Semper 6 *Plus*

APPLICATION NOTES

Synoptics

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Semper 6 Application Notes Index

Semper 6 Application Notes provide practical examples of using Semper to solve image processing problems. There are 20 titles in this series; the available titles are listed below.

❑ Available Titles

1. Printing Pixel Values

The Semper command **print** can be used to examine the numerical value of pixels in a image.This can be very useful when debugging image processing algorithms.

```
display 1 to display:1
```

Picture 1 is displayed in display partition 1. (Figure 1).

```
xwires
```

A mouse driven cursor is displayed on the image and the mouse button clicked on the centre of the area of interest of the image. The Semper variables *x* and y are set when this is done.

```
print 1  @xy
```

A region of pixel values centred on the cursor is printed out on the console. The expression `@xy` is a macro that sets up the *x,y* co-ordinates for the **print** command. (Figure 2).

The origin of the picture, point (0,0) is at the centre.

Figure 1. Original image.

	-47	-46	-45	-44	-43	-42	-41	-40	-39
48 |	201	203	197	200	201	202	202	204	204
47 |	206	199	200	202	198	200	205	206	207
46 |	202	201	201	201	204	205	206	206	207
45 |	199	201	204	205	206	206	207	208	208
44 |	202	204	204	204	204	205	206	208	209
43 |	205	206	201	204	205	206	207	208	208
42 |	208	205	202	205	206	204	205	208	208
41 |	207	204	202	203	203	204	205	207	207
40 |	203	204	202	203	202	204	205	205	204

Figure 2. Numerical values of pixels around cursor.

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2. Extracting and Displaying a Single Line from an Image

The Semper command **extract** can be used to extract and display in graphical form a sample from a picture along an arbitrary line.

```
display 1 to display:1
```

The image of a T.V. test chart is displayed. (Figure 1).

```
xwires section
```

The line along which the samples are to be taken is drawn with the mouse.

```
extract from 1 to 2 @section
```

The marked line from the image is extracted and stored in picture number 2.

```
display 2 to display:1
```

The extracted one dimensional picture is displayed in partition 1. One dimensional pictures are displayed in graphical form. This particular example illustrates how Semper might be used to evaluate the resolution performance of a T.V. Camera. (Figure 2).

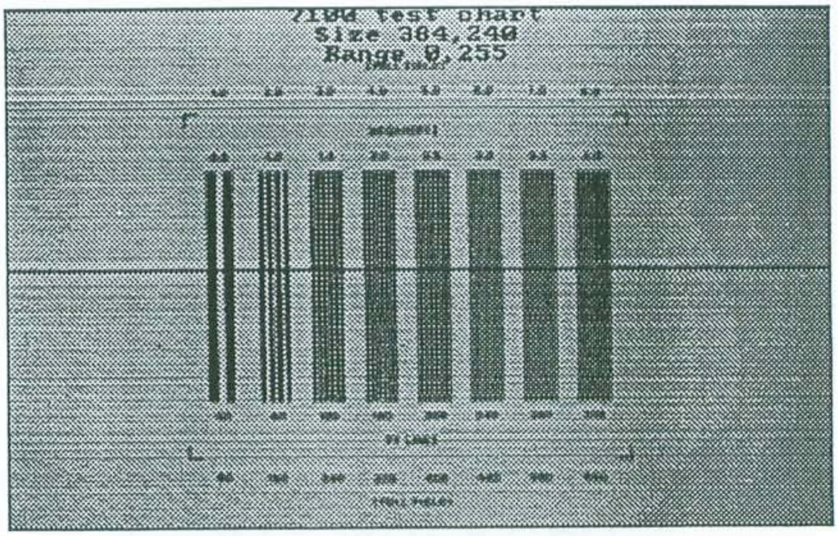

Figure 1. Image of a T.V. test chart.

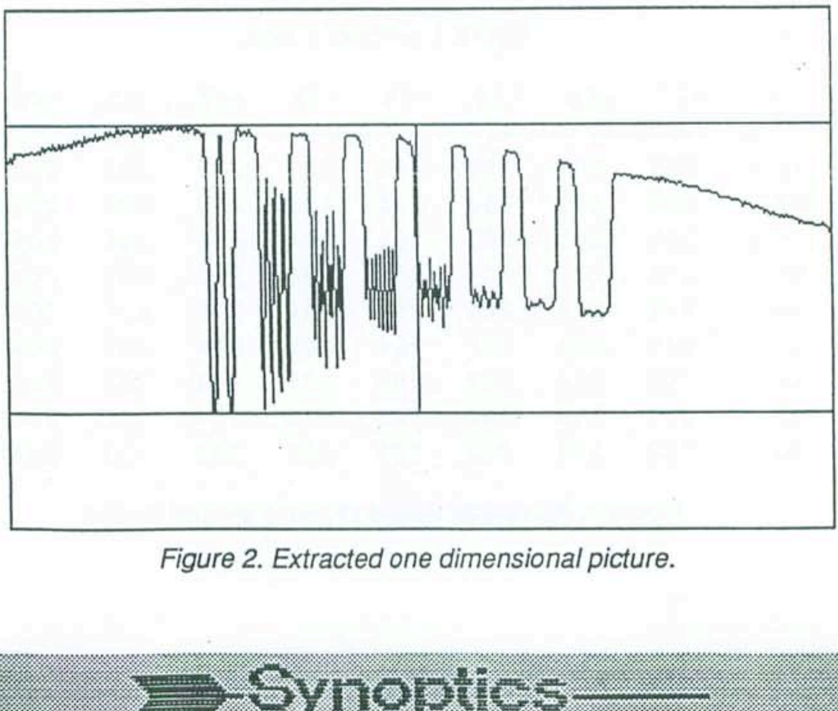

Figure 2. Extracted one dimensional picture.

3. Picture Masking

The Semper command **mask** allows pixel values to be overwritten over a defined region. This region may be defined interactively using a mouse and the Semper **xwires** command.

```
display 1 to display:1
```

The image stored in picture 1 is displayed in partition 1. (Figure 1).

```
xwires curve closed to 2
```

A mouse driven cursor is displayed on the screen and used to outline the area of interest. The resulting list of co-ordinates is stored in picture 2. The picture class used is called a points list (*Plist*). The keyword **closed** ensures that the region defined by the mouse is a closed curve.

```
mask 1 with 2 outside value max to dis:2
```

The original image is masked and displayed in partition 2. (Figure 2). The masked region has its pixel values set to maximum brightness level in the original picture. The Semper variable *max* is set to the maximum intensity in the picture when it is first displayed.

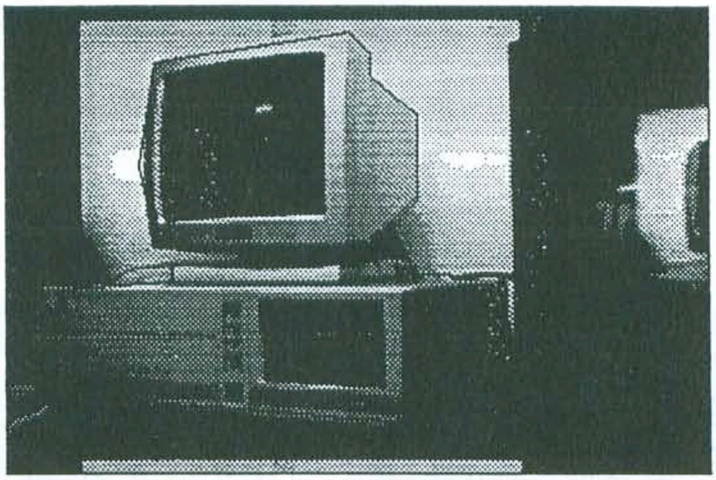

Figure 1. Original image.

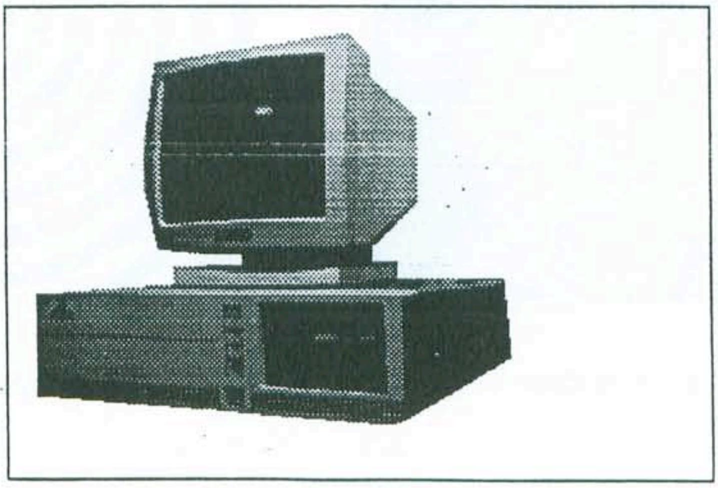

Figure 2. Masked image.

4. Synthetic Picture Calculations

The Semper **calculate** command can be used to produce a synthetic picture whose intensity values are a quite arbitrary mathematical function of the pixel co-ordinates.

```
create 1 size 256,256 integer
```

A 256 pixel square picture is created. Note that the picture type has to be integer to accommodate negative pixel values.

```
calculate 255*sin(x/10)*sin(y/10)
```

The intensity values in the picture are calculated according to the mathematical expression.

```
display 1 to display:1
```

Picture 1 is displayed in display partition 1. (Figure 1).

```
create 2 size 256,256 byte
```

Create a second blank picture.

```
calc (sin(root(x*x/8+y*y/8))>0)
```

The pixel intensity is set to zero or one depending on whether the inequality is satisfied.

```
display 2 to display:2
```

The second picture is displayed. This type of synthetic image could be used as a test pattern for image processing algorithms or equipment.

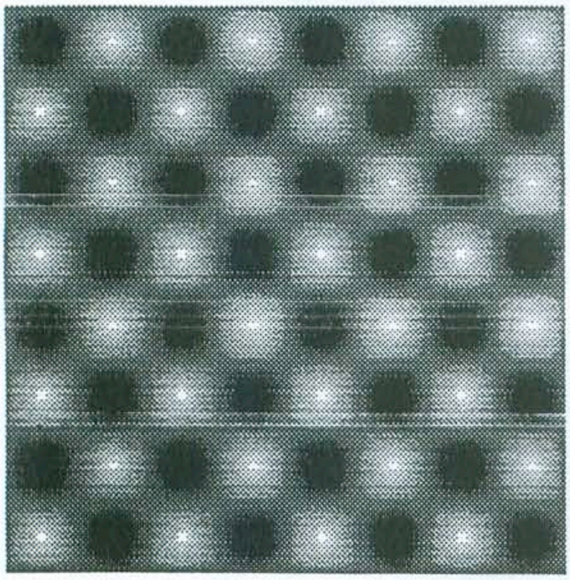

Figure 1. Synthetic image 1.

Figure 2. Synthetic image 2.

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5. Cutting and Pasting Images

Semper 6 contains a comprehensive set of commands for geometrical manipulation of images. Cutting and pasting may be performed using the Semper commands **cut** and **paste**.

```
display 1 to display:1
```

The image stored in picture 1 is displayed in partition 1. (Figure 1).

```
display 2 to display:2
```

The image stored in picture 2 is displayed in partition 2. (Figure 2).

```
xwires region
```

A mouse driven cursor is displayed on the screen and moved to define the region from which the image is to be cut. The mouse button is clicked on the two corners.

```
cut display:2 to 3 @region
```

The region defined by the cursor is extracted and stored in picture 3.

```
origin reset
```

The origin of the extracted region is reset.

```
paste 3 to display:1 position 0,0
```

The region that is stored in picture 3 is pasted into the centre of the image that is displayed in partition 2. (Figure 3).

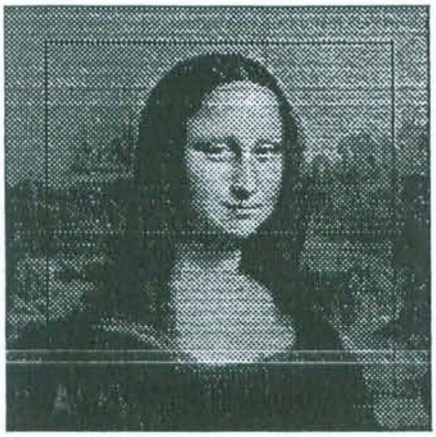

Figure 1. Original image.

