Electronic Supplementary Material (ESI) for Lab on a Chip.

Supplementary Information for

Deep imaging flow cytometry

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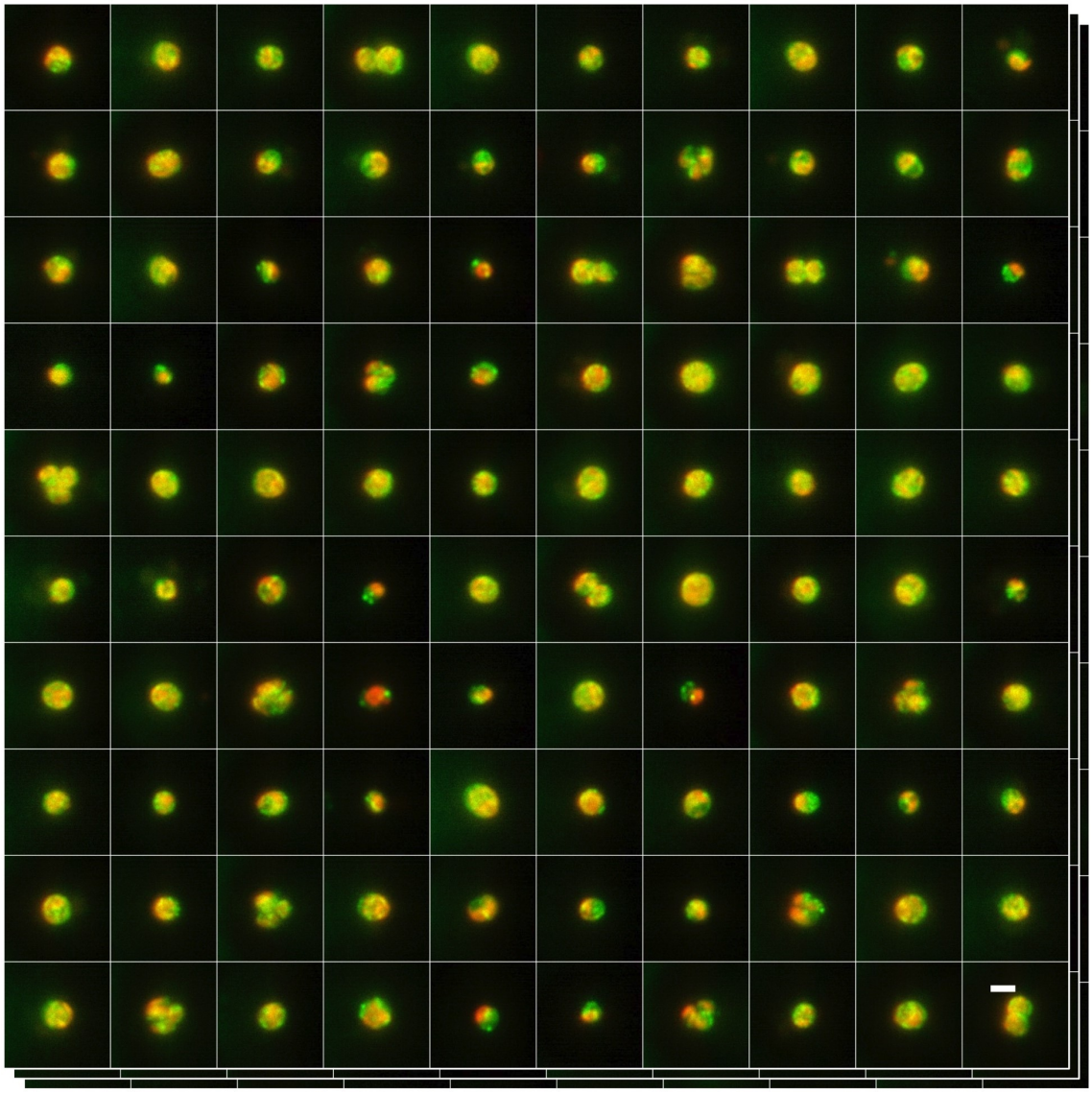

Figure S1 *C. reinhardtii* images obtained by VIFFI with the high-magnification lens. Scale bar: 5 µm.

