OPTICAL IMAGING FOR LYMPH NODE SURVEILLANCE IN BREAST CANCER PATIENTS: INTRAOPERATIVE DETECTION AS A STEP-UP APPROACH TOWARDS TUMOR-TARGETED IMAGING
ABSTRACT

INTRODUCTION
The sentinel lymph node (SLN) procedure is widely accepted as a method for lymph node staging in cT1-2 N0 breast carcinoma. In the case of a positive SLN, remaining axillary lymph nodes (ALNs) are generally removed. However, these ALNs appear to be negative in 50-80% of the patients, indicating substantial overtreatment. In this paper, near-infrared fluorescence (NIRF) imaging is outlined as a technique for intraoperative detection of the SLN. Moreover, the potential of intraoperative detecting of tumor load within the SLN for a one-step SLN/ALN dissection is outlined.

METHODS
Seven women with biopsy-proven cT1-2 N0 breast carcinoma underwent the standard SLN-procedure (preoperative injection with radiolabeled colloid and intraoperative injection with patent blue). Additionally, all patients received a peritumoral injection with 1 ml (0.5mg/ml) indocyanine green (ICG) intraoperatively. The SLN was visualized with a customized multispectral fluorescence camera system.

RESULTS
The SLN was successfully identified in all patients. Total numbers of lymph nodes detected with radiolabeled colloid, patent blue and ICG were 15, 9 and 13, respectively. The use of the NIRF optical imaging system did not interfere with the standard operative procedure. No adverse reactions were encountered.

CONCLUSION
The intraoperative detection of the SLN with a NIRF optical imaging system is technically feasible. In the near future, tumor-targeted optical contrast agents may allow for a one-stage intraoperative evaluation of lymph node tumor status and intraoperative decision making towards an ALN dissection.
INTRODUCTION

Breast cancer is the leading type of cancer in women with an estimated 1.4 million new cases worldwide in 2010. Treatment of primary breast cancer depends on the resection of the primary tumor and identification of cancer spread to the lymph nodes (LNs). Both are independent prognostic factors for survival and recurrence of the disease. For identification of tumor involvement in axillary lymph nodes (ALNs), the first-draining node, also denominated as the sentinel lymph node (SLN), is analyzed by histology. The best results for the sentinel node biopsy and lymphatic mapping are obtained with a combination of preoperative lymphoscintigraphy with radiolabeled colloid and intraoperative injection with blue dye (e.g. patent blue). In 97% of the cases, the SLN can be traced intraoperatively with the combination of a gamma probe guided by blue-colored discoloration of lymph vessels and LNs. However, there are disadvantages to both the blue dye and the radiolabeled colloid, mainly concerning detection problems in obese patients, allergy to the blue dye, the shine-through effect of the injection site of the radiocolloid and the need for appropriate logistics for radio-active matter.

As an alternative, several groups have shown high accuracy and sensitivity with indocyanine green (ICG) enhanced optical imaging with a handheld or laparoscopic fluorescence or infrared camera in breast cancer patients. ICG has been used for the last three decades in patients for the study of organ perfusion and ophthalmology and has a safe and well-known pharmacological profile. When excited at the appropriate wavelength, ICG emits photons in the near-infrared fluorescence (NIRF) range of around 800 nm. Because near-infrared (NIR) light is invisible to the human eye, a special optical imaging system is needed to visualize the NIR signal in the surgical field. Such a system was recently presented by Troyan et al in a study of lymphatic mapping in breast cancer. In addition to showing the fluorescence signal, the described camera is able to show the signal in real-time, depicted on the anatomy of the surgical field.

