

Submission ID #: 69341

Scriptwriter Name: Sulakshana Karkala

Project Page Link: <https://review.jove.com/account/file-uploader?src=21149053>

Title: Pulsed Wave Doppler Assessment of Diastolic Dysfunction in the ZSF-1 Rat Model of Pulmonary Hypertension Due to Left Heart Disease

Authors and Affiliations:

Maria Bauer¹, Lakshmi Santhanam², Jochen Steppan¹

¹Department of Anesthesiology and Critical Care Medicine, Johns Hopkins University

²Department of Anesthesiology and Critical Care Medicine, Department of Biomedical Engineering, Department of Chemical and Biomolecular Engineering, Johns Hopkins University

Corresponding Authors:

Jochen Steppan (J.Steppan@jhmi.edu)

Email Addresses for All Authors:

Maria Bauer (mbauer14@jhmi.edu)

Lakshmi Santhanam (lsantha1@jh.edu)

Jochen Steppan (J.Steppan@jhmi.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**
- 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: 31

Number of Shots: 57

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**.

INTRODUCTION:

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

- 1.1. **Maria Bauer:** Our research focuses on heart failure with preserved ejection fraction, and the significant sex differences the disease manifests.
 - 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. **Maria Bauer:** Diastolic dysfunction is a hallmark of HFpEF with indices that can be assessed using Doppler sonography without 2D echocardiography which is expensive and requires sophisticated equipment.
 - 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. **Maria Bauer:** This protocol allows you to study established echocardiographic indices of HFpEF including E/A ratio, IVRT or E-wave deceleration time.
 - 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. **Maria Bauer:** This technique lacks 2D visualization of the heart, and some markers of diastolic function are load dependent, a condition that is difficult to control for.

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. **Maria Bauer:** This is a low-cost technique that reliably detects disease progression over time in cardiopulmonary diseases that manifest diastolic dysfunction.

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

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Johns Hopkins University

Protocol

NOTE: Screen recordings and timestamps are added at the postshoot stage, please contact Poornima for any doubts (Poornima.g@jove.com)

Videographer's NOTE:

Most of the slates should be correct. There were a couple times where talent started before I could slate - so put the slate at the tail.

C012.mov was a false start on the camera - so didn't include that one.

Since we had a nice shot of the work area with the rat and software on the monitor, I decided to film the shots marked SCREEN while we were already there. I advised talent to still do the screen recordings as originally planned, but it didn't take us a lot of extra time so I figured it might give the editor more options.

~~2. Preparation and Setup of Electrocardiogram Monitoring in a Rodent Model~~

Preparation and Acquisition of Electrocardiogram (ECG) Tracings in Rodent

NOTE: Title changed during postshoot

Demonstrator: Maria Bauer

NOTE: The screen recordings are a bit hazy, but this is the best authors can record in their old system

- 2.1. To begin, turn on the tablet connected to the ECG tracing pad [1]. Select **RSMonitor** from the available applications [2]. Then turn on the ECG pad by pressing the round button in front [3].
 - 2.1.1. Talent powering on the tablet placed beside the electrocardiogram tracing pad.
 - 2.1.2. SCREEN: SCREEN-2.1.2.mp4 00:04-00:10.
 - 2.1.3. Talent pressing the round power button on the front of the electrocardiogram pad.
- 2.2. Tap **SETTINGS** in the right lower corner of the user interface [1]. Select **PRESETS** from

the menu of experimental settings [2]. Tap the dropdown menu under **Saved Presets** [3]. Then choose **Rat CV** and click **OK** in the bottom right corner [4].

2.2.1. SCREEN: SCREEN-2.2.1-to-2.2.4-Screen_Recording_20260304-124731_RSMonitor.mp4 00:04-00:07.

2.2.2. SCREEN: SCREEN-2.2.1-to-2.2.4-Screen_Recording_20260304-124731_RSMonitor.mp4 00:15-00:18.

2.2.3. SCREEN: SCREEN-2.2.1-to-2.2.4-Screen_Recording_20260304-124731_RSMonitor.mp4 00:19-00:22.

2.2.4. SCREEN: SCREEN-2.2.1-to-2.2.4-Screen_Recording_20260304-124731_RSMonitor.mp4 00:23-00:29.

2.3. Now clean the ECG pad with 70 percent ethyl alcohol to remove any ECG gel residue [1]. Obtain highly conductive electrode gel for the electrocardiogram and water-soluble hypoallergenic ultrasound gel for the Doppler [2].

