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BASIC PRINCIPLES OF IMAGE ANALYSIS BY A COMPUTER

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ABSTRACT

This article reviews the main principles of image analysis by a computer. The picture is stored in computer memory as a collection of picture elements (pixels), each located in 2-dimensional space and characterized by a grey value. A histogram of grey levels (grey level histogram) can be calculated by the computer and applied in thresholding. Other operations described include background noise smoothing, boundary enhancement, erosion, dilatation, and size distribution analysis.

Numerous applications have been found for computerized image analysis. These include automatic reading of written text, analysis of X-ray images, analysis of ultrasound images, analysis of remote sensing satellite images, robotic vision and analysis of photographic images of all kinds. Histopathologists are just learning to live with the analysis of microscope images. Under pattern analysis we also place the analysis of patterns obtained by any measuring device, e.g. those produced by electrophysiological processes, sounds, voice and even spoken language; in fact a pattern may be defined as any set of measurements or signals with characteristic relationships between them. The pattern produced by the results of various laboratory tests can also be analysed for diagnostic purposes and the patterns of tests with reference to disturbance variables can be analysed by pattern recognition methods. Much has been published in the last ten years on image and pattern analysis and recognition. A few recent papers are listed as references (Preston and Onoe 1976, Oja and Simula 1981, Proc ISMII 1982, Proc ICPR 1982, Serra 1982, Johansen and Becker 1983). We also refer to research into computer-assisted diagnostic histopathology and cytopathology (Bartels et al. 1972, Stenkvist et al. 1979, Baak et al. 1982, Simon et al. 1980, Kunze et al 1978, Preston 1980, Gamel et al. 1982, Goerttler and Stöhr 1982).

In this paper we concentrate on the basic principles of computerized visual image processing with emphasis on applications in medical morphometry.

USES OF IMAGE ANALYSIS

Biomedical image analysis has several uses:

- enhancement of image features e.g. grey tone balancing, detection of boundaries of objects

- detection of useful features, e.g. areas with special color or grey tone, lines with certain orientation
- data compression in storing the image in computer memory
- measurement of interesting features, e.g. areas, line lengths, perimeter lengths, morphometric measurements

A PICTURE IN COMPUTER MEMORY

The objects in our environment are extremely complicated; at least in theory detection of finer and finer detail by using instruments with better and better resolution (eye, hand lens, microscope, electron microscope, X-ray analysis) to atomic particle level is possible, although one cannot imagine such reproduction in visual images. Also in the images there are the smallest resolvable units - in the retina these are determined by the size of rods and cones and neural circuits linked with these, in newspapers by the density of screen used by the printer. Computer analysis of visual images is based on digitization of the picture into a set of discrete picture elements (pixels, or pels), resembling a half tone newspaper picture. In computer memory each pixel is located by two coordinates which determine the position of the pixel in the (2-dimensional) image and represented by the grey level (darkness or brightness) of the pixel. The above applies for black and white images. If colour images are analysed 3 intensity levels need be stored (one each for green, red, and blue). In the following we consider black and white images only.

To the computer a black and white picture is thus a collection of numbers, which for this presentation can be described as follows (see Fig. 1):

p (1,1), p (1,2), p (1,3) ..... p (1,m), p (2,1) .....
p (n,m)

where p presents the grey level and the location coordinates are given in the parentheses.

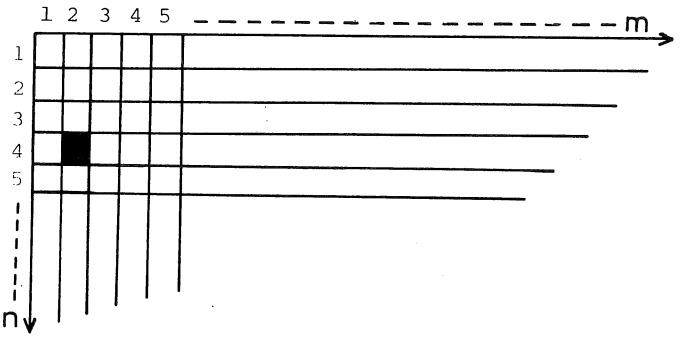


Fig. 1. Principle of image presentation in the computer. The pixel stained black could be p (4,2) in computer memory because it is the second pixel of the 4th row. p is the grey level of the pixel. The screen image contains altogether n times m pixels.

