FINAL SCRIPT: APPROVED FOR FILMING

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Title: *In Vivo* Confocal Microscopy: A Standard Operating Procedure for The Detection of *Demodex* Mites at The Eyelid Margin

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: 14
Number of Shots: 33

Introduction

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

1.1. **Guanshun Yu:** We are developing a standardized *in vivo* confocal microscopy protocol for detecting eyelid *Demodex* mites, to expand clinical adoption **[1]**.

1.1.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 2.13*

What research gap are you addressing with your protocol?

1.2. **Guanshun Yu:** We are addressing the lack of a standardized IVCM protocol, which limits detection of *Demodex* mites *in vivo* in clinical practice **[1]**.

1.2.1. INTERVIEW: Named Talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B. roll: 3.1.1*

What advantage does your protocol offer compared to other techniques?

1.3. **Guanshun Yu:** It provides non-invasive, painless, and rapid *in vivo* detection with easy follow-up, eliminating epilation pain and residual mite issues **[1]**.

1.3.1. 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 Review Board (IRB) of the Eye Hospital, Wenzhou Medical University

Protocol

2. Setup and Execution of *In Vivo* Eyelid Imaging Using Scanning Laser Ophthalmoscopy

Demonstrators: Guanshun Yu, Chenghao Wang

2.1. To begin, clean the lens of the device using lens cleaning wipes **[1]**. Use alcohol-soaked cotton balls to disinfect the headrest and chinrest thoroughly **[2]**.

2.1.1. WIDE: Talent cleaning the microscope lens with a lens wipe.

2.1.2. Talent wiping the headrest and chinrest with alcohol-soaked cotton.

2.2. Press the two black levers together and pull the chinrest forward until it catches to ensure it is in the correct horizontal position **[1]**. Now, turn the adjustment screw to move the camera away from the patient before the examination **[2]**.

2.2.1. Talent pressing the black levers and pulling the chinrest forward.

2.2.2. Talent turning the adjustment screw to shift the camera backwards.

2.3. Apply approximately 0.02 milliliters of Carbomer-based ophthalmic gel on the front surface of the microscope lens of the device **[1]**.

2.3.1. Talent applying a small drop of gel to the microscope lens using a dropper.

2.4. Next, remove a sterile, disposable polymethylmethacrylate cap from its protective cover **[1]**. Press the sides of the cap firmly onto the microscope lens without touching the anterior surface **[2]**. Then apply one drop of gel tear substitute on the front surface of the cap **[3]**.

2.4.1. Talent unsealing the PMMA cap from its protective packaging.

2.4.2. Talent pressing the cap onto the microscope lens carefully.

2.4.3. Talent applying tear substitute gel onto the cap.

2.5. Create a **New Patient** File in the software **[1]**. Now, apply a drop of Proparacaine hydrochloride at the margin of the eyelid to be examined **[2]**.

2.5.1. SCREEN: 68534_screenshot_1.mp4 00:00-00:10

2.5.2. Talent applying anesthetic drop to the eyelid margin.

2.6. Move the scanning camera to the left from the operator's point of view **[1]**. Then turn the CCD *(C-C-D)* camera objective lens to adjust the control image perpendicularly to the optical axis of the scanner laser camera **[2]**. Start a new examination using the software **[3]**.

2.6.1. Talent shifting the scanning camera laterally to the left.

2.6.2. Talent turning the CCD camera objective lens to align the image orientation.

2.6.3. SCREEN: 68534_screenshot_2.mp4. 00:00-00:06

2.7. In the **Examination Data** tab, select **Heidelberg Retina Tomograph-Cornea** from the **Device** dropdown list and observe the **Cornea Module Settings** dialog box **[1]**. Choose **FOV 400** *(F-O-V-Four-Hundred)* from the **Field Lens** dropdown list and click **OK** to confirm **[2]**. Then wait for the acquisition window to open and verify the display of the scanning laser camera live image and control image **[3]**.

2.7.1. SCREEN: 68534_screenshot_3.mp4. 00:00-00:03

2.7.2. SCREEN: 68534_screenshot_3.mp4. 00:04-00:09

2.7.3. SCREEN: 68534_screenshot_3.mp4. 00:10-00:18

2.8. Next, set the factors in the acquisition window. Select **1x** *(One-Ex)* from the **CCD Zoom Factor** dropdown to set the control image size **[1]**. Check the **Automatic Brightness** and **Auto** boxes to enable automatic brightness control **[2]**. Ensure the **Section** scan type is selected **[3]**.

2.8.1. SCREEN: 68534_screenshot_4.mp4. 00:00-00:03

2.8.2. SCREEN: 68534_screenshot_4.mp4. 00:04-00:10

2.8.3. SCREEN: 68534_screenshot_4.mp4. 00:11-00:15

2.9. To acquire a reference image, turn off the automatic brightness control, then adjust the focal plane with the adjusting pin until the live image darkens and subsequently a bright reflection appears, indicating the anterior surface of the polymethylmethacrylate cap **[1]**. Click **Reset** in the **Focus Position [µm]** *(Micrometer)* section and wait for the value to reach 0 **[2]**. Then check the **Automatic Brightness** box, and switch off **Auto Reset [3]**.

2.9.1. SCREEN: 68534_screenshot_5.mp4. 00:00-00:09

2.9.2. SCREEN: 68534_screenshot_5.mp4 00:10-00:13

2.9.3. SCREEN: 68534_screenshot_5.mp4 00:14-00:17

2.10. Now, adjust the height of the examination table **[1].** Place the patient's chin on the chinrest and forehead against the forehead rest **[2]**. Adjust the chin rest elevation using the black screw so that the patient's upper eyelids align with the red marks on the headrest column **[3]**.

