Supporting Material

Independent synchronized control and visualization of interactions between living cells and organisms

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Content

SUPPORTING MATERIALS AND METHODS

Cell culture

mCD4 3A9 T cells (1,2) were grown in RPMI (Gibco, Life technologies, Saint-Aubin, France) supplemented with 5 % heat-inactivated fetal bovine serum (Gibco), sodium pyruvate 1 mM (Gibco) and HEPES 10 mM (Gibco) at 5 %CO_2 and 37°C in a humidified atmosphere.

Labeling method

For cell labeling, mCD4 3A9 T cells (10^5 in 100 µl) were incubated in presence of FITC-labelled mAb anti-CD45 (Becton Dickinson Company, Le Pont-de-Claix, France) or PE-labelled mAb anti-CD45 (Becton Dickinson) at final concentration of 0.5 µg/µL for 10 min at 4°C. Cells were washed three times by centrifugation (3 min at 200 × *g*) in complete medium. Cells were imaged in HBSS (Gibco) supplemented with 10 mM HEPES (Gibco) in Lab-Tek well chamber (Thermo scientific, Dominique Dutscher, Brumath, France). Acquisition was performed using 40 focal planes separated by 500 nm, with 200 ms exposure time.

SUPPORTING TABLE

Category of equipment	Company	Reference
Microscope	**Nikon**	*assembled by Nikon*
Inverted microscope		TI-E/B
Objectives		
for trapping & imaging		CFI Plan Fluor 40 × oil, 1.3 NA
		CFI Plan Fluor 100 × oil, 1.3 NA
for HOT alignment		CFI 4 ×, 0.1 NA
		CFI Plan 10 ×, 0.25 NA
Mercury lamp		C-HGFIE
M2 lamphouse adapter		TI-AT
Imaging Module	**Visitron Systems**	*assembled by Visitron Systems*
Cameras	Hamamatsu	ImagEM 512 C9100-13
Confocal spinning disk head	Yokogawa	CSU-X1-A1-5000rpm
Laser sources	Toptica	405 nm - 405S-10367
	"	440 nm - 445S-10368
	"	473 nm - 473S-10369
	"	488 nm - 488S-10370
	"	640 nm - 640S-10371
	Cobolt	561 nm - 0561-04-01-0075-400
Laser collimator (imaging)	AMS Technologies	HPUC-23AF-400/700-P-6AC-2
Optical fiber (imaging)	Yokogawa	CSU-X1 fiber
FRAP module	Visitron Systems	2D-VisiFRAP
Laser collimator (FRAP)	AMS Technologies	HPUC-23AF-400/700-P-6AC-2
Optical fiber (FRAP)	AMS Technologies	QPMJ-3AF3S-488-3.5/125-3-3-1
HOT Module		*home made*
IR laser	IPG Photonics	PYL-2-1050-LP
Half-wave plate	Thorlabs	WPH05M-1053
L1 to L4 lenses	Thorlabs	LA1805-B
	"	LA1911-B
	"	LA1050-C
	"	LA1417-C
Spatial light modulator	Hamamatsu	X8267-15
D2 infrared dichroic mirror	Rocky Mountain Instrument Co	SWP2607U0734
IR filters	Thorlabs	FM01
CCD camera	JAI	CV-A60
Synchronization		
Linear motor LM	Physik Instrumente	M683.2U4
XY motorized stage	Applied Scientific Instrumentation	ASI S22121010
Z-top plate	"	ASI PZ-2150

