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Sectional Anatomical Atlas of Sprague-Dawley Rat

主编 刘谦 龚辉　　主审 李云庆

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主　　编：刘　谦　龚　辉
副 主 编：骆清铭　李　和　唐　雷
编　　委：骆卫华　王　珍　贾向红　杨世明　刘秀平
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Reviewer-in-chief: Li Yunqing

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责任编辑:余　强　　封面设计:秦　茹
责任校对:朱　霞　　责任监印:熊庆玉

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内容简介 Introduction

本图谱是华中科技大学武汉光电国家实验室（筹）Britton Chance生物医学光子学研究中心构建的数字大鼠研究成果。内容包括数字SD大鼠全身的横断面、矢状面和冠状面三个方向的断层标本彩图、解剖结构信息标注和三维可视化图片，整套数据集来源于同一只实验鼠。数字SD大鼠数据集的构建，是通过获取SD大鼠的三维断层结构信息实现的，并标注解剖结构信息。全书结构清晰，内容全面，本书另附一张包含所有断层图谱的光盘。该书既可以满足从事相关专业的老师和学生学习实验鼠解剖学的需要，又可以为以实验鼠为动物模型的研究者们提供实验帮助。

This atlas is composed of transverse, coronal and sagittal sectional atlas as well as three-dimensional (3D) visualization models, produced by Britton Chance Center for Biomedical Photonics, Wuhan National Laboratory for Optoelectronics, Huazhong University of Science and Technology. Anatomical structures of the whole SD rat are labeled in transverse sections as well as coronal and sagittal sections from the same one. This book is written clearly and comprehensively, with a CD including all the atlas, it not only benefits the students and teachers who study the anatomy of the whole rat, but also provides digitization information for the researchers using rats as animal models.

序 Preface

大鼠常作为生命科学研究中的实验动物。随着实验需求的不同，相继培育出各种优良的品种，而SD大鼠是较为常用的实验动物之一，在解剖生理性、病理免疫性、疾病防治性等不同的研究领域，都被广泛应用。

当前，信息科学领域的计算机技术日益成熟，为生命科学领域的研究提供了数字化、可视化和模拟仿真的技术支持。但是，“巧妇难为无米之炊”，为实现生物的分子、细胞、组织、器官、系统的数字化实验研究，首先要建立相关实验动物的解剖数据集。由华中科技大学武汉光电国家实验室（筹）生物医学光子学研究部等单位编写的这本《大鼠断层解剖彩色图谱》，是国际上第一套整体实验鼠的数据集中精选出来的代表性断面图像，填补了以往的空白区，难能可贵。

“工欲善其事，必先利其器”，本研究采用170 g的成年雄性SD大鼠，经过冷冻、切削、拍照、配准、分割、重建得到整套断层解剖数据集。可以运用这些数据集中的原始资料，精心设计，构建拟进行研究的大鼠疾病模型，为数字化实验研究提供重要的形态学依据。

数字化实验动物研究，毕竟是一项新的尝试。“千金敝帚有定价，周玉郑鼠难强名”，对待新技术方法的出现，如何认识？如何使用？将会有不同的理解和取向，见仁见智在所难免。本书及其相关的数据集，对提高生命科学实验研究水平，能得到多大效益呢？贵在用其所长、补其所短，盼能灵活巧用，通过实践检验，得出预期的良好评价。

中国工程院资深院士

钟世镇

2010年春

Rats are essential animals in life science research. There are various kinds of rats nurtured with different experimental demands. SD rats, one of the most common animals, have been widely used in anatomical physiology, immunopathogenesis, and diseases prevention etc.

With the development of the computer and information technology, it is now possible to realize digitalization, visualization and simulation in life science research. However, anatomic data sets of animals must be established first for the digitalization of biological molecular, cells, tissues, organs and system. As a result, this book is published to fill the blank in this area, which also provides the first whole-body data set of SD rat in the world.

To obtain the whole cryosectional anatomical data set, a 170 g adult male SD rat was frozen, cut, photographed, registered, segmented and finally reconstructed. After post processing, this data set can be applied to many research areas, such as establishing models for diseases of rats, which provides an important morphological basis for animal digitalization research.

Digitalization of animals is a new attempt in today's research. Thus, there exits challenges brought by different kinds of understanding and applications. How much contribution will this atlas and its associated data sets make to improve experimental research in life science? However, we believe that this book will not only have broad range of applications but also make us excited in the future.

Zhong Shizen

April , 2010

前 言 Preview

实验小动物是目前遗传学研究及药物研究等领域最基本的研究工具之一。大鼠作为生物学与医学中最为常用的实验动物，已应用到生理、生化、病理和免疫等方面的科学研究与实验教学中。为了方便各种生物学信息的共享和整合，同时，出于对动物生存权利及动物福利的考虑，小动物的结构与功能信息的数字化已经成为国内外各研究机构的研究热点。数字解剖数据集以其更加准确的、直观的、图形化表示方法等具有广泛应用价值的特点，引起了越来越多的科研人员的关注。本图谱原始图片通过冰冻铣削断层解剖成像系统获取，经过后期图像处理构建高分辨率SD大鼠的断层解剖数据集，对SD大鼠横断面、矢状面和冠状面的解剖结构信息进行标记。

本书由图谱简介、横断面断层图谱、冠状面断层图谱、矢状面断层图谱和三维可视化图像组成。本简介主要介绍大鼠样品的制备、图像获取系统、图像获取过程、图像数据信息以及图像的三维可视化。

