The successful diagnosis and treatment of cancerous growths requires as timely and non-invasive a procedure as possible. Epithelial tumors are often more readily accessible as they are on or adjacent to the lumen wall of the GI and vocal tract. The endomicroscopy of these cells' lesions can provide both the localization and the type of cancer, if any. The more conventional technique for this is endoscopic ultrasound fine needle aspiration (EUS-FNA). With the ultrasound probe and cytology sampling from this apparatus, this technique can perform relatively non-invasive sampling with fewer sampling passes. With the ancillary additions to the EUS-FNA device, improvements are consistently being done: better needles, more sensitive probes, improved cytology samplers, enhanced endomicroscopy techniques, and introduced accessory microscopic techniques. Despite these current and ongoing improvements EUS-FNA is not a stand-alone "gold standard" cancer diagnosis technique. Such problems as false negative frequencies, difficulty of the technique, and difficulty of lesion identification make EUS-FNA a complementary technique to increase the sensitivity of cancer diagnosis only [10, 15].

Ultrasound, audio frequencies above human hearing, can be used at levels up to around 30 MHz for diagnostic medical imaging. The physical properties of the media that the sound pulses traverse give signature reflection and absorbance picked up by the transducer/receiver. Tissue media have "acoustic impedances" that are related to the density of the tissue. Often deviations from the echoic consistencies of tissue cells (hypoechoic, hyperechoic) give suggestion of lesions [32]. These lesions can be inflammations, injuries, pathogenic infections, or artefacts in addition to cancerous cells [41, 50]. In the case of cancer, to be able to diagnose such a finding in a noninvasive way is critical for successful treatment. For this reason, the technique of endoscopic ultrasound fine needle aspiration
(EUS-FNA) holds much potential; Figure 1 shows direct intracystic biopsy and pancreatic cystoscopy through a modified 19-gauge (G) endoscopic ultrasound (EUS) needle [1]. This is recorded and depicted in Video 1: Pancreatic Cyst Endomicroscopy.

![Figure 1: EUS-FNA of a pancreatic cyst using a forceps-equipped, 19-G EchoTip Ultra Needle. A sonograph of the pancreatic head cyst with needle visible (A). Visualization inside of the cyst with the Spyglass fiberoptic probe through the needle (B).](image)

EUS is a technique officially recognized in the early 1980's. With high frequency ultrasound probes coupled with Doppler imaging, EUS is useful in imaging epithelial cells' vascular structures and hemodynamics: pancreas, gastrointestinal tract, kidney, liver, spleen, stomach, lymph nodes, lings, and adrenal glands [13]. The pancreas is a very convenient target as it can provide the most cell sample with the least number of passes [10, 22]. As Rambam Healthcare Campus statistical records Chart 1 shows, the pancreas is a frequent target for EUS-FNA applications [5]. This is very important for diagnosing pancreatic cancer, the 4th leading death-causing cancer [10, 16]. The attachment of a biopsy needle to the ultrasound transducer enables EUS-FNA apparatuses as shown in Figure 2 [46].
The initial identification of anomalous tissue is done by the abnormal echoing picked up by the receiving probe. Upon this identification, sampling of the lesion is then done to further identify it. The quality of the image is essential in order to identify the area to be sampled. Figure 3 shows a strong demarcation in a pancreatic lesion from EUS which suggests a pancreatic neuroendocrine tumor (PNT). A comparison of

**Figure 2:** Some early models of EUS-FNA apparatuses (A, B, C). A conventional EUS-FNA device (D) with a grided, 19-G nitinol needle for greater flexibility (E).

**Figure 3:** Pancreatic lesion.
EUS-FNA to EUS in the diagnosing of this pancreatic lesion, shown in Figure 4, shows some discrepancy of the sonogram results [2]. As the pancreas is one of the most easy and efficient sampling regions for EUS-FNA, cytologic assays providing false negative results are very possible. The limitation of EUS-FNA becomes apparent with isoechoic cell lesions or lack of clear demarcations of the area of diagnostic interest. Sonographic images of pancreas [2, 3, 14, 17].

