ESRF	Experiment title: Non-destructive investigate 3D characters of the oldest fossil evidence found by Phase-contrast microtomography with sub-micron resolution	**Experiment number**: EC609
Beamline: ID19	**Date of experiment**: **from: 16/05/2010 at 8:00 to: 24/05/2010 at 8:00**	**Date of report**: 2011-02-06
Shifts:12	Local contact(s)**:** Dr. Paul TAFFOREAU	*Received at ESRF:*

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Report:

During the beam time assigned to EC609, more than 170 three dimensional preserved microfossils (300-700micron diameter) from the Precambrian Weng'an fauna, were investigated non-destructive using synchrotron x-ray phase contrast micro-tomography (SR-μCT) with sub-micron resolution (0.28, 0.56 and 0.7 microns) at ID19. The Weng'an fauna have attracted great attention as the oldest fossil evidence yet found for multicellular animal life on Earth. The radiometric age dating indicates that the Doushantuo is older than 580my. SR-μCT permits non-destructive computational examination of the specimen from any vantage point, visualization of internal characters in virtual sections in any plane, as well as 3D virtual extractions of internal structures. In the case of the Weng'an fossils it can reveal complex internal features of structures that might have been ignored or misinterpreted because of their deceptive exterior forms. Our studies of Weng'an embryos based SR-μCT images have the following two discoveries.

First, this three dimensional digital analysis of SR-μCT data has resulted in the discovery of new kinds of fossil embryos, which form two different types of developmental sequence comparable to duet spiral cleavage, like that found in modern acoel flatworms, as well as quartet spiral cleavage with a giant polar macromere, which among the extant phyla is known from prostomian bilaterians including many lophotrochozoans and crustacean ecdysozoans. Duet and quartet spiral embryos in modern animals undergo an invariant sequence of cell divisions to give rise to identifiable progeny cells, of which the early cleavage stages are under both maternal and zygotic control. Unequal cleavage is a common mechanism for generating intrinsic differences among the blastomeres, and it occurs in many extant Bilateria, especially protostomians, many of which give rise to macromere/micromere polarity in very early cleavage stages, beginning at the 4-cell stage in acoel flatworms and the 8-cell stage in many protostomian bilaterians. This study provides novel information on embryonic pathways during the early evolution of metazoans, and suggests that a number of developmental programs found in extant Bilateria had evolved by Precambrian Doushantuo time. Please see Figure 1 and its caption for detail information. This research will be submitted to Nature Geoscience[1].

Second, our studies of Doushantuo embryos based on SR-μCT images demonstrate the existence of two groups ornamented spherical fossils. The first have an envelope consisting of at least two thick capsules with various surface typical Megasphaera ornaments and a single internal body, which can be interpreted as

resting eggs. The second have an envelope consisting of a thin capsule with cell-like surface structure and internal blastomeres (amount is equal to 2n, n=0, 1, 2, 3…), which are probably the developing blastulae at various holoblastic cleavage stages. The related results have been submitted to Bulletin of Geosciences.

