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Presenting Human Embryology in an International Open-Access Reference Centre (HERC)

Beate Brand-Saberi[1], Edgar Wingender[2], Otto Rienhoff[2] and Christoph Viebahn[2]
[1]*Ruhr-Universität Bochum,*
[2]*Georg-August-Universität Göttingen,*
Germany

1. Introduction

Specimens from early human embryonic development largely originate from chance findings in material collected for pathological analysis following spontaneous or induced abortions, and minimally invasive gynecological surgery for termination of pregnancy (suction curettage) emerging in the early 1990s has now made it almost impossible to procure new intact specimens. Although the absolute number of specimens collected world-wide may be quite high due to the intrinsic fascination held by human embryos, few concerted long-term projects have managed to organise the complex and labour-intensive logistics of acquiring, processing and safely storing specimens for morphological (i.e. histological) analysis. As a result, only four centres world-wide house collections with an appreciable number of scientifically useful specimens.

2. Major embryo collections of the world

2.1 Washington D.C. (USA)

The Carnegie Collection of Human Embryos in Washington D.C. (USA) is the largest collection of embryos (some 10 000) cut into serial histological sections. Because many of these specimens stem from the time before optimal histological fixation protocols were available, only relatively few of them are suitable for high-resolution histological analysis. Nevertheless, this collection formed the basis for the definition of the 23 stages of human development during the first 8 weeks (O'Rahilly and Müller, 1987), which serves as the international standard. For further information on the Carnegie Collection see http://nmhm.washingtondc.museum/collections/hdac/index.htm in this book.

2.2 Kyoto (Japan)

The Congenital Anomaly Research Centre at Kyoto University (Japan) is at present the largest human embryology collection with some 40 000 embryos and fetuses. Emphasis here lies on nuclear magnetic resonance (NMR) analysis of intact specimens; however, 1,000 specimens of this collection are serially sectioned, with one half of them being diagnosed as

normal and the other half as abnormal. Further information on the Kyoto Collection may be found in Yamada et al. (2010) and in the chapter by Yamada et al. in this book.

2.3 Göttingen (Germany)

The embryo collection at the centre of Anatomy, Göttingen University (Germany), is unique as it has probably the largest number of excellently preserved specimens of the latter half of the embryonic period (weeks 5 to 8 post conception) world-wide; this was achieved by a combination of a special fixation procedure adjusted by Erich Blechschmidt (1904-1992) to the then "state-of-the-art" gynecological practice (mechanical curettage or hysterectomy) for gynecological operations including termination of pregnancy. As a result, the quality of paraffin histological sections of the more than 120 embryos comprising this collection is unsurpassed and reveals valuable morphological detail of organ development in early human development (cf. Fig. 1). Unfortunately, microscopical glass slides used to hold histological sections are delicate and in constant danger of destruction during use; even under optimal storage conditions they have a finite useful life (in the order of decades) due to gradual deterioration such as evaporation of cover glass glue and bleaching of histological stains. Photomicrographs of individual histological sections from several specimens were published in Blechschmidt's embryology textbook (Blechschmidt, 1960) but the only chance to preserve for posterity morphological information contained in these specimens consisted, at that time, in building large-scale polymer plastic reconstruction models (cf. Fig. 2C) from camera-lucida drawings at an intermediate magnification of regularly spaced histological sections (Blechschmidt 1954). Unique in his approach was the strategy that using the same series of serial sections several times over, Blechschmidt made reconstructions of the surface anatomy and the morphology of several organ systems of the same embryo, thereby

