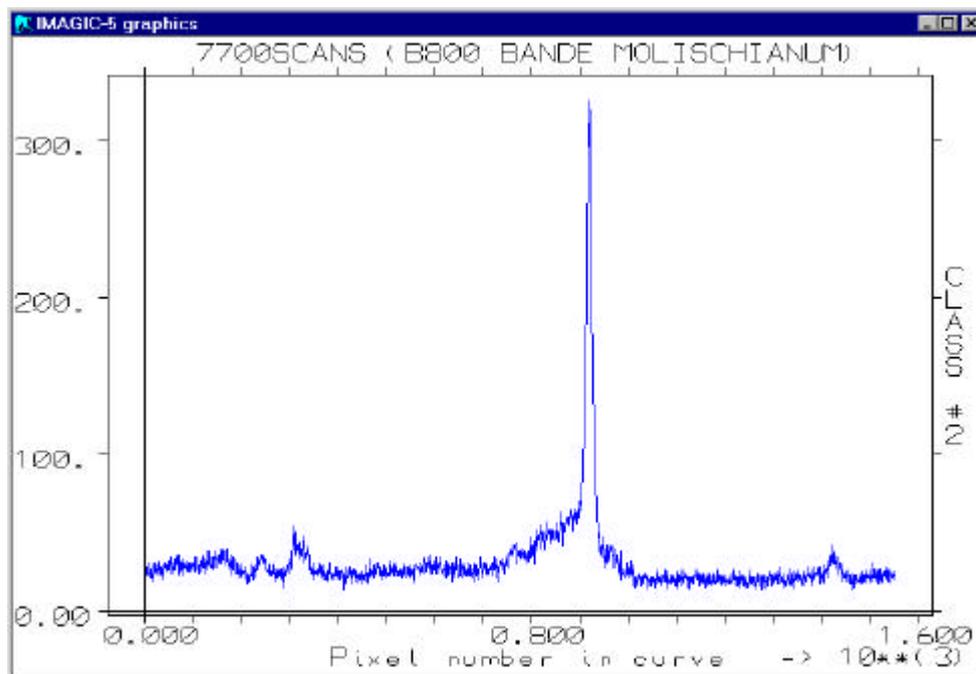

IMAGIC-5 Spectra Analysis

A Little Introduction



This manual is a little "hands-on" first introduction to work with IMAGIC-5 in which the new user will get to know the generalities of the system and will classify spectra.

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This manual contains the following chapters:

- ◆ INTRODUCTION

A little introduction for new users to learn the generalities of IMAGIC-5 image analysis system.

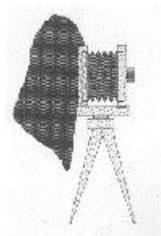
- ◆ CLASSIFICATION OF SPECTRA

Doing some classification on spectra (1D images).

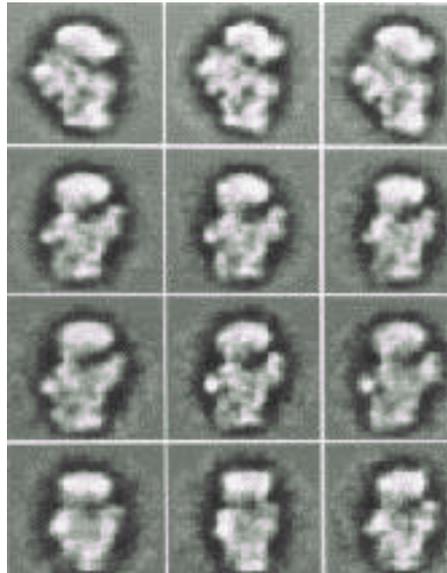
- ◆ RELATED REFERENCES

Some references

WELCOME TO THE
MAGIC WORLD
OF IMAGIC



Introduction



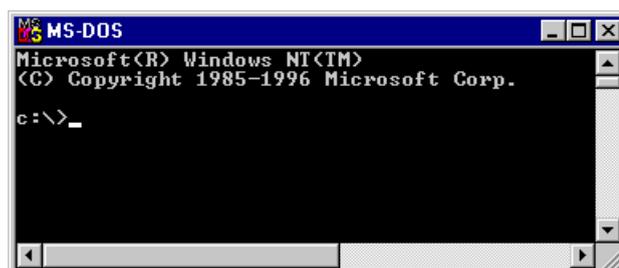
Starting IMAGIC-5:

