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Improving quantification of intravascular fluorescence imaging using structural information

Georgios Mallas^{*,1,2}, Dana H. Brooks¹, Amir Rosenthal^{2,3}, R. Nika Nudelman³, Adam Mauskopf², Farouc A. Jaffer², and Vasilis Ntziachristos³

¹Communications and Digital Signal Processing Research Center, Department of Electrical and Computer Engineering, 409 Dana Building, Northeastern University, 360 Huntington Avenue, Boston, MA 02115, USA

²Cardiovascular Research Center, Cardiology Division, Massachusetts General Hospital, Harvard Medical School, Boston, MA, USA

³Institute for Biological and Medical Imaging (IBMI), Technische Universität München and Helmholtz Zentrum München, Munich, Germany

Abstract

Intravascular near-infrared fluorescence (iNIRF) imaging can enable the in vivo visualization of biomarkers of vascular pathology, including high-risk plaques. The technique resolves the bio-distribution of systemically administered fluorescent probes with molecular specificity in the vessel wall. However, the geometrical variations that may occur in the distance between fibre-tip and vessel wall can lead to signal intensity variations and challenge quantification. Herein we examined whether the use of anatomical information of the cross-section vessel morphology, obtained from co-registered intravascular ultrasound (IVUS), can lead to quantification improvements when fibre-tip and vessel wall distance variations are present. The algorithm developed employs a photon propagation model derived from phantom experiments that is used to calculate the relative attenuation of fluorescence signals as they are collected over 360 degrees along the vessel wall, and utilizes it to restore accurate fluorescence readings. The findings herein point to quantification improvements when employing hybrid iNIRF, with possible implications to the clinical detection of high-risk plaques or blood vessel theranostics.

1. Introduction

Atherosclerosis often leads to serious health complications including cardiac arrest and stroke (Lloyd-Jones et al., 2010). Early clinical diagnosis of atherosclerotic plaques that are prone to rupture (termed “vulnerable plaques”) is thought to be critical to prevent catastrophic events. For the smaller coronary arteries, various intravascular imaging modalities have been developed and tested clinically, including optical coherence tomography (OCT) and intravascular ultrasound (IVUS) (Garcia-Garcia et al., 2010, Chen and Wu, 2011, Jang et al., 2005, Yabushita et al., 2002). However, although these methods provide information about the structural characteristics of the imaged vessel, they do not directly characterize biological aspects of disease (Calfon et al., 2010, Kim and Jaffer, 2010), such as inflammation or angiogenesis (Sanz and Fayad, 2008, Jaffer et al., 2007). Intravascular fluorescence imaging (Jaffer et al., 2007, Sanz and Fayad, 2008, Calfon et al., 2010, Jaffer et al., 2009) on the other hand, can make use of appropriate molecular and

*Corresponding author’s mallasgeo@gmail.com.

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cellular fluorescence probes that are sensitive to cellular or molecular and metabolic processes of interest, such as inflammation, to obtain information about the underlying biology. Other molecular imaging modalities employed for detection and characterization of atherosclerosis in larger vessels include the iodinated nanoparticulate contrast agent, N1177 in computed tomography (CT) (Hyafil et al., 2007), positron emission tomography (PET) with F-fluorodeoxyglucose (F-FDG) (Chen and Wu, 2011) and magnetic resonance imaging (MRI) using an ultrasmall superparamagnetic iron oxide (USPIO) probe (McLachlan et al., 1994, Kooi et al., 2003, Tang et al., 2009). However, none of these modalities is currently sufficient for imaging in coronary arteries or other small vessels. In contrast, intravascular near infrared fluorescence (iNIRF) imaging is suitable for imaging in those critical vessels. Our group has previously reported on iNIRF systems able to sense fluorescence signals through blood (Jaffer et al., 2011). The potential of iNIRF imaging to sense plaque inflammation in small diameter vessels has been already demonstrated (Jaffer et al., 2008). We have more recently developed a 2D iNIRF imaging system (Razansky et al., 2010) and have displayed its ability to image plaque inflammation in vivo in rabbit models (Jaffer et al., 2011).

