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Installation and Imaging Protocol for the

Phoenix Retinal Imaging System

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1. **Shipping**

Please store the boxes in a safe place when they arrive and leave them unopened. Phoenix Research Laboratories will supply installation support.

Micron IV | Slit Lamp Imaging | Image-Guided Laser | Focal ERG | Image-Guided OCT | Ganzfeld ERG

2. **Lab set up**

The Micron III imaging system will require approximately 1.5m of linear lab bench space.

3. **Power**

The system is configured for the country specific voltage, and supplied with its own power strip. Therefore, only one wall plug is required.

4. **Training**

The system is extremely easy to use. In most instances two to four hours to of training are required operate the system. The Phoenix Research Labs' installer will be available for one full day of training. An additional day is available if requested in advance for larger working groups without additional charge.

5. **Animal Preparation**

Successful imaging is obtained by properly preparing the animal, which will improve the transparency of the mouse eye. The following protocol is recommended:

a) **Dilation**

For best results, dilate the animal *before* sedation. A sedated animal does not dilate as well, and the dilation drops do not reach the eye if there is Genteal or Gonak present.

1. Fully wet each eye with Atropine (Tropicamide can alternately be used).
2. Wait 2 minutes.
3. Fully wet each eye with Phenylephrine.
4. Wait 5-10 minutes.
5. Check response to light stimulation.
6. Apply additional drops as necessary.

Shine a flashlight directly into the eye to check the animal's response to light stimulation. If the iris constricts, apply more dilators and wait.

b) **Sedation**

We recommend a Ketamine Hydrochloride/Xylazine Hydrochloride mixture (Sigma Aldrich K113. 80mg/ml Ketamine, 12mg/ml Xylazine).

FOR MICE:

1. Dilute the K113 stock solution by 10x.

2. Inject your diluted solution intraperitoneally at 10µl/g body weight (0.1ml/10g body weight)(i.e. for a 30g mouse, inject a 0.3ml bolus of your diluted solution).

FOR RAT S ≤ 200g :

1. Dilute the K113 stock solution by 5x.

2. Inject your diluted solution intraperitoneally at 5µl/g body weight (0.1ml/20g body weight) (i.e. for a 160g rat, inject a 0.8ml bolus of your diluted solution).

FOR RAT S ≥ 200g :

1. Dilute the K113 stock solution by 2.5x.

2. Inject your diluted solution intraperitoneally at 2.5µl/g body weight (0.1ml/40g body weight)(i.e. for a 200g rat, inject a 0.5ml bolus of your diluted solution).

Inject diluted solution into the abdomen or just under the skin. Do not inject into the leg muscle, as this can cause very quick sedation that will likely be too deep. Do not over sedate; this is not a surgical procedure. The cornea MUST be kept moist with an eye wetting agent such as Genteal or Gonak as soon as the animal is sedated. Dry eyes are a major contributor to sedation induced cataracts. Best results are obtained if the imaging session begins as soon as the animal is quiet. Keep the animal warm at all times.

We do not recommend gas or Avertin. These substances can cause the animal's eye lids to close.

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Ketamine/Xylozene Dosage Tables for Mice and Rats

For Mice	
Dilute Ketamime stock by 10x	
Inject 0.1ml/10g body weight	
Mouse Weight	**Diluted Dose**
10 g	0.1
12 g	0.12
14 g	0.14
16 g	0.16
18 g	0.18
20 g	0.2
22 g	0.22
24 g	0.24
26 g	0.26
28 g	0.28
30 g	0.3
32 g	0.32
34 g	0.34
36 g	0.36
38 g	0.38
40 g	0.4
50 g	0.5

For Rats < 200g	
Dilute Ketamime stock by 5x	
Inject 0.1ml/20g body weight	
Rat Weight	**Diluted Dose**
100 g	0.5
115 g	0.575
120 g	0.6
125 g	0.625
130 g	0.65
135 g	0.675
140 g	0.7
145 g	0.725
150 g	0.75
155 g	0.775
160 g	0.8
165 g	0.825
170 g	0.85
175 g	0.875
180 g	0.9
185 g	0.925
190 g	0.95
195 g	0.975

For Rats >200g		
Dilute Ketamime stock by 2.5x		Dilute Ketamime stock by 1.25x
Inject 0.1ml/40g body weight		
Rat Weight	**Diluted Dose by 2.5 x**	**Diluted Dose by 1.25 x**
200 g	0.5	0.25
250 g	0.63	0.32
275 g	0.69	0.35
300 g	0.75	0.38
325g	0.81	0.41
350g	0.88	0.44
375 g	0.94	0.47
400 g	1	0.5
425 g	1.06	0.53
450 g	1.13	0.56
500 g	1.25	0.63
525 g	1.32	0.66
550 g	1.38	0.69
575 g	1.44	0.72
600 g	1.5	0.75
625 g	1.56	0.78
650 g	1.63	0.82
675g	1.69	0.85
700 g	1.75	0.88

c) **Fluorescein Dosing**

We recommend AK-FLUOR® (fluorescein injection, USP) 10% w/v, 100 mg/mL, in a 5 mL vial. (Akorn NDC 17478-253-10).

FOR MICE:

1. Dilute 10% stock solution by 10x.

2. Inject the diluted solution intraperitoneally at 10µl/g body weight (0.1ml/10g body weight) (i.e. for a 30g mouse, inject a 0.3ml bolus of your diluted solution).