In the University Medical Center Groningen (UMCG), we recently introduced a novel multispectral normalized near-infrared fluorescence (NIRF) imaging device in the field of breast-conserving surgery. This camera can provide near-real-time video-rate NIRF imaging of subcutaneous lymphatic vessels and LNs in the surgical field. In addition, the system is capable of multi-spectral imaging. The current study is aimed at lymphatic mapping with ICG alongside the routine technique using blue dye and radiolabeled colloid in early-stage breast cancer. First, we describe the camera system and its use in a clinical setting. Subsequently, we introduce the methodology for a future concordance study that will determine whether NIRF-guided lymphatic mapping with ICG is equally sensitive compared to the commonly used technique, consisting of radiocolloid and patent blue. Future applications toward intraoperative detection of tumor load within the SLN are elucidated in order to design a one-step SLN/ALN dissection procedure.
PATIENTS AND METHODS
A total of seven women diagnosed with biopsy-proven stage I-II breast cancer who were scheduled to undergo sentinel lymph node biopsy (SLNB) for staging and treatment of their disease, were included in this study. Exclusion criteria were defined as pregnant or breast feeding condition, age younger than 21, significant renal (serum creatinin ≥ 400 μmol/l), cardiac or pulmonary disease (ASA III-IV), history of iodine allergy or anaphylactic reactions to insect bites or medication, present or former hyperthyroidism and recent surgery on the armpit. Informed consent was obtained prior to surgery and all information regarding the patients was anonymized. The study was approved by the Institutional Review Board (IRB) of the University Medical Center Groningen (UMCG) and the National Committee for Clinical Research (CCMO). This study was performed in accordance with the ethical standards of the Helsinki Declaration. The study was registered prior to execution at the Dutch Trial Registry no. 2083.

PREOPERATIVE PROCEDURE
Technetium-99m labeled colloid was routinely administered in four equally-divided peritumoral injections (total volume: 0.4 ml) one day prior surgery. Additionally, a preoperative lymphoscintigram was obtained to determine the definitive position of the SLN and its lymphatic drainage pattern from the primary tumor.

SURGICAL PROCEDURE
After the induction of general anaesthesia, the patient was injected peritumorally into the breast parenchyma with a 2 ml mixture of patent blue and ICG, consisting of 1 ml undiluted patent blue (Guerbet, Roissy-Charles-de-Gaulle, France) added to 0.5 mg (0.65 mmol) indocyanine green (Pulsion, Munich, Germany) dissolved in 1 ml sterile water (B. Braun Medical, Hillcross, Martindale). The total volume of the mixture was equally divided over four peritumoral injections. Immediately after injection, fluorescence images were acquired with the NIRF camera and displayed on two wide-screen monitors, of which one was in direct vision of the operating surgeon (Figure 1). Images of the injection site and the axillary area were acquired with video-rate acquisition. During the surgical procedure, the SLN was identified following the standard procedure with the combination of a hand-held gamma probe and visual inspection for blue discoloration. After an incision was made on the site with the highest count, as determined by the gamma probe, the NIRF camera system was directed at the surgically exposed area to capture an image and quantify fluorescence activity. The radioactive and/or blue SLNs were subsequently excised. After excision, the axillary basin was reassessed for radioactivity and blue spots as well as for NIRF signals. Residual radioactive and/or blue nodes were removed when necessary. In case of remaining fluorescence hot spots, a maximum of one additional SLN was excised by the surgeon. Nodes that were solid and highly suspicious for metastatic tumor on tactile inspection were also removed, irrespective of radioactivity or patent blue staining. The excised SLNs were im-
aged *ex vivo* with the NIRF camera system immediately after removal. All SLNs were sent in for histopathological analysis. Postoperatively, patients were monitored for 24 hours for an adverse reaction to ICG.

**NEAR-INFRARED FLUORESCENCE (NIRF) CAMERA SYSTEM**

For this study, fluorescence imaging was performed with a multispectral intraoperative fluorescence camera system that was designed and built within the Institute for Biological and Medical Imaging (IBMI) at the Technical University of Munich, Germany (Figure 1). The system contains a halogen white light source, which allows for the illumination of the operating field without interference with the fluorescence channels. Also, the system contains a laser diode, providing light with a specific wavelength for excitation purposes. After excitation, photons emitted by the fluorescent optical contrast agents can be detected with a highly sensitive charged-coupled device camera (CCD camera 1). Intrinsic fluorescence signals can be detected with a second camera (CCD camera 2). A customized software system permits simultaneous near-real-time acquisition of images from both CCD cameras at up to 12 frames per second. For intraoperative use, the entire camera system was covered in sterile drapes. The entire system was granted approval by the Institutional Review Board (IRB) of the UMCG. A detailed description of the technical characteristics of the NIRF camera system used in this study was published previously by Themelis et al.

