Light scattering spectroscopy identifies the malignant potential of pancreatic cysts during endoscopy

Lei Zhang1, Douglas K. Pleskow2, Vladimir Turzhitsky1, Eric U. Yee3, Tyler M. Berzin2, Mandeep Sawhney2, Shweta Shinagare3, Edward Vitkin1, Yuri Zakharov1, Umar Khan1, Fen Wang2, Jeffrey D. Goldsmith3, Saveli Goldberg4, Ram Chuttani2, Irving Itzkan1, Le Qiu1* and Lev T. Perelman1,2,5*

Pancreatic cancers are usually detected at an advanced stage and have poor prognosis. About one-fifth of these arise from pancreatic cystic lesions. Yet not all lesions are precancerous, and imaging tools lack adequate accuracy for distinguishing precancerous from benign cysts. Therefore, decisions on surgical resection usually rely on endoscopic ultrasound-guided fine-needle aspiration (EUS-FNA). Unfortunately, cyst fluid often contains few cells, and fluid chemical analysis lacks accuracy—which has dire consequences, including unnecessary pancreatic surgery for benign cysts and the development of cancer. Here, we report an optical spectroscopic technique, based on a spatial gating fibre-optic probe, that predicts the malignant potential of pancreatic cystic lesions during routine diagnostic EUS-FNA procedures. In a double-blind prospective study in 25 patients, with 14 cysts measured in vivo and 13 postoperatively, the technique achieved an overall accuracy of 95%, with a 95% confidence interval of 78–99%, in cysts with definitive diagnosis.

Pancreatic cancer has the lowest survival rate among all major cancers, typically six months from diagnosis. This is due to an inability to detect it early, while still treatable, largely because of the inaccessible location of the pancreas deep in the abdomen. Also, the disease often metastasizes while it is still asymptomatic. About one-fifth of pancreatic cancers arise from cystic lesions that can potentially be identified in early, treatable stages with non-invasive imaging techniques such as computed tomography (CT) and magnetic resonance imaging (MRI). Cystic lesions are relatively common, occurring in at least 2% of adults, with some studies describing the incidental finding of pancreatic cysts in more than 10% of abdominal MRIs obtained for non-pancreatic indications. It should therefore come as no surprise that cystic lesions account for one-third of all pancreatic surgeries. However, while CT and MRI could be used to screen for cystic lesions, they have limited accuracy with regard to identifying the type of pancreatic cyst. Currently, there is no sufficiently accurate diagnostic technique that can reliably distinguish cancerous and precancerous cysts from benign cysts. The resulting uncertainty in diagnosis of pancreatic cystic lesions can lead to a delay in surgical resection of precancerous lesions, as well as unnecessary surveillance and even surgery for benign cysts. Considering the high mortality and morbidity of pancreatic surgeries and the even higher mortality from untreated pancreatic cancers, there is an obvious need for the development of new diagnostic methods to accurately identify pancreatic cysts that need surgical intervention.

The best currently available diagnostic method for identifying malignancy in pancreatic cyst lesions is based on the minimally invasive EUS-FNA procedure, which is performed in at least 90% of cases when the decision to undergo surgery is required. This procedure has an overall sensitivity of less than 50% for detecting malignancy, with the majority of results being non-diagnostic. During the EUS-FNA procedure, the cyst fluid is collected and then analysed both for tissue evaluation (cytopathology), and also for the presence of certain molecular markers or glycoproteins, such as carcinoembryonic antigen (CEA).

There are two primary types of precancerous pancreatic cystic lesions, intraductal papillary mucinous neoplasms (IPMNs) and mucinous cystic neoplasms (MCNs), that could be treated surgically, achieving a high cure rate. However, the majority of cystic pancreatic lesions have no malignant potential and do not require surgery. Certain types of precancerous cysts can be safely monitored over years, and may not require surgical resection. Higher-risk precancerous cysts should be removed surgically before cancer development. Pancreatic surgery is complex and is associated with significant morbidity and mortality. For instance, pancreatoduodenectomy, also known as the Whipple procedure, involves removing the head of the pancreas, two-thirds of the duodenum and one-third of the stomach, and has a mortality rate of more than 11% when averaged over all US hospitals. Therefore, the decision to consider surgery for a pancreatic cyst requires the treating physician to weigh data from potentially inaccurate EUS-FNA results with several even less conclusive imaging tests, as well as with the patient’s ability to tolerate the surgery. As a result, of the pancreatoduodenectomies that are performed on cystic lesions, only about 42% are later confirmed as featuring precursor lesions with malignant potential. On the other hand, precancerous and small resectable cancerous cysts, when left untreated, have the risk of progressing to incurable cancer.

