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

Deep learning-assisted sensitive detection of fentanyl using a bubbling-microchip

Hui Chen[1], Sungwan Kim[1], Joseph Michael Hardie[1], Prudhvi Thirumalaraju, Supriya Gharpure, Sahar Rostamian, Srisruthi Udayakumar, Qingsong Lei, Giwon Cho, Manoj Kumar Kanakasabapathy, and Hadi Shafiee*

Division of Engineering in Medicine, Department of Medicine, Brigham and Women's Hospital and, Harvard Medical School, Boston, Massachusetts 02139, United States;

[1] These authors contributed equally.

*Email Address correspondence to: hshafiee@bwh.harvard.edu

Supporting Information

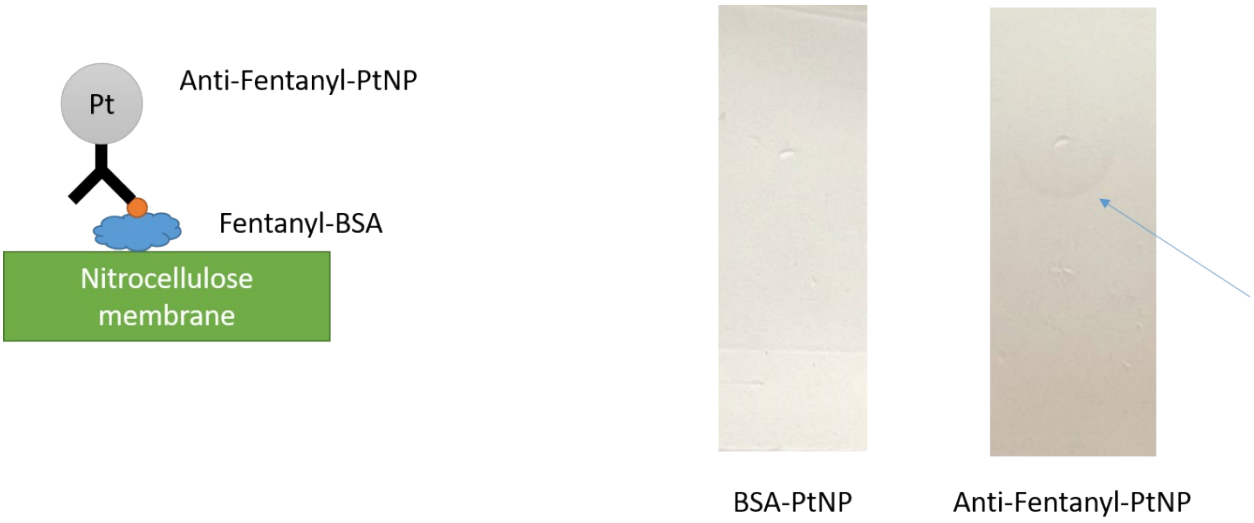

Figure S1. Characterization of platinum nanoparticles conjugated with anti-fentanyl antibodies (FenAb-PtNP). Fentanyl conjugated bovine serum albumin (Fen-BSA) was spotted on cellulose ester membranes (Hi-Flow Plus HF135, MilliporeSigma, Inc. St. Louis, MO, USA). Then PtNPs were flowed through the membrane, followed by washing with PBS. As indicated by the arrow, a gray circle appeared on the Fen-BSA spot while no gray circles formed with the BSA coated PtNPs, indicating that the FenAb-PtNPs but not BSA-PtNPs bound to the Fen-BSA on the membrane.

