Electronic Supplementary Material (ESI) for Integrative Biology

Electronic Supplementary Information (ESI)

Preparation, Characterization, and Surface Immobilization of Native Vesicles Obtained by Mechanical Extrusion of Mammalian Cells

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SUPPLEMENTARY FIGURES

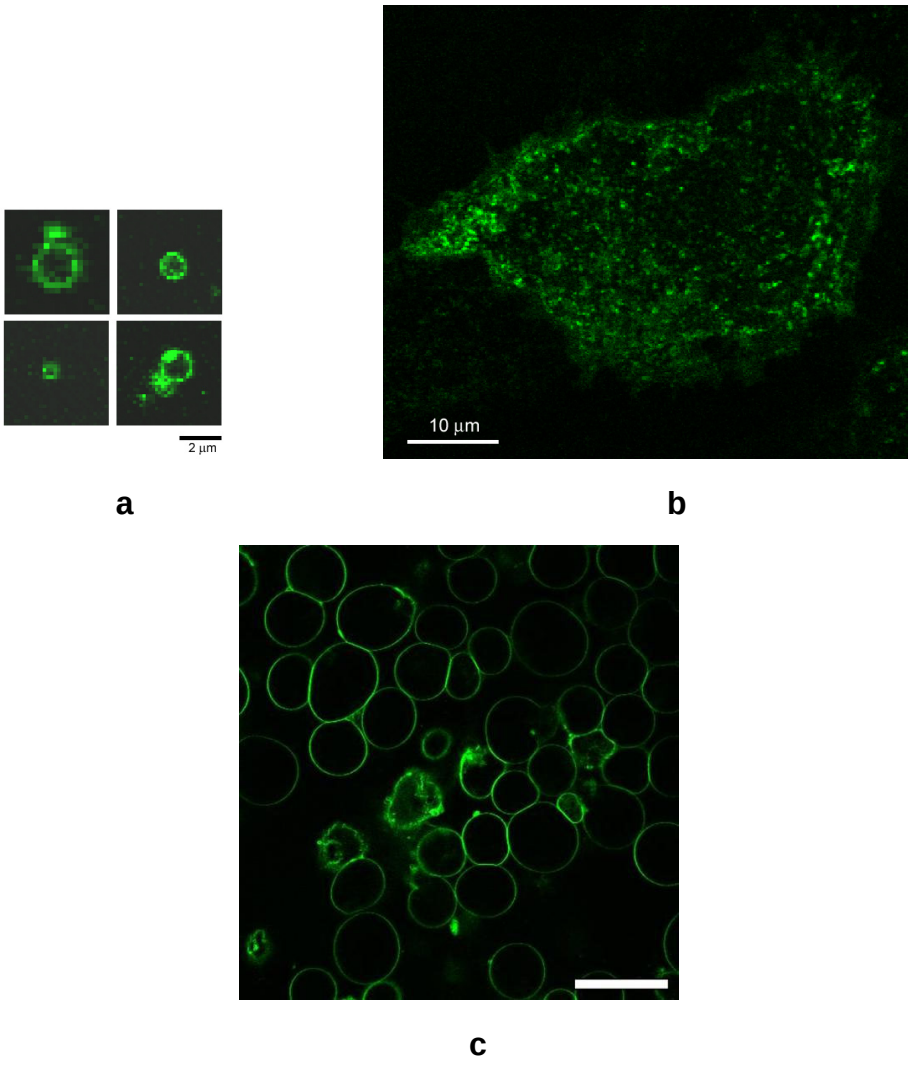


Figure S1 Qualitative comparison of the CTB-488 labeled reduced cells and whole cells, imaged by laser scanning confocal fluorescent microscopy. (**a**) The boundary of some of the reduced cells imaged shows almost continuous fluorescence stain of CTB-488, suggesting a considerable concentration of Gm1, typically associated with lipid rafts. (**b**) Bottom surface of an ARPE-19 cell shows the punctate fluorescence stains typically seen for raft domains in live ARPE-19 cells. (**c**) Section image of CTB-488 labeled ARPE-19 cells shows that the CTB-488 decorates only the plasma membrane (bar = 40 µm).

