Activity	Author	Activity Date
Major Version Incremented	Morsi, Nora	10/5/2020 3:48 PM
Amendment AMEND202001094 closed (Approved)	Morsi, Nora	10/5/2020 3:48 PM

Amendment Approved: AMEND202001094

Opened Amendment	Morsi, Nora	10/5/2020 3:43 PM

Amendment: AMEND202001094

PI Proxy Assigned	Sabesan, Ramkumar	10/5/2020 11:03 AM

PI Proxies Added: Xiaoyun Jiang

Letter Sent	Brot, Michelle	9/15/2020 11:37 AM

Correspondence_for_PROTO201800103.doc

Letter Prepared	Brot, Michelle	9/15/2020 11:37 AM

Correspondence_for_PROTO201800103.doc

Private Comment Added	Brot, Michelle	9/15/2020 11:33 AM

Note that I received an e-mail from the Occ Health Nurse saying that this is fine to approve.

Designated Member Review Submitted	Sullivan, Jane	9/12/2020 12:22 PM
Response Submitted	Sabesan, Ramkumar	9/11/2020 4:08 PM

The reviewer's comments were addressed.

Comment Added	Huang, Stephanie W	9/8/2020 11:01 AM

Hi Dr. Sabesan,

This is a reminder that the IACUC has additional questions on this submission which require a response. Please revise/respond as necessary and submit back to our office.

If you have any questions at all, please feel free to contact your liaison, Michelle Brot (mbrot@uw.edu).

Thank you,
Stephanie

Clarification Requested by IACUC Member	Administrator, System	8/24/2020 10:21 AM

Hello,

The IACUC had additional questions regarding this item. Please revise/respond as necessary and submit back to our office.

Thank you,
Stephanie

Clarification by Designated Reviewer Requested	Huang, Stephanie W	8/24/2020 10:21 AM

Hello,

The IACUC had additional questions regarding this item. Please revise/respond as necessary and submit back to our office.

Thank you,
Stephanie

Designated Reviewers Assigned	Huang, Stephanie W	8/24/2020 10:21 AM
Assigned to Designated Review	Huang, Stephanie W	8/24/2020 10:20 AM
Agenda Item Removed	Huang, Stephanie W	8/24/2020 10:20 AM

	Ancillary Review Submitted	Cashman, Judy L	8/24/2020 8:09 AM
	Ancillary Reviews Managed	Cashman, Judy L	8/24/2020 8:09 AM
	Tags Managed	Cashman, Judy L	8/24/2020 8:09 AM
	OHRs attached	Cashman, Judy L	8/24/2020 8:09 AM
	Private Comment Added	Sullivan, Jane	8/21/2020 3:47 PM

Correction: I have a question related to funding support for this protocol.

	Private Comment Added	Sullivan, Jane	8/21/2020 3:38 PM

No questions or comments for this minimally invasive imaging protocol

	Meeting Assigned	Brot, Michelle	8/14/2020 10:54 AM
	Pre-Review Submitted	Brot, Michelle	8/14/2020 10:54 AM
	Vet Consult Submitted	Stocking, Kim	8/14/2020 7:21 AM

Do you accept the submission? yes

	Vet Consult Sent	Brot, Michelle	8/13/2020 9:41 PM

The PI has (finally!) responded to the vet questions. The answers seemed okay to me but see what you think.

Activity	Author	Activity Date
Response Submitted	Sabesan, Ramkumar	8/13/2020 4:27 PM
Responses to vet review made.		
Tags Managed	Nguyen, Tony	7/22/2020 1:55 PM
SLU 3.1 E286 inspected and approved for animal use. Room Hold removed.		
Tags Managed	Nguyen, Tony	3/31/2020 11:41 AM
Room hold for SLU 3.1 E286 inspected on 2/19/20 with 8 concerns. E286 to be re-inspected for final approval.		
Clarification by Pre-Reviewer Requested	Brot, Michelle	2/26/2020 3:14 PM
The vet had several questions for you to address. Thanks, Michelle		
Vet Consult Submitted	Stocking, Kim	2/26/2020 1:27 PM
Do you accept the submission? no Some questions/comments		
Vet Consult Sent	Stocking, Kim	2/26/2020 12:31 PM
Vet Consult Sent	Brot, Michelle	2/17/2020 9:52 AM
This one is FINALLY ready for review!		
Response Submitted	uwhover	2/17/2020 9:17 AM
Submitting on behalf of the PI		
Clarification by Pre-Reviewer Requested	Brot, Michelle	9/13/2019 4:53 PM
Hi Ram, Reviewed your responses and just had one follow-up question on the Animal Details page. Also, I'm curious where things stand wrt this project. If you have time to give me a call, it might be helpful to chat for a couple minutes. Thanks, Michelle (221-0891).		
Response Submitted	Sabesan, Ramkumar	7/18/2019 3:52 PM
The responses to the reviewer's questions have been addressed and changes made in the protocol. Thanks		
Coordinator Assigned	Schoenleben, Aubrey	5/19/2019 3:09 PM
Assigning back to Michelle. Assigned to Michelle Brot		
Coordinator Assigned	Schoenleben, Aubrey	4/30/2019 9:20 AM
Re-assigning to Aubrey to look after amendment while Michelle is out of town. Assigned to Aubrey Schoenleben		
Tags Managed	Kunsman, Robyn	1/28/2019 9:23 AM
Clarification by Pre-Reviewer Requested	Brot, Michelle	1/27/2019 6:04 PM
There are reviewer questions to address scattered in various sections throughout the protocol. Please make the relevant changes within the document and then indicate in your response what modifications you made.		
Coordinator Assigned	Brot, Michelle	1/18/2019 10:11 AM
Assigned to Michelle Brot		
Coordinator Assigned	Huang, Stephanie W	1/18/2019 10:09 AM
Assigned to OAW Purple Team		
Coordinator Assigned	Huang, Stephanie W	1/18/2019 8:22 AM
Assigned to OAW Blue Team		

	Assigned to OAW Blue Team		
	Assigned Portfolio ID	Huang, Stephanie W	1/18/2019 8:16 AM
	Submitted	Sabesan, Ramkumar	1/17/2019 2:47 PM
	Protocol Created	Sabesan, Ramkumar	10/2/2018 6:02 PM

From: Michelle Brot <mbrot@uw.edu>
Sent: Thursday, September 3, 2020 9:57 AM
To: Ramkumar Sabesan
Subject: IACUC Protocol

Hi Ram,
We are really close to getting your protocol approved but the IACUC reviewer had a couple really minor comments that you could probably address in <5 min. They've been there for a couple weeks so I thought I'd let you know in case you overlooked that they were waiting for your response.

Take care,
Michelle

UNIVERSITY of WASHINGTON

APPROVAL OF NEW PROTOCOL SUBMISSION

September 15, 2020

Dear Ramkumar Sabesan,

This email serves as written notice of animal use approval by the Institutional Animal Care and Use Committee (IACUC).

*To help us better serve you, please take this **3 question survey** about your experience with the review process.*

Type of Review:	Designated Member Review
Short Title of Protocol:	4462-01: In vivo retinal imaging in mice
Investigator:	Ramkumar Sabesan
HoverBoard ID:	PROTO201800103

Please note the approval and expiration date listed. All animal use protocols must be renewed annually from the date of IACUC approval, independent of project or funding dates. Please refer to the assigned protocol number for all animal orders and future correspondence with the IACUC.

Protocol Approval Dates: 9/15/2020 to 9/14/2023

Next Annual Expiration Date:

Next Triennial Expiration Date: 9/14/2023

If you have any questions, contact OAWRSS at oawrss@uw.edu.

Sincerely,

Office of Animal Welfare

OFFICE of ANIMAL WELFARE

Basic Information

1. *** Select research team:**
   Sabesan team
2. *** Title of protocol:**
   Imaging the mouse retina
3. *** Short title:**
   4462-01: In vivo retinal imaging in mice
4. *** Summary of research:**
   The mouse is an excellent model for studying the retina. This research is aimed at testing the feasibility of imaging the cellular structures within the mouse retina in vivo using a specialized camera.

   The primary objective is to investigate the feasibility of visualizing different retinal cell types and their connections without and with fluorescent markers.

   The general approach is to use a custom-built camera to take high resolution pictures of the mouse retina through the intact eye and in a non-contact fashion.
5. *** Principal investigator:**
   Ramkumar Sabesan
6. *** What is the intention of the animal protocol?**
   Experimental Research

Experimental Research Protocol Addition

1. * **Will the protocol include breeding?**
   ○ Yes ● **No**

Protocol Team Members

1. Identify each additional person involved in the design, conduct, or reporting of the research:

Name	Role	Involved in Animal Handling	Authorized To Order Animals	E-mail	Phone
Ethan Buhr	Co-Investigator	yes	yes	buhre@uw.edu	
Xiaoyun Jiang	Postdoctoral Fellow	yes	yes	jxyun@uw.edu	+1 425 499-0713
Owen T Lawrence	Research Scientist	yes	yes	olawr@uw.edu	+1 206 616-2883
Maureen Neitz	Co-Investigator	yes	yes	mneitz@uw.edu	+1 206 543-7998
Ramkumar Sabesan	Other	no	yes	rsabesan@uw.edu	+1 206 221-4925
Rachel O Wong	Co-Investigator	yes	yes	wongr2@uw.edu	+1 206 616-3275

2. If veterinary care will be provided by individuals outside of DCM or WaNPRC, provide the name, credentials and contact information below:

Funding Sources

1. Identify each organization supplying funding for the protocol:

	Funding Organization	eGC1 Number(s)
View	Ophthalmology	

Scientific Aims

1. * Scientific aims of the research:

The goal of this research is to

1) modify an existing adaptive optics retinal camera for in vivo retinal imaging in mice. The mouse eye presents unique challenges for retinal imaging. Modifying our existing camera to suit these requirements and testing/refining them is the first aim of this project.

2) investigate the feasibility of in vivo imaging of retinal structures in mice with and without fluorescent reporters. These structures are otherwise obscured by the optics of the intact mouse eye. Currently, the only way to visualize these structures are via ex vivo preparations.

2. * Using language understandable to non-scientists, describe the goals and significance of the protocol to humans, animals and science:

The goal of this project is to a) equip a non-contact, non-invasive retinal camera with capabilities to image the mouse retina in vivo and b) test the feasibility of this camera to capture cellular level detail in the morphology and function of retinal cells.
The mouse is a widely used animal model for diseases of the visual system. Developing and testing technology that allows visualizing mice retinal cells non-invasively in health, in disease and to monitor effectiveness of potential therapies is critical. Achieving this capability extends the scope of basic research into mechanism of vision as well as clinical translation, especially in cases where a live model and longitudinal follow up is required.

3. * Provide a statement to address the potential harm to the animals on this study (e.g., pain, distress, morbidity, mortality) relative to the benefits to be gained by performing the proposed work:

The benefit of this research is that it will lead to new insight into mechanisms of normal vision, help design, test and refine new therapies for preventing and restoring vision from human retinal diseases.

Mice will not experience any more pain or distress than that associated with administering anesthesia and dilation drops. To prevent drop in body temperature following anesthesia, a heating pad will be used to regulate temperature. The heating pad will not be in direct contact with the animal. The camera that we will use to take pictures of the mice retina is non-contact and routinely used for human retinal imaging. Therefore the potential harm to the animal is minimal.

Experiments

Note: If you will be administering cells, cell lines, sera or other biologicals to rodents, contact the Rodent Health Monitoring Program (RHMP, rhmp@uw.edu). Testing may be required prior to administration to rodents.

1. * Define the experiments to be used in this protocol:

Name	Species	USDA	Count	Count by Pain Category	Procedures	Husbandry Exception Types
Determining the effect of anesthesia and fluorescent labels for imaging cellular structures in living mice retina	Mice	no	45	B: 0 C: 0 D: 45 E: 0	▪ Euthanasia: Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine (Standard) ▪ Euthanasia: Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia (Standard) ▪ Imaging: Sabesan team - Micron II fundus imaging (Team) ▪ Imaging: Sabesan - imaging (Team) ▪ Substance Administration: Sabesan team : Administering topical eye drops (Team) ▪ Substance Administration: Anesthesia, Isoflurane, Long Duration (>1 hour) (Standard) ▪ Substance Administration: Anesthesia, Isoflurane, Short Duration (<1 hour) (Standard) ▪ Substance Administration: Anesthesia, Terminal, Tribromoethanol (Avertin) (Standard)	Mice - No husbandry or enrichment exceptions.

2. Will any single animal undergo more than one survival surgery? (include any animal that underwent surgery prior to use on this protocol) ○ Yes ● No

Procedure Personnel Assignment

1. * Select the team members who will be performing each procedure:

Procedure	Species	Is USDA Species	Team Members
Euthanasia: Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine , ver. 1 (Standard)	Mice	no	Xiaoyun Jiang
Euthanasia: Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia , ver. 1 (Standard)	Mice	no	Xiaoyun Jiang
Imaging: Sabesan - imaging, ver. 1 (Team)	Mice	no	Maureen Neitz Ethan Buhr Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Imaging: Sabesan team - Micron II fundus imaging, ver. 1 (Team)	Mice	no	Maureen Neitz Ethan Buhr Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Substance Administration: Anesthesia, Isoflurane, Long Duration (>1 hour), ver. 2 (Standard)	Mice	no	Maureen Neitz Ethan Buhr Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Substance Administration: Anesthesia, Isoflurane, Short Duration (<1 hour), ver. 2 (Standard)	Mice	no	Maureen Neitz Ethan Buhr Owen T Lawrence Xiaoyun Jiang Rachel O Wong

Procedure	Species	Is USDA Species	Team Members
Substance Administration: Anesthesia, Terminal, Tribromoethanol (Avertin), ver. 2 (Standard)	Mice	no	Maureen Neitz Ethan Buhr Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Substance Administration: Sabesan team : Administering topical eye drops, ver. 1 (Team)	Mice	no	Maureen Neitz Ethan Buhr Owen T Lawrence Xiaoyun Jiang Rachel O Wong

2. Team member training:

First Name	Last Name	Training
Ethan	**Buhr**	

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	7/31/2019	7/31/2022
SLU 3.1 Facility Orientation	Orientation	In Person	Basic Course	Stage 1	5/17/2013	
Lab-Managed Animal Care & Records	General	In Person	Basic Course	Stage 1	10/6/2009	
Cervical Dislocation, Mouse Anesthetized	Procedure	In Person	Basic Course	Stage 1	10/7/2009	
Rabbit Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/18/2016	
Lab-Managed Sick Rodent Recognition	General	In Person	Basic Course	Stage 1	10/6/2009	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	8/26/2020	8/26/2025
Brotman Facility Orientation, Rodent Users	Orientation	In Person	Basic Course	Stage 1	5/18/2016	

No experience data to display

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
6th Floor Facility Orientation, Non-Rodent Users	Orientation	In Person	Basic Course	Stage 1	10/27/2009	
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	9/23/2009	
Cervical Dislocation, Mouse Unanesthetized	Procedure	In Person	Basic Course	Stage 1	11/16/2011	
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	5/6/2020	5/31/2021
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	10/7/2009	

Xiaoyun Jiang

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	6/14/2019	6/30/2022
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	10/4/2018	10/31/2019
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	7/6/2016	
Cervical Dislocation, Mouse Anesthetized	Procedure	In Person	Basic Course	Stage 1	5/23/2016	
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	6/1/2016	
SLU 3.1 Facility Orientation	Orientation	In Person	Basic Course	Stage 1	10/9/2018	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/23/2016	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	5/13/2016	5/13/2021

No experience data to display

Owen T Lawrence

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	4/15/1999	

No experience data to display

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	5/21/2018	
6th Floor Facility Orientation, Rodent Users	Orientation	In Person	Basic Course	Stage 1	1/16/2002	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	11/9/2019	11/9/2024
Lab-Managed Aquatic Animal Care & Records	General	In Person	Basic Course	Stage 1	5/27/2014	
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	11/13/2019	11/30/2020
Lab-Managed Animal Care & Records	General	In Person	Basic Course	Stage 1	3/2/2018	
Cervical Dislocation, Mouse Anesthetized	Procedure	In Person	Basic Course	Stage 1	11/17/2017	
Lab-Managed Sick Rodent Recognition	General	In Person	Basic Course	Stage 1	3/2/2018	
Cervical Dislocation, Mouse Unanesthetized	Procedure	In Person	Basic Course	Stage 1	1/30/2018	
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	8/21/2019	8/31/2022
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/14/2013	

Maureen Neitz

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
WaNPRC Necropsy Room Clearance	Orientation	In Person	Basic Course	Stage 1	10/1/2018	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	12/28/2010	
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	2/26/2020	2/28/2023
Lab-Managed Sick Rodent Recognition	General	In Person	Basic Course	Stage 1	10/21/2009	

No experience data to display

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
WaNPRC Surgery Suite Clearance	Orientation	In Person	Basic Course	Stage 1	2/1/2018	
Lab-Managed Animal Care & Records	General	In Person	Basic Course	Stage 1	10/21/2009	
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	7/8/2020	7/31/2021
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	1/30/2009	
SLU 3.1 Facility Orientation	Orientation	In Person	Basic Course	Stage 1	9/23/2013	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	7/24/2018	7/24/2023

Ramkumar Sabesan

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	1/5/2018	1/5/2023
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	10/21/2019	10/31/2022

No experience data to display

Ramkumar Sabesan

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	1/5/2018	1/5/2023
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	10/21/2019	10/31/2022

No experience data to display

Rachel O Wong

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Lab-Managed Aquatic Animal Care & Records	General	In Person	Basic Course	Stage 1	8/15/2006	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	8/23/2011	

No experience data to display

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	8/18/2014	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	1/4/2016	1/4/2021
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	12/21/2017	12/31/2020
6th Floor Facility Orientation, Rodent Users	Orientation	In Person	Basic Course	Stage 1	7/18/2006	
Annual DCM Facility Access Training (Rodent)	General	Online	Basic Course	Stage 1	7/27/2020	7/31/2021
T-Wing Facility Orientation	Orientation	In Person	Basic Course	Stage 1	8/6/2013	

Animal Details

1. * How are animals acquired?