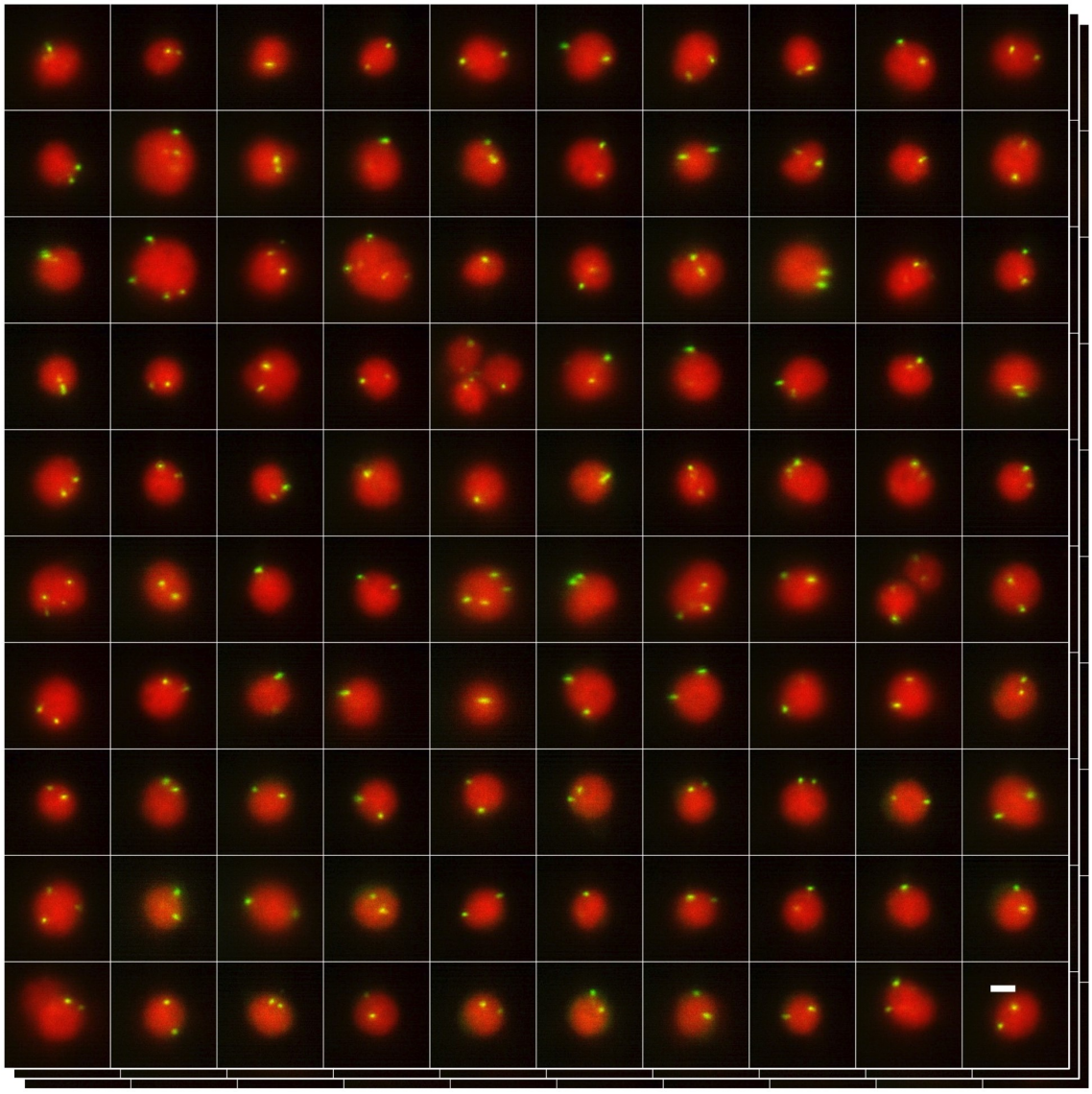

Figure S2 FISH images of Jurkat cells obtained by VIFFI with the high magnification lens. Scale bar: 5 µm.

