Near-infrared fluorescence catheter system for two-dimensional intravascular imaging in vivo

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Abstract: Detection of high-risk coronary arterial plaques prior to rupture remains an unmet clinical challenge, in part due to the stringent resolution and sensitivity requirements for in vivo human coronary arterial imaging. To address this need, we have developed a near-infrared (NIR) fluorescence imaging catheter system for intra-vascular molecular imaging of atherosclerosis in coronary artery-sized vessels, capable of resolving two-dimensional fluorescence activity in hollow organs, such as blood vessels. Based on a rotational fiber design, the catheter system illuminates and detects perpendicular to the rotational axis, while an automated pullback mechanism enables visualization along blood vessels with a scan speed of up to 1.5 mm/sec. We demonstrate the previously undocumented capacity to produce intravascular NIR fluorescence images of hollow organs in vivo and showcase the performance metrics of the system developed using blood vessel mimicking phantoms. This imaging approach is geared toward in vivo molecular imaging of atherosclerotic biomarkers and is engineered to allow seamless integration into the cardiac catheterization laboratory.

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1. Introduction

Myocardial infarction due to atherosclerotic plaque rupture is a leading cause of morbidity and mortality worldwide. The earliest possible detection of high-risk plaque instability is therefore likely to be critical in preventing plaque rupture and consequent myocardial infarction [1,2]. A number of intravascular catheter systems (i.e. intravascular ultrasound, optical coherence tomography, NIR spectroscopy) [3,4] have been tested in human subjects for detecting structural features of high-risk coronary plaques, such as the presence of a thin fibrous cap and large lipid pool [5,6]. However at present, current intravascular imaging approaches do not assay the underlying biology of coronary atheroma, and in particular plaque inflammation, a critical factor underlying the progression and rupture of high-risk plaques [7,8].

Molecular imaging technology attempts to illuminate the underlying biology of atherosclerosis using a combination of molecular and cellular targeted imaging probes in concert with sensitive, clinically applicable hardware detection modalities. Notably, several solutions (e.g. 18F-fluorodeoxyglucose enhanced positron emission tomography, ultrasmall superparamagnetic iron oxide enhanced MRI) have demonstrated clinical potential for molecular imaging of inflammation in large arteries such as the aorta, carotid, and iliac arteries [9–12]. Yet to date, scant data is available demonstrating the ability to image plaque inflammation in human coronary or coronary-sized arteries in vivo, in part due to the stringent sensitivity and resolution requirements of coronary atherosclerosis imaging.

Recently, the use of near-infrared (NIR) fluorescent molecular probes with sensitivity to inflammation has shown promise for sensitive and specific detection of inflammatory
proteolytic activity in atherosclerosis [9,13]. By operating in the NIR region, fluorescence imaging can avoid limitations common to both visible and infrared light due to biological absorbers, hemoglobin in the visible light and water/lipids in the infrared [14,15].

Detection of NIR fluorescence from hollow organs, such as vessels, has been reported previously, based on a preliminary one-dimensional pull-back intravascular wire system for molecular fluorescence sensing of atherosclerosis [13,16]. While this one-dimensional manual pullback system has showcased the feasibility of detecting fluorescence in-vivo in animal models, it did not generate anatomically accurate vessel images and it is overall not appropriate for clinical intervention. We now report on the development of an automated rotational 2D NIR fluorescence (NIRF) catheter based system that can visualize fluorescent probes in hollow vessels utilizing 360° scans. The system operates within clinical grade intravascular ultrasound (IVUS) catheters to enable rapid integration into the cardiac catheterization laboratory. In this article, we describe key design and performance characteristics of the new NIRF catheter, and demonstrate the ability for accurate fluorescence imaging of hollow organs.

2. Materials and methods

2.1 Experimental setup

The experimental setup developed is shown in Fig. 1. The system includes a continuous-wave laser source operating at 750 nm (B&Wtek Inc, Newark, DE, USA) with electronically adjustable output power of up to 250mW. The input light is guided through a beamsplitter arrangement into a high-speed rotational stage (Oriental motors Inc, Tokyo, Japan) that incorporates a rotational fiber coupler (Princetel Inc, Pennington, NJ, USA) and enables rotation of the imaging fiber within the vessel with a speed of up to 200 rounds per minute. The close up of pullback and rotational mechanism is shown in Fig. 2. All the fibers utilized had a numerical aperture of 0.12 and a core/clad diameter of 100/140 µm. The experimental data shown herein were obtained using 25mW excitation light at the catheter tip.

