**Editorial Comments:**

* Passive voice is used in the second part of step 4.5; this should be changed to imperative tense.

The second part of step 4.5 has been changed to imperative tense:

“Place the probe gently on the flank skin making sure to avoid dispersing the local vasculature. Hold the probe by hand for the duration of the measurement.”

* “Animal use concern: The Note after 5.3 indicates that mice sometimes regain consciousness and "wiggle free" during irradiation. Veterinary reviewers are likely to question the lack of anesthesia maintenance during this procedure, so the authors should be prepared to offer experimental justification and appropriate documentation to support this practice.”

Step 5.3 and the subsequent NOTE have been modified to clarify the use of restrainers and anesthesia during the irradiation procedure. Step 5.3 now emphasizes the importance of using a custom lead jig that also immobilizes the mouse (added: “The custom lead jig should be small enough to immobilize the mouse.”). The subsequent NOTE now describes the measures that ensure that the mouse is immobilized during the procedure through additional restraints or ketamine administration (added to NOTE: “If the custom jig does not completely immobilize the mouse, then use additional restrainers and/or ketamine administration to keep the mouse immobilized throughout the entire irradiation procedure.”).

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**Reviewer 1 Comments:**

* The figure legends should be understandable without reading the main text. This is not the case in Figure 4 where the legend should mention what the three boxes mean. Also, figure 6 is not at all mentioned in the main text and the legend should describe better what the boxes represent.

We have improved the resolution of the figure and also clarified the legend caption in Figure 4:

**Figure 4:** Change **in the oxygenation fraction of mouse skin following 40 Gy irradiation.** The baseline-normalized mean difference between the two groups (per mouse) is significant for Days 6 (Box 1), 9 (Box 2) and 12 (Box 3). With permission from Yohan et al 20145.

We have added a statement to the last paragraph of the Results section alluding to Figure 6: “Changes in A and k can also be monitored over time (Figure 6), and these indicate that the scattering properties of the skin are changing in response to the radiation.”

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**Reviewer 3 Comments:**

* Major Concerns: How about the chronic response of ionizing radiation induced skin toxicity, does it can be quantitative assessed by diffuse optical spectroscopy?

Chronic radiation toxicity is the primary concern during cancer radiotherapy, and this is currently scored subjectively as well. Late radiation skin toxicity is clinically characterized by telangiectasia and fibrosis, which are not as amenable to characterization by DOS as the strong inflammatory response in acute skin toxicity. In our manuscript, we cite Chin et al, 2013 (REF 4), who evaluated tissue oxygenation and total hemoglobin both acutely and up to 56 days post irradiation. They found that the skin was slightly hypoperfused (as determined by a modest decrease in total hemoglobin) by the 56-day timepoint, suggesting that a more robust DOS measure than hemoglobin may be needed to quantify chronic toxicity. Characterizing a new target (e.g. fibrosis) would be outside of the scope of this methodology. To point out this potential avenue, we have added a statement to the last Discussion paragraph: “In the future, it may be interesting to expand DOS-based quantification to the features of chronic radiation induced skin damage (e.g. fibrosis).” Even still, our method may be beneficial by predicting acute radiation skin toxicity *very early on* in treatment so that appropriate interventions can be applied.

* Minor Concerns: For clinical translation, any differenes between human skin and mouse skin involved in this study?

In the 3rd Discussion paragraph, we already highlight the variables that would need to be considered when applying DOS to other skin types (accounting for melanin in various skin colours, variable skin thickness, and hair removal). In our final Discussion paragraph, we additionally state that when applying DOS to human skin, “the probe design may require minor modifications with slightly larger optode separations to account for the increased thickness of human skin”, highlighting the major structural difference between mouse and human skin.

Furthermore, our experimental model employs a single large fraction of radiation applied to murine skin, which is known to mimic the pathophysiology of accelerated severe human acute radiotoxicity (Williams et al, 2010).  We agree that this is not completely representative of the clinical situation where patients are typically irradiated daily over several weeks.  However, we are planning to use our DOS system to monitor acute skin reaction in human patients undergoing radiotherapy, and validate our preclinical findings. We have added a statement to the last Discussion paragraph to address this aspect of our model: “Although our model employs a large single radiation dose (rather than multiple small fractionated doses that are used in the clinical setting), this mimics the pathophysiology of acute human skin radiotoxicity21.”)

**LONG ABSTRACT:**

**-“…** skin toxicity via functional response that is easily translatable to the clinical setting…**” Remove “easily”**

**INTRO:**

**-last paragraph: bedside (not “**bed side**”)**

**METHODS:**

**4.2 – “**pinching a toe on the hind limb**”. Change on🡪of**

**4.4 – Moved the sentence “**Do not sterilize the skin**” from step 4.5 to 4.4.**

**5.1 – “**4.1-4.3**.” Change 4.3🡪4.2**

**REPRESENTATIVE RESULTS:**

**-1st paragraph: “…**both the magnitude and shape of the measured reflectance spectra,\*\*\* which are related..**” Change\*\*\* to period.**

**DISCUSSION:**

**-1st paragraph: “**The DOS system and analysis software is simple to use\*\*\* requires minimal training and returns objective functional parameters for interpreting physiological changes in skin.**” Add comma to \*\*\*  
-1st paragraph: “**Sections 1 and 7 highlights the main processing steps…” **Remove “s”  
-1st paragraph: “**Finally, Section\*\*\* 3-5 describes…**” Add “s” to \*\*\*, and remove “s”  
-1st paragraph: “**Care must be made to avoid disrupting the local vasculature by only gently placing the DOS probe on the mouse surface.**” Reword : “To avoid disrupting the local vasculature, use gentle force to place the DOS probe on the mouse skin surface.”**

**-2nd paragraph: “**Despite the lack of spatial resolution, previous work in mice5 have shown**” Change to “has”**

**-3rd paragraph: “**the application of the same model to dark skinned mice skin may require**” Change to “darker skin” (to accommodate non-murine models)**

**-5th: “**As shown in Figure 6, the initial results from Ref [5] indicate that A and K…” **Change to Yohan et al 2014; use lowercase “k”**

**-5th: end with period.**

**REFERENCES:**

**-last one remove extra “n” from “annd"**

**-add to end: 21. “Williams et al 2010”**

21. Williams, J.P. et al. Animal models for medical countermeasures to radiation exposure. *Radiat. Res.* **173** (4), 557-578 DOI: 10.1667/RR1880.1 [doi] (2010).