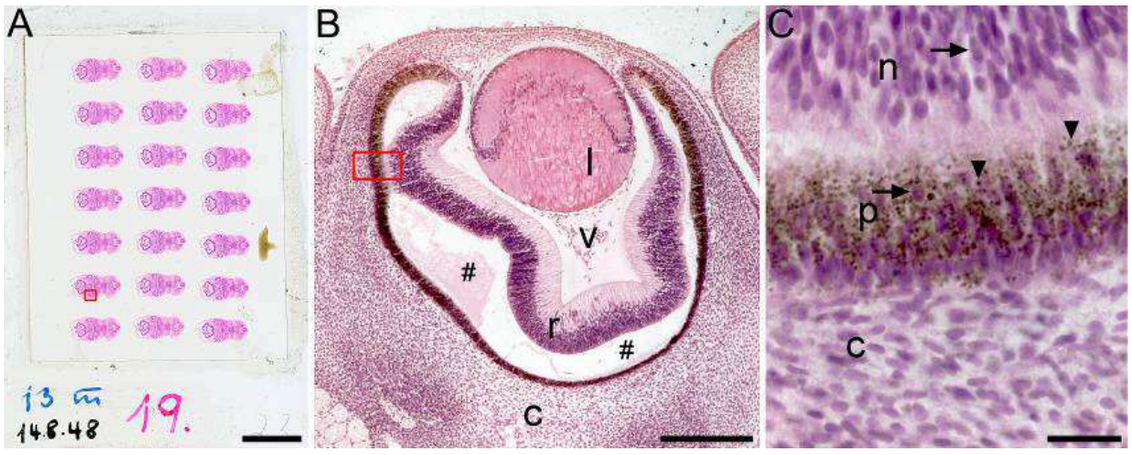

Fig. 1. **A:** Microscopical glass slide with three rows of seven hematoxylin-eosin stained transverse paraffin sections each from a 13-mm human embryo (stage 18) and original inscriptions of specimen details and section number. **B:** Magnification of area marked with the red box in A showing anlage of the eye bulb with lens (l), vitreous body (v), neural layer of the immature retina (r), choriocapillaris layer (c) and typical shrinkage artefacts (#) between the neural (inner) and pigmented (outer) layers of the retina. **C:** Highest magnification of choriocapillaris (c) and neural (n) and pigmented (p) layers of the retina in the area marked with the red box in B showing cellular details such as nuclei (arrows) and pigment granules (arrowheads). Magnification bars: 10 mm (A), 0.2 mm (B), 0.02 mm (C).

Fig. 2. Views of the large-scale reconstruction models of the "Humanembryologische Dokumentationssammlung Blechschmidt" in the exhibition hall of the Centre of Anatomy in Göttingen (**A**), a selection of three different models reconstructed from the same series of serial sections from a 4.2-mm embryo (**B**) and a close-up (**C**) of one of these models highlighting, amongst other features, the developing arterial and venous vascular systems (orange and blue, respectively), digestive system (green) and nervous system (beige).

enabling direct comparison of topographical characteristics and their dynamic changes during development, even though the cellular detail detectable at high magnification (cf. Fig. 1C) remained unexplored with this method. However, over the course of several decades (from 1946 to 1979) more than 64 models were created, which, to this day, form the basis of the "Humanembryologische Dokumentationssammlung Blechschmidt", a permanent exhibition housed at the Centre of Anatomy of Göttingen University (Fig. 2).

Detailed documentation on individual specimens of the Blechschmidt Collection is sparse. Collectively these specimens are known to be chance findings from pathological material obtained after gynecological operations including legal terminations of pregnancies for medical indications, but there is a catalogue of technical entries on both the histological sections and the large-scale reconstructions. Some of the specimens are depicted as colour drawings in Blechschmidt (1960).

2.4 Bochum (Germany)

Principles for high-quality tissue preservation similar to those successfully practised in Göttingen were applied by Klaus V. Hinrichsen, a pupil of Blechschmidt, after he took the chair of Anatomy and Embryology at the Ruhr University Bochum in 1970. As a result of improved fixative solutions for electron microscopy developed since the start of Blechschmidt's project, many excellent specimens, some of them suitable for subcellular analysis previously unknown from human specimens (cf. Figs. 1 and 3), were collected by Hinrichsen's team between 1969 and 1994 and are now housed in the Department of Anatomy and Molecular Embryology at the Ruhr-Universität Bochum, Germany. Details of

many of these specimens in the Hinrichsen Collection (total number n = 70) were published in Hinrichsen's textbook on human embryology (Hinrichsen, 1990) and in many original publications (e. g. Hinrichsen et al. 1994) but reconstructions have not been attempted from these specimens and many specimens have likewise remained unexplored, to date.