Fluorescence amplitude quantification is an important parameter for reliably identifying disease and evaluating its extent. The ability to quantify relates not only to retrieving the amount of fluorescence present in a lesion, in an absolute sense, but importantly also to the overall accuracy in retrieving the relative concentration of fluorescence throughout the vessel imaged. Even in this relative sense, fluorescence quantification is challenging when performing intravascular optical imaging, especially in the presence of blood. This is because the light intensity recorded by an iNIRF imaging system does not only reflect fluorescence concentration but is instead a mixed contribution that depends on the fluorescence concentration, the depth of the activity, tissue autofluorescence and importantly also on the optical properties of the blood (attenuation, scattering) present in the vessel and the distance between the fibre detector and the vessel wall. In particular, the signal strength decreases, in a non-linear fashion, with the distance between the source and the detector. As the diameter of the human coronary arteries is neither constant (it typically ranges from 2.5 to 3.5 mm, and varies even more in the presence of atheroma), nor symmetric, and the fibre cannot reliably be placed in the centre of the vessel, it becomes imperative to account for the variations of the fluorescence signal intensity as a result of these dependencies.

Otherwise, the consequence of the non-linear dependence of fluorescence strength on the distance between the fibre tip and the vessel wall is that it becomes impossible to disambiguate a higher concentration of fluorophore which happens to lie in a portion of the vessel wall farther from the probe from a lower concentration closer to the probe. To address this problem, we report here on a methodology for *quantitative* intravascular fluorescence imaging, which shares features with other hybrid methods developed for tomographic fluorescence imaging (Schulz et al., 2010, Ale et al., 2012). This method uses structural information captured from IVUS images of the same vessel and combines the anatomical information with a model of photon propagation in blood. This information is then employed to correct the iNIRF signal for the non-linear dependence of light intensity with distance from the source. We demonstrate the approach proposed with phantoms and display its performance by an example from an in vivo imaging measurement.

2. Methods

2.1. iNIRF and IVUS imaging

The iNIRF imaging catheter system utilized (figure 1) has been previously described and its performance demonstrated in phantoms and in vivo imaging of atherosclerotic plaque and arterial stent inflammation in New Zealand white rabbits (Razansky et al., 2010, Jaffer et al.,

2011). Briefly, the imaging system consisted of an optical fiber of numerical aperture (NA) 0.37 housed in a catheter made from polyethylene tubing (PE50, Becton Dickinson), emitting continuous wave laser light centered at 750nm (B&W Tek Inc, Newark, DE, USA) perpendicularly to the fiber's axis. The emitted beam is a diverging cone, with the spot's full width half maximum (FWHM) measured to be 0.12 mm in air at a 1mm distance from the fibre, a relevant distance for coronary arteries. During pullback the fibre is automatically rotated at a rotation speed of 100 rotations per minute and translated with a step size of 0.5 mm per rotation inside the catheter, while data are collected at a sampling frequency of 1 kHz. For each pullback an image map $I(z, \phi)$ of the fluorescence signal collected from the fibre is generated as a function of the longitudinal position z of the translational motor and of the angular position ϕ of the rotational motor. iNIRF imaging was performed in conjunction with a Galaxy IVUS imaging system (Boston Scientific, Natick, MA) connected to an Atlantis SR Pro 40MHz imaging catheter (Boston Scientific, Natick, MA). A pullback along a vessel with the IVUS imaging system provides 30 cross-sectional frames per second at a pullback speed of 0.5 mm per second, from which the structural information of the vessel per pullback position can be extracted. The IVUS catheter was filled with saline before imaging for optimal coupling of ultrasound signals.

