High resolution X-ray micro-CT imaging of fibrin scaffold using large area single photon counting detector

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Abstract: This paper deals with the high-resolution X-ray micro-computed tomographical (micro-CT) visualization of a fibrin scaffold intended to be used during medical repairs of various types of human tissue. Due to the cellular nature of scaffolds, it is important to inspect their microstructure in high detail on a volumetric basis. In this work, we demonstrate the micro-CT measurement of a fibrin-based bone scaffold performed using a proprietarily developed tomographical scanner equipped with a large-area imaging device (LAD) composed of $10 \times 10$ Timepix silicon pixel detectors without any gaps between the individual tiles. The fibrin scaffolds are based on organic materials, which may be reinforced by various additives to improve their mechanical characteristics, and their dimensions are generally very small (i.e. micrometer to millimeter scale). As the organic material used in fibrin scaffolds exhibits very low X-ray attenuation, low-energy X-ray radiation is desirable to achieve sufficient contrast in the projections. Moreover, a high resolution is needed to visualize the fine features in the scaffolds. Here, conventional scintillation detectors suffer two problems that make the aforementioned LAD superior for the imaging of the investigated scaffolds: a wide point-spread function and low sensitivity at low energies. Despite the high LAD sensitivity to low-energy photons, it was necessary to apply several correction procedures to achieve the highest possible resolution. Here, a computational procedure was developed to compensate for the drift of the tube’s focal spot, geometrical imperfections of the LAD detector assembly, and the effects of its border pixels with different responses and sizes. We demonstrate the results on the final reconstructed images based on uncorrected and corrected projections, where we achieved a $1 \mu m$ voxel size.

Keywords: Computerized Tomography (CT) and Computed Radiography (CR); Pixelated detectors and associated VLSI electronics; Inspection with x-rays
1 Introduction

Tissue engineering has become a very attractive approach to the treatment of various injuries and diseases which formerly resulted in irreparable damage to human tissue. Here, tissue scaffolds of different types can be implanted into the body to temporarily take over the function of the original tissue, while being simultaneously subjected to the ingrowth of natural tissue over time. During and after full restoration of the natural tissue, the scaffold degrades, leaving only natural tissue in a way that no signs of the treated defect are apparent. Such ideal scaffolds then fulfill all the requirements for bioactivity with the potential to support and stimulate the regeneration of the natural tissue and its full bioreabsorbability, which can be considered the highest level of biocompatibility. Several different types of scaffolds have been recently proposed including the gellan-gum-based bone scaffolds and multifunctional fibrin scaffolds.

Fibrin scaffolds are formed by an interlinked network of proteins that can support a variety of living human tissues including adipose tissue, bone tissue, and cardiac tissue. Because the fibrin proteins are naturally produced by the body after an injury and the engineered scaffolds act as both the replacement tissue and a tissue substitute accelerating healing [1], the fibrin scaffolds can be considered a typical example of biomimicking technology. As the proteins for manufacturing of the scaffold can be extracted directly from the patient’s blood (i.e. autologous nature), the main advantage of this technology is particularly the initial stability of the grafted stem cells without an aggressive response by the immune system. However, almost irrespective of its final application, research in this field is concentrated on the production of scaffolds with controlled stiffness, strength, and permeability by means of reinforcements in the scaffold microstructure. This involves not only mechanical experiments, but particularly the inspection of synthesized scaffolds to determine their microstructural characteristics and internal defects that can be coupled with finite element simulations, for example, for an inverse estimation of their mechanical properties.

