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Scriptwriter Name: Pallavi Sharma

Project Page Link: https://review.jove.com/files_upload.php?src=21743498

Title: Real-Time, Noninvasive Evaluation of Mitochondrial Function Using Resonance Raman Spectroscopy

Authors and Affiliations:

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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**

- 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: 26

Number of Shots: 50

Interviews

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

INTRODUCTION:

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

- 1.1. **Yasemin Altug:** My research focuses on utilizing mitochondrial redox states and mitochondrial health as indicators of organ viability during cold-storage and machine perfusion.
 - 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. **Yasemin Altug:** Current methods inadequately assess organ viability during cold preservation, whereas mitochondrial redox measurements provide a temperature-independent indicator of viability.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B roll: 3.5.1*

CONCLUSION:

~~What does this protocol allow researchers to study or measure?~~

- 1.3. **Yasemin Altug:** This protocol measures mitochondrial redox states, which in turn allows the users to measure organ viability.
 - 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. **Yasemin Altug:** A critical consideration while performing this protocol is the creation and validation of tissue- and wavelength-specific spectral libraries, as new libraries may be required for each application.

1.4.1. 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?~~

- 1.5. **Yasemin Altug:** Additional analyses that can be performed following this procedure include ATP quantification, inflammatory biomarkers, and liver injury markers.

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 Massachusetts General Hospital

Protocol

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

2. Setting up the Resonance Raman Spectroscopy (RRS) System

Demonstrator: Yasemin Altug

- 2.1. To begin, connect the spectrometer unit to the direct current power supply [1] and connect it to the power adapter using the supplied cables [2]. Identify the spectrometer unit with the round plug that contains alignment indentations [3].
 - 2.1.1. WIDE: Talent connecting the spectrometer unit to the direct current power supply. ***Videographer:** Please obtain CU shot as well for this shot.*
 - 2.1.2. Talent connecting the spectrometer unit to the universal serial bus port using the supplied cable.
 - 2.1.3. Close-up of the spectrometer unit showing the round plug with alignment indentations.
- 2.2. Align the indentations of the round plug with those of the cable connected to the power adapter [1]. Connect the ports without cleaning them beforehand [2].
 - 2.2.1. Talent aligning the indentations of the round plug with the cable connector.
 - 2.2.2. Talent connecting the ports together.
- 2.2A. Connect the computer running the analysis to the same power adaptor that powers the spectrometer and power supply, using the supplied cables. **NOTE: Added step**
 - 2.2.3. Talent connecting the computer running the analysis software to the universal bus port.
- 2.3. Place the laser probe in the probe holder [1]. Then adjust the probe's position at the start of the experiment [2].
 - 2.3.1. Talent placing the laser probe in the probe holder.
 - 2.3.2. Talent adjusting the position of the laser probe.
- 2.4. Open the spectrometer control software. This screen displays the percent Resonance Raman reduced mitochondrial ratio or 3RMR (*Three-R-M-R*), tissue oxygen saturation, and myoglobin saturation if selected, in real time throughout the measurements [1]. Click the **Settings** tab at the bottom left of the page [2].
 - 2.4.1. SCREEN: 2.4.1 & 2.4.2 Opening Software- SCREEN.mp4: 00:14-00:25
 - 2.4.2. SCREEN: 2.4.3 Settings Tab- SCREEN.mp4: 00:00-00:04

