

Submission ID #: 72041

Scriptwriter Name: Vipasha Sharma

Project Page Link: <https://review.jove.com/account/file-uploader?src=21972553>

Title: Ultrasound-Controlled Blood-Brain Barrier (BBB) Opening in a BBB-Glioblastoma Microfluidic Chip for Drug Delivery Studies

Authors and Affiliations:

Yihong Li^{1,2,3*}, Liyang Wu^{1,2,3*}, Huadong Fan^{1,2,4}

¹Ningbo No.2 Hospital

²Innovation Center for Diagnosis and Treatment of Neurological Diseases, Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences

³Lab of Nanopharmacology Research for Neurodegeneration, Department of Research and Development of Science and Technology, Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences

⁴Lab of Dementia and Neurorehabilitation Research, Department of Research and Development of Science and Technology, Ningbo Institute of Life and Health Industry, University of Chinese Academy of Sciences

*These authors contributed equally

Corresponding Authors:

Huadong Fan (fanhuadong@ucas.ac.cn)

Yihong Li (yihongli@ucas.ac.cn)

Email Addresses for All Authors:

liyangw214@gmail.com

fanhuadong@ucas.ac.cn

yihongli@ucas.ac.cn

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? **Yes**

If **Yes**, how far apart are the locations? Filming will take place in three locations. One is on the **5th floor**, where the interviews will be conducted. The other two are on the **2nd floor**, where two separate labs will be used for the experiments. The distance between these two labs is approximately 20 meters. There is an elevator that directly connects the 5th and 2nd floors for easy transitions.

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. **No**

Current Protocol Length

Number of Steps: 21

Number of Shots: 58

Interviews

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

Authors: Statements have been edited for length and clarity. 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. **Yihong Li:** Our research focuses on developing a microfluidic glioblastoma model to investigate ultrasound-controlled transient blood-brain barrier opening and its potential for drug delivery.

- 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. **Yihong Li:** Existing microfluidic chips mainly assess drug permeability and lack physical stimulation. This protocol integrates ultrasound stimulation with nanoparticle-based drug delivery.

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

~~Describe the research contexts or systems in which this protocol can be applied.~~

- 1.3. **Yihong Li:** This protocol can be applied to screen BBB-penetrating nanomedicines, investigate ultrasound-mediated BBB disruption, and evaluate therapies targeting the central nervous system.

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

CONCLUSION:

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

- 1.4. **Huadong Fan:** Future studies could evaluate other drugs for blood-brain barrier penetration and investigate mechanisms responsible for ultrasound-induced transient blood-brain barrier opening.

- 1.4.1. 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 Institutional Animal Care and Use Committee (IACUC) of Ningbo Institute of Life and Health Industry

Protocol

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

Author: For better continuity and flow, Steps 5.1 in the script have been included from the unhighlighted portions of the manuscript.

Note: With the authors' permission, steps for HCMEC preparation that are similar to the astrocyte preparation steps demonstrated earlier have been omitted to avoid repetition.

2. Photomask Preparation and Patterning

Demonstrator: Liyang Wu

- 2.1. To begin, arrange the required materials on the workbench[1]. Place a blank silicon wafer on the spin-coater vacuum chuck and activate the vacuum to immobilize it [2]. Slowly dispense 10 milliliters of SU-8 (*S-U-eight*) photoresist onto its center [3].
 - 2.1.1. Talent arranging the material on the workbench
 - 2.1.2. Talent placing the blank silicon wafer on the spin-coat and activating the vacuum
 - 2.1.3. Talent dispensing the 10 mL photoresist onto the wafer
- 2.2. Spin-coat at 500 rpm for 10 seconds, followed by 2,500 revolutions per minute for 90 seconds, to obtain a uniform layer [1]. Transfer the coated wafer to a hot plate and soft-bake it at 95 degrees Celsius for 30 minutes to evaporate the solvent [2]. Allow it to cool to room temperature, then measure the SU-8 thickness with a thickness gauge [3].
 - 2.2.1. Talent spin coating at 500 rpm for 10 seconds, followed by 2,500 rpm for 90 seconds
 - 2.2.2. Talent transferring the coated wafer to a hot plate for soft-baking at 95°C
 - 2.2.3. Talent measuring the SU-8 thickness after cooling to room temperature
- 2.3. For photolithographic patterning, set the mask aligner to an ultraviolet intensity of 14 to 15 milliwatts per square centimeter [1]. Align the chromium photomask with the SU-8-coated wafer and expose it to ultraviolet light for 10 seconds [2]. Immediately transfer it to a hot plate for post-exposure baking at 65 degrees Celsius for 10 seconds, followed by 95 degrees Celsius for 300 seconds [3].

