Electronic Supplementary Material (ESI) for ChemComm.

Supporting Information (SI) for

Dean flow assisted-cell ordering system for lipid profiling in single-cells using mass spectrometry

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Experimental Section

Reagents.

RPMI medium 1640 and trypsin−EDTA were purchased from Gibco (Grand Island, NY, U.S.A.). RPMI medium MEM was purchased from Macgene (Beijing, China). Penicillin, streptomycin, and fetal bovine serum (FBS) were purchased from Invitrogen (Carlsbad, CA, U.S.A.). All other reagents used in this experiment were of analytical reagent grade and used without further purification.

Apparatus.

The capillary used in the experiments was 100μm inner diameter and 363 μm outer diameter with 20μm standard polyimide coating，which was purchased from Innosep (11 Changchun Road, Zhengzhou High New-Tech Zone, China). The tip of capillary was firstly disposed in 300 °C fire and then washed with acetone to peel off the coating of the tip. Next the tip was polished by a sand disc of 3000 mesh to a smooth-faced hollow cone. Finally, the tip was dealt with silanization treatment, during which the dimethyldichlorosilane was used to react with the hydroxy on the surface of the capillary, which made the surface become more hydrophobic. The sample pool in the device was made of plastic connected with the capillary with polydimethylsiloxane seamlessly. The polyimide capillary was used, which can be easily curved. Thus, an insulation-tape was used to immobilize curved capillary.

A 500x portable USB digital microscope (B011, purchased from Shenzhen Supereyes Co. Ltd) was used to observe the cells and Taylor cone generated on the tip of capillary. A laboratory-made high-voltage power supply (0−10 kV) was used for the formation of Taylor cone and electrospray ionization. Images of the electrospray were obtained by using the high-speed microscope (VW-9000, Keyence Corporation of America). ESI-TOF was conducted on the the SCIEX TripleTOF 5600+ mass spectrometer (AB Sciex Pte. Ltd.), and the mass spectra were obtained in the positive mode and analyzed using the data analysis software package provided by Sciex.

Cell Culture and Sample Preparation.

HepG2, Caco-2, and HUVEC cells (Cancer Institute and Hospital, Chinese Academy of Medical Science, Beijing, China) were cultured with RPMI medium 1640 supplemented with 10% FBS, 100 U/mL penicillin, and 100 U/mL streptomycin in the incubator with 5% CO2 at 37 °C. U87 cells were cultured with RPMI medium MEM while the other condition is the same. The cells were trypsinized and reseeded every 2 days. Before the experiment, the cells were treated with trypsin−EDTA and removed from the petri dish. Then, the cells were centrifuged and resuspended by 50% methanol aqueous solution to obtain a cell suspension of appropriate cell concentration. Then, the cells were diluted in 50% methanol aqueous solution at a concentration of 150 cells/mL. The cell suspension was kept in the sample pool before the experiment.

Simulation and Calculation of Dean flow in curved capillary.

Comsol Multiphysics 5.3 (Comsol) was used to carry out 3-D simulations on a four-core, 64-bit computer (Asus) with 16 GB of RAM. The geometry of the tool was set as same as the that in the experiment. The capillary is 50 μm inner diameters which has a curved segment of 3 loops with a total length of 14 cm. Injection flow rate was 40 μL/min to simulate the flow rate under the condition of electrospray. The injection solution was assumed to be 50% methanol aqueous solution with a density of 895.9 kg/m3 and a viscosity of 0.001 Pa·s. The simulations were run under steady-state conditions with the flow boundary conditions at the edges of the capillary perimeter sides set as open boundaries (equal to atmospheric pressure).

