Hybrid techniques for intraoperative sentinel lymph node imaging: early experiences and future prospects

Sentinel lymph node biopsy (SLNB) has become widely accepted as a standard of care in the surgical staging of the axilla in breast cancer patients, and it has been increasingly used as a staging procedure for various other malignancies such as melanoma, head and neck cancer, and gynecological and urogenital malignancies. SLNB accurately predicts regional nodal status, determines prognosis and is helpful in devising therapeutic strategies. In spite of these achievements, there is a need to advance the intraoperative identification beyond the current state-of-the-art. This will help facilitate surgeons to find sentinel lymph nodes (SLNs) more accurately. Accurate visualization appears to be essential during surgeries in complex areas, as frequently observed in malignancies with deep lymphatic drainage or with drainage to multiple basins. By placing the SLNs within their anatomical context, the introduction of SPECT/CT, in addition to lymphoscintigraphy, has significantly improved the generation of preoperative 2D and 3D surgical roadmaps. In addition, dedicated intraoperative tools, such as portable γ-cameras, help provide real-time imaging to surgeons regarding the location of the radioactive SLN. In recent years, fluorescent dyes were introduced to enable (superficial) fluorescence guidance towards the SLNs. However, in order to combine the two approaches, hybrid tracers are required to enable simultaneous radioactive and fluorescence detection. The initial experiences using these novel hybrid tracers, which preserve preoperative mapping with lymphoscintigraphy and SPECT/CT, for SLNB in patients with breast cancer, prostate cancer, melanoma and head and neck cancer, are discussed in this review. The hybrid approach for SLNB is confronted with both the conventional modalities and the use of single fluorescent tracers. Following the initial clinical experience, further validation of hybrid tracers must follow on larger series of patients on the basis of a protocol combining preoperative mapping (lymphoscintigraphy and SPECT/CT) with intraoperative hybrid signal detection using separate imaging devices for the detection of radioactive and fluorescent signals. It is expected that the use of hybrid tracers will lead to improved SLN detection; principally in complex anatomical areas or when SLNs are localized close to primary lesions.

Nodal status remains the single most important prognostic variable in the management of cancer. Cabañas was the first person who proposed the significance of cancer treatment management in relation to the sentinel lymph node (SLN) [1]. SLNs are defined as the first lymph nodes (LNs) in the regional lymphatic basins that receive lymph flow from the primary tumor. Assuming the orderly spread of metastasis through the lymphatic system, SLNs will be the first nodes to contain metastasis and their biopsy will accurately predict regional node status. SLN biopsy (SLNB) is accompanied by a low morbidity and for many patients with malignancies, such as breast cancer and melanoma, complete regional lymphadenectomy has been replaced by SLNB.

Currently, SLNB is widely accepted as an accurate predictor of regional nodal status, and, as such, in determining prognosis and devising therapeutic strategies in a variety of malignancies, such as breast cancer and melanoma. It has also been increasingly used as a staging procedure for head and neck cancer, and gynecological and urogenital malignancies.

In spite of these achievements, the evolution of the SLNB demands new technological improvements. The SLN concept assumes the function of LNs as effective tumor cell filters on direct drainage pathways. Therefore, all LNs with direct drainage from the site of the primary tumor are considered to be SLNs. In many cases, this factor does increase the complexity of the procedure because of the existence of aberrant routes of lymphatic drainage or due to the direct drainage from the primary tumor to multiple LN basins. For instance, in breast cancer SLNB, areas of alternative drainage, such as the internal mammary chain or around the clavicle,
often require a different approach compared with the axilla. In addition, in melanoma, the routes of lymphatic drainage are frequently multidirectional and sometimes unpredictable in areas such as the head, neck and trunk. The need to localize SLNs in complex anatomical areas has significantly increased with the introduction of the SLNB in head and neck cancer, prostate cancer, cervical cancer and other malignancies.

To facilitate the procedure adequately, in recent years preoperative lymphatic mapping has significantly improved with the introduction of SPECT/CT. Using this technique in addition to lymphoscintigraphy, surgeons may receive 2D and 3D information about the anatomical SLN localization. This is important, not only to adequately plan operations in the expected areas of drainage, but also to include other draining lymphatic basins to the operative strategy. Another significant practical advantage of SPECT/CT is that no additional tracer injections are required.

The necessity to preserve preoperative mapping led to the development of hybrid tracers for SLNB. Due to the radioactive component of these tracers, preoperative lymphoscintigraphy and SPECT/CT remains unmodified. In addition, the intraoperative SLNB procedure can significantly be improved by the incorporation of high-resolution images based on the fluorescent component of the tracer. Moreover, in the operating room, hybrid tracers are able to facilitate the combination of $\gamma$-probes and/or portable $\gamma$-cameras with devices adjusted to detect the fluorescent SLN signals.

