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Title: Grossing of Non-Neoplastic Globes, Including Fetal Eyes

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? **Yes**

If **Yes**, can you record movies/images using your own microscope camera?

No

If your protocol involves microscopy but you are not able to record movies/images with your microscope camera, JoVE will need to use our scope kit.

If your microscope does not have a camera port, the scope kit will be attached to one of the eyepieces and **you will have to perform the procedure using one eye.**

Videographer: Authors will indicate the scope shot numbers on the spot. Please film the shots, that they indicate, with the scope kit. This will be decided based on the specimen they obtain on the day of shoot. So, please carry the scope kit.

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

3. Filming location: Will the filming need to take place in multiple locations? **Yes**

If **Yes**, how far apart are the locations? Two rooms away

Current Protocol Length

Number of Steps: 19

Number of Shots: 33

Introduction

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

NOTE: All statements were filmed in English and German. Ignore the German versions of statements

- 1.1. **Martina Herwig-Carl:** My research focuses on ophthalmic pathology which includes clinico-pathologic correlations and basic research on uveal melanoma.
 - 1.1.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 2.3.1*

What significant findings have you established in your field?

- 1.2. **Martina Herwig-Carl:** I have described several clinico-pathologic correlations e.g. on Schnabel's optic nerve atrophy as well as post-surgery findings after corneal crosslinking and subretinal implants.
 - 1.2.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1*

NOTE: This may have been slated as 1.3

What research gap are you addressing with your protocol?

- 1.3. **Martina Herwig-Carl:** The macroscopic inspection and sectioning of a globe is quite challenging. Our protocol should help pathologists to identify critical ocular structures and properly section a globe without a tumor.
 - 1.3.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.7.1*

NOTE: This may have been slated as 1.5

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

Testimonial Questions (OPTIONAL):

Videographer: Please ensure that all testimonial shots are captured in a wide-angle format, while also maintaining sufficient headspace, given that the final videos will be rendered in a 1:1 aspect ratio.

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

1.6. Martina Herwig-Carl: Grossing of non-tumour eyes is challenging in particular if not carried out by an ophthalmic pathologist. It is crucial to familiarize yourself with the ocular history and any previous ocular surgical procedures. Based on this information and the macroscopic findings, the globe can be sectioned in such a way that the most important ocular structures are represented in the PO section. Publishing with JoVE will hopefully help to improve grossing of globes for diagnostic purposes by increasing visibility of our standardized procedure.

1.7. German version of 1.4: Der Zuschnitt eines Auges birgt - insbesondere bei Nicht-Tumoraugen – auf Grund der komplexen Anatomie Herausforderungen, insbesondere wenn das Auge nicht durch einen Ophthalmopathologen zugeschnitten wird. Es ist wichtig, die okulären Vorerkrankungen des Patienten incl. vorausgegangene chirurgische Eingriffe zu kennen. Basierend auf diesen Informationen und der makroskopischen Inspektion, wird das Auge so zugeschnitten, dass die wichtigsten Strukturen im Schnitt liegen. Wir erhoffen uns, dass die Publikation unseres Protokolls mit JoVE dazu beitragen wird, den Zuschnitt von Augen für die Diagnostik zu verbessern.

1.7.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 3.7.1*

Can you share a specific success story or benefit you've experienced—or expect to experience—after using or publishing with JoVE?

1.8. Martina Herwig-Carl: We have experienced in the past that globes received for a second opinion are often mis-sectioned, preventing a proper evaluation. I hope we can encourage pathologists to use our protocol. We are, of course, open to provide special training.

1.9. German version: Wenn wir Augen zur Zweitbefundung erhalten, erleben wir oft, dass die Augen nicht ordnungsgemäß zugeschnitten sind. Dadurch wird die Befundung deutlich erschwert und durch die Zuschnittbedingten Artefakte ist eine vollständige Diagnosestellung nicht immer möglich. Wir hoffen, dass wir viele Pathologen erreichen, damit sie unser Protokoll benutzen. Selbstverständlich stehen wir auch für Rückfragen gern zur Verfügung.

