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ESRF

Experiment Report Form

The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

http://193.49.43.2:8080/smis/servlet/UserUtils?start

Reports supporting requests for additional beam time

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

Reports on experiments relating to long term projects

Proposers awarded beam time for a long term project are required to submit an interim report at the end of each year, irrespective of the number of shifts of beam time they have used.

Published papers

All users must give proper credit to ESRF staff members and proper mention to ESRF facilities which were essential for the results described in any ensuing publication. Further, they are obliged to send to the Joint ESRF/ ILL library the complete reference and the abstract of all papers appearing in print, and resulting from the use of the ESRF.

Should you wish to make more general comments on the experiment, please note them on the User Evaluation Form, and send both the Report and the Evaluation Form to the User Office.

Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

Instructions for preparing your Report

? fill in a separate form for each project or series of measurements.

? type your report, in English.

? include the reference number of the proposal to which the report refers.

? make sure that the text, tables and figures fit into the space available.

? if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

ESRF	**Experiment title:** Relating ultrastructure to shape in normal and pathological human cornea	**Experiment number:** SC-2583
Beamline:	**Date of experiment**: from: 29/10/2008 to: 01/11/2008	**Date of report**: January 2009
Shifts: 9	**Local contact(s)**: Dr. Manfred Burghammer	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Prof. Keith M. Meek (Main applicant), Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

Dr. Craig Boote (Co-applicant), Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

Dr. Christina S. Kamma (Co-applicant), Structural Biophysics Research Group, School of Optometry and Vision Sciences, Cardiff University, Cardiff, UK

Report:

Aims and background information:

- To examine collagen orientation in the periphery of the human cornea as a function of the tissue depth and subsequently build a 3-D model of corneal structure that will contribute to the formation of biomechanical models aimed at predicting the outcome of corneal surgical procedures and hence reducing the risk of post-surgery complications.
- To investigate short term collagen orientation changes in the cornea after a severe case of wounding and assess their effect on the biomechanics and stability of the tissue.
- To measure collagen intermolecular spacing changes after UVA/Riboflavin treatment, a popular procedure used for the treatment of keratoconus and to compare it to other cross-linking agents.

Methodology:

Two human eye-bank corneas provided by Bristol Eye Bank, UK were examined. Serial sections taken from the periphery of the cornea at 100μm intervals, parallel to the surface and ranging from the anterior towards the posterior part of the tissue were scanned in two dimensions at Station ID-13, ESRF. For this mapping, an X-ray 8μm microfocus beam was used and scattering patterns were recorded at 200μm intervals for each section.

Bovine corneas were severely injured using a 5mm biopsy punch and left to heal in culture for up to one week. Whole corneas were mounted in air-tight chambers and, using the same experimental set-up described above,

