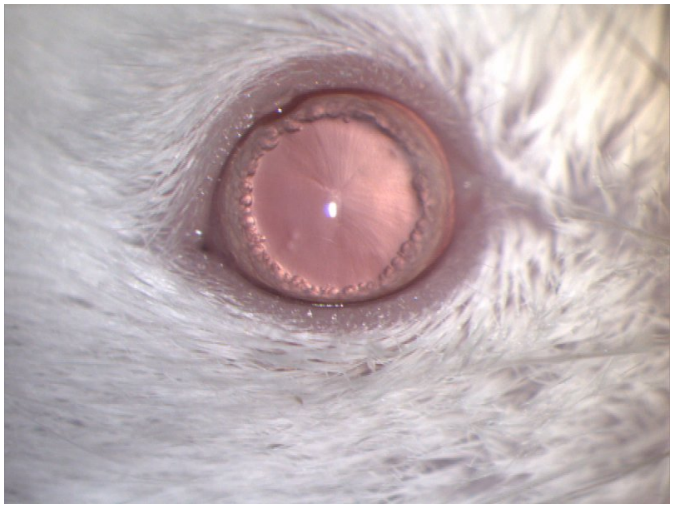
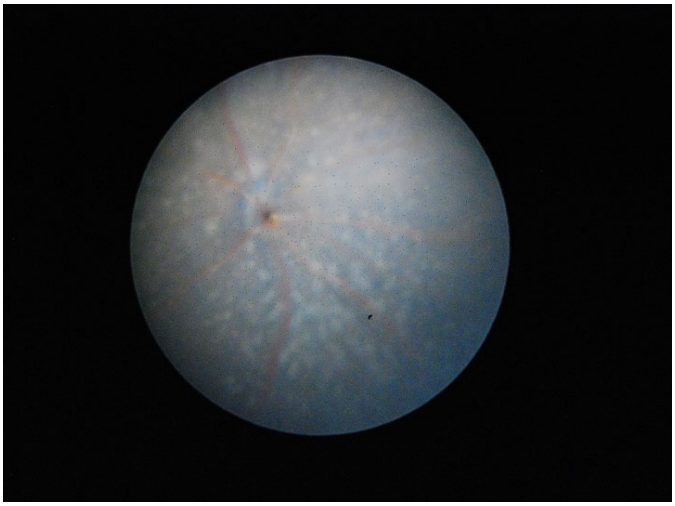
# WELLCOME SANGER INSTITUTE

# STANDARD OPERATING PROCEDURE PACKET







# SANGER INSTITUTE
# STANDARD OPERATING PROCEDURE

**SUBJECT: Eye Morphology Screen – V1**

| | | |
|---|---|---|
| **SOP Number: SOP0094** | **To be reviewed:** | |
| **Author(s):** | Signed: | Date: |
| **Editor:** | Signed: | Date: |
| **Risk Approver:** | Signed: | Date: |
| **Date of Implementation:** | | |

## INTRODUCTION:

The purpose of this procedure is to detect abnormalities in eye morphology using slit lamp and indirect ophthalmoscope.

## ABBREVIATIONS:

**DCF** = Data Capture Form
**IVC** = Individually Ventilated Cage
**LAA** = Laboratory Animal Allergens
**PAF** = Project Authorisation Form
**PIL** = Procedure Individual Licence
**PPE** = Personal Protective Equipment
**PPL** = Procedure Project License
**QC** = Quality Control
**RA** = Risk Assessment
**RSF** = Research Support Facility
**SMP** = Sick Mouse Procedure
**SOP** = Standard Operating Procedure
**WT** = Wild-Type

## QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.
If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

**In-Life Procedures:**

| Problem / Issue | QC fail reason |
|---|---|
| At any point during the procedure the mouse is deemed sick and processed | Fail whole DCF as 'Sick mouse' – for all tests that day |

| | |
|---|---|
| through Sick Mouse Procedure (SMP) | |
| Mouse incorrectly scheduled at wrong week | Fail whole DCF as 'Scheduling Issue' |
| Insufficient anaesthesia level affects the whole test DCF | Fail whole DCF as 'Anaesthesia Issue' |
| Insufficient anaesthesia level affects specific parameter(s) | Fail parameter(s) as 'Anaesthesia issue' |
| A welfare issue makes it impossible to collect specific parameters | Fail parameter(s) as 'Welfare issue' |
| Parameters affected by delays or noise due to fire alarms | Fail parameter(s) as 'Fire alarm' |
| An equipment failure affecting specific parameters | Fail parameter(s) as 'Equipment failure' |
| A software issue affecting specific parameters | Fail parameter(s) as 'Software failure' |
| A procedural error which affects data collection | Fail parameter(s) as 'Manual error' |
| Parameter cannot be assessed | Fail parameter(s) as 'Readout not possible' |
| Wrong value has been entered which cannot be re-measured or accounted for | Fail parameter(s) as 'Erroneous data' |
| Glucose meter unable to record high blood values | Fail parameter(s) as 'Meter reading HI' |
| Fighting occurs prior to or during data collection | Fail parameter(s) as 'Fighting during procedure' |
| Parameter on the current DCF is not required for that specific test/pipeline | Fail parameter(s) as 'Not required' |

## HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

**Name:** WTSI-1190
**Assessment Title:** Eye Morphology Assessment and Imaging
**Assessor:**
**Approver:**

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
  - Mask
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or down flow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.

## RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual License (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

## RESOURCES:

**Equipment:**

1. Balance
2. 70% Ethanol and tissues – **Hazardous substance: highly flammable**
3. Tecniplast mobile Individually Ventilated Cage (IVC) rack
4. Transport rack
5. Diet (as defined by pipeline)
6. Zeiss SL130 Slit Lamp
7. Minims 1% tropicamide
8. Heine Omega 500 ‘unplugged’ ophthalmoscope
9. Volk NC Superfield lens
10. Heated mat
11. Lens cleaning cloth
12. Two corkboards
13. Stop watch
14. Scraper
15. Hydrex Pink - **Hazardous substance: highly flammable**
16. Hydrex Hard Surface Spray - **Hazardous substance: highly flammable**
17. “Post procedure check required” labels
18. “mice awaiting ophthalmic measurement” labels
19. Disinfectant wipes
20. 1% Trigene/detergent
21. “Phenotyping in progress” laminated sign
22. “Eye phenotyping in progress” laminated sign

**Associated SOPs/Documentation:**

- **SOP0045 - Weigh Mice**
- **SOP0101 - Taking and Returning Cages for Procedures**
- 1) Leica image register
- Eye Catalogue
- \Slit Lamp  What am I looking at
- 1) Glossary of Terms
- Imaging decision tree for hypopigmentation

**Staff:** This test can be completed with one phenotyper but is optimally run with two.

## NOTE:

Always use the lowest light setting giving a clear view of the eye. Albino eyes will be more sensitive to light.

## PROCEDURE:

**Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).**

1. Ensure heated mat is on and lower lights to “low” level.

2. Collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (refer to SOP0101 – Taking and Returning Cages for Procedures).

3. Put on the correct PPE, place “Phenotyping in progress” and “Eye phenotyping in progress” signs on the outside of the door.

4. Select one staff member to carry the stop watch on their person from this point forward.

5. Place the cages to be tested and tropicamide ampoules on the warmed heat mat.

6. Examine undilated eyes using the slit lamp:
   - 6.1. Set magnification to x20 using the wheel located at either side of the slit lamp, adjacent to the eye piece.
   - 6.2. Test mice in cage card order. Identify mouse to be tested.
   - 6.3. Observe mouse for abnormalities in eye size or eyelid closure. Observe for presence of blood in or around the eyes.
   - 6.4. Scruff the mouse.
   - 6.5. Seated at the slit lamp, focus on the eye of interest and observe for abnormalities in the following tissues:
     - 6.5.1. Eyelid
     - 6.5.2. Presence of blood
     - 6.5.3. Discharge
     - 6.5.4. Cornea
     - 6.5.5. Lens
     - 6.5.6. Iris
     - 6.5.7. Pupil
   - 6.6. Test for an Iris/Pupil light response: dim the light wait a few seconds then return to the previous light level. Observe for any abnormalities in this response.
   - 6.7. Repeat steps 6.4-6.6 for the second eye.
   - 6.8. Record results and observations according to current DCF, using the Glossary of Terms document to copy and paste comments.
   - 6.9. If abnormalities are to be imaged, add details to the Leica image register. This does not need to be done for WT mice as they do not require an image.
   - 6.10. Apply 1-2 drops of Minims 1% tropicamide to both eyes (ensure the drops are still within the used by date).
   - 6.11. Return mouse to home cage.
   - 6.12. Repeat steps 6.3-6.11 for each mouse in the cage.