Fluorescence light, emitted from within the blood vessel after excitation, is captured by the same fiber and guided back through the rotational coupler onto a dichroic mirror with 765 nm cut-off wavelength (AHF analysentechnik AG, Tübingen, Germany). The dichroic separates the light that back-propagates in the fiber into the excitation and the emission spectral components, which are subsequently detected with the two photomultiplier tubes (PMT1 and PMT2, Models H5783-20, Hamamatsu Photonics, Shizuoka Pref., Japan). The second PMT is used herein to measure the excitation light intensity, in parallel with the fluorescence measurements, in order to correct the fluorescence measurements for possible excitation light variations. In addition to using dichroic mirrors, the signals, detected at the excitation and emission wavelengths, were further filtered using appropriate band-pass filters placed in front of the corresponding PMT’s. The signals recorded from the PMT’s were digitized at 1Ksps using an analog to digital converter sampling card (Model NI PCI-6014, National Instruments Co., Austin, TX) and stored on the PC.
Fig. 1. NIR fluorescence imaging system catheter. Schematics of the experimental setup: laser light propagated through the optical cube into multimodal (MM) fiber, travels through rotational coupler and, finally, propagate into MM fiber that has angle-polished, mirror-coated front end. The delivered light excites fluorochromes in the ROI and collects corresponding emission signal, which propagates through the same MM fiber to the detection cube and measured by two PMTs. Signal is then digitized, stored and analyzed.

Fig. 2. Photograph of the rotating pullback device with imaging fiber mounted inside of modified IVUS catheter. Rotational stage with coupler inside ensure uniform rotation of the fiber inside of the IVUS housing, while linear stage provides automatic pullback of the rotational stage together with sensor.

2.2 Catheter and fiber system

A 2.6F (0.87mm diameter) IVUS catheter housing (Boston Scientific Co., Natick, MA), standard for cardiac catheterization lab IVUS imaging, was custom-modified and used for fiber protection and guiding (Fig. 2). The custom modification of the catheter included the removal of the back-end connectors, the IVUS wire and detectors, and the subsequent insertion of a bare 100/140 µm multimodal optical fiber with coating diameter of 250 µm (AFW Technologies Pty Ltd, Hallam, Victoria, Australia). The bare optical fiber was angle polished at the front end and covered with foil to create side light emission at a 90 degree angle (WT&T Inc., Lachine, Quebec, Canada). The fiber tip was further covered with radio-opaque lead material so that it could also be visible on X-ray angiography. The fiber is
therefore flexible enough to be guided inside blood vessels, while at the same time being protected by the IVUS catheter housing from damaging the arteries. A custom made connector was introduced between the fiber ferrule and the housing input to optimally interface the catheter system to the experimental prototype developed and prevent liquid leakage. By utilizing different focusing optics and numerical apertures, different illumination-length dependent point spread functions (PSF) can be constructed, which overall determine spatial resolution of the system. To enable visualization along vessels, an automatic pull back mechanism was custom assembled using industrial stages (Oriental Motor (Europa) GmbH, Düsseldorf, Germany) with minimal step size of 0.02 mm. When the maximal rotational speed was applied (200 rpm), the linear stage maximal pull-back speed was 1.5 mm/sec - comparable with the rates currently used in IVUS imaging.

2.3 Image formation

Images were formed by registering the intensity of each measurement onto a pixel in a two-dimensional space defined by the angle of rotation and the pull-back distance. Assuming an image with m by n pixels, formed after the pull back of the fiber over a distance d, each pixel position in the image can be expressed as p(φ, z), where the dimension of each discrete step along the angular axis φ is 3600/m (m being the number of discrete points collected in a single full rotation) while the pixel dimension along the pullback axis z is d/n. The formulated image is therefore a “raw data” image. This image can be further improved using a deconvolution process or a similar process that corrects for the point spread function (psf) of the system used. However this study presents the unprocessed images obtained, independently of those additional processing steps that can improve image appearance.

