Electronic Supplementary Material (ESI) for RSC Advances.

SUPPLEMENTARY INFORMATION

Single-cell intracellular nano-pH probes

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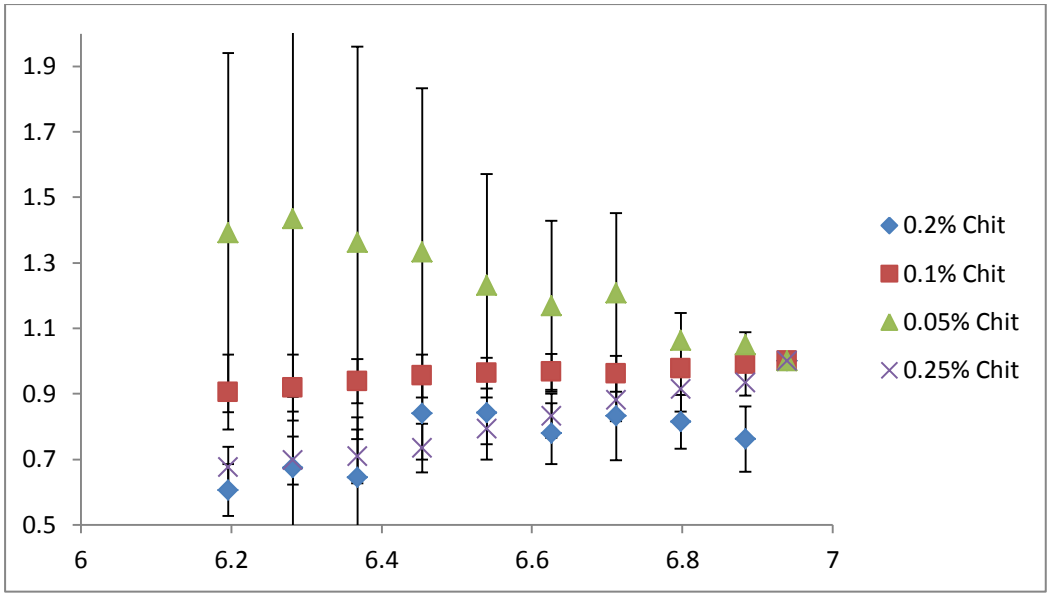

Figure SI1: The effect of chitosan concentration on pH sensing capabilities.

Chitosan solutions below 0.2% were not pH sensitive and performed similar to a bare nanopipette. Concentrations higher than 0.25% did not reach to the nanopipette's tip due to the geometrical hindrance and high viscosity of the chitosan solution. Taking into consideration these aspects, 0.25% chitosan solution was selected to coat the inner walls of nanopipettes.

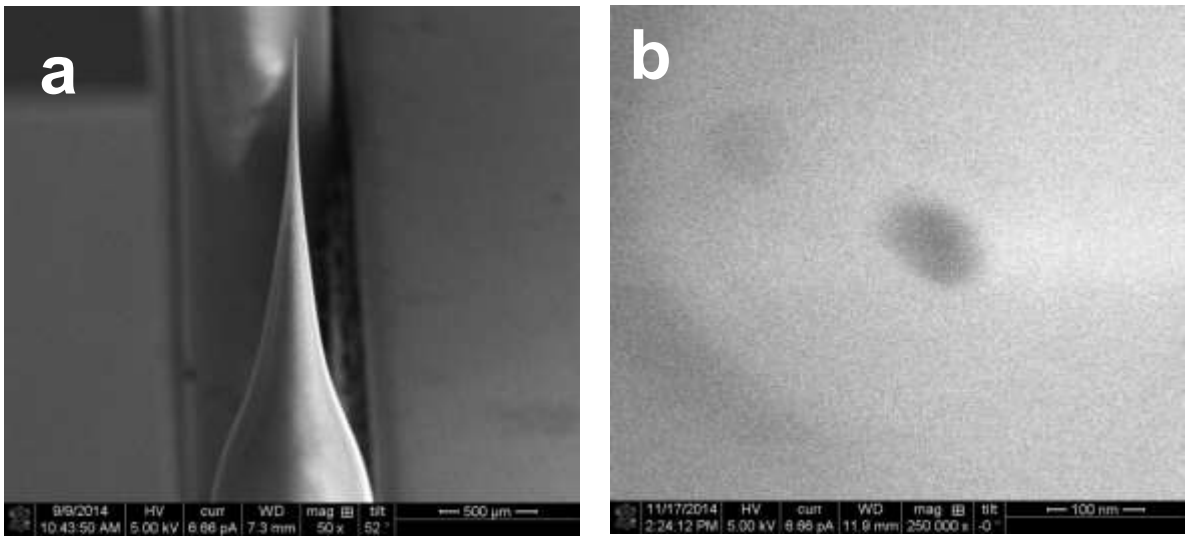

Figure SI2: SEM characterization of quartz nanopipette. Scanning electron micrographs of **(a)** the side view of a nanopipette tip, and **(b)** the pore of chitosan-modified nanopipette.