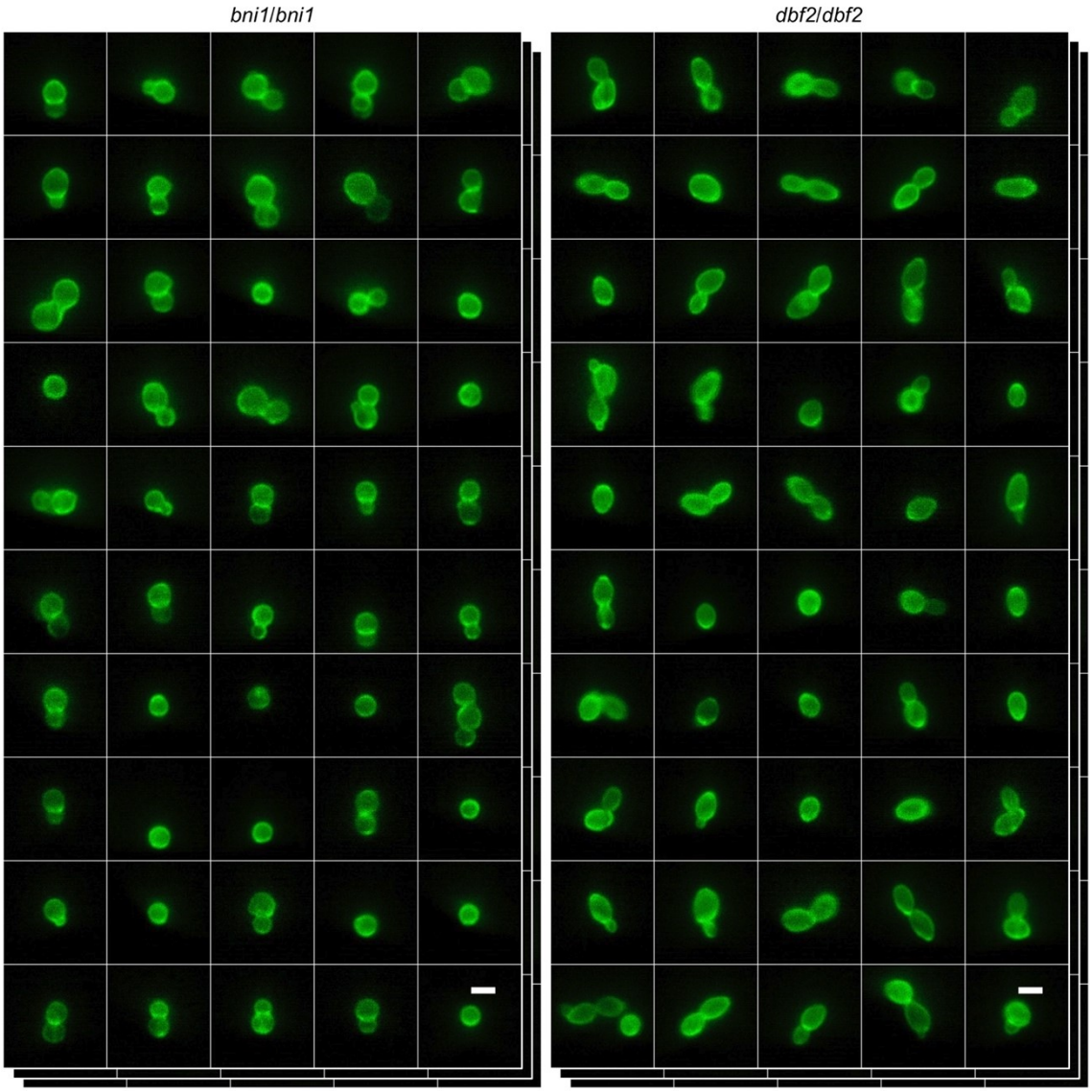

Figure S3 *S. cerevisiae* images obtained by VIFFI with the high-magnification lens. Scale bars: 5 µm.

