

Some Reflections On How Much Information Is There On A Piece Of Film, On How Film Compares With CCD Cameras And What Features A Scanner Would Need To Digitize TEM Negatives.

A personal point of view by Alwyn Eades, Lehigh University.

Introduction

The world of electron microscopy is in a period of transition from acquiring images on film to acquiring images digitally, using CCD cameras, for example. It would be useful to know how much information there is on a piece of film, in order to know how film compares with digital methods and to be able to make good judgements on the optimum moment to change from one technology to the other.

This is an attempt to use simple arguments to estimate just how much information there is in an image exposed on film in the transmission electron microscope. The main reason for addressing this issue is that, while many people are affected by it, there seems to be little agreement on the answer.

Background

When photographic film is exposed to high-energy electrons, it behaves quite differently from when it is exposed to light. Although this has long been known, the debate on film versus CCD does not always take it properly into account. There is a good review on the exposure of film to electrons by Valentine (1966). Despite its age, this still seems to be the best introduction to the subject. There is also valuable information in a paper by Zeitler (1992) although he draws heavily on work that predates Valentine's review.

Measurements on film are made in terms of the optical density. If the transmission of the film is T (expressed as a fraction of the incident intensity), then the optical density is defined as:

$$D = \log_{10}(1/T)$$

This needs two qualifications. First, the transmission T is usually measured, not with respect to no film present, but with respect to a piece of completely unexposed film. Second, the optical density is not a well defined quantity since the transmission depends on the details of the measurement (in particular, how much of the diffusely scattered light is included).

When an electron hits the emulsion of the film, it renders many grains developable. Since the electron is scattered in the emulsion these grains are spread about the initial point of impact. The region exposed by each electron (on the order of $10 \mu\text{m}$) is much larger than the size of the individual grains (on the order of $1 \mu\text{m}$).

The relation between the optical density and the exposure of the film is:

$$D = D_{\text{MAX}}(1 - \exp(-sd/D_{\text{MAX}}))$$

Where D_{MAX} is the saturation optical density, s is the speed of the film and d is the exposure (or dose) in electrons per unit area (usually electrons per micron²). The initial part of this relation between dose and optical density is linear and the speed is just the slope of this linear part of the curve.

$$s = dD/dd \text{ at low dose.}$$

Speed is usually measured in units of μm^2 per electron. Typical values for electron microscope film are about 3 for D_{MAX} and about 1 for s . There is significant variation from film to film and the values will depend on processing. This means that, in the middle of the exposure range, a typical dose is a few electrons per μm^2 (the dose at the film, not the dose at the sample).

Calculating the Information

The amount of information, which can be carried by the medium used to acquire an image, can be calculated by multiplying the number of pixels by the number of bits at each pixel. For a CCD camera this is easy to calculate. The number of pixels is defined by the manufacturer and the number of bits (b) is derived from the number of distinguishable grey levels (g):

$$b = \log_2 g$$

The number of grey levels is, in turn, determined from the dynamic range and the noise. The information content calculated this way is the information limit of the CCD chip. In practice it will be reduced by the other elements of the system.

Information on the film

In making the corresponding estimate of the information which can be carried by a piece of film, the problem is that there is no obvious definition of the pixel size. If the film is divided into little squares and each square is treated as a pixel, the result obtained for the information content depends on the size of the square. A larger square can have more distinguishable grey levels, and hence more bits, but the number of bits increases more slowly than the reduction in the number of squares. Therefore the amount of information estimated gets bigger as the size of the square is reduced. The maximum amount of information would appear to be estimated by finding the smallest area which can have two distinguishable grey levels (one bit) and treating that as the pixel. However the size of the square which can give two grey levels is well below the size of the region exposed by a single electron.

The logical choice of the "pixel" size in estimating the information carrying power of film is, therefore, related to the point spread function of the electrons in the emulsion. A proper treatment would model the shape of the point spread function and do a detailed calculation involving the modulation transfer function as a function of the spatial frequencies in the image (much of the basis for such a calculation is given in Zeitler, 1992). This note will make a much simpler approximation.

