Peer Review File
Article information: https://dx.doi.org/10.21037/jtd-22-244

Reviewer A

This is a well designed ex vivo model of the use of ICG in localising deep seated lung tumour. However, there are several points I wish the authors could clarify.

Comment 1: Who are the assessor of the ICG camera image or spectrometer? Were the results assessed by a single assessor and was the assessor blind to the nature of the specimen i.e. not knowing it is pseudo-tumour with ICG v.s. negative control.

Thank you for your comments. All near-infrared camera and spectral system wavelengths were evaluated by three surgeons. We have added our text as advised (see Page 10, line 14-15 and Page13, line 2-4)

Comment 2: On P.9 line 15 in the pseudo-tumour preparation paragraph, please clarify if PBS is a typo (FBS instead) or not, otherwise please define PBS

Thank you for your comments. we have corrected our text as advised (see Page 10, line 12)

Comment 3: It was mentioned that the spectra were measured by pressing the probe on top of the lung/sponge, to extrapolate this to real life situation, can the probe undergone sterilisation? what is the diameter of the probe and does it need a bigger wound when used in VATS/thoracoscopic operation?

Thank you for your comments. The probe is sterilizable and 10 mm in diameter. For the probe details, We have added our text as advised (see Page 9, line 4-8)

Comment 4: The fact that the near-infrared camera was placed at arbitrary distance render bias in the interpretation of result, as the distance directly affect the possibility of acquiring positive ICG image from reviewer's own experience.

Thank you for your comment. We were aware that the closer the distance from the probe to the pseudo-tumor, the easier it would be to capture the luminescence. However, since the thickening of the intervening material, even slightly, often made it invisible, we investigated at various distances to see if it was really invisible. Therefore, we described it as an arbitrary distance, but we have modified our text as advised (see Page 12, line 6-9 and Page 12, line 18-Page13, line1)

Comment 5: For "wet-lab experiments using porcine lungs" on P.11 line 11-12 What do you mean by "lung were fixed at distanced 10mm, 15mm and 20mm from the organ"? Do you mean the lung were marked at different thickness or do you mean the lung was fixed at different distance from the camera? Please clarify.

Thank you for your comments. The lungs were not sliced and lung thickness was measured. we have modified our text as advised (see Page 12, line 13-15)

Comment 6: It was mentioned in the last sentence of discussion that it does not require the use of expensive equipment; would the authors like to take this chance to clarify the cost of using this novel system?

Thank you for your comments. As for the cost, detailed equipment prices have not yet been determined, but could be at least less expensive than the ICG cameras currently in clinical use, and there are no consumables. In addition, it can be used in conjunction with a thoracoscopic camera, thus eliminating the need to purchase a new endoscopy system. Furthermore, although it has not yet been examined, it could be used for sentinel node biopsies, blood flow measurements, and other procedures in many other departments and organs, and considering the overall cost of these procedures, it is not expensive. we have added our text as advised (see Page 19, line 3-10)

Reviewer B

Dr. Chiba et al:
Describe their findings on utilizing a new spectroscopy system to detect NIR fluorochrome ICG at significantly increased depths which now includes 30-40 mm. This is one of the areas of significant challenges in molecular imaging guided lung cancer resections. They demonstrate that using their NIR fluorescence spectrum system that is optimized for ICG emission wavelength, they can now detect lung lesions at depth of 2-3 cm which addresses current challenges in NIR-I based intraoperative molecular imaging guided cancer resections. The claims made by the authors are interesting.

Comment 1: Overall: The introduction and discussion of the paper primarily discuss current literature of known challenges in this field. While the authors make substantial claims, the results section and description of their findings is lacking. For example The results section is almost less than a page. The authors do not demonstrate spectrophysical properties of their tracer in different media and how their spectral device performs in various conditions.

Secondly, I do not understand the importance or the purpose of the sponge experiment. The optical density and light penetration through is significantly different than a solid sponge. Unless the authors demonstrate similarity, then the findings are not significant.

Rely 1: Thank you for your comments. As for the reason for using sponges, we considered sponges to be the closest to the lungs. We used two different types of sponges to simulate two real-world surgeries with lungs of different densities.
We believe that in real-world clinical practice, there are problems with blood flow and background lung problems such as emphysema and interstitial pneumonia. However, we thought it would be better to simplify first in order to conduct the experiment. Therefore, this time we conducted the experiment using pig lungs under two conditions: collapsed lung and inflated lung. Since it is difficult to add more conditions than those in this experiment, we would like to study them in actual clinical situations in future clinical trials.

