Long-term culture and electrophysiological characterization of human brain organoids

Paola Arlotta (paola_arlotta@harvard.edu)
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
Giorgia Quadrato^*^
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA
John Lawrence Sherwood^*^
Department of Stem Cell and Regenerative Biology, Harvard University, Cambridge, MA 02138, USA

Method Article

Keywords: Human brain organoids, long-term culture, single cell enzymatic dissociation, extracellular recordings

Posted Date: April 27th, 2017

DOI: https://doi.org/10.1038/protex.2017.049

Abstract

Here we describe a modified version of the culturing protocol first described by Lancaster et al.,[1] that supports prolonged development of self-organizing 3D human brain organoids for over 9 months. Prolonged culture allows maturation of organoids that acquire a high degree of cellular diversity and neuronal maturation, including formation of dendritic spines and spontaneously active neuronal networks. In this model, photoreceptor-like cells mature substantially and become responsive to non-invasive, light-based sensory stimulation that appears capable of affecting neuronal activity. In Quadrato et al.[2] we have used large-scale, single cell RNA sequencing to provide a first measure of the diversity of cells generated across organoids grown in different bioreactors. Here, we describe in detail the protocol for dissociation and cell isolation in brain organoids for large-scale single-cell droplet sequencing[3]. We also describe an electrophysiological rig specialized for performing extracellular recordings in whole organoids with controlled light stimulation, and methods for recording spontaneously active neuronal networks in organoids.

Reagents

Long-term culture of human brain organoids • Geltrex, LDEV-Free, hESC-Qualified, Reduced Growth Factor Basement Membrane Matrix \(ThermoFisher Scientific, #A1413301) • DMEM/F-12 medium \(ThermoFisher Scientific, #11330-057) • mTeSR \(Stemcell Technologies, #05850) • Gentle Cell Dissociation Reagent \(Stemcell Technologies, #7174) • StemPro Accutase Cell Dissociation Reagent \(ThermoFisher Scientific, #A1110501) • ROCK inhibitor Y-27632 \(EMD Millipore, #SCM075) • Trypan blue solution, 0.4% \(ThermoFisher Scientific, #15250-061) • KnockOut Serum Replacement \(ThermoFisher Scientific, #10828-028) • Hyclone Fetal Bovine Serum \(ThermoFisher Scientific, #SH30070.03) • GlutaMAX Supplement \(ThermoFisher Scientific, #35050-061) • MEM Non-Essential Amino Acids Solution \(Invitrogen, #11140-050) • 2-mercaptoethanol \(ThermoFisher Scientific, #21985023) • Penicillin Streptomycin Solution 100X \(VWR, #30002CI) • Recombinant human FGF-basic \(Peprotech, #100-18B) • N-2 supplement \(100X) \(Life Technologies, #17502048) • Heparin \(Sigma, #21103-049) • Neurobasal Medium \(Invitrogen, #21103-049) • Insulin \(Sigma, #I9278) • B27 without Vitamin A \(Invitrogen, #12587010) • B27 \(Invitrogen, #17504044) • Matrigel matrix gel \(#356234, Corning) • Parafilm \(Bemis, #52858-076) • Papain vials \(Worthington, #LK003176) • DNase vials \(Worthington, #LK003170) • Inhibitor BSA vial \(Worthington, #LK003182) • Earle's Buffer Vial \(Worthington, #LK003188) • Recombinant Human BDNF \(Peprotech, #450-02B) _Embryoid body medium - **EBM** \(quantity per ~500 mL medium)_ DMEM-F12 \(400 mL) KOSR \(100 mL) Fetal Bovine Serum \(15 mL) GlutaMAX \(5 mL) MEM-NEAA \(5 mL) 2-mercaptoethanol \(3.5 µL) Penicillin-Streptomycin \(5 mL) _Intermediate induction medium - **IIM** \(quantity per ~500 mL medium)_ DMEM-F12 \(500 mL) KOSR \(30 mL) FBS \(4.5 mL) N2 supplement \(3.5 mL) GlutaMAX \(5 mL) MEM-NEAA \(5 mL) Heparin \(350 µg) Penicillin-Streptomycin \(5 mL) _Neural induction medium - **NIM** \(quantity per ~500 mL medium)_ DMEM-F12 \(480 mL) N2 supplement \(5 mL) GlutaMAX \(5 mL) MEM-NEAA \(5 mL) Heparin \(500 µg) Penicillin-Streptomycin \(5 mL) _Cerebral differentiation medium - **CDM** \(quantity per

~500 mL medium)_ DMEM F-12 \(250 mL) Neurobasal medium \(250 mL) N2 supplement \(2.5 mL) GlutaMAX \(5 mL) MEM-NEAA \(2.5 mL) Insulin \(125 µL) 2-mercaptoethanol \(1.75 µL) Penicillin-Streptomycin \(5 mL) B27 with or without vitamin A \(5 mL) **Electrophysiological Recordings** • NaCl \(Sigma, #S7653-1KG) • D-Glucose \(Sigma, #49158-1KG-F) • $NaHCO_3$ \(Sigma, #S5761-1KG) • KCl \(Sigma, #60130-250G) • NaH_2PO_4 \(Sigma, #S8282-500G) • $MgCl_2$ \(Sigma, #M1028-100ML) • $CaCl_2$ \(Sigma, #21115-100ml) • L-Ascorbic acid \(Sigma, #A5960-100G) • Na-Pyruvate \(Sigma, #P5280-25G) • Carbogen, 95% O_2/5% CO_2 \(Airgas) _Artificial CSF - **aCSF** \(in mM)_ NaCl \(125.0 mM) D-Glucose \(10.0 mM) $NaHCO_3$ \(26.2 mM) KCl \(3.5 mM) NaH_2PO_4 \(1.3 mM) $MgCl_2$ \(1.0 mM) $CaCl_2$ \(1.2 mM) L-Ascorbic acid \(1.0 mM) Na-Pyruvate \(1.0 mM) Bubbled with 95% O_2/5% CO_2

Equipment

Long-term culture of human brain organoids • 100 mm tissue culture dishes \(Corning, #353003) • Cell scraper \(Corning, #3008) • Freezing container \(Sigma, #C1562) • 96-well plate, ultra-Low attachment, polystyrene, round bottom \(Corning, #CLS7007) • Reagent reservoir \(ThermoScientific, #95128095) • 24 well plate, Ultra-Low attachment, polystyrene, flat bottom \(Corning, #CLS3473) • 60 mm TC-Treated Cell Culture Dish \(Corning, #353002) • 125 mL spinner flasks \(Corning, #3152) • Razor blades \(VWR, #55411-050) • CO_2 incubator • Magnetic stirrer \(ChemGlass, #CLS-4100-09) • Digital rocker \(Chemglass Life Sciences, #CLS-4033-100) • Hemocytometer • Centrifuges • 15 mL Falcon Centrifuge Tubes, Polystyrene \(VWR, #21008-929) **Electrophysiological Recordings** _Isolation Table_ • CleanBench \(TMC, #63-7590E) • Full Perimeter enclosure for CleanBench \(TMC, #81-7590) • Faraday cage \(TMC, #81-333-03) • Grounding Block \(McMaster-Carr, #2450K2) • Dark box enclosure \(ThorLabs, built from 25 mm square construction rails and accessories) _Silicon Probe, micro-manipulation, & data acquisition_ • Mounted silicon probe \(for assembly see Figure 1) 1. Dovetail Probe Holder \(Scientifica, #PH-1000) 2. Silicon probe mount \(a) and post \(b) 3. SmartLink32 chronic headstage \(Neuronexus) 4. Silicon probe breakout board, AB3: AF-64-FFC/2x32 \(Jorg Scholvin, Boyden Lab, MIT) 5. Premo-Flex FFC Jumper \(Molex, #15015-0433) 6. 64 channel high density silicon probe[4] \(Jorg Scholvin, Boyden Lab, MIT; Justin P. Kinney, LeafLabs) 7. Steel round head #4-40 thread screw, length 0.25" \(McMaster-Carr, #96880A216) 8. Nylon 6/6 Male-Female threaded standoff, #4-40 thread, length ¼" \(McMaster-Carr, #92745A320) 9. Brass flanged knurled-head thumb nut, #4-40 thread, height ¼" \(McMaster-Carr, #92741A100) 10. SmartLink cables \(Neuronexus) 11. Miniature cable ties, length 4" \(McMaster-Carr, #71295K62) 12. Miniature cable ties, length 4" \(McMaster-Carr, #71295K62) • PatchStar Micromanipulator \(Scientifica, #PS-7000) • PatchPad Touch \(Scientifica, #PT) • PatchStar steep bracket \(Scientifica, #PS-7550) • SmartBox \(Neuronexus) • Computer running SmartBox software version 1.0 \(Neuronexus) _Organoid recording chamber_ • Custom recording chamber \(for assembly see Figure 2) 1. Kwik-Sil, low viscosity silicon adhesive \(World Precision Instruments, #KWIK-SIL) 2. Kwik-Sil mixing tip \(World Precision Instruments, #600022) 3. Glass microscope slides, 3" x 2" \(Ted Pella, #260439) 4. Tissue culture dish, 60 x 15 mm \(Corning, #353002) 5. Tubing, Teflon® PTFE, 1" OD x 1/2" ID \(McMaster-Carr, #8547K45) 6. Anchor, Floating microelectrode array \(MicroProbes for Life Science, #FMA-PI-16-\[1.0-1.2]) 7. Heater elements \(Warner Instruments, #64-0274) a. Power film resistor b.

