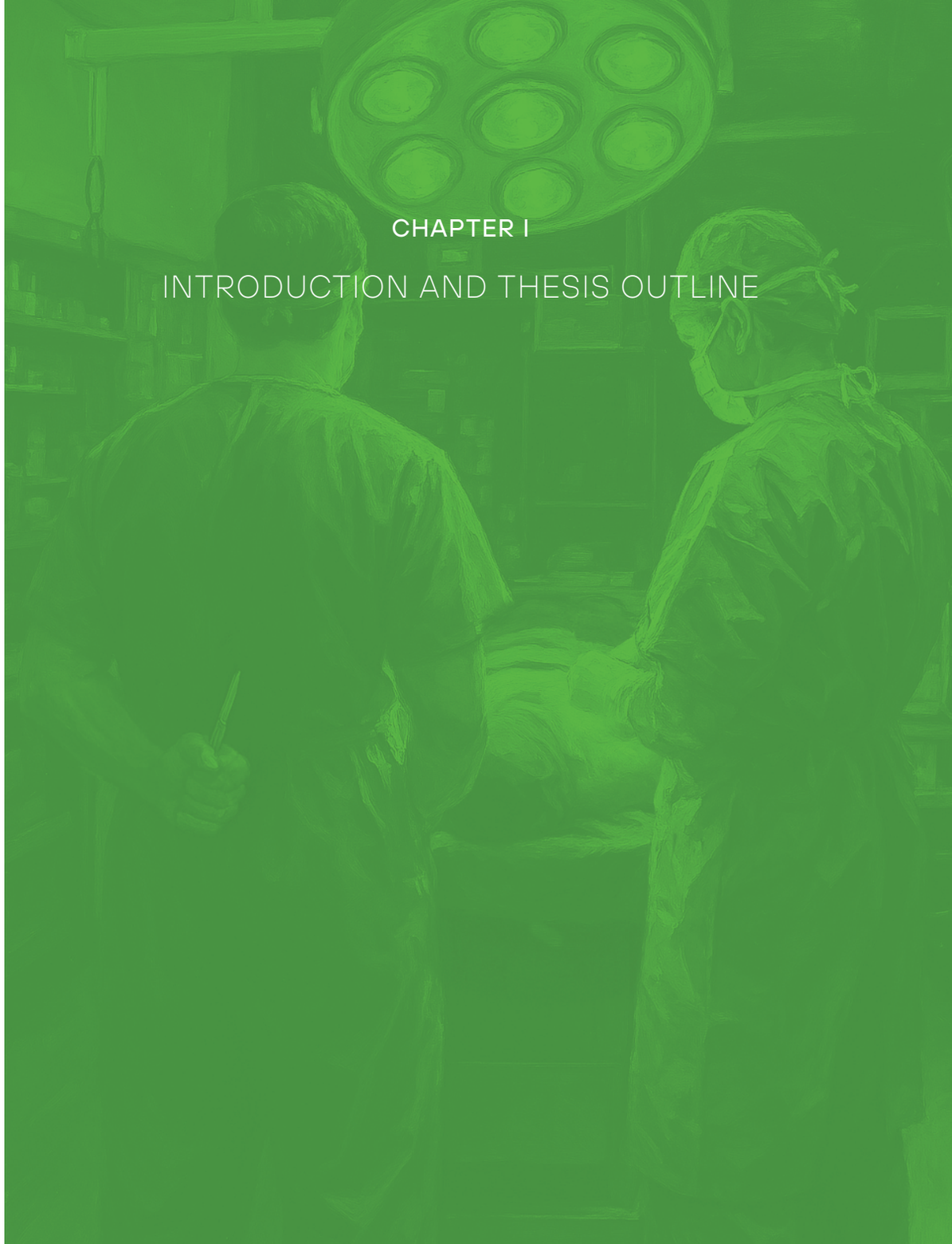
Near-infrared fluorescence imaging in colorectal cancer and its metastases

Meijer, R.P.J.



CHAPTER I

INTRODUCTION AND THESIS OUTLINE

Colorectal cancer

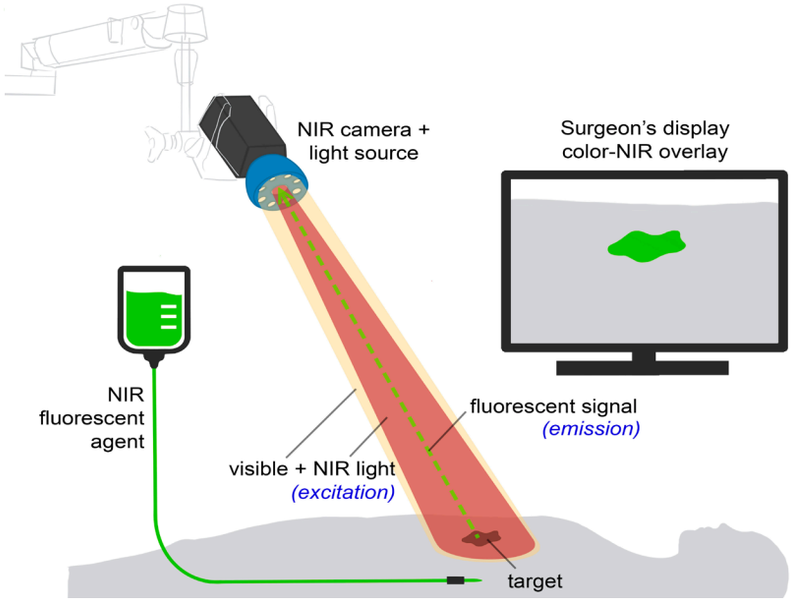
With almost 2 million new cases annually worldwide, colorectal cancer (CRC) is one of the most frequent diagnosed malignancies and the second cause of cancer related deaths.[1] Despite implemented screening programs and improved non-invasive treatment options, surgery is still the primary approach for colorectal cancer. Surgical success is based on two major outcome measures: complete tumor resection (including metastases) and surgical complications. Ensuring tumor-negative resection margins is of utmost importance, as tumor-positive resection margins are associated with a significant decrease in overall survival.[2,3] In addition, surgical complications, like anastomotic leakage (AL) and nerve damage are dreaded because of their impact on quality of life and, in the case of AL, the risk of reoperations, with increased mortality.[4,5] Therefore, it is crucial to identify comprised tissue perfusion at the anastomosis site and to differentiate between tumor tissue and vital structures. Although, the integration of minimally invasive and robotic surgery had great advantages, it came at the expense of this differentiation, given the absence of tactile feedback.

Moreover, identifying tumor tissue becomes even more challenging in cases that have undergone prior resection or neoadjuvant treatment. This results from the development of scar tissue, making it challenging to distinguish between fibrosis and remaining tumor tissue, both visually and tactically. Novel intraoperative identification techniques could provide the surgeon with a solution.

Near-infrared fluorescence imaging

Near-infrared (NIR) fluorescence imaging offers the potential of improved visual feedback during surgery. By employing fluorescence imaging principles, it enhances surgical outcomes by providing real-time intraoperative visual feedback of the surgical field. This technology can aid surgeons in discriminating malignant tissue from healthy tissue effectively. It can potentially improve oncological outcome and protect vital structures. NIR fluorescence imaging relies on the administration or application of a fluorescent contrast agent that selectively accumulates in the target tissue. NIR fluorescence imaging is a real-time imaging technique that combines a NIR fluorescent agent with a specialized imaging system (figure 1). These systems can capture light emitted by a fluorescent agent after excitation with an appropriate light source. NIR light (650-900 nm) is favorable for intraoperative imaging compared to visible light because of its better depth penetration in tissue (up to 10 mm). Moreover, the fluorescent agents will not interfere with the standard surgical field, as the human eye is unable to detect light within these NIR wavelengths. During surgical procedures a NIR light source can be strategically placed above the patient or be integrated within the laparoscopic system. The resulting fluorescence can be instantly visualized in real-time on the camera system screens in the form of images with color, overlay, and NIR representations, allowing surgeons to interpret the information seamlessly. NIR fluorescent agents are predominantly injected intravenously and can be divided into two groups: targeted (binding to a specific ligand or activated by the tumor-specific environment) and non-targeted. Currently, various targeted fluorescent agents are tested in phase I-III clinical trials.[6] In this thesis the focus was on SGM-101.

FIGURE 1 The basics of near-infrared fluorescence imaging.
Near-infrared (NIR) fluorescent agents can be administered either intravenously or locally, and their imaging is conducted using a specialized fluorescence imaging system. This system comprises a white light source, a standard camera, a dedicated NIR excitation light, collection optics and filtration, and a camera specifically for capturing NIR fluorescence emission. The NIR fluorescence output is displayed on a screen in the operating theatre. Ideally, a simultaneous visible light image is captured and can be merged with the NIR fluorescence image for enhanced visualization.



Only a handful of tumor-targeted fluorescent agents have been tested in early phase clinical trials for colorectal cancer. One of these agents is SGM-101, a monoclonal antibody targeting the carcinoembryonic antigen (CEA) bound to the fluorophore BM-104.[7] CEA is notably overexpressed in numerous solid tumors. Specifically, CEA expression is observed in approximately 90% of colorectal adenocarcinomas and 70% of pancreatic adenocarcinomas.[8,9] A phase II study, consisting of 37 patients, showed the first promising clinical results of SGM-101.[10] Based on fluorescence assessment, the surgical plan was changed in nine (24%) patients. In seven patients, fluorescence led to resection of malignant lesions that were not identified with white light only. In two patients, clinically suspected but non-fluorescent tissue was proven to be benign, which resulted in a less extensive resection. These promising results are the basis of the multiple clinical trials described in this thesis.

PERFUSION ASSESSMENT

AL is among the most serious complications in CRC surgery, frequently necessitating additional surgical or radiological interventions, leading to a prolonged hospital stay. AL is reported up to 20% of patients undergoing CRC surgery with associated mortality rates as high as 27%.[11,12]

Inadequate bowel perfusion is considered a significant contributing factor to AL.[13,14] Indocyanine green (ICG) fluorescence-angiography can provide real-time feedback of bowel perfusion and aid to determine in the optimal location for the anastomosis.[15] During the procedure ICG is injected intravenously, and its fluorescence allows for dynamic monitoring of blood flow. This information helps surgeons identify regions with compromised blood supply, which may indicate areas at risk of ischemia or necrosis. By incorporating NIR fluorescence perfusion assessment into standard of care, surgeons could optimize surgical outcomes by preserving well-perfused tissues and minimizing the risk of postoperative complications like anastomotic leakage. Pooled analysis of cohort studies has demonstrated that ICG fluorescence angiography reduces anastomotic leakage, but high-quality evidence is currently lacking. This has led to our initiation of a Dutch multicenter randomized controlled trial assessing the influence of NIR fluorescence perfusion assessment on AL, the AVOID trial.

LYMPH NODE ASSESSMENT

In CRC patients, accurate lymph node staging is crucial for determining prognosis and treatment decisions. Especially the sentinel lymph node (SLN), the first lymph node draining the tumor, seems important for nodal staging because it is believed to be the first place for lymphogenic metastases. Moreover, approximately one-third of patients with stage I and II colon cancer, who are staged as lymph node-negative, still develop distant metastases.[16] This may result from understaging by histopathology, attributed to the presence of lymph nodes with occult malignant cells and micrometastases. Current routine histopathological analysis typically involves the examination of a single paraffin-embedded slide per lymph node, which increases the likelihood of missing tumor cells not located at the slide's cutting surface. More extensive histopathological analysis of all resected lymph nodes could enhance nodal staging, but this process is time-consuming and costly.[17-19] However, extensive analysis of only the SLN is feasible, and thus unfolds a niche for SLN mapping in CRC. Fluorescent dyes like ICG, offer promise for SLN mapping, both *in vivo* and *ex vivo*.[20,21]

Though techniques like ICG mapping show high success rates, they also face issues such as false negatives due to skip metastases.[22] Despite these challenges, fluorescence-guided SLN mapping shows potential for improving staging and guiding treatment decisions in CRC, warranting further exploration and optimization of techniques.

Over the past decade NIR fluorescence-guided surgery has developed from the preclinical stage to initial small-scale (first-in-human) clinical trials. A significant challenge now lies in transitioning these promising results to more extensive clinical trials, as presented in this thesis. These large-scale trials must unequivocally demonstrate patient benefit, such as enhanced complete tumor resections and reduced complication rates, for the technique to be integrated into standard care surgical practices.

Thesis outline

In this thesis, clinical applications of fluorescence guided surgery in colorectal cancer and its metastases are described. It focusses on the clinical use of both targeted and non-targeted fluorescent agents with the use of dedicated open and minimal invasive NIR camera systems. As a result, this thesis will provide insights into the future development and implementation of this technology within the realm of colorectal surgery. Part I of this thesis starts with a review of the current status of fluorescence-guided surgery in colorectal cancer **(chapter 2)**. Subsequently the use of ICG in sentinel node detection **(chapter 3)** and perfusion assessment **(chapter 4, chapter 5)** in colorectal cancer surgery will be

explored. In part II several clinical studies using the CEA-targeted fluorescence tracer SGM-101 will be highlighted. The first chapter **(chapter 6)** will be an outline of the development of this targeted fluorescent agent. This will be followed by chapters about the detection of distant liver metastases of colorectal and pancreatic cancer **(chapter 7)**, the detection of colorectal lung metastases **(chapter 8)** and the detection of both primary lung cancer and colorectal lung metastases **(chapter 9)**. In part III a study for the identification of novel targets for fluorescence-guided lung surgery with the use of data driven software was performed **(chapter 10)**.

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