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Title: A Digital Microfluidic Electroporation Platform for Low-input CRISPR Genome Editing and mRNA Transfection in Suspension T Cells and 3D Cell Models

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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**
- 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: 24

Number of Shots: 56

Introduction

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

INTRODUCTION:

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

- 1.1. **Hugo Sinha:** Our research develops digital microfluidic platforms for efficient CRISPR editing using fewer cells than conventional methods.
 - 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. **Hugo Sinha:** Standard electroporation requires millions of cells. This platform achieves high editing efficiency with just ten thousand cells per condition.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

CONCLUSION:

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

- 1.3. **Hugo Sinha:** This protocol enables measurement of CRISPR knockout efficiency, post-electroporation viability, and mRNA delivery across multiple parallel conditions.
 - 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. **Hugo Sinha:** A key challenge is achieving consistent guide RNA spotting on hydrophobic surfaces, where PGA polymer additives are essential for reproducibility.
 - 1.4.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

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

- 1.5. **Hugo Sinha:** Future studies can explore allelic editing rates, cytokine function, and in vivo engraftment to advance therapeutic translation.

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.

Protocol

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

NOTE: LAB MEDIA/SCREEN timestamps for protocol were added at the postshoot stage. Please contact the postshoot note integrator (Sri Lakshmi Priya) for queries regarding lab media.

2. Preparation of Primary Human T Cell Suspension

Demonstrator: Hugo Sinha

- 2.1. To begin, obtain a T75 (*T-seventy-five*) flask from the incubator containing CD3+ (*C-D-three-positive*) human primary T cells [1]. In the tissue culture hood, transfer the cells to a 15-milliliter Falcon tube [2] and centrifuge at a relative centrifugal force of 200 for 5 minutes [3]. Discard the supernatant and resuspend the pellet in 5 milliliters of fresh medium [4]. NOTE: The VO is edited as per the revised shot
 - 2.1.1. WIDE: Talent taking out the T75 flask containing CD3+ human primary cells from the incubator.
 - 2.1.2. Talent transferring the cells to a 15 mL tube in tissue culture hood.
 - 2.1.3. Talent placing the tube into a centrifuge.
 - 2.1.4. Talent removing the supernatant and resuspending the cell pellet in 5 mL fresh medium.
- 2.2. Pipette 10 microliters of cell suspension into a Countess cell counting chamber [1]. Using an automated cell counter, determine the cell concentration and viability [2]. NOTE: VO is added for the added shot.
 - 2.2.1. Talent loading 10 µL cell sample into an automated cell counter.
Added Shot: Shot of the automated cell counter display showing the cell concentration and viability
- 2.3. Pipette 100,000 cells into 4 to 5 wells of a V-bottom well plate [1] and place on ice [2].
 - 2.3.1. Close-up shot of pipetting cells into a V-bottom well plate.
 - 2.3.2. Talent placing the plate on ice.

3. Preparation of Guide RNA Spots on the DMF Substrate

- 3.1. Reconstitute 1 lyophilized TRAC knock out or KO (*TRAC-K-O*) targeting single guide RNA or sgRNA (*S-G-RNA*) and 1 lyophilized non-targeting control or NTC (*N-T-C*) sgRNA in nuclease-free water to a stock concentration of 100 micromolar [1]. After preparing

a 100 milligram per milliliter stock solution of poly-L-glutamic acid or PGA (*P-G-A*) in nuclease-free water in 2 tubes, label the tubes as TRAC and NTC [2]. Combine the sgRNA and PGA at a 5 to 4 volume ratio [3] and ~~vortex briefly~~ mix by pipetting [4].

