

This online training is required for any researchers or staff who wish to use Comparative Medicine's ultrasound to image small animals for diagnostic or surgical purposes in the small animal vivarium. It is meant to be completed before in person training and also as a reference for future use of the equipment.

Overview

Scientific Background

Imaging Procedure

Further Resources

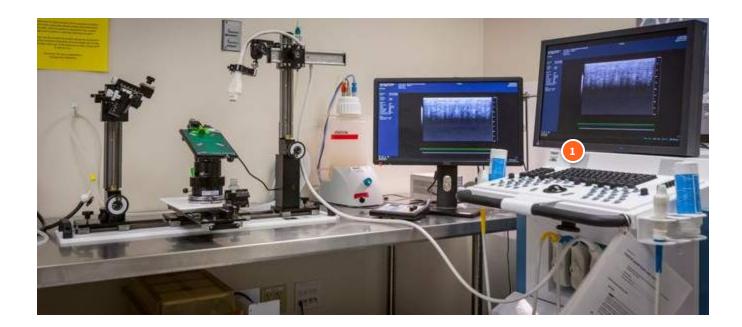
Lesson 1 of 4



Preclinical Imaging

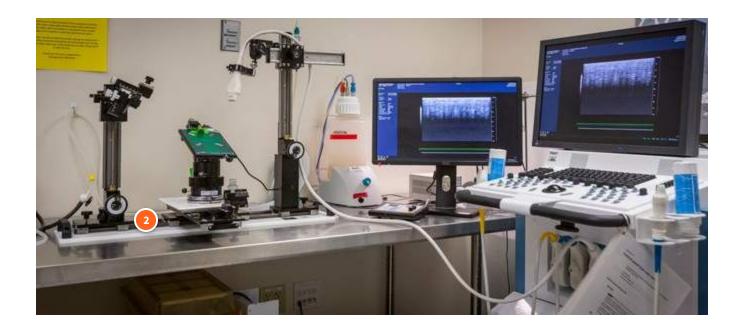
Reservations on the Vevo 2100 ultrasound can be made up to 30 days in advance in iLab. The machine can be reserved on weekends and holidays, with 24/7 access. Full access is granted after completing a Comparative Medicine facility tour, this online training, and a brief in-person ultrasound training.





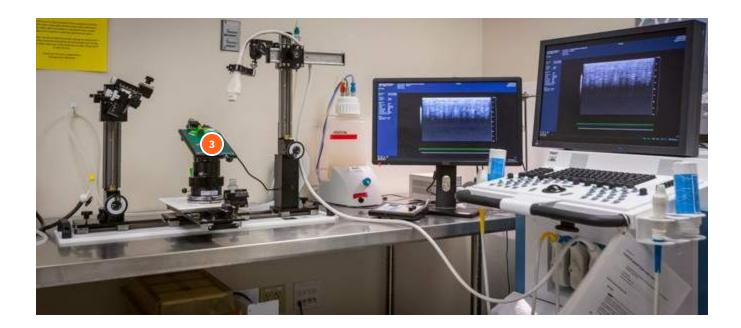
Ultrasound machine

Vevo 2100 Ultrasound Machine from VisualSonics.



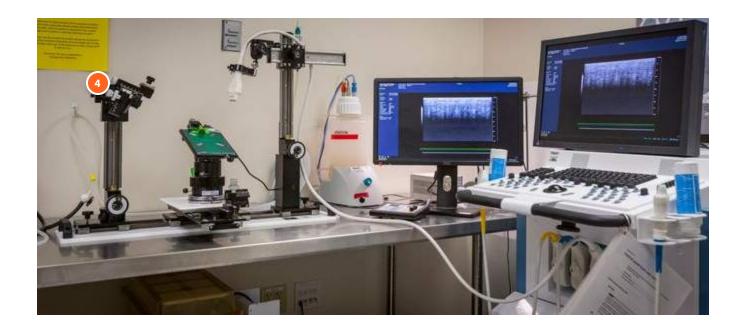
Stereotaxic Frame

Allows for precise positioning of the animal on the heated platform, precise positioning of the ultrasound transducer, and excellent control of a syringe mount system for guided injections.



