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One way to improve upon the detection limits for digital images involves increasing the number of counts per pixel. Eight bits, or 1 byte (256 counts), per pixel is most commonly used for monochrome optical, electron and x-ray imaging. This number is well matched to the human eye which can discern approximately 128 gray values, while photographic and some laser printer outputs have the capability of displaying nearly 256 gray values. When the counts exceed 8 bits/pixel the recorded image file size will likely double to 16 bits/pixel. With the better imaging detection limits we are faced with the technical problem of how to view more than 256 gray levels. One solution to both problems is to store and display the square root of the image intensity. While the compressed data cannot be reconstructed exactly to match the original numbers, it will be shown that no statistically significant data will be lost. This compression scheme reduces storage requirements by eliminating the statistical variations in the data. The square root display of the data into 256 levels also enhances low concentration visibility since the brightness change per gray level is greatest at low concentrations.

The time required to record an image with 334 X 180 picture elements (60,120) with a maximum of 255 counts/pixel at 20K cps is 12.8 minutes. For x-ray images this high count rate can only be achieved using wavelength dispersive spectrometers (WDS). With the limited counting rate and poorer P/B ratio of EDS detectors<sup>1</sup> x-ray images with comparable statistics would take more than 10 times longer. The examples in this article illustrate CaK $\alpha$  x-ray images of a silicate mineral section whose maximum calcium concentration is 10 wt%. The count rate from one of the PET crystals on our EPMA at 20 keV with a Faraday current of 100nA is 20K cps with a P/B of 202. For an 8 bit x-ray image the background intensity is 1, which is not statistically significant. With more counts the background becomes significant. The minimum detection limit (MDL<sup>2</sup>) for the 8 bit case under the conditions above is 0.13%, while

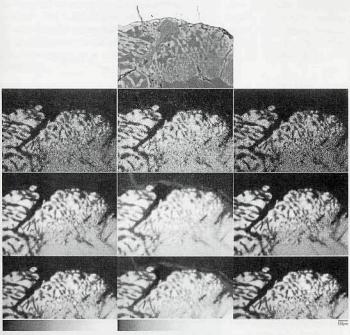


FIGURE: Top Center: Backscattered electron image of silicate mineral. Row 2: Left-CaKα x-ray image with a maximum of 8 counts/pixel. Center-Square root image. Right-Reconstruction.

Row 3: Left-CaKα x-ray image with a maximum of 255 counts/pixel. Center-Square root image. Right-Reconstruction.

Row 4: Left-CaKa x-ray image with a maximum of 12.7K counts/pixel. Center-Square root Image. Right-Reconstruction.

Bottom: Gray level representation. Left (and right)-linear. Center-square root

the concentration for each of 256 gray levels would be 10%/256, or 0.04%. As such, the gray levels 0-2 are not meaningful. Increasing the number of counts to 12.7K (considering the background) would result in a MDL of 0.021%. Using a linear quantizer<sup>3</sup> to compress the 12.7K to 256 levels would result in a loss of information since the MDL is about half of the concentration change per gray level.

It is not easy to see these lowest brightness levels in a linear display, especially if using photographic film where the resultant density from low exposure (toe region) has lower contrast than in the linear portion. Laser printers have a more linear response to exposure and show lower brightness levels with more contrast. The square root intensity display (a non-linear quantizer) can further enhance the contrast at low count at the expense of contrast in the higher levels since the change in lower counts rate has a larger brightness increase. For instance, the concentrations for the first several gray levels are: 0.000, 0.008, 0.032, and 0.071%. It is not until the 3rd gray level that the image with 12.7K counts reaches the MDL. We should therefore re-normalize the image to maximize the useful display brightness range.

The maximum data reconstruction error using the square root technique depends upon the # counts/pixel. At 225 counts there is no error since  $225^{0.5}$  is exactly 15. The integer of the root for 255 counts is 15. The error is  $255 \cdot 15^2$ , or 30 counts (12%). The 2 sigma error (assuming Poissonian statistics) is 2 \*  $225^{0.5}$ , or 30 counts. At higher count rates the error is reduced considerably. At 65,536 counts the reconstruction error is only 0.4%.

We may not wish to use 65,535 counts/pixel due to time considerations (in this paper we used 12,700). Collection time for this image was 11 hours. The data presented was acquired on a JEOL JXA-733 using "dPict", our Windows based digital imaging program that can store 9 images simultaneously. The images were printed on a Hewlett Packard Laserjet IV printer with an XLI "Laserpix" imaging board which has an effective resolution of 2400 dots/inch. The images are printed using a dot density of 106/inch. The illustrations were prepared for printing without using the normal "screen" that is placed over continuous tone photographs.

