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# Experiment Report Form

**The double page inside this form is to be filled in by all users or groups of users who have had access to beam time for measurements at the ESRF**.

Once completed, the report should be submitted electronically to the User Office using the **Electronic Report Submission Application:**

*http://193.49.43.2:8080/smis/servlet/UserUtils?start*

***Reports supporting requests for additional beam time***

Reports can now be submitted independently of new proposals – it is necessary simply to indicate the number of the report(s) supporting a new proposal on the proposal form.

The Review Committees reserve the right to reject new proposals from groups who have not reported on the use of beam time allocated previously.

***Reports on experiments relating to long term projects***

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## Deadlines for submission of Experimental Reports

- 1st March for experiments carried out up until June of the previous year;
- 1st September for experiments carried out up until January of the same year.

## Instructions for preparing your Report

- fill in a separate form for each project or series of measurements.
- type your report, in English.
- include the reference number of the proposal to which the report refers.
- make sure that the text, tables and figures fit into the space available.
- if your work is published or is in press, you may prefer to paste in the abstract, and add full reference details. If the abstract is in a language other than English, please include an English translation.

| ESRF | **Experiment title:**<br>X-ray nanodiffraction on intracellular keratin bundles | **Experiment number**:<br>SC3188 |
|---|---|---|
| **Beamline**:<br>ID 13 | **Date of experiment**:<br>from: 30.06.2011 to: 05.07.2011 | **Date of report**: |
| **Shifts:**<br>15 | **Local contact(s)**:<br>Michael Reynolds | *Received at ESRF:* |

**Names and affiliations of applicants** (* indicates experimentalists):

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## Report:

We have studied the structure of the keratin intermediate filament (IF) network in eukaryotic SK8/K18 (IF free SW13 cells transfected with fluorescent keratin protein [1]) at the nano-beam set-up at EH3 of ID13. The keratin IF is one component of the cytoskeleton. It provides a pronounced tensile strength to epithelial cells and thus prevents cell rupture as a consequence of external stress. In cells, keratin IFs form bundles and dense networks (see fluorescence micrograph in Figure 1a) with bundle diameters between a few tens and a few hundreds of nm (measured by electron microscopy). Our goal was to probe the structure of individual bundles in the cell with a nano-focussed beam to obtain knowledge about characteristic length scales in the arrangement of keratin filaments to a bundle.

Samples of freeze-dried cells grown on silicon nitride membranes were prepared and characterized beforehand. During sample characterization, the keratin network in SK8/K18 cells was visualized using fluorescence microscopy (Figure 1a) and interesting cells for x-ray measurements were selected.

X-ray measurements were carried out in transmission geometry at a photon energy of 15.25 keV. The beam was focussed to 140 x 110 nm² (*h* x *v*) using the crossed nano-focusing refractive lens system, yielding a primary beam intensity of ~$3x10^9$ photons/s. Taking advantage of the high-magnification beamline microscope and the precise sample stage, the samples were positioned in the focus of the beam. After a coarse mesh-scan over a large sample area (not shown), region-of-interest-scans with a small step size on the order of 200 nm with long illumination times were performed. Figure 1b shows a dark field image reconstructed from a mesh scan on the keratin-rich "arm" of the cell in Figure 1a with a step size of 250 nm. The total diffraction signal on the cell's "arm" is significantly higher than the background scattering. A composite image of all diffraction patterns from this scan (see Figure 1c and the magnified region in Figure 1d) shows a high degree of oriented scattering in each diffraction pattern on the cell's "arm" corresponding to an aligned arrangement of keratin filaments in the bundles. In the magnified image the pronounced scattering at the outermost edge of the cell is clearly visible. Further steps in analysis include radial integration of diffraction pattern to determine intensity maxima corresponding to characteristic length scales or distances in the sample [2].

We also performed measurements using the same setup on a different biological sample, auditory stereocilia isolated from the mammalian inner ear. These hair cells´ protrusions are filled with a highly ordered array of actin filaments and our goal is to characterize these structures. By running a mesh scan with a step size of 200 nm on the freeze-dried stereocilia visible in the fluorescent image (Figure 2a, inset) we have reconstructed the correspondent dark field image (Figure 2a). The total diffraction signal on the structures is not as high as for the SK8/K18 cells (figure 1). Nonetheless, oriented scattering that varies between different regions of the

sample is observed (Figure 2b shows the magnified composite image of region E from Figure 2a). Single diffraction patterns from this scan reflect the predicted predominant direction of the actin structure in the selected spots (Figure 2c) [3].

