THE USE OF A VIDEO CAMERA AND PC FOR CRYSTAL IMAGE ANALYSIS

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Abstract

The use of a video camera attached to a Personal Computer (PC) fitted with a frame grabber card is described. Microscopic images of sugar crystals for record purposes can replace the more expensive use of Polaroid film. Images can be stored on disk or printed on high resolution printers. Software to measure crystals manually for crystal size distribution is described. Efforts to automate the measurements of size distribution are summarised.

Introduction

Felixton and Amatikulu mills spend in total about R50 000 annually on Polaroid film for keeping records of their C-massecuites. The Sugar Technology Department of Tongaat-Hulett Sugar decided to investigate the use of a video camera and PC as a cheaper method for recording images of sugar crystals. The ability to have video images in a computer format made it possible to investigate the use of image analysis software for automatic crystal size measurement. Two different types of 'frame grabber' cards (which interface a video camera to a PC) were tested in conjunction with two different software packages for analysis. Various combinations of microscope and video camera configuration and various lighting and crystal darkening techniques were tried to enhance the stored image and assist image analysis techniques.

Theory

Frame grabbing

Image acquisition involves the use of a frame grabber card, a fast PC to process and display incoming information without flicker, a suitable operating system and manipulation software. The equipment used for this paper is listed in Table 1.

Table 1

Equipment used for this work

Camera	CCD Video camera	
Computer	386-33 PC with 4 Mb RAM	
Video display	VGA Monitor	
Frame grabber card	Scorpion	Video Plus
Software package	Image Pro Plus	iPhoto Plus
Operating system	MS DOS 5.0	Windows 3.1

Figure 1 illustrates the mechanism of image acquisition. Information from an external source (input) that adheres to the NTSC or PAL video standard (e.g. video recorders, TV) is digitised at 12,6 MHz by the analogue to digital (A/D) convertor. The Phase Lock Loop (PLL) synchronises the horizontal and vertical components of the Video signal. This information is converted into an equivalent grey level by the look up table (LUT) and stored in the Frame Memory. The Frame Memory stores the image in a pixel-by-pixel manner which is arranged so that it represents the 2-D display surface (Simon and Slater, 1987). Each location in the Frame Memory represents the brightness of a single pixel of the image. A second look up table and D/A is used for converting these digital values into analogue equivalents which are displayed on the monitor. The displayed image size is 640 by 480 pixels, each of which has 256 possible shades of grey.
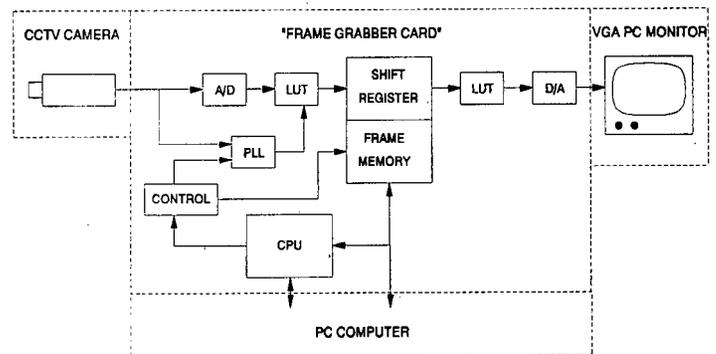

FIGURE 1 Frame grabber flow diagram.

When the image processing software is running, the Shift Register usually 'mirrors' the contents of the Frame Memory creating a real time display mode. If however the software is instructed to freeze the current display, then the values stored in the Shift Register are displayed while the Frame Memory continues capturing data from the external source. It is this image that can be stored to disk or 'grabbed'.

Operation of Image Pro Plus software

When the equipment is initially set up, the moving image appears on the monitor like a TV picture. The desired frame can be captured through the *acquire* feature of the menu. Once this has been done the following operations are some of the image processing features that can be performed.

Images can be *analysed* in terms of their intensity or optical density contents using histograms and frequency analysis on points, line profiles, or areas.

The *contrast* in an image can be changed. The image can also be 'thresholded' which converts it into a high contrast image of pure black and white. Thresholding is the technique of selecting a grey level below which all pixels are made black, and above which all pixels are made white. The image can also be inverted.

Images can be *filtered* to enable one to sharpen an image, reduce noise, or extract features (edges, contours, etc.) present in an image. Different types of filters are available.

The *Count/Size* feature permits one to count automatically and measure the number of objects in an image and

classify them according to a set of size and shape criteria that are specified. The outlines of the measured images are shown on screen in a different colour. The results can be viewed or saved to disk in ASCII or Lotus formats. This was one of the important features that was investigated in the package.

