



Technical Brief

A goniolens for clinical monitoring of the mouse iridocorneal angle and optic nerve

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Purpose: Due to the increasing importance of mouse models and mouse genetics in ophthalmic research, we have developed a goniolens for mice.

Methods: The size and basic design parameters of a human goniolens were adapted to the mouse eye.

Results: The goniolens is straightforward to use and allows non-invasive visualization of the structures of the anterior chamber angle, including Schlemm's canal, trabecular meshwork, iris and anterior surface of the peripheral ciliary body. Goniophotography using the mouse lens allows documentation of anterior chamber angle abnormalities, and facilitates comparisons of changes to these structures in the same eye over time. In addition, high quality magnified views of the optic nerve, retinal vessels and posterior retina are easily obtained.

Conclusions: This goniolens is an important new tool for studying the genetic and clinical etiology of anterior segment dysgenesis and glaucoma in mice.

Due to the established genetic tools and resources of mouse genetics, mouse models are assuming increasing importance in biomedical research [1-4]. The search for mouse models of ocular diseases has been productive, and experimental studies in mice are proving useful in understanding disease mechanisms [3,5-11].

The development and adult structure of the eye are similar in rodents and primates [12-19]. The relatively small size of the mouse eye makes its clinical examination more challenging than for larger animals. However, useful methods to meet these needs exist [10,18,20-22] (reviewed in Smith [23]). From a clinical standpoint, it is now possible to perform high quality slit lamp photography, fundus photography, fluorescein angiography, intraocular pressure measurement, electroretinography, and ophthalmic ultrasound in mice. Anterior chamber angle abnormalities, including trabecular meshwork hypoplasia, anterior synechiae and accumulation of pigmented macrophages occur in mouse models with mutations in orthologs of human glaucoma associated genes and contribute to elevated intraocular pressure (IOP) and glaucoma in mice and humans [10,24,25]. Routine slit lamp biomicroscopy cannot demonstrate these abnormalities because the angle is hidden from direct observation due to internal reflection. This problem is overcome in human eyes by utilizing specialized corneal contact lenses with angled mirrors that reveal structural details of the anterior chamber angle [26]. These goni-

scopic lenses permit a magnified view of the anterior iris surface, trabecular meshwork, and peripheral internal surface of the cornea. A mouse goniolens would be an important tool for finding and studying mouse models of anterior segment dysgenesis and glaucoma. To date, the only method to assess the severity and extent of mouse angle lesions is histologic processing of eyes and subsequent evaluation of sections. This is a useful approach but is costly and time-consuming due to the need to evaluate serial sections from different ocular locations [24,27]. Additionally, the magnitude of work required to analyze the entire angle is prohibitive and it is not possible to analyze the same eye at different stages of disease.

We report here the development of a mouse goniolens and provide photo-documentation of its utility for non-invasive clinical analysis of mouse eyes. This mouse goniolens will permit new avenues for experimental investigation that include: 1. Clinical assessment of the severity and extent of angle abnormalities that associate with high IOP, 2. Clinical evaluation of the angle in high-throughput mutagenesis screens [28-32] or in mice with known mutations in different genes, 3. Evaluation of progressive angle changes in the same glaucomatous animal over time. It also permits documentation of the optic nerve without a fundus camera.

METHODS

Animal care guidelines comparable to those established by the Institute for Laboratory Animal Research (Guide for the Care and Use of Laboratory Animals) were observed during this study.

Basic goniolens parameters: The most common goniolens configuration includes a corneal contact lens with attached

