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Application of small angle X-ray scattering (SAXS) for differentiation between normal and cancerous breast tissues

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ABSTRACT

Background: Small angle X-ray scattering (to angles less than 10°) is predominantly coherent. Coherent scattering leads to diffraction effects and especially constructive interferences. These interferences carry some information about the molecular structure of the tissue. As breast cancer is the most widespread cancer in women, this project evaluated the application of small angle X-ray scattering (SAXS) for differentiation between normal and cancerous breast tissues.

Materials and Methods: The energy dispersive method with a set up including X-ray tube, primary collimator, sample holder, secondary collimator and HP Ge detector was used. The best constructive interference was found to be at 6.5° after doing experiments on adipose breast tissue at several angles of 4, 5, 6, 6.5 and 7.3 degrees. The total number of 99 breast tissue samples, including normal and tumor were studied at the 6.5°. The corrected intensity versus momentum transfer was obtained for each sample.

Results: Adipose tissue shows a sharp peak in low momentum transfer region. It is easy to separate adipose tissue and mixed tissue (adipose & fibroglandular) from tumor in peak positions (each coherent scattering spectrum has a peak that its position is determined by momentum transfer). Furthermore adipose tissue has shown significantly higher peaks than other breast tissues. Benign and malignant breast tissues were differentiated by both peak positions and peak heights (each peak has a height in coherent scattering spectrum). Preservation of samples nitrogen tank had no effects on molecular structure of the breast tissue.

Conclusion: By energy dispersive small angle X-ray scattering, it is possible to differentiate between normal, benign and malignant breast tissues. *Iran. J. Radiat. Res., 2005; 2 (4): 205-210*

Keywords: *Small angle X-ray scattering, breast tissues, normal, cancerous.*

INTRODUCTION

Breast cancer is the most widespread cancer in women. The incidence rates are continuously increasing in many parts of the world, and in the industrial world the rate is close to about one in 12 women. It is a major cause of death in middle-aged women of 33-55 years (Parking *et al.* 1988). The need for early and more accurate diagnosis methods for breast cancer has been identified as a major factor that could help to save the lives of many women.

Scattering of X-rays at low angles is dominated by coherent (elastic) scattering process. There are interference effects due to the coherence phenomenon of scattered photons, mainly at small angles. This effect gives rise to a unique scattering signature characterized by each material. Thus, the dependence of coherent scattering on molecular composition of the target could

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potentially be used to characterize the tissue. Kosanetzky *et al.* (1987), presented diffraction patterns for some plastics and several biological samples. The characteristic nature of scattering was reported by Evans *et al.* (1991). The sensitivity of small angle X-ray scattering (SAXS) due to molecular level changes was also reported by Kidane *et al.* (1999). Malden *et al.* (2000), used CdZnTe detectors to collect energy dispersive diffraction spectra at a range of scatter angles, obtained from sheets of explosive materials hidden in baggage. It was shown that the combined information from these 'signatures' can be used to determine whether an explosive sample is present or not. By using SAXS, systematic differences in the intensities and D-spacing have been clearly demonstrated between the collagen of normal, malignant and benign breast tissues by Lewis *et al.* (2000). Fernandez *et al.* (2002), showed the average intensity of scattering from cancerous regions is an order of magnitude higher than the intensity from healthy regions. Desouky *et al*. (2002), presented low angle X-ray scattering profiles of five different spices, namely anise, coriander, cumin, fennel and nigella. These profiles showed characteristic peak position for each spice.

