Supplementary Information: High-Accuracy Label-Free Classification of Cancerous Extracellular Vesicles with Nanoaperture Optical Tweezers and Deep Learning

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Supplementary Information

Supplementary materials accompanying this paper are available and provide additional details and data that support the findings reported in the main text. These materials include the following information:

- Finite Difference Time Domain (FDTD) Simulations on DNHs

- DNH Samples
- EVs Transmission Electron Microscopy
- Optical tweezers setup
- Analysis of Single EV In-Situ Trapping Events
- Gradient-weighted Class Activation Mapping (Grad-CAM) Interpretations of Trapping Signals

These materials provide additional details and data supporting the findings reported in the main text.

Appendix A Finite Difference Time Domain Simulation on DNHs

Assuming the trap to be well described by a harmonic potential and considering the effects of the thermal/Brownian motion, the dynamics of the trapped particle can be described by the Langevin equation [1–3]:

$$m\ddot{\mathbf{r}}(\mathbf{t}) = -\gamma\dot{\mathbf{r}}(\mathbf{t}) - \mathbf{k_p} \bigodot \mathbf{r}(\mathbf{t}) + \sqrt{2k_B T_\gamma}\mathbf{W}(\mathbf{t}) \tag{A1}$$

where r and m are the position and mass of the particle, k_p is the trap stiffness and W_t is a stochastic contribution term of the random collisions with other particles in the fluid.

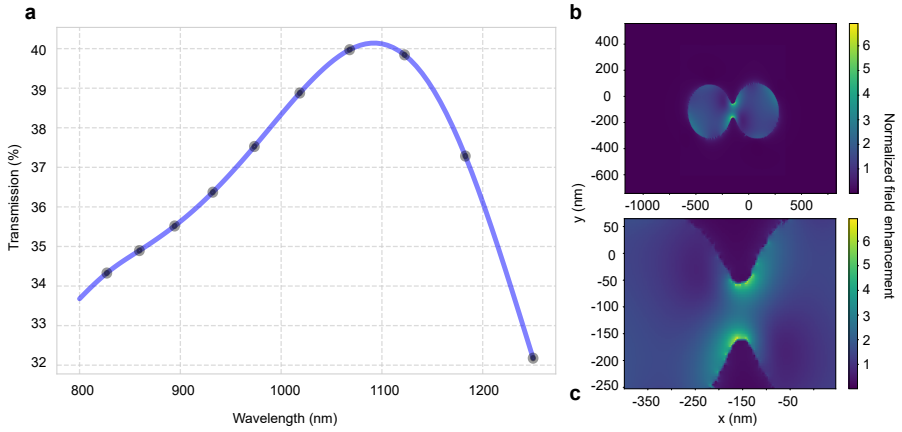

Fig. A1 FDTD simulations of the gold DNH sample: (a) the normalized electric field enhancement relative to the incident intensity for DNH aperture characterized by a 470 nm hole diameter and 102 nm cusp separation; (b) a zoom-in view of the field enhancement at the cusps; and (c) the transmission spectrum of the aperture, ranging from 800 nm to 1250 nm.

The DNHs we fabricated enhance the gradient force, allowing for more stable trapping at lower probe laser power compared to traditional optical tweezers. This

configuration also provides spatial confinement, effectively reducing the trapping volume. We performed FDTD simulation via Ansys Lumerical software (2020 R2) and figure A1 presents the properties of the DNH samples utilized in our experiments. As shown in Figure A1a, significant gap resonances appear around the near-IR region. Figures A1b and c demonstrate the normalized electric field enhancement at the DNH aperture, featuring a 102 nm cusp size and a 470 nm hole diameter. At the cusp, where the trapping occurs, the electric field is enhanced by nearly 7 times than the incident field.

