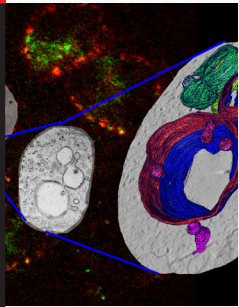
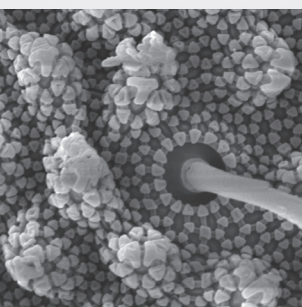


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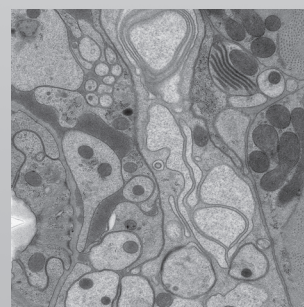
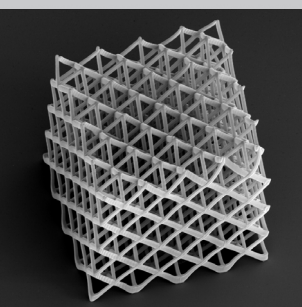
Life Science
Research

Application Booklet

Micro-CT Analysis

related instrument Leica EM CPD300

Medical
Research



Industrial
Manufacturing



Natural
Resources



CRITICAL-POINT DRYING FOR THE PREPARATION OF BIOLOGICAL SAMPLES FOR MICRO-CT ANALYSIS

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X-ray micro-computed tomography (micro-CT) is a routinely applied non-invasive technique for the investigation of the internal anatomy and morphology of organisms. As a result of a micro-CT scan a stack of grey-scale images is generated from a series of projections taken at defined angles during sample rotation. Since several years the number of lab-based micro-CT imaging systems is constantly growing making this technique available to a broad spectrum of researchers and applications.

Similar to other imaging techniques such as scanning electron microscopy, micro-CT allows to study biological samples in nearly every condition (e.g. fresh, dried or within preservatives). Micro-CT is the ideal technique for studying bones, teeth and shells in 3D, but the analysis of soft tissue is significantly influenced and hindered by its low absorption contrast based on the presence of compounds with low-atomic number elements. In order to overcome this limitation, several approaches can be applied including different staining and/or drying techniques as well as phase-related contrast imaging. However, whenever possible samples should be analyzed in dry condition as it provides a significantly higher signal to noise ratio compared to samples scanned in liquid. In order to dry delicate biological samples, critical point drying was proofed to be the best method compared to e.g., chemical or air drying as it preserves the structures while minimizing artifacts such as shrinkage of tissue and distortion.

MICRO-COMPUTER TOMOGRAPHY PROTOCOLS

1. Micro-CT of Book Scorpion Musculature

Introduction:

Species: Book scorpion (*Neobisium* sp.)

Critical point drying of book scorpion with subsequent X-ray micro-computed tomography (micro-CT) to detect anatomical features with special regard to the musculature.

Procedure:

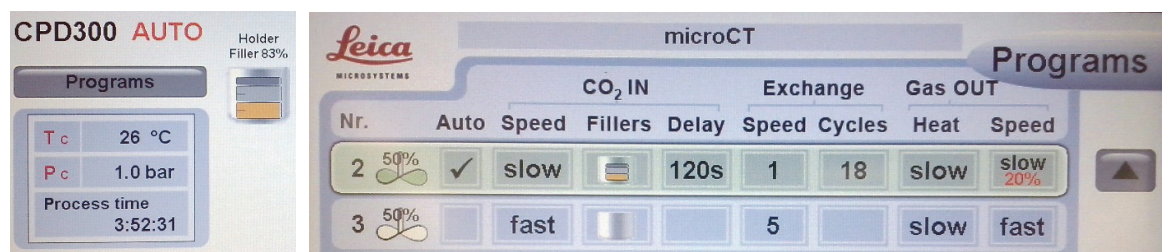
Sample Holder:

Sample was transferred to microporous specimen pot and placed into chamber of Filter Discs and Porous Pots holder.

