Developing cross-correlation as a method for microvessel imaging using clinical intravascular optical coherence tomography systems

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Abstract: Current clinical intravascular optical coherence tomography (IV-OCT) imaging systems have limited in-vivo flow imaging capability because of non-uniform catheter rotation and inadequate A-line scan density. Thus any flow-localisation method that seeks to identify sites of variation within the OCT image data-sets, whether that is in amplitude or phase, produces non-representative correlation (or variance) maps. In this study, both mean and the variation within a set of cross-correlation maps, for static OCT imaging was used to differentiate flow from non-flow regions. Variation was quantified by use of standard deviation. The advantage of this approach is its ability to image flow, even in the presence of motion artifacts. The ability of this technique to suppress noise and capture flow maps was demonstrated by imaging microflow in an ex-vivo porcine coronary artery model, by nailfold capillary imaging and in-vivo microvessel imaging from within the human coronary sinus.

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OCIS codes: (170.4500) Optical coherence tomography; (170.3880) Medical and biological imaging.

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#225726 - $15.00 USD Received 27 Oct 2014; revised 17 Jan 2015; accepted 21 Jan 2015; published 3 Feb 2015
(C) 2015 OSA 1 Mar 2015 | Vol. 6, No. 3 | DOI:10.1364/BOE.6.000668 | BIOMEDICAL OPTICS EXPRESS 668
1. Introduction

Intravascular optical coherence tomography (IV-OCT) provides high spatial resolution, in-vivo images of coronary arteries and is widely used for atherosclerotic plaque characterization [1,2]. Neo-vessels within the coronary vessel wall arise from the vasa vasorum and proliferate during plaque growth. Animal models have shown that microvessels are critical to the pathophysiology of plaque progression and vulnerability [3].

Commercially available clinical IV-OCT systems have been applied for imaging plaque-associated microvessels arising from the coronary lumen which appear as a signal void during coronary blood clearance [4]. However, the majority of microvessels arise from the adventitia of the artery and may not be cleared during luminal imaging.

Application of flow imaging approaches based on amplitude (Cross-Correlation, Speckle Variance, Split spectrum decorrelation etc.) or phase (Doppler, phase variance, etc.) to clinical IV-OCT data-sets has significant challenges. The mechanical rotation of both clinical and research-grade OCT catheters introduces a Non-Uniform Rotation Distortion (NURD) in the...
OCT images [5]. This type of distortion causes a random shift in A-line scanning position, resulting in image distortion [5]. A number of strategies have been described to correct for the NURD. These include correction by image registration [5, 6] or motion tracking [7]. Also, current commercial clinical IV-OCT systems allow only limited variability in the scanning protocol. Therefore, over sampling of A-lines in the radial and pullback direction is not possible with the current clinical systems.

To identify microvessels from the IV-OCT images, a higher-order statistical analysis - Kurtosis method, was recently reported [8]. This approach exploited sudden changes in intensity due to blood flow within the microvasculature, across OCT frames, to identify the adventitial vasa vasorum of a carotid artery. However, this method is not readily applicable to coronary cases because of the high degree of physiological movement of the heart.

Microvessel imaging using a Doppler technique has also been reported, for a modified clinical IV-OCT system [9]; where 3D vascular mapping of an airway segment was presented. The rotation and translation speeds of the OCT system were reduced to oversample the A-lines and improve the Doppler signal [9, 10]. A minimum ≈ 2 mm/s resolvable velocity was reported [10]. To improve the Doppler measurements a split-spectrum method was also applied [11]. Improved sensitivity and specificity of flow mapping was reported. However, these improvements remain inadequate to resolve physiological microvascular flow velocities, which are typically around 200 µm/s. Also due to the low frame-rate (≤20 Hz) [9–11], this approach would have limited application within coronary arteries.

The current commercially available clinical IV-OCT systems are primarily designed and optimized to carry out “high-speed” structural imaging of coronary arteries. Thus, the systems generate undersampled cross-sectional images in the pullback direction [12]. Over-sampling by high-density imaging is required to enable accurate flow mapping and reduce noise effects, as demonstrated by the Doppler studies [9–11]. To increase image density, new catheter designs, involving distal-end high-speed micromotors (12,500–210,000 rpm) [12–14] or otherwise enhanced rotation speeds (21000 rpm) [15], have been reported. These research-grade OCT systems employ laser scanning speeds ≥ 1 MHz [12, 14]. Very high frame-rates up to 3200 Hz have been reported [12]. By controlling the pullback speeds, frame-separation either less than [13, 14] or very close to [12, 15] the transverse resolution (30 µm) have been reported. However, these capabilities are not currently available in commercially clinically approved IV-OCT systems.

This study demonstrates a flow mapping technique that employs an intensity based Cross-Correlation (CC) analysis method. While the NURD of the catheter influences the CC coefficient, a statistical analysis of CC values are proposed. On the basis of statistical analysis, appropriate masks were developed to localize the flow regions. These masks were determined and optimised using phantom flow imaging methods. Proof of concept in-vivo flow mapping of nailfold capillary and human coronary sinus microvessels are also presented. Because of undersampling in the pullback direction of the clinical IV-OCT system, a zero pullback (stationary dataset [8]) mode was used in these experiments. The demonstration of effective microvessel mapping suggests that this analysis technique coupled with advances in OCT catheter technologies focused on increasing image density (and therefore over-sampling) may enable future microvessel imaging during a coronary pullback.

2. Method

2.1. Analysis technique

The undesired effects of the catheter NURD on OCT images are readily observed in clinical IV-OCT zero pullback datasets. In situations where OCT systems are unstable and there is unreliable phase measurement, amplitude/intensity based methods have been utilized [16]. Intensity
based correlation approaches have been reported for transverse flow measurements [17, 18]. Recently vascular imaging using the correlation approach has also been reported [19–22].

Pearson Correlation Coefficient (PCC), is a widely used flow detection method [17–20, 22] as shown in equation 1

\[
X = \frac{\sum_{m,n}(I^{(1)}_{mn} - \bar{I}^{(1)})(I^{(2)}_{mn} - \bar{I}^{(2)})}{\sqrt{(\sum_{m,n}(I^{(1)}_{mn} - \bar{I}^{(1)})^2)(\sum_{m,n}(I^{(2)}_{mn} - \bar{I}^{(2)})^2)}}
\]  

(1)

where, ‘m’ and ‘n’ are dimensions of a rectangular window applied to two consecutive frames \(I^{(1)}\) & \(I^{(2)}\); \(\bar{I}^{(1)}\) and \(\bar{I}^{(2)}\) respectively are the mean of the intensity values within the sub-region. As the \((m \times n)\) window is shifted across the entire image, a 2D correlation map – \(X\) is generated. PCC is a measure of the linear dependence between the two selected sub-regions \(I^{(1)}_{mn}\) and \(I^{(2)}_{mn}\). The resulting correlation value is in the range of \(-1\) to \(+1\), where \(+1\) signifies positive correlation and 0 denotes no correlation, while \(-1\) implies negative correlation.

