Experimental Report

Aims and background information

To examine the structure and organization of human corneal collagen as a function of tissue depth and to see how this changes between the cornea and the limbus. Our long-term aim is to build a 3-D model of corneal structure that will explain how the cornea maintains its precise shape, and what are the causes of shape changes following surgery.

Methodology

Healthy human corneas were obtained from Eye Banks in the UK and USA. Strips of tissue extending from limbus to limbus were cut from central parts of the cornea and blocks of tissue were cut from different sites across the limbus. All these were sectioned into 100micron slices from anterior to posterior. The sections were scanned in two dimensions at Station ID-13, ESRF.

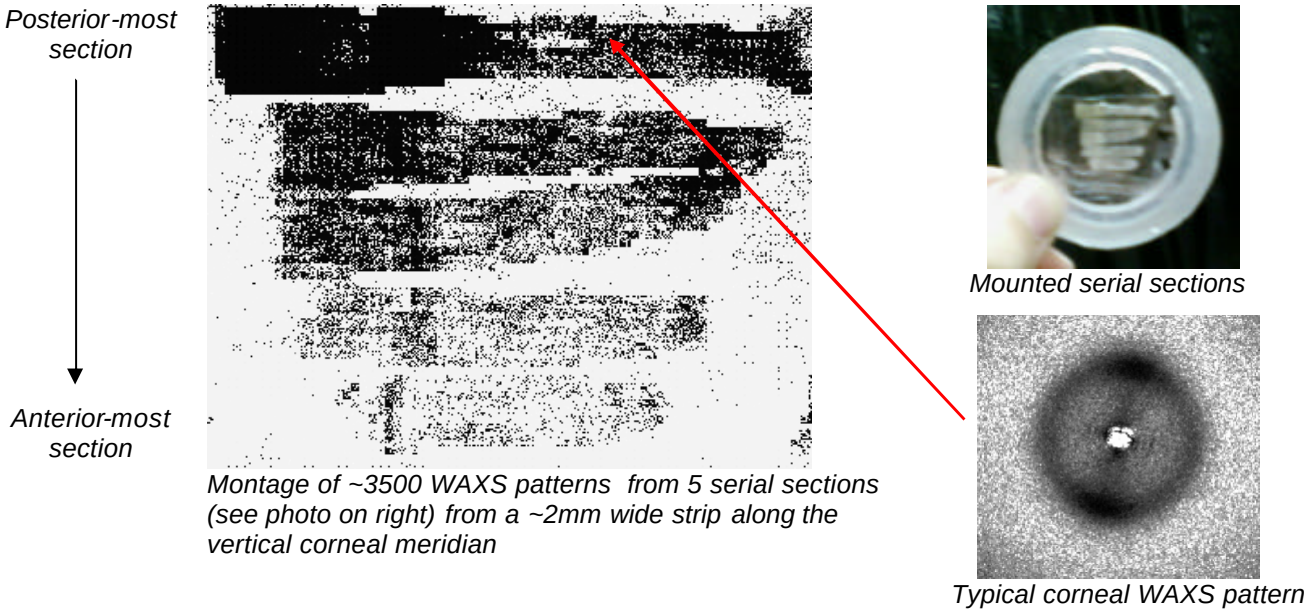
Whole corneas were mounted in air-tight chambers and using the same experimental set-up described above, microfocus x-ray scattering data were recorded at 25μm intervals along each of the 8 principal corneal meridians, in order to examine in detail the integration of the predominantly orthogonal collagen in the cornea, with the tangential/annular collagen at the limbus.

The remaining cornea was used to study the molecular structure of collagen as a function of tissue depth by cutting a thin vertical strip (≈1mm) and mounting it such that the beam crossed it edge-on. The microfocus beam allowed ~30 images to be taken from the front (epithelial) to the back (endothelial) surface of the cornea at 25μm intervals. When analysed, the data will allow us to determine the intermolecular spacing of the collagen as a function of depth.

