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Title: Intravital Imaging of Attenuated Auxotrophic *Mycobacterium tuberculosis* in Murine Lung during Early Intravascular Infection

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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**

4.5.1, 4.6.1

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **No**

Videographer: Please film the computer screen for 4.5.1 and 4.6.1

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

The locations are in the same building: Rooms 208, 211, and 569.

4. Testimonials (optional): Would you be open to filming two short testimonial statements **live during your JoVE shoot**? These will **not appear in your JoVE video** but may be used in JoVE's promotional materials. **Yes**

Current Protocol Length

Number of Steps: 40

Number of Shots: 80

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

INTRODUCTION:

~~REQUIRED: Summarize your research focus and the main questions it aims to answer.~~

- 1.1. **Yookyung Jung:** Our research visualizes early tuberculosis bacilli and host-cell interactions in the living mouse lung.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the research gap or limitation in existing methods and how this protocol addresses them.~~

- 1.2. **Yookyung Jung:** Previous imaging methods required strict biosafety containment. Our protocol enables imaging by using a safe, attenuated strain and a permanent lung window.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 4.2.1*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Yookyung Jung:** Using this protocol, researchers can directly track bacterial dissemination, aggregation, and macrophage uptake at single-cell resolution over multiple days.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Describe the most important consideration or challenge when performing this protocol.~~

- 1.4. **Yookyung Jung:** The key challenge while performing this protocol is achieving window transparency with minimal glue while firmly stabilizing the mouse to prevent motion artifacts.

- 1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.14.2.*

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

- 1.5. **Yookyung Jung:** Future studies can explore bacterial mutants, immune clearance mechanisms, and real-time visualization of immune-mediated bacterial killing.

- 1.5.1. 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.

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

What problem in your field of research does your JoVE article address?

- 1.6. **Yookyung Jung, Research Assistant Professor:** (authors will present their testimonial statements live)
 - 1.6.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

How does the video format of your article help you to share your work with the broader scientific community?

- 1.7. **Yookyung Jung, Research Assistant Professor :** (authors will present their testimonial statements live)
 - 1.7.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera.

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Albert Einstein College of Medicine

Protocol

***Videographer:** Please always record an audio slate while filming each shot mentioned in the shotlist.*

2. Construction of the BSL-2-Approved tdTomato-Expressing Mtb Strain mc²8471

Demonstrator: Catherine Vilchèze

- 2.1. To begin, inoculate 100 microliters of mc²7902 (*em-see square-seven-nine-zero-two*) transformed with plasmid pYUB1169 (*P-Yub-Eleven-Sixty-Nine*) into 10 milliliters of sterile Middlebrook 7H9 supplemented [1] with the presented components in a 30-milliliter inkwell bottle [2].
 - 2.1.1. WIDE: Talent inoculating mc²7902 into the supplemented sterile Middlebrook 7H9 in a 30-milliliter inkwell bottle. ***Videographer:** Please obtain a CU shot as well for this shot.*
 - 2.1.2. TEXT ON A PLAIN BACKGROUND
OADC enrichment (10% (v/v))
Glycerol (0.2% (v/v))
D-pantothenate (24 mg/L)
L-leucine (50 mg/L)
L-arginine (200 mg/L)
Sodium propionate (0.1 mM)
Tyloxapol (0.05% (v/v))
- 2.2. Incubate the culture with slow shaking at 37 degrees Celsius until it reaches an optical density at 600 nanometers of 0.7 to 1.0 [1].
 - 2.2.1. Talent placing the culture for incubation with slow shaking.
- 2.3. Then, transfer the cells into a 15-milliliter conical centrifuge tube [1]. To wash the cells, centrifuge the tube at 3,000 *g* for 10 minutes at room temperature [2]. Once the supernatant is discarded, resuspend the cells in 10 milliliters of sterile 10 percent glycerol containing 0.05 percent tyloxapol [3].
 - 2.3.1. Talent transfers the cells into a 15-milliliter conical centrifuge tube.
 - 2.3.2. Talent placing the tube into the centrifuge.
 - 2.3.3. Talent shows the pellet and resuspending it in sterile 10 percent glycerol containing 0.05 percent volume per volume tyloxapol.