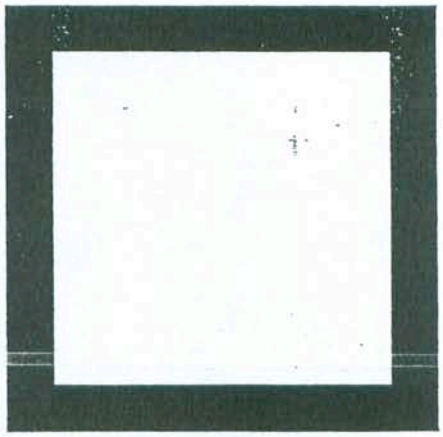

Figure 2. Second image.

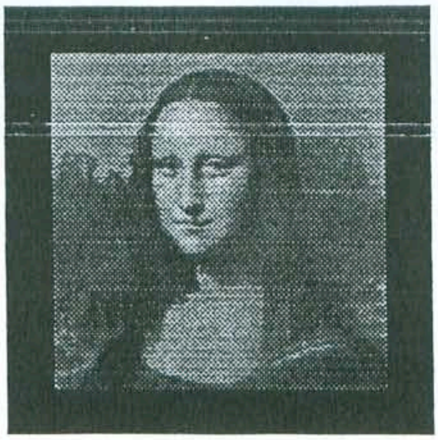

Figure 3. Result of cut and paste operation.

6. Rank Filtering

Semper 6 has a comprehensive set of commands which modify a pixel according to its nearest neighbours. One such command is **rank** which ranks the pixels in the neighbourhood of a given pixel in order of brightness and replaces it by the mid intensity value.

```
display 1 to display:1
```

The picture which has been corrupted by impulsive interference is displayed in partition 1. (Figure 1).

```
rank 1 to 2
```

The rank filter is applied and the result stored in picture 2.

```
display 2 to display:2
```

The filtered image is displayed in partition 2. It can be seen that the impulsive noise has been removed without significant blurring. (Figure 2).

Figure 1. Original corrupted image.

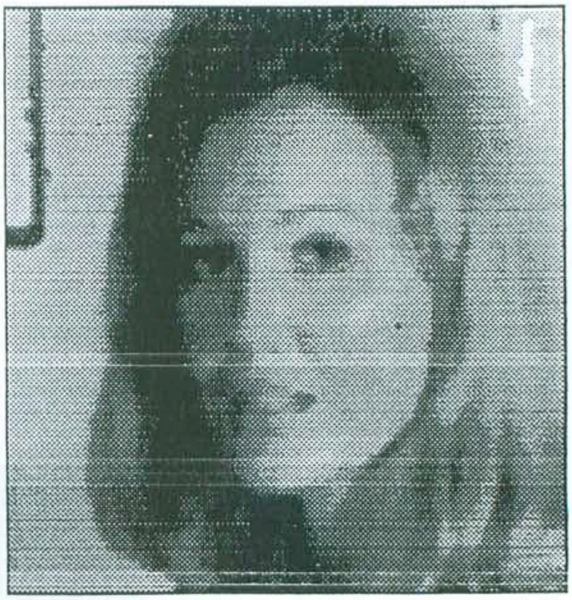

Figure 2. Result of rank filter.

7. Local Mean Filtering

Semper has a comprehensive set of commands which modify a pixel according to its nearest neighbours. The **lmean** command sets a given pixel to the mean value of its neighbours in an arbitrary sized block.

```
display 1 to display:1
```

Picture 1 of a cell dividing is displayed in partition 1. (Figure 1).

```
lmean 1 to 2 over 5
```

The local mean operator is applied to picture 1 over a 5 by 5 region and the result is stored in picture 2.

```
display 2 to display:2
```

The filtered image is displayed in partition 2. (Figure 2). This shows the effect of the local mean filtering. The noise level has been reduced at the expense of fine detail.

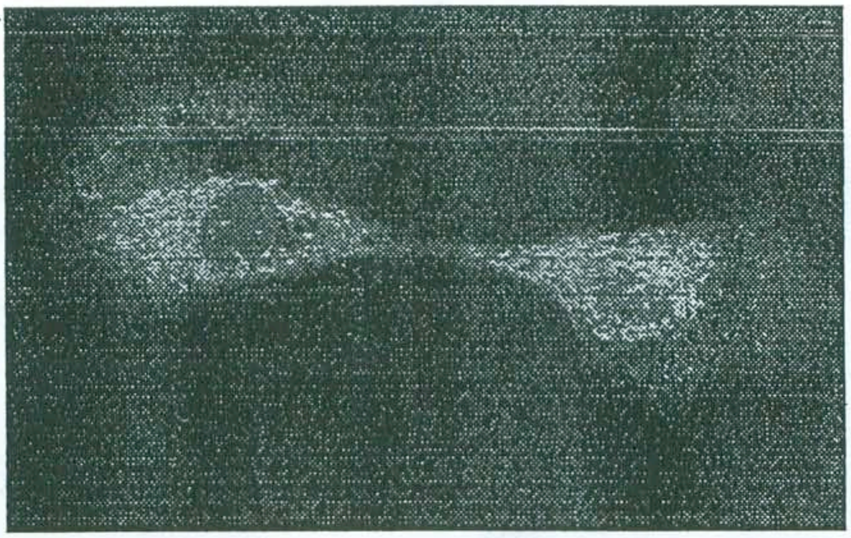

Figure 1. Original noisy image.

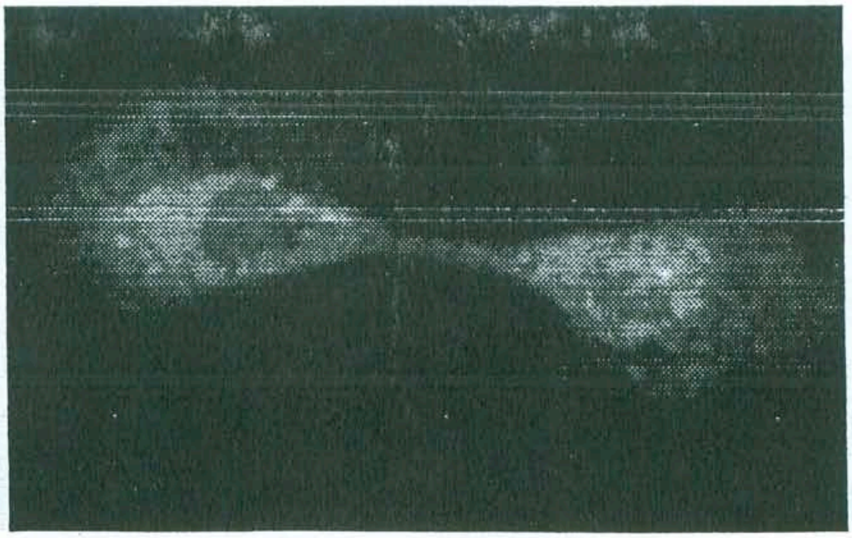

Figure 2. Result of local mean filter.

8. Picture Rotation and Reflection

Semper 6 contains a comprehensive set of commands for geometrical manipulation of images. Image rotation and reflection may be performed using the commands **rotate** and **turn**.

```
display 1 to dis:1
```

Picture 1 is displayed in display partition 1. (Figure 1).

```
rotate dis:1 to dis:2 angle pi/3
```

This picture is rotated through 60 degrees and displayed in partition 2. (Figure 2).

```
turn dis:1 to dis:3 over
```

The picture in partition 1 is turned over and displayed in partition 3. (Figure 3).

```
turn dis:1 to dis:4 upsidedown
```

The picture in partition 1 is turned upside down and is displayed in partition 4. (Figure 4.)

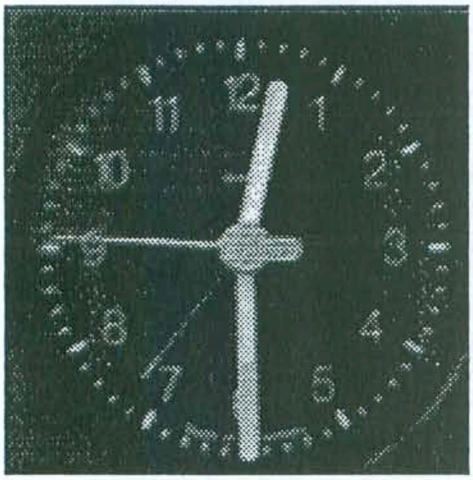

Figure 1. Original image.

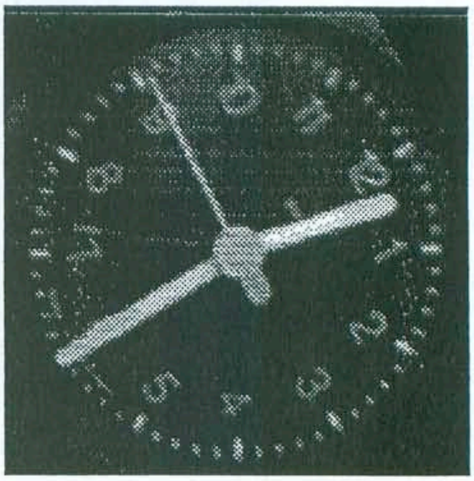

Figure 2. Original rotated by pi/3.

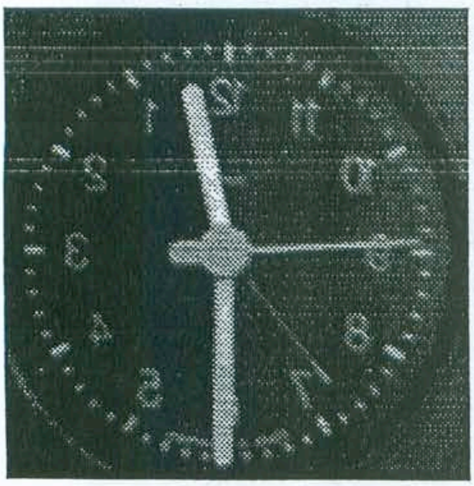

Figure 3. Original reflected.

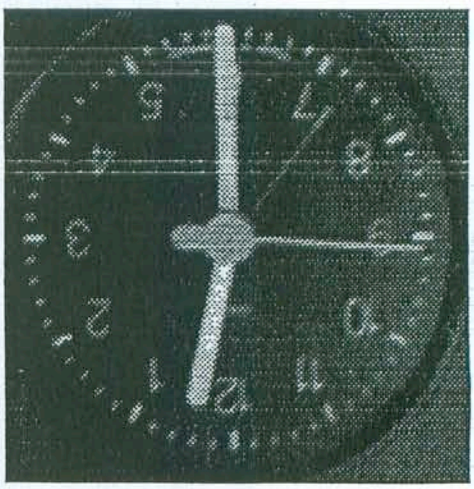

Figure 4. Original inverted.

9. Image Histograms and Histogram Equalisation

The Semper **histogram** command enables considerable information about the subjective qualities of an image to be obtained. The histogram may then be used with the Semper **map** command to perform contrast enhancement.

```
display 1 to display:1
```

Picture 1 of a cell obtained from a confocal scanning microscope is displayed in partition 1. (Figure 1).

```
histogram 1 to 999
```

The grey level histogram of the image is calculated and stored.

```
display 999 to display:2
```

The histogram stored on disk in picture 999 is displayed. (Figure 2). It can be seen from the graph that the majority of the grey levels are concentrated on the left hand side of the histogram giving a subjectively "dark" appearance to the image.

```
map display:1 with 999 to display:3
```

Histogram equalisation is performed on the image of the cell and the result put in partition 3. (Figure 3).

```
histogram display:3 to display:4
```

The histogram of the equalised image is displayed in partition 4. This shows that the distribution of grey levels is more uniform giving a subjective improvement in image quality. (Figure 4).

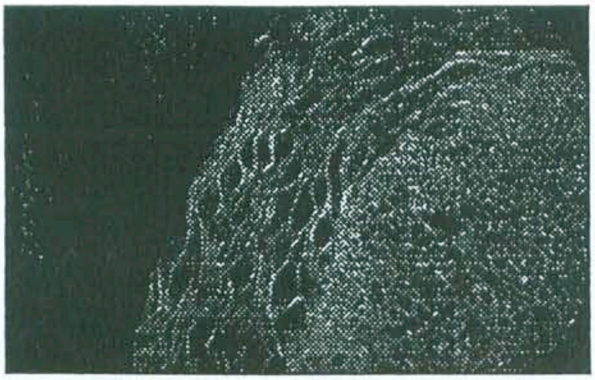

Figure 1. Original image.

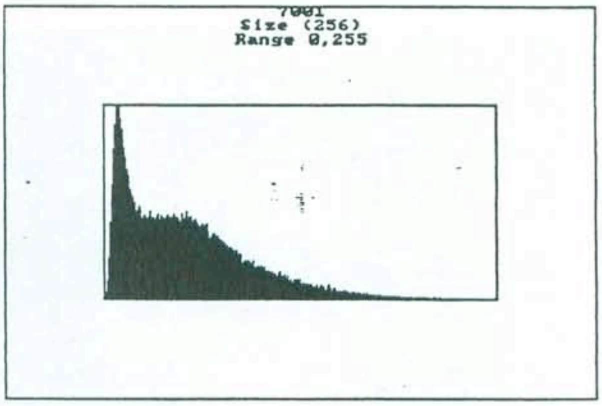

Figure 2. Grey level histogram of original.