- 2.3.1. CU: Talent setting the ultraviolet intensity of the mask aligner to 14-15 mW/cm²
- 2.3.2. Talent aligning the chromium photomask with SU-8-coated wafer and exposing it for 10 seconds
- 2.3.3. Talent transferring the wafer to the hot plate for baking at 65 °C for 10 s, followed by 95 °C for 300 s

- 2.4. After cooling the silicon wafer to room temperature, immerse the wafer in propylene glycol monomethyl ether acetate, or PGMEA, to develop for 4 to 6 minutes [1]. Rinse the silicon wafer with isopropanol to terminate development, then wash with deionized water [2].
 - 2.4.1. Talent immersing the wafer in PGMEA to develop
 - 2.4.2. Talent rinsing the silicon wafer with isopropanol and washing with deionized water
- 2.5. Dry the wafer with nitrogen gas [1] and inspect the patterned microchannels under an optical microscope [2]. Then bake the completed master at 200 degrees Celsius for 1 hour to improve adhesion between the SU-8 and silicon [3].
 - 2.5.1. Talent drying the wafer with nitrogen gas
 - 2.5.2. SCREEN: 72041_screenshot_1.mp4 00:07-00:14
 - 2.5.3. Talent keeping the wafer at 200 °C for 1 hour

3. PDMS Chip Fabrication

- 3.1. To cast the chip, mix the polydimethylsiloxane base prepolymer and curing agent at a 10-to-1 weight ratio for 30 minutes [1]. Pour the mixture over the silicon master mold until the microstructures are fully covered [2]. Degas the mold containing the mixture in a vacuum desiccator to remove air bubbles [3], then cure it in an oven at 65 degrees Celsius for 1.5–2 hours [4].
 - 3.1.1. Talent mixing the base polymer and curing agent
 - 3.1.2. Talent pouring the mixture over the silicon master
 - 3.1.3. Talent placing the mold in the desiccator for degassing it
 - 3.1.4. Talent placing the mold in the oven for curing at 65 °C

- 3.2. Using a craft knife, cut the cured PDMS layer along the chip boundaries and gently peel it from the silicon master mold [1]. Immerse the chip in 70% to 75% ethanol for 30 minutes for preliminary sterilization, then rinse it with deionized water and allow it to air-dry [2]. Make 2-millimeter inlet and outlet ports with a biopsy punch [3].
 - 3.2.1. CU: Talent cutting and gently peeling the PDMS layer
 - 3.2.2. Talent immersing the chip in 70-75% ethanol, rinsing with water and keeping it aside for air drying
 - 3.2.3. Talent punching the inlet and outlet ports
- 3.3. For plasma bonding, place the PDMS chip and a clean glass slide in the plasma cleaner with the microchannel side facing upward [1]. Evacuate the chamber below 30 pascals, then introduce oxygen for 90 seconds while maintaining the chamber pressure below 40 pascals [2]. Apply 55% radio-frequency power for 90 seconds to activate PDMS and glass surfaces [3].
 - 3.3.1. Talent placing the PDMS chip and glass slide into the plasma cleaner
 - 3.3.2. Talent evacuating the chamber below 30 Pa and introducing the oxygen flow for 90 seconds
 - 3.3.3. Talent setting the radio-frequency power to 55 % for surface activation
- 3.4. Immediately bring the activated PDMS chip and glass slide into contact and gently press them together to achieve irreversible bonding [1]. Place the assembled device in an oven at 65 degrees Celsius for 24 hours to stabilize the bond and restore hydrophobicity [2]. Before cell seeding, sterilize it by ultraviolet irradiation for 30 minutes. Maintain sterility by storing the chips in sealed petri dishes inside a biosafety cabinet until use [3].
 - 3.4.1. CU: Talent bringing the PDMS chip in close contact with the glass slide and gently pressing them
 - 3.4.2. Talent placing the device in an oven at 65 °C
 - 3.4.3. Talent sterilizing the device by ultraviolet irradiation and keeping it inside a biosafety cabinet in a sealed petri dish