Mounting screw c. Heat transfer pads 8. Heating stage \(Figure 3) 9. Heating stage clamp \(Figure 4); and #4-40 thread socket head screw for mounting \(b) 10. Heating stage mounting screw, flat head, #4-40 thread 11. Heating stage thermal isolation block \(Figure 5) 12. Magnetic clamp for wires \(Warner Instruments, #64-0360) 13. Stainless steel discs \(Ø 0.5") • TC-344C dual automatic temperature controller \(Warner Instruments, #64-2401) • Cable assembly for heater controllers, CC-28 \(Warner Instruments, #64-0106) • Carbogen manifold \(for assembly see Figure 6) 1. 1/4"-20 low-profile channel screws \(ThorLabs, #SH25LP38) 2. Ø1/2" Post Holder, length 1" \(ThorLabs, #PH1) 3. Ø1/2" Post Holder, length 2" \(ThorLabs, #PH2) 4. Ø1/2" Optical Post, SS, 8-32 Setscrew, 1/4"-20 Tap, L = 0.75" \(ThorLabs, #TR075) 5. Ø1/2" Optical Post, SS, 8-32 Setscrew, 1/4"-20 Tap, L = 2" \(ThorLabs, #TR2) 6. Miniature V-Clamp, 0.42" Long, 8-32 Tapped Hole \(ThorLabs, #VH1) 7. 25G x 7/8" BD PrecisionGlide needle \(BD Worldwide, #305124) 8. Luer connector \(Harvard Apparatus, #72-1443) 9. Tygon Tubing, ID 0.02", OD 0.06" \(Cole-Parmer, #06419-01) 10. Needle Valves \(Air Logic, #Nylon F-2822-40-B80-SS-KSS) 11. Tygon Tubing, ID 1/16", OD 3/16" \(Harvard Apparatus, #72-1016) 12. Mounting Base \(ThorLabs, #BA2) 13. 1/4"-20 Stainless Steel Cap Screw, 3/8" Long, Pack of 25 \(ThorLabs, #SH25S038) 14. 1/4" Washer, M6 Compatible, Stainless Steel, Pack of 100 \(ThorLabs, #W25S050) _Organoid visualization and manipulation_ • Night vision goggles, night optics D-2MV \(http://www.bhphotovideo.com, #NID2MV) • Near IR digital microscope, 940 nm, 10X-220X \(Dino-Lite, # Edge AM4115-FJT) • IR digital microsope mount 1. Dino-Lite camera mount \(microscope.com, #MS35B) 2. Custom mounting post 3. Studded pedestal base adapter \(ThorLabs, #BE1) 4. Clamping fork \(ThorLabs, #CF125) • Tissue chute \(for assembly see Figure 7) 1. Three-dimensional coarse manipulator \(Narishige, #U-3C) 2. One-dimensional coarse manipulator with a pivot and tilt mechanism \(Narishige, #U-12C) 3. Adapter with external M4 thread and external M3 thread \(ThorLabs, #AP4M3M) 4. Ø12.7 mm Optical Post, SS, M4 Setscrew, M6 Tap, L = 20 mm \(ThorLabs, #TR20/M) 5. Ø1/2" Post Holder, length 1" \(ThorLabs, #PH1) 6. Studded pedestal base adapter \(ThorLabs, #BE1) 7. Clamping fork \(ThorLabs, #CF125) 8. Custom eppendorf mount a. Organoid Chute Mount \(Figure 8) b. Organoid Chute Post \(Figure 9) 9. Eppendorf microcentrifuge tube, 1.5 mL _Light source, driver and calibration_ • LED light source \(for assembly see Figure 10): 1. 530 nm mounted LED \(ThorLabs, #M530L3) 2. Optional second LED light source \(ThorLabs) 3. SM1 Lens Tube, 0.3" Thread Depth \(ThorLabs, #SM1L03) 4. Collimation a. Ø1" SM1 lens tube, 1/2" long external threads \(ThorLabs, #SM1V05) b. Aspheric condenser lens with diffuser \(ThorLabs, #ACL2520U-DG6-A) 5. Fast-Change Lens Tube Filter Holder \(ThorLabs, #SM1QP) a. Slotted Lens Tube \(ThorLabs, #SM1QB) b. Filter Carriage \(ThorLabs, #SM1QT) c. 530/11 nm BrightLine® single-band bandpass filter \(Semrock, #FF01-530/11-25) 6. SM1 coupler \(Thorlabs, #SM1T2) 7. 4-Way mounting 30 mm cage cube \(ThorLabs, #C4W) 8. Blank cover plate with rubber o-ring for C4W/C6W \(ThorLabs, #B1C) 9. Externally SM1-threaded end cap \(ThorLabs, #SM1CP2) 10. SM1 Lens Tube, 0.30" \(ThorLabs, #SM1L03) 11. SM1 Lens Tube, 3.00" \(ThorLabs, #SM1L30) 12. Dichroic Filter a. 30 mm Cage Cube with Dichroic Filter Mount \(ThorLabs, #CM1-DCH) b. 25 mm x 36 mm Longpass Dichroic Mirror, 650 nm Cutoff \(ThorLabs, #DMLP650R) 13. Kinematic cage cube platform \(ThorLabs, #B4C) 14. 30 mm Cage-Compatible dichroic filter mount \(ThorLabs, #FFM1) 15. Longpass dichroic mirror, 25 x 35mm, 490 nm cutoff \(ThorLabs, #DMLP490R) 16. Ø1/2" Optical Post, SS, 8-32 Setscrew, 1/4"-20 Tap, L = 4" \(ThorLabs, #TR4) 17. Slip-On Post Collar for Ø1/2" Posts \(ThorLabs, #R2) 18. Ø1/2" Post Holder,

length = 4" \(ThorLabs, #PH4) 19. Mounting Base, 2" x 3" x 3/8" \(ThorLabs, #BA2) 20. 1/4"-20 Stainless Steel Cap Screw, 3/8" Long, Pack of 25 \(ThorLabs, #SH25S038) 21. 1/4" Washer, M6 Compatible, Stainless Steel, Pack of 100 \(ThorLabs, #W25S050) • LED Driver and TTL pulse generation: 1. T-Cube LED Driver, 1200 mA Max Drive Current \(ThorLabs, #LEDD1B) 2. 15 V Power Supply Unit for a Single T-Cube \(ThorLabs, #TPS001) 3. Matlab R2014, 32-bit \(Mathworks) 4. Matlab Data Acquisition Toolbox \(Mathworks) 5. NI PCIe-6323, X Series Data Acquisition board \(National Instruments) 6. NI BNC-2090A, Sheilded rack-mount BNC connector block \(National Instruments) 7. BNC cables \(ThorLabs, #2249-C-XX) 8. BNC T adapter \(ThorLabs, #T3285) • LED Power Calibration: 1. Digital Power & Energy Meter \(ThorLabs, #PM100D) 2. Slim Si Sensor, 400-1100 nm, 500 pW - 500 mW \(ThorLabs, #S130C) • Additional items: 1. Laser Safety Glasses \(ThorLabs, #LG1B) 2. Adjustable spanner wrench \(ThorLabs, #SPW801) _Data analysis_ • Spike detection and clustering software \(Klusta[5])

Procedure

Long-term culture of human brain organoids _Thawing human pluripotent stem cells \(hPSCs)_ Maintain hPSCs in mTeSR medium in feeder-free 10 cm tissue culture dishes coated with Geltrex membrane. 1. Coat a 100 mm tissue culture dish using a 1% Geltrex solution in DMEM/F-12; hold prepared Geltrex on ice until transfer to the dish. 2. Place coated dish in a 37°C incubator for at least 30 minutes. 3. Thaw cells by placing cryovial in 37°C water bath \(for preparation of the cryovial refer to section “freezing hPSCs”). 4. Immediately transfer contents to a 15 mL conical tube containing 4 mL of pre-warmed mTeSR to dilute freezing medium. Minimize pipetting of thawed cells. 5. Centrifuge at 200 g for 4 min. Aspirate the supernatant. Gently resuspend the pellet in mTeSR with ROCK inhibitor \(12.5 μM). 6. Transfer to coated 100 mm tissue culture dishes and culture in a 37°C incubator. 7. Replenish the medium daily by completely aspirating the mTeSR and replacing it with 10 mL of fresh mTesR. _Passaging hPSCs_ 1. Passage PSCs upon 70% confluency. 2. Prepare a fresh 10 cm Geltrex-coated dish as described above. 3. Aspirate mTeSR from the culture dish and add 5 mL of Gentle Cell Dissociation Reagent \(for a 10 cm dish). 4. Incubate at room temperature for 5-6 minutes. Under the microscope, colonies should appear to separate into pieces but not detach from the plate. 5. Aspirate reagent and add 5 mL mTeSR. 6. Use a cell lifter to gently scrape off colonies, using a circular motion. 7. Slowly triturate colonies four times using a 5 mL pipet, trying not to break up colonies. Some degree of colony splitting is unavoidable, and beneficial to the protocol. However, creating single-cell suspension should be prevented. 8. Pipette off 2.5 mL of cell aggregates, transfer to a 15 mL conical tube, and make a 1:4 dilution of aggregate suspension with mTeSR. 8. Aspirate excess Geltrex solution from the 10 cm coated dish. 9. Add the 10 mL of the diluted cell aggregate suspension onto the new dish. _Freezing hPSCs_ Freeze cells at optimum density \(70% confluency). Prepare an appropriate number of cryovials; plan to put the equivalent of one 10 cm plate into each cryovial. Label each cryovial with the cell line, passage number, date, operator’s initials, and culture size per vial. Place vials on ice and pre-chill the freezing container at 4°C. 1. Dissociate colonies as in passaging protocol \(steps 1- 7) and collect into a 15 mL conical tube. 2. Centrifuge at 200 g for 4 minutes. 3. Aspirate the supernatant and resuspend the pellet in 0.5 mL of ice-cold freezing media \(90% mTESR / 10% DMSO by volume) per cryovial. 4. Immediately pipet cell

suspension into cryovials. Immediately transfer cryovials to the freezing container and place it in a -80°C freezer. 5. After 24 hours, transfer cryovials to liquid nitrogen for long-term storage. _Generation of embryoid bodies_ 1. Grow hPSC colonies in a 100 mm dish until 70% confluent. Colonies should have no evidence of differentiation and should display optimal features. Clusters of differentiated cells should be designated with a marker on the plate while inspecting the dish under a tissue culture microscope and then aspirated off before proceeding. 1. Aspirate mTeSR medium and wash plate with 3 mL of PBS. 2. Aspirate off PBS, then add 5 mL of Accutase and place the dish back in the incubator for 10 minutes. 3. Cells should detach from the plate. Use a pipettor fitted with a P1000 pipette tip to triturate and wash the plate to detach the remaining cells. 4. Add cell suspension to a 15 mL conical tube with 5 mL pre-warmed mTESR. 5. Triturate up and down with a P1000 pipette a few times to ensure a single-cell suspension. 6. Use a hemocytometer to count a sample of the hPSCs. 7. Meanwhile, centrifuge cells at 200 g for 3 minutes. Aspirate and resuspend cells in 5 mL of embryoid body medium \(EBM). 8. Add a volume of the single-cell suspension to a tube of 15 mL of EBM so as to create a suspension with 2,500 cells per 150 µL of EBM. 9. Pipet hPSC suspension into a reservoir. 10. Using a multichannel pipette, add 150 µL of cell suspension into each well of a low-attachment, round-bottom 96-well plate. 11. Replenish EB medium every 48 hours by removing 75 µL of EB medium from each well and adding 150 µL of fresh EBM. Include Rock inhibitor \(50 µM) and bFGF \(4 ng/mL) in the EBM medium for the first 4 days. **Tip:** Before removing the 75 µL of EB medium, gently agitate the medium to remove debris accumulated around the embryoid body \(typically due to cell death). To agitate, use a multichannel pipette set at 30 µL and gently pipette up and down. To prevent the debris from resettling at the bottom of the wells, resuspend and change media for the first six columns of the 96-well plate before proceeding to the next six. _Neural Induction_ 1. The development of the organoids is counted from the day of EB seeding \(Day 0). On Day 6, transfer EBs from low-attachment, round bottom 96-well plates to intermediate induction medium \(IIM) in low-attachment, flat-bottom 24-well plates. a. Fill each well of the 24-well plates with 500 µL of IIM. b. To avoid disrupting the EBs, use a cut 200 µL pipette tip to individually transfer each EB to a well of the 24-well plate. 2. On Day 8, add an additional 500 µl of Neural Induction Medium \(NIM) to each well. _Embedding in Matrigel_ 1. On Day 10, thaw Matrigel at 4°C and keep on ice for the rest of the procedure. 2. Use Parafilm to make a grid of indents on which to shape the Matrigel droplets. a. Cut a piece of Parafilm using sterile scissors. Spray Parafilm with 70% ethanol before placing it in the tissue culture hood. b. Stretch the piece of Parafilm over an empty tip tray for 200 µL sized tips. c. Use a gloved finger to push the Parafilm partway into the holes in the tip tray, creating dimples in the Parafilm sheet. Make sure each dimple is a smooth hemisphere. d. Detach the Parafilm sheet from the empty tip tray and use sterile scissors to cut it into 4x4 dimple sheets. Place one 4x4 dimple Parafilm sheet in each 60 mm tissue culture dish. 3. Use a cut-tip 200 µL pipette set at 35 µL to individually transfer each tissue from the low-attachment, 24-well plate to a Parafilm dimple. Use an uncut 200 µL pipette to aspirate off most of the medium used in transferring the tissues, leaving the tissue surrounded by minimal medium. 4. Add 35 µL of thawed Matrigel to each dimple. Immediately after adding the Matrigel droplet, gently reposition each tissue in the middle of the Matrigel droplet using a pipette tip. Complete embedding as soon as possible after aspirating off excess medium in step 3 to avoid drying out the tissue. 5. Place the dish back in 37°C incubator for 20 minutes to allow the Matrigel droplets to solidify. Then rinse each Parafilm

