# Alignment of tilt series

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# Outline

- Introduction to Electron Tomography (ET)
- Image alignment problem
- Proposed image alignment technique
  - Preproccessing
  - Alignment
- Results

# Electron Tomography

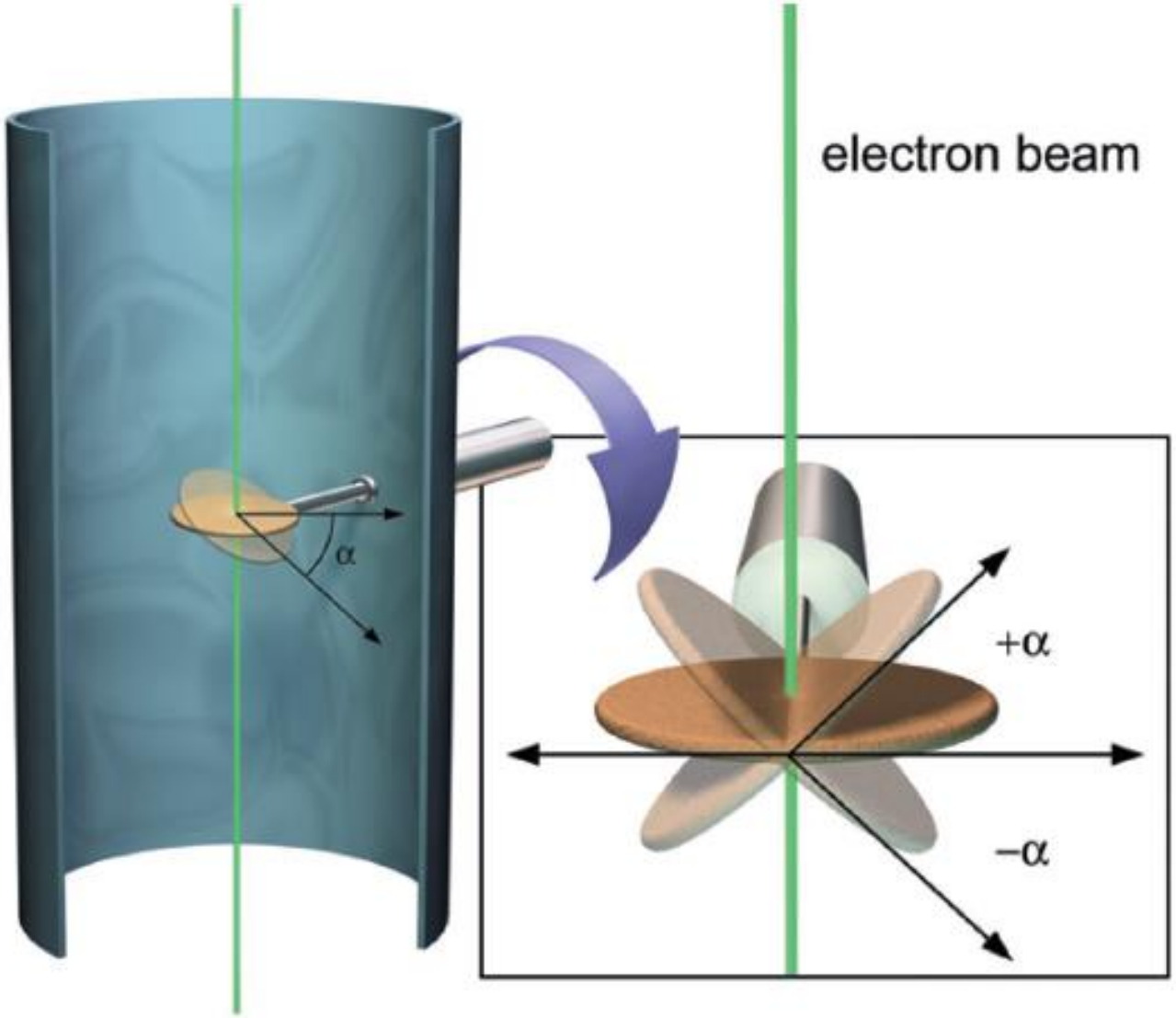
- Electron tomography is an imaging technique that allows to recover 3D structure of low atomic number specimen
- Involves similar principles to those used in light microscopy
  - Electrons are used for illumination instead of photons
- Electron microscopy (EM) reveals basic specimen structures in details, 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 ➔ 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

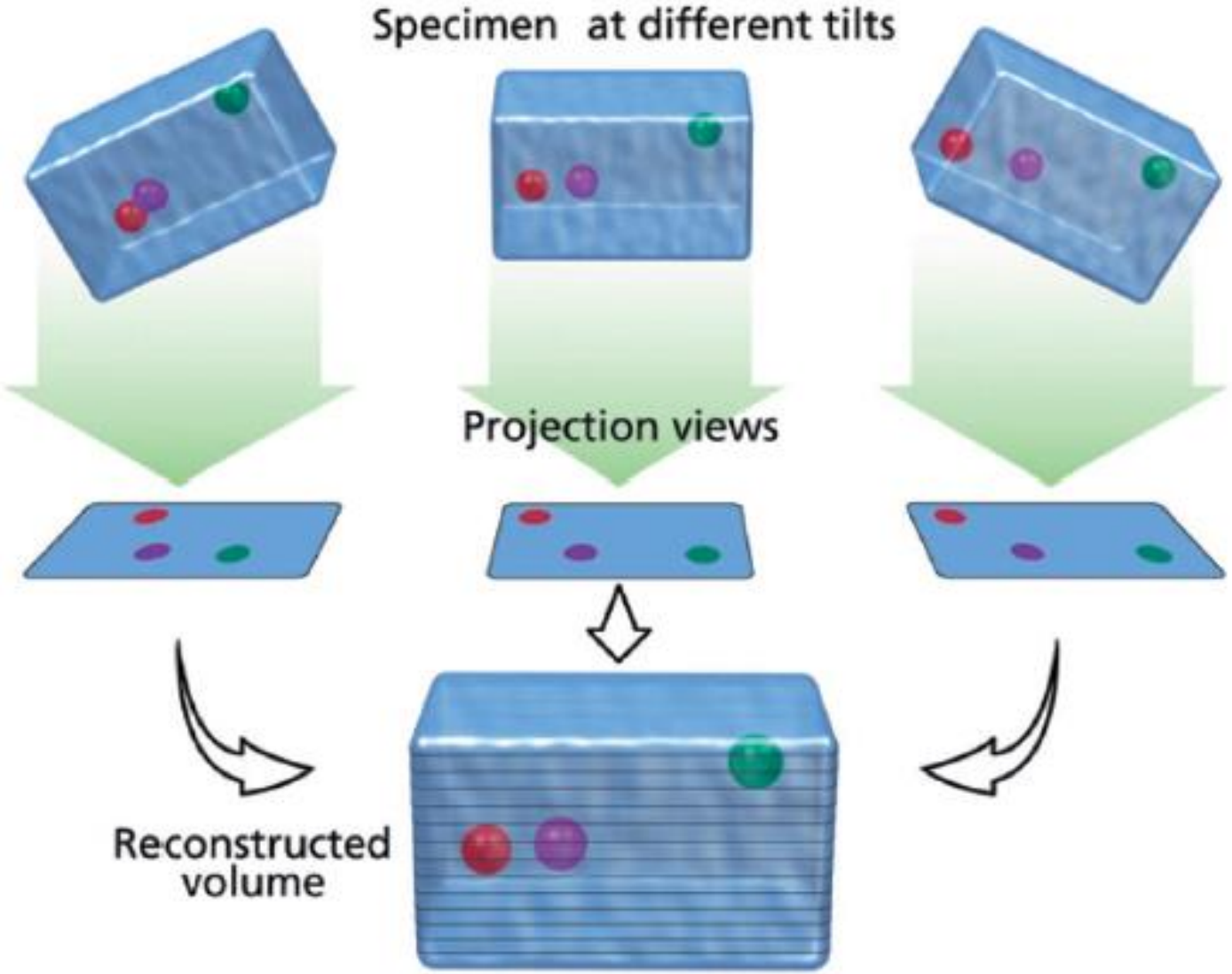
# Electron Tomography

- 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

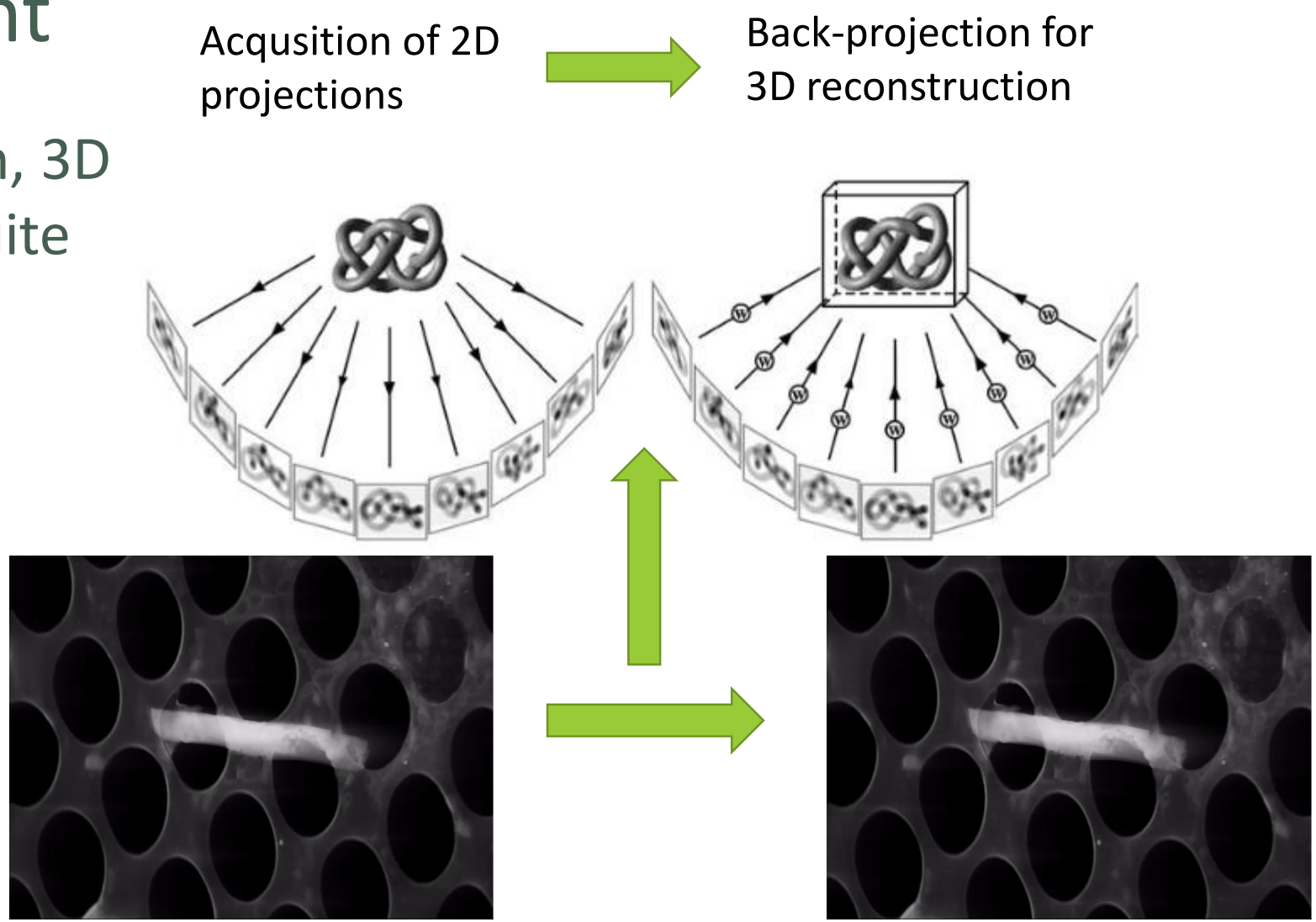


# Electron Tomography

- 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 alingment

- 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 ➔ minimize the alignment error as a function of the lateral trasnlations 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 grafically 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

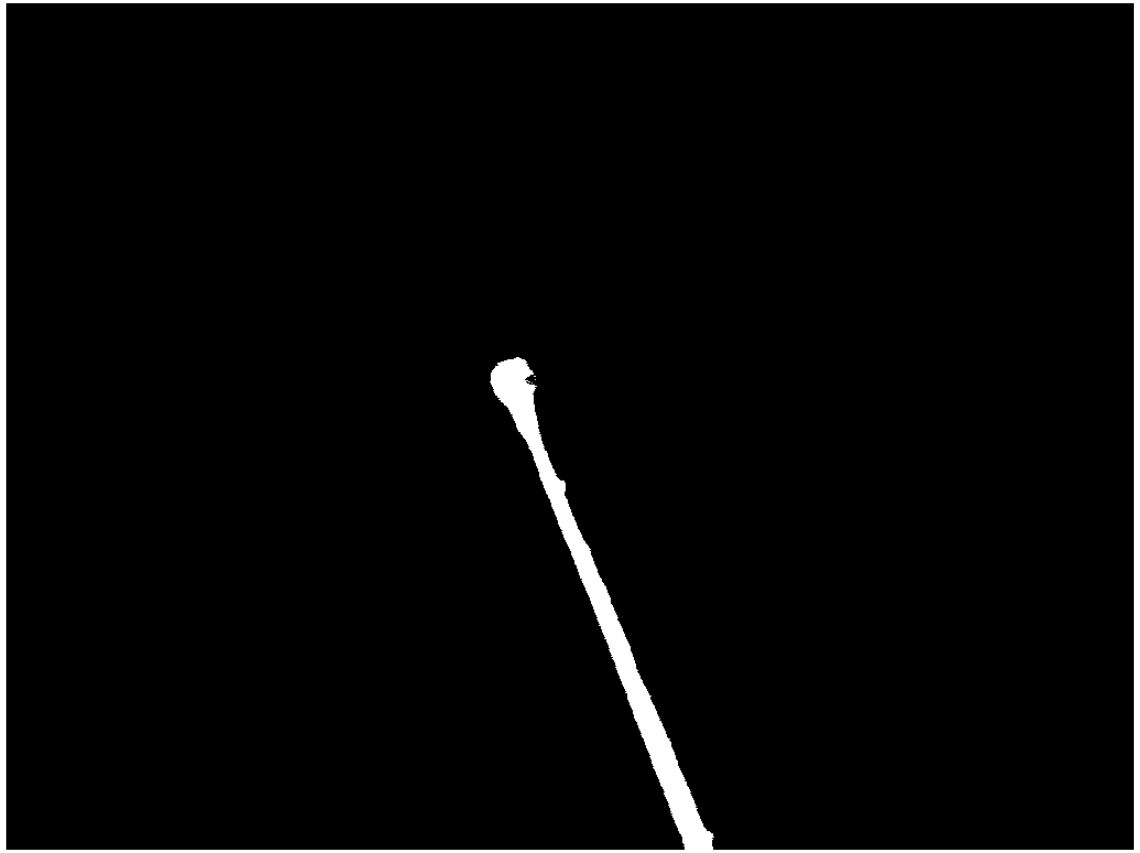
- Pre-processing
  - Filtering, segmentation
- Image transformation
  - Affine transformation, composition of translation and rotation
- Alignment
  - According to correlation values between the transformed image and a reference image

