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Technical note

Submersion method for neuroanatomical photography: visualizing arachnoid membranes and cisterns

Isaías Henrique Martins Vasconcelos Norte Bezerra[1], Guilherme Cristiano Garcia[1], Florisvaldo José Morais Vasconcelos Junior[2], Gabriel Melo Duarte Costa[3], Antonio Cavalcanti de Albuquerque Martins[3], Marcelo Moraes Valença[2], Mateus Reghin Neto[4], Eduardo Carvalhal Ribas[5], Hung Tzu Wen[5], Carolina Martins[2]

[1]Pernambuco State University, Recife, Pernambuco, Brazil
[2]Federal University of Pernambuco, Recife, Pernambuco, Brazil
[3]Medical School of Pernambuco, Recife, Pernambuco, Brazil
[4]Institute of Neurological Sciences, São Paulo, São Paulo, Brazil
[5]University of São Paulo, São Paulo, São Paulo, Brazil

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Juliana Ramos de Andrade

Introduction
The subarachnoid space expands into cisterns, limited by arachnoid membranes. Dissection within these compartments is the hallmark of microneurosurgeons, working *in-vivo*, within the head, with the brain bathed in cerebrospinal fluid, under magnification, and extra-illumination. Other study methods cannot reproduce these conditions, and *ex-vivo* anatomy proves inadequate, as cisterns collapse, impairing recognition of delicate sheaths, limiting comprehension, and creating a knowledge gap that starts during graduation.

Objective
This technical report (CAAE: 94582325.1.0000.5208) presents a photographic method that restores cisternal configuration through submersion.

Methods
Twenty-two brains were obtained with informed consent during routine autopsies. After intravascular rinsing and fixation in formalin, specimens were submerged in water within black containers to re-expand subarachnoid spaces. Arachnoid membranes were marked with color-coded pins. High-resolution photographs were acquired using a dual 12 MP camera system (wide f/1.6 with sensor-shift stabilization; ultra-wide f/2.4, 120° field of view, Apple Inc., Cupertino, USA). Two light sources minimized glare and reflection.

Results
Submersion restored the three-dimensional configuration of cisterns, allowing visualization of the arachnoid membranes including: Liliequist's, lateral pontomesencephalic, anterior pontine and medial and lateral pontomedullary and carotid membranes. The photographic protocol produced detailed images that facilitated recognition of cisternal boundaries and membrane orientation.

Conclusion
This submersion-based photographic method provides a simple, low-cost, and reproducible approach for neuroanatomical documentation and is particularly useful in depicting the arachnoidal cisterns. By approximating intraoperative conditions through submersion, it enables students and residents to visualize and comprehend complex arachnoid structures. This approach may improve anatomical proficiency and facilitate neurosurgical training.

Keywords: Subarachnoid space, Arachnoid, Anatomy, Cerebral cisterns, Neurosurgical procedures, Education, Photography, Methods

Carolina Martins
cmrecife@hotmail.com

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Introduction

The subarachnoid space expands into cisterns, limited by arachnoidal membranes. Dissection within these compartments is the hallmark of microneurosurgeons, working *in-vivo,* within the head, with the brain bathed in cerebrospinal fluid, under magnification, and extra-illumination. Other study methods cannot reproduce these conditions, and ex-vivo anatomy proves inadequate, as cisterns collapse, impairing recognition of delicate sheaths, limiting comprehension, and creating a knowledge gap that starts during graduation.

Several landmark papers have focused on the study of the arachnoidal membranes separating these compartments (1–5), but they have relied on traditional photography and depicted specimens outside of a fluid environment.

This technical report - part of a broader study using donated human cadaveric specimens, introduces a photographic protocol that restores cisternal configuration through submersion and its potential in clarifying this anatomy. It highlights the surgical relevance and overall importance of understanding subarachnoid compartments early in medical training.

Method

Twenty-two brains were obtained with registered informed consent during routine autopsies at Federal University of Pernambuco (IRB/CAAE: 94582325.1.0000.5208). After intravascular rinsing and fixation in formalin, specimens were submerged in water within black containers to re-expand the subarachnoid spaces (Figure 1).

