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ORIGINAL ARTICLE

First-in-human evaluation of a hybrid modality that allows combined radio- and (near-infrared) fluorescence tracing during surgery

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Abstract
Purpose The clinical introduction of the hybrid tracer indocyanine green (ICG)-^{99m}Tc-nanocolloid, composed of a radioactive and a near-infrared (NIR) fluorescence component, has created the need for surgical (imaging) modalities that allow for simultaneous detection of both signals. This study describes the first-in-human use of a prototype opto-nuclear probe during sentinel node (SN) biopsy using ICG-^{99m}Tc-nanocolloid.
Methods To allow for fluorescence tracing, a derivative of the conventional gamma probe technology was generated in which two optical fibers were integrated to allow for excitation (785 nm) and emission signal collection (> 810 nm). The ability of this opto-nuclear probe to detect the fluorescence signal of the hybrid tracer ICG-^{99m}Tc-nanocolloid was firstly determined ex vivo in (non)SNs samples obtained from 41 patients who underwent hybrid tracer-based SN biopsy in the head and neck or urogenital area. In an in vivo proof-of-concept study in nine of these 41 patients, SNs were localized using combined gamma and fluorescence tracing with the opto-nuclear probe. Fluorescence tracing was performed in a similar manner as gamma tracing and under ambient light conditions.
Results Ex vivo, the gamma tracing option of the opto-nuclear probe correctly identified the SN in all 150 evaluated (non)SN samples. Ex vivo fluorescence tracing in the low-sensitivity mode correctly identified 71.7 % of the samples. This increased to 98.9 % when fluorescence tracing was performed in the high-sensitivity mode. In vivo fluorescence tracing (high-sensitivity mode) accurately identified the SNs in all nine patients (20 SNs evaluated; 100 %).
Conclusion This study demonstrates the first-in-human evaluation of a hybrid modality capable of detecting both gamma and fluorescence signals during a surgical procedure. Fluorescence tracing could be performed in ambient light.

Keywords Radioguided surgery · Fluorescence · Sentinel node · Multimodal · Hybrid imaging · Gamma probe

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Introduction

In Europe, sentinel node (SN) biopsy is routinely performed via a radioguided surgery approach that involves a technetium-99m-labeled colloid (standard in Europe: ^{99m}Tc-nanocolloid) [1]. For some indications, radio guidance is accompanied by the intraoperative use of a blue dye that aids visual identification of the SNs and their draining lymphatic vessels. Although this procedure can be considered relatively straightforward, e.g. for the identification of SNs in the axilla, for the identification of SNs in the head and neck or in the pelvic area several issues remain to be resolved: *i*) the

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complexity of the anatomy and the abundance of delicate anatomical structures that surround the SN may complicate intraoperative SN detection; *ii*) blue dye is of limited value in these areas and may obscure resection margins [2]; and *iii*) detection of an SN located in close proximity to the injection site may be hampered due to the high radioactive background signal coming from the injection site [3]. The integrated use of the near-infrared (NIR) dye indocyanine green (ICG) and ^{99m}Tc-nanocolloid in the form of the hybrid tracer ICG-^{99m}Tc-nanocolloid [4] was shown to extend the conventional radioguided technology. For example, it showed promise in optical identification of SNs in the parotid gland [5] and near the injection site [5, 6]. Moreover, fluorescence imaging of the hybrid tracer demonstrated improved detection sensitivity compared to blue dye [7, 8].

It was recently reported that a conventional gamma probe could be modified to allow for fiber-based illumination and detection of light. This initial modification allowed optical tracing of patent blue V via light absorption measurements [9]. For the current study, we modified the optical properties of this set-up in order to excite and detect ICG (laser-λ_{ex}: 785 nm and λ_{em}: > 810 nm). We evaluated this prototype opto-nuclear probe for gamma tracing and NIR fluorescence tracing during ICG-^{99m}Tc-nanocolloid-based SN biopsy in patients with head and neck, penile, and prostate cancer.

Materials and methods

Opto-nuclear probe

From an engineering perspective, the prototype opto-nuclear probe (Eurorad S.A., Eckbolsheim, France) itself is highly similar to the conventional gamma probe. However, for excitation of ICG, a narrow-band 785 nm laser excitation source was integrated into the device. In addition, for detection of the fluorescent emission signal of ICG, and to exclude reflected laser light, a broadband cut-off filter (> 810 nm) was placed in front of the detector. An adjustable photomultiplier tube (PMT; range: 0.7 V (1.0×10^5; low-sensitivity mode) to 0.9 V (1.0×10^6; high-sensitivity mode)) was integrated into the opto-nuclear probe to fine-tune the sensitivity for fluorescence tracing. The resulting prototype opto-nuclear probe is shown in Fig. 1.

