Characterizing human pancreatic cancer precursor using quantitative tissue optical spectroscopy

Seung Yup Lee,1 William R. Lloyd,1 Malavika Chandra,2 Robert H. Wilson,2 Barbara McKenna,3 Diane Simeone,4 James Scheiman,5,6 and Mary-Ann Mycek1,2,6,*

1Department of Biomedical Engineering, University of Michigan, Ann Arbor, MI 48109-2099, USA
2Applied Physics Program, University of Michigan, Ann Arbor, MI 48109-1040, USA
3Department of Pathology, University of Michigan, Ann Arbor, MI 48109-0602, USA
4Department of Surgery, University of Michigan, Ann Arbor, MI 48109-5331, USA
5Department of Internal Medicine, University of Michigan, Ann Arbor, MI 48109-0362, USA
6Comprehensive Cancer Center, University of Michigan, Ann Arbor, MI 48109-0944, USA

*mycek@umich.edu

Abstract: In a pilot study, multimodal optical spectroscopy coupled with quantitative tissue-optics models distinguished intraductal papillary mucinous neoplasm (IPMN), a common precursor to pancreatic cancer, from normal tissues in freshly excised human pancreas. A photon-tissue interaction (PTI) model extracted parameters associated with cellular nuclear size and refractive index (from reflectance spectra) and extracellular collagen content (from fluorescence spectra). The results suggest that tissue optical spectroscopy has the potential to characterize pre-cancerous neoplasms in human pancreatic tissues.

©2013 Optical Society of America

OCIS codes: (170.6510) Spectroscopy, tissue diagnostics; (170.4580) Optical diagnostics for medicine.

References and links

1. Cancer Facts & Figures 2013” (American Cancer Society, 2013), retrieved www.cancer.org.
2. R. H. Hruban, K. Takaori, D. S. Klimstra, N. V. Adsay, J. Albores-Saavedra, A. V. Biankin, S. A. Biankin, C. Compton, N. Fukushima, T. Furukawa, M. Goggins, Y. Kato, G. Klöppel, D. S. Longnecker, J. Lüttges, A. Maitra, G. J. Offerhaus, M. Shimizu, and S. Yonezawa, “An illustrated consensus on the classification of pancreatic intraepithelial neoplasia and intraductal papillary mucinous neoplasms,” Am. J. Surg. Pathol. 28(8), 977–987 (2004).
3. T. Baba, T. Yamaguchi, T. Ishihara, A. Kobayashi, T. Oshima, N. Sakaue, K. Kato, M. Ebara, and H. Saisho, “Distinguishing benign from malignant intraductal papillary mucinous tumors of the pancreas by imaging techniques,” Pancreas 29(3), 212–217 (2004).
4. C. R. Ferrone, C. Correa-Gallego, A. L. Warshaw, W. R. Brugge, D. G. Forcione, S. P. Thayer, and C. Fernández-del Castillo, “Current Trends in Pancreatic Cystic Neoplasms,” Arch. Surg. 144(5), 448–454 (2009).
5. F. Maire, A. Couvelard, P. Hammel, P. Ponsot, L. Palazzo, A. Aubert, C. Degott, A. Dancour, M. Felce-Dachez, D. O’toole, P. Lévy, and P. Ruszniewski, “Intraductal papillary mucinous tumors of the pancreas: the preoperative value of cytologic and histopathologic diagnosis,” Gastrointest. Endosc. 58(5), 701–706 (2003).
6. M. A. Eloubeidi and A. Tamhane, “EUS-guided FNA of solid pancreatic masses: a learning curve with 300 consecutive procedures,” Gastrointest. Endosc. 61(6), 700–708 (2005).
7. V. T.-C. Chang, P. S. Cartwright, S. M. Bean, G. M. Palmer, R. C. Bentley, and N. Ramanujam, “Quantitative physiology of the precancerous cervix in vivo through optical spectroscopy,” Neoplasia 11(4), 325–332 (2009).
8. M.-A. Mycek, K. T. Schomacker, and N. S. Nishioka, “Colonial polyp differentiation using time-resolved autofluorescence spectroscopy,” Gastrointest. Endosc. 48(4), 390–394 (1998).
9. Z. Volynskaya, A. S. Haka, K. L. Bechtel, M. Fitzmaurice, R. Shenk, N. Wang, J. Nazemi, R. R. Dasari, and M. S. Feld, “Diagnosing breast cancer using diffuse reflectance spectroscopy and intrinsic fluorescence spectroscopy,” J. Biomed. Opt. 13(2), 024012 (2008).
10. R. H. Wilson and M.-A. Mycek, “Models of light propagation in human tissue applied to cancer diagnostics,” Technol. Cancer Res. Treat. 10(2), 121–134 (2011).
11. S. C. Kanick, C. van der Leest, J. G. J. V. Aerts, H. C. Hoogsteden, S. Kasciková, H. J. C. M. Sterenborg, and A. Amelink, “Integration of single-fiber reflectance spectroscopy into ultrasound-guided endoscopic lung cancer staging of mediastinal lymph nodes,” J. Biomed. Opt. 15(1), 017004 (2010).
1. Introduction

