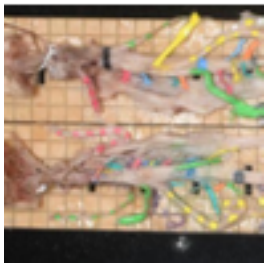
Sep 08, 2025

REVA #7: Excision of Cadaveric Human Vagus Nerves



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Noa B Nuzov[1], Leina M Lunasco[2], Brandon A.S. Brunsman[2], Katharine Workman[2], Valerie Lam[1], Zeyna Samba[2], Rebecca Prince[2], Nicole A Pelot[3], Andrew J. Shoffstall[1,4], Andrew R. Crofton[2,5]

[1]Department of Biomedical Engineering, Case Western Reserve University, Cleveland, OH, USA, 44106;
[2]Department of Anatomy, Case Western Reserve University, Cleveland, OH, USA, 44106;
[3]Department of Biomedical Engineering, Duke University, Durham, NC, USA, 27708;
[4]APT Center, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, OH;
[5]Department of Pathology and Cell Biology, University of South Florida, Tampa, FL

Noa B Nuzov: ORCID: 0000-0001-8187-2115;
Leina M Lunasco: ORCID: 0000-0003-3403-7429;
Brandon A.S. Brunsman: ORCID: 0009-0005-1162-2870;
Katharine Workman: ORCID: 0009-0007-8323-2913;
Valerie Lam: ORCID: 0009-0005-6777-3184
Zeyna Samba: ORCID: 0009-0000-2205-901X
Rebecca Prince: ORCID: 0009-0000-4802-5854
Nicole A Pelot: ORCID: 0000-0003-2844-0190
Andrew J. Shoffstall: ORCID: 0000-0002-0881-2180
Andrew R. Crofton: ORCID: 0000-0002-1105-3971

Nicole A Pelot
Duke University

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Abstract

This protocol outlines the process of removing the vagus nerve, dissected in an embalmed human cadaver, and adhering it to acrylic grid boards. This provides sample stability in transit and during downstream processing, and the grids provide a reference coordinate system to clearly label portions of the nerve after grossing.

Image Attribution

SR030-Whole-Nerve-Removed_DSC_2080.JPG. Taken August 1, 2024.

Guidelines

N/A

Materials

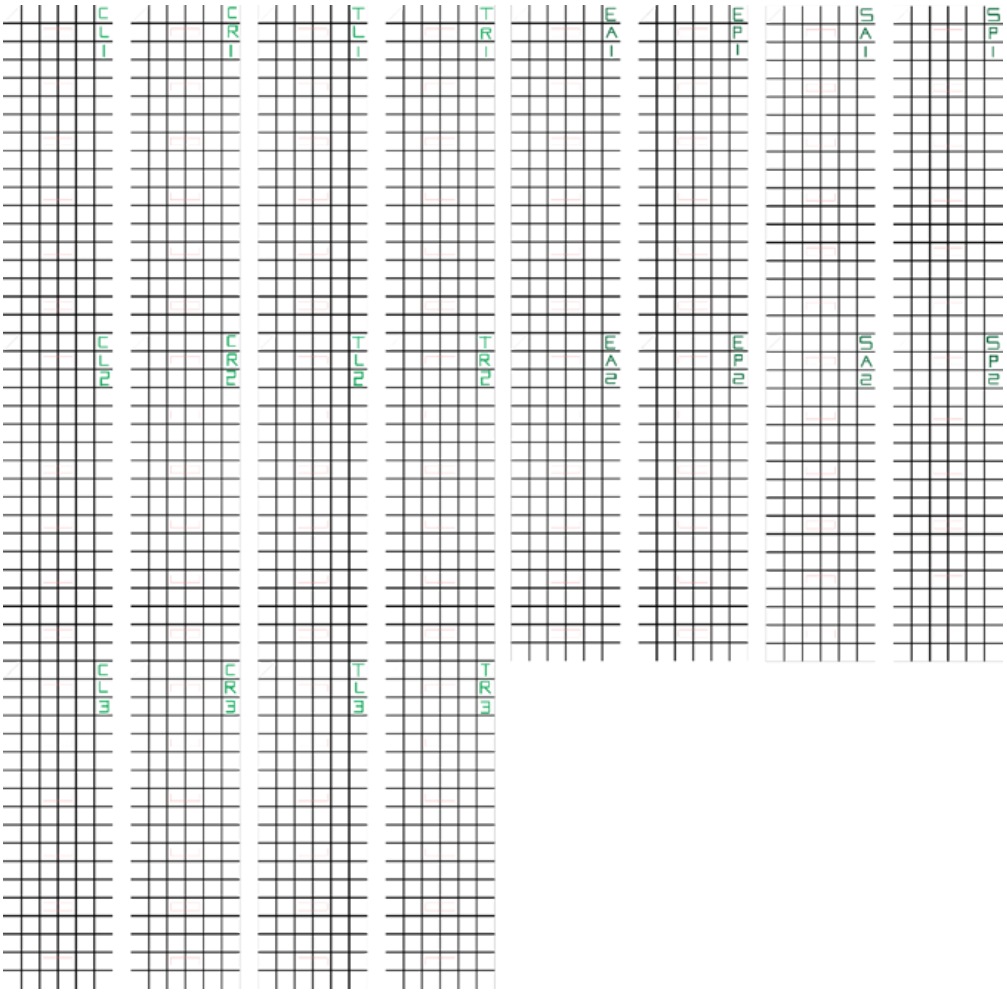
1. Large, enclosed container to store the nerve.
- Use “Hefty Large 13-gallon (52-quart) Clear Case with White Lid Underbed Tote with Latching Lid” from Lowe’s. (**Link**)