When the PCC approach is applied to intensity images for flow detection, the correlation coefficients are dependent on a change in intensity. Since, flow regions have a propensity to change in intensity, so they will have low coefficient values. Correlation based flow detection techniques take advantage of the intensity variation in flow regions, which decorrelate across A-lines [17, 18] or image frames [19, 20, 22]; while non-flow regions have high correlation. In flow mapping methods [19, 20], correlation coefficients in the range \((\geq -0.6 \& \leq 0.6)\) were considered low correlation values and so treated as flow regions; while non-flow regions had high correlation \((< -0.6 \ or \ > 0.6)\).

However, in the IV-OCT images, the pixel intensity at non-flow regions fluctuates in the same way as in flow regions. This intensity fluctuation (or, speckle “blinking”) at non-moving locations can be attributed to the erratic movement of the A-scan; to-and-fro around the preferred fixed scanning location. This type of OCT image distortion affects any image correlation based flow detection methods. As a result of intensity noise, uncharacteristic CC maps are obtained, where non-flow regions exhibit low correlation values, similar to flow regions. Thus, it becomes difficult to distinguish flow from non-flow regions and flow maps become redundant.

The present study proposes a method to enable a CC approach for flow mapping, especially for IV-OCT systems. The differences in the correlation profiles for flow and non-flow regions have been investigated. It is demonstrated that in combination, an assessment of the mean and variation within correlation values, can be used to extract flow regions from a set of structural OCT zero-pullback images generated by a commercially available rotational IV-OCT system.

Due to motion artifact (or NURD), when any two corresponding A-lines from any consecutive OCT image pair lay beyond the resolving limit, low CC values were obtained for both flow and non-flow regions. However, when the corresponding A-lines were within the resolving limit, higher CC values were obtained for non-flow regions while flow regions still had low CC values. Thus for a zero pullback scan, where a given A-line tries to scan a preferred location \(N\)-times, the non-flow regions will exhibit higher fluctuation in CC values. Whereas, the variation for a flow region will be muted. The differences in the degree of CC variation for flow and non-flow regions were quantified either by means of standard deviation (SD) using equation 2 [22], or using both mean (or average) and SD of the fluctuation.

\[
s(x,y) = \left[ \frac{1}{(N - 1)} \sum_{i=1}^{N-1} (X(x,y,i) - \bar{X}(x,y))^2 \right]^{1/2}
\]

(2)
where, $\bar{X}(x,y) = \frac{1}{N-1} \sum_{i=1}^{N-1} X(x,y,i)$.

It was also experimentally observed that the mean and SD of the CC fluctuations, for flow regions were less than the non-flow regions. Therefore to extract flow regions from a stack of CC maps, a threshold value for mean and SD of CC fluctuation was experimentally determined for respective flow and non-flow regions. Based on the threshold values mean and SD binary masks were generated, which were then applied to the stack of CC maps for obtaining the flow maps. While in ref. [22] only SD binary masks were used, here both mean and SD masks are applied.

Though suppression of the non-flow regions was the main focus of this study, an equally important factor to incorruptible flow imaging is the removal of background intensity noise. The background noise implies regions with low signal-to-noise ratio (SNR). When SNR values are low the pixel intensities fluctuate randomly and the CC variation profile are similar to flow regions. Therefore, these background regions need to be suppressed. In the present study the background regions were suppressed using an intensity threshold [19], where pixels having intensity below a threshold were suppressed. The background regions can also suppressed using the Wiener filtering method [23].

2.2. Flow mapping procedure

The approach taken to extract flow map from IV-OCT dataset, were as follows:

**Step-1 CC mapping** - Consecutive images within the $N$-frame OCT were cross-correlated using equation-1. Throughout this study, the CC analysis was performed over a $(8 \times 4)$ kernel window, unless otherwise mentioned. As a result of this cross-correlation process, $(N-1)$ CC maps were obtained.

**Step-2 Intensity Mask** - Masks were generated from the OCT images, to suppress the background intensity signals. The intensity threshold required for creating the masks was mainly determined using the Otsu method. This method employs a normalized histogram distribution of pixel intensities and determines the optimum global threshold value on the basis that the inter-class variance between background and signal pixel intensities is maximised [24]. To improve the quality of the Intensity Masks, an $(8 \times 4)$ averaging filter was applied to the OCT image, prior to the determination of the normalized intensity threshold value. The averaging filter smooths the random pixel intensities. Image smoothing and threshold value determination were performed for the first $(N-1)$ OCT images, to generate respective binary masks. Thus obtained masks were applied on to the $(N-1)$ CC maps (from Step-1), to suppress the background pixels. However, when flow regions had low intensities values (close to background intensities), the Otsu based threshold values were modified in order to avoid masking out of flow regions.

**Step-3 SD Mask** - An SD map was obtained from the $(N-1)$ CC maps generated. From section-2.1, this SD map was converted into a binary mask using the experimentally determined SD threshold value. Pixels having SD value above the threshold were set to zero, while pixels below the threshold were set to one.

**Step-4 Mean Mask** - Likewise a mean map was obtained from the $(N-1)$ CC maps. As in the case of the SD Mask, a Mean Mask was generated using the mean threshold, with pixels having a mean value below the threshold assigned one, or otherwise zero.

Each of the Masks were sequentially applied to the $(N-1)$ CC maps. Firstly, the $(N-1)$ Intensity Masks were applied, then the Mean Mask and finally the SD Mask. All the image processing was performed using Matlab (MathWorks, Inc.).
3. Materials

A Clinical system – St Jude C7-XR IV-OCT (St Jude inc., USA, “ILUMIEN”) was used as a representative imaging tool for rotational OCT systems. This system has a fixed A-scan rate of 50 kHz and frame-rate of 100 Hz. Though the St Jude IV-OCT offers both pullback and zero pullback (or, static) mode, the latter was used in this study. The zero pullback mode provides high density data for a certain cross-section, which is currently impossible to obtain using the pullback mode. Each circumferential scan consists of 504 A-lines, which were then transformed into polar form as cross-sectional images. However for CC analysis (see, section 2.1), the original raw format data were used. In the raw-format structural OCT images, the radial A-lines form the columns of the image while the row embodies the radially outward depth. A raw image from the St Jude system consisted of $968 \times 504$ (rows and columns, respectively) pixels. A clinical grade catheter (C7 Dragonfly, St Jude inc., USA, “ILUMIEN”) was used throughout the study. While the IV-OCT system offers a 15 $\mu m$ axial resolution, the catheter is designed to generate a spot size of 25 $\mu m$ and a working distance of $\sim 1.5$ mm [10].