One important objective of the use of hybrid tracers is to enable SLNB in complex areas of drainage such as the parotid/periauricular region (periauricular melanomas), the submandibular LNs (oral cavity cancer, melanomas of the cheek/skin) or in pelvic LN stations (prostate cancer). Failure to find the SLN in these areas may lead to an increase in the false-negative rate and clinical recurrences. For instance, in head and neck melanomas, a median false-negative rate of 20.4% has been determined in a meta-analysis of 32 studies and 3442 patients [2]. In another study concerning oral cavity cancer, the negative predictive value for carcinoma of the floor of the mouth was lower than for other malignancies (88.5 vs 95.4%); this was due to the difficulties in identifying SLNs in levels I and IIa of the neck [3].

The background of the hybrid approach, as well as its initial clinical validation in various malignancies, will be discussed in the present review. In order to better understand the properties and function of hybrid tracers, the application of single fluorescent tracers for SLNB will also be discussed. Furthermore, the hybrid approach will be evaluated in light of other technological advances for intraoperative procedures, such as mobile $\gamma$-cameras and sophisticated fluorescence cameras.

Preoperative SNL mapping

Lymphoscintigraphy

Almost 20 years after its introduction for the SLNB procedure, lymphoscintigraphy still remains the standard for preoperative lymphatic mapping. Based on its ability to identify LN basins at high risk for metastases, lymphoscintigraphy became a useful roadmap for surgeons, leading to both the improvement of the accuracy and the reduction of morbidity related to the use of handheld $\gamma$-probes for SLNB in the operating room.

Based on the use of large-field $\gamma$-cameras, lymphoscintigraphy is usually performed following the injection of radiocolloids (diameter ranging between 10 and 600 nm) in or surrounding the tumor. For breast cancer, tracer injection may be related to the skin (subcutaneous, intradermal), the aureola (periaurelar, subaureolar) or the tumor (around or into the tumor). For melanoma, head and neck cancers and other malignancies (e.g., penile, vulva and cervix), several injections are given, tracer dose is divided in three to four injections intradermally around the tumor. In deeply located malignancies (e.g., prostate), or when tumors are not palpable (e.g., breast), multiple injections are usually guided by ultrasound.

In line with the SLN concept, an important aspect of lymphoscintigraphy relies on its ability to detect LNs directly draining from the injection site. This is accomplished by the visualization of the lymphatic vessels. For this purpose, image acquisition must be sequential and, in cases with expected fast drainage (e.g., melanoma, penile cancer and head and neck cancer), a dynamic study, started as soon as possible after tracer administration, is mandatory. Current protocols are further based on the acquisition of early (in the first 30 min after injection) and late (mostly after 2 h) static planar images. This sequential protocol frequently leads to differentiate early draining LNs (the SLNs) from other higher echelon nodes, as is illustrated in Figure 1.

SPECT/CT

The standard conventional 2D lymphoscintigraphy does not always define the exact anatomical location of the SLNs. In particular,
in head and neck and intra-abdominal areas, localization of SLN is more difficult than elsewhere due to the complex anatomy and the presence of several vital structures which must be spared.

With the advent of SPECT/CT cameras, these problems can be fixed. SPECT is a tomographic version of conventional lymphoscintigraphy and the images have better contrast and resolution. When fused with CT, it provides anatomical information facilitating the preoperative planning of the surgical approach. In addition, SLNs close to the injection site, where high signal in the injection depots can hamper SLN detection on lymphoscintigraphy, SPECT/CT can help. 

SPECT/CT is principally orientated to the anatomical localization of SLNs. This is the principal reason why SPECT/CT is acquired using a low-dose CT. The use of a diagnostic high-dose CT, with or without intravenous contrast, is not necessary because the SLNB procedure primarily aims to detect subclinical metastases in unenlarged LNs. This category mainly includes small macrometastases (>2 and ≤5 mm) and micrometastases (>0.2 and ≤2 mm).

However, for SLN localization, the CT component of the SPECT/CT must be able to provide optimal anatomical information. This became possible with the second generation of SPECT/CT cameras and it enables the evaluation of the LNs corresponding with the radioactive nodes on fused SPECT/CT images with a low-dose (40 mAs) CT. For superficial areas such as the groin and the axilla, 5-mm slides are recommended. For more complex anatomical areas (e.g., head and neck, pelvis and abdomen) 2-mm slides may be necessary. With this approach SPECT/CT can accurately localize SLNs in relation to the vascular structures in deep anatomical areas.

The CT is also used to correct the SPECT signal for tissue attenuation and scattering. After these corrections, SPECT is fused with CT. A gray scale is used to display the morphology in the background image (CT), whereas a color scale is

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**Figure 1. Sentinel lymph node identification using lymphoscintigraphy and SPECT/CT in a patient with a melanoma of the left cheek.** (A) Early anterior and (B) lateral planar images show tracer drainage from the injection site with visualization of lymphatic ducts towards three lymph nodes (LNs) in the neck; another LN is seen on (B) the early lateral view very close to the injection site, and on (C) the delayed planar anterior image a fifth LN is depicted. (D) All these sentinel LNs are further localized on SPECT/CT with volume rendering. Axial fused SPECT/CT images show (E) the injection site, (F) the sentinel LNs submandibular, (G) contralateral in level II of the neck and (H) left supraclavicular.
used to display the SLN in the foreground image (SPECT) [5].