Transferred from another UW Protocol

2. Describe the acquisition for:

a. * Transferred from another UW Protocol, provide Protocol Number:

4206-01 (Neitz), 4122-01 (Wong), 4184-03 (Van Gelder)

b. * If animals were transferred, please provide an explanation of all procedures the animal(s) experienced prior to transfer (i.e substance administrations, surgeries, dietary restrictions, etc.):

No procedures.

Wong lab animals will be B6.Cg-Tg(Thy1-YFP) mice. Their strain/stock information is contained in protocol 4122-01.

The Neitz protocol 4206-01 includes breeding and some of the animals not needed for their studies are euthanized at the time of weaning. Instead of euthanizing them, these animals (which have not undergone any procedures) would be transferred.

The Van Gelder lab mice are generated with standard laboratory breeding and are weaned at 21 days. No other procedures are performed. Protocol : 4184-03

3. Identification of individual animals (other than cage cards):

a. Method(s) (e.g., ear punch/tag, tattoo, tagging/banding, radio collar, etc.) (Note: If method is implantation (e.g. PIT tag), create or select an Implant procedure to describe the details. If method is surgical (e.g., satellite tag), create or select Survival Surgery procedure to describe the details):

Neitz lab ear tag and Wong lab have toe-tattoos.

Van Gelder lab animals are identified by 2 mm ear punches, and are genotyped using DNA extracted from the 2 mm ear biopsy resulting from the ear punches.

b. Will external identification be replaced if it falls off/out? If yes, describe the plan for replacement:

Ear tags will be replaced if they fall out.

c. Will external identification be removed as part of the protocol (e.g., radio collars on field animals)? If yes, describe the plan for removal:

No

4. Identify strain/stock for rodents and genetically modified animals:

Species	Is USDA Species	Strain	Genetically Modified Strain	Phenotype Description

	Species	Is USDA Species	Strain	Genetically Modified Strain	Phenotype Description
View	Mice	no	C57BL/6J, C57BL/6NTac, C57BL/6NCrl (C57BL/6)	yes	Thy1-YFP on C57Bl/6J bckg - From Rachel Wong's protocol :4122-01 Phenotype- subset of cells in retina and nervous systm expressing YFP, no adverse phenotypes. Opn5Cre;Ai14 on C57Bl/6J bckg .From Ethan Buhr's protocol : 4184-03 Phenotype - Opn5 expressing cells fluoresce red in the presence of green light. There is no behavioral or health phenotype. C57Bl/6J - Wild type from Maureen Neitz's protocol.

Animal Number Adjustments

"Animals Identified in Experiments" is the total number of animals per pain category listed in all experiments on this protocol. If more or fewer animals will be used on the protocol (see Help Text for examples), click Update to enter this new number in the corresponding "Adjusted Animal Count" column. **Only input numeric values in this field; 0 is acceptable.**
If no adjustment is required, the values in the "Animals Identified in Experiments" and "Adjusted Animal Count" columns must match. Click Update in each Pain Category row to input the matching value.
For questions about adjusting animal numbers, contact OAW.

1. * Click Update to adjust the number of animals to be used or produced for this protocol:

	Species	USDA Covered Species	Pain Category	Animals Identified in Experiments	Adjusted Animal Count
View	Mice	no	Pain Category B	0	0
View	Mice	no	Pain Category C	0	0
View	Mice	no	Pain Category D	45	45
View	Mice	no	Pain Category E	0	0

2. If you adjusted the number of animals for this protocol, explain why:

N/A

3. If you will be using animals to train personnel or to practice procedures included in this protocol, describe below:

N/A

4. Supporting documents:

Document Name	Date Modified
There are no items to display	

Alternatives and Duplication Searches

Display Procedures that cause pain or distress: none

1. **Record all searches for any previous research that this protocol might duplicate:**

	Search Date	Searched Databases	Other
View	1/17/2019	EMBASE (searches multiple databases) PubMed/Medline	

2. **Briefly describe the results of your searches and why you can or cannot incorporate the findings. Or, if a literature search was not performed, describe the methods used to determine that alternatives are not available or feasible:**

   The goal of this research is to develop an imaging tool that can be used in live animals to monitor over time disease mechanisms and responses to therapies. By necessity, this must be done in live animals.

   The search results demonstrated a dearth of live imaging modalities for mice retina. They also support our methods as the state-of-the-art for imaging mice retina.

3. **Confirm that you have made every effort to ensure that this protocol is not unnecessary duplication of previous research:** ☑

Housing and Use

Housing and use outside of the vivarium is not allowed without strong scientific justification.

1. Identify each location where animals will be housed:

	Facility	Species	Justification for Housing Outside Vivarium
View	SLU3.1 ABSL1	Mice	

2. Identify each location where animals will be used:

	Facility	Use	Species	Justification for Use Outside Vivarium
View	SLU3.1 E279	This location will be used for fundus imaging	Mice	This location is in the Neitz lab and houses the micron II camera to be used for fundus imaging. This imaging needs to be conducted in conjunction with high-resolution imaging in E286 under the same anesthetic event. E286 and E279 are along the same hallway and separated by 3 rooms. The instrument in E286 is not portable.

Disposition

1. **Disposition plans for the animals when this research is complete:**
   (check all that apply)
   Euthanasia
2. **If other, provide an animal disposition description:**
3. **If protocol involves fixing tissues, list agents (e.g., paraformaldehyde, formalin):**

Refinement, Replacement and Reduction

1. Describe below how the three R's (refinement, replacement and reduction) have been employed on this project. Include alternatives that were considered for the procedures above that cause pain or distress:

* Refinement (use of methods to decrease animals' sensitivity to pain)

We have proposed methods that rely on non-contact imaging of mouse retina. Experimental methods and anesthesia are optimized to decrease animals' sensitivity to pain. We propose to use a heating pad along with anesthesia to regulate body temperature.

* Replacement (include in vitro tests, use of less sentient animals)

We propose to use mice as a live animal model for studying vision in health and disease. Mice are the lowest vertebrates where photoreceptor and ganglion cell morphology and physiology is accessible. Their visual system is sufficiently similar to that of humans, such that the results can be feasibly correlated to studies involving diseases and their treatments relevant to humans. There remain scientific and clinical questions related to retinal development and repair that are possible only in live models while in vitro and ex vivo assays provide only cross-sectional information. Secondly, the process of excising and fixing tissue for histology can affect its structure and physiology. In vivo imaging in mice overcomes both these problems and opens up a wide range of studies that were otherwise impossible.

* Reduction (use of fewer animals to attain statistical significance)

Our goal is to obtain useful and statistically significant information with minimum number of animals.

2. Describe the rationale for using animals and the appropriateness of the species proposed:

Photoreceptors and ganglion cells are among the most important cell types in the retina for vision and their dysfunction make up the most debilitating of eye diseases. To track the mechanistic implications and time-course of diseases/ treatments, a live animal model is needed. In vitro and ex vivo assays provide only cross-sectional information. The process of excising and fixing tissue for histology can affect its structure and physiology.

The visual system of the mouse has numerous similarities to that of human, and many cell types are homologous. Furthermore, the possibility of transgenic variants make mice one of the most widely used animal model for vision research. For instance, there exist mice models of all major retinal diseases and ones where specific cell types are fluorescently labeled making them exceedingly amenable for live imaging.

Supporting Documents

1. **Attach supporting files:**

Document Name	Date Modified
There are no items to display	

Procedures Appendix:

View: Custom SF: Procedure Identification

Procedure Identification: Sabesan - imaging

1. *** Name of the procedure or surgery:**
   Sabesan - imaging
2. *** Select procedure type:**
   Imaging
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

   If yes,

   i. **Identify expected symptoms from administering this procedure:**
   N/A

   ii. **Identify criteria under which animals will be removed from research:**
   N/A

Imaging

1. **Imaging types:**
   Optical Imaging (e.g., IVIS, 2-Photon)
2. **If Other, specify:**
3. **Select the anesthesia and analgesia procedures to be used:**

Anesthesia, Isoflurane, Long Duration (>1 hour)	Substance Administration	2 Standard
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2 Standard
Anesthesia, Terminal, Tribromoethanol (Avertin)	Substance Administration	2 Standard

4. **Frequency, including minimum time between imaging sessions and the maximum number of sessions (enter specific, detailed procedure timing in the Experiment):**
   Maximum 3 times per animal, 3-7 days apart
5. **Duration of imaging session:**
   Up to 2 hours
6. **Purpose:**
   The purpose of this procedure is to test the feasibility of imaging mice retina in vivo at high resolution using adaptive optics imaging systems.
7. **Will supportive care of animals be necessary during the imaging session?**
   Yes No
8. **If yes, describe:**
   Heating pad

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

Procedure Identification: Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine

1. *** Name of the procedure or surgery:**
   Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

Euthanasia

1. *** Method of euthanasia:**
   Cervical Dislocation

2. **Describe procedure:**

   The mouse is placed in an induction chamber and 1-5% isoflurane (pharmaceutical grade) is administered until the mouse is recumbent. If more than momentary anesthesia is required, the mouse is removed from the chamber and positioned in a nosecone or intubated, with 1-5% isoflurane administered to maintain anesthesia.

   A surgical plane of anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and rate.

   Then, cervical dislocation will be performed. This procedure will only be performed by certified protocol personnel.

   Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

   Waste gas is scavenged using either an activated charcoal canister (e.g., F/Air), active scavenging system, or by conducting the work within a certified fume hood.

   Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

3. *** Will anesthesia be used?** **Yes** No

4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.

5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

Procedure Identification: Anesthesia, Isoflurane, Long Duration (>1 hour)

1. *** Name of the procedure or surgery:**
   Anesthesia, Isoflurane, Long Duration (>1 hour)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Isoflurane	Standard	Inhalation	1-5%	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

The mouse is placed in an induction chamber and 1-5% isoflurane is administered until the mouse is recumbent. The mouse is removed from the chamber and positioned in a nose cone or intubated, with 1-5% isoflurane administered to maintain anesthesia. Adequate depth of anesthesia is monitored by respiratory rate, corneal reflex, and response to toe pinch. Heat support and eye lubrication will be provided. Fluid support will be administered subcutaneously at a rate of approximately 10 microliters of fluid/gram of body weight/hour of anesthesia (10 µL/g/hr). Fluids will consist of pharmaceutical grade isotonic saline or Lactated Ringer's Solution (LRS), warmed to body temperature.

3. Describe the intended effects of administering the substance(s):

General anesthesia

4. Describe any potential adverse reactions to administering the substance(s):

Respiratory depression, hypotension, cardiac arrhythmia

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

Waste gas is scavenged using either an activated charcoal canister (e.g. F/Air), active scavenging system, or by conducting the work within a certified fume hood.

Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create Substance

1. *** Substance:**
   Isoflurane
2. **Route:**
   Inhalation

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   1-5%
4. **Frequency and duration of dosages:**
   Continuous for ≥1 hour (estimated)
5. **Volume (for rodents or intracranial injections):**
   N/A
6. **Concentration:**
   N/A
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Isoflurane is pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

View: Custom SF: Procedure Identification

Procedure Identification: Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia

1. *** Name of the procedure or surgery:**
   Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
N/A

ii. **Identify criteria under which animals will be removed from research:**
N/A

Euthanasia

1. *** Method of euthanasia:**
   Cervical Dislocation
2. **Describe procedure:**

   The mouse is anesthetized with ≥500 mg/kg avertin IP in a volume of not greater than 26 microliters per gram of body weight.

   A surgical plane of anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and rate.

   Then, cervical dislocation will be performed. This procedure will only be performed by certified protocol personnel.
3. *** Will anesthesia be used?** **Yes** No
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

Procedure Identification: Anesthesia, Terminal, Tribromoethanol (Avertin)

1. *** Name of the procedure or surgery:**
   Anesthesia, Terminal, Tribromoethanol (Avertin)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Tribromoethanol (Avertin)	Standard	Intraperitoneal	≥500 mg/kg	N/A	Total volume will not exceed 26 microliters per gram of body weight.	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Avertin is administered IP to induce anesthesia appropriate for a short (<20 minutes) terminal procedure such as perfusion.

Deep anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and decreased respiratory rate.

3. Describe the intended effects of administering the substance(s):

Anesthesia for short (<20 minutes) terminal procedure

4. Describe any potential adverse reactions to administering the substance(s):

N/A

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Needles must not be recapped unless a recapping device is used.

Gloves must be worn when handling this agent.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create Substance

1. *** Substance:**
   Tribromoethanol (Avertin)
2. **Route:**
   Intraperitoneal

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   ≥500 mg/kg
4. **Frequency and duration of dosages:**
   Once
5. **Volume (for rodents or intracranial injections):**
   Total volume will not exceed 26 microliters per gram of body weight.
6. **Concentration:**
   N/A
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Avertin is not available pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

View: Custom SF: Procedure Identification

Procedure Identification: Anesthesia, Isoflurane, Short Duration (<1 hour)

1. *** Name of the procedure or surgery:**
   Anesthesia, Isoflurane, Short Duration (<1 hour)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
N/A

ii. **Identify criteria under which animals will be removed from research:**
N/A

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Isoflurane	Standard	Inhalation	1-5%	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

The mouse is placed in an induction chamber and 1-5% isoflurane is administered until the mouse is recumbent. If more than momentary anesthesia is required, the mouse is removed from the chamber and positioned in a nose cone or intubated, with 1-5% isoflurane administered to maintain anesthesia. Adequate depth of anesthesia is monitored by respiratory rate, corneal reflex, and response to toe pinch. Heat support and eye lubrication will be provided.

3. Describe the intended effects of administering the substance(s):

General anesthesia

4. Describe any potential adverse reactions to administering the substance(s):

Respiratory depression, hypotension, cardiac arrhythmia

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

Waste gas is scavenged using either an activated charcoal canister (e.g., F/Air), active scavenging system, or by conducting the work within a certified fume hood.

Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create Substance

1. *** Substance:**
   Isoflurane
2. **Route:**
   Inhalation

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   1-5%
4. **Frequency and duration of dosages:**
   Continuous for <1 hour (estimated)
5. **Volume (for rodents or intracranial injections):**
   N/A
6. **Concentration:**
   N/A
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Isoflurane is pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

View: Custom SF: Procedure Identification

Procedure Identification: Sabesan team - Micron II fundus imaging

1. *** Name of the procedure or surgery:**
   Sabesan team - Micron II fundus imaging
2. *** Select procedure type:**
   Imaging
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**

ii. **Identify criteria under which animals will be removed from research:**

Imaging

1. **Imaging types:**
   Optical Imaging (e.g., IVIS, 2-Photon)
2. **If Other, specify:**
3. **Select the anesthesia and analgesia procedures to be used:**

Anesthesia, Isoflurane, Long Duration (>1 hour)	Substance Administration	2 Standard
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2 Standard
Anesthesia, Terminal, Tribromoethanol (Avertin)	Substance Administration	2 Standard

4. **Frequency, including minimum time between imaging sessions and the maximum number of sessions (enter specific, detailed procedure timing in the Experiment):**
   Once for every "Adaptive optics imaging in mice in vivo" procedure
5. **Duration of imaging session:**
   5 minutes
6. **Purpose:**
   Frequently, mice spontaneously develop cataracts and retinal degeneration. Both impede the ability to perform high-resolution adaptive optics imaging. The micron II is a commercially available fundus camera that provides a rapid and wide-field view of the mouse retina.
   Before undergoing high-resolution imaging, the mouse eye will be inspected for cataractogenesis and retinal degeneration in the micron II camera. If both are deemed to be optimal (clear ocular media and standard retinal morphology), we will progress forward to high-resolution with the same mice.
7. **Will supportive care of animals be necessary during the imaging session?**
   Yes **No**
8. **If yes, describe:**

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Procedure Identification

Procedure Identification: Sabesan team : Administering topical eye drops

1. *** Name of the procedure or surgery:**
   Sabesan team : Administering topical eye drops
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain or distress?** Yes **No**

   If yes,

   i. **Identify expected symptoms from administering this procedure:**

   ii. **Identify criteria under which animals will be removed from research:**

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Atropine	Standard	Ophthalmic	5-20 microliter of 0.5-1%	0.5-1%	5-20 microliter	N/A
View	Phenylephrine (Metaoxedrin, Metasympatol, Mezaton, Neo-Synephrine)	Standard	Ophthalmic	5 - 20 microliter of 2.5%	2.5%	5 - 20 microliter	N/A
View	Tropicamide (Mydriacyl)	Standard	Ophthalmic	5 - 20 microliter of 0.5-1%	Tropicamide 0.5-1%	5 - 20 microliter of 0.5-1%	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Medication will be transferred to a sterile 1.5 ml microfuge tube and applied topically with a pipetteman using a sterile filter tip.

Tropicamide and Phenylephrine will be mixed together in equal proportion to make 20uL total before applying to the eye. Atropine will not be mixed with the other agents and constitute a maximum of 20ul by itself for applying to eye.

3. Describe the intended effects of administering the substance(s):

Atropine, Phenylephrine, and Tropicamide : Eye dilation

4. Describe any potential adverse reactions to administering the substance(s):

light sensitivity, brief stinging/irritation. No toxic affects are expected for any agents.

5. If working with hazardous agents, protocol personnel will read and follow the Occupational Health Recommendations (OHRs) and Biological Use Authorization letter (BUA), if applicable. The OHRs and the BUA can be found on the protocol workspace.

N/A

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA)**

paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu**.**

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
Sabesan team - atropine	1/16/2019 4:18 PM
Sabesan team - Phenylephrine	1/16/2019 4:14 PM
Sabesan team - tropicamide	1/16/2019 4:14 PM

1. *** Substance:**
   Atropine
2. **Route:**
   Ophthalmic

   If you indicated Other, specify the route:
3. **Dose:**
   5-20 microliter of 0.5-1%
4. **Frequency and duration of dosages:**
   once per procedure
5. **Volume (for rodents or intracranial injections):**
   5-20 microliter
6. **Concentration:**
   0.5-1%
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Pharmaceutical grade
8. **Complication remediation:**
   No expected complications. Visually monitor eye irritation/redness once at the end of procedure
9. **Substance order for the procedure:**
   N/A

1. *** Substance:**
   Phenylephrine (Metaoxedrin, Metasympatol, Mezaton, Neo-Synephrine)
2. **Route:**
   Ophthalmic

   If you indicated Other, specify the route:
3. **Dose:**
   5 - 20 microliter of 2.5%
4. **Frequency and duration of dosages:**
   Once per procedure
5. **Volume (for rodents or intracranial injections):**
   5 - 20 microliter
6. **Concentration:**
   2.5%
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   Pharmaceutical grade
8. **Complication remediation:**
   No expected complications. Visually monitor eye irritation/redness once at the end of procedure
9. **Substance order for the procedure:**
   N/A

View: Custom: Create Substance

1. *** Substance:**
   Tropicamide (Mydriacyl)
2. **Route:**
   Ophthalmic

   If you indicated Other, specify the route:
3. **Dose:**
   5 - 20 microliter of 0.5-1%
4. **Frequency and duration of dosages:**
   Once per procedure
5. **Volume (for rodents or intracranial injections):**
   5 - 20 microliter of 0.5-1%
6. **Concentration:**
   Tropicamide 0.5-1%
7. **Confirm the agents used will be pharmaceutical grade. If you must use non-pharmaceutical grade agents, provide scientific justification for their use and describe how the agent will be prepped and sterilized prior to use:**
   This is pharmaceutical grade
8. **Complication remediation:**
   No expected complications. Visually monitor eye irritation/redness once at the end of procedure
9. **Substance order for the procedure:**
   N/A

Substances Appendix:

View: Custom SF: Substance Information

Substance Information: Isoflurane

1. *** Name:**
   Isoflurane
2. *** Substance types:** (select all that apply)
   Anesthetic
   Reproductive Hazard/Teratogen
3. *** Is this a hazardous agent:** Yes No

   NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions,**

contact EH&S Research and Occupational Safety at 206-221-7770 or ehsbio@uw.edu**.**

4. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Substance Information

Substance Information: Atropine

1. *** Name:**
   Atropine
2. *** Substance types:** (select all that apply)
   Reproductive Hazard/Teratogen
3. *** Is this a hazardous agent:** **Yes** No

   NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**
4. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Substance Information

Substance Information: Phenylephrine (Metaoxedrin, Metasympatol, Mezaton, Neo-Synephrine)

1. *** Name:**
   Phenylephrine (Metaoxedrin, Metasympatol, Mezaton, Neo-Synephrine)
2. *** Substance types:** (select all that apply)
   Reproductive Hazard/Teratogen
   Other
3. *** Is this a hazardous agent:** **Yes** No

   NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

4. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Substance Information

Substance Information: Tribromoethanol (Avertin)

1. *** Name:**
   Tribromoethanol (Avertin)
2. *** Substance types:** (select all that apply)
   Anesthetic
   Reproductive Hazard/Teratogen
3. *** Is this a hazardous agent:** Yes **No**

   NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**
4. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Substance Information

Substance Information: Tropicamide (Mydriacyl)

1. *** Name:**
   Tropicamide (Mydriacyl)
2. *** Substance types:** (select all that apply)
   Paralytic Agent
   Reproductive Hazard/Teratogen
3. *** Is this a hazardous agent:** **Yes** No

   NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**
4. **Supporting documents:**

Document Name	Date Modified

Document Name	Date Modified
There are no items to display	

1. *** Select the funding organization:**
   Ophthalmology

   If Other was selected in question 1, provide Funding Organization:

2. *** All animal use projects must be reviewed for scientific merit prior to initiating animal use. Choose the required reviews for this project:**
   Has been conducted by my department or school and has been found to be scientifically meritorious

3. **Provide name of the committee or the department reviewer** (Required if "Has been conducted by my department or school and has been found to be scientifically meritorious" was selected):
   Jennifer Chao, Vice-Chair Research, UW Ophthalmology

4. **eGC1 Number(s):**(assigned internally)

Experiments Appendix:

Determining the effect of anesthesia and fluorescent labels for imaging cellular structures in living mice retina

1. *** Experiment name:**
   Determining the effect of anesthesia and fluorescent labels for imaging cellular structures in living mice retina
2. *** Species:**
   Mice
3. **If other was selected, provide a species:**
4. **What is the scientific goal of this experiment:**
   This study will use a specialized retina camera to investigate whether the morphology and function of individual retinal cells and their connections are resolvable and visualizable in vivo in mice. Currently, imaging technologies do not have access to cellular level detail in mice in vivo. Therefore, in order to visualize cellular morphology, animals need to be sacrificed and the tissue is thereafter sent for histology . Our goal is to develop and refine imaging technology such that the same level of cellular detail is achievable in vivo.

   In this experiment, we will test how imaging cellular structures in the mouse retina is a) affected by different anesthetics (isoflurane vs avertin) and b) affected by fluorescently labeling specific cell types for imaging and compare against wild-type mouse.
5. *** Describe the animal experience in the experiment, from enrollment in the study to the final endpoint, including all procedures in chronological order and the minimum time between procedures. We encourage using bullet points, timeline, table, or a flow chart as appropriate:**

   There will be two experimental groups. Both groups will follow the same experimental protocol except for anesthesia. One group will be anesthetized using Avertin and the other using Isofluorane. This is required because depending on the anesthetic, the physiology of the mouse eye may be variable and present different challenges for imaging. Second, we plan to longitudinally follow up imaging in the same animal using isofluorane in future studies while employing Avertin for a one-time imaging session before euthanasia.

   Most important of all physiological changes following anesthesia administration is the development of cataracts that can obscure non-invasive in vivo optical imaging in mice through the intact eye. According to this conference abstract (https://www.aaopt.org/detail/knowledge-base-article/cataractogenesis-anesthetized-mice), Avertin leads to similar cataractogenesis as ketamine/xylazine after 10-20 minutes of administration. No published records exist following up cataractogenesis after Avertin with care given to hydration and regulating body temperature. This information will be important for future studies to gauge whether for one-time imaging, Avertin is a suitable anesthetic.

   Step 1: Mice will be obtained from the protocols of Rachel Wong, Russell Van Gelder (Ethan Buhr) and Maureen Neitz. These mice will vary between wild type and mice where cone photoreceptors and retinal ganglion cells are tagged with

fluorescent labels. Cones will be tagged with red fluorescent proteins (RFP) while ganglion cells are labeled with yellow fluorescent protein (YFP).

Step 2: Animals will be first dilated with topical eye drops and then anesthetized with one of either Avertin or isofluorane. We will use a heating pad along with anesthesia to regulate body temperature. The heating pad will not be in direct contact with the animal. We will have a surgical drape or blue pad between the mouse and heating pad.

Step 3: genteal or other artificial tears will be applied to the mouse cornea to keep it moist.

Step 4: Take retinal images with a commercial mouse fundus camera

Step 5: Take retinal images with custom-built adaptive optics camera. Apply genteal on the eye when required as deemed by image quality degradation. Retinal images include images of photoreceptors, ganglion cells, nerve fiber, optic nerve head, blood vessels.

Step 6: If isofluorane was used to anesthetize, place animal back in the vivarium. Follow up from step 2, for a maximum of 3 times, 3-7 days apart. Once the imaging is complete, carry out euthanasia with cervical dislocation. If Avertin was used to anesthetize, carry out euthanasia with cervical dislocation once the imaging is complete.

Animal Sex:
Female
Male

Animal Ages:
6 weeks or older

Animal Size:

6. Select experimental procedures:

Name	Type	Version	Scope
Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine	Euthanasia	1	Standard
Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia	Euthanasia	1	Standard
Sabesan - imaging	Imaging	1	Team
Sabesan team - Micron II fundus imaging	Imaging	1	Team
Anesthesia, Isoflurane, Long Duration (>1 hour)	Substance Administration	2	Standard
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2	Standard
Anesthesia, Terminal, Tribromoethanol (Avertin)	Substance Administration	2	Standard
Sabesan team : Administering topical eye drops	Substance Administration	1	Team

7. **Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):**
The mice will be monitored daily for eye irritation, activity levels and posture for 3 weeks. Vet services will be consulted for concerns.

Signs to note include lethargy, failure to eat and drink leading to greater than 20% weight loss, open wounds, scruffy fur or development of non-experimental conditions such as tumors and/or illnesses. If these signs are observed (regardless of the duration), we will contact Vet Services and euthanize if advised. Method of euthanasia is cervical dislocation under anesthesia.

8. If there is expected mortality (spontaneous death) in this experiment:

a. Procedure/condition associated with mortality:
No

b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):
N/A

c. Explain why euthanasia is not possible or appropriate:
N/A

9. **Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.**
No

10. *** Total number of animals used in this experiment:**(including all the animals to be produced)
45

a. Justify total number of animals used in this experiment:

The three variables we need to account for in deducing the optimal number of animals is 1) cataractogenesis in mice, b) fluorescence (red-fluorescent - RFP and yellow-fluorescent protein (YFP) labels) vs. non-fluorescence imaging, c) inter-mice variability in imaging parameters.

The natural prevalence of cataracts in mice is 5% while under Ketamine/xylazine, the frequency of corneal opacities is reported to be as high as 42% (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4519051/). With Avertin, the results are similar. Mice are significantly less prone to cataract formation under Isoflurane. For establishing instrument parameters for single time point imaging, we will require mice with clear optical media (cornea and lens). The mouse eye has substantially different optical properties than that of humans. In particular, the numerical aperture (NA) of the mouse eye is 2.5x that of humans. This larger NA is advantageous for obtaining better spatial resolution, although, it also leads to large aberrations that need to be measured and compensated appropriately to obtain sharp images of cellular features within the retina. The eyeball is significantly smaller than a human and requires careful opto-mechanical alignment to the instrument in order to ensure good performance. Due to the propensity of developing cataracts soon after anesthesia, temperature and corneal hydration need to be maintained at levels that allow for imaging with relatively clear ocular media.

A sample size analysis based on the anticipated mean and standard deviation of previously obtained measurements based on inter-mice variability in imaging parameters indicate that 4 mice will be sufficient to establish significance ($p \leq 0.001$) based on 90% power. This analysis does not account for 42% cataractogenesis under Ketamine/xylazine and avertin. Accounting for cataractogenesis, this leads to 8 animals each for fluorescence and non-fluorescence conditions. Therefore in total, we expect to use 24 animals under single time point imaging under Avertin anesthesia (8 wild type, 8 YFP, 8 RFP, 24 total).

For longitudinal imaging, using isoflurane, we expect that the incidence of cataracts would be reduced. However, we expect two challenges that may govern animal numbers. First, the nose cone for administering isoflurane may impede imaging by imposing mechanical constraints on the light entering the mouse eye's pupil. Second, for following up the same retinal areas with time, we expect that we may face challenges in positioning the mice retina at the same spatial coordinates with time. We have considered the use of 3 different nose cone designs and plan to test each for optimality. These designs are based on achieving the least invasive footprint and interference with the imaging light. Additionally, we have designed and built an optomechanical mount with fine adjustment capability for navigation across the retinal field. In this pilot experiment, we plan to test one nose cone design on one animal within each group (3 wild type, 3 YFP, 3 RFP, 9 total). While we expect that the small anatomical variation in the facial structure of the mice may require more animals to be tested, we consider this first round of experiments with the nose cones as a pilot study with the goal to refine the design further in the future based on observations made here. Once the parameters are finalized, we estimate needing 12 animals (4 wild type, 4 YFP, 4 RFP, 12 total) for training additional lab personnel on conducting the entire procedure. Therefore, in summary, we estimate needing 45 animals total.

11. Number of animals by pain and distress category:(include each animal only once in the highest pain category)

B: 0
C: 0
D: 45
E: 0

a. Justify the need for any animals in pain category E:

12. * Identify husbandry exceptions:

	Exception Type	Description and Justification
View	Mice - No husbandry or enrichment exceptions.	

13. Supporting documents:

Document Name	Date Modified
There are no items to display	

1. * Exception type:

Mice - No husbandry or enrichment exceptions.

2. Description and justification:

1. * Identify the location where animals will be used:

SLU3.1 ABSL1

a. For locations that are lab managed, provide justification for housing outside of the vivarium:

2. * What species will be housed in this location?

Common Name	Scientific Name
Mice	Mus

1. Campus:

Vivarium

2. Vivarium:

SLU 3.1: 0 Level Vivarium

3. * BSL Level:

SLU3.1 ABSL1

1. * Identify the location where animals will be used:

SLU3.1 E279

a. For locations that are outside of the vivarium, provide justification for the use of this space:

This location is in the Neitz lab and houses the micron II camera to be used for fundus imaging. This imaging needs to be conducted in conjunction with high-resolution imaging in E286 under the same anesthetic event. E286 and E279 are along the same hallway and separated by 3 rooms. The instrument in E286 is not portable.

2. * What species will be used in this location?

Common Name	Scientific Name
Mice	Mus

3. Describe how this location will be used:

This location will be used for fundus imaging

4. * If animals are left unattended in this location, provide an explanation and include maximum duration:

Not left unattended.

5. Describe how animals will be transported to and from this location, including container and route. (Note: use of private vehicles requires IACUC approval):

Mice will be transported from the vivarium in covered cages with filter tops. The shortest route up to E279 is via the direct elevator from the vivarium up to second floor. Then we will follow the north-south hallway on the eastern side of SLU3.1 to E279.