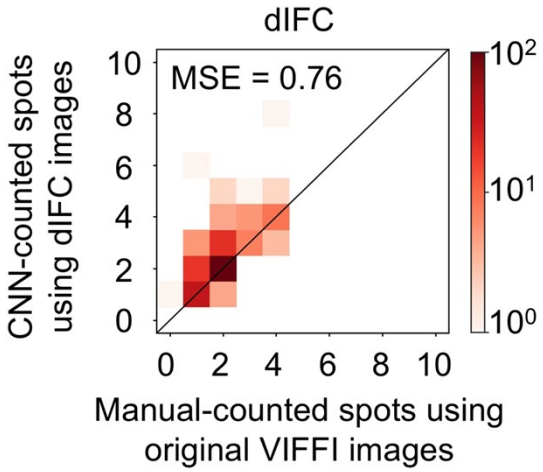

Figure S4 FISH spot counting based on a HR-image generator trained by a paired training dataset. The heat map shows the relation between CNN-counted FISH spots using dIFC images and manual-counted FISH spots using original VIFFI images. Original VIFFI images are images obtained by VIFFI with the low-magnification lens. N = 200. It is noted that this figure corresponds to the right panel in Figure 4b in the main text, but with a different training dataset.

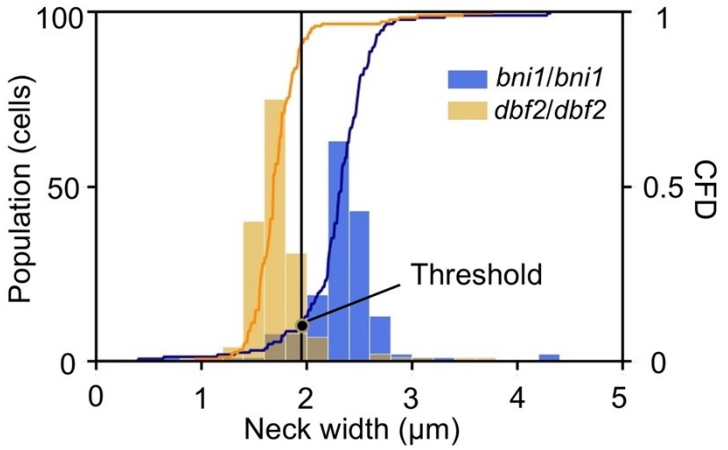

Figure S5 Neck-width-based classification of *bni1*/*bni1* and *dbf2*/*dbf2* using images obtained by a commercially available microscope (AxioImager M1, Carl Zeiss AG, Germany). The neck width analysis was performed by an algorithm implemented on CalMorph[38]. The classification accuracy was 0.903 by a neck width threshold of 1.95 μm. CFD, cumulative frequency distribution. N = 165.

