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Abstract. The morphogenetic relationship between early patterning and polarity formation is of fundamental interest and remains a controversial issue in preimplantation embryonic development. We use a label-free three-dimensional (3-D) imaging technique of full-field optical coherence tomography (FF-OCT) successfully for the first time to study the dynamics of developmental processes in mouse preimplantation lives. Label-free 3-D subcellular time-lapse images are demonstrated to investigate 3-D spatial relationship between the second polar body (2PB) and the first cleavage plane. By using FF-OCT together with quantitative study, we show that only 25% of the predicted first cleavage planes, defined by the apposing plane of two pronuclei, pass through the 2PB. Also only 27% of the real cleavage planes pass through the 2PB. These results suggest that the 2PB is not a convincing spatial cue for the event of the first cleavage. Our studies demonstrate the feasibility of FF-OCT in providing new insights and potential breakthroughs to the controversial issues of early patterning and polarity in mammalian developmental biology. [DOI: 10.1117/1.JBO.18.1.010503]

Keywords: label-free imaging; preimplantation mouse embryos; full-field optical coherence tomography; early patterning and polarity.

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1 Introduction

The morphogenetic relationship between early patterning and the polarity formation is of fundamental interest. In contrast to most nonmammalian species, early mammalian embryos have been thought to be highly regulative in response to the developmental perturbations and hence lack of polarity until blastocyst stage.[1,2] However, recent studies have proposed that spatial factors of the first cleavage, such as pronuclei in zygote,[3] location of the second polar body (2PB),[4] and the first cleavage plane,[5,6] etc., are associated with each other as well as the polarity formation in several ways. But some of these findings are not consistent with each other. Several groups suggested that the embryonic–abembryonic (Em–Ab) axis of the mouse blastocyst was perpendicular to the first cleavage plane.[5,6] Also, the 2PB was used as a stationary marker for animal pole and the first cleavage plane was assumed to be meridional with reference to the animal-vegetal (A-V) axis, leading to the conclusion that polarity of the mouse embryo was predetermined as early as the zygote stage. Other groups have disputed the claims that a considerable amount of the first cleavage planes did not pass through the 2PB and the 2PB was not stationary, but moved toward the first cleavage furrow during cleavages.[3,4] Nonetheless, whether these factors play decisive roles in the establishment of the polarity has not been fully understood. Early patterning and polarity of the mammalian preimplantation embryos still remains a controversial subject.

Besides the remarkable regulating capacity, the controversies also arise from experimental methods used in the related studies. Most of the studies are based on the two-dimensional (2-D) imaging methods[3–6] such as a conventional microscope, which has barely axial resolution, or differential interference contrast microscopy, which is sensitive to the optical path differences within the sample but not a real three-dimensional (3-D) imaging. As a result, many embryos are often excluded from analysis[3] because of the improper orientation, causing statistical discrepancy so that the results are not fully reliable. Therefore, 3-D imaging techniques are utilized such as confocal and multi-photon excitation laser scanning microscopy.[6,7] But preprocessing and dye labeling are required at the same time. Furthermore, the optical damage to the sample is not avoidable,[8] as the optical power density at the focal point is as high as 10^6 to 10^9 W/cm^2. Therefore, label-free 3-D imaging techniques with low optical power are of great importance to the study of early patterning and polarity.

Full-field optical coherence tomography (FF-OCT) is an emerging 3-D imaging technology.[9–11] It is analogous to ultrasound imaging except that it uses light instead of sound. Due to its unique characteristics, such as label-free, non-invasive, 3-D subcellular resolution and low optical dose, FF-OCT has become a valuable imaging modality in biological and medical studies.[12,13] Recently, our group optimized protocols for static mouse embryo culture for FF-OCT imaging[14] and demonstrated that FF-OCT could be successfully applied to live embryonic 3-D morphological imaging at various typical preimplantation embryonic stages. Although time-lapse live imaging study is very important, there was no such dynamic study on early patterning and polarity of mouse embryo at its very early life with FF-OCT.

In this paper, we extended our previous work and focused our imaging on the event of the first cleavage to investigate 3-D spatial morphogenetic relationship between the 2PB and the first cleavage plane. By time-lapse FF-OCT imaging of the embryos before and after their first cleavages, we found that the first cleavages did not strongly depend on the spatial

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positions of the 2PB of the zygote. Our results revealed the potential of FF-OCT as an imaging tool capable of 3-D time-lapse morphogenetic analysis for understanding early patterning and polarity of mammalian embryos.

2 Experimental Design and Discussion

The schematic of our FF-OCT setup is shown in Fig. 1. It was based on the Linnik interference microscope geometry with a tungsten-halogen lamp in a Köhler illumination system. The light source had its central wavelength of 600 nm, output optical power density of 0.2 W/cm^2 and spectral bandwidth of 180 nm, corresponding to an axial resolution of 0.7 μm in water. Water-immersion microscope objectives (Nikon 20×, 0.5 NA) were used in both arms, achieving a transverse resolution of 0.7 μm. The interferograms were digitized by a CCD camera (IMPERX, MDC-1004, 12 bit, 48 Hz). A stack of 2-D tomographic images at successive depths were obtained by moving samples on the motorized translation stage with a step of 1.2 μm. A custom-designed incubator on the translation stage provides 37°C, 5% CO_2 to keep the embryos alive. Three-dimensional measurements, reconstruction and segmentation were performed with Amira 5.2.0 and 3D med 3.0.

