Report of the Inquiry into the Allegation of Research Misconduct by Dr. Lei Yao and Dr. Huabei Jiang

Submitted to Dr. David Norton, Vice President for Research

February 16 , 2016

Executive Summary

The Inquiry addressed an allegation brought against Drs. Lei Yao and Dr. Huabei Jiang (Respondents) by a Complainant, who wished to remain anonymous, that the Respondents falsified data in a published paper.

According to federal policy and the University of Florida (UF) policy, "falsification means manipulating research materials, equipment or processes, or changing or omitting data or results such that the research is not accurately represented in the research record." Further, a finding of research misconduct, according to the UF policy for Dealing with Conduct in Research requires that: 1) there is a significant departure from accepted practices of the relevant research community; and 2) the misconduct be committed intentionally, or knowingly or recklessly; and 3) the allegation be proven by a preponderance of evidence.

Preliminary information-gathering and preliminary fact-finding from the inquiry indicated that the allegation may have substance. Thus, it was determined that the allegation warranted further investigation.

<u>Description of the Allegation</u>: According to the Complainant, many data sets of photo-acoustic measurements were recorded and provided to Dr. Yao to develop a finite element based algorithm for the reconstruction of absolute temperature distribution in tissue. According to the Complainant, and supported by emails from Dr. Yao (Attachment 1), Dr. Yao chose specific data sets, to show that the algorithm worked and published a paper. Specifically, figure 3 of the paper supposedly supports the contention that the algorithm works when in fact the Complainant alleges figure 3 is incorrect. In addition, the Complainant alleges that he discussed this with Drs. Yao and Dr. Jiang, but the paper was published and has not been retracted. The paper is titled "Finite-element-based photoacoustic imaging of absolute temperature in tissue", and was published September 10, 2014 in Optical Society of America (Attachment 2).

<u>Name and Position of the Respondent</u>: Dr. Yao now lives in China. He was a post-doctoral Associate at UF from 2013 through 2015 under the mentorship of Dr. Jiang. The Respondent, Dr. Jiang, is a professor in the Department of Biomedical Engineering.

<u>Support Information</u>: The research was supported by the J. Crayton Pruitt Family Endowment.

<u>Applicable Regulations</u>:

1. UF Regulation 6C1-1.0101; Policy for Dealing with Conduct in Research found at http://www.admin.ufl.edu/DDD/attach06-07/R10101-0704.pdf

<u>Inquiry Process:</u>

The inquiry process was conducted by Dr. David Hahn, Professor and Department Chair, Mechanical and Aerospace Engineering, Dr. Irene Cooke and Mr. Michael Scian, Director and Assistant Director, respectively, of the Division of Research Compliance, Office of Research, UF.

1. Dr. Irene Cooke and Mr. Michael Scian met with the Complainant, and reviewed emails between the Complainant and the Respondent, Dr. Yao dated January 27, 2015.
2. Dr. Hahn and Mr. Scian met with the Respondent, Dr. Yao.
3. Dr. Hahn reviewed data from the Complainant and the Respondent, Dr. Yao.
4. Dr. Hahn met with the Complainant, and reviewed emails between the Complainant and the Respondent, Dr. Yao dated January 27, 2015.

Results of the Inquiry Process:

1. The paper in question claims to have developed an algorithm for the reconstruction of absolute temperature distribution in tissue using photoacoustic measurements.
2. In discussions with the Complainant by Dr. Cooke and Mr. Scian, the Complainant said he applied the algorithm to the data and it did not yield data consistent with figure 3 in the paper. Rather, the data was much more random. The Complainant produced an email exchange between him and the Respondent, Dr. Yao dated January 27, 2015. Dr. Yao wrote that there were problems processing the data due to laser instability, thermometer resolution, the contrast level of velocity, and that stable results could not be obtained. Dr. Yao further wrote: "Thus in order to get expected results from experiments I had to try different parameters to do the calculations, and selected the best ones step by step. In other words, I ignored bad results and only picked those good ones, based on the assumption that the parameters in the good results were somehow offsetting the negative impact of the hardware limitations."
3. During the meeting with the Respondent, Dr. Yao, Dr. Hahn and Mr. Scian, Dr. Yao stated he used a qualitative test to select data for figure 3 in the paper. He looked for trends in the data and rejected data that did not line up with his algorithm.
4. Summary of preliminary inquiry by David Hahn:
   Dr. Hahn met with the Complainant in his office on January 27, 2016 to discuss this case. The Complainant explained the overall data collection process, namely, that each experiment involved data collection from 120 transducers. For each experimental condition (concentration and laser power), multiple runs were made. For the actual data reported in the paper, the concentration was fixed at 0.06 mg/ml, and experiments were performed for three power cases (Case 1 = 3 W, Case 2 = 2 W and Case 3 = 1 W). The Complainant showed Dr. Hahn all the raw data files, which corresponded to the following replications:

   Case 1: 4 runs
   Case 2: 3 runs
   Case 3: 6 runs

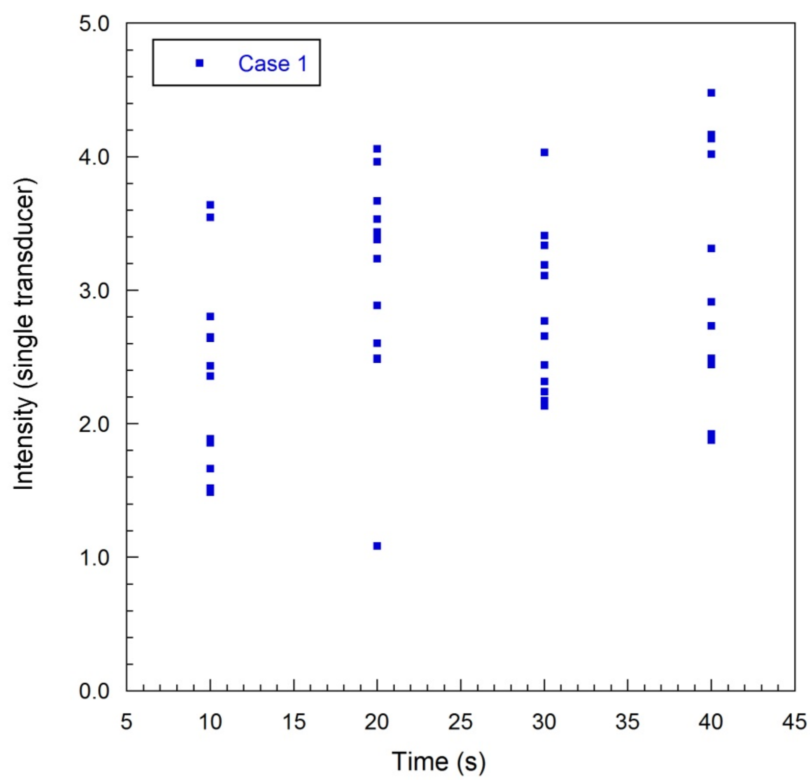
   The Complainant further explained that the data was noisy (i.e. poor signal-to-noise ratio on a given transducer) and single transducers with the greatest signal-to-noise ratios were selected. Noise was attributed to variations in the pulse-to-pulse stability of the laser, transducer-to-transducer variance, and overall low signal levels (i.e. at the signal detection limit).

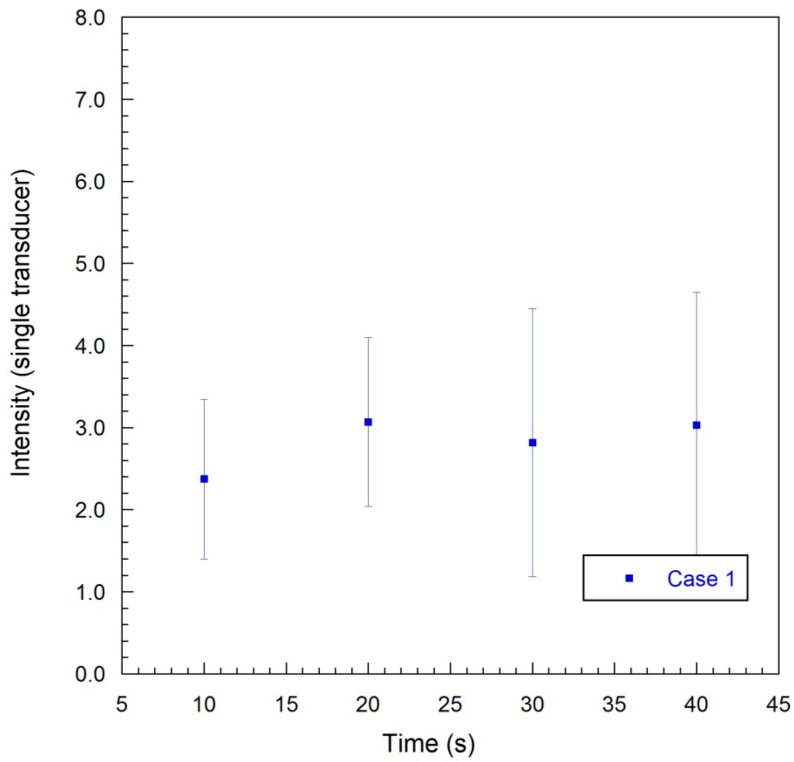
   This approach provided an absolute temperature measurement using signal intensity data and time delay data. The latter provided the acoustic velocity which then used a calibration curve (e.g. figure 1 in paper) to provide absolute temperature.

   The Complainant related that the overall data was noisy and did not provide the relationship as reported in figure 3 of the paper, noting that figure 3 plotted experimental data from the TC (thermocouple) versus the recovered experimental data from the inversion algorithm. Therefore, figure 3 was reported to provide an experimental validation of their method by comparing against a known temperature measurement, the TC data. The Complainant claimed that individual data was cherry-picked from a broad range of data to fit the plot.

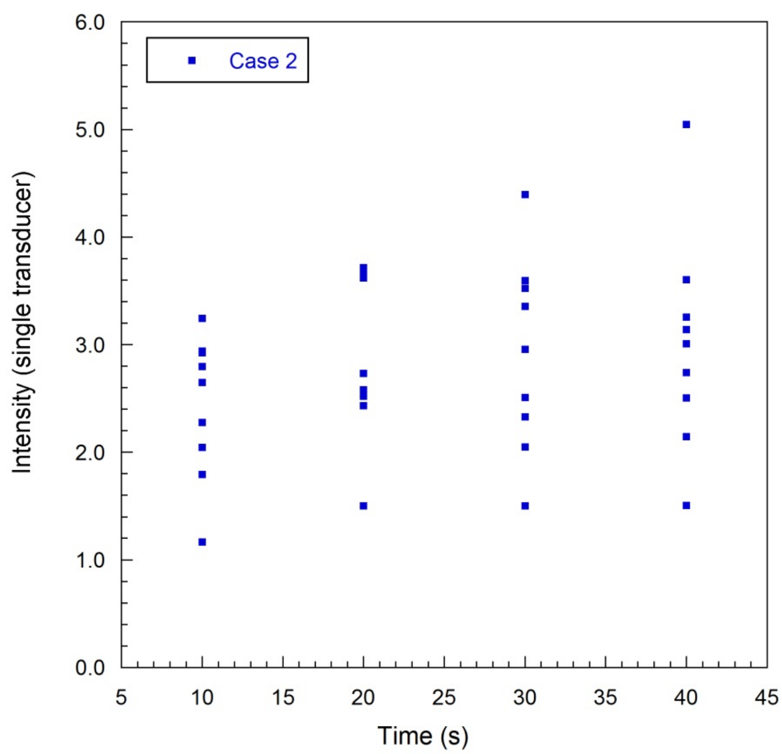
The Complainant provided a set of all data that was processed as follows. For each of the runs summarized above, the Complainant selected the three transducers with the best signal-to-noise ratio and processed all data to yield intensity versus time. While the intensity data could be further processed into an absolute temperature measurement with the acoustic data, the intensity data represented a monotonic relationship with temperature. Exploring intensity data was a sufficient means to explore the primary question, namely, does the experimental data support the figure 3 plot. Dr. Hahn plotted below all the data provided by the Complainant for the three experimental cases:

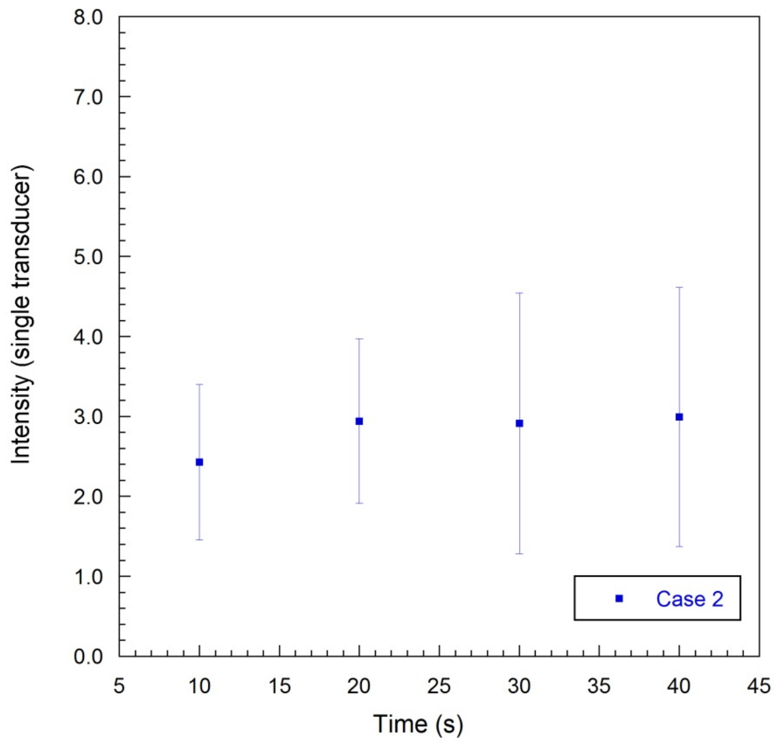
Individual data points from 3 highest SNR transducers vs. time, along with averages and SD
Case 1



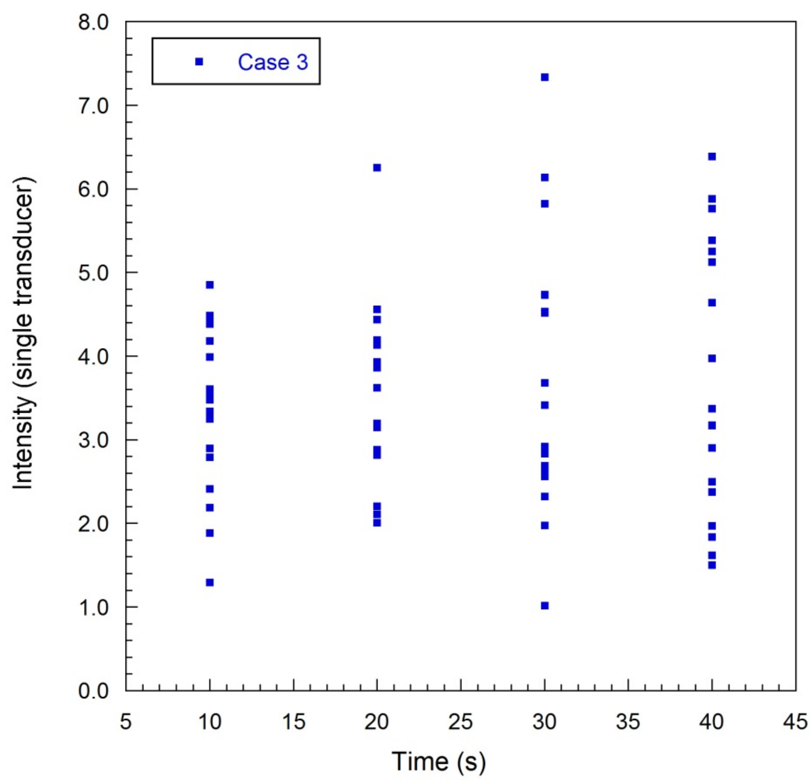


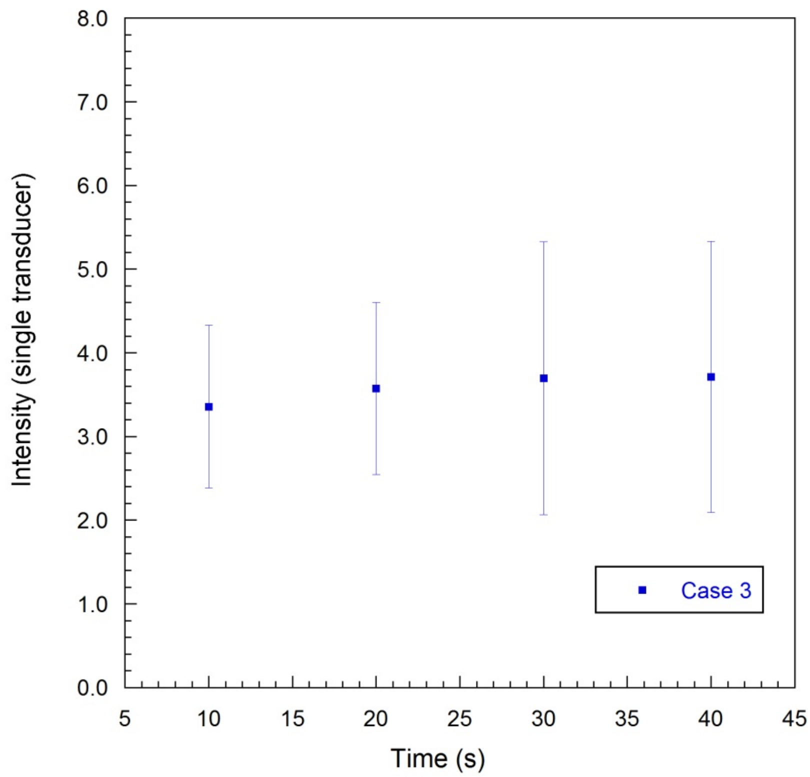
Individual data points from 3 highest SNR transducers vs. time, along with averages and SD
Case 2





