Characterization of ovarian tissue based on quantitative analysis of photoacoustic microscopy images

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Abstract: In this paper, human ovarian tissue with malignant and benign features was imaged ex vivo using an optical-resolution photoacoustic microscopy (OR-PAM) system. The feasibility of PAM to differentiate malignant from normal ovarian tissues was explored by comparing the PAM images morphologically. Based on the observed differences between PAM images of normal and malignant ovarian tissues in microvasculature features and distributions, seven features were quantitatively extracted from the PAM images, and a logistic model was used to classify ovaries as normal or malignant. 106 PAM images from 18 ovaries were studied. 57 images were used to train the seven-parameter logistic model, and a specificity of 92.1% and a sensitivity of 89.5% were achieved; 49 images were then tested, and a specificity of 81.3% and a sensitivity of 88.2% were achieved. These preliminary results demonstrate the feasibility of our PAM system in mapping microvasculature networks as well as characterizing the ovarian tissue, and could be extremely valuable in assisting surgeons for in vivo evaluation of ovarian tissue during minimally invasive surgery.

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OCIS codes: (110.5120) Photoacoustic imaging; (170.3880) Medical and biological imaging.

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Ovarian cancer is the fifth most common cancer among women, and it has the lowest survival rate among all of the gynecologic cancers because it is predominantly diagnosed in late stages due to the lack of early symptoms as well as the lack of effective screening techniques [1]. As a result, it is necessary to develop more sensitive tools to evaluate ovarian tissue. Photoacoustic imaging has emerged as a promising biomedical imaging modality [2,3] and demonstrated great potential for imaging ovarian tissue [4–7]. PAM in particular, is capable of mapping microvasculature networks in biological tissue and resolving blood vessels with much higher resolution than conventional photoacoustic images obtained with ultrasound array transducers [8–20]. Guo et al. performed the quantification of total hemoglobin concentration and hemoglobin oxygen saturation in a mouse using PAM [20]. Xie et al. studied the feasibility of PAM in differentiating malignant from benign bladder tissues [18]. In their study, the comparison of malignant and benign images was based on visual observations. Alqasemi et al. have introduced a recognition algorithm using a support vector machine for assisting ovarian cancer diagnosis, and they used features extracted from ultrasound and photoacoustic images obtained from array transducers of 5-6 MHz central frequency [6]. However, photoacoustic images obtained with conventional ultrasound array transducers in the central frequency range of 3-7 MHz have lower resolution in resolving microvasculature networks and distributions in ovarian tissue than that of PAM. In this paper, we imaged ex vivo human ovaries with malignant and benign features using a newly developed OR-PAM system with lateral resolution of 6µm. We extracted seven features from high resolution PAM images, and used a logistic model to classify the normal and malignant ovarian tissues. We also evaluated the diagnostic sensitivity, specificity, positive and negative predictive values (PPV, NPV) and the area under the receiver operating characteristic (ROC) curves (AUC). To the best of our knowledge, this study is the first one reporting quantitative analysis and feature extraction of PAM images for classifying normal and malignant ovarian tissues. Quantitative analysis of PAM images is extremely valuable in assisting physicians to characterize and diagnose normal and malignant processes.

2. Materials and methods

2.1 Ovary sample

Human ovaries were extracted from patients undergoing prophylactic oophorectomy at the University of Connecticut Health Center (UCHC). These patients were at risk for ovarian cancer or they had ovarian mass or pelvic mass suggesting malignancy. This study was approved by the Institutional Review Boards of UCHC, and informed consent was obtained from all patients. Ovaries were kept in the 0.9% wt/vol NaCl solution and imaged within 24 hours after oophorectomy. After PAM imaging, the ovaries were fixed in 10% formalin solution and returned to the Pathology Department for histological processing.
2.2 PAM system

The OR-PAM system configuration is shown in Fig. 1. A Ti:sapphire laser pumped by a Q-switched Nd:YAG laser delivers 15ns laser pulses at 745nm with a repetition rate of 15Hz. The laser beam is spatially filtered by an iris, and then focused on the ovary by using a 10X objective lens (NA = 0.25). Ultrasound (US) gel is used to couple the photoacoustic signal to a single element transducer (Echo, BI933) with a center frequency of 3.5MHz and a bandwidth of 60%. The acquired photoacoustic signal is amplified by a Panametrics receiver and then sampled by a data acquisition (DAQ) PC. A 3D motor is used to scan the transducer together with the ovary to obtain PAM images, and the distance between the objective lens and the ovary can be adjusted to achieve optimal resolution.

