Guideline

## Guide for Data Acquisition in Vivo Microscopy for Small Animal Slit Lamps (2025)

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## **ABSTRACT:**

The slit-lamp microscope is one of the most commonly utilized diagnostic tools in ophthalmology, widely employed to examine ocular structures such as the eyelid, conjunctiva, sclera, cornea, anterior chamber, iris, lens, and anterior vitreous body. Performing a slit-lamp examination on small animals requires a complex series of steps, which may be influenced by inter-operator variability, potentially compromising data comparability and the accuracy of results. Therefore, establishing standardized operational guidelines is essential for ensuring consistency and reliability. This standardized operational guide outlines clear procedures and criteria to maintain methodological uniformity across experimental trials, thereby improving data reliability, reproducibility, and cross-study validity.

This guide holds critical importance for facilitating rigorous scientific inquiry, standardizing data acquisition protocols, advancing disciplinary methodologies, and enhancing training efficacy in ophthalmic practice. It is anticipated to function as a foundational resource within ophthalmological research domains, establishing a robust methodological framework to ensure experimental reproducibility and drive innovation in subsequent investigations.

### THE BACKGROUND AND METHODS DEVELOPED

The eye constitutes a vital organ characterized by its compact spatial dimensions and delicate anatomical architecture within the human body. Most ocular pathologies evade detection through unaided visual inspection, necessitating specialized optical instrumentation such as biomicroscopes for diagnostic confirmation. Contemporary classification of ocular surface disease assessment systems categorizes diagnostic imaging modalities into two distinct paradigms: corneal contact and non-contact methodologies, each with specific clinical applications and resolution capabilities (Shao et al., 2023). The slit-lamp biomicroscope (SLB) constitutes a critical non-contact diagnostic modality in ophthalmic practice, representing the most widely utilized instrumentation for anterior segment evaluation. Its illumination system projects a variable-width optical section onto ocular structures, enabling detailed visualization of corneal and lenticular interfaces. Through binocular observation, clinicians systematically assess pathologies involving the eyelid, conjunctiva, sclera, cornea, anterior chamber, iris, lens, and anterior vitreous. This technique permits precise quantification of lesion dimensions - including location, size, and depth - while characterizing pathological features of diverse ocular surface disorders such as blepharitis, entropion, pterygium, conjunctival neoplasms, corneal ulcers, and keratitis (Chandrakanth et al., 2022; Perkins, 1988; Qi, 2013).

Slit-lamp biomicroscopy demonstrates superior diagnostic capabilities in the clinical assessment of ocular surface pathologies. Conventional diagnostic approaches lack the resolution required for comprehensive evaluation of conjunctival microvascular networks. In contrast. functional slit-lamp biomicroscopy (FSLB) enables precise quantification of morphological and hemodynamic parameters within the ocular microcirculatory system. This modality proves clinically practical due to its operational simplicity, reproducibility across operators, and cost-effectiveness compared to advanced imaging technologies (Chen et al., 2018). The research demonstrates that during implantable collamer lens (ICL, ICL V4c) implantation, measurements obtained via the digital slit-lamp imaging system and the digital caliper are interchangeable. Furthermore, the digital slit-lamp imaging system offers a safer and more streamlined measurement protocol while exhibiting superior repeatability and reliability compared to conventional methods (Clover, 2018).

The integrative application of laser scanning confocal microscopy (LSCM) and slit-lamp biomicroscopy demonstrates superior diagnostic performance compared to fungal culture for filamentous fungal-induced infectious keratitis. This multimodal approach not only enables preliminary identification of fungal morphotypes but also delivers targeted therapeutic guidance for antifungal agent selection. Furthermore, it facilitates longitudinal monitoring of treatment efficacy through serial slit-lamp examinations, allowing clinicians to optimize therapeutic

regimens in real time based on dynamic pathological changes (Burrow, Gurnani, & Patel, 2025; Toumasis, Tsantes, Tsiogka, Samonis, & Vrioni, 2024).

