The Chesapeake Cytometry Consortium

of the Mid-Atlantic Area

CCC Fall 2026 Seminar

Date:
September 22, 2026

Time:
08:30 am-5:00 pm

Location:

Montgomery College

Bioscience Education Center

20200 Observation Dr,

Germantown, MD 20876

Chesapeake Cytometry Consortium- Annual Cytometry Seminar- September 22, 2026
Montgomery College, Germantown Campus, Biosciences Education Center
For information about the meeting, please visit www.cccflow.org.

8:30-9:00 **Registration/Breakfast**
9:00-9:15 **Opening Remarks & Welcome Address**

9:15- 10:00 Serial Flow Cytometry: A measurement device for estimating uncertainty, elasticity, and kinetics of individual cells, organelles, and particles.
Gregory A. Cooksey, Ph.D., National Institute of Standards and Technology
10:00 -10:15 **Coffee Break**

10:15-11:00 Quirks and All: unlocking comprehensive biological discovery out of your spectral flow cytometry datasets
David Rach, Ph.D., University of Maryland Greenebaum Comprehensive Cancer Center
11:00-11:30 PARP inhibition plus a selective bifunctional T-cell agonist drives a polyclonal antitumor immunity and tumor regression: Relevance of a 30-color Spectral Flow Cytometry Panel
Ginette S. Santiago-Sanchez, Ph.D., NCI/NIH
11:30-12:00 Using Imaging Flow Cytometry to Quantify Bispecific Immune Cell Engagers
Nicholas Battaglia, Ph.D.
Dynamic Omics, CGR, Biopharmaceuticals R&D, AstraZeneca, Gaithersburg, MD, USA

12:00-13:00 **Lunch/ Visit with Vendors**

13:00 -13:30 AI Model Training on the Attune CytPix for Macrophage Characterization
Zuleima Munoz, Ph.D.
Dynamic Omics, CGR, Biopharmaceuticals R&D, AstraZeneca, Cambridge, UK
13:30- 14:00 Neuromorphic Label-free Imaging for Kinetic Serial Flow Cytometry of Cell Dynamics
Matthew DiSalvo, Ph.D.
National Institute of Standards and Technology

14:00-14:30 **Afternoon Coffee Break**

14:30-14:55 Building Robust Unmixing in High-Parameter Spectral Flow Cytometry
Ben Janoschek, Ph. D, Sony Biotechnology
14:55-15:20 What lies beyond arbitrary fluorescence standardization?
Joshua Welch, Ph. D, Waters Biosciences
15:20-15:45 A Practical Spectral Flow Cytometry Approach to Classify Functional Subsets of Human T Cells
Jessica Gucwa, Ph.D., Cytek Biosciences
15:45-16:10 More Than Markers— Unlocking Cellular Function with Fluorescent Probes
Andrea Luker, Ph.D. Revvity (Biolegened)
16:10-16:35 Authentic Spectral Signatures for Reproducible Results: How Cell Mimics Improve Spectral Flow Cytometry
Sarah Kotanchiyev, Ph.D.
16:30-17:00 When Cells Meet: Functional Cell–Cell Interaction Screening with Nanovials
Sean Maynard, Ph.D., Partillion Bioscience

Serial Flow Cytometry: A measurement device for estimating uncertainty, elasticity, and kinetics of individual cells, organelles, and particles.

***Gregory A. Cooksey*[1], *Matthew DiSalvo*[1], *Eric W. Esch*[1,2], *Brandon K. Stacks*[1], *Graylen R. Chickering*[3], *Eric M. Darling*[3], *and Paul N. Patrone*[1]**

[1]National Institute of Standards and Technology (NIST), Gaithersburg, MD
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[3]Brown University, Providence, RI

We have developed serial cytometry as a technique for uncertainty quantification through repeated measurements of objects as they pass through multiple interrogation regions in a microfluidic chip [DiSalvo et al., Lab Chip 2022]. The system provides many opportunities to study and optimize measurement uncertainty while also enabling novel high-throughput measurement modes. I will discuss our recent efforts to estimate elastic modulus from individual particles under flow, to track per-cell kinetics of membrane permeabilization, and to measure uncertainty of isolated mitochondria.

