IMAGIC-4D

Version: June 2010

Ribosome pictures kindly provided by Prof van Heel, Imperial College, London

IMAGIC-4D: Introduction:

Whereas IMAGIC programs (since 1980) will automatically loop over stacks of 2D images, the new program versions will also automatically loop over stacks of 3D volumes present in an IMAGIC input "4D file".

All 3D operations are now in-core ensuring fast processing avoiding unnecessary input/output operations. The extended IMAGIC-4D file format remains compatible with the previous IMAGIC-5 format. All time-critical programs exploit MPI parallelization allowing for millions of input images to be processed efficiently.

2D / 3D commands:

There are commands working on stacks of 2D images, and there are commands working on input stacks 3D volumes.

Examples:

2D

```
IMAGIC-COMMAND : copy-image

  ** COPYIM welcomes you **

Input file, image loc#s                    : my_image,3,5
Output file, image loc#s                   : my_2d_copy
```

Command COPY-IMAGE copies a stack of 2D images. Location numbers are 2D image locations and the output file is a 2D image file.

3D

```
IMAGIC-COMMAND : threed-copy

  ** INC3DMENU welcomes you **

Option used for current IMAGIC command    : DENS_COPY
Input file, 3D loc#s                       : my_volume,7,10
Output file, 3D loc#s                      : my_3d_copy
```

Command THREED-COPY copies a stack of 3D volumes. The specified locations are 3D location numbers and the output file is a 3D volume file.

2D or 3D

Some IMAGIC commands are created to manipulate 3D volumes

```
IMAGIC-COMMAND : auto-mask

  ** AUTOMASK3D welcomes you **

Automasking options:
  DO_IT_ALL   REFINE_THRESHOLD
Please specify option [DO_IT_ALL]         :

Input 3D volume file                      : my_3d

...
```

but they also work on 2D images:

```
IMAGIC-COMMAND : auto-mask

  ** AUTOMASK3D welcomes you **

Automasking options:
  DO_IT_ALL   REFINE_THRESHOLD
Please specify option [DO_IT_ALL]         :

Input 3D volume file                      : my_image

...
```

2D and 3D

Some IMAGIC commands can use both, 2D images and 3D volumes. For example, IMAGIC command EXCLUSIVE-COPY option EXTRACT can copy 2D image(s) into an output 2D image file, can copy 2D images into a 3D volume file… and can copy 3D volume(s) into a 3D output file.

For example

```
IMAGIC-COMMAND : exclusive-copy

  ** EXCOPY welcomes you **

What should be copied:
  2D_IMAGES/SECTIONS  3D_VOLUME
Please specify option                      :

Exclusive copy operations:
   EXTRACT   EXCLUDE   SELECT   SORT   REVERSE   RANDEX
Please specify option                      :
Input file, NO loc#s                       : my_images
Output file, image loc#s                   : my_3d,10

Get image loc#s from:
  PLT_FILE   INTERACTIVE
Please specify option [INTERACTIVE]        : inter
Location number(s) of image(s) wanted      : 3-5

...
```

In which case some sections in the output 3D are replaced.

Some related 2D and 3D IMAGIC commands

2D	3D
APPEND-IMAGE	THREED-APPEND
ARITHMETIC-WITH-IMAGE	THREED-ARITHMETIC
BLOW-UP	THREED-BLOWUP
CENTER-IMAGE	THREED-CENTER
COPY-IMAGE	THREED-COPY
CUT-IMAGE / SUBFILE	THREED-CUT / THREED-SUBFILE
INCORE-MENU	THREED-MENU
NORM-VARIANCE	THREED-NORM-VARIANCE
ROTATE-IMAGE	THREED-ROTATE
TEST-IMAGE-CREATION	THREED-TEST
TWO-IMAGE-OPERATIONS	THREED-TWO-VOLUMES-OPERATIONS
More…	More…

Some IMAGIC commands that work on both, 2D and 3D:

EXLUSIVE-COPY:	Exclusive copy of 2D images or 3D volumes
MSA-RUN, MSA-CLASSIFY and MSA-SUM	MSA classification of either a stack of 2D images or 3D volumes
THREED-FILTER	Band-pass or low-pass filter 2D image(s) or 3D volume(s)
AUTOMATIC-MASK	Automatically mask 2D image(s) or 3D volume(s)

IMAGIC-4D:

RELATED IMAGIC COMMANDS:

MSA-RUN / MSA-CLASSIFIY / MSA-SUM:

MSA classification of 3D volumes

M-R-A

Multi-reference alignment with respect to references created from multiple 3D volumes. The alignment can be done "within" a given 3D membership or to determine a 3D membership (the 3D membership of the best reference is taken over). M-R-A can thus be used for refining towards multiple 3D structures.

