

Submission ID #: 69742

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Project Page Link: <https://review.jove.com/account/file-uploader?src=21271358>

Title: Multiplexed Immunofluorescence Assay for Spatial Assessment of Senescence Markers *in vivo*

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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: 16

Number of Shots: 48

Introduction

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**

Note: The statements have been edited for length and clarity

Videographer's Note: Ryan changed a few words in the introduction and conclusion.

INTRODUCTION:

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

- 1.1. **Dr Ryan Wallis:** Spatial analysis of senescent cells is important for understanding processes like aging, cancer, immunity and normal tissue homeostasis.

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. **Dr Ryan Wallis:** Detection of senescence relies on profiling multiple biomarkers. Multiplex immunofluorescence imaging enables high throughput profiling within a single tissue section.

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. **Dr Ryan Wallis:** This protocol allows probing a combination of senescence, structural, and immune biomarkers within a single microscopy slide.

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. **Dr Ryan Wallis:** By detecting combination markers within a single sample, spatial analysis of tissue architecture and senescent cell distribution can be performed.

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. **Dr Ryan Wallis:** Future applications include large-scale profiling of senescence biomarkers across tissue types and integration with spatial omics to better resolve cellular heterogeneity.

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.

Ethics Title Card

~~The tissue samples used in this protocol were obtained in compliance with all relevant institutional and regulatory guidelines.~~

Note: This protocol involves the use of tissue microarray mounted with human tissue samples obtained as surplus to diagnostic requirements from the Barts Health NHS archive. Since the video protocol doesn't show the direct use of human tissue samples, the ethics card can be ignored.

Protocol

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

2. Slide Preparation for Multiplex Immunofluorescence

Demonstrator: Dr Federica Mossa

- 2.1. To begin, verify that the five-micrometer thick formalin-fixed paraffin-embedded, or FFPE (*F-F-P-E*) tissue sections with a maximum size of 2 cm x 2 cm (*two-by-two centimeter square*) are centrally mounted on positively charged microscopy slides [1] and are clearly visible within the Coverslipless Slide Holder's viewing window [2-TXT].

2.1.1. WIDE: Talent holding up and examining the central positioning of FFPE tissue sections on charged microscopy slides

2.1.2. Talent confirming the visibility of FFPE tissue section through window. **TXT:**
Tissue section: Cores of 34 tissue types

Videographer's Note: Shot 2.1.3 is an extra shot to show the holes.

- 2.2. Perform [1] the sample preparation steps [2] either manually using a decloaking chamber [3] or automatically using an automated stainer [4].

~~2.2.1. Talent preparing the samples for loading into Coverslipless Slide Holder~~ **Note:**
Shot 2.2.1 was not filmed and will be covered by the following shots in this step.

2.2.2. TEXT ON A PLAIN BACKGROUND

Sample Preparation Steps:

- 1) Dewaxing, rehydration and permeabilization of FFPE sections
- 2) Antigen Retrieval
- 3) Blocking and
- 4) DAPI Counterstaining

2.2.3. Talent prepares the sample manually with a decloaking chamber.

2.2.4. Talent preparing the sample automatically using an automated stainer.

Videographer's note: Shot 2.2.4 has a fair amount of reflections.

- 2.3. Place the prepared slides into the Coverslipless Slide Holder [1]. Align the top and right edges against the metal stops [2]. Lock the slide in place using both hands at the same time to turn the plastic cams to the closed padlock position [3].

2.3.1. Talent placing the prepared slides into Coverslipless Slide Holder.

Videographer's Note: Shots 2.3.1 & 2.3.2 were combined.