1Center for Advanced Biomedical Imaging and Photonics, Department of Medicine and Department of Obstetrics, Gynecology and Reproductive Biology, Beth Israel Deaconess Medical Center, Harvard University, Boston, Massachusetts 02215, USA. 2Division of Gastroenterology, Department of Medicine, Beth Israel Deaconess Medical Center, Harvard University, Boston, Massachusetts 02215, USA. 3Division of Pathology, Beth Israel Deaconess Medical Center, Harvard University, Boston, Massachusetts 02215, USA. 4Division of Biostatistics and Biomathematics, Massachusetts General Hospital, Harvard University, Boston, Massachusetts 02114, USA. 5Biological and Biomedical Sciences Program, Harvard University, Boston, Massachusetts 02115, USA. *These authors contributed equally to this work. e-mail: lqiu@caregroup.harvard.edu; lperelman@fas.harvard.edu
There is a critical need for the development of a diagnostic method that improves the accuracy of cyst evaluation and can be employed during the EUS-FNA procedure. The basic physical principle that elastic light scattering can distinguish precancerous and early cancerous lesions has already been demonstrated. There are three main components of tissue light scattering spectra. The largest is a diffuse background signal from submucosal tissue; next is scattering by small organelles; and, lastly, a relatively small backscattered component from epithelial cell nuclei. The submucosal background can be excluded by one of several gating techniques and the smaller organelles have a very different scattering spectral dependence than that of the nuclei. Elastic light scattering can also be used to measure other cellular compartments, such as mitochondria, whose spectra are sufficiently different from that of nuclei to be distinguished. The combination of gating and difference in spectral behaviour allows the epithelial nuclear scattering spectrum to be isolated in the processed light scattering spectroscopy (LSS) signal. A significant contribution from nuclear backscattering and clear correlation of dysplasia with nuclear size has been demonstrated in earlier studies. Direct comparison of the nuclear size distribution extracted from the backscattering signal to that of histological examination of the corresponding haematoxylin and eosin (H&E)-stained sections has also been demonstrated.

Now we have developed a new instrument that uses this principle to solve the difficult problem of identifying precancerous and early cancerous lesions in the pancreas. The instrument can probe the internal surface of pancreatic cysts, obtaining multiple noninvasive optical ‘biopsies’ from each cyst in a matter of seconds, performing significantly better than existing cytology and cyst fluid CEA markers. Our results indicate that this technology has significant potential to aid in identifying both precursor lesions and early-stage pancreatic cancers.

### Results

To develop an in vivo LSS system and diagnostic algorithm, we first performed an ex vivo pilot study to evaluate the ability of LSS to differentiate cystic neoplasms with varying grades of malignancy from benign cysts. We then designed a needle-based LSS instrument for in vivo use during EUS-FNA procedures and have collected spectra from the pancreatic cysts of 14 consecutive subjects who satisfied the study enrollment criteria. The diagnostic cut-offs were determined prospectively and the experimenters performing the data collection and analysis were blinded to the patient diagnosis, while the gastroenterologists making the patient diagnosis were blinded to the LSS results.