The number of grey levels for each pixel can be determined by the program used. In general these are powers of 2:

$2^3 = 8$; $2^4 = 16$; $2^5 = 32$; ... $2^8 = 256$

For example: In one system the number of grey levels is $2^5 = 32$. The grey level of a single pixel is one of the numbers

0, 1, 2, 3, 4 ..... 31.

In the computer these are presented as binary digits:

Grey level	Corresponding binary number
0	00000
1	00001
2	00010
3	00011
4	00100
5	00101
6	00110
7	00111
8	01000
.	
10	01010
.	
.	
.	
31	11111

In a computer each picture needs lots of memory space. If we have a picture screen with 256 x 256 = 65536 pixels and each pixel needs 8 bits (which is the same as a byte) - as presented e.g. by the digit 00110101, it takes 65536 bytes or 64 kilobytes (kB) to store the picture in the computer memory (1 kilobyte = 1024 bytes). In microprocessors there are variable amounts of memory. The smallest personal computers have 1 kB of random access memory (RAM) suitable for picture storage. Such computers are not suited for image analysis (and are not intended for that purpose either). Larger personal computers have memories of 64 kB. In principle one could store one picture of the above kind in such a memory, but nothing else. So computers for image analysis need larger memories to store several pictures at a time.

Thus, within the computer the image is presented by a matrix or array of numbers. Individual pixels are presented in the form of numbers of type p (i,j)

where i attains values 1,2...m (e.g. m = 256) and
j attains values 1,2...n (e.g. n = 256)
and p itself attains values 0,1 ... r-1 (e.g. r = 32).

Usually the image is input to the computer with the help of an "optical scanning digitizer" which may be, e.g., a digitizing TV-camera or a micro-densitometer connected to a scanning device.

The digitized image, made of pixels, is output on a graphic terminal or an ordinary terminal. It can also be plotted by a printer.

GREY LEVEL HISTOGRAM

A grey level histogram is a histogram which gives the number of pixels of each grey level in a picture. This can be calculated from the picture as a whole, or along a row of pixels in the picture. The grey level histogram is used to extract relevant objects from an image.

At each grey level the computer counts the number of pixels with that grey level and the resulting number determines the height of the histogram at that grey level. Histograms are not usually uniform, but there are maximum values (peaks) and minimum values (valleys) between them. The histogram is easily computed and plotted by the computer on a terminal.

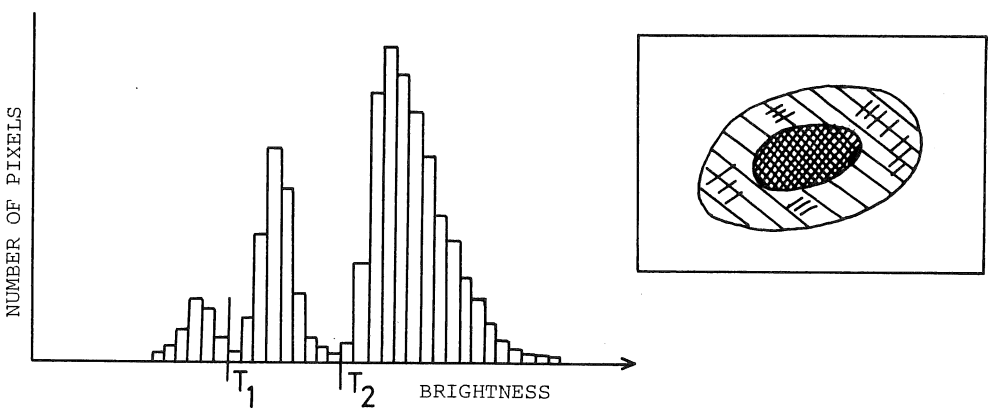


Fig. 2 Grey level histogram of an image of a cell (right).

THRESHOLDING

Thresholding of the grey level histogram is used to divide the picture into parts or segments, each having an approximately uniform brightness. The minimum values (valleys) are well suited to being used as threshold values. We refer to Fig. 2. T_1 and T_2 are such threshold values. Thresholding means that e.g. if a pixel p (i,j) is lighter than T_2, i.e. p (i,j) > T_2, the pixel p (i,j) belongs to a certain segment of the image that may be called the "background".

Further in the histogram of Fig. 2:

if $T_1 < p\ (i,j) \leq T_2$, the pixel belongs to "cell cytoplasm"

and if $p\ (i,j) \leq T_1$, the pixel belongs to the "nucleus"

The program user may set the threshold values as he likes. Through thresholding one can visualize the cell better and it is also possible for the computer to calculate the area of the nucleus in the image. The area is the number of the pixels with p (i,j) $\leq T_1$, times the area of a pixel. In histopathology relative values (nucleus versus cytoplasm) may be valuable and are now easily calculated.