sheet holding the tissues with 5 mL CDM without vitamin A. If necessary, use sterile forceps to dislodge the Matrigel droplets from the Parafilm sheet. Remove the Parafilm sheet and make sure each tissue is completely enveloped in a Matrigel droplet. Otherwise, repeat steps 3-5 for any organoids that failed to be completely embedded. 6. Place dish with organoids back in incubator and continue culturing. 7. On Day 12, replace the CDM in each dish by aspirating off the medium and adding 5 mL of fresh CDM without vitamin A. _Transferring to spinner flasks and long-term culture_ 1. On Day 14, use a 50 mL pipette to gently transfer the tissues to a 125 mL spinner flask containing 80 mL of CDM with vitamin A. Transfer up to 40 organoids per spinner flask. Maintain cultures on a magnetic stirrer set at a speed of 35 rpm. 2. Replenish the medium once every six days by completely aspirating the medium and adding fresh CDM with vitamin A. 3. On Day 30, add freshly-reconstituted brain-derived neurotrophic factor \(BDNF) to the flask at a concentration of 14 ng/mL. From this point on, use media with BDNF to change the media every 6 days. **Tip:** When changing media, remove the cap from one of the arms of the spinner flasks to reach an aspirating pipette into the flask; avoid removing the main top cap of the spinner flask as a preventative measure against contamination. **Tip:** Make sure to remove any debris that may accumulate over time around the paddle of the flask, using an aspirating pipette. Check the caps periodically and replace caps if necessary. Periodically replace the spinner flasks as needed. _Single-cell dissociation of brain organoids for droplet sequencing_ 1. Transfer one organoid from the spinning bioreactor into a 60 mm cell culture dish containing 5 mL of prewarmed Worthington papain solution \(PAP2) with 250 µL of Worthington DNase solution \(D2). 2. Cut the organoid into small pieces with a blade. 3. Place the 60 mm dish that contains the tissue onto a digital rocker in a primary cell culture incubator, and incubate with rocking speed set at 27 rpm for 60 minutes at 37°C. 4. Collect digested tissues into a 15 mL tube and add 5 mL of RT EBSS. 5. Triturate the mixture with 10 mL plastic pipette 20 times, by pipetting up and down. Allow any pieces of undissociated tissue remaining after trituration to settle to the bottom of the tube. 6. Carefully transfer the cloudy cell suspension to a 15 mL tube. Be careful to avoid including any pieces of undissociated tissue. 7. Add 2.7 mL of EBSS, 3 mL of reconstituted Worthington inhibitor solution \(OI.BSA), and 250 µl DNAse solution to the 15 mL tube with the tissue. Mix gently, avoiding bubbles. 8. Pellet cells by centrifuging at 300 g for 5 minutes at room temperature. 9. Resuspend cells in 1 mL of ice cold neurobasal medium, and filter through a 20 µm Nitex mesh. 10. Count cells using trypan blue. 11. Dilute cells in ice cold DPBS+ 0.2%BSA to a concentration of 100 cells/µL. 12. Place cells on ice until ready for droplet collection. Total time required for dissociation: 1 hr and 45 min. **Electrophysiological Recordings** _Building the electrophysiology rig_ 1. Assemble a dark box inside the Faraday cage. 2. Mount the silicon probe and wire up the SmartBox system \(Figure 1). **Tip:** Accurate probe maps will need to be constructed for data analysis. We recommend that the user makes a careful note of all connections between the probe and the SmartBox. Paying close attention to the position of the FFC jumpers, the orientation of the SmartLink32 headstages relative to the AB3 breakoutboard, and port assignments at the SmartBox. **Tip:** During setup, mark SmartLink cables with colored cable ties, at both ends. These will help to trace wires. **Tip:** Use cable ties to secure the SmartLink Cables to the silicon probe mounting post. This will reduce stress on the connection between the SmartLink32 headstages and the AB3 breakout board. 3. Assemble and position the recording chamber inside the darkbox \(for assembly refer to Figure 2). a. Detach the floating micro-electrode array

from its leads. b. Make some holes in the side of the PTFE teflon tube. These are required to join the inner and outer chambers \(the inner chamber holds the organoid; the outer chamber holds the AgCl ground/ref, the bath thermistor, the carbogen line, and receives drugs). c. Use Kwik-Sil to: i. Fix the tissue culture dish to the glass slide. ii. Mount the PTFE Teflon tube inside the culture dish. This will form inner chamber used to mount the organoid. iii. Fix the stainless steel disks to the heating stage isolator. iv. Mount the detached floating micro-electrode array inside the inner chamber. This will be used to anchor the organoid. d. Make 5 holes in the culture dish lid \(one large central hole, Ø 27 mm; four small radial holes, Ø 3 mm). Holes in the recording chamber lid are used to access the inner and outer chamber by key elements of the recording setup \(see Figure 2c). 4. Assemble and position carbogen manifold inside the darkbox \(for assembly refer to Figure 6). 5. Assemble the organoid chute \(see Figure 7). 6. Assemble and collimate the light source \(for assembly refer to Figure 10: the collimation procedure is described on the "ThorLabs":https://www.thorlabs.com/newgrouppage9.cfm?objectgroup_id=2692 website) 7. Position the light source over the recording chamber, and center on the organoid anchor \(Figure 11). 8. Calibrate LED power with power meter and sensor \(perform calibration in the dark). 9. Position the micro-manipulator and set approach angle for the silicon probes; the target is 3-4 mm above the center of the recording chambers anchor. Ensure that the approach angle does not obscure the light path \(to avoid costly accidents we advise using dummy silicon probes during the alignment process). 10. Position digital microscopes. 11. Microscope #1 is positioned to give a bird’s eye view of the organoid; it will be used during the placement of the organoid onto the anchor. Position microsope #1 above the longpass dichroic mirror \(item 12 in Figure 10). 12. Microscope #2 is positioned to view the silicon probe and organoid at high magnification; it will be used during the implantation of the silicon probe. Position microsope #2 close to the recording chamber, being careful not to obscure the light path or the approach angle of the silicon probe. _Setting up the DAQ_ 1. Wire up and ground the DAQ according to the user manual \(Neuronexus SmartBox). 2. Suggested amplifier settings: a. Sampling rate = 30 kHz b. DSP cutoff frequency = 1.00 Hz c. Lower bandwidth = 0.1 Hz d. Upper bandwidth = 7.5 kHz 3. Checklist: a. Confirm all analog channels are active. b. Confirm the digital channels, receiving TTL pulses, are active. **Tip:** On-line spike detection can be facilitated by applying an additional software filter to the displayed data \(for example, a 100 Hz high-pass filter). **Tip:** Set file save format appropriate for the analysis procedure. A flat binary file with no header is appropriate for analysis with Klusta software \(for SmartBox software, this format is provided by the *.dat file option). **Tip:** If TTL pulses are going to be used to drive an LED, send the signal to one of the DAQ digital inputs. _Mount the Organoid_ 1. Prepare aCSF \(see Reagents – Electrophysiology) and bubble with 95%O_2/5%CO_2. Warm aCSF to 30°C in a water bath. 2. Position the recording chamber lid. 3. Position the bath thermistor, the carbogen line, and the AgCl ground/ref into the outer chamber of the organoid bath. 4. Turn on the heating stage. 5. Add 15 mL of aCSF to the recording chamber, maintaining the recording bath at 30°C. **Tip:** In the described setup, the heating stage temperature is under homeothermic control, NOT the recording chamber. Therefore, the temperature of the heating stage will need to be set higher than the target temperature for the recording chamber. Make sure that you are monitoring the bath temperature. **Tip:** If pharmacological compounds will be added to the chamber during the experimental protocol, accurately record the total volume of aCSF dispensed into the bath during this and subsequent steps. 6. In the

culture room, transfer the organoid from the culture media into a tube with prewarmed aCSF \(bubbled with 95%O_2/5%CO_2). **Tip:** Exposure of the organoid to light should be avoided. 7. Move the organoid chute into position over the organoid anchor, and lower into position. **Tip:** IR digital microscope #1 can be used to visualize the chute and anchor. 8. Turn off the room lights, and put on the night vision goggles. 9. Use a pipette to gently drop the organoid down the chute. **Tip:** While raising the tissue chute using the manipulator, pipette a few drops of aCSF down the chute. This will help to secure the organoid onto its anchor. 10. Let the organoid acclimatize for 30 minutes at 30°C. 11. Over a further 30 minutes, slowly raise the temperature of the recording chamber to 36°C. _Implanting the Silicon Probe_ 1. Position the silicon probe over the recording chamber. 2. Connect the recording chamber AgCl ground/ref to the headstage. **Tip:** There are alternative connectors on the breakout-board; these are closer to the chamber and require a shorter jumper wire. 3. Seal the dark box and turn on the room lights. 4. Submerge the silicon probe into the aCSF. 5. Measure the probe impedance at 1000 Hz in aCSF. **Tip:** These values should be recorded before and after the experiment, and used to remove bad channels from analysis. 6. While viewing the organoid and silicon probe tip on IR digital microscope #2, position the probe tip over the organoid using the three orthogonal axes \(x, y, z) of the micro-manipulator. 7. Zero the axes distance counter on the micro-manipulator controller. 8. Implant the probe into the organoid. **Tip:** During implantation, set the micro-manipulator speed to slow. **Tip:** To avoid stress to the silicon probe shaft and organoid, the probe should be advanced along the axis of the silicon shaft. This can be done by using the 4^{th} virtual “approach” axis of the micro-manipulator. 9. While advancing the silicon probe, use the micro-manipulator controller to monitor the distance advanced along the approach axis. 10. Adjust the probe position until spikes are isolated. 11. Acquire spontaneous activity. 12. Trigger light stimulus by sending TTL pulses to the LED driver. _Analysis_ 1. Generate a probe map for the recording set up. 2. Identify the probe channels with either a high or low impedance. Remove these defective probe sites from the list of channels to be analyzed. 3. Process the raw data file\(s) using spike detection and clustering software designed for high density microelectrode arrays, Klusta[5].

