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Project Page Link: <https://review.jove.com/account/file-uploader?src=20481103>

Title: Using 16 α -[18F]-Fluoro-17 β -Estradiol PET to Visualize Estrogen Receptor α Expression in Human Breast Cancer Xenografts in Female Ovariectomized Mice

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, all done**

- 3. Filming location:** Will the filming need to take place in multiple locations? **No**

Current Protocol Length

Number of Steps: 26

Number of Shots: 56

Introduction

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

AUTHORS: Due to a change in template, all statements must now be delivered in 20 words

~~**REQUIRED:** What is the scope of your research? What questions are you trying to answer?~~

- 1.1. **Sadia Quazi**: This study demonstrates PET imaging using fluoroestradiol (FES) to detect estrogen receptor alpha in breast tumor tissue of mice.
 - 1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. **Videographer's NOTE: End of take 2**

~~What are the most recent developments in your field of research?~~

- 1.2. **Sadia Quazi**: FES PET was recently FDA-approved for clinical use to detect estrogen receptor alpha in primary and metastatic breast tumor lesions.
 - 1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. **Videographer's NOTE: Take 2**

CONCLUSION:

~~How will your findings advance research in your field?~~

- 1.3. **Sadia Quazi**: Visualizing this technique enables preclinical monitoring of estrogen receptor alpha during drug treatment, supporting translation of FES PET into clinical practice.
 - 1.3.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. **Videographer's NOTE: End of Take 2**

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

Testimonial Questions (OPTIONAL):

Videographer: Please capture all testimonial shots in a wide-angle format with sufficient headspace, as the final videos will be rendered in a 1:1 aspect ratio. Testimonial statements will be presented live by the authors, sharing their spontaneous perspectives.

- Testimonial statements will **not appear in the video** but may be featured in the journal's promotional materials.
- **Provide the full name and position** (e.g., Director of [Institute Name], Senior Researcher [University Name], etc.) of the author delivering the testimonial.
- Please **answer the testimonial question live during the shoot**, speaking naturally and in your own words in **complete sentences**.

What is the key takeaway you hope viewers remember after watching your video article?

1.4. **Sadia Quazi, Researcher:** (authors will present their testimonial statements live)

1.4.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. **Videographer's NOTE: Use last take**

How does the video format of your article help you to share your work with the broader scientific community?

1.5. **Sadia Quazi, Researcher:** (authors will present their testimonial statements live)

1.5.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. **Videographer's NOTE: Use last take**

AUTHORS: Please note that there is a change in the template. Authors are now required to answer the testimonial questions spontaneously and naturally. Please fill in the author title

Ethics Title Card

This research has been approved by the Institutional Animal Care and Use Committee (IACUC) at the Austin Hospital

Protocol

2. 18F-FES PET/MRI Imaging for Assessing Tumor Uptake in MCF-7 Mouse Models

Demonstrators: Sadia Quazi, Angela Rigopolous and Ingrid Burvenich

- 2.1. To begin, put on double gloves with a ring monitor between the two gloves [1]. Record the weight of a mouse with MCF-7 (*M-C-F-seven*) breast cancer in the experiment log [2].
 - 2.1.1. Talent putting on gloves with a ring monitor in between the two pairs of gloves. Videographer's NOTE: 50 FPS, Take 1
 - 2.1.2. Talent noting the weight in a laboratory notebook. Videographer's NOTE: 50 FPS, Take 1
- 2.2. Dilute synthesized 18F-FES (*F-E-S*) in a 10% ethanol-saline solution [1-TXT].
 - 2.2.1. Talent diluting the 18F-FES in ethanol-saline solution. **TXT: 18F-FES: Fluorine-18 Fluoroestradiol; Decay-corrected injection concentration: 150–300 $\mu\text{Ci}/100 \mu\text{L}$** Videographer's NOTE: Take 1 WIDE, CU, Take 2 CU
- 2.3. Next, use an insulin syringe with a 29-gauge needle to draw 100 microliters of the 18F-FES solution [1]. Measure and record the radioactivity dose and time with a dose calibrator [2]. Then place the syringe behind a lead shield until it is time for injection [3].
 - 2.3.1. Talent drawing 100 microliters of 18F-FES solution into the insulin syringe. Videographer's NOTE: Take 1 is unmarked
 - 2.3.2. Shot of dose calibrator showing the recorded dose and time. Videographer's NOTE: Take 1 12:50, 11.7 MBq
 - 2.3.3. Talent placing the syringe behind a lead shield. Videographer's NOTE: Tail Slate, Take 1
- 2.4. To dilate the tail veins for intravenous injections, place the mouse under a heating element positioned at 30 centimeters for 2 minutes [1]. Wipe the tail with 70% ethanol to disinfect it [2].
 - 2.4.1. Talent positioning the mouse under a heating element. Videographer's NOTE: Take 1 WIDE-CU-WIDE
 - 2.4.2. Talent cleaning the mouse's tail with an ethanol-soaked tissue Videographer's NOTE: Take 2 with mouse
- 2.5. Now, administer 100 microliters of 18F-FES solution with a bolus injection via the lateral tail vein [1-TXT]. Record the time of injection [2] and press the injection site with tissue to stop bleeding [3].