Individual data points from 3 highest SNR transducers vs. time, along with averages and SD
Case 3





As noted above, the data presented above were not absolute temperature data, but per discussions with the Complainant and from Dr. Hahn's reading of the paper, they were representative of the data trends and importantly, represented the *precision* of the data. In two of the three cases, the average data point at 30 seconds was less than the value at 20 or 40 seconds; hence it was not representative of the monotonic increase reported in figure 3. Although Dr. Hahn did not perform a test (e.g. t-test) for statistical significance, based on his extensive experience with such analyses, Dr. Hahn concluded that the intensity data as reported above would not show any statistical differences in support of the positive and monotonic temperature increase with time as reported in figure 3, which supported the Complainant's statement that the experiments did not support the figure 3 data. Simply put, the data was far too noisy.

Dr. Hahn then had a detailed discussion with the Respondent and co-author Dr. Yao on February 5, 2016, about the paper. Mr. Scian was also present. Dr. Yao was very forthcoming in his discussion of the manuscript. He explained that the data was very noisy, noting the poor signal-to-noise ratios and the laser pulse-to-pulse fluctuations. He explained that he did a two-part test to include data from a particular run. He first screened to see if the resulting data produced a monotonic increasing temperature value with time. If not, the data was rejected. For this, he used a "qualitative" back-projection to do this first screening, which only used intensity information. Dr. Hahn considered this similar to the concept reported above by Complainant, namely, that intensity data was representative of temperature data, notably so in trends and precision.

Once this first screening was performed, Dr. Yao then processed the passing data with his FEM algorithm. He stated that he had strong confidence in the algorithm itself, which was his main contribution and that he "expected the (experimental) results to fit" the thermocouple data, that the "points should be close". If several data sets passed his first test (i.e. back-projection) as noted above, he stated that he selected the single data point "closest to the TC data".

Dr. Hahn asked Dr. Yao if he had any experience with reporting data rejection (i.e. outlier rejection) and explained that there were standard methods for such rejection, but that simply taking the closest data was not the standard. Dr. Yao stated that he "never previously dealt with data rejection".

Dr. Yao stated that he discussed the overall data and method with Dr. Jiang, and that they concluded in view of the hardware limitations and the sensitivity limitations, that the algorithm worked.

Overall, based on the data, as well as discussions with Dr. Yao and with the Complainant, it was concluded that the actual data was simply too noisy (i.e. poor signal-to-noise) and at the signal detection limits to make any clear statement in validation of the algorithm via comparisons with the TC data. Dr. Hahn indicated he had no reason to believe that the FEM method was flawed.

It was also clear that Dr. Yao rejected data based on trends that did not fit the expected results, namely, did not fit the TC data. By his own admission, he selected the results that fit the TC data. Of great significance, the paper made no mention of the rejection of data. To any scientist or engineer reading the paper, the conclusion would be an excellent fit of experiments with TC data. By scientific standards, it is both unacceptable to simply "cherry-pick" the desired results to match other data and equally unacceptable to make no mention of data rejection.

The Ethical Practices guidelines from Optics Letters, the journal in question, state in part:

- A research paper should contain sufficient detail and reference to public sources of information to permit the author's peers to repeat the work. Adequate information should be provided with numerical data to allow comparison with other research. Specifically, data should include sources and magnitudes of uncertainties, and graphs representing numerical data should display error bars where appropriate.

- It is an author's responsibility to submit an erratum for publication when a significant error is discovered in one of her or his published reports.

In conclusion, with this published paper, the authors have collectively:

1. Omitted experimental data, and made no mention of such omitted data in violation of the ethical standards stated for OSA for Optics Letters.
2. Such omission of data is a significant departure from common practices in the field of optics and spectroscopy.
3. Per discussions with Dr. Yao, the data was intentionally screened and data that did not fit the TC data trends was omitted.
4. There is no indication that their FEM method is incorrect.

Conclusion of the Inquiry Process:

Based on the above, there is a reasonable basis for concluding that the allegation falls within the definition of research misconduct per the UF regulation; and the preliminary information-gathering and preliminary fact-finding from the inquiry indicates that the allegation may have substance. Thus, it was determined that the allegation warranted further investigation.

Charges to Consider for the Investigation:

1. Determine whether the allegation meets the definition of research misconduct; in this case, falsification.
2. Determine whether there was a significant departure from the accepted practices of the relevant research community.
3. Determine whether the falsification (if present) was intentional or unintentional, knowing or reckless based on the facts of the case.
4. Determine whether the allegation can be proven by a preponderance of evidence.

Submitted February 16, 2016

Inquiry Committee Members (alphabetical order)

Irene Cooke

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Assistant Vice President for Research and Director,
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Michael Scian

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From:	
To:	Scian,Michael P
Cc:	Cooke,Irene; Moore,Christina S
Subject:	Re: Emails about the paper
Date:	Thursday, January 14, 2016 3:02:34 PM

From:
Sent: Tuesday, January 27, 2015 11:52 PM
To:
Cc:
Subject: Re: about the OL paper

Dear Prof. Jiang,

I think it is not appropriate for Dr. Yao Lei to publish this paper based on the experimental results, which he claims that the experimental results can not be used for reconstruction. My role in this work is not just only collecting the experimental data. It took more than one year to conduct this experiment. It is necessary for Dr. Yao to show his respect to people who cooperate with him. If he still want to keep this paper for publishing, I want to remove my name from that paper. Hope to have suggestions from you. Thanks a lot!

