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Review

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Use of fluorescence image-guided surgery and autofluorescence in thyroid and parathyroid surgery

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Abstract

Fluorescence-guided surgery (FGS) has seen increased interest in recent decades. Technological advances have made it more widely accessible for a variety of applications, including thyroid and parathyroid surgery. Parathyroid autofluorescence can be utilized to help identify parathyroid glands during thyroid or parathyroid surgery and reduce rates of postoperative hypocalcemia after thyroidectomy. Fluorescent dyes such as indocyanine green (ICG) may be used to evaluate perfusion of parathyroid glands during thyroid or parathyroid surgery and help guide decision-making about auto-transplantation or which gland to leave as a remnant. As an emerging technology, additional research is needed to determine the optimal use of FGS in thyroid and parathyroid surgery, including the developing field of molecularly targeted fluorophores. FGS is an exciting and promising field that may help make endocrine surgery safer, faster, and more effective.

Keywords: Indocyanine green, near-infrared fluorescence, fluorescence-guided surgery, autofluorescence, parathyroid, thyroid, endocrine surgery

INTRODUCTION

Surgeons have been exploring fluorescence-guided surgery (FGS) since the 1950s. Some of the earliest defined uses were in ophthalmology and cataract surgery, though applications in gynecologic and hepatobiliary surgery soon followed[1-3]. Additional surgical fields found use for fluorescence in the ensuing decades (including vascular surgery, otolaryngology, and others), but the development of high-quality commercially available near-infrared (NIR) imaging systems in the 2000s led to rapid expansion and interest in almost all surgical specialties, including endocrine surgery[4-13]. Potential applications in this field include the use of indocyanine green (ICG) fluorescence guidance in parathyroidectomy and thyroidectomy, the role of autofluorescence in parathyroid gland identification and preservation, and more. This review will focus on the use of indocyanine green (ICG) fluorescence guidance in parathyroidectomy and thyroidectomy, as well as the role of autofluorescence in parathyroid gland identification.

Background

Successful endocrine surgery relies on the identification and preservation of critical structures while removing pathologies that are often difficult to distinguish or separate from normal structures. There has therefore been genuine interest amongst endocrine surgeons in utilizing NIR fluorescence imaging as the technology has become more widespread. Several properties of ICG fluorescence guidance make it an intuitive choice in endocrine surgery.

It has been discovered that the thyroid and parathyroid glands possess inherent fluorescence (peak 820-830 nm) when excited by NIR light (785 nm). NIR light is chosen because of improved tissue penetration compared to other wavelengths, as well as more selective autofluorescence[14,15]. Paras *et al*.[16] in 2011 described the use of custom software intraoperatively to assess this autofluorescence of the thyroid and parathyroid glands in real time and found that it could be used to distinguish thyroid and parathyroid tissue from surrounding structures as well as to differentiate the higher fluorescence parathyroid glands from the thyroid (this remained true whether the parathyroid glands were normal or hyperfunctioning). There now exist commercially available systems for detecting autofluorescence with either a probe [Figure 1] or a NIR imaging system [Figure 2]. Utilization of this natural property of parathyroid glands may be performed independently of or in combination with contrast-enhanced fluorescence angiography (FA) to take advantage of the properties unique to each system. These autofluorescence imaging systems can detect ICG fluorescence as well as the autofluorescence of the glands, so the technologies may be combined and simultaneously utilized without changing to a different imaging set-up.

ICG is a water-soluble tricarbocyanine dye used in humans since the 1950s[17]. It has an excellent safety profile, with the primary concern being potential cross-reactivity in patients with iodine allergies[18]. It fluoresces in the NIR spectrum (835 nm) when excited by NIR light (805 nm). When given intravenously, it binds avidly to plasma proteins and circulates intravascularly until it is excreted by the biliary system[19,20]. Fluorescence therefore primarily depends on blood supply or the presence of biliary tissue. Endocrine organs require a rich blood supply and tend to be hypervascular compared to surrounding tissue. This property allows fluorescence imaging with ICG to differentiate endocrine organs from surrounding tissues, with theoretically increased distinction from hyperfunctioning and hypervascular glands. Unfortunately, the design of these systems does not usually result in provocation/visualization of thyroid/parathyroid auto-fluorescence. Though other compounds such as methylene blue, fluorescein green, and aminolevulinic acid have been used as adjuncts for imaging of parathyroid glands, concerns over safety and usability have limited widespread adoption[21-24].

While initial studies required customized equipment and software to take advantage of these properties, the increased interest in and demand for NIR intraoperative imaging has resulted in standardized integration into newer models of laparoscopic and robotic imaging systems as well as the development of systems specific to open surgery [Figure 3]. As there is no longer a need to utilize unwieldy and complex systems, the barriers to using this technology have decreased. Fluorescence imaging may now be seamlessly

Figure 1. PTeye™ (Medtronic, Minneapolis, MN, USA) near-infrared autofluorescence probe system. Image from PTeye™ brochure https://www.medtronic.com/us-en/healthcare-professionals/products/ear-nose-throat/parathyroid-detection-systems/pteye.html.

