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# Spin-echo small-angle neutron scattering study of the structure organization of the chromatin in biological cell

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**Abstract.** Spin-echo small-angle scattering (SESANS) technique is a method to measure the structure of materials from nano- to micrmeter length scales. This method could be important for studying the packaging of DNA in the eukaryotic cell. We measured the SESANS function from chicken erythrocyte nuclei which is well fitted by the exponential function $G(z) = \exp(-z/\xi)$, where $\xi$ is the correlation length of a nucleus (in experimental data $\xi = 3,3$ $\mu$m). The exponential decay of $G(z)$ corresponds to the logarithmic pair correlation function $\gamma(r) = \ln(\xi/r)$. As the sensitivity of the SESANS signal depends on the neutron wavelength, we propose the SESANS setup with the changeable wavelength in the range from 2 to 12 Å. Such option allows one to study in great detail the internal structure of the biological cell in the length scale from $10^{-2}\mu$m to 10 $\mu$m.

## 1. Introduction

It is well known that the cell is the basic structural and functional unit of all known living organisms. The cell nucleus acts like the control center of the cell. In this nucleus, a complex of macromolecules can be found which consists of DNA and proteins is called the chromatin. All the information about the biological organism is written in the double-helix DNA sequence.

The large-scale chromatin organization in interphase nuclei is fundamentally different from the small-scale chromatin structure [1-6]. There are several levels of chromatin organization. At the simplest level, chromatin is a double-helical structure of DNA. After that DNA is complexed with histones to form nucleosomes. The nucleosomes fold up to produce a 30-nm fiber. One of the most exciting questions that scientists are trying to answer is how DNA is packed inside the nucleus of a cell. Although DNA macromolecules extend up to a few meters in unraveled condition, they are packed really tight in the nucleus. The most mysterious feature of DNA in the nuclei is the way it is packed so thatany part can be accessed, read, transcripted and translated at any time in the process of replication.

Nowadays the multiscale fractal model of DNA arrangement inside the nuclei [3, 4] is the most promising to explain this mystery. Experimental proof of this theory is complicated firstly

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because of the necessity to investigate nuclei under conditions as close as possible to those inside the organism and secondly by the simultaneous coexistence of DNA together with proteins inside the nuclei. Neutron scattering seems to be the most suitable instrument for such investigation due to such advantages of neutrons as the high penetration depth, the non-damaging and the possibility to use the contrast variation method. The last is very important as it allows one to illuminate a separate structure of DNA and proteins in the nuclei.

Previously, the arrangement of chromatin in intact chicken erythrocyte nuclei was investigated by small (and ultra-small) angle neutron scattering [4, 3]. The experimental results demonstrated the power-law character of the neutron scattering intensity versus the scattering vector $I \sim Q^{-D}$ with parameter $D \approx 2.5$ in the Q-range from $1.5 \cdot 10^{-2}$ to $4 \cdot 10^{-1}$ nm$^{-1}$ and $D \approx 3$ in the Q-range from $3 \cdot 10^{-3}$ to $1.5 \cdot 10^{-2}$ nm$^{-1}$. It means that at lenght scales between 15 nm and 1 $\mu$m the nucleus exhibites properties of bi-fractal structure. On the length scales between 15 and 400 nm chromatin exhibites properties of a mass fractal with a fractal dimension of approximately 2.5.

In recent years, the data obtained via novel experimental techniques to study the genome structure, in particular Hi-C methods [7, 8], seem to provide data supporting the topological fractal globule approach. The fractal globule is a compact polymer state that emerges during polymer condensation as a result of topological constraints which prevent one region of the chain from passing across another one [8, 9]. The majority of scientists suggest that the chromatin structure is a fractal globule.

