

Submission ID #: 71124

Scriptwriter Name: Poornima G

Project Page Link: <https://review.jove.com/account/file-uploader?src=21692868>

Title: A Web Platform for Automated Image-Based Quantification of Human and Rodent Isolated Pancreatic Islets

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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? **Yes**

2. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **Yes, section 2, 5, 6, 7 done**

Videographer: Please record the computer screen for the shots labeled as SCREEN. The screen should be in focus and the fonts should be clearly visible.

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. **Yes**

Current Protocol Length

Number of Steps: 33

Number of Shots: 76 (55 SC)

Interviews

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

Authors: Please note that **the questions will not appear on screen**. Please answer in **stand-alone full sentence with sufficient context**

We have chosen the 5 best answers

INTRODUCTION:

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

1. **David Habart:** We are developing accessible and accurate image analysis methods for islet quantification as an indirect measure of β -cell mass in transplantation.
 - 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.~~

2. **David Habart:** Existing methods rely on subjective thresholding that struggle with the inherent sample variability. We provide efficient deep learning-based image analysis with improved accessibility through an online service.
 - 2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera

CONCLUSION:

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

3. **David Habart:** IsletNet analyses single 2D microscopic projections and returns islet count, size histogram, and volume estimates using several existing methods with side-by-side comparison.
 - 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.~~

4. **David Habart:** Reliable performance requires microscopy images consistent with those used during IsletNet training. If imaging conditions change, retraining with additional images is recommended.

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

~~How can future studies build on this work? Describe the questions or research directions that can be investigated next.~~

5. **David Habart:** IsletNet can support future standardized multicenter studies using isolated islets and unbiased comparisons with re-aggregated pseudo-islets and stem cell-derived islets.
 - 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 Ministry of Health of the Czech Republic

Protocol

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

NOTE to RE: Please contact me before starting this project. This is a special case.

NOTE: I had to re-write/revise the script 8 to 9 times as the authors were not ready to skip any step and added new content with every version. An approval was taken from Swati, and Anastasia was informed that we are accommodating more shots in the script than we usually do.

Authors: Our shot limit per video is 55. We are considering your case an exception after a discussion and allowing 20 extra shots. Please note that it will be difficult for us to accommodate more.

2. Software Setup

2.1. To begin, prepare the software environment for image acquisition, storage, and analysis [1]. After launching the software, create folders for the expected image files [2]. Open the IsletNet (*islet-net*) website and confirm the displayed training date [3].

2.1.1. WIDE: Talent opening the live-view window of the Quick Photo software on the computer.

2.1.2. **SCREEN:** 2.1.2-2.1.3 00:00-00:08.

2.1.3. **SCREEN:** 2.1.2-2.1.3 00:10-00:18

***Videographer:** Please record the computer screen for the shots labeled as **SCREEN**. The screen should be in focus and the fonts should be clearly visible.*

2.2. Access the image submission page through the Clinical Isolation link or the Upload button [1]. Start the session by entering the operator initials and the current session title [2].

2.2.1. **SCREEN:** 2.2.1-2.2.2 00:00-00:08.

2.2.2. **SCREEN:** 2.2.1-2.2.2 00:09-00:14 and 00:19-00:21.

3. Staining Islet Samples with Dithizone

3.1. After islet purification, place the 250-milliliter conical tubes containing the high-,

medium-, and low-purity fractions into a cooling box inside the laminar flow hood [1].

3.1.1. Talent placing the cooling box, labelled tubes, pipettes, dithizone working solution, and four marked Petri dishes inside the hood.

3.2. Label four Petri dishes for the high-purity replicates. Dispense 25 microliters of 2.5 milligrams per milliliter dithizone working solution into the center of each dish, avoiding bubble formation [1-TXT]. Replace the tip after each dish [2].

3.2.1. Talent dispensing dithizone into the center of the dishes. **TXT: Label the dishes as H1, H2, H3 and H4; Prepare the dithizone in an alkaline alcoholic solution**

3.2.2. Talent replacing the pipette tip.

3.3. Immediately before sampling, gently invert the high-purity tube five times [1] and open the lid [2].

3.3.1. Talent inverting the high-purity tube.

3.3.2. Talent opening the lid.

3.4. Using a large-bore pipette tip, collect one replicate sample from the midpoint of the suspension [1]. Dispense the islet suspension into the dithizone drop. Mix by pipetting up and down three times, using only the first plunger stop to avoid bubbles [2]. Replace the tip after dispensing [3].