Supplementary Figures and Legends

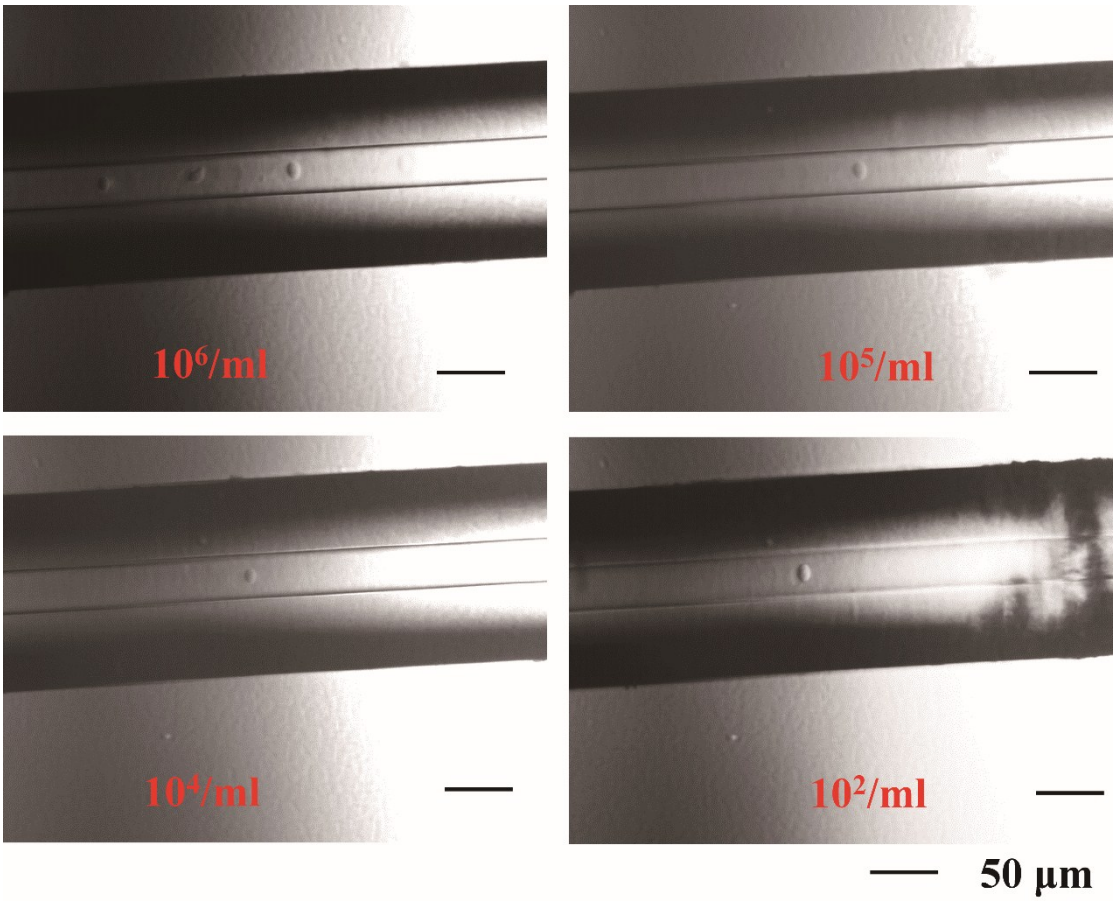


Fig. S1 Cells in cell suspension observed near the outlet of capillary. Different concentrations from 10^2-10^5 /ml are proved to be suitable for this device to separate single-cells.