To answer the question how DNA is packed inside the interphase nucleus at the micron scale, the new method spin-echo SANS (or SESANS) was used. The technique allows one to measure a full pair correlation function in samples over distances from 20 nm to 20 $\mu$m. The ability to measure the correlation at the micron scale is incredibly important in biological cell studies. In the present study we report the result of the spin-echo SANS measurements from chicken erythrocyte nuclei. Analyzing these results we propose to optimize the parameters of the SESANS setup in order to obtain the full information on the pair correlation function of the chromatin inside a biological cell.

## 2. Principles of spin-echo SANS

The method is based on the Larmor precession of polarized neutrons transmitted across two successive precession devices before and after the sample, which encodes the scattering angle into a precession angle [10].

Spin-echo SANS technique is a method to measure structures of materials in real space. These structures can be determined over three orders of magnitude in length scale from 20 nm to 20 $\mu$m. The measured SESANS signal G(z) depends on the spin-echo (SE) length $z$, which is equal along the beam axis coordinate in real space

$$z = C\lambda^2 BL \cot(\theta_0)/2\pi, \tag{1}$$

where $C$ is a constant, $\lambda$ is the neutron wavelength, $\theta_0$ is the angle of inclination of the precession devices front and end faces towards beam axis, $B$ is the magnetic field in the precession device, and $L$ its length [10].

As shown by Kruglov [11] the SESANS function G(z) is the projection of the spatial pair correlation function $\gamma(r)$ along the beam axis

$$G(z) = \frac{2}{\xi} \int_z^\infty \frac{\gamma(r) r}{\sqrt{r^2 - z^2}} dr, \tag{2}$$

where $\xi$ is the correlation length. The inverse transformation is found as

$$\gamma(r) = -\frac{\xi}{\pi} \int_r^\infty \frac{G'(z)}{\sqrt{z^2 - r^2}} dz. \tag{3}$$

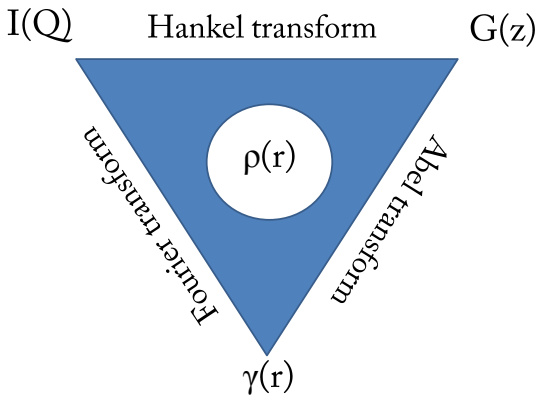


**Figure 1.** The relationship between the correlation function $\gamma(r)$, the scattering cross section I(Q) and the SESANS correlation function G(z) [11].

In addition, the SESANS correlation function $G(z)$ in terms of the differential scattering cross section $I(Q)$ of a sample is

$$G(z) = \frac{1}{k_0^2 \sigma} \int_{R^2} \cos(zQ_z) I(Q) dQ_y dQ_z, \tag{4}$$

where $k_0$ is a neutron wave number, $\sigma$ is the total scattering cross section and $Q = (0, Q_y, Q_z)$ is the scattering vector. The so-called Fourier – Abel – Hankel cycle relates the three functions $I(Q)$, $G(z)$ and $\gamma(r)$ to each other. The relationships are illustrated schematically in Fig.1 for isotropic distributions, where the figure shows the inverse related Abel – Hankel – Fourier transformation [12]. Nevertheless, in SANS measurements only the scattering intensity is measured (and not the phase of the scattered neutron wave), so critical information is lost which prevents the full retrieval of the original density (the 'phase problem'). The same situation occurs in the SESANS method. It should be noted that both methods give information about the spatial pair correlation function.

