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Automated, 100-Specimen Loading and Imaging System for Transmission Electron Microscopy

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The screening of multiple specimens using a transmission electron microscope (TEM) is time consuming as a user must manually load and unload samples, operate the microscope, and record images. We have developed an automated imaging system that has fully automated these processes with a device that can (i) load up to 100 specimens into the TEM, (ii) acquire images from each specimen, and (iii) let the user screen selected regions of a specimen. Our automated system includes a multi-specimen loader called the Gatling which has been combined with a modified version of our in-house data acquisition software, AutoEM [1], which controls the operation of both the Gatling and the electron microscope.

The Gatling utilizes cartridges to hold each specimen, and up to 100 cartridges can be contained and loaded in a single imaging session. Once the chamber containing the cartridges has been mounted on the microscope, cartridges can be loaded into the microscope column. Automated cartridge loading is achieved by using a tweezer assembly which picks up the desired cartridge from the cartridge holder and moves it into the microscope column where it is mounted using a motorized holder. After the cartridge has been loaded, data acquisition steps are launched and images are recorded on the CCD camera mounted below the projection chamber. The parameters used for cartridge specification and data acquisition are completely handled through AutoEM user interfaces.

The procedure for screening specimens involves: (i) recording a low magnification image (60-100 X) from each specimen, (ii) drawing markers around regions-of-interest (ROI) using the Digital Micrograph (DM) graphical user interface, (iii) converting the markers to microscope stage displacements relative to the center of the specimen grid, and (iv) acquiring images from each ROI and saving them to a local or network computer.

The use of asymmetrically patterned grids allows flexibility in loading grids in any orientation. The unique features of the patterned grids are used to define a common reference frame for image acquisition. The use of robust centering algorithms ensures that the same regions can be correctly addressed when grids are re-inserted into the microscope for further analysis.

For 96 sequential specimens, it takes 10.1 hours to load, acquire an overview image at 60X magnification, and unload each cartridge in the present implementation. A significant advantage of the Gatling is that it mounts on the side of the TEM without altering the microscope or the room that houses it. Additionally, standard operation of the microscope remains unaffected when the Gatling is not used. The automated data acquisition software can also be used with a conventional specimen holder for acquiring images from a single specimen.

