## TECHNICAL NOTE

# Magnetic Resonance Microscopy of Chemically Fixed Human Embryos at High Spatial Resolution

Yosuke OTAKE<sup>1</sup>, Shinya HANDA<sup>1</sup>, Katsumi Kose<sup>1\*</sup>, Kohei SHIOTA<sup>2</sup>, Shigehito YAMADA<sup>2</sup>, and Chigako UWABE<sup>2</sup>

<sup>1</sup>Institute of Applied Physics, University of Tsukuba 1–1–1 Tennodai, Tsukuba 305–8573, Japan <sup>2</sup>Congenital Anomaly Research Center, Kyoto University Graduate School of Medicine (Received March 17, 2014; Accepted September 19, 2014; published online February 12, 2015)

We acquired magnetic resonance (MR) microscopic images of chemically fixed human embryos of Carnegie stages 16 to 22 with a large image matrix  $(256 \times 256 \times 512)$  using an MR microscope that we developed with a 9.4-tesla vertical wide-bore superconducting magnet and a dual-channel receiver system to extend the dynamic range of the MR signal. The images showed clear anatomical structures at spatial resolutions of  $(40 \,\mu\text{m})^3$  to  $(60 \,\mu\text{m})^3$ . We concluded that the experimental technique we developed will aid construction of the next anatomical database of the collection of chemically fixed human embryos.

Keywords: anatomical database, human embryo, MR microscopy

## Introduction

Only a few studies have reported on magnetic resonance (MR) microscopy of chemically fixed human embryos because acquisition of specimens is very difficult.<sup>1,2</sup> In 1999, Duke University reported on 3-dimensional (3D) MR microscopic images of several chemically fixed human embryos at various developmental stages (Carnegie Stages; CSs) with a  $128 \times 256 \times 256$  matrix using a 9.4-tesla vertical bore superconducting magnet.

In 2007, our group reported on 3D MR microscopic images of 1,204 chemically fixed human embryos selected from the Kyoto Collection of Human Embryos ("Kyoto Collection") that were collected from 1961 to 1974,<sup>3</sup> and were acquired with the superparallel MR microscope.<sup>2,4</sup> The 3D image datasets included about 100 embryos for each developmental stage from CS11 to CS23 and were used to study the development of various organs of the embryos.<sup>5–8</sup> However, the number of image matrices for these images was limited to  $128 \times 128 \times 256$ voxels primarily because of the limitation of the signal-to-noise ratio (SNR) of the MR signal determined by the magnetic field strength (2.34T) used for MR microscopy. Because the SNR of the MR signal is proportional to the 7/4th power of the magnetic field strength, the use of a higher magnetic field was thus the most straightforward approach to increase the spatial resolution or matrix size. In the present study, we developed a 9.4T MR microscope and demonstrated its capability to acquire 3D MR microscopic images of human embryos in a 256  $\times$  256  $\times$  512 image matrix for the next anatomical database of the Kyoto Collection.

### **Materials and Methods**

We carefully selected 7 human embryos of CS16 to CS22 from the Kyoto Collection, chemically fixed with Bouin solution or formalin, and stored in a 10% formalin water solution. We transferred the specimens from preservation tubes to nuclear magnetic resonance (NMR) sample tubes filled with the formalin solution to prevent them from drying and from magnetic susceptibility effect. The outer diameters of the sample tubes were 8 mm (CS16 and CS17), 10 mm (CS18), and 15 mm (CS19 to C22) (Fig. 1a).

We developed the MR microscope using a 9.4T vertical wide bore (89-mm diameter) superconducting magnet (Japan Superconductor Technologies, Inc. [JASTEC], Kobe, Japan), a cylindrical gradient

<sup>\*</sup>Corresponding author, Phone: +81-29-853-5335, Fax: +81-29-853-5205, E-mail: kose@bk.tsukuba.ac.jp

coil set, radiofrequency (RF) probes, and an MR imaging console developed in our laboratory. The gradient coil set consisted of Golay-type transverse gradient coils (Gx and Gy) and a Maxwell-type axial gradient coil (Gz). Table lists the specifications of the gradient coils. We developed 3 saddle-shaped RF coils with inner diameters of 8, 10, and 15 mm to optimize the SNR of the MR signal of the embryos.