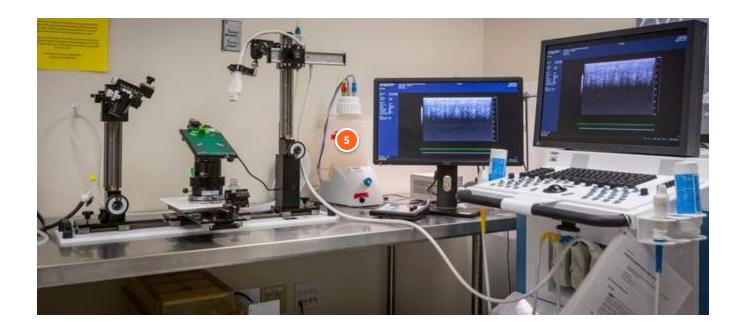
Heated Animal Monitoring Stage

Keeps animals warm during imaging or surgery and prevents hypothermia



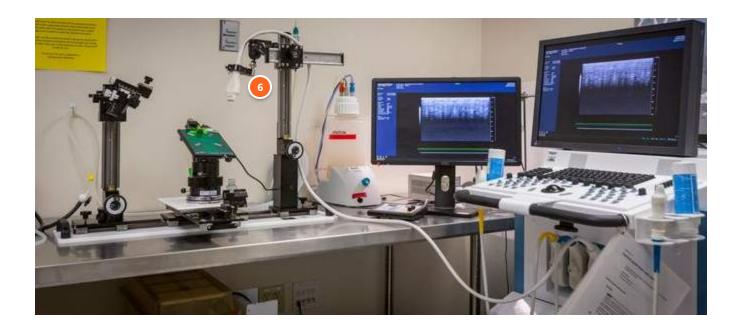
Needle Injection Mount

Allows for precise positioning and delivery



Vacuum pump

Can be used to suction ethanol and other fluids used for ultrasound-guided surgical procedures

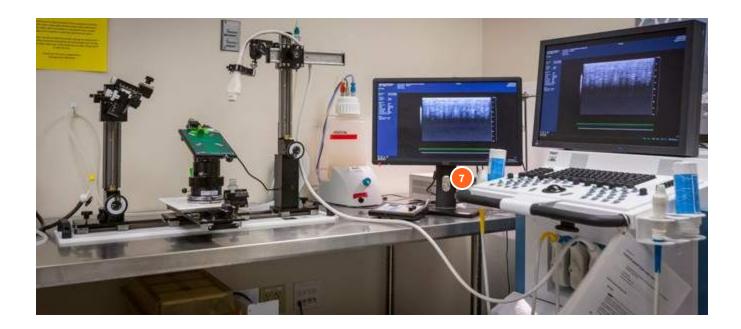


55 MHz transducer

This is the most commonly used transducer in the vivarium. It is optimized for mouse/rat embryology, mouse abdominal, reproductive, epidermal imaging, tumor imaging (up to 13 mm in diameter), mouse vascular, small rat vascular, some abdominal (kidney), and ophthalmology.

Specifications:

- broadband frequency: 26 MHz 52 MHz
- axial image resolution: 40 microns
- image size: 14.1 mm (w) x 15 mm (d)
- max frame rate: 557 fps

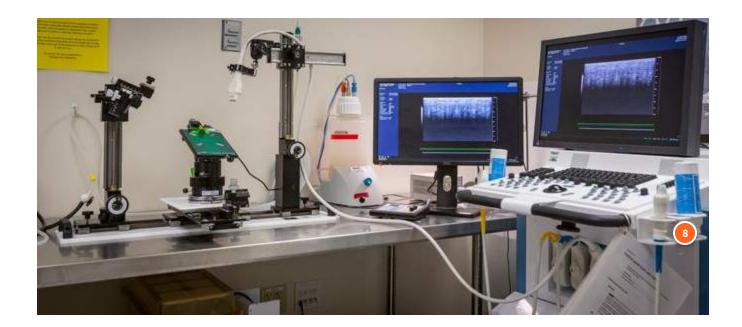


24 MHz Transducer

Alternative transducer less commonly used, but used for rat cardiology and abdominal imaging (less than 400 g), large tumor imaging (up to 23 mm in diameter), and all contrast applications.

Specifications:

- broadband frequency:14 MHz 28 MHz
- axial image resolution: 75 microns
- image size: 23 mm (w) x 30 mm (d)
- max frame rate: 367 fps

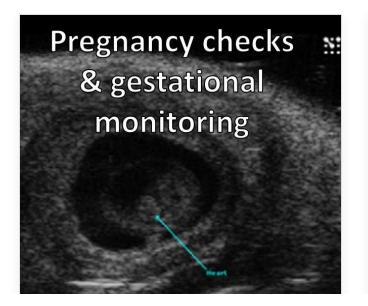


Ultrasound Gel

Provided in the room and restocked daily

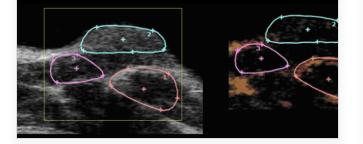
Click on the items in the image below to see more information about each of the ultrasound components.

