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

Supplementary Materials

Dielectrophoretic enrichment of live chemo-resistant circulating-like pancreatic cancer cells from media of drug-treated adherent cultures of solid tumors

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A. Supplementary Results

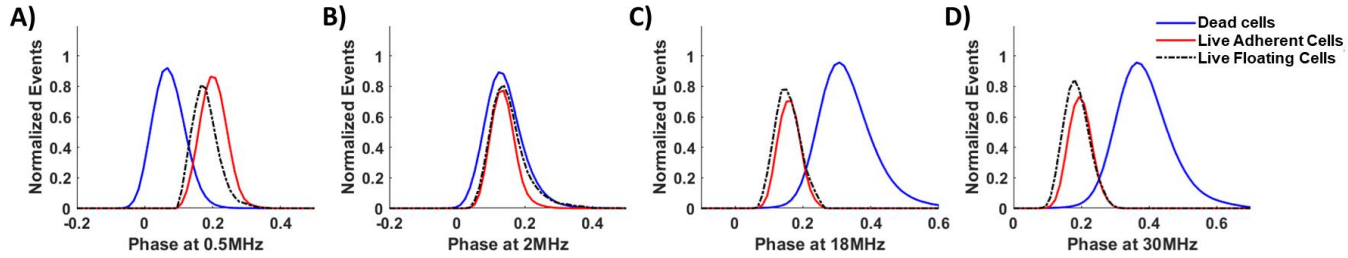

Fig S1: Impedance metrics of live adherent, live floating cells and dead cells: Live adherent and live floating cells have the same impedance characteristics in terms of phase at A) 0.5 MHz ($\phi Z_{0.5\,MHz}$), B) 2 MHz ($\phi Z_{2\,MHz}$), C) 18 MHz ($\phi Z_{18\,MHz}$) 18 MHz and D 30 MHz ($\phi Z_{30\,MHz}$). Live cells can be distinguished from dead cells as they have lower $\phi Z_{0.5\,MHz}$ and show larger phase at higher frequencies ($\phi Z_{18\,MHz}$ and $\phi Z_{30\,MHz}$).

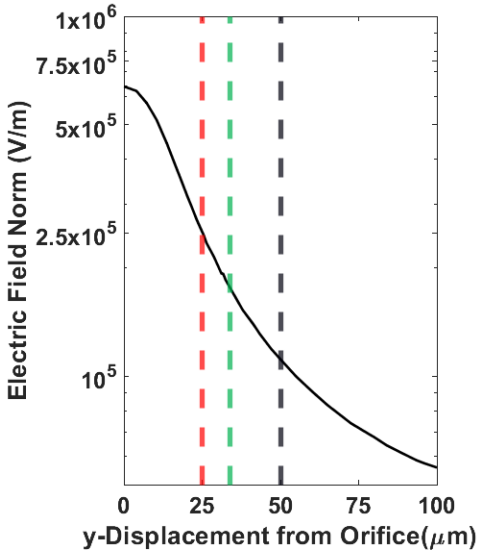

Fig S2: Electric field extent for optimal focusing of sample to maximize pDEP without cell entrapment at orifices: Focusing of the cells was optimized (~ 35 µm away from orifice or 15 µm from center of cross-sectional width, green) to maximize pDEP force while preventing trapping. Focusing of the cells in the center of the channel (50 µm away from orifice, black) leads to cells experiencing insufficient electric field causing low pDEP, while focusing closer to the orifice (25 µm away from orifice, red) leads to exposure to high electric field leading to trapping and subsequent cell death.