Fluorescence sensitivity measurements in a phantom set-up

^{99m}Tc-nanocolloid was prepared by adding 400 MBq pertechnetate in saline to a vial of nanocolloid (GE Healthcare, Leiderdorp, The Netherlands). To form the hybrid tracer, 50 μL of a 5 mg/mL ICG/sterile water for injection solution (ICG-Pulsion, 25 mg vial; Pulsion Medical Systems, Munich, Germany) was added to the ^{99m}Tc-nanocolloid vial.

To obtain an indication for the optimal concentration range wherein fluorescence tracing can be performed, and to determine the correlation between gamma and fluorescence tracing using the opto-nuclear probe, 121.91 MBq of the hybrid tracer ICG-^{99m}Tc-nanocolloid (containing 0.125 mg/mL ICG) was diluted with saline in 11 steps down to 0.22 MBq (containing 0.061 ng/mL ICG). The dilution range was prepared in Eppendorf tubes containing a total volume of 0.4 mL. After each dilution step, the amount of radioactivity present in the Eppendorf tube was counted in a dose calibrator (CII radioisotope calibrator CRC®-5, Capintec Inc., Montvale, NJ, US). Subsequently, gamma tracing was performed by placing the head of the opto-nuclear probe directly in front of the Eppendorf tube. The 5-s and 2-s count rates were then measured in triplicate.

Thereafter, 10 μL of each step of the dilution range was pipetted onto a paper towel and air-dried (in the dark). Fluorescence tracing was performed by placing the tip of the opto-nuclear probe on the surface of the paper towel. Both the 5-s and 2-s count rates were measured with the PMT set at 0.7 and at 0.9 V (low- and high-sensitivity mode, respectively). Measurements were performed in triplicate. The correlation (r^2 value; linear regression analysis) between the results obtained with the gamma tracing and fluorescence tracing mode of the opto-nuclear probe was calculated in the linear range of the measured hybrid tracer dilution range.

To provide a quantitative reference for the fluorescence emission signal, 100 μL of each step of the hybrid tracer dilution range was transferred into a black 96-well plate (Cellstar, Greiner Bio-One GmbH, Frickenhausen, Germany), and a fluorescence emission curve was measured with a PerkinElmer LS 55 fluorescence spectrophotometer (λ_{ex}: 760 nm and λ_{em}: 800 nm; PerkinElmer Health Sciences B.V., Groningen, The Netherlands). The correlation (r^2 value; linear regression analysis) between the results obtained with fluorescence tracing using the opto-nuclear probe and the spectrophotometry results was calculated in the linear range of the measured hybrid tracer dilution range.

To determine the responsiveness of the opto-nuclear probe to blue dye, a dilution range of patent blue V dye (Laboratoire Guerbet, Aulnay-Sous-Bois, France) was generated by diluting 25 mg/mL patent blue V dye with sterile water for injection in 13 steps down to a final concentration of 3.1 ng/mL. The dilution range was prepared in a black 96-well plate (Cellstar), and the fluorescence emission signal was measured with the LS 55 fluorescence spectrophotometer (λ_{ex}: 610 nm and λ_{em}: 670 nm; PerkinElmer). Subsequently, 10 μL of each step of the dilution range was pipetted onto a paper towel and air-dried (in the dark). Thereafter, fluorescence tracing was performed as described above.

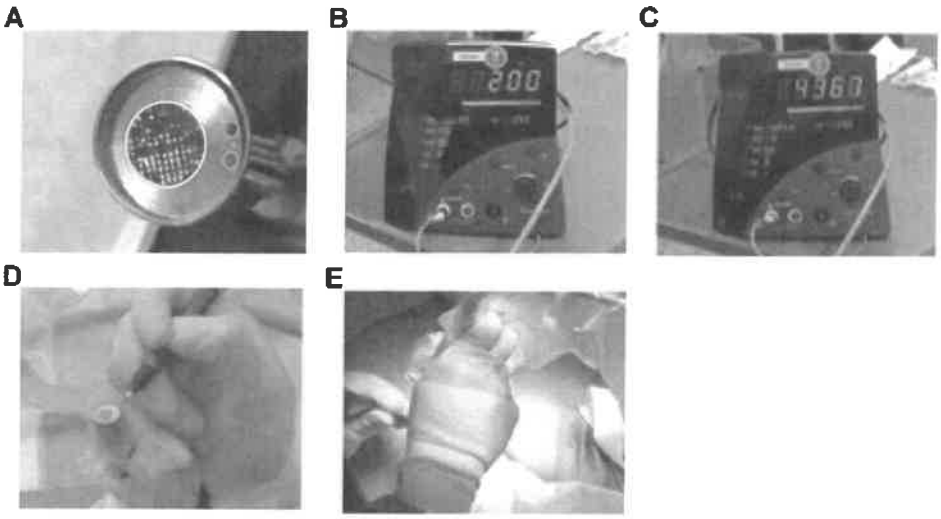


Fig. 1 Prototype opto-nuclear probe. (**a**) In the head of the prototype opto-nuclear probe, next to the crystal for gamma detection (yellow circle), two optical fibers are embedded (green circles). (**b**, **c**) Traced gamma (**b**) and near-infrared fluorescence (**c**) signals are presented to the surgeon via an acoustic output. (**d**) Intraoperatively, to effectively perform near-infrared fluorescence tracing using the opto-nuclear probe, the location of the optical fibers is marked on the probe. (**e**) Intraoperative fluorescence tracing is performed in a similar way as conventional gamma tracing