Appendix B EVs Tranmission Electron Microscopy

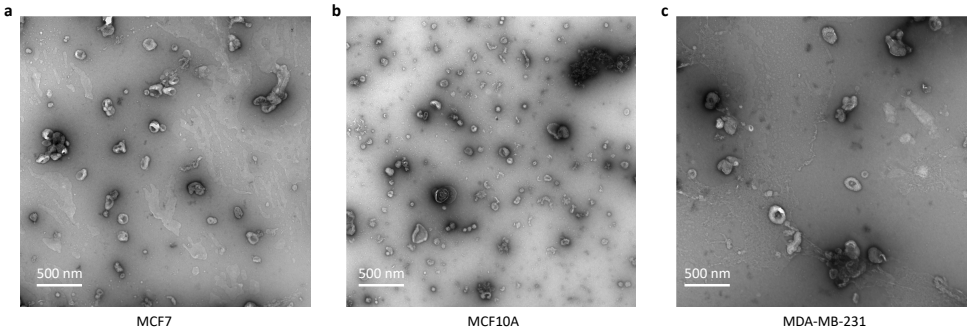

Fig. B2 TEM images for isolated EVs.

Appendix C DNH Samples

We fabricated DNHs and characterized them by using scanning electron microscope (SEM) images, shown in Fig C3. The average cusp size of the DNH is around 100 nm.

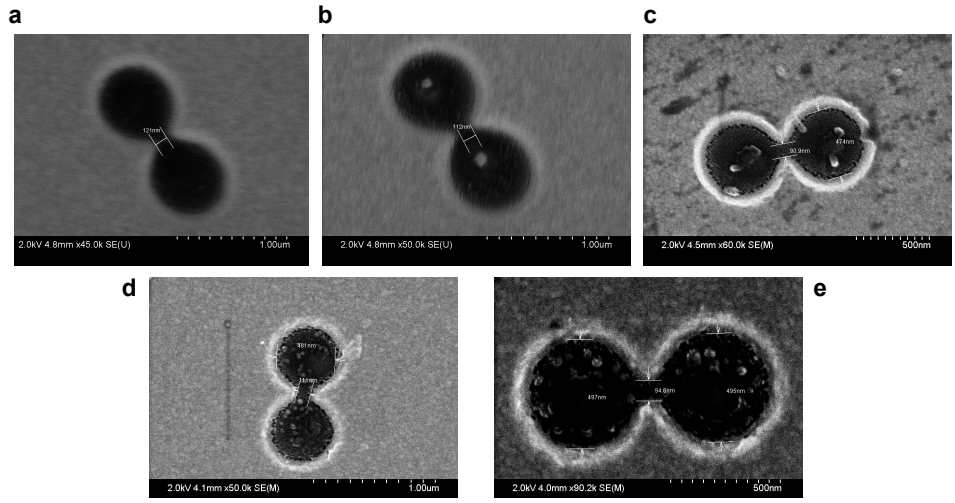

Fig. C3 Five colloidally fabricated DNH samples used in single EV trapping experiments, with cusp sizes of 121 nm, 112 nm, 90.9 nm, 111 nm, and 94.6 nm, respectively.

