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Detecting the Near Infrared Autofluorescence of the Human Parathyroid: Hype or Opportunity?

CC Solórzano[1,*], **G Thomas**[2,*], **N Baregamian**[1], **A Mahadevan-Jansen**[2]

[1]Division of Surgical Oncology & Endocrine Surgery, Department of Surgery, Vanderbilt University Medical Center, Nashville, TN 37232 USA

[2]Vanderbilt Biophotonics Center, Department of Biomedical Engineering, Vanderbilt University, Nashville, TN 37232 USA

Abstract

Objective: With the recent approval of two near infrared autofluorescence (NIRAF)-based devices for label-free identification of parathyroid glands (PG) by the Food and Drug Administration, it becomes crucial to educate the surgical community on the realistic scope of this emerging technology. Here, we have compiled a review of studies that utilize NIRAF and present a critical appraisal of this technique for intraoperative PG detection.

Background: Failure to visualize PGs could lead to accidental damage/excision of healthy PGs or inability to localize diseased PGs, resulting in postsurgical complications. The discovery that PGs have NIRAF led to new avenues for intraoperatively identifying PGs with high accuracy in real-time.

Methods: Using the following key terms: ‘parathyroid, near infrared, autofluorescence’ in various search engines such as PubMed and Google Scholar, we identified various publications relevant to this review of NIRAF as a technique for PG identification. Articles were excluded if they focused solely on contrast agents, served as commentaries/overviews on NIRAF or were not written in English.

Results: To date, studies have investigated the potential of NIRAF detection for (i) identifying PG tissues intraoperatively, (ii) locating PGs prior to or after dissection, (iii) distinguishing healthy from diseased PGs and (iv) minimizing post-operative hypocalcemia after total thyroidectomy.

Conclusions: Since NIRAF-based identification of PG is non-invasive and label-free, the popularity of this approach has considerably surged. As the present limitations of various technologies capable of NIRAF detection are identified, we anticipate that newer device iterations will continue to be developed enhancing the current merits of these modalities to aid surgeons

Corresponding Author: Carmen C. Solórzano, MD, Professor of Surgery, Chair, Department of Surgery, Director, Endocrine Surgery, Vanderbilt University Medical Center, 2220 Pierce Avenue, 597 Preston Research Building, Nashville, TN 37232 USA, Phone: (615) 322-2391, FAX:(615) 936-6535, carmen.solorzano@vumc.org.

*Solórzano CC and Thomas G contributed equally to this work

Disclosures

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in identifying and preserving PGs. However, more concrete and long-term outcome studies with these modalities are essential to determine the impact of this technique on patient outcome and actual cost-benefits.

Keywords

Parathyroid; autofluorescence; hypoparathyroidism; near infrared; imaging; fiber probe

Background

Identification and preservation of the parathyroid glands (PGs) is a critical and challenging step during neck surgeries. Permanent hypoparathyroidism is a devastating sequela of such procedures and can result from the surgeon's inability to identify the PGs, preserve their blood supply and/or avoid accidental removal or damage.[1, 2] Incidental parathyroid removal occurs in 5% to 22% of thyroid surgeries, causing transient hypocalcemia in about 13% of these cases[3-5]. These side effects could further lead to permanent hypocalcemia, observed in 2 to 5% of adults[3, 6] to as high as 7% in children.[7] Consequently, patients that develop permanent hypoparathyroidism following total thyroidectomy may have associated increased mortality rates.[6] In contrast, during parathyroidectomies, failure to identify and remove diseased PGs occurs in up to 10% of cases leading to persistent hyperparathyroidism, which frequently requires repeat excisional procedures that may be associated with greater complication rates and costs.[8-10]

Surgeons that perform endocrine neck procedures typically rely on visually identifying PGs, which can be subjective and inaccurate, particularly when the surgeon lacks experience. Intraoperative PG tissue confirmation is conventionally performed by frozen section analysis and in parathyroidectomies also through intraoperative parathyroid hormone assays.[11] Both of these tests are invasive, often require repeat sampling and add to the overall time of surgery, while biopsies can additionally injure a healthy PG. Therefore, a noninvasive, rapid and highly accurate intraoperative tool could be practice changing for the surgeon in identifying PGs in real time. Moreover, such a tool has the potential to avoid permanent postsurgical hypoparathyroidism.

Discovery of near infrared autofluorescence (NIRAF) in PGs

Various light-based technologies have been developed for discriminating between tissue types in a non-invasive and rapid manner for oncological and non-oncological applications.[12-14] However, the application of such techniques for anatomical detection is novel. Previous studies have evaluated the scope of optical imaging with exogenously administered contrast agents such as indocyanine green (ICG), methylene blue and other labels for localizing PGs. However, these agents may be associated with toxicity, non-specific localization of the dye and photobleaching.[15-19] On the other hand, label-free optical modalities can rely on intrinsic tissue properties and circumvent the limitations associated with contrast agents. Techniques like optical coherence tomography (OCT), confocal reflectance imaging and Raman spectroscopy among many others, have been applied for PG identification with variable degrees of success[20-22], but have not gained

wider acceptance with surgeons, as translating these modalities for intraoperative PG localization can be challenging due to technical complexities.

Circa 2008, researchers at Vanderbilt University discovered that PGs exhibit strong autofluorescence under near-infrared (NIR) light.[23] Autofluorescence in simple terms can be described as the process by which biological molecules/tissues can be excited using light at certain wavelengths; these molecules return to its inherent state by emitting light at longer wavelengths without requiring additional dyes/contrast. This early report found that both healthy and diseased PGs consistently exhibit 'bright' near infrared autofluorescence (NIRAF) compared to surrounding soft tissues in the neck.[24] Since current preoperative and intraoperative modalities can only localize hyperfunctioning PGs and not the healthy ones, presence of elevated NIRAF in both healthy and diseased PGs make this finding even more remarkable. Following this unique discovery and successful demonstration of this approach *in vivo* in a clinical setting[25, 26], the field of label-free NIRAF intraoperative PG identification exploded with various laboratory-based prototypes and commercially available devices being evaluated for the identification of PGs (Figure 1), during thyroid and parathyroid surgeries.

While several studies have now reported that PGs emit NIRAF with a peak emission wavelength at ~820 nm, the underlying origin of NIRAF in PGs remains unknown. To date, no biomolecules have been known to possess intrinsic fluorescence beyond 700 nm. Physico-chemical characterization of PG tissues have so far concluded that the PG NIRAF is resilient to extreme temperatures and proteinase activity.[27, 28] Other studies unanimously observed that NIRAF levels were markedly lower in PGs in patients with secondary hyperparathyroidism (SHPT) from renal-failure and that PGs with predominantly oxyphil cell distribution had higher NIRAF.[26, 29] Several candidates including calcium-sensing receptors that are highly localized to parathyroid and thyroid tissues have been proposed as potential candidates[24, 29]. However, more research is needed to definitively uncover the fluorophore responsible for the observed NIRAF in PGs.

Types of modalities that use NIRAF detection for identifying PGs

Probe-based modalities: When Paras *et al.* first reported about utilizing NIRAF for identifying PGs in real-time, they utilized a portable fluorescence spectroscopy system, which consists of a NIR laser source for excitation, a spectrometer for signal collection, a laptop for data processing and a fiber optic probe to deliver and collect the light from the sample (Figure 2a)[24]. In early iterations of this approach, measurements were performed in patients during surgery with operating room (OR) lights turned off. Each measurement provided a plot of fluorescence intensity as a function of emission wavelength (spectrum), where the peak intensity was found to be stronger in PGs compared to thyroid glands (Figure 2b). These early studies predominantly relied on NIRAF spectroscopy for PG detection.[24-26, 30] This 'lab-based' system was subsequently developed into a user-friendly clinical device called PTeye (by AiBiomed, USA) that provides visual, quantitative and auditory feedback when the handheld fiber probe touches a PG, akin to a nerve-monitoring device.[31, 32]. The aforementioned devices that rely on a fiber optic probe for NIRAF detection will hereafter be referred to as 'probe-based' modalities in this review.