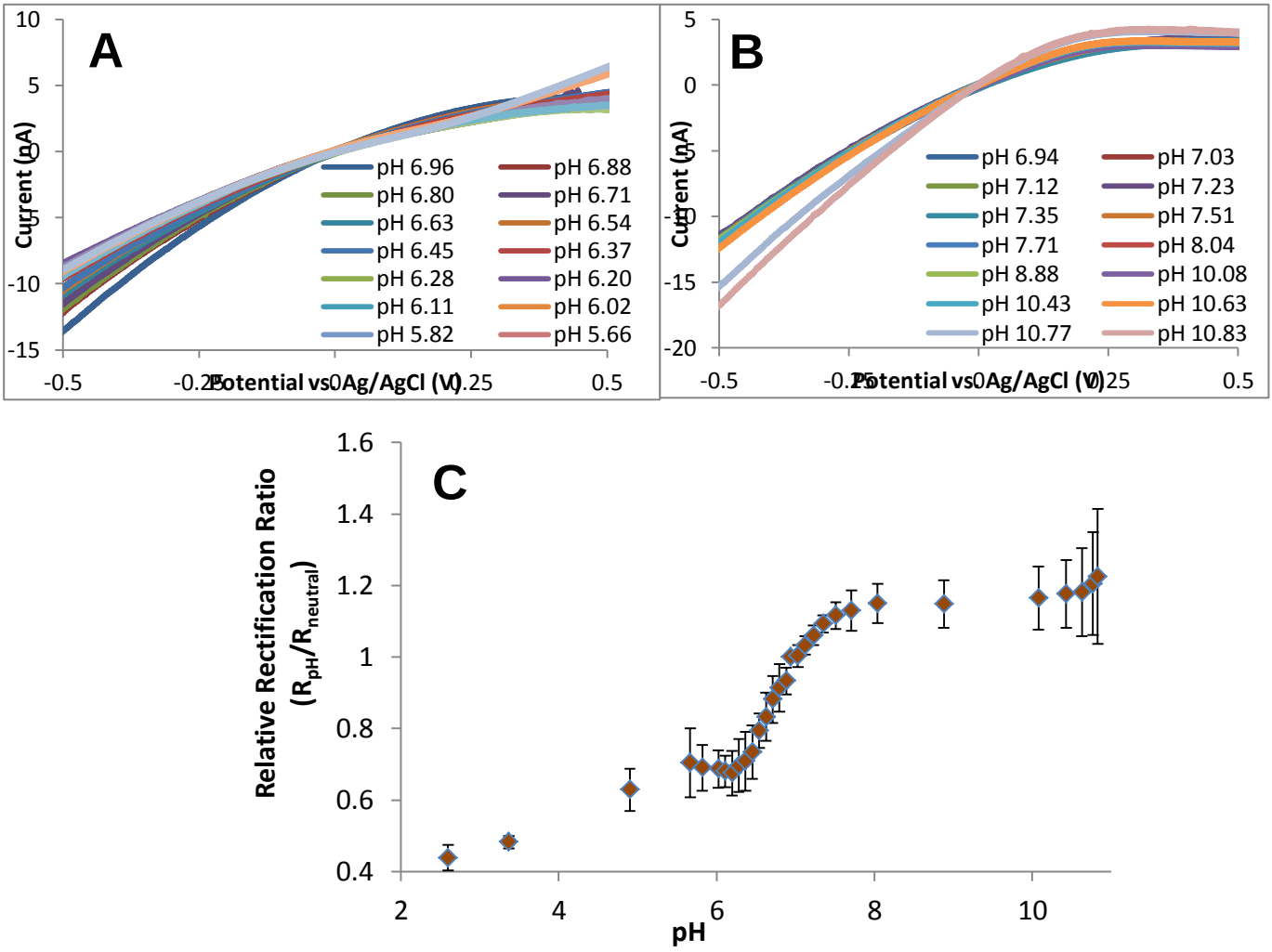

Figure SI3: Wide range calibration of nano-pH probe. Typical linear sweep voltammograms of **(a)** acid and **(b)** base titration of a chitosan-modified nanopipette. **(c)** The corresponding calibration nano-pH probe between 2.59 and 10.83.