*The workflow will benefit if performed by 2 people from this point forward: Person A and Person B*

7. Person A: Examine dilated eyes using the slit lamp, dilation expected 5 minutes after tropicamide application:
   - 7.1. Set magnification to x20.

7.2. Test mice in cage card order. Identify mouse to be tested.
7.3. Scruff the mouse.
7.4. Seated at the slit lamp, focus on the eye of interest and observe for abnormalities in the following tissues. At this stage, commonly occurring abnormalities include:
  7.4.1. Cornea
  7.4.2. Lens (anterior and posterior)
  7.4.3. Iris
  7.4.4. Pupil
7.5. Distinguish between cornea, anterior lens and posterior lens by adjusting light source angle to extreme left/right and adjusting light source width to a slim beam. This will result in 3 distinct areas of light:
  7.5.1. Outer arc = brightest = corneal surface
  7.5.2. Inner arc = anterior lens
  7.5.3. Past inner arc = darkest = posterior lens
7.6. Repeat steps 7.3-7.5 for the second eye.
7.7. Record results and observations according to current DCF using the Glossary of Terms document to copy and paste comments.
7.8. If abnormalities are to be imaged, add details to the Leica image register. This does not need to be done for WT mice as they do not require an image.
7.9. Weigh mouse (refer to SOP0045 - Weigh Mice).

8. Person B: Examine dilated eyes using the indirect ophthalmoscope:
  8.1. Set-up ophthalmoscope:
    8.1.1. Ensure unit is secure and comfortable on head
    8.1.2. Set to the smallest pupil setting using lever on underside
    8.1.3. Correct light brightness using knob on right side of head unit
    8.1.4. A single, central, clearly defined circle of white light can be seen through the eye pieces
  8.2. Identify the mouse to be tested.
  8.3. Scruff the mouse.
  8.4. Seated at a bench with support for both arms, position mouse eye in beam of light.
  8.5. Hold Volk NC Superfield lens in free hand, in beam of light and move it slowly back and forth to view the fundus. Observe for abnormalities in the following tissues:
    8.5.1. Retina
    8.5.2. Blood vessels
    8.5.3. Optic disc
  8.6. Repeat steps 8.3-8.5 for the second eye.
  8.7. Record results and observations according to current DCF using the Glossary of Terms document to copy and paste comments.
  8.8. If abnormalities are to be imaged, add details to the Leica image register. This does not need to be done for WT mice as they do not require an image.

9. Repeat steps 7 & 8 for each mouse in the cage.

10. Repeat steps 5-9 for all cages to be tested. Ensure cages where testing is complete cages display updated cage cards and ‘POST PROCEDURE CHECK REQUIRED’ label.

11. House mice at lowered light level for minimum of 1 hr. Start the stop watch once the last drops of Minims 1% tropicamide have been applied. This watch MUST be kept by someone at all times until the cages have been returned to the animal room.

12. Clean equipment, surfaces and clean up any obvious waste on the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

13. **Register cages to the correct rack whilst returning them to their destination/home rack in the animal room (refer to SOP0101 – Taking and Returning Cages for Procedures).**

# SANGER INSTITUTE
# STANDARD OPERATING PROCEDURE

**SUBJECT: Eye Imaging – V1**

| | |
|---|---|
| **SOP Number: SOP0042** | **To be reviewed:** |
| **Author(s):** Signed: Date: <br> **Editor:** Signed: Date: | |
| **Risk Approver:** Signed: Date: | |
| **Date of Implementation:** | |

## INTRODUCTION:

The purpose of this procedure is to record variations in eye morphology by acquisition of photographs using the slit lamp camera or Topical Endoscopic Fundus Imaging (TEFI) Fundus camera.

