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Progress on multimodal molecular / anatomical intravascular imaging of coronary vessels combining near infrared fluorescence and ultrasound

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Abstract— **The use of intravascular imaging modalities for the detection and assessment of atherosclerotic plaque is becoming increasingly useful. Current clinical invasive modalities assess the presence of plaque using anatomical information and include Intravascular Ultrasound (IVUS) and Optical Coherence Tomography (OCT). However, such modalities cannot take into account underlying functional biological information, which can however be revealed with the use of molecular imaging. Consequently, intravascular molecular imaging is emerging as a powerful approach. We have developed such a Near-Infrared Fluorescence (NIRF) imaging system and showcased, in both phantom and *in-vivo* (rabbit) experiments, its potential to successfully detect inflamed atherosclerotic plaques, using appropriate fluorescent probes. Here, we discuss some limitations of the current system and suggest the combined use of the NIRF and IVUS imaging systems as a means for more accurate assessment of atherosclerotic plaque. We include some results and models that showcase the potential power of this kind of hybrid imaging.**

I. Introduction

CORONARY arterial disease is a leading cause of death in the western world. As a result, the last fifteen years have seen great development in the field of intravascular imaging, focused on imaging modalities which provide the anatomical structure of the imaged vessel, such as intravascular ultrasound (IVUS) and optical coherence tomography (OCT) [1-4]. IVUS in particular has found extensive clinical use in the anatomical detection of atherosclerotic plaque, as it combines high resolution with sufficient imaging depth, while, in contrast to OCT, not requiring flushing the blood to obtain an image. However, it is usually not possible to obtain information on the underlying molecular and cellular aspects of atherosclerotic plaques through the use of IVUS or OCT. On the contrary, recent developments have shown near-infrared fluorescent probes to be highly sensitive to inflammation processes associated with atherosclerosis [5, 6]. The use of such probes with emission and excitation spectra in the near-infrared (NIR) region is advantageous because of the ability to achieve reasonably long imaging depth without flushing due to the relatively low absorption of blood in this region.

To take advantage of those developments, we have developed a Near-Infrared Fluorescence (NIRF) imaging system and showcased its ability to detect appropriate fluorescent probes that are sensitive to inflammation, both in phantoms and in *in-vivo* rabbit models while imaging through blood [7]. In particular, our system has been used to detect inflamed atherosclerotic plaque *in-vivo*, as well as coronary stent induced inflammation. Our results were confirmed with the use of IVUS and ex-vivo NIRF and fluorescence reflectance imaging (FRI).

However, although the afore-mentioned blood absorption in the NIR range is relatively low, it introduces a dependence of the fluorescence signal with distance, which prevents the acquisition of quantitative images. To respond to that limitation, we have suggested the use of anatomical information to correct for the distance-dependence of the NIRF signal [8]. In particular, an algorithm which uses a light propagation model in conjunction with IVUS structural information was developed to correct for the distance-dependence of the fluorescence signal. The algorithm has been tested with appropriate phantoms, which were imaged sequentially with the IVUS and NIRF systems. However, the use of sequential imaging is not attractive clinically, due to both clinically relevant factors---it would require the sequential insertion of the two catheters, thus prolonging procedure duration and increasing the chance of adverse complications---and technical factors on the imaging side, in particular the difficult co-registration of two catheters *in-vivo*. This lack of capability for accurate co-registration between the two separate catheters in the vessel limits accurate conclusions on the risk of vulnerable plaque.

Thus, overcoming these limitations requires the

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development of a hybrid NIRF/IVUS system. We detail here the background which motivates the need for such a system and describe its requirements, as supported by evidence from our preliminary studies.

II. Methods

A. The NIRF system

The NIRF catheter system was designed for fluorescence imaging through blood. The catheter was made of polyethylene (PE-50) tubing with size (3F) comparable to that of clinical IVUS catheters (2.9F). A multimode fiber with numerical aperture 0.37 and core diameter 100μm, which was used to transmit laser light centered at 750nm was inserted into the catheter. In order to illuminate the vessel walls, the tip of the fiber was cleaved so that the laser light was emitted at a 90 degree angle compared to the fiber's axis. The fiber was rotated and translated while in the catheter under automatic control, using a rotational stage coupled on top of a translational stage. The same fiber was used to collect the fluorescent light, which was transmitted to a photomultiplier tube (PMT) through an arrangement of optical filters and dichroic mirrors (Fig. 1). The time series data received from the PMT were recorded as a function of both relative longitudinal and angular position.