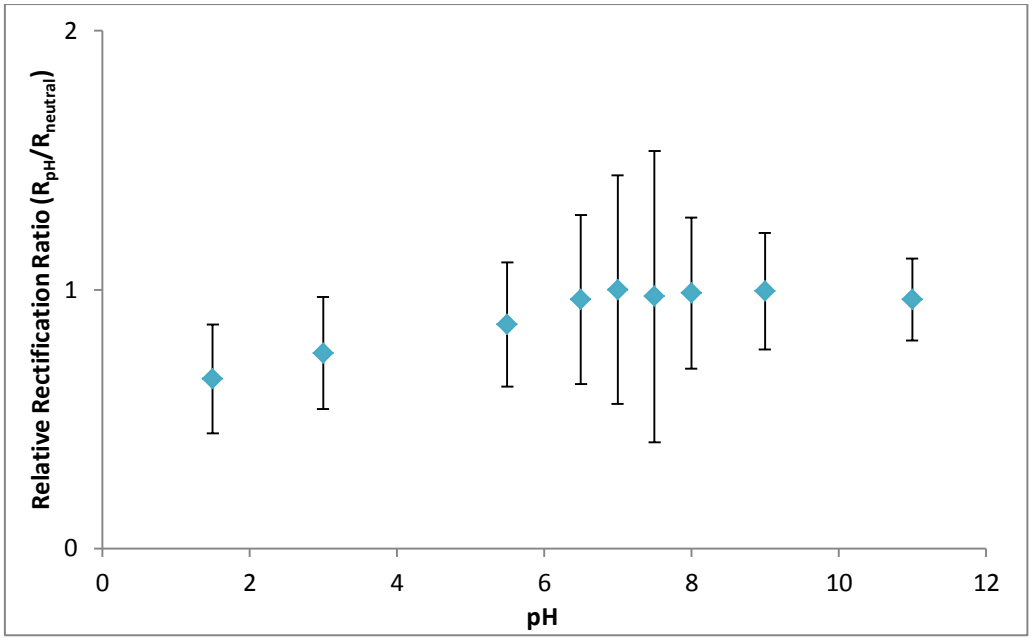

Figure SI4: pH response of a bare nanopipette. The error bars indicate standard deviation for *n = 3* replicate measurements.

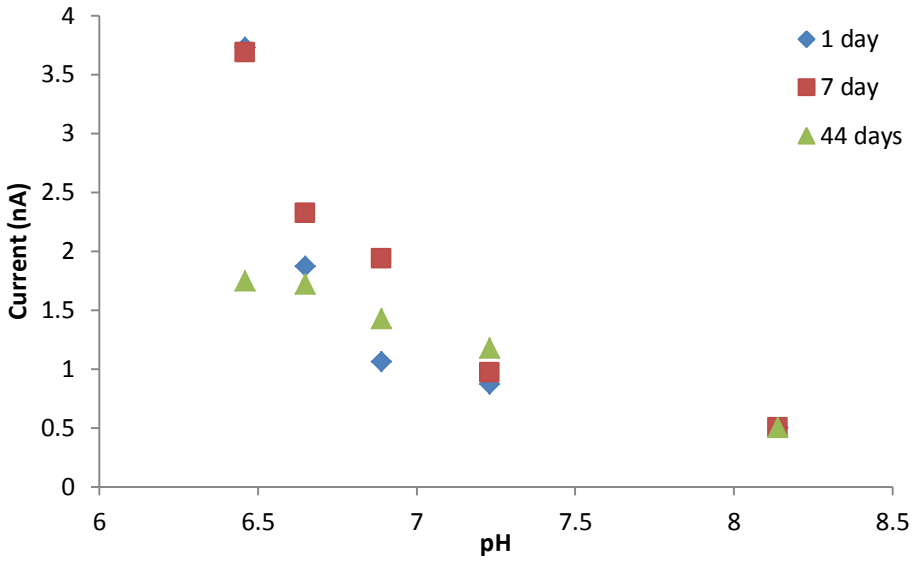

Figure SI5: Storage stability of the chitosan modified quartz nanopipettes.

