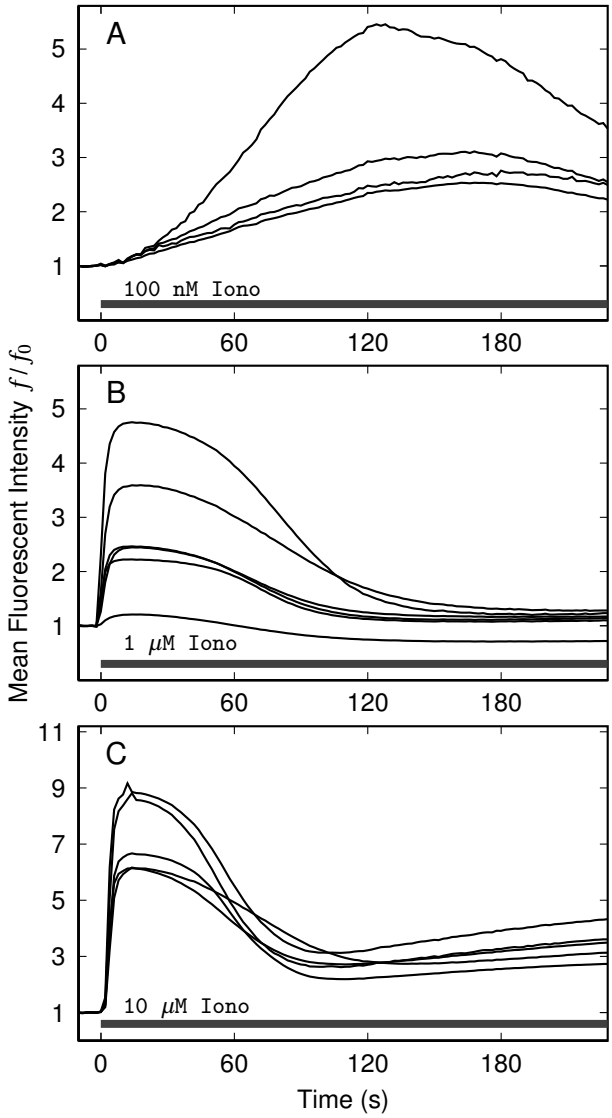

Figure 1: Step Response of fibroblasts to ionomycin at the specified concentration. Each panel shows a single experiment with multiple cells in the field of view. Results are typical of 29 experiments including 148 cells.