**RESULTS**

In all patients, a total of 15 SLNs could be identified with an average of 2 SLNs (range, 1-4 SLNs) per patient. Patient characteristics are listed in Table 1. The mean age was 65 years. Six patients (86%) were diagnosed with invasive ductal carcinoma (IDC), one patient with adenoid cystic carcinoma.

Clear transcutaneous visualization of the SLN was not found feasible in any of the patients, in part due to spillage of ICG at the injection site. The number of SLNs detected intraoperatively with radiolabeled colloid, patent blue and ICG were 15 (100%), 9 (60%) and 13 (86.7%), respectively. Figure 2 shows the integration of fluorescence signals and color video images of the surgical field as seen by the surgeon on a wide-screen monitor in the operating room. Importantly, NIRF imaging could detect 3 out of 3 SLNs containing (micro)metastases, revealed by conventional histopathology.

The total duration of the surgical procedure combined with intraoperative NIRF optical imaging was prolonged by 30 minutes. This was mainly due to the learning curve for handling the camera system, together with the time necessary for data acquisition during the operative procedure. The surgeons judged the camera system in combination with the wide-screen monitors convenient for intraoperative use, and comparable to the use of monitors in laparoscopic surgery. It was regarded fairly easy to detect the NIR highlighted
SLNs guided by the images on the screen. In addition, the surgeons appreciated the real-time, ‘live’ images, which gave them a sense of direct feedback on the presence of residual SLNs during the operation.

No adverse reactions or wound infections were encountered after the administration of ICG.

DISCUSSION

Systematic studies have shown that tumor cells migrating from the primary tumor metastasize to the first draining lymph node (sentinel lymph node, SLN) before spreading to other ALNs.\(^1\) When histopathological evaluation of the SLN indicates the presence of (micro)metastases, the standard treatment is to perform a complete ALND.\(^19\) Histopathological evaluation of excised SLNs at the time of dissection is considered to be a highly accurate method for assessing the spread of the disease.\(^20\)

However, in a large meta-analysis of over 7500 breast cancer patients who underwent SLNB, no additional positive ALNs could be identified on ALND in 47% of the patients who had a positive SLN.\(^21\) Furthermore, in 65 to 80% of patients with micrometastases (>0.2 ≤ 2 mm) in the SLN, no metastases were found in the remaining ALNs.\(^22, 23\) In case of presence of isolated tumor cells (metastases ≤0.2 mm) in the SLN, the percentage of patients with tumor-free ALNs even increases towards 90%.\(^23\) Consequently, non-affected ALNs are often needlessly removed due to the fact that the surgeon lacks real-time intraoperative feedback on LN tumor load status. As such, the SLN procedure, although highly specific and sensitive, leads to substantial overtreatment in breast-conserving surgery (BCS) with the known accompanying risks of nerve injury, lymph edema, shoulder dysfunction and seroma formation.\(^19, 24-26\) There seems to be a clear need for non-invasive assessment of the SLN tumor burden status. This would significantly reduce co-morbidity associated with the unnecessary removal of non-metastatic ALNs, but could also implicate a one-step procedure in case of a positive SLN for an ALND if the tumor burden is larger than 2 mm. NIRF-imaging with a tumor-specific probe may provide such a lymph node surveillance tool for detecting and assessing the actual SLN status.