## ABBREVIATIONS:

**DCF** = Data Capture Form
**IVC** = Individually Ventilated Cage
**LAA** = Laboratory Animal Allergens
**PAF** = Project Authorisation Form
**PIL** = Procedure Individual Licence
**PPE** = Personal Protective Equipment
**PPL** = Procedure Project License
**QC** = Quality Control
**RA** = Risk Assessment
**RSF** = Research Support Facility
**SMP** = Sick Mouse Procedure
**SOP** = Standard Operating Procedure
**TEFI** = Topical Endoscopic Fundus Imaging

## QUALITY CONTROL (QC) DURING PROCEDURE:

Refer to the table below for approved QC fail comments steps to be used during procedures.
If a value has been collected leave on the Data Capture Form (DCF) and then apply the fail reason from below;

**In-Life Procedures:**

| Problem / Issue | QC fail reason |
|---|---|
| At any point during the procedure the mouse is deemed sick and processed | Fail whole DCF as 'Sick mouse' – for all tests that day |

| | |
|---|---|
| through Sick Mouse Procedure (SMP) | |
| Mouse incorrectly scheduled at wrong week | Fail whole DCF as 'Scheduling Issue' |
| Insufficient anaesthesia level affects the whole test DCF | Fail whole DCF as 'Anaesthesia Issue' |
| Insufficient anaesthesia level affects specific parameter(s) | Fail parameter(s) as 'Anaesthesia issue' |
| A welfare issue makes it impossible to collect specific parameters | Fail parameter(s) as 'Welfare issue' |
| Parameters affected by delays or noise due to fire alarms | Fail parameter(s) as 'Fire alarm' |
| An equipment failure affecting specific parameters | Fail parameter(s) as 'Equipment failure' |
| A software issue affecting specific parameters | Fail parameter(s) as 'Software failure' |
| A procedural error which affects data collection | Fail parameter(s) as 'Manual error' |
| Parameter cannot be assessed | Fail parameter(s) as 'Readout not possible' |
| Wrong value has been entered which cannot be re-measured or accounted for | Fail parameter(s) as 'Erroneous data' |
| Glucose meter unable to record high blood values | Fail parameter(s) as 'Meter reading HI' |
| Fighting occurs prior to or during data collection | Fail parameter(s) as 'Fighting during procedure' |
| Parameter on the current DCF is not required for that specific test/pipeline | Fail parameter(s) as 'Not required' |

## HEALTH & SAFETY:

This procedure is covered by the following Risk Assessment (RA):

**Name:** WTSI-1190
**Assessment Title:** Eye Morphology Assessment and Imaging
**Assessor:**
**Approver:**

- Appropriate Personal Protective Equipment (PPE) is to be worn at all times when handling animals. This includes:
  - Overshoes
  - Gown
  - Gloves
  - Mask
- In addition to the above, when sources for Laboratory Animal Allergens (LAA) (animals or soiled cages) are not contained within Local Exhaust Ventilation Systems (change stations, fume hoods or down flow tables), a respiratory mask, for which you have passed a face fit test, must be worn.
- The cold light fountain is a source of intense visible light. The phenotyper assisting the photographer should wear tinted glasses at all time when the cold light fountain is On.
- Lone worker alarms should be used when working alone.
- This procedure can only be performed during Research Support Facility (RSF) core hours (7:30am-7:30pm).
- All electrical equipment is to be inspected for damage before use.

## RESPONSIBILITIES:

All staff performing this procedure are responsible for ensuring that this Standard Operating Procedure (SOP) and accompanying Risk Assessment have been read, understood and where applicable is followed in accordance with the relevant Procedure Project License (PPL). All staff should be trained and competent to perform the procedure, where applicable they should also be licensed to perform the procedure with a valid Procedure Individual License (PIL).