Table S1 – References of the unusual instruments used

SUPPORTING FIGURES

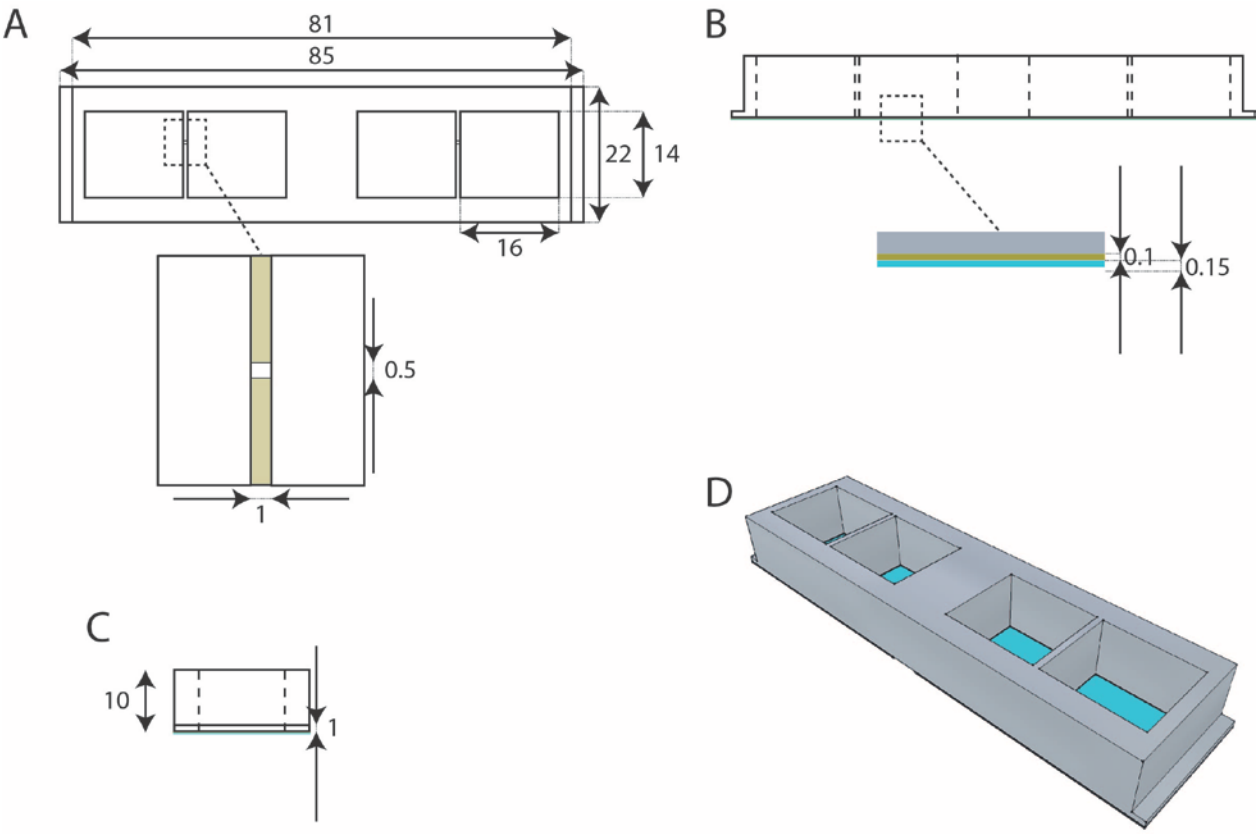

Figure S1 – Observation chamber designed for controlled *C. elegans* infection experiments

A two well chamber slide linked by a small channel has been designed with a reservoir for the spores and the other for the tests of adhesion. Wells were fabricated in an aluminum piece. The bottom face was covered by a double-sided tape in which an incision makes the channel. A size #1 cover glass seals the chamber. (*A*) Top view with zoom on the channel. (*B*) Lateral view with zoom on the different layers: aluminum (grey), double-sided tape (yellow) cover glass (blue). (*C*) Front view. (*D*) 3D illustration. All of the values are in mm.