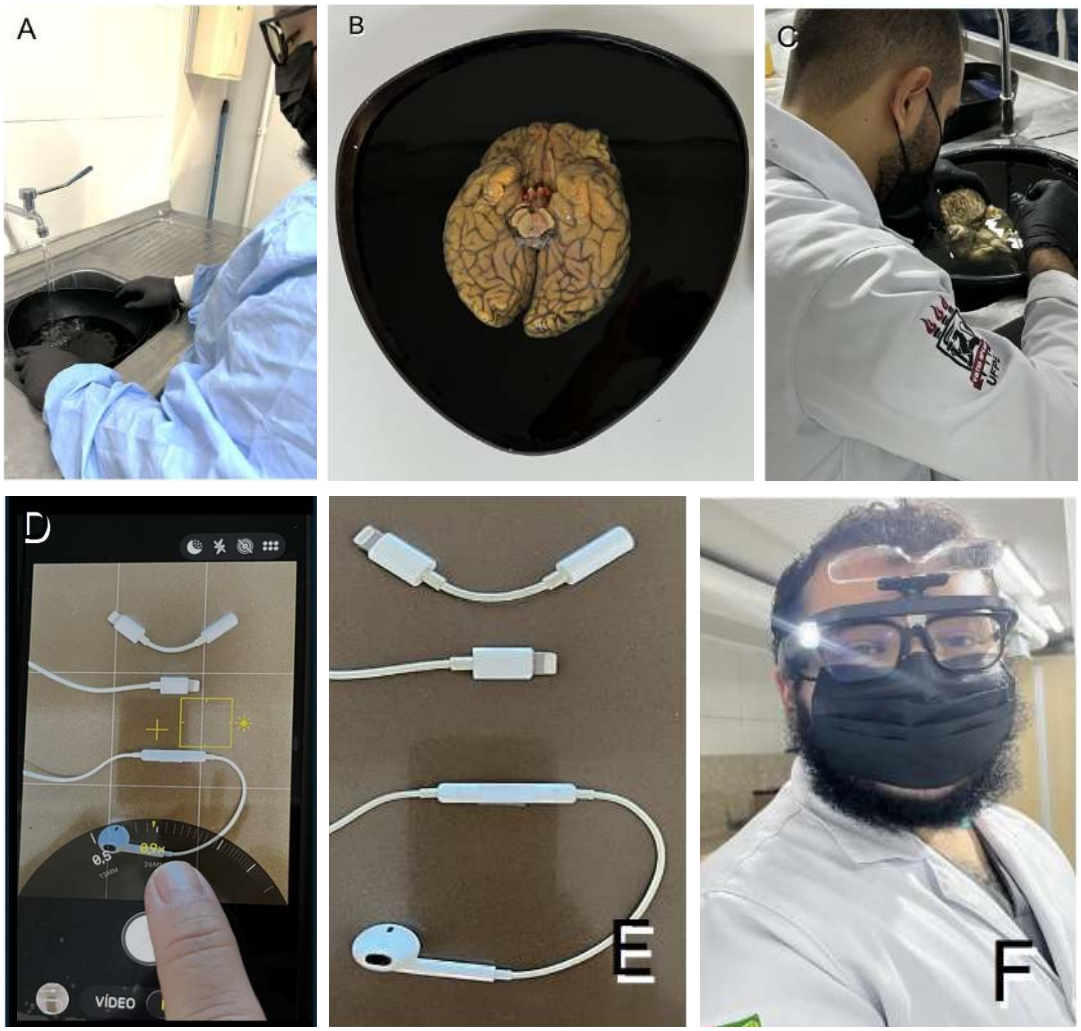


Figure 1. Submersion-based photography using cadaveric specimens. A. A black basin is filled with water; while researchers wear black gloves. B. The preserved brain specimen is submerged to restore cisternal configuration. Light room conditions are kept at minimum. C. One researcher handles the specimen to provide exposure and optimize visualization of specific targets. D. Several onboard camera features including grid, level, digital zoom, focus lock and earbud switch help in acquiring images. Grid (Settings-Camera-Composition-Grid On) is useful for composition and image cropping. Photographic framing is guided by the camera level feature (Settings-Camera-Composition-Level On), using crosshair alignment (white to yellow) to ensure perpendicular orientation to the specimen's surface. This guarantees perpendicular positioning of the camera in relation to the water surface and minimizes distortion and glare when combined with tangential illumination. Digital zoom (below1,5x) (Camera App-Zoom Controls-Slide dial) and focus lock (Camera App-Tap screen for AE/AF lock) and exposure adjustment (Camera App-Tap target area-Slide Sun Icon up/down) were also employed. E. The earbud switch is programmed (Plug or Bluetooth earbuds-Camera App-Trigger shutter using volume control) to shutter camera. F. The second researcher, wearing magnification glasses fitted with LED lamps for tangential illumination, conducts the photographic capture.