Some ultrasound applications at Fred Hutch are...



Verifying pregnancy and monitoring embryonic development. Image shows an embryonic mouse heart, non-invasively imaged through the mother's abdomen.

Tumor monitoring and response to treatment



Monitoring the development and response of tumors to new forms of treatment.

Image shows quantification of tumor size using non-linear contrast agents.

Ultrasoundguided surgical techniques

Right Lobe of Live

Ultrasound-guided surgical techniques to improve animal health outcomes.

Image shows an image-guided injection into the portal vein of an adult mouse.

CONTINUE

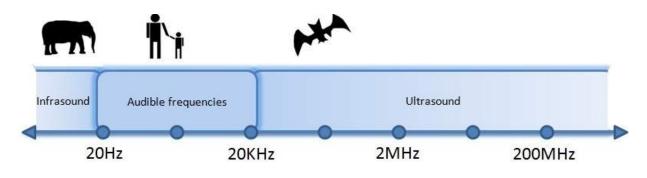
Lesson 2 of 4



PI Preclinical Imaging

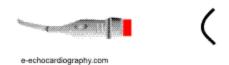
Ultrasonic Waves & Transducers

The ultrasound transducer sends out very high frequency soundwaves above 20 kilohertz, these soundwaves cannot be heard by the human ear.

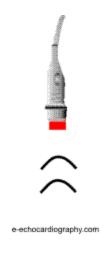


Sound frequency range. Image courtesy of teachengineering.org

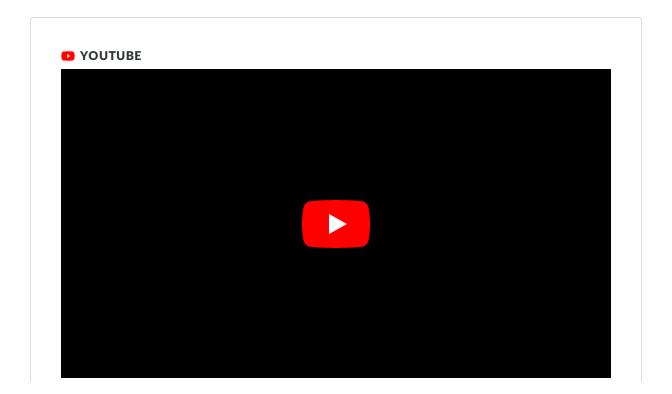
As these ultrasonic waves travel through a medium, they reflect off the different membranes and edges that they encounter. Some of these reflected soundwaves are re-detected by the transducer and are compiled together to form an image.



The more material the ultrasound waves encounter in a medium, the more those waves will be attenuated. Thus, the intensity of the images farther into a sample are going to be lower than they are at the top of the image. The imaging depth limit of our ultrasound machine is around 10 mm, but this number can vary with tissue type, the transducer used, and the application.



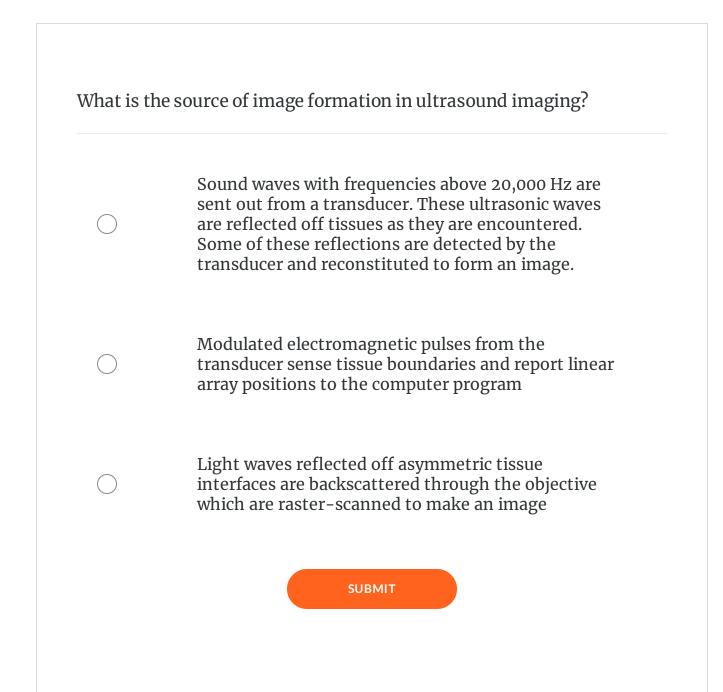
Further detail on how ultrasound imaging works:



Ultrasound medical imaging | Mechanical waves and sound | Physics | Khan Academy

You can actually use sound to create images of the inside of the body. Wild! Created by David SantoPietro.Watch the next lesson: https://www.khanacademy.org/...