Fig. 1. Configuration of the OR-PAM system.

2.3 Feature extraction

Several features were quantitatively extracted from the PAM images to classify normal and malignant ovaries, based on the observed differences between the PAM images of normal and malignant ovarian tissues in terms of microvasculature features and distributions. For example, the photoacoustic signal distribution is more scattered and diffuse in malignant cases, whereas the distribution is more clustered and the microvasculature networks are more clearly recognized in normal cases. These results suggest that the spatial frequency components, and the spatial spread of the PAM images are important. This observation also suggests that the statistical properties of the PAM images are of great importance to account for the photoacoustic signal fluctuation. In Ref. 6, both statistical mean and variance were used as features to characterize normal and malignant ovarian tissues. However, the difference of statistical variance between normal and malignant PAM images was not significant (p = 0.618), and the diagnostic results based on PAM images were getting worse by adding this feature. Therefore, the statistical variance was not used in this study. Overall, seven parameters were extracted from PAM images: low frequency components, high frequency components, Gaussian fitting standard deviation (SD) of the mean Radon transform, Gaussian fitting error of the mean Radon transform, statistical mean, Gamma distribution mean and variance. Similar to the method used to extract features from B-scan ultrasound and photoacoustic images in Ref. 6, all the 1.5mm x 1.5mm PAM images were normalized to their own maximum. The low frequency and high frequency components were calculated by selecting a low-pass window of the 2-D fast Fourier transform (FFT) with half of the sampling frequency. The average absolute value within that window was considered as low frequency component, while the average absolute value outside that window was considered as high frequency component. The average Radon transform from 0 degree to 90 degree was computed, and then fit to a Gaussian distribution. The Gaussian fitting SD was used to describe the spatial spread of the images, and the fitting error was used to describe the uniformity of the tissue absorption. The statistical properties were studied by calculating the statistical mean of the images. In addition, Gamma distribution mean and variance were calculated to account for those images that were not symmetrically distributed.
2.4 Logistic model

Logistic regression belongs to the class of generalized linear model (GLM) based on the exponential distribution family. It is a statistical model that can describe the relationship of several predictor variables to a dichotomous response variable (0 or 1). The logistic model was used to classify normal and malignant ovarian tissues. The seven parameters extracted from PAM images were used as predictor variables, and actual diagnosis results were used as the response variable (1 represents malignant and 0 represents normal). The MATLAB GLMFIT function was used to estimate the coefficients of the linear model, and then those coefficients were applied to the MATLAB GLMVAL function to calculate the responses. The quality of the logistic model was evaluated using ROC curve and AUC.

3. Results and discussion

3.1 Lateral resolution test

The lateral resolution of the PAM system was tested by imaging a 7µm diameter carbon fiber. Figure 2(a) shows the PAM maximum amplitude projection (MAP) image, and Fig. 2(b) shows normalized cross-sectional profile of the carbon fiber along the dotted line in Fig. 2(a). The full width at half maximum (FWHM) was estimated to be 13µm. The subtraction value of FWHM and the carbon fiber diameter was used to estimate the lateral resolution of the system [10]. Therefore, the lateral resolution of the PAM system is ~6µm. The axial resolution is ~360µm, which is limited by the bandwidth of the transducer.

