Optics Letters

Nakagami statistics-based photoacoustic spectroscopy used for label-free assessment of bone tissue: supplement

TING FENG,[1,*,†] YIHAN ZHU,[1,†] XIAOXIANG GAO,[2] WEIYA XIE,[3] (iD)
HAIGANG MA,[1] LIMING CHENG,[4] DEAN TA,[5] AND QIAN CHENG[3,4,6]

[1]*School of Electronic and Optical Engineering, Nanjing University of Science and Technology, Nanjing 210094, China*
[2]*Department of Nanoengineering, University of California San Diego, La Jolla, California 92093, USA*
[3]*Institute of Acoustics, School of Physics Science and Engineering, Tongji University, Shanghai 200092, China*
[4]*The Key Laboratory of Spine and Spinal Cord Injury Repair and Regeneration, Ministry of Education, Department of Orthopaedics, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065, China*
[5]*Center for Biomedical Engineering, Fudan University, Shanghai 200433, China*
[6]*e-mail: q.cheng@tongji.edu.cn*
Corresponding author: fengting@njust.edu.cn
[†]*These authors contributed equally to this work.*

Nakagami statistics-based photoacoustic spectroscopy used for label-free assessment of bone tissue: supplemental document

S1. Theoretical analysis

As the biological tissue samples used in this study were very thin (~1 mm), the light attenuation in the tissue (i.e., $\exp(-\mu_{\text{eff}}\mathbf{z})$) was neglected. In this case, Eq. (1) in the manuscript can be simplified as follows:

$$p(t,\lambda,\mathbf{r}) \sim \Gamma_G F_0 \mu_a(\lambda,\mathbf{r})\exp(-\alpha ct),\ t>0, \tag{S1}$$

In this study, the Nakagami distribution is used to describe the statistics of the PA signal envelope. For the Nakagami distribution, the probability density function (PDF) of the envelope can be expressed by:

$$F(R) = \frac{2m^m R^{2m-1}}{\Gamma(m)\Omega^m}\exp(-\frac{m}{\Omega}R^2)U(R), \tag{S2}$$

where R is the envelope of the signal, which is obtained by applying the Hilbert transform and can be approximately considered as the initial PA pressure $p(t,\lambda,\mathbf{r})$; $\Gamma(\cdot)$ represents the gamma function; and $U(\cdot)$ represents the unit step function. The parameter m is determined by the shape of Nakagami distribution, and is independent of the system and the signal's absolute amplitude [1s,2s]. The parameter m can be estimated as follows [1s]:

$$m = \frac{E(R^2)}{E[R^2 - E(R^2)]}, \tag{S3}$$

The scaling parameter Ω is related to the average energy or power of the PA signal [2s], and can be estimated as follows:

$$\Omega = E(R^2), \tag{S4}$$

The properties of the parameter m indicate that it is independent of the signal amplitude. Therefore, it is also independent of F_0. Thus, it is reasonable to assume that parameters F_0 and Ω of each PA signal can be expressed as constants. Hence, parameters F_0 and Ω in Eqs. (S1) and (S2) can be considered as 1. Thus, the above equations can be simplified as follows:

$$p(t,\lambda,\mathbf{r}) \sim \Gamma_G \mu_a(\lambda,\mathbf{r})\exp(-\alpha ct),\ t>0, \tag{S5}$$

$$F(R) = \frac{2m^m R^{2m-1}}{\Gamma(m)}\exp(-mR^2), \tag{S6}$$

By definition, the PDF of signal R can also be expressed as follows:

$$F(R) = \frac{d\int_{-\infty}^{t} R\,dt}{dt},\ t>0, \tag{S7}$$

Therefore, an equation connecting the parameter m and the signal R can be obtained from Eqs. (S6) and (S7) as follows:

$$\frac{d\int_{-\infty}^{t} R\,dt}{dt} = \frac{2m^m R^{2m-1}}{\Gamma(m)}\exp(-mR^2), \tag{S8}$$

where R can be considered as the PA signal $p(t,\lambda,\mathbf{r})$. Then, after integrating both sides of Eq. (S8) and substituting it into Eq. (S5), we obtain:

35 $$\Gamma_G \mu_a(\lambda, \mathbf{r}) \exp(-\alpha ct) \sim \frac{2m^m (\Gamma_G \mu_a(\lambda, \mathbf{r}) \exp(-\alpha ct))^{2m-1}}{\Gamma(m)} \exp(-m(\Gamma_G \mu_a(\lambda, \mathbf{r}) \exp(-\alpha ct))^2), \quad \text{(S9)}$$

36 From Eq. (S9) we can obtain the expression of m at different optical wavelengths as follows:

37 $$m(\lambda) \sim \frac{1}{1 + \dfrac{\Gamma_G^2 \mu_a^2(\lambda, \mathbf{r}) \exp(-2\alpha ct)}{2\alpha ct}}, \quad \text{(S10)}$$

38 Eq. (S10) shows that the parameter $m(\lambda)$ is inversely proportional to the optical absorption as a function
39 of $\mu_a^2(\lambda, r)$. where $\mu_a(\lambda, \mathbf{r}) = \sum_{i=1}^{k} \mu_{a_i}(\lambda, \mathbf{r}_i)\delta_i$, i represents different components, δ_i represents the
40 corresponding content of each chemical component, k represents the number of components types, and
41 $\sum_{i=1}^{k} \delta_i = 1$. In addition, the parameter $m(\lambda)$ is affected by Γ_G, α and c in tissues. As the variation range
42 of Γ_G is wavelength-independent and much smaller than that of μ_a in bone tissue (see Supplement1,
43 Section S4), while the variation range of c is much smaller than that of α, the influence of Γ_G and c is
44 ignored hereafter. To further study the relationship between features of NSPS curve $m(\lambda)$ and chemical
45 properties of tissue, we conducted the numerical simulation and experimental studies on different bone
46 models.
47

48 **S2. Biological tissue preparation**

49   Female New Zealand rabbits aged approximately 4 months were randomly divided into a
50 control group (N = 6) and an osteoporosis group (N = 6). Ovariectomy was performed on the
51 rabbits in the osteoporosis group, which led to osteoporosis due to the cessation of estrogen.
52 All rabbits were euthanized 20 weeks after the operation, and the femur was dissected as an
53 experimental sample to collect PA signals. To validate the PA measurement results obtained
54 using the two types of bone specimens, all the bone specimens were scanned using a micro-CT
55 system (SCANCO, vivaCT 80), and the results verified that the mean BV/TV value of the
56 osteoporosis group significantly decreased, with a 3.3% reduction from 34.55% to 31.2% with
57 respect to that of the control group. In addition, histological analysis was applied to examine
58 the collagen in rabbit bone specimens. After collagen staining was performed on each bone
59 specimen section, the average collagen staining area was calculated by MATLAB. The
60 histological results showed that the collagen content in the osteoporosis group decreased
61 significantly by 14.5% compared with that in the normal group. This study was approved by
62 the ethics committee of the Nanjing University of Science and Technology (No. 202100129).