4. Establishment of the BBB-Glioblastoma Model

- 4.1. Culture the primary astrocytes previously isolated from neonatal rat cerebral cortices in T25 flasks containing astrocyte culture medium [1]. On day 1, add 1 milliliter of 0.25%

trypsin-EDTA to the astrocytes, gently swirl for even coverage [2], and incubate at 37 degrees Celsius for 1 to 2 minutes until the cells detach [3].

- 4.1.1. Talent culturing the previously isolated primary astrocytes in the astrocyte culture medium
- 4.1.2. Talent adding 1 mL of 0.25 % trypsin-EDTA to the astrocytes and gently swirling it
- 4.1.3. Talent placing the flask at 37 °C
- 4.2. Neutralize the trypsin with 3 milliliters of astrocyte culture medium [1-TXT] and gently pipette to obtain a single-cell suspension and transfer it to a centrifuge tube [2].
 - 4.2.1. Talent adding 3 mL of astrocyte culture medium **TXT: See text protocol for astrocyte culture medium preparation**
 - 4.2.2. Talent gently pipetting to collect the cell suspension
- 4.3. Centrifuge the cell suspension at 300 *g* for 3 minutes at 4 degrees Celsius [1], remove the supernatant and resuspend the pellet in 200 microliters of medium [2]. Using an automated cell counter, determine the cell concentration [3] and adjust the density to 2.5×10^6 cells per milliliter [4].
 - 4.3.1. Talent placing the tube into a centrifuge and closing the lid
 - 4.3.2. Talent removing the supernatant and resuspending the pellet in 200 μ L of medium
 - 4.3.3. Shot of the talent inserting the counting slide into the automated cell counter, with the cell concentration visible on the instrument display
 - 4.3.4. Talent diluting the cell suspension to adjust the cell density
- 4.4. Mix the astrocyte cell suspension with Matrigel at a 3:5 volume ratio [1]. Using a micropipette, dispense 10 microliters of the Matrigel-Astrocyte mixture into the brain channel while keeping the chip on ice [2]. Incubate it at 37 degrees Celsius with 5% carbon dioxide for 30 minutes to promote Matrigel polymerization and cell stabilization [3].
 - 4.4.1. Talent mixing the cell suspension with Matrigel
 - 4.4.2. Talent dispensing 10 μ L of the mixture into the brain channel
 - 4.4.3. Talent placing the chip at 37 °C with 5% CO₂

4.5. Carefully introduce 10 microliters of HCMECs (H-C-M-E-Sees) suspension into the vascular channel of the chip [1-TXT]. Position the chip sideways in the incubator for 30 minutes to promote endothelial cell attachment along the channel wall [2].

4.5.1. Talent dispensing 10 μ L of the HCMECs suspension into the vascular channel

TXT: See text protocol for preparation of HCMECs suspension

4.5.2. Talent positioning the chip sideways for 30 minutes

4.6. Seed an additional 10 microliters of HCMECs suspension into the channel to increase endothelial density and incubate the chip for an additional 30 to 60 minutes to allow HCMECs adhesion [1]. Add 100 microliters of a 1:1 mixture of astrocyte and HCMECs media to the chip inlet [2]. After two days of co-culture, seed 10 microliters of U87-MG cells into the glioma channel of the chip [3] and incubate the device at 37 degrees Celsius with 5% carbon dioxide for an additional 48 hours [4].

