Standard Operating Procedure for MicroCT or Quantum GX2

Overview:

The Micro CT, or *Quantum GX2*, is located in the 3D Imaging Lab, Room 307, CRNTS department, IIT-B, Powai, Mumbai - 400 076. This document outlines the procedure for its users and operator.

Contact Information: For further inquiries or booking, please contact the lab operator, Mr. Vishal (8554823511) at 3dbioimaginglab@gmail.com

Equipment description: The Quantum GX2 is a basic unit consisting of a Cathode Ray Tube mounted onto a gantry rotor along with detector and emitter, a control and displaysystem, a power supply unit. The main outlet is able to incorporate various bed and bed covers for appropriate sizes of samples. Power supply unit is placed at the back of the system.

Principle: There are cathode ray tubes present in the upper dome of the instrument, whereas below that there is a rotor gantry with detector, emitter, and sample holder, anddown in the working mechanics of the equipment. When a particular current with a highvoltage hits the cathode tubes, X rays are generated which are focused toward the sample passing through the particular filter. Once the X rays are focused onto the sample,it will absorb, scatter the x ray. The aligned detector is a CMOS camera which will convert the X ray beam into a displayable image onto the screen area. Further this imageis analyzed using a particular software.

Applications: Micro-Computed tomography is a useful tool to get sharper images of a sample based on absorption of X-ray for better visualization and representation at a micro level. Micro CT uses a PMT tube and variety of filter for a wide range of sample type and size. Micro CT can be used to image and measure bone density and fat contents of the small animals

Standard Operating Procedure for IVIS Spectrum

Overview:

The IVIS Spectrum, or *In vivo* imaging system spectrum, is located in the 3D Imaging Lab, Room 307, CRNTS department, IIT-B, Powai, Mumbai - 400 076. This document outlines the procedure for its users and operator.

Contact Information: For further inquiries or booking, please contact the lab operator, Mr. Vishal (8554823511) at 3dbioimaginglab@gmail.com

Equipment description: The IVIS Spectrum comprises a laser, a bed, an anesthesiaunit, a set of filters, and a CCD camera detector. Additionally, it includes a PC for display and dedicated software called Living Images for image analysis.

Principle: The IVIS emits laser beams across a range of wavelengths from the UV to far-infrared region. When a sample is placed within the field of view (FOV), the laser passes through an excitation filter to obtain desired wavelength light, which interacts with the sample and emits photons. The emitted light passes through an emission filter, allowing the desired wavelength to form an image using the CCD camera.

Applications: IVIS Spectrum facilitates various applications, including:

- Luminescence and fluorescence in vivo sampling
- Bio-distribution studies
- Long-term monitoring of drug effects

Standard Operating Procedure (SOP) for Multiphoton confocal microscope and Laser Doppler imaging and monitoring system

Mutiphoton Confocal Microscope

To analyze samples such as small animals, cells, 3D cell seeded scaffolds etc. with as little disturbance as possible, you must use low labelling density for your biological models. This requires excellent imaging performance combined with low phototoxicity and high speed. LSM 980, your platform for confocal 4D imaging, is optimized for simultaneous spectral detection of multiple weak labels with the highest light efficiency.

Laser Doppler imaging and monitoring system

The moor VMS-LDF laser Doppler monitor for blood flow and temperature monitoring is a high performance, medical grade module for clinic or laboratory. Use of DSP technology brings you a portable, lightweight module featuring uncompromised specification, quality and reliability at a breakthrough price.

The moorLDI2 laser Doppler imager is suitable for a wide range of pre- clinical research investigations, more typically where smaller areas are under investigation. The system features unique focused optics to provide 50 micron pixel size and 512 x 512 pixel resolution for high resolution blood flow images. The scan areas range from just 2.5cm x
2.5cm up to 25cm x 25cm with scan times typically less than 5 minutes. Use of a focussed laser provides a deeper measurement depth, optimal for angiogenesis studies such as hind limb ischemia and tumour modelling and pre-clinical cerebral blood flow imaging. Highly refined image measurement and analysis software allows for flexibility in measurement set up and comprehensive analysis functions. The moorLDI2 features a colour photo image of the scanned area and automatic distance measurement, making the positioning and comparison of images easier.

Pre-Imaging Requirements for small animals:

1. Equipment Slot Booking:

- All users must book equipment slots online in advance. This is crucial to ensure availability and prevent conflicts with other users.

2. Form B Clearance:

Before booking, the user must have a Form B approved by the Animal Ethics Committee. This document should detail:

- The treatment or procedure to be performed on the sample.
- The method of animal transportation.
- The model/species of animal used.
- Cage occupancy information (number of animals per cage, etc.).

3. Cleanliness and Hygiene:

- Animals must be clean and free of contaminants when brought to the facility. Care should be taken to ensure that the animals are transported under proper conditions to avoid distress or contamination.

4. Lab Attire:

- All users are required to follow lab attire protocols, which include wearing:
  - Lab coat or apron provided by the facility.
  - Footwear provided by the lab, or suitable lab shoes that meet hygiene standards.
- Personal protective equipment (PPE) such as gloves and masks should also be worn at all times when handling animals or equipment.

5. Accompaniment Protocol:

- Only one additional person may accompany the primary user during the experiment. This is to prevent overcrowding and maintain a clean and safe environment.

Post-Imaging Protocols:

6. Cleaning and Sanitization:

- Users must maintain cleanliness throughout the experiment, and after completion, they should sanitize all working areas thoroughly.
- Disinfection procedures include using 70% alcohol to wipe down surfaces and equipment used during the procedure.

7. Restoring Equipment Settings:

- Before leaving, users should ensure that all equipment is returned to its default settings. This includes:

▪ Restoring software settings.
▪ Returning physical components like lenses or attachments to their original positions.

▪ Ensuring that equipment is powered down correctly.

8. Waste Disposal:

- Dispose of animal waste, used PPE (gloves, masks), and consumables in the designated biohazard bins inside the lab.
- Animal carcasses, if applicable, must be disposed of in the designated biohazard bags and handled according to the lab 307 disposal protocols.

Consumables:

Each user must bring their own consumables for the experiment.

These include but are not limited to:

- Lab coat for personal protection.
- Surgical equipment such as scalpels, scissors, or forceps.
- Gloves to maintain hygiene and safety.
- Saline solution for cleaning or sample preparation.
- Syringes for injections or sample collection.
- Alcohol (70% concentration) for sterilizing surfaces and equipment.
- Cotton for cleaning or sample preparation.
- Petri plates for sample handling or storage.
- Catheter (if required for the procedure).

- Any other specific items required based on the nature of the experiment.

Additional Safety Guidelines:

- Training Requirements: Only users who have completed the necessary training for animal handling and equipment use are permitted to perform live imaging procedures.
- Animal Welfare: Users must adhere to strict animal welfare guidelines, ensuring that the animals are treated humanely and with minimal stress.

By adhering to these guidelines, users help maintain the safety, cleanliness, and ethical standards of the 3D Imaging Lab while ensuring accurate and reliable experimental results.

For booking or queries, contact 3dbioimaginglab@gmail.com .

Please adhere to these guidelines for efficient and safe operation of the Multiphoton confocal microscope and Laser Doppler imaging and monitoring system.