1. **Campus:**
   Lab-Managed
2. **Vivarium:**
   South Lake Union
3. *** BSL Level:**
   SLU 3.1 Laboratory Spaces
4. *** Room:**
   SLU3.1 E279

From: Michelle Brot <mbrot@uw.edu>
Sent: Tuesday, February 26, 2019 9:52 AM
To: Ramkumar Sabesan
Subject: Protocol reminder

Hi Ram,
Have you made any progress on your protocol? No rush on my end, but just wanted to remind you in case you meant to submit it back.

Thanks,
Michelle

From: Michelle Brot <mbrot@uw.edu>
Date: Thursday, February 14, 2019 at 10:03 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Subject: Re: PROTO201800103: Notification of Requested Clarifications

Hi Ram,
Thanks for checking in about your questions/comments. The approach you suggested will work but I like a different approach better:

1. Using the reviewer notes section, note which pages contain the questions. Most of the questions (maybe all) will be on your Experiments page, so I¹ll use that as an example. So click ³Edit Protocol² on the left side of the ³flow chart page" and then use the Jump To menu to get to the Experiments page (be aware that you probably have to click through two Jump To menus to get to it).
2. Then, you¹ll see the Questions at the top of the Experiments page. To make it easy to see the questions and to address them in the experiment, I recommend opening a second copy of the protocol and have them side-by-side. You can look at the questions on the left and address them in the protocol on the right, for example. It won¹t mess anything up by having two open at once.
3. Make whatever changes you need to in the text of the protocol, remembering to hit OK at the bottom of any pop-up window, such as the experiment pop‹up and Save at the top of any page once you¹ve made changes.
4. Then indicate in the ³Click here to respond² section what changes you made to address the question.
5. Once you¹re done responding to the questions in all sections, click ³Submit² on the left on the ³flow chart page.² You get there by clicking ³Exit² from any page.

One thing I¹ve found very helpful is to right click to open a procedure in a new tab. Otherwise, it replaces the screen you¹re working in and you have to hit the back button to return to that screen. This way, you can close the procedure and still have the other screen there (or you can click back and forth between them).

Feel free to call me if any questions arise as you¹re working on this: 206-221-0891.

Take care,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Thursday, February 14, 2019 at 9:34 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Fwd: PROTO201800103: Notification of Requested Clarifications

Hi Michelle,

I am looking into these clarification. Before I get too far ahead into the corrections, I just want to clarify if I am following the right process in Hoverboard to do this.

Let me know if this is the right approach to address these :
- I click on Reviewer Notes.
- For each clarification point, I click on the section, say "Experiments", "Procedural Personnel assignment", "animal details" and address the clarification.
- Once the clarification is addressed within its section, I click on "click here to respond" and say what steps I followed to respond to the clarification.

Does this seem like it is the correct approach ? I am not all that familiar with Hoverboard , just wanted to clarify before I get too deep into it.

Thanks

-Ram

---------- Forwarded message ---------
From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

--

Ramkumar Sabesan, Ph.D.
Assistant Research Professor
Ophthalmology
Bioengineering (Adj.)
Biological Structure (Adj.)
University of Washington
750 Republican St, E213
Seattle WA 98109
Email : rsabesan@uw.edu
Web: depts.washington.edu/sabaolab
Tel: 206-221-4925 Fax:206-685-9315

From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Tuesday, October 23, 2018 7:10 PM
To: Michelle Brot; Rachel O. Wong
Subject: Protocol
Attachments: Protocol_AO_imaging_mice_v2.pdf

Hi Michelle,

I am attaching a draft of the protocol for conducting these imaging studies in my lab at SLU.

We think that at this point, some feedback from you would be very helpful. Really appreciate it ! Please let me know if there are questions or concerns.

-Ram

--

Ramkumar Sabesan, Ph.D.
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Tel: 206-221-4925 Fax:206-685-9315

HoverBoard

Date: Tuesday, October 23, 2018 7:04:01 PM Print Close

View: SF: Basic Information

Basic Information

1. *** Select research team:**
   Sabesan team
2. *** Title of protocol:**
   Testing the feasibility of in vivo high-resolution retinal imaging in mice
3. *** Short title:**
   In vivo retinal imaging in mice
4. *** Summary of research:**
   The mouse is an excellent model for studying the retina. This research is aimed at testing the feasibility of imaging the cellular structures within the mouse retina in vivo using a specialized camera.

   The primary objective is to investigate the feasibility of visualizing different retinal cell types and their connections without and with fluorescent markers.

   The general approach is to use a custom-built camera to take high resolution pictures of the mouse retina through the intact eye and in a non-contact fashion.
5. *** Principal investigator:**
   Sabesan Ram Kumar
6. *** What is the intention of the animal protocol?**
   Experimental Research

View: SF: Experimental Research Protocol Addition

Experimental Research Protocol Addition

1. * **Will the protocol include breeding?**
   ○ Yes ● **No**

View: Custom SF: Protocol Team Members

Protocol Team Members

1. Identify each additional person involved in the design, conduct, or reporting of the research:

Name	Role	Involved in Animal Handling	Authorized To Order Animals	E-mail	Phone
Xiaoyun Jiang	Postdoctoral Fellow	yes	yes	jxyun@uw.edu	+1 425 499-0713
Sabesan Ram Kumar	Other	no	yes	rsabesan@uw.edu	+1 206 221-4925
Owen T Lawrence	Research Scientist	yes	yes	olawr@uw.edu	+1 206 543-6651
Maureen Neitz	Co-Investigator	yes	yes	mneitz@uw.edu	+1 206 543-7998
Rachel O Wong	Co-Investigator	yes	yes	wongr2@uw.edu	+1 206 616-3275

2. If veterinary care will be provided by individuals outside of DCM or WaNPRC, provide the name, credentials and contact information below:

View: Custom SF: Funding Sources

Funding Sources

1. Identify each organization supplying funding for the protocol:

Funding Organization	eGC1 Number(s)
There are no items to display	

View: Custom SF: Scientific Aims

Scientific Aims

1. * Scientific aims of the research:

The goal of this research is to

1) modify an existing adaptive optics retinal camera for in vivo retinal imaging in mice. The mouse eye presents unique challenges for retinal imaging. Modifying our existing camera to suit these requirements and testing/refining them is the first aim of this project.

2) investigate the feasibility of in vivo imaging of retinal structures in mice with and without fluorescent reporters. These structures are otherwise obscured by the optics of the intact mouse eye. Currently, the only way to visualize these structures are via ex vivo preparations.

2. * Using language understandable to non-scientists, describe the goals and significance of the protocol to humans, animals and science:

The goal of this project is to a) equip a non-contact, non-invasive retinal camera with capabilities to image the mouse retina in vivo and b) test the feasibility of this camera to capture cellular level detail in the morphology and function of retinal cells.
The mouse is a widely used animal model for diseases of the visual system. Developing and testing technology that allows visualizing mice retinal cells non-invasively in health, in disease and to monitor effectiveness of potential therapies is critical. Achieving this capability extends the scope of basic research into mechanism of vision as well as clinical translation, especially in cases where a live model and longitudinal follow up is required.

3. * Provide a statement to address the potential harm to the animals on this study (e.g., pain, distress, morbidity, mortality) relative to the benefits to be gained by performing the proposed work:

The benefit of this research is that it will lead to new insight into mechanisms of normal vision, help design, test and refine new therapies for preventing and restoring vision from human retinal diseases.

Mice will not experience any more pain or distress than that associated with administering anesthesia and dilation drops. To prevent drop in body temperature following anesthesia, a heating pad will be used to regulate temperature. The heating pad will not be in direct contact with the animal. The camera that we will use to take pictures of the mice retina is non-contact and routinely used for human retinal imaging. Therefore the potential harm to the animal is minimal.

View: Custom SF: Experiments

Experiments

Note: If you will be administering cells, cell lines, sera or other biologicals to rodents, contact the Rodent Health Monitoring Program (RHMP, rhmp@uw.edu). Testing may be required prior to administration to rodents.

1. * Define the experiments to be used in this protocol:

Name	Species	USDA	Count	Count by Pain Category	Procedures	Husbandry Exception Types
Adaptive optics imaging in mice in vivo	Mice	no	30	B: 0 C: 0 D: 30 E: 0	▪ Euthanasia: Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine (Standard) ▪ Euthanasia: Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia (Standard) ▪ Euthanasia: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution (Standard) ▪ Euthanasia: Cervical Dislocation, Unanesthetized (Standard) ▪ Imaging: Sabesan - imaging (Team) ▪ Imaging: Sabesan team - Micron II fundus imaging (Team) ▪ Substance Administration: Anesthesia, Isoflurane, Long Duration (>1 hour) (Standard) ▪ Substance Administration: Anesthesia, Isoflurane, Short Duration (<1 hour) (Standard) ▪ Substance Administration: Anesthesia, Terminal, Tribromoethanol (Avertin) (Standard)	Mice - No husbandry or enrichment exceptions.

2. Will any single animal undergo more than one survival surgery? (include any animal that underwent surgery prior to use on this protocol) ○ Yes ● No

View: SF: Procedure Personnel Assignment

Procedure Personnel Assignment

1. * Select the team members who will be performing each procedure:

Procedure	Species	Is USDA Species	Team Members
Euthanasia: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution, ver. 1 (Standard)	Mice	no	Maureen Neitz Xiaoyun Jiang
Euthanasia: Cervical Dislocation, Unanesthetized, ver. 1 (Standard)	Mice	no	Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Euthanasia: Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine , ver. 1 (Standard)	Mice	no	Xiaoyun Jiang
Euthanasia: Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia , ver. 1 (Standard)	Mice	no	Xiaoyun Jiang
Imaging: Sabesan - imaging, ver. 1 (Team)	Mice	no	Maureen Neitz Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Imaging: Sabesan team - Micron II fundus imaging, ver. 1 (Team)	Mice	no	Maureen Neitz Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Substance Administration: Anesthesia, Isoflurane, Long Duration (>1 hour), ver. 2 (Standard)	Mice	no	Maureen Neitz Owen T Lawrence Xiaoyun Jiang Rachel O Wong

Procedure	Species	Is USDA Species	Team Members
Substance Administration: Anesthesia, Isoflurane, Short Duration (<1 hour), ver. 2 (Standard)	Mice	no	Maureen Neitz Owen T Lawrence Xiaoyun Jiang Rachel O Wong
Substance Administration: Anesthesia, Terminal, Tribromoethanol (Avertin), ver. 2 (Standard)	Mice	no	Maureen Neitz Owen T Lawrence Xiaoyun Jiang Rachel O Wong

2. Team member training:

First Name Last Name Training

Xiaoyun Jiang

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	5/13/2016	5/13/2021
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	5/1/2016	5/1/2019
Annual Animal Use Training for Rodent Users	General	Online	Basic Course	Stage 1	10/4/2018	10/31/2019
Cervical Dislocation, Mouse Anesthetized	Procedure	In Person	Basic Course	Stage 1	5/23/2016	
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	7/6/2016	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/23/2016	
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	6/1/2016	
SLU 3.1 Facility Orientation	Orientation	In Person	Basic Course	Stage 1	10/9/2018	

No experience data to display

Sabesan Kumar Ram

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	1/5/2018	1/5/2023
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	1/5/2018	1/31/2021

No experience data to display

Sabesan Kumar Ram

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	1/5/2018	1/5/2023
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	1/5/2018	1/31/2021

No experience data to display

Owen T Lawrence

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
6th Floor Facility Orienation, Rodent Users	Orientation	In Person	Basic Course	Stage 1	1/16/2002	
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	3/10/2016	3/10/2021
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	9/26/2016	9/30/2019
Annual Animal Use Training for Rodent Users	General	Online	Basic Course	Stage 1	11/11/2017	11/30/2018
Cervical Dislocation, Mouse Anesthetized	Procedure	In Person	Basic Course	Stage 1	11/17/2017	
Cervical Dislocation, Mouse Unanesthetized	Procedure	In Person	Basic Course	Stage 1	1/30/2018	
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	5/21/2018	
Lab-Managed Animal Care & Records	General	In Person	Basic Course	Stage 1	3/2/2018	
Lab-Managed Aquatic Animal Care & Records	General	In Person	Basic Course	Stage 1	5/27/2014	
Lab-Managed Sick Rodent Recognition	General	In Person	Basic Course	Stage 1	3/2/2018	

No experience data to display

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	5/14/2013	
Rat Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	4/15/1999	

Maureen Neitz

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	7/24/2018	7/24/2023
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	4/25/2017	4/30/2020
Annual Animal Use Training for Rodent Users	General	Online	Basic Course	Stage 1	8/2/2018	8/31/2019
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	1/30/2009	
Lab-Managed Animal Care & Records	General	In Person	Basic Course	Stage 1	10/21/2009	
Lab-Managed Sick Rodent Recognition	General	In Person	Basic Course	Stage 1	10/21/2009	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	12/28/2010	
SLU 3.1 Facility Orientation	Orientation	In Person	Basic Course	Stage 1	9/23/2013	
WaNPRC Necropsy Room Clearance	Orientation	In Person	Basic Course	Stage 1	9/5/2016	9/5/2017
WaNPRC Surgery Suite Clearance	Orientation	In Person	Basic Course	Stage 1	7/21/2016	7/21/2017

No experience data to display

Rachel Wong O

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
6th Floor Facility Orienation, Rodent Users	Orientation	In Person	Basic Course	Stage 1	7/18/2006	

No experience data to display

Course	Category	Source	Stage	Stage Number	Completion Date	Expiration Date
Animal Use Laws & Regulations	General	Online	Basic Course	Stage 1	1/4/2016	1/4/2021
Animal Use Medical Screening	General	Online	Basic Course	Stage 1	12/21/2017	12/31/2020
Annual Animal Use Training for Rodent Users	General	Online	Basic Course	Stage 1	7/30/2018	7/31/2019
Foege Facility Orientation	Orientation	In Person	Basic Course	Stage 1	8/18/2014	
Lab-Managed Aquatic Animal Care & Records	General	In Person	Basic Course	Stage 1	8/15/2006	
Mouse Hands-On Laboratory	Animal Handling	In Person	Basic Course	Stage 1	8/23/2011	
T-Wing Facility Orientation	Orientation	In Person	Basic Course	Stage 1	8/6/2013	

View: Custom SF: Animal Details

Animal Details

1. * How are animals acquired?

Transferred from another UW Protocol

2. Describe the acquisition for:

a. * Transferred from another UW Protocol, provide Protocol Number:

4206-01(Neitz) and 4122-01(Wong)

b. * If animals were transferred, please provide an explanation of all procedures the animal(s) experienced prior to transfer (i.e substance administrations, surgeries, dietary restrictions, etc.):

No procedures.

Wong lab animals will be B6.Cg-Tg(Thy1-YFP) mice. Their strain/stock information is contained in protocol 4122-01

Netiz protocol includes breeding and some of the animals are euthanized at the time of weaning. These animals would be transferred, not the animals that have been involved in other procedures.

3. Identification of individual animals (other than cage cards)

a. Method(s) (e.g., ear punch/tag, tattoo, tagging/banding, radio collar, etc.) (Note: If method is implantation (e.g. PIT tag), create or select an Implant procedure to describe the details. If method is surgical (e.g., satellite tag), create or select Survival Surgery procedure to describe the details):

Neitz lab ear tag and Wong lab toe-tattoos

b. Will external identification be replaced if it falls off/out? If yes, describe the plan for replacement:

Ear tags will be replaced if they fall out.

c. Will external identification be removed as part of the protocol (e.g., radio collars on field animals)? If yes, describe the plan for removal:

No

4. Identify strain/stock for rodents and genetically modified animals:

Species	Is USDA Species	Strain	Genetically Modified Strain	Phenotype Description

There are no items to display

View: Custom SF: Animal Number Adjustments

Animal Number Adjustments

"Animals Identified in Experiments" is the total number of animals per pain category listed in all experiments on this protocol. If more or fewer animals will be used on the protocol (see Help Text for examples), click Update to enter this new number in the corresponding "Adjusted Animal Count" column. **Only input numeric values in this field; 0 is acceptable.**
If no adjustment is required, the values in the "Animals Identified in Experiments" and "Adjusted Animal Count" columns must match. Click Update in each Pain Category row to input the matching value.
For questions about adjusting animal numbers, contact OAW.