Lines, traces, areas, angles, and distances between objects can be *measured*. Measurements can be performed manually or automatically.

The *Fast Fourier Transform* feature enables one to convert and filter an image in the frequency domain, and then reconvert it to a spatial image. This enables, for example, noise to be removed from an image by manipulation in the frequency domain.

Text and simple graphic enhancements can be added to an image by *annotating*.

Relationship between particle separation and contrast

If particles are separate from each other and the *whole* of the boundary is distinguishable from the background by being either darker or lighter, then Image Pro Plus will easily count the particles and draw a line on their perimeters and thus analyse them. It can then calculate a number of criteria which can be specified (e.g. length, perimeter, area etc.). When an image touches another one Image Pro Plus cannot distinguish the separation but measures along the boundary where there is a change in intensity from the background i.e. it measures the two objects as one. The average background level and the boundary intensity levels can be specified by the user. To illustrate the requirements of sample preparation and photographic techniques versus image processing techniques, a hypothetical model of the process of getting the right image quality is shown graphically in Figure 2, which shows the relationship between contrast and separation of particles. Ideally the image characteristics should be such that the contrast and separation are good, i.e. in the top right corner of the graph. In this region technique A, which is simply letting Image Pro Plus count and size the

particles, is successful. Image enhancement techniques can extend the ability of the system to accommodate poorer contrast and sample separation. This is illustrated in the following examples.

If the separation is good but the contrast is poorer, then image contrasting techniques (B) can be used to improve contrast. This is relatively difficult if the optimum contrast has already been obtained by optical means. Methods B + A.

If the contrast is good and the separation is poorer and crystals overlap, then statistical methods (C) can be used to discard those with odd shapes. This is relatively easy and quick. Methods A + C.

If both separation and contrast are poor, i.e. in the bottom left hand corner of the graph, then sample preparation and photographic techniques must be applied to the samples to improve separation and contrast. It is possible to improve separation for example by mechanical means, such as dropping the crystals from a height onto a sticky or matt surface and by keeping the crystal density low. The contrast can be somewhat improved by polarised light, less so in C-massecuite (C M/C), and by using a matt black background and incident light from several directions.

Method

A compact black and white surveillance camera was obtained which had a ½" Charge Coupled Device (CCD) chip with an image size of 6,4 × 4,8 mm. A standard C-mount 25 mm telephoto lens with various extension tubes from 5 to 40 mm were used with which the magnification could be altered.

Graded quartz sand (as used in water filtration), refined sugar and C-massecuite were all used to obtain images and experiment with counting and sizing. The sand was used as it is not as transparent as sugar and therefore gives better contrast. This would thus give an indication of the count/size feature under good contrast conditions. Some samples of refined sugar were dyed with indicator dissolved in alcohol. In particular eosin, methylene blue and methyl red produced dark sugar although not very effectively as the sugar is dyed unevenly. The use of dye with a colour camera and software might be more successful. Some samples of white sugar were slurried in glycerine to try to separate the crystals. The standard way of producing an image is to place the sample on a slide and view it through diffuse transmitted plain or polarised light. Some samples of sugar were stuck to black sticky tape and viewed with incident light.

Results

Printing of images for record purposes

The Image Pro Plus package uses the method of printing images known as dither, of which there are several variations. Since most printers can only print in black and white, dithering is the selection of the half tone pattern used to represent greys on the printer. From the 24 alternatives available, three of the best examples of a small portion of an image using the dither processes are shown in Figure 3. Classic dot 8 appears to give one of the best results. The area of the test square is 160 × 170 pixels.