2.4 Phantom imaging

Experimental measurements were performed using five different phantom configurations described below and intended to showcase different system performance characteristics.

2.4.1 Imaging of hollow vessel phantoms

To showcase the basic ability for visualizing hollow organs we utilized a 3 mm diameter plastic tube, mimicking the typical diameter of a human coronary artery. Two small glass tubes (1 mm diameter, length 30 mm) were attached in parallel onto the plastic tube, as shown in Fig. 3a, and were filled with 1 μM of the near-infrared fluorescent probe Alexa Fluor 750 (AF750; Invitrogen, Carlsbad, CA), which attains peak excitation at 750 nm and peak emission at 775 nm. AF750 has an extinction coefficient of ε = 0.24 μM−1 cm−1 and quantum yield (QY) of 0.12. (Invitrogen Inc. Molecular Probes. The Handbook. Section 1.3) Imaging
was performed using an automated fiber pull-back over a 6 cm distance. The rotation speed was 30 RPM and the pull back speed was 0.25 mm/s, corresponding to a linear step size in the axial direction of 0.5 mm per round.

An additional phantom constructed consisted of four glass tubes (1mm diameter) containing 1µM concentration of AF750. The glass tubes were perpendicularly attached to a 2mm diameter glass cuvette (Fig. 3b). This phantom was similarly imaged over total length of 6 cm with 30 rpm rotation and pull-back speed of 0.5mm/s.

2.4.2 Spatial resolution characterization

To investigate the spatial resolution achieved by the system we utilized the resolution phantom shown in Fig. 3c. For this, two flexible and thin (150µm inner diameter) tubes were filled with 1µM of AF750 and arranged in a crossed pattern on the outer surface of the 2 mm glass cuvette. The catheter was inserted inside the cuvette and imaging was performed with 30 RPM rotation and pull-back speed of 0.5mm/s.

2.4.3 Signal to noise ratio (SNR)

To quantify the system’s SNR, a 3 mm – diameter plastic tube was used, to which three 0.4 mm diameter glass tubes with varying concentration of the fluorochrome (10µM, 1µM and 100nM) were attached perpendicularly (Fig. 3d). The phantom was then immersed into solution containing 1.2% Intralipid and 1200 ppm of black India ink, resembling the typical optical parameters of whole blood [17] at 750 nm (reduced scattering coefficient µ’s = 20cm-1, absorption coefficient µa = 6cm-1). Imaging was done at 10 RPM rotation speed. The signal to noise ratio in (dB) was calculated as a ratio of the mean intensity of the detected signal after background subtraction, divided by the background standard deviation.

2.4.4 Sensitivity in blood-like media

Finally, in order to accurately determine the sensitivity as a function of the separation between fluorochrome and fiber tip, a glass tube (inner diameter 0.75mm, 5cm length) containing 10µM AF750 was fixed at an angulated position relatively to the catheter, shown on Fig. 2e, so the distance between the tip of the fiber and the tube varied linearly from 1 to 2 mm during pull-back. A relatively high fluorochrome concentration was used herein in order to study the effects of attenuation with distance. Since a linear relation exists between fluorochrome concentration and fluorescence signal intensity (for the light intensity and fluorochrome concentrations used) [18] the findings herein can be used in a straightforward manner in order to extrapolate the sensitivity limits at smaller concentrations. This arrangement was immersed into the blood-mimicking solution described above and imaging was performed at 15 RPM.

2.4 In vivo clearance of indocyanine green (ICG) from the blood

To showcase the basic ability of the NIRF catheter to sense fluorochromes in a rotating mode intravascular manner, we performed indocyanine green ICG blood clearance test measurements, while the catheter was inserted in the iliac artery of a living rabbit. ICG is a common fluorochrome that has been used for different clinical indications, for example measurements of plasma volume [19] or in quantitatively assessing liver function [20] and cardiac output [21]. It has peak absorption at 805 nm and peak emission at 830 nm in blood in vivo. During the first few (3-5) minutes after the bolus injection the ICG concentration in the blood is rising, to be followed by clearance through the hepatobiliary tract [22].