SD大鼠三维可视化模型的构建全过程如图1所示。首先对大鼠进行预处理并冰冻包埋后，用高精度数控铣床对大鼠样本进行磨削，获得高分辨率横断面断层图片。对断层图片进行配准、分割等数据准备工作后，进行三维重建。

SD大鼠为雄性成年大白鼠，体长146.90 mm（不含尾长），体高42.20 mm，体重170 g。由于大鼠全身披毛，因此样本处理的第一步就是要去除大鼠皮肤表面的披毛。脱毛采用手工剪毛和化学脱毛相结合的方式，其中脱毛剂为新鲜配制的8%的Na_2S溶液。大鼠脱毛后的图像如图2所示。将脱毛后的大鼠按照正常的解剖学姿势置入冷冻盒中进行整形处理，使得大鼠头、背中线和尾巴在同一直线上，身体两侧基本对称，整个整形过程控制在10 min以内。

Currently, laboratory animal is one of the essential research tools in genetics and pharmaceutics etc. Rats, the commonest animals used in biology and medicine, have been widely utilized in experimental teaching and scientific research areas, such as physiology, biochemistry, pathology and immunology. The digitization of structural and functional information of small animals has become very popular for various research institutions all over the world in order to better share and integrate the biological information, and protect animal rights and welfare as well. As a result of several practical qualities, such as its accurate intuitive and graphical representation, the digital anatomical data set is attracting interests of more and more researchers. Original images of this atlas are acquired by using the frozen cryosection-milling imaging system. After processing, these images form a high resolution cryosection anatomical data set of SD rat, which describe the anatomical information in transverse section as well as in reconstructed coronal and saggital sections. This book is composed of transverse sectional atlas, coronal sectional atlas, sagittal sectional atlas and three-dimensional (3D) visualization. Three-dimensional computerized models of SD rat were constructed as shown in Figure 1. Rat was pretreated and frozen embedded. Then, a high-precision CNC milling machine is used to grind at sample in order to capture high-resolution transverse sectional images. Cryosectional images were registered, segmented and then reconstructed into 3D computerized models.

