Generation and Mixing of Subfemtoliter Aqueous Droplets On Demand

SUPPLEMENTAL INFORMATION

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Supplemental information for this manuscript includes (1) A description of the optics, (2) instructions for preparing glass micropipettes, and (3) video files demonstrating droplet generation.

Optical Apparatus: The experimental setup for optical manipulation and fluorescence detection is described in Figure 1A. The apparatus is built around a Zeiss microscope body (Zeiss, Axiovert S100TV). IR light for the optical trap is provide by a fiber YAG laser (IPG Photonics, YLD-5, 5W, λ=1064 nm). Fluorescence excitation light at 488 nm is provided by an Argon ion laser and at 532 nm by a Nd:YAG diode laser (Crystal Laser Inc. λ=532 nm). Collimated light from the IR trapping and excitation lasers is combined (using a dielectric IR mirror that passes visible light, M2) and directed through the objective lens OBJ (Olympus UPlanFl, 100X, numerical aperture NA=1.30), using steering mirrors, to a focused spot in the focal plane of the microscope. Separate sets of alignment mirrors and collimation optics on the IR and excitation beams permit the IR and excitation light to be aligned with respect to the microscope field-of-view and each other. Alignment mirror AM1 (AM2) is imaged by L1 (L2) and L3 onto the back focal plane of OBJ to permit translation of the laser focus in the field of view. A dual-band dichroic mirror (Chroma Technology, z488-1064 rpc for 488 nm excitation, or Omega Optical, XF2017 for 532 nm excitation) is used in the microscope slider to reflect IR and excitation lasers to OBJ and to pass fluorescence to the detector. Fluorescence is collected through the OBJ and focused onto the active area of a detector, APD (avalanche photo diode, SPCM-14, PelkinElmer Optoelectronics, active area diameter 170 μm). Filters used to remove the remaining excitation and IR light include two IR filters F1, (FGS900, Thorlabs), a notch filter F2 (488 nm or 532 nm, Kaiser Optical Systems Inc.), and bandpass filters F3 (Omega Optical, 3^{RD} millennium, 500 nm-550 nm or 550 nm-600 nm for different excitation lasers and dyes).

For the dual-trap setup, the 1064 nm laser beam is split into two beams by a polarizing beamsplitter to generate two independent optical traps, one with a picomotor-driven steering mirror (SM) imaged (using L1 and L3) onto the back focal plane for automated translation of the trap in the focal plane. The two IR beams are recombined by another polarizing beamsplitter. A half-wave plate in front of the first polarizing beamsplitter is used to modulate the relative laser intensity of these two traps. The distance between two traps can be adjusted to tens of microns.

A CCD video camera (SONY, XC-ST50 or Cohu 4990 series RS-170) is used to record a brightfield image and movie of the field of view. Fluorescence photons detected at the APD are reported as TTL pulses that are counted by a data acquisition board (National Instruments, PCI6602), and images from the camera are collected by a frame grabber card (National Instruments, PCI1405). All data and imaging acquisition processes are controlled by Labview software (National Instruments).

Preparation of the micropipette: Microcapillary glass tube (OD 1.0 mm, ID 0.25 mm, A-M Systems, Inc. or Borosilicate tube with filament with OD 1.0 mm and ID 0.50 mm, Sutter Instrument) is pulled by a micropipette puller (P-2000, Sutter Instrument Company). The internal diameters of the micropipette tips are nominally around 0.5 μm. The micropipette tips are dip-coated in SigmaCote (Sigma-Aldrich Co.) to render them hydrophobic. To prevent clogging, a small backing pressure is applied to force air through the tip before it is removed from the Sigmacote. Tips are then dried in 90 °C oven for 1 hour. The quality of each coated tip is visually inspected under an optical microscope before use.

Two video files are included in this supplement:

(1) In Movie S1, uniform droplets are generated from the injector tip at a rate of 2 per second. The tip is prepared as described in the Methods section. The tip can continuously generate droplets for at least 48 hours. A gravity-driven flow from a larger microcapillary tube (upper left) drags the droplets down and to the right.

(2) Movie S2 is a video of tip motion and droplet formation taken at 16,000 frames per second. From this video it is apparent that the droplets are generated with zero velocity. In this video it is also apparent that the droplet is fully formed, and the tip fully retracted, by the third frame (190 μs) after tip retraction initiates.