Testing The Feasibility of Using Ptychographical Methods in Determining the Structure in the D-Banding From Rat-Tail Tendon Collagen.

Kris Robert Wallace
March 2009

1st Supervisor – Ian Robinson
2nd Supervisor – Franco Cacialli

Table of Contents

Acknowledgements

I would like to thank Ian Robinson for providing this project and supplying me a stipend through a UCL Studentship throughout summer 2008. This allowed me a greater amount of time to grasp the project. I would like to thank Franco Cacialli for agreeing to be my second supervisor, Richard Bean for matlab guidance, Cat McCallion for AFM images and Felisa Berenguer for many hours of helpful guidance.

Testing The Feasibility of Using Ptychographical Methods in Determining the Structure in the D-Banding From Rat-Tail Tendon Collagen.

K.R. Wallace

Department of Physics and Astronomy, University College London, Gower Street, London, WC1E 6BT

March 2009

Elastic scattering of X-rays can provide molecular structure and axial arrangement of collagen molecule in a fibril. The 67 nm D-banding distribution of electron density along a collagen fibril gives rise to X-ray scattering in the meridional direction. Inverse Fourier transformation allows the distribution of electron density to be determined from measurement of the intensities of the diffraction maxima in combination with the corresponding phase. Unfortunately, use of conventional X-ray scattering techniques inherently loses all phase information by the measurement of intensity. Here the feasibility of ptychography is studied which uses overlapping diffraction patterns measured only in intensity to solve for both amplitude and phase. A simple implementation of the method is modelled and demonstrated, showing how the algorithm uses overlapping data in real space to resolve ambiguities in the solution. Inputting real data into the algorithm shows promising results in both phase and amplitude reconstruction.

Introduction

The scattering of X-rays to determine the biological structure of collagen has occupied biophysicists for over half a century (Astbury, 1940)(Kratky & Serkora, 1943). Herzog and Jancke first examined it by means of X-ray scattering in 1920, where an indistinct fibre diagram was obtained.(Herzog & Jancke, 1920) In the 1940s Bear (Bear, 1942) was able to show that a periodicity corresponding to orders of 640A spacing could be detected using X-ray diffraction, which was in good agreement with electron microscope investigations carried out by Hall, Jakus & Schmitt. (Schmitt, Hall, & Jakus, 1942) The collagen fibrils examined by both groups were thoroughly dried in a vacuum so that comparisons could easily be made. Such a pattern is now known as the low angle meridional reflections.

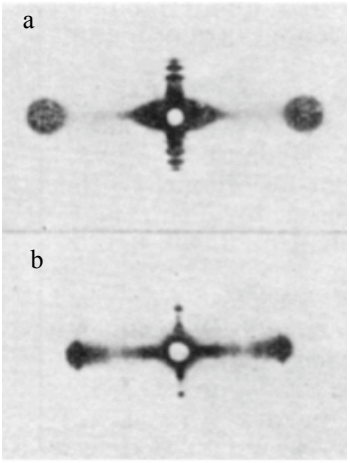

Figure 1: a) X-ray photograph of vacuum-dried rat tail tendon produced with nickel-filtered copper Kα radiation and b) the central region of an X-ray photograph of moist rat tail tendon collagen sealed in thin walled glass capillary tubes. Showing both equatorial and meridional reflections. (North, Cowan, & Randall, 1954)

Additional investigations showed that in the equatorial direction perpendicular to the tendon axis, there existed a set of sharp maxima, overlaying some diffuse scatter.(North, Cowan, & Randall, 1954) The presence of these sharp reflections indicated the existence of an ordered lateral packing of molecules in some fibrils. A number of models have been proposed for this lateral packing and the two which give the best agreement with observed data are the microfibril and the quasi-hexagonal model. (Miller & Wray, 1971) However the interest in this study is in reflections produced in the meridional direction to determine electron density parallel to the fibre and so equatorial reflections have been neglected.

In the 1950s collagen fibrils stained with heavy metal salts were shown to exhibit a characteristic-repeating pattern of ridges with alternating dark and light bands, each period D containing 10 dark bands. (Hoffman, 1952) This with other X-ray and electron microscope evidence suggested that the fibril might not be made up of one complete unit, but rather an aggregation of smaller parts of quite

definite size. (North, Cowan, & Randall, 1954) The uptake of stain which gives rise to the dark bands was recognised to be due to the presence of charged amino acids in this region. Hodge and Schmitt (Hodge & Schmitt, 1963) first established the relationship between the fibril pattern and the molecular pattern and later Hodge and Petruska (Hodge & Petruska, 1963) showed that a collagen molecule of length L, which was slightly less than 3000A extended over 4.4 periods in a fibril.

