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Supplementary information

Optofluidic Raman-activated cell sorting for targeted genome retrieval or cultivation of microbial cells with specific functions

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Supplementary Information: Optofluidic Raman-activated cell sorting for targeted genome retrieval or cultivation of microbial cells with specific functions

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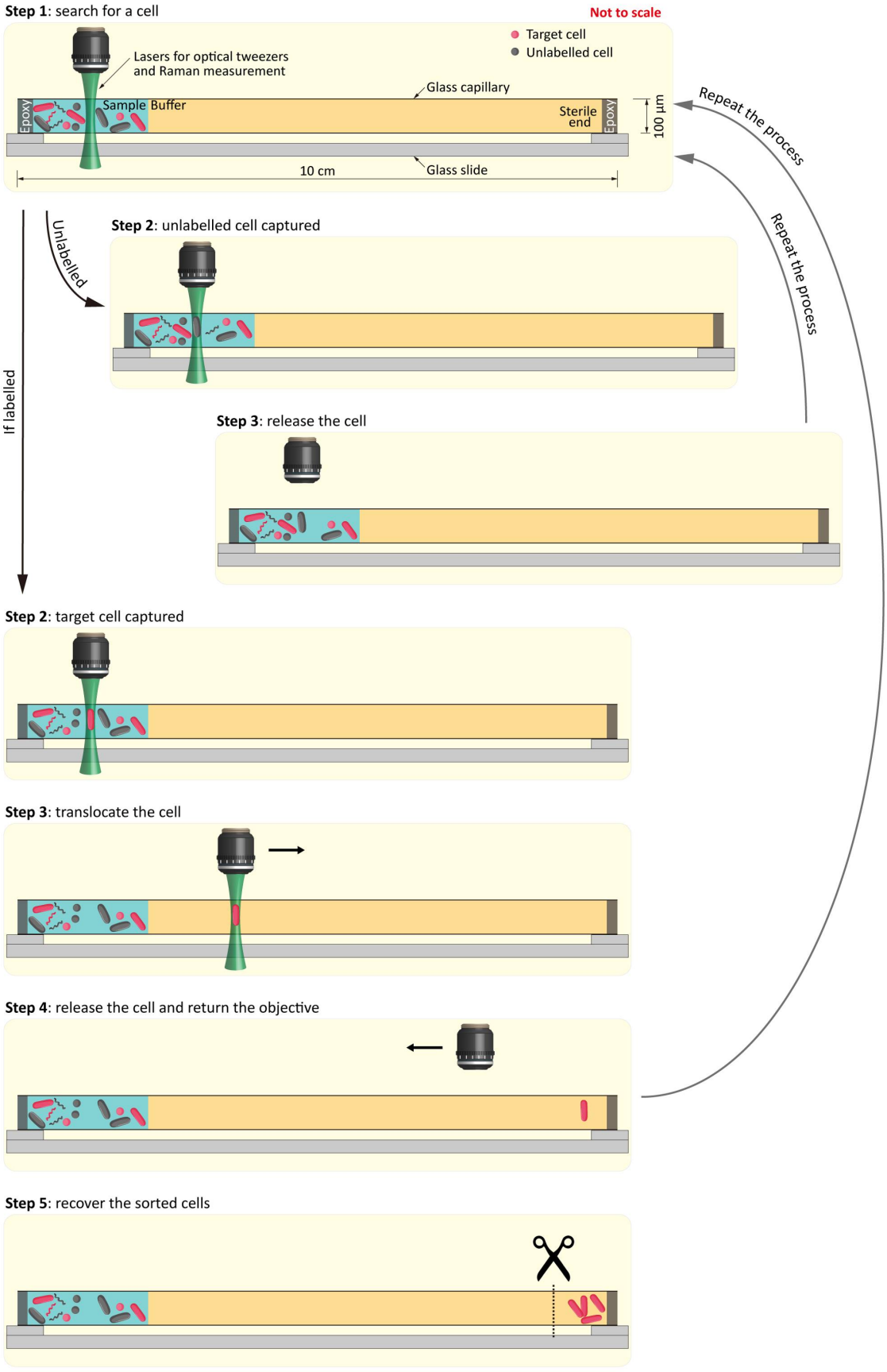
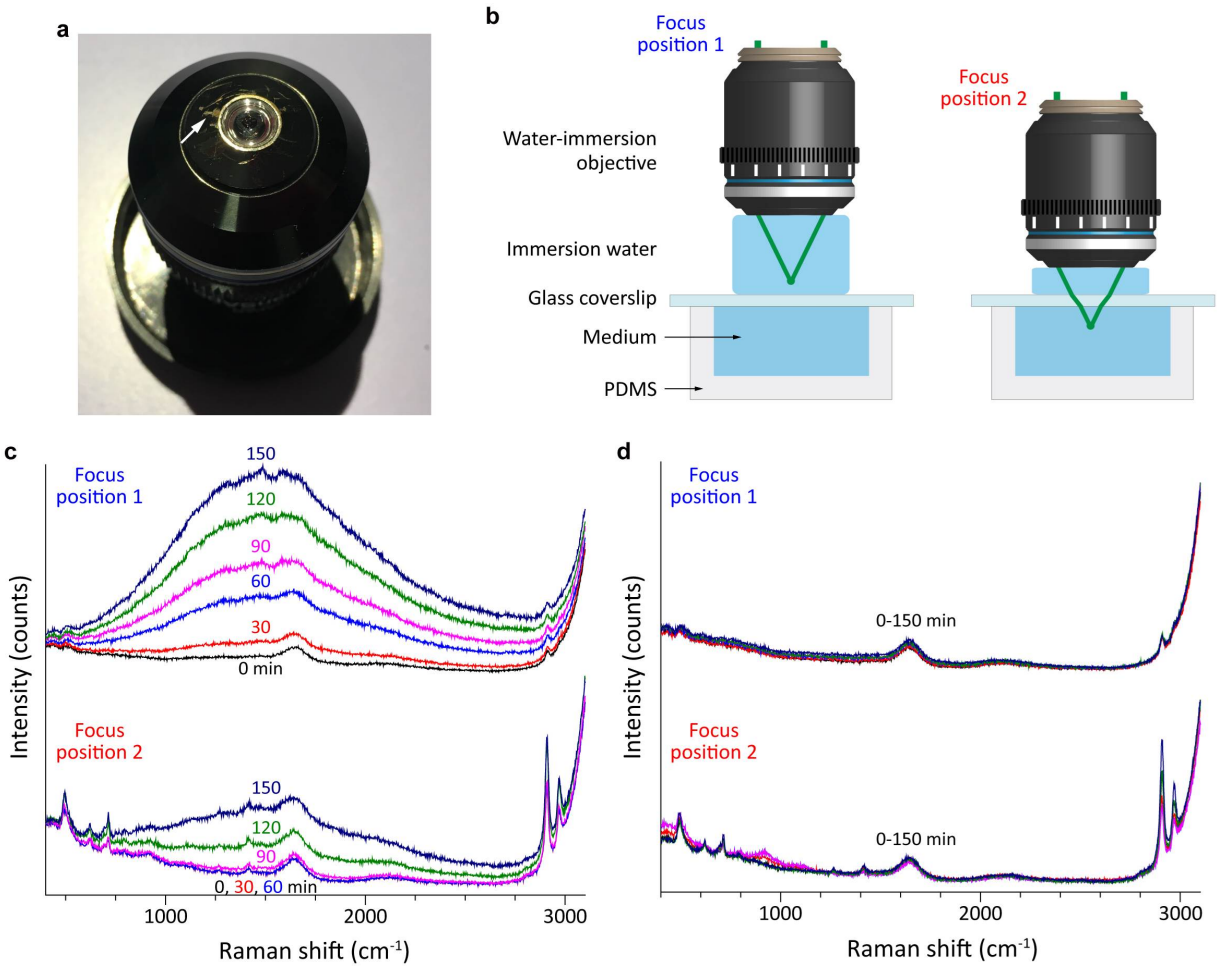
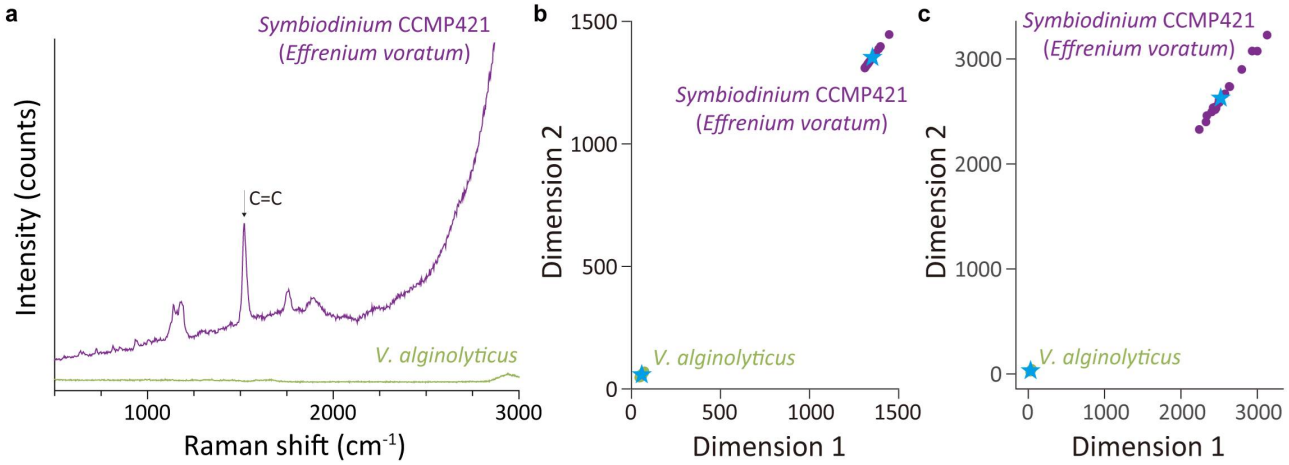