2.3.2. Talent aligning the top and right edges of the slide against metal stops.

- 2.3.3. Talent using both hands to turn the plastic cams to lock the slide in place.
- 2.4. Verify the slide is seated completely flat and does not rock within the holder [1]. Place the Click Well insert chamber over the slide [2] and pinch the side bars with two hands to limit pressure and prevent breakages [3].
 - 2.4.1. Talent checking if the slide is seated flat and steady without moving.
 - 2.4.2. Talent placing the Click Well insert chamber over the slide. Videographer's Note: Shots 2.4.2 & 2.4.3 were combined.
 - 2.4.3. Talent pinching the side bars with two hands to limit pressure and prevent breakages.
- 2.5. Load the Click Well with two milliliters of mounting media containing fifty percent glycerol in phosphate-buffered saline via the inlet port [1]. Add a barcode label to the left-hand side of the slide in either orientation, then proceed for imaging [2].
 - 2.5.1. Talent loading the Click Well with 2 mL of mounting media. Videographer: Obtain multiple reusable takes of 2.5.1 as these will be used later in the protocol.
 - 2.5.2. Talent adding barcode label to the left-hand side of the slides on both orientations.

3. Multiplex Imaging Workflow

Demonstrator: Dr. Luke Gammon

- 3.1. For region selection and autofluorescence analysis, open the Mx (M-X) workflow version 4.2.0.4 (four-two-zero-four) multiplex imaging acquisition software [1]. Click on **Tools** to select **Protocol Publisher** [2]. In the **Multiplex Protocol Publisher** pop-up, enter the **Protocol name** [3]. Click on **Create Setup Round** [4], followed by **Add Next Protocol Round** to create multiple rounds of biomarker labelling [5]. Click **Publish**, then select **OK** in the pop-up window [6]. Note: The narration and numbers are adjusted to accommodate the added shots.
 - 3.1.1. SCREEN: 3.1.1_Screen_Recording_Updated.mp4. 00:08–00:19
 - 3.1.2. SCREEN: 3.1.2_Screen_Recording_Updated.mp4. 00:06–00:10
 - Added shot: SCREEN: 3.1.2_Screen_Recording_Updated.mp4. 00:13–00:21
 - Added Shot: SCREEN: 3.1.2_Screen_Recording_Updated.mp4. 00:28-00:31
 - Added Shot: SCREEN: 3.1.2_Screen_Recording_Updated.mp4. 00:39–00:49; 1:03–1:10
 - Added Shot: SCREEN: 3.1.2_Screen_Recording_Updated.mp4. 1:21– 1:27
- 3.2. Acquire the Cy3 (Sigh-three) channel for virtual hematoxylin and eosin or H&E (H and E) images [1]. Click on **Slide**, then **Create New Batch** [2]. After selecting the protocol

name, enter the **slide ID [3]**. Click on **Add Slide** and then select **Create Batch** for creating a multiplex experiment workflow [4]. **Note: The narration and numbers are adjusted to accommodate the added shots.**

3.2.1. SCREEN: 3.2.1_Screen_Recording_Updated.mp4. 00:15–00:17; 00:21–00:26

3.2.2. SCREEN: 3.2.2_Screen_Recording_Updated.mp4. 00:04–00:09

Added Shot: SCREEN: 3.2.2_Screen_Recording_Updated.mp4. 00:20–00:29

Added Shot: SCREEN: 3.2.2_Screen_Recording_Updated.mp4. 00:30–00:39

- 3.3. Open the Cell DIVE acquisition software [1], click on Load [2] to load the slide into the microscope [3]. Under the Tools drop-down menu, click on the grid icon to define the desired regions [4] and use the move tool to reposition the defined regions [5]. Next, use the draw tool to select 4 fields of view in the selected range, ensuring each field of view has at least two neighbors to prevent stitching issues, [6] and click Save regions [7]. In the Mx Workflow window, click on **Viewer QC** to open the Cell Dive Viewer page and visualize the tissue image in 10X magnification [8]. Click on the Image correction icon to adjust the image brightness, and click **Submit QC**, to select **Pass and Reload Slide** in the pop-up window [9]. Once the image reloads, click on ADD ROI to draw and adjust the regions of interest [10]. Select the desired fields of view and repeat the addition of ROI [11]. Next, click **Send to Lab [12]**. **Note: The narration and numbers are adjusted to accommodate the added shots.**

3.3.1. SCREEN: 3.3.1_Screen_Recording_Updated.mp4. 00:06–00:10

Added Shot: SCREEN: 3.3.1_Screen_Recording_Updated.mp4. 00:14–00:16

Added Shot: SCREEN: 3.3.1_Screen_Recording_Updated.mp4. 00:29–00:33

3.3.2. SCREEN: 3.3.2_Screen_Recording_Updated.mp4. 00:10–00:18

Added Shot: SCREEN: 3.3.2_Screen_Recording_Updated.mp4. 00:18–00:28

Video Editor: Please emphasize the text “Edit Scan Plan: Click on a region and move the selected region” at the bottom left side of the screen.