Best,

On Wed, 28 Jan 2015 04:29:37 +0000, Yao,Lei wrote:
> Dear Prof. Jiang,
>
> Today send me several emails about our paper published on
> Optics Letter on the absolute temperature imaging. I think we should
> sit down together to talk about this problem. Please first review the
> emails between us. Thank you.
>
>

From:
Sent: Tuesday, January 27, 2015 11:05 PM
To:
Subject: Re: About the paper

Who do you think you are? You definitely faked this paper, no matter
how you justify your excuses. Obviously, you picked up data
intentionally to justify your reconstruction! Hope you did not
manipulate all your data for published papers.

In any case, if your result is based on experimental results, you have
to get repeatable reconstruction results for publishing! If the
experimental data are not stable due to the inevitable reason, your so
called perfect theoretical model can not be used in this real situation.
Then just give it up. You are not supposed to pick up any my
experimental data for reconstruction for publishing. Who taught you to
do research in this way? Don't play tricks with people. Academic
misconduct is a serious problem. Shame on you!

On Tue, 27 Jan 2015 22:41:29 -0500, Yao,Lei wrote:
> I think you must have not understand this project and the results,
> since you never studied this work carefully and you missed most of my
> reports on the lab meeting. This is definitely not fake work!
>
> The simulation results confirm that the algorithm is reliable and
> correct (I can send you the results tomorrow). Even when the contrast
> level is very low (like the experimental situation, 1:1.03), we still
> can get good reconstructed absolute temperature images. The problem
> is, when the noise level increases (for example, more than 10%), or
> the variation of the laser source intensity increase (for example,
> more than 5%), we can hardly get good images when the contrast level
> of velocity is very low (1:1.03). In other situations, even with high
> noise level or very unstable laser source intensity, the
> reconstruction results were still reasonable.
>
> As for the experimental data processing, there are following
> problems:
> 1) the laser source intensity is very unstable;
> 2) the resolution of thermometer is very low;
> 3) the contrast level of velocity is very low (the heating time is

> short, or the changing of temperature is small);
> 4) your results is non-repeatable (for same case, you could not get
> stable results. each time the results were different, so that we had
> to average them, which you called statistic method). Even we used
> this
> statistic method, the curve we obtained was still not fit the linear
> relationship well.
>
> These problems limited our capability of recovering STABLE results
> from your experiments. Thus in order to get expected results from
> your
> experiments, I had to try different parameters to do the
> calculations,
> and selected the best ones step by step. In other words, I ignored
> bad
> results and only picked those good ones, based on the assumption that
> the parameters in the good results were somehow offsetting the
> negative impact from the hardware limitations. This is not a perfect
> solution, of course, because though we could get expected results on
> the phantom experiments (since we knew the real value and structure
> in
> advance), this approach can hardly be extended on the future animal
> experiments or clinical cases. And even for phantom experiments, this
> work is very time consuming and sometimes we still could not get good
> results with many possible parameters. That's why we could not move
> forward on this project now. Again, the algorithm itself is
> absolutely
> correct.
>
> If we want to solve this problem rigorously, we had to solve the
> hardware issues (stable laser source and high quality thermometer),
> and do the experiments more carefully (the experiments should be
> applied by experienced person and the data should be repeatable and
> stable). Please remember, if all these problems could be solved (by
> sufficient money and enough time), our project can move forward
> hopefully, and the core method is still the same one: the developed
> FEM algorithm (maybe I'll add a calibration pre-processing strategy
> later).
>
> I hope this helps. And I suggest you really understand this project
> and my work before you draw any silly conclusions.

>
> On Tue, 27 Jan 2015 17:19:02 -0500, wrote:
>> Can you tell me that the reconstructed results are correct or not?
>> Because my name is on a paper, I need to know the results and I can
>> not put my name on a faked paper.
>>
>>
>>
>>
>> On Tue, 27 Jan 2015 14:56:52 -0500, Yao,Lei wrote:
>>> Your contribution here was only collecting the experimental data.
>>> You will not be responsible for the FEM algorithm, data processing,
>>> the discussion or the conclusion in the paper.
>>>
>>> On Tue, 27 Jan 2015 14:05:17 -0500, wrote:
>>>> Hi Dr. Yao,
>>>>
>>>> I saw the paper of Finite-element-based photoacoustic imaging of
>>>> absolute temperature in tissue was published on Optics Letters.
>>>> Because the absolute temperature can not be reconstructed based on
>>>> the
>>>> experimental data as you mentioned, it is not appropriate to
>>>> publish
>>>> this result. It will ruin our lab's reputation, if someone can not
>>>> replicate this result. It's better to withdraw it. But if you
>>>> don't
>>>> want to do this, can you contact the editor to remove my name from
>>>> it?
>>>> Thanks!
>>>>
>>>> Best,
>>>>

Finite-element-based photoacoustic imaging of absolute temperature in tissue

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posted August 18, 2014 (Doc. ID 212008); published September 10, 2014

We describe a finite element based algorithm for the reconstruction of absolute temperature distribution in tissue using photoacoustic measurements. Assuming a linear temperature dependence of the Grueneisen parameter in tissue, the algorithm aims to recover the temperature dependent acoustic speed while a heating mechanism is used. The absolute temperature over time is then calculated using the recovered temperature dependent acoustic speed. To validate our method, photoacoustic measurements were conducted using a graphene nanosheet containing tar get embedded in a 20 mm diameter tissue like phantom with varied heating power. The results obtained suggest that quantitatively accurate temperature images can be produced, suggesting that our method may serve as a tool to guide and monitor the temperature distribution in tissue in real time noninvasively and to improve the safety and efficacy of thermotherapy.

