Spatial-domain low-coherence quantitative phase microscopy to improve the cytological diagnosis of pancreatic cancer

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ABSTRACT

Use of endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) cytology to detect pancreatic cancer is limited, with a high false negative rate mainly due to the relatively fewer number of completely cancerous cells. To improve the accuracy of EUS-FNA cytological diagnosis, we evaluated a novel optical system—spatial-domain low-coherence quantitative phase microscopy (SL-QPM)—to analyze nanoscale nuclear architecture on original cytology samples, especially those diagnosed as indeterminate for malignancy, with the goal of maintaining high specificity and reducing false positive rate. We performed SL-QPM on original cytology samples obtained by EUS-FNA from 40 patients with suspicious pancreatic solid lesions (27 adenocarcinomas, 5 neuroendocrine tumor, 8 chronic pancreatitis), including 13 cases that were cytologically indeterminate. Each diagnosis had been confirmed by follow-up surgical pathology. The SL-QPM-derived nanoscale nuclear architectural parameters distinguished pancreatic cancer from cytologically indeterminate cells. A logistic regression model using nuclear entropy and SD increased the sensitivity of cytology in identifying pancreatic cancer from 72% to 94% while maintaining 100% specificity. The SL-QPM-derived nanoscale nuclear architecture properties show great promise in improving the cytological diagnosis of EUS-FNA for pancreatic cancer and could be used when traditional cytopathology does not get an accurate diagnosis, and can be easily translated into a traditional clinical device.

Significance of this study

What is already known about this subject?

- Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is often used for suspicious pancreatic lesions in order to get an accurate diagnosis, and while cytology has a relatively high specificity (approaching 100%), sensitivity remains suboptimal, in part because of the relatively fewer number of completely cancerous cells.
- The reported diagnostic accuracy of EUS-FNA cytology varies widely from 64% to 90%.
- The biggest challenge in diagnosing pancreatic cancer from EUS-FNA cytology is determining a specific number of cells that meet the cytological criteria of malignancy.
- The sensitivity of EUS-FNA cytology for pancreatic solid lesions can be significantly compromised by the difficulty in obtaining a diagnostic specimen, a limited number of needle passes, and the absence of an onsite cytopathologist.

What are the new findings?

- A novel optical microscope—spatial-domain low-coherence quantitative phase microscope (SL-QPM)—can be used to examine unmodified clinical cytology specimens without any additional processing and can detect subtle structural changes as small as 0.9 nm, well beyond what conventional microscopy reveals.
- SL-QPM-derived nanoscale-sensitive nuclear architectural parameters are shown to be capable of identifying patients with pancreatic cancer from EUS-FNA cytology specimens.

INTRODUCTION

A definitive preoperative diagnosis of pancreatic adenocarcinoma (PDAC) is critical for clinical management, particularly for the appropriate use of neoadjuvant treatment strategies. Endoscopic ultrasound-guided fine needle aspiration (EUS-FNA) is performed on a routine basis for suspicious pancreatic lesions, and while cytology has a relatively high specificity (approaching 100%), sensitivity remains suboptimal,[1–3] in part because of the relatively fewer number of completely cancerous cells.

Ideally, a cytopathologist should be in the room to ensure that an accurate diagnosis is achieved while limiting the number of needle passes if diagnostic tissue is obtained with an early aspiration. Otherwise, five to seven passes are needed to ensure that a diagnostic material is obtained. In this latter scenario, the patient's diagnostic accuracy is compromised by a 10%–15% reduction in definitive cytological diagnoses, longer procedure time, and increased risk for complications.[4 5]

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Significance of this study

- SL-QPM-derived nuclear architectural analysis of EUS-FNA cytology specimens could potentially improve the diagnostic accuracy of pancreatic solid lesions in those cells that are labeled as 'indeterminate' or 'negative' by an expert cytopathologist, and could reduce healthcare resources by limiting the number of required needle passes into a pancreatic lesion and the need for onsite cytopathology.

How might these results change the focus of research or clinical practice?

- The nanoscale nuclear structure parameters that derived from the SL-QPM system show great prospect in increasing the accuracy of cytological diagnosis of EUS-FNA for pancreatic malignant lesions, and could be performed when common cytological diagnosis is not definitive or when an expert pathologist specialized in cytological diagnosis is not on site.
- The SL-QPM system can be easily translated into a traditional clinical device because it can be performed on the original cytological samples directly.
- Further, use of this novel optical technology could reduce the times of needle passes for aspiration biopsy, thus can reduce the complications of EUS-FNA.

