Development of TV-rate CCD Cameras for *in-situ* Electron Microscopy

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Introduction

We have all heard the quote "A picture is worth a thousand words" when describing how much information can really be represented by a single image. Many of you may have written and published pages of results all based sometimes on a single piece of important data such as an electron micrograph. Of course, if one image is worth a thousand words, as the saying goes, how much would 30 or more be worth? With the advent of better performing electronics and development of newer CCD (charge couple device) technology, it is now possible to extend the famous saying and complete it with "but a movie tells the whole story". By extending the single acquired image into a series of fast-acquired frames, movie creation is possible. This capability allows us to record dynamic events in many different applications, leading towards sharing the sequence of images and telling a more complete story that cannot be done with one image alone. A brief background on how this technique is used in electron microscopy follows.

In-Situ Experiments in TEM

Observing dynamic events through *in-situ* experiments in TEM (transmission electron microscopy) is not a recent or new technique. Doing an internet search just on "*in-situ* transmission electron microscopy" will give you over 269,000 results alone. Some of the *in-situ* topics range from: heating thin metals, stress-relief experiments, nano-indentation of metals, melting and freezing of crystalline materials, growing of nanotubes, to biological cryo imaging or using environmental cells for biological events. These types of electron microscopy techniques are on an upswing as can be seen by their popularity in the many recent symposiums and conferences held at key scientific meetings in the U.S (ASU Jan 2006, FEMMS meeting Sept 2007, AVS meeting in Seattle Oct.2007).

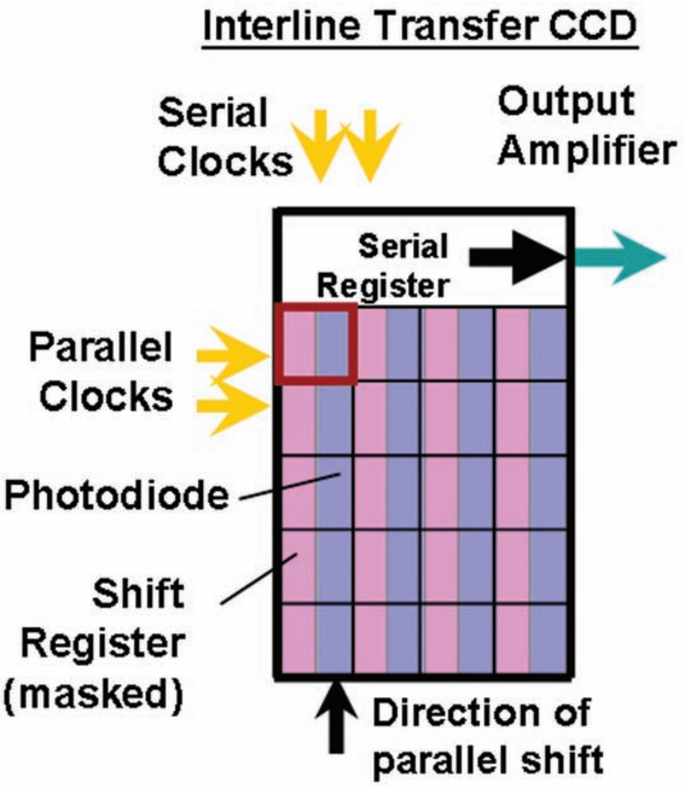


Fig. 1 - A schematic drawing of a part of an interline CCD array. The marked red box outlines a square pixel.

The major requirements for *in-situ* microcopy studies on samples during heating, deformation, straining and chemical reaction require appropriate specimen holders and also a camera with a recording system interfaced to the TEM. The first commercially available camera for the TEM was introduced by Gatan in the mid 1980s. The camera offered real-time observation with standard TV rate – *i.e.*, 30 frames per second - ideal for sample search and manual adjustment of the TEM and also for recording dynamic events on tapes. However, the cameras were analog-based systems for which corrections for camera defects and gain non-uniformity across the scintillator were nearly impossible because of the analog signal. The system for recording video data from this analog camera system has been with some type of semi-permanent media such as magnetic recording tape or the early forms of laser disk media. Due to the technology at hand at the time, the analog TV cameras and recording system that entered the market were not exactly perfect. It, however, contributed greatly to *in-situ* microscopy.