OCIS codes: (110.5120) Photoacoustic imaging; (110.6820) Thermal imaging; (110.6960) Tomography.
http://dx.doi.org/10.1364/OL.39.005355

Photoacoustic tomography (PAT) is an emerging noninvasive imaging technique for visualizing the internal structure of soft tissue with excellent spatial resolution and satisfactory imaging depth [1 3]. Since tissue acoustic properties such as thermal-expansion coefficient and acoustic speed are temperature dependent [4,5], PAT has been applied to obtain temperature changes in tissue [6,7], taking advantage of its rich optical absorption contrast, high detection sensitivity, and deep penetration depth. The current methods, however, can provide only the information of temperature changes compared to a baseline and cannot measure the absolute temperature in tissue, because these methods extract the temperature information directly from the measured photoacoustic signals themselves [8]. It is known that the knowledge of absolute temperature is critical for effective thermal therapies, because such knowledge provides the necessary control of the boundary of the heated abnormal tissue and minimizes thermal damage to the surrounding normal tissue. Initial effort has been made to quantify absolute photoacoustic thermometry using the temperature dependence of the acoustic speed in tissue [9]. However, in this initial effort, a nonmodel-based technique was used where the optical fluence was assumed as uniformly distributed across the object and background media, which could be satisfied only when temperature-induced change in optical attenuation is small and localized. In addition, the assumption that both the Grueneisen parameter and acoustic speed can be approximated as a linear function of temperature is not correct apparently, and the two equations used to quantify the absolute temperature should not be considered independently, since the Grueneisen parameter is actually a function of acoustic speed, thermal expansion coefficient, and specific heat at constant pressure. Therefore, there is a clear need for developing new model-based PAT methods to provide truly accurate photoacoustic temperature imaging.

Here we describe a reconstruction method that can provide absolute temperature distribution in tissue by rigorously solving the Helmholtz-like photoacoustic wave equation coupled with an inverse strategy using the finite element method (FEM). While we have previously reported the development of FEM-based reconstruction algorithms for recovery of tissue optical and acoustic properties [10,11], in this work we extend these PAT algorithms to include the ability of recovering temperature distribution in tissue. As a result, absolute temperature in tissue can be quantified accurately without any estimation or measurement at a known baseline temperature, which means that this technique could be a powerful tool to monitor temperature in deep tissue, especially for internal organs where the temperature might be significant different from other parts of the body during thermotherapy.

Our reconstruction algorithm consists of three steps. The first step is to recover the distribution of absorbed energy density through a time-domain finite-element-based PAT algorithm previously developed [10]. Here, the following time-domain Helmholtz-like equation is used to describe the propagation of photoacoustic wave in tissue:

$$\nabla^2 P(\mathbf{r},t) - \frac{1}{v^2}\frac{\partial^2 P(\mathbf{r},t)}{\partial t^2} = -\frac{\beta\Phi(\mathbf{r})}{C_P}\frac{\partial J(t)}{\partial t}, \tag{1}$$

where P is the pressure of the generated acoustic wave; v is the reference acoustic speed of the medium, which is homogenous and can be determined experimentally; $\Phi(\mathbf{r})$ is the absorbed optical energy density; β is the thermal expansion coefficient; C_P is the specific heat at constant pressure; and $J(t) = \delta(t - t_0)$ is assumed in our study, which is the short laser pulse that irradiates the medium.

The finite-element discretization of Eq. (1) can then be written as

$$\sum_{i=1}^{N} P_i\left[\int_S \nabla\psi_i \cdot \nabla\psi_j \mathrm{d}S\right] + \sum_{i=1}^{N} \ddot{P}_i\left[\int_S \frac{1}{v^2}\psi_i\psi_j \mathrm{d}S\right] + \oint_l \psi_i\psi_j\left[\frac{1}{v}\dot{P}_i + \frac{1}{2r}P_i\right]\mathrm{d}l = \sum_{i=1}^{N}\Phi_i\left[\int_S \frac{\beta}{C_P}\frac{\partial J}{\partial t}\psi_i\psi_j \mathrm{d}S\right], \tag{2}$$

where ψ_j is the basis function. Here the following first-order absorbing boundary conditions are used:

$$\nabla P \cdot \hat{n} = -\frac{1}{v}\frac{\partial P}{\partial t} - \frac{P}{2r}, \quad (3)$$

where $\hat{n}$ is the unit normal vector.

The matrix form of Eq. (2) can be expressed as

$$[K]\{P\} + [C]\{\dot{P}\} + [M]\{\ddot{P}\} = [B]\{\Phi\}, \quad (4)$$

where

$$K_{ij} = \int_S \nabla\psi_i \cdot \nabla\psi_j dS + \frac{1}{2r}\oint_l \psi_i\psi_j \mathrm{d}l,$$
$$C_{ij} = \oint_l \frac{1}{v}\psi_i\psi_j \mathrm{d}l,$$
$$M_{ij} = \int_S \frac{1}{v^2}\psi_i\psi_j \mathrm{d}S,$$
$$B_{ij} = \int_S \frac{\beta}{C_P}\psi_i\psi_j \mathrm{d}S \cdot \frac{\partial J}{\partial t},$$
$$\{P\} = \{P_1, P_2, \cdots P_N\}^T; \{\dot{P}\} = \{\dot{P}_1, \dot{P}_2, \cdots \dot{P}_N\}^T,$$
$$\{\ddot{P}\} = \{\ddot{P}_1, \ddot{P}_2, \cdots \ddot{P}_N\}^T; \{\Phi\} = \{\Phi_1, \Phi_2, \cdots \Phi_N\}^T.$$