Currently, IMAGIC-5 is only running as a console application in an MS-DOS window. You must therefore first open such a MS-DOS window. Use the corresponding icon



or start a Command Prompt using Start / Programs / Command Prompt

You will get an MS-DOS window like this:



IMAGIC-5: Spectra Analysis

Then change to your working directory:

```
c:\> cd c:\<my directory>
```

you specify <my directory>

To start IMAGIC-5 type:

```
c:\> c:\imagic\imagic
```

IMAGIC-5 will respond

```
** IMAGIC-5 welcomes you **
```

```
IMAGIC-COMMAND:
```

and is waiting for your command(s).

Some helpful hints when working in an MS-DOS window:

First of all, it is a good idea to activate the so called DOSKEY feature which is available for most systems. When you enter and activate the DOSKEY command, you will later be able to use the arrow keys for scrolling through the list of commands you have entered before.

If you are running Windows NT, another helpful feature is the number of buffered lines of your MS-DOS console window. Use the right mouse button and click into the title bar of the MS-DOS window to activate the properties window. Please check out more details yourself.

Panic and worry: Don't !

- You can always give **?** or **HELP** to get help at any level of IMAGIC-5.
- You can ask **?** for all questions that appear on the screen to get helpful explanation of what is being asked.
- You can always use the default answers (in brackets) by just giving **CR**, but, of course, you can also type your own ones.
- IMAGIC-5's general quit command is the *****. It is a legitimate answer to any question that appears on the screen and its effect is to leave the current operation.
- At any level of IMAGIC-5 you can give an operating system command by typing **!** or **\$** followed by the system command.

Looking for a specific IMAGIC-5 command:

Since IMAGIC-5 responds to over one-hundred different commands, it is clear that we cannot go into details. If you need assistance on valid commands you can retrieve a list of all commands by typing **MENU**.

Example: try to find a command to import the raw spectra into IMAGIC-5:

```
IMAGIC-COMMAND: MENU *import
```

You will get a listing of all command names that have to do with "import":

```
IMAGIC-COMMAND: MENU *import

&IMPORT-EXPORT-IMAGIC-FORMAT
&IMPORT-PDB-TO-PLT
```

Now you can ask for specific **HELP** by typing:

```
IMAGIC-COMMAND: HELP *import
```

How to run an IMAGIC-5 command:

Example: creating test images with command **TEST-IMAGE-CREATION**

```
IMAGIC-COMMAND: TEST-IM
```

you can always use abbreviations of the command name

```
** TESTIM welcomes you **
```

```
Output filename, image loc#s      [test] : ?
```

you can give a ? to get help:

```
The filename consists of any string of characters that is compatible with the file system on your computer. The file filename may be followed by location numbers as in:
```

```
myimage,1,20
```

```
in which case a total of 20 test images are generated by this program which occupy the first 20 locations of the image file.
```

```
The file will be stored in the default directory you are working in. If you want another directory specify that by using the correct prefixes.
```

```
Output filename, image loc#s      [test] :
```

you can give a system command (for example to list the directory) by using a ! or \$:

```
Output filename, image loc#s      [test] : $ dir *.img
```

**(MS-DOS style)
(Unix style: ! ls *.img)**

```
my_file1.img  
my_file2.img
```

```
Output filename, image loc#s      [test] :
```

if you only give RETURN then the default value in brackets (here "test") is used as your answer

```
Image dimensions X,Y [128,128]    : 64,64
```

your own answer

etc.

The DISPLAY command:

One of the most frequently used IMAGIC-5 commands is **DISPLAY-IMAGE**. In an XWindows / Windows NT environment, you can (and often will) start multiple display windows:

```
IMAGIC-COMMAND: DISP

** DISPLAY welcomes you **

Input image file, image loc#s      :
```

The first question that will appear on the screen concerns the choice of the file you wish to display; go ahead and try to get one of the test images you just created on the screen. If you have forgotten the names of the images type:

```
Input image file, image loc#s      : $ DIR *.img    MS-DOS
```

or:

```
Input image file, image loc#s      : ! ls *.img    UNIX
```

This is just an operating system call to get a list of the files you own. Look for files with the extension ".img".