Patients

Between January 2013 and April 2014, 150 nodal samples from 41 patients who underwent hybrid tracer-based SN biopsy (head and neck [n=13], penile [n= 23], and prostate cancer [n=5]) were collected for ex vivo analysis using the opto-nuclear probe. Of these 41 patients, the opto-nuclear probe was evaluated in vivo in nine patients (head and neck [n=2] or penile cancer [n=7]). The study protocol was approved by the local medical ethical committee, and written informed consent was obtained from all patients in whom the opto-nuclear probe was evaluated intraoperatively.

Clinical tracer preparation and sentinel node biopsy procedure

For illustration, a schematic overview of the pre- and intraoperative SN biopsy procedure is provided in Fig. 2. ICG-^{99m}Tc-nanocolloid preparation, administration, preoperative SN mapping, routine surgical guidance (gamma tracing and fluorescence imaging) and (histo)pathological evaluation for head and neck [5, 6], penile [7], and prostate [10] cancer were performed as previously described.

In patients with penile cancer, an injection with patent blue V dye was given intraoperatively [7]. In patients with head and neck or prostate cancer, no patent blue V dye was used [5, 6, 10].

Ex vivo sentinel node measurements

Similar to our previous studies, during the operation, nodal samples were evaluated by the surgeon as being radioactive (yes or no), fluorescent (yes or no) and, in patients in whom blue dye was used, blue (yes or no) [5–7, 10].

To determine the sensitivity of the opto-nuclear probe on human tissue, after excision, nodal samples were evaluated using the opto-nuclear probe. Firstly, gamma tracing was performed with 5-s count rates measured. After switching the settings to the fluorescence mode, fluorescence tracing was performed with the PMT set at 0.7 V (low-sensitivity mode). In the case that a node positive with gamma tracing could not be fluorescence-traced, the PMT was increased to 0.9 V (high-sensitivity mode) and measurements were repeated. All measurements were performed in triplicate. The results were compared to those obtained with our conventional approach (gamma tracing [Neoprobe; Johnson & Johnson Medical B.V., Amersfoort, The Netherlands, or Europrobe; Eurorad S.A.] and fluorescence imaging [PDE; Hamamatsu Photonics K.K., Hamamatsu, Japan]) and scored for correct or incorrect prediction by the opto-nuclear probe.

In all experiments, fluorescence tracing with the opto-nuclear probe was performed in ambient light.

Intraoperative sentinel node identification using the opto-nuclear probe

The opto-nuclear probe was evaluated intraoperatively in nine patients scheduled for SN biopsy (Table 1). Operations were performed by six different surgeons.

During the operation, the SN was first pursued with the gamma tracing option of the opto-nuclear probe. After localization of the node, the opto-nuclear probe was switched to the fluorescence tracing mode. Here, the PMT was initially set at