- 2.4. After completing the 2nd wash, resuspend the cell pellet in 0.175 milliliters of 10 percent glycerol containing tyloxapol [1] and add 2 microliters of pYUB1169 DNA [2].
 - 2.4.1. Talent discards the supernatant, shows the pellet, and adds 10 percent glycerol containing 0.05 percent volume per volume tyloxapol.
 - 2.4.2. Talent adding pYUB1169 deoxyribonucleic acid to the resuspended cells.
- 2.5. Next, transfer mc²7902 and pYUB1169 into a 0.2-centimeter electroporation cuvette and wait 10 minutes [1]. After removing air bubbles, electroporate at 2,500 millivolts, 1,000 ohms, 25 microfarads, and a time constant of 20 milliseconds at room temperature [2].
 - 2.5.1. Talent transferring mc²7902 and pYUB1169 into a 0.2-centimeter electroporation cuvette.
 - 2.5.2. Talent placing the cuvette in the electroporator and sets the parameters.
- 2.6. Then, add 1 milliliter of sterile enriched Middlebrook 7H9 medium [1]. Transfer the contents of the cuvette to a 15-milliliter conical centrifuge tube [2] and incubate on a shaker at 37 degrees Celsius overnight [3].
 - 2.6.1. Talent adding sterile supplemented Middlebrook 7H9 to the electroporation cuvette.
 - 2.6.2. Talent transferring the contents of the cuvette into a 15-milliliter conical centrifuge tube. Videographer's NOTE: 2.6.2 clip C112_C338_0521VJ_001 slate labeled as 2.6.1-.2 but 2.6.1-.3 was actually filmed.
 - 2.6.3. Talent placing the tube for incubation with shaking at 37 degrees Celsius overnight.
- 2.7. Plate 0.1 milliliters of the transformation mixture onto an enriched Middlebrook 7H10 agar plate containing kanamycin [1]. Centrifuge the remaining transformation mixture [2], discard 0.9 milliliters of the supernatant [3], and resuspend the cell pellet in the residual supernatant [4].
 - 2.7.1. Talent plating the transformation mixture onto a supplemented Middlebrook 7H10 agar plate.
 - 2.7.2. Talent placing the remaining transformation mixture into the centrifuge.
 - 2.7.3. Talent discarding 0.9 milliliters of supernatant from the tube.
 - 2.7.4. Talent resuspending the cell pellet in the residual supernatant.
- 2.8. Plate the resuspended cells onto a second Middlebrook 7H10 agar plate containing the

same supplements and antibiotics [1]. Incubate the plates at 37 degrees Celsius for 3 to 4 weeks [2].

2.8.1. Talent plating the resuspended cells onto a second supplemented Middlebrook 7H10 agar plate. **NOTE: 2.8.1 includes A and B shots**

2.8.2. Talent placing the agar plates for incubation at 37 degrees Celsius.

2.9. Using a sterile loop, pick one isolated pink colony and inoculate it into 5 milliliters of sterile Middlebrook 7H9 supplemented with the same components [1].

2.9.1. Talent picking an isolated pink colony and adding it to a 30-milliliter inkwell bottle.

2.10. To prepare mc²8471 for intravenous infection, grow 10 milliliters mc²8471 in enriched sterile Middlebrook 7H9 medium in a 30-milliliter inkwell bottle until mid-log phase [1].

2.10.1. Talent placing a 30-milliliter inkwell bottle into the incubator.

2.11. Transfer the cells into a 15-milliliter conical centrifuge tube [1] and wash them two times by centrifugation as demonstrated earlier [2].

2.11.1. Talent transferring the culture into a 15-milliliter conical centrifuge tube.

2.11.2. Talent placing the tube into the centrifuge.

2.12. Then, resuspend the washed cell pellet in 4 milliliters of PBS containing 0.05 percent tyloxapol [1]. Sonicate the cells at 100 watts with 2 pulses of 1,000 joules [2].

2.12.1. Talent resuspending the cell pellet in phosphate-buffered saline containing 0.05 percent volume per volume tyloxapol.