ICG clearance rate measurements were performed in a healthy New Zealand white rabbit (weight 4 kg; Charles River Laboratories, Wilmington, Mass). After an overnight fast, anesthesia was induced with intramuscular ketamine (35 mg/kg) and xylazine (5 mg/kg) injection. Anesthesia was continued with inhaled isoflurane (1% to 2% vol/vol, Baxter, Deerfield, III) and supplemental 2L O2. A 4F introducer was placed in the right carotid artery and then delivered to the descending thoracic aorta under fluoroscopic guidance. Intravenous heparin (150 U/kg) was subsequently administrated. The constructed 2D NIRF catheter was introduced to the distal iliac artery through the sheath and over a standard 0.014” coronary
guidewire. 2D, automated NIRF pullback scans were performed at baseline (pre-ICG injection), and at 5, 15 and 25 minutes after ICG intravenous injection (2 mg/kg diluted in 5 mL aqueous solution injected as a bolus). The scanning was done over 5 cm pullback distance at 30 rpm and with step size of 0.5 mm, resulting in pullback speed of 0.25 mm/sec.

Upon completion of the experiment, the animal was euthanized. The institutional Subcommittee on Research Animal Care approved the animal protocols.

3. Results

3.1 Imaging of hollow organs

The ability to visualize fluorochromes arranged in the periphery of hollow structures is summarized in Fig. 4. The first phantom, consisting of two parallel fluorochromes-containing tubes, was clearly imaged while the fluorescence is visualized across the entire measured length of about 12 mm (Fig. 4a). Imaging of the four-tube phantom also showcased the ability to visualize more spatially complex signals, which confirmed the constructed location of the fluorescence signal in the phantom of Fig. 3b.

![Fig. 4. 2D NIRF imaging experiments. (a) Reconstruction of a two-tube phantom shown in Fig. 3a; (b) Reconstruction of a 4-tube phantom shown in Fig. 3b.](image)

3.2 Spatial resolution characterization

Imaging of the phantom in Fig. 3c was used herein to characterize the spatial resolution. Figure 5a depicts an image showing a detail of the intersection point between the two tubes, while the entire reconstructed image is shown in Fig. 5b having a good correspondence to the phantom constructed (Fig. 3c). The resolution calculated based on the minimal resolvable distance between the two peaks corresponding to the two tubes in Fig. 5a was ~300 µm, a value that matches the spot size of the fiber (280µm) measured at a distance of 1 mm from the tip.
3.3 SNR and sensitivity measurements

The results of the SNR and sensitivity measurements are presented in Fig. 6. The results simulate a measurement scenario in a 3mm diameter vessel, and measure signal intensity from volumes that are defined by the intersection of the volume excitation capacity of the fiber utilized, i.e. for a spot size of ~300 microns, and the tube diameter. The approximate excitation volume in this case was ~0.04 mm³. The smallest concentration of 100 nM was detected with contrast-to-noise (CNR) of 7.5 dB. While this measurement is representative of imaging in a relatively small diameter vessel, the general system sensitivity also depends on the distance between the fiber tip and the blood vessel wall.

Figure 6b summarizes the measured effects of fiber – phantom wall separation on the signal intensity. The results shown in this case originate from an approximate excitation volume of ~0.085 mm³. The measurements were obtained both in the presence of a fluid with blood-like attenuation properties and in the presence of saline, the latter simulating conditions where blood vessel flushing with a clear medium is applied – a common practice for instance in intravascular OCT imaging and Angioscopy. As expected, the results demonstrate stronger attenuation of the signal intensity with distance when a highly absorbing blood-like fluid is employed. This strong attenuation sets limitations for the achievable sensitivity through blood, especially as the blood vessel diameter increases, which might direct the use of a saline bolus within the vessel in order to offer temporary dilution of the local blood concentration and reduce light attenuation. Correspondingly, the measurements through saline exhibit higher detected fluorescence that does not attenuate at such a high rate as was obtained for...
blood-like medium. Overall, the experimental evidence suggests herein that the detection limits of the system are on the order of 100nM of an organic fluorochrome dye.

It is foreseeable that while no flushing will be required for imaging smaller diameter vessels, imaging in larger diameter vessels will require either flushing or further increasing the system sensitivity. Nonetheless, with typical human coronary arterial luminal diameter of 2.5-3.5mm, the presented measurements provide initial estimation of fluorochrome detection sensitivity at distances relevant for the target application.