Added Shot: SCREEN: 3.3.2_Screen_Recording_Updated.mp4. 00:28–00:35

Video Editor: Please emphasize the text “Edit Scan Plan: Click and/or drag to select fields of view in the range” at the bottom left side of the screen.

Added Shot: SCREEN: 3.3.2_Screen_Recording_Updated.mp4. 00:37–00:40

Added Shot: SCREEN: 3.3.3_Screen_Recording_Updated.mp4. 00:07– 00:19

Added Shot: SCREEN: 3.3.3_Screen_Recording_Updated.mp4. 00:21– 00:33

Added Shot: SCREEN: 3.3.3_Screen_Recording_Updated.mp4. 00:36– 00:47

Added Shot: SCREEN: 3.3.3_Screen_Recording_Updated.mp4. 00:50– 00:54;
1:11–1:18

Added Shot: SCREEN: 3.3.3_Screen_Recording_Updated.mp4. 1:30– 1:35

- 3.4. Load the slide to start the 20x (*twenty-X*) autofluorescence image acquisition [1]. Under the Imaging QC menu, select the checkbox corresponding to the specific slide ID, then click **Viewer QC [2]**. Zoom in to the loaded image to scan for out of focus regions or artefacts [3]. Generate background images for subtraction from the final

biomarker signal and repeat for each multiplex round [4]. Click **Submit QC**, then select **Pass and Exit** to generate background images [5]. **Note: The narration and numbers are adjusted to accommodate the added shots.**

- 3.4.1. SCREEN: 3.4.1_Screen_Recording_Updated.mp4. 00:14–00:16; 1:34–1:40.
Video Editor: Please emphasize the text “Estimated time remaining and Scanning Slide” at the bottom left of the screen.
- 3.4.2. SCREEN: 3.4.2_and_3.4.3_Screen_Recording_Updated.mp4. 00:03–00:07
Added shot: SCREEN: 3.4.2_and_3.4.3_Screen_Recording_Updated.mp4. 00:14– 00:21
Added shot: SCREEN: 3.4.2_and_3.4.3_Screen_Recording_Updated.mp4. 00:27– 00:34
- 3.4.3. SCREEN: 3.4.2_and_3.4.3_Screen_Recording_Updated.mp4. 00:35–00:39
- 3.5. Prior to sequential biomarker staining, centrifuge the antibodies at 13000 *g* for one minute at room temperature to limit artefacts [1]. Prepare the antibody solution at a 1:200 (*one-in-two hundred*) dilution in phosphate-buffered saline or PBS (*P-B-S*) containing 0.3 (*zero-point three*) percent bovine serum albumin or BSA (*B-S-A*) [2]. Remove the mounting media by pipetting out from the Click Well port [3] and wash thrice with two milliliters of PBS containing 0.01 (*zero-point zero one*) percent Tween twenty or PBST (*P-B-S-T*) for five minutes [4].
 - 3.5.1. Talent centrifuging the antibody sample. *Videographer: Please capture the digital meter on the centrifuge showing the centrifuge speed and time.*
 - 3.5.2. Talent preparing the antibody solution in PBS containing 0.3% BSA.
 - 3.5.3. Talent is removing the mounting media from the well. *Videographer: Obtain multiple reusable takes of 3.5.3 and 3.5.4 as these will be used later in the protocol.*
 - 3.5.4. Talent washing the well with 2 mL PBST.
- 3.6. Add three hundred and fifty microliters of antibody solution into the port to ensure full sample coverage within the staining chamber [1]. Incubate for one hour at room temperature [2]. Next, pipette out the antibody solution from the Click Well port [3] and wash thrice with two milliliters of PBST for five minutes [4].
 - 3.6.1. Talent adding 350 μ L antibody solution to the well port. **Videographer’s Note: Shots 3.6.1 & 3.6.2 were combined.**
 - 3.6.2. Talent incubating the slides for one hour at RT.
 - 3.6.3. Talent removing the antibody solution from the well port.
 - 3.6.4. *Reuse 3.5.4*
- 3.7. Add two milliliters of mounting media [1]. Insert Click Well holder into the microscope [2] and load the slide to start 20X biomarker imaging [3]. Perform the imaging QC

using the **Blend Editor and Image Correction** options and submit the image QC evaluation [4].