1.9.1. INTERVIEW: Named talent says the statement above in an interview-style shot, looking slightly off-camera. *Suggested B-roll: 4.4.1*

Authors: Could you please also deliver the above statements in German?

Videographer: Please film the testimonials in both English and German

Protocol

2. Orienting and Grossing the Eye Globe

Demonstrator: Martina Herwig-Carl

2.1. To begin, gather all the required items for the procedure [1]. First, identify the four rectus muscles in the eye globe sample [2] and then identify the superior oblique muscle by its tendinous appearance, as it inserts into the sclera on the posterotemporal surface of the globe [3-TXT].

2.1.1. WIDE: Talent arranging the required materials on the procedure table.

2.1.2. Talent exploring to locate the four rectus muscles on the globe.

2.1.3. Talent pointing to the superior oblique muscle, showing its tendinous insertion near the superior rectus. **TXT: Look near the insertion of the superior rectus muscle Videographer's NOTE: 2.1.3 AND 2.2.1 there is a normal and a scope version**

2.2. Next, identify the inferior oblique muscle, which inserts temporal inferiorly into the sclera between the lateral rectus muscle and the optic nerve [1].

2.2.1. Talent pointing to the inferior oblique muscle.

2.3. After identifying the long posterior ciliary artery [1], determine the laterality of the globe accurately based on the positions of the superior oblique, inferior oblique, and long posterior ciliary artery [2].

2.3.1. Talent locating and pointing to the long posterior ciliary artery on the specimen.

2.3.2. Talent orienting the globe and based on anatomical landmarks.

2.4. Using a caliper, measure the anterior-posterior, horizontal, and vertical diameters of the globe in millimeters [1]. Examine and describe any abnormalities on the external surface of the globe, including scars, sutures, foreign materials or deformations [2].

2.4.1. Talent measuring the globe dimensions. **TXT: The current globe measures 27 x 26 x 27 mm**

2.4.2. Talent inspecting and writing the observations down on the ophthalmic pathology submission form.

- 2.5. Next, measure the cornea in its horizontal and vertical diameters in millimeters [1]. Describe the corneal characteristics, including transparency, presence of scars, signs of keratoplasty, sutures, or vascularization [2-TXT]. In this globe, there was a superior staphyloma and a small staphyloma underneath the medial rectus muscle [3]. **NOTE: VO added shot the extra shot**
 - 2.5.1. Talent using a caliper to measure the corneal dimensions.
 - 2.5.2. Close-up of cornea with talent pointing to any character. **TXT: The cornea is cloudy and indented; It measures 9 x 12 mm**
Videographer's NOTE: there is a normal and a scope version
ADDED SHOT: Talent pointing to the staphyloma present superiorly.
- 2.6. Describe the visible iris in terms of color, pupil configuration, and abnormalities [1] ~~and use transillumination to detect iris defects from trauma or congenital issues [2].~~
 - 2.6.1. Talent noting the iris features.
- 2.7. Measure the optic nerve for its length and note any abnormalities such as hemorrhages in the sheaths [1-TXT].
 - 2.7.1. Talent measuring the optic nerve. **TXT: The atrophic optic nerve in this globe measures 8 mm**
- 2.8. Make a cross section through the optic nerve if possible [1], and submit it separately for histological examination [2].
 - 2.8.1. Talent cutting a cross section of the optic nerve.
 - 2.8.2. Talent placing the optic nerve into a labeled container.
- 2.9. Now, transilluminate the globe using an LED pipe to highlight iris defects [1] and scleral shadows potentially indicating tumors, hemorrhages, or foreign bodies. In this case, the staphyloma can be visualized and there is a shadowing nasal inferiorly [2].
 - 2.9.1. Talent transilluminating the globe.
 - 2.9.2. Talent pointing to shadows or defects on the sclera.
- 2.10. Perform photo documentation of the entire globe [1].
 - 2.10.1. Talent capturing high-resolution images of the intact globe.
Videographer: This may have to be filmed first in a separate room. Authors will be able to guide you about this shot

