In vivo Vertebrate Morphogenesis Study Using Noninvasive Harmonic Generation Microscopy

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Abstract: Due to its noninvasive nature and sub-micrometer 3-D sectioning power, harmonic generation microscopies is most suitable in studying vertebrate morphogenesis *in vivo* with no need of extrinsic or intrinsic fluorophores.

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Recently, several techniques have been used in the field of developmental biology, e.g., optical coherence tomography (OCT) [1] and two-photon fluorescence microscopy (2PFM) [2]. OCT has demonstrated ~ 1μm axial resolution [3], which is good enough for subcellular imaging but still inferior to that of nonlinear microscopy. Due to the nonlinear nature of two-photon process and the near-infrared source used, 2PFM provides a sub-micron optical sectioning power at a few hundred μm depths. However, the on-focus strong absorption and the usual need of exogenous fluorophores make this technique not truly 'noninvasive'. Harmonic-generations (HGs) are known to leave no energy deposition due to their virtual level transition characteristic, providing the optical "noninvasive" nature [4]. Similar to multi-photon induced fluorescence process, the nonlinear dependence of HGs allows intrinsic optical sectioning. It is important to utilize these nonlinear signals for imaging purpose to replace unnecessary usage of invasive and toxic fluorophores. This is especially important for *in vivo* animal subject since most vertebrate tissues are not autofluorescent. Here we demonstrate an *in vivo* vertebrate morphogenesis study using HG microscopy based on a femtosecond Cr:forsterite laser, providing deepest penetration and least photodamage possibility [5]. By using a numerical aperture (NA) 1.2 objective and a NA 1.2 achromatic collection lens, the

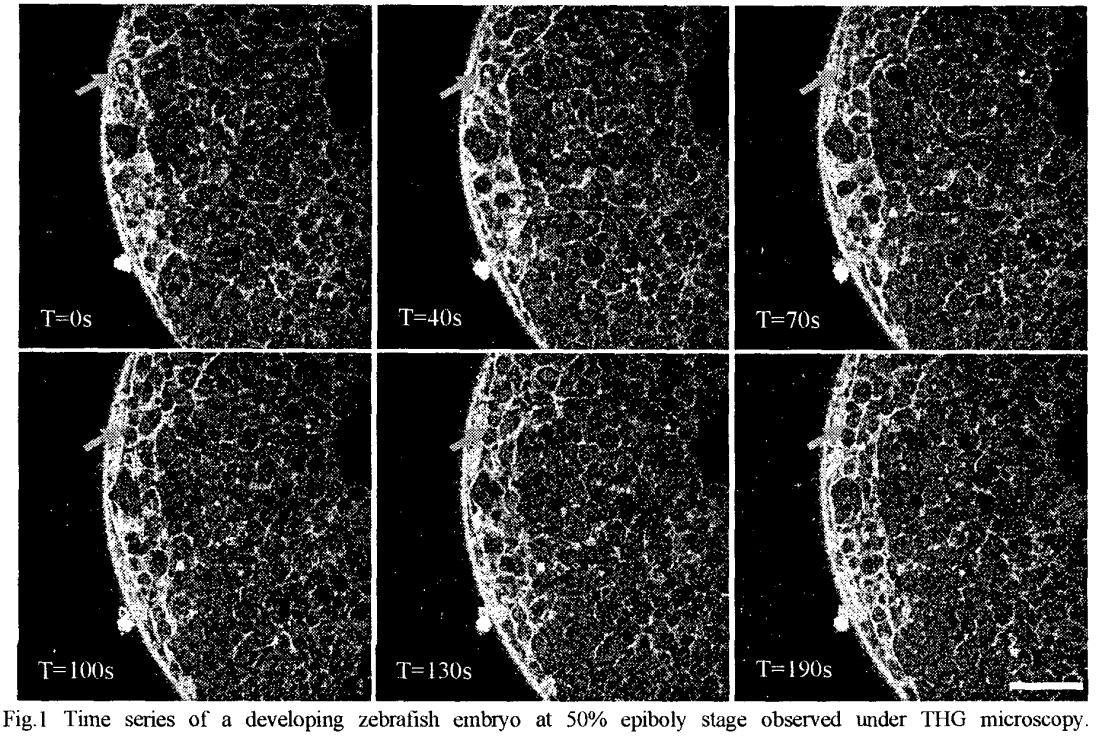

Fig.1 Time series of a developing zebrafish embryo at 50% epiboly stage observed under THG microscopy. Scale bar: 50μm

