

NEW FRAME-TRANSFER WIDE-ANGLE SLOW-SCAN CCD CAMERA ALLOWS RECORDING OF DISTORTION-FREE IMAGES FOR DIGITAL MONTAGES

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Modern SSCs combine low noise with high sensitivity and provide digital images with high dynamic and excellent linearity. Since these images are immediately available in a PC, SSCs have become a non-dispensable tool for on-line image processing and software driven automated TEM tuning [1]. Many experiments performed nowadays in the area of high resolution, low dose, holographic reconstruction, and EFTEM [2] would not be possible without the digital input coming from SSCs.

Despite their excellent characteristics SSCs provide a restricted number of individual image points in respect to film. This turned out to be the most prominent disadvantage of SSCs especially for applications where highly resolved large specimen areas need to be captured. Tab. 1 shows a typical example for a biological application where an image detail of 4 nm (plasma membrane) needs to be resolved. Depending on the film material used, this resolution is matched more or less by a TEM magnification of $\sim 8,000\times$. Based on a 3-fold over-sampling for various SSCs and their respective position on the TEM, tab. 1 lists the maximum imaged area of the specimen and the magnification needed to achieve the same resolution in the digital image. Tab. 1 clearly shows how small the digitally imaged specimen areas are in respect to the negative. To overcome this situation, either larger CCDs (e.g. 2kx2k) can be used, or individual images can be spliced together to form a "digital montage". Nowadays this process is automated by software controlling the TEM image shift coils to achieve the desired overlaps. However, when splicing images together the spiral distortions of the projector lens system become of significant importance. The smaller the magnification and thus the larger the field of view on the SSC detector in respect to the negative, the more prominent are the distortions in the recorded images (fig. 1). Since these distortions are proportional to the 3rd power with the radius, this effect becomes most apparently visible in images recorded by wide angle SSCs.

In contrast to all other wide angle camera systems, which are inserted and retracted horizontally, the ProScan camera [3] is inserted by tilting the camera head from a vertical into a horizontal position (fig. 1). By this, the distance of the camera scintillator to the last projector lens cross-over is enlarged, enabling the camera to "see" a considerably smaller area (45 x 45 mm) than imaged by the film sheet (78x78 mm). As a consequence, the spiral distortions are limited to 20% of the distortions recorded by the negative (fig 1). With the distortions in the negative $<1\%$ in the 906E, digital images coming from the ProScan camera are almost distortion-free ($<0.2\%$). An image shift range of >25 cm in the film plane of the LEO 906E - independently from the TEM magnification - allows recording of distortion-free, perfectly matching digital montages of up to 5000x5000pixel by the ProScan wide angle SSC. Fig. 2 gives an example of a 4x3 montage recorded with the MIA (Multiple Image Alignment) software module from the AnalySIS 3.0 software package. Acquiring the 12 individual sub-images and splicing them together to form the final image took less than 2 minutes.

References:

- [1] O.L. Krivanek and P.E. Mooney, Applications of slow-scan CCD cameras in transmission electron microscopy, *Ultramicroscopy* 49 (1993), 95-108.
- [2] W. Jäger and J.Mayer, Energy-filtered transmission electron microscopy of Si_mGe_n superlattices and Si-Ge heterostructures. I. Experimental results, *Ultramicroscopy* 59 (1995), 33-45.
- [3] S.A. Hiller, et al., New SSC Cameras with frame/interline CCD architecture avoid TEM shutter control, provide excellent image quality and can be easily retrofitted, *Proc. MSA* (1999).

Tab 1: Maximum specimen areas imaged by different detectors for a detail resolution of 4nm (e.g. plasma membrane). Calculations are based on a 3-fold over-sampling on the SSC cameras.

Detector characteristics	Camera factor	TEM magnification	Magnification on target	Imaged specimen area [μm]
Sheet film 8x10cm (78x78mm used image format)	1	8,000	8,000	9.75 x 9.75
SSC bottom mount camera 1kx1k pixel (19x19 μm)	~ 1.2	12,000	14,250	1.37 x 1.37
Conventional wide-angle SSC 1kx1k pixel (24x24 μm)	~ 0.27	67,000	18,000	1.33 x 1.33
ProScan wide-angle SSC 1kx1k pixel (14x14 μm)	~ 0.31	33,800	10,500	1.33 x 1.33

Fig. 1. Scheme explaining the projected image areas from a normal wide angle SSC and the ProScan frame-transfer SSC to the film sheet plane of the LEO 906E TEM. By the lower position of the ProScan camera, the spiral distortions are reduced by a factor of 7 in respect to standard wide angle SSCs. This allows the ProScan camera to record distortion-free images for perfect montages by maintaining its "wide-angle" character.

