Brief Scientific Overview

Conference broadly centered around the following topics interspersed throughout the week

Topic I - From Labels & Tagging to Fluorescence:

Aguilera
Widom
Ishii
Kisley
Jaqaman

Topic II - Chromosomal Dynamics

Beliveau
Taniguchi

Topic III - Dynamics, Energy Landscapes, and Molecular Cooperativity

Leitner
Makarov
Morozov

Topic IV - Label-free: Cryo, Photothermal, and Tomographic Imaging

Shepherd
Sander
Prigozhin

Topic V - Simulation-based Inference

Covino
Pressé

Topic VI - Raman and Beyond

Katsumasa
Clément
Komatsuzaki
Oh
Hiramatsu

Schedule

The workshop will be held at the Telluride Science and Innovation Center located at 300 S Townsend St in the Gallery Room.

June 8
17:00-18:30
Meet and Greet for badge pick up.
Located at the Alibi (121 S Fir St, Telluride, CO 81435).

For those interested, meeting at New Sheridan Historic Bar (231 W Colorado Ave, Telluride, CO 81320) at 18:30 after badge pickup

June 9
7:30-8:30 Breakfast
8:30-8:40 Brief Introduction by Organizers
8:40-9:30 Shepherd
9:30-10:20 Sander
10:20-10:40 Break
10:40-11:30 Komatsuzaki
11:30-12:20 Taniguchi
12:20- Free time (Hiking or other activities)

For those interested, piano evening in planning (still securing piano)

June 10
7:30-8:30 Breakfast
8:30-9:20 Jaqaman
9:20-10:10 Beliveau
10:10-10:30 Break
10:30-11:20 Kisley
11:20-12:10 Leitner
12:10- Free time until Town talk (Hiking or other activities)

18:30-19:30-Town talk. Located in Mountain Village at the Telluride Conference Center (580 Mountain Village Blvd, Mountain Village, CO 81435). Free and open to the public.

June 11
7:30-8:30 Breakfast
8:30-9:20 Covino
9:20-10:10 Prigozhin
10:10-10:30 Break
10:30-11:20 Aguilera
11:20-12:10 Widom
12:10- Free time (Hiking or other activities)

17:30-19:00 - BBQ/Picnic located in the tent behind the Telluride Intermediate School (721 W Colorado Ave, Telluride, CO 81435). Free and open to all Telluride Science participants and their families.

June 12
7:30-8:30 Breakfast
8:30-9:20 Morozov
9:20-10:10 Katsumasa
10:10-10:30 Break
10:30-11:20 Makarov
11:20-12:10 Pressé
12:10- Free time until Conference dinner (Hiking or other activities)

17:00- Group dinner at 221 S Oak

June 13
7:30-8:30 Breakfast
8:30-9:20 Oh
9:20-10:10 Ishii
10:10-10:30 Break
10:30-11:20 Clément
11:20-12:10 Hiramatsu

Abstracts

Topic I-From Labels & Tagging to Fluorescence

Title: UTag, a novel tagging system for tracking single-molecule translation live

Luis U. **Aguilera**[1], Ashlyn Chen[2], Jake Yarbro[1], Rhiannon Sears[1], Jacob DeRoo[2], Hunter Ogg[5], Brian J Geiss[2,4], Chris Snow[2, 3], Timothy J Stasevich[5], Ning Zhao[1]

[1] Department of Biochemistry and Molecular Genetics, University of Colorado-Anschutz Medical Campus, Aurora, CO, USA.
[2] School of Biomedical Engineering, Colorado State University, Fort Collins, CO, USA.
[3] Department of Chemical & Biological Engineering, Colorado State University, Fort Collins, CO, USA.
[4] Department of Microbiology, Immunology, & Pathology, Colorado State University, Fort Collins, CO, USA.
[5] Department of Biochemistry and Molecular Biology, Colorado State University, Fort Collins, CO, USA.