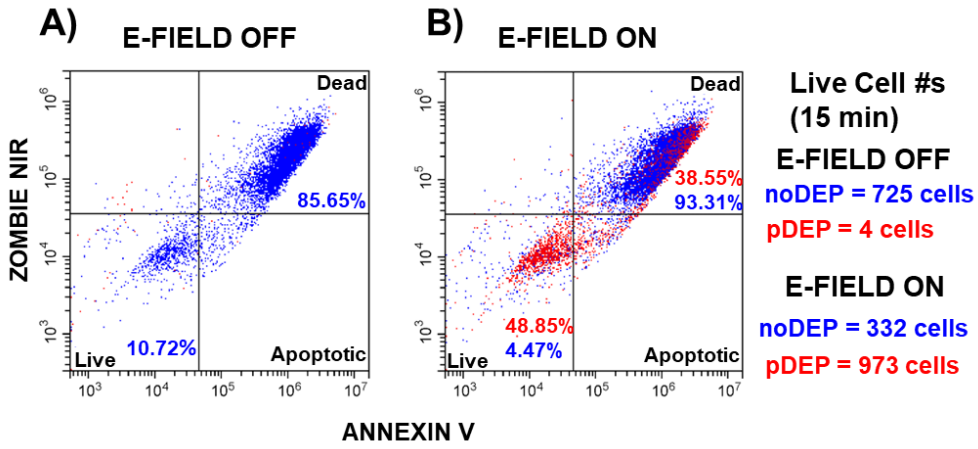

Fig S3: Live-Dead Staining of cells in each outlet with E-Field OFF and E-Field ON: A) Under E-field off conditions the 10.72% live cells were all collected in the no-DEP outlet (725 cells) with very few (4 cells) in the pDEP outlet. B) With the E-field on, the input of 10.72% was enriched to 48.85% in the pDEP outlet with ~75% of the live cells (973) collected in the pDEP outlet.

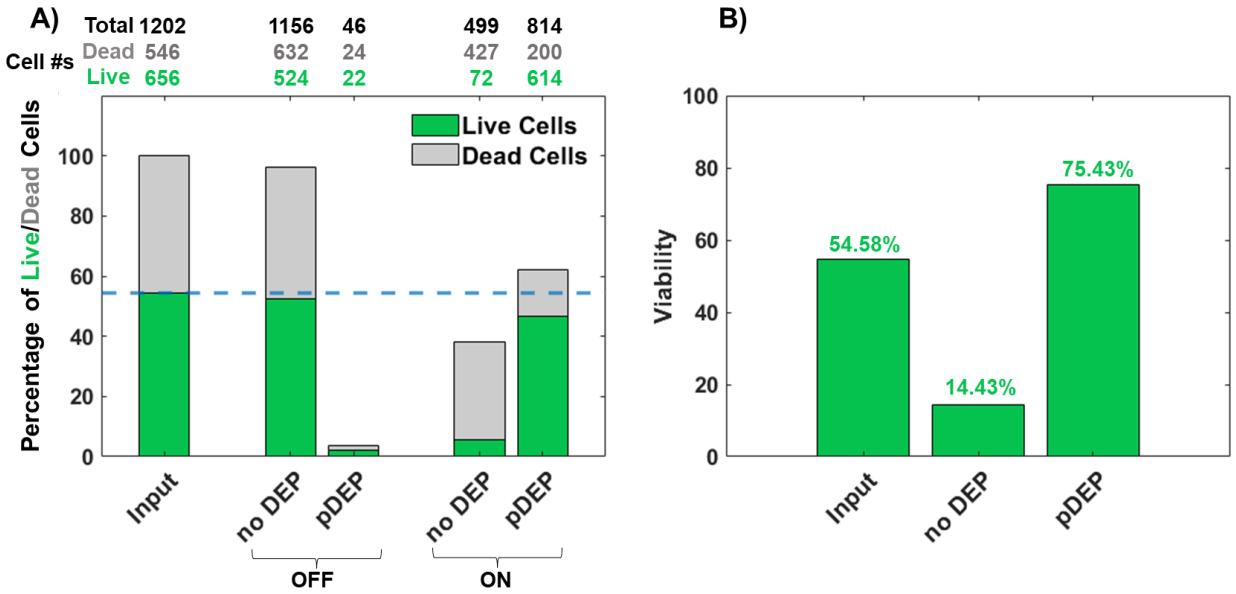

Fig S4: Representative distribution and GFP based viability of adherent and circulating cells (2 well plates of floating and adherent) in each outlet after collection for 15 mins at the optimized conditions of 25 V, 1MHz: A) The distribution of live (GFP+) and dead cells (GFP-) in each outlet shows that majority of live cells are deflected to the pDEP outlet and at 25 V, 1 MHz. B) The viability of collected cells (GFP+) is enriched from 55% in the input to 75% in the pDEP outlet.