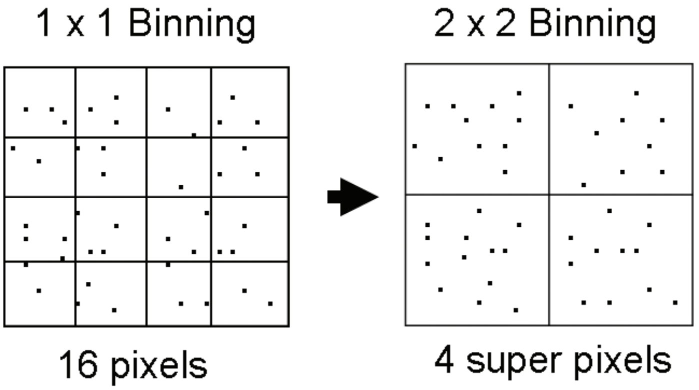
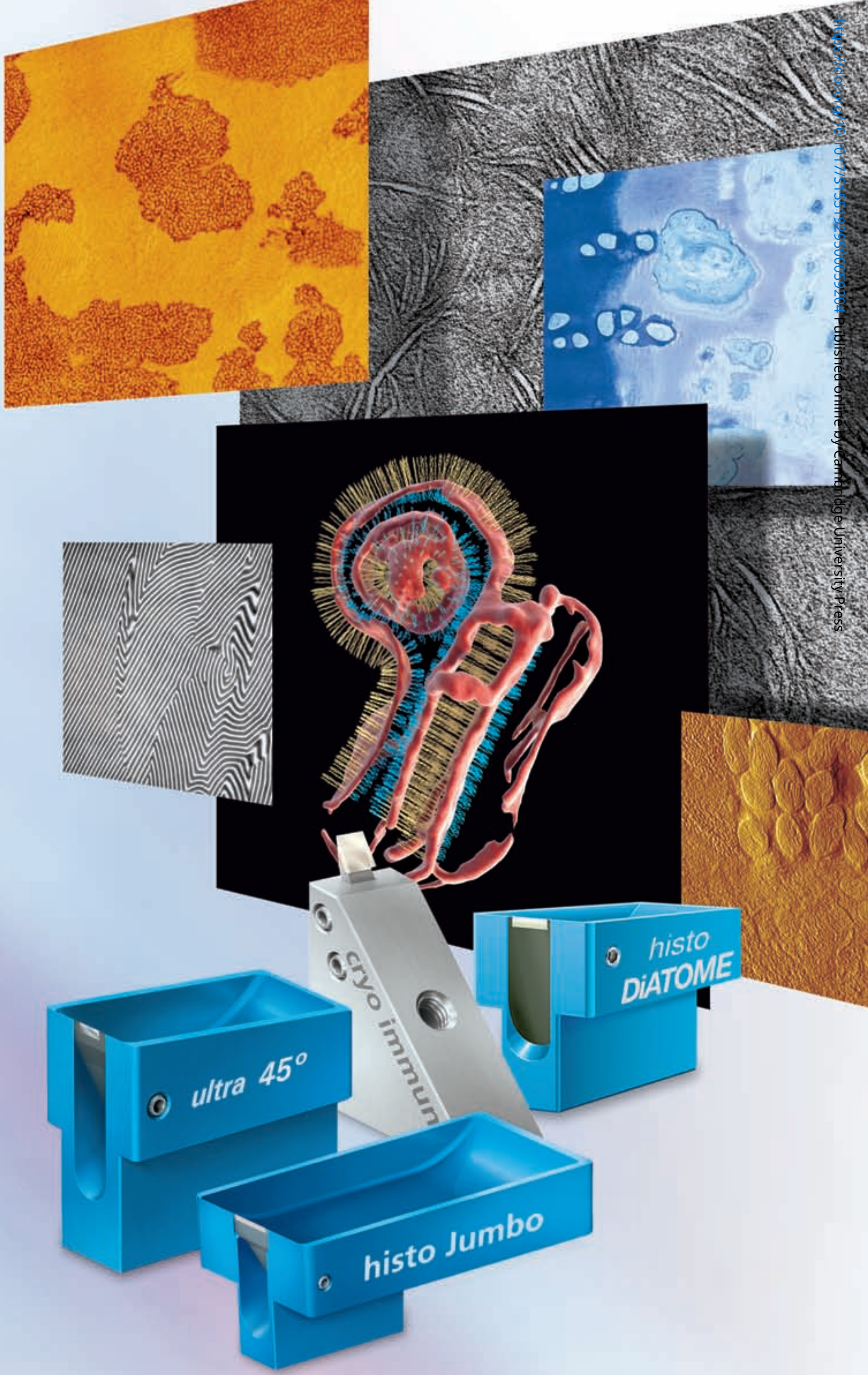