For secondary phenotyping, seek confirmation with project manager for deviations from this SOP. Any deviation will be detailed in the Project Authorisation Form (PAF).

## RESOURCES:

**Equipment:**

1. 70% Ethanol – **Hazardous substance: highly flammable**
2. Tissues
3. Scraper
4. Hydrex Pink - **Hazardous substance: highly flammable**
5. Hydrex hard surface spray - **Hazardous substance: highly flammable**
6. 1% Trigene/detergent solution
7. Disinfectant wipes
8. Tecniplast mobile Individually Ventilated Cage (IVC) rack
9. Transport rack
10. Heat mat
11. Corkboard
12. Stop watch
13. Grey background for white balance.
14. 3d barcode sheet
15. Leica LAS software
16. Zeiss SL130 Slit Lamp with attached Leica DFC420
17. Minims 1% Tropicamide
18. Materials for fundus photography
19. Nikon (D40x, later D3300) camera with Nikkor 85mm lens, lens attachmentand SD card reader or USB cable
20. Tele-otoscope - **Physical hazard: tinted glasses must be worn whilst turned On**
21. Cold light fountain
22. Fibre optic cable
23. Tripod
24. Tinted glasses
25. Minims 2.5% Phenylephrine Hydrochloride
26. Minims 0.4% Oxybuprocaine Hydrochloride
27. Viscotears
28. Template for TEFI Imaging
29. “Post procedure check required” labels
30. “Phenotyping in progress” laminated sign
31. “Eye phenotyping in progress” laminated sign

**Associated SOPs/Documentation:**

- **SOP0094 – Eye Morphology Screen**
- **SOP0101 – Taking and Returning Cages for Procedures**
- Leica image register

**Staff:** One phenotyper is required for slit lamp photography**,** two phenotypers are required for fundus photography.

## PROCEDURE:

**Before performing any tests verify this is the correct set of procedures at this time point in the pipeline or project, by consulting the cage card(s).**

1. Turn on the heat mat and lower lights to “low” level.

2. Open the Leica image register and print a copy of the mice to be imaged. Use these details to collect scheduled mice from the animal room, transport them to the procedure room and register them to the correct rack (refer to SOP0101 – Taking and Returning Cages for Procedures).

