Supplementary Information

Figure SI shows that the cell in the retention structure was continually inspected using a television monitor, as the images were captured by a video camera using red light. A detection window which was defined by an adjustable rectangular aperture (or spatial filter) was used for fluorescent measurement using a photomultiplier tube (PMT) in a photometric system (Photon Technology International). An electronic shutter was installed to either allow or block the excitation light (483 nm) to reach the cell.

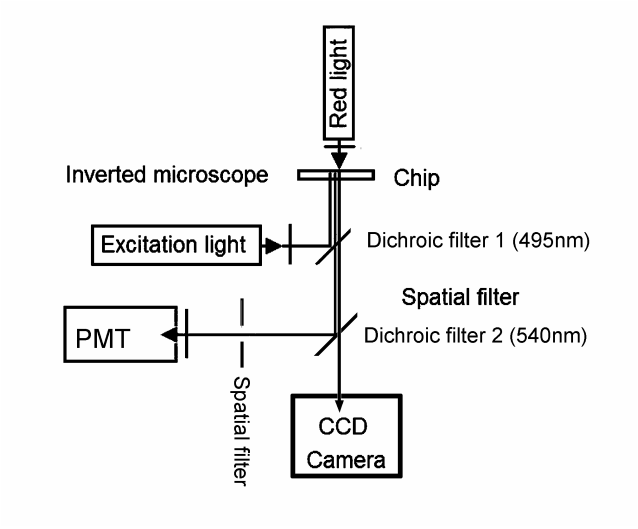

Fig. S1 The optical observation and fluorescent measurement system. The red light passed straight through to the CCD camera for optical observation. The excitation light (483 nm) was reflected at dichroic mirror 1 to excite fluorescein. The fluorescein emission was reflected at dichroic mirror 2 to the PMT for fluorescent measurement.

Figure S2 shows the results of the cell being scanned at 2 different speeds. For the left set of peaks, a faster scanning speed is obtained because a higher differential voltage (500 V) is applied across a and b (see Fig 1 for notations). These 5 peaks are spaced closer to each other, and the peak widths are smaller, as compared to the right set of peaks which are obtained using a lower differential voltage of 200 V. The inset shows the details about the difference in the fluorescent intensities of the mother cell and its bud, obtainable only at a slower scan speed.

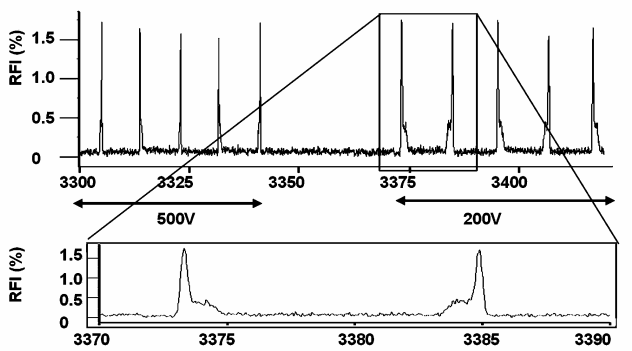

Fig. S2 The scanning results of a budding yeast cell using a narrow detection window and 2 different scanning speeds. The left five peaks were generated by 500 V, resulting in a faster scanning speed, and the right five peaks were generated by 200V, resulting in a slower scanning speed. The inset shows the 2 mirrored peaks depicting the fluorescent intensities of the mother cell and its bud.

Figure S3A depicts the gradual increase in the fluorescent intensity of the FDA-containing H4 buffer over 8000s (or about 2h). This increase was caused by the slow hydrolysis of FDA in aqueous solutions. This increase is smaller in an aqueous solution at pH 4 of the H4 buffer, as compared with pH 7 of the G7 buffer. By continually switching the buffer between H4 and G7, it was apparent that H4 had a lower fluorescent background (Fig.S3B), presumably due to a lower FDA hydrolysis rate at a lower pH in the H4 buffer. In a complex experiment on a single cell, the switching of reagent is performed many times, resulting in a complex baseline of background.

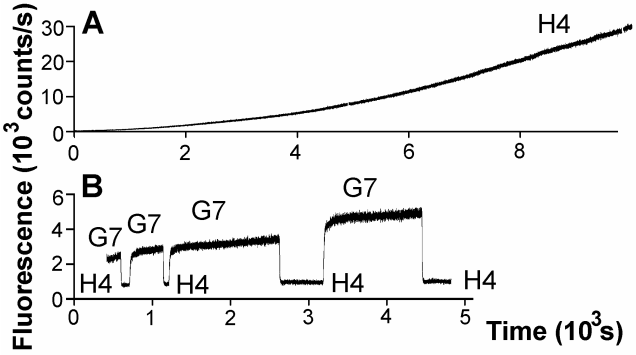

Fig. S3 The background fluctuation due to reagent switch. (**A**) The fluorescent intensity of H4 over a long period of time. (**B**) The fluorescent intensity due to the switch between H4 and G7, which were two different FDA-containing buffers (at 2 pH values) used in the single cell experiments.