Fixation and Dehydration:

| | |
|--|------------|
| Ethanol (70%) | overnight |
| Ethanol series: 80%, 90%, 96%, 100% | 2x 10 min. |
| Iodine staining (1% iodine solution in 100% ethanol) | overnight |
| Ethanol: 100% | 2x 10 min. |

CPD300 auto Program:



Mounting and Scanning:

Dried sample was glued on an insect pin and scanned with an Xradia MicroXCT-200 X-ray imaging system (Carl Zeiss X-ray Microscopy Inc., Pleasanton, USA).

Results:



Volume reconstruction of a book scorpion prosoma (outer view and inner view to visualize the musculature).

Courtesy of Elisabeth Lipke and Dr. Peter Michalik, University of Greifswald, Germany.

2. Micro-CT of Insect Brain Protocol

Introduction:

Species: Blow fly (*Lucilia caesar*)

Critical point drying of the blow fly with subsequent X-ray micro-computed tomography (micro-CT) to detect neuroanatomical features.

Procedure:

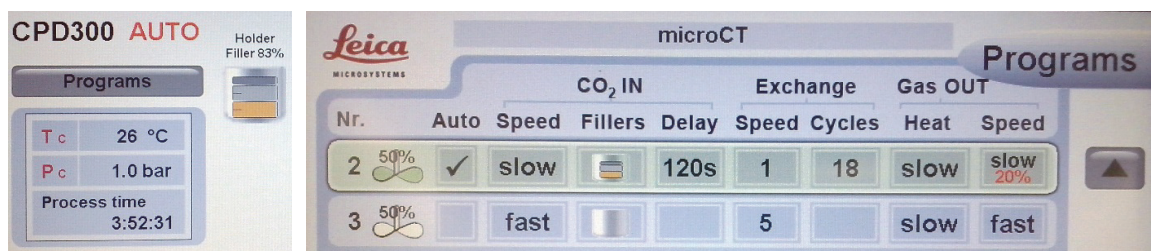
Sample Holder:

Samples were placed individually in the chambers of Arthropoda holder.

Fixation and Dehydration:

| | |
|--|------------|
| Bouin's fixative | overnight |
| 0.1 M phosphate buffer (1.8% Sucrose, pH 7.2) | 3x 10 min. |
| Ethanol series: 60%, 70%, 80%, 90%, 96%, 100% | 2x 10 min. |
| Iodine staining (1% iodine solution in 100% ethanol) | overnight |
| Ethanol: 100% | 2x 10 min. |