3. Write details of which mouse/mice, which eye(s) and what type of images is needed on the cage cards.

4. Put on overshoes, disposable gloves, gown and mask.

5. Place “Phenotyping in progress” and “Eye phenotyping in progress” signs on the outside of the door.

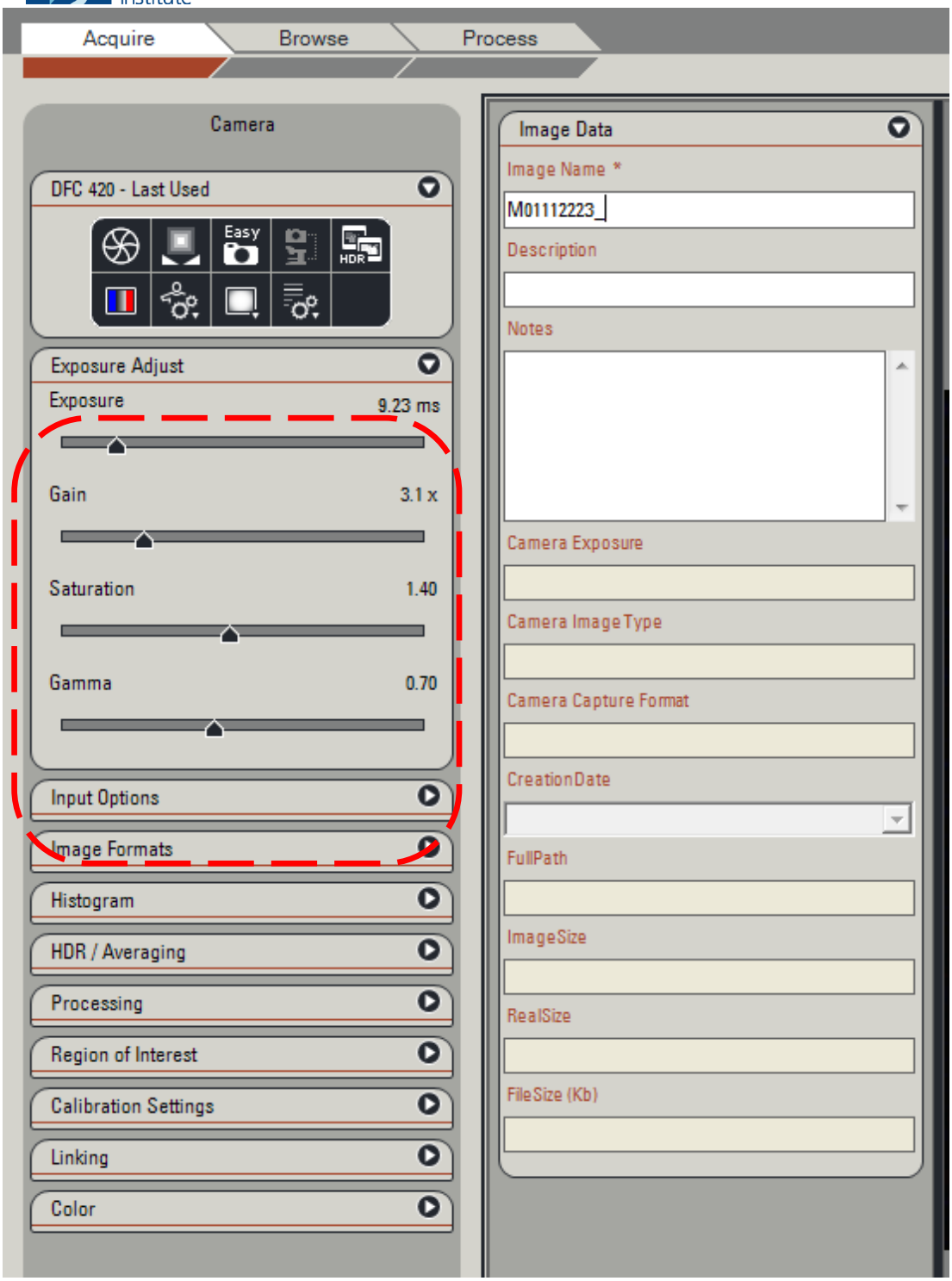
6. Select one staff member to carry the stop watch on their person from this point forward.

7. Place the first cages to be imaged along with the tropicamide, phenylephrine and oxybuprocaine ampoules on the warmed heat mat.

8. Person A: can start taking undilated slit lamp images.

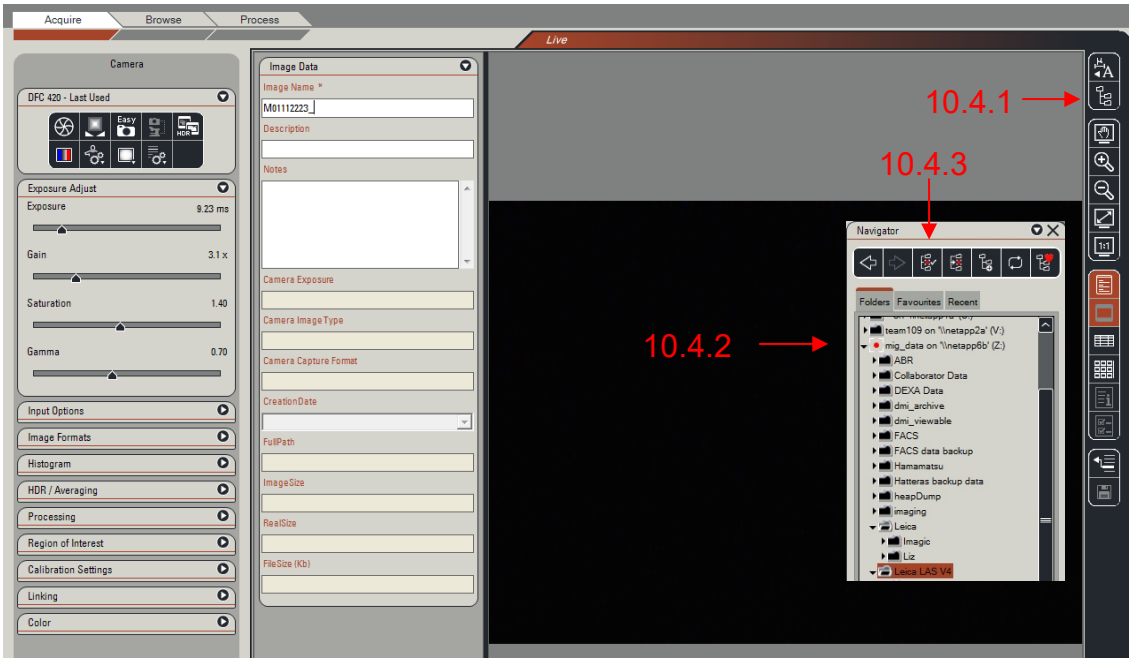
9. Person B: can start applying the necessary drops for dilated slit lamp images and TEFI imaging to those mice that do not require an undilated slit lamp image. According to expected workflow.

