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A Portable Fluorescence Camera for Testing Surgical Specimens in the Operating Room: Description and Early Evaluation

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Abstract

Purpose—Clinical translation of novel optical probes requires testing of human specimens *ex vivo* to ensure efficacy. However, it may be difficult to remove human tissue from the operating room due to regulatory/privacy issues. Therefore, we designed a portable fluorescence camera to test targeted optical imaging probes on human specimens in the operating room.

Procedures—A compact benchtop fluorescence camera was designed and built in-house. A mouse xenograft model of ovarian cancer with an activatable imaging probe based on rhodamine green was used to test the device. Comparison was made to commercially available imaging systems.

Results—The prototype camera produced images comparable to images acquired with commercially available, non-portable imaging systems.

Conclusion—We demonstrate the feasibility of a specimen-based portable fluorescence camera for use in the operating room. Its small size ensures that tissue excised from patients can be tested promptly for fluorescence within the operating room environment, thus expediting the testing of novel imaging probes.

Keywords

Fluorescence imaging; Surgery assistance; Portable camera; Surgical specimen

Introduction

Optical molecular imaging has the potential to assist surgeons and endoscopists by enhancing their ability to detect otherwise invisible lesions [1–4]. This requires the synthesis

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and testing of novel optical imaging probes. The development of such probes involves the translation from pre-clinical to clinical testing. Pre-clinical testing can be completed in the laboratory using commercially available benchtop fluorescence cameras and can be performed *in vitro*, *in vivo*, and *ex vivo*. However, once clinical specimen testing begins with human tissues, there are a number of important issues that arise [5,6]. For instance, there are regulations concerning the transport and storage of human tissues, privacy concerns, and the requirement for many probes that the specimen must be tested rapidly after being removed from the body. Taken together, there is a strong impetus to develop a portable fluorescence camera that can image the specimen directly in the operating room or endoscopy suite and then return the specimen for normal processing [7].

No such commercial fluorescence imaging device exists. Therefore, we undertook to design and build a portable narrow-band (*i.e.*, limited spectral window) fluorescence camera for specimen imaging in the operating room. This device is designed to be easily carried into the operating room or endoscopy suite and then set up within minutes using a laptop computer. Once the specimen is available, it is treated with the experimental molecular imaging probe and assessed for fluorescence under the camera. After the imaging has been completed, the tissue is returned for normal processing or storage, and the images are available for immediate evaluation. In this manner, the specimen never leaves the environment where it was procured, thus reducing regulatory concerns. Herein, we describe the development of a portable fluorescence camera and preliminary testing of the device using specimens obtained from mice.

Materials and Methods

Design Goals

As mentioned, the primary design goal was portability so a single person could easily handle and transport the fluorescence imaging system (Fig. 1) via car or public transportation. During a specific imaging session, the process requires the ability to individually image multiple samples, as well as to acquire consecutive images from any given sample at multiple predetermined time points. This process requires handling multiple tissue specimens at a time; therefore, it was important for the camera system design to maintain user-friendliness and minimize errors due to user setup or tissue handling while acquiring images. This was accomplished in the design by incorporating fixed lens magnification and focus, fixed specimen orientation (*i.e.*, keyed tray), multiple specimen trays, and fixed fluorescence excitation light intensity. For this application, the tissue samples fit into a volume approximately 2 in.² by 0.5 in. high. For thin samples, a specimen tray spacer is used to raise the tissue within the imaging depth of field (*i.e.*, focus). All electronics (*e.g.*, charge-coupled device (CCD) camera, light-emitting diodes (LEDs), LED driver) are entirely self-contained within the portable camera enclosure with external cable connections for power and USB interface to a laptop computer.

The required spectral region of interest corresponds to the rhodamine green fluorophore, with a peak excitation wavelength of 480 nm, and a peak emission wavelength of 532 nm. The CCD camera was specified to have a minimum image resolution of 800× 600 pixels and programmable exposure times from several milliseconds to several seconds. The imaging system depth of field needed to accommodate a reasonably thick tissue sample in order to maintain focus from the top to bottom surfaces. Although designed for a relatively narrow fluorescence spectral range, a color CCD camera was required in order to facilitate tissue visualization and to provide design flexibility for potential future system enhancements or modifications (*e.g.*, bright-field image). In the case where a new molecular imaging probe study would require the overall portable camera to have different spectral specifications, the

method of mounting of optical components should facilitate substitution (not by end user) of the excitation source (LEDs), excitation filters, and emission filter.

Major Subsystems

The portable narrow-band fluorescence camera system (Fig. 2a) consists of a LED-based excitation light source, fluorescence excitation and emission filters, CCD camera, custom plastic enclosure and mechanical components, and custom instrument software.