Fig. 2 - A schematic drawing demonstrating on-chip binning from 1×1 binning to 2×2 binning.

CCD cameras for TEM

The charge coupled device (CCD) was invented in 1969 by Willard Boyle and George E. Smith at AT&T Bell Labs. Due to its high sensitivity in converting light to electrons, CCD was soon used for light detection in astronomy in 1974 and in electron microscopy through a use of scintillator to convert electrons to light before entering the CCD in the mid-1980s [1,2]. In 1990, Gatan, Inc. made the first commercial CCD digital camera for TEM [3,4]. The first generation of CCD cameras was called slow scan CCD because they had a very slow frame rate due to the large pixel size (24 um) and the full frame readout technology available at that time. The large pixel size gives a large full well capacity (the ability to collect CCD electrons), which is necessary for generating high quality images. In addition, the images acquired from a computer are conveniently acquired in a digital format that can be viewed, processed, and analyzed immediately. It was quickly realized that



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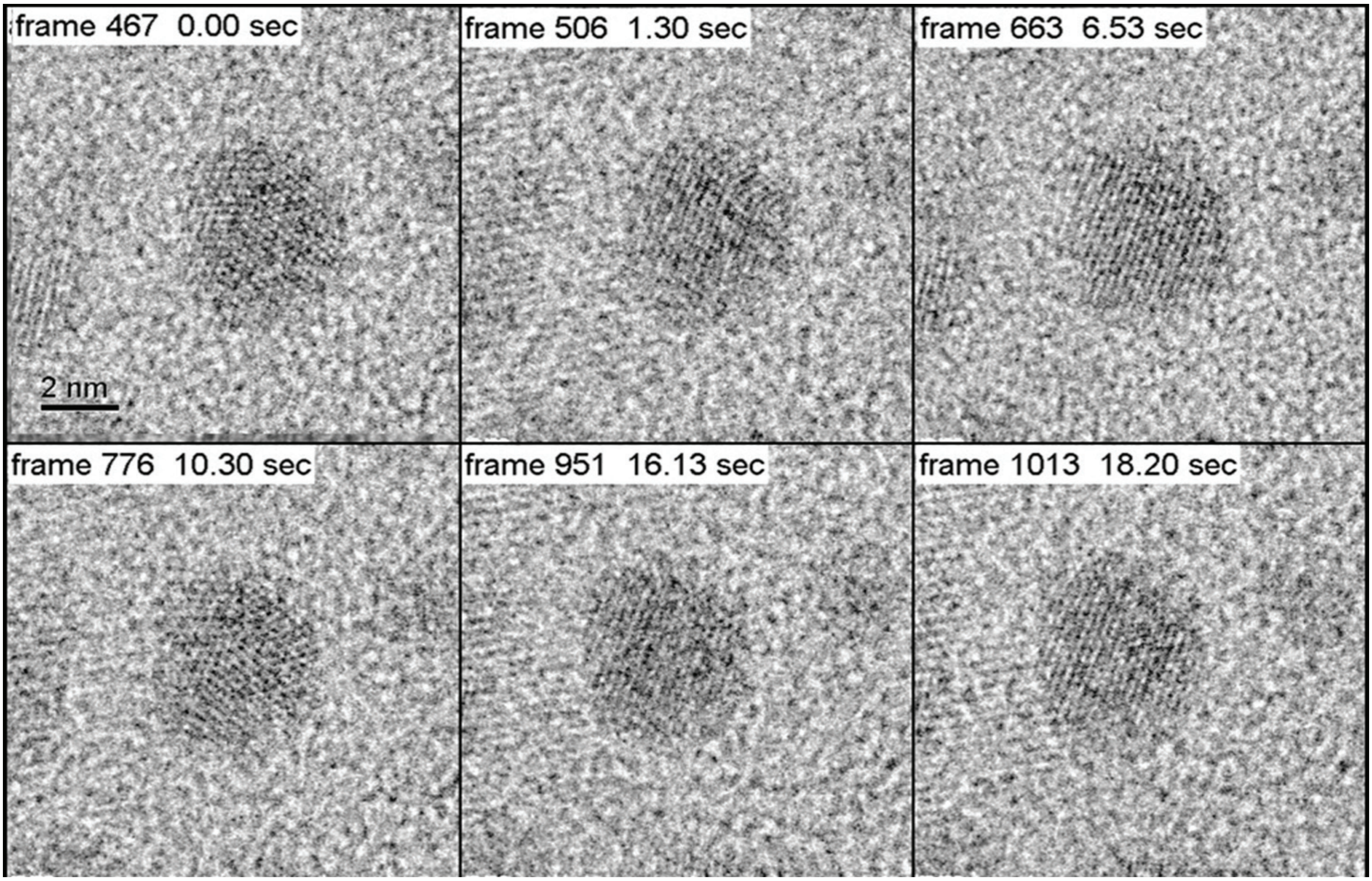

Fig. 3 - Individual time-lapsed frames from an in-situ movie (30fps) showing an Au nano-particle on Si substrate. The movie was recorded using the ORIUS SC200 on a 400kV TEM (Images courtesy of Prof. David Smith and Karl Weiss, Center for Solid State Science (CSSS), Arizona State University).

the CCD camera could eventually replace the photographic film traditionally used for recording TEM images.