To illustrate the effects of rotational artifacts on the correlation mapping methods and to experimentally determine the threshold parameters (discussed in section 2.1), for flow extraction a flow phantom model was used. The flow phantom model consisted of a capillary tube, with inner diameter (ID) approximately 900 $\mu m$ and outer diameter (OD) about 1.5 mm, embedded in a solidifying mixture of (1.0% v/v) Agar and (1.0%) Intralipid, at an approximate depth of 900 $\mu m$. The solidifying mixture represented the coronary tissue, while the embedded capillary tube symbolized the micro-vessels developing during plaque progression. Intralipid solution concentrations of 1% and 0.05% were used as flow media and imaging was performed with and without flow (where Brownian motion was expected). However, results for Brownian flow are presented here. In order to determine and optimize the mean and SD threshold values, the 1.0% concentration Intralipid solution was used as the flow media. The results and its analysis are presented in section-4.1.

To highlight the effects of NURD on CC mapping, a phantom flow imaging study was carried out using a commercial research grade SS-OCT system (OCM1300SS, Thorlabs Inc., Newton, NJ, USA.). The system provided a scan-rate of 16 kHz and axial resolution of $\sim 12 \mu m$. The sample arm of the SS-OCT was fixed and focused with a LSM003 (Thorlabs Inc.) scanning lens which provided a lateral resolution of 25 $\mu m$. Only X-scan was allowed, with a travel length of 5 mm. In the X-scan a depth cross-section is repeatedly scanned over time; thus it resembles a zero pullback of the IV-OCT system. The XZ-plane image consisted of 1024 A-lines (or columns) and 1024 rows; while the imaging was performed at $\sim 15$ Hz. A separate phantom model was used for imaging with the SS-OCT system. The phantom model consisted of a capillary tube (with ID $\sim 500 \mu m$ and OD $\sim 1.5$ mm). The capillary tubes, in a loop fashion were inserted into a synthetic clay (Blu-Tack®, Bostik, Wauwatosa, Wisconsin)) to mimic the background optical heterogeneity observed in tissues [20]. The capillary tube was filled with 2% intralipid solution, with no flow. Comparative study of the CC maps obtained for both IV-OCT and SS-OCT systems are presented in section-4.1.

To demonstrate the applicability of the proposed method for a coronary artery, a porcine Left Anterior Descending (LAD) coronary artery of a freshly excised pig heart was imaged. A 100 $\mu m$ ID capillary tube was adhered externally and in close proximity to the Left Anterior Descending (LAD) artery of the heart. For imaging the St Jude C7-XR IV-OCT system was used and the C7 Dragonfly catheter was inserted into the coronary lumen mimicking clinical coronary imaging. The capillary tube was filled with 3% intralipid solution and zero pullback imaging performed. Flow imaging results are presented in section-4.2.

To investigate the applicability of the proposed method for in-vivo cases, zero pullback imaging of human nailfold capillaries, using the St Jude C7-XR IV-OCT system, were also carried
out. The results are presented in section-4.3.

Finally, the clinical application of the proposed flow imaging technique in an in-vivo coronary scenario was demonstrated by imaging of microvascular flow in the wall of the coronary sinus. This model was used to enable tracking of definite microvessels arising from the main coronary sinus and imaging without blood clearance (to mimic the situation in plaque associated coronary microvessels arising from the adventitia, where blood clearance does not occur during coronary imaging). In brief, full ethical approval was obtained in accordance with national and institution regulations. A consenting patient undergoing cardiac catheterisation on clinical grounds, underwent coronary sinus intubation. A C7 Dragonfly catheter was introduced into the coronary sinus and zero-pullback imaging performed without blood clearance. A coronary vein branching from the main vessel was identified and this area selected for flow analysis. Flow mapping results are provided in section-4.4.

4. Results
4.1. Phantom imaging

The flow phantom analyses are used here to:

1. Illustrate that the effects of rotational artifact on CC maps,
2. Define the threshold values for the key parameters - SD and Mean, required for flow extraction,
3. Investigate the working range of these threshold parameters.

4.1.1. Cross correlation variation comparison between standard and rotational OCT systems

To illustrate the effects of NURD on CC mapping method, a comparison of the CC dataset, for SS-OCT and IV-OCT systems was carried out. A CC dataset for the SS-OCT system acted as a template for the IV-OCT dataset. Zero pullback imaging of the respective phantom models, elaborated in section-3, were carried out. In both cases, \( N = 31 \) zero pullback OCT images were used and using the Step-1 (also see, equation 1) in section-2.2, \( (N - 1) = 30 \) CC maps were obtained. The CC mapping results for both systems are presented in Fig. 1 and the raw-format data used in the case of IV-OCT system is shown in Fig. 1(e). One of the CC maps obtained for the SS-OCT and IV-OCT systems respectively, is shown in Fig. 1(b) and 1(f). By comparing both CC maps, the effect of NURD becomes evident. In Fig. 1(b) the flow and background regions show low correlation while non-flow regions exhibit high correlation [19, 20, 23]. However, in Fig. 1(f) all regions exhibit low-correlation.

This low correlation in Fig. 1(f) can be attributed to the rotational artifact of the IV-OCT system that causes the A-scan beam to randomly move in and beyond the resolving limit of the system, during each rotational cycle. Thus, the corresponding A-lines in successive frames may correlate or decorrelate leading to fluctuation in correlation values across the CC stack. This is confirmed by Fig. 1(g), which plots the variation of CC values for a known flow and non-flow pixel, across the 30 CC maps. Comparing Fig. 1(g) with a similar plot for SS-OCT (Fig. 1(c)), it becomes apparent that flow regions in both cases show a similar type of CC variation. However, non-flow regions for the IV-OCT dataset (Fig. 1(g)) show a relatively higher degree of CC variation than the non-flow region (Fig. 1(c)) in the SS-OCT dataset.

The variation in the CC values for the flow regions are due to the “fluidic” nature of the speckle pattern in the flow regions. Hence, it can be inferred that the CC values for the flow regions are less affected by the rotation distortion. However for non-flow regions, changes in CC values are not expected and in the absence of movement, a CC profile such as that shown in (Fig. 1(c)), would be anticipated. Therefore, the high degree of variation observed in (Fig. 1(g)), for the non-flow region is due to NURD.
Fig. 1. Comparison of application of the CC mapping method to a zero pullback dataset of SS-OCT (top row) and IV-OCT systems (bottom row). SS-OCT results are a template for IV-OCT data. (a) and (d-e) Structural OCT images from the respective systems. (b) and (f) CC map for (a) and (e), respectively. The colormap in both cases indicates coefficient values. (c) and (g) variation in the CC values across the 30 CC stack for SS-OCT and IV-OCT systems. OCT images obtained from IV-OCT systems are in polar form (d), while raw-format image (e) was used for CC mapping. The markings □ and □ in (a and e) are respectively the non-flow and flow regions, selected for CC variation analysis shown in (c and g). The dotted green circle in (a and d-e) highlights the flow regions. While flow and non-flow regions are distinguishable in (b), a broad low CC map is obtained for (f). This is due to the rotational artefact of the IV-OCT system. The distortion also introduces correlation variation which primarily affects the non-flow region CC values as shown in (g). Scale bar in (a) and (d) represents 1 mm.