Figure 2. Fluobeam® LX (Fluoptics, Grenoble, France) near-infrared autofluorescence imaging system; this system is also compatible with ICG for fluorescence angiography. Image from Fluobeam® LX brochure https://fluoptics.com/en/fluobeam-lx/. ICG: indocyanine green.

integrated into parathyroid and thyroid operations with the ability to transition instantaneously between fluorescent overlays, grayscale images, and traditional white light to allow the surgeon to customize their operation for the safest and most effective operation possible.

Figure 3. The SPY Portable Handheld Imaging (SPY-PHI) System by Stryker (Kalamazoo, MI). The image on the left shows the handheld imaging device in the foreground with the associated imaging tower in the background. The image on the right shows the SPY-PHI System in use intraoperatively. Figure and caption reproduced from Matson *et al.*[51].

THYROID SURGERY

Thyroidectomy may be indicated for a variety of benign or malignant conditions, including Graves' disease, goiter, or thyroid cancer. The parathyroid glands are four pea-sized endocrine glands primarily responsible for calcium balance in the body and located in close proximity to the thyroid gland (occasionally even embedded within it). Failure to identify and protect the parathyroid glands is associated with postoperative hypocalcemia following thyroidectomy[25]. The most common complication following thyroid surgery is postoperative hypocalcemia, occurring transiently in approximately 10%-30% of patients, with a permanent deficiency of about 1%-10%[25,26]. When hypocalcemia is mild, it is typically asymptomatic. However, when hypocalcemia is symptomatic, it can have a significant impact on patients' quality of life as well as the duration of hospitalization and healthcare costs. Typical symptoms are related to the crucial role of calcium in muscle contraction and neurotransmitter release and include paresthesias, perioral numbness and tingling, and muscle spasms/tetany (such as the classic Chvostek sign- contraction of the ipsilateral facial muscles when tapping the facial nerve- and Trousseau sign- carpopedal spasm with inflation of a blood pressure cuff over the upper arm)[27-29]. Life-threatening symptoms include seizures, laryngospasm, cardiac arrhythmias, and cardiomyopathy[27]. When permanent, even asymptomatic hypocalcemia can be debilitating, requiring lifelong supplementation with calcium and vitamin D and routine follow-up, particularly for patients with comorbidities or women of childbearing age.

Parathyroid glands can be challenging to identify even for experienced surgeons in ideal circumstances; they can be highly variable in their location (particularly the inferior glands), preoperative imaging often lacks sensitivity and/or specificity (especially for normal glands), they can be difficult to distinguish from thyroid tissue, lymph nodes, or fat tissue, and they can vary in number. This task may be even more challenging in the case of a large goiter or invasive thyroid cancer. Even when identified, preserving the delicate blood supply of parathyroid glands and ensuring adequate postoperative function presents another challenge. If devascularized, the need to auto-transplant one or more parathyroid glands can also be unclear. The use of NIR imaging to visualize the presence and function of the parathyroid glands during thyroid surgery offers surgeons an intraoperative adjunct.

Autofluorescence

In 2016, Falco *et al.*[30] published a feasibility study demonstrating that the parathyroid glands could be reliably identified using autofluorescence with NIR imaging in various pathologies of the thyroid and parathyroid glands, and that the parathyroid glands could be distinguished from the thyroid gland using fluorescence intensity [Figure 4]. This led to increased interest in the practical implications of using this technology, and a randomized controlled trial by Dip *et al.*[31] in 2019 included 170 patients and demonstrated that the use of NIR autofluorescence (NIRAF) imaging of the parathyroid glands increased the number of parathyroid glands identified prior to initiating the thyroid gland dissection from 2.6 to 3.5 ($P < 0.001$) when compared to white light, including at least one previously missed gland in 2/3 of patients. More importantly, it reduced the rate of hypocalcemia (defined as calcium level ≤ 7.5 mg/dL) by 90% compared to the control group undergoing dissection under white light alone ($P = 0.005$). They did not find a significant difference between the total number of parathyroid glands identified after thyroid gland dissection under white light and pre-dissection with NIR light (3.6 *vs.* 3.5, respectively).

These findings were supported by the PARAFLUO multicenter randomized trial comparing rates of hypocalcemia (corrected calcium < 8.0 mg/dL) on postoperative day (POD) 1 or 2. In that study, Benmiloud *et al.*[32] randomized 241 patients to conventional thyroidectomy *vs.* NIRAF-assisted thyroidectomy and found that the rates of temporary hypocalcemia were 9.1% for the NIRAF group and 21.7% for the controls ($P = 0.007$), though they did not detect a significant difference in permanent hypocalcemia (0% *vs.* 1.6%, respectively). These findings persisted in multivariate analysis, with an odds ratio of 0.35 for hypocalcemia in the NIRAF group. Secondary outcomes further demonstrated that the rates of auto-transplantation (3.3% *vs.* 13.3%; $P = 0.009$) and inadvertently resected glands (2.5% *vs.* 11.7%; $P = 0.006$) were also lower for the NIRAF-assisted compared to traditional thyroidectomy.

