Micron 103 (2017) 29–33

Contents lists available at ScienceDirect

Micron

journal homepage: www.elsevier.com/locate/micron

Full Length Article

Automatic system for electron tomography data collection in the ultra-high voltage electron microscope

Meng Cao[a,b,*], Ryuji Nishi[b], Fang Wang[a]

[a] Key Laboratory for Physical Electronics and Devices of the Ministry of Education, Department of Electronic Science and Technology, Xi'an Jiaotong University, Xi'an 710049, PR China
[b] Research Centre for Ultra-High Voltage Electron Microscopy, Osaka University, 7-1 Mihogaoka, Ibaraki, Osaka 567-0047, Japan

ARTICLE INFO

Keywords:
Electron tomography
Auto tracking
Auto focus
Data collection
Image stitching

ABSTRACT

In this study, we report an automatic system for collection of tilt series for electron tomography based on the ultra-HVEM in Osaka University. By remotely controlling the microscope and reading the observation image, the system can track the field of view and do focus in each tilt angle. The automatic tracking is carried out with an image matching technique based on normalized correlation coefficient. Auto focus is realized by the optimization of image sharpness. A toolkit that can expand the field of view with technique of image stitching is also developed. The system can automatically collect the tilt series with much smaller time consumption.

1. Introduction

Electron tomography (ET) is a powerful three-dimensional (3D) microscopic image tool for obtaining internal structures of many kinds of samples of materials science, nano-science and biology. (Andersson et al., 2012; Hayashida and Malac, 2016; Suh et al., 2013; Vanhecke et al., 2011). The ultra-high voltage electron microscope (ultra-HVEM) can provide a remarkable capability of ET observation for micron-thick samples (Cao et al., 2013; Murata et al., 2014; Takaoka et al., 2008; Wang et al., 2016). In ET, the 3D structure is calculated from a set of 2D projection images of the sample recorded at different tilt angles. The collection of the tilt series is the basic step in ET. Manual collection of tilt series is usually a consuming work. Due to the limitation of mechanical accuracy of the goniometer, the tilt axis of a specimen holder cannot be exactly stable. It is very difficult to keep the observation position on the specimen on the tilt axis during tilting. Image position often changes with tilt increment. At the same time, focus position will also changes with tilt increment. Thus, several operations such as re-centering the image and focusing are required, almost for each tilt angle. The time consuming manual collection not only means a slow, laborious work to the operator, but only increase the risk of radiation damage to the sample (Egerton, 2013; Egerton et al., 2004). Furthermore, fast collection can achieve more original data in a certain time and provide more information for further analysis, which could be helpful for noise decreasing and artifacts reduction (Cao et al., 2010; Crowther et al., 1970; Mastronarde, 1997). An automatic system for collection of tilt series is therefore high desired.

In this work, we report our new developed automatic data collection system for ET based on the ultra-HVEM in Osaka University. The system controls the microscope and reads the observation image from a remote control computer. More importantly, it can track the field of view and do focus in each tilt angle. The automatic view position tracking is carried out with an image matching technique based on the normalized correlation coefficient (NCC). Auto focus is performed by maximizing the image sharpness using an optimal object current. Observation images for position tracking and auto focus are captured by a high speed digital complementary metal–oxide–semiconductor (CMOS) camera (Hamamatsu ORCA-Flash 4.0 V2), increasing the speed for operation. After position tracking and auto focus, images are then captured by a high resolution charge-coupled device (CCD) camera (TVIPS F486BK). The system can automatically collect the tilt series with a time consumption of less than 30 s for each tilt angle.

2. Overall structure of the system

The overall structure of the system hardware is given in Fig. 1. The transmission electron microscope (TEM), cameras and data storage device are connected to the operation terminal via TCP/IP channels. Operation on the TEM is performed by sending ASCII commands to the TEM server. Responses from the TEM server are also ASCII encoded. The digital CMOS camera with 2048 × 2048 pixels resolution is used for the observation. Operation commands and responses for the camera

---

* Corresponding author at: Key Laboratory for Physical Electronics and Devices of the Ministry of Education, Department of Electronic Science and Technology, Xi'an Jiaotong University, Xi'an, 710049, PR China.
E-mail address: mengcao@mail.xjtu.edu.cn (M. Cao).

http://dx.doi.org/10.1016/j.micron.2017.09.006
Received 24 July 2017; Received in revised form 7 September 2017; Accepted 11 September 2017
Available online 12 September 2017
0968-4328/ © 2017 Elsevier Ltd. All rights reserved.

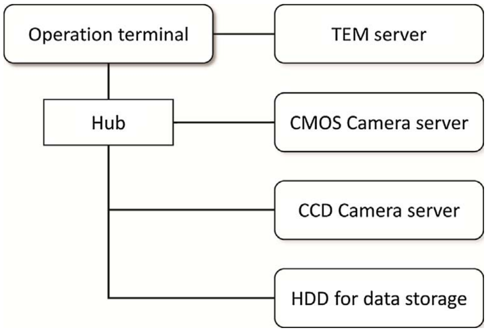

Fig. 1. The overall structure of the hardware.

are both ASCII encoded and transported with a TCP/IP channel. Binary image data are transported by another individual channel. The frame rate of the CMOS camera is normally set to about 8.3–10 frames per second (100–120 ms/frame). The slow scan CCD camera with 4096 × 4096 pixels resolution is used for high quality image capture. Asynchronous programming has been utilized for sending commands and reading response. The programming language is C# which allows for language-level asynchronous programming. Basically, a new asynchronous task is generated for each new operation. The main thread will not be blocked by the asynchronous task and can still response to the operator. After the asynchronous task is done, the corresponding return value will be sent back to the main thread. The asynchronous programming provide a possibility of parallel operation.