#### Studies in freshly resected pancreatic samples

Measurements on freshly resected pancreatic ductal adenocarcinoma and distal pancreatoduodenectomy samples from 11 subjects including a total of 13 pancreatic cystic lesions were obtained. The LSS spectra from pancreatic resection samples were measured with the clinical LSS system and polarization gated probe, developed previously for Barrett’s oesophagus (BE) studies. The spectra were collected from multiple locations with the measured sites marked with India ink, and photographed to ensure proper co-registration with the subsequent histopathology examination. To differentiate various cystic neoplasm histopathologies, we employed the diagnostic parameter $\Delta$ introduced in our BE studies, with the only difference in that the root mean square normalized spectrum, employed in the diagnostic algorithm, was now calculated using all cystic lesion measurements. In our BE studies, if this diagnostic parameter was greater than 0.1 (10% of the mean squared spectrum summed over all spectral points), the site was considered to be dysplastic. Following the same logic, our diagnostic criteria for cystic lesions classified $\Delta < 0.1$ as benign, $0.1 < \Delta < 0.2$ as low-grade dysplasia (LGD), and $\Delta > 0.2$ as high-grade dysplasia (HGD). The cut-offs $\Delta = 0.1$ and $\Delta = 0.2$ correspond to 25% and 50% enlarged nuclei according to our earlier work. The use of the same diagnostic criteria as in the BE studies is rationalized by the fact that the two most common types of precancerous pancreatic cystic lesions, IPMNs and MCNs, are characterized by a similar type of lining as in the BE — columnar epithelium. The data analysis was performed in a double-blind manner, before postoperative histopathology results were available. The preoperative cytology results and CEA levels were not taken into account.

To check if LSS would improve the diagnosis of cystic neoplasms, we compared our findings with preoperative imaging results, cytology results and cyst fluid CEA levels as well as postoperative histopathology, which is considered the gold standard. These results are summarized in Table 1 and discussed below.

In all cases, LSS diagnosis of benign and dysplastic cysts agreed with histopathology. When dysplasia grades were taken into account, two benign cases, four LGD cases and six HGD cases were correctly identified, while one HGD case was identified as LGD. Figure 1e

### Table 1 | Ex vivo differentiation of cystic neoplasms.

| Cyst | MRI/CT | CEA (ng ml$^{-1}$) | Cytology | Histopathology cyst type | Histopathology diagnosis | LSS ($\Delta$) | LSS diagnosis |
|------|--------|------------------|----------|-------------------------|-------------------------|--------------|--------------|
| 1*   | CNET   | 686              | -        | IPMN                    | LGD                     | 0.11         | LGD          |
| 2*   |        |                  | -        | Serous                  | Benign                  | 0.07         | Benign       |
| 3    | Serous | 67               | -        | IPMN                    | LGD                     | 0.12         | LGD          |
| 4    | IPMN   | 142              | -        | IPMN                    | HGD                     | 0.74         | HGD          |
| 5    | IPMN   | 430              | -        | LGD IPMN                | LGD                     | 0.19         | LGD          |
| 6    |        |                  | -        | Pseudocyst              | Benign                  | 0.08         | Benign       |
| 7    |        |                  | -        | HGD IPMN                | HGD                     | 0.76         | HGD          |
| 8    | IPMN   | 151              | -        | IPMN                    | HGD                     | 0.19         | LGD          |
| 9    | IPMN   | 151              | -        | LGD                     | HGD                     | 0.23         | HGD          |
| 10†  | IPMN   | 151              | -        | IPMN                    | HGD                     | 0.17         | LGD          |
| 11†  | IPMN   | 151              | -        | Adenocarcinoma          | IPMN                    | 0.22         | HGD          |
| 12   | IPMN   | 122              | -        | Carcinoma               | IPMN                    | 0.29         | HGD          |
| 13   | IPMN   | 122              | -        | LGD IPMN                | HGD                     | 0.26         | HGD          |

Polarization gated LSS optical spectroscopic technique versus MRI/CT, CEA level, preoperative cytology and postoperative histopathology. The two last columns present $\Delta$ parameter and the LSS diagnosis. MRI includes both abdominal MRI and MRCP. CNET, cystic neuroendocrine tumour; ITPN, intraductal tubulopapillary neoplasm. Dashes indicate no information due to lack of imaging classification, cellular material or absence of data on CEA level. *Cysts 1 and 2 are from the same subject. †Cysts 10 and 11 are from the same subject.
Fig. 1 | Ex vivo optical spectroscopic differentiation of cystic neoplasms. 

a, Abdominal and pelvic CT angiography in subject 1. b, Magnetic resonance cholangiopancreatography (MRCP) in subject 6. c, d, Cross-sectional cut photographs of the corresponding pancreatic resection samples with cysts clearly seen (red circles in a, b). e, Diagnostic parameter \( \Delta \) for 13 cyst measurements, with red bars indicating cysts diagnosed by histopathology as HGD, blue as LGD IPMN and green as benign. Green and red horizontal lines represent diagnostic algorithm LGD and HGD/cancer cut-offs, respectively. Cysts 1 and 2 are from the first subject, and cysts 10 and 11 are from the ninth subject.

