ESRF	**Experiment title:** Unveiling the topmost layers of spider silk by ultra-high resolution mapping of sections	**Experiment number**: LS-3087
Beamline: ID13	**Date of experiment**: from: 08 June 2022 to: 12 June 2022	**Date of report**:
Shifts: 12	**Local contact(s)**: Jiliang Liu	*Received at ESRF:*

Names and affiliations of applicants (* indicates experimentalists):

Karolina Peter* (1), Leon Ploszczanski* (1), Arno Frank (2), Christian Riekel* (3), Sarah Stadlmayr (4), Aida Naghilou (4), Hannes Schniepp (5), Christine Radtke (4), Helga Lichtenegger (1)

1) University of Natural Resources and Life Sciences, Vienna, Department of Material Sciences and Process Engineering, Institute of Physics and Materials Science, Peter-Jordan-Straße 82, 1190 Vienna, Austria
2) Technical University of Vienna, X-ray center, Getreidemarkt 9, 1060 Vienna, Austria
3) ESRF, The European Synchrotron, 71 Avenue des Martyrs, CS40220, 38043 Grenoble Cedex 9, France
4) Department of Plastic, Reconstructive and Aesthetic Surgery, Medical University of Vienna, Spitalgasse 23, 1090 Vienna, Austria
5) Department of Applied Science, William & Mary, P.O. Box 8795 Williamsburg, VA 23187-8795, USA

Report:

A series of nanobeam X-ray diffraction measurements (nanoXRD) on different samples of spider silk (SPSI) were carried out in context of experiment LS-3087. The on-site experimental team comprising Karolina Peter, Leon Ploszczanski, Arno Frank and Dr. Christian Riekel was supported by ID13 staff members: Jiliang Liu (local contact) and Manfred Burghammer. Thanks to the stable ID13 beamline operation and excellent local organization, all prepared samples could be measured. .

Experiments were performed on whole fibers and cryosections of SPSI. For cryosectioning, a bundle of silk fibres was embedded in OCT (Opt. Cutting Temperature Comp.) and cut with a cryo immuno knife (Diatome) to 1-3 μm thickness. Species used for sectioning were *Nephila inaurata* (*N. inaurata*), *Nuctenea Umbratica* (*N. umbratica*), *Phidippus regius* (*P. regius*), *Peucetia lucasi* (*P. lucasi*). The larger diameters of *N. inaurata* and *N. umbratica* fibers allowed preparing and probing longitudinal and perpendicular sections to the fiber axis. We also probed longitudinal and transverse sections of *N.inaurata* dragline silk as well as from the two morphologically distinct silks of the egg sac, in addition to whole fibers. Cross-sections and whole fibers were also probed for *P. regius* and *P. lucasi*. All sections and most whole fibres were mounted on Si_3N_4 membranes (Norcada). For *P. regius*, the very fragile fibres had to be mounted on a plastic frame with a central hole. Despite the challenging sample preparation,

which has not yet been illustrated in the literature for silk fibers, we have succeeded with cryosectioning in preparing sections of silk in such a way that they can be probed by nanoXRD.

The samples were probed with a 70 nm fwhm beam. Systematic tests of sample stability up to sectioning of fibers suggested exposure times of 5-10 ms/pattern for limiting radiation damage. We performed fly scans with e.g. 20x20 µm mesh at a position previously chosen on the microscope. A step size of 100 nm was also chosen based on stability tests. Calibrated sample detector distances of 741.6 mm and 194.7 mm respectively allowed optimizing the patterns for the SAXS and WAXS range. Here we show preliminary composite SAXS intensity maps based on pixels with specific SAXS features extracted from individual patterns. Indeed, optical images of SPSI cross sections are shown in Fig. 1 together with the corresponding microscope images. Detoriation of the OCT embedding material could be observed after the experiments. The possibility of a more radiation resistant embedding medium is currently explored for future experiments. Composite SAXS intensity maps of SPSI longitudinal sections with the corresponding microscope images are shown in Fig. 2. Whole fibres of different species were also sucessfully probed. Fig. 3 shows composite SAXS intensity maps of *P. regius* dragline silk and *P. lucasi* dragline silk.

