Electronic Supplementary Material (ESI) for Lab on a Chip.

Spectral reading of optical resonance-encoded cells in microfluidics

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Interpretation of the correlation matrix

In the processing identifying barcodes and comparing cells, many different detection scenarios can be encountered. Here we analyze some of the cases we have observed and illustrate how they presented themselves in the correlation matrix.

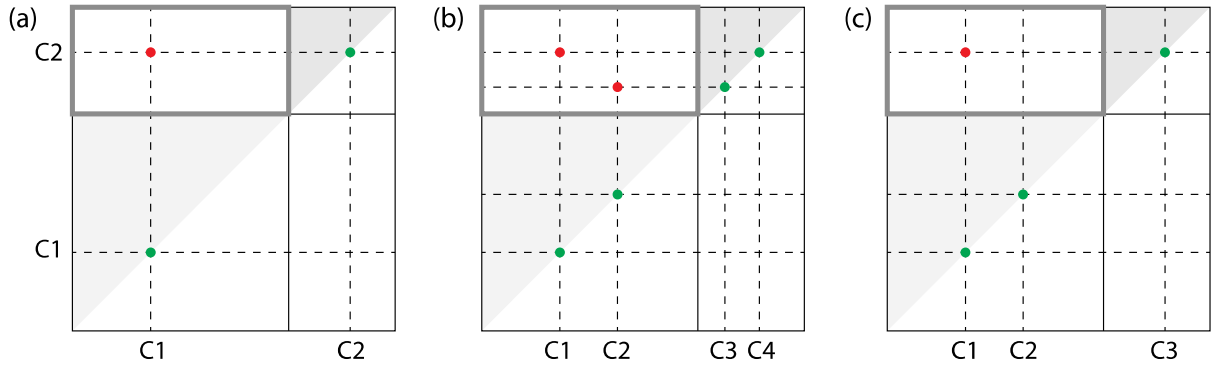

Fig. S1 *Normal events. (a) One cell is flown in both directions and successfully identified. (b) Two cells are flown in both directions and successfully identified. (c) Out of two cells flown in the forward direction only one returns.*

(a) In this most common example, for a cell C1 detected in the forward flow and a cell C2 (with its identity yet to be determined) detected in the backward flow, and the protocol decides that these two cells have the same barcode within the maximum spectral shift $\Delta\lambda_{max}$. Then a correlation mark (red filled circle) is represented in the cross-correlation domain (boxed region), and trivial self-correlation marks (green circles) appear in the reference and sample domain (shaded triangular regions).

(b) An extended example of the same type as (a): for two cells, C1 and C2, detected in the forward run and two cells, C3 and C4, detected in the backward scan, C3 is identified to be C2, and C4 is identified to be C1, and this make two correlation marks (red) in the cross-correlation domain.

(c) A cell, C2, detected in the forward scan but not detected in the backward scan. Typically, this situation happens when the cell gets trapped in the tubing or syringe and never returns to the channel.

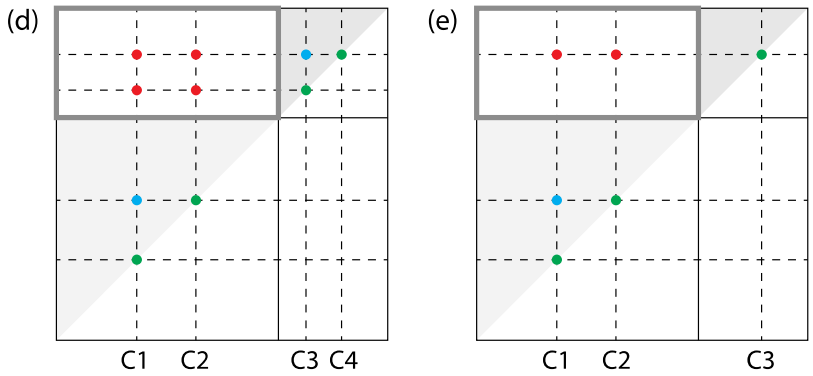

Fig. S2 *Scenarios leading to non-unique correlation and false-positive errors: (d) Two cells with identical barcodes are flown in forward direction and both return to the channel in the backward flow. (e) Same as previous example, but only one returns.*

(d) Two cells happen to have very similar beads and their barcodes are considered identical. These cells detected in the forward run, C1 and C2, produces a non-unique correlation hit (cyan-filled circle) in the non-diagonal space (bottom-left area). When these cells return in the backward flow, C3 and C4, they mark a correlation hit in the self-correlation domain (top-right area). In the cross-correlation domain, four correlation marks (red circles) appear.

(e) Same example as (d) except that now only one of the two cells, C3, return to the channel in the backward scan. Then, C3 matches with both C1 and C2, leaving two correlation marks (red circles).

Note. In the case of (d), unfortunately there is no way to tell whether C3 (or C4) was C1 or C2. And, in (e) we cannot identify whether C3 is C1 or C2. Therefore, in both cases, in post processing we remove all the non-unique cells from reference and sample, by deleting rows and columns C1 through C4 in (d) and rows and columns C1 through C3 in (e), from the dataset. This is how we generated re-processed map in Fig. 4e. In principle it would be enough to remove only cells C1 and C2, which would simulate a sample with only unique-barcodes. However, since barcodes can be altered (Fig. S3) it is better to remove also non-unique cells in the sample to get rid of errors such as in Fig. S3g.

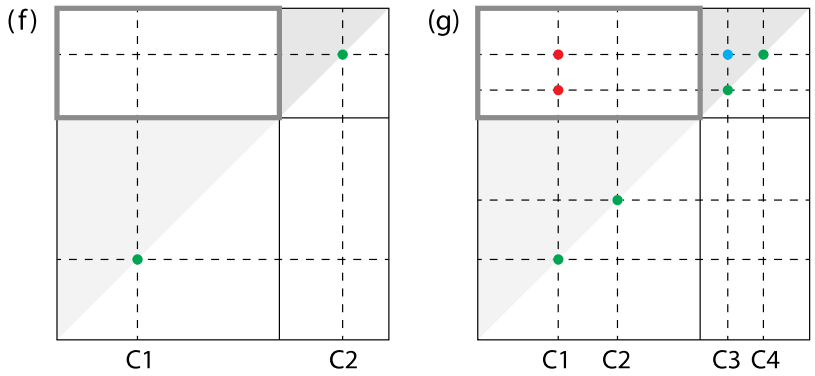

Fig. S3 *Scenarios for false-negative errors due to altered barcodes: (f) The barcode of a single cell changes during the reversal of the flow and the cell is not identified. (g) Two unique cells flown in forward direction change their barcode in such way that they become identical.*

(f) The spectrum from the beads in a cell, C2, measured in the backward flow is different from the spectrum of the same cell, C1, measured in the forward flow. This can occur if the effective refractive index of the intracellular environment surrounding the beads has changed between the two measurements. This situation leaves no cross-correlation mark.

(g) A rare case. The spectra of two cells, C1 and C2, are similar to each other but distinguished in the forward flow, but for any reason, such as cellular or environment changes, the spectra of the two cells, C3 and C4, in the backward direction are changed such that they are no longer distinguished from each other. This makes C3 and C4 non-unique cells. Moreover, suppose C3 and C4 are identified to be the same as C1 but not C2; in this case two correlation marks (red) appear in the cross-correlation domain. C1, C3 and C4 were removed from the dataset when the re-processed map in Fig. 4(e) was generated.