Supplementary Information

Instantaneous extracellular solution exchange for concurrent evaluation of membrane permeability of single cells

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1. Signal changes of stained cells in fluorescent images

We show the green/red signal changes up to 15 minutes at 5-minute intervals after the osmotic shock and before the osmotic shock. The first fluorescence image after the osmotic shock was taken 10 seconds after the osmotic shock. As we mentioned in the main manuscript, a live cell is defined as the grayscale intensity of the G channel is larger than that of the R channel, dead cell is defined as vice versa. The data were classified into live and dead cell groups at 10 minutes after osmotic shock, as cell type 1 and 2. As shown in Fig. S1 and S2, the grayscale intensity of the G channel of the cell type 1 group was always higher than that of the R channel. On the other hand, as shown in Fig. S3 and 4, the grayscale intensity of the G channel of the cell type 2 group was not higher than that of the R channel after osmotic down shock. In particular, the principal intensity was changed from the R channel to the G channel between before and 10 seconds after osmotic shock. At 5 minutes after osmotic shock, the principal fluorescent intensity for all of the cells was switched from the R channel to the G channel. Thereafter, the grayscale intensity of both R and G channels decreases due to the effect of photobleaching. As it takes time for PI to diffuse completely after cell rupture, data from 10 minutes after osmotic shock were used in this study.

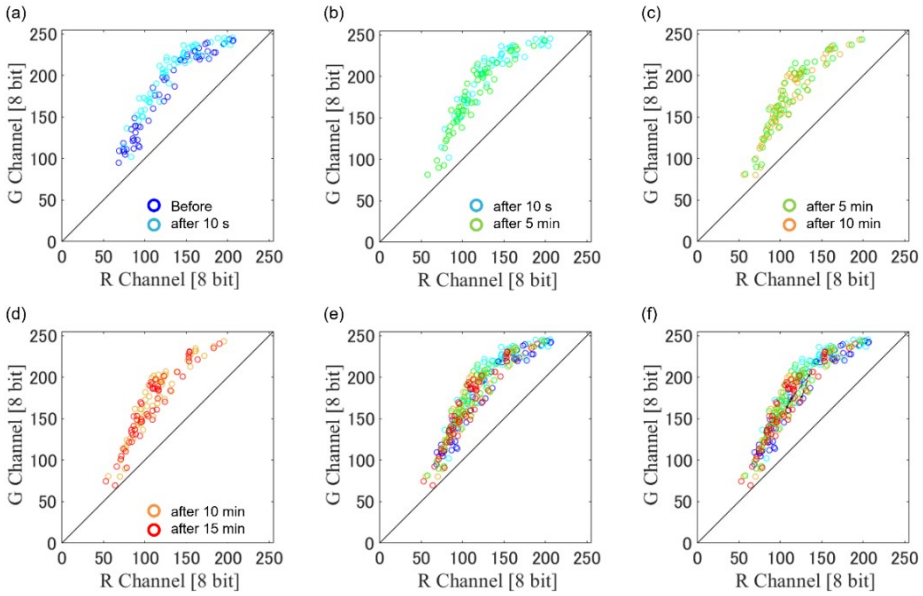

Fig. S1 Relationship between average intensity within cell boundary of Red channel and Green channel with regard to live cells at 10 minutes after 1M to 0.5M osmotic down shock. Comparison between (a)before osmotic shock and 10 seconds after osmotic shock, (b)10 seconds and 5 minutes after osmotic shock, (c) 5 minutes and 10 minutes after osmotic shock, (d) 10 minutes and 15 minutes after osmotic shock. (e) Before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock. (f) Plots of (e) with a trajectory of the mean of before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock.