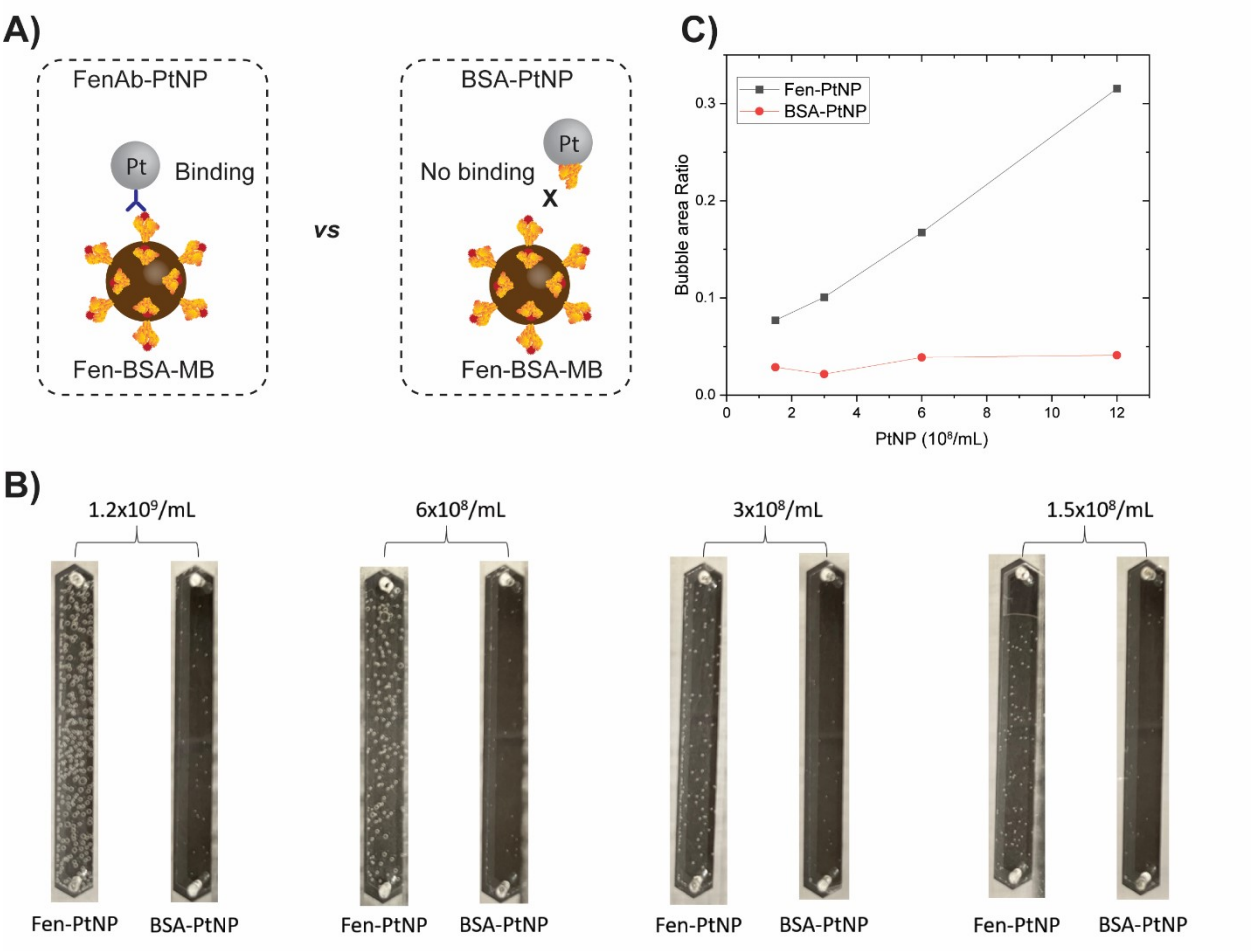

Figure S2. Optimization of the concentration of platinum nanoparticles conjugated with anti-fentanyl antibodies (FenAb-PtNP) for fentanyl bubbling assay. Different concentrations of FenAb-PtNP and bovine serum albumin conjugated PtNPs (BSA-PtNPs) were separately interacted with magnetic beads coated with fentanyl conjugated bovine serum albumin (Fen-BSA-MB). Then the MB captured PtNPs were analyzed with bubbling microchips. A) Design of the test. The left scenario represents the blank signal of fentanyl bubbling assay. The right scenario represents the maximum signal of fentanyl bubbling assay. B) Images of the microchips of Fen-BSA-MBs capturing FenAb-PtNPs and BSA-PtNPs at different concentrations. C) Comparison of the bubble areas of bubbling microchips with Fen-BSA-MBs capturing FenAb-PtNPs and BSA-PtNPs at different concentrations.