2. Cast acrylic sheet cut to fit inside the large bin. (**Link**)
- Cut an 1/8 inch thick acrylic sheet to a rectangle that is 30 inches by 12.5 inches.
- Company: McMaster-Carr. Color: black is recommended. Item number: 8505K745.

3. Waterproof Velcro, 1” wide (**Link**)
- Company: VELCRO, sold by Amazon. Size: 20 feet long, 1 wide.

4. Waterproof Velcro, 2” wide (**Link**)
- Company: VELCRO, sold by Staples. Size: 20 feet long, 2 inches wide. Item number: 464675.

5. Cast acrylic sheet cut into individual grid boards to put samples on. (**Link**)
- The acrylic sheet is laser cut into connected 3 cm x 9 cm boards (Figure 1) for each sample (cervical left, cervical right, thoracic left, thoracic right, esophageal anterior, esophageal posterior, sub-esophageal anterior, and sub-esophageal posterior sections).
- Each resulting board is etched with a 5 mm x 5 mm grid, and with the sample label in the top-right and on the back. Centered 5 mm x 10 mm holes are cut every 12 mm so that staining solutions can more uniformly access the nerve. See protocol **dx.doi.org/10.17504/protocols.io.n92ld6r3xg5b/v1** for sample label standards.


CL1
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TL1
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EA1
EP1
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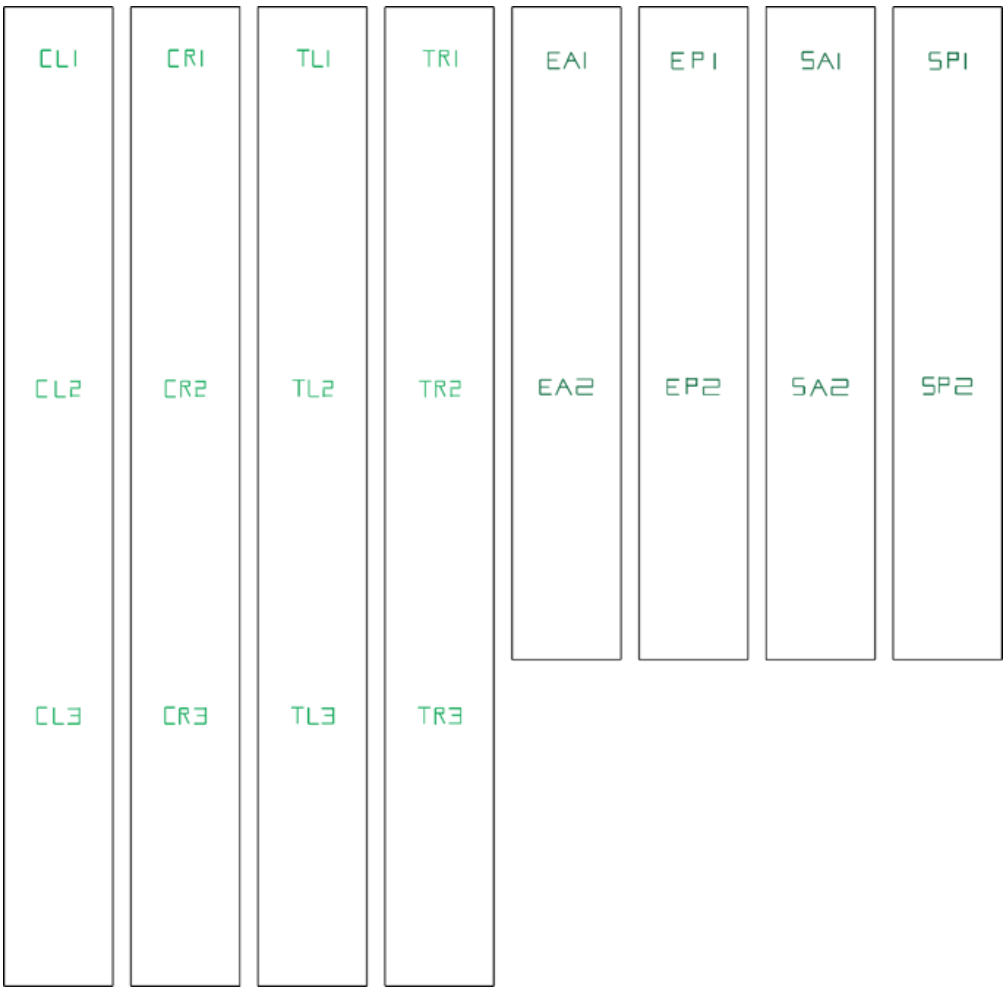