Live-cell imaging and intracellular antibody fragments (intrabodies) tagging systems have revolutionized our understanding of gene expression at single-molecule resolution. A current problem this technology is facing is the limited number of tagging systems that restricts the versatility of these experiments. **We aim** to contribute to solving this problem by introducing a novel disulfide-bond free tagging system (UTag, intrabody) to efficiently visualize single-molecule gene expression. We benchmarked UTag against the well-established SunTag and the recently introduced ALFA-tag systems using KDM5B, a nuclear reporter protein. **Our results show** that UTag has rapid cytosolic diffusion and efficient nuclear import. Western blot confirmed full-length protein expression, demonstrating that UTag does not perturb translation or protein stability. Treatment with translation inhibitors eliminated discrete translation foci, validating that UTag detects active mRNA translation. To quantify UTag's performance in dynamic translation assays, we combined long-term imaging, particle tracking, correlation analysis, and a Totally Asymmetric Simple Exclusion Process (TASEP) model. Integrating TASEP simulations with live-cell measurements via parameter inference, **we conclude** that ribosomal initiation occurs at ~0.04 1/sec and elongation occurs at ~4.5 aa/sec, across the three tested tagging systems.

Title: Understanding and circumnavigating the impacts of fluorophore labeling on RNA folding

Julia R. **Widom**[1,2], Taylor L. Coulson[1], Janson E. Hoeher[1], Natalie E. Sande[1]

[1] University of Oregon Department of Chemistry and Biochemistry, Eugene, OR 97403
[2] Oregon Center for Optical, Molecular and Quantum Science, University of Oregon

Fluorescence-based methods have provided significant insight into biomolecular structure and dynamics, but most measurements require chemical modification of the biomolecule in order to generate the desired spectroscopic signals. I will present my lab's work characterizing the impacts of fluorophore labeling on the sensing of small molecule ligands by two different RNA aptamers. In these studies, we combine bulk spectroscopy and single-molecule FRET to analyze the impacts of two classes of fluorophore: a fluorescent base analogue that substitutes for one of the native bases in RNA, and a cyanine dye. We found that significant insight could be gained even from experiments using labeling sites that strongly perturbed ligand binding, as long as the results were interpreted in the context of appropriate controls. These challenges are bypassed when the intrinsic fluorescence of the biomolecule or biomolecular complex is the topic of interest. I will present unpublished work on fluorogenic RNA aptamers in which we are using steady-state and time-resolved fluorescence spectroscopy to analyze the mechanism by which the fluorescence of the ligand is activated upon binding to the aptamer. We present evidence that increased rigidity of the ligand's environment is not always necessary or sufficient to activate its fluorescence, and that the electronic environment created in its binding pocket plays a key role.

Title: Independent component analysis disentangles fluorescence signals from diffusing single molecules

Author: Kunihiko **Ishii**, Miyuki Sakaguchi, Tahei Tahara

Molecular Spectroscopy Laboratory, RIKEN; RIKEN Center for Advanced Photonics

Multiparameter single-molecule fluorescence measurement is a promising technique for detecting and quantifying heterogeneity in freely diffusing molecules, albeit with challenges in data analysis due to limited photon numbers. In this talk, we will introduce a universal analytical framework called IFCA (Independent Fluorescence Component Analysis) that leverages independent component analysis for unmixing multiparameter fluorescence signals. Applications to static and dynamic mixture systems will be presented to demonstrate its potential allowing model-free separation of subpopulations with microsecond time resolution in the nanomolar concentration regime.

Title: The analytical imaging conditions required to accurately super-resolve complex dynamics with correlation analysis

Lydia **Kisley**

Case Western Reserve University, Department of Physics and Chemistry, Cleveland, OH USA