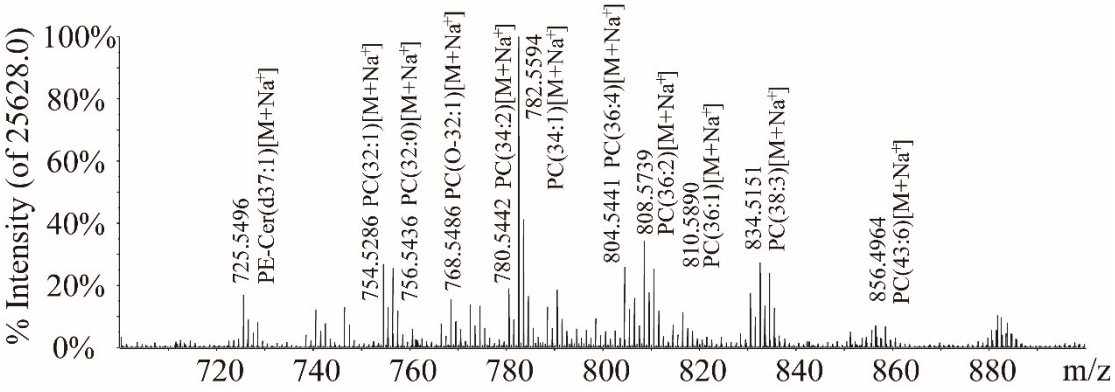


Fig. S2 Mass Spectrum of detectable lipids in cells

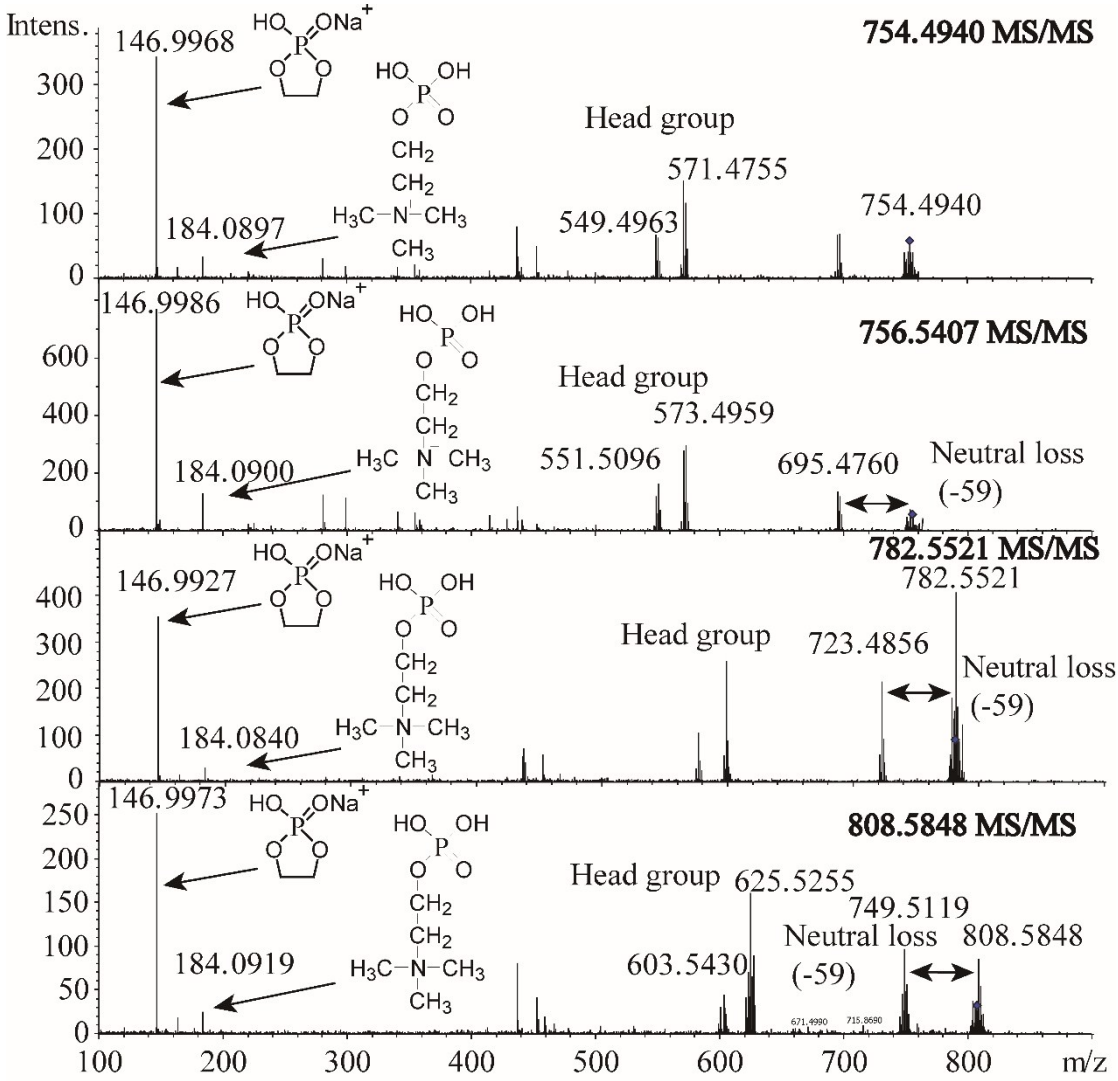


Fig. S3 Several MS/MS spectra providing head group information to confirm the lipid classes[1,2].