Figure 1. Front (top) and back (bottom) laser engraving and cutting designs for creating grid boards that nerve samples will be glued to.

6. Anatomical dissection tools. See protocol **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1** for details.

7. Camera and tripod or stand for photos. See protocol **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1** for details.

8. Black marker with a thin tip, like the Thermo Scientific Cryoware Permanent Marker in black. (**Link**)
- This marker was tested and found to withstand rehydration and the chemicals used in the downstream processing of samples.

9. White ruler for measurements and as a scale bar in photos. (**Link**)
- Custom-printed laser-cut acrylic boards with 5 mm x 5 mm engraved grids are also used (the marker in Item 7 is used to mark the engraved grid lines so they show up clearly in photos).

10. Digital calipers for measuring diameters. See protocol **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1** for details.

11. Paint and tissue dye. See protocol **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1** for details.

12. Phosphate-buffered saline (PBS).
- Prepared from 10x concentrated PBS diluted with DI water. (**Link**)
- Company: Fisher BioReagents, sold by Fisher Scientific. Name: PBS 10X solution. Size: 20L cube.

13. Cadaver wetting solution (0.01% phenol, 40% laundry bleach (7.5% sodium hypochlorite), 59.99% water).

14. Spray bottles for hydrating nerves.
- A trigger spray bottle is helpful when the nerve is excised. (**Link**). Company: Qorpak, sold by Avantor. Name: Trigger spray bottle. Size: 16 ounces.
- A continuous mist spray bottle is helpful when the nerve is still in the body. (**Link**). Company: Beautify Beauties Store, sold by Amazon. Size: 24 ounces.

15. Micro precise super glue. (**Link**)
- Company: Gorilla Glue, sold by Staples and Amazon. Name: Micro precise super glue. Color: clear. Size: 6 grams.

16. Duct tape.

17. Cheesecloth or gauze.

18. Standard hand-held fan or hair dryer.

19. ColorBond™ Tissue Marking Dye Mordant spray. (**Link**)

20. Autopsy headrest or other wooden or plastic block for elevating the cadaver's head. (**Link**)

21. Overhead lights (**link**) with c-stands (**link**)

Safety warnings

This protocol might include items and/or substances that may pose hazards (e.g., chemical, physical, biological, or otherwise) to your health upon use or exposure. Before engaging in the processes described in this protocol, familiarize yourself with and follow the safety data sheets, manufacturer safety recommendations, and local regulations.

Ethics statement

Be sure to seek approval for or an exemption from human subjects research from your local regulatory body(ies) as required by local and/or institutional regulations before initiating studies.

This study was determined to be exempt from IRB oversight by the Case Western Reserve University Institutional Review Board (IRB) because it involved de-identified cadaveric tissue and no protected health information was collected from the donors.