The receiver system consisted of 2 parallel MR imaging receivers (DTRX4, MRTechnology, Tsuku-



**Fig. 1.** (a) Chemically fixed human embryos stored in NMR sample tubes filled with formalin solution. The outer diameters of the sample tubes are 8 mm for CS16 and CS17, 10 mm for CS18, 15 mm for CS19 to 22. The pictures of the embryos are distorted by the refraction of the formalin solution. (b) Midsagittal cross sections of the chemically fixed human embryos. The geometrical scale is identical for all embryos. The voxel size for the embryos was  $(40 \,\mu\text{m})^3$  for CS16 and CS17,  $(45 \,\mu\text{m})^3$  for CS18,  $(50 \,\mu\text{m})^3$  for CS19, and  $(60 \,\mu\text{m})^3$  for CS20 to CS22.

ba, Japan) used with a different gain setting (typically 32-dB difference). To extend the dynamic range of the receiver system, the MR signals were acquired simultaneously using the 2 receiver channels with different receiver gains, and k-space datasets were synthesized from those acquired with the 2 channels. The details of the technique are described elsewhere.<sup>9</sup>

A 3D gradient echo pulse sequence (repetition time [TR], 100 ms; echo time [TE], 5 ms; 90° flip angle [FA]) was used with  $256 \times 256 \times 512$  voxel image acquisition. The voxel size was  $(40 \,\mu\text{m})^3$  for CS16 and CS17,  $(45 \,\mu\text{m})^3$  for CS18,  $(50 \,\mu\text{m})^3$  for CS19, and  $(60 \,\mu\text{m})^3$  for CS20 to CS22. The MR signals were accumulated 12 times for each phaseencoding step, and the total image acquisition time for each embryo was about 22 hours. For the CS22 embryo, a 3D spin echo pulse sequence (TR, 100 ms; TE, 10 ms) was used with the same imaging parameters as those for the gradient echo sequence.

The image datasets were synthesized to obtain  $256 \times 256 \times 512$  point k-space components, zero-filled to  $512 \times 512 \times 1024$  point datasets, and Fourier-transformed to obtain  $512 \times 512 \times 1024$  voxel MR images. The interpolated image datasets were rotated in the 3D real space to obtain the midsagittal plane.

## Results

Figure 2 shows midsagittal cross sections of the CS22 embryo acquired with the (a)  $T_1$ -weighted gradient echo sequence, (b)  $T_1$ -weighted spin echo sequence (TR, 100 ms; TE, 10 ms) at 2.35T (128 × 128 × 256 voxel image) acquired in the previous study.<sup>2</sup> The signal-to-noise ratio (SNR) measured in the square region of the brain was 19.9, 23.3, and 101 for Fig.2a, Fig.2b, and Fig.2c, respectively. The spin echo image acquired at 9.4T demonstrated signal loss due to short  $T_2$  relaxation times that was remarkable in the liver. From this result, we decided to measure all the embryos using the gradient echo sequence despite the magnetic susceptibility effect and sensitivity to magnetic field inhomogeneity.

Table. Specifications of the cylindrical gradient coils developed for imaging of chemically fixed human embryos

	Coil diameter (mm)	Number of turns	Wire diameter (mm)	Resistance (Ω)	Inductance (µH)	Efficiency (mT/m/A)
Gx	47	10	0.6	0.61	128	15
Gy	46	10	0.6	0.62	134	16
Gz	37	12	1.0	0.21	12	24

Gx and Gy, Golay-type transverse gradient coils; Gz, Maxwell-type axial gradient coil



**Fig. 2.** Midsagittal cross sections of the CS22 embryo acquired with (**a**) the  $T_1$  weighted gradient-echo sequence (TR = 100 ms, TE = 5 ms, NEX = 12, total data acquisition time = 22 h) at 9.4T ( $256 \times 256 \times 512$  voxel image), (**b**)  $T_1$  weighted spin echo sequence (TR = 100 ms, TE = 10 ms, NEX = 12, total data acquisition time = 22 h) at 9.4T ( $256 \times 256 \times 512$  voxel image), and (**c**)  $T_1$  weighted spin echo sequence (TR = 100 ms, TE = 10 ms, NEX = 16, total data acquisition time = 7.3 h) at 2.35T ( $128 \times 128 \times 256$  voxel image) acquired in the previous study. The dotted squares in the brain show regions in which the SNR was evaluated. Signal decay in the liver caused by the short  $T_2$  value (~10 ms) in the spin echo sequence at 9.4T is remarkable.