VIEW ON YOUTUBE >



P

 $Complete \ the \ content \ above \ before \ moving \ on.$

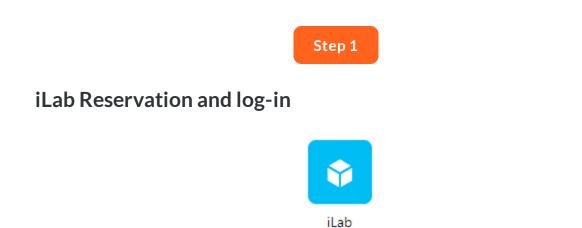
Lesson 3 of 4



Preclinical Imaging

Getting Started with the Ultrasound





Reserve time on iLab and log in at the start of your session to turn the monitor on.

For instructions on how to register for iLab if you have not yet done so, <u>click here.</u>

Step 2

Anesthesia System Set-Up

- Check isoflurane level, ensure you have brought your own isoflurane to refill at the end of your session
- Open oxygen tank and check oxygen tank level
- Turn on heat monitoring stage (35 40 celsius)
- Place mouse in induction box and turn isoflurane vaporizer level between 1.5-2.5% to put the mouse to sleep. Make sure air flow to box is open.

Step 3

Mouse positioning for imaging

- Once mouse is anesthetized, transfer to heated platform in a supine configuration. Make sure air flow to nose cone is open.
- Use tape to hold arms and legs securely so the mouse does not shift while imaging, being careful not to over-extend limbs.
- If necessary, shave mouse in area where imaging will occur, or use Nair to remove hair to improve image quality.
- Place a generous amount of ultrasound gel over the region of interest for scanning.

You are ready to begin imaging!

See the next section on how to operate the ultrasound machine

Keyboard layout and Control Panel

The seven most commonly used features of the control panel are labeled in the below image.





Mouse/Scroll bar

Moves the cursor around on the screen



Prefs, also known as "User Preferences"

Go here to add a new user, or Map to Network Drive to save and transfer your data



Select/Click

Select button effectively 'clicks' on whatever the cursor is hovering over



Study Management

Takes the user to the data overview page, where they can begin a new study or add a series to their existing studies. Here users can see images that they have just acquired and review their data.



B-Mode

This is the main method for imaging. Shows the 2-D image acquired live from the transducer in use.



Cine Store

Saves a video of the previous 100 frames (NOT the following 100 frames) after it is pressed.

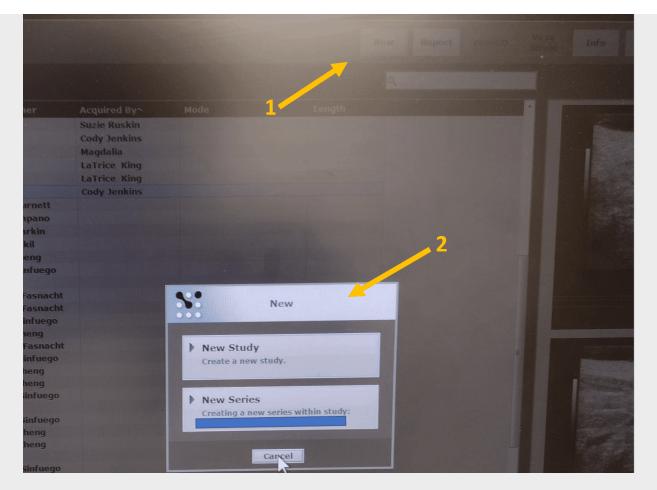


Frame Store

Saves a single image (1 frame) while in B-mode.

Further information on additional keys are on laminated sheets in the room and can be discussed at the in-person component of the ultrasound training.