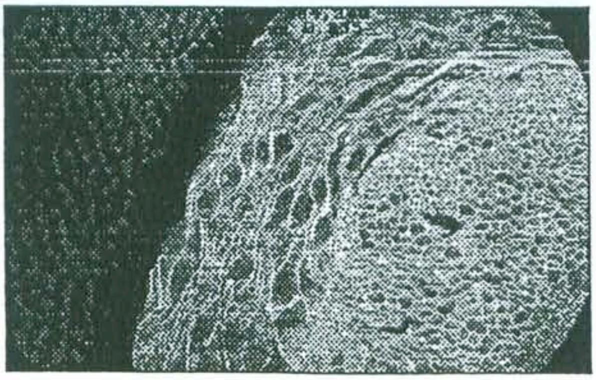

Figure 3. Image after histogram equalisation.

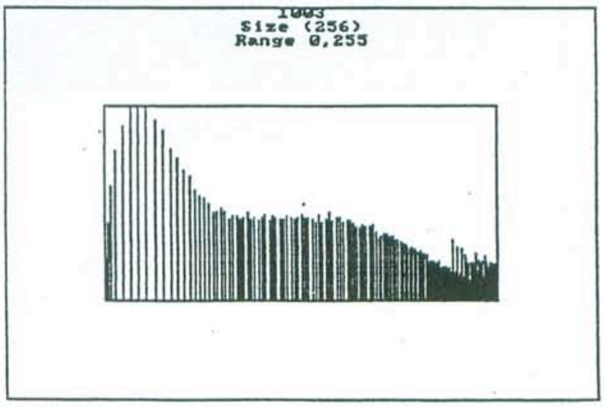

Figure 4. Histogram of equalised image.

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10. Edge Detection

Semper 6 has a comphehensive set of local operators which modify a pixel according to its nearest neighbours. One such operator is **edge** which can be used to delineate the edges of an object.

```
display 1 to display:1
```

Picture number 1 is displayed in partition 1. (Figure 1).

```
edge 1 to 3
```

Apply edge detection operator to picture 1 and store the result in picture 2.

```
display 2 to display:2
```

Display picture 2 in partition 2. (Figure 2).

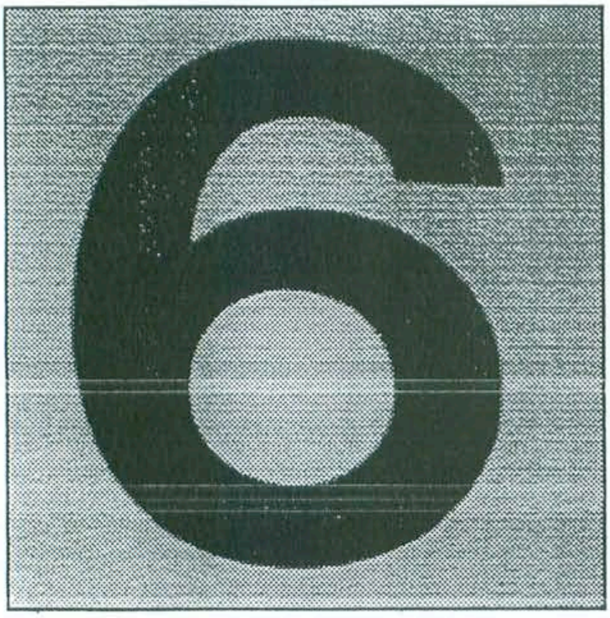

Figure 1. Original image.

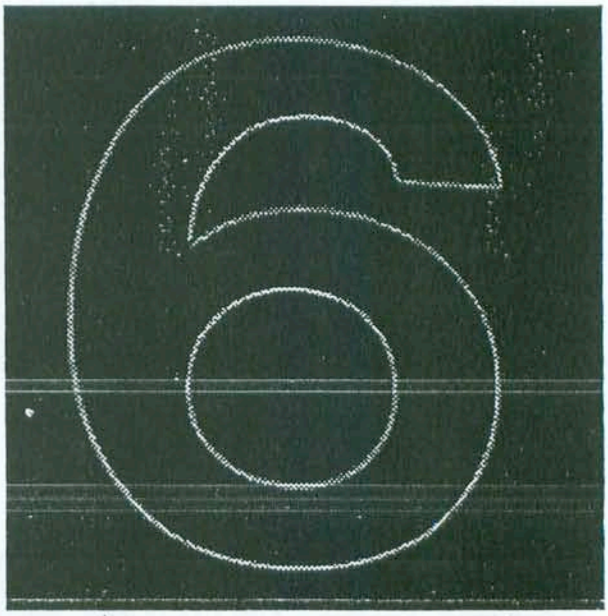

Figure 2. Image after edge detection.

11. Exporting Images to Desktop Publishing Systems

Semper provides a means of exporting images to desktop publishing systems. This is particularly useful when writing reports and papers on imaging work. The Semper application notes are examples of this technique. The Semper **postscript** command generates a PostScript™ description of an image. This can be printed directly using any PostScript™ compatible printer. When used with the **encapsulated** option it creates an encapsulated PostScript™ description of the picture. Images in this form are recognised by many desktop publishing packages and may be imported into documents.

`display 1 to display:1`	Display image in partition 1.
`postscript 1 name 'Image' encapsulated black`	Generate encapsulated PostScript™ image with a black boundary. Figure 1 shows an example of an image incorporated into a document.
`postscript 1 name 'Picture' black`	Generate a PostScript™ image with a black boundary. The PostScript™ file created here can be sent directly to a PostScript™ hard copy device.

Many Semper commands generate information in the overlay plane. Examples of this include histogram calculation and image annotation produced during image analysis. The information in the overlay plane can also be incorporated into the PostScript™ description. **postscript** used with the **partition** key generates a PostScript™ description of the image in the partition plus the overlay. It is usually preferable to have a black annotation against a white background. This can be done by creating a picture in the display with all the pixels set to the peak white value of 255. The overlay is set to black to provide contrast. Figure 2 shows the results of this procedure.

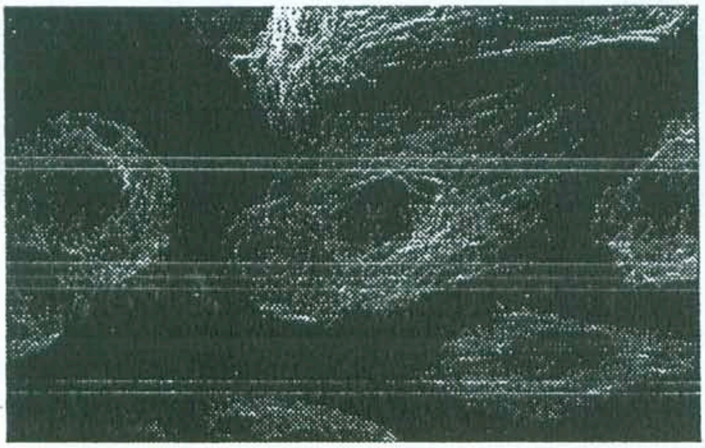

Figure 1. Exported image.

Figure 2. Overlay information.

`histogram 1 to display:2`	Calculate and display histogram.
`overlay black`	Set overlay to black.
`create display:2 size 256 byte value 255`	Create white background.
`post partition 2 name 'Hist' enc black`	Generate a PostScript™ description of display partition with the overlay set to black.

12. Diffraction Pattern Display (1)

The display of image diffraction patterns is a common requirement in transmission electron microscopy. However, the large dynamic range of typical diffraction patterns mean that some form of processing is required to display the image. Semper provides a number of means to display diffraction patterns. Consider the following example:

`display 1 to display 1`	Display source image. (Figure 1).
`mask 1 to 2 radius 110 width 10`	Smooth away picture edges.
`ps 2 to 3`	Calculate power spectrum.
`fullplane 3`	Calculate full plane.

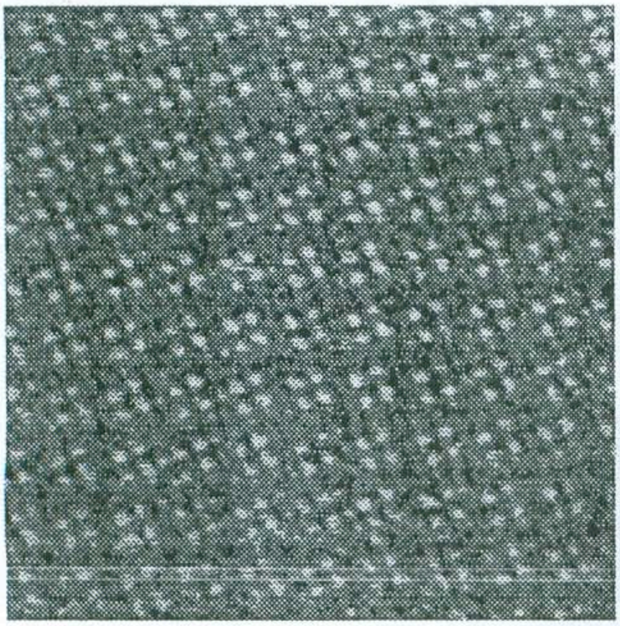

Figure 1. Original image.

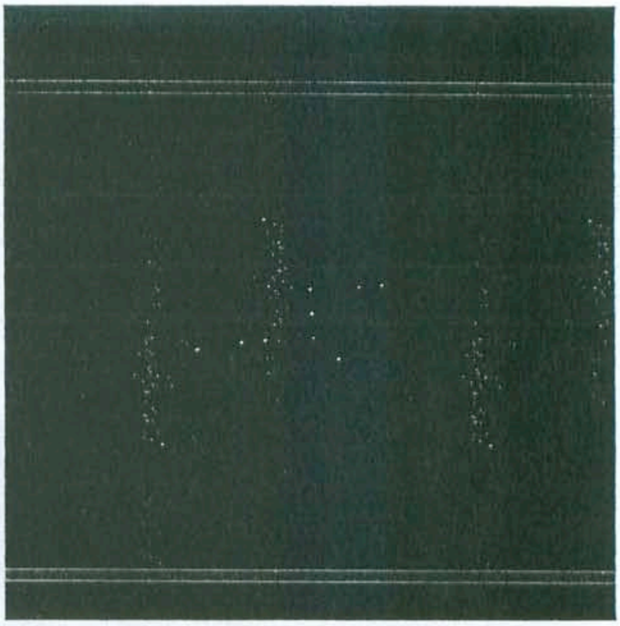

Figure 2. Diffractogram

As the power spectrum of a real image is symmetrical, initially only the half plane is calculated. The command **fullplane** reconstructs the full plane image. Any attempt to display the power spectrum directly at this point results in a single white dot in the centre of the image, so some form of dynamic range compression is required. One commonly used technique is to cause the image to saturate above some given threshold. To determine a sensible threshold the **survey** command is used. This reports the maximum pixel value present. This can then be divided by a large number, typically 1000, and this new value can be used to override the automatic display scaling by using the **preset** option with the **display** command.

`survey 3`	Report maximum and minimum pixel values.

```
Size=129,256,
Range 56.683,5.126765e13
```

`max=max/1000`	Set saturation threshold.

12. Diffraction Pattern Display (2)

`display 3 to display:1 preset`	Display using new maximum value. (Figure 2).
`max=max/100`	Try a lower threshold.
`display 3 to display:1 preset`	Display again. (Figure 3).

An alternative technique is to use logarithmic compression which can be further improved by saturating the lower half of the intensity range to black:

`ps 2 to 3 ln`	Calculate log power spectrum.
`fullplane 3`	Calculate full plane.
`survey full`	Calculate mean pixel value.

```
size 129,256
range 3.334897, 31.5523
mean 13.7179 Sd 2.0813
```

`min=mean`	Set lower threshold
`display 3 to display:3 preset`	Display result saturating lower half of the intensity range to black. (Figure 4).