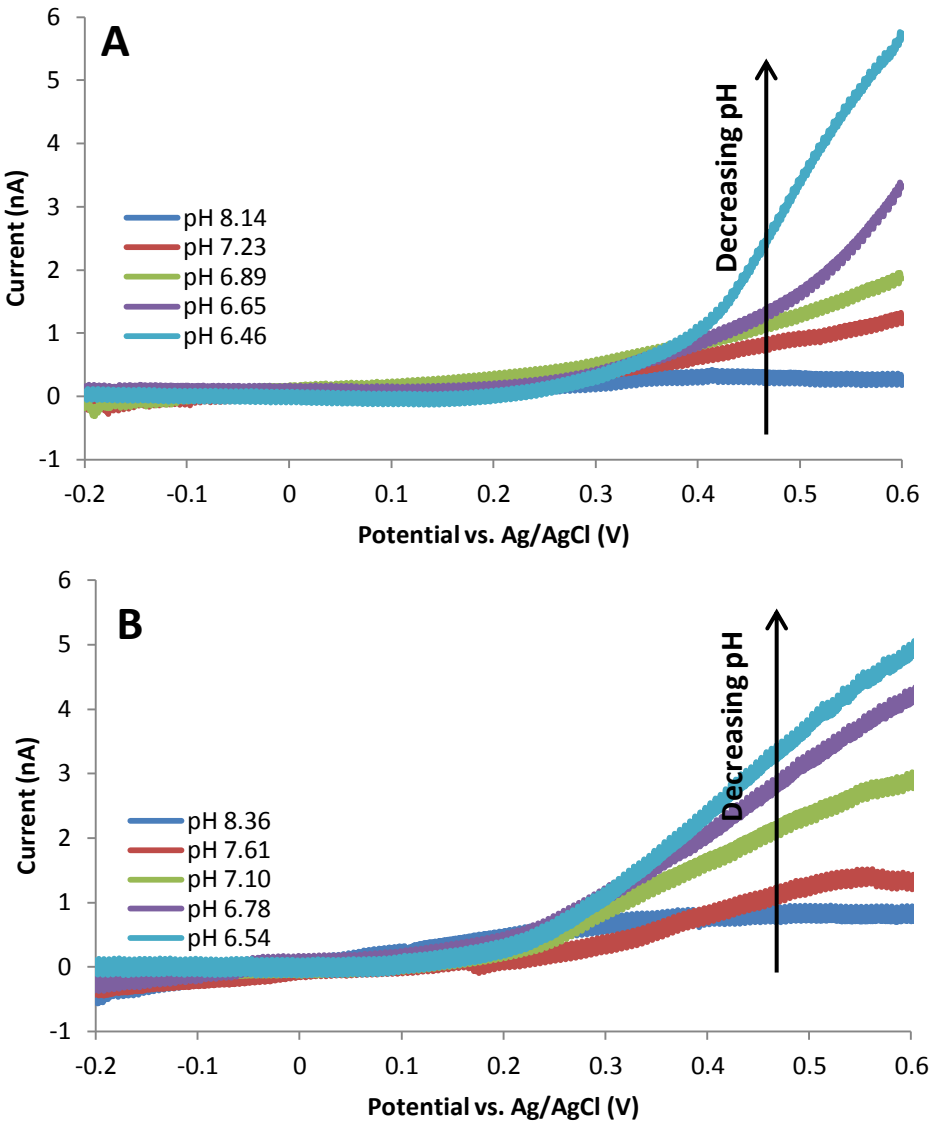

Figure SI6: Current-potential curves of chitosan-modified nanopipette for acid titration in cell culture media **(a)** MEM and **(b)** DMEM.