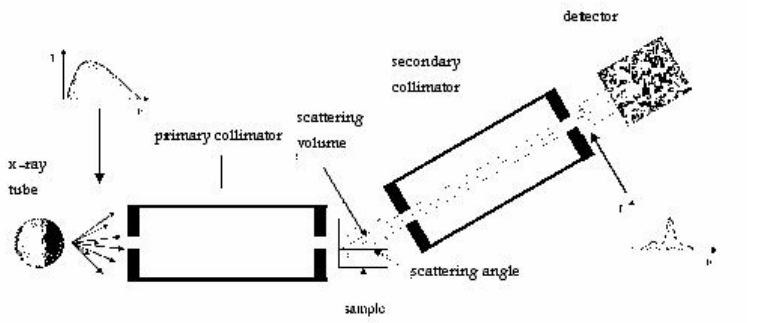

Figure 1. Schematic diagram of an EDXRD experiment. The x-ray spectrum, produced by the tube is modified by the structure of the sample so a spectrum unique to its crystalline structure is detected.

In order to evaluate the possible application of the small angle X-ray scattering in diagnostic setting, we compared the results of coherent X-ray scattering measurements obtained from normal, benign and malignant breast tissues.

MATERIALS AND METHODS

The X-ray diffraction measurements were made by using an energy dispersive X-ray diffraction (EDXRD) approach. In this approach diffraction patterns can be obtained by using polyenergetic photons at a certain scattering angle. Figure 1 shows a schematic diagram of the small angle X-ray scattering set up used in this study. The source is a tungsten target X-ray source capable of producing photons up to 60 keV. To obtain a pencil beam, we have used two collimators made of lead with 6 cm length and 1 mm hole. Primary collimator was attached to another collimator with 3 cm length inserted into the X-ray tube orifice close to its window. Secondary collimator was placed into a hole in the middle of a lead block in front of the detector center. A planar HPGe detector (model GEM, Ortec EG&G) was also used with energy resolution of 850 eV at 59.7 keV. In order to minimize the background several lead rings and lead bricks were placed around the detector. The output pulses were fed to a PC – based multichannel analyzer (92X Spectrum Master, EG&G Ortec). The multichannel analyzer (MCA) has three functions: to digitize the analogue information contained in each pulse, to process and store the data in memory section of the MCA (buffer), and finally to display the contents of the processed data in the form of counts against channel number.

The channel number was converted into energy of the scattered photons by performing a calibration procedure using an isotope of known energy (^{241}Am).

In order to obtain the scatter signatures, all the samples with 5 mm diameter and 5 mm height were positioned at the center of the scattering volume, defined by the collimation geometry. During the scattering measurements, the samples were rotated around the scattering center, by using a step motor. This ensured that

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all parts of tissue were exposed uniformly to X-ray.

The total exposure time was set to 500 seconds, which provided a good counting statistics for the measurements.

Due to limitation of the X-ray tube, 10 mA and 55 kVp were set for all exposures. The choice of the X-ray energy for a given scattering angle affects the range of measured momentum transfer.

In our setting, slit width of collimators was set to 1mm, object to detector distance to 170 mm, and X-ray tube focal spot to object distance at 150 mm. All system parts and sample holder were mounted on an isolated optical table. Sample holder was fixed between two collimators.

In order to obtain the optimum scattering angle, scatter angles between 4° to 8° were tested on adipose tissues. The best result found to be at angle of 6.5°. Therefore, all examinations were performed at 6.5° and at a temperature of $18^{\circ}C \pm 1^{\circ}C$.

Ninety samples were collected from women who underwent mastectomy procedures in Imam Khomeini hospital (Tehran, Iran). Following surgery, the samples were placed in a nitrogen tank and frozen at $-196^{\circ}C$ for storage prior to measurements. A piece of each sample and stored was sent for histological analysis. Each sample was brought into equilibrium at room temperature prior to measurement. These tissue samples were selected to be, 20 adipose tissues, 17 mixed (adipose plus fibroglandular) normal tissues, 41 carcinoma and 21 fibrocystic changes.

In order to investigate the coherent scattering signatures of breast tissue, all samples of normal and cancerous tissues needed to be irradiated in their fresh form, therefore, the tissues were transferred into a nitrogen freezer immediately after being collected from the original site, and stored at $-196^{\circ}C$. No fixative materials were added to the samples.