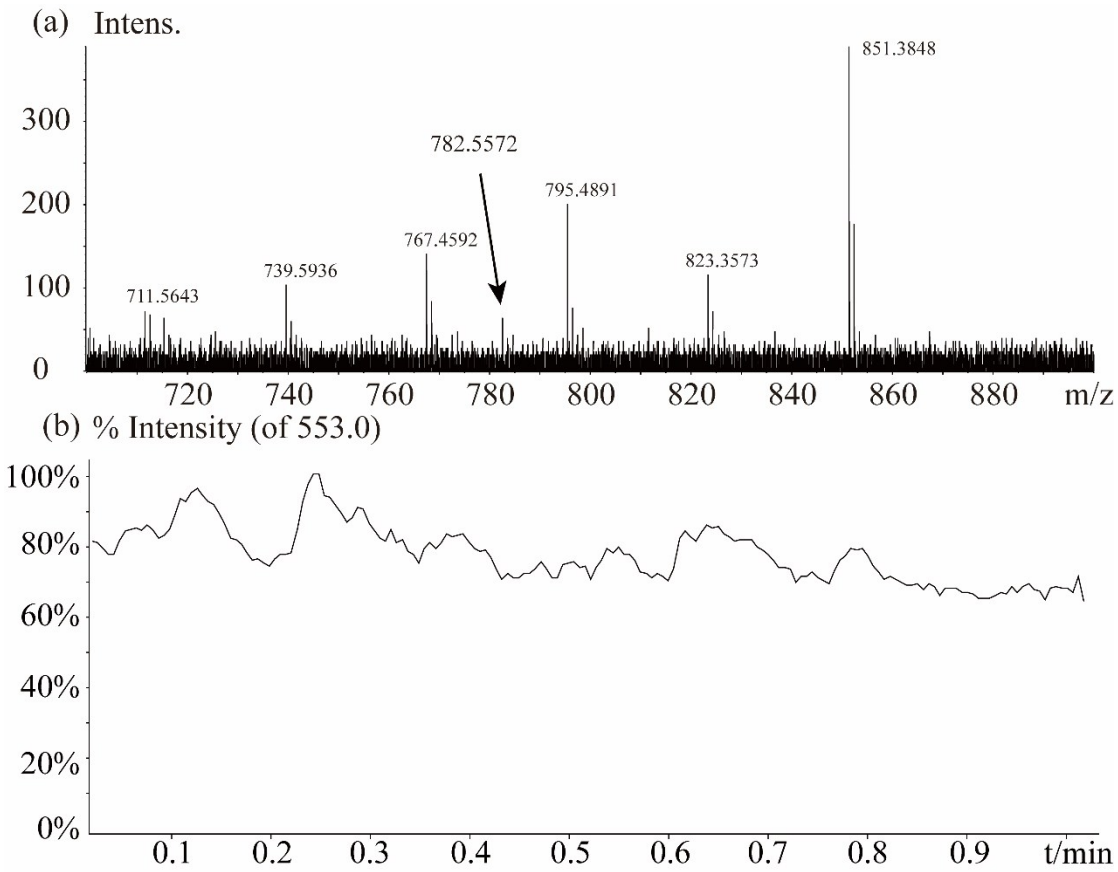


Fig. S4 (a) Contrast test done by centrifugal treatment of cell suspension after the experiment and collect the supernate for MS analysis. (b) XIC diagram at m/z = 851.38, which is from the background peaks.