3. Sectioning of Routine Specimens from Eye Globe

- ~~3.1. Place the globe cornea side down on a stable surface [1].~~
 - ~~3.1.1. Talent positioning the globe cornea down on a cutting board.~~
- 3.2. Using a pen, mark the vertical meridian or desired section plane so that the section passes close to the optic nerve and peripheral cornea [1-TXT]. Using a microtome blade, section the globe along the marked line while securing it with the other hand [2].
 - 3.2.1. Talent marking the section line along the vertical meridian in order to have the staphyloma on the section. **TXT: Avoid cutting through areas of interest** **NOTE: 3.2.1 file number is DSCF8495**
 - 3.2.2. Talent carefully slicing the globe along the marked path.
- 3.3. Remove the calotte and inspect for abnormalities [1]. Store it in formalin or submit for histological processing [2-TXT].
 - 3.3.1. Talent removing the calotte and examining it.
 - 3.3.2. Talent placing the sample in formalin. **TXT: If silicon oil is present, take precautions due to its sticky nature**
- 3.4. Now, inspect internal ocular structures including the anterior chamber, lens status, iris, ciliary body, choroid, retina, optic nerve, [1] and sclera for abnormalities like atrophy or thickening [2]. The staphyloma can be visualized on the section [3] along with the back of the iris [4]. The ciliary body has a cyclitic membrane [5-TXT]. The retina is typically detached after enucleation [6]. The atrophic optic nerve can be visualized [7], along with the macula and the lighter choroid [8]. **NOTE: VO added for the extra shots**
 - 3.4.1. Talent systematically examining internal structures with a probe. **NOTE: 3.4.1 and 3.4.2 are in 3 files (DSCF8501,2 and 3)**
 - 3.4.2. Talent pointing to the sclera. **TXT: No intraocular lens**
Added shot: Talent pointing to staphyloma. NOTE: All these added shots may be slated as 3.4.2. The author mentions that she has verbally explained and pointed out to each part
Added shot: Talent pointing to the back of iris.
Added shot: Talent pointing to the ciliary body.
Added shot: Talent pointing to the retina.
Added shot: Talent pointing to optic nerve.
Added shot: Talent pointing to macula and choroid.
- 3.5. Hold the now opened globe and section it parallel to but above the optic nerve, again aiming through the peripheral cornea [1-TXT].

- 3.5.1. Talent making the final section, removing of the second calotte, while stabilizing the specimen. **TXT: Maintain stability as the globe is more fragile**
- 3.6. Then, remove the calotte and inspect for any remaining abnormalities
 - [1]. Store or submit the sample for histological analysis [2].
 - 3.6.1. Talent removing and examining the final calotte.
 - 3.6.2. Talent placing the sample in formalin.
- 3.7. Finally, perform photo documentation of the posterior-optic section and submit it for further processing [1].
 - 3.7.1. Talent taking images of the posterior-optic section.

Results

4. Results

4.1. Multiple external foreign material applications for historic retinal detachment surgery was performed for sample consisting of plates and several scleral buckles [1].

4.1.1. LAB MEDIA: Figure 4C

4.2. The corresponding Hematoxylin and Eosin stained section showed the intrascleral location of a buckle [1] as well as the negative image of an extra-scleral plate [2].

