ADAPTIVE ENHANCEMENT OF PERCEIVED CONTRAST IN DIFFUSE IMAGES;

CASE STUDY: S.E.M. ELECTRON MICROSCOPE IMAGES

Harvey A. Cohen and David Suter

Computer Science, La Trobe University,
Bundoora, Melbourne, Victoria, Australia 3083

This paper is concerned with the use of various forms of adaptive histogram equalization (a.h.e.), also known as local area histogram equalization (l.a.h.e.), and as rank filtering, as the method of merit for the enhancement of biological images. The particular images studied here were generated by a scanning electron microscope (S.E.M.) and involved specimens, which were flash frozen, leading to low contrast diffuse images, but for which the structural features were well-known, from S.E.M. examination of normally prepared specimens. A comparative study was made of filtering over various sized windows, and various versions of contrast-limited ("clipped") l.a.h.e. This study complements studies made by Pizer and co-workers on the application of variants of 1.a.h.e. on medical X-rays and tomographic images. The paper includes a full discussion of implementation issues.

Introduction

This study extends the work initiated by Pizer and co-workers [1][2][3][4][5] and extended by Dhawan et al [6][7] (see also [11]) in which various X-ray and tomographic images were transformed by a.h.e. to enhance the diagnostic skill of radiologists, especially in determination of small malignant features and unusual conditions. The particular images studied here were generated by a scanning electron microscope (S.E.M.) and involved specimens whose structural features were well-known, as determined by S.E.M. examination of normally prepared specimens. The specimens used in the S.E.M. were flash-frozen, [8][9] so as to avoid any chemical change, being intended for use in (local) elemental analysis for the study of *transport* between cells. As in the mammograms studied by Dhawan et al such images have significant average density variations. These S.E.M. images were very diffuse due to electron scattering from the prevalent frozen water. It is a general feature of biological images that features of interest are often emersed within background clutter. The study thus was directed at a new class of biological image of significant interest.

Introduction

Digital image enhancement is the process of making a digitised image more amenable to interpretation, that is, for human interpretation, though similar enhancing is required in automated interpretation (see Powley [12]-[14]) Any such interpretation is primarily a segmentation of the image into regions primarily of moderately varying grey-scale. In natural scenes the human viewer can easily see some regions as distinct, despite continuous grey scale variation, by virtue both of contrast from surroundings and by comparison with largely scale illumination gradients. For instance a person standing partly in shadow is perceived as a single entity despite major variations in illumination between shaded/unshaded regions.

A digital image may not be well tuned for human interpretation. Firstly, the human viewer can not readily discriminate between closely spaced grey-scale levels, so that an image of low contrast is difficult interpret. Nor can the human visual system respond well to grey-scale levels that are extremely low or high, due to the limited dynamic range of the human visual system. Secondly, where a digital image has substantial variation in local statistics, the human system of compensation may lead to irrelevant inter-pretations in terms of depth and lighting. To aid interpretation, grey scale levels must be altered to enhance the contrast between adjoining regions. A third characteristic defect in images is noise. In discussing an enhancement procedure its susceptibility to noise is of vital significance.

Traditional means of enhancing contrast in digital images involve global transformations of the entire picture. These methods prove inadequate where there is significant variation in image statistics across the image.

Adaptive image processing involves the application of image transforms to an image that adapt to the local image statistics in a small region about each pixel.

The plan of this paper is to first explain adaptive histogram equalization, its problem with speckle adjacent to extensive uniform area. We introduce variants of adaptive histogram equalization (a.h.e.), termed contrast limited' or 'clipped' Such filters involve in their application some variation in window size and clipping number utilised is described. In the second section the nature of the S.E.M. images involved is described and images before and after enhancement are presented. These images, of oblique sections of the secretary tubule in the nasal gland of a duck, involve unusual preparations modes, but the nature of the structures actually present is well known, and is indicated via a sketch of detail suitable for the potential readers of this paper. Effect of variation of clipping number is indicated, as well as the use of regional approximations to such a.h.e.

The final section of the paper discusses the computational issues involved.

Adaptive Histogram Equalization

The global statistics of a digital image is summarised in the image histogram, which plots the number of pixels with grey scale q, h(q), against q.

Where the range of pixel values is shorter than the range of available pixel values, a simple scaling and sliding of pixel grey scales, termed histogram stretching, will increase contrast.

Because of the eye's poor ability to discriminate small grey scale differences, it is preferable if grey scale values are distributed across the available range. Insofar as the eye's response is approximately logarithmic, grey scale levels with logarithmic spacing would be appropriate to enable the eye to equally
distinguish all grey-scale graduations.

