High-throughput phenotyping of *Chlamydomonas* swimming mutants based on nanoscale video analysis

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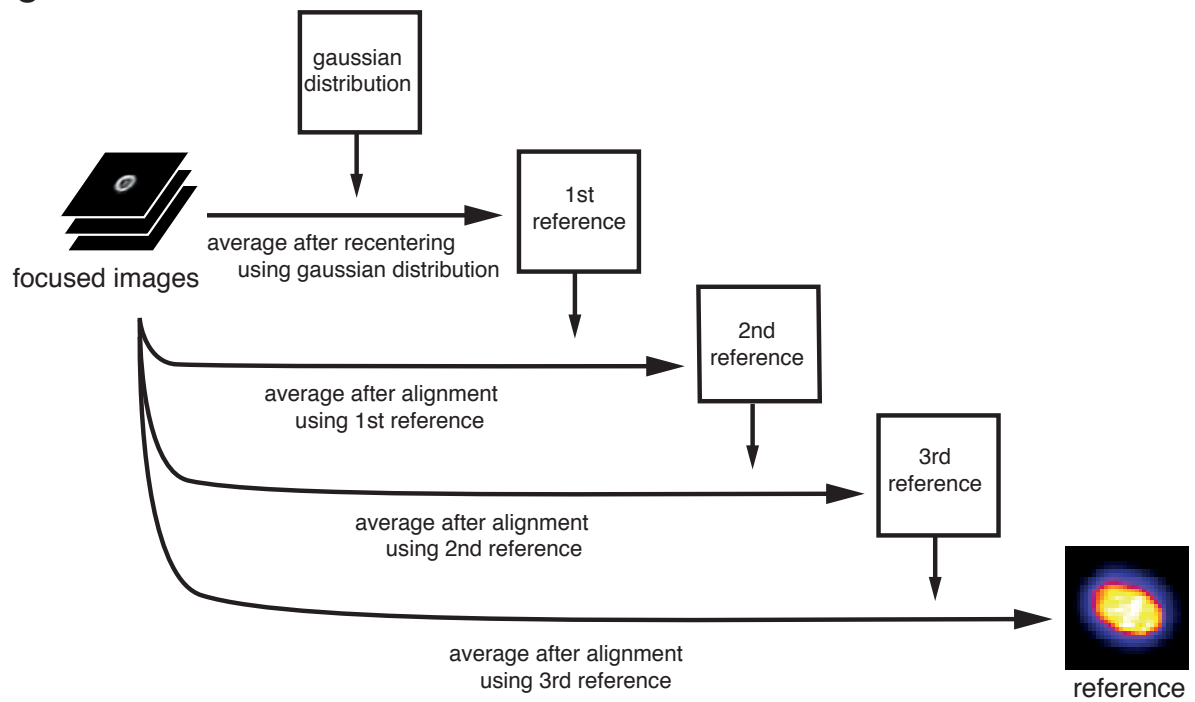## Supporting Material

Figure S1

Figure S1: Flow chart of making a reference image. The best 30 in-focus images were selected from the image stack, recentered using two-dimensional Gaussian fit [1], and averaged to produce an reference image. The final reference image was obtained by iterating this procedure two to three times using the most recently generated reference image.

