Materials List for:

Ratiometric Calcium Imaging of Individual Neurons in Behaving *Caenorhabditis Elegans*

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Materials

Name	Company	Catalog Number	Comments
***C. elegans* growth, cultivation, and mounting**			
Escherichia coli bacterial strain, OP50	Caenorhabditis Genetic Center	OP50	Food for C. elegans. Uracil auxotroph. E. coli B. Biosafety Level 1
HSN GCaMP5+mCherry worm strain	Caenorhabditis Genetic Center	LX2004	Integrated transgene using *nlp-3* promoter to drive GCaMP5 and mCherry expression in HSN. Full genotype: *vsIs183 [nlp-3p::GCaMP5::nlp-3 3'UTR + nlp-3p::mCherry::nlp-3 3'UTR + lin-15(+)], lite-1(ce314), lin-15(n765ts)* X
lite-1(ce314), lin-15(n765ts) mutant strain for transgene preparation	author	LX1832	Strain for recovery of high-copy transgenes after microinjection with pL15EK *lin-15(n765ts)* rescue plasmid. Also bears the linked *lite-1(ce314)* mutation which reduces blue-light sensitivity. Available from author by request
pL15EK *lin-15a/b* genomic rescue plasmid	author	pL15EK	Rescue plasmid for recovery of transgenic animals after injection into LX1832 *lite-1(ce314), lin-15(n765ts)* X strain. Available from author by request
pKMC299 plasmid	author	pKMC299	Plasmid for expression of mCherry in the HSNs from the *nlp-3* promoter. Has *nlp-3* 3' untranslated region
pKMC300 plasmid	author	pKMC300	Plasmid for expression of GCaMP5 in the HSNs from the *nlp-3* promoter. Has *nlp-3* 3' untranslated region
Potassium Phosphate Monobasic	Sigma	P8281	For preparation of NGM plates
Potassium Phosphate Dibasic	Sigma	P5655	For preparation of NGM plates
Magnesium Sulfate Heptahydrate	Amresco	0662	For preparation of NGM plates
Calcium Chloride Dihydrate	Alfa Aesar	12312	For preparation of NGM plates
Peptone	Becton Dickinson	211820	For preparation of NGM plates
Sodium Chloride	Amresco	0241	For preparation of NGM plates
Cholesterol	Alfa Aesar	A11470	For preparation of NGM plates

Agar, Bacteriological Type A, Ultrapure	Affymetrix	10906	For preparation of NGM plates
60 mm Petri dishes	VWR	25384-164	For preparation of NGM plates
24 x 60 mm micro cover glasses, #1.5	VWR	48393-251	Cover glass through which worms are imaged
22 x 22 mm micro cover glasses, #1	VWR	48366-067	Cover glass that covers the top of the agar chunk
Stereomicroscope with transmitted light base	Leica	M50	Dissecting microscope for worm strain maintenance, staging, and mounting
Platinum iridium wire, (80:20), 0.2mm	ALFA AESAR	AA39526-BW	For worm transfer
Calcium imaging microscope			
Anti-vibration air table	TMC	63-544	Micro-g' Lab Table 30" x 48" anti-vibration table with 4" CleanTop M6 on 25mm top
Inverted compound microscope	Zeiss	431007-9902-000	Axio Observer.Z1 inverted microscope
Sideport L80/R100 (3 position)	Zeiss	425165-0000-000	To divert 20% of output to brightfield (CMOS) camera, 80% to fluorescence (sCMOS) camera
Tilt Back Illumination Carrier	Zeiss	423920-0000-000	For infrared/behavior imaging
Lamphousing 12V/100W w/ Collector	Zeiss	423000-9901-000	For infrared/behavior imaging
Halogen lamp 12V/100W	Zeiss	380059-1660-000	For infrared/behavior imaging. White-light LEDs do not emit significant infrared light, so they will not allow brightfield imaging after the infrared bandpass filter
32 mm Infrared bandpass filter (750-790 nm) for Halogen lamp	Zeiss	447958-9000-000	BP 750-790; DMR 32mm, for infrared illumination for brightfield and behavior
6-filter Condenser Turret (LD 0.55 H/DIC/Ph), Motorized	Zeiss	424244-0000-000	For infrared/behavior imaging
Condenser & Shutter	Zeiss	423921-0000-000	For infrared/behavior imaging
Binocular eyepiece with phototube for infrared CMOS camera	Zeiss	425536-0000-000	For infrared/behavior imaging
Eyepiece 10x, 23mm	Zeiss	444036-9000-000	For worm localization on the agar chunk
C-Mount Adapter 2/3" 0.63x demagnifier	Zeiss	426113-0000-000	Mount for infrared CMOS camera
CMOS camera for infrared brightfield and behavior (1" sensor)	FLIR (formerly Point Grey Research)	GS3-U3-41C6NIR-C	Camera for brightfield imaging
USB 3.0 Host Controller Card	FLIR (formerly Point Grey Research)	ACC-01-1202	Fresco FL1100, 4 Ports
8 pins, 1m GPIO Cable, Hirose HR25 Circular Connector	FLIR (formerly Point Grey Research)	ACC-01-3000	Cable for TTL triggering. The green wire connects to GPIO3 / Pin 4 and the brown wire connects to Ground / Pin 5
Plan-Apochromat 20x/0.8 WD=0.55 M27	Zeiss	420650-9901-000	Best combination of magnification, numerical aperture, and working distance
6-cube Reflector Turret, Motorized	Zeiss	424947-0000-000	For fluorescence imaging
Fluorescence Light Train, Motorized	Zeiss	423607-0000-000	For fluorescence imaging
Fluorescence Shutter	Zeiss	423625-0000-000	For fluorescence imaging