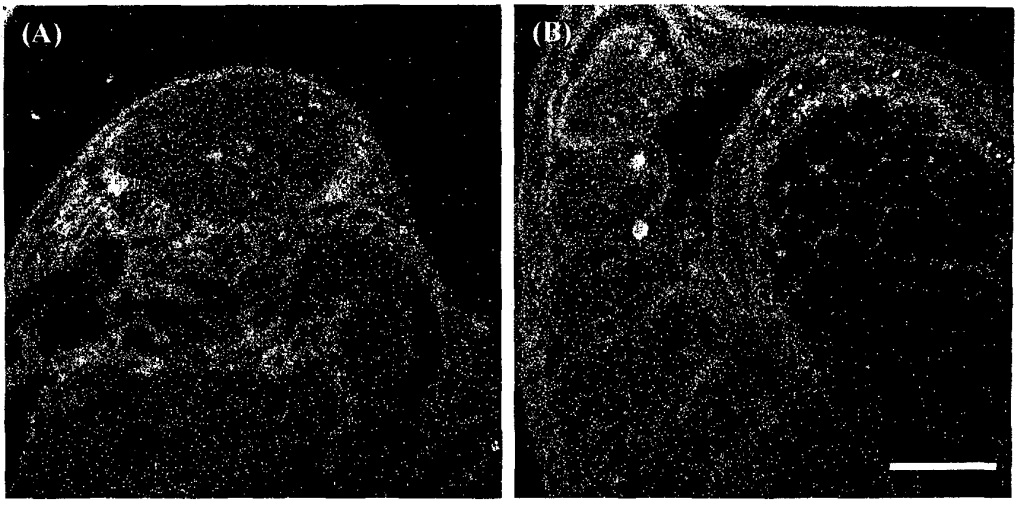

Fig. 2 Scanning THG images of a 20-somite period zebrafish embryo at (A) head and (B) somites. Scale bar: 50μm

resolution can be improved to 610nm in axial and 210nm in radial direction with THG microscopy.

The optical sectioning power and noninvasive nature of SHG and THG allow us to observe the three-dimensional processes of cell proliferation in embryo *in vivo*. Fig. 1 shows a time series of the cell proliferation in a zebrafish embryo at 5-hour-postfertilization (5hpf or 50% epiboly stage). Each cell and its organelle can be identified through THG signals, which is sensitive to optical interfaces. Every phase during cell duplication can be visualized through THG signals, which is sensitive to optical interfaces. (see arrows in Fig. 1), Namely, (i) interphase (t=0s): the complete nucleus, (ii) prometaphase (t=40s): the dissolution of the nuclear membrane, (iii) anaphase (t=70s): the elongation of cell and separation of chromosomes, (iv) telophase (t=100s): the membrane formation around two daughter cells, and (v) cytokinesis (t=130s): the actin contraction leads the cells to divide into two daughter cells. The motion of sub-cellular organelles and the generation of daughter cells can be clearly observed in this sectioned plane with the sub-μm resolution of THG microscopy.

Fig. 2 shows the THG optical section at the head and somites of a zebrafish embryo at 20-somite period. The early stage during neural system formation can be studied by HG microscopy based on a Cr:forsterite laser due to its non-invasive and highly-penetrative characters. Fig. 3 is SHG and THG sectioned microscopic images at the dorsal side of a 5-dpf zebrafish larva. The muscle fibers and individual sacromeres on them are revealed by SHG signals, which is sensitive to the bio-crystalline structure such as myofibrils that formed by the actin and myosin filaments

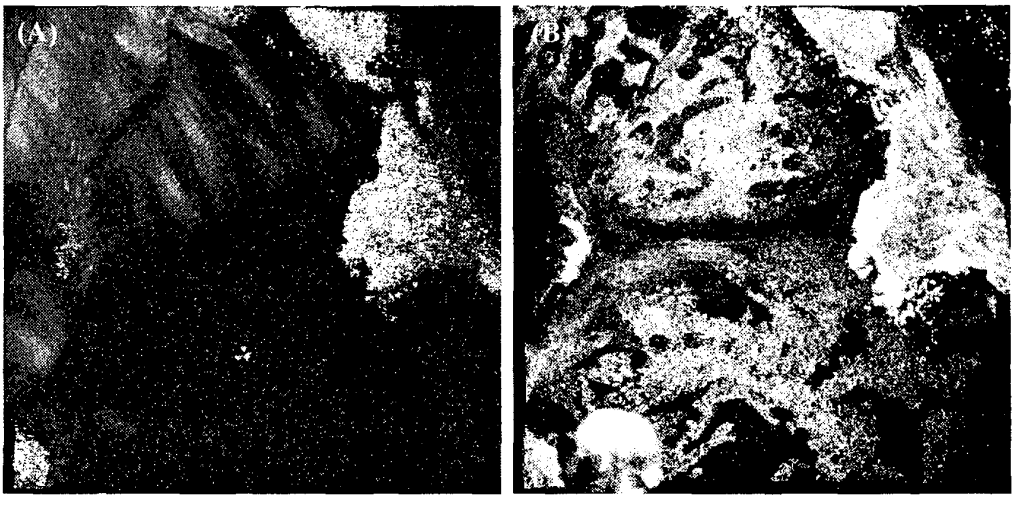

Fig. 3 Scanning (A) SHG and (B) THG of a 5-dph zebrafish larva at the dorsal side. Scale bar: 50μm.

[6]. Due to its high sensitivity to this orderly arranged bio-crystalline structure, SHG microscopy can be used to determine when does the actin and myosin filaments form regularly distributed sacromeres and then muscle fibers, which remains an unknown question. The fully developmental dynamics of somites and skeletal muscle of zebrafish will be given during the conference.

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