- 2.5. Under the **Info** tab, enter the session information in **Session Name [1]** and any necessary details in **Session Description [2]**.
 - 2.5.1. SCREEN: 2.5.1 Info Tab- SCREEN.mp4: 00:00-00:06.
 - 2.5.2. SCREEN: 2.5.1 Info Tab- SCREEN.mp4: 00:25-00:35 *Video editor: Please speed it up!*
- 2.6. Under **Settings File**, select the protocol appropriate for the project [1].
 - 2.6.1. SCREEN: 2.6.1 Protocol Selection- SCREEN.mp4
- 2.7. To import a new settings file, click the drop-down menu under **Settings File**. Select **Import New Settings File** and click the new settings file [1].
 - 2.7.1. SCREEN: 2.7.1 Import Settings- SCREEN.mp4: 00:00-00:11
- 2.8. Once all session information is entered, click the **System** tab [1].
 - 2.8.1. SCREEN: 2.8.1 System Tab- SCREEN.mp4: 00:01-00:05
- 2.9. Select **Continuous Mode** as the data collection mode for the experiment. Confirm that spectra will be recorded continuously until the stop button is pressed [2].
 - 2.9.1. SCREEN: 2.9.1 Continuous Mode-SCREEN.mp4
- 2.10. In the **Regression Libraries** section, select and confirm the libraries for data acquisition and analysis. The libraries for reduced and oxidized mitochondria were selected for this experiment [1]. Other users may select to use libraries that also include hemoglobin, myoglobin, or other complexes found under “Advanced Library” [2].
 - 2.10.1. SCREEN: 2.10.1 Regression Libraries- SCREEN.mp4
 - 2.10.2. SCREEN: ADVANCED Regression Libraries- SCREEN”: 00:03-00:18
- 2.11. Select and confirm the preferred **Regression Window Mode (Adaptive or Fixed Mode)** (*Regression-Window-Mode-Adaptive-or-Fixed-Mode*) [1].
 - 2.11.1. SCREEN: 2.11.1 & 2.12.1 Regression Window- SCREEN.mp4: 00:00-00:04
- 2.12. In adaptive mode, individual spectra are recorded and averaged until the maximum sigma target is reached [1]. Allow the recording to stop at the upper limit of the regression window if the sigma target is not reached [2]. Display the measurement results on screen after the minimum sigma target is reached [3].
 - 2.12.1. SCREEN: 2.11.1 & 2.12.1 Regression Window- SCREEN.mp4: 00:06-00:20
 - 2.12.2. SCREEN: 2.12.2 Regression Window-SCREEN.mp4: 00:05-00:23
 - 2.12.3. SCREEN: 2.12.3 Sigma Target- SCREEN.mp4: 00:05-end
- 2.13. During fixed-width mode, individual spectra are recorded and averaged until the target number of spectra set under average length is included. If using fixed-**width** mode, select as such under the “Regression Window Mode” tab [1].

2.13.1. SCREEN: 2.13.1 A Fixed width- SCREEN.mp4: 00:02-00:07, 2.13.1 B Fixed width- SCREEN.mp4: 00:04-end

2.14. Once the system settings are complete, click the **Plot** tab [1]. Under **Main Series**, select the components to be plotted and displayed on screen during the measurements [2]. ~~Confirm that all selected measurement components are consistent with the protocol of interest [1].~~ **NOTE: The VO for the deleted shot is removed**

2.14.1. SCREEN: 2.14.1 Plot Settings- SCREEN.mp4 00:02-00:05

2.14.2. SCREEN: 2.14.1 Plot Settings- SCREEN.mp4: 00:06-00:13

~~2.14.3. SCREEN: Selected measurement components being reviewed and confirmed.~~ **NOTE: Not filmed**

2.15. To create a new settings file, adjust the desired settings parameters. Then, click the **Save** button to save the parameters as a separate settings file [1].

2.15.1. SCREEN: 2.15.1 Save Settings- SCREEN.mp4: 00:00-00:16

3. Data Acquisition

3.1. Select the appropriate pre-saved settings, recording parameters, and library files [1].

3.1.1. SCREEN: 3.1.1 & 3.4.1 Pre-saved settings- SCREEN.mp4: 00:03-00:12

3.2. Place the laser probe directly above the rat liver or any other organ of interest [1]. Position the probe as perpendicular as possible and 1 centimeter away from the tissue [2]. Adjust the probe to a distance less than 1 centimeter if the signal is unclear, while ensuring the probe does not touch the tissue [3]. **NOTE: The VO is edited for the deleted shot**

3.2.1. Talent positioning the laser probe directly above the tissue or organ.

3.2.2. Talent adjusting the laser probe to a perpendicular position 1 centimeter from the tissue. **NOTE: combined and slated as 3.2.2 and includes 3.2.4**

~~3.2.3. Talent measuring 1 centimeter from the organ surface with a ruler.~~ **NOTE: Not filmed**

3.2.4. Talent adjusting the probe position closer to the tissue without making contact.

3.3. Confirm that the laser spot is located on the left lateral lobe as close to the portal vein as possible [1].

3.3.1. Shot of the laser spot positioned on the left lateral lobe near the portal vein.

3.4. Confirm that all selected libraries, regression settings, target sigma, regression window, and acquisition modes are correct and as desired [1].