Imaging-based modalities: NIRAF detection based on the 'imaging' approach typically uses a NIR light source in conjunction with a camera and appropriate filters to visualize a tissue of interest on a display monitor (Figure 2c, 2d). With the camera held at a defined distance from the surgical field, this approach does not require tissue contact and can help a surgeon spatially localize PGs within the surgical field as demonstrated in 2014 (Figure 2e – 2g).[30] NIRAF imaging also requires that the OR lights be turned off during use. Easy access to commercial NIR imaging systems developed for ICG fluorescence (FDA-approved for other applications – tissue perfusion and transfer circulation in free-flaps, plastic and reconstructive surgery) such as Fluobeam-800 (Fluoptics, France), PDE Neo II (Hamamatsu, Japan) and Karl Storz cameras (Karl Storz, Germany) further popularized this method of PG identification.[33-35]

FDA approval: Within a decade following the discovery of the NIRAF in PGs, the FDA granted approval to two of the aforementioned devices - the PTeye (probe-based) and Fluobeam-800 (imaging-based) – for label-free intraoperative PG identification.[36, 37] Despite approval, many questions and misconceptions remain about the capability of NIRAF and its application for thyroidectomy and parathyroidectomy. We therefore present a review of the published literature prior to and after FDA approval of these two NIRAF detection devices. We will also discuss how surgeons might be able to use these tools in their practice.

What can the current label-free technology that relies on NIRAF do for surgeons during neck endocrine procedures?

The expression "conserve each PG as if it's the last one" continues to hold true today. When surgeons step into the operating room to perform neck procedures, they are cognizant that every PG will be at risk for injury. The most common neck endocrine surgical procedures performed today are thyroidectomy and parathyroidectomy. Localization, identification and preservation of the PG during such procedures remains highly challenging. Furthermore, since PG blood supply could be variable in every patient, the surgeon may lack specific preoperative knowledge to predict anatomy of PG vasculature. So, how can NIRAF help surgeons improve on the performance and outcomes of these procedures?

A number of techniques have been described for intraoperative localization of PGs using contrast injectables, but these approaches have their limitations as mentioned before. On the basis of all relevant studies that have not relied on contrast agents as reported to date (Table 1), the benefits of label-free technologies that detect NIRAF during neck endocrine procedures mainly include: 1) Helping the surgeon identify PG tissue intraoperatively in real time; 2) Visualizing and mapping the location of PGs prior to or after dissection 3) Distinguishing healthy from diseased PGs and 4) Preventing temporary hypocalcemia after total thyroidectomy.

1) Helping the surgeon identify PG tissue intraoperatively in real time—In the operating room, identification of PG tissue has traditionally been based on a surgeon's visual assessment with a frozen section subsequently confirming the surgeon's impression. Using a probe-based approach, McWade *et al.* first relied on spectroscopy to detect NIRAF and noted a 100% PG detection rate across 45 patients.[25] The authors extended the scope

of this technique across 137 patients, where NIRAF was analyzed to be higher in 97% (256/264) of PGs as compared to surrounding tissue.[26] An interesting observation made in this study was the presence of intra-glandular spatial heterogeneity in NIRAF signal with the authors recommending at least three measurements per PG in different locations. The study further found that high calcium, low 25-hydroxyvitamin D, disease state of PG and high body mass index led to varied NIRAF intensities in PGs, but ultimately did not affect the capability of NIRAF detection in identifying PGs during surgery. The authors concluded that NIRAF detected with spectroscopy using a fiber probe was capable of reliable, label-free and real-time detection of PGs, regardless of the clinical and demographic characteristics of the patients. Subsequently, Thomas *et al.* compared the now FDA-approved 'PTeye' (Figure 3a – 3d) against the 'lab-built' , and found that the PTeye had an accuracy of 96.1% compared to 92.5% with the lab-built system in the same patients. [31] They also noted that presence of blood on tissues did not affect the performance of either system in detecting NIRAF for PG identification.

The feasibility of an 'imaging' approach for NIRAF detection to spatially localize PGs in thyroid or parathyroid operations was first studied by McWade *et al.*, where 100% PG detection rate was achieved.[30] For this modality, a modified Karl Storz camera was placed 15 cm over the surgical field and NIRAF images were captured to be displayed in real-time on a separate monitor after OR lights were turned off. Raw images were processed *post hoc* to quantify the ratio of NIRAF intensity of PGs to its surrounding tissues. Based on these images, the authors inferred that high levels of NIRAF from PGs could offer a unique opportunity for PG-selective imaging, without administering injectable contrast agents.

These early results ushered the era of NIRAF imaging for label-free intraoperative PG identification. A series of manuscripts that utilized imaging modalities for NIRAF detection to visualize PGs using commercially available or custom-built systems validated the original findings (Table 1).[33, 34, 38-42] In 2016, Falco *et al.* were the first to utilize a commercially available NIR imaging system (Fluobeam 800) to visualize and confirm PG tissues in the OR, where the device detected 100% of PGs.[43] Around the same time, De Leeuw *et al.* also reported on the feasibility of Fluobeam 800 for PG detection with 94% sensitivity and 80% specificity in 28 ex-vivo specimens[38] and observed positive NIRAF in 98.8% of in-situ PGs. Kahramangil *et al.* later observed that NIRAF imaging could visualize PGs earlier than ICG-based fluorescence.[44] The same authors later reported the performance of NIRAF detection via imaging in the first multi-centric study across three sites that yielded a PG identification rate of 98% in 210 patients (Figure 3e – 3g).[40] In parallel, imaging of NIRAF in PGs was successfully demonstrated with another commercially available device – the PDE system (Hamamatsu, Japan) showing 100% sensitivity and 97.3% specificity[34], while several other studies relied on modified iterations of Karl Storz systems to obtain PG detection rates ranging from 86.4 to 92.3% (Table 1).[35, 45-48]

Thus, the available studies strongly support that NIRAF detection can accurately identify and confirm both healthy and diseased PG tissues in real-time without injectable labels, using either probe-based or imaging-based platforms. These modalities that rely on NIRAF detection could in theory be used in lieu of frozen section to confirm PG tissue and could save operating room time by facilitating PG identification and avoiding excessive dissection.

As with any new technology, surgeon will need to prospectively study the tool in his/her clinical practice and assess how it performs against the current standard of care.

2) Visualize and "Map" the location of the PG prior to or after dissection— Early detection of PGs during neck dissection could, in concept, help avoid damage to healthy PGs and guide in their localization during thyroidectomy and/or parathyroidectomy. De Leeuw *et al.* was the first to suggest that imaging-based approach to detect NIRAF could guide in localizing PGs prior to surgeon visualization.[38] The authors indicated that imaging for NIRAF aided in finding the PG in five instances before the surgeon could observe it with the naked eye. Similarly, Kahramangil *et al.* reported that NIRAF imaging aided in localizing 37 to 67% of all PG candidates prior to the surgeon's eye.[40] Falco *et al.* noted that the mean number of PGs identified per patient with plain white light increased significantly from 2.5 to 3.7 PGs with NIRAF imaging.[33] Recently Squires *et al.* stated that imaging with the PDE Neo II improved the surgeon's ability by 20%, in detecting NIRAF and identifying PGs not initially visible.[49] The need for frozen sections was obviated in 29% of PGs as the authors felt confident in having correctly identified PGs with NIRAF detection.