Fluorescence correlation spectroscopy super-resolution optical fluctuation imaging (fcsSOFI) is a wide field super-resolution imaging method that quantifies both diffusion constants and structure in heterogeneous materials. Samples consist of fluorescent diffusing emitters within porous materials, such as hydrogels, liquid crystals, or the extracellular matrix, which are imaged on a 2D camera over time. The correlation analysis of intensity signal over time provides super-resolution spatial SOFI information, where the amplitude of the correlation curve resolves the excluded volume of the material and diffusion, and FCS data, from a fit to an empirical model of diffusion to obtain D at each individual pixel. fcsSOFI can, therefore, produce nanoscale images of molecular dynamics within materials in situ without requiring the drawbacks of freezing (electron microscopy), high forces and surface structure only (atomic force microscopy), averaging (rheology), or low sampling (confocal FCS) or resolution (FRAP).3 While statistical parameters and experimental conditions to achieve super-resolution images from static, blinking emitters for SOFI or accurate diffusion for diffraction-limited, confocal FCS have been established, the limits of fcsSOFI have not been analytically or experimentally established. I will discuss our progress in establishing the key parameters for accurate fcsSOFI imaging including minimum and maximum lag time, signal-to-background ratio, emitter concentration, along with the effect of non-Brownian diffusion dynamics and heterogeneous species.

Title: Inference of VEGFR2 dimerization kinetics on the cell surface by integrating single-molecule imaging and mathematical modeling

Khuloud **Jaqaman**

Department of Biophysics and Lyda Hill Department of Bioinformatics, UT Southwestern Medical Center, Dallas, TX 75390

Inter-receptor interactions play a key role in receptor signaling, the first step in cell signaling. In the case of Vascular Endothelial Growth Factor Receptor 2 (VEGFR2), dimerization is necessary for activation. But VEGFR2 undergoes reversible interactions also in the absence of ligand. Therefore, for a quantitative understanding of transmembrane signal transduction, it is necessary to quantify VEGFR2's interaction kinetics. Live-cell single-molecule imaging (SMI) has the powerful ability to capture receptor interaction events in their native cellular environment with high spatiotemporal resolution. However, it reveals these interactions for only the labeled subset, which is a small fraction of the full population of receptors. We have previously shown that mathematical modeling, combined with SMI data, offers a route to compensate for this lost information to infer the population-level receptor interaction kinetics from SMI data (de Oliveira & Jaqaman 2019). Here, we applied this appraoch to VEGFR2, both full length WT VEGFR2 (FLR2) and a truncated mutant consisting of the extracellular and transmembrane domains (ECTM), which has been shown to undergo little homotypic interactions in the absence of ligand (Sarabipour et al. 2016). We developed a stochastic mathematical model that mimicked VEGFR2 diffusion and interactions, and determined the unknown model parameters through a combination of direct experimental measurements and stochastic model calibration. We found that a model of dimerization was sufficient to describe VEGFR2 interactions in the absence of ligand (i.e., there was no evidence for higher order oligomerization). While ECTM was primarily monomeric, FLR2 exhibited a substantial fraction of dimers. Our inference revealed that the difference between FLR2 and ECTM was primarily in the dimer association rate constant, which was about an order of magnitude lower for ECTM than for FLR2. To our knowledge, this is the first time that the interaction kinetics of VEGFR2 have been calculated in live cells.

Topic II- Chromosomal Dynamics

Title: From single molecules to bulk—trying to make sense of 3D chromosome folding

Brian J. **Beliveau**[1,2,3]

[1]Department of Genome Sciences, University of Washington, Seattle WA 98195, USA
[2]Brotman Baty Institute for Precision Medicine, Seattle WA 98195, USA3
[3]Institute of Stem Cell and Regenerative Medicine, University of Washington, Seattle WA 98109, USA

Mammalian cells spatially confine a ~2 M linear genome into nuclei whose diameters generally only span a few microns. Despite this massive compaction, cells are faithfully able to execute the essential DNA transactions of replication, transcription, and repair. While both the folding of chromosomes in 3D space and chromosome dynamics are known to be critical for proper nuclear function, the exact mechanisms that sculpt nuclear architecture remain poorly understood. Here, I will present efforts to build imaging and biochemical tools to characterize the folding conformations adopted by individual chromosomes at the single molecule level and to identify the molecular factors that influence their structure and function in an unbiased manner. Particular emphasis will be placed on challenges shared by both the single molecule and bulk biochemical methods, most notable of which is the staggering amount of heterogeneity present in both types of measurement.