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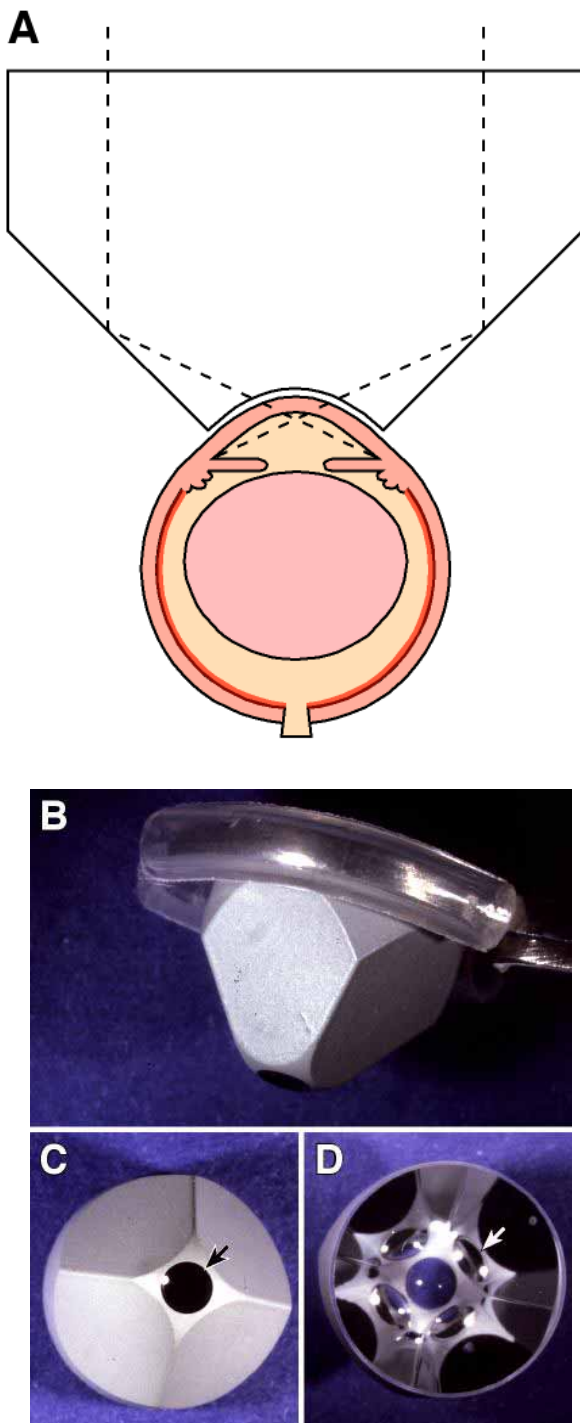


Figure 1. Design of goniolens. **A:** Schematic of mouse goniolens on an eye. The observer views the eye through the plano surface of the lens. The dotted lines trace the image path. The angled surfaces of the lens direct light into the anterior chamber angle. The corneal surface of the lens is directly tangent to the cornea and separated from it only by the tear film. **B:** Goniolens held in forceps that are modified by covering the gripping surface with polyethylene tubing. **C:** Corneal surface of goniolens (arrow). All other portions of the goniolens seen from this surface are silvered. **D:** Plano surface of goniolens. The four mirrors of the lens are visible as darker semicircles adjacent to the corneal contact lens (white arrow points to one). The corneal contact lens is the central area that appears blue because of the background.

mirrors set at an angle. The configuration of contact lens and mirrors directs light into the anterior chamber angle and allows viewing of the angle in the mirrors (Figure 1). The corneal contact surface of the lens should conform closely to the curvature of the cornea for maximum image clarity. This curvature creates a lens power that varies with the radius of curvature of the lens. The diameter of the corneal contact surface should be no greater than the corneal diameter. The external surface of the angled portion of the lens is silvered to produce a high-quality mirror surface (Figure 1). A plano (no optical power) polished front surface of the goniolens allows a full view of the mirrored surfaces (Figure 1). The region just below the plano surface where the sides of the lens are parallel to each other provides a place for attachment of a handle for lens manipulation during clinical examination (Figure 1).