In this study, the effect of freezing was also investigated. First of all, scatter measurements were made on fresh adipose and cancerous samples about 1.5 hours after the collection. Then the samples were stored at $-196^{\circ}C$ for 2 months, and all the measurements were repeated. Figure 3 compares the measured signatures between the fresh and the frozen tissues. As shown in this figure, there was no difference between fresh and frozen sample in term of scatter measurements. As shown in figure 3, there was no difference between fresh and frozen sample in term of scatter measurements. As a matter of fact two curves have the same peak position and shape.

Data analysis

Two types of corrections were done on raw data shown by the EDXRD spectra: The background spectrum due to scatter from the sample holder was subtracted from raw data. The incident beam for normalizing the spectral distribution was divided by intensity from each sample.

The energy values were converted into momentum transfer by using the following relationship between wavelength λ and the scatter angle θ:

$$x = \frac{1}{\lambda}\sin(\frac{\theta}{2})$$

Finally, baseline subtraction was performed before obtaining peak.

RESULTS

Table 1 summarizes the results obtained from different types of breast tissues. Table 2 shows statistical parameters, obtained by *t-test* examination in relation to peak position. As

Table 1. The results for 99 breast tissue samples classified as histologically including information related to peak position, FWHM and peak height at 6.5° scattering angle.

Tissue type	Number of samples	Peak position (nm^{-1})	FWHM (nm^{-1})	Peak Height (Normalized intensity)
Carcinoma	41	1.53 ± 0.03	0.3 ± 0.05	0.09 ± 0.01
Fibrocystic changes	21	1.38 ± 0.04	0.29 ± 0.03	0.07 ± 0.02
Adipose	20	1.09 ± 0.03	1.56 ± 0.03	0.125 ± 0.015
Mixed (adipose+ Fibroglandular)	17	1.09 ± 0.03 1.25 ± 0.05	0.25 ± 0.02	0.12 ± 0.01 0.08 ± 0.01

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Table 2. The results for 99 breast tissue samples histologically classified, including information related to SPSS statistical values of peak position in *t-test* examination.

Tissue type	Numbers	Peak position (nm^{-1})	T value	P- value	95% confidence interval of the difference	
					lower	upper
Carcinoma	41	1.53 ± 0.03	13	<0.0001	0.13	0.18
Fibrocystic changes	21	1.38 ± 0.04				
Carcinoma	41	1.53 ± 0.03	65	<0.0001	0.43	0.46
Adipose	20	1.09 ± 0.03				
Fibrocystic changes	21	1.38 ± 0.04	25	<0.0001	0.27	0.32
Adipose	20	1.09 ± 0.03				
Carcinoma	41	1.53 ± 0.03	47	<0.0001	0.27	0.3
Fibroglandular	17	1.25 ± 0.05				
Fibrocystic changes	21	1.38 ± 0.04	12	<0.0001	0.11	0.16
Fibroglandular	17	1.25 ± 0.05				

shown in table 2 using peak position is a reliable way to differentiate between normal and cancerous breast tissues. Moreover, it is possible to differentiate between fibrocystic change and carcinoma using this parameter. In this regard, the difference between adipose and tumors is very highlighted. Fibroglandular peak is close to fibrocystic change peak, but they can be well differentiated.

Figure 2 shows diffraction curves for adipose, mixed (adipose and fibroglandular), carcinoma and fibrocystic changes. Adipose tissue presents sharper peak at lower momentum transfer values compared with tumor.

The width of the curve for tumors is noticeably wider than adipose tissue. Peak height (0.11 ± 0.01 normalized intensity count) in adipose is more than tumors (0.09 ± 0.01 for carcinoma and 0.07 ± 0.02 for fibrocystic change) and fibroglandular (0.08 ± 0.01).