A third randomized trial sought to evaluate the combined use of near-infrared autofluorescence and ICG FA in parathyroid gland identification and evaluation. Yin *et al.*[33] enrolled 180 patients in their study and randomized them to either fluorescence image assistance or naked-eye assessment of parathyroid glands during thyroidectomy and central lymph node dissection. NIRAF was used to aid in the identification of the parathyroid glands and ICG was used to assess their perfusion *in situ*. They found significantly decreased transient hypoparathyroidism (27.8% *vs.* 43.3%; $P = 0.029$) and improved identification of parathyroid glands (3.6 ± 0.5 *vs.* 3.2 ± 0.4; $P < 0.001$) with fluorescence assistance. Additionally, they found that fluorescence imaging assessment of parathyroid glands was more accurate than naked-eye assessment as just 4.5% of patients with at least one parathyroid gland deemed to be adequately perfused on ICG FA developed hypoparathyroidism compared to 34.6% ($P < 0.001$) of those examined with the naked eye.

While other studies have had conflicting results regarding the ability of NIRAF imaging to reduce hypocalcemia, these three large randomized trials provide level II evidence that NIRAF can improve the identification and preservation of parathyroid glands during thyroid surgery[31,32,34-36]. Additionally, a meta-analysis of eight studies by Lu *et al.*[37] found that utilizing NIRAF during thyroidectomy compared to naked-eye assessment reduced the incidence of postoperative hypocalcemia from 22.40% to 7.11% ($P < 0.001$). These results were consistent with those found by Wang *et al.*[38] (relative risk of hypocalcemia on postoperative day 1 was 0.49; $P < 0.001$), who additionally found that the relative risk of inadvertent parathyroid gland resection was 0.48 ($P = 0.023$) compared to naked-eye assessment. This may increase surgeon confidence in identification of glands and allow them to proceed more freely with thyroid gland dissection, or it could reduce the need for frozen section analysis or intraoperative parathyroid hormone (PTH) measurement, thereby saving time in the operating room, as well as potentially reducing the rates of transient postoperative hypocalcemia and incidental parathyroidectomy. While NIRAF can assist in identification of parathyroid glands, it does not provide information regarding adequate parathyroid gland perfusion, which is essential for proper function in the perioperative period.

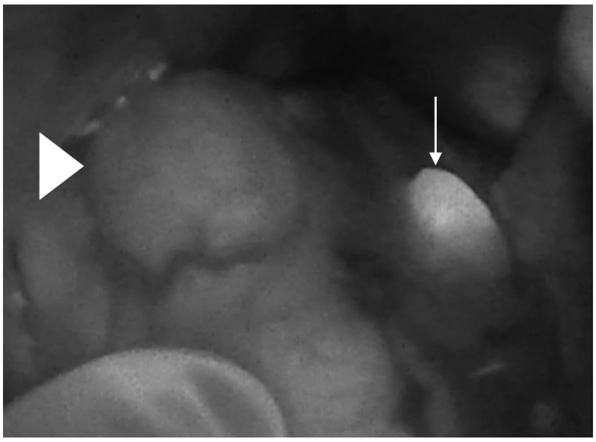

Figure 4. Near-infrared autofluorescence viewed using Fluobeam® LX (Fluoptics, Grenoble, France) of a normal parathyroid gland (long arrow) demonstrating clear differentiation from the thyroid gland (arrowhead).

Fluorescent dyes

One of the advantages of incorporating the use of fluorescent dyes is that they can give real-time information about the blood flow to various tissues, as demonstrated in the trial by Yin *et al.*[33] above. Early studies on the use of ICG FA during thyroid surgery suggested that having well-vascularized parathyroid glands on NIR imaging was associated with normal PTH levels following thyroid surgery[39-41]. Case series' summarized by Spartalis *et al.*[42] further supported the idea that adequate perfusion of parathyroid glands on ICG FA correlated with postoperative PTH levels and even that poor perfusion was associated with an increased risk of hypocalcemia. However, there was significant heterogeneity in the doses of ICG and protocols summarized in this review. This was also one of the limitations mentioned by Kim *et al.*[43] in their meta-analysis of 21 studies, which demonstrated that low fluorescence scores with ICG FA were predictive of postoperative hypoparathyroidism (with improved accuracy when using the imaging-based fluorescence intensity).