NOTE: The VO is edited as per the revised shot

- 3.1.1. Talent adding nuclease-free water to lyophilized RNA.
- 3.1.2. Talent labelling the tubes as TRAC and NTC.
- 3.1.3. Talent mixing the sgRNA and PGA in a tube.
- 3.1.4. Talent ~~vortexing the tube briefly~~ pipetting the mixture.
- 3.2. Add the guide PGA mixture to a low-dead-volume acoustic source in wells corresponding to the desired reaction site layout and seal the plate [1]. NOTE: The VO is edited as per the revised shot.
 - 3.2.1. Talent pipetting the mixture into a well of an acoustic source plate and sealing the plate.
- 3.3. Using an acoustic dispenser, transfer 40 nanoliters of the guide or PGA mixture to each target spotting region of the Digital microfluidic or DMF (*D-M-F*) substrate [1]. Monitor droplet dispensing in the Echo software [2]. After droplet transfer is complete, eject the consumable from the acoustic dispenser [3].
 - 3.3.1. LABMEDIA: Nanodispersing.mp4: 00:00–00:39
 - 3.3.2. LABMEDIA: echo-software-raw.mp4: 00:08–00:21
 - 3.3.3. LABMEDIA: echo-spot.mp4: 01:41–01:50
- 3.4. In a biological safety cabinet, let the spotted droplets air dry completely at room temperature until no visible liquid remains [1].
 - 3.4.1. Talent placing the substrate inside a biological safety cabinet for drying.
- 3.5. Place the Echo plate, sgRNA tubes, and PGA tube at minus 80 degrees Celsius [1].
 - 3.5.1. Talent placing Echo plate, sgRNA tubes and PGA Tube back into the –80°C.
- 3.6. Align the top plate over the spotted substrate by matching the asymmetrical snapping features with the notches [1]. Apply even pressure along the short edges until a mechanical snap confirms engagement [2].
 - 3.6.1. Talent aligning two plates carefully.
 - 3.6.2. Talent pressing the plates together until a snap is observed.
- 3.7. Store the unused assembled cartridges in a sealed package with desiccant at room temperature for up to 48 hours [1].
 - 3.7.1. Talent placing the unused assembled cartridges in a sealed package with desiccant.

4. Preparation of the Liquid Handler

- 4.1. To prepare the liquid handler deck, rerack the tip box and position it on the deck [1]. ~~After keeping the spare tips into an empty box, place the cooling block in position A of the liquid handler [2]. Add two empty 8-strip tubes to the cooling block [3] and pipette liquid electrode and cells into the wells of one strip [4]. Once 15 milliliter of complete media is added into the reservoir, pipette 130 microliters into each well of the other strip for quenching [5].~~
- 4.1.1. Close-up of a tip box being re-racked and placed on the deck.
- 4.1.2. Close-up of a cooling block being placed onto the slot labeled position A on the liquid handler.
- 4.1.3. Close-up of two empty 8-strip tubes being placed onto the cooling block.
- 4.1.4. ~~Close-up of pipetting liquid electrode solution followed by cell suspension into the well.~~
- 4.1.5. ~~Close-up of pipetting media into the well of the other strip.~~ Author's NOTE: Remove it.
- 4.2. Using a multichannel pipette, fill up the well plate in position B with 100 microliters of complete media per well [1] and place in position B [2]. Add 125 microliters of complete media to the right tube strip [2]. Position the dock onto the liquid handler in position C [3]. Position the custom bin in the bin position and route cables carefully [4]. NOTE: The sentence numbers are adjusted to accommodate the added shot.
- 4.2.1. Close-up of position B in the well plate being filled with complete media using a multichannel pipette.
- 4.2.2. ~~Close-up of plate being placed in position B.~~ Author's NOTE: Remove it. The well plate is already in place in the previous shot
- Added shot: Close-up of complete media being added to the right tube strip.
- 4.2.3. Close-up of dock being placed in position C.
- 4.2.4. Close-up of custom bin being placed in bin position and cables are being routed.

5. Preparation of Cells

- 5.1. Prepare 1 milliliter of complete transfection buffer by adding DMF-compatible electroporation buffer into a tube containing non-ionic surfactant at a 20 to 1 volume ratio [1]. After mixing it by flicking the tube, keep it on ice [2].
- 5.1.1. Talent pipetting DMF compatible electroporation buffer into a tube containing non-ionic surfactant.
- 5.1.2. Talent placing the tube on ice.
- 5.2. Centrifuge the V-bottom well plate at ~~180~~ 200 g for ~~2~~ 5 minutes [1]. Aspirate the supernatant and resuspend the cell pellet in DMFfection (D-M-F-ection) compatible ~~electroporation~~ buffer [2]. Centrifuge at 200 ~~180~~ g for 2 minutes, and repeat washing

two more times as previously demonstrated [3]. Resuspend the cells in 100 microliters of complete transfection buffer containing surfactant [4], and place them on ice [5].