Results

Our previous visit to ID13 was not successful as far as data collection is concerned, but did allow us to work out what experimental set-up was required for subsequent trips. However, the wide-angle camera provided during the current trip yielded excellent results which, because a key team member went on Maternity leave, are still being analysed. Although we have not fully analysed all the data, the following preliminary results were obtained:

1. Mapping sections cut from different depths in the cornea



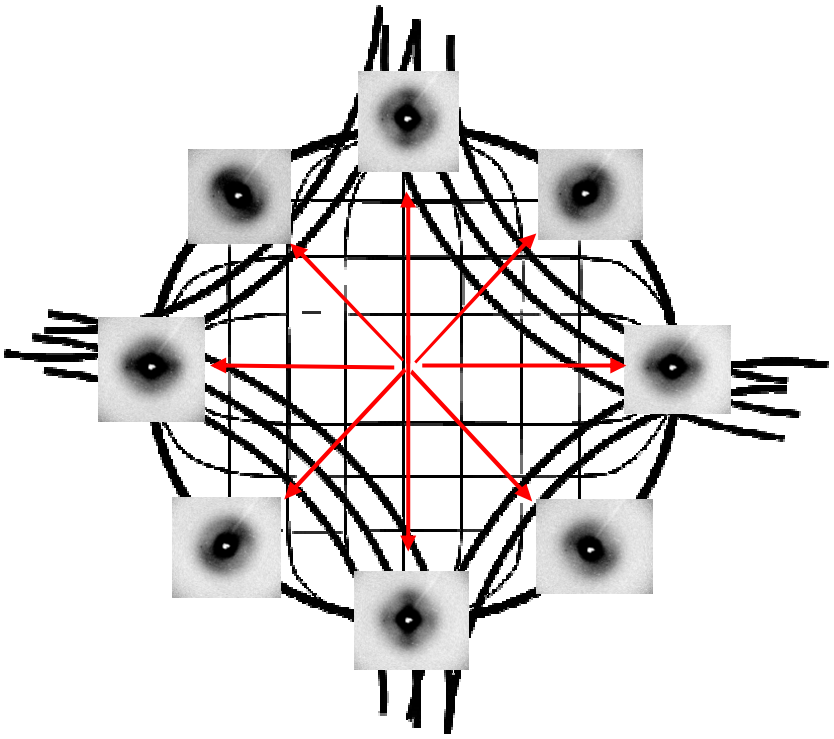
Montage of ~3500 WAXS patterns from 5 serial sections (see photo on right) from a ~2mm wide strip along the vertical corneal meridian

Mounted serial sections

Typical corneal WAXS pattern

We will use our established WAXS analysis protocol to determine dominant collagen orientations throughout the sections. By analyzing the azimuthal distribution of intensity of the principal equatorial WAXS reflection (intermolecular collagen reflection), we will be able to quantify the relative number of collagen molecules lying in each direction within the corneal plane and thereby determine preferred directions of stromal collagen lamellae.

