Carl Zeiss SMT – NTS Division

Enhancing resolution for digital imaging in the **LIBRA® 120**

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Market:	All markets using digital image recording
Samples:	The sample shown has been conventionally prepared. Glutaraldehyde and $Os0_4$ simultaneous fixation according to Shigenaka, dehydration and embedding in EPON 812. Ultra thin sectioned to a thickness of about 50 nm. The sections have been conventionally stained (uranyl acetate and lead citrate). Specimen is *Pseudomicrothorax sp.* a freshwater ciliate. The presented images are showing cross sections through the nematodesmal rods built up by highly ordered and interlinked microtubules aligning the “mouth funnel” of this protozoon.
Purpose of investigation:	Acquisition of digital images to resolve the tubulin subunits by covering at the same time a large specimen area.
Background information:	Documentation of results in TEM is always related to the properties of the used detector. The relevant parameters here are detector size, detector resolution (pixels), camera length, TEM magnification and magnification on target. In addition it must be clear what is the smallest detail, which should be resolved in the recorded image. Depending on the used detector and its position within the TEM these parameters define (limit) the maximum recordable specimen area. This dependency for various detectors in different positions on the TEM column is summarized in the following table:

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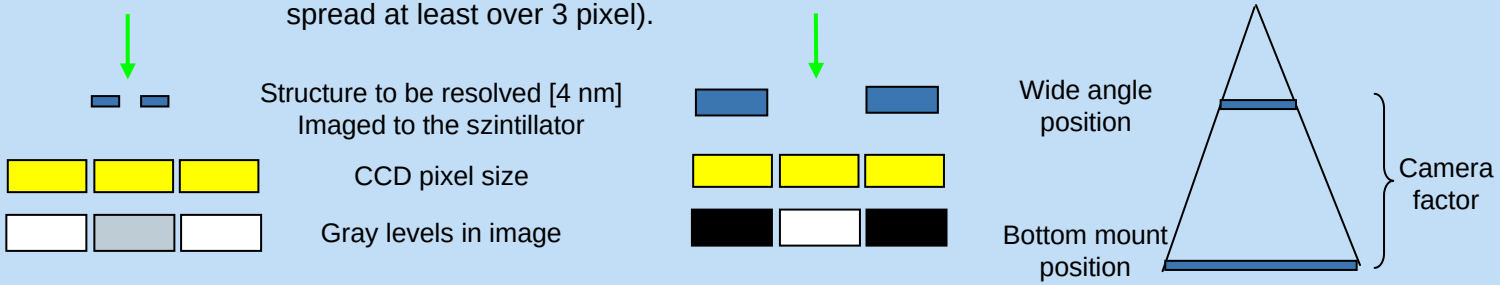
Background information:

Detector characteristics	Camera factor	Magnification on target	TEM magnification	Imaged specimen area [µm]
Negative 8x10cm (78x78mm used) 9,75kx9,75k pixel (8x8µm)	1	6 000	6 000	**13,0 x 13,0**
ProScan wide-angle SSC 1kx1k pixel (14x14µm)	~ 0,32	**10 500**	32 813	**1,37 x 1,37**
Conventional wide-angle SSC 1kx1k pixel (24x24µm)	~ 0,27	18 000	66 667	**1,37 x 1,37**
SSC bottom mount camera 1kx1k pixel (19x19µm)	~ 1,22	14 250	11 680	**1,37 x 1,37**
Proscan bottom mount camera 2kx2k pixel (14x14µm)	~ 1,22	**10 500**	8 607	**2,73 x 2,73**
SSC bottom mount camera 2kx2k pixel (30x30µm)	~ 1,35	22 500	16 667	**2,73 x 2,73**

Initial conditions: Image detail to be resolved: 4 nm (membrane bi-layer). Magnification on target is too low in the first example given below**.** 3 x undersampling on CCD is necessary (image detail to be spread at least over 3 pixel).



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Background information:	**Consequences:** • The negative records the largest specimen area by resolving the requested detail • the TEM magnification has to be adapted according to the camera factor and the pixel size • the imaged specimen area is only given by CCD's or negatives pixel (grain size) number • for a given pixel size, the magnification on target (scintillator) is constant independently of the CCD's position in the TEM • the magnification on target (scintillator) is proportional to the pixel size (negative <-> CCD !!) • for a given pixel size (and a given S/N ratio) the dose in the sample is always the same independently of the CCD's position. This means there is no preferred position of the CCD for low dose applications • A wide angle camera system is not recommended to record images at the resolution limit of the TEM
Solution:	**Three possibilities to overcome the problem of the small imaged areas by CCDs:** • Use camera with more pixel: 1k -> 2k -> 4k -> 8k (rather expensive for 4k & 8k cameras) • Use smaller pixels (not recommended -> point spread function, S/N, reduced saturation capacity) • **Shift the sample over the detector and acquire multiple images which are aligned and spliced together automatically (montages)**
Parameter settings:	LIBRA® 120, 100 kV, 60000x primary magnification. Frame transfer wide angle slow scan CCD camera (1024x 1024 pixel; pixel size: 14 µmx14µm). iTEM image processing system. Acquisition was done automatically by using the LIBRA®'s image shift deflection system. Automontaging done by multiple image alignment (MIA) of the image processing system.