Using Film

The resolution of the unaided eye is about $100 \mu\text{m}$. If focussing were done

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looking at the final screen of the microscope, the image on the film would rarely have information below 100 μm , which would require a pixel of 50 μm . However, focussing is usually done through a binocular with a magnification of perhaps 10 X or, increasingly, with a TV system. The limit of resolution might be taken to be 10 μm for a binocular system and 25 μm for a TV system (the typical size of a pixel on the CCD chip). My own experience suggests that binocular focussing does not go below 25 μm - it is rare for a negative to have information which cannot be seen with a 4X loupe.

This conclusion may not apply in certain circumstances. For example, at high voltages the point spread function is reduced and biologists can often focus using minimum contrast so that the film records detail below what the eye can see. Jim Pawley tells me that, under these conditions, the "pixel" size can be as small as 6 μm . However, I have the impression that most of the time microscopists work at magnifications which are higher than those needed theoretically. I am tolerably sure that most images taken in materials science (which is what I know) do not have useful information below 25 μm (a spatial frequency of 50 μm). For example, in high resolution imaging, at a magnification of 400,000X and an instrument which can resolve 0.2 nm, a pixel size of 40 μm would match the information limit.

Digitizing Film

If images on film are digitized, the performance of the scanner is usually quoted as dpi - dots per inch. This translates to pixel size as follows:

dpi	Pixel Size in Microns
600	42
1200	21
5080	5

This suggests that a 1200 dpi scanner is perfectly adequate for (most) transmission electron microscopy negatives.

Pixel Size

The practical argument just given suggests the use of a 25 μm pixel size. This is also close to a typical value for the point spread function of the film. While it is true that information can be carried by the film at spacings below the characteristic length of the point spread function (because the function is cusped), this length is a reasonable place to set the pixel size.

Grey Levels

Film exposed to electrons does not generally add significant noise to the image. The noise comes from the variation in the number of electrons - shot noise. This is the only significant contribution to the noise except for the small variations in the transparency in the unexposed film, which are only relevant at very low exposures. Take the number of electrons in a given pixel to be N. Then the number needed to get a distinguishable grey level can be taken to be

$$N - N^{1/2}$$

This can be used to get the relation between the number of electrons in a pixel and the number of distinguishable Grey levels.

Maximum Number Of Electrons	Distinguishable Grey Levels	Bits
2	2	1
7	4	2
25	8	3
89	16	4
319	32	5
1178	64	6

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In a pixel of side 25 μm , the area is 625 μm^2 and the number of electrons at optical density 2 (taken as a convenient practical limit) is about

2000/s typically about 2000.

A factor of two in the speed of the film will not affect the result significantly. There will still be 6 bits per pixel. A piece of film is 9 x 8 cm. This corresponds to 10^7 pixels and a total information content of 6×10^7 bits (8×10^6 bytes).

The effect of Contrast.

The extent to which a recording medium can handle a particular image depends on the contrast of the image. Suppose that we need to detect a pixel which shows a contrast of C % with respect to its neighbors. If the number of electrons which gave rise to the signal in that pixel is n, then this must differ from the neighboring pixels by dn. Where $dn/n = C/100$. The difference, dn, must be greater than the noise for detection, so

$$dn > n^{1/2} \quad \text{thus:}$$

$$C > 100/n^{1/2}$$

This means that to see contrast at the 1% level in an image the pixels must receive about 10,000 electrons each. But for 10% contrast 100 electrons per pixel are sufficient. In the typical fibre-optically coupled CCD camera the number of electrons required to saturate the device is in the range 1500 to 8000 (Krivanek and Mooney 1993). This means that, if the exposure is chosen to almost saturate the chip, this kind of camera can work at contrast levels of

2.5% to 1% at the resolution of the pixels, depending on the design of the camera. If a camera designed to saturate at 1500 electrons per pixel were used to make an image where the contrast was only 1 %, the effective pixel size would be increased to several times the actual pixel size and the effective number of pixels on the chip correspondingly reduced.

The case of film is similar. The "pixel" size from considerations given above is about 25 μm . With this pixel size, a film speed of 1 and a saturation density of 3, the number of electrons per pixel to reach a density fairly close to saturation is about 4000 – corresponding to imaging at contrast levels of 1.5%. In practice, film is usually exposed to levels far below saturation. Then the contrast levels which can be distinguished in a 25 μm pixel will be correspondingly higher.

In materials science samples, the contrast is usually strong and these considerations will not normally limit the performance of the recording medium. In the case of biomedical samples, where contrast is weaker, they may be relevant.