2.3.1. Talent wiping the electrocardiogram pad surface with 70 percent ethyl alcohol.

2.3.2. Shot of the electrode gel and ultrasound gel on the workspace.

2.4. Place the anesthetized animal supine on the noninvasive ECG pad with the nose positioned in the appropriately sized nose cone with a Vaporizer Output set to 1.5 to 2% [1].

2.4.1. Talent positioning the anesthetized animal supine on the electrocardiogram pad with the nose secured in the nose cone.

2.5. Using a cotton-tipped applicator, apply a small amount of electrode gel to the dorsal surface of the forepaws and to the plantar side of the hind paws [1]. Secure the paws with tape on the electrode contact surface of the ECG pad, ensuring sufficient contact between the gel and the pad [2-TXT].

2.5.1. Talent applying electrode gel to the forepaws and hind paws with a cotton-tipped applicator.

2.5.2. Talent taping the paws onto the electrode contact surfaces of the electrocardiogram pad.

2.6. Then use a gentle depilatory cream to remove the chest and abdominal hair [1]. Wipe off any residual cream to avoid skin irritation [2].

2.6.1. Talent applying depilatory cream to the chest and abdomen of the animal.

2.6.2. Talent wiping off the depilator cream from the skin surface.

2.7. Ensure that the isoelectric baseline and the P, QRS, and T waveforms are clearly displayed on the computer monitor [1].

2.7.1. SCREEN: SCREEN-2.7.1-Screen_Recording_20260304-142206_RSMonitor.mp4.

3. ~~Noninvasive Doppler Echocardiography in Rodent Model~~

Configuring the Parameters for Doppler Measurements

NOTE: Title changed during postshoot

- 3.1. Create a new folder identifying the animal and the data of interest [1].
 - 3.1.1. SCREEN: SCREEN-3.1.1-2026-03-04-13-58-16.mp4 01:49-01:58.
- 3.2. Turn on the Pulsed Doppler Transceiver device using the power switches on the front and the back of the large blue box [1]. Then turn on the Doppler Signal Processor [2].
 - 3.2.1. Talent switching on the front and rear power buttons of the large blue Pulsed Doppler Transceiver device.
 - 3.2.2. Talent turning on the small black Doppler Signal Processor.
- 3.3. Now open the Doppler Signal Processing Workstation software [1]. Click on **Setup** and select **System Setup** [2].
 - 3.3.1. SCREEN: SCREEN-3.3.1---3.3.2,-3.4.1.mp4 00:10-00:18.
 - 3.3.2. SCREEN: SCREEN-3.3.1---3.3.2,-3.4.1.mp4 00:19-00:26.
- 3.4. Select the 20 megahertz probe frequency for the experiment [1-TXT]. Plug in the 20-megahertz probe when investigating a young rat or mouse. Use a 10 megahertz probe for older, larger, or obese rats [2]. If both probes are connected, use the toggle switch on the front of the transceiver module to switch between frequencies as needed [3].
 - 3.4.1. SCREEN: SCREEN-3.3.1---3.3.2,-3.4.1.mp4 00:31-00:37.
 - 3.4.2. Talent connecting the appropriate handheld probe to the transceiver module.
 - 3.4.3. Shot of the toggle switch on the front panel being used to alternate between probe frequencies.
- 3.5. Ensure that the handheld probe transducer is connected to channel A or B receptacle on the transceiver module [1]. Select the corresponding channel to operate the handheld probe [2]. Set the **DIRECTION** switch to **TWD (T-W-D)** to display flow toward the probe above the zero-flow baseline [3].
 - 3.5.1. SCREEN: Shot of the probe cable connected to channel A or B receptacle. **NOTE:** The entire steps 3.5 and 3.6 were covered by the videographer as per the authors
 - 3.5.2. SCREEN: Shot of the channel selection switch set to 10 megahertz or 20 megahertz.
 - 3.5.3. SCREEN: the **DIRECTION** switch being set to **TWD**.