Figure 1(g), also shows that there is higher degree of CC variation in non-flow regions than in flow regions. The following conclusions can therefore be drawn from Fig. 1(g):

1. Flow regions, in the IV-OCT dataset, tend to have low CC values (≤ 0.6 from [19, 20]),

2. Whereas, non-flow regions have highly variable CC values; sometimes high CC values (> 0.6 from [19, 20]), or otherwise low CC values.

The higher degree of CC variation, in non-flow regions, can therefore be utilized. As proposed in section-2.1, the extent of CC variation can be better quantified either by taking both mean and SD or just the SD [22] of the 30 CC maps.

4.1.2. Standard deviation threshold value determination

We have previously described the CC variation analysis using the SD method for flow extraction [22]. Step-3 in section 2.2 was applied to the stack of 30 CC maps, for the SS-OCT and IV-OCT datasets. Like in section-4.1.1, the SS-OCT dataset was used for comparison. The resultant of the SD calculation, was a map where each pixel value corresponds to the SD of the CC values for that pixel, across the 30 CC maps. From the SD map for each OCT dataset, the SD values at the selected flow and non-flow regions (in Fig. 1(a) and 1(e)), were derived to estimate the distribution in each case. In the case of SS-OCT dataset, the selected flow region has (37 × 54) (row and column respectively) pixel area, whereas the non-flow region has (127 × 274) pixel area. While for IV-OCT dataset the selected flow region consisted of (95 × 35) pixel area and
Flow (SS-OCT) Non-flow (SS-OCT)

Flow (IV-OCT) Non-flow (IV-OCT)

Fig. 2. Histogram plot showing the distribution of the SD values at selected flow and non-flow regions (marked in Fig. 1(a) and 1(e)) in the SD map, obtained from the 30 CC map stack. (a-b) flow regions and (c-d) non-flow regions in the SS-OCT and IV-OCT dataset. While (a) and (b) have similar profile, the non-flow region histogram profile in (d) resembles more a flow SD profile than to (c). From (b) and (d) a optimum threshold value for SD ($\sigma = 0.22$) can be obtained. Due to the overlapping of the SD histogram plots in (b and d), at this threshold value, some of the non-flow regions can be mislabelled as flow regions.

non-flow region comprised of $(336 \times 170)$ pixels. While selecting flow and non-flow regions, care was taken to select the maximum number of pixels, so that proper SD distribution could be obtained. Histogram plots of the distribution of the SD values for flow and non-flow regions in each OCT dataset are shown in Fig. 2.

From section-2.1 and equation 2, it is known that the SD quantifies the degree of variation. Since, flow regions in both OCT datasets have a similar type of CC variation (see, section-4.1.1), so the respective SD values and their distribution will also have a similar profile. Figure 2(a) and 2(b) shows a similar histogram profile for flow regions in SS-OCT and IV-OCT datasets. The stability of SS-OCT system enables a steady CC value for non-flow regions (see, Fig. 1(c)), so the histogram plot for non-flow regions shows a low SD distribution in Fig. 2(c). However, a contrasting histogram profile was obtained for the IV-OCT dataset in Fig. 2(d). A very high SD distribution with a profile similar to the flow region was obtained. This is consistent with Fig. 1(g) where the flow and non-flow pixels have similar CC variation profiles. Because the degree of variation is higher for the non-flow region, so a higher SD distribution was obtained.

Comparing Fig. 2(b) and 2(d) reveals that there is a separation between the two histogram profiles. Based on this separation, a threshold value was determined; which will enable suppression of non-flow regions. Since flow regions have lower SD compared to non-flow regions, so the SD value at the half maximum ($SD_{half\ max} = 0.22$) for flow regions was determined from (Fig. 2(b)). From Fig. 2(d) it is evident that more than 60% of non-flow regions would be suppressed at this threshold value. However, still some non-flow regions will be erroneously marked as flow regions. As described in Step-3, an SD Mask was produced from the SD map of the $(N - 1) = 30$ CC maps, using this SD threshold value.
Fig. 3. Histogram plot showing the distribution of the mean values at selected flow and non-flow regions (marked in Fig. 1(a) and 1(e)) in the mean map, obtained from the 30 CC map stack. (a-b) flow regions and (c-d) non-flow regions in the SS-OCT and IV-OCT dataset. Though (a) and (b) have similar profile, non-flow regions shown in (d) have lower mean value distribution compared to (c). This drop in mean value is a result of the random fluctuation of the CC values in the non-flow regions of the IV-OCT phantom dataset. Comparing (b and d) with (a and c) shows that, for IV-OCT dataset a threshold value of mean = 0.12 can be used to discriminate non-flow regions.

4.1.3. Mean threshold value determination

From section 2.1, the mean method can also be applied to the stack of CC maps, to differentiate flow from non-flow regions. Step-4 in section 2.2 was applied to the stack of \((N - 1) = 30\) CC maps for the SS-OCT and IV-OCT datasets. A mean map, akin to the SD map was obtained; where each pixel value corresponds to the mean of the CC variation at that pixel over the 30 CC maps. Like, Fig. 2, histogram plots for the mean value at the selected flow and non-flow regions are shown in Fig. 3. Same pixels used in section 4.1.2, were also analyzed here. As in Fig. 2(a) and 2(b), flow regions for SS-OCT (Fig. 3(a)) and IV-OCT (Fig. 3(b)) datasets have a similar type of mean distribution. Because it is an average of the CC values for a particular pixel, so the mean distribution for non-flow regions in the SS-OCT dataset (Fig. 3(c)) is above 0.6 [19, 20]. However in the case of the IV-OCT dataset, due to the NURD the mean distribution was spread across a broad range (see, Fig. 3(d)).

Nevertheless, comparing Fig. 3(a) and 3(c) versus Fig. 3(b) and 3(d) it is observed that even though there is a drop in separation between the histogram profile for flow and non-flow regions in the IV-OCT dataset, a threshold value can still be determined to suppress the non-flow regions. Thus from Fig. 3(b) and 3(d) a threshold value of mean = 0.12 was determined, which will suppress most non-flow regions. As described in Step-4, a Mean Mask was produced from the mean map of the \((N - 1) = 30\) CC maps, using this mean threshold value.
4.1.4. Application of mean and SD masks for flow phantom imaging with IV-OCT system

The Correlation profile for background regions is similar to the flow regions and therefore the Mean and SD Masks are ineffective in suppressing background regions. So Intensity Masks were first applied to every \( (N-1) = 30 \) CC maps, in order to suppress the background regions. For the first 30 OCT images, an Intensity Mask was generated using Step-2 in section-2.2. Intensity threshold value of 0.22 was used. This value was lower than the threshold value determined with the Otsu method. A lower threshold value was used because the flow regions had low intensity and would be partly masked out if Otsu threshold were used. However, as the intensity threshold value is lowered, it also increases the possibility of having background regions marked falsely as flow regions.

After Intensity Masking, the Mean and SD Masks were sequentially applied to the \( (N-1) = 30 \) CC maps. Figure 4(a) shows the result of applying the Intensity and Mean Masks on Fig. 1(f). A further improvement was achieved when the SD Mask was applied to Fig. 4(a) and the result is shown in Fig. 4(b). The resulting \( (N-1) = 30 \) flow maps were binarized and an image filtering method was applied to remove flow pixels that were smaller than the kernel size \((8 \times 4\) pixels). The resultant binarized flow map for (Fig. 4(b)) is shown in Fig. 4(c).

