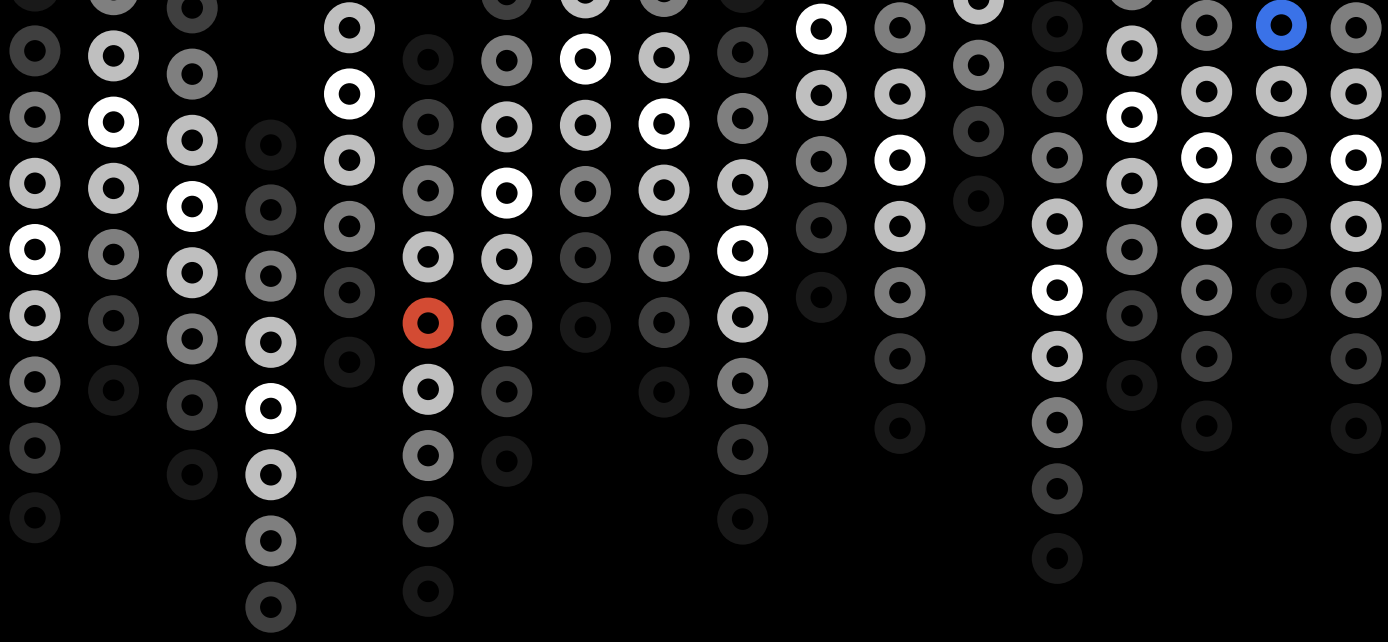


YOUR GROUND CONTROL IN HIGH-THROUGHPUT BIOLOGY

TECHNICAL NOTE

DG-TN0001 – Rev A – Technical Note

CONTROLLABLE DROPLET GENERATION ENHANCES THE ACCURACY AND SENSITIVITY OF CELLULAR AND MOLECULAR ASSAYS

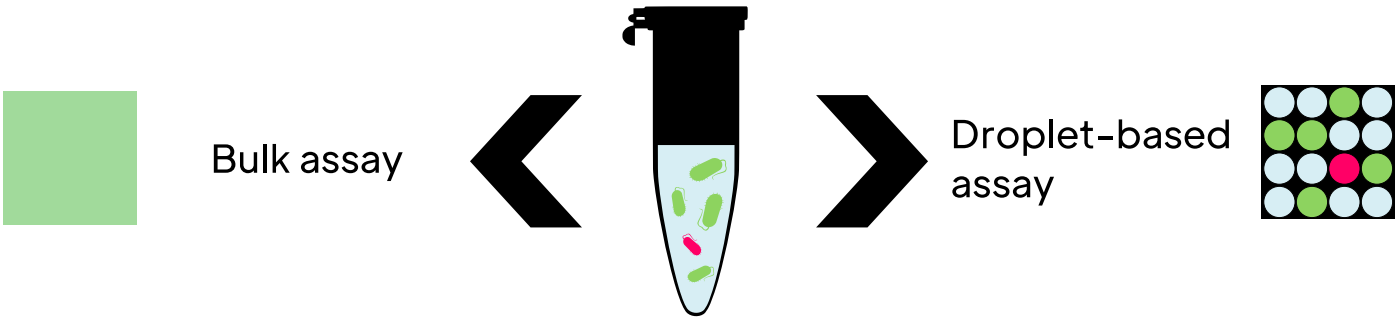
Droplet Genomics

DROPLETGENOMICS.COM

Controllable droplet generation enhances the accuracy and sensitivity of cellular and molecular assays

