

Inflammation, Infection Immunity and Immunotherapy (I-4ward) Progress Report October 2024

Members of the 2024 I-4ward Task force included: Frances Lund PhD (Microbiology, Task Force Chair), Andre Ballesteros-Tato (Medicine, Rheumatology), David Kimberlin MD (Pediatrics, ID), Khurram Bashir MD MPH (Neurology), Mark Banaszak Holl PhD (Mechanical and Materials Engineering), Jessica Scofield PhD (Microbiology), Rakesh Patel PhD (Pathology), Jennifer Pollock PhD (Medicine, Nephrology), Carlos Orihuela PhD (Microbiology).

The major 2024 goal of I-4ward has been to support the development and launch of spatial single-cell technologies at UAB.

Background. Experimental approaches that examine population-level characteristics of cells in healthy and diseased tissues often obscure crucial differences between individual cells. A recent report from Berkshire Hathaway indicated that “Single cell genomics is driving an enormous new research area with wide health care applications and opening up a whole new world of biotechnology”. While UAB has excellent single cell (sc) RNA-seq capabilities within the Flow Cytometry and Single Cell Core (FCSC, [UAB Flow Core webpage](#)), there was no core location on campus where investigators could elucidate the transcriptome and proteome of cells with single cell resolution *within tissue sections*. Moreover, UAB did not have the ability to link the molecular and proteomic data from single cells to their location within a tissue. This linkage is crucial for analysis of tissue-specific “neighborhoods” or niches. For example, one could imagine that if we could identify the unique transcriptional programs that differ between immune cells that invade the tumor microenvironment versus the immune cells that are excluded from the tumor microenvironment, it might be possible to identify immune modulating therapeutics that would convert the excluded cells into cells that can enter the tumor and exert anti-tumor activity.

Progress. Our goal was to support needed infrastructure (equipment, data management tools, software, informatics) for UAB faculty to address fundamental questions about interactions between neighboring cells within a tissue with single cell resolution. I-4ward partnered with the entities listed below, including the Brain Health Task Force, to purchase, install and host:

- (i) Lunaphore COMET ([COMET webpage](#)), which can be used to measure 40 proteins in each cell at single cell spatial resolution. Partners included O’Neal Comprehensive Cancer Center (OCCC) and the Immunology Institute (II).
 - a. The COMET is available for assisted use in the FCSC Core ([UAB COMET Services](#)) with more than 20 UAB labs now using the COMET for their research projects.
 - b. I-4ward provided financial support for optimizing the staining of tissue slides with ~120 different antibodies (60 antibodies specific for mouse proteins and 60 antibodies specific for human proteins ([List of optimized antibodies](#)). Currently, there are 14 different antibody panels ([list of optimized panels](#)) that are available for “off-the-shelf” analysis of tissues.
 - c. I-4ward provided logistical support for the recent Spatial Day Workshop that included presentations by Lunaphore and Visiopharm software ([Spatial Day](#))
 - d. I-4ward partnered with II to launch a COMET Voucher RFA ([COMET RFA](#)) that will support up to 8 COMET-based projects. The deadline for applications is Nov. 15, 2024, and the awards (up to \$20K/project) will be made by January 2025.

- (ii) 10X-Genomics Xenium instrumentation ([Xenium webpage](#)), which is used to obtain single cell spatial resolution of 1000s of different mRNA species/cell. Partners providing support for this purchase and installation included the HSOM Brain Health Task Force, 15 UAB Centers and Institutes, and 11 Departments and Divisions.
 - a. I-4ward and the Brain Health were awarded an HSF-GEF grant in Fall 2023. The Xenium was purchased in January 2024 and installed in the FCSC Core in early Summer 2024. Training for FCSC Core staff by 10X technical group was completed in late Summer 2024.
 - b. The first test experiments were designed and runs on the Xenium are scheduled for November 2024. I-4ward provided financial support for purchasing 10X reagents for the first test run.
 - c. Xenium will be available for assisted use by UAB investigators in early 2025.

Infrastructure to support storage and analysis of very large datasets is critical to any project using COMET

and/or Xenium technologies. I-4ward and II established a working group of investigators with expertise in computational biology, information technology, software development, single cell informatics, spatial biology and single cell analysis. This group reports weekly and meets monthly to ensure the establishment and **integration** of each of these critical infrastructure pieces. Infrastructure accomplishments include:

- (iii) Establishment of Visiopharm ([Visiopharm webpage](#)) software platform at UAB. Visiopharm is an AI-driven analytic platform that can be used to integrate and quantitate data from tissue sections at single cell resolution. Visiopharm training modules and support are available for UAB users.
- (iv) Establishment of Microsoft Azure Cloud Computing seats. This platform is required for analysis of the large imaging datafiles. Partners included FCSC Core, II, Research Computing and members of D-TECH.
- (v) Construction of a data pipeline to facilitate the movement (ie Globus) of big data files to LTS (long term storage) and to supercomputer (CHEAHA) for data analysis. Partners included FCSC Core, II, Research Computing and members of D-TECH.
- (vi) Dedicated effort support for informatics staff and faculty in the Biological Data Sciences Core (U-BDS, ([U-BDS webpage](#))) and the Dept of Radiology to develop standardized Xenium analytic pipelines and advanced analytic tools for nearest neighbor and neighborhood/niche analyses using Xenium and COMET datasets.

2025 Goals

1. Financial support for 4 COMET vouchers that will be selected from the applications due Nov. 15 2024
2. Logistical support for quarterly Spatial Workshop to introduce new users to Spatial Biology approaches and to cover topics of interest by current users. Next workshop will focus on Xenium
3. Financial support (effort) for informatics staff who will refine Xenium data pipelines AND develop new tools to integrate Xenium and COMET datasets from the same tissue sample
4. Financial support for Xenium voucher RFA that will be released in early 2025. Expect to support 4 projects
5. Logistical support for recruitment awards for graduate student trainees ([GBS recruitment awards](#))
6. Financial support for the development and launch of a NEW Spatial Biology and Inflammation Prime Program that will be closely associated with the Brain Prime program already launched by the Brain Health Taskforce
7. Financial support to acquire additional instrumentation (e.g. spatial metabolomics or spatial genomics) that will be used to understand how cellular/tissue “neighborhoods” affect inflammatory processes in settings of health and disease
8. Design certificate program in “Spatial Biology” for graduate and post-doctoral trainees

Description of the NEW Spatial Biology and Inflammation Prime Program: I-4ward is establishing a **Postdoctoral Research Initiative for Multidisciplinary Exploration (Prime)** program that will be modeled after the successful Brain Prime program ([Brain-PRIME](#)) started by the Brain Health Task force. Briefly, our goal is to increase success rates in recruiting outstanding postdoctoral fellows (PDF) interested in I-4ward relevant research and spatial biology to UAB labs. I-4ward will provide selected PDF candidates with financial incentives (augmented salary and career development funds for 2 years) to join I-4ward labs at UAB. These PDFs will be part of a cohort-based program that will provide the PDFs with a built-in network of peers. The program will offer career mentoring and career development training that is specifically tailored for fellows interested in understanding how spatial biology contributes to the molecular and cellular drivers of infectious and inflammatory-mediated diseases. The I-4ward task force will release an RFA in November 2024 to identify a *Faculty Director* for the new **Spatial Biology and Inflammation Prime Program**. We expect that the new Faculty Director will be identified by January 2025. The new director will interface with the leaders of other HSOM Prime programs to establish UAB as a true national and international destination for post-doctoral fellows. We plan to advertise for the first cohort of Prime PDFs by April 2025 and begin interviewing and enrolling PDFs into the program by end of Summer 2025.