

Multispectral Fluorescence Imaging of Ovarian Surface for Oncologic Tissue Characterization



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Introduction

Women diagnosed with ovarian cancer have an overall 5-year survival rate of 45% [1]. In cases when ovarian cancer is discovered at the early, localized stage, the 5-year survival rate is a much higher 92%. Early ovarian cancer has no obvious symptoms, and, consequently, only 19% of ovarian cancer cases are diagnosed at the localized stage [1]. Women are not routinely screened for this disease because no sufficiently-reliable screening test exists.

Difficulty in Screening: The location of the ovaries in the pelvic wall prevents access for routine examination. While abnormalities can be detected by transvaginal ultrasound, MRI, or CT, these expensive procedures can determine only that an ovary is enlarged. Early detection of ovarian cancer and the differentiation of malignancies from benign neoplasms and dysplastic growths seem to require direct interrogation of the ovary surface. Laparoscopic surgery can thus be warranted in a high-risk individual for this purpose.

Probe-Based Solution:

Laparoscopic procedures are commonly used for exploration of serious pelvic problems and for ovary removal. Small incisions in the abdomen allow insertion of slender tools (See Fig. 1). Fiber optic bundles incorporated into a rigid probe can be used for light delivery and collection. Probe-based imaging is common. Although this study is not probe-based, the method can be adapted for such a device.



Figure 1. Ovarian Laparoscopy Illustration

Disease Origins: About 90% of ovarian cancers arise in the thin outermost layer of the ovary, the epithelium. Below this is a dense layer of fibrous, collagenous tissue called tunica albuginea. Optical methods, such as fluorescence imaging, are well-suited for evaluating the ovarian surface epithelium where tumors originate.

Materials and Methods

Fluorescence Imaging: The ovarian surface is imaged in this study without the application of contrast agent. Narrowband ultraviolet light is applied to the tissue. Light is absorbed in the tissue, and some components re-emit the energy in a broad spectrum of visible light. Sources of the autofluorescence include the extracellular protein collagen and the metabolic cofactors NADH and FAD found in the mitochondria and cytosol.

Clinical Study: Human ovary images were obtained for this *in vitro* study from tissue of 30 consented women undergoing oophorectomy either due to disease presence or for prophylactic purpose. 90% of these were between the age of 46 and 75 years. 83% were post-menopausal. Ovaries were placed into shallow dish in a dark room and imaged immediately after surgical removal and about 15 minutes after loss of blood supply. A moist ovarian surface was maintained using phosphate-buffered saline.

Instrument: Fluorescence images of whole ovaries were taken using a multispectral tissue imager from Apogen Technologies, a subsidiary of QinetiQ North America in San Diego, California. The device excites with 365 nm light (FWHM 6 nm) and collects emission using 8 filter bands from 400 to 600 nm. Quad Prism Aperture Sharing allows collection of 4 independently-filtered images at once in a quad-image. An image for filter bands centered at 418, 437, 457 and 485 nm is collected first, and image collection for 507, 530, 562 and 601 nm bands immediately follows. The device also has a white light source and collects reflectance images. (See Fig. 2)



Figure 2. Multispectral Tissue Imager

Pathology Groups: Ovarian specimens were grouped into 1 of 4 designated pathologies: Normal, Cancer, Endometriosis, or Benign Neoplasm. Only measurements of Normal and Cancer were considered for this initial study.

References

[1]. Cancer Facts & Figures 2008. Atlanta: American Cancer Society, 2008.

Multispectral Data: Each image capture sequence produces 8 uniquely-filtered fluorescence images of the ovary (See Fig. 3) (and 8 reflectance images). After image registration and spectral sensitivity calibration, corresponding pixel intensities from each fluorescence image can be plotted together to view the emission spectra of small areas of the ovary. Reflectance images can be used to produce RGB "true color" images.

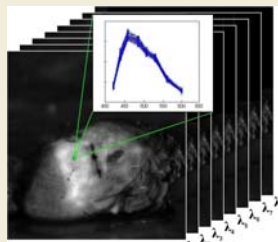


Figure 3. Grayscale Fluorescence Emission Image Stack and Plot of 8-point spectra corresponding to small, boxed, image region

Data Analysis via Classification: In a previous single-point spectroscopic study, excitation emission data were collected via spectrofluorometer from ovarian biopsies of 64 patients. For comparison, these data were transformed to the same wavelength space as the multispectral imager using knowledge of the imager's filter transmissions and of its spectral sensitivity (See Fig. 4). The transformed data (of known pathology) then could be used as a training set to classify the new image data. Jackknife analysis was used to determine that the unnormalized data (Fig. 4, Left) would be more effective training data than normalized data (Fig. 4, Right).