The procedure for data collection with the system is sketched in the follow chart in Fig. 2. Recording an image at certain tilt angle consists of steps of tilting, position tracking, auto focus and capture. Tilt angle can be changed by simply sending a command with certain parameters to the TEM server. An automatic position tracking is used to keep the region of interest (ROI) in the observation image center. The optimal focus depth is achieved by maximizing the image sharpness. Both the position tracking and auto focus are based on images obtained by the high speed CMOS camera. After re-centering and auto focus, the image can be captured either by the CCD camera or by the CMOS camera. The former provides higher resolution while the latter is of high speed. The procedure of recording image repeats until all data are achieved. The tilt direction should not be changed for the whole tilt series to avoid the mechanical errors associated with direction reversal.

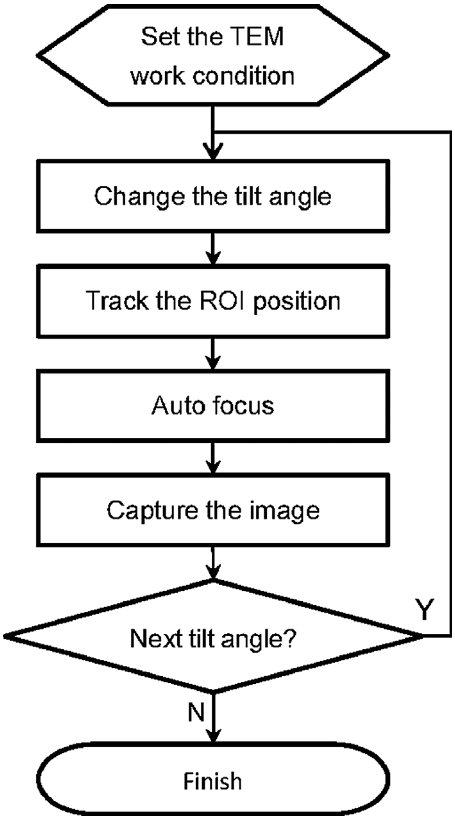

Fig. 2. The flow chart for the auto data collection.

2.1. Auto position tracking technique

Auto tracking is based on the technique of image matching using the NCC. In this method, the image shift can be determined by the NCC between the images before and after tilting. Note that the image shift due to the change of tilt angle is normally along the direction perpendicular to the tilt axis. We can enhance the image in that direction to increase the sensitivity and accuracy of position tracking. The method for enhancement is to filter the image with a differential operator in the moving direction

$$I_{\mathrm{en}} = |\cos\theta I_x + \sin\theta I_y|, \tag{1}$$

where θ is the angle between the direction of image shift and x axis. I_x and I_y are the differential of the image I in x and y directions, respectively. The NCC between two enhanced images before and after tilting is calculated and the images shift is determined by the position of the maximum point of the NCC.

Fig. 3 demonstrates the determination of image shift by enhanced images. Figs. 3a and b give the original image and Fig. 3c is their NCC distribution. Figs. 3d–f are the results with enhancements. The vector from center of the NCC to the maximum value point indicates the image shift. We can see that the bright point on Fig. 3f is less blurry than that on Fig. 3c, providing an accurate result for image shift.

There are two methods to re-center the ROI of the image: using the electromagnetic deflector for small image shift or adjusting the specimen stage position. The deflector can control the image position fast and accurately. However, the adjustable range is limited and can be used only for a small image shift. The stage position adjustment will be used for a large image shift. Once the image shift is determined, the system first tries to re-center the image using the electromagnetic deflector currents. If the accumulated image shift is beyond the adjustable range, the system will reset the deflector currents and then compensate the image shift by changing the specimen stage position. A fine image position adjustment after the stage position changing is still required because of the hysteresis and relaxation effect of stage movement due to the mechanical gap. A waiting of about 10 s is required for a stable response for stage movement. A careful stage adjustment that make the ROI close to the tilt axis could release the burden of tracking.

2.2. Auto focus technique

Auto focus is based on the method of image sharpness optimization (Nishi et al., 2014; Nishi et al., 2013). The sharpness of images obtained by the CMOS camera was calculated. To reduce the effect of impulsive X-ray noise, the image was filtered by a median filter. The image was then filtered with four differential operators with respect to different directions of horizontal, vertical, diagonal and anti-diagonal. The standard deviation values of the differential images were calculated. The square root of the sum of the four standard deviations was defined as the image sharpness.

The optimal focus is determined by maximizing the image sharpness. The defocus value is controlled by the object lens current I_{obj} of the TEM. The image sharpness can be seen as a function of object current. Several images with different object currents $I_{\mathrm{obj}} = I_0 + t\Delta I$ were captured by the CMOS camera, where I_0 is the initial object current value, ΔI is the defocus step, t is the defocus amount. The sharpness value $S(t)$ was then calculated for these images. Seven object currents

Download English Version:

https://daneshyari.com/en/article/5456903

Download Persian Version:

https://daneshyari.com/article/5456903

Daneshyari.com