shows a summary of the diagnostic parameter values as bars that are coloured according to final histopathology diagnosis. In vivo CT and magnetic resonance cholangiopancreatography (MRCP) images of cystic lesions 1 and 6 are shown in Fig. 1a,b, circled in red. Photographs of the same cysts are shown in Fig. 1c,d, respectively. For example, the first subject had a 1.3 × 2.3 cm cystic lesion within the pancreatic tail, which was detected via abdominal and pelvic CT angiography (Fig. 1a) and described as a possible side-branch IPMN. EUS-FNA cyst fluid resulted in a CEA of 686 ng ml⁻¹, significantly higher than the 192 ng ml⁻¹ cut-off suggestive of a mucinous lesion.⁶,⁷ Cytopology reported scant acellular debris that could not be further categorized. Although the CEA level and cytolology results were inconclusive for cancer, these results, along with the size of the cyst and clinical findings, were considered worrisome enough to warrant pancreatic surgery. LSS spectroscopy performed on the freshly resected cyst diagnosed all 7 locations within the cyst as LGD and later postoperative histopathology findings for all 7 locations were indeed IPMN with LGD. The other cyst measurements showed similar correlations with histopathology.

To summarize, this double-blind ex vivo study in cysts, representing 3 out of 4 primary types of pancreatic lesions (IPMN, serous cystadenoma and pseudocyst, but not MCN), demonstrated 92% accuracy, with a 95% confidence interval (CI) of 67–99% when dysplasia grades are taken into account, and 100% accuracy (95% CI: 77–100%) when identifying dysplastic versus benign cysts, suggesting that the proposed technique is accurate. By comparison, the accuracy of MRI/CT imaging, as determined from the patient cohort within our study, was only 54% for identifying dysplasia grade when compared with postoperative histopathology. Note that the percentage of premalignant cysts in our study is higher than that in the general population because our cohort was composed of pancreatic resection samples.

Fig. 2 | In vivo spatial gating fibre optic probe for use with EUS-FNA. 

a, The probe inserted in the FNA needle. Three SMA connectors at the proximal end for coupling groups of fibres with 120 μm, 220 μm and 240 μm distal-end source–detector separations with three individual spectrometers and another SMA connector for coupling the delivery fibre with the broadband light source. b, Probe extended by 2 mm from the beveled needle tip with the source on and a US penny for scale. c, Distal tip of the probe. The 450 μm outer diameter probe consists of seven 100 μm core diameter fibres with a numerical aperture (NA) of 0.21. The probe jacket is made of a robust medical-grade biocompatible polyimide. The delivery fibre in the outer ring is illuminated. Scale bar, 100 μm. d–f, Probe latching mechanism and fixed-length tube. The mechanisms can be locked with the position locking button (d) and toggled to extend (e) or retract (f) the probe tip from the needle. g, Fixed-length tube locked on the needle handle with Luer lock connection.
work\textsuperscript{27} solves this longstanding problem in radiative transport\textsuperscript{28,29}, and provides a highly accurate analytic expression for the spatially dependent reflectance near the point of entry, as well as a means to evaluate the backscatter signal from the signals measured by the spatial gating probe. This allows obtaining the same diagnostic parameter $\Delta$ from spatially gated data that we used with polarization gated data (see Methods).

Differentiating cystic lesions \textit{in vivo}. We performed clinical \textit{in vivo} measurements using spatial gating LSS during routine EUS-FNA procedures in 14 consecutively enrolled subjects with pancreatic cysts. Before the procedure, the spatial gating probe was inserted in the 22-gauge or 19-gauge endoscopic ultrasound aspiration needle (Expect, Boston Scientific) and secured with a fixed-length tube and probe latching mechanism to ensure that its distal end was completely inside the FNA needle. The subject was administered sedation and supplemental oxygen was used. The echoendoscope was introduced through the mouth and advanced to the duodenum (Fig. 3). After pancreatic EUS examination, the FNA needle was inserted into the echoendoscope, and the cyst was punctured under ultrasound guidance. The spatial gating probe was then extended 2 mm beyond the tip of the needle (Fig. 3a) with the probe latching mechanism and locked in that position by the locking button. By moving and angling the needle tip slightly, from 7 to 31 locations were measured (depending on the size of the cyst), covering a portion of the forward hemisphere of the internal cyst surface under EUS guidance (Fig. 3b and Supplementary Videos 1 and 2).

