# Spatiotemporal encoding for multiplexing of droplet assays

Stanford inventors have devised a method of multiplexing droplet reactions to analyze and identify many reactions in parallel on a single microfluidic chip using off-the-shelf flow control and valving.

Droplet microfluidics systems rely on creating droplets within a carrier, immiscible fluid, and are widely used in analytical detection in biomedical research and the clinic. Multiplexing droplet multiplexed systems isuseful for increasing throughput and required for some custom assays, and several strategies have been implemented. These include multiple fluorescence dyes (e.g., using multiple emission wavelengths and color filters), barcoding of droplets using barcoded particles, and barcoding using gel beads containing barcoded synthetic DNA. When samples are labeled by color, a one-to-one relationship between color and a particle or reaction is common, limiting the degree of multiplexing. This is due to the finite bandwidth of the emission spectrum of commonly used fluorophores, and the limited range of the visible spectrum. There is a need for higher multiplexing capabilities for droplet microfluidics systems.

Therefore, Stanford inventors developed a novel multiplexing method that uses fluorescence and encoding of multiple samples by the spatial and temporal sequence of droplet formations and the subsequent spatial positioning of droplets in a constrained, poorly mixed output region. This new method can be applied in varying configurations, one of which is to use four different dyes in combination with spatial positioning to achieve 48-sample multiplexing.

Stage of development Research - in vitro

### Applications

• Multiplexed droplet-based assays in biomedical research, drug discovery and cancer screening.

#### **Advantages**

- Improved degree of multiplexing by one or two orders of magnitude relative to current techniques.
- There currently exists no assay that uses both fluorescent signal and droplet position to differentiate samples in droplet-based microfluidics assays.

#### Innovators

- Juan Santiago
- Alexandre Avaro

## **Licensing Contact**

#### Chris Tagge

Technology Licensing Program Manager

<u>Email</u>