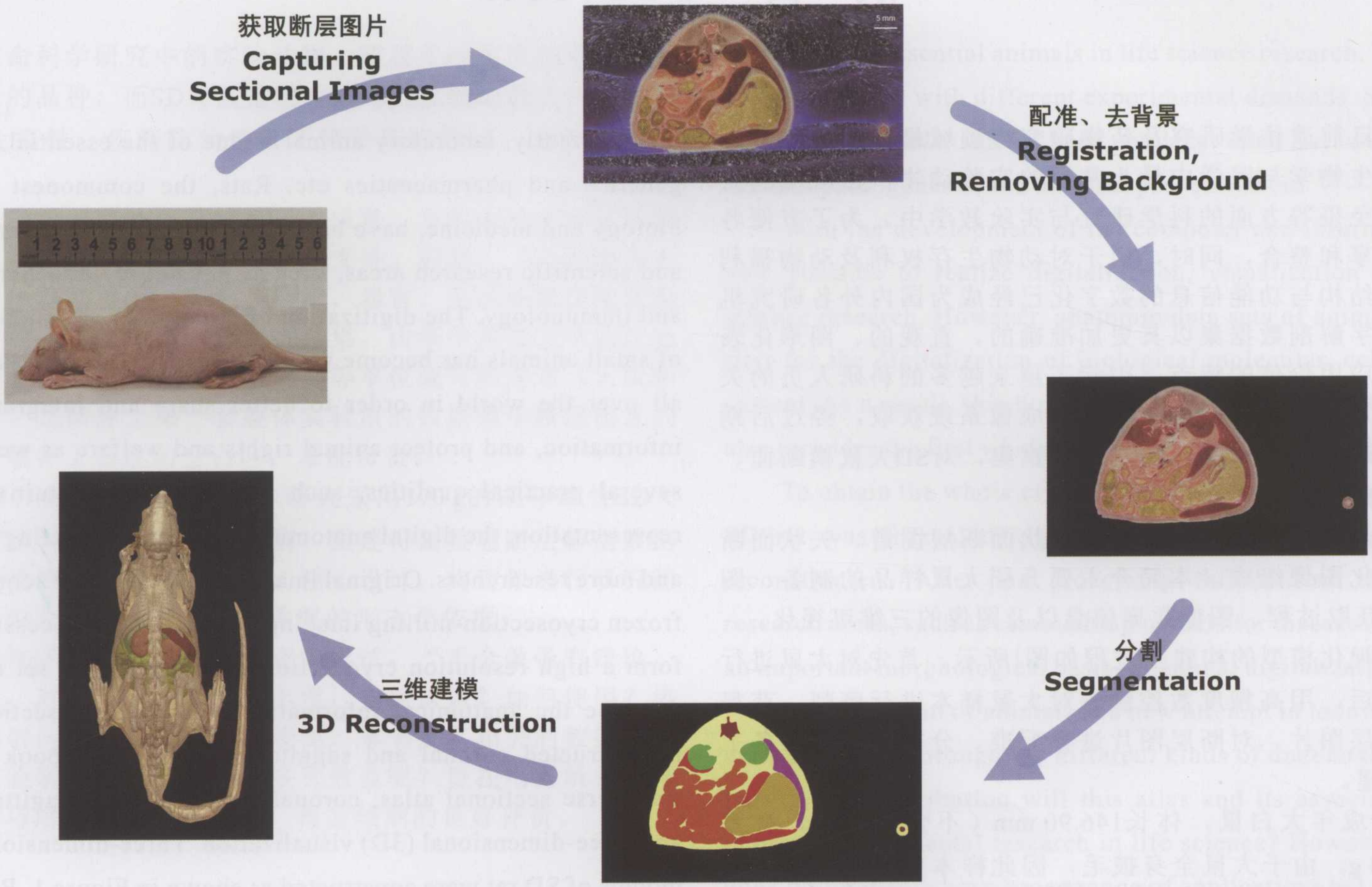

图1 大鼠三维可视化模型的构建过程

Figure 1 The reconstruction of rat 3D computerized models

图2 大鼠全身脱毛后

Figure 2 Depilated rat

将整形后的大鼠和密封的冷冻盒一起放入－85℃冰箱内冷冻固定24 h以上，以确保大鼠被完全冷冻。样本制作的最后一步是对整只大鼠进行包埋处理，包埋剂采用的是新鲜配制的3%的蓝色明胶溶液，即3 g明胶加入100 ml双蒸水中加热溶解为3%的明胶溶液，再按0.25 g亚甲蓝/100 ml明胶溶液的比例加入染色剂亚甲蓝。

由于本实验中所使用的小动物数控铣床是立式铣床，而大鼠的正常姿势是四肢着地的趴姿，采用立式包埋的方法不好固定大鼠的正常体态，因此采用卧式包埋和立式铣削的工艺，即图3左图是用于大鼠样本包埋采用的倒立姿势，图3右图是大鼠样本被铣削时的竖立姿势。包埋盒由底座和包埋仓构成，底座两侧各有一个凹槽，用于固定待铣削的样本。

整个包埋过程采用逐层包埋逐层冷冻的方式进行，步骤如下：首先，在预先装夹好的包埋盒（如图3左图所示）中加入1/4体积的包埋剂，并将包埋盒放入－85℃冰箱冷冻；其次，待包埋剂变硬后，将冷冻固定后的大鼠样本移至包埋盒，并往包埋盒内再次加入1/4体积的包埋

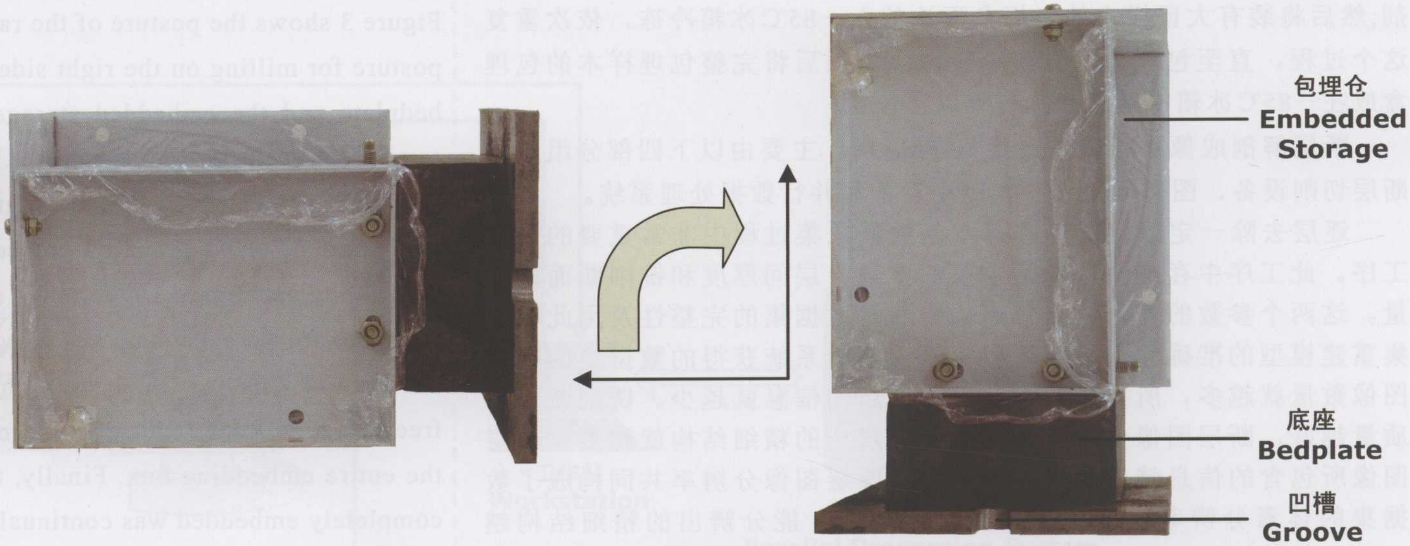

图3 包埋盒

Figure 3 Embedding box

SD rat was a healthy adult male rat, which has the body length of 146.90 mm (excluding tail length), body height of 42.20 mm, and weight of 170 g. The first step of treatment is to remove the hair manually by shaving and using freshly prepared 8% Na_2S solution as depilating agent. The depilated rat is shown in Figure 2. The specimen remained in the normal anatomical posture with rats head, dorsal midline and tail staying in the same line and both sides symmetrical, it was then placed into the frozen box. The time for this treatment was restricted less than 10 min.

The reshaped rat was frozen completely in a -85℃ ultra freezer together with the frozen box for at least 24 hours. Then, the whole rat was embedded with 3% fresh blue gelatin solution, which is 3 g gelatin with 100 ml double distilled water, and also mixed with 0.25 g methylene blue.

The CNC milling machine used in this experiment is vertical, in which the rat cannot keep the normal posture of all fours on the ground. Thus, horizontal embedding and vertical milling were used in the experiment.