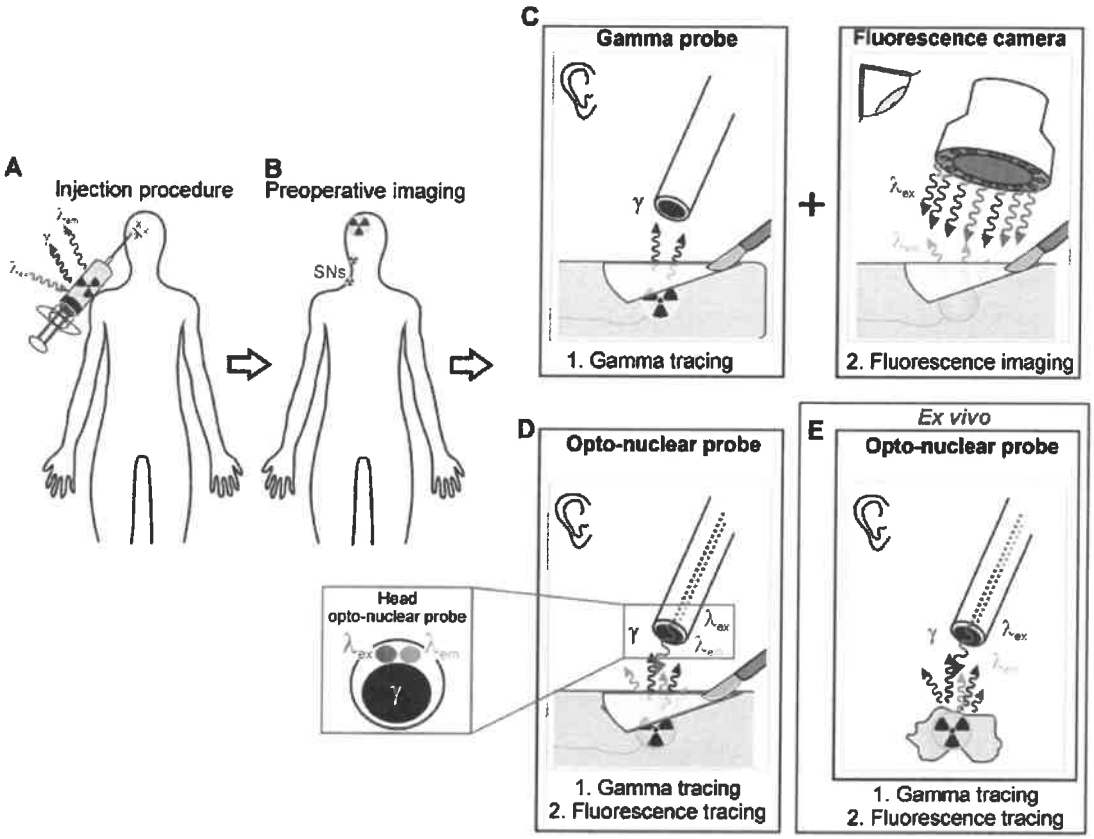


Fig. 2 A hybrid approach for intraoperative lesion identification. Schematic overview of the sentinel node biopsy procedure using a hybrid tracer composed of a radioactive and a near-infrared fluorescence moiety. Following hybrid tracer injection (**a**), preoperative imaging (lymphoscintigraphy and single photon emission computerized tomography [SPECT-CT] imaging) (**b**) is performed to identify the lesion of interest. Intraoperatively, the conventional approach (**c**) consists of the use of a gamma probe (gamma tracing of the lesion) and a near-infrared fluorescence camera (visualization of the fluorescence signal in the lesion). With the newly proposed approach, one modality is used that allows both gamma tracing and near-infrared fluorescence tracing (**d**). The opto-nuclear probe can be used both in vivo and ex vivo and (**e**) to evaluate the gamma and near-infrared fluorescence signal

0.9 V (high-sensitivity mode). When a signal was obtained, the PMT voltage was reduced to 0.7 V (low-sensitivity mode). Fluorescence tracing with the opto-nuclear probe was performed in ambient light.

Validation of the in vivo fluorescence signal was performed using a handheld fluorescence camera (PDE; Hamamatsu Photonics K.K.). For the detection of the fluorescence signal using the fluorescence camera, the lights in the operation theatre had to be dimmed. Obtained results were scored for correct or incorrect prediction by the opto-nuclear probe.

After excision of the SN(s), ex vivo SN measurements were performed as described in the "Ex vivo SN measurements" section above.

Results

Since gamma tracing of radiolabeled colloids (e.g., the hybrid tracer ICG-^{99m}Tc-nanocolloid) is already a well-known and thoroughly proven surgical guidance method, the current study focused on the evaluation and validation of the novel fluorescence tracing mode of the opto-nuclear probe. Initially, the system was evaluated in a phantom set-up. These findings provided the basis for the evaluation of the opto-nuclear probe technology on resected nodal samples. As a last step, we evaluated the technology in an image-guided surgery set-up during SN biopsy.

Sensitivity measurements in a phantom set-up

In the phantom experiments, where a dilution range of ICG-^{99m}Tc-nanocolloid was measured using the opto-nuclear probe, a strong correlation was found between gamma and fluorescence tracing intensities (Fig. 3a and b, respectively). With the PMT set at 0.7 V (low-sensitivity mode), r^2 values of 0.73 and 0.91 were found for the measured 5-s and 2-s count rates, respectively (Fig. 3c). At 0.9 V (high-

Table 1 Characteristics of the patients included for intraoperative opto-nuclear probe evaluation

	Age	Sex	Primary tumor	Time from injection to surgery (hours)	No. SNs evaluated with ONP/total no. preoperatively defined SNs	Location of ONP-evaluated SNs	Opto-nuclear probe			Blue dye	Pathology: SN+ or SN−
							Gamma tracing	Fluorescence tracing			
								0.9 V	0.7 V		
1	73	M	SCC penis	7.25	3/3	L: 2x groin R: groin	3	3	1	2	Negative
2	65	M	SCC penis	5.25	2/3	L: 2x groin	2	2	0	2	Negative
3	71	M	SCC penis	6.25	1/2	L: groin	1	1	0	1	Negative
4	53	M	SCC penis	4	2/2	L: groin R: groin	2	2	1	2	Negative
5	59	M	SCC penis	22.75	4/4	L: 2x groin R: 2x groin	4	4	2	-	Negative
6	63	M	SCC penis	18	2/2	L: groin R: 2x groin	2	2	2	-	Negative
7	66	M	SCC tongue L	4	2/4	L: level IA, IB	2	2	1	-	Positive (2x)
8	54	F	Merkel cell cheek L	4.5	1/1	L: level II	1	1	0	-	Negative
9	64	M	SCC penis	20.5	3/3	L: 2x groin R: groin	3	3	0	3	Negative
Average	64			9.75							
Total					20/24		20/20 (100 %)	20/20 (100 %)	7/20 (35.0 %)	10/11 (90.9 %)	

SNs sentinel nodes, *ONP* opto-nuclear probe, *SN+* tumor-positive sentinel node, *SN−* tumor-negative sentinel node, *SCC* squamous cell carcinoma, *M* male, *F* female, *L* left, *R* right

sensitivity mode), however, continuous oversaturation of the opto-nuclear probe prevented us from obtaining an r^2 value.