Corneal curvature and lens specifics: The radius of corneal curvature of C57BL/6J mouse eyes was initially estimated by measuring glutaraldehyde/paraformaldehyde fixed, plas-

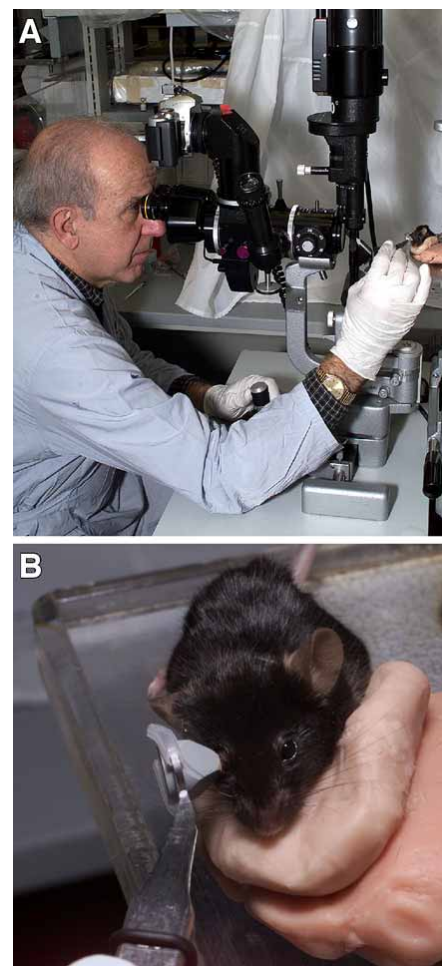


Figure 2. Use of goniolens. **A:** Gonioscopy on an anesthetized mouse. The operator's hand rests for stability on the modified slit lamp platform. **B:** Close-up view shows the goniolens in contact with the cornea. The mouse is supported and positioned on a moldable platform to avoid movement. Stainless steel holders [18] keep the mouse in position (not visible).

tic-embedded sections. This suggested a radius of curvature of approximately 3.00 mm. An initial series of trial contact lenses 4.5 mm in diameter were obtained. The base curves of the available lenses were 3.66, 4.00, and 4.50 mm. After topical proparacaine anesthesia and instillation of sodium fluorescein, a lens was placed on the eye and the fit of the lens evaluated, as it would be in a human contact lens patient. All of the initial lenses were much flatter than the curvature of the mouse cornea. This was evident by the presence of a large peripheral air pocket between the cornea and lenses, as well as central corneal touch of the trial lenses (contact of the center of the lenses with the central cornea). Based on these ob-

servations, the corneal curvature of mice was estimated to be between 2.00 and 2.5 mm. Using this information, a prototype goniolens was produced, tested, and found to correctly match the corneal curvature. Although a view of anterior chamber angle structures was obtained, the diameter of the corneal surface of the prototype (3.00 mm) was too large. Based on these observations we generated a final lens design (Figure 1). This goniolens was produced by Ocular Instruments (Bellevue, WA, USA). The diameter of the corneal surface of this lens is 2.00 mm and the radius of curvature of the corneal surface is 2.00 mm. The corneal contact lens has a power of approximately 78 diopters when in contact with goniosol-like material. The diameter of the plano surface is 9.0 mm. The mirrors are set at an angle of 45° to the corneal contact lens. This final lens is available from Ocular Instruments. It works