Before start

See the protocol for dissecting the human vagus nerve from embalmed cadavers (**dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1**).

Section 1: Container Preparation

1 Ensure the nerve is enclosed in an air-tight container for transport and whole-nerve imaging to avoid dehydration or damage. The container listed in the materials is sized to fit the largest possible vagus nerve.

2 Laser-cut or trim the cast acrylic sheet to fit into the bottom of the container.

2.1 The acrylic enables the sample to be laid flat and easily moved in and out of the container. The bottoms of the large containers are often curved.

3 Cover the “top” of the acrylic sheet with 2”-wide Velcro (loop side).

4 Velcro can also be used on the “bottom” of the acrylic sheet and the bottom of the large container for stable adherence between the two components.

Section 2: Nerve Removal

5 Once dissection, painting, and 3D nerve tracing are completed and the vagus nerve is accessible along its entire length (see **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1** & **dx.doi.org/10.17504/protocols.io.dm6gpm98pgzp/v1**), the nerve is ready for removal.

6 If the nerve can be removed in one piece, it should be removed from superior to inferior slowly and hydrated every 00:10:00 - 00:15:00 with cadaver wetting solution in a spray bottle.

6.1 Prop up the cadaver’s head by placing an autopsy headrest behind the head to access the brainstem.

6.2 Leaving the medulla intact is optimal as it provides tension to the left and right vagal nerves.

7 Using dissection tools, carefully release the nerve from the inside of the jugular foramen by scraping with blunt dissection tools until it is loose.

8 Cut each branch of the left and right vagus nerves as distally as possible. Each branch should be at least 2-3 cm long if possible.

9 If there are interconnections between the vagus and other nerves (e.g., glossopharyngeal, hypoglossal, sympathetic trunk, carotid sinus nerve, etc.), keep as much of the other nerve as possible, but at a minimum 1 cm in either direction from the branch connection.

10 Once all branches of the left and right vagus nerves are cut in the cervical and thoracic regions, and both vagus nerves are separated from the body in the neck and chest, remove the medulla.

10.1 Cut the medulla in the axial plane above and below the roots of the vagus nerve.

10.2 If the whole vagus complex is kept in one piece, the medulla needs to be lifted over the head towards the anterior side of the body.

10.3 If the left and right sides of the vagus need to be separated, make a vertical midline cut (in the sagittal plane) in the medulla so the left and right nerves are separated at their superior ends.

11 Move the cranial aspects of the vagus nerve (with the medulla attached) to the front of the body. Optionally, remove the head from the autopsy headrest.

12 Cut the esophagus superior to the start of the esophageal plexus and at the inferior border of the cardia of the stomach to remove it with the esophageal plexus.

13 Cut the branches in the abdominal region at the termination of the dissected length to separate them from their target organs.

14 Once the entire sample is mobile, remove it from the body and place it on a dissection table or flat surface.

15 Use dissection tools to separate the esophagus from the esophageal plexus. The plexus needs to be flattened with the anterior and posterior sides next to each other (placed on the left and right, respectively).

16 Although the nerve was painted during dissection to mark levels of anatomical structures and branches (see dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1), paint should be reapplied during the removal process as needed to ensure preservation of the paint markings.

16.1 Some branches and structures are uncovered during the removal process and should be painted and noted during removal.

Section 3: Grid Board Trimming and Alignment

17 Spray the nerve with 1x PBS every 00:05:00 - 00:07:00 during the board trimming and alignment process.

18 Locate the following levels along the vagus nerve: superior border of the clavicle on the left and right sides (paint color: blue), tracheal bifurcation on the left and right sides (paint color: black), 1 cm superior to the start of the esophageal plexus on the left and right sides (paint color: white), esophageal hiatus on the anterior and posterior abdominal trunks (paint color: black).

Note

In some cadavers, the level of the jugular angle (paint color: green) or tracheal bifurcation (paint color: black) may be inferior to 1 cm superior to the start of the esophageal plexus (paint color: white). Choose the most inferior of these three levels.

19 Line up the left and right cervical grid boards (CL and CR) with the most superior part of the left and right vagus nerve. Place the superior end of the nerve sample as high as possible (but it does not need to align with the top edge).

19.1 Mark the gridline with the black marker at the level of the superior border of the clavicle (paint color: blue) for both sides.