The reported diagnostic accuracy of EUS-FNA cytology varies widely from 64% to 90%.[1–3 6 7] The biggest challenge in diagnosing pancreatic cancer from EUS-FNA cytology is determining a specific number of cells that meet the cytological criteria of malignancy. Due to sampling error and the presence of a desmoplastic response, the small number of sampled cells may not necessarily come directly from the tumor. Furthermore, the cytopathologist identifies features of malignant cells using a conventional microscope with a resolution limited by diffraction that detects structural alterations at the scale of $\sim 1\,\mu$m.

Spatial-domain low-coherence quantitative phase microscopy (SL-QPM) is an emerging optical technique that is capable of detecting architectural changes in the cells at a nanoscale well below the resolution limit of conventional light microscope.[8–10] SL-QPM uses the nanoscale sensitivity of light interference effect, while effectively suppressing multiple noise sources in conventional interferometer-based microscopy, and quantifies the nanoscale-sensitive optical path length (OPL) distribution within each individual cell nucleus. Importantly, this technique is suitable for analyzing nanoscale architecture of cell nuclei on original unmodified cytology and histology specimens prepared with the standard clinical protocols without any additional processing. We have previously demonstrated the enormous potential of this technique for identifying various types of malignancies from cells that appear normal to experienced cytopathologists.[8–10]

In this pilot study of 40 patients, we investigated the feasibility of evaluating SL-QPM-derived nanoscale architecture properties in the nuclei of EUS-FNA cytology samples. We assumed that cells distant from or at an early stage of cancer development may show nanoscale structural alterations that appear non-cancerous to conventional cytopathology but could be detected by SL-QPM. Our goal is to integrate this system into a strategy that improves the diagnostic accuracy of EUS-FNA cytology with fewer needle passes.

Table 1 Characteristics of patient population

	Case (n=40)	
Characteristics	**Benign (n=8)**	**Malignant (n=32)**
Age (years)		
40–49	3	0
50–59	2	8
60–69	2	6
>70	1	18
Sex		
Male	2	14
Female	6	18

MATERIALS AND METHODS

Human specimens

Archived cytology specimens at the University of Pittsburgh Medical Center were obtained by EUS-FNA from 40 patients with suspicious pancreatic solid lesions. Each diagnosis had been confirmed by follow-up surgical pathology.

Table 1 shows the characteristics of the patient cohort. The mean age range was 50–60 years for the 8 patients with chronic pancreatitis (CP) (75% female) and 60–70 years for the 32 patients diagnostic with pancreatic malignancy (63% female). Cytology had correctly identified 23 of 32 patients with malignancy: 18 of 27 PDACs and all 5 neuroendocrine tumors (NET).

The cytopathologist identified frankly malignant cells and non-malignant-appearing epithelial cells from PC patients and non-malignant-appearing epithelial cells from benign patients for SL-QPM analysis. Since cytology is only considered to be truly positive when a cytopathologist can provide a definitive diagnosis of malignancy, the cytological diagnoses of atypical, suspicious, and negative were all categorized as 'indeterminate'. All samples were prepared as air-dried smears followed by Diff-Quik stains. The slides were identified by an expert cytopathologist who marked the lesions of interest. We performed around 30–40 cell nuclei per sample for SL-QPM analysis.

Spatial-domain low-coherence quantitative phase microscopy

We have previously published a detailed description of the SL-QPM instrument and data analysis algorithms.[8 9] Briefly, SL-QPM produces a two-dimensional OPL map of the cell nucleus with subnanometer sensitivity. The unique aspect of this system is that it uses a low spatial-coherence thermal light source and common-path interferometer configuration to eliminate the multiple noise coming from conventional interferometric microscopy, bringing about superior stability. Notably, the SL-QPM-derived nanoscale-sensitive OPL can be obtained from a subcellular structure (ie, nucleus) from the original unmodified clinical cytology specimens without any additional sample processing. The intrinsic arrangement of the clinical cytology specimen is effectively used in the common optical path configuration,

with the glass substrate serving as a reference, and the cellular components on the cytology slides were used as samples without bringing additional optical components.

