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Title: Intravital Longitudinal Imaging of Vascular Dynamics in the Calvarial Bone Marrow

Authors and Affiliations:

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Author Questionnaire

- 1. Microscopy:** Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **No**
- 2. Software:** Does the part of your protocol being filmed include step-by-step descriptions of software usage? **YES, all done**
- 3. Filming location:** Will the filming need to take place in multiple locations? **NO**

Current Protocol Length

Number of Steps: 29

Number of Shots: 57

Introduction

Videographer: Obtain headshots for all authors available at the filming location.

NOTE: Videographer's notes had the protocol as the 1st section. Hence Script is retained that way

- 1.1. **Diana Passaro:** This research focuses on understanding vascular dynamics in the calvarial bone marrow using intravital microscopy, aiming to reveal how blood vessels respond to normal and pathological hematopoiesis, tumor growth, and therapeutic interventions.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What technologies are currently used to advance research in your field?

- 1.2. **Pierre Bourdoncle:** We use advanced imaging technologies like two-photon microscopy, transgenic animal models and live cell tracers to visualize and quantify dynamic vascular changes in living tissue over time.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research gap are you addressing with your protocol?

- 1.3. **Julie Lesieur:** A key advancement is the refinement of our protocol to improve animal welfare, in line with the 3Rs. Our longitudinal approach minimize the surgical steps and significantly reduces the number of animals needed for a study..

1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What advantage does your protocol offer compared to other techniques?

- 1.4. **Thomas Guilbert:** Our protocol addresses the need for non-toxic, long-term intravital imaging by using a two-photon microscope, which allows deep tissue penetration and high-resolution visualization of cell behavior and interaction.

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What significant findings have you established in your field?

- 1.5. **Aleria Duperray Susini:** Using a 3D-printed head implant makes it very easy to find the area of interest during longitudinal imaging sessions, without having to change the height or angle of the holder.

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

What research questions will your laboratory focus on in the the future?

- 1.6. **Diana Passaro:** We are currently using this protocol to trace specific endothelial subtypes and their response to leukemia and drug treatment, to better understand their contribution to disease pathogenesis.

1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Videographer: Obtain headshots for all authors available at the filming location.

Ethics Title Card

This research has been approved by the French Institutional Animal Care and Use Committee (IACUC) “Ministère de l'enseignement supérieur, de la recherche et de l'innovation” under the ethical agreement APAFIS#27215-2020041513522374 v6

Protocol

NOTE: Script Numbering maintained as per videographer's edits

1. Segmentation and Design of Skull Implant Components

Demonstrator: Jozsua Fodor

- 1.1. To begin, launch the segmentation software ~~on a computer system [1]~~, and import the micro-CT (*micro-C-T*) DICOM (*Die-com*) files into to isolate the skull [1].

1.1.1. ~~WIDE: Representative shot of talent launching the software.~~

NOTE: Shot not provided

1.1.2. SCREEN: movie_1.1.2.mp4 00:04-00:33

Video Editor: Please speed up the video

- 1.2. Perform threshold-based segmentation to separate the bone tissue from surrounding structures. Manually adjust complex or noisy regions to refine the segmentation [1].

1.2.1. SCREEN: movie_1.2.1-1.2.2.mp4 00:00-00:24

~~1.2.2. SCREEN: movie_1.2.1-1.2.2.mp4.~~

- 1.3. In 3D Slicer, import the DICOM files and load the dataset [1]. Observe the CT (*C-T*) slices in the **axial**, **sagittal**, and **coronal** slice views [2].

1.3.1. SCREEN: movie_1.3.1-1.3.2.mp4. 00:03-00:10

1.3.2. SCREEN: movie_1.3.1-1.3.2.mp4 00:18-00:27

- 1.4. Open the **Segment Editor** and click **Add** to create a new segmentation then click **Add** again to create a new segment for thresholding [1].

1.4.1. SCREEN: movie_1.4.1.mp4 00:00-00:30

- 1.5. In the **Segment Editor** panel, click on the **Threshold** effect. Adjust the **Lower** and **Upper** threshold sliders or enter numeric values to highlight the segmented region in red [1].