Examples of images

A stored full image is shown in Figure 4 of white sugar taken with the 20 mm extension tube illustrating image quality. The sugar was placed loosely on a slide to promote separation and the polariser was on bright. This was one of the better images that could be obtained.
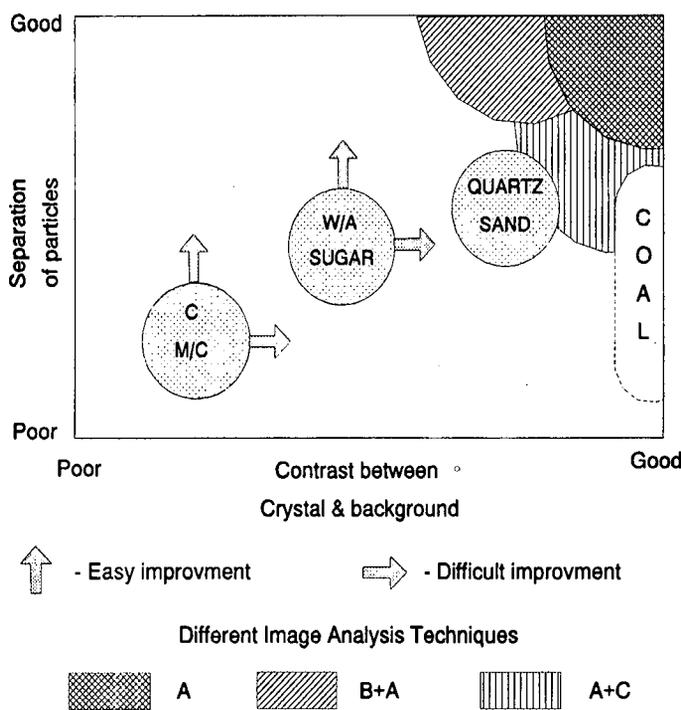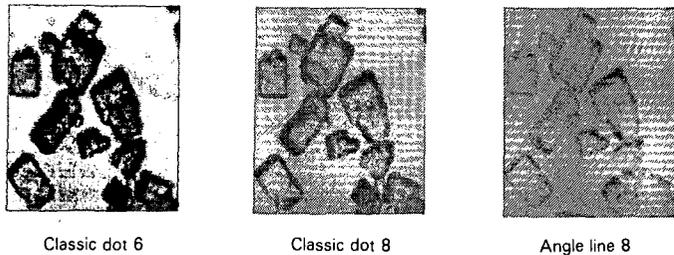

FIGURE 2 Image quality graph.

Classic dot 6   Classic dot 8   Angle line 8

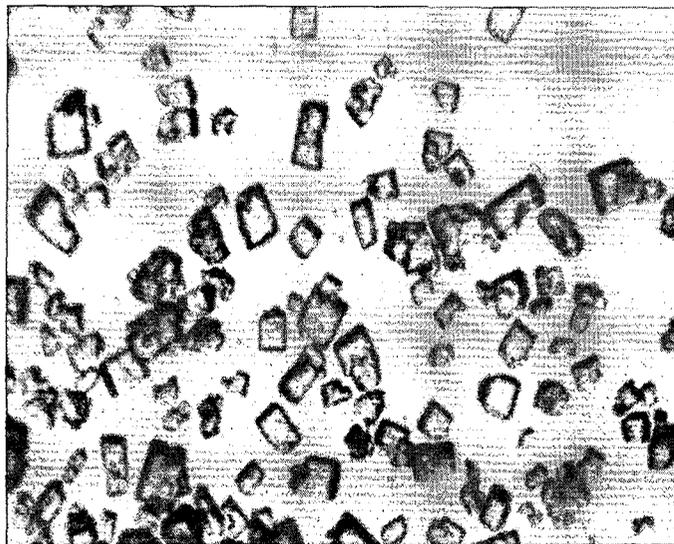**FIGURE 3** Examples of different dither techniques.

FIGURE 4 Image of white sugar crystals taken with 25 mm lens and 10 mm extension tube.

A sample of C-massecuite with the best polariser position is illustrated in Figure 5. Image Pro Plus cannot count any crystals here, but the image could be used for record purposes although very small crystals cannot be seen.
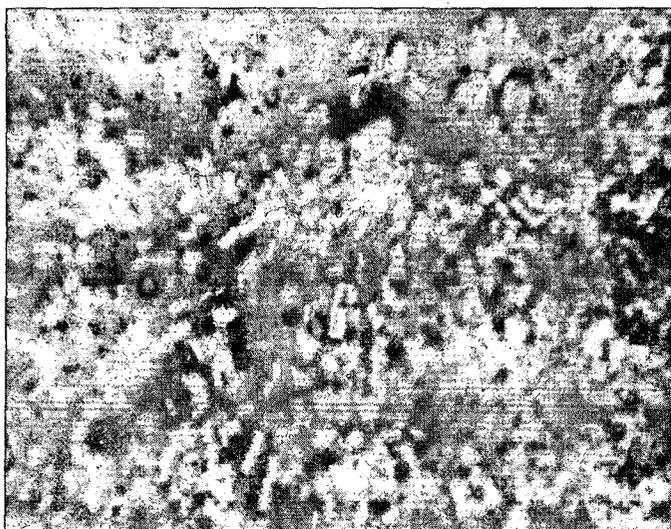

FIGURE 5 Image of C-massecuite with 25 mm lens and 20 mm extension tube.