Setting up the computer for an experiment



Choose new study or new series

- Select study management
- Click "New" in top banner
- A Study is a group of Series
- You can later add more images or videos from a series to a study
- Once a series is closed, you cannot add more to the series

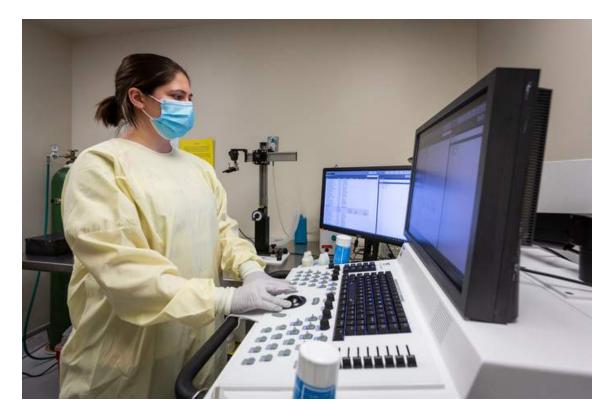
Series	
dy Information	
Study Name Ongoing Study or New Study title here	Study Notes
Owner PI or Researcher name here	
Granting Institution	
ries Information	
Series Name Series 1 200 Series Series Series 1 200 Series S	
	Series Notes
Name of person collecting the data here	e
· · · · · · · · · · · · · · · · · · ·	
Animal ID	
Type	Anesthetic Type
	Anesthetic On Management and Anesthetic On
Color Strain Strain	Anesthetic Off
Source Weight Weight	Protocol ID
Heart Rate	Protocol Name
Date of Birth mm/dd/yyyy	Injectable Contractor Contractor
Sex Male	Injection Site
© Female	Injection Amount

- Input Study or Series info
- Input your Study and/or Series name
- "Owner" and "Acquired by" can be different users (e.g. the PI name and then technician name)
- Most of the time "General Imaging" and "Abdominal Package" should be used. When ready to begin, hit "OK"
- Press B-mode to start scanning

How could you record a 100-frame video of an ultrasound-guided injection?

\bigcirc	Appropriately position transducer, perform injection, then quickly press 'Cine store'
\bigcirc	Appropriately position transducer, press 'Cine Store', then perform injection
\bigcirc	Appropriately position transducer, perform injection, then quickly press 'Frame store'
	SUBMIT

Wrapping Up





Recover animals



If applicable, let each mouse wake up in a warm cage. Once awake, return it to its home cage.

Step 2

Save data to network drive

- 1. Click "Prefs"
- 2. Select the "Network" tab.
- 3. Click on "Map Network Drive".
- 4. Fill in "network location" with: \\file.fhcrc.org\home "Z:" drive should be selected. Box labeled "log on with different credentials" should be checked.
- 5. Log on with your hutch user ID (followed by <u>@fhcrc.org</u>) (your ID should look like username@fhcrc.org.) and password
- 6. Once connected to the network, exit out of window by clicking "ok" in the top right corner of the screen.
- 7. In the study browser, select datasets by holding [Ctrl] and clicking the desired studies.
- 8. To save, click "copy to." Select your mapped network and desired folder then click "ok".
- 9. Once finished, go back to "Prefs" key, Network tab, select your network drive and click "delete network drive" to disconnect.

These instructions are also printed out in the room.

Please note that Comparative Medicine is not responsible for saved data. It is the responsibility of the user to copy and store their data in a different location.



Clean up!



- 1. Clean gel off transducer with T-spray and kim wipes (do *not* use C-Dox on the ultrasound as it can rust or damage the materials)
- 2. Remove magnetic mount and clean anything that may have accumulated under it! (see photo above)
- 3. Wipe up water and ethanol mess that may have accumulated under stage (NOT doing this results in the equipment rusting)
- 4. Turn off oxygen tank
- 5. Turn off vaporizer and refill with isoflurane
- 6. Turn off heat stage and heated platform
- 7. Clean stage and any tape residue
- 8. If you used the vacuum pump bottle, empty it in sink (nearby sinks are in necropsy, BE-351, or BE-157)



Log out of iLab to avoid being charged for further time.

Summary

Wrap up complete!

See the next section for further organ-specific imaging guides and information on how to view the images that you have just acquired!

Which of the following should you *always* do when finished imaging with the ultrasound? Select all that apply:

Turn off heat stage

Top off isoflurane in the vaporizer

Turn off oxygen tank

Clean ultrasound gel off transducer with T-spray and Kim Wipe
Clean induction box
Clean heated bed
Wipe up any water or ethanol that has accumulated on the platform, under the magnetic mount, or under the rails
spray everything with C-DOX
tell someone from Preclinical Imaging that you are done using the machine
tell CM staff that you are done in the room
Log out of iLab
Report any issues , including low isoflurane or that the previous user did not clean up after themselves
SUBMIT

?

Complete the content above before moving on.