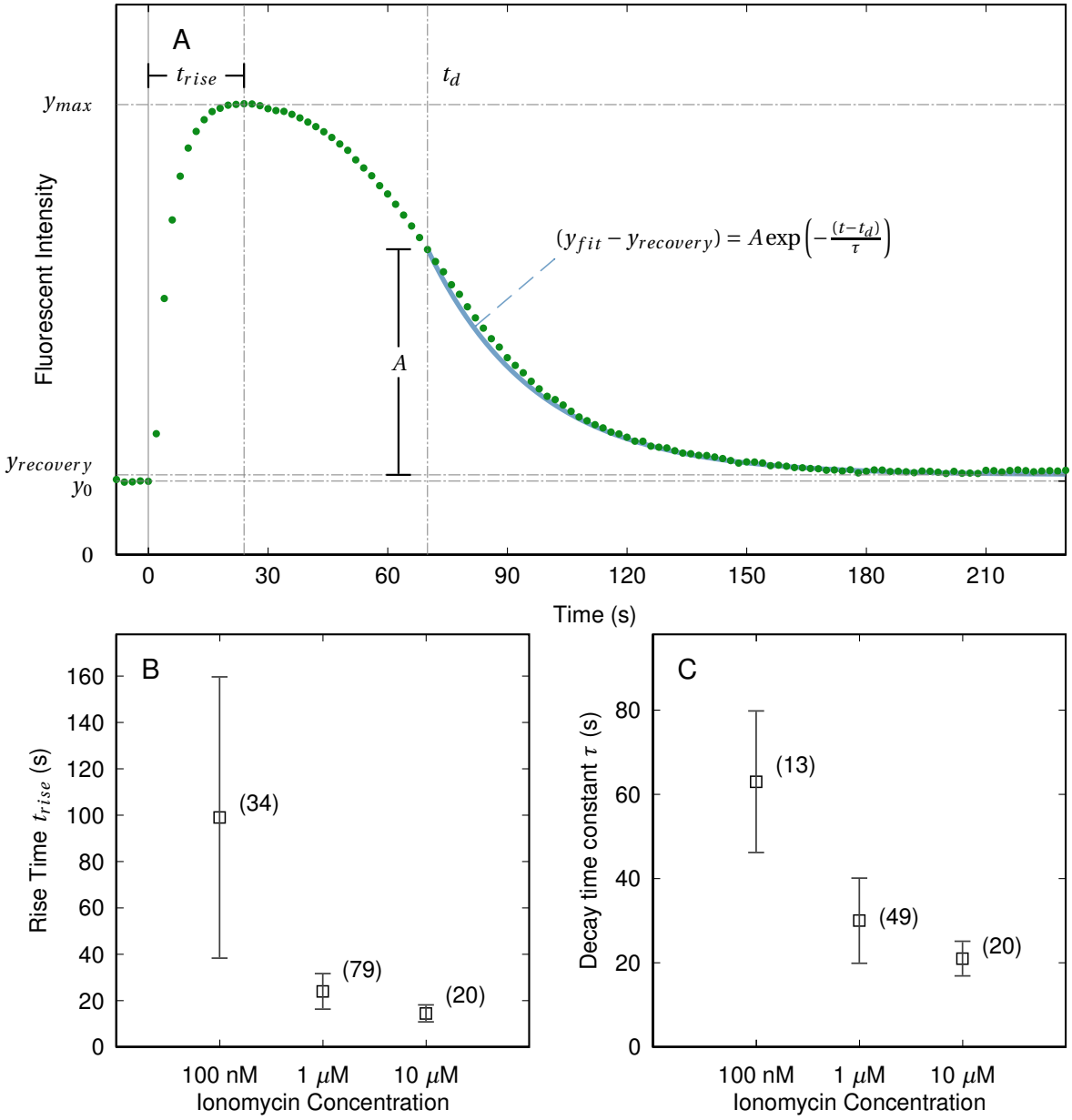

Figure 2: Step Response Modeling. All cells were monitored until they reached a peak intensity, after which they began their recovery towards the baseline intensity. After the rate of recovery reached a maximum, the subsequent recovery was approximated as an exponential decay. The cells were characterized in terms of the time to rise to peak intensity and the characteristic time constant of the exponential decay. (A) A diagram showing salient figures in the step response analysis. (B) Rise time as a function of ionomycin concentration. Each datapoint shows mean and standard deviation. The number of cells in each sample is shown in parentheses. For 100 nM, $t_{rise} = 99 \pm 61$ s; for 1 μM, $t_{rise} = 26 \pm 8$ s; for 10 μM, $t_{rise} = 14.5 \pm 4.0$ s. (C) Exponential decay time constant as a function of ionomycin concentration. Results shown as in (B). For 100 nM, $\tau = 64 \pm 16$ s; for 1 μM, $\tau = 28 \pm 9$ s; for 10 μM, $\tau = 21 \pm 4.3$ s.

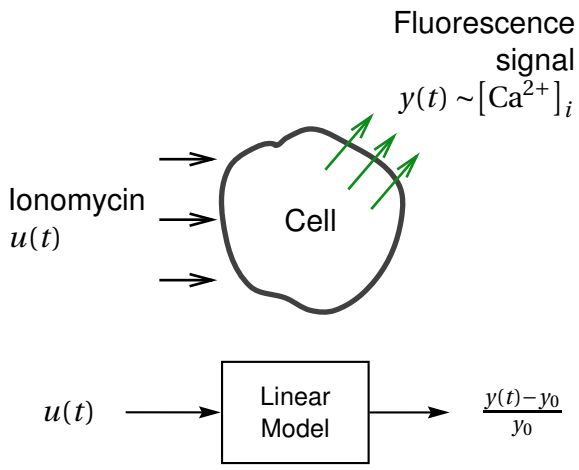

Figure 3: Identifying linear characteristics of cellular response to ionophore. Estimates of cellular exposure to ionomycin were compared with measurements of cell fluorescence to ascertain whether the cellular response can be described by a linear model. The input was the mean concentration of ionomycin over the cell's apical surface versus time. The output was the change from baseline levels of the cell's mean fluorescent intensity.