The classic method algorithm for global contrast enhancement is histogram equalization. The transformation is a mapping of grey scales, from grey scale g to grey scale p. The mapping is dependent on H(g) the cumulative histogram, which is the total number of pixels in the whole image with a grey scale less than or equal to g. For every pixel of grey scale g the new grey scale is

$$p = \frac{g_{max}\, H(g)}{p_{max}}$$

After histogram equalization, the cumulative histogram becomes linear in p in the continuous limit.

Standard texts give impressive examples of the utility of histogram equalization. However, whereas histogram equalization gives reasonable enhancement for an image such as Fig (iia), no satisfactory image is derived from images such as Fig(iiia) or Fig(iva).

The notable defect of histogram equalization is that it tends to over-emphasise noise spikes, and is also prone to produce similar speckles, where none existed in the original, in regions of one predominant gray scale.

Adaptive Histogram Equalization Variants

Adaptive histogram equalization, a.h.e., (Pizer 1982) involves applying histogram equalization to a pixel by performing histogram equalization over a rectangular region centred on that pixel; region sizes of 16* 16 or 32*32 are typical. It is computationally expensive, as this operation has to be performed for each pixel of the image, apart from the boundary pixels. Inherently pixels of the same grey scale will be mapped onto a similar grey scale if close together, but far apart pixels of the same grey scale can be mapped onto very different grey scales. Adaptive histogram equalization is also termed *rank* filtering. This term is most readily appreciated in the special case where the local region centred on a pixel is 16* 16, and the grey scale range is 0 to 255. In this case, adaptive histogram equalization amounts to the determination of the rank of the pixel within that 256 pixel neighbourhood, and replacing its grey scale by that rank. In general, the transformed grey scale is proportional to its rank.

Unfortunately, a.h.e. inherits one of the defects of ordinary histogram equalization: if a large number of neighbouring pixels have a particular grey scale then adjoining grey-scale values on either side will be mapped into vastly differing grey-scale values. The solution to this problem, proposed by Pizer et al (1986), is to 'clip' peaks in the histogram, to distribute the surplus numbers through the histogram uniformly, and to use the histogram so produced with the usual adaptive histogram algorithm. This 'clipping' limits the contrast between adjoining regions, so the procure is most appropriately termed 'contrast limited' a.h.e.

In mathematical detail, in adaptive histogram equalization, the grey scale mapping of g is to p given by

$$p = g_{max}\frac{H(g)}{H(g_{max})}$$

where H(g) is the cumulative histogram corresponding to the histogram value h(g) integrated from g=0 to g. The total range of allowed g values is from 0 to g_{max} In 'clipped' histogram equalization, the histogram function h(g) is clipped so that it never exceeds value h_{max}.. The clipped histogram function is termed hclip(g)

Note that if $h(g) < h_{max}$, $h_{clip}(g) = h(g)$, and the maximum value of $h_{clip}(g)$ is h_{max}. After clipping, there will be $m(h_{max})$ pixels that will need to be redistributed. Distributing these clipped pixels uniformaly gives a new histogram function

$$h_{ca}(g) = h_{clip}(g) + \frac{m(h_{max})}{g_{max}}$$

with corresponding cumulative histogram $H_{ca}(g)$. The clipped adaptive histogram algorithm involves mapping the grey scale g into the cumulative histogram $H_{ca}(g)$.

$$p = g_{max} * \frac{H_{ca}(g)}{H_{ca}(g_{max})}$$

The point to be appreciated is that the clipping has eliminated oversized jumps of the cumulative histogram.

Electron microscope images

The research described in this paper describes the application of adaptive filters to the enhancement of scanning electroscope images of oblique sections of the secretary tubule in the nasal gland of a duck. Using conventional metal staining techniques the structure is known. Such a conventional chemical treatment changes somewhat the chemical constitution of the specimen. In of to perform an X-Ray microanalyis of diffusible elements a different specimen preparation technique is essential. The method used, freeze-substitution [8],[9] involves rapid freezing followed by substitution of a resin for frozen water. Scattering of electrons in a scanning electron microscope (S.E.M.) depends on atomic number of scattering nuclei. Hence after this preparation there is little contrast between the protein structures and the enveloping resin. Because an intense electron beam will destroy a freeze substituted specimen, beam current is limited. Accordingly, a temporal filter, the Australian designed ARLUNYA TF 4110,

[10] is used to provide what is essentially a time-averaged micrograph. In this paper we deal exclusively with the enhancement of such images produced in a JOEL JEM 1 200EX scanning transmission electron microscope with video output to an ARLUNYA temporal filter, with actual digital image captured by an IMAGING TECHNOLOGY PCVision framegrabber in an IBM XT clone. The framegrabber digitises the image to 6 bits, 512 pixels per line of the 60Hz image.