Title: Nucleosome-level 3D organization of the genome

Yuichi **Taniguchi**

Quantitative Biology Center (QBiC), RIKEN, Suita, Osaka 565-0874, Japan

Resolving the global and local spatial organization of the genome in the nucleus is crucial to understand mechanisms on regulation of gene expressions. Current high-throughput chromosome conformation capture (Hi-C) technologies have identified large-scale chromatin structural motifs, such as topologically-associating domains and looping. However, structural rules at the smallest or nucleosome scale remain poorly understood. Here, we coupled nucleosome-resolved Hi-C technology with simulated annealing-molecular dynamics (SA-MD) simulation to reveal 3D spatial distributions of nucleosomes and their genome-wide orientation in chromatin. Our method, called Hi-CO, revealed distinct nucleosome folding motifs across the yeast genome. Our results uncovered two types of basic secondary structural motifs in nucleosome folding: α-tetrahedron and β-rhombus analogous to α-helix and β-sheet motifs in protein folding. Using mutants and cell-cycle synchronized cells, we further uncovered motifs with specific nucleosome positioning and orientation coupled to epigenetic features at individual

loci. By illuminating molecular-level structure-function relationships in eukaryotic chromatin, our findings establish organizational principles of nucleosome folding.

Topic III-Dynamics, Energy Landscapes, and Molecular Cooperativity

Title: Vibrational energy landscapes and energy flow in proteins
David **Leitner**

Dept. of Chemistry, University of Nevada, Reno

When a protein is vibrationally excited, excess energy flows through the protein anisotropically and in a subdiffusive fashion. Time-resolved spectroscopic measurements and molecular dynamics simulations continue to map out pathways for energy flow following photoexcitation or chemical reaction and characterize the nature of energy flow. I will discuss some recent work on identifying structural and dynamic properties of proteins that mediate the flow of energy, with focus on the underlying energy landscape. As an example, I will discuss energy landscapes and transport in a G-protein coupled receptor (GPCR). The influence of specific amino acids that contribute to GPCR plasticity and function on energy transport can be seen in graphical representations of the vibrational energy landscape. Striking differences between inactive and active states are found where structural and dynamic changes occur upon activation, contributing to allosteric regulation.

Title: Diffusion in rough, fluctuating energy landscapes

Dmitrii E **Makarov**[1], Peter Sollich[2]

[1]Department of Chemistry and Oden Institute for Computational Engineering and Sciences, University of Texas at Austin, Austin, TX, 78712)
[2]Institute for Theoretical Physics, Georg-August-Universität Göttingen, 37077, Göttingen, Germany

Molecules in dense environments, such as biological cells, are subjected to forces that fluctuate both in time and in space. While spatial fluctuations are captured by Lifson-Jackson-Zwanzig's model of ``diffusion in a rough potential'', and temporal fluctuations are often viewed as leading to additional friction effects, a unified view where the environment fluctuates both in time and in space is currently lacking. We introduce a discrete-state model of a landscape fluctuating both in time and in space. Importantly, the model accounts for the back-reaction of the system. As a result -- surprisingly -- we find that many features of the observable dynamics do not depend on

the temporal fluctuation timescales and are already captured by the model of diffusion in a rough potential despite the fact that it assumes a static energy landscape.

Title: Emergence of cooperativity in the game of Prisoner's Dilemma

Alexandre **Morozov**
Department of Physics and Astronomy, Rutgers University, Piscataway, NJ 08854, USA

Complex life would be impossible without cooperation at all levels of biological organization: genes cooperate to create regulatory networks and signaling pathways, cells become parts of multicellular organisms, animals enter into complex cooperative arrangements both within and between species. Cooperation is ubiquitous in modern human societies and is credited with the development of modern civilization. However, Darwinian evolution is believed to promote selfish behavior - societies of cooperators are vulnerable to cheaters that ultimately take over, destroying cooperation in biological populations. This apparent paradox has puzzled evolutionary biologists for over half a century. A well-known model of the paradox is provided by the celebrated Prisoner's Dilemma, an elegant mathematical framework which favors cheaters, even though being a part of a cooperative society brings about greater rewards. Here we demonstrate that it is possible to achieve high levels of cooperativity in the game of Prisoner's Dilemma without developing any multi-step game strategies or introducing additional assumptions about genetic relatedness and spatial population structure. The only requirement is for the probability of cooperation to vary depending on the opponent, instead of being blind to the opponent's physical appearance and patterns of behavior. Opponent-specific responses drive the population to persistent partially cooperative states - a potential foundation for more specific modes of cooperation observed in animal and human societies.