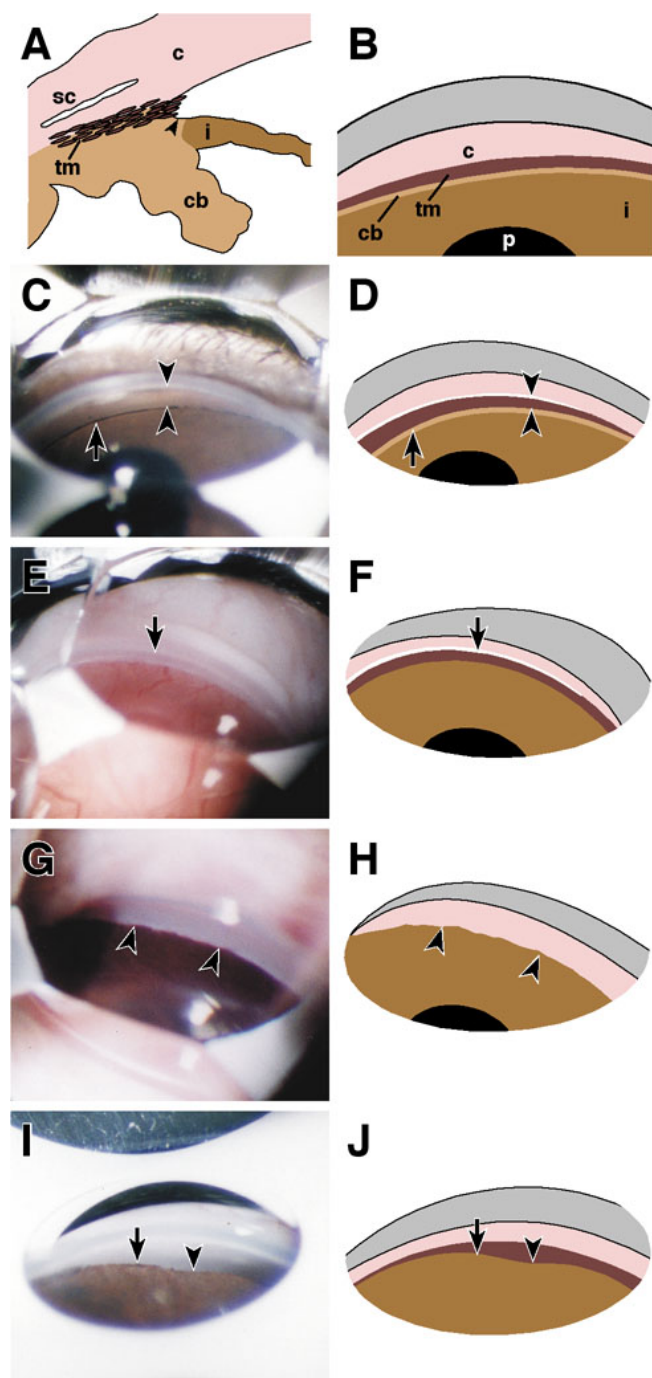


Figure 3. Goniophotographs of normal and abnormal angles. **A:** Schematic cross-section of a mouse eye showing the angle region. Indicated structures are the cornea (c), Schlemm's canal (sc), trabecular meshwork (tm), iris (i), and ciliary body (cb). The region immediately next to the iris root that is often referred to as the ciliary band is marked by the arrowhead. The same colors are used to designate these structures throughout this figure and are labeled only where needed for clarity. In the schematic gonioscopic views (**B, D, F, H, J**) Schlemm's canal is represented by a white band and the most peripheral cornea in pink. The upper gray area represents the out of focus image of more central corneal tissue and other surface structures that always appear at this location when performing gonioscopy on mice or humans. **B:** Schematic gonioscopic view of the normal mouse angle. A portion of the anterior surface of the ciliary body (cb, often referred to as the ciliary band) is sometimes apparent between the trabecular meshwork and iris, but is not always evident. Schlemm's canal is not represented in this schematic. The visibility of Schlemm's canal depends on contrast provided by pigmentation or by the presence of blood in the canal. Occasionally, a reflux of blood into Schlemm's canal also occurs during human gonioscopy [37]. "p" indicates the pupil. **C:** Normal adult C57BL/6J angle. The lightly pigmented trabecular meshwork is located between the arrowheads. The tip of the upper arrowhead touches Schlemm's canal, which appears white. Due to the presence of blood in a similar structure at the same location during some examinations, we interpret this structure to be Schlemm's canal and not Schwalbe's line. A small portion of the face of the ciliary body is evident (arrow). **D:** Schematic gonioscopic view corresponding to panel C. **E:** Adult albino C57BL/6J mouse (C57BL/6J^{Tyr^{-2/c-2J}}). Though not evident in the photograph blood was visible in Schlemm's canal. Schlemm's canal is indicated by the arrow. The TM is evident, but appears semitransparent due to lack of pigmentation. **F:** Schematic gonioscopic view corresponding to panel E. Schlemm's canal is indicated by the white line. The ciliary body is not seen because of the lack of pigment. The iris and trabecular meshwork are depicted with the same colors as those used for pigmented mice to keep the diagram analogous with the others. Panels **G-J** demonstrate anterior synechiae in 11 month old DBA/2J mice. The synechiae are of different severity as is reminiscent of the disease in this strain [10]. **G:** Pigmented broad anterior synechiae (arrowheads) conceal the trabecular meshwork and have a scalloped appearance. **H:** Schematic gonioscopic view corresponding to panel G. **I:** Anterior synechiae have progressed more centrally and obscured more of the trabecular meshwork on the left side of the view (arrow) than on the right (arrowhead). **J:** Schematic gonioscopic view corresponding to panel I.

well on different mouse strains and in glaucomatous DBA/2J mice.