Arachnoid membranes were marked with color-coded pins. High-resolution photographs were acquired using a dual 12 MP camera system (wide f/1.6 with sensor-shift stabilization; ultra-wide f/2.4, 120° field of view, Apple Inc., Cupertino, USA). Two light sources minimized glare and reflection.

Onboard device features as grid, level, digital zoom, focus lock, and earbud switch were incorporated into the protocol to guarantee a perpendicular view towards the specimen surface being depicted, maintain focus at the target area, and minimize undue movement while activating the camera shutter.

The submersion-based photographic protocol is summarized in Box 1.

Results

Submersion restored the three-dimensional configuration of cisterns, allowing consistent visualization of the arachnoid membranes including: Liliequist's membrane (diencephalic and mesencephalic sheets), lateral pontomesencephalic, anterior pontine and medial and lateral pontomedullary and carotid membranes (Figures 2-4).

It has also revealed new aspects of the variability of arachnoidal compartments and unexpected attachments of their membranes, all of which justify further anatomical studies employing this methodology (Figures 2-4). By reconstituting these spaces in conditions closer to normal in vivo (Figure 4), its application facilitates the understanding of the natural shapes, internal contours, and divisions of the cisterns—an enduring gap in anatomical studies during graduation that carried lasting repercussions into postgraduate training.

By restoring the volume of the subarachnoid compartment through submersion and using a photographic method designed to reduce distortions, it was possible to obtain detailed images of the arachnoid membranes and subarachnoid spaces. These images made it easier to identify cisternal boundaries and membrane orientation, while also highlighting the clinical and surgical relevance of these structures early in medical training.

Box 1. Protocol for submerged photography

Specimen Preparation and Positioning
Place specimens in black basin filled with water to reconstitute arachnoidal compartments
Choose black gloves for handling and positioning specimens
Maintain specimens close to the water surface

Camera Settings

Activate grid and level at device settings
Activate focus and exposure adjustments at camera app
Connect earbuds (plug or Bluetooth) to activate volume controls as camera shutter

Camera Positioning

Maintain shortest possible working distance to minimize refraction and distortion.
Use level feature to guarantee 90-degree position of camera in relation to the surface being depicted

Alignment and Stability

Use grid and level functions for precise orientation
Use AE/AF lock to stabilize focus and exposure
Prepare remote shutter camera release by programing earbuds to ensure stability

Exposure and Zoom

Adjust brightness manually with the exposure slider to counter glare
Apply moderate digital zoom (no more than 1,5x, fine tuning with dial) to highlight structures without pixelation

Lighting Control

Ensure diffuse, even illumination to suppress reflections
Avoid direct overhead light that produces glare
Include tangential LED lighting

Image Capture

Use remote shutter release to prevent vibration.