10. **Set-up for slit lamp imaging:**
    10.1. Start Leica LAS v4.1 software.
    10.2. Use the Acquire tab to confirm settings are set as shown below:




10.3. Set white balance:
  10.3.1. Open the camera and set light level as marked on the dial of the slit lamp.
  10.3.2. Position the grey background card in the field of view.
  10.3.3. Left click and drag the curser over the field of view to draw a box containing consistent mid-tone grey colour.
  10.3.4. Right click over this box and select 'White balance' from the drop down menu
10.4. Set 'save to', selecting location.

10.4.1. Open the navigator using the quick access toolbar on the right of the screen.




10.4.2. Select desired folder.

10.4.3. Set this as the save to location using the select location button. A red dot will appear over this file to confirm the save location has been set.

11. **<u>Acquiring slit lamp images:</u>**

11.1. If dilated slit lamp images are required, please see steps 12.2-12.5 for dilation.

11.2. Select x12 magnification using the wheel located at either side of the slit lamp, adjacent to the eye pieces.

11.3. If not already selected, select 'Show form' from the toolbar at the extreme right of the screen and enter image specifics and enter Image name = 'Mxxxxxxxx_'

11.4. Whilst wearing the correct PPE, hold the mouse in position (refer to SOP0094 – Eye Morphology Screen) and position it in order to have the eye in focus. To achieve good positioning and focus, use the live camera view on the computer screen.

11.5. With the eye in focus and as stationary as possible, press the acquire image button in Leica LAS or press the yellow footswitch to take the photo. Multiple images can be captured consecutively and the image name will remain unique with the mouse barcode unchanged and a number appended to subsequent images.

11.6. In the "Browse" tab view acquired images. Repeat steps 11.4-11.5 until a passable image is acquired or mouse welfare dictates you stop the procedure.

11.7. Click on chosen image and if not already selected, select 'Show form' from the toolbar at the extreme right of the screen and enter image specifics (Eye Morphology, slit lamp/TEFI, dilated/non-dilated, eye left/right…).

11.8. Once the mouse has started the procedure, start the DCF and print the updated cage card. If performing slit lamp and TEFI on the same mouse, this will be recorded in one DCF.

12. **Acquiring TEFI Fundus Camera-*requires 1 person to start and then 2 for imaging*:**

12.1. Place cages containing mice to be imaged on heat mat and select first mouse.

12.2. Whilst wearing the correct PPE, apply 1 drop of Minims 2.5% Phenylephrine Hydrochloride to eyes to be imaged (ensure the drops are still within the used by date). For mice marked as WT control images or mutant representative images, only dilate the eyes of a maximum of 2 mice even if more are indicated on the Leica image register. Only dilate the eyes of another 2 mice if no good quality image is attained from the first 2 mice.