Finally, the regions beyond the 2.5 mm depth (approximately halfway through the image
Fig. 6. Comparing the effectiveness of the Mean and SD Masks using the CC map in Fig. 1(f). (a and b) Binary version of Fig. 4(a) and 4(b), respectively. Regions below 2.5 mm were masked out, as there was no quantitative information available. (c) shows the differences between (a and b). (d) CC fluctuation for certain non-flow regions. Most non-flow regions are suppressed with Mean Mask, however some pixels still appear as flow regions. From (d) these pixels (represented by ▲ Non-Flow2) have low mean value and therefore escape the Mean Mask. Roughly, 13% improvement was obtained when SD Mask was used along with Mean Mask.

in the vertical direction (Fig. 4)) were masked out (see, Fig. 4(d)) because these regions have low SNR value and do not contain any quantitative information. The green arrow in Fig. 4(a) indicates certain background regions that were falsely identified as flow regions. These regions which also include the walls of the capillary tube, can be masked out by adjusting the intensity threshold (in Step-2, section-2.2). However, due to the low intensity of the flow regions, a lower intensity threshold was used in the present case. The flow map thus obtained was transformed into image coordinates and superimposed on to the IV-OCT image (in Fig. 1(d)), as shown in Fig. 5.

To highlight the improvement in segmenting the flow region with the SD Mask, the difference between the outcomes before and after the SD Mask application, was studied. Figure 6(a) and 6(b), shows a respective binarized CC map before and after the SD Mask was applied. The resultant of the difference is shown in Fig. 6(c). While most non-flow pixels (□ Non-Flow1 in Fig. 6(c)) are suppressed with the Mean Mask, some pixels (▲ Non-Flow2 in Fig. 6(c)) show CC variations which have low mean value. These pixels can be suppressed with the SD Mask as they retain the higher degree of CC fluctuation. From the difference map (Fig. 6(c)) it can be concluded that an approximately 13% improvement in suppressing the non-flow regions was achieved with an SD Mask.

4.1.5. Effect of number of cross correlation frames on mean and SD maps

In Fig. 1-6, \( (N-1) = 30 \) CC maps were used to obtain the mean and SD maps. Using these maps the threshold values for the mean and SD parameters were determined, in order to extract the flow regions. Because mean and SD values are statistical parameters, so the accuracy of the threshold values and thus misidentification of pixels as a flow or non-flow region, will be dependent upon the number of CC maps used. However, a larger number of CC maps require more computational time and bring minimal improvement.

To optimize the proposed flow extraction method and to verify its repeatability, the phantom flow imaging (Fig. 1(d)) experiment was repeated 20 times, in zero pullback mode of the IV-OCT system. No SS-OCT imaging were performed. From each experimental dataset, \( N = 91 \) raw-format IV-OCT images (Fig. 1(d)) were used and Step-1 in section-2.2 was applied to
obtain \(N - 1 = 90\) CC maps. To assess the effects of number of CC maps (or CC frames), using Step-4 and Step-3 in section-2.2 respectively, the mean and SD maps were calculated for various number of CC maps \((N - 1 = 5, 10, 15, ..., 90)\), for a given experimental dataset. This procedure was repeated for all the 20 phantom flow measurements.

For every \((N - 1) = 5 - 90\) CC map set, of a given phantom data, the histogram distributions of SD and mean values (similar to Fig. 2 and 3) were obtained for the selected flow and non-flow regions. The selected regions consisted of similar order number of pixels as used in section-(4.1.2 and 4.1.3). The exact number of pixels analysed is different for the 20 phantom experiments because the location of flow and non-flow region shifts within the image, as the starting location of scan beam changes for every experiment. Angular shift in the initial location of the scan beam and subsequent rotational shift in the OCT image is commonly observed in the current commercially available clinical IV-OCT systems.

From every histogram of flow and non-flow region, the percentage number of pixels that have mean and SD values beyond the threshold value (0.12 and 0.22, respectively) were determined. Any pixels having parametric values beyond these limits will be falsely identified. For flow regions, the percentage number of pixels above the threshold value was determined, while for non-flow regions the number of pixels below the threshold values was assessed. Thus obtained values for flow and non-flow region, in each of the \(N = 6 - 91\) cases, were averaged over 20 IV-OCT phantom datasets. Results for the respective mean and SD cases are respectively shown in Fig. 7(a) and 7(b). The plots describe the percentage number of pixels that were falsely identified as flow or non-flow regions, within the selected region, as a function of the number of CC maps. The error-bars in the plots show the variation in the determined values across the 20 data sets.

From Fig. 7(a), it is evident that the erroneous identification of flow/non-flow regions will be greatly reduced when the number of CC maps used to obtain the Mean Masks are increased. It is a consequence of the narrowing of mean histogram, which occurs when the CC map number \((N - 1)\) is increased. Thus, in comparison to \((N - 1) = 5\) CC maps, a much closer distribution of mean values is achieved when \((N - 1) = 90\) CC maps is used to calculate the mean map. Consequently, the amount of overlapping between the mean histograms, for flow and
non-flow regions, also drops with the increasing number of CC maps. This implies that, the 
area of histogram that lies beyond the mean threshold (=0.12) is reduced and the amount of 
misidentification is also reduced. Therefore, in Fig. 7(a), the percentage number of pixels that 
are misidentified drops off with increasing number of CC maps.

The optimum number of CC maps required to obtain unambiguous mean maps, using mean 
threshold value (=0.12), can also be inferred from Fig. 7(a). Beyond \((N - 1) = 30\) CC maps 
very few pixels are misidentified. Thus, with \(30\) CC maps and a mean threshold of 0.12, unam-
biguous identification of flow regions was possible. Therefore, usage of any larger number of 
CC maps was not required.

Also from Fig. 7(a) it is evident that, for any given \((N - 1)\) CC maps, the percentage number 
of mislabelled pixels is always less in non-flow regions than in flow regions. Thus, with 0.12 
mean threshold value, a better suppression of non-flow regions would be achieved. Figure 7(a) 
also shows that the variation in misidentification (indicated by the errorbar), across 20 phan-
tom experiments, is very small and this is further reduced with the increased number of CC 
maps. This indicates that even in the presence of NURD, it may be possible to generate highly 
reproducible flow maps, using a mean threshold (=0.12) and \((N - 1) \geq 20\) CC maps.

Figure 7(b) too shows a diminishing trend in erroneous identification of the flow (or non-
flow) regions, with the increasing number of CC maps. An SD threshold of 0.22 was used here. 
The flow profile shows that flow regions can be identified with better accuracy and that beyond 
30 CC-frames there is negligible error in identification. However, non-flow regions were more 
susceptible and beyond 30 CC maps, no appreciable improvement in mislabelling is achieved. 
Thus from Fig. 7(b) it can be deduced that, about 30 - 40\% of non-flow region pixels will be 
mistakenly marked as flow regions, if only the SD Mask were used. Therefore, throughout this 
study the Mean Mask is applied before the SD Mask.

