**Editor:**

Questions/Concerns:

1 - Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

We have proofread the manuscript and we are sure that there are no spelling or grammar issues.

2. Please abbreviate all journal titles.

We have abbreviated all the journal titles.

3. Length is at maximum limit.

We added concise information to the manuscript while making suggested revisions.

4. Grammar: Line 51 – “the Microfluidic CODES”

We have modified this sentence. (See lines 51-52).

5. Additional detail is required:

-Section 1 – Please provide a citation as insufficient detail is supplied to replicate the design.

We have added a citation about designing Gold codes in the first step of section 1. (See line 106).

-2.7 – What settings are used for deposition?

We used an automated e-beam evaporator (Denton Explorer) for metal deposition in our work. Denton Explorer was given deposition rate and target metal thickness as only inputs. We have added the deposition rate and base pressure recorded during deposition to the manuscript in lines 153-155.

-2.8 – What constitutes “mild sonication”?

We used a conventional normal ultrasonic bath machine for sonication, with non-controllable power setting. In lines 157-159, we have revised the sentence into “…sonication with frequency 40 kHz at 100% amplitude for 30 min at room temperature…” to describe the sonication process conditions.

-3.10 – How is this done?

We dispense 200 μL of trichlorosilane in a petri dish and placed it in a vacuum desiccator together with the SU-8 mold at room temperature. The SU-8 mold was kept under vacuum (94.8 kPa) for 8 hours to facilitate coating of the mold surface with trichlorosilane molecules in vapor-phase. (See lines 203-205).

-4.3 – Approximately what size should the pieces be?

The sizes of the cut PDMS pieces are approximately 20 mm × 7 mm. We revised the text to provide this information. (See lines 215-218).

-4.7 – How is bonding performed?

Plasma-activated glass substrate and PDMS bonds spontaneously when brought in physical contact. We revised the text to clarify this point. (See lines 228-229).

-6.3 – What flow rate is used?

In our experiments, we used flow rates ranging from 50 μL/h to 1000 μL/h. We revised the text to provide this information. (See lines 274-275).

-Section 6 – Please indicate how different pieces are connected to the chip. Should these connections be performed prior to flowing cells into the chip? If so, Step 6.3 should appear much later in the section.

Yes, electrical connections to the chip should be performed prior to flowing cells into the chip. We revised the order of procedures to clarify this point. (See lines 262-275).

-Section 7 – How are these things done in the software? Please provide the code as a supplemental file or include a citation containing instructions on how to do these analyses in the software.

We have added a more detailed description about the signal processing part and explicitly stated built-in MATLAB functions used in our mathematical calculations. We also included references for specific decoding or optimization algorithms mentioned in the protocol. (See lines 296-338).

6. Results: Figure 4 legend – Please describe what is shown in each panel. What are the differences among them?

We revised the figure caption to describe plots in each panel. (See lines 422-430).

7. Discussion: Please discuss the significance with respect to alternative methods. Please also discuss the limitations of the method.

We revised the manuscript to discuss the significance of our technology with respect to alternative methods in the first paragraph of the discussion. (See lines 457-463).

**Reviewer #1:**

Questions/Concerns:

I recommend that in the introduction that the authors make sure to highlight and mention previous works from different groups which have also made use of multiple electrodes and corrugated channels prior to CODES to make the readers familiar with the field and to know how the idea of using multiple electrodes for improvements in performance has evolved over the last 5-7 years. This will also be useful so that the readers will know the different applications of these various multi-electrode schemes. Here are references I recommend the authors include:

1. Spencer D, Caselli F, Bisegna P, Morgan H. High accuracy particle analysis using sheathless microfluidic impedance cytometry. Lab on a Chip. 2016.

