ESRF	**Experiment title:** **High-resolution non-destructive 3D imaging of oldest known fossil multicellular animal from China by phase-contrast microtomography**	**Experiment number**: ME1239
Beamline: ID19	**Date of experiment**: **from:** 03/10/2005 at 8:00 **to:** 07/10/2005 at 8:00	**Date of report**: 2006-08-08
Shifts:12	Local contact(s)**:**Dr. Paul TAFFOREAU	*Received at ESRF:*

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Report:

During the beam time assigned to ME1239, about 90 Weng'an fauna small (300-700micron diameter) fossil samples, from Precambrian Doushantuo phosphates in Weng'an, Guizhou Province, China, were investigated using x-ray synchrotron micro-tomography (SR-μCT) in phase contrast mode at ID19. The Weng'an fauna contains likely fossil representatives of the oldest known metazoans, radiometrically age-dated as at least 580 million years old. These fossils can be classified into two groups by the scientific problem they are related.

First, the fossil embryos, whose gross external morphology superficially resembles the modern lobe-forming embryos, were investigated using SR-μCT with micron or sub-micrometer resolution (0.56, 0.7 or 1.4 μm depending on the size of the samples). The SR-μCT examinations (Fig.1b) suggest that the three blastomeres **(embryonic cell)** are homologous with the CD cell, AB cell, and PL of trefoil embryos respectively. Among the three blastomeres (Fig.1b), the second largest one is interpreted as the CD cell because it is separated completely from the likely AB cell (the largest lobe) by two layers thin whole membrane but connected to the likely PL (smallest lobe) by a narrow cytoplasmic neck (Fig.1b$^{2-3}$).

When using traditional method, such as SEM, it is impossible to discriminate between asymmetric cleavage and unhealthy cleavage only on the external morphology. SEM images can only provide scanty details about the inner structures and the accurate volume of the cells and presumptive polar lobes.

Using SR-μCT, the volumes of the embryo's sub-units were calculated precisely on the voxel volume and the voxel number per sub unit. The relationship between the sub unit's volumes was deeply analyzed. In Fig. 1b, the volume of the PL is 0.036 mm^3; AB cell is 0.082 mm^3; and CD cell is 0.042 mm^3. From our analysis on 20 this kind samples, the volume ratio of the CD cell plus PL and AB cell is about 1:1 or 2:1. It strongly supports the existence of polar lobes in the studied specimens.

The discovery of 580 million-year-old lobe-forming embryos indicates that lobe

formation is an evolutionarily ancient process of embryonic specification, and the developmental mechanisms similar to those of modern bilaterians existed at least 40 million years before the Cambrian. This research was published in Science[1].

Second, we investigated the fossil embryos with enigmatical micron-tunnel discovered at the same place. Using SR-μCT, we nondestructively studied the characters of the micron-tunnel inside the embryos in detail. One example result is shown in Fig2. The 3D distribution of micron-tunnels with different diameters can be seen very clearly. We also found some very interesting fine structure of the tunnels, such as longitudinal ridges, and spire structures. We have obtained some very interesting clews on the reason of these tunnels. More 3D-reconstruction work and deeper analysis are still in process that should lead rapidly to important publications.