Specify the image file you want to display:

```
IMAGIC-COMMAND: DISP

** DISPLAY welcomes you **

Input image file, image loc#s      : test
```

IMAGIC-5: Spectra Analysis

Before continuing IMAGIC-5 first shows you some information about the specified image(s), like:

```
Image name and parameters [and history]:
TEST IMAGE
SIZE: 64,64  LOC: 1  TYPE: REAL  CREA.DATE:21-10-1999
TESTIM:SIEMENS STAR,
```

In a windows environment IMAGIC-5 will ask you to give the display window size, for example:

```
Give size of the display window [512,512]  : 800,600
```

Then IMAGIC-5 will show a list of all parameters (display settings) that are currently set for displaying your images, for example:

```
Current DISPLAY settings:

Input image FILE name           : test
LOCATION numbers                  : 1 to: 3
Output DEVICE                    : XWINDOW
SCALE factor                     : 1.0000
MINX, MAXX                      : 1 to: 64
MINY, MAXY                      : 1 to: 64
GREYVALUES                      : : AUTOMATIC
ERASE screen                    : : NO
STARTing point (top left)       : 1 , 1
Display of NAME and location    : LOCATION
Video lookup table (VLT)       : STANDARD BLACK/WHITE

Change parameter settings
(ERASE, SCALE, LOC, START, SCAN, SAVE, BYE ...)? [NO] :
```

If you do not understand the meaning of a parameter, don't hesitate to answer ? .

IMAGIC-5: Spectra Analysis

Then just give RETURN, which means "NO MORE CHANGES". IMAGIC-5 will display your image(s) and will return to the parameter setting as shown above.

```
Change parameter settings
(ERASE,SCALE,LOC,START,SCAN,SAVE,BYE ...)? [NO].: NO
```

If you want to change some of the parameters, please look at the words written in uppercase. If you answer the question "Change parameter settings ?" by typing such a word you can change the corresponding parameter(s).

Example:

In the parameter setting above the scale factor is 1.0. If you want a scaling of 2.3, answer:

```
Change parameter settings
(ERASE,SCALE,LOC,START,SCAN,SAVE,BYE ...)? [NO] : SCALE
```

IMAGIC-5 will show you the current image size and ask you the next question:

```
Image size is :          64 x 64
Give scale factor for display [Def=1]          :
```

You can specify the desired value, like:

```
Image size is :          64 x 64
Give scale factor for display [Def=1]          : 2.3
```

IMAGIC-5: Spectra Analysis

IMAGIC-5 will change the parameter and show you the new settings:

```
Current DISPLAY settings:
Input image FILE name      : test
LOCATION numbers            : 1 to: 3
Output DEVICE              : XWINDOW
SCALE factor               : 2.3000
MINX, MAXX                 : 1 to: 64
MINY, MAXY                 : 1 to: 64
GREYVALUES                 : AUTOMATIC
ERASE screen               : NO
STARTing point (top left)  : 1 , 1
Display of NAME and location : LOCATION ONLY
Video lookup table (VLT)   : STANDARD BLACK/WHITE

Change parameter settings
(ERASE,SCALE,LOC,START,SCAN,SAVE,BYE ...)? [NO] :
```

You don't have to worry if you want to change more than just one parameter, the program will loop back to this question after you changed something. Once the setting will be correct and you want to see the images. Answer "NO CHANGES" by giving RETURN:

```
Change parameter settings
(ERASE,SCALE,LOC,START,SCAN,SAVE,BYE ...)? [NO].: NO
```

IMAGIC will display the images #1, #2 and #3 of file **test** on the monitor using the specified parameters.

If you want to leave the **DISPLAY** program give BYE or * . Also try to use command EXIT and see what happens.

```
Change parameter settings
(ERASE,SCALE,LOC,START,SCAN,SAVE,BYE ...)? [NO].: BYE
```

The PLOT command:

This command is working (in a similar way) like **DISPLAY** but is used to display curves, profiles, spectra stored in either IMAGIC-5 plt format or as one-dimensional (1D) IMAGIC-5 image files. The design of **PLOT** is similar to the one of **DISPLAY**. There are also many options which you can choose and which are shown in "Current PLOT settings". Option **NO CHANGE** also means everything is specified, go ahead and display the curve. The command can be started by giving **PLOT**.