Troubleshooting

Long-term culture of human brain organoids • We recommend pilot experiments to find the optimal number of starter cells for embryoid body formation, testing cell seeding densities between 2,000 and 3,000 cells per well. The optimal starter cell number may vary depending on the proliferation rates of different PSC lines. • For embryoid body formation, use only cells that do not display spontaneous differentiation. • Troubleshooting using different PSC lines may be necessary if the specific PSC line has not been used to generate brain organoids, as there are differences in efficiency of neuroectodermal differentiation across different PSC lines. • In order to reduce variability between organoids cultured long-term, screen organoids after 5 days in neural induction conditions before embedding in Matrigel. Select for embedding only organoids with visible formation of neuroepithelium. Neuroepithelial formation is demonstrated by the presence of translucent tissue around the edges of the organoid. • The incubation time in papain solution required for dissociation may vary with organoid age and between organoids generated from different cell lines. **Electrophysiological Recordings** • _How do I know that the silicon

probe is submerged in the chamber?_ If you are using an audio amp, you will hear a distinctive change in sound when the probe hits the solution surface and the circuit closes. You can also monitor the level of channel noise using the SmartBox software, or do a quick impedance measurement. A probe that is not in contact with the aCSF will have an open circuit and a very large impedance. • _Problems Anchoring the Organoid_ The majority of organoids will fall through the chute and come to rest on the anchor with little additional assistance. Some organoids may roll off the anchor. These troublesome organoids can be carefully manipulated onto the anchor using two fine paintbrushes. • _Chamber temperature keeps rising_ Check that the heat platform / feedback thermistor is in-place. If the thermistor has poor contact with the aluminum heating plate or fits loosely, it can respond poorly and lead to excessive heating of the recording chamber. Thermal transfer can be improved by adding a drop of mineral oil or vacuum grease to the thermistor and its mounting hole. Note that high temperature can be detrimental to the tissue. • _Chamber temperature is low_ Check that the bath thermistor is submerged in the recording chamber. Note that this thermistor only monitors temperature. • _Heating stage is not warming up_ Check that the heating element \(power film resistor) is functional, and that it has good thermal contact with the heating stage. Thermal transfer can be improved by placing a heat transfer pad between the resistor and the stage. • _What is the maximum temperature that can be used to drive the heating stage?_ The heat stage thermistor has a maximum temperature rating of 60°C, and this should not be exceeded by the power film resistor / heating element \(the power film resistor has a maximum temperature of 150°C). If the setup is functioning correctly, there should be no need to supply temperatures beyond the thermistor's rating. Check that all component of the homeothermic feedback system are functional, and that there is good thermal contact at all sites. • _Chamber pH drifts_ 1. Check that the carbogen bubbling is adequate for bicarbonate buffering. 2. Check that the chamber lid is in place. The lid will help to minimize evaporation and maintain salt concentrations. **Tip:** With long recordings a drift in the pH with the described setup is unavoidable. Long recording will benefit from adapting the recordings chamber to a perfusion system. Intermediate recording lengths might benefit from switching to a HEPES-based buffer. • _The wide-band signal is dominated by 50-60 Hz line noise_ If the rig is optimally configured, 50-60 Hz noise should be negligible / invisible by eye in the time-domain. If you experience prominent line noise in the wide-band signal, the rig will require some additional optimization: 1. clean up wiring to remove ground loops 2. check that the system is grounded correctly 3. check that the AgCl ground / ref is in good condition 4. check the quality of soldering 5. check that aCSF is not overflowing from the recording chambers • _Intermittent low frequency noise in the wide-band signal, synchronized across probe sites_ Instability in the baseline signal could be related to the carbogen flow. Bursting of large bubbles can produce surface ripples that are capable of producing recording artifacts. To fix, adjust the flow rate and the positioning of the gas line. • _What is the Expected Noise?_ Using the system described here, for new high density silicon probes, the typical signal root-mean-square \(rms) is 3-4 μV. Conservatively, with on-line peak thresholding, it is possible to isolate spikes >24 μV. • _Channel noise is larger than expected_ With repeated reuse of silicon probes there is a build up of detritus, paralleled by increase in probe impedance and the rms. This will negatively impact the signal to noise ratio. We recommend replacing the probe when the majority of channels have >8 μV_{rms}. • _How do I extend the life of the silicon probes?_ The lifespan of probes can be extended by rinsing in dd.H_2O immediately after use, and then storing the

silicon shaft in a mild protease or detergent \(for example, a contact lens cleaning solution suitable for silicon lenses). Use of alcohol is NOT recommended, as it will cause tissue debris to dehydrate and adhere strongly to the probe. Old probes can be reconditioned by chemical stripping and re-coating with PEDOT. • _Channel Impedance is low \(<0.1 MΩ, measured at 1000 Hz in aCSF)_ This is indicative of a short-circuit. Channels with low impedance should be discarded from analysis. **Tip:** Disabling acquisition of data from defective channels might produce changes to file mapping. If memory is not an issue it can be simpler to acquire all channels and remove defective channels during analysis. • _Channel impedance is high \(>1 MΩ, measured at 1000 Hz in aCSF)_ High impedance channels should be discarded from analysis. We have often observed spike-like events that are isolated to high-impedance channels; these events will not appear on neighboring sites with an expected impedance. As these events / artifacts often occur synchronously at multiple high-impedance probe sites, and do so irrespective of their spatial geometry, it is not believed that they are spikes. **Tip:** Disabling acquisition of data from defective channels might produce changes to file mapping. If memory is not an issue it can be simpler to acquire all channels and remove defective channels during analysis. • _During recordings, spikes drift across probe sites_ Probe implantation can lead to some small compression of the surrounding tissue. Compressed tissue will settle back / rebound over time, and can cause spikes to exhibit a time-related drift across probe space. If this movement is large it can hamper the clustering of spikes during analysis. Drift is particularly troublesome when there are multiple neuronal units whose spikes overlap in probe space. The greater the overlap, and the more numerous the units, the greater the problem in spike sorting. If this is a problem, position the probe and then let the tissue settle/recover for 10-15 minutes before beginning the experiment proper. • _The spikes signal to noise ratio is small_ 1. Check the quality of the probe. 2. Try repositioning the probe \(recorded spikes are larger when the probe is proximal to the cell soma[6]). **Note:** Some contributing factors are intrinsic to the neuron being recorded from[6], and outside of the experimentalist's control. It should also be noted that key features of a neuron change during maturation \(for example: soma size, dendritic morphology, ion channel expression and distribution), and will likely produce a recording bias towards mature neurons. **Tip:** All errors caused by the assignment of spikes to the wrong neuronal unit, or the dropping of spikes that fall below a detection threshold, propagate detrimentally down the analysis stream. Therefore, analysis should only be done on well-isolated spike trains. • _How deep should I advance the probe tip?_ If the aim is to record from this superficial population of neurons, the probe should be advanced by a distance equal to the vertical spread of the probe sites. The high-density silicon probe described in this protocol are composed of 64 9x9 µm probe sites, organized in 2 columns of 32 rows, with a 12 µm pitch[2]; to record from neurons at the organoid's surface, these 2x32 probes should be advanced 380 µm. • _Is the composition of aCSF important?_ The composition of aCSF, and the recording temperature, was selected to be close to physiological [7,8]. Modifications to aCSF composition might change the level / structure of spontaneous network activity. • _How do I perform acute pharmacological studies?_ To avoid the effects caused by the dilution of aCSF it is recommended that negligible volumes of stock solution are added to a known volume of aCSF \(15 mL in this protocol). TTX, applied to the external chamber, typically attenuates neuronal spike rate within 2-5 minutes \(this is based on recordings made from probes implanted <380 µm below the organoids surface). **Tip:** Internal control experiments should always be performed. For

example, add an equivalent volume of the vehicle without drug. **Tip:** Consider adapting the described setup to a perfusion-based recording chamber; this will enable more elegant pharmacological protocols. • _What is the best way to clean the recording chamber?_ We recommend rinsing immediately after use with dd.H_2O. Repeat the rinse to remove salts and tissue debris. **Note:** Calcium deposits will form if the rinsing procedure is insufficient. **Tip:** The process of removing solution from the recording chamber can be simplified by setting up a vacuum aspirator. For precision aspiration, we recommend using a 200 µL micropipette tip. To increase the speed of aspiration trim back the end of the micropipette tip.

References

1. Lancaster, M.A. et al. Cerebral organoids model human brain development and microcephaly. Nature. 501, 373-379 \(2013). 2. Quadrato, G., et al. Cell diversity and network dynamics in photosensitive human brain organoids. Nature. In Press \(2017). 3. Macosko, E.Z. et al. Highly Parallel Genome-wide Expression Profiling of Individual Cells Using Nanoliter Droplets. Cell 161, 1202-1214, \(2015). 4. Scholvin, J. et al. Close-packed silicon microelectrodes for scalable spatially oversampled neural recording. IEEE Trans. Biomed. Eng. 63, 120-130 \(2016). 5. Rossant, C. et al. Spike sorting for large, dense electrode arrays. Nat. Neurosci. 19, 634-641 \(2016). 6. Gold, C. et al. On the origin of the extracellular action potential waveform: a modeling study. J. Neurophysiol. 95, 3113-3128 \(2006). 7. Sanchez-Vives, M. and McCormick, D.A. Cellular and network mechanisms of rhythmic recurrent activity in neocortex. Nature Neurosci. 3, 1027-1034 \(2000). 8. Bar-Yehuda, D. and Korngreen, A. Cellular and network contributions to excitability of layer 5 neocortical pyramidal neurons in the rat. PLoS One. 11, 1209 \(2007).

Acknowledgements

We are grateful to Natalie Maria and Juliana Brown for editing of the manuscript. We thank Ema Zuccaro, Frank Yates and Serena Pavoni for valuable advice on the culturing protocol. We thank Ziv Williams for advice and insightful discussion in the development of the electrophysiology rig. We thank Edward Boyden, Jorg Scholvin and Justin Kinney for providing probes and for creating an interface to the Neuronexus SmartBox DAQ. We thank Mike McKenna and his team at Harvard SEAS for assistance refining designs and machining custom parts for the electrophysiology rig.

Figures

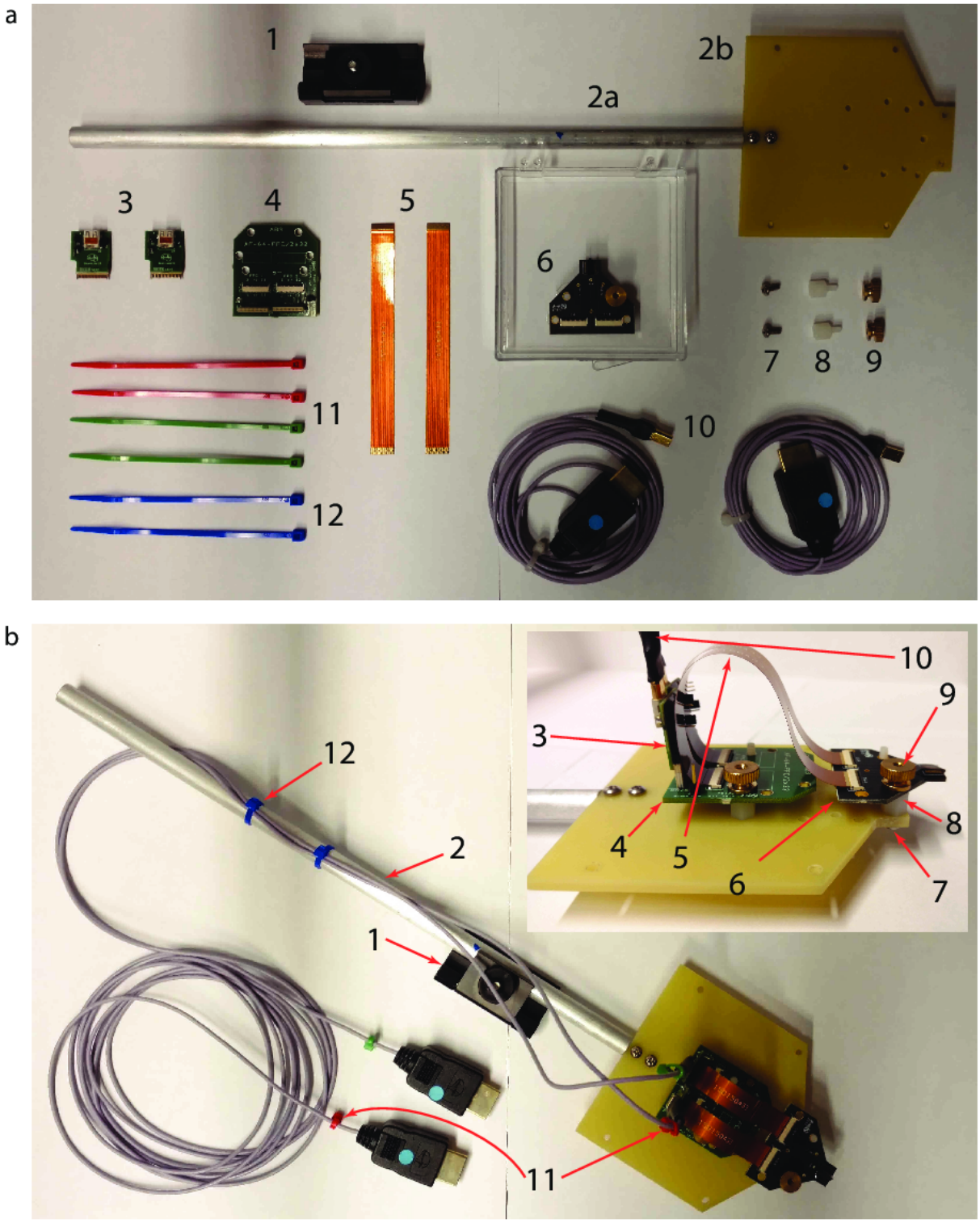


Figure 1

Silicon Probe and Manipulator Mount Assembly a) Image of the parts required to assemble the mounted silicon probe (part specifications and suppliers are detailed in the protocols Equipment section); b) Image of the mounted silicon probe, assembled with SmartBox headstage and required connectors. The parts are numbered according to panel a. Part#: 1. probe holder, this is used to mount the assembly onto the micro-manipulator; 2. Silicon probe mount and its post (parts 2a and 2b respectively); 3. SmartLink32