- 3.6. Then adjust the **RANGE** to 2.5 to 3.5 millimeters for mice or 4 to 6 millimeters for rats [1]. Set **FILTER** to 15 to smooth the phasic velocity output to a damped signal [2].
- 3.6.1. SCREEN: The **RANGE** dial adjusted to the specified millimeter depth. **NOTE: The entire steps 3.5 and 3.6 were covered by the videographer as per the authors, these involved physical knobs etc**
- 3.6.2. SCREEN: The **FILTER** control is being set to 15.
- 3.7. Press **GO** in the top left corner of the user interface [1]. In the pop-up window, enter the relevant acquisition information including animal type, gender, date of birth, age on acquisition date, and weight [2].
- 3.7.1. SCREEN: SCREEN-3.7.1---3.8.2-2026-03-04-14-05-28.mp4 00:09-00:12.
- 3.7.2. SCREEN: SCREEN-3.7.1---3.8.2-2026-03-04-14-05-28.mp4 00:15-00:27.
- 3.8. Select the desired pre-created folder from the **Directory** dropdown menu and click **OK** [1]. Adjust the ECG baseline by dragging the axis scale bar up or down and until the R waves are clearly visible [2].
- 3.8.1. SCREEN: SCREEN-3.7.1---3.8.2-2026-03-04-14-05-28.mp4 00:28-00:30 and 00:55-01:00.
- 3.8.2. SCREEN: SCREEN-3.8.2-2026-03-04-14-09-28.mp4 00:10-00:21.

Noninvasive Pulsed Doppler Assessment

NOTE: Section title added at postshoot stage

- 3.9. To perform Doppler measurements, first apply a small amount of ultrasound gel to the substernal and epigastric region of the anesthetized animal's chest and abdomen [1].
- 3.9.1. Talent applying ultrasound gel to the substernal and epigastric area of the animal.
- 3.10. Place the Doppler probe midline under the diaphragm just below the sternum with the head pointing cephalad toward the left ear of the animal [1]. Slowly scan for blood flow by moving the probe in small circular motions, with increasing radius [2].
- 3.10.1. Talent positioning the Doppler probe under the diaphragm with correct orientation.
- 3.10.2. Shot of the probe being moved in small circular motions over the gelled area.
AND
SCREEN: SCREEN-3.10.2-2026-03-04-14-13-19.mp4 00:08-00:16.
Video Editor: Please play both shots side by side in a split screen

3.11. Maintain gentle pressure of the probe against the skin under the diaphragm at all times [1].

3.11.1. Talent holding the probe steadily with gentle pressure applied to the skin.

3.12. If mitral flow is not detected, adjust the **RANGE** dial in increments of 0.5 millimeters [1] until the transmitral waveform is identified [2].

3.12.1. SCREEN: SCREEN-3.12.2---3.13.1-2026-03-04-14-41-17.mp4 01:00-01:15.

3.12.2. SCREEN: Figure 1A. **NOTE:** The recordings and figure are a little blurry and that is the best they can provide

3.13. Ensure that the Doppler waveform and the electrocardiogram are displayed simultaneously on the screen [1].

3.13.1. SCREEN: SCREEN-3.12.2---3.13.1-2026-03-04-14-41-17.mp4 01:18-01:25.

3.14. Create snapshots using the foot pedal once the correct flow is identified and Repeat until optimal data quality is recorded [1]. Stop data acquisition by clicking **STOP** when satisfied with the data quality [2].

3.14.1. Talent pressing the foot pedal to capture a snapshot.

3.14.2. SCREEN: SCREEN-3.14.2-2026-03-04-17-21-39.mp4 00:05-00:10.

4. ~~Electrocardiogram Acquisition in the Rodent Model~~

Doppler Ultrasonography Data Processing

NOTE: Title changed during postshoot

4.1. Click on **File** and select **Open** from the dropdown menu [1]. Choose the highest quality data file from the pre-created folder for analysis [2].

4.1.1. SCREEN: SCREEN-4.1-2026-03-04-15-13-43.mp4. 00:00-00:10

4.1.2. SCREEN: SCREEN-4.1-2026-03-04-15-13-43.mp4. 00:15-00:25

4.2. Now press on **Analysis** and then **Analysis Mode**, choose **Step 2: General Setup** and set **Data type** to **Doppler** [1]. Then set **Signal** to **Mitral Inflow**, select the appropriate animal model [2] and choose **All measurement names** under default measurement names [3].