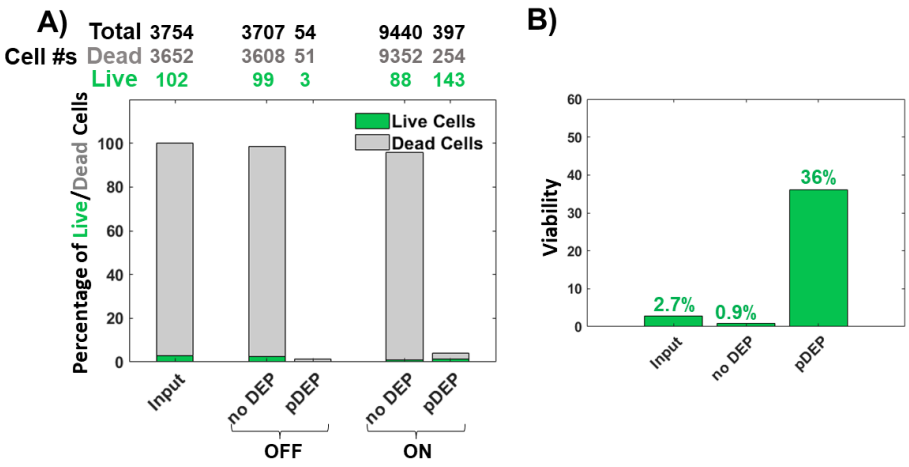

Fig S5: Representative distribution and GFP based viability of floating cells only in each outlet after collection for 25 mins at the optimized conditions of 25 V, 1MHz: A) The distribution of live (GFP+) and dead cells (GFP-) in each outlet shows that majority of live cells are deflected to the pDEP outlet and at 25 V, 1 MHz. B) The viability of collected cells (GFP+) is enriched from ~3% in the input to 36% in the pDEP outlet.

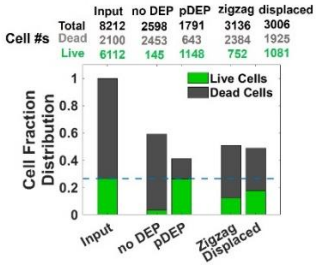

Fig. S6: Collected cell #s in 15 minutes to compare live cell enrichment after pDEP vs. DLD methods.

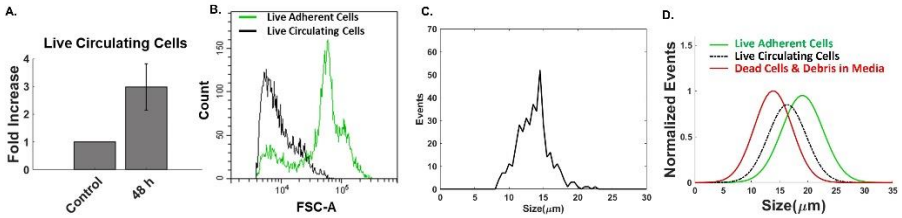

Fig. S7: Circulating cell numbers and sizes. A. Number of live circulating cells in media increases after 48 h gemcitabine treatment (1 μg/mL) vs. control (untreated with ~500 live events). Size comparison: B. FSC Flow Cytometry; C-D. Electrical size from impedance cytometry of 3% live circulating cell input sample used for results in Figure 5 of manuscript.

Movie 1: Movie showing the inlets, active region, cell deflection into pDEP and noDEP outlets under E-Field OFF and E-Field ON: Cells are focused using sample and sheath flows to enter the active region consisting of posts and orifices. Under E-Field OFF, cells pass undeflected into the no DEP outlet with no cells entering pDEP outlet, while with E-Field ON, cells are deflected to the pDEP outlet based on viability status.

B. Supplementary Methods

1. Machine learning for optimizing impedance metrics to gate live vs. dead PDAC cells: The support vector machine (SVM) supervised learning model was utilized to train impedance metrics with known samples of dead (heat treated) and live (untreated) PDAC cells. Per the gates and associated confusion matrices in Fig. S6, the specific metrics that distinguish live *vs.* dead cells were identified to arise from comparison of impedance phase (ϕZ) at low frequency (0.5 MHz) to that at high frequency (18 MHz or 30 MHz); i.e., Fig. S6 A and S6B. These metrics were used to compute the hyperplane (line in 2D plot) for label-free gating of live *vs.* dead PDAC cells.