In contrast to commercial imaging systems, Kim *et al.* described a custom-built Canon camera specifically designed to identify and locate PGs.[41, 42] The authors noted 100% NIRAF detection rate in the PGs examined, where the system was able to localize 10 PGs that were 'veiled' by connective tissue, fat or blood vessels and not apparently visible to the surgeon's naked eye. While it was noted that unexposed PGs tended to have lower NIRAF intensity than in an exposed state, their location could still be detected by the described instrument. The same authors coined the term 'parathyroid mapping' based on a systematic three-stage NIRAF detection approach which was applied in their subsequent studies.[41, 50] The first stage (P1) involved aiming the camera at predicted locations of PGs prior to dissection or identification with surgeon's plain sight (Figure 4a – 4c). For PGs not visualized with NIRAF detection in P1 stage, the imaging protocol was repeated after the surgeon dissected or identified PGs with the naked eye for the second (P2) stage. For the 3rd stage (P3), ex-vivo NIRAF images were taken from excised surgical specimens looking for PG not detected in P1 or P2 stage. Using this approach, the authors were able to detect 82.7 to 92.8% of PGs within P1 stage, 96.2 to 98.6% PGs by P2 stage and 98.1 to 100% by P3 stage, indicating that NIRAF-based parathyroid mapping may guide in early PG localization.

On the other hand, Ladurner *et al.* reported that the authors were unable to visualize NIRAF from PGs in 8 cases when using an optimized Karl Storz camera, because the PG were embedded in adipose tissue.[35] Unlike the earlier described studies, the authors recommended dissecting and partially freeing potential PGs from its connective tissue sheath for NIRAF imaging, as overlying tissue would obstruct NIRAF detection. In conformity with this finding, other studies have also observed an inability to detect NIRAF when PGs were covered with fat.[29, 34, 46, 51]

There are currently no studies that report using probe-based modalities for NIRAF detection to spatially 'map' PGs. However, it is plausible that the PTeye could provide comparable results to NIRAF detection with imaging in terms of PG mapping/localization. For instance, once the thyroid lobe is systematically mobilized during surgery, the fiber optic probe can

be used to ‘scan’ the surgical field for PGs and could notify the surgeon with an auditory feedback, analogous to a ‘metal detector’ or ‘nerve monitor’. The surgeon could then repeat the process after further dissection, in a similar manner to the ‘parathyroid mapping’ technique described by Kim *et al.* (see above).

The FDA approval granted for Fluobeam 800 and PTeye explicitly state that these two devices are meant to solely ‘assist’ and ‘not replace’ experienced visual assessment by the surgeon in identifying PG tissues. Moreover, since NIR light typically has a penetration depth of 0.4 to 5 millimeters in soft tissues[52], the ability to localize ‘hidden’ PGs would be contingent on (i) sensitivity, exposure time and related optics of the detector used, and (ii) optical properties of the tissues overlying the PG. Therefore, current FDA-approved devices – Fluobeam 800 and PTeye – may be limited in NIRAF detection beyond a 5 mm depth, due to limitations of their existing system design. As a result, these devices would be unlikely to localize deep-seated PGs (intrathyroidal or ectopic). Currently the surgeon should fully or partially expose PG candidates prior to NIRAF detection. It is however quite possible that future technical advances and iterations for technologies that can detect NIRAF, could definitively ensure spatial mapping for ‘unseen’ PGs, as described by Kim *et al.*[41, 42]

3) Distinguish healthy PGs from diseased PGs—The ability of a surgeon to differentiate between normal and abnormal PGs plays a pivotal role in the success of parathyroid surgery. Preoperative ultrasound imaging, ^{99m}technetium-sestamibi scintigraphy and computed tomography (CT) can aid in localizing hyperfunctioning/abnormal PG, but have yielded subpar results in detecting multiglandular disease present in 5 to 33% patients with primary hyperparathyroidism.[9, 53-55] With the advent of NIRAF detection for identifying PGs, researchers have attempted to identify discriminant traits in NIRAF signals between normal and abnormal PGs. McWade *et al.* was the first to observe that PGs of SHPT patients had relatively weaker NIRAF intensity than normal or adenomatous PGs, which was later confirmed by other studies.[26, 29, 31] Using PTeye, Thomas *et al.* found no significant difference in NIRAF intensity detected between healthy and diseased PGs associated with primary hyperparathyroidism, which was in agreement with observations by others who relied on NIRAF imaging.[31, 45, 49]

In stark contrast to these findings, Falco *et al.* found that adenomas showed higher NIRAF intensity than normal PGs using Fluobeam 800 during cases of primary hyperparathyroidism.[33] They hypothesized that the higher NIRAF intensity of PG adenomas could be related to increased cellularity and lower fat concentration in adenomas. Interestingly, the exact opposite findings were recorded by Kose *et al.*, where hyperfunctioning PGs displayed lower NIRAF intensity than normo-functioning PGs upon using the same Fluobeam 800 device.[56] The authors further determined that a normalized NIRAF intensity ratio of 2.0 or above could serve as an optimal cutoff to differentiate normo-functioning from hyperfunctioning PGs. In addition, the authors observed that hyperfunctioning PGs often displayed heterogenous patterns of NIRAF as compared to the normo-functioning ones. While McWade *et al.* also reported about intraglandular heterogeneity of NIRAF with fiber-optic probe measurements, this study did not report if the assessed PGs were healthy or diseased.[26]

In summary, due to inconsistent findings in the various reported studies, there is currently no clear-cut consensus on whether NIRAF can definitively distinguish between normal and abnormal PGs. Further studies are necessary to determine whether the degree of NIRAF intensity can accurately predict the presence of hyperfunctioning PGs.

4) Prevention of hypocalcemia after total thyroidectomy.—The question of whether routine use of NIRAF detection to identify and localize PGs can improve patient outcome after total thyroidectomy was recently investigated in three studies. Benmiloud *et al.* evaluated the effect of NIRAF imaging on patient outcome by comparing the results of such procedures by two surgeons where one surgeon used the Fluobeam 800 to evaluate the surgical field during total thyroidectomy while the other did not. [39] This study revealed that NIRAF detection with imaging reduced the incidence of inadvertent PG removal, PG auto-transplantation rates and transient hypocalcemia (Calcium <8 mg/dL at post-operative day 1 or 2). However, the surgeon that did not use NIRAF detection also showed reduction in transient hypocalcemia, presumably due to the Hawthorne or observer effect. In another study, Dip *et al.* compared postsurgical outcomes with use of imaging (NIR light) vs. the surgeon's naked eye (white light).[57] For the study design, patients were block randomized to two equal groups. White light and anatomical landmarks were used to localize PGs in Group 1, while imaging with Fluobeam 800 was used in Group 2. While there was no significant difference in the incidence of transient hypocalcemia (defined as serum calcium < 8.0mg/dL) between groups, the authors reported that the incidence of severe transient hypocalcemia (defined as serum calcium < 7.6mg/dL) was significantly decreased in Group 2 as compared to Group 1. In another study, DiMarco *et al.* agreed that NIRAF imaging may aid in detecting accidentally excised PGs and allow timely PG auto-transplantation, but failed to find a significant reduction in missed inadvertent parathyroidectomies or postsurgical hypocalcemia (transient or permanent) upon using Fluobeam 800 during thyroidectomy.[51]

The present data suggest that NIRAF imaging during total thyroidectomy may help visualize PGs more readily and consequently avoid their injury. However, more clarity is required to fully determine if NIRAF imaging can minimize accidental injury or removal of PGs or reduce the incidence of postoperative hypocalcemia. To our knowledge, probe-based detection of NIRAF has not been evaluated in patient outcome studies yet, although studies are currently in progress to evaluate the role of PTeye in affecting patient outcome.