3.4.1. Talent collecting the islet suspension using a large-bore pipette tip.

3.4.2. Shot of dispensing the suspension into the dithizone drop and mixing slowly three times.

3.4.3. Talent discarding the pipette tip and attaching another tip.

3.5. Before collecting each additional replicate, gently invert the original tube five times to maintain representative sampling [1]. Repeat the sampling and dispensing procedure for the next dish [2].

3.5.1. Talent inverting the tube before the next replicate.

3.5.2. Talent dispensing another sample into the dithizone drop in the next dish.

3.6. Transfer the tray containing the four high-purity replicate samples to the microscope bench [1].

3.6.1. Talent transferring the sample tray to the microscope bench with the computer,

camera software, and IsletNet visible.

3.7. After 1- to 2-minutes of incubation, place dish H1 under the microscope [1] and visually monitor the staining until the islets appear bright red [2].

3.7.1. Talent placing dish H1 on the microscope stage.

3.7.2. Close-up or SCREEN shot of well-stained islets.

3.8. Then, add 4 milliliters of albumin-supplemented PBS directly to the drop in the dish H1, avoiding bubble formation [1]. Swirl each dish vigorously to disperse the dithizone and keep the tissue near the center [2].

3.8.1. Talent adding albumin-supplemented PBS to the dish.

3.8.2. Talent vigorously swirling the dish.

3.9. Immediately, inspect dish H1 under the microscope and rotate the dish to locate any islets outside the camera field [1]. Use fluid movement from a 200-microliter pipette tip to guide displaced islets toward the center without touching them directly [2]. Place the dish on the bench without disturbing the islets [3]. Repeat the procedure for dishes H2-H4 without any delay [4].

3.9.1. Talent inspecting and rotating the dish under the microscope.

3.9.2. SCREEN: moving displaced islets toward the center using the pipette tip.
Author's NOTE: Use videographer's footage for this

3.9.3. Talent placing the dish back on the bench.

3.9.4. *Reuse 3.8.1*

Authors: We will film and show only 1 replicate as per our journal guidelines. I have added the above text in bold. This text will be displayed on screen so that the viewers know that they have to repeat the procedure for other replicates

Author's NOTE: 3.9, 4.1 might sound repetitive, but it is essential and part of our workflow. Hence do **NOT** delete these steps. While we review other dishes, the incubation of H1 dish will be over. So we place the dish on stage, check, and remove. And examine other dishes. Then put H1 dish on the stage again in 4.1

4. Imaging the Stained Islets

4.1. Immediately after staining, place dish H1 under the microscope [1]. Turn off the room lights [2] and adjust the halogen light to the standardized intensity [3].

- 4.1.1. Talent placing the H1 dish under microscope.
- 4.1.2. Talent turning off the room lights.
- 4.1.3. Talent adjusting the halogen light source.

4.2. ~~After confirming that all the islets are within the camera field of view, gently shake the dish to separate adjacent islets and exocrine tissue particles [1]. Confirm redistribution of islets and exocrine tissue within the camera field of view [2].~~

- ~~4.2.1. Talent viewing the islets on screen and gently shaking the dish.~~
- ~~4.2.2. SCREEN: Showing redistribution of islets and exocrine tissue.~~

NOTE: Please contact Poornima before starting section 4

4.3. Select the pre-saved automatic camera settings and acquire the image [1]. Save it in the high-purity folder. Use the JPG format to reduce analysis time when appropriate. Include magnification, dilution, and pellet information in the file name [2].

- 4.3.1. SCREEN: 4.3.1-4.3.2 00:03-00:10.
- 4.3.2. SCREEN: 4.3.1-4.3.2 00:23-00:32.

4.4. Repeat the sampling, staining, and imaging procedure for the medium- and low-purity fractions [1]. Save each image in the corresponding folder [2] and verify the expected increase in exocrine tissue content as purity decreases [3].

- 4.4.1. Talent placing the medium-purity sample dish on the microscope stage.
- 4.4.2. SCREEN: 4.4.2-4.4.3 00:20-00:30.
- 4.4.3. SCREEN: 4.4.2-4.4.3 00:31-00:38.