Excitation Light Source and Filter

Four blue LEDs (Philips, Model Luxeon Rebel LXML-PB01-0023; 466 ± 16 nm, see Fig. 3) are mounted in an equally spaced circular pattern with an approximately 1.5-in. radius and coaxial with the centerline axis of the CCD camera. The location and slight inward-facing angle of these LEDs maximizes light intensity in the system field of view, while also providing adequate intensity uniformity (4% over the entire 2-in.² viewing area). The LEDs are soldered to aluminum Metal Core Printed Circuit Boards (Luxeon Star, Model LXB-RS20A) which are each attached to 27 mm L $\times$ 27 mm W $\times$ 18 mm H heat sinks (Aavid Thermalloy, Model 374424b00032g) using thermal adhesive. The entire LED assembly mounts face down on the top plate holes of the imaging chamber using custom plastic clips. The LEDs are powered by an LED driver evaluation board (Renesas, Model EV-K0-HCD). The LED driver board features a microcontroller (Renesas, Model μ PD78F8024) with a four-channel constant-current LED driver. In conjunction with a small potentiometer mounted next to the LED driver board, the microcontroller is used to provide excitation intensity control. The potentiometer is tool accessible from outside the enclosure for calibration purposes but is not intended for frequent user intensity adjustments. The LED driver board was modified to allow for increased 700-mA current drive for each output channel. The LED assemblies are wired serially in two pairs, so only two driver channels are used and overall current requirements are reduced. The other two LED driver board channels could be used to drive additional LEDs to meet future requirements for other wavelengths (*e.g.*, a second fluorophore color or bright-field imaging).

A narrow bandpass optical filter (Omega Optical, Model XF3075) was mounted in front of each LED assembly. The filter response peaks at 480 nm with a bandwidth of 30 nm, which limits the wider spectral bandwidth of the LED to effectively eliminate overlap with the emission filter described below. Without this excitation filter, the longer wavelength components of the LED emission passed through the emission filter, resulting in unacceptably high image background intensity and a low signal-to-noise ratio. Focusing and diffuser lenses specifically designed to mate with the LED lens are commercially available, but tests indicated no substantial benefit from the added complexity. Therefore, a custom plastic holder was designed to mount the excitation filter very close to the LED lens to minimize light leakage and the corresponding image background levels. The angular dependence of the filter cutoff leads to a slight broadening of the excitation bandpass, but even with this effect, there is no overlap with the emission filter.

Emission Filter and Camera

The off-the-shelf color CCD camera (IDS Imaging, Model IDS-UI-2240-ME-C; ~\$1,200) provides an image resolution of 1,280 $\times$ 1,024 pixels with a 1/2-in. detector. The camera allows for exposures from 66 μ s to 10 min using a software trigger, generated by the custom instrument application program. The CCD camera has a right-angle form factor which reduces the overall height of the portable system enclosure by several inches. The CCD camera USB interface provides the necessary power which eliminates the need for an additional power cable.

A 2/3-in., 8-mm F1.4 lens (Kowa, Model LM8JCM) attaches to the CCD camera C-mount. A long-pass emission filter (Chroma, Model HQ520LP) was mounted directly in front of the lens. Because the filter could not be procured with a holder compatible with direct mounting to the lens body, a custom emission filter holder was fabricated which mounts just under the top plate of the imaging chamber. During initial testing, a bright ring artifact was centered in the acquired images. This image artifact was caused by light entering the filter at acute angles, leading to suboptimal filter performance. A modified emission filter holder with a cylindrical tube extension (1-in. inner diameter, 0.75-in. length) in front of the filter (*i.e.*, tissue sample side) eliminated the image ring artifact by reducing the amount of scattered light reaching the filter. The CCD camera and lens assembly is mounted on top of the imaging chamber pointing in the downward direction. The lens surface is approximately level with the top of imaging chamber (Fig. 2b). The lens magnification and focus are adjusted to provide a 2×2-in. field of view at an approximate working distance of 3.5 in. In conjunction with a specimen tray spacer, the depth of field of the CCD camera and lens configuration provides good focus for very thin to 0.5-in.-thick tissue specimens.

Enclosure, Mechanical, Tissue Handling

All mechanical designs, such as the enclosure, optics mounting brackets, and specimen trays, were completed using 3D computer-aided design software (Dassault Systèmes SolidWorks) (Fig. 2c). A noteworthy detail on this portable camera development process is that all mechanical components were modeled and fabricated in black ABS plastic using a 3D printer (Stratasys, Model Dimension Elite). This method of rapid prototyping minimized the engineering development time by streamlining the iterative design process, as well as decreased fabrication and component costs. The portable camera total parts cost is approximately \$4,000, making this design very cost effective relative to commercially available fluorescence imaging solutions. Admittedly, these commercial systems including the FluorVivo 300 (INDEC BioSystems, Santa Clara, CA), which is one of the least expensive systems of approximately \$50,000, typically provide a larger range of wavelengths, selectable filters, and other functionality. On the other hand, if this portable fluorescence camera were to be commercialized, the plastic enclosure and other mechanical components could be injection-molded to further control costs.