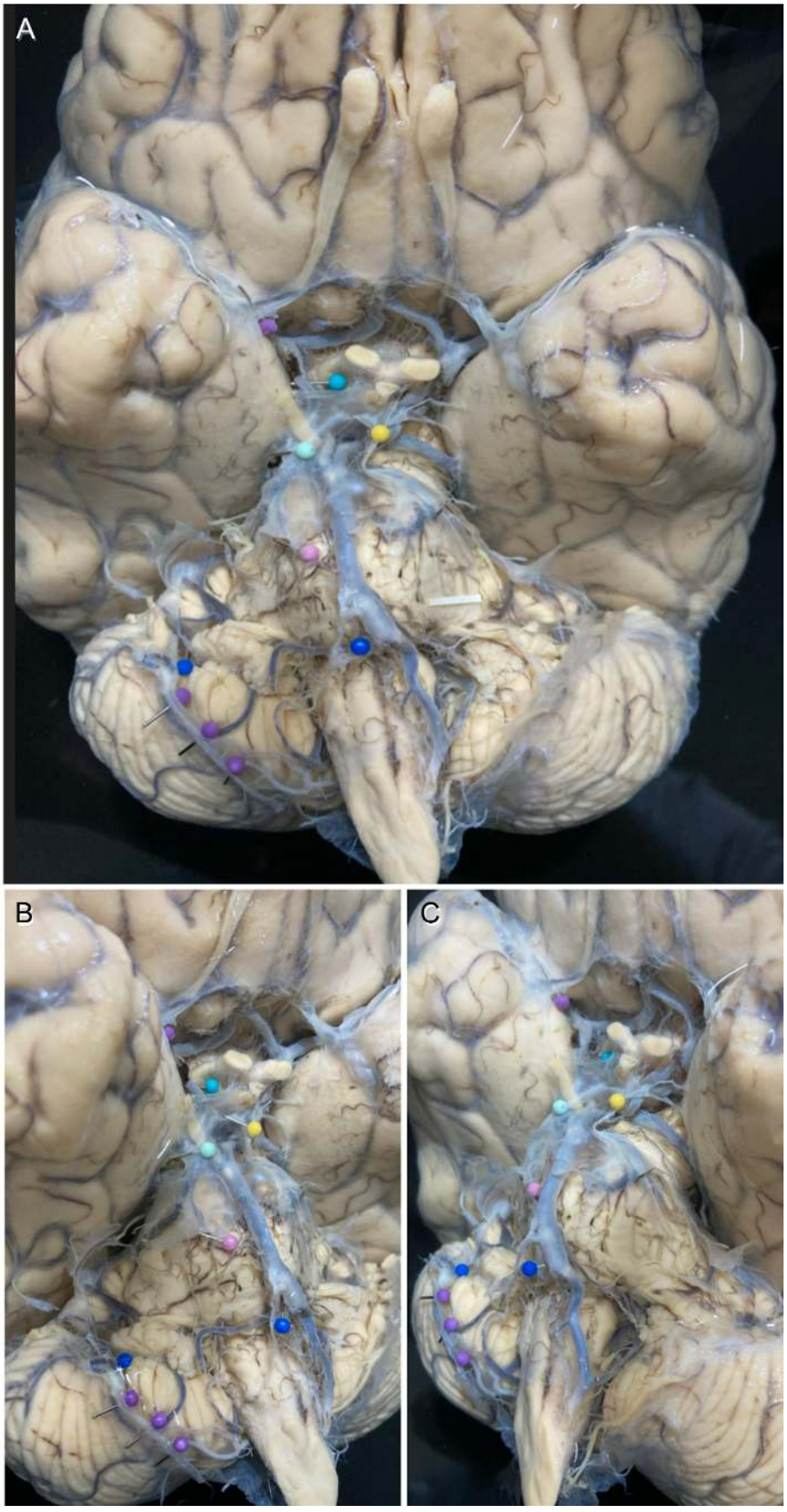

A
B
C

Figure 2. A–C. Non-injected brain specimen is submersed to reconstitute the arachnoidal compartments, and its basal surface is photographed, with arachnoidal layers highlighted using colored pins. The medial sylvian arachnoidal membrane (purple pin), also called proximal sylvian membrane (3), separates the sphenoidal part of the sylvian cistern from the carotid cistern at the anterior incisural space, forming the medialmost limit of the sylvian cistern (6) just lateral to the MCA origin at the ICA. The medial carotid membrane (cyan pin) extends from the lower surface of the chiasm to the oculomotor nerve, partially separating the carotid and chiasmatic cisterns. The Liliequist membrane (yellow pin) is a horizontal sheet surrounding the infundibulum, attaching laterally to the oculomotor nerve and toward the uncus (see also B) (2), separating the carotid and interpeduncular cisterns, while an inner mesencephalic sheet defines the boundary between interpeduncular and prepontine cisterns. The pontomesencephalic membrane (aqua pin) is a horizontal trabecula separating the ambiens and cerebellopontine cisterns, enveloping the trigeminal root proximally and continuous with Meckel's cave. The anterior pontine membrane (pink pin) lies medial to the trigeminal root, vertically oriented, and forms a competent limit between the cerebellopontine angle and prepontine cisterns, arising from its superior attachment at the oculomotor nerve. The medial and lateral cerebellomedullary membranes (blue pins) are horizontal sheets; the medial membrane is best seen at the cerebellomedullary sulcus, sparing the vertebrobasilar junction, while the lateral membrane extends toward the cerebellopontine angle, separating cerebellopontine and cerebellomedullary cisterns. The outer arachnoidal layer of the cisterna magna (three purple pins) attaches along the biventral lobules, separating lateral cisterns from the cisterna magna posterior to the medulla, where the accessory nerve can be seen coursing along its posterolateral extension. Pin color code: purple: medial sylvian membrane; cyan: medial carotid membrane; yellow: Liliequist membrane; aqua: lateral pontomesencephalic membrane; pink: anterior pontine membrane; blue: medial and lateral cerebellomedullary membranes: purple (×3): outer arachnoidal layer of cisterna magna.

