Automatic assessment of stent neointimal coverage by intravascular optical coherence tomography

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Aims
This study aimed to validate automatic intravascular optical coherence tomography (IVOCT) analysis for the evaluation of neointimal coverage in response to stent implantation.

Methods and results
Fourteen stented segments in common iliac arteries, acquired from a total of seven adult male New Zealand White rabbits, were interrogated in vivo by IVOCT. Durable polymer everolimus-eluting stents (EES; Xience V, Abbott Vascular, Santa Clara, CA, USA) were used exclusively. Comparison with histology was made in a total of 63 pairs of images, where neointimal thickness over corresponding individual stent struts was assessed. A high correlation coefficient ($R = 0.85, P < 0.001$) was obtained by comparing automatic IVOCT analysis with histology. Moreover, Bland–Altman statistics showed good limits of agreement (LOAs) of $\pm 45\, \mu m$, with an average difference of $-10\, \mu m$. In addition, manual IVOCT assessment presented very similar results when compared with histology ($R = 0.83, P < 0.001$ and LOA $= \pm 48\, \mu m$ with an average difference of $-8\, \mu m$). Therefore, a very high correlation value was found, comparing manual to automatic IVOCT measurements ($R = 0.95, P < 0.001$) together with good LOAs ($\pm 27\, \mu m$) and an average difference of $-2\, \mu m$.

Conclusion
The results of the study suggest that automatic IVOCT analysis is a reliable and accurate tool able to speed up current IVOCT analysis procedures. This would potentially allow for a better integration of IVOCT in clinical practice and clinical studies assessing vascular response to stent implantation in a large series of patients.

Keywords
optical coherence tomography • OCT • stent • neointimal coverage • image analysis

Introduction
The accurate in vivo assessment of neointimal coverage after drug-eluting stent (DES) implantation is a critical parameter to assess their safety and efficacy.1 Human pathology studies suggested that late DES thrombosis is associated with the lack of strut coverage due to delayed vascular healing.2,3 As a tomographic image technique, intravascular optical coherence tomography (IVOCT) allows for the in vivo visualization of coronary arteries.4,5 Due to its very high axial resolution (<20 μm), IVOCT is currently the only technique able to evaluate neointimal formation following DES implantation in vivo.6 Despite this potential, IVOCT currently requires a very time-consuming manual analysis to quantify individual stent strut coverage.7 Given that state-of-the-art Fourier-domain imaging systems record pullbacks with very high frame rates,8 the analysis of a single stent typically requires the manual assessment of thousands of struts, hampering its widespread introduction in clinical practice and studies including a large number of patients.

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Automatic assessment of IVOCT images allows for a fast and detailed analysis of stent strut coverage. Previous studies by our group have demonstrated its potential to assess stents in vivo, drastically reducing the time required by current analysis. However, such algorithms are typically validated through comparison with IVOCT manual analysis by expert image readers, while histopathology is actually considered the gold standard for the evaluation of tissue morphology and composition. Therefore, the goal of this study was to compare automatic quantification of stent strut coverage with matched histological cross-sections.

IVOCT and histological data were collected in an animal model at different time-points following DES implantation. This allowed for the validation of IVOCT automatic stent strut coverage quantification in vivo over different degrees of neointimal coverage, mimicking real-life clinical data.

Methods

Data were acquired from 14 common right and left iliac arteries from a total of seven adult male New Zealand White rabbits, weighting 2.8–3.1 kg. Durable polymer EES (Xience V, Abbott Vascular, Santa Clara, CA, USA) were used exclusively. The study protocol was approved by the ethical committee of the University of Antwerp (Belgium). The experiments were performed in accordance with the standard guidelines for the care and use of laboratory animals.

Histological data

The set-up of the experimental animal model was done as indicated by previous studies. All animals were subjected to an atherogenic diet consisting of 1% high cholesterol and 6% peanut oil. After 7 days, the animals underwent endothermal denudation of both iliac arteries and 4 weeks later, the diet was adjusted to a lower cholesterol level (0.025%). At Week 9, all rabbits underwent bilateral stent implantation (left and right iliac arteries) with Xience V (Abbott Vascular) EES with diameter and length of 3.0 and 18.0 mm, respectively. General anaesthesia was induced using Domitor (0.3 mL/kg, intramuscularly) and Anesketin (5 mg/kg, intravenously).

