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Development of Computer Assisted Electron Microscopy

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Advance in computer control of contemporary electron microscopes either by internal or external computer has made possible a computer assisted electron microscopy (CAEM) [1]. The ability to set all lenses' currents and record images on-line in digital form provide the basis of the CAEM. The objective of CAEM will be three-fold: (1) to free inexperienced microscopists from technical details of operating an electron microscope [2], where focusing, stigmating, and aligning the microscope are crucial to acquire high quality images; (2) to assist experienced microscopists in making the critical and yet difficult adjustment for human operators such as aligning the incident beam direction in the presence of 3-fold astigmatism in objective lens [3,4]; (3) to automate in searching and recording objects under very low dose conditions with full information on image conditions which will support further off-line data processing.

There are four key elements needed to achieve the CAEM: (1) image pick-up devices for acquiring images for evaluation purpose and for recording final high quality images; (2) communication with microscope to read and set parameters of the microscope; (3) image acquisition and analysis software to evaluate imaging parameters such as focus, residual astigmatism, and beam alignment; (4) a suitable algorithm for the CAEM and a fast computer to perform the CAEM tasks.

First two of the objectives of CAEM mentioned above have been almost achieved. On the other hand, the third one is still a challenging problem because a computer is still not enough to replace a human operator in controlling a microscope. We are seeking an efficient way for a computer to control the microscope. One of the major problems for the reproducibility and reliability of CAEM is hysteresis in microscope lenses. For example, the beam position at a given spot size may depend upon the approach of the spot size from larger or smaller spot size due to the hysteresis in condenser lens. Hysteresis is intrinsic in magnetic materials used in the lenses and therefore it can not be corrected. A human operator always correct hysteresis by re-adjusting a beam position by using a deflector before the objective lens. One way to control hysteresis may be by bringing a lens current always in a fixed route after changing the current between maximum and minimum several times. Then a particular state is always realized in the same manner, hence the hysteresis is minimized to a practical extent.

In electron microscopy, we have to find a good specimen area to be recorded at a final magnification. At present a computer is not so good as a human being to find a good specimen area. Therefore, a human operator should assist a computer by appraising a specimen. However, the way of searching a good specimen area adopted by a human operator is not suited for the CAEM. Normally, a specimen is searched by driving a stage controller at a low magnification on a main screen and a center area will be checked with a binocular. If a good area is found, the area is examined by increasing a magnification up to the one to be used to take a final image. After taking some images, the search process is repeated at a low magnification. Since such a process always requires a continuous commitment by a human operator to appraise a specimen quality, it is not suited for the CAEM. Therefore, we implemented a new specimen search algorithm (a progressive specimen search or magnification) in the CAEM different from the one taken by a human operator. In the CAEM a low magnification survey image (a parent image) is taken, areas of interest are then marked on it. An image (a daughter image) is taken automatically at higher magnification from each marked areas. Normally, the magnification ratio between a parent image and its daughter image is about 10 times. This process is repeated from a whole mesh image taken at a few 10x to a final image taken at a few 100 kx. The whole magnification range will thus be covered by four progressive magnification increments as shown in the Table. This table shows an image size, a required alignment accuracy, a camera to be used,

magnifications at the screen and a detector and an image/sample alignment scheme.

In the present study we use two cameras: a wide-angle TV camera, which is installed at 35-mm port of the microscope, and a MultiScan CCD (MSC) camera attached at the end of a Gatan Imaging Filter (GIF). The wide-angle TV camera is used for a real time image acquisition for an alignment purpose and to acquire images at lower magnifications which can not be attained at the GIF camera. Thus, a whole grid image is acquired by the wide-angle TV camera in the Low Mag mode at 15x on the screen. The images at the next magnification are taken also in the Low Mag mode at 150x by controlling the mechanical stage drive. We have found a stage drive accuracy is about 0.3 μm. Thus, the first three steps require only controlling the stage drive to align a sample area to be imaged. Contrary to this, at the forth step both the stage drive and deflector control are needed to take an image from each sample area of an order of 1 μm. Images at the final magnification is taken by the MSC in the Mag mode at 13 kx on the screen, which corresponds to 250 kx on the detector. Here, the whole area is positioned by controlling the stage drive, and then the electron beam is scanned onto each sample area and its image is shift back into the fixed MSC camera.

The ability to control and adjust microscopes by computer can significantly reduce the electron dose on the sample. Therefore, the CAEM is useful for examining beam sensitive materials such as biological specimens. It should be pointed out that the CAEM will facilitate a remote control of microscope (tele-microscopy), since the progressive specimen search is especially suitable for it. Furthermore, the volume of information to be sent over the network is much less in the CAEM. For example, a command to focus replaces a whole series of images necessary for a human operator to find an optimum focus.

Another example of CAEM in adjusting defocus, removing astigmatism and further determining sample tilt angles are reported in this conference by Pan, Ishizuka and Kimura [5].

Table Typical data acquisition scheme with progressive magnification

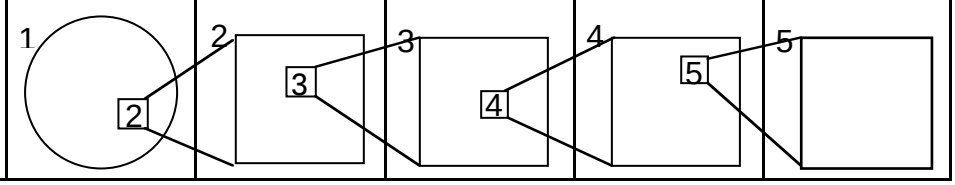
Step	1	2	3	4	5
Image Area	1 mm	0.1 mm	10 μm	1 μm	0.1 μm
Required Accuracy	0.1 mm	10 μm	1 μm	0.1 μm	10 nm
Camera	WA-TV	WA-TV	WA-TV	MSC/GIF	MSC/GIF
Mag Mode	Low Mag	Low Mag	Low Mag	Mag	Mag
Mag (detector)	5 x	50 x	500 x	25 kx	250 kx
Mag (screen)	15 x	150 x	1500 x	1300 x	13 kx
Image Alignment	Stage	Stage	Stage	Stage + Deflector	Deflector
					

Image area is assumed to be always divided into 10x10 sub-areas. An image area is thus always decreased by ten times.

References

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