For this study, a broadband white light xenon arc lamp was collimated by a 4f imaging system and focused onto the sample by a low numerical aperture (NA) objective (NA=0.4). The reflected mode image was gathered by a spectrometer (Acton Research, Massachusetts) coupled with a CCD camera (Andor Technology, Connecticut). The CCD camera records a three-dimensional intensity cube *I(x, y, k)*, where *x* and *y* denote the spatial position of each pixel in the microscopic image, and *k* denotes the wave number. The transmission mode optical devices are also employed to record traditional cytological images. The measured sensitivity of the SL-QPM system was characterized to be 0.9 nm.[8 9]

OPL map

The SL-QPM was performed to obtain a spatial distribution of OPL map, which is subsequently used to compute the nanoscale nuclear structural parameters. The reflectance spectrum *I(k)* from each pixel (*x, y*) is first normalized by the spectral profile of the optical system to account for the wavelength-dependent response of the light source and optical components. The Fourier-transformed data at the protruding peak according to the OPL of interest are chosen for data analysis. It has been confirmed that the OPL of interest is not affected by the absorption curve of the cytological staining process.[9] After performing the discrete Fourier transform, a complex-valued F is obtained, and the phase can be depicted by the following equation: $\varphi(x,y) = \tan^{-1}\left\{\frac{Im(F(x,y)|_z)}{Re(F(x,y)|_z)}\right\}$. The phase value of each pixel can be calculated by the Fourier analysis of *I(k)* and is turned to the related axial OPL by $\delta(OPL) = \frac{\lambda_0}{4\Pi}\varphi(x,y)$, where λ_0 is the source central wavelength (λ=550 nm) and $\varphi(x,y)$ is the phase at each pixel. The OPL is defined by the equation $OPL(x,y) = n(x,y)L(x,y)$, where *n(x, y)* is the average refractive index along the axial direction (ie, z-direction) at a specific pixel *(x, y)*, and *L(x, y)* is the physical thickness. As a result, we obtained a two-dimensional spatial distribution of nanoscale-sensitive OPL (ie, OPL map).

Quantitative analysis of OPL map

For quantitative characterization of the nanoscale-sensitive nuclear architecture map, *OPL(x, y)*, we consider two statistical texture descriptors of *OPL(x, y)*: SD σ_{OPL} and entropy E_{OPL}.[11] Both parameters characterize the architectural heterogeneity within each cell nucleus and are unaffected by the average staining levels. The SD is derived from the second statistical moment and measures the average spread of the OPL values of *OPL(x, y)*; the entropy E_{OPL} is a measure of structural randomness (or the average per pixel) of *OPL(x, y)*. To calculate the entropy, we use the well-known Shannon entropy equation, $E_{OPL} = -\sum_i p_i \log_2 p_i$, where p_i is derived from the normalized histogram of *OPL(x, y)*. The histogram comes from considering 1000 sample bins over a range of appropriate OPL values. These bins were selected to confirm that the histogram details are captured without bringing any sampling noise. Therefore, the nuclear SD σ_{OPL} and the nuclear entropy E_{OPL} describe the intranuclear architectural heterogeneity from different perspectives: σ_{OPL} quantifies an overall spread of the intranuclear architectural variation, and the nuclear entropy E_{OPL} quantifies the intranuclear architectural randomness. To obtain a diagnostic parameter for each sample, we analyze the average value of the above-described nanoscale nuclear architectural heterogeneity parameters—σ_{OPL} and E_{OPL}—over around 30–40 cell nuclei for each sample, described as $(\sigma_{OPL})_p$ and $(E_{OPL})_p$, respectively.

Statistical analysis

We performed statistical analyses using GraphPad Prism V.7.0. The statistical comparison between two patient groups was performed based on Mann-Whitney U test (https://www.sciencedirect.com/topics/biochemistry-genetics-and-molecular-biology/mann-whitney-u-test), and two-tailed p values of less than 0.05 were considered statistically significant. To generate receiver operating characteristic (ROC) curve, we used logistic regression using one of the two variables $(E_{OPL})_p$ and $(\sigma_{OPD})_p$ or combined. Logistic regression constructs the probability of a positive diagnosis (ie, cancer) using the following equation: $I = In\left(\frac{p}{1-p}\right) = \beta_0 + \beta_1(E_{OPD})_p + \beta_2(\sigma_{OPD})_p$, where I is the constructed index, p is the probability of a positive diagnosis (ie, cancer), and β_0, β_1 and β_2 are the coefficients determined by the logistic regression model fit provided by GraphPad Prism V.7.0.