GFP and mCherry dual excitation and emission filter cube (for microscope)	Zeiss	489062-9901-000	FL Filter Set 62 HE BFP+GFP +HcRed for fluorescence imaging
LED illumination system	Zeiss	423052-9501-000	Triggerable Colibri.2 LED system for fluorescent illumination
GFP LED module (470 nm)	Zeiss	423052-9052-000	Colibri.2 LED for GFP fluorescence excitation
mCherry LED module (590 nm)	Zeiss	423052-9082-000	Colibri.2 LED for mCherry fluorescence excitation
Iris stop slider for incident-light equipment	Zeiss	000000-1062-360	Field aperture iris to limit LED illumination to the camera field of view
C-Mount Adapter 1" 1.0x	Zeiss	426114-0000-000	Adapter for image-splitter and sCMOS fluorescence camera
Image splitter	Hamamatsu	A12801-01	Gemini W-View, other image splitters may be used, but they may not be optimized for the large sensor size of the sCMOS cameras
GFP / mCherry dichroic mirror (image splitter)	Semrock	Di02-R594-25x36	Splitting GCaMP5 from mCherry and infrared signals
GFP emission filter (image splitter)	Semrock	FF01-525/30-25	Capturing GCaMP5 fluorescence
mCherry/ emission filter (image splitter)	Semrock	FF01-647/57-25	This filter is necessary to exclude the infrared light used for brightfield imaging
sCMOS camera for fluorescence (1" sensor)	Hamamatsu	A12802-01 / C11440-22CU	Orca FLASH 4.0 V2. Newer models allow for separate image acquisition settings on separate halves of the sensor, allowing acquisition of two-channel images in combination with an image splitter
Motorized XY Stage	Märzhäuser	SCAN IM 130 x 100	Stage movement; the XY resolution of this stage is 0.2µm per step
XY Stage controller with joystick	LUDL	MAC6000, XY joystick	Manual tracking of worms. MAC6000 controller should be connected to the PC through the serial (RS-232) port configured to 115200 baud
Digital Acquisition board (DAQ)	Arduino	Uno	Receiving TTL triggers from sCMOS camera. The Uno should be loaded with the standard Firmata package, and the computer USB port configured to 57600 baud
BNC Male to BNC Male Cable - 6 ft	Hosa Technology	HOBB6	BNC connectors for TTL triggering
Gold-Plated BNC Male to SMA male coaxial cable (8.8")	uxcell	608641773651	To connect the fluorescence camera trigger outputs
BNC turn head adapter	Hantek	RRBNCTH21	BNC to Banana Plug Adapter (4mm)
BNC female to female connector	Diageng	20130530009	Female to female BNC adapter to connect the BNC output from the camera to the Banana Plug
Solderless flexible breadboard jumper wires	Z&T	GK1212827	To connect the BNC trigger outputs to the Arduiono DAQ. Male to male.
High performace workstation	HP	Z820	Windows 7, 64GB RAM, Dual Xeon processor, solid state

			C: drive, serial (RS-232) port, multiple PCIe3 slots for ethernet connectivity, USB 3.0 cards, and additional solid state drives
M.2 Solid state drive	Samsung	MZ-V5P512BW	High-speed streaming and analysis of image data
M.2 Solid state drive adapter for workstations	Lycom	DT-120	M.2 to PCIe 3.0 4-lane adapter
Network attached storage	Synology	DS-2415+	Imaging data storage and analysis
Hard disk drives	Western Digital	WD80EFZX	RED 8 TB, 5400 RPM Class SATA 6 Gb/s 128MB Cache 3.5 Inch. Storage of imaging data (10 drives + 2 drive redundancy)
Software			
Fluorescence Acquisition	Hamamatsu	HCImage DIA	Recording of two channel (GCaMP5 and mCherry) fluorescence image sequences at 20 fps
Brightfield Acquisition	FLIR (formerly Point Grey Research)	Flycapture	Recording of brightfield JPEG image sequences
Stage Serial Port Reader	Bonsai	https://bitbucket.org/horizongir/bonsai	Facilitates tracking of worms during behavior
LED controller software	Zeiss	Micro Toolbox Test 2011	To set up the intensity and trigger inputs for the different LEDs in the Colibri.2 unit
ImageJ	NIH	https://imagej.net/Fiji/Downloads	Simple review of image sequences and formatting changes for import into Ratiometric Quantitation software
Excel	Microsoft	2002984-001-000001	For generating subsets of comma-separated value data from Volocity for MATLAB analysis
Peak Finding	MATLAB	R2017a	Script used for Ratio peak feature calculations
Ratiometric Quantitation	Perkin Elmer	Volocity 6.3	Facilitates calculation of ratiometric image channels, image segmentation for object finding, and ratio measurement of found objects
Scripts			
XY-stage-final.bonsai	Bonsai	TTL-triggered DAQ and stage position serial port reader	Records X and Y stage position (in microns) when the attached Arduino receives a positive TTL signal from sCMOS camera during frame exposure. Script writes a .csv file with four columns: frame number, X position (microns), Y position (microns), and the time elapsed between frames (typically ~50 msec when recording at 20 fps). X and Y stage position from this output (columns 2 and 3, respectively) are added to the X and Y centroid positions from the AnalyzeGCaMP_2017.m MATLAB script (columns 4 and 5, respectively), to give the final X and Y position of the fluorescent object for the recording.