Supplementary Figures

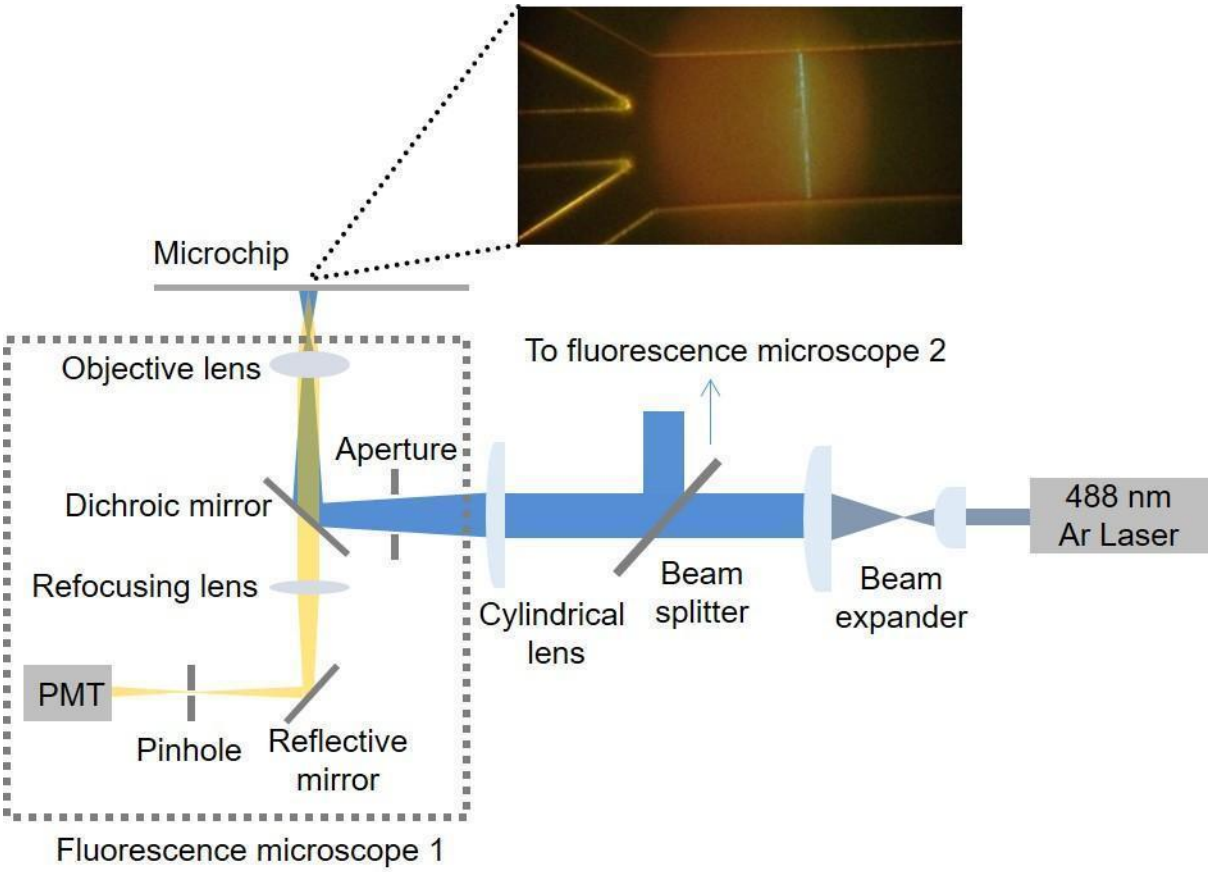

Figure S1. Illustration of the optical setup for detection system. Fluorescence microscope 2 is same with fluorescence microscope 1 and not represented in the figure. Also shown is a microscopic image of the linear focused beam on the chip near the inlet channels.