2.12.2. Talent soniciating the cells using a sonicator.

2.13. Measure the optical density of the culture [1]. Dilute the culture in PBS containing 0.05 percent tyloxapol to a final concentration of 5×10^6 colony forming units per injection [2].

2.13.1. Talent measuring the optical density of the culture. **NOTE: 2.13.1 includes A and B shots.**

2.13.2. Talent diluting the culture to the specified final concentration.

3. Permanent Lung Window Implantation in Mice for Intravital Imaging

- 3.1. Before starting the procedure, verify that the anesthetized mouse has reached an adequate depth of anesthesia [1-TXT].
 - 3.1.1. Talent assessing the depth of anesthesia before proceeding. **TEXT: Anesthesia: 5% Isoflurane**
- 3.2. After injecting pre-operative analgesia and applying ophthalmic ointment to both eyes [1], tie a 2-0 silk suture around the base of a 22-gauge catheter, leaving approximately 2-inch tails for securing the catheter [2].
 - 3.2.1. Shot of the mouse after applying ophthalmic ointment.
 - 3.2.2. Talent tying a silk suture around the base of a 22 gauge catheter with extended tails.
- 3.3. Then, intubate the mouse with the suture-tied catheter [1]. Visualize the tracheal opening by transillumination [2] and confirm placement using an inflation bulb by observing bilateral chest rise [3].
 - 3.3.1. Talent inserting the suture-tied catheter into the mouse for intubation.
 - 3.3.2. Talent visualizing the tracheal opening using transillumination.
 - 3.3.3. Talent confirming catheter placement using an inflation bulb while observing chest rise.
- 3.4. To secure the intubation catheter, tie the 2-0 silk suture around the snout after successful intubation [1]. Connect the catheter to the ventilator [2].
 - 3.4.1. Talent tying a 2-0 silk suture around the snout to secure the intubation catheter.
 - 3.4.2. Talent connecting the catheter to the ventilator.
- 3.6. Place the mouse in right lateral decubitus on a heated surgical platform to expose the left thorax [1]. Secure the forelimbs, hindlimbs, and back with tape to maintain proper positioning and stability throughout the procedure [2]. **NOTE: This step is placed here as per the author's request**
 - 3.6.1. Talent positioning the mouse in right lateral decubitus on a heated surgical platform.
 - 3.6.2. Talent taping the hindlimbs and the back to maintain stable positioning.
- 3.5. Next, remove hair from the left upper thorax using depilatory cream under 3%

isoflurane anesthesia for the remainder of the procedure [1].

3.5.1. Talent applying depilatory cream to the left upper thorax to remove hair.

~~3.6. Place the mouse in right lateral decubitus on a heated surgical platform to expose the left thorax [1]. Secure the forelimbs, hindlimbs, and back with tape to maintain proper positioning and stability throughout the procedure [2]. (Please move whole 3.6 (shot 3.6.1 and 3.6.3 before 3.5.)~~ **NOTE: This step is moved before 3.5**

~~3.6.1. Talent positioning the mouse in right lateral decubitus on a heated surgical platform.~~

~~3.6.2. Talent taping the hindlimbs and the back to maintain stable positioning.~~

3.7. Prepare the surgical field with an antiseptic solution [1]. Make a 5–10-millimeter circular incision with a biopsy punch [2], 7 millimeters left of the sternum and above the subcostal margin, centered over the 6th–7th intercostal space [3].

3.7.1. Talent applying an antiseptic solution to the surgical field.

3.7.2. Talent lifting the skin at the surgical site and creating a circular incision. **3.7.2-.3 The author thought these were the "same step".**

3.7.3. Shot of the created incision at the specified location over the 6th–7th intercostal space

3.8. Inspect the exposed field for superficial vessels and remove the overlying soft tissue to clearly expose the ribs before entering the thoracic cavity [2].

3.8.1. Talent examines the exposed field for superficial vessels and removes overlying soft tissue.

3.9. Grasp and elevate the sixth or seventh rib with forceps [1]. Then, gently pierce the intercostal muscle between them using blunt micro-dissecting scissors, facing the lung to enter the thoracic cavity [2].