Lesson 4 of 4



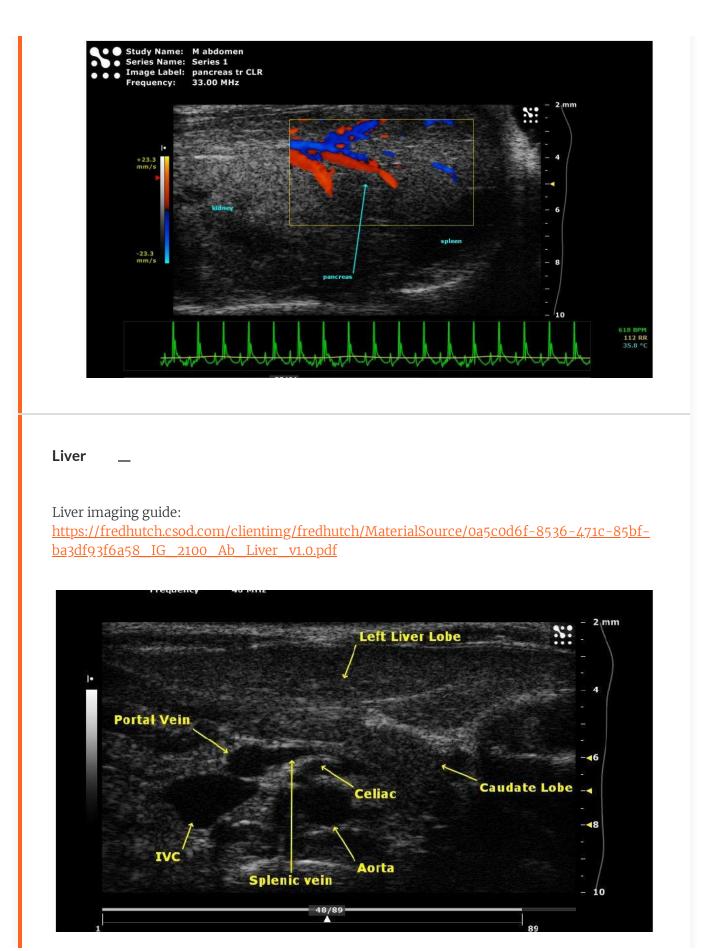
Preclinical Imaging

Organ-Specific Imaging Guides and examples

Select from below the organ(s), areas, and applications for your own research to explore the further guides available and see example images, all courtesy of VisualSonics.

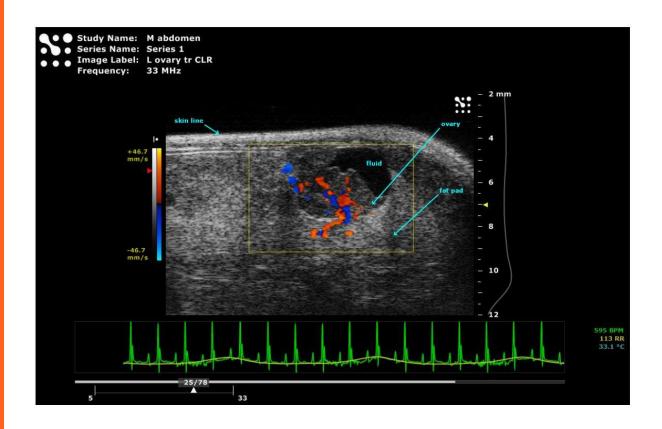
Pancreas

Pancreatic Imaging guide: <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/949f3932-72e9-493b-be3f-</u> <u>17f480ed018f_IG_2100_Ab_Pancreatic_v1.0.pdf</u>



Reproductive Organs & Embryonic Development Monitoring

- Embryonic Mouse Imaging
 <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/5356ede8-45fd-40e8 ac37-fa1a6cdece66 IG 2100 Db Embryonic Mouse v1.0.pdf</u>
- Ovarian Imaging guide: <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/f392466f-27d9-4f41-</u> <u>8601-93a10496e1ad_IG_2100_Re_Ovarian_v1.0.pdf</u>
- Testicle and epididymus guide: <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/8befbe57-f100-4a52-</u> <u>90a4-b666e896b986_IG_2100_Re_Testicle_and_Epididymus_v1.0.pdf</u>
- Prostate Gland and Surrounding Structures
 <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/179cfc76-9273-4c36 aa35-545e87961fd6_IG_2100_Re_Prostate_v1.0.pdf</u>