Discussion

Photography has long been valuable in producing evidence (9,10). This remains as important in microneurosurgical anatomy today as it was in the nineteenth century (11) when photography was first described. Several techniques now in use—including three-dimensional imaging (12) and underwater photography (9)—were not merely envisioned but already described and applied at that time. However, because technology was not yet mature, these innovations largely disappeared for decades, only to resurface and flourish once technological progress caught up with those early discoveries.

On the face of it, submerging specimens to photograph something like the arachnoidal cisterns seems obvious, but the reasons why it hasn't been standard practice are a combination of tradition, technical bias, and lack of accessible tools.

Traditional neuroanatomical photography was built around SLR cameras, macro lenses, tripods, and flash lighting. Those setups were optimized for dry-photography of specimens and became the standard practice in microneurosurgical labs around the world. While suitable for delivering reliable results in dry conditions (13), this methodology is inherently incapable of overcoming Snell's law, which is often used to describe the technical difficulties of picturing immersed objects at the surface, by describing how light bends when passing between media of different refractive indices (11). In anatomy, the phenomenon of distorting structures explains why even potentially submersible, camera-bearing instruments (ie. endoscopes) have been used in dry-photography settings (3,13) .

Nevertheless, this understanding has motivated a "fully subaquatic" technique were both, camera and specimen are submerged. Although based on sound reasoning—which involves the understanding that water and saline solutions share a refractive index close to that of human tissue, thereby reducing light scattering and improving penetration into deeper structures—immersion also allows delicate fibers, ligaments, fascia (and arachnoidal membranes) to separate and fan out naturally, producing high-definition macro images with enhanced clarity. In addition, clinical evidence indicates that saline immersion can improve visualization of peripheral structures and help evaluate tissue rigidity (14). Outside the scenario of the fish-eye endoscopic perspective, fully subaquatic photography has specific technical demands, that are accentuated in microneuroanatomy. This might include the need for submersible housings for cameras that are pressure-resistant while allowing manual control of focus and exposure underwater, as well as for macro lenses adapted for underwater use, and the requirement that the medium itself receive treatment with dyes or buffers to highlight structures. Besides, lighting is considerably more complex, since water absorbs and shifts wavelengths (reds and oranges especially). Therefore, the fully subaquatic workflow tends to be complex, as handling a fully submerged camera introduces challenges in maneuverability, stability, and fine adjustments requiring training and preparation – and not only this, but steep costs should be listed as the main reasons for its limited usability.

Therefore for decades, the prevailing belief was that immersion would introduce optical complications (reflections, scattering, difficulty controlling lighting), rather than solve them, and researchers trying to confront Snell's law at the surface (camera outside and objects inside fluid) used external lighting tricks, instead of trying to reduce the air–fluid boundary. In truth, traditional camera systems were not as flexible as modern smartphone cameras. These powerful, portable and minimized tools allow for adjusting exposure, locking focus, or compensating for glare without requiring extra gear. The idea of using a smartphone camera with onboard features (grid, level, AE/AF lock, manual exposure, digital zoom, earbud shutter release) simply was not available.