# 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

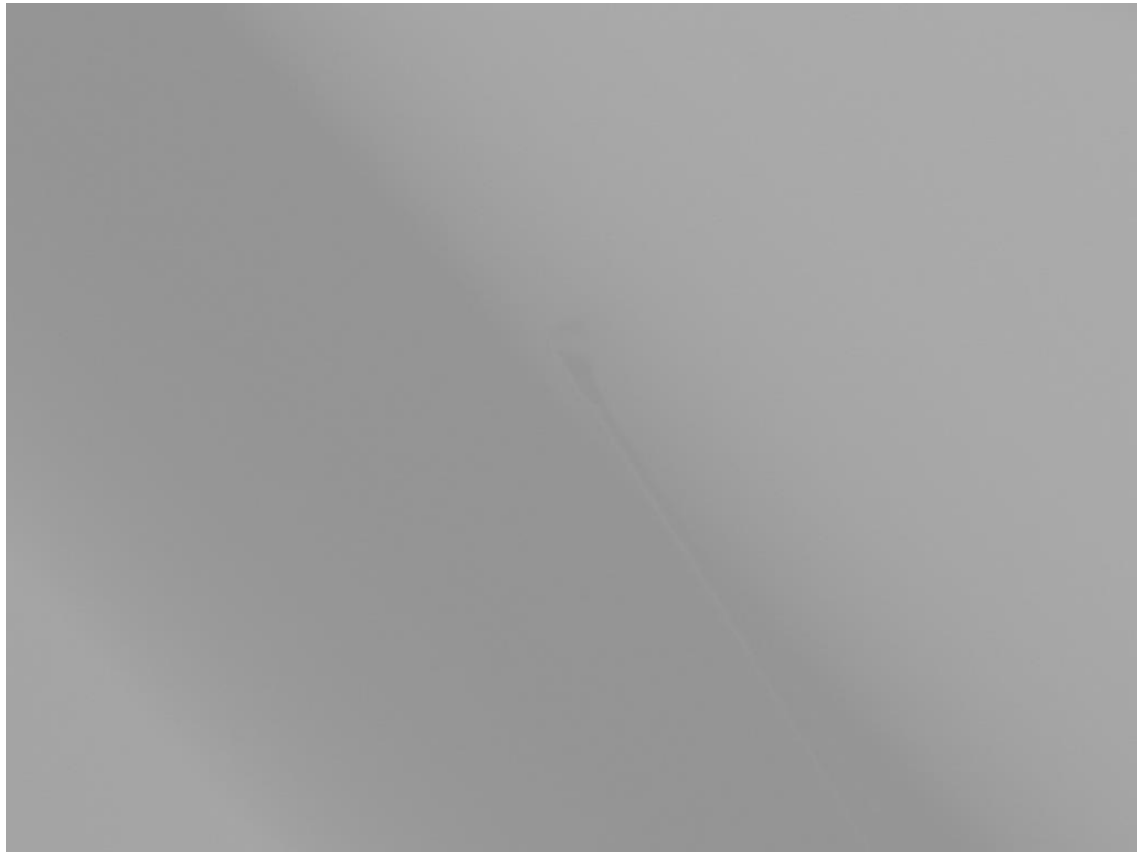
# Pre-processing

- A black and white image helps to solve the problem
- Segmentation obtained using Otsu's method for optimal threshold.
- For few images was necessarly 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 tansformation

- 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

- $c = \cos(\theta)$
- $s = \sin(\theta)$

$$T_2 = \begin{bmatrix} (1-c)u_x^2 + c & (1-c)u_x u_y - s u_z & (1-c)u_x u_z + s u_y & 0 \\ (1-c)u_x u_y + s u_z & (1-c)u_y^2 + c & (1-c)u_y u_z - s u_x & 0 \\ (1-c)u_x u_z - s u_y & (1-c)u_y u_z + s u_x & (1-c)u_z^2 + c & 0 \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

- Rotation axis
- $u = [u_X \; u_y \; u_z]$

# Image tansformation

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

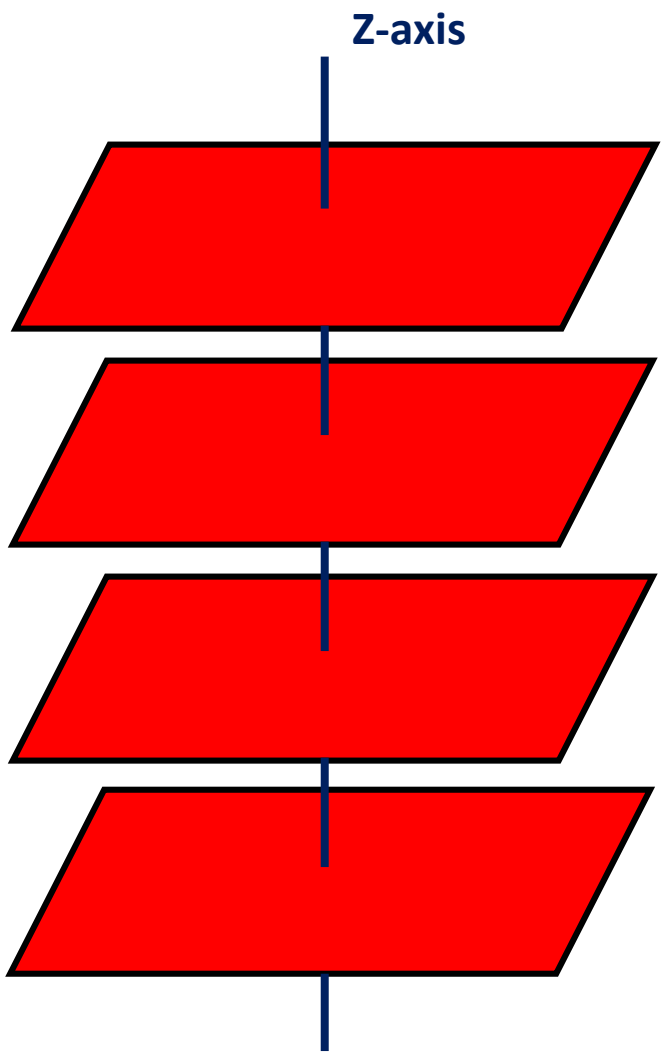
$$T_3 = \begin{bmatrix} 1 & 0 & 0 & t_x \\ 0 & 1 & 0 & t_y \\ 0 & 0 & 1 & t_z \\ 0 & 0 & 0 & 1 \end{bmatrix}$$

- The overall transformation matrix is

$$T = T_1 T_2 T_3$$

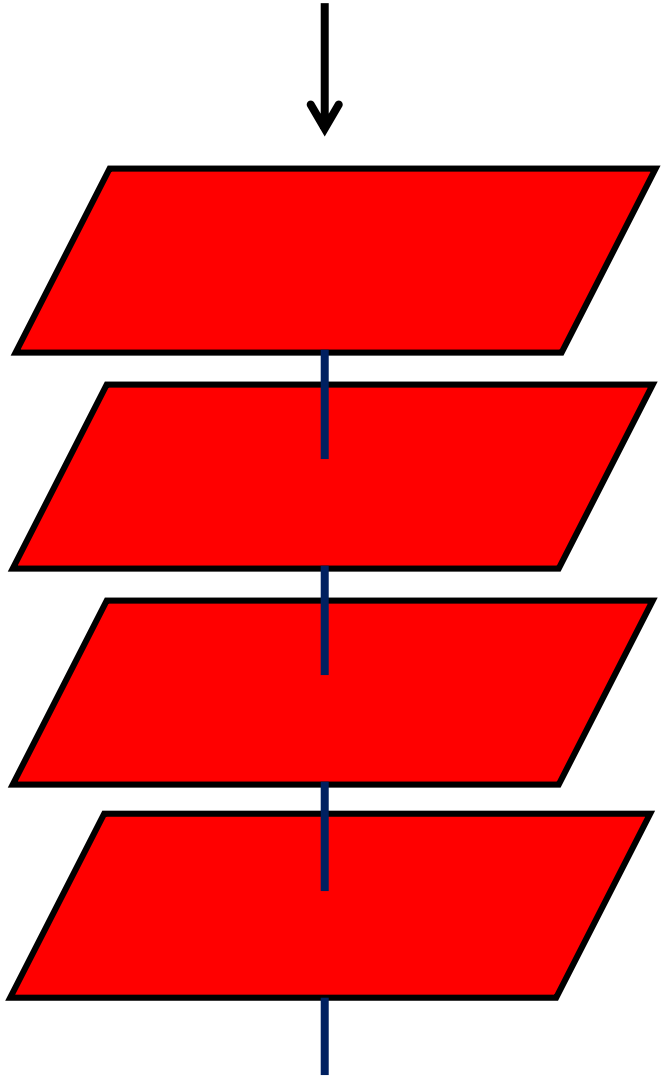
# Image transformation

- 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



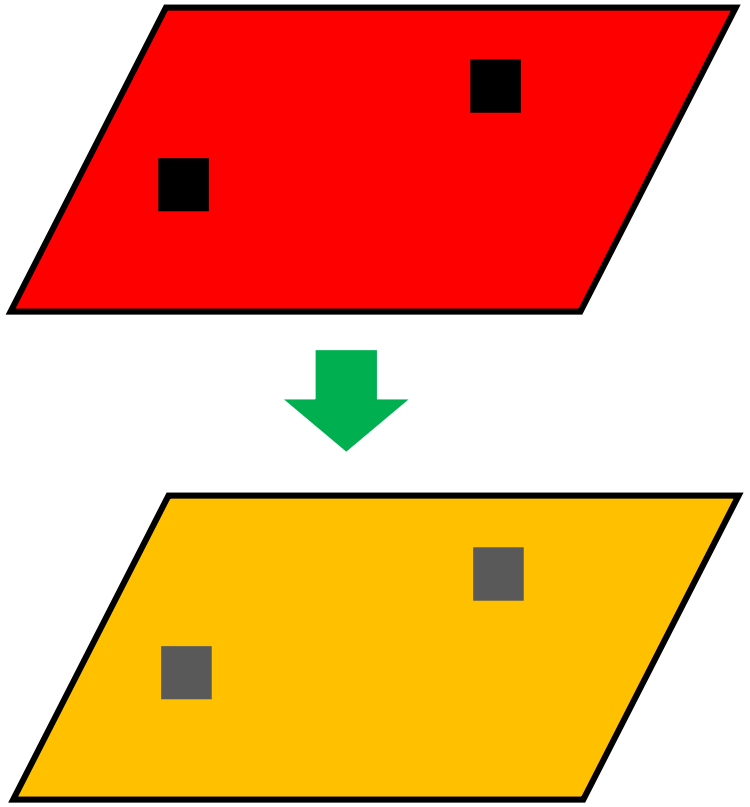
# Image space

- 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



# Modification of the range

- 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 correspondly 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

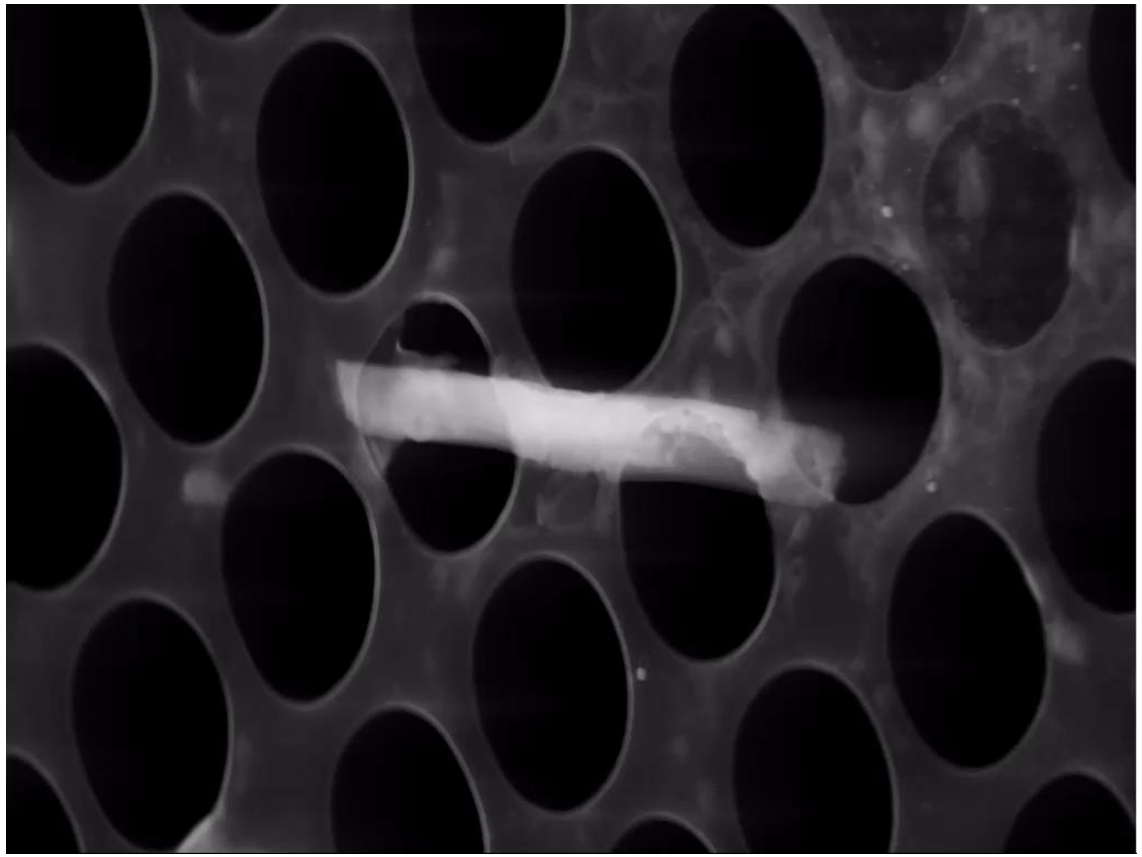
# How to evaluate the alignment?

- 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

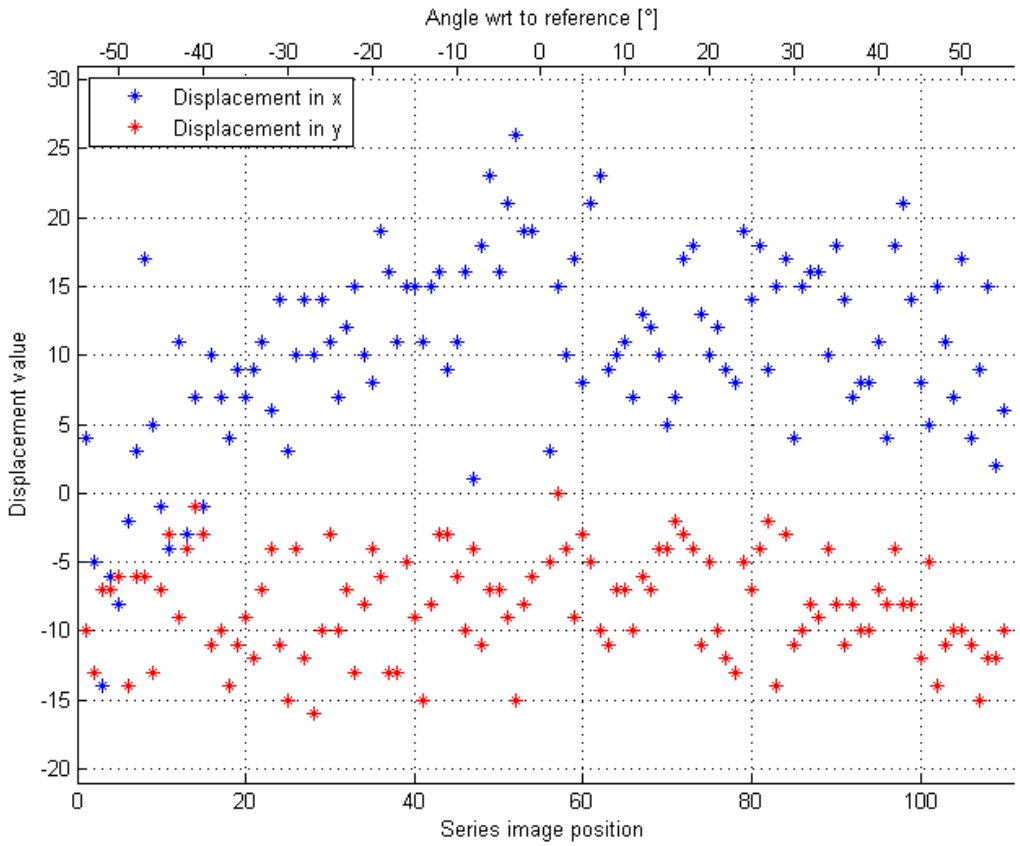
- Global correlation coefficient

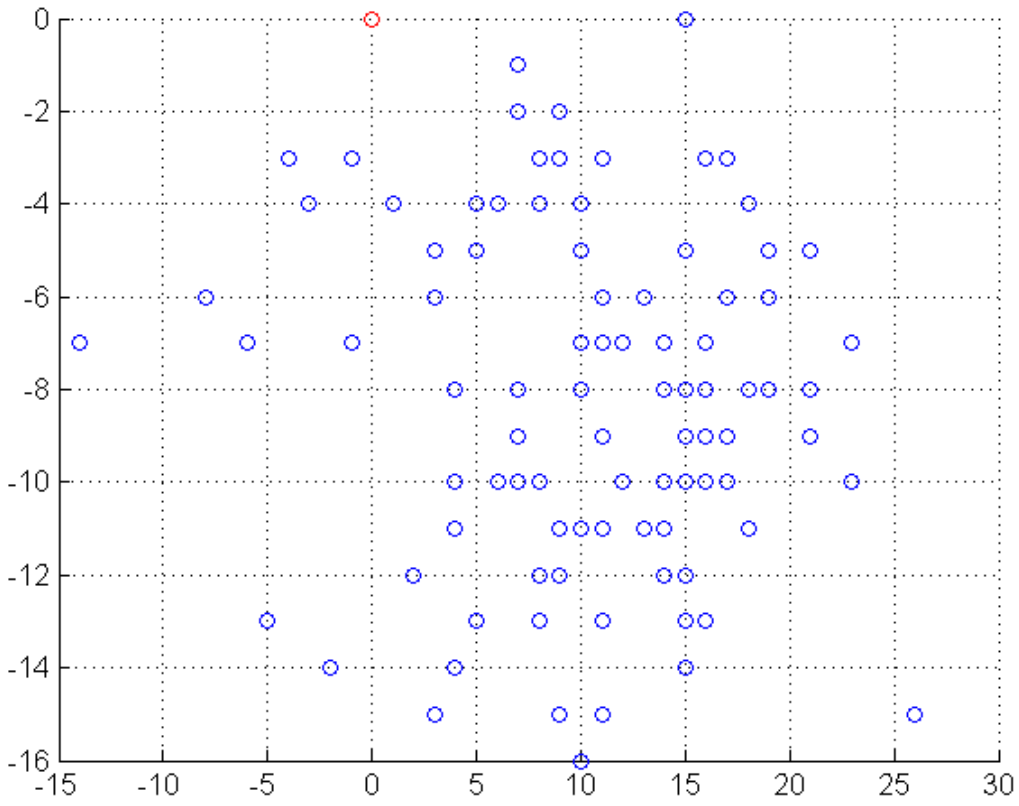
# Tilt series Fiber

- 110 images



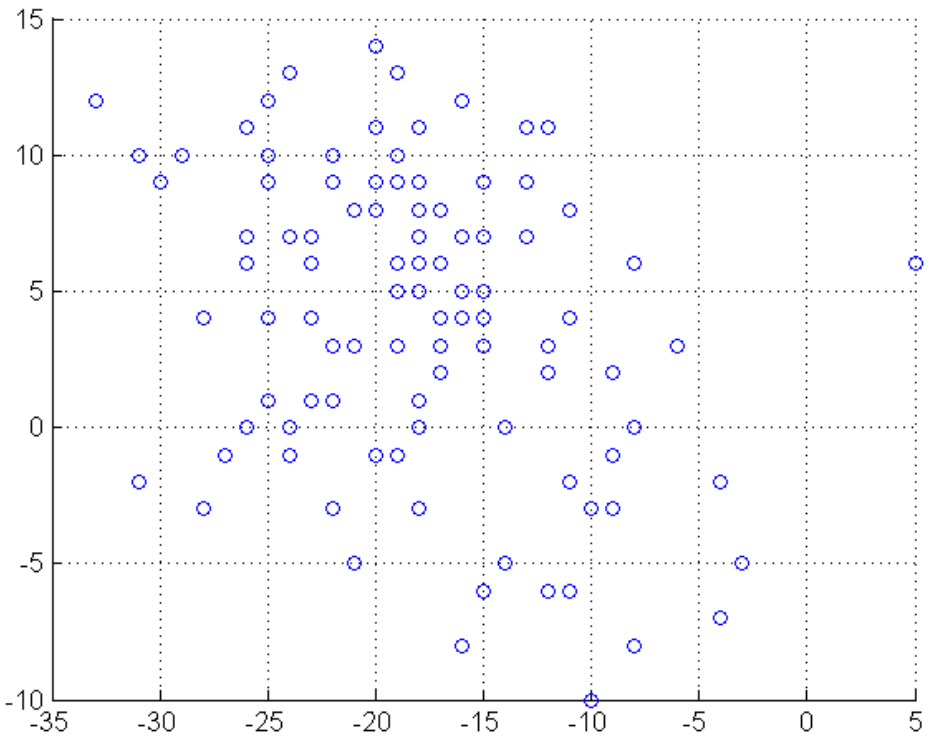
# Misalignment distribution – Fixed reference



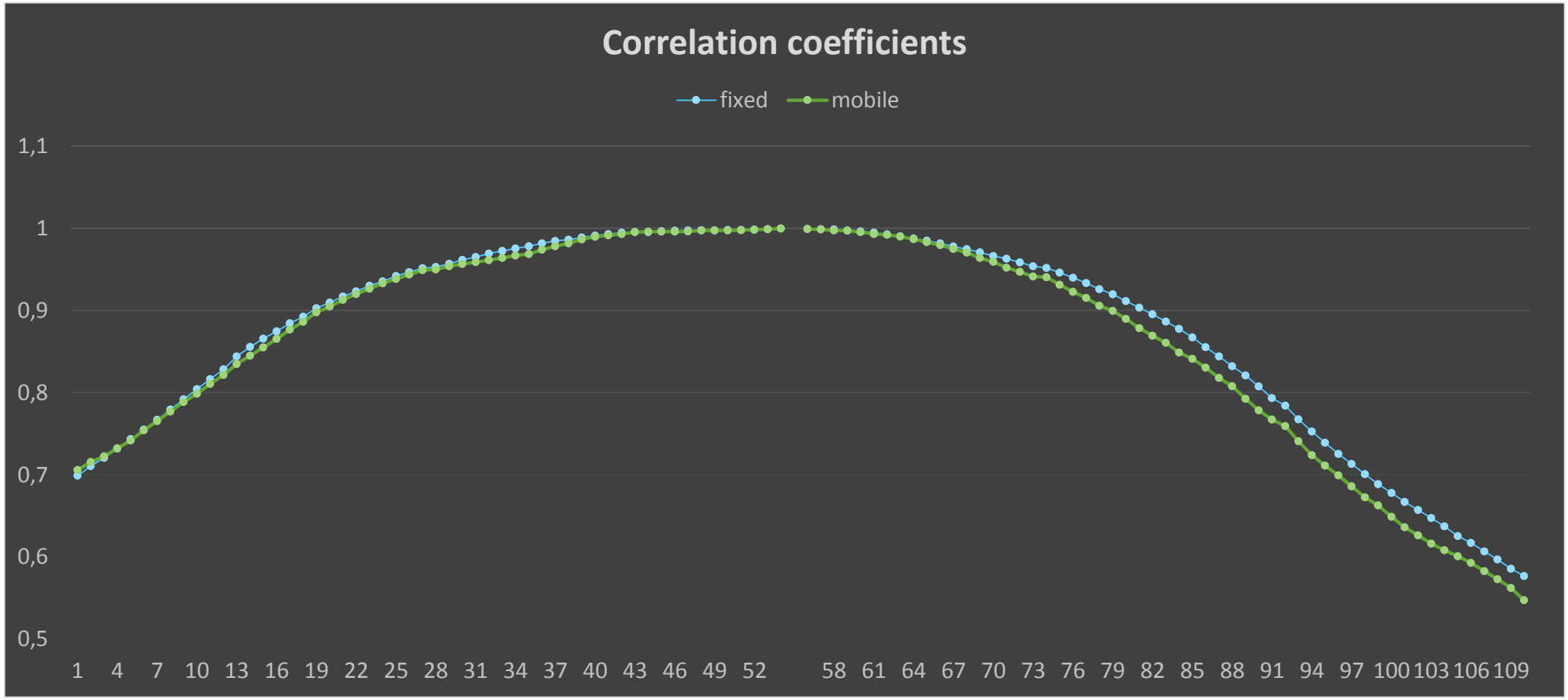
# Misalignment distribution – Mobile reference

- The image shows the distribution of the displacements of images wrt reference image



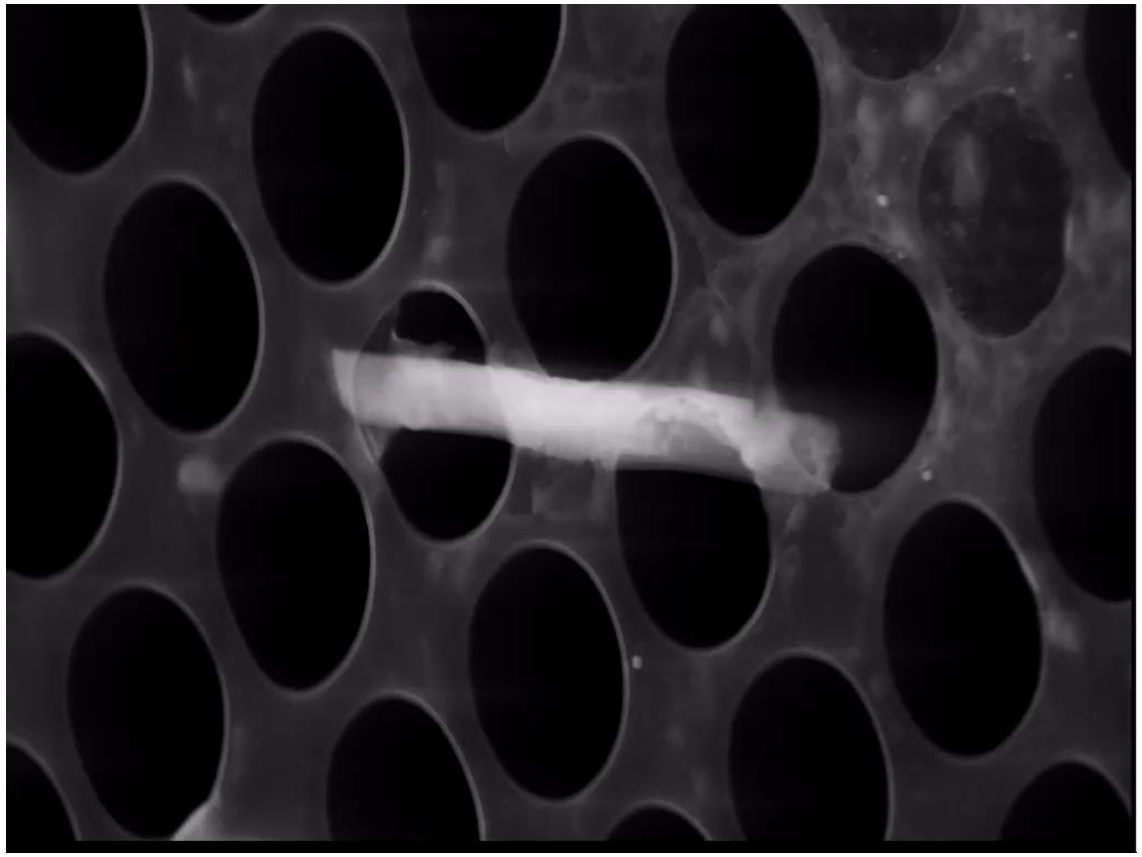
# Correlation coefficients – Consecutive images

# Correlation coefficients – Fixed reference



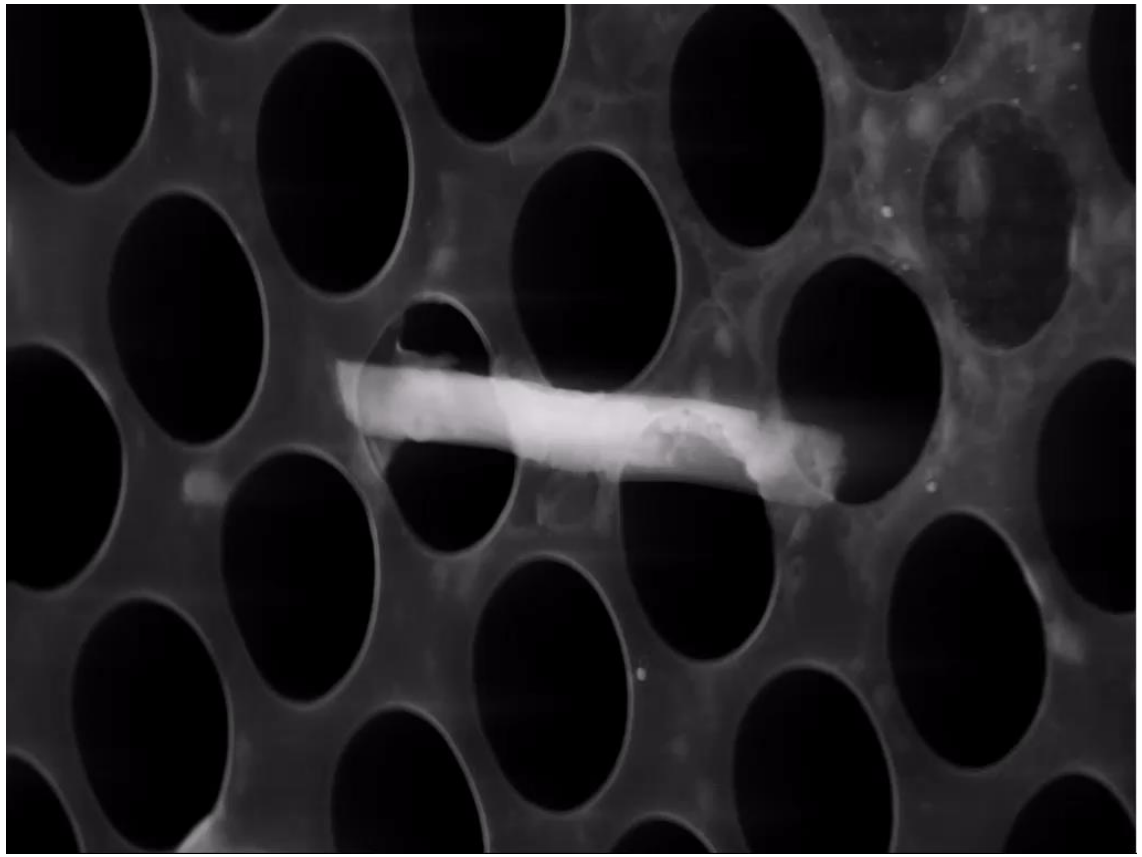
# Alignment – Fixed reference

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



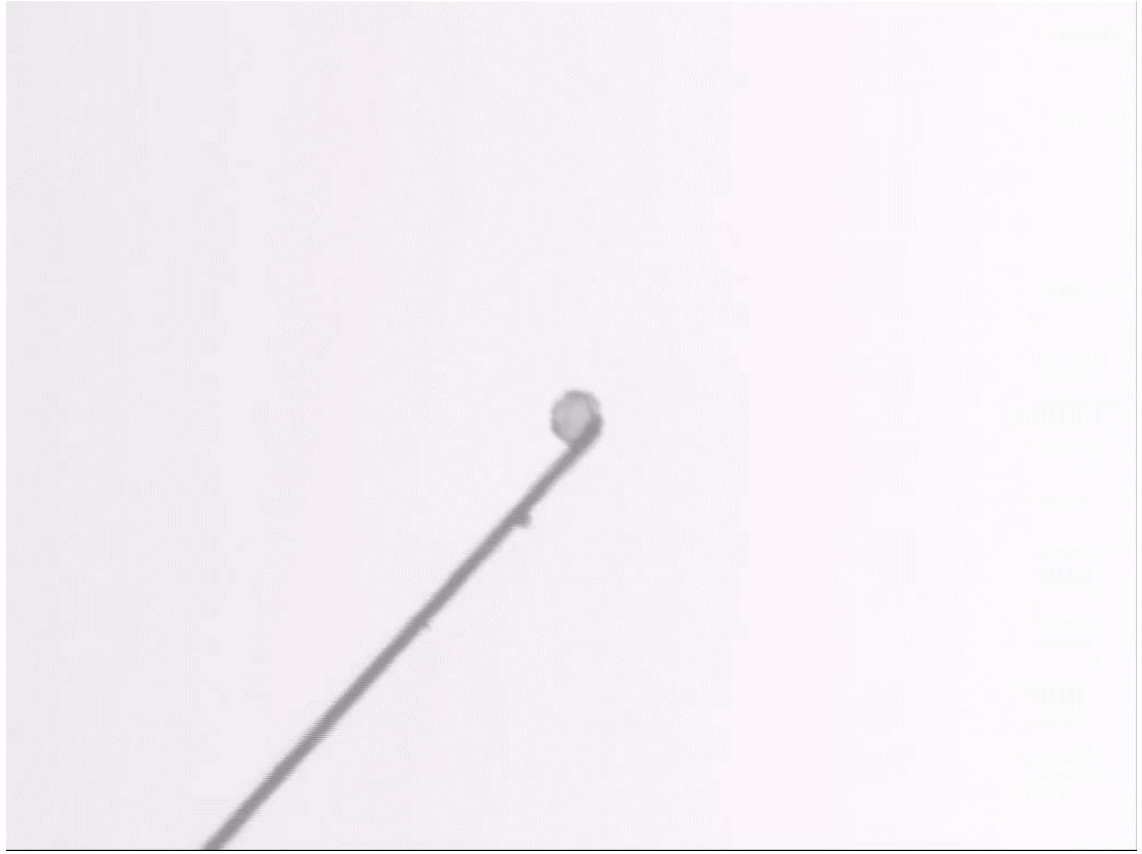
# Alignment – Mobile reference

- Global correlation coefficient: 0.9920

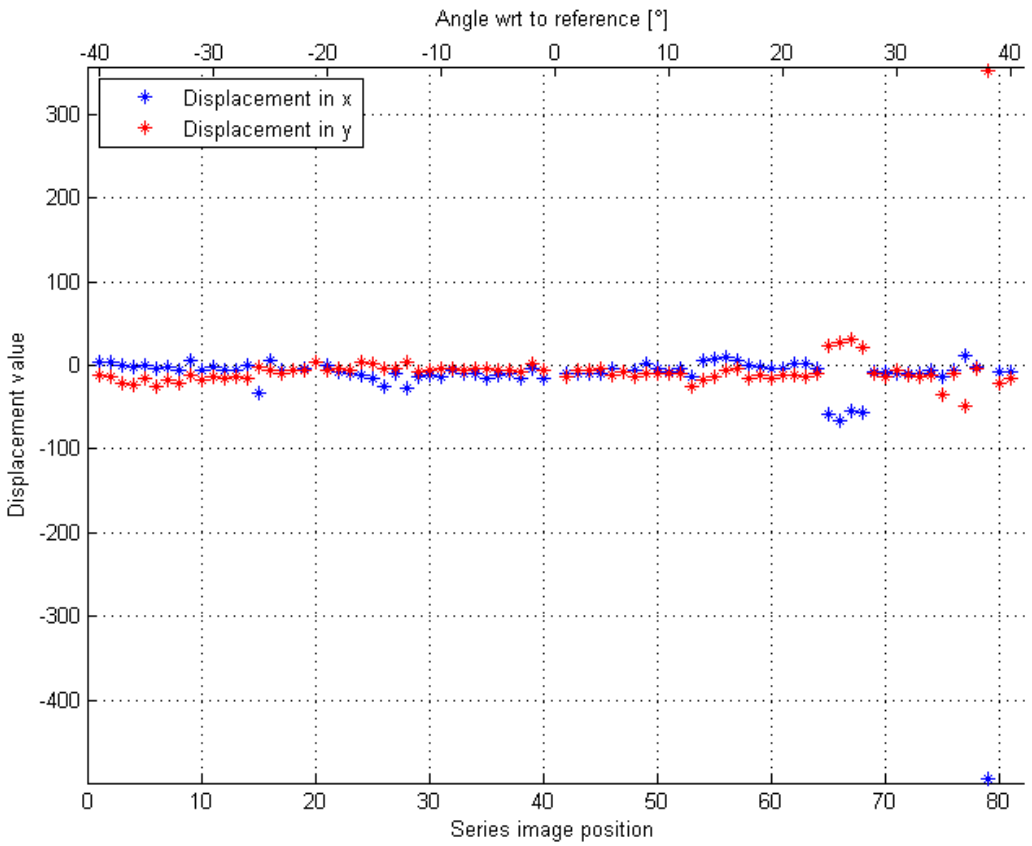


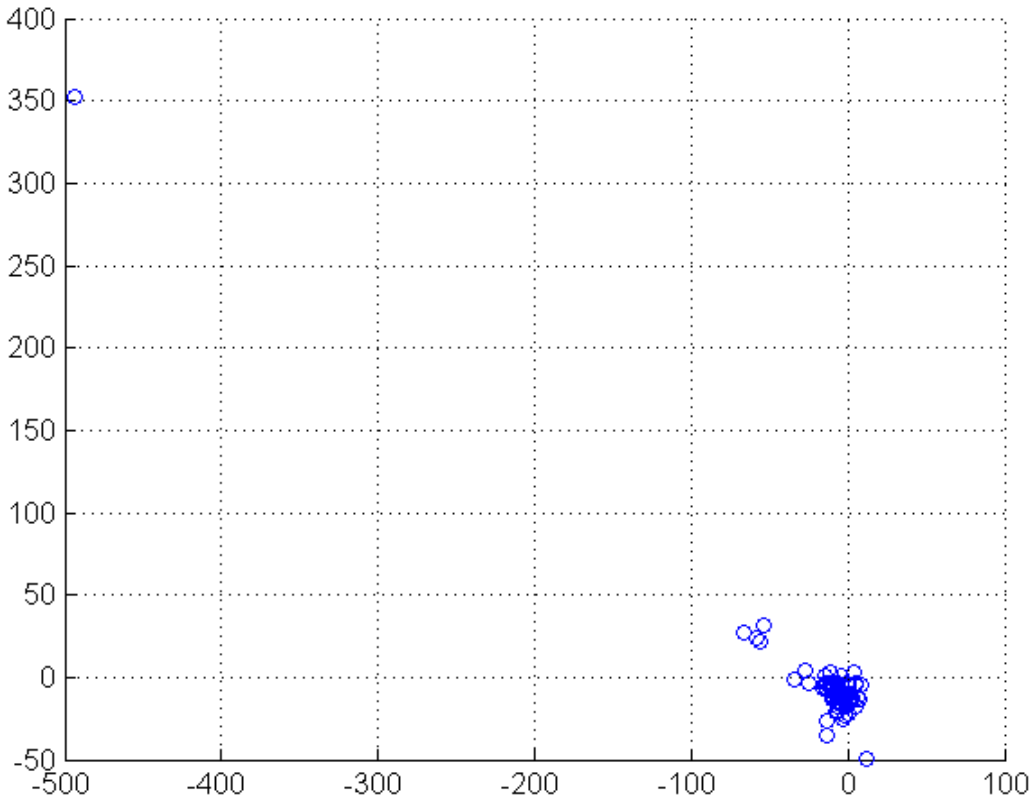
# Tilt series 06

- 81 images
- Global correlation coefficient: 0.6539

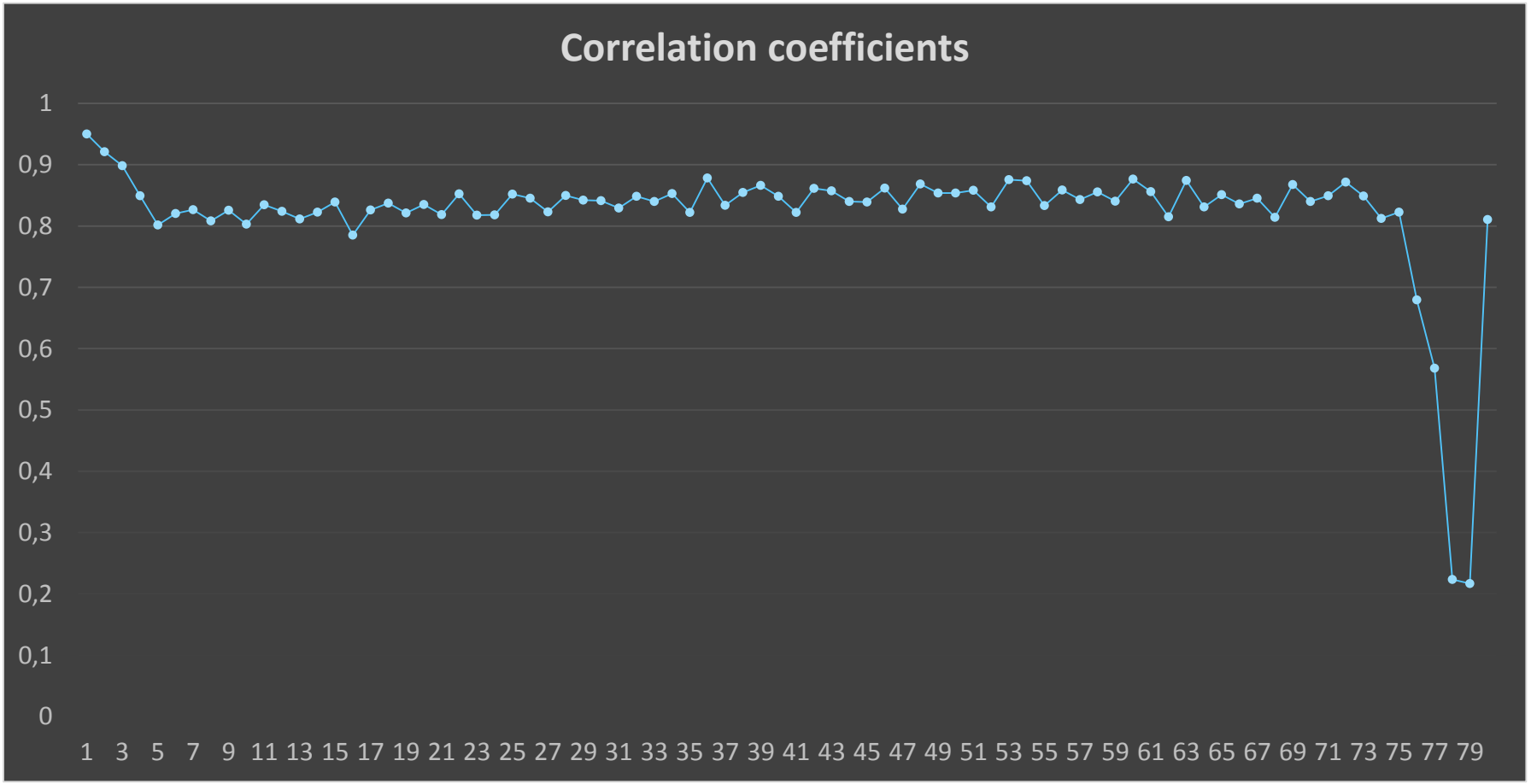


# Misalignment distribution



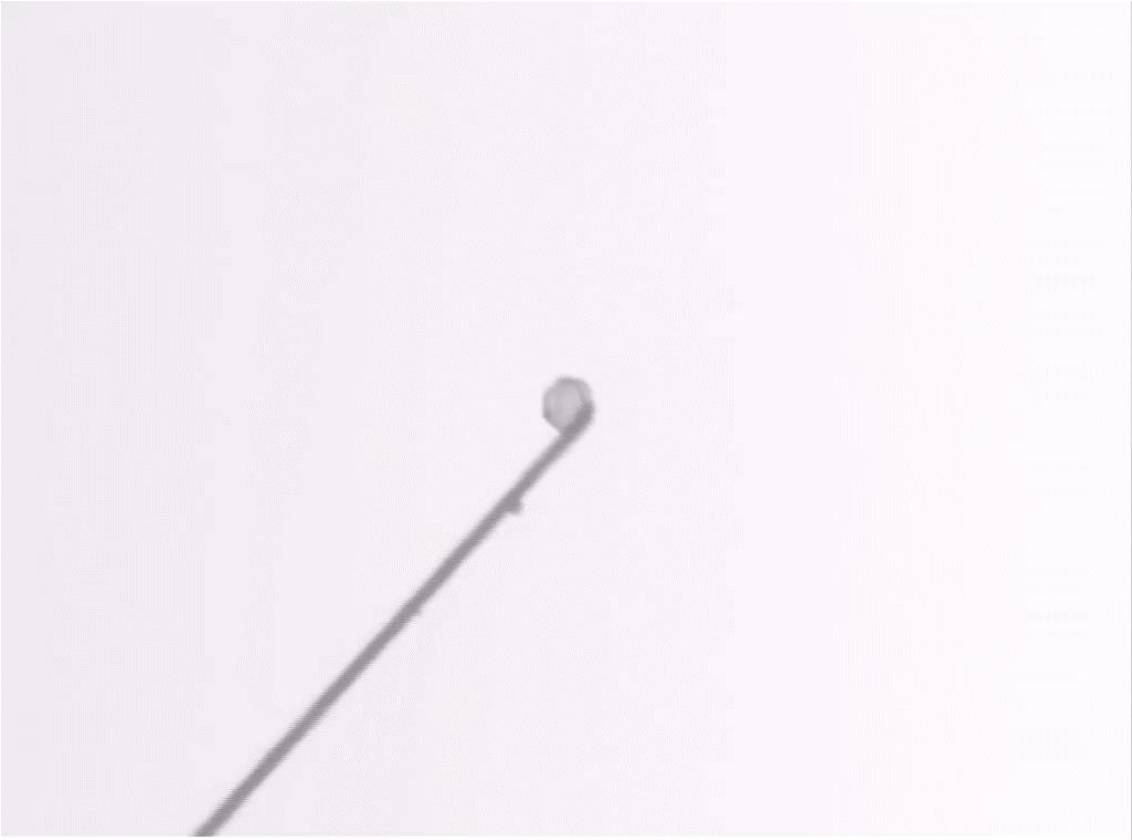



# Correlation coefficients



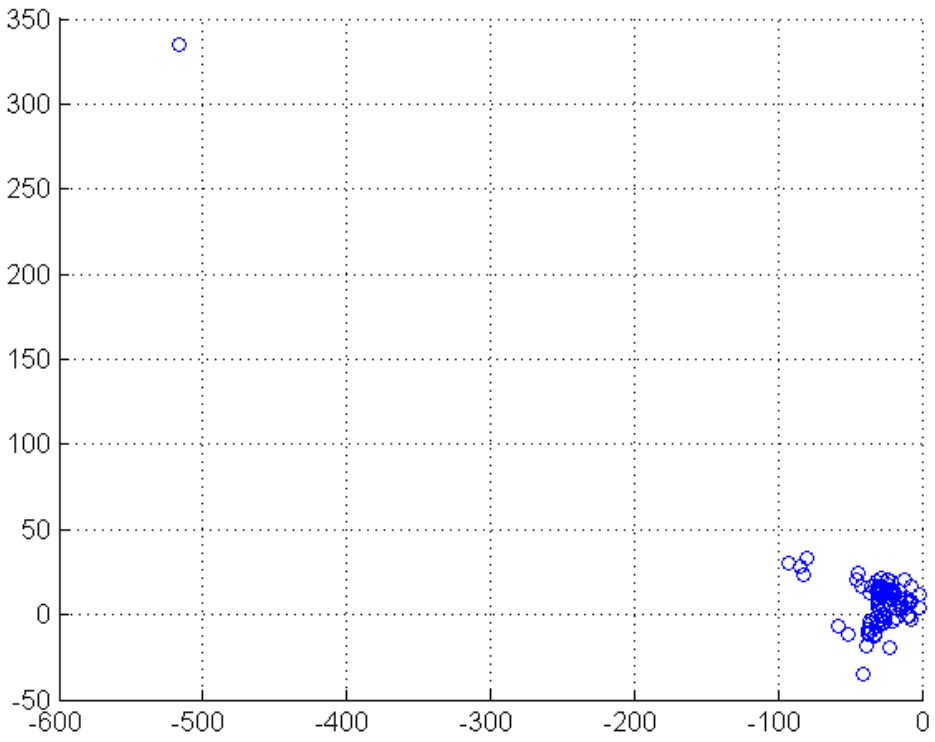
# Alignment – Fixed reference

- 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

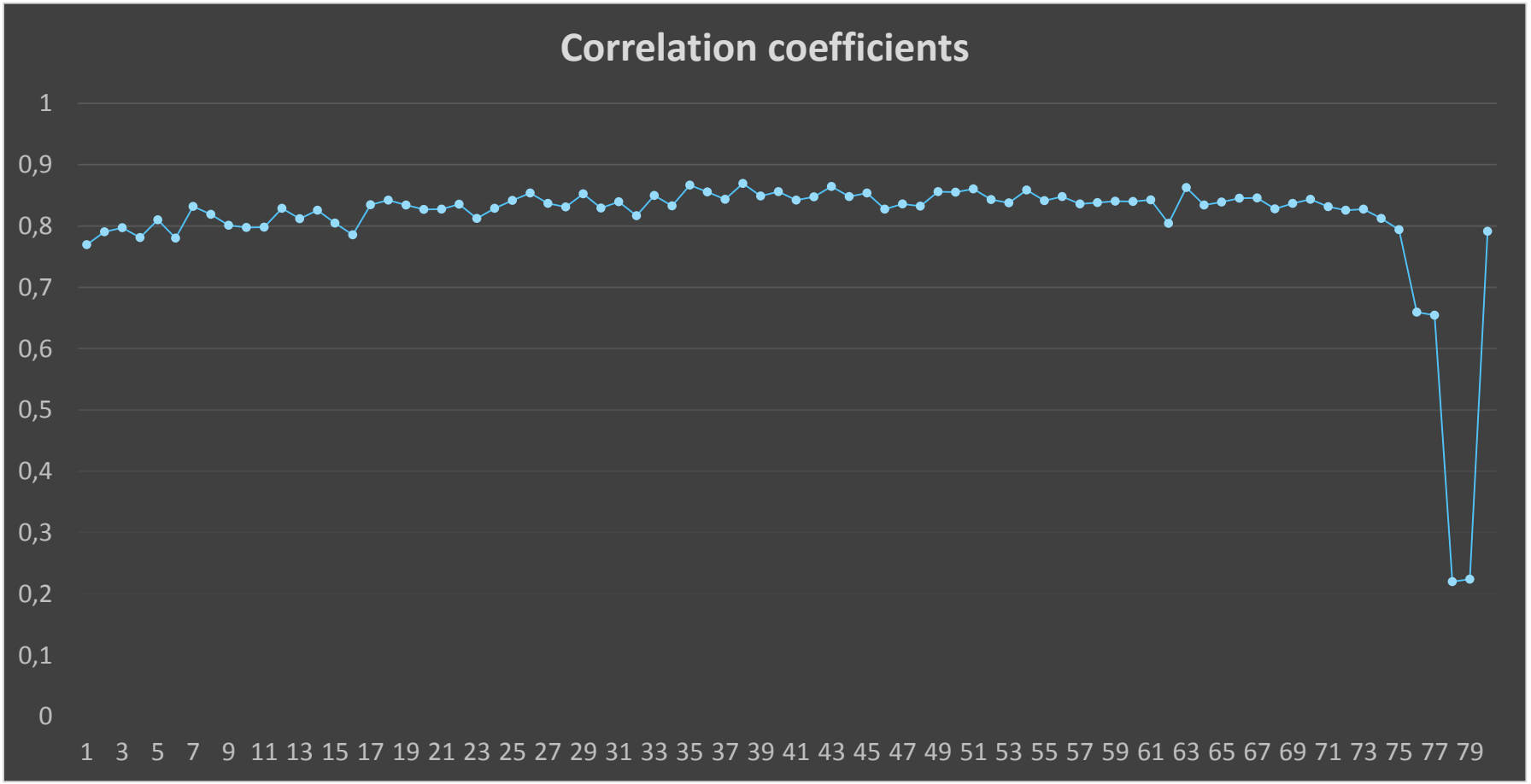


# Misalignment distribution

- The image shows the distribution of the displacements of images wrt reference image
- One image present a very large displacements in both directions

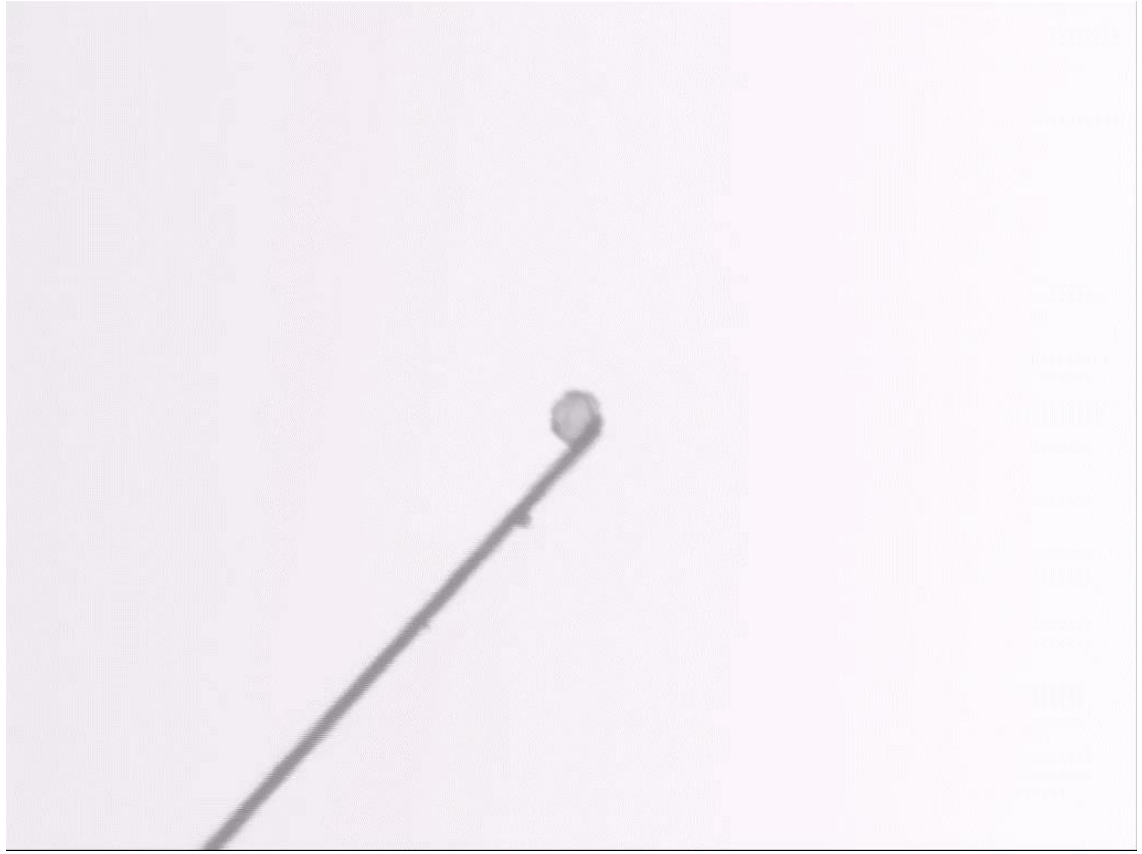


# Correlation coefficients



# Alignment – Mobile reference

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

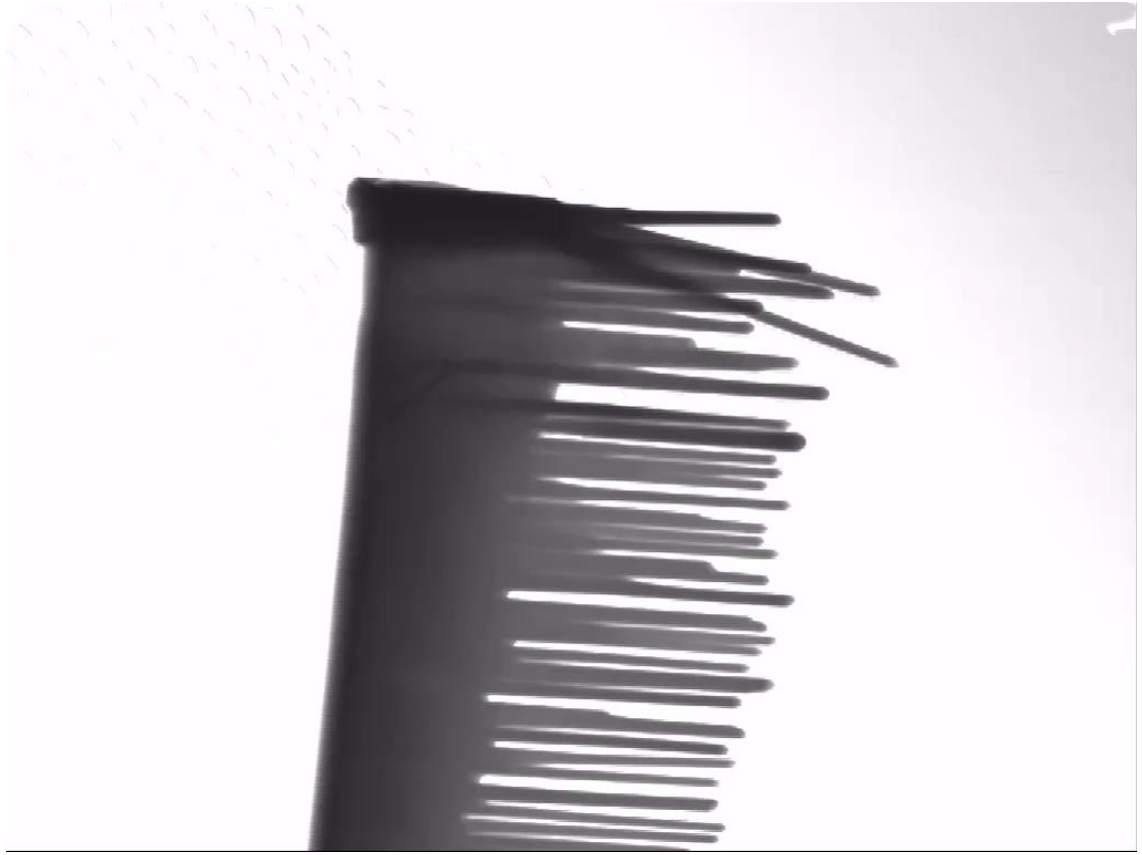


# Tilt series 07

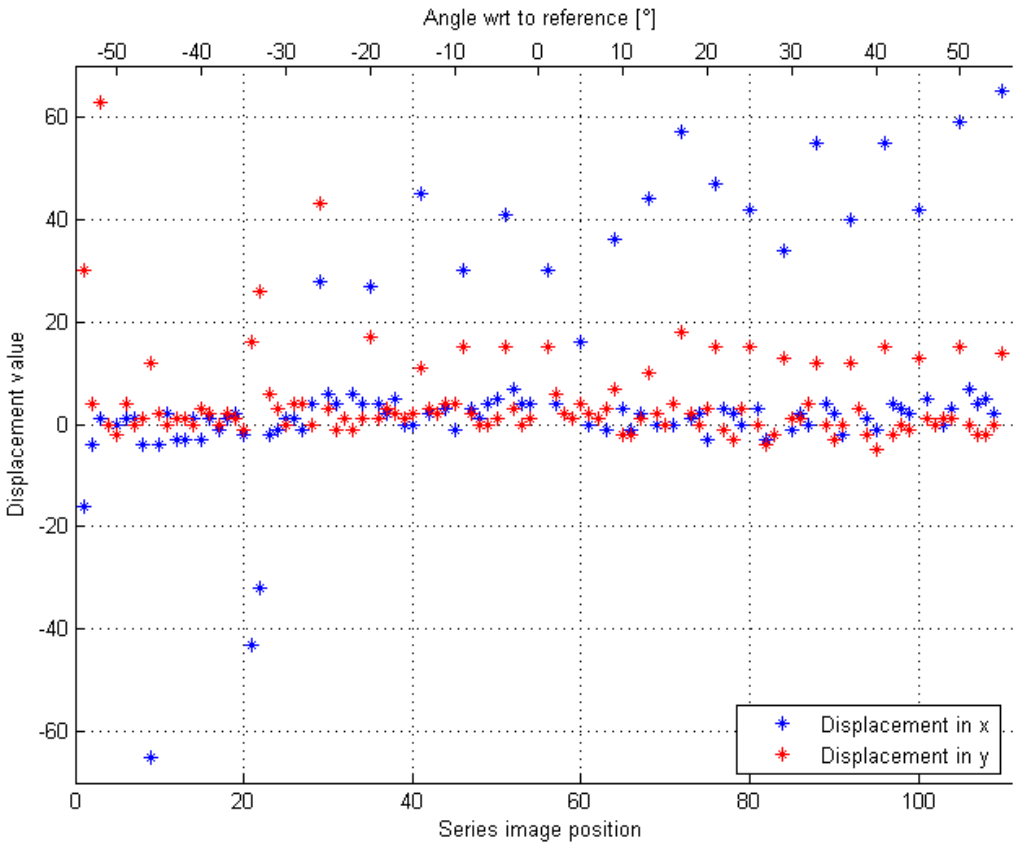
- ZnO comb
- 16 Bit gray

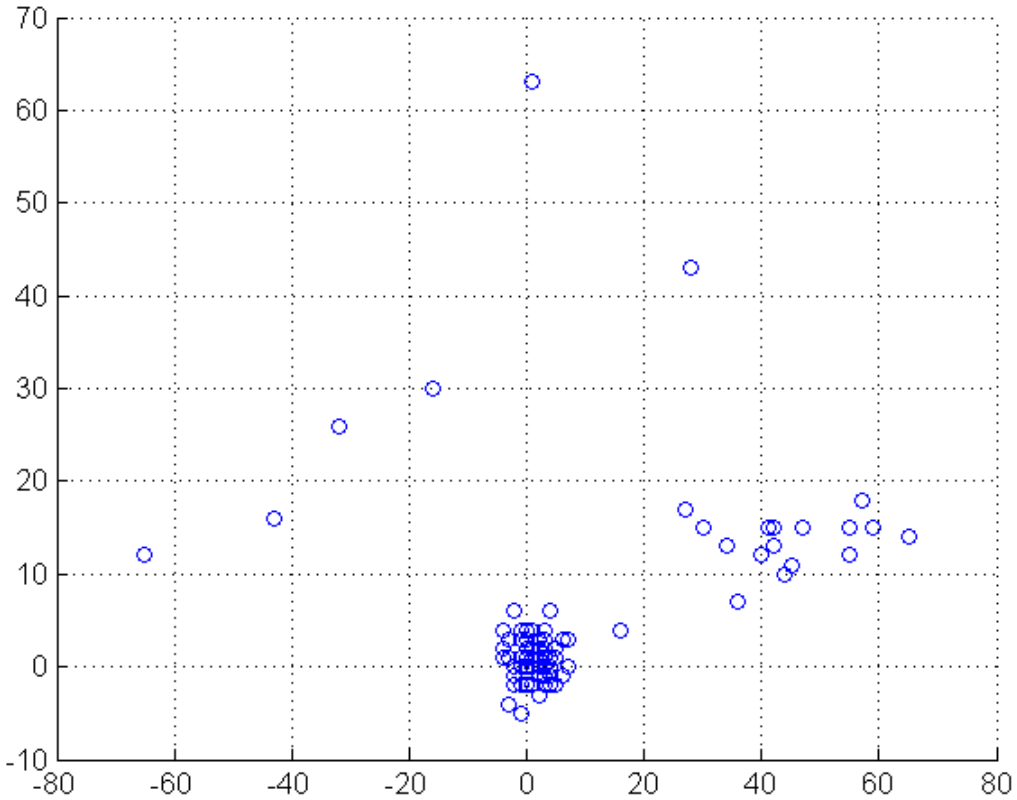
- From 120 degrees to 229 degrees
- 110 images

- Global correlation coefficient: 0.8665

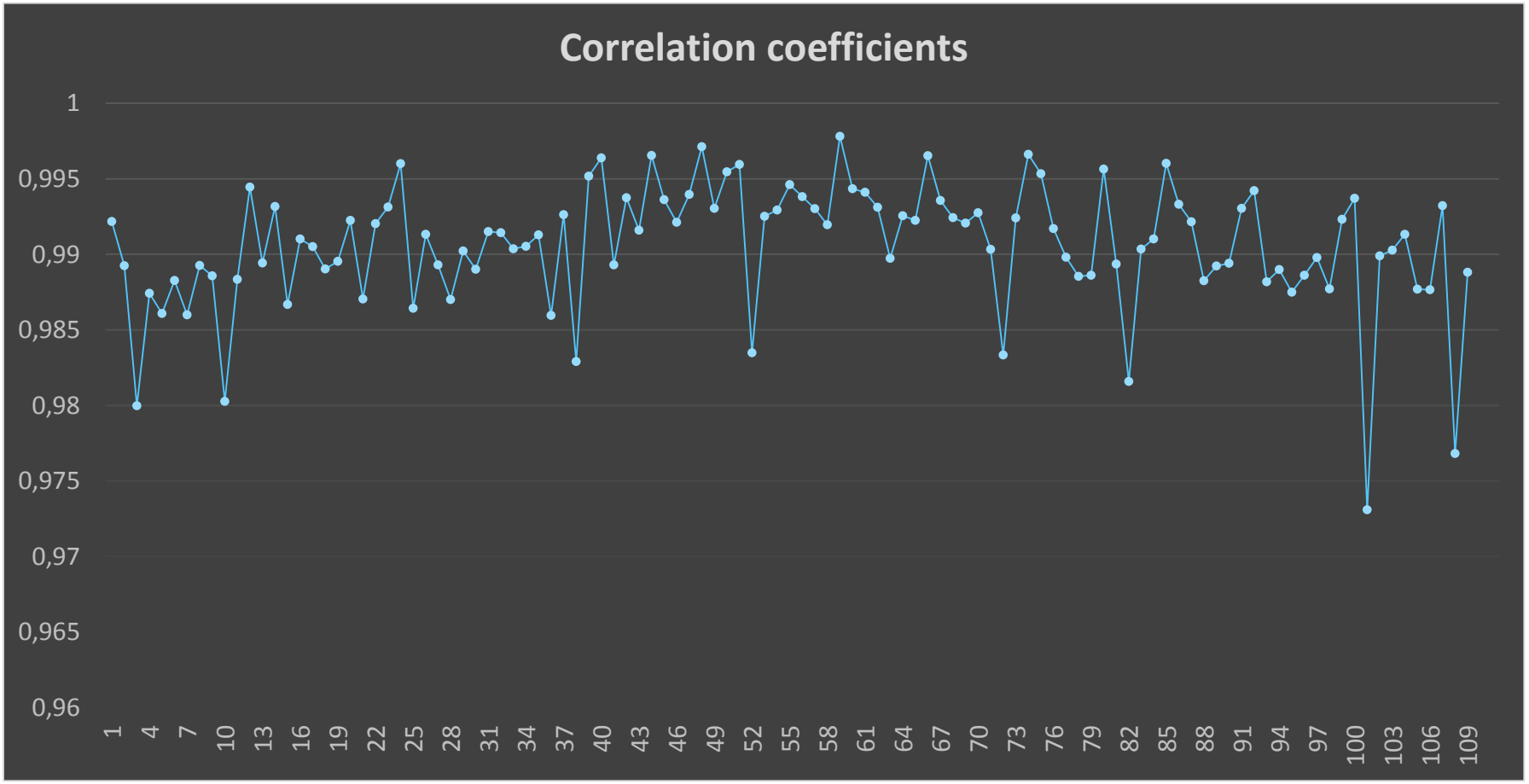


# Misalignment distribution



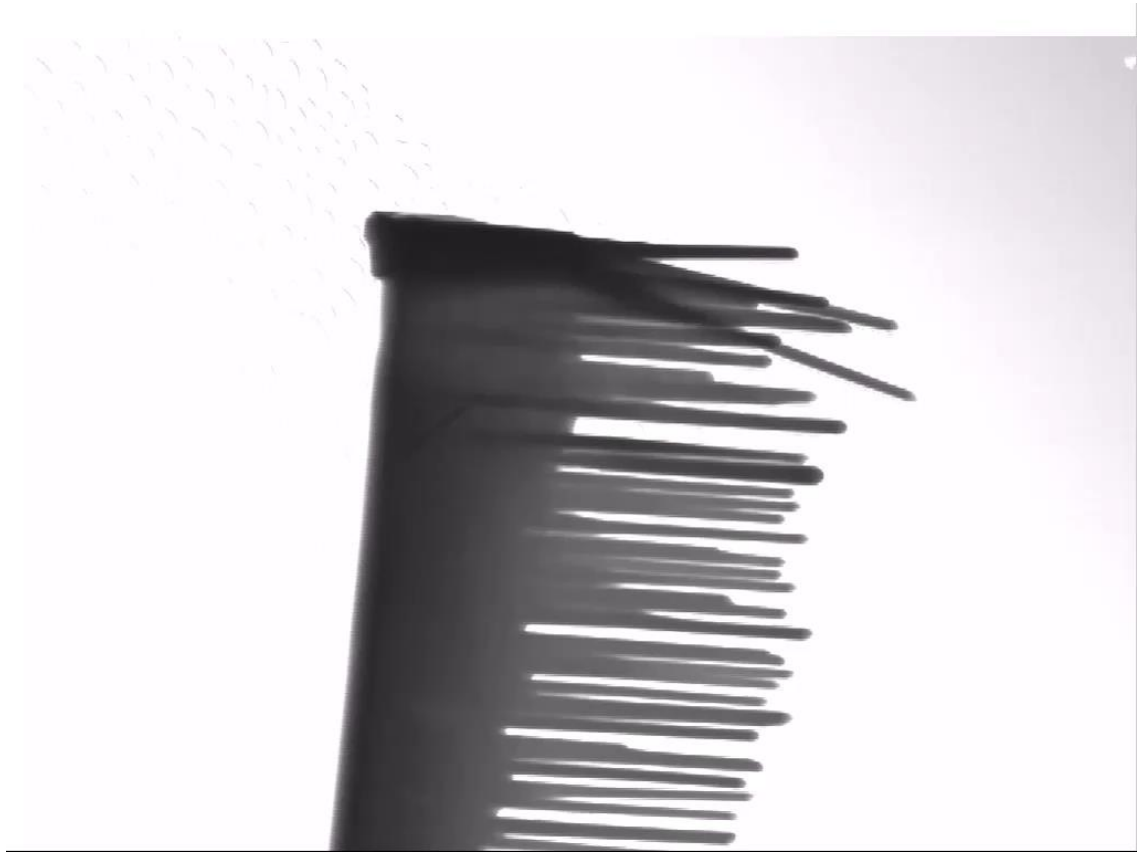



# Correlation coefficients



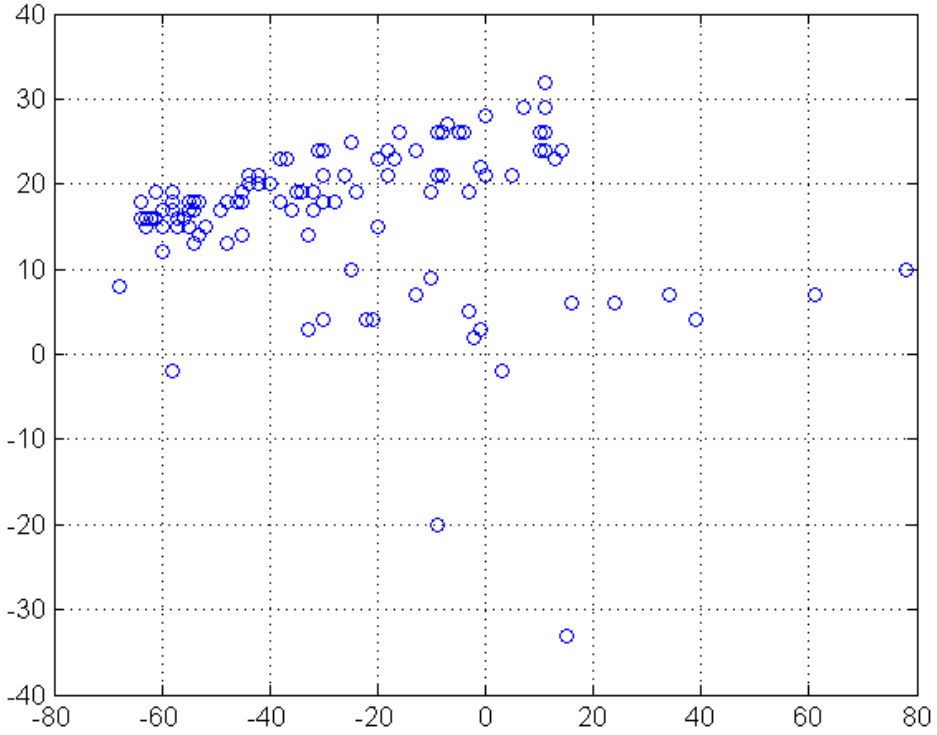
# Alignment – Fixed reference

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

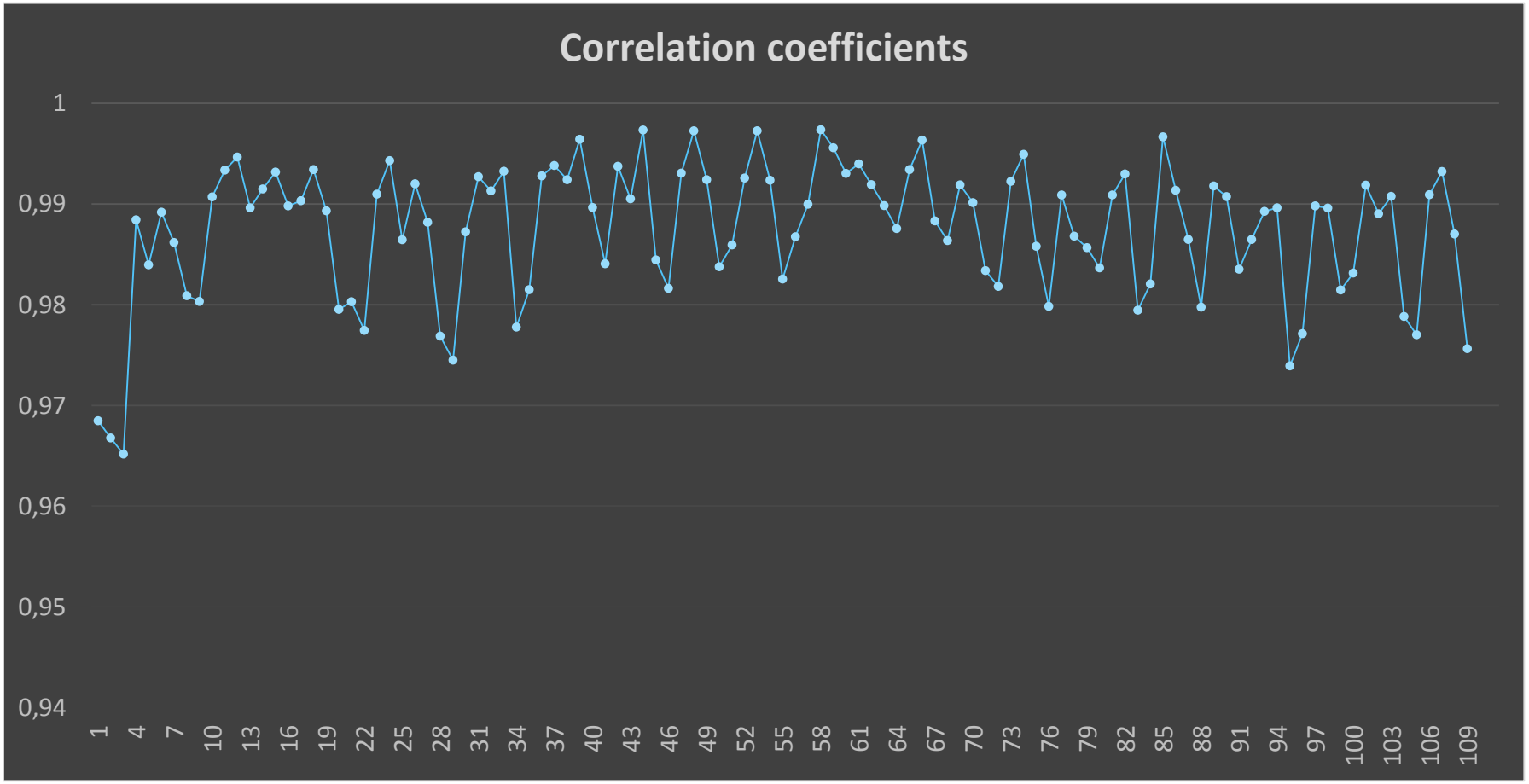


# Misalignment distribution

- The image shows the distribution of the displacements of images wrt reference image

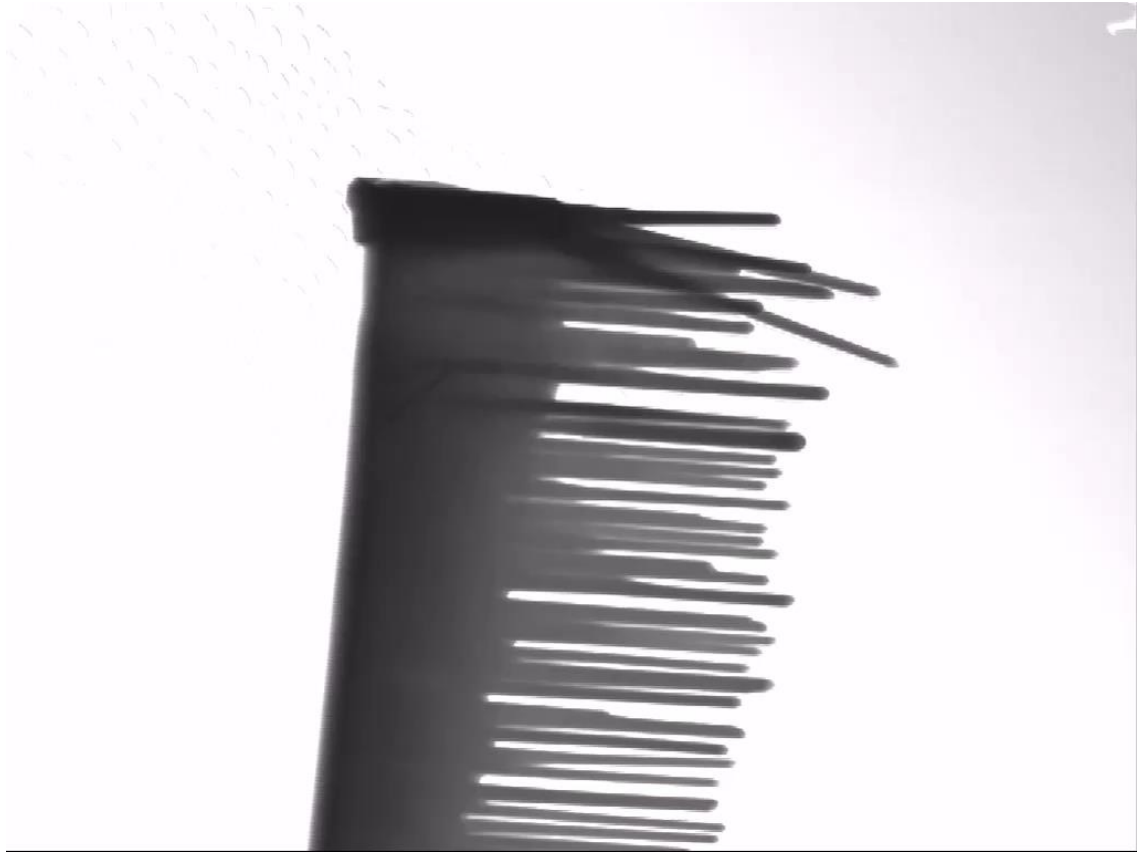


# Correlation coefficients



# Alignment – Mobile reference

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



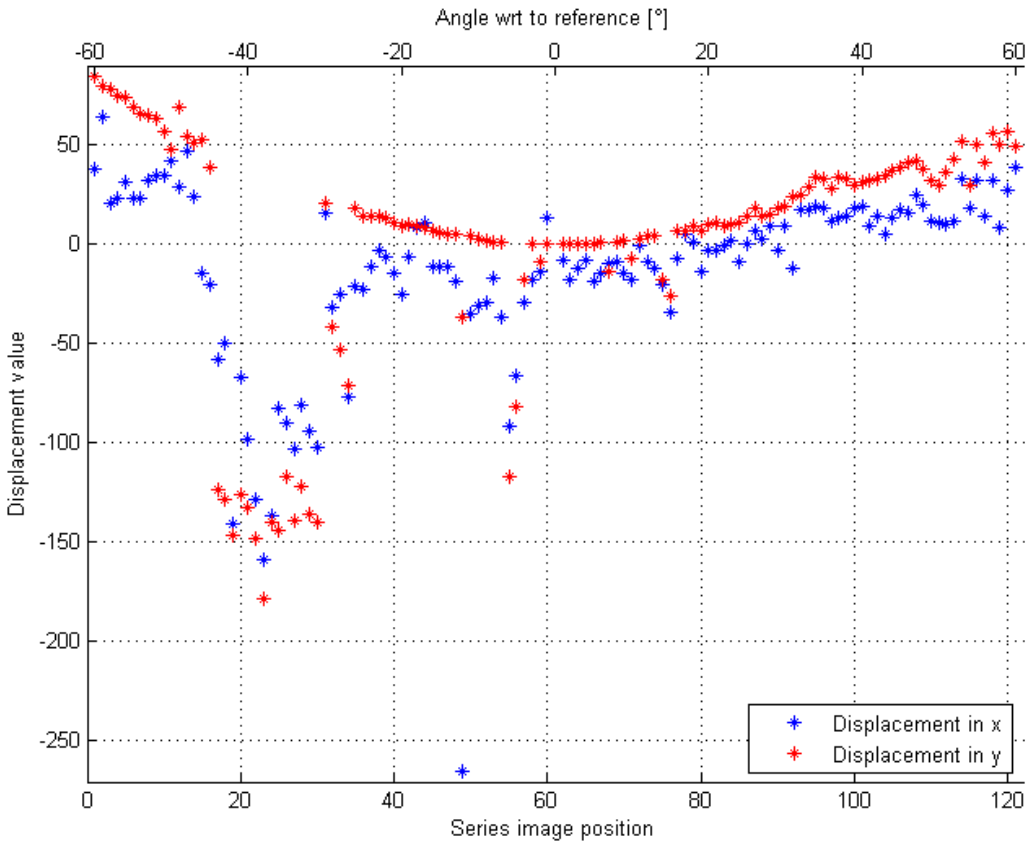
# Tilt series 08

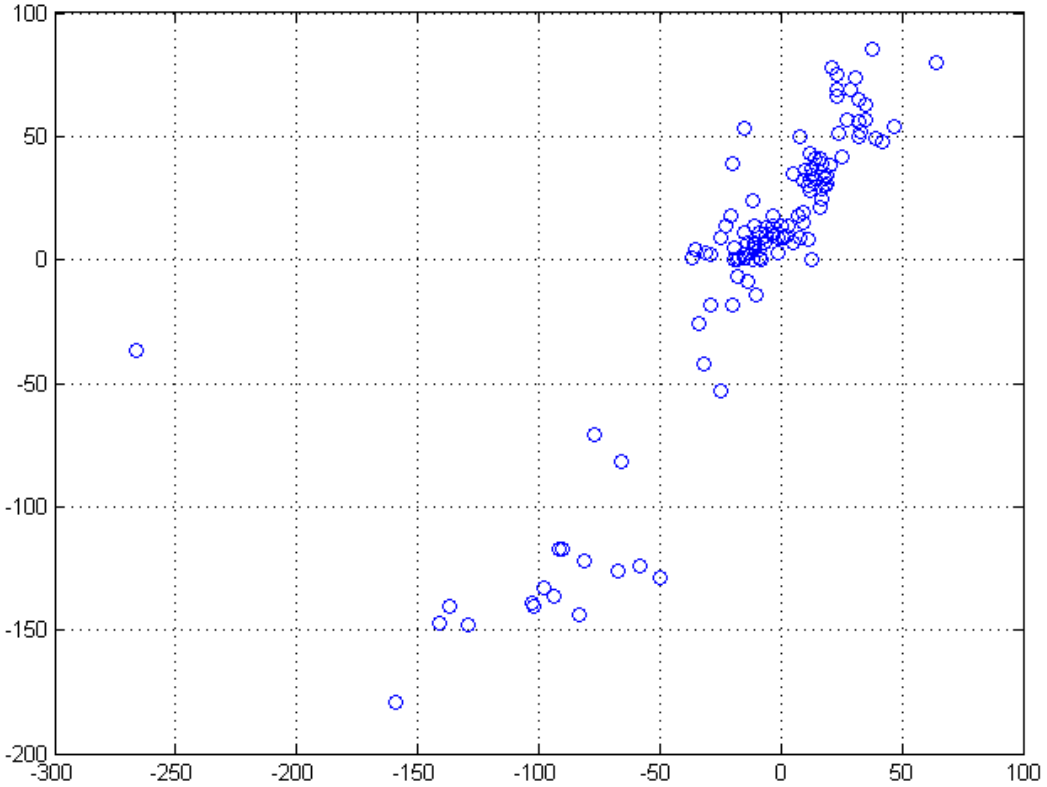
- ZnO comb
- 16 Bit grey

- From 122 degrees to 242 degrees
- 121 images

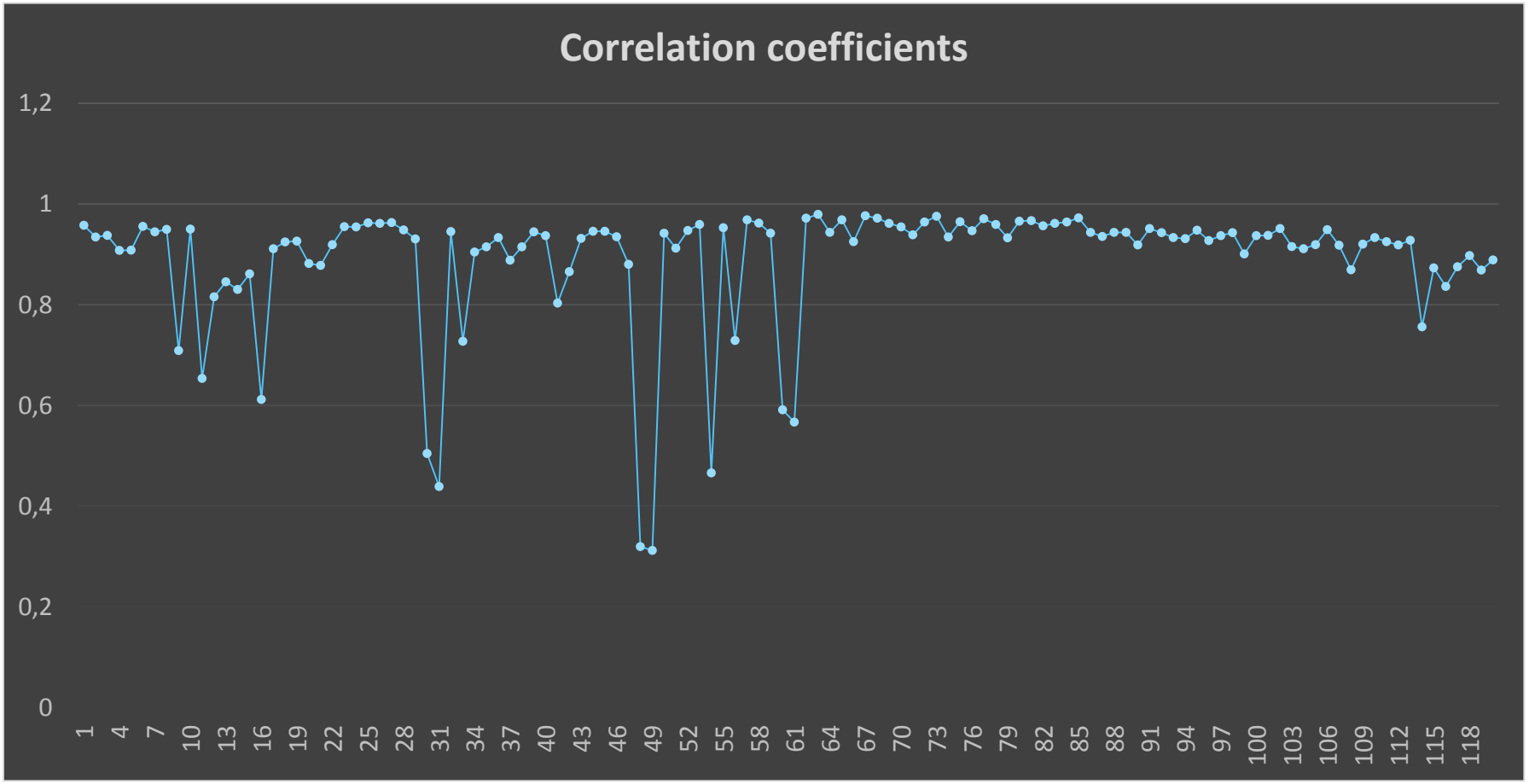
- Global correlation coefficient: 0.6970

# Misalignment distribution



# Correlation coefficients

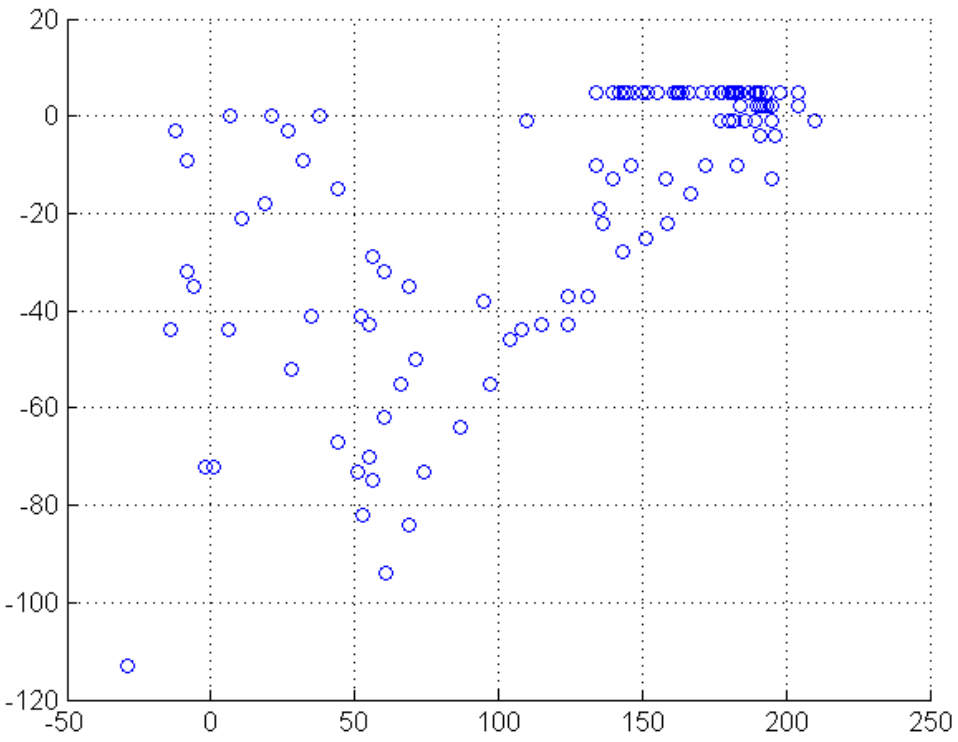


# Alignment – Fixed reference

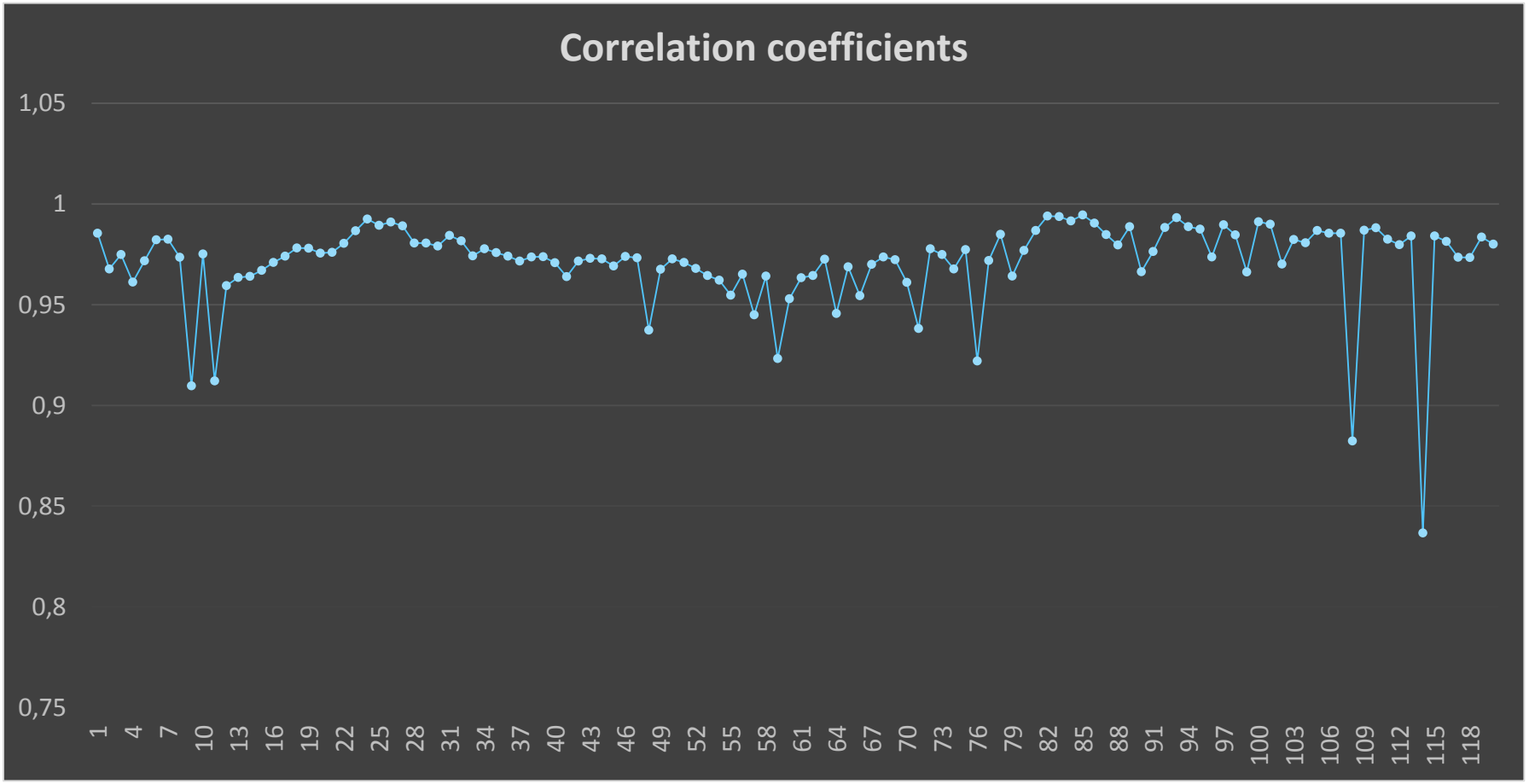
- Central image taken as the reference
- Global correlation coefficient: 0.8906
- Min: 0.3116

# 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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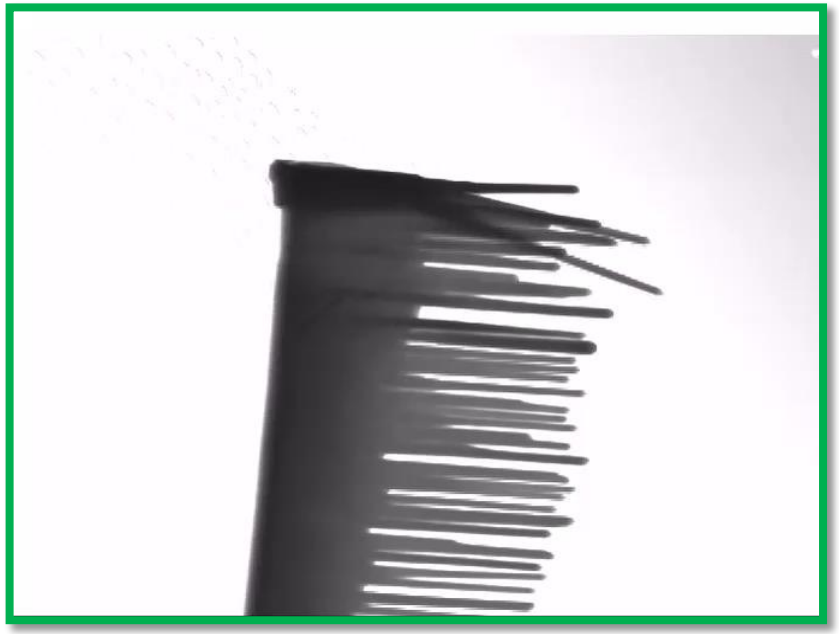
# Results comparison

Manual alignment

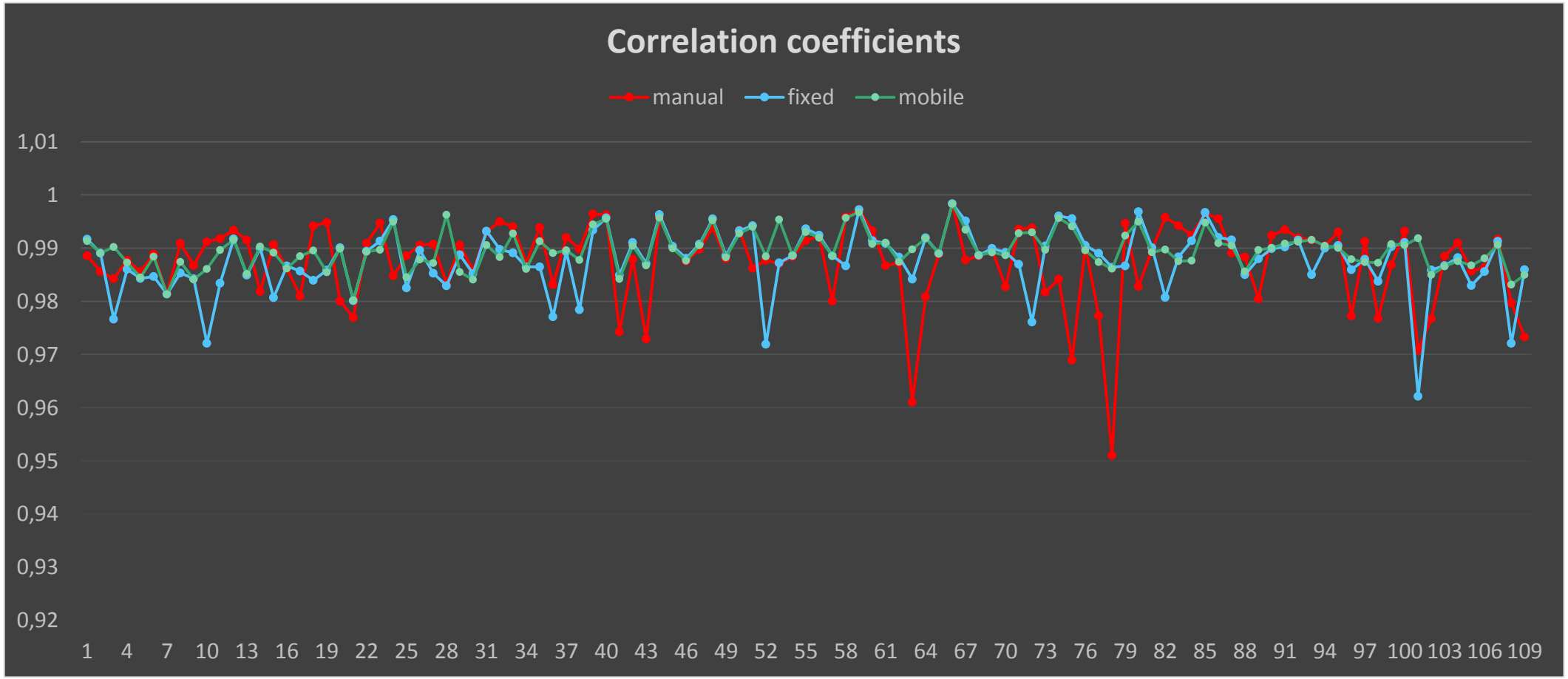
Global correlation coefficient: 0.9875



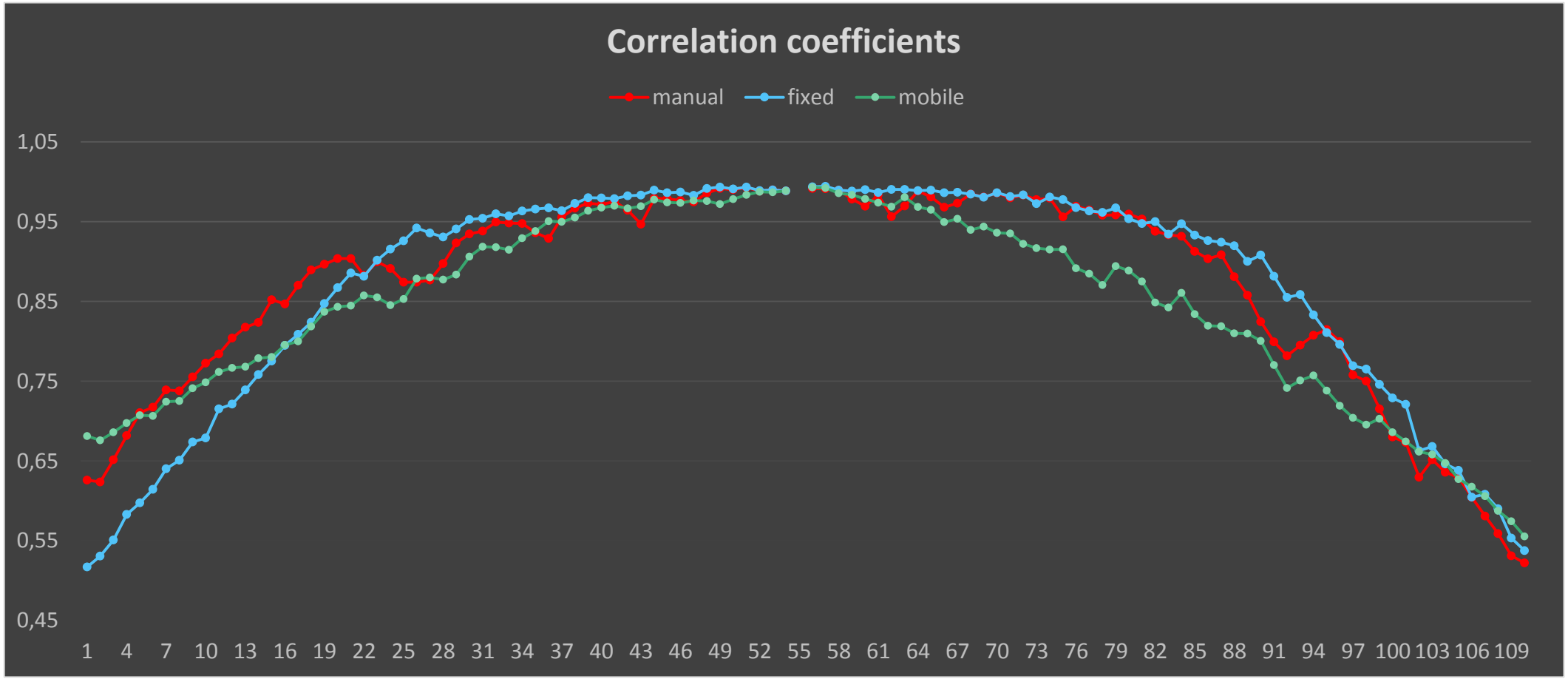
Automatic alignment with fixed reference
Global correlation coefficient: 0.9878

Automatic alignment with mobile reference
Global correlation coefficient: 0.9896

# Correlation coefficients – Consecutive images

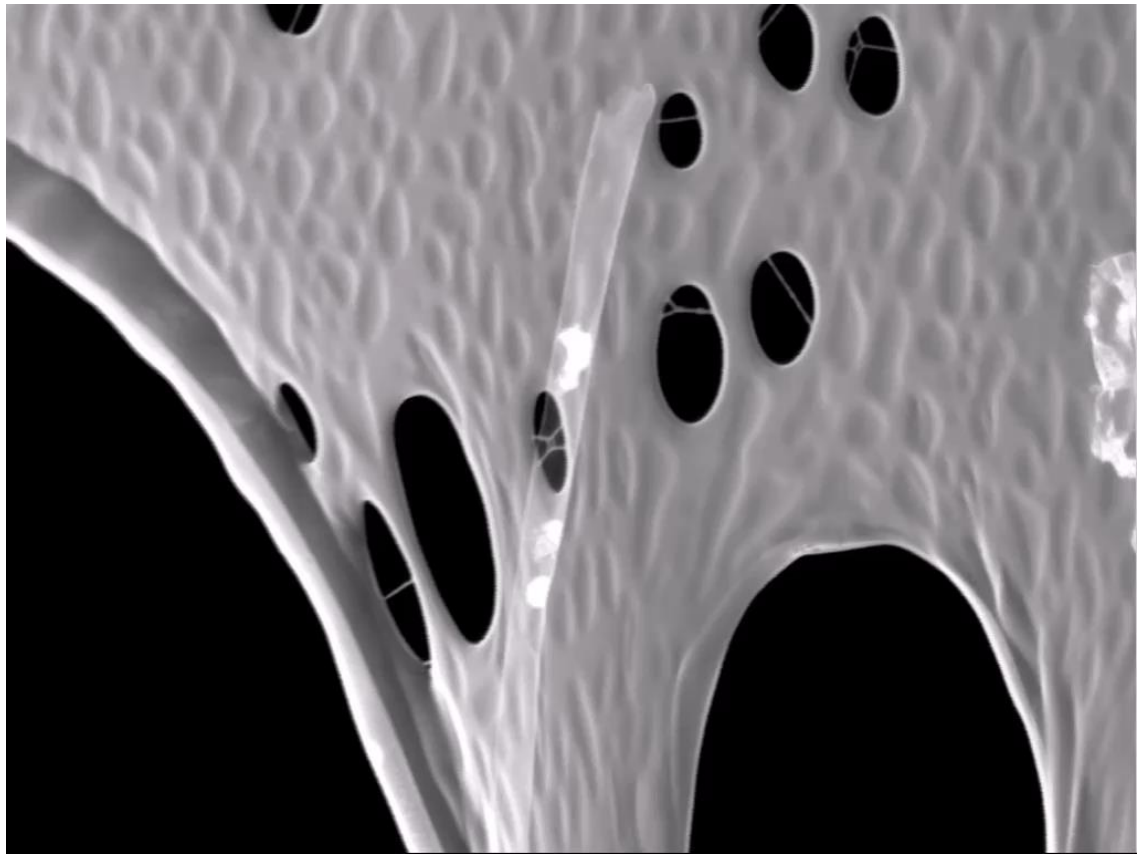


# Correlation coefficients – fixed reference

