Supplementary information

Label-free microfluidic impedance cytometry for acrosome integrity assessment of boar spermatozoa

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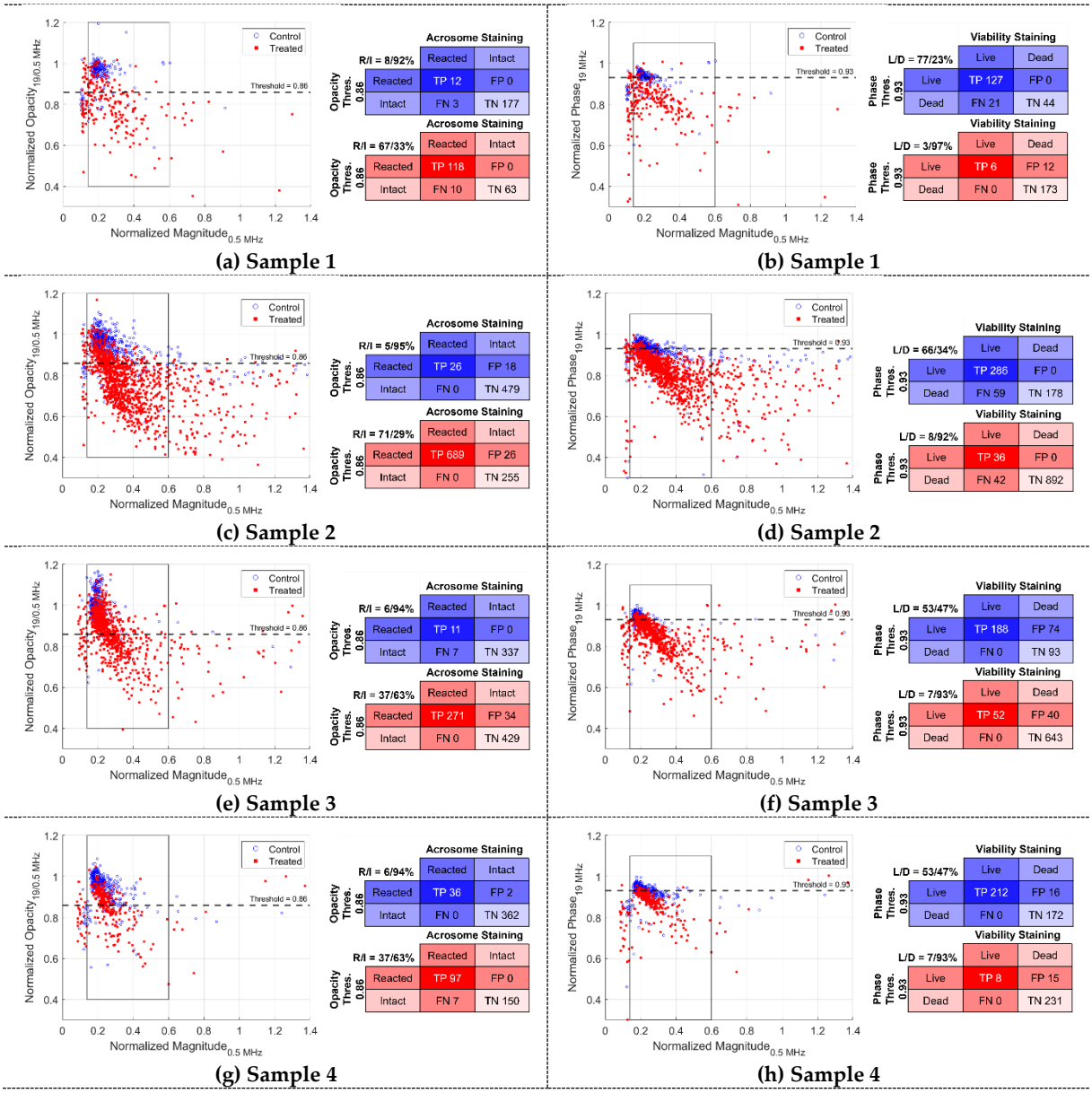
Supplementary figures



(a) Sample 1 **(b) Sample 1**

(c) Sample 2 **(d) Sample 2**

(e) Sample 3 **(f) Sample 3**

(g) Sample 4 **(h) Sample 4**

Figure S1: Scatterplots of normalized magnitude (0.5 MHz) vs. opacity (19/0.5 MHz) and normalized magnitude (0.5 MHz) vs. Phase (19 MHz) with their corresponding confusion matrix at a threshold of 0.86 and 0.93, respectively. The black square indicates the window of interest. a-b) sample 1. c-d) sample 2. e-f) sample 3. g-h) sample 4.