SUMMARY:

- Assays performed on samples partitioned into microdroplets enable to obtain single-cell or single-molecule level insights.
- Microfluidic technologies allow the controllable generation of monodisperse emulsions.



INTRODUCTION

Cellular heterogeneity has important implications for tissue biology and disease states such as cancer[1]. Conventional experimental techniques that interrogate samples comprising millions of cells (bulk assays) provide an average readout for the cell population, thus making them not suitable to study genetic and/or epigenetic differences between the cells. Droplet-based methods enable partitioning the assay into many isolated compartments, each of which contains, on average, less than one cell. The small volume of the compartments means that cellular contents are at high concentration and can be easily detected or manipulated, which in turn substantially improves the sensitivity of "omics" techniques. Among many exciting examples, droplet-based assays allowed to discover novel rare cell types[2] and to gain valuable insights into precision oncology[3].

Furthermore, compartmentalization of individual molecules improves the linearity of quantitative assays. Many popular conventional methods, such as quantitative PCR, are essentially analog, e.g., average signals from mutant and wild-type molecules present in the sample are obtained. Partitioning ensures the "purification" of target molecules from the surrounding background. Discrete counting of individual DNA molecules powered by droplet-based digital assays demonstrated superior sensitivity over analog

methods by detecting the mutant *KRAS* oncogene in the presence of 200,000–fold excess of wild-type *KRAS*[4]. In addition, DNA compartmentalization can solve the amplification bias issue as each molecule within the droplet is amplified approximately to the same level once amplification reaches a plateau. This notion was practically verified by obtaining superior single-cell genome coverage upon sequencing of emulsion-amplified gDNA[5].

The formation of water-in-oil droplets might be performed by bulk homogenization or using microfluidic devices. Bulk emulsification includes mixing two immiscible fluids and applying force to break down larger micelles into smaller droplets. This approach leads to droplets that are polydisperse due to inhomogeneous turbulent flows[6]. Variation in droplet size results in increased measurement errors and substantially restricts the repertoire of feasible applications. In contrast, microfluidics relies on interfacial tension and viscous shear stresses that depend on the geometry of microfluidic chips and fluid flow rates[7]. Since these parameters are kept constant, all generated drops exhibit narrow size distribution resulting in a monodisperse emulsion.

Microfluidic droplet formation approaches can be classified into active and passive. Active methods use external energy, such as electric, magnetic or centrifugal, while simpler passive droplet formation relies on cross-flowing, flow-focusing, co-flowing, or step emulsification[8]. In flow-focusing design, immiscible fluids are hydrodynamically focused while flowing in the same direction. Droplets are formed with high frequencies at a narrower nozzle region (Figure 1). Flow-focusing geometry is often preferred as such microfluidic devices can be readily fabricated with soft lithography and offer tight control over droplet diameter and generation rate.

Droplet Genomics has developed user-friendly solutions for controlled droplet generation. The **Onyx system** equipped with 4 syringe pumps, high-speed microscopy, and in-line droplet analysis capabilities as well as a wide selection of microfluidic chips provide unprecedented control over your droplet-based experiments while keeping the flexibility to accommodate custom designs.