If the apparatus gives positive abnormal sonographic imaging and samples of tissue cells can be collected, additional tests are done to identify the lesion: histochemistry, immunostaining, and biomarkers. This is essential to differentiate such conditions as pancreatic cancer from chronic pancreatitis. Most common histochemistry staining techniques include Figure 5a: H&E (hematoxylin and eosin) for staining of the nuclei from the sample [2]. Another common histochemical stain for cancerous cell identification is Papanicolaou-staining as shown in Figure 5b [40, 53]. Immunostaining may also be employed for diagnosis: Synaptophysin and insulin immunostaining are often employed for the pancreas shown in Figure 6 and Figure 7, respectively [3]. The Ki-67 index is an antibody titer technique that indirectly suggests cancerous growth. The active proliferation of cells by the cell division cycle (CDC) produce high amounts of the Ki-67 and immunohistochemical staining provides this quantification. This is a technique showing potential for giving the cancer grade of PNT
Figure 5: Pancreatic cell staining. The area of interest is a hypoechoic nodule found by EUS-FNA (A). H&E staining with x400 magnification shows an intrapancreatic splenosis nodule (B). Branching papillary fragments shown from Papanicolaou-staining with x400 magnification (C). Strong synaptophysin immunostaining in pancreatic tumor cells at x200 magnification (D). Strong and diffuse insulin immunostaining in pancreatic tumor cells with x200 magnification (E).
In addition to cell staining, biomarker assays may also be employed. This often relies on genomic mutation detection of oncogenes and/or tumor suppressor genes. One important type of tumor suppressor are the mismatch excision repair (MMR) genes. As MMR genes are essential in maintaining the genomic fidelity of replicating cells, their levels of expression can indicate cancerous cells. This is done by detecting lesions and sampling the tissue with EUS-FNA. The sample is then utilized for quantitative reverse transcription polymerase chain reaction (qRT-PCR).

Signature levels of various MMR genes can be used to confirm or reject a cancer diagnosis [16]. Apart from the tumor-suppressor biomarkers, oncogene biomarkers can be used to support or reject the possibility of pancreatic cancer. Mutations in the K-Ras oncogene are very common in pancreatic cancer such as pancreatic ductal adenocarcinoma (PDAC). The K-Ras gene can be sequenced from the cDNA of the RTPCR on the total RNA (tRNA) of the sample taken. Cycleave PCR compliments this procedure to find K-Ras mismatches in as few as 5% of tumor cells among normal cells [16, 33]. One advantage to using biomarkers over cytological staining is the less amount of sample, therefore the less invasive.

Figure 6: Ki-67 index in PNT where numbers indicate the Ki-67 subsection indices. A digital slide from a resected specimen showing a Grade 2 tumor (a). A digital slide from an EUS-FNA showing a Grade 2 tumor (b).
Enhancements to EUS-FNA are essential in making this technique more efficient. The ongoing development of advanced techniques and equipment bring promise for this outcome. Advanced equipment includes higher quality needles, needle ancillary components, and advanced endoscopes. The needles attached to the EUS-FNA apparatus are identified by their gauge (G) or needle size. For access to hard-to-reach regions, both durability and flexibility are a must [20]. For this reason, better materials must be used to fabricate the EUS-FNA needles [10].

A high quality needle is further advanced by the ancillary additions to it. Such designs as the EchoBrush, a through-the-needle cytologic brush system, for advanced tissue sampling have been done (Future12(1)). This EchoBrush shows its advantage in the sampling of pancreatic tissue but it can be used to collect solid and cystic lesions [6]. Other enhancements include new designs to improve upon this tissue sampling as the needle forceps in Figure 7. H&E staining provides support to the reliability of this new design, and a cystic wall biopsy is shown in Video 2: Cystic Biopsy [1].

In addition to EUS-FNA apparatus material and design improvements, the inclusion of advanced endomicroscopic techniques is crucial. Figure 8 shows a needle confocal laser endomicroscopy (nCLE) EUS-FNA apparatus [8, 26]. Confocal laser endomicroscopy (CLE) is based on tissue illumination of potential molecular markers for cancer with a low-power laser. Subsequent detection
of the fluorescent light reflected on cellular samples is obtained by EUS-FNA that could be from the tissue. Confocal imaging can be based on tissue reflectance or tissue fluorescence [23]. CLE based on fluorescence uses local and/or intravenous contrast agents. A confocal mini probe is passed through the biopsy channel of the endoscope. Figure 9 shows a comparison of normal cells to carcinoma cells using the nCLE technique [26].
As Video 3: nCLE Application shows, EUS examination demonstrates a cystic lesion in the pancreas head. The lesion has septations and a mural nodule. A 19-gauge EUS-FNA needle is preloaded with the needle-based confocal laser endomicroscopy probe. From Video 4: Cystic Wall Endoscopy, confocal laser endomicroscopic imaging is performed within the cyst via the needle with endosonographic guidance. Imaging of the cyst wall reveals dense and dark cells, consistent with pancreatic acinar cells and villous structures. This is consistent with all of the papillary projections in an intraductal papillary mucinous neoplasm or in other words, a pancreatic tumor [26].