Appendix D Optical tweezers setup

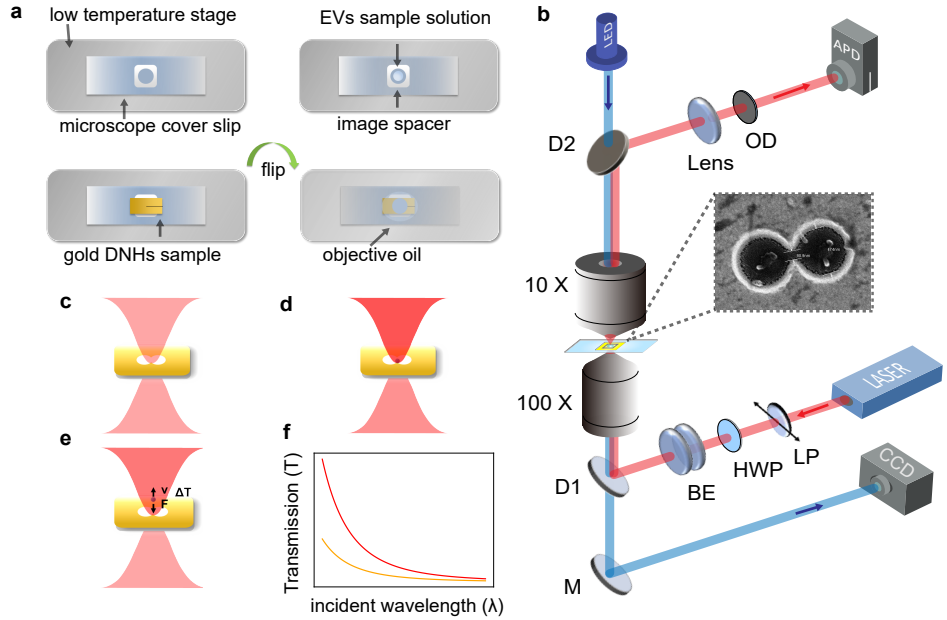

Fig. D4 Optical trapping workflow and working principle. (a) Schematic of the Low-Power Optical Trapping Setup: This setup uses a continuous 980 nm laser and includes a linear polarizer (LP), half-wave plate (HWP), beam expander (BE), dichroic mirror (D), objective lenses with 10x and 100x magnification, optical density filter (OD), avalanche photodetector (APD), mirror (M), and a charge-coupled device (CCD). (b) Procedure for EV Sample Preparation: Begin by placing the microscope cover slip on a metal stage chilled to 4°C, using a double-sided image spacer. Deposit the diluted EV solution in the center of the spacer and immediately cover it with the gold DNH sample to isolate the solution. Flip the cover slip over and apply microscope oil directly in the middle of the sample. (c-f) Illustration of the optical tweezer working principle: (c) no particle loaded: the aperture without any particle loaded. (d) Particle trapping: a particle trapped near the aperture enhances transmission through dielectric loading. (e) Decrease in transmission: the transmission signal decreases as the particle moves away from the trapping aperture. (f) Signal shift: demonstrates the shift in the transmission signal between the trapped (red) and un-trapped (black) states.

Appendix E Single EVs in-situ trapping events analysis

We randomly selected 10 trapping events for different EVs in Fig E5 (MDA-MB-231), Fig E6 (MCF7) and Fig E7 (MCF10A), respectivevly.