3.4.1. SCREEN: 3.1.1 & 3.4.1 Pre-saved settings- SCREEN.mp4: 00:12-end

3.9. Make sure the room is as dark as possible [1]. Verify that the probe window is free of

contamination, especially blood [2]. Take a lint-free, non-abrasive wipe, spray it with 70% isopropyl alcohol [3], and clean the quartz window if contamination is present [4]. Make sure to dry it thoroughly with a dry wipe before proceeding [5]. **NOTE: The VO is edited for the additional shot**

3.9.1. Shot of the darkened experimental room.

3.9.2. Talent inspecting the probe window for contamination.

Added Shot: Talent spraying non-abrasive wipe with 70% isopropyl alcohol

3.9.3. Talent cleaning the quartz window with a lint-free, non-abrasive wipe and 70% isopropyl alcohol.

3.9.4. Talent drying the quartz window with a dry wipe.

3.5. On the main page, click the **Start** button to begin data acquisition [1].

3.5.1. SCREEN: 3.5.1-A-Start-button---SCREEN.mp4: 00:00-00:03

3.10. Afterward, subject the acquired rat liver to varying levels of ischemia and oxygen reperfusion by pausing and resuming perfusion flow as a stress test [1]. Take RRS measurements throughout the stress test **and** plot the 3RMR values [2].

3.10.1. Representative shot of the liver undergoing stress test.

3.10.2. SCREEN: 3.10.2 Stress Test.mp4: 02:54-03:15

3.6. Once data acquisition and the experimental protocol are complete, discard the entire liver or tissue into a biohazard bin [1]. Clean any system or surface that came into contact with the organ or tissue using 70% ethanol [2].

3.6.1. Talent discarding the whole liver or tissue into a biohazard bin.

3.6.2. Talent cleaning the contacted system and surfaces with 70% ethanol.

3.7. If working with a perfusion system, run deionized water mixed with an enzymatic cleanser. Then, run clean deionized water through the system [1].

3.7.1. Talent running the enzymatic cleanser solution through the perfusion system.

3.8. Infer the quality of the RRS signal from the plots and values displayed by the software [1]. If the RRS signal does not meet the quality control criteria, adjust the probe to be as perpendicular as possible and approximately 9 millimeters from the surface to the probe window [2].

3.8.1. SCREEN: 3.8.2.-Signal-Quality-Improvement--SCREEN 03:10-03:20

3.8.2. Talent adjusting the probe position relative to the tissue surface.

~~3.9. Make sure the room is as dark as possible [1]. Verify that the probe window is free of contamination, especially blood [2]. Using a lint free, non-abrasive wipe, clean the quartz window with 70% isopropyl alcohol if contamination is present [3], and dry it thoroughly with a dry wipe before proceeding [4]. **NOTE: This step is moved before 3.5**~~

~~3.9.1. Shot of the darkened experimental room.~~

~~3.9.2. Talent inspecting the probe window for contamination.~~

~~3.9.3. (ADDED SHOT) Talent spraying non-abrasive wipe with 70% isopropyl alcohol~~

~~3.9.4. Talent cleaning the quartz window with a lint free, non-abrasive wipe and 70% isopropyl alcohol.~~

~~3.9.5. Talent drying the quartz window with a dry wipe.~~

~~3.10. Afterward, subject the acquired rat liver to varying levels of ischemia and oxygen-reperfusion by pausing and resuming perfusion flow as a stress test [1]. Take RRS measurements throughout the stress test, and plot the 3RMR values [2].~~ NOTE: This step is moved before 3.6

~~3.10.1. Representative shot of the liver undergoing stress test.~~

~~3.10.2. SCREEN: The RRS values are being recorded.~~

Results

4. Results

- 4.6. The 3RMR values at T0 (*T-Zero*) and T5 (*T-Five*) were within the optimal range of 10% to 30% in continuously oxygenated livers [1].
 - 4.6.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the data point between T0 and T5*
- 4.7. The 3RMR value at the end of the ischemic period, T10, was outside the optimal range [1]. At the end of the reperfusion period, T15, the 3RMR value returned to the optimal range [2].
 - 4.7.1. LAB MEDIA: Figure 5A. *Video editor: Highlight the data point at T10 above the shaded optimal range*
 - 4.7.2. LAB MEDIA: Figure 5A. *Video editor: Highlight the data point at T15 within the shaded optimal range*
- 4.8. Raw RRS spectra at T0 [1] and T15 [2] showed spectral characteristics of oxidized mitochondria, including a spectral marker peak at 1371 inverse centimeters [3].
 - 4.8.1. LAB MEDIA: Figure 6A.
 - 4.8.2. LAB MEDIA: Figure 6C.
 - 4.8.3. LAB MEDIA: Figure 6D and F. *Video editor: Highlight the marked peak*
- 4.9. The RRS spectra at T10 showed a reduced marker peak at 1357 inverse centimeters [1].
 - 4.9.1. LAB MEDIA: Figure 6B and E
- 4.10. An increase in 3RMR levels was observed for complex III (*Three*) and complex IV (*Four*) during ischemia, and both returned to optimal levels after oxygen was reintroduced [1].
 - 4.10.1. LAB MEDIA: Figure 5B.
- 4.11. Outflow lactate levels [1] and potassium levels remained relatively consistent throughout the stress test despite changes in oxygenation [2].
 - 4.11.1. LAB MEDIA: Figure 5D.
 - 4.11.2. LAB MEDIA: Figure 5C.
- 4.12. Oxygen consumption rates showed no signs of damage even after a period of no-flow ischemia [1].
 - 4.12.1. LAB MEDIA: Figure 5E.

