nature portfolio

Peer Review File

Imaging and sensing of pH and chemical state with nuclear-spin-correlated cascade gamma-rays via radioactive tracer

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The work presented is a very innovative approach to readout microenvironmental features (in solution) from decay properties of single photon emitters with cascade decay events. Overall, this reviewer found the approach and the presented data to be of a very high quality. I have some specific comments, below.

Claims are made throughout regarding the potential value for this approach for clinical imaging. This reviewer feels that the merits of this work stand on their own - this is a nice advance on the technical detection side alone - but if such medical claims are being made, then more realistic medical application evaluation needs to be made. This specifically impacts two areas: the range of pH that the authors evaluate, and the presence of scatter and attenuation medium in their setup. For the pH question, it would be most interesting to measure a sample's angular correlation going from pH 4 through 8 which is the most relevant biological pH range (extending from endosomes through the basic intracellular environments). Second, it would be important to show, even at this early prototype-detector stage, what is the impact of attenuation and photon scatter in water surrounding the microtubes of activity. What impact does scatter have on the ability to make any accurate measurements? The time frame of coincidence here is large - so it may be a small effect - but unlike 511keV photons, there will be a lot of interaction events in water/tissues. This should be addressed.

An experiment that is presented is confusing - using the biotin-chelate labeled DOTA. This reviewer understands the concept behind the pretargeting approach - but not how it applies as an example for chelation in this study. What is the effect on angular correlation for DOTA alone versus the psyche-construct. What is the magnitude of such a difference versus free isotope in solution. Most importantly, what is the proposed justification for the change in the angular correlation for the chelated agent's difference?

The authors should ask for some editorial assistance in the preparation of the manuscript as there are areas where the writing is unclear. There are numerous (small) grammatical errors, including in the first line of the abstract.
This reviewer was also confused by the supplemental section on methodological improvements. Why are the authors interested in MeV gamma ray detection using this system?

Reviewer #2 (Remarks to the Author):

This is nicely written manuscript that proposes the application of two existing techniques for imaging and sensing pH, and chemical state with nuclear spin correlated cascade gamma-rays for nuclear medicine imaging. The manuscript suggest to use DPECT, an already published method, exploiting the simultaneous detection of cascade gamma rays emitted by some radio-nuclides, some of them actually being used in nuclear medicine. The chemical sensing technique proposed is the perturbated angular correlation (PAC) technique, which is known and has been applied to study pure samples with samples of high-activity. These two techniques are not novel but the innovation of the manuscript is in the joint usage of these techniques to bring a new horizon in nuclear medicine imaging. Unfortunately the manuscript sends the wrong message that the technique may work but a

careful look at the data suggests important difficulties.

The difficulties reside in the follow aspects:

1. The activity required and/or the acquisition time needed to achieve the count statistics is several orders of magnitude higher than what is encountered in nuclear medicine imaging practice. A sample of 1 MBq/100 uL corresponds to 10 MBq/mL while organs/tumors would have at most 50-100 KBq/mL. This is two orders or magnitude less activity and imaging system would need to have much higher detection sensitivity and scan time would be prohibitively too long.

2. The pH range employed in the samples are not of physiological significance. The pH of the human body is physiologically maintained at values of around 7.4. The samples were prepared with pH of the order 1-3 or 9-12, so either very acidic or very basic. No chemical sensitivity was demonstrated at the physiological pH range of 6.5 - 7.5.

3. The distinction of InCl3 from In-DOTA-Psyche is a non-issue. In-DOTA-Psyche will likely bond to receptors on tumor cell surface while InCl3 will circulate and either be trapped in the liver or excreted in urine. Free 111In will strongly bind to plasma blood protein transferrin.

Finally, the application of this technique in vivo presents more challenges as the human body is a complex chemical environment with various ions, proteins and other molecules circulating and it is unlikely small pH difference could be detected even at high concentration of activity.

A number of other issues are noted below:

line 118: with 8 layers... replace by 'with 8 detector of 8x8 arrays...'

line 122: I think you refer to the wrong figure

line 130: The graph show no distinction at pH higher than 5. The physiological pH are in the order of 7.4

Line 138: This is actually 10 MBq/mL. This is in fact much higher that anything else encountered in Nuclear Medicine. SPECT and PET are known to have pico-molar sensitivity. The p mol (pico mole) is in fact obtained when integrating over the whole half-life of the nuclide. More appropriately, the mentioned activity should be expressed in [pico-]molar concentration values.

Line 154: The pH of 4.5 is before administration in the body and represents the solution it is incorporated in. That matters is the pH in the body. The amount of tracer injected will not affect body pH.

Line 189: Again, this is two extreme pH range and is not physiological.

Line 222: What physiological phenomena would you investigate with longer half life nuclides?

Figure 3 caption: add pH scale values in addition to the color scale.

Line 440: Add references to Geant4. A better description of the source and geometry that was simulated with the Geantt4 code is needed.

Line 436: What was the resulting radiochemical purity?

Line 471: add the collimator septal thickness.

Reviewer #3 (Remarks to the Author):

The authors propose to explore to use double-emissive isotopes as quantum sensor for local environment quantum sensing (pH; a concept previously reported for quantum sensing) in combination with SPECT imaging (routine medical use of such isotopes). Their approach is supported by vary basic/preliminary lab experiments and value/impact of the work is not apparent from the manuscript.

Comments:
- Manuscript requires major English editing checking of the figure references. In the current form it is hard to follow.
- How does DSPECT relate to quantum sensing? Why did the authors use conventional SPECT in their measurements?
- How would quantum sensing add value in nuclear medicine? The molecular state of the radioisotope is already predefined by the tracer administered and anatomy specific tracer accumulation seems to be more predictive (and straightforward) than pH. How does the pH sensitivity correlate to that of the radioisotope detection?
- It not always clear what value presented details and or formulae bring. More focus on actual results/findings would be desirable. As is, it seems the results obtained are limited (figures 1-3 do not seem to present more than basic/preliminary data)
- It seems there is more than pH that can influence quantum sensing, how sure are the authors there

are no other environmental parameters that impact their results.
- Authors seem to switch in detector technology why?
- To assess the potential of the technology presented the authors should include extensive in vivo experiments in animal models wherein the added value of quantum sensing is apparent. This should then be done using clinically relevant applications

Reviewer #4 (Remarks to the Author):