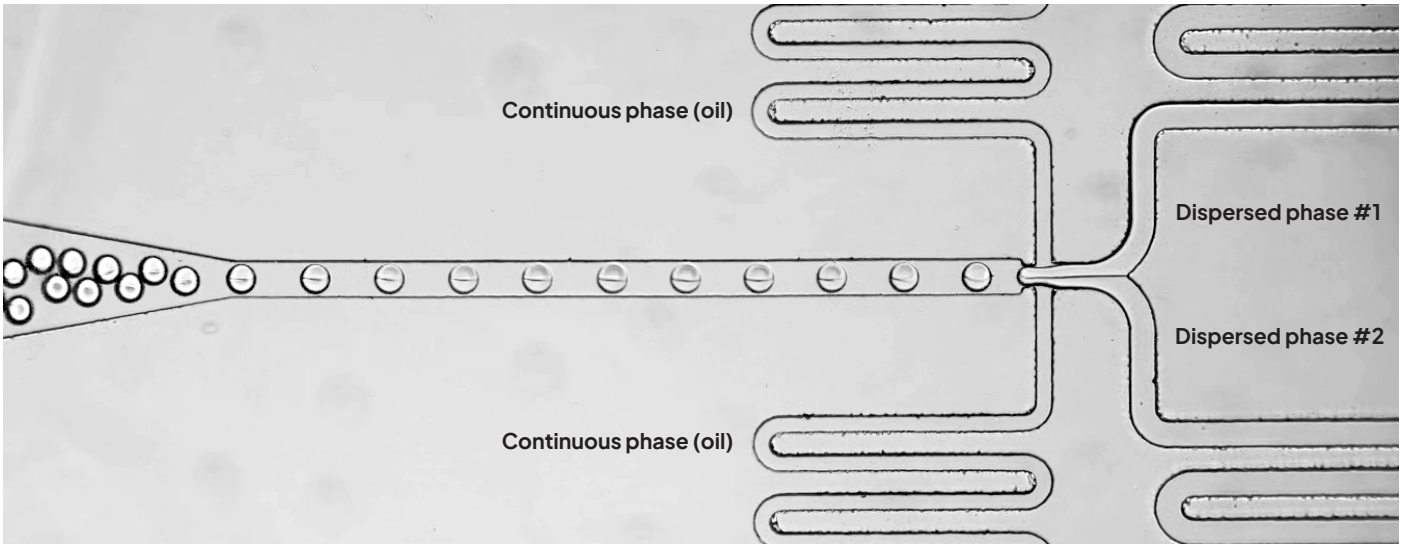


Figure 1. Droplet generation with Onyx system using a flow-focusing microfluidic chip.

CONSIDERATIONS

What droplet size is right for my assay? The sensitivity of droplet-based assays is limited by the number of individual droplets that can be analyzed. The smallest possible droplets are typically desired for molecular analysis applications as they enable higher local concentrations of analytes and higher throughputs without inflating the analysis cost. On the other hand, cell culturing requires larger droplets[9,10] to ensure that a sufficient amount of nutrients is available to cells. Droplet Genomics offers a selection of droplet generation chips to produce uniform pL- to nL-scale droplets.

Will my emulsion remain stable throughout the experiment? Poor emulsion stability leading to premature droplet coalescence may drastically decrease assay reliability. Emulsion stability depends on many factors, such as droplet size distribution, type and concentration of surfactant, the composition of an aqueous phase, and temperature. Emulsions generated with Droplet Genomics Droplet Stabilization Oil are suitable for most of the standard molecular biology assays including PCR.

Table 1. Modulating flow rates can lead to the formation of droplets of various sizes. Exemplary flow rates and the size of resulting droplets produced with Droplet Genomics droplet generation chips.

	Droplet diameter, µm	Droplet volume, pL	Aqueous phase 1 flow rate, µL/h	Aqueous phase 2 flow rate, µL/h	Oil phase flow rate, µL/h	Drops per second
DG-CF-35	30	14.2	50	50	500	2000
	35	22.9	50	50	200	1200
	40	33.9	50	50	125	800
DG-CF-60	63	128.1	200	200	800	900
	69	173.2	200	200	600	600
	77	239.3	200	200	400	500