Lens manipulation and care: The small size of the mouse goniolens necessitates the use of a handle for its manipulation. The specimen forceps (catalog number 76802) manufactured by Electron Microscopy Sciences (Fort Washington, PA, USA) work well for holding the goniolens. The forceps come with a small rubber O-ring that slides down near the curved blades, holding them in the closed position. We have added two short segments of polyethylene tubing to cover the curved blades, thereby improving the grip and decreasing the risk of damage to the silvered surfaces of the lens (Figure 1). After use the lens is rinsed with warm water, sprayed with 70% ethanol, and gently blotted with an absorbent wipe, taking care to avoid damage to the silvered surfaces.

Use of the lens: The lens is used in conjunction with a slit lamp, modified by replacing the chin rest with a platform [23]. The mouse is anesthetized (mix of 99 mg/kg ketamine and 9 mg/kg xylazine) and supported on a molded clay cradle in front of the slit lamp beam (Figure 2). A drop of balanced salt solution placed on the cornea helps remove any debris and keeps the cornea moist. The goniolens is gently placed in contact with the cornea. In most cases, broad beam illumination provides the best view. The lens is slowly moved until the angle structures become visible. A small amount of pressure on the goniolens is frequently helpful, as it causes slight chamber deepening and helps reveal the angle structures. Excessive pressure must be avoided to prevent distortion of the angle structures. More importantly, excessive pressure can collapse the orbital venous sinus, causing the globe to sink into the orbit and making examination impossible. Goniophotography is performed using 40x slit lamp magnification with an attached 35 mm camera. The goniolens can be used to view the optic nerve, retinal vessels, and posterior pole retina. To view these structures, the slit lamp and beam are focused directly through the corneal contact surface of the goniolens. In most cases, this can be done through an undilated pupil, although dilation with a drop of 1% atropine or cyclopentolate enlarges

the viewable area. Broad beam illumination is optimal for most of the retinal examination, but a medium narrow slit beam directed at the optic nerve is helpful in judging depth of the optic nerve cup.

Terminology: Previously, the aqueous collecting system of all lower mammals was believed to consist of a scleral venous plexus, with only primates having a true Schlemm's canal. It is now known that rats and mice have a single circular drainage structure that appears very similar to Schlemm's canal by both light and electron microscopy, and that its developmental origin and pattern is very similar in primates and mice. The rodent drainage canal also connects to collector channels and aqueous veins in the same way as the primate Schlemm's canal, responds to pressure changes in the same way as the primate canal and its development is affected by mutations in the same human and mouse genes [14,17,19,24,33-35]. For these reasons, and following other studies, we refer to the mouse aqueous collecting structure as Schlemm's canal.

RESULTS & DISCUSSION

We report development of a four mirror goniolens that fits the radius of curvature of the mouse corneal surface. The lens allows direct visualization and photography of the iridocorneal angle of the anterior chamber (Figure 3). Normal mice have an anterior chamber that is shallow compared to that of humans. For this reason, careful tilting of the lens is needed to visualize the angle structures. The situation is analogous to viewing these structures in a human eye with anatomically narrow angles.

In an adult C57BL/6J mouse (Figure 3C), the trabecular meshwork (TM) is lightly pigmented. The iris and trabecular meshwork are separated by a darker line that is a portion of the anterior face of the ciliary body. Because the ciliary body extends slightly more anteriorly in mice than in humans [36], its visualization is expected. In an 11 month old DBA/2J mouse (Figure 3G) with glaucoma, most of the angle structures are obscured by peripheral anterior synechiae (compare to Figure 3C). The structure of the TM is more difficult to observe in albino mice (Figure 3E) because of the lack of contrast. However, when filled with blood, Schlemm's canal is easily seen in albinos. Focal changes, such as developing synechiae (Figure 3I) are easily identified and could be observed over time to evaluate progression. Lower magnification (25x) is useful in evaluating anterior iris morphology. The iris is best viewed through the corneal contact surface, rather than the mirrors. When the lens is tilted properly, structural features of the iris surface such as focal swellings caused by collections of phagocytic cells around the pupil (10) can be demonstrated (Figure 4A).