1. * Click Update to adjust the number of animals to be used or produced for this protocol:

	Species	USDA Covered Species	Pain Category	Animals Identified in Experiments	Adjusted Animal Count
View	Mice	no	Pain Category B	0	0
View	Mice	no	Pain Category C	0	0
View	Mice	no	Pain Category D	30	5
View	Mice	no	Pain Category E	0	0

2. If you adjusted the number of animals for this protocol, explain why:

N/A

3. If you will be using animals to train personnel or to practice procedures included in this protocol, describe below:

N/A

4. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom SF: Alternatives

Alternatives

Display Procedures that cause pain or distress:
none

1. **Date of alternative search for procedures causing pain or distress:**
2. **Databases searched (select more than one):**
   There are no items to display
   If other, provide databases searched:
3. **Describe the search strategy used:**
4. **Time period covered by search (for triennial reviews, the start date should be the date of your last approval):**
   Start Date:

   End Date:
5. **Briefly describe the results of your searches and why you can or cannot incorporate the findings. Or, if a literature search was not performed, describe the methods used to determine that alternatives are not available or feasible:**
   The goal of this research is to develop an imaging tool that can be used in live animals to monitor over time disease mechanisms and responses to therapies. By necessity, this must be done in live animals.

View: Custom SF: Refinement, Replacement and Reduction

Refinement, Replacement and Reduction

1. Describe below how the three R's (refinement, replacement and reduction) have been employed on this project. Include alternatives that were considered for the procedures above that cause pain or distress:

* Refinement (use of methods to decrease animals' sensitivity to pain)

We have proposed methods that rely on non-contact imaging of mouse retina. Experimental methods and anesthesia are optimized to decrease animals' sensitivity to pain. We propose to use a heating pad along with anesthesia to regulate body temperature. The heating pad will not be in direct contact with the animal.

* Replacement (include in vitro tests, use of less sentient animals)

We propose to use mice as a live animal model for studying vision in health and disease. Mice are the lowest vertebrates where photoreceptor and ganglion cell morphology and physiology is accessible. Their visual system is sufficiently similar to that of humans, such that the results can be feasibly correlated to studies involving diseases and their treatments relevant to humans. There remain scientific and clinical questions related to retinal development and repair that are possible only in live models while in vitro and ex vivo assays provide only cross-sectional information. Secondly, the process of excising and fixing tissue for histology can affect its structure and physiology. In vivo imaging in mice overcomes both these problems and opens up a wide range of studies that were otherwise impossible.

* Reduction (use of fewer animals to attain statistical significance)

Our goal is to obtain useful and statistically significant information with minimum number of animals.

2. Describe the rationale for using animals and the appropriateness of the species proposed:

Photoreceptors and ganglion cells are among the most important cell types in the retina for vision and their dysfunction make up the most debilitating of eye diseases. To track the mechanistic implications and time-course of diseases/ treatments, a live animal model is needed. In vitro and ex vivo assays provide only cross-sectional information. The process of excising and fixing tissue for histology can affect its structure and physiology.

The visual system of the mouse has numerous similarities to that of human, and many cell types are homologous. Furthermore, the possibility of transgenic variants make mice one of the most widely used animal model for vision research. For instance, there exist mice models of all major retinal diseases and ones where specific cell types are fluorescently labeled making them exceedingly amenable for live imaging.

View: Custom SF: Housing and Use

Housing and Use

Housing and use outside of the vivarium is not allowed without strong scientific justification.

1. Identify each location where animals will be housed:

	Building	Room	Species	Justification for Housing Outside Vivarium
View	SLU 3.1: 0 Level Vivarium	Vivarium ABSL1	Mice	

2. Identify each location where animals will be used:

	Building	Room	Use	Species	Justification for Use Outside Vivarium
View		SLU3.1 E286	This location will be used to administer anesthesia and carry out the imaging experiments.	Mice	The specialized instrument to image the mice retina in vivo is built on a 4 ft by 8ft optical table in Rm E286, SLU3.1. It has components and design constraints that govern its size and hence restrict it from transported to the vivarium.
View	SLU 3.1 Laboratory Spaces	E279	This location will be used for fundus imaging	Mice	This location is in the Neitz lab and houses the micron II camera to be used for fundus imaging. This imaging needs to be conducted in conjunction with high-resolution imaging in E286 under the same anesthetic event. E286 and E279 are along the same hallway and separated by 3 rooms. The instrument in E286 is not portable.

View: Custom SF: Disposition

Disposition

1. **Disposition plans for the animals when this research is complete:**
   (check all that apply)
   Euthanasia
2. **If other, provide an animal disposition description:**
3. **If protocol involves fixing tissues, list agents (e.g., paraformaldehyde, formalin):**

View: SF: Supporting Documents

Supporting Documents

1. **Attach supporting files:**

Document Name	Date Modified
There are no items to display	

Procedures Appendix:

View: Custom SF: Procedure Identification

Procedure Identification: Sabesan - imaging

1. *** Name of the procedure or surgery:**
   Sabesan - imaging
2. *** Select procedure type:**
   Imaging
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

   If yes,

   i. **Identify expected symptoms from administering this procedure:**
   N/A

   ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Imaging

Imaging

1. **Imaging types:**
   Optical Imaging (e.g., IVIS, 2-Photon)
2. **If Other, specify:**
3. **Select the anesthesia and analgesia procedures to be used:**

Anesthesia, Isoflurane, Long Duration (>1 hour)	Substance Administration	2 Standard
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2 Standard
Anesthesia, Terminal, Tribromoethanol (Avertin)	Substance Administration	2 Standard

4. **Frequency, including minimum time between imaging sessions and the maximum number of sessions (enter specific, detailed procedure timing in the Experiment):**
   Maximum 3 times per animal, 3-7 days apart
5. **Duration of imaging session:**
   2 hours
6. **Purpose:**
   The purpose of this procedure is to test the feasibility of imaging mice retina in vivo at high resolution using adaptive optics imaging systems.
7. **Will supportive care of animals be necessary during the imaging session?**
   Yes No
8. **If yes, describe:**
   Heating pad

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
Artificial tears - generic	10/11/2018 12:36 PM
Sabesan Team - cyclopentolate	10/11/2018 12:37 PM
Sabesan team - phenylephrine	10/11/2018 12:37 PM
Sabesan Team - tropicamide	10/11/2018 12:36 PM
Sabesan team- artifical tears	10/11/2018 12:35 PM

View: Custom SF: Procedure Identification

Procedure Identification: Anesthesia, Isoflurane, Long Duration (>1 hour)

1. *** Name of the procedure or surgery:**
   Anesthesia, Isoflurane, Long Duration (>1 hour)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Administration of Substances

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Isoflurane	Standard	Inhalation	1-5%	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

The mouse is placed in an induction chamber and 1-5% isoflurane is administered until the mouse is recumbent. The mouse is removed from the chamber and positioned in a nose cone or intubated, with 1-5% isoflurane administered to maintain anesthesia. Adequate depth of anesthesia is monitored by respiratory rate, corneal reflex, and response to toe pinch. Heat support and eye lubrication will be provided. Fluid support will be administered subcutaneously at a rate of approximately 10 microliters of fluid/gram of body weight/hour of anesthesia (10 µL/g/hr). Fluids will consist of pharmaceutical grade isotonic saline or Lactated Ringer's Solution (LRS), warmed to body temperature.

3. Describe the intended effects of administering the substance(s):

General anesthesia

4. Describe any potential adverse reactions to administering the substance(s):

Respiratory depression, hypotension, cardiac arrhythmia

5. For hazardous agents, describe potential danger to humans, precautions to protect personnel and containment requirements:

Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

Waste gas is scavenged using either an activated charcoal canister (e.g. F/Air), active scavenging system, or by conducting the work within a certified fume hood.

Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

View: SF: Procedure Documents

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create Substance

1. *** Substance:**
   Isoflurane
2. **Route:**
   Inhalation

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   1-5%
4. **Frequency and duration of dosages:**
   Continuous for ≥1 hour (estimated)
5. **Volume (for rodents):**
   N/A
6. **Concentration:**
   N/A
7. **Non-pharmaceutical justification, if appropriate:**
   Isoflurane is pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

View: Custom SF: Procedure Identification

Procedure Identification: Anesthetic Overdose, Pentobarbital or Pentobarbital Solution

1. *** Name of the procedure or surgery:**
   Anesthetic Overdose, Pentobarbital or Pentobarbital Solution
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

   If yes,

i. Identify expected symptoms from administering this procedure:

N/A

ii. Identify criteria under which animals will be removed from research:

N/A

View: Custom SF: Euthanasia

Euthanasia

1. *** Method of euthanasia:**
   Anesthetic Overdose
2. **Describe procedure:**
   Mice will be injected IP with pentobarbital (Nembutal) or a pentobarbital solution at a dose of at least 270 mg/kg.

   Pentobarbital solution may be diluted with sterile pharmaceutical grade saline, but administered volume will not exceed 10 microliters per gram of body weight.

   Examples of pentobarbital solutions include Beuthanasia, Euthasol and similar solutions containing a mixture of pentobarbital and phenytoin. Dosing is based on the pentobarbital component of the solution.
3. *** Will anesthesia be used?** **Yes** No
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Procedure Identification

Procedure Identification: Cervical Dislocation, Unanesthetized

1. *** Name of the procedure or surgery:**
   Cervical Dislocation, Unanesthetized
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Euthanasia

Euthanasia

1. *** Method of euthanasia:**
   Cervical Dislocation
2. **Describe procedure:**
   Only certified protocol personnel will perform this procedure.
3. *** Will anesthesia be used?** Yes **No**
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Procedure Identification

Procedure Identification: Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine

1. *** Name of the procedure or surgery:**
   Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Euthanasia

Euthanasia

1. *** Method of euthanasia:**
   Cervical Dislocation

2. **Describe procedure:**

   The mouse is placed in an induction chamber and 1-5% isoflurane (pharmaceutical grade) is administered until the mouse is recumbent. If more than momentary anesthesia is required, the mouse is removed from the chamber and positioned in a nosecone or intubated, with 1-5% isoflurane administered to maintain anesthesia.

   A surgical plane of anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and rate.

   Then, cervical dislocation will be performed. This procedure will only be performed by certified protocol personnel.

   Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

   Waste gas is scavenged using either an activated charcoal canister (e.g., F/Air), active scavenging system, or by conducting the work within a certified fume hood.

   Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

3. *** Will anesthesia be used?** **Yes** No

4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.

5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Procedure Identification

Procedure Identification: Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia

1. *** Name of the procedure or surgery:**
   Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia
2. *** Select procedure type:**
   Euthanasia
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Euthanasia

Euthanasia

1. *** Method of euthanasia:**
   Cervical Dislocation
2. **Describe procedure:**

   The mouse is anesthetized with ≥500 mg/kg avertin IP in a volume of not greater than 26 microliters per gram of body weight.

   A surgical plane of anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and rate.

   Then, cervical dislocation will be performed. This procedure will only be performed by certified protocol personnel.
3. *** Will anesthesia be used?** **Yes** No
4. **Describe how death will be confirmed:**
   Death will be confirmed by lack of respirations and heartbeat.
5. **Is this method approved by the AVMA Guidelines on Euthanasia (2013)?**
   Yes No

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Procedure Identification

Procedure Identification: Anesthesia, Isoflurane, Short Duration (<1 hour)

1. *** Name of the procedure or surgery:**
   Anesthesia, Isoflurane, Short Duration (<1 hour)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Administration of Substances

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Isoflurane	Standard	Inhalation	1-5%	N/A	N/A	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

The mouse is placed in an induction chamber and 1-5% isoflurane is administered until the mouse is recumbent. If more than momentary anesthesia is required, the mouse is removed from the chamber and positioned in a nose cone or intubated, with 1-5% isoflurane administered to maintain anesthesia. Adequate depth of anesthesia is monitored by respiratory rate, corneal reflex, and response to toe pinch. Heat support and eye lubrication will be provided.

3. Describe the intended effects of administering the substance(s):

General anesthesia

4. Describe any potential adverse reactions to administering the substance(s):

Respiratory depression, hypotension, cardiac arrhythmia

5. For hazardous agents, describe potential danger to humans, precautions to protect personnel and containment requirements:

Isoflurane is administered using an anesthesia machine that has been adequately tested and certified.

Waste gas is scavenged using either an activated charcoal canister (e.g., F/Air), active scavenging system, or by conducting the work within a certified fume hood.

Isoflurane is an irritant and may cause reproductive problems in women. Refer to Occupational Health Recommendations.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

View: SF: Procedure Documents

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create Substance

1. *** Substance:**
   Isoflurane
2. **Route:**
   Inhalation

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   1-5%
4. **Frequency and duration of dosages:**
   Continuous for <1 hour (estimated)
5. **Volume (for rodents):**
   N/A
6. **Concentration:**
   N/A
7. **Non-pharmaceutical justification, if appropriate:**
   Isoflurane is pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

View: Custom SF: Procedure Identification

Procedure Identification: Sabesan team - Micron II fundus imaging

1. *** Name of the procedure or surgery:**
   Sabesan team - Micron II fundus imaging
2. *** Select procedure type:**
   Imaging
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

   If yes,

i. Identify expected symptoms from administering this procedure:

ii. Identify criteria under which animals will be removed from research:

View: Custom SF: Imaging

Imaging

1. **Imaging types:**
   Optical Imaging (e.g., IVIS, 2-Photon)
2. **If Other, specify:**
3. **Select the anesthesia and analgesia procedures to be used:**

Anesthesia, Isoflurane, Long Duration (>1 hour)	Substance Administration	2 Standard
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2 Standard
Anesthesia, Terminal, Tribromoethanol (Avertin)	Substance Administration	2 Standard

4. **Frequency, including minimum time between imaging sessions and the maximum number of sessions (enter specific, detailed procedure timing in the Experiment):**
   Once for every "Adaptive optics imaging in mice in vivo" procedure
5. **Duration of imaging session:**
   5 minutes
6. **Purpose:**
   Frequently, mice spontaneously develop cataracts and retinal degeneration. Both impede the ability to perform high-resolution adaptive optics imaging. The micron II is a commercially available fundus camera that provides a rapid and wide-field view of the mouse retina.
   Before undergoing high-resolution imaging, the mouse eye will be inspected for cataractogenesis and retinal degeneration in the micron II camera. If both are deemed to be optimal (clear ocular media and standard retinal morphology), we will progress forward to high-resolution with the same mice.
7. **Will supportive care of animals be necessary during the imaging session?**
   Yes **No**
8. **If yes, describe:**

View: SF: Procedure Documents

Procedure Documents

1. **Supporting documents:**

Document Name	Date Modified
There are no items to display	

View: Custom SF: Procedure Identification

Procedure Identification: Anesthesia, Terminal, Tribromoethanol (Avertin)

1. *** Name of the procedure or surgery:**
   Anesthesia, Terminal, Tribromoethanol (Avertin)
2. *** Select procedure type:**
   Substance Administration
3. *** Species:**
   Mice
4. *** Will administering this procedure cause any more than momentary pain and distress?** Yes **No**

If yes,

i. **Identify expected symptoms from administering this procedure:**
   N/A

ii. **Identify criteria under which animals will be removed from research:**
   N/A

View: Custom SF: Administration of Substances

Administration of Substances

1. * Substances:

	Substance	Substance Scope	Route	Dose	Concentration	Volume	Substance Order for the Procedure
View	Tribromoethanol (Avertin)	Standard	Intraperitoneal	≥500 mg/kg	N/A	Total volume will not exceed 26 microliters per gram of body weight.	N/A

2. * Describe step-by-step the procedure for administering the substance(s):

Avertin is administered IP to induce anesthesia appropriate for a short (<20 minutes) terminal procedure such as perfusion.

Deep anesthesia is confirmed by lack of response to toe pinch, change in respiratory character and decreased respiratory rate.

3. Describe the intended effects of administering the substance(s):

Anesthesia for short (<20 minutes) terminal procedure

4. Describe any potential adverse reactions to administering the substance(s):

N/A

5. For hazardous agents, describe potential danger to humans, precautions to protect personnel and containment requirements:

Needles must not be recapped unless a recapping device is used.

Gloves must be worn when handling this agent.

6. * Does this procedure include the use of a paralytic agent?

Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

View: SF: Procedure Documents

Procedure Documents

1. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create Substance

1. *** Substance:**
   Tribromoethanol (Avertin)
2. **Route:**
   Intraperitoneal

   If you indicated Other, specify the route:
   N/A
3. **Dose:**
   ≥500 mg/kg
4. **Frequency and duration of dosages:**
   Once
5. **Volume (for rodents):**
   Total volume will not exceed 26 microliters per gram of body weight.
6. **Concentration:**
   N/A
7. **Non-pharmaceutical justification, if appropriate:**
   Avertin is not available pharmaceutical grade.
8. **Complication remediation:**
   N/A
9. **Substance order for the procedure:**
   N/A

Substances Appendix:

View: Custom SF: Substance Information

Substance Information: Isoflurane

1. *** Name:**
   Isoflurane
2. *** Substance types:** (select all that apply)
   Anesthetic
   Reproductive Hazard/Teratogen
3. *** Is this a hazardous agent:** **Yes** No

   NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

4. Supporting documents:

Document Name Date Modified

There are no items to display

View: Custom SF: Substance Information

Substance Information: Tribromoethanol (Avertin)

1. * Name:

Tribromoethanol (Avertin)

2. * Substance types: (select all that apply)

Anesthetic
Reproductive Hazard/Teratogen

3. * Is this a hazardous agent: Yes **No**

NOTE: Working with biohazardous agents requires a separate approval from the Institutional Biosafety Committee (IBC). Submit the Biological Use Authorization **(BUA) paperwork to initiate this process. If you have questions, contact EH&S Research and Occupational Safety at 206-221-7770 or** ehsbio@uw.edu**.**

4. Supporting documents:

Document Name Date Modified

There are no items to display

View: Custom: Create and Edit

Experiments Appendix:

Adaptive optics imaging in mice in vivo

1. *** Experiment name:**
   Adaptive optics imaging in mice in vivo
2. *** Species:**
   Mice
3. **If other was selected, provide a species:**
4. **What is the scientific goal of this experiment:**
   This study will use a specialized retina camera to investigate whether the morphology and function of individual retinal cells and their connections are resolvable and visualizable in vivo in mice. Currently, imaging technologies do not have access to cellular level detail in mice in vivo. Therefore, in order to visualize cellular morphology, animals need to be sacrificed and the tissue is thereafter sent for histology . Our goal is to develop and refine imaging technology such that the same level of cellular detail is achievable in vivo.
5. *** Describe the experiment:** (include the animal experience from enrollment in the study to the final endpoint on this protocol, including order and minimal time between procedures):

   There will be two experimental groups. Both groups will follow same experimental protocol except for anesthesia. One group will be anesthetized using Avertin and the other using Isofluorane. This is required because depending on the anesthetic, the physiology of the mice eye may be variable and present different challenges for imaging. Second, we plan to longitudinally follow up imaging in the same animal using isofluorane in future studies while employ Avertin for a one-time imaging before euthanasia.

   Most important of all physiological changes following anesthesia administration is the development of cataracts that can obscure non-invasive in vivo optical imaging in mice through the intact eye. According to this conference abstract(https://www.aaopt.org/detail/knowledge-base-article/cataractogenesis-anesthetized-mice) Avertin leads to similar cataractogenesis as ketamine/xylazine after 10-20 minutes of administration. No published records exist following up cataractogenesis after Avertin with care given to hydration and regulating body temperature. This information will be important for future studies to gauge whether for one-time imaging, Avertin is a suitable anesthetic.

   Step 1: Mice will be obtained from the protocols of Rachel Wong and Maureen Neitz. These mice will vary between wild type and mice where cone photoreceptors and retinal ganglion cells are tagged with fluorescent labels.

   Step 2: Animals will be first dilated and then anesthetized with one of either Avertin or isofluorane.

   Step 3: genteal or other artificial tears will be applied to the mouse cornea to keep it moist.

   Step 4: Take retinal images with a commercial mouse fundus camera

   Step 5: Take retinal images with custom-built adaptive optics camera. Apply genteal on the eye when required as deemed by image quality degradation. Retinal images include images of photoreceptors, ganglion cells, nerve fiber, optic nerve head, blood vessels.

Step 6: If isofluorane was used to anesthetize, place animal back in the vivarium. Follow up from step 2, for a maximum of 3 times, 3-7 days apart. Once the imaging is complete, carry out euthanasia with cervical dislocation. If Avertin was used to anesthetize, carry out euthanasia with cervical dislocation once the imaging is complete.

Animal Sex:
Female
Male

Animal Ages:
6 weeks or older

Animal Size:

6. * Select experimental procedures:

Name	Type	Version	Scope
Anesthetic Overdose, Pentobarbital or Pentobarbital Solution	Euthanasia	1	Standard
Cervical Dislocation, Unanesthetized	Euthanasia	1	Standard
Cervical Dislocation, Under Isoflurane Anesthesia, Anesthesia Machine	Euthanasia	1	Standard
Cervical Dislocation, Under Tribromoethanol (Avertin) Anesthesia	Euthanasia	1	Standard
Sabesan - imaging	Imaging	1	Team
Sabesan team - Micron II fundus imaging	Imaging	1	Team
Anesthesia, Isoflurane, Long Duration (>1 hour)	Substance Administration	2	Standard
Anesthesia, Isoflurane, Short Duration (<1 hour)	Substance Administration	2	Standard
Anesthesia, Terminal, Tribromoethanol (Avertin)	Substance Administration	2	Standard

7. Monitoring protocol, including frequency and specific behavioral and clinical signs to be monitored. Include humane endpoints (criteria for euthanasia):
The mice will be routinely monitored for eye irritation, activity levels and posture. Vet services will be consulted for concerns.

Criteria for euthanasia include lethargy, failure to eat and drink, open wounds,scruffy fur or development of non-experimental conditions such as tumors and/or illnesses. Method of euthanasia is cervical dislocation under anesthesia.

8. If there is expected mortality (spontaneous death) in this experiment:

a. Procedure/condition associated with mortality:
No

b. Estimated mortality rate, i.e. percentage of animals expected to die spontaneously (not via euthanasia) or need to be euthanized as a result of the procedure. (Be sure to account for this in your animal number calculations):
N/A

c. Explain why euthanasia is not possible or appropriate:
N/A

9. Will some animals live out their natural lifespan as part of this experiment? If so, indicate their use and describe the monitoring plan for aged animals (e.g., rodents >18 months of age), including frequency, behavioral and clinical signs to be monitored and criteria for euthanasia.
No

10. * Total number of animals used in this experiment: (including all the animals to be produced)
30

a. Justify total number of animals used in this experiment:

The three variables we need to account for in deducing the optimal number of animals is 1) cataractogenesis in mice, b) fluorescence vs. non-fluorescence imaging, c) inter-mice variability in imaging parameters.

The natural prevalence of cataracts in mice is 5% while under Ketamine/xylazine, the frequency of corneal opacities is reported to be as high as 42% (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4519051/). With Avertin, the results are similar. Mice are significantly less prone to cataract formation under Isoflurane. For establishing instrument parameters for single time point imaging, we will require mice with clear optical media(cornea and lens). The mouse eye has substantially different optical properties than that of humans. In particular, the numerical aperture of the mouse eye is 2.5x that of humans. This larger NA is advantageous for obtaining better spatial resolution, although, it also leads to large aberrations that need to be measured and compensated appropriately to obtain sharp images of cellular features within the retina. The eyeball is significantly smaller than a human and requires careful opto-mechanical alignment to the instrument in order to ensure good performance. Due to the propensity of developing cataracts soon after anesthesia, temperature and corneal hydration need to be maintained at levels that allow for imaging with relatively clear ocular media. For these reasons, we estimate requiring 4 imaging sessions to tune all instrument parameters for repeatable and reliable performance based on human studies under the same imaging system. Accounting for cataractogenesis, this leads to 8 animals each for fluorescence and non-fluorescence conditions. Therefore in total, we expect to use 16 animals under single time point imaging under Avertin anesthesia (8 wild type, 8 YFP, 16 total).

For longitudinal imaging, using isoflurane, we expect that the incidence of cataracts would be reduced. However, we expect two challenges that may govern animal numbers. First, the nose cone for administering isoflurane may impede imaging by imposing mechanical constraints on the light entering the mouse eye's pupil. Second, for following up the same retinal areas with time, we expect that we may face challenges in positioning the mice retina at the same spatial coordinates with time. We have considered the use of 3 different nose cone designs and plan to test each for optimality. These designs are

based on achieving the least invasive footprint and interference with the imaging light. Additionally, we have designed and built an optomechanical mount with fine adjustment capability for navigation across the retinal field. We plan to test the 3 different nose cone designs on 2 animals each (1 wild type, 1 YFP, 6 total). Once the parameters are finalized, we estimate needing 8 animals (4 wild type, 4 YFP, 8 total) for training additional lab personnel on conducting the entire procedure. Therefore, in summary, we estimate needing 30 animals total.

11. Number of animals by pain and distress category: (include each animal only once in the highest pain category)

B: 0
C: 0
D: 30
E: 0

a. Justify the need for any animals in pain category E:

12. * Identify husbandry exceptions:

	Exception Type	Description and Justification
View	Mice - No husbandry or enrichment exceptions.	

13. Supporting documents:

Document Name	Date Modified
There are no items to display	

View: Custom: Create and Edit

1. * Exception type:

Mice - No husbandry or enrichment exceptions.

2. Description and justification:

View: Custom: Add Vivarium Location

1. *Identify the location where animals will be housed:

Vivarium ABSL1

a. For locations that are lab managed, provide justification for housing outside of the vivarium:

b. If you cannot find the location above, identify it here and provide justification for housing outside of the vivarium:

2. * What species will be housed in this location?

Common Name	Scientific Name
Mice	Mus

View: Custom: Add Animal Use Location

1. * Identify the location where animals will be used:

a. For locations that are outside of the vivarium, provide justification for the use of this space:

The specialized instrument to image the mice retina in vivo is built on a 4 ft by 8ft optical table in Rm E286, SLU3.1. It has components and design constraints that govern its size and hence restrict it from transported to the vivarium.

b. If you cannot find the location above, identify it here and provide justification for the use of animals outside of the vivarium:

SLU3.1 E286

2. * What species will be used in this location?

Common Name	Scientific Name
Mice	Mus

3. Describe how this location will be used:

This location will be used to administer anesthesia and carry out the imaging experiments.

4. * If animals are left unattended in this location, provide an explanation and include maximum duration:

Animals will not be left unattended in this location.

5. Describe how animals will be transported to and from this location, including container and route

(Note: use of private vehicles requires IACUC approval):

Mice will be transported from the vivarium in covered cages with filter tops. The shortest route up to E286 is via the direct elevator from the vivarium up to second floor. Then we will follow the north-south hallway on the eastern side of SLU3.1 to E286.

View: Custom: Add Animal Use Location

1. * Identify the location where animals will be used:

E279

a. For locations that are outside of the vivarium, provide justification for the use of this space:

This location is in the Neitz lab and houses the micron II camera to be used for fundus imaging. This imaging needs to be conducted in conjunction with high-resolution imaging in E286 under the same anesthetic event. E286 and E279 are along the same hallway and separated by 3 rooms. The instrument in E286 is not portable.

b. If you cannot find the location above, identify it here and provide justification for the use of animals outside of the vivarium:

2. * What species will be used in this location?

Common Name	Scientific Name
Mice	Mus

3. Describe how this location will be used:

This location will be used for fundus imaging

4. * If animals are left unattended in this location, provide an explanation and include maximum duration:

Not left unattended.

5. Describe how animals will be transported to and from this location, including container and route

(Note: use of private vehicles requires IACUC approval):

Mice will be transported from the vivarium in covered cages with filter tops. The shortest route up to E279 is via the direct elevator from the vivarium up to second floor. Then we will follow the north-south hallway on the eastern side of SLU3.1 to E279.

From: Michelle Brot <mbrot@uw.edu>
Sent: Wednesday, July 22, 2020 3:15 PM
To: Tony Nguyen; Ramkumar Sabesan; Xiaoyun Jiang
Subject: Re: E-286 Wednesday, July 22 at around 1pm

Thanks, Tony! Nice job, Ram!! Does this mean your laser mouse retina studies will begin soon? I am excited about them....

☺ Michelle

From: Tony Nguyen <tonytn@uw.edu>
Sent: Wednesday, July 22, 2020 2:03 PM
To: Ramkumar Sabesan <rsabesan@uw.edu>; Xiaoyun Jiang <jxyun@uw.edu>
Cc: Michelle Brot <mbrot@uw.edu>
Subject: RE: E-286 Wednesday, July 22 at around 1pm

Hi Ram and Xiaoyun,

There were no issues in E286. It was noted the space was really clean and well kept. E286 is approved for animal use. I'm adding E286 to the IACUC site visit list and inspected along with Dr. Brockerhoff's lab E268.

You may edit and add E286 to your new protocol. Please let me know if you have any questions.

Thanks,
Tony

From: Tony Nguyen
Sent: Monday, July 13, 2020 10:42 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Cc: Xiaoyun Jiang <jxyun@uw.edu>; Michelle Brot <mbrot@uw.edu>
Subject: RE: E-286 Wednesday, July 22 at around 1pm

Thanks, Ram. The concerns are complete and we are set for the inspection on 7/22.

Thanks,
Tony

From: Ramkumar Sabesan [mailto:rsabesan@uw.edu]
Sent: Monday, July 13, 2020 10:26 AM
To: Tony Nguyen <tonytn@uw.edu>
Cc: Xiaoyun Jiang <jxyun@uw.edu>; Michelle Brot <mbrot@uw.edu>
Subject: Re: E-286 Wednesday, July 22 at around 1pm

Hi Tony,

I just uploaded the SOP for curtain cleaning onto Hoverboard, and responded to the concern.

Xiaoyun and I will meet Jeanot Muster at around 1pm on 7/22 outside E-286.

Thanks

Ram

On Fri, Jul 10, 2020 at 5:43 PM Tony Nguyen <tonytn@uw.edu> wrote:

> Thanks, Ram. Jeanot Muster will meet you and Xiaoyun in SLU 3.1 E286 on 7/22 at around 1pm.
>
> Once you have the curtains cleaning SOP finalized, please respond to the concern in HoverBoard and upload the SOP.
>
> Thanks,
> Tony
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Sent:** Friday, July 10, 2020 5:23 PM
> **To:** Tony Nguyen <tonytn@uw.edu>; Xiaoyun Jiang <jxyun@uw.edu>; Michelle Brot <mbrot@uw.edu>
> **Subject:** E-286 Wednesday, July 22 at around 1pm
>
> Hi Tony,
>
> Yes, July 22 around 1pm should be fine for the inspection. I am cc-ing Xiaoyun Jiang, postdoc, who will be around with me to answer any questions.
>
> Thanks
> Ram
>
> --
> ---
> Ramkumar Sabesan, Ph.D.
> Assistant Research Professor
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From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Thursday, September 3, 2020 10:03 AM
To: Michelle Brot
Subject: Re: IACUC Protocol

Hi Michelle,

Sorry this is taking so long. I am yet to hear back from the chair of our department approving the study - I will ping him again now. Besides that, we are all set.

I will let you know once I hear back and turn it in. Thanks so much!

-Ram

On Thu, Sep 3, 2020 at 9:57 AM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> We are really close to getting your protocol approved but the IACUC reviewer had a couple really minor comments that you could probably address in <5 min. They've been there for a couple weeks so I thought I'd let you know in case you overlooked that they were waiting for your response.
>
> Take care,
> Michelle

--

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From: Michelle Brot <mbrot@uw.edu>
Sent: Thursday, February 14, 2019 10:03 AM
To: Ramkumar Sabesan
Subject: Re: PROTO201800103: Notification of Requested Clarifications

Hi Ram,
Thanks for checking in about your questions/comments. The approach you suggested will work but I like a different approach better:

1. Using the reviewer notes section, note which pages contain the questions. Most of the questions (maybe all) will be on your Experiments page, so I¹ll use that as an example. So click ³Edit Protocol² on the left side of the ³flow chart page" and then use the Jump To menu to get to the Experiments page (be aware that you probably have to click through two Jump To menus to get to it).
2. Then, you¹ll see the Questions at the top of the Experiments page. To make it easy to see the questions and to address them in the experiment, I recommend opening a second copy of the protocol and have them side-by-side. You can look at the questions on the left and address them in the protocol on the right, for example. It won¹t mess anything up by having two open at once.
3. Make whatever changes you need to in the text of the protocol, remembering to hit OK at the bottom of any pop-up window, such as the experiment pop‹up and Save at the top of any page once you¹ve made changes.
4. Then indicate in the ³Click here to respond² section what changes you made to address the question.
5. Once you¹re done responding to the questions in all sections, click ³Submit² on the left on the ³flow chart page.² You get there by clicking ³Exit² from any page.

One thing I¹ve found very helpful is to right click to open a procedure in a new tab. Otherwise, it replaces the screen you¹re working in and you have to hit the back button to return to that screen. This way, you can close the procedure and still have the other screen there (or you can click back and forth between them).

Feel free to call me if any questions arise as you¹re working on this: 206-221-0891.

Take care,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Thursday, February 14, 2019 at 9:34 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Fwd: PROTO201800103: Notification of Requested Clarifications

Hi Michelle,

I am looking into these clarification. Before I get too far ahead into the corrections, I just want to clarify if I am following the right process in Hoverboard to do this.

