

# Micro-CT as a tool in invertebrate and plant palaeontology studies

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## Aims

Usage of micro-CT as a non-invasive method for study of palaeontological objects started in the early 1980s, when the medical technology of x-ray computed tomography was first applied to vertebrate fossils (Tate and Cann, 1982). Its intensive application continued through the beginning of the twenty-first century, with an increasing number of papers focusing on fossil plants (Friis et al., 2015), trace fossils (Tapanila, 2008; Wisshak et al., 2017), and animals (Pleissis and Broeckhoven, 2019). Additionally, some papers compared the permeability of x-rays in different types of rocks (Carlson et al., 2003; Heřmanová et al., 2020). Micro-CT is now being applied in several studies focusing on bioerosion of recent and fossil shell materials (Wisshak et al., 2017; Heřmanová et al., 2020). The recent enormous growth of its use in paleontological studies is mainly due to the increasing availability of relatively affordable micro-CT devices.

The aim of this presentation is to present some selected results of fossil invertebrate and plant fossils investigations in which computer tomography was used.

## Method

In our Palaeontological Department (National Museum, Prague, Czech Republic) we use a SkyScan 1172 x-ray micro-tomograph with a tungsten source, a 5 µm focal spot, and a CCD-based x-ray camera with an 11-megapixel sensor (4000 × 2664 pixels, 9 µm pixel pitch). We have worked with a wide range of fossils, including very small Cretaceous or Tertiary plant mesofossils like seeds, flowering parts, insect eggs, fossil conifer cones, lower Palaeozoic invertebrate body fossils, specimens with diverse borings in shells or burrows in the internal moulds etc. However, we are limited by the chamber size, which allows us to work with specimens maximally 6 cm high and 4 cm in diameter.

For the subsequent reconstruction, we use N-Recon software, and for a complete 3D representation of internal or external micro and/or macrostructures, we apply Avizo software. For successful scan results, an optimal specimen holder is needed. Larger samples we mostly place in individually custom-made polystyrene foam (Styrofoam) holders, without the use of adhesive. Polystyrene foam is completely transparent to x-rays and therefore invisible in the subsequent reconstruction. It is readily available as scrap in essentially unlimited quantities, and can easily be cut to any desired shape with a (not too) hot knife. Small objects like microfossils (about 1 mm in diameter) we usually mount on custom-made thin metal stubs using nail polish, which can be afterwards dissolved with acetone. Another possibility is placing specimens in plastic tubes, which allow more samples to be scanned at once.

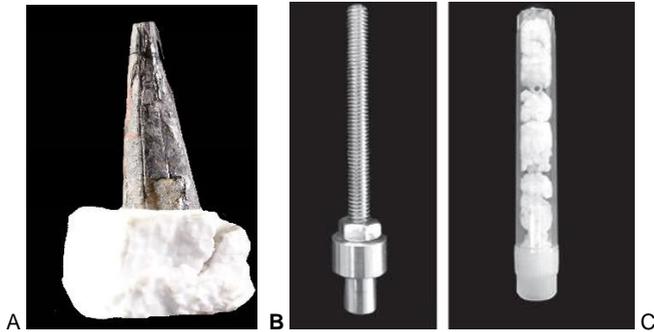


Figure 1: Custom-made holders specially made or adapted for each scanned specimen. (A) Polystyrene foam holder. (B) Aluminium holder for small specimens. (C) Plastic tube filled with polystyrene foam pieces.

Since any material is defined by its density and atomic number, the results from micro-CT depend critically on x-ray penetrability through matter, and therefore on proper filter selection. In addition to the inner Al 0.5 mm and combined Al+Cu filters distributed standardly with the SkyScan 1172, we use specially made 1 mm copper and 0.25 mm aluminum filters. No filter is typically used for specimens with a low x-ray absorption, like Tertiary seeds and insects in amber. On the other hand, a 1 mm copper filter is typically used for specimens with high x-ray absorption, like fossils in siliceous nodules, sandstones and limestones.

We normally work with samples ranging in size from 1 mm to 6 cm and with a resolution of pixel size 0,2-27  $\mu\text{m}$  (1K, 2K and 4K), voltage source 40-100 KV, current source 100-250  $\mu\text{A}$ .

