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Title: Enhanced Spatial Mapping of Mouse Gastric Muscle Layers Using a Modified Swiss Roll Technique

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Author Questionnaire

1. We have marked your project as author-provided footage, meaning you film the video yourself and provide JoVE with the footage to edit. JoVE will not send the videographer. Please confirm that this is correct.

√ Correct

2. Microscopy: Does your protocol require the use of a dissecting or stereomicroscope for performing a complex dissection, microinjection technique, or something similar? **NO**

3. Software: Does the part of your protocol being filmed include step-by-step descriptions of software usage? **NO**

4. Proposed filming date: To help JoVE process and publish your video in a timely manner, please indicate the proposed date that your group will film here: **N/A**

When you are ready to submit your video LAB MEDIAs, please contact our Content Manager, Utkarsh Khare.

Current Protocol Length

Number of Steps: 11
Number of Shots: 23

Introduction

NOTE: Authors provided pre-recorded interviews

INTRODUCTION:

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

1.1. **Srinidhi Babu:** Our research focuses on improving how we prepare and analyze the mouse stomach for imaging. We've adapted the "Swiss roll" technique, originally developed for the murine intestine, so that all major gastric regions including the fundus, corpus, and antrum can be embedded together in a single cryosection. This allows us to visualize the stomach's structure and cellular composition side by side, while maintaining accurate spatial orientation and high image quality.

1.1.1. LAB MEDIA: Intro-1-27s 00:00 – 00:27

~~What advantage does your protocol offer compared to other techniques?~~

1.2. **Srinidhi Babu:** The stomach is a complex organ with delicate tissues. Small mistakes can damage cells or distort their arrangement, which makes high-resolution imaging and immunostaining inconsistent. Our Swiss roll technique solves this by keeping all regions including duodenum and esophagus intact in a single cryoblock, making experiments more reliable and reproducible.

1.2.1. LAB MEDIA: Intro-2-22s 00:00-00:22

NOTE: This was moved here to facilitate a longer introduction section

CONCLUSION:

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

1.3. **Srinidhi Babu:** The Swiss roll technique can advance research on region-specific changes in gastric tissue. Unlike traditional approaches that embed regions separately, the Swiss roll technique processes all gastric regions under identical conditions, saving both time and reagents. This makes it a practical and scalable platform for comprehensive analysis of gastric tissue in both health and disease.

1.3.1. LAB MEDIA: Intro-3-22s 00:00 – 00:22

Ethics Title Card

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

Protocol

NOTE: Scripted from footage

2. Preparation, Rolling, and Confocal Imaging of Mouse Gastric Tissue Using the Swiss Roll Technique

Demonstrator: Srinidhi R. Babu

2.1. To begin, use scissors to cut along the ventral mesentery of the duodenum and stomach up to the pin at the corpus **[1]**. Using a transfer pipette filled with PBS, flush out any residual food from the gastric lumen **[2]**.

2.1.1. LAB MEDIA: Surgery-1 00:00-00:22

2.1.2. LAB MEDIA: Surgery-1 01:44-01:2:04

2.2. Gently stretch the opened stomach to achieve a flat orientation **[1-TXT]**. Pin the tissue flat again after the brief fixation **[2]**.

2.2.1. LAB MEDIA: Surgery-1 02:45-02:50, 03:21-03:28
TXT: Fix in cold 4% PFA for 2 min

2.2.2. LAB MEDIA: Surgery-1 04:06-04:28

2.3. Using scissors, trim the stomach along the dashed outline to create a uniform shape suitable for rolling **[1]**. Place the trimmed gastric tissue on PBS-moistened filter paper **[2]**.

2.3.1. LAB MEDIA: Surgery-2 00:00-00:18

2.3.2. LAB MEDIA: Surgery-3 00:02-00:13

2.4. Roll the tissue into a Swiss roll configuration and secure it by inserting a toothpick through the center **[1]**.

2.4.1. LAB MEDIA: Surgery-4 00:08-00:22

2.5. Once rolled, insert a 30-gauge needle to secure its shape **[1]**. Then, bend the needle at a 90-degree angle to stabilize the rolled stomach **[2]**. Fix the rolled tissue in 4 percent paraformaldehyde at 4 degrees Celsius for 6 hours **[3]**. Then gently remove the stabilizing needle **[4]**.

