Encoded Hydrogel Microparticles for High-throughput Molecular Diagnostics and Personalized Medicine

The ability to accurately detect and quantify biological molecules in a complex mixture is crucial in both basic research and clinical settings. Advancements in molecular diagnostics and personalized medicine require robust detection technologies that can obtain high-density information from biological samples in a rapid and cost-effective manner. Suspension (particle-based) arrays offer several advantages over conventional planar microarrays in the multiplexed detection of biomolecules, including the use of smaller sample volumes, more favorable probe-target binding kinetics, and rapid probe-set modification. The work presented in this thesis focuses on the development of a graphically-encoded, hydrogel-based microparticle array for the highly sensitive, multiplexed detection of biological molecules that may be used as markers for disease diagnosis, prognosis, and treatment selection.

The Doyle Lab has previously described a microfluidic-based photolithography process (stop-flow lithography, SFL) for rapidly polymerizing geometrically and chemically complex hydrogel microparticles from liquid monomer streams at rates up to $10^4$ per hour. In this work, we used the SFL process to develop a suite of microparticle-based techniques for the high-throughput expression profiling of medically-relevant biomolecules. Utilizing SFL’s ability to polymerize across several laminar co-flowing monomer streams, we created particles consisting of multiple chemical sections, with one “code” region and one or more “probe” regions for biomolecule capture. In the code region, unpolymerized holes in the wafer structure of the particle (created with an appropriate transparency mask) were used to construct a graphical code that could then be used to identify the DNA capture probe(s) covalently incorporated within the other region(s) of the gel particle for analysis purposes after multiplexed assays.

Prior to assay development, we created a microfluidic system for the rapid flow alignment and fluorescence scanning of these multifunctional hydrogel microparticles in order to enable high-throughput analysis of particles. Using high-speed imaging, we developed and optimized a flow-through system that allowed for a high particle throughput and ensured proper particle alignment for decoding and target quantification. A tapered channel flanked by side focusing streams was used to orient the flexible, tablet-shaped particles into a single-file, well-ordered flow in the center of the channel. The ability to align particles in the channel with high precision enabled photomultiplier tube (PMT)-based detection with a simple one-dimensional line scan that integrated particle fluorescence intensity along a thin
laser excitation beam established perpendicular to the flow direction. The resulting fluorescence profile was then used to determine the code identity of a passing particle, ascertain the probe(s) it carries, and measure the extent of the binding events on probe regions at rates up to 25 particles per second.

MicroRNAs (miRNAs) are short, non-protein-coding nucleic acids that have recently been shown to exert a significant amount of control over the translation of proteins from mRNA, and as a result, they have rapidly become a focal point in the search for effective biomarkers for disease diagnosis, prognosis, and treatment efficacy. Despite advances in technologies for the multiplexed assay of nucleic acids, it has proven challenging to accurately and efficiently profile miRNAs due to their small size, wide range of abundance, secondary structure, and high degree of sequence homology. In this work, we established the encoded gel particle system as an efficient miRNA profiling platform through the introduction of a novel ligation-based fluorescent labeling scheme that selectively attached fluorophores only to those targets captured on gel-embedded probes. Using our microfluidic scanner, we validated the system by performing a 12-plex assay of clinically relevant miRNA targets and demonstrated efficient miRNA expression profiling over four human cancer types in only three hours using low-input total RNA and a simple workflow.

We further enhanced assay performance on our encoded hydrogel microparticles through the adoption of a signal amplification scheme that provided sub-femtomolar sensitivity and single-molecule reporting resolution in miRNA assays. Rolling circle amplification (RCA) of a single sequence that was ligated to all captured targets provided the ability to label each target with multiple fluorescent reporters and eliminated the possibility of amplification bias. The high degree of sensitivity achieved by the RCA scheme and the resistance to fouling afforded by the use of gel particles were leveraged to directly detect miRNA in small quantities of unprocessed human serum samples without the need for RNA extraction or target-amplification steps, an achievement with powerful implications for the development of rapid, non-invasive diagnostic assays.

We also applied our particle-based detection scheme to the analysis of raw cellular lysate, achieving efficient detection of miRNA targets from HeLa lysate without the need for RNA extraction. In addition, seeking to probe the miRNA expression profiles of single cells, we developed a microwell-based system that enabled the colocalization of gel particles and cells in 3-nL reaction volumes, which dramatically lowered the limit of detection of the particles and allowed for interrogation of miRNAs from fewer than ten cells in preliminary experiments.

This platform's unprecedented combination of sensitivity, flexibility, and throughput offers exciting possibilities for future discovery and clinical applications, particularly in the rapid quantification of low-abundance miRNA and other biomolecules in readily-accessible media like serum and raw lysate.