Examples of statistical analysis of count/size feature

Table 2 is an example of Image Pro Plus output in a spreadsheet format. The spreadsheet has been sorted in ascending order on the Roundness column. The roundness is $\frac{Perimeter^2}{Area \cdot 4\pi}$ and can be regarded as a shape factor related to a circle. A circle would have a value of 1 and a square a value of 1,27 while more irregular shapes would have higher values. In Figures 6 and 7 the sorted roundness has been plotted for sand and white sugar size/counts. It can be seen that sorting in this way can provide a value to choose for rejecting data. In the sand example the roundness value gradually increases from nearly round to just under 1,8. Particle No. 10 could thus be rejected as it is one with a piece projecting from itself. In the white example the roundness value increases gradually from under 1,6 to 2,4, and then a jump occurs where one or more particles are touching. Similarly a plot of a count from a white slide is shown in Figure 7. Here particles 18, 9, 5, 10, 13, 12, and 19 were out of line with the average count and could possibly be rejected.

Use of imaging to replace photographic film

An important aspect of these tests has been to see whether it is practical to let image gathering and storage replace the existing method of Polaroid photography as used by FX and AK and what cost saving could be achieved. Examples of the printout from a laser printer have already been given. Image Pro Plus has only one size for a full screen and that is A5 printed in the middle of an A4 page. Doubtless other packages suitable only for data capture and storage will have the ability to vary the output format.

Table 2

Example of output from size/count of sand

Experiment: Sand
Date: Oct 14, 1992
Time: 3:05:18 pm
Total number of objects: 12

Obj. #	Area	Perim.	Round.	Max.Dia.	Min.Dia.
8	16,08	15,25	1,15	5,11	3,91
11	21,91	18,51	1,24	5,65	4,27
4	10,06	12,68	1,27	4,09	2,81
5	11,89	13,99	1,31	5,02	2,92
3	20,34	18,40	1,32	6,97	3,52
2	25,32	20,54	1,33	6,24	4,79
9	5,70	9,75	1,33	2,93	2,12
7	18,28	17,57	1,34	5,55	3,44
1	20,83	19,04	1,39	6,64	3,71
6	37,79	25,93	1,42	9,60	4,98
10	22,68	20,29	1,44	6,35	3,92
	35,26	27,67	1,73	8,28	4,69
Statistics					
Min	5,70	9,75	1,15	2,93	2,12
Max	37,79	27,67	1,73	9,60	4,98
Range	33,90	18,92	1,58	7,67	3,86
Sum	246,4	219,61	16,27	72,43	45,07
Mean	1	18,30	1,36	6,04	3,76
Std Dev	20,51	4,90	0,13	1,70	0,83
	9,01				
Ranges:					
Min:	0,10	0,10	0,10	0,10	0,10
Max:	1E4	1E4	1E4	1E4	1E4

Calibration
Spatial Unit Name: Pixel
X-Axis. Pixel   10
Y-Axis. Pixel   10
Intensity Unit Name: Grey Level
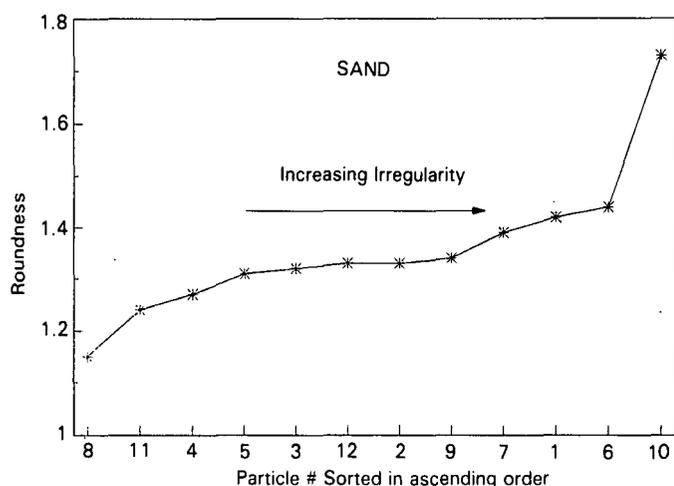Angle. Unit: Degrees
Axis. of Refer   0