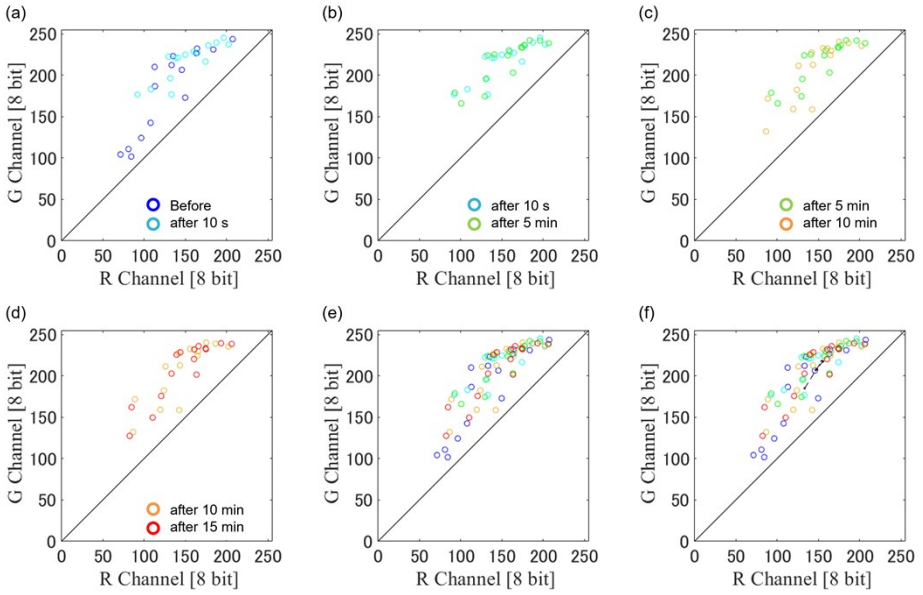

Fig. S2 Relationship between average intensity within cell boundary of Red channel and Green channel with regard to live cells at 10 minutes after 1M to 0.25M osmotic down shock. Comparison between (a)before osmotic shock and 10 seconds after osmotic shock, (b)10 seconds and 5 minutes after osmotic shock, (c) 5 minutes and 10 minutes after osmotic shock, (d) 10 minutes and 15 minutes after osmotic shock. (e) Before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock. (f) Plots of (e) with a trajectory of the mean of before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock.

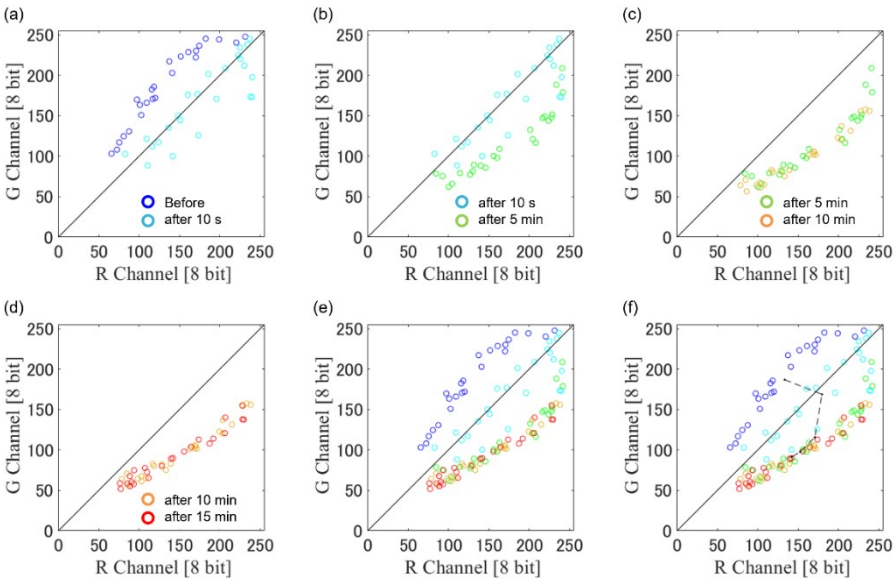

Fig. S3 Relationship between average intensity within cell boundary of Red channel and Green channel with regard to dead cells at 10 minutes after 1M to 0.5M osmotic down shock. Comparison between (a)before osmotic shock and 10 seconds after osmotic shock, (b)10 seconds and 5 minutes after osmotic shock, (c) 5 minutes and 10 minutes after osmotic shock, (d) 10 minutes and 15 minutes after osmotic shock. (e) Before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock. (f) Plots of (e) with a trajectory of the mean of before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock.

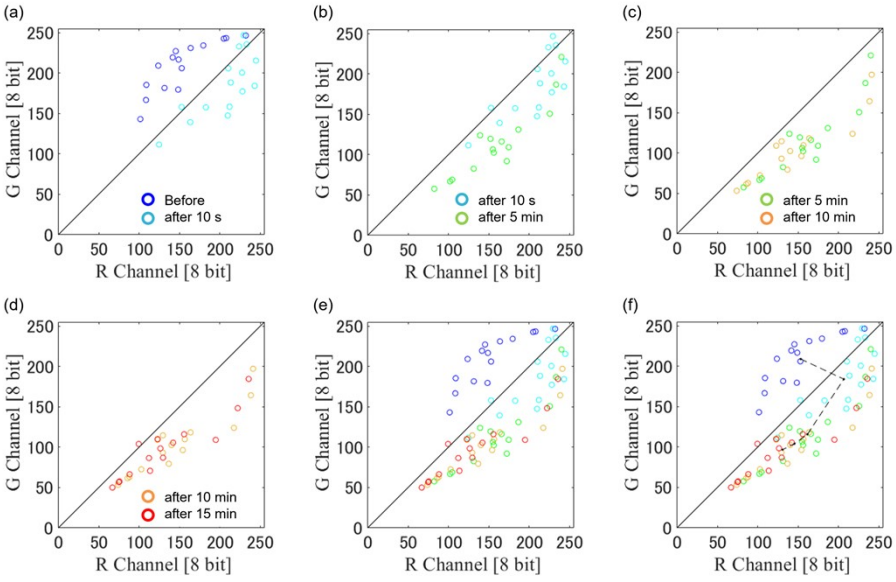

Fig. S4 Relationship between average intensity within cell boundary of Red channel and Green channel with regard to dead cells at 10 minutes after 1M to 0.25M osmotic down shock. Comparison between (a)before osmotic shock and 10 seconds after osmotic shock, (b)10 seconds and 5 minutes after osmotic shock, (c) 5 minutes and 10 minutes after osmotic shock, (d) 10 minutes and 15 minutes after osmotic shock. (e) Before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock. (f) Plots of (e) with a trajectory of the mean of before osmotic shock, 10 seconds, 5 minutes, 10 minutes, and 15 minutes after osmotic shock.