As the mouse is an excellent model to investigate early patterning and polarity, wild-type ICR/CD1 mice (Laboratory Animal Facility, Tsinghua University, China) were used. Females (eight-week-old) were mated overnight with males. Zygote stage embryos whose two pronuclei apposed closely were obtained on late E0.5. Embryos were then imaged in a droplet of human tubal fluid (HTF) medium with 20% FBS (Hyclone, USA) covered with mineral oil (M4080, Sigma, USA). As the embryos were sensitive to the prolonged and frequent light exposure,[7] we only imaged the 3-D structures of the 2PB and two pronuclei rather than the entire embryos. The time needed to image all the 3-D structures of 2PB, two pronuclei of each embryo ranged from 20 to 60 s, depending on their spatial orientations. Also, embryos were imaged only twice, before and after the first cleavages to minimize the optical dose. With this kind of time-lapse imaging, the normal development was ensured and the 3-D structural images were sufficient to reveal important 3-D spatial relations between the 2PB and the first cleavage plane.

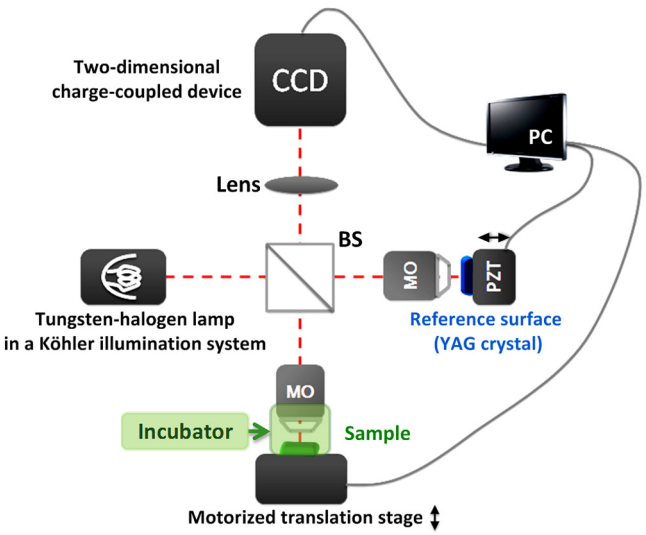

Fig. 1 Schematic diagram of FF-OCT based on the Linnik interference microscope. BS, beam splitter; MO, microscope objectives (water-immersion, 20×, 0.4 NA); Lens (300 mm focal length); PZT, piezoelectric transducer; CCD, 2-D silicon charged-coupled device; PC, personal computer.

First, according to the angular classification methods used in Ref. 3 and on the basis of the conclusions that the first cleavage planes can be defined (or predicted) by the two apposing pronuclei,[3] we classified embryos into two categories without excluding any embryo from analysis: Type A, in which the predicted cleavage plane (PCP) occurs within 30 deg of the 2PB; type B, between 30 and 90 deg. According to Ref. 3, the PCPs that belonged to type A were considered to pass through the 2PB and the PCPs which belonged to type B were considered not to pass through the 2PB. The schematic model, the percentages of each type and 3-D reconstructions of two typical embryos of each type are shown in Fig. 2. For visualization of the two pronuclei, a part of the zygotes were removed with clipping planes. Based on our previous work,[14] the dark areas in Fig. 2(c) and 2(d) were the areas of nuclei: the biggest one is the male pronucleus (MPN); the one of middle size is female pronucleus (FPN) and the smallest is the 2PB's. In Fig. 2(a), the points are the 3-D geometry centers of the 2PB, FPN and MPN. The point in the center is the middle point of the 3-D geometry centers of two pronuclei. The PCP which was concluded to be perpendicular to apposing direction of two pronuclei,[3] and the angles were measured with these points. Only 25% (13 out of 51, type A) of the PCPs were consistent with the assertion that the first cleavage plane coincides with A-V axis whose animal pole is assumed to be marked with the 2PB, while 75% for type B (38 out of 51: 23 between 30 and 60 deg; 15 between 60 and 90 deg) were not, as shown in Fig. 2(b). Our results suggest that the PCP does not pass through the 2PB. Instead, it tends to occur in a random way, but a little more frequently between 30 and 60 deg.