4.2.1. LAB MEDIA: Figure 4D *Video editor: Please highlight the area pointed by the big thick black arrow*

4.2.2. LAB MEDIA: Figure 4D *Video editor: Please highlight the area pointed by the asterisk*

4.3. The target result of the sectioning was a proper pupil-optic nerve section [1].

4.3.1. LAB MEDIA: Figure 5 and Figure 6.

Pronunciation guide :

1. Rectus

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/rectus>
 - **IPA:** /ˈrɛk.təs/
 - **Phonetic Spelling:** rek-tuhs([merriam-webster.com](https://www.merriam-webster.com), [merriam-webster.com](https://www.merriam-webster.com))
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2. Oblique

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/oblique>
- **IPA:** /əˈbli:k/
- **Phonetic Spelling:** uh-bleek([merriam-webster.com](https://www.merriam-webster.com))

3. Sclera

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/sclera>
 - **IPA:** /ˈsklər.ə/
 - **Phonetic Spelling:** sklehr-uh([merriam-webster.com](https://www.merriam-webster.com))
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4. Optic Nerve

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/optic%20nerve>
 - **IPA:** /ˈɑp.tɪk nɜːrv/
 - **Phonetic Spelling:** op-tik nurv([merriam-webster.com](https://www.merriam-webster.com), [merriam-webster.com](https://www.merriam-webster.com))
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5. Staphyloma

- **Pronunciation link:** <https://www.merriam-webster.com/medical/staphyloma>
 - **IPA:** /ˌstæf.əˈloo.mə/
 - **Phonetic Spelling:** staf-uh-loh-muh([merriam-webster.com](https://www.merriam-webster.com))
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6. Keratoplasty

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/keratoplasty>
 - **IPA:** /ˈker.ə.təˌplæs.ti/
 - **Phonetic Spelling:** keh-ruh-tuh-plas-tee
-

7. Iris

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/iris>
 - **IPA:** /ˈaɪ.rɪs/
 - **Phonetic Spelling:** eye-ris
-

8. Transillumination

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/transilluminate>
 - **IPA:** /ˌtræn.sɪ.luː.məˈneɪ.ʃən/
 - **Phonetic Spelling:** tran-sih-loo-muh-nay-shun([merriam-webster.com](https://www.merriam-webster.com))
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9. Atrophic

- **Pronunciation link:** <https://www.howtopronounce.com/atrophic>
 - **IPA:** /əˈtroʊ.fɪk/
 - **Phonetic Spelling:** uh-troh-fik([merriam-webster.com](https://www.merriam-webster.com))
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10. Calotte

- **Pronunciation link:** <https://www.howtopronounce.com/calotte>
 - **IPA:** /kəˈlɒt/
 - **Phonetic Spelling:** kuh-lot
-

11. Ciliary Body

- **Pronunciation link:** <https://www.howtopronounce.com/ciliary-body>
 - **IPA:** /ˈsɪl.i.ər.i ˈbɒd.i/
 - **Phonetic Spelling:** sil-ee-air-ee bod-ee
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12. Choroid

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/choroid>
 - **IPA:** /ˈkɔːrɔɪd/
 - **Phonetic Spelling:** kor-oyd
-

13. Macula

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/macula>
- **IPA:** /ˈmæk.jʊ.lə/
- **Phonetic Spelling:** mak-yuh-luh

14. Cyclitic Membrane

- **Pronunciation link:** <https://www.howtopronounce.com/cyclitic-membrane>
 - **IPA:** /saɪˈklɪt.ɪk ˈmem.breɪn/
 - **Phonetic Spelling:** sigh-klit-ik mem-brayn
-

15. Retina

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/retina>
 - **IPA:** /ˈret.ɪ.nə/
 - **Phonetic Spelling:** ret-uh-nuh([merriam-webster.com](https://www.merriam-webster.com))
-

16. Scleral Buckle

- **Pronunciation link:** <https://www.howtopronounce.com/scleral-buckle>
 - **IPA:** /ˈsklər.əl ˈbʌk.əl/
 - **Phonetic Spelling:** sklehr-uhl buk-uhl
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17. Hematoxylin

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/hematoxylin>
 - **IPA:** /ˌhiː.məˈtɒk.sɪ.lɪn/
 - **Phonetic Spelling:** hee-muh-tok-suh-lin
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18. Eosin

- **Pronunciation link:** <https://www.merriam-webster.com/dictionary/eosin>
 - **IPA:** /ˈiː.ə.sɪn/
 - **Phonetic Spelling:** ee-uh-sin
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