Image sequences in IMAGIC-5

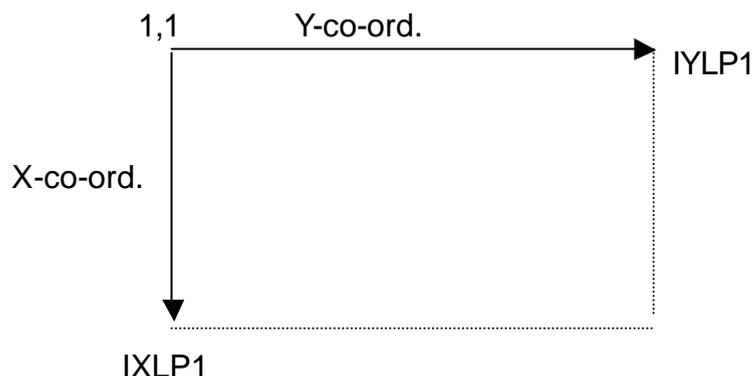
Every IMAGIC-5 image file can contain a sequence of images. All images in the sequence will automatically be treated by the procedure unless the user specifies a set of image location numbers explicitly, like in:

```
IMAGIC-COMMAND: COPY-IM
** COPYIM welcomes you **
Input file, image loc#s           : test,10,64
                                etc.
```

in which case the images number 10 to 64 are used-

The IMAGIC-5 co-ordinate system

The IMAGIC-5 co-ordinate system is a right-handed system with its (1,1) origin in the top-left corner of the image. The length of the lines (number of rows/columns) is IYLP1 and the number of lines is IXLP1:



The centre of the image, for almost all operations on the image, is given by:

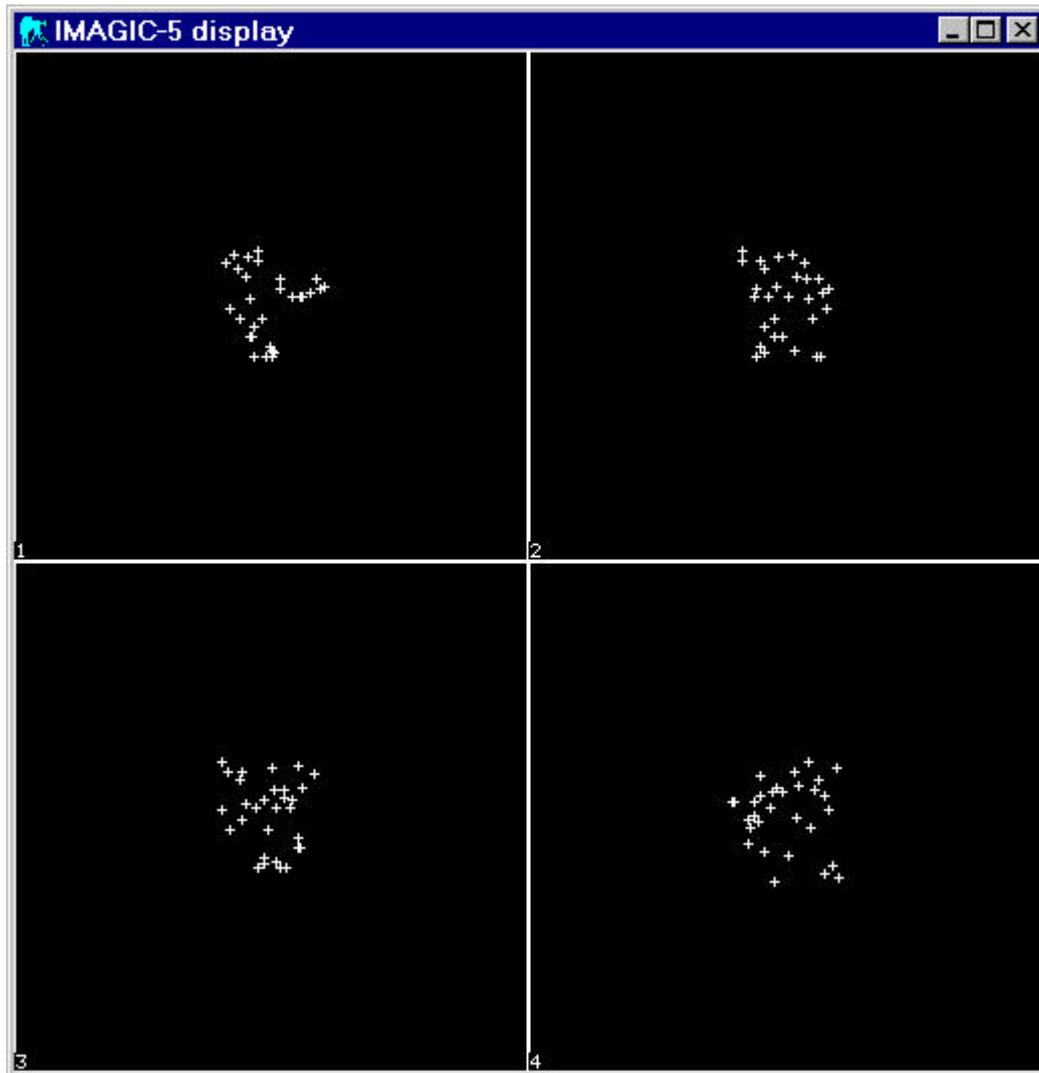
$$(IXMID,IYMID) = (IXLP1/2 + 1 , IYLP1/2 + 1).$$