RESULTS

SL-QPM-derived nanoscale nuclear architecture

To define the characteristics of SL-QPM-derived nanoscale-sensitive nuclear architectural heterogeneity, three groups of pancreatic epithelial cells were analyzed: (1) cells from patients who were subsequently confirmed to have CP (indeterminate/CP); (2) cells labeled as 'indeterminate' by the expert cytopathologist from patients who were subsequently confirmed to have PDAC on surgery (indeterminate/PDAC); and (3) malignant cells from patients with PDAC (malignant/PDAC). Figure 1 compares the cytological images and the corresponding OPL maps of representative cell nuclei in these three groups. The OPL maps exhibit a distinct spatial distribution in these three groups of cell nuclei, with malignant cells showing more heterogeneous spatial distribution. It is encouraging that for those cells identified as indeterminate by cytopathologists, OPL maps can still distinguish PDAC from CP, suggesting its potential to improve the cytological diagnosis of pancreatic cytology.

Nuclear architectural parameters are capable of distinguishing benign pancreatic lesions from malignant pancreatic lesions

To quantify the nuclear architectural changes from these OPL maps, we extracted nuclear heterogeneity parameters—σ_{OPL} and E_{OPL}—over around 30–40 cell nuclei for each sample, described as $(\sigma_{OPL})_p$ and $(E_{OPL})_p$, respectively. We first evaluated whether the SL-QPM-derived nuclear architectural heterogeneity can distinguish different pancreatic lesions that can be accurately diagnosed by cytopathologists. We analyzed the nuclear architectural heterogeneity parameters from 26 patients with correct cytological diagnosis, consisting of 3 CP cases, 5 NET cases and 18 PDAC

