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Hyperspectral Imaging and Analysis for the Diagnosis of Pigmented Skin Lesions during Gross Pathology

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1 Introduction

More people are diagnosed with skin cancer each year in the U.S. than all other cancers combined [1]. Crude mortality rates of malignant melanoma and other skin cancers increased twofold in Japan from 1999 to 2014, independent of the aging of the Japanese population. The increased workload demands increased effectiveness in the diagnosis and management of malignant lesions. Skin lesions of concern are evaluated clinically and/or histopathologically. The purpose of histopathology includes the accurate delineation of tumor margins. The final diagnosis is produced by combining dermatological with histopathological findings. The whole process takes 2-3 days to complete. If the histological examination shows that malignant tumor margins are compromised, i.e., the tumor extends further than the excised area, then a follow-up surgery is required. Gross pathology is a manual stage between the clinical and the histopathological evaluation of lesions of concern. Despite recent advances in digital pathology, gross pathology remains an analog, labor-intensive and non-standardized step in the diagnostic workflow. Hyperspectral Imaging (HSI) is an imaging technique that captures spectral signatures dependent on substance concentrations in the target. HSI is emerging as a useful modality for staining and standardization in histopathology [2]. In the past decade, several research groups have investigated applications of HSI and Multispectral Imaging (MSI) at the grossing stage as well, towards diagnosis and tumor classification of skin lesions, aiming to achieve optical biopsies and increase diagnosis speed.

Discordant signatures can describe atypical concentrations of skin chromophores and be an indicator of abnormal growths [3]. HSI or MSI systems are one of the means to record such signatures. RGB cameras mimic the behavior of cone cells in the human eye, showing three distinct wide-band responses to visible light. In contrast, HSI and MSI use narrow-band filters with a width of a few nm. HSI systems sample the entire spectrum with a fixed step and provide continuous spectral signatures. In problems where target wavelengths are unknown, the continuous spectral signature might reveal a spectral pattern that is concealed in the MSI. On the other hand, the increase in the number of channels adds additional complexity to data storage and processing, as well as increases processing time. MSI systems are usually customized to the absorbance features of the target tissue. Depending on the imaging equipment, it is possible to capture a 2D surface instantly, with good spatial accuracy.

HSI offers an alternative way to observe tissue, so that a non-invasive or an optical biopsy can be achieved. Currently, the problem of delineating tumor margins during gross pathology has not been extensively investigated. HSI-based segmentation in medicine has only recently gained research traction. Firstly, it is a non-contact, non-invasive, non-ionizing imaging method. HSI does not modify the physical (cellular- and tissue-level) properties of the tissue while preserving the spatial dimension of the distribution of tissue chromophores. Secondly, HSI is fast compared to histopathology. An image can be acquired and processed in a few seconds or minutes instead of days. Coupled with a semi- or fully automated processing tool, HSI-based tumor segmentation can be implemented easily and with minimal training of the medical personnel. In turn, associated costs can be reduced, and resources can be reserved for the diagnosis of more difficult cases. Skin tissue, which is characterized of an increased presence of chromophores and surface

inconsistencies, is an ideal target for colorimetric and texture analysis. HSI has been investigated for applications in tumor detection, dermoscopy and temporal monitoring. Previous studies on commercial MSI-based diagnostic tools showed a considerable increase in sensitivity and specificity for histology guidance by dermatologists and non-dermatologist clinicians. Other studies have attempted HSI-based tissue classification and margin detection with mixed performance. However, the particulars regarding the implementation of an HSI-based system for skin lesions are still unknown.

To achieve an optical biopsy at the gross pathology stage, the suitability of HSI and associated analysis techniques need to be investigated. This work aims to propose a HSI-based framework for the task of tumor margin evaluation of pigmented skin lesions (PSL) during gross pathology. The end goal is to determine the appropriateness of HSI data, tissue condition, preprocessing schemes, and artificial intelligence classifiers for the task, as well as to provide patho-physiological explanations for the results. The proposed framework should provide fully automated predictions of the tumor margin.