What are the advantages and limitations of NIRAF for PG identification?

1 – Comparing probe-based vs. imaging-based platforms for PG identification—Table 2 summarizes the capabilities of the current FDA-approved devices for intraoperative PG identification using NIRAF detection. The pen-like configuration of the fiber optic probe (Figure 2b) utilized in the PTeye allows for it to be easily handheld and used in very small incisions potentially reaching into "nooks and crannies" within a surgical field. The device is compatible with ambient OR lights and gives real-time quantitative information with an immediate auditory feedback to the surgeon when the PG is detected. The PTeye however does not provide information of PG viability/perfusion or a "field view" of the operative site. Since the PTeye probe currently needs to be in contact with the PG

in question, the fiber optic probe utilized at present is essentially a disposable. While the contact-based approach requires probe sterility, it was observed that probe-based NIRAF detection was more sensitive in identifying PGs (Detection rate: 97%) as compared to a non-contact based imaging approach (Detection rate: 90.9%) in a pilot study where both probe- and imaging-based approaches of NIRAF were tested concurrently.[58]

The Fluobeam 800 is a reusable non-contact camera that is typically handheld by the surgeon above the neck incision. Since it is a camera-based modality, it provides a "field view" that can be very valuable for spatially "mapping" locations of PGs, as discussed above. The Fluobeam 800, along with other imaging devices such as the PDE systems or Karl Storz cameras, have the added benefit of being used for multiple applications in surgery, as these instruments were originally approved to evaluate tissue perfusion in plastic surgery and lymphatic mapping,[59, 60] while also being able to assess PG perfusion when used in conjunction with ICG administration. Current imaging systems such as Fluobeam 800 require that the OR lights be turned off during NIRAF measurements. To achieve optimum visualization of PGs with these camera-based systems, the surgeon may need to make wider neck incisions or ensure that the camera is held at a fixed distance from the target, as NIRAF intensities can fluctuate with varying distance between target tissue and the camera held by the surgeon.[29] Since there is no real-time quantitative information of NIRAF intensity from PGs, the surgeon would have to subjectively judge NIRAF intensity on display monitors for presence of potential PGs.

2 – False positives and false negatives with NIRAF detection modalities— Using Fluobeam 800, De Leeuw *et al.* observed 3 false positive cases which were attributed to colloid nodules in thyroid or brown fat.[38] The false positives typically presented as bright spots in the images with a normalized NIRAF intensity higher than that of the thyroid. The authors also noted that although brown fat exhibited strong NIRAF, its bright autofluorescence declined rapidly after resection while that of the PGs did not. Interestingly, the bright spots of brown fat could also be observed with plain white light illumination with the NIR laser turned off, unlike the PGs. The sole false negative result obtained in this study was that of an intra-thyroidal parathyroid. In comparison, McWade *et al.* noted that while some PGs emitted lower NIRAF, these glands could still be detected as these signals were higher relative to the thyroid. However reduced sensitivity was recorded particularly with PGs of SHPT patients, where elevated NIRAF was observed in only 54% of PGs confirmed by histology.[26] Thomas *et al.* also observed that SHPT patients were predominantly responsible for false negatives obtained with the PTeye.[31] In addition, false negatives were observed for parathyroid cysts with PTeye, while false positives were found to occur in fibroadipose tissues, brown fat and occasionally in lymph nodes. Lowered NIRAF intensity or detection rate were also reported in PGs of SHPT patients with NIRAF imaging in other studies.[29, 47] Using Fluobeam 800 for parathyroidectomy, DiMarco *et al.* observed that 9.5% of PGs lacked NIRAF signal (false negatives) with no false positives being observed.[29] In addition, 3 potential PGs could not be localized neither by the surgeon nor by NIRAF imaging. In another report only 50% of patients with primary hyperparathyroidism associated with multiple endocrine neoplasia type 1 (MEN1) syndrome exhibited detectable NIRAF in PGs when viewed with PDE Neo II.[61]

False NIRAF positives in non-parathyroid tissues – brown fat, lymph nodes – should thus be interpreted with necessary care by surgeons. Findings with NIRAF in such scenarios should be inferred in conjunction with either intraoperative PTH levels during parathyroidectomy or frozen section biopsies in total thyroidectomy for malignant thyroid disease.[31] The former ensures that the diseased PG is removed instead of brown fat (additionally distinguished from PG using white light illumination), while the latter ensures preservation of healthy PGs and removal of metastatic lymph nodes. In comparison, surgeons should exert attention if they were to use NIRAF detection for PG localization in SHPT and MEN1 cases, because of the associated false negative rates – possibly due to the unique histopathogenesis in PGs for these diseases. It will be cogent to note that 'non-tissues' such as purple vicryl suture may also have strong NIRAF due to its dye, causing false positives and interference with imaging.[34] Similarly, surgical kittner sponges may strongly 'glow' under NIR cameras as a false positive, due its radiopaque dye.[62]

3 – Inability of NIRAF to assess parathyroid gland viability—NIRAF modalities do not provide information regarding PG blood supply or their viability. The PG's ability to emit NIRAF remains intact even after it loses its blood supply. The NIRAF of PGs persists after excision from the body and thus can be detected by probe or imaging-based systems in an *ex vivo* setting. Current clinical efforts to evaluate perfusion/viability is focused on exogenously administered contrast agents like ICG to assess perfusion/viability of PGs. As a result, imaging systems such Fluobeam 800, PDE Neo or Novadaq (Stryker, US) among others have been successfully used to assess perfusion and viability of PGs or other tissues, but only after injection of ICG.[59, 63] Recently it was demonstrated that PG perfusion can now be assessed in a label-free manner as well by using laser speckle contrast imaging with 91.5% accuracy (Figure 5a – 5e).[64] However, no study has been reported to date where probe-based NIRAF detection was applied for assessing PG viability using ICG.

Potential impact of NIRAF detection modalities and future prospects

The ability of detecting NIRAF to identify PGs reliably during neck endocrine surgical procedures is groundbreaking, as the technique is essentially an 'optical biopsy' using NIR light, analogous to a frozen section biopsy, but capable of providing results in real-time without disruption or removal of the PG. To date, highly experienced surgeons have painstakingly learned to identify PGs over time, by repeatedly observing its morphological characteristics – color, size, consistency – and studying its relation to adjacent anatomical landmarks. As a result, younger or less experienced surgeons require time to become as proficient in identifying PGs and thus may have a higher rate of postsurgical complications.[65, 66] More importantly, it is neither practical nor cost-effective to send frozen sections on all tissues that appear to be a PG.[67] Identification with NIRAF could help avoid unnecessary frozen sections, while allowing surgeons of varying skillset and experience to immediately locate PGs in the surgical field and avoid injuring it. This new technology could also serve as an educational/training tool to identify PGs for trainees, residents or less-experienced surgeons, where the device can assist in confirming if a tissue is PG in real-time (confirming the opinion of the senior/experienced surgeon), thereby shortening their learning curve at academic institutions or high-volume centers. With about 72,344 total thyroidectomies and 9,934 parathyroidectomies being performed in the US annually[68, 69],

minimizing even a fraction of postsurgical complication rates due to failure in PG identification/localization could considerably reduce the socio-economic burden on patients and the healthcare industry. While certain studies have already demonstrated the ability of NIRAF detection to minimize post-surgical hypocalcemia following thyroidectomies[39, 57], additional large scale and/or long-term studies with these devices are warranted in the near future to assess the true benefits on the patients. However, the benefit of this approach to aid the surgeon is amply demonstrated in the literature.