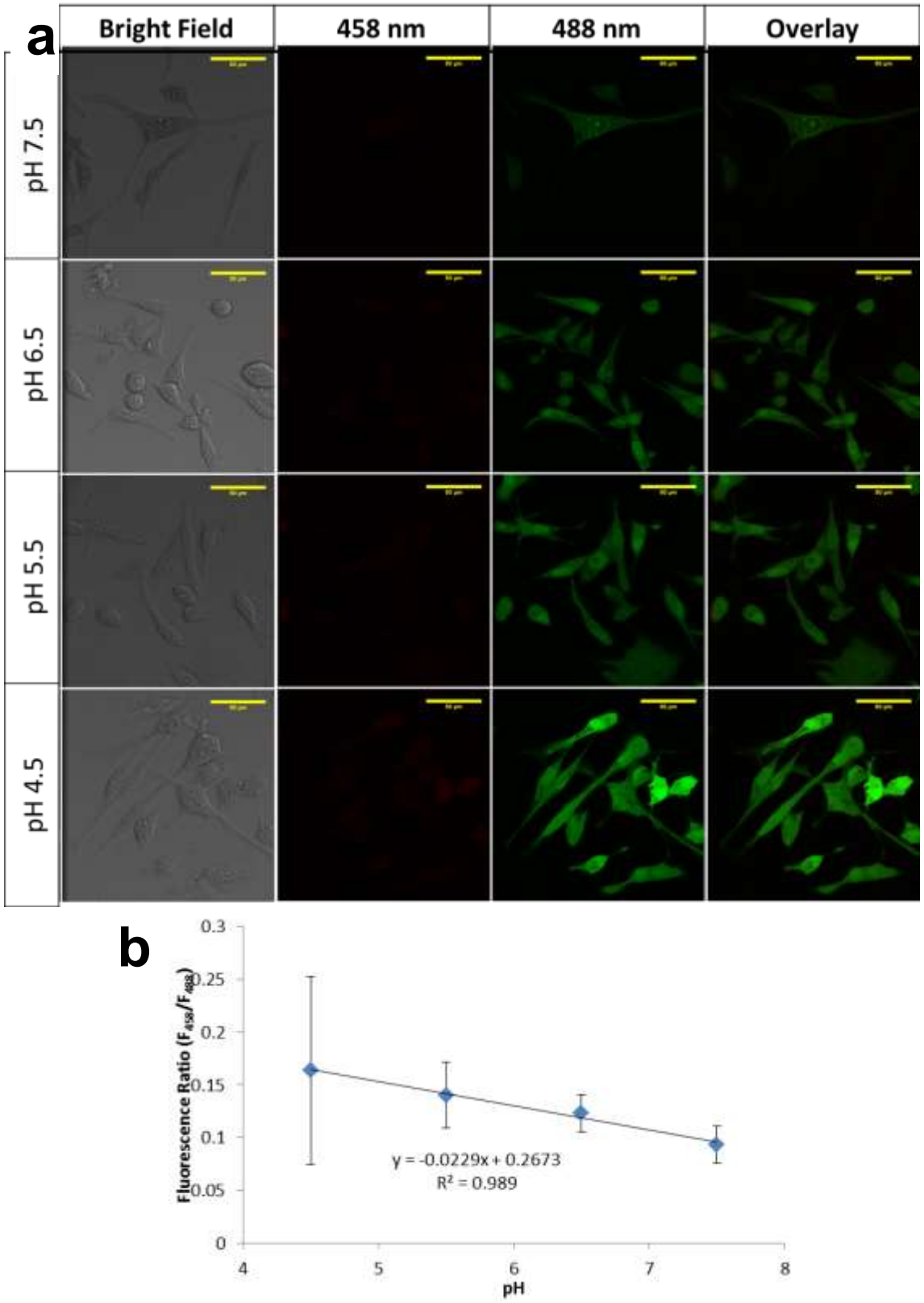

Figure SI7: Intracellular pH measurement with a conventional fluorescence dye. **(a)** Fluorescence images of MDA-MB-231 cells exposed to standard pH solutions of 7.5, 6.5, 5.5 and 4.5 in the presence of BCECF, AM. The first (left) column shows bright field micrographs, while second and third show the fluorescence images at 458 and 488 nm

excitation wavelength, respectively. The last column (on right) exhibits the overlay of the two excitation wavelengths. **(b)** Corresponding intracellular pH calibration curve of BCECF-AM. Error bars represent the standard deviation for *n = 16 – 23* cells across three replicate cell cultures.