1.5.1. SCREEN: movie_1.5.1.mp4 00:00-00:30

- 1.6. To export the segmented skull as a STL (*S-T-L*) file, switch to the **Segmentation Module** [1]. Ensure the thresholded segment is selected [2]. In the **Export/Import Models** (*Export-Import-Models*) and **Labelmaps** (*Label-maps*) section, set **Export type** to **Models** and **File Format** to **STL** (*S-T-L*) then click **Export** to save the file [3].

1.6.1. SCREEN: movie_1.6.1-1.6.3.mp4 00:00-00:05

1.6.2. SCREEN: movie_1.6.1-1.6.3.mp4 00:06-00:08

Video Editor: Please freeze frame

1.6.3. SCREEN: movie_1.6.1-1.6.3.mp4 00:09-00:35

- 1.7. For preparation of the skull model in CAD (*cad*), first import the simplified skull model in STL format into the CAD software as a new part or model [1]. In the median plane, create an axis tangential to the calvaria then save the prepared skull model [2].

1.7.1. SCREEN: movie_1.7.1-1.7.2.mp4 00:00-00:17

1.7.2. SCREEN: movie_1.7.1-1.7.2.mp4 00:19-00:45, 01:31-01:39

Video Editor: Please speed up the video

- 1.8. Create a sketch in the calvarial plane and design a pear-shaped spline from AP (*A-P*) plus 6.5 to minus 2, with a width of 6 millimeters at AP 0.0 (*A-P-Zero-point-Zero*). This will be the **Observable Surface Contour** [1].

1.8.1. SCREEN: movie_1.8.1.mp4 00:08-00:15, 00:46-01:03, 01:23-01:31, 02:46-02:55, 04:04-04:16

Video Editor: Please speed up the video if necessary

- 1.9. Design the tail of the implant, ensuring it conforms to the skull structure for proper fixation while respecting the available volume [1]. Finally, create a protective cover to safeguard the observation window when not in use [2].

1.9.1. SCREEN: movie_1.9.1.mp4 01:53-02:14

Video Editor: Please speed up the video if necessary

1.9.2. SCREEN: movie_1.9.2.mp4 00:14-00:29, 02:26-02:46, 04:15-04:28

Video Editor: Please speed up the video

2. Preparation of the Mouse for Imaging and Head Fixation

Demonstrator: Aleria Duperray Susini, Julie Lesieur

- 2.1. Place an anesthetized mouse on a heating pad set to 37 degrees Celsius and visually monitor the respiratory rate [1-TXT]. Shave the mouse's head with an electric razor [2]. Then, apply a drop of ophthalmic gel to the eyes of the mouse [3].

2.1.1. Talent places an anesthetized mouse on a heating pad set to 37 °C. **TXT:**

Anesthesia: 4% Isoflurane inhalation; Maintenance: 2% Isoflurane

2.1.2. Talent carefully shaving the fur from the head of the mouse.

2.1.3. Shot of ophthalmic gel being applied to the eyes of the mouse.

- 2.2. Next, swab the top of the scalp with a cotton tissue dipped in disinfectant [1]. Ensure all hair is removed to prevent imaging artifacts and reduce the risk of wound infection [2].

2.2.1. Talent swabbing the scalp with disinfectant.

2.2.2. Shot of completely hair-free scalp after cleaning.

AUTHOR'S NOTE: End of shot

- 2.3. Now use a pair of sterile forceps and scissors to make a small incision in the central portion of the scalp to expose the central bone scar [1-TXT]. Remove the connective tissue between the skull and the scalp [2].
 - 2.3.1. Shot of sterile forceps and scissors being used to make an incision in the central scalp. **TXT: Follow scar to define correct length and width of imaging area**
 - 2.3.2. Talent carefully removing connective tissue with forceps.
- 2.4. Mix sufficient dental cement in a Petri dish to make a paste [1]. Quickly apply it to the bottom of the head fixation implant [2]. Without allowing dental cement to enter the imaging area, place the head holder onto the exposed skull and let it set [3].
 - 2.4.1. Talent mixing dental cement in a Petri dish.
 - 2.4.2. Shot of the dental cement being applied to the bottom of a head fixation implant.
 - 2.4.3. Talent positioning the head holder onto the skull.