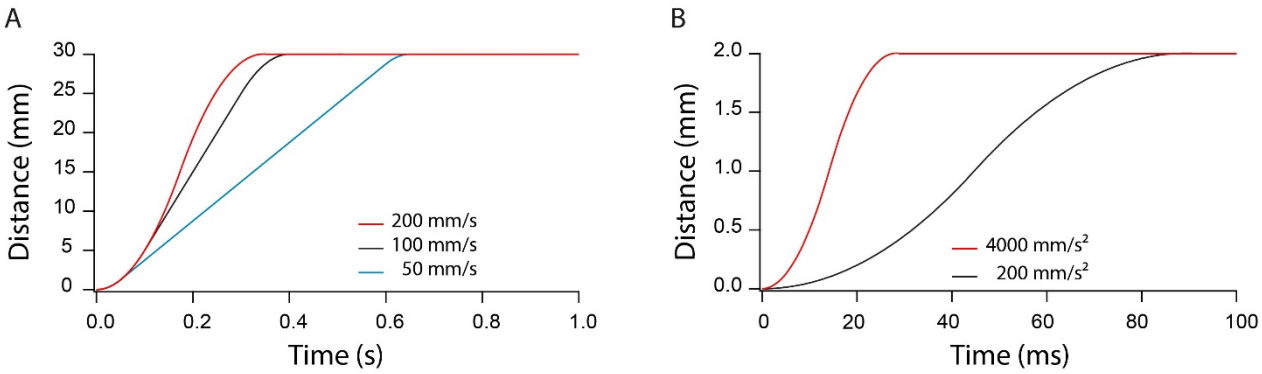

Figure S2 – Speed and acceleration characteristics of the linear motor supporting L3

All of the data were recorded and visualized with the software controlling the linear motor (LM) (PI France SAS, Montrouge, France). (*A*) The acceleration closed-loop control was set at its default value (200 mm/s^2) to test motor speeds ranging from 50 to a maximum of 200 mm/s. Considering the constraints due to the weight of the L3 lens, the latter speed value fits our requirement. (*B*) The speed control was set at its maximum value (200 mm/s) for comparison of the default and maximum closed-loop acceleration values, 200 and 4000 mm/s^2, respectively. By reaching 2 mm displacement in less than 30 ms, the LM characteristics fit our requirement to synchronize the trapping and imaging modules. To avoid inconsistent error message due to small overshot at the deceleration, the maximum position error has to be set at 5 µm.

