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研究課題名（和文）ゲッティンゲン大学ヒト胚子コレクションの組織学的解析およびデジタルアトラス作成

研究課題名（英文）Digitization, Histological Analyses and Making Web Atlas of Blechschmidt Collection

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研究成果の概要（和文）：再生医学の発展に伴い、ヒトの発生学の重要性は増してきている。胚子・胎児の組織標本はその最大の情報源であるが、ヒト標本の新規獲得は困難であり、既存標本の保存・活用が必須となっている。Blechschmidtコレクション（ドイツ）は世界的に見ても量・質ともに大変貴重なヒト胚子連続組織切片標本群であるが、経年劣化が進み、研究利用もしにくいといった問題が生じていた。そこでこの重要な試料を保存し、研究へ活用するためにデジタル画像化を行った。得られた２次元画像から３次元再構成を試み、その有用性が確かめられた。加えて、画像を整理しウェブで公開したことで、更なる研究の活発化にも寄与できたと考える。

研究成果の概要（英文）：Regenerative medicine is now developing, and it is important to understand the mechanism of human development. Sets of serial sections of embryos and fetuses are the largest source of information, but it is difficult to acquire human embryo specimens newly. Therefore, preservation and utilization of existing specimens are required. The Blechschmidt collection (Germany) is one of various collection including many sets of serial histological sections, however, glass slides of histological specimens normally deteriorate over time and staining by hematoxylin and eosin (HE), typically fade. Now we saved this important samples by digitization for research use. Three-dimensional reconstruction was attempted from the obtained two-dimensional image. In addition, we arranged the images and publishing them on the web, thereby contributing to the further activation of research.

研究分野：　解剖学、発生学

キーワード：　Human Embryo　Blechschmidt Collection　digitization

１．研究開始当初の背景

Morphogenesis is key to understanding the morphology of human organs in the postnatal period and crucial for fields, including regenerative medicine, developmental biology, plastic surgery for congenital anomalies, and evolutionary biology. Traditional human embryology has increased our understanding of morphological and developmental processes, and several major collections of human embryos and fetuses have been maintained worldwide with this aim.

However, the expansion of human embryo collections has become extremely difficult in recent years due to the changes in clinical techniques. Therefore, existing embryo collections should be preserved and utilized effectively. Glass slides of histological specimens normally deteriorate over time. Furthermore, old specimens, mainly stained with hematoxylin and eosin (HE), typically fade (Fukunaga & Yashiro, 1979). Therefore, the need to digitize and preserve these slides has become an imperative. Advances in digital image acquisition have made it much easier to obtain 2D and 3D images and reconstruct 3D models to understand human development. Specific organs and tissues, such as the heart and bronchial tubes, can also be extracted using 3D images (Yamada et al., 2007). Similar 3D imaging of development has been performed using magnetic resonance imaging (MRI) for the brain (Yamaguchi et al., 2017) and nasal region (Katsube et al. 2017).

The Blechschmidt Collection is a major embryo collection famous for its reconstructed models and fine histological slices. Erich Blechschmidt (1904-1992) began the systematic collection of human embryos at the end of 1945, and the collection is now preserved in the Centre of Anatomy, University Medical Centre Göttingen (Georg-August-University of Göttingen, Germany). The Blechschmidt Collection contains high-quality serial sections from more than 120 specimens. In addition, it includes some larger, valuable specimens of earlier fetuses. The Centre of Anatomy also houses a unique collection of 64 enlarged plastic (Leguval Bayer) models of human embryos. The three-dimensional reconstructions were built based on serial sections of well-preserved human embryos. The sophisticated models show the entire embryo surface and some organ systems, including the circulatory system, skeletal system, and central nervous system (CNS). Many textbooks use figures of the models.

The Blechschmidt Collection has not been subjected to a digitization project thus far. In 2014, some of the present researchers from Kyoto University scanned sections in Göttingen and found the specimens to be in suitable condition for research (Ueno et al., 2016). On this basis, the decision was made to scan the remaining slides of the Blechschmidt Collection, and the digitization of the collection commenced in 2015.