The total LSS measurement time was less than 2 minutes. The spatial gating probe was then removed, a 10 ml syringe was attached to the proximal end of the needle and the aspirated fluid was collected in the standard fashion. In 13 out of 14 procedures performed, the aspirated cyst fluid was found to be clear; however, in one case it appeared turbid on visual examination. In that case a separate 10 ml syringe with isotonic saline solution was used to replace the cyst contents\textsuperscript{30}, thereby expanding it back to the original size, and the data was retaken. After the procedure, the aspirated fluid was sent for cytological and biochemical analysis.

The \textit{in vivo} results are summarized in Table 2 and Fig. 4. The only available gold standard for pancreatic cyst lesion \textit{in vivo} malignancy diagnosis is either histopathology or survival with follow-ups, showing no indication of cancer development. Untreated cystic malignancy has a median survival of three months and a one-year survival rate of less than 10\textsuperscript{\%}\textsuperscript{31}. Thus, a one-year follow-up after LSS measurement would identify the vast majority of previously undetected malignancies due to the rapid progression of this disease. Within our 14 \textit{in vivo} patient set, two had definitive histopathology diagnoses, one was classified by our technology as cancer but misdiagnosed by cytology as negative for malignancy (and the patient has since died of metastatic cancer), one had a definitive adenocarcinoma cytology diagnosis (although cytology has poor sensitivity, it is very accurate when identifying cancer\textsuperscript{32}), and five have survived for more than a year with follow-ups showing no evidence of malignancy. We consider the diagnosis of these nine patients as reliable according to the above gold standard. Five remaining patients were only recently measured by our technique, and therefore do not have sufficient survival follow-up after the measurement. For those subjects, an independent assessment of the cysts by two expert gastroenterologists was obtained, who took into account the clinical history (four of the five cysts had a long history of cystic lesions with no malignancy progression), cytology results, CEA levels and imaging results on interval growth, but were blinded to the LSS findings. If the resulting diagnosis was in agreement, the consensus assessment was used as a secondary endpoint.

The diagnostic parameter $\Delta$ for the malignant category is significantly higher than that for the non-malignant category ($P < 0.05$). All cysts with definitive diagnosis were identified correctly by LSS for the presence of malignancy (differential diagnosis between cancer, HGD and cystic neuroendocrine tumor (CNET) lesions\textsuperscript{33} was not evaluated, as the therapeutic choice would be the same). The accuracy for all 14 patients with both definitive diagnosis and consensus assessment is 93% (95% CL: 69\%–99\%).

Discussion

Pancreatic cysts are now being discovered in large numbers of patients due to the increased use of high-resolution CT and MRI diagnostic imaging, with as many as 14\% of MRI scans and 3\% of CT scans incidentally discovering their presence\textsuperscript{34–36}. Because some pancreatic cysts are precancerous, and because pancreatic cancer is such a deadly condition, diagnosing the type of pancreatic cyst lesion accurately is a high-stakes challenge. Despite the recent improvements in CT and MRI methodologies, these imaging approaches are unable to distinguish cancerous, premalignant and benign cysts reliably, in part due to a lack of sensitivity to cellular structure and biochemical properties, with wide variations in the reported accuracy ranging from 20\% to 80\%\textsuperscript{37}. EUS is still the most sensitive technique currently available for the detection of small (<2–3 cm) pancreatic cysts\textsuperscript{38,39}, but its accuracy for distinguishing mucinous from non-mucinous cysts is only 51\%\textsuperscript{6}. Because imaging techniques have limited ability to identify the type of pancreatic cyst, there has been a major effort to identify the cyst type with cyst fluid obtained during EUS-FNA procedures.

