The Digital Precision Imaging Laboratory
Part I: The New Tools of Differential Contrast Imaging

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In recent years, many developments in imaging instrumentation and support technologies have put in question the conventional way of working with images. Conversion from analog to digital imaging is pursued at all levels of microscopy driven by the benefits of new forthcoming digital precision imaging technologies for visualization and quantification of the image information. These developments will revolutionize microscopy by extending the information retrieval from the level of the full-frame image to the level of individual pixels.

Why convert from analog to digital?

The two prime reasons for converting to digital image data handling are found as follows in: I. Independence from Spread Functions, and II. Mathematical Pixel-Accurate Data Treatment. Both aspects define a new level of image data handling which values the image data pixel-by-pixel with respect to spatial resolution as well as contrast resolution. Many precision imaging technologies are already available that require a "pixel-accurate" display and measurement of captured information. Foremost, the atomic force microscope is a high precision instrument which can routinely deliver 16-bit imaging data. The transmission electron microscope also reveals high resolution power in combination with precision CCD cameras for digital data acquisition. Other imaging instruments, like the scanning electron microscope, have the potential of pixel-level accuracy. It was recently shown (Peters, MAS 1995:393-394) that inherent problems of signal collection deficiencies can be overcome by reconstitution of digital image data, elevating these instruments to the level of precise scanned electron beam microscope.

ELECTRON MICROSCOPE SERVICE ENGINEER

The University of Kentucky anticipates hiring a service engineer to perform routine and emergency repairs on the University's electron microscopes. The successful candidate should have a minimum of three years experience in field service of electron-optics instrumentation and expertise in trouble-shooting solid state electronic, electro-mechanical, high vacuum, and microprocessor systems. The position involves working independently to solve all instrument failures, and performing field upgrades, and modifications so as to maintain these instruments in state-of-the-art condition. The successful applicant will work with a consortium of faculty and staff who utilize twelve electron microscopes in six sites including tungsten, LaB_6, and field emission cathode instruments; oil diffusion, turbo-molecular, ion getter, and molecular drag pumping systems; energy dispersive X-ray detectors and various supporting systems from a variety of manufacturers.

Candidates for this position should send a copy of their vita that details their expertise and experience as well as three letters of recommendation to David Watt, Vice Chancellor for Research and Graduate Studies, University of Kentucky, 201 Gillis Building, Lexington, KY 40536-0033.

The position will be available as early as July 1, 1996, and a competitive salary and benefits package is offered. The University of Kentucky is an EEO/AA employer

I. INDEPENDENCE FROM SPREAD-FUNCTIONS

While digital imaging technologies have been well established for over 30 years, only recently was the "precision imaging" potential of microscopes demonstrated by Oho and Kanaya with a comparison of digital versus conventional analog image handling (Scanning 12, 1990:141-146). Scaled full-frame views of both types of image data revealed no dramatic visual differences. However, if viewed at 16-times enlargement (photographically or digitally by bicubic zoom, respectively) at the level of individual pixels, the differences became very obvious. The spread-functions of the phosphorous layer of the CRT and the photographic emulsion blurred individual pixels and spread higher intensities over a larger area than the display lines were wide, increasing the pixel's area and reducing the intensity. Additionally, the noise of the photographic emulsion became evident. These problems in information storage and display seriously limit the precision imaging capabilities of modern microscopes. Digitally handled data are free of these limitations since they can be stored and displayed without spread function limitations. Digital imaging can preserve the pixel character of data as generated by digital imaging sensors and thus can maintain the data precision. Such "pixel-accurate" preservation is of critical importance for the visualization and quantification of the high resolution information in image data.

II. MATHEMATICAL PIXEL-ACCURATE DATA TREATMENT

Digital precision images contain much more information than the eye can perceive and recognize. Therefore digital imaging requires a careful distinction between the digital data set (**raw data**), the displayed image (**original image**) and the visually perceived and recognized part of the displayed image (visual image or **image**). The information of the visual image is structured as contrast patterns which represent the various spatial components. Contrast patterns and spatial components are bound to each other by a dual principle, i.e., an image without contrast contains no spatial information and vice versa. The contrast patterns are discretely recognizable and are distinct from each other by different contrast levels. All contrast patterns are additive and constitute the total image information within a contrast range equal to the intensity range of the image data set. If the image would be composed of non-discrete contrast patterns, no discrete spatial components would be recognizable. *It is the art of microscopy to prepare specimens and to operate the microscopes in such a way that discernible contrast patterns are produced* and that they reflect pertinent spatial properties of the sample (the same holds true in all other imaging technologies). All microscopies generate high resolution contrast patterns, but in general these patterns are so small in size (only a few pixels wide) or so weak in contrast (only a few intensity steps high) that they remain unrecognizable in the image. This loss in precision information is not acceptable in microscopy that aims at "seeing" the smallest possible sample details.