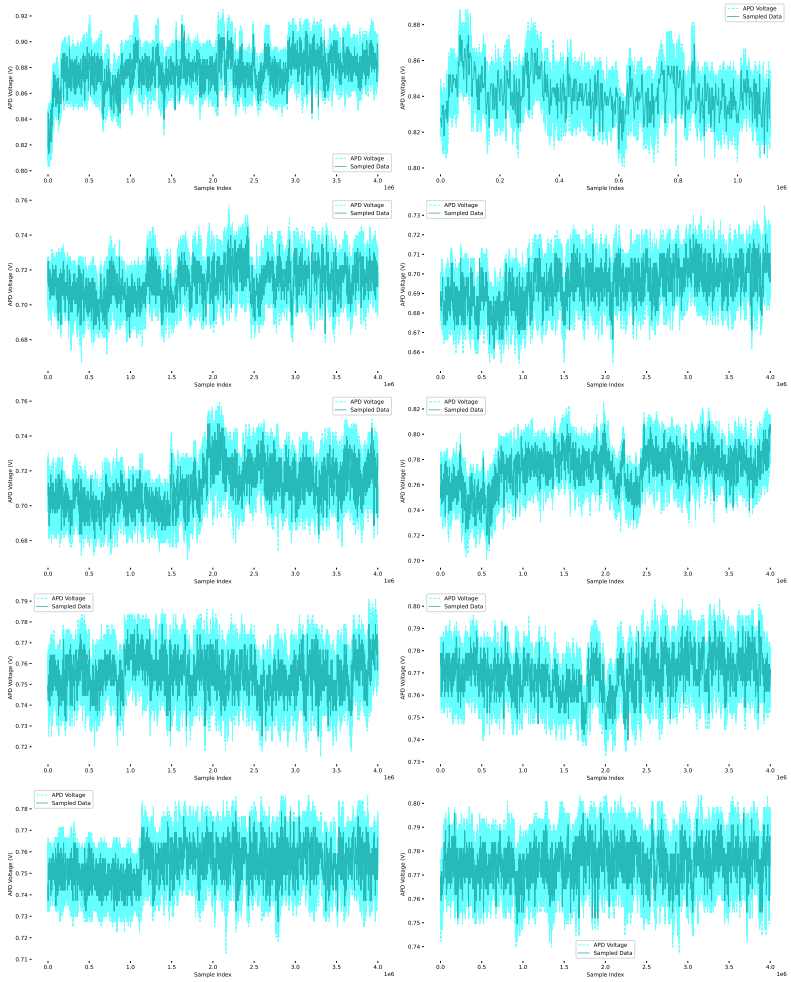

Fig. E5 Examples of MDA-MB-231 trapping events recorded by APD.

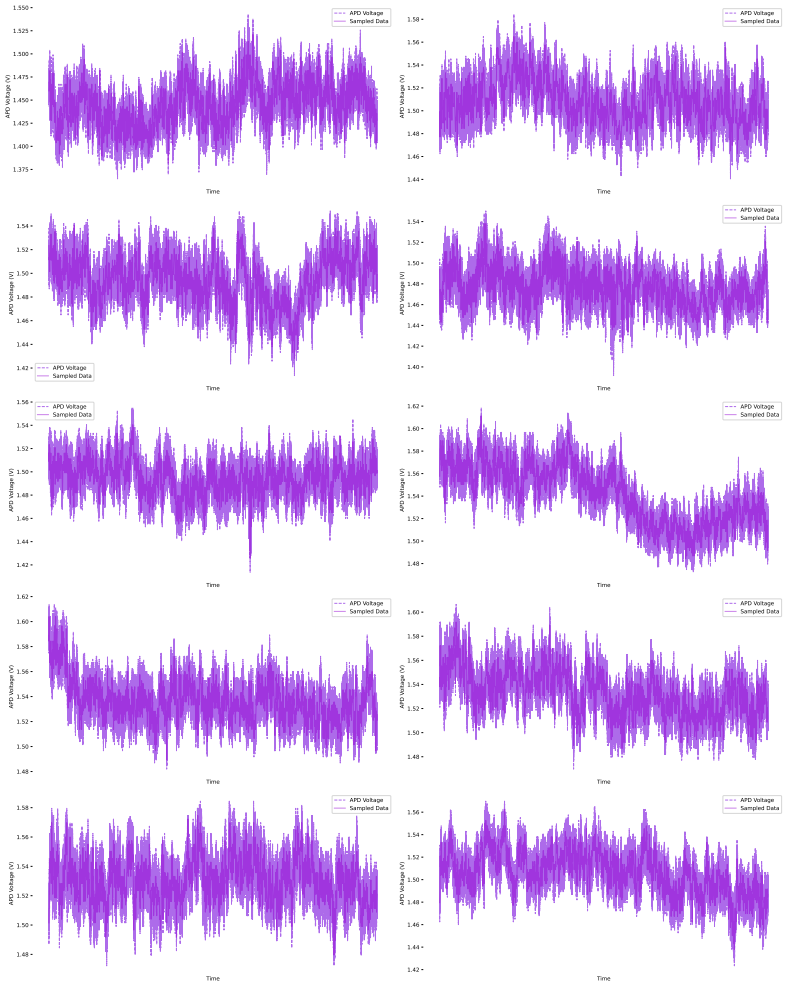

Fig. E6 Examples of MCF7 trapping events recorded by APD.

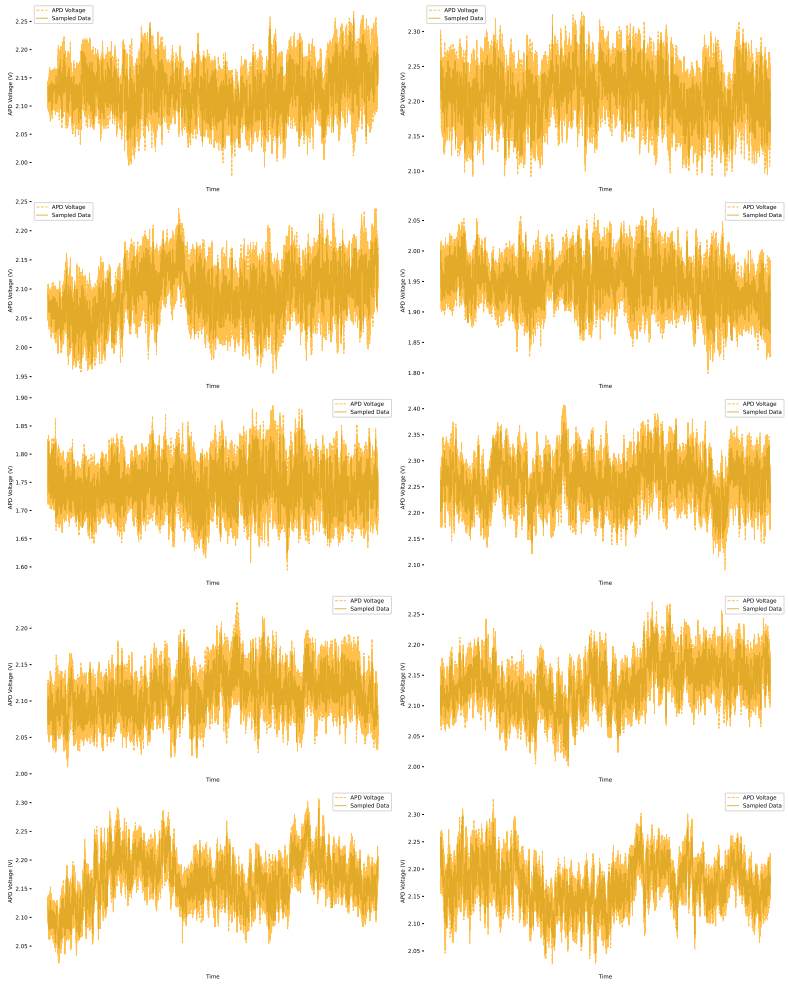

Fig. E7 Examples of MCF10A trapping events recorded by APD.

Appendix F Grad-CAM interpretation with trapping signals

16 randomly selected trapping events overlapped with GradCAM were shown in Fig F8 (MDA-MB-231), Fig F9 (MCF7), Fig F10 (MCF10A), respectively.

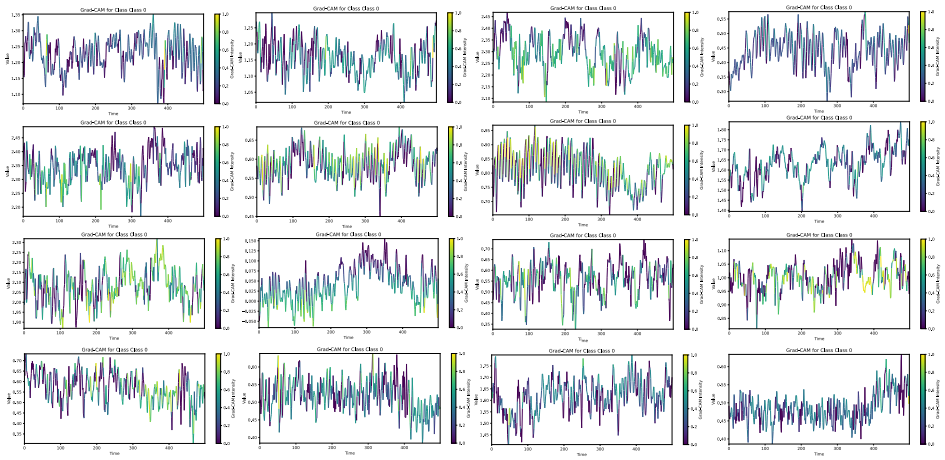

Fig. F8 Examples of MDA-MB-231 trapping events overlapped with GradCAM intensity.