All animals (n = 7) were divided into four groups to evaluate different stages of neointima formations. Stents were harvested at 2, 3, 4 (n = 2), and 10 weeks (n = 1) post-implantation. Before being harvested, the stented iliac arteries were interrogated in vivo by IVOCT. Immediately after the intravascular imaging, vessels were excised and processed for histopathology.

For the purpose of light microscopic analyses, the stented vessels were fixed in 10% formalin up to 24 h, and embedded in Technovit 9100 (methyl methacrylate, Heraeus Kulzer GmbH, Wehreim, Germany). Six-micrometre-thick slices were cut on an automated microtome (Leica, Milan, Italy) using a tungsten Wolfram Carbide metal knife every 0.6 mm. All sections were stained with haematoxylin–eosin to identify coverage above stent struts. Images were digitalized for later analysis with an Axioskop light microscope at a 5× magnification (Zeiss, Oberkochen, Germany).

OCT imaging protocol

OCT pullbacks were recorded in vivo using the C7-XR™ IVOCT system and Dragonfly™ catheters (St Jude Medical, St Paul, MN, USA). The C7 system acquires images at a frame rate of 100 images per second of a segment ~54 mm long. Imaging specifications, according to the manufacturer, are an axial resolution <20 μm and a lateral resolution of 25–60 μm. All pullbacks were acquired at the default speed of 20 mm/s. IVOCT imaging was performed during manual flush with a contrast medium (Iomeron®, Bracco SPA, Milan, Italy) for blood clearance. In case, the initial pullback was not of adequate quality, additional pullbacks were acquired until an optimal imaging sequence was achieved. All images were stored for off-line analysis.

Data analysis

Histological cross-sections were registered to IVOCT frames by using the distal stent edge as a reference. Therefore, image matching was attempted every 0.6 mm; however, as multiple effects can make a correct registration challenging (e.g. IVOCT image acquisition procedure and movement artefacts affecting image acquisition), in case a sub-optimal registration was obtained images were discarded. This procedure was followed in order to obtain the best data possible. However, it is important to notice that IVOCT data were acquired in vivo and, in case they presented sub-optimal image quality, images were not discarded but included in this validation study. This procedure resulted in a total of 63 pairs of images (i.e. 63 IVOCT images registered to 63 histological cross-sections). Subsequently, individual struts appearing in both IVOCT and histological cross-sections were located and a one-by-one correspondence created. Overall, a one-to-one correspondence for a total of 323 stent struts (for all images) was obtained. It is also important to notice that both frames and individual strut selection were done prior to any image quantification in order to avoid a selection bias.

Once the registration procedure was completed, individual strut coverage was quantified on the histological cross-sections using the software ImageJ. At the same time, IVOCT strut coverage was both automatically and manually quantified. Manual analysis was made through the use of the Lightlab/St Jude offline review workstation. Note that manual analysis of both IVOCT and histological data was obtained by image readers blinded to previous results in order to avoid a bias.

Automatic image analysis

Automatic quantification of IVOCT stent strut coverage was obtained using previously developed routines, such that vessel lumen and stent struts were automatically segmented and strut coverage quantified. The software analyses raw data (polar domain) 16-bit IVOCT images prior to any image processing (e.g. log-compression, eight-bit compression, and RGB conversion) typically applied for visualization purposes. In brief, automatic image segmentation is obtained by analysing A-scan line-intensity profiles (illustration in Figure 1). As metallic stent struts in IVOCT images appear as very high reflecting objects casting a shadow, three properties of the A-scan lines are able to unravel the presence of a strut: (1) maximum intensity, (2) rapid rise and fall of energy, and (3) the shadow a strut generates. More in detail, the rapid rise and fall of energy is quantified as the number of pixels above the full-width ‘partial’ maximum of the signal and the shadow generated by a strut is assessed using a sliding window (Figure 1). This allows for a robust detection of stent struts, not only based on the presence of an image shadow, but also on other properties of the OCT trace such as signal energy. Once such properties are quantified, segmentation is obtained as follows: first, A-scan line classification is applied to identify lines containing a strut; secondly, contours delineation is obtained through common image processing techniques using two-dimensional (2D) spatial continuity of vessel wall and struts over multiple lines. Once the different structures are correctly delineated, neointimal tissue covering the stent is automatically quantified after scan conversion of polar IVOCT data as detailed in Figure 1. All the specific details about this procedure can be found in a former publication.
Validation methods

Automatic quantification of neointimal coverage was validated using histology data as the ground truth. All individual struts that were previously registered with a one-to-one correspondence were taken into account. In case a strut was not properly detected by the automatic procedure, it was labelled as a false negative. Regression analysis and Bland–Altman statistics were used to quantify algorithm accuracy, and the percentage of false negatives was reported. In addition, manual IVOCT analysis was also considered and it was compared both with histology and with automatic IVOCT quantification results. All statistical analyses reported in this study were made by the means of software Matlab v7.12 R2011a (MathWorks, Natick, Massachusetts, USA) and the dedicated Statistics Toolbox v7.5 (MathWorks).