4.5. Next, turn on the transmitted light. Align the stage micrometer scale with the long edge of the camera frame and bring it into focus. Acquire the image using the same magnification as for the islet samples [1]. Save it in the calibrator folder, including the magnification information in the file name [2].

- 4.5.1. SCREEN: 4.5.1-4.5.2 00:04-00:12.
- 4.5.2. SCREEN: 4.5.1-4.5.2 00:26-00:32.

5. Clinical Isolation Module: On-Site Use

- 5.1. Upload the high-purity sample images using the **Add Images** button or by dragging the files from the folder **[1]**.

5.1.1. SCREEN: 5.1.1-5.6.2 00:08-00:14.

- 5.2. Note the magnification specified in the uploaded image file names **[1]**. Open the pixel size calibration link and upload the corresponding stage micrometer image **[2]**.

5.2.1. SCREEN: 5.1.1-5.6.2 00:15-00:18

5.2.2. SCREEN: 5.1.1-5.6.2 00:21-00:23 and 00:26-00:30

- 5.3. Position the endpoints of the red measurement line across a substantial distance on the digital ruler **[1]**. Enter the selected distance in micrometers **[2]**, then click Submit to accept the calculated pixel size **[3]**.

5.3.1. SCREEN: 5.1.1-5.6.2 00:32-00:00:35 and 00:41-00:44.

5.3.2. SCREEN: 5.1.1-5.6.2 00:47-00:50.

5.3.3. SCREEN: 5.1.1-5.6.2 00:51-00:53.

- 5.4. Back in the batch submission page, enter the minimum islet size threshold, conventionally 50 micrometers **[1]**.

5.4.1. SCREEN: 5.1.1-5.6.2 00:54-00:55. *Video editor: Highlight the minimum islet size = 50 box*

- 5.5. Enter the dilution listed in the image file name **[1]**, the estimated pellet volume from the corresponding conical tube, and the batch ID **[2]**.

5.5.1. SCREEN: 5.1.1-5.6.2 00:56-00:59.

5.5.2. SCREEN: 5.1.1-5.6.2 01:00-01:07.

- 5.6. Click **Submit Batch [1]** and wait until the numerical and graphical results appear in the Inspection window **[2]**.

5.6.1. SCREEN: 5.1.1-5.6.2 01:08-01:10.

5.6.2. SCREEN: 5.1.1-5.6.2 01:25-01:28 and 01:57-01:59..

- 5.7. Review the preliminary individual sample values and batch averages for islet count,

volume, and purity [1]. To view additional details for the current image, examine the information panel outlined in red on the left [2].

5.7.1. SCREEN: 5.7.1. 00:06-00:10.

5.7.2. SCREEN: 5.7.1. 00:11-00:17.

6. Visual Quality Control

6.1. Open the Visual Quality Control window by clicking the image [1]. Enable the legend to view keyboard shortcuts, including zoom and view-toggle controls [2]. Zoom in and out using the “i” and “o” (*eye and oh*) keys [3].

6.1.1. SCREEN: 6.1.1-6.2.3. 00:00-00:06

6.1.2. SCREEN: 6.1.1-6.2.3. 00:07-00:11.

6.1.3. SCREEN: 6.1.1-6.2.3. 00:18-00:25.

6.2. Evaluate the automated contours by toggling between the original image and contour views. Use the number keys to switch views while keeping the islet border in focus [1]. Toggle to the islet segmentation view to confirm that the islets below the counting threshold are visible but do not have corresponding contours. Use the down-arrow key to proceed to the next sample image [2].

6.2.1. SCREEN: 6.1.1-6.2.3. 00:36-00:42.

6.2.2. SCREEN: 6.1.1-6.2.3. 00:43-00:49.

6.3. After validating the high-purity batch, click on **Add Another** [1] and upload the medium-purity fraction images [2]. Enter the dilution and pellet volume. Adjust calibration if necessary [3]. Then, type in the batch ID and submit it for analysis [5]. Confirm that exocrine tissue has not been incorrectly classified as islets [6].