Let me know if this is the right approach to address these :
- I click on Reviewer Notes.
- For each clarification point, I click on the section, say "Experiments", "Procedural Personnel assignment", "animal details" and address the clarification.
- Once the clarification is addressed within its section, I click on "click here to respond" and say what steps I followed to respond to the clarification.

Does this seem like it is the correct approach ? I am not all that familiar with Hoverboard , just wanted to clarify before I get too deep into it.

Thanks

-Ram

---------- Forwarded message ---------
From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

--

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From: Michelle Brot <mbrot@uw.edu>
Sent: Wednesday, October 31, 2018 5:11 PM
To: Ramkumar Sabesan
Subject: Re: Protocol

Great, Ram. Also, I forgot to mention on that list that you need to fill out the Alternatives page. This is where you do your literature review to see if there are alternatives to using animals in your research or any literature doing similar work with different methodologies that you could consider. I know you[1]re currently just planning on anesthetizing mice and not doing much that[1]s stressful, etc, but you should do your best to fill it out anyway.

Thanks,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Wednesday, October 31, 2018 at 4:44 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol

Hi Michelle,

Thanks so much for this feedback. All of these make sense and are easy to change.
I should be able to send it in latest by the end of the week.

-Ram

On Wed, Oct 31, 2018 at 1:12 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Generally, it looks really good. Here are a few comments:
>
> 1. It sounds like this experiment is determining the feasibility for imaging the mouse with your equipment and set-up, but I[1]m assuming if it works, you will want to study this more. Therefore, I think the title of the protocol should be more generic, like "Imaging the Mouse Retina" or even what you used as the title of your experiment: "Adaptive optics imaging in mice[2] while the title of this experiment, should reflect what you[1]re doing now, which is [3]Determining the effect of anesthetics and fluorescence on imaging feasibility", or [3]Testing methodological parameters of in vivo mouse imaging[2] or something along those lines. If this is successful, you will likely add more experiments to compare timing or determine ways to improve the image quality or whatever, so those will use similar procedures but have a different goal (Q4). Thus, I would think that this feasibility testing, such as comparing two anesthetics, should be described in Q4 for this experiment.
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> Hope these make sense and you[1]re able to integrate them. Let me know if you need any assistance or clarification. Otherwise, once you make these changes, it should be good to go to the vets!

Take care,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Wednesday, October 31, 2018 at 9:53 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol

Hi Michelle,

I am following up on my email from last week.
Please let me know if I can clarify anything. If you think that nothing major needs to be changed at the moment, I might think about submitting the protocol as such for the next review round.

Thanks

-Ram

On Tue, Oct 23, 2018 at 7:10 PM Ramkumar Sabesan <rsabesan@uw.edu> wrote:

> Hi Michelle,
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> I am attaching a draft of the protocol for conducting these imaging studies in my lab at SLU.
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> We think that at this point, some feedback from you would be very helpful. Really appreciate it !
> Please let me know if there are questions or concerns.
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Take care,
Michelle

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From: Michelle Brot <mbrot@uw.edu>
Sent: Wednesday, October 31, 2018 10:40 AM
To: Ramkumar Sabesan
Subject: Re: Protocol

Hi Ram,
Thanks for the follow-up. Glad you did as this slipped through the cracks as I was busily addressing the items in my Inbox (which is where it goes once you submit it!) Anyway, I'll take a look at it right now and provide feedback.

Cheers,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Wednesday, October 31, 2018 at 9:53 AM
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From: Ramkumar Sabesan <rsabesan@uw.edu>
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From: Michelle Brot <mbrot@uw.edu>
Sent: Thursday, January 17, 2019 4:48 PM
To: Ramkumar Sabesan
Subject: Re: Protocol

Great, RamŠI'll plan to review it soon and give you feedback.

Regards,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Thursday, January 17, 2019 at 3:23 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol

Hi Michelle,

I finally submitted my protocol this afternoon. Incorporated all the changes that you had suggested. It took a lot longer than I had anticipated, in a large part due to other deadlines etc.

Thanks so much for your help in the process!! Really appreciate it.

-Ram

On Wed, Oct 31, 2018 at 5:11 PM Michelle Brot <mbrot@uw.edu> wrote:

> Great, Ram. Also, I forgot to mention on that list that you need to fill out the Alternatives page. This is where you do your literature review to see if there are alternatives to using animals in your research or any literature doing similar work with different methodologies that you could consider. I know you're currently just planning on anesthetizing mice and not doing much that's stressful, etc, but you should do your best to fill it out anyway.
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> Hi Michelle,
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> I am attaching a draft of the protocol for conducting these imaging studies in my lab at SLU.
>
> We think that at this point, some feedback from you would be very helpful. Really appreciate it !
> Please let me know if there are questions or concerns.
>
> -Ram
>
> --
> _____

Ramkumar Sabesan, Ph.D.
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From: Michelle Brot <mbrot@uw.edu>
Sent: Friday, July 12, 2019 6:26 PM
To: Ramkumar Sabesan
Subject: Re: Protocol reminder

Hi Ram,
Sure, feel free to give me a call Monday morning. I have a meeting until 10 am but should be in my office the rest of the morning. I'm sure we can figure out a way to write it up so you have flexibility to test out the various nose cone designs and allow for improvement between testing.

Have a nice weekend,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Friday, July 12, 2019 at 6:22 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Thanks Michelle.

I have gone through the questions and seems like they should be straightforward to address for us.

The key thing I am uncertain about is #3 under "Also in Q10a" which pertains to the nose cone testing and the numbers chosen according to the text in the protocol below

.
"We have considered the use of 3 different nose cone designs and plan to test each for optimality. These designs are based on achieving the least invasive footprint and interference with the imaging light. Additionally, we have designed and built an optomechanical mount with fine adjustment capability for navigation across the retinal field. We plan to test the 3 different nose cone designs on 2 animals each (1 wildtype, 1 YFP, 1 RFP, 9 total). "

There are no papers which report any of these nose cone designs(I will try to dig more). And without trying them out, I am just a little unsure what the challenges we might face with them.

Second question pertains to the 3 week study duration for the monitoring section. It is indeed correct that once we start imaging, the study would be finished for one mouse in 3 weeks. However, we would like to stagger the imaging with different mice, so as to be able to tweak the system in the interim in order to improve it for the next round of imaging. Perhaps discussing this over a phone call might help me describe my understanding of how this may happen in practice and see what other alternatives exist. Would sometime Monday morning work for you ?

Thanks

Ram

On Fri, Jul 12, 2019 at 3:51 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Welcome back, hope the travels were wonderful. And yes, you need to address the questions by making the changes to the text and responding about what the changes were. I don't think there's anything else you're missing. If you have any trouble navigating places within Hoverboard or making the edits, feel free to shoot me an e-mail or call.
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Michelle Brot, PhD
Scientific Reviewer
Office of Animal Welfare, UW
Seattle, WA 98195

206-221-0891 phone

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Friday, July 12, 2019 at 3:46 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

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I had to go back to our earlier correspondence to remind myself on the most optimal way to navigate hoverboard and it seems simple after your explanation.

I just want to clarify I am answering everything - There are 9 questions in the reviewer notes that need to be addressed, is that correct ? Is there anything else I am missing ?

Thanks

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From: Michelle Brot <mbrot@uw.edu>
Date: Thursday, February 14, 2019 at 10:03 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Subject: Re: PROTO201800103: Notification of Requested Clarifications

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From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

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Sent: Friday, July 12, 2019 3:52 PM
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Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

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Notification of Requested Clarifications

To: Ramkumar Sabesan

Link: 4462-01: In vivo retinal imaging in mice
PI: Ramkumar Sabesan

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From: Michelle Brot <mbrot@uw.edu>
Sent: Monday, July 1, 2019 4:30 PM
To: Ramkumar Sabesan
Subject: Re: Protocol reminder

Hi Ram,
This is a final reminder that this protocol will have to be withdrawn in 2 weeks if you don't address the reviewer questions. Since you put a lot of work into preparing the protocol, if there is any chance you plan to do the work in the next 3 years, it would be much easier to respond now than to have to discard the protocol. If you have concerns about navigating through Hoverboard since it's been a while, please let me know and I'll be happy to help you find your way to making your responses.

Take care,
Michelle

Michelle Brot, PhD
Scientific Reviewer
Office of Animal Welfare, UW
Seattle, WA 98195

206-221-0891 phone

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Friday, May 31, 2019 at 5:21 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Thanks for the reminder Michelle. I will plan to certainly get it in before the withdrawal deadline. Thanks so much for your patience !

-Ram

On Fri, May 31, 2019 at 5:11 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Hope you're doing well. This is just another reminder about your protocol. It would be a good idea to finish up your responses in the next few weeks as we're getting close to the 6 month mark since you submitted it and that's our withdrawal timepoint. Let me know if you need any help with getting the clarifications submitted.
>
> Thanks,
> Michelle
>
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>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Tuesday, February 26, 2019 at 11:15 AM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** Re: Protocol reminder
>
> Hi Michelle,
>
> Thanks so much for the reminder!
> I am behind with a grant deadline fast approaching. Hope to have the revisions in soon.

Best,
Ram

On Tue, Feb 26, 2019 at 9:53 AM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
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> **From:** Michelle Brot <mbrot@uw.edu>
> **Date:** Thursday, February 14, 2019 at 10:03 AM
> **To:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Subject:** Re: PROTO201800103: Notification of Requested Clarifications
>
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> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Thursday, February 14, 2019 at 9:34 AM
> **To:** Michelle Brot <mbrot@uw.edu>

Subject: Fwd: PROTO201800103: Notification of Requested Clarifications

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Thanks

-Ram

---------- Forwarded message ---------
From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

--

Ramkumar Sabesan, Ph.D.
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750 Republican St, E213
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Web: depts.washington.edu/sabaolab

Tel: 206-221-4925 Fax:206-685-9315

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From: Michelle Brot <mbrot@uw.edu>
Sent: Friday, May 31, 2019 5:11 PM
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From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Tuesday, February 26, 2019 at 11:15 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Hi Michelle,

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I am behind with a grant deadline fast approaching. Hope to have the revisions in soon.

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Date: Thursday, February 14, 2019 at 10:03 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Subject: Re: PROTO201800103: Notification of Requested Clarifications

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From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To: Ramkumar Sabesan
Link: 4462-01: In vivo retinal imaging in mice
PI: Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

--

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From: Michelle Brot <mbrot@uw.edu>
Sent: Thursday, March 14, 2019 2:12 PM
To: Ramkumar Sabesan
Subject: Re: Protocol reminder

Hi Ram,
Hope you got your grant in! It's been a few weeks so I'm just sending another friendly reminder about responding to the questions about your protocol. If you need any help, feel free to call me when you start working on it.

Take care,
Michelle

From: Michelle Brot <mbrot@uw.edu>
Date: Tuesday, February 26, 2019 at 10:17 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Subject: Re: Protocol reminder

Sure, no problem. Good luck getting the grant completed!!

Michelle Brot, PhD
Scientific Reviewer
Office of Animal Welfare, UW
Seattle, WA 98195

206-221-0891 phone

From: Ramkumar Sabesan <rsabesan@uw.edu>
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Date: Sun, Jan 27, 2019 at 6:05 PM
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To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

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Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To: Ramkumar Sabesan
Link: 4462-01: In vivo retinal imaging in mice
PI: Ramkumar Sabesan

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From: Michelle Brot <mbrot@uw.edu>
Sent: Thursday, July 18, 2019 4:30 PM
To: Ramkumar Sabesan
Subject: Re: Protocol reminder

Sounds good, I'll take a look!

:) Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Thursday, July 18, 2019 at 3:53 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Hi Michelle,

I addressed the questions and resubmitted the protocol.

Again, thanks a ton for all your help through the process and for your patience. I really appreciate it!

Best,

Ram

On Mon, Jul 15, 2019 at 10:47 AM Michelle Brot <mbrot@uw.edu> wrote:

> Sure, noon is fine.
>
> ~Michelle
>
> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Monday, July 15, 2019 at 10:26 AM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** Re: Protocol reminder
>
> Hi Michelle,
>
> I am in the middle of a meeting - Sorry I missed your call.
>
> Can I call you at noon today ?
>
> Thanks
>
> -Ram
>
> On Fri, Jul 12, 2019 at 6:26 PM Michelle Brot <mbrot@uw.edu> wrote:
>
>> Hi Ram,
>> Sure, feel free to give me a call Monday morning. I have a meeting until 10 am but should be in my office the rest of the morning. I'm sure we can figure out a way to write it up so you have flexibility to test out the various nose cone designs and allow for improvement between testing.

Have a nice weekend,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Friday, July 12, 2019 at 6:22 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Thanks Michelle.

I have gone through the questions and seems like they should be straightforward to address for us.

The key thing I am uncertain about is #3 under "Also in Q10a" which pertains to the nose cone testing and the numbers chosen according to the text in the protocol below

.

"We have considered the use of 3 different nose cone designs and plan to test each for optimality. These designs are based on achieving the least invasive footprint and interference with the imaging light. Additionally, we have designed and built an optomechanical mount with fine adjustment capability for navigation across the retinal field. We plan to test the 3 different nose cone designs on 2 animals each (1 wildtype, 1 YFP, 1 RFP, 9 total). "

There are no papers which report any of these nose cone designs(I will try to dig more). And without trying them out, I am just a little unsure what the challenges we might face with them.

Second question pertains to the 3 week study duration for the monitoring section. It is indeed correct that once we start imaging, the study would be finished for one mouse in 3 weeks. However, we would like to stagger the imaging with different mice, so as to be able to tweak the system in the interim in order to improve it for the next round of imaging. Perhaps discussing this over a phone call might help me describe my understanding of how this may happen in practice and see what other alternatives exist. Would sometime Monday morning work for you ?

Thanks

Ram

On Fri, Jul 12, 2019 at 3:51 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Welcome back, hope the travels were wonderful. And yes, you need to address the questions by making the changes to the text and responding about what the changes were. I don¹t think there¹s anything else you¹re missing. If you have any trouble navigating places within Hoverboard or making the edits, feel free to shoot me an e-mail or call.
>
> Have a nice weekend,
> Michelle
>
> Michelle Brot, PhD
> Scientific Reviewer
> Office of Animal Welfare, UW
> Seattle, WA 98195
>
> 206-221-0891 phone

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Friday, July 12, 2019 at 3:46 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Hi Michelle,

I am back from my travels and hoping to have the protocol back to you quickly in the next few days.

I had to go back to our earlier correspondence to remind myself on the most optimal way to navigate hoverboard and it seems simple after your explanation.

I just want to clarify I am answering everything - There are 9 questions in the reviewer notes that need to be addressed, is that correct ? Is there anything else I am missing ?

Thanks

Ram

On Tue, Jul 2, 2019 at 9:35 PM Michelle Brot <mbrot@uw.edu> wrote:

> OK, sounds good.
>
> Hope you enjoy the rest of your travels,
> Michelle

> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Sent:** Tuesday, July 2, 2019 9:32:30 PM
> **To:** Michelle Brot
> **Subject:** Re: Protocol reminder
>
> Hi Michelle,
>
> I'll plan to have it in within two weeks. I'm traveling up until early next week and once I'm back, I'll be in touch with questions. I'm sure there will be some
>
> Thanks so much
> Ram
>
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Date: Thursday, February 14, 2019 at 10:03 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Subject: Re: PROTO201800103: Notification of Requested Clarifications

Hi Ram,
Thanks for checking in about your questions/comments. The approach you suggested will work but I like a different approach better:

1. Using the reviewer notes section, note which pages contain the questions. Most of the questions (maybe all) will be on your Experiments page, so I'll use that as an example. So click ³Edit Protocol² on the left side of the ³flow chart page" and then use the Jump To menu to get to the Experiments page (be aware that you probably have to click through two Jump To menus to get to it).
2. Then, you'll see the Questions at the top of the Experiments page. To make it easy to see the questions and to address them in the experiment, I recommend opening a second copy of the protocol and have them side-by-side. You can look at the questions on the left and address them in the protocol on the right, for example. It won't mess anything up by having two open at once.
3. Make whatever changes you need to in the text of the protocol, remembering to hit OK at the bottom of any pop-up window, such as the experiment pop‹up and Save at the top of any page once you've made changes.
4. Then indicate in the ³Click here to respond² section what changes you made to address the question.
5. Once you're done responding to the questions in all sections, click ³Submit² on the left on the ³flow chart page.² You get there by clicking ³Exit² from any page.