2.10.1. Talent adjusting table height.

2.10.2. Talent helping patient position their face.

2.10.3. Talent turning the black screw to align the eyelids with red marks.

2.11. Instruct the patient to look downward toward the fixed light source and maintain a steady gaze **[1]**.

2.11.1. Talent guiding patient to fix gaze at the light source.

2.12. With a cotton swab, flip the upper eyelid and fully expose the eyelash root and palpebral margin **[1]**. Turn the adjustment screw to align the laser beam reflection at the upper eyelid level **[2]**. Then move the scanning laser camera until the upper eyelid margin gently contacts the center of the polymethylmethacrylate cap **[3]**.

2.12.1. Talent flipping the upper eyelid using a cotton swab.

2.12.2. Talent adjusting the screw to align laser beam reflection.

2.12.3. Shot of the PMMA cap being moved to contact the upper eyelid margin.

2.13. Carefully scan the eyelash root from the temporal to nasal side using minute horizontal movements of the palpebral margin **[1]**. Turn the microscope lens clockwise or counterclockwise to modify the focus and observe entire follicles and roots **[2-TXT]**.

2.13.1. SCOPE/SCREEN: 68534_screenshot_6.mp4. 00:00-00:12

2.13.2. SCOPE/SCREEN: 68534_screenshot_6.mp4. 00:13-00:19

TXT: Note the different image depths in the scanning laser camera live feed

2.14. Record all suspected *Demodex* images using the foot pedal **[1]**. After quitting the examination, select **Sort by acquisition time (fast)** *(sort-by-acquisition-time- Fast)* in the **Options** dialog to display images from temporal to nasal side **[2]**.

2.14.1. Talent pressing foot pedal to record and saving images on screen.

2.14.2. SCREEN: 68534_screenshot_7.mp4 00:00-00:06

Results

3. Results

3.1. In 2024, the number of subjects undergoing *In Vivo* confocal microscopy for *Demodex* detection increased significantly across all age groups compared to 2023 **[1]**, with the highest number observed in the 21 to 30 year age group **[2]**.

3.1.1. LAB MEDIA: Figure 5.

3.1.2. LAB MEDIA: Figure 5. *Video editor: Highlight the green bar corresponding to the 21–30 year age group.*

3.2. The standard operating procedure introduced in 2024 enabled the inclusion of subjects from as young as 4 years old to over 90 years of age **[1]**.

3.2.1. LAB MEDIA: Figure 5. *Video editor: Highlight the green bars at both extremes of the age range—1–5 years and >90 years.*

Pronunciation Guide:

1. polymethylmethacrylate
Pronunciation link:
https://www.howtopronounce.com/polymethyl-methacrylate
youtube.com+14howtopronounce.com+14wordpanda.net+14
IPA: /ˌpɑliˈmɛθəl məˈθækrəˌleɪt/
Phonetic spelling: pol-ee-METH-uhl muh-THA-kruh-layt

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2. carbomer
Pronunciation link:
https://www.howtopronounce.com/carbomer
youtube.com+9howtopronounce.com+9pronouncehippo.com+9
IPA: /kɑrˈboʊmər/
Phonetic spelling: kar-BOH-mur

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3. proparacaine
Pronunciation link:
https://www.howtopronounce.com/proparacaine
medindia.net+10howtopronounce.com+10pronouncekiwi.com+10
IPA: /proʊˈpærəkeɪn/
Phonetic spelling: proh-PAR-uh-kayn

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4. ophthalmic
Pronunciation link:
https://dictionary.cambridge.org/us/pronunciation/english/ophthalmic
youglish.com+15dictionary.cambridge.org+15oxfordlearnersdictionaries.com+15
IPA: /ɑfˈθæl.mɪk/
Phonetic spelling: off-THAL-mik

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5. palpebral
Pronunciation link:
https://dictionary.cambridge.org/us/pronunciation/english/palpebral merriam-webster.com+7dictionary.cambridge.org+7dictionary.cambridge.org+7
IPA: /ˈpæl.pə.brəl/
Phonetic spelling: PAL-puh-bruhl

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6. Demodex
Pronunciation link:
https://www.howtopronounce.com/demodex
youtube.com+15howtopronounce.com+15howtopronounce.com+15
IPA: /ˈdiːməˌdɛks/
Phonetic spelling: DEE-muh-deks

7. Heidelberg Retina Tomograph
Pronunciation link:
https://www.youtube.com/watch?v=s0Gzd4-pJpw youtube.com
IPA (for "Tomograph"): /ˈtɑməˌgræf/
Phonetic spelling: TAH-muh-graf

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8. chinrest
Pronunciation link:
https://dictionary.cambridge.org/pronunciation/english/chin-rest glosbe.com+15dictionary.cambridge.org+15howtopronounce.com+15
IPA: /ˈtʃɪnˌrɛst/
Phonetic spelling: CHIN-rest

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9. headrest
Pronunciation link:
(No direct audio found) — No confirmed link found
IPA: /ˈhɛdˌrɛst/
Phonetic spelling: HEAD-rest

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10. palpebral fissure
Pronunciation link:
https://www.merriam-webster.com/medical/palpebral%20fissure youtube.com+11merriam-webster.com+11merriam-webster.com+11
IPA: /ˈpæl.pə.brəl ˈfɪʃʊr/
Phonetic spelling: PAL-puh-bruhl FISH-ur

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11. microscope
Pronunciation link:
https://www.merriam-webster.com/dictionary/microscope — (standard)
IPA: /ˈmaɪkrəˌskoʊp/
Phonetic spelling: MY-kruh-skohp