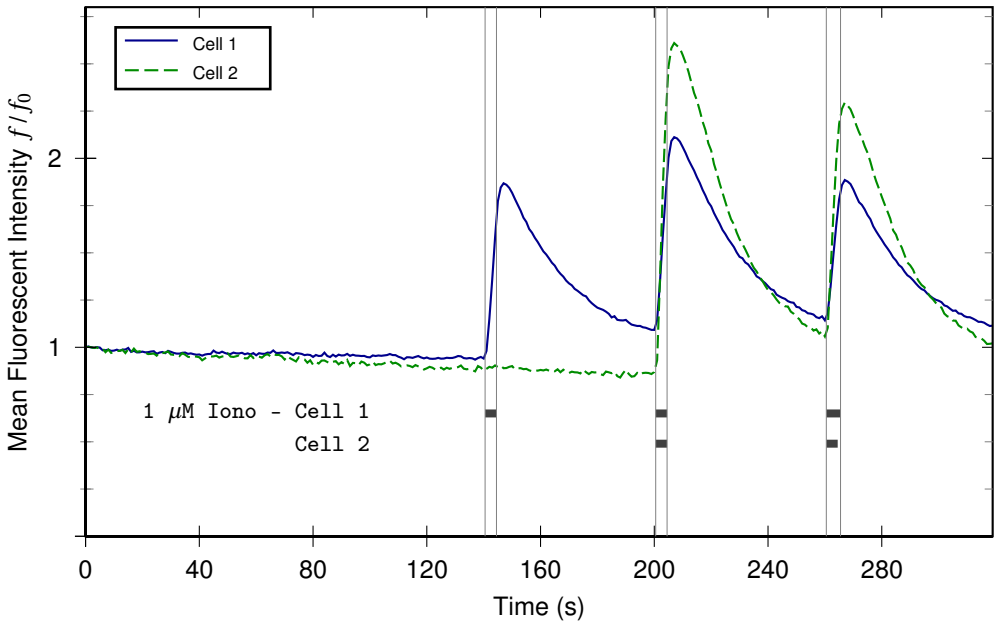

Figure 4: Measuring Impulse Response. (some data was previously published in PLoS, figure 4b.)

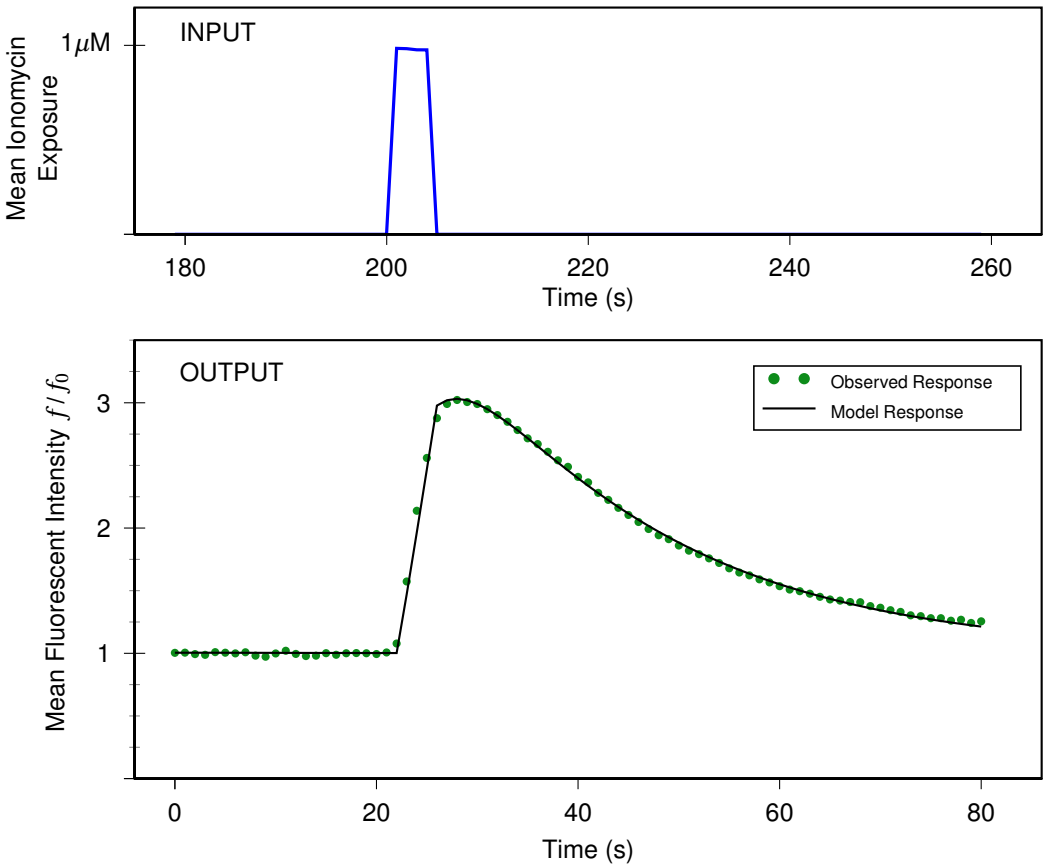

Figure 5: Pulse Response Identification. An iterative parameter estimation algorithm was used to identify potential models. A second-order linear model was found to fit the data consistently in 23 of 31 trials. The remaining 8 resulted in ill-conditioned models.