剂;然后将装有大鼠样本的包埋盒再次放入－85℃冰箱冷冻，依次重复这个过程，直至包埋剂填充整个包埋盒。最后将完整包埋样本的包埋盒放在－85℃冰箱中继续冷冻三天以上。

断层解剖成像系统整体结构如图4所示，主要由以下四部分组成：断层切削设备、图像采集设备、制冷设备和并行数据处理系统。

逐层去除一定厚度的冷冻标本是数据采集过程中非常重要的一道工序。此工序中存在两个非常重要的参数：层间厚度和铣削断面的质量。这两个参数的变化将直接影响所获得数据集的完整性及用此数据集重建模型的准确性。层间厚度越小，利用系统获得的数据集的断层图像数量就越多，所损失的小动物精细结构信息就越少。铣削断面的质量越好，断层图像分辨率越高，能分辨出的精细结构就越多，断层图像所包含的信息越丰富。层间厚度与断层图像分辨率共同构成了数据集的体素分辨率，因此体素分辨率越高，能分辨出的精细结构越多，获得的样品的结构信息也就越准确，在计算机中重建的模型就越真实。

断层切削设备是一台高精度数控铣床，如图5所示。铣床*X*方向工作行程为600 mm，*Y*方向工作行程为280 mm，*Z*方向工作行程为480 mm，最小进给量0.001 mm，重复定位精度0.01/300 mm，最大刀盘直径120 mm，已加工表面的表面粗糙度R_a 1.6，平面度0.02 mm。利用这样的精度进行切削完全可以达到对系统的设计要求，即以每层0.02 mm甚至更高的精度切削，获得高空间分辨率的实验小动物数据集。

断层图像采集是影响到整个数据集质量的一道非常重要的工序。如何才能不失真地采集断层图像并且完整地采集到断层图像的精细结构信息是选择图像采集设备的重要依据。图像采集设备通常有几种选择：数码相机、扫描仪、CCD。数码相机和扫描仪都是通过光电器件CCD来获取图像信息的。但是，由于数码相机和扫描仪结构原理不同，使得两者获取图像所需的时间不同。扫描仪的光电器件是条状的，它识别图形的过程是运动的，光电器件从图像的一端运动到另一端，完成扫描。而数码相机的光电器件是矩阵状，其工作的过程是瞬间的。由此可见用扫描仪扫描一幅断层图像的时间要大于用数码相机

Figure 3 shows the posture of the rat for embedding on the left side, and the posture for milling on the right side. The embedding box is consisted of the bedplate and the embedded storage. There is a groove for fixing milling samples on each side of the bedplate.

The specimen was embedded and frozen layer by layer in the following way: first of all, a prepared embedding box (Figure 3 left) was filled by embedding medium to 1/4 volume , and was frozen in the -85℃ ultra freezer; then, the frozen specimen was moved to the embedding box, together with another 1/4 volume of embedding medium. The box was put in -85℃ ultra freezer again. Repeat this process in turn until the embedding medium filled the entire embedding box. Finally, the embedding box filled with specimen completely embedded was continually frozen in -85℃ ultra freezer for more than three days.

The overall structure of the cryosection-milling imaging system shown in Figure 4 is mainly consisted of the following four components: milling equipment, image capturing equipment, refrigeration equipment, and parallel data processing system.

Removing the frozen sample with a certain thickness layer by layer is an essential process for the data acquisition. There are two important parameters: layer thickness and the quality of milling section. Changes of these two parameters will have a directly influence on the integrity of the data set and the accuracy of the models reconstructed. The thinner the layer, the higher vertical resolution of the image will have. The better the quality of the section, the higher horizontal resolution of the image will have. High resolution brings more information, resulting in a more accurate reconstructed model.

Milling equipment is a high-precision CNC milling machine, shown in Figure 5, whose working stroke is 600 mm in *X*-direction, 280 mm in *Y*-direction, and 480 mm in *Z*-direction. The minimum feeding rate is 0.001 mm.