Histograms are also used to help in visualization. This is done through equalization or balancing the grey levels so that the range of the grey levels in the histogram is better suited to human visual appreciation.

BACKGROUND NOISE REMOVAL BY SMOOTHING

Often the pictures contain artefacts introduced by the inaccuracy of the measuring device, analog-to-digital conversion, etc. This means that the grey values p (i,j) of the pixels are not exact but contain a random error. This error can be largely removed by smoothing.

When the computer operates with the grey level histogram, as explained above, each pixel is handled irrespective of the grey levels of its surroundings. On the other hand, when background noise smoothing takes place the computer considers local neighbourhoods or groups of pixels, such as groups of 9 pixels (3 x 3).

Take, for example, one such neighbourhood of the picture

0	0	1
1	7	2
2	0	1

in which digits 0-7 are grey levels. In the above situation the high value of the most central pixel could be a background noise artefact.

Smoothing takes place with the help of smoothing filters. They are arrays of 3 x 3 weight values such as

1	2	1
2	4	2
1	2	1

These are so-called convolution filters. What happens in the computer is that the computer takes a group of 9 pixels at a time and applies the smoothing filter to it. To simplify, if the centermost pixel is p_5 = p (i,j), where i and j are the row and column in which the pixel is located, the other pixels can be numbered as shown below:

p_1 = p (i-1, j-1)	p_2 = p (i,j-1)	p_3 = p (i+1, j-1)
p_4 = p (i-1, j)	p_5 = p (i,j)	p_6 = p (i+1, j)
p_7 = p (i-1, j+1)	p_8 = p (i,j+1)	p_9 = p (i+1, j+1)

The computer now gives a new filtered value to the centermost pixel which is dependent on the neighbouring pixels in a way determined by the filter. The grey tone value of each pixel is first multiplied by the weight number given by the filter:

1 x p_1	2 x p_2	1 x p_5
2 x p_4	4 x p_5	2 x p_6
1 x p_7	2 x p_8	1 x p_9

and thereafter the results are summed and the sum is divided by 16 (the sum of weights of the filter). The result is the smoothed value for the centermost point p' (i,j), which then must be rounded to integer value.

The above can be described by the following formula:

$$p'(i,j) = \frac{1}{16}\left[p(i-1, j-1) + 2p(i, j-1) + p(i+1, j-1) + 2p(i-1, j) + 4p(i,j) + 2p(i+1, j) + p(i-1, j+1) + 2p(i, j+1) + p(i+1, j+1)\right]$$

For the 9 pixels shown on page the computer makes the following calculations:

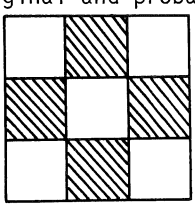
0	0	1	1	2	1
1	7	2	2	4	2
2	0	1	1	2	1

Neighbourhood to be smoothed — Smoothing filter

$p(i,j) = 7$

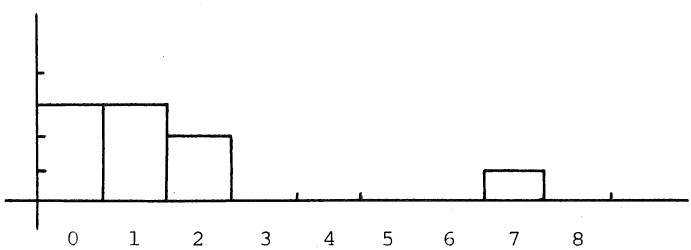
$$p'(i,j) = \frac{1}{16}(0 + 0 + 1 + 2 + 28 + 4 + 2 + 0 + 1) = \frac{38}{16} = 2{,}375$$

The value 2,375 is rounded to 2, which may be closer to truth than the original and probably erroneous value of 7.



The computer performs the above procedure for one group of pixels at a time so that the next centermost pixel could be any of the shaded pixels shown on the left (depending on how the computer is programmed to do its job).

Another alternative is median filtering in which the new grey level of the centermost pixel is the median of the grey levels of the 9 pixels to be considered in the image. For instance, the 9 pixels of the previous example give the following histogram:



The median of the grey levels will be = 1 because there are as many pixels on the darker side as there are on the lighter side of 1.