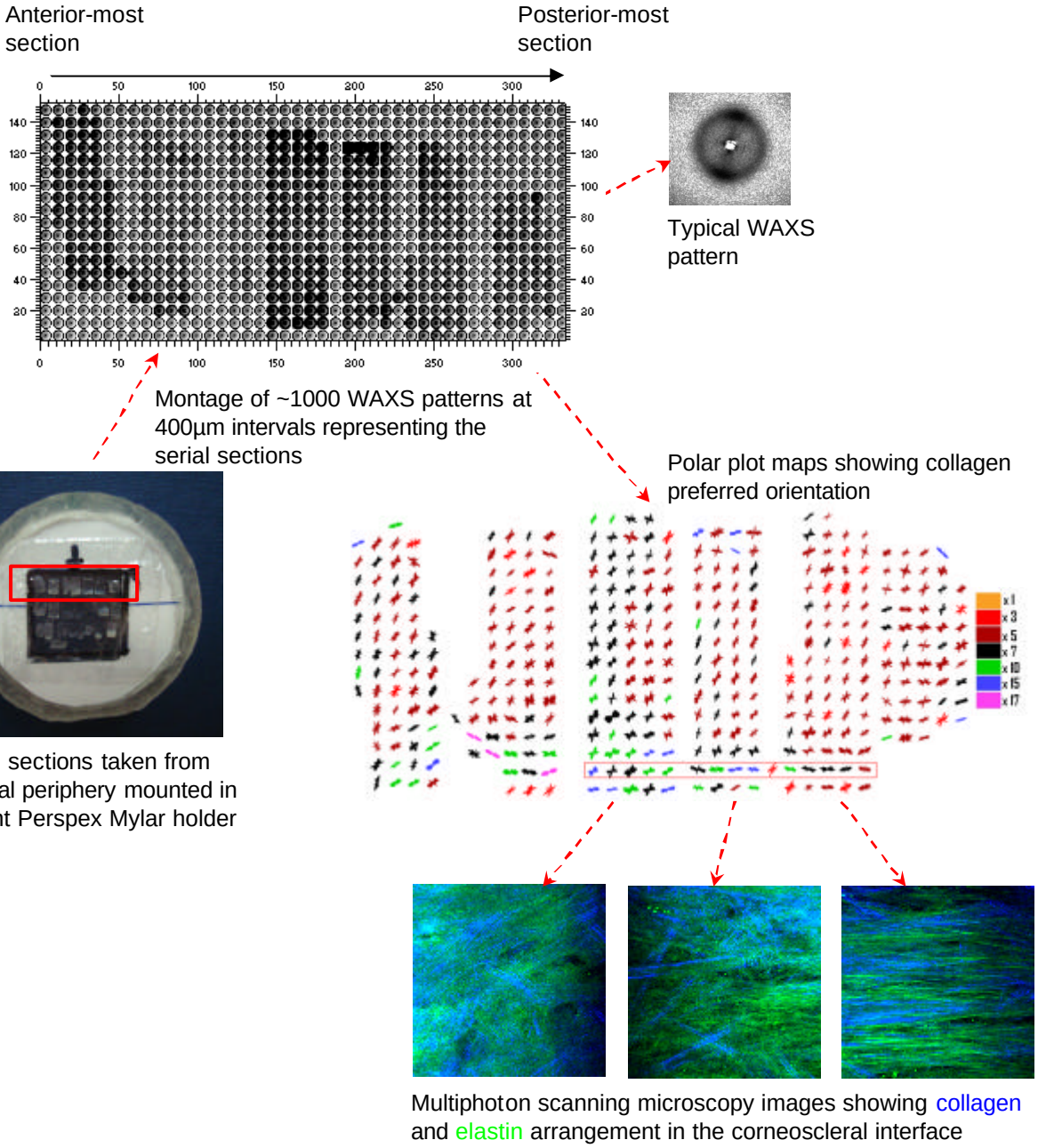
experimental X-ray data was collected at 150, 300, 600 and 1000µm intervals in a central cornea area of 7.3x7.3mm that included the 5mm wound.

Normal and keratoconus buttons were initially scanned. Immediatelly after both groups received treatment with UVA/Riboflavin and were rescanned.

Results:

1. Collagen preferred orientation throughout different depths in peripheral human cornea

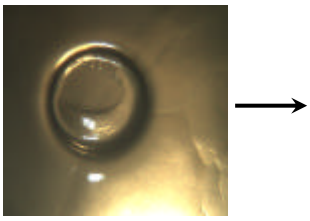
The wide angle camera provided during the current trip yielded excellent results for the collagen orientation studies. The additional samples that we scanned for the 3-D corneal study gave us the oportunity to complete the existing set of data that we obtained in our previous trip (SC-2246). Additionally, using confocal microscopy (second harmonic generation and two photon fluorescence) we were able to image regions of interest highlighted by the XRD data. We are ready to submit a publication to a scientific journal in the immediate future; as soon as the last parts of the X-ray data analysis are finalised. Part of these results have already been presented at major international ophthalmology conferences such as the International Society for Eye Research (September 2008, Beijing, China) and the European Association for Vision and Eye Research (October 2008, Portoroz, Slovenia). In addition, the complete data will be presented at the annual meeting of the Association of Research in Vision and Ophthalmology (May 2009, Fort Lauderdale, Florida USA).



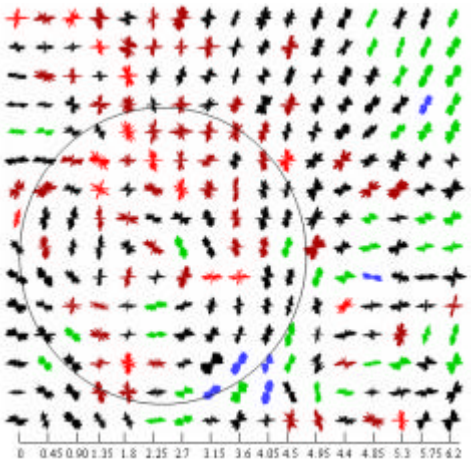
Frozen sections from the limbus (the edge of the cornea) were cut from anterior to posterior, mounted in a perspex holder, and scanned. By analyzing the azimuthal distribution of intensity of the principal equatorial WAXS reflection (intermolecular collagen reflection), we were able to quantify the relative number of collagen molecules lying in each direction within the corneal plane and thereby determine preferred directions of stromal collagen lamellae. In addition, by using multiphoton scanning microscopy we visualised collagen and elastin arrangment in the regions that WAXS showed the strongest collagen allignment. The circumcorneal collagen structure, which is thought to be implicated in the maintenance of corneal shape and curvature, resides in the mid-posterior part of the tissue. With increasing proximity to the anterior surface of the cornea collagen arrangement becomes more isotropic.