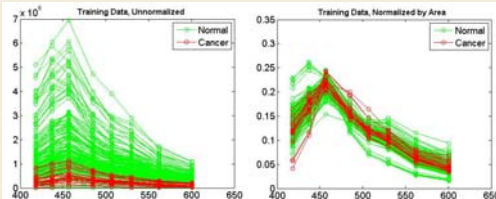


Figure 4. Transformed Emission Data from single-point study

Intensity Scaling: Unnormalized training data were scaled to match the intensity of the multispectral imager data. The scale factor was determined by building histograms of the 457-nm intensities from Normal ovary measurements of the two studies (See Fig. 5), fitting these histograms to gamma distributions, locating the two maxima, and calculating their ratio.

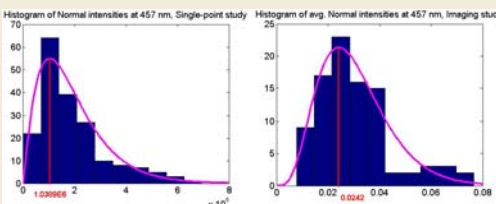


Figure 5. Intensity Histograms used to find scale factor

Principal Component Analysis: PCA was used to prepare the spectral data for classification by reducing the number of variables. Eigenvector decomposition showed that only 3 variables were significant for classification. These highest scoring eigenvectors are shown in Fig. 6.

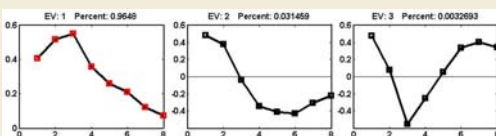


Figure 6. Eigenvectors (EV) given highest scores. Note that EV1 resembles an average fluorescence spectrum, while EV2 weights high and low portions of the spectrum. EV3 is more unusual.

Results

ROI Selection: A region of interest (ROI) was manually selected in each ovary image. In attempt to minimize the effect of intensity change with viewing angle, only ovarian surface viewed en-face by the camera was selected.

Classification: Linear discriminant analysis was applied using the unnormalized but scaled training data (Shown in Fig. 4, Left). Every pixel in the ROI of each ovary image was classified as Normal or Cancer. Results were displayed in color-coded classification maps (See Fig. 7).

Criteria: When an ovary had 90% or more of its ROI pixels classify as normal, the ovary itself was classified as normal. If 90% or more of the ROI pixels classified as cancer, the ovary was classified as cancer. For all other pixel classification percentages, classification was considered indeterminate (See Fig. 7, Bottom Image Set).

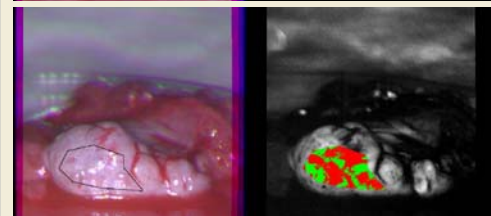
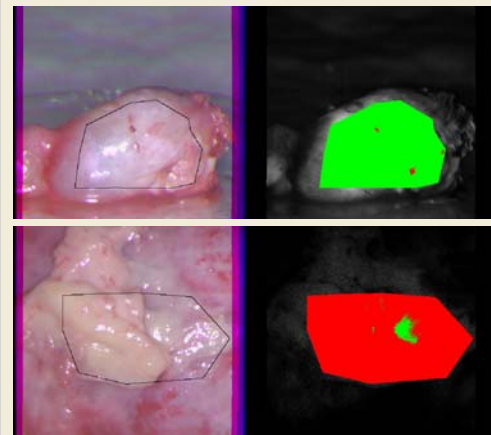


Figure 7. "True Color" Reflectance images are shown on the left with outlined ROI's. Grayscale Fluorescence images are shown on the right with color-coded classification maps overlaid. Green = Normal; Red = Cancer. Top ovary was correctly classified as Normal. Middle ovary was correctly classified as Cancer. Bottom ovary Normal but classification indeterminate.

Results: Multispectral fluorescence images of twenty-five normal and cancerous ovaries were processed (23 normal and 2 cancerous). Benign and Endometriosis ovaries were excluded. The presence of blood on the ovarian surface was found to interfere with classification. Blood-coated ovary images were therefore excluded.

The criteria used resulted in a sensitivity of 100% and a specificity of 95% when omitting the indeterminate classifications. If the indeterminate ovaries were included as false positives, then a specificity of 83% resulted.

Conclusions: The sensitivity of the classification was high. Although the number of cancerous ovaries in the study was small, sensitivity is believed to be truly high as evidenced by the 100% sensitivity realized in jackknife analysis of the unnormalized training data, which included a larger number of cancerous measurements. The specificity is lower partially because of the apparent sensitivity of classification to small blood vessels near the ovary surface and the presence of these vessels in some normal ovaries.

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