2.5.1. LAB MEDIA: Surgery-5 00:27-00:39

2.5.2. LAB MEDIA: Surgery-6 00:02-00:11

2.5.3. LAB MEDIA: Surgery-6 00:21-00:28

2.5.4. LAB MEDIA: Surgery-7 01:05-01:10

2.6. Embed the tissue in optimal cutting temperature compound using disposable base molds **[1]**. Then, freeze the mold on dry ice **[2]**. Once fully frozen, store the mold at minus 80 degrees Celsius **[3]**.

2.6.1. LAB MEDIA: Surgery-7 01:10-01:16, 01:35-01:42

2.6.2. LAB MEDIA: Surgery-7 02:05-02:11

2.6.3. LAB MEDIA: Surgery-8 00:00-00:03

NOTE: 2.7 to 2.11 were deleted at author's request

~~2.7. To acquire a stitched image, center the Swiss roll on the confocal microscope at 10X magnification **[1]**.~~

~~2.7.1. LAB MEDIA: Confocal-1min48s~~

~~2.8. Ensure proper laser power and gain to avoid pixel saturation **[1]**. Confirm that the sample is clearly in focus **[2]**. On the Ti2 Eclipse confocal microscope, use the AI autosignal feature to optimize the signal **[3]**.~~

~~2.8.1. LAB MEDIA: Confocal-1min48s~~

~~2.8.2. LAB MEDIA: Confocal-1min48s~~

~~2.8.3. LAB MEDIA: Confocal-1min48s~~

~~2.9. To define the acquisition parameters, open **Applications**, and select **Define/Run ND Acquisition** **[1]**. Use the given settings in the **Large Image** tab **[2]**.~~

~~2.9.1. LAB MEDIA: Confocal-1min48s~~

~~2.9.2. LAB MEDIA: Confocal-1min48s~~

~~2.10. In the **Z** tab, set the top and bottom coordinates of the sample **[1]**. Select 8 steps for image acquisition **[2]**.~~

~~2.10.1. LAB MEDIA: Confocal-1min48s~~

~~2.10.2. LAB MEDIA: Confocal-1min48s~~

~~2.11. Once done, switch the laser scanner to the **Galvano** setting, and click **Run now** to begin acquisition **[1]**.~~

~~2.11.1. LAB MEDIA: Confocal-1min48s~~

Results

3. Results

3.1. The new method enabled inclusion of the fundus, corpus, antrum, a portion of the esophagus, and duodenum within a single murine gastric Swiss roll section **[1]**.

3.1.1. LAB MEDIA: Figure 1C and D. *Video editor: Highlight the labeled areas "Fundus," "Corpus," "Antrum," "Esophagus," and "Duodenum" in panel C and D.*

3.2. Hematoxylin and eosin staining of the gastric Swiss roll revealed well-preserved architecture in the fundus, corpus, antrum, esophagus, and duodenum **[1]**, with clearly visible mucosal, submucosal, and muscularis layers **[2]**.

3.2.1. LAB MEDIA: Figure 3. *Video editor: Highlight the labeled regions "Fundus," "Corpus," "Antrum," "Esophagus," and "Duodenum" in the stained cross-section.*

3.2.2. LAB MEDIA: Figure 3.

3.3. Immunofluorescent staining showed expression of KIT *(Kit)* and ANO1 *(An-oh-one)* in interstitial cells of Cajal (*ka-HAHL*) **[1]**, and expression of TUBB3 *(beta-TOO-buh-lin three)* in enteric neurons, confirming cellular preservation in the gastric Swiss roll **[2]**.

3.3.1. LAB MEDIA: Figure 4. *Video editor: Highlight the bottom image in KIT and ANO1 panels*

3.3.2. LAB MEDIA: Figure 4. *Video editor: Highlight the bottom image in red-stained TUBB3 panels*

3.4. KIT expression was also detected in Paneth and goblet cells in the gastric mucosa **[1]**, while ANO1 was highly expressed in fundic columnar epithelial cells **[2]**.

3.4.1. LAB MEDIA: Figure 4. *Video editor: Highlight the white arrows in the bottom image of KIT panel*

3.4.2. LAB MEDIA: Figure 4. *Video editor: Highlight the white arrows in the bottom image of ANO1 panel*

3.5. TUBB3 expression was observed in gastric endocrine and mucus-secreting cells, in addition to enteric neurons **[1]**.

3.5.1. LAB MEDIA: Figure 4. *Video editor: Highlight the red arrows in the bottom image of the TUBB3 panel*

3.6. MYH11*(myosin HEH-vee chain eleven)* staining confirmed the presence of smooth muscle cells and myofibroblasts in the gastric mucosa **[1]**, while E-cadherin *(ee-kad-HEER-in)* staining showed epithelial cells **[2]**, and KIT staining again marked interstitial cells of Cajal **[3]**.