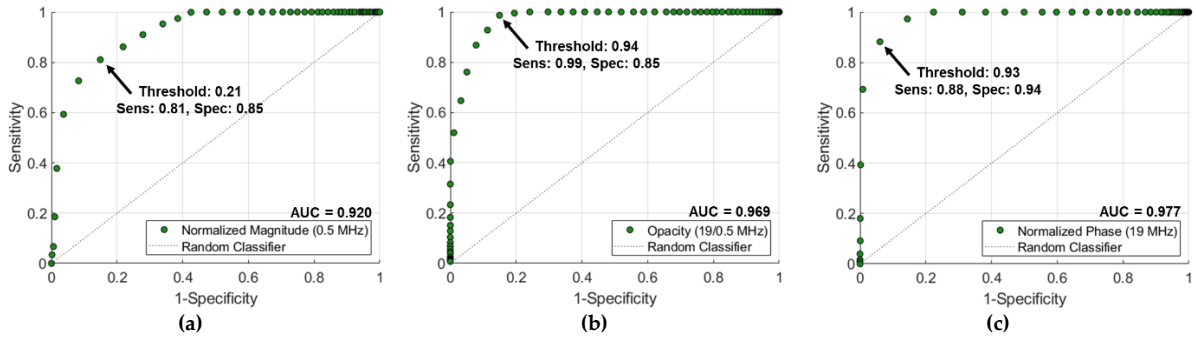


Figure S2: ROC curves to determine the sensitivity and specificity towards viability for (a) normalized magnitude (0.5 MHz), (b) phase (19 MHz) and (c) opacity (19/0.5 MHz). Phase at 19 MHz is found to be the most sensitive and specific for measuring viability.

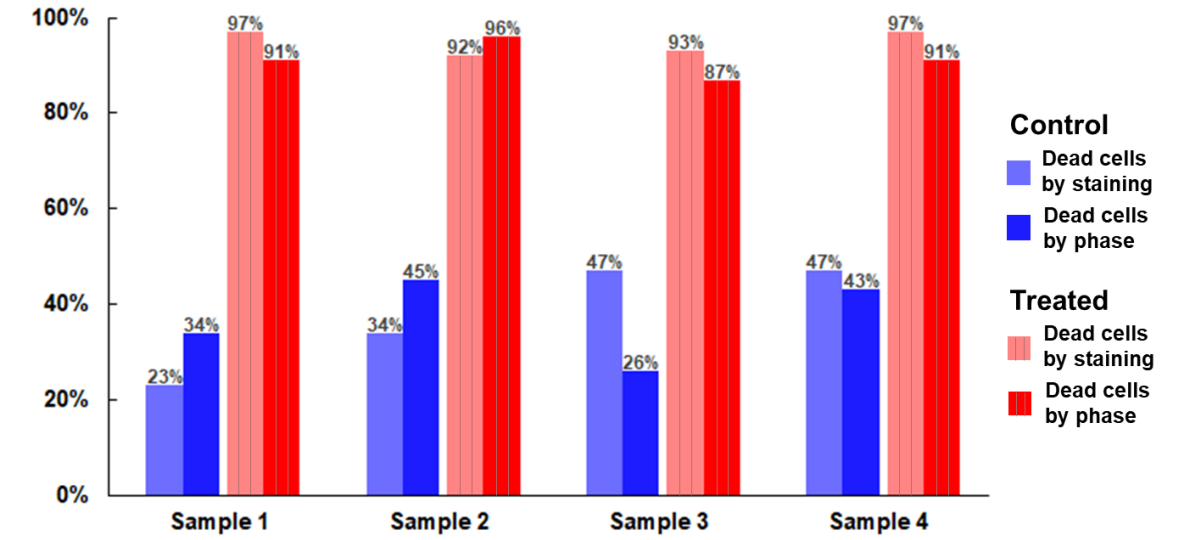


Figure S3: Determined viability by impedance phase at 19 MHz versus viability (PI positive) staining. A threshold of 0.93 is given as the compromise between sensitivity (88%) and specificity (94%) to give the best result for all control and treated samples.