The display of SPECT/CT is similar to that of conventional tomography. Multiplanar reconstruction enables 2D display of fusion images in relation to CT and SPECT. The use of cross-reference lines allows the navigation between axial, coronal and sagittal views. At the same time, this tool correlates radioactive SLNs seen on fused SPECT/CT with LNs seen on CT. This information may be helpful for the intraoperative procedure and the postexcision control using portable γ-cameras or probes.

Fused SPECT/CT images may also be displayed using maximum intensity projection (MIP). MIP is a specific type of rendering in which the brightest voxels are projected into a 3D image, allowing improvement of anatomical SLN localization and recognition by the surgeon. One limitation of MIP is that the presence of other high-attenuation voxels on CT may obscure the recognition of the vasculature and other anatomical structures. Another limitation is that the MIP display is a 2D representation that cannot accurately depict the actual relationships of the vessels and other structures [6].

Using volume rendering for 3D display, different colors are assigned to anatomical structures such as vessels, muscle, bone and skin. This allows better anatomical reference points to be obtained and an additional dimension in the recognition of SLNs to be incorporated. By incorporating a color display, volume rendering improves the visualization of complex anatomy and 3D relationships (Figure 1).

**Comprehensive image interpretation in preoperative lymphatic mapping**

SPECT/CT does not replace lymphoscintigraphy and is mostly performed following delayed planar images (in general 2 h following tracer administration). SPECT/CT aims to anatomically localize SLNs already visualized on planar images. In some cases, SPECT/CT can detect additional SLNs. van der Ploeg et al. found that up to 14% of SLNs were only detected by SPECT/CT [7]. To understand the combined use of lymphoscintigraphy and SPECT/CT, it is necessary to specify the criteria for SLN identification on preoperative images. The major criteria in identifying LNs as SLNs are the visualization of lymphatic ducts, the time of appearance, the LN basin and the intensity of LN uptake [8,9].

Following these criteria, visualized radioactive LNs may be classified in three categories: definitely SLNs (all LNs draining from the site of the primary tumor through an own lymphatic vessel or a single radioactive LN in a LN basin); highly probable SLNs (LNs appearing between the injection site and a first draining node, or nodes with increasing uptake appearing in other LN stations); and less probable SLNs (all higher echelon nodes – in trunk and extremities – or lower echelon nodes – head and neck).

Early planar images of lymphoscintigraphy are essential to identify first-draining LNs as SLNs by the visualization of lymphatic ducts. First-draining nodes (category one) can be distinguished from secondary LNs (category three), which are mostly appearing on delayed planar images. In other cases, a single LN is seen on early and/or delayed images. This node is also considered as a definitively SLN (category one). However, in some cases, SPECT/CT can detect additional LNs in other basins. These nodes can be considered as definitively (category one) or highly probable (category two) SLNs. Less frequently, a radioactive LN may appear between the injection site and a first draining node; its increasing uptake can confirm this node as a highly probable SLN (category two), and it helps to differentiate this node from prolonged valve activity in a lymphatic duct.

The clinical relevance of SPECT/CT has recently been evaluated in melanoma. Among patients with clinically LN-negative melanoma, the use of SPECT/CT for SLNB was associated with a significantly higher frequency in the detection of metastatic SLN involvement and a higher rate of disease-free survival when compared with SLNB without SPECT/CT [10]. In addition, Covarelli et al. and Klode et al. previously demonstrated that SPECT/CT will significantly reduce operating time and costs [11,12].

SPECT/CT in breast cancer is indicated in cases with no SLN visualization on planar images of lymphoscintigraphy, and also to anatomically localize SLN in localizations (intramammary, interpectoral, intercostal and periclavicular) other than level I of the axilla [13]. In melanoma, SPECT/CT was also demonstrated to localize SLN in cases with aberrant drainage, as seen on lymphoscintigraphy or in cases with no visualization on planar images [14]. However, Stoffels et al. extended the indication to all patients receiving SLNB, due to the benefit in disease-free interval observed when SPECT/CT is incorporated into the protocol [10]. For head and neck malignancies, prostate cancer, cervical cancer and other malignancies, the use of SPECT/CT for SLN localization is
always indicated due to the essential anatomical information obtained by this modality [15].

**Conventional intraoperative SLN localization**

Over the past few decades, the SLNB procedure in the operating room has been based on the combination of visual and acoustic signals. Since the introduction of SLNB, the visual component is represented by the use of blue dyes, which enables both lymph duct visualization and the identification of the SLN. The acoustic component is the contribution of the γ-probe, which is able to instantaneously transform count-rates in auditory signals.