NOTE: The sentence numbers are adjusted to accommodate the added shot.

NOTE: The author's VO and shots are not aligning.

5.2.1. Talent placing the V-bottom well plate into a centrifuge.

5.2.2. Talent aspirating the supernatant and adding buffer to the cells.

5.2.3. Talent placing the plate into a centrifuge.

Added shot: Talent resuspending the cells in complete transfection buffer.

Author's NOTE: This shot is 5.2.3, and the numbering was changed during the shoot.

5.2.4. Talent placing the plate on ice.

5.3. Before loading onto the cartridge, add CRISPR-associated protein 9 or Cas9 (*Cas-9*) nuclease directly to the 100 microliters of T cell suspension [1]. Mix by gentle pipetting [2] and keep on ice [3]. load 45 microliters into 2 tubes of the left 8-strip [3]. Fill 4 wells with liquid electrode [4]. NOTE: VO is added for additional shots.

5.3.1. Talent adding Cas9 to the cell suspension.

5.3.2. Talent gently pipetting to mix.

~~5.3.3. Talent placing the plate on ice.~~ Videographer's NOTE: Remove it.

Added Shot: Talent adding suspension to 2 separate tubes of the left 8-strip

Added Shot: Talent filling 4 wells with liquid electrode.

6. Electroporation and Flow Cytometry Analysis

6.1. Power on the system [1]. Place the LED board on the DropGenie dock [2]. After booting up the system, select the plate type and orientation, and run a system health check [3].

NOTE: VO is added for the added shots, and the sentence numbers are adjusted to accommodate the added shots.

Added shot: Talent turning on the system by pressing the ON switch on the front of the DropGenie instrument, and the light of the DropGenie dock starts blinking. Note: The author numbered it as 6.0

Added Shot: Talent placing the LED board on the DropGenie dock. Note: The author numbered it as 6.0.1

6.1.1. SCREEN: JoVE-Screen-Record.mp4: 00:00-00:10

6.2. After turning on the Voyager pipette, pair it to the liquid handler [1]. Run the vialab script [2], then initiate the run [3]. After allowing the liquid handler to idle, position the

pipette on the liquid handler [4]. NOTE: VO is added for the added shots, and the sentence numbers are adjusted to accommodate the added shots.

6.2.1. ~~SCREEN:~~ Show the pipette screen where the Voyager pipette is being connected to the liquid handler. NOTE: The screenshots are changed to talent shots from

6.2.1 to 6.2.3

6.2.2. ~~SCREEN:~~ Show the pipette screen where the Vialab script is being selected from the list and opened.

6.2.3. ~~SCREEN:~~ Show the pipette screen where the **Run** or **Start** button is being selected.

Added shot: Shot of a pipette being positioned on the liquid handler tower.

6.3. Enter the experiment name and consumable ID, select the wells, and set the electroporation parameters [1]. Then, initiate the electroporation [2].

6.3.1. SCREEN: JoVE-Screen-Record.mp4: 01:27- 01:51

6.3.2. SCREEN: JoVE-Screen-Record.mp4: 02:10-02:16

6.4. Position the consumable on the DropGenie dock and close the dock to clamp the consumable [1]. ~~Observe the hardware indicator light flashing blue, indicating the system is idle and ready for loading [2].~~ NOTE: The VO is edited as per the revised shot.

6.4.1. Close-up of positioning the consumable on the DropGenie dock and the dock closing and clamping the consumable.

~~6.4.2. Close up of the hardware indicator light flashing blue on the device.~~ Author's NOTE: Remove it.