2. Collagen orientation changes in the cornea during the healing process.

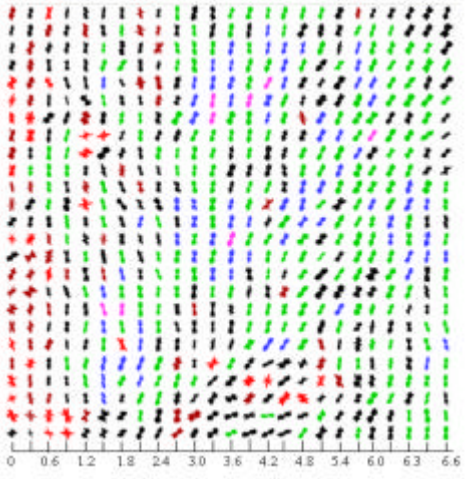
Using the same analysis methods as described aboved we investigated short term collagen orientation changes after severe injury. Polar plot maps show collagen preferred orientation in a centrally located area from a trephine wounded cornea and from a control/uninjured cornea after 1 week in culture. Collagen in normal/uninjured bovine corneas essentially has a uniaxial arrangement. Collagen fibrils in the trephine wounded cornea form a radial arrangement both outside and inside the wound area after the first week in culture. This is an indication of wound contraction, an essential part of the wound healing process. Changes in collagen orientation can be related to changes in the biomechanical stability of the tissue after injury or refractive surgery. These results have already been submitted to Molecular Vision and we are currently awaiting the reviewers' comments (please find abstract attached at the end of this report).



Focused image showing a 5mm wound in a bovine cornea



Polar plot maps at 450µm intervals showing collagen preferred orientation changes 1 week after injury in a 5mm wound in a bovine cornea.



Polar plot maps at 0.3µm intervals showing collagen preferred orientation in a normal/uninjured bovine cornea after 1 week in culture.

Note: Numerical scale in mm - circle in last picture indicates approximate position of the 5mm wound.

3. Corneal crosslinking

For the UVA/Riboflavin experiments this trip to the ESRF was not successful as far as data collection is concerned, but did allow us to identify the correct experimental protocols, and we now intend to apply for extra beamtime so we can repeat and extend these experiments.

Abstract of the paper that was submitted in Molecular Vision:

Effects on collagen orientation in the cornea after trephine injury

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Purpose: Structural changes are well known to occur in the cornea after injury. The aim of the study was to investigate collagen orientation changes in the cornea during a short term wound healing process.
Methods: Seven bovine corneas were injured using a penetrating 5mm biopsy punch and organ cultured for up to 2 weeks. Six uninjured corneas acted as controls. The trephine wounded samples were removed from culture at 0, 1 and 2 weeks and snap frozen in liquid nitrogen. Control/uninjured samples were also snap frozen at 0hrs or 1 week. Wide angle x-ray diffraction data were collected from each cornea at the UK Synchrotron Radiation Source (Daresbury, Warrington, UK) or at the European Synchrotron Radiation Facility (ESRF, Grenoble, France). Data analysis revealed information about collagen orientation and distribution in the corneal stroma during wound healing. For histology, two trephine wounded corneas at 0hrs and 1 week and one control/uninjured cornea at 0hrs were fixed in 10% neutral buffered formalin and processed for wax embedding. Wax sections were subsequently counterstained with Haematoxylin and Eosin to observe tissue morphology and the time course of complete re-epithelialisation.
Results: Immediately after injury, collagen organisation was altered in a small area inside the wound, but remained similar to the control/uninjured sample in the remainder of the tissue. After 1 week, the trephine-wounded corneas showed complete re-epithelialisation and evidence of swelling, while collagen adopted a radial arrangement inside and outside the wound.
Conclusions: Remarkable changes in collagen fibril orientation were observed in trephine wounded corneas. Orientation changes immediately after wounding are likely to be due to the mechanical deformation of the tissue during the wounding process. However, tissue swelling and changes in collagen orientation at later stages probably reflect the processes of tissue repair. These differences will determine corneal stability and strength following trauma and, possibly, refractive surgery.