This study provides a base framework and dictates good practices, which can be used to build robust systems for the computer-assisted diagnosis of skin lesions. To the author's knowledge, this is the first work that focuses on HSI-based tumor margin delineation of pigmented skin lesion. A common drawback in HSI research for medicine is the lack of labeled datasets, so this study provides a new in-house HSI dataset of PSL. Moreover, this is the first study to investigate the effects of formalin-fixing on the concentration of tissue chromophores. Additionally, the effects of dimension reduction on classification performance for skin HSI are investigated for the first time, comparatively evaluated, and associated with patho-physiological factors. The analysis in this study is more complex than previous discrimination approaches that focused on manual thresholding. Two novel frameworks based on machine learning and deep learning for HSI-based margin delineation of PSL tumors are proposed and tested with a multitude of core models. Gross pathology practice can be significantly accelerated by incorporating the findings of this study, with immediate effects on improving the work conditions of the medical personnel and the treatment quality of patients. Insights about margin detection on ex-vivo tissue could be extended to the intra-operative assessment of tumor margins.

2 Literature Review

Hyper- and Multispectral Imaging (HMSI) has the potential to standardize, accelerate and facilitate diagnosis, by a) producing tumor segmentation in the form of optical biopsies (intra-operatively), or by b) clarifying cancer margins at the clinical stage (pre-operatively). The current trends and proposed systems in skin tissue analysis at the macroscopic level need to be investigated, to develop a viable HSI-based system for margin detection. A systematic review of HSI and MSI applications from 2010-2020 on skin tissue diagnosis, including acquisitions systems, preprocessing schemes and diagnostic models was performed. The effects and limitations of each method are also summarized. In this systematic review, the methodology proposed in the updated 2020 Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement was followed.

Since HMSI is still a developing technology, there is great variability in the

imaging equipment used in each eligible study. The bulk of the reports used either general HMSI cameras or research prototypes. All acquisition systems captured information in at least the visible range. Some systems acquired information up to the near-infrared range. The maximum spectral resolution for HSI was approximately 0.6nm and the maximum number of channels was 10 for MSI and 1127 for HSI. For applications in gross pathology of the skin, experimentation started with MSI prototypes and gradually expanded to HSI. Most studies used immobile HMSI systems. Only a few studies reported on the capture area, spatial resolution, or imaging speed. Capture duration ranged from seconds to minutes. Regarding applications on in-vivo tissue, breathing or unconscious movement should be taken into consideration. While it is noted that a lightweight hand-held device is easier to use in a clinical setting, this should be limited to small ($\leq 10mm$) capture areas. Additionally, fast capture time is preferable to avoid data noise due to movement. Systems that try to emulate the function of a dermoscope are attached to the skin surface or positioned a few mm away. However, most systems were positioned at a distance of a few cm away from the target. This affects the spatial resolution as well as the maximum area that can be imaged. In this regard, a larger distance is preferable, but a macroscopic lens can be used to improve spatial resolution. Another parameter is the illumination condition. Studies were split in half between those that used LED lamps and those that used halogen lamps. Fiber-optic lighting is also an option. The use of polarizers helps to reduce noise and saturation, therefore is suggested. Additionally, a dark box for measurements should be considered, to avoid the influence of ambient light.

Various preprocessing steps can be used sequentially for HMSI analysis. Dataset-wide normalization using a fully reflective and a dark reference is an essential step, especially if complex models are used later. Quality enhancement with average filtering should be used (if used at all) with caution, because it may erase spectral features in HMSI data with low spatial resolution. Dimension reduction is optional, while it depends on the size of the dataset. Feature Extraction or Selection can alleviate ill-posed problems with a large input vector compared to the available number of data samples. However, it is an optional step and if used, methods with different assumptions about the data should be compared. Additionally, Feature Extraction is not recommended when a complex decision model is used, to avoid overfitting. In studies where texture information was incorporated in segmentation, it assisted performance.

Many of the eligible studies emphasized feature extraction, resulting in simple visual evaluation of index images or semi-automatic thresholding. There were a few studies that used traditional machine learning classifiers and only five that used deep learning. For clearly identified tasks such as Melanoma versus Nevus classification, the use of thresholding on a discrimination index seems sufficient [4]. In this regard, an MSI with a few channels is enough. However, for a multi-class problem, a more complex approach is necessary. Staple classifiers such as SVM can provide good results [5]. It should be noted that Synthetic Minority Oversampling TEchnique (SMOTE) can alleviate the problem of unbalanced training classes for an SVM model. Integration of concepts prepared for remote sensing HSI, such as SAM and endmembers, can improve performance. On the other hand, ANN systems generally perform poorly compared to simple methods. This could be attributed both to the relatively small size of the training dataset, the large number of training parameters,

and the unsuccessful learning of rare features.