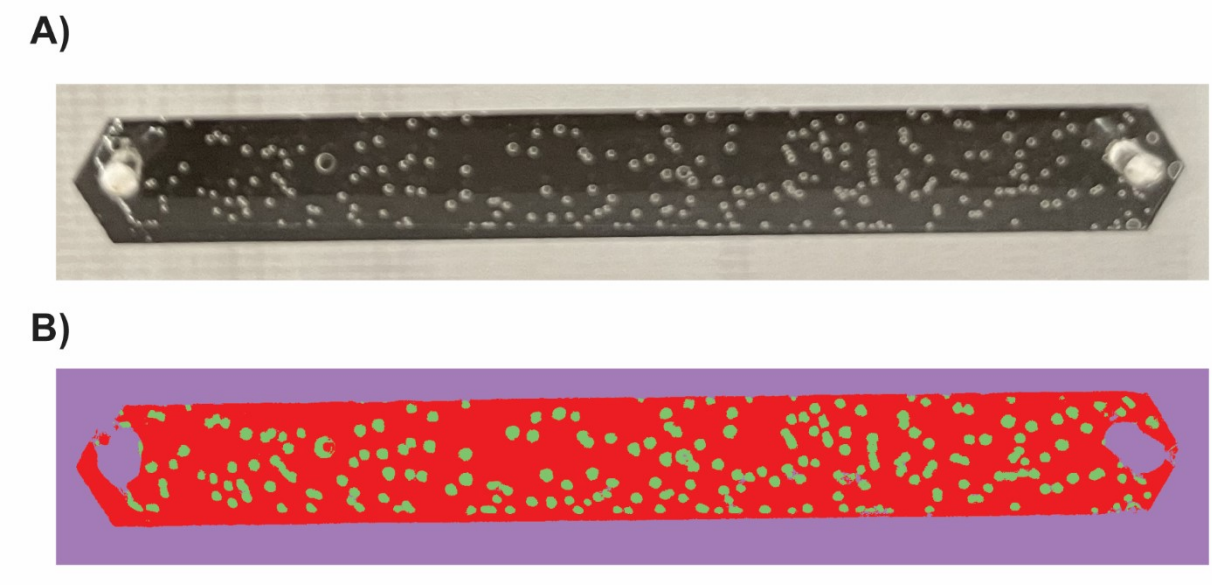

Figure S3. Processing microchip images to measure bubble areas. A) An example image of a fentanyl bubbling chip. B) Processed image via Fiji to identify pixels corresponding to bubbles in the image. Bubble-associated pixels (green), chamber background pixels (red) and microchip side pixels (purple).