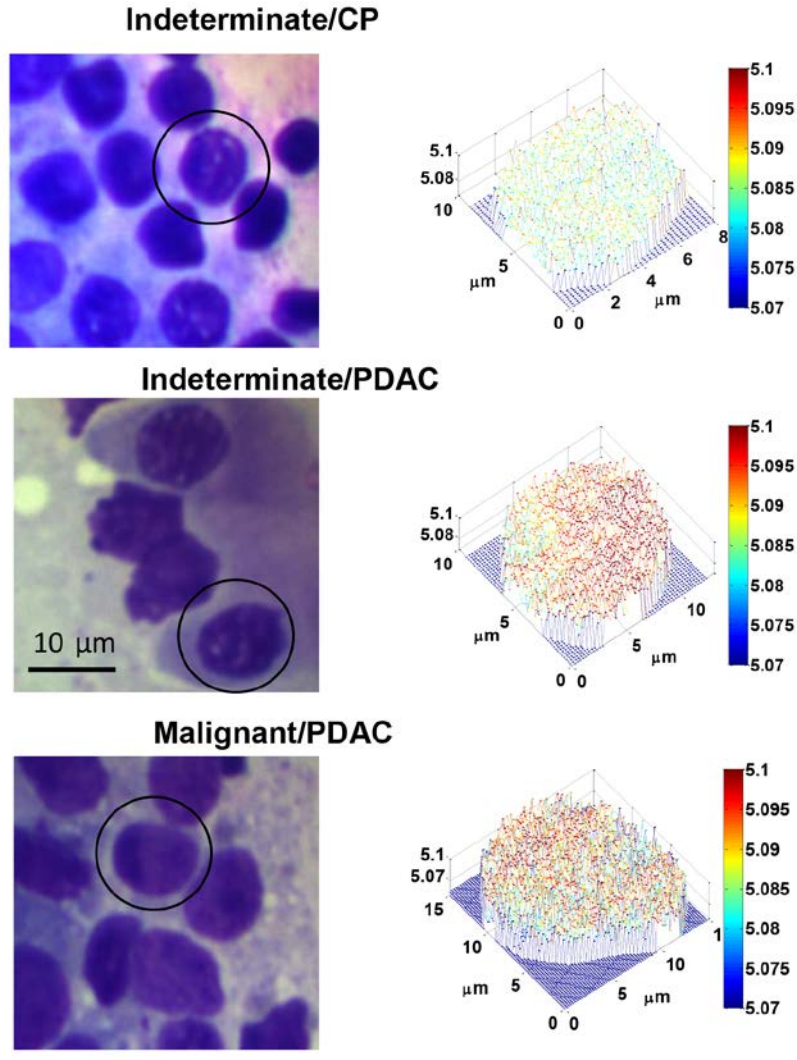

Figure 1 Representative cytological images and corresponding optical path length (OPL) maps of a cytologically indeterminate epithelial cell from a patient with chronic pancreatitis (CP), a cytologically indeterminate cell from a patient with pancreatic adenocarcinoma (PDAC), and a cytologically malignant cell from a patient with PDAC. Scale bars in the image indicate 10 µm. Color bar represents the magnitude of OPL in microns. The images show the progressively increased nuclear heterogeneity in frankly malignant cells and cytologically indeterminate cells from patients with pancreatic cancer compared with those cells from patients with chronic inflammation.

cases. Figure 2 shows the statistical analysis from the 26 patients with correct cytological diagnosis consisting of CP, NET and PDAC. Out of three nuclear architectural parameters, the nuclear heterogeneity parameters ($(\sigma_{OPL})_p$ and $(E_{OPL})_p$) are increased in malignant cells (NET and PDAC) compared with those from CP, with statistical significance ($p<0.05$). This result suggests the potential of quantitative nuclear architectural heterogeneity parameters to distinguish benign from malignant pancreatic lesions. This result also indicates that these nuclear heterogeneity parameters cannot separate NET from PDAC cases.

SL-QPM-derived nuclear heterogeneity parameters can improve the accuracy of detection of malignancy from indeterminate cytological diagnosis

Next, we evaluate whether the SL-QPM-derived nuclear architectural parameters can improve the detection of pancreatic malignancy from those with indeterminate cytological diagnosis. We compared nuclear architectural parameters from four groups of patients: (1) patients who received 'indeterminate' cytological diagnosis but were surgically confirmed to have CP (indeterminate/CP); (2) patients who received 'indeterminate' cytological diagnosis but were subsequently found to have PDAC (indeterminate/PDAC); (3) patients who were cytologically diagnosed and surgically confirmed to have NET; and (4) patients who were cytologically diagnosed and surgically confirmed to have PDAC. In those patients who received indeterminate cytological diagnosis, the nuclear heterogeneity parameters—$(\sigma_{OPL})_p$and $(E_{OPL})_p$—from patients with PDAC are significantly higher than those from CP ($p=0.004$ and $p=0.002$, respectively), even though the average values of $(\sigma_{OPL})_p$and $(E_{OPL})_p$ from cytologically indeterminate cells are slightly lower than those from frankly malignant cells. This result also indicates the importance of quantitative nuclear heterogeneity measure in distinguishing pancreatic cancer

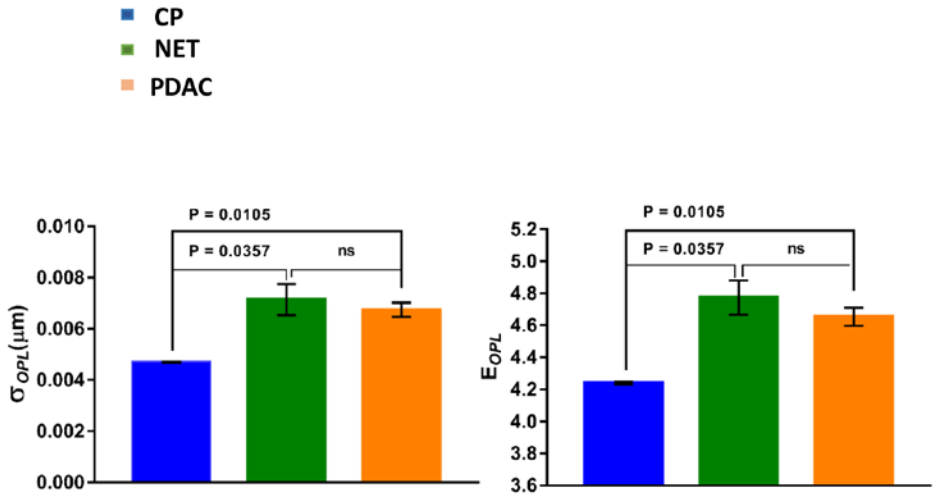

Figure 2 Statistical analysis of σ_{OPL} and E_{OPL} performed on different pancreatic lesions with correct cytological diagnosis consisting of 26 patients (18 pancreatic adenocarcinomas (PDAC), 5 neuroendocrine tumor (NET) and 3 chronic pancreatitis (CP)). Approximately 30–40 cells were analyzed for each patient. The nuclear heterogeneity parameters ($(\sigma_{OPL})_p$ and $(E_{OPL})_p$) are capable of distinguishing chronic pancreatitis from malignant pancreatic lesions with statistical significance ($p<0.05$). ns, no statistical significance.

from CP from cases that cannot be correctly identified by cytopathologists (figure 3).