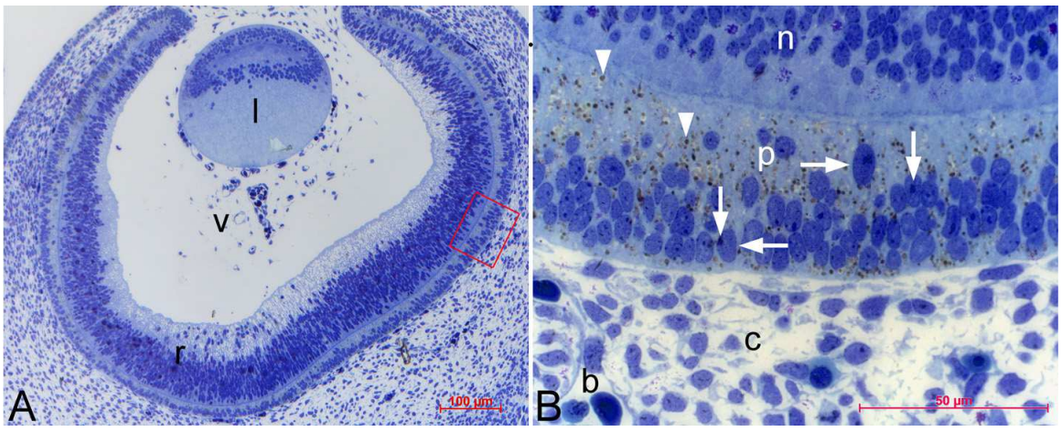

Fig. 3. Semithin plastic section of the eye anlage of a stage 18 embryo. **A** Survey photograph showing lens (l), vitreous body (v), retina (r) and surrounding connective tissue of the eye bulb. **B** Higher magnification taken from border between retina and choriocapillaris (c) layer (similar area marked with red box in A) with blood vessels (b) and the pigmented (p) and nervous (n) layers of the immature retina. Visible subcellular details include dark pigment granules (arrowheads) in the cytoplasm in the pigment layer (cf. Fig. 1C); within the cell nuclei nucleoli (vertical arrows) and the nuclear lamina (horizontal arrows) can be distinguished. Magnification bars: 0.1 mm (A), 0.05 mm (B).

Derivation of the Bochum specimens was in the tradition of the Blechschmidt collection, i.e. they were chance findings in the pathological material derived from legal abortions, as well as from spontaneous abortions. Documentation includes hospital names and dates, but further specimen details are missing. The approval of the ethics committee of Bochum University was recently obtained for the use of specimens from the Hinrichsen collection in medical dissertations (Reg. No. 3791-10).

3. Current methods for digitisation of microscopical specimens

3.1 Digitisation of sectional embryonic morphology

With the advent of digital microphotography serial sections of human embryos from the Carnegie Collection at Washington DC were scanned at high resolution at Louisiana State University Health Center in New Orleans (USA) as part of the Virtual Human Embryo (VHE) project funded by the National Institutes of Health, Maryland, USA. Because the stitching of neighbouring high-magnification microphotographs had to be carried out manually after the scanning was complete, this initial project ran for many years and a complete set of one specimen each for the first 17 Carnegie stages of the first 5 weeks of prenatal development is now available in DVD format (http://virtualhumanembryo.lsuhsc.edu/). The remaining stages up to stage 22 are due to be completed by April 2012 (R. Gasser, pers. inf.). Technological advances during the close of the VHE project brought about a major breakthrough in automatic scanning of

microscopical slides for seamless virtual microscopy, 3D-construction and on-line usage which, however, could not yet be used for the VHE project.

In their clinically oriented approach, the Congenital Anomaly Research Centre at Kyoto University (Japan) created a web-accessible annotated 3-D Human Embryo Atlas using their extensive data base of nuclear magnetic resonance (NMR) and episcopic fluorescence capture (EFIC) images of first trimester human embryos (Yamada et al. 2010; http://apps.devbio.pitt.edu/HumanAtlas).

3.2 Virtual microscopy

Virtual microscopy uses digitally stored histological slides previously scanned at high resolution and stored in an open format, to browse through all parts of the histological specimen and zoom in *ad libitum* to any part of the section at the highest light-microscopical resolution, in a manner close to conventional (physical) microscopy. Powerful and versatile light microscopy scanning systems are presently produced by a few leading manufacturers only (Olympus and Zeiss/Metasystems) and consist of a light microscope, digital camera, motorised scanning microscope table, a computer workstation and software for scanning, archiving and viewing whole histological slides. Virtual microscopy includes visualisation of the specimens at continuous intervals of magnification (up to 40x) and fine focusing of the specimen along the z-axis at a given magnification (Fig. 4). Initial tests with the Olympus system carried out on a microscopic slide containing 3 rows of 7 histological sections each from a 13-mm human embryo from the Blechschmidt collection (cf. Fig. 1) provided the proof of principle for scanning serial sections mounted on over-sized glass slides and, most importantly, gave an approximation for the scanning time of about 20 min when using the 40x lens on an individual histological section with a tissue surface of about 2 cm² (cf. Fig. 4).

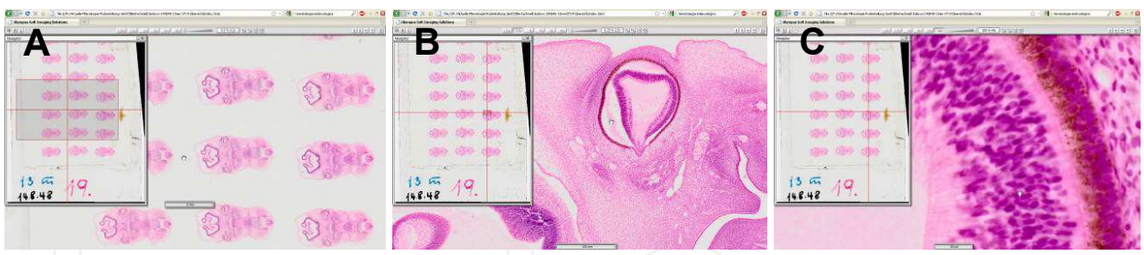

Fig. 4. Screen shots of the Olympus virtual microscopy viewer applied to the slide shown in Fig. 1 containing the developing eye ball at stage 18 at low (0.2x, **A**), middle (2.5x, **B**) and maximal (40x, **C**) digital zoom level. The high zoom level (**C**) shows subcellular detail such as position of cell nuclei and pigment granules (brown) in the nervous and pigmented layers of the immature retina, respectively.

