

Submission ID #: 71048

Scriptwriter Name: Sri Lakshmi Priya

Project Page Link: <https://review.jove.com/account/file-uploader?src=21669688>

Title: Characterizing Riboglow Probes *In Vitro* as the Basis for Fluorescence Lifetime Imaging In Live Mammalian Cells and Three-Dimensional Cellular Models

Authors and Affiliations:

Gareth M. Francis*, Luke K. Shafik*, Olayinka Barnes*, Kosonike Barnes*, Giulia Chitu*, Sydney Darensburg*, Isabel Early*, Jordan Elarfari*, Elana Elder*, Hayden Giles*, Nathan Ho*, Manya Reddy*, Andrew Shen*, Adelle Snider*, Erin N.L. Tran*, Esther Braselmann

Department of Chemistry, Georgetown University

* These authors contributed equally

Corresponding Authors:

Esther Braselmann esther.braselmann@georgetown.edu

Email Addresses for All Authors:

Gareth M. Francis	gf373@georgetown.edu
Luke K. Shafik	lks60@georgetown.edu
Olayinka Barnes	odb7@georgetown.edu
Kosonike Barnes	kdb109@georgetown.edu
Giulia Chitu	gvc16@georgetown.edu
Sydney Darensburg	srd93@georgetown.edu
Isabel Early	ise8@georgetown.edu
Jordan Elarfari	jse44@georgetown.edu
Elana Elder	eae58@georgetown.edu
Hayden Giles	heg34@georgetown.edu
Nathan Ho	ndh29@georgetown.edu
Manya Reddy	mmr181@georgetown.edu
Andrew Shen	als433@georgetown.edu
Adelle Snider	ars381@georgetown.edu
Erin N.L. Tran	ent19@georgetown.edu
Esther Braselmann	esther.braselmann@georgetown.edu

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**

If **Yes**, can you record movies/images using your own microscope camera?

N/A

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye**.

Nikon Ti2 Eclipse

If a dissection or stereo microscope is required for your protocol, please list all shots from the script that will be visualized using the microscope (shots are indicated with the 3-digit numbers, like 2.1.1, 2.1.2, etc.).

Scope shots: 3.7.2, 3.9.1, 3.9.2

***Videographer:** Please film above mentioned shots using a scope kit.*

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. **Yes**

If **Yes**, please provide the **full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author willing to participate.

Luke Shafik (Graduate Student Researcher, Georgetown University), Esther Braselmann (Assistant Professor, Department of Chemistry, Georgetown University).

Current Protocol Length

Number of Steps: 24

Number of Shots: 55

Interviews

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

Authors: Answers are slightly edited in accordance with journal guidelines. 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. **Gareth M. Francis:** Our research focuses on visualizing RNA in live cells to investigate the spatiotemporal dynamics of RNA.
 - 1.1.1. 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.~~

- 1.2. **Gareth M. Francis:** This Riboglow platform uses quantitative fluorescence lifetime imaging microscopy in multicellular systems applicable to biological systems in health and disease.
 - 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. **Luke Shafik:** This protocol allows researchers to determine the fluorescence lifetime of fluorescent molecules in different biological contexts.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

~~Are there any additional methods or analyses that can be performed following this procedure?~~

- 1.4. **Luke Shafik:** Researchers can perform quantitative data analysis and assess variables of interest following this procedure, including the addition of RNA ligands.
 - 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. **Gareth M. Francis:** Future studies can apply Riboglow-FLIM to disease models, expand multiplexing capabilities, and track RNA dynamics in live animal systems.
- 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.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What value do you expect this video article to have for other researchers working in your field?

- 1.6. **Enter author name, Enter author title:** (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. **Enter author name, Enter author title:** (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.

Protocol

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

NOTE: Timestamps for SCREEN shots were added at the postshoot stage. Please contact post shoot integrator (Sri Lakshmi Priya) for any discrepancies

2. FLIM Imaging System Setup and Initialization

Demonstrator: Luke K. Shafik

- 2.1. To begin, obtain the RNA (*R-N-A*) samples and bead-loaded live cells with Riboglow probes [1]. Switch on the confocal laser scanning microscope system [2], followed by the microscope control unit [3]. Power on the time-correlated single photon counting based fluorescence lifetime imaging microscopy or FLIM (*flim*) system, including the laser combining unit, the photomultiplier detector, and the event timer [4].