ANGULAR-RECONSTITUTION:

Euler Angle assignments and refinements with respect to multiple 3D volumes as anchor sets. Various 4D options related to 3D membership (options are: OWN_ANCHOR_SET, EACH_TO_BEST_ANCHOR_SET…).

THREED-RECONSTRUCTION:

Calculate multiple 3D reconstructions. Depending on the 4D option this IMAGIC command can create stacks of 3D reconstructions. Different 3D volumes get different 3D memberships.

THREED-FORWARD:

Create forward projections from multiple 3D volumes. The forward projections get different 3D memberships according to the different 3D memberships of the 3D volumes.

NEW 4D
3D RECONSTRUCTION / EULER ANGLES DETERMINATION SCHEME

With the ANGULAR-RECONSTITUTION command one typically assigns an Euler angle orientation to a 2D image by finding its best overall sinogram correlation peaks with respect to an "anchor set" of 2D forward projection from one single 3D volume.
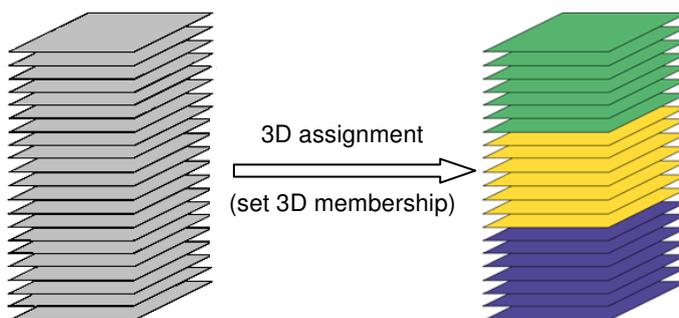
ANGULAR-RECONSTITUTION has now been extended to loop over multiple anchor sets and has thus been upgraded to a "4D" level. As a consequence, the command is now capable of competitively choosing the best anchor set among anchor sets generated from different 3D volumes (different 3D memberships).

ANGULAR-RECONSTITUTION can now thus also be used for refining towards multiple 3D structures as was already the case with the MULTI-REFERENCE-ALIGN command.

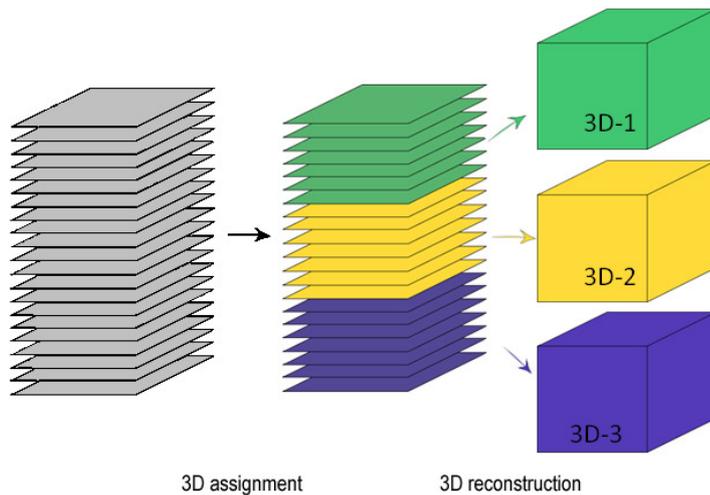The 4D scheme:

a) 3D assignment

One starts with the (a priori) assignment of the images in a stack (original images or class averages) to a number of 3D memberships:

Depending on the strategy pursued this assignment can be of various origins:

- MULTI REFERENCE ALIGNMENT: The references are forward projections from different 3Ds. Take over the 3D membership of the reference to which the best alignment was archived.