3.6.1. LAB MEDIA: Figure 5. *Video editor: Highlight the green MYH11 staining in the lower left panel indicating smooth muscle and mucosal regions*.

3.6.2. LAB MEDIA: Figure 5. *Video editor: Highlight yellow E-cadherin signal marking epithelial cells in the lower middle-right panel.*

3.6.3. LAB MEDIA: Figure 5. *Video editor: Highlight KIT staining with white arrows in the middle-lower panel labeled "KIT."*

Pronunciation Guide:

 Laparoscopic
Pronunciation link: https://www.merriam-webster.com/dictionary/laparoscopic
IPA: /ˌlæp.ə.rəˈskɑː.pɪk/
Phonetic Spelling: lap-uh-ruh-SKAH-pik
 Gastrectomy
Pronunciation link: https://www.merriam-webster.com/dictionary/gastrectomy
IPA: /gæˈstrɛk.tə.mi/
Phonetic Spelling: gas-TREK-tuh-mee
 Mesentery
Pronunciation link: https://www.merriam-webster.com/dictionary/mesentery
IPA: /ˈmɛz.ənˌtɛr.i/
Phonetic Spelling: MEZ-uhn-ter-ee
 Duodenum
Pronunciation link: https://www.merriam-webster.com/dictionary/duodenum
IPA: /ˌduː.əˈdiː.nəm/
Phonetic Spelling: doo-uh-DEE-nuhm
 Paraformaldehyde
Pronunciation link: https://www.merriam-webster.com/dictionary/paraformaldehyde
IPA: /ˌpær.ə.fɔːrˈmæl.də.haɪd/
Phonetic Spelling: pair-uh-for-MAL-duh-hide
 Cryosection
Pronunciation link: https://www.merriam-webster.com/dictionary/cryosection
IPA: /ˌkraɪ.oʊˈsɛk.ʃən/
Phonetic Spelling: kry-oh-SEK-shuhn
 Confocal
Pronunciation link: https://www.merriam-webster.com/dictionary/confocal
IPA: /kɑnˈfoʊ.kəl/
Phonetic Spelling: kon-FOH-kuhl
 Immunofluorescent
Pronunciation link: https://www.merriam-webster.com/dictionary/immunofluorescent
IPA: /ˌɪm.jə.noʊ.flʊˈrɛs.ənt/
Phonetic Spelling: im-yuh-noh-floor-ESS-uhnt
 Hematoxylin
Pronunciation link: https://www.merriam-webster.com/dictionary/hematoxylin
IPA: /ˌhiː.məˈtɑː.k.sɪ.lɪn/
Phonetic Spelling: hee-muh-TAHK-suh-lin
 Eosin
Pronunciation link: https://www.merriam-webster.com/dictionary/eosin
IPA: /ˈiː.oʊ.sɪn/
Phonetic Spelling: EE-oh-sin

 Muscularis
Pronunciation link: https://www.merriam-webster.com/dictionary/muscularis
IPA: /ˌmʌs.kjəˈlɛr.ɪs/
Phonetic Spelling: mus-kyuh-LAIR-iss
 Submucosal
Pronunciation link: https://www.merriam-webster.com/dictionary/submucosal
IPA: /ˌsʌb.mjuːˈkoʊ.səl/
Phonetic Spelling: sub-myoo-KOH-suhl
 Interstitial
Pronunciation link: https://www.merriam-webster.com/dictionary/interstitial
IPA: /ˌɪn.tɚˈstɪʃ.əl/
Phonetic Spelling: in-ter-STISH-uhl
 Cajal
Pronunciation link: https://www.merriam-webster.com/dictionary/Cajal
IPA: /kəˈhɑːl/
Phonetic Spelling: kuh-HAHL
 Myosin
Pronunciation link: https://www.merriam-webster.com/dictionary/myosin
IPA: /ˈmaɪ.oʊ.sɪn/
Phonetic Spelling: MY-oh-sin
 Myofibroblast
Pronunciation link: https://www.merriam-webster.com/dictionary/myofibroblast
IPA: /ˌmaɪ.oʊˈfaɪ.brəˌblæst/
Phonetic Spelling: my-oh-FY-broh-blast
 E-cadherin
Pronunciation link: https://www.merriam-webster.com/dictionary/E-cadherin
IPA: /ˌiːˈkæd.hɪr.ɪn/
Phonetic Spelling: ee-KAD-hair-in