The diagnostic consistency of slit-lamp biomicroscopy remains significantly influenced by operator expertise, frequently resulting in substantial inter-operator variability even when examining identical subjects under standardized conditions. To address these limitations, the Chinese Medical Education Association's Ophthalmic Imaging and Intelligent Medical Branch, in collaboration with the Translational International Society for Medicine's Committees on Intelligent Medicine and Ophthalmology, convened a multidisciplinary task force to develop the Small Animal Slit Lamp Living Microscope Data Acquisition Operation Guide (2024). This initiative systematically analyzed procedural protocols and quality control measures for small animal anterior segment imaging through hybrid consensus-building sessions involving refractive surgery specialists and ophthalmic imaging experts. The task force developed an initial draft through iterative Delphi rounds, incorporating peer reviews via digital platforms. Following multiple revisions informed by clinical validation studies, the finalized guidelines were established.

This guide delineates standardized imaging protocols for corneal and lenticular assessment while emphasizing their translational implications for preclinical research. Implementation of these guidelines facilitates harmonized data acquisition across institutions, enhances dataset interoperability for meta-analyses, and enables evidence-based clinical decision-making in surgical planning, therapeutic monitoring, and prognostic evaluation.

### **FOREWORD**

The slit-lamp biomicroscope (SLB) constitutes an indispensable optical imaging system in clinical ophthalmology and biomedical research. Primarily employed to non-invasively visualize ocular structures, this modality enables high-resolution assessment of anterior segment anatomy and pathological alterations (Bernard et al., 1981; Qi, 2013). Its operational principle involves projecting a focused light beam through an adjustable slit

aperture, coupled with a stereoscopic magnification system to detect micron-level structural changes.

As a cornerstone instrument in ocular diagnostics, the SLB facilitates comprehensive preoperative evaluations, therapeutic monitoring, and disease management (Kaur & Gurnani, 2024). Clinicians utilize this modality to detect diverse pathologies through meticulous evaluation of anatomical deviations, including corneal dystrophies

(Wirbelauer et al., 2002), cataracts(van den Berg, 2018), iridociliary abnormalities (Zhao, Zhang, Han, & Zhang, 2021) and so on. In addition, the slit lamp proves particularly valuable for detecting and characterizing anterior segment trauma, intraocular foreign bodies, and infectious infiltrates (Ahmed, House, & Feldman, 2015; Sampaio et al., 2022).

In preclinical ophthalmic research, the small animal slit-lamp biomicroscope serves as a critical investigative platform, enabling researchers to diagnose experimental ocular pathologies, quantify postoperative therapeutic efficacy, and systematically characterize traumatic corneal injuries. The system's stereoscopic magnification capability achieves micron-level resolution of anterior segment microanatomy, facilitating detailed analysis of structural biomarkers. This high-fidelity visualization supports the development of hypothesis-driven experimental frameworks, enhances reproducibility in translational studies, and provides quantitative benchmarks for comparative interventional analyses.

#### SMALL ANIMAL SLIT-LAMP INTRAVITAL MICROSCOPY

#### **Principle of operation**

Slit-lamp biomicroscopy is mainly composed of a slit-lamp illumination system and a binocular microscope. Its working principle is to use a concentrated light(Sadagopan & Periasamy, 2021; X. M. Zhang, Shen, Shen, Zhang, & Wang, 2002). The slit-lamp biomicroscope emits light with collimated beam geometry, enabling precise optical sectioning through ocular tissues. As this structured illumination traverses refractive-media interfaces, it selectively illuminates discrete anatomical planes while maintaining adjacent structures in optical darkness. This

spatial selectivity generates high-contrast optical sections that delineate micron-level structural details against unilluminated adjacent regions. Transparent ocular components-including the cornea, lens, and vitreousexhibit distinct light-scattering properties under physiological versus pathological states. By systematically analyzing these differential light interactions through stereoscopic observation, researchers can characterize tissue biomechanical properties at cellular resolution, facilitating non-invasive detection of subclinical pathologies in preclinical models and enabling longitudinal monitoring of disease progression (Elsner & Muller, 2008).

#### Standard operation procedure



Prior to initiating data acquisition, researchers must validate environmental parameter stability (e.g., ambient illumination, humidity, and temperature) and rigorously adhere to ethical animal handling protocols (Cordero, 2010). The standardized protocols comprise the following core procedural phases, as exemplified by OPTOPROBE: (1) Preparation: Confirm that the slit-lamp biomicroscope is properly connected to a grounded power source and initiate system calibration. Verify that the light source intensity and ergonomic control interface operate within manufacturer-specified parameters. Perform pre-procedural operational stability validation to minimize motion artifacts during prolonged examinations.