If one would like to use smoothing based on averages the filter would be

1	1	1
1	1	1
1	1	1

EDGE OR BOUNDARY ENHANCEMENT

An alternative to grey level histogram thresholding in extracting relevant regions, such as nuclei, from an image is the detection of the boundaries of objects. A boundary is detected by a steep gradient in grey levels.

Again matrices of 3 x 3 pixels may be used. They are called gradient filters.

One of these is Sobel's operator, which includes two matrices:

$$d_x = \begin{matrix} 1 & 2 & 1 \\ 0 & 0 & 0 \\ -1 & -2 & -1 \end{matrix} \quad \text{and} \quad d_y = \begin{matrix} -1 & 0 & 1 \\ -2 & 0 & 2 \\ -1 & 0 & 1 \end{matrix}$$

These are applied in the same way as the smoothing filters we have already described. We get two values $p_x'(i,j)$ and $p_y'(i,j)$ and the final value for the centermost pixel is

$$p''(i,j) = \alpha \sqrt{p_x'(i,j)^2 + p_y'(i,j)^2} \quad \text{where}$$

α = constant. The role of α is to bring the value of $p''(i,j)$ to the allowed range of grey levels.

For example:

Across the following group of nine pixels there is a borderline going from the lower left corner to the upper right corner:

7	7	7
6	7	1
5	0	0

The above calculations give:

$p_x'(i,j) = 23$

$p_y'(i,j) = -15$

$p''(i,j) = \alpha \cdot 27{,}46$

The following group of nine pixels shows no borderline:

1	2	2
6	2	3
1	5	5

$$p_x' \ (i,j) = -9$$

$$p_y' \ (i,j) = -1$$

$$p'' \ (i,j) = \alpha \cdot 9{,}06$$

In the former case there was enhancement three times as strong as in the latter case.

After Sobel's operator, thresholding can be applied and by combining these methods pictures with borderlines only can be created (binary pictures).

The so-called Laplace operators have a corresponding effect. Here are two of these operators:

$$L_1 = \begin{matrix} 1 & 0 & 1 \\ 0 & -4 & 0 \\ 1 & 0 & 1 \end{matrix}, \quad L_2 = \begin{matrix} 1 & -2 & 1 \\ -2 & 4 & -2 \\ 1 & -2 & 1 \end{matrix}$$

Robert's operator also enhances the borderlines. It consists of two separate matrices

$$d_1 = \begin{matrix} 1 & 0 \\ 0 & -1 \end{matrix}, \quad d_2 = \begin{matrix} 0 & 1 \\ -1 & 0 \end{matrix}$$

With these we calculate $p_1' \ (i,j)$ and $p_2' \ (i,j)$

and thereafter

$$p'' \ (i,j) = \beta \sqrt{p_1' \ (i,j)^2 + p_2' \ (i,j)^2}$$

The pixel (i,j) is now in the left upper corner of the 2 x 2 field.

MEASURING

The borderlines produced by the operators are usually not smooth. Curves can be fitted to them and reliable measurements achieved. One can use circles and ellipses of various sizes, eggshaped curves and other convex closed curves, usually consisting of so-called polynomial splines.

EROSION AND DILATATION

Erosion and dilatation are operations by which regions with a uniform grey level but ragged boundaries can be "smoothed" without using any curve fitting. Following Serra (1982), both are based on a "structure element" which is a small set of pixels with a characteristic shape. Consider a structure with a ragged form and holes (Fig. 3).

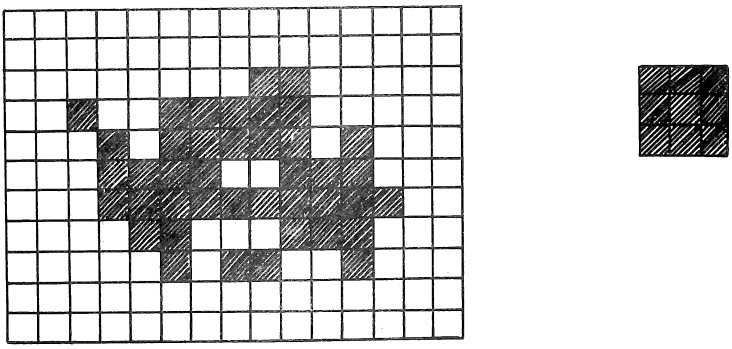


Fig. 3. Region R in a picture to be subjected to the operation called dilatation. The structure element S, which will be applied on the picture is shown on the right. The rules defining the operation are given in the text.