FOR RATS:

1. Do not dilute the 10% stock solution.

2. Inject the 10% stock solution intraperitoneally at 1.5µl/g body weight (0.1ml/67g body weight) (i.e. for a 150g rat, inject a .225ml bolus of the 10% stock solution).

Ensure both filter wheels are in position 2 for fluorescein angiography or green fluorescent protein (GFP) imaging. The light intensity may need to be increased. Software gain and exposure control (see customer manuals on desktop) can also be used to increase signal.

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6. Keys to reliable quality imaging

Maximal dilation, a clear cornea, a clear crystalline lens, bubble-free coupling gel, and alignment to the camera all contribute to image good quality. Due to the small pupil, these factors are much more critical in the mouse than in the rat.

a) **Proper dilation:** Pupil size of 1mm or more in mouse; 2.5mm or more in rat are encouraged for best resolution and contrast. See last page, Fig. 1 for typical dilation.

b) **Keep the cornea moist:** This will reduce the chance of cataracts, and reduce corneal scarring.

c) **Inspect the cornea:** Observe the cornea before contact to determine if there is damage. A damaged cornea will greatly degrade resolution of the retina.

d) **Trim whiskers:** Whiskers should be trimmed, and eyelashes pushed away from front surface of the eye. Alternatively, they can be stuck down to the surrounding fur with extra coupling gel.

e) **Proper alignment:** Visualize the front of the eye with the camera prior to contact to ensure direct alignment. The retina can be seen prior to contact if dilation and alignment are correct.

f) **Use of Gonak/GenTeal:** Generous application works best. This fills any voids between the eye and the lens, and allows off axis imaging.

7. Imaging without sedation

Mice can be imaged without sedation. Use of a topical anesthetic is recommended. The images are often not as well resolved as when the animal is sedated, but this technique is often sufficient for animal screening. Sufficient dilation is critical with this approach.

The non-sedated animal is “scruffed” by lifting the animal from the skin at the back of the head. If done correctly the scruffing pulls back the skin around the eye and exposes the eye for examination.

This procedure will require some practice and modest eye-hand coordination but most researchers tell us that with a few mice the technique can be mastered.

This approach is especially valuable for screening large numbers of animals.

8. Image contrast

The **Micron** acquires data in a “diagnostic” or “linear” mode. This means that the digital values recorded are a linear function of the light level. Nearly all consumer and ophthalmic cameras operate in a “high contrast mode” meaning that the output is a non-linear function of the input. This greatly aids in improving contrast.

The linear mode is selected so that the user can do analytical analysis post imaging. That is, if a spot is measured to be larger or brighter the measurement of values will be true. This does not produce the best visualization of the image.

Most eyes are, however, of very high transparency so that the image naturally has high contrast. However, with lower dilation some light will pass through the iris and this scatter will reduce image contrast. Some eyes have cataracts or other corneal scattering centers. Less than ideal alignment will also lower contrast.

It is recommended that some image processing be applied to all images that will be presented and that the native image is saved for future reference.

9. Saving the data: digital video vs. digital stills

The data is recorded as a digital still or a digital video. The easiest method for recording the session is digital video. Every frame in the video is a full resolution, 24bit RBG image. There is no information loss with the video compared to still image capture. While there is very little need to record dynamic phenomena in mice the purpose of the digital video is to allow the user to hand select the best still frames from the digital video.

The camera has transverse resolution the order of 5 microns and this implies a depth of focus (DOF) of around 20 microns. Mouse motion induced by breathing or the user searching about the retina causes motion and lack of best image focus. The digital video enables recording a continuous digital record and embedded in the software is a convenient means to extract the best images. The export enables saving these as BMP, TIFF, or PNG. Video can also be extracted as an AVI file.

Note that each frame in the video is 2MB; therefore a 30 second video will be approximately 1.8 GB. Accordingly, after an animal study, the pertinent frames should be extracted from the video file and saved to prevent the disk from becoming full. The video files should then be deleted and removed from the trash. If this file maintenance is not followed, the computer will run out of memory and will cease to function reliably. The most common cause of computer instability is insufficient memory due to too many saved video files.

10. Care of the instrument

The instrument is robust and should require very little maintenance.

The fiber optic cable, which connects the light box to the camera, is susceptible to damage if kinked or coiled too tightly. If the system is to be moved, disconnect the fiber from the light box to reduce the chance of damage.

When finished with the imaging session any remaining Gonak or coupling gel needs to be carefully cleaned away from the tip of the lens. Use water and the supplied lens tissue or other soft tissue to clean the lens.

The light box contains a high voltage and high UV output lamp. Do not attempt to open this box or service this light source. Service of the light source can only be completed at the factory.

The lifetime of the lamp is limited and we recommend that the light be turned off when not in use.

11. Obtaining service

For general service questions contact the Phoenix Research Labs by phone (+1-925-485-1100) or email (service@phoenixreslabs.com); seewww.phoenixreslabs.com for the best contact. If you are outside the US and want phone support, contact us by email and suggest a time when we can contact you by phone.

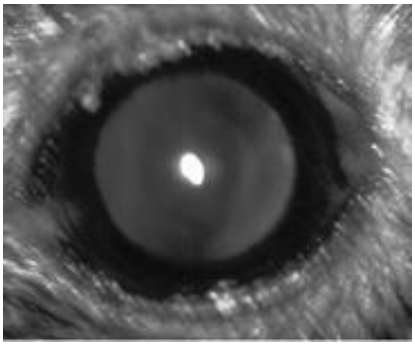

Fig. 1 Typical required dilation.