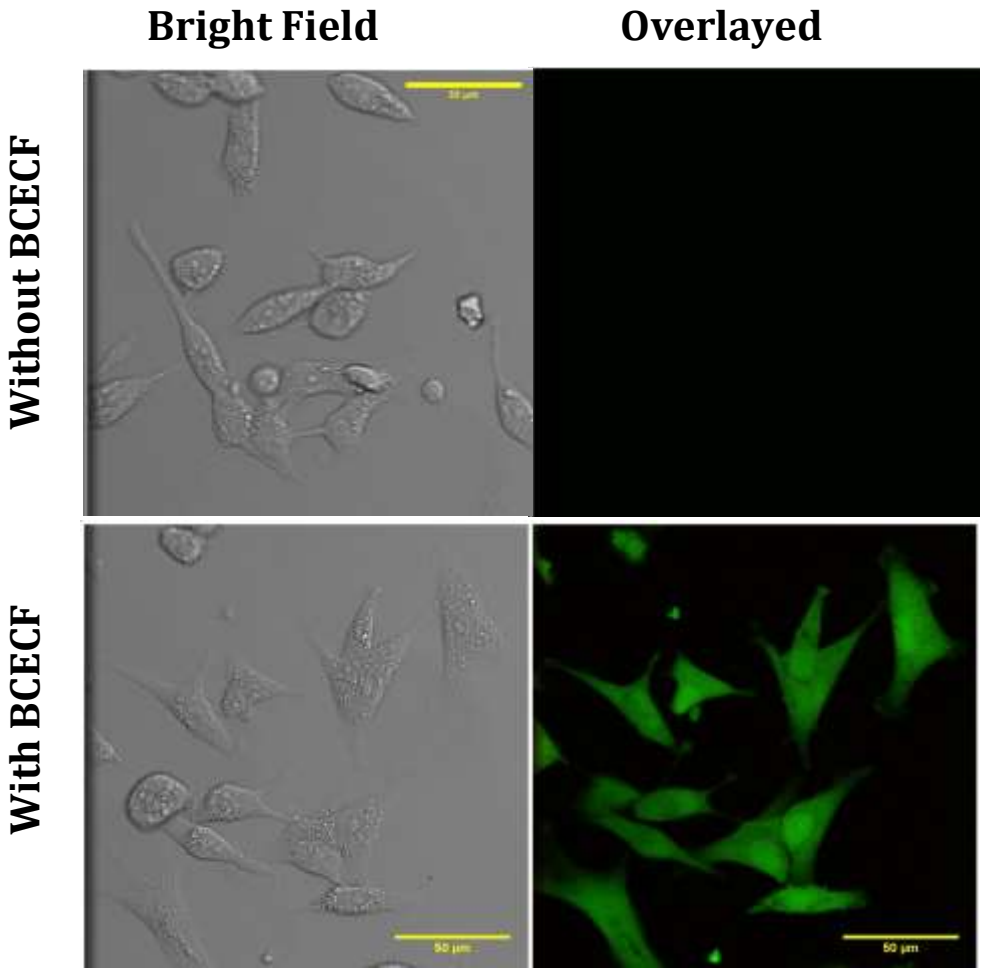

Figure SI8: Fluorescence micrographs of MDA-MB-231 cells in the absence (first row) and in the presence (second row) of BCECF-AM exposures.

Ratiometric calibration of intracellular cells:

To compare the sensitivity and performance of the nano-pH probes, we used BCECF-AM, a conventional pH dye, for intracellular pH measurements. MDA-MB-231 cells were selected

for the fluorimetric intracellular pH measurements. Cells were exposed to BCECF-AM and incubated for 15 min. Then, cells were washed and exposed to nigericine containing cellular pH calibration buffer (pH 7.5, 6.5, 5.5 and 4.5) for 10 min. BCECF-AM has dual excitation wavelengths; therefore, images were taken at 458 and 488 nm. **Figure SI7a** shows the bright field and fluorescence micrographs obtained for the two excitation wavelengths of each pH value. A ratiometric calibration curve was obtained using fluorescence intensities of 16 to 23 individual cells (**Figure SI7b**). One group of cells served as negative control (without BCECF-AM) to evaluate the presence of intracellular autofluorescence (**Figure SI8**). In the absence of the pH dye, there was no observable fluorescence for MDA-MB-231. Cells exposed to BCECF-AM were used to estimate the intracellular pH values of individual cells. The average intracellular pH value obtained from 10 individual cells was calculated to be 6.78 (±0.83). However, the micrographs taken after BCECF-AM exposure revealed that fluorescence intensity over the cell body varies (**Figure SI8**). Fluorescence intensity was higher where cells were thicker. Additionally, at any two regions in close proximity to one another in an individual cell was found to have large variation in pH values. These variations can be attributed to (i) unequal distribution or accumulation of the fluorescence dye; (ii) cross-reactivity of the fluorescence dye with another molecule. Another drawback of fluorescence measurements is the sample preparation step that requires the frequent change of media, which can stress cells and alter the basal intracellular levels. Moreover, the use of fluorescence dyes does not allow continuous interrogation of a single-cell over the course of treatment, such as drug testing, or toxicity measurements, because the presence of these dyes along with the compound of interest may cause false experimental conclusions by changing the physiology of a cell or by cross-reacting with the compound to be tested.