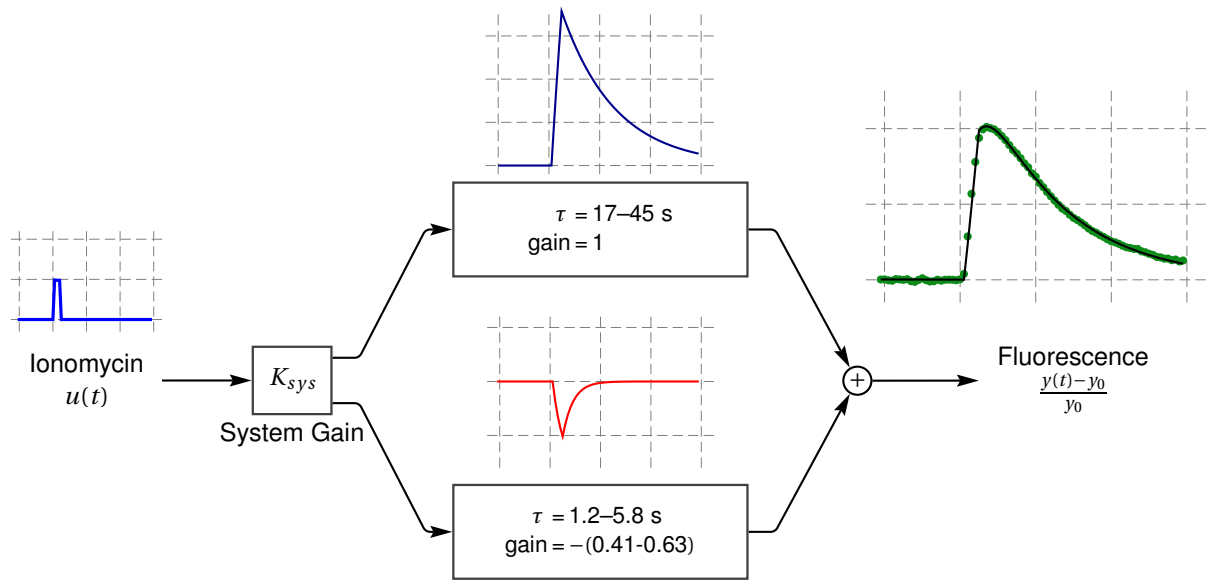

Figure 6: Pulse Response Modeling. The pulse responses were found to be well-described as two first-order systems working in parallel and acting in opposite directions ($n = 23$). The slower system, which dominated the response, had a time constant of 31±14 sec. The faster system had a time constant of roughly 1–6 s and was roughly half as effective at influencing fluorescent intensity as the dominant system (relative gain -0.52 ± 0.11). K_{sys} represents system gain, which is correlated to the magnitude of the increase in fluorescence and was wide-ranging (from 1.17 to 70.2).

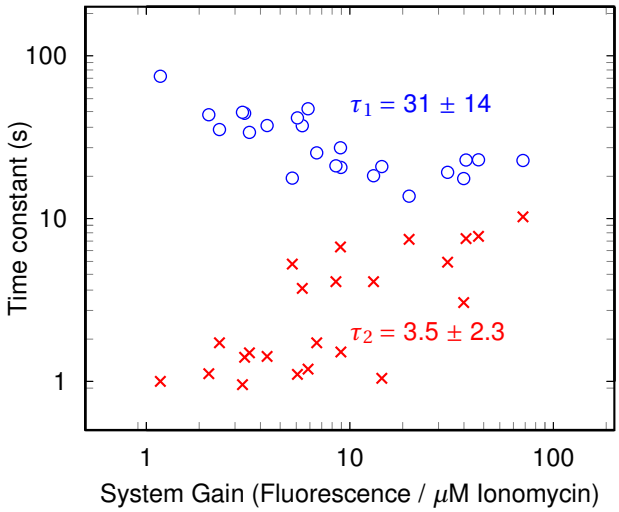

Figure 7: Pulse response time constants as a function of cell responsiveness. Horizontal axis is the system gain computed for each trial (see Figure **??** above). Vertical axis shows the time constants of the two first-order systems. These are the numerical inverses of the eigenvalues of the characteristic equation of the linear model.