Cyst fluid can be analysed for cytological findings, protein constituents, molecular markers, viscosity and DNA. The accuracy of...
cyst fluid analysis depends on the volume of cyst fluid obtained and therefore the size of the cyst. Recently, a large multicentre prospective clinical study evaluated both cytology and CEA for their ability to diagnose mucinous cystic lesions based on EUS-FNA in 341 patients. Pancreatic surgical resections of 112 of these patients found that cytology of cyst fluid has a sensitivity of 35% and a specificity of 83% for diagnosing mucinous versus non-mucinous cysts, and just 22% sensitivity for detecting mucinous cystic cancers. Apart from CEA, the diagnostic potential of other molecular markers (including amylase	extsuperscript{17}, cancer antigen (CA) 19-9	extsuperscript{18,19}, DNA	extsuperscript{20} and fluid viscosity	extsuperscript{21}) have been investigated, with CEA being the only marker that achieves enough accuracy to be of clinical utility. However, CEA addition provides only a slight improvement over cytology alone in distinguishing between benign and mucinous cysts. Due to the limited performance of existing cytological and molecular markers, there is a strong need to augment existing cyst fluid analysis approaches with an accurate diagnostic test.

Recently, mutations in genes such as GNAS (guanine nucleotide binding protein, alpha stimulating), and mutational profiles of targeted next-generation sequencing of cancer genes, have been suggested as an adjunct to cytology and CEA to improve the diagnosis of mucinous cysts and to identify early malignancy within lesions by analysing cyst fluid	extsuperscript{22-27}. These studies are quite promising in substantiating the feasibility of detecting DNA mutations in IPMN using cyst fluid, even when these molecules are at low concentrations—although the performance of these genetic markers needs to be further evaluated in prospective in vivo clinical studies.

In our in vivo and ex vivo pilot studies, native contrast LSS correctly identified the malignant potential of 21 out of 22 cystic lesions from 20 subjects in a double-blind comparison with either postoperative histopathology or survival outcomes achieving 95% accuracy (95% CI: 78%–99%) for identifying the presence of malignancy. This result is sufficiently powered to demonstrate a significant improvement over cytology ($P = 0.002$), which has an accuracy of 58% (95% CI: 50%–65%). The resulting sensitivity is 90% (95% CI: 60–98%) and specificity is 100% (95% CI: 76–100%). In the in vivo studies, the technique demonstrated the capability of obtaining data in part of the forward hemisphere of the internal cyst surface with a point probe and showed excellent agreement with the definitive diagnosis. To improve accuracy of the in vivo measurements, sampling of a larger fraction of the cyst wall could be beneficial.

We conclude that the LSS technique, which identifies malignant potential of pancreatic cystic lesions during regular EUS-FNA procedure, is rapid and inexpensive, offers great promise for distinguishing cancerous and precancerous cysts from benign cysts, and accurately identifies those pancreatic cysts that need surgical intervention. If this technique was to be used routinely, unnecessary pancreatectoduodenectomies for benign lesions may be avoided and malignant cysts that otherwise could be missed may be identified.

Table 2 | In vivo differentiation of cystic neoplasms in 14 subjects.

| Subject | MRI/CT | CEA (ng/ml) | Size (mm) | Cytology | Source of diagnosis | Diagnosis | LSS (Δ) | LSS diagnosis |
|---------|--------|-------------|-----------|----------|-------------------|----------|---------|-------------|
| 1       | IPMN   | 7.8         | 11        | LGD IPMN | CD                | LGD IPMN | 0.16    | LGD         |
| 2       | MCN    | 21          | 49        | Degenerated glandular debris | CD | Benign | 0.08    | Benign      |
| 3       | Serous | 370         | 27        | Acellular specimen | CD | Benign | 0.05    | Benign      |
| 4       | Pseudocyst | 7.3     | 51        | ACC or CNET | Histopathology | CNET | 0.43    | HGD/cancer  |
| 5       | IPMN   | 212         | 20        | Benign paucicellular sample | CD | Benign | 0.05    | Benign      |
| 6       | IPMN   | 3,676       | 22        | LGD IPMN | CD | Benign | 0.26    | HGD/cancer  |
| 7       | Serous | 226         | 32        | Negative for malignant cells | CD | Benign | 0.07    | Benign      |
| 8       | IPMN   | <1          | 37        | Insufficient cellular material | GCA | IPMN | 0.19    | LGD         |
| 9       | IPMN   | 9           | 20        | Virtually acellular specimen | GCA | IPMN | 0.25    | HGD/cancer  |
| 10      | -      | 7,290       | 57        | Adenocarcinoma | Cytology | Cancer | 0.56    | HGD/cancer  |
| 11      | IPMN   | -           | 50        | Negative for malignant cells | Histopathology | Pseudocyst | 0.09    | Benign      |
| 12      | Serous | -           | 29        | Serous cystadenoma | GCA | Benign | 0.03    | Benign      |
| 13      | Serous | -           | 28        | Insufficient material | GCA | Benign | 0.08    | Benign      |
| 14      | IPMN   | 2,364       | 21        | IPMN | GCA | LGD IPMN | 0.11    | LGD         |