Videographer's NOTE: The first wide take, talent did a bit at the end of miming working at the computer console, figured it would be good for b-roll if needed. Also did some closer options of turning on the pieces of equipment.

- 2.1.1. WIDE: Talent walking into the room with a tube rack containing RNA samples and bead-loaded live cells. *Videographer: Please obtain CU shot as well for this shot.*
- 2.1.2. Talent powering on the confocal laser scanning microscope.
- 2.1.3. Talent powering on the microscope control unit.
- 2.1.4. Talent powering on the TCSPC based FLIM system components.
- 2.2. After opening the image acquisition and analysis computer software, under the boot-up options, select **AX with PicoQuant** (*A-X*) [1].
 - 2.2.1. SCREEN: 71048_2.2.1-and-2.3.1_t1.mp4: 0:08-0:16
- 2.3. Once the software has booted up, select **AX FLIM and FCS** (*F-C-S*) [1].
 - 2.3.1. SCREEN: 71048_2.2.1-and-2.3.1_t1.mp4: 1:09 – 1:29
- 2.4. To set up the microscope for fluorescence lifetime data acquisition, confirm that **Galvano** is selected in the AX pad section [1]. Set the averaging value to 1 [2] and dwell time to 2 [3].
 - 2.4.1. SCREEN:71048_2.4,-2.5,-and-2.6_t1.mp4: 0:01-0:08
 - 2.4.2. SCREEN:71048_2.4,-2.5,-and-2.6_t1.mp4: 0:09-0:15
 - 2.4.3. SCREEN:71048_2.4,-2.5,-and-2.6_t1.mp4: 0:15-0:23

- 2.5. In the laser power section, lower the individual laser sliders to 3 to 4 percent [1]. Set the **All Lasers** slider to zero [2].

2.5.1. SCREEN: 71048_2.4,-2.5,-and-2.6_t1.mp4: 0:22-0:33

2.5.2. SCREEN: 71048_2.4,-2.5,-and-2.6_t1.mp4: 0:33-0:40

- 2.6. On the TI2 Pad (*T-twelve*), after selecting the 20 times objective, in the scan area settings, select 512 by 512 [1] and turn on the detectors [2].

2.6.1. SCREEN: 71048_2.4,-2.5,-and-2.6_t1.mp4: 0:41-0:48

2.6.2. SCREEN: 71048_2.4,-2.5,-and-2.6_t1.mp4: 0:49-0:57

- 2.7. To define the data file path, go to the bottom of the controller section and select **Configure** [1]. After clicking the **PicoQuant or FLIM FCS** tab, under **Workspace path**, select the folder symbol on the right [2]. At storage location, create a new folder for the experiments for the day and close the configure window [3].

2.7.1. SCREEN: 71048_2.7_t1.mp4: 0:03-0:06

2.7.2. SCREEN: 71048_2.7_t1.mp4: 0:07-0:11

2.7.3. SCREEN: 71048_2.7_t1.mp4: 0:12-0:18

- 2.8. Once **Live** is selected and the image acquisition window appears, select **Live** again to pause photon collection [1].

2.8.1. SCREEN: 71048_2.8-and-2.9_t1.mp4: 0:02-0:08

- 2.9. To set up fluorescence lifetime imaging microscopy or FLIM data collection windows, right-click on the live image window and select **PicoQuant Plugin** [1]. Enable **Show Decay Curve** [2], **Show Time trace** [3] and **Show FLIM Statistics** [4].

2.9.1. SCREEN: 71048_2.8-and-2.9_t1.mp4: 0:09-0:14

2.9.2. SCREEN: 71048_2.8-and-2.9_t1.mp4: 0:15-0:16

2.9.3. SCREEN: 71048_2.8-and-2.9_t1.mp4: 0:17-0:28

2.9.4. SCREEN: 71048_2.8-and-2.9_t1.mp4: 0:29-0:41

3. 2D Cellular FLIM Data Collection and Acquisition Setup

Demonstrator: Gareth M. Francis

- 3.1. To set up laser arrangement for the cellular experiment, go to **Experiment Settings** at the top right of the page next to the lasers [1]. After clicking the **Add** next to the multichannel dye box, add the desired dyes [2].

3.1.1. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 0:01-0:07

3.1.2. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 0:08-0:029

- 3.2. Select **Expert mode** [1]. Adjust the detector assignments for each emission spectrum [2]. Route overlapping excitation and emission spectra to different detectors [3].