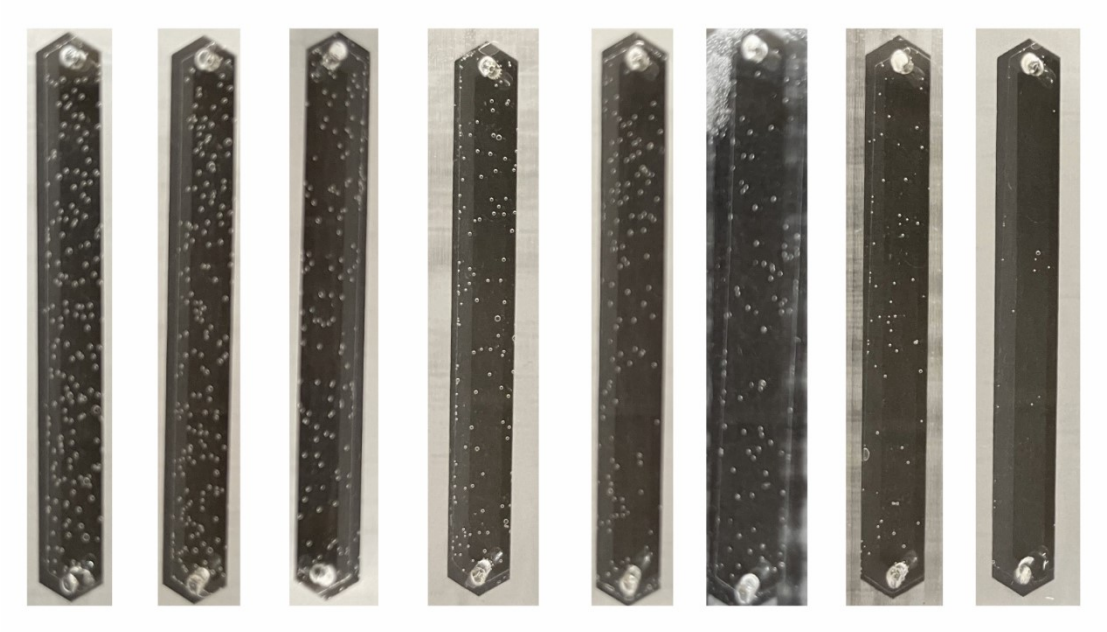

Figure S4. Images of fentanyl bubbling microchips with different concentrations of fentanyl. From left to right: 0, 0.1, 0.5, 1, 2, 5, 10, and 100 ng/mL fentanyl in buffer (PBS, pH 7.4, containing 1% BSA).

