GENERAL DISCUSSION AND FUTURE PERSPECTIVES
MOLECULAR IMAGE-GUIDED ABDOMINAL SURGERY

During surgical procedures the surgeon still relies mainly on inspection and palpation to identify structures. It is often very difficult to distinguish malignant from healthy tissue, or to distinguish between fibrotic and inflamed tissue while avoiding injury to structures that should be spared. The introduction of laparoscopic and robotic surgery, which both minimize intraoperative tactile feedback, has accelerated the need for additional perioperative imaging modalities. Molecular image-guided surgery, using radionuclides or optical tracers, has the potential to improve current surgical performance. After administration of radionuclides, the radioactive signal can be used for preoperative nuclear imaging and for intraoperative tumor localization by identification of an acoustic signal produced by a hand-held detection probe. Intraoperative guidance, using near-infrared (NIR) fluorescence imaging in particular, allows for real-time visualization of tissue and vital structures.

Depending on the specific pathology and the aim of tissue visualization, imaging modalities can be used either separately or in combination. Real-time NIR fluorescence imaging has found applications in the imaging of nerves, bile ducts, ureters and in the evaluation of adequate blood flow. Optical imaging, alone or combined with nuclear imaging (PET or SPECT), is preferred in oncological surgery as it can be utilized for sentinel lymph node (SLN) identification or to localize tumor tissue in order to determine disease extent and improve radical resection.

In this thesis, we illustrate the application and feasibility of molecular image-guided surgery in abdominal surgical procedures. Our research focused on 1) the identification of the SLN in colon cancer, and 2) bile duct imaging during laparoscopic cholecystectomy. The technique was suitable for both technical applications. To improve the diagnostic and clinical performance of molecular image-guided surgery, several technical hurdles first need to be tackled, and a better understanding is required of disease pathology and patient characteristics in order to improve perioperative visualization. In general, molecular image-guided surgery has the potential to dramatically improve the treatment and prognosis of the individual patient.

TECHNICAL CHALLENGES IN MOLECULAR IMAGE-GUIDED ABDOMINAL SURGERY

Intraoperative real-time imaging

The use of high definition (HD) and 3-dimensional (3D) imaging systems has improved intraoperative imaging dramatically in the last few years. NIR fluorescence imaging has advantages compared to conventional white light imaging due to the emission of light in the
NIR range (700-1000nm), resulting in deeper penetration depth and less autofluorescence from surrounding tissue. A fluorescent fluorophore is required to produce a NIR-fluorescent signal, and currently only Indocyanine Green (ICG) and methylene blue are approved for clinical use by the Food and Drug Administration and the European Medicines Agency.

ICG is the most widely used fluorophore in clinical studies. Due to its short serum half-time of only a few minutes, combined with exclusive clearance by the liver, intravenous administration of ICG allows visualization of micro-perfusion of the bowel and imaging of the bile duct. Peritumoral injection of ICG facilitates SLN mapping and is preferred over methylene blue due its favorable excitation peak of around 800 nm and its larger particle size. 

In this thesis, we used ICG as a fluorescent agent for SLN mapping and bile duct imaging. NIR fluorescence imaging proved to be feasible for both applications. However, a major drawback is the limited penetration depth of 5-10 mm, meaning that fluorescent structures located beneath a thick layer of (fatty) tissue, severe edema or dense adhesions are difficult to identify. To improve NIR fluorescence imaging, new dyes with better fluorescent properties might improve intraoperative imaging quality. The fluorescent dye IRDye800CW is currently the best NIR-fluorescent dye available and is close to FDA approval. Animal studies have shown an excellent contrast ratio between SLNs stained with IRDye800CW and surrounding tissue. Additionally, conjugation of IRDye800CW to a nanocolloid improved retention time in the node to up to 24 hrs. ZW800-I is another interesting new dye which is currently being clinically tested. ZW800-I has low toxicity and is excreted through the kidneys, enabling visualization of the ureters and perfusion of anastomoses. It also has a favorable emission wavelength of 788 nm, which allows for visualization with current camera systems adjusted for ICG. Dyes that emit light in the NIR-II range from 1000 to 1700 nm rather than the NIR-I range (700-1000 nm) might also improve imaging. These dyes have longer wavelengths and therefore less attenuation and scattering, which is especially favorable when imaging deeply located tissue. However, these favorable properties are accompanied by increased autofluorescence. Furthermore, these dyes have a lower quantum yield compared to NIR-dyes in current use, they show different pharmacokinetics, and they require visualization adjustment in current clinical imaging systems.