## Results

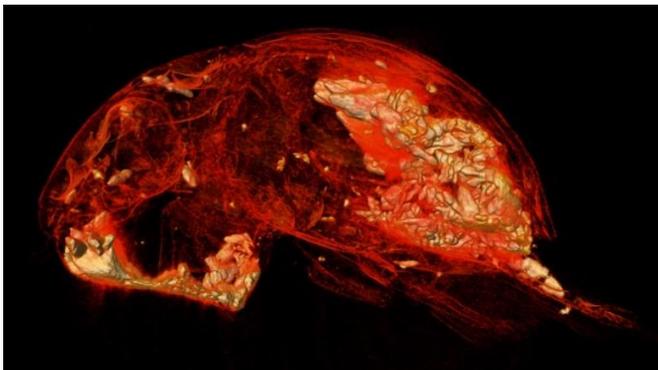


Figure 2: Tertiary beetle preserved in Baltic amber with highlighted fecal pellets in the posterior part of body.

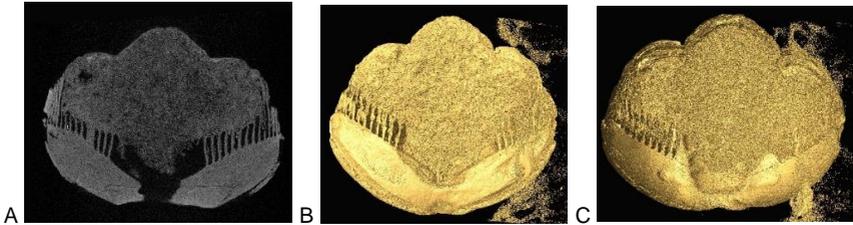


Figure 3: Ordovician enrolled trilobite from siliceous nodule. (A) Transversal section (ortho slice) through trilobite. (B) Visualization of surface (isosurface, front face). (C) Visualization of internal and external surfaces (isosurface, both faces).

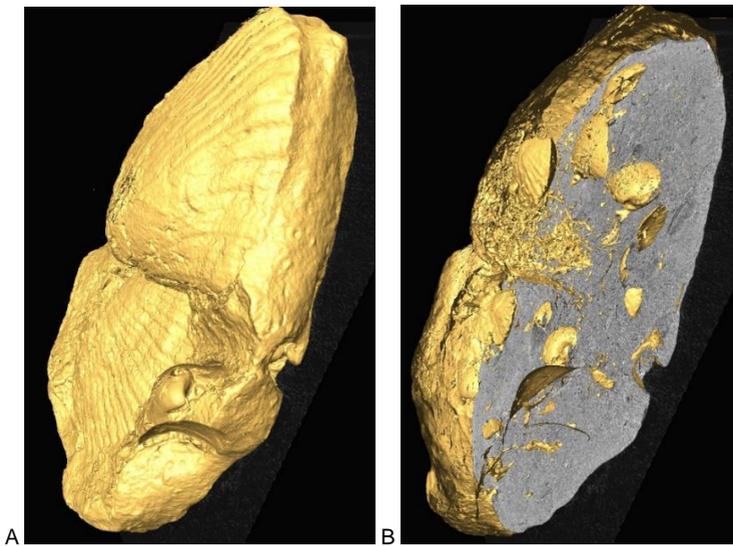


Figure 4: Ordovician brachiopods preserved in siliceous nodules. (A) Visualization of surface with two shells (isosurface, front face). (B) Interior of siliceous nodule showing additional minute fossil shells like brachiopods inside (combination of ortho slice and isosurface, both faces).

