

# Identificação de patologias mamárias através do espalhamento elástico de raios X

## Identification of human breast pathologies by x-ray elastic scattering

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### Resumo

Neste trabalho foram determinados os perfis de espalhamento de amostras normais, benignas e malignas de tecido mamário no intervalo de momento transferido  $0,07\text{nm}^{-1} \leq q \leq 70,55\text{nm}^{-1}$ , resultante da combinação dos dados de WAXS (*wide angle x-ray scattering*) e SAXS (*small angle x-ray scattering*). Os resultados obtidos mostram que cada tipo de tecido mamário estudado apresenta seu próprio perfil de espalhamento. Baseado neste fato, alguns parâmetros, que representam características estruturais, foram extraídos dos perfis de espalhamento e submetidos à análise de discriminante. A partir da análise estatística, a razão entre as intensidades dos picos em  $q=19,8\text{nm}^{-1}$  e  $q=13,9\text{nm}^{-1}$  e a intensidade do pico de espalhamento de 3ª ordem das fibras de colágeno surgiram como dois potenciais classificadores de tecidos mamários e, combinando-os foi possível diferenciar entre normal, benigno e maligno.

**Palavras-chave:** câncer de mama, espalhamento de raios X, WAXS, SAXS, radiologia.

### Abstract

In this paper we determine the scattering profiles of normal, benign and malignant human breast samples in a momentum transfer range of  $0.07\text{nm}^{-1} \leq q \leq 70.55\text{nm}^{-1}$ , resulted from combining WAXS (wide angle x-ray scattering) and SAXS (small angle x-ray scattering) data. The results showed considerable differences between the scattering profiles of each tissue type. Based on this fact, some parameters, representing structural features, were extracted from these scattering profiles and submitted to a discriminant analysis. From statistical analysis, the ratio between the peak intensities at  $q=19.8\text{nm}^{-1}$  and  $q=13.9\text{nm}^{-1}$  and the intensity of 3<sup>rd</sup> order axial collagen peak arose as two potentials breast tissue classifiers and, from combining them it was possible differentiate among normal, benign and malignant lesions.

**Keywords:** breast cancer, x-ray scattering, WAXS, SAXS, radiology.

### Introduction

Breast cancer is the second most frequently incident type of cancer and the most common in women. According to projections of breast cancer incidence in Brazil in 2010 will must arise about 49,240 new cases of this disease<sup>1</sup>. Nowadays, mammography is the principal technique for early detection of breast cancer, however, due to its inherent limitation, some cases of false diagnoses and inappropriate biopsies have occurred. Then, new spectroscopic<sup>2-3</sup> and imaging<sup>4,5</sup> techniques have been studied in order to complement the information provided by the mammography. Recent researches have demonstrated that the x-ray coherent scattering techniques appear as a potential alternative to enhance the mammography, since that the coherent scattering distribution (scattering profile) carries information about the tissue structures

providing details about possible structural changes due to cancer progression. Usually two techniques are used to measure scattering profiles from human breast tissues: WAXS (wide angle x-ray scattering) and SAXS (small angle x-ray scattering). WAXS technique allows obtaining a spatial distribution of smallest cell structures that compose the tissues, as for example water and fatty acid<sup>6</sup>, while the SAXS technique allows determining supramolecular system features, for example the collagen fibrils<sup>7</sup>. In this sense, combining the scattering profiles at WAXS and SAXS regions allows correlate changes at molecular level with those occurred at supramolecular scale and then, could provide a mean of differentiate the human breast tissues<sup>8,9</sup>.

Therefore, in this study, both techniques were applied on each sample (normal and neoplastic breast tissues) in order to determine their total scattering profiles; and to

study which parameters can be used to classify the human breast tissues.

## Material and Methods

The breast tissue samples analyzed in this work were obtained from mastectomy and reduction mammoplasty procedures. The samples were histopathologically classified as: normal tissue, benign lesion and malignant lesion. However, due to heterogeneity of the normal tissue, it was subdivided into: adipose and fibroglandular. Subsequently to collection and classification, the samples were stored within suitable cases and fixed in formalin (4% formaldehyde in water). At the moment of the measurements, the samples were cut to 1mm thick to fit into the circular sample holder with 10 mm of diameter and sandwiched between thin mica foils and positioned to carry out the measurements.