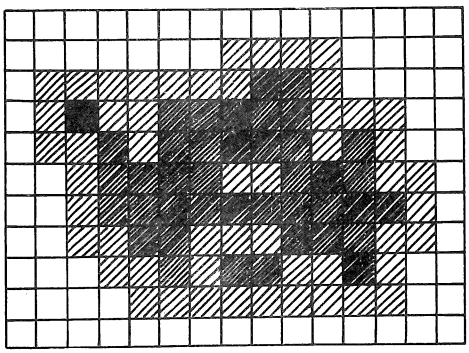


Fig. 4. Region R after dilatation (the pixels of the original region are shaded more darkly to indicate which shaded pixels are old, and which are new).

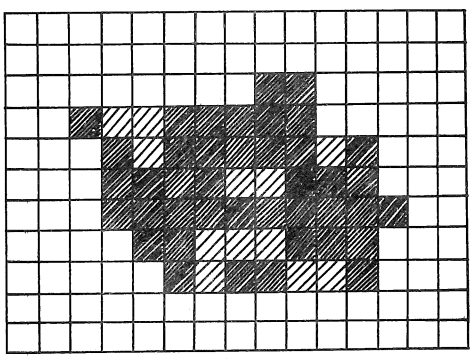


Fig. 5. Region R (Fig. 3) after dilatation and erosion. This should be compared to the original region R (Fig. 3); the holes and dents have been filled, but otherwise the regions are very similar.

It is assumed that the picture in Fig. 3 is the outcome of histogram thresholding and is therefore a binary picture (grey values 0 or 1).

In dilatation, the center point of the structure element S is thought to be placed on a pixel in region R. Then some of the pixels of the structure element may fall on pixels of R and some on pixels of the background, not belonging to R. All those pixels of S that do not overlap any pixel of R are added to R. Extra pixels are thus added to the original region especially near boundaries and holes. This procedure is repeated for all the pixels of the original object R. Dilatation applied to the region R of Fig. 3 produces the dilated region shown in Fig. 4.

In erosion, the center point of the structure element S is again thought to be placed on each pixel of the region R in turn. Call the pixel that the center point overlaps p. If now all the pixels of S fall on pixels of R, then p is preserved. If one or several of the pixels of S fall on the background, not on R, then p is removed (eroded) from R.

Applied to the dilated region above, erosion produces the image shown in Fig. 5.

Both dilatation and erosion are easily programmed on a computer, which performs them fully automatically once the form of the structure element is given to it.

SIZE DISTRIBUTION OF GRAINS

After the coherent united darker regions (grains) of the picture are found by either thresholding or gradient filtering and the boundaries are smoothed by either curve fitting or dilatation and erosion (there are other methods, too) their relative sizes can be directly measured by the computer. Thereafter a size distribution histogram can be computed and plotted. Such information can be most relevant to the diagnosis of cancer. This is because nuclear size distribution reflects nucleic acid contents and class of polyploidy (Sandritter 1981).

FULLY AUTOMATIC OPERATION

There are two main reasons why fully automatic image analysis by computer is desirable. The first is the high level of invariance and reproducibility in the analysis by a computer. The second is replacement of human resources in routine work, for example, fully automatic analysis systems for tens of millions of cytological samples examined every year worlwide (Bartels et al. 1982, Goenttler and Stöhr 1982), and automatic differential blood counts. On the other hand, in more complicated nonroutine investigations full automation is probably neither desirable nor possible in the near future. Fully automatic operation as a concept also conveys a misconception in itself. Even though replacement of humans may motivate research in this area because of financial benefits, medical diagnostics is a field in which human beings cannot be replaced. Instruments can help us in diagnostics but the physician remains necessary to interpret the results and to decide - with the patient - on further action.

INTERACTIVE OPERATION

A recent trend in image analysis is to develop easy-to-use program packages which can be applied by the researcher himself. Of course, such packages should be accessible to users that are new in the computer field. This kind of software is used either with a menu-based technique, in which the user chooses the item he wants from a number of present possibilities, or uses a

command language, in which there is a set of commands that can be application-oriented. In a typical software package, there are commands for the following operations:

- storing and erasing pictures and moving them to the display from the disc memory
- scaling, rotating and choosing subpictures for consideration
- computing and plotting grey level histograms, changing grey levels, histogram equalization, thresholding
- gradient filters (Sobel, Roberts, Laplace)
- smoothing, median filters
- erosion, dilatation
- Fourier (spectrum) operations
- overlaying, adding, subtracting several pictures
- measurements
- extraction of texture parameters.

Most of these operations have been clarified above.

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