INTRODUCTION AND PERSPECTIVES OF THE THESIS
GENERAL INTRODUCTION

Image-guided surgery refers to any procedure in which a surgeon uses preoperative or intraoperative images in order to directly or indirectly guide resection. Image-guided surgery helps surgeons to perform a safer and less invasive procedure, while obtaining more effective removal of affected tissue. Preoperative and intraoperative imaging modalities have gained enormous interest over the last decades, along with general improvements in healthcare that have resulted in new diagnosis and treatment options, healthy aging and longer survival of patients with previously deadly diseases.

In this thesis we will focus on perioperative imaging during abdominal surgery. Excluding bariatric surgery, more than 55,000 abdominal surgical procedures are performed in the Netherlands annually (source Centraal Bureau voor Statistiek; www.statline.cbs.nl). More than half of these procedures consist of malignant colonic resections or laparoscopic cholecystectomy for symptomatic cholecystolithiasis or gallstone disease. In both surgical procedures perioperative imaging can decrease surgical harm, while improving diagnosis and therapy outcomes. In colon cancer, preoperative imaging may lead to more accurate assessment of tumor spread, while intraoperative imaging may improve complete tumor resection. During laparoscopic cholecystectomy, improvement of optical intraoperative imaging could help to avoid bile duct injury, which would otherwise require re-intervention, a prolonged hospital stay and incur an increased risk of permanent physical damage.

MOLECULAR IMAGING

Next to traditional imaging techniques such as X-ray, ultrasonography, computed tomography (CT) and magnetic resonance imaging (MRI), molecular imaging based on exogenously administrated tracers is now available. Single photon emission computed tomography (SPECT) imaging and positron emission tomography (PET) are both highly sophisticated imaging techniques, visualizing metabolically active tissue by measuring the concentration of administrated radiopharmaceuticals. Both imaging modalities can be combined with a CT scan, thus integrating metabolic information with detailed anatomical information. In addition, SPECT and PET allow three-dimensional imaging, which facilitates the dynamic imaging of deeply lying intra-abdominal organs or of organs above or below other organs with significant amounts of activity. This characteristic contrasts with classic scintigraphy, which yields planar data and can only be used to create a two dimensional image. The ability to combine functional...
and anatomical data has contributed enormously to the better differentiation of physiological and pathological uptake, more accurate localization of pathology and better characterization of small or equivocal uptake foci.

The most commonly used radionuclide in SPECT is the gamma-photon $^{99m}$Tc-nanocoll which has a half-life ($t_{1/2}$) of 6 hrs and can be coupled to various compounds. The most commonly used tracer in PET imaging is $^{18}$F-fluorodeoxyglucose ($^{18}$FDG), which is a marker for tissue uptake of glucose. The great advantage of PET over SPECT is the superior sensitivity and spatial resolution. Additionally, dynamic 3D imaging provides detailed anatomical information on the location of radiopharmaceutical and tracer distribution. These favorable characteristics have expanded the application of PET from imaging of tumor metabolism to selective tumor targets. Nowadays, targeted therapies based on the conjugation and radiolabelling of monoclonal antibodies with PET isotopes are being investigated. These therapies can be aimed exclusively at tumor cells while sparing healthy cells. At the same time new PET-radiocolloids, especially $[^{89}$Zr]$^{89}$Zr-nanocoll are under development. The prolonged $[^{89}$Zr]$^{89}$Zr-nanocoll half-life ($t_{1/2}$) of 78.4 hrs improves the quantitative investigation of tracer distribution and therefore allows for a more detailed analysis of metastatic spread of a primary tumor, especially regarding drainage of tumor cells toward lymph nodes $^{1,2}$.

A limitation of radiocolloids is their colorless appearance, which means that they cannot be visualized intraoperatively. Intraoperative guidance towards highly radioactive structures can be achieved by imaging with a portable gamma camera but these cannot differentiate radioactivity between structures $^{3}$. Optical tracers are clearly needed to facilitate intraoperative image-guided surgery. The most frequently used optical tracers are blue dyes (e.g. methylene blue, isosulfan blue or patent blue). Blue dyes are safe when administered locally or in low doses. However, they can induce severe adverse effects such as arrhythmias, coronary vasoconstriction, and hemolytic anemia in patients with renal insufficiency or after intravenous administration of higher doses $^{4}$. Visualization of blue dye through tissue with the naked eye is limited and therefore restricts applications to the identification of superficial structures.