4.6.1. Talent transferring 10 μ L of the HCMECs suspension into the vascular channel and keeping it aside

4.6.2. Talent adding 100 μ L mixture of astrocyte and HCMECs media to the chip inlet

4.6.3. Talent adding 10 μ L U87-MG cells into the glioma channel of the chip

4.6.4. Talent placing the device at 37 °C with 5% CO₂

5. Ultrasound Stimulation

5.1. Before ultrasound treatment, evaluate blood-brain barrier integrity by introducing 40-kilodalton FITC-dextran into the vascular channel [1] and acquire fluorescence images of both the vascular and adjacent brain channel at 20-minute intervals to monitor dextran diffusion across the endothelial barrier [2]. Perform zonula occludens-1 immunofluorescence staining [3]. Acquire confocal Z-stack images to confirm continuous tight-junction localization before proceeding to ultrasound stimulation [4].

5.1.1. Talent introducing the 40 kDa FITC-dextran solution into the vascular channel

5.1.2. SCREEN: 72041_screenshot_2.mp4

5.1.3. Talent adding the zonula occludens-1 antibody solution to the microfluidic channels

5.1.4. SCREEN: 72041_screenshot_3.mp4 00:10-00:13 and 00:17-00:20

5.2. Transfer the chip from the culture dish to a biosafety cabinet and prepare a therapeutic ultrasound system equipped with a 1-megahertz transducer with a 5-square-centimeter effective radiating area [1]. Place a 3-millimeter medical-grade acoustic coupling pad

between the chip and the probe [2], position the probe directly above the vascular channel, and ensure that no air bubbles remain between the transducer and the device [3].

5.2.1. Talent transferring the chip from culture to the biosafety cabinet and preparing a therapeutic ultrasound system

5.2.2. CU: Talent placing the acoustic coupling pad between the chip and probe

5.2.3. CU: Talent positioning the probe directly above the vascular channel while checking for air bubbles

5.3. Set the ultrasound system to a 60% duty cycle, a frequency of 1 megahertz, an intensity of 1 watt per square centimeter, a pulse repetition frequency of 100 hertz, and an exposure time of 30 seconds [1]. Apply the treatment to the ultrasound group [2]. For the untreated sham control, position the inactive transducer identically to ensure the same handling without acoustic exposure [3]. After treatment, immediately remove the coupling pad and return the chip to a sterile culture dish [4].

5.3.1. Talent setting the ultrasound parameters

5.3.2. Talent applying the 30-second treatment

5.3.3. Talent positioning the inactive transducer for the untreated control group

5.3.4. Talent removing the coupling pad and returning the chip to a sterile culture dish

5.4. Following ultrasound exposure, perform zonula occludens-1 immunofluorescence staining [1]. Acquire sequential confocal Z-stack images, reconstruct the image stacks in three dimensions, and assess changes in tight-junction localization [2].

5.4.1. Talent adding the zonula occludens-1 antibody solution to the microfluidic channels

5.4.2. SCREEN: 72041_screenshot_4.mp4 00:07-00:10 and 00:16-00:18

Authors: Step 5.4 has been added to better connect the protocol with the Results section.

Results

6. Results

6.1. Zonula occludens-1 showed a continuous distribution along the endothelial cell junctions, indicating tight-junction formation [1]. FITC-dextran permeability across the barrier significantly decreased over time [2].

6.1.1. LAB MEDIA: Figure 1C *Video Editor: Please highlight the red ZO-1 staining in the left figure labeled as ZO-1*

6.1.2. LAB MEDIA: Figure 1F *Video Editor: Please highlight the 20-, 40-, and 60-min permeability bars*

6.2. Following ultrasound exposure, zonula occludens-1 intensity remained comparable immediately and at 24 hours, indicating preserved tight-junction integrity [1]. However, ultrasound significantly increased permeability immediately after exposure [2]. By 24 hours post-ultrasound exposure, permeability substantially decreased, indicating recovery of barrier function [3].

