Materials List for:
Ratiometric Calcium Imaging of Individual Neurons in Behaving Caenorhabditis Elegans

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<table>
<thead>
<tr>
<th>Name</th>
<th>Company</th>
<th>Catalog Number</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>C. elegans growth, cultivation, and mounting</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Escherichia coli</em> bacterial strain, OP50</td>
<td>Caenorhabditis Genetic Center</td>
<td>OP50</td>
<td>Food for C. elegans. Uracil auxotroph. E. coli B. Biosafety Level 1</td>
</tr>
<tr>
<td>HSN GCaMP5+mCherry worm strain</td>
<td>Caenorhabditis Genetic Center</td>
<td>LX2004</td>
<td>Integrated transgene using nlp-3 promoter to drive GCaMP5 and mCherry expression in HSN. Full genotype: vs1s183 [nlp-3:::GCaMP5::nlp-3 3'UTR + nlp-3:::mCherry::nlp-3 3'UTR + lin-15(+)], lite-1(ce314), lin-15(n765ts) X</td>
</tr>
<tr>
<td><em>lite-1(ce314), lin-15(n765ts)</em> mutant strain for transgene preparation</td>
<td>author</td>
<td>LX1832</td>
<td>Strain for recovery of high-copy transgenes after microinjection with pl15EK lin-15(n765ts) rescue plasmid. Also bears the linked <em>lite-1(ce314)</em> mutation which reduces blue-light sensitivity. Available from author by request</td>
</tr>
<tr>
<td>pL15EK <em>lin-15a/b</em> genomic rescue plasmid</td>
<td>author</td>
<td>pL15EK</td>
<td>Rescue plasmid for recovery of transgenic animals after injection into LX1832 <em>lite-1(ce314), lin-15(n765ts) X</em> strain. Available from author by request</td>
</tr>
<tr>
<td>pKMC299 plasmid</td>
<td>author</td>
<td>pKMC299</td>
<td>Plasmid for expression of mCherry in the HSNs from the nlp-3 promoter. Has nlp-3 3’ untranslated region</td>
</tr>
<tr>
<td>pKMC300 plasmid</td>
<td>author</td>
<td>pKMC300</td>
<td>Plasmid for expression of GCaMP5 in the HSNs from the nlp-3 promoter. Has nlp-3 3’ untranslated region</td>
</tr>
<tr>
<td>Potassium Phosphate Monobasic</td>
<td>Sigma</td>
<td>P8281</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Potassium Phosphate Dibasic</td>
<td>Sigma</td>
<td>P5655</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Magnesium Sulfate Heptahydrate</td>
<td>Amresco</td>
<td>0662</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Calcium Chloride Dihydrate</td>
<td>Alfa Aesar</td>
<td>12312</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Peptone</td>
<td>Becton Dickinson</td>
<td>211820</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Sodium Chloride</td>
<td>Amresco</td>
<td>0241</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Cholesterol</td>
<td>Alfa Aesar</td>
<td>A11470</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>Item</td>
<td>Supplier</td>
<td>Model/Part Number</td>
<td>Notes</td>
</tr>
<tr>
<td>----------------------------------------------------------------------</td>
<td>---------------</td>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Agar, Bacteriological Type A, Ultrapure</td>
<td>Affymetrix</td>
<td>10906</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>60 mm Petri dishes</td>
<td>VWR</td>
<td>25384-164</td>
<td>For preparation of NGM plates</td>
</tr>
<tr>
<td>24 x 60 mm micro cover glasses, #1.5</td>
<td>VWR</td>
<td>48393-251</td>
<td>Cover glass through which worms are imaged</td>
</tr>
<tr>
<td>22 x 22 mm micro cover glasses, #1</td>