Another interesting new technique that might improve intraoperative imaging is optoacoustic imaging. A major advantage of optoacoustic imaging is better spatial resolution in deep tissue, with less photo bleaching and autofluorescence compared to NIR-imaging. Optoacoustic imaging is a method based on the absorption of tissue when illuminated by NIR-light. The
absorbed optical energy is converted into heat, causing a transient thermoelastic expansion that generates ultrasound waves from the tissue that absorbs the NIR-light. These ultrasound waves can be detected, resulting in visualization of NIR-absorbing tissue. NIR-dyes (ICG and IRDye800CW) can be used as contrast agents with optoacoustic imaging to visualize tissues of interest. Optoacoustic imaging studies in mouse models have shown favorable results regarding the sensitivity of detection and the differentiation of fluorescent targets, suggesting that this technique could overcome the problem of restricted penetration depth of current NIR fluorescence imaging modalities.\(^7\)

The performance of intraoperative imaging may also be enhanced through improvement of imaging systems. First, the sensitivity of imaging systems can be improved by modification of software, emission filters and background corrections. Secondary to these camera system improvements, it also appears to be essential to keep the imaging probe perpendicular during inspection in order to obtain an optimal fluorescent signal and better distinguish the fluorescent target from normal tissue.\(^8\) Although seemingly simple, due to the limited flexibility of the laparoscopic camera this can be quite challenging during surgery. A DROP-IN NIR camera, which can be inserted through a trocar port and picked up by the surgeon with laparoscopic devices, could overcome this problem. A prototype DROP-IN gamma probe has already been investigated for use in SLN imaging in prostate cancer. This study demonstrated an enlarged probe maneuverability to freely scan in every direction in the abdominal cavity and increased the autonomy of the operating surgeon.\(^9\)

**Perioperative molecular nuclear imaging**

Preoperative molecular nuclear imaging is used to plan the surgical strategy and define the extent of disease. The radioactive signal can subsequently be used for intraoperative localization of the tissue of interest using a handheld radionuclide probe.

For preoperative nuclear imaging, positron emission tomography (PET) combined with conventional computed tomography (CT) scans is currently the most sophisticated nuclear imaging modality. Compared to single photon emission computed tomography (SPECT), PET scanners have a higher sensitivity and much better temporal resolution, producing high-quality whole-body 3D images. PET characteristics are favorable regarding SLN identification in colon cancer, since lymphatic drainage patterns are unknown. However, it often appears that the majority of SLNs are located near the injection site, and the limited resolution of SPECT and a shine-through effect from the tracer depot may preclude accurate identification when a SLN is close to the tumor.\(^10,11\) PET/CT lymphoscintigraphy is able to detect SLNs near the
primary tumor and can provide a detailed localization of these nodes in oral cancer patients. PET/CT imaging has the potential to improve the SLN procedure in colon cancer patients and has therefore been investigated in this thesis as a nuclear imaging technique for this application.

PET/CT is a relatively new technique in the molecular nuclear imaging field. Specific PET tracers are needed for PET imaging and over the last decade 89-Zirconium has garnered a great deal of attention as an emergent radionuclide for PET imaging. Its favorable half-life of 78.4 hrs facilitates quantitative analysis of tracer distribution over time. Secondly, it can be easily coupled to various compounds, in particular to a (nano)colloid resulting in the radiocolloid $[^{89}\text{Zr}]\text{Zr-Nanocoll}$. Nanocoll enlarges the radionuclide molecule and therefore improves retention of the radiocolloid in the SLN. Drawbacks of PET imaging are the high associated costs and the limited availability of PET/CT cameras. Additionally, a handheld PET probe for intraoperative localization of the radioactive signal would be desirable but is not yet available. The development of this type of PET-probe is challenging and expensive due to high-energy photons that need a large collimated and shielded detector, which is probably unsuitable for laparoscopic surgery. Meanwhile, improved hand-held gamma cameras and SPECT imaging systems have been developed. Additionally, new SPECT radionuclides such as indium-111, with a half-life of 67.32 days, are now available. However, it is unclear if these imaging probes and dyes will be able to overcome the limited temporal resolution of SPECT and associated shine-through, and the diminished visualization of adjacent tissues of interest.