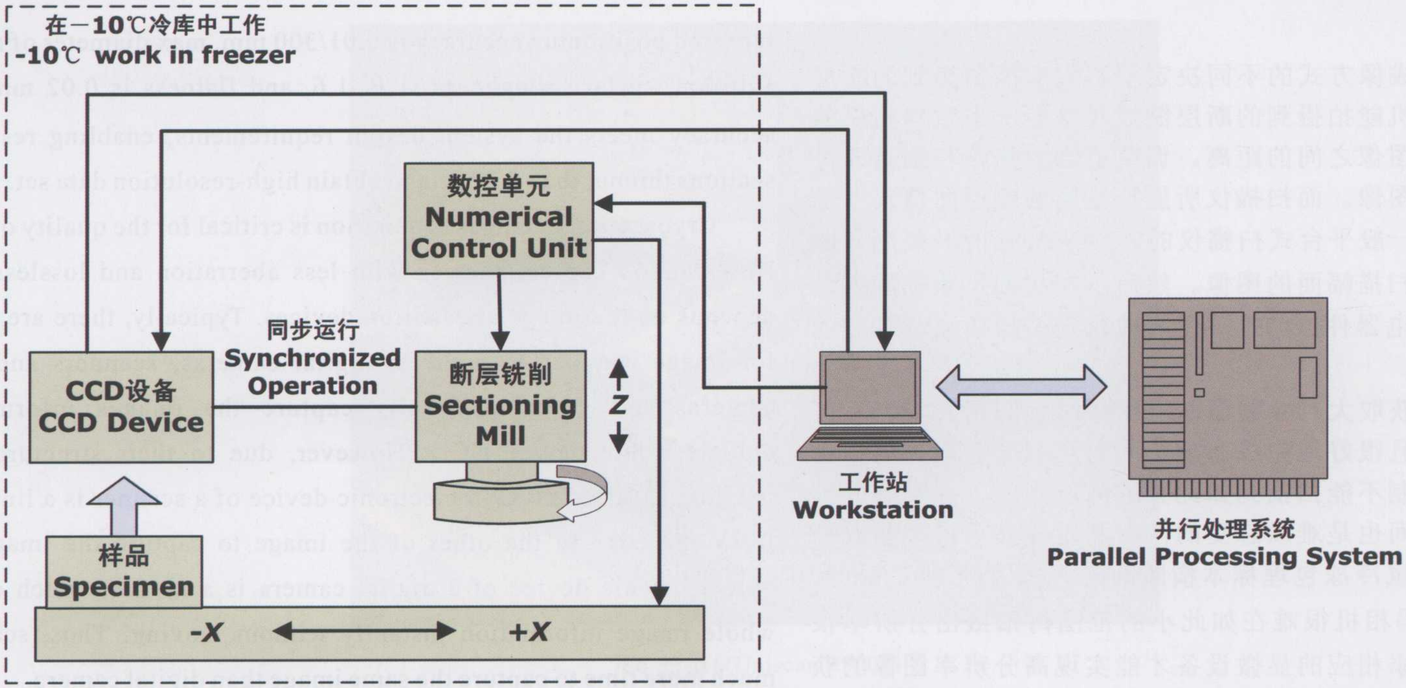

图4 系统结构图
Figure 4 System diagram

图5 高精度数控铣床
Figure 5 High-precision CNC milling machine

拍摄的时间。

数码相机和扫描仪成像方式的不同决定了它们所能拍摄到的断层图像尺寸不同。数码相机能拍摄到的断层图像尺寸取决于数码相机的焦距和数码相机与断层图像之间的距离，调整适当的焦距和距离即可拍摄到不同尺寸的断层图像。而扫描仪所能扫描到的断层图像尺寸受到扫描仪的扫描幅面（一般平台式扫描仪的为A4或A3幅面）限制，即扫描仪不能扫描超出其扫描幅面的图像。然而，在相同尺寸范围内，数码相机和扫描仪的光电器件CCD的排列方式决定了扫描仪可达到的分辨率要高于数码相机。

在利用较高分辨率获取大尺寸截面断层图像时，利用数码相机可以瞬间获取断层图像并且很好地完成高质量的断层图像获取，而扫描仪由于其扫描幅面的限制不能扫描到如此大的断层图像，并且高分辨率扫描大尺寸图像的时间也是难以忍受的；在利用较高分辨率获取小尺寸截面断层图像（大鼠冷冻包埋标本横断面几何尺寸约为90 mm × 60 mm）时，单独用数码相机很难在如此小的范围内拍摄出分辨率很高的断层图像，必须增添相应的显微设备才能实现高分辨率图像的获取。（数码相机要根据体像素精度（断层图像精度）及照相区域进行选择，如果体像素精度为0.02 mm × 0.02 mm × 0.02 mm，照相区域按90 mm × 90 mm计算，数码相机的要求为：90 × 50 × 9 0 × 50 = 2025万像素。）然而即使利用低端的扫描仪在此范围内也可扫描出高分辨率的断层图像，扫描图像所用的时间也很短。

因此，根据小动物标本的横断面几何尺寸较小、结构精细等具体特点，综合考虑上述各因素，我们采用1200 DPI分辨率扫描仪来进行断层图像采集，如图6所示。

数据集的获取是一项非常巨大的工程，一只成年大鼠的切削工作（假设层间厚度为0.02 mm）长达数周之久，为了使得整个数据集的性质均一，必须保证连续不间断地获取整个小动物的断层图像数据集，减少因为异地冻存、多次装夹而造成的数据损失和样本反复冻融造成的小动物标本组织形状和颜色变化而引起的误差。另外，断层解剖成像系统是基于冰冻铣削技术设计的，在长时间的切削过程中为

repeated positioning accuracy is 0.01/300 mm, max diameter of milling cutter is 120 mm, surface roughness is R_a 1.6, and flatness is 0.02 mm. Such cutting accuracy meets the system design requirements, enabling researchers to cut sections thinner than 0.02 mm to obtain high-resolution data set.

Cryosectional images acquisition is critical for the quality of entire data set. How can we capture images with less aberration and lossless fine structure depends on the image acquisition devices. Typically, there are several options for image acquisition devices: digital cameras, scanners and CCD. Digital cameras and scanners usually capture the images information through optoelectronic device CCD. However, due to their structure, the time for imaging is different. Optoelectronic device of a scanner is a line, which moves from one edge to the other of the image to capture the image information; optoelectronic device of a digital camera is a matrix, which can capture the whole image information instantly without moving. Thus, scanner will take much more time to capture the same image than digital camera.

Different ways of imaging for digital cameras and scanners make different cryosectional image sizes. The size of cryosectional image depends on the focal length of digital camera and the distance between digital camera and the section. Images in different sizes can be obtained by adjusting the focal length and distance appropriately. The size of cryosectional image obtained from scanner is limited by the scanning dimension (general flatbed scanner with A4 or A3 format), that is, the scanner can't capture images beyond its scan dimension. However, in the same size, scanners can achieve higher resolution than digital cameras because of the different arrangement of optoelectronic devices CCD.