A randomized controlled trial by Vidal Fortuny *et al.*[44] compared the need for calcium and PTH measurements as well as vitamin D and calcium supplementation if patients had at least one well-perfused parathyroid gland by ICG FA. Patients were randomized to either standard measurement of calcium and PTH on POD 1 with vitamin D and calcium supplementation *vs.* neither blood tests nor supplementation. Rates of hypocalcemia on POD 10-15 were compared. Of the 196 patients undergoing ICG FA, 146 had at least one well-perfused gland and were randomized; of the 50 patients who did not have a well-perfused parathyroid gland, eleven had hypoparathyroidism on POD 1, with six having persistent hypoparathyroidism on POD 10-15, while none of the randomized patients developed hypoparathyroidism (P = 0.007). The intervention group (no supplementation or measurement of PTH or calcium levels) was therefore found to be non-inferior, with only two patients developing symptoms of hypocalcemia requiring supplementation, suggesting that patients with at least one well-vascularized parathyroid gland by ICG FA during thyroidectomy do not need routine supplementation or lab draws. However, there are no randomized trials comparing the use of ICG FA during thyroidectomy to traditional surgery. The most impactful results would be a demonstration of reduced complications such as hypocalcemia and hypoparathyroidism with NIR imaging; while transient hypocalcemia and hypoparathyroidism are relatively common and may be observed with a single-institution study, the infrequent nature of permanent hypocalcemia will likely require a large, multicenter trial to reach adequate statistical power.

In some cases, not only the global perfusion of the glands may be seen, but specific feeding vessels can be identified and preserved. Benmiloud *et al.*[45] performed a prospective study on 47 patients who underwent 34 total thyroidectomies and 13 lobectomies with the assessment of a total of 76 parathyroid glands. Parathyroid glands were first identified using autofluorescence then ICG was utilized to identify the feeding vessels. A second injection was utilized to assess perfusion after dissection. The first injection was scored on a 0-2 scale (0 being not informative, one a general vascular pattern visible, and two identifications of a clear vascular pedicle entering the parathyroid). 31.6% received a score of 2, 60.5% a score of 1, and just 7.9% a score of 0. This indicates that in the vast majority (over 90%) of cases, ICG angiography provides useful information about the blood supply feeding the parathyroid glands.

It has also been theorized that ICG FA may allow for more selective auto-transplantation, preventing unnecessary auto-transplantation and encouraging auto-transplantation of compromised glands, but the literature in this field is limited. Rudin *et al.*[46] found that the auto-transplantation rate was 36% with ICG FA compared to 12% in the control group (P = 0.001), while Razavi *et al.*[47] concluded that low-flow patterns with ICG FA were not associated with postoperative hypoparathyroidism or hypocalcemia and may result in unnecessary auto-transplantation.

Other dye alternatives to ICG have been examined. Enny *et al.*[23] evaluated the use of fluorescein green (FG) dye as a low-cost alternative to ICG. FG has long been used for ophthalmologic purposes but has not previously been applied to the identification of parathyroid glands. It absorbs light in the blue spectrum (465-490 nm) and fluoresces in the green spectrum (520-530 nm). Enny *et al.*[23] administered 500 mg (2 mL of 25% FG) of dye after resection of the thyroid gland, followed by visualization of the parathyroid glands under white light *vs.* fluorescent light. They found that FG fluorescence did appear to be correlated with function postoperatively, as patients who had 3-4 fluorescent glands did not experience hypocalcemia, whereas visualization of 3 or 4 glands with the naked eye still resulted in rates of hypocalcemia of 23% and 28%, respectively. They also found that postoperative PTH and calcium levels correlated with the number of fluorescent parathyroid glands seen with FG. While additional studies are required, this may be a viable option in low-resource areas to predict the need for postoperative supplementation and laboratory monitoring in patients undergoing thyroid surgery. As newer base models of laparoscopic and robotic imaging systems incorporate NIR capabilities and less unwieldy devices for open surgical approaches become more widespread, this is likely irrelevant for first-world countries. However, it may allow for the application of similar principles without the required investment in expensive imaging systems in resource-limited situations.

Potential pitfalls

While both NIRAF and dye-enhanced NIR fluorescent imaging appear to offer some advantages over traditional thyroid surgery, there are important limitations of the technologies of which surgeons must be aware while using them [Table 1].

For NIRAF, the fluorescence signal can only be detected through two to three millimeters of soft tissue. This limited tissue penetration can result in missed glands if the overlying tissues have not been sufficiently dissected. However, excessive dissection can also result in devascularization if care is not taken to protect the blood supply during exposure. Intrathyroidal glands pose a particular challenge in this regard. There is also significant variability in the relative fluorescence of parathyroid and thyroid glands, so it may lack utility in certain patients. There have been reports of false positives with brown fat, colloidal thyroid nodules, purple-dyed vicryl sutures and even metastatic lymph nodes[48,49]. The lattermost situation is particularly concerning since the consequences of mistaking a metastatic lymph node for an inadequately