6.5. After starting the liquid handler, allow it to load the reagents, Cas9 suspension, and liquid electrode from the left 8-strip [1]. Start the electroporation run [2]. Observe the movement of droplets across the consumable during operation [3]. Once the software run is completed, offload the samples [4]. ~~to quench the samples [4] and~~ Transfer contents into a 96-well plate containing 100 microliters of prewarmed basal medium with interleukin 2 [5]. NOTE: For the removed shot, VO is concisely added in the previous step. VO is added for the added shots, and the sentence numbers are adjusted to accommodate the added shots. The VO is edited as per the revised shot.

Added Shot: Closeup showing the liquid handler injecting liquid into the cartridge and the liquid being taken in by the cartridge (author numbered this shot as 6.5.0B)

6.5.1. SCREEN: JoVE-Screen-Record.mp4: 02:28-02:32

6.5.2. Close-up of droplets moving across the consumable surface. NOTE: If this shot is not filmed, skip the VO.

6.5.3. SCREEN: JoVE-Screen-Record.mp4: 02:50-02:54

- 6.5.4. ~~Close-up of the liquid handler being enabled and operating as it transfers liquid into a 96-well plate.~~ Close-up showing the liquid handler aspirating the liquid out of the cartridge and transferring it into the 96-well plate **NOTE: This shot is replaced**
- 6.6. Place the plate cover onto the plate [1] and discard the well strips [2]. Complete the run by pressing **Next** on the software [3]. Once the dock opens, remove and discard the consumable [4].
- 6.6.1. Close-up of placing a lid onto the 96-well plate.
 - 6.6.2. Close-up of discarding the used well strips into waste.
 - 6.6.3. SCREEN: JoVE-Screen-Record.mp4: 02:55-03:02
 - 6.6.4. Close-up of removing the consumable and placing it into a disposal pouch.
- 6.7. Review the post-run quality control report immediately after the run and note the wells to exclude from the analysis [1].
- 6.7.1. SCREEN: JoVE-Screen-Record.mp4: 03:17-03:24
- 6.8. Incubate the cells for 72 hours at 37 degrees Celsius and 5 percent carbon dioxide [1].
- 6.8.1. Talent placing the plate into an incubator.
- 6.9. After removing the plate from the incubator, view it under a microscope [1]. Using a flow cytometer, perform downstream analysis and acquire fluorescence data from cells [2].
- 6.9.1. Talent placing the plate under microscope.
 - 6.9.2. Close-up of plate being placed into the flow cytometer.

Results

7. Results

- 7.1. Droplet deposition variability was most evident at intermediate droplet volumes ranging from 90 to 120 nanoliter [1].
 - 7.1.1. LAB MEDIA: Figure 2A. *Video editor: Emphasize the blue bars along with the vertical lines on top of the blue bars with the labels 90 nL H₂O and 120 nL H₂O.*
- 7.2. Addition of PGA resulted in fluorescein deposition that scaled linearly with absorbance [1] and reduced variance across cartridges [2].
 - 7.2.1. LAB MEDIA: Figure 2A. *Video editor: Emphasize all the purple bars labeled as PGA.*
 - 7.2.2. LAB MEDIA: Figure 2A. *Video editor: Emphasize the vertical lines on top of all the purple bars labeled as PGA.*
- 7.3. Free sgRNA exhibited a small hydrodynamic radius [1], while Cas9 titration formed a larger ribonucleoprotein complex [2].
 - 7.3.1. LAB MEDIA: Figure 2B. *Video editor: In the panel labeled 0 mg/ml PGA, emphasize the curve from x-axis 0.001 up to 0.01.*
 - 7.3.2. LAB MEDIA: Figure 2B. *Video editor: In the panel labeled 0 mg/ml PGA, emphasize the curve from x-axis 0.01 to near 1000.*
- 7.4. Addition of PGA reduced the apparent affinity between Cas9 and sgRNA and decreased complex size to approximately 10 nanometers [1].
 - 7.4.1. LAB MEDIA: Figure 2B. *Video editor: Emphasize the curve in the panel labeled 0.005 mg/ml PGA.*
- 7.5. Without polymer, Cas9–sgRNA mixtures showed aggregation [1], whereas addition of PGA produced uniform dispersion profiles [2].
 - 7.5.1. LAB MEDIA: Figure 2C. *Video editor: Emphasize the graph in the panel labeled 0 mg/ml PGA.*
 - 7.5.2. LAB MEDIA: Figure 2C. *Video editor: Emphasize the graph in the panel labeled 0.0025 mg/ml PGA.*
- 7.6. Editing efficiency remained high at 40, 20, and 10 nanoliter input [1].
 - 7.6.1. LAB MEDIA: Figure 2D. *Video editor: Emphasize the blue and purple bars at 40 nL, 20 nL, and 10 nL.*
- 7.7. A standard gating strategy identified live singlet cells [1], followed by CD4 [2] and CD8 positive subsets [3], and T cell receptor expression [4].

