



This online training is required for any researchers or staff who wish to use the EchoMRI to measure the whole-body composition of awake small animals in the vivarium of Comparative Medicine.

Access to the EchoMRI requires a two-part training: This online component and a brief in-person component with a Preclinical Imaging staff member.

Overview

Scientific Background

Operating the Echo

Logging off and Saving Data

Echo Quiz

Lesson 1 of 5



PI Preclinical Imaging

The EchoMRI performs whole body composition measurements of fat and water composition in live, awake small animals



Mouse body composition measurements that can be obtained via the EchoMRI

- Whole body composition measurements of fat and water composition in live, awake animals
- No need for anesthesia & no radiation or toxicity concerns
- Not a form of imaging, just whole-body <u>measurements</u> of fat tissue, lean tissue, free water & total water
- Soft tissue measurements only, does not account for bone weight



Example of some data that can be acquired using the EchoMRI







Preclinical Imaging

The EchoMRI operates under the same principles that guide Nuclear Magnetic Resonance (NMR) spectroscopy and Magnetic Resonance Imaging (MRI). However it does NOT acquire images.

The EchoMRI acquires four principle measurements from the mouse (rather than images or a spectrum).



The Four Principal Measurements obtained with EchoMRI

FAT: The mass of all the fat molecules in the body expressed as the equivalent weight of canola oil



LEAN: Muscle tissue mass of all the body parts containing water, but excluding fat, bone minerals, and other substances like hair, claws, etc.



FREE WATER: Contributions come mostly from the bladder. In experiments, for a limited time, water can be detected after it is injected into abdominal cavity of an animal, until it gets absorbed.



TOTAL WATER: Includes both the Free Water and the water contained in Lean Fat, which is all the water in the body. For normal animals, the hydration ratio (Total Water - Free Water) / Lean is



typically within a few percent of 80%.

How does the animal's weight compare with the sum of the above measured components?

(Weight) = (Lean) + (Fat) + (Free Water) + (Undetected substances) + (Systemic Errors)

(Total Water) = (Free Water) + (Lean_[water only])

CONTINUE

Lesson 3 of 5



Preclinical Imaging

Set-up for Procedure

(Etho EchoMRI 2020 Body Composition Analyser ٥ × Folder - System Test Repeat Scans Enter Template Review - Help -EchoMRI Body Composition Analyzer E26-379-M R EC Start Scan (F5) **MRI That Counts** Stop (F6) Scan: Primary Stage 1/2 Scan: Primary Stage 2/2 Scan: Recovery 15 sec before Water Stage 1/1 Darken Scan: Water Stage 1/1 Water Stage Completed Primary Accumulation 1 Mice Completed scan 1/1 Scan ALL DONE Water Stage Yes \sim



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

Step 2

Computer configurations



- Wake up computer by pressing computer power button (I/O, top level) once.
- Open the EchoMRI software on the computer desktop.
- Set your data path in the software
 - click Folder>Browse/New
 - Follow the datapath format C:\Echo Scans\PI name\date
 - New folders must be empty otherwise you will receive an error message.
 You may need to open File Explorer and make a new folder for today's date first before doing this.



Canola Oil System Test



- Assemble canola oil system test sample as shown above
 - All materials are housed in the bottom drawer of the EchoMRI stand assembly. Use the canola oil vial labeled "35.1".
- Place in machine with black arrow pointing towards back of machine.
- Click "System Test" in software (or "Alt + Y")
 - After System test completes if the Scan Stages Report window says "System Test Done" proceed with experiment.
 - "System test failed", two possible events have occurred:
 - A small deviation in allowed error was measured, which prompts a new Canola calibration to be started automatically.
 - A large deviation was measured and a warning message will display, prompting you to check that the sample was prepared properly. Verify that the machine has been on for more than >3 hours.



Choosing the appropriate mouse holder



Weigh each mouse. Round the weight of the mouse to the nearest multiple of 10. Use this number to select the appropriate labeled sample holder from the white mesh cart.

For example, a 23 gram mouse should go into the sample holder labeled '20'.



Be careful!



Echo Tubes are fragile. Please be careful when handling.

Summary

The system is now ready to measure the body composition of small animals!

Acquiring Animal Measurements





Unattach Velcro tab & remove clear inner tube

Put the mouse into the red tube and position it at the end by the air holes. It is easiest to do this if the tube is held vertically while scruffing the mouse.