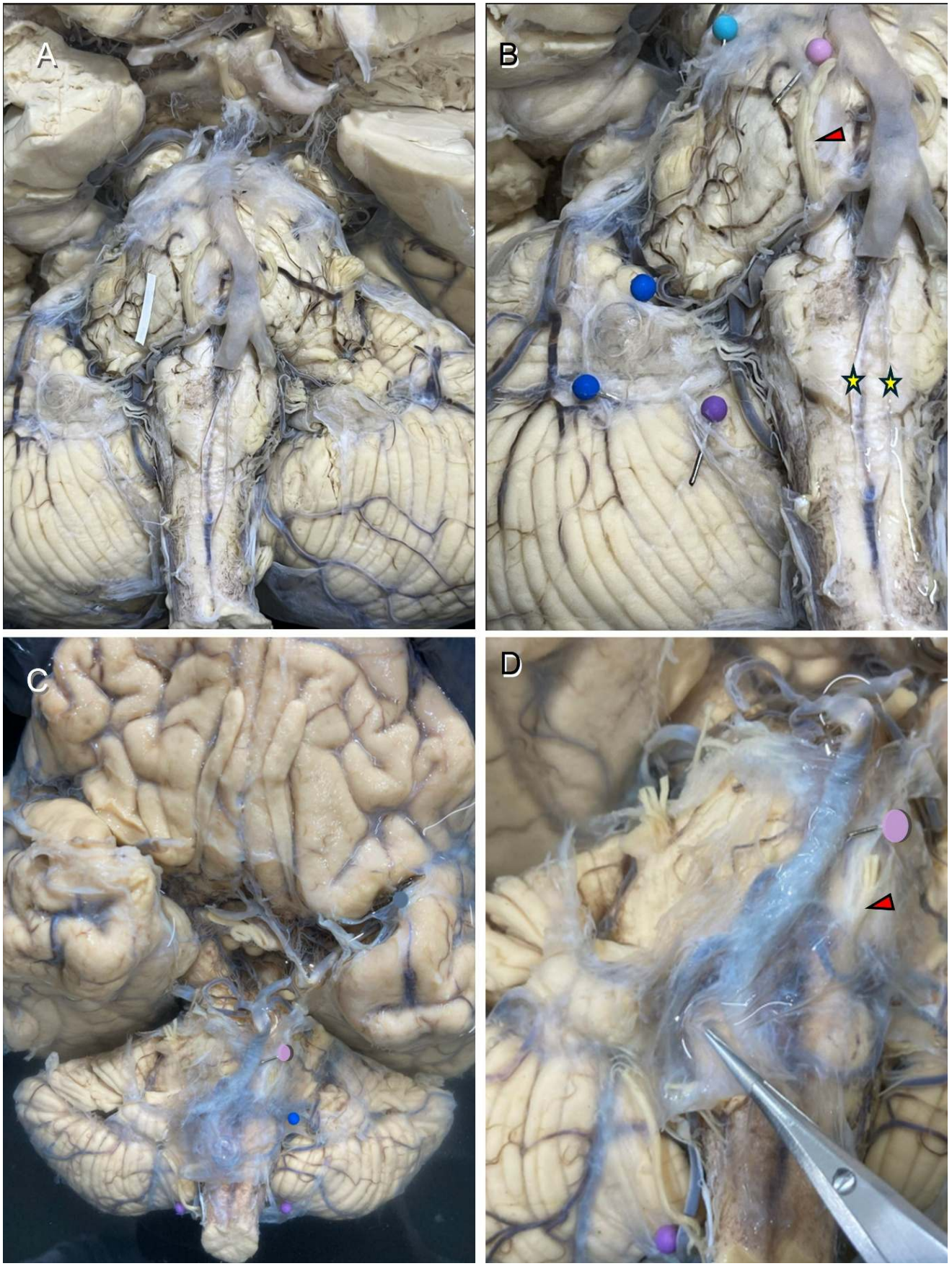

A
B
C
D

Figure 3. A–D. Submersion-based photography of the cisterns has revealed new and significant anatomical information. A-B. Cisterns have been reconstituted by submersion. Note the lateral cerebellomedullary membrane (blue pins) in this specimen. It divides into two leaflets and completely surrounds the cornucopia (7), foramen of Luschka and flocculus, forming an additional arachnoidal compartment (not previously described) just posterior to the cerebellomedullary cistern. An air bubble (between the blue pins) is seen trapped within this additional compartment. Comparison with Figure 1 highlights differences in the attachment of the outer cisterna magna membrane, showing how the size and shape of arachnoidal spaces may influence intracranial pathways and clinical symptoms. C-D. A third specimen with cisterns reconstituted by submersion. In this case a thick outer arachnoidal membrane allowed exploration of the inner limits of the spaces anterior and lateral to the brainstem. The anterior medullary membrane separates the anterior medullary from the cerebellomedullary cistern. This usually thin, vertically oriented membrane has a disputed attachment. It is considered to attach at the dorsal margin of the olive, in front of the CN IX, X and XI (2). Here it attaches superiorly at the level of the abducens nerve (red triangle on D), a fact confirmed by the impossibility to progress the tips of a jeweller's forceps beyond this arachnoidal wall (D). In C, the anterior medullary membranes (yellow stars) lie more medially, unrelated to the abducens nerve or dorsal olive, reflecting the narrow corridor which is the anterior pontine cisterna in this specimen. In both cases, note the relationship of the abducens nerve with the anterior pontine membrane (pink colored pin), as this membrane, in turn, separates