Virtual microscopy - with or without annotation - is widely used for teaching normal (http://www.mikroskopie-uds.de, http://mirax.net-base.de/Home.uni-ulm.0.html) and pathological anatomy (http://patho.med.uni-magdeburg.de/Virtuelle_Pathologie/goea.shtml). Solutions suitable for embryological research purposes are only beginning to be established but will have to connect to existing digital atlases containing conventional images (of defined but fixed magnification) of human development (i.e. NMR and EFIC data at the Kyoto collection: http://apps.devbio.pitt.edu/HumanAtlas) or of various animal model organisms for developmental biology (mouse, chick, frog, zebrafish, fruit fly): http://www.sdbonline.org/index.php?option=content&task=view&id=17&Itemid=22).

3.3 Annotation

Prerequisites for teaching and, most importantly, for reconstruction are annotation systems for individual structures and organ systems within a given histological section. Due to the high-quality conservation methods used by Blechschmidt and Hinrichsen, the cellular structure in the tissue sections is generally so well preserved that annotating substructures of embryos and cells will be possible at different high-resolution levels.

However, annotation may create problems in case of ambiguous terminology or unforeseen tissue artefacts which may occur during cutting and staining of histological sections. The former problem can be minimised (1) by closely adhering to the Terminologia Embryologica (TE) which has been completed in 2010 by an international committee of embryologists (FIPAT), and (2) by use of the ontology databases (s. Burger et al. 2008). In these databases material entities and immaterial concepts of a knowledge domain are interconnected with a set of specified relationships (e.g. “partOf” or “developsInto”), which makes an automatic interpretation of entities possible and embeds them into a semantic web. For the HERC project the "Cytomer" database (Michael et al. 2005) will be used which is the only known ontology of anatomical entities directly connected to (Carnegie) stage-related information such as precursor structures (“anlagen”) and germ layer origin of tissues: the entity 'heart', for example, is defined as (1) being an organ, (2) being part of the cardiovascular system, (3) developing from the heart tube and (4) having parts such as the heart valves. This information will then be linked to the annotated structures in serial sections and whole embryos. If earmarked with persistent or unique object identifiers (PID and UOI, respectively) such metadata enables multiple search and filtering functions and facilitates the integration of other resources, such as specimens and structures defined in other embryo collections and anatomical atlases, and in databases for molecular biology (e.g. UniProt, GenBank) and for scientific publications (e.g. PubMed).

3.4 Metadata handling

Handling of the scientific data created by annotation presents an increasing challenge to data management. The latest tendencies in scientific information management, therefore, deal with establishing well integrated virtual research environments (VRE) across organizational boundaries of academic institutions. The Department of Medical Informatics of Göttingen University has accumulated ample experience in the BMBF joint project WissGrid with regard to VREs being built on a distributed IT infrastructure. This experience will serve to integrate and optimise the software used in the project presented here. WissGrid is linking knowledge of several key research partners (e.g. State and University Library Göttingen and e.g. the Astronomy Center in Potsdam). Therefore image-handling as well as annotation have formed part of BMBF funded research in the Department. The results of WissGrid were discussed with the DFG in September 2010; the recommendations from those discussions will be the basis of the work programme for this project.

3.5 Longterm archiving

Preserving research data is paramount for all sciences (cf. Vorschläge zur Sicherung guter wissenschaftlicher Praxis, DFG, 1998-2010). The Dept. of Medical Informatics has been constantly involved in several projects which foster this goal: A research oriented web based image database (Chili PACS) has been hosted since 2005 (this database contains data of 8 projects including the national competence networks for congenital heart defects, dementia,

and multiple sclerosis). Digital preservation has also been established within the DFG joint project KoLaWiss from 2008 to 2009, and in the BMBF joint project WissGrid generic biomedical requirements are investigated with regard to digital preservation. Finally, the department is a leading partner in the digital preservation related DFG joint project LABIMI/F, expected to start in March 2011.