2.2. Light attenuation measurement

To experimentally study the dependence of iNIRF signal attenuation on distance in a blood-like medium, we constructed the tank phantom shown in figure 2a. Pairs of holes were drilled in the opposite ends of the tank such that the catheter was positioned at a 7 degree angle with respect to a plastic straw containing a solution of the NIR fluorochrome Alexa Fluor 750 (Alexa Fluor 750 carboxylic acid, succinimidyl ester, 1 mg, Invitrogen) (figure 2a). Measurements were performed at three fluorochrome concentrations: 50, 5 and 0.5 μM . The straw was intentionally chosen to have a white colour to emulate the scattering in a vessel wall. The wall thickness of the straw was 200 μm , comparable to that of a normal rabbit aorta, which is typically between 200 μm and 300 μm as measured on our IVUS datasets (data not shown). The minimum distance between the catheter and the straw was 0.5 mm. The tank was filled with a blood-simulating solution made from a mixture of India ink, saline and 10% Intralipid (Liposyn III, Hospira Inc., Lake Forest, IL), yielding anisotropy, absorption and reduced scattering coefficients equal to $g = 0.995$, $\mu_a = 1 \text{ cm}^{-1}$, and $\mu_s' = 4.5 \text{ cm}^{-1}$ respectively, to match the optical properties for light propagated through in vivo human blood at a wavelength of 750 nm, as reported in (Roggan et al., 1999). Ex-vivo blood was not used in our phantom experiments, as the optical properties of in vivo and ex vivo blood differ (Roggan et al., 1999).

iNIRF measurements were performed by inserting the catheter and fibre system inside plastic tubing, simulating blood vessel measurements. Images collected over a 2 cm length in the plastic tubing, after fibre pullback and rotation, are displayed as two dimensional images, with the z -axis representing the pullback dimension and the ϕ -axis representing the angle scanned within each pullback position in the vessel (figure 2b).

Following the measurement, a light attenuation function $A(z)$ was computed as follows: we first identified the full width half maximum (FWHM) of the fluorescence signal across the angle dimension at each longitudinal position z . We then computed the mean of the fluorescence image intensity map $I(z, \phi)$ for the signal recorded within the FWHM. Following iNIRF imaging, the same phantom was imaged with the IVUS system in the same way as with the iNIRF. A longitudinal slice through the acquired IVUS cylinder (figure 2c) was then employed to record the geometry and compute the distance $D(z)$, representing the minimum distance found between the catheter and the straw as a function of z . As the IVUS data are oversampled compared to the iNIRF data, the IVUS data were first sub-sampled along the z -axis with a step of 0.5 mm, as in the iNIRF measurement. The iNIRF and the

subsampled IVUS data were then co-registered by aligning the location of the peak iNIRF signal with the location of minimum distance in the IVUS pullback. After co-registering the iNIRF and IVUS measurements, the function $A(z)$ recorded the drop in signal intensity as a function of $D(z)$, yielding a model for the light attenuation in blood as a function of source-detector distance. The idea of using such an attenuation function to correct fluorescence signal was first introduced in (Mallas et al., 2011a).

2.3. In vitro phantom for algorithmic validation

To evaluate the effectiveness of the correction algorithm, a second phantom, shown in figure 3a, was constructed. A tank was filled with the same scattering and absorbing medium as in the phantom of figure 2. Three pairs of holes were drilled in the tank, so that the imaging catheter and two straws containing the same concentration of the fluorescent solution used in the first experiment could be inserted in the tank. The phantom was designed such that the distance between the lower straw and the catheter was 1.3 mm and the distance between the upper straw and the catheter 1.8 mm (figure 3a). iNIRF and IVUS measurements were then performed taking care to accurately register the starting position and the relative angle between the iNIRF and IVUS scans for post-processing data registration. Angle registration was achieved by aligning the fluorescent source with the highest signal in the iNIRF data with the closest straw imaged in the IVUS data.

2.4. Correction algorithm

For IVUS-based correction of the iNIRF images, as above the IVUS data were sub-sampled to a 0.5 mm pullback step to match the iNIRF pullback resolution. For each z -pullback position iNIRF cross-sectional images were radially projected onto the corresponding IVUS cross-sections. To create the iNIRF cross-sectional image, the iNIRF data from a single rotation were interpolated across the angle dimension to match the size of the IVUS cross-section image, 480 pixels on a side. In other words, we assigned the value of the iNIRF signal at each angle to the entire radial line that started from the centre of the projected image at an angle from the positive horizontal axis equal to the data point's corresponding angle, as illustrated in figure 3b.