3. Microscope Setup, Imaging Acquisition, and Dynamic Feature Measurement

Demonstrator: Aleria Duperray Susini

- 3.1. Switch on the isoflurane flow toward the stereotaxic mask of the microscope [1]. Now, bring the mouse rapidly to the microscope [2]. Carefully insert the mouse's teeth into the mask to ensure proper isoflurane penetration by lifting its nose with one hand [3].
 - 3.1.1. Talent switching on the isoflurane toward the stereotaxic mask.
 - 3.1.2. Talent transporting the mouse to the microscope.
AUTHOR'S NOTE: Use the 2nd part
 - 3.1.3. Shot of the mouse's nose being lifted to fit into the mask.
- 3.2. While holding the mouse with one hand, gently slip the dovetail of the head fixation implant into the fixation holder using the other hand [1]. Secure it with a half-turn of the screw knob [2].
 - 3.2.1. Talent inserting the dovetail into the fixation holder.
AUTHOR'S NOTE: Use Take 2, 2nd part
 - 3.2.2. Shot of the screw knob being turned to secure the implant.
AUTHOR'S NOTE: Shot with 3.2.1
- 3.3. Next, introduce a rectal probe pre-embedded with a water-based gel to monitor the mouse's temperature [1]. Fill the head imaging chamber with a large amount of water-based gel or PBS [2]. Lower the water immersion objective until it is fully immersed for optimal excitation and signal detection during two-photon excitation [3].

- 3.3.1. Talent inserting a rectal probe coated with gel.
AUTHOR'S NOTE: Use Take 2, 2nd part
- 3.3.2. Talent applying water-based gel or PBS into the head imaging chamber.
AUTHOR'S NOTE: Slated 3.3.3, take 2
- 3.3.3. Shot of the Objective lens being lowered into the gel or PBS.
AUTHOR'S NOTE: Use Take 1
- 3.4. Move the x-y (X-Y) stage and the z drive to focus on the central bone in the scaffold scar [1-TXT].
3.4.1. SCREEN: movie_3.4.mkv 00:09-00:37
TXT: Use the lamp as a light source, filtered by tri-band excitation emission filter
- 3.5. Switch off the light in the room and close the box around the microscope stand [1]. After activating the non-descanned detectors, set PMT1 (P-M-T-one) to detect second SHG (S-H-G) at 423 to 461 nm (nanometers), HyD2 (High-D-2) for GFP at 485 to 548 nm and HyD3 for tdTOMATO (t-d-TOMATO) at 551 to 645nm. Leave the offset at 0 [2].
3.5.1. Talent switching off the light and closing the box around the microscope stand.
3.5.2. SCREEN: movie_3.5.2.mkv 00:20-00:58, 01:03-end
- 3.6. Identify the tissue, marked by the bone surface and central vein [1]. Switch off the metal halide lamp and close the microscope cage windows to protect the hybrid detectors and allow the infrared laser to pass [2].
AUTHOR'S NOTE: Move step before 3.5; one shot in dark room and another in plain light, to use accordingly
- 3.6.1. **Added shot: Talent looking at the microscope eyepiece**
AND
~~SCREEN: Tissue with the central vein and bone surface is being seen.~~
NOTE: Shot not provided. Use Added shot instead
- 3.6.2. Talent switching off the metal halide lamp and closing cage windows.
- 3.7. Now set the PMT/SHG (P-M-T-S-H-G) gain to 850 V (volts) and the HyD (high-D) gains at 100% [1]. Increase the infrared laser power until an image with a dynamic range of 200 gray levels is visible on the lowest of the three channels [2-TXT].
3.7.1. SCREEN: movie_3.7.1.mkv 00:00-00:12
3.7.2. SCREEN: movie_3.7.2.mkv 00:04-00:33
TXT: If emission gains are significant, use LUT mode to reduce it
- 3.8. In the acquisition software, locate a region containing bone marrow pockets, indicated encased within the bone surfaces [1].