Supplementary Fig. 1 | The procedure for manual cell sorting. One side of a glass capillary is filled with the sterile buffer and then the sample is placed into the other end. Both ends of the

capillary are sealed by epoxy to prevent evaporation of liquid during sorting. The lasers for optical tweezers and Raman measurement are focused at the same position using a single objective and a user searches for a cell. When a single cell is trapped in the optical tweezers, its Raman spectrum is measured. If the cell is identified as unlabelled, it is immediately released and the user searches for a new cell. On the other hand, if the cell is identified as labelled, it is translocated to the sterile end of the capillary and released. The objective is then moved back to the sample region and the search for a new cell begins. Once the required number of cells have been collected, the sterile end of the capillary is broken and the collected cells are recovered for downstream analysis. This manual sorting procedure yields 1–2 cells per hour, whereas the platform in this protocol enables an automated procedure with more than 100-fold higher throughput (up to 500 cells per hour).



Supplementary Fig. 2 | Fluorescence signal generated from the surface of a damaged water-immersion objective. a, Objective whose surface is damaged (white arrow). **b,** Two focus positions of the 532-nm (Raman) laser: one is within the immersion water (focus position 1) and the other within the microfluidic device (focus position 2). **c,** Time series of Raman spectra at the two positions measured using a damaged objective. Fluorescence emerging at the 'focus position 1' influences the Raman spectra in the microfluidic device from 90 min onwards. **d,** Time series of Raman spectra using an undamaged objective. Spectra are consistent over time at both focus positions, although the slight change in the spectra at focus position 2 (in the spectral region <1,200 cm^{-1}) is due to slight drift of the focus position 2 in depth and thus an increase in the effect of the glass coverslip.



Supplementary Fig. 3 | RACS based on K-means clustering algorithm. a, Representative Raman spectra of carotenoid-containing (*Effrenium voratum*) and non-carotenoid-containing (*Vibrio alginolyticus*) microorganisms. **b,c,** Classification of a mixture of cells of *E. voratum* (n = 20) and *V. alginolyticus* (n = 80) into two clusters when based on a wide spectral region (400–3,300 cm^{-1}) (**b**) and on a narrow region characteristic of the C=C bond (1,500–1,560 cm^{-1}) (**c**). The blue star represents the centroid of each cluster.