4.2.1. SCREEN: SCREEN-4.2-USE-THIS-2026-03-04-15-25-32.mp4 00:07-00:00:25.

4.2.2. SCREEN: SCREEN-4.2-USE-THIS-2026-03-04-15-25-32.mp4 00:26-00:36

4.2.3. SCREEN: SCREEN-4.2-USE-THIS-2026-03-04-15-25-3200:42-00:48

4.3. Select **Step 5: R peak editor** from the **Analysis Control** dropdown menu [1]. Enable

Display beat and R peak markers and click **Auto calculate [2]**.

4.3.1. SCREEN: SCREEN-4.3.1---4.3.2-2026-03-04-14-57-24.mp4 00:03-00:08.

4.3.2. SCREEN: SCREEN-4.3.1---4.3.2-2026-03-04-14-57-24.mp4 00:15-00:21

- 4.4. Now choose **Step 6: Beat editor** from the Analysis Control dropdown menu, enable **Display beat and R peak markers** and click **Select all beats [1]**. Next, choose **Step 7: Marker editor**, click **Display analysis markers** and choose the desired parameter under **Current Marker Selected [2]**. Ensure the given parameters are available for the completeness of data **[3]**.

4.4.1. SCREEN: SCREEN-4.4.1-USE-THIS-2026-03-04-15-27-14.mp4 00:03-00:14

4.4.2. SCREEN: SCREEN-4.4.3-2026-03-04-15-27-52.mp4 00:03-00:11

4.4.3. SCREEN: SCREEN-4.4.3-2026-03-04-15-27-52.mp4 03:10-03:20

AND

TEXT ON PLAIN BACKGROUND:

Parameters:

ES: Early Start

EPV: Early Peak Velocity

EEAS: Early End, Atrial Start

APV: Atrial Peak Velocity

AE: Atrial End

IVCTE: Isovolumic Contraction Time End

IVRT: Isovolumic Relaxation Time Start

Video Editor: Please play both shots side by side in a split screen

- 4.5. Select **Step 8: Measurement results**, click **Copy Results to Clipboard** to copy the data into an Excel file and save it **[1]**. ~~After closing the **Analysis Control** window, press **File** and select **Save** to store the performed measurements **[2]**. Exit the Doppler Signal Processing Workstation **[3]**.~~

4.5.1. SCREEN: SCREEN-4.5-2026-03-04-15-31-26.mp4 00:05-00:09, 00:13-00:16 and 01:22-01:26

~~4.5.2. SCREEN: Click on **File** and select **Save** to save the measurements.~~

~~4.5.3. SCREEN: Exit the Doppler Signal Processing Workstation.~~

- 4.6. Remove the tape from the animal's paws after turning off the vaporizer **[1]**. Then remove the animal from the electrode pad **[2]**.

4.6.1. Talent gently removing tape from the animal's paws.

4.6.2. Talent lifting the animal carefully from the electrocardiogram pad.

4.7. Clean off any remaining depilator cream and electrode gel from the animal [1]. Then transfer the animal back into its cage with free access to water and food for recovery [2].

4.7.1. Talent wiping the animal's skin to remove residual cream and gel.

4.7.2. Talent placing the animal back into its cage with visible water and food sources.

Results

5. Results

5.1. For the most accurate assessment of diastolic function, antegrade and retrograde flow patterns related to the opening and closing of the mitral and aortic valve needed to be visualized within the same cardiac cycle over multiple cycles [1].

5.1.1. LAB MEDIA: Figure 1. **NOTE:** The figure is a little blurry. Authors said they cannot provide a new figure 1 as their system is old/technical limitations and they informed that is how the data will look. Plus the blurry background and edges in the waves of figure 1 are inherent to the experiment and that is how they should look.

5.2. Multiple indices were determined from the biphasic transmitral flow pattern, including the early filling peak [1], the atrial contraction peak [2], the ratio between these two peaks [3], and the deceleration time of the early filling peak [4].

5.2.1. LAB MEDIA: Figure 2. *Video editor: Highlight the top left plot labeled “E max (cm/s)”.*

5.2.2. LAB MEDIA: Figure 2. *Video editor: Highlight the top right plot labeled “A max (cm/s)”.*

5.2.3. LAB MEDIA: Figure 2. *Video editor: Highlight the bottom left plot labeled “E/A”.*

5.2.4. LAB MEDIA: Figure 2. *Video editor: Highlight the bottom right plot labeled “E decel time”.*

5.3. The isovolumic relaxation time or IVRT was a good indicator of diastolic dysfunction in rodents [1]. IVRT increased during aging in ZSF1 rat model of Pulmonary hypertension-Left heart disease [2]. This change could be detected as early as 16 weeks of age [3]. 24-weeks old obese male rats showed higher IVRT [4] compared to earlier timepoints [5] and lean controls [6].