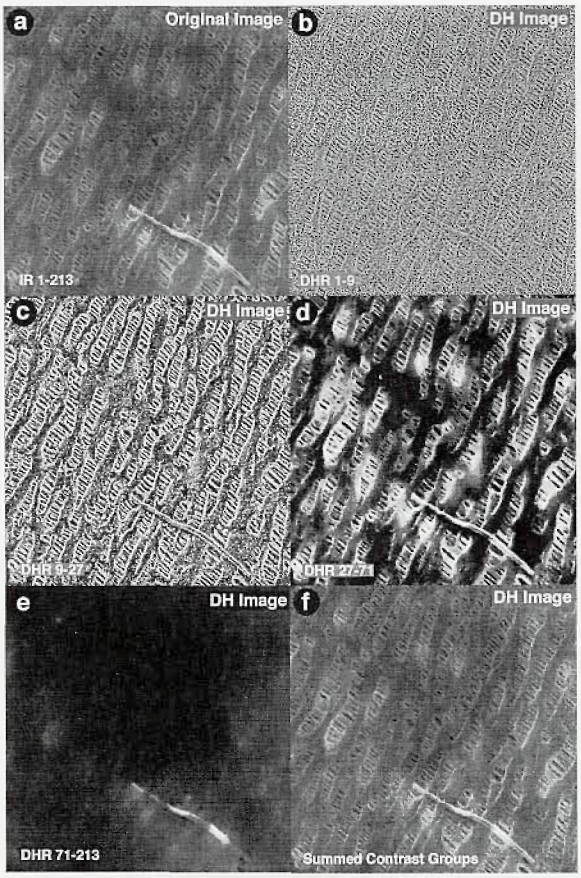
Revertible Mathematical Segmentation into Contrast Patterns

A mathematical definition of contrast groups became possible through differential hysteresis (DH) processing (Peters, MSA 1995:642-643; Scanning 1996: in press; URL http:panda.uchc.edu/htklaus/index.html). The raw data image can be interactively segmented into discrete contrast patterns which, in return, can be summed to generate the raw data set (see figure below). This revertible mathematical image treatment is distinct from conventional image processing since it applies to all images independent of size, depth, and content, and requires only one universal processing parameter, the differential hysteresis range (DHR). It defines the differential contrast range of an extracted pattern with two intensity values, i.e., contrasts larger than the first value and smaller than the second value. The ease of segmentation is demonstrated for a SEM image data set of a polymer filter which was acquired at 512x512x8-bit at 20 kV (tungsten) and 20,000X magnification from a specimen thinly coated with platinum. The raw data image (a, IR = intensity range) can be mathematically segmented by differential hysteresis processing into distinct contrast patterns of increasing DHR (b-e), and then again be reconstituted by arithmetic summation (f) of these patterns which each represent a different contrast component of the data. Distinct contrast patterns of this data set include the DH noise pattern (b), the DH contrast pattern of evenly collected SE produced in the Pt coating (c), the

https://doi.org/10.1017/S1551929500067523 Published online by Cambridge University Press

DH contrast pattern of charging contrasts (d, unevenly collected SE produced in the Pt), and the DH contrast pattern of charge-induced SE. (Raw data by courtesy of Judy Propst, Hoechst Celanese Corp., Charlotte, NC).

The imaging of interactively chosen contrast patterns provides a simple but effective tool for visual analysis of the image data. Pertinent spatial components may be found in certain contrast patterns which are solely defined by the microscopy, e.g., the SEM imaging example aimed at seeing the pore structures in a secondary electron contrast.