Pancreatic adenocarcinoma, the 4th leading cause of cancer death in the United States with a five-year survival rate of less than 6%, is often detected at late-stages of development when treatment is ineffective [1]. Intraductal papillary mucinous neoplasm (IPMN, Fig. 1) is a precursor lesion of pancreatic cancer, characterized by an intraductal proliferation of neoplastic cells with mucin production [2].

![Fig. 1. Representative histological images of (A) normal pancreatic ductal tissue (BPC: benign pancreatic cell), (B) IPMN (MPC: malignant pancreatic cell with enlarged nuclei), and (C) pancreatic adenocarcinoma (AC with enlarged nuclei). The nuclei and stroma have been stained purple (hematoxylin stain) and pink (eosin stain), respectively. Note that IPMN and AC tissues have similar biophysical features, including nuclear enlargement and abundant collagen surrounding cells, relative to normal tissues. These features can be analyzed by quantitative multimodal optical spectroscopy.](image)

IPMN diagnosis, which could offer an opportunity to treat patients before they develop a potentially incurable pancreatic malignancy, may be challenging; identifying those IPMN with progression to malignancy remains an unmet medical need. Of the main imaging modalities employed to detect IPMN (computed tomography, endoscopic retrograde cholangiopancreatography, magnetic resonance cholangiopancreatography, and endoscopic ultrasonography (EUS)), EUS offers the highest resolution [3] and enables a cytologic
diagnosis of IPMN via fine-needle aspiration (EUS-FNA). However, the sensitivity of EUS-FNA cytology diagnosis is low (44-75%) [4, 5] and the technique suffers from a steep learning curve and high inter-operator variability [6]. Thus, the effectiveness of identifying cancer precursor lesions such as IPMN could be improved by developing a more sensitive and quantitative detection method.

Optical spectroscopy has shown promise as a potential diagnostic tool for pre-cancer detection in various human tissues, including the cervix [7], the colon [8] and the breast [9]. Advantages of optical spectroscopy compared to current imaging modalities (listed above) include quantifying tissue morphological and biochemical alterations occurring at the molecular and cellular levels during neoplastic progression [10] and clinical compatibility with EUS-FNA procedures [11]. Previously, our group successfully distinguished human pancreatic diseases, including pancreatic adenocarcinoma (AC) and chronic pancreatitis, from normal tissues with multimodal optical spectroscopy and a mathematical photon-tissue interaction (PTI) model [12–15].

Here, we report the first, to our knowledge, optical spectroscopic (reflectance and fluorescence) measurements from human pancreatic malignant precursor, IPMN. Because the study employed tissues obtained surgically, typically from patients with advanced disease, the opportunity to study IPMN (a cancer precursor) in patients was limited. For freshly excised pancreatic tissues (Fig. 1), we developed quantitative tissue-optics models to assess the biophysical similarities and differences between normal tissues, IPMN, and AC tissues.