Serial cytometry utilizes novel 3D fluid focusing to achieve consistent particle velocity, which enables measurements to be tracked through multiple regions along a flow path. From repeated measurements, we get estimates of per-particle measurement imprecision, which we have demonstrated can be below 2% for fluorescence. A serial cytometer can also estimate the size and elastic modulus of objects by combining analysis of the pulse shape with measurements of changes in region-to-region time of flight caused by deformation. Cell-like polyacrylamide microparticles with a range of known sizes (8.9 µm to 23 µm diameter) and stiffnesses (0.1 kPa to 9.1 kPa) were used to generate a model, which was then applied to estimate the elastic modulus of live MG-63 osteosarcoma cells [Chickering et al., Lab Chip 2026]. The serial cytometer is also being used for kinetic measurements by integrated features that increase the time delay between measurement regions. As proof-of-concept, we studied membrane permeabilization of individual Jurkat T-lymphocytes over about 3 s following exposure to Triton X-100 in the sheath fluid, which caused decrease in brightness of cells labeled with calcein. Finally, we are utilizing the serial cytometer to characterize inherent heterogeneity in mitochondria labeling. The mitochondria of THP-1 monocytes were stained with the membrane potential indicator JC-1 and determined to have a median per-cell uncertainty in JC-1 intensity of 4% within a population distribution spanning roughly a 50% coefficient of variation (CV; population standard deviation divided its mean). We also isolated mitochondria labeled with MitoTracker Deep Red from THP-1 cells using a nitrogen bomb and found per-mitochondrial intensity uncertainty to be roughly 12%, which is a good benchmark given their small size and relatively low intensity. Ultimately, our goal is to use the kinetic aspect of the serial cytometer to track dynamics such as individual mitochondrial function and calcium signaling following in-flow chemical perturbation.

Quirks and All: unlocking comprehensive biological discovery out of your spectral flow cytometry datasets

David Rach, Ph.D.
University of Maryland Greenebaum Comprehensive Cancer Center
Flow Cytometry Shared Resource

Spectral flow cytometry (SFC), with its capacity to resolve remarkably similar fluorophores while rapidly acquiring millions of events, has permitted the profiling of immune systems at breadth and depths unimaginable just a few years ago. For researchers working with limited biospecimen, this has been a game changer, allowing for wider understanding of human immune heterogeneity, and the response to infection and immunization.

While the analytical process remains essentially unchanged if only one cell subset is of interest, the increased dimensionality of the datasets compared to conventional flow cytometry necessitate the use of unsupervised or semi-supervised analytical approaches if researchers wish to characterize the majority of cell populations within their acquired samples. However, many of these existing tools, originally developed for use with mass cytometry and single-cell RNA-seq, struggle due to both the increased number of events, as well as the inherent uncertainty in the unmixing process, with the resulting variation in MFI frequently introducing batch effects.

To address these unique quirks, over the last two years, we have been working on developing open-source tools in both R and Rust, facilitating troubleshooting of unmixing errors due to tandem degradation and additional autofluorescence; pre-emptive identification of issues arising from failures of instrument quality control; and enabling more thorough and reproducible analysis to profile all cell populations present within our datasets. We highlight these approaches in context of an on-going analysis of a rare clinical cohort of HIV-exposed uninfected (HEU) neonates, using 32-fluorophore SFC panel for innate-like and conventional T cells, acquired on a 5-laser Cytek Aurora. All the while, weighing the question: "What good is any tool if the community as a whole is unable to use it?"

PARP inhibition plus a selective bifunctional T-cell agonist drives a polyclonal antitumor immunity and tumor regression: Relevance of a 30-color Spectral Flow Cytometry Panel

Ginette S. Santiago-Sanchez[1], Francesca Rosato[1], Kellsye P. Fabian[1], Michelle R. Padget[1], Jung-Min Lee[3], Fatima Karzai[4], Jeffrey Schlom[1], James L. Gulley[1], Duane H. Hamilton[1], Jonelle K. Lee[1], Margaret R. Pruitt[1], Andrew Bayliffe[2], Zhen Su[2], Jacques Moisan[2], Madan Katragadda[2], James W. Hodge[1]

[1] Center for Immuno-Oncology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. [2]Marengo Therapeutics, Cambridge, MA, USA. [3]Women's Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA. [4]Genitourinary Malignancies Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA.