5.3.1. LAB MEDIA: Figure 3

5.3.2. LAB MEDIA: Figure 3 *Video editor: Highlight the data sequentially for baseline, interim, and final timepoints*

5.3.3. LAB MEDIA: Figure 3. *Video editor: Highlight the red ‘interim’ data*

5.3.4. LAB MEDIA: Figure 3. *Video editor: Highlight the red data points in the ‘Final’ group that are positioned higher*

5.3.5. LAB MEDIA: Figure 3. *Video editor: Highlight the red data points in the baseline and interim*

5.3.6. LAB MEDIA: Figure 3. *Video editor: Highlight the blue data points in the 'Final'*

1. Electrocardiogram
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrocardiogram>
IPA: /ɪˌlɛk.troʊˈkɑːr.di.ooˌgræm/
Phonetic Spelling: ihˌlekˌtrohˌkarˌdeeˌohˌgram
2. Rodent
Pronunciation link: <https://www.merriam-webster.com/dictionary/rodent>
IPA: /ˈroʊ.dənt/
Phonetic Spelling: rohˌduhnt
3. Tracing
Pronunciation link: <https://www.merriam-webster.com/dictionary/tracing>
IPA: /ˈtreɪ.sɪŋ/
Phonetic Spelling: trayˌsing
4. Conductive
Pronunciation link: <https://www.merriam-webster.com/dictionary/conductive>
IPA: /kənˈdʌk.tɪv/
Phonetic Spelling: kuhnˌdukˌtiv
5. Electrode
Pronunciation link: <https://www.merriam-webster.com/dictionary/electrode>
IPA: /ɪˌlɛk.troʊd/
Phonetic Spelling: ihˌlekˌtrohd
6. Hypoallergenic
Pronunciation link: <https://www.merriam-webster.com/dictionary/hypoallergenic>
IPA: /ˌhaɪ.pouˌæl.əˈdʒen.ɪk/
Phonetic Spelling: hyˌpohˌalˌerˌjenˌik
7. Ultrasound
Pronunciation link: <https://www.merriam-webster.com/dictionary/ultrasound>
IPA: /ˈʌl.trəˌsaʊnd/
Phonetic Spelling: ulˌtruhˌsownd
8. Doppler
Pronunciation link: <https://www.merriam-webster.com/dictionary/doppler>
IPA: /ˈdɑː.plə/
Phonetic Spelling: dopˌler
9. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetized>
IPA: /əˈnɛs.θə.taɪzd/
Phonetic Spelling: uhˌnesˌthuhˌtyzd
10. Noninvasive
Pronunciation link: <https://www.merriam-webster.com/dictionary/noninvasive>

- IPA: /ˌnɑːn.ɪnˈveɪ.sɪv/
Phonetic Spelling: non·in·vay·siv
11. Vaporizer
Pronunciation link: <https://www.merriam-webster.com/dictionary/vaporizer>
IPA: /ˈveɪ.pəˌraɪ.zər/
Phonetic Spelling: vay·puh·ry·zer
12. Dorsal
Pronunciation link: <https://www.merriam-webster.com/dictionary/dorsal>
IPA: /ˈdɔːr.səl/
Phonetic Spelling: dor·suhl
13. Plantar
Pronunciation link: <https://www.merriam-webster.com/dictionary/plantar>
IPA: /ˈplæn.tɑːr/
Phonetic Spelling: plan·tar
14. Depilatory
Pronunciation link: <https://www.merriam-webster.com/dictionary/depilatory>
IPA: /dɪˈpɪl.əˌtɔːr.i/
Phonetic Spelling: dih·pil·uh·tor·ee
15. Isoelectric
Pronunciation link: <https://www.merriam-webster.com/dictionary/isoelectric>
IPA: /ˌaɪ.sou.ɪˈlek.trɪk/
Phonetic Spelling: eye·soh·ih·lek·trik
16. Baseline
Pronunciation link: <https://www.merriam-webster.com/dictionary/baseline>
IPA: /ˈbeɪs.laɪn/
Phonetic Spelling: bays·line
17. Waveforms
Pronunciation link: <https://www.merriam-webster.com/dictionary/waveform>
IPA: /ˈweɪvˌfɔːrm/
Phonetic Spelling: wave·form
18. Echocardiography
Pronunciation link: <https://www.merriam-webster.com/dictionary/echocardiography>
IPA: /ˌek.ouˌkɑːr.diˈɑː.grə.fi/
Phonetic Spelling: ek·oh·kar·dee·og·ruh·fee
19. Transceiver
Pronunciation link: <https://www.merriam-webster.com/dictionary/transceiver>
IPA: /trænˈsiː.vər/
Phonetic Spelling: tran·see·ver
20. Processor
Pronunciation link: <https://www.merriam-webster.com/dictionary/processor>
IPA: /ˈprɑː.ses.ər/
Phonetic Spelling: prah·sess·er
21. Frequency
Pronunciation link: <https://www.merriam-webster.com/dictionary/frequency>