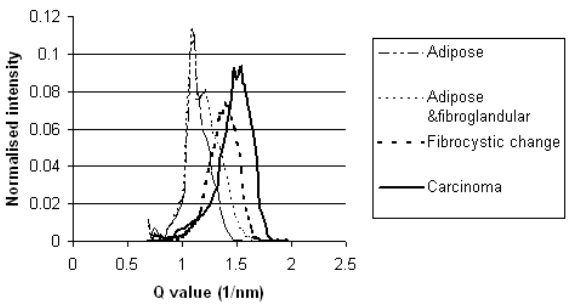

Figure 2. Measured coherent diffraction curve for the classification of tissue type listed in table 1. Each of the curves is the average value of corrected intensity of samples within its classification versus momentum transfer.

Figure 3 shows adipose coherent scattering spectra in both fresh and stored conditions.

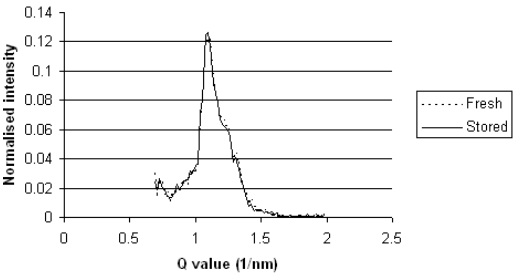

Figure 3. Adipose tissue sample after mastectomy and then after preserving in nitrogen thank for 2 months.

DISCUSSION

A noticeable shaper peak was obtained in adipose tissue as compared to benign and carcinoma. This can be as the results of existing high fat cells in adipose tissue.

Figure 2 shows that, there are separate peaks with respect to tissue in momentum transfer values, higher than 1 nm^{-1}. The peak value is dependant on material characteristics, therefore in Q values, over 1 nm^{-1}; differentiation is mainly due to the type of sample. Peak position values shown in table 1 are close to the results, obtained by Kidane *et al.* (1999).

The peak positions for the adipose and carcinoma were 1.09 ± 0.03 nm^{-1} and 1.53 ± 0.03 nm^{-1}, respectively. The connective tissue that supports the malignant cells varies in composition from fibroblastic to densely hyaline and contains varying amounts of collagen, extra cellular mucin and elastic tissue (Mills *et al.*1994). This tissue replaces the fat, as the tumor invades. So, carcinoma is typically characterized by the lack of isolated pockets of fat within its mass. Hence, there was lack of adipose peak in coherent scattering spectrum of carcinoma.

The peak positions for the adipose and carcinoma were 1.09 ± 0.03 nm^{-1} and 1.38 ± 0.04 nm^{-1}, respectively. On the other hand, adipose was replaced by tumor. Fibrocystic change arise as lobular lesions in which the individual acini or

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terminal ductules dilate, untwist, and unfold to produce a solitary lobule that then enlarges as a cyst. Fibrosis is often reported by pathologists in an attempt to explain clinical palpability or mammographic density Harris *et al.* (1999). But adenocarcinoma including ductal carcinoma, lobular carcinoma and in-situ ductal carcinoma studied in this research is referred to glandular tissue. Therefore, it is expected to have different peak positions between carcinoma and fibrocystic changes. According to the above explanations it is expected to have separate diffraction peaks for fibrocystic change. Our results supported this expectation.

The peak position of fibrocystic change was close to the carcinoma peak position. However, *t-test* examination revealed that they could be separated well in to two different groups. In all cases P value is less than 0.0001 and reasonable condition was obtained for the differentiation.

By using equation $n\lambda=2d\sin(\theta/2)$ it is possible to calculate d spacing. It was found that the d- spacing for carcinoma region is 0.32 nm, which is similar to the water d- spacing, reported by Morin *et al.* (1982). It can be concluded that there is water accumulation in carcinoma samples. Because the coherent spectrum of fibrocystic change has a considerable area overlap with the carcinoma sample, it also has water accumulation.