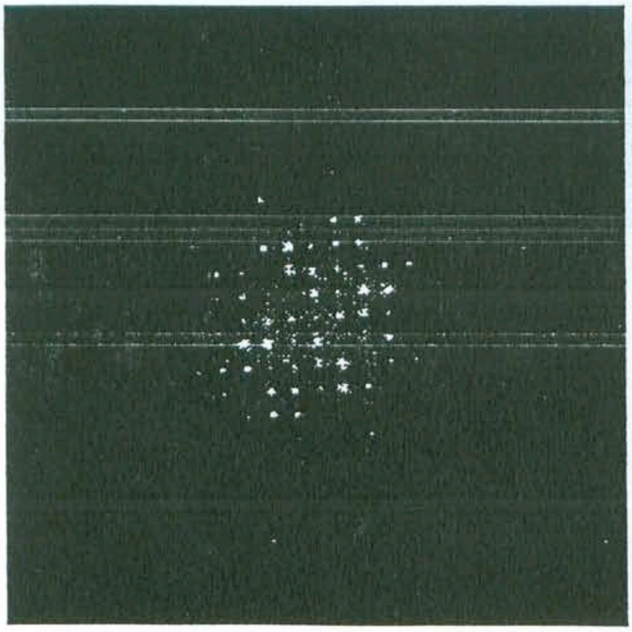

Figure 3. Diffractogram – lower saturation threshold.

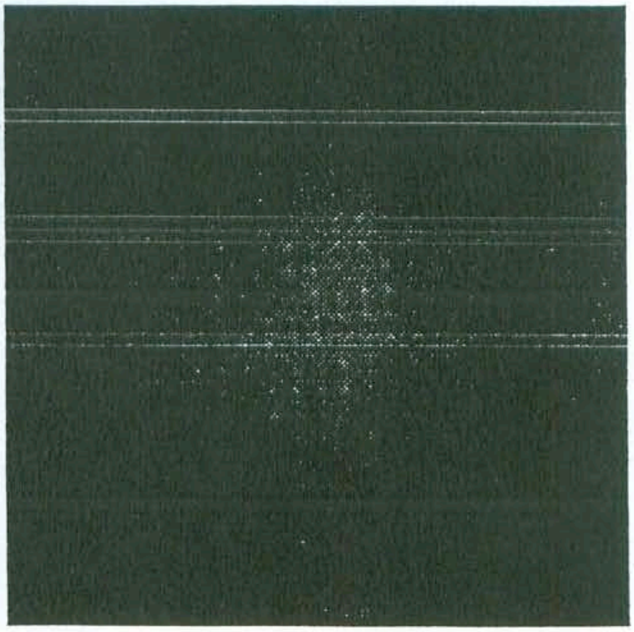

Figure 4. Log compression and saturation.

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13. Lattice Averaging using Real Space Superposition: The Imperfect Lattice (1)

The Semper 6 Image processing language has a very powerful set of features that can be used to exploit the properties of periodic structures that are often encountered in T.E.M. microscopy applications.

Repeated instances of a noisy unit cell may be averaged in various ways to obtain a clear image of the cell. There are two broad classes of technique, those based on real space averaging and those based on fourier domain methods. In this note we discuss the real space methods. Real space superposition is conceptually the simplest way of effecting a lattice average and is as efficient a way as any.

The first stage in any spatial averaging process is to obtain the positions of the unit cell of the lattice. In near perfect structures these are determined by the lattice vectors. However in many cases the lattice is distorted to such an extent that this approach is not appropriate. In these situations one technique that gives good results is to extract the unit cell motif and then cross correlate this with the original source image. The resultant correlation peaks give the cell positions for averaging. Consider this example of a negatively (PTA) stained RS layer protein from the cell wall of deinococcus radiodurans.

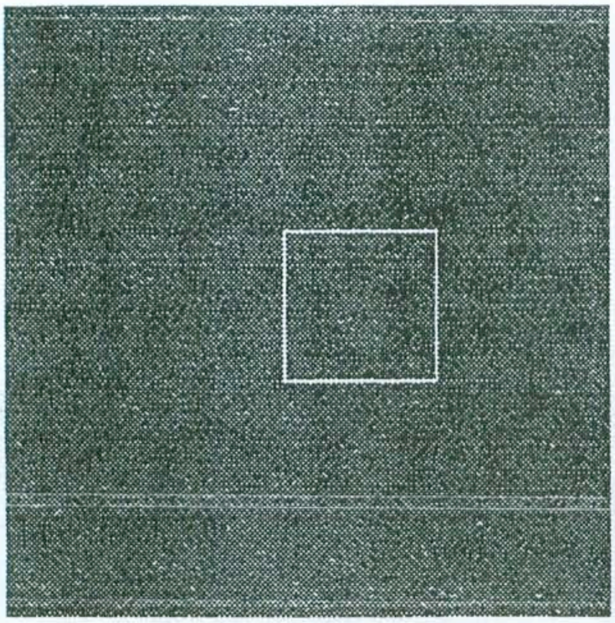

Figure 1. Original image.

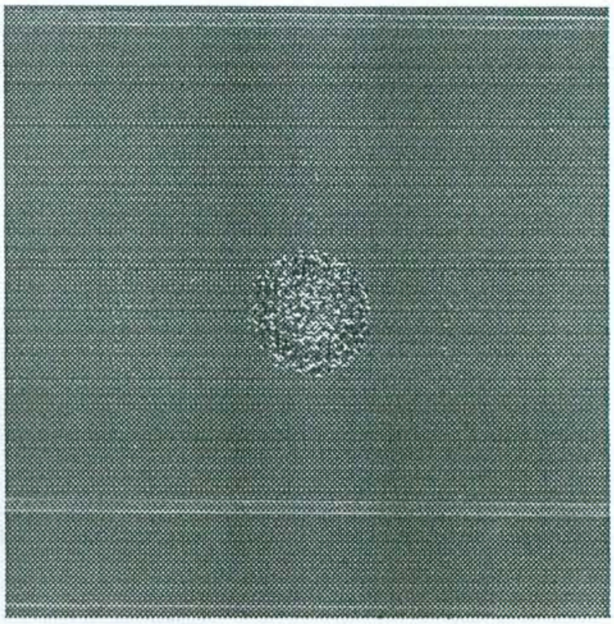

Figure 2. Extracted motif.

`display 1 to display:1`	Display image (Figure 1).
`xwires region`	Identify motif using mouse.
`create 2 size 512 byte value 0`	Create an empty picture.
`extract 1 to 3 @region`	Extract motif.
`origin 3 reset`	Reset origin.
`paste 3 to 2`	Paste into blank picture.
`mask 3 out radius 50 width 3`	Apply circular mask.
`display 3 to display:1`	Display motif. (Figure 2).

13. Lattice Averaging using Real Space Superposition: The Imperfect Lattice (2)

We have now generated the two images which when cross correlated will locate the positions of the motif sites. The display of the *xcf* image is improved if the central peak is masked out. **xcf** sets Semper variables *x,y* to the position of the central peak.

`mask 1`	Mask.
`xcf 2 with 1 to 4`	Cross correlate images.
`mask 4 inside radius 10 value 0 @xy`	Mask out main peak.
`display 4 to display:1`	Display *xcf* image. (Figure 3).

The command **peaks** may be used to compile a list of peaks in the image. In this particular case they are visually identifiable but not particularly well defined, so some of the refinements of the **peak** search command should be used. The **radius** key will position them more accurately by doing a centre of mass calculation and the **sradius** key suppresses secondary peaks within a specified radius of the main peak. The selection of the peaks is made according to a threshold which can be conveniently set to around 2.5 times the intensity standard deviation.

`survey 4 full`	Calculate standard deviation.
`peaks 4 to 5 threshold 2.5*sd +` `+radius 10 sradius 60`	Locate peaks.
`mark display:1 with 5`	Check peak detection. (Figure 4).

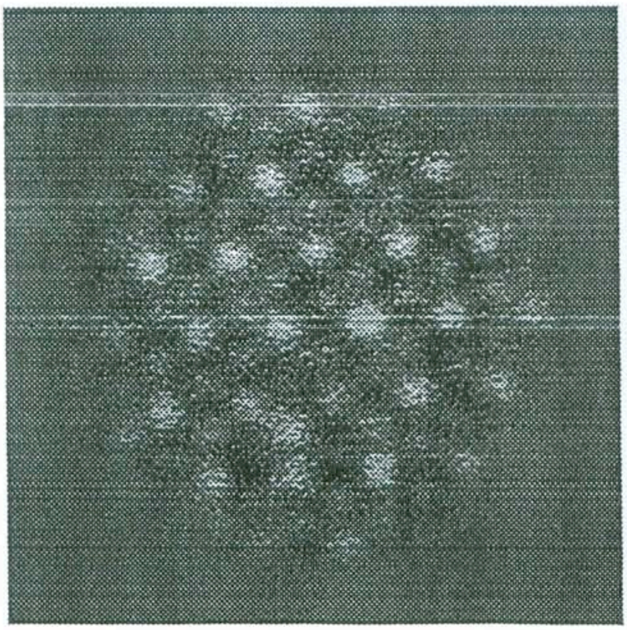

Figure 3. Cross correlated image.

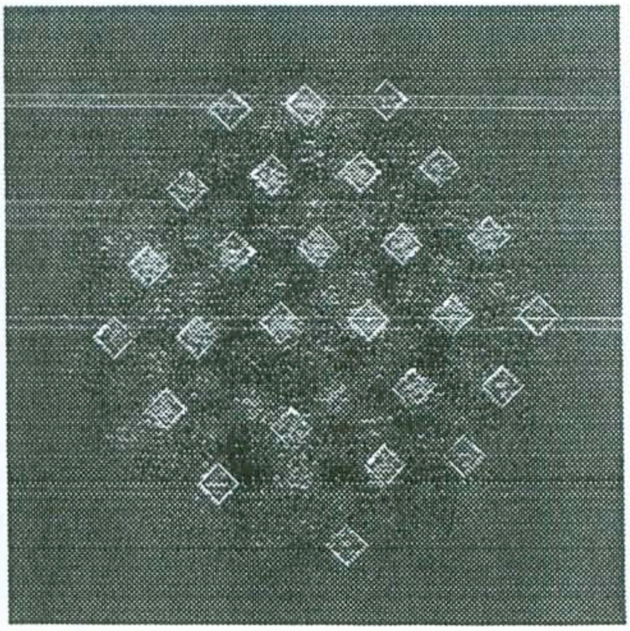

Figure 4. Verification of peak search.

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13. Lattice Averaging using Real Space Superposition: The Imperfect Lattice (3)

Having compiled a list of peaks we can now use this as a basis for the spatial averaging process.

`motif 1 to 6 with 5 size 128`	Carry out spatial average.
`display 6 to display:1 time 4`	Display and magnify result. (Figure 5).

Here we see a considerable improvement in image quality. In this particular case the sample is known to be rotationally symmetric so a further stage of averaging may be performed to exploit this fact. In order to use this property of the image we need to find the centre of rotation of the motif. This can be conveniently done by rotating the motif through 180 degrees and then cross correlating the two images. The resulting peak is twice the displacement from the centre of rotation.

`mask 6 to 7 radius 50 width 3`	Mask off edges.
`extract 6 to 8 angle pi`	Form image rotated by 180 degrees.
`mask 8 radius 50 width 3`	Mask of edges.
`xcf 8 with 7`	Cross correlate images.
`extract 6 to 9 position x/2,y/2`	Centre motif.
`extract 9 to 10 angle pi/3` `extract 9 to 11 angle 2*pi/3` `extract 9 to 12 angle pi` `extract 9 to 13 angle 4*pi/3` `extract 9 to 14 angle 5*pi/3`	Form rotated versions.
`calculate :9+:10+:11 to 100 integer`	Add first three rotated versions.
`calculate :100+:12+:13+:14`	Add next three rotated images.
`mask 100 outside radius 64`	Mask.
`display 100 to display:1 times 4`	Display magnified image. (Figure 6).

13. Lattice Averaging using Real Space Superposition: The Imperfect Lattice (4)

The rotational averaging has further improved the detail in the image.

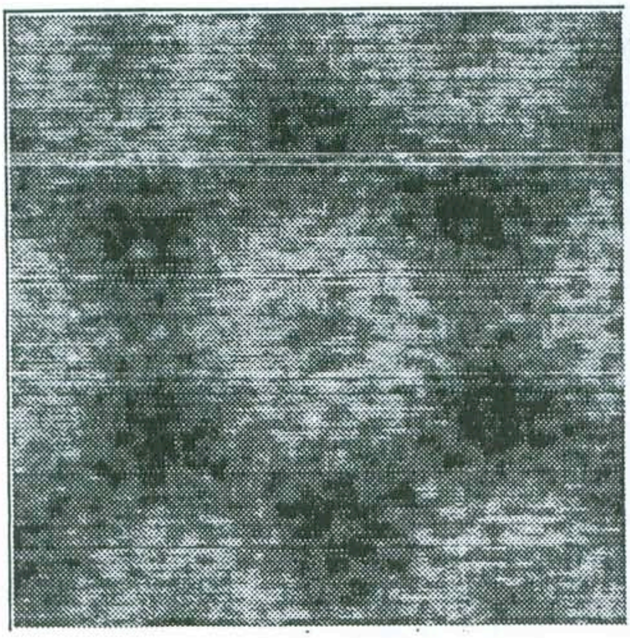

Figure 5. Spatially averaged image.