Topic IV-Label-free: Cryo, Photothermal, and Tomographic Imaging

Title: Exploring the 'rules of life' through computational imaging

Peter T. Brown[1,2], Nikta Jabbarzadeh[1,2], Luis Meneses[3], Kaia Swanson[4], Aidan Pintuff[4], Ekaterina Monakhova[4], Rory Kruithoff[1,2], Navish Wadhwa[1,2,3], Domenico F. Galati[4], Douglas P. **Shepherd**[1,2]

[1] Center for Biological Physics, Arizona State University, Tempe, AZ 85287, USA.
[2] Department of Physics, Arizona State University, Tempe, AZ 85287, USA.
[3] Biodesign Center for Mechanisms of Evolution, Arizona State University, Tempe, AZ 85287, USA.
[4] Biology Department, Western Washington University, Bellingham, WA 98225, USA.

Many biological and soft-matter processes occur at high speeds in complex 3D environments, and developing imaging techniques capable of elucidating their dynamics is an outstanding challenge. We recently developed Fourier Synthesis Optical Diffraction Tomography (FS-ODT), a novel approach for quantitative phase imaging capable of recording the 3D refractive index at kilohertz rates. FS-ODT introduces pattern generation and inverse computational strategies that multiplex tens of illumination angles in a single tomogram, dramatically increasing the volumetric imaging rate. We demonstrate the capabilities of FS-ODT for probing complex systems by studying hindered diffusion of colloids and motility of bacterial swimmers. We further integrate FS-ODT into a multimodal microscope combining phase imaging with multicolor structured illumination microscopy. The resulting multimodal imaging platform unlocks challenging imaging regimes in biophysics and soft-matter that have been little explored, including the interactions of microswimmers with their viscous 3D environment and the interplay between these stimuli and the molecular response of biological systems.

Title: Label-free time-resolved molecular mid-infrared photothermal imaging

Michelle Y. **Sander**

Department of Electrical and Computer Engineering, Department of Biomedical Engineering, Division of Materials Science and Engineering, Boston University
BU Photonics Center and BU Neurophotonics Center

Mid-infrared (mid-IR) photothermal imaging has proven a powerful label-free chemical imaging technique. Relying on a pulse quantum cascade laser as a pump beam combined with a shorter wavelength near-infrared probe beam, sub-micron spatial resolution can be achieved for imaging. With time-resolved mid-IR photothermal imaging, heat transfer dynamics across interfaces can be studied simultaneously with molecular composition. Hence, beads in aqueous environments, cells and axons (e.g. their lipids and proteins) can be analyzed based on vibrational signatures. The residual water background in the mid-IR can be separated based on its inherently different transient response captured with boxcar detection. Hyper temporal imaging stacks from extracted crayfish axon-bundles are presented. It is demonstrated that at the interface of an axon-bundle with surrounding tissue compared to a more isolated axon-bundle, less heat transfer is observed during the diffusion window. Different diffusion behavior for beads (with 100 nm and 500 nm) in water and their heat exchange with the surrounding environment are further characterized. Hence, chemical imaging is combined with studies of interface dynamics, offering insights into the transient responses and thermal resistance of biological samples.

Title: Dynamic and Multicolor Electron Microscopy

Max **Prigozhin**

Department of Molecular and Cellular Biology; Department of Applied Physics
Harvard University

My lab is developing biophysical methods to achieve multicolor and dynamic biological imaging at the molecular scale. Our approach to capturing the dynamics of cellular processes involves

cryo-vitrifying samples after known time delays following stimulation using custom cryo-plunging, microfluidic freezing, and high-pressure freezing instruments. In a separate thrust, to achieve multicolor electron imaging, we are exploring the property of cathodoluminescence -- optical emission induced by the electron beam. We are developing “cathodophores” that will be used as luminescent protein tags in electron microscopy. We are applying these new methods to study the formation of biomolecular condensates in single cells and visualize the organization of complex multicellular assemblies.