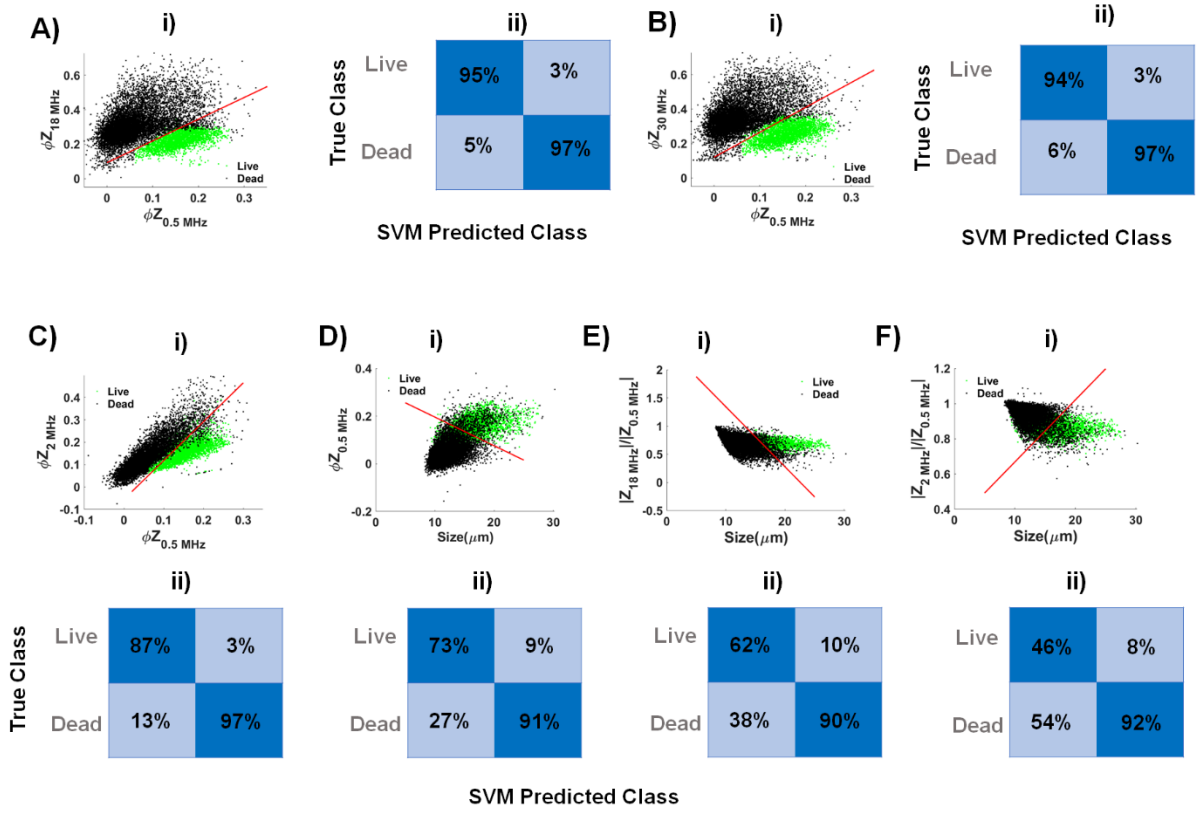

Fig S8: Using Supporting Vector Machine(SVM) with linear kernel to classify dead vs live cells. A) Using phase at 18 MHz and 0.5 MHz, B) Phase at 30 MHz and 0.5 MHz, C) Phase at 2 MHz and 0.5 MHz, D) Electrical size vs phase at 0.5 MHz, E) Opacity ($|Z_{18\ MHz}|/|Z_{0.5\ MHz}|$) vs electrical size, and F) Opacity ($|Z_{2\ MHz}|/|Z_{0.5\ MHz}|$) vs electrical size.

2. Dielectric Shell Modelling

For a cell suspended in a dielectric medium, the dielectric properties of the suspension can be determined using Maxwell's Mixture theory by calculating the complex permittivity of the suspension ($\tilde{\varepsilon}_{mix}$). To calculate the dielectric properties of the suspended cell, MMT-based shell models can be used. For the sake of simplification, the cell is modelled as a series of concentric shells, each with its own defined dielectric properties. The simplest model of a cell, a single shell model has two dispersions at its interfaces (medium-membrane and membrane-interior). The complex permittivity of the suspension ($\tilde{\varepsilon}_{mix}$) is:

$$\tilde{\varepsilon}_{mix} = \tilde{\varepsilon}_{medium}\frac{1+2\varphi f_{CM}}{1-\varphi f_{CM}} \qquad (1)$$

where $\tilde{\varepsilon}_{medium}$ is the complex permittivity of the surrounding medium, φ is the volume fraction of the particle in the medium and f_{CM} is the Clausius-Mossotti factor of the cell in the mixture. $\tilde{\varepsilon}$ can be defined as:

$$\tilde{\varepsilon} = \varepsilon_0\varepsilon - j\frac{\sigma}{\omega} \qquad (2)$$

where ε is the permittivity, ε_0 is the constant permittivity in vacuum, σ is the conductivity, ω is the frequency of the applied electric field and j^2= -1.