Figure S2

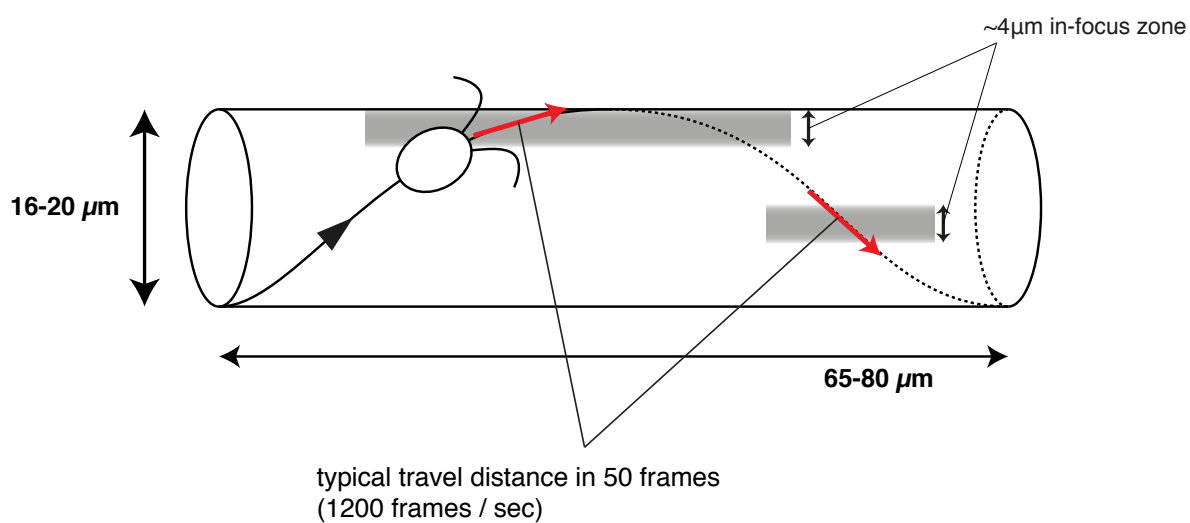typical travel distance in 50 frames
(1200 frames / sec)

Figure S2: Relationship between the swimming path and in-focus zone. Although the swimming of *Chlamydomonas* is a 3-D helical movement [2], we have selected the beat cycles in which the cell swims parallel to the in-focus zone (gray area) by using a focus index. A *Chlamydomonas* cell typically swims about 9 μm (red arrows) in the time window (50 frames) used for the focus index. Since the thickness of in-focus zone is about 4 μm, the contribution of the perpendicular movement is less than 10%.

Table S1: Details of the 38 *Chlamydomonas* strains used in the screening

| Strain	Candidate gene(s) Protein ID	Name	motility defect	Known Function
roc9	-	-	-	-
roc11	No gene models	-	-	-
roc12	21422	*VPS11*	-	-
roc15	No gene models	-	-	-
roc22	186225	*FAP256*	-	Flagellar associated protein 256, a cilia tip protein
roc23	173322	-	+	-
roc28	-	-	-	-
roc30	24252	*ODA4*	++	Flagellar outer dynein arm heavy chain β
roc34	-	*PF9*	++	Flagellar inner arm dynein 1 heavy chain α
roc39	108167	*MFT10*	-	-
roc40	No gene models	-	-	-
roc54	24252	*ODA4*	++	Flagellar outer dynein arm heavy chain β
roc55	No gene models	-	-	-
roc56	94281	-	-	-
roc59	186304	-	-	-
roc63	137559	*DLC7a*	++	Flagellar outer dynein arm light chain LC7a
roc66	No gene models	-	-	-
roc69	187830	*ATR1*	-	-
roc74	No gene models	-	-	-
roc75	No gene models	-	-	-
roc76	144235	-	-	-
roc77	194623	*ALIX*	+	-
roc78	127161	*MAPKKKK1*	+	-
roc80	128004	*SKP1*	-	Subunit of E3 ubiquitin ligase
roc81	176522	-	-	-
roc86	95232	*XRN1*	-	N-terminus of single-stranded RNA 5'-3' exoribonuclease
roc88	-	-	+	-
roc93	153922	-	-	-
roc94	15736	*MUT-9*	-	Ser/Thr protein kinase involved in transcriptional gene silencing
roc97	Several	Several	-	-
roc103	131208	*FAP131*	-	Flagellar associated protein 131
roc104	No gene models	-	-	-
roc105	194623	*ALIX*	+	-
roc106	194837	*FLA14*	++ (immotile)	Flagellar outer dynein arm light chain 8
roc108	186976	-	-	-
roc110	195428	*DRP2*	-	-
roc112	173322	-	+	-
roc114	186976 (SC5)	-	-	-