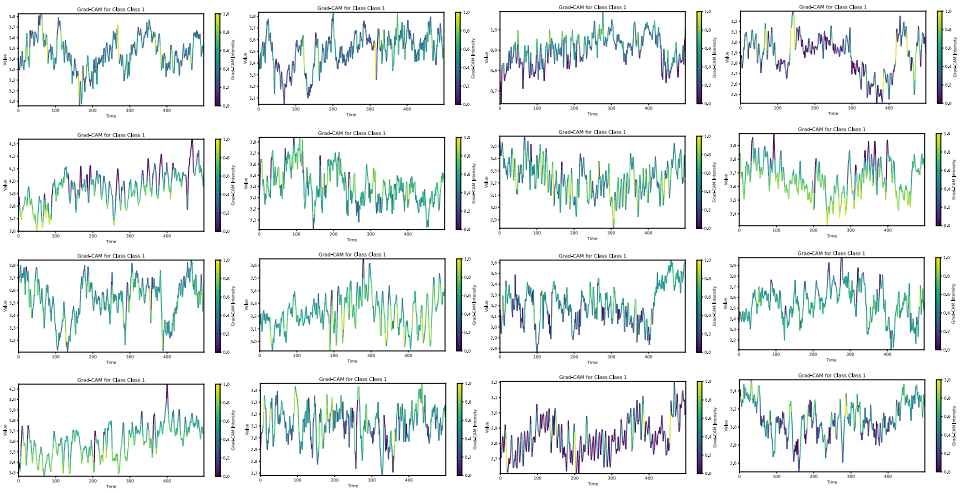

Fig. F9 Examples of MCF7 trapping events overlapped with GradCAM intensity.

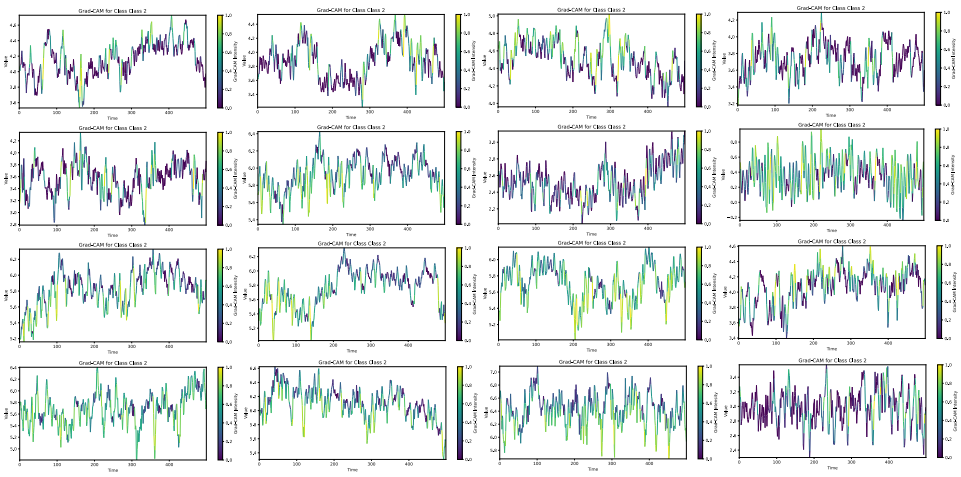

Fig. F10 Examples of MCF10A trapping events overlapped with GradCAM intensity.

References

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