Metastatic castration-resistant prostate cancer (mCRPC) remains an aggressive disease with limited response to systemic therapies despite androgen deprivation. Immune-excluded prostate tumors are typically resistant to immunotherapy, highlighting the need for strategies that promote immune infiltration and antigen spreading. STAR0602 is a selective bifunctional T-cell agonist composed of an antibody targeting Vβ6 and Vβ10 T-cell receptor (TCR) chains fused to human interleukin-2 that selectively expands $Vβ6^+$ $CD8^+$ memory T cells and has demonstrated clinical activity as a monotherapy in anti-PD(L)-1–resistant tumors (NCT05592626). We hypothesized that combining PARP inhibition with Vβ-directed T-cell activation would enhance antitumor immunity and promote polyclonal T-cell responses in immune-excluded prostate cancer. We evaluated the antitumor activity of the PARP inhibitor olaparib combined with mSTAR1302, a murine surrogate for STAR0602, in the TRAMP-C2 prostate tumor model. We assessed tumor growth, survival, and immune responses by multi-parameter flow cytometry and functional depletion studies. Combination therapy with olaparib and mSTAR1302 induced significant tumor regression and improved survival compared with either agent alone. Treatment increased tumor-infiltrating lymphocytes, expanded activated $Vβ13^+$ $CD4^+$ and $Vβ13^+$ $CD8^+$ T cells, reduced immunosuppressive populations, and enriched stem-like progenitor exhausted $CD8^+$ T cells. Depletion studies demonstrated that $Vβ13^+$ $CD4^+$ T cells, $Vβ13^+$ $CD8^+$ T cells, natural killer (NK) cells, and interferon-γ were required for therapeutic efficacy. TRAIL-R2 knockout experiments demonstrated that tumor-intrinsic TRAIL-R2 signaling contributes to the antitumor activity of the combination. Mechanistically, these findings support a model in which PARP inhibition sensitizes tumors while mSTAR1302-mediated $Vβ13^+$ T-cell expansion initiates a broader polyclonal antitumor immune response associated with antigen spreading and recognition of multiple tumor antigens and neoepitopes. Together, these results provided a mechanistic rationale for developing a clinical trial at NIH to evaluate olaparib in combination with STAR0602 in patients with mCRPC who have progressed on androgen deprivation therapy.

Using Imaging Flow Cytometry to Quantify Bispecific Immune Cell Engagers

Nicholas Battaglia[1], Margaret Costanzo[2], Zuleima Munoz[1], Vasudev Kantae[3], Steven Eck[2], Daisy Yuill[3], Adrian Freeman[3], Nikki Heller[1], Raffaello Cimbro[4]

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[4]Dynamic Omics, CGR, Discovery Sciences, BioPharmaceuticals R&D, AstraZeneca, Cambridge, UK

Background: Quantification of bispecific T-cell engager (TCE) interactions at the single-cell level remains a major challenge for optimizing pharmacodynamic (PD) characterization and dose selection. Conventional flow cytometry cannot accurately quantify cell-cell interaction complexes formed when a TCE simultaneously binds T cells and target cells, while microscopy lacks the throughput needed to assess rare cell populations. These limitations restrict accurate measurement of target engagement, development of robust PD models, and validation of target depletion across non-clinical species.

Methods: We developed a first-in-class spectral imaging flow cytometry panel on the BD FACSDiscover A8 platform to directly visualize and quantify TCE trimer formation and associated cell-cell interactions. The assay was applied to human samples to evaluate trimer formation across concentrations and assess species translatability.

Results: The platform enabled direct quantification of TCE-mediated trimers at single-cell resolution. Trimer frequencies containing intended effector cells were highest at lower Effector:Target ratios, suggesting a potentially important window for maximizing productive target engagement while limiting excessive T-cell activation and reducing risk of cytokine release syndrome.

Conclusions: Imaging flow cytometry provides a novel and quantitative way to assess TCE trimer formation and cell-cell interactions in biologically relevant matrices. The approach has potential to strengthen PD modeling, improve prediction of in vivo responses, and guide efficacious dose selection across the TCE portfolio. It may also be extended to additional TCE molecules in immunology and hematology indications.