During the systematic review, the following gaps in the research were identified. Many studies proposed imaging systems with relatively small spatial resolution. There is a need for imaging systems with a higher field of view and higher resolution. Datasets focused on melanoma versus nevus detection, ignoring other rare or common pathologies. More diverse datasets should be prepared. There are no comparative studies or guidelines about effective preprocessing or feature extraction on HSI data for skin gross pathology. Most studies focus either on pixel-wise analysis or on aggregating information from manually selected regions or the entire image. More research is needed on utilizing effectively both the spatial and the spectral dimensions of an HSI data cube. There was limited research on the robustness and explainability of the proposed systems. Both criteria are important for applications in a clinical setting.

A variety of HMSI-based methodologies for skin tumor segmentation and margin detection of skin lesions have been proposed. Most studies applied simple image processing or machine learning, due to small training datasets. Methodologies have been evaluated on heavily curated datasets, with a major focus on melanoma detection. Evaluation of larger datasets, comparison of a variety of methodologies, and estimation of robustness to unusual lesions is necessary. The choice of preprocessing scheme greatly influences the performance of the classifier. Dimension reduction is required to avoid redundancies that are inherent in HSI systems. The incorporation of both spatial and spectral information shows potential. In order to use HMSI for tumor margin detection in practice, the focus of system evaluation should shift toward explainability of the decision-making process.

3 Materials

This section focuses on the acquisition of spectral information and the original datasets that were prepared for this study. There are two ways to design an MSI or HSI camera. The first is to use timed illumination at specific wavelengths in a dark box and retrieve reflectance information with a standard camera. However, this method is limited to the available wavelengths for LED lights. Moreover, it is slow, cannot achieve great spectral resolution, and allows only MSI data acquisition. The alternative is to use white illumination and a monochromatic camera with a filter for selected frequencies, or alternatively an HSI camera. An HSI acquisition system has a considerable cost and complexity. Therefore, the feasibility of HSI-based analysis should be investigated beforehand to warrant the investment. Two independent datasets of tissue samples were prepared and labeled, to assist. The labels were a binary categorization of areas on the tissue as either tumorous tissue (positive class) or non-neoplastic tissue (negative class).

Based on the above, the first stage of this research and proof-of-work experiments were performed with a custom MSI system, capable of 2D snapshot capture. HSI spectral signatures were retrieved from the MSI data via a reconstruction step during the analysis. After confirming the first two research questions, the camera system was upgraded to another custom system capable of 2D snapshot HSI. The HSI system was designed and calibrated in detail, according to the preliminary findings of the experimentation with the MSI system. Both systems captured information in the visible range.

In order to further research in HSI gross pathology of the skin, the present study focused not only on melanocytic lesions but also on skin cancers and other types of skin tumor with increased pigmentation. Data collection was performed in the main hospital of Saitama Medical University (Hidaka, Saitama, Japan). Expert dermatologists and pathologists clarified the criteria of what constitutes a PSL of interest for imaging with HSI. The criteria include melanoma, melanocytic nevus, basal cell carcinoma (BCC), squamous cell carcinoma (SCC), Paget's disease, and Bowen's disease, among others. All samples are obtained from Japanese subjects, with skin type II–IV in the Fitzpatrick classification. All experiments passed review and obtained approval according to the ethical regulations of Saitama Medical University. The experiments in this study were approved by the Saitama Medical University Hospital Institutional Review Board (IRB) (17-128) and the Ethical Committee of Saitama Medical University (977).

For the MSI acquisition system, a total of 10 skin samples were excised from 10 patients. Each sample was imaged with the MSI system and the RGB system three times. Specifically, immediately after excision (unfixed tissue), after formalin fixing (fixed tissue), and sectioning (cross-tissue). The exact same tissue samples were captured with both the HSI and the RGB camera, without any movement of the sample, so images correspond one to one. White balance correction is applied to each MSI data cube. Scene illumination is estimated with the Gray World assumption on a calibration image of a Macbeth color chart. HSI signatures with a 5nm spectral resolution were reconstructed by Wiener reconstruction using the corrected MSI data and the reference signatures.