Performance characteristics of SL-QPM-derived nuclear architectural parameters

To identify the property of SL-QPM-derived parameters, we showed the scatter plots of the two nuclear architectural heterogeneity parameters in figure 4A. Most patients with CP and those with cytologically malignant cells are well separated. Importantly, those cases originally labeled as 'indeterminate' by the expert cytopathologist show similar distribution to malignant cells that are distinct from those benign cells. We then developed a logistic regression model using each of the two nuclear heterogeneity parameters—nuclear architectural randomness or entropy $(E_{OPL})_p$ and the SD σ_{OPL} $(\sigma_{OPL})_p$ and combined—with maximal specificity (minimized false positives) from the 32 patients with pancreatic malignant lesions and the 8 patients with CP. Figure 4B shows the ROC curve for using $(E_{OPL})_p$ alone, which has an area under ROC (AUC) of 0.965. The parameter $(E_{OPL})_p$ is selected based on our calculation of AUC for σ_{OPL} $(\sigma_{OPL})_p$ alone (AUC=0.957) or $(E_{OPL})_p$ alone (AUC=0.965) and combined $(E_{OPL})_p$ and σ_{OPL} $(\sigma_{OPL})_p$ (AUC=0.965). Because the same AUC for nuclear entropy and combined nuclear entropy and SD and less number of variables mitigate the potential for overfitting, we selected a single variable $(E_{OPL})p$ alone in our model. By optimizing specificity (minimizing false positives), this

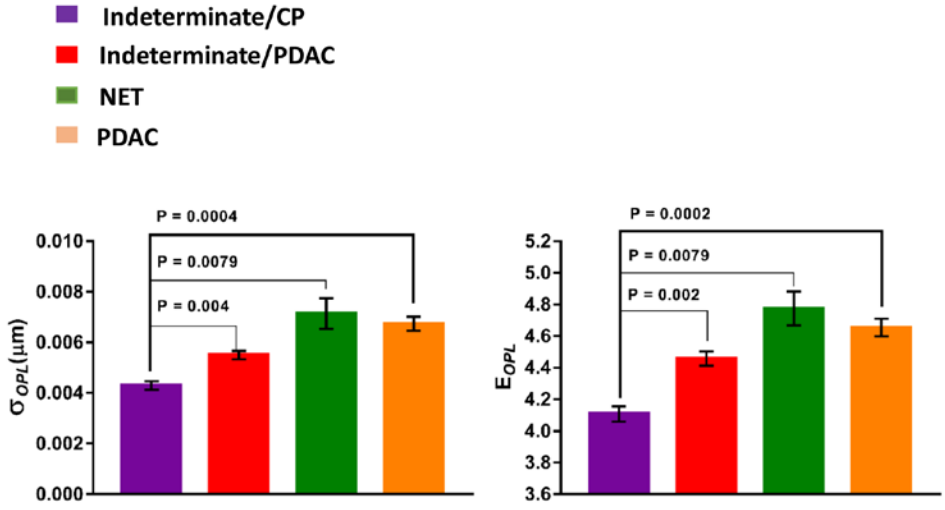

Figure 3 Statistical analysis of σ_{OPL} and E_{OPL} was performed on 37 patients (18 pancreatic adenocarcinoma (PDAC), 5 neuroendocrine tumor (NET), and 17 indeterminate cases in whom 9 were subsequently found to have adenocarcinomas (PDAC) and 4 were chronic pancreatitis (CP)). Approximately 30–40 cells were analyzed for each patient, and the statistical average of each parameter was used as the diagnostic parameter for each patient. The nuclear heterogeneity parameters ($(\sigma_{OPL})_p$ and $(E_{OPL})_p$) can distinguish pancreatic malignancy from cytologically indeterminate patients. OPL, optical path length.