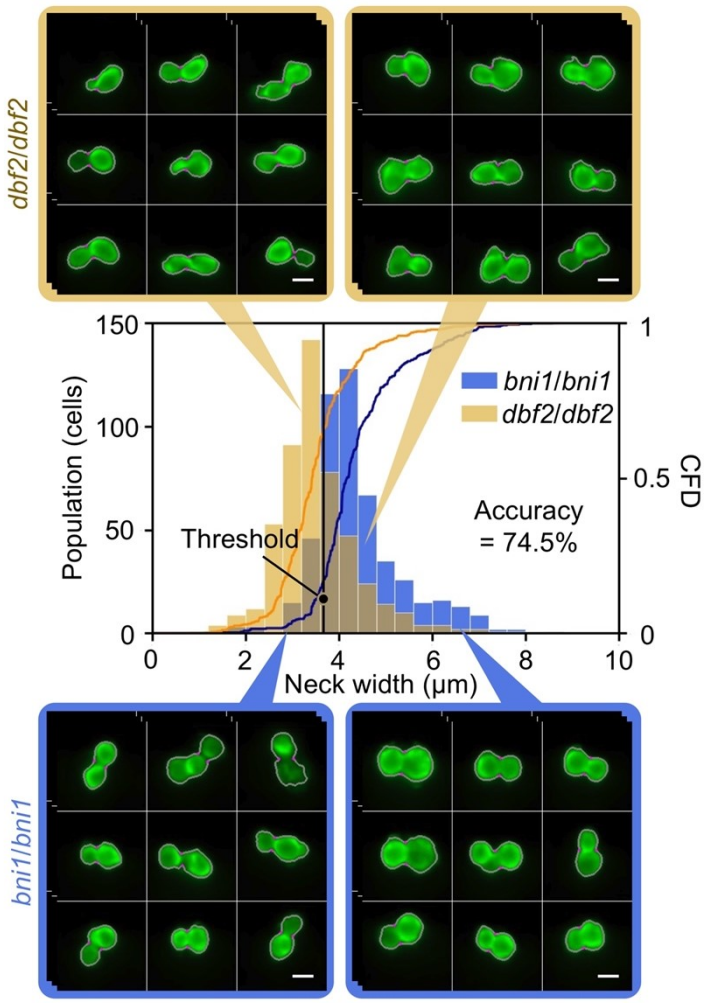

Figure S6 Analysis of neck widths of budding yeast cells in dIFC images acquired by a HR-image generator trained by an unpaired training dataset. The histogram shows the neck width (distance between the pink circles) of budding yeast of two different strains. Image panels at the top and bottom show typical images for each strain with the small neck width (left) and large neck width (right), respectively. The black line at a neck width of 3.67 μm indicates the classification threshold which optimally separates the two strains. The blue and orange curves show the cumulative frequency distribution. CFD, cumulative frequency distribution. N = 500 for each. Scale bars: 5 μm. It is noted that this figure corresponds to Figure 5c in the main text, but with a different training dataset.

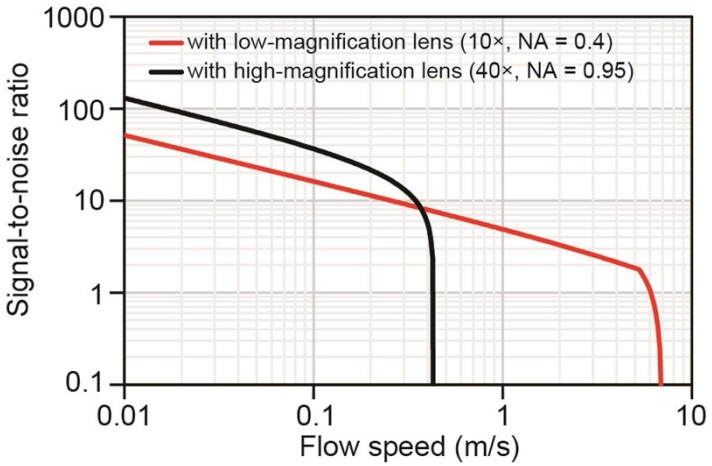

Figure S7 Theoretically estimated relation between the flow speed and SNR. The SNRs were calculated using equations described in our previous paper as a basis, but with specific parameters for this paper (See Table S1).

