Identification of Neoplasias of Breast Tissues Using a Powder Diffractometer

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Mammography/Breast cancer/X-ray scattering/Scattering profiles.

An investigation was carried out to study the potential use of the angular distribution of scattered photons by human breast samples for a rapid identification of neoplasias of breast tissues. This technique has possible applications as diagnostic aid for breast cancer. In this work, a commercial powder diffractometer was used to obtain the scattering profiles from breast tissues histopathologically classified as normal breast tissues, fibroadenomas (benign breast diseases) and carcinomas (malignant breast diseases), in the interval $0.02\text{Å}^{-1} < \chi < 0.62\text{Å}^{-1}$. The experimental methods and data corrections are discussed in detail, and they included background subtraction, polarization, self-attenuation and geometric effects. The validation of the experimental procedure was achieved through an analysis of water sample. The results showed that the scattering profile is a unique impression of each type of tissue, being correlated with their microscopic morphological features. Multivariate analysis was applied to these profiles in order to verify if the information carried by these scattering profiles allow the differentiation between normal and neoplastic samples. The statistical analysis results showed that a correct identification of 75% of the analyzed samples is accomplished. The values of sensibility and specificity of this method in correctly differentiating between normal and neoplastic samples were 95.6% and 82.3%, respectively, while the values for differentiation between benign and malignant neoplasias were 78.6% and 62.5%. These initial results indicate the feasible use of commercial powder diffractometer to provide a rapid diagnostic with a high sensitivity.

INTRODUCTION

X-ray diffraction is commonly used to determine the spatial structure of matter at the atomic and molecular level. This technique is based on the fact that the angular distribution of the number of coherently scattered photons (scattering profiles) exhibits peaks, and their positions and shape depend on the interatomic and intermolecular structures of the scatterers, being characteristics of the particular material under study.

Harding and co-workers1) first demonstrated that X-ray diffraction and medical imaging techniques could be successfully combined to provide a powerful new technique able to differentiate tissues with similar x-ray attenuation characteristic. This technique was later extended to investigate breast tissues.2–10) In these works, a clear difference in scattering profiles has been demonstrated between normal and malignant breast tissues, although none of these works studied or ascertained the possibility to differentiate between benign and malignant breast tissues. All of these studies were performed in a research laboratory with different irradiation facilities, for example using conventional x-ray diffractometer,4,5,10) synchrotron radiation6,7) and a broad beam x-ray source at a fixed scattering angle,3,8,9) or with a stationary position sensitive detector.3) Recently, the feasibility of measuring the scattering profile of biological and amorphous materials using commercial powder diffractometers has been assessed.11,12) Both groups developed different methods in order to obtain the scattering profiles. Tartari et al.11) studied three different powder diffractometers with different irradiation facilities, and although their methodology provides accurate data, the main relevant correction factors were evaluated by means of Monte Carlo and computational algorithm approaches, being more complex and time consuming. On the other hand, Johns and Wismayer12) studied two powder diffractometers and considered three principal corrections: background, angle-dependent effect and nor-
nalization. The two first corrections were obtained in an experimental way and the last one using a theoretical model (Independent Atomic Model, IAM). This method is simpler, but its accuracy is insufficient for detailed studies. Both works do not display applications on breast tissues and therefore they do not show the potentialities of the technique in distinguishing diverse types of breast tissues, specifically between benign and malignant. To our knowledge, the degree of accuracy achievable by using a commercially powder diffractometer as a diagnosis technique has not been investigated yet. Therefore, the present work attempts firstly to describe a method of measuring the scattering profiles of small samples of normal and neoplastic breast tissues using a commercial powder diffractometer, and finally to show that a multivariate analysis of the experimental scattering profiles could successfully differentiate samples according to their histological classifications.

**MATERIALS AND METHODS**

**Experimental aspects**

**Samples**

The breast tissue samples measured were obtained from mastectomies, biopsies and breast reduction procedures. A total of 40 breast samples were collected, 17 of them being histologically diagnosed as healthy tissues (comprising different contents of adipose and glandular tissue), 9 as fibroadenomas (benign breast disease), and 14 as carcinomas (mostly invasive ductal carcinomas). The samples were stored at room temperature in recipients containing formaldehyde (10% formaldehyde in water).