WissGrid addresses bitstream preservation and generic technical aspects of content preservation within the world-wide available and standardized Grid Computing infrastructure. Additional biomedicine related aspects of digital preservation will be investigated in the DFG joint project LABIMI/F which can also deal with data from magnetic resonance imaging (Kyoto website) and other research oriented databases (Edinburgh mouse atlas: http://genex.hgu.mrc.ac.uk/) and can therefore intersect with the present project on the generic level of digital preservation of digital image data. The Open Archival Information System (OAIS) provides a generic digital preservation approach (CCSDS, Consultative Committee for Space Data Systems: http://public.ccsds.org/publications/archive/650x0b1.pdf). Against this background Göttingen provides an ideal research environment for the digital preservation envisaged here due to a number of highest-level cooperative IT projects: **- GoeGrid:** interdisciplinary cooperation of grid computing communities: http://goegrid.de. **- GWDG:** experienced IT service provider for bitstream preservation: http://www.gwdg.de /index.php?id=898. **- SUB:** strong experience in digital preservation and leader of the TextGrid joint project: http: //rdd.sub.uni-goettingen.de.

4. The project

4.1 General aims

Bringing together four partners from two universities and with a view to providing the basis for an international human embryonic reference centre (HERC), this pilot project aims at developing methods and standards for the preservation and open-access presentation of microscopic preparations of early human development. Cutting-edge computer technology in image acquisition, digital annotation and cross-border information management will be developed and applied to selected specimens from two large collections of irreplaceable human embryonic specimens. Strategic long-term commitment to follow the first project period in both Universities will eventually enable the complete digitisation and open-access provision of the two collections as an indispensable step to secure this unique resource for scientific use.

Separable goals are as follows:

1. creating a virtual research environment (VRE) across organizational boundaries of the academic institutions involved.
2. implementing a system of high-resolution digitisation of histological serial sections of human embryology taken from representative organogenesis stages of development (Carnegie stages 12 to 23).
3. development of an ontology based annotation client optimised for a high number of related histological sections.
4. annotation of embryos, relevant structures and landmarks with persistent identifiers (PIDs) using international terminology standards (e.g. the TE) and the Cytomer ontology.

5. use of PIDs defined in the annotation process (goal 3) for complex cross-linking to similar entities in neighbouring histological sections, different specimens, developmental stages and research databases.
6. close cooperation with databases of (1) The Human Developmental Anatomy Collection (Carnegie Collection) at the National Museum of Health and Medicine at Washington DC, USA and (2) The Congenital Anomaly Research Center in Kyoto, Japan.
7. establishing a platform for intelligent search functions for PIDs.
8. development and implementation of hardware and software for mass data handling and long-term archiving.
9. open access presentation at the official website of Göttingen University.

Future perspectives consist in (1) detailed comparison of individual variations between embryos of the same stage digitised in the two other human embryology centres (Washington DC and Kyoto), (2) cross-referencing with gene expression databases such as Mouse Genome Informatics of The Jackson Laboratory, Bar Harbor, ME, (USA), and (3) 3D-reconstruction of whole embryos complete with annotation and open-access presentation.

4.2 The schedule

The complex aims set out in this enterprise can only be met if a plausible time schedule is followed so that initial technical problems can be solved and methods come to fruition in the long term and for further projects on a similar line. The 2-year time frame shown at the end of this chapter (s. Table 3) is based deliberately on a set of 4 defined work packages (WP1 – WP4) and a subset of 2 – 5 milestones (M) using a given number of specimens from which extrapolation may be deduced for other specimens, collections and cooperative set-ups.

4.2.1 Digitisation (WP1)

Göttingen. Digitisation of histological sections requires dedicated scanning software. A virtual microscopy system provided either by Olympus or Zeiss/Metasystems will be installed in a room close to the safe store of the microscopical slide collection in the Centre of Anatomy. After capture files will be transferred for further use and backup storage to the two servers housed in different departments.

Stage	Embryo no.	embryo size (greatest length)	approx. no. of sections à 10µm
11 (13 somites)	HERC11-1	3.1 mm	310
12 (23 somites)	HERC12-1	2.5 mm	250
12 (27 somites)	HERC12-2	3.4 mm	340
13 (30 somites)	HERC13-1	4.2 mm	420
14	HERC14-1	6.3 mm	630
15	HERC15-1	7.5 mm	750
16	HERC16-1	10.0 mm	1000
17	HERC17-1	13.5 mm	1350
19	HERC19-1	17.5 mm	1750
		total:	6800

Table 1. List of 9 representative embryos from the Blechschmidt Collection.

Paraffin sections:				
Stage	Embryo no.	embryo size (gr. length)	plane	approx. no. sections à 10µm
19	HERC19-2	19 mm	sag.	800
20	HERC20-1	21 mm	sag.	800
22	HERC22-1	26 mm	transv.	1600
23	HERC23-1	29 mm	front.	1500
			total:	4700
Semithin sections:				
1. Heart				
Stage 13	HERC13-2	5.5 mm	front.	400
2. Eye				
Stage 18	HERC18-1	16 mm	sag.	600
Stage 18	HERC18-2	15.5 mm	sag.	300
			total:	1300

Table 2. List of representative embryos of the Hinrichsen Collection.

