Reply to Pouw et al.

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We have read with great interest the comments by Pouw and Bergman regarding our recent case report on three-dimensional optical coherence tomography (3D-OCT) imaging of the normal esophagus, Barrett's esophagus, and the post-radiofrequency ablation (RFA) gastroesophageal junction (GEJ) region [1]. While we appreciate many of the points raised by the readers, we disagree with their assessment of our pilot study as biased, irrelevant, and misleading. Furthermore, we believe that it would be irresponsible to summarily disregard 3D-OCT for studying ablative therapies due to the many advantages provided by this rapidly developing visualization technique.

The readers' criticisms focus on three main issues. First, there is concern that the structures labeled as "? Barrett's esophagus" in our publication are not buried Barrett's esophagus glands, but are actually undermined gastric mucosal glands. Second, there is a lack of histological correlation between the post-RFA 3D-OCT findings of buried glands and our excisional biopsy samples from the same region. Third, the readers question the fundamental need for rigorous imaging studies of post-RFA patients, and suggest that conventional imaging and biopsy techniques are sufficient for detecting residual Barrett's esophagus following RFA.

First, we will address the concern that the scattered subsquamous glands observed in our post-RFA case are not actually buried Barrett's glands. Based on our group's decade-long experience with OCT imaging of the human gastrointestinal tract [2–5], including histology correlation studies, we can assert that these structures are consistent with Barrett's esophagus and are not consistent with any normal glands found near the GEJ or in the esophagus. As additional support, **Fig. 1** provides OCT images and corresponding histology from an ex vivo endoscopic mucosal resection (EMR) nodule and an in vivo region near the GEJ. Normal esophageal glands in a well circumscribed lobular form are large (1 mm) with a heterogeneous moderately scattering center. Barrett's glands are irregular, smaller (100–300 µm), have sharp borders with OCT, and are devoid of signal in their centers. Gastric glands are found underneath gastric pits and may have slightly more diffuse borders with OCT.

The readers suggest in their commentary that "there is a mean overlap of 4 mm of squamous and gastric mucosa at the GEJ," and that therefore "the structures detected on 3D-OCT may actually be undermined gastric mucosal glands." In our opinion, this is highly unlikely. Undermined gastric mucosa was not detected with our biopsy samples. The 3D-OCT images in our original Fig. 6 also did not show gastric pits underneath squamous tissue. Finally, our original Fig. 6 showed scattered buried glands extending at least 12 mm proximal of the GEJ, far beyond the expected squamous-gastric overlap region. In fact, one advantage of 3D-OCT compared with prior 2D-OCT is the ability to precisely register individual cross-sectional images to anatomic landmarks such as the GEJ or proximal border of the gastric folds. This can be appreciated in our original Fig. 6 as a clear division between a distal region containing gastric pits and a proximal region containing squamous tissue. The only glandular structures with the same size, sharp boundaries, and signal-devoid centers as those observed in our post-RFA case are Barrett's glands.

Further evidence that the structures observed in the post-RFA case are buried Barrett's glands can be found in a recent independent publication [6]. The authors conducted ex vivo two-dimensional OCT and co-registered histology analysis of esophagectomy specimens from 14 patients undergoing resection due to high-grade dysplasia or adenocarcinoma. The histologically confirmed buried Barrett's glands shown in their publication correlate very well with our findings. In total, 10/14 specimens showed buried Barrett's glands detected with OCT. Although buried Barrett's esophagus is known to be more common in patients with high-grade dysplasia and adenocarcinoma, none of the patients had previously shown buried Barrett's esophagus under endoscopic biopsy alone. This additionally suggests that current biopsy protocols may be ill-suited for detecting buried Barrett's glands.

Next, we will address the lack of histological correlation between our post-RFA 3D-OCT findings and our excisional biopsy samples. The readers point out that "no endoscopic resection was performed to correlate the 3D-OCT findings with histopathology." We agree that performing EMR on the post-RFA patient following 3D-OCT imaging would have been ideal in terms of generating corresponding histology data. Unfortunately our experimental protocol precluded us from altering the standard of care, and there was no compelling reason to conduct EMR based on the video endoscopy findings or the patient's clinical presentation. We were therefore limited to obtaining jumbo biopsy specimens from the region imaged with 3D-OCT.

As we stated in the case report, the biopsy specimens from the post-RFA case did not show buried Barrett's glands. However, given the sparse distribution of buried glands detected under 3D-OCT, this lack of correlation is not surprising. The diameter of a typical adult esophagus is 25 mm, giving a circumference of 79 mm. An "aggressive post-ablation biopsy regimen," as recommended by the readers, would obtain four biopsy samples for every 10 mm of esophageal length. Only 1–2 biopsy samples would therefore fall within a region of the esophagus 20 mm in circumference and 10 mm in length, which is the same size as the 200 mm^2 area analyzed by our 3D-OCT system. As the glandular density in our post-RFA case is approximately one buried gland per 20 mm^2, and as each jumbo biopsy covers only 4 mm^2, an aggressive biopsy regimen is not expected to reliably detect these sparsely-distributed buried glands. These findings underscore the relatively poor sampling density of current biopsy protocols, and highlight the benefits of 3D-OCT as a method for obtaining comprehensive, volumetric image data over a continuous surface area 50 times larger than that of a jumbo biopsy specimen.