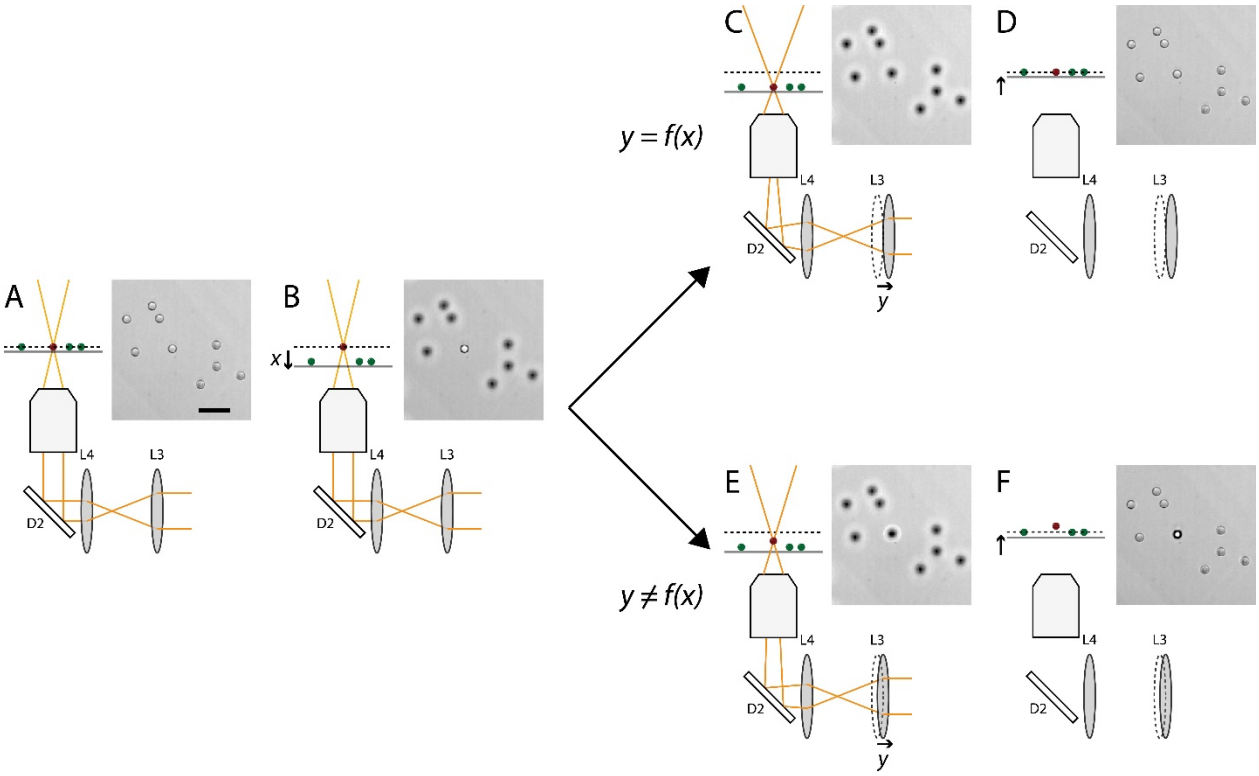

Figure S3 – Schematic representation of the calibration procedure that allows compensation of the sample holder displacement, but maintenance of the laser trap at a fixed position

(*A*) Latex beads are settled on a cover glass and imaged in brightfield. Among them, one bead is trapped without any change in its position. This gives the initial zero position, where both the imaging plane (dotted line) and the trapping plane are exactly overlapping (i.e. the zero reference plane). (*B*) The sample holder is moved by a distance *x* along the optical axis whereas the trapped bead stays in the focal plane. (*C*) The L3 lens is moved a distance *y* in order to move back the trapped bead to its initial position on the cover glass. (*D*) The IR laser is turned off and at the same time, the sample holder is moved back to recover the reference imaging plane. We validate the position of the trapped bead in the initial reference image by verifying that the shape of the trapped bead is equivalent to that of the other beads. As seen in Fig. 3, the shape criterion is particularly efficient around the zero reference plane. (*E*) and (*F*): same as (*C*) and (*D*) but with unsuitable compensation of the L3 displacement. In that case, the shape of the reference bead and the ones that have settled are not identical (see (*F*)). Scale bar, 10 µm. Here, bead imaging was performed with the 40 × objective.