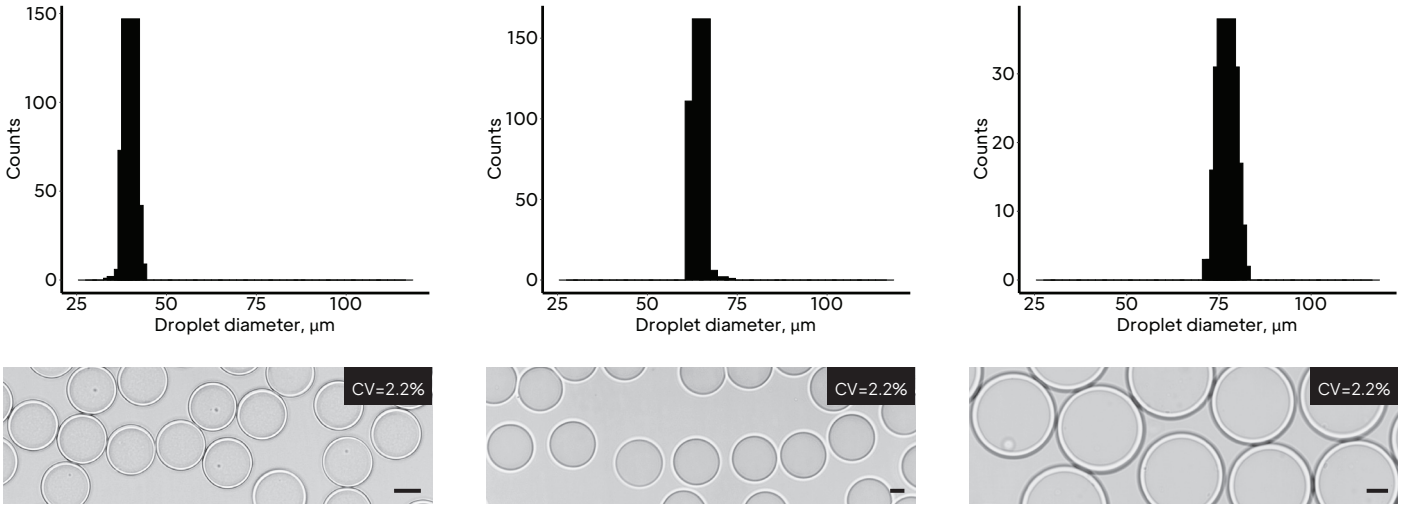


Figure 2. Droplet size distribution measured after emulsion generation with Onyx system. All scale bars represent 20 µm.

DROPLET GENERATION

Producing droplets of controlled sizes as well as at controlled generation rates are key factors to obtain consistent and robust results of droplet-based assays. Droplet size is determined by the balance between interfacial tension and shearing force. Flow rate ratio variation is an established technique for adjusting droplet size. The increase in the continuous phase (oil) flow rate enables the reduction of droplet size while the decrease in oil flow rate will result in the formation of larger droplets. Table 1 demonstrates how droplet size can be modulated by flow rate variation for two different co-flow droplet generation devices.

Uniform emulsions allow for predictable experimental design and minimize the uncertainty of digital assays. Partitioning the sample into droplets results in a statistical distribution of targets (cells or molecules) among the compartments. The probability that a droplet will contain k targets is governed by the binomial and Poisson distributions[11]. Variation in droplet volume can skew the distribution of targets, especially at higher concentrations. Emulsions generated with Droplet Genomics Onyx system exhibit droplet size CV of <3% (Figure 2) making Onyx a reliable tool for the production of monodisperse emulsions for a broad range of downstream applications.

CONCLUSIONS

- Sample partitioning into microdroplets substantially increases the sensitivity of “omics” techniques.
- Droplet-based assays preserve cellular heterogeneity and enable discoveries that bulk analyses miss.
- Compartmentalization of individual molecules offers a discrete counting opportunity that considerably improves the sensitivity of rare target detection.
- Uniform emulsions allow minimizing measurement errors of droplet-based assays.
- Droplet Genomics Onyx system enables the controllable generation of monodisperse emulsions making it an ideal choice for droplet-based cellular and molecular assays.

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ORDERING INFORMATION

Product	Quantity	Cat. No.
Instruments		
Onyx	1 instrument	DG-ONYX
Stand-Alone Microfluidic Pump	1 instrument	DG-PMP
Kits		
Droplet Generation Kit	5 runs	DG-KIT-DM
Co-Flow Droplet Generation Kit	5 runs	DG-KIT-CF
Reagents & Consumables		
Droplet Stabilization Oil	15 mL	DG-DSO-15
Sample Loading Oil	15 mL	DG-SLO-15
Emulsion Breaker	1 mL	DG-EB-1
Microfluidic Tubing	30 m (OD 0.76 mm)	DG-MT0.3-30
	30 m (OD 1.07 mm)	DG-MT0.56-30
Microfluidic Chips	1 chip	Please inquire

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TECHNICAL NOTE

DG-TN0001 – Rev A – Technical Note

For technical assistance, contact
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