- 2.5.1. Talent injecting the 18F-FES solution into the lateral tail vein of a mouse in a restrainer. **TXT: Inject mice in staggered batches of two to allow sequential imaging** Videographer's NOTE: Take 1 WIDE-CU-WIDE
- 2.5.2. Talent recording the time of injection.
- 2.5.3. Talent applying tissue to the injection site to stop bleeding. Videographer's NOTE: Take 1 -CU
- 2.6. Now, use the dose calibrator to measure and record the remaining dose in the syringe and the tissue after injection [1]. Record the remaining radioactivity dose in the syringe and tissue [2].
 - 2.6.1. Talent measuring the remaining dose in the syringe with a dose calibrator. Videographer's NOTE: 50 FPS, still frame, Take 1: 12:54 0.9MB9
 - 2.6.2. Talent recording the remaining radioactivity dose.
- 2.7. Following injections, transfer the mice to a cage labelled appropriately with a radioactive sticker on the cage card [1] and place the box behind a lead shield [2].
 - 2.7.1. Shot of cage card appropriately labelled with a radioactive sticker. Videographer's NOTE: Clip 30 unlabeled WIDE
 - 2.7.2. Talent placing cage on a cage rack behind a lead shield. Videographer's NOTE: Take 1 Clip 31, Take 2 and 3 good
- 2.8. Activate the respiratory monitoring system in a multi-chamber setup [1].
 - 2.8.1. SCREEN: [2.8.1-2.8.2.mp4](#). 00:03-end
- 2.9. Place two anesthetized mice in a prone position in the imaging chamber [1-TXT].
 - 2.9.1. Shot of 2 mice being placed in a prone position in the imaging chamber. **TXT: Anesthesia: Induction: 5% isoflurane, 5L/min O₂; Maintenance: 2% isoflurane, 2L/min O₂**
- 2.10. To monitor the breathing of the mouse throughout image acquisition, use a respiration chart to ensure a rate of 60–80 breaths per minute [1].
 - 2.10.1. SCREEN: 2.10.1.mp4: 00:05-00:20.
- 2.11. To start acquisition, open **New Protocol** in Nucline (*New-Kline*) Nanoscan [1]. Under subject information, fill out the name and study ID [2].
 - 2.11.1. SCREEN: [2.11.1.mp4](#) 00:00-end
 - 2.11.2. SCREEN: 2.11.2.mp4 00:12-end
- 2.12. For a multichamber setup, input the bodyweight of the mouse in the left position in the multichamber [1]. Then choose a pre-defined PET protocol under **Study Information**, by pressing **New**, followed by **Acquire Study** [2].
 - 2.12.1. SCREEN: [2.12.1.mp4](#) 00:00-end

2.12.2. SCREEN: [2.12.2.mp4](#) 00:00-00:16

2.13. Now set the mouse position to prone and define the radiopharmaceutical details by pressing Modify to open the **Radiopharmaceutical editor [1]**. Fill out the details under **Full Syringe, Application, and Empty Syringe**, and then press **Open [2]**.

2.13.1. SCREEN: [2.13.1.mp4](#) 00:00-end

2.13.2. SCREEN: [2.13.2.mp4](#) 00:02-00:16, 00:40-00:48, 00:50-00:52

2.14. To ensure the mouse is correctly positioned for imaging, capture a 2D Scout sequence at the front of the mouse **[1]**. Activate the sequence by highlighting the acquisition step, pressing **Prepare**, followed by **Start [2]**. Once the bed holder is in position, Press **Go** to begin acquisition **[3]**.

2.14.1. SCREEN: 2.14.1.mp4 00:00-00:21,

2.14.2. Shot of bd holder moving into the camera. Videographer's NOTE: Take 1 clip 39

2.14.3. SCREEN: 2.14.3.mp4 00:00-00:11

2.15. Check if the front of the mouse is captured correctly **[1]**. Then capture a 2D scout sequence at the back of the mouse **[2-TXT]**.