Due to the nature of cross-linked protein networks and the millimetric dimensions of specimens, the microstructure can be studied with the required level of detail only on a volumetric basis, which
can be provided using tomographical methods. It has been shown in this field that such organic biomaterials with the micrometric elements of a microstructure exhibit very low attenuation to X-rays and are practically transparent at higher X-ray energies, yielding laboratory imaging with scintillating detectors problematic. It has been also demonstrated that instrumentation based on Medipix2 hybrid silicon pixel detector can be used to perform high-resolution X-ray imaging of biological samples [2]. Furthermore, assembly of silicon pixel detectors of the Timepix family has been successfully used for micro-computed tomography (micro-CT) of organic scaffolds under loading (i.e. a 4D micro-CT) as an on-the-fly experiment [3]. However, to use the reconstructed volumes as a tool for an inspection of the materials’ microstructure, the achievable spatial resolution has to be improved to a micrometric level. Such a micro-CT measurement is then influenced by detector pixel size, for example, and its point-spread function and also by the long-term properties of the X-ray source, including the stability of the focal spot and mechanical stability of the imaging device. These factors are particularly important in long-time CT measurements with a high number of angular projections and long acquisition times per projection required to achieve sufficient image statistics. Theoretically, the tomographical scan is carried out by rotation of the investigated sample, while other components of the tomographical device including the detector, radiation source and its focal spot remain stationary. Although the electro-mechanical nature of the positioning systems guarantees their stability from a control point of view, other factors, including thermal expansion, have to be taken into consideration in practice. Thermal expansion and other drifts of the tube lead to geometrical movement of the origin of the cone beam, which in turn causes an undesirable sample projection drift (SPD). This effect considerably affects the achievable spatial resolution in the reconstructed 3D volumes. Several papers dealing with the quantification of SPD effects and the means for its compensation, including the use of calibration datasets used for several subsequent scans, have been published [4, 5]. Many studies treat the SPD effect as stochastic, though. Methods employing a static reference object placing a sample sideways into the detector field of view or using a sparsely-sampled reference dataset are considered the most reliable techniques for its compensation [6, 7].

In this paper, we demonstrate the effects of compensation procedures on the spatial resolution of the reconstructed volume of a fibrin-based tissue scaffold. The high-resolution of a cross-linked fibrin specimen with a millimeter size was achieved using the large-area imaging device (LAD) developed by the CTU in Prague [8]. The detector was used due to its combination of high resolution necessary to visualize the fine features in the scaffolds and the low X-ray attenuation coefficient of the organic fibrin material. The superior quality of the LAD for the imaging of low attenuation objects was demonstrated in [9] and we have already shown that conventional scintillation detectors suffer from a wide point-spread function and low sensitivity at low X-ray energies [3]. The correction procedure developed compensates for drift of the tube’s focal spot and the electron beam, geometrical imperfections of the LAD assembly, and the different sizes and responses of border pixels in the edgeless Timepix detectors used. The results are demonstrated on the final reconstructed volumes based on corrected and uncorrected projections showing the behavior of micrometer-sized clusters of fibrin fibers in the scaffold investigated. It is shown that the spot movement taken into account during the data processing procedures leads to significantly sharper results compared to non-corrected reconstruction.
2 Methods

2.1 X-ray imaging and CT reconstruction

Tomographic imaging of the fibrin scaffold was performed using the TORATOM (Twinned Orthogonal Adjustable Tomograph) scanner. TORATOM is composed of two perpendicular imaging lines with the capability of performing dual-source and dual-energy measurements. Imaging geometry is fully adjustable thanks to complex 13-axis positioning systems. The scanner is equipped with two different X-ray tubes, which can be operated in an identical mode. For imaging, various detectors are available, for instance flat panels with a Gd- or CsI-based scintillator or pixelated silicon detectors from the Medipix family. For the purpose of this paper, the measurements were carried out using a combination of a XWT 160 TCHR microfocus transmission type X-ray tube (X-RayWorX, Germany) and a semiconductor large-area photon counting detector (LAD). The LAD detector was composed of a matrix of $10 \times 10$ tiles of Timepix silicon pixel detectors operated in synchronous mode [10]. Each Timepix detector has a $256 \times 256$ pixel resolution with $55 \mu m$ pitch, resulting in a $2560 \times 2560$ pixel resolution of the LAD device on a sensitive area of $14.3 \times 14.3 \text{ cm}^2$ without any gaps between the tiles. The arrangement of the radiographical setup is depicted in figure 1.

The setup geometry was set to a source-object distance of $28.5 \text{ mm}$ and a source-detector distance of $987.5 \text{ mm}$ leading to approximately $34 \times$ geometrical magnification (image pixel size $0.9 \mu m$). The X-ray tube was operated at an accelerating voltage of $40 \text{ kV}$ and a target current $240 \text{ mA}$ leading to a target power of $9.6 \text{ W}$. Tomographical scanning of the object was performed in 1200 projections with a $0.3^\circ$ angular step. CT reconstruction was performed in VG Studio Max (Volume Graphics, Germany) using a cone-beam filtered backprojection algorithm.