3.7.1. *Reuse 2.5.1*

3.7.2. Talent inserting the Click Well holder into the microscope. *Videographer: Obtain multiple reusable takes of 3.7.2 as these will be used later in the protocol.*

3.7.3. SCREEN: 3.7.3_Screen_Recording_Updated.mp4. 00:12–00:14; 1:30–1:35

3.7.4. SCREEN: 3.7.4_Screen_Recording_Updated.mp4. 00:06–00:08; 00:32–00:40; 00:46–00:51

3.8. Pipette out the mounting media from the Click Well port [1] and wash thrice with two milliliters of PBST for five minutes [2]. Prepare [3] the dye inactivation solution freshly before use [4].

3.8.1. *Reuse 3.5.3*

3.8.2. *Reuse 3.5.4*

3.8.3. Talent preparing the dye-inactivation solution.

3.8.4. TEXT ON A PLAIN BACKGROUND

Double distilled water 7 mL

0.5 M NaHCO₃ 2 mL

30% H₂O₂ 1 mL

Prepare the solution freshly before use and apply immediately without delay.

3.9. After removing PBST, add two milliliters of dye inactivation solution for each slide [1]. Incubate for fifteen minutes in the slide holder [2]. Pipette out the dye inactivation solution from the Click Well port [3] and wash with two milliliters of PBST for five minutes [4].

3.9.1. Talent adding 2 mL dye inactivation solution. **Videographer's Note: Shots 3.9.1 & 3.9.2 were combined.**

3.9.2. Talent placing the slides for 15 minutes in slide holder.

3.9.3. Talent removing the dye-inactivation solution from the well port.

3.9.4. *Reuse 3.5.4*

3.10. Add two milliliters of mounting media and insert Click Well holder into the microscope [1] to initiate automatic round specific 20x autofluorescence imaging [2]. Progress the workflow by passing QC steps as directed on screen upon completion [3].

3.10.1. *Reuse 2.5.1 and 3.7.2.*

3.10.2. SCREEN: 3.10.2_Screen_Recording_Updated.mp4. 00:14–00:16; 1:43–1:47

3.10.3. SCREEN: 3.10.3_Screen_Recording_Updated.mp4. 00:05–00:08; 00:14–00:17;
00:23–00:25; 00:33–00:35

3.11. Repeat the sequential staining, dye inactivation, and multiplex imaging for as many markers as required and continue until completion [1]. Use **HALO** (*halo*) software to select various channels for visualizing the localization of markers at different regions of the tissue core [2]. Click **Analyze** and select **Entire Image** to analyze the quantitative metrics for individual markers [3]. Click on **Results** to view the total number of cells in the analyzed tissue core and the subset of cells expressing specific markers [4]. **Note:** The narration and numbers are adjusted to accommodate the added shots.

3.11.1. SCREEN: 3.11.1_Screen_Recording_Updated.mp4. 00:05–00:14; 00:19–00:22

3.11.2. SCREEN: 3.11.2_Screen_Recording_Updated.mp4. 00:03–00:08

Added Shot: SCREEN: 3.11.2_Screen_Recording_Updated.mp4. 00:16–00:24

Added Shot: SCREEN: 3.11.2_Screen_Recording_Updated.mp4. 00:28–00:30;
00:41–00:48