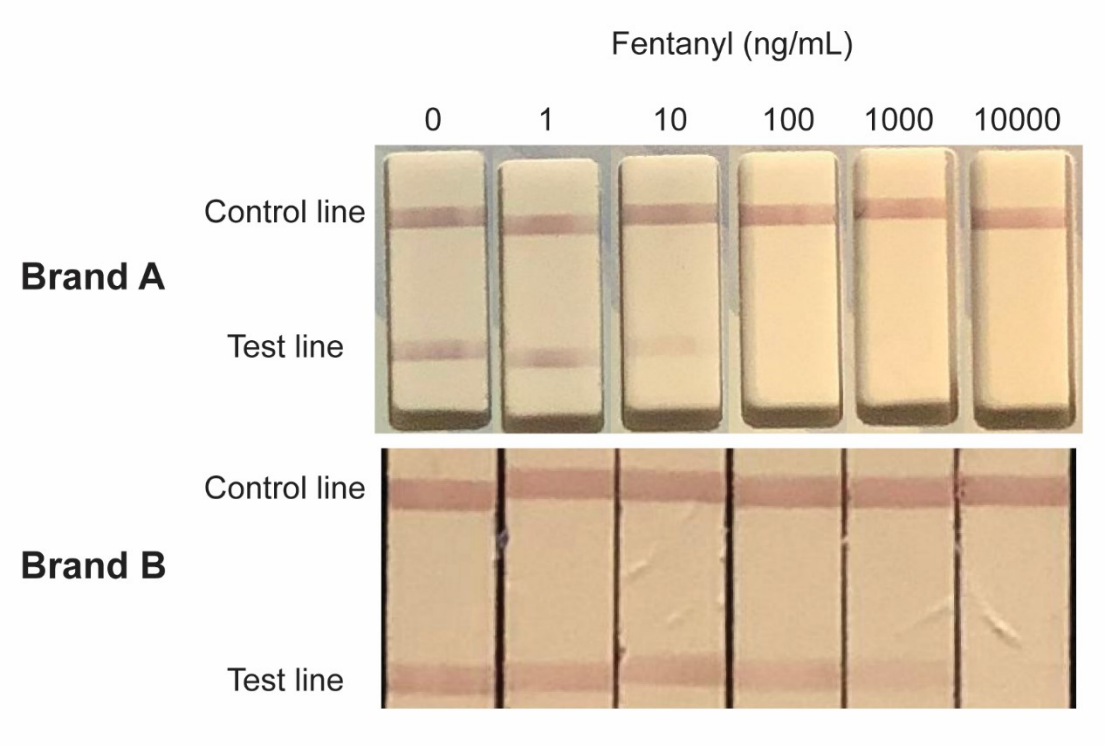

Figure S5. Performances of commercially available fentanyl lateral flow strips. From left to right: 0, 1, 10, 100, 1000 and 10000 ng/mL of fentanyl in buffer (PBS, pH 7.4, containing 1% BSA). Brand A, One Step Fentanyl Test Dip Card (Healgen Scientific LLC, TX, USA). Brand B, Rapid Response Fentanyl Test Strips (BTNX Inc, ON, Canada).

Figure S6. Images of fentanyl bubbling microchips with different concentrations of norfentanyl. From left to right: 0, 10, 100, and 1000 ng/mL norfentanyl in buffer (PBS, pH 7.4, containing 1% BSA).

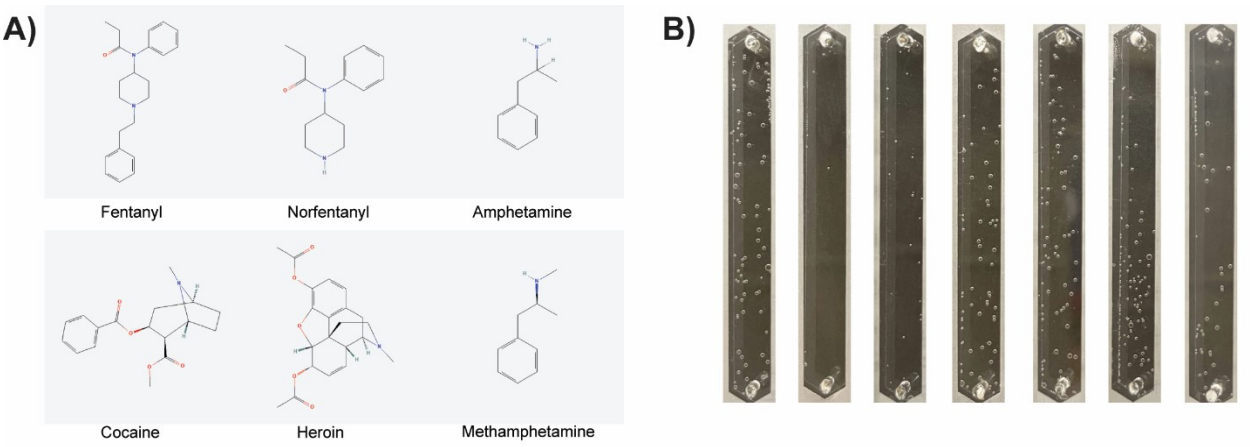

Figure S7. Images of fentanyl bubbling microchips with different drugs at a concentration of 100 ng/mL. A) Structures of different drugs. B) From left to right: buffer (PBS, pH 7.4, containing 1% BSA), fentanyl, norfentanyl, amphetamine, cocaine, heroin, methamphetamine.