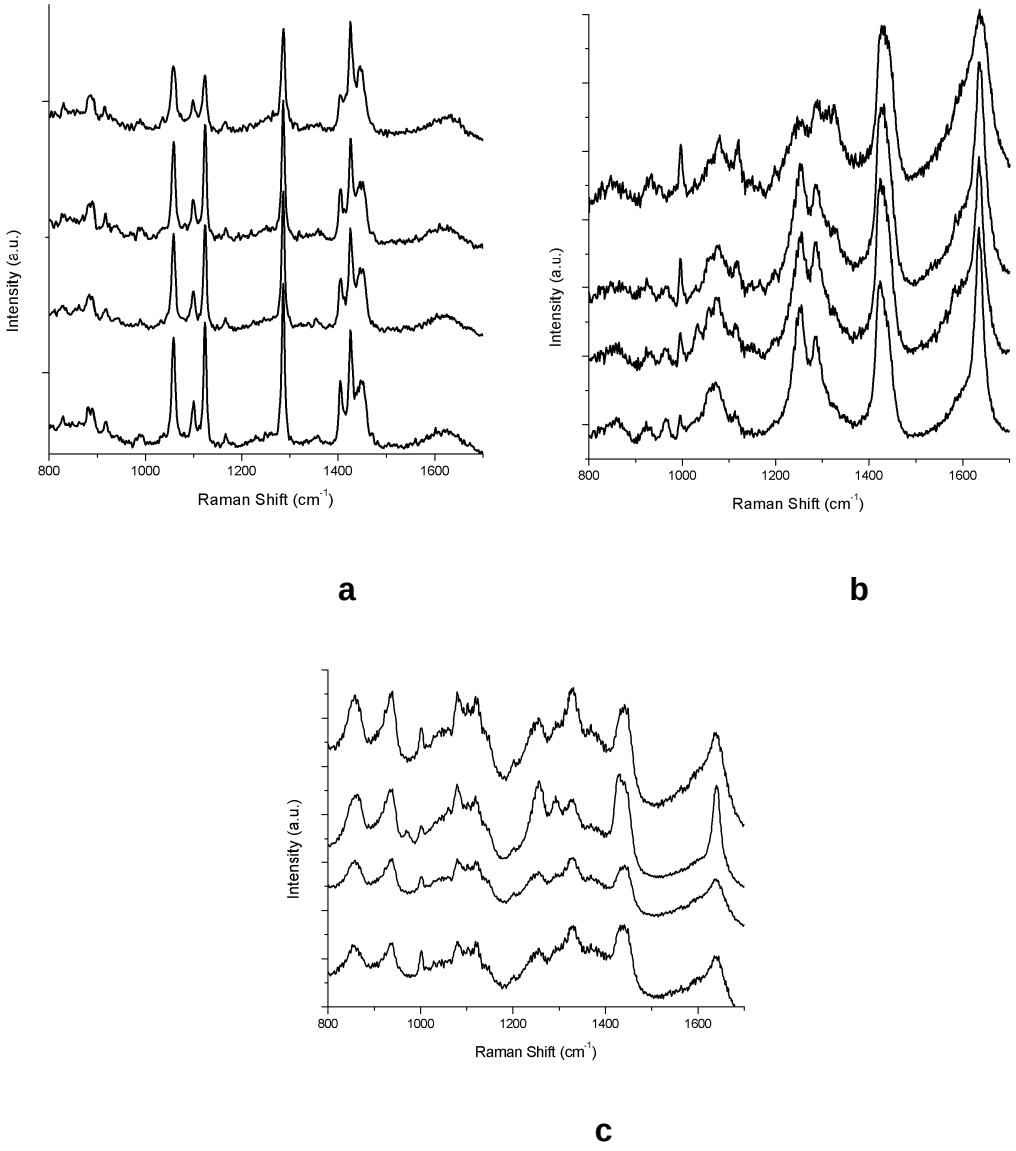


Figure S2 Laser-trapping Raman spectra of four independent particles of (**a**) lipid-rich reduced cells, (**b**) protein-rich reduced cells, and (**c**) whole ARPE-19 cells.