<td>VWR</td>
<td>48366-067</td>
<td>Cover glass that covers the top of the agar chunk</td>
</tr>
<tr>
<td>Stereomicroscope with transmitted light base</td>
<td>Leica</td>
<td>M50</td>
<td>Dissecting microscope for worm strain maintenance, staging, and mounting</td>
</tr>
<tr>
<td>Platinum iridium wire, (80:20), 0.2mm</td>
<td>ALFA AESAR</td>
<td>AA39526-BW</td>
<td>For worm transfer</td>
</tr>
<tr>
<td>Calcium imaging microscope</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-vibration air table</td>
<td>TMC</td>
<td>63-544</td>
<td>Micro-g’ Lab Table 30” x 48” anti-vibration table with 4” CleanTop M6 on 25mm top</td>
</tr>
<tr>
<td>Inverted compound microscope</td>
<td>Zeiss</td>
<td>431007-9902-000</td>
<td>Axio Observer.21 inverted microscope</td>
</tr>
<tr>
<td>Sideport L80/R100 (3 position)</td>
<td>Zeiss</td>
<td>425165-0000-000</td>
<td>To divert 20% of output to brightfield (CMOS) camera, 80% to fluorescence (sCMOS) camera</td>
</tr>
<tr>
<td>Tilt Back Illumination Carrier</td>
<td>Zeiss</td>
<td>423920-0000-000</td>
<td>For infrared/behavior imaging</td>
</tr>
<tr>
<td>Lamphousing 12V/100W w/ Collector</td>
<td>Zeiss</td>
<td>423000-9901-000</td>
<td>For infrared/behavior imaging</td>
</tr>
<tr>
<td>Halogen lamp 12V/100W</td>
<td>Zeiss</td>
<td>380059-1660-000</td>
<td>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</td>
</tr>
<tr>
<td>32 mm Infrared bandpass filter (750-790 nm) for Halogen lamp</td>
<td>Zeiss</td>
<td>447958-9000-000</td>
<td>BP 750-790; DMR 32mm, for infrared illumination for brightfield and behavior</td>
</tr>
<tr>
<td>6-filter Condenser Turret (LD 0.55 H/DIC/Ph), Motorized</td>
<td>Zeiss</td>
<td>424244-0000-000</td>
<td>For infrared/behavior imaging</td>
</tr>
<tr>
<td>Condenser &amp; Shutter</td>
<td>Zeiss</td>
<td>423921-0000-000</td>
<td>For infrared/behavior imaging</td>
</tr>
<tr>
<td>Binocular eyepiece with phototube for infrared CMOS camera</td>
<td>Zeiss</td>
<td>425536-0000-000</td>
<td>For infrared/behavior imaging</td>
</tr>
<tr>
<td>Eyepiece 10x, 23mm</td>
<td>Zeiss</td>
<td>444036-9000-000</td>
<td>For worm localization on the agar chunk</td>
</tr>
<tr>
<td>C-Mount Adapter 2/3° 0.63x demagnifier</td>
<td>Zeiss</td>
<td>426113-0000-000</td>
<td>Mount for infrared CMOS camera</td>
</tr>
<tr>
<td>CMOS camera for infrared brightfield and behavior (1&quot; sensor)</td>
<td>FLIR (formerly Point Grey Research)</td>
<td>GS3-U3-41C6NIR-C</td>
<td>Camera for brightfield imaging</td>
</tr>
<tr>
<td>USB 3.0 Host Controller Card</td>
<td>FLIR (formerly Point Grey Research)</td>
<td>ACC-01-1202</td>
<td>Fresco FL1100, 4 Ports</td>
</tr>
<tr>
<td>8 pins, 1m GPIO Cable, Hirose HR25 Circular Connector</td>
<td>FLIR (formerly Point Grey Research)</td>
<td>ACC-01-3000</td>
<td>Cable for TTL triggering. The green wire connects to GPIO3 / Pin 4 and the brown wire connects to Ground / Pin 5</td>
</tr>
<tr>
<td>Plan-Apochromat 20x/0.8 WD=0.55 M27</td>
<td>Zeiss</td>
<td>420650-9901-000</td>
<td>Best combination of magnification, numerical aperture, and working distance</td>
</tr>
<tr>
<td>6-cube Reflector Turret, Motorized</td>
<td>Zeiss</td>
<td>424947-0000-000</td>
<td>For fluorescence imaging</td>
</tr>
<tr>
<td>Fluorescence Light Train, Motorized</td>
<td>Zeiss</td>
<td>423607-0000-000</td>
<td>For fluorescence imaging</td>
</tr>
<tr>