2.2 Drift compensation

X-ray imaging of organic materials with a fine microstructure, such as the fibrin-based scaffolds, requires relatively low energies. At the same time, high resolution is usually required, which leads to the need for low tube power in order to maintain a reasonable X-ray tube spot size, and thus avoid related image blurring. Such requirements lead to a prolongation of the X-ray image acquisition time and consequently long-time CT scans. Due to this, many factors affecting the
quality of tomographic reconstruction, including the fluctuating power on the tube target, cooling of the tube head and ambient temperature, have to be taken into account. The main factor influencing high resolution CT quality is focal-spot movement due to thermo-elastic deformations of the tube and electron beam drift. We demonstrated this in a previous study that, in the case of our X-ray tube, the focal-spot movement is one order of magnitude higher than the effect of the electron beam drift [6]. For this reason, we have developed a measurement protocol which can be used for significant improvement in the quality of the high resolution CT reconstruction correcting tube spot movement [4, 6]. During the CT data acquisition procedure performed in this work, an object was subjected to 20 sets of 60 tomographic projections, covering a full 360° rotation by 1200 projections with a 0.3° angular step, and 40 reference images were also recorded.

In detail, the process is as follows. For the first projection set: the reference image and concurrent first projection is acquired at 0°; the other 60 projections are recorded at 6° angular steps, i.e. the last projection is acquired at 354°; the second reference projection is acquired at 360° (i.e. 0°). The next projection set is done in a similar manner. The first reference image is recorded at 0°; projections are recorded with a 0.3° angular shift from the first projection set, i.e. the first projection is recorded at 0.3° and the last projection at 354.3° respectively. For each additional projection set, a 0.3° angular shift is successively added, i.e. the last set is started at 5.7° and ended at 359.7°. In addition, two reference images at 0° and at 360° are recorded for each projection set.

Note that the reason for recording the reference image at the end and beginning of the consecutive set is that a tube refreshment is necessary to regularly conduct long-time measurements, and it should be done between projection sets (although it is not necessary to do it between all projection sets). Obviously, the number of projection sets can be changed for another measurement. As mentioned above, 40 reference projections are recorded during CT data acquisition. The movement of the objects within these projections caused by the tube spot movement is analyzed at subpixel image resolution in both perpendicular directions (in the detector plane). Digital image correlation (DIC) based on the Lucas-Kanade tracking algorithm [11] implemented by the proprietarily developed Matlab procedure is used for this purpose. Reference movements are linearly interpolated over all projections for every projection set. Calculated projection movements are corrected by shifting them in opposite directions using bicubic interpolation.

2.3 LAD correction procedure

X-ray imaging using the LAD device offers significant advantages over detectors equipped with a scintillator when imaging organic materials that generally yield a low achievable signal-to-noise ratio in the projections. Here, performance of the Timepix-type detectors is superior thanks to their sensitivity to low energy photons. However, imaging using devices composed of multiple detectors may also cause problems not encountered when using other detector types as the tiled design of a LAD leads to the need for using advanced correction procedures for data processing before reconstruction. Geometrical imperfections of the LAD assembling was solved in [12] utilizing reference measurement with fine grid made from Cu wires. An example of an uncorrected projection showing the fibrin scaffold captured using LAD is shown on the left side of figure 2.

It can be seen that the matrix of individual detector tiles is clearly apparent on the projection. This effect is caused by several reasons. Firstly, the LAD device is composed of 100 individual detectors that inevitably exhibit variations in their properties, particularly their sensitivity. Secondly,
the number of columns and rows of the detector matrix also increases the probability and magnitude of geometrical imperfections of the detector assembly, including its flatness. The final source of artefacts are the border pixels of the edgeless Timepix detectors that are larger than others and exhibit different responses. To improve CT performance, the detector was tilted by $0.2^\circ$ around its horizontal axis of symmetry, which resulted in the significant suppression of artefacts in the tomographical reconstruction arising from the edges between the lines of chips. Moreover, a four-step projection-level procedure was developed at MATLAB. As a first step, the identification of the false signal on the chips’ boundaries is conducted using a high pass filter, and summation in the vertical direction is performed to distinguish significant intensity variations. Then, gaps between the rows of chips are determined using a high-pass filter on the signal from within the object. After that, the equalization of pixel level responses is carried out by fitting a polynomial surface of 2 degrees over individual LAD tiles. As a last step, interpolation of the missing data in the gaps between every two rows of chips from the signal outside of the sample is performed.