1. Spectrometer
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrometer>
IPA: /spek'trɑ:.mə.tə/
Phonetic Spelling: spek·trah·muh·ter
2. Resonance Raman
Pronunciation link: <https://www.merriam-webster.com/dictionary/resonance>
IPA: /'rez.ə.nəns rə'mɑ:n/
Phonetic Spelling: rez·uh·nuhns ruh·mahn
3. Mitochondrial
Pronunciation link: <https://www.merriam-webster.com/dictionary/mitochondrial>
IPA: /,maɪ.tə'kɑ:n.dri.əl/
Phonetic Spelling: my·tuh·kon·dree·uhl
4. Myoglobin
Pronunciation link: <https://www.merriam-webster.com/dictionary/myoglobin>
IPA: /,maɪ.oo'gloo.bɪn/
Phonetic Spelling: my·oh·gloh·bin
5. Regression
Pronunciation link: <https://www.merriam-webster.com/dictionary/regression>
IPA: /rɪ'ɡref.ən/
Phonetic Spelling: ree·gresh·uhn
6. Adaptive
Pronunciation link: <https://www.merriam-webster.com/dictionary/adaptive>
IPA: /ə'dæp.tɪv/
Phonetic Spelling: uh·dap·tiv
7. Sigma
Pronunciation link: <https://www.merriam-webster.com/dictionary/sigma>
IPA: /'sig.mə/
Phonetic Spelling: sig·muh
8. Perpendicular
Pronunciation link: <https://www.merriam-webster.com/dictionary/perpendicular>
IPA: /,pə:.pən'dɪk.jə.lə/
Phonetic Spelling: per·pun·dik·yuh·ler
9. Ischemia
Pronunciation link: <https://www.merriam-webster.com/dictionary/ischemia>
IPA: /ɪ'ski:.mi.ə/
Phonetic Spelling: iss·kee·mee·uh
10. Reperfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/reperfusion>
IPA: /,ri:.pə'fju:.ʒən/
Phonetic Spelling: ree·per·fyoo·zhuhn
11. Biohazard
Pronunciation link: <https://www.merriam-webster.com/dictionary/biohazard>
IPA: /'baɪ.oo'hæz.əd/
Phonetic Spelling: bye·oh·haz·erd

12. Enzymatic
Pronunciation link: <https://www.merriam-webster.com/dictionary/enzymatic>
IPA: /ˌen.zəˈmæt.ɪk/
Phonetic Spelling: en·zye·mat·ik
13. Isopropyl
Pronunciation link: <https://www.merriam-webster.com/dictionary/isopropyl>
IPA: /ˌaɪ.souˈproʊ.pəl/
Phonetic Spelling: eye·soh·proh·pul
14. Quartz
Pronunciation link: <https://www.merriam-webster.com/dictionary/quartz>
IPA: /kwɔrts/
Phonetic Spelling: kworts
15. Oxidized
Pronunciation link: <https://www.merriam-webster.com/dictionary/oxidize>
IPA: /ˈɑːk.səˈdaɪzd/
Phonetic Spelling: ok·suh·dyzd
16. Lactate
Pronunciation link: <https://www.merriam-webster.com/dictionary/lactate>
IPA: /ˈlæk.teɪt/
Phonetic Spelling: lak·tayt
17. Oxygenation
Pronunciation link: <https://www.merriam-webster.com/dictionary/oxygenation>
IPA: /ˌɑːk.sɪ.dʒəˈneɪʃən/
Phonetic Spelling: ok·sih·juh·nay·shuhn
18. Mitochondria
Pronunciation link: <https://www.merriam-webster.com/dictionary/mitochondrion>
IPA: /ˌmaɪ.təˈkɑːn.dri.ə/
Phonetic Spelling: my·tuh·kon·dree·uh
19. Perfusion
Pronunciation link: <https://www.merriam-webster.com/dictionary/perfusion>
IPA: /pəˈfjuː.ʒən/
Phonetic Spelling: per·fyoo·zhuhn
20. Oxygenated
Pronunciation link: <https://www.merriam-webster.com/dictionary/oxygenate>
IPA: /ˈɑːk.sɪ.dʒəˈneɪ.tɪd/
Phonetic Spelling: ok·sih·juh·nay·tid