A strategy for HSI system building and data extraction to assist gross pathology of PSL was presented. The two experiments during system building resulted in a configuration with single light source and polarizer pair being selected as the optimal configuration. Measurements were normalized Pixelwise, because not only provides higher similarity to expected spectra, but also is superior to the alternatives in terms of preserving the spectral curve characteristics. Fast imaging at 618ms, well below the 6min threshold, allows for additional recapturing in cases of failure. Smaller region of interest (ROI) selection results in an even smaller imaging duration. A shorter duration means that raw tissue properties are preserved and that the influence of LED light flickering on noise is limited. It is demonstrated that by tweaking the angle of the light source, average goodness-of-fit and error values slightly improved. Despite the faster imaging duration, bias from the target position is effectively suppressed. The proposed HSI system faces limitations regarding minimum distance from the target, for sharp focus to be preserved. The proposed HSI system was used to create an original database of PSL, for the development of a tumor margin detection system.

For the finalized HSI acquisition system, the wavelength range was limited to 420-750nm with step 1nm, in order to match polarizer response and reduce noisy bands. Data capture was controlled by customized software, provided by the camera maker. Integration time was optimized at 618 ms, resulting in an average imaging duration of 3 minutes per HSI cube. A single LED illumination source at 40° was used. The acquisition system was moved inside the pathology laboratory. Pathology experts performed HSI capture during their usual medical practice. Each HSI data cube was cropped spatially to include only the tissue sample. The largest HSI data cube had spatial dimensions of 500x700 pixels and corresponded to a skin sample

with a length 8cm. The data collection process resulted in a total of 28 HSI data cubes of PSL from 21 patients. An HSI data cube, a tumor segment mask, disease name, and malignancy status were recovered for each sample. The captured dataset was split into two individual parts, the Validation and the Testing dataset. A total of 384,014 spectral signatures were collected for validation, 33% of which were within the tumor margin detected by histology. Another set of 283,206 spectral signatures was collected in the same manner, and used for testing.

4 Effectiveness of Hyperspectral Imaging for Tumor Classification

This experiment aims to answer the following research question: "Are HSI more effective compared to standard RGB images for the classification of tumors versus non-neoplastic tissue?". The hypothesis under consideration is whether spectral features derived from HSI achieve better classification performance compared to equivalent features from standard RGB images or not (null hypothesis). To this end, two datasets were prepared from the same tissue samples: a) an RGB image dataset and b) a multispectral image (MSI) dataset. HSI spectral signatures were retrieved from the MSI data via additional processing. Afterward, three classifiers were trained on features derived from each dataset to classify spectral signatures as tumorous or not (non-neoplastic) and the results were compared.

For this experiment, a novel semi-automated framework based on color and texture analysis of MSI is proposed. The framework is expected to classify critical regions on excised tissue as tumorous or not, to assist tumor margin identification for skin lesions during gross pathology. In terms of image format, MSI is tested against conventional RGB images. The framework attempts to mimic the pathologist's visual assessment of color and texture. This is achieved by combining handcrafted features from reconstructed spectral reflectance and local binary patterns, respectively. The aim is two-fold. First, to investigate the effectiveness of features derived from an original MSI dataset compared to RGB. Secondly, to examine the performance of MSI input to traditional machine learning classifiers.

A MSI-based framework for tumor detection on ex-vivo skin gross tissue was proposed. The framework incorporated both spectral reflectances and texture features. MSI data was more effective compared to conventional RGB images. The importance of using unfixed tissue during analysis is highlighted. Visualization of the classification results depicted fairly accurately both regions with similar tissue characteristics as well as their tumor probabilities. A Random Forest classifier with a feature vector consisting of reconstructed spectral reflectance and Multispectral Multiscale Local Binary Pattern (MMLBP) texture features was the optimal model choice. Although further investigation on a larger dataset is necessary, the proposed MSI framework is capable of enhancing the current pathology practice.

5 Influence of Formalin Fixing on Chromophore Concentrations

This experiment aims to answer the following research question: "Do chromophore concentrations change when the tissue is formalin-fixed? In other words, are both untreated (raw) and formalin-fixed tissue suitable for further experimentation?". The hypothesis under consideration is whether estimated concentrations of skin chromophores are altered after formalin-fixing or not (null hypothesis). To this end, two images were prepared from each tissue sample: a) an unfixed MSI and b) a formalin-fixed MSI. Chromophore concentration Maps (CrMaps) were prepared from each MSI. Chromophore concentrations before and after fixing were compared visually and by distance metrics.

