Supplementary Material: Multicolor iLIFE (m-iLIFE) Volume Cytometry for High-Throughput Imaging of Multiple Organelles

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Supplementary Notes 1-2

Supplementary Note 1. Actual Photograph of the m-iLIFE Imaging Cytometry System

Supplementary Note 2. Correlation and Shift Correction Analysis

Supplementary Videos 1-4

Supplementary Video 1: Fluorescent beads (size~1 μm) flowing through microfluidic channels at varying frow rates (500 – 2000 nm/min).

Supplementary Video 2: Mixed fluorescent beads suspended in triple-distilled water are flown through the microfluidic channels, and independently recorded by dual channel detector system (green channel (channel 1), red channel (channel 2)). Alongside merged multicolor video is also shown.

Supplementary Video 3: Fluorescently-labelled cells simultaneously flowing through single, double and four microfluidic channels. The cells are optically sectioned by the system PSF.

Supplementary Video 4: Reconstructed multicolor volume of sample cells (cell #1 and cell #2).

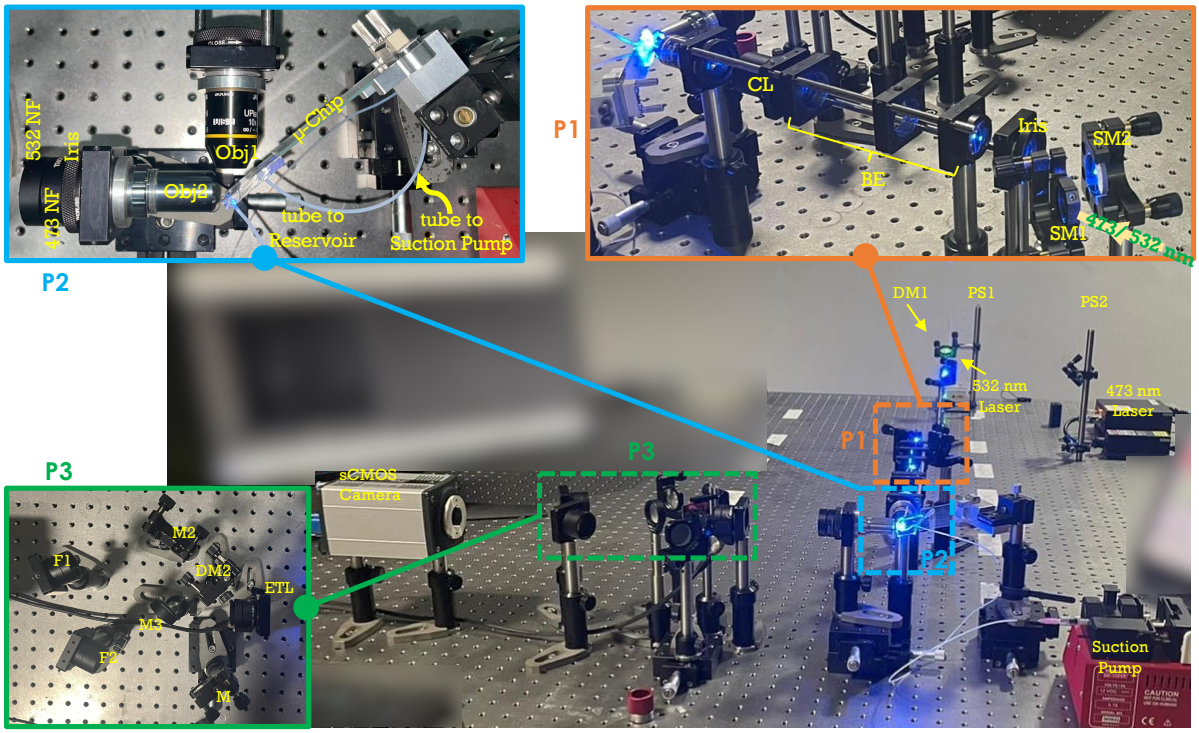

Fig. S1. Actual Experimental Setup: The photograph of actual experimental setup. Note that some of the items such as isolation boxes and separation sheets are removed to take the photograph. Three critical parts (P1: illumination, P2: Sample holding and orthogonal lightsheet configuration, and P3: Dual-color widefield 4f detection) of the setup are also shown. Key optical elements are: Objectives: Obj1, Obj2; Notch Filters: 532 NF, 473 NF; Electrical Tunable Lens: ETL; Mirrors: M1, M2, M3, SM1, SM2; Dichroic Mirrors: DM1, DM2; Beam Expander: BE; Periscope: PS1, PS2, Cylindrical Lens: CL; Filters: F1, F2.