(2) Anesthetic administration: Select speciesand weight-appropriate protocols to ensure humane immobilization. For C57BL/6 mice, administer tribromoethanol (1.25% w/v) via intraperitoneal injection at a standardized dosage of 0.2 mL/10 g body weight(Lee et al., 2018; Meyer & Fish, 2005). All procedures must be conducted under the supervision of certified personnel trained in rodent handling to minimize iatrogenic complications and ensure compliance with institutional animal care protocols.

(3) Illumination calibration: Activate the slit-lamp biomicroscope's illumination system and utilize the calibrated intensity dial with beam angulation controls to achieve optimal coaxial illumination alignment. For enhanced detection of epithelial defects or fluorescein staining patterns, employ blue excitation filters to optimize visualization of corneal microstructures and pathological changes.

(4) Focal calibration: Position the subject securely on the stabilized platform. Utilize ergonomic positioning controls to align the slit-lamp biomicroscope's optical axis orthogonally to the corneal apex, iteratively adjusting the focal length to achieve parfocal alignment. This ensures micron-level resolution of anterior segment microstructures through optimal optical sectioning, enabling precise visualization.

(5) Use of slit lamps: Slit-lamp biomicroscopes feature

dynamically adjustable slit apertures to modulate beam geometry. By iteratively adjusting the slit angle, researchers can systematically interrogate ocular microstructures. This technique enhances depth-resolved visualization, facilitating quantitative analysis of pathological changes.

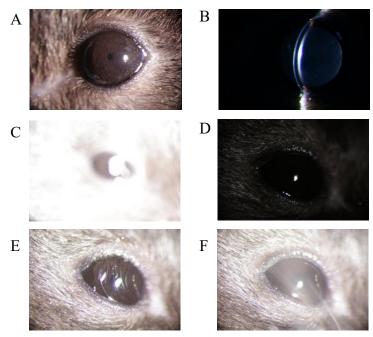
(6) Observation: The slit-lamp biomicroscope enables precise control of slit dimensions through calibrated adjustment knobs. Rotating the width selector (range: 0.1–8.0 mm) and light-bar handwheel allows continuous modulation of slit geometry. This dynamic adjustability facilitates differential diagnosis of pathologies such as stromal edema, synechiae, or inflammatory infiltrates.

(7) Record the results: press the button on the rocker to shoot, record and save the results.

(8) Cleaning and disinfection: Following experimental procedures, thoroughly irrigate ocular surfaces with sterile normal saline (0.9% NaCl) to remove residual debris. Apply levofloxacin ophthalmic drops bilaterally to mitigate infection risks, adhering to aseptic technique. Clean the instrument surface with soft cloth, but avoid the use of corrosive cleaners.

The slit lamp images with standard quality have clear focus, moderate brightness, no eyelashes or eyelid occlusion, as shown in Figs.1A and 1B.

Comparison of the anesthetic effects of three anesthetic drugs on mice



#### Fig.1.Slit lamp photos of C57BL / 6 mice

A, B: Standard slit lamp photos of C57BL / 6 mice. A is a plane map and B is a fissure map. C: non-standard images caused by excessive exposure; D: non-standard images caused by too weak background light intensity; E: non-standard images caused by occlusion; F: non-standard images caused by beard occlusion.

#### Pictures under the slit lamp with irregular quality:

(1) Too much exposure (Fig.1C).

Solution: manually adjust the light intensity knob to reduce the light intensity;

(2) Weak background light (Fig.1D).

Solution: manually improve the background light intensity;

## Slit lamp data acquisition operation specification for eye diseases:

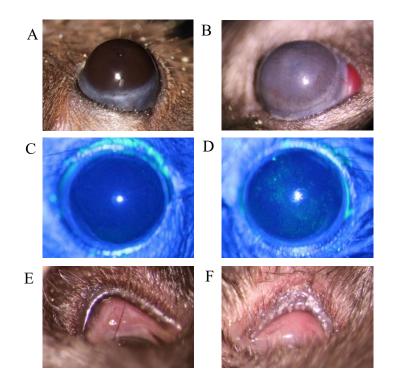
(1) When photographing corneal neovascularization (Fig.2A,2B), fully expose the conjunctival and corneal areas. Tilt the eyeball about  $30^{\circ}-60^{\circ}$  to ensure that the light reflection point on the cornea does not affect the imaging

(3) Eyelash occlusion (Fig.1E).Solution: use a wet cotton swab or a cotton swab dipped in a gel to gently remove or sort the eyelashes;

(4) Focal blurring caused by beard occlusion (Fig.1F).

