FINAL SCRIPT: APPROVED FOR FILMING

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# Title: In Vivo Confocal Microscopy in the Diagnosis and Management of Dry Eye: A Focus on Imaging Protocols and Interpretation

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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**

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: 22
Number of Shots: 47 (13 SC)

# Introduction

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

**INTRODUCTION:**

1.1. **Fen Chen:** This research demonstrates how to use IVCM to observe the microscopic ocular surface structure of patients with dry eye disease.

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

1.2. **Fen Chen:** The main experimental challenges are the absence of marker-based positioning and a limited field of view.

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

**CONCLUSION:**

1.3. **Fen Chen:** This protocol achieves an integrated multimodal assessment of dry eye through a single procedure at the cellular level.

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

1.4. **Fen Chen:** Future research may focus on AI-assisted distinction between different cells of IVCM images and expanding the observation field of view.

**1.4.1.** 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.***

**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**.

How do you think publishing with JoVE will enhance the visibility and impact of your research?

1.5. **Fen Chen, Attending Ophthalmologist of Hankou Aier Eye Hospital** : (Testimonial statement: JoVE provides a massive platform. It pairs the written paper with experimental video, so your research can be shared more directly. This gives a full picture to a global audience. JoVE 提供了很广阔的平台，它通过论文和操作视频相结合的形式、让我们的研究能更直观更全面的展现给全世界对此感兴趣的人。)

1.5.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:2.3.1*

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE? (This could include increased collaborations, citations, funding opportunities, streamlined lab procedures, reduced training time, cost savings in the lab, or improved lab productivity.)

1.6. **Fen Chen, Attending Ophthalmologist of Hankou Aier Eye Hospital** : (Since my paper came out in JoVE, I've been watching the view count slowly climb. It feels really satisfying, and I hope our work can actually help someone out there. 在 JoVE 上发表论文后，我一直关注着文章的浏览量在不断的、慢慢的增加，心里很有成就感，希望我们的研究能对他们有帮助)

1.6.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll:4.3.1*

Authors: Could you please also deliver the above statements in Chinese?
*Videographer: Please film the testimonials in both English and Chinese*

**Ethics Title Card**

This research has been approved by the Ethics Committee of Hankou Aier Eye Hospital

# Protocol

## 2. Preparation of the Instruments and the Patient's Eyes

**Demonstrators:** Fen Chen, Ying Wang, Lisha Ke

2.1. To begin, prepare all required materials required for the procedure **[1]**.

2.1.1. **WIDE:** Talent arranging all the listed materials neatly on a clean laboratory table, ensuring clear visibility of each item.

2.2. Examine and understand the key components of the in vivo confocal microscopy device **[1]** and wear a protective face mask and wear disposable gloves **[2]**.

2.2.1. Talent showing the in vivo confocal microscopy device . **Authors**: We need not list all the equipment here. The videographer will simply cover the setup

2.2.2. Talent putting on a face mask and wearing disposable gloves.

2.3. Wipe the forehead and chin rests of the device thoroughly with alcohol swabs to disinfect them **[1]**.

2.3.1. Talent cleaning the forehead and chin rests with alcohol swabs in a slow, deliberate motion.

2.4. On the connected computer, open the accompanying IVCM software **[1]**, verify existing patient information, and create a new patient record if required **[2]**.

2.4.1. Talent opening the software.

2.4.2. Talent reading the patient data displayed on the monitor.

2.5. Administer two drops of ophthalmic topical anesthetic in the lower fornix of the patient's eye, maintaining a 3-minute interval between each drop **[1]**.

2.5.1. Talent instilling one drop of anesthetic into the lower fornix of the patient's eye.

2.6. Then, prepare the Rostock cornea module by coating its laser-emitting area with coupling gel, ensuring that no air bubbles are present **[1]** and attach a disposable corneal contact cap securely onto the Rostock cornea module **[2]**.

2.6.1. Close-up shot of talent evenly spreading coupling gel over the laser-emitting

area, checking for air bubbles.