20 Line up the top edges of the left and right thoracic grid boards (TL and TR) with the corresponding levels of the superior border of the clavicle (paint color: blue).

20.1 Mark the gridline with a black marker where the lowest thoracic level is located. Repeat on both sides.

20.2 The most inferior thoracic location on the TL and TR grid boards is either at the 1 cm superior to the start of the esophageal plexus (paint color: white), tracheal bifurcation (paint color: black), or jugular angle (paint color: green), whichever is most inferior.

21 Line up the top edge of the anterior and posterior esophageal grids (e.g., EA and EP) with the lowest thoracic level. The left cervical-thoracic vagus nerve goes primarily to the anterior esophageal plexus, and the right cervical-thoracic vagus nerve goes primarily to the posterior esophageal plexus.

21.1 Mark with the black marker the gridline at the level of the esophageal hiatus (paint color: black).

22 Use the Dremel to trim all the grids at their marked lines. If any marks are at the natural division between two grids, gently break the grid by snapping it with your hands.

22.1 Use the cutting attachment of the Dremel.

22.2 Then use the sanding attachment of the Dremel to smooth the cut surface.

23 Put 1″-wide Velcro on the back (i.e., non-engraved grid side) of all acrylic engraved grid boards. Make sure to have at least one piece at the top and bottom of each 9 cm grid (e.g., CR1, CR2, etc.). Put the hook side on the acrylic grid boards.

23.1 Put Velcro on the sub-esophageal pieces (e.g., SA and SP). These will be cut later.

23.2 Peel off the paper covering from the acrylic on the underside before adhering to the Velcro.

23.3 Do not cover the grid name label (e.g., CL1, CR1, etc.) on the back of the grid.

24 Adhere grid pieces using Velcro to the large acrylic sheet that fits into the large white transportation bins. Center the nerve on the large acrylic sheet to make CT scanning faster.

24.1 Start with the esophageal pieces side by side in the center of the board. Ensure grid lines always line up for side-by-side pieces (e.g., if a grid board is shifted down relative to the board next to it, shift it in increments of 5 mm so gridlines are continuous).

24.2 Align the nerve's lowest thoracic level with the top of the esophageal grid pieces and press the grids into the acrylic sheet so the Velcro adheres. Then align the bottom of the cervical grids with the top of the thoracic grids and press into the sheet so the Velcro adheres.

24.3 The cervical and thoracic grids may not be directly abutted side-by-side. If the esophageal plexus is very wide, then the cervical and thoracic grids can be spaced out in increments of 5 mm to allow the trunks to naturally align with the center of the grid board.

25 The anterior sub-esophageal grid board (SA1) connects to the bottom of the anterior esophageal (EA) grid board, and the posterior sub-esophageal grid board (SP1) connects to the bottom of the posterior esophageal (EP) grid board.

25.1 Use as many sub-esophageal grids as needed. These can be lined up vertically and/or side-by-side.

25.2 Once the nerve is laid down, the sub-esophageal grids can be snapped at their 9 cm mark, or the Dremel can be used to trim the inferior side of the grid up to the end of the nerve.

Section 4: Nerve Gluing

26 Ensure that each vagus nerve segment is centered on its respective grid board, such that they cross the center of the grid board holes.

27 Spray the sample with 1x PBS every 00:05:00 - 00:07:00 during the gluing process.

28 When gluing the vagus and its branches to the grid boards, (1) the glue should make the sample sufficiently stable to ensure that nothing moves if the grids are shaken, but (2) do not add more glue than is necessary.

29 Apply glue to the top center and bottom center of the grid board, use a probe to gently move the vagus nerve to the center of the grid board, and press the vagus down into the glue. The vagus nerve should only be glued on the upper and bottom-most edges of each grid board. The nerve may twist in some areas, typically around the cervical and thoracic regions. Identify any twisting before gluing and accommodate the twist in a branch-free region to reduce twisting at branching points and to improve downstream processing. Glue the nerve in the following order:

29.1 Left and right levels of the superior border of the clavicle (paint color: blue)

29.2 Whichever is most inferior: left and right 1 cm superior to the esophageal plexus (paint color: white), left and right jugular angle (paint color: green), or left and right tracheal bifurcation (paint color: black).