- 7.7.1. LAB MEDIA: Figure 3A. *Video editor: Emphasize plots labeled as cells F7 and singlets F7 under control and cells C3 and singlets C3 under experimental.*
- 7.7.2. LAB MEDIA: Figure 3A. *Video editor: Emphasize the orange box in the plot labelled Live F7 under control and Live C3 under experimental.*
- 7.7.3. LAB MEDIA: Figure 3A. *Video editor: Emphasize the green box in the plot labelled Live F7 under control and Live C3 under experimental.*
- 7.7.4. LAB MEDIA: Figure 3A. *Video editor: Emphasize the plot CD4-F7, CD8-F7 under control and CD4-C3, CD8-C3 under experimental.*

- 7.8. High-efficiency TRAC KO was achieved in CD4 and CD8 positive T cells [1] compared to non-targeting and off-cartridge controls [2].
 - 7.8.1. LAB MEDIA: Figure 3B. *Video editor: In the graph with X-axis CD4+ and CD8+ and Y-axis %TCRa/b KO, emphasize the two tall bars with blue dots labeled as TRAC KO under CD4+ and CD8+.*
 - 7.8.2. LAB MEDIA: Figure 3B. *Video editor: In the graph with X-axis CD4+ and CD8+ and Y-axis %TCRa/b KO, emphasize the bars with orange dots labelled as non targeting control and grey dots labeled as off chip control under CD4+ and CD8+.*

- 7.9. Cell viability exceeded 96 percent across all conditions [1].
 - 3.16.1. LAB MEDIA: Figure 3B. *Video editor: In the graph with X-axis viability (singlets) and Y-axis % live, emphasize all three bars labeled as TRAC KO, NTC, and OCC.*

Pronunciation guides:

1. Centrifuge
 Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
 IPA: /ˈsentrəˌfjuːdʒ/
 Phonetic Spelling: sen·truh·fyooj
2. Supernatant
 Pronunciation link: <https://www.merriam-webster.com/dictionary/supernatant>
 IPA: /ˌsuːpərˈneɪtənt/
 Phonetic Spelling: soo·per·nay·tuht
3. Resuspend
 Pronunciation link: <https://www.merriam-webster.com/dictionary/resuspend>
 IPA: /ˌriːsəˈspend/
 Phonetic Spelling: ree·suh·spend
4. Pellet
 Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>