**Diffractometer**

The angular distribution of photons scattered by each sample was measured using a powder diffractometer Siemens D5005 operating in back-reflection mode. The X-ray tube was equipped with a Cu anode \((Z = 29, K_{\alpha} = 8.04 \text{ keV} \text{ e } K_{\beta} = 8.91 \text{ keV})\) and Ni added filtration. A compensating divergence slit was used to keep the irradiated area constant at 6 mm × 10 mm. A Söller slit was positioned between the compensating divergence slit and the sample in order to minimize incident beam divergence. Each sample was located in a cylindrical sample-holder consisting of an acrylic plate with a 4 mm deep and 18 mm of diameter cylindrical central cavity and covered with a 1.5 μm polyvinyl chloride film. Between the sample and the detector system, another set of Söller and compensating divergence slit were used to allow measurements within a small angular range around the desired scattering angle. The detector system consisted of a graphite monochromator and a scintillation detector of NaI. The monochromator was positioned at an angle \(\theta_0 = 26.57^\circ\) to the scattered beam in order to accept scattered photons with the \(K_{\alpha}\) energy of Cu and to exclude others energies (like Compton in large angle and multiple scatter). Scattering angles from 5° up to 150° were scanned in steps of 1/3°, corresponding to a interval of momentum transfer \(x\) between 0.02 Å\(^{-1}\) and 0.62 Å\(^{-1}\), where \(x = (E/12.398) \times \sin(\theta/2)\), being \(E\) the incoming photon energy (in keV). A counting time of 20 s for each angular position was selected in order to provide a statistical uncertainty less than 5%.

**Methodology**

**Data corrections**

The scattering profile, or linear differential scattering coefficient, is defined as \(\mu_s = n_v \cdot d\sigma/d\Omega\), where \(n_v\) is the number of molecules per unit of volume, and \(d\sigma/d\Omega\) is the total molecular differential cross-section\(^{11,13}\).

There are many necessary steps in obtaining the scattering profile. The first one is to remove statistical fluctuations from the scattering distribution data, to do this the acquired data were processed using a zero-phase forward and reverse filter. The second step is to subtract the number of photons originated from every other spurious scattering sources from the original data. Third, the resulting data have to be corrected for polarization factor in order to compensate for the partial polarization effect induced by the use of the monochromator, for the self-attenuation and geometric factors. This latter accounts for the angular intensity variation of the incident beam, so that the irradiated area on the sample was maintained constant during the acquisition time. Finally, the last step consists in to normalize the data, by using a scaling factor, in order to obtain the absolute linear differential scattering coefficient. The procedure adopted in this work to obtain the latter three steps is detailed below.

**Correction of the collected data for spurious scattering sources:** due to the experimental setup, three spurious scattering sources contributed to the total photon counting at the detector: the air above the sample holder, the polyvinyl chloride film and the bulk sample holder. Therefore additional measurements were performed in order to quantify these contributions and subtract them from the original data\(^{11,12}\). The corrected data, \(N_{corr}\), was obtained from \[ N_{corr} = N_{tot} - \sum_i T_i N_i, \] where \(N_{tot}\) is the total photon counting, \(N_i\) represent each others sources counting (air, film and bulk sample-holder), and \(T_i\) the appropriate transmission factor to be applied to each \(N_i\).

**Correction due to polarization \(P\), self-attenuation \(A\), and geometric effect \(D\):** the first two effects were calculated using standard analytical functions\(^{14,15}\) and the latter effect was determined with an ordered calibration material (quartz crystal).