Due to the brightness of the LED excitation light source (measured 3.9 mW/cm² at the center of tray opening), two safety devices were integrated into the design to protect the user. A miniature plunger-style (Panasonic, Model ESE-11HS2) switch is installed on the bottom plate of the enclosure near the specimen tray port. This switch shorts out the excitation intensity control signal, forcing the LEDs off, when the enclosure is lifted off the specimen tray. In addition, a tilt sensor (NKK, Model DSBA1P) is installed inside the enclosure which also shorts out the intensity control signal when the enclosure is tilted more than 30°. Due to an instability in the tilt sensor, the LEDs will turn back on if the enclosure is tilted more than 60°. However, the light simply flickers at a very low level which is eye safe.

Since the LEDs generate a significant amount of heat, four vents were added to the enclosure to assist in heat dissipation. These vents are positioned in line with the four LED heat sinks, and miniature fans (Sunon, Model GM1203PFV2-8) were added at two of the vents to force air flow over the heat sinks. Even if the LEDs are not turned on, the fans run continuously once power is applied.

Multiple specimen trays are included to provide the surface area for tissue placement and to enable an assembly-line-type processing of several specimens. The trays simply rest on the table work surface during imaging. The trays are keyed with a tab to prevent accidental rotation of a given tissue sample orientation when cycling through different tissue samples

on their corresponding trays when acquiring images at multiple time points. The tray perimeter edge is chamfered to mate with the enclosure bottom plate specimen port, which enables the specimen tray to be self-centering when the camera enclosure is lowered down on top of the tray (Fig. 4). A typical imaging session, involving multiple tissue samples on separate specimen trays, requires the user to move the portable camera between trays by simply lifting the enclosure off one tray and lowering it down on another tray. This overall specimen handling and imaging process have been proven to be light-tight (*i.e.*, blocking room lighting) and user-friendly.

Software

The portable fluorescence camera application-specific software was written in C++ and used the wxWidgets framework for the graphical user interface. For ease of use and to minimize the potential for user error in a stressful operating room environment, the software interface offers limited functionality, including limited gain and exposure control, image capture, and image saving in TIFF or JPEG formats. When saving an image, the filename can be auto-generated or entered manually. The target platform was an Apple laptop computer, but the CCD camera manufacturer does not provide compatible drivers. The custom software was developed for the Microsoft Windows platform and is run under a Windows 7 virtual machine through the use of the Parallels Desktop 5 software. Using the Coherence mode, the application can be run as if it is a standard Mac application.

Quantification Validation with Phantom Solutions

To determine the sensitivity of the camera, rhodamine green–succinimidyl ester (Invitrogen Co., Carlsbad, CA) was dissolved in dimethyl sulfoxide and 10-mM stock solution was prepared. The stock solution was diluted to concentrations of 10-, 1- μ M, and 100-nM concentrations in phosphate-buffered saline (PBS). Same-sized droplets (10,750 $\pm$ 346 pixel on the image) of 100 μ L of each dilution were placed onto a non-fluorescent specimen tray for imaging. Images were taken and regions of interest (ROIs) were drawn over each droplet for quantification.

Testing in a Mouse Tumor Model

All *in vivo* procedures were carried out in compliance with the Guide for the Care and Use of Laboratory Animal Resources (1996), National Research Council, and approved by the National Cancer Institute Animal Care and Use Committee. A human ovarian adenocarcinoma cell line, SHIN3, was grown in RPMI-1640 medium containing 10% FBS, 0.03% L-glutamine, 100 U/mL penicillin, and 100 μ g/mL streptomycin at 37°C in 5% CO₂. SHIN3 cells (4 $\times$ 10⁶) suspended in 200 μ L of PBS were injected intraperitoneally into female nude mice, and experiments imaging tumor xenografts were carried out 2 weeks after injection, when tumors typically develop.

Tumor-bearing mice received an intraperitoneal injection of 25 μ g of galactosamine-conjugated human serum albumin (GSA) conjugated to rhodamine green (RG) resuspended in 300 μ L PBS [8,9]. The mice were then euthanized by CO₂ inhalation 4 h after GSA-RG injection. The peritoneum was then exposed with an abdominal incision, and the mesentery was removed. Immediately afterwards, the tumor nodules were excised and removed. Tumor nodules were placed on a non-fluorescent specimen tray and imaged with the portable camera.

