



	<b>Experiment title:</b> Phase contrast-enhanced tomography of spiders mechano-sensors in their working state	<b>Experiment number:</b> SC-3725
<b>Beamline:</b> ID19	<b>Date of experiment:</b> from: 13 Nov 2013 to: 18 Nov 2013	<b>Date of report:</b> 01 July 2015
<b>Shifts:</b> 12	<b>Local contact(s):</b> Alexander Rack	<i>Received at ESRF:</i>
Names and affiliations of applicants (* indicates experimentalists): Y. Politi*, P. Fratzl, M. Erko* Max Planck Institute of Colloids and Interfaces, Department of Biomaterials, 14424 Potsdam, Germany		

## Report:

Mechano-sensing in spiders is well recognized for its high sensitivity and specificity [1]. The widespread spiders' strain detectors (slit sensilla) are elongated openings within the exoskeleton, innervated by sensory cells. The metatarsal lyriform organ (HS10) of the Central American wandering spider *Cupiennius salei* forms an array of 21 slits (figure 1). Substrate vibrations deflect the most distal leg segment, the tarsus, which in turn transmits the signal to the vibration-sensitive slit sensilla by pressing against the metatarsus. A cuticular pad (figure 1b–d) is located just in front of the slit lyriform organ, at the contact area with the tarsus. The pad plays a major role in the mechanical filtering of vibrational stimuli by forming an effective high-pass filter. Here we report our last experiment at ESRF at the micro-CT beamline ID19 in which we obtained detailed 3D models of the slit sensing organ and especially the cuticular pad by micro-CT employing phase-contrast enhanced radiographs [2] in hydrated state upon compressions mimicking reception of a mechanical signal. In order to stationary simulate the “working” state of the organ we used the ID19 beamline Tomopress device [3] dedicated to in-situ compression tests during  $\mu$ CT measurements. A self-built sample holder was adjusted to the Tomopress allowing us to control the tarsus to meta-tarsus deflection angle (figure 2).

The experiments were performed at beamline ID19. In order to find a compromise between high imaging sensitivity and low radiation damage, the samples were measured at 26 keV photon energy. A single harmonic undulator (u13, gap 11.5 mm) with a narrow bandwidth was used as radiation source with a diamond filter and a Be window as the only optical elements in the beam path, leading to a homogeneous wavefront at the position of the experiment and therefore excellently suited for X-ray phase contrast imaging in parallel-beam geometry. Approximately 35 mm downstream of the specimen a high-resolution indirect imaging detector was placed (ESRF custom made CCD camera FReLoN (type: A7899)) operating with a nominal effective pixel size of 0.7 mm. The exposure time was set to 0.2 s for each of 1000 projection images while rotating the sample through 180°. Pad morphology data were reconstructed using the ESRF software package PyHST\_2, which includes a phase-retrieval using Paganin's approach [4]. For data visualization and segmentation, ZIBAmira software (Zuse Institute, Berlin, Germany; FEI Visualization Science Group, Burlington MA, USA) was used.

The results of this experiment show the compression of the pad at high tarsal deflections levels. These results were central for our publication on the spiders signal transmitting mechanisms published in the journal of the Royal Society Interface [5] – see figure 3. The data also show the compression of the slits, however here we intend to repeat these experiments with smaller angular step size at the small deflection levels.

## References:

[1] Barth FG. (2004) Spider mechanoreceptors. *Curr. Opin. Neurobiol.* 14, 415–422. (doi:10.1016/j.conb.2004.07.005)

- [2] Weitkamp, T., Haas, D., Wegrzynek, D., Rack, A., ANKPhase: software for single-distance phase-retrieval from inline X-ray phase contrast radiographs. *Journal of Synchrotron Radiation* **18** (2011).
- [3] Dario P, Chiara Carrozza M, Beccai L, Laschi C, Mazzolai B, Menciassi A (ed.). Design, fabrication and applications of biomimetic sensors in biorobotics. In *International Conference on Information Acquisition; 2005-2006 Hong Kong, China*. Piscataway.
- [4] Weitkamp T, Haas D, Wegrzynek D, Rack A. 2011 ANKPhase: software for single-distance phase retrieval from inline X-ray phase-contrast radiographs. *J. Synchrotron Radiat.* **18**, 617–629.
- [5] M. Erko, O. Younus-Metzler, A. Rack, P. Zaslansky, S. Young, G. Milliron, M. Chyasnovichyus, F. G. Barth, P. Fratzl, V. Tsukruk, I. Zlotnikov and Y. Politi. (2015) Micro- and nano-structural details of a spider's filter for substrate vibrations. Relevance for low frequency signal transmission. *Royal Soc. Interface.* **12**: 20141111.