63 **S3. Details of the experimental setup**

64   The experimental device is shown in Fig. 4 in the manuscript. The laser was generated by
65 a Nd:YAG laser pumped optical parametric oscillator (vibrant B, Opotek) and then split into
66 two beams by a beam splitter, one of which was focused on the surface of the bone sample
67 through a lens with 90% laser intensity to generate PA signals, as shown in Fig. 4 (a). The beam
68 diameter was kept at approximately 8 mm, and the total luminous flux on the bone surface was
69 kept below 20 mJ/cm^2, which was within the safety limit of the American National Standards
70 Institute (ANSI). The bone specimen and PA signal receiver were coupled by ultrasound
71 coupling gel. The PA signal receiver used a needle hydrophone (hnc-1500, Onda Co.,
72 Sunnyvale, CA, USA) with a bandwidth of 0~10 MHz for high accuracy. The PA signal
73 generated from the other beam by the black rubber illuminated by another laser beam was
74 received by an ultrasonic transducer (FC = 1 MHz, V302, Olympus, Tokyo, Japan) to determine
75 the laser energy for energy calibration as described earlier. Because the signals used for energy
76 calibration was focused on the energy change of PA signal, and do not need high frequency
77 information of PA signal, we used a transducer with a lower center frequency less affected by
78 the high frequency noise to receive them. To further improve the SNR of the PA signal, more
79 than 50 measured values of the PA signal were averaged.

S4. The preliminary study of Grüneisen parameter in experimental study

The Grüneisen parameter is one of the important parameters of bone tissue in NSPS method, as shown in Eq. (S5). The expression of Grüneisen parameter is

$$\Gamma_G = \frac{\beta \cdot c^2}{C_p}, \qquad (S11)$$

where β is the thermal coefficient of volume expansion, c is the speed of sound (SOS) of target tissue, C_p is the heat capacity at constant pressure. In order to investigate the effect of Grüneisen parameter to m curve, we calculated difference of the Grüneisen parameter between control bone group and osteoporosis bone group. We considered the BV/TV of two groups of rabbit bone samples used in this research has a 3% difference, for example, the average BV/TV of control group is about 34.5%, and average BV/TV of experiment group is about 31.2%. With the parameters as shown in Table 1s [**Error! Reference source not found.-Error! Reference source not found.**], we estimated and obtained the Grüneisen parameters for those two groups. The calculated results show that the Grüneisen parameters of control group and osteoporosis group are 0.55 and 0.54 respectively, with change less than 2%.

Therefore, in this study, we didn't take the Grüneisen parameter into account. However, in clinical related study, the differences of BV/TV for human bones and animal bones may larger than the rabbit bone samples we used in this study, which may lead to large affect generated from Grüneisen parameter and cannot be ignored. Besides, since the method we used to calculate the Grüneisen parameter for heterogeneous tissue here is preliminary study, it should be further studied with more factors considered in future works.

Table 1s. The physical properties of the major components in bone tissue [**Error! Reference source not found.-5s**]

	Trabecular bone (mostly Tricalcium phosphate)	Water in marrow	Lipid in marrow
B (1/K)	14.2×10^{-6}	2.1×10^{-4}	1.9×10^{-3}
c (m/s)	1886	1480.0	975.0
C_p (J/(kg·K)) at about 20 ℃	730.4	4.2×10^3	2.7×10^3
content in control group (%)	34.5	36.0	29.5
content in osteoporosis group (%)	31.2	37.8	31.0

References:
1s. X. Gao, N. Dai, C. Tao, and X. Liu, "Quantification of number density of random microstructure from a photoacoustic signal by using Nakagami statistics," Opt. Lett. 44/12, 2951–2954 (2019)
2s. C. Liu, R. Dong, B. Li, Y. Li, F. Xu, D. Ta, and W. Wang, "Ultrasonic backscatter characterization of cancellous bone using a general Nakagami statistical model," Chin. Phys. B 28/2 (2019).
3s. Da-Kang Yao, Chi Zhang, Konstantin Maslov, Lihong V. Wang, "Photoacoustic measurement of the Grüneisen parameter of tissue," J. Biomed. Opt. 19(1), 017007(2014).
4s. J. C. Southard and R. T. Milne, "Low Temperature Specific Heats. V. The Heat Capacity of Tricalcium Phosphate between 15 and 298'K.," Chem. Soc. 57, 983 (1935).
5s. M. Milosevski, J. Bossert, D. Milosevski, N. Gruevska, "Preparation and properties of dense and porous calcium phosphate," Ceram. Int. 25, 693 (1999).