2.6.2. Talent fitting the disposable corneal contact cap onto the module with precision.

2.7. Next, retract the Rostock cornea module completely **[1]**, secure the forehead rest in a horizontal position **[2]**, and adjust the monitoring camera toward the temporal side of the subject **[3]**. Identify the camera's stop point that aligns perpendicular to the optical axis of the module **[4]**.

2.7.1. Talent retracting the module to its full position.

2.7.2. Talent aligning and tightening the forehead rest horizontally.

2.7.3. Talent repositioning the monitoring camera to the temporal side.

2.7.4. SCREEN: 69156-2.7.4.mp4.mp4 .

2.8. Rotate the focus knob until the software displays a maximized white facula with the image quality value reading exactly 100 **[1]**. Then, reset the depth value to zero to complete calibration **[2]**.

2.8.1. SCREEN: 69156-2.8.1.mp4.mp4.

2.8.2. SCREEN: 69156-2.8.2.mp4.mp4. 00:07-00:14

2.9. Now, select **Section Mode** as the default scan mode unless another mode is specifically indicated **[1]**.

2.9.1. SCREEN: 69156-2.9.1.mp4.mp4.

2.10. Ask the patient to rest their chin firmly on the chin rest **[1]**, press their forehead gently against the forehead rest **[2]**, and adjust the examination table and chin rest to match the patient's height **[3]**.

2.10.1. Talent guiding the patient to position their chin on the chin rest. **Authors**: The shots 2.10.1 and 2.10.3 can be normal shots that can be filmed by the videographer.

2.10.2. Show the fully aligned patient's forehead against the forehead rest.

2.10.3. Talent adjusting the examination table height and chin rest for a comfortable fit.

## 3. Corneal and Eye Examination Protocol

3.1. Insert the eyelid speculum carefully to keep the patient's eyelids for the procedure **[1]**.

3.1.1. Talent inserting the eyelid speculum gently into the patient's eye.

3.2. Begin macroscopic alignment by visually adjusting the Rostock cornea module to approximate the corneal apex **[1]**. Switch to the monitoring camera to fine-tune alignment as the module approaches the cornea **[2]**. Instruct the patient to rotate their gaze when examining lesional corneas, centering the lesion under the module **[3]**. Maintain gentle contact between the cap and cornea to avoid damage **[4]**.

3.2.1. Talent aligning the RCM visually toward the corneal apex.

3.2.2. SCREEN: 69156-3.2.2.mp4.mp4. *Video editor: Please speed up*

3.2.3. SCREEN: 69156-3.2.3.mp4.mp4 00:10-00:25.

3.2.4. Close-up shot of the RCM maintaining gentle contact with the corneal surface.

3.3. Now, depress the foot pedal to perform prolonged scanning, capturing sequential depth images to ensure no critical data are missed **[1]**.

3.3.1. Talent pressing the foot pedal and maintaining it in a depressed position.

3.4. Then, perform layered corneal assessment by scanning progressively from the superficial to the deep layers from the corneal epithelial cells to the subbasal nerve fibers and subepithelial inflammatory cells **[1]**.

3.4.1. SCREEN: 69156-3.5.1.mp4.mp4. 00:05-00:25

3.5. Focus on the inferior whorl region, the recommended anatomical landmark, and examine the adjacent superior and inferior areas **[1]**.

3.5.1. SCREEN: 69156-3.5.1.mp4.mp4. 00:05-00:10 and 00:22-00:32

3.6. Next, reset the focal plane to a depth value of zero **[1]** and apply coupling gel evenly onto the corneal contact cap **[2]**. Instruct the patient to direct their gaze temporally or inferotemporally **[3]** and gently advance the RCM toward the conjunctival surface **[4]**.