- MSA CLASSIFICATION: 2D MSA manifold separation

- MSA-CLASSIFICATION: 3D MSA classification

- SET RANDOM: Option(s) can be found in command THREED-RECONSTRUCTION
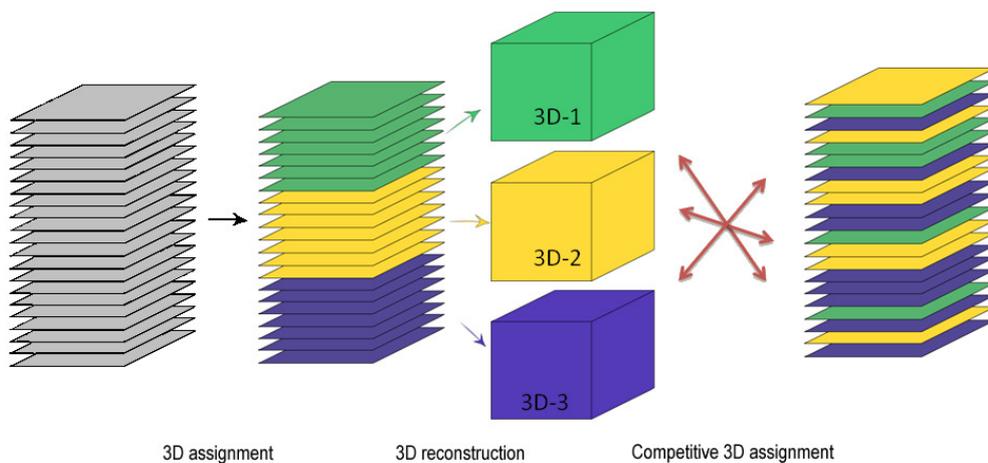
b) Create multiple 3D reconstructions

3D assignment   3D reconstruction

Related options in command THREED-RECONSTRUCTION are:

- HEADER_3D_MEMBERSHIP:	Other programs have already assigned a 3D membership to the input images. For example commands: M-R-A, ANGULAR-RECONSTITION, or THREED-FORWARD.
- SPLIT_3D_MEMBERSHIP:	Other programs have already assigned a 3D membership to the input images. For example the commands: M-R-A, ANGULAR-RECONSTITION, or THREED-FORWARD. However, you want to increase the number of 3Ds by splitting each 3D membership further.
- SEQUENTIAL_ASSIGNMENT:	Each image of a sequence of input images will be assigned to one of a user-defined number of 3D reconstructions in sequential order. For example: for 1000 input images, choosing the number of ten 3D reconstructions, the first 100 images are used for the first 3D, the second 100 images for the second 3D, etc.
- RANDOM_3D_MEMBERSHIP:	Each image from a sequence of input images will be assigned at random to one of a of the (user-defined number) output 3D reconstructions. Each input image is used only once.
- MULTIPLE_RAND_ASSIGN:	For creating one (of possible many) 3Ds, a random choice is made of input images. Each image of a sequence of input images

may thus be assigned to more than one of a (user-defined) number of 3D reconstructions. An input image can even show up more than once in the same output 3D.