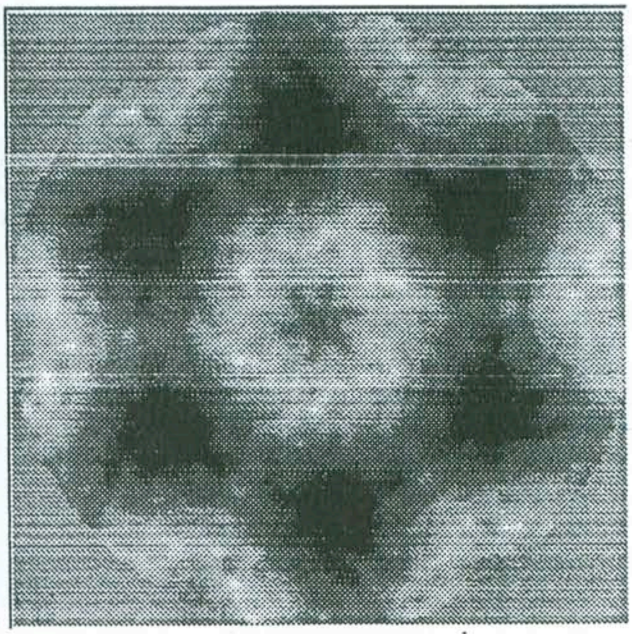

Figure 6. Image after rotational averaging.

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14. Lattice Averaging Using Real Space Superposition Techniques (1)

The Semper 6 image processing language has a very powerful set of features that can be used to exploit the properties of periodic structures that are often encountered in materials science T.E.M microscopy applications.

Repeated instances of a noisy unit cell may be averaged in various ways to obtain a clear image of the cell. There are two broad classes of technique, those based on real space averaging and those based on fourier domain methods. In this note we discuss the real space method. Real space superposition is conceptually the simplest way of effecting a lattice average and is as efficient a way as any.

We first consider an example of real space averaging in the case of the near perfect lattice of a block oxide near $GeNB_9O_{25}$ in composition. The first step in this process is to obtain an estimate of the lattice vectors *u,u2* and *v,v2*. In a perfect lattice all the positions of the instances are determined by the lattice vectors. If the periodicity is visible then the library program **lattice** can be invoked. This asks the user to identify and index two independent lattice sites using the mouse. It calculates the lattice vectors *u* and *v* from this information.

```
display 1 to display:1
```

Display the image of GeN_9B0_{25}. (Figure 1).

```
library lattice
mark an origin
mark some other point you can index
and enter its indices ie(4,1): 1,0
mark another independent point
and enter its indices : 0,1
```

Run the library program called *lattice*.

```
Base Vectors U=26,1.5 length=26.0432
V=-1.5,2 length=26.0432
```

Semper reports the lattice vectors and the lattice is superimposed. (Figure 2).

```
angle (U to V)= 1.5708
```

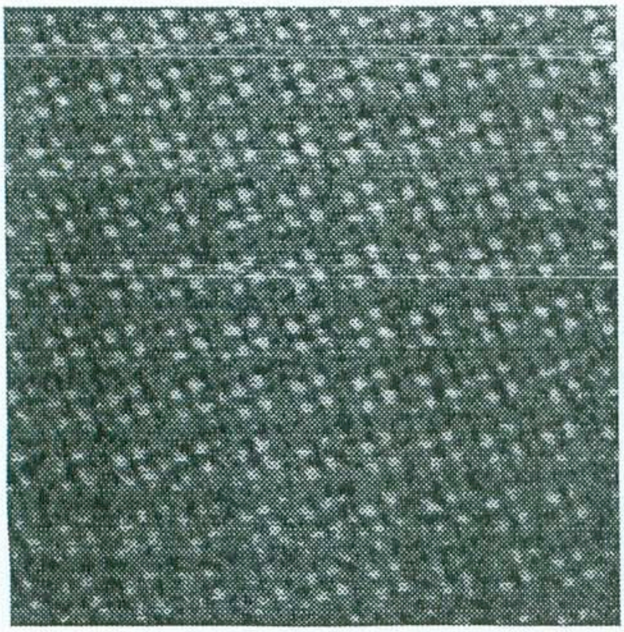

Figure 1. Original image.

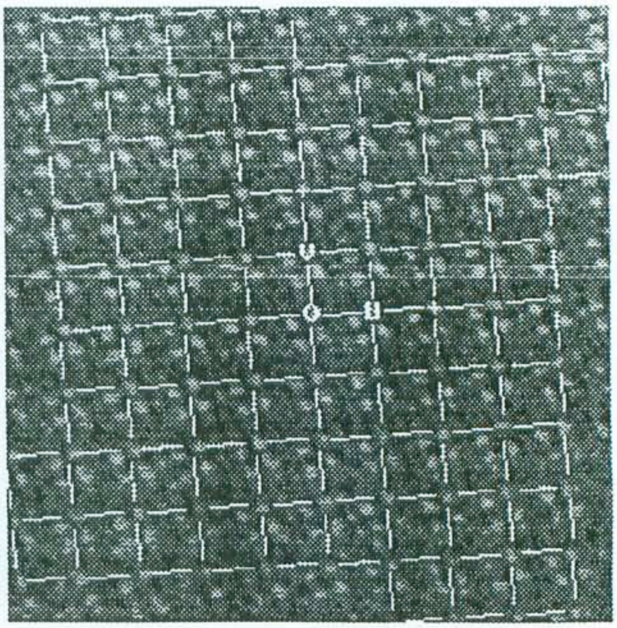

Figure 2. Image with superimposed lattice.

14. Lattice Averaging Using Real Space Superposition Techniques (2)

If the image quality is poor it is often not possible to identify accurately the position of the lattice sites by eye. One technique that can be used with a perfect lattice is to calculate the autocorrelation function of the picture. This reveals the position of periodicities as peaks in the *acf* image. Prior to calculating the *acf* the image should be masked to two thirds radius to prevent spurious peaks due to the correlation process wrapping around at the edges of the picture.

`mask 1 to 2`	Mask the original image.
`acf 2 to 3`	Calculate the autocorrelation function.
`display 2 to display:1`	Display the masked image. (Figure 3).
`display 3 to display:2`	Display the autocorrelation function. (Figure 4).

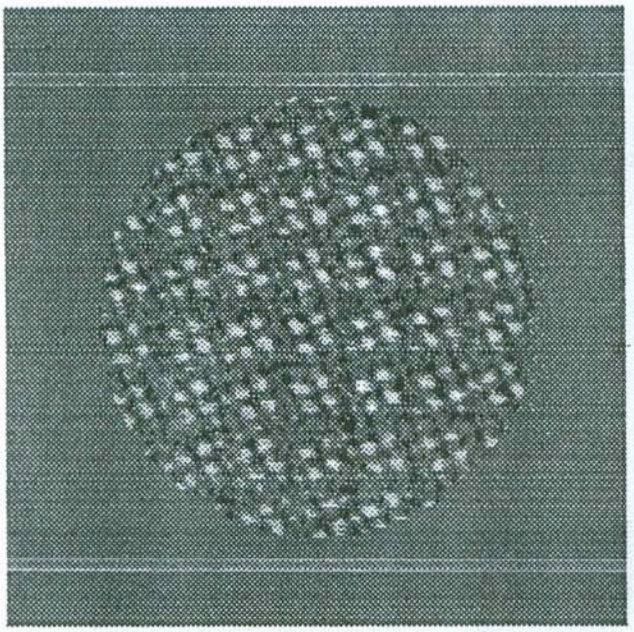

Figure 3. Masked image.

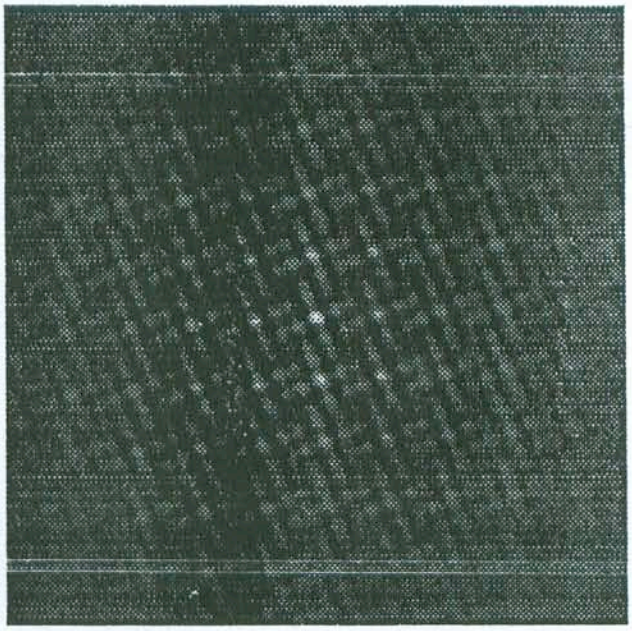

Figure 4. Autocorrelation image.

The display of the *acf* can be improved by masking out the dominant central peak. In addition high pass filtering removes background variations.

`mask 3 inside radius 5 value 0`	Mask out the central peak.
`hp 3 over 15 to 4`	Remove background variations.
`display 4 to display:1`	Display the masked filtered result. (Figure 5).

In the case of the near perfect lattice, the peaks in the autocorrelation picture may be directly identified with the lattice sites. The library routine **lattice** may then be invoked which asks the user to identify and index independent peaks in the same manner that was used previously. Or alternatively an automatic process may be used to identify the lattice vectors *u* and *v*.

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14. Lattice Averaging Using Real Space Superposition Techniques (3)

The Semper command **find** can be used to locate the dominant peak. This sets the variables *x,y* to the co-ordinates of the dominant peak. If we assume that the lattice symmetry is known we can rotate this vector through the appropriate angle to obtain an estimate for the second lattice vector.

`find 4`	Find the largest peak and set to *x,y*.
`mark display:1 @xy`	Mark the largest peak.
`u=x;u2=y`	Set *u*.
`th=pi/2`	Set the angle.
`v=u*cos(th)+u2*sin(th)`	Rotate *u* to find an estimate for *v*.
`v2=-u*sin(th)+u2*cos(th)`	

The Semper command **peaks** compiles a list of peaks in the image when given a threshold value. Typically this would be set to around half the maximum intensity value in the image.

The estimate of the lattice vectors may be refined by using a least squares curve fitting technique. The command **base** can then be used to perform a least squares fit to adjust the initial estimates of *u* and *v*. A tolerance value is given to **base** whereby it rejects peaks that fall outside a specified range. The command **base** produces an edited peaks list that contains the peaks that fall within the fitting tolerance. This is expressed as a proportion of the lattice vector. This list can be used to mark the image to check the process of fitting.

`peaks 4 to 5 threshold max*0.4`	Compile a list of peaks.
`base 5 to 6 tolerance 0.1`	Edit out peaks not on lattice sites.
`display 4 to display:1`	Display the *acf* picture.
`mark display:1 with 6`	Mark the fitted lattice sites. (Figure 6).

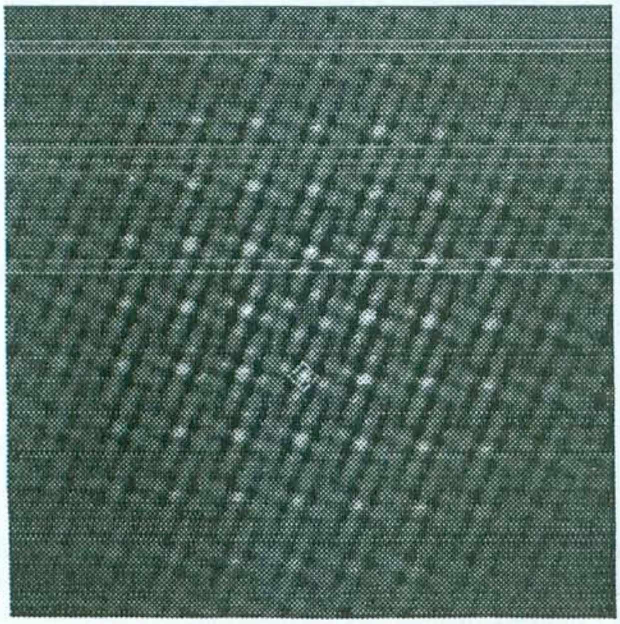

Figure 5. Acf image marked with largest peak.