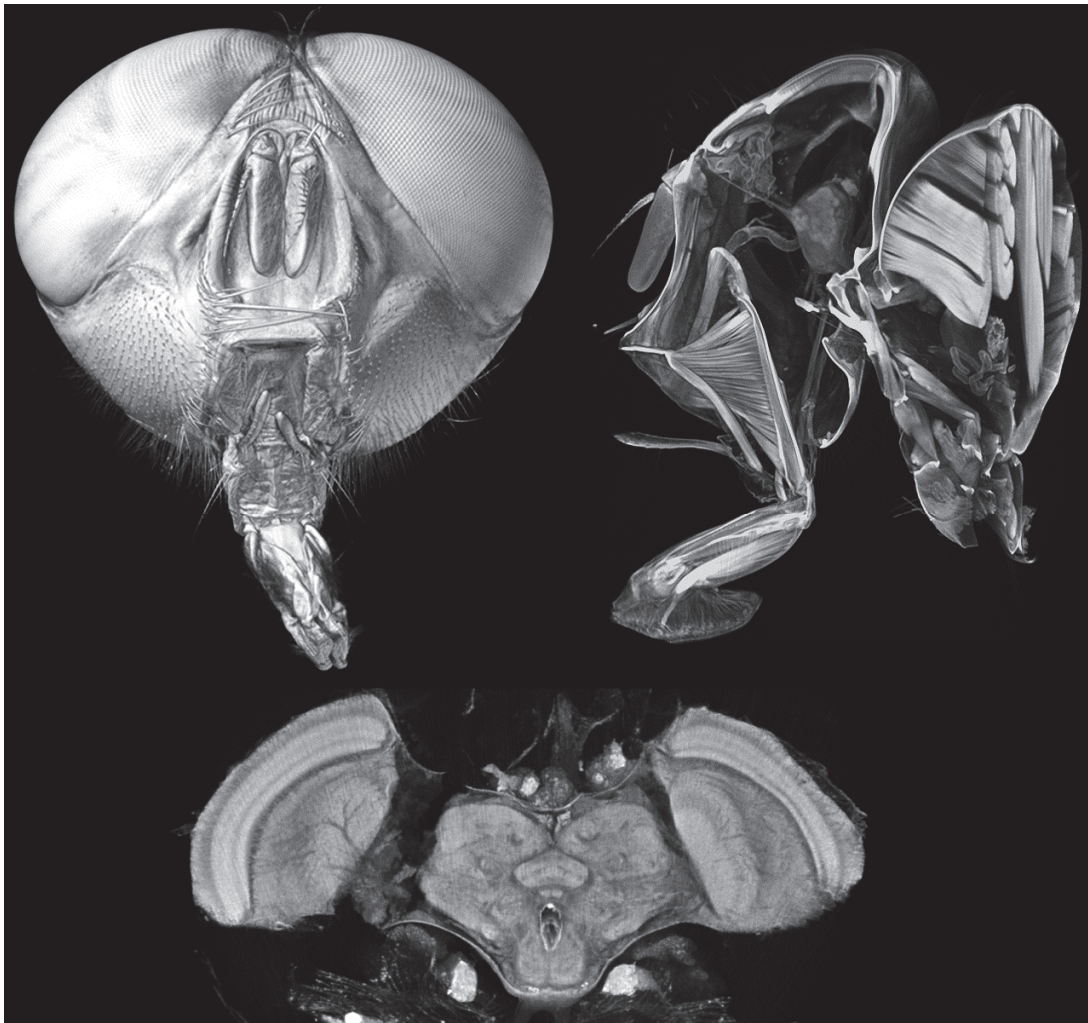
CPD300 auto Program:



Mounting and Scanning:

Dried samples were glued on insect pins and scanned with an Xradia MicroXCT-200 X-ray imaging system (Carl Zeiss X-ray Microscopy Inc., Pleasanton, USA).

Results:



Volume reconstructions and virtual sections of the head and brain of a blow fly.

Courtesy of Elisabeth Lipke and Dr. Peter Michalik, University of Greifswald, Germany.

3. Micro-CT of Insect Larva Protocol

Introduction:

Species: red blood worm (midge larva)

Critical point drying of midge larvae with subsequent X-ray micro-computed tomography (micro-CT) to reconstruct the inner anatomy.

Procedure:

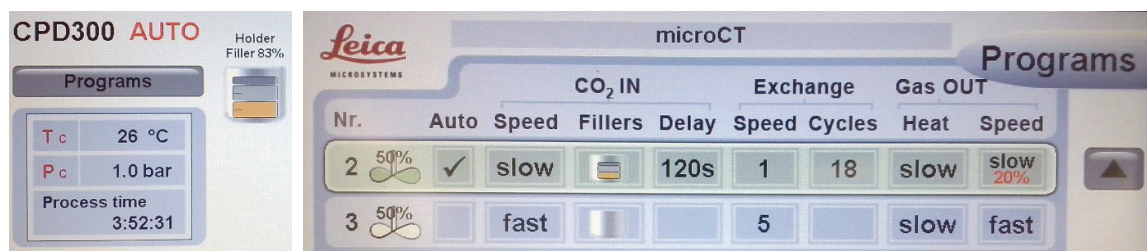
Sample Holder:

Sample was placed individually in the chambers of Arthropoda holder.