c) Competitive 3D membership assignment

Once different 3Ds have been generated, various "competitive" 3D membership assignment schemes can be applied to the 2D images in the stack.
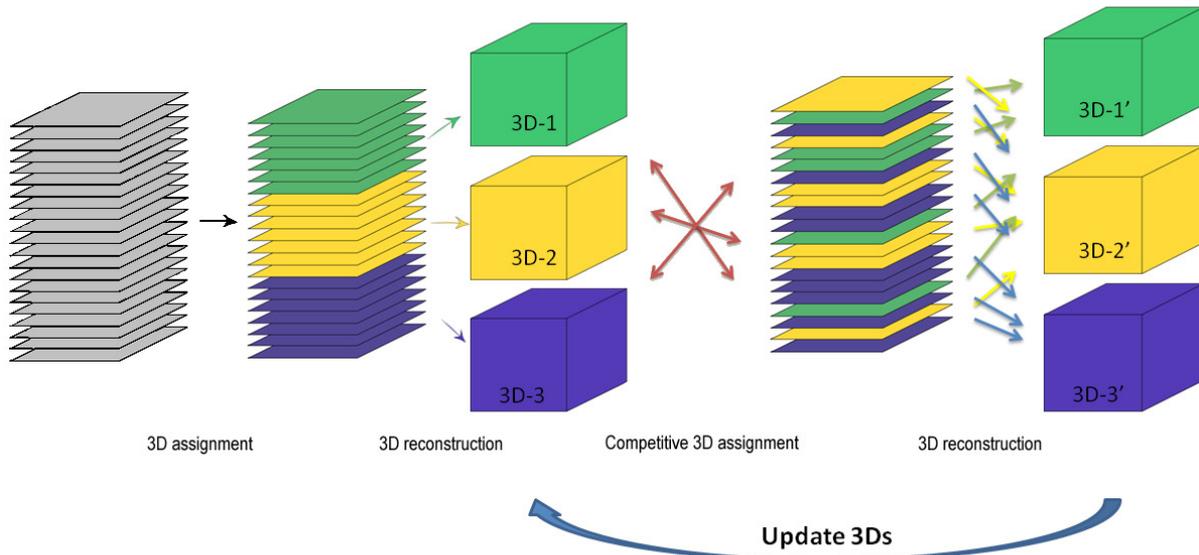

3D assignment   3D reconstruction   Competitive 3D assignment

Competitive 3D membership assignments are:

- MULTI-REFERENCE-ALIGNMENT: A classical one is the multi-reference alignment with respect to re-projections of the different 3D volumes. The related M-R-A option is OWN_REF3D_REFERENCES

- MULTI-ANCHOR-SET
  ANGULAR-RECONSTITUTION: Another approach is the multi-anchor set Euler angle assignment by ANGULAR-RECONSTITUTION (ANCHOR_SET with option EACH_TO_BEST_ANCHOR_SET). The best anchor set among anchor sets generated from different 3D structures is chosen competitively. For each image the 3D membership of its best best anchor set is taken over.

d) Iteration

The resulting new 3Ds (named 3D-1' to 3D-3') take the place of the earlier 3Ds in this generic iterative refinement scheme:

All figures are based on a scheme of C. Linnemayr (ETH Zurich)

The iterations are stopped once convergence criteria like the Fourier shell correlation (FSC) resolution of the 3Ds reach stability.

d) A "real science" 4D image procedure

A "real science" 4D image procedure can therefore look like this:

1. Go for a usual 2D image processing (starting with alignment-by-classification, and an iterative procedure of MSA classification and M-R-A).

2. Start with 1000 class-averages (out of let's say 4000).

3. Assign random Euler angles to each selected class average and assign them to one of about ten 3Ds at random. Use THREED-RECONSTRUCTION with option RANDOM_3D_MEMBERSHIP and RANDOM_EULER_ANGLES when asked for "Source of Euler angles".

4. Refine iteratively using THREED-FORWARD, ANGULAR-RECONSTITUTION (option EACH_TO_BEST_ANCHOR_SET) and THREED-RECONSTRUCTION (option 3D_MEMBERSHIP).

5. Kill poor 3D reconstructions from 4D stack by specifying a "Minimum number of images per 3D") in THREED-RECONSTRUCTION).

6. Extend refinement to all class averages.

7. Assign Euler angles to all single images using the 3D information of their classes. Use HEADER with option TAKEOVER and CLASS_SUM_ANGREC to take over Euler angles and 3D membership of the class averages into the headers of the related single images.

8. Maybe doing a refinement: ALIGN-PARALLEL between images and related re-projections from the 3Ds.

9. Calculate the final 3Ds using all single images.

Some references

IMAGIC-4D:

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M van Heel, G Harauz, EV Orlova, R Schmidt and M Schatz (1996) A new generation of the IMAGIC image processing system. J. Struct. Biol. 116: 17-24.

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4D methodology

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AE Leschziner and E Nogales (2007) Visualizing flexibility at molecular resolution: Analysis of heterogeneity in single-particle electron microscopy reconstruction. Annu. Rev. Biophys. Biomol. Struct. 36: 62-43.

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WebPages:

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