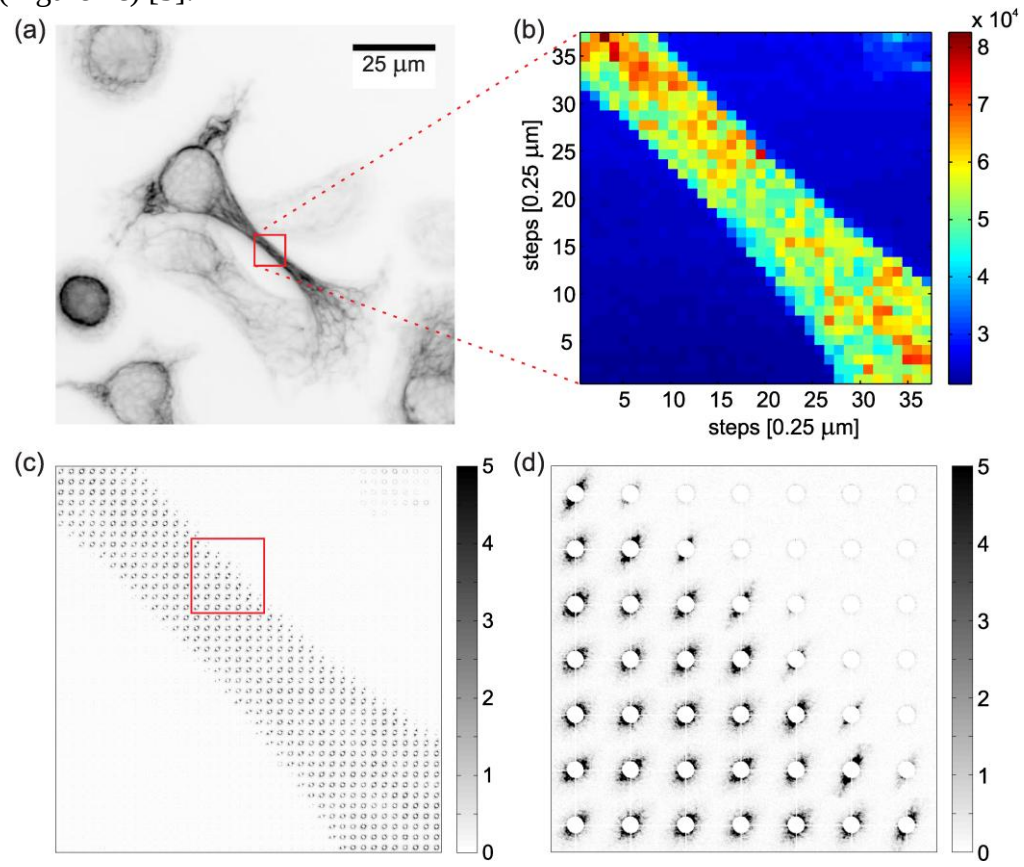


**Figure 1. Keratin networks in eukaryotic cells.** (a) Fluorescence microscopy image of the keratin network in SK8/K18 cells. This image was taken in on PFA-fixed cells, before plunge-freezing and freeze-drying. On the keratin-rich "arm" (red box) of one cell, a mesh scan was performed with a step size of 250 nm. (b) Dark field image of the mesh scan indicates strong scattering on the cell's "arm". (c) Composite image of all diffraction patterns from the same scan (4x4 binning) and (d) magnification of the diffraction patterns in the red box in (c) indicate a high degree of orientation of the keratin bundles in the measured regions, especially at the outermost edge of the cell.

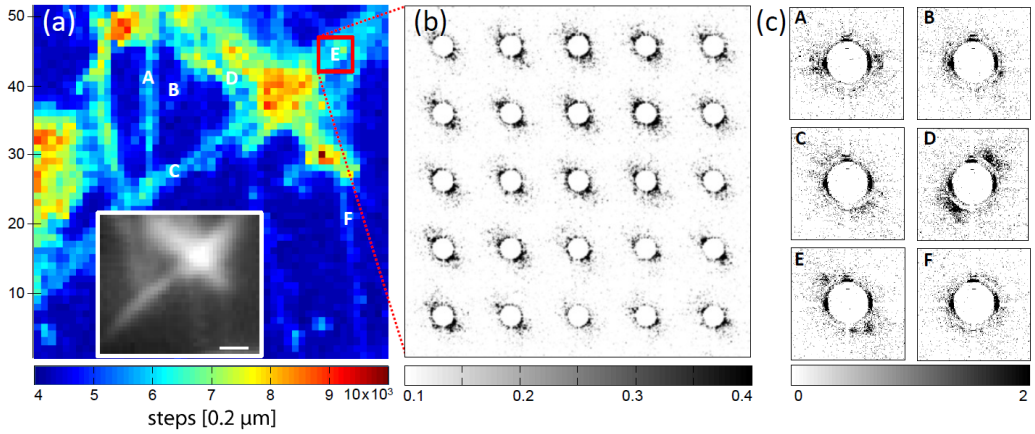


**Figure 2. Actin structure in stereocilia of hair cells.** (a) Dark field image of a mesh scan (step size 200 nm) of the stereocilia shown in the fluorescence micrograph (inset, bar: 5 µm). (b) Zoomed region E from panel a. (c) Representative diffraction patterns from selected spots (A – F) in panel a, showing the predominant direction of the respective actin structures.

## References


[1] A. Leibovitz, W. B. McCombs, D. Johnston, C. E. McCoy, and J. C. Stinson. New human cancer cell culture lines. I. SW-13, small-cell carcinoma of the adrenal cortex. *J. Natl. Cancer Inst.*, 51, 691 (1973); S. Wöll, R. Windoffer, and R. E. Leube. Dissection of keratin dynamics: different contributions of the actin and microtubule systems. *Eur. J. Cell Biol.*, 84, 311 (2005)

[2] B. Weinhausen et al., unpublished work

[3] V. Piazza et al, unpublished work