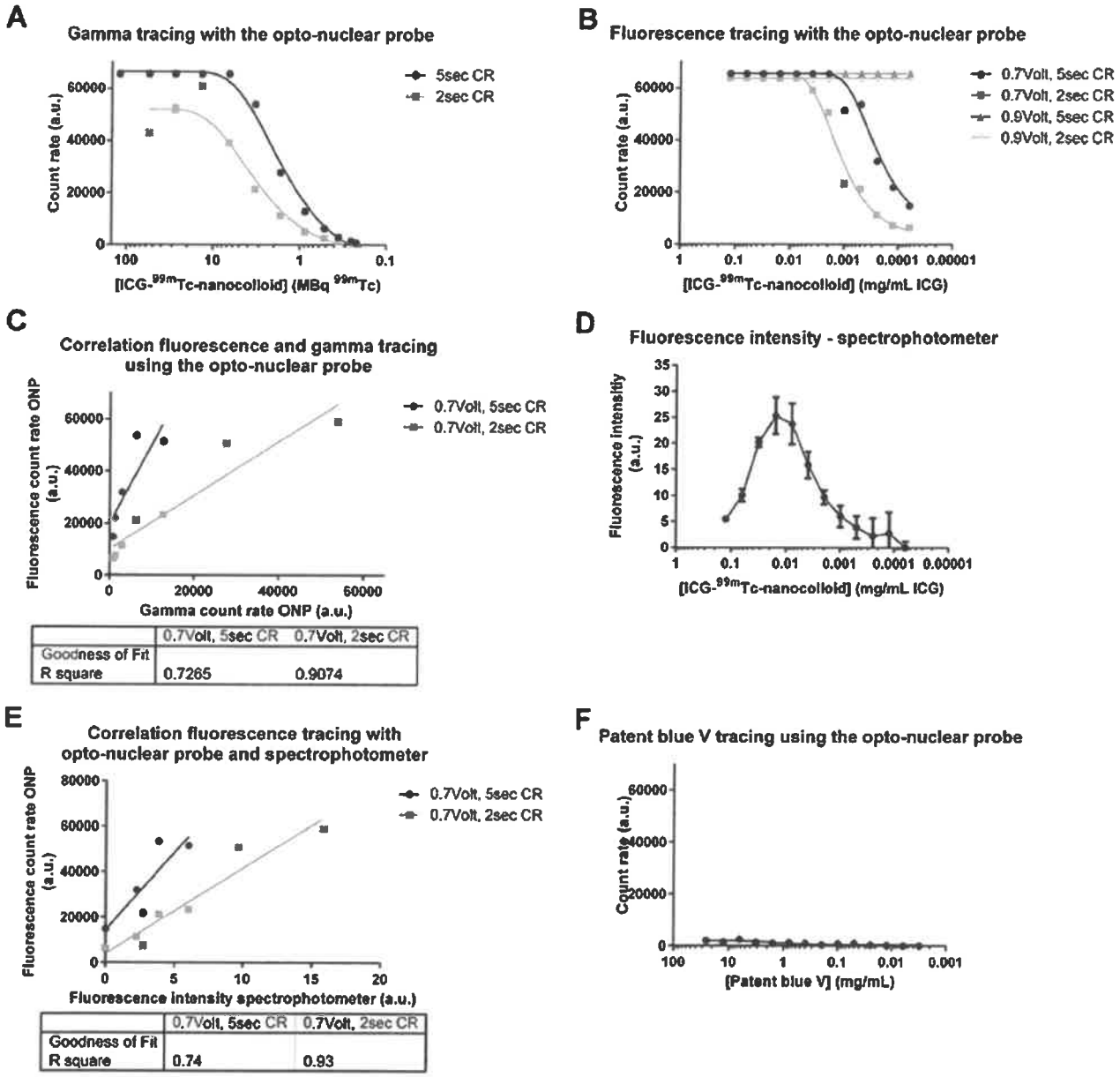
A comparison between the fluorescence count rates obtained with the opto-nuclear probe (Fig. 3a) and the signal intensities measured with a fluorescence spectrophotometer (the standard method for fluorescence intensity quantification) (Fig. 3d) gave an r^2 of 0.74 at a 5-s count rate, which increased to 0.93 at the 2-s count rate (Fig. 3e). Again the r^2 could not be calculated at 0.9 V (high-sensitivity mode) due to signal oversaturation. The fluorescence spectrophotometer measurements showed that the fluorescence peak intensity was highest at the concentration range 7.0-30.0 μg/mL ICG-^{99m}Tc-nanocolloid (Fig. 3e).