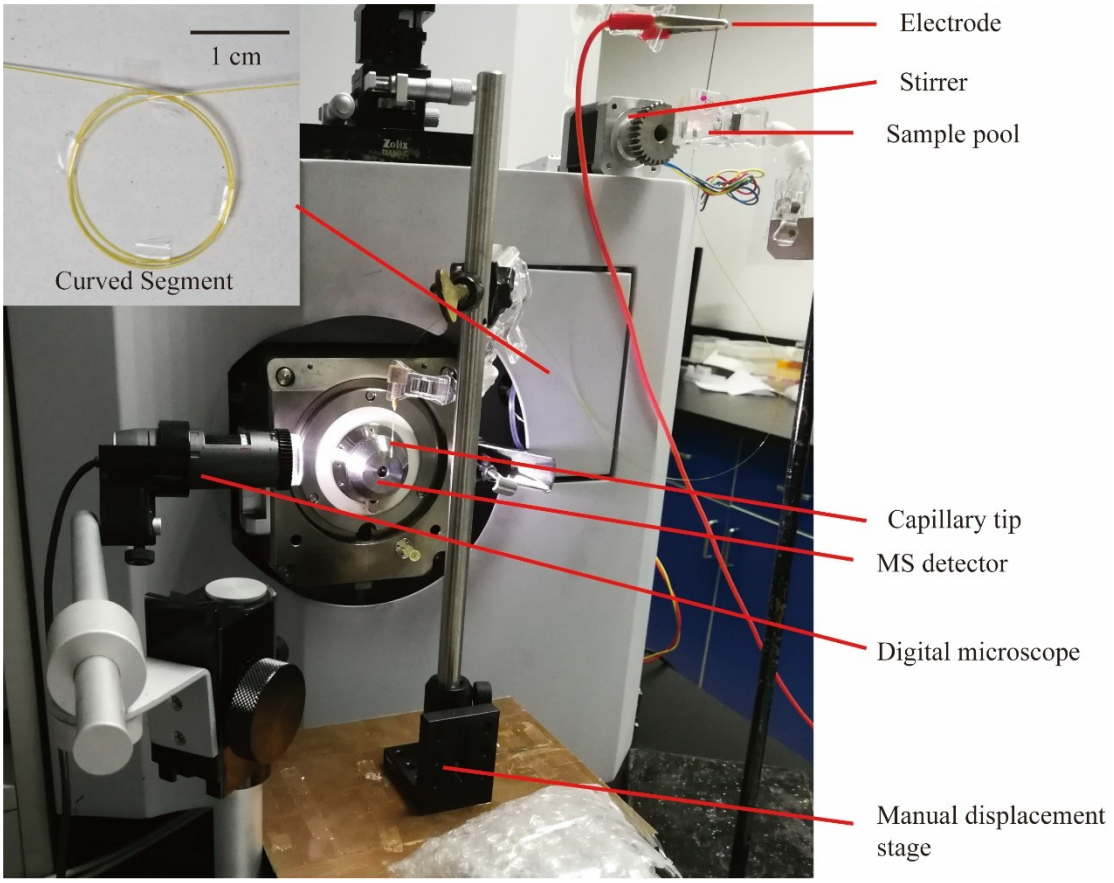
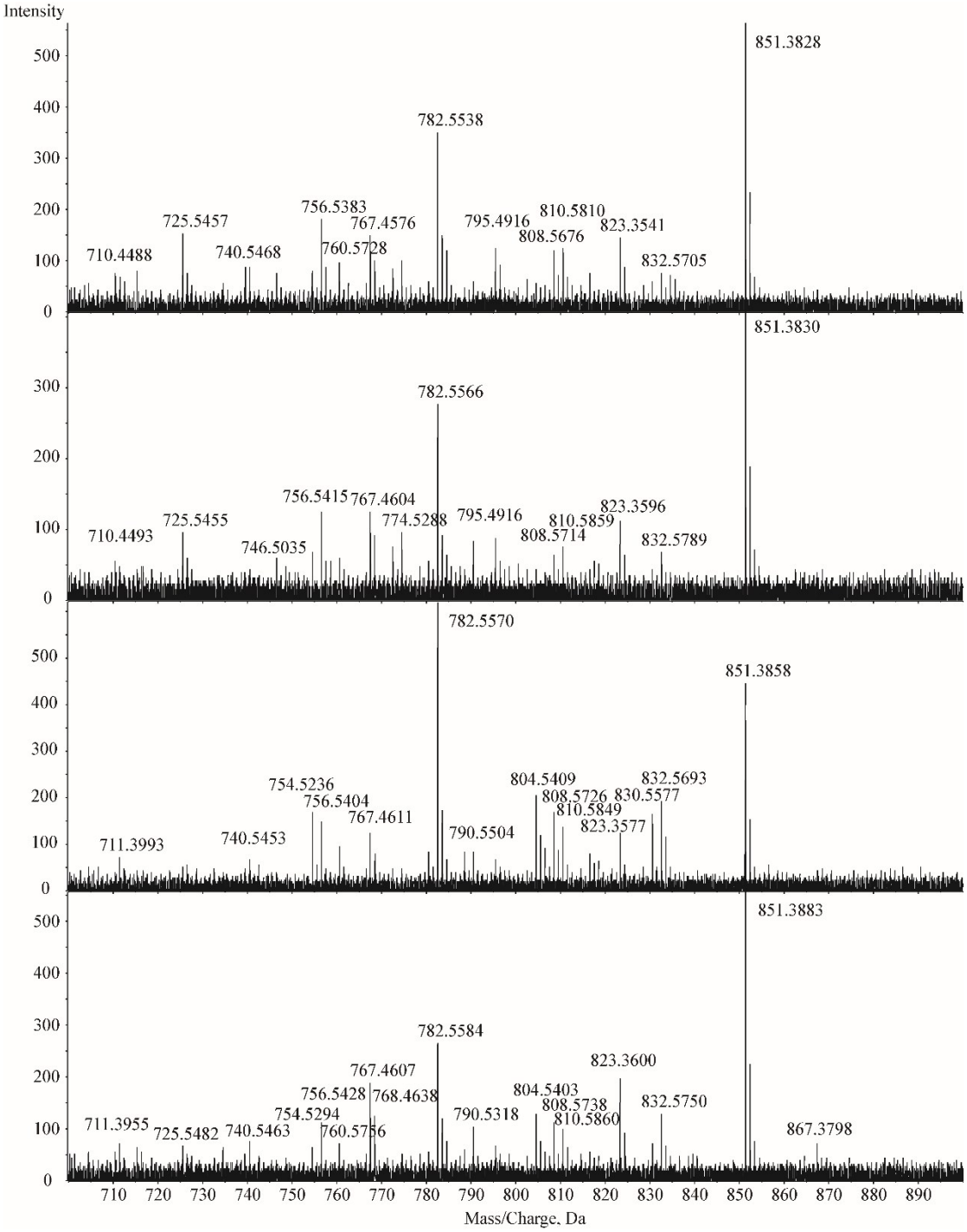