3.9.1. Talent grasping and elevating a rib with forceps.

3.9.2. Talent inserting blunt micro-dissecting scissors to pierce the intercostal muscle and enter the thoracic cavity.

3.10. Apply brief, gentle bursts of compressed air at the intercostal opening to temporarily deflate the adjacent lung and separate it from the chest wall before creating the window [1].

3.10.1. Talent deflating the lung and separating it from the chest wall.

3.11. Next, position the biopsy punch on the cutting tool and guide it through the intercostal opening while keeping it parallel to the chest wall [1]. Punch a 5-millimeter circular defect in the rib cage [2].

3.11.1. Talent positioning the biopsy punch on the cutting tool and guiding it through the intercostal opening parallel to the chest wall.

3.11.2. Talent creating a circular defect in the rib cage using the biopsy punch.

3.12. Place a 5-0 silk purse-string suture approximately 1 millimeter from the edge of the circular defect, passing it between adjacent ribs to encircle the opening [1].

3.12.1. Talent placing a purse-string suture around the circular defect and threading it between adjacent ribs to encircle the opening.

3.13. Then, seat the window frame so the edges of the chest wall defect fit into the groove of the frame [1]. Tighten and secure the purse-string suture to lock the frame in place [2].

3.13.1. Talent positioning the window frame onto the chest wall defect.

3.13.2. Talent tightening and securing the suture around the frame.

3.14. Dry the lung surface with a continuous, gentle stream of compressed air until it appears matte [1]. Using a 1-milliliter syringe, apply a thin layer of cyanoacrylate adhesive to the undersurface of the frame [2]. Gently lift the frame to separate it from the lung [3].

NOTE: The VO is edited for the rearranged shots

3.15.1. Talent directing a gentle stream of compressed air to dry the lung surface. **NOTE: This shot is moved here as per the authors' request**

3.14.1. Talent applying adhesive to the underside of the frame.

3.14.1 Talent lifting the window frame slightly away from the lung surface. **NOTE: NOTE: This shot is moved here as per the authors' request**

~~3.15. Dry the lung surface with a continuous, gentle stream of compressed air until it appears matte [1].~~

3.15.1. Talent directing a gentle stream of compressed air to dry the lung surface.

3.16. Using positive end expiratory pressure, inflate the lung and attach the window to the

frame [1]. Release the positive end expiratory pressure and keep pressure on the window frame for 20 to 30 seconds [2].

3.16.1. Talent inflating and adhering the window to the frame.

3.16.2. Talent releasing the PEEP and pressing on the window frame.

3.17. Place a second 5-0 silk purse-string suture less than 1 millimeter from the skin incision edge [1]. Tuck the skin beneath the frame, ensuring excess skin is captured under the window frame or trimmed away [2]. Cinch the purse-string suture tightly and tie securely [3].

3.17.1. Talent placing a purse-string suture near the skin incision edge.

3.17.2. Talent tucking the skin beneath the window frame and adjusting excess skin under the frame.

3.17.3. Talent tightening and securing the suture.

~~3.18. Apply a small amount of liquid cyanoacrylate at the interface between the coverslip and the metal frame to reinforce the airtight seal [1].~~

~~3.18.1. Talent applying liquid cyanoacrylate between the coverslip and the metal frame.~~

NOTE: This step is moved after 3.20

3.19. Using a vacuum pickup, apply a very thin layer of adhesive to the underside of the 5-millimeter coverslip and scrape off excess on the edge of a rectangular cover glass [1]. Dry the lung surface again with a continuous, gentle stream of compressed air [2].

3.19.1. Talent applying adhesive to the underside of the coverslip with a vacuum pickup and removing excess on a cover glass edge.

3.19.2. Talent directing compressed air to dry the lung surface.

3.20. Position the circular coverslip within the recess of the window frame at an angle above the lung surface [1]. Briefly increase airway pressure to inflate the lung, then rotate the coverslip parallel to the glass so the lung contacts the glass [2]. Hold gentle pressure for 25 seconds until the adhesive sets, then detach the vacuum pickup [3].