3.8.1. SCREEN: movie_3.8.1.mkv 00:00-00:15

- 3.9. To identify different regions of interest or ROIs (*R-O-eyes*), activate **LAS NAVIGATOR** (*Las-Navigator*) and create an overview using the **spiral mode** [1-TXT]. Click on the **single image** icon to record selected ROI positions and rename each position in the task list [2].

3.9.1. SCREEN: movie_3.9.1.mkv. 00:02-00:30

TXT: Stop acquisition when scanned area is sufficiently large

3.9.2. SCREEN: movie_3.9.2.mkv 00:01-00:24

- 3.10. To acquire a z-stack volume, select **z-stack mode** and define the **step size** at **3 μ m** (*micrometers*) [1]. Deselect the **same stack size for all regions** option then press **Begin** to start and **End** to finish. After capturing, click **Redefine Stack** [2].

3.10.1. SCREEN: movie_3.10.1-3.10.2.mkv 00:04-00:08, 00:34-00:37

3.10.2. SCREEN: movie_3.10.1-3.10.2.mkv. 00:09-00:11, 01:08-01:20

- 3.11. Press **Start** to start image collection and save the images in the appropriate folder [1]. To measure a dynamic feature, such as vascular permeability, change the **acquisition mode** to **xyzt** (*x-y-z-t*) to acquire a timelapse. In the **t module**, adjust the **time interval** to **3 min** (*minutes*) and the **duration** to **1 h** (*hour*) [2-TXT].

3.11.1. SCREEN: movie_3.11.1.mkv 00:27-00:36

3.11.2. SCREEN: movie_3.11.2.mkv 00:15-00:48

TXT: Do this before dextran injection

- 3.12. Now inject 100 microliters of 70 kilodalton Dextran-TRITC (*Try-T-C*) [1] at a concentration of 4 milligrams per milliliter intravenously and start the acquisition [2-TXT].

3.12.1. Talent injecting Dextran intravenously. **TXT: Monitor the mouse's breathing frequency and temperature frequently**

AUTHOR'S NOTE: Just show needle insertion

3.12.2. SCREEN: movie_3.12.2.mkv. 00:00-00:12

- 3.13. ~~For mouse recovery, transfer the mouse to a surgery mask on a heat pad [1].~~ After transferring mouse to a heated surgery mask, use a sterile swab to gently remove the water-based gel or PBS from the skull [1-TXT]. Close the imaging area of the head implant with the specific cover and carefully secure the cover with a screw [2].

~~3.13.1. Shot of the mouse being moved to a surgery mask on a heat pad-~~

NOTE: Shot deleted by authors

Added shot: gel was added onto skull

3.13.2. Talent using a sterile swab to remove gel or PBS from the skull. **TXT: Apply intrasite gel to skull to maintain moisture between sessions**

3.13.3. Talent placing the specific cover over the imaging area and securing it with a screw.

3.14. Place the mouse into the heating box set to 37 degrees Celsius and wait for it to awaken [1]. Once the mouse has fully recovered, transport it to the animal facility and house it in a clean cage with hydrogel and environmental enrichment [2].

3.14.1. Talent placing the mouse in the heating box.

3.14.2. Shot of Mouse inside a clean cage with hydrogel and enrichment materials.

Added shot: Mouse wandering inside cage

3.15. For longitudinal acquisitions, place a small drop of ophthalmic gel on the eyes of a mouse with a head holder already installed [1-TXT]. Obtain the overview image as demonstrated earlier [2].

3.15.1. Talent applying ophthalmic gel to the mouse's eyes. **TXT: The titanium head implant makes it easy to find the overall area of interest**

3.15.2. SCREEN: movie_3.15.2-3.16.1-3.16.2.mkv. 00:00-00:02

Video Editor: Please freeze frame here

3.16. If necessary, realign the previous and new images using the **Load and Align Image** module [1]. Open the screen capture image to mark positions in their original place [2].