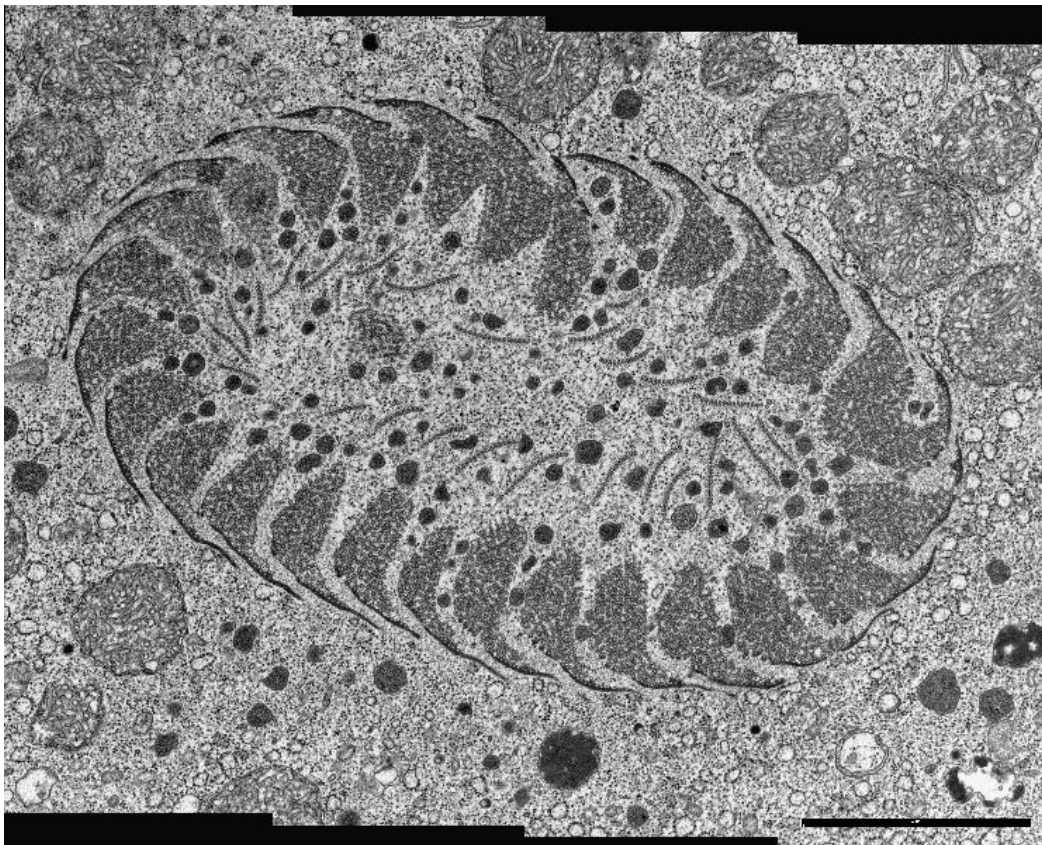
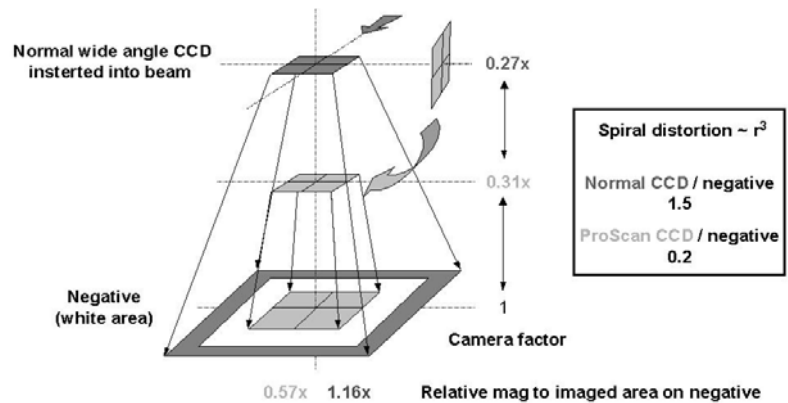


Fig. 2. 4 x 3 digital montage image showing a cross section of the oral basket of the ciliate *Pseudomicrothorax dubius*. Image was taken with the ProScan wide angle camera on LEO 906E at 21 560x. Final image size is 3,729 x 3,006 pixel. Note the perfect match of the single images and the good contrast of the digital image from this unstained sample. Specimen courtesy Inst. Spez. Zoologie, Universität Tübingen, EM Labor. Scale bar = 2 μm .