headstages; 4. probe breakout board; 5. FFC Jumpers; 6. 64 channel high density silicon probe; 7. screw; 8. nylon standoff; 9. brass thumb nut; 10. SmartLink cables; 11. red and green cable ties (these are attached to both ends of the SmartLink cables, with the intended purpose of color coding the wires); 12. blue cable ties (these are used to attach the SmartLink cables to the mounting post, and reduce stress on the connection between the SmartLink headstages (part #3) and the probe breakout board (part #4)

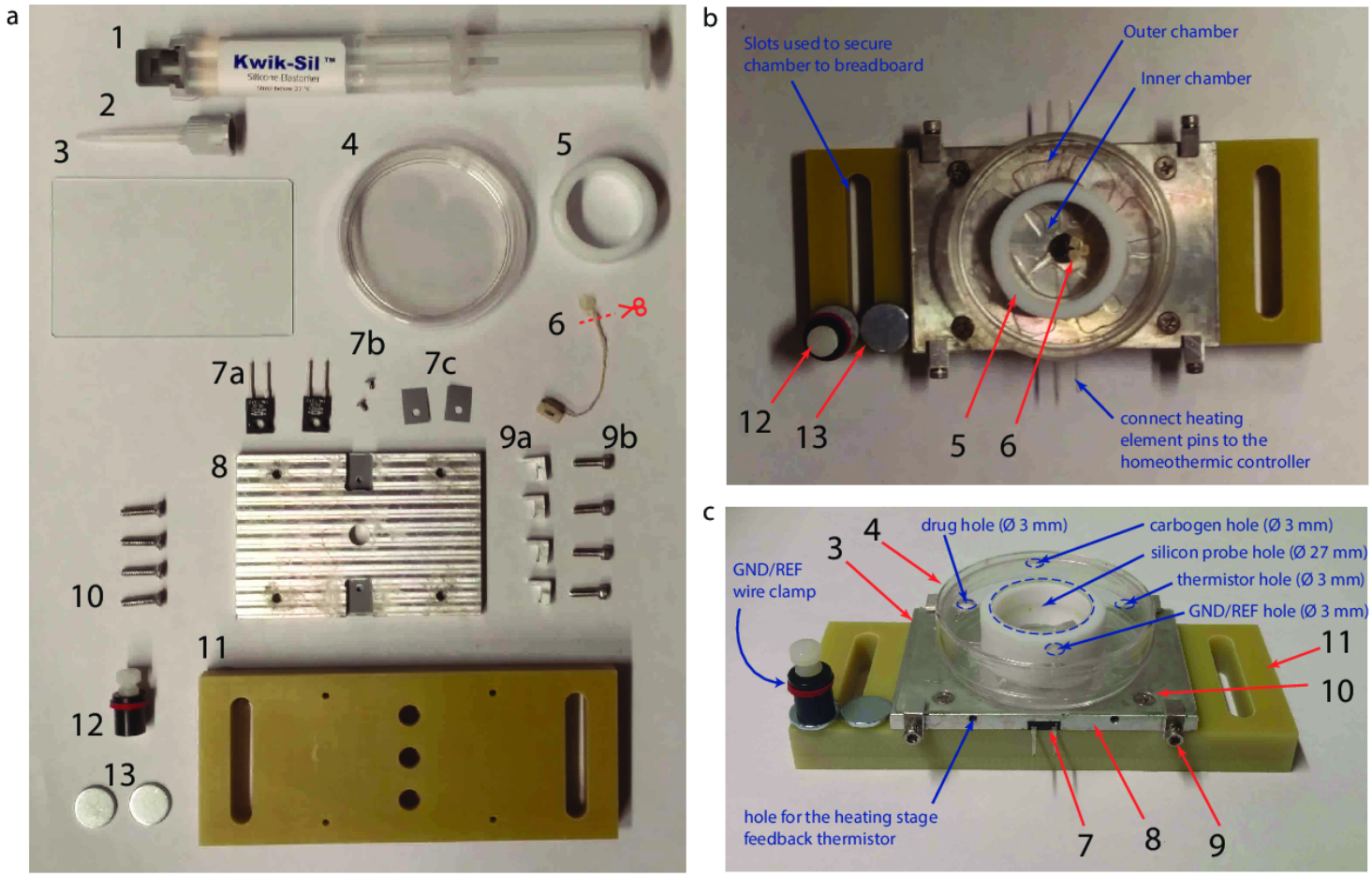


Figure 2

Recording Chamber Assembly a) Parts required to assemble the recording chamber (part specifications and suppliers are detailed under the Equipment section); b) top view and c) side view of the assembled recording chamber. The assembled parts are numbered according to panel a. Part#: 1. Silicon adhesive (and 2. mixing tip); 3. Glass microscope slides; 4. Tissue culture dish; 5. PTFE tubing; 6. Floating microelectrode array (detach electrode array to use as organoid anchor); 7. Heater elements (7a. resistor / heating element; 7b. mounting screw; and 7c. heat transfer pads); 8. Heating stage (for technical drawing see Figure 3); 9a. Heating stage clamp (for technical drawing see Figure 4); 9b. clamp mounting screw; 10. Heating stage mounting screw; 11. thermal isolation block for the heating stage (for technical drawing see Figure 5); 12. magnetic wire clamp used to secure the AgCl ground wire; 13. Stainless steel discs. The silicon adhesive (parts #1-2) is used to assemble the recording bath (parts #3-6), and secure some stainless steel discs (part #13) to the thermal isolation block (part #11). The inner chamber of the recording bath, made by the PTFE tube (part #5), is used to mount the organoid. The outer chamber is

used to monitor recording chamber temperature, bubble with carbogen, and add drugs. Holes made in the side of the PTFE tube, allow the free movement of solutions between the inner and outer chambers. The five holes made into the culture dish lid (one large central hole, and four small radial holes) are used to access the inner and outer chambers by key elements of the recording setup (see panel c).

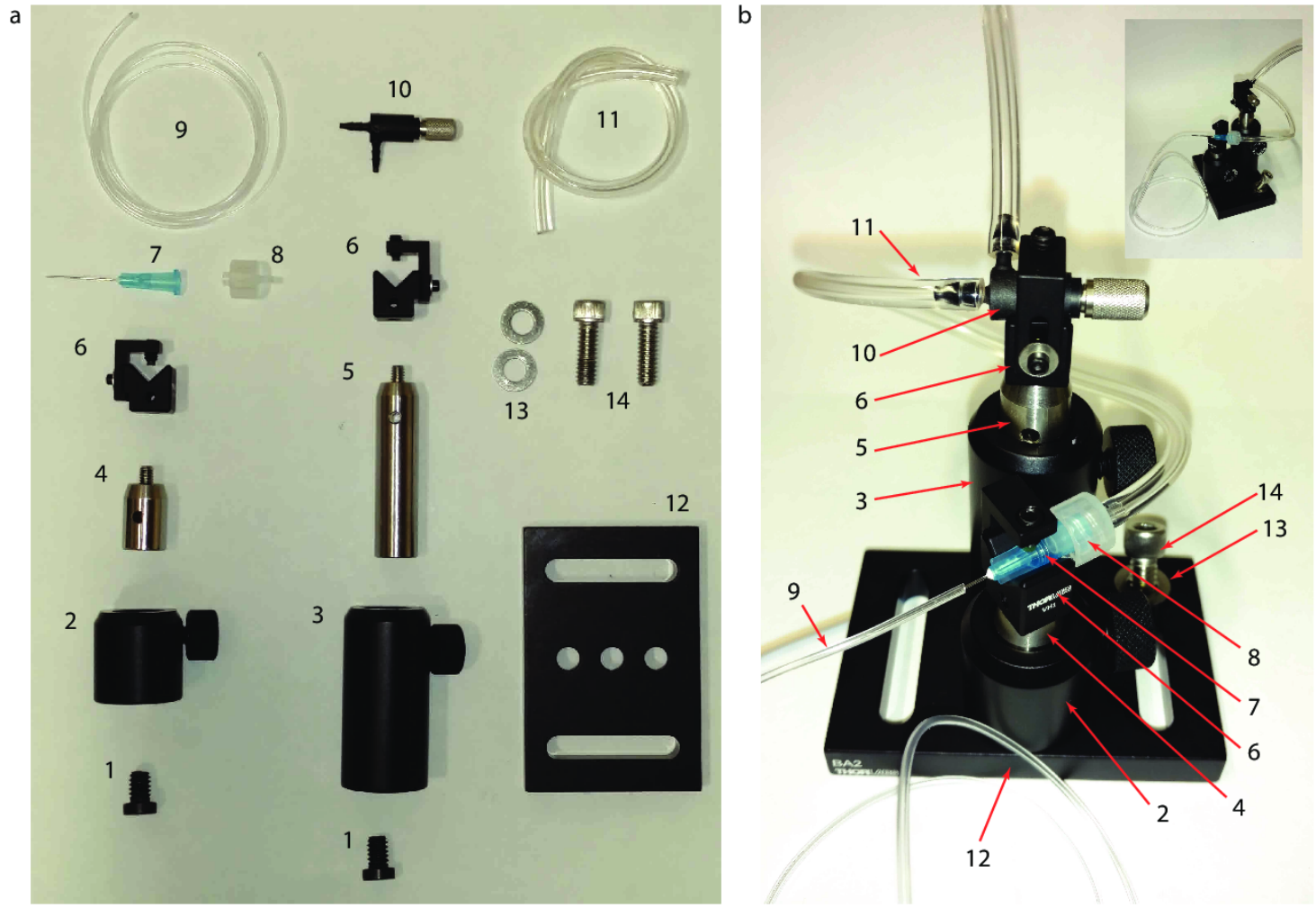


Figure 3

Figure 6 Carbogen Manifold Assembly a) Parts required to assemble the carbogen manifold (part specifications and suppliers are detailed under the Equipment section); b) view of the assembled manifold. The assembled parts are numbered according to panel a. Part#: 1. Screws; 2. Post holder (length 1"); 3. Post holder (length 2"); 4. Post (length 0.75"); 5. Post (length 2"); 6. Miniature v-clamps; 7. 25G needle; 8. Luer connector; 9. Tubing; 10. Needle valve; 11. Tubing; 12. Mounting base; 13. Screws; 14. Washers.