First results of a phase I study in renal cell carcinoma using indium-111 as a tracer, combined with preoperative SPECT imaging, are promising. However, it must be emphasized that the study only included solitary tumors of at least 1.5 cm. Future studies must demonstrate whether indium-111, combined with SPECT imaging modalities, is capable of detecting signals from smaller, adjacent and more widely-spread tumor tissues. It seems likely that an optimal radionuclide should be chosen in light of the precise clinical question for each specific disease pathology.

FUTURE PERSPECTIVES OF IMAGE-GUIDED ABDOMINAL SURGERY

The sentinel lymph node procedure in colon cancer

As shown in Chapter 3, the sensitivity of the SLN procedure (SNP) in colon cancer is with 56% much lower compared to breast cancer and melanoma. It seems to be difficult to identify the correct SLN(s) in colon cancer probably due to technical and anatomical properties which are unique for colon cancer. This combined with the wide variation of the used SLN mapping...
methods, patients selection and histopathological analysis of lymph nodes, we have to conclude that the performance of SNP in colon cancer is still insufficient. To improve the SNP, several patient, tumor and procedure-related factors should be optimized and standardized.

The first step is to select only those patients with stage I and eventually stage II disease, who have potentially the greatest benefit of the SLN procedure. More advanced tumor stages already meet criteria for adjuvant chemotherapy and their treatment will not be changed by a SLN procedure. It is difficult to distinguish early and more advanced tumor stages with current preoperative imaging techniques (CT scan). Therefore, future studies should integrate patient selection as part of the research protocol.

Subsequently, refinement of the preoperative and intraoperative SLN method should be further investigated with imaging techniques other than planar lymphoscintigraphy, gamma-counting and blue dye. In Chapter 6 we demonstrated that preoperative PET/CT lymphoscintigraphy, combined with NIR fluorescence imaging, could guide the surgeon to the location of a number of SLNs in early-staged colon cancer, even when they were located near the injection site. However, physical movement of the bowel that changes the anatomical position of SLNs, combined with a minimized but not entirely absent ‘shine-through effect’ from the injection depot of both tracers, still impedes perioperative SLN identification. Additionally, the limited penetration depth of NIR fluorescence imaging and the fast migration of ICG to higher echelon nodes hampered intraoperative localization of the SLNs. To improve perioperative SLN identification, a handheld PET-probe would be desirable but as mentioned is not yet clinically feasible. However, the lack of a handheld PET-probe, combined with the limiting properties of NIR fluorescence imaging, means that an additional ex vivo PET/CT scan of the specimen is unavoidable. When the only aim of SLN mapping in colon cancer is improvement of lymph node staging, this technique should be simplified and investigated in future large volume studies. However, if the purpose of the SNP is to diminish the extent of surgery through SLN biopsy and local excision of the primary tumor, it is doubtful whether preoperative SLN identification using PET/CT lymphoscintigraphy combined with intraoperative NIR fluorescence imaging will be sufficient. Several studies, in multiple types of cancer, have shown promising results using the hybrid tracer ICG-99mTc-Nanocolloid, which allows preoperative SPECT/CT lymphoscintigraphy combined with intraoperative NIR-imaging and gamma-probe guided SLN detection. Since 99mTc-Nanocolloid has exactly the same behavior in vivo as 89Zr-Nanocolloid, the applicability of ICG-99mTc-Nanocolloid as an SLN mapping technique should be reinvestigated in colon cancer using insights derived from our results. An additional advantage of ICG-99mTc-Nanocolloid is the wide availability in several countries, whereas 89Zr-Nanocolloid is not yet FDA approved.
mentioned earlier, it is uncertain whether SPECT actually contributes to improvement of the SNP, since it has an unfavorably low resolution compared to PET/CT.