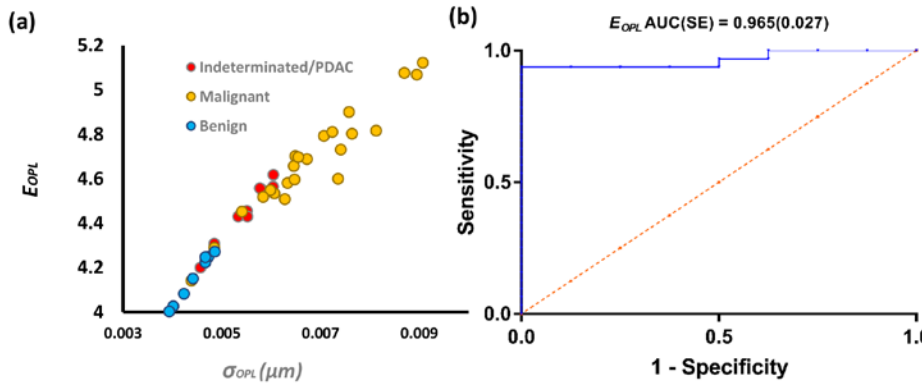

Figure 4 (A) Scatter plot of two statistical parameters derived from nuclear optical path length (OPL) maps ($(\sigma_{OPL})_p$ and $(E_{OPL})_p$) from 40 patients. Each symbol represents a representative patient. Blue dots: patients with confirmed chronic pancreatitis; red dots: patients with pancreatic adenocarcinoma (PDAC) diagnosed as 'indeterminate' by cytology (indeterminate/PDAC); and yellow dots: frankly malignant cells diagnosed as a pancreatic malignancy by cytology (malignant). (B) Receiver operating characteristic curve to distinguish benign from malignant pancreatic lesions using $(E_{OPL})_p$ alone. The area under the curve (AUC) is calculated to be 0.965±0.027 (mean±SE).

model achieves 94% sensitivity for $(E_{OPL})_p$ compared with 87.5% sensitivity for $(\sigma_{OPL})_p$ in detecting pancreatic malignancy at 100% specificity. Overall, the nanoscale nuclear entropy increased the sensitivity of cytological diagnosis in distinguishing pancreatic cancer from 72% (23/32) to 94% (30/32) while maintaining 100% specificity. Among patients who had indeterminate cytological diagnosis (n=13), the nuclear entropy correctly identified eight out of those nine patients with PDAC and all four patients with CP.

DISCUSSION

Here we demonstrate that the changes in the nanoscale-sensitive nuclear architectural parameters derived from SL-QPM not only differentiate benign and malignant cells in EUS-FNA cytology specimens from pancreatic solid lesions, but even more importantly we show the ability of these nanometer-sensitive nuclear architecture characteristics to detect pancreatic cancer even in cells labeled as 'indeterminate' by expert pathologists that were confirmed at surgery to be from a patient with cancer.

Enormous research has identified that EUS-FNA cytology has a relatively high specificity (approaching 100%), but remains to have suboptimal sensitivity in the diagnosis of malignant tumors in solid pancreatic lesions.[12 13] EUS-FNA cytology does not always make a definite diagnosis for all solid pancreatic lesions, and those cases which did not receive a definite diagnosis (8%–17%) are subject to an 'indeterminate' diagnosis, which is divided into 'atypical' or 'suspicious' for malignancy.[14 15] There are some reasons for the diagnosis of 'atypical' or 'suspicious' in the EUS-FNA solid pancreatic lesions.[16] The first reason is technical factors, including the endosonographer's skill, ability of distinguishing representative lesions and acquiring adequate cells, and the availability of rapid onsite assessment. The second reason is lesion-related factors, such as well-differentiated tumors with almost no atypical cells, and tumors showing serious desmoplastic response, necrosis, and cystic change, all of which may affect the quality of cells in the samples. The third factor that may impact the accuracy of diagnosis is pathologist-related, such as the pathologist's experience, expertise, and other factors.[17 18]

Compared with conventional cytology, SL-QPM offers an emerging technique to assess the in-situ nuclear architectural properties with a nanoscale sensitivity, which can be extremely responsive to even minute changes in the subcellular structures, well beyond what conventional microscopy reveals. Notably, this technique can be directly applied to the original unmodified format (ie, cytology slides) without any special sample preparation. Additionally, this technique requires only a small number of cell nuclei (~30–40 cells per patient) and is thus ideally suited for the FNA cytology specimens for which a limited number of cells are available for analysis. Therefore, the ability to analyze the subtle, nanoscale alterations in nuclear architecture on the original cytology specimens represents a significant technical advancement, which allows us to target the biggest challenge in FNA cytology of pancreatic lesions: sampling error.