When capturing high-resolution cryosectional images in large size, digital camera can finish it instantly, while scanner is time-consuming for scanning. When capturing small size high-resolution cryosectional images (e.g. the geometry of frozen embedded specimen in this study is 90 mm × 60 mm),

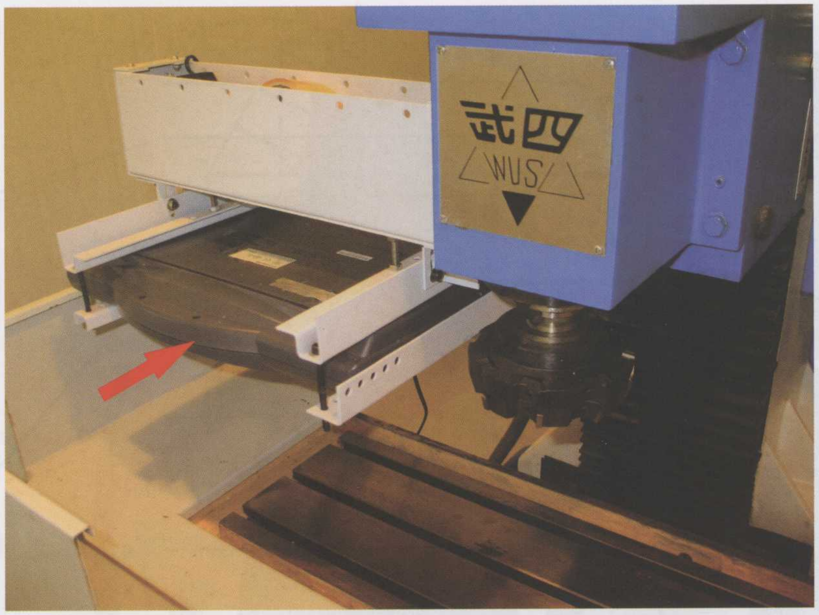

图6 扫描仪Uniscan B800型号

Figure 6 Scanner of Uniscan B800

了防止因标本的解冻失去支撑而导致小动物的器官移位和形体变化，必须要保持标本的冷冻状态，确保最终获得的小动物数据集的准确性。因此将整个的切削环境置于－10℃冷库中，并且利用干冰辅助降低冷冻样本表面温度，减少由于切削过程中刀具和标本断面摩擦产生的热量，从而消除由于断面温度变化而引起的组织性质的变化，使得所有断层图像的获取环境条件相同，保证整个数据集的断层图像的性质均一性。

大鼠切削工作在3周内完成，最终获取的断层图像数据集的每张图像分辨率为4600 × 2580 × 24 bit，体素大小为0.02 mm × 0.02 mm × 0.02 mm，共获得9475张二维横断面解剖图像，总容量达314.68 Gbyte。由于在采集过程中时间比较长，而且存在机械振动，因此最后得到的断层切片会发生二维空间位移，而此位移会引起重建模型的不准确。为了减小这一误差，需要对原始彩色断层切片进行医学图像配准。

digital camera cannot obtain high-resolution images without corresponding micro-devices (Digital cameras must be chosen according to the voxel precision (cryosectional precision) and the region. E.g., if voxel precision is 0.02 mm × 0.02 mm × 0.02 mm, the region photographed is 90 mm × 90 mm, the requirement of a digital camera should be 90 × 50 × 90 × 50 = 20.25 million pixels). However, in such small size, even if low-end scanner can scan a high-resolution image in a very short time.

Thus, considering the small animal having small cross-sectional geometry and fine structures, a 1200 DPI resolution scanner was adopted to capture cryosectional images, as shown in Figure 6.

The data set acquisition is a huge project. Completing cutting an adult rat will take several weeks. In order to make the entire data set uniform,