Comment 2: Perhaps using agarose or gelatin based pseudo tumor system would be better.

Reply 2: Indeed, there are scattered reports of pseudo-tumors created with agarose or gelatin. We considered these to be soft and difficult to handle because they would be crushed. Therefore, we thought that it would be easy to use when injecting into the lung or through a bronchus, but when placing under the lung as in this case, we thought it would be better to create a pseudo-tumor with a somewhat harder material. We used silicon because it meets these conditions, is readily available on the market, and can be used to create a pseudo-tumor.
In its current form, the claims made by the authors are not supported by their limited description of their results. However, if substantial changes are made, this can be informative for the molecular imaging community. The images of the devices are not pertinent for the manuscript but can be incorporated into the supplementary file. Additionally, a video demonstration would be useful as well.

We treated it as a Fig because we thought it was important to know what kind of machine it actually is. In addition, a video of the actual experiment was added as supplemental data (Supplemental Video 1). We have added our text as advised (see Page 9, line 4-8). Also, data for a 40 mm low-density sponge has been added to Fig. 3b.

Comment 3: Introduction:

Authors need to clearly define hypothesis, and their aims. In this current form the introduction while highlighting important challenges in thoracic surgery, does not

delineate why their method would be innovative in this realm.

Thanks for your comment. we have added our text as advised (see Page 7, line 18 - Page 8, line 6)

Lines 5-7: authors describe surgical management of early stage lung disease but make comment on improving survival for metastatic lung lesions.

Thanks for your comment. we have added our text as advised (see Page 5, line 5-8)

11: What do you mean relative safety, you should highlight the aspects that you believe are important and supported by evidence.

> Thanks for your comment. we have added our text as advised (see Page 5, line 11-13)

Page 7: line 6-8, It is hard to follow authors arguments in this context as it is not clear what is mean by intravenous demarcation and ICGs use in clinical context. Multiple trials have been performed with ICG based and NIR tracers in surgical and thoracic oncology.

It is certainly a digression from the previous context. Here, I wanted to describe how ICG is currently used in thoracic surgery in actual practice, and then describe the novelty and usefulness of this method. we have modified our text as advised (see Page 7, line 9-10)

Comment 4
Discussion:

Overall authors do not highlight how their results and device implementation are different compared to current techniques. This section reads more like a review paper of ICG in cancer detection rather than discussion of pertinent findings.
I would argue that the authors have not described and/or presented results to make substantial claims of finding deep parenchymal lesions.

Reply 4: Thank you for your comment. The difference from the current device is that it performs fluorescence spectrum measurement. Fluorescence spectrum measurement allows the fluorescence wavelength to be confirmed and even weak fluorescence signals to be measured. In fact, the paper by Ebihara et al. examines this at the thickness of the stomach wall, and the ability to observe fluorescence at greater depths will enable ICG fluorescence identification at greater depths.

Ebihara Y, Li L, Noji T, et al. A novel laparoscopic near-infrared fluorescence spectrum system with indocyanine green fluorescence overcomes limitations of near-infrared fluorescence image-guided surgery. J Minim Access Surg 2022;18:125-128.

Comment 5: Page 14 line 8: ICG accumulates in tumors shortly after infusion, however, it also accumulates in the normal parenchyma and vessels as well. However, after 24 hours, ICG is drained from normal visceral organs due to unaltered capillary system but not the tumor. Would rephrase to make it clearer.

Reply 5: Thanks for your comment. we have added our text as advised (see Page 15, line 13-18)

Comment 6: Page 14, line 18: the purpose of utilizing ICG is to detect lesions not amenable to visual or tactile feedback regardless of the camera/spectral system used.

Reply 6: Thanks for your comment. we have modified our text as advised (see Page 16, line 10)

Reviewer C

Comment: Please add more details of a novel imaging system for NIR detecting in terms of the mechanisms of this machine and differences with conventional NIR systems. Thanks for your contribution.

Reply: Thank you for your comment. In conventional ICG cameras, ICG is activated by near-infrared light emitted from the probe, and luminescence must be confirmed with the naked eye from the camera image, which can project light in the near-infrared light region. On the other hand, this spectral system is similar to the conventional ICG camera in that ICG is excited by near-infrared light irradiated through the probe, but the emitted fluorescence is separated into a spectrum and the peak fluorescence values can be measured. Therefore, even very weak fluorescence that cannot be recognized by conventional ICG cameras can be detected as wavelengths, facilitating the detection of lesions at deeper locations.

In addition, although not evaluated in this study, it may be possible to recognize weak wavelengths even for small tumors that cannot be recognized by conventional ICG cameras.