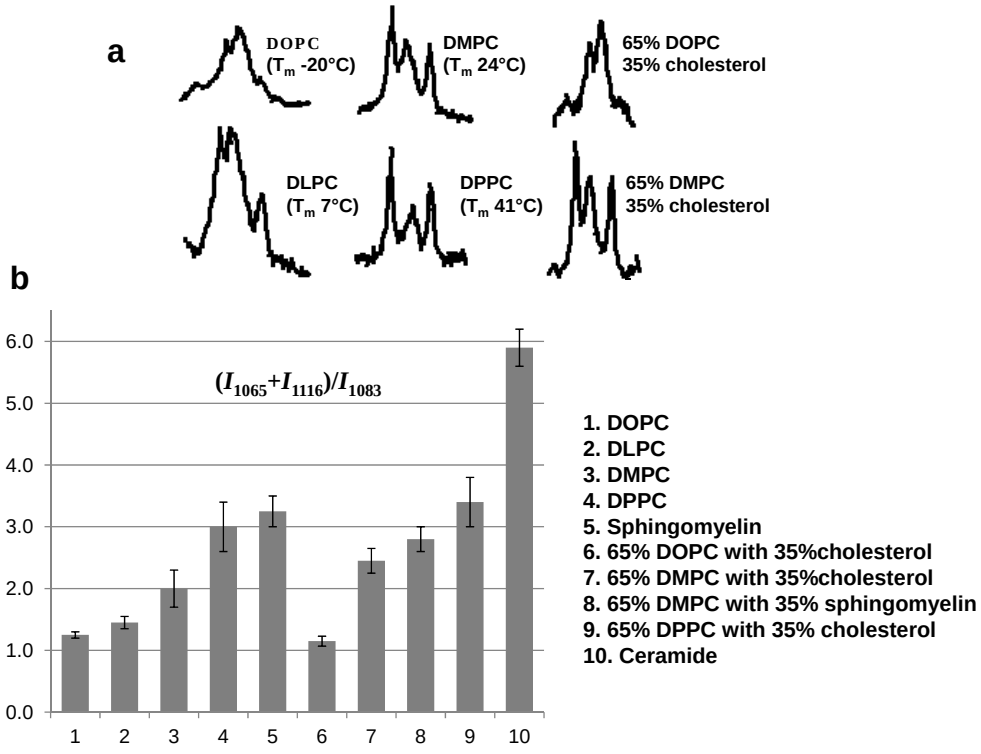


Figure S3 (a) Raman spectral conformation of C-C *trans*- (1065 and 1116 cm^{-1}) and *gauche*- (~1083 cm^{-1}) stretches associated with the acyl chains in various pure lipid vesicles or particles. (b) Ratios of *trans to gauche* C-C stretch intensities (I_{1065}/I_{1083} and I_{1116}/I_{1083}) provide a measure of relative degree of saturation (stiffness).

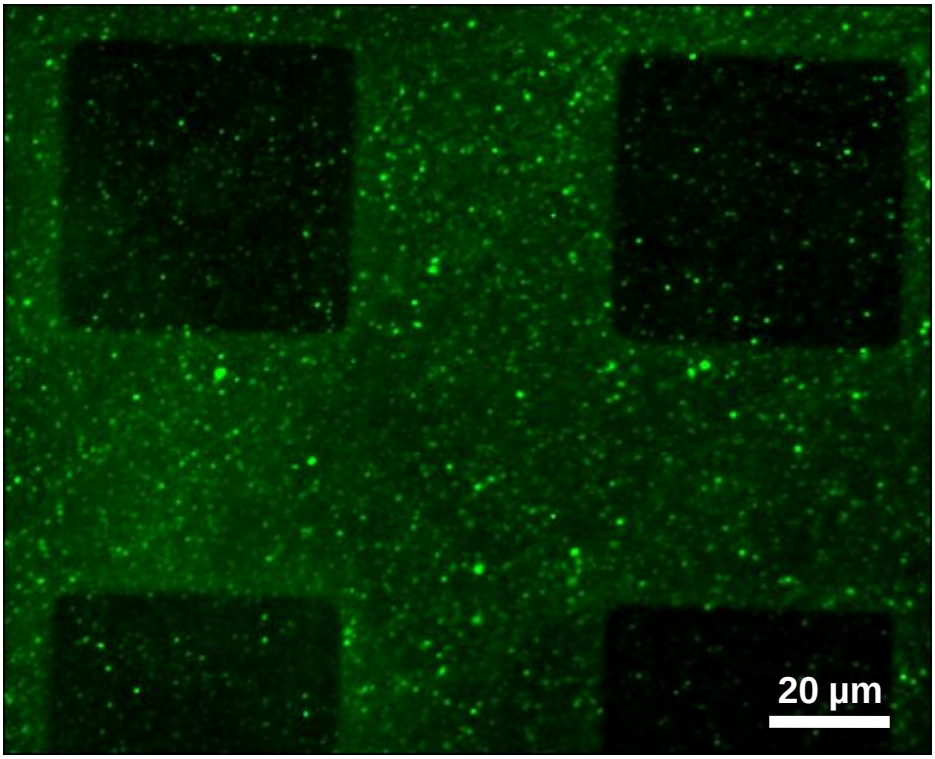
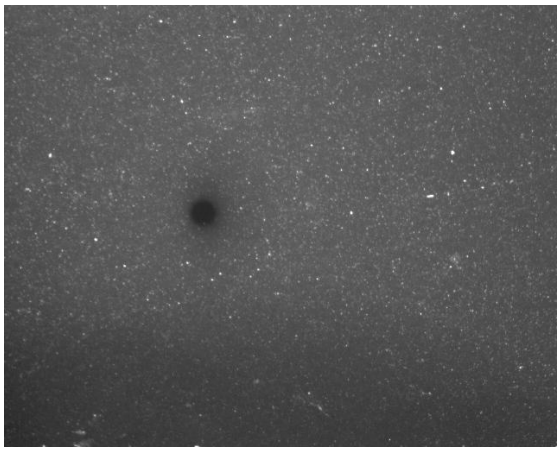
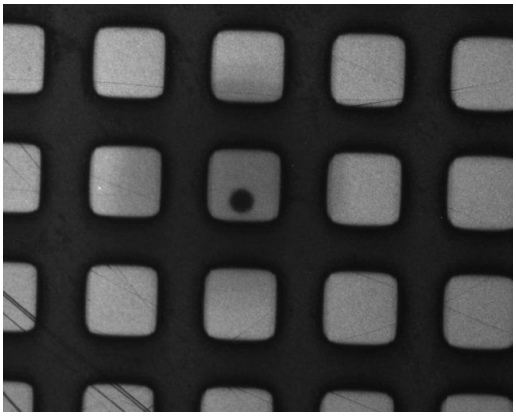