2. Experimental methods

2.1 Instrumentation

A Reflectance and Fluorescence Lifetime Spectrometer (RFLS) [16], was employed to obtain reflectance and fluorescence spectra of human pancreatic tissues. A tungsten halogen lamp (HL 2000FHSA, Ocean Optics) provided white light (400-750 nm) for reflectance measurements; a pulsed laser at 355 nm (PNV001525-140, JDS Uniphase) provided fluorescence excitation light. Lamp and laser light were delivered to tissue via two separate optical fibers with core diameters of 600 μm. Tissue reflectance and fluorescence light were collected by a third identical fiber and delivered to the detectors. Collected light was passed to a spectrograph (MS 125, Oriel Instruments) coupled to an intensified charge-coupled device camera (ICCD 2063, Andor Technology) to detect tissue reflectance (400-750 nm) and fluorescence (360-700 nm). The RFLS also measured time-resolved fluorescence decays, which are not discussed here. Data acquisition time for each modality was less than 1 s. All measured spectra were background subtracted, corrected, and normalized as in [16].

2.2 Human studies and data set

The study was approved by the Institutional Review Board of the Univ. of Michigan (U of M) Medical Center. Informed written consent was obtained from each patient. Optical data were acquired from freshly-excised human pancreas within 30 minutes of resection during pancreatic surgery. Measurements were taken at up to 10 sites on each tissue specimen, depending on specimen size. At each site, two reflectance and two fluorescence spectra were collected. For each tissue site, immediately after optical measurements were acquired, the site was biopsied by a clinical pathologist for histopathological tissue analysis to confirm diagnosis.

| Tissue Type | Number of Patients | Number of Analyzed Tissue Sites |
|-------------|--------------------|-------------------------------|
|             | Reflectance        | Fluorescence                  |
| Normal      | 3                  | 11                            |
| IPMN        | 2                  | 8                             |
| AC          | 4                  | 17                            |

#194944 - $15.00 USD Received 31 Jul 2013; revised 29 Sep 2013; accepted 31 Oct 2013; published 14 Nov 2013
(C) 2013 OSA 1 December 2013 | Vol. 4, No. 12 | DOI:10.1364/BOE.4.002828 | BIOMEDICAL OPTICS EXPRESS 2830
Table 1 summarizes the study. Two measurements on each site were averaged for analysis. The IPMN diagnoses were “IPMN with moderate dysplasia,” as shown in Fig. 1. The 22 normal and 33 AC reflectance and fluorescence spectra from 11 and 17 sites, respectively, used for comparison with the IPMN data, were reported previously [13]. For one IPMN patient, fluorescence spectra were not recorded due to misalignment. Among 18 measured IPMN reflectance spectra from 9 sites, two spectra from one site were excluded by the exclusion criteria $\frac{R_{550\ nm}}{R_{650\ nm}} < 0.1$ [12, 14] due to excessive blood absorption.

2.3 Direct Fit PTI model for steady-state reflectance and fluorescence

A PTI model for reflectance and fluorescence spectra was reported [12, 13]. The model employed a semi-empirical reflectance equation to extract absorption- and scattering-related tissue parameters from measured reflectance spectra. The empirical model was described as a function of the tissue scattering coefficient (related to the cellular nuclear diameter $L$, nuclear refractive index $n_s$, and cellular density $[\text{Cell}]$) and the tissue absorption coefficient (related to a linear combination of oxygenated $[\text{oxy-Hb}]$ and deoxygenated $[\text{deoxy-Hb}]$ hemoglobin concentration). In this model, nuclear diameter, total hemoglobin concentration and oxygen saturation were freely varied for fitting with remaining variables fixed.

Here, a Direct Fit PTI (DF-PTI) model was developed and employed with seven main modifications to the previous model [12]: 1) to improve fit quality at all wavelengths, the semi-empirical model was fit directly to the measured data without a “canonical normal” spectrum, 2) the wavelength range for fitting was expanded from 700 nm to 750 nm to more accurately account for the effect of cellular nuclear size and refractive index on spectrum shape [17], 3) the cost function was minimized with a nonlinear least-squares iterative algorithm, 4) bilirubin $[\text{Bilirubin}]$ was added as an absorber [11], 5) collagen concentration $[\text{Collagen}]$ and cellular density $[\text{Cell}]$ were freely varied, 6) nuclear refractive index was freely varied, as the refractive index is related to tissue malignancy [18], and 7) the mean vessel radius $R_v$ was freely varied [19]. In the original PTI model, mean vessel radius was fixed at 7 µm and the absorption coefficient of whole blood was employed for the vessel packaging correction factor. In the DF-PTI model, the blood absorption in the capillary network was modeled with a varying vessel radius size and blood volume fraction ($\rho$) [20]. The ranges of varied tissue parameters employed in the DF-PTI model are shown in Table 2.