- IPA: /'fri:.kwən.si/
Phonetic Spelling: free·kwuhn·see
22. Receptacle
Pronunciation link: <https://www.merriam-webster.com/dictionary/receptacle>
IPA: /rɪ'sɛp.tə.kəl/
Phonetic Spelling: ruh·sep·tuh·kuhl
23. Phasic
Pronunciation link: <https://www.merriam-webster.com/dictionary/phasic>
IPA: /'feɪ.zɪk/
Phonetic Spelling: fay·zik
24. Velocity
Pronunciation link: <https://www.merriam-webster.com/dictionary/velocity>
IPA: /və'leɪ.sə.ti/
Phonetic Spelling: vuh·lah·suh·tee
25. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /,æk.wə'zɪʃ.ən/
Phonetic Spelling: ak·wuh·zish·uhn
26. Directory
Pronunciation link: <https://www.merriam-webster.com/dictionary/directory>
IPA: /də'rek.tə.ri/, /dɑ'rek.tə.ri/
Phonetic Spelling: duh·rek·tuh·ree
27. Substernal
Pronunciation link: <https://www.merriam-webster.com/dictionary/substernal>
IPA: /sʌb'stɜ:.nəl/
Phonetic Spelling: sub·stur·nuhl
28. Epigastric
Pronunciation link: <https://www.merriam-webster.com/dictionary/epigastric>
IPA: /,ɛp.ɪ'gæs.trɪk/
Phonetic Spelling: ep·ih·gas·trik
29. Diaphragm
Pronunciation link: <https://www.merriam-webster.com/dictionary/diaphragm>
IPA: /'daɪ.ə fræm/
Phonetic Spelling: dye·uh·fram
30. Cephalad
Pronunciation link: <https://www.merriam-webster.com/dictionary/cephalad>
IPA: /'sɛf.ə.læd/
Phonetic Spelling: sɛf·uh·lad
31. Mitral
Pronunciation link: <https://www.merriam-webster.com/dictionary/mitral>
IPA: /'maɪ.trəl/
Phonetic Spelling: my·truhl
32. Transmitral
Pronunciation link: <https://www.merriam-webster.com/dictionary/transmitral>

IPA: /ˌtrænzˈmaɪ.trəl/

Phonetic Spelling: tranz·my·truhl

33. Antegrade

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

IPA: /ˈæn.tiˌɡreɪd/

Phonetic Spelling: an·tee·grayd

34. Retrograde

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

IPA: /ˈrɛ.troʊˌɡreɪd/

Phonetic Spelling: reh·troh·grayd

35. Biphasic

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

IPA: /baɪˈfeɪ.zɪk/

Phonetic Spelling: by·fay·zik

36. Isovolumic

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

IPA: /ˌaɪ.sou.vəˈluː.mɪk/

Phonetic Spelling: eye·soh·vuh·loo·mik

37. Echocardiographic

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

IPA: /ˌɛk.oʊˌkɑːr.di.ooˈɡræf.ɪk/

Phonetic Spelling: ek·oh·kar·dee·oh·graf·ik

38. Hypertension

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

IPA: /ˌhaɪ.pəˈtɛn.ʃən/

Phonetic Spelling: hy·per·ten·shuhn

39. Aortic

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

IPA: /eɪˈɔːr.tɪk/

Phonetic Spelling: ay·or·tik

40. Clipboard

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

IPA: /ˈklɪpˌbɔːrd/

Phonetic Spelling: klip·bord