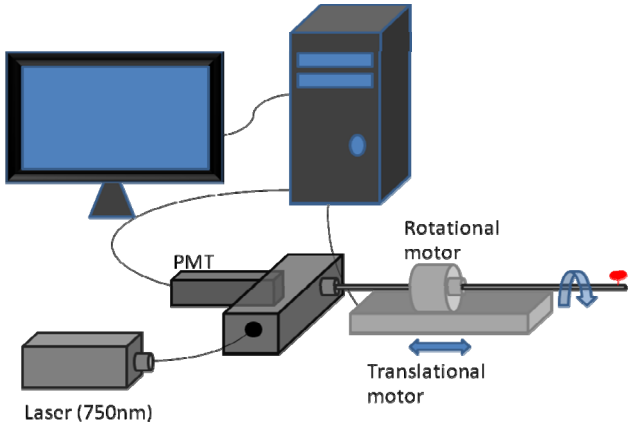

Fig. 1. NIRF system schematic; Laser light is transmitted, through a filter and mirror arrangement, to a rotational junction which is connected to a fiber with a cleaved tip. The collected fluorescent light is guided to a PMT through the same arrangement

B. Sequential acquisition of NIRF and IVUS data

The NIRF imaging system has been successfully used to detect NIR fluorescent probes associated with plaque inflammation in phantom experiments [9]. Additionally, in a previous study [7] the NIRF system was used to *in-vivo* image the aorta of rabbits with atherosclerosis which had been injected with an NIR fluorochrome sensitive to inflammation. Herein, we present the results of such an *in-vivo* experiment. A New Zealand white rabbit with atherosclerosis was sequentially imaged *in-vivo* with the IVUS and NIRF systems. Atherosclerosis was induced by balloon injury across the rabbit's aorta, combined with high cholesterol diet, as in a previous study [10]. An activatable NIR fluorescent probe specific to cysteine protease activity (Prosense VM110, excitation/emission spectra, 750nm/780 nm; VisEn Medical, Woburn, MA) was injected twenty four hours prior to imaging. The peak NIRF signal locations were shown to be highly correlated to plaque locations along the vessel, as determined from IVUS imaging (Fig. 2, Galaxy IVUS system, Boston Scientific, Natick, MA). These findings were supported by ex-vivo NIRF and FRI imaging along the excised vessels. Ex-vivo NIRF imaging was performed by inserting the catheter in the excised vessel, which was stretched so that its length resembled its length before excision. In that study, the NIRF and IVUS pullbacks were co-registered using the starting position of each catheter as shown from an angiogram recorded during the procedure (Fig. 2a).

C. Combining the information from NIRF and IVUS for quantitative imaging in a phantom

As confirmed from our *in-vivo* study, the absorption of light from the blood is a critical factor preventing the acquisition of quantitative NIRF data. We have shown [7] that the signal to noise ratio (SNR) of the NIRF signal received decreases exponentially as the distance between the fiber and the fluorescent source increases. To compensate for this attenuation, we have developed an algorithm which uses anatomical information extracted from the IVUS to correct the NIRF signal for its distance dependence [8]. In particular, a propagation model of light through blood was developed through phantom studies. This model was used in conjunction with distance information that can be extracted from the IVUS anatomical images to correct for the dependence on distance of the fluorescence signal. The algorithm's effectiveness was validated with the use of appropriate phantoms, different from the ones used to obtain the depth-dependent attenuation factors.

D. Development of a Monte Carlo propagation model

To validate our experimental light propagation model, we developed a Monte Carlo (MC) [11] simulation which computed the fluorescent light attenuation as a function of distance. Propagation of photon packets at 750nm was simulated in a scattering and absorbing medium with the optical properties of oxygenated blood [12]. The geometry of the simulation was similar to the phantom experiments described in our previous studies [7, 8], where a fluorescent target was assumed at increasing distances from a source. The simulation included two steps. In the first step, photon packets were emitted from the source towards the fluorescent target. The polar angle of the emitted photons was modeled using a Gaussian probability distribution function (pdf) with its parameters defined experimentally, while the azimuthal angle was modeled using a uniform pdf in $[0, 2\pi]$. The number of photon packets reaching the fluorescent target was recorded as a function of distance between source and target. In the second step, a number of

fluorescent photon packets equal to that recorded in the first step were launched from the fluorescent target, here assuming isotropic emission. Then the number of fluorescent photon packets reaching the simulated detector (light collector) at the fiber tip was again logged as a function of distance. The two steps of the simulation were repeated for increasing distances between the source and the fluorescent target. The attenuation function computed from the simulation closely matched the experimental one. One such result is illustrated in Fig. 3, where the good agreement can easily be visually assessed.

III. Results

To demonstrate the advantages of multimodal imaging, we report on results from an *in-vivo* imaging experiment (Fig. 2). As it is evident from the IVUS pullback (Fig. 2b), the highest areas of NIRF signal were detected where the vessel was narrower, where the attenuation from blood was less significant.