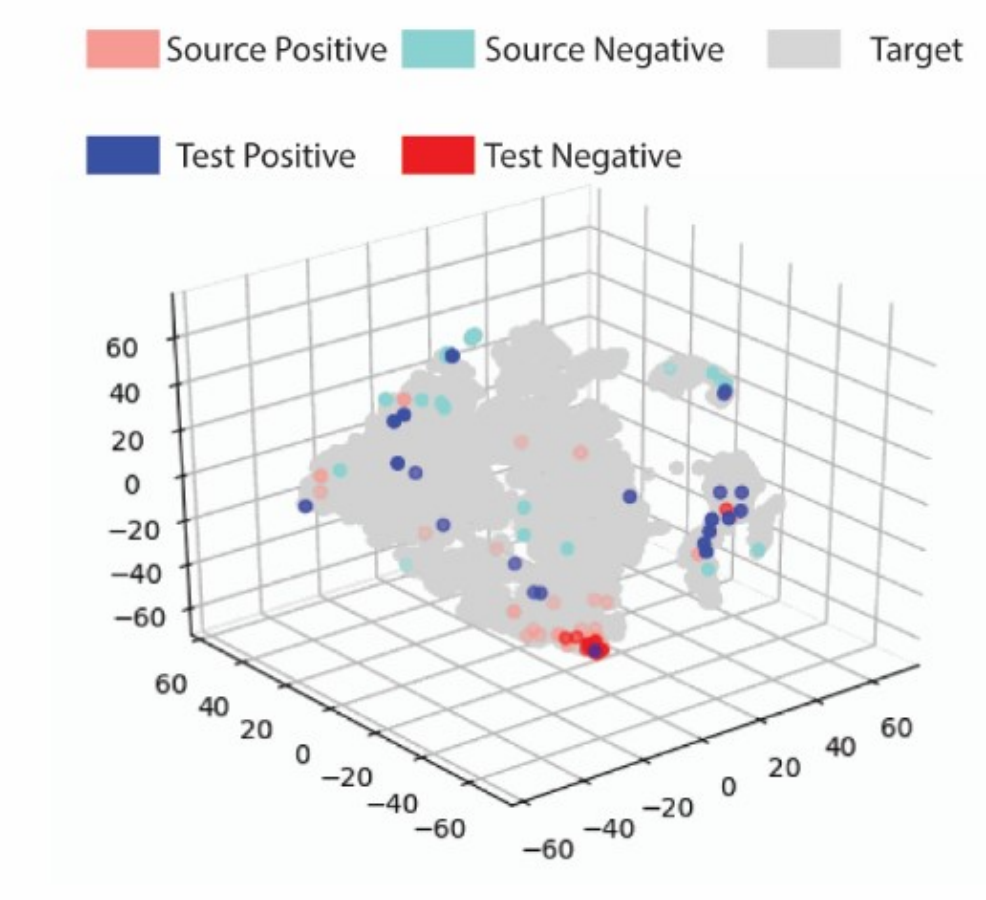

Figure S8. t-SNE plots demonstrating the ability to distinguish between positive and negative samples in both source and test data points, as well as the association between source, test distribution with target distribution.