Figure S3
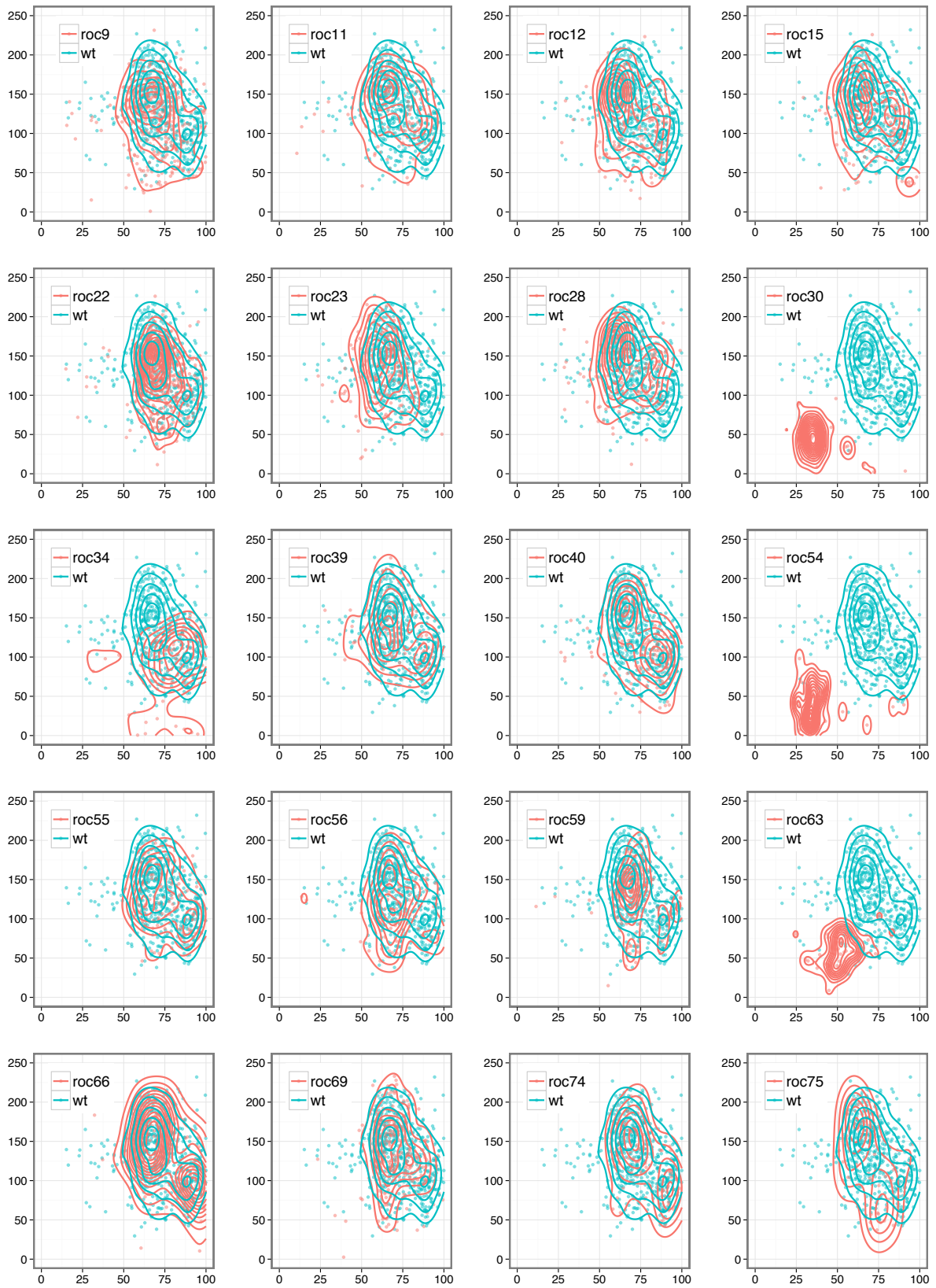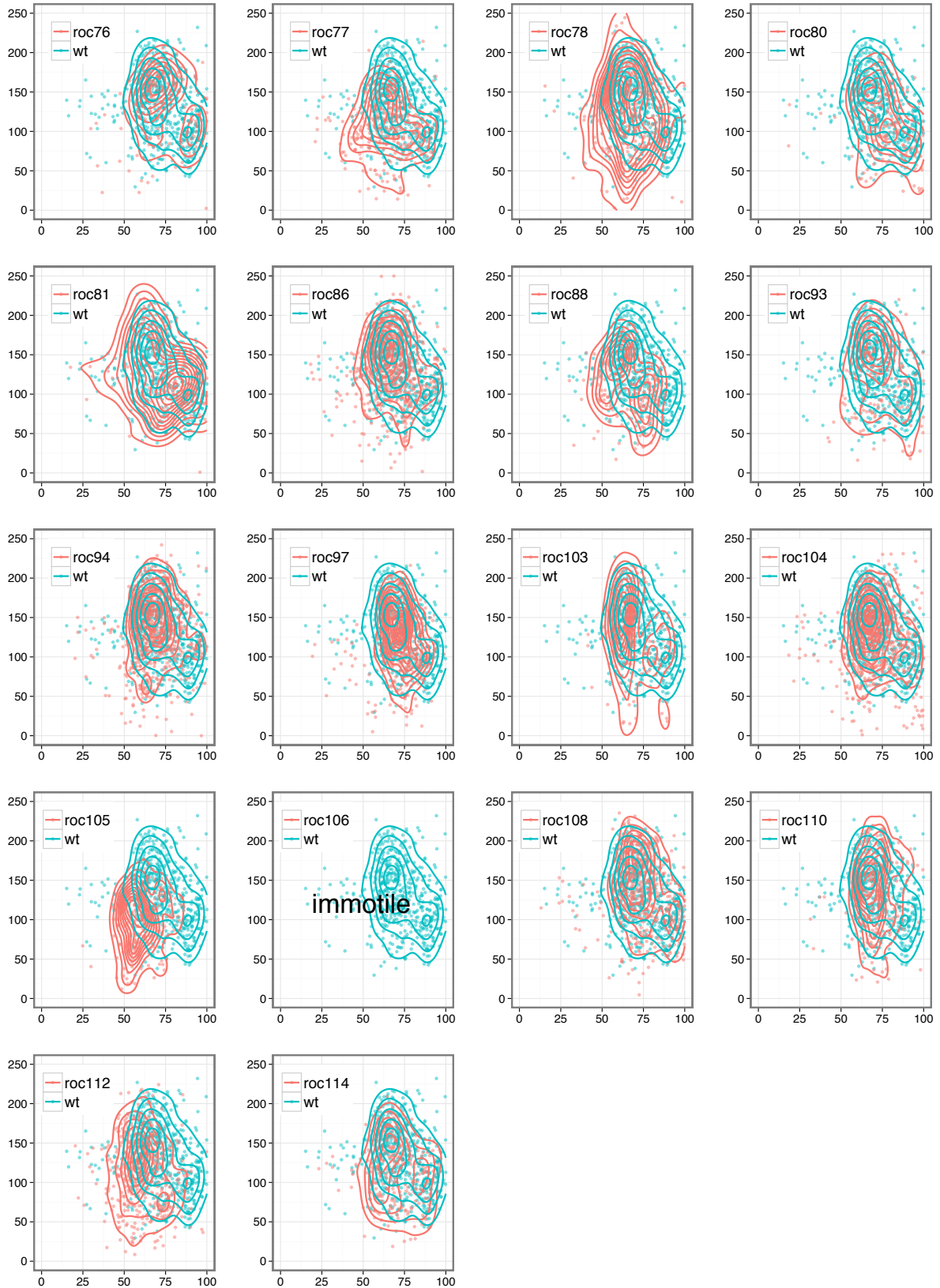
Figure S3: Screening of 38 *Chlamydomonas* strains for motility-deficient mutants using the CLONA method. Scatter plots of beat frequency f (x axis) versus average swimming velocity $\overline{V}$ (y axis) of the beat cycles. The blue contour lines show the characteristic cluster of the control. Each dot represents one beat cycle. Focus index > 80.

Movie S1, S2, S3, and S4 (S1) 1200 fps high speed video used for the plots shown in Fig. 3A and B. (S2) 1200 fps high speed video used for the plot shown in Fig. 3C. (S3) 1200 fps high speed video of the control strain, CBR$^+$, used in the screening test. (S4) 1200 fps high speed video of a newly detected motility-deficient mutant, *roc105*.

Supporting References

[1] R. E. Thompson, D. R. Larson, and W. W. Webb. Precise nanometer localization analysis for individual fluorescent probes. *Biophys J*, 82(5):2775–2783, 2002.

[2] K. W. Foster and R. D. Smyth. Light Antennas in phototactic algae. *Microbiol Rev*, 44(4):572–630, 1980.