<td>Fluorescence Shutter</td>
<td>Zeiss</td>
<td>423625-0000-000</td>
<td>For fluorescence imaging</td>
</tr>
<tr>
<td>Component</td>
<td>Manufacturer</td>
<td>Model</td>
<td>Notes</td>
</tr>
<tr>
<td>------------------------------------------</td>
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<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>GFP and mCherry dual excitation and emission filter cube (for microscope)</td>
<td>Zeiss</td>
<td>489062-9901-000</td>
<td>FL Filter Set 62 HE BFP+GFP +HcRed for fluorescence imaging</td>
</tr>
<tr>
<td>LED illumination system</td>
<td>Zeiss</td>
<td>423052-9501-000</td>
<td>Triggerable Colibri.2 LED system for fluorescent illumination</td>
</tr>
<tr>
<td>GFP LED module (470 nm)</td>
<td>Zeiss</td>
<td>423052-9052-000</td>
<td>Colibri.2 LED for GFP fluorescence excitation</td>
</tr>
<tr>
<td>mCherry LED module (590 nm)</td>
<td>Zeiss</td>
<td>423052-9082-000</td>
<td>Colibri.2 LED for mCherry fluorescence excitation</td>
</tr>
<tr>
<td>Iris stop slider for incident-light equipment</td>
<td>Zeiss</td>
<td>000000-1062-360</td>
<td>Field aperture iris to limit LED illumination to the camera field of view</td>
</tr>
<tr>
<td>C-Mount Adapter 1&quot; 1.0x</td>
<td>Zeiss</td>
<td>426114-0000-000</td>
<td>Adapter for image-splitter and sCMOS fluorescence camera</td>
</tr>
<tr>
<td>Image splitter</td>
<td>Hamamatsu</td>
<td>A12801-01</td>
<td>Gemini W-View, other image splitters may be used, but they may not be optimized for the large sensor size of the sCMOS cameras</td>
</tr>
<tr>
<td>GFP / mCherry dichroic mirror (image splitter)</td>
<td>Semrock</td>
<td>DI02-R594-25x36</td>
<td>Splitting GCaMP5 from mCherry and infrared signals</td>
</tr>
<tr>
<td>GFP emission filter (image splitter)</td>
<td>Semrock</td>
<td>FF01-525/30-25</td>
<td>Capturing GCaMP5 fluorescence</td>
</tr>
<tr>
<td>mCherry/ emission filter (image splitter)</td>
<td>Semrock</td>
<td>FF01-647/57-25</td>
<td>This filter is necessary to exclude the infrared light used for brightfield imaging</td>
</tr>
<tr>
<td>sCMOS camera for fluorescence (1&quot; sensor)</td>
<td>Hamamatsu</td>
<td>A12802-01 / C11440-22CU</td>
<td>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</td>
</tr>
<tr>
<td>Motorized XY Stage</td>
<td>Märzhäuser</td>
<td>SCAN IM 130 x 100</td>
<td>Stage movement; the XY resolution of this stage is 0.2µm per step</td>
</tr>
<tr>
<td>XY Stage controller with joystick</td>
<td>LUDL</td>
<td>MAC6000, XY joystick</td>
<td>Manual tracking of worms. MAC6000 controller should be connected to the PC through the serial (RS-232) port configured to 115200 baud</td>
</tr>
<tr>
<td>Digital Acquisition board (DAQ)</td>
<td>Arduino</td>
<td>Uno</td>
<td>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</td>
</tr>
<tr>
<td>BNC Male to BNC Male Cable - 6 ft</td>
<td>Hosa Technology</td>
<td>HOBB6</td>
<td>BNC connectors for TTL triggering</td>
</tr>
<tr>
<td>Gold-Plated BNC Male to SMA male coaxial cable (8.8&quot;)</td>
<td>ucell</td>
<td>608641773651</td>
<td>To connect the fluorescence camera trigger outputs</td>
</tr>
<tr>
<td>BNC turn head adapter</td>
<td>Hantek</td>
<td>RRBNCTH21</td>
<td>BNC to Banana Plug Adapter (4mm)</td>
</tr>
<tr>
<td>BNC female to female connector</td>
<td>Diageng</td>
<td>20130530009</td>
<td>Female to female BNC adapter to connect the BNC output from the camera to the Banana Plug</td>