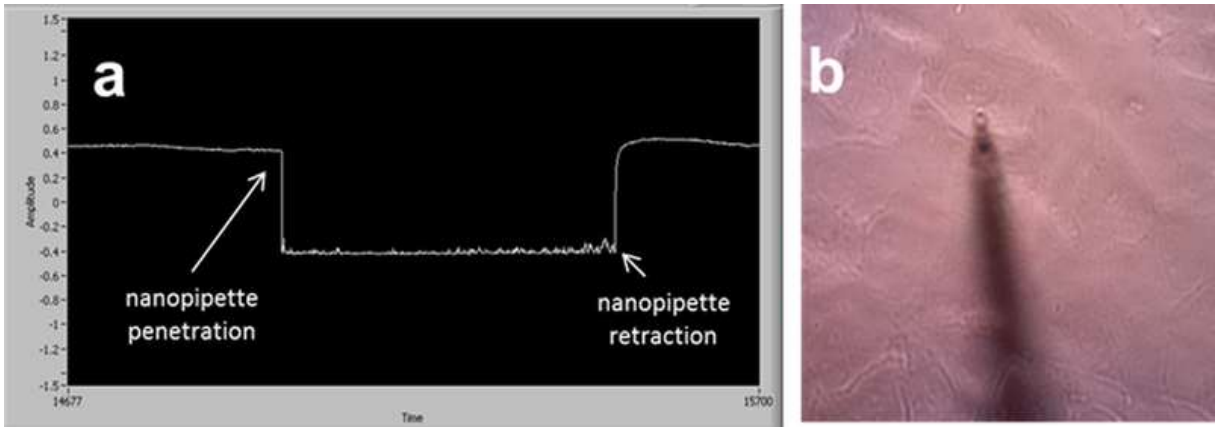

Figure SI9: Nano-pH probe insertion and retraction signal. **(a)** Customized scanning ion conductance microscopy current-feedback signal recorded before, during and after cell penetration using an Axopatch 200B amplifier. Amplitude at y-axis is nanoamperes. **(b)** The corresponding micrograph of the inserted chitosan-modified nanopipette.

Nanopipette penetration and retraction signals were collected by a custom LabView program. The decrease in the current-feedback is due to the penetration of the chitosan-modified nanopipette into the cell. After the retraction of the nanopipette from the cell, current feedback returned to the original extracellular level (**Figure SI9a**). These results, also, suggest that the chitosan-modified nanopipette remains intact during the single-cell intracellular pH measurements.