**Scaling factor:** or normalization constant, \(K\), was obtained by averaging the ratio between the theoretical data computed within the independent atomic model (IAM) and the corresponding experimental data correcting by all previous steps for \(x\) between \(x_{critical}\) (\(x\) value above which there is no more intermolecular interference) and \(x_{max}\) (the maximum value \(x\)
measured in the experiment). In the IAM approach, each atom scatters independently of the others so that no interference effects are produced, and the scattering profile is given by 
\[ \mu_x = N_x \rho \left( F'_x/M \right) d\sigma/d\Omega_x + (S_x/M) d\sigma/d\Omega_{kg} \]
with 
\[ F'_x(x)/M = \sum_{i} \left( w_i/m_i \right) f_i(x) \] and 
\[ S_x(x)/M = \sum_{i} \left( w_i/m_i \right) S_i(x) \]
where \( w_i, m_i, f_i \) and \( S_i \) are respectively the mass fraction, the atomic mass, the atomic form factor and the incoherent scattering function of the \( i \)-th element in the sample; \( d\sigma/d\Omega_x \) and \( d\sigma/d\Omega_{kg} \) are the well-known Thomson and Klein-Nishina differential cross sections, respectively. For amorphous materials, this approximation is considered valid for \( x_{\text{critical}} > 0.4 \AA^{-1} \). The mass fraction and physical densities of samples were determined from previous works. The atomic form factor \( f \) and incoherent scattering function \( S \) were obtained from Hubbell et al. 

The experimental scattering profile is then obtained from:
\[ \mu_x = KA^{\text{meq}} C_{(1)}^{-1} \] (1)
where \( C_{(1)} = A_{(1)} \times P_{(1)} \times D_{(1)} \) is the resulting factor due to polarization \( P \), self-attenuation \( A \), and geometric effects \( D \).

Multivariate Data Analysis

As pointed out in previous works, multivariate analysis of the experimental scattering profiles could successfully differentiate samples according to their histological classification.

The discriminant analysis is a multivariate technique for feature extraction, and it consists in obtaining the discriminant functions \( Z_i \), which are linear combinations of variables and by minimizing \( \min(p, g-1) \), where \( p \) is the number of independent variables and \( g \) the number of groups, two discriminant functions \( Z_1 \) and \( Z_2 \) were obtained. Also, the coordinates of samples at these functions (sample scores) were computed.

Classification and Validation

The classification of samples was performed applying the \( k \)-means clustering method, using the Euclidean distances of their scores to the nearest centroid, in the two-dimensional discriminant space. These centroids correspond to the mean coordinates of each function computed for each group. The leave-one-out cross validation procedure was applied to data in order to ascertain the efficiency of this model in classifying new samples.

RESULTS AND DISCUSSION

Diffraction curves for water

To test the methodology of collection and correction of experimental data, a sample of liquid water was measured and its scattering profile corrected and compared with literature. Figure 1 illustrates the data correction procedures for this case. Figure 1a shows the results of measurements performed to obtain the number of photons scattered just in the sample. The curve corresponds to the total number of detected photons, comprising the scattered photons by the sample and by all spurious scattering sources. The dot curve corresponds to the empty container (polyvinyl chloride film, air and bulk sample holder signals), and it shows a pattern typical of amorphous materials, since there are no sharp peaks, but only a few broad peaks corresponding to the polyvinyl chloride film signal. The dashed curve shows the contribution from scattering on the air above the sample holder (structureless pattern), and finally, the dot-dot-dashed curves shows the bulk sample holder signal (without covering film). It can be seen from Fig. 1 that the most important contribution of all spurious scattering signals lies in the region of low angles and it comes from the polyvinyl chloride film. The contribution of the bulk sample holder is practically null, by the fact that the incident beam is attenuated by the sample before reaching it, and moreover, due to irradiating geometric condition, only photons scattered by the bulk sample holder with \( x > 0.52 \AA^{-1} \) could reach the detector. The contribution of air for the scattering is significant only for very small angles. Figure 1b shows the contribution from scattering on the air above the sample holder
biological samples of small size, as those obtained from biopsies. Figure 1c shows the corrected and normalized scattering profile compared with data obtained by using the molecular form factor tabulated by Morin.24) As can be seen from Fig. 1d, our data agree very well with the gold standard pattern obtained from Morin data, in the interval of momentum transfer measured, considering the experimental uncertainties (which were always smaller than 10%, and mostly around 7%). The close agreement between the two curves shows that the measurement and correction procedures are both suitable, allowing their application to other samples.