WAXS and SAXS experiments were carried out at the D12A-XRD1 and D02-SAXS2 beam lines in the National Synchrotron Light Laboratory (LNLS) in Campinas, Brazil. For WAXS experiment the x-ray beam energy was fixed at 11 keV and the irradiation area on the sample was 3.0 mm x 1.0 mm. The sample was assembled on a rotative table inside of the Huber three-circle diffractometer operating in transmission mode. The detector system consists of a graphite monochromator, which was positioned in order to select only photons scattered with 11 keV and exclude other energies, and a fast scintillation detector NaI(Tl). Coherent scattered intensities were scanned covering a momentum transfer range of  $0.7 \text{ nm}^{-1} \leq q = 4\pi \sin(\theta/2) / \lambda \leq 70.5 \text{ nm}^{-1}$  where  $\theta$  is the scattering angle and  $\lambda$  the wavelength. While for SAXS experiment was used an x-ray beam of energy of 7.7keV, whose size on the sample was 1.0 x 0.5 mm, and a two-dimensional MarCCD 165 camera detector of 2048 x 2048 pixels, with resolution of 79  $\mu\text{m}$  per pixel. Two sample-detector distances were used (641 mm and 2043 mm), allowing to record the momentum transfer interval of  $0.07 \text{ nm}^{-1} \leq q \leq 4.20 \text{ nm}^{-1}$ . Three SAXS images were acquired on different places of the same sample and were summed in order to obtain an average scattering profile for the whole sample. Standard sample of Silver Behenate was used as a calibrant, in order to establish the correct reciprocal space scale of each scattering profile.

The differential linear coherent scattering coefficient,  $\mu_{CS}$ , was obtained from WAXS measured intensity,  $I_M(q)$ , by<sup>2</sup>:

$$\mu_{CS} = [I_M(q) - B(q)T] A(q)^{-1} P(q)^{-1} K \quad (1)$$

where  $B(q)$  represents the background signal, which correspond to photons originated from every other spurious scattering sources, in this case were from three sources: the layer of air between sample and detector, the mica foils and the bulk sample holder;  $T$  is the transmission factor;  $A(q)$  is the sample self-attenuation and geometric factor;

$P(q)$  is the polarization factor and  $K$  is a normalization constant.  $A(q)$  and  $P(q)$  both were calculated using standard analytical functions<sup>10</sup>.

The software FIT2D<sup>11</sup> was used to process all SAXS images in order to extract the one-dimensional scattering coherent intensity distribution from 2D images by radial averaging. The relative intensity scattered from the sample ( $I_s$ ) is obtained after applying some corrections on the coherent scattered intensity measured ( $I_M$ ). This procedure is summarized in equation 2<sup>5</sup>:

$$I_s(q) = I_M^*(q) \cdot A_M(q) - B^*(q) \cdot A_B(q) \quad (2)$$

where  $I_M^*(q)$  and  $B^*(q)$  are the total scattering intensity measured (sample+background) and background signal, respectively, normalized by incident intensity;  $A(q)$  represent the same factors shown in WAXS experiment, however for SAXS experiment were considered constant for all  $q$  range (since  $\cos(\theta) \approx 1$ ). These correction factors were experimental measured during the SAXS experiment. The indexes  $M$  and  $B$  corresponding to measured and background respectively.

Additionally, in order to obtain the total scattering profile, the  $\mu_{CS}(q)$  from each sample at WAXS region was used to normalize the  $I_s(q)$  from the correspondent sample at SAXS region, in a common interval ranging from 0.7 to  $4.20 \text{ nm}^{-1}$ <sup>7</sup>. Finally, from the total scattering profiles were extracted some parameters that representing structural information and submitted to discriminant analysis in order to verify what these parameters could be statistically significant to differentiate between the groups of breast tissues based on their structural features.

## Results and Discussion

Figure 1 shows the experimental differential linear coherent scattering coefficient (scattering profile), resulting from fusion of the SAXS and WAXS spectra of each breast tissue type analyzed in this work.