More recently, intraoperative imaging using the near-infrared (NIR) light spectrum has been introduced, using light at wavelengths invisible to the naked eye (between 700-900 nanometers). Advantages of NIR light include high tissue penetration of up to 1 cm, and low autofluorescence. As a result, optimal signal-to-background ratios can be achieved, improving both contrast and the identification of different tissue types $^{5}$. NIR imaging requires a NIR fluorescent agent or so-called fluorophore, combined with an imaging system that is able to both excite and detect
the fluorescent signal. The fluorescent signal can be visualized immediately and most imaging systems are able to combine fluorescence signals with conventional color videos, allowing direct anatomical orientation.

Two fluorophores are currently approved by the US Food and Drug Administration (FDA) and the European Medicines Agency (EMA) for clinical applications; methylene blue and Indocyanine Green (ICG). Both are nonspecific contrast agents but with different characteristics. Indocyanine Green has an excitation peak around 800 nm, while methylene blue has an excitation peak of approximately 700 nm. ICG shows better tissue penetration depth and less autofluorescence. Another advantage is the better safety profile of ICG, whether injected locally or systematically. Adverse events have been reported in fewer than 1 in 40,000 patients and most relate to hypersensitivity reactions.

Methylene blue and ICG can be used for different surgical applications. Methylene blue is mainly cleared renally and is therefore very useful for ureter mapping to prevent iatrogenic injuries. Indocyanine Green is cleared exclusively by the liver and is excreted into the bile after intravenous administration. It may help reduce bile duct injuries by imaging the biliary tree during laparoscopic cholecystectomy. Additionally, ICG has favorable characteristics that may improve imaging of tumor spread by visualization of lymph node drainage patterns, identification of malignant tissue and finally, to determine adequate resection margins. A third promising fluorophore is IRDye800CW (LI-COR Bioscience, Lincoln, NE), which has an additional NHS ester and can be easily coupled to an antibody without changing its fluorescent properties. Referred to as tumor-targeted imaging, this approach allows for very specific pre- and intraoperative detection of tumor tissue. IRDye800CW is currently available for conjugation to targeted biomolecules for investigational use in clinical trials. However, it has not been studied for diagnostic or therapeutic applications in humans, and has not been FDA approved for this use. Use of IRDye800CW therefore remains limited in current routine treatment of surgical patients.

**IMAGE-GUIDED SURGERY IN COLON CANCER**

Colorectal cancer is currently the second most frequently diagnosed cancer in the Western world, and is the third most common malignancy, after prostate and lung cancer, in men in the Netherlands. In women, only breast cancer is diagnosed more frequently. As a result of the introduction of a nationwide screening program, increased life expectancy and an aging population, the number of colorectal cancer patients has increased dramatically, from 7100 in
1990 to 14000 in 2017 (source: www.cijfersoverkanker.nl). About two thirds of these patients are diagnosed with colon cancer.

Colon cancer is classified according to stage at diagnosis, as defined by invasion depth of the tumor (T-stage; T1-T4), lymph node and distant metastases (TNM classification) (Figure 1). Early detection is crucial to maximize the chance of cure. Currently, five-year survival ranges from 97% to as low as 8%, depending on disease stage at time of diagnosis. Of newly diagnosed colon cancer cases, early stage T1 and T2 tumors (stage I disease) account for 24% and 34.5%, respectively (source Dutch ColoRectal Audit DCRA-DICA 2017). By this stage a tumor has grown through the muscularis mucosa into the submucosa (T1), and may also have penetrated into the muscularis propria (T2). The general incidence of tumor spread to nearby lymph nodes (N-stage) or distant sites (M-stage) is low in these T1 and T2 tumors. More advanced tumors grow into the outermost layers of the colon (T3) or though the visceral peritoneum (T4), increasing the risk of metastatic spread dramatically.

