UNIVERSITY OF NORTH CAROLINA
CHARLOTTE

Animal Care and Use Program

Guidelines: *In Vivo* Optical Imaging of Mice and Rats

Objective:	To describe procedures for *in vivo* optical imaging of mice and rats using the IVIS imaging system
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Purpose

The University of North Carolina at Charlotte Research Imaging provides small animal imaging services for preclinical research. Animal handling procedures for optical imaging using the Xenogen IVIS offered by the Vivarium are described below.

In Vivo Optical Imaging

A. General Anesthesia Procedures

Animals are anesthetized (described below) for the duration of the imaging study to prevent motion. A heated animal bed, heating pads and, if necessary, a heating lamp, will be used to ensure that body temperature is maintained both pre-imaging and during the procedure. For non–terminal studies animals are monitored during recovery. After recovery, animals are returned to the animal room. Special handling applies to animals injected with short-lived non-radioactive contrast agents as described below (refer to **C. Injections**).

B. Anesthesia Protocols

Mice and rats will be anesthetized with an inhalation anesthetic (isoflurane). Anesthesia will be induced in an induction chamber (2-5% isoflurane), after which the animal will be placed in the imaging instrument and fitted with a nose cone connected to a vaporizer to maintain isoflurane (1.0-2.5%) during the procedure. This range of concentrations produces a level of anesthesia that prevents animal movement during scanning. If respiratory rate begins to accelerate or slow down, the isoflurane concentration will be increased or decreased, respectively.

C. Injections

Animals may be injected with a contrast agent for some imaging procedures, or with an injectable anesthetic. In most cases injections will be intravenous (tail vein) or intraperitoneal. Intramuscular injection will be avoided if possible.

Injection volumes and needle sizes

Method of Injection	Mice	Rats
IV	<0.25 ml / 20g (25-30 ga)	<5 ml / kg (22-25 ga)
IP	<0.4 ml / 20g (25-27 ga)	<10 ml / kg (25 ga)
SC	<0.4 ml / 20g (23-25 ga)	<10 ml / kg (23-25 ga)
IM	<0.05 ml / site (25-27 ga)	<0.1 ml / site (25 ga)

Spectral information for common fluorescent probes

Fluorophore	Excitation (nm)	Emission (nm)
GFP	445-490	515-575

DSRed	500-550	575-650
Cy5.5	615-665	695-770
ICG	710-760	810-875

Source: IVIS Spectrum Protocol Manual

D. Optical Imaging Procedures

For optical imaging, anesthetized animals may be imaged in a Xenogen IVIS system, typically for bioluminescence imaging or fluorescence imaging. In some cases, animals will be shaved in the regions where signal is anticipated and a commercial hair removal cream may be applied briefly to remove stubble in order to optimize transmission of the light signal emitted from within the animal's body. For bioluminescence imaging, anesthetized animals carrying a bioluminescent reporter gene are injected ip or iv with the substrate for luciferase (luciferin, 150 mg/kg iv/ip or coelenterazine, 3 mg/kg iv/ip). Animals are placed on a warmed surface in a light-tight box (IVIS imaging chamber) and imaged with a sensitive CCD camera for typically 20 min or less. For fluorescence imaging, anesthetized animals are injected (iv, ip, sc, or im) with a small quantity of fluorescently-labeled murine antibody, fluorescently-labeled microspheres, or red/near-infrared emitting optical contrast agent. Animals are placed on a warmed surface in a light-tight box and the fluorescent light is imaged with a sensitive CCD camera for periods typically ranging from seconds up to sixty (60) minutes.

E. Animal Housing

Following the scan, the animals may be euthanized (see below) or returned to the animal room. To minimize the risk of contamination of the Vivarium, animal cages will be sprayed with Quatricide or other approved disinfectant before they are returned to the animal room.

F. Euthanasia

In some cases, animals will be euthanized at the end of their imaging studies. Euthanasia will be by cervical dislocation under anesthesia (mice); or by CO_2 (mice or rats) followed by a physical method. Refer to the Guidelines: Euthanasia of Mice and Rats.

References

IVIS Spectrum Protocol Manual

Revision History

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