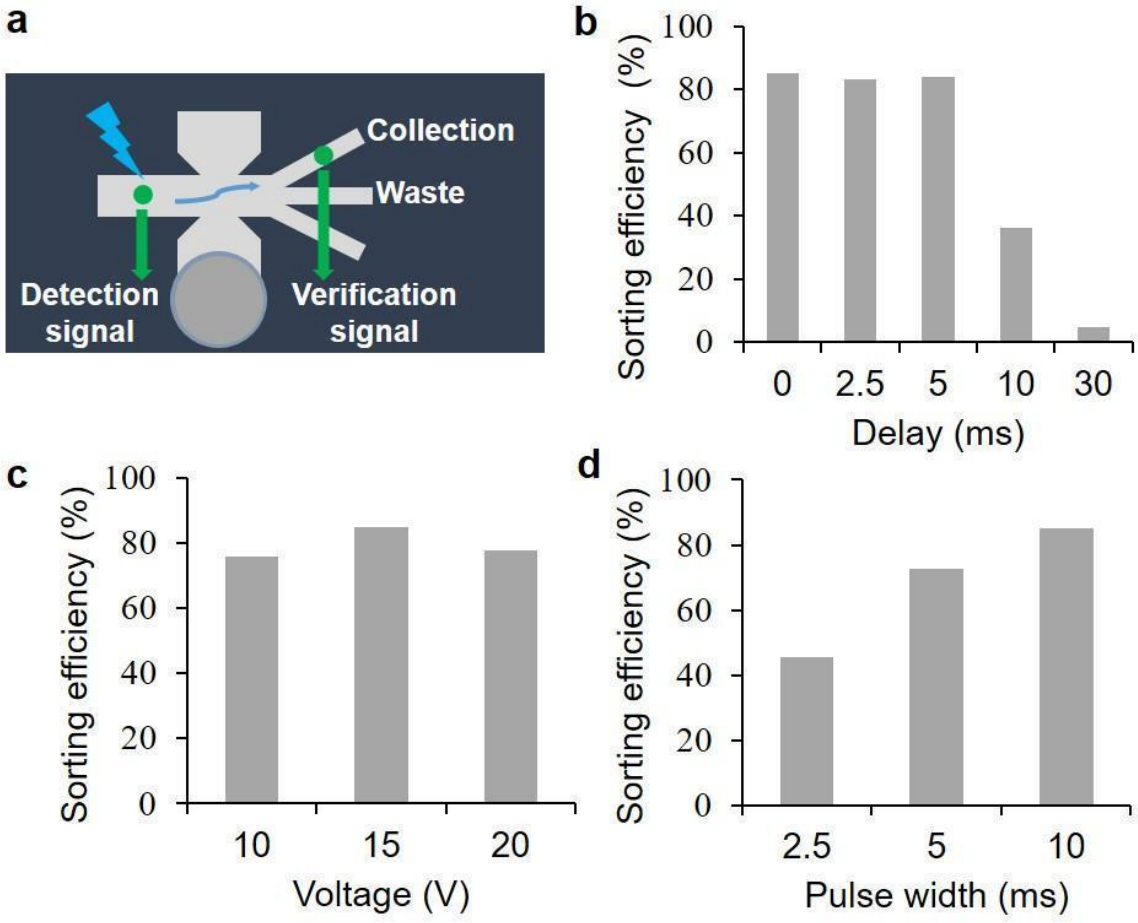

Figure S2. Optimization of pulse delay, voltage, and width for actuator activation. (a) Schematic representation of detection setup for measurement of sorting efficiency. Sorting efficiency was optimized at a linear velocity 2.89 cm/s. (b) Sorting efficiency at different delay times to trigger the sorter. The voltage applied to the sorter is 15 V and the pulse width is 10 ms. (c) Sorting efficiency with different voltages applied to the sorter. The delay time is 0 and the pulse width is 10 ms. (d) Sorting efficiency at different pulse widths. The voltage applied to the sorter is 15 V and the delay time is 0.