Independent of T-stage, accurate pathological examination of resected lymph nodes is a prerequisite, since lymph node metastasis (LNM) serves as the strongest prognostic factor and most important criterion for adjuvant chemotherapy. In the absence of LNM (stage I-II), the 5-year survival of exceeds 95% for stage I and approximately 80% for stage II disease. The incidence of LNM in T1/T2 tumors is relatively low, at 8-20%. The proportion of these early staged tumors is expected to increase to approximately 50% due to the introduction of nationwide screening programs. In patients with T1/T2 tumors a complete resection of the primary tumor can be easily achieved with a small resection of the affected colon or endoscopic guided resection. This is an attractive treatment option that avoids exposure to unnecessary surgery-related morbidity and mortality, which are currently as high as 13.5% and 1-5%, respectively. Moreover, previous studies indicate that endoscopic treatment of T1 tumors is safe in patients with low-risk tumors. However, endoscopic resection is insufficient in the majority of T1 tumors categorized as high-risk (71-81%). In these patients an endoscopic resection is insufficient since LNM are present in 8% of T1 tumors. For T2 tumors the risk of lymph nodes is even higher up to 20%. Currently, it is not possible to distinguish patients with or without lymph node metastases prior to surgery. Therefore, large segmental resection, with en-block excision of all lymph nodes, is unavoidable in the majority of patients with T1 tumors and in all patients with T2 colon cancer.
On the other hand, up to 20-30% of patients without lymph node metastases show disease recurrence and eventually die within five years of initial treatment, despite complete surgical resection 19. This high recurrence rate in lymph node-negative patients is probably the result of understaging due to overlooked occult tumor cells (micrometastases or isolated tumor cells) during routine histopathological examination 20. To improve lymph node staging while decreasing the extent of surgery in node-negative patients, detection of the sentinel lymph node (SLN) could offer a solution.

The sentinel lymph node procedure

Some history
Lymph node involvement as a consequence of the metastatic spread of cancer was already described by Hippocrates, who mentioned a disease similar to currently known lymph node metastases 21. The investigation of the lymphatic system has a long and fascinating history, with important contributions from several medical scientists. Although some medical pioneers had already described the lymphatic system, probably accidentally, it is generally accepted that it was first properly recognized by the Italian professor Gasparo Asselius (1581-1626) in 1622, who was then Professor of Anatomy at Pavia University in Italy 22. He noticed ‘white vessels’ in the
mesentery during dissection of a living dog just after eating. He also observed leakage of white fluid from the vessels after cutting through them and therefore described them as 'lacteals'. The anatomy and physiological role of the lymphatic structures was a mystery but began to receive attention from many investigators following Asselius’s description. In 1651 the Dutch professor Johannes van Horne (1621-1670) described a main lymphatic collecting vessel which is currently known as the thoracic duct. It was van Horne’s student, Frederik Ruysch (1638-1731) (Figure 2), who first demonstrated the drainage of fluids from the organs towards the lymphatic system and finally into the venous blood stream. Concurrently, the Danish physician, mathematician and theologian Thomas Bartholin (1616-1680) studied the lymphatic system of two executed criminals. He confirmed that the lymphatic system was a circulatory system distinct from blood circulation and proposed that lymph originated from blood by filtration. He named the lymph vessels ‘vasa lymphatica’, a term derived from the Latin word ‘lympha’ which means clear spring water. Anthony Nuck (1650-1692), a professor of anatomy in Leiden, the Netherlands, visualized lymphatics by injection of a mixture of mercury, tin and lead. As a result, the function of the lymph nodes as filters of the lymphatic system was slowly uncovered. The German pathologist, Rudolf Virchow (1821-1902), can be considered the founding father of our modern understanding of the lymphatic system and lymph nodes. He suggested that lymph nodes function as filters in the lymphatic system and also proposed that lymphatic fluid from any given area in the body drains through the lymphatics to specific lymph nodes and subsequently to other lymph nodes. These hypotheses were supported by his own observations during the autopsy of a sailor, in whom carbon pigment from a tattoo on the arm had migrated to a single axillar lymph node.