The errorbar for non-flow regions in Fig. 7(b) shows that the amount of misidentification 
varies for the 20 phantom experiments. This variation occurs due to both shifting and change 
in SD histogram shape, over the 20 phantom experiments. While, for the same dataset, the 
flow regions had a steady SD distribution. Since, the SD value quantifies the fluctuation in CC 
values, so it can be concluded that this large amount of variability is caused by the NURD of 
the catheter which varies between measurements.

Thus, for the consecutive-frame CC mapping approach, \((N - 1) = 30\) CC maps were the 
optimum number required for generating accurate mean and SD maps. These maps can then 
be converted to respective masks using threshold values 0.12 and 0.22. Though a very good 
masking can be achieved with the Mean Mask alone, sometimes some pixels behave randomly 
(as shown in Fig. 6) and these can be suppressed with the SD mask.

4.1.6. Effect of cross correlation mode on the flow mapping

From section 4.1.1-4.1.5, cross-correlation was performed between consecutive OCT frames, 
for \((N)\) images. An alternative approach could be to cross-correlate every \((N)\) images with 
each other. Since, the OCT images were obtained using zero-pullback mode and are essentially 
from the same anatomical location, so such a CC mapping approach can be employed. Because 
the purpose of obtaining enough CC maps was to generate accurate Mean and SD Masks, so 
an every-frame CC mapping approach will generate more CC maps, for a given \((N)\) images, 
when compared to the consecutive CC mapping approach. Therefore, fewer images will be 
required to generate the Mean and SD Masks. This approach is particularly suitable for in-vivo 
clinical IV-OCT images, where only a few images can be obtained for the same anatomical 
location, with the zero-pullback mode.

Determination of the mean and SD threshold values and optimization of the every-frame 
CC mapping approach were carried out using the same phantom dataset used in section 4.1.5. 
However, only 5 phantom experimental datasets and upto \((N = 31)\) OCT frames were used.
Fig. 8. Analysis of every-frame CC mapping approach and determination of threshold value for corresponding mean and SD maps. (a-b) respectively shows histogram plot of mean and SD values of the selected flow and non-flow regions, obtained from CC maps for $N = 31$ images. There is minimal overlap between flow and non-flow distribution in (a). A mean threshold value 0.06 - 0.08 can be determined. Likewise, from (b) an SD threshold value of 0.19 was obtained. Though there is an overlap between flow and non-flow histogram profiles it is considerably less than in Fig. 2(b and d). (c-d) exhibits the effect of frame numbers on respective mean and SD maps and improvement in reduction of flow/non-flow identification ambiguity. The data presented here are averaged over 5 phantom experiments and the errorbar shows the variability across these experiments.

With the every-frame CC mapping approach, 465 CC maps would be generated for a given ($N = 31$) OCT images.

Figure 8(a) and 8(b), respectively shows the mean and SD distribution for selected flow and non-flow regions, obtained from the CC maps of ($N = 31$) images. From these histogram plots the threshold values for the Mean and SD masks were determined. The number of pixels used for generating the respective histogram were of a similar order to the ones used in section-4.1.2 and 4.1.3.

As observed in Fig. 8(a), for the mean value there is minimal overlap between the histogram plot for the flow and non-flow regions at 0.06 - 0.08. This value therefore can be used as a mean threshold to discriminate non-flow regions. Compared to section-4.1.3, there is a drop in the mean threshold value. This is attributed to the differences in the CC mapping approaches used in both cases, which also alters the histogram profile of the flow and non-flow regions. In the every-frame CC mapping approach there is an additional effect of correlation time along with the correlation dependence on flow. It can be assumed that this leads to a narrowing of the histogram profile of the flow regions, while for non-flow regions it gets broadened.

Similar type of changes, in histogram profiles of flow and non-flow regions for SD values can
be seen in Fig. 8(b). Although there is overlap between flow and non-flow histogram profiles, these are much less compared to the overlap of profiles shown in Fig. 2(b) and 2(d). Because there is overlap, so the SD threshold cut-off was the SD value at half maximum, which is $SD_{halfmax} = 0.19$.

The mean and SD threshold values thus obtained were then used to optimize the number of OCT images required to obtain the unambiguous flow maps. The every-frame CC mapping approach was applied to a combination of $(N = 5, 6, 7, ... 30)$ images and repeated for all the 5 phantom experimental datasets. In order to determine the exact number of frames, required to generate accurate Mean and SD masks, the effect of inclusion of every additional OCT image $(\Delta N = 1)$ was considered here.

Optimization was carried out on the basis that there was a minimal amount of overlap between the flow and non-flow region histograms, for both mean and SD cases. The description in section-4.1.5, for obtaining Fig. 7(a) and 7(b), were followed and results for each case are respectively shown in Fig. 8(c) and 8(d).

Figure 8(c) shows the effect of improvement in the Mean Mask as the number of frames were increased. The misidentification of pixels was reduced and for the mean threshold limit of 0.08, 0.5% misidentification was achieved with $N = 15$ OCT images. Like, in Fig. 7(a), a very good suppression of non-flow regions is achieved. However, fewer OCT images were required. A better reproducibility of flow maps can also be achieved with every-frame CC mapping approach, as shown by the small errorbars in Fig. 8(c).

The SD Mask quality also improves with increasing image volume and comparing Fig. 8(d) with Fig. 7(b) it is evident that a better SD Mask is generated when the every-frame CC mapping method is used. This can be attributed to the better separation between the flow and non-flow histograms, when the every-frame CC mapping method is used. From Fig. 8(d), it can be predicted that for a larger number of CC maps $(N - 1) = 30$, the SD Mask alone could suppress the non-flow regions and nearly no-loss flow maps can be obtained.

When a limited number of frames are available, then the every-frame CC mapping method could be used with mean and SD threshold respectively at 0.08 and 0.19. This is especially suitable for clinical images where often a few matching frames can be obtained.

### 4.1.7. Effect of flow concentration on the flow mapping

The CC mapping method (equation 1) which employs intensity variation in speckle patterns to map flow, is dependent upon intensity range. That is, in an OCT image, the regions with background intensities will exhibit artificial speckle intensity variation due to the random noise effect. Whereas, signal intensity speckles display variation in accordance with the sample behaviour, unless affected by systemic noise. Because low CC values are obtained for background regions, so an Intensity Mask (see, Step-2 in section-2.2) was used to suppress the background regions. Therefore a flow region should have speckle intensities above the background noise intensity, to avoid being suppressed. The lowest speckle intensity required for uncorrupted flow mapping by the proposed CC mapping approach, was assessed.

While the signal intensity drops-off with depth, the measurement depth depends upon the tissue type. The flow concentration in the flow region also influences the signal intensity. The phantom model (see, Fig. 1(d)), described in section-3 for the IV-OCT system, was used. Instead of 1% intralipid, 0.05% conc. was used as the flow media to evaluate the lowest speckle intensity that can be unambiguously extracted.