An initial six-week training phase will be arranged to develop time-efficient handling of the virtual slide system and the data management. This training period will also be used to establish an efficient work-flow within the local network of project partners. Subsequently, the whole series of histological sections from nine embryos chosen for their excellent tissue preservation (Table 1) will be scanned continuously over the two-year period of the project. Preliminary tests using the Olympus virtual slide system VS110-S5-E showed that the scanning time in one z-layer per average histological section of the collection is about 20 minutes. Following an estimation of the number of histological sections per embryo at different stages (s. Table 1) and taking the variable surface area of sections in different parts of the embryo and at different stages of development into account, a minimum of time needed for scanning all histological sections of the nine selected embryos, some of them at several z-layers per section, is calculated as follows: 6800 sections : 3 = 2268 hours; using a working time of 20 h per week for a 0,5 - technician position this results in just over 110 working weeks pure scanning time, which can be accommodated in a project period of two years.

Scanning will start with the youngest embryo (No. HERC11-1, stage 11, 3.1 mm, 13 somites) and will end with the embryo of the most advanced stage (No. HERC19-1, stage 19, 17.5 mm). This approach will optimize the efficiency of the annotation procedure (see 3.2.2) as work will proceed along the normal developmental time-line from simple to complex and more numerous morphological structures. Only continuous interactions between the departments involved will guarantee efficient and scientifically correct definition of annotations.

Bochum. After installing a virtual microscopy system with the same manufacturer's specifications as in Göttingen, four complete series of embryos of the Hinrichsen Collection ranging between 5 and 29 mm (Table 2) will be scanned with a view to comparing digitisation conditions and requirements on the basis of different sectional planes (sagittal, frontal and horizontal), and three alternating staining procedures: Trichrome, Hematoxylin-Eosin and Azan. In addition, semithin (1 µm thick) sections of one embryonic heart specimen and of two embryonic eye specimens (Fig. 3) will be digitised.

Scanning time for these paraffin sections is as calculated above for the Blechschmidt specimens. Scanning times for single organ anlagen such as heart and eye are expected to be

similar to those for scanning complete embryonic cross sections. For 6000 sections 2000 hours will be needed; at 20 h per week of a 0.5 technician's position this results in about 100 weeks' pure scanning time spread over a two-year project period.

4.2.2 Annotation (WP2)

Development of annotation tools and the annotation itself will be carried out as a basis for a virtual research environment (VRE) about to be established. An annotation format and a procedure for data exchange will be defined. A software engine will be built to dynamically operate on the extracted slides and sections, to allow an on-the-flight visualisation of the observed image part without loading every single image. This requires stream loading processes in order to transfer only the involved tiles which increased performance for optimal usability. During the project the annotation client will be constantly enhanced based on the outcomes of the semi-automatic image processing (s. below).

The annotation client will expand on features implemented in existing tools for annotation of teaching material (e.g. the Netbase solution for MyMicroscope: http://mirax.net-base.de/UK-Ulm.411.0.html) to meet the requirements of the present project:

- definition of persistent identifiers (PIDs) and unique object identifiers (UOIs)
- semi-automatic edge detection (segmentation) in preparation for manual annotation
- continuous access to changes in annotation with respect to advances in knowledge
- solutions for concurrent annotations in cases of conflicting terminology
- persistent annotation while focussing through Z-stacks of the same histological section,
- fast search through sections of the same embryo along contours of a defined structure (individual organ anlagen)
- intelligent searches for specimens, sections, defined edges, terminology, items defined in ontology databases (e.g. Cytomer ontology)
- crosslinking to international databases in prospect of 3-D reconstructions.

Systematic annotation will start at a basic level, i.e. with the technical details of each specimen, to render scanned images efficiently accessible and to uniquely identify each data point defined thereafter. This content-based metadata will already be defined with a view to being interlinked with other information available on the world wide web, such as the data provided by cooperation partners at Washington DC and Kyoto.

At the next level, annotations which guide the user to specific points of interest or explain basic facts will be assigned using arrows and (pop-up) labels to important morphological structures. A standardised vocabulary derived from the terminologia embryologica (TE) will help to name structures in a stereotype, unambiguous way. Frequent reconciliation with partner departments will help to increase annotation quality. As with the technical data of each specimen morphology-related annotation data will be stored alongside the images to render specific structures retrievable from the archive. To optimize and accelerate the annotation process images will be processed by application of an edge detection algorithm (segmentation) for the embryonic structures within neighbouring sections to be developed as part of this project. Adjacent sections will then inherit annotation from each other in a semi-automatic manner.

Annotation information for certain structures and organs (e.g. the eye anlage) will be linked in multiple ways to the same morphological entity (1) in different sections, (2) in different embryos of the same stage, (3) in embryos of the same stage but fixed or stained with

alternative methods (e.g. embryos sectioned at 1 and 10µm, Kyoto embryos analysed by NMR), (4) in embryos of different stages, (5) in embryos from different collections, etc.. Multiple search and filtering functions will be implemented to integrate other resources, embryonic collections or other anatomical data, based on their distinct annotation.