During gross pathology, surgically excised tissue is stabilized with a process called formalin fixing, to preserve and harden the tissue. Since formalin is targeting blood hemoglobin, formalin fixing facilitates oxygen de-saturation in the blood vessels and causes tissue colors to appear washed out, especially red hues. Nevertheless, formalin fixing is necessary to proceed to diagnosis under the microscope. In some instances, the only remaining record after excision is a fixed specimen, which is processed and visually discrepant from the original raw tissue. Moreover, under emergency protocols to prevent disease spreading, such as the recent SARS-CoV-2 pandemic, only fixed specimens may be available for processing.

In this experiment, maps of the relative concentrations of hemoglobin and melanin in ex-vivo gross skin specimens were produced. This was achieved using a capture system with multispectral illumination and an RGB camera. The novelty lies in the creation of MSI-based Chromophore maps (CrMaps) of melanin and hemoglobin from both untreated and formalin-fixed tissue. Three different methods for generating CrMaps were used to produce saliency maps of relative melanin and relative total hemoglobin concentrations. During gross pathology, areas with high chromophore content were manually identified on the tissue. The influence of formalin fixing was quantified using two similarity measures and a distance metric. Comparison of similarities and distance for CrMaps for unfixed and formalin-fixed tissue showed that the content of melanin CrMap is not altered as much as the content of hemoglobin CrMap. This shows that in cases when raw tissue samples are unavailable, fixed samples can be used for the determination of melanin concentrations without severe loss of information. The decrease of hemoglobin CrMap values after formalin-fixing is consistent with reduced oxyhemoglobin and deoxyhemoglobin concentrations. A portion of total hemoglobin is converted to another compound, Hematin, during the chemical process of formalin fixing. In terms of CrMap generation, the Variational Index method was more appropriate for the task according to both visual-based and metric-based comparisons.

Results show dissimilarity between hemoglobin maps of unfixed and fixed tissue pairs in terms of correlation and histogram intersection. This highlights the influence of formalin fixing on hemodynamics. On the other hand, melanin maps are affected by formalin fixing to a lesser extent. Therefore, MSI-based melanin concentration maps can be a useful tool in cases of gross pathological diagnosis where only formalin-fixed specimens are available.

6 Effect of Dimension Reduction on Tumor Margin Detection

Spectral signatures can help detect different skin pathologies using imaging with narrow-band filters. However, dozen- or hundred-point long spectral signatures increase calculation complexity and harm classification performance. For this reason, dimension reduction is commonly proposed as a preprocessing step for machine learning with spectral data. It can both reduce classification complexity and discard noise or redundant information from the spectral signature. Moreover, dimension reduction is commonly used to employ machine and deep learning models that are commonly prepared for three-channel images.

This experiment aims to answer the following research question: "Does the choice of dimension reduction scheme and number of retained dimensions affect classification performance when using HSI data?". The hypothesis under consideration is whether the choice of dimension reduction scheme affects tissue segmentation result or not (null hypothesis). To this end, different dimension reduction schemes were applied to the HSI dataset. A custom HSI acquisition system and an original HSI dataset of ex-vivo gross pathology tissue were prepared. A variety of common dimension reduction techniques, plus one specifically targeting HSI images were applied. The advantages and disadvantages of each method are presented, together with quantified results regarding signal preservation. For each scheme, a classifier was trained and its performance was evaluated. The aim is to provide suggestions for the preprocessing of skin HSI data to facilitate computer-assisted diagnosis of skin tumors.

The choice of dimension reduction scheme and retained dimensions affects HSI-based tumor classification during skin gross pathology. While some methods can extract features relevant to the absorbance of skin chromophores, final classification suffers from low sensitivity and low generalization. Further investigation of dimension reduction methods suitable for HSI is necessary.

7 Implementation and Feasibility of HSI-based Tumor Margin Detection

The end goal of histopathology is to determine the existence or absence of malignancy and to evaluate the status of tissue margins. Knowledge of the tumor margins at an earlier stage, either during clinical examination, during surgery or during gross pathology, can greatly enhance treatment outcomes. Optical margin detection can be interpreted as a segmentation problem of recovering healthy and tumor segments on the tissue surface.

This experiment aims to answer the following research question: "Can an optical tumor margin detection be achieved with HSI in combination with machine learning?". The hypothesis under consideration is that tumor margin detection of pigmented skin lesions can be achieved with HSI data. Both machine learning and deep learning solutions will be investigated. This problem has not been investigated as of yet (according to the author's knowledge). This experiment investigates the problem of tumor margin detection on PSL during gross pathology using HSI data. The goal is to propose a framework for margin detection, which has good enough per-

formance to facilitate computer-assisted diagnosis. This is the first work according to our knowledge to investigate this problem for ex-vivo gross PSL tissue samples. The Validation and Testing datasets are used for experimentation. Performance was evaluated with emphasis on the two-dimensional structure of the predicted tumor segments and investigation of the parameters that affect the model's decision.