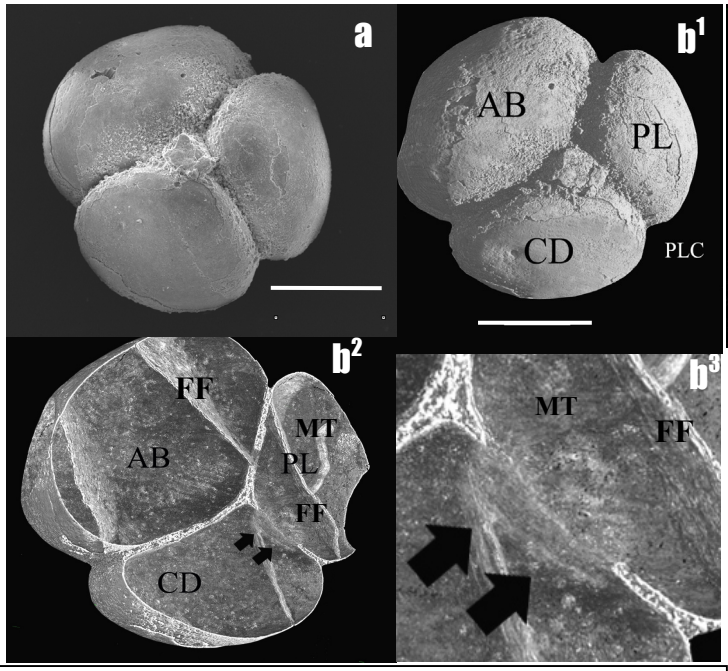

Fig.1 the SEM (a) and SR-μCT (b) images of lobe-forming embryo in trefoil stage from Weng'an fauna. The Fig1b[2] is dissecting images of Fig1b[1], showing preservation of a connected neck (black arrow), b[3], a magnified image of b[2]. Abbreviations: PLC, polar lobe constriction; MT, biogenetic micro-tunnel; FF, diagenetic fissure. Scale bar is 250μm.

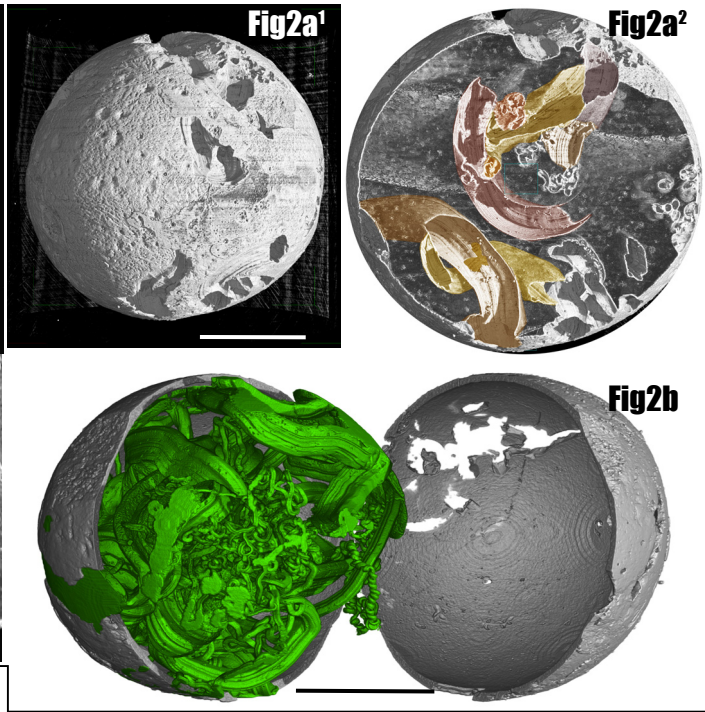

Fig.2a the SR-μCT external image (a[1]) and its virtual opened image to show the cross point different tunnels and the tunnel's inner structures (a[2]) of 026 sample. Fig2b is the tunnel's 3D image of 038 sample shown the tunnels 3D distribution inside the embryo. Scale bar is 250μm.

We also investigated some test type sample fossil, such as the fossil embryos with likely spiral cleavage mode, and also obtained very interesting result.

For traditional paleontological studies, one of the main problems to study these unique fossils is the scarcity of the nondestructive imaging method to obtain the inner structure information with high spatial resolution. The work reported here shows that SR-μCT provides a non-invasive method of analysis of small fossil materials to unlock the finest details of preserved anatomy from fossilized remains. This report is the preliminary form for our new application of the beam time at ID19.

Reference:

1. Jun-Yuan Chen, David J. Bottjer, Eric H. Davidson, Stephen Q. Dornbos, Xiang Gao, Yong-Hua Yang, Chia-Wei Li, Gang Li, Xiu-Qiang Wang, Ding-Chang Xian, Hung-Jen Wu, Yeu-Kuang Hwu, Paul Tafforeau, Phosphatized Polar Lobe–Forming Embryos from the Precambrian of Southwest China, VOL 312 SCIENCE, P1644-1646 (16 June 2006)

The Abstract of our publication in the journal Science

REPORTS

Phosphatized Polar Lobe–Forming Embryos from the Precambrian of Southwest China

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In developing embryos of some extant spiralian animals, polar lobe formation is one of the symmetry-breaking mechanisms for segregation of maternal cytoplasmic substances to certain blastomeres and not others. Polar lobe formation leads to unique early cleavage morphologies that include trilobed, J-shaped, and five-lobed structures. Fossil embryos similar to modern lobe-forming embryos are recognized from the Precambrian Doushantuo Formation phosphates, Weng'an, Guizhou Province, China. These embryos are abundant and form a developmental sequence comparable to different developing stages observed in lobe-forming embryos of extant spiralians. These data imply that lobe formation is an evolutionarily ancient process of embryonic specification.