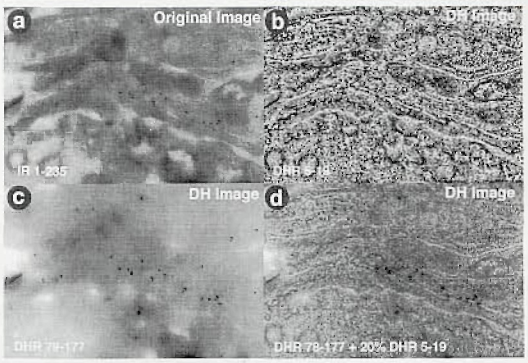


Contrast Patterns Present in a Digital SEM Image

The contrast was generated by elastic scattering of probe electrons in the small platinum grains of the coating and was found undisturbed from collection deficiencies at DHR 9-27. This demonstrators the superior capabilities of this objective mathematical imaging which has never before been provided by conventional image processing technologies. DH imaging allows data visualization as well as contrast quantification, and maintains the data character with regard to contrast resolution as well as spacial resolution since it is "pixel-accurate".

Imaging of selected Contrast Groups

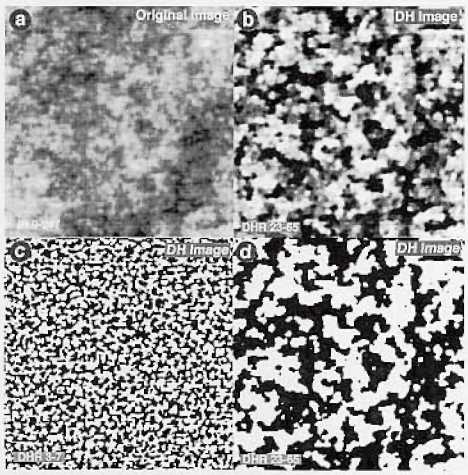
DH imaging provides a general tool for precision data handling. It extracts specific contrasts from an image data set and reassembles them at any desired proportion into a new image. This is especially important if pertinent image information cannot be visually perceived and recognized or if some information is obscured by other insignificant contrast patterns (e.g., gold labeling patterns in TEM cryo-sections) are often obscured by mass density contrasts from unevenness of the section or condensations of ice crystals (following figure: a). The important differential contrasts of this cryo-section were found in the contrast patterns of the cytoplasm (b) and of the label (c). Both can be combined for a clearer imaging of the labeling without loosing any precision of the data (d). (Data by courtesy of James O'Rourke, University of Connecticut Health Center, Farmington, CT). This selective imaging of pertinent contrast patterns provides a new tool for digital imaging that is essential for expansion of our limited visual perception and recognition capabilities to match the contrast resolution level of the microscopes.



TEM Cryo-Section Data Set Represented as Composite DH Image.

Quantification of Spatial Components

Quantification of image components is another essential part of microscopy. Conventional image processing and analysis provides many strategies for segmentation of images into discrete measurable components. However, these processing routines must be tediously adapted to each type of datum and do not allow artifact free reconstitution of segmented spatial components



AFM Image Set Reduced to Binary Contrast Patterns of Unit-Particles and their Aggregates.

(because of inherent spatial processing artifacts). Quantification is facilitated with DH contrast patterns because they are selected independently from the image content and the intensity level of the background, and because they maintain the maximum contrast of selected image components. When measuring in an AFM image of a silica sol/gel surface coating on polymer substrate (figure above, a) the size of unit particle (20 nm) and their aggregates (80-120 nm), the appropriate differential contrast patterns(b) imaged these components with highest possible spatial and contrast precision and without any interference from the very uneven background (data by courtesy of Rong T. Cheng, Hoechst Celanese Corp., Summit, NJ). DH imaging in binary display mode (c.d) facilitated an optimization of the "DHR for selected contrast information measurements with conventional software.

Summary

Contrast imaging is a new way of "seeing" microscope data. It provides intuitive access to the contrast functions of the microscope for objective "knowledge-based" image understanding. Working with contrast patterns offers multiple benefits, i.e., visualization and quantification. Since differential hysteresis processing is very computer intensive, massive parallel processing is required for real-time on-line imaging (JEOL USA, Inc., Peabody, MA: URL http://www.jeol.com/pixision/pixi.html; PiXISON Inc. Lakeville, CT: URL http://trunpike.net/emporium/P/Pixison/index.html). But, since computer technology is rapidly evolving, high volume computing becomes more affordable and now readily available to microscopy. Differential contrast imaging will soon become an essential tool in today's modern precision microscopy. ■

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