Several studies have reported on a near-infrared fluorescence (NIRF) optical imaging system, with which the SLN can be detected non-invasively after subcutaneous injection with the fluorescent optical contrast agent indocyanine green (ICG).\(^9, 11, 13-15, 27\) ICG flows along with the lymph fluid and accumulates in the SLNs within minutes, enabling rapid detection and visualization of SLNs as fluorescent hot spots. No adverse reactions were reported in any of the conducted trials. The NIRF camera system at the University Medical Center Groningen enables near-real-time intraoperative fluorescence imaging of subcutaneous lymphatic vessels and LNs while capturing color video images of the surgical field simultaneously. It is technically possible to overlay the fluorescence image on the color images for the anatomical positioning of the fluorescence signal. Additionally, the system is capable of multispectral imaging, enabling normalized fluorescence and the
simultaneous detection of different optical contrast agents.\textsuperscript{16}

NIRF optical imaging enhanced with optical contrast agents in the near-infrared (NIR) range (e.g. ICG) offers considerable advantages to the current SLN procedure: the technique offers a high resolution, is relatively cheap, makes use of non-ionizing radiation and offers high sensitivity and specificity rates.\textsuperscript{28} In this pilot study, the surgeons appreciated the near-real-time feedback on the presence of residual SLNs and found the system easy to work with. It was very well possible to excise the NIR highlighted LNs guided by the NIR signal on the screen.

The most important limitation of fluorescence imaging is the limited penetration depth (1-2 cm) due to the absorption and scattering of photons when propagating through tissue.\textsuperscript{29} This might cause visualization problems in obese patients. However, tissue autofluorescence in the NIR range is minimal, with a maximal signal-to-background ratio.\textsuperscript{29}

In our study, the total operation time was prolonged mainly due to the intraoperative use of the NIRF optical imaging system. However, in the course of these surgeries, we experienced a decrease in the time necessary to set up and install the system. We anticipate that in the near future the total prolongation of the surgical procedure will not exceed 30 minutes.

In this study, we have confirmed that lymphatic mapping with ICG next to the routine blue dye/radiolabeled colloid technique is feasible in early breast cancer, as also recently reported by Troyan et al\textsuperscript{14}, Murawa et al\textsuperscript{13}, Hirche et al\textsuperscript{12} and various Japanese groups.\textsuperscript{9-11}

At our institution, NIRF imaging will be expanded towards NIR tumor-targeted optical imaging for intraoperative image-guided detection of (micro)metastases in the SLN using NIR dyes like IRDye CW800 (Li-Cor Inc, Lincoln, Nebraska) conjugated to tracers of interest. Several studies have shown the feasibility of tumor-labeling with targeted optical contrast agents.\textsuperscript{30-33} Sevick-Muraca et al have shown the feasibility of NIR optical imaging following microdose administration of ICG.\textsuperscript{15} Although ICG is non-specific, these findings suggest that comparable concentrations can be used for tumor-targeted NIR optical contrast agents for non-invasive SLN status assessment in humans.

In the future, it may be possible to visualize a tumor-targeted optical contrast agent and a non-specific optical contrast agent with different wavelengths for simultaneous detection and assessment of the SLN. This might enable non-invasive evaluation of the SLN status both for lymph node guidance as surveillance.

Multi-spectral NIRF optical imaging may improve therapeutic outcome of BCS in the future by creating a one-step SLN/ALN dissection procedure based on the tumor burden within the SLN, which will additionally reduce the number of unnecessary ALN dissections and associated morbidity. This needs to be confirmed by multicenter studies using uniformly designed and calibrated camera systems and optical agents for tumor-targeting.
ACKNOWLEDGEMENTS
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Table 1 - Patient characteristics

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ACC = adenoid cystic carcinoma; ALND = axillary lymph node dissection; IDC = invasive ductal carcinoma; LUQ = lateral upper quadrant; MUQ = medial upper quadrant; n.a. = not applicable; n.p. = not performed.

a. Skin types I-VI in accordance with the American Academy of Dermatology.
b. White skin type common in Central European women: sometimes burns; tans gradually.
c. Yes (+), weak (0), no (-).
d. Total number of positive lymph nodes/ total number of lymph nodes identified during ALND.
REFERENCE LIST


