In vivo Study of Early Development of Vertebrate Nervous System Using Noninvasive Harmonics Optical Microscopy

Szu-Yu Chen and Shi-Wei Chu
Graduate Institute of Electro-Optical Engineering, National Taiwan University, Taipei, 10617 TAIWAN, R.O.C.
Tel:+886-2-23635251 ext. 406. FAX:+886-2-23677467. Email: r92941004@ntu.edu.tw.

Cheng-Yung Lin and Huai-Jen Tsai
Institute of Molecular and Cell Biology, National Taiwan University, Taipei 10617, TAIWAN, R.O.C.

Chi-Kuang Sun
Graduate Institute of Electro-Optical Engineering and Department of Electrical Engineering, National Taiwan University, Taipei, 10617 TAIWAN, R.O.C

Abstract: Due to the noninvasive and functional natures of higher-harmonics generation, harmonics optical microscopy provides an excellent tool to study the early developmental dynamics of vertebrate nervous system without any extrinsic or intrinsic fluorophores.

OCIS codes: (170.3880) Medical and biological imaging, (190.4160) Multiharmonic generation, (170.6900) Three-dimensional microscopy

Among many physiological systems of vertebrates, nervous system is one of the most important research subjects and lots of aspects of nervous system patterning and the relationship between embryonic and adult nervous system morphology remains unknown. Due to the simplicity of the developing nervous system, zebrafish embryos are amenable to the studies of vertebrate nervous system development. In addition, zebrafish is also an ideal model for studying veterbrate somite development because of clearly recognized stages of somite development [1]. Different from other modalities, harmonics optical microscopy (HOM) provides a truly "noninvasive" way to observe the dynamics of embryonic development without using any invasive and toxic fluorophores while proving sub-micrometer 3D spatial resolution with ~1mm penetration capability [2]. Based on the nonlinear nature, harmonics generation are known to leave no energy deposition due to the virtual-level-transition characteristic [2] and have high 3D optical-sectioning power (~1μm axial resolution). In this presentation, we demonstrate an *in vivo* HOM study of developmental dynamics in embryonic nervous system and somites in live zebrafish embryos. Based on a femtosecond Cr:forsterite laser, which provides the deepest penetration and least photodamage [2], complete developing processes of the nervous system, somites and other related organs such as eyes and otic can be non-invasively observed inside one embryo.

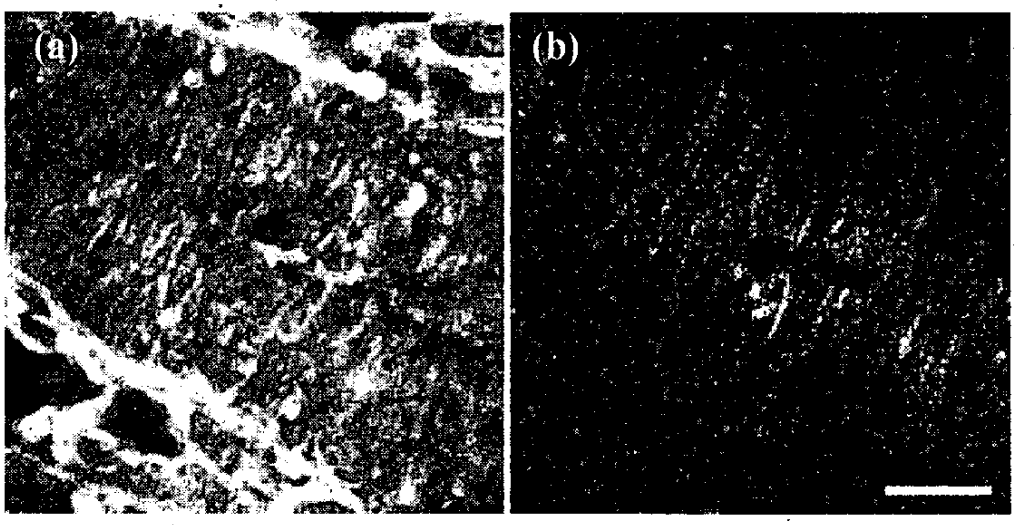

Fig.1 Scanning images of the midbrain of the zebrafish at 15-somite stage. (a) THG and SHG signals;(b) SHG signals. Scale bar:20μm