Implementation Issues

To perform rank filtering within a window containing W pixels, the centre pixel must be compared with the other W-1 pixels, and a count kept of the number of times the centre pixel is larger. That is, despite our use of the cumulative histogram in explaining adaptive histogram equalization, no histogram data needs to be collected to determine the rank, and hence the gray scale, of the output pixel. The computational cost is thus largely the cost of fetching from memory each pixel in the window to the processor. For a window of 256 pixels, this involves 256 memory accesses.

In contrast, to perform clipped a.h.e. Inherently requires the production of a histogram for the window involved. To simplify discussion, suppose there are 256 grey scale values, and also 256 pixels within the window. To compute a histogram within a window, the histogram data must first be set to zero, in 256 accesses. Then each pixel within the window must be accessed (256 accesses), and the pixel value used as a pointer offset to increment the corresponding count. Note that to perform the incrementation, the current value must be loaded into the processor, and then stored, in all, 512 accesses. To produce the cumulative histogram within that window, a single sweep read of the histogram is needed followed by a write to the cumulative histogram, in all 512 accesses. Thus in all 1536 memory accesses need to be performed for cumulative histogram preparation. Clipped a.h.e. will require further computations to yield the output grey scale - a sixfold increase over rank filtering. For the images discussed, the application of clipped adaptive histogram equalization on 512*512 8-bit image using 33*33 mask took approximately 60 minutes of elapsed time on a Pyramid MX-90 mainframe. The program written in C was essentially as described above. However, significant savings could be achieved by noting that when a window slides

just one pixel, the histogram needs to altered by dropping 16 pixels (48 accesses) and adding 16 pixels to the histogram (another 48 pixels). Hence the number of accesses to produce the cumulative histogram for the next position by this recursive strategy is just 96 + 512 = 608.

Pfizer has suggested [1] that rather than use the exact clipped a.h.e. operation, to perform a useful approximation. Pizer examined the results of computing the clipped cumulative histogram at only 16 representative points in the image, and to use at each pixel a linear interpolation to these histograms. For a 512*512 image the number of clipped histograms computed is reduced from 256K to 16. Pizer [2] reports a 60-fold speed-up for the interpolative technique, with output not markedly different from that of true clipped a.h.e. Our general conclusion is that after the determination of appropriate filter size and clipping number for a class of images, clipped a.h.e. has been shown to be a powerful method for contrast enhancement. Its widespread application depends on the availability of specialised hardware, or the validation of reasonable and fast interpolative approximations.

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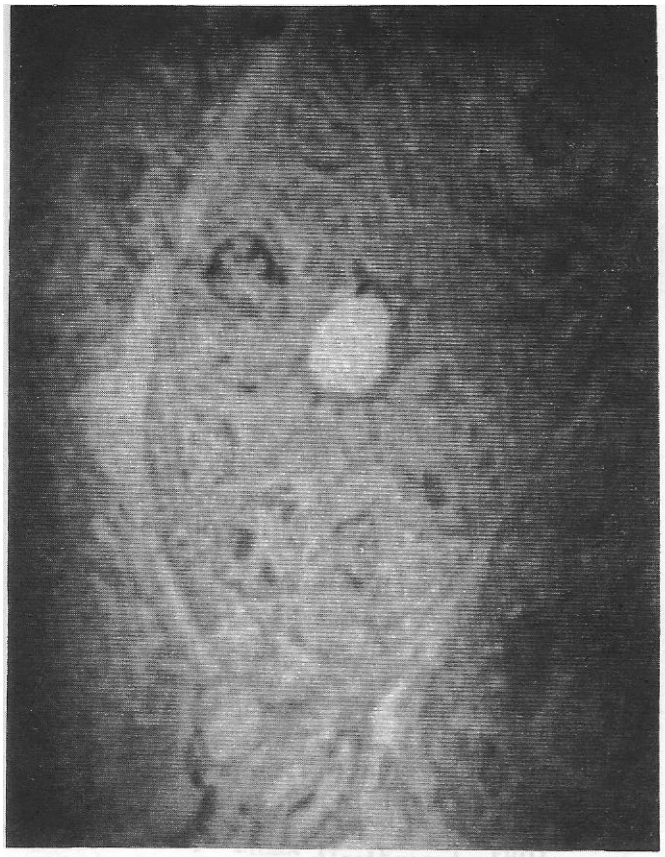