II Blue dye

Blue dyes are able to anatomically display lymphatic ducts and LNs. Following injection, blue dyes migrate from the injection site by diffusion. The chemical structure of methylene blue is illustrated in Figure 2. The transport through the lymphatic system can be accelerated by massaging the injection site. Depending on the distance between the injection site and the draining LN basin, SLNs are colored within 5–15 min. SLN washout often occurs after 30–45 min. In some centers without nuclear medicine facilities available for SLN detection with radioactive tracers, intraoperative SLN detection is based exclusively on vital blue dyes. In hospitals where radioactive tracers are available, intraoperative SLNB is mostly performed using the combination of blue dye and a γ-probe. Over the recent years, many studies demonstrated that the combined use of blue dye and radiocolloid is more accurate compared with the dye method alone. Radovanovic et al. found a sensitivity of 83% for SLNs found with blue dye, with respect to radiocolloid, with a sensitivity of 95% [16]. The prediction of SLNs using combined techniques had a significantly higher accuracy and facilitated quicker identification of the SLN intraoperatively [5,16,17]. A 10-year study of SLNB in head and neck tumors also showed a complementary success of the combined approach for SLNB; however, the radiocolocalization-based biopsy alone had the highest success rate (95%) [18].

The use of blue dyes has some limitations. For instance, the synchronization between injection and SLNB identification may be critical, often depending on the experience of the surgeon. In addition, blue staining of the skin can occur; although mostly temporary, it can lead to a cosmetic limitation, principally in the head and neck. Furthermore, in a small number of patients, blue dye was found to be associated with the occurrence of anaphylactic reactions; this is also the reason for its contraindication in pregnancy. Finally, blue dyes appear to be of limited value in identifying aberrant-draining SLNs.

II γ-probes

Intraoperative real-time γ-probe use allows acoustic detection of the tracer accumulated in the SLN. Radioactive tracers have no problems with tissue penetration because of their low tissue attenuation. In line with these properties, γ-probes must be able to combine high spatial resolution, to more precisely localize small LNs, with high sensitivity, to promptly guide surgeons to the SLN location in the surgical field. However, this technique can suffer from background signals from the injection site, especially when the SLN is located close to the injection site [19,20]. Vidal-Sicart et al. demonstrated that the conventional γ-probe sometimes failed to detect the SLN because of weak uptake of the tracer in the SLNs close to the injection site. SLNs at unclear locations and in case of a cluster of SLNs [21].

![Figure 2. Schematic representation of the various compounds allowing image-guided surgery.](image)
Advances in intraoperative SLN imaging

The introduction of SPECT/CT further improved preoperative SLN mapping and SLN identification. SPECT/CT is of great value in difficult anatomic areas such as the neck and abdomen and in patients with no visualization of a SLN on the planar lymphoscintigraphy. However, the localization of SLNs in complex anatomical areas requires real-time imaging technologies in the operating room to enable surgical navigation. In this context, there is an increasing use of intraoperative devices for image-guided surgery [22].

■ Intraoperative portable γ-cameras

The incorporation of portable γ-cameras (Figure 3) for the SLNB procedure is principally based on the need to facilitate real-time imaging and navigation during surgery by correlating portable γ-camera findings with those of preoperative lymphoscintigraphy and SPECT/CT. This enables surgeons to more accurately localize LNs previously identified as SLNs. By using a portable γ-camera, it has become possible to reproduce the findings of preoperative SLN mapping in the operation room, giving not only a general orientation of the field to be operated, but also of the target (SLN) within that field. In light of this, portable γ-cameras are more complementary to the γ-probe than its alternative.

Vidal-Sicart et al. preoperatively tested a portable γ-camera for the detection of the SLN(s) in patients with breast cancer [23]. The portable γ-camera showed less SLNs than the conventional γ-camera, but when they used a lead shield to mask the injection area, the detection rate raised to 88% for the portable γ-camera versus 95% for the conventional one, although this was not statistically significant [23]. The findings of Vidal-Sicart et al. led to the an important software adjustment in the new version of the used portable device (Sentinella, Oncovision, Valencia, Spain); currently it is possible to electronically mask the injection site in order to obtain a better, high-resolution, image of the radioactive SLNs. Dengel et al. reported a SLN detection sensitivity of 90% using a mobile γ-camera versus 97% with the fixed γ-camera [24]. A study by Kerrou et al. showed a good prediction and localization of SLNs when using a handheld γ-camera in breast cancer patients, it was found to be not inferior to the standard procedure based on conventional lymphoscintigraphy [25].

Another study by Vidal-Sicart et al. studied the value of intraoperative real-time imaging using a portable γ-camera in comparison with the conventional γ-probe [21]. They evaluated 20 patients with various malignancies (e.g., melanoma, breast and gynecologic cancers) with SLNs which were difficult to localize due to, for example, weak tracer uptake or being located near the injection site or at unclear locations. They found a higher intraoperative detection rate of the SLN in patients where the combination of both techniques was used. The portable γ-camera also helped to find the precise location of the SLN in four out of 20 patients [21].

The portable γ-camera can also be used to check the excision site for remaining radioactivity after the excision of the SLNs. Vermeeren et al. [22] found a 100% identification rate of all preoperatively identified SLNs in five patients with head and neck melanoma or oral cavity carcinoma. Intraoperatively, they found nine additional SLNs at difficult sites after excision of the primary tumor, of which one SLN contained tumor mass [4]. A feasibility study by Brouwer et al. also showed additional value of the intraoperative use of a portable γ-camera, in which the portable γ-camera identified additional SLNs in two patients compared with preoperative SLN mapping with lymphoscintigraphy and SPECT/CT [26]. With these results, it can be concluded that a portable γ-camera can find additional LNs, which are not found by the γ-probe; therefore, this technique can reduce false-negative LNs. False-negative rates may be reduced by the detection of additional SLNs during operations [27,28].

