

# ENCODING OF LIQUID CAPPED MICROCAPSULE AND HETEROGENEOUS ASSEMBLY FOR MULTIPLEXED ASSAY

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## ABSTRACT

In this paper, we have demonstrated a technology for encoding of liquid capped microcapsule and dispensing thousands of heterogeneous encoded liquid drops into microwell array with only a single pipetting step, which would normally require thousands of individual pipetting. With our technology, we can take advantages of both high coding capacity and liquid-liquid reaction, which is impossible in conventional droplet and bead-based assay platforms.

## KEYWORDS

Encoded particle, Liquid microcapsule, Heterogeneous assembly, multiplexed assay

## INTRODUCTION

Nowadays, many researchers have tried to utilize droplet- and bead-based assay platform for multiplexed assay [1, 2]. The droplet has advantage in analyzing liquid-liquid reaction and isolation, but has limitation in encoding method. On the other hand, the bead-based assay has high coding capacity, but has limitation in that it is unable to analyze liquid-liquid reaction itself. Here, we propose a novel multiplexed assay platform based on encoded liquid capped microcapsules that can accomplish both of high coding capacity and liquid-liquid reaction. The liquid drops are encapsulated with Teflon microshell using microfluidic devices and the microcapsules are encoded without special coding materials. The encoded microcapsules are assembled in microwell array and the liquid are released by breaking the Teflon shell with pulse laser.

## PARTICLE GENERATION

Figure 1 shows the schematic view of generating of liquid capped microcapsule. Using microfluidic device generating double emulsion droplet by 3D coaxial flow with simple PDMS hillock structure [3], we generate stable water/perfluoropolyether(PFPE)/water double emulsion droplets. Core liquid droplets are generated by the PFPE phase and the outer water phase then breaks the PFPE phase which contains the core chemical droplets, generating double emulsion droplets. After UV irradiation, the PFPE phase is polymerized and microcapsules are generated. The PFPE has water and oil repellence property and chemical resistance, and does not swell in organic solvent. These properties enable to encapsulate core liquid stably in PFPE microcapsule. As a proof of concept, we make 10 different colored liquid capped microcapsules that represent 10 different chemical compounds.

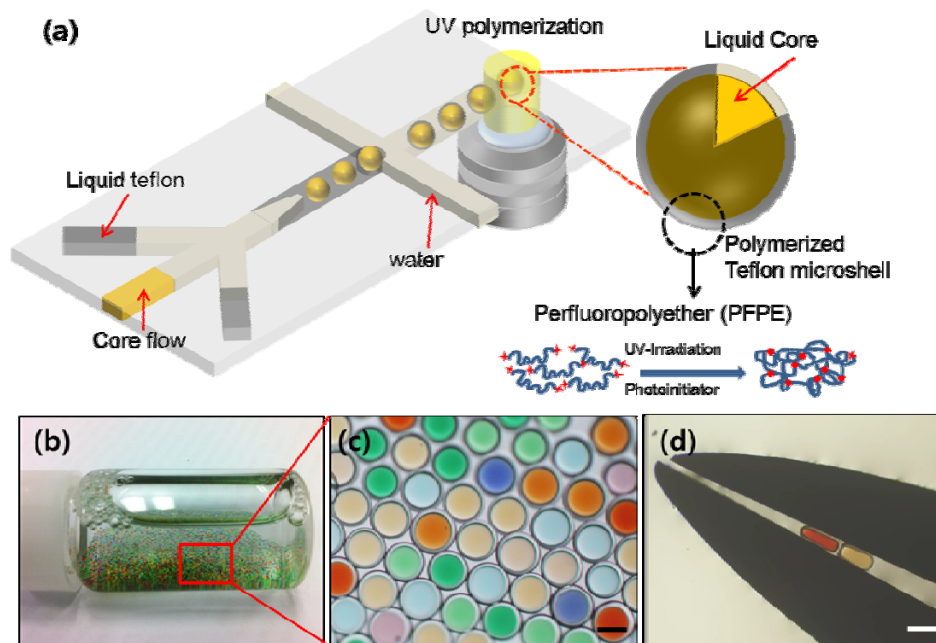


Figure 1. Liquid capped microcapsule generation. (a) Schematic image of liquid capped microcapsule generation using PDMS hillock structure. Core liquids are encapsulated by photocurable PFPE polymer and the PFPE polymer is then polymerized by UV exposure. (b-c) 10 different color liquid capped microcapsules. Core liquids are mixed with various food dyes to represent different liquid chemicals. (d) Core liquid is stably encapsulated by PFPE polymer

## PARTICLE ENCODING

The polymerized microcapsules are closely packed to consist monolayer of microcapsules then patterned UV light is illuminated on the PFPE shell as shown in figure 2. We found that the initiator we mixed with PFPE has photoluminescence(PL) property when exposed to UV light. The PL intensities also vary according to change of UV intensity and exposure time. Using digital micromirror device(DMD), patterned UV light is illuminated and the particle has PL pattern in polymerized PFPE shell. By changing the pattern of the DMD, various codes can be simply encoded without any physical mask and it enables a lot of particles to have distinct codes corresponding to their chemicals, which is necessary for identification of molecules in bead-based multiplexed assay system.