![Fig. 2. (a) PAM MAP image of a 7µm carbon fiber, scale bar: 50µm; (b) normalized cross-sectional profile of the carbon fiber along the dotted line in (a).](image)

3.2 Ovarian tissue characterization

Some PAM images of normal and malignant ovarian tissues are presented in Fig. 3(a) and 3(b), respectively. As shown by the MAP images, PAM was capable of imaging detailed microvasculature maps in ovarian tissue with much higher resolution than that of conventional photoacoustic images obtained with ultrasound array transducers [5–7]. In the normal ovarian tissue, the microvessel network consists of a larger vessel and several branching small vessels. The network shows more regular shape and better continuity, and these vessels are well organized. However, in the malignant ovarian tissue, the photoacoustic imaging features are diffuse and scattered which are likely caused by the extensive angiogenesis associated with malignancy of the ovary. The corresponding histology images of 3(a) and 3(b) are shown in Fig. 3(c) and 3(d), respectively. The PAM images of both the normal and malignant ovaries match the histology. Based on the histology, the blood vessels in normal ovarian tissue form structured microvasculature networks, from large vessels to smaller ones, which are different from the scattered distributions seen in malignant ovary. For the malignant case, PAM image shows more blood vessels than histology image. The reason is that the PAM image is the maximum amplitude projection from multiple depths, while the histology image shows only one of the projected planes at a certain depth.
Fig. 3. PAM images of (a) normal ovarian tissue and (b) malignant ovarian tissue; (c) H&E corresponding to (a); (d) H&E corresponding to (b); scale bar: 300µm; arrows: blood vessels.

In order to investigate the differences of morphological features and statistical properties between normal and malignant ovarian tissues, we quantitatively extracted above-mentioned seven parameters from PAM images. 106 images (70 normal and 36 malignant) were acquired ex vivo. Figure 4 shows the boxplots and p values of seven parameters of normal and malignant ovary groups. To provide readers with statistical performance of these parameters, data from both training and testing sets were used in these plots. The differences of some parameters were highly statistically significant between normal and malignant groups. Note that for Gaussian fitting SD, the normal group had higher value than the malignant group, and the standard deviations of both normal and malignant groups were large. Perhaps this was due to the diverse normal samples, for the normal group, the range of patient age was 43-77; for the malignant group, the range of patient age was 58-71. The seven parameters were used as predictor variables of the logistic model to classify normal and malignant ovaries. We separated all images into two groups, 57 images (38 normal and 19 malignant) were used as a training set to train the logistic classifier, and 49 images (32 normal and 17 malignant) were tested using our trained logistic prediction model. Figure 5 shows the ROC curves and AUC of training and testing set. For the training set, we could achieve 92.1% specificity, 89.5% sensitivity, 85.0% PPV, 94.6% NPV, and AUC (95% confidence interval) equals to 0.940 (0.869-1); for the testing set, we could achieve 81.3% specificity, 88.2% sensitivity, 71.4% PPV, 92.9% NPV, and AUC (95% confidence interval) equals to 0.886 (0.792-0.980).

This work has several limitations. First, the training and testing results are based on a limited sample pool, so more data will be acquired to validate these initial results. As a preliminary study, all ovarian tissue imaging was conducted ex vivo. For translating this technique from bench to bedside, a new PAM system based on a laser diode excitation is being developed in order to reduce the size and cost of the system, and a fiber catheter will replace free-space imaging for in vivo evaluation of ovarian tissue. In addition, the data acquisition speed of the current system is limited by the laser repetition rate of 15Hz; by using the laser-diode based PAM system, the data acquisition speed can be increased by modulating the laser diode to ~kHz or even to ~MHz level.
4. Summary

In this report, *ex vivo* ovarian tissue was imaged by using an OR-PAM system, and quantitative analysis was performed by extracting features from PAM images. The initial results have demonstrated that PAM was capable of imaging microvasculature maps in ovarian tissue. By utilizing a seven-parameter logistic model to classify PAM images of normal and malignant ovaries, we could achieve 92.1% specificity and 89.5% sensitivity in the training set, and 81.3% specificity and 88.2% sensitivity in the testing set. The high resolution microvasculature network features extracted from PAM images could be extremely valuable in assisting and guiding surgeons for *in vivo* evaluation of ovarian tissue during minimally invasive surgery.

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