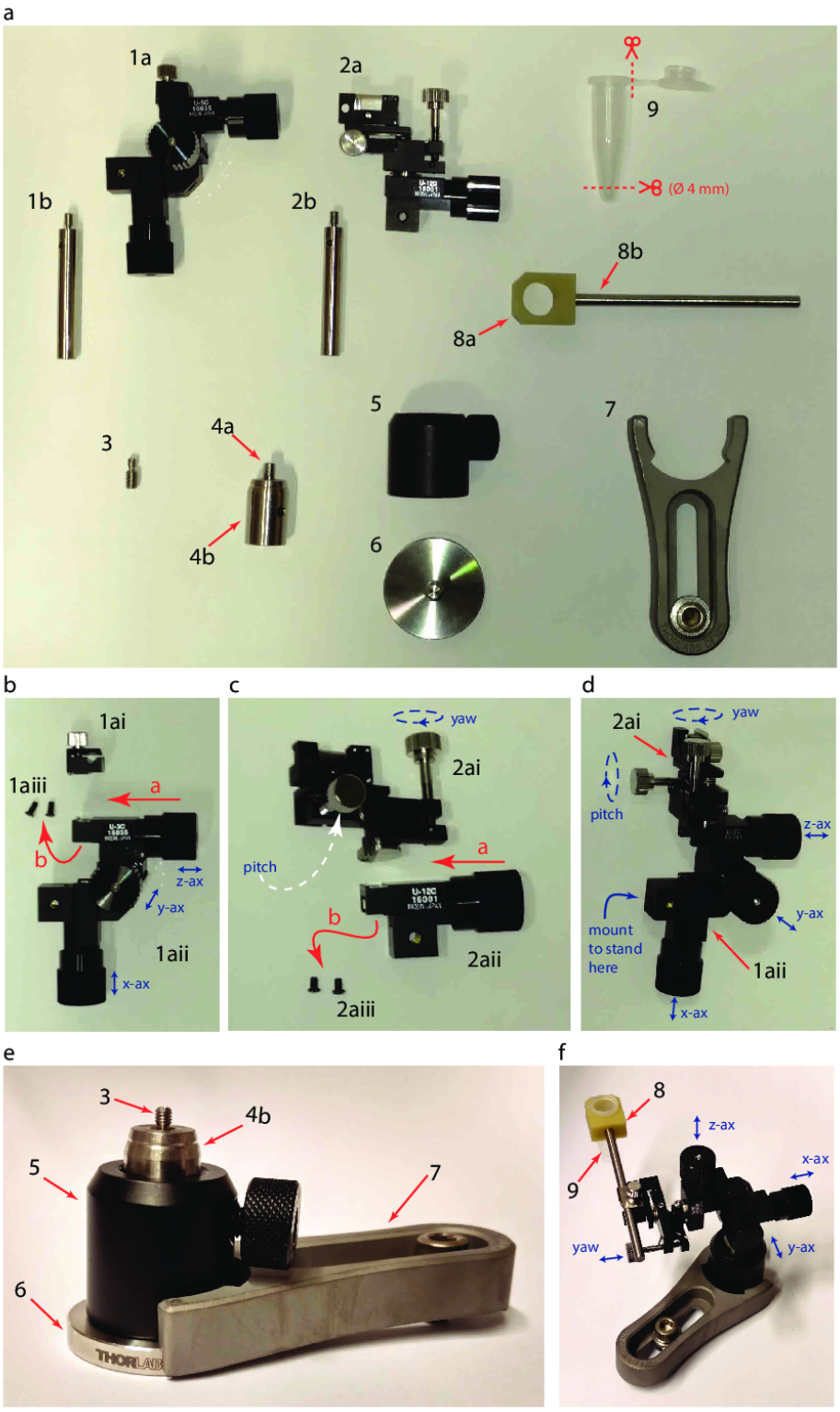
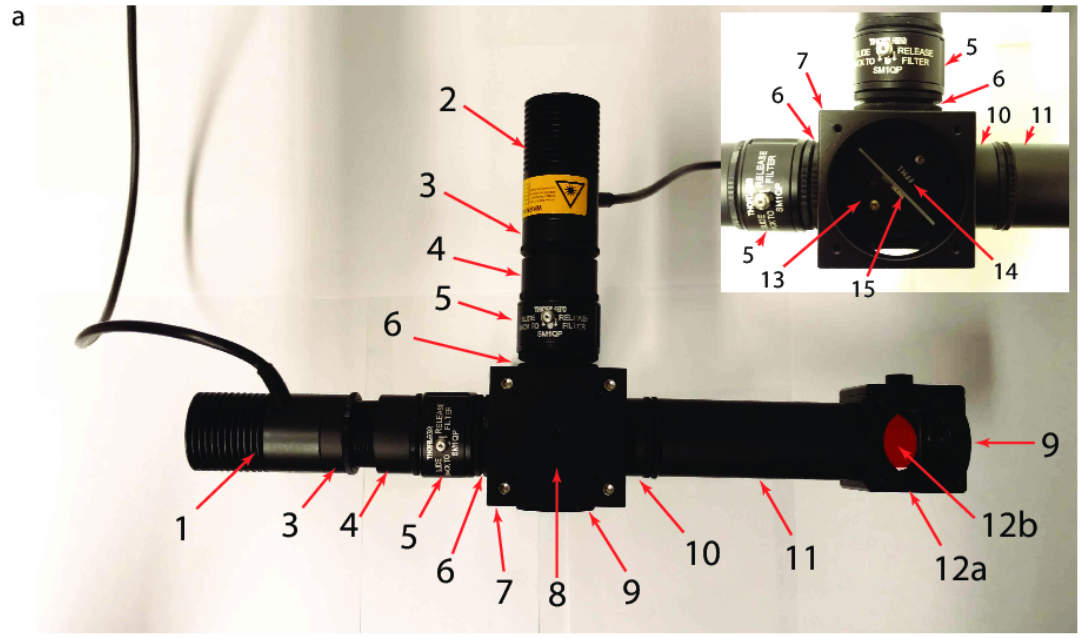


Figure 4

Figure 7 Organoid Chute Assembly a) Parts required to assemble the organoid chute (part specifications and suppliers are detailed under the Equipment section); b-d) The steps required to assemble the organoid chute manipulator; e) a view of the assembled manipulator stand; f) a view of the assembled organoid chute and it manipulator. The assembled parts are numbered according to panel a. Manipulator axes are labeled according the assembled organoid chute, shown in panel f. To make the organoid chute

proper, cut the lid from the 1.5 mL microcentifuge tube (part #9), and remove approximately 3.5 mm from the bottom (as illustrated by dashed red lines in panel a; the internal diameter at the bottom of the chute should be approximately 4 mm). Part#: 1. Three-dimensional coarse manipulator; 2. One-dimensional coarse manipulator with a pivot and tilt mechanism; 3. Thread adapter; 4. Post; 5. Post holder; 6. Pedestal base adapter; 7. Clamping fork; 8. Custom eppendorf mount (8a. Organoid chute mount, Figure 8; b. Organoid chute post, Figure 9); 9. Microcentrifuge tube (1.5 mL).



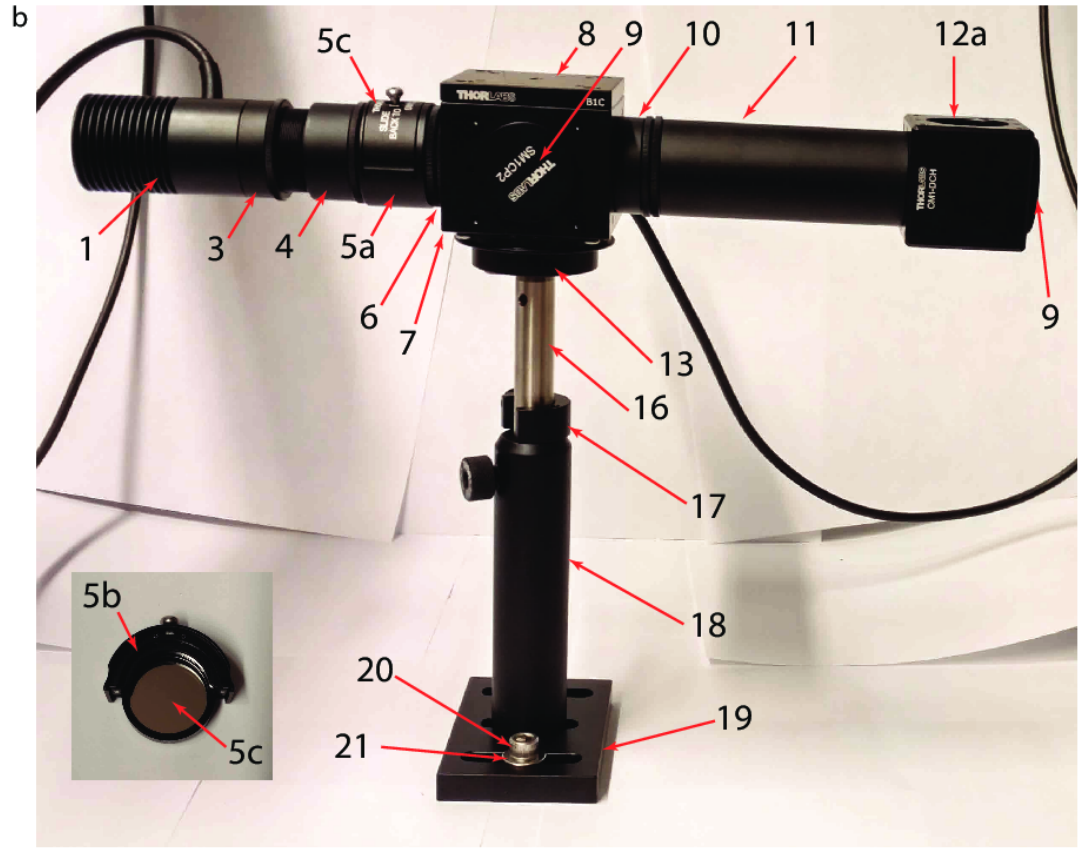


Figure 5

Figure 10 LED Light Source Assembly a) Top view of the assembled light source. Panel a inset) a close up of the cage cube (part #7) used to align two independent LED light sources (part #1 & 2) into a single light path; the cover plate (part #8) has been removed to expose the dichroic mirror and its mount (part #13-15). b) Side view of the assembled light source, providing a clearer view of the mounting stand. Panel b inset) a view of the band-pass filter (part #5b) and its filter carriage (part #5c). The IR digital microscope #1, used to view the organoid during its placement on the anchor, should be positioned above the long-pass dichroic mirror, 650 nm cut-off (part #12). Part#: 1. 530 nm mounted LED; 2. Optional second LED; 3. Lens tube (length 0.3"); 4. Collimation lens (aspheric condenser lens with diffuser is mounted inside the lens tube); 5. Fast-change lens tube filter holder (5a. Slotted Lens Tube; 5b. Filter Carriage; 5c. 530 nm band-pass filter); 6. Coupler; 7. Four-way mounting cage cube; 8. Cover plate; 9. End cap; 10. Lens tube (length 0.30"); 11. Lens Tube, (length 3.00"); 12. Dichroic filter (Long-pass dichroic mirror, 650 nm cut-off); 13. Kinematic cage cube platform; 14. Dichroic filter mount; 15. Long-pass dichroic mirror (490 nm cut-off); 16. Optical post; 17. Post collar; 18. Post holder; 19. Mounting base; 20. Screw; 21. Washer. More detailed part specifications, and suppliers are provided in the Equipment section.