3.6.1. SCREEN: 69156-3.5.1.mp4.mp4.

3.6.2. Talent applying coupling gel evenly to the cap surface.

3.6.3. Talent guiding the patient to look temporally or inferotemporally.

3.6.4. Close-up of the RCM approaching the conjunctival surface slowly.

3.7. With the right hand, adjust the RCM position for an optimal field of view **[1]**. With the left hand, rotate the focus knob to locate goblet cells in the outermost conjunctival layer **[2]**.

3.7.1. Talent using the right hand to finely position the RCM over the ocular surface.

3.7.2. Talent focusing and locating the goblet cells.

3.8. Remove the eyelid speculum carefully after the imaging procedure is complete **[1]**.

3.8.1. Talent gently releasing and removing the eyelid speculum from the patient's eye.

3.9. Reset the device by releasing and elevating the forehead rest **[1]**, zeroing the focus knob **[2]**, and reapplying coupling gel to the RCM surface **[3]**.

3.9.1. Talent releasing and lifting the forehead rest into its original position.

3.9.2. Shot of zeroing the focus knob.

3.9.3. Talent applying a fresh layer of coupling gel to the RCM.

3.10. For meibomian gland and eyelash follicles evaluation, instruct the patient to look downward **[1]**. Using one hand, gently elevate and press the upper eyelid skin inward to align the lid margin parallel to the corneal cap plane **[2]**. Move the RCM horizontally to capture multiple images of similar structural features **[3]**, and vertically from bottom to top to transition from meibomian gland orifices to gland acini and eyelash roots **[4]**.

3.10.1. Talent guiding the patient's downward gaze.

3.10.2. Close-up of the examiner lifting and pressing the upper eyelid skin for proper alignment.

3.10.3. SCREEN: 69156-3.10.3.mp4.mp4. 00:04-00:20

3.10.4. SCREEN: 69156-3.10.4.mp4.mp4 00:05-00:25.

3.11. Perform post-procedure hand hygiene by disinfecting hands thoroughly **[1]**. Gently wipe away residual coupling gel, tears, or debris from the ocular surface with a medical cotton swab **[2]**. Instill antibiotic eye drops to the patient to prevent infection **[3]**.

3.11.1. Talent disinfecting hands using alcohol-based gel.

3.11.2. Talent wiping the patient's ocular surface gently with a cotton swab.

3.11.3. Talent instilling antibiotic drops into the patient's eye.

3.12. Finally, document and report findings by including at least one image per examined tissue structure **[1]**. Arrange all selected images in the order of examination sequence and depth value, followed by detailed descriptions and quantitative measurements **[2]**.

3.12.1. SCREEN: 69156-3.12.1.mp4.mp4 00:05-00:20.

3.12.2. SCREEN: 69156-3.12.3.mp4.mp4. 00:48-01:00

# Results

## 4. Results

4.1. When the central optical zone achieved perfect contact with the target tissue, clear and complete images of ocular structures were obtained **[1]**.

4.1.1. LAB MEDIA: Figures 9–17. *Video editor: Show these figures sequentially.*

4.2. Corneal epithelial cell pathology in dry eye disease exhibited hyper-reflective borders and altered cell morphology **[1]**.

4.2.1. LAB MEDIA: Figure 12A.

4.3. Sub-basal nerve fiber degeneration in dry eye disease showed terminal swellings and beading **[1]**, and axonal tortuosity with reduced density **[2]**.

4.3.1. LAB MEDIA: Figure 12B. Video editor: Zoom in on the bright round bead-like structures along the nerve lines.

4.3.2. LAB MEDIA: Figure 12C. Video editor: Highlight the irregularly curved fibers with sparse distribution.

4.4. Conjunctival goblet cell density was markedly reduced to around 25 cells per square millimeter, showing only four goblet cells per field **[1]**.

4.4.1. LAB MEDIA: Figure 13A.

4.5. In other dry eye cases, goblet cells were absent, and conjunctival epithelium showed enhanced reflectivity **[1]**.

4.5.1. LAB MEDIA: Figure 13B and 13C.

4.6. Meibomian gland orifices in dry eye disease were dilated and completely obstructed **[1]**.

4.6.1. LAB MEDIA: Figure 14A.

4.7. Some orifices were occluded by multiple *Demodex brevis* mites, visible with characteristic tail projections **[1]**.