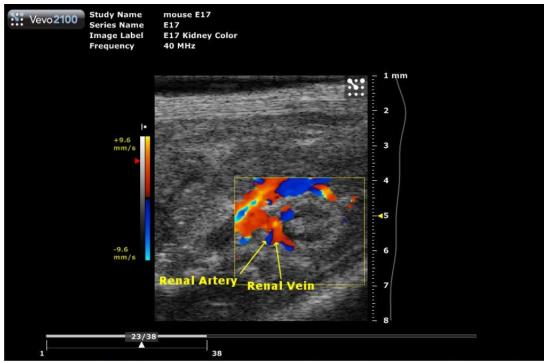


- Adrenal gland imaging guide:

https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/83048a32-0b48-4c71-abeeatea80601087__IG__2100__Ab__Adrenal__Gland__v1.0.pdf

- Renal System Imaging

https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/6bde8c6f-2176-4e21-8294e79ddc332113__IG__2100__Ab__Renal__System__v1.1.pdf

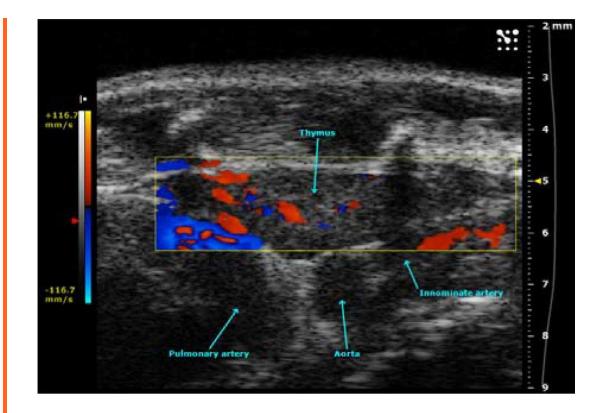


Images 6 & 7: E17 Renal Artery from the Aorta above and E16 below

Thymus

Thymus imaging guide:

https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/62d4a79e-4e15-4a4f-a666ac85d6a2bc66 IG 2100 Cv Thymus v1.0.pdf



Heart & Vasculature

- Carotid Imaging
 <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/a5eab1c2-5478-4210 9352-16d85a379fb1_IG_2100_Cv_Carotid_v1.0.pdf</u>
- Powerpoint on How to do Mouse EchoCardiography <u>https://fredhutch.csod.com/ui/lms-learning-details/app/material/3dc236ee-e2d5-4808-b6da-foab20d53757</u>
- Vascular Imaging
 <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/820e4002-8903-42fb-</u>
 <u>a75e-5da0a114cof9_IG_2100_Cv_Vascular_v1.0.pdf</u>
- Echocardiography Imaging (PDF) <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/45a486ca-5599-4d10-</u> <u>ae53-dof5f77f1ac7_IG_2100_Cv_Echocardiography_v1.1.pdf</u>

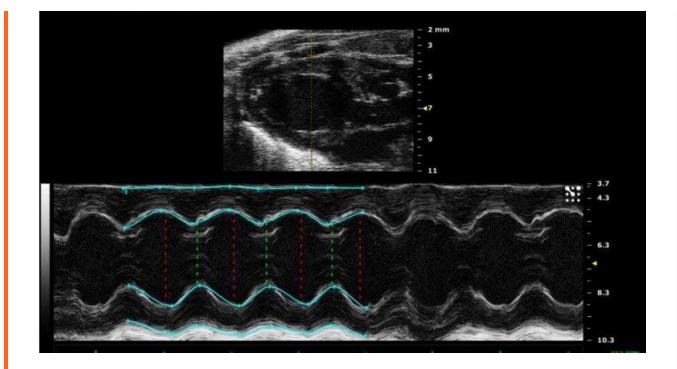
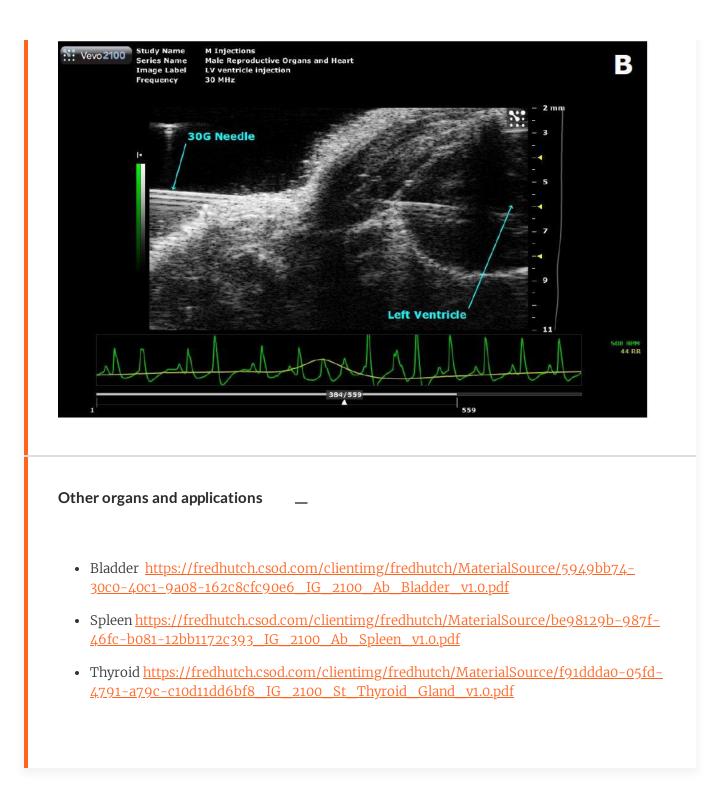


