

Calcium Imaging in Freely Behaving *Caenorhabditis elegans* with Well-Controlled, Nonlocalized Vibration

 Kazuki Shigyou¹, Haruka Maeoka¹, Ryuji Igarashi^{2,3,4}, Takuma Sugi¹

¹Program of Biomedical Science, Graduate School of Integrated Sciences for Life, Hiroshima University ²Institute for Quantum Life Science, National Institute for Quantum and Radiological Science and Technology ³National Institute for Radiological Sciences, National Institute for Quantum and Radiological Science and Technology ⁴JST, PRESTO

*These authors contributed equally

Corresponding Authors

Ryuji Igarashi

igarashi.ryuji@qst.go.jp

Takuma Sugi

sugit@hiroshima-u.ac.jp

Citation

Shigyou, K., Maeoka, H., Igarashi, R., Sugi, T. Calcium Imaging in Freely Behaving *Caenorhabditis elegans* with Well-Controlled, Nonlocalized Vibration. *J. Vis. Exp.* (170), e61626, doi:10.3791/61626 (2021).

Date Published

April 29, 2021

DOI

10.3791/61626

URL

jove.com/video/61626

Materials

Name	Company	Catalog Number	Comments
Data analysis software			
DualViewImaging.nb	author		For analysis of acquired data
Mathematica12	Wolfram		For running data analysis software DualViewImaging
<i>Escherichia coli</i> and <i>C. elegans</i> strains			
<i>E. coli</i> OP50	Caenorhabditis Genetics Center	OP50	Food for <i>C. elegans</i> . Uracil auxotroph. <i>E. coli</i> B.
<i>lite-1(ce314)</i> strain	Caenorhabditis Genetics Center	KG1180	Light-insensitive mutant
<i>lite-1(ce314)</i> strain expressing NLS-GCaMP-NLS and TagRFP under the control of the AVA-specific promoter	author	ST12	<i>lite-1(ce314)</i> mutant carrying the genes expressing NLS-GCaMP5G-NLS (NLS; nuclear localization signal) and TagRFP under the control of the <i>flp-18</i> promoter as an extrachromosomal arrays
Laser Doppler vibrometer			
Lase Doppler vibrometer	Polytec Japan	IVS-500	For quantifying frequency and displacement generated by the acoustic transducer
Mouse macro system			
Assay.txt	Author		Script for temporally and specially controlling mouse cursor in Windows
HiMacroEx	Vector	https://www.vector.co.jp/download/file/winnt/util/fh667310.html	Free download software for controlling mouse cursor based on a script
Nematode growth media plate			
Agar purified, powder	Nakarai tesque	01162-15	For preparation of NGM plates
Bacto pepton	Becton Dickinson	211677	For preparation of NGM plates
Calcium chloride	Wako	036-00485	For preparation of NGM plates

Cholesterol	Wako	034-03002	For preparation of NGM plates
di-Photassium hydrogenphosphate	Nakarai tesque	28727-95	For preparation of NGM plates
LB broth, Lennox	Nakarai tesque	20066-95	For culture of <i>E. coli</i> OP50
Magnesium sulfate anhydrous	TGI	M1890	For preparation of NGM plates
Potassium Dihydrogenphosphate	Nakarai tesque	28720-65	For preparation of NGM plates
Sodium Chloride	Nakarai tesque	31320-05	For preparation of NGM plates
Petri dishes (60 mm)	Nunc	150270	For preparation of NGM plates
Nonlocalized vibration device			
Amplifier	LEPY	LP-A7USB	For stimulation with controllable vibration
Acoustic transducer	MinebeaMitsumi	LVC25	For stimulation with controllable vibration
WaveGene Ver. 1.5	Thrive	http://efu.jp.net/soft/wg/down_wg.html	Free download software for controlling vibration property
Noninvasive calcium imaging			
2-Channel benchtop 3-phase brushless DC servo controller	Thorlabs	BBD202	Compatible controller for MLS203-1 stages
479/585 nm BrightLine dual-band bandpass filter	Semrock	FF01-479/585-25	For acquisition of two channel images (GCaMP and TagRFP)
505/606 nm BrightLine dual-edge standard epi-fluorescence dichroic beamsplitter	Semrock	FF505/606-Di01-25x36	For acquisition of two channel images (GCaMP and TagRFP)
512/25 nm BrightLine single-band bandpass filter	Semrock	FF01-512/25-25	For acquisition of two channel images (GCaMP and TagRFP)
630/92 nm BrightLine single-band bandpass filter	Semrock	FF01-630/92-25	For acquisition of two channel images (GCaMP and TagRFP)
Computer	Dell	Precision T7600	Windows7 with Intel Xeon CPU ES-2630 and 8 GB of RAM
High-speed x-y motorized stage	Thorlabs	MLS203-1	Fast XY scanning stage
Image splitting optics	Hamamatsu photonics	A12801-01	For acquisition of two channel images (GCaMP and TagRFP) generated by W-VIEW GEMINI Image splitting optics
LED light source	CoolLED	pE-4000	For generating 470 nm and 560 nm excitation light
Microscope	Olympus	MVX10	
sCMOS camera	Andor	Zyla	
x 2 Objective lens	Olympus	MVPLAPO2XC	Working distance 20 mm and numerical aperture 0.5
Plasmid			
pKDK66 plasmid	author	pKDK66	Co-injection marker
pTAK83 plasmid	author	pTAK83	Plasmid for expression of TagRFP under the control of the <i>flp-18</i> promoter
pTAK144 plasmid	author	pTAK144	Plasmid for expression of NLS-GCaMP5G-NLS under the control of the <i>flp-18</i> promoter
Tracking software			
homingback.vi	author		SubVi file for tracking a fluorescent spot of a worm through feedback control of sCMOS camera and x-y motorized stage
LabVIEW	National instruments		For running tracking software
Zyla Control ver.2.6Cl.vi	author		For tracking a fluorescent spot of a worm through feedback control of

		sCMOS camera and x-y motorized stage
--	--	--------------------------------------