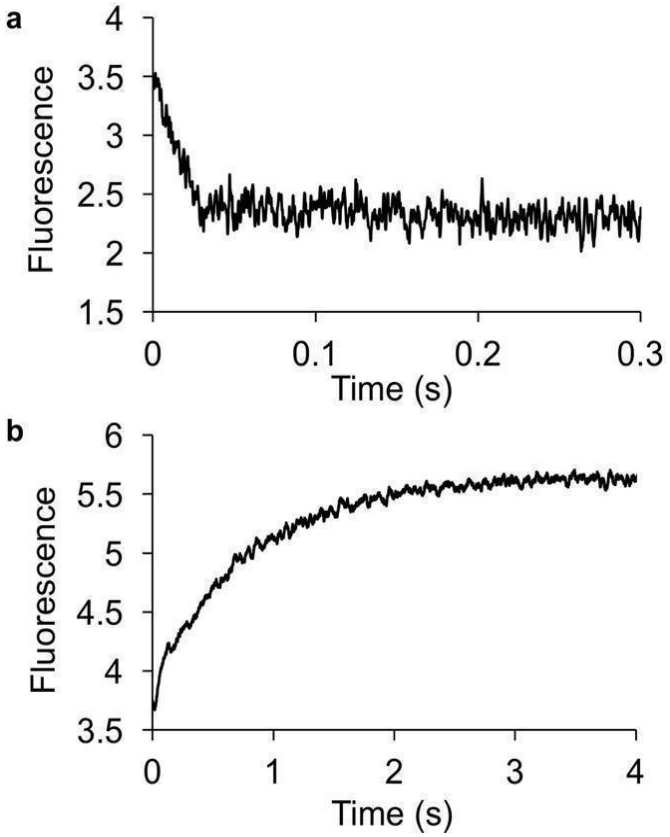

Figure S3. Ca^{2+} association and dissociation kinetics of Y-GECO1f in intact *E. coli* cells. (a) Fluorescence changes versus time for Ca^{2+}-association. (b) Fluorescence change versus time for Ca^{2+}-dissociation.

Supplementary Tables

Table S1. Distribution of the cell-to-cell time difference at different throughput at the flow rate of 5.25 µL/min

Throughput (cells/s)	Percentage of cells at different cell-to cell time difference					Total number of cells
	< 0.05 s	< 0.1 s	< 0.2 s	< 0.3 s	< 0.5 s	
3	6%	15%	31%	46%	61%	2144
5	13%	31%	53%	68%	84%	2143
10	27%	51%	77%	90%	97%	2051
20	59%	86%	97%	99%	100%	2149

Table S2. Mutations in Y-GECO variants.

Variant	Mutations
Y-GECO1m	-
Y-GECO1f	ΔG13[1]
Y-GECO2f	ΔG13, M1T, T30S, S128P

[1] ΔG13 represents that G13 is deleted.

Table S3. Properties of Y-GECO variants.

Protein variant	Ca^{2+}	λ_{abs} (nm) with ε ($mM^{-1}cm^{-1}$) in parenthesis	ϕ [1] with λ_{ex}, λ_{em} (nm) in parentheses	ε * ϕ [2]	Ca^{2+}-dependent change in brightness[3] with λ_{ex} (nm) in parentheses	pK_a[4]
Y-GECO1f	-	412 (17) 522 (26)	0.05 (412, 538) 0.75 (522, 538)	0.9 19.5	↑4× (412) ↓6× (522)	6.3
	+	412 (23) 522 (6)	0.16 (412, 538) 0.58 (522, 538)	3.7 3.5		8.5
Y-GECO2f	-	412 (20) 522 (31)	0.06 (412, 538) 0.77 (522, 538)	1.2 23.9	↑2× (412) ↓24× (522)	6.4
	+	412 (30) 522 (2)	0.09 (412, 538) 0.51 (522, 538)	2.7 1.0		9.1

[1] Quantum yield ϕ for emission (m) at the 538 nm peak measured at the two excitation (x) peaks.

[2] The product of ε and ϕ is proportional to the overall fluorescent brightness in units of $mM^{-1}cm^{-1}$.

[3] Up and down arrows indicate fluorescence increases and decreases, respectively.

[4] The pK_a is defined as the pH at which the ratio fluorescence response (excitation at 526 nm/ excitation at 416 nm) is 50% of maximum.

Table S4. *In vitro* k_{off} and K_d' of Y-GECO variants

Protein variant	k_{off} (s^{-1})	K_d' (nM) with Hill coefficient in parenthesis
Y-GECO1f	11.65	2500 (2.7)
Y-GECO2f	5.96	2200 (1.4)

Table S5. Oligonucleotides used in this work

Name	5’ to 3’ sequence
FW_XbaI_6His	GCGATGTCTAGAGGTTCTCATCATCATCATCATCATGGTATGGCTAGC
RV_stop_HindIII	GCGATGAAGCTTCTACTTCGCTGTCATCATTTGTACAAACTCTTCGTAGTTT
FW_BamHI_Kozak_6His	AAACAGGAGGAATTAAGCTTGGGATCCACCATGGGTTCTCATCATCATCATCATCATGGTATGGC
RV_CaM_stop_EcoRI	CGCGAATTCCTACTTCGCTGTCATCATTTGTAC