Response to P. Suortti *et al.*'s and K. D. Rogers *et al.*'s *Comments on Synchrotron fibre diffraction identifies and locates foetal collagenous breast tissue associated with breast carcinoma by V. J. James (2002). J. Synchrotron Rad. 9, 71–76*

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1. Addressing the comments of Suortti *et al.*

(i) While I agree with Suortti *et al.* that the ring resulting from fat is found in this region of the diffraction pattern, Fig. 1 shows clearly the presence of sharp rings emanating from the wider 'fat' arcs here only present in the equatorial direction. A plot of intensities in the equatorial direction of a different sample shows that there is more than one reflection in the region of interest (Fig. 2). Furthermore, the spacings for these sharp rings as given in my paper are not the spacings determined from one ring but rather, as stated clearly in my paper, they are the spacings calculated from the series of reflections observed for each of the samples which for some locations showed no fat at all. (The histopathology slides in Figs. 3 and 4 here confirm that there is no fat present in the flat areas surrounding the cancer. Fat is visible as the gold areas in the slides under a polarizing microscope.) Up to four orders were recorded for some samples and three for many others. The spacings were calculated from data obtained at the Photon Factory BL15A and verified by examining the same samples and further sets at different beamlines. Variations were also noted in the orders recorded for the individual samples depending on the condition of samples. Both the condition of the samples and the beam condition must be optimized to obtain such diffraction patterns. For this reason, I excised ducts for these samples. Since the fibrillar collagen is aligned along the ducts, the quality of the images was much improved when compared with earlier samples taken from other sections of the breast or from random biopsy material. We found that a certain deterioration occurred to some samples when stored in liquid nitrogen as were the samples studied in the report of Fernández *et al.* (2002). Such samples still gave a pattern but the patterns were not as sharp. All samples used in our experiments were kept at 100% humidity for the duration of the experiment and stored in phosphate buffered saline between excision and the X-ray exposures. Tests on methods of storage confirmed that these storage and experimental procedures gave the best results for all collagen samples. The focusing of the three beamlines used for my experiment is excellent, the backgrounds very low and the wide dynamic range of the imaging plates allow the measurement simultaneously of very weak and very strong reflections. In our comparative studies (still being monitored at least twice yearly) we have found that these plates have advantages over CCD detectors, the first of which was actually built in my laboratory (Dalglish *et al.*, 1984).

(ii) The figures actually used on the right-hand side of Fig. 2 of the paper under discussion are in fact from different samples from those published earlier. Such plots are made routinely for all samples being analysed. These were added to the paper on the suggestion of the referee to indicate the origin of the results. The first plot does indeed show the Bessel functions. The fine structure does exist. It is not, as Suortti *et al.* suggest, SAXS noise. The method of verification of this was given in great detail in the paper by James *et al.* (1993). We have continued to observe orders of this lattice in all two-dimensional collagen tissues and also in much better oriented one-dimensional and two-dimensional tissues which are predominately collagen type IV (unpublished results). This lattice is never observed in tendon, neither in human tendon from persons over 3 months nor from animals, even when placed in the same cell as the two-dimensional tissue and examined sequentially. Both the samples and the results must be of the highest quality to observe this lattice. I have not had the opportunity to work at ESRF so cannot comment on whether or not such a lattice can be obtained there, given samples are of the correct quality, but I have not been able to obtain data of this quality at Daresbury during my two visits there whether because of the conditions of the samples used or the beamline set-up at that time.

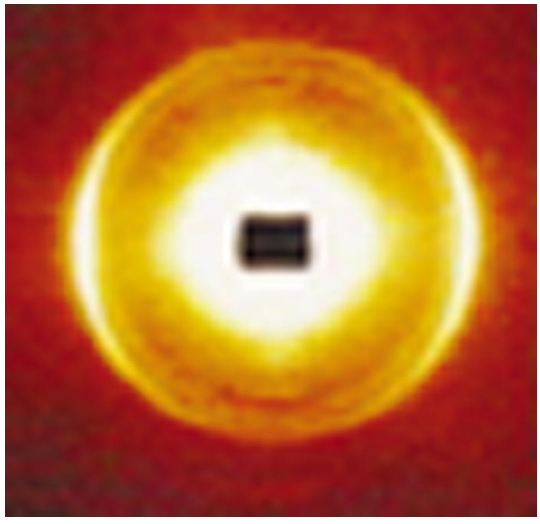

Figure 1
A diffraction pattern of ductal tissue showing the remnant of a fat ring in the equatorial direction with a fine breast cancer related ring emanating from it.

2. Addressing the comments of Rogers *et al.*

(i) While I am not conversant with the present UK terminology, the paper under discussion was shown to histopathologists and medical doctors both in Australia and the USA and none had any problem with the wording of this section of the paper. In this answer I will use the term 'elastotic' for the very dense tissue to which I refer in the paper. It is indeed hyalinized collagen. Two slides taken from results

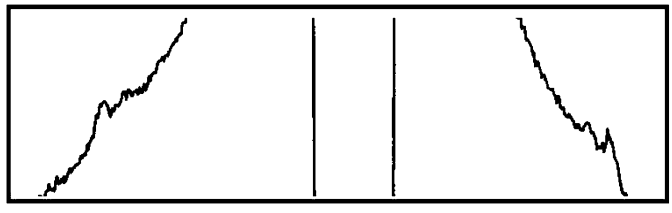

Figure 2
This graphical section of the intensity *versus* equatorial position of ductal collagen shows two sharp peaks on top of the broad 'fat' peak.