Spatially gated LSS optical spectroscopic technique versus MRI/CT, CEA level, cyst size, cytology and the resulting diagnosis. The source of the resulting diagnosis is either histopathology, gastroenterologists’ consensus assessment (GCA), or conclusive diagnosis (CD), combining more than one-year follow-up with CEA. The two last columns present Δ parameter and LSS diagnosis. MRI includes both abdominal MRI and MRCP. ACC, acinar cell carcinoma; CNET, cystic neuroendocrine tumor. Dashes indicate no information due to lack of imaging classification or absence of data on CEA level. *Positive predictive value (PPV) of cytology when identifying cancer is 100%.

![Figure 4](https://example.com/figure4.png)
Methods
Spatial gating probe. The spatial gating probe is designed to obtain a shallow single-scattering signal by collecting light at very small source–detector separations. Measurements at these sub-diffusion spatial separations have shown to have a penetration depth of a few hundred micrometres. The 0.45 mm outer diameter spatial gating probe (Fig. 2) consists of seven 100 μm core diameter fibres with a numerical aperture (NA) of 0.21 (Fig. 2c). A fibre in the outer ring of the probe is selected as the delivery fibre and is connected to a dedicated SMA connector, while three groups of collection fibres are selected to provide source–detector separations of 120, 220 and 240 μm and are terminated in three SMA connectors coupled to individual spectrometers. All of the fibre trunks are connected to a metal ferrule. The probe jacket is made of a robust medical grade biocompatible polyimide.

The spatial gating fibre optic probe inserted in the EUS-FNA needle is shown in Fig. 2a. To precisely control the 2 mm extension of the probe tip beyond the bevelled needle tip (Fig. 2b), we designed and 3D-printed the probe latching mechanism (Supplementary Video 3). The mechanism can be toggled to extend (Fig. 2e) or retract (Fig. 2f) the probe tip from the needle and locked in those positions with the locking button. One of the sides of the probe latching mechanism has a Luer lock connection for attaching it to the probe ferrule (Fig. 2d). The other side is attached to the fixed length tube, which can be locked on the needle handle with a similar Luer lock (Fig. 2g). The probe is connected to the optical spectroscopy clinical system with the delivery fibre coupled to a 75 W Xenon arc lamp source (Apex, Newport) at the proximal end and the collection fibres are coupled to fibre optic spectrometers (AvSpec, Avantes).

Diagnostic algorithm. To obtain the diagnostic parameter $\Delta$ (Fig. 3c) collected with the spatial gating probe, we utilize the fact that the contribution of backscattering to the total spatially resolved reflectance decreases with the increase in source–detector separation $r$, significantly faster than that of the multiple scattering signal. Supplementary Fig. 1 shows the contribution of the single large-angle backscattering component and the diffuse reflectance component in epithelial tissue with a reduced scattering coefficient $\mu_s' = 3$ mm$^{-1}$ for the closest ($r = 120$ μm) and farthest ($r = 240$ μm) fibres in the spatial gating probe. From here it is clear that while total reflectance should be calculated as a sum of the diffuse reflectance and single large-angle backscattering for the closest fibre, it can be accurately approximated with the diffuse reflectance from the farthest one alone.