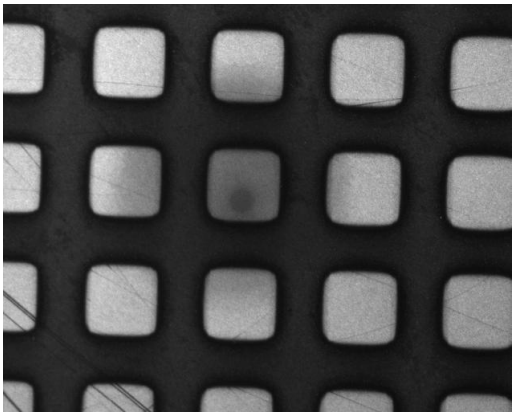
Figure S4 Surface presentation of the reduced cells on OTS-patterned glass substrates, in which outside of squares is coated with OTS, and the OTS inside the squares is removed by UV/ozone treatment. Reduced cells derived from CTB-488 labeled cells are deposited on the substrate, showing a higher preference for the OTS regions.



a (t = 30 min)



b (t = 0)



c (t=30 min)

Figure S5 Fluorescent recovery after photobleaching (FRAP) experiments reveal that the reduced cells are presented on surfaces as discrete particles (simply sitting on the surface). (**a**) TR-DHPE-labeled post-extruded particles were spread on a glass surface. The photobleached spot had no recovery after 30 minutes. (**b**) TR-DHPE labeled POPC small unilamellar vesicles (SUVs) were mixed with post-extruded particles and then spread on an OTS-patterned glass substrate, in which outside of squares (50 μm x 50 μm)

is coated with OTS, and the OTS inside the squares is removed by UV/ozone treatment. TR-DHPE was photobleached, showing a dark spot. (**c**) After 30 minutes, the bleached spot's fluorescence intensity was partially recovered, vs. full recovery of pure POPC bilayer.[1]

SUPPLEMENTARY METHODS

Quantitative Analysis for Pattern Fidelity

Quantitative comparison of intensity values from original images (8-bit TIFF) captured by the CCD in the epi-fluorescence microscope setup were used to give a quantitative idea of the pattern fidelity when samples were presented on the OTS patterned substrates.

For comparison of the reduced cells' pattern fidelity between the single-sample condition (post-extruded particles only) and the mixed-sample condition (post-extruded particles mixed with POPC SUVs), a representative rectangular area was chosen for each image, as shown in the figures (**Figure S6**) below.