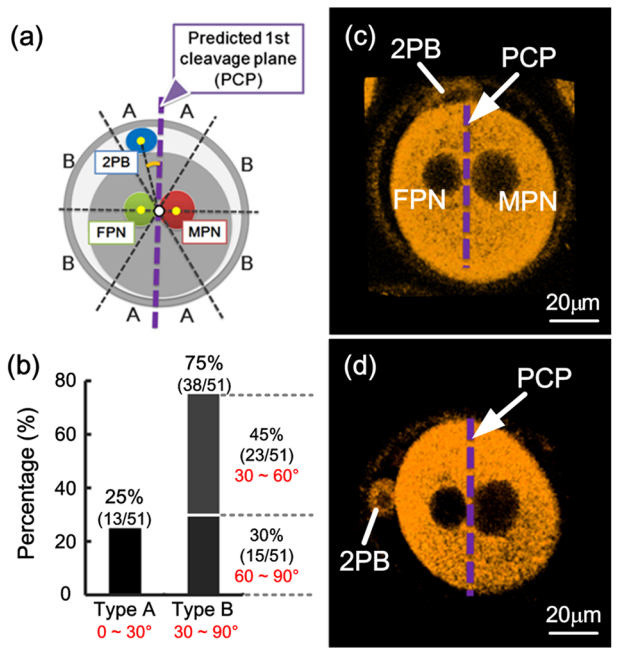

Fig. 2 (a) The schematic model of types A and B. The points are the 3-D geometry centers of the 2PB, female pronucleus (FPN) and male pronucleus (MPN). The point in the center is the middle point of the 3-D geometry centers of two pronuclei. (b) Percentages of each type. (c) 3-D reconstruction of a typical embryo of type A. (d) 3-D reconstruction of a typical embryo of type B.

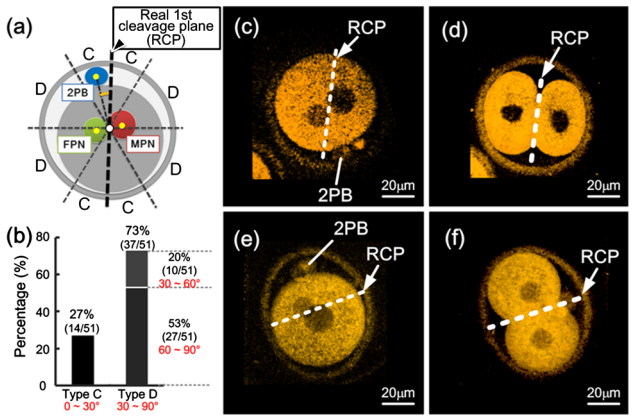

Fig. 3 (a) The schematic model of types C and D. (b) Percentages of each type. (c), (d) 3-D reconstruction of a typical embryo of type C before and after the first cleavage. (e), (f) 3-D reconstruction of a typical embryo of type D before and after the first cleavage.

For further analysis, to address whether the real first cleavage plane (RCP) passes through the 2PB, the embryos were kept incubating in the incubator and imaged again after their first cleavages. To determine the occurrences of the first cleavages, we used the conventional 2-D imaging every 1 h. The embryos were imaged for the second time right after the determination. For our samples, the average time interval between the first FF-OCT imaging and the occurrences of the first cleavage was 8 h. Then we quantified every RCP using the 3-D datasets obtained with the second imaging at 2-cell stage by FF-OCT. Because the two areas of nuclei of each future blastomere move apart during division in the direction that is perpendicular to the first cleavage plane, the 3-D RCP was characterized by 3-D geometry centers of two nuclei of each 2-cell stage blastomere. Also, we classified the embryos into two categories: type C, in which the RCP occurs within 30 deg of the original 2PB at the zygote stage, but not the one which has moved to another position at 2-cell stage; type D, between 30 and 90 deg. According to Ref. 3, the RCPs which belonged to type C were considered to pass through the 2PB and the RCPs which belonged to type D were considered not to pass through the 2PB. Figure 3(a) shows the schematic model and Fig. 3(b) shows the percentages of each type. 3-D reconstructions of a typical embryo of type C before and after its first cleavage are shown in Fig. 3(c) and 3(d), while Fig. 3(e) and 3(f) shows this for type D. For visualization of the two pronuclei and the nuclei of the blastomeres, a part of the embryos were removed with clipping planes. Only 27% (14 out of 51, type C) of the RCPs were formed within 30 deg of the original 2PB, whereas 73% (37 out of 51, type D: 10 between 30 deg and 60 deg; 27 between 60 deg and 90 deg) were not, as shown in Fig. 3(b). These results suggest that the RCP does not pass through the 2PB and does not strongly depend on the 2PB. On the other hand, if there still assumed to be predetermined A-V axis and the first cleavage plane is assumed to be meridional with reference to this axis, the 2PB is not a good spatial cue that marks the animal pole. At the opposite point of view, if the 2PB does mark animal pole, only a little proportion of the real first cleavage planes would align with A-V axis. Instead, the real first cleavage planes tend to occur perpendicular (between 60 deg and 90 deg) to the A-V axis more frequently.

3 Conclusion

In conclusion, for the first time, we demonstrated the successful use of FF-OCT to study the dynamics of developmental processes in mouse preimplantation lives. Label-free 3-D time-lapse images are demonstrated to investigate 3-D spatial relationship between the 2PB and first cleavage plane. With quantitative study, we show that the 2PB is not a reliable morphogenetic determinant for the event of the first cleavage. Our studies demonstrate the feasibility of FF-OCT in providing new insights and potential breakthrough to the controversial issues of early patterning and polarity in mammalian developmental biology.

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