To enable multi-organelle interrogation, m-iLIFE imaging cytometry system is developed, the actual picture of the setup is as shown in Fig. S1. Here, two organelles (here, mitochondria and lysosomes) are labelled by two spectrally-distinct dyes (Lysotracker Green DND-26 and MitoTracker Red FM), for which the signals are separated by the dual-channel detection system (Fig. S1, P3). The illumination is achieved by a couple of light sources which are combined in the illumination sub-system. The combined beams are expanded and focussed by a combination of cylindrical lens – objective lens to generate overlapping PSFs which is used to section the flowing the cells (Fig. S1, P1). The cell flowing sample holder connected to suction pump along with the illumination and detection objective (configured in an orthogonal position) are shown in Fig. S1, P2. All the sub-systems (P1, P2 and P3) are coupled together and operated in a synchronized manner to record the sectional images of flowing cells by the sensitive sCMOS camera (Zyla 4.2, Andor, UK) [1].

Supplementary Reference

1. https://andor.oxinst.com/products/scmos-camera-series/zyla-4-2-scmos

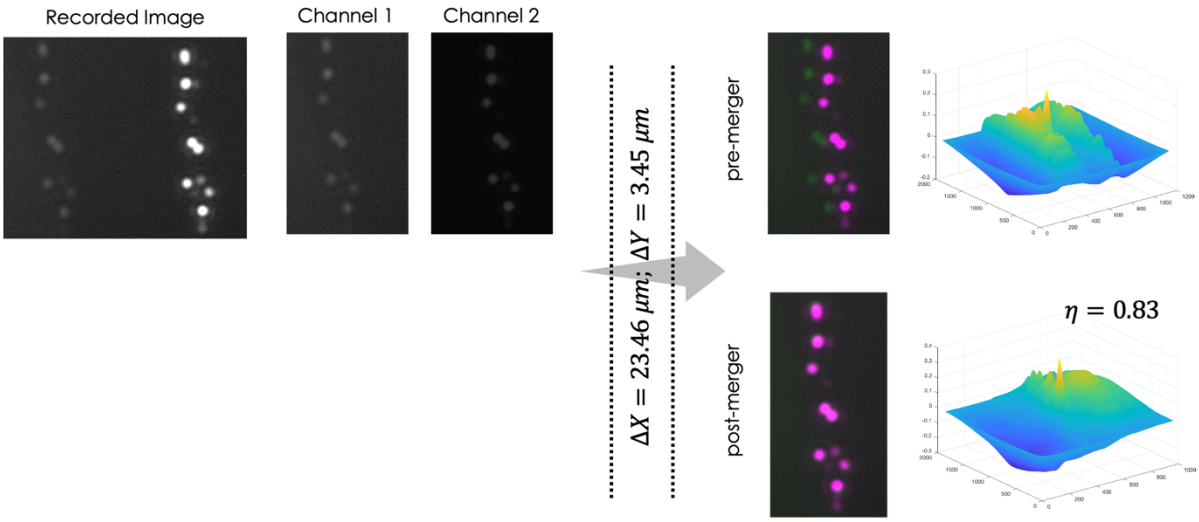

Fig. S2. Correlation and Shift: Recorded image, segregation into channel 1 and 2, shift along X and Y along with pre- and post- merger. Surface plots showing a correlation coefficient factor of 0.83.

Two organelles (here, mitochondria and lysosomes) are labeled by two spectrally distinct dyes (Lysotracker Green DND-26 and MitoTracker Red FM), for which the signals are separated and collected in two different regions of the camera chip. In the calibration experiment, the fluorescent beads with broad emission spectra (470-650 nm) are flown through a microfluidic channel, and the emission is detected in both channels (see, Fig.S2). Subsequently, the images are recorded, and segmented into two parts (window 1 and 2). These sub-images are spatially correlated using inbuilt Matlab scripts to determine the shift [1]. The correlation study shows a shift of ΔX = 23.46 μm and ΔY=3.45 μm. Finally, these shift values are incorporated into the images before merging them to form the multicolor image. Post-merger, the sub-images show a correlation coefficient of 0.83 (see, Fig.S2). All the merged sectional multicolor images are then stacked together to reconstruct multicolor cell volume. In addition, the surface plot with and without shift correction is also shown.

Supplementary Reference

1. https://www.mathworks.com/help/matlab/ref/corrcoef.html