Results

Figure 2 shows an example of matched IVOCT and histological cross-sections. Overall, a total of 63 IVOCT images could be directly registered to histological cross-sections. One-to-one stent struts correspondence was obtained for a total of 343 struts with different degrees of neointimal coverage ranging, according to histology, from 0 (uncovered) to 315 μm. Histology measured a mean neointimal thickness of 52 ± 40 μm; automatic and manual IVOCT analysis measured a mean neointimal thickness of 62 ± 43 and 60 ± 42 μm, respectively.

Figure 3 reports regression analysis and Bland–Altman statistics for neointimal coverage quantification as assessed by automatic IVOCT image analysis, manual IVOCT analysis, and histological cross-sections analysis. A Pearson correlation coefficient $R = 0.85$ ($P < 0.001$) was found when comparing individual stent strut coverage by IVOCT automatic analysis with histology data. Bland–Altman statistics reported limits of agreement (LOAs) of ± 45 μm, with an average difference of −10 μm (obtained subtracting IVOCT measurements to histology data). Automatic strut detection presented a total ratio of false negatives of 6%.

In the same way, comparison of IVOCT manual analysis with histological data showed a Pearson correlation coefficient $R = 0.83$ ($P < 0.001$), LOAs (Bland–Altman statistics) of ± 48 μm were found with an average difference of −8 μm. Therefore, comparison of IVOCT automatic analysis with manual IVOCT quantifications showed a Pearson correlation coefficient $R = 0.95$ ($P < 0.001$). LOA resulted to be equal to ± 27 μm with an average difference of −2 μm (obtained subtracting automatic measurements to the manual ones). Results are summarized in Table 1. Automatic analysis of a single cross-sectional image (comprehending polar image segmentation, scan conversion, and automatic neointimal coverage quantification) took, on average, ~0.6 s.

Discussion

This study aimed to validate automatic IVOCT stent strut coverage analysis. Data from multiple animals collected at different time-points after DES implantation were used to generate different degrees of neointimal coverage ranging from 0 to 315 μm.

Comparison of automatic IVOCT results with histological data showed a high correlation value ($R = 0.85$, $P < 0.001$) and good LOA of ± 45 μm together with a low ratio of false negatives of 6% (corresponding to approximately one ‘missed’ strut every four images). Moreover, a very high correlation value ($R = 0.95$, $P < 0.001$) and an excellent agreement were found comparing automatic with manual measurements. The average difference between histology and both automatic and manual IVOCT measurements (of 8 and 10 μm, respectively), revealed by Bland–Altman statistics, can be attributed to tissue shrinkage due to histology processing. It is also important to notice that the difference between the two
measures appears to be constant for different levels of neointimal coverage as illustrated in Figure 3. Given that IVOCT data were acquired in vivo, thus including realistic clinical imaging conditions such as incomplete vessel flushing and eccentricity of the imaging catheter, and frame selection was blinded to subsequent analysis the results of this study suggest that automated IVOCT analysis is an accurate and reliable tool for the assessment of stent strut neointimal coverage, potentially able to replace manual analysis. As such, the use of automatic algorithms allows for a more time-efficient analysis of entire IVOCT pullbacks, potentially allowing for a better integration of IVOCT in clinical practice and research.