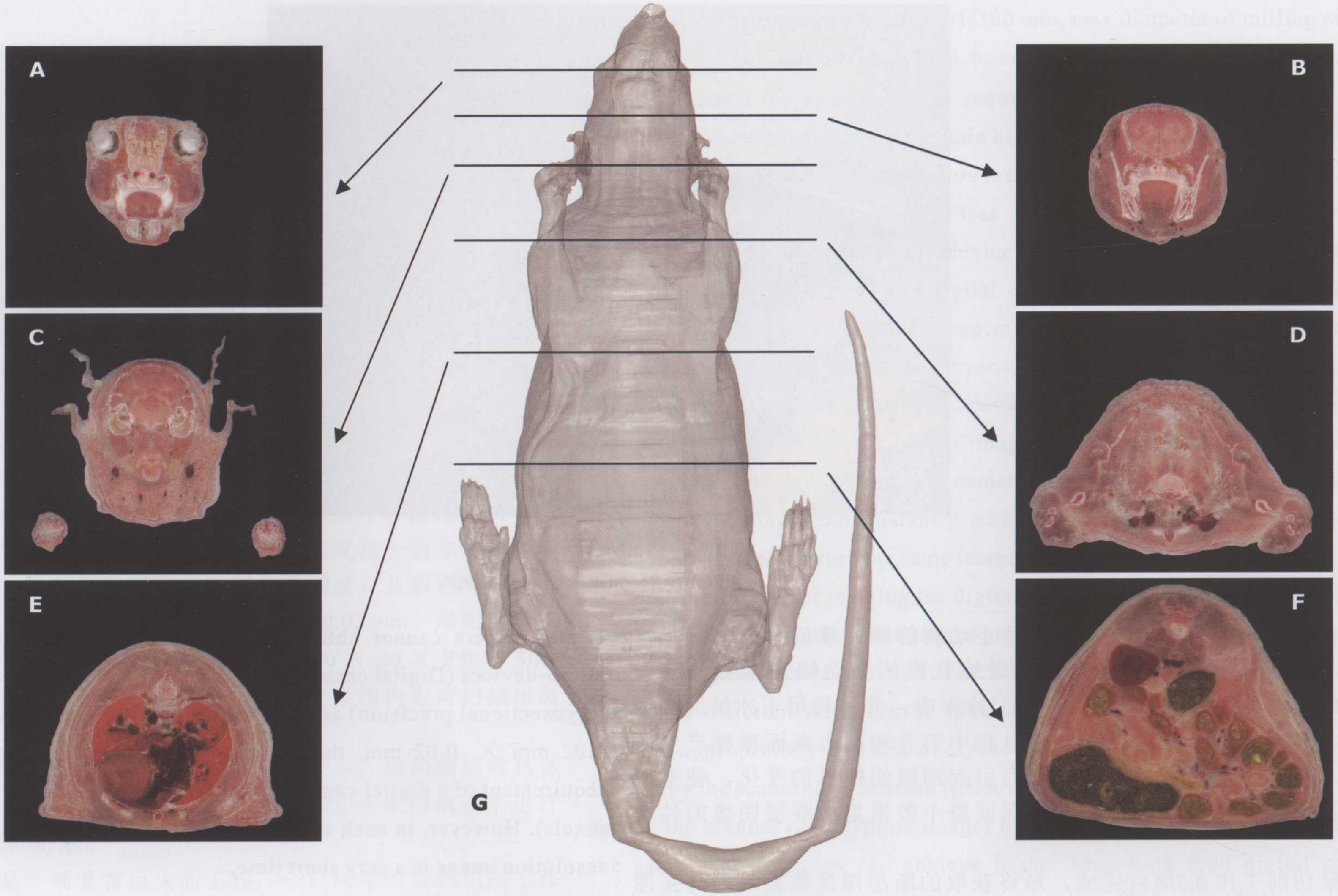

图7 大鼠不同部位的横断面断面解剖图像

A-F：分别为经过眼部、大脑、耳、第一肋、胸部及腹部的横断面断层解剖图像；G：大鼠表面重建模型

Figure 7 Cryosectional images of different parts

A-F: Cryosectional image through eye, brain, ear, first rib, thorax, abodomen; G: Rat surface reconstruction model

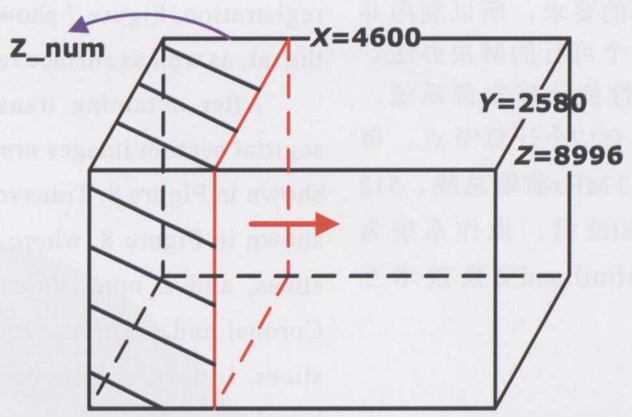

图8 断层图谱重建示意图

Figure 8 A schematic drawing of sections reconstructed

图7展示了大鼠从头到尾各个不同部位的横断面断层解剖图谱以及大鼠表面绘制重建模型。

在获取SD大鼠横断面断层解剖图谱后，还需重建冠状面和矢状面断层解剖图谱，冠状面、矢状面断层图片的重建思想如图8所示。将SD大鼠横断面断层图片（$X=4600$，$Y=2580$代表横断面断层图片分辨率为4600 × 2580）依次摆放，便形成了图8所示的长方体，其中$Z=8996$代表的是横断面断层图片的数量，Z_num代表程序读取一次原始数据时，重建断层图片的数量。根据横断面的断层图片重建出冠状面和矢状面的断层图片,其中冠状面断层图片分辨率为4600 × 8996,矢状面断层图片分辨率为2580 × 8996。打个不太恰当的比方，三个方向上的断层图片其实就像将一块长方形的面包在三个不同方向切成的面包片一样。图9、图10、图11分别展示了横断面断层图片以及重建得到的冠状面断层图片和矢状面断层图片。

小动物三维模型的重构是小动物研究中重要的环节之一，其数据属于三维空间体数据类型。通常将体数据表达成立方体，即层间厚度与断层图像像素尺寸相同。如果以每层0.02 mm的精度切削（即体素尺寸为0.02 mm × 0.02 mm × 0.02 mm），获得的小动物数据集的数据量

cryosectional images of the entire rat must be obtained uninterruptedly to reduce data loss caused by off-site frozen and multiple setup, and changes in shape and color caused by freezing and thawing. In addition, in order to prevent animal organs shift and physical changes caused by samples thawing; samples must keep frozen in the long cutting process. Therefore, the whole cutting process is completed in -10℃ freezer. Other measures were also taken, such as using dry ice to reduce the temperature on the surface of the sample, to decrease the heat of friction between the cutting tool and the cryosection during the cutting process, to eliminate the aberration of the tissue caused by cross-sectional temperature changes, and to make the environmental condition of cryosectional images same to ensure the nature of sectional images homogeneous in the whole data set.