From Figure 1 it is easily seen that the scattering profiles are a typical signature of each breast tissue type, and their behavior show several features strongly dependent on the momentum transfer values. At low region, it is possible observe the influence of the large-scale arrangement, mainly due to collagen fibrils (peaks from  $0.25$  to  $1.20 \text{ nm}^{-1}$ ) for fibroglandular and pathological samples, as well as triacylglycerides ( $q=1.38 \text{ nm}^{-1}$ ) for adipose tissues<sup>7</sup>. At high region, the scattering profiles reflect effects of molecular interference related to fatty acids ( $q=13.9 \text{ nm}^{-1}$ ) for adipose samples and water ( $q=19.8 \text{ nm}^{-1}$ ) for fibroglandular and pathological tissues<sup>6,9</sup>.

From the discriminant analysis of the parameters extracted from the scattering profiles only the ratio between the peak intensities at  $q=19.8 \text{ nm}^{-1}$  and  $q=13.9 \text{ nm}^{-1}$  and the intensity of 3<sup>rd</sup> order axial collagen peak were statistically significant ( $p < 0.001$ ) and allows classifying the breast tissues as shown in Figure 2.

## Conclusion

This work shown that x ray elastic scattering experiments applied in human breast tissues provide a unique signature of each tissues type. Using WAXS technique it is possible to find features at molecular level, fatty acid and water, for example, while changes in a supramolecular level, as collagen fibrils, can be observed employing SAXS technique. Combining both techniques allows correlate changes at molecular and supramolecular levels.

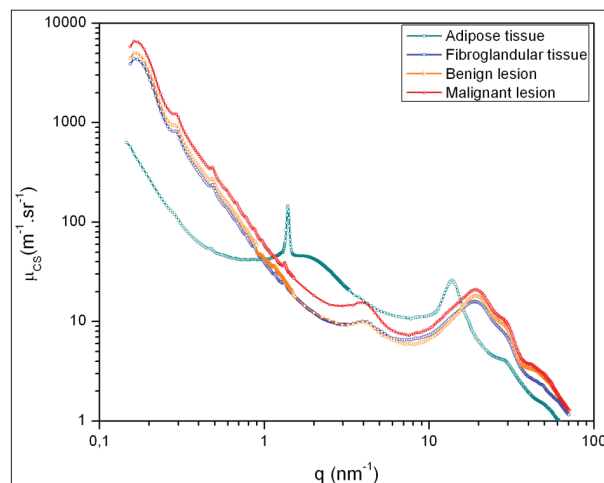
Moreover, statistical analysis of the scattering profiles has shown that two parameters, the ratio between the peak intensities at  $q=19.8 \text{ nm}^{-1}$  and  $q=13.9 \text{ nm}^{-1}$  and the intensity of the third-order axial collagen peak, can be considered valuable histological classifiers of the human breast tissues. Therefore, combining these two parameters is possible the differentiating among normal adipose, benign and malignant tissues.

## Acknowledgments

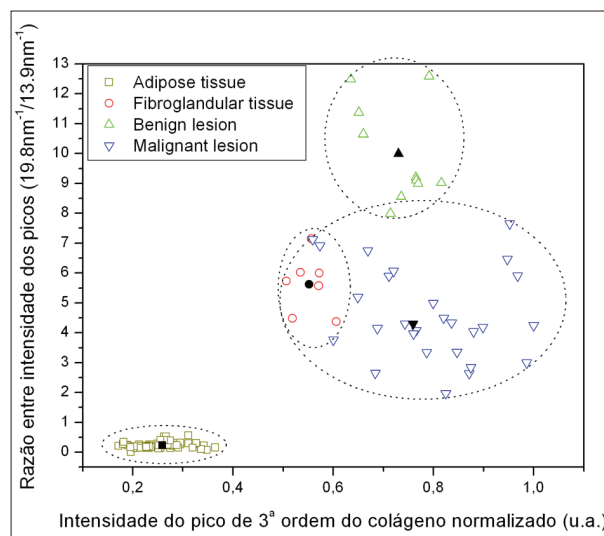
The authors would like to acknowledge the support by the Brazilian agencies Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), as well as the D02A-SAXS2 and the D12A-XRD1 beam lines staffs for the help during the experiments in the National Synchrotron Light Laboratory (LNLS). In addition, we also would like to thank the Department of Pathology of the Clinics Hospital, Faculty of Medicine of Ribeirão Preto, Brazil, for allow to collect the human breast samples.

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**Figure 1.** Scattering profile of each breast tissue group analyzed in this work



**Figure 2.** Scatter plot of the ratio of water-like and fatty acid peak intensities versus the third-order peak intensity. Ellipses are shown to illustrate the clustering.