Additional confirmations were provided by the fact that reported results are comparable with previous studies assessing the accuracy of IVOCT measurements. Furthermore, automatic to manual IVOCT analysis validation resulted in comparable values to the ones previously reported by our research group. In this study, a wide range of neointimal thickness was evaluated. A large number of struts was taken into account (n > 300), with neointimal thickness ranging from very thick neointima to a complete lack of coverage. Despite the excellent results obtained, one particular aspect of IVOCT coverage analysis merits further consideration. Recent studies aimed to assess reproducibility and accuracy of IVOCT for strut coverage assessment. Although, in general, a satisfying reproducibility was reported, more specific studies assessing histological correlation for very thin layers of neointimal coverage and for the discrimination of slightly covered from uncovered struts reported lower reproducibility and accuracy. The main challenge is that discrimination between a very thin layer of neointimal tissue and a total lack of coverage intrinsically deals with the measurement of distances of the same order of the axial resolution of IVOCT (\(\sim 20 \mu m\)). Moreover, a strut blooming of \(\sim 37 \mu m\) in thickness (i.e. blooming artefact), extending of \(\sim 18 \mu m\) from the strut surface towards the imaging catheter, further complicates the measurement of neointimal coverage \(< 20 \mu m\). The possible use of a threshold for the discrimination of uncovered struts by IVOCT (not by accident usually equal to 20 \(\mu m\)) is still a matter of concern and debate. Our final recommendation is that automatic quantification of strut coverage would better be supported by the visual inspection of a trained user, especially in case of lack of coverage. Therefore, the software was incorporated in a user friendly interface allowing for a rapid visual inspection and possible correction of the final results.

With this system, a very fast analysis of entire IVOCT pullbacks can be achieved. As a matter of fact, the automatic analysis of a single IVOCT cross-sectional image takes \(\sim 0.6\) s, while manual analysis of a single IVOCT cross-sectional image may vary between 2 and 6 min (depending on image quality, number of struts that need to be analysed and with some variations from one image reader to another). As such, the analysis of an entire stent (e.g. analysing IVOCT frames with an interval of 0.6 mm, resulting, for example, in...
50 images for a 30-mm stent) may take up to 2–3 h when done manually, while automatic analysis can be obtained in a much shorter time. Although we suggested that automatic analysis needs to be supported by visual inspection of the results, given that few corrections are usually needed only in case of uncovered stent struts (as detailed above) and inadequate image quality (i.e. image artefacts, for example, due to a very high amount of blood in the lumen9), final results for the example above can be obtained in $\approx 15–20$ min, thus resulting in a procedure which is up to 10 times faster than ‘classic’ manual analysis.

An additional consideration is that the registration of histological tissue to corresponding IVOCT images acquired in vivo remains a significant challenge and a very common issue with respect to the validation of any imaging modality. Extreme care was taken in this study to ensure precise matching between IVOCT images and histological cross-sections. However, multiple effects and intrinsic limitations, such as helicoidal IVOCT data acquisition, catheter movements due to cardiac motion, IVOCT longitudinal sampling, catheter positioning, and orientation in the vessel lumen, could have deteriorated matching accuracy. Moreover, tissue damage due to the tissue procedure preparation could have influenced histological quantification accuracy. As such, in order to optimize matching accuracy, images presenting only a sub-optimal registration were discarded from this study, resulting in 63 pairs of well-registered images.

**Table 1** Comparison of histological, automatic, and manual IVOCT morphometric analysis

| N = 343 struts (63 image pairs) | Automatic IVOCT/histology | Manual IVOCT/histology | Automatic/manual IVOCT |
|--------------------------------|---------------------------|------------------------|-------------------------|
| Correlation                    | 0.85                      | 0.83                   | 0.95                    |
| Average difference (µm)        | $-10$                     | $-8$                   | $-2$                    |
| Limits of agreement (µm)       | $\pm 45$                  | $\pm 48$               | $\pm 27$                |
| Measurement (µm)               | Automatic                  | Manual                  | Histology |
|                                | $62 \pm 43$               | $60 \pm 42$            | $52 \pm 40$ |

Values for all comparisons were $P < 0.001$.  

Figure 3  Regression analysis and Bland–Altman statistics for the quantification of neointimal thickness over stent struts. Automatic IVOCT analysis was compared with histology data (first raw) and with manual IVOCT analysis (last raw). Comparison between manual IVOCT analysis and histology data is also reported (middle raw). A total of $n = 323$ struts (excluding the false negative ratio of 6%) were analysed over a total of 63 pairs of IVOCT and histological cross-sectional images.
Finally, it is important to notice that, although there was not a completely homogeneous distribution of the selected frames among the different vessels (as frame selection was driven by registration feasibility as described above), such selection was kept free of bias, as it was performed prior to any image quantification (i.e. blinded to stent strut coverage quantification results).

**Conclusion**

In summary, automatic IVOCT analysis is a reliable tool to speed up data analysis, potentially able to replace manual assessment. Although a final visual inspection of the results is recommended to ensure the final quality of the analysis with respect to uncovered struts, the proposed methodology allows to significantly speed up current IVOCT manual analysis. This would potentially contribute to a better integration of IVOCT in clinical practice and cardiovascular research.

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**Conflict of interest:** none declared.

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