With the PMT set at 0.7 V (low-sensitivity mode) the presence of patent blue V dye in the samples did not interfere with fluorescence tracing. However, when the PMT was set to 0.9 V 9 (high-sensitivity mode), a continuous number of background counts was measured at various concentrations of blue dye (ranging 25 mg/mL-3.1 ng/mL; Fig. 3f). Surprisingly the fluorescence spectrophotometer did not record any additional fluorescence signal for patent blue V dye.

Ex vivo sample evaluation

A total of 150 nodal samples from 41 patients were analyzed ex vivo. All SNs (n=131) were radioactive and fluorescent. The 19 additionally excised samples (referred to as non-SNs) were not radioactive and not fluorescent and functioned as negative control.

During ex vivo evaluation (Fig. 4a, b), all radioactivity-containing nodes were able to be gamma traced with the opto-nuclear probe. The fluorescence tracing efficacy, however, was dependent on the PMT settings. With the PMT set at 0.7 V (low-sensitivity mode), merely 70.7 % of the evaluated nodes could be correctly staged for their fluorescent content (Fig. 4c). A strong increase in detection sensitivity to 98.9 % was achieved by adjusting the PMT voltage to 0.9 V (high-sensitivity mode; Fig. 4c). Most of the nodal samples



	0.7Volt, 5sec CR	0.7Volt, 2sec CR
Goodness of Fit R square	0.7265	0.9074

	0.7Volt, 5sec CR	0.7Volt, 2sec CR
Goodness of Fit R square	0.74	0.93

Fig. 3 Phantom experiments evaluating the opto-nuclear probe. (**a**) A dilution range of the hybrid tracer ICG-^{99m}Tc-nanocolloid was prepared (x-axis), after which gamma tracing using the opto-nuclear probe was performed. The 2-s and 5-s count rates were measured during performance of opto-nuclear probe-based gamma tracing (y-axis) of the hybrid tracer. (**b**) A dilution range of the hybrid tracer ICG-^{99m}Tc-nanocolloid was prepared (x-axis), after which near-infrared fluorescence tracing using the opto-nuclear probe was performed. For near-infrared fluorescence tracing, 2-s and 5-s count rates were measured with the PMT set at 0.7 V (low sensitivity) and 0.9 V (high sensitivity) (y-axis). (**c**) The correlation between the counts measured via gamma tracing (x-axis) and near-infrared fluorescence tracing (y-axis) was calculated. (**d**) The hybrid tracer dilution range (x-axis; concentration hybrid tracer) was also measured on a fluorescence spectrophotometer (y-axis, fluorescence intensity in a.u.). (**e**) Correlation between opto-nuclear probe-based near-infrared fluorescence tracing (x-axis) and measurements of the fluorescence spectrophotometer (y-axis) when evaluating the hybrid tracer dilution range. (**f**) Opto-nuclear probe-based near-infrared fluorescence tracing of a dilution range of patent blue V dye (x-axis) with the PMT set at 0.9 V (high-sensitivity mode). Five-second count rates were measured (y-axis). In the low-sensitivity mode (PMT set at 0.7 V), no fluorescence signal was detected with the opto-nuclear probe. ICG indocyanine green, ONP opto-nuclear probe, a.u. arbitrary units, sec seconds, CR count rate, PMT photomultiplier tube

contained some additional dissected tissue (see Fig. 4b), which influenced the fluorescence tracing efficacy. For example, in contrast to the above-described phantom measurements, the influence of the tissue in the nodal samples resulted in a poor correlation between the signal intensities measured using gamma and fluorescence