This point is also the default centre of rotational symmetric masks and the like.

Starting IMAGIC-5 from many windows

You can start IMAGIC-5 in more than only one terminal window. For example: it can be very helpful to use one window for command **DISPLAY** while playing around / testing filter parameters with command **BAND-PASS-FILTER** in an other window.

Classification of Spectra



The purpose of this chapter is to provide the user with guidance in a practical hands-on to classify spectra.

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Import Spectra Data

The spectra are stored as lines of a two-dimensional (2D) image, which is stored as "raw data" in integer 16bit format and which was created a Silicon Graphics workstation. First convert this image into IMAGIC-5 format:

Call **IMPORT-EXPORT-IMAGIC-FORMAT** or **EM2EM**, respectively:

```
IMAGIC-COMMAND: EM2EM

** EM2EM welcomes you **

Data format of the input to be converted : RAW
Export to which data format [?]         : IMAGIC
Input = set of 2D sections of a 3D volume: NO
Input image file (WITH EXT.)           : 7700scans.dat

Mode of input                           : 2D

Give image dimensions X,Y)              : 7700,1552
Data type of the input file              : INTG
Move the input image upside down         : NO
Workstation of input file                 : Silicon
Output image file (NO EXT.)              : scans

Pixel spacing in Angstroem (X,Y,Z)      : 0,0,0
```

You select

The spectra are stored as 2D image

You select

Not important here

In the example we called the IMAGIC-5 file "scans".

Check the converted image

Use command **DISPLAY** to check the imported image. Since the monitor screen is smaller than the image size, the image will automatically be scaled with a scale factor smaller than 1. To display part(s) of the image 1:1 (**SCALEFACTOR 1.0**) please use the options **MINX**, **MINY** or **SCAN**. Use **?** if you need help.

Create spectra (1D images)

What we really need is not one single image but a series of 1D images representing all spectra. The easiest way to do this is to simply create a new **IMAGIC-5** header:

Call **CREATE-HEADER**:

```
IMAGIC-COMMAND: CREATE-HEAD

** CREAHEAD welcomes you **

Output header filename, loc#s           : scans,1,7700
                                           Number of spectra
Give image dimensions X,Y               : 1,1552
                                           Length of spectra
Data type                                : INTG
                                           Same as before
Give title                               : B800 BANDE
                                           You can give a
                                           headers text (info)
```

Now **IMAGIC-5** will interpret the input file (**scans**) as a series (of **7700**) 1D images

You can display (plot) the various curves by using command **PLOT**. The design of **PLOT** is similar to the one of **DISPLAY**. There are also many options which you can choose and which are shown in "Current **PLOT** settings". Option **NO CHANGE** also means everything is specified, go ahead and display the curve.

Normalisation and band-pass filter:

There are a number of IMAGIC-5 commands to normalise, filter, manipulate 1D images (curves, spectra). If you want to do so try to play around with commands like:

ARITHM-WITH-CURVE
CUT-CURVE
FILTER-CURVE
MSA-CURVE-MASK
PRETREAT-CURVE

As usual use **HELP *ARITHM** (for example) for details and/or **?** if you need help to answer a certain question.