Topic V-Simulation-based Inference

Title: Simulation-based inference of single-molecule experiments

Roberto **Covino**

Professor of Computational Life Science, Goethe University Frankfurt
Senior Fellow, Frankfurt Institute for Advanced Studies

Single-molecule experiments are a unique tool to characterize the structural dynamics of biomolecules. However, reconstructing molecular structure and dynamics from noisy and partial single-molecule data is challenging. Simulation-based inference (SBI) is a powerful framework for analyzing complex experimental data, integrating statistical inference, physics-based simulators, and machine learning. Recent advances in deep learning have enabled the development of new SBI methods, empowering the application of Bayesian inference to an ever-increasing number of scientific problems. In this talk, I will describe our ongoing work on applying SBI to analyze single-molecule experiments. I will overview emerging deep learning–based SBI methods to perform Bayesian inference for complex models encoded in computer simulators. I will illustrate the first applications of SBI to single-molecule force spectroscopy and cryo-electron microscopy experiments. I will discuss how SBI allows us to leverage powerful computer algorithms modeling complex biomolecular phenomena to connect scientific models and experiments in a principled way.

Title: Simulation-based inference for likelihood approximation with conditional normalizing flows

Pedro Pessoa[1,2], Juan Andres Martinez[3], Vincent Vandenbroucke[3],
Frank Delvigne[3], Steve **Pressé**[1,2,4]

[1]Center for Biological Physics, Arizona State University, Tempe, AZ, USA
[2]Department of Physics, Arizona State University, Tempe, AZ, USA
[3]Terra Research and Teaching Centre,
Microbial Processes and Interactions (MiPI),
Gembloux Agro-Bio Tech, University of Liège, Gembloux, Belgium
[4]School of Molecular Sciences, Arizona State University, Tempe, AZ, USA

Performing physics-inspired inference often relies on formulating likelihoods understood as the probability of observing the data given the model. Likelihoods can then be directly maximized for the purposes of parameter estimation or integrated into a Bayesian framework. However, many models, even models very simple to formulate, lack tractable likelihoods. For example, estimating protein production from snapshot observations of actively dividing cells presents a fundamental challenge in formulating the likelihood. This is because cell divisions occur at intervals that are not exponentially distributed, breaking Markovian assumptions, and each division event partitions proteins between daughter cells, thus making the number of proteins within a cell dependent on its complex division history. This prohibits a straightforward formulation of a likelihood derived from a solution to, for instance, a master equation. Here we extend rigorous likelihood-based inference to those problems for which likelihoods cannot be written down. We choose to tackle the simple-to-formulate yet pathological problem just described as an illustrative example. To perform inference, we use the family of neural network methods known as conditional normalizing flows developed to learn probability distributions to approximate likelihoods from simulated data though the notion of approximating likelihoods is general and can be achieved with other neural net architectures. Applying this approach, we quantify protein production across cell cycles, overcoming challenges posed by the process' inherent non-Markovian nature. More broadly, our framework proposes a scheme for direct model inversion for systems where large-scale simulations are feasible and likelihoods are otherwise difficult to compute.