Figure 1. (a) Adult female spider *Cupiennius salei*. (b) Magnification of the two last (distal) leg segments; the metatarsus and the tarsus. The cuticular pad is situated at joint between the two segments (arrow). (c) Optical light microscope image of the cuticular pad and the vibration receptor of the spider. Top view on the dorsal side of the pad. figure adapted from Ref [5]



Figure 2. the spider tarsus-metatarsus joint placed in the Tomopress in-situ compression tester. The wedge on the left allows to control the angle between the tarsus and the meta-tarsus without application of additional external load. The magnetic sample holder on the right allows to control the orientation of the leg-joint in the beam path. The chamber was sealed with kapton foil and a humidity generator was used to maintain constant humidity level.

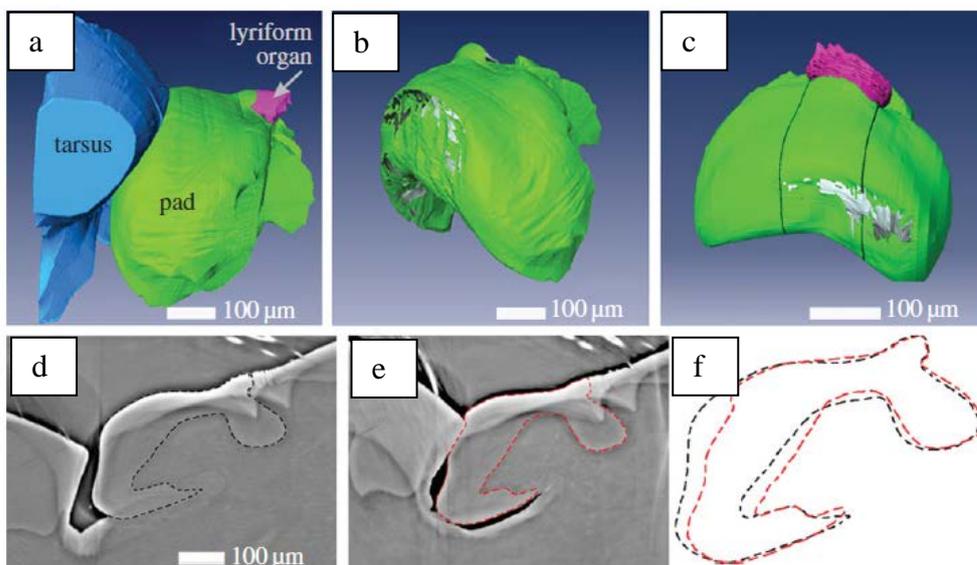


Figure 3. (a) Surface rendering of reconstructed mCT data showing three selected components of the metatarsal vibration receptor including the tarsus (blue), the pad (green), and the slit-sensilla lyriform organ (pink) measured during contact. The deflection angle between the tarsus and metatarsus was 98. (b) Three-dimensional shape of the cuticular pad extracted from (a). Grey regions at the distal side of the pad indicate the contact area with the tarsus. (c) Three-dimensional shape of the cuticular pad under load with a slight lateral component. The tarsus-metatarsus angle was 88. Grey regions at the distal side of the pad indicate the contact area with the tarsus. (d-f) mCT virtual slices of the sample in a-b sectioned in the sagittal plane in the centre of the pad in relaxed (d) state (less than 08), and deflected by 98 (e). The dashed lines indicate the outline of the cuticular material of the pad. The white arrows indicate one slit of the metatarsal lyriform organ. Darker region below the ventral side of the pad is caused due to reduction in vapour pressure; the pad itself however is still moist. (f) An overlay of the pad shape from d and e. figure adapted from Ref [5]