2. Relative volume change of single cells based on viability

Figures. S5 and S6 show the relative-volume changes in live and dead single cells for 20 samples based on the viability at 10 minutes after osmotic shock, respectively. As shown in Fig. S5, live cells converged to a steady state. On the other hand, as shown in Fig. S6, depending on the cell, the timing and gradient of the decrease in the relative volume time curve following the initial increase were heterogeneous. We used the sample that has most sharp gradient of slope in relative volume change after the initial increase as shown in the main manuscript Fig. 4(c) to emphasize the peak of relative volume change for the reader. As shown in Fig. 4(d) in the main manuscript, there is a clear correlation between the phenomena corresponding to membrane rupture and cell death.

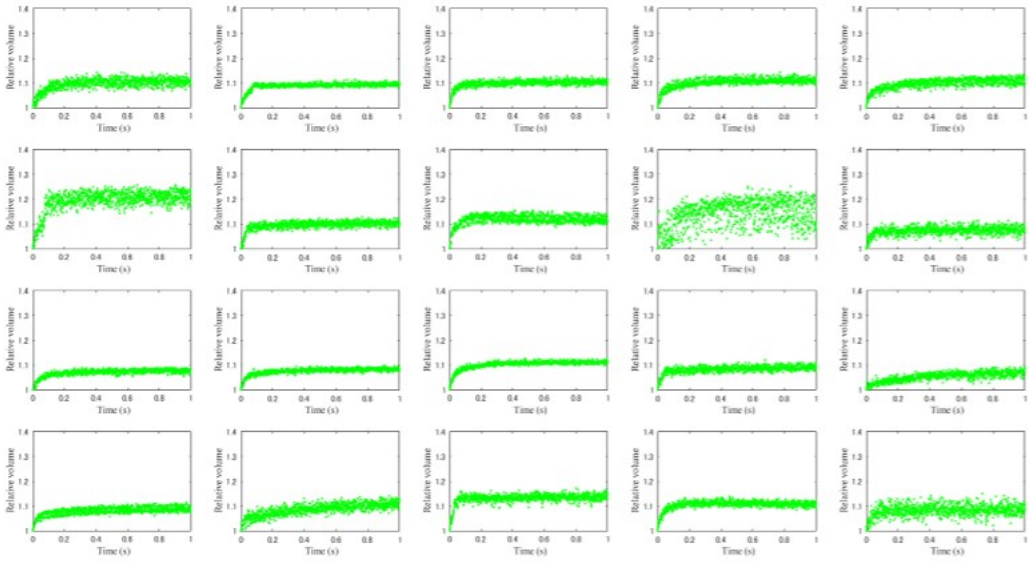

Fig. S5 Relative volume-time curve of live single cells at 10 minutes after osmotic down shock.

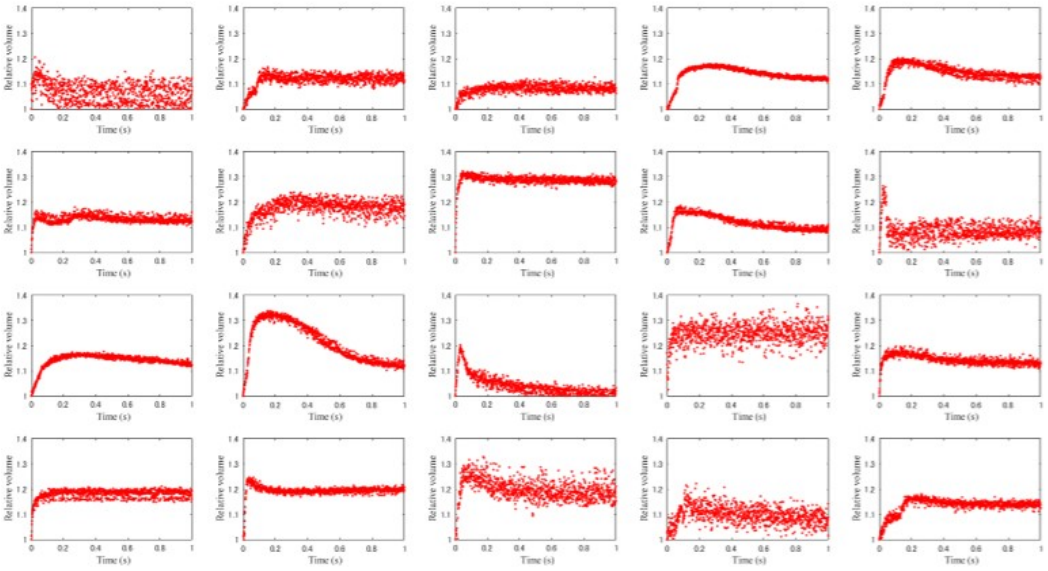

Fig. S6 Relative volume-time curve of dead single cells at 10 minutes after osmotic down shock.