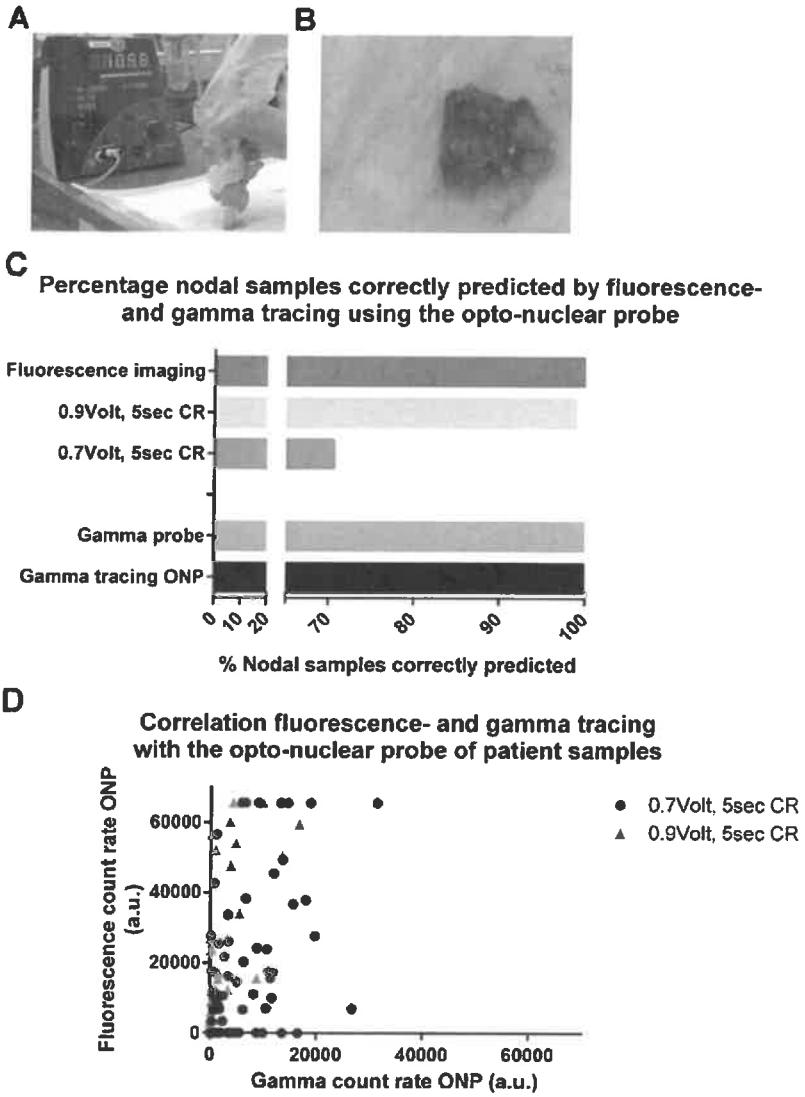


Fig. 4 Patient sample evaluation with the opto-nuclear probe. (**a**) Illustration showing ex vivo fluorescence tracing of a sentinel node. (**b**) Sentinel node illustrating that not only the sentinel node is excised but also some surrounding (fatty) tissue. (**c**) Percentage of sentinel nodes correctly predicted with the opto-nuclear probe. Of the 150 evaluated nodal samples, gamma tracing and near-infrared fluorescence tracing results obtained with the opto-nuclear probe were compared to the results obtained with the conventional gamma probe and near-infrared fluorescence camera, respectively. (**d**) 150 Nodal samples were evaluated with the opto-nuclear probe. The graph shows the correlation between count-rates measured for gamma tracing and near-infrared tracing using the opto-nuclear probe. ONP opto-nuclear probe, a.u. arbitrary units, CR count rate, sec seconds

tracing (Fig. 4d). The presence of patent blue V dye in the nodal samples did not influence the fluorescence tracing.

Intraoperative sentinel node identification using the opto-nuclear probe

We set out to evaluate the potential of the modality to provide in vivo guidance in the form of gamma and fluorescence tracing in nine patients scheduled for SN biopsy. Findings are specified in Table 1.

Intraoperatively, 20 SNs were evaluated with the opto-nuclear probe (Fig. 1). Similar to the results of the ex vivo situation, the gamma tracing option of the opto-nuclear probe allowed localization of 100 % of the SNs, and fluorescence tracing of all of these SNs (100 %) was possible when the PMT was set to 0.9 V (high-sensitivity mode). At low-sensitivity mode (0.7 V), however, accurate fluorescence tracing with the opto-nuclear probe was achieved for only seven of the 20 nodes (35.0 %). This detection percentage is markedly lower than that obtained at these settings in the above-described ex vivo measurements. In contrast to the findings in the phantom set-up, in vivo, the presence of patent blue V dye did not lead to false-positive results at 0.9 V (high-sensitivity mode).

The surgeons found the use of the fluorescence tracing option of the opto-nuclear probe to be intuitive. The handling, method of fluorescence tracing, and acoustic read-out (Fig. 1) were similar to that of the conventional gamma probe that is routinely used for SN identification. To improve the ease of probe placement during the operation, the location of the optical fibers was marked on the opto-nuclear probe with a marker pen (Fig. 1d). Making circularly rotating movements while

performing fluorescence tracing allowed scanning of a larger area and easier detection of the fluorescence signal. This effect was strengthened in the high-sensitivity mode (PMT set at 0.9 V). Importantly, the fact that the technology was effective in ambient light conditions had a minimal influence on surgical logistics. The ability to get a quantitative feel of the fluorescence signal intensity (counts) in relation to the gamma tracing findings was considered advantageous. However, the inability to visualize the SNs was considered a disadvantage by some surgeons.

Discussion

To the best of our knowledge, this is the first clinical study reporting on a hybrid modality that allows for combined gamma and NIR fluorescence tracing during a surgical procedure. Following the positive results of the initial phantom and ex vivo evaluation experiments, our first-in-human study in nine patients nicely illustrated the clinical feasibility of this technology. The integration of the fluorescence tracing option into an existing modality saves both additional investment and valuable space in the operation theatre. It should be apparent, however, that larger clinical evaluation studies are needed to determine the true clinical potential of this technology.