In addition to viewing the angle structures, the goniolens provides an excellent view of the optic nerve and posterior pole retina. These structures are in sharp focus when viewed through the corneal contact surface of the goniolens (Figure 4B). This feature should be useful in clinical detection of subtle optic nerve and retinal changes and for following and documenting optic nerve cupping in mice with glaucoma. The

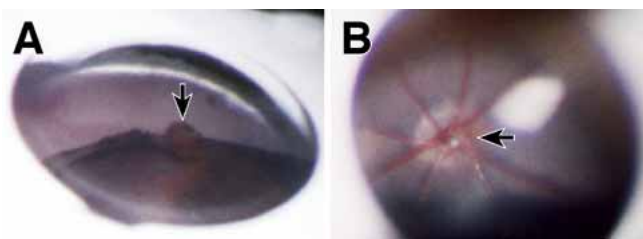


Figure 4. Additional uses of goniolens. **A:** DBA/2J mouse, 12 months of age. In a slightly oblique view through the center of the goniolens (corneal contact lens), an elevation of the iris surface due to a focal collection of pigmented cells (arrow) is present at the pupillary margin. **B:** The optic nerve, retinal vessels, and posterior pole retina of a normal two month old C57BL/6J mouse as seen through the center of the goniolens. The nerve is indicated by the arrow. The retinal vessels pass over the edge with a slight curvature that indicates the shallow physiological cup.

goniolen has several advantages over indirect ophthalmoscopy and fundus photography. The optic nerve magnification is greater than we have achieved by indirect ophthalmoscopy and fundus photography, and the binocular view of the slit lamp allows the depth of the optic nerve cup to be easily evaluated. Narrowing of the illumination from broad beam to a slit may be useful in evaluating subtle details of the optic nerve and retina.

In summary, due to the increasing importance of mouse models and mouse genetics in ophthalmic research, we have developed a goniolen sized for mice. The goniolen is straightforward to use and provides clear non-invasive views of the angle and optic nerve. It is easier to view the structures than it is to photograph them but with practice high quality images can be captured. Furthermore, the goniolen is inexpensive and can be used to rapidly classify mutants in mutagenesis screens. The goniolen is an important new tool for assessing mouse eyes and will both enhance and facilitate studies of anterior segment dysgenesis and glaucoma in mice.