Fig. S5 Photograph of the single-cell operation system. The device consists of a platinum electrode connected to the laboratory-made high-voltage power supply, video capture device (a digital microscope focused on the capillary tip showing the cell condition in the capillary and the Taylor cone), a magneton and stirrer to induce homogeneous cell suspension, the specially immobilized capillary a MS detector.

Mass spectrum of different single cells of HUVEC



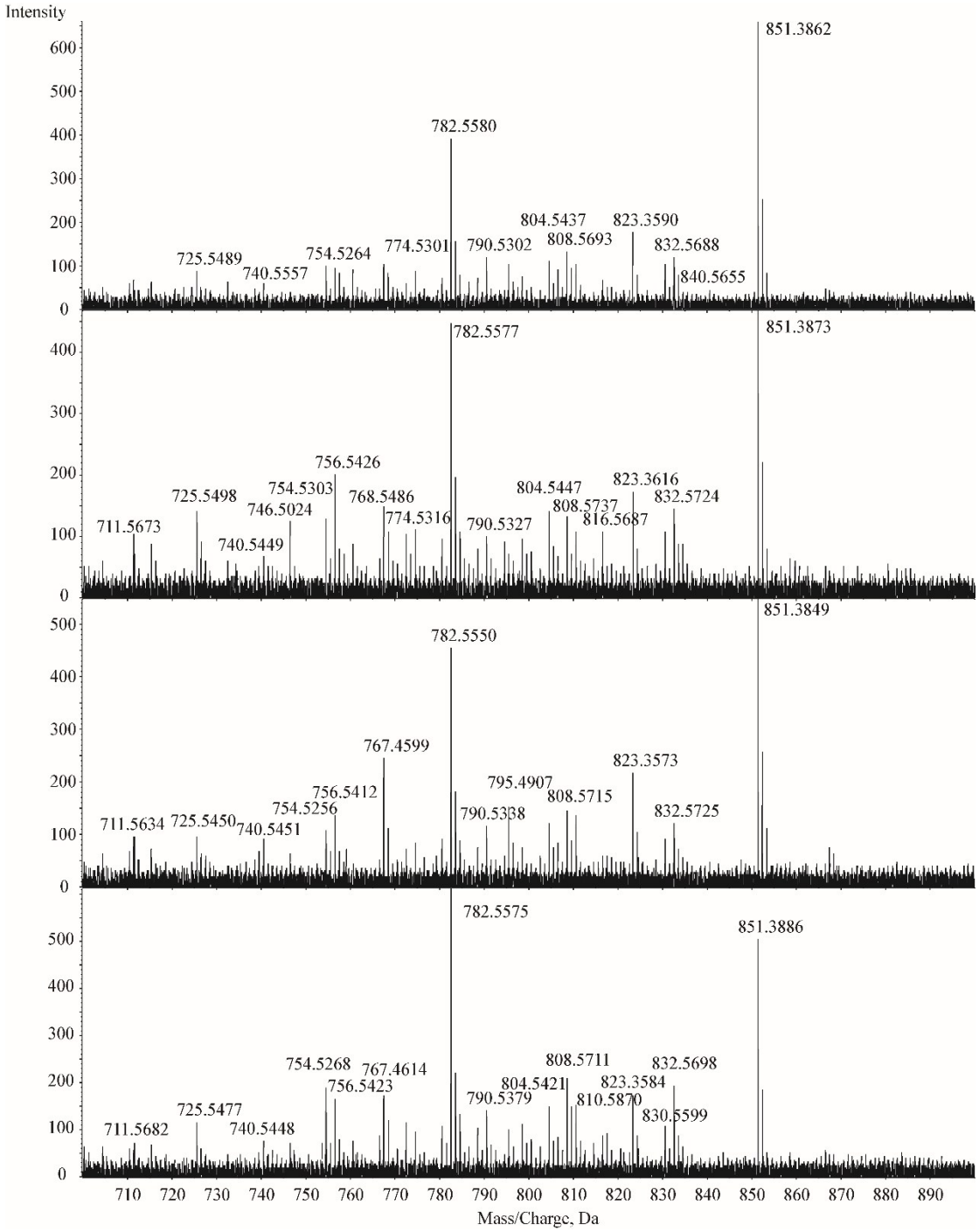


Fig. S6 Mass spectrum of different single cells of HUVEC

In this period, 8 different single HUVEC cells are picked out as examples of single-cell mass spectra. The main ion peak of cell is 782.5 m/z, accompanied with 725, 756 and 810 m/z.

Table

Table S1. Different cell with the intensity of 4 different phosphatidylcholine

	Intensity, m/z			
Cell number	PC(32:1)	PC(34:1)	PC(36:2)	PC(36:1)
Caco2-1	537	819	297	108
Caco2-2	445	763	185	125
Caco2-3	229	537	193	84
Caco2-4	398	621	216	97
Caco2-5	229	537	169	84
Caco2-6	368	655	212	100
Caco2-7	320	436	162	105
Caco2-8	303	590	225	92
Caco2-9	381	622	224	101
Caco2-10	412	626	189	80
HepG2-1	233	724	313	84
HepG2-2	240	645	294	104
HepG2-3	181	552	281	84
HepG2-4	165	506	193	92
HepG2-5	272	645	301	100
HepG2-6	277	793	365	120
HepG2-7	273	734	353	100
HepG2-8	265	695	321	141
HepG2-9	229	554	241	88
HepG2-10	257	682	293	104
U87-1	72	293	92	72
U87-2	60	301	116	72
U87-3	72	378	112	104
U87-4	76	309	92	88