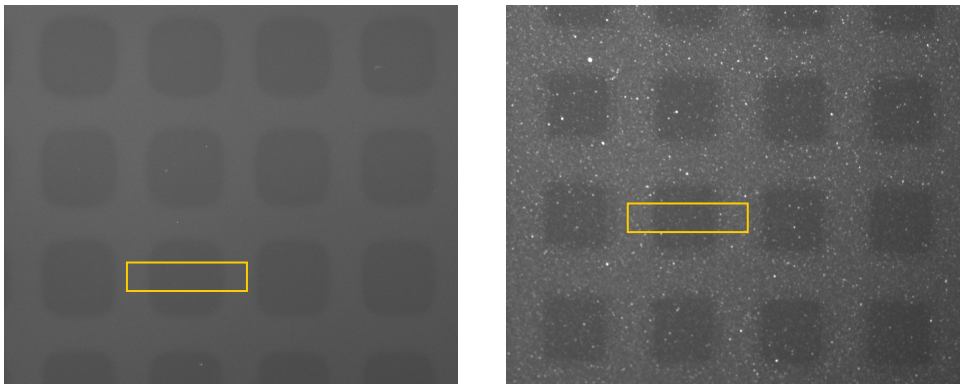


Figure S6-1 Unprocessed images in the CTB-488 channel, captured from single-sample condition (*left*) and mixed-sample condition (*right*). A representative area was chosen in each image to read the intensity values for the quantitative analysis.

The images were converted into ASCII files by the Winspec32 software (Roper

Scientific). Intensity values of vertical lines of pixels in the selected areas were averaged. Hydrophilic area is in the rough range of x = [220-280], while hydrophobic OTS areas are in the horizontal range of about x = [160-200] and [300-340]. The intensity averages in these ranges were plotted in the following figure, for both single-sample condition and mixed-sample condition.

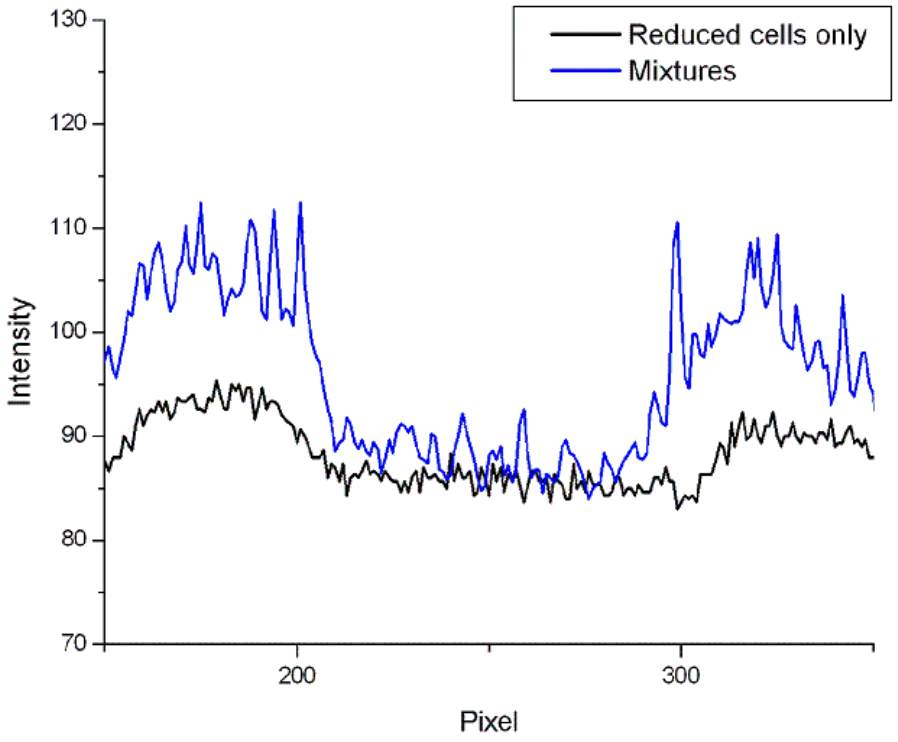


Figure S6-2 Intensity trace of a representative square in the CTB-488 channel.

Further, the averages of intensities in each sample condition, and in each area (hydrophobic and hydrophilic) were computed. We have, for the single sample condition, on hydrophilic area and hydrophobic, respectively:

$I_{ss\_avg\_glass\_CTB} = 85.7$; $I_{ss\_avg\_OTS\_CTB} = 93.0$.

And for the mixed sample condition:

$I_{ms\_avg\_glass\_CTB} = 87.9$; $I_{ms\_avg\_OTS\_CTB} = 105.9$.

