Radiation Dose and its Effects

Radiation doses for scans done using the in vivo microCT are given below (Table A.1). Importantly, **these doses are localized to the region being scanned**; the regions of the animal not being scanned are shielded from this full dose. Note that at "higher resolutions", i..e, smaller voxel sizes, the radiation dose increases.

Table A.1 The CTDI (CT dose index) produced by the Scanco VivaCT system; information provided by the manufacturer.

Voltage [(1)]	Resolution [(2)]	Field of view (mm)	Nominal Voxel Size (μm)	CTDI (cGy) [(3)]
70 kV	High	38.9	19	26
55 kV	High	38.9	19	22
45 kV	High	38.9	19	19
70 kV	High	30.7	15	42
55 kV	High	30.7	15	35 [(4)]
45 kV	High	30.7	15	31
70 kV	High	21.5	10.5	85
55 kV	High	21.5	10.5	72
45 kV	High	21.5	10.5	62
70 kV	Standard	38.9	38	6.5
55 kV	Standard	38.9	38	5.5
45 kV	Standard	38.9	38	4.8
70 kV	Standard	30.7	30	10.5
55 kV	Standard	30.7	30	8.8
45 kV	Standard	30.7	30	7.8
70 kV	Standard	21.5	21	21.3
55 kV	Standard	21.5	21	18
45 kV	Standard	21.5	21	15.5

Notes:
(1) Higher voltage will produce higher power and give greater penetration and image contrast. Typically we image bones ex vivo at 55 kV, and we expect to use this as our default value as has been used by others in vivo [Boyd, 2006 #1650].
(2) High resolution is 2048 x 2048 image size; standard resolution is 1048 x 1048 image size. There is a 4-fold greater dose at high resolution due to greater number of projections and thus collection time.
(3) In this proposal we use *centigray* (cGy) as the unit of radiation dose, where 1 cGy = 0.01 Gy, and 1 cGy = 1 rad .
(4) Likely settings for our applications are shown in the shaded rows. The choice of settings will depend on the application. We will carefully consider the trade-off between greater radiation exposure and higher resolution.

Lethality: In mice, a **whole-body** dose of ~1000 cGy will lead to 100% lethality within 30 days due to failure of the hematopoietic system, while a dose of ~600 cGy will result in 50% lethality [mccullock and till]. The exact dose for lethality is likely to depend on mouse strain and age. In our experience, a dose of 1000 cGy (produced by a Cesium-137 irradiator that generates gamma rays; an equivalent exposure to *x-rays* and *gamma rays* will result in similar tissue doses and similar effects) resulted in 100% lethality within 10 days for 6-month male C57Bl/6

mice, and doses of 750 cGy result in 0% lethality (unpublished data). Note that these data relate to total-body irradiation, whereas the **Scanco VivaCT does not cause total body irradiation**. Our applications will always involve scanning of targeted areas of the mouse or rat (typically a region of several mm in one of the extremities). Thus, the radiation dose caused by exposing part of one extremity to in vivo microCT, even at the highest likely dose of 72 cGy and for multiple imaging sessions, is **extremely unlikely to result in lethality**.

Sub-lethal effects: While lethality is not an issue, cell damage/death and related biological effects at the local site being scanned must be carefully considered. A whole-body dose of 50 cGy resulted in a 24% reduction in colony-forming units granulocyte-macrophase (CFU-GM, a measure of hematopoietic progenitors) cultured from femoral bone marrow [Grande, 2006 #1651]. Extrapolating this result (based on the exponential curve reported) to 18 cGy (55 kV, standard, 21 μm voxel size) gives an estimated reduction in CFU-GM of 9%. Thus, using parameters that we will tentatively use as default values for mouse scans, there will be a reduction in hematopoietic progenitors of ~10% **at the local site of interest** (e.g., proximal tibia) for each scan. (The effect may be even less than this, as the reference cited [Grande, 2006 #1651] used a relatively high dosing rate of 103 cGy/minute, while we estimate a low dosing rate on the order of 1-5 cGy/min. Slower dosing rates are less damaging to some cell types [FitzGerald, 1986 #1648].) It remains to be seen whether or not this will cause a change in local bone cell activity, such as a reduction in osteoclast number, although these effects will have to be carefully considered when planning experiments. Regarding the rate of recovery after irradiation exposure, the number of leukocytes in peripheral blood recovered to baseline levels within ~2 weeks of partial-body irradiation [Grande, 2006 #1651]. We have observed a partial recovery of marrow CFUs nine days after gamma irradiation at 250 cGy (unpublished). Thus, scanning intervals less than 2 weeks will have to be carefully weighed against the effects of repeated radiation doses. If recovery time is not allowed, the effects of multiple scans is expected to be that of a single scan of the same total dose [xu 1986].