２．研究の目的

The invaluable specimens of the Blechschmidt Collection were digitized with a commercial flatbed scanner and reconstructed into 3D images by using modern techniques. From these 3D images, the specimens could be classified into CSs, and several organ systems could be observed clearly.

３．研究の方法

(1) Digitization of the specimens

To create digital images from histological sections, flatbed scanners (CanoScan 9000F, Canon, Tokyo, Japan) and PhotoStudio software (Version 6.0.1.148, ArcSoft, Fremont, CA) were used. The sections on the glass slides were scanned at 4800 dots per inch (dpi), and saved in BMP and JPG formats. The glass slides carrying information about the histological sections, such as the slice thickness, missing slices, and stain, were also scanned to acquire metadata.

(2) Analysis of digital data

Among the 125 scanned specimens, two were selected for additional analyses, based on the completeness and intactness of the slices. The larger specimen was a fetus of crown-rump length (CRL) 64.0 mm that was processed on April 25, 1950; the CNS was extracted, and 3D images were reconstructed. The sagittal slices of this specimen are complete from the right temporal tip of the head to the left end; however, the lower extremities were cut off at the middle of the thighs, and the upper extremities were cut off at the middle of the brachia. The smaller specimen was an intact embryo of CRL 17.5 mm, processed on June 22, 1949, and selected in an attempt to classify the CS (Figure 1). The eight separate slices in the BMP images of each glass slide from the specimen with a CRL of 17.5 mm were cut out and saved as separate files. The CNS of the larger specimen was segmented with Photoshop Elements and Photoshop (Adobe, San Jose, CA). Segmented areas were painted on a separate new layer. For computer analysis, pixel resolutions were reduced to 10% for the larger specimen and 25% for the smaller embryo. The images were converted into TIFF format and stacked with ImageJ software (Wayne Rasband [National Institutes of Health], Bethesda, MD).

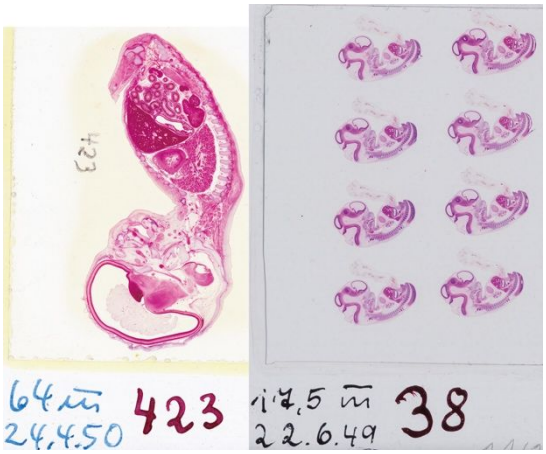

Figure 1. Images of glass slides of the larger specimen with a crown-rump length (CRL) of 64.0 mm (left) and one of the smaller specimens with a CRL of 17.5 mm (right). The date processed, CRL, and serial number of the glass slide of the specimen are written on each glass slide. For the larger specimen, one slice was mounted on one glass slide, whereas for the smaller specimen, eight slices were aligned on a glass slide. The cut at the middle of the thigh of the larger specimen is visible.

Distortions introduced during processing and sectioning were retained. Finally, to register the stacked TIFF three-dimensionally and render a 3D model, Amira 6.0.1 software (FEI, Hillsboro, OR) was used. Registrations were made first with the AlignSlices module of Amira, and then they were adjusted manually.

(3) Constructing a website

The digitalized images of the 125 specimens were released in a website. Pixel resolution of all the images was reduced by Photoshop software. A representative image for every specimen was picked up and arranged in the size (CRL) order. On the information page for the specimens with a lot of slides, selected images were uploaded such as every 5, 10, 20, or 50 slides. In addition, the movies of 3D reconstruction from the slides were processed using Amira suitable for the website.