One thing I've found very helpful is to right click to open a procedure in a new tab. Otherwise, it replaces the screen you're working in and you have to hit the back button to return to that screen. This way, you can close the procedure and still have the other screen there (or you can click back and forth between them).

Feel free to call me if any questions arise as you're working on this: 206-221-0891.

Take care,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Thursday, February 14, 2019 at 9:34 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Fwd: PROTO201800103: Notification of Requested Clarifications

Hi Michelle,

I am looking into these clarification. Before I get too far ahead into the corrections, I just want to clarify if I am following the right process in Hoverboard to do this.

Let me know if this is the right approach to address these :
- I click on Reviewer Notes.
- For each clarification point, I click on the section, say "Experiments", "Procedural Personnel assignment", "animal details" and address the clarification.

- Once the clarification is addressed within its section, I click on "click here to respond" and say what steps I followed to respond to the clarification.

Does this seem like it is the correct approach ? I am not all that familiar with Hoverboard , just wanted to clarify before I get too deep into it.

Thanks

-Ram

---------- Forwarded message ---------
From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To:	Ramkumar Sabesan
Link:	4462-01: In vivo retinal imaging in mice
PI:	Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

--

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From: Michelle Brot <mbrot@uw.edu>
Sent: Monday, July 15, 2019 10:47 AM
To: Ramkumar Sabesan
Subject: Re: Protocol reminder

Sure, noon is fine.

~Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Monday, July 15, 2019 at 10:26 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Hi Michelle,

I am in the middle of a meeting - Sorry I missed your call.

Can I call you at noon today ?

Thanks

-Ram

On Fri, Jul 12, 2019 at 6:26 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Sure, feel free to give me a call Monday morning. I have a meeting until 10 am but should be in my office the rest of the morning. I'm sure we can figure out a way to write it up so you have flexibility to test out the various nose cone designs and allow for improvement between testing.
>
> Have a nice weekend,
> Michelle
>
> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Friday, July 12, 2019 at 6:22 PM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** Re: Protocol reminder
>
> Thanks Michelle.
>
> I have gone through the questions and seems like they should be straightforward to address for us.
>
> The key thing I am uncertain about is #3 under "Also in Q10a" which pertains to the nose cone testing and the numbers chosen according to the text in the protocol below
> .
> "We have considered the use of 3 different nose cone designs and plan to test each for optimality. These designs are based on achieving the least invasive footprint and interference with the imaging light. Additionally, we have designed and built an optomechanical mount with fine adjustment capability for navigation across the retinal field. We plan to test the 3 different nose cone designs on 2 animals each (1 wildtype, 1 YFP, 1 RFP, 9 total). "
>
> There are no papers which report any of these nose cone designs(I will try to dig more). And without trying them

out, I am just a little unsure what the challenges we might face with them.

Second question pertains to the 3 week study duration for the monitoring section. It is indeed correct that once we start imaging, the study would be finished for one mouse in 3 weeks. However, we would like to stagger the imaging with different mice, so as to be able to tweak the system in the interim in order to improve it for the next round of imaging. Perhaps discussing this over a phone call might help me describe my understanding of how this may happen in practice and see what other alternatives exist. Would sometime Monday morning work for you ?

Thanks

Ram

On Fri, Jul 12, 2019 at 3:51 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Welcome back, hope the travels were wonderful. And yes, you need to address the questions by making the changes to the text and responding about what the changes were. I don't think there's anything else you're missing. If you have any trouble navigating places within Hoverboard or making the edits, feel free to shoot me an e-mail or call.
>
> Have a nice weekend,
> Michelle
>
> Michelle Brot, PhD
> Scientific Reviewer
> Office of Animal Welfare, UW
> Seattle, WA 98195
>
> 206-221-0891 phone
>
> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Friday, July 12, 2019 at 3:46 PM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** Re: Protocol reminder
>
> Hi Michelle,
>
> I am back from my travels and hoping to have the protocol back to you quickly in the next few days.
>
> I had to go back to our earlier correspondence to remind myself on the most optimal way to navigate hoverboard and it seems simple after your explanation.
>
> I just want to clarify I am answering everything - There are 9 questions in the reviewer notes that need to be addressed, is that correct ? Is there anything else I am missing ?
>
> Thanks
>
> Ram
>
> On Tue, Jul 2, 2019 at 9:35 PM Michelle Brot <mbrot@uw.edu> wrote:
>
> > OK, sounds good.

Hope you enjoy the rest of your travels,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Tuesday, July 2, 2019 9:32:30 PM
To: Michelle Brot
Subject: Re: Protocol reminder

Hi Michelle,

I'll plan to have it in within two weeks. I'm traveling up until early next week and once I'm back, I'll be in touch with questions. I'm sure there will be some

Thanks so much
Ram

On Tue, Jul 2, 2019, 2:30 AM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> This is a final reminder that this protocol will have to be withdrawn in 2 weeks if you don't address the reviewer questions. Since you put a lot of work into preparing the protocol, if there is any chance you plan to do the work in the next 3 years, it would be much easier to respond now than to have to discard the protocol. If you have concerns about navigating through Hoverboard since it's been a while, please let me know and I'll be happy to help you find your way to making your responses.
>
> Take care,
> Michelle
>
> Michelle Brot, PhD
> Scientific Reviewer
> Office of Animal Welfare, UW
> Seattle, WA 98195
>
> 206-221-0891 phone
>
> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Friday, May 31, 2019 at 5:21 PM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** Re: Protocol reminder
>
> Thanks for the reminder Michelle. I will plan to certainly get it in before the withdrawal deadline. Thanks so much for your patience !
>
> -Ram
>
> On Fri, May 31, 2019 at 5:11 PM Michelle Brot <mbrot@uw.edu> wrote:
>
> > Hi Ram,
> > Hope you're doing well. This is just another reminder about your protocol. It would be a good idea to finish up your responses in the next few weeks as we're getting close to the 6 month mark since you submitted it and that's our withdrawal timepoint. Let me know if you need any help with getting the

clarifications submitted.

Thanks,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Tuesday, February 26, 2019 at 11:15 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol reminder

Hi Michelle,

Thanks so much for the reminder!
I am behind with a grant deadline fast approaching. Hope to have the revisions in soon.

Best,
Ram

On Tue, Feb 26, 2019 at 9:53 AM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Have you made any progress on your protocol? No rush on my end, but just wanted to remind you in case you meant to submit it back.
>
> Thanks,
> Michelle

From: Michelle Brot <mbrot@uw.edu>
Date: Thursday, February 14, 2019 at 10:03 AM
To: Ramkumar Sabesan <rsabesan@uw.edu>
Subject: Re: PROTO201800103: Notification of Requested Clarifications

Hi Ram,
Thanks for checking in about your questions/comments. The approach you suggested will work but I like a different approach better:

1. Using the reviewer notes section, note which pages contain the questions. Most of the questions (maybe all) will be on your Experiments page, so I¹ll use that as an example. So click ³Edit Protocol² on the left side of the ³flow chart page" and then use the Jump To menu to get to the Experiments page (be aware that you probably have to click through two Jump To menus to get to it).
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3. Make whatever changes you need to in the text of the protocol, remembering to hit OK at the bottom of any pop-up window, such as the experiment pop‹up and Save at the top of any page

once you've made changes.

4. Then indicate in the "Click here to respond" section what changes you made to address the question.
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One thing I've found very helpful is to right click to open a procedure in a new tab. Otherwise, it replaces the screen you're working in and you have to hit the back button to return to that screen. This way, you can close the procedure and still have the other screen there (or you can click back and forth between them).

Feel free to call me if any questions arise as you're working on this: 206-221-0891.

Take care,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Thursday, February 14, 2019 at 9:34 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Fwd: PROTO201800103: Notification of Requested Clarifications

Hi Michelle,

I am looking into these clarification. Before I get too far ahead into the corrections, I just want to clarify if I am following the right process in Hoverboard to do this.

Let me know if this is the right approach to address these :
- I click on Reviewer Notes.
- For each clarification point, I click on the section, say "Experiments", "Procedural Personnel assignment", "animal details" and address the clarification.
- Once the clarification is addressed within its section, I click on "click here to respond" and say what steps I followed to respond to the clarification.

Does this seem like it is the correct approach ? I am not all that familiar with Hoverboard , just wanted to clarify before I get too deep into it.

Thanks

-Ram

---------- Forwarded message ---------
From: <HBNoResp@uw.edu>
Date: Sun, Jan 27, 2019 at 6:05 PM
Subject: PROTO201800103: Notification of Requested Clarifications
To: <rsabesan@uw.edu>

Notification of Requested Clarifications

To: Ramkumar Sabesan
Link: 4462-01: In vivo retinal imaging in mice

PI: Ramkumar Sabesan

Clarifications have been requested on this submission. This requires a response from you. For additional details, click the link above to review and provide clarification.

--

Ramkumar Sabesan, Ph.D.
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From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Thursday, January 17, 2019 3:24 PM
To: Michelle Brot
Subject: Re: Protocol

Hi Michelle,

I finally submitted my protocol this afternoon. Incorporated all the changes that you had suggested. It took a lot longer than I had anticipated, in a large part due to other deadlines etc.

Thanks so much for your help in the process!! Really appreciate it.

-Ram

On Wed, Oct 31, 2018 at 5:11 PM Michelle Brot <mbrot@uw.edu> wrote:

> Great, Ram. Also, I forgot to mention on that list that you need to fill out the Alternatives page. This is where you do your literature review to see if there are alternatives to using animals in your research or any literature doing similar work with different methodologies that you could consider. I know you're currently just planning on anesthetizing mice and not doing much that's stressful, etc, but you should do your best to fill it out anyway.
>
> Thanks,
> Michelle
>
> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Date:** Wednesday, October 31, 2018 at 4:44 PM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** Re: Protocol
>
> Hi Michelle,
>
> Thanks so much for this feedback. All of these make sense and are easy to change.
> I should be able to send it in latest by the end of the week.
>
> -Ram
>
> On Wed, Oct 31, 2018 at 1:12 PM Michelle Brot <mbrot@uw.edu> wrote:
>
> > Hi Ram,
> > Generally, it looks really good. Here are a few comments:
> >
> > 1. It sounds like this experiment is determining the feasibility for imaging the mouse with your equipment and set-up, but I'm assuming if it works, you will want to study this more. Therefore, I think the title of the protocol should be more generic, like "Imaging the Mouse Retina" or even what you used as the title of your experiment: "Adaptive optics imaging in mice[2] while the title of this experiment, should reflect what you're doing now, which is [3]Determining the effect of anesthetics and fluorescence on imaging feasibility", or [3]Testing methodological parameters of in vivo mouse imaging[2] or something along those lines. If this is successful, you will likely add more experiments to compare timing or determine ways to improve the image quality or whatever, so those will use similar procedures but have a different goal (Q4). Thus, I would think that this feasibility testing, such as comparing two anesthetics, should be

described in Q4 for this experiment.

2. Based on what you describe in Step 1, it looks like you are also comparing WT and fluorescently labeled mice (of two backgrounds?). Can this variable also be mentioned in Q4 as part of your goals?
3. In Step 2, can you say how you are dilating the mice, and add a Substance Administration procedure if it's using a substance.
4. In Q7 of the experiment, can you provide a bit more specific detail on how frequently you intend to monitor the mice? Right now it says routinely monitored. Also, the vets will want you to say for how long you would need to observe the euthanasia criteria before you would euthanize sick animals.

Hope these make sense and you're able to integrate them. Let me know if you need any assistance or clarification. Otherwise, once you make these changes, it should be good to go to the vets!

Take care,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Date: Wednesday, October 31, 2018 at 9:53 AM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Protocol

Hi Michelle,

I am following up on my email from last week.
Please let me know if I can clarify anything. If you think that nothing major needs to be changed at the moment, I might think about submitting the protocol as such for the next review round.

Thanks

-Ram

On Tue, Oct 23, 2018 at 7:10 PM Ramkumar Sabesan <rsabesan@uw.edu> wrote:

> Hi Michelle,
>
> I am attaching a draft of the protocol for conducting these imaging studies in my lab at SLU.
>
> We think that at this point, some feedback from you would be very helpful. Really appreciate it !
> Please let me know if there are questions or concerns.
>
> -Ram
>
> --
> ---
> Ramkumar Sabesan, Ph.D.
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From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Monday, July 13, 2020 10:15 AM
To: Michelle Brot
Subject: Re: SOP - curtains

Hi Michelle,

Thanks so much. Will make this modification you suggested and submit the SOP.

Best,
Ram

On Fri, Jul 10, 2020 at 8:21 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Yes, something simple like this is just fine. The only thing is that you might modify the first part as follows:
>
> There are 4 curtains in Room E-286 that will be cleaned on a 6-month cycle or twice a year, *provided that the room is in use*. Frequencies will be increased in case of any adverse events such as spills or if we notice excess dust in the rooms.
>
> Thanks,
> Michelle
>
> ---
>
> **From:** Ramkumar Sabesan <rsabesan@uw.edu>
> **Sent:** Friday, July 10, 2020 5:44 PM
> **To:** Michelle Brot <mbrot@uw.edu>
> **Subject:** SOP - curtains
>
> Hi Michelle
>
> I am at a bit of a loss as to what to put down in the SOP other than to say the following - would this suffice ? Let me know what you think.
>
> - There are 4 curtains in Room E-286 that will be cleaned on a 6-month cycle or twice a year. Frequencies will be increased in case of any adverse events such as spills or if we notice excess dust in the rooms
> - The curtains will be pulled down by qualified building maintenance personnel with a work order submitted a few days in advance of the requisite date.
> - The curtains will be taken to a drycleaner and cleaned.
> - They will then be installed back by the same building maintenance personnel.
>
> What do you think?
>
> -Ram

--

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From: Michelle Brot <mbrot@uw.edu>
Sent: Friday, July 10, 2020 8:22 PM
To: Ramkumar Sabesan
Subject: Re: SOP - curtains

Hi Ram,
Yes, something simple like this is just fine. The only thing is that you might modify the first part as follows:

There are 4 curtains in Room E-286 that will be cleaned on a 6-month cycle or twice a year, *provided that the room is in use*. Frequencies will be increased in case of any adverse events such as spills or if we notice excess dust in the rooms.

Thanks,
Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Friday, July 10, 2020 5:44 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: SOP - curtains

Hi Michelle

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- The curtains will be taken to a drycleaner and cleaned.
- They will then be installed back by the same building maintenance personnel.

What do you think?

-Ram

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From: Michelle Brot <mbrot@uw.edu>
Sent: Thursday, August 13, 2020 3:02 PM
To: Ramkumar Sabesan
Subject: Re: Vet reviewer questions on your protocol

Hi Ram,
Those responses look good to me. Submit it back and I'll forward it to the vets for their final approval and then it goes to the IACUC.

Thanks!
~Michelle

From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Thursday, August 13, 2020 12:12 PM
To: Michelle Brot <mbrot@uw.edu>
Subject: Re: Vet reviewer questions on your protocol

Hi Michelle,

Just took care of these. Please let me know if the responses are ok and if there is anything else required.

Thanks

Ram

On Fri, Aug 7, 2020 at 4:57 PM Michelle Brot <mbrot@uw.edu> wrote:

> Hi Ram,
> Now that the room issue is resolved, I wanted to remind you that there are still several vet review questions from February that you need to respond to in order to get this protocol approved. Let me know if you need any assistance with them.
>
> Have a nice weekend,
> Michelle

--

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From: Ramkumar Sabesan <rsabesan@uw.edu>
Sent: Friday, July 10, 2020 5:45 PM
To: Michelle Brot
Subject: SOP - curtains

Hi Michelle

I am at a bit of a loss as to what to put down in the SOP other than to say the following - would this suffice ? Let me know what you think.

- There are 4 curtains in Room E-286 that will be cleaned on a 6-month cycle or twice a year. Frequencies will be increased in case of any adverse events such as spills or if we notice excess dust in the rooms
- The curtains will be pulled down by qualified building maintenance personnel with a work order submitted a few days in advance of the requisite date.
- The curtains will be taken to a drycleaner and cleaned.
- They will then be installed back by the same building maintenance personnel.

What do you think?

-Ram

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From: Michelle Brot <mbrot@uw.edu>
Sent: Friday, August 7, 2020 4:57 PM
To: Ramkumar Sabesan
Subject: Vet reviewer questions on your protocol

Hi Ram,
Now that the room issue is resolved, I wanted to remind you that there are still several vet review questions from February that you need to respond to in order to get this protocol approved. Let me know if you need any assistance with them.

Have a nice weekend,
Michelle