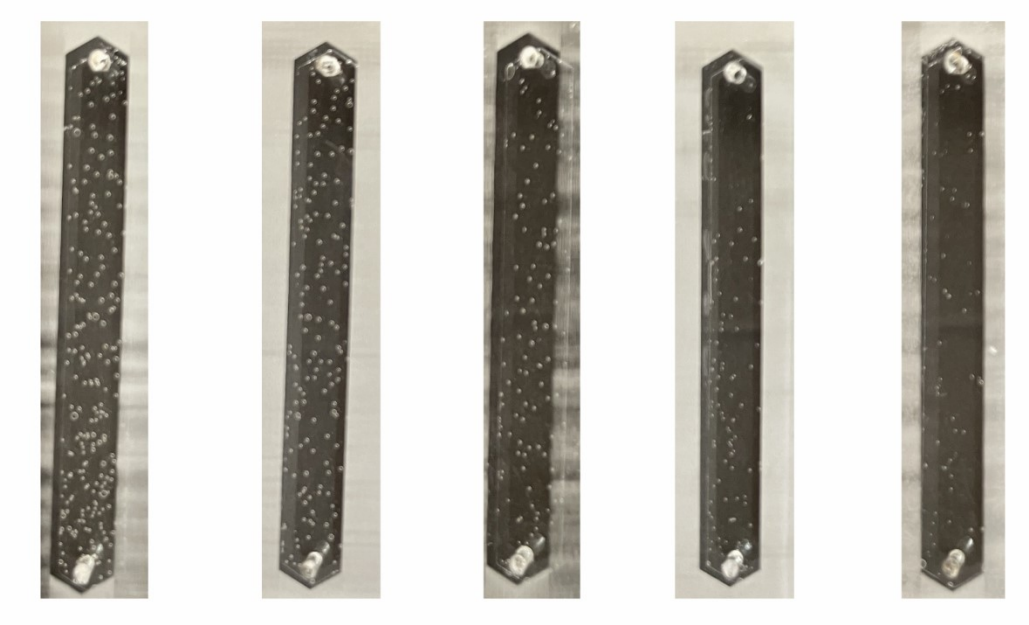

Figure S9. Images of fentanyl bubbling microchips of artificial urine samples with different concentrations of spiked fentanyl. From left to right: artificial urine only, 0.1, 1, 10, and 100 ng/mL spiked fentanyl in artificial urine.

Figure S10. Images of fentanyl bubbling microchips of human serum samples with different concentrations of spiked fentanyl. From left to right: human serum only, 0.1, 1, 10, and 100 ng/mL spiked fentanyl in human serum.

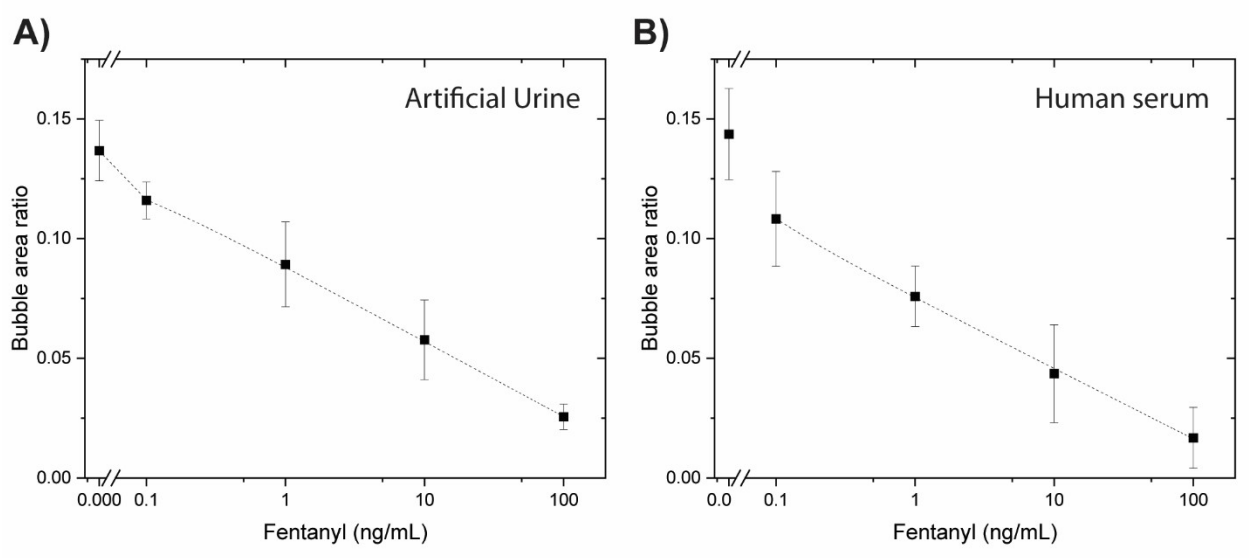

Figure S11. Dose response curves of fentanyl bubbling assay with spiked artificial human urine and human serum samples. A) Dose response curve of bubble area ratio of fentanyl bubbling chips against artificial urine samples with different concentrations of spiked fentanyl (0, 0.1, 1, 10, and 100 ng/mL). B) Dose response curve of bubble area ratio of fentanyl bubbling chips against human serum samples with different concentrations of spiked fentanyl. Mean ± standard deviation; n=3.

Table S1. Coefficient of variations of the fentanyl bubbling assay

Fentanyl (ng/mL)	CV
0	7%
0.1	11%
0.5	16%
1	8%
2	19%
5	2%
10	29%
100	39%
Average CV	16%