Figure 1b shows the midsagittal cross sections of CS16 to CS22 human embryos selected from the 3D image datasets acquired with the 3D gradient echo sequence. Although voxel size varied from (40  $\mu$ m)<sup>3</sup> to (60  $\mu$ m)<sup>3</sup>, the geometrical scale was normalized for all the embryos. The development of internal structures is clearly seen.

Figures 3 and 4 show maximum intensity projections (MIP) from 9 different directions calculated from the 3D image datasets of the CS18 and CS22 embryos. The structures of internal organs are clearly visualized through such low intensity parts as the skin and other tissues, the signal of which decreased due to relatively short  $T_2$  relaxation times and/or low proton densities.

Figure 5 shows cross sections selected from the 3D image dataset of the CS22 embryo acquired with the gradient echo sequence (TR, 100 ms; TE, 5 ms). The fine structures of the choroid plexus, eyes, arms, hands, and liver are clearly visualized.

## Discussion

#### Pulse sequence and image contrast

In a previous study,<sup>2</sup> we acquired all MR images using a  $T_1$ -weighted spin echo sequence (TR, 100 ms; TE, 8–12 ms), whereas in this study, we acquired images using the  $T_1$ -weighted gradient echo sequence. We used the  $T_1$ -weighted sequence for 2 reasons– because saturation effects suppress the MR signal of the protons in the formalin solution and because  $T_1$ -weighted contrast resembles that obtained with Nissl staining, a widely used method for visualization of embryo sections.<sup>2,10</sup>

We also used the gradient echo sequence instead of the spin echo sequence for 2 reasons. First, the  $T_2$ relaxation time of water protons in the embryo specimen is around 10 ms, and the shorter echo time is better from the viewpoint of SNR. Second, the dynamic range of the MR signal is narrower for the gradient echo signal than the spin echo signal because the peak amplitude of the gradient echo is lower as a result of the inhomogeneity of the local magnetic field and/or magnetic susceptibility effect.

From the above discussion, we conclude that the  $T_1$ -weighted gradient echo sequence is optimal for the acquisition of large matrix and high resolution images of the chemically fixed human embryos.

#### SNR of the MR images

We employed a 2.35T magnetic field with solenoid RF coils in the previous study<sup>2</sup> and a 9.4T field with saddle-shaped RF coils in the present study. Because the SNR of the MR signal is proportional to the 7/4th power of the magnetic field strength,<sup>11</sup> if the dominant noise source is the loss of the RF



**Fig. 3.** Maximum intensity projections from 9 different directions calculated from the three-dimensional image datasets of the CS18 embryos. Voxel size is  $(45 \,\mu\text{m})^3$ .

coil, such as in our case, the SNR gain caused by the magnetic field strength is  $4^{7/4} \sim 11.3$ . However, the solenoid RF coils have about a 3-fold SNR advantage over the saddle-shaped coils when they are compared for the same diameter.<sup>11</sup> Therefore, an SNR gain of about 4 times is expected for the present system compared with the previous system.<sup>2</sup>

As shown in Fig. 2, the SNR of the brain observed in the T<sub>1</sub>-weighted spin echo image at 2.35T ( $[120 \,\mu\text{m}]^3$  voxel volume) was 101, and that at 9.4T ( $[60 \,\mu\text{m}]^3$  voxel volume, 1/8 of that for 2.35T) was 23.3. The repeated time of the pulse sequences at 2.35T was  $128 \times 128$  (phase encoding) × 16 (NEX) = 262,144, and that at 9.4T was  $256 \times 256 \times 12$  (NEX) = 786,432. The frequency bandwidth per voxel for the images acquired at 2.35T and 9.4T was identical (195.3 Hz). Therefore, the SNR advantage for the measurements themselves at 9.4T was  $\sqrt{3}$  times.