Fibroglandular was differentiated well from the other studied tissues in this study in relation to the peak position. It was found that the intensity of scattering from cancerous are higher than normal adipose tissue. This is similar to the report of Fernandez *et al.* (2002), even though there were different diffraction patterns. As a matter of fact, in this study energy dispersive method was used, whereas Fernandez *et al.* used angular dispersive method (The diffraction patterns were obtained by using monoenergetic photons and scanning the various scattering angles).

CONCLUSION

Small angle X-ray scattering can cause constructive interferences between coherent X-rays. By this type of interferences, it might be possible to obtain characteristic intensity spectrum versus momentum transfer for normal and cancerous breast tissues. As a result, differentiation between normal and cancerous breast tissue was obtained in the present work. Distinct peak positions were obtained for different tissues including adipose (1.09 ± 0.03 nm^{-1}), fibroglandular (1.25 ± 0.05 nm^{-1}), carcinoma (1.53 ± 0.03 nm^{-1}) and fibrocystic changes (1.38 ± 0.04 nm^{-1}). Also, peak height could help to differentiate between normal and cancerous breast tissues. Therefore this method can be used in mammography for early detection of breast cancer. There were no significant differences in peak position, peak height and area between diffraction curves obtained from a tissue before and after storing in nitrogen tank. Therefore, it can be concluded that freezing has no effect on tissues at molecular level.

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REFERENCES

Desouky O.S., Ashour, A.H., Abdullah M.I., Elshemey W.M. (2002). Low angle X-ray scattering from spices. *Radiation Physics and Chemistry,* ***64:*** *267-271.*

Evans S.H., Bradley D.A., Dance D.R., Bateman J.E., Jones C.H. (1991). Measurement of small angle photon scattering for some breast tissues and tissue substitute materials. *Phys. Med. Biol.,* ***36:*** *7-18.*

Fernandez M., Keyrilainen J., Serimaa R., Torkkeli M., Karjalainen-Lindsberg M.L., Tenhunen M., Thomilnson W., Urban V., Suortti P. (2002). Small angle X-ray scattering studies of human breast tissue samples. *Phys. Med. Biol.,* ***47:*** *577-592.*

Harris J.R., Lippman M.E., Morrow M. (1999). Diseases of the breast, second edition.

[Downloaded from ijrr.com on 2024-04-25]

http://www.thebreastclinic.com/breast.htm

Kidane G., Speller R.D., Royle G.J., Hanby A.M. (1999). X-ray scatter signatures for normal and neoplastic breast tissues. *Phys. Med. Biol., **44:** 1791-1802.*

Kosanetzky J., Knoerr, B., Harding G., Neitzel U. (1987). X-ray diffraction measurement of some plastic materials and body tissues. *Med. Phys., **14:** 1811-1816.*

Lewis R.A., Rogers K.D., Hall C.J., Towns-Andrews, Slawson S., Evan A., Pinder S.E., Ellis I.O., Boggis C.R.M., Hufton A.P. , Dance D.R. (2000). Breast cancer diagnosis using scattered X-rays. *J. Synchrotron Rad., **7:** 348-352.*

Malden C.H. and Speller R.D. (2000). A CdZnTe array for the detection of explosives in baggage by energy dispersive X-ray diffraction signatures at multiple scatter angles. *Nuclear Instruments and Methods in Physics Research, A **449:** 408-415.*

Mills R.R., Hanby A.M., Girling A.C. (1994). Diagnostic Surgical Pathology. Vol. 1 (ed. Sternberg S S), New York: Raven 323.

Morin L.R.M. (1982). Molecular form factors and photon coherent scattering cross sections of water. Data Ref. *J. Phys. Chem., **11:** 1091.*

Parking D.M., Laara E., Muri C.S. (1988). Estimates of the world wide frequency of sixteen major cancers in 1980. *Int. J. Cancer, **41:** 184.*

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