the anterior pontine and cerebellopontine cisterns. In D, this membrane is clearly seen as a white linear streak at the site it attaches at the outer membrane and located medial to the nerve. These findings emphasize the need for further studies on the arachnoidal compartments that can benefit from the submersion-based protocol presented here. Pin color code: aqua: lateral pontomesencephalic membrane; pink: anterior pontine membrane; blue: lateral cerebellomedullary membranes; purple: outer arachnoidal layer of cisterna magna.

Our photographic protocol which has been based on fundamentals of shallow-water macrophotography, therefore leverages the smartphone built-in features with careful attention to details as shooting at a 90-degree angle, by positioning the lens perfectly perpendicular (straight down) to the water's surface to minimize light refraction, preventing specimens from looking warped or blurry; to hold specimens near the surface of water and mind the lens distance, keeping it as close to the water's surface as safely possible (to minimize the amount of air space the light has to travel through); and choose a black water container and gloves to create a high-contrast background that - while softening reflection and glare - makes pink and white tissue structures pop visually.

This submersion-based photography protocol minimizes refraction and suppresses reflections. This integrated approach not only stabilizes imaging but also preserves the natural appearance of neuroanatomical structures and bundled together minimized the optical limitations that hindered traditional methods. Our results provide a glimpse of new and exciting neuroanatomical aspects that might have direct impact on how we understand and perform neurosurgery and it helps correct gaps in knowledge advancing the early training of medical professionals.

