TOMOSAXS: MULTIMODAL VOLUMETRIC ANALYSIS OF COLLAGEN NANO-TO-MICROSTRUCTURE

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Introduction

We here present the results of initial validation studies and proof-of-concept for the "TomoSAXS" multimodal X-ray analysis technique for characterization of collagenous tissues. Collagenous structures are key constituents in a range of biomechanically important soft tissues, and form a hierarchy ranging in size from interlocking fibrils (nm), to collagen fibres (µm), and lamellae (10's-100's µm). A range of X-ray probing and imaging techniques exist to study the morphology and mechanics of these structures individually; Small Angle X-ray Scatter (SAXS) provides information on orientation and D-spacing between collagen fibrils [1], while microcomputed X-ray tomography (µCT) permits volumetric imaging of collagen fibres and lamellae [2]. TomoSAXS presents the first technique to correlatively employ these modalities, providing information on nm-µm structures with a volumetric context.

Following development of the TomoSAXS algorithm using digital phantom data, validation has been performed on collagenous tissues at a range of resolutions, upon samples of varying complexity. Analysis of tendon collagen using varying probing resolutions from laboratory and synchrotron X-ray sources provides proof-of-concept for the quantitative analysis of nm-to-µm scale characterization of collagen structures using TomoSAXS.

Materials and Methods

Unlike other volumetric SAXS techniques (e.g. SAXS tensor tomography [3]), TomoSAXS uses complimentary µCT data to provide information on collagen fibril orientation. This information is subsampled and centred to mirror the (coarser) SAXS voxel map, and orientation values saved as separate matrices comprising azimuthal and lateral angles (respectively). These matrices are used to provide information for 3D collagen diffraction models [4] to simulate meridional SAXS peaks from interaction between X-ray beams and collagenous structures of discrete 3D orientations. These models are employed across a simulation of the TomoSAXS scan (SAXS maps sampled at differing angular orientations), providing estimates of per-voxel occupation of "χ-space" and overlap.

Voxel interactions are analysed in a cascading sequence, starting with those with the highest proportion of independent χ-space. The measured fibril D-period (Q position of respective peak in 1D azimuthal integration plots) and fibril morphology (fibril thickness inversely related to the ratio between χ-space/Q-space occupation) from 2D SAXS maps sampled at n angles are used to deconvolute signals from overlapping interactions until a 3D estimate is provided for every scanned voxel.

This methodology (developed in the Python [3.8] environment and validated upon digital phantoms of known per-voxel D-period and orientation) was first employed to study eight chicken foot tendon samples cured under differing tare loads and arranged in differing orientations, probed using DL-SAXS (Xenocs Xeuss 3.0; 450 µm^3 voxel size) and µCT (Phoenix Tomo X;14.5 µm^3 voxel size) (Fig.). These same samples, alongside 12 additional samples, were further characterized at higher resolutions at the I22 (SAXS; 20 µm^3 voxel size) and I13 (µCT; 1.63 µm^3 voxel size) beamlines at Diamond Light Source.

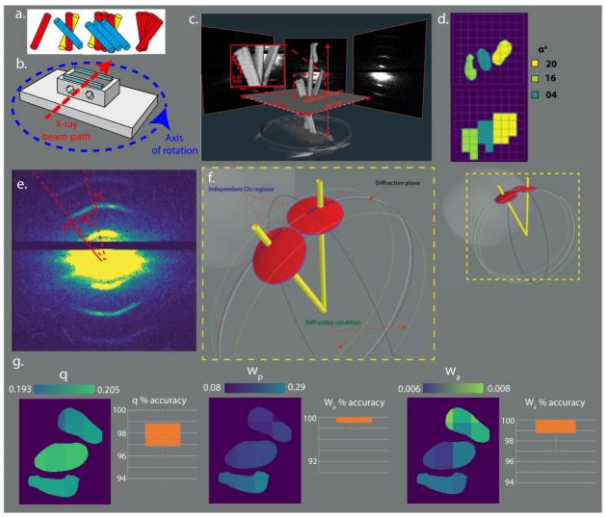

Figure 1. a-b) sample orientation and mounting. c-d) µCT data calibration. e-f) TomoSAXS simulation and modelling. g) Results of Q-parameter estimation for tendon samples (maps) and digital phantoms (graphs).

Results and discussion

Validation of the TomoSAXS algorithm using digital phantoms suggests it estimates D-period and fibril morphology to >95% accuracy. Estimation of these properties in chicken foot tendon samples reflect differing tare loads and are comparable between scans of differing resolution. These results highlight the potential of the TomoSAXS method for characterizing the interplay between nm-scale and µm-scale collagenous structures. This provides a new opportunity for assessing a host of biomechanical enquiries including the effects of ageing and disease.

References

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