This study shows simultaneous imaging and quantum sensing in a phantom study. The claim is that this ability could lead to a new direction in nuclear medicine diagnosis. While I find the idea intriguing, for me in its current form the paper is not convincingly showing that this is a realistic application.
The authors mention several applications of quantum sensing such as pH, chemical state and chelating molecule sensing. However, I don't see any concrete medical applications where quantum sensing could really make an impact. Most probably, a scanner which can do quantum sensing and also image the activity distribution (conventional imaging) would be inferior to normal SPECT for the conventional imaging part (usually if you combine methods you cannot optimize for both methods). So, to be applicable in nuclear medicine quantum sensing should really add value and I don't see any clear examples worked out.
Typically in clinical SPECT the sensitivity of the scanner (for single photon detection) is about 0.02%. This means that the probability to detect both photons would be extremely small (4e-6%) and almost all detected signal would be from single-photon events which would provide a huge background on the interesting two-photon events. Of course you could increase sensitivity, but this would be at the cost of resolution which would make the imaging part very inferior to conventional SPECT. And then it is still not clear if you could increase sensitivity sufficiently to extract any quantum sensing information. What I miss is any analysis of this. All though I miss some information on the dimensions of the scanner you use, it seems to be a much smaller set-up than what is needed for patients which would make the resolution-sensitivity trade off far better than what could be reached in a patient. I would like to see an analysis of how many counts you can realistically expect in a patient scanner (given normal activity levels and scan time), how much of these are two-photon events and if it is possible to get any information from these. You could at least have reduced the number of counts in your experiment to a realistic level and assumed a realistic background to see what would happen.
Scatter and attenuation play a huge part in clinical SPECT and are not considered at all here. I don't understand why you didn't use a more realistic phantom with water-like material surrounding the sources.
In your experiments, you use very localized sources. In reality, there is always a lot of activity present in other organs. I am not convinced that if you could do this for a localized source (even under realistic conditions), you could also do this for a more realistic activity distribution.
Abstract
The abstract L. 24-27 suggest that in this paper you already applied this method to humans. Please make very clear that this is not the case, that this is a phantom experiment with localized sources.

In the abstract you talk about 'with the energy of MeV', but actually the energy of photons here is much lower than 1 MeV (about a factor 5). Please write this differently.

L. 40-43: I don’t understand what the sentence “,which corresponds to the concentration of molecules itself” refers to. If you write it in this way, it is unclear what ‘which’ refers to.
L. 43-46: You first write that SPECT is used for diagnosis. Then in the next sentence you write for example.. and then you mention an isotope that is therapeutic. That is not a logical way of writing.
-I think the level of English of this manuscript is not sufficient at the moment. There are many small errors/typos. I name a few below, but cannot correct the whole manuscript. I strongly advise to let a native English speaker check it:

- L. 7: remove ‘and’ after (PET)
- L. 10 its accumulation -> the accumulation
- L. 15 via radioactive tracer -> via a radioactive tracer-
- L. 19: carries significant chemical and physical state -> carries significant information on the chemical and physical state-
- L. 25 with the energy of MeV -> with an energy in the MeV range
- L. 26: penetrating human body: penetrating the human body
- L. 54: quantum sensor -> a quantum sensor

L. 68, equation, please write what the sum is over (k runs from .. till ..)

Comments from Reviewer #1

The work presented is a very innovative approach to readout microenvironmental features (in solution) from decay properties of single photon emitters with cascade decay events. Overall, this reviewer found the approach and the presented data to be of a very high quality. I have some specific comments, below.

Thank you very much for the positive comments and your insightful understanding of the method. We revised the manuscript to describe the method and its limitations, and necessary future work for the actual applications according to your comments.

"We propose and demonstrate quantum sensing capability of local micro-environment (pH and chelating molecule) in solution along with radioactive tracer accumulation imaging, by using multiple gamma-rays time-and-energy detection."

Claims are made throughout regarding the potential value for this approach for clinical imaging. This reviewer feels that the merits of this work stand on their own - this is a nice advance on the technical detection side alone - but if such medical claims are being made, then more realistic medical application evaluation needs to be made.
We completely agree with your statements and changed the manuscript to explain only the advance on the technical detection. We added the current limitations for future work in medical applications.

"Further investigation would be necessary for in-vivo imaging including scatter effect and sensitivity."

This specifically impacts two areas: the range of pH that the authors evaluate, and the presence of scatter and attenuation medium in their setup. For the pH question, it would be most interesting to measure a sample's angular correlation going from pH 4 through 8 which is the most relevant biological pH range (extending from endosomes through the basic intracellular environments).

Yes, we completely agree this range will be of great interest if we directly use this method. We observed a radical change around pH 5 according to the chemical state change. We will need further optimization if we would like to use it in the pH 6-8 range, which I clarified in the below sentence. Another application could be pH-controlled delivery combined with other techniques, such as liposome (ref [42,43].). We added explanations to those points as future necessary improvements.

"The measured data shows the transition around pH 5 and the typical value of pH in the body is approximately pH 6.5 to 7.5. It would be difficult to use direct pH detection and

requires further investigation. Another possible way is the combination of other techniques, such as using liposomes, to enable controlled delivery[42,43]."

Second, it would be important to show, even at this early prototype-detector stage, what is the impact of attenuation and photon scatter in water surrounding the microtubes of activity. What impact does scatter have on the ability to make any accurate measurements? The time frame of coincidence here is large - so it may be a small effect - but unlike 511keV photons, there will be a lot of interaction events in water/tissues. This should be addressed.
In principle, photon scattering will be also important in our method as well as in the current SPECT system, which will decrease the number of photo absorption events and increase the false coincidence events. In practical application, in our method the normalization of the correlated photo-absorption events by un-correlated photo-absorption events eliminates the effect of attenuation as well as the geometry effect, so the main effect of scattering will result in the decrease of counts. This should be investigated in future work by changing the thickness of the water.
We added the sentences in the discussion section.

"The normalization of correlated events by the uncorrelated events will work to eliminate the effect of geometry including the scattering in the target region."

"Further investigation would be necessary for in-vivo imaging including scatter effect and sensitivity."

An experiment that is presented is confusing - using the biotin-chelate labeled DOTA. This reviewer understands the concept behind the pretargeting approach - but not how it applies as an example for chelation in this study. What is the effect on angular correlation for DOTA alone versus the psyche-construct. What is the magnitude of such a difference versus free isotope in solution. Most importantly, what is the proposed justification for the change in the angular correlation for the chelated agent's difference?

In this context, we only wanted to report the effect of angular correlations by DOTA (Psyche-Cupid is one of the examples for applications). In figure 2, we showed the difference between pH 7 solution (close to free RI in body) and chelated state (RI is captured in DOTA with antibody-system), which shows the capability to know if RI is captured in DOTA or not. Thus, the pre-targeting system is only a possible application and has no relation with the angular correlation except DOTA. We changed the manuscript to make this point clear.