AI Model Training on the Attune CytPix for Macrophage Characterization

Zuleima Munoz[1], Giulia Riparini [1], Nikki Heller [1], Raffaello Cimbro[2]

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Macrophage polarization is highly dynamic and encompasses functionally distinct states that are often difficult to resolve using conventional phenotyping alone. In this study, we are developing a label-free AI model using Attune CytPix image-enabled flow cytometry to characterize macrophages across the major polarization states. Human monocyte-derived macrophages were differentiated in vitro for a week from healthy donor blood CD14+ monocytes. The macrophages were then polarized to generate unpolarized (M0), M1 (IFN-g+LPS), and M2(IL-4) macrophages, with the M2 compartment further differentiated into M2a(IL-4), M2b (heat-aggregated+LPS), M2c (IL-10, TGF-b), and M2d (LPS+ A2AR agonist) subsets using cytokine- and agonist-specific polarization media. After 48 h of polarization, cells were lifted and stained with labelled characteristic macrophage surface markers for each polarization state. Both fluorescence data and cell images were acquired on the Attune CytPix platform. Using the cellular features captured by the instrument, including morphology, light scatter, and image-derived phenotypic signatures, we used the built-in AI to train a model to learn multidimensional patterns associated with the different macrophage states. Expression of the characteristic surface markers validated that the model was able to recognize inflammatory and alternatively activated macrophage states, and to distinguish closely related M2 subtypes that are traditionally challenging to separate. Future work will focus on using the model to identify macrophage phenotypes from clinically relevant, in vivo settings, such as tumor-associated macrophages, inflammation resolving and tissue reparative macrophages, or macrophages associated with fibrosis. Overall, the experiment establishes a workflow for combining standardized macrophage polarization protocols with AI-based label-free classification on the Attune CytPix, supporting scalable, reproducible, and high-resolution macrophage characterization for immunology research and translational applications.

Neuromorphic Label-free Imaging for Kinetic Serial Flow Cytometry of Cell Dynamics

Matthew DiSalvo[1], Gregory A. Cooksey[1]

[1] National Institute of Standards and Technology

The temporal dynamics of cellular behaviors within flow cytometers are critical dimensions for measurement that distinguish functional phenotypes. We recently developed serial kinetic flow cytometers that track and repetitively measure single cells to map dynamics in cell deformability and membrane integrity. Here, we report the incorporation of neuromorphic event-based imaging to flow cytometers to provide continual motion monitoring in support of time-resolved measurements. Event sensors operate as an ensemble of asynchronously triggering pixels and offer a highly data-efficient, low power, and high temporal resolution alternative for imaging flow cytometry (IFC). The self-triggering nature of event signals, novel readout mechanism, and sparse data format together mark a substantial paradigm shift in IFC. We report a complete pipeline for neuromorphic transmitted light IFC that extracts particle trajectories and shapes from event data. This pipeline bypasses traditional image generation and analysis and instead uses novel algorithms to filter, segment, and extract particle trajectories from raw event streams as sparse 3D (x-y-t) data at their full timestamping resolution (1 μs). Neuromorphic IFC was applied to continually monitor cells and particles under perturbations in hydrodynamic and inertial flow focusing. We anticipate that these methods for neuromorphic IFC open new opportunities for instrument design, tuning, and quality control and will support the development of advanced kinetic serial flow cytometers.

Ben Janoschek

Regional Field Application Lead, Sony Biotechnology Inc.

Building Robust Unmixing in High-Parameter Spectral Flow Cytometry

As high-parameter spectral flow cytometry expands, reliable unmixing has become a key challenge. The technological and experimental factors that determine unmixing quality are not yet systematically understood. In this talk, we present a framework for robust unmixing based on two key principles: separation - the correct identification of fluorochrome emission spectra, and orthogonality - the consistency of unmixing across different reference controls. Using the Sony ID7000™ Spectral Cell Analyzer, we discuss how instrument components and design principles contribute to each principle, enabling robust unmixing regardless of single-stained control type (beads or cells) or sample brightness.

Josh Welsh

Waters Biosciences

What lies beyond arbitrary fluorescence standardization?

The increasing adoption of high-parameter and spectral flow cytometry has increased the need for fluorescence measurements that are reproducible across instruments, sites, and platforms. As dimensionality increases, instrument-dependent differences in fluorescence scaling can affect many parameters simultaneously, while panel composition and fluorophore selection increasingly influence assay sensitivity, spectral complexity, and data concordance. Systematic fluorophore screening and informed panel design are therefore important components of assay standardization, particularly when transferring high-parameter panels between instruments or platforms.

This presentation outlines the tiers of flow cytometry standardization to facilitate cross-platform standardization e.g. conventional to spectral. The practical implications of commonly used fluorescence units, and considerations including the dynamic nature fluorophore photo-characteristics using spectral cytometry and the growing need for reproducible, concordant high-parameter data.

