeneous expression between ND samples for certain targets in the exocrine compartment, for instance the metabolic enzyme CBS, as in the pancreatic islets where RBP4 expression is not present in all delta cells. Further analysis is going to be focused on addressing the intrapancreatic heterogeneity based on region and the comparison with T2D donors.

Eleanor O’Brien

Senior Research Engineer, SciLifeLab & KTH Royal Institute of Technology

DRP-PhenoCycler: Establishing simultaneous detection of DNA, RNA, and proteins on the PhenoCycler platform

Recent advances in spatial characterisation of protein expression has greatly improved our understanding of tissue architecture, cell-cell interactions and their function. In this work, we aim to further improve cell characterisation by combining the detection of DNA, RNA and proteins in the same sample in an automated and cyclic manner. Single molecule DNA FISH can identify cells with genetic aberrations and RNA FISH can reveal transcript expression linked to different tumour types or aggressiveness, however these techniques lack spatial context. We set out to use the microfluidic PhenoCycler platform to detect single molecule DNA and RNA FISH signals, as well as protein, in order to gain multiple layers of spatial information from one sample.

Our results in cells have been promising; after optimisation we have been able to develop an integrated protocol for DNA FISH probe hybridisation and antibody staining followed by detection of both DNA and protein using the Phenocycler-Fusion system. We then applied this method to a user project where the aim is to spatially map cell-cell interactions between immune and tumour cells in neuroblastoma. In this case, DNA FISH identifies regions of malignancy and RNA FISH aids to subtype the tumour, while protein expression elucidates the tumour microenvironment. Using tissue samples, we have been able to sequentially hybridise RNA probes, DNA probes and stain with antibodies but imaging is done after each step on separate microscopes. Visualisation therefore requires co-registration of images for each molecule type. We now hope to fully integrate and automate the protocol combining detection of RNA, DNA and protein on tissue.

Pranauti Panshikar

Research Engineer, SciLifeLab & KTH Royal Institute of Technology

Establishing a protocol to integrate standard IF with PhenoCycler Fusion system

Oligonucleotide-conjugated antibodies have gained importance for their application in understanding the spatial biology of complex tissues.The phenocycler-fusion integrated system uses this barcoding technology to facilitate highly multiplexed immunofluorescence imaging of tissues. While Akoya's custom conjugation protocol enables conjugation of antibodies against unique targets of interest, the degree of reproducibility and to some extent the efficiency of the conjugation reaction itself is not consistent - resulting in the failure of some antibody-barcode conjugations. A potential solution is to first perform standard immunofluorescence staining and image acquisition with antibodies against targets that are difficult to conjugate and then perform the phenocycler run with the complete antibody panel on the same sample. Preliminary optimisations of the protocol have successfully demonstrated we can image a sample on the PhenoImager using 1xCODEX buffer and elute the primary-secondary antibody complex prior to the phenocycler run. The next steps will be to fully integrate the standard IF protocol followed by a phenocycler run and to overcome the challenge of overlaying images to visualize the full panel.

Carolina Oses

Researcher, Spatial Proteomic Unit, SciLifeLab & KTH Royal Institute of Technology

Proof of concept study for lung cancer diagnosis

Lung cancer only constituting 6% of cancer cases in Sweden in 2019 it is the most deadly form of cancer in Sweden and worldwide. A small biopsy or cytology can be obtained to further pathological analysis, which results in a very limited amount of tissue. When hematoxylin and eosin stain and tumor morphology does not provide a definite answer, immunohistochemistry (IHC) staining for certain markers can provide clues about what cancer type it is. The problem with IHC is that only one or two markers (plus counterstaining of all nuclei in the tissue) can be visualized on each tissue sample. If several markers are to be stained for, the amount of samples needed quickly add up. To get around this limitation of the current method, we used a multiplex immunofluorescence (IF) approach using COMET instrument from Lunaphore. Seven markers commonly used in clinic were sucessfully adressed usign COMET metodology. These seven markers allows to identify neuroendocrine tumors and the non-small cell carcinoma tumor into adenocarcinoma, squamous cell carcinoma and large cell carcinoma. This study showed that it is possible to translate singleplex IHC analyses into multiplex IF with some alterations. Developing a functioning panel for sequential immunofluorescence on lung cancer tissue would mean that less biopsy material is required to subtype the cancer, leaving more material to do molecular and genetic analysis and customize the treatment.