6.2.1. LAB MEDIA: Figure 2B *Video Editor: Please highlight the 0 h and 24 h ZO-1 signals/profiles in the US+ group*

6.2.2. LAB MEDIA: Figure 2D *Video Editor: Please highlight the 0 h bars for US- and US+*

6.2.3. LAB MEDIA: Figure 2D *Video Editor: Please highlight 0 h and 24 h bars within the US+ group-*

1. Photoresist

Pronunciation link: <https://www.merriam-webster.com/dictionary/photoresist>

IPA: /ˌfoʊtoʊrɪˈzɪst/

Phonetic Spelling: foh·toh·rih·ZIST

2. Photolithographic

Pronunciation link: <https://www.merriam-webster.com/dictionary/photolithographic>

IPA: /ˌfoʊtoʊˌlɪθəˈɡræfɪk/

Phonetic Spelling: foh·toh·lith·uh·GRAF·ik

3. Chromium

Pronunciation link: <https://www.merriam-webster.com/dictionary/chromium>

- IPA: /'kroʊmiəm/
Phonetic Spelling: KROH·mee·uhm
4. Propylene
Pronunciation link: <https://www.merriam-webster.com/dictionary/propylene>
IPA: /'proʊpəˌliːn/
Phonetic Spelling: PROH·puh·leen
 5. Glycol
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycol>
IPA: /'glaiˌkɔːl/
Phonetic Spelling: GLY·kaal
 6. Monomethyl
Pronunciation link: No confirmed link found
IPA: /ˌmɑːnoʊ'mɛθəl/
Phonetic Spelling: mon·oh·METH·uhl
 7. Isopropanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropanol>
IPA: /ˌaɪsoʊ'proʊpəˌnɑːl/
Phonetic Spelling: eye·soh·PROH·puh·naal
 8. Deionized
Pronunciation link: <https://www.merriam-webster.com/dictionary/deionize>
IPA: /diː'arəˌnaɪzd/
Phonetic Spelling: dee·EYE·uh·nyzd
 9. Microchannels
Pronunciation link: No confirmed link found
IPA: /'maɪkroʊˌtʃænəlz/
Phonetic Spelling: MY·kroh·chan·uhlz
 10. Polydimethylsiloxane
Pronunciation link: No confirmed link found
IPA: /ˌpɑːliˌdaɪˌmɛθəl'sɪləkˌseɪn/
Phonetic Spelling: pol·ee·dye·meth·uhl·SIL·uhk·sayn
 11. Prepolymer
Pronunciation link: No confirmed link found
IPA: /priː'pɑːlɪmər/
Phonetic Spelling: pree·POL·ih·mer
 12. Desiccator
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccator>
IPA: /'dɛsɪˌkɛtər/
Phonetic Spelling: DESS·ih·kay·ter
 13. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'ɛθəˌnɔːl/
Phonetic Spelling: ETH·uh·naal
 14. Hydrophobicity
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrophobicity>

- IPA: /ˌhaɪdrəfoʊˈbɪsəti/
Phonetic Spelling: hy·droh·foh·BISS·uh·tee
15. Astrocytes
Pronunciation link: <https://www.merriam-webster.com/dictionary/astrocyte>
IPA: /ˈæstrəˌsaɪts/
Phonetic Spelling: AS·troh·syts
16. Neonatal
Pronunciation link: <https://www.merriam-webster.com/dictionary/neonatal>
IPA: /ˌniːoʊˈneɪtəl/
Phonetic Spelling: nee·oh·NAY·tuhl
17. Cerebral
Pronunciation link: <https://www.merriam-webster.com/dictionary/cerebral>
IPA: /səˈriːbrəl/, /ˈserəbrəl/
Phonetic Spelling: suh·REE·bruhl / SAIR·uh·bruhl
18. Cortices
Pronunciation link: <https://www.merriam-webster.com/dictionary/cortex>
IPA: /ˈkɔːrtəˌsiːz/
Phonetic Spelling: KOR·tuh·seez
19. Trypsin
Pronunciation link: <https://www.merriam-webster.com/dictionary/trypsin>
IPA: /ˈtrɪpsɪn/
Phonetic Spelling: TRIP·sin
20. EDTA
Pronunciation link: No confirmed link found
IPA: /ˌiːdiːtiːˈeɪ/
Phonetic Spelling: E·D·T·A
21. Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /ˈsentrəˌfjuːdʒ/
Phonetic Spelling: SEN·truh·fyooj
22. Supernatant
Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
IPA: /ˌsuːpərˈneɪtənt/
Phonetic Spelling: soo·per·NAY·tuht
23. Resuspend
Pronunciation link: No confirmed link found
IPA: /ˌriːsəˈspend/
Phonetic Spelling: ree·suh·SPEND
24. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪkroʊˌliːtərz/
Phonetic Spelling: MY·kroh·lee·terz
25. Matrigel
Pronunciation link: No confirmed link found