2. D. Polling, S. C. Deane, M. R. Burcher, C. Glasse and C. H. Reccius, Proceedings of uTAS (The 14th International Conference on Miniaturized Systems for Chemistry and Life Sciences), October 3-7, 2010, Groningen, The Netherlands

3. M. Javanmard and R. W. Davis, IEEE Sens. J., 2013, 13, 1399-1400.

4. K. R. Balakrishnan, G. Anwar, M. R. Chapman, T. Nguyen, A. Kesavaraju and L. L. Sohn, Lab Chip, 2013, 13, 1302-1307 .

5. S. Emaminejad, S. Talebi, R. W. Davis and M. Javanmard, IEEE Sens. J., 2015, 15, 2715-2716

6. P. Kiesel, M. Bassler, M. Beck and N. Johnson, Appl. Phys. Lett., 2009, 94, 041107

7. J. Martini, M. I. Recht, M. Huck, M. W. Bern, N. M. Johnson and P. Kiesel, Lab Chip, 2012, 12, 5057-5062 .

Also, I think it would be good to include 5-10 more references on more contemporary applications of impedance cytometry for proteomics, smart electroporation, nucleic acid analysis, virus detection, etc... so that readers will appreciate the broader impacts that CODES can have.

Aside from these minor points, this is excellent work.

We revised to text to add additional references as suggested by the Reviewer.

**Reviewer #2:**

Questions/Concerns:

1 - What is the scalability of the SIC process when more and more channels are added for large-scale cell sorting architectures? Because the correlation of each channel must be done in succession, i.e. serially, it is not a parallel process and for large number of channels the time to process signals from all channels will add up. How does this limit the overall sorting speed per number of channels? Or is signal processing not a limitation due to fast computing speeds available?

We thank the Referee for pointing to the need to explain this point. Signal processing speed is not a limitation in our system. We revised the text to discuss this issue. (See lines 493-499).

2. Can the fluidic channels be fabricated to control the cell flow so they are in each channel one by one? E.g. size dependent cell sorter. If not, in the event of 2 similar cells passing parallel to each other in a channel, are they distinguishable or would they show up as a strong correlation signal, hence a big cell? This seems like a problem as this is mentioned in lines 453-461 in discussion.

We thank the Referee for pointing to the need to clarify this important point. In our device, the cross section of the microfluidic channels is designed to be close to the cell size so that two cells cannot simultaneously pass over a sensor in parallel. We revised the text to clarify this point. (See lines 342-345).

3. How orthogonal do the codes have to be before signal differentiation by correlation peak detection becomes poor? Ex. For 1010110 and 1010111, how distinguishable will they be in this system? I would imagine when scaling to more channels, highly orthogonal codes (such as 1010110 and 0111111 as used here) would be ideal as mentioned in lines 446-451. When the codes are increased in length to keep them highly orthogonal, does this have negative consequences? What is a practical limit on this code length to make the system speedy yet easy to signal process?

Orthogonal codes are easier to distinguish due to minimal cross-correlation. However, the codes should also be chosen such that they remain orthogonal under random phase shifts when sensors asynchronously interfere. Gold codes have this feature. We revised the text to clarify this point. (See lines 472-474). The Reviewer is right, to obtain more orthogonal codes to multiplex more channels, we need longer codes. However, longer codes lead to higher sensing volume and increase the likelihood of interference for a given sample density. We revised the to explain this trade-off. (See lines 478-486). The longer codes will not affect the signal processing speed directly.

4. Figure 6 legend should be corrected: singal -> signal

We thank the Referee for pointing out this error. We now revised the legend of Figure 6.

5. What is the main difference between this paper and work presented in Lab Chip?

Lab Chip paper was the first introduction of the Microfluidic CODES technology. In that paper, we mainly focused on the principle of operation of the sensor, and presented our experimental results. In this paper, our goal is to make this technology available to our researchers so that they can use it for their applications. Therefore, we present a detailed protocol that explains individual steps in design of devices, microfabrication process, culturing of cell lines, constructing the experimental setup and signal processing. Some of the figures in the paper were adapted from the Lab Chip paper as supportive materials for explaining the protocol. Moreover, due to its video paper format, Journal of Visualized Experiments provides the optimum channel to fully demonstrate the capabilities of our method. We revised the manuscript to explicitly state this point. (See lines 93-100).