The slow readout rate, however, is not suitable for *in-situ* microscopy where dynamic events can take place faster than the CCD camera readout rate. To improve readout rate, CCD arrays are divided into several sectors and each sector has its own readout output (multiport readout design). CCD pixel size is also shrinking, for example from 24 µm to 14-15 µm or smaller, due to the advancements in the chip making industry. Additionally, CCD output amplifiers have evolved to provide low readout noise at significantly higher speeds. Because of all these, the subsequent generation of CCD cameras for TEM can achieve about 5 frames per second (fps) with an adequate image size and resolution.

New Solutions in Digital CCD TV Imaging

To further improve readout rate, Gatan launched a new camera platform by using a progressive scan interline transfer CCD array in 2006. Larger format interline CCDs have square pixels like the full frame CCDs already in use in TEM. However, in these interline CCDs, each pixel is divided into two regions which perform different functions. One side is a photodiode that detects and converts light during the exposure and the other side is for charge shifting, which is isolated from the first side so that shifting out of the previous exposure can happen while charge is being accumulated for the next exposure (Fig. 1). After readout of the previous exposure, the newly acquired image can be transferred almost instantaneously into the shifting side, whereupon the next exposure can begin without delay. With the sensor-designed speed of 30 MHz in fast mode, a single transfer step can be performed in about 30 milliseconds. Accurate readout timing control allows very short exposure intervals without the need of physical shutters on the TEM. In addition to the fast mode of 30 MHz for viewing/video recording, the CCD also operates at slow mode of 5 MHz for acquiring high quality images.