In the Hodge & Petruska model the molecular length is not an exact multiple of the D period. It was shown that a stagger between adjacent collagen molecules existed, which resulted in, a gap region and an overlap region in each D repeat.(Hodge & Petruska, 1963) Each period D, comprises an overlap zone of length 0.4D in which five molecular staining patterns contribute to the fibril pattern and a gap zone of 0.6D in which four molecular staining patterns contribute to the fibril pattern. (Chapman J. A., 1974)(Orgel, Wess, & Miller, 2000)

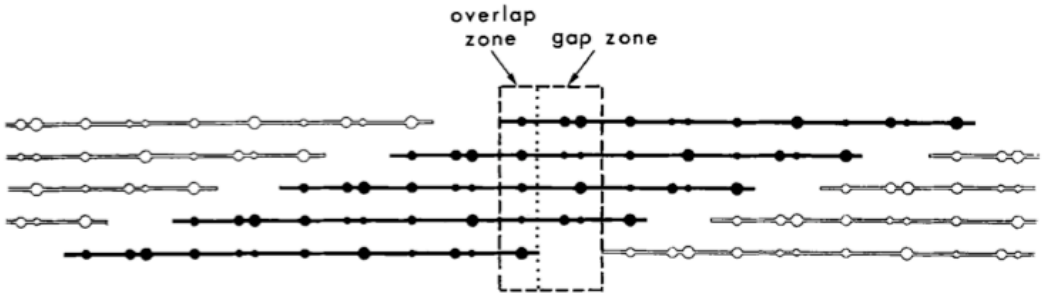

Figure 2: Schematic representation of the packing of collagen molecules in a staggered array in a native type collagen fibril, showing the division of the period D into overlap and gap zones. This shows the overlap zone contributing five molecular microfibrils and the gap zone contributing four. (Chapman J. A., 1974)

Furthermore, measurement of the breadth of the Bragg reflections indicated that only approximately half of the unit cell was contributing to this as a coherent unit. This indicated that the overlap region was the main contributor to the Bragg peak sampling and that the less electron dense gap region does not contribute significantly due to the increased lateral molecular motion. (Fraser, MacRae, Miller, & Suzuki, 1983) The existence of the gap and overlap regions is the major low resolution feature of collagen fibrils and dominates both the image of negatively stained electron micrographs and the low resolution X-ray pattern.

X-rays scattered through large angles, arbitrarily greater than two degrees, give rise to the wide angle pattern (WAXS). At smaller scattering angles, the small angle pattern is recorded (SAXS). Both patterns are present in the overall X-ray scatter and in practice small angle patterns are recorded with a specimen-detector distance of several meters and the wide angle with a specimen-detector distance of several tens of centimeters. Investigations involving the wide angle pattern occur when the concentration of collagen fibrils is low or their orientation is poor. (Aspden & Hukins, 1979). However in this investigation, the rat tail tendon is well orientated and consists of a high density of collagen fibrils and thus will be examined using small angle X-ray scattering.

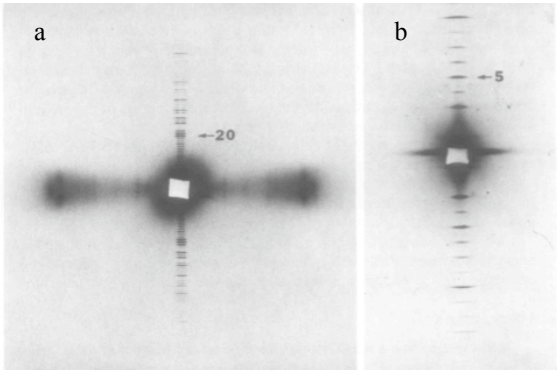

Figure 3: X-ray diffraction of rat tail tendon immersed in buffered saline and slightly stretched a) wide angle scatter showing diffuse equatorial reflections and b) the low-angle scatter where lower orders of reflection can be seen with the absence of equatorial reflections. (Brodsky & Eikenberry, 1980)

The precise nature of the packing of collagen in tissues such as tendon have been widely investigated. X-ray diffraction of rat-tail tendon reveals closely spaced Bragg peaks as a series of parallel lines in the

direction of the meridian. This indicated that tendons are composed of crystalline molecules of type I collagen.(North, Cowan, & Randall, 1954) Collagen from tendon has the added advantage of containing well-orientated fibrils. (Brodsky & Eikenberry, 1980) It is these properties that make this tissue an excellent specimen for the study of collagen structure, especially by X-ray diffraction.