Interestingly, the different experiments performed illustrated a small discrepancy in findings among the phantom, ex vivo and in vivo setting. For example, at 0.9 V (high-sensitivity mode), the phantom experiments indicated that detection of the fluorescent signal was oversaturated, making 0.7 V (low-sensitivity mode) the preferred setting for the fluorescence tracing measurements. Moreover, at 0.9 V, the presence of patent blue V dye induced a background signal during fluorescence tracing. Ex vivo, however, we found that the fluorescence tracing ability was improved when switching from low-sensitivity (PMT set at 0.7 V) to high-sensitivity (0.9 V) mode (from 70.7 % to 98.9 %, respectively), and no influence of patent blue V dye was observed at either setting. During the in vivo evaluation, this effect was even more profound, resulting in an improvement of detection, from 35 to 100 % (0.7 and 0.9 V, respectively). Evidently, the attenuation of the fluorescence signal in the nodal sample tissue influences the detection of the fluorescence signal with the opto-nuclear probe. Moreover, the limited penetration depth of the fluorescence signal as a result of tissue attenuation also influenced the correlation between the signal intensities obtained in the ex vivo set-up. In the phantom experiments, a strong correlation was found between gamma and fluorescence tracing, underscoring the utility of the technology, while measurements in clinical samples yielded a poor correlation. Distribution of the tracer through the node, and thus the degree of tissue attenuation at different points of measurement, may also vary; earlier histopathological evaluation of fluorescent SNs also indicated that the tracer is not homogeneously distributed through the node [7]. This result supports previous findings indicating that tissue attenuation of the fluorescent emission signal [11] severely limits the ability to acquire a quantitative read-out in tissue specimens.

Existing, routinely used clinical procedures such as the SN biopsy procedure provide advantages for the valorization of new techniques. Initially, we demonstrated the value of this concept by the evolution of the radiotracer ^{99m}Tc-nanocolloid into the hybrid tracer ICG-^{99m}Tc-nanocolloid that allows for both radio- and fluorescence-based SN detection [4]. In the current study, we showed that the extension of a gamma probe with fluorescence tracing capabilities resulted in a unique hybrid surgical guidance modality which could also be rapidly evaluated in a clinical trial. Because the opto-nuclear probe is directly derived from the existing gamma probe technology, its use was considered intuitive for surgeons with experience in SN biopsy. Implementation of this hybrid tracing technology in clinical routine may therefore have a short learning curve. Since the read-out is acoustic rather than visual, the technology can easily be used in combination with fluorescence imaging. For the detection of gamma photons, a combination of acoustic (gamma tracing) and visible (gamma imaging using portable camera systems) feedback has already proven its value [12, 13].

Importantly, with the opto-nuclear technology, fluorescence tracing can be performed in ambient light, which is a significant improvement to the application of the current generation of surgical (NIR) fluorescence cameras that require the lights in the operating theatre to be dimmed or switched off completely [14–16]. This means that with the opto-nuclear probe technology, the impact that fluorescence detection has on operation theatre logistics is minimized.

Although we specifically focused on the combined use of a hybrid tracer and opto-nuclear tracing technology, use of the latter is not confined to ICG-^{99m}Tc-nanocolloid. The probe settings can easily be switched between gamma and fluorescence tracing, thus allowing for the use of tracers other than the hybrid tracer, e.g., other ^{99m}Tc-labeled colloids [1], or separately administered ICG. This makes it a versatile technology that is easy implement. For future purposes, one may also want to apply the fluorescence tracing technology during ICG perfusion measurements (e.g. kidney ischemia [17]), ICG-based tumor tracing (e.g. exploring the liver for the presence of colorectal metastasis [18]), or even in combination with tumor receptor targeting tracers functionalized with a fluorescence dye like ICG [19] or alternative NIR dyes such as Cy7 (λ_{ex}: 750 nm; λ_{em}: 773 nm; Luminoprobe GmbH, Hannover, Germany),

CW800 (λ_{ex}: 778 nm; λ_{em}: 794 nm; LI-COR Biosciences, Lincoln, NE, USA), or ZW800 (λ_{ex}: 772 nm; λ_{em}: 788 nm [20]). Here it should be noted that using optical tracing alone will severely limit the surgical guidance provided to superficial structures with a known location, due to the limited penetration depth of the fluorescent dye [11].

Conclusion

This proof-of-concept study demonstrated the first clinical evaluation of a hybrid surgical modality capable of detecting both gamma and NIR fluorescence signals. Because NIR fluorescence tracing is performed in a similar manner as conventional gamma tracing, the technology could be easily adopted by surgeons.

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Compliance with ethical standards

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Disclosure The authors each declare that they have no conflicts of interest.

Research involving human participants All procedures performed in studies involving human participants were in accordance with the ethical standards of our institution and with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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