We also expect that, in addition to the imaging technique used, procedure-related factors may play an important role in improving current SLN performance in colon cancer. First, injection into the submucosal layer shows favorable results compared to the subserosal injection technique. This is probably the result of more accurate injection close to the tumor after submucosal injection, with consequent improvement of uptake by all tumor-draining lymphatic vessels. Secondly, we found that an ex vivo performed SNP showed better sensitivity. We hypothesized that this is probably explained by a more aggressive dissection of the mesocolon to identify SLNs. However, ex vivo SLN identification after extraction of the specimen disrupts natural lymphatic pathways and may fail to identify SLNs when these are located outside the resection area. Meanwhile, an in vivo executed SNP could identify aberrant lymph node drainage patterns, which may change the mesocolonic resection margins when SLNs are located outside the conventional surgical field. Furthermore, the in vivo technique has the potential to perform SLN picking, combined with local excision of the primary tumor when no metastases are found in the SLN. We therefore advocate that the SNP should be performed in vivo using an endoscopic submucosal injection technique.

In addition to technical improvements of SLN biopsy in colon cancer, the procedure would be greatly improved by better knowledge concerning lymphatic drainage patterns and thus understanding of lymphatic metastatic spread. In contrast to breast cancer and melanoma, SLNs of the colon are not located at a prespecified intra-abdominal location. Moreover, aberrant lymphatic drainage outside the standard resection margins has been described in up to 22% of patients, accompanied by lymph node metastases in 10% of these cases. Better exploration and determination of lymphatic drainage patterns would help guide the surgeon to the location and number of SLNs that need to be retrieved. This knowledge would also be very helpful when SLNs are difficult to assign due to retention of fluorescent dye in fat tissue or when radioactive stool is mistaken for a SLN following tracer leakage into the lumen of the colon.

The potential benefit of the SNP in colon cancer is to improve staging, patient management and survival. The widely accepted practice for lymph node assessment is H&E-staining of a single or a few sections cut from each lymph node block representing only a small volume of the lymph node in a single axis, potentially producing sampling error. Serial sectioning increases detection of (macro)metastases and especially of the "occult tumour cells", i.e. isolated tumour cells and micrometastases. As shown in Chapter 3, true prevalence of LNM as derived from
the high quality concept validation studies was 48% (after extensive histopathology) versus 36% after conventional H&E-staining. These missed LNM after initial surgical treatment could explain the disease recurrence in patients considered to have no lymph node metastases after primary surgery with standard histopathology. However, the prognostic relevance of occult tumour cells and treatment of these patients with adjuvant chemotherapy to improve survival is still unclear and must be established. It is suggested that only micrometastases are associated with a significant reduction in 5-year survival while their presence is much lower compared to isolated tumour cells. The EnRoute study (NCT01097265) was designed to determine the benefits of adjuvant chemotherapy in those patients with SLNs revealing micrometastases. Unfortunately, this study had to be terminated prematurely due to low patient numbers and slow inclusion. Saha et al. recently showed an improved survival in patients treated with chemotherapy if only one lymph node with micrometastases was found compared to those who refused adjuvant treatment. In patients treated with chemotherapy, an average survival of 108 months was reported compared to 50 months in those not treated with chemotherapy. However, only 30 patients were included in the study and the authors did not stratify for tumor stage. Future studies should investigate the prognostic relevance of isolated tumour cells and micrometastases separately, as identified by SLN procedures. Future randomized trials should establish if adjuvant chemotherapy improves survival in these patients. When a reliable SNP has been developed and the clinical relevance of occult tumour cells is known, studies which investigate SLN-picking combined with local excision of the primary tumour when no metastases are found in the SLNs can be designed as final step. This treatment approach would dramatically change therapy options for patients with early staged tumours, and could potentially decrease surgery-related morbidity rates while improving patient survival.