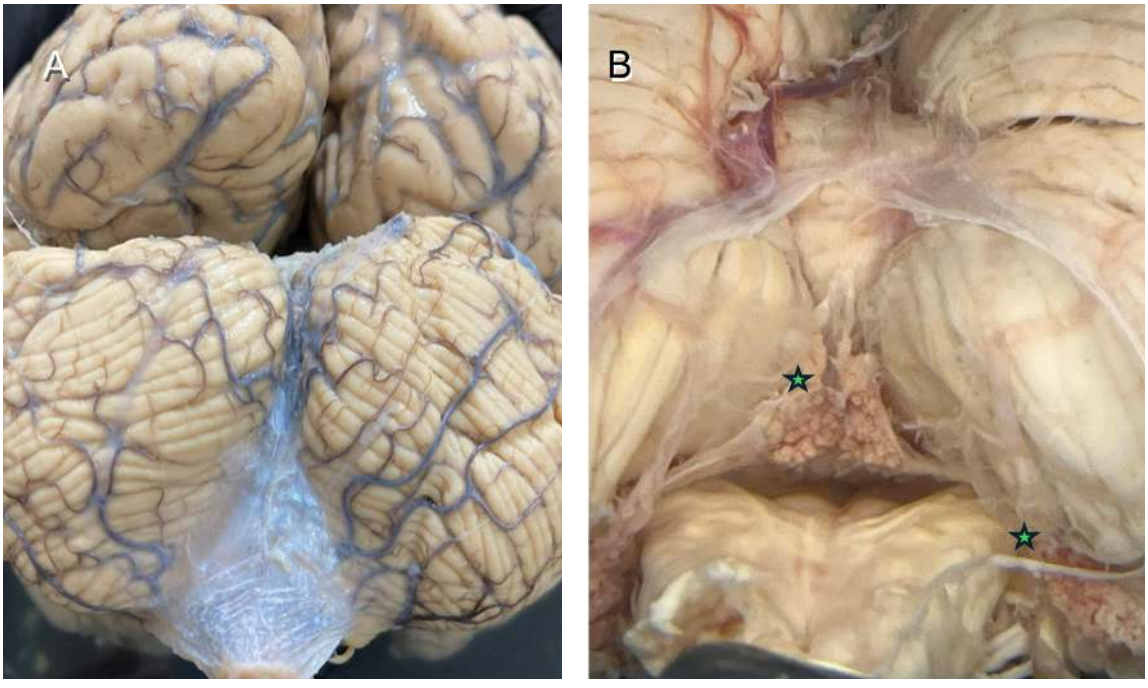


Figure 4. A–B. The cisterna magna is considered a space posterior to the medulla, but when reconstituted by submersion, new understandings of this area arise. Its outer arachnoidal membrane attaches medially at the vermis at the level of the pyramid (as in B). It can also extend superiorly (as in A), to communicate with the superior cerebellar cistern encompassing the tentorial surface of the cerebellum, depending on the prominence of the falx cerebelli (2). The medial attachment of the outer arachnoidal membrane therefore creates a vertical space posterior to the vermis (at the posterior cerebellar incisura) and the posterior surface of the medulla (this being continuous with the posterior spinal cistern). The area between the medial surfaces of the tonsils in this space is called the cerebellar vallecula (8). Laterally the outer arachnoidal membrane attaches to the biventral lobe and approximates the laterally located cerebellomedullary cistern. The lateral dimension of this space may not be fully appreciated in surgery - when bony prominences such as the occipital condyles and jugular tubercles impinge on the area. The inner space within the cisterna magna is further subdivided by the presence of the inferior medullary velum and tela, bearing on its inner side the strands of the choroid plexus of the IV ventricle (green stars). Between the cerebellum and the tela and velum is the cerebellomedullary fissure. Between the floor of the IV ventricle and the tela and velum is the inferior portion of the IV ventricle. This surgically crucial understanding is lost at graduation level – another area greatly benefited by the submersion-based study of the cisterns.

Conclusion

This submersion-based photographic method provides simple, low-cost, and reproducible approach for neuroanatomical documentation and is particularly useful in depicting the arachnoidal cisterns. By approximating intraoperative conditions through submersion, it enables students and residents to visualize and comprehend complex arachnoid structures. This approach may improve anatomical proficiency and facilitate neurosurgical training.

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Isaías Henrique Martins Vasconcelos Norte Bezerra
https://orcid.org/0009-0002-0102-0022
Guilherme Cristiano Garcia
https://orcid.org/0000-0002-7647-3408
Florisvaldo José Morais Vasconcelos Junior
https://orcid.org/0009-0003-0605-1044
Gabriel Melo Duarte Costa
https://orcid.org/0009-0005-8398-458X
Antonio Cavalcanti de Albuquerque Martins
https://orcid.org/0000-0002-1249-8622
Marcelo Moraes Valença
https://orcid.org/0000-0003-0678-3782
Mateus Reghin Neto
https://orcid.org/0000-0003-4448-8231
Eduardo Carvalhal Ribas
https://orcid.org/0000-0003-3054-5661
Hung Tzu Wen
https://orcid.org/0009-0007-4852-3512
Carolina Martins
https://orcid.org/0000-0002-0197-3520

Author contributions: IHMVNB, GCG, FJMVJ, GMDC, CM, ACAM: Data collection and organization, Draft version of the manuscript, literature review; IHMVNB, GCG, FJMVJ, GMDC, MRN, ECR, HTW, MMV, CM, ACAM: agreement to be accountable for all aspects of the work; MRN, ECR, HTW, MMV, CM, ACAM: Reviewing the work critically for important intellectual content, final approval of the version to be published; CM, ACAM: Substantial contributions to the conception of the work; IHMVNB, CM: contributed equally as first authors of this work.
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