Topic VI-Raman and Beyond

Title: Cryo-optical microscopy with on-stage rapid freezing

Katsumasa **Fujita**

Department of Applied Physics, University of Osaka

Capturing rapid cellular dynamics with high spatial resolution remains a major challenge in bioimaging, especially when seeking to preserve transient states such as ion fluxes or molecular conformations. In this talk, I will introduce a novel technique we developed—time-deterministic cryo-optical microscopy—that enables millisecond-scale cryofixation of living cells directly on the microscope stage. By integrating a rapid cryogen injection system with precise temporal control (±10 ms), our method immobilises dynamic cellular processes such as calcium ion waves at specific timepoints, preserving both molecular distribution and chemical state. This approach combines the advantages of live-cell imaging and cryofixation, offering high signal-to-noise ratio, improved quantitative accuracy, and compatibility with super-resolution and Raman microscopy. I will demonstrate its capabilities through multimodal imaging of subcellular structures and ion dynamics in cardiomyocytes and HeLa cells, including 3D-SIM and Raman mapping. The technique's potential for visualising chemically labile states, such as redox or pH distributions, and its compatibility with correlative light and electron microscopy (CLEM), will also be discussed. This method establishes a new paradigm for spatiotemporally resolved, multimodal analysis of living systems with unprecedented precision.

Title: Accelerated Raman Imaging for Biomedical Analysis

Jean-Emmanuel **Clément**[1], Shunsuke Ono[2] , Zannatul Ferdous[3] , Peter Dedecker[1,4],Fujita Katsumasa[4], Jian Ping Gong[7], Masumi Tsuda[7], Shinya Tanaka[7], Tamiki Komatsuzaki[6,7]

[1] CNRS, LASIRe (UMR 8516), University of Lille, Fran[ce]
[2]Department of Computer Science , Institute of Science Tokyo, Japan
[3]Department of Physics, Ecole Normale Supérieure Paris, France
[4]Department of Chemistry, KU Leuven, Leuven, Belgium
[5]Departement of Applied Physics, Osaka University, Japan
[6]Research Institute for Electronic Science , Hokkaido University, Japan
[7]The Institute for Chemical Reaction Design and Discovery, Hokkaido University, Japan

Raman imaging is a powerful technique for revealing the biochemical states of biological samples. However, its capacity to probe fast cellular dynamics is limited by acquisition speeds that cannot capture events occurring on timescales shorter than a few minutes. To overcome this limitation, we introduce a compressed-sensing framework that acquires undersampled spatio-spectral or spatio-temporal data using random patterns and shear transformations. The resulting images are reconstructed by solving an inverse problem regularized with a novel penalty that enforces both low-rank structure and local smoothness in Raman images. Additionally, we present a recent work on cell–hydrogel interactions, supported by a data preprocessing pipeline that extracts single-cell Raman information from overlapping cellular and hydrogel signals.

Title: Real-Time AI in Biology: Optimizing Measurements and Detecting Indirect Interactions

Tamiki **Komatsuzaki**

Research Center of Mathematics for Social Creativity
Research Institute for Electronic Science
The Institute for Chemical Reaction Design and Discovery
(WPI-ICReDD), Hokkaido University

I present our recent researches engaging information science into biology, one is how AI can adaptively optimize the measurement condition for phenotype discrimination, and the other is how one can infer typical interaction length between agents such as cells and whether they interact directly or indirectly via third agent(s).

When the aim of a measurement such as classifying phenotypes is set, the conventional paradigm that separates data acquisition from analysis becomes inefficient. Our key lies in embedding AI-driven analysis directly into the measurement loop, dynamically guiding the process toward optimal acquisition conditions. We present our recent studies in AI-integrated measurement science, where artificial intelligence intervenes in real-time to accelerate data acquisition while preserving the accuracy of measurement objectives. When the aim of a measurement—such as detecting tumors, identifying anomalies, or classifying phenotypes—is defined, the conventional paradigm that separates data acquisition from analysis becomes increasingly inefficient. Our key component lies in embedding AI-driven analysis directly into the measurement loop, dynamically guiding the process toward optimal acquisition conditions.
This is particularly critical in applications constrained by time and cost, such as label-free phenotypic classification using spontaneous Raman microscopy. While Raman microscopy offers chemically rich information, its inherently slow acquisition—due to the weak scattering cross-section—limits practical throughput. We address this bottleneck by introducing a reinforcement learning-based illumination strategy, which adaptively determines where and how to measure during the imaging process. This method selectively focuses on regions likely to

carry diagnostically relevant signals, drastically reducing acquisition time while maintaining discrimination accuracy [1].

