Preparation	Compensation	Unstimulated	BzATP Stimulated	Fold-increase	Stimulated - Unstimulated
Ficoll prep PBMC, A	Bead-adjusted	2.26	292	129.20	290
Whole blood, A	Bead-adjusted	5.84	276	47.26	270
Whole blood, B	Uncompensated	8.27	30.7	3.71	24.4
Whole blood, B	Y0-PRO-PE set at 2%	4.59	27.4	5.97	22.8
Whole blood, C	Uncompensated	5.97	1071	179.40	1065
Whole blood, C	Y0-Pro-PE set at 2%	2.61	1067	408.81	1064

Table 1 (Supplement) . Effects of Monocyte Preparation and Compensation Method on Measured YO-PRO-1 Fluorescence. Blood was drawn from one donor (A) and the sample was divided so that a portion of the same blood could be ficolled as described or was left whole. The assay was performed on both samples and the data was collected on a FACScan flow cytometer using bead-adjusted settings as described. Blood samples were drawn from two other donors (B, C) and the PMT sensitivities were determined with the Rainbow beads but were acquired uncompensated. Off line analysis and compensation were performed using FlowJo (Treestar) analysis software. Data is expressed as MFI of YO-PRO-1 in live monocytes. Fold-increase is the ratio of unstimulated MFI to stimulated MFI.

Figure 1 (Supplement). Histograms of the fluorescence of Spherotech Rainbow Beads (mid-range) when set to the indicated target values in each of the PMTs used in the assay. These target values were used on the analog flow cytometers, FACScan, and FACSCalibur.

Figure 2 (Supplement). Representative spectral overlap corrections performed using FITC or PE hard-dyed Calibrite Beads (Becton Dickinson) and the sensitivities determined for each PMT as illustrated in Figure 1. Mean intensities of unlabelled beads in YO-PRO-1 PMT, PE PMT and PI PMT were 1.01 for all PMTs. The spectral overlap of the FITC beads into the PE PMT was compensated to yield a matching intensity of the FITC beads to the unstained beads in that channel. The spectral overlap of the PE beads into the YO-PRO-1 PMT and into the PI PMT was also removed from these detectors until the mean intensities matched the unstained beads in those channels.

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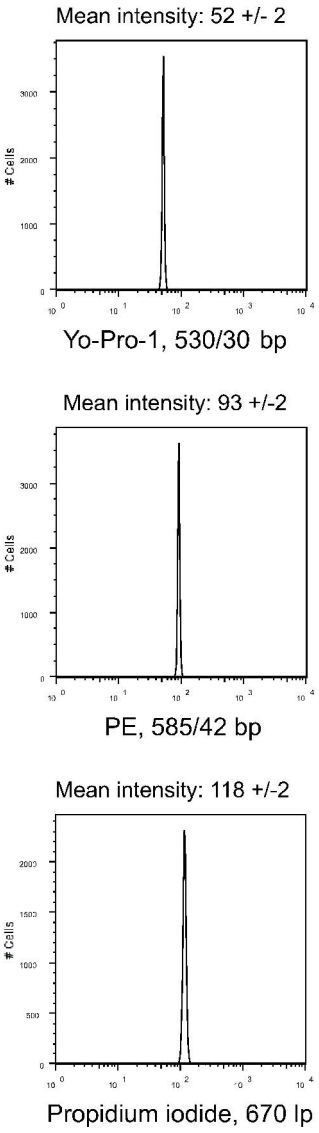
Supplementary Figure 1



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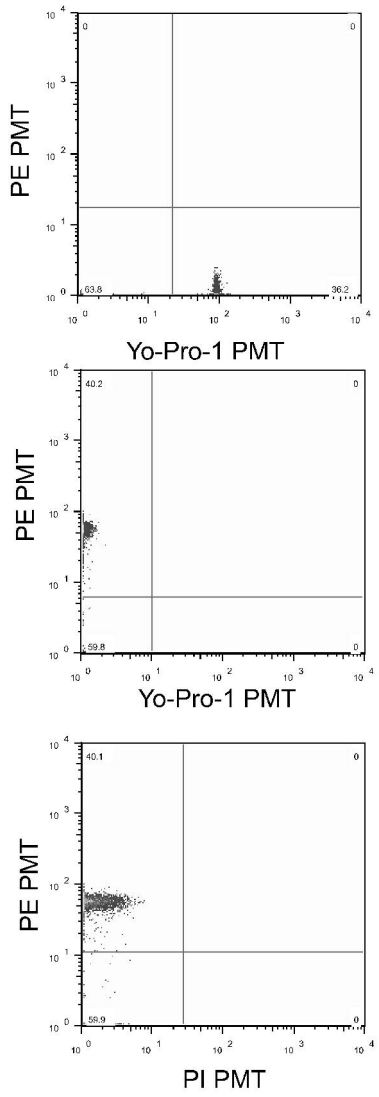
Supplementary Figure 2



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