Table 1. Utility and limitations of fluorescence guidance during thyroid surgery

System	Benefits/Utility	Limitations
Autofluorescence	Does not require any exogenous contrast agent	Occasionally contrast between thyroid and parathyroid glands is not significant
	Distinguishes between thyroid and parathyroid glands	Does not provide information on perfusion/viability
	Distinguishes between thyroid/parathyroid glands and surrounding tissues	Limited tissue penetration
	Improves identification of parathyroid glands before dissection	Potential false positives with metastatic lymph nodes, brown fat, colloidal thyroid nodules
	Reduces rate of inadvertently resected parathyroid glands	Requires purchase of imaging system
	Reduces rate of auto-transplantation	Unclear effect on operative duration
	Helps identify inadvertently removed parathyroid glands in resection specimens for auto-transplantation	
	Reduces rates of short- and medium-term hypocalcemia	
Contrast-enhanced	Provides real-time information on parathyroid gland perfusion	Limited ability to distinguish between thyroid and parathyroid glands
	May help identify and preserve the parathyroid gland blood supply	Limited tissue penetration
	Can guide decision-making regarding the need for auto-transplantation	Subjective interpretation of the degree of fluorescence/perfusion
	Helps determine which patients are at risk for postoperative hypocalcemia	ICG contraindicated in patients with iodine allergy
	Can help determine the need for postoperative labs and calcium and vitamin D supplementation	Requires purchase of imaging system and dye
	Rapid cycling between fluorescence imaging, white light, and fluorescence overlay	Unclear effect on operative duration

ICG: Indocyanine green.

perfused parathyroid gland and auto-transplanting it could be catastrophic. Another limitation is that NIRAF does not offer any information on the perfusion status of the parathyroid glands. While this limits the utility in predicting postoperative function, since it is not dependent on perfusion, it does allow for more ready identification of inadvertently removed glands in the resection specimen that could then be auto-transplanted. Finally, there is an upfront cost to acquire the imaging system. There is conflicting data on the effect on the operative duration and the ability to prevent hypocalcemia and no cost-effectiveness studies have been published, so it is unclear what the overall financial impact of incorporating this technology into a thyroid surgery practice would be.

ICG FA has several similar limitations. The penetration is also restricted to only several millimeters, so overlying soft tissue can obscure the parathyroid glands. There is also rapid enhancement of the thyroid gland by ICG as it, too, is a hypervascular organ. Therefore, ICG FA should not be used to distinguish between the thyroid and parathyroid glands. Furthermore, current systems largely depend on subjective interpretation of fluorescence, which limits standardization and the ability to determine cutoffs associated with postoperative hypocalcemia and hypoparathyroidism. Quantitative fluorescence is just beginning to be integrated into imaging systems and, until it is more broadly available, requires customization of equipment for real-time visualization or is limited to later analysis of videos with appropriate software, which does not provide immediate feedback to the surgeon. While the capability for NIR fluorescence imaging is being integrated into newer laparoscopic and robotic imaging systems, purchasing these newer models requires significant investment. Additionally, the greatest utility in thyroid surgery is likely using systems specifically designed for open surgery that do not require using unwieldy laparoscopic or robotic cameras to visualize the neck (and which also do not require sterilization of the camera between cases). Also, ICG dye is contraindicated in patients with allergies to iodine. Finally, like NIRAF, there is conflicting data on the effect of the technology on duration of the operation. There is undeniably a requirement to wait for fluorescence after administration of the dye, but most studies have shown this to range from less than a minute to no more than 3 min[50,51]. This may also result in time-savings if it allows surgeons to proceed with the operation and wound closure without waiting for intraoperative PTH levels or frozen section analysis or avoids unnecessary auto-transplantation.

Summary and vision for application

Based on early results, there has been a lot of excitement and numerous publications about the use of fluorescence image guidance in thyroid surgery. While the vast majority of studies are retrospective, single-institution case series or cohort studies, there are several large, randomized trials that seem to show the superiority of NIRAF visualization of the parathyroid glands compared to traditional surgery[31,32]. ICG FA may provide additional insight into the perfusion status of identified glands to help guide auto-transplantation and the need for postoperative monitoring and supplementation. The systematic review by DeMarchi *et al.*[50] summarized 25 studies utilizing either NIRAF or dye-enhanced NIR imaging in thyroid surgery; they concluded that the use of the technology was feasible and safe and had several benefits, including improved identification and preservation of the parathyroid glands as well as early identification of mistakenly resected glands that might allow auto-transplantation. Together, this may result in reduced postoperative hypoparathyroidism and hypocalcemia. This is supported by the findings of Barbieri *et al.*[52]; the authors performed a meta-analysis of 13 studies looking at fluorescence imaging in total thyroidectomy and included 1,484 operations. They found that fluorescence-guided surgery resulted in improvements in both short- and medium-term hypocalcemia rates compared to traditional surgery (8% *vs.* 15% and 1% *vs.* 5%, respectively).