"This shows the feasibility of detecting chelated and unchelated state of a radioisotope in solution through the gamma-ray correlation data. The detection of chelation state of a radioisotope provides important information as to whether the radioisotope is captured in DOTA of Psyche-Cupid system or not."

The authors should ask for some editorial assistance in the preparation of the

manuscript as there are areas where the writing is unclear. There are numerous (small) grammatical errors, including in the first line of the abstract.
Thank you for pointing this out. The document was proofread by a native English speaker from a professional scientific English editing service. A certificate of editing is attached.

This reviewer was also confused by the supplemental section on methodological improvements. Why are the authors interested in MeV gamma ray detection using this system? This sentence only intended to emphasize the use of gamma-rays with much higher energy than visible photons in comparison with NV center. We revised the sentence as “sub-MeV” gamma-ray photons. In this energy region, other imaging methods, such as Compton cameras, can be applied for increased sensitivity. We changed the manuscript as follows.

“However, the actual practical imaging method should be further optimized in terms of sensitivity and spatial resolution by choosing appropriate angular resolving methods, such as multi-pinhole collimated imaging[27], Compton imaging[28,29,30], or coded aperture imaging.”

Reviewer #2 (Remarks to the Author):

This is nicely written manuscript that proposes the application of two existing techniques for imaging and sensing pH, and chemical state with nuclear spin correlated cascade gamma-rays for nuclear medicine imaging. The manuscript suggest to use DPECT, an already published method, exploiting the simultaneous detection of cascade gamma rays emitted by some radio-nuclides, some of them actually being used in nuclear medicine. The chemical sensing technique proposed is the perturbated angular correlation (PAC) technique, which is known and has been applied to study pure samples with samples of high-activity. These two techniques are not novel but the innovation of the manuscript is in the joint usage of these techniques to bring a new horizon in nuclear medicine imaging. Unfortunately the manuscript sends the wrong message that the technique may work but a careful look at the data suggests important difficulties.

Thank you very much for the accurate comments and your insightful understanding of the method. As suggested, we revised the manuscript to focus on describing the technical development. We also add explanation to clarify the limitations and future necessary work for actual medical applications. We substantially revised the manuscript to clarify the points raised as follows.

"Since ^{111}In is an already available radioactive SPECT tracer in clinical applications, the additional functionalities of detecting the micro-environment surrounding the radioisotope could be useful. However, the actual practical imaging method should be further optimized in terms of sensitivity and spatial resolution by choosing appropriate angular resolving methods, such as multi-pinhole collimated imaging[27], Compton imaging[28,29,30], or coded aperture imaging. In particular, Compton imaging will be useful in the sub-MeV range to increase sensitivity."

"We believe that this new method of imaging and quantum sensing the local micro-environment in addition to its radio labeled molecule accumulation will contribute to the nuclear medicine imaging field."

The difficulties reside in the follow aspects:

1. The activity required and/or the acquisition time needed to achieve the count statistics is several orders of magnitude higher than what is encountered in nuclear medicine imaging practice. A sample of 1 MBq/100 uL corresponds to 10 MBq/mL while organs/tumors would have at most 50-100 KBq/mL. This is two orders or magnitude less activity and imaging system would need to have much higher detection sensitivity and scan time would be prohibitively too long.

Thank you for raising these important points. Improvements of detection sensitivity will certainly be important in our future work comparing with the state-of-the art SPECT technology in human. Currently we are using two collimations method for this proof-of-concept study for localizing the position. However, single collimation and coincidence detectors could be a future possible design to increase the sensitivity. (Here I show preliminary results for

future publication.) As for the efficiency, we added a reference paper mentioning the detection efficiency of the double photon imaging method, which is similar to SPECT (single ~$2x10^{-4}$, double ~$4x10^{-8}$). The proposed detection method is validated in this condition. Another important aspect is that DPECT will not require the image reconstruction compared with SPECT, because the double photon method localizes the RI at one point and not in a line, so the meaning of detection efficiency will be different in the two methods. Some explanations are added as follows with references [44,45].

"An optimized collimation design in a DPECT system or further system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging (50 – 100 kBq/mL in organs). In practical applications the consideration of scatter effect is important as shown in the state-of-the-art SPECT imaging, and further development will be necessary to address this issue."

"Double photon detection decreases the efficiency significantly as described in the multi-nuclide imaging demonstration[44]. Although we used double photon imaging to visualize the accumulation because of the capability of accumulation determination in one point, the rotating method with the same configuration could be investigated in future. Use of active collimators is also proposed in a study[45] for increasing the sensitivity."

44. Uenomachi, *et al.* Simultaneous multi-nuclide imaging via double-photon coincidence method with parallel hole collimators. *Sci Rep* **11,** 13330 (2021). 10.1038/s41598-021-92583-4

45. Omata, A. *et al.* Performance demonstration of a hybrid Compton camera with an active pinhole for wide-band X-ray and gamma-ray imaging. *Sci. Rep.* **10**(1), 1–9 (2020). 10.1038/s41598-020-71019-5

We also added the method of Compton imaging for possible increase of sensitivity as above.

"A multi-pinhole collimation method with higher sensitivity[46] or the Compton imaging[47] technique could be investigated in a future study to achieve higher sensitivity for sub-MeV gamma-rays. An optimized collimation design in a DPECT system or further system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging."

2. The pH range employed in the samples are not of physiological significance. The pH of the human body is physiologically maintained at values of around 7.4. The samples were prepared with pH of the order 1-3 or 9-12, so either very acidic or very basic. No chemical sensitivity was demonstrated at the physiological pH range of 6.5 - 7.5.

Thank you for raising another important point. Figure 1 describe the change of angular correlation in all degrees and indicates the significant change that happened around pH 5 (In above case pH 3-7). Our purpose is to show the detection capability of micro-environment in solution, not direct pH information; thus, we revised the manuscript to clarify this point. In the proof-of-concept study we used pH 1-3 and pH 9-12 to show the clear difference. We are of course aware the physiological pH is around 6.5 to 7.5 and requires more investigation if we use direct pH information. The purpose of this imaging is to show the PoC study to discriminate the micro-environment surrounding RI. The application of this micro-environment detection should be further considered. In the DOTA experiment, it shows the capability to discriminate the difference in chelated state or pH 7 state, which could be useful to know RI is chelated (explained in next response). Another possible application is the use of liposome to encapsulate the radioisotope with the controlled environment. To resolve these issues, we clarified the limitations and possible applications in the manuscript. We also added a reference regarding the use of liposome with RI.