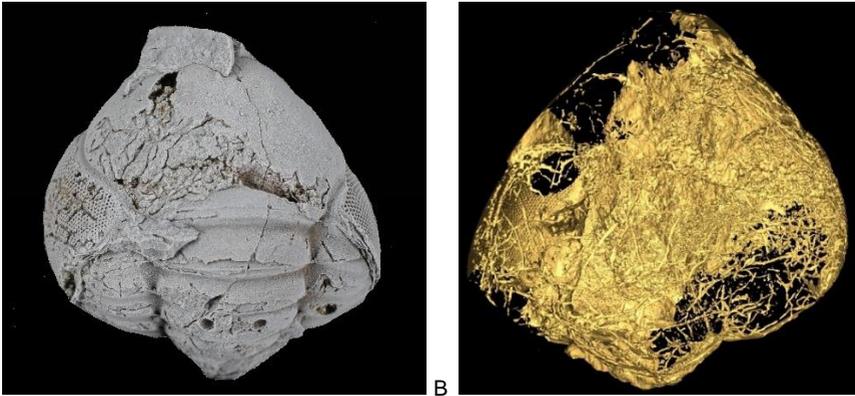


Figure 5: Ordovician enrolled trilobite with highlighted burrows inside its interior mould, siliceous nodule. (A) Photo of specimen coated with ammonium chloride. (B) Visualization of burrows (empty holes) inside of trilobite mould (isosurface, back face).



Figure 6: Tertiary spider and insect visualized from amber. Specimens preserved as empty holes (isosurface, back face).

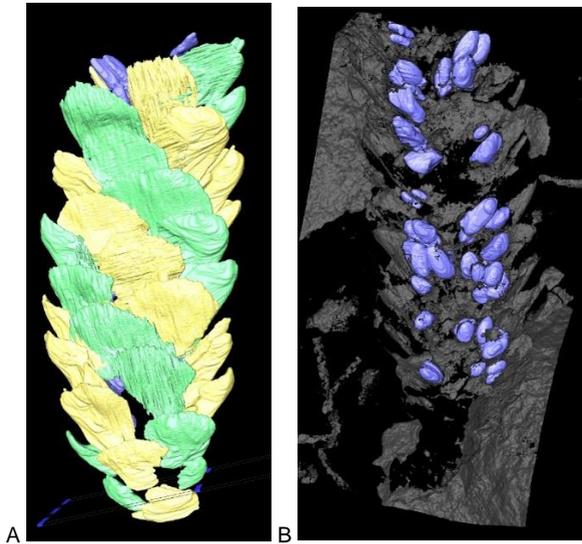


Figure 7: Mesozoic conifer cone. (A) Visualization of cone with helically arranged bract-scale complex (green and yellow) and seeds (blue) (segmentation). (B) longitudinal section of cone with segmented seeds (ortho slice, segmentation).

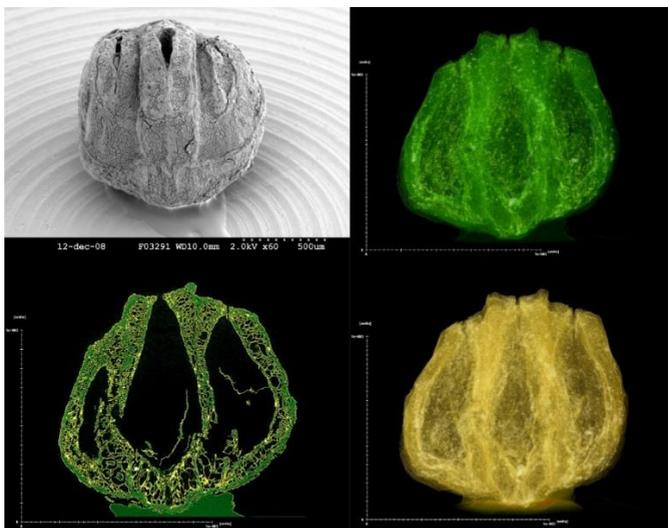


Figure 8: Mesozoic nut. Photo from SEM microscope, visualized surface (volume rendering) and ortho slice.

## Conclusion

Micro-CT is an ideal tool for non-destructive study of fossils, especially in palaeontology, where there is a lot of pressure to preserve the investigated specimen for further studies. The reconstructed virtual fossil can be transected in any direction. Internal and external structures can be studied and visualized with volume rendering, ortho slices and isosurfaces, which we found very useful (especially in trace fossils like borings or burrows, which form an empty space inside the fossil or shell). Segmentation uncovers important details of investigated objects, but is time-consuming.

The most serious limits of micro-CT in paleontology are related to resolution and permeability of x-rays due to different types of fossil preservation. Usage of metal filters in combination with different resolutions often extends the scanning time for several days, which makes this method very expensive and brings a higher risk of various noises and other errors. Camera in offset mode and oversize samples also progressively increase the scan time several times, and increase the possibility of digital artefacts in the results.

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