ESRF	**Experiment title:** Nanoimaging of diseased intracellular desmin	**Experiment number**: SC4893
Beamline: ID13	**Date of experiment**: from: 05.10.2018 to: 08.10.2018	**Date of report**:
Shifts: 9	**Local contact(s)**: Manfred Burghammer	*Received at ESRF:*

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Report:

Overview: The aim of this experiment was to apply our recently developed method for X-ray micro-diffraction on large ensembles of cells to the expression of a disease mutant of desmin intermediate filament proteins in cells. The biological relevance of this question lies in protein aggregates in the cytoplasm when desmin carries certain mutations and is probably related to severe muscle diseases. We took advantage of the fast scanning mode at the ID13 beamline to image hundreds of cells in a single measurement. Thus, structural information from the intracellular aggregates was collected for a statistically significant number of cells, from which we expect to shed some light on the mechanisms of misfolding.

Experimental setup and data collection: Three different cell lines were examined: mouse embryonic fibroblasts (MEFs) lacking vimentin [1], MEFs lacking vimentin and expressing wild type human desmin, and MEFs lacking vimentin and expressing a mutation of human desmin (R406W) known to cause the formation of abnormal protein aggregates in the cytoplasm and associated with muscular diseases (desminopathies [2]). Samples containing cells from one of the three cell lines were prepared by growing cells on silicon rich nitride windows with a total membrane area of 1.5 x 1.5 mm^2; the cells were then chemically fixed and freeze-dried at our home laboratory (as described in [3-5]). The experiments were performed at the EHII of ID13 using a microfocused beam (3 x 1.5 μm^2). The diffraction patterns were collected by an Eiger 4M detector (Dectris) placed 0.96 m away from the sample. Five windows, each containing one of the three cell types, were scanned in fast scanning mode [6] with a step size of 0.5 µm in both the horizontal and vertical direction, and an exposure time for each position of 3 ms. A step size smaller than the beam size was deliberately chosen, in order to better resolve cells in the dark field contrast (oversampling approach). Complete windows were scanned in single runs, amounting to more than 6,900,000 diffraction patterns each, with a total scan time of about 7.5 hours each. About 200 cells could be scanned on each window.

Results: Because of the large amount of data collected, data analysis is still in progress. From the already computed dark field contrast images (Figure 1a and 1c show examples for cells expressing the mutant and cells expressing the wild type desmin, respectively), a necessity to correct for variations in the incoming X-ray beam intensity (due to beam refills and to vibrations in the upstream apertures) emerges. Despite the short exposure time and comparatively large beam size, the spatial resolution of the dark field image allows to clearly distinguish the cells from the background and the nuclear regions from the cytoplasm, as shown in the enlargements in Figure 1b and 1d. Conversely, there are no evident differences between the cells expressing wild type desmin and those expressing mutant desmin. However, we expect differences to emerge from the analysis of individual diffraction patterns, which carry structural information in the nm to tens of nm range. The diffraction pattern analysis we will use has already been described in [4-5]. Self-written Matlab scripts will enable us to speed up the segmentation of the dark field images into regions of interest, so that a statistically relevant analysis of each cell population will be possible in reasonable times, and the results for different cell lines will be compared.

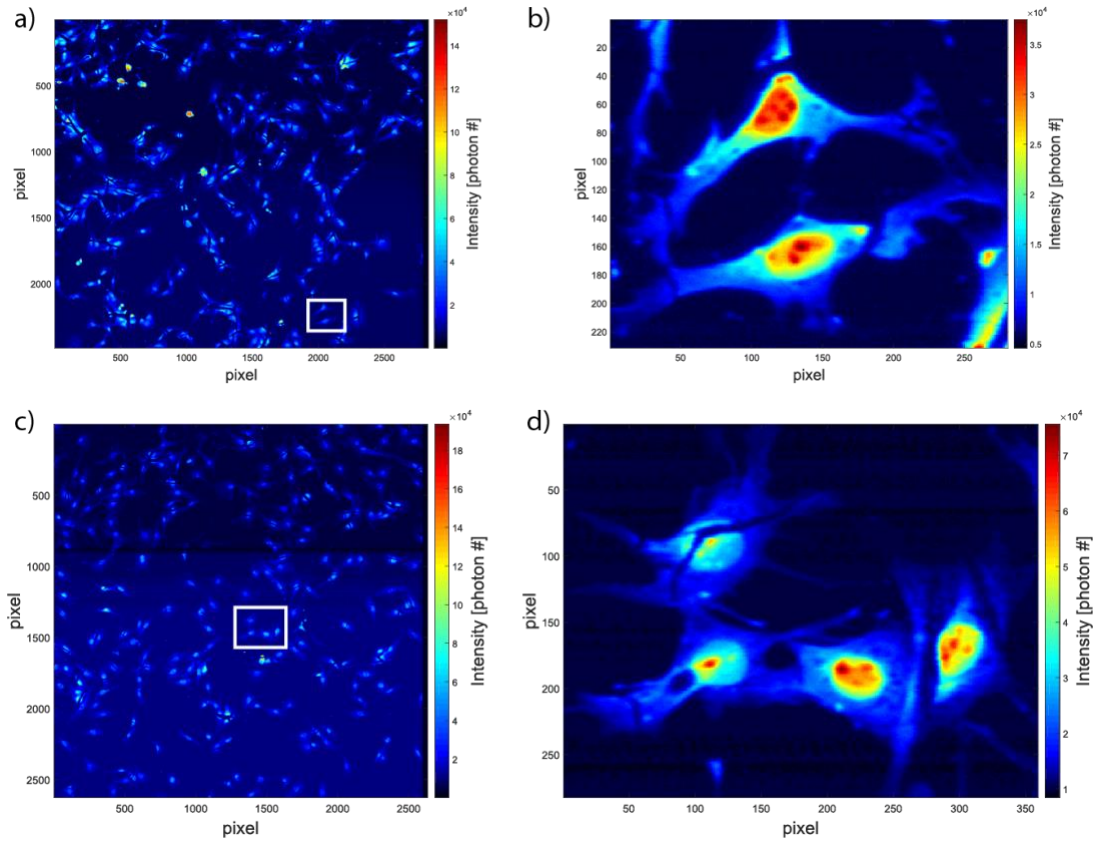

Figure 1: a) Dark field image of a silicon nitiride window carrying MEFs lacking vimentin and expressing R406W human desmin. **b)** Enlargement of the area contained in the white box in a: the cells and their nuclear regions are clearly visible. **c)** Dark field image of a silicon nitiride window carrying MEFs lacking vimentin and expressing wild type human desmin. **c)** Enlargement of the area contained in the white box in c.

References:

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[3] Hémonnot, Clément YJ, et al. "X-rays reveal the internal structure of keratin bundles in whole cells." *ACS Nano* 10.3 (2016): 3553-3561.
[4] Hémonnot, Clément YJ, et al. "Following DNA Compaction During the Cell Cycle by X-ray Nanodiffraction." *ACS Nano* 10.12 (2016): 10661-10670.
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[6] Nicolas, J-D., et al. "Scanning X-ray diffraction on cardiac tissue: automatized data analysis and processing." *Journal of Synchrotron Radiation* 24.6 (2017): 1163-1172.