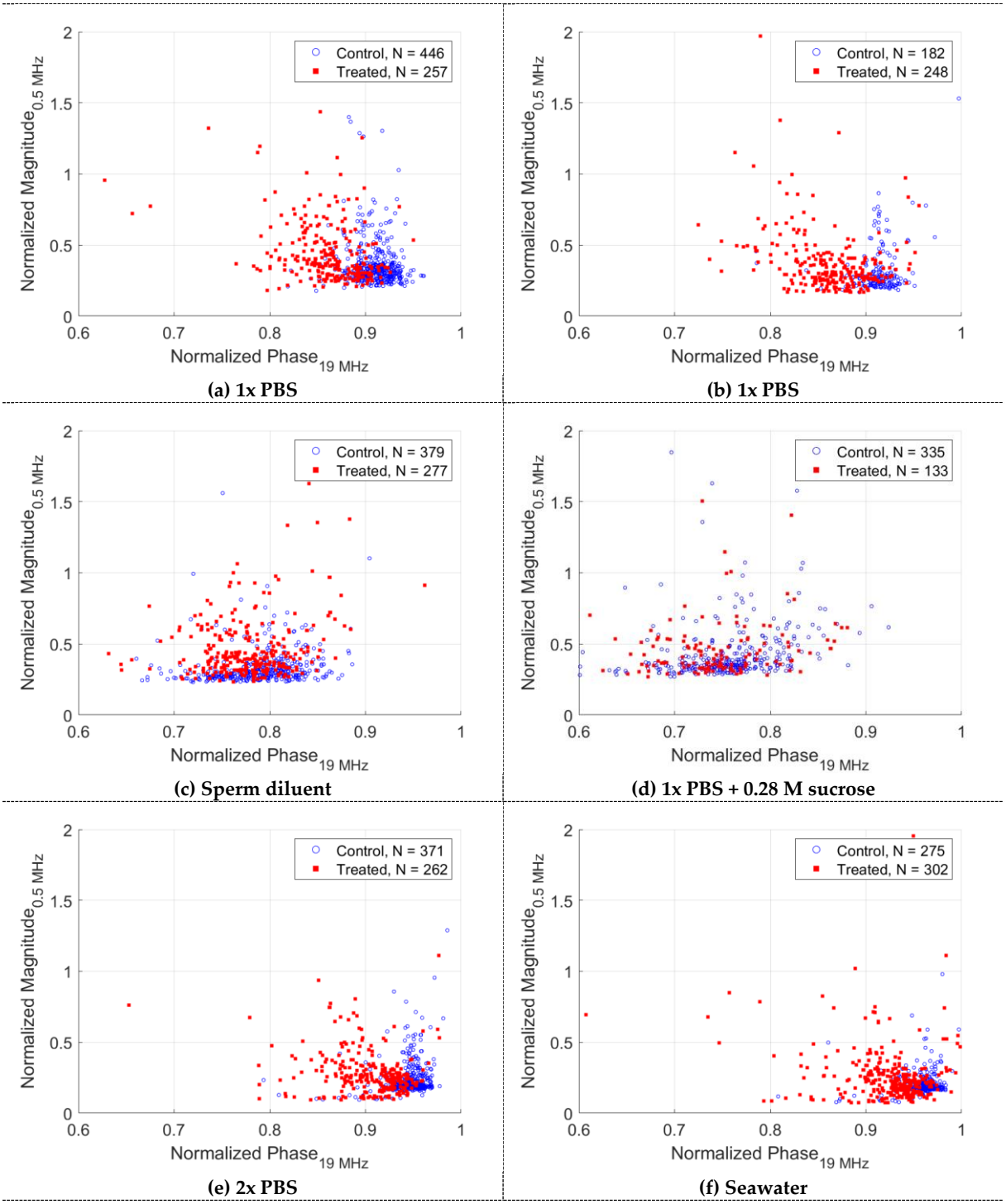


Figure S4: Scatterplots of normalized phase (19 MHz) vs. normalized magnitude (0.5 MHz) in buffers of varied conductivity and analyte composition. Impedance measured of control and treated sample in a-b) 1x PBS, c) sperm diluent ($\sigma = 0.6$ S/m), d) 1x PBS + 0.28 M sucrose ($\sigma < 0.6$ S/m), e) 2x PBS ($1.6 < \sigma < 4$ S/m), and f) seawater ($\sigma = 4$ S/m).