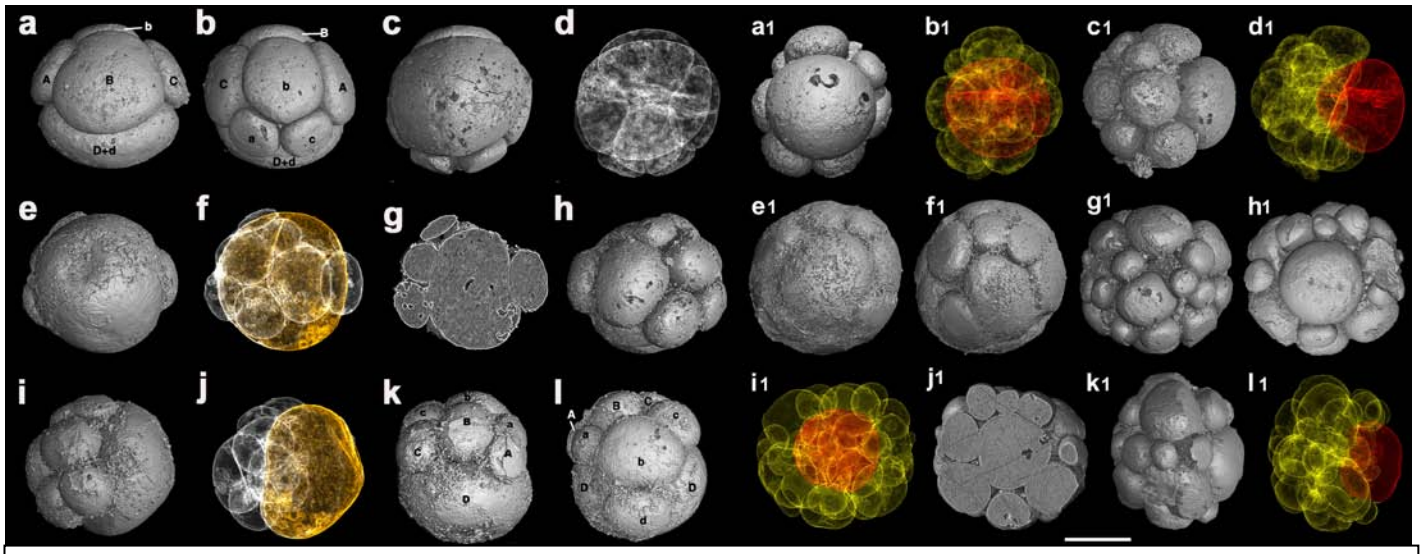

Fig.1, the spiral cleavage embryos with a giant macromere at 8, 16 and 32 cell stage from Weng'an fauna discovered using SR-μCT at ID19 during the beamtime of ec609. a-d, an 8-cell stage embryo (023) showing: digital external view from macromere pole on one side(a); on bottom (b), top (c) and its transparent view (d), which reveal that the constituent 7 cells are arranged in a perfect bilateral symmetry on both sides. e-j, an 8-cell stage embryo showing: digital external from macromere on top (e) and its transparent view (f); an internal section down deep 51% from micromere pole, digital external view from micromere pole (h); digital external (i) and its transparent view (j) from macromere on right side. k-l, an 12-cell stage embryo showing external view with bilateral symmetry. a1-d1, a 16 cell stage embryo (077), showing digital external (a1) and its transparent (b1) views from a giant polar macromere on top and external (c1) and transparent (d1) views from the macromere pole on right side. e1-f1, a 16-cell stage embryo (04-4), showing digital external views from giant polar macromere side (e1) and micromere cap (f1). g1-l1, a 32-cell stage stereo blastula (019) showing SR-μCT digital external views from micromere pole (g1), the external (h1) and its transparent view (i1) from giant polar macromere view, an internal section down deep 33% from the surface of the polar macromere pole (1j), external (k1) and transparent (l1) views from a surface with a 90^0 rotation from macromere pole. Scale bar: 250μm.

We also investigated the sub-cell fine structure of the embryo fossils from Weng'an fauna using holotomography with 0.28 micron to study the evolutionary biology at the organelle level. We have scanned about 20 exceptional preserved embryos during this proposal. Many interesting fine sub-cell structure can be distinguished clearly but the data processing of these samples need more time.

Traditional methods to study Weng'an embryos are by petrologic thin section, or by examination of external form with SEM. But reconstruction of the whole form is difficult by the first of these methods, while examination of internal structure is impossible by the second. The work reported here shows SR-μCT is an very powerful method to address both these problems. It is not only non-destructive but also can reveal many structures that are invisible, or hardly visible, by the classical absorption contrast-based imaging technique. This report is the preliminary form for our new application of the beam time at ID19.

Reference:

1. Jun-Yuan Chen, et.al. Critical Assessment of combined evidence for animal origin of Doushantou Micron fossil Embryos, will be submitted to Nature Geosciences.
2. ZJ Yin, et.al., Reassessment of Ediacaran embryo from the Weng'an biota, have been submitted to Bulletin of Geosciences