By the end of the 19th century European surgeons were starting to discuss local treatment of cancer, supplemented with regional lymph node therapy to improve curative efficacy. Supported by the work of Virchow, the prominent British surgeon Herbert Snow (1847-1930) published an article in 1882 in which he advocated elective lymph node dissection in patients with melanoma. Simultaneously, the American surgeon William S. Halsted (1852-1922) developed a mastectomy procedure with en-block axillary resection in breast cancer. From then on, excision of the primary tumor combined with regional node surgery became the standard of care in the surgical treatment for a wide variety of malignancies.
In 1960, Gould et al. described the metastatic drainage patterns of parotid tumors towards a lymph node located at the junction of the anterior and posterior facial and referred to this node as the ‘sentinel node’\(^24\). In the same year, the American surgeon Ramon Cabanas studied lymph node metastases in penile carcinoma and noticed that a specific lymph node near the pubic tubercle often harbored tumor cells\(^25\). Similarly to Gould, he called this node the ‘sentinel node.’ From then on, the imaging of lymphatic drainage patterns using blue dyes and radiocolloid began to be widely investigated. In that period, lymphatic drainage was proposed as a static process occurring in an orderly fashion towards the same fixed location of the sentinel node. Interindividual variability in lymphatic drainage patterns was not taken into account and techniques for SLN identification proved to be unreproducible. As recently as 1992, the American oncologist Donald M. Morton (1934-2014) and pathologist Alastair Cochran finally demonstrated the dynamic patterns of lymphatic drainage, which were found to be variable between patients. This discovery by Morton and Cochran has led to our current concept of the Sentinel lymph node, in which migration of metastatic cells from the primary tumor to a lymph node or nodes occurs in an orderly spread via lymph fluid to the first node or nodes in their paths. This first node or nodes are the so-called Sentinel Lymph Nodes (SLNs) (Figure 4). In 1982 Cochran described an additional advantage of SLN identification, demonstrating that the...
presence of small metastases in these nodes is an essential element in the accurate staging of melanoma. Currently, the primary aim of the SLN procedure is to improve the staging of disease by determination of metastases after detailed histopathological assessment of this specific node. In breast cancer and melanoma, the SLN procedure shows high accuracy in the prediction of metastatic spread and is used routinely. However, the SLN procedure appears more challenging in colon cancer and is still under debate.

Figure 4. The sentinel lymph node concept

Challenges of sentinel lymph node imaging in colon cancer

The first step of the SLN procedure is identification of the node, followed by excision and extensive histopathological examination consisting of conventional hematoxylin and eosin staining and additional serial-sectioning combined with immunohistochemistry. In melanoma and breast cancer, SLN biopsies are routinely performed using a combination of preoperative colloid planar or SPECT lymphoscintigraphy, intraoperative guidance by gamma-probe and optical guidance of blue-stained nodes after preoperative injection with blue dye. Preoperative (SPECT) lymphoscintigraphy informs the surgeon about the number and localization of radioactive nodes. Intraoperative localization of SLNs is guided by gamma counting of the gamma probe and real-time visualization of blue-stained nodes. In breast cancer and melanoma, detection rates greater than 95% are reported. False negative rates are low, at between 4.6-16% and an overall diagnostic accuracy of 93-97.6% is reported for both malignancies. Results for colon cancer vary widely between studies. Additionally, diverse and non-standardized methods mean that results and their interpretation are both of questionable reliability. In general, while high detection rates of over 95% are reported, reported false negative rates are also high at up to 30%, resulting in a lower diagnostic accuracy (88.2%) and pooled sensitivity (76%). Several patient, tumor and procedure-related factors are offered as possible causes for the currently disappointing performance of the SLN procedure in colon cancer.
As in breast cancer and melanoma, it has been proposed that SLN identification in colon cancer is most effective in early disease stages. Higher staged disease, with more advanced transmural tumors and increased risk of lymph node metastases, could theoretically destroy efferent lymphatic pathways. Secondly, large longitudinal tumors can involve adjacent lymphatic patterns, which may increase false negative rates. The number of patients with early staged tumors (T1/T2) is low in the majority of published studies. This is unsurprising, since colon cancer is often asymptomatic until more advanced tumor stages (T3/T4) are reached. With the introduction of nationwide screening programs, the number of early staged tumor is expected to increase. This shift in tumor-stage presentation will facilitate the validation of the SLN technique in colon cancer. Another worrying feature of current SLN performance is the inclusion and combined presentation of results for colon and rectal cancer. The SLN procedure in rectal cancer is questionable, as neoadjuvant chemoradiation therapy may change lymphatic drainage patterns and influence performance of the SLN procedure.