Even faster readout can be achieved by providing the array with two readout channels and by reducing the number of pixels to be read with binning. Binning is a way to combine charge from neighboring pixels prior to readout (Fig. 2). It allows a significant speedup in terms of frame rate in exchange for a reduction in spatial resolution. For instance, 4x4 binning reduces a 2K×2K array to an effective 512×512 array but boosts the frame rate from about 7fps to 30fps. It does this without a loss of sensitivity, since all of the charge that hits the detector gets concentrated into the binned pixels. Binning uses the whole CCD array, therefore keeping the same large field of view as no binning. This is different from using a sub-region of the CCD (*i.e.*, smaller field of view) to achieve more frames per second.

All these efforts in developing a system suitable for *in-situ* applications has resulted in a totally digital CCD imaging system capable of acquiring images at true TV rate, *i.e.* 30 fps, at 512×512 (bin 4×4) image size (Gatan Orius^{TM} SC200 CCD camera). The SC200 is a 2k×2k CCD (4M pixels) camera capable of achieving high quality images at TV readout speed for imaging applications in both life and materials science.

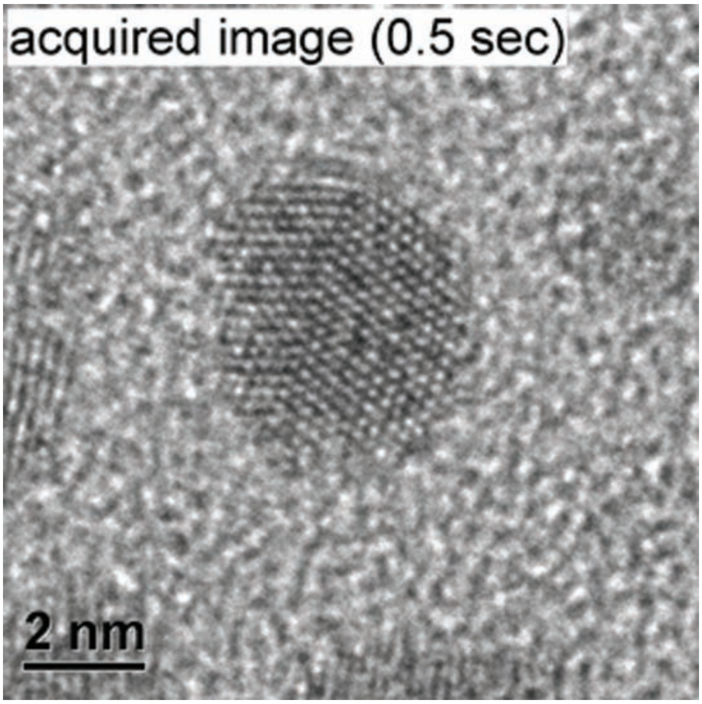

Fig. 4 – High resolution image of a Au nano-particle recorded in high quality mode during video recording.

Video Recording from TV rate CCD cameras

Creating a movie of a dynamic event or from a sequence of fast displayed images can be easily done with the Gatan Digital Streaming Video (DSV) software that comes with the camera. Streaming video can be created using a Windows™ standard method for handling live video feeds; it is compatible with widely used webcast (webcam) and home video applications. The video stream is generated simultaneously while viewing and can be recorded into a digital movie via video authoring software as uncompressed AVI or other popular movie formats (mpeg, DVD, WMV).

The recorded movie cannot be displayed in this article due to the paper format, so to illustrate this capability, we show individual frames captured in a movie. Figure 3 shows 6 individual frames extracted from a digital movie of a small Au nano-particle in a 400kV TEM. The high-speed imaging allows the user to observe rapid structural changes (single and multiple twinning, moving of the twinning plane, creation and disappearance of multiple twinning, *etc.*) of this nanometer-scale Au particle under the electron beam. The ability to be able to examine details or use computer software to analyze the image data in each time stamped frame is very important for quantifying changes in a dynamic event. If desired, a full resolution, single frame image can be acquired at any time using the camera's high quality mode of operation (Figure 4).