Collective behaviors in groups emerge from complex webs of direct and indirect interactions among individuals. While pairwise interactions are fundamental to understanding group dynamics, each agent's historical trajectory confounds causal inference, making it challenging to disentangle direct interactions from those mediated by hidden intermediaries. Here, we address the question: given observational tracking data from only a single pair of agents, can we determine whether their interaction is direct or mediated by an unseen third agent? and in what spatial scale they are interacting each other. We propose a framework based on information theory, modified transfer entropy, across delay times to detect the presence of a hidden intermediary. Our approach reveals a distinct signature: direct interactions exhibit a consistently decreasing modified transfer entropy with increasing delay time, whereas indirect interactions deviate from this trend. This method provides a simple yet versatile tool for uncovering hidden structures in complex systems, with broad implications for networked dynamics in biological, social, and artificial systems. [2-4]

[1] K. Tabata et al. On-the-fly Raman microscopy guaranteeing the accuracy of discrimination. PNAS 121(12), e2304866121, (2024)
[2] U. S. Basak, S. Sattari, M. M. Hossain, K. Horikawa, T. Komatsuzaki. An information-theoretic approach to infer the underlying interaction domain among elements from finite length trajectories in a noisy environment. J. Chem. Phys. 154,034901 (2021)
[3] U. S. Basak, S. Sattari, M. M. Hossain, K. Horikawa, M. Toda, T. Komatsuzaki. Comparison of particle image velocimetry and the underlying agents dynamics in collectively moving self propelled particles. Sci. Rep. 13(12566), (2023)
[4] S. Sattari, U. S. Basak, R. G. James, L. W. Perrin, J. P. Crutchfield, T. Komatsuzaki. Modes of information flow in collective cohesion Sci. Adv. 8(6), (2022) sciadv.abj1720

Title: Quantitative SRS Microscopy for Measuring Cytoplasmic Composition and Density in Live Cells

Seungeun **Oh**

Department of Physics, University of California San Diego, La Jolla, CA 92093, USA
Department of Cellular and Molecular Medicine, University of California San Diego, La Jolla, CA 92093, USA

The physical state of the intracellular environment—particularly macromolecular concentration and density—profoundly affects molecular crowding, diffusion, reaction dynamics, and phase behavior. To quantitatively interrogate these properties, my lab develops and applies Stimulated Raman Scattering (SRS) microscopy for label-free, absolute measurement of protein, lipid,

water, and nucleic acid concentrations in live cells and tissues. Our implementation, Normalized Raman Imaging (NoRI), corrects for light scattering and optical aberrations, enabling accurate quantification even in thick, heterogeneous specimens.
Using NoRI, we investigate how cells regulate cytoplasmic concentration during growth, stress, and differentiation, and how these changes impact biochemical function. I will present recent findings on cytoplasmic density homeostasis and its cell type specificity, highlighting how quantitative vibrational imaging can reveal fundamental principles of intracellular organization and control.

Title: High-Throughput Broadband Raman Fingerprint Cell Sorting

Kotaro **Hiramatsu**

Department of Chemistry, School of Science, Kyushu University, Fukuoka 819-0395, Japan

The ability to isolate and study specific cell populations is foundational in both basic biology and applied biomedical research. Traditional approaches often rely on fluorescent labels, which can perturb native cell states or be limited by label availability. Raman-activated cell sorting (RACS) offers a powerful, label-free alternative by probing the intrinsic vibrational signatures of biomolecules within cells. Despite its potential, a longstanding trade-off between chemical specificity (bandwidth) and processing speed (throughput) has constrained its broader use.
Here, we present a major advance in RACS by enabling broadband detection across the entire Raman fingerprint region (300–1,600 cm−1) at a record throughput of ~50 cells per second. This represents a 25-fold improvement over previous broadband systems, achieved outside the resonance Raman regime.
To showcase the practicality of our RACS, we conduct real-time label-free sorting of microalgal cells based on their accumulation of carotenoids and polysaccharide granules. The outcomes of this research have promising implications for medical, biofuel, and bioplastic applications, potentially broadening the reach and impact of RACS technology.