Just as with any evolving new technologies, there are abundant avenues to further advance the capabilities of NIRAF for identifying PGs. An aspect of PG detection that could be enhanced is the manner in which PGs are visualized by surgeons with current optical modalities. At present the surgeons need to stare at remote display monitors and simultaneously correlate the image with the surgical field under her/his view, which can be challenging and lead to faulty image interpretation. Recent technological progress can now enable successful merging of NIRAF images from a camera directly with the surgeon's field of vision. NIR fluorescence goggles is a prime example of one such advanced modality, where the surgeon can wear a binocular goggle (Figure 5e) to directly visualize NIR fluorescence within the surgical field of view, without needing to divert attention to a display screen.[70, 71] While these goggles have not yet been tested for NIRAF-based visualization of PGs, the visual gadget appears promising and has been successfully demonstrated for contrast-based lymph node surveillance and tumor margin demarcation.[72] Another promising device called the Overlay Tissue Imaging System (OTIS)[73], which detects NIRAF from the surgical field and projects it back as a visible green image directly onto the same field, was recently shown to accurately localized the PG without needing contrast agents or display monitors (Figure 5f – 5h).[32, 73] Further device optimization would however be required for the eventual clinical use of these technologies.

In conclusion, we believe that the application of detecting NIRAF for PG identification is not hype, but rather an opportunity to improve the performance and outcomes of neck endocrine surgery. The eventual scope of NIRAF detection for identifying and preserving PGs would ultimately depend on technologic innovations made periodically, so as to meet the ever-changing needs of the surgeon and the patient in an intraoperative setting. As with any disruptive innovations in the field of surgery, it is likely that optical modalities used for PG identification based on NIRAF detection may traverse across a similar trajectory as that of the 'nerve-monitoring' devices, before gaining wide acceptance among surgeons. Furthermore, developing a cost-effective, yet user-friendly device interface to aid in intraoperative PG identification by surgeons can ensure simple, easy and wide-scaled implementation of these modalities into the existing surgical workflow. Strategic steps in this direction can ensure a realized future for this decade-old, novel technology that is slated to aid a wide range of surgeons and ultimately improve patient outcomes.

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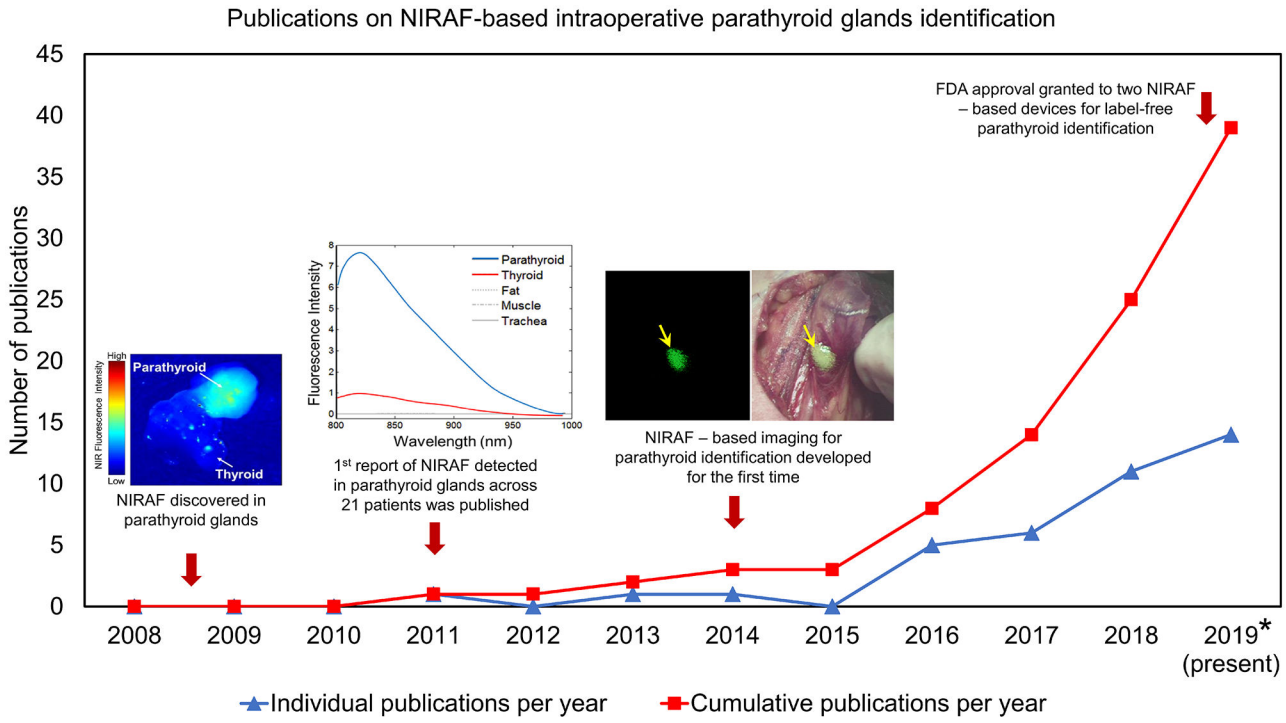

Figure 1:

Trend line of publications based on NIRAF detection for intraoperative PG identification since 2011. (* – Relevant studies considered up to September 2019). Note the exponential rise in research related to label-free intraoperative PG localization after 2014 when the feasibility of NIRAF imaging was established. (Figures adapted from the works of Paras *et al.*[23] and McWade *et al.*[74])

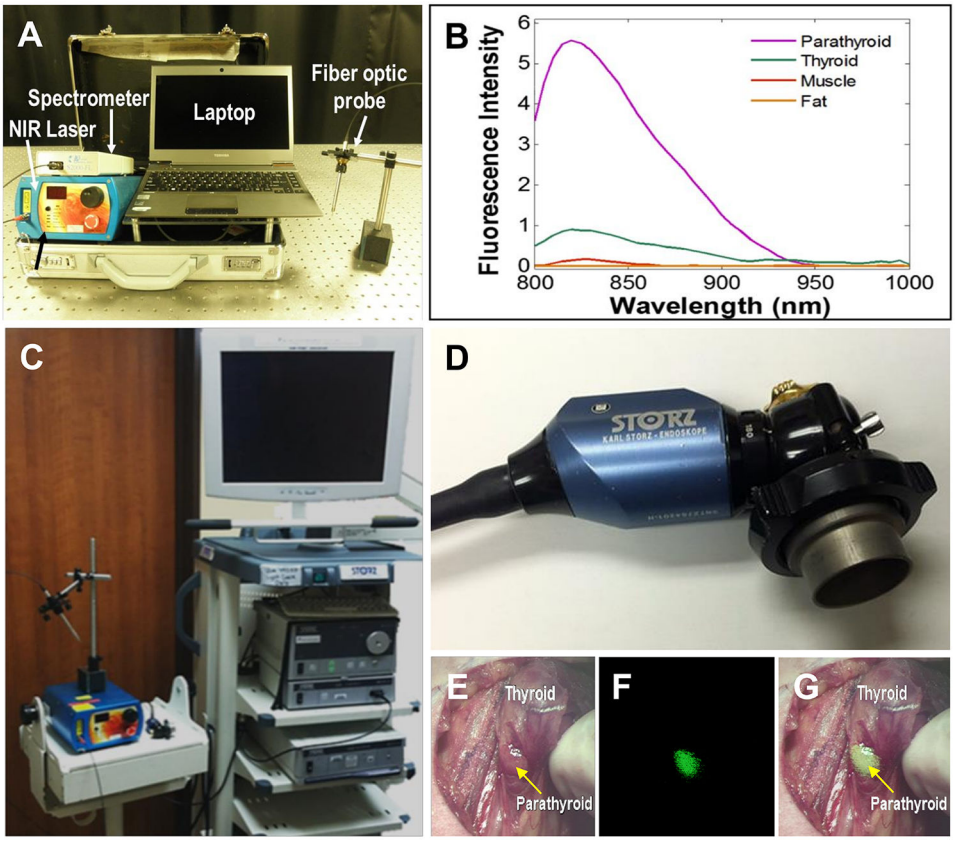

Figure 2:

Lab-built spectroscopy system vs imaging systems used for NIRAF detection to identify PGs. (A) Components of the lab-built system. The system consists of a NIR laser, a spectrometer, a fiber-optic probe and a data-processing laptop. (B) Tissue NIRAF typical output as fluorescence spectra in the laptop, when using the spectroscopy system. Note the stronger intensity of the NIRAF spectra for parathyroid glands compared to other tissues. (C) A lab-built imaging system consisting of a NIR laser source, a display monitor and (D) a modified Karl Storz endoscope camera. (E) White light image of parathyroid and thyroid tissue in situ. (F) NIRAF image with stronger intensity appearing to originate from the parathyroid gland. (G) Co-registration of white light and NIRAF image validating that stronger NIRAF intensity is present in the parathyroid gland, compared to its surrounding tissues NIRAF – near infrared autofluorescence; NIR – Near Infrared (Figures adapted from the work of McWade *et al.*[25, 74])

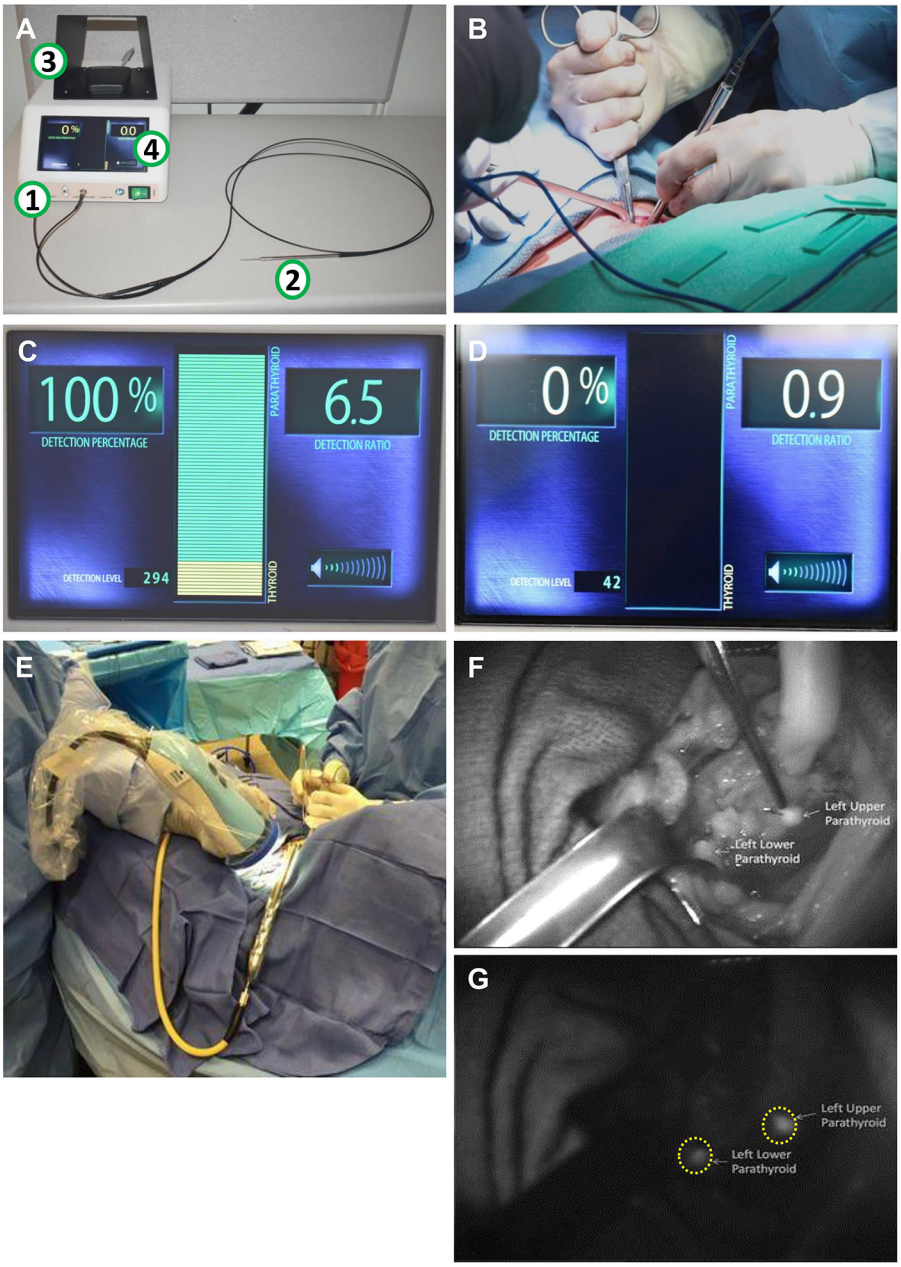

Figure 3:

Commercial technologies capable of NIRAF detection that are currently available for label-free parathyroid identification. (A) PTeye, a probe-based commercial device designed for NIRAF detection is composed of 1) a console that encloses a NIR laser, a detector and internal circuitry, 2) A detachable fiber-optic probe, 3) A foot-pedal to activate the laser and 4) A display interface to inform the surgeon if the tissue is a parathyroid or not with quantification of NIRAF intensity. (B) Hand-held fiber optic probe of PTeye being kept in contact with tissue for NIRAF detection. (C & D) Display interface when the tissue is a parathyroid (left) and when it is not (right). (E) Fluobeam, a commercial imaging system used for NIRAF detection, with the handheld camera being held over the surgical field, (F

& G) White light and corresponding NIRAF images of an exposed left superior and inferior parathyroid glands. Note the higher NIRAF intensity for these two glands in yellow dotted circles. NIRAF – near infrared autofluorescence; NIR – Near Infrared (Figures adapted from the works of Thomas *et al.*[32], Kahramangil *et al.*[40])

