ADVANCING THE TECHNOLOGY AND APPLICATIONS OF SURGICAL FLUORESCENCE IMAGING WITH TARGETED FLUOROCHROMES

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ABSTRACT

A new concept aiming to improve surgical outcome by providing real-time surgical feedback on tumor margin delineation is presented. We are using a novel multispectral imaging system for real time measurement of fluorescence probes with molecular specificity to tumor biomarkers. Mice bearing xenograft tumors were used to simulate surgical operations of tumor excision guided by real time fluorescence imaging. Bioluminescence imaging and histopathology were used as gold standards to confirm the in-vivo findings. Results demonstrate the capability of the method to identify tumor negative margins with high specificity and provide real time feedback to the surgeon.

Index Terms— Intraoperative imaging, multispectral imaging, fluorescent molecular probe, tumor

1. INTRODUCTION

Optical imaging offers a powerful modality for intra-operative imaging since it relates directly to the surgeon’s vision and offers highly attractive characteristics, including high flexibility in contrast mechanisms, portability, small form factor and fast image acquisition. Especially, fluorescence imaging consists an appealing approach for intra-operative because of the increasing availability of fluorescence probes that can be intravenously administered and identify otherwise invisible cellular and sub-cellular function indicative of specific cell types or physiological responses. Moreover, imaging of fluorescence probes can provide high contrast and sensitivity. Despite these attractive advantages, the imaging performance in resolving superficial fluorescence activity can be compromised by two major parameters, i.e. tissue auto-fluorescence and the variation in tissue optical properties. Systems that utilize imaging at multiple wavelengths [1] have been developed to differentiate auto-fluorescence from a fluorochrome of interest. Another strategy is to utilize near-infrared fluorochromes, since tissue auto-fluorescence in the near-infrared is significantly reduced compared to the visible [2]. The variation of tissue optical properties can also compromise the performance of photographic fluorescence imaging. This variation is typically corrected in tomographic systems [3-5] but corresponding corrections have not so far been described in systems developed for photographic imaging applications and intra-operative applications.

We present a multispectral fluorescence imaging system that implements image correction for tissue optical attenuation, developed for intra-operative surgical imaging [6]. The system developed herein is indented to operate as a fluorescence platform that can examine a variety of diseases and a variety of fluorescence methods while correcting for optical property variation. For this reason attention has been given to collect fluorescence, attenuation and color images simultaneously, from the same field of view, while operating in real-time mode, i.e. at video rate capacity. We demonstrate the performance of the developed system in phantoms and in vivo mouse measurements. Taken together, the imaging of anatomy in the color channel combined with corrected fluorescence measurements provides an imaging system with the potential to offer superior intra-operative quantification ability compared to previous implementations.

2. MATERIALS AND METHODS

We developed an imaging system for intraoperative use with the following key parameters: 1) the capability of simultaneous video-rate imaging in excitation, emission
and visible spectrum for color imaging, 2) the capability of providing fluorescence normalization and finally 3) the ability to be adapted to any optical imaging system such as surgical microscope or endoscope.

Figure 1: Experimental setup of multispectral fluorescence imaging for intraoperative use

To reach these functional specifications, a three imaging channel system was developed as shown in Figure 1. Illumination was provided by two 250 W halogen lamps, one for white-light color imaging and the other for fluorochrome excitation by means of appropriate filters. Light from the surgical field was collected using a zoom lens. The setup employs two mirrors that split into three imaging channels, i.e. color, emission and excitation (intrinsic) spectral bands. CCD cameras are used at the color and intrinsic channels while a very sensitive EMCCD camera is used to capture fluorescence so that all imaging channels can acquire data at video rate. At the fluorescence and intrinsic channels we are using a new method for multispectral imaging that allows concurrent capturing of application-defined spectral bands using single-chip color CCD cameras. The principle of operation relies on the combination of color CCD cameras and multiple-bandpass filters. Appropriate calibration of the system enables the transformation of the color measurements of the camera into spectroscopic information, i.e. light intensity at multiple spectral bands [7]. Thereby the system is capable to measure simultaneously more than one fluorophores together with the color image of the tissue under examination.

Measurements on phantoms and post mortem animals were performed in order to test the multispectral performance of the system and the ability to correct fluorescence measurements for the variation of the tissue optical properties.

In vivo measurements were performed on mice (nu/nu) with subcutaneous and intraperitoneal tumors developed after injection of tumor cells. A variety of different specific probes, including both visible and infrared fluorescent probes were tested.

In vivo measurements were also performed intraoperatively in humans using tumor specific fluorescence probes approved for clinical tests in patients.

3. RESULTS AND DISCUSSION

Figure 2 shows in vivo measurements from a mouse with subcutaneous tumor (4T1 cell line), 24 hours after injection of a fluorescently labeled non-peptide small molecule aimed at the $\alpha_v\beta_3$ integrin receptor (IntegriSense™ 680; VisEn Medical Woburn MA, USA). This probe is used as a generic cancer marker, since the $\alpha_v\beta_3$ integrin receptor is known to be regulated in cancer cells and hardly expressed in normal tissues except kidney tissue [8-11].

Fluorescence and color images were fused to create pseudocolor images where the real color is altered only at the areas where fluorescence signal is detected. This results in the tumor to appear green so that it creates a noticeable contrast to the healthy tissue which is illustrated with its original color. This pseudocolor illustration makes the tumor areas easily noticeable while the image retains all the visual information.

The surgical procedure was performed in stages to simulate the standard surgical tumor excision and thereto demonstrate how real time intraoperative fluorescence imaging can guide the surgeon and improve the surgical outcome. First, the tumor was exposed by removing the skin (Figure 2a), then ~90% of the tumor was removed under visual guidance, leaving behind a small rim of tissue of 10% simulating residual disease (Figure 2b). At the next stage, the all residuals were removed (Figure 2c). Subsequently, normal (non-fluorescent) tissue that surrounded the tumor was also excised and sent to pathological examination. Surgical removal of the tumor was performed by a surgeon guided by the fluorescence image which was displayed in real time.
Overall, hematoxilin and eosin (H&E) examination confirmed the presence of tumor in all of the excised fluorescent positive samples (i.e. primary tumor, residual tumor and other fluorescent lesions (spots) that appeared, as in Fig. 3. None of the samples with a negative (non-detectable) fluorescent signal showed any tumor deposits. In addition, the appearance of a rim on histological examinations was also identifiable on the fluorescence and confirming bioluminescence images.

4. CONCLUSIONS

We have showed for the first time the potential of a practical next generation real-time multi-spectral fluorescence-imaging platform to intra-operatively image targeted fluorescent probes and identify microscopic residual disease during surgery. This imaging strategy can lead to improving the surgical outcome and comes with significant dissemination potential in operating rooms, due to the operational advantages it attains.

5. REFERENCES