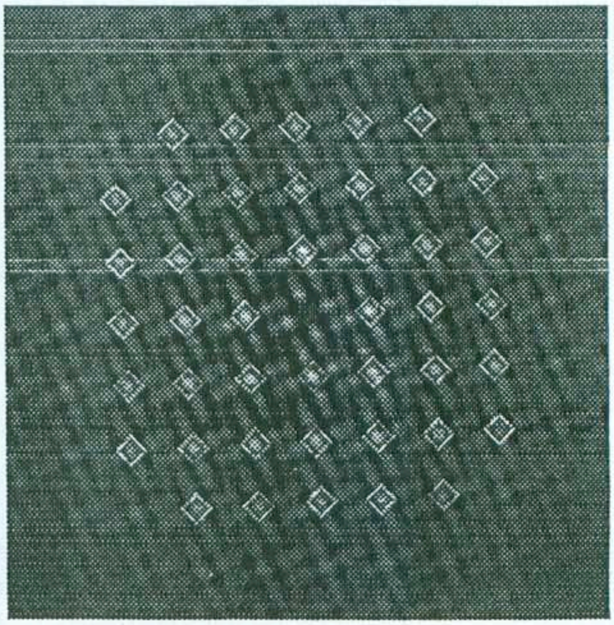

Figure 6. Verification of peak search.

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14. Lattice Averaging Using Real Space Superposition Techniques (4)

Having obtained an accurate estimate of the lattice vectors *u* and *v* we are now in a position to perform the spatial averaging on the perfect lattice. The Semper command **lattice** will generate a list of lattice sites given *u* and *v*. This list can be used by the **motif** command to perform spatial averaging on the picture.

`lattice to 7`	Generate a list of lattice sites from *u* and *v*.
`motif 1 to 8 with 7 size 32`	Perform spatial averaging.
`display 8 to display:1`	Display the result. (Figure 7).

The small motif image shows considerable improvement in detail. We can reconstruct the image of the whole crystal by fitting together unit cells. This is done using the Semper command **extract** with the **uv** option. The unit vectors for the extraction process are calculated by dividing *u* and *v* by the motif size, in this case 32. The whole crystal is then reconstructed by re-interpolating over a large area again using **extract** with the **uv** option. The initial extraction distorts the image. The sampling grid required to reverse the distortion is calculated using the library program **invert.**

`u=u/32,u2/32;v=v/32,v2/32;`	Calculate unit vectors for resampling grid.
`extract 8 to 9 uv size 32`	Extract the unit cell.
`library invert` **`unit cell dim(s) ? 1`** **`new vectors: u=1.27592,-0.0510367`** **`v=0.0510367,1.27592`**	Calculate a new sampling grid. Answer 1 when prompted for the dimensions of the unit cell.
`extract 9 to 10 uv size 256`	Reconstruct the crystal from the unit cell
`display 10 to display:1`	Display the result. (Figure 8).

Figure 7. Averaged unit cell.

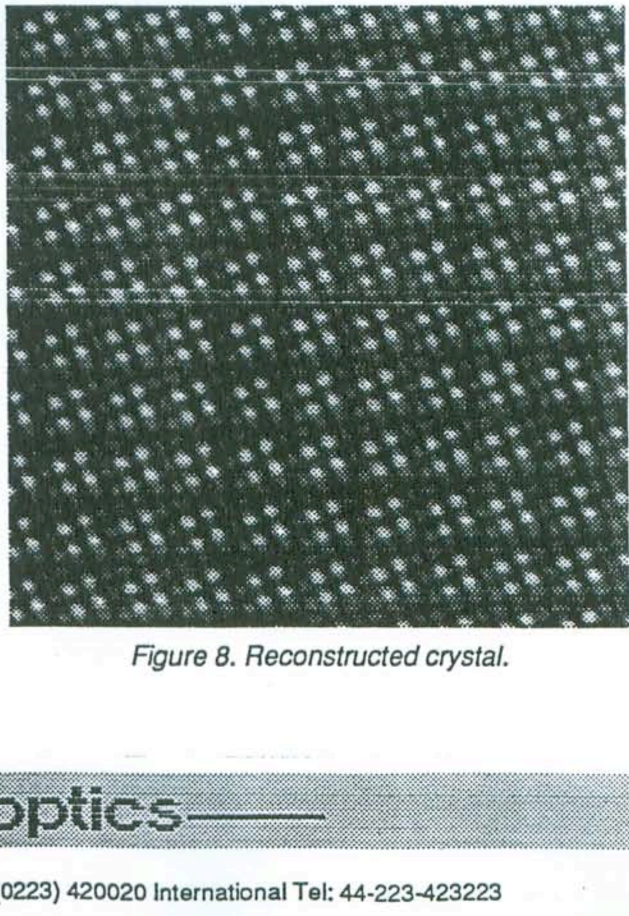

Figure 8. Reconstructed crystal.

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15. Visualisation of Scanning Tunnelling Microscope Images (1)

Semper 6 provides the facilities to visualise the images of surface topography produced by the scanning tunnelling microscope.

Figure 1 shows a synthetic S.T.M. image displayed conventionally with intensity proportional to surface height.

The Semper command **sheet** enables this to be visualised as a surface which can be viewed and lit from a specified angle.

`display 1 to display:1`	Display the conventional image. (Figure 1).
`sheet 1 to display:2`	Display the surface using default viewing angles and illumination conditions. (Figure 2).

The surface is first rotated clockwise about the *z* axis through the angle specified by the key **theta.** It is then rotated about the *x* axis through an angle specified by the angle **psi**. If these angles are not specified then the angles **theta** and **psi** default to pi/4.

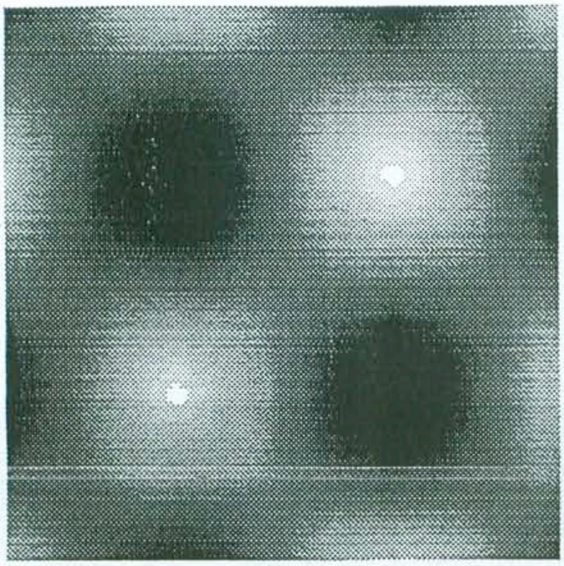

Figure 1. Synthetic S.T.M. image.

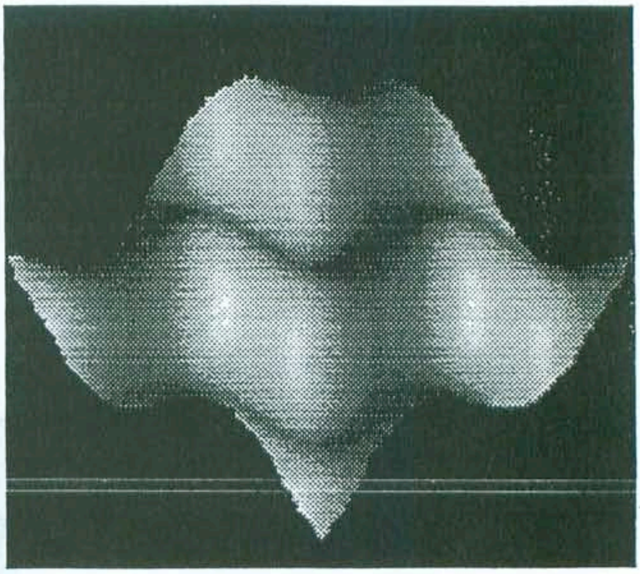

Figure 2. Visualisation of the surface.

sheet allows you to specify intensities of three sources of illumination. These are a fixed source, a non directional ambient source and a movable source.They are identified by the keywords **main, ambient** and **forward.** The position of the forward illumination may be specified by the angle keys **lphi** and **ltheta.**

```
sheet 1 to display:3 theta pi/9 psi pi/3
+ main 50 ambient 100 forward 50
+ ltheta pi lphi pi
```

Display the surface at the specified orientation using the selected lighting conditions. (Note that a plus sign (+) denotes a Semper continuation line).

15. Visualisation of Scanning Tunnelling Microscope Images (2)

Figure 3 shows the image displayed under these new conditions.

Another factor that is under the control of the user is the scaling and origin of the *z* values in the picture. The **range** key specifies the range of heights to be represented by actual pixel values. Figure 4 shows the original image displayed with a modified range setting.

`sheet 1 to display:4 range 1,100`	Display with heights between 1 and 100. (Figure 4).

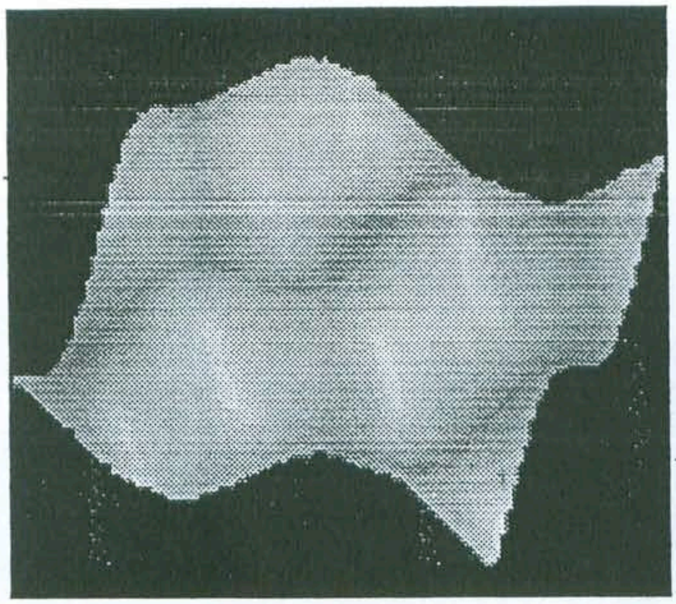

Figure 3. Change of viewing conditions.

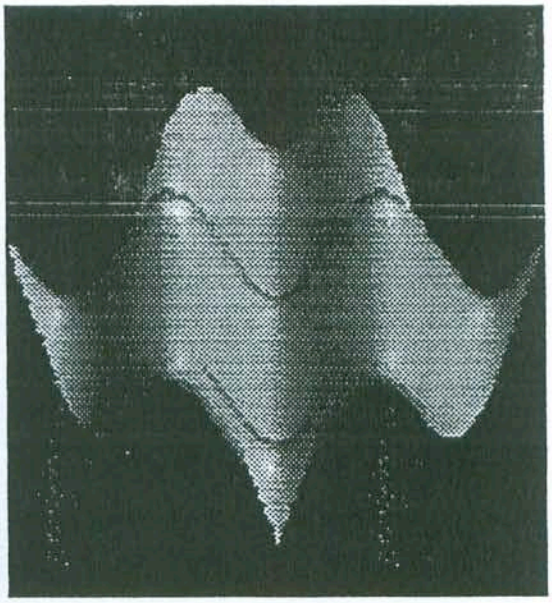

Figure 4. Change of scaling.

16. Interference Pattern Analysis (1)

Semper 6 provides various means to analyse the interference fringes produced by interferometer equipment. A typical interference pattern is shown here in Figure 1.

A typical requirement would be to count and position fringes defined along a line centered on the fringe pattern. The original image has significant intensity shading across it. This is a problem because the peak searching algorithms that might be used to detect the fringe positions require a fixed threshold to be specified. In addition the image is marked by a number of artefacts which could confuse any direct counting process. A number of processing operations can be applied to overcome these problems.

`display 1 to display:1`	Display the fringe pattern. (Figure 1).
`hp 1 over 25 to 2 integer`	High pass filter to remove background.
`display 2 to display:2`	Display the filtered result. (Figure 2).