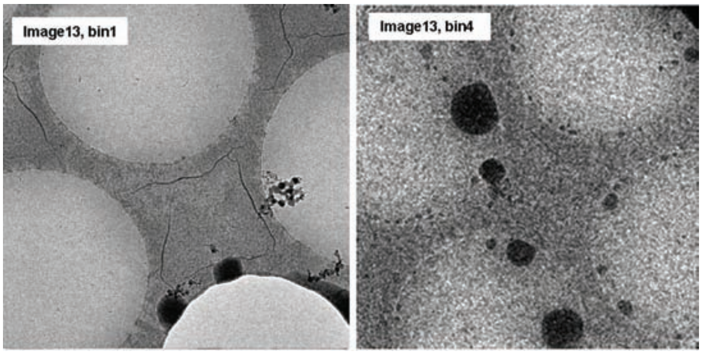

Fig. 5 - Low magnification survey images of frozen hydrated sample recorded in high quality mode (image L) and TV rate mode (image R) under low electron beam dosage. Exposure times for the left and right images were 1 sec. and 0.05 sec., respectively (Image courtesy of Drs. James Conway and Dalaver Anjum, University of Pittsburgh School of Medicine).

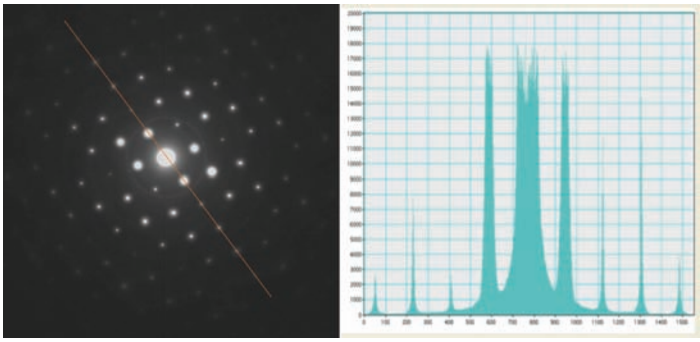

Fig. 6 - SAED Diffraction pattern of Si with adjoining line plot showing high resolution and high dynamic range of the SC200 CCD camera.

In cryo-biological applications, the amount of electrons, or beam dosage, interacting with the sample is critical and needs to be very closely maintained so as not to introduce specimen damage that could alter the morphology or 3D model being investigated. As shown in Figure 5, a set of cryo sample images were obtained under controlled electron beam conditions with minimal dosage of approximately 0.04 (bin 1×1) and 0.06 (bin 4×4) electrons/Å^2/second respectively using a 200kV FETEM at 2500x TEM magnification. At such a low magnification, the electron beam is spread over a very large area that helps minimize the radiation damage. This type of surveying at lower magnifications is the first step of low dose imaging for judging the ice condition and distribution of the sample that will be viewed and captured in later steps at higher magnifications. The use of the SC200 camera with its fast 30fps TV rate makes the surveying very easy and quick even in the lowest of beam conditions. The combination of this TV-rate digital camera with a high performance large format CCD camera (for example, a 4K×4K) provides a powerful digital imaging system for cryo-biological applications.

The camera has built-in anti-blooming performance to release excess charges in saturated pixels before they spill over to the surrounding pixels. This is necessary for recording electron diffraction patterns because of the presence of very bright center diffraction spot generated from thin samples. Figure 6a shows a selected area electron diffraction (SAED) pattern from a silicon crystal in [110] orientation. The sample is thin as is evidenced by the weak (200) and (-200) diffraction spots. The intensity line scan of the diffraction pattern (Fig. 6b) shows excellent resolution and dynamic range (14-bit). The ability of recording diffraction patterns with this camera is helpful to observe structure changes during *in-situ* experiments that are hard to detect when viewed in real space imaging mode. ■

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