Another improvement for EUS-FNA cancer diagnosis is the availability of software for analysis of lesion characteristics such as elastography. This can be transferred to graphical software as Image J to observe the relative levels of elasticity. This allows for the hardness of a lesion to be determined, giving the lesion's physiological characteristics and enabling kappa values from the coordination of multiple specialists' analyses, see Figure 10. This is important for distinguishing between cancer and
non-cancer as Video 5: Chronic Pancreatitis provides endoscopic ultrasound elastography of a patient with chronic pancreatitis, and Video 6: Adenocarcinoma provides endoscopic ultrasound elastography of a patient with adenocarcinoma at the pancreas head [42].

Immediate clinical treatment advances with EUS-FNA are also being developed. The forward view (FV)-EUS is essentially a real-time EUS-FNA procedural technique with much potential for immediate lesion treatment. Argon Laser Coagulation (ALC) is used in complementation to limit internal hemorrhaging of the patient. This advancement is making immediate surgical treatment with EUS-FNA more of a feasibility for treatment of multiple pathogenic conditions.

Figure 10: Using Image J software on pancreatic lesions. Patient with chronic pancreatitis and an average hue histogram of 162.81 (a). Patient with pancreatic cancer and an average hue histogram of 219.87 (b).
Despite all of these enhancements, clinical statistics of the successful diagnosis of patients’ diseases with EUS-FNA vary considerably. The technique success is judged by the sensitivity and the specificity of EUS-FNA. Some clinical summaries with only EUS-FNA used have sensitivity and specificity as high as 80% and 90%, respectively. The sensitivity of only using EUS-FNA can be lower than 50%. The reason for these deviations is due to lesion type and location. There is a difference between a cystic and a solid mass lesion just as there is a difference between accessibility of a pancreatic lesion of the pancreas head and the pancreas tail. Despite the varying statistics of the EUS-FNA sensitivity and specificity, one admonition of EUS-FNA is recognized by proponents and critics alike: these values increase significantly when used with other complementary diagnostic techniques. EUS with complementary techniques holds promise as shown in Figure 11 [31], but EUS-FNA can provide a more thorough analysis. Figure 12 shows a cytology and histology complimenting EUS-FNA to give rates of 100% (Pitfalls 59, Future (8-13). [1, 3, 6, 8, 16, 30, 46].

Figure 11: Pancreatic cyst found by EUS (a), computerized tomography (CT) (b), and magnetic resonance imaging (MRI) (c).
The knowledge and training of the practitioners in EUS-FNA analysis (sonographers, cytologists, spectroscopists, graphics specialists) cannot be compromised. Depending on the lesion type and location, the most advanced EUS-FNA device may not be the appropriate choice for diagnosis. Consider something as simple as the needle gauge: in a study, EUS-FNA was performed for pancreatic mass lesions (>30) using both 25-G and 22-G needles. The differences in accuracy rate, needle visibility, ease of puncture, and quantity of obtained specimen were evaluated. The 22-G had slightly greater accuracy (sensitivity) over the 25-G when sampling. The 22-G also had higher quantity of specimen obtained outside of the pancreas. The visibility of both needles were similar. The 25-G needle, however, was far easier to use for puncturing and had higher quantity of pancreatic specimen

Figure 12: Through diagnosis of a hypoechoic pancreatic mass using EUS (A), EUS-FNA (B), CT (C), and H&E stain (D) the mass is diagnosed as a tumor.
(specificity) obtained over the 22-G needle. On the whole, the 25-G needle seems more appropriate for hard lesions and pancreatic masses [20]. Therefore, the effective application of EUS-FNA is greatly dependent on the practitioner's skill and judgment of the appropriate EUS-FNA device to employ in addition to the enhancement of the EUS-FNA apparatus, itself [15].

In conclusion, EUS-FNA is very unlikely to be a stand-alone "gold standard". The success of EUS-FNA depends on the improvement of the apparatus, advancement of software, condition of the patient, training of specialists, and the coordination among these specialists [47]. Even with all of these factors met, it has never enabled 100% sensitivity and specificity [15]. Only with complementary analyses techniques has the diagnosis of pancreatic cancer been without question [10]. Nevertheless, the technological advancements of hardware and software do make of EUS-FNA as a stand-alone "gold standard" a slim possibility.

Video References

Video 1: Pancreatic Cyst Endomicroscopy- http://www.youtube.com/watch?v=RBMKSHiExt8
Video 2: Cystic Biopsy- http://www.youtube.com/watch?v=nIZijIDdgoU
Video 3: nCLE Application- http://www.youtube.com/watch?v=fcAM6A4hgNs
Video 4: Cystic Wall Endoscopy- http://www.youtube.com/watch?v=3SezDS0JhXY
Video 5: Chronic Pancreatitis- http://www.youtube.com/watch?v=SgHzDQVRYrk
Video 6: Adenocarcinoma- http://www.youtube.com/watch?v=h-AwHGIIxxo

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