The OCT image obtained with the IV-OCT system is shown in Fig. 9(a) and its polar counterpart in Fig. 9(b). Comparison of the intensity profile along an A-line (marked --- in Fig. 9(b)) for 0.05% conc. and 1% conc. intralipid solution is shown in Fig. 9(c)). While the intensity at the flow region for the 0.05% conc. was similar to its background intensity, the signal intensity for 1% conc. was above its corresponding background intensity. The Intensity Mask (discussed...
Fig. 9. Dependence of flow intensity on the flow mapping method. (a) Cross-sectional OCT image of the flow phantom, with (○) indicates the 900 μm capillary tube. The flow area is filled with 0.05% intralipid solution, with brownian flow. (b) Polar form raw-format image of (a) and --- highlights an A-line. (c) Intensity profile along the highlighted A-line for 0.05% and 1% intralipid concentration. (inset) shows the intensity profile at the flow region, which is marked by □. The profile shows how the intralipid concentration affects the flow intensity. The intensity for 1% conc. is clearly above the background intensity range, whereas for 0.05% conc. the flow intensity is very close to the background intensity. Intensity threshold values of (d) 0.18, (e) 0.2 and (f) 0.22 were used to generate respective Intensity Masks and applied to CC maps. From (c), at 0.18 intensity threshold limit most background regions are visible in the flow map as shown in (d). As the threshold limit is increased to 0.2 and 0.22, the presence of background is also reduced, which is respectively shown in (e) and (f). Increase in the threshold value also masks out lower intensity flow regions, as in (f).

in Step-2 in section-2.2) utilizes the normalized intensity pixel values to determine the threshold value, for distinguishing the measurement from the background intensity. Thus for 0.05% conc., most of the flow regions were masked out (see Fig. 9(f)), when an intensity threshold of 0.22, used in section 4.1.4, was applied. As the intensity threshold value is reduced to 0.2 and 0.18, the flow regions become more visible (respectively shown in Fig. 9(e) and 9(d)). However, the background region also gets falsely highlighted as the threshold value is reduced.

Thus, proper selection of an intensity threshold value enables suppression of background regions. Because, pixel intensity at a flow location is dependent upon the flow concentration and its depth, so the threshold value should be determined prior to the implementation of the flow extraction method.

Ability to measure low concentration flow at a location (at 0.9 mm depth) very close to the boundary limit of measurement range of the OCT system (1 mm depth), shows that the current technique can be applied for a wide range of flow types. For the given 0.05% conc., a better
signal intensity could be achieved if the capillary tube was closer to the surface.

Although the proposed CC mapping method is not dependent upon the intensity of the flow, an appropriate intensity threshold is required for suppressing the background regions. The Mean and SD Masks would then conceal the non-flow regions.

4.1.8. Effect of flow speed on the flow mapping

The flow phantom analysis using brownian flow (Fig. 1–9) shows that the proposed flow mapping method utilizes the dynamics of the scatterer rather than the flow. Thus when there is actual flow and the dynamics of the scatterer becomes stronger, the current flow mapping method remains still effective. The results are not presented here but experimentally this has been verified.

4.2. Ex-vivo imaging of the porcine coronary artery

The applicability of the proposed CC mapping method for a coronary artery case was next demonstrated. The arrangement described in section-3, was imaged using C7-XR FD-OCT system. Figure 10(a) shows the cross-sectional image of the artery, with the intralipid filled 100µm capillary tube applied to it. The procedure laid out in section-2.2, was followed to extract the flow map.

As described in Step-1 (in section-2.2) \( N = 31 \) raw-format OCT images were used and consecutive-frame CC mapping method was employed. The resulting \( (N-1) = 30 \) CC maps were used to obtain Mean and SD Masks, constructed using respective threshold values of 0.12 and 0.22. Intensity Masks, created using the intensity threshold value pre-determined for the OCT images (Step-2 in section-2.2), were first applied to each of the corresponding \( (N-1) = 30 \) CC maps. This was followed by the sequential application of Mean (Step-4 in section-2.2) and SD (Step-3 in section-2.2) Masks on the concerned CC maps. The Intensity Masks suppresses the low-correlation background region, the Mean and SD Mask combination conceals the non-flow regions. To further improve the quality of the flow maps, the image processing-based spatial filtering was applied to suppress flow locations having pixel area less than \( (8 \times 4) \) kernel window.

Finally, the flow map was transformed to image coordinates and superimposed on to the OCT image, as shown in Fig. 10(b). The flow region was adequately extracted from the noisy OCT data-set, using the proposed CC mapping method. The NURD issue that affected the
Nailfold capillary imaging [20] was carried out to demonstrate the identification of flow locations in live tissues using this method. Zero-pullback mode imaging of dorsal nail-fold area over the distal phalanx of the little finger, was performed using the same St. Jude C7-XR FD-OCT system. Figure 11(a) shows the cross sectional view of the structural OCT image. Flow extraction procedures listed in section-2.2, namely - CC mapping, Intensity Masking, Mean and SD Masking, were sequentially applied to the raw data for Fig. 11(a).

The consecutive-frame CC mapping was applied to the \(N = 31\) raw-format OCT images. While Intensity Masks were created on the basis of the signal intensities of the images, the global threshold values for mean (0.12) and SD (0.22) were used for making the respective masks. Finally the spatial image filtering method described in sections-4.1.4 and 4.2, was applied on to the resulting flow maps. The obtained flow image was then superimposed on to the structural OCT image (see, Fig. 11(b)).

Through the proposed flow extraction approach, capillary locations visible on the anatomical image were successfully identified.

4.4. In-vivo coronary sinus imaging

To demonstrate the capability of the proposed flow extraction method to image flow from in-vivo coronary cases, microvessel flow within the Coronary Sinus was imaged. The procedure followed for imaging has been detailed in section-3. In this approach, the microvessels imaged could be tracked to confirm lumenal continuity with the coronary sinus. Also no blood clearance was required, as the imaging catheter was very close to the vessel wall.