Results

4. Results

- 4.1. Compared to the automated slide stainer-based method, the manual, declocking chamber-based method resulted in a higher frequency of tissue artifacts, characterized specifically by tissue detachment [1], as well as lost [2] or slipped cores [3].
 - 4.1.1. LAB MEDIA: Figure 3: *Video Editor: Please emphasize the row "Tissue detachment" in BOND and Manual columns sequentially.*
 - 4.1.2. LAB MEDIA: Figure 3: *Video Editor: Please emphasize the row "Missing Cores" in BOND and Manual columns sequentially.*
 - 4.1.3. LAB MEDIA: Figure 3: *Video Editor: Please emphasize the row "Slipped Cores" in BOND and Manual columns sequentially.*
- 4.2. Quantification performed with digital pathology software on representative pancreas tissue revealed that p16-positive cells were associated with lower LB-1 levels [1] and vice versa, demonstrating a profile consistent with senescence-associated marker patterns [2].
 - 4.2.1. LAB MEDIA: Figure 6B: *Video Editor: Please emphasize the orange region in the image.*
 - 4.2.2. LAB MEDIA: Figure 6C: *Video Editor: Please emphasize the green region in the image.*
- 4.3. A Z-score (*Zee-score*) normalized heatmap illustrates the distribution of the p16+/LB1-ve (*P-sixteen-positive-Lamin-B-one negative*) senescence phenotype across various tissues, revealing high concentrations of senescent cells in the lung, adrenal and pituitary glands [1] corresponding with elevated p16 [2] and diminished LB1 levels [3].
 - 4.3.1. LAB MEDIA: Figure 6D: *Video Editor: Please emphasize the boxes corresponding to "Lung, Adrenal and Pituitary" and the right-side label "Senescent".*
 - 4.3.2. LAB MEDIA: Figure 6D: *Video Editor: Please emphasize the boxes corresponding to "Lung, Adrenal and Pituitary" and the right-side label "p16".*
 - 4.3.3. LAB MEDIA: Figure 6D: *Video Editor: Please emphasize the boxes corresponding to "Lung, Adrenal and Pituitary" and the right-side label "Lamin B1".*
- 4.4. Conversely, the appendix, lymph node and spleen exhibited low senescence [1], maintaining high LB1 [2] and low p16 expression [3].
 - 4.4.1. LAB MEDIA: Figure 6D: *Video Editor: Please emphasize the boxes corresponding to "Appendix, Lymph node and spleen" and the right-side label "Senescent".*

- 4.4.2. LAB MEDIA: Figure 6D: *Video Editor: Please emphasize the boxes corresponding to “Appendix, Lymph node and spleen” and the right-side label “Lamin B1”.*
- 4.4.3. LAB MEDIA: Figure 6D: *Video Editor: Please emphasize the boxes corresponding to “Appendix, Lymph node and spleen” and the right-side label “p16”.*

Pronunciation Guide:

- FFPE
Pronunciation link: <https://www.merriam-webster.com/dictionary/FFPE>
IPA: /ˌɛf,ɛf,piˈi:/
Phonetic Spelling: ef·ef·pee·ee
- Formalin-fixed
Pronunciation link: <https://www.merriam-webster.com/dictionary/formalin>
IPA: /fərˈmælin/
Phonetic Spelling: fer·mal·in
- Paraffin-embedded
Pronunciation link: <https://www.merriam-webster.com/dictionary/paraffin>
IPA: /ˈpærəfɪn/
Phonetic Spelling: par·uh·fin
- Microscopy
Pronunciation link: <https://www.merriam-webster.com/dictionary/microscopy>
IPA: /maɪˈkrɑːskəpi/
Phonetic Spelling: my·krah·skuh·pee
- Immunofluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/immunofluorescence>
IPA: /ɪˌmjuːnoʊflɔːresəns/
Phonetic Spelling: ih·myoo·noh·floor·ess·uhns
- Permeabilization
Pronunciation link: <https://www.merriam-webster.com/dictionary/permeabilize>
IPA: /ˌpɜːmiəˈbɪləˌzeɪʃən/
Phonetic Spelling: per·mee·uh·bil·uh·zay·shuhn
- Antigen
Pronunciation link: <https://www.merriam-webster.com/dictionary/antigen>
IPA: /ˈæntɪdʒən/
Phonetic Spelling: an·tuh·juhn
- Counterstaining
Pronunciation link: <https://www.merriam-webster.com/dictionary/counterstain>
IPA: /ˈkaʊntərˌsteɪnɪŋ/
Phonetic Spelling: kown·ter·stay·ning
- DAPI
Pronunciation link: <https://www.howtopronounce.com/dapi>