**Diffraction curves for normal and neoplastic breast tissues**

Figure 2 shows the mean scattering profiles as a function of \( x \) for the various histological classifications studied. These curves were averaged for all tissues measured within each histological classification and therefore they represent a general characteristic for each type of tissue. Scattering profiles from normal tissues were divided in their principal components: adipose and glandular tissues. The scattering profile for normal adipose tissue (solid curve) is different from the profile for normal glandular tissue (dashed curve). Its first peak appears at lower \( x = 0.11 \text{Å}^{-1} \) and is narrower than the peak for glandular tissue. This behavior is due to the large number of fatty acids within the adipose cells, and because the molecular spacing between the first neighbors in these cells is larger and correlated over a large spatial range, resulting in a higher degree of order.13) The scattering profile for normal glandular breast tissue is similar in shape to water, with a broad scattering peak at \( x = 0.17 \text{Å}^{-1} \), indicating that the major contribution to the scattering distribution of glandular tissues at small angles probably comes from its water content.19) The difference found in low \( x \) value indicates the presence (probably just a few percent) of adipose

![Fig. 1](image1.png)

**Fig. 1.** a) Relative number of photons scattered by the sample and by all spurious scattering sources; b) correction factor as a function of the momentum transfer; c) comparison between experimental data and previous data and d) relative percent difference between experimental data and previous data.

![Fig. 2](image2.png)

**Fig. 2.** Mean scattering profiles of samples with different histological classification.
tissue. The scattering profiles for benign and malignant breast tissue are quite similar from the pattern for glandular tissue (and consequently for water, Fig. 1). The only difference between them is the intensities of the peaks. The profile shape and the peak positions found in this work for each type of tissues are in good agreement with the ones found in literature.\(^2\)\(^{-9}\) Our experimental measurements show that each tissue presents a unique profile.

**Statistical model**

**Discriminant Analysis**

Figure 3 shows the sample scores in the two-dimensional space defined by the discriminant functions \(Z_1\) and \(Z_2\). It can be seen that a very good separation of groups N, B and M is achieved, since samples corresponding to the same histological type tend to be clustered in specific regions. A One-way MANOVA test applied to these scores showed that the group centroids are statistically different at the \(p = 0.04\) level of significance.

**Classification**

Table 1 shows the result of the cross validation procedure, using the \(k\)-means clustering method for classification. The total percentage of correctly classified samples is 75%. The values of sensibility and specificity of this method in correctly differentiating between normal and diseased samples were 95.6% and 82.3%, respectively. The values for differentiation between benign and malignant diseases were 78.6% and 62.5%. These values showed that samples from the healthy normal group are successfully differentiated from diseased tissues, and vice-versa. On the other hand, fibroadenomas tend to be often misclassified as malignant, while some malignant diseases are also misclassified as benign. Nevertheless, the malignant group comprises different types of carcinomas, being actually a very heterogeneous group. A more detailed investigation is still necessary in order to explain the possible similarities between the profiles of fibroadenomas and some kinds of carcinomas.

**CONCLUSION**

Normal, benign and malignant breast tissues have been characterized in term of the scattering profile obtained using a commercial powder diffractometer and the ability to differentiate between these types of tissues has been statistically demonstrated.

These initial results are encouraging and indicate acceptable outcome for clinical purpose, for example, for a rapid diagnostic test using excised breast tissues with a small sample (typical form of biopsies). Another advantage is that this analysis does not require sample preparation procedure, allowing the possibility to complement this study with standard histopathological analysis. The statistical model used for classification of breast tissues is simple and it can be automatized to quickly identify benign and malignant breast lesions with high sensibility and specificity. Alternatively, other data analysis and classification procedures can be applied, in order to optimize the histological identification of samples.

**ACKNOWLEDGMENTS**

This work was supported by the Brazilian agency Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

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Received on February 27, 2008
Accepted on June 2, 2008
J-STAGE Advance Publication Date: July 31, 2008

J. Radiat. Res., Vol. 49, No. 5 (2008); http://jrr.jstage.jst.go.jp