3.4 In vivo indocyanine green (ICG) blood clearance

Figure 7 summarizes the results of ICG clearance rate measurements. All the measurements were processed with identical signal filtering settings and thresholds. The mean intensity of the fluorescence counts (arbitrary scale) was calculated in each scan and subsequently normalized to the first (and strongest) count, measured 5 minutes after the ICG injection. The relative fluorescence intensity decay, measured intravascularly with the NIRF catheter, approximately follows a clearance rate that correlates well with ICG blood clearance dynamics described in the literature [23]. Despite the small number of measurements available, we describe ICG concentration by the following bi-exponential model in Eq. (1):

\[ C(t) = a \exp(-k_1 \cdot t) + b \exp(-k_2 \cdot t), \]  

where a and b are weighting factors, t is the post injection time in minutes and k1 and k2 are time constant factors. By using curve fitting technique, the concentration decay of ICG in blood can be estimated.

![Fig. 7. In vivo measurements of ICG blood clearance time dynamics. Normalized fluorescence count was taken at 4 time points (in red asterisks) and the bi-exponential fit (blue line) was based on them.](image)

4. Discussion

In this work, we developed an intravascular catheter-based imaging system for rapid detection and two-dimensional visualization of near-infrared fluorescent agents and probes in hollow organs. The system is targeted for in vivo catheterization procedures in human coronary-sized arteries, showcased here by detecting local variations of fluorescence signal intravenously in abdominal aorta of a living rabbit with a diameter of 3-4 mm, the typical diameter of human coronary arteries. The current design operates in a manner similar to intravascular ultrasound (IVUS) and uses the same catheter housing and guiding system. As such, the NIRF system developed could offer a platform system for further integration with IVUS catheters.

As opposed to previous one-dimensional near-infrared catheter sensing implementations [13,16], the current system offers full 360-degrees view and resolves information in both axial and angular directions, allowing two-dimensional assessments of fluorescence distribution in blood vessel-like phantoms. The spatial resolution of the system was determined to be of the
order of 300 µm for vessels with up to 3 mm diameter, a specification that is adequate for the first imaging applications foreseen for the system, i.e. in the intravascular imaging in rabbit models of atherosclerosis. The resolution can deteriorate however for larger vessels when using the fiber employed herein. For improving the overall resolution and ensuring high-quality imaging of larger vessels, focusing optics with front illuminating and GRIN lenses, would be necessary. The resolution achieved will be also determined by imaging through whole blood, a scattering medium that can blur the images, or by utilizing flushing to locally remove the blood at the imaged location. Likewise, the current implementation achieved detection sensitivity of 100nM in volumes of 0.04mm3, which translates to about 4 femtomoles when a saline medium was employed. This sensitivity performance worsens as expected when measurements are performed through blood and as a function of distance.

There is an ongoing effort to improve technical characteristics and feasibility of the system in order to meet requirements of clinical intravascular arthrosclerosis detection. In the next step we aim to engineer focusing optics at the sensor’s end to reduce the beam diameter at 0.5-2 mm distance from the sensor tip. This will improve spatial resolution and sensitivity and simultaneously allow increasing the rotational speed of the system. Quantification can be further improved by introducing light intensity correction algorithms that utilize morphological maps from other imaging modalities, like IVUS or micro-CT. Similar methods have already been considered for fluorescence tomography applications, as described elsewhere [24], [25].

Clearly, the issue of imaging contrast between different plaque types and normal tissue demands further attention. As implied from previous studies [13], there is negligible autofluorescence from non-inflamed blood vessel. However, since the accuracy of the lesion localization depends also on the binding specificity of fluorescence agent, specificity of the detected fluorescence signal is yet another challenge in preclinical studies of atherosclerotic lesions. Vulnerable plaque assessment can overall become more accurate if multi-spectral detection is utilized. Multiple biomarkers, targeting different biological processes in the atherosclerotic lesion, e.g. macrophages, protease or myeloperoxidase activity, can efficiently bind to fluorochromes with distinct spectra. This will enable simultaneous detection of multiple targets and, as a result, improve the accuracy of vulnerable plaque detection and provide additional tools for plaque assessment.

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