Solution: use a wet cotton swab or use a cotton swab dipped in a gel to remove the beard.

of neovascularization(Huang et al., 2022; X. Wang, Tang, Zhang, Li, & Chen, 2020). The imaging protocol centers on the neovascularization interface, ensuring high-resolution visualization of pathophysiological features such as angiogenic trajectory, spatial propagation dynamics, and quantitative metrics including vascular density indices.



#### Fig.2. Slit lamp images of different eye diseases in C57 mice

A: Corneal side images of normal mice; B: picture of corneal neovascularization; C: picture of normal mouse corneal fluorescein sodium staining; D: picture of corneal fluorescein sodium staining of dry eye mouse model; E: picture of meibomian gland in normal mice; F: Picture of mouse meibomian gland opening blockage.

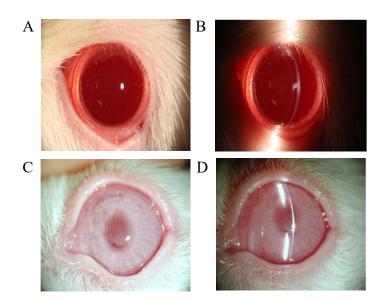
(2) Dry eye fluorescein sodium staining (Fig.2C,2D). Gently retract the murine eyelids to expose the cornea, and administer 1-2µL of 1% sodium fluorescein (NaFl) into the inferior conjunctival fornix using a calibrated micropipette. Return the mouse eyeball to natural position to ensure that the cornea is in full contact with fluorescein sodium for 10-15 seconds. Re-expose the ocular surface and irrigate with 10 mL phosphate-buffered saline (PBS, pH 7.4) at 5-10 mL/min to eliminate residual NaFl and debris. Wick excess fluid from the medial/lateral canthi using sterile absorbent surgical spears, then observe using a blue filter. In dry eye mice, punctate or flaky fluorescence will appear at the corneal injury site, while it does not appear in the normal group(Y. C. Wang et al., 2016; X. Zhang et al., 2017). Experimental conditions between dry eye models and control cohorts require strict standardization to ensure reproducibility, such as the amount of fluorescein sodium, the time of contact between the cornea and fluorescein sodium, the degree of PBS buffer flushing (including flushing speed, flushing fluid volume, flushing site, etc.) and the unity of the experimental operator to ensure the accuracy of the experimental results.

(3) Meibomian gland obstruction (Fig.2E, 2F). Using sterile microsurgical forceps, apply controlled vertical traction to evert the murine eyelid while maintaining globe position via atraumatic technique. This prevents corneal compression and preserves optical clarity for slit-lamp biomicroscopy (Bu et al., 2019). The picture should focus on the opening of the meibomian gland duct, and blocked or enlarged duct openings can be observed. At the same time, the eyelid conjunctiva can also be observed.

#### SLIT-LAMP PHOTOGRAPHS OF OTHER EXPERIMENTAL ANIMALS

Using the OPTOPROBE slit lamp microscope, you can also collect images of SD rats and New Zealand white

rabbits (Figure 3).



**Figure 3. Slit-lamp photographs of other experimental animals** A: front view of SD rat taken by slit lamp; B: slit-lamp view of SD rat; C: front slit lamp shot of New Zealand white rabbit; D: slit-lamp diagram of a New Zealand white rabbit.

#### **RESEARCH APPLICATION**

The slit-lamp biomicroscope (SLB) is a diagnostic imaging system that non-invasively visualizes anterior segment microstructures, including the cornea, iris, lens, and conjunctiva. As a cornerstone modality in ophthalmic practice, it facilitates clinical diagnosis and longitudinal monitoring of anterior segment pathologies such as corneal dystrophies, uveitis, and glaucoma(Kaur & Gurnani, 2024). This system enables systematic evaluation of ocular integrity, differential diagnosis of inflammatory/infectious conditions, optimization of therapeutic strategies, and longitudinal tracking of pathological trajectories (Martin, 2018; Yuan et al., 2015). The slit lamp is widely used in ophthalmology. It can be used for anterior chamber and corneal examination(Koyama et al., 2021; Pathak Ray, Ramesh, & Rathi, 2021; Ursea & Silverman, 2010), iris and lens assessment (Cook & Koretz, 1991; He et al., 2007), conjunctival examination (Oellers et al., 2014; Veres, Tapasztó, Kosina-Hagyó, Somfai, & Németh, 2011), ocular trauma assessment (Babineau & Sanchez, 2008; Ghasemi et al., 2009) and so on.