To characterize the nuclear architecture in a quantitative and objective manner, we extracted two nanoscale-sensitive statistical texture descriptors for the nuclear heterogeneity described by the SD of the optical path length $(\sigma_{OPL})_p$ and the nuclear architectural randomness described by the entropy $(E_{OPL})_p$. Our results show that nuclear heterogeneity is elevated in pancreatic cancer cells, as we found in other tumor types (ie, colorectal cancer).[9] In particular, the nuclear heterogeneity parameters, including $(\sigma_{OPL})_p$ and $(E_{OPL})_p$, are most significant in detecting pancreatic cancer in patients who received indeterminate cytological diagnosis, as indicated by the high level of statistical significance between benign patients and cytologically indeterminate patients whose pancreatic cancer was confirmed at surgery (p=0.004 and p=0.002, respectively). In particular, the nuclear architectural entropy has the best performance characteristics. The nuclear entropy alone can achieve 94% sensitivity and 100% specificity, even in cells with an indeterminate cytological diagnosis, showing a superior sensitivity than standard cytopathology. Although the number of patients in this study was relatively small, the effect size was enough to achieve statistical significance. Furthermore, the application of only one parameter (ie, nuclear entropy) in our study reduces the possibility of overfitting.

The nanoscale nuclear architecture properties are quantified by the product of refractive index and the physical thickness of the cell ($OPL=nL$). If assuming a single layer of cells has consistent physical thickness for the same cell type, the alterations in OPL are essentially due to the changes in nuclear refractive index. The increased refractive index has previously shown to arise from the increased mass density (ie, macromolecular concentration). Our finding of increased average OPL (ie, $(<OPL>)_p$) in the cell nuclei of malignant cells is likely due to the increased density of the nuclear components,[19 20] such as chromatin and nuclear matrix. Indeed, the increased nuclear density (ie, hyperchromasia) in cancer cells has been well documented as one of the important pathological criteria for cancer diagnosis.[21]

Unlike cells diagnosed as benign lesions, the alteration of nuclear structural parameters from cytologically indeterminate pancreatic cancer cells is similar to that of complete malignant cells, suggesting that these cytologically indeterminate cells from patients with cancer may have certain common biological features with malignant cells. This result is in line with a recent pan-cancer study that found that patients with chromatin heterogeneous tumors had worse survival than those with chromatin homogeneous tumors.[22] The nuclear heterogeneity may also serve as a marker to improve cancer diagnosis. Although specific biological events that contribute to increased nuclear structural parameters in malignant cells are not well understood, these subtle alterations in nuclear structure may also be the result of complex genetic and molecular events from multiple molecular pathways, such as genomic instability.[23–25]

Clinically, the SL-QPM-derived nanoscale nuclear structural parameters can be performed to enhance the accurate detection of malignancy in EUS-FNA samples that would otherwise be missed by conventional cytopathology. SL-QPM-based nuclear analysis could be performed in cases for which traditional cytopathology cannot make an accurate diagnosis. This technique could also reduce the number of needle passes required, thus potentially reducing FNA-associated complications, procedure time, and cost. The SL-QPM system can be easily translated into a traditional clinical device because it can be performed on the original cytological samples directly.

Although this study shows the potential of SL-QPM-derived nuclear architectural parameters to improve the diagnosis of pancreatic malignancy, it also has several limitations. First, this study was based on archived cytology specimens, rather than a prospectively recruited cohort. Second, our sample size is rather limited, even though we are encouraged by the statistically significant results. Third, the average age of patients with pancreatic malignancy is significantly higher than that of CP. The diagnostic utility of this technique must be further validated in a larger independent patient population. Ultimately, a large multicenter study would need to determine whether our proposed strategy of limiting needle passes can maintain or even improve the accuracy of FNA in diagnosing patients with pancreatic cancer.

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Contributors HM and PW performed the experiments and analyzed the data. HM wrote the draft. DS and YL revised the article and supported the study. DS and YL jointly designed, oversaw, and directed the study.

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Competing interests The authors declare that a US patent was issued to spatial-domain low-coherence quantitative phase microscopy (SL-QPM) (US Patent 10,156,479), which could be construed as a potential conflict of interest.

Patient consent for publication Not required.

Ethics approval All studies were performed with the approval of the institutional review board at the University of Pittsburgh.

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