The polarization $P/P_0$ in a SESANS experiment is measured as a function of the total scattering cross section $\sigma$, the thickness of the sample $l$ and the spin-echo length $z$

$$P(z)/P_0 = \exp(l\sigma[G(z) - 1]). \tag{5}$$

## 3. Study of the interphase chicken erythrocyte nuclei

The sample of isolated chicken erythrocyte nuclei was investigated at the SESANS-instrument at the HOR reactor of the Delft University of Technology, The Netherlands. To maximally increase the neutron scattering intensity we used the densest solution possible for these samples. To avoid the problem of agglomeration of nuclei, the detergent Triton X-100 was added to the solution, then clusters of order of a micron and more were mechanically destroyed by passing the solution through a syringe.

The SESANS instrument uses a constant wavelength $\lambda = 2$Å. The measurements were carried out in solution of a $D_2O > 95$ % to reach the maximum contrast for the scattering. Three different thicknesses of the sample (2, 4, 10 mm) were used to obtain the SESANS signal from the chromatin in the nucleus. The noticeable function had been measured for the sample of 10

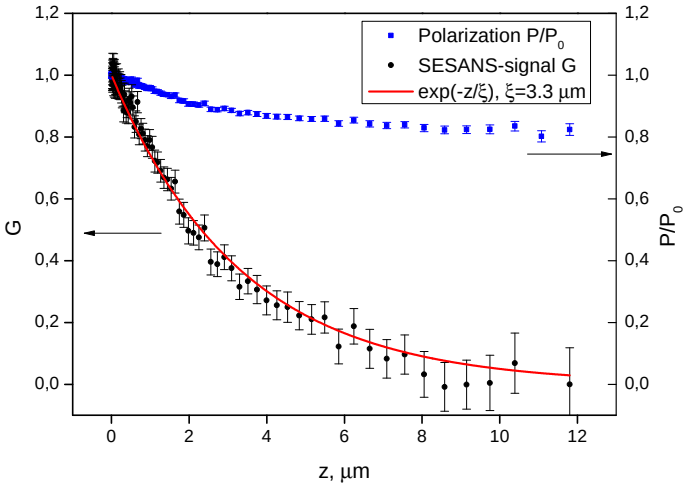


**Figure 2.** The polarization $P/P_0$ and the SESANS function $G$ in dependence on the SE length $z$ from the isolated chicken erythrocyte nuclei. The line is an exponential decay with the best fitted parameter $\xi = 3.3 \pm 0.1$ $\mu$m.

mm only. Fig.2 presents the polarization $P/P_0$ as a function of the SE length $z$. As one can see the polarisation changes from $P/P_0 = 1.00 \pm 0.01$ at small $z$ to $P/P_0 = 0.85 \pm 0.01$ at $z = 8$ $\mu$m. The constant level of polarisation $P/P_0$ at large $z$ determines the value of the product $\sigma l$ in Eq. (5). Thus the SESANS function of $G(z)$ can be definitely determined from the measured function $P/P_0(z)$ using Eq.(5). The SESANS function $G(z)$ is also shown in Fig.2. We were able to fit this function using the simple exponential decay $G(z) = \exp(-z/\xi)$, where $\xi = 3.3 \pm 0.07$ $\mu$m is the correlation length of a nucleus.

The pair correlation function can be obtained using the Abel transform Eq.(3)

$$\gamma(r) = \frac{1}{\pi} \int_r^\infty \frac{\exp(-z/\xi)}{\sqrt{z^2 - r^2}} dz = \frac{1}{\pi} K_0(r/\xi), \tag{6}$$

where $K_0(x)$ is the Macdonald function of the 0th order. In its turn, the Hankel transform of exponential decay of $G(z)$ gives for the neutron cross section $I(Q) = A \cdot (1 + (Q\xi)^2)^{-3/2}$, which was observed in [3] for the large scale chromatin organization. However the upper border with fractal structure had not been found with SANS experiment because of an instrument resolution. Thus, the complementarity of SANS and SESANS can be realized via the suitable spacial ranges for SANS from 1 to $5 \cdot 10^2$ nm and that for SESANS from $5 \cdot 10^1$ to $2 \cdot 10^4$ nm. As we are interested in the correlation inside rather than outside the sample, we can restrict ourself by a small value of $r/\xi$. With this focus to the internal structure of the nucleus, we can simplify Eq.(6) when $r/\xi << 1$. We obtained the simplified expression of the pair correlation function using Eq.(6) and it's asymptotic form gives

