Chewing on biology, one bit at a time – high throughput assays at single-cell resolution using Drop-Based Microfluidics reveal novel variations in heterogeneous populations.

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Populations are inherently heterogeneous yet most biological assays treat them as a whole, therefore averaging out their internal variations. Drop-Based Microfluidics overcomes the technical difficulties in observing variations between individuals. Micron size drops of water immersed in an inert carrier fluid act as minute reaction vessels where biological assays are performed at a single cell resolution without compromising throughput, scalability and flexibility. When applied to both viral evolution and single-cell epigenetics novel variations between individuals emerge from a seemingly homogenous population.

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POPULATIONS of organisms have substantial heterogeneity that is important for their function and understanding. Such variations between individuals in the population are reflected in both genotype and phenotype. When characterizing a pool of individuals by conventional methods, most variations are lost in the population background and cannot be detected. Instead, isolating individuals in separate compartments enables the observation of single organism variation ns; however, this is challenging since the effective concentration of an individual is orders of magnitude smaller than that of a typical population sample, so that the chance for detection and the rate of reactions becomes impractically low. Thus, a method for isolating single organisms at a high concentration is essential for understanding the behavior and function of heterogeneous biological systems. Drop-Based Microfluidics (DBM) restores the effective concentration in single organism assays by drastically decreasing the reaction volume without compromising assay throughput, scalability and flexibility. We use micron size drops of water immersed in an inert carrier fluid as minute reaction vessels that can be precisely controlled by microfluidic devices. Drops can be formed, refilled, thermo-cycled, merged, split and sorted at rates of millions per hour with exquisite control over individual drops. Thus, using drops we can compartmentalize and assay millions of single organisms in an effective volume, time and cost equivalent to that of assaying a population of a million individuals in a micro-liter sample.

I will describe the application of DBM to both viral evolution and single-cell epigenetics. To study viral evolution, individual viruses are encapsulated in drops together with their hosts. Isolating viruses in drops reduces competition and allows the replication of viruses that would otherwise be driven to extinction by the fittest individuals that typically take over the population. Thus, by compartmentalizing viruses, DBM assays hundreds of times more evolutionary pathways than conventional assays, revealing adaptive phenotypes that were otherwise inaccessible. To analyze cell-cell epigenetic variability of diverse populations, cells are encapsulated one per drop, and then each drop is fused with another drop containing high concentrations of a unique barcode used to tag the contents of the cell. After tagging each cell, drops are pooled and merged and downstream assays are performed on the mix of barcoded cellular information before it is sequenced. Upon sequencing, the cell origin of each fragment is identified by reading the attached barcode. The platform is compatible with both DNA and RNA, uses ligation or hybridization to attach the barcodes and can be scaled up to a large number of cells ultimately limited only by the capacity and cost of Next-Gen sequencing. When applying this method to single-cell ChIP-Seq of mouse Embryonic Stem cells, novel cell-cell variations are observed, revealing two subpopulations that emerge from an a-priori homogenous cell-line.

Conclusion

These examples illustrate how DBM is used to efficiently observe biology one individual at a time, promising novel insight on biological systems with underlying heterogeneity.

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