Reviewer's report

Title: Comparison of mammary gland imaging techniques and applications: reflectance confocal microscopy, GFP Imaging, and ultrasound

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Reviewer: Rebecca Richards-Kortum

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Summary:
This manuscript presents a comparative study of three imaging techniques (reflectance confocal microscopy (RCM), wide-field microscopy with green fluorescent protein (GFP), and high-frequency ultrasound (US)), applied to mammary gland imaging. Imaging in all three modalities was based on commercially-available systems, alongside conventional histopathology of suitably prepared tissue sections. While the findings of the study are based on a relatively small number of animals, the experimental work appears to have been rigorously conducted and is well presented, and as such could serve as a useful reference for researchers using animal models in the field of breast cancer biology. The manuscript can be accepted for publication provided the following comments are addressed to the Editor's satisfaction.

General comments:
The manuscript would benefit from a thorough proof-reading to eliminate minor grammatical errors. The authors present and discuss GFP as one of their three imaging techniques when really this is one specific example of the more general methods of fluorescent protein labeling and epi-illumination microscopy. In contrast, confocal microscopy and ultrasound are distinct imaging techniques in their own right, and perhaps this distinction could be made clearer. Given the "review / overview" style of this manuscript, the relative merits of epi-fluorescence imaging of GFP-labeled mammary tissue versus alternative fluorescent proteins or fluorescent dyes could be discussed for the reader.

Specific comments:
Title: It should be made clear in the title that the imaging modalities studied here are applied to small animal models, to avoid the interpretation that these techniques can be directly applied (at this stage) in living humans.

Abstract, Results: RCM may have provided the highest resolution in this study, but is not intrinsically superior to GFP imaging, since in the latter case, the resolution is dependent only on the microscope system (objective) used.

Introduction, paragraphs 1 & 2: References that describe quite specific experimental studies are cited here as examples of much broader concepts. Could the authors provide references that represent a wider view of their field in
this context?

Introduction: I suggest removal of sentence 1, since the focus of this work is on imaging of animal models for breast cancer development, rather than human imaging.

Introduction, para's 4, 5, 8: A few sentences indicating the typically imaging parameters for each of the three modalities would be helpful for the reader unfamiliar with these technologies (eg. field-of-view, imaging depth, and resolution).

Page 7, line 3: Can the RCM imaging of whole organ cultures be described as in vivo?

Does fig. 2d contain bubbles appearing as high contrast circular artifacts? The conclusion that the tissue remains viable following application of 1% acetic acid but not after 3% is interesting, but not rigorously supported by studies on only three samples for each group.

Figure Legends: Perhaps this is consistent with the journal's style, but the figure legends appear to contain more descriptive detail than the main body of text (eg. the legend for fig. 7 is 1 page long).