The image series with a maximum of 8 counts/pixel has been scaled by a factor of 30 such that 8 counts is bright. Notice that the reconstructed image appears identical to the original data. Note that the root image shows a noisy band of Ca in the upper center. This band can be seen better as the number of counts/pixel increases to 256, however, there is still some "noise" above the band. At 12,700 counts the noise is not evident and the band appears clearly. In the square root display the image clearly shows higher contrast in the low concentration areas, and lower contrast in the high concentration areas.

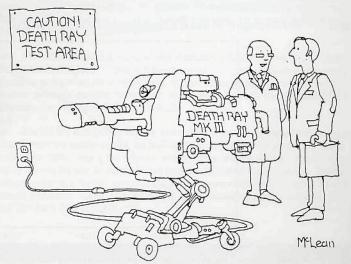
In summary, we have shown that it is possible to reduce the file space needed by a factor of 2 for images without sacrificing statistically significant data. By using a non-linear display function, such as the square root, we can enhance the contrast in low concentration levels at the expense of contrast at other concentrations.

J.D. Geller, Scanning Electron Microscopy/1977, Vol.1, 281.

2. T. Ziebold, Anal. Chem., 39(1967)28.

 R.C. Gonzalez and P. Wintz, *Digital image processing*, Reading, MA: Addison-Wesley Pub. (1977)234.

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A rapid staining method using Sudan IV and Oil Red O Staining fats with osmium tetroxide vapour

## FOOD MICROSCOPY

O. Flint, University of Leeds, U.K.

A practical guide to using optical microscopy for examining the microstructure of food products and obtaining information to complement chemical and physical analyses. The book covers a range of practical techniques with the emphasis on rapid methods and includes sufficient theoretical background information to understand the mechanisms involved. After reading this book, a microscopist should be able to select and modify the techniques to make them suitable for individual food products.

### - CONTENTS -

	Combined osmium and iodine vapour staining References	4
8	Food Starches	
•	Physical structure of commercial starches	
	Chemical properties of commercial starches	
	The effects of moist heat on starch	
	Starch gelatinization	
	The gelatinization process	
	The microscopy of chemically modified starches	
	Other modified starches	
	The microscopy of Turkish jellies	
	Starch in baked products	
	The microstructure of baked products	
	Preparation of baked foods for the microscope	
	Further reading	
9	Meat, Fish and their Products	
	Meat	
	Structure of skeletal muscle	
	The skeletal muscle fibres of meat	
	Preparation of wholemounts of striated muscle	
	fibres from meat	
	Cryosections of meat	
	Connective tissues associated with meat	
	Fat	
	Skin and rind in meat products	
	Tendons	
	Elastic tissue and ligamentum nuchae	
	Starch constituents of meat products	
	Experiments in sausage microscopy using simple	
	equipment	
	Materials needed	
	Apparatus	
	Experimental technique	
	Fish and fish products	
	Fish muscle tissue	
	Fat is fish	
	Fish skin	
	Fish scales	1
	"Crustacean fish products"	
	References	
	Further reading	
10	Vegetable Proteins	
	Introduction	
	The soya bean and soya products	
	Processed soya beans	
	Microstructure of soya grits and flours	
	Microstructure of soya protein isolate	1
	Textured vegetable protein (TVP)	
	Microstructur of TVP	
	Wheat protein	
	Microstructure of wheat gluten powder	(
	Mycoprotein	9
	Microstructure of Quorn	
	References	
	Further reading	(

11 The Howard Mould Count of Tomato Products Introduction The Howard mould count and its limitations Tomato histology Skin cells Flesh cells Fibrovascular tissue Seed "hairs" Mould histology Parallel filament walls Branching of filaments Septation Granulation Practical mould counting Preparing the sample for the Howard cell Mounting the sample Counting technique Mould count tolerances References 12 Food Gums Introduction Reactions of gums with toluidine blue Reactions of gums with iodine Birefringence of stained bums The identification of commercial food gums Reagents needed Toluidine staining of hydrated gums Method for pectin, gum arabic and pre-gelled starches Toludine blue staining of acidified gums lodine staining of gums Identification of powdered gum mixtures Identification of gums in food products Dry products Moist products Further reading 13 Food Emulsions Introduction Microscopy of food emulsion constituents Solid fat in butter, margarines and low-fat spreads Liquid fat and comparison of o/w and w/o emulsions Identification of the stabilizer in a low-calorie salad cream and a low-fat spread Emulsion microstructure Further reading Appendix Equipment Materials Order From Microscopy Today:

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