3.20.1. Talent positioning the coverslip at a shallow angle within the window frame.

3.20.2. Talent increasing airway pressure and rotating the coverslip into a parallel position against the lung surface.

3.20.3. Shot of the lung under airway pressure and talent removing the vacuum pickup.

- 3.18. Apply a small amount of liquid cyanoacrylate at the interface between the coverslip and the metal frame to reinforce the airtight seal [1]. **NOTE: This step is placed here as per author's request**

3.18.1. Talent applying liquid cyanoacrylate between the coverslip and the metal frame.

- 3.21. Insert a 1 milliliter insulin syringe below the xiphoid process, advance toward the left shoulder through the diaphragm into the thoracic cavity, and gently aspirate to remove residual air [1].

3.21.1. Talent inserting the needle below the xiphoid process, advancing toward the left shoulder and aspirating the air.

- 3.22. Turn off isoflurane and continue ventilation with 100 percent oxygen until a near-normal waking respiratory rate is resumed [1].

3.22.1. Talent turning off isoflurane and maintaining ventilation with 100 percent oxygen.

- 3.23. Remove the securing suture, extubate the mouse [1], and place it in a clean, warmed cage for recovery [2]. Monitor the animal until fully ambulatory and euthanize according to institutional guidelines if normal respiration is not regained [3].

3.23.1. Talent removing the securing suture and extubating the mouse.

3.23.2. Talent placing the mouse into a clean, warmed cage.

3.23.3. Talent monitoring the animal during recovery.

4. Intravital Imaging of *Mycobacterium tuberculosis* Strain in Murine Lung

- 4.1. One to five days after surgery, prepare the animal for intravital imaging [1-TXT]. ~~Maintain body temperature at approximately 37 degrees Celsius using a forced air heated chamber with a temperature sensor feedback loop [2].~~

4.1.1. Shot of the anesthetized mice placed on the surgical area. **TXT: Anesthesia: 3% isoflurane**

4.1.2. ~~Talent positioning the mouse in a forced air heated chamber with temperature control.~~ **NOTE: This shot is moved after 4.2.2**

- 4.2. Insert the custom stage plate-window frame adapter between the skin and the outer rim of the lung window frame [1]. Secure a standard microscope stage plate using adhesive tape [2]. Maintain body temperature at approximately 37 degrees Celsius

using a forced air heated chamber with a temperature sensor feedback loop [3]. **NOTE: The VO is edited for the moved shot**

4.2.1. Talent inserting the custom stage plate-window frame adapter between the skin and window frame.

4.2.2. Talent securing the microscope stage plate with adhesive tape.

4.1.2. Talent positioning the mouse in a forced-air heated chamber with temperature control. **NOTE: This shot is placed here as per the authors' request**

4.3. Place the mouse with the attached stage plate-window frame adapter onto the microscope translation stage [1]. ~~Fix the stage plate-window frame adapter in place using paper tape [2].~~ **NOTE: The VO is edited for the deleted shot**

4.3.1. Talent positioning the mouse on the microscope translation stage. **NOTE: 4.3.1 includes A and B shots.**

~~4.3.2. Talent securing the adapter with paper tape.~~ **NOTE: This shot was not filmed. This procedure was already done in the previous steps.**

4.4. Gently pull back the skin around the eye on the injection side to expose the medial canthus [1]. Inject 100 microliters of fluorescein isothiocyanate dextran retro-orbitally to label the vasculature [2].

4.4.1. Talent retracting the skin around the eye to expose the medial canthus.

4.4.2. Talent performing retro-orbital injection of fluorescein isothiocyanate dextran.

4.5. Acquire snapshot, z-stack, or time-lapse images of the lung vasculature prior to bacterial injection using appropriate parameters [1].

4.5.1. SCREEN: Display acquisition of lung vasculature images showing snapshot, z-stack, and time-lapse capture modes.

Videographer: Please film the computer screen for all shots labeled as SCREEN

4.6. Similarly, acquire images of tuberculosis, fluorescently labeled cells, and vasculature following injection of 5×10^6 CFU of tdTomato-expressing mc²8471. Adjust detector gain to maximize signal without saturation [1].

4.6.1. SCREEN: Display images of tuberculosis, labeled cells and vasculature. Show adjustment of detector gain settings to optimize signal without saturation.

4.7. After imaging, carefully remove the mouse from the microscope, maintain it in a warm

environment, and continuously monitor it until it has fully recovered [1].

