Scanning Small-Angle-X-Ray Scattering for Imaging Biological Cells.

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Scanning small angle X-ray scattering (SAXS) bridges two worlds of X-ray imaging: We use highly focused beams to spatially resolve the different constituents inside biological cells. Additionally, each individual scattering pattern contains a wealth of information about the internal structure on molecular length scales. X-rays provide high resolution due to their small wave length and high penetration power, allowing for imaging of large three-dimensional objects. For these reasons, X-rays have been established as complementary probes for bio-imaging, besides well-established methods like visible light fluorescence microscopy and electron microscopy (EM). Scanning SAXS, in particular, is well suited for systems with some degree of order, such as bundles of parallel filaments, or high-density aggregates [1].

Here, we present scanning SAXS experiments that were performed at dedicated synchrotron beamlines providing a small beam between 100 nm and 2 µm diameter, high flux, high-end pixel detectors and a sample environment suitable for cell samples, e.g. ID13 at the European Synchrotron Radiation Facility (ESRF), P10 at Deutsches Elektronen-Synchrotron (DESY) or cSAXS at Swiss Light Source (SLS). We use X-ray energies between 7 and 15 keV. A schematic of a typical setup is shown in figure 1a. In the following, we will summarize the most important results we recently obtained on different biological systems, such as components of the cytoskeleton and the DNA in the nucleus.

The cytoskeleton of eukaryotic cells is a "composite" biopolymer network consisting mainly of three filament types, actin filaments, microtubules and intermediate filaments, along with a large number of cross linkers and molecular motors. These filaments possess differing mechanical properties, in terms of bending and stretching resistance and form complex structures like bundles and networks. It is well accepted that these networks are to a great part responsible for the mechanical properties of the cell and it is likely that these properties are encoded in the specific architecture of filaments and their superstructures. Thus, we have imaged keratin networks in epithelial cells (see figure 1b) [2-4] and actin parallel bundles in stereocilial hair cells from mouse inner ears (see figure 1c) [5] by this technique. Typically, we compute dark field images, where we integrate the total scattered intensity in each scattering pattern and plot the resulting value on a color scale as a "pixel" in the respective position, for real space overview images. If the individual scattering patterns are plotted in a composite image, they reveal the degree and the direction of the orientation of subcellular structures, which is then further quantified. An important point is that in this case the size of the scatterer (filament bundle with a diameter on the order of 100 nm) and the beam size are very similar. In order to interpret the scattering patterns correctly, we have therefore simulated the situation [6,7] in order to fit the two-dimensional patterns or the azimuthally integrated $I(q)$ curves (see figure 1d). For the keratin bundles we were thus able to derive values for filament diameter, bundles diameter, filament distance and "lattice type" – very much in agreement with EM data, albeit without the need of slicing the cell sample.

We also applied scanning SAXS to the nucleus in eukaryotic cells (see figure 2 a,c), with special attention to changes during the cell division cycles [8]. In this case, as the DNA strands are tightly packed rather

than forming parallel bundles, we exploit the one-dimensional $I(q)$ data by applying a generalized Porod's law, $I(q) = K\,q^{\alpha} + B$. The Porod exponent α and the Porod constant K provide information about the aggregation state of the DNA in the nucleus. We observe that before the cells divide, the DNA is duplicated, thus the density increases, whereas after division the (smaller) cells grow, including the nucleus, and the DNA decompacts.
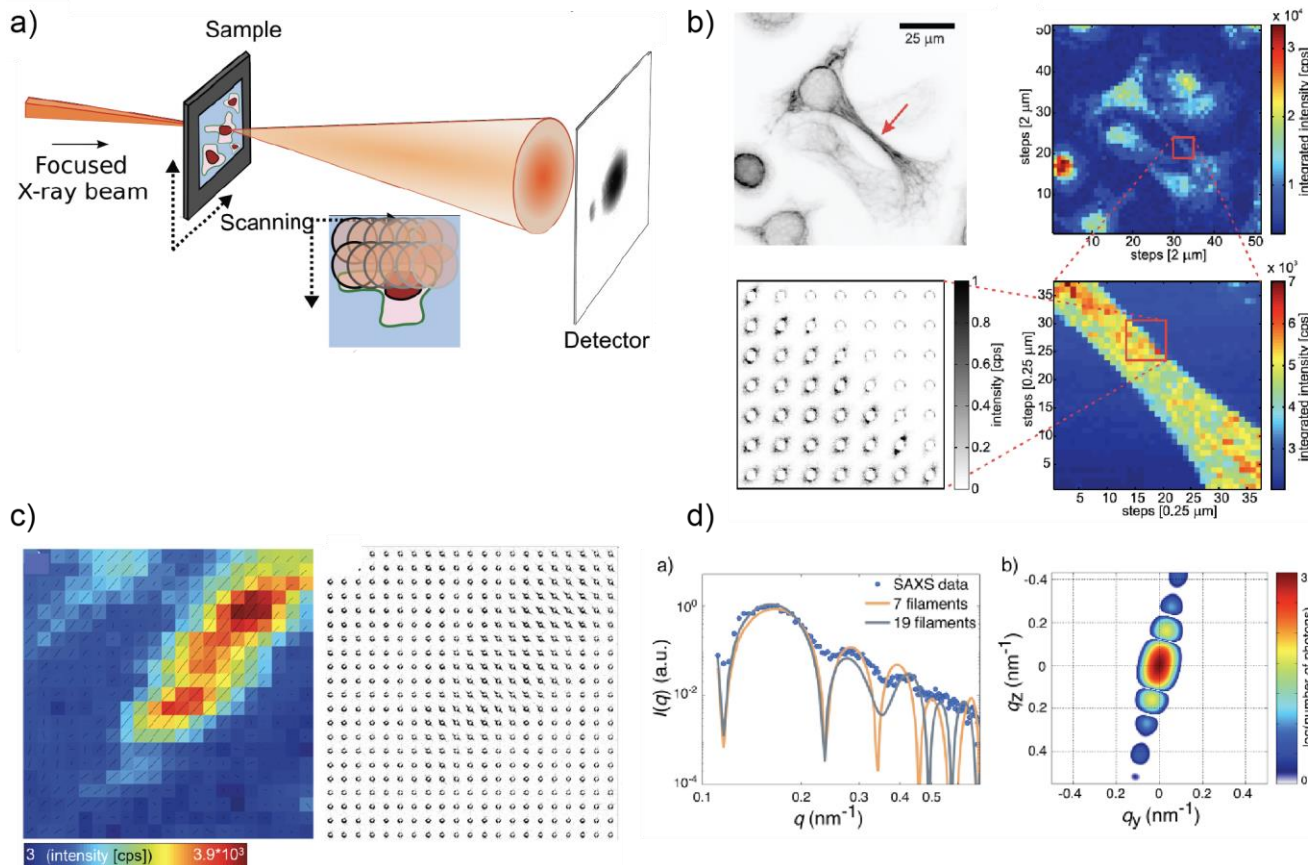
Figure. 1. a) Typical scanning SAXS setup (from [1]). b) Scanning SAXS on cytoskeletal keratin networks, including dark field and composite images showing anisotropic, oriented scattering patterns (from [2]). c) Scanning SAXS on hair cell stereocilia (inner ear, reprinted with permission from [5]. Copyright (2014) American Chemical Society). d) Fits to scanning SAXS data of keratin bundles, which reveal structural data such as filament diameter and distances (from [7]).