Vermeeren et al. also showed that a laparoscopic γ-probe in combination with a portable γ-camera enabled adequate real-time SLN identification in 90% of the patients with urological malignancies. They used an I-125 seed fixed on the tip of the laparoscopic γ-probe as a pointer. By matching the signals of the seed and the portable γ-camera, the exact location of the SLN was found. The portable γ-camera allowed the surgeon to check the removal of the radioactive SLNs and was found to be complementary to the intraoperative laparoscopic γ-probe [27,29].

In summary, the intraoperative use of a portable γ-camera can facilitate the SLNB procedure in all cases, but especially for SLNs at more difficult sites, such as near the injection area, next to another SLNs or deeper-lying SLNs which gives attenuation of the γ-signal.

■ Freehand SPECT (3D)

The need to generate imaging in the operating room led to the development of other alternative devices to portable γ-cameras, such as a freehand
SPECT probe in combination with a tracking system (Figure 4). From this, 3D reconstructions of radioactivity distributions are possible, which enables 3D imaging and navigation in the operating room. For this objective, surgeons need to move the γ-probe around the structure of interest (tumor or SLN). This movement of the probe is tracked by the tracking system. 3D data are acquired and allow display of depth of the target structure [29,30]. Owing to this 3D feature, which acquires display of depth, this camera has advantages in comparison with planar imaging generated by the portable γ-camera. A study by Wendler et al. showed that freehand SPECT can be integrated into the operating room without considerably changing the standardized workflow of finding the SLN with radiocolloid [31]. Second, after SLN excision, this technique could be used to confirm that all structures were resected. They found an accuracy of freehand SPECT of 77.8% (seven out of nine nodes) in scans with good quality, while for intermediate and poor quality scans, the accuracy reduced to 34.3 and 12.8%, respectively, when using SPECT/CT as reference. False-negative findings were related to insufficient scanning time, insufficient coverage of the axillary region, close proximity of the SLN to the injection site and low tracer uptake in the SLNs [31].

- **Single near-infrared dyes**

In some circumstances, the search for the SLN is challenging and time-consuming when using radiocolloids and/or blue dyes. Sometimes the SLN is difficult to localize, for example in head and neck cancer and melanoma where the SLN can be located close to the injection site where background activity may interfere with the tracer signal in the SLN. New techniques have been developed to deal with the limitations of planar lymphscintigraphy and γ-probe, especially for these challenging cases, and to deal with the disadvantage tattooing effect of the blue dyes. The introduction of hybrid imaging, such as SPECT/CT, and intraoperative portable devices are a step in the direction aiming to provide a better anatomical mapping for surgeons. For a similar objective, recently single fluorescent agents such as indocyanine green (ICG) and hybrid tracers such as ICG–99mTc nanocolloid were recently introduced.

Motomura et al. was the first group who described the technique near-infrared fluorescence imaging with ICG for detection of the SLN in breast cancer patients with an identification rate of 73.8% [17]. Once injected, the fluorophore ICG is strongly bound to serum proteins and takes several minutes to be transferred by the lymphatic vessels to the SLN. Owing to the small size of the particle, ICG has a velocity of several minutes.

![Figure 3. Mobile γ-camera detection of the sentinel lymph node(s) during sentinel lymph node biopsy for head and neck cancer. (A) With the mobile γ-camera (Sentinella, Oncovision, Valencia, Spain), (B) a pre-excision overview of the area of interest can be generated, allowing the localization of the radioactive hotspots (SLNs) with regard to T. (C) Following excision of the SLNs, postexcision γ-camera imaging confirms the removal of both SLNs. SLN: Sentinel lymph node; T: Injection site.](image-url)
The fluorophore ICG has a tissue penetration of <1 cm and, in the case of superficial laying SLNs, it enables a real-time transcutaneous and intraoperative visualization of both lymphatic vessels and the SLN(s). Owing to the dye is invisible to the naked eye, the surgical field will not be altered and the tattooing effect seen with the blue dye does not occur \[32–34\]. However, a dedicated imaging system (a fluorescence camera) is required to visualize the near-infrared fluorescence signal \[35\].