"The measured data shows the transition around pH 5 and the typical value of pH in the body is approximately pH 6.5 to 7.5. It would be difficult to use direct pH detection and requires further investigation. Another possible way is the combination of other techniques, such as using liposomes, to enable controlled delivery[42,43]."

"This shows the detection feasibility of chelated and unchelated state of a radioisotope in solution through the gamma-ray correlation data. The detection of chelation state of a radioisotope provides important information as to whether the radioisotope is captured in DOTA of Psyche-Cupid system or not."

42. van der Geest, T., Laverman, P., Metselaar, J. M., Storm, G. & Boerman, O. C. Radionuclide imaging of liposomal drug delivery. *Expert Opin. Drug Deliv.* **13**(9), 1231-42 (2016). 10.1080/17425247.2016.1205584.

43. Goel, S.et al. Positron emission tomography and nanotechnology: A dynamic duo for cancer theranostics. *Adv. Drug Deliv. Rev.* **113,** 157-176 (2017). 10.1016/j.addr.2016.08.001

3. The distinction of InCl3 from In-DOTA-Psyche is a non-issue.
In-DOTA-Psyche will likely bond to receptors on tumor cell surface while InCl3 will circulate and either be trapped in the liver or excreted in urine. Free 111In will strongly bind to plasma blood protein transferrin.

We think whether the In atom is trapped in DOTA or not is important information, as well as the accumulations, for providing additional knowledge of the 111In state in the body. As you know, in this case we will not inject InCl3 directly; we inject the Psyche-DOTA chelated 111In for pre-targeting. The discrimination of

chelated state or non-chelated (free) state will be important information if it is detectable. We added an explanation regarding this point.

"This shows the feasibility of detecting chelated and unchelated state of a radioisotope in solution through the gamma-ray correlation data. The detection of chelation state of a radioisotope provides important information as to whether the radioisotope is captured in DOTA of Psyche-Cupid system or not."

Finally, the application of this technique in vivo presents more challenges as the human body is a complex chemical environment with various ions, proteins and other molecules circulating and it is unlikely small pH difference could be detected even at high concentration of activity.
We agree it is more challenging to apply this method to the human body. Thus, we focus on describing the method as mentioned by reviewer 1 and clarify the limitations of the method so as not to send the wrong message. We also added discussion of the necessary future works.

"This study shows utility for nuclear medical imaging by detecting the local chemical and physical environment, such as pH or molecular state, surrounding molecules. Further investigation would be necessary for in-vivo imaging including scatter effect and sensitivity."

"An optimized collimation design in a DPECT system or further system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging (50 – 100 kBq/mL in organs). In practical applications, the consideration of scatter effect is important as shown in the state-of-the-art SPECT imaging, and further development will be necessary to address this issue."

A number of other issues are noted below:
Thank you for pointing out these other issues, we revised and fixed them as follows.

line 118: with 8 layers... replace by 'with 8 detector of 8x8 arrays...'
The words are changed according to the advice.
line 122: I think you refer to the wrong figure
Thank you for pointing this out, we fixed it.

line 130: The graph show no distinction at pH higher than 5. The physiological pH are in the order of 7.4
As noted in our responses above, we added an explanation in the manuscript
Line 138: This is actually 10 MBq/mL. This is in fact much higher that anything else encountered in Nuclear Medicine. SPECT and PET are known to have pico-molar sensitivity. The p mol (pico mole) is in fact obtained when integrating over the whole half-life of the nuclide. More appropriately, the mentioned activity should be expressed in [pico-]molar concentration values.
As noted in our responses above,, we mentioned this in the manuscript.
Line 154: The pH of 4.5 is before administration in the body and represents the

solution it is incorporated in. That matters is the pH in the body. The amount of tracer injected will not affect body pH.
As explained above, our purpose is to show the detection capability of the micro-environment, not direct pH information. We revised the manuscript to clarify this point.
Line 189: Again, this is two extreme pH range and is not physiological.
As noted in our responses above, we added an explanation for this point in the manuscript
Line 222: What physiological phenomena would you investigate with longer half life nuclides?
Tumor-specific imaging is now available using SPECT with 111In-labeled ligands, such as the anti-CD20 antibody that targets CD20 positive lymphoma, or pentetreotide that targets somatostatin receptors

Iagaru, A., Gambhir, S. S., & Goris, M. L. "90Y-ibritumomab therapy in refractory non-Hodgkin's lymphoma: observations from 111In-ibritumomab pretreatment imaging. J Nucl Med. 49, 1809-1812, doi: https://doi.org/10.2967/jnumed.108.052928 (2008).

Delpassand, Ebrahim, S., et al. Safety and efficacy of radionuclide therapy with high-activity In-111 pentetreotide in patients with progressive neuroendocrine tumors. Cancer. 23, 292-300, doi: https://doi.org/10.1089/cbr.2007.0448 (2008).

Figure 3 caption: add pH scale values in addition to the color scale.
The color indicates the value of anisotropic parameters estimating pH (not direct pH). The relationship between pH and anisotropic parameters is shown in the extended figure 4(b). An explanation has been added to the captions.

“The color indicates the value of the anisotropic parameters estimating pH value extracted by coincidence detection. The relation of pH and anisotropic parameters are shown in the extended Figure 4(b).”

Line 440: Add references to Geant4. A better description of the source and geometry that was simulated with the Geantt4 code is needed.
References of Geant4 codes are added in the manuscript and a description of simulation geometry is added as follows. The geometry is the same as that used in the experiment described in Extended Data Figure 2(a).

37. Agostinelli, S. et al. GEANT4—a simulation toolkit. *Nucl. Instrum. Methods Phys. Res. A* **506**(3), 250-303 (2003).

Line 436: What was the resulting radiochemical purity?

The labeling rate of Psyche-DOTA is approximately 80%. We added a description of this.

Line 471: add the collimator septal thickness.
The hole pitch is 3.2 mm, so the wall thickness is around 1.2 mm. We clarified the pitch information.

“(2 mm diameter, 3.2 mm pitch)”

Comments from Reviewer #3

The authors propose to explore to use double-emissive isotopes as quantum sensor for local environment quantum sensing (pH; a concept previously reported for quantum sensing) in combination with SPECT imaging (routine medical use of such isotopes). Their approach is supported by vary basic/preliminary lab experiments and value/impact of the work is not apparent from the manuscript.

Our originality and purpose is the first demonstration of micro-environment imaging in solution of pH and chemical state with 111In RI probes. This was noted by the other reviewers. We have now revised the manuscript to clarify the point of the paper as follows.