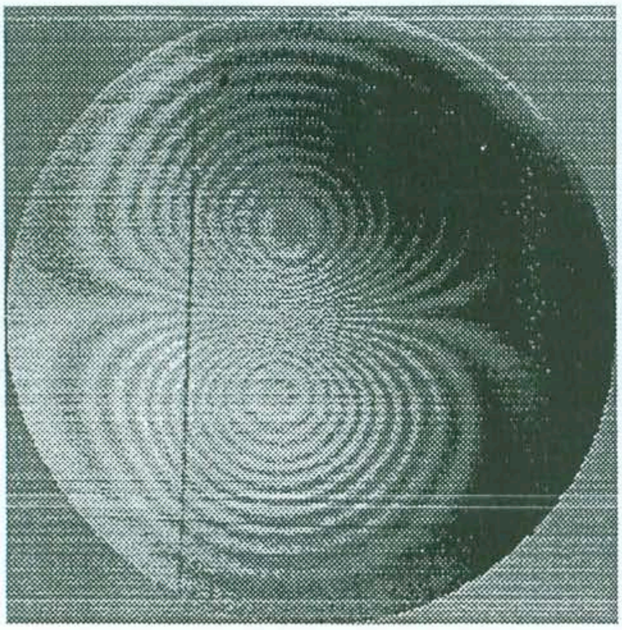

Figure 1. Original image.

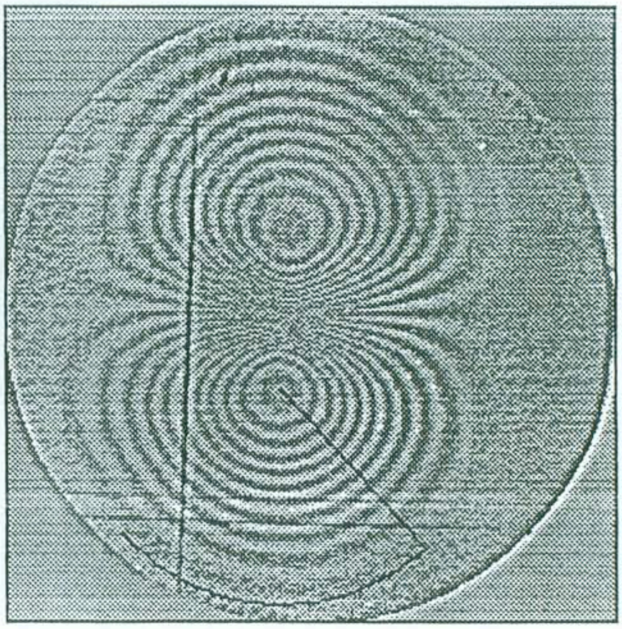

Figure 2. High pass filtered image.

The high pass filtering operation removes the background intensity variations but it has the undesirable effect of increasing the noise level in the image. The signal to noise ratio may be improved by means of a radial averaging process over a defined arc. The starting position for this process is defined by drawing a line using the mouse.

`xwires line`	Define a line using the mouse.
`mark display:2 position x,y radius r` `+ angle theta-pi/2,theta`	Mark swept area for averaging. (Note that a plus sign (+) denotes a Semper continuation line).
`section 2 to 3 fp position x,y` `+ angle theta width pi/2`	Perform radial averaging.

16. Interference Pattern Analysis (2)

The sectioning process generates a one dimensional picture with a size twice that of the line used to define the arc. The lower half of this picture contains the radial average of interest. The upper section contains the radial average of the upper region defined by the section.

`extract 3 to 4 left size 170,1`	Extract region of interest in the 1-D picture.
`display 4 to display:3`	Display the 1-D picture. (Figure 3).

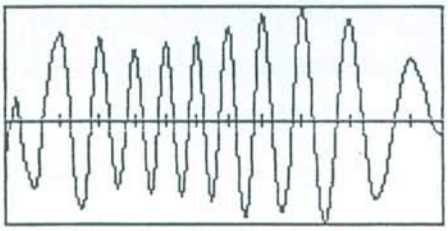

Figure 3. Result of radial average showing fringes.

This image clearly shows a noise free representation of the fringes. A peak search procedure (**peaks** command) can be applied to this picture to locate the position and number of peaks. This information may be used to mark the display to cross check the peak detection procedure.

`peaks 4 to 5 threshold max*0.4`	Count and locate the fringes.
`mark display:3 with 4`	Mark the display with detected positions. The peak search command returns the number of peaks detected in the variable *n*.
`type n`	Report the total number of fringes counted.

17. Extracting a 2-D Slice from a 3-D Image

A common requirement when working with 3-D data sets is to be able to extract an arbitrary two dimensional slice and display it. Such three dimensional data sets could be produced for example, whilst observing how some processes evolve in time. The initial data is a set of two dimensional pictures each separated by a certain time interval. For the purposes of this example the sequence of pictures range from 1 to 32 and are of size 30,30. These images may be stacked together to form a single three dimensional image.

`stack 1,32 to 100`	Stack 2-D pictures together.

The section of interest may be identified by displaying the top layer of the picture and then using the mouse to identify the section of interest. Each layer is displayed below in Figure 1.

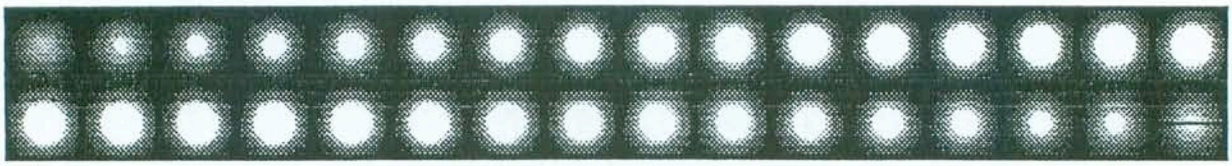

Figure 1. Individual layers of 3-D picture. Section line shown in bottom right hand corner.

`display 100 layer 1 to display:1`	Display the top layer of a 3-D picture.
`xwires section`	Draw out a section of interest with the mouse.
`extract 100 to 101 @section`	Extract a 2-D slice to make a picture with the dimensions 30,1,32.

The extraction has been performed but the image is the 'wrong way round' to be displayed. It needs to be of dimensions 30,32,1. The picture can be turned around by successive applications of the **paste** command.

`pcb 101`	The **pcb** command sets variables *ncol* and *nlay* to the number of columns and rows of picture 101.
`create 103 size ncol,nlay`	Create an empty picture.
`for n=1,nlay` `paste 101 to 103 top near pos 0,1-n,n-1` `loop`	Copy each layer of the transposed picture to the output picture.
`display 103 to display:2`	Display a picture of dimensions 30,32,1 (Figure 2).

Figure 2. 2-D picture extracted along section line.

18. Particle Analysis with Semper 6 (1)

Semper 6 incorporates an extensive set of image analysis capabilities that can be used to extract information about objects or particles in an image.

Consider the image in Figure 1 of a mylinated nerve fibre. A typical requirement would be to obtain measurements of the outer and inner region of each fibre in the image.

The analysis procedure identifies each particle according to an upper and lower threshold and on the basis of this information counts and measures them.

The method used to identify the best threshold levels to use involves highlighting the grey level between the upper and lower thresholds to full intensity on the display. Semper provides the means to carry out this procedure interactively using the mouse. The **ladjust** command can be used to couple mouse movement in one direction to the lower threshold and movement in the other direction to the upper threshold. The initial range to be highlighted is specified by *a* and *b*. These are calculated from the minimum and maximum intensity values that are found when the source picture is displayed.

`display 1 to display:1`	Display source image (Figure 1).
`a=min+(max-min)/4` `b=min+3*(max-min)/4`	Calculate initial highlight range.
`ladjust upper lower scale display` `+ initial range a,b`	Enable interactive threshold (Note that a plus (+) sign denotes a Semper continuation line)

Terminating the thresholding procedure with a press of the mouse key sets the Semper variables *r*,*r2*. The scale option causes these values to be scaled correctly to be used as thresholds for the **analyse** command. Figure 2 shows highlighting in operation.

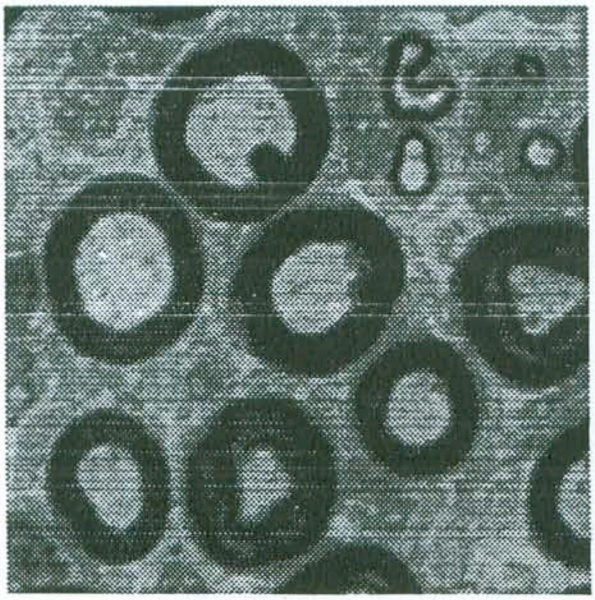

Figure 1. Original image.

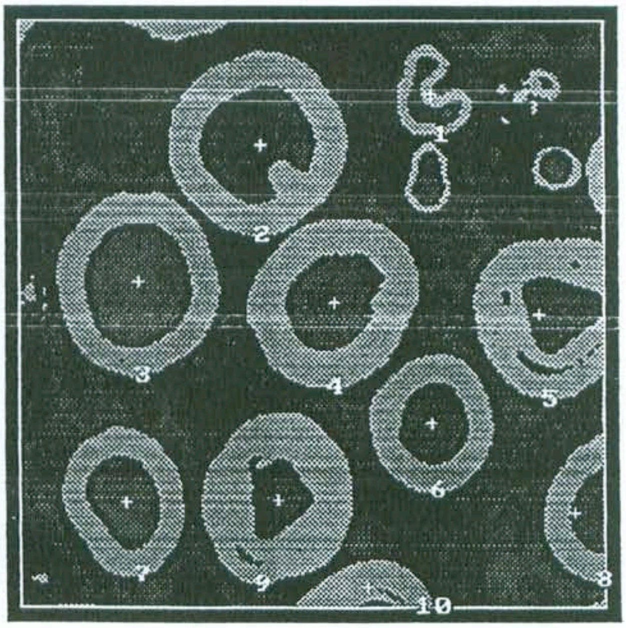

Figure 2. Highlighting grey levels between thresholds.

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18. Particle Analysis with Semper 6 (2)

`analyse 1 segment 100 ge r le r2` `+ area 400`	Perform image analysis.
`pmark display:1 cm id`	Annotate the image.
`type 'index area feret diameter'`	Annotate the table.

In the above example an area threshold has been applied so that particles with an area of less than 400 pixels are ignored. The analysis procedure generates two sets of information, a list of particle parameters and a segmented image, where the outline of each particle is stored. This information may be used to annotate the image. In this particular example we wish to measure and tabulate the area and inner and outer diameters of the cells of interest. As cells are not perfectly circular we need to be more specific about the meaning of diameter. One useful measurement that can be done in these circumstances is to determine the angle of the major axis of the particle and then calculate the feret diameter at this angle.

The Semper command **pferet** calculates the feret diameter at an arbitrary specified angle using the particle outline in the segmented image. The diameter is returned in the variable *f*. The angle of the major axis, *theta* is one of the parameters calculated by the **analyse** command. A set of criteria can be applied to filter the data generated so that only particles of interest are selected. In this particular case the selection criteria used are that the particle does not touch the edge of the frame and that it has only one hole. This process is carried out by examining the edge contact flag and the number of holes reported for each particle. These are stored in the Semper variables *ec* and *h* .

In the following Semper program we step through each particle in the parameter list and access the image analysis parameter for each particle using the **pset** command. The total number of particles in the list may be obtained by setting *count* with the command **pset** . This returns the number of particles in the variable *n*. The feret diameter is calculated and the properties of particles of interest are printed out.

`type 'index area feret diameter'`	Annotate the table.
`pset count`	Access the total number of particles.
`for i=1,n`	Particle count in *n*.
`pset all index i`	Access the data for each particle.
`pferet angle theta nover`	Calculate the feret diameter.
`if ec=0 & h=1 type i,' ',f,' ',a`	Type the index, area and diameter.
`loop`	

```
index area   feret diameter
1     540    40.5577
2     3040   82.1073
3     2415   81.1594
4     2932   80.2968
6     1672   61.0407
7     1625   66.0853
```

The text printed on the screen may be directed to a text file to retain a permanent record of the results.

`assign new file name 'results'` **`device 6 assigned`**	Assign a log file for the results.
`echo console device 6`	Send console output to the logfile.

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19. Image Geometry Correction (1)

Many imaging devices based on raster scanning systems, such as scanning electron microscopes or tube cameras, suffer some form of geometrical image distortion. Some of the mechanisms that give rise to this can be differing scan system sensitivities in the two scan directions or non-orthogonal alignment of the scanning coils. In some situations, such as scanning electron microscope operation under low magnification conditions, non-linear distortion becomes significant.