4．研究成果

(1) *Digitization*

125 specimens of the Blechschmidt Collection were digitized with flatbed scanners. The CRL ranged from approximately 2.5 to 140.0 mm; it was also included valuable specimens such as fetus of post-embryonic stage. As a whole serial section, CRL 64.0 mm was the maximum. Almost all specimens except for extremely historic (processed in 1939) and recent (processed in 1977 and 1995) ones were processed from 1945 to the 1960s.

The obtained data were sufficiently detailed for macroscopic morphological observation. Figure 1 presents a glass slide of the largest specimen and one of the finest preparations of a histological section. The largest specimen of this study had a CRL of 64.0 mm, which is unavailable in the Kyoto Collection. By traditional definition, it is not an embryo but a fetus of post-embryonic stage, an exceptionally large-sized sample in histological serial sections of human embryology, and each single section is mounted on one glass slide. The sagittal sections were complete, finely prepared, and preserved from right end to the left of the body of the specimen. The slice shown to the left of Figure 1 is slightly off the midline of the fetus; therefore, the lateral ventricle of the cerebrum, heart, digestive canal, liver, vertebral column, lung, some bones around the hip joint, and other organs are clearly observed. The smaller specimen, with a CRL of 17.5 mm, to the right of Figure 1, shows the CNS, vertebral column, digestive canal, liver, and other organs clearly. Eight sections of this specimen were mounted on one glass slide.

(2) *Analysis of the digitized data*

To test the utility of the data for 3D reconstruction and analyzing the 3D shape, the two specimens shown in Figure 1 were further assessed. Segmentation and 3D reconstruction of the CNS were achieved with the largest specimen (CRL 64.0 mm). Segmentation was performed manually. From the reconstructed 3D images, the detailed outline of the CNS was apparent (Figure 2). The Sylvian fissure had emerged, and the cerebellum was differentiated.

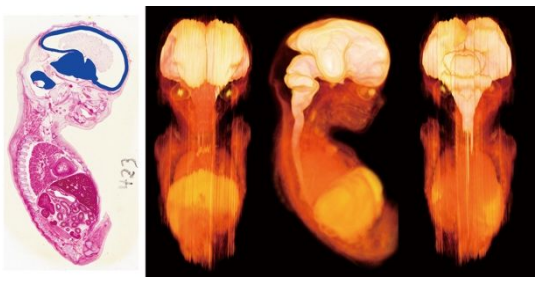

Figure 2. Left image shows the central nervous system (CNS) of the larger specimen (CRL 64.0 mm) segmented manually in blue with Photoshop. Right images show 3-dimensional reconstruction of the larger specimen with the segmented CNS highlighted. Shown left to right are the ventral, right, and dorsal views. Note that some distortions are due to section deformations during tissue processing.

Three-dimensional reconstruction and classification were performed with the smaller specimen (CRL 17.5 mm). Images of the slices were stacked serially and reconstructed into 3D images (Figure 3). The 3D images clearly represented the intact outer shape of the

specimen. True to the models made from the photograph before sectioning, this 3D model showed the neural tube curves according to the heads and tails, which move leftward. The CS was determined without difficulty. This specimen was classified as the latter half of CS 20, with the features of the upper limbs bent at the elbows and interdigital notches apparent on the rim of the foot plates. The maximum intensity projection rendering mode revealed some fine inner structures with exceptional clarity, such as the neural ganglia and bronchi.

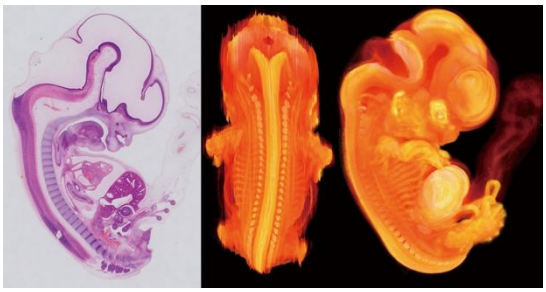

Figure 3. Left image shows a section of the smaller specimen (CRL 17.5 mm). Shown right are two 3-dimensional renderings in the dorsal and right views. Note that the distortions from processing appear to be less pronounced in the smaller specimen.

