ESRF	**Experiment title:** Chitin fiber orientation in locust wing membranes investigated using scanning nanodiffraction	**Experiment number**: SC-5008
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Report:

Using scanning X-ray nanodiffraction we investigated the membrane cells and their connection with the wing veins of the desert locust *Schistocerca gregaria* hind wing in different regions. These membranes and veins are located along the span and chord of the wing, show different deformation and bear different loading, while providing efficient performance during flying. A specific variation of fiber orientation and organization of the wing cuticle is assumed to be responsible for the structural strength and stiffness stability at dynamic deformation and loading during flight.

In order to gain information on the orientation and distribution of the chitin fibers, pieces from selected regions of the wing were mounted on silicon windows and scanned in the X-ray beam by means of a nanometer precise manipulator. Mapping different regions of the wing membrane and veins, as well as the merging regions between membranes and veins and vein joints were performed using the combined small angle X-ray scattering (SAXS)/wide-angle X-ray scattering (WAXS) setup including a helium-filled flight tube available for the nanofocus scanning setup at ID13. Mapping the diffraction patterns was aimed for the reconstruction of changes in fiber orientation in the wing.

The mapping X-ray diffraction experiments was performed at the nanofocus experimental hutch EH III of the beamline ID13 using a beam size of 200 nm at a spatial resolution of 250 nm. Square-shaped patches were mapped in areas of 400 μm^2 and in line scans up to 600 µm

long and 9 µm wide. The beam energy was 14.85 keV. Overall, ten different samples of the wing were scanned. For each sample, at least twelve square patch-scans (ps) on the membrane and two line-scans (ls) across the longitudinal and cross veins were performed (Fig. 1a). In total, we were able to collect the data of 912,000 diffractograms.

The WAXS-signals contain information about the orientation of the chitin fibers. An original diffraction pattern of a single exposure location is shown in Fig. 1b. Converting the original diffraction pattern to 2D azimuth integral (Fig. 1c) enables further analysis of the oriented angle of chitin fibers and other structures that still have to be identified (for example at q = 1.45). Furthermore, the oriented angle of chitin from each exposure location was summed up to plot orientation maps for the whole patch (Figs 1d,e). The analysis of the angle of orientation shows that at least the majority of the chitin fibers from the membrane were oriented along the span of the wing. Further analysis focusing to compare fiber orientation in different regions is underway.

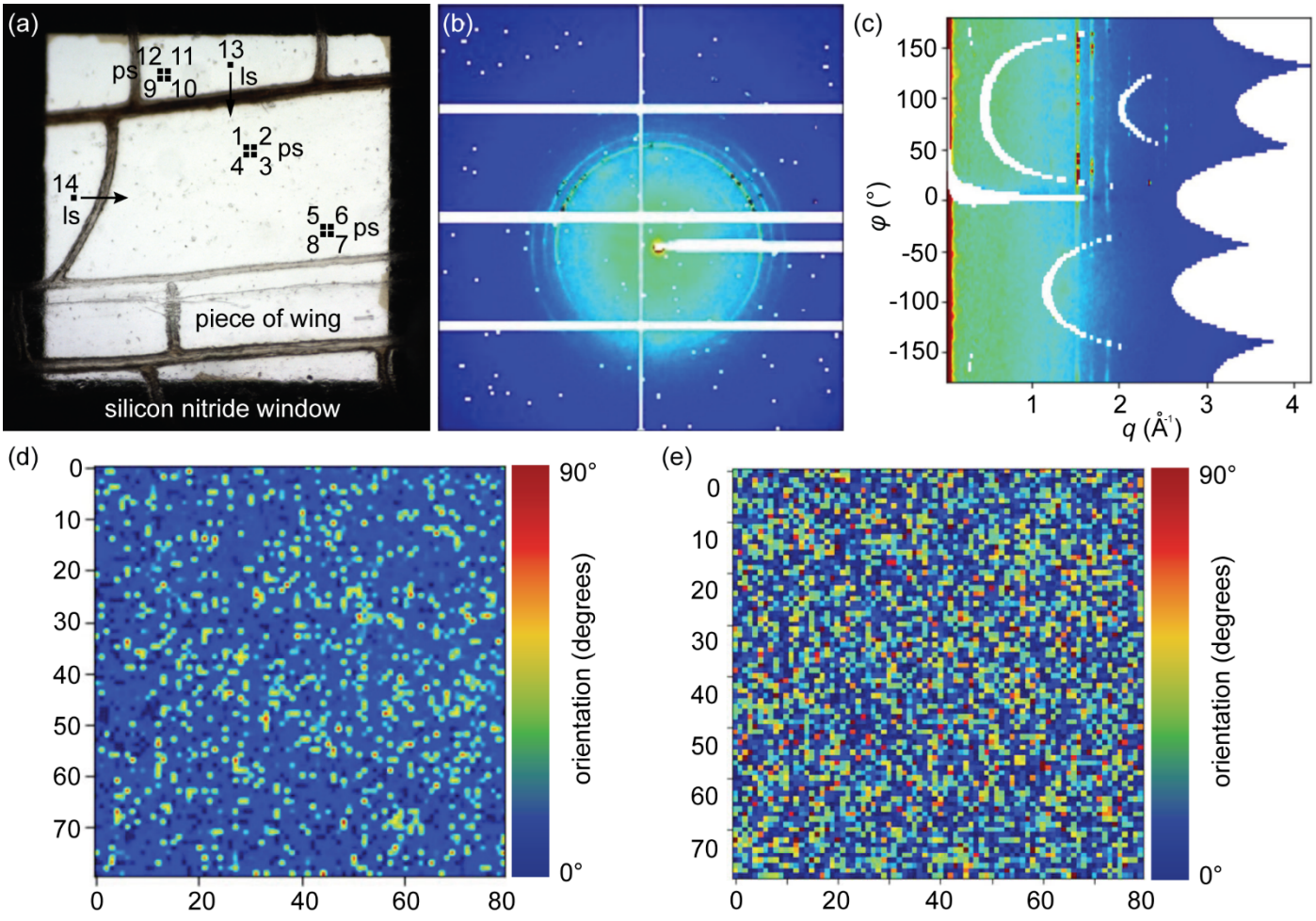

Figure 1 (a) Light microscopy image indicating the locations of patch-scans (ps) and line-scans (ls) of a locust wing sample. (b) Original diffractogram of the SAXS-signals of an exposure location. (c) 2D azimuth integral of the diffractogram in (b) using a software routine in pyFAI. (d) 2D orientation map of the chitin fibers from patch 1 in (a), showing the color-coded fiber orientation in the membrane plane. Dark blue indicates a horizontal orientation of the chitin fibers (from the chitin specific signal at 1.86 Å), dark red indicates a vertical orientation of the fibers. (e) Out-of-plane orientation map of the chitin fibers corresponding to (d) Blue indicates that the chitin fibers were oriented within the membrane plane, while red indicates that the fibers were oriented perpendicular.