**Intraoperative real-time optical molecular imaging**

Since ICG is exclusively cleared by the liver and excreted into the bile, it can be used for imaging of the biliary tree during laparoscopic cholecystectomy. The main advantage of intraoperative NIR fluorescence imaging of the extrahepatic biliary structures is the potential to avoid bile duct injuries in patients. For patients with complicated cholecystolithiasis this application could be of particular value because identification of structures is often difficult due to adhesions or severe edema caused by an acute or post inflammatory state. Currently, only a few studies have included patients with complicated gallstone disease, percutaneous gallbladder drainage or after endoscopic retrograde cholangiopancreatography (ERCP). In contrast to patients with uncomplicated gallstone disease, fluorescent bile duct imaging seems to be difficult in patients with complicated gallstone disease, see Chapter 8.
The cause of this difference in performance between gallbladder pathologies is unclear. We hypothesized that a suboptimal dosage, concentration or inappropriate time interval between administration of ICG and surgery contributes to this diminished visualization. In patients with uncomplicated cholecystitis the optimal time-frame between ICG administration and imaging seem to be between 3 to 24 hrs. In patients with complicated cholecystitis a decreased liver function could cause a delayed hepatic clearance of ICG. As a consequence this time-interval should be exceeded, although this is probably not feasible in daily practice since patients with complicated cholecystitis are often operated in an unplanned acute setting. Secondly, thickened (post) inflammatory tissue may also impede the fluorescent signal. Future studies must show whether these limitations can be overcome, as NIR fluorescence cholangiography has real potential in the prevention of bile duct injuries in patients with complicated cholecystolithiasis.

One strategy to improve extrahepatic bile duct visualization is injection of the fluorophore directly into the gallbladder. A major advantage of this technique is the absence of background fluorescence in the liver due to accumulation after intravenous injection. Current published results regarding visualization of the bile duct using this method are similar to those for intravenous injection. An additional complex surgical procedure consisting of intraoperative needle puncture, preparation of a purse-string at the gallbladder fundus and injection of ICG into the purse-string requires advanced surgical skills and can be time-consuming. The major limitation of this technique is the optical contamination of the operative field at NIR visualization due to ICG leakage. The technique could be interesting for patients who already have a transhepatic drain or in difficult cases when additional imaging would increase the safety of the cholecystectomy. Future studies should seek to refine the technique and compare systematic versus intragallbladder injection in patients with complicated cholecystolithiasis.

Based on our results in this thesis, we believe that NIR-fluorescent cholangiography with ICG is a potentially promising technique for the avoidance of bile duct injuries or to replace IOC for biliary mapping. However, future research focusing on optimization of the technique and standardization of doses, concentration and timing of administration is necessary to gain wide clinical acceptance. Moreover, the low rate of bile duct injury in uncomplicated cholecystolithiasis indicates that a very large number of patients would need to be monitored. Practical clinical efficacy of the technique will probably not be found in the prevention of bile duct injuries but rather in the earlier establishment of CVS, resulting in cost reductions. The first multicenter randomized controlled study is currently underway with this alternative endpoint as its main objective. Meanwhile, there could be a tremendous potential benefit of early visualization of the extrahepatic bile ducts in patients with complicated cholecystolithiasis using fluorescent
cholangiography. However, NIR fluorescence cholangiography in patients with more severe
gallbladder pathologies appears to create a complex situation which influences the efficacy
of the technique. The technique, dosage and timing in these patients should be investigated
separately from patients with uncomplicated cholecystolithiasis.

Another interesting application of NIR fluorescence imaging in abdominal surgery not
yet discussed in this thesis is the visualization of micro-perfusion of the bowel to prevent
anastomotic leakage. Anastomotic insufficiency leading to anastomotic leakage is a serious
complication during colorectal (cancer) surgery. Currently, the selection of an optimal site
for anastomosis with adequate perfusion is determined by the intraoperative opinion of
the surgeon. Several studies have suggested that fluorescence ICG-mediated angiography
improves the outcome of laparoscopic anastomotic bowel surgery by changing the surgical
plan \cite{35,36}. Although NIR fluorescence imaging in this application appears to be easy, safe and
effective, there is still a lack of large, well-designed randomized trial evidence for its routine
use in colorectal surgery. Furthermore, most studies have not used an objective quantification
of the fluorescent signal to assess optimal tissue perfusion. Imaging software that measures
fluorescence intensity is currently under investigation in several studies \cite{36-38}. Tools that quantify
bowel perfusion will help develop the technique as a reliable instrument and should be integrated
in future studies.