"This study shows utility for nuclear medical imaging by detecting the local chemical and physical environment, such as pH or molecular state, surrounding molecules. Further investigation would be necessary for in-vivo imaging including scatter effect and sensitivity."

Comments:

- Manuscript requires major English editing checking of the figure references. In the current form it is hard to follow.

Thank you for your comments. The document was proofread by a native English speaker from a professional scientific English editing service. A certificate of editing is attached.

- How does DSPECT relate to quantum sensing? Why did the authors use conventional SPECT in their measurements?

In addition to the conventional SPECT, we added extra detectors to localize and sense the angular correlation for pH sensing. These additional detectors are necessary to detect two photons simultaneously. DPECT will enable the detection of accumulation in one point, not in a line as done in SPECT. We improved the explanation to clarify this. We also added one reference.

"In the imaging experiment, four modules with physical collimations are used to surround the two $^{111}InCl_3$ solutions for detecting the double photons emitted from radioisotope in simultaneously."

44. Uenomachi, *et al.* Simultaneous multi-nuclide imaging via double-photon coincidence method with parallel hole collimators. *Sci. Rep.* **11,** 13330 (2021). 10.1038/s41598-021-92583-4

- How would quantum sensing add value in nuclear medicine? The molecular state of the radioisotope is already predefined by the tracer administered and anatomy specific tracer accumulation seems to be more predictive (and straightforward) than pH. How does the pH sensitivity correlate to that of the radioisotope detection?

The basic idea is the simultaneous detection of RI accumulation (current) and "additional" micro-environment detection (proposed). We added explanation regarding the possible application of the proposed method. One is the combination of liposome-based RI delivery system, and the other is monitoring the chelation of the RI. In the DOTA experiment, the capability to discriminate the difference in chelated state or pH 7 state is shown, which could be useful to know if the RI is chelated.

"This shows the capability of determining the chelation state of a radioisotope. Lastly, the simultaneous imaging and pH sensing capability is demonstrated using the physical collimation method as a proof-of-concept study using a phantom."

"The detection of chelation state of a radioisotope provides important information as to whether the radioisotope is captured in DOTA of Psyche-Cupid system or not."

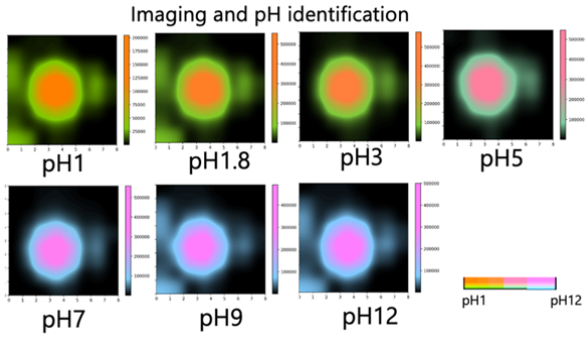
- It not always clear what value presented details and or formulae bring. More focus on actual results/findings would be desirable. As is, it seems the results obtained are limited (figures 1-3 do not seem to present more than basic/preliminary data)

Thank you for pointing this out. Figure 1 shows the first simultaneous measurement of angular correlation in different pH values and Figure 2 shows that of a chelated molecule, which have not been previously reported anywhere. Figure 3 shows the first imaging results of pH through the proposed method. We added text to explain this more clearly.

"We propose and demonstrate quantum sensing capability of local micro-environment (pH and chelating molecule) in solution along with radioactive tracer accumulation imaging, by using multiple gamma-rays time-and-energy detection."

- It seems there is more than pH that can influence quantum sensing, how sure are the authors there are no other environmental parameters that impact their results.

As you pointed out, what we detect is mainly the hyperfine electric quadrupole interaction of nuclear spin with micro-environment. It is known that temperature (~100deg order) will also have an effect; thus, we control the temperature. (In addition, there are magnetic dipole interactions, but it is negligible here. We did another experiment to change it with ~Tesla order). Other factors, such as viscosity, might have an effect, but we used pH as an indicator this time. The below repeated experiment showed similar results.



- Authors seem to switch in detector technology why?

The Extended Figure 2 setup is used to characterize the response of pH sensitive angular correlation, which is the basis of the second experiment. Extended Figure 4 shows the setup of the imaging experiment using the results confirmed by the first setup. We added more explanations to clarify this.

"In the pH and chelating molecule characterization experiment, eight detector modules are used to surround the measurement target solution for covering the solid angle to characterize the relation between micro-environment and gamma-ray emissions."

"In the imaging experiment, four modules with physical collimations are used to surround the two $^{111}InCl_3$ solutions for detecting the double photons emitted from radioisotope in simultaneously."

- To assess the potential of the technology presented the authors should include extensive in vivo experiments in animal models wherein the added value of quantum sensing is apparent. This should then be done using clinically relevant applications

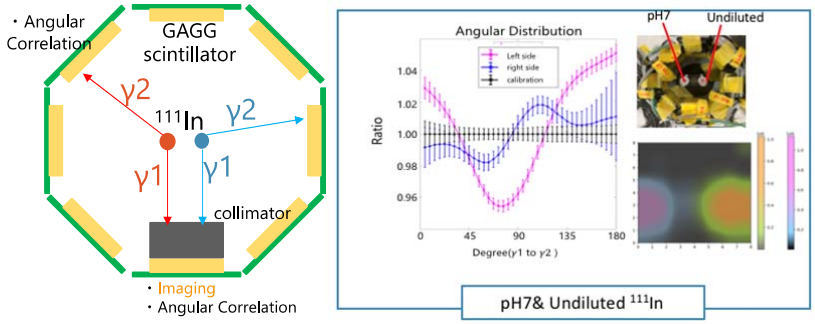
Thank you for your comments. Certainly, the in-vivo imaging application and clinical trials are our final goals and should be done in the future follow-up works. This paper shows the first proof-of-concept demonstration of a proposed method. As per the points raised by you and other reviewers, we revised the manuscript focusing on reporting the novel result. We also added an explanation of current limitations and future necessary works.

"This study shows utility for nuclear medical imaging by detecting the local chemical and physical environment, such as pH or molecular state, surrounding molecules along with its highly sensitive (~ p mol) localization of radio-labeled molecules itself through nuclear spin and successive gamma-rays simultaneously. Further investigation would be necessary for in-vivo imaging including scatter effect and sensitivity."