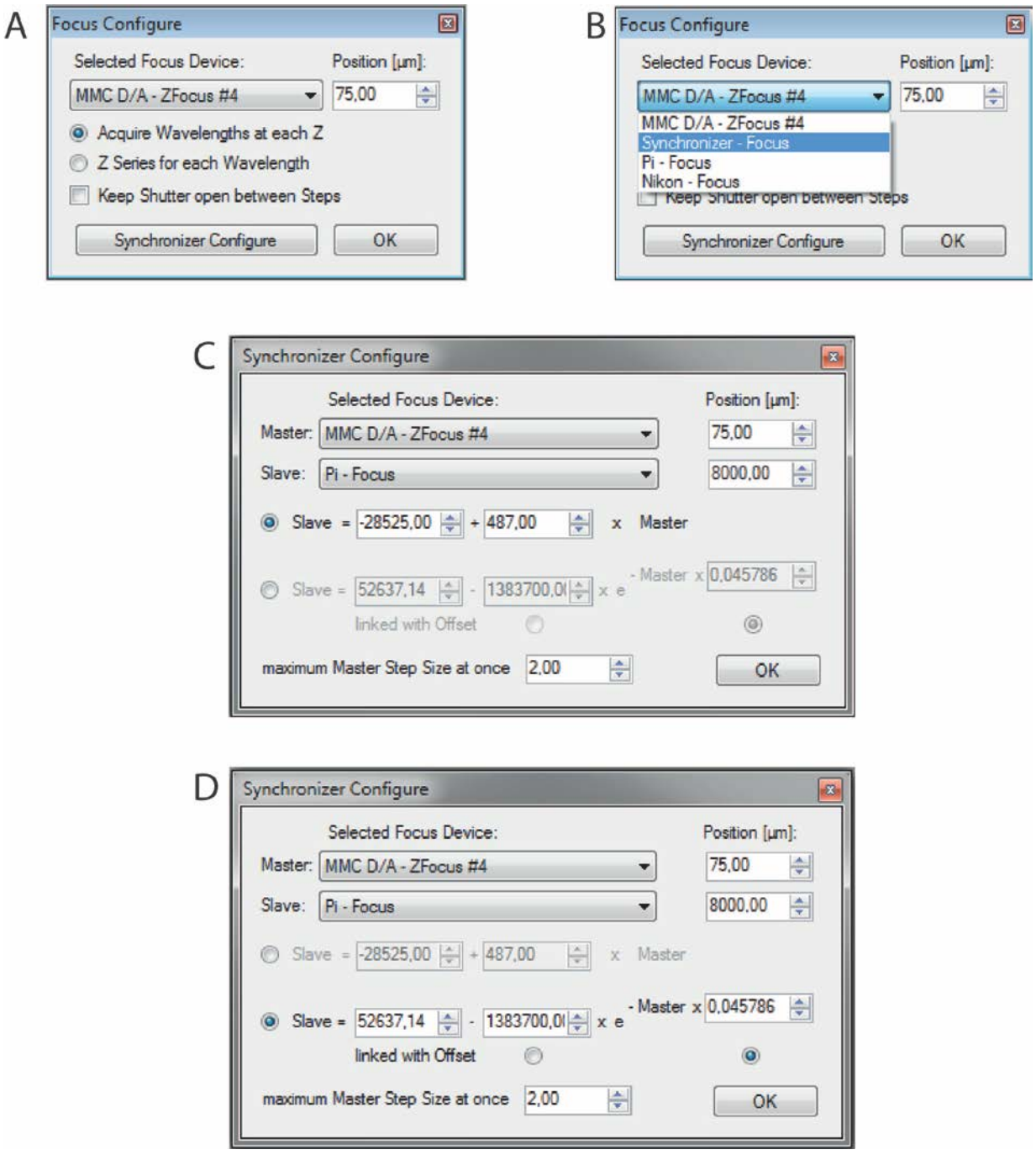

Figure S4 – Synchronization module implemented in the Visiview software

The windows for focus configuration display different options in the mode of acquisition requested (*A*) and a list of controlled devices with adjustable position settings. The list includes the synchronized mode between both motors (Synchronizer – Focus), the LM (Pi – Focus) and the microscope control of the objective position (Nikon – Focus). (*B*). In "Synchronizer – Focus" mode, a second window is open to determine master and slave and their initial positions. This window allows to choose a linear (*C*) or exponential (*D*) model for synchronization and to introduce appropriate equation parameters.

