

Millions of parallel reactions enabled by centrifugal microfluidic array technology for high-dynamic-range digital assays in liquid biopsy

Abstract Submitter: Peter Juelg, Germany*

Co-Authors: Marvin Heyer, Salman Murad, Fabian Lickert, Judith Schlanderer, Helena Gmoser, Tobias Hutzenlaub, Nils Paust

*Hahn-Schickard

Abstract

Background: Digital assays are a key method for various liquid biopsy applications, such as monitoring minimal residual disease using digital PCR (dPCR). These assays typically involve partitioning samples into tens of thousands of parallel reactions, enabling absolute quantification of target molecules, such as cell-free DNA (cfDNA). However, compared to real-time quantitative PCR (qPCR) and next-generation sequencing (NGS), the number of partitions constrains the achievable dynamic range of digital assays. To address this limitation, we propose Centrifugal Microfluidic Array Technology (CM-ArT), which enables rapid and robust partitioning of reaction mixes into millions of parallel reactions.

Objective: We introduce CM-ArT as a microfluidic method capable of fast and massively parallel partitioning. As a proof of concept, we demonstrate the partitioning of various reaction mixes into 3.8 million reaction wells in under 90 seconds, achieving a dynamic range exceeding six logs for digital assays.

Methods: We designed and fabricated a microfluidic chip, incorporating millions of nanoimprinted picoliter wells. The robustness of CM-ArT partitioning was evaluated using fluorescence microscopy across three representative reaction mixes for PCR, loop-mediated isothermal amplification (LAMP), and recombinase polymerase amplification (RPA). Additionally, we demonstrated a digital LAMP assay within the chip.

Results: The current CM-ArT chip design features 3.6 pL wells arranged in six arrays, each containing 630,000 wells, resulting in 3.8 million reactions per chip. Further optimization of well size and chamber alignment could increase this number beyond 10 million reactions per chip. Homogeneous partitioning was achieved within 90 seconds across all three reaction mixes. The integration of a digital LAMP assay successfully differentiated positive and negative partitions at varying target molecule concentrations.

Conclusions: We demonstrate rapid and massively parallel liquid partitioning in high-density well arrays using CM-ArT. Its compatibility with various reaction mixes makes it a versatile platform for digital assays, while also mitigating sample preparation variations. The partitioning capability of CM-ArT enables applications requiring exceptionally high dynamic ranges, such as the quantification of cfDNA mutations at parts per million (ppm) levels or of NGS libraries. Future work will focus on expanding the range of digital assays and integrating on-chip sample preparation to facilitate sample-to-answer liquid biopsy testing.

Do you have any conflicts of interest?

No, I do not have a conflict of interest.