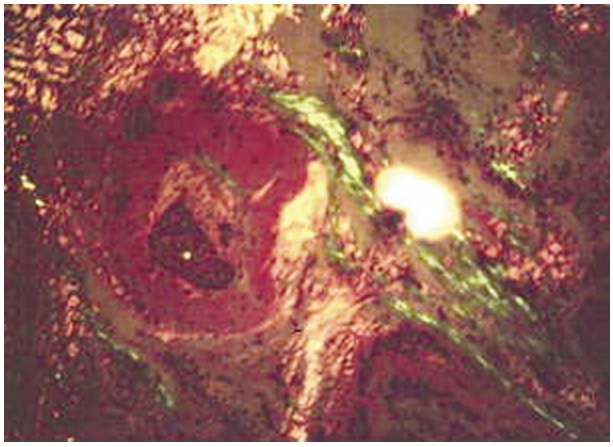

Figure 3
This pathology slide of a frozen section of breast cancer tissue viewed under cross polarization shows the large tumour (green-coloured) surrounded by elastotic areas where there is no fat (gold-coloured).

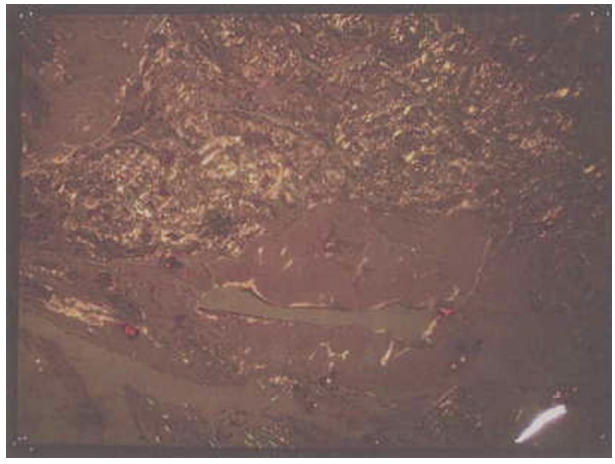

Figure 4
The very small tumour (bright white) surrounded by vast areas of elastotic tissue in this pathology slide of a frozen section of breast confirms that the collagen changes precede the proliferation of the tumour.

obtained when I was studying the pathology of breast tissue are given here in Figs. 3 and 4. Fig. 3 shows that the ductal breast cancer within the duct (green colour) is surrounded by the 'elastotic' tissue. When this study was in its infancy there was still some controversy as to whether the 'elastotic' tissue preceeded the cancer or *vice versa*. A large number of reports would indicate the former because of the histopathology slides of very small tumours. Fig. 4 shows the histopathology slide from a woman who presented with a palpable lump. Subsequent minor surgery revealed that the lump was a calcified duct and the woman was sent home. However, when the frozen section was further examined by histopatholgy, extensive 'elastotic' tissue was revealed. On taking further sections the slide pictured in Fig. 4 was obtained showing the complete very tiny cancer embedded in the 'elastotic' tissue (white). It was carefully explained to me when I was studying this pathology that the sign of such a change should be investigated carefully to avoid the possibility of missing a tumour. This still applies even if the terminology has changed.

(ii) It is certainly possible to discern this changed collagen with a hyperdermic needle.

(iii) This point has been answered above in §1(ii).

(iv) What is more important than the size of the sample is the sample itself and the ordering of the collagen. Different beamlines were used with totally different focusing techniques. Details of these are given in earlier papers. The methods for obtaining peak positions are given in this paper. All three methods were used for each sample. Three different persons in my laboratory were each given the data. Working quite independently, each person used a different method to locate the peaks. The three individual sets of results were then handed to me for the final analysis. The similarity of the results was very convincing. The blind statement of Rogers *et al.* that second-order derivatives and boxcar techniques are not appropriate is ridiculous. It is certainly not true of good data sets but perhaps indicates that this stems from considerations of inferior data. The advantages of this technique have been discussed in detail by Nie *et al.* (2002). With the excellent background of the pictures we obtained, both of these techniques work very well. Boxcars of various sizes and shapes were used for different areas of the pattern, depending on the pattern being studied. I would question that this comment was made from lack of experience with the use of boxcars. It is certainly provided for use in most data-analyzing programs. Second-order derivatives are extremely useful for locating peaks if used on good quality data. It is also routinely used in ascertaining true peak positions in other fields such as infrared, when peaks are overlapping or poorly defined in otherwise good data. An illustration of the use of boxcars and double derivative in determining peaks was given in James *et al.* (1996).

(v) This point has been answered in §1(ii) above.

(vi) Changes in the Bessel function 'equatorial spots' implies changes in radii. A full discussion of these reflections was given by Eikenberry *et al.* (1982).

(vii) This point has been discussed in §1(i) above.

References

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