We use a regularized Newton's method to update the initial distribution of the absorbed energy density $\Phi(\mathbf{r})$ iteratively to minimize an object function composed of a weighted sum of the squared difference between computed and measured data at the medium's surface. The core procedure in this reconstruction algorithm is the iterative solution to the following regularized matrix equation [11]:

$$(\mathbf{J}^T\mathbf{J} + \lambda\mathbf{I})\Delta\chi = \mathbf{J}^T(\mathbf{P}^o - \mathbf{P}^c), \quad (5)$$

where $\mathbf{J}$ is the time-dependent Jacobian matrix formed by $\partial P/\partial\Phi$ at the boundary measurement sites at each time step, $\mathbf{P}^{o,c}$ is the time-dependent measured and calculated acoustic fields for $i = 1, 2, \cdots M$ locations, $\Delta\chi = (\Delta\Phi_1, \Delta\Phi_2, \cdots \Delta\Phi_N)^T$ is the updating vector, $\mathbf{I}$ is the identity matrix, and λ is the regularization parameter determined by combined Marquardt and Tikhonov regularization schemes. The distribution of the absorbed energy density $\Phi(\mathbf{r})$ can then be reconstructed iteratively.

The second step of our method is to recover the distribution of the acoustic speed during the heating process. Previous experimental studies confirmed that almost no changes in optical properties from 25°C to 50°C were observed [7,8,12], until the tissue temperature was raised to more than 60°C when protein started to denature [12]. Thus, we assume that the optical properties are temperature insensitive in this study, which allows us to apply the reconstructed absorbed energy density, $\Phi(\mathbf{r})$ (from the first step) as known values during the heating process to further our investigation.

Based on the time-domain Helmholtz-like equation [Eq. (1)] and its boundary conditions, we have the following new finite-element discretization during the heating process:

$$\sum_{i=1}^{N} P_i^t\left[\int_S \nabla\psi_i \cdot \nabla\psi_j \mathrm{d}S\right] + \sum_{i=1}^{N} \ddot{P}_i^t\left[\int_S \frac{1}{v_t^2}\psi_i\psi_j \mathrm{d}S\right] + \oint_l \psi_i\psi_j\left[\frac{1}{v_t}\dot{P}_i^t + \frac{1}{2r}P_i^t\right]\mathrm{d}l = \sum_{i=1}^{N}\int_S \hat{\Phi}\frac{\beta}{C_P}\frac{\partial J}{\partial t}\psi_i \mathrm{d}S, \quad (6)$$

where P^t and v_t are the acoustic pressure and acoustic speed at time point t, respectively, and $\hat{\Phi}$ is the absorbed optical energy density obtained from the first step. In this study we neglected the temperature-dependency of tissue thermal expansion β, since it is typically more than two orders of magnitude smaller than that induced by the change of acoustic speed [13]. The temperature dependency of the specific heat capacity at constant pressure C_P is also ignored, since for most solids this dependency is very weak at room temperature [14].

The inverse procedure to obtain the acoustic speed iteratively is based on the following regularized matrix equation:

$$(\mathbf{J}_t^T\mathbf{J}_t + \lambda\mathbf{I})\Delta\chi_t = \mathbf{J}_t^T(\mathbf{P}_t^o - \mathbf{P}_t^c), \quad (7)$$

where $\mathbf{J}_t$ is the Jacobian matrix formed by $\partial P/\partial v$ at the boundary measurement sites at the time point t, $\mathbf{P}_t^{o,c}$ is the measured and calculated acoustic fields for $i = 1, 2, \cdots M$ locations at the time point t, $\Delta\chi_t = (\Delta v_{t,1}, \Delta v_{t,2}, \cdots \Delta v_{t,N})^T$ is the updating vector, I is the identity matrix, and λ is the regularization parameter determined by combined Marquardt and Tikhonov regularization schemes. By solving Eq. (7) at each time point, the distribution of the acoustic speed $v(\mathbf{r}, t)$ during the whole heating process can then be reconstructed iteratively, which leads us to obtain the distribution of absolute value of temperature in the third step.

In PAT, the Grueneisen parameter Γ, which is the conversion efficiency of optical energy deposition to pressure, is defined as

$$\Gamma = \frac{\beta v^2}{C_p}. \quad (8)$$

Linear dependence of the Grueneisen parameter Γ on temperature in water or water-based and fatty tissues has been demonstrated [4,5,15,16]. Since we neglected the effect of tissue thermal expansion, the acoustic speed v can be expressed by an empirical equation:

$$v = \sqrt{A + BT}, \quad (9)$$

where A and B are constants that can be determined experimentally and T is the temperature. Thus, the distribution of absolute temperature can be expressed as $T(\mathbf{r}, t) = (v^2(\mathbf{r}, t) - A)/B$.

We demonstrate our reconstruction algorithm using several tissue-like phantom experiments under various practical scenarios. The photoacoustic imaging system we used in this study has been described in detail elsewhere [17]. For PAT, a pulsed light from a tunable Ti:Sapphire laser (690 950 nm) was vertically delivered to a 20-mm-diameter rounded background phantom (prepared with agar). A graphene nanosheet solution with a concentration of 0.06 mg/ml (fabricated by microwave-assisted oxidation reaction) was injected into a 2-mm-diameter hole located at almost the center of the phantom. The intensity of each laser pulse was monitored by a photodiode for calibration. A 120-element transducer array was used to record the photoacoustic (PA) signals, and a 64-channel data acquisition system coupled with a 2-1 electronic multiplexer was used to collect the PA signals with a temporal resolution of 150 ms. The 6-mm-diameter ultrasonic transducers had a central frequency of 5 MHz and a 70% nominal bandwidth (Blatek, Inc.), and were located inside a water tank to receive the acoustic signals. To simulate thermal therapy, an 808-nm continuous-wavelength (CW) laser (Apollo Instruments, Inc.) was used to heat the phantom [18]. Three different laser powers (3, 2, and 1 $\mathrm{W/cm^2}$, respectively) were used in these experiments to illuminate the graphene nanosheets for 2 min to evaluate the performance of the photothermal efficiency of the nanographene, while as the reference a thermometer was used to measure the temperature of the nanographene in real time during the whole heating process. In this study, the initial temperature of the phantom was around 22°C, the thermal-expansion coefficient was 6.9×10^{-5}/°C, and the specific heat at constant pressure C_P was 1.0 cal/g · °C.