Comparing these two 3D reconstructions, the smaller specimen provided a better result because the distortions during the slice preparations were less pronounced. The larger specimen showed more noticeable distortions of the slices, especially on the regions of neck and cranium.

The images from all sections of a given specimen were stacked according to their inner structures, and 3D images were reconstructed from the sections.

The CNS from the specimen of CRL 64.0 mm was segmented directly on the sections, and the outer surface and CNS were reconstructed into 3D images by stacking the slices with Amira software (Figure 2). Other systems or organs, such as the circulatory system or liver, can also potentially be segmented and reconstructed into 3D images to observe their shape and quantify their volumes.

From reconstructed 3D images, specimens in serial sections could be classified into CSs. Based on observation of its surface on 3D images, the 17.5-mm specimen was classified into CS 20. With reconstructed 3D images, characteristics such as bent elbows and notched rims of the footplate are easily observed, and classification of a specimen into a CS is simple. If observation had been limited to the sliced sections alone, such classification would have been difficult. The CS classification represents a new staging for the Blechschmidt Collection, which has heretofore tagged their specimens only with CRL and the date of sectioning. Classification with the CS will be complementary to comparisons with specimens in other collections such as those in Kyoto.

The resolution used in the present study (4800 dpi, that is 5.29 micrometers per dot), is comparatively low in the field of histology. With such a low resolution, each cell cannot be distinguished. To overcome such a limitation, high power field scanning for human embryos is underway worldwide (Digital Embryology Consortium, 2015).

Comparing the two specimens analyzed in this study, the smaller embryo (CRL 17.5 mm) achieved a more satisfactorily result of 3D reconstruction. Slices of the larger specimen (CRL 64.0 mm) had become more deformed during the course of section preparation. This could have been compensated for with non-rigid deformation techniques (Kajihara et al., 2017).

In this study, digital images were acquired from histological sections of the Blechschmidt Collection, which is valuable to the study of human embryology. Furthermore, the outer surfaces of two specimens were reconstructed into 3D images. The CNS of a large specimen (in fetus stage) was also extracted and reconstructed. A smaller specimen in the embryonic stage was classified according to CSs from the 3D surface reconstruction. These applications are highly valuable to the further progress of human embryology. I am continuing to digitize and analyze the specimens of the Blechschmidt Collection to revisit in future research.

(3) *Website "Blechschmidt Collection"*

A website about this study was constructed in Japanese and English. It also plays the role as an atlas of the Blechschmidt Collection. On atlas page, we can browse all the specimens sorted by the size of CRL. By clicking on the cover image or the CRL button, we can open information page of each specimen, where we can see information about the specimen, such as the processed date, slice thickness, missing slices, stain, and so on. And, by clicking on the name of each image, the enlarged views of several scanned images are observed.

In addition, 3D-reconstructed images of 4 specimens were also released on this page. Moving on the information page, enlarged images and information about the specimen are observed. The specimen in the movie is rotated 360 degrees, and it can be operated go back and forth at will.

By releasing the website of the digitalized Bleshschmidt Collection, everyone can access the precious specimens from

anywhere in the world at any time via the internet. Since the precious specimens should not be moved from the University of Göttingen, we had to visit Göttingen and we could observe them only during staying there. The released website is helpful for the researchers who want to study the human embryology but who don't have enough materials. The website will greatly contribute to the sharing of the samples and activation of human embryonic research.

５．主な発表論文等
（研究代表者、研究分担者及び連携研究者には下線）

〔雑誌論文〕（計２件）

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② Yamada S, Nakano S, Makishima H, Motoki T. Novel Imaging Modalities for Human Embryology and Applications in Education. Review Article. Review Article. *Anat Rec (Hoboken)*. 2018 Jun;301(6): 1004-1011.. doi: 10.1002/ar.23785. [Epub ahead of print]

〔学会発表〕（計０件）

〔図書〕（計１件）

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〔産業財産権〕

○出願状況（計０件）

○取得状況（計０件）

〔その他〕
ホームページ等
http://atlas.cac.med.kyoto-u.ac.jp/BC/index.html

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