- IPA: /'mertri,dʒəl/
Phonetic Spelling: MAY·trih·jel
26. Polymerization
Pronunciation link: <https://www.merriam-webster.com/dictionary/polymerization>
IPA: /pə,lɪmə'reɪʃən/
Phonetic Spelling: puh·lim·er·uh·ZAY·shuhn
27. HCMECs
Pronunciation link: No confirmed link found
IPA: /,ertʃsi:ɛm i:'si:z/
Phonetic Spelling: H·C·M·E·seez
28. Vascular
Pronunciation link: <https://www.merriam-webster.com/dictionary/vascular>
IPA: /'væskjələr/
Phonetic Spelling: VAS·kyuh·ler
29. Endothelial
Pronunciation link: <https://www.merriam-webster.com/dictionary/endothelial>
IPA: /,endou'θi:liəl/
Phonetic Spelling: en·doh·THEE·lee·uhl
30. Glioma
Pronunciation link: <https://www.merriam-webster.com/dictionary/glioma>
IPA: /glai'oomə/
Phonetic Spelling: gly·OH·muh
31. Glioblastoma
Pronunciation link: <https://www.merriam-webster.com/dictionary/glioblastoma>
IPA: /,glaioublæ'stoomə/
Phonetic Spelling: gly·oh·blas·TOH·muh
32. Kilodalton
Pronunciation link: No confirmed link found
IPA: /'kɪləʊ,də:ltən/
Phonetic Spelling: KIL·oh·daal·tuhn
33. FITC-dextran
Pronunciation link: No confirmed link found
IPA: /,ɛf ai ti:'si:'dɛkstræn/
Phonetic Spelling: F·I·T·C DEK·stran
34. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'resəns/, /flɔ:'resəns/
Phonetic Spelling: floo·RESS·uhns / flaw·RESS·uhns
35. Dextran
Pronunciation link: <https://www.merriam-webster.com/dictionary/dextran>
IPA: /'dɛkstræn/
Phonetic Spelling: DEK·stran
36. Zonula
Pronunciation link: No confirmed link found

- IPA: /'zounjələ/
Phonetic Spelling: ZOH·nyuh·luh
37. Occludens
Pronunciation link: No confirmed link found
IPA: /ə'klu:dənz/
Phonetic Spelling: uh·KLOO·denz
38. Immunofluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunofluorescence>
IPA: /ɪ,mju:nɒʊflʊ'resəns/
Phonetic Spelling: ih·MYOO·noh·floo·RESS·uhns
39. Confocal
Pronunciation link: No confirmed link found
IPA: /kən'foukəl/
Phonetic Spelling: kuhn·FOH·kuhl
40. Localization
Pronunciation link: <https://www.merriam-webster.com/dictionary/localization>
IPA: /,ləukələ'zeɪʃən/
Phonetic Spelling: loh·kuh·luh·ZAY·shuhn
41. Transducer
Pronunciation link: <https://www.merriam-webster.com/dictionary/transducer>
IPA: /trænz'du:sər/
Phonetic Spelling: tranz·DOO·ser
42. Megahertz
Pronunciation link: <https://www.merriam-webster.com/dictionary/megahertz>
IPA: /'megə'hɜ:rts/
Phonetic Spelling: MEG·uh·hurts
43. Irradiation
Pronunciation link: <https://www.merriam-webster.com/dictionary/irradiation>
IPA: /ɪ'reɪdi'eɪʃən/
Phonetic Spelling: ih·ray·dee·AY·shuhn
44. Microfluidic
Pronunciation link: No confirmed link found
IPA: /,maɪkroʊflu'ɪdɪk/
Phonetic Spelling: MY·kroh·floo·ID·ik
45. Permeability
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeability>
IPA: /,pɜ:rmɪə'bɪləti/
Phonetic Spelling: per·mee·uh·BIL·uh·tee