Concerning filter parameters (in command **FILTER-CURVE**, for example): If you want to calculate the frequency cut-off in terms of spatial frequency, then here is an example. Say that the image is scanned such that each pixel is of size x . Then in the Fourier transform, the edge of the image corresponds to the maximum spatial frequency $1 / (2 * x)$. Remember that the centre of the transform is zero spatial frequency. Thus a fraction f (between 0 and 1) corresponds to a spatial frequency of

$$\frac{f}{2 * x}$$

Multivariate statistical analysis (MSA):

Input images are the original or manipulated 1D images (**scans**). Call **MSA-RUN**:

```
IMAGIC-COMMAND: MSA-RUN

** MSA welcomes you **

Choose mode: FRESH-MSA, REFINE [F] : fresh
Distance:
EUCLIDIAN, CHISQUARE, MODULATION : modulation
                                     use modulation distance

Filename input aligned "images" : scans
Are there any inactive images? [NO] : no
                                     if wanted some spectra can be excluded

Give input MSA mask file [none] : none
Eigenimages output file : scans_eigen
Pixel coordinates output file : scans_pixcoos
Eigenpixel vectors output file : scans_eigenpix
Give number of iterations (<64) : 24 "real science": >23
Give number of eigenimages (<69) : 68
Overcorrection factor (0< ocf< 0.9) : 0.8
Rootname for registration files : scans_msa
```

The results of **MSA-RUN** are mainly stored in the header file of the input images, in an ASCII registration file (**scans_msa.lis**) and in the (output) eigenimages (**scans_eigen**), which you can visualise with **PLOT**.

Classification:

The next step is the classification within the hyper-space created by **MSA-RUN**. Call **MSA-CLASSIFY**:

```
IMAGIC-COMMAND: MSA-CLASSIFY

** CLASSIFY welcomes you **

Input to be classified [IMAGES] : images
Input images file (treated by MSA) : scans
Percentage of images to be ignored [0] : 0
Active factors for classification? [69] : 69
                                     this number must be less/equal
                                     the one used in MSA-RUN
```

IMAGIC-5: Spectra Analysis

```
Try a tricky normalisation? [NO TRICKS] : no
Weight the factorial coordinates [NO] : no
Plot the classification trees [YES] : no
Maximal amount of other print output : no
Classification (MSA) maps stored [NO] : no
What NUMBER of classes do you wish ? : 50 you select
Name for output result files : scans_classes50
Mass threshold parameter ? [0] : 0
```

The most important results file is an ASCII text file with extension ".lis" ([scans_classes50.lis](#)). Please have a look at the last table to get an idea, which classes are best, etc.

Class-sums of the spectra (averages):

Call **MSA-SUM**:

```
IMAGIC-COMMAND: MSA-SUM

** CLASSUM welcomes you **

Input aligned images : scans
Rootname of input classification files : scans_classes50
Output class averages [classsums] : scans_clssums50
Downweight small classes? [YES] : yes
Produce summing statistics: : no
Fraction of worst class members to ignore : 0 you select
```

Please look at the class-sum images with **PLOT**. If necessary you can re-do the classification with less or more classes.

To get an idea how the MSA hyperspace looks like you can use command **MSA-MAP**, which creates projections onto two hyperspace axes. These projections can be created for the single images/spectra or the (centre of the) classes. Use **DISPLAY** for visualisation.

Publication

Write a nice paper for Nature, Science, etc. This writing you have to do yourself as, unfortunately, the corresponding IMAGIC-5 commands ([WRITE-FOR-NATURE](#), etc.) are still in preparation.

Related References



Some image processing references for your further reading enjoyment:

M van Heel, B Gowen, R Matadeen, EV Orlova, R Finn, T Pape, D Cohen, H Stark, R Schmidt, M Schatz and A Patwardhan (2000) Single-particle electron cryo-microscopy: towards atomic resolution. Q. Rev. Biophys. 33: 307-369.

WebPages:

www.ImageScience.de
www.ImageScience.de/imagic

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M van Heel (1984) Multivariate statistical classification of noisy images (of randomly oriented biological macromolecules). Ultramicroscopy 13: 165-183.

M van Heel (1989) Classification of very large electron microscopical data sets. Optik 82: 114-126.

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M Schatz, EV Orlova, P Dube, H Stark, F Zemlin and M van Heel (1997) Angular reconstitution in 3D electron microscopy: Practical and technical aspects. *Scanning Microsc.* 11 (1997) 179-193.

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FT-IR classification

D Naumann, V Fijala and H Labischinski (1988) The differentiation and identification of pathogenic bacteria using FT-IR and multivariate statistical analysis. *Mikrochim. Acta I*, 373-377