4.7.1. LAB MEDIA: Figure 14B.

4.8. Meibomian acini in dry eye disease were dilated, fused, and structurally disrupted **[1]**, with signs of acinar atrophy and periacinar fibrosis **[2]**.

4.8.1. LAB MEDIA: Figure 15A.

4.8.2. LAB MEDIA: Figure 15B.

4.9. Eyelash follicles infected with mites contained visible *Demodex* organisms and secretory debris **[1]**.

4.9.1. LAB MEDIA: Figure 16A–C.

4.10. Intact mites on the eyelid skin showed distinct mouthparts and legs **[1]**.

4.10.1. LAB MEDIA: Figure 16D. .

4.11. Inflammatory and immune cells were abnormally distributed in ocular tissues in dry eye disease **[1]**.

4.11.1. LAB MEDIA: Figure 17A–I.

- **in vivo**

Pronunciation link: https://www.merriam-webster.com/dictionary/in+vivo
IPA: /ˌɪn ˈviˑvoʊ/
Phonetic Spelling: in- VEE-voh

- **confocal** *(as in "confocal microscopy") *

Pronunciation link: https://www.merriam-webster.com/dictionary/confocal
IPA: /kənˈfoʊˑkəl/
Phonetic Spelling: kuhn-FOH-kul

- **microscopy**

Pronunciation link: https://www.merriam-webster.com/dictionary/microscopy
IPA: /maɪˈkrɒpˑskəˑpi/ or /maɪˈkrɑːpˑskəˑpi/ (Am. often /maɪˈkrɑːpˑskəˑpi/)
Phonetic Spelling: my-KROP-skuh-pee

- **ophthalmic** *(as in "ophthalmic topical anesthetic")*

Pronunciation link: https://www.merriam-webster.com/dictionary/ophthalmic
IPA: /ˌɒfˑθælˈmɪk/ or /ˌɑfˑθælˈmɪk/ (Am. often /ˌɑf-THAL-mik/)
Phonetic Spelling: af-THAL-mik

- **topical**

Pronunciation link: https://www.merriam-webster.com/dictionary/topical
IPA: /ˈtɒp·ɪ·kəl/ or /ˈtɑːp·ɪ·kəl/ (Am. often /ˈTAA-pi-kul/)
Phonetic Spelling: TOP-i-kul

- **anesthetic**

Pronunciation link: https://www.merriam-webster.com/dictionary/anesthetic
IPA: /ˌæn·əsˈθɛt·ɪk/
Phonetic Spelling: an-es-THET-ik

- **coupling** *(as in "coupling gel")*

Pronunciation link: https://www.merriam-webster.com/dictionary/coupling
IPA: /ˈkʌp·lɪŋ/
Phonetic Spelling: KUP-ling

- **module** *(as in "cornea module / Rostock cornea module")*

Pronunciation link: https://www.merriam-webster.com/dictionary/module
IPA: /ˈmɒd·ʒuːl/ or /ˈmɑːd·ʒu·l/ (Am. often /ˈMAH-jool/)
Phonetic Spelling: MAH-jool

- **laser**

Pronunciation link: https://www.merriam-webster.com/dictionary/laser
IPA: /ˈleɪ·zɚ/
Phonetic Spelling: LAY-zer

- **optical** *(as in "optical axis")*

Pronunciation link: https://www.merriam-webster.com/dictionary/optical
IPA: /ˈɑp·tɪ·kəl/ or /ˈɒp·tɪ·kəl/ (Am. often /ˈOP-ti-kul/)
Phonetic Spelling: OP-ti-kul

- **calibration**

Pronunciation link: https://www.merriam-webster.com/dictionary/calibration
IPA: /ˌkæl·əˈbreɪ·ʃən/
Phonetic Spelling: kal-uh-BRAY-shuhn

- **speculum** *(as in "eyelid speculum")*

Pronunciation link: https://www.merriam-webster.com/dictionary/speculum
IPA: /spəˈkju·ləm/
Phonetic Spelling: spuh-KYOO-luhm

- **macroscopic**

Pronunciation link: https://www.merriam-webster.com/dictionary/macroscopic
IPA: /ˌmæk·rəˈskɒp·ɪk/ or /ˌmæk·rəˈskɑːp·ɪk/ (Am. often /ˌMAK-ruh-SKAHP-ik/)
Phonetic Spelling: MAK-ruh-SKOP-ik

- **epithelial** *(as in corneal epithelial cells, subepithelial inflammatory cells)*

Pronunciation link: https://www.merriam-webster.com/dictionary/epithelial
IPA: /ˌɛp·əˈθi·li·əl/
Phonetic Spelling: ep-uh-THI-lee-uhl

- **subbasal** *(as in "subbasal nerve fibers")*

Pronunciation link: No confirmed link found on Merriam-Webster for "subbasal".
IPA (approximate): /sʌbˈbeɪ·zəl/
Phonetic Spelling: sub-BAY-zuhl

- **conjunctival** *(as in "conjunctival surface / conjunctival epithelium")*

Pronunciation link: https://www.merriam-webster.com/dictionary/conjunctival
IPA: /kənˈdʒʌŋk·tɪ·vəl/
Phonetic Spelling: kun-JUNK-ti-vuhl

- **goblet** *(as in "goblet cells")*

Pronunciation link: https://www.merriam-webster.com/dictionary/goblet
IPA: /ˈgɒb·lət/ or /ˈgɑːb·lət/ (Am. often /ˈGOB-lət/)
Phonetic Spelling: GOB-lət

- **meibomian** *(as in "meibomian gland/orifices") *

Pronunciation link: https://www.merriam-webster.com/dictionary/meibomian
IPA: /ˌmi·bəˈmi·ən/ or /maɪ·ˈboʊ·mi·ən/ (Am. often /my-BOH-mee-uhn/)
Phonetic Spelling: my-BOH-mee-uhn

- **orifice** *(as in "gland orifices") *

Pronunciation link: https://www.merriam-webster.com/dictionary/orifice
IPA: /ˈɔr·ə·fɪs/ or /ˈɔr·əˌfɪs/ (Am. often /ˈOR-uh-fis/)
Phonetic Spelling: OR-uh-fis

- **acini** *(plural of acinus — as in "gland acini") *

Pronunciation link: https://www.merriam-webster.com/dictionary/acini
IPA: /əˈsaɪ·naɪ/ or /əˈsi·naɪ/ (Am. often /uh-SYE-nye/)
Phonetic Spelling: uh-SYE-nye

- **fibrosis** *(as in "periacinar fibrosis") *

Pronunciation link: https://www.merriam-webster.com/dictionary/fibrosis
IPA: /faɪˈbroʊ·sɪs/
Phonetic Spelling: fy-BROH-sis

- **Debris**

Pronunciation link: https://www.merriam-webster.com/dictionary/debris
IPA: /dəˈbriː/
Phonetic Spelling: duh-BREE

- **shaving** *(as in "wiping ... swabs to disinfect" — "swab" is common, but "swabs" as plural)*

Pronunciation link: https://www.merriam-webster.com/dictionary/swab
IPA: /swɑb/ or /swɒb/ (Am. often /swab/)
Phonetic Spelling: SWAB

- **ethanol**

Pronunciation link: https://www.merriam-webster.com/dictionary/ethanol
IPA: /ˈɛθ·əˌnɑl/
Phonetic Spelling: ETH-uh-nol

- **software**

Pronunciation link: https://www.merriam-webster.com/dictionary/software
IPA: /ˈsɔftˌwɛr/ or /ˈsɒfˌtwɛr/ (Am. often /ˈSAWFT-wair/)
Phonetic Spelling: SAWFT-wair