- 3.2.1. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 0:29-0:34
- 3.2.2. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 0:34-0:39
- 3.2.3. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 0:40 – 0:57
- 3.3. On the left panel, adjust the pass order to minimize crosstalk between dyes with overlapping excitation and emission spectra [1]. After saving and naming the new laser configuration, select the saved configuration for the experiment [2].
 - 3.3.1. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 0:58-1:07
 - 3.3.2. SCREEN: 71048_3.1,-3.2,-and-3.3_t1.mp4: 1:08-1:20
- 3.4. To open the XYZ (X-Y-Z) Overview window, select **View**, choose **Acquisition Controls**, and then select **XYZ Overview** [1].
 - 3.4.1. SCREEN: 71048_3.4_t1.mp4: 0:02-0:14
- 3.5. Add live-cell nuclear stain to the cells [1].
 - 3.5.1. Talent adding live-cell nuclear stain to the cells.
- 3.6. After 30 seconds, place the imaging dish onto the microscope stage [1]. Push the dish toward the bottom left corner of the microscope stage [2]. Tape the sample to the stage when using non-air objectives to prevent xy drift [3].
 - 3.6.1. Talent placing the imaging dish onto the microscope stage.
 - 3.6.2. Talent positioning the dish at the bottom left of the stage.
 - 3.6.3. Talent taping the imaging dish to the microscope stage.
- 3.7. Switch to the 4' 6 diamidino 2 phenylindole or DAPI (Dapi) channel [1]. Using the knob on the side of the controller, slowly raise the Z height until the cell nuclei come into focus [2]. Adjust the microscope controller carrots to modify the sensitivity of the Z height knob [3] and if cells do not appear in view, adjust the XY coordinates [4].
 - 3.7.1. SCREEN: 71048_3.7_t1.mp4: 0:02-0:13
 - 3.7.2. SCOPE: Show the cell nuclei come into focus.
 - 3.7.3. Talent adjusting the microscope controller carrots.
 - 3.7.4. SCREEN: 71048_3.7-and-3.8_t1.mp4: 0:20-0:24
- 3.8. On the microscope control panel, after enabling the Perfect Focus System or PFS (P-F-S) automatic focus, on the right side of the AX (A-X) panel, select the **magnifying glass** next to the PFS button [1]. Once the microscope has automatically normalized the Z height, confirm that the PFS button illuminates and displays **in focus** [2]. **NOTE: The VO is adjusted as per SC.**
 - ~~3.8.1. SCREEN: Show the cursor selecting PFS automatic focus On button.~~
 - 3.8.1. SCREEN: 71048_3.7-and-3.8_t1.mp4: 0:24-0:28. **NOTE: This shot number is changed from 3.8.2 to 3.8.1**
 - 3.8.2. SCREEN: 71048_3.7-and-3.8_t1.mp4: 0:29-0:34 **NOTE: This shot number is changed from 3.8.3 to 3.8.2**

3.9. After searching the cells using AX detector, find the areas of the cells using the DAPI channel [1]. Switch to the ATTO 590 (*AT-TOH-five ninety*) channel to effectively identify bead-loaded cells [2].

3.9.1. SCOPE: Show cells visualized in the DAPI channel.

3.9.2. ~~SCOPE: Show bead loaded cells visualized in the ATTO 590 channel.~~

SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:03-0:08 **NOTE: This scope shot is changed to SC.**

3.10. Once a cell is identified and centered by double clicking, switch to **AX FLIM and FCS** [1].

3.10.1. SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:09-0:18

3.11. Ensuring that **512 by 512** is selected, adjust the frame size by clicking and dragging the corners to fit the cell [1]. Allow the resized frame to turn red [2]. Right-click inside the frame or select **Live** until the frame turns green to confirm the adjustment [3].

3.11.1. SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:19-0:22

3.11.2. SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:23-0:24

3.11.3. SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:25-0:27

3.12. After adjusting the number of captures to the desired value, select **Capture** [1]. Observe the new capture window and the capture progress box on the right side of the screen [2].

3.12.1. SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:28-0:30

3.12.2. SCREEN: 71048_3.9,-3.10,-3.11,-and-3.12_t1.mp4: 0:31-0:40

3.13. Once the FLIM analysis software is opened, navigate to the correct file path [1]. Observe the acquisitions automatically populating the analysis software window [2]. Rename the acquisitions as they are collected [3].