3.16.1. SCREEN: movie_3.15.2-3.16.1-3.16.2.mkv 00:03-00:11

3.16.2. SCREEN: movie_3.15.2-3.16.1-3.16.2.mkv 00:12-00:36, 00:48-00:52

Results

4. Representative Results

- 4.1. The CAD (*cad*) model of a titanium head fixation implant was designed to conform to the anatomical structure of the skull, ensuring stable attachment to the microscope stage for cellular-level imaging [1].
 - 4.1.1. LAB MEDIA: Figure 1. *Video editor: Highlight panel A*
- 4.2. The implant was successfully attached to the mouse skull and enabled stable imaging over time while allowing the mouse to maintain normal activities [1].
 - 4.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight panels A and C*
- 4.3. The calvarial bone marrow vasculature was visualized using a tile scan, revealing a complex network of arterioles, transition capillaries, and sinusoids [1].
 - 4.3.1. LAB MEDIA: Figure 3 A-B. *Video editor: Highlight each white box of A and simultaneously show the corresponding box of B.*
- 4.4. Quantitative vessel analysis demonstrated segmentation of vascular structures, allowing measurement of vessel diameter, length, and straightness, and their correlation [1].
 - 4.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight the vessel segmentation in panel A, then sequentially show graphs of B when VO says "vessel diameter, length and straightness", and their correlation in C*
- 4.5. Longitudinal imaging captured progressive remodeling of calvarial vasculature during leukemia progression, with increased vascular density observed over time [1].
 - 4.5.1. LAB MEDIA: Figure 5. *Video editor: Sequentially show the images of "slice" (but not the z-stack) of Day 4, Day 7 and Day 10 in both Position 1 and Position 2*
- 4.6. Vascular permeability was assessed dynamically, showing differential retention of fluorescent dye over time, indicating changes in barrier integrity [1].
 - 4.6.1. LAB MEDIA: Figure 6B. *Video editor: Sequentially show the images from T1 to T54*

Pronunciation Guide:

1. Intravital

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/intravital>
- **IPA:** /ˌɪn.trəˈvaɪ.təl/
- **Phonetic Spelling:** in-truh-VY-tuhl [Merriam-WebsterMerriam-Webster+4Merriam-Webster+4Merriam-Webster+4](#)

2. Calvarial

- **Pronunciation link:** No confirmed link found
- **IPA:** /kælˈvɛər.i.əl/
- **Phonetic Spelling:** kal-VAIR-ee-uhl [Merriam-Webster+1Merriam-Webster+1Merriam-WebsterLifewire+5Merriam-Webster+5science-products.com+5](#)

3. DICOM

- **Pronunciation link:** No confirmed link found
- **IPA:** /ˈdaɪ.kəm/
- **Phonetic Spelling:** DYE-kom [Harvard Apparatus+4Delta Microscopies+4Adobe Stock+4](#)

4. Sagittal

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/sagittal>
- **IPA:** /ˈsædʒ.ɪ.təl/
- **Phonetic Spelling:** SAJ-ih-tuhl [Merriam-WebsterMerriam-Webster+15Merriam-Webster+15Merriam-Webster+15](#)

5. Coronal

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/coronal>
- **IPA:** /kəˈroʊ.nəl/
- **Phonetic Spelling:** kuh-ROH-nuhl [Merriam-Webster+2Merriam-Webster+2Merriam-Webster+2](#)

6. STL

- **Pronunciation link:** No confirmed link found
- **IPA:** /ˌɛs.tiːˈɛl/
- **Phonetic Spelling:** ess-tee-ELL [Merriam-Webster+1Merriam-Webster+1](#)

7. CAD

- **Pronunciation link:** No confirmed link found
- **IPA:** /ˌsiː.ərˈdiː/

- **Phonetic Spelling:** see-ay-DEE [Merriam-Webster+27Merriam-Webster+27Merriam-Webster+27](#)

8. Calvaria

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/calvaria>
- **IPA:** /kæl'vɛər.i.ə/
- **Phonetic Spelling:** kal-VAIR-ee-uh [Merriam-Webster](#)

9. Ophthalmic

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/ophthalmic>
- **IPA:** /ɑf'θæl.mɪk/
- **Phonetic Spelling:** ahf-THAL-mik [Merriam-Webster+4Merriam-Webster+4Merriam-Webster+4Merriam-Webster+3Merriam-Webster+3Merriam-Webster+3](#)