Taking this information into consideration, we believe that these techniques can be used in a complementary fashion for optimal results. An optimal protocol is likely similar to that developed by Yin *et al.*[33] whereby NIRAF is used early in the case to help with the identification of the parathyroid glands and ICG FA is used to either identify and preserve the blood supply of the parathyroid glands prior to removal of the thyroid gland and/or used to assess their viability after completion of the thyroidectomy and aid with decisions regarding auto-transplantation and postoperative supplementation.

PARATHYROID SURGERY

The utility of fluorescence-guided surgery in identifying parathyroid glands and determining their perfusion status may also be applied to parathyroid surgery. Parathyroid surgery is primarily performed to prevent complications or alleviate symptoms related to hypercalcemia secondary to elevated PTH levels (i.e., kidney stones, fatigue, depression, osteoporosis, and other symptoms). The most common indication for parathyroidectomy is primary hyperparathyroidism, or abnormally elevated PTH and calcium levels, which is usually due to a single hyper-functional parathyroid adenoma. Less common causes of primary hyperparathyroidism are multiple adenomas, diffuse hyperplasia, and parathyroid carcinoma. In patients with single or multiple adenomas, their disease process can typically be cured and calcium/PTH normalized by removal of the pathologic gland(s). Patients with diffuse hyperplasia as well as those with secondary (caused by constant stimulation from low calcium and elevated phosphate levels in renal disease) or tertiary (hyperparathyroidism from a renal disease that persists after kidney transplant) hyperparathyroidism require subtotal parathyroidectomy, or removal of most parathyroid glands and leaving about $^1/_2$ gland in situ or auto-transplanted to a more easily accessible area such as the forearm or sternocleidomastoid. Failure to remove diseased glands may result in persistent hyperparathyroidism, while leaving or auto-transplanting inadequately perfused glands during subtotal parathyroidectomy could result in hypoparathyroidism and hypocalcemia. Preoperative imaging is most effective at localizing single adenomas but has far less sensitivity and specificity for multiple adenomas or diffuse hyperplasia; even in the case of a single adenoma, however, intraoperative localization may present a challenge[53-56]. Using near-infrared imaging either to elicit autofluorescence of the parathyroid gland or in combination with a fluorescent dye such as ICG may facilitate localization of the abnormal glands and the latter may be used to help determine which gland is most appropriate for preservation/auto-transplantation.

Autofluorescence

Parathyroid gland autofluorescence has been found to be helpful in identifying parathyroid glands during parathyroid surgery for various indications as well as during thyroid surgery. Paras *et al.*[16] found a significant difference in autofluorescence intensity between parathyroid and thyroid glands ($P = 0.00000235$). McWade *et al.*[57] corroborated this (parathyroid gland autofluorescence is between 1.2 and 29 times higher than thyroid autofluorescence) and found a sensitivity for intraoperative parathyroid gland detection of 97%. The clinical significance of autofluorescence in parathyroidectomy is to help identify parathyroid glands and thereby prevent persistent hyperparathyroidism due to a missed adenoma or incomplete resection in diffuse hyperplasia. Given current high cure rates at specialist centers (up to 97%), superiority over traditional surgery is difficult to demonstrate[58]. In their 2019 review of NIRAF in thyroid and parathyroid surgery, DiMarco and Palazzo[35] determined that 902 patients would need to be included to demonstrate an improvement in persistent hyperparathyroidism from 2% to 0.1%. Thus far, no studies have been performed looking at that outcome. However, Kose *et al.*[59] found a statistically significant difference in autofluorescence intensity between normal and pathologic parathyroid glands with mean normalized fluorescence 1.8 *vs.* 2.6 ($P < 0.001$), respectively. Demarchi *et al.*[60] also recently described a characteristic auto-fluorescent pattern for parathyroid adenomas (heterogeneous fluorescence with a more highly fluorescent cap that correlated with normal parathyroid tissue in 74% of specimens); all patients in their study demonstrated a cure for their primary hyperparathyroidism. If this finding is confirmed in larger studies, the characteristic pattern may permit the use of NIRAF in further distinguishing normal gland(s) from abnormal one(s) and obviating the need to wait for confirmation with a 50% reduction in PTH levels after resection, as is the current standard.

Fluorescent dyes

The use of dye-based techniques to help identify the parathyroid glands has existed for half a century[21]. The combination of dye administration with NIR fluorescence imaging has seen a significant uptick in interest and use in the last decade. While various compounds have been explored for his purpose, including methylene blue and aminolevulinic acid, the dye that has been the most studied is ICG[12,22,24,42].