The cutting work was completed in three weeks. The final data set is composed of 9475 cryosectional images captured in 4600 × 2580 × 24-bit format, whose total capacity is 314.68 gigabytes, with voxel size of 0.02 mm × 0.02 mm × 0.02 mm. Because of the long working time and mechanical vibration, the final slices have space displacement. To eliminate this error, the original slices need

是相当惊人的，普通的计算机已经不能满足运算的要求，所以利用并行体系结构加快对其三维重建的计算速度，是一个可行的解决办法。本系统采用的并行计算环境是浪潮天梭10000高性能计算集群系统，配置了符合MPI标准的消息传递库。该集群系统有17个计算节点，每个节点的配置为：2个Intel Xeon 2.4 GHz CPU、533 MHz前端总线、512 kB L2 Cache、2 G DDR RAM、18 GB 10000转SCSI硬盘，操作系统为RedHat Linux 7.2（Kernel 2.4）。节点间采用InfiniBand交换技术互联。

registration. Figure 7 shows transverse anatomical images from different parts of the rat, as well as surface reconstruction model of the rat's skin.

After obtaining transverse anatomical images, reconstructed coronal and sagittal section images are also obtained. Their frameworks of reconstruction are shown in Figure 8. Transverse section slices are placed in order, forming a cuboid shown in Figure 8, where $Z = 8996$ represents the quantity of transverse section slices, and Z_num represents the quantity of cryosection reconstructed once. Coronal and sagittal section images were computerized from transverse section slices. In fact, cryosectional slices of the three directions are like the pieces of bread cut in three different directions. Figure 9, Figure 10, Figure 11, show the transverse section image, reconstructed coronal and sagittal section image.

The reconstruction of three-dimensional models for small animals is an important part of small animal studies. These models are described by three-dimensional volume data. Usually volume data is expressed as a cube, namely, the inter-layer thickness is in same size as cross-sectional image pixel. At 0.02 mm inter-layer thickness,the data of the animal will have excellent quantity. However, ordinary computers cannot meet the computing requirement, the Langchao Tiansuo 10000 high-performance computing cluster system is introduced to generate the 3D computerized model of rat anatomy , using a parallel reconstruction algorithm. The cluster system has the standard MPI message passing library, and 17 computing nodes with each node configured as follows: two Intel Xeon 2.4 GHz CPU, 533 MHz front-side bus, 512 kB L2 Cache, 2 G DDR RAM, 18 GB 10000 switch SCSI hard drive, RedHat Linux 7.2 (Kernel 2.4) operating system. Connection from node to node is completed by InfiniBand switching technology.

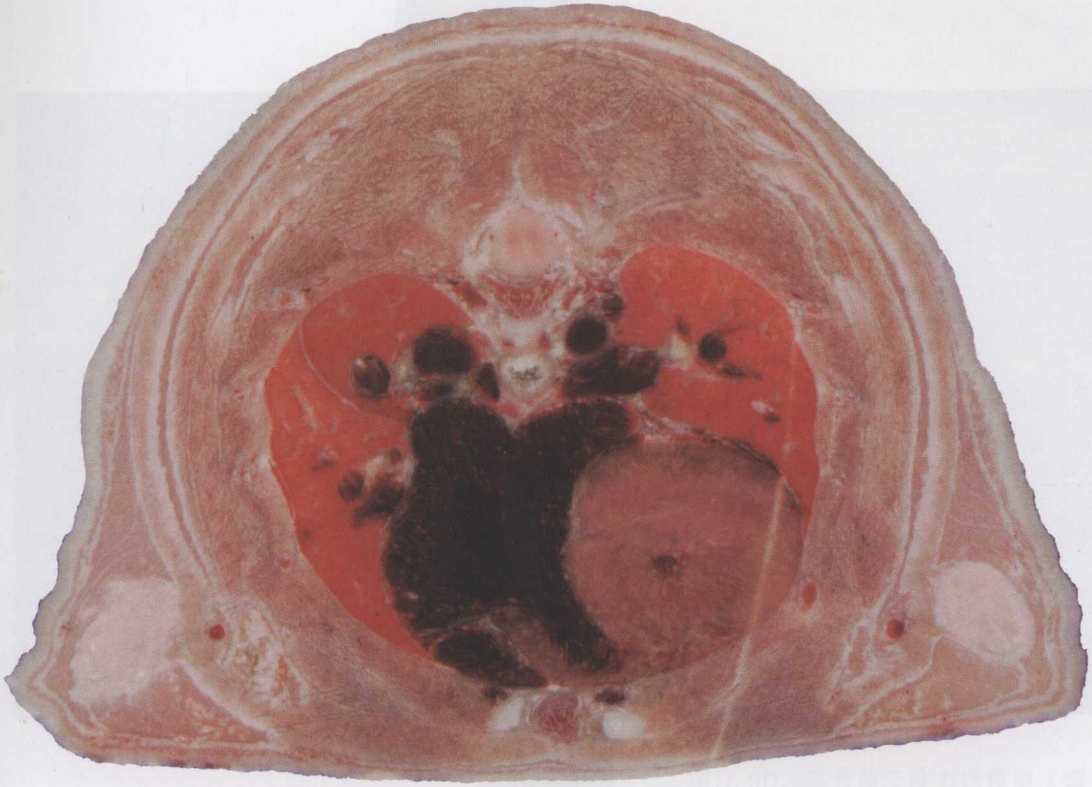

图9 横断面断层图片
Figure 9 The transverse image

图10 冠状面断层图片

Figure 10 The coronal image

图11 矢状面断层图片

Figure 11 The sagittal image