Reposition clear inner tube

Carefully put clear inner tube back, being very careful not to squish the mouse! Leave enough room for the mouse to turn around, but not enough so that it can run back and forth through the tube. Reattach the Velco strap to secure the placement of the clear tube.



Push red tube into chamber

The mouse-end of the tube goes in first.

The clear collar will bump up against the surface of the machine.

Be careful not to bump against the clear inner tube, as it can squish the mouse if pushed upon.



"Start Scan" (or F5) in software

Input label, comments, and other identifiers like weight if necessary

See the next section for further data acquisition options!

Further Interface Options



Output Folder = C:\EchoScans\Animal Imaging\20210928\



Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		

Start Scan

Yields a prompt to input the mouse label, weight (in grams), and any other comments.



Output Folder = C:\EchoScans\Animal Imaging\20210928\

Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		

Primary Accumulation

How many measurements to take.

- "1" takes 1 measurement (default setting)
- "3" takes the same measurement 3 times in a row, then averages the results together

Scan: Primary Stage 1/2 Scan: Primary Stage 2/2 Scan: Recovery 15 sec before Water Stage 1/1 Scan: Water Stage 1/1 Water Stage Completed	
Scan: Primary Stage 1/2 Scan: Primary Stage 2/2 Scan: Recovery 15 sec before Water Stage 1/1 Scan: Water Stage 1/1 Water Stage Completed	Start Scan (F5)
Completed scan 1/1 Scan ALL DONE	Stop (F6) Darken Primary Accumulation 1 Water Stage Yes

Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		
A									

Data Table

Shows results from each scan.

This data can be exported to an excel sheet at end of experiment.



Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.water (g)	Time, Date, Duration (S), User	ALL	weight	Comments
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		

Water Stage

'Yes' is default setting and yields total water and free water measurements.

'No' will only collect Fat and Lean values.

olde	er - Sys	stem Tes	st Repe	at Scans	Enter '	Template Review	Help	•		
(Ecl		RI	•	Ec	choMRI Body Compositi	on An	alyzer	E26-379-M Start Scar	n (F5)
Scar Scar Scar Scar Wate Com Scar	n: Priman n: Priman n: Recov n: Water er Stage npleted s n ALL DC	y Stage 1/ y Stage 2/ ery 15 sec Stage 1/1 Complete can 1/1 DNE	2 2 : before W d	ater Stage	1/1	Mice		Prim	Stop (F Darken nary Accumulatio Water Stage	F6) n 1 → Yes →
Out	out Fold	er = C:\Ec	hoScans	\Animal Im	aging\202	210928\				
Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments	
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1			

Repeat Scans

Automatically repeats any number of scans of the same sample without requiring re-input of sample information each time.

In comparison to "Primary Accumulation = 3", Repeat Scans inserts automatic wait times between scans (at least 20 seconds between each scan) but you get each measurement individually rather than averaged together.



Enter Template

Best for situations where many mice are being imaged on many different occasions. Saves settings so that labels don't need to be re-input every time.



Output Folder = C:\EchoScans\Animal Imaging\20210928\

Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		

Stop

Aborts a scan mid-acquisition



Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		

Status Box

Yields what the status of a scan is as it is being acquired.



Output Folder = C:\EchoScans\Animal Imaging\20210928\

abei Fat(g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Duration (s); User	Acc	Weight	Comments
35.15	0.00	0.02	1.41	12:41:22; 28 Sep 2021; 67; ems	1		
	35.15	35.15 0.00	35.15 0.00 0.02	35.15 0.00 0.02 1.41	35.15 0.00 0.02 1.41 12:41:22; 28 Sep 2021; 67; ems	35.15 0.00 0.02 1.41 12:41:22; 28 Sep 2021; 67; ems 1	35.15 0.00 0.02 1.41 12:41:22; 28 Sep 2021; 67; ems 1

Review

Options to save or export data, generate statistics, or edit data that has already been acquired.

CONTINUE

Lesson 4 of 5

Logging off and Saving Data

PI Preclinical Imaging

Saving Data & logging off

A	А	В	С	D	E	F
1	RecNumb	Label	Fat	Lean	FreeWater	TotalWater
2	1	mouse1	1.93	18.02	0.07	15.57
3	2	mouse2	2.06	17.55	0.03	15.16
4	3	mouse3	1.66	17.84	0.08	15.78
5	4	mouse3	1.52	17.87	0.13	16.63
6	5	mouse3	1.54	18.44	0.16	16.23
7						
8						

This process will describe how to back up your data to the homelink network drive then log off from the computer



Export data table

EchoMRI	2020 Body Com	position Analyser										-	٥
olde	er · Sys	stem Te	st Repe	eat Scans	Enter '	Template	Re	view · I	Help	•			
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	EG		ТМ			1000		Extract	Tabl	e nlata		F8	
		MRI Thai	Counts		1000				iem	plate	-		_
Sca	n [.] Prima	rv Stage 1	12			^		Selecte	d Sta	ITISTIC	S	F3	
Sca	n: Prima	ry Stage 2	/2			Zip for Inspection				ו	Alt+Z Alt+D		
Sca	n: Recov	very 15 sec	c before W	ater Stage	1/1		Auto Diagnostics						
Sca Wat	n: Water er Stage	Stage 1/1 Complete	d				Calibration					Ctrl+B	
Con	pleted s	can 1/1	-					Parame	ters			Alt+R	-
Sca	n ALL DO	ONE						Usage \$	Stats			Alt+U	
Out	put Fold	ler = C:\Ec	choScans	Animal In	aging\202	210928\							
Num	Label	Fat (g)	Lean (g)	F.Water (g)	T.Water (g)	Time, Date; Dur	ration	n (s); User	Acc	Weight	Comments		
1	Tst	35.15	0.00	0.02	1.41	12:41:22; 28 Sep	2021;	; 67; ems	1				
Num 1	Label Tst	Fat (g) 35.15	Lean (g) 0.00	F.Water (g) 0.02	T.Water (g) 1.41	Time, Date; Dur 12:41:22; 28 Sep	2021;	n (s); User ; 67; ems	Acc 1	Weight	Comments		

- 1. Select the data you wish to save from the Scan Results table (click + shift on each entry)
- 2. Click Review>Extract Table OR press F8 and save to the desired file location
- 3. If desired, save statistics (mean and standard deviation values) via Review>Selected Statistics



Transfer data onto homelink



- 1. Open file explorer (the folder icon), go to "This PC", click and select "Map network drive" from the banner on top.
- 2. Select "Drive: Z:" and input "\\file.fhcrc.org\home"
- 3. Check "Connect using different credentials".
- 4. Log in with your Hutch email address and password
 - a. it is very important that for the email address you put yourHutchUsername@fhcrc.org, not @fredhutch.org!
- 5. Transfer data from the folder on computer to the selected folder in your home drive
- 6. When finished, right click on the mapped drive, and click disconnect.

7. When back at your personal work computer, back up the data from the Z: drive to an additional location.



Close software, disconnect from network drive, and log out of iLab



- To close the software simply X out of the software
- Disconnect from your network drive by going to "This PC" and right clicking on the Z: drive. Then select 'Disconnect'.
- Log out of iLab so that you are not charged for more time.

Step 4

Clean up

- Clean the inner clear tube with T-Spray or LABSAN (no ethanol)
- Use long tube with paper towels and T-Spray or LABSAN to clean the inside of the red tube

Thank you for completing this module!



Please continue on to the quiz if you have not completed the in-person training yet.

We recommend revisiting this module whenever a refresher on how to use the Echo is needed!

i Please note you will not be able to use the EchoMRI until you have completed a brief inperson training. You should receive an email to schedule this soon, but feel free to contact <u>preclinicalimaging@fredhutch.org</u> with any questions. Click below if you would like to save a PDF of this training for future reference or to print out for your records





Lesson 5 of 5



PI Preclinical Imaging

Complete the following quiz to complete the online-training portion of the EchoMRI training

01/04

Why is this machine called an EchoMRI even though it does not take images?

- The EchoMRI operates upon the same principles that guide MRI and NMR spectroscopy
- O The EchoMRI uses echolocation just like MRIs do
- O It is called an MRI because it can take pictures of the mice

02/04

Why should you run a system test before taking any other measurements?



03/04

What does the label on the red tubes indicate?



- O The length of the tube
- The circumference of the magnet used to take the measurements
 - The approximate weight (in grams) of the mouse that should be put inside the tube
 - The approximate weight (in pounds) of the mouse that should be put inside the tube

04/04

What do you need to do when you are finished using the EchoMRI? (Select all that apply)

Thoroughly clean mouse housing tubes
Alert Preclinical Imaging that you are done using the machine
Export data table
Alert Comparative Medicine that you are done using the machine
Save data to Homelink then disconnect from network
Log out of FOM