Comments from Reviewer #4

This study shows simultaneous imaging and quantum sensing in a phantom study. The claim is that this ability could lead to a new direction in nuclear medicine diagnosis. While I find the idea intriguing, for me in its current form the paper is not convincingly showing that this is a realistic application. Thank you for your comments and interest in this work. As the points raised by the other reviewers, we revised the manuscript to focus on the proof-of-concept demonstration work. We now also clearly describe the limitations regarding realistic medical clinical application and necessary work for that as noted below.

The authors mention several applications of quantum sensing such as pH, chemical state and chelating molecule sensing. However, I don't see any concrete medical applications where quantum sensing could really make an impact. Most probably, a scanner which can do quantum sensing and also image the activity distribution (conventional imaging) would be inferior to normal SPECT for the conventional imaging part (usually if you combine methods you cannot optimize for both methods). So, to be applicable in nuclear medicine quantum sensing should really add value and I don't see any clear examples worked out.
In the revised manuscript we describe the current limitations to apply this method to the medical clinical situation, which requires a certain sensitivity. Although it is not included in the manuscript, here I show the two-dimensional imaging results by simply adding extra non-collimated detectors to the conventional SPECT like system. This method could be the possible solution to your question. I added an explanation regarding this in the manuscript.



"Since ^{111}In is an already available radioactive SPECT tracer in clinical applications, the additional functionalities of detecting the micro-environment surrounding the radioisotope could be useful. However, the actual practical imaging method should be further optimized in terms of sensitivity and spatial resolution by choosing appropriate angular resolving methods, such as multi-pinhole collimated imaging[27], Compton imaging[28,29,30], or coded aperture imaging. In particular, Compton imaging will be useful in the sub-MeV range to increase sensitivity."

"A multi-pinhole collimation method with higher sensitivity[42] or the Compton imaging[43] technique could be investigated in a future study to achieve higher sensitivity for sub-MeV gamma-rays. An optimized collimation design in a DPECT system or further

system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging."

Typically in clinical SPECT the sensitivity of the scanner (for single photon detection) is about 0.02%. This means that the probability to detect both photons would be extremely small (4e-6%) and almost all detected signal would be from single-photon events which would provide a huge background on the interesting two-photon events. Of course you could increase sensitivity, but this would be at the cost of resolution which would make the imaging part very inferior to conventional SPECT. And then it is still not clear if you could increase sensitivity sufficiently to extract any quantum sensing information. What I miss is any analysis of this. All though I miss some information on the dimensions of the scanner you use, it seems to be a much smaller set-up than what is needed for patients which would make the resolution-sensitivity trade off far better than what could be reached in a patient. I would like to see an analysis of how many counts you can realistically expect in a patient scanner (given normal activity levels and scan time), how much of these are two-photon events and if it is possible to get any information from these. You could at least have reduced the number of counts in your experiment to a realistic level and assumed a realistic background to see what would happen.

Regarding efficiency, we added a reference paper 44 mentioning the detection efficiency of the double photon imaging method (single ~$2x10^{-4}$, double ~$4x10^{-8}$). The proposed detection method is validated in this condition. The detector configuration is described in Extended Figure 2 as 69 mm diameter, similar to a small animal scanner. One way to improve these aspects is using active collimators as mentioned in reference 45. In addition, although we are using double photons to detect micro-environment information, the configuration of one detector is similar to a SPECT system, which does not lose the function to detect single photons. In addition to the two-dimensional imaging, single events are always acquired and can be used for conventional accumulation imaging in a properly designed system. This possible improvement is mentioned using a different detection method.

"Double photon detection decreases the efficiency significantly as described in the multi-nuclide imaging demonstration[44]. Although we used double photon imaging to visualize the accumulation because of the capability of accumulation determination in one point, the rotating method with the same configuration could be investigated in future. Use of active collimators is also proposed in a study[45] for increasing sensitivity."

44. Uenomachi, *et al.* Simultaneous multi-nuclide imaging via double-photon coincidence method with parallel hole collimators. *Sci. Rep.* **11,** 13330 (2021). 10.1038/s41598-021-92583-4

45. Omata, A. et al. Performance demonstration of a hybrid Compton camera with an active pinhole for wide-band X-ray and gamma-ray imaging. *Sci. Rep.* **10**(1), 1–9 (2020). 10.1038/s41598-020-71019-5

"A multi-pinhole collimation method with higher sensitivity[43] or the Compton imaging[44] technique could be investigated in a future study to achieve higher sensitivity for sub-MeV gamma-rays. An optimized collimation design in a DPECT system or further system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging."

Scatter and attenuation play a huge part in clinical SPECT and are not considered at all here. I don't understand why you didn't use a more realistic phantom with water-like material surrounding the sources.
In your experiments, you use very localized sources. In reality, there is always a lot of activity present in other organs. I am not convinced that if you could do this for a localized source (even under realistic conditions), you could also do this for a more realistic activity distribution.
In principle, photon scattering will be also important in our method, as well as the current SPECT, which will decrease the number of photo absorption events and increase the false coincidence events. This should be investigated in future work by changing the thickness of the surrounding water. In practical application, the normalization of the correlated photo-absorption events by un-correlated photo-absorption events eliminates the effect of attenuation as well as the geometry effect, so the main effect of scattering will be the decrease of counts. Further study to increase the sensitivity will be required and those discussions are added in the draft. We also added text in the discussion section.
We also repeated the experiment with 2D configuration and obtained similar results. Thus, the detection itself will be possible.

"An optimized collimation design in a DPECT system or further system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging (50 – 100 kBq/mL in organs). In practical applications, the consideration of scatter effect is important as shown in the state-of-the-art SPECT imaging, and further development will be necessary to address this issue."

Abstract
The abstract L. 24-27 suggest that in this paper you already applied this method to humans. Please make very clear that this is not the case, that this is a phantom experiment with localized sources.
Thank you for raising this important point. We made it very clear in the revised manuscript that this is only a proof-of-concept study of the proposed method and requires substantial work before practical application.

"This study shows utility for nuclear medical imaging by detecting the local chemical and physical environment, such as pH or molecular state, surrounding molecules."

"Lastly, the simultaneous imaging and pH sensing capability is demonstrated using the physical collimation method **as a proof-of-concept study using a phantom**."