4.7.1. Talent removing the mouse from the microscope and placing the mouse in a warm environment.

Results

5. Results

- 5.1. Baseline images of the lung vasculature were acquired before infection [1]. Within a few minutes following intravenous infection with mc²8471, bacilli were observed within blood vessels, illustrating rapid localization in the lung vasculature [2].
 - 5.1.1. LAB MEDIA: Figure 1A.
 - 5.1.2. LAB MEDIA: Figure 1B-D. *Video editor: Highlight the bright pink spots indicated by arrows inside the vessel structures.*
- 5.2. At 1-hour post-infection, bacillary fluorescent signal remained stationary and associated with the pulmonary vasculature, appearing either as discrete puncta or as larger clustered structures [1].
 - 5.2.1. LAB MEDIA: Figure 2A. *Video editor: Highlight the pink signal.*
- 5.3. By 6 hours post-infection, bacilli were widely disseminated throughout the lung parenchyma in regions where no macrophages or blood vessels were visible [1]. By 3 days post-infection, bacilli were markedly reduced compared with the 6-hour time point [2].
 - 5.3.1. LAB MEDIA: Figure 2B.
 - 5.3.2. LAB MEDIA: Figure 2C.
- 5.4. At 3 days post-infection, bacteria were found predominantly within macrophages, with multiple bacilli present per cell [1].
 - 5.4.1. LAB MEDIA: Figure 3A-C. *Video editor: Highlight the pink signals located inside the blue cell shapes.*
- 5.5. High-resolution imaging confirmed bacteria within macrophages in complementary datasets acquired using two-photon microscopy [1].
 - 5.5.1. LAB MEDIA: Figure 4A-C. *Video editor: Highlight the pink signals inside the blue cell regions indicated by arrows.*

1. Inoculate
Pronunciation link: <https://www.merriam-webster.com/dictionary/inoculate>
IPA: /ɪˈnɑː.kjəˌleɪt/
Phonetic Spelling: ih·nah·kyuh·layt
2. Plasmid
Pronunciation link: <https://www.merriam-webster.com/dictionary/plasmid>
IPA: /ˈplæz.mɪd/
Phonetic Spelling: plaz·mɪd
3. Middlebrook
Pronunciation link: No confirmed link found
IPA: /ˈmɪd.əl.brʊk/
Phonetic Spelling: mid·uhl·brook
4. Supplemented
Pronunciation link: <https://www.merriam-webster.com/dictionary/supplement>
IPA: /ˈsʌp.ləˌmen.tɪd/
Phonetic Spelling: sup·luh·men·tuhd
5. OADC
Pronunciation link: No confirmed link found
IPA: /oo ɛɪ diː siː/
Phonetic Spelling: oh·ay·dee·see
6. Glycerol
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycerol>
IPA: /ˈglɪs.əˌrɔːl/
Phonetic Spelling: gliss·uh·rawl
7. D-pantothenate
Pronunciation link: <https://www.merriam-webster.com/dictionary/pantothenate>
IPA: /pænˈtɑː.θəˌneɪt/
Phonetic Spelling: pan·tah·thuh·nayt
8. L-leucine
Pronunciation link: <https://www.merriam-webster.com/dictionary/leucine>
IPA: /ˈluː.siːn/
Phonetic Spelling: loo·seen
9. L-arginine
Pronunciation link: <https://www.merriam-webster.com/dictionary/arginine>
IPA: /ˈɑːr.dʒəˌniːn/
Phonetic Spelling: ar·juh·neen
10. Sodium propionate
Pronunciation link: <https://www.merriam-webster.com/dictionary/propionate>
IPA: /proʊˈpiː.əˌneɪt/
Phonetic Spelling: proh·pee·uh·nayt
11. Tyloxapol
Pronunciation link: <https://www.howtopronounce.com/tyloxapol>
IPA: /taɪˈlɒk.səˌpɒl/
Phonetic Spelling: tie·lok·suh·pol