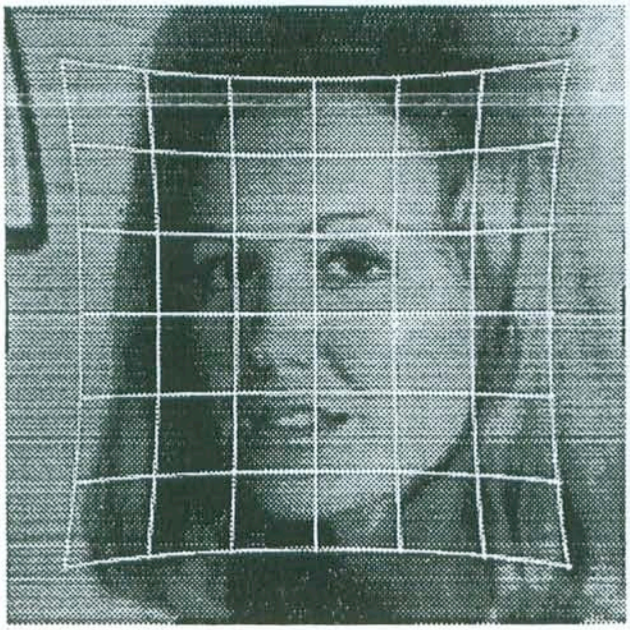

Figure 1. Pincushion distortion.

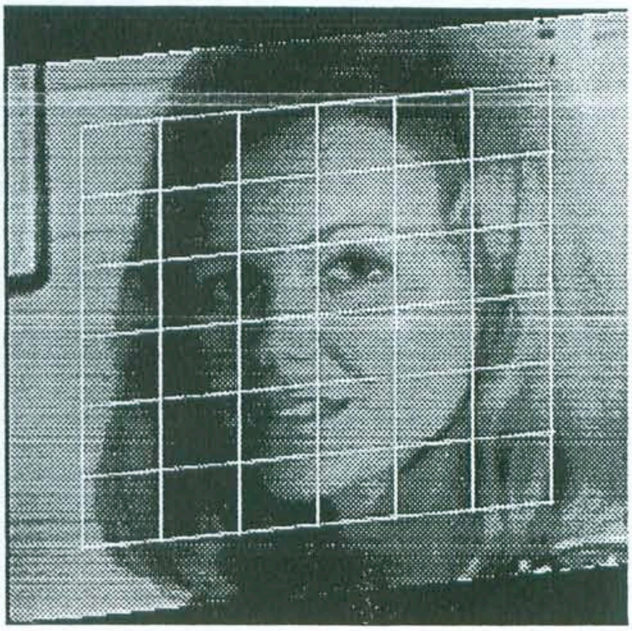

Figure 2. Skew distortion.

Figures 1 and 2 show skew and pincushion distortion in a tube camera. A grid is superimposed on the image to show the effect more clearly.

The Semper command **warp** can be used to correct for image distortions in such situations. This command warps the picture using a polynomial, the coefficients of which are calculated from two sets of control points that are supplied in the form of points list pictures. The *map* points list contains the co-ordinates of a set of objects in the image whose position is accurately known. The *image* points list contains the positions of the same set of objects expressed in terms of the image co-ordinate system.

Before the process of correction can be carried out for an arbitrary image, control point lists have to be generated. One way to do this is to produce a test pattern with a grid of points of known spacing. The positions of points on the grid form one of the lists of control points. The second set of points are obtained from measuring where these points appear in terms of the uncorrected image co-ordinates when the test pattern is viewed through the distorting imaging system. The Semper **analyse** command provides a convenient way of doing this.

Figure 3 overleaf shows a test image that could be used for the purposes of distortion calibration. The analysis procedure identifies individual particles according to an upper and lower grey level threshold level and on the basis of this information counts and measures them. The method used to identify the best threshold levels is to highlight the grey levels between the upper and lower threshold to full intensity.

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19. Image Geometry Correction (2)

Semper provides the means to do this procedure interactively using the mouse using the **ladjust** command. Horizontal movement of the mouse is used to change the upper threshold, and vertical movement is used to alter the lower threshold. The initial range of values to be highlighted is specified by *a* and *b*. These are calculated from the maximum and minimum intensity values that are found when the source picture is displayed.

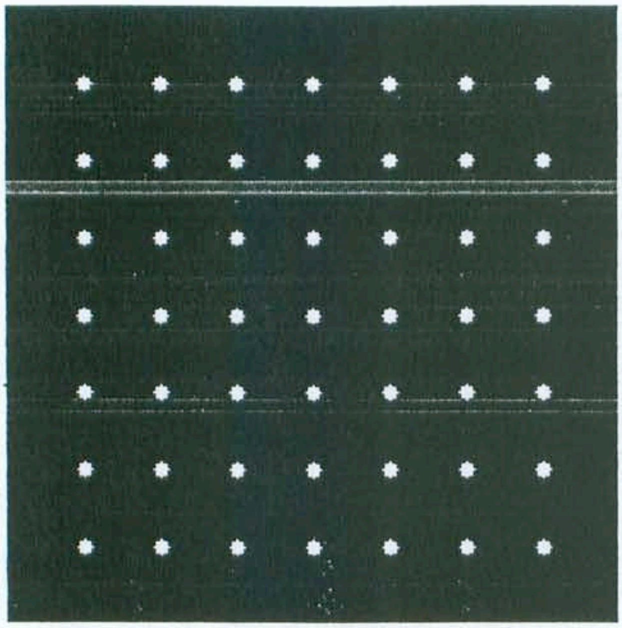

Figure 3. Distortion measurement test pattern.

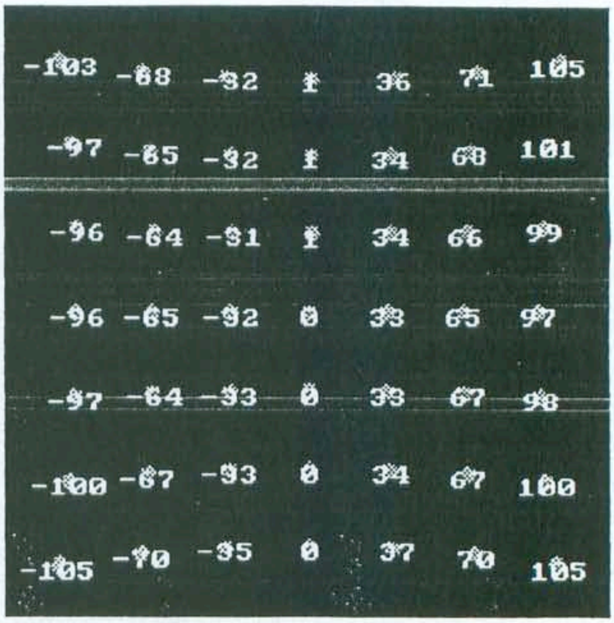

Figure 4. Pattern viewed through distorting camera.

Figure 4 shows the test pattern viewed through the distorting imaging system. It has been thresholded prior to analysis and annotated with the measured *x* coordinates of the dots.

`display 1 to display:1`	Display the image of a test chart.
`a=min+1/4*(max-min);b=min+3/4*(max-min)`	Calculate the initial highlight values.
`ladjust upper lower scaled display` `+ initial range a,b`	Enable interactive thresholding. (Figure 4).

Once the optimum thresholds have been determined the thresholding process is terminated using a mouse key. This sets the Semper variables *r,r2* to the correct highlight values.

The following Semper program generates the two sets of points lists,one measured and the other calculated. To keep track of which dot is which the analysis is performed on small subregions of the image that each contain one dot. This can be done because the approximate position of each dot is known. The **position** and **size** keys in the **analyse** command are used for this.

`create 500 size 49,1,2 plist value 0`	Create empty points list.
`create 501 size 49,1,2 plist value 0`	Create empty points list.
`x=-128`	Set up subregions for analyse.
`y=-128`	
`s=32`	

19. Image Geometry Correction (3)

`for i=1,7;for j=1,7;xp=x+j*s;yp=y+i*s;` `analyse 5012 position xp,yp size 32` `+ ge r le r2`	Run **analyse** for each subregion inside two nested loops. (Note that a plus sign (+) denotes a Semper continuation line).
`ptype xcen ycen`	Report the dot positions.
`pset xcen ycen`	Set the positions in the variables *xc* and *yc*.
`pmark xcen`	Annotate the display with *x* position.
`select 500`	
`p (i-1)*7+j-1,0,0=xc` `p (i-1)*7+j-1,0,1=yc`	Insert the image point data in points list 500.
`select 501`	
`p (i-1)*7+j-1,0,0=xp` `p (i-1)*7+j-1,0,1=yp`	Insert the map point data in points list 501.
`loop` `loop`	

The Semper variables *xc* and *yc* contain the centre of mass for each of the dots. The command **pset** is used to access them. They are stored in the points list (*Plist*). After this program has run the map co-ordinates are in *Plist* 501 and the image co-ordinates are in *Plist* 500. As the map and image co-ordinates that characterise the distortion are known it is possible to correct an arbitrary image.

`display 1 to display:1`	Display the original image. (Figure 5).
`warp 1 to display:2 map 500` `+ image 501 order 3 bicubic`	Correct and display the image. (Figure 6).

The order of the polynomial used to do the correction can be specified using the **order** key. It will default to 2. Pincushion distortion is at least of an order of 3.

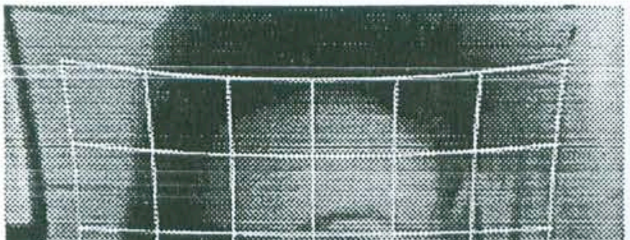

Figure 5. Image viewed through a distorting camera.

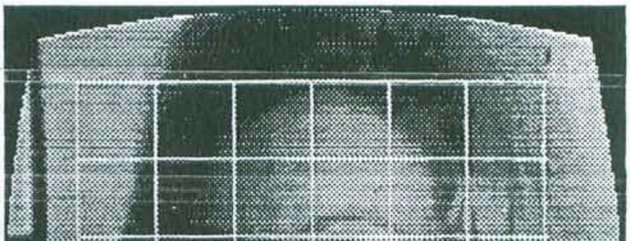

Figure 6. Geometry correction applied.

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20. Pixel Aspect Ratio and Framestore Video Sampling Frequency

In choosing a framestore device on which to run any image processing software such as Semper 6 one of the most important considerations is that of the pixel aspect ratio. It is very desirable to have equal sampling rates in the *x* and *y* directions across the image, or in other words square pixels.

There is a very simple way to cross check the claims of framestore vendors regarding the pixel aspect ratio of their framestores. For each of the two T.V. standards there exists just one correct sampling frequency for perfectly square pixels.

Consider first the C.C.I.R. T.V. standard. The following characteristics are important when calculating the sampling frequency required for square pixels.

C.C.I.R . Standard	
Number of lines:	625
Number of blanking lines:	50
Line time:	64µs
Active line time:	52µs
Screen aspect ratio:	4/3
Frame time:	1/25 sec

The C.C.I.R. system scans 625 lines of which 50 are blanked making 575 lines visible. The 4/3 screen aspect ratio means that there should be 575 x 4/3=767 pixels along the active video line. This number of samples has to be taken in the 52 µs of the active line. The sampling rate is thus 767/52µs=14.74Mhz.

Consider the N.T.S.C. standard .

N.T.S.C. Standard	
Number of lines:	525
Number of blanking lines:	38 or 42
Line time:	63.5555µs
Active line time:	52.6555µs
Screen aspect ratio:	4/3
Frame time:	1/30 sec

The N.T.S.C. system scans 525 lines of which 487 or 483 are visible. The 4/3 aspect ratio means that there should be 487x4/3=649 or 483x4/3=644 pixels along the active line. This number of samples has to be taken in the 52.6555µs of the active line giving a sampling frequency of 649/52.6555µs=12.33 Mhz or 644/52.655µs=12.23 Mhz.

In practice it is not always convenient to use the exact frequency given by this calculation. The *Synoptics'* **Synergy** framestore samples at 15 Mhz which means that 780 pixels are sampled in the active line period. 768 pixels are stored and displayed. The 15 Mhz sampling rate gives a pixel aspect ratio of 1 : 1.017 in C.C.I.R. mode. In N.T.S.C. mode the pixel aspect ratio is 1 : 1.216 or 1 : 1.226.

To summarize, check the sampling frequency of your vendor's framestore and make sure that it is consistent with the pixel aspect ratio specification. In several cases we have noticed that inconsistent figures are quoted.

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