"In practical applications, the consideration of scatter effect is important as shown in the state-of-the-art SPECT imaging, and **further development will be necessary to address this issue."**

In the abstract you talk about 'with the energy of MeV', but actually the energy of photons here is much lower than 1 MeV (about a factor 5). Please write this differently.
We changed the word 'MeV' to 'sub-MeV'.
L. 40-43: I don't understand what the sentence ",which corresponds to the concentration of molecules itself" refers to. If you write it in this way, it is unclear what 'which' refers to.
We deleted the sentence 'which …' to clarify the sentence.
L. 43-46: You first write that SPECT is used for diagnosis. Then in the next sentence you write for example.. and then you mention an isotope that is therapeutic. That is not a logical way of writing. We added 'evaluation of therapeutic nuclides' in the first sentence to make it logical.
-I think the level of English of this manuscript is not sufficient at the moment. There are many small errors/typos. I name a few below, but cannot correct the whole manuscript. I strongly advise to let a native English speaker check it:
Thank you for checking for typos and errors. The document has now been proofread by a native English speaker from a professional scientific English editing service. A certificate of editing is attached.
We fixed the errors as follows.
- L. 7: remove 'and' after (PET) 'and' is removed
- L. 10 its accumulation -> the accumulation "its" is changed to "the"
- L. 15 via radioactive tracer -> via a radioactive tracer- 'a' is inserted
- L. 19: carries significant chemical and physical state -> carries significant information on the chemical and physical state- the sentence is changed accordingly
- L. 25 with the energy of MeV -> with an energy in the MeV range 'an' is inserted and 'sub' is inserted to indicate the correct energy range for SPECT
- L. 26: penetrating human body: penetrating the human body 'the' is inserted
- L. 54: quantum sensor -> a quantum sensor 'a' is inserted
L. 68, equation, please write what the sum is over (k runs from .. till ..)
Explanation added as follows.
The highest term of this expansion is decided by the selection rule: k_{max} = Min ($2I$, $2l_1$, $2l_2$).

Reviewers' comments:

Reviewer #1 (Remarks to the Author):

The authors have addressed all of this Reviewer's comments.

Reviewer #2 (Remarks to the Author):

The authors have mostly addressed my concerns.
Th abstract should clearly state that this is a proof of concept and clinical applications will require the development of high absolute sensitivity nuclear medicine cameras beyond what exist today.
The last statement of the abstract should say:

"This work demonstrates a proof of concept and further work is necessary to increase sensitivity of the technique for in vivo imaging and study the effect of scattered radiation for possible application in nuclear medicine."

And also, mention again mention at the end of the introduction.

Line 125: Add the information that no correlation is observed at pH larger than 3.

Line 148: You say Psyche-DOTA has a pH of 4.5. Is this in the vial? What is the PH once in vivo? It should still be around 7 since the amount of tracer will not change the body's pH.

Figure Caption 2: point (a) and (b) needs to be rewritten.
"This is provided by Medi-Physics..."
What is provided by Medi Physics?
The rest of the sentence should say
... other samples are produced by mixing with other solutions...
What are those solutions?
(b) The chemical structure of Psyche... , add word chemical

Again this data points to my comments above, only extremely acidic conditions can be detected, and point to the 'proof-of-concept' nature of this work.

Reviewer #4 (Remarks to the Author):

I still feel the authors should analyze better the practicality of their method. I understand that this is a new technique and that they cannot solve everything at once, but they can at least analyze what is needed. E.g.
- The authors now acknowledge that sensitivity is an important aspect and that it should be

improved. As they also state there selves, current results are obtained at activity concentration levels that are much higher than what is typical in organs. From the data they have, they could easily reduce the number of measured counts and analyze what would happen at realistic activity concentrations. Is the technique still usefull then? And if not, at which activity level does it break down with the current SPECT equipment? That gives an idea of how much sensitivity improvement is needed.

-As I already stated, it would be very easy to at least put the separate sources in Fig. 3 in water to see what happens when scatter occurs. According to the authors, scatter would just reduce the number of counts because they have a scatter correction method. However, in general such a scatter correction method does not completely eliminate the scatter effect, because scatter is also noisy. It would be relatively easy to check what happens when there is more scatter.

Comments from Reviewer #2

The authors have mostly addressed my concerns.
Th abstract should clearly state that this is a proof of concept and clinical applications will require the development of high absolute sensitivity nuclear medicine cameras beyond what exist today. The last statement of the abstract should say:
"This work demonstrates a proof of concept and further work is necessary to increase sensitivity of the technique for in vivo imaging and study the effect of scattered radiation for possible application in nuclear medicine."
And also, mention again mention at the end of the introduction.
We thank the reviewer for the insightful comments and suggestions on our manuscript. We are glad that the authors found the previous changes satisfactory. We have added the suggested sentence at the end of abstract as well as introduction to clarify the necessity of further improvement.
"This work demonstrates a proof of concept, and further work is necessary to increase sensitivity of the technique for in vivo imaging and to study the effect of scattered radiation for possible application in nuclear medicine."

Line 125: Add the information that no correlation is observed at pH larger than 3.
As suggested, we have added the sentence “No correlation is observed at pH larger than the transition around 3.” in the suggested line.

Line 148: You say Psyche-DOTA has a pH of 4.5. Is this in the vial? What is the PH once in vivo? It should still be around 7 since the amount of tracer will not change the body's pH.
We understand the concern raised by the reviewer. It is the measured value in the tube. We have added the following part: “has a measured pH value of 4.5 in the tube.”

Figure Caption 2: point (a) and (b) needs to be rewritten.
"This is provided by Medi-Physics..."
What is provided by Medi Physics?
We have clarified the sentence as “The raw $InCl_3$ solution is provided by Medi-Physics”.

The rest of the sentence should say
... other samples are produced by mixing with other solutions...
What are those solutions?
Based on the suggestion of the reviewer, we have revised the sentence as "Other samples with different pH are produced by mixing the raw solution with other solutions." Those solutions are listed in the supplementary table 1.

(b) The chemical structure of Psyche... , add word chemical
The word "chemical" is added as suggested.

Again this data points to my comments above, only extremely acidic conditions can be detected, and point to the 'proof-of-concept' nature of this work.
As suggested, we have added the suggested "proof-of-concept" sentence at the end of abstract as well as introduction to clarify the necessity of further improvement. We will work on further improvement in future.

Comments from Reviewer #4

I still feel the authors should analyze better the practicality of their method. I understand that this is a new technique and that they cannot solve everything at once, but they can at least analyze what is needed. E.g.

We thank the reviewer for the careful review and analysis of our manuscript. We have conducted an additional experiment with water to see the effect of scattering and reducing the counts for evaluating the sensitivity.

- The authors now acknowledge that sensitivity is an important aspect and that it should be improved. As they also state there selves, current results are obtained at activity concentration levels that are much higher than what is typical in organs. From the data they have, they could easily reduce the number of measured counts and analyze what would happen at realistic activity concentrations. Is the technique still usefull then? And if not, at which activity level does it break down with the current SPECT equipment? That gives an idea of how much sensitivity improvement is needed.