Tony Ullman

Research Engineer, SciLifeLab & KTH Royal Institute of Technology

Individualized therapy selection for invasive bladder cancer

Bladder cancer is the tenth most common cancer in the world and is more prominent in the male population. The disease is categorised as non-muscle invasive (NMIBC), or Muscle invasive bladder cancer (MIBC) depending on the tumor aggressiveness. The latter is normally diagnosed at 55 years of age or older and the 5 year survival is 50%, with half of the patient group seeing a recurrence within 2 years after surgical or chemotherapeutic intervention. In the past, the clinical approach to treat cancer, including bladder cancer, has usually been met with a one-size-fit-all approach, which could compromise the efficacy of the treatment and cause unwanted side effects in the individual patient. In recent years efforts have been made to create a more individual-focused treatment in order to overcome these issues. As of now, clinical prognostic and treatment predictive biomarkers for MIBC are lacking and therefore needs to be adressed. With the advancement of multiplex immunofluorescence imaging we can now reveal spatial proteomic identity of tissues in various conditions. Together with multiomics data acquired from patient samples, this offers hope in identifying potential candidate biomarkers for MIBC and a better treatment outcome for the individual patient. In this three-part research project, I will briefly introduce you to two parts of this project: Checkpoint200 and Biobladder and my involvement in them.

Wei Ouyang

Researcher, SciLifeLab & KTH Royal Institute of Technology
Visiting Scholar, Stanford University
January 2023: Assistant Professor, SciLifeLab & KTH Royal Institute of Technology

Building AI-powered data-driven whole-cell models

Abstract

Advances in single-cell and genomic measurement techniques, such as high-throughput fluorescence microscopy, scRNA-seq and in situ sequencing are making it possible to rapidly generate massive datasets that can be used to model the entire cell. Whole-cell modeling enables a holistic and quantitative view of cell biology and allows performing in silico experimentation which has a great potential in revolutionizing system biology, synthetic biology, medicine and other applications in life science. However, modeling the entire cell, such as for predicting cellular stress response, is an extremely complex task and is heavily limited by our understanding of the biological systems, especially when using manually curated data and cellular reactions networks. As a result, these bottom-up modeling approaches suffer from scalability issues and existing whole-cell modeling work is mostly carried out with simple organisms such as M. genitalium (Tomita et al., 1999) or E. coli (Macklin et al., Science 2020).

Here we present our plan to tackle the grand challenge of building a simulator for the human cells. We plan to build a deep-learning based whole-cell model for modeling cellular state transition during normal or perturbed conditions. We will use the cell images from the Human Protein Atlas and various other data sources to train Generative Adversarial Networks (GAN) and Diffusion Models for generating protein images conditioned on cell types, protein sequence and other data. To obtain enough data for training, a self-driving live cell imaging system will be developed in order to obtain a dataset that covers cell populations with different cellular states. The cells will be treated with environmental stressors, such as toxins and mechanical damage in human cells, which will cause stress responses and eventually lead to various cell fates, such as cell cycle arrest, apoptosis, or senescence.

Emmie Pohjanen

PhD Student, SciLifeLab & KTH Royal Institute of Technology

Mapping the micronuclear proteome in ecDNA-rich cells

Circular extrachromosomal DNA (ecDNA) is common in many human cancers much like micronuclei. We have therefore started a joint effort with Chang and Mischel labs at Stanford to try to decipher the connection between the two phenomena. Chang and Mischel labs have discovered that cells with ecDNA hubs generally contain more micronuclei than cells that lack these hubs. Moreover, around 30% of micronuclei contain ecDNA (unpublished data). In enDNA+ cells, micronuclei can be induced by treatment with hydroxyurea, which in low doses causes replication stress and DNA double-strand breaks. For ecDNA- cells however, micronuclei cannot be induced with hydroxyurea whereas reversine is used instead. In low doses, reversine induces chromosome mis-segregation and thus produces lagging chromosomes and whole-chromosome micronuclei. We could therefore expect that the proteins recruited to the micronuclei could differ between the induction methods used.

Here I will present our experimental plan and show some results from our first effort of screening 120 genes involved in DNA damage repair and DNA replication in tumor cells with either ecDNA (COLO320-DM) or homogeneously staining regions (HSRs)(COLO320-HSR).

Sanem Sariyar

PhD Student, SciLifeLab & KTH Royal Institute of Technology

High-parametric protein maps reveal the spatial organization in developing human lung

The respiratory system, consisting of the lungs, trachea and the associated vasculature, is essential for terrestrial life. Recent studies on lung development generated new insights into the origins of different cell lineages existing in the lung, and the molecular pathways regulating these lineages, which in turn provide new insights into congenital lung diseases, lung abnormalities and acquired lung diseases. But, most of these insights into lung development is still inferred from animal models, and little is known about human lung development. The recently initiated Human Developmental Cell Atlas (HDCA) project is one of the focus areas of the international Human Cell Atlas project, and it aims to create a comprehensive molecular atlas of human prenatal development by identifying and visualizing present cells across anatomical space and developmental time, including the lung. This study, within the HDCA framework, focuses on molecular and spatial characterization of the main cell types like epithelial, endothelial, mesenchymal and immune cells and their interactions during the first trimester of human lung development using single-cell RNA sequencing and highly-multiplexed tissue imaging technologies (CO-DEtection by indeXing, CODEX).