| Parameters | $L$ (µm) | $n_s$ (cm$^{-1}$) | $[\text{Cell}]$ (µM) | $[\text{Collagen}]$ (µM) | $[\text{oxy-Hb}]$ (µM) | $[\text{deoxy-Hb}]$ (µM) | $R_v$ (µm) | $\rho$ | $[\text{Bilirubin}]$ (µM) |
|------------|----------|-----------------|----------------------|---------------------------|-----------------------|-------------------------|-----------|------|------------------------|
| Min. Value | 8        | 1.37            | 5.6 × 10$^0$         | 1.0 × 10$^0$              | 0 µM                  | 7 µM                    | 0         | 7    | 0                      |
| Max. Value | 14       | 1.40            | 8.4 × 10$^0$         | 1.0 × 10$^0$              | 50 µM                 | 20 µM                   | 0.15      | 40   | 40 µM                  |

For each tissue measurement, the scattering coefficient calculated via the best DF-PTI reflectance fit was employed to correct the corresponding fluorescence spectrum for attenuation [12]. As measurements were ex vivo and local blood content could vary over time, only the extracted scattering, and not absorption, coefficient was employed to calculate intrinsic fluorescence, while hemoglobin and bilirubin concentrations were varied when calculating intrinsic fluorescence. The resulting intrinsic fluorescence spectra were fit to a linear combination of basis spectra of three endogenous tissue fluorophores: nicotinamide adenine dinucleotide (NADH, 450-500 nm emission peak), flavin adenine dinucleotide (FAD, 500-600 emission peak) and collagen (400-450 emission peak) [12]. The best fit provided the fractional contribution of each fluorophore to the intrinsic fluorescence.
3. Results and discussion

3.1 Reflectance analysis

Figure 2(A) shows the average of all normalized reflectance spectra from human pancreatic tissues: normal, IPMN with moderate dysplasia, and AC tissues. The features in the 400-440 nm and 540-580 nm ranges were attributed mainly to hemoglobin absorption. The higher reflectance in the 450-530 nm range in IPMN and AC sites was attributed to the cellular density and nuclear size [12, 13]. Figure 2(B) shows wavelength-dependent standard errors of the mean spectra for each tissue type. It is noted that standard errors are greatest in the wavelength range related hemoglobin absorption. Figure 2(C) shows a representative DF-PTI model fit to IPMN reflectance. Mean percent error between measured data and fit for all the measurements was less than 5% in the range 450-750 nm. The mean values of extracted nuclear diameters and refractive indices from IPMN measurements were larger than those from normal tissues (Table 3.)

The mean extracted nuclear sizes are consistent with histology (Fig. 1), indicating nuclear enlargement both in IPMN and AC relative to normal pancreas. Additionally, the ratio of the extracted nuclear size of AC to normal tissue (11.64/8.89 = 1.30) is consistent with the previously reported nuclear dilation factor L/L0 (1.27 ± 0.01) [13]. The result for mean extracted refractive index of normal pancreas (1.372) was consistent with previous reports [12, 13]. The extracted nuclear refractive indices of IPMN and AC were larger than that of normal pancreas. This finding is consistent with studies reporting that dysplastic cell nuclei have larger refractive indices than normal nuclei due to the higher DNA content and concentrations of nucleic acids in dysplastic cells relative to normal cells [18, 21].

