Submission ID #: 62538
Scriptwriter Name: Gaurav Vaidya
Supervisor Name: Anastasia Gomez
Project Page Link: https://www.jove.com/account/file-uploader?src=19074138

Title: Stabilized Longitudinal In Vivo Cellular-Level Visualization of the Pancreas in a Murine Model with a Pancreatic Intravital Imaging Window

Authors and Affiliations:
Inwon Park[1], Pilhan Kim[2,3]

[1]Department of Emergency Medicine, Seoul National University Bundang Hospital, 82, Gumi-ro 173 Beon-gil, Bundang-gu, Seongnam-si, Gyeonggi-do, Republic of Korea.
[2]Graduate School of Medical Science and Engineering, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Yuseong-gu, Daejeon, Republic of Korea.
[3]KI for Health Science and Technology, Korea Advanced Institute of Science and Technology, 291 Daehak-ro, Yuseong-gu, Daejeon, Republic of Korea.

Corresponding Authors:
Inwon Park (emresuscitation@gmail.com)

Email Addresses for All Authors:
pilhan.kim@kaist.ac.kr
emresuscitation@gmail.com

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? **No**

3. **Interview statements:** Considering the COVID-19-imposed mask-wearing and social distancing recommendations, which interview statement filming option is the most appropriate for your group? **Please select one**.

   ☒ Interviewees wear masks until videographer steps away (≥6 ft/2 m) and begins filming, then the interviewee removes the mask for line delivery only. When take is captured, the interviewee puts the mask back on. Statements can be filmed outside if weather permits.

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

Current Protocol Length

Number of Steps: 18
Number of Shots: 32

Introduction

1. Introductory Interview Statements

REQUIRED:

1.1. **Inwon Park:** This protocol describes stabilized and repetitive cellular-level in vivo imaging of the pancreas with the novel pancreatic intravital imaging window.

1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 3.6.1.*

1.2. **Inwon Park:** The main advantage of this technique is that it is possible to perform motionless 3-dimensional imaging with resolution up to the cellular level over three weeks in the pancreas of a live mouse.

1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *B-roll: 3.9.1.*

Ethics Title Card

1.3. Procedures involving animal subjects have been approved by the Institutional Animal Care and Use Committee at the Korea Advanced Institute of Science and Technology (KAIST) and Seoul National University Bundang Hospital (SNUBH).

Protocol

2. Surgery

2.1. Begin by preparing the surgical platform **[1]** and sterilizing the surfaces with 70 percent ethanol **[2-TXT].** After anesthetization, use a rectal probe with a homeothermic controlled heating pad to monitor the body temperature **[3]**.

2.1.1. WIDE: Establishing shot of the talent preparing the surgical platform.

2.1.2. Talent sterilizing the surfaces. **TEXT: Use aseptic techniques for longitudinal imaging sessions**

2.1.3. Talent monitoring the body temperature.

2.2. Shave the left flank of the mouse **[1]** and apply an alcohol and iodine-based scrub in three alternating rounds **[2]**.

2.2.1. Talent shaving the left flank of the mouse.

2.2.2. Talent applying alcohol and iodine-based scrub.

2.3. Then, make a 1.5-centimeter incision on the left flank of the mouse, dissecting the skin and the muscle **[1]**. Use a black or nylon 4-0 suture to perform a purse-string suture in the incision margin **[2]**.

2.3.1. Talent making an incision and dissecting the skin and muscle.

2.3.2. Talent performing the purse-string suture.

2.3.3. Talent using a micro retractor to expose the spleen.
NOTE: 2.3.3. has been dropped on author's instructions.

2.4. Pool the spleen carefully with ring forceps and identify the pancreas **[1]**. Placing the window at the flank of the mouse **[2]**, pass the spleen and the pancreas through the open space of the window **[3]**. *Videographer: This step is important!*

2.4.1. Talent pooling the spleen and identifying the pancreas.

2.4.2. Talent placing the window at the flank of the mouse.

2.4.3. Talent passing the pancreas and spleen through the window.

2.5. Then, gently place the pancreas on the plate of the imaging window **[1]** while placing the spleen on the open space of the window **[2]**. *Videographer: This step is important!*

2.5.1. Talent placing the pancreas on the plate of the imaging window.

2.5.2. Talent placing the spleen on the open space of the window.

2.6. Inject PANC-1 NucLight *(pronounce 'Nuke-light')* Red directly into the pancreas **[1-TXT]**.

2.6.1. Talent injecting the cells into the pancreas. **TEXT: 1.0 x 10^6 cells**

2.7. Use a 31-gauge catheter needle to apply drops of N-butyl cyanoacrylate glue to the margin of the imaging window, ensuring a minimal amount of glue is applied **[1].** Gently apply a 12-millimeter round cover glass to the margin of the imaging window **[2]**. *Videographer: This step is important!*

2.7.1. Talent applying glue to the margin of the window.
2.7.2. Talent applying the cover glass to the imaging window.

2.8. Then, pull the suture loop to fit into the lateral groove of the window **[1]** and tie it three times **[2]**. *Videographer: This step is important!*

2.8.1. Talent pulling the suture loop into the lateral groove of the window.
2.8.2. Talent tying the suture thrice.

2.9. Finally, to prevent the interruption of these tight stitches when the mice are awake, cut the maximal proximal site of the tie **[1]**.