Jessica Gucwa, Ph.D

Senior Field Applications Specialist, Cytek Biosciences

A Practical Spectral Flow Cytometry Approach to Classify Functional Subsets of Human T Cells

T-cell profiling is essential to translational research and biomarker discovery, there is growing demand for standardized assays that enable deep and reproducible characterization of T-cell populations. Spectral flow cytometry, combined with high-quality cFluor reagents, provide a powerful approach to resolve T-cell functional and memory heterogeneity.
The Cytek® cFluor® 21-Color Human T-Cell Kit was developed to support comprehensive identification of major $CD4^+$ T-helper (TH), including TH1, TH2, Th17, Th22, ThGM-CSF, and T follicular helper (Tfh), as well as CD8 T-cytotoxic and polarization subsets, while enabling robust parallel evaluation of the memory T-cell compartment. Careful selection of markers, clones, and conjugates, together with panel optimization, ensure strong resolution across all populations. In addition, validated staining, and acquisition protocols, including flexible overnight staining options, help reduce hands-on time and improve consistency across experiments and laboratories.
We have also demonstrated how this panel can be extended to 40 colors to evaluate polarization and activation states in other immune subsets. Together, these features illustrate how standardized reagent kits can simplify panel implementation, enhance reproducibility, and accelerate in-depth characterization of human T-cell biology for immune monitoring and biomarker discovery.

Andrea Luker

Technical Application Scientist – Maryland, DC, Revvity (Biolegened)

More Than Markers— Unlocking Cellular Function with Fluorescent Probes

Spectral flow cytometry has recently transformed the field by expanding the degree of multiparametric analysis possible within a single sample. In keeping pace with these advances in instrumentation, BioLegend continues to develop novel fluorescent reagents that push flow applications beyond conventional immunophenotyping. This seminar will focus on BioLegend's portfolio of fluorescent probes that introduce a deeper layer of analysis—including tools to study subcellular organelles and a variety of cell health metrics. Additionally, I will highlight assay kits that allow researchers to collect data on metabolic/effector functions (ex. NETosis, phagocytosis, fatty acid transport, etc)

Sarah Kotanchiyev, Ph.D.

Field Application Scientist, SlingshotBio

Authentic Spectral Signatures for Reproducible Results: How Cell Mimics Improve Spectral Flow Cytometry

Spectral flow cytometry has expanded panel sizes well beyond 40 colors, but conventional controls, such as polystyrene beads and donor-derived cells, often fail to reproduce the true autofluorescence and spectral signatures needed for accurate unmixing. Slingshot Bio's precision-engineered synthetic cell mimics have cell-like scatter and display the authentic spectral signature of all fluorophores in an experiment, addressing these challenges while drastically improving convenience in real-world flow cytometry workflows. FluoroCytes™ contain fluorescent proteins to improve reference spectra accuracy relative to polystyrene beads, without the need to create biological single-color controls. Similarly, Slingshot will present data showing how its ViaComp® Ultra™ XT - Amine, a viability control with multi-species antibody capture and amine-reactive viability reduces spectral spreading error while simplifying setup for a 36-color panel. Finally, Slingshot's immunophenotyping controls, such as TBNK Mimic™, contain biological antigen densities for T cell, B cell, and NK cell populations to provide reproducible support for assay development and longitudinal performance monitoring. Together, these controls strengthen the reproducibility and reliability of spectral cytometry workflows across instruments and panels.

Sean Maynard

Manager, Scientific Solutions & Platform Adoption, Partillion Bioscience

When Cells Meet: Functional Cell–Cell Interaction Screening with Nanovials

Many important cellular behaviors emerge only when cells interact, yet conventional flow cytometry typically analyzes cells as independent events. Nanovials provide defined microenvironments that maintain interacting cell pairs as a single sortable event, enabling cell–cell interaction assays within standard flow cytometry workflows.

This presentation will describe practical approaches for establishing two-cell microenvironments, analyzing and sorting Nanovial–cell complexes using conventional flow cytometers and cell sorters, and recovering viable cell pairs for downstream single-cell sequencing. Applications will include investigating immune cell–tumor cell interactions through secretion, target engagement, and cellular response, as well as discovering antibodies against antigens presented in their native cellular context.