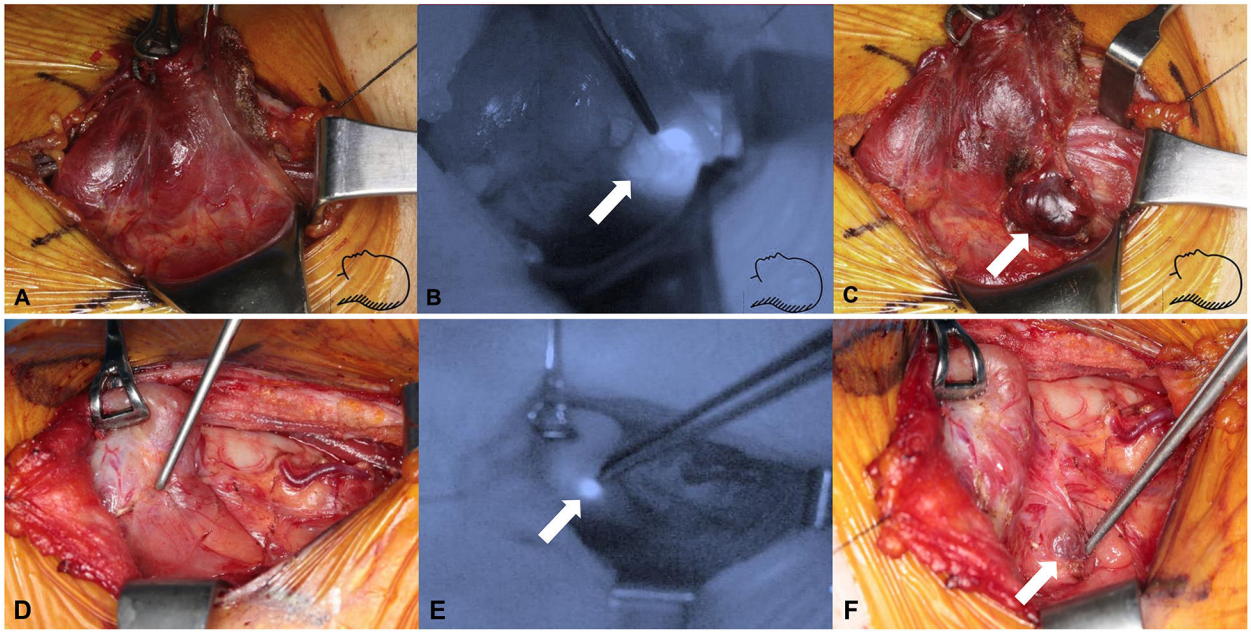

Figure 4:

Concept of Parathyroid Gland Mapping based on NIRAF visualization. (A & D) A white light image of the surgical field, without dissecting out the parathyroid gland. (B & E) Parathyroid gland mapping using NIRAF reveals the parathyroid gland lying underneath a sheath of connective tissue. (C & F) The parathyroid gland was localized by 'mapping' and dissected out accordingly. NIRAF – near infrared autofluorescence. (Figure adapted from the work of Kim et al.[41] and unpublished images courtesy of Dr. Sung-Won Kim and Dr. Kang-Dae Lee, Department of Otolaryngology – Head and Neck Surgery, Kosin University College of Medicine, Busan, South Korea)

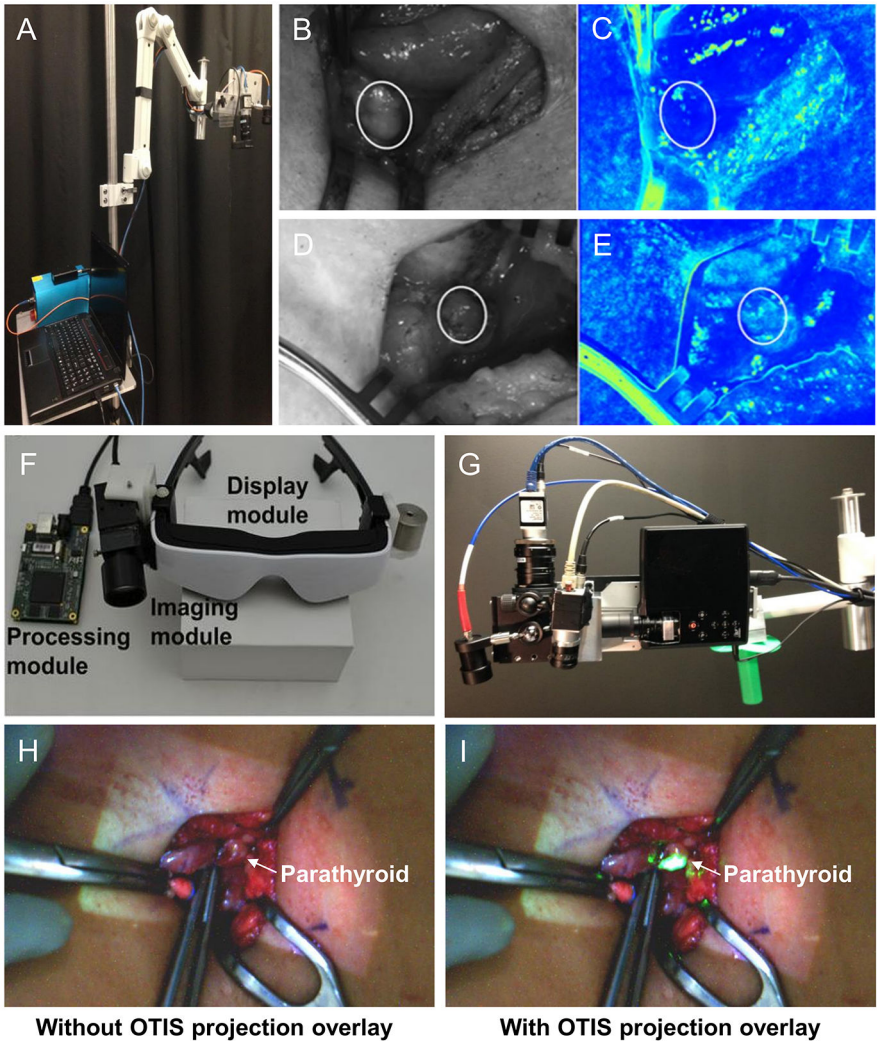

Figure 5:

Future technological advancements that could enhance a surgeon's ability to localize and preserve parathyroid glands during neck operations. (A) A laser speckle contrast imaging system that can assess parathyroid gland perfusion in a label-free manner. (B & C) White light (left) and laser speckle image (right) for a well-vascularized parathyroid gland (encircled), as compared to (D & E) a devascularized/non-viable parathyroid gland (encircled). Note that the laser speckle contrast is lower for a well-vascularized parathyroid gland than a devascularized one. (F) A wearable goggle system that can aid a surgeon to visualize NIR fluorescence directly in the surgical field for sentinel lymph node mapping and tumor resection with contrast agents. While not tested yet for label-free parathyroid localization, this technology may be a promising tool. (G) An overlay tissue imaging system (OTIS) that was developed to back-project tissue NIRAF directly onto the surgical field. (H) A white light image of an exposed parathyroid gland. (I) NIRAF of the parathyroid gland is back-projected onto it as visible green light, enhancing its visibility to the surgeon directly

in the surgical field. NIRAF – near infrared autofluorescence; NIR – near infrared (Figures adapted from the results of Mannoh *et al.*[64], Mondal *et al.*[71] and Thomas *et al.*[32])

Table 1:

Overview of relevant studies that have utilized optical modalities relying on near infrared autofluorescence detection for label-free intraoperative identification of parathyroid glands (NIRAF – Near Infrared Autofluorescence; PG – Parathyroid gland; SHPT – secondary hyperparathyroidism; MEN1 – Multiple Endocrine Neoplasia 1; OR – operation room; N/A – Not applicable or not reported)