2.15.1. SCREEN: 2.15.1.mp4. 00:00-00:10

2.15.2. SCREEN: 2.15.1.mp4. 00:11-00:23

TXT: Similarly scout the back position

2.16. Before starting PET acquisition, ensure the mice are centered within the field of view **[1]**. Perform a 15-minute static PET acquisition with a coincidence mode of 1 to 5, then press **Prepare** and **Start** to begin acquisition **[2]**. If the acquisition is running properly, peaks will appear between 400 to 600 in the energy spectrum and the total prompt rate and delayed random rate will increase **[3]**.

2.16.1. SCREEN: 2.16.1-2.16.2.mp4. 00:00-00:15

2.16.2. SCREEN: 2.16.1-2.16.2.mp4. 00:49-00:54

2.16.3. SCREEN: [2.16.2b.mp4](#) 00:00-00:04.

2.17. For MRI acquisition, first verify that the field-of-view frame aligns with the position of the mice by ensuring that the mice are centered within the FOV on the view box **[1]**. Choose geometrical parameters by setting the **number of slices to 102, slice thickness to 0.3 millimeters, frequency and phase resolution to 0.25 millimeters, the number of field of views to two, and the field of view overlap to 12% [2]**.

2.17.1. SCREEN: 2.17.1-2.17.2.mp4 00:00-00:20

2.17.2. SCREEN: 2.17.1-2.17.2.mp4 01:27-01:50

2.18. Then press **Prepare, Start, and Go [1]**. At the end of the acquisition, change the intensity to check whether the image has been successfully captured **[2]**.

2.18.1. SCREEN: 2.17.1-2.17.2.mp4 01:51-02:00
2.18.2. SCREEN: 2.17.3.mp4 01:20-01:33

2.19. Next, input the subject ID in the database [1] and select the PET and MRI files for reconstruction [2]. Choose the **PET reconstruction** protocol, followed by **static normal**, and then click **Open** [3]. Adjust the intensities to make the objects visible [4-TXT].

2.19.1. SCREEN: [2.18.1.mp4](#) 00:04-00:10.
2.19.2. SCREEN: [2.18.2.mp4](#) 00:00-00:15
2.19.3. SCREEN: [2.18.3.mp4](#). 00:00-00:15
2.19.4. SCREEN: [2.18.4.mp4](#) 00:00-00:10

TXT: Use geometric planner to modify area of reconstruction

2.20. Now, navigate to the **Results Identification** Window, switch random corrections, **attenuation correction (AC) + scatter** (*attenuation-correction-plus-scatter*) and positron range to **On**, then **Set the decay reference time to ADMIN** (*add-min*) [1]. Press **Prepare** and **Go** to commence reconstruction. The estimated reconstruction time can be seen in the bar below [2]. Following this, change the intensity to make the reconstructed PET visible, and then **Close protocol** [3].

2.20.1. SCREEN: [2.19.1.mp4](#). 00:00-00:21
2.20.2. SCREEN: 2.19.2.mp4 00:00-00:10, 00:14-00:22, 00:38-00:41
2.20.3. SCREEN: [2.19.3.mp4](#) 00:00-00:15

2.21. For co-registration, open interview fusion, select the PET/MRI (*Pet- -M-R-I*) images and press **Load** [1]. Drag the images to the window and adjust intensity [2].

2.21.1. SCREEN: [2.20.1.mp4](#) 00:15-00:24
2.21.2. SCREEN: [2.20.2.mp4](#) 00:00-00:21

2.22. To realign the MRI to fit the PET, press **Manual Registration** and drag the MRI to the correct position, then unclick **Manual Registration** [1]. Save the aligned MRI image as a new DICOM (*die-com*) file [2]. Navigate back to Nucline NanoScan, then select the aligned MRI file and repeat PET reconstruction [3].

2.22.1. SCREEN: 2.21.1a.mp4 00:04-00:16
2.22.2. SCREEN: [2.21.1b.mp4](#) 00:03-00:12
2.22.3. SCREEN: [2.21.2.mp4](#) 00:00-00:20

2.23. For hotel separation, open **InterView Fusion** and open the realigned MRI and reconstructed PET image. Load, drag images to the window, and adjust intensity to make images visible [1]. To separate the multi-chamber images, click on **Image Processing** followed by **Segmentation** and **Hotel Separator** [2].