For a shell model, the Claussius-Mosotti factor of the cell in the mixture (f_{CM}) is given by:

$$f_{CM} = \frac{\tilde{\varepsilon}_{cell} - \tilde{\varepsilon}_{medium}}{\tilde{\varepsilon}_{cell} + 2\tilde{\varepsilon}_{medium}} \qquad (3)$$

The complex permittivity of the cell ($\tilde{\varepsilon}_{cell}$) in a single shell model can be modelled as:

$$\tilde{\varepsilon}_{cell} = \tilde{\varepsilon}_{membrane}\frac{\gamma^3 + 2\left(\frac{\tilde{\varepsilon}_{interior} - \tilde{\varepsilon}_{membrane}}{\tilde{\varepsilon}_{interior} + 2\tilde{\varepsilon}_{membrane}}\right)}{\gamma^3 - \left(\frac{\tilde{\varepsilon}_{interior} - \tilde{\varepsilon}_{membrane}}{\tilde{\varepsilon}_{interior} + 2\tilde{\varepsilon}_{membrane}}\right)} \qquad (4)$$

with;

$$\gamma = \frac{r_{cell}}{r_{cell} - d_{membrane}} \qquad (5)$$

where r_{cell} is the radius of the cell and $d_{membrane}$ is the thickness of the cell membrane. With the calculation of the complex permittivity of the suspension ($\tilde{\varepsilon}_{mix}$), the impedance of the mixture ($\tilde{Z}_{mix}$) can be calculated as:

$$\tilde{Z}_{mix} = \frac{1}{j\omega\tilde{\varepsilon}_{mix}G} \qquad (6)$$

where G is the geometric constant of the system, and can be approximated as:

$$A_{electrode}/d_{electrode} \qquad (7)$$

where $A_{electrode}$ is the surface area of the electrode and $d_{electrode}$ is the distance between the electrodes. Since $\tilde{Z}_{mix}$ is frequency dependent, relaxation curves for impedance magnitude ($|Z|$) and phase (ϕZ) can be calculated using:

$$|Z| = \sqrt{Re\left(\tilde{Z}_{mix}\right)^2 + Im\left(\tilde{Z}_{mix}\right)^2} \qquad (8)$$

$$\phi Z = \tan^{-1}\frac{Im\left(\tilde{Z}_{mix}\right)}{Re\left(\tilde{Z}_{mix}\right)} \qquad (9)$$

where $Re\left(\tilde{Z}_{mix}\right)$ and $Im(\left(\tilde{Z}_{mix}\right)$ are the real and imaginary parts of the complex impedance of the mixture $\left(\tilde{Z}_{mix}\right)$.

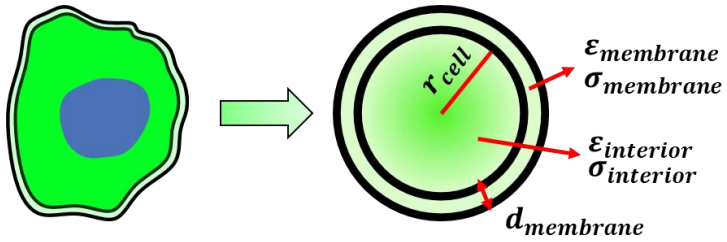

Fig S9: Single shell model of cell based on Maxwell's mixture theory.

Table S1: Fitting parameters for simulating cell dielectric dispersions

Parameter	Value
Vaccuum permittivity (ε_0)	8.85× 10^{-12} F m^{-1}
Medium conductivity (σ_{medium})	1.6 S/m
Medium permittivity (ε_{medium})	80
Membrane Conductivity ($\sigma_{membrane}$)	10^{-3}-10^{-6} S/m
Membrane Permittivity ($\varepsilon_{membrane}$)	5.87
Interior Conductivity ($\sigma_{interior}$)	0.005-0.5 S/m
Interior Permittivity ($\varepsilon_{interior}$)	60
Cell Radius (r_{cell})	8.5 um
Membrane Thickness ($d_{membrane}$)	14
Surface Area of electrode ($A_{electrode}$)	10^{-9} m^2
Distance between electrodes ($d_{electrode}$)	60 × 10^{-6} m

Based on this, the dielectrophoretic force (F_{DEP}) experienced by the cells due to their polarization in a non-uniform electric field can be calculated as:

$$F_{DEP} = 2\pi\varepsilon_{medium}{r_{cell}}^3 Re(f_{CM})(\nabla E^2) \qquad (10)$$

where ∇E^2 is the Laplacian operator of the applied electric field squared.