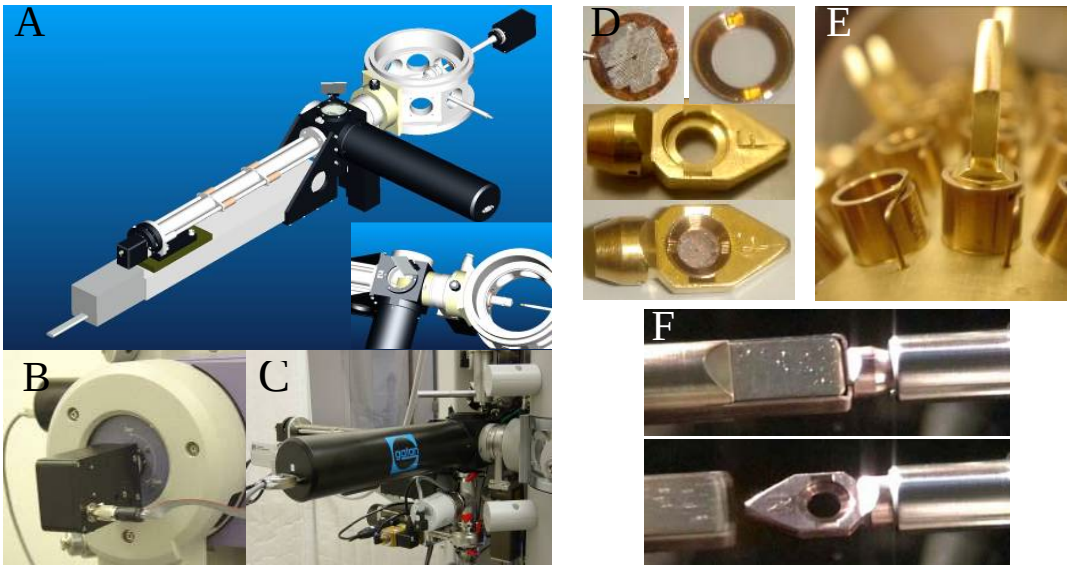

Fig. 1. Sequence of events for operating the Gatling. A. Schematic representation of the Gatling. B. Motorized holder used to mount cartridges is inserted into microscope stage. C. Gatling system mounted to Tecnai 12 TEM. D. Cartridge holds a single specimen grid. E. Cartridges loaded onto 100 position drum. F. Tweezers bring the cartridge into the microscope column where it is mounted onto the holder.

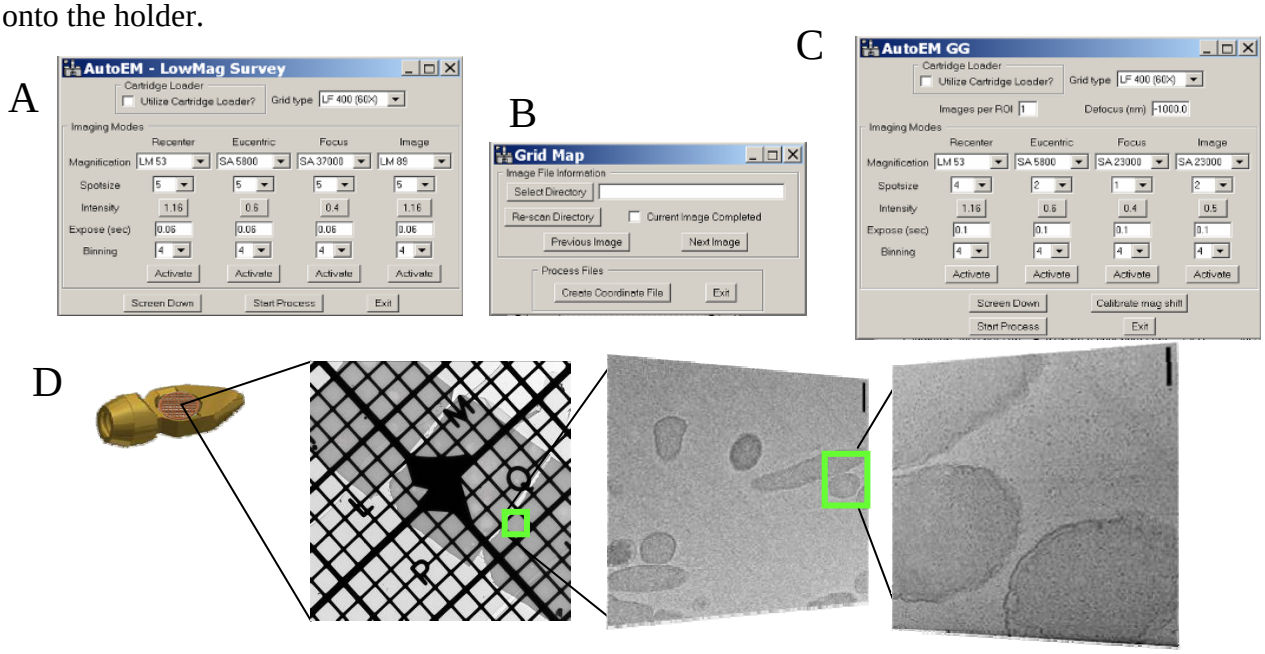

Fig 2. Graphical user interfaces used for collecting data using the Gatling (A,B,C). D. Images collected from a typical specimen at low (60X), medium (3300X), and high (26 kX) magnifications.

References

[1] P. Zhang, et al. J Struct Biol. 135 (2001) 251-61.
[2] The work was supported by the intramural program at the National Cancer Institute.

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