Charlotte Stadler

Co-Director Spatial and Single Cell Biology Platform and Head of Spatial Proteomics at SciLifeLab & KTH Royal Institute of Technology

The Spatial Proteomics Unit – a journey from 2017 and beyond

I will go back to 2017 when I started working in the facility and tell you how the unit has evolved since then. I will give some general updates on the ongoing work in our team and also tell you a little bit of what´s ongoing in the rest of the Spatial and Single Cell Biology platform at Scilifelab. Our missions from Scilifelab will be presented and I will also share my visions for where I hope we will be in a few years from now.

Kalliopi Tzavlaki

Research Engineer, SciLifeLab & KTH Royal Institute of Technology

Regulation of cell differentiation by members of the TGFβ family

Transforming growth factor-β (TGFβ) represents an evolutionary conserved family of 33 secreted polypeptide factors, including TGFβs as well as bone morphogenetic proteins (BMPs), that play important roles during embryonic development and in the regulation of adult tissue homeostasis. TGFβ is a potent inducer of epithelial to mesenchymal transition (EMT), a vital developmental process, while BMP signaling is involved in bone formation and neural cell differentiation. As anticipated, TGFβ and BMP signaling are tightly regulated at multiple levels in order to perform their physiological functions. Perturbations in TGFβ/BMP signaling have been linked to various diseases such as cardiovascular diseases and cancer.

TGFβ exerts dual roles during cancer development. It acts as tumor suppressor at the onset of the disease by promoting cell cycle arrest and apoptosis, while it promotes cell migration and metastasis in advanced malignancies. Similar to TGFβ, BMP signaling can also have pro-tumorigenic or tumor suppressive roles in different types of cancer.

Liver kinase B1 (LKB1) is a tumor suppressor protein kinase involved in the regulation of cell metabolism, proliferation and polarity. We investigated the role of LKB1 as a regulator of BMP signaling and we demonstrated that LKB1 interacts with one of the BMP type I receptors and mediates its degradation, leading to the inhibition of BMP-induced cell differentiation. We then focused on the role of LKB1 in the establishment of mammary epithelial polarity. Upon LKB1 depletion, normal mammary epithelial cells lost the ability to form polarized acini and displayed enhanced TGFβ responses. Chemical inhibition of TGFβ type I receptor kinase restored acini formation in LKB1 KO cells, suggesting that the contribution of LKB1 to this process is dependent on the regulation of TGFβ signaling.

In the context of glioblastoma (GBM), we investigated the role of different effectors downstream of TGFβ/BMP signaling. TGFβ enhances the self-renewal potential of GBM stem cells (GSCs), while BMP promotes their differentiation towards the astrocytic lineage. Identifying target genes of TGFβ/BMP signaling in GBM cells can provide insight on how these pathways regulate fate choices between stemness and differentiation. We demonstrated that SNAIL was induced by BMP7 in GBM, promoting astrocytic differentiation and suppressing stemness. We also focused on CXXC5, a TGFβ/BMP target gene and found that its expression was enriched in tissue sections of GBM tumors that express high levels of stem cell markers NESTIN and SOX2. Mass spectrometry analysis revealed chromatin remodelers as interacting partners of CXXC5 which suggested epigenetic regulation of gene expression. Further analysis indicated that the recruitment of CXXC5 to regulatory elements of different genes, fine-tunes the expression of genes linked to stem-like identity in GBM.

Collectively, with this work our aim was to provide evidence on mechanisms that regulate cell differentiation by interfering with TGFβ/BMP signaling.

Casper Winsnes

PhD Student, SciLifeLab & KTH Royal Institute of Technology

Thoughts and results from the Cytodata Hackathon

At the Cytodata 2022 conference, several of us attended a Hackathon where we worked with a 3D dataset of segmented cells. We trained a variational autoencoder on mitotic cells in the data. We then applied a pseudo-time model on the latent-space from the encoder. I will present what we did and the results that we managed to achieve during the hackathon.

Attendees

Name	Institution	In Person / Virtual
Ulrika Axelsson	KTH	In Person
Burcu Ayoglu	KTH	Virtual
Frederic Ballllosera Navarro	Stanford	In Person
Frida Björklund	KTH	In Person
Anna Bäckström	KTH	In Person
Anthony Cesnik	Stanford	In Person
Nicolai Dorka	KTH	In Person
Trang Le	Stanford	In Person
Jenny Fall	KTH	Virtual
Christian Gnann	KTH	In Person
Ishan Gaur	Stanford	In Person
Jan Hansen	KTH	In Person
Emma Lundberg	KTH & Stanford	In Person
Mariya Mardamshina	KTH	Virtual
Anna Martinez Casals	Stanford	In Person
Eleanor O'Brien	KTH	Virtual
Carolina Oses	KTH	Virtual
Wei Ouyang	KTH & Stanford (visiting)	In Person
Pranauti Panshikar	KTH	Virtual
Emmie Pohjanen	KTH	In Person
Sanem Sariyar	KTH	Virtual
Charlotte Stadler	KTH	In Person
Kalliopi Tzavlaki	KTH	Virtual
Casper Winsnes	KTH	In Person