The ratios of the intensities in each type of surface are:

$R_{ss\_CTB} = I_{ss\_avg\_OTS\_CTB} / I_{ss\_avg\_glass\_CTB} = 1.085$;

$R_{ms\_CTB} = I_{ms\_avg\_OTS\_CTB} / I_{ms\_avg\_glass\_CTB} = 1.205$.

Therefore, the ratio of the intensity in the hydrophobic surface over the intensity in the hydrophilic surface, in CTB channel (only reduced cells are visualized), is increased about 12%, from the single-sample condition to the mixed sample condition.

Similar calculations were done in the images from both sample conditions in Texas red channel.

$R_{ss\_TR} = I_{ss\_avg\_OTS\_TR} / I_{ss\_avg\_glass\_TR} = 103.1/157.0 = 0.657$;

$R_{ms\_TR} = I_{ms\_avg\_OTS\_TR} / I_{ms\_avg\_glass\_TR} = 45.0/201.4 = 0.223$.

The ratio of the intensity in the hydrophobic surface over the intensity in the hydrophilic surface, in Texas red channel (only POPC SUVs are visualized) is decreased about 66%, from the single-sample condition to the mixed sample condition.

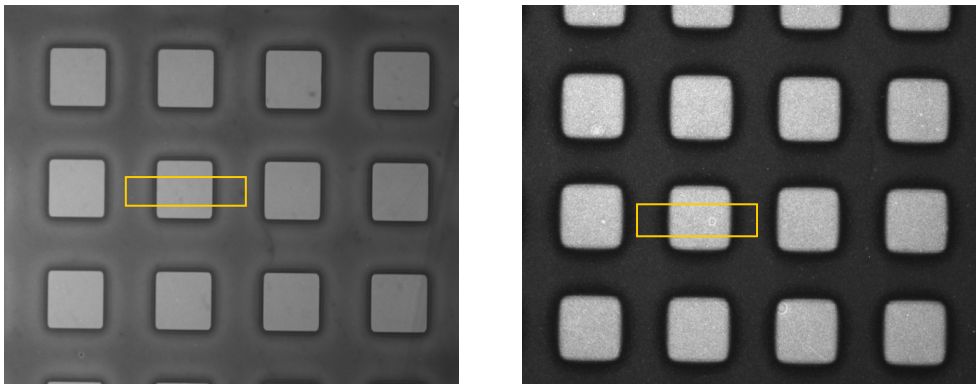


Figure S6-3 Unprocessed images in the Texas red channel, captured from single-sample condition (*left*) and mixed-sample condition (*right*). A representative area is chosen in each image to read the intensity values for quantitative analysis.

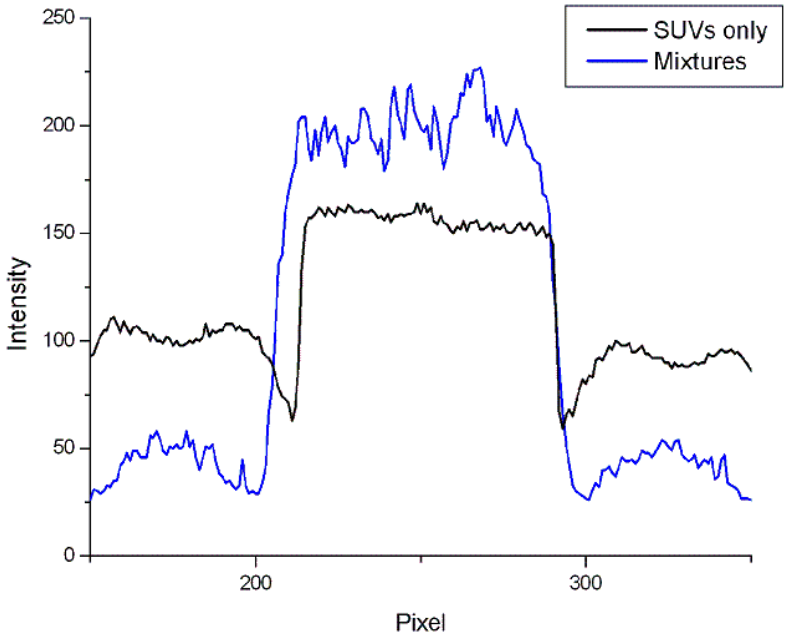


Figure S6-4 Intensity trace of a representative square in Texas red channel.