12.3. Return mouse to home cage and wait 10-15 mins.

12.4. Apply a second application of Minims 2.5% Phenylephrine Hydrochloride and wait a further 10-15 mins.

12.5. Apply 1 drop of Minims 1% Tropicamide (ensure the drops are still within the used by date) and wait 2-5 mins.

12.6. Apply a second application of Minims 1% Tropicamide and wait 2-5 mins.

12.7. Apply 1 drop of Minims 0.4% Oxybuprocaine Hydrochloride (ensure the drops are still within the used by date) and wait a maximum of 2 mins.

12.8. Repeat steps 12.2-12.7 for all mice to be imaged, typically starting the next mouse when the previous mouse is given its second set of Phenylephrine drops.

12.9. During breaks between applications of dilating agents, set up TEFI equipment and prepare the TEFI spreadsheet:

12.9.1. Plug in the cold light fountain.

12.9.2. Insert fibre optic cable into plug on front right of cold light fountain.

12.9.3. Screw other end of fibre optic cable in to tele-otoscope.

12.9.4. Insert tele-otoscope into adaptor on lens of camera.

12.9.5. Attach camera to tripod and check that there is adequate battery power and space for images.

12.9.6. Move small trolley into centre of room. Place camera on it with tripod legs extended to maximum and an upturned empty cage base and corkboard for arm support.

12.9.7. Ensure camera and light fountain are at correct settings:

- **Pigmented eyes:**

| | |
|---|---|
| Light setting: | **3** |
| Mode: | S |
| ISO: | **3600** |
| Exposure time: | 1/15 |
| Exp. Compensation: | +0.0 |
| Flash Compensation: | +0.0 |
| White balance: | Incandescent |
| Release mode: | Continuous |
| Metering: | Center-weighted metering |

- **Albino/Red Eyes:**

| | |
|---|---|
| Light setting: | **2** |
| Mode: | S |
| ISO: | **800** |
| Exposure time: | 1/15 |

| | |
|---|---|
| Exp. Compensation: | +0.7 |
| Flash Compensation: | +0.0 |
| White balance: | Incandescent |
| Release mode: | Continuous |
| Metering: | Center-weighted metering |

12.9.8 To alter light setting, use the dial on the front of the cold light fountain.

12.9.9 To alter the camera settings, press the i button to the left of the camera view screen, use the arrow buttons to navigate.

**Two people are required from this point:**

12.10. Person A: Scruff mouse and apply a generous blob of Viscotears to eye to be imaged. Hold mouse stationary in front of otoscope whilst resting arms on the trolley (use cage base and corkboard for support if needed).

12.11. Person B: **put the tinted glasses on** and turn on light fountain.

12.12. Person A: Starting with the mouse close to (but not touching) light source, look through the camera view finder and bring the mouse towards the light until the light source touches the Viscotears & the retina can be seen. If needed, subtly adjust positioning of mouse to gain an image which fills the view area, has the optic disc in centre, adequate light and is in focus. Press and hold the image capture button to capture repeated images. Person B: Observe the mouse/light source contact and monitor mouse welfare. Person A will not be fully aware of how close the light source is from the surface of the eye. If moving too close, or if mouse welfare is in question at any point, advise person A to stop.

12.13. Images taken can be viewed directly from the camera by pressing the play button. Once a satisfactory image has been obtained return mouse to home cage.

12.14. Record images taken for each eye with corresponding mouse barcode in custom TEFI spreadsheet.

12.15. Once the mouse has started the procedure, start the DCF and print the updated cage card.

13. **Image upload:**

13.1. QC the images as described in the QC section, and move all passed images to the appropriate folder.

13.2. Upload images to the database.

14. **Finishing the Procedure:**

14.1. Once all procedures are completed store mice at lowered light level for minimum of 2 hr if dilated images have been taken. Place a 'POST PROCEDURE CHECK REQUIRED' label on each cage. Start the stop watch once the last drops have been applied. This watch MUST be kept by someone at all times until the cages have been returned to the animal room.

14.2. Clean all equipment, surfaces and the floor. **Transfer all waste to a yellow offensive waste bag or clearly labelled waste container.**

14.3. **Register cages to the correct rack whilst returning them to their destination/home rack in the animal room. (Refer to SOP0101 – Taking and Returning Cages for Procedures).**