By combining the almost 4-fold SNR advantage originated from the hardware discussed in the first



**Fig. 4.** Maximum intensity projections from 9 different directions calculated from the three-dimensional image datasets of the CS22 embryos. Voxel size is  $(60 \,\mu\text{m})^3$ .

paragraph of this subsection and taking into consideration the 1/8 of the voxel volume,  $(4 \times \sqrt{3})/8$ – 0.87 times, or comparable SNR *per voxel* is expected for the measurement at 9.4T. However, the observed SNR at 9.4T (23.3) is about 4 times worse than that (101) observed at 2.35T shown in Fig. 2. This SNR degradation is caused by elongation of the T<sub>1</sub> that is roughly proportional to 1/3rd power of the resonance frequency (about 1.6 times loss at 9.4T compared with that at 2.35T) and the insufficient receiver dynamic range pointed out in the previous study.<sup>9</sup>

#### Image contrast of the liver

As shown in Fig. 2b and 2c, image contrast of the liver is quite different between the 2 magnetic field strengths. This difference is caused mainly by shortening of the  $T_2$  and  $T_2^*$  of protons in the liver at 9.4T compared with those at 2.35T. This shortening is considered to be attributable to hematopoietic stem cells (HSC) in the liver because the

#### MR Microscopy of Fixed Human Embryos



**Fig. 5.** Axial cross sections selected from the three-dimensional image dataset of the CS22 embryos acquired with the gradient echo sequence (TR = 100 ms, TE = 5 ms). (a) The level of the choroid plexus. (b) The level of eyes. (c) The level of arms and hands. (d) The level of the liver.

HSCs produce red blood cells that contain hemoglobin and related paramagnetic compounds.

## The next anatomical database of the Kyoto Collection

This study solved several technical problems for constructing the next (higher spatial resolution) anatomical database for the Kyoto Collection. However, the measurement time for a single sample was about one day, so it would take about 4 years to measure 1,200 embryos if we repeat measurements of specimens identical to those of the previous study. Therefore, more efficient techniques, such as use of the superparallel MR microscope at high magnetic fields (>7.0T) and/or the compressed sensing technique,<sup>12</sup> are desired.

## Conclusion

We developed a fundamental technique that could be used to construct the next anatomical database of the Kyoto Collection, experimentally demonstrated its feasibility for acquiring images with matrix of  $256 \times 256 \times 512$  of embryos of Stages CS16 to 22 within a practical imaging time, and clarified the problems to be solved for the next project. Although measurement time is a major problem, we think future developments in hardware or software could overcome this problem.

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#### References

- Smith BR. Visualizing human embryos. Sci Am 1999; 280:76–81.
- 2. Matsuda M, Ono S, Otake Y, et al. Imaging of a large

collection of human embryo using a super-parallel MR microscope. Magn Reson Med Sci 2007; 6:139–146.

- Nishimura H, Takano K, Tanimura T, Yasuda M. Normal and abnormal development of human embryos: first report of the analysis of 1,213 intact embryos. Teratology 1968; 1:281–290.
- 4. Matsuda Y, Utsuzawa S, Kurimoto T, et al. Superparallel MR microscope. Magn Reson Med 2003; 50: 183–189.
- Nakashima T, Hirose A, Yamada S, Uwabe C, Kose K, Takakuwa T. Morphometric analysis of the brain vesicles during the human embryonic period by magnetic resonance microscopic imaging. Congenit Anom (Kyoto) 2012; 52:55–58.
- 6. Kagurasho M, Yamada S, Uwabe C, Kose K, Takakuwa T. Movement of the external ear in human embryo. Head Face Med 2012; 8:2.
- 7. Hirose A, Nakashima T, Yamada S, Uwabe C, Kose K, Takakuwa T. Embryonic liver morphology and

morphometry by magnetic resonance microscopic imaging. Anat Rec (Hoboken) 2012; 295:51–59.

- Kaigai N, Nako A, Yamada S, Uwabe C, Kose K, Takakuwa T. Morphogenesis and three-dimensional movement of the stomach during the human embryonic period. Anat Rec (Hoboken) 2014; 297:791– 797.
- 9. Otake Y, Kose K, Haishi T. A solution to the dynamic range problem in MRI using a parallel image acquisition. Concepts in Magn Reson Part B 2006; 29B:161–167.
- 10. Drews U. Taschenatlas der embryologie. 1st ed. Stuttgart: Georg Thieme Verlag, 1993.
- 11. Hoult DI, Richards RE. The signal-to-noise ratio of the nuclear magnetic resonance experiment. J Magn Reson 1976; 24:71–85.
- 12. Lustig M, Donoho D, Pauly JM. Sparse MRI: the application of compressed sensing for rapid MR imaging. Magn Reson Med 2007; 58:1182–1195.