2.23.1. SCREEN: [2.22.1.mp4](#) 00:00-00:30
2.23.2. SCREEN: [2.22.2.mp4](#) 00:00-00:11

2.24. Now activate **Scientific Mode** and adjust the threshold and volume parameters to identify two objects, then press **OK [1]**.

2.24.1. SCREEN: [2.23.1.mp4](#) 00:00-00:20

2.25. To adjust the weight and injected dose for the second mouse, right-click the PET image of mouse two, then press **Modify tags [1]**. Change the bodyweight and injected dose, then press **OK [2]**. Save each mouse as a separate DICOM file by saving the PET and MRI images separately **[3]**.

2.25.1. SCREEN: [2.24.1.mp4](#) 00:00-end

2.25.2. SCREEN: [2.24.2.mp4](#) 00:00-end

2.25.3. SCREEN: [2.24.3.mp4](#) 00:10-00:34

2.26. Next, change the display unit from **SUV (S-U-V)** to **%ID/g (Percent-Injection-dose-Per-Gram)** by clicking the **SUV** button and changing to percentage of injected dose **[1]**.

2.26.1. SCREEN: [2.25.1.mp4](#) 00:00-end

2.27. Lastly, draw regions of interest on the tumor sections by selecting the **Measurements** tab **[1]**. Use the **polygon** or **freehand** tool to carefully outline the tumor while the PET image is active **[2-TXT]**.

2.27.1. SCREEN: [2.26.1.mp4](#) 00:00-end

2.27.2. SCREEN: [2.26.2.mp4](#) 00:05-00:28

TXT: The result is a value of ROI as % injected dose/g

Results

3. Results

- 3.1. Uptake of 18F-FES was highest in MCF-7 tumors implanted in the shoulder [1]. The shoulder tumors were clearly visible due to their distance from organs showing significant uptake [2].

3.1.1. LAB MEDIA: Figure 4A *Video Editor: Please emphasize the area pointed at by the red arrows*

3.1.2. LAB MEDIA: Figure 4B *Video Editor: Please emphasize the area pointed at by the red arrows*

- 3.2. Uptake of 18F-FES in MCF-7 tumors grown in the mammary fat pad was partially obscured by intestinal excretion [1].

3.2.1. LAB MEDIA: Figure 4C and D *Video Editor: Please emphasize the area pointed at by the red arrows*

1. Synthesized
Pronunciation link: <https://www.merriam-webster.com/dictionary/synthesize>
IPA: /'sɪnθəˌsaɪzd/
Phonetic Spelling: sin·thuh·syzed
2. Ethanol
Pronunciation link: <https://www.merriam-webster.com/dictionary/ethanol>
IPA: /'e-thəˌnɒl/
Phonetic Spelling: eh·thuh·nawl
3. Saline
Pronunciation link: <https://www.merriam-webster.com/dictionary/saline>
IPA: /'seɪˌliːn/
Phonetic Spelling: say·leen
4. Fluoroestradiol
Pronunciation link: <https://www.howtopronounce.com/fluoroestradiol>

- IPA: /ˌflɒr.oʊ.ˌɛs.trəˈdaɪ.əl/
Phonetic Spelling: floor·oh·es·truh·dye·awl
5. Microliters
Pronunciation link: <https://www.merriam-webster.com/dictionary/microliter>
IPA: /ˈmaɪ.kroʊ.liː.tər/
Phonetic Spelling: my·kroh·lee·ter
6. Radioactivity
Pronunciation link: <https://www.merriam-webster.com/dictionary/radioactivity>
IPA: /ˌreɪ.di.əʊ.ækˈtɪv.ə.t̬i/
Phonetic Spelling: ray·dee·oh·ak·tiv·uh·tee
7. Calibrator
Pronunciation link: <https://www.merriam-webster.com/dictionary/calibrator>
IPA: /ˈkæl.əˌbreɪ.t̬ər/
Phonetic Spelling: kal·uh·bray·ter
8. Intravenous
Pronunciation link: <https://www.merriam-webster.com/dictionary/intravenous>
IPA: /ˌɪn.trəˈviː.nəs/
Phonetic Spelling: in·truh·vee·nuhs
9. Dilate
Pronunciation link: <https://www.merriam-webster.com/dictionary/dilate>
IPA: /ˈdaɪ.lert/
Phonetic Spelling: dye·layt
10. Disinfect
Pronunciation link: <https://www.merriam-webster.com/dictionary/disinfect>
IPA: /ˌdɪs.ɪnˈfekt/
Phonetic Spelling: dis·in·fekt
11. Administer
Pronunciation link: <https://www.merriam-webster.com/dictionary/administer>
IPA: /ədˈmɪn.ə.stər/
Phonetic Spelling: ad·min·uh·ster
12. Bolus
Pronunciation link: <https://www.merriam-webster.com/dictionary/bolus>
IPA: /ˈboʊ.ləs/
Phonetic Spelling: boh·luhs
13. Lateral
Pronunciation link: <https://www.merriam-webster.com/dictionary/lateral>
IPA: /ˈlæt̬.əl/
Phonetic Spelling: lat·er·uhl
14. Respiratory
Pronunciation link: <https://www.merriam-webster.com/dictionary/respiratory>
IPA: /ˈres.pə.rəˌtɔːr.i/, /rɪˈspɪr.əˌtɔːr.i/
Phonetic Spelling: res·puh·ruh·tor·ee; ri·speer·uh·tor·ee
15. Anesthetized
Pronunciation link: <https://www.merriam-webster.com/dictionary/anesthetize>