In the 600 nm to 800 nm wavelength range, tissue absorption can be ignored and the diffuse reflectance for the detector fibre $\text{i}$ can be written as

$$R_d^{\text{i}}(\lambda | \lambda_i) = \frac{1}{\pi r_i^2} \int_A dA \int R_i^{\text{f}}(\lambda | \text{r}_i - \text{r})$$  \hspace{1cm} (1)

where $R_d^{\text{i}}(\lambda | \lambda_i)$ is the well-known diffuse reflectance density.\(^4\) The integrals here are numerically calculated over the area of the source fibre $A$, with radius $r_i$ and collection fibres $A_i$ with radius $r_i$ $(i = 1, 2)$. Therefore, using spectral measurements $S_i(\lambda)$ and $S_d(\lambda)$ by collection fibres 1 and 2, respectively, we get the following system of equations:

$$R_d^{\text{i}}(\lambda | \lambda_i) + R_d^{\text{i}}(\lambda | \lambda_i) = S_d(\lambda)$$  \hspace{1cm} (2a)

$$R_d^{\text{i}}(\lambda | \lambda_i) = S_d(\lambda)$$  \hspace{1cm} (2b)

where $R_d^{\text{i}}(\lambda | \lambda_i)$ is the single large-angle backscattering component (Fig. 3d). This component carries diagnostic information and has been previously evaluated from the polarization gated data.\(^5\)

We used phantom experiments to isolate $R_d^{\text{i}}(\lambda | \lambda_i)$ by removing the multiple scattering contribution in the system of equations (2a,b). This contribution, in the case of weak absorption, has the same spectral dependence for both fibres. This can be understood by considering that multiple scattering is primarily governed by $\mu_s(\lambda)$ dependent near the point of entry. Therefore, using phantoms, we can calibrate the multiple scattering component in both fibres to make sure it can be cancelled. Phantoms with scattering coefficients close to that of tissue from 0.5 μm and 0.99 μm diameter polystyrene beads (Polysciences) in agarose gel (Sigma) were measured. These phantoms had the same $\mu_s(\lambda)$ but different phase functions, and produced a nearly identical calibration coefficient for balancing the multiple scattering component in the two fibres.

Human subjects. The feasibility of LSS in identifying precursor pancreatic cystic lesions and early-stage pancreatic cancers was tested in vivo in freshly resected pancreatic samples of 11 human subjects who underwent surgery for high-risk pancreatic cysts and then in vivo during standard EUS-FNA procedures in another 14 subjects who were undergoing initial EUS evaluation for pancreatic cysts. Both protocols were reviewed by the Institutional Review Board of Beth Israel Deaconess Medical Center and the requisite approvals were obtained.

In the in vivo study, consecutive patients undergoing EUS-FNA procedures for known pancreatic cystic lesions were enrolled. The inclusion criteria were as follows: (i) males and females older than 21 years with pancreatic cyst(s); (ii) referred for EUS-FNA procedure; (iii) willing and able to provide written informed consent. We explained the procedure, indications, preparation and potential complications to the subjects, who indicated their understanding and signed the corresponding consent forms.

We reviewed medical records of the patients within our study for the purpose of comparing the accuracy of the developed technique with the standard of care. The medical records, reviewed retrospectively after LSS diagnosis, included reports from MRI and CT imaging, cytology, histopathology and cyst fluid biochemistry.

Statistical analyses. Significance between two groups of pancreatic cysts with and without malignant potential for in vivo and ex vivo data sets was determined by a twailed Wilcoxon rank sum test (IBM SPSS Statistics 23). Data were inferred as statistically significant if $p < 0.05$. Confidence intervals were calculated according to the Wilson score method.\(^11\) The chi-square test was used for comparing diagnostic accuracy with cytology. No statistical test was used to predetermined the sample size. The investigators were double-blinded during the measurements and outcome assessment.

Code availability. The diagnostic algorithm is described in detail in the Methods. We have opted not to make the data acquisition and processing code available because the code is proprietary and used for other projects.

Data availability. The data that support the findings of this study are available in figshare with the identifier http://dx.doi.org/10.6084/m9.figshare.4496039 (ref. 29). The authors declare that all other data supporting the findings of this study are available within the paper and its Supplementary Information.

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Author contributions
L.Q., D.K.P. and L.T.P. conceived the method and initiated the project; L.Q. and L.T.P. wrote the manuscript; L.T.P. wrote the manuscript. L.Z., E.U.Y., V.T., J.D.G., F.W., L.Q. and L.T.P. conceived the method and initiated the project; L.Q. and L.T.P. wrote the manuscript.

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Correspondence and requests for materials should be addressed to L.Q. and L.T.P.

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