It has been hypothesized that ICG FA may be beneficial in identifying abnormal parathyroid glands because of their theoretically increased blood flow. The initial case study by Chakedis *et al.*[61] demonstrated that ICG FA could effectively assist with the localization of a recurrent parathyroid adenoma in redo parathyroidectomy. Several case series confirmed its utility in preoperative parathyroidectomy as well as in initial parathyroid surgery[62-66]. These studies showed superior sensitivity of ICG FA compared to traditional techniques such as ultrasound or Sestamibi, and even compared to newer imaging modalities such as 4D CT. Compared to NIRAF, ICG FA can also give insight into the perfusion status of the parathyroid glands [Figure 5]. This is important in subtotal parathyroidectomy to ensure that an adequately perfused remnant is selected for either preservation or auto-transplantation. Studies have demonstrated that ICG FA may be useful in this regard, as none of the patients in these case series developed hypoparathyroidism[42,65,67]. This was one of the proposed uses for contrast-enhanced NIR fluorescence imaging in parathyroidectomy described by Solórzano *et al.*[12] in their recent review of NIR fluorescence in endocrine surgery of the neck.

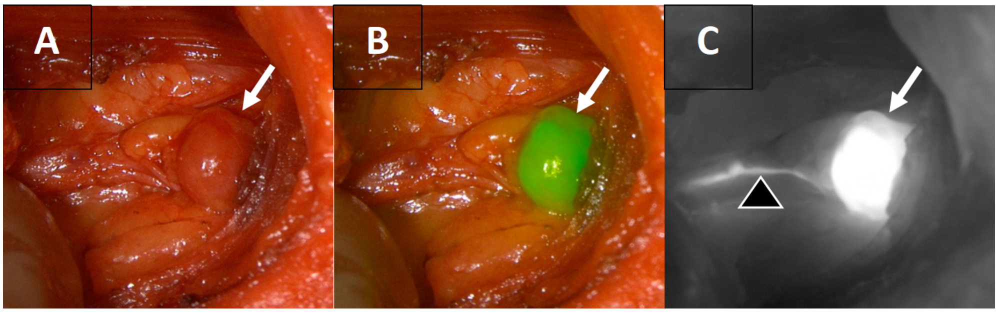

Figure 5. A representative image of parathyroid adenoma fluorescence following injection of ICG and excitement with NIR light. All are from the same patient and injection of ICG. (A) Parathyroid adenoma (white arrow) under white light prior to injection of ICG; (B) the adenoma (white arrow) with computer-generated green overlay after peak fluorescence was achieved; (C) fluorescence of the adenoma (white arrow) and its feeding vessel (black triangle) seen in grayscale viewing mode. Figure and caption reproduced from Matson *et al.*[51]. ICG: indocyanine green; NIR: near-infrared.

Doses of ICG administered for the detection of parathyroid glands during parathyroidectomy were variable[42]. As little as 2.5 mg can be used effectively and repeat administration of ICG can still provide adequate fluorescence visualization, such as for bilateral neck exploration, with ICG FA first performed on one side of the neck before repeating the procedure on the contralateral side[42,51]. The parathyroid glands will typically reach peak fluorescence in less than a minute; the authors' recently published study found an average time to initial fluorescence of 26.7 seconds and an average time to peak fluorescence of 38.0 seconds, with a significant association between time to fluorescence and how quickly the anesthesia provider flushed the IV following injection of the dye[42,51].

Potential pitfalls

Like thyroid surgery, both NIRAF and dye-enhanced fluorescent imaging have a potential role in parathyroid surgery. This role and the limitations are variable depending on the exact indication for surgery [Table 2].

The primary role of NIRAF in parathyroid surgery is in the identification of the parathyroid glands, similar to its role in thyroid surgery. Therefore, some of the possible issues with its use are the same: the limited depth of penetration (just a couple of millimeters) could result in missed glands, potentially causing persistent hyperparathyroidism and requiring reoperation, or could result in excessive dissection and devascularization of glands that should be preserved, potentially resulting in hypoparathyroidism and hypocalcemia; the lack of information about perfusion status could result in selection for preservation or auto-transplantation of a suboptimally perfused gland and hypocalcemia. In parathyroid surgery, it is also important to be aware of the variable autofluorescence of pathologic parathyroid glands (often less brightly fluorescent than normal glands or heterogenous in enhancement, and sometimes even hyperfluorescent compared to surrounding tissues), which is particularly true for secondary hyperparathyroidism and MEN1.

Dye-enhanced NIR fluorescent imaging may be used for either the identification of normal or abnormal parathyroid glands during parathyroidectomy or for the assessment of perfusion status of the remnant gland in subtotal parathyroidectomy. The limitations are essentially the same as for thyroid surgery: limited tissue penetration and ability to detect intrathyroidal or other "buried" glands, inability to differentiate thyroid and parathyroid tissue, and potentially increased costs and OR time associated with its use. However, in parathyroid surgery, there may be a better delineation between thyroid and parathyroid tissue since the parathyroid gland being localized is usually hyperplastic and therefore should theoretically have increased uptake of ICG and greater relative fluorescence.