- IPA: /'dæpi/
Phonetic Spelling: dap·ee
- Autofluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/autofluorescence>
IPA: /,ɔːtoʊflɒ'resəns/
Phonetic Spelling: aw·toh·floor·ess·uhns
 - Biomarker
Pronunciation link: <https://www.merriam-webster.com/dictionary/biomarker>
IPA: /'baɪoʊ mɑːrkər/
Phonetic Spelling: by·oh·mar·ker
 - Hematoxylin
Pronunciation link: <https://www.merriam-webster.com/dictionary/hematoxylin>
IPA: /,hi:mə'tɑːksəlɪn/
Phonetic Spelling: hee·muh·tok·suh·lin
 - Centrifuge
Pronunciation link: <https://www.merriam-webster.com/dictionary/centrifuge>
IPA: /'sentrəˌfjuːdʒ/
Phonetic Spelling: sen·truh·fyooj
 - Glycerol
Pronunciation link: <https://www.merriam-webster.com/dictionary/glycerol>
IPA: /'glɪsəˌrɔːl/
Phonetic Spelling: gliss·uh·rawl
 - Phosphate-buffered
Pronunciation link: <https://www.merriam-webster.com/dictionary/phosphate>
IPA: /'fɑːsˌfeɪt/
Phonetic Spelling: foss·fayt
 - Bovine
Pronunciation link: <https://www.merriam-webster.com/dictionary/bovine>
IPA: /'boʊvaɪn/
Phonetic Spelling: boh·vine
 - Albumin
Pronunciation link: <https://www.merriam-webster.com/dictionary/albumin>
IPA: /æl'bjʊːmɪn/
Phonetic Spelling: al·byoo·min
 - Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /'maɪkrəʊˌliːtər/
Phonetic Spelling: my·kroh·lee·ter
 - NaHCO₃
Pronunciation link: <https://www.howtopronounce.com/sodium-bicarbonate>
IPA: /'soʊdiəm baɪ'kɑːrbəneɪt/
Phonetic Spelling: soh·dee·uhm by·kar·buh·nate

- Hydrogen peroxide
Pronunciation link: <https://www.merriam-webster.com/dictionary/hydrogen%20peroxide>
IPA: /'haɪdrədʒən pə'rɔːksaɪd/
Phonetic Spelling: hy·druh·juhn puh·rok·side
- Pathology
Pronunciation link: <https://www.merriam-webster.com/dictionary/pathology>
IPA: /pə'θɔːlədʒi/
Phonetic Spelling: puh·thol·uh·jee
- Localization
Pronunciation link: <https://www.merriam-webster.com/dictionary/localization>
IPA: /ˌlɒskələ'zeɪʃən/
Phonetic Spelling: loh·kuh·luh·zay·shuhn
- Quantitative
Pronunciation link: <https://www.merriam-webster.com/dictionary/quantitative>
IPA: /'kwɑːntə'tetɪv/
Phonetic Spelling: kwon·tuh·tay·tiv
- Artefacts
Pronunciation link: <https://www.merriam-webster.com/dictionary/artifact>
IPA: /'ɑːrtə'fækt/
Phonetic Spelling: ar·tuh·fakt
- Multiplex
Pronunciation link: <https://www.merriam-webster.com/dictionary/multiplex>
IPA: /'mʌltɪ'pleks/
Phonetic Spelling: mul·tuh·pleks
- ROI
Pronunciation link: <https://www.howtopronounce.com/roi>
IPA: /ˌɑːr.oʊ'aɪ/
Phonetic Spelling: ar·oh·eye
- Autofluorescence
Pronunciation link: <https://www.merriam-webster.com/dictionary/autofluorescence>
IPA: /ˌɔːtɒʃlʊ'reɪsəns/
Phonetic Spelling: aw·toh·floor·ess·uhns