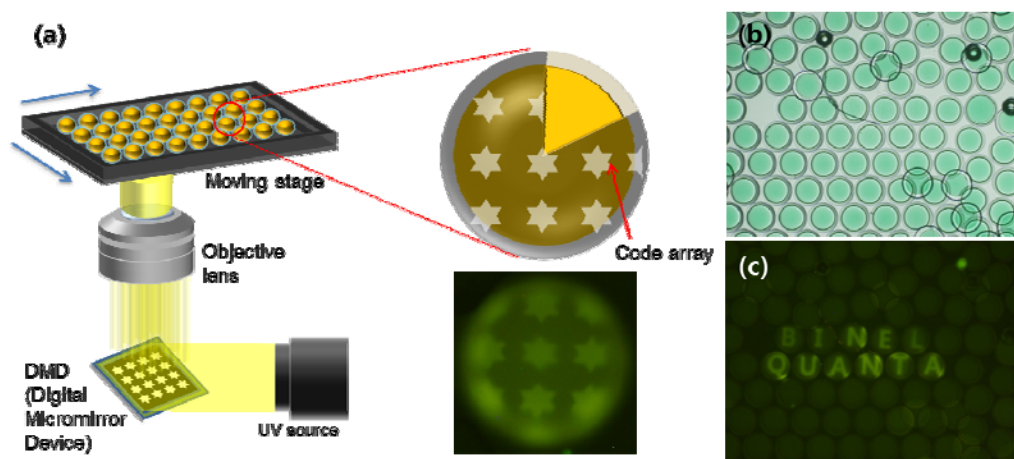


Figure 2. Encoding of liquid capped microcapsule. (a) Schematic image of encoding process. Microcapsules are monolayer-packed and patterned UV light is illuminated. After UV exposure, the photoinitiator has photoluminescence property and various code can be encoded (b,c) Bright and fluorescent images of encoded microcapsule.

## PARTIPETTING : HETEROGENEOUS ASSEMBLY

For dispensing of thousands of heterogeneous liquid drops, we introduce a new method of liquid handling called 'partipetting'. We illustrate the concept of 'partipetting' in Figure 3. Various liquid capped microcapsules are collected in one vial and assembled in PDMS microwell array. The PDMS microwell is made by conventional soft-lithography process. Each well consists of two different size of space, larger one is for microcapsule assembly and the other one is for reaction of core and outer liquid. The PDMS microwell is treated with oxygen plasma to make hydrophilic surface and immersed into water to maintain hydrophilic property. Then, the microcapsules are dispensed on the PDMS microwell array by one pipetting and swept by cover glass. Because of high density of PFPE material, the microcapsules tend to be submerged by gravitational force and we can easily assemble even by one sweeping process.

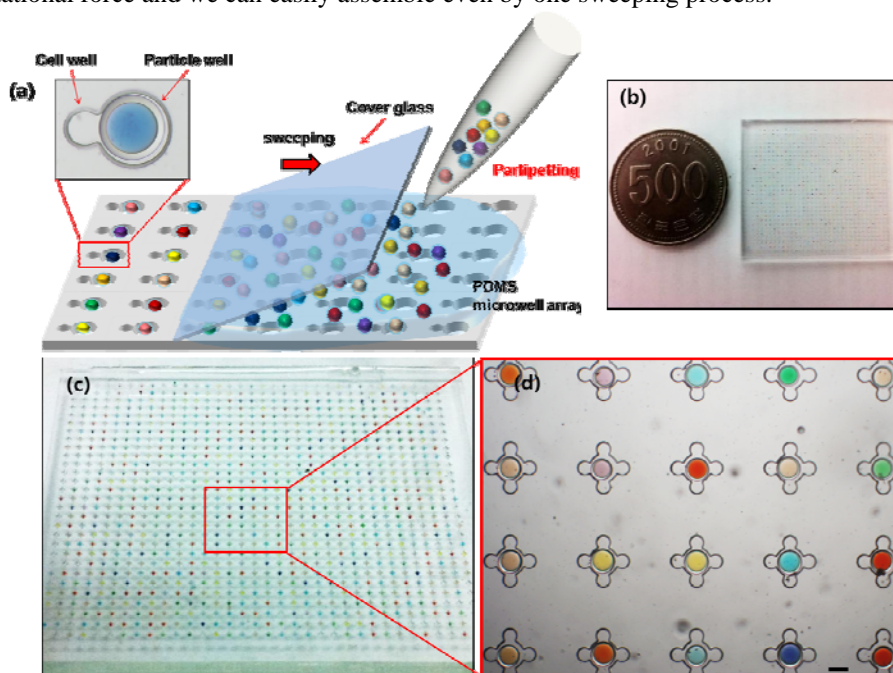


Figure 3. Heterogeneous assembly of liquid capped microcapsules. (a) Schematic image of heterogeneous assembly of particles in microwell array. (b) PDMS microwell array chip. The microwell chip is as small as a coin (c,d) Various microcapsules are assembled in microwell array. The assemble efficiency is more than 99.5%