3.13.1. SCREEN: 71048_3.13_t1.mp4: 0:01-0:13

3.13.2. SCREEN: 71048_3.13_t1.mp4: 0:14-0:32

3.13.3. SCREEN: 71048_3.13_t1.mp4: 0:33-0:48

3.14. When the capture is complete, switch back to the AX detector [1]. Select **Live** to search for a new cell [2]. Adjust the size of the AX FLIM and fluorescence correlation spectroscopy or FCS (*F-C-S*) window as needed for larger or smaller cells [3].

3.14.1. SCREEN: 71048_3.14_t1.mp4: 0:02-0:15

3.14.2. SCREEN: 71048_3.14_t1.mp4: 0:16-0:18

3.14.3. SCREEN: 71048_3.14_t2.mp4: 0:41-0:48

3.15. After acquiring new cells during the 3-hour imaging period, export the created .sptw (*dot-S-P-T-W*) file [1].

3.15.1. SCREEN: 71048_3.15_t1.mp4: 0:09-0:28

3.16. Proceed with FLIM data processing and analysis to extract fluorescence lifetime measurements from the collected samples [1].

3.16.1. SCREEN: 71048_3.16_t1.mp4: 0:20-0:23, 0:32-0:50

Results

4. Results

- 4.1. In vitro fluorescence lifetime measurements demonstrated that Riboglow probes Cbl-3xGly-ATTO590 or 3xGly (*three-X-gly*) and Cbl-5xGly-ATTO590 or 5xGly (*Five-X-gly*) were identical except for the number of glycine repeats in their linker segments [1], with 3 repeats in 3xGly [2] and 5 repeats in 5xGly [3].
 - 4.1.1. LAB MEDIA: Figure 3D.
 - 4.1.2. LAB MEDIA: Figure 3D. *Video editor: Emphasize the pink structure labelled as 3xGly.*
 - 4.1.3. LAB MEDIA: Figure 3D. *Video editor: Emphasize the blue structure labelled as 5xGly.*
- 4.2. Representative FLIM acquisition windows with normalized false color scales [1] aligned with the extracted τ AvAmp (*Tau-average-amplitude*) values for each sample type [2].
 - 4.2.1. LAB MEDIA: Figure 2A.
 - 4.2.2. LAB MEDIA: Figure 2B.
- 4.3. Representative decay curves showed that the presence of the RNA A (*R-N-A-A*) Tag increased the extracted fluorescence lifetime in vitro [1], while samples without the RNA A Tag showed left-shifted decay curves indicating shorter average lifetime values [2].
 - 4.3.1. LAB MEDIA: Figure 2C. *Video editor: Emphasize the pink curve labeled as 3xGly + RNA and the green curve as labeled 5xGly + RNA.*
 - 4.3.2. LAB MEDIA: Figure 2C. *Video editor: Emphasize the purple curve labelled as 3xGly and the light blue curve labelled as 5xGly.*
- 4.4. Cellular fluorescence lifetimes of cells bead-loaded with 3xGly [1] were significantly lower than those loaded with 5xGly [2].
 - 4.4.1. LAB MEDIA: Figure 3D. *Video editor: Emphasize the pink dots labelled as 3xGly.*
 - 4.4.2. LAB MEDIA: Figure 3D. *Video editor: Emphasize the blue dots labelled as 5xGly.*
- 4.5. Representative decay curves showed that cells loaded with 3xGly had left-shifted decay curves [1] compared to cells loaded with 5xGly, indicating lower average fluorescence lifetime values [2].
 - 4.5.1. LAB MEDIA: Figure 3C. *Video editor: Emphasize the pink curve labelled as 3xGly.*
 - 4.5.2. LAB MEDIA: Figure 3C. *Video editor: Emphasize the blue curve labelled as 5xGly.*

- 4.6. The nucleus of cells loaded with Riboglow probe consistently showed higher fluorescence lifetimes [1] than the cytosol of the same cell [2].
 - 4.6.1. LAB MEDIA: Figure 5F. *Video editor: Emphasize the light blue dots and light pink dots along with the horizontal line labelled as nucleus in 3xGly and 5xGly.*
 - 4.6.2. LAB MEDIA: Figure 5F. *Video editor: Emphasize the dark blue and dark pink dots along with the horizontal line labelled as cytosol in 3xGly and 5xGly.*
- 4.7. The false color scale acquisition window [1], decay curves [2], and extracted τ_{AvAmp} values all showed the same fluorescence lifetime trend [3].
 - 4.7.1. LAB MEDIA: Figure 5C. *Video editor: Emphasize the bright area in the middle of the first image labelled as 5xGly.*
 - 4.7.2. LAB MEDIA: Figure 5D and 5E. *Video editor: Emphasize the light blue curve in 5D and light pink curve in 5E.*
 - 4.7.3. LAB MEDIA: Figure 5F. *Video editor: Highlight the light pink and light blue dots labeled as nucleus.*