Other difficulties regarding colon SLN identification are the unpredictable number and location of the SLNs. In contrast to breast cancer and melanoma, it seems that more than one node is frequently assigned as the SLN and they appear to be located near the primary tumor. Since injection of tracer is administrated peritumorally, uptake of tracer in a SLN close to the primary tumor may be hidden by the highly radioactive injection site. This phenomenon is known as the ‘shine-through effect’. Such SLNs may not be visualized at preoperative imaging. Currently, the gamma-photon $^{99m}$Tc–nanocoll is used for SLN identification, a radiocolloid that only allows imaging using planar lymphoscintigraphy or SPECT. The limited resolution of gamma cameras may also hamper precise localization of the SLN. Furthermore, intraoperative detection of the SLNs is difficult since handheld gamma probes cannot differentiate radioactivity arising from the SLN versus the injection site.

Sentinel lymph nodes of the colon are also more often smaller (< 1 cm) and located beneath a thick layer of (fat) tissue. As the penetration depth and particle size of blue dyes are both limited, intraoperative detection of SLNs using blue dye is suboptimal since SLNs are located in an often fatty mesocolon. Secondly, small particle size results in fast migration of blue dye from the SLNs to second echelon nodes, which increases the false negative rate. New technologies that improve the preoperative and intraoperative identification of true SLNs are under investigation. PET/CT lymphoscintigraphy is one such new imaging technique. In oral cancer patients, PET/CT lymphoscintigraphy showed superior results regarding visualization of SLNs near the primary tumor. Additionally, NIR fluorescence imaging using ICG has shown favorable results for SLN identification in several types of cancer. The combination of these new techniques may have a significant impact in the development and implementation of the SLN procedure in colon cancer.
Laparoscopic cholecystectomy is the treatment of choice in patients with symptomatic gallstone disease. Around 23,000 laparoscopic cholecystectomies are performed in the Netherlands annually (source Centraal Bureau voor Statistiek; www.statline.cbs.nl). The complication rate after laparoscopic cholecystectomy is 2-12%, with a mortality rate of 0.2%. General complications include wound infection, intra-abdominal abscess formation and postoperative bleeding from the cystic artery.

The most feared complication is bile duct injury (BDI), which has an incidence of 0.5-1.0%. Bile duct injury leads to bile leakage that in turn may cause sepsis, multiple organ failure and even death. It can also lead to obstruction, causing "obstructive jaundice," potentially leading to a need for liver transplantation. Bile duct injuries play a significant role in morbidity and mortality rates, lower quality of life and extra costs. The main cause of BDI is misidentification of anatomy, which can occur when a surgeon mistakes the common bile duct or an aberrant right hepatic duct for the cystic duct. To ensure that the cystic duct has been identified correctly, it is important to use Strasberg’s Critical View of Safety (CVS) (Figure 5). At CVS, the cystic duct and cystic artery are clearly identified and can be clipped and divided safely. Mobilization of the infundibulum is essential to reach CVS. In this procedure a window is created between the cystic duct and cystic artery, and between the cystic artery and liver bed (Calot’s triangle).

Figure 5: The critical view of safety (Picture adapted from: from Strasberg SM et al. Rationale and use of the critical view of safety in laparoscopic cholecystectomy. J Am Coll Surg. 2010, source 42)
In some cases mobilization of the infundibulum is difficult or cannot be reached due to retraction of the gallbladder against the liver, resulting in an increased risk of bile duct injury. Several factors such as male gender, co-morbidity, complexity, urgency of surgery and conversion are associated with an increased risk of BDI during laparoscopic cholecystectomy. Local risk factors include acute cholecystitis, aberrant anatomy, severe fibrosis after previous inflammation, and bleeding that disturbs the intraoperative view during the procedure. In the Netherlands, conversion to an open procedure is recommended when CVS cannot be reached. However, conversion to an open procedure requires experience, and since laparoscopic cholecystectomy is now the standard surgical approach few surgeons are familiar with open cholecystectomy.