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REFERENCES

- Paigen K. A miracle enough: the power of mice. *Nat Med* 1995; 1:215-20.
- Batley J, Jordan E, Cox D, Dove W. An action plan for mouse genomics. *Nat Genet* 1999; 21:73-5.
- John SWM, Anderson MG, Smith RS. Mouse genetics: a tool to help unlock the mechanisms of glaucoma. *J Glaucoma* 1999; 8:400-12.
- Hamilton BA, Frankel WN. Of mice and genome sequence. *Cell* 2001; 107:13-6.
- Smith RS, Sundberg JP, Linder CC. Mouse mutations as models for studying cataracts. *Pathobiology* 1997; 65:146-54.
- Cayouette M, Smith SB, Becerra SP, Gravel C. Pigment epithelium-derived factor delays the death of photoreceptors in mouse models of inherited retinal degenerations. *Neurobiol Dis* 1999; 6:523-32.
- Kedzierski W, Nusinowitz S, Birch D, Clarke G, McInnes RR, Bok D, Travis GH. Deficiency of rds/peripherin causes photoreceptor death in mouse models of digenic and dominant retinitis pigmentosa. *Proc Natl Acad Sci U S A* 2001; 98:7718-23.
- Swank RT, Novak EK, McGarry MP, Rusiniak ME, Feng L. Mouse models of Hermansky Pudlak syndrome: a review. *Pigment Cell Res* 1998; 11:60-80.
- Naf D, Wilson LA, Bergstrom RA, Smith RS, Goodwin NC, Verkerk A, van Ommen GJ, Ackerman SL, Frankel WN, Schimenti JC. Mouse models for the Wolf-Hirschhorn deletion syndrome. *Hum Mol Genet* 2001; 10:91-8.
- John SWM, Smith RS, Savinova OV, Hawes NL, Chang B, Turnbull D, Davisson MT, Roderick TH, Heckenlively JR. Essential iris atrophy, pigment dispersion, and glaucoma in DBA/2J mice. *Invest Ophthalmol Vis Sci* 1998; 39:951-62.
- Anderson MG, Smith RS, Hawes NL, Zabaleta A, Chang B, Wiggs JL, John SWM. Mutations in genes encoding melanosomal proteins cause pigmentary glaucoma in DBA/2J mice. *Nat Genet* 2002; 30:81-5.
- Pei YF, Rhodin JA. The prenatal development of the mouse eye. *Anat Rec* 1970; 168:105-25.
- Tripathi RC, Tripathi BJ. The mechanism of aqueous outflow in lower mammals. *Exp Eye Res* 1972; 14:73-9.
- Reme C, Urner U, Aeberhard B. The development of the chamber angle in the rat eye. Morphological characteristics of developmental stages. *Graefes Arch Clin Exp Ophthalmol* 1983; 220:139-53.
- Morrison J, Farrell S, Johnson E, Deppmeier L, Moore CG, Grossmann E. Structure and composition of the rodent lamina cribrosa. *Exp Eye Res* 1995; 60:127-35.
- Smith RS, Bechtold L, John SWM. Ultrastructure of Mouse Trabecular Meshwork. *Invest Ophthalmol Vis Sci* 1998; 39:S705.
- Smith RS, Zabaleta A, Savinova OV, John SWM. The mouse anterior chamber angle and trabecular meshwork develop without cell death. *BMC Dev Biol* 2001; 1:3.
- John SWM, Savinova OV. Intraocular Pressure Measurement in Mice: Technical Aspects. In: Smith RS, John SWM, Nishina PM, Sundberg JP, editors. *Systematic Evaluation of the Mouse Eye: Anatomy, Pathology and Biomethods*. Boca Raton, FL: CRC Press; 2002. p. 313-20.
- Morrison JC, Fraunfelder FW, Milne ST, Moore CG. Limbal microvasculature of the rat eye. *Invest Ophthalmol Vis Sci* 1995; 36:751-6.
- John SWM, Hagaman JR, MacTaggart TE, Peng L, Smithes O. Intraocular pressure in inbred mouse strains. *Invest Ophthalmol Vis Sci* 1997; 38:249-53.
- Hawes NL, Smith RS, Chang B, Davisson M, Heckenlively JR, John SWM. Mouse fundus photography and angiography: a catalogue of normal and mutant phenotypes. *Mol Vis* 1999; 5:22.
- Savinova OV, Sugiyama F, Martin JE, Tomarev SI, Paigen BJ, Smith RS, John SWM. Intraocular pressure in genetically distinct mice: an update and strain survey. *BMC Genet* 2001; 2:12.
- Smith RS, Hawes NL, Miller J, Sundberg JP, John SWM. Photography and Necropsy. In: Smith RS, John SWM, Nishina PM, Sundberg JP, editors. *Systematic Evaluation of the Mouse Eye: Anatomy, Pathology and Biomethods*. Boca Raton, FL: CRC Press; 2002. p. 251-64.
- Smith RS, Zabaleta A, Kume T, Savinova OV, Kidson SH, Martin JE, Nishimura DY, Alward WL, Hogan BL, John SWM. Haploinsufficiency of the transcription factors FOXC1 and FOXC2 results in aberrant ocular development. *Hum Mol Genet* 2000; 9:1021-32.
- Chang B, Smith RS, Peters M, Savinova OV, Hawes NL, Zabaleta A, Nusinowitz S, Martin JE, Davisson ML, Cepko CL, Hogan BL, John SWM. Haploinsufficient Bmp4 ocular phenotypes include anterior segment dysgenesis with elevated intraocular pressure. *BMC Genet* 2001; 2:18.
- Palmberg P. Gonioscopy. In: Ritch R, Shields MB, Krupin T, editors. *The Glaucomas*. St. Louis: Mosby-Year Book; 1996. p. 455-68.
- Smith RS, Zabaleta A, John SWM. Light Microscopy. In: Smith RS, John SWM, Nishina PM, Sundberg JP, editors. *Systematic Evaluation of the Mouse Eye: Anatomy, Pathology and Biomethods*. Boca Raton, FL: CRC Press; 2002. p. 266-71.
- O'Brien T. Mutagenesis and genetic screens in the mouse. In:

- Smith RS, John SWM, Nishina PM, Sundberg JP, editors. Systematic Evaluation of the Mouse Eye: Anatomy, Pathology and Biomethods. Boca Raton, FL: CRC Press; 2002. p. 93-108.
29. Munroe RJ, Bergstrom RA, Zheng QY, Libby B, Smith R, John SWM, Schimenti KJ, Browning VL, Schimenti JC. Mouse mutants from chemically mutagenized embryonic stem cells. *Nat Genet* 2000; 24:318-21.
 30. Nolan PM, Peters J, Strivens M, Rogers D, Hagan J, Spurr N, Gray IC, Vizor L, Brooker D, Whitehill E, Washbourne R, Hough T, Greenaway S, Hewitt M, Liu X, McCormack S, Pickford K, Selley R, Wells C, Tymowska-Lalanne Z, Roby P, Glenister P, Thornton C, Thaug C, Stevenson JA, Arkell R, Mburu P, Hardisty R, Kiernan A, Erven A, Steel KP, Voegeling S, Guenet JL, Nickols C, Sadri R, Nasse M, Isaacs A, Davies K, Browne M, Fisher EM, Martin J, Rastan S, Brown SD, Hunter J. A systematic, genome-wide, phenotype-driven mutagenesis programme for gene function studies in the mouse. *Nat Genet* 2000; 25:440-3.
 31. Hrabe de Angelis MH, Flaswinkel H, Fuchs H, Rathkolb B, Soewarto D, Marschall S, Heffner S, Pargent W, Wuensch K, Jung M, Reis A, Richter T, Alessandrini F, Jakob T, Fuchs E, Kolb H, Kremmer E, Schaeble K, Rollinski B, Roscher A, Peters C, Meitinger T, Strom T, Steckler T, Holsboer F, Klopstock T, Gekeler F, Schindewolf C, Jung T, Avraham K, Behrendt H, Ring J, Zimmer A, Schughart K, Pfeffer K, Wolf E, Balling R. Genome-wide, large-scale production of mutant mice by ENU mutagenesis. *Nat Genet* 2000; 25:444-7.
 32. Favor J, Neuhauser-Klaus A. Saturation mutagenesis for dominant eye morphological defects in the mouse *Mus musculus*. *Mamm Genome* 2000; 11:520-5.
 33. McMenamin PG, al-Shakarchi MJ. The effect of various levels of intraocular pressure on the rat aqueous outflow system. *J Anat* 1989; 162:67-82.
 34. Tripathi RC. Comparative physiology and anatomy of the aqueous outflow pathway. In: Davson H, Graham LT, editors. *The Eye*. Vol. 5. 2nd ed. New York: Academic Press; 1974. p. 163-356.
 35. van der Zypen E. Experimental morphological study on structure and function of the filtration angle of the rat eye. *Ophthalmologica* 1977; 174:285-98.
 36. Smith RS, Sundberg JP, John SWM. The anterior segment and ocular adnexae. In: Smith RS, John SWM, Nishina PM, Sundberg JP, editors. Systematic Evaluation of the Mouse Eye: Anatomy, Pathology and Biomethods. Boca Raton, FL: CRC Press; 2002. p. 3-24.
 37. Campbell DG, Netland PA. The Normal Anterior Segment. In: Campbell DG, Netland PA, editors. *Stereo Atlas of Glaucoma*. St. Louis: Mosby-Year Book; 1998. p. 5-11.