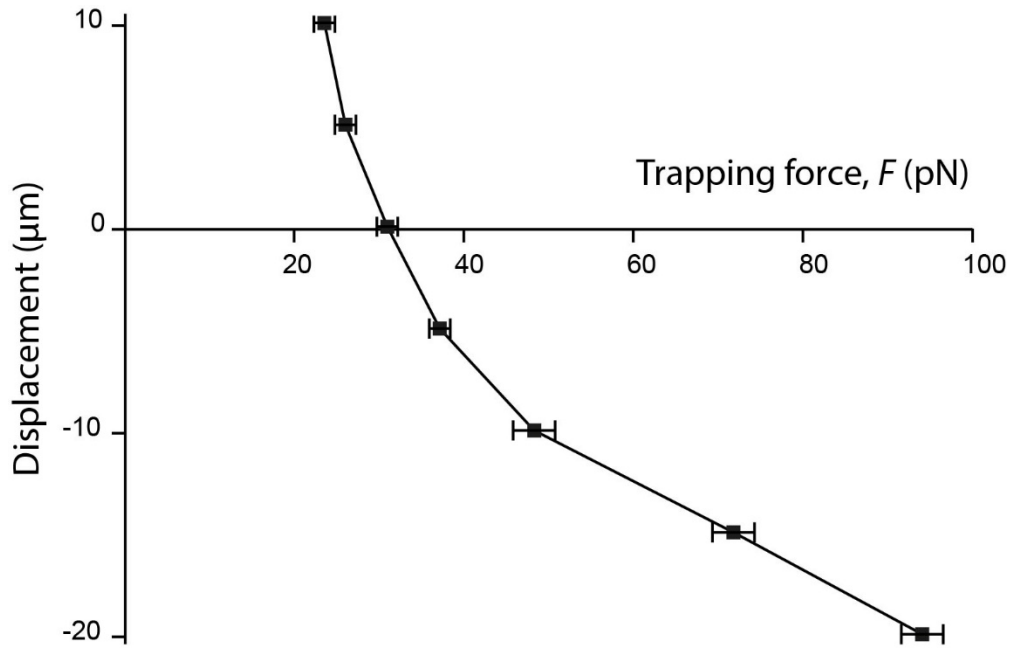

Figure S5 – Variation of the trapping force during the IR laser decollimation

A latex bead of 3 µm in diameter is initially trapped in the imaging plane, then moved by the linear motor above or below this plane from a given distance as reported on the ordinate axis. Next, the trapping force *F* is experimentally quantified by dragging the bead at increased speeds until its release from the trap. All measurements were performed in 50% glycerol medium at 30°C. The IR laser power at the reference position was set at 60 mW at the focalization point.