Digitization and information.

As indicated above, the number of electrons per pixel to saturate the medium is on the order of several thousand whether we are talking about film or CCD. If the digitization is on a linear scale, as it will be for the CCD - it is necessary to digitize to a precision of one electron. This is because the grey levels are separated by one or two electrons for the lowest exposures. This means that the digitization must be done to at least 12 bits, even though there are less than 8 bits of information present. The discrepancy is because, at the higher exposure, the spacing between the grey levels is much bigger (measured in electrons).

For film the situation is similar although the discrepancy between the number of digitization levels required and the number of bits of information present is much less. This is because the scanner which digitizes the film measures transmission not number of electrons.

Conclusions

For film and CCD cameras the pixel sizes are much the same - 25 μm . For a typical CCD camera in which each electron in the beam generates about 100 electrons in the CCD, the dynamic range, the number of grey levels and the number of electrons to saturate a pixel are all about the same for film as for the CCD camera. The ratio of the amount of information in the two will then be the ratio of the areas.

What then is the advantage of CCD? Linearity, better vacuum, convenience, feedback (i.e., the FFT for a HREM alignment check is immediate and knowing that the recorded image is what is wanted without delay)...

Many of us will continue to be forced to use film for some time to come because of the cost of CCD cameras. A good solution for this period seems to be to use film in the TEM but to digitize the negatives with a scanner for subsequent processing. Scanners for digitizing TEM film should digitize at about 1200 dpi, they should digitize at, at least, 12 bits and they should be able to handle an optical density of at least 3. ■

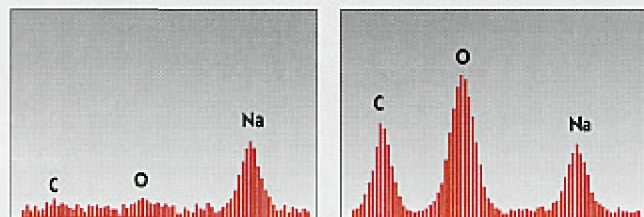
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Note: The above article was first published in the January/February 1966 issue of this publication. On reading it through prior to this reprinting, there is one thing I would modify. In specifying a scanner for TEM negatives, I would be more demanding than in the original article. I believe I was a little too conservative in my estimates of pixel size for film and a little too optimistic about just how a scanner specification translates to practical digitization. We have found that TEM negatives can have detail which does not show on the images scanned on a scanner with nominal 1200 dpi. To be able to scan TEM negatives and get all the detail from all the negatives, a better scanner is needed.

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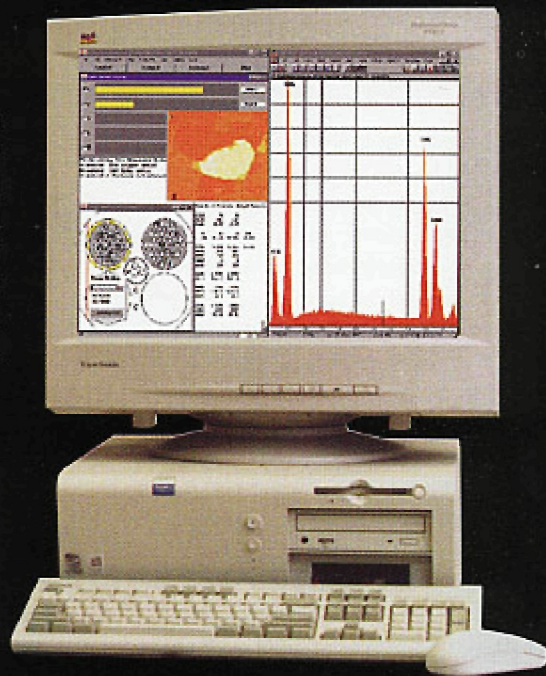
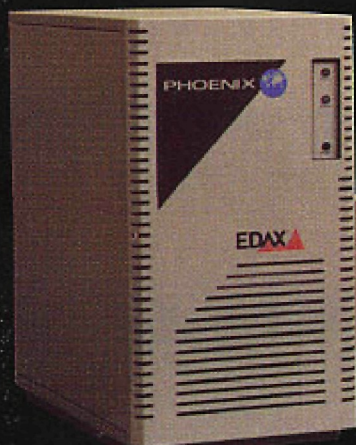
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