2.9.1. Talent cutting the maximal proximal site of the tie.

3. Intravital Imaging

3.1. Begin by switching on the intravital microscope, including the laser power **[1]**.

3.1.1. Talent switching on the intravital microscope.

3.2. To insert a vascular catheter, apply pressure on the proximal side of the tail with the index and third finger. If necessary, heat the tail with a lamp **[1]**. Sterilize the tail vein with a 70 percent ethanol spray **[2]**.

3.2.1. Talent by applying pressure on the proximal side of the tail.

3.2.2. Talent sterilizing the vein.

3.3. Then, insert a 30-gauge catheter into the lateral tail vein **[1]** and visualize the regurgitation of blood in the PE10 tube **[2]**. Apply silk tape on the catheter to stabilize it **[3]**.

3.3.1. Talent inserting the catheter into the lateral tail vein.

3.3.2. A shot of blood regurgitating in the PE10 tube.

3.3.3. Talent applying silk tape to the catheter.

3.4. Inject FITC-dextran and TMR-dextran or other fluorescent probes, as appropriate, according to the combination of fluorescent probes **[1-TXT]**.

3.4.1. Talent injecting the fluorescent probes. **TEXT: 25 µg of anti-CD31 conjugated with Alexa 647**

NOTE: Shot number 3.4.2. and corresponding VO narration moved to after 3.6.1.

3.5. Insert a rectal probe to automatically control the body temperature with the homeothermic heating pad system **[1]**.

3.5.1. Talent inserting the rectal probe.

3.6. Then, insert the pancreatic imaging window prepared during the intravital microscopy setup into the window holder **[1]**. Transfer the mouse from the surgical platform to the imaging stage **[2]**.

3.6.1. Talent inserting insert the pancreatic imaging window into the window holder.

3.6.2. Talent placing the mouse on the imaging stage. NOTE: 3.4.2. and its corresponding VO narration was moved, and it is shot number 3.6.2. now

3.7. To perform intravital imaging, start with imaging the pancreas at a low magnification such as 4x to scan the whole view of the pancreas in the pancreatic imaging window **[1-TXT]**.

3.7.1. SCOPE: Talent imaging the pancreas at a low magnification **TEXT: Recommended field of view: 2500 x 2500 µm**

3.8. Once the region of interest has been determined, switch to a higher magnification objective lens like 20x or 40x to perform cellular level imaging with lateral and axial resolution approximately 0.5 micrometers and 3 micrometers, respectively **[1-TXT]**.

3.8.1. Talent imaging the pancreas at a high magnification. **TEXT: Recommended field of view: 500 x 500 µm or 250 x 250 µm**

3.9. Perform z-stack or time-lapse imaging to observe the 3D structure or cellular-level dynamics, such as cell migration **[1]**.

3.9.1. Talent acquiring the images.

Results

4. Results: In Vivo Cellular-Level Visualization of the Pancreas using a Pancreatic Intravital Imaging Window

4.1. Intravital microscopy combined with the pancreatic intravital imaging window **[1]** provides long-term tissue stability that enables the acquisition of high-resolution imaging to track individual islets for up to 3 weeks **[2]**.

4.1.1. LAB MEDIA: Figure 1A

4.1.2. LAB MEDIA: Figure 6

4.2. The window implanted in a C57BL/6N mouse **[1]** with intravenously injected anti-CD31 antibody conjugated with an Alexa 647 fluorophore facilitated wide-area imaging **[2]** and magnified 3D imaging of the pancreas **[3]**.

4.2.1. LAB MEDIA: Figure 2.

4.2.2. LAB MEDIA: Figure 3A

4.2.3. LAB MEDIA: Figure 3B, 3C, and 3D.

4.3. Acinar cells were identified in the averaged images of pancreatic tissue and the adjacent vasculature, visualized using autofluorescence and anti-CD31 antibody, respectively **[1].**

4.3.1. Figure 4.

4.4. Using the mosaic imaging method, which combines a wide-field view with high-resolution imaging **[1]**, approximately 40 to 50 islets with the adjacent vasculature were visualized in a MIP-GFP mouse **[2]**. A previous study showed that islets can be tracked for up to 3 weeks using this stable imaging method **[3]**.

4.4.1. LAB MEDIA: Figure 5.
4.4.2. LAB MEDIA: Figure 5. *Video Editor: Emphasize the image inset.*
4.4.3. LAB MEDIA: Figure 6.

4.5. To visualize cancer cells, PANC-1 NucLight Red cells were directly implanted into the mouse pancreas during surgery and nearby vessels were stained with anti-CD31 conjugated with Alexa 647 **[1]**.

4.5.1. LAB MEDIA: Figure 7A

4.6. Using this protocol wide-field images of pancreatic cancer were generated **[1]**. This helped delineate the margin of the tumor **[2]** as well as achieve high-resolution 3D images at the single-cell level **[3]**.

4.6.1. LAB MEDIA: Figure 7A and 7B.
4.6.2. LAB MEDIA: Figure 7C and 7D.

Conclusion

5. Conclusion Interview Statements

5.1. **Inwon Park:** The most critical step in this method is the skillful implantation of the pancreatic imaging window in the mouse.

5.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested b-roll: 2.4.2 , 2.5.1., 2.7.2*

5.2. **Inwon Park:** Using other combinations of the fluorescent mouse, cells, and antibody probes, dynamic interaction of nearby cells with either islets or cancer cells could be identified.

5.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.

5.3. **Inwon Park:** This method can be widely applied by those exploring the change of the islets in various pathophysiological conditions and microenvironments of the pancreatic cancer in situ.

5.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera.