Alignment of tilt series

**Students:**
Andrea Sanzogni
Ledion Daja
Alessandro Gnutti

**Professors:**
Alberto Signoroni
Matteo Ferroni
Outline

• Introduction to Electron Tomography (ET)
• Image alignment problem
• Proposed image alignment technique
  ▫ Preprocessing
  ▫ Alignment
• Results
Electron Tomography

- Electron tomography is an imaging technique that allows to recover 3D structure of low atomic number specimen
- Involves similar principles to those used in light microscopy
  - Electrons are used for illumination instead of photons
- Electron microscopy (EM) reveals basic specimen structures in details, reaching a better resolution than light microscopy
- However, Electron Microscopy provides only 2D projection views of the specimen
- The recent development of Electron Tomography (ET) allows to determine the 3D structure at a resolution of a few Å
Electron Tomography

- Electron microscopes record projection images
  - A single image $\Rightarrow$ information from all heights of the specimen collapsed onto a single plane
- A series of images is acquired, by varying the orientation of the specimen relative to the incident beam
  - Such series contain all the information required to describe the 3D structure of the imaged object
Electron Tomography

• Specimen is rotated around an axis perpendicular to the electron beam
Electron Tomography

- For each angle, the projection images are saved
- The 3D reconstruction is done by backprojecting the projection views
Electron Tomography

• Result of acquisition process:
  ▫ A stacked series of 2D images, each representing a projection of the 3D structure.

• Rotation of specimen is not ideal
  ▫ Mechanical inaccuracies of the specimen holder

• This causes a disaligned set of images
  ▫ IMAGE ALIGNMENT needed
Image Alignment

• For ideal acquisition, 3D reconstruction is quite simple
• Alignment process
Image alignment

- It is a crucial step in electron tomographic reconstruction
- Includes rotational and translational alignment
  - Also identification of the tomographic tilt axis
- Alignment of tomographic series approached in several ways, classified as:
  - Fiducial markers
  - Markerless alignment
Fiducial Markers Alignment

- Fiducial markers are added to the specimen
  - E.g: colloidal gold particles
  - Assumed to be immobile wrt specimen structure
- Coordinates of each marker:
  - Automatically
  - Manually
- 3 markers are needed at least
  - Alignment model calculated using least-squares procedures \( \rightarrow \) minimize the alignment error as a function of the lateral translations and the angle or the tilt axes
Fiducial Markers Alignment

• Advantages:
  ▫ Markers can be localized accurately
  ▫ If positioned outside the volume, they can be removed graphically from the final reconstruction

• Disadvantages:
  ▫ Time-consuming for the user
  ▫ In low SNR images, it is difficult to detect markers
Marker-Less methods

- Maximize a “similarity” function (i.e. cross correlation, mutual information)
- The maximum value has a different position in respect of the ideal case
- Compensate this shift to align image
- Attention: cross correlating two images give a wrong result, it’s important to transform the image simulating the behaviour of the microscope

- Sensible to noise
Alignment Procedure

- Pre-processing
  - Filtering, segmentation
- Image transformation
  - Affine transformation, composition of translation and rotation
- Alignment
  - According to correlation values between the transformed image and a reference image
Pre-processing

- Using correlation on this type of images does not give a correct result
- Background pixel contributes to correlation values
- Peak position is wrong
- 2-level segmentation is useful in these type of situations
Pre-processing

• A black and white image helps to solve the problem

• Segmentation obtained using Otsu's method for optimal threshold.

• For few images was necessary to manually find the optimal threshold.
Pre-processing

• If the initial image is too noisy, segmentation fails and the results are not satisfactory

• The alignment procedure fails
Reference image

• Fixed reference
  ▫ Every image is aligned in respect of the same image
  ▫ High tilt degrees can give problems
  ▫ Computationally demanding

• Mobile reference
  ▫ Every image is aligned in respect of the previous image of the stack
  ▫ Faster procedure
  ▫ Risk of errors propagation
Image transformation

- First apply a translation to move the rotation center

\[ T_1 = \begin{bmatrix} 1 & 0 & 0 & -t_x \\ 0 & 1 & 0 & -t_y \\ 0 & 0 & 1 & -t_z \\ 0 & 0 & 0 & 1 \end{bmatrix} \]
Image transformation

- Rotation in a 3D space
- \( c = \cos(\theta) \)
- \( s = \sin(\theta) \)
- Rotation axis
- \( u = [u_x \ u_y \ u_z] \)

\[
T_2 = \begin{bmatrix}
(1 - c)u_x^2 + c & (1 - c)u_xu_y - su_z & (1 - c)u_xu_z + su_y & 0 \\
(1 - c)u_xu_y + su_z & (1 - c)u_y^2 + c & (1 - c)u_yu_z - su_x & 0 \\
(1 - c)u_xu_z - su_y & (1 - c)u_yu_z + su_x & (1 - c)u_z^2 + c & 0 \\
0 & 0 & 0 & 1
\end{bmatrix}
\]
Image transformation

- At the end apply a translation to move the rotation center back

$$T_3 = \begin{bmatrix} 1 & 0 & 0 & t_x \\ 0 & 1 & 0 & t_y \\ 0 & 0 & 1 & t_z \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

- The overall transformation matrix is

$$T = T_1 T_2 T_3$$
Image transformation

- From a 2-D matrix to a 3-D matrix
- Each plane represents a different z-component
- Each plane contains a “piece” of the 3-D object
Image space

- In order to obtain the new image we analyze the last plane on high
- In particular we scan each pixel:
  - ✔ If we find an “image” pixel, we consider it as a new image pixel
  - ✔ Else we move in the lower planes on the pixel axis until we found a valid value
In order to don’t confuse a null pixel with a black image pixel

- We add up ‘1’ to each pixel of the referenced image before to compute the transformation
- We subtract ‘1’ to each pixel of the new obtained image
Alignment

- R - reference image
- W - image to align
- F - reference image transformed using the matrix T

- Cross correlation between F and W

- Ideally peak value would be located in the position correspondingly to the maximum superposition of the two images.
Alignment

• The peak position is located and it’s calculated the difference in respect of the expected position for both rows and columns

• Using these values the image is shifted along the two directions

• Then the steps are repeated for another image, changing the transformation parameters
How to evaluate the alignment?

• The eye is quite good at detecting small changes in position of image features as a tilt series is played through as a movie

• In situations of a poor alignment there is obvious “jitter” as images are displayed

• The position of the tilt axis is easily discernable as the series is played back and forth as a movie

• Global correlation coefficient
Tilt series Fiber

- 110 images
Misalignment distribution – Fixed reference
Misalignment distribution – Mobile reference

- The image shows the distribution of the displacements of images with respect to the reference image.
Correlation coefficients – Consecutive images
Correlation coefficients – Fixed reference
Alignment – Fixed reference

- Central image taken as the reference
- Global correlation coefficient: 0.9918
Alignment – Mobile reference

- Global correlation coefficient: 0.9920
Tilt series 06

- 81 images
- Global correlation coefficient: 0.6539
Misalignment distribution
Correlation coefficients
Alignment – Fixed reference

- Image 78 is completely wrong

- The noise present in the images, even after the pre-processing, makes hard to obtain good results for the final part of the stack

- Global correlation coefficient: 0.8218

- Min: 0.2165
Misalignment distribution

- The image shows the distribution of the displacements of images wrt reference image.
- One image presents very large displacements in both directions.
Correlation coefficients
Alignment – Mobile reference

- Image 78 is completely wrong
- Global correlation coefficient: 0.8117
- Min: 0.2165
Tilt series 07

- ZnO comb
- 16 Bit gray
- From 120 degrees to 229 degrees
- 110 images
- Global correlation coefficient: 0.8665
Misalignment distribution

![Graph showing misalignment distribution with angle relative to reference and displacement values.](image-url)
Correlation coefficients
Alignment – Fixed reference

- Central image taken as the reference
- Global correlation coefficient: 0.9906
- Min: 0.9731
Misalignment distribution

- The image shows the distribution of the displacements of images wrt reference image.
Correlation coefficients
Alignment – Mobile reference

- Global correlation coefficient: 0.9875
- Min: 0.9652
- Loss of information at the borders of the images
Tilt series 08

- ZnO comb
- 16 Bit grey
- From 122 degrees to 242 degrees
- 121 images
- Global correlation coefficient: 0.6970
Misalignment distribution
Correlation coefficients
Alignment – Fixed reference

- Central image taken as the reference
- Global correlation coefficient: 0.8906
- Min: 0.3116
Misalignment distribution

- The image shows the distribution of the displacements of images wrt reference image
Correlation coefficients
Alignment – Mobile reference

• Global correlation coefficient: 0.9716
• Min: 0.8367
Alignment of tilt series – Additional Results

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Ledion Daja
Alessandro Gnutti

Professors:
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Matteo Ferroni
Results comparison

Manual alignment
Global correlation coefficient: 0.9875

Automatic alignment with fixed reference
Global correlation coefficient: 0.9878

Automatic alignment with mobile reference
Global correlation coefficient: 0.9896
Correlation coefficients – Consecutive images
Correlation coefficients – fixed reference
Cobalt particles in carbon tube

- 53 images
- Global correlation coefficient: 0.876694
Misalignment distribution – Fixed reference
Misalignment distribution – Mobile reference

- The image shows the distribution of the displacements of images wrt reference image
Correlation coefficients – Consecutive images
Correlation coefficients – reference image
Results comparison

Manual alignment
Global correlation coefficient: 0.9462

Automatic alignment with fixed reference
Global correlation coefficient: 0.9280

Automatic alignment with mobile reference
Global correlation coefficient: 0.9398
Results comparison: cropped images

Manual alignment
Global correlation coefficient: 0.9549

Automatic alignment with fixed reference
Global correlation coefficient: 0.9316

Automatic alignment with mobile reference
Global correlation coefficient: 0.9502
Collagen Fibers

- 91 images
- The original images are not aligned if we apply directly the algorithm
- It is necessary to exploit a threshold in the pre-processing step
Misalignment distribution – Fixed reference
Misalignment distribution – Mobile reference

• The image shows the distribution of the displacements of images wrt reference image
Correlation coefficients – Consecutive images
Correlation coefficients – Consecutive images
Correlation coefficients – reference image
Correlation coefficients – reference image
Results comparison

Automatic alignment using dedicated software
Global correlation coefficient: 0.8747

Automatic alignment with fixed reference
Global correlation coefficient: 0.8471

Automatic alignment with mobile reference
Global correlation coefficient: 0.8751
Results comparison (central axis): cropped images

Automatic alignment using dedicated software
Global correlation coefficient: 0.9957

Automatic alignment with fixed reference
Global correlation coefficient: 0.9585

Automatic alignment with mobile reference
Global correlation coefficient: 0.9925
Results comparison (10.8° axis): cropped images

Automatic alignment using dedicated software
Global correlation coefficient: 0.9957

Automatic alignment with fixed reference
Global correlation coefficient: 0.9442

Automatic alignment with mobile reference
Global correlation coefficient: 0.9926