12. Optical density
 Pronunciation link: <https://www.merriam-webster.com/dictionary/optical>
 IPA: /'ɑ:p.tɪ.kəl/ ; /'den.sə.ti/
 Phonetic Spelling: op·tih·kuhl ; den·suh·tee
13. Supernatant
 Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
 IPA: /,su:.pər'nei.tənt/
 Phonetic Spelling: soo·per·nay·tuht
14. Electroporation
 Pronunciation link: <https://www.merriam-webster.com/dictionary/electroporation>
 IPA: /ɪ.lek.troʊ.pə'reɪ.fən/
 Phonetic Spelling: ih·lek·troh·puh·ray·shuhn
15. Cuvette
 Pronunciation link: <https://www.merriam-webster.com/dictionary/cuvette>
 IPA: /kju:'vet/
 Phonetic Spelling: kyoo·vet
16. Deoxyribonucleic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/deoxyribonucleic%20acid>
 IPA: /di.ɑ:k.si.raɪ.boʊ.nu:'kli:.ɪk/
 Phonetic Spelling: dee·ok·see·rye·boh·noo·klee·ik
17. Kanamycin
 Pronunciation link: <https://www.merriam-webster.com/dictionary/kanamycin>
 IPA: /kæn.ə'maɪ.sɪn/
 Phonetic Spelling: kan·uh·my·sin
18. Intravenous
 Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>
 IPA: /,ɪn.trə'vi:.nəs/
 Phonetic Spelling: in·truh·vee·nuhs
19. Sonicate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/sonicate>
 IPA: /'sɑ:.nə,kert/
 Phonetic Spelling: sah·nuh·kayt
20. Colony-forming units
 Pronunciation link: <https://www.merriam-webster.com/dictionary/colony>
 IPA: /'kɑ:.lə.ni/ ; /'fɔ:r.mɪŋ/ ; /'ju:.nɪts/
 Phonetic Spelling: kah·luh·nee ; for·ming ; yoo·nits
21. Anesthetized
 Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>
 IPA: /ə'nes.θə.taɪzɪd/
 Phonetic Spelling: uh·ness·thuh·tyzed
22. Isoflurane
 Pronunciation link: <https://www.merriam-webster.com/dictionary/isoflurane>
 IPA: /,aɪ.sou'flʊr.eɪn/
 Phonetic Spelling: eye·soh·floor·ayn

23. Analgesia
 Pronunciation link: <https://www.merriam-webster.com/dictionary/analgesia>
 IPA: /, æn.əlˈdʒiː.zi.ə/
 Phonetic Spelling: an·uhl·jee·zee·uh
24. Ophthalmic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ophthalmic>
 IPA: /ɑːfˈθæl.mɪk/
 Phonetic Spelling: off·thal·mik
25. Catheter
 Pronunciation link: <https://www.merriam-webster.com/dictionary/catheter>
 IPA: /ˈkæθ.ə.tər/
 Phonetic Spelling: kath·uh·ter
26. Intubate
 Pronunciation link: <https://www.merriam-webster.com/dictionary/intubate>
 IPA: /ˈɪn.tuː.beɪt/
 Phonetic Spelling: in·too·bayt
27. Tracheal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/tracheal>
 IPA: /ˈtreɪ.ki.əl/
 Phonetic Spelling: tray·kee·uhl
28. Transillumination
 Pronunciation link: <https://www.howtopronounce.com/transillumination>
 IPA: /, træn.zɪ.luː.məˈneɪ.ʃən/
 Phonetic Spelling: tranz·ih·loo·muh·nay·shuhn
29. Ventilator
 Pronunciation link: <https://www.merriam-webster.com/dictionary/ventilator>
 IPA: /ˈven.tə.leɪ.tər/
 Phonetic Spelling: ven·tuh·lay·ter
30. Decubitus
 Pronunciation link: <https://www.merriam-webster.com/dictionary/decubitus>
 IPA: /dɪˈkjuː.bə.təs/
 Phonetic Spelling: dih·kyoo·buh·tuhs
31. Depilatory
 Pronunciation link: <https://www.merriam-webster.com/dictionary/depilatory>
 IPA: /dɪˈpɪl.ə.təːr.i/
 Phonetic Spelling: dih·pill·uh·tor·ee
32. Antiseptic
 Pronunciation link: <https://www.merriam-webster.com/dictionary/antiseptic>
 IPA: /, æn.tiˈsep.tɪk/
 Phonetic Spelling: an·tee·sep·tik
33. Intercostal
 Pronunciation link: <https://www.merriam-webster.com/dictionary/intercostal>
 IPA: /, ɪn.təˈkɑː.stəl/
 Phonetic Spelling: in·ter·kah·stl