Distribution of the absorbed energy density was first reconstructed through our FEM-based algorithm, and the images are shown in Fig. 1(a). We see that the target (graphene nanosheet solution) can be identified clearly with correct location. We then tried to determine the parameters A and B in Eq. (9) based on recorded photoacoustic signals and temperatures measured by the thermometer [9]. The acoustic speed of the target can be calculated by $v = d/\Delta t$, where d is the dimension of the target and Δt is the photoacoustic wave propagation duration inside the target, which could be quantified from the acoustic signals since the front and backward boundaries of the target could be clearly imaged. Figure 1(b) shows the results on quantification of these two parameters in Eq. (9), and it confirms that it is the square of the acoustic speed, not the speed itself, that can be approximated as a linear function of temperature within the range from 22°C to 33°C in these experiments. We used $A = 0.016 \times 10^{12}\ \mathrm{mm^2/(s^2 \cdot {}^\circ C)}$ and $B = 1.6 \times 10^{12}\ \mathrm{mm^2/s^2}$ in the following calculations.

In Fig. 2, we present the reconstructed temperature images with different CW laser powers (3, 2, and 1 $\mathrm{W/cm^2}$) at four different time points (10 s, 20 s, 30 s, and 40 s) after the CW laser was on. We can see that during the heating process, the increase in temperature over time for the target is clearly notable, especially for the first case [Figs. 2(a) 2(d)] with CW laser power 3 $\mathrm{W/cm^2}$, which means that higher laser power leads to higher performance of the photothermal efficiency for the nanographene. A moderate temperature increase for the background can also be observed. We also note that the artifacts in the background were smaller with higher laser power. Extensive quantitative analysis can be found in Fig. 3, where we provide comparisons between the temperatures recovered by our method and those measured by the thermometer. For the high laser power (3 $\mathrm{W/cm^2}$ in case 1), the temperature of the target increased from 22.0°C to 33.0°C in 40 s during the heating process, while for the moderate laser power (2 $\mathrm{W/cm^2}$ in case 2) it reached to 29.7°C in 40 s, and for the low laser power (1 $\mathrm{W/cm^2}$ in case 3) it could only reached to 26.7°C in 40 s. The relative error between the temperature recovered by our method and that measured by the thermometer is in the range of 0.6% 1.6% for high laser power (3 $\mathrm{W/cm^2}$ in case 1), 1.4% 2.0% for moderate laser power (2 $\mathrm{W/cm^2}$ in case 2), and 2.0% 2.7% for low laser power (1 $\mathrm{W/cm^2}$ in case 3). Thus, it is clear that higher CW laser power (as long as it remains in the safety range) will provide more accurately recovered temperature distribution with larger photothermal efficiency.

In summary, we have demonstrated that our PAT-based temperature reconstruction algorithm is capable of imaging absolute temperature distribution quantitatively. The experimental results obtained have shown that the relative error between the FEM-calculated temperature and the actual value can be less than 1%, which

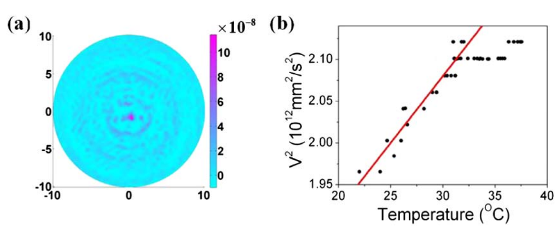

Fig. 1. (a) Reconstructed absorbed energy density image and (b) experimental determination of the parameters in Eq. (9).

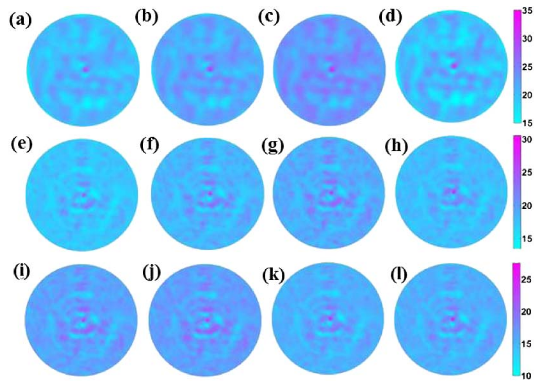

Fig. 2. Reconstructed temperature images for case 1 [(a) (d), CW laser power: 3 $\mathrm{W/cm^2}$], case 2 [(e) (h), CW laser power: 2 $\mathrm{W/cm^2}$], case 3 [(i) (l), CW laser power: 1 $\mathrm{W/cm^2}$] at four different time points after the CW laser was on: 10 s (a), (e), (i); 20 s (b), (f), (j); 30 s (c), (g), (k); and 40 s (d), (h), (l).

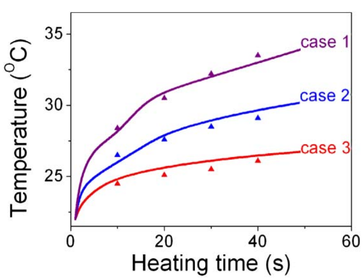

Fig. 3. Comparison between the temperature calculated by our method (scattered points) and that measured by thermom eter (solid lines).

indicates that this method has the potential to be used for noninvasive, real-time temperature monitoring during thermotherapy. Further evaluation of the technique using *in vivo* animal experiments is underway in our laboratory and the results will be reported in the future.

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