In a further phase of annotation terms from the Cytomer ontology will be linked to structures visible and annotated in the individual sections; depending on the resolution available in individual embryos (e.g. in embryos sectioned at 1µm), the annotation client will consider subcellular structures also.

A final version of the annotation client will enable the annotator to trace individual structures with a polygon line instead of the simple arrows used as the standard method. Semi-automatic edge detection for images will be included to suggest borders of anatomical structures to the annotator in an editable manner. The confirmed or corrected structures are returned to support the image processing of the adjacent sections. To foster consistent annotation among human embryology centres world-wide the client will finally be made available to the international partners in Washington DC and Kyoto and to future partners providing relevant data.

4.2.3 Data handling (WP3 and WP4)

Data handling in this project contains at least two aspects, i.e. data presentation and data preservation, which will be dealt with separately below:

Data presentation (WP3). After the first scan is complete, image files - containing all layers and sections - will be disassembled into genuine sections of the defined graphics format for digital preservation. The digital data originates in a free (Zeiss/Metasystems) or proprietary image format (VSI, Olympus) with about 1.7 terabyte per embryo (each image file scales at about one gigabyte). The images are named and stored in a unique and structured way in a file system, compressed if applicable, and managed by a database that is assembled after the export process by the Olympus or Zeiss/Metasystems scanning system. This process will be repeated for each scan over the project period. This aims to implement a solution which guarantees that it will be possible to access all relevant information of the unique embryology collection presented in HERC, once it has been transformed into digital image data.

In close cooperation between partner departments, the portal will integrate the ability to segment the image data as well as edit and adjust the intellectual metadata. This will significantly influence the development of the annotation tool itself. In order to facilitate the annotation process, a semi-automatic segmentation algorithm will be developed to find structural borders in the embryonic section. This will significantly improve the annotation process - if segments can be re-identified in neighboring layers, the annotation can also be re-used. A suitable segmentation algorithm as well as the software engine for segmentation will have to be developed: The algorithm will comprise colour level windowing followed by edge detection, thus giving isolated objects for annotation. Finally, the annotated slides will be disseminated in an online open-access portal for other researchers and student teaching (s. below). Depending on the quality of the image sections (fragments, contrast, noise etc.) the segmentation solution may later be utilized to generate a 3-D-reconstruction of the whole embryo to be displayed in the web based portal.

The visualisation component has to consider performance issues due to the large data size for both the VRE as well as the open access microscopy. Therefore, the computational and

major data analysis has to be performed server-sided. Users will be provided with optimized data streams to their client computers. The internet portal for the embryo collection data will implement the working environment for the anatomy and molecular biology researchers and it will combine the annotation tools in combination with a visualisation component. The portal will thus have two areas of application: a) virtual research environment, and b) open access microscopy.

The VRE part will use a role-based access in order to ensure data protection. In this area researchers will have full or limited access to the research data and will be able to create their own annotation information sets. In this context the established national Public Key Infrastructure (PKI) of the local D-Grid infrastructure will be able to support the cross-institutional integration of the VRE. The open access portal will be available to national as well as international users but limited to read-only access. Primary aim of the portal is to offer an initial working solution with the possibility of later extension. A dedicated visualisation related infrastructure for a portal prototype with high performance computational capabilities will be provided. This will allow world-wide provision of the embryo collection data backed up by profound data preservation in combination with the digital preservation concept to be developed (s. below).

Digital preservation (WP4). The Open Archival Information System (OAIS) provides a generic digital preservation approach. This includes the three primary layers of digital preservation: a) bitstream preservation – digital layer, b) content preservation – logical layer, and c) data curation – intellectual / conceptual layer. Besides aspects of preservation, possible threats to the digital images will be considered in a bottom-up approach based on digital preservation best practises (Rosenthal et al. 2005).

The major data structure of the digital embryology collection is presently determined by digital images and related annotation data. According to digital preservation best practices, it is vital to convert any proprietary data into a standardized and open format. Thus, an image data format needs to be specified as well as the process of format and technical metadata conversion. In addition to the content preservation mechanisms it is necessary to define a concept for data curation. While content preservation deals with technical metadata and data format specifications, data curation is necessary to provide researchers with profession-related metadata – intellectual metadata – of the digital content. In that context, a persistent identifier (PID) for digitized embryonic data will be defined together within the departmental consortium to ensure non-ambiguous data access. A digital object identifier (DOI) related solution, as established for geo science data (http://www.tib-hannover.de/de/spezialsammlungen/forschungsdaten) is a possible solution. Furthermore, until released for publication, intellectual property rights (IPR) potentially apply to images in combination with intellectual metadata. Therefore, it is necessary to consider data protection aspects in the VRE.