A disadvantage of ICG is its limited tissue penetration to a maximum of approximately 1 cm. This depth is further diminished in patients with a high BMI; Polom \textit{et al.} found only adequate percutaneous SLN visualization in patients with a BMI below 23 \[32\]. A second disadvantage is its poor retention in the SLNs because of its small hydrodynamic diameter. As such, ICG can easily pass through to second echelon nodes \[33\]. Hojo \textit{et al.} compared two methods for SLNB: the combination of ICG and radioisotope and the combination of ICG and blue dye \[36\]. They demonstrated that when using radiocolloid and ICG, 93.1\% of SLNs were fluorescent, whereas 100\% were radioactive. When comparing blue dye and ICG, 100\% of nodes were fluorescent. Only 92.9\% of these nodes were blue. From this study, the authors concluded that fluorescence was particularly beneficial when SLNs could not be appropriately identified with blue dye. However, on a per-patient basis, a larger number of SLNs were identified with ICG (3.8 vs 2.0 and 1.9 for ICG, radiocolloid and blue dye, respectively). Although this was suggested as a limitation of ICG, the minimal invasive nature of the SLNB was not affected. The authors did not report on statistical significance of their findings.

\begin{itemize}
  \item Single fluorescent agents in combination with other techniques
\end{itemize}

The use of ICG alone has the disadvantage of a lack of preoperative visualization of SLNs in different basins as it is possible with lymphoscintigraphy; this may lead to larger incisions in order to gain access to the affected area, and also to the inability to visualize the affected SLN in the presence of more than 1 cm of soft tissue coverage \[37\].

Motomura \textit{et al.} compared the use of ICG with the use of a combination of ICG and radioactivity in two separate injections \[17\]. The study showed a significantly higher success rate in SLNB when using the combination of both techniques compared with ICG alone, with a detection rate of 94.9\% versus 83.9\% when only ICG was used \[17\]. Several studies followed to optimize the ICG concentration for its use in SLNB, in order to achieve a better retention in the SLNs. Preclinical work demonstrated that adsorption of ICG to human serum albumin (HSA; ICG–HSA) increased fluorescence intensity and hydrodynamic diameter, therefore, providing improved detection in signal-to-background ratio (SBR) and better retention in the SLN \[30,34,35,38\].

Polom \textit{et al.} compared ICG and ICG–HSA with the standard of care and radiolabelled colloid, in patients with breast cancer \[32\]. In the patients who received ICG alone, ten more SLNs were compared with the standard. It is important to note that this increase in the amount of SLNs was not associated with upstaging of the disease.
When using ICG–HSA only, three more SLNs found compared with the standard. Binding ICG with HSA increased the molecular weight and, therefore, improved retention in the SLNs, which will reduce the number of unnecessarily removed second echelon nodes during surgical procedure. Furthermore, they found less interfering spread of ICG in the surgical field (on gloves and surrounding the resection area) when using ICG–HSA compared with ICG alone [32]. The group of Gioux et al. confirmed the improved kinetics with ICG bound to HSA [38]. By contrast, a study by Hutteman et al. did not find any significant improvement in the SBR of ICG–HSA compared with ICG alone (SBR: 8.4 vs 11.3, respectively; p = 0.18) [39].

Mieog et al. searched for the optimal concentration of ICG–HSA in patients with breast cancer and found a optimal concentration between 400 and 800 µM. There was a significantly lower SBR for concentrations below 400 µM and above 800 µM [34]. Similar findings were shown for melanoma [40].

In a randomized study by van der Vorst et al., ICG and 99mTc nanocolloid were compared with patent blue in 24 patients with breast cancer [41]. The surgeon did not use the γ-probe for the first 15 min. After 15 min, the γ-probe was used to identify the SLN(s) if the SLN was not localized, using only ICG or ICG in combination with patent blue. In 25% of patients, the γ-probe was needed to identify the SLN. The average BMI of patients in whom the γ-probe was needed was significantly higher than in patients in whom the probe could be omitted. No benefit of using patent blue for SLN mapping was found owing to a low detecting rate for SLNs of only 84% [41]. A recent review by Schaafsma et al. reported similar findings when comparing ICG (with radiocolloid) with blue dye [33].

- Near-infrared fluorescence cameras for intraoperative imaging

Near-infrared fluorescence is invisible to the naked human eye and, therefore, a dedicated near-infrared fluorescence camera is required. The camera excites the fluorophore with a one-wavelength light and, the fluorophore emits a different near-infrared wavelength in response, which is detected by the intensifier and displayed on the screen.

However, due to the interference between light and picture quality (and visualization ability), operating room lighting has to be dimmed as the near-infrared light is present in the operating theatre lights [1,32]. This can be overcome by using special light-emitting diode lights in the operating room, for example, the light-emitting diode Twin Beam (designed for Vision Inc., Ronkonkoma, NY, USA), which can be placed on the head of the surgeon to allow visualization of the operation field, while performing near-infrared fluorescence imaging.

There are several fluorescence cameras on the market (e.g., [mini]-FLARE™ [Beth Israel Deaconess Hospital, MA, USA], Fluobeam® [Fluoptics, Grenoble, France], SPY™ [Novadaq Technologies, ON, Canada], Storz™ [Karl Storz Endoskopes, Tuttlingen, Germany] and PDE™ [Photonics, Hamamatsu, Japan]), which can differ in the spectrally resolved light source and transfer camera and can serve for different types of open and laparoscopic surgeries (Figure 5) [35].

In addition, intraoperative navigation based on the optical tracking of a fluorescence endoscope may help to improve the accuracy of hybrid surgical guidance. Rieger et al. used reference targets fixed on the patient and on a endoscope and to generate a 3D view of the SPECT/CT data from the perspective of the endoscope, which allowed the endoscope to be navigated to the target lesions identified by SPECT/CT [42]. Although near-infrared fluorescence is only suboptimal for the localization of deep lesions, the hybrid navigation approach aims to combine navigation based on preoperative SPECT/CT data with real-time intraoperative near-infrared fluorescence detection.