10. Isoflurane

- **Pronunciation link:** No confirmed link found
- **IPA:** /,aɪ.sʊ'flʊr.eɪn/
- **Phonetic Spelling:** eye-so-FLOOR-ane [Merriam-Webster+15Merriam-Webster+15Merriam-Webster+15science-products.com+5Pinterest+5Merriam-Webster+5](#)

11. Stereotaxic

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/stereotaxic>
- **IPA:** /,stɛr.i.ʊ'stæk.sɪk/
- **Phonetic Spelling:** STER-ee-oh-TAK-sik [Merriam-Webster](#)

12. Dovetail

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/dovetail>
- **IPA:** /'dʌv.teɪl/
- **Phonetic Spelling:** DUHV-tail

13. PBS

- **Pronunciation link:** No confirmed link found
- **IPA:** /piː.biː'ɛs/
- **Phonetic Spelling:** pee-bee-ESS

14. SHG

- **Pronunciation link:** No confirmed link found

- **IPA:** /,ɛs.ertʃ'dʒi:/
- **Phonetic Spelling:** ess-aych-JEE

15. GFP

- **Pronunciation link:** No confirmed link found
- **IPA:** /,dʒi:.ɛf'pi:/
- **Phonetic Spelling:** gee-eff-PEE

16. tdTOMATO

- **Pronunciation link:** No confirmed link found
- **IPA:** /,ti:.di:.toʊ'meɪ.toʊ/
- **Phonetic Spelling:** tee-dee-toh-MAY-toh [Merriam-Webster+6ResearchGate+6MDPI+6](#)

17. HyD

- **Pronunciation link:** No confirmed link found
- **IPA:** /,haɪ'di:/
- **Phonetic Spelling:** high-DEE [Harvard Apparatus+4Delta Microscopies+4Adobe Stock+4Merriam-Webster](#)

18. PMT

- **Pronunciation link:** No confirmed link found
- **IPA:** /,pi:.ɛm'ti:/
- **Phonetic Spelling:** pee-em-TEE [Merriam-Webster](#)

19. Infrared

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/infrared>
- **IPA:** /,ɪn.frə'red/
- **Phonetic Spelling:** in-fruh-RED [Merriam-Webster](#)

20. ROI

- **Pronunciation link:** No confirmed link found
- **IPA:** /,ɑ:r.oʊ'aɪ/
- **Phonetic Spelling:** ar-oh-EYE

21. LAS Navigator

- **Pronunciation link:** No confirmed link found
- **IPA:** /'læs 'næv.ɪ.geɪ.tər/
- **Phonetic Spelling:** lass NAV-ih-gay-tor [Merriam-Webster+1Merriam-Webster+1](#)

22. Z-stack

- **Pronunciation link:** No confirmed link found
- **IPA:** /'zi: stæk/
- **Phonetic Spelling:** zee stack

23. xyzt

- **Pronunciation link:** No confirmed link found
- **IPA:** /,ɛks.waɪ.zi:'ti:/
- **Phonetic Spelling:** eks-why-zee-TEE

24. Dextran-TRITC

- **Pronunciation link:** No confirmed link found
- **IPA:** /'dɛk.stræn 'traɪ.tɪk/
- **Phonetic Spelling:** DEK-stran TRY-tik

25. Kilodalton

- **Pronunciation link:** No confirmed link found
- **IPA:** /'kɪl.ɒʊ.dəl.tən/
- **Phonetic Spelling:** KILL-oh-dawl-tuhn

26. Intravital

- **Pronunciation link:** No confirmed link found
- **IPA:** /'ɪn.trəˌsaɪt/
- **Phonetic Spelling:** IN-truh-syte

27. Arterioles

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/arteriole>
- **IPA:** /ɑːr'tɪr.i.əʊlz/
- **Phonetic Spelling:** ar-TEER-ee-ohlz

28. Sinusoids

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/sinusoid>
- **IPA:** /'sɪn.ju.sɔɪdz/
- **Phonetic Spelling:** SIN-yoo-soydz

29. Leukemia

- **Pronunciation link:** <https://www.merr>