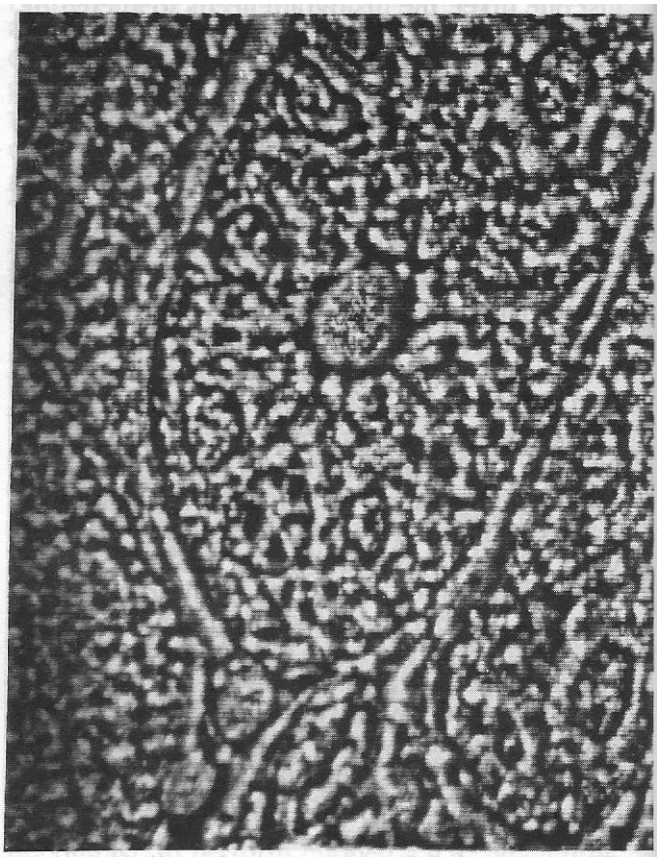

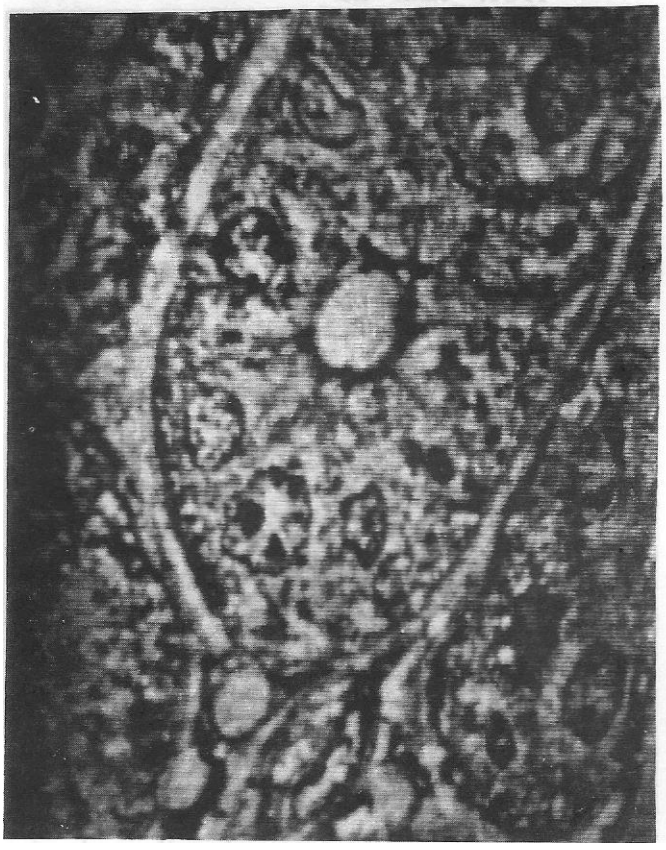

Figure 1: Top left, oblique section through secretary tubule in the nasal gland of a duck., a S.E.M. image of a frozen section. The section includes the tubule perimeter, and some exterior connective tissue. The region between the round near central lumen and the perimeter is demarcated by radial cell membranes into cells, each of which has a speckled nucleus.

The original is diffuse, lacks contrast, and suffers from local variation of average gray scale.

Above right. Rank filtered image, 16* 16 region. Note the significant enhancement of features marred by speckle.

Bottom left, after application of clipper a.h.e. on 33*33 region, clipped at 100. Marked clarity of features.