Then, for each IVUS cross-section, the luminal surface was segmented so that the distance between the lumen and the catheter could be computed (figure 3c). For the purposes of this paper segmentation was performed manually, but algorithms exist for automatic segmentation of IVUS images (Klingensmith et al., 2000, Cardinal et al., 2006, Brusseau et al., 2004, Giannoglou et al., 2007).

Finally, the distance information extracted from the IVUS cross-sections was employed to allow for distance-dependent fluorescence signal correction. We treated herein the fluorescence signal as originating entirely from the near edge of the lumen, that is, the same edge segmented in the IVUS image. At every pixel of the back-projected and interpolated iNIRF image that corresponds to the edge of the lumen, the distance to the edge of the IVUS catheter was calculated from the corresponding pixel in the IVUS image. The fluorescence signal value at that pixel was then divided by the attenuation function at that specific distance obtained from the phantom experiment. Finally, the corrected iNIRF image was reconstructed into a single 2D image in a straightforward fashion (figure 3d).

2.5. In vivo experiment for algorithmic validation

To further test the potential utility of the algorithm in a more realistic setting, we applied it to data collected in an in vivo experiment performed on a New Zealand white rabbit. The Subcommittee on Research Animal Care at Massachusetts General Hospital approved this experimental protocol for all procedures. The abdomen of the animal was surgically opened

in order to reveal its aorta. The area surrounding the aorta was filled with a mixture of saline and ultrasound transmission gel (Aquasonic 100 Ultrasound Transmission Gel, Parker Laboratories, NJ, USA) and three identical straws, each containing the Alexa Fluor dye 750 at a concentration of 50 μM , were implanted adjacent to the aorta (figure 4a). The iNIRF and IVUS catheters were consecutively inserted in the aorta, with care so as to accurately register their positions in the vessel for post-processing registration, guided by using the fluorescent straws as fiducial markers. Subsequently, images of the fluorescent straws, imaged by both systems in vivo, were corrected for distance variations by the algorithm described. We note that the attenuation curve reconstructed from phantom data was applied unchanged to correct the in vivo data.

3. Results

Figure 2 summarizes the light attenuation measurement procedure and the corresponding findings. Figure 2d shows measurements from the three concentrations, normalized to the maximum value obtained from each concentration scanned. As expected, all curves attenuate with the same rate; however, as the concentration drops the noise is more evident on the measurements. As observed, the fluorescence signal in the blood-like solution decreased more than 65% in amplitude as the distance increased from 0.5 mm to 1 mm. This is a considerable change that challenges quantitative performance. The signal decay in this case is a complex process that depends on the overall geometry of the vessel and fibre location, the distance from the fibre tip to the different parts of the vessel wall contributing to signal generation, the optical properties of the interfering diffusive medium and the particulars of the illumination beam as to its propagation outward the fibre. For example, the decay may differ for tightly focused vs. unfocused beams, even through a few millimeters propagation in a diffusive medium. Therefore, while the curves can be fit by an exponential decay for demonstration reasons (for example for the 50uM case one finds $A(d) = 2.83 \exp(-2.20d)$), this should not be construed as a model that describes all the relevant complexity of the underlying physical problem.

Figure 3 depicts the results from the application of the correction algorithm, including intermediate steps. From figure 3b it can be seen that the signal from the straw located 1.8 mm away from the catheter is considerably weaker than the signal originating from the straw located 1.3 mm from the catheter, as expected. In figure 3d, the overlay of the iNIRF cross-section on the corresponding segmented IVUS cross-section is shown, before and after the correction. Finally, in figure 3e the iNIRF image is displayed before and after the application of the correction algorithm. As can be seen, the distance-based correction improves the accuracy of the iNIRF image by equalizing the iNIRF intensity recorded for the two straws, as expected, compared to the non-corrected image that can erroneously interpret fluorescence concentration differences between the two straws, based on the intensities recorded on the raw image. The difference between the signals recorded for each straw was quantified before and after correction as follows: first, the FWHM of the fluorescence signal across the angle dimension was computed for each straw, and then the mean signal across the z-dimension and within the FWHM interval was calculated. A *difference factor* was then defined as the difference between the mean signal of each straw, divided by the mean intensity of the straw with the highest signal. Before correction, the difference factor was 61%, while after correction it was only 5%.

