Electronic Supplementary Material

Spectrophotometric Analysis at the Single Cell Level:

Elucidating Dispersity within Melanic Immortalized Cell Populations

Luis Polo-Parada,^{*,†‡} Gerardo Gutiérrez-Juárez,^{*§} David Cywiak,[€] Rafael Pérez-Solano,[§] and Gary A. Baker^{*∥}

⁺ Department of Medical Pharmacology and Physiology. University of Missouri.

[‡] Dalton Cardiovascular Research Center, University of Missouri.

§ División de Ciencias e Ingenierías-Campus León, Universidad de Guanajuato, México.

€ Centro Nacional de Metrología. km 4.5 Carretera a Los Cués, Municipio El Marqués, Qro. C.P.

76246 México.

|| Department of Chemistry, University of Missouri.

This general figure caption applies to all six supporting figures (Figs. S1–S6) that follow. The cell type(s) simulated are identified specifically within each supporting figure:

Simulated photoacoustic signal generation by two different cells. Using data obtained experimentally from real cells (*i.e.,* cell sizes and single cell extinction coefficients measured at 532 nm) we randomly created two (or four, in the case of Fig. S2) artificial cells using Monte Carlo methods. The first cell is always positioned at the center of the simulation field—a condition ensuring maximal photoacoustic signal (PAS)—and the second cell is randomly positioned anywhere within the stimulation field. Using previously-developed mathematical models described in the literature, ^{S1,S2} we estimated the PAS generated by these cellular arrangements for these laser excitation characteristics: t = 10 ns, fluency 20 mJ cm⁻², r = 0.5 mm. We then estimated the magnitude of the maximal peak-to-peak PAS and the corresponding area integrated for the entire PAS. This process was repeated for a total of 100 times. The resulting data clusters are presented in: A) a histogram of the distribution of the extinction coefficients at 532 nm for all the cells tested; B) cell positions randomly generated for PAS analysis; C) A plot of the PAS generated by each set of cells; D) a histogram of the maximal peak-to-peak amplitude of each PAS; and E) a histogram of the integrated area for PAS.



Fig. S1 Simulated PAS generation for two RBCs.



Fig. S2 Simulated PAS generation for one HS936 cell and one RBC.



Fig. S3 Simulated PAS generation for one HS936 cell and three RBCs.



Fig. S4 Simulated PAS generation for one B16F1 cell and one RBC.



Fig. S5 Simulated PAS generation for one B16F10 cell and one RBC.



Fig. S6 Simulated PAS generation for one T47D cell and one RBC.

References

- S1 R. P. Solano, F. I. Ramirez-Perez, J. A. Castorena-Gonzalez, E. A. Anell, G. Gutiérrez-Juárez and L. Polo-Parada, An experimental and theoretical approach to the study of the photoacoustic signal produced by cancer cells. *AIP Advances* 2012, **2**, 011102 (15 pp.).
- S2 F. I. Ramirez-Perez, G. Gutierrez-Juarez, S. Bok, K. Gangopadhyay, S. Gangopadhyay, G. A. Baker and L. Polo-Parada, Dye-doped organosilicate nanoparticles as cell-preserving labels for photoacoustic signal generation. *J. Biomed. Nanotechnol.* 2014, **10**, 3337–3350.