$$\gamma(r) = \ln(\xi/r). \tag{7}$$

Thus the exponential law of the SESANS function corresponds to the logarithmic pair correlation function. The correlation function increases with an additive rather than multiplicative constant, upon reducing the ruler length by a fixed rescaling factor. This leads to a logarithmic law instead

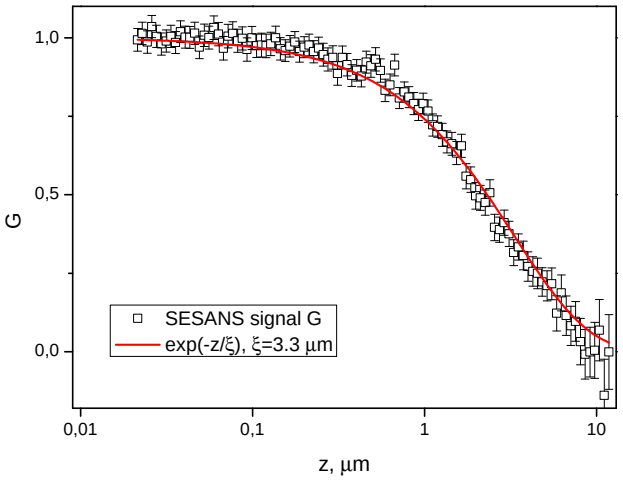


**Figure 3.** The lin-log plot of the SESANS function $G$ as a function of the SE length from isolated chicken erythrocyte nuclei.

of the usual power law for the fractals. The logarithmic correlation function correspond to a hierarchically branched structure. A good sample of such structures are trees. Such a structure provides high accessibility of enzymes to specific gene site.

## 4. Criteria for SESANS measurements of a biological cell

The possibilities to extract the SESANS function $G(z)$ appear to be limited for the biological cell due to weak neutron scattering attributed to the nuclei of the cell. Fig.2 demonstrates the maximum of the possible signal determined by the maximum value of $\sigma l$ with a thickness of $l = 10$ mm and maximal $\sigma$ provided by the maximum contrasting of the $D_2O$. However, as it is shown in Fig.3, the obtained meaningful data set for the SESANS function $G(z)$ is limited by the range of $z$ from 1 to 12 $\mu$m, in spite of the fact that polarization data $P/P_0$ were measured within 3 decades of the SE length $z$ from 0.02 to 12 $\mu$m. This limitation is caused by the experimental errors of the measurements of the polarisation $P/P_0$, which can be at the best measured with a accuracy of 1 %. This value is related not with the statistical errors of the neutron measurements but with the stability of many devices used to manipulate the orientation of the neutron spin in the SESANS setup. This limitation can be overcome if one is able to vary not only the thickness of the sample $l$ but also the total neutron cross section via the neutron wavelength. The cross section for the small-angle neutron scattering depends of the wavelength as $\lambda^2$. Therefore the change of the wavelength from $\lambda = 2$ Å to $\lambda = 12$ Å will change the sensitivity of the SESANS method by 36 times. In the other words, as it is impossible to increase the thickness of the specimen to infinity, one should change the other parameter, $\sigma$, which can be easily done via variation of the neutron wavelength $\lambda$.