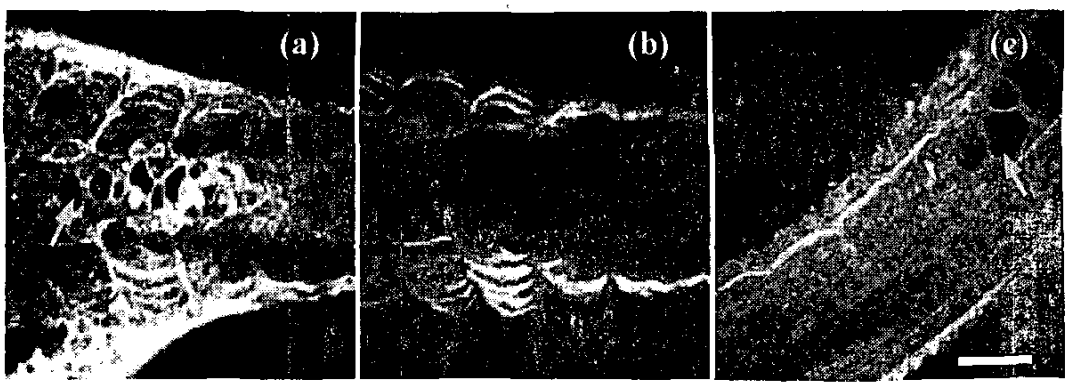

Fig. 2 HOM images in somites of a zebrafish embryo (dorsal view) at its (a, b) a 20-somite stage and (c) 12-prim stage. SHG and THG modalities are both shown in (a) and (c) while (b) shows the SHG image in (a). Scale bar:50 μm

Fig.1 shows example HOM images optically sectioned at the midbrain [3] of a live developing zebrafish embryo (animal pole view) at its 15-somite-stage. Due to different characteristics, third-harmonic-generation (THG, Figure 1(a)) signals provide the general morphalogy of the midbrain while second-harmonic-generation (SHG, Figure 1(b)) signals reflect the distribution of the microtubule ensembles in neurons and during mitosis [4]. Time-series HOM images at different section depth and developing period (will be presented in conference) provide detailed information on the developing dynamics of the nervous system.

Fig.2 shows sectioned HOM images in somites of a zebrafish embryo (dorsal view) at its (a,b) 20-somite-stage and (c) 12-prim-stage. SHG and THG are both shown in Figure 2(a) and (c). While THG provides clear outline of the somites and the notochord (arrow in Fig.2), individual sacromeres formed by actin and myosin filaments can be revealed by SHG signals [5]. Fig.3 shows sectioned HOM images in the left eye of a zebrafish embryo at its (a) 8-somite-stage and (b,c) 25-somite-stage. Figure 3(a) and (b) correspond to the THG modality while Figure 3(c) is the corresponding SHG image of Figure 3(b). The highly organized disk membrane breaks the spatial symmetry and should be responsible for the SHG observation in the retina. Through time-series studies of the same embryo, the dynamic developments of the rod and cone cells in the bottom of the eye can be clearly revealed.

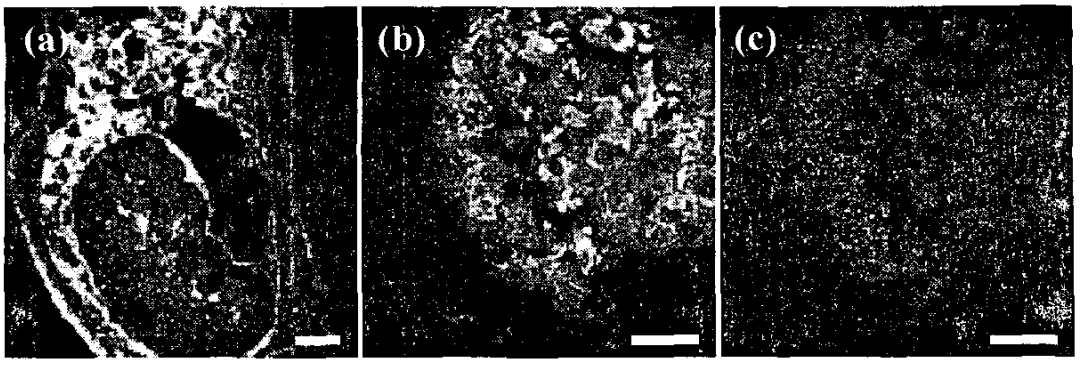

Fig.3 Scanning images at the left eye of (a) a 8-somite stage (only THG signals) and (b) a 25-somite stage zebrafish embryo including SHG and THG signals; (c) SHG signals separated from (b). Scale bar:30μm

Without the need of extrinsic or intrinsic fluorophores, misgivings of mutation resulting from fluorophores is thus completely eliminated in the HOM studies. Combining with its capability of high penetration and high cell viability, noninvasive HOM can thus provide an unique optical tool for dynamic and functional imaging of early developments in the vertebrate nervous system, which is highly sensitive to external stimulations.

References

[1] *H. L. Stickney et al.* "Somite Development in Zebrafish." *Dev. Dyn.* **219**, 287-303 (2000)

[2] S.-W. Chu *et al.* "In vivo developmental biology study using noninvasive multi-harmonic generation microscopy." *Opt. Express* **11**, 3093-3099 (2003).

[3] *C. B. Moens et al.* "Constructing the Hindbrain : Insights From the Zebrafish." *Dev. Dyn.* **224**, 1-17(2002)

[4] *Daniel A.Dombeck et al.* "Uniform polarity microtubule assemblies imaged in native brain tissue by second-harmonic generation microscopy." *PNAS* **100,** 7081-7086 (2003)

[5] S.-W. Chu *et al.* "Nonlinear bio-photonic crystal effects revealed with multi-modal nonlinear microscopy." *J. Microscopy* **208**, 190-200 (2002).