6.3.1. SCREEN: 6.3.1-6.3.6 00:02-00:06.

6.3.2. SCREEN: 6.3.1-6.3.6 00:07-00:13.

6.3.3. SCREEN: 6.3.1-6.3.6 00:14-00:22.

6.3.4. SCREEN: 6.3.1-6.3.6 00:23-00:26.

6.3.5. SCREEN: 6.3.1-6.3.6 00:27-00:29.

6.3.6. SCREEN: 6.3.1-6.3.6 00:45-00:50

6.4. If adjacent islets are not correctly separated, left-click outside the islets to initiate a separation line [1]. Add intermediate points with left-clicks to guide the separation line between the adjacent islets crossing the contour. Complete the line with a right-click [2]. To remove manually added incorrect separation lines, right-click any point on the line and select the remove option from the pop-up menu [3].

6.4.1. SCREEN: 6.4.1-6.5.2. 00:21-00:25.

6.4.2. SCREEN: 6.4.1-6.5.2. 00:26-00:30 and 00:36-00:39

6.4.3. SCREEN: 6.4.1-6.5.2. 00:44-00:51.

6.5. Submit the separation line and wait for the Inspection window to show the updated analysis [1], marked with the blue check [2].

6.5.1. SCREEN: 6.4.1-6.5.2. 00:52-00:55 and 00:59-01:05.

6.5.2. SCREEN: 6.4.1-6.5.2. 01:06-00:09.

6.6. Proceed sequentially with the next sample images in the Visual Quality Control window. Use the switch at the top of the window to exclude the images with missed or partially detected islets [1] and the images in which exocrine tissue, background artifacts, or bubbles are classified as islets [2].

6.6.1. SCREEN: 6.6.1-6.6.2. 00:00-00:09.

6.6.2. SCREEN: 6.6.1-6.6.2. 00:10-00:14.

6.7. Use **Edit Batch** to correct batch information if needed [1]. Modify the available parameters as needed, but retain the existing session code. Assign new names to any replacement images rather than reusing the names of deleted files [2].

6.7.1. SCREEN: 6.7.1-6.7.2. 00:12-00:16

6.7.2. SCREEN: 6.7.1-6.7.2. 00:20-00:26.

7. Report Download

7.1. After reviewing and correcting all the batches, proceed to the session **summary** [1]. Check the session totals and batch averages for islet count, volume, purity, and pellet measurements calculated from the included images [2].

7.1.1. SCREEN: 7.1.1-7.2.1. 00:02-00:05

7.1.2. SCREEN: 7.1.1-7.2.1. 00:06-00:12.

7.2. Download the Complete PDF report for review and records [1].

7.2.1. SCREEN: 7.1.1-7.2.1. 00:13-00:16.

7.3. Finally, open the report for validation. Confirm whether manual editing was performed, and verify the batch results, number of images included, and the pixel sizes [1]. Review the yield, pellet, islet size index, and islet size histogram [2]. If the Report is valid, close the current Islet-Net session [3].

7.3.1. SCREEN: 7.3.1-7.3.2. 00:01-00:05 and 00:08-00:15.

7.3.2. SCREEN: 7.1.1-7.2.1. 00:18-00:24.

7.3.3. SCREEN: 7.3.3. 00:40-00:47

NOTE: The authors strongly recommend that we skip figures as the relevant figures are already shown while performing the steps in the screen recordings.