Image-guided needle injections

- Embryo image-guided needle injections <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/06af9b1b-b63c-4424-</u> <u>9cb0-7585a1dcf65e_IG_2100_Ot_IGNI_Embryo_v1.0.pdf</u>
- Adult small animal image-guided needle injections
 <u>https://fredhutch.csod.com/clientimg/fredhutch/MaterialSource/8bda3eaa-fabd-48b8-b1ef-8dfb952be962_IG_2100_Ot_IGNI_Adult_v1.0.pdf</u>

Preclinical Imaging offers in-person trainings on performing image-guided needle injections. Contact us if you'd like to schedule a training!



Click below if you would like to save a PDF of this training for future reference or to print out for your records

PDF

preclinical-vevo-2100-ultrasound-online-training-nWahS3Fx.pdf

How do I view my ultrasound images after I have taken them?

After you transfer your data to your personal network drive, the images can be viewed in several different types of software. See below for possible options:

Some ultrasound image analysis software options:

FIJI / Image J

FIJI (also known as ImageJ) is a free and open source software developed by the NIH. FIJI is the recommended analysis method for viewing basic ultrasound images, as it can be downloaded to any computer without a license. <u>However</u>, you will need to export your ultrasound images to a different file format (.tiff is preferable) before transferring them off of the ultrasound computer. Some more advanced ultrasound imaging applications should be viewed with VevoLab.

All Arnold Library windows computers have ImageJ installed for use as well.



VevoLab

Vevo Lab is the image analysis software from VisualSonics, who is the manufacturer of the Comparative Medicine Vevo 2100 ultrasound.

This software is available for use on Arnold Library Computer #5 and on the Arnold Fellows Office Computer.

Analyze 14

Analyze 14 is an advanced data viewing software. If users already are familiar with it, it can be a powerful tool for more complex image analysis. However, if you are not already familiar with Analyze 14 or do not need very advanced analysis, we <u>do not</u> recommend using this modality for viewing ultrasound images. We rather refer users to ImageJ or VevoLab.

Analyze 14 is available on Arnold Library Computer #5 or on the Steam Plant PC.

ANALYZE 14 INFO

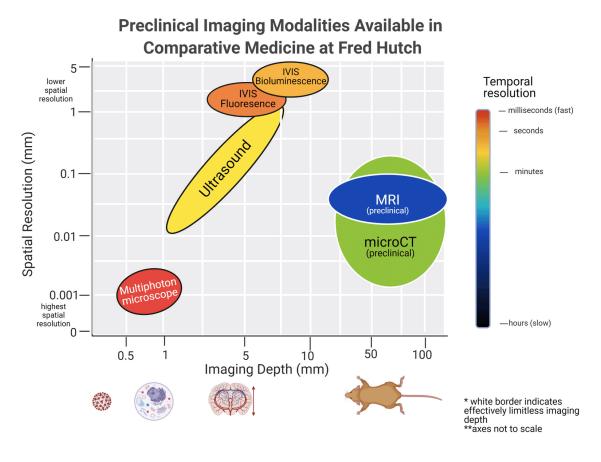
Thank you for completing this part of the ultrasound training!

You will need to complete a brief in-person component of this training before being able to access the ultrasound. Please contact <u>preclinicalimaging@fredhutch.org</u> with any questions or feedback regarding this training.

Have questions or want further information?

Preclinical Imaging offers an array of imaging services, including one-on-one consultations and analysis advice!

See below charts for further information regarding the services provided by Preclinical Imaging and feel free to send an inquiry to us at <u>preclinicalimaging@fredhutch.org</u>.



Spatial, temporal, and depth imaging capabilities of the Modalities available in Preclinical Imaging