Recently, the effects of repeated in vivo scanning on trabecular microstructure in mice has been reported in abstract form [browers; klinck ORS 2006]. Brouwers et al. performed weekly scanning of the right tibia in adult rats for 8 weeks at a dose of 49 cGy. Comparing irradiated (right) to non-irradiated (left) controls, they reported no effect on the total number of viable marrow cells at the end of the experiment. Moreover, they found no significant differences in trabecular morphology between right versus left proximal tibiae at the end of the experiment (except for an aberrant difference in connectivity density that was attributed to high variability). In slight contrast, Klinck et al. reported that weekly scans (at a dose of 100 cGy) for 3 weeks in juvenile mice led to a significant reduction of 3% in trabecular bone volume in irradiated versus non-irradiated limbs in two of three mouse strains. These findings suggest that **weekly imaging at high dose levels (>50 cGy) may lead to confounding effects on trabecular bone morphology, whereas weekly scans at lower dosages may not alter bone morphology**. Other effects have been noted as well; notable papers are listed under the section “Radiation Dose Effects” on our website. In general, it is appropriate to use a control set of mice who are scanned at the beginning and end of your study only to determine the effects of radiation when high-resolution or very frequent scanning must be performed.

Given the possibility of sub-lethal effects at the doses we will use, careful preliminary studies and appropriate use of controls will be an import part of any study design. An internal control group of mice who are only scanned as their treatment may help mitigate the effects of radiation on bone parameters when higher resolution *in* vivo scanning is necessary. We will advise careful consideration of the tradeoff between smaller voxel sizes and frequent scanning intervals versus total radiation dose. Adequate resolution for trabecular bone morphology can

be achieved at 15-30 μm voxel size [Muller, 1998 #1075; Thomsen, 2005 #1652; Nagele, 2004 #1653], which will result in < 35 cGy. **We will advise a nominal voxel size of 21 μm as the default (18 cGy)**. Higher resolution imaging (10.5 μm) will be recommended only for applications that require higher resolution and can accommodate less frequent (~monthly) scan intervals, or if the biological effects are shown to be negligible or unimportant. Given the wide number of projects and applications, there is no single approach that we can describe, nor do we propose an exhaustive set of experiments aimed at evaluating all the effects of irradiation associated with in vivo scanning. Nonetheless, we will work with investigators to devise preliminary experiments appropriate to each project. Important elements of these preliminary experiments will include the following:

- repeated scanning at the resolution (radiation dose) and interval desired on a small set of animal (n = 5);
- comparisons between irradiated versus control limbs based on microCT morphology as well as cellular histomorphometry (i.e., osteoblast and osteoclast surface measurements from toluidine blue and tartrate resistant acid phosphatase (TRAP) stained sections as described [Baron, 1983 #462; Parfitt, 1987 #242; Liu, 1987 #943]);
- dynamic histomorphometry based on double fluorochrome labeling will also be done to determine if bone formation rates are altered by the imaging protocol;
- consideration of site of interest, e.g. periosteal effects may be less than endocortical/endosteal effects given the differences in radiosensitivity of non-hematopoietic cells versus hematopoietic marrow cells;
- if radiation effects are observed, changes in the scanning protocol to lower total radiation dose will be made and the preliminary experiments repeated.

As we gain experience with use in vivo scanning, we will develop general guidelines to advise users.

In **summary**, scans done using the Scanco VivaCT scanner will not impact animal survival but may cause sub-lethal cellular effects that must be considered. We will carefully consider the tradeoff between scanning resolution and frequency versus total radiation dose, and will advise users to perform preliminary studies to quantify possible cellular effects. Nevertheless, available evidence indicates that repeated scanning at ~20 μm voxel size will not produce significant changes in bone morphology. Thus, we anticipate that informed use of the scanner will generate accurate, artifact-free data for many applications.