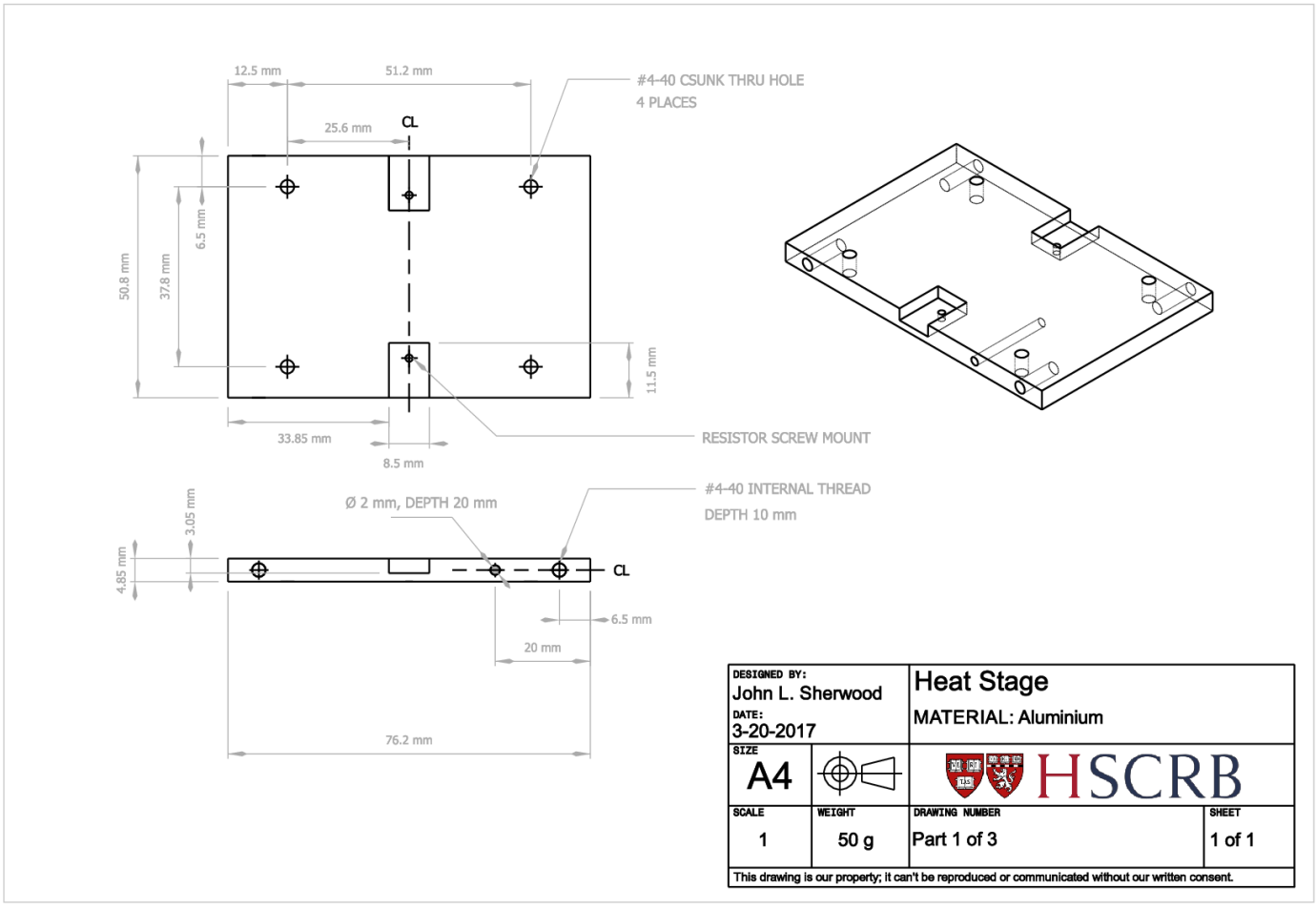


Figure 6

Figure 3 Heat Stage Proper Technical drawing of the heat stage.

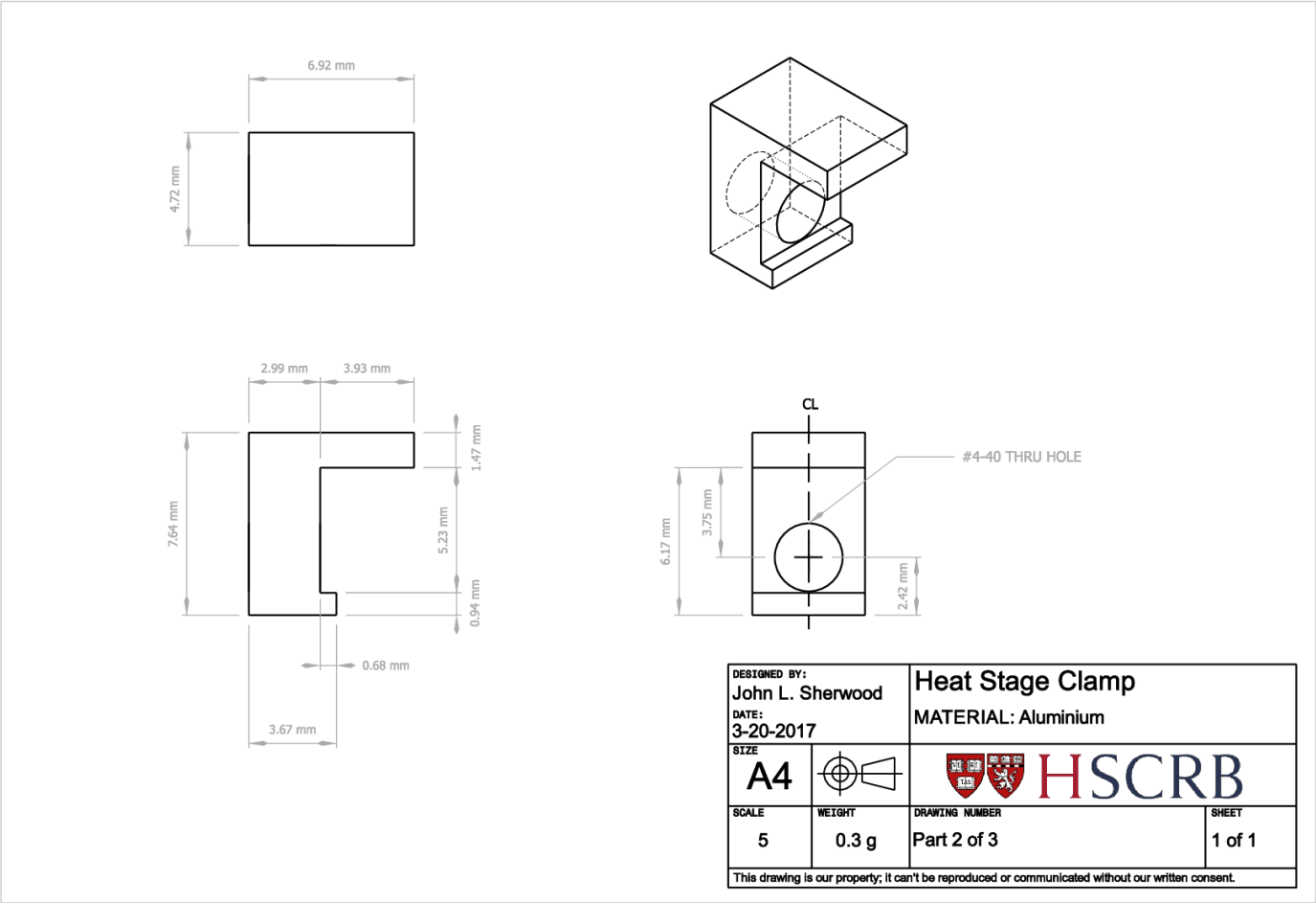


Figure 7

Figure 4 Heat Stage Clamp Technical drawing of the heat stage clamp. The assembled recording chamber requires 4 clamps. The clamps are used to secure the recording chamber to the heat stage.

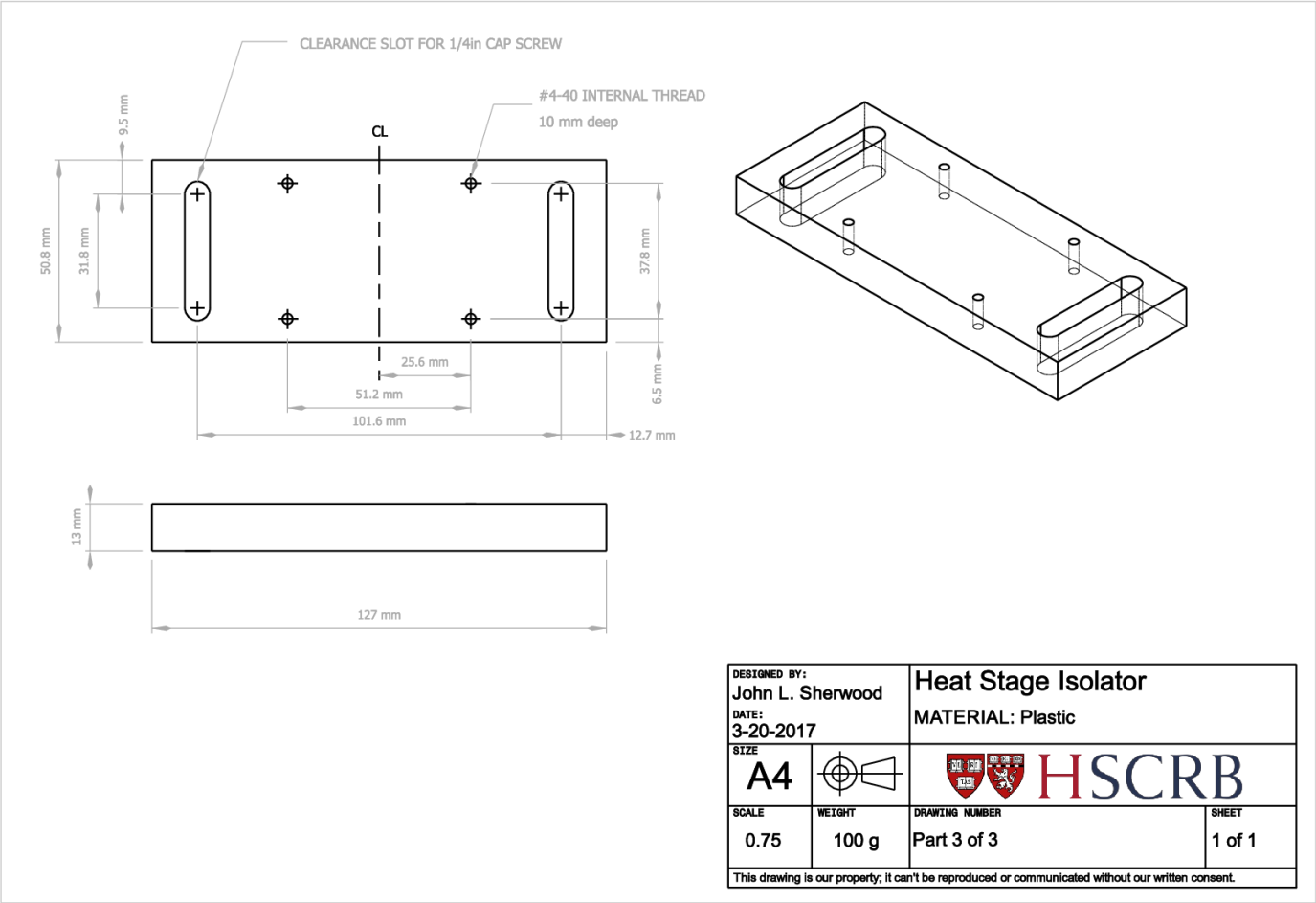


Figure 8

Figure 5 Heat Stage Thermal Isolator Technical drawing of the heat stage thermal isolator. The purpose of the block is to isolate the heat stage from the setup, without the isolator the rig breadboard would act as a heat sink.