More recently, we have also started to combine scanning SAXS with other X-ray or non-X-ray techniques for complementary imaging. Whereas we routinely characterize the samples by bright field of visible light fluorescence microscopy prior to synchrotron measurements, we have recently combined different X-ray modalities such as holography and ptychography (see figure 2 a, b). Both methods impose a much-reduced dose on the sample than scanning SAXS and provide quantitative phase contrast. By contrast, scanning SAXS provides the full wealth of structural data when analyzing the reciprocal space data. Additionally, we have combined super-resolution microscopy (stimulated emission depletion, STED) with these X-ray methods, which adds yet another layer of information gained from the samples, that is the exact location of specifically labelled cellular components (figure 2c). Future experiment will exploit the novel "fast scanning" option to allow for imaging of ensembles of cell, thus providing statistically relevant data sets.
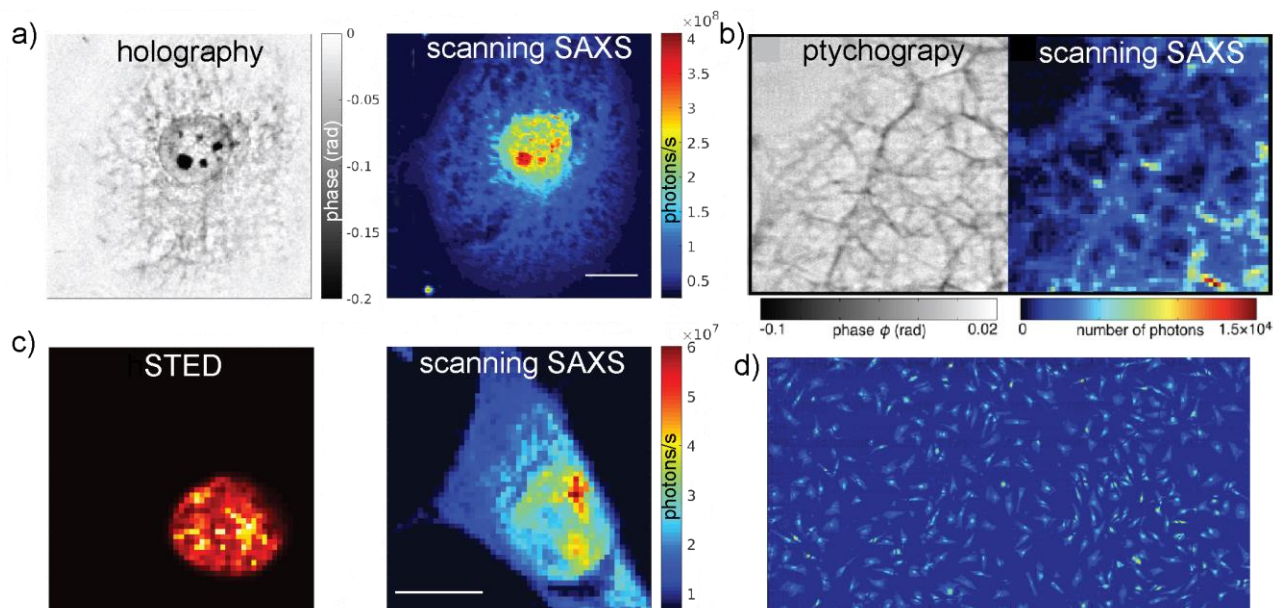
Figure. 2. a) Combination of scanning SAXS and holography on a whole cell. b) Combination of scanning SAXS and ptychography (reprinted with permission from [7]. Copyright (2016) American Chemical Society). c) Combination of scanning SAXS and STED on a whole cell with a fluorescently labelled nucleus. All scale bars: 10 µm. d) Scanning SAXS of a large cell ensemble, each bright spot represents a mouse fibroblast.

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