

## Overview/Abstract

The spatial organization of cells within tissues is critical to establish physiological functions. Tissues are complex environments that comprise many different cell types that interact to provide functions beyond the cellular scale. Cellular interactions drive this scale integration (from cell to tissue to organ) and play a critical role in healthy and diseased tissues. Most organs are not fully understood, comprehensive gene expression data at high spatial resolution could really improve their understanding. **Our overarching goal is to develop an easy-to-use discovery platform to contextualize genomic information in-tissue by integrating high-resolution sequencing and spatial information.** Our platform will reveal critical information about how cells interact, which is critical in understanding how healthy tissues function and how diseases develop. It will give easy access to this information by providing high resolution and high depth data and lower the barriers to entry for this groundbreaking technology. Providing a novel discovery tool to decipher the functional organization of tissues has a general and direct biological and medical relevance.

Sequencing technologies can generate a large amount of information in an unbiased and cost-effective manner. However, in-tissue cellular interactions cannot be resolved without spatial information, typically discarded during sequencing. Recent methods have strived to deliver spatial genomics. Still, they fall short because of low throughput (laser capture) [1], gene bias (Fluorescence *In Situ* Hybridization (FISH) techniques) [2, 3], high complexity (in tissue sequencing) [4], and low data quality (capture microarrays) [5, 6]. Capture microarrays are promising because their simple format makes them easily adoptable by the broader scientific community. **Two limitations** currently impede the performance of capture microarrays: 1) a low read count that precludes deep analysis of the transcriptome, and 2) the difficulty in decoding the pattern of the randomly arrayed barcodes used to capture and spatially tag mRNA molecules.

In contrast, our platform will enable high data quality to support discovery and a low entry barrier to spatial genomics. Our project is based on **three hypotheses**: 1) mRNA capture in closed microwells will increase the number of molecules collected without sacrificing spatial resolution, 2) enabling multistep workflows, including washing steps, will provide for more efficient cDNA synthesis and a higher number of reads per barcode, 3) neighboring information between beads, instead of the beads' absolute positions, is sufficient to decipher the barcode spatial pattern. We are uniquely qualified to perform this interdisciplinary project thanks to our background in physics, engineering, microfabrication, molecular biology, and assay development. In addition, our collaborator Prof. Mike Wigler will provide support with sequencing generation and analysis.

**We will address limitations of current approaches by developing a two-prong strategy:**

**1-** We will adapt our novel device, an array of microwells on top of a filter, to perform spatial transcriptomics. The microwells will 1) contain the barcoded capture beads; 2) act as independent microreactors. The closed microwells will allow optimization of mRNA capture without sacrificing spatial resolution. The filter will enable washing steps to improve cDNA conversion. Together, those advances promise higher-quality data from tissue sections while maintaining spatial information at single-cell resolution.

**2-** We will simplify barcode mapping by developing a strategy where pattern deciphering is performed *in silico* using sequencing data. Currently, barcode maps are obtained *via* repeated cycles of FISH [5] or SoliD sequencing [6]; however, both require specialized skills and equipment. In contrast, we hypothesize that neighbor information between beads, as opposed to the absolute positions of beads, is sufficient to decipher the spatial barcode pattern. Our approach links neighboring beads using a unique location barcode shared locally to create a connectivity map that leverages sequencing and computing capabilities. Location barcodes are provided by a random array of donor beads on top of the capture beads. Our strategy relies on the counter-intuitive idea that a random pattern can be used to decipher a random pattern.