In figure 4a, the details of the in vivo experiment can be seen. Straws A, B and C were placed adjacent to the aorta of the animal with the nearest tips at distances from the imaging catheter of 0.86 mm, 0.8 mm and 1 mm, respectively, as measured with the IVUS, and at as close to the same angle with respect to the vessel as feasible given experimental factors. The iNIRF signal intensity detected from the straws was comparable to that detected from

atherosclerotic plaques when in vivo imaging the fluorophore Prosense VM110, injected at a dose of 3.75 mg/kg in an atherosclerotic rabbit, as described in (Jaffer et al., 2011). To ensure that the iNIRF and IVUS catheters were at approximately the same position in the vessel during imaging, the iNIRF and IVUS catheters were advanced over the same fixed guidewire. Figure 4b shows the iNIRF signal acquired from each straw. As can be seen from the experiment schematic, the peaks of the fluorescence signal originated from the tips of the straws that were near the vessel. The decay of the signal along the rest of each straw was quite rapid. Moreover, since the tip of straw C was further from the catheter than for straws A and B, the corresponding measured peak iNIRF signal was weaker (figure 4b). The iNIRF and IVUS data were aligned in the radial dimension using the three straws as markers. Using the correction factors obtained from the phantom, the correction algorithm brought the peak signal from each straw to a similar level (figure 4c). In order to quantitatively assess the performance of the correction algorithm, we defined the *peak signal area* of each straw as the 2 mm-long area closest to the vessel according to the IVUS pullback. Only these peak signal areas were corrected, while the signal along the rest of each straw was discarded. The mean signal along the z-axis for each peak signal area was calculated for each straw before and after correction (figure 4d) and was used to calculate a *difference factor*, as earlier, with the peak signal area of Straw A as a reference; the FWHM was computed first for each peak signal area, and then the mean signal for each such area across that FWHM. The difference factor before correcting was 4.5% for Straw B and 15.7% for Straw C, while after correcting it decreased to only 2.4% for Straw B and 6.1% for Straw C.

4. Discussion

In this work we experimentally studied the effects of distance in measurements emulating fluorescence intravascular imaging, which are shown to lead to considerable signal intensity fluctuations that challenge quantification. Correspondingly, we concluded that accounting for geometrical factors is important for accurate performance. We therefore proposed a hybrid imaging method which can correct for such effects by utilizing structural information from an anatomical modality which is co-registered.

Quantification improvements can be also achieved by saline flushing during the measurements in order to physically clear the blood between the fiber tip and the vessel wall. This approach is utilized for example in OCT measurements, where it is necessary for achieving acceptable signal-to-noise ratio in the corresponding measurements. In iNIRF imaging this is not always necessary, especially when imaging small diameter vessels (2–4 mm). Flushing is not always desirable as it complicates the imaging procedure and in addition it does not completely solve the quantification problem, since varying amounts of blood may be still present in the measurement, and this contamination may change with time, i.e. increasing amounts of blood may be present during the pullback procedure. In addition, geometrical changes can influence the intensity recorded even at the absence of an absorber. The magnitude of these changes will depend on the particulars of the illumination beam shape and photon collection acceptance angle.

Despite the apparent procedural benefits of performing iNIRF through blood without flushing, the correction algorithm proposed herein comes with its own limitations. First, accurate estimates of blood attenuation are a pre-requisite. Second, in the experiments reported here, we assumed that the fluorescence signal originated from the surface of the fluorescent source, when in practice the whole volume of the source contributes to the fluorescence signal. Third, if the distance between the fluorescent source and the probe exceeds a certain threshold (about 2.5–3 mm with our current intravascular imaging system), the signal from the source will be below the noise floor and thus undetectable. Finally, perhaps the most important limitation of our approach comes from the fact that we employ

measurements from two different, non-integrated, imaging systems, iNIRF and IVUS. In particular, for accurate correction the two probes need to be accurately aligned and co-registered in both angular and longitudinal dimensions (Mallas et al., 2011b). Without such a hardware improvement, our correction algorithm can be readily applied in a phantom, even an in vivo phantom such as the one presented here, but would find significant challenges in more demanding in vivo applications.