**Tumor-targeted molecular imaging**

Tumor-targeted molecular imaging has only briefly been discussed in this thesis so far but will
probably prove to be the most important and revolutionary application in abdominal surgery,
especially in the treatment of malignancies.

Visualization of tumor boundaries and tumor spread is pivotal to complete tumor resection,
while preventing excision of unnecessary tissue that may cause surgery-related complications.
Tumor-targeted molecular imaging exploits tumor-specific biomarkers that are overexpressed
by the tumor of interest and which can be targeted by molecules (e.g. monoclonal antibodies
or small peptides) \cite{39} easily conjugated to fluorophores, radioactive isotopes or both.

In abdominal surgery, tumor-targeted molecular imaging could improve surgical outcomes
in patients with peritoneal metastases and might help achieve complete resection in organ-
sparing surgery. Treatment of patients with peritoneal metastases consists of cytoreduction
surgery (CRS) combined with hyperthermic intraperitoneal chemotherapy (HIPEC). Survival of
patients depends on the extent of disease and completeness of the cytoreduction. Therefore,
accurate patient selection and macroscopically complete cytoreduction are of the utmost importance. Current preoperative imaging modalities have limited value in the detection of peritoneal metastases since most lesions are too small to be identified with PET, CT or MRI. Tumor-targeted molecular imaging would be desirable in patients with PM in terms of selecting those patients who will ultimately benefit from surgery and achieving complete cytoreduction. In pancreatic and rectal cancer the technique can be used to improve radical resection and diminish local recurrence or distant metastases. Additionally, it could help to evaluate response to chemoradiotherapy in rectal cancer patients, which in turn might improve selection of patients who could benefit from additional surgery. Several phase I and II studies with fluorophore-labeled tumor-specific antibodies are underway in patients with colon, rectal and pancreatic cancer (NTR5673; NCT02743975; NCT03384238; NCT02973672; NCT03659448; NCT01972373). Furthermore, a study using a combination of the SPECT/CT nuclide Indium-III, fluorescent tracer IRDye800CW and the carcinoembryonic antigen-specific antibody, Labetuzumab, is presently being conducted in patients with peritoneal metastases of colorectal cancer (NCT03699332). Tumor localization and resection will be defined by a combination of preoperative imaging with intraoperative targeted radio- and fluorescent-guided surgery. The results of this study will be very interesting with regard to the impact of tumor-targeted imaging on perioperative clinical decision making in these patients. Eventually, future phase III studies should focus on the impact of tumor-targeted imaging on clinical endpoints such as morbidity rates, quality of life and progression-free or overall survival rates.

**General conclusion**

In this thesis we described the application of image-guided molecular imaging techniques for SLN identification in colon cancer and during laparoscopic cholecystectomies. Intraoperative NIR fluorescence imaging proved feasible for SLN mapping in colon cancer and bile duct imaging, and in both applications has the potential to improve surgical outcomes, patient survival and safety. The development of new fluorescent tracers and clinical imaging systems that improve the penetration depth of the NIR-signal are important to overcoming current limits of visualization. To improve SLN mapping in colon cancer, a highly sensitive preoperative nuclear imaging technique would bring additional benefit by creating a road-map of the number and location of SLNs. Moreover, careful patient selection regarding disease pathology would help identify those who would potentially derive the most benefit from additional pre-and/or intraoperative molecular imaging. In the future, the characteristics of the patient and pathology should be matched to the specific properties of an imaging technique, thus optimizing molecular image-guided surgery for each indication. This should be followed by large, well-designed
randomized trials to assess the clinical implementation of these techniques in abdominal surgery.
REFERENCES