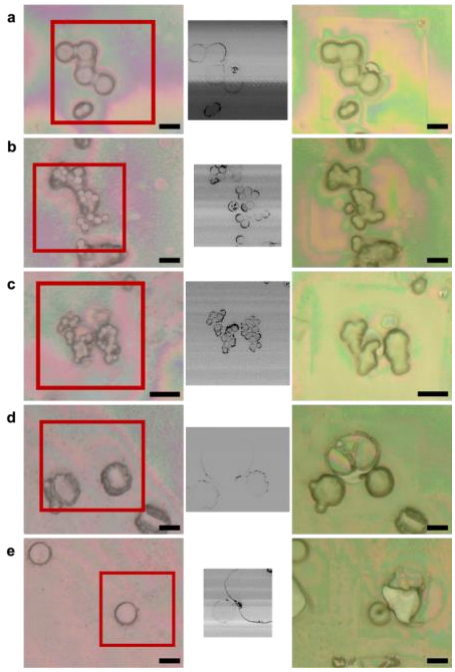

Fig. 1 Examples of cross-sections used for scanning nanoXRD. Left column: Pre-measurement microscope image with region of interest (roi - red frame). Middle: SAXS intensity maps of roi. Right: Post-measurement microscope images. Bar corresponds to 10 µm.

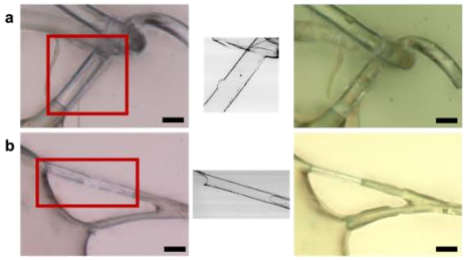

Fig. 2 Longitudinal sections of SPSI from N. inaurata (a) and N .umbratica (b). Left:Pre-measurement microscope image. Middle: Composite SAXS intensity map from nanoXRD measurement. Right: Post-measurement microscope image.

An interesting observation during sample preparation was, that the egg sac silk of spider *N. inaurata* shows two types of silk different in morphology. A "smooth" looking silk and a "grooved" appearing silk. In Figure 4 composite SAXS maps of these two different silk types are shown.

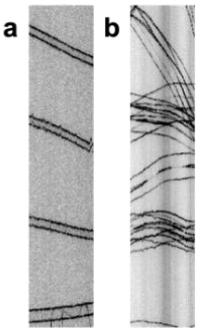

Fig. 3 Composite SAXS intensity maps of P.regius (a) and P.lucasi (b).

We are currently in the process of data integration with pyFAI, generating first composite maps and identifying regions of interest for further analysis. WAXS as well as SAXS features will be analyzed for all samples probed. We are particularly interested in analyzing the top most layers of the silk and reveal possible skin-core variations in ultrastructure. For this purpose the ultra-high resolution will help a lot.

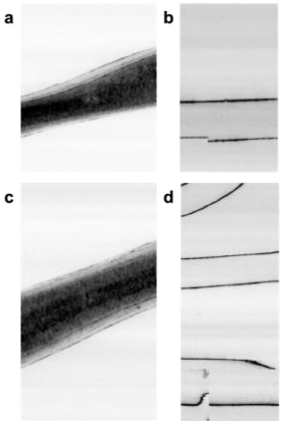

Fig. 4 Composite SAXS intensity maps of two different silk types of N.inaurata egg sac silk. Morphologically "grooved" (a,c) and "smooth" silk (b,d). WAXS experimental setup (a,b) and SAXS setup (c,d).

In summary, extensive data could be obtained in these 12 shifts of beamtime. Preliminary data analysis already shows interesting features (like the two different types of silk in the *N.inaurata* egg sac silk) which will be further investigated.