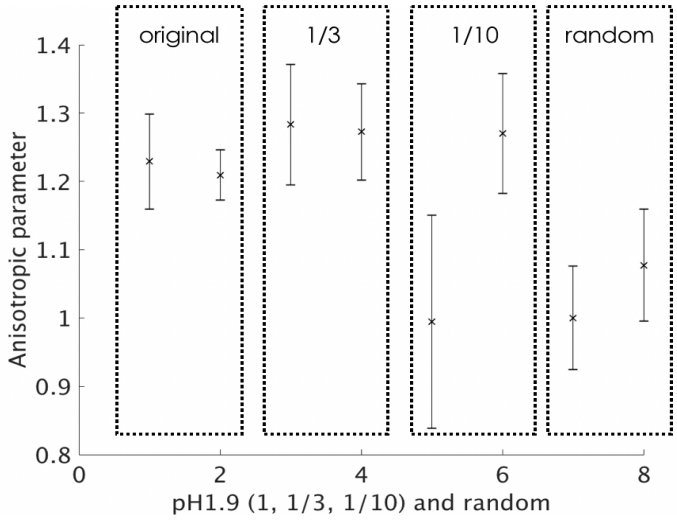
The bottom figure shows the results of anisotropy with decreased counts (1/3, 1/10). 1/3 concentration still shows the correlation. However one of the 1/10 lost the correlation, which indicates sensitivity higher by an order of 2-3, will be required.

Recently, another group published a work using both pinhole and slit collimators to improve the sensitivity of cascade gamma-ray imager in around two orders.

[48]. Liu, X., Liu, H., Cheng, L., Wu, J., Bao, T., Yao, R., & Liu, Y. (2021). A 3-dimensional stationary cascade gamma-ray coincidence imager. Physics in Medicine & Biology.

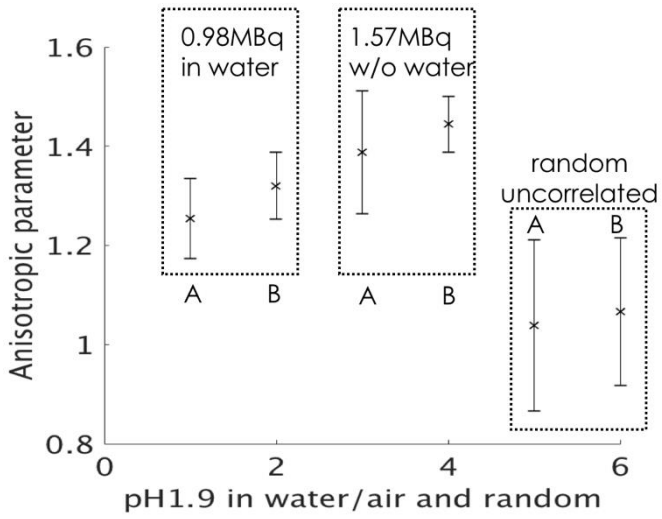
The adoption of such a method and its future development will make our method more practical. We have added the reference and the explanation on this point in the methodological improvement section.

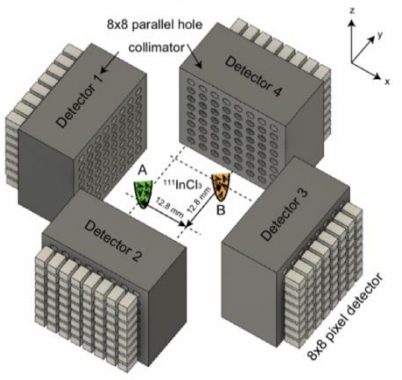
“An optimized collimation design in a DPECT system or further system design consideration will be necessary to increase the sensitivity for practical nuclear medical imaging (50 – 100 kBq/mL in organs). Recently, a research group also showed the improvement of detection efficiency using both pin-hole and slit collimators in coincidence imaging[48].”



-As I already stated, it would be very easy to at least put the separate sources in Fig. 3 in water to see what happens when scatter occurs. According to the authors, scatter would just reduce the number of counts because they have a scatter correction method. However, in general such a scatter correction method does not completely eliminate the scatter effect, because scatter is also noisy. It would be relatively easy to check what happens when there is more scatter.

As suggested, we have conducted the experiment with water and without water to observe the effect of scattering. The bottom figure shows the results of anisotropy of raw solution (around pH 1.9) as compared to the uncorrelated random events. Both showed a similar value in this case, which confirmed the robustness of the method. This work shows the proof-of-concept nature study. However, as suggested by the reviewer, in the future, a more precise scatter correction will be necessary. We added those sentences to the methodological improvement section. “In practical applications, the consideration of scatter effect is important as shown in the state-of-the-art SPECT imaging, and further development will be necessary to address this issue, which includes the scatter correction method.”
"This work demonstrates a proof of concept, and further work is necessary to increase the sensitivity of the technique for in vivo imaging and to study the effect of scattered radiation for possible application in nuclear medicine" in the introduction section.


0.98MBq
in water
1.57MBq
w/o water
random
uncorrelated
A
B
A
B
A
B
Anisotropic parameter
1.6
1.4
1.2
1
0.8
0
2
4
6
pH1.9 in water/air and random


8x8 parallel hole
collimator
Detector 1
Detector 4
Detector 2
Detector 3
A
B
^{111}InCl$_3$
12.8 mm
12.8 mm
8x8 pixel detector
z
y
x

REVIEWERS' COMMENTS:

Reviewer #2 (Remarks to the Author):

No further comments.

Reviewer #4 (Remarks to the Author):

I am happy with the reviewer's answers to my concerns. I especially like that the authors have quantified how much more sensitivity would be needed in order to apply their technique to realistic activity concentrations. I think the authors should also state this result in their paper (not merely in their rebuttal letter). With that, I would recommend acceptance of this work.

Reviewer #2:
Remarks to the Author:
No further comments.

Response:
Thank you for your evaluation of our work. We are pleased to know that our revisions were satisfactory.

Comments from Reviewer #4:
Remarks to the Author:
I am happy with the reviewer's answers to my concerns. I especially like that the authors have quantified how much more sensitivity would be needed in order to apply their technique to realistic activity concentrations. I think the authors should also state this result in their paper (not merely in their rebuttal letter). With that, I would recommend acceptance of this work.

Response:
Thank you very much for your insightful suggestion. Accordingly, we have added the necessary figures and statements in the manuscript. We believe our manuscript can now be accepted.