# *NANOCT IMAGING OF AN ONYCHOPHORAN LIMB*

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**Summary:** X-ray microCT has established itself as a valuable imaging technique to reveal fine inner structures in 3D. However, most laboratory CT systems struggle, when it comes to resolutions below the micrometre range. We present a table-top nanoCT system foregoing any X-ray optics that reaches resolutions down to 120 nm. To explore the exceeding potential of the nanoCT, we investigate a limb of *Euperipatoides rowelli*, a representative of the invertebrate group Onychophora.

## 1. INTRODUCTION

In recent years, the field of microCT imaging has evolved striving for higher resolutions below the micrometre range. At synchrotrons, due to the high brilliance of the radiation, resolutions in the nanometre range are already possible [1]. Laboratory-based setups are required to use different approaches to resolve structures below the micrometre range. One of the two main approaches is to use X-ray optics, such as Fresnel zone plates, combined with high flux sources. Thereby resolutions below 100 nm are accomplished [2]. Another technique is based on the combination of mere geometrical magnification and an X-ray source featuring small X-ray focal spots. Until recently, this approach was limited to a maximum resolution of about 400 nm. Despite the lower resolving power, setups using solely geometrical magnification have proven themselves valuable since they allow larger FOVs, shorter acquisition times and work with broad energy spectra [3].
We present an innovative table-top setup, which is based on geometrical magnification and is comprised of a nano-focus X-ray source and a single photon counting detector. The setup achieves a resolution down to 120 nm. This allows non-destructive imaging of inner structures in 3D with very high resolution. These features have been applied for investigating and tracking the hitherto understudied muscular structures of the limb in a representative of Onychophora, or velvet worms.

## 2. EXPERIMENTAL METHOD

The setup is comprised of a nanofocus X-ray source, an overhead rotation stage and a single-photon counting detector. The system does not contain any X-ray optics and is solely based on geometrical magnification.
The nanofocus X-ray tube (Excillum AB, Sweden) provides full control of the spot size. Advanced electron optics in combination with a thin tungsten transmission target enables the source to reach focal spots with FWHM values down to 200 nm in its current state. The X-ray camera is a PILATUS 300K-W 20 Hz detector system with a 1.0 mm thick silicon sensor with an image area of 1475 x 195 square pixels with a side length of 172 µm. It was operated in single-photon counting mode, which allows signal acquisition without readout or dark current noise. Thereby, it ensures good image quality even with low X-ray flux [4]. The FOV is given as 1400 x voxel size in horizontal direction and 190 x voxel size in the vertical direction. The FOV can be extended vertically by combining several CT scans.
The maximum resolution in the reconstructed CT volume was determined sampling a highly refracting glass microsphere with a diameter ≈ 30 µm. An isotropic FWHM of 120 nm was calculated by deriving the LSF from the edge profiles from the reconstructed CT volume of the sphere.

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For the velvet worm data of the entire limb, 10 datasets were acquired and subsequently combined vertically to the presented volume data. Each dataset was measured over 360° and the source was operated at an acceleration voltage of 60 kV, resulting in a mean spectral energy of 20 keV.
The acquired projections were further processed with a Richardson-Lucy deconvolution algorithm [5, 6] to enhance the sharpness of the image. A phase-retrieval algorithm was applied to dampen the image noise, to correct for edge enhancement effects and to improve the soft tissue contrast [7]. The sample was reconstructed using a filtered backprojection algorithm (FBP).
The onychophoran limb sample was fixed, contrasted in 2% osmium tetroxide, dehydrated in an ethanol series, dried in a critical-point dryer and mounted onto sample holders for the nanoCT.

## 3. RESULTS

To explore potential applications in the field of biology, we imaged a 400 µm long walking appendage of the onychophoran species *Euperipatoides rowelli* with a voxel size of 400 nm.
The resulting data set exhibits a high level of detail of the surface structures, such as the claw and the spinous pad (Figure 1). Moreover, it allows single muscle fibers to be followed internally and a precise reconstruction of the entire limb musculature. Herein, we reconstructed, for example, two of the prominent muscles: the claw retractor muscle and the leg remoter muscle (Figure 1). The reconstructed volume provides new information about the position, shape and attachment sites of these muscles. Thereby, biologists are now able to draw new conclusions about the function and the role of each limb muscle, and previous studies can be now revised and improved.

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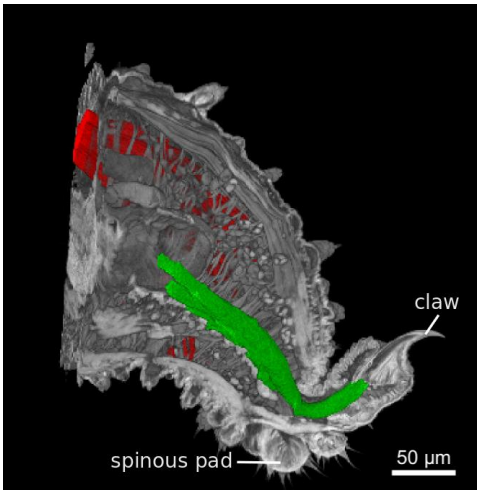


**Figure 1:** Digital sagittal section through the limb of the onychophoran species *E. rowelli* showing the leg musculature. The claw retractor muscle is segmented in green and the leg remoter in red.