29.3 Anterior and posterior esophageal hiatus (paint color: black).

30 Identify all branches on the cervical grid boards and spread them out.

31 Glue down the medulla and the large tissue mass that was inside the jugular foramen.

32 Spread out each branch on the thoracic grid board.

33 Glue the distal ends of each branch on the cervical and thoracic grid boards. Maximize the straight length of the branch present on the grid board. Additional lengths can be looped upward or downward along the grid board, glued to the side of the grid board, or cut.

33.1 Do not glue each branch too close to the vagus, they must each be visually discernible.

34 Add glue underneath ~5 mm of the vagus at every major gridline that separates the 9 cm-long grid boards (e.g., CL1, CL2, CL3, CR1, CR2, etc.).

35 Glue down any other nerves present on the sample, including but not limited to the glossopharyngeal nerve, hypoglossal nerve, spinal accessory nerve, carotid sinus nerve, and sympathetic chain.

36 Stretch out the esophageal plexus so it is as thin as possible. Place glue underneath the anterior and posterior abdominal vagal trunks at the level of the esophageal hiatus (paint color: black) and broadly under all branches of the esophageal plexus.

37 Stretch out the sub-esophageal plexus, with the painted branches and aortic hiatus (paint color: white or blue) visible and stretched as vertically as possible. Place glue broadly under all sub-esophageal branches and plexus.

37.1 Gluing of painted branches and anatomical levels of the sub-esophageal plexus should be prioritized over gluing of unpainted branches, if necessary.

Section 5: Nerve Repainting

38 Using the subject-specific ordered list of branches along the vagus nerve recorded during dissection, check that all branches are present and repaint any that are not visually clear after gluing.

39 Check that all anatomical levels along the right and left vagus nerve are present and repaint any that are not visually clear.

40 Ensure other cranial nerves are painted with dotted paint (see **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1**).

41 Esophageal and abdominal trunks are painted with dotted paint (see **dx.doi.org/10.17504/protocols.io.yxmvmb976g3p/v1**).

42 Label one 5 mm x 5 mm square on the edge of each 9 cm-long grid board with the cadaver number using the permanent black thin-tip marker.

43 After the paint has dried for ~ 00:02:00 , spray with ColorBond Tissue Marking Dye Mordant.

43.1 Use a fan or hairdryer to dry the paint for ~ 00:00:30 before and after using the Mordant spray.

44 Once all paint has completely dried, spray the entire sample with 1x PBS. The sample is now ready for final photographs (Figure 2).

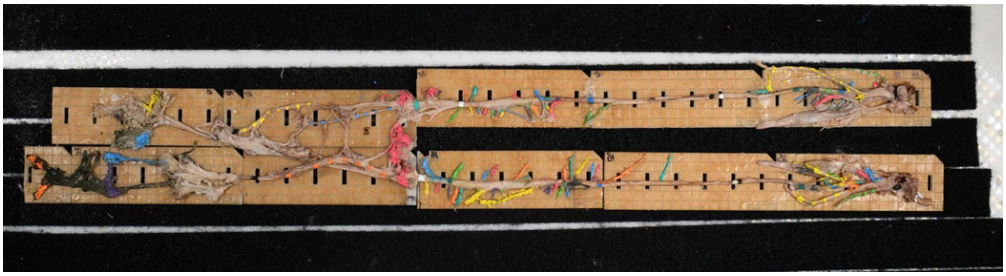

Figure 2: Example of the vagus nerve on grid boards after gluing and painting. The superior end of the sample is on the right, and inferior end of the sample is on the left. The right-side of the vagus nerve is on the top, and the left-side is on the bottom

Section 6: Completion of Nerve Removal and Gluing

45 Take photos of the nerve to document painted landmarks and branches and for reference during downstream processing. Use small levels on the camera to ensure that photos are taken parallel to the ground. Position multiple overhead lights parallel to the ground or aligned with the camera's viewing angle to uniformly illuminate the nerve and to prevent backlight.

46 Once completed, lay 1x PBS-soaked gauze or cheesecloth over the nerve to keep it hydrated.

47 Place the lid on the container.

48 If the container is not airtight, rehydrate the nerve frequently and/or use duct tape to seal the edges of the container.

> 49 If the container is too large to fit into the fridge, the sample can remain at room temperature for up to 8 hours overnight, and then promptly divided into smaller sections, as described in **dx.doi.org/10.17504/protocols.io.5qpvod95xg4o/v1**.

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