Results

Development Testing of the Portable Fluorescence Camera Device

The camera was able to detect a 100- μ L droplet of 100 nM rhodamine green, and quantification following normalization of ROI mean intensity from serial dilutions of rhodamine green demonstrated strong linear correlation ($r^2=0.9994$) between the concentration of the GSA rhodamine green compound and the fluorescence intensity ranging between 10 μ M and 100 nM.

Ex Vivo Findings in Excised Tumor Nodules

Ex vivo tumor nodules were clearly identified with the portable camera (Fig. 5a). Further, when imaging tumor dissemination to explanted mesentery, tumors were clearly visualized and nodules were readily distinguished from bowel autofluorescence (Fig. 5c). Images were comparable to those obtained using a commercially available device (Fig. 5b, d), which, due to its size, is not easily transported.

Discussion

The ability to provide portable fluorescence imaging in the operating room and endoscopy suite is important for the clinical translation of optical molecular imaging probes [10,11]. An intermediate step in the clinical translation of such agents is the proof of efficacy in excised tissues. This step poses no risk to the patient yet clearly employs relevant human tissue. However, a number of important regulatory and legal issues pertain to the use of such cameras in this setting [6]. Such studies must receive Investigative Review Board approval because the device is experimental. However, in addition, clinicians are justifiably concerned if tissue samples, potentially important for diagnosis or management, are removed from the hospital for testing with experimental agents and therefore made unavailable for routine processing in the Pathology Department. While sufficient amounts of tissue are usually harvested, occasionally, there is a problem with the primary specimen, and it is desirable to have the secondary specimen available for processing. Moreover, concerns about patient privacy, potential storage of human tissues, and the need to image the human specimen rapidly after procurement encouraged us to develop this portable fluorescence camera. In our case, the camera was designed for frequent transport to and from an out-of-state hospital. Without the portable camera, the tissue would need to be shipped (one way) by certified courier from the remote hospital to our laboratory. Obviously, imaging the tissue at the surgery hospital avoids the possibility of many additional hours of uncertain temperature conditions between tissue harvesting and testing, with potentially detrimental effects on the reproducibility and accuracy of the fluorescence imaging and subsequent data analysis.

Using a mouse model of ovarian cancer, we demonstrate that harvested tissue can be assessed rapidly with the portable fluorescence camera. The tissue can then be immediately fixed and stained for routine histopathology. The agent employed in this study requires up to 4 h of physiological washout of unbound probes before the tumor can first be visualized. At this 4-h time point, the portable camera provided tumor visualization with a signal-to-noise ratio comparable to that obtained with less portable commercial fluorescence imaging systems. These results provide preliminary evidence of efficacy for this agent, which can then be translated to human specimens.

This portable fluorescence camera system could be useful even after fluorescence agents are approved for use in clinical practice. For fluorescence-guided biopsy or surgery, it is clearly advantageous, for both patients and surgeons, if we can immediately confirm correct tissue

sampling or clear margin in cancer nodule resection without sending specimens to the pathology department for processing.

In summary, we have designed and built a portable narrow-band fluorescence camera for use with human specimens in order to test novel molecular imaging probes in the operating room or endoscopy suite immediately after tissue procurement. The portability of this device enables it to be transported readily and placed in the room where the tissue is procured while occupying minimal space due to its diminutive footprint. The camera digital images are transferred and stored on a laptop computer. While tailored for a limited spectral window or set of probes, this device can be assembled for significantly lesser cost (~\$4,000) while still providing imaging performance comparable to benchtop full-sized *in vivo* fluorescence imaging systems (above \$50,000). The fluorescence spectral window of interest, corresponding to the molecular imaging probe under study, can be modified by simply installing an appropriate excitation LED and excitation and emission filter set, although cameras could be more than tenfold more expensive when using probes in the NIR range. We believe such portable fluorescence imaging devices will accelerate the development and testing of optical molecular imaging probes and allow for their more rapid introduction into routine clinical care.

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Fig. 1.
System photo. The camera (*right*) can be connected with a laptop Macintosh computer via USB connection.

Fig. 2.
a Camera design transparent model, **b** portable fluorescence camera design approximate dimensions (inches and 3 lbs 3 oz), **c** camera 3D rendering outside view.

Fig. 3. Spectra of a single LED (*blue solid*), camera LEDs with excitation filters (*red solid*), rhodamine green excitation (*blue dashed*), and rhodamine green emission (*green dashed*).

Fig. 4.
Demonstrating lowering camera onto specimen tray (safety interlocks temporarily disabled enabling LEDs to be on).

Fig. 5. Comparison of images obtained using our portable camera system (**a** and **c**) and images obtained from a commercially available benchtop system, FluorVivo 300 (**b** and **d**). The smallest scale indicates 1 mm.