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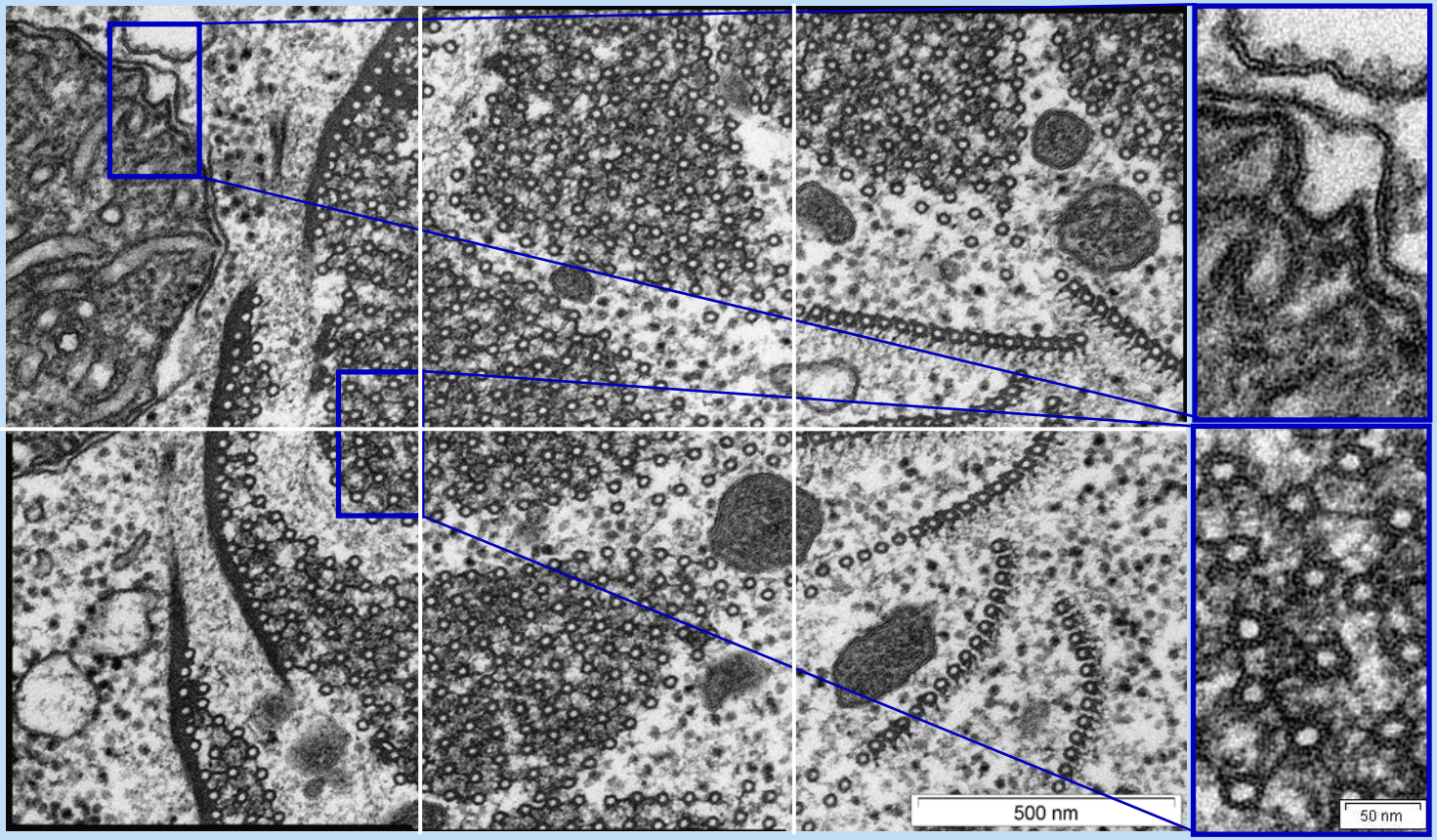
Solution:



3x2 montage showing cross sectioned nematodesmal rods of *Pseudomicrothorax*. The highlighted enlarged areas clearly show the membrane bi-layers and the tubulin subunits forming the single microtubules with their cross-linked bridges. Note the perfect image match even in overlapping corners.

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Customer benefit:	**Digital montaging is a very elegant and cost effective method to overcome the common problem of limited pixel resolution of CCD cameras.** **The LIBRA® TEM allows control of almost every function from a remote system. Thus an image shift producing orthogonally perfect aligned images with a predefined overlap is done simply via a mouse click in the image processing system. Since the montaging is done with pixel accuracy by an automated cross correlation routine, the whole acquisition and aligning process is fully automated and takes only a few seconds up to a minute.** **Montages of up to 10x10 images (depending on the used camera system) are possible. The covered areas - and resolutions - from such montages can be even larger than by sheet film image. Limitations currently are more due to the available memory instead to limitations of the image shift range of the LIBRA® TEM.** **These images may be used for extra high post magnifications (posters) or simply to document a whole structure at a very high resolution.** **The montages are available as digital image files in various formats to be further processed or exchanged over network.**

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Summary:	Montaging of images with the LIBRA® TEM is as simple as counting from 1 to 3. By considering the pecularities of digital cameras, images can be obtained which show excellent resolution by not compromising the imaged area. Applying Zero Loss filtering techniques the brilliance of these images is even increased by eliminating chromatic aberration of inelastically scattered electrons in the LIBRA® 120. Köhler illumination provides in addition strictly parallel illumination and excellent evenness of the illuminated specimen what allows to record outstanding images even by means of a 1kx1k CCD camera.
Special Comments:	By the special design of the LIBRA® wide angle ports distortion free montages are even obtained from wide angle cameras. This will be the topic of an other application gallery.

We make it visible.