Because the digital preservation infrastructure to be implemented relies on profession related requirements, three workshops will be organised in the first 6 months of the project period: A first workshop with experts from the areas of molecular biology, anatomy and embryology, bioinformatics as well as digital imaging will be organized to define basic requirements and standards for digitisation and annotation. In a second workshop the digital preservation related aspects and requirements will be investigated with the help of experts from the area of digital preservation. Relevant frameworks such as the FedoraCommons repository (http://fedora-commons.org) will be discussed and a first

recommendation derived. Furthermore, by including international experts in both workshops, aspects of information interchange and metadata standardisation will be addressed. In that context it will also be possible to address the aspect of financial necessities, software sustainability and further preservation threats based on a broad perspective. A third workshop with experts from both groups will pool the facts from the previous workshops, international cooperation and produce recommendations for the digital preservation concept. Based on the feedback of the first two workshops potential digital preservation frameworks will be investigated. The results of the primary investigation will be used to propose a selection of relevant frameworks within the third workshop in the course of which a selection recommendation will be determined.

Based on the clarification of the profession related requirements in the workshops a first prototype will be developed on a Grid Computing cluster located at the Göttingen University. This prototype will implement a metadata and image data preservation concept in a provided test environment. The prototype will be validated on the basis of a developed parameter set within the project. This will also include the international access perspective of the digital preservation infrastructure as well as the definition of a profound persistent identifier definition.

The final stage of development of the digital preservation infrastructure will be achieved as an operational implementation. The operational implementation will be available for the designated laboratory environment in Bochum and Göttingen as a proof of concept. An expansion to further users and the transfer to production environments are not part of the development, but it will be possible to achieve this by application of the concept to those areas.

4.2.4 Time frame

To arrive at a manageable time frame for this project the four work packages defined in this chapter are subdivided further into two to five milestones (M1.1, M1.2, M2.1, …, M3.1, etc.; s. Table 3). The time needed (in the order of months) for each milestone is meant to be an approximation which may have to be adjusted as the project proceeds. The closest correlation exists, of course, with the time periods calculated in connection with the digitisation process (s. 4.2.1).

Month / Work package	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
WP0 - Coordination																								
WP1 - Digitisation						M1.1																		M1.2
WP2 - Annotation																								
WP2.1 - Requirements		M2.1																						
WP2.2 - Implementation												M2.2						M2.3						M2.4
WP2.3 - Data Interchange																								M2.5
WP3 - Data Presentation																								
WP3.1 - Requirements	M3.1																							
WP3.2 - Viewer Prototype						M3.2																		
WP3.3 - Segmentation engine																M3.3								M3.4
WP4 - Digital Preservation																								
WP4.1 - Requirements		M4.1	M4.2		M4.3																			
WP4.2 - Prototype												M4.4												
WP4.3 - Production System																								M4.5

Table 3. Time schedule for work packages (WP) and milestones (M).

5. Conclusion

Open access to digitised, annotated and extensively cross-linked high-resolution microscopy of irreplaceable specimens of human embryonic development can be expected to be widely used in the international biomedical scientific community. Preservation and presentation of this data will help to reduce vexing uncertainties about methodological artifacts and inter-individual variations during normal and abnormal human development, which still represent a serious hindrance for determining the significance of morphological and molecular results obtained in animal models for the human condition. After standards and procedures are established by this pilot project, the database is likely to expand quickly to become an internationally recognised reference centre, through the long-standing commitment of the cooperation partners and through cooperation with other centres with a research interest in human development and disease.

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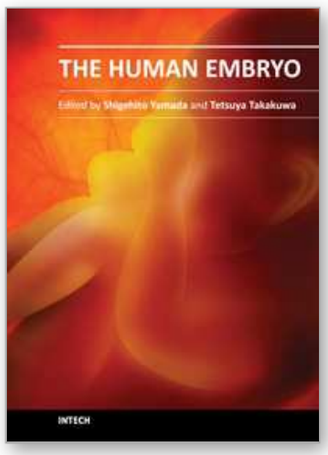

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Human embryology is now rapidly moving to a new phase due to recent innovation and advances of life science including ES and iPS technology. This new era also directs a difficult challenge for scientists in terms of technological and ethical issues for future human embryology. However, human embryology is difficult to research due to ethics involved in the collection of human materials. This book traces the early history and provides knowledge on demonstration of principles from ancient to the most recent embryo studies amidst the unresolved scientific and ethical issues. We hope this book will help the readers to understand human embryo development better.

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Slavka Krautzeka 83/A
51000 Rijeka, Croatia
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No.65, Yan An Road (West), Shanghai, 200040, China
中国上海市延安西路65号上海国际贵都大饭店办公楼405单元
Phone: +86-21-62489820
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