IPA: /'pɛlət/

Phonetic Spelling: pel·uht

5. Microliter

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

IPA: /'maɪkroʊˌli:tər/

Phonetic Spelling: my·kroh·lee·ter

6. Viability

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

IPA: /ˌvaɪəˈbɪləti/

Phonetic Spelling: vy·uh·bil·uh·tee

7. Lyophilized

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

IPA: /laɪˈɑːfəˌlaɪz/

Phonetic Spelling: ly·off·uh·lyze

8. Nuclease

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

IPA: /'nuːkliˌeɪs/

Phonetic Spelling: noo·klee·ays

9. Micromolar

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

IPA: /ˌmaɪkroʊˈmoʊlər/

Phonetic Spelling: my·kroh·moh·ler

10. Poly-L-glutamic

Pronunciation link: <https://www.merriam-webster.com/dictionary/glutamic%20acid>

IPA: /ˌgluːˈtæmɪk/

Phonetic Spelling: gloo·tam·ik

11. Vortex

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

IPA: /'vɔːrˌtɛks/

Phonetic Spelling: vor·teks

12. Acoustic

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

IPA: /əˈkuːstɪk/

Phonetic Spelling: uh·koos·tik

13. Nanoliter

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

IPA: /'nænoʊˌli:tər/

Phonetic Spelling: nan·oh·lee·ter

14. Microfluidic

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

IPA: /ˌmaɪkroʊfluˈɪdɪk/

Phonetic Spelling: my·kroh·floo·id·ik

15. Biological
Pronunciation link: <https://www.merriam-webster.com/dictionary/biological>
IPA: /ˌbaɪəˈlɑːdʒɪkəl/
Phonetic Spelling: by·uh·loj·i·kuhl
16. Desiccant
Pronunciation link: <https://www.merriam-webster.com/dictionary/desiccant>
IPA: /ˈdɛsɪkənt/
Phonetic Spelling: dess·ih·kuhnt
17. Electroporation
Pronunciation link: <https://www.merriam-webster.com/dictionary/electroporation>
IPA: /ɪˌlɛktroʊpəˈreɪʃən/
Phonetic Spelling: ih·lek·troh·puh·ray·shuhn
18. Surfactant
Pronunciation link: <https://www.merriam-webster.com/dictionary/surfactant>
IPA: /sərˈfæktənt/
Phonetic Spelling: ser·fak·tuhnt
19. Transfection
Pronunciation link: <https://www.merriam-webster.com/dictionary/transfection>
IPA: /trænsˈfɛkʃən/
Phonetic Spelling: trans·fek·shuhn
20. CRISPR
Pronunciation link: <https://www.merriam-webster.com/dictionary/CRISPR>
IPA: /ˈkrɪspər/
Phonetic Spelling: kris·per
21. Nuclease
Pronunciation link: <https://www.merriam-webster.com/dictionary/nuclease>
IPA: /ˈnuːkliˌeɪs/
Phonetic Spelling: noo·klee·ays
22. Consumable
Pronunciation link: <https://www.merriam-webster.com/dictionary/consumable>
IPA: /kənˈsuːməbəl/
Phonetic Spelling: kuhn·soo·muh·buhl
23. Incubator
Pronunciation link: <https://www.merriam-webster.com/dictionary/incubator>
IPA: /ˈɪŋkjəˌbeɪtər/
Phonetic Spelling: ing·kyuh·bay·ter
24. Microscope
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscope>
IPA: /ˈmaɪkrəˌskoʊp/
Phonetic Spelling: my·kruh·skohp
25. Flow Cytometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytometer>

IPA: /saɪˈtɑːmətər/

Phonetic Spelling: sy·tom·uh·ter

26. Fluorescence

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

IPA: /flʊˈresəns/

Phonetic Spelling: floor·ess·uhns

27. Fluorescein

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

IPA: /ˌflʊrəˈsiːɪn/

Phonetic Spelling: floor·uh·see·een

28. Absorbance

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

IPA: /əbˈzɔːrbəns/

Phonetic Spelling: ub·zor·buhns

29. Hydrodynamic

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

IPA: /ˌhaɪdrəʊdaɪˈnæmɪk/

Phonetic Spelling: hy·droh·dy·nam·ik

30. Ribonucleoprotein

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

IPA: /ˌraɪboʊˌnuːkliooˈproʊtiːn/

Phonetic Spelling: rye·boh·noo·klee·oh·proh·teen

31. Aggregation

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

IPA: /ˌægrɪˈgeɪʃən/

Phonetic Spelling: ag·rih·gay·shuhn

32. Dispersion

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

IPA: /dɪˈspɜːʒən/

Phonetic Spelling: dih·spur·zhuhn

33. Gating

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

IPA: /ˈgeɪtɪŋ/

Phonetic Spelling: gay·ting

34. Singlet

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

IPA: /ˈsɪŋɡlət/

Phonetic Spelling: sing·gluht

35. Receptor

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

IPA: /rɪˈseptər/

Phonetic Spelling: ruh·sep·ter