Table 2. Utility and limitations of fluorescence guidance during parathyroid surgery

System	Benefits/Utility	Limitations
Autofluorescence	Does not require any exogenous contrast agent	Occasionally contrast between thyroid and parathyroid glands is not significant
	Distinguishes between thyroid and parathyroid glands	Does not provide information on perfusion/viability
	Distinguishes between thyroid/parathyroid glands and surrounding tissues	Limited tissue penetration
	May improve intraoperative identification of parathyroid glands over traditional surgery	Potential false positives with metastatic lymph nodes, brown fat, colloidal thyroid nodules
	Potential to identify parathyroid adenomas based on characteristic fluorescence pattern in some cases	Requires purchase of imaging system
	Potential to reduce rates of persistent hyperparathyroidism	Unclear effect on operative duration
Contrast-enhanced	Provides real-time information on parathyroid gland perfusion	Limited ability to distinguish between thyroid and parathyroid glands
	May help guide the selection of remnant gland for subtotal parathyroidectomy	Limited tissue penetration
	May reduce hypoparathyroidism/hypocalcemia after subtotal parathyroidectomy	Subjective interpretation of the degree of fluorescence/perfusion
	May improve the localization of parathyroid adenomas over traditional preoperative imaging techniques	ICG contraindicated in patients with iodine allergy
	Dosing may be safely repeated for bilateral neck exploration	Requires purchase of imaging system and dye
	Uptake of ICG is rapid in both normal and pathologic glands	Unclear effect on operative duration
	Rapid cycling between fluorescence imaging, white light, and fluorescence overlay	

ICG: Indocyanine green.

Summary and vision for application

Overall, the data supporting the use of both NIRAF and ICG FA in parathyroid surgery is limited, with no randomized trials or meta-analyses. There are several case series that seem to demonstrate superior sensitivity compared to traditional methods of preoperative localization and multiple systematic reviews that highlight the potential uses of these technologies. There remains a need for large cohort studies and randomized controlled trials to better support the widespread adoption of these techniques.

Because the data is limited, the optimal use of either or both NIRAF and ICG FA in parathyroid surgery is unclear. For surgeons who already use one or both technologies in their thyroid operations, they may prefer to continue integrating that technology into their parathyroid operations. However, the authors believe that ICG FA offers more advantages in parathyroid surgery than autofluorescence. First, pathologic parathyroid glands have variable autofluorescence, which may include heterogenous enhancement or global hyperenhancement[35]. This is particularly true in the diffuse hyperplasia of secondary hyperparathyroidism and Multiple Endocrine Neoplasia type 1 (MEN1). Second, the use of ICG can provide information on the perfusion status of the parathyroid glands and guide auto-transplantation or subtotal parathyroidectomy to reduce the risk of postoperative hypoparathyroidism.

DISCUSSION

Safe and successful endocrine surgery requires a detailed understanding of the relevant anatomy. However, even experienced endocrine surgeons sometimes experience difficulty identifying and preserving essential structures while removing the pathology. Fluorescence-guided surgery provides an adjunct that can aid surgeons in identifying relevant structures and minimizing complications.

In thyroid and parathyroid surgery, autofluorescence has been shown to reliably improve identification of the thyroid glands and differentiate between the parathyroid glands and surrounding tissues, including the thyroid gland, with randomized controlled trials demonstrating decreased rates of hypocalcemia following thyroidectomy[31,32]. Meanwhile, ICG FA may provide surgeons with information on the perfusion status of the parathyroid glands in addition to helping to identify them during parathyroidectomy. A randomized controlled trial has shown that prophylactic vitamin D and calcium supplementation may be safely foregone in patients with at least one well-perfused parathyroid gland by ICG FA post-thyroidectomy[44]. Other applications include selecting an appropriate gland for preservation during subtotal parathyroidectomy to minimize the risk of postoperative hypoparathyroidism and hypocalcemia.

Overall, the use of various fluorescence imaging techniques in endocrine surgery shows promise but requires further investigation. It has been shown to be safe and helpful in several applications, but optimal procedures and uses are only beginning to be defined. Large, well-designed randomized trials with long-term follow-up are needed to determine the utility of preventing permanent hypocalcemia in thyroid surgery and subtotal parathyroidectomy. Quality data regarding operative duration with fluorescence guidance compared to traditional surgery in all fields will also help to define its optimal use and cost-effectiveness, while leveraging artificial intelligence and advances in technology may decrease subjectivity[68]. Additionally, the potential of tissue-targeted fluorophores, such as for the identification of peripheral nerves in head and neck surgery, is still an emerging technology but has the potential to radically alter the landscape of FGS[69]. As FGS becomes more accessible and integrated into imaging platforms, perhaps the barriers to adopting the technology will fall, and fluorescence imaging will become even more broadly utilized by endocrine surgeons worldwide.

DECLARATIONS

Authors' contributions

Made substantial contributions to the conception of the project, oversaw the development of the review, and performed edits of the manuscript: Bouvet M

Performed literature search and review, drafted and edited the manuscript: Matson J

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Dr. Michael Bouvet serves as a consultant for Stryker, Inc. Dr. Jared Matson has no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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