Pronunciation guides:

1. Riboglow
Pronunciation link: No confirmed link found
IPA: /'raɪboʊˌɡloʊ/
Phonetic Spelling: rye·boh·gloh
2. Confocal
Pronunciation link: <https://www.merriam-webster.com/dictionary/confocal>
IPA: /kən'fəʊkəl/
Phonetic Spelling: kuhn·foh·kuhl
3. Fluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorescence>
IPA: /flʊ'reɪsəns/
Phonetic Spelling: floor·eh·suhns
4. Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪ'krɑːskəpi/
Phonetic Spelling: my·krah·skuh·pee
5. Photomultiplier
Pronunciation link: <https://www.merriam-webster.com/dictionary/photomultiplier>
IPA: /ˌfoʊtoʊ'mʌltəˌplaɪər/
Phonetic Spelling: foh·toh·muhl·tuh·ply·uhr
6. Detector
Pronunciation link: <https://www.merriam-webster.com/dictionary/detector>

- IPA: /dɪ'tektər/
Phonetic Spelling: dih·tehk·tuhr
7. PicoQuant
Pronunciation link: No confirmed link found
IPA: /'pi:kɒs,kwɑːnt/
Phonetic Spelling: pee·koh·kwahnt
8. Fluorophore
Pronunciation link: <https://www.merriam-webster.com/dictionary/fluorophore>
IPA: /'flʊrə,fɔːr/
Phonetic Spelling: floor·uh·for
9. Lifetime
Pronunciation link: <https://www.merriam-webster.com/dictionary/lifetime>
IPA: /'laɪf,tʌɪm/
Phonetic Spelling: lyfe·tyme
10. Calibration
Pronunciation link: <https://www.merriam-webster.com/dictionary/calibration>
IPA: /,kælɪ'breɪʃən/
Phonetic Spelling: kal·uh·bray·shuhn
11. Emission
Pronunciation link: <https://www.merriam-webster.com/dictionary/emission>
IPA: /ɪ'mɪʃən/
Phonetic Spelling: ih·mish·uhn
12. Excitation
Pronunciation link: <https://www.merriam-webster.com/dictionary/excitation>
IPA: /,eksɪ'teɪʃən/
Phonetic Spelling: ehk·sy·tay·shuhn
13. Photon
Pronunciation link: <https://www.merriam-webster.com/dictionary/photon>
IPA: /'fɒʊ,tɑːn/
Phonetic Spelling: foh·tahn
14. Cytoplasm
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytoplasm>
IPA: /'saɪtə,plæzəm/
Phonetic Spelling: sy·tuh·plaz·uhm
15. Autofluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/autofluorescence>
IPA: /,ɔːtɒʃlɒ'resəns/
Phonetic Spelling: aw·toh·floor·eh·suhns
16. Nanoparticle
Pronunciation link: <https://www.merriam-webster.com/dictionary/nanoparticle>
IPA: /,nænɒs'pɑːrtɪkəl/
Phonetic Spelling: nan·oh·par·tuh·kuhl

17. Spectroscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectroscopy>
IPA: /spek'trɑːskəpi/
Phonetic Spelling: spek·trah·skuh·pee
18. Photobleaching
Pronunciation link: <https://www.merriam-webster.com/dictionary/photobleaching>
IPA: /ˌfoʊtoʊˈbliːtʃɪŋ/
Phonetic Spelling: foh·toh·blee·ching
19. Cytotoxicity
Pronunciation link: <https://www.merriam-webster.com/dictionary/cytotoxicity>
IPA: /ˌsaɪtəʊtɪˈkʰsɪtɪ/
Phonetic Spelling: sy·toh·tok·siss·uh·tee
20. Quantification
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantification>
IPA: /ˌkwɑːntəfəˈkeɪʃən/
Phonetic Spelling: kwahn·tuh·fuh·kay·shuhn