By providing high-resolution stereoscopic documentation, the SLB serves as an indispensable tool for evidence-based management of ocular diseases. It enables clinicians to detect subclinical pathologies, refine treatment protocols through serial comparisons, and establish baseline datasets for multicenter research studies.

#### **CONCLUSION AND PROSPECT**

The implementation of standardized operating protocols for slit-lamp biomicroscopy holds critical importance for enhancing diagnostic accuracy in ophthalmic practice and mitigating diagnostic discrepancies. These guidelines enable clinicians to maximize the SLB's diagnostic potential while ensuring intra- and inter-observer reproducibility of imaging findings. Although contemporary clinical practice has successfully integrated standardized SLB protocols across diverse diagnostic applications-including glaucoma monitoring, corneal dystrophy classification, and anterior uveitis staging-persistent inter-operator variability in technical proficiency compromises procedural standardization, thereby impacting data reliability in multicenter studies.

This comprehensive standardized protocol for slit-lamp biomicroscopy in preclinical ophthalmic evaluations ensures reproducible acquisition of high-fidelity data. Through this operation guide, we have established a detailed, standard and standardized process to ensure that accurate and reliable data are collected through slit lamp in small animal eye tests. In the experimental preparation stage, we emphasize the importance of clear research objectives and reasonable experimental design to ensure the scientific nature of the experimental results. At the same time, we also emphasize the key to the device setup process to ensure accurate control of light, angle and position during data acquisition. In the process of animal operation, reasonable arrangement of adaptation stage can reduce the influence of environmental change on animal behavior, so as to obtain more reliable experimental data.

The protocol serves as a foundational tool for preclinical ocular pathophysiology studies, enabling expansion into mechanistic investigations of anterior segment pathobiology. Its implementation reduces inter-operator variability and enhances inter-laboratory reproducibility, meta-analyses harmonized while enabling across institutions. Future integration with adaptive optics, OCT systems, and machine learning-driven diagnostic pipelines promises to refine longitudinal data uniformity and automate pathological grading, bridging preclinical findings to clinical applications.

The establishment of standardized operating guidelines for preclinical slit-lamp biomicroscopy represents a pivotal advancement in ophthalmic research, ensuring reproducible data acquisition and harmonized methodologies across studies. These protocols not only enhance the diagnostic precision of SLB technology but also facilitate clinical translation by bridging preclinical findings to therapeutic development for conditions such as corneal dystrophies and glaucoma. Crucially, this initiative drives progress in three key domains: (1) enabling methodologically robust preclinical studies through quantitative imaging metrics, (2) fostering novel imaging modalities such as adaptive optics integration, and (3) informing evidence-based clinical decision-making via standardized pathological grading systems [38,39].

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consortiums in validating these protocols under diverse experimental conditions. Looking ahead, advancements in preclinical ocular imaging — particularly AI-integrated diagnostic frameworks and molecular contrast agents—are anticipated to redefine longitudinal disease modeling and personalized therapeutic screening in ophthalmology.