Finally, we will address the readers' belief that there is no need for rigorous imaging studies in post-ablation patients. The readers state that "we do not have evidence to support the premise that occult buried glands are a common occurrence after ablation" based on prior biopsy-based studies. They also state that there is no "clinical need for such second-tier detection", and believe that "it is more efficient and more effective to use high-quality endoscopes with narrow-band imaging or comparable techniques along with biopsy to detect residual or recurrent Barrett's mucosa during post-ablative follow-up." As discussed above, pilot data from our study and from Cobb et al. [6] suggest that aggressive biopsy protocols may not reliably detect sparsely distributed buried Barrett's glands due to sam-

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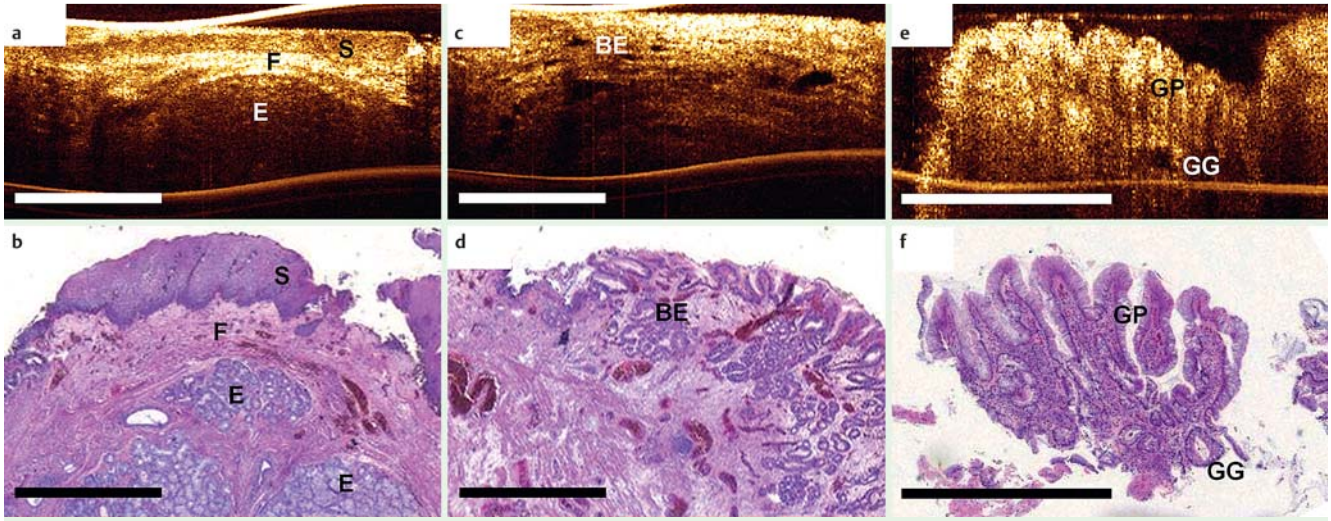

Fig. 1 Optical coherence tomography (OCT) images and corresponding histology of esophageal, Barrett's, and gastric glands obtained from an ex vivo esophageal endoscopic mucosal resection nodule (**a – d**) and an in vivo image near the gastroesophageal junction (GEJ) (**e, f**). **a, b** OCT and corresponding histology demonstrate representative esophageal glands (E) with a diameter of about 1 mm underneath squamous epithelium (S) and fibrous tissue (F). **c, d** Barrett's esophagus glands (BE) were also observed from the same nodule. Barrett's esophagus glands had smaller diameters (~ 100 – 300 µm) and were closer to the mucosal surface compared with the esophageal gland. **e, f** A gastric gland (GG) was observed underneath hyperplastic gastric pits (GP). Gastric glands are much smaller than normal esophageal glands and have a more diffuse border than Barrett's esophagus glands. Scale bars, 1 mm.

pling limitations. Larger studies with much larger patient numbers are required to fully understand this issue, but it would be irresponsible to disregard the current body of OCT data and to rely solely on biopsy as a method to detect residual or recurrent Barrett's esophagus following ablation. As narrow-band imaging (NBI) does not provide any depth-resolved information and is incapable of visualizing 3D glandular structures beneath neosquamous epithelium, we do not see how it could possibly provide any benefit in detecting residual or recurrent buried Barrett's esophagus during post-ablative follow-up. We are unsure what the readers mean by "comparable techniques" to NBI, since other methods such as endoscopic confocal microscopy also suffer from a lack of depth resolution and small imaging area compared with 3D-OCT.

In conclusion, we agree that our limited sample size prevents us from drawing statistically significant conclusions based on a single post-RFA case. This was certainly not the intention of our case report. However, we disagree with readers' characterization of our study as irrelevant and misleading. We have clearly shown that 3D-OCT detects buried post-RFA glandular structures that are consistent with Barrett's esophagus, based on morphological features previously established in published OCT/histology correlation studies and further supported by a recent publication from an independent group. We fully acknowledged the lack of histological correlation between our post-RFA biopsy samples and the 3D-OCT images, although we presented a reasonable explanation for this based on the sparse distribution of the buried glands and the limited sampling density of the biopsy protocol. These results, at the very least, provide motivation for future studies of post-RFA cases using 3D-OCT to assess healing or to guide biopsy. We stress that the clinical relevance of sparse buried glands following RFA is currently unknown, and that we share the readers' enthusiasm for the ablation device manufactured by their research sponsors at BARRX Medical. However, we do believe that 3D-OCT can become an invaluable tool for studying ablative therapies, as it may significantly reduce the sampling error associated with unguided biopsy and, as shown here, has the unique potential to locate buried glands that may otherwise go undetected.

Competing interests: Yes J.G. Fujimoto receives royalties for intellectual property licensed to LightLab Imaging. J.M. Schmitt is an employee of LightLab Imaging. D.C. Adler had no competing interests at the time of the study, but is now an employee of LightLab Imaging.

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