Relevant Studies (Published Year)	Device (Manufacturer)	Imaging (I) or Probe (P)	Number of Patients	PG Detection Rate	Sensitivity (histology) for excised PGs (SHPT incl.)	Sensitivity (histology) for excised PGs (SHPT excl.)	Key Notes
Paras *et al.* (2011)[24]	Lab-built fiber optic probe system	P	21	100%	N/A	100%	1st device based on NIRAF detection for PG identification
McWade *et al.* (2013)[25]	Lab-built fiber optic probe system	P	45	100%	N/A	100%	
McWade *et al.* (2014)[30]	Lab-built fiber optic probe & imaging systems with modified Karl Storz camera	I + P	6 (I) 110 (S)	100%	N/A	100%	1st NIRAF imaging system for PG localization
McWade *et al.* (2016)[26]	Lab-built fiber optic probe system	P	137	97% (SHPT incl.) 99.2% (SHPT excl.)	91.3%	98.2%	1st study to assess NIRAF variability in patients. Found SHPT cases as outliers for NIRAF detection
Falco *et al.* (2016)[43]	Fluobeam 800 (Fluoptics)	I	28	100%	N/A	100%	1st commercial imaging device used for NIRAF detection in PGs
De Leeuw *et al.* (2016)[38]	Fluobeam 800 (Fluoptics)	I	35 (*in vivo*), 28 (*ex vivo*)	98%	N/A	94.1%	
Kim *et al.* (2016)[42]	Lab-built imaging system with Canon camera	I	8	100%	N/A	N/A	Only thyroidectomy
Ladurner *et al.* (2017)[35]	Karl Storz (Karl Storz Endoskope)	I	25	81%	N/A	100%	Modified Karl Storz endoscopy camera used.
Shinden *et al.* (2017)[34]	PDE (Hamamatsu)	I	17 (*ex vivo*) incl. 1 *in vivo*	100%	N/A	100%	
Falco *et al.* (2017)[33]	Fluobeam 800 (Fluoptics)	I	74	N/A	N/A	N/A	NIRAF detection with imaging increased PG visualized per patient.
Kahramangil *et al.* (2017)[44]	Fluobeam 800 (Fluoptics)	I	44	98%	N/A	N/A	Only thyroidectomy. 1st comparison study between NIRAF and ICG-based fluorescence in PGs
Ladurner et al. (2018)[45]	Karl Storz (Karl Storz Endoskope)	I	20	90.2%	N/A	N/A	Only thyroidectomy.
Benmiloud *et al.* (2018)[39]	Fluobeam 800 (Fluoptics)	I	93	76.3%	N/A	N/A	1st outcome-based study for only total thyroidectomy. NIRAF detection reduced postsurgical hypocalcemia

Relevant Studies (Published Year)	Device (Manufacturer)	Imaging (I) or Probe (P)	Number of Patients	PG Detection Rate	Sensitivity (histology) for excised PGs (SHPT incl.)	Sensitivity (histology) for excised PGs (SHPT excl.)	Key Notes
Kim *et al.* (2018)[41]	Lab-built NIRAF imaging system with Canon camera	I	38	100%	N/A	N/A	Only thyroidectomy. Coined 'parathyroid mapping'.
Kahramangil *et al.* (2018)[40]	Fluobeam 800 (Fluoptics)	I	210	98%	N/A	N/A	1st multicenter study. 3 centers participated.
Alesina *et al.* (2018)[46]	Karl Storz (Karl Storz Endoskope)	I	5	92.3%	N/A	100%	1st study to use NIRAF detection to visualize PGs during video-assisted neck surgeries
Thomas *et al.* (2018)[31]	PTeye (AiBiomed) Commercial fiber optic probe system	P	35	97.0% (SHPT incl.) 99.2% (SHPT excl.)	89.2%	97.3%	1st commercial probe-based system for NIRAF detection. Functions with OR lights. Tested with SHPT cases
Thomas *et al.* (2019)[32]	PTeye (AiBiomed) Commercial fiber optic probe system + Lab-built imaging system	I + P	6 (I) 20 (P)	100% (I) 98.2% (P)	N/A	100% (I) 95.6% (P)	1st study that compared between imaging and probe-based NIRAF detection.
Kose *et al.* (2019)[56]	Fluobeam 800 (Fluoptics)	I	50	96.5%	N/A	96.5%	1st prospective study for only parathyroidectomy. Heterogeneity noted in NIRAF of diseased PGs.
Wolf *et al.* (2019)[47]	Karl Storz (Karl Storz Endoskope)	I	39	86.4%	86.4%	90.0%	1st study where Karl Storz system was tested in SHPT cases.
Squires *et al.* (2019)[49]	PDE Neo II (Hamamatsu)	I	59	87% *(only ex vivo)*	N/A	87%	2nd prospective study for only parathyroidectomy. NIRAF detection helped avoid frozen section analysis in 29% of PGs.
McWade *et al.* (2019)[73]	Lab-built NIRAF Projection overlay system (OTIS)	I (Projection Overlay)	30	97%	95.8%	95.7%	NIRAF of PGs was overlaid directly onto surgical field. No need for display monitors.
Dip *et al.* (2019)[57]	Fluobeam 800 (Fluoptics)	I	170	N/A	N/A	N/A	2nd outcome-based study for only total thyroidectomy. NIRAF detection increased PG visualized per patient. NIRAF used reduced postsurgical hypocalcemia.
DiMarco *et al.* (2019)[29]	Fluobeam 800 (Fluoptics)	I	96	90.5%	98.3%	97.8%	3rd prospective study for only parathyroidectomy. 1st study where Fluobeam system was tested in SHPT cases.
DiMarco *et al.* (2019)[51]	Fluobeam 800 (Fluoptics)	I	269	N/A	N/A	N/A	3rd outcome-based study for thyroidectomy. NIRAF did not minimize inadvertent parathyroidectomy or postsurgical hypocalcemia in this study.
Ladurner et al. (2019)[48]	Karl Storz (Karl Storz Endoskope)	I	117	87.3%	N/A	N/A	Compilation of data over 5 years.
Squires *et al.* (2019)[61]	PDE Neo II (Hamamatsu)	I	71 incl. 6 MEN1	91.2% (non-MEN1) 25.0% (MEN1)	N/A	N/A	High false negative rates observed in MEN1 patients.

Relevant Studies (Published Year)	Device (Manufacturer)	Imaging (I) or Probe (P)	Number of Patients	PG Detection Rate	Sensitivity (histology) for excised PGs (SHPT incl.)	Sensitivity (histology) for excised PGs (SHPT excl.)	Key Notes
Kim *et al.* (2019)[50]	Lab-built NIRAF imaging system with Canon camera	I	26	98.1	N/A	N/A	Only thyroidectomy. High resolution video imaging for PG mapping.
Thomas *et al.* (2019)[58]	PTeye (AiBiomed) + PDE Neo II (Hamamatsu)	I + P	20 (I+P)	90.9% (I) 97.0% (P)	N/A	90.9% (I) 97.0% (P)	1st study that concurrently compared commercial imaging-based and probe-based systems for PG identification using NIRAF detection.

Table 2:

Advantages and limitations of current FDA approved near infrared autofluorescence detection devices for identifying parathyroid glands

	Probe-based NIRAF detection (PTeye)	Imaging-based NIRAF detection (Fluobeam 800)
Auditory feedback when the PG is identified	+	
Not affected by ambient light (Operating room lights on)	+	**
Real time quantitative intensity measurements	+	
Easier access to PG in deeper planes or aberrant locations	+	
Easier access to PG in a small incision or crevice	+	
Compact and easier to hold	+	
PG detection does not depend on detector distance to target	+	
Provides spatial information or “field view”		+
No contact required		+
Reusable	§§	+
Multifunctional and multipurpose		+
Short learning curve	+	+
Can be used as a teaching tool	+	+
Can be used to detect incidentally removed PG in the resected specimen	+	+
Does not provide information on viability of the PG	+	+
Can be used in conjunction with injectable fluorophores like ICG	+	+
Provide information regarding PG vascularity/viability on its own		

PG: Parathyroid gland

**: the Fluobeam-LX (model unveiled in February 2019, advertised as compatible with OR lights; device performance unknown at present); ICG: indocyanine green.

§§: Fiber optic probe is sold as a disposable