ESRF	**Experiment title:** Assessing bundling events in keratins by scanning micro-diffraction	**Experiment number**: SC4195
Beamline: ID13	**Date of experiment**: from: 04/12/2015 to: 08/12/2015	**Date of report**:
Shifts: 12	**Local contact(s)**: Britta Weinhausen	*Received at ESRF:*

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Report:

Motivation: The proposed experiment involved studying the ion induced bundling of keratin filaments. Keratin belongs to the intermediate filament (IF) protein family that constitutes the cytoskeleton of eukaryotes together with actin filaments and microtubules. In cells, the keratin bundles (lateral association of several filaments) provide a biophysical example of a highly ordered natural structure. Epithelial cells are continuously exposed to pressures and shear forces and these shear forces are to a great part being born by the keratin network within the cells. *In vitro*, the bundles can be formed by adding monovalent and/or multivalent ions to the keratin filament solution [1] and are visible by light microscopy (see Fig. 1a, c). However, investigating the internal structure of the bundles requires nanoscale imaging methods, such as x-ray diffraction using a micro-focused beam as we had previously demonstrated on keratin structures in whole freeze-dried and living cells [2, 3]. In this present study, we investigated a purified *in vitro* protein system with the goal to decipher the keratin signal without obscuring influences from other cellular components.

Experimental setup and data collection: The experiments were performed at ID13/EHII using a micro-focused beam (3.5 x 2.5 μm^2 horizontal x vertical). Keratin bundles were prepared on Topas (cyclic olefin copolymer, COC) windows (50 µm thickness) by a droplet-fusion technique. A droplet of protein solution was deposited on one window, a second drop of assembly buffer (KCl, $MgCl_2$ of different concentrations) was placed on another window and the two droplets were merged by putting the windows in contact [1, 4]. Bundles were localized and imaged for further reference using the microscope of the beamline laboratory. We scanned the sample through the beam and scattering patterns were recorded with an Eiger 4M detector placed at 0.75 m downstream of the sample.

Results: We recorded the scattering signal of keratin bundles assembled in presence of 8 different concentrations of $MgCl_2$, 6 concentrations of KCl and 2 samples in presence of both substances. For each concentration, several scans were performed at different positions. Similarly to our preliminary experiments (SC3960 and SC3188), we could observe anisotropy of the signal in some of the diffraction patters (see Fig.

1). The orientation angle of this anisotropic signal is automatically detected for all the frames, and black lines are plotted (perpendicular to the anisotropy thus displaying the real space orientation) to show the orientation of the fibers inside the bundles. Two examples are shown in Fig. 1a-d. Isotropic and anisotropic signals can be seen in Fig. 1e-f, which correspond to the pixels indicated by the white circles in Fig. 1b. At the merging point of different fibers (see point f) in Fig. 1b), the signal is isotropic whereas for an extended fiber, the signal is anisotropic (see point e) in Fig. 1b). We performed an azimuthal integration of the diffraction patterns. To do so, we used the major angle of the streak in the detector plane and performed the integration in 8 equal segments, with the streak aligned with two of these segments. The radial intensities of these "main" segments are represented in Fig. 1g for the isotropic signal (blue curve) and anisotropic signal (orange curve). We observe a change in the curvature of the signal at low q (steeper for the anisotropic signal). This hints structural differences between the extended fibers and merging points of different fibers. However, we are still in the process or analyzing the data as each dark field image contains up to 15,000 scattering patterns and the Eiger detector provides a wealth of data. We are currently searching for the most efficient way to analyze the data, also concerning the novel data format. One idea would be to compare the average signal of the different concentrations and ions, but this would lead to a loss of the anisotropic information, as the signal would be averaged over different orientations of different fibers. Another idea would be to study the signal from the cross-section and longitudinal section of the different bundles and compare them. Already visually, we can observe that KCl ions form more extended bundles, while $MgCl_2$ ions are cross-linking different fibers together.

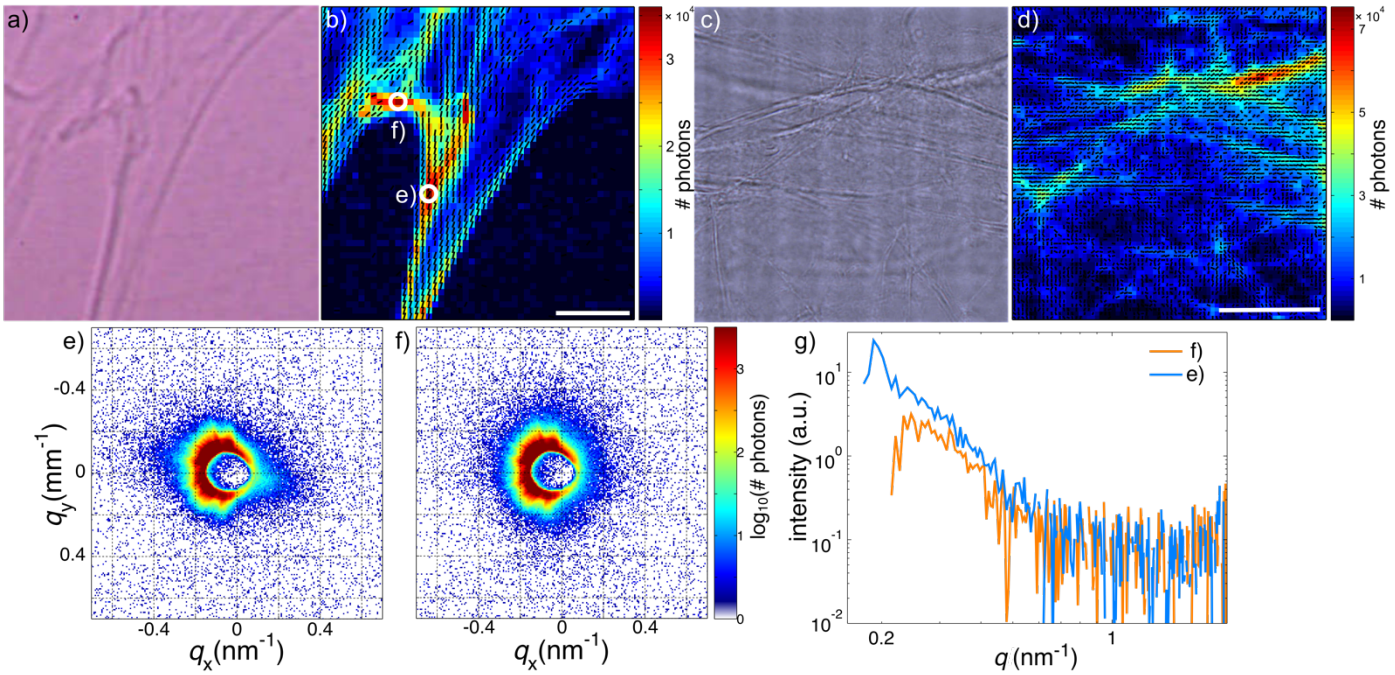

Figure 1: a) Micrograph of keratin bundles assembled in the presence of 1 mM $MgCl_2$. b) Dark-field representation of the same bundle, with the black lines representing the orientation of the fibers inside the bundles. c) Micrograph of keratin bundles assembled in the presence of 0.5 mM $MgCl_2$. d) Dark-field representation of the same bundle, with the black lines representing the orientation of the fibers inside the bundles. e) Diffraction pattern from b) showing an anisotropic signal. f) Diffraction pattern from b) showing an isotropic signal. g) Radial intensity of e) and f).

References

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