1. IsletNet

Pronunciation link: No confirmed link found

IPA: /'aɪlətˌnɛt/

Phonetic Spelling: eye-luht-net

2. Dithizone

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

IPA: /'daɪθɪˌzoʊn/

Phonetic Spelling: dye-thih-zohn

3. Islet

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

IPA: /'aɪlət/

Phonetic Spelling: eye-luht

4. Purification

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

IPA: /ˌpjʊərəfə'keɪʃən/

Phonetic Spelling: pyur-uh-fuh-kay-shuhn

5. Conical

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

IPA: /'kɑ:nɪkəl/

Phonetic Spelling: kon·ih·kuhl

6. Laminar

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

IPA: /'læmənər/

Phonetic Spelling: lam·uh·ner

7. Pipette

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

IPA: /paɪ'pet/

Phonetic Spelling: pie·pet

8. Petri

Pronunciation link: <https://www.merriam-webster.com/dictionary/Petri%20dish>

IPA: /'pi:tri/

Phonetic Spelling: pee·tree

9. Alkaline

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

IPA: /'ælkə laɪn/

Phonetic Spelling: al·kuh·line

10. Alcoholic

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

IPA: /,ælkə'hɑ:lɪk/

Phonetic Spelling: al·kuh·hol·ik

11. Suspension

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

IPA: /sə'spenʃən/

Phonetic Spelling: suh·spen·shuhn

12. Incubation

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

IPA: /ˌɪnkjəˈbeɪʃən/

Phonetic Spelling: in·kyuh·bay·shuhn

13. Microscope

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

IPA: /ˈmaɪkrəˌskoʊp/

Phonetic Spelling: my·kruh·skohp

14. Albumin

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

IPA: /ælˈbjuːmɪn/

Phonetic Spelling: al·byoo·min

15. PBS

Pronunciation link: No confirmed link found

IPA: /ˌpiː biː ˈɛs/

Phonetic Spelling: pee·bee·ess

16. Exocrine

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

IPA: /ˈeksəˌkraɪn/

Phonetic Spelling: ek·suh·krine

17. Halogen

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

IPA: /ˈhælədʒən/

Phonetic Spelling: hal·uh·juhn

18. Micrometer

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

IPA: /maɪ'krɑ:mətər/
Phonetic Spelling: my·krom·uh·ter

19. Magnification

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

IPA: /,mægnəfə'keɪʃən/
Phonetic Spelling: mag·nuh·fuh·kay·shuhn

20. Calibration

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

IPA: /,kælə'breɪʃən/
Phonetic Spelling: kal·uh·bray·shuhn

21. Clinical

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

IPA: /'klɪnɪkəl/
Phonetic Spelling: klin·ih·kuhl

22. Pixel

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

IPA: /'pɪksəl/
Phonetic Spelling: pik·suhl

23. Micrometers

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

IPA: /maɪ'krɑ:mətərz/
Phonetic Spelling: my·krom·uh·terz

24. Graphical

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

IPA: /'græfɪkəl/
Phonetic Spelling: graf·ih·kuhl

25. Contours

Pronunciation link: <https://www.merriam-webster.com/dictionary/contour>
IPA: /'kɑ:n.tər/
Phonetic Spelling: kon·toor

26. Segmentation

Pronunciation link: <https://www.merriam-webster.com/dictionary/segmentation>
IPA: /,segmən'teɪʃən/
Phonetic Spelling: seg·muhn·tay·shuhn

27. Spurious

Pronunciation link: <https://www.merriam-webster.com/dictionary/spurious>
IPA: /'spjʊəriəs/
Phonetic Spelling: spyoor·ee·uhs

28. Histogram

Pronunciation link: <https://www.merriam-webster.com/dictionary/histogram>
IPA: /'hɪstə,græm/
Phonetic Spelling: hiss·tuh·gram

29. Yield

Pronunciation link: <https://www.merriam-webster.com/dictionary/yield>
IPA: /'ji:ld/
Phonetic Spelling: yeeld

30. Pellet

Pronunciation link: <https://www.merriam-webster.com/dictionary/pellet>
IPA: /'pelət/
Phonetic Spelling: pel·uht

31. Dilution

Pronunciation link: <https://www.merriam-webster.com/dictionary/dilution>
IPA: /daɪ'lu:ʃən/
Phonetic Spelling: dye·loo·shuhn

32. Replicate

Pronunciation link: <https://www.merriam-webster.com/dictionary/replicate>
IPA: /'rep.lə.kət/ (noun), /'rep.lə.keɪt/ (verb)
Phonetic Spelling: rep·luh·kut (noun); rep·luh·kayt (verb)

33. Representative

Pronunciation link: <https://www.merriam-webster.com/dictionary/representative>
IPA: /,reprɪ'zentətɪv/
Phonetic Spelling: rep·ruh·zen·tuh·tiv

34. Visual

Pronunciation link: <https://www.merriam-webster.com/dictionary/visual>
IPA: /'vɪʒuəl/
Phonetic Spelling: vizh·oo·uhl

35. Validation

Pronunciation link: <https://www.merriam-webster.com/dictionary/validation>
IPA: /,vælə'deɪʃən/
Phonetic Spelling: val·uh·day·shuhn