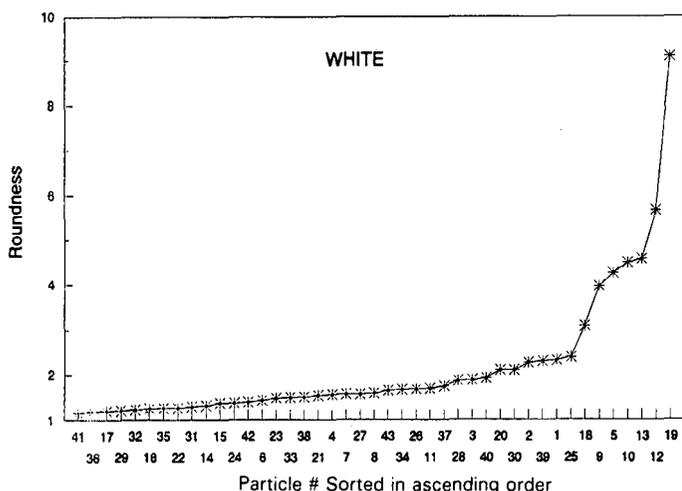**FIGURE 6** Plot of Shape factor of Sand vs Particle No.

FIGURE 7 Plot of Shape factor of White vs Particle No.

The variable cost of image storage (film versus disks) are shown for comparison in Table 3. ACT diskettes are amongst the cheapest available. TIF files can be compressed by about 25% with PKXARC.

Table 3

Comparison of film and disk storage costs

	Price each (R)	No of images (with 25% compression)	Cost/image (cents)
Polaroid 667 film	3,30	1	330
ACT diskettes			
5¼" 360 kb	2,70	1	270
5¼" 1.2 Mb	3,25	5	65
3½" 720 kb	3,25	3	92
3¼" 1,44 MB	5,00	6	83
Laser printer (approx)			15

A total of R47 000 was spent by AK and FX in the 1991/92 season on Polaroid film. If this were to be replaced by ¼" 1,2 Mb floppy disks then a saving of R38 000 would be realised if the images were stored permanently. If the disks were reused every year then the price of the disks would be a once off cost. If all the images were printed on a laser printer the cost would be around R2 000.

The cost of setting up the equipment would depend on whether a PC and printer need to be purchased. A CCTV camera and tele-photo lens would cost under R2 000. Image Pro Plus costs around R6 000. iPhoto Plus costing around R1 000 cannot do count/sizing but is all that is required to store and print images only.

Discussion

Data capture for record purposes

It is certainly very practical and cheap to capture and store images with a PC and CCTV camera. The quality of the image is not as good as with film, but the image is larger. The images reproduced here are not as good as the images on the monitor. Considerable definition is lost in the dither process where definition is sacrificed for producing grey tones.

The SMRI has a graphics printer which prints all the grey shades seen on the monitor on special film-like paper about 100 × 80 mm. Each print costs about 30 cents to produce. The printer costs R16 000.

Image analysis

The automatic image analysis has been successful where there is clear separation of crystals and sufficient contrast exists between crystal and background to produce separate images when 'thresholded'. The thresholding does not improve the contrast but merely allows one to make a very distinct cut-off at a chosen level of grey so that the darker values are made black and the lighter ones white. If there is an overlap between the crystal and the background then thresholding would not overcome this but merely make it more visible to the operator. The other image processing techniques for closing holes etc. operate at a very specific level and are not broad enough to cover the large variations encountered in a real crystal image. There exists scope for improving the contrast by using a very matt black background and incident light from several directions. The incident light used in these tests was from one direction only and dark edges sometimes occur which blend in with the background.

The possibility of using manual methods of counting and measuring crystals was not pursued here, but it certainly is a feasible alternative to automatic counting and will work as well as the skill of the operator allows. Crystals which are touching or overlapping can easily be differentiated visually, whereas software has great difficulty in distinguishing them. Image Pro Plus does have a method of manually measuring lines or areas, but it is a bit clumsy compared with the Kontron method.

The use of statistical cut-off criteria does offer some scope for rejecting out of line data, but its use would have to be evaluated carefully for each situation and does not lend itself to routine analysis. Furthermore the rejected data could be correlated with the size of the crystals concerned thus skewing the results.

Conclusions

The system to store crystal images for record purposes is feasible.

Manual crystal sizing with a pointing device is possible and cheaper than the Kontron apparatus purchased by the SMRI. The SMRI's apparatus cost R26 000 for the PC, digitiser tablet and software.

A fully automatic method of counting needs further investigation as it will require improved sample preparation and close-up photography to obtain images suitable for automatic processing for routine work. The automatic processing of these enhanced images will need to be verified by comparing with other techniques.

REFERENCES

Simon, R and Slater, M (1987). Computer Graphics Systems and Concepts. Addison Wesley Publications.