Often the SESANS method is applied to measure the size of the particle ($10^{-1}$-10 $\mu$m). However, the key information about the fractal properties of the particle is hidden at the small values of $z$. As was argued above for the biological cell the small values of $z$ are inaccessible. Therefore we formulated the criteria necessary to perform a complete study of such biological objects using the SESANS method:

- one must have the possibility to change the neutron wavelength;

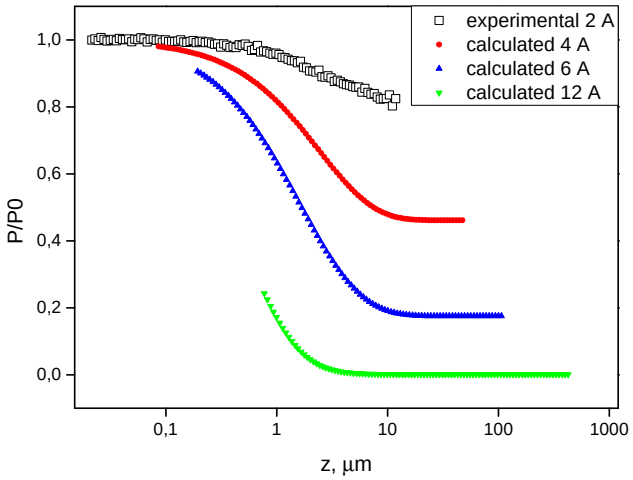


**Figure 4.** The lin-log plot of the SESANS correlation function from chicken erythrocyte nuclei at the different wavelengths: $\lambda = 2$ Å (experiment), $\lambda = 4, 6, 12$ Å (calculations) taken the same parameters of the setup such of B, inclination angle $\theta_0$ and the lenth of the SE arm $L$ in Eq.1.

- one must use different thickness;
- one must use the contrast-variation technique.

Here we introduce the sensitivity of SESANS is an important parameter which is independent on the polarization efficiency or statistics. The sensitivity is the difference between $P_0$ and $P_{inf}$ normalized to $P_0$. The bigger is this number (which runs from 0 ($P_0 = P_{inf}$)) to 1 ( $P_{inf} = 0$), the higher is sensitivity of the measurements of G(z) for those z at which $P/P_0 > 0$. Of course, we do not want to set $P_{inf} = 0$, as we must know its true level, but not the one which is restricted by the measurement conditions. To demonstrate the sensitivity of the SESANS signal on the neutron wavelength, the polarisation $P/P_0$ was calculated for the three different wavelengths $\lambda = 4, 6, 12$ Å, using Eq.(5) and the SESANS function from chicken erythrocyte nuclei ($G(z) = \exp(z/\xi)$).

Fig.4 presents the calculated values of the polarization $P/P_0(z)$ ($\lambda = 4, 6, 12$) Å and the experimental polarization $P/P_0$ for $\lambda = 2$ Å. The experimental polarization $P/P_0$ ($\lambda = 2$ Å) approaches a constant ($P/P_0 \cong 1$) for $z < 1$ $\mu$m. The sensitivity of the calculated polarization increases with increasing wavelength $\lambda$ in the same z-range. However, the polarization $P/P_0$ with $\lambda = 12$ Å can be well measured for relatively small $z$ only, since it tends to zero at $z$ larger than 1 $\mu$m. In this case the value of $\sigma l$ can not be determined. It is clear from this consideration that the optimum measurements can be obtained with $\lambda = 6$Å. In general, in order to really cover the 3 decades (from $10^{-2}\mu$m to 10 $\mu$m) in measurements of the SESANS function $G(z)$, one must perform such measurements with at least 3 different values of the neutron wavelength. Such protocol of SESANS measurements is analogous to the protocol of the SANS measurements at 3 different sample-detector positions (2, 10, 18 m) aiming to provide a wide Q-range from $10^{-3}$ to 1 nm$^{-1}$.

## 5. Conclusions

In summary: the use of the beam with $\lambda = 6$ Å leads to an increase of the sensitivity of the SESANS method and opens the range of $z$ [$10^{-2}$ - 10] $\mu$m for the study of biological cells.

A SESANS setup with a changeable wavelength in the range from 2 to 12 Å will be highly appreciated by the community of life sciences.

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