Intraoperative cholangiography (IOC) was introduced to improve intraoperative visualization of relevant anatomical procedures. A small catheter is placed into the cystic duct during surgery and after injection of a small amount of contrast fluid an X-ray is taken. Lower rates of BDI are reported with routine use of IOC but selective use has been discouraged. In several countries, including the Netherlands, IOC is not routinely used during laparoscopic cholecystectomy due to the drawbacks of radiation exposure, need for additional equipment and additional costs. An additional serious drawback of IOC is the incision that has to be made in the cystic duct. This can be easily confused with the common bile duct, resulting in bile duct injury. Interpretation of IOC is also difficult when it is not frequently used and it should therefore only be performed by experienced professionals.

The use of NIR fluorescence imaging during laparoscopic cholecystectomy, with ICG as contrast agent, is a relatively new technique. After intravenous injection ICG is rapidly cleared by the liver and almost completely excreted in the bile. When NIR fluorescence imaging is used, the outflow of ICG from the gallbladder through the cystic duct can be visualized and may prevent misidentification of the biliary structures. In addition, it could improve procedural efficiency and shorten operation time due to early intraoperative anatomy navigation.
OUTLINE OF THIS THESIS

This thesis consists of two parts. In the first part we outline the current performance of the SLN procedure in colon cancer and describe the limitations and difficulties of the procedure. To improve the preoperative and intraoperative guidance towards the SLN, we investigated the use of NIR fluorescence imaging and PET/CT lymphoscintigraphy as SLN mapping techniques. In the second part, NIR fluorescence imaging is investigated as an intraoperative imaging technique for visualization of biliary structures during laparoscopic cholecystectomy in patients with mild to severe cholecystolithiasis.

PART I  MOLECULAR IMAGE-GUIDED SURGERY IN COLON CANCER

In Chapter 2, a general overview is given of the SLN procedure in colon cancer combined with an introduction of NIR fluorescence imaging as a technique for SLN biopsy. Results of the SLN procedure according to current literature in terms of sensitivity, negative predictive value, detection rate and upstaging are evaluated in Chapter 3. These results are additionally stratified for several tumor and procedure-related factors to assess their influence on current SLN performance.

In Chapter 4 and Chapter 5 we evaluate several variants of the SLN procedure using NIR fluorescence imaging. Additionally, based on current literature we outline the performance and pitfalls of NIR fluorescent SLN mapping in colon cancer.

To improve the SLN procedure in colon cancer, we evaluate the identification and visualization of SLNs using preoperative PET/CT lymphoscintigraphy and intraoperative NIR fluorescence imaging in Chapter 6. The combination of these highly sophisticated imaging techniques, further combined with restricted selection criteria, provided essential information regarding number and location of the SLNs. The results of this study could be fundamental to the development of a standardized and accurate SLN procedure in colon cancer.
PART II MOLECULAR IMAGE-GUIDED SURGERY DURING LAPAROSCOPIC CHOLECYSTECTOMY

The use of NIR fluorescence imaging during laparoscopic cholecystectomy has the potential to become a standard part of the surgical procedure and might offer an alternative to the intraoperative cholangiogram.

In Chapter 7 we investigated the additional value of intraoperative NIR fluorescence imaging of the cystic duct and common bile duct during elective laparoscopic cholecystectomy in patients with uncomplicated gallstone disease. Since early visualization of the biliary anatomy may increase the safety of laparoscopic cholecystectomy in patients with an increased risk of BDI, we investigated this population in Chapter 8. An overview of the current performance of imaging of the bile duct with NIR fluorescence imaging is presented as a systematic review in Chapter 9. In this chapter we evaluated the NIR fluorescence imaging technique with regard to dosage, timing of ICG administration and patient pathology. Additionally, we performed a meta-analysis of visualization of the cystic duct, common bile duct and common hepatic duct between NIR fluorescence imaging and intraoperative cholangiography.
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