Micrographs of shadowed fibrils show that the density maxima (the bands) represent small elevations of the fibril profile, but it will be assumed that the diffraction effects due to the varying radius may be neglected in comparison with those due to variations in fibril density. (Burge & Randall, 1955) The subsequent determination of the cell parameters passed through a number of stages as data interpretation and data quality improved and has resulted in the currently wide held view that the axial packing of collagen molecules involves the staggered nature of neighbouring molecules by integer multiples of 670A.

Diffraction has the distinct advantage in comparison with electron microscopy in that it may be carried out on intact, hydrated specimens. The experimental arrangement for X-ray diffraction, using either an enclosed cell or a sealed capillary tube, permits the specimen to be kept continuously hydrated by contact with water vapor or by immersion in a buffer.(Brodsky & Eikenberry, 1980)

Analyzing dried collagen samples including parchment using X-ray diffraction has shown that the sharp interface between the gap and overlap regions is less apparent. The drying of the collagen fibres in a vacuum has the effect of removing water from the individual collagen molecules. This brings about a degree of tilting in portions of the collagen chains, most likely in the centre of the gap region where molecular shear may occur after molecular collapse brought about by dehydration. The D-period of collagen is reduced which indicates that a molecular level contraction of the staggered array. (Kennedy & Wess, 2003) Thus the D-period in type I collagen is reduced from the widely accepted 67nm to ~64nm, which is consistent with initial observations of the periodicity first observed in the 1940s.

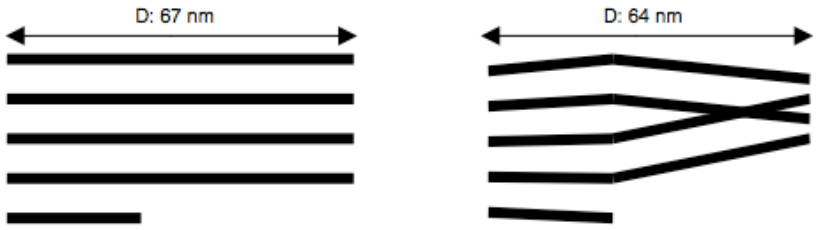

Figure 4: Illustration of the effect of dehydration on the D periodic stagger of collagen fibrils. Upon dehydration the areas of the molecule in the gap and overlap regions undergo tilting. This has the effect of shortening the length of the D period. (Kennedy & Wess, 2003)

Muscles, collagen and membranes belong to a group of biological macromolecules that are insoluble in water. These molecules exhibit a pronounced tendency to structure in one or two dimensions. There are over twenty different types of collagen molecules known to exist. Collagen molecules from types I, II, III, V and XI are capable of self-assembling to form fibrils.(Hulmes, 1992) These have been characterized biochemically and each type of tissue is found to have a characteristic composition of collagen fibres. The alignment of the fibres is a key factor in the overall mechanical nature of the tissue. The arrangement of collagen molecules into fibrils is characteristic of the hierarchical structure that is key to its success as a biomaterial. At the structural level collagen fibres exist which are composed of fibrils. The fibrils are made up of collagen molecules which in turn are made of individual peptide chains.(Kennedy & Wess, 2003)

Each lamella of the rat tail tendon may be reasonably approximated as an array of parallel cylinders of uniform size. The *jth* cylinder is displaced from an (arbitrary) origin by a position vector $\boldsymbol{r}_j$. If a plane wave is incident on the cylinders perpendicular to their axes, the field observed at some distance z, is a superposition of the waves scattered from all the cylinders. The diffraction pattern from an assemblage of collagen fibrils in a tissue is simply the sum of the intensities scattered by each individual fibril. The multiple copies of the collagen fibrils amplify the signal and their regular arrangement concentrates the far-field diffraction pattern into Bragg disks.(Brodsky & Eikenberry, 1980) Inter-fibril interference may be ignored.(Aspden & Hukins, 1979)

When illuminated by a beam of X-rays, each point in the collagen sample experiences an oscillating electric field. This induces a dipole moment whose size depends on the square of the refractive index at that point. Each oscillating dipole then re-radiates electromagnetic radiation in all directions. Although