- IPA: /əˈnes.θəˌtaɪzd/
Phonetic Spelling: uh·nes·thuh·tyzed
16. Isoflurane
Pronunciation link: <https://www.howtopronounce.com/isoflurane>
IPA: /ˌaɪ.souˈflʊr.eɪn/
Phonetic Spelling: eye·soh·floor·ayn
17. Acquisition
Pronunciation link: <https://www.merriam-webster.com/dictionary/acquisition>
IPA: /ˌæk.wəˈzɪʃ.ən/
Phonetic Spelling: ak·wuh·zish·uhn
18. Protocol
Pronunciation link: <https://www.merriam-webster.com/dictionary/protocol>
IPA: /ˈproʊ.təˌkɔːl/
Phonetic Spelling: proh·tuh·kawl
19. Radiopharmaceutical
Pronunciation link: <https://www.merriam-webster.com/dictionary/radiopharmaceutical>
IPA: /ˌreɪ.di.ouˈfɑːr.məˈsuː.tɪ.kəl/
Phonetic Spelling: ray·dee·oh·far·muh·soo·tuh·kuhl
20. Coincidence
Pronunciation link: <https://www.merriam-webster.com/dictionary/coincidence>
IPA: /koʊˈm.sə.dəns/
Phonetic Spelling: koh·in·suh·duhns
21. Spectrum
Pronunciation link: <https://www.merriam-webster.com/dictionary/spectrum>
IPA: /ˈspek.trəm/
Phonetic Spelling: spek·truhm
22. Attenuation
Pronunciation link: <https://www.merriam-webster.com/dictionary/attenuation>
IPA: /əˌten.juˈeɪ.ʃən/
Phonetic Spelling: uh·ten·yoo·ay·shuhn
23. Positron
Pronunciation link: <https://www.merriam-webster.com/dictionary/positron>
IPA: /ˈpɑː.zəˌtraːn/
Phonetic Spelling: pah·zuh·tron
24. Geometrical
Pronunciation link: <https://www.merriam-webster.com/dictionary/geometrical>
IPA: /ˌdʒiː.əˈme.trɪ.kəl/
Phonetic Spelling: jee·uh·met·ri·kuhl
25. Reconstruction
Pronunciation link: <https://www.merriam-webster.com/dictionary/reconstruction>
IPA: /ˌriː.kənˈstrʌk.ʃən/
Phonetic Spelling: ree·kuhn·struk·shuhn
26. DICOM
Pronunciation link: <https://www.howtopronounce.com/dicom>

IPA: /'daɪ,kɑ:m/

Phonetic Spelling: dye·kom

27. Segmentation

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

IPA: /,seg.mən'teɪ.ʃən/

Phonetic Spelling: seg·muhn·tay·shuhn

28. Threshold

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

IPA: /'θref,hoʊld/

Phonetic Spelling: thresh·hohld

29. Mammary

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

IPA: /'mæm.ə.ri/

Phonetic Spelling: mam·uh·ree

30. Intestinal

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

IPA: /ɪn'tes.tə.nəl/

Phonetic Spelling: in·tes·tuh·nuhl

31. Excretion

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

IPA: /ɪk'skri:.ʃən/

Phonetic Spelling: ik·skree·shuhn

32. Tumor

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

IPA: /'tu:.mə/

Phonetic Spelling: too·mer

33. Intravenous

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

IPA: /,ɪn.trə'vi:.nəs/

Phonetic Spelling: in·truh·vee·nuhs