Future work is currently targeted to address the limitations outlined above. In the area of more accurate determination of attenuation functions, we believe that we need a more grounded understanding of attenuation in the scenarios of interest to be able to determine sensitivities to various experimental parameters. For example, we have started exploring Monte Carlo calculations to model the appropriate geometry (Mallas et al., 2011b). We anticipate that either direct application of these results, perhaps based on case-specific determination of blood parameters (for example through a hematocrit or by using a small blood quantity from the patient to directly measure attenuation in the preferred wavelength), or model-based methods guided by similar parameters, will allow a more flexible approach to the determination of a wide variety of attenuation profiles. To address the second limitation we plan to use schemes which explicitly take into account the volume of the plaque region detected in the IVUS image, including deconvolution with the point spread function of the imaging system. Such an approach would also require light attenuation models appropriate for photon propagation in tissue. In parallel, we will address the third limitation. One approach will be to simply use fluorescent probes with higher quantum efficiency to increase our distance threshold. We may also be able to increase it by improving the sensitivity of the imaging system, for example by using a focused fibre instead of a non-focused one, but we may still face dynamic range tradeoffs to measure both nearby and farther off sources with sufficient accuracy.

Important in the more general application of the technique presented herein is the development of appropriate hybrid catheters that combine iNIRF and IVUS imaging into accurately registered geometrical arrangements. Two initial approaches towards the integration of fluorescence and IVUS imaging have been recently published, the first combining iNIRF and IVUS imaging (Abran et al., 2011) and the second combining fluorescence lifetime and IVUS imaging (Bec et al., 2011). However, complete integration in devices suitable for use in human coronary arteries remains an unresolved issue, as in these reports the optical and ultrasound imaging sensors are separate and attached together externally, making the footprint prohibitively large for intracoronary use. As with integrated IVUS and OCT (Yin et al., 2010, Li et al., 2010, Yang et al., 2010), the future of fluorescence-based intravascular imaging seems likely to lie in multimodality approaches, where the information from integrated complementary imaging modalities will be analyzed by algorithms such as the one presented in this paper for a more accurate and quantitative assessment of the imaged process.

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Figure 1.
iNIRF System Schematic. Excitation and emission light are coupled into a fibre which can be inserted in a catheter system through a rotational and translational stage, to allow fibre rotation and pull-back. The front end of the fibre is modified using a 45 degree prism so that fluorescence readings are obtained perpendicular to the translation axis.

Figure 2.

Experiment for the determination of the light attenuation function: (a) Phantom schematic: Fluorescent straw set at an angle with the catheter; (b) iNIRF longitudinal image of the straw; (c) IVUS longitudinal image of the straw at an appropriate angle; (d) Light attenuation as a function of distance, as reconstructed from (b) and (c), for three concentrations and with an exponential fit.

Figure 3.

Experiment for validation of the algorithm: (a) Phantom schematic: two straws containing the same concentration of fluorescent dye at different distances from the catheter; (b) iNIRF longitudinal image of the straws A and B and cross-section formation of a particular pullback position of interest; (c) Corresponding IVUS cross-section of the straws and their corresponding edges; (d) iNIRF cross-section overlaid on the segmented IVUS cross-section is used for the correction of the iNIRF signal; (e) Correction of the iNIRF image along the entire pullback.

Figure 4.

In vivo correction experiment: Three straws filled with fluorochrome were placed adjacent to a rabbit aorta, as described in the text, with Straws A and B slightly closer to the catheter than Straw C, and were imaged sequentially with both the iNIRF and IVUS systems; (a) Experimental schematic; (b) Acquired iNIRF image; (c) Corrected iNIRF image; (d) Mean signal for each straw across the area to be corrected, before and after correction. Note the difference in scale of the vertical axis between the two graphs.