Here, variations in local tissue blood content arising from the experimental protocol (tissue dissection prior to optical measurement) preclude the reporting of tissue absorption-related parameters. We note that mean estimated vessel radii were 11 to 13 µm for all tissue types. Larger estimated vessel radius (compared to previously employed 7 µm), with the inclusion of fractional blood volume, produced more accurate fits within the Soret absorption band.

| Tissue Type | Nuclear diameter | Refractive index | Cell Density | Collagen Density |
|-------------|------------------|-----------------|--------------|-----------------|
| Normal      | 8.89 ± 0.13 µm   | 1.372 ± 0.002   | 8.07 ± 0.12 × 10⁷/cm³ | 1.28 ± 0.16 × 10⁶/cm³ |
| IPMN        | 11.50 ± 0.88 µm  | 1.394 ± 0.004   | 7.15 ± 0.09 × 10⁷/cm³ | 6.15 ± 1.56 × 10⁶/cm³ |
| AC          | 11.64 ± 0.37 µm  | 1.396 ± 0.002   | 7.21 ± 0.09 × 10⁷/cm³ | 8.58 ± 0.62 × 10⁶/cm³ |
3.2 Fluorescence analysis

Figure 3(A) shows the average of all normalized fluorescence spectra from human pancreatic normal, IPMN, and AC tissues. Fluorescence spectra included contributions from intracellular NADH and FAD, and from stromal collagen, with emission peaks around 470 nm, 540 nm, and 430 nm, respectively. Although measured fluorescence spectra were attenuated by hemoglobin absorption, notable spectral differences between normal and diseased pancreas were apparent. Figure 3(B) shows the wavelength-dependant standard error of the mean spectra for each tissue type, with higher variation in the wavelength band attributed to hemoglobin absorption. Intrinsic fluorescence was calculated from measured fluorescence (Fig. 3(C)). Mean percent error of fitting intrinsic fluorescence in the range 400-550 nm was under 7%.

![Fig. 3. Quantitative analysis of measured fluorescence spectra can distinguish IPMN and AC from normal pancreas. (A) Mean of normalized fluorescence spectra obtained from human pancreatic normal tissues, IPMN with moderate dysplasia, and AC. (B) Wavelength-dependent standard error of mean spectra for each tissue type. (C) Representative PTI model fit for IPMN intrinsic fluorescence.](image)

The extracted percentage contribution from collagen emission to the intrinsic fluorescence for normal, IPMN, and AC tissues were 45.4 ± 7.3%, 92.2 ± 3.4% and 76.1 ± 5.4%, respectively. The collagen contribution to the intrinsic tissue fluorescence was greater for IPMN and AC tissues than for normal pancreas. This result is consistent with histological findings (Fig. 1), which indicate a collagen-rich stroma in IPMN and AC relative to normal pancreas. The increase of extracellular collagen content in IPMN and AC relative to normal pancreas arises from a fibrotic response during disease development, with collagen surrounding IPMN being more mature and with denser organization, since it is formed more slowly than collagen surrounding pancreatic AC, which is immature and newly formed in the process of tumor-induced desmoplasia (abundant fibrotic stroma) [22].

4. Conclusion

The results suggest that multimodal tissue optical spectroscopy coupled with quantitative tissue-optics models can characterize intraductal papillary mucinous neoplasm (IPMN), a cancer precursor in human pancreatic tissues. The analysis developed here assessed parameters associated with cellular nuclear size, nuclear refractive index, and tissue collagen content, and the results were consistent with known histopathology for these tissues.

Parameters extracted from model fits to reflectance and fluorescence data distinguished IPMN tissues from normal pancreas and closely associated IPMN tissues with pancreatic cancers. Thus, optical characterization of IPMN tissues, which have similar optical scattering properties to adenocarcinoma, could enable early clinical intervention, including follow-up examinations to monitor progression to malignancy with the goal to intervene prior to development of an incurable pancreatic cancer.
Acknowledgments

This work was supported in part by the National Institutes of Health (NIH CA-114542), the National Pancreas Foundation, the Wallace H. Coulter Foundation, the U of M Comprehensive Cancer Center, and grants from the U of M Medical School Translational Research Program and the American Society for Gastrointestinal Endoscopy. We thank Drs. M. Mulholland, R. Minter, and K. Nguyen for allowing us to collect optical data from their patients and Sheryl Korsnes for assistance in recruitment of patients.