3 Results

The tomographic reconstructions were performed using the spot drift corrected and uncorrected datasets to evaluate the developed procedures. In both cases, corrections for compensation of LAD-specific effects were performed, and the right side of figure 2 shows the result after application of the LAD correction procedure.

During the subsequent tomographic reconstructions, the voxel size was set to 1 $\mu$m by slight oversampling during reconstruction. Figure 3 shows reconstructed projection with dimensions $2 \times 2.5$ mm of the investigated specimen without correction of the spot movement.

It can be seen that the reconstruction is still influenced by the tiling of the LAD detector, and the ring artefact is apparent in the horizontal cross-section. From a microstructural point of view, the clusters of fibrin fibers are significantly blurred. This demonstrates the importance of spot movement corrections to achieve micrometric resolution in the reconstructions. During the
spot movement evaluation procedure based on 20 sets of calibration projections, the drifts in both perpendicular directions in the detector plane were calculated with sub-pixel precision using the implemented in-house DIC algorithm. The resulting drift in both directions (hereby denoted as $x$ and $y$ for horizontal and vertical drift, respectively) is shown in figure 4.

It shows that spot movement in both directions is significantly different as the vertical movement converges after scatter during the initial sets of projections to a value in the region of units of pixels, whereas the horizontal movement gradually increases during the 20 sets of projections presented to its asymptotic value of approximately 40 pixels. The calculated characteristics of the spot movement were then used during the correction procedure, where the calculated spot movement at a given
time from the beginning of the spot movement evaluation procedure was applied in the opposite direction to the projection with the respective time from beginning of the tomographical scanning of the investigated sample. The corrected dataset was then used in the tomographic reconstruction. Figure 5 shows the reconstructed projection of the investigated specimen with the correction of the spot movement applied.

The figure shows that the applied correction procedure leads to the suppression of artefacts caused by the tiling of the LAD detector and also the ring artefact, which can be seen on the left side of figure 6 (see interior of the reconstructed scaffold at the bottom of the image) depicting the reconstructed volumes. The individual clusters of fibrin fibers are clearly apparent after the correction, and the achieved spatial resolution enables the study of the microstructure of the scaffold on a micrometric scale and the use of the reconstructed volumes (see figure 6) for quantitative microstructural analyses.

4 Conclusions

We performed a high-resolution micro-CT of a cross-linked fibrin-based scaffold with a very fine microstructure using a proprietarily developed tomographical scanner equipped with an large-area single photon counting detector. Due to the combination of high resolution and relatively low energies for the imaging of such very low attenuating organic material, a long-time CT scan was necessary in order to maintain a reasonable size of the spot. During the scanning, we encountered the sample projection drift effect caused by thermal expansion and other drifts of the tube, which resulted in strong artefacts in the reconstructed volume. The in-house developed correction procedure based on the experimental evaluation of the drifts on the detector plane using the digital image correlation procedure with sub-pixel resolution was used for compensation of the sample projection drift effect.
Figure 6. Reconstructed volumes of fibrin scaffold based on drift uncorrected (left) and corrected (right) dataset.

Furthermore, compensation for the detector-specific effects leading to artefacts from the tiling of the detector was carried out, including tilting of the detector which significantly improved the quality of the reconstruction. The volume reconstructed from the dataset with all the correction procedures applied allowed the identification of clusters of fibrin fibers in the scaffold microstructure thanks to the 1 µm voxel size achieved. Such a reconstructed volume can be then used for further various analyses, including advanced microstructural characterization methods, digital volume correlation of time-lapse experiments, and inverse finite element simulations of mechanical properties and permeability characteristics using a computational flow dynamics approach.

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