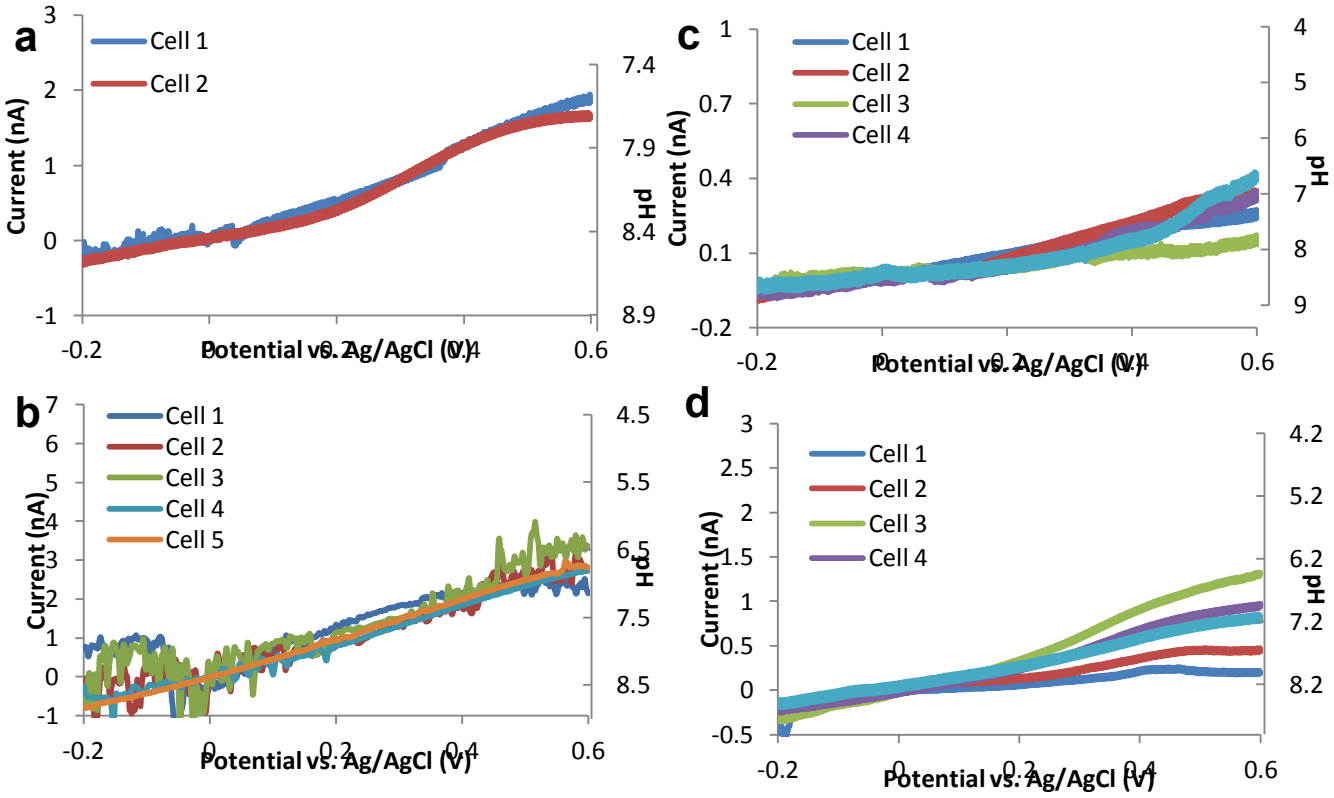

Figure SI10: Representative current-potential curves of intracellular pH measurements with the chitosan-modified nanopipette for different cell types: **(a)** human fibroblast, **(b)** HeLa, **(c)** MCF7 and **(d)** MB231. All readings for each type of cell lines were obtained with a single pH nanoprobe.

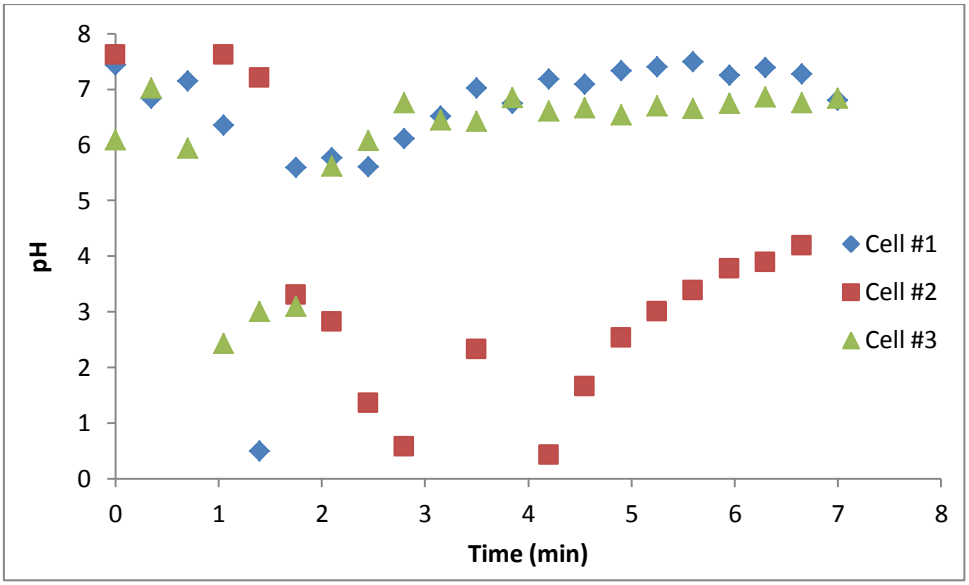

Figure SI11: Graph representing pH changes over time of three MDA-MB-231 cells as a result of 100 μM NPPB (Cl^- channel blocker) exposure. Readings were obtained every 21 sec post channel blocker exposure.