OFF-CHIP MONODISPERSE DROPLET GENERATION FOR DIGITAL PCR AND DIGITAL LAMP BY CENTRIFUGATION

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Stable micro-emulsion droplets as miniaturized reactors have enabled a wide range of synthetic and bioanalytical applications, and still promise to give rise to many innovations to come. Partitioning plays an essential part in digital bioanalytical assays, which requires uniformity, stability and high throughput. Dividing reaction mix into hundreds or thousands of microcentrifuge tubes/vials was first employed in the early stages of digital PCR[1] which being costly and laborious was soon replaced. Later came nanoliter to femtoliter reaction chambers in polymer or glass made microfluidic devices, [2-4] or water-in-oil (w/o) emulsion droplets. [5, 6] Currently the emulsion based approaches have become the most popular method used in research and medical laboratories. However, the cost of microfluidic chip-based emulsion generating devices, as well as their control instruments, are still relatively high. Not only are the in-house designed microfluidic devices too complex to be adapted by other labs, but also many commercially available instruments require extra skills to process properly.

Using bench-top centrifuge, we develop a novel method of producing monodisperse emulsion droplets by micro-channel array (MiCA). Subjected to the centrifuge, aqueous liquid ejects out at the nozzles of the micro-channel into monodispersed droplets, which then are stabilized by the receiving oil underneath. Within few minutes, $>3x10^5$ pico-liter droplets can be generated without complicated handling of microfluidics devices and control system. By tuning the spinning speed and changing the MiCA with different channel number or sizes, we are able to generate droplets of various size. We demonstrate digital PCR and LAMP assays through MiCA approach. The digital PCR result is highly concordant with commercial equipment (Bio-Rad QX200). Our newly formulated digital LAMP protocol has also see favorable linearity in dilution quantification experiments.

MiCA-enabled emulsion generation is facile and robust, exhibiting great advantage over conventional lab equipment. With the cost-effective and highly precise micro-channel array (MiCA) the aqueous solution can be dispersed into stable picoliter-droplets and then perform PCR thermal cycling without extra liquid transfer in microcentrifuge tubes, significantly reducing the difficulty and complexity of performing droplet-based biological and chemical assays, and minimizing the loss of rare input materials by eliminating the dead volume. By the virtue of centrifuge this novel emulsion generation method is intrinsically highly parallel given that many samples can be processed simultaneously without contamination.

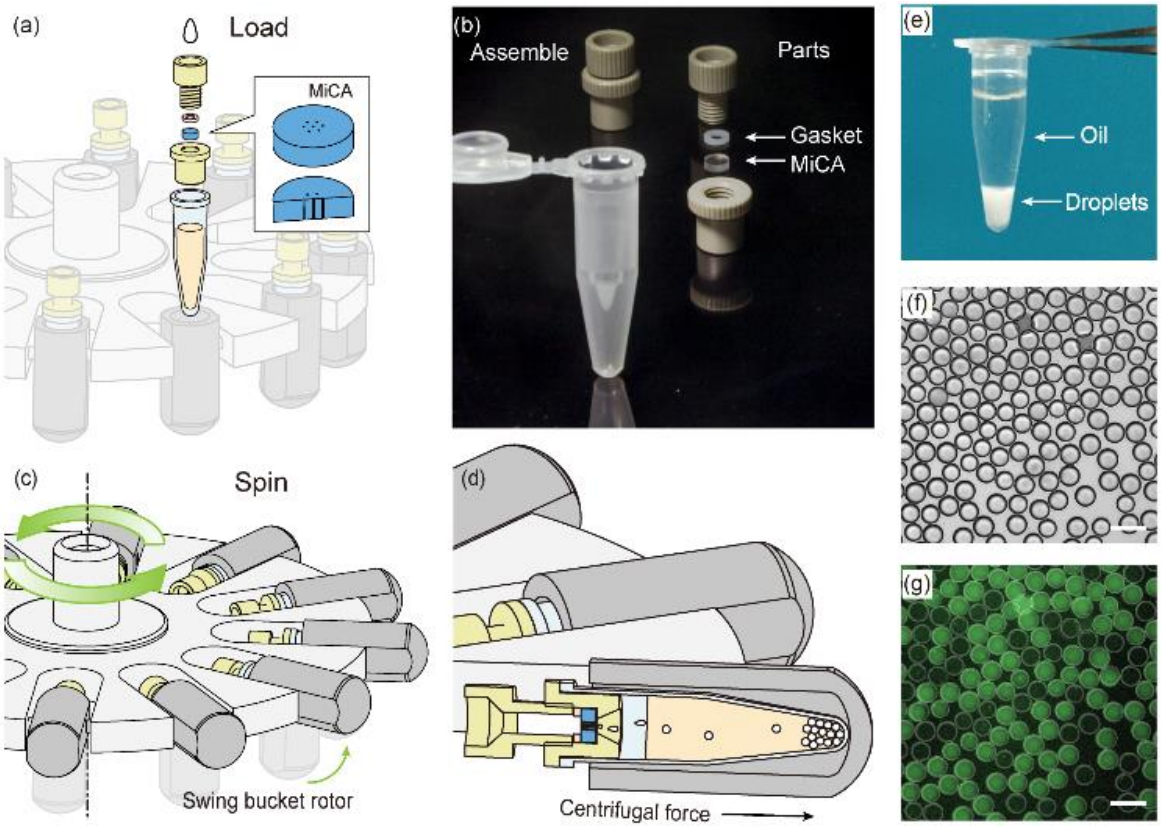

Fig. 1 Construction and operation principle of the MiCA-emulsifier. (a) Assembly of a container with MiCA. The main body was made of PEEK with a PTFE gasket ring. (b) The components. (c) The swing buckets with microcentrifuge tubes and MiCA inserts will flip centripetally when spinning. (d) During spinning the centrifugal force is perpendicular to the MiCA plate, breaking the solution into small droplets which then form emulsion in the receiving oil. (e) Emulsion is stably sitting at the bottom of a microcentrifuge tube after centrifugation. (f) Microphotograph of emulsion droplets after 40 thermal cycles of PCR. (g) Fluorescence microphotograph indicating the digital amplification within the emulsion. Scale bars: 100 μm.

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