34. Thoracic
Pronunciation link: <https://www.merriam-webster.com/dictionary/thoracic>
IPA: /θəˈræs.ɪk/
Phonetic Spelling: tuh·rass·ik
35. Cyanoacrylate
Pronunciation link: <https://www.merriam-webster.com/dictionary/cyanoacrylate>
IPA: /ˌsaɪ.ə.noʊˈæk.rəˌleɪt/
Phonetic Spelling: sigh·uh·noh·ak·ruh·layt
36. Coverslip
Pronunciation link: <https://www.merriam-webster.com/dictionary/coverslip>
IPA: /ˈkʌv.əˌslɪp/
Phonetic Spelling: kuv·er·slip
37. Xiphoid
Pronunciation link: <https://www.merriam-webster.com/dictionary/xiphoid>
IPA: /ˈzaɪ.fɔɪd/
Phonetic Spelling: zy·foyd
38. Extubate
Pronunciation link: <https://www.merriam-webster.com/dictionary/extubate>
IPA: /ˈeks.tuˌbeɪt/
Phonetic Spelling: eks·too·bayt
39. Intravital
Pronunciation link: <https://www.merriam-webster.com/medical/intravital>
IPA: /ˌɪn.trəˈvaɪ.təl/
Phonetic Spelling: in·truh·vye·tuhl
40. Mycobacterium
Pronunciation link: <https://www.merriam-webster.com/dictionary/mycobacterium>
IPA: /ˌmaɪ.koʊ.bækˈtɪr.i.əm/
Phonetic Spelling: my·koh·bak·teer·ee·uhm
41. Tuberculosis
Pronunciation link: <https://www.merriam-webster.com/dictionary/tuberculosis>
IPA: /tuˌbɜː.kjəˈloo.sɪs/
Phonetic Spelling: too·ber·kyuh·loh·suhs
42. Murine
Pronunciation link: <https://www.merriam-webster.com/dictionary/murine>
IPA: /ˈmjʊr.ən/
Phonetic Spelling: myoor·ine
43. Fluorescein
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescein>
IPA: /flʊˈres.iːn/
Phonetic Spelling: floor·ess·een
44. Isothiocyanate
Pronunciation link: <https://www.merriam-webster.com/dictionary/isothiocyanate>
IPA: /ˌaɪ.souˌθaɪ.ouˈsaɪ.əˌneɪt/
Phonetic Spelling: eye·soh·thye·oh·sigh·uh·nayt

- 45. Retro-orbitally
Pronunciation link: No confirmed link found
IPA: /ˌret.roʊˈɔːr.bi.təl.i/
Phonetic Spelling: ret·roh·or·bih·tuh·lee
- 46. Vasculature
Pronunciation link: <https://www.merriam-webster.com/dictionary/vasculature>
IPA: /ˈvæs.kjə.lə.tʃə/
Phonetic Spelling: vas·kyuh·luh·cher
- 47. Bacilli
Pronunciation link: <https://www.merriam-webster.com/dictionary/bacillus>
IPA: /bəˈsɪl.aɪ/
Phonetic Spelling: buh·sil·eye
- 48. Parenchyma
Pronunciation link: <https://www.merriam-webster.com/dictionary/parenchyma>
IPA: /pəˈreŋ.kə.mə/
Phonetic Spelling: puh·reng·kuh·muh
- 49. Macrophages
Pronunciation link: <https://www.merriam-webster.com/dictionary/macrophage>
IPA: /ˈmæk.rəˌfeɪdʒɪz/
Phonetic Spelling: mak·ruh·fay·jiz
- 50. Two-photon
Pronunciation link: <https://www.merriam-webster.com/dictionary/photon>
IPA: /ˈtuː ˈfoʊ.tən/
Phonetic Spelling: too·foh·ton