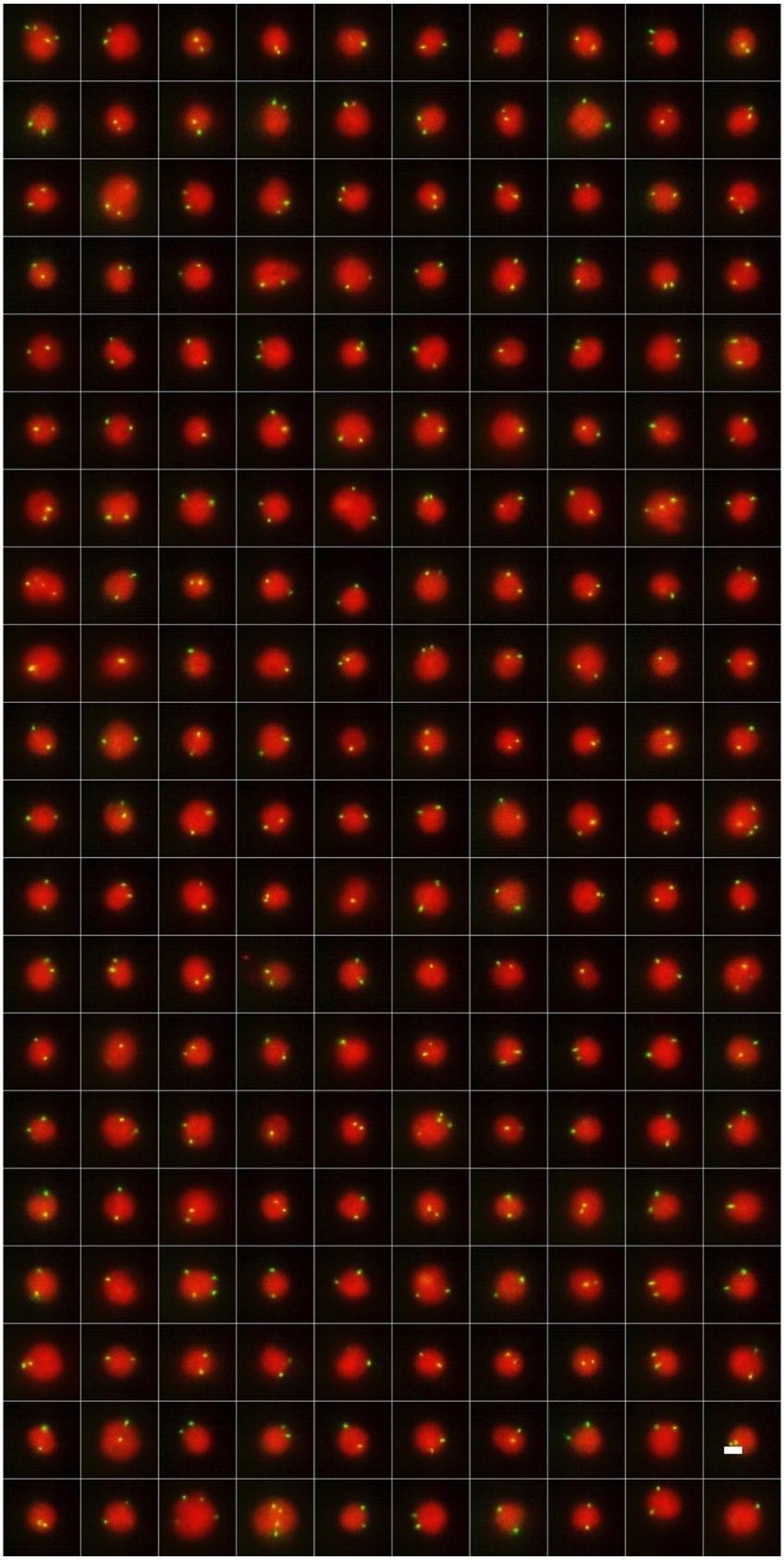

Figure S8 FISH images having FISH spots that are well visible obtained by VIFFI with the high-magnification lens. Scale bar: 5 μm.