Fixation and Dehydration:

| | |
|--|------------|
| 2.5 % Glutardialdehyde (in 0.1 M phosphate buffer) | overnight |
| 0.1 M phosphate buffer (1.8% Sucrose, pH 7.2) | 3x 10 min. |
| Ethanol series: 60%, 70%, 80%, 90%, 96%, 100% | 2x 10 min. |
| Iodine staining (1% iodine solution in 100% ethanol) | overnight |
| Ethanol: 100% | 2x 10 min. |

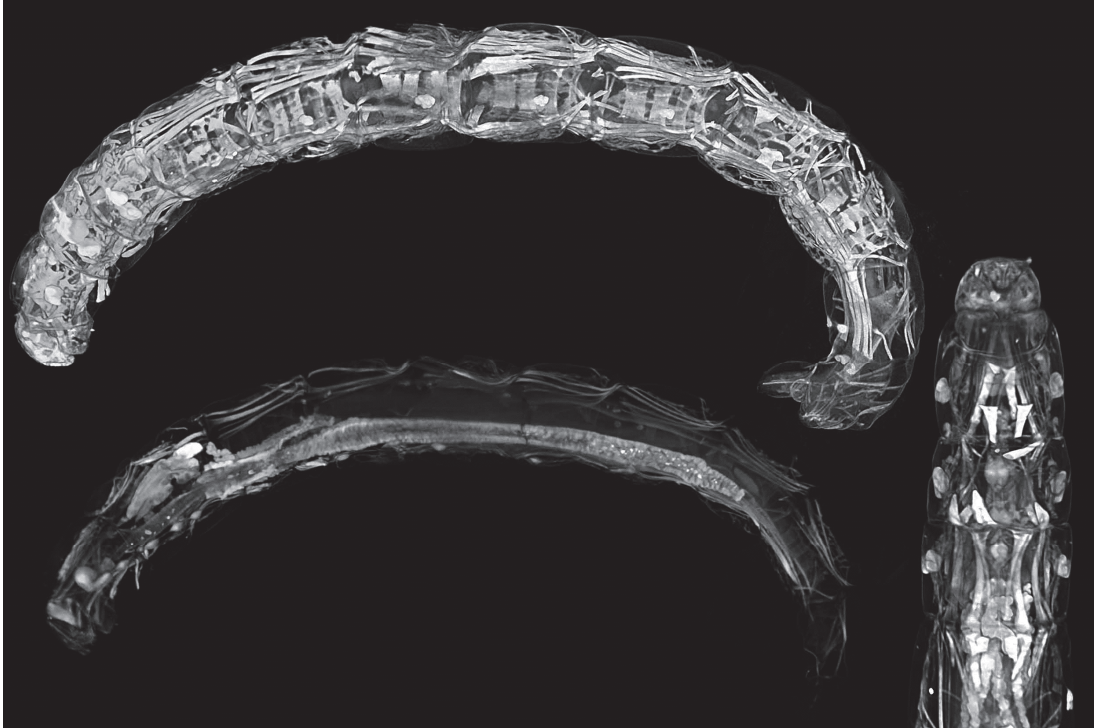
CPD300 auto Program:



Mounting and Scanning:

Dried sample was glued on an insect pin and scanned with an Xradia MicroXCT-200 X-ray imaging system (Carl Zeiss X-ray Microscopy Inc., Pleasanton, USA).

Results:



Volume reconstructions and virtual section of the red blood worm showing a variety of organ systems.

Courtesy of Elisabeth Lipke and Dr. Peter Michalik, University of Greifswald, Germany.



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