</tr>
<tr>
<td>Solderless flexible breadboard jumper wires</td>
<td>Z&amp;T</td>
<td>GK1212827</td>
<td>To connect the BNC trigger outputs to the Arduino DAQ. Male to male.</td>
</tr>
<tr>
<td>High performance workstation</td>
<td>HP</td>
<td>Z820</td>
<td>Windows 7, 64GB RAM, Dual Xeon processor, solid state</td>
</tr>
<tr>
<td>Component</td>
<td>Vendor</td>
<td>Model/Version</td>
<td>Description</td>
</tr>
<tr>
<td>---------------------------------------------------------------------------</td>
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<td>-----------------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
</tr>
<tr>
<td>C: drive, serial (RS-232) port, multiple PCIe3 slots for ethernet connectivity, USB 3.0 cards, and additional solid state drives</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M.2 Solid state drive</td>
<td>Samsung</td>
<td>MZ-V5P512BW</td>
<td>High-speed streaming and analysis of image data</td>
</tr>
<tr>
<td>M.2 Solid state drive adapter for workstations</td>
<td>Lycom</td>
<td>DT-120</td>
<td>M.2 to PCIe 3.0 4-lane adapter</td>
</tr>
<tr>
<td>Network attached storage</td>
<td>Synology</td>
<td>DS-2415+</td>
<td>Imaging data storage and analysis</td>
</tr>
<tr>
<td>Hard disk drives</td>
<td>Western Digital</td>
<td>WD80EFZX</td>
<td>RED 8 TB, 5400 RPM Class SATA 6 Gb/s 128MB Cache 3.5 inch. Storage of imaging data (10 drives + 2 drive redundancy)</td>
</tr>
<tr>
<td><strong>Software</strong></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Fluorescence Acquisition</td>
<td>Hamamatsu</td>
<td>HCImage DIA</td>
<td>Recording of two channel (GCaMP5 and mCherry) fluorescence image sequences at 20 fps</td>
</tr>
<tr>
<td>Brightfield Acquisition</td>
<td>FLIR (formerly Point Grey Research)</td>
<td>Flycapture</td>
<td>Recording of brightfield JPEG image sequences</td>
</tr>
<tr>
<td>Stage Serial Port Reader</td>
<td>Bonsai</td>
<td><a href="https://bitbucket.org/horizongir/bonsai">https://bitbucket.org/horizongir/bonsai</a></td>
<td>Facilitates tracking of worms during behavior</td>
</tr>
<tr>
<td>LED controller software</td>
<td>Zeiss</td>
<td>Micro Toolbox Test 2011</td>
<td>To set up the intensity and trigger inputs for the different LEDs in the Colibri.2 unit</td>
</tr>
<tr>
<td>ImageJ</td>
<td>NIH</td>
<td><a href="https://imagej.net/Fiji/Downloads">https://imagej.net/Fiji/Downloads</a></td>
<td>Simple review of image sequences and formatting changes for import into Ratiometric Quantitation software</td>
</tr>
<tr>
<td>Excel</td>
<td>Microsoft</td>
<td>2002984-001-000001</td>
<td>For generating subsets of comma-separated value data from Volocity for MATLAB analysis</td>
</tr>
<tr>
<td>Peak Finding</td>
<td>MATLAB</td>
<td>R2017a</td>
<td>Script used for Ratio peak feature calculations</td>
</tr>
<tr>
<td>Ratiometric Quantitation</td>
<td>Perkin Elmer</td>
<td>Volocity 6.3</td>
<td>Facilitates calculation of ratiometric image channels, image segmentation for object finding, and ratio measurement of found objects</td>
</tr>
<tr>
<td><strong>Scripts</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>XY-stage-final.bonsai</td>
<td>Bonsai</td>
<td>TTL-triggered DAQ and stage position serial port reader</td>
<td>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.</td>
</tr>
</tbody>
</table>