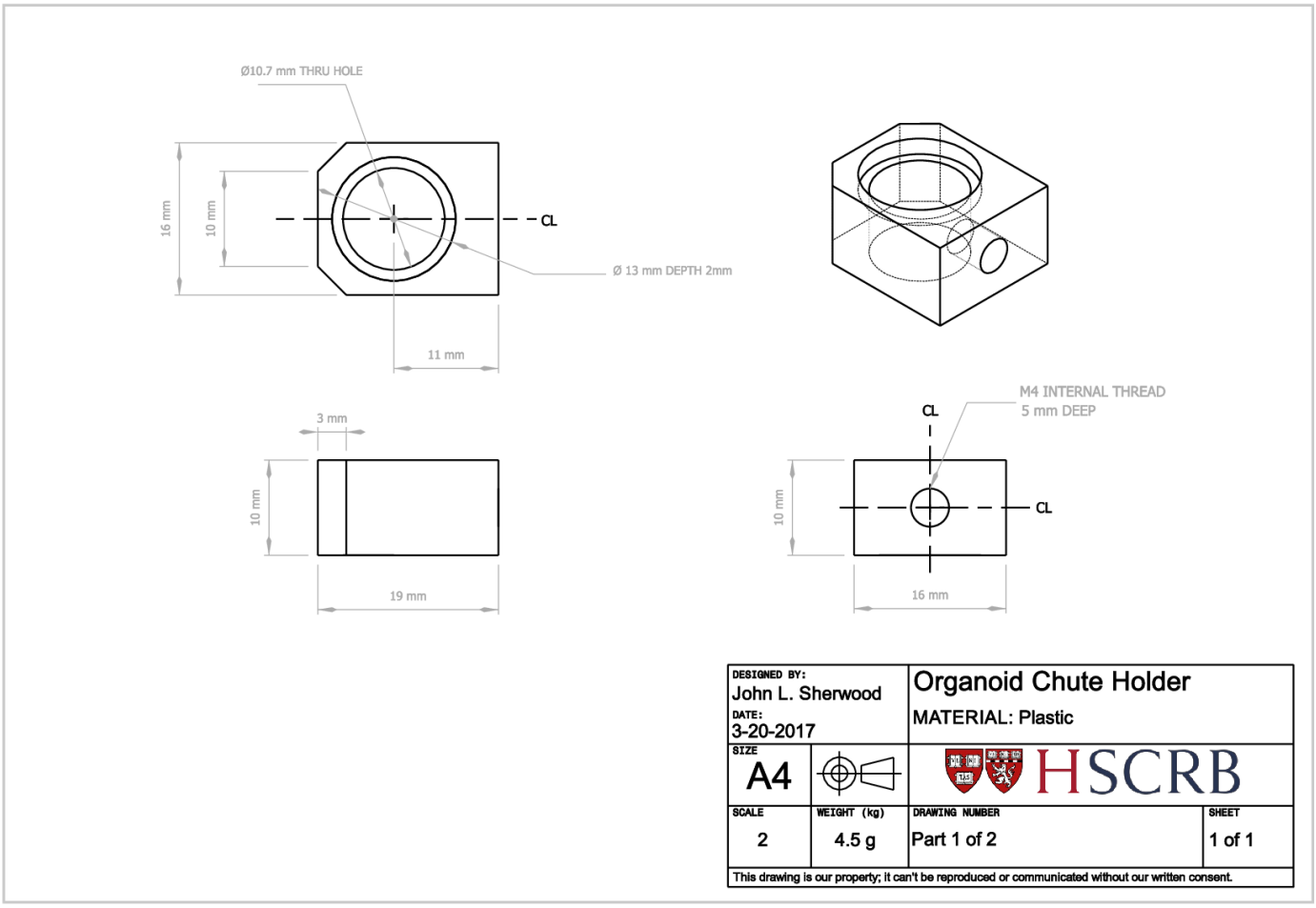


Figure 9

Figure 8 Organoid Chute Mount Technical drawing of the organoid chute mount.

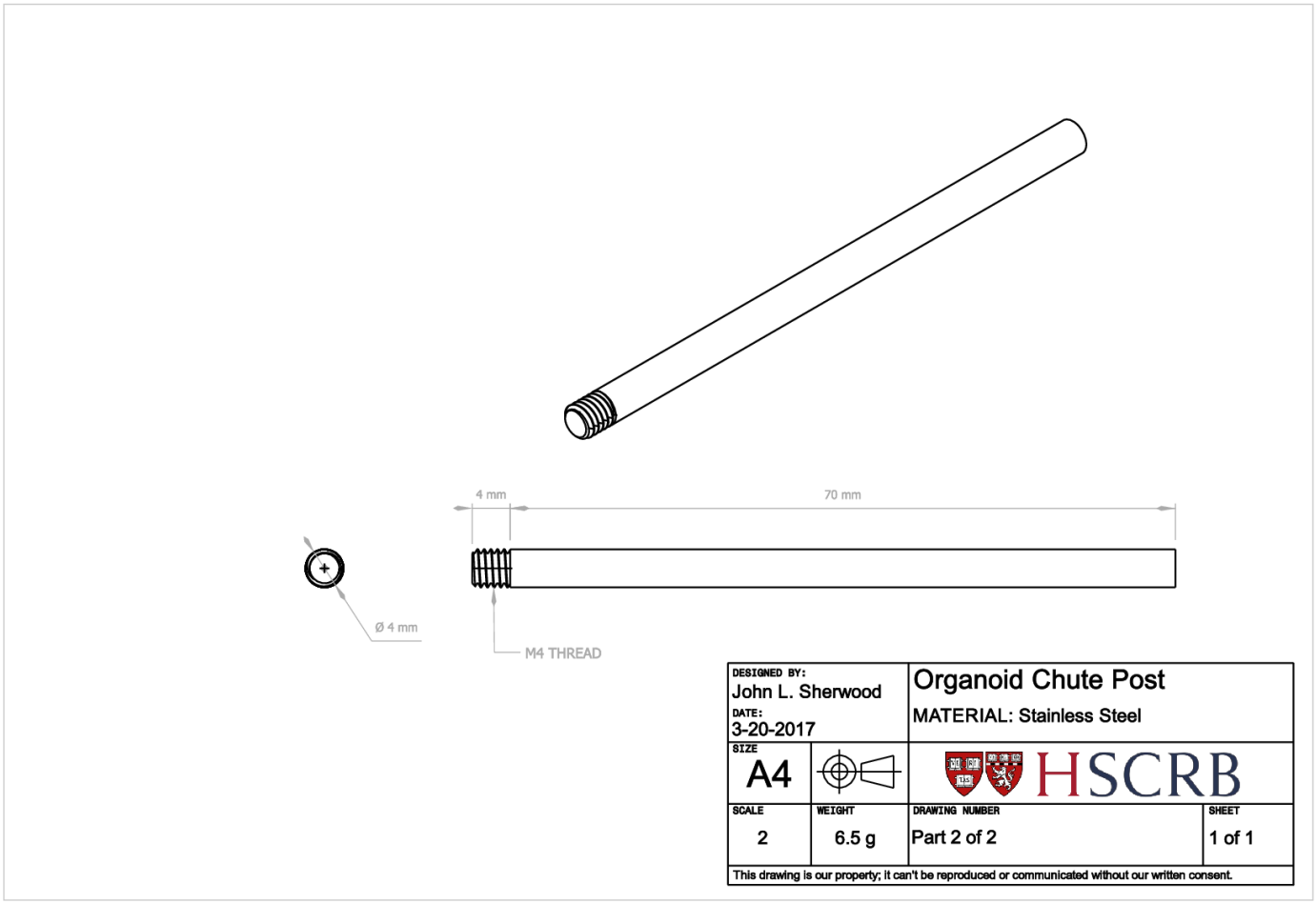


Figure 10

Figure 9 Organoid Chute Post Technical drawing of the organoid chute post.

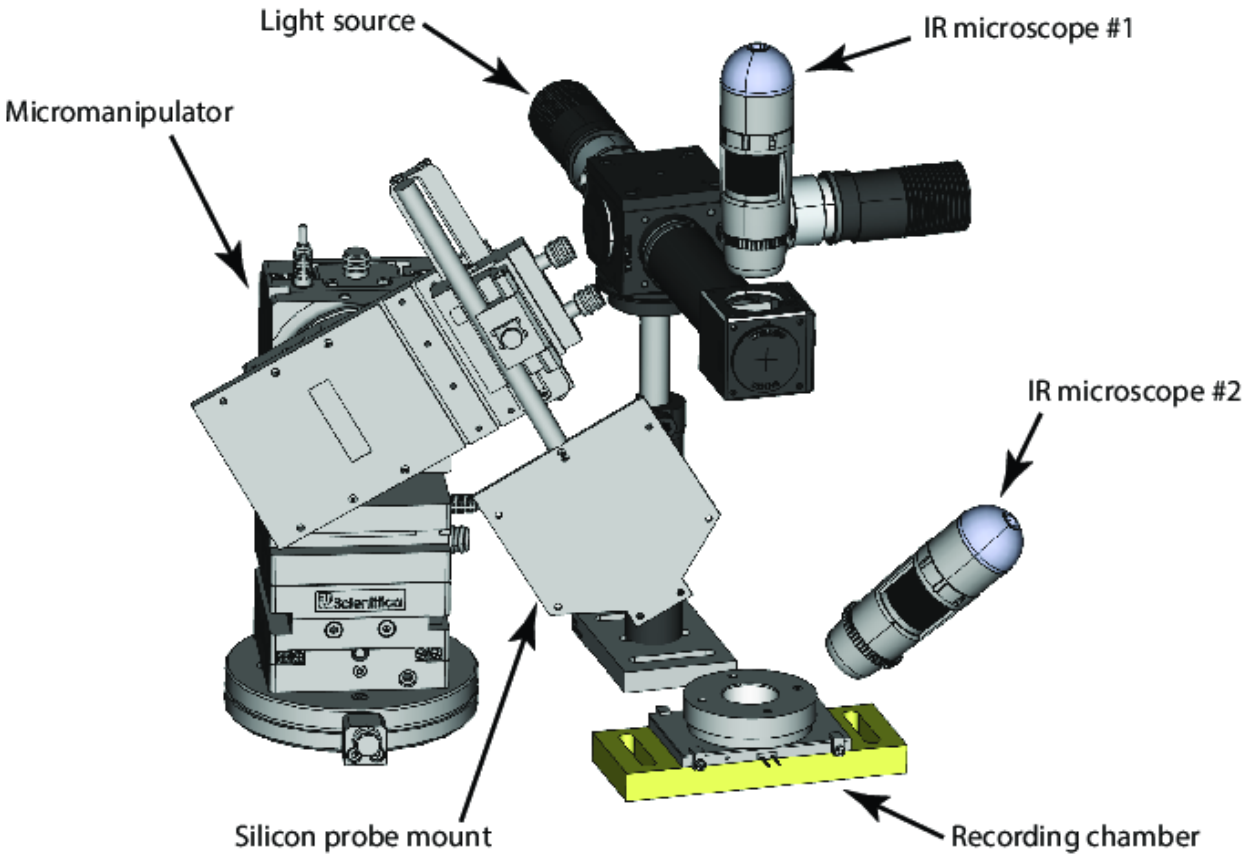


Figure 11

Organoid electrophysiology rig setup A three dimensional rendering of the electrophysiological rig. The rendering highlights the relative position of the recording chamber, the micromanipulator and silicon-probe mount, the LED light source, and the two infrared digital microscopes. For clarity, the microscope stands, the carbogen manifold, and the organoid chute assembly are not rendered.