Although in phantom studies (section-4.1) and nailfold capillary imaging (section-4.3), \(N = 31\) raw-format OCT images were used, for in-vivo coronary imaging it is difficult to achieve this number of consecutive frames without movement distortion. The physiological movement of the heart alters the imaging location. Thus, only few frames can be obtained for the same cross-sectional location. Therefore, the every-frame CC mapping approach presented in section-4.1.6 has to be applied, instead of the consecutive-frame method. The in-vivo OCT images were affected by both NURD and physiological movement of the heart. Figure 12(a)-12(c) illustrates the effect of the distortions on the CC maps, for a set of OCT images (discussed below). Comparing, Fig. 12(a) and Fig. 1(g) shows that the flow regions - venous and microvessel blood flow, will always exhibit a similar type of CC variation. That is, the flow...
induced speckle variation effect on CC variation, will be dominant. However, the non-flow or tissue regions will have a CC profile dependent upon the distortion effects. As there was an additional distortion effect - heart movement, so a select flow and probable non-flow region from a 21 CC map set, were analyzed. The flow region consisted of \((26 \times 22)\) row and column respective pixel area while non-flow

![Diagram](image)

Fig. 12. *In-vivo* microvessel imaging through the Human Coronary Sinus using the every-frame CC mapping method. (a) CC coefficient variation across 21 CC maps obtained from 7 IV-OCT images. Non-Flow, Microvessel flow, Venous blood flow. (b - c) Respectively, are the histogram of mean and SD values for flow and non-flow regions. (d and f) Cross-sectional OCT images obtained with zero pullback. Bold red arrows indicate the vessels. (e and g) Flow maps corresponding to (d) and (f) superimposed onto the respective OCT images. Flow regions are marked red.

region has \((7 \times 28)\) pixel area. The histogram of mean and SD of the CC coefficients, in the selected regions are respectively plotted in Fig. 12(b and 12(c)). Comparing Fig. 8(a) and Fig. 12(b), it is evident that the heart's movement causes a broader distribution of mean values for flow regions, while shifting the mean distribution for non-flow region towards a lower mean
value range. Whereas, the SD distribution for the flow region is shifted upward in the SD scale. When compared to Fig. 8(a) and 8(b), a different mean and SD histogram were obtained, so using Fig. 12(b) and 12(c) new threshold values were determined. A mean threshold = 0.12 and the SD threshold = 0.19 were thus obtained.

A visual inspection of the zero pullback dataset was first carried out to select a set of images having structural similarity. Two sets of images were obtained. The first set-Fig. 12(d), consisted of \( N = 7 \) and the second set-Fig. 12(f), consisted of \( N = 9 \) consecutive OCT images. The thin coronary sinus wall is visible at around 4 – 11 ‘o’ clock portion in Fig. 12(d) and at 6 – 11 ‘o’ clock portion in Fig. 12(d). While the intravenous blood flow filled the rest of the portion and enveloped the catheter (see, Fig. 12(d) and 12(f). The guidewire and the accompanying shadow can also be seen in both Fig. 12(d) and 12(f). The target microvessel locations have been indicated by bold red arrows.

The polar-type raw-format OCT images were used and the every-frame CC mapping approach (section-4.1.6) followed. For CC mapping, a kernel size of \((10 \times 10)\) was used (see, equation-1). The data for Fig. 12(a) - 12(c) were obtained from \((N - 1) = 21\) CC maps, for the first set of \((N = 7)\) OCT images (see, Fig. 12(d)). The CC map volume thus generated, for each OCT image set (Fig. 12(d) and 12(f)), was processed to obtain respectively a mean and SD map. The threshold values determined above, were used to generate Mean and SD Masks from the respective maps. As in section- 4.1-4.3, the Intensity Mask was generated for every image, using the pre-determined intensity threshold value. Firstly, the Intensity Mask and then Mean and SD Masks were applied to each of the \((N - 1)\) CC maps dataset, to extract the flow map for each case. Thereafter image processing based spatial filtering was performed on the flow maps; flow pixels that are smaller than the \((10 \times 10)\) kernel window were suppressed. The flow maps thus obtained were transformed from polar coordinates to image co-ordinates. The flow maps for Fig. 12(d) and 12(f) are respectively shown in Fig. 12(e) and 12(g). The flow maps have been superimposed on to the respective OCT images, to illustrate the correspondence between the flow locations in the flow map and the OCT image.

The target microvessels are clearly delineated along with a number of smaller vessels traversing the coronary sinus adventitia. The movement during scanning results in imaging of different cross-sections of the coronary sinus. Thus, Fig. 12(d) and 12(f) represents a different location within the coronary sinus.

The intravenous blood flow and the vascular blood regions have been mapped appropriately, by the proposed flow extraction method. It should be noted that the movement associated with coronary sinus imaging is worse than our experience in intracoronary cases. Even in such a highly moving scenario, the flow mapping method was able to image the vascular flow. This vascular flow, which acts as a known flow location, closely resembles microvascular structures within arterial walls.

This coronary intravenous imaging demonstrates that the correlation variation analysis based flow mapping method is not only able to overcome the NURD but also perform adequately in a highly-moving scenario.

5. Discussion

Our previous study [22] had also used \((N - 1 = 30)\) CC maps to overcome NURD effects and to generate authentic flow maps. However, in the present study we have elaborately analyzed the effects of number of CC maps on flow extraction. It demonstrates that 30 CC maps are indeed the optimum number of frames required for obtaining accurate mean and SD maps. The current study also demonstrates that a combination of Mean and SD maps can perform better than our previous approach [22] where SD Mask was only used to extract flow maps. SD threshold value = 0.22, obtained in our previous study [22] has been validated; while mean
threshold = 0.12 was obtained. The 20 phantom experiments also show that these optimized parameters are consistent.

Structural noises in OCT images can deteriorate the flow map, even if a good quality Mean and SD Masks were used. This effect is observed in Fig. 4(a) and 4(b), where localised high density noisy pixels are seen (marked by green arrow). These noisy pixels are background regions and can be removed with proper structural masks.

Two types of CC mapping approaches have been presented - consecutive-frame and every-frame CC mapping. Although the every-frame approach requires a lesser number of OCT images, the computational time would be higher than the consecutive-frame approach, as the every-frame approach generates a higher number of CC maps. However, the CC calculation time can be reduced by implementing this approach in a GPU framework. Figure 7 and Fig. 8 can also be used to determine the oversampling density required for a pullback dataset to generate accurate flow maps, using respective CC mapping approaches. The physiological movement of the heart also introduces additional movements issues. However, as demonstrated in Fig. 12 with oversampling and high-speed imaging, intravascular flow mapping can be performed.

In the present study, it was demonstrated that motion artifacts can be reduced by performing mean and SD analysis of inter-frame CC maps. Where the OCT images have a high density of A-lines (tens of A-lines for a given lateral resolution) and enough time separation, this approach can potentially be extended to an inter-A-line CC dataset (correlation is performed between columns of the OCT images). However, commercially available current clinical IV-OCT systems lack enough oversampling of A-lines within an OCT image, thus limiting the application of inter-A-line CC mapping approach at present. Thus, to pursue any of the CC approaches for a pullback mode requires that A-lines and images should be oversampled. Developments in IV-OCT technologies [11–15] promises that in future it may be possible to acquire coronary images with sufficiently high density and at high-speed.

6. Conclusion
Feasibility to image micro-flows with IV-OCT system was demonstrated. As the NURD of the catheter corrupts the CC map, both flow and non-flow regions exhibit similar CC coefficients. It was shown that flow regions can still be extracted from the corrupted CC maps by taking mean and SD of the CC maps. Two types of CC mapping approaches - consecutive-frame and every-frame, were presented. The performance of the proposed methods have been demonstrated using phantom model, nailfold capillary imaging and \textit{in-vivo} microvessel imaging.

Acknowledgments
The work reported in this paper is supported by St Jude Medical UK Limited and NIHR Leicester Cardiovascular Biomedical Research Unit, UK.