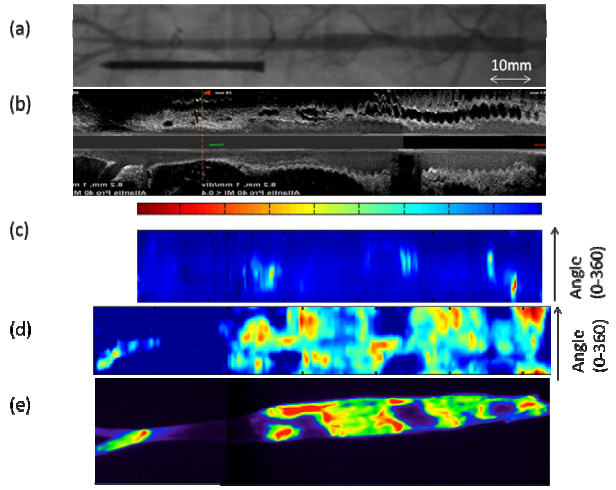

Fig. 2: In-vivo imaging of a rabbti's aorta with multiple modalities; (a) Angiogram; (b) IVUS; (c) In-vivo NIRF imaging; (d) Ex-vivo NIRF imaging; (e) Ex-vivo FRI of opened vessel

Additionally, we report on the comparison between the light propagation models derived experimentally and from our MC simulation. As it is showcased in Fig. 3, our experimentally derived model was closely approximated by the MC computed model.

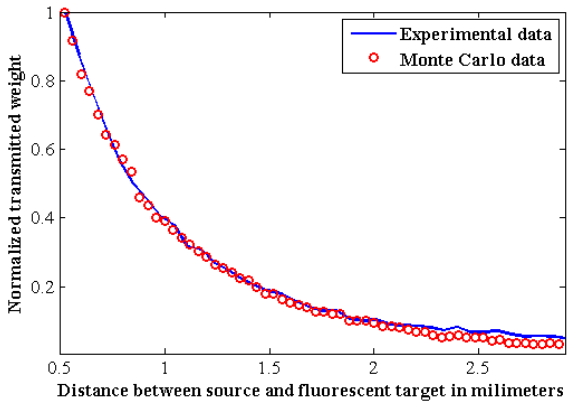

Fig. 3: Comparison between the experimentally derived light propagation model and the MC computed model

IV. Discussion

As it can be seen from Fig. 2, the *in-vivo* and ex-vivo NIRF images differed, while the ex-vivo NIRF and FRI images were similar. This difference between *in-vivo* and ex-vivo pullbacks was the result of strong light attenuation through blood. *In-vivo* imaging was performed through blood, thus NIRF signal was more likely to be detected where the vessel was narrower, as it can also be seen from the IVUS pullback of Fig. 2b. However, ex-vivo NIRF was performed through air, where the only attenuation factor was the divergence of the beam, which was negligible for short distances. Additionally, after excision the vessel collapsed, thus the distance between the vessel wall and the catheter was decreased compared to *in-vivo* imaging.

The correction algorithm developed currently cannot be applied successfully on *in-vivo* data, as it requires accurate co-registration between the NIRF and IVUS catheters in three dimensions. Although it is possible to achieve such co-registration for sequential imaging in a controlled phantom environment, co-registration in *in-vivo* imaging presents many complications. Most significantly, the path that each catheter follows in the hollow vessel with respect to its center is unknown. Further complications include the unknown initial relative angular phase difference between the two catheter systems and the movement of the catheters during imaging, because of the blood flow and cardiac and respiratory motion of the subject. Only the relative initial longitudinal phase difference can be determined, with some error, using x-ray angiography.

The development of an integrated multimodal imaging system should be able to resolve such issues. Nevertheless, the development of such a system is not a simple task. Several groups have taken steps towards the development of hybrid systems combining either IVUS with NIRF imaging [13], or IVUS with fluorescence lifetime imaging [14]. However, in these approaches separate ultrasound and optical sensors were attached together, leading to catheter sizes unacceptable for clinical imaging.

An alternative would be to integrate an IVUS transducer with an optical fiber sensor in the same probe. However, this

is not a simple task; there are complications in both manufacturing and size constraints. Several designs are under consideration to achieve a clinically acceptable sized catheter for a hybrid system. Assuming an acceptable size could be achieved, the integration of the two probes in one would allow the acquisition of co-registered data. Moreover, possible motion of the catheter inside the vessel would affect both probes in the same way and thus it would be taken properly into account by the correction algorithm.

V. Conclusions

Atherosclerosis and its complications may lead to sudden cardiac death if not diagnosed in an early stage. Towards that goal, we suggested the development of a hybrid NIRF/IVUS imaging system, and demonstrated, through results obtained from our preliminary studies, the potential of such a system. The imaging results presented showcase the advantages of multimodality imaging.

Our NIRF imaging system was able to detect signal at locations where plaques were present, as validated from the co-registered IVUS images. However, because of light attenuation in the blood, the signal detected had peaks where the vessel was narrower; hence, accurate quantitative analysis of the entire NIRF image was not possible. Moreover, as the NIRF/IVUS imaging was performed sequentially, it was not possible to reliably co-register the two catheters in the angular axis. Such complications should be overcome with the use of a single NIRF/IVUS imaging system in conjunction with correction algorithms developed for blood attenuation.

Hence, building a truly integrated NIRF/IVUS imaging system remains an open problem. In spite of that fact, these developments showcase that the future of intravascular imaging lies in multimodal approaches, a prediction which is confirmed by additional efforts to integrate NIRF with OCT [15-17] or with optical frequency domain imaging [18].

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