The hybrid approach

One important shortcoming concerning the use of single fluorescent agents is the lack of preoperative mapping. The need to preserve preoperative mapping as an essential part of the SLN procedure led to the design of hybrid tracers for SLNB. Due to the radioactive component of these tracers, preoperative lymphoscintigraphy
and SPECT/CT are still possible. Additionally, the fluorescent component of the tracer enables high-resolution images in the operating room. Moreover, during the surgical act, hybrid tracers facilitate the combination of $\gamma$-probes and/or imaging portable $\gamma$-devices with fluorescence cameras. All these aspects led to the validation of a hybrid approach for SLNB.

**The hybrid tracer ICG–$^{99m}$Tc nanocolloid**

Small dyes such as ICG (ICG–HSA) and blue dyes do not accumulate in SLNs or have a very short lymph node retention; hence, these compounds can only be used to visualize real-time lymphatic transport at the operation room [43]. To overcome this shortcoming, the authors developed a multimodal colloid called ICG–$^{99m}$Tc nanocolloid. To form this compound, ICG was noncovalently bound to $^{99m}$Tc nanocolloid, resulting in a complex that is both radioactive and fluorescent (Figure 2). This hybrid complex allows SLN detection using preoperative visualization with lymphoscintigraphy or SPECT/CT images, and intraoperative radiotracing using a $\gamma$-probe, as well as intraoperative visualization with a fluorescence camera in just one injection. Enhanced signal intensity, with an 86-fold increase in SBR for the multimodal imaging agent over ‘free’ ICG was found in preclinical studies [25,30,44]. Moreover, preoperative SPECT/CT imaging demonstrated the same lymphatic dynamics, distribution and specificity as found with $^{99m}$Tc nanocolloid alone. The preclinical results were recently confirmed in a clinical study by Brouwer et al. comparing drainage patterns of this hybrid tracer ICG–$^{99m}$Tc nanocolloid with the current standard tracer in Europe, $^{99m}$Tc nanocolloid, in 25 patients with melanoma or penile carcinoma [45]. On the first day, standard SLN identification with $^{99m}$Tc nanocolloid (dynamic study and planar images at 10 min and 2 h after injection followed by SPECT/CT) was performed. In the same afternoon, or the following day, the procedure was repeated after injection of the hybrid tracer ICG–$^{99m}$Tc nanocolloid. The same SLNs were identified during the second scintigraphic procedure, yielding a high correlation between the radioactive counting rates in the SLNs of both scintigraphic studies. Intraoperatively, all preoperatively identified SLNs were localized using radio and fluorescence guidance. Of all SLNs, only 54% of the SLN were stained blue [45].

Van der Poel et al. published the first human study with the integrated multimodal method in 11 patients with prostate carcinoma with an increased risk of nodal metastasis, who underwent robot-assisted laparoscopic prostatectomy [19]. During surgery, the prostate and SLNs were excised followed by a retroperitoneal LN dissection. At 15 min and 2 h after the tracer injection, static planar $\gamma$-camera images were acquired, followed by SPECT/CT 2 h postinjection. At 15 min after injection, only 55% of the SLNs were visualized. The visualization rate of the SLNs increased to 91% after 2 h. During the surgical resection, the SLNs were identified in real time using a combination of a $\gamma$-probe and a near-infrared optimized fluorescence laparoscope. In this study, SLNs located in the prostatic fossa were hard to find owing to the high background signal of the radioactivity from the injection site, which prevented accurate $\gamma$-probe-based navigation. Those SLNs could, however, be identified via...

<table>
<thead>
<tr>
<th>Study (year)</th>
<th>Tumor type</th>
<th>$^{99m}$Tc (MBq)</th>
<th>Volume (ml)</th>
<th>Deposits (n)</th>
<th>Injection before operation (h)</th>
<th>Intraoperative detection rate using radioactivity (%)</th>
<th>Intraoperative detection rate using fluorescence (%)</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td>van der Poel et al. (2011)</td>
<td>Prostate</td>
<td>280</td>
<td>0.4</td>
<td>4 (two injection deposits in each prostate lobe)</td>
<td>3</td>
<td>96</td>
<td>89</td>
<td>[19]</td>
</tr>
<tr>
<td>Brouwer et al. (2012)</td>
<td>Melanoma and penile carcinoma</td>
<td>54–88</td>
<td>0.4</td>
<td>3–4</td>
<td>4–23</td>
<td>94</td>
<td>95</td>
<td>[45]</td>
</tr>
<tr>
<td>Brouwer et al. (2012)</td>
<td>Head and neck melanoma</td>
<td>70</td>
<td>0.4</td>
<td>4</td>
<td>4–6</td>
<td>93</td>
<td>93</td>
<td>[26]</td>
</tr>
<tr>
<td>van den Berg et al. (2012)</td>
<td>Oral cavity carcinoma</td>
<td>67–94</td>
<td>0.4</td>
<td>3–4</td>
<td>3–19</td>
<td>88</td>
<td>96</td>
<td>[47]</td>
</tr>
</tbody>
</table>
near-infrared fluorescence imaging. In areas where there was a less prominent radioactive signal, the fluorescent signal provided guidance during the last centimetres of surgical exploration. Due to the limited tissue penetration of the fluorescent signal, fluorescence imaging did not directly identify the SLNs in 15% of the cases. Here, \( \gamma \)-tracing helped to find these SLNs [19,46].