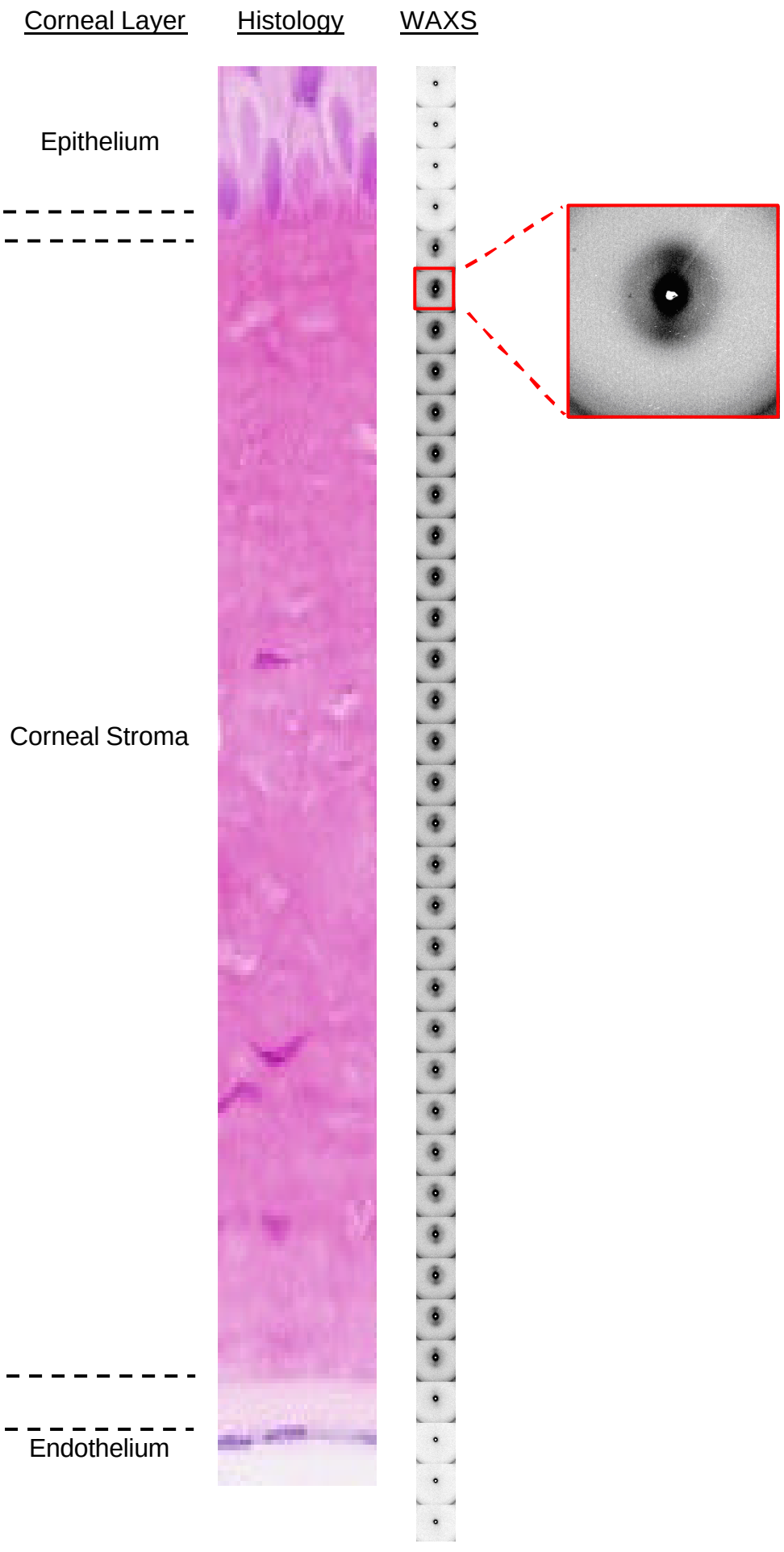
2. Collagen orientation along the principal meridians



Model of principal, depth-averaged, collagen orientations in the human cornea (black lines). Microfocus WAXS data was collected at 25μm intervals along 8 corneal meridians (red arrows). Limbal (radius = 5mm) WAXS patterns are shown.

Analysis of WAXS patterns using an identical protocol to that for serial sections will enable us to quantify preferred fibril orientations in the interesting transition region between the orthogonal collagen of the cornea and the tangential/annular collagen of the limbus.

3. Intermolecular spacing as a function of depth



WAXS images taken from the front (epithelial) to the back (endothelial) surface of the cornea at 25μm intervals, showing variation in preferred collagen orientation as a function of depth.

When the data is further analysed we will calculate the average collagen intermolecular spacing at each sampled depth in the stroma from the radial position of the principal equatorial corneal WAXS reflection, using the 0.304nm reflection from powdered calcite as a calibrant,.