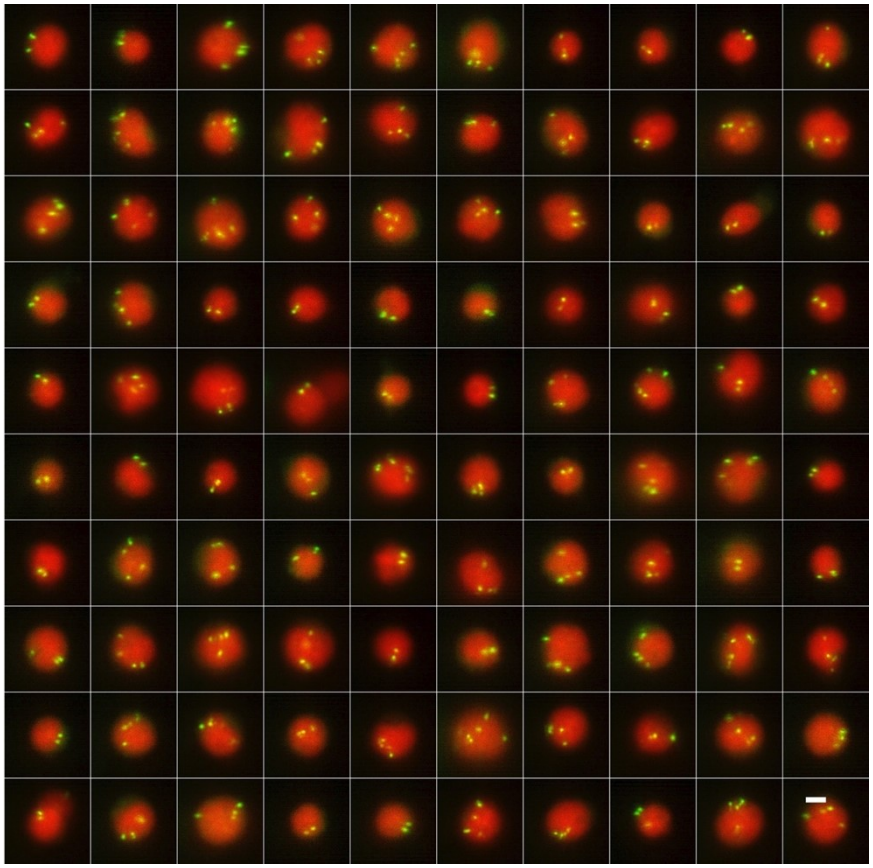

Figure S9 FISH images having close-spaced FISH spots obtained by VIFFI with the high-magnification lens. Scale bar: 5 μm.

Table S1 Parameter settings for the estimation of the SNRs.

Parameter	Value	Note
Magnification of objective lens	10 (low-NA), 40 (High-NA)	Olympus UPLSAPO10X (low-NA), UPLSAPO40X (high-NA)
Numerical aperture of objective lens	0.4 (low-NA), 0.95 (high-NA)	Olympus UPLSAPO10X (low-NA), UPLSAPO40X (high-NA)
Excitation beam power (mW)	180	Accounting for ~10% loss at beam scanner
Excitation beam diameter in depth direction (μm)	4	Current setting
Excitation beam diameter in flow direction (μm)	26	Current setting
Cross section of excitation beam (μm^2)	104	Approximated by $\pi D_x D_z/4$
Time of excitation beam illumination	(variable)	$\min(t_{exp1}, t_{exp2})D_x/FOV_x$ (see Eqs. 5 and 6 of Ref. [17])
Absorption cross section of fluorescent molecules (cm^2)	1.16×10^{-16}	Fluorescein
Quantum yield of fluorescent molecules	0.8	Fluorescein
Photon collection efficiency of imaging system	0.0478 (low-NA), 0.00737 (high-NA)	Estimated values from calibrated value of setup at NA = 0.75
Quantum efficiency of image sensor	0.55	Specification of pco.edge 5.5 (λ = 530 nm)
Readout noise (e^- rms)	1.7	Specification of pco.edge 5.5 (λ = 530 nm)
Number of fluorescent molecules	10	