In two other studies by Brouwer et al. and van den Berg et al., the hybrid tracer was evaluated in the head and neck region during open SLNB [26,47]. Here, the addition of fluorescence imaging proved most valuable for SLNs located near the injection site; these nodes could not be accurately identified via \( \gamma \)-tracing because of the shine-through effect of the radioactive signal. However, the limited tissue penetration of the fluorescent signal of \(<0.5\) cm blocks background signals and, thus, the SLN could accurately be identified. These studies also underlined the additional value of the use of a portable \( \gamma \)-camera next to the \( \gamma \)-probe: additional, deeper lying SLNs that were not instantly picked up by fluorescence imaging could be detected. \textit{Ex vivo}, those SLNs were also fluorescent [26,47]. Table 1 gives an overview of the studies reported with the hybrid tracer ICG–\(^{99m}\)Tc nanocolloid in various malignancies.

**Conclusion**

Both the incorporation of SPECT/CT for preoperative lymphatic mapping and portable imaging devices for intraoperative surgical navigation have contributed to the extension of the SLN procedure to other malignancies. In many cases, SLNs must be excised in complex anatomical areas. To improve identification of these SLNs, single fluorophore agents and fluorescence imaging devices have been developed and validated in various malignant tumors. The need to preserve preoperative mapping as an essential part of the SLN procedure led to the design of hybrid tracers for SLNB. Preclinical and clinical studies have demonstrated that these tracers have a similar pattern of distribution and SLN uptake as conventional radiocolloids. This approach maintains the preoperative lymphoscintigraphy and SPECT/CT at the nuclear medicine department. During surgery, the combination of \( \gamma \)-probes and/or imaging portable \( \gamma \)-devices with fluorescence cameras has been shown to be feasible and particularly useful when the SLNs are close to the injection site. In other areas, such as intra-abdominal, fluorescence helps to identify the exact location of the SLNs with a higher precision. Following this initial clinical study, further clinical validation of hybrid tracers must be extended to larger series of patients on the basis of a protocol combining preoperative mapping (lymphoscintigraphy and SPECT/CT) with intraoperative hybrid signal detection using separate imaging devices for detection of radioactive and fluorescent SLN signals. It is expected that the use of hybrid tracers will lead to improved SLN detection, principally in complex anatomical areas or when SLNs are localized close to primary lesions. This will help to reduce false-negative rates and the number of recurrences without an extension of the procedure.

**Future perspective**

Hybrid tracers using both acoustic and visual modalities will improve intraoperative imaging for SLNB. The technique is especially valuable in areas where the SLN is located near the primary tumor (the injection site) or when the SLN is
Papers of special note have been highlighted as:

**of interest**
- Describes the use of indocyanine green for sentinel lymph node (SLN) mapping in breast, gastric, colorectal, anal and lung cancer.
- Discusses the additional value of SPECT/CT compared with the conventional lymphoscintigraphy. Where lymphoscintigrams are not always clear, SPECT/CT allows anatomical localization of the SLNs. SPECT/CT also allows the identification of sentinel lymph nodes missed with lymphoscintigraphy.

**Financial & competing interests disclosure**

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**References**

Papers of special note have been highlighted as:

* of interest
** of considerable interest


Following the preclinical papers of van Leeuwen et al. [30] and Buckle et al. [44], this is the first clinical paper describing the use of the hybrid tracer indocyanine green–99mTc nanocolloid for the detection of SLNs during robot-assisted laparoscopic prostatectomy.


Hybrid techniques for intraoperative sentinel lymph node imaging


39 Discusses the ‘ideal’ near-infrared contrast agent and near-infrared camera system. In addition, the authors discuss the indications that could benefit from near-infrared imaging, such as SLN biopsy, bile duct visualization and flap reconstructions.


44 Discusses the various types of radioactive- and fluorescence-labeled nanoparticles that have been developed in a preclinical setting for image-guided surgery.


46 The application of indocyanine green–99mTc nanocolloid for SLN biopsy was studied and compared with ‘free’ indocyanine green in an orthotopic mouse model for breast cancer.


48 Compared the hybrid tracer indocyanine green–99mTc nanocolloid with the conventional 99mTc nanocolloid and showed that the drainage pattern of both is similar. By using indocyanine green–99mTc nanocolloid, preoperative SLN mapping can be combined with intraoperative radio- and fluorescence-guided SLN biopsy.


51 In 14 patients, the use of indocyanine green–99mTc nanocolloid for sentinel lymph node biopsy of oral cavity carcinoma was evaluated. The use of the fluorescent signature of the hybrid tracer was found to be especially valuable when nodes were located close to the injection site.