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|                | University   | Hai-Jun Yang   | Nanchang Bright Eye Hospital                               |
| Wei-Jie Ouyang | The Affiliated Hospital of Guizhou Medical   | Qi-Chen Yang   | West China Hospital of Sichuan University                  |
|                | University   | Shu Yang       | The First People's Hospital of Kunming                     |
| Kun-Liang Qiu  | Joint Shantou International Eye Center of  | Yi-Ran Yang    | Henan Eye Hospital   |
|                | Shantou University and the Chinese University                                      | Yu-Li Yang     | The Southwest Hospital of Army Medical                     |
|                | of Hong Kong   |                | University   |
| Wei-Qiang Qiu  | Peking University Third Hospital   | Yi-Feng Yu     | The Second Affiliated Hospital of Nanchang                 |
| Sheng-Wei Ren  | Henan Eye Hospital   |                | University   |
| Yi-Lei Shao    | Eye Hospital of Wenzhou Medical University   | Qing Yuan      | Jiujiang First People's Hospital                           |
| Ting Su        | Renmin Hospital of Wuhan University  | Yan-Mei Zeng   | The First Affiliated Hospital of Nanchang                  |
| Lei Tang       | The Third People's Hospital of Yibin   |                | University   |
| Li-Ying Tang   | Zhongshan Hospital, Xiamen University  | Qing Zhang     | The Second Hospital of Anhui Medical                       |
| Li-Yang Tong   | Wenzhou Medical University Ningbo Eye  |                | University   |
|                | Hospital   | Yu-Qing Zhang  | The Second Affiliated Hospital of Chongqing                |
| He Wang        | The Affiliated Hospital of Xuzhou Medical  |                | Medical University   |
|                | University   | Yu-Jie Zhang   | Xiamen Eye Center of Xiamen University                     |
| Shen Wang      | The First Affiliated Hospital of Xinxiang  | Zhen Zhang     | The First Affiliated Hospital of Xiamen                    |
|                | Medical University   |                | University   |
| Shao-Pan Wang  | Institute of Artificial Intelligence, Xiamen                                       | Zhen-Hao Zhang | Shanghai University of Medicine and Health                 |
|                | University   |                | Sciences Affiliated Zhoupu Hospital                        |
| Shu-Rong Wang  | The Third Bethuen Hospital of Jilin University                                     | Jing Zhong     | Zhongshan Ophthalmic Center, Sun Yat-sen                   |
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|                | University   | Pei-Wen Zhu    | Eye and ENT Hospital of Fudan University                   |
| Xue-Lin Wang   | The First Affiliated Hospital of Jiangxi Medical                                   | Zhuo-Ting Zhu  | Eye Center of the University of Melbourne                  |
|                | College  | Jie Zou        | The First Affiliated Hospital of Nanchang                  |
| Hong Wei       | The First Affiliated Hospital of Nanchang  |                | University   |
|                | University   |                |  |
|                |  |                |  |

### **CONFLICT OF INTEREST**

All authors declare that there is no conflict of interest. The formulation of this consensus does not accept the

### **STATEMENT OF CONSENSUS**

All experts who participated in the formulation of this consensus stated that they adhered to an objective position, based on professional knowledge, research data and clinical experience. After full discussion, all experts agreed to form this consensus. This consensus was drafted by

#### **DISCLAIMER OF LIABILITY**

The contents of this consensus only represent the guidance opinions of the experts involved in the development of this consensus for the reference of ophthalmic medical workers. Despite extensive consultation and discussion among experts, there are still some incomprehension. The advice provided in this consensus is not mandatory, and the practice inconsistent with this consensus does not mean

### **DISSEMINATION AND IMPLEMENTATION**

After the release of the consensus, the following methods will be used for dissemination, implementation and evaluation: (1) The full text of the consensus will be published in Chinese Journal of Experimental Ophthalmology, including the specific methods and steps of developing the consensus and the members and division of labor of the consensus working group. (2) To preach the consensus in national academic conferences, interpret the consensus and provide relevant training for ophthalmic medical workers, technicians and graduate students engaged in ophthalmic laboratory work; (3) In some

#### REFERENCE

[1] Shao Y, Jie Y, Liu ZG, Expert Workgroup of Guidelines for the application of artificial intelligence in the diagnosis of anterior segment d, sponsorship of any enterprise.

some experts of the Ophthalmic Imaging and Intelligent Medical Branch of the Chinese Medical Education Association and the Ophthalmic Special Committee of the World Society of Translational Medicine.

that it is wrong or improper. There are still many problems to be explored in clinical practice, and ongoing and future clinical diagnosis and treatment will provide further evidence. With the accumulation of clinical experience and the emergence of treatment methods, this consensus needs to be revised and updated regularly in the future to bring more clinical benefits to the examined patients.

provinces (cities) of China, we plan to organize promotional meetings on the contents of the consensus to promote the comprehensive and accurate application of the consensus by clinical ophthalmic medical workers, technicians and graduate students. (4) To promote the contents of this consensus through online multimedia; (5) Relevant studies should be carried out regularly in the next two years to evaluate the current situation in China, and to further understand the dissemination and application value of this consensus and its role in clinical decision-making after implementation.

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