U87-5	64	253	96	104
U87-6	60	317	116	80
U87-7	88	556	165	153
U87-8	96	416	133	149
U87-9	88	571	149	149
U87-10	76	277	92	120

The intensity of each of ions from PC(32:1), PC(34:1), PC(36:2) and PC(36:1) is picked out in each cell. In the PCA analysis, with each individual cell as a variable, the data matrix is treated by linear transformation and then dimensionality reduced into 2-dimension. And finally, the result is shown in the graph.

Movie Description

Movie 1: Single-cells come out from Taylor cone and are detected by mass spectrometry
Description: Cells are labeled by Hoechst 33342 (HOE) and are observed under 365 nm wavelength laser. It is indicated that cells are ordered well by Dean flow after going through the curved segment of capillary.

Movie 2: Movement of a single-cell in Taylor cone
Description: Cells are labeled by Hoechst 33342 (HOE) and are observed under 365 nm wavelength laser. A single-cell is observed in Taylor cone with no other companies. The movement can be observed clearly.

Movie 3: Electrospray carried out by the device
Description: A high-speed microscope (VW-9000, Keyence Corporation of America) is used to observe the stability and condition of electrospray.

Supplementary References

1 H. J. Yang, K. H. Park, W. L. Dong, H. S. Kim and J. Kim, Rapid Communications in Mass Spectrometry Rcm, 2012, 26, 621.
2 F. Xu, L. Zou, Q. Lin and C. N. Ong, Rapid Communications in Mass Spectrometry Rcm, 2009, 23, 3243–3254.