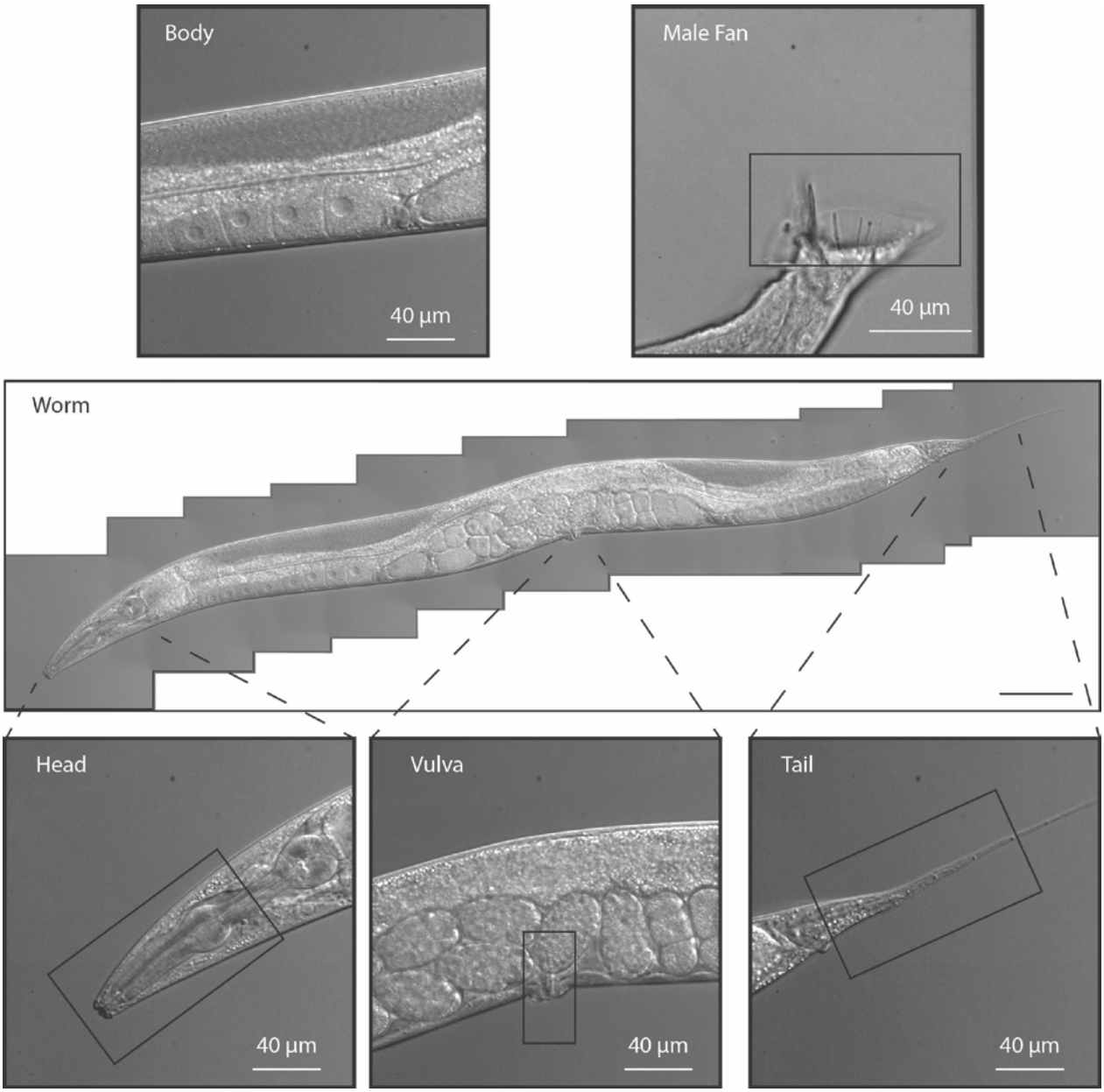

Figure S6 – Different regions of *C. elegans* visualized by light microscopy

Stitching reconstruction of a hermaphrodite N2 wild-type worm imaged by differential interference contrast (DIC) microscopy; scale bar, 100 µm. The vignettes detail the head, vulva and tail of the worm. The black boxes delineate the area of adhesion tests. The body area is defined by excluding tail head or vulva with a security distance of around 100 µm. The upper right image illustrates the fan of a male wild-type strain N2.

SUPPORTING REFERENCES

1. Allen, P. M. and E. R. Unanue. 1984. Differential requirements for antigen processing by macrophages for lysozyme-specific T cell hybridomas. Journal of immunology 132:1077-1079.

2. Babbitt, B. P., G. Matsueda, E. Haber, E. R. Unanue, and P. M. Allen. 1986. Antigenic competition at the level of peptide-Ia binding. Proceedings of the National Academy of Sciences of the United States of America 83:4509-4513.

LEGENDS OF THE SUPPORTING MOVIES

Movie S1 – 3D image of a bead with or without synchronization

A single latex bead (red dot) from the ones settled down on the cover glass is trapped and imaged in brightfield (stack of 10 images at 1 μm intervals). Sample was moved by the z-top plate without synchronization (left panel), or with the trapped bead maintained in its initial position by synchronization (right panel). The z position of the image focal plane is specified in the bottom left corner of the movie. In the right panel, the persistency of the trap is demonstrated by controlled displacement of the bead to the left.

Movie S2 – 3D image of a cell with or without synchronization

A membrane-stained T cell hybridoma (CD4 3A9 T cell) was trapped and observed by confocal imaging (stack of 20 images at 1 μm intervals). Cell was imaged with OFF or ON synchronization between trap and imaging, left and right panels, respectively. The *z* position of the image focal plane is specified in the bottom left corner of the movie.

Movie S3 – Independent 3D displacement of fluorescent spores

D. coniospora spores were stained by DiI. The movie illustrates the confocal images recording the independent motion of five spores in *xyz* direction.

Movie S4 – Adhesion test performed by displacement of a sedated worm

The movie illustrates confocal imaging recorded during an adhesion test in which a sedated worm is moved by the *xyz* stage of the microscope to contact in head area three stationary trapped spores. *D. coniospora* spores are stained by DiI. The *C. elegans* strain expressed the GFP in the pharynx and epidermis.

Movie S5 – Adhesion test performed by displacement of multiple independent trapped spores

The movie illustrates confocal imaging recorded during an adhesion test in which three trapped spores are moved to contact one at a time the head area of a worm: the first spore instantly adheres but disappears from the focal plane (it is still adherent as illustrated at the end of the movie recording defocused images); the second one adheres also immediately to the head and stays in the focal plane; the third one becomes adherent after two consecutive contacts. This is mainly due to a defective orientation of the conidium part of the spore contacting the cuticle surface. *D. coniospora* spores are stained by DiI. The *C. elegans* strain expressed the GFP in the pharynx and epidermis.

Movie S6 – 3D multicolor confocal imaging during adhesion of a single spore on *C. elegans*

The movie illustrates 3D confocal images in three colors recorded as in Fig. 5, during and after the adhesion of a spore on the head area of a worm. *D. coniospora* spores are stained by DiI. The *C. elegans* strain expressed the GFP in the epidermis (green) and the RFP in the pharynx (yellow). Its cuticle and neurons are stained by DiD (red).

Movie S7 – 3D multicolor confocal imaging of T cells moved by HOT

Two subsets of mCD4 3A9 T cells were stained by mAb anti-CD45 labeled by FITC or PE and then mixed together. Cells were trapped and moved in 3D independently from each other.