## LIQUID RELEASING

For multiplexed assay using liquid capped microcapsule particle, we need to release core liquid from the shell of the microcapsule to the surrounding liquid of the particle. To break the particle, we set up the particle breakage equipment with pulse laser as shown in Figure 5. The pulse laser equipment consists of motorized moving stage, CCD camera and nanosecond pulse laser (Minilite ND:YAG laser, Continuum Inc.). The microcapsules are assembled in microwell array and the microwell is sealed with slide glass to prevent evaporation of surrounding liquid filled in reaction well. The microwell is then placed on the motorized stage with pulse laser equipment. By applying one pulse laser, the shell is broken and the liquid inside the microcapsule is released to the microwell and mixed with surrounding liquid. Figure 4 (c) shows the breakage and releasing results of three microcapsules which have different color-core liquids. After breakage, core liquid is released into microwell and diluted by outer liquid of reaction well, which represent the reaction between core liquid chemical of microcapsule and reagent of reaction well. Using motorized stage and simple programming, we can accomplish breakage and liquid releasing of whole particles in microwell array within a few minute automatically. The releasing result suggests its possibility of multiplexed liquid assay.

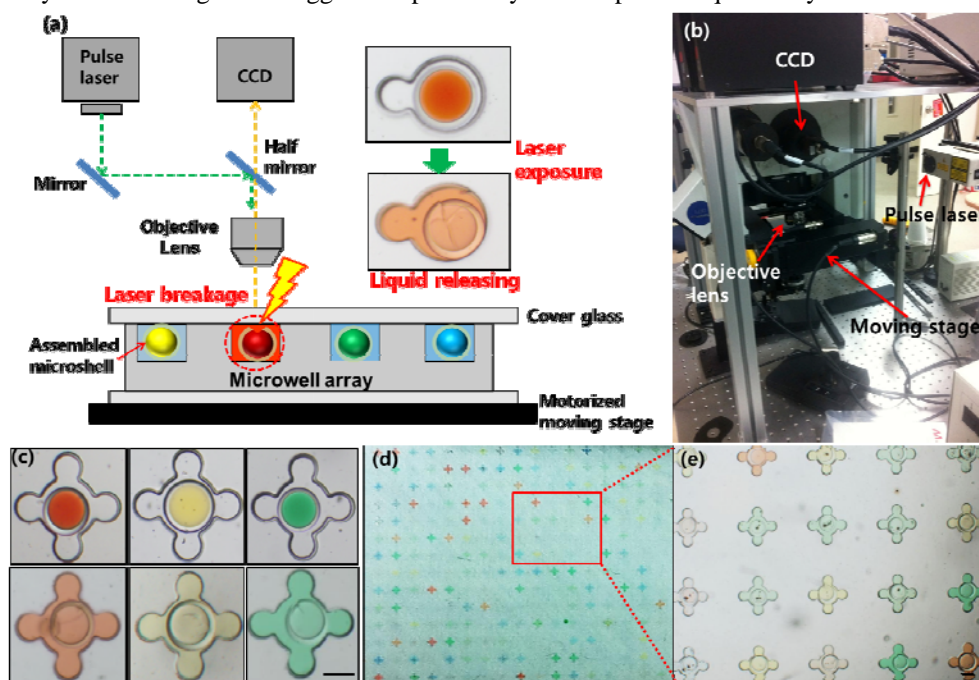


Figure 4. Particle breakage and liquid releasing results (a) Schematic image of pulse laser equipment for particle breakage. The PFPE shell is broken by pulse laser inducement. (b) The equipment is consists of motorized moving stage, CCD camera and nanosecond laser. (c) After applying pulse laser, the core liquid is released and diluted in microwell. (d,e) Images of microwell array after particle breakage and releasing.

## CONCLUSION

We introduced encoding of liquid capped microcapsule, assembly and releasing technologies as a platform for the multiplexed assay. We believe our technology would enable multiplexed assay with liquid-phase assay, whereas current multiplexed assay technologies are limited to multiplexing of surface bound molecules such as DNA or protein.

## ACKNOWLEDGEMENTS

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