Tumor segmentation of HSI data is a new field in gross pathology. This work proposed two novel frameworks, a pixel-wise and a patch-based framework for the segmentation of HSI data from PSL. Results showed that Support Vector Machines (SVM) can achieve good performance but produce noisy tumor masks. The joint inclusion of spatio-spectral information by deep learning models produces more comprehensive tumor segments. Xception networks with 3D Convolution can achieve performance similar to state of the art models, while allowing for further customization in terms of convolution in the spectral direction. Good performance was achieved for melanocytic lesions, but margins were difficult to detect in some cases of basal cell carcinoma. This study showed that tumor segmentation of skin PSL using HSI information is feasible, but further investigation is needed until it is implemented in clinical practice to facilitate fast and optical delineation of tumor margins.

8 Conclusions

This study provides valuable insights for the implementation and evaluation of HSI-based models for skin tumor margin detection. Specifically, the following contributions are listed. Two new fully-labeled databases, one with MSI and one with HSI data, were prepared. A variety of melanocytic and non-melanoma tumors and PSL is included. Two-dimensional mapping of the tumor area generated by expert pathologists after histology evaluation is provided as labels. The final diagnosis is also attached to the image data. A custom system capable of snapshot HSI in two dimensions, with spectral resolution at 1nm was designed and calibrated in detail. Insights are provided about the design process, calibration scheme, and signal normalization. Moreover, the effectiveness of the use of HSI instead of RGB data was verified. Alternative schemes of dimension reduction for HSI data in gross pathology applications for PSL were investigated. The influence of dimension reduction in tumor margin detection was evaluated comparatively. Detailed insights about the correct application of dimension reduction on gross tissue samples of the skin were provided.

This is one of the first works that investigate the task of tumor margin detection of a variety of PSL using HSI data. Two frameworks for tumor margin detection were proposed and tested. The frameworks include complex models at their core, based on machine learning and deep learning. The benefits and limitations of pixel-wise and spatio-spectral analysis are investigated. The proposed methods were evaluated with ground truth labels from cross-section histology slides, which is the standard means of evaluation in a pathology laboratory. This way the actual effect is measured. The feasibility of using formalin-fixed tissue for imaging investigated, for cases when emergency protocols do not allow the use of raw tissue. Different techniques to prepare maps of relative concentrations of skin chromophores were adapted and compared. ClusterPCA, a new dimension reduction technique suitable for gross-level skin HSI data, that performs better than principal component analysis

(PCA). Open-source MATLAB and Python packages for analysis of gross pathology HSI data, including documentation.

Researchers, engineers, and doctors can use the framework proposed in this study as a base to build more advanced models for intra-operative and in-situ margin evaluation. The results of this study can function as a benchmark for the performance of future models. Details about system design and calibration, data collection protocols, and evaluation metrics can guide future studies. Although the dataset is closed, the developed code and models are available open source on GitHub. Since this is one of the first attempts at HSI-based tumor margin detection during skin gross pathology, the insights in this study can significantly accelerate future research.

HSI-based schemes for skin analysis have been investigated for the past two decades. This work focused on the problem of HSI-based optical biopsy for tumor margin detection for PSL. The big question remains whether such an approach is feasible for practical application during the clinical practice. At least two HSI-based commercial systems have been produced and obtained approval from the Food and Drug Administration (FDA) of the United States. These are MelaFind and SIAscope, both tools to facilitate dedrmatology. The biggest bottleneck during investigation of HSI is the large data size and the cost of the HSI camera equipment. In the past few years, advancements in sensor technology, computer components, hardware and software have decreased costs and allowed faster processing. Therefore, the potential of applications during clinical pathology is high. Especially, the use of HSI in a framework for bimodal imaging can further facilitate computer-assisted diagnosis.

While applications of HSI during gross-pathology still require surgery, the proposed method allows only for optical biopsy i.e. without a need to cut the tissue into cross-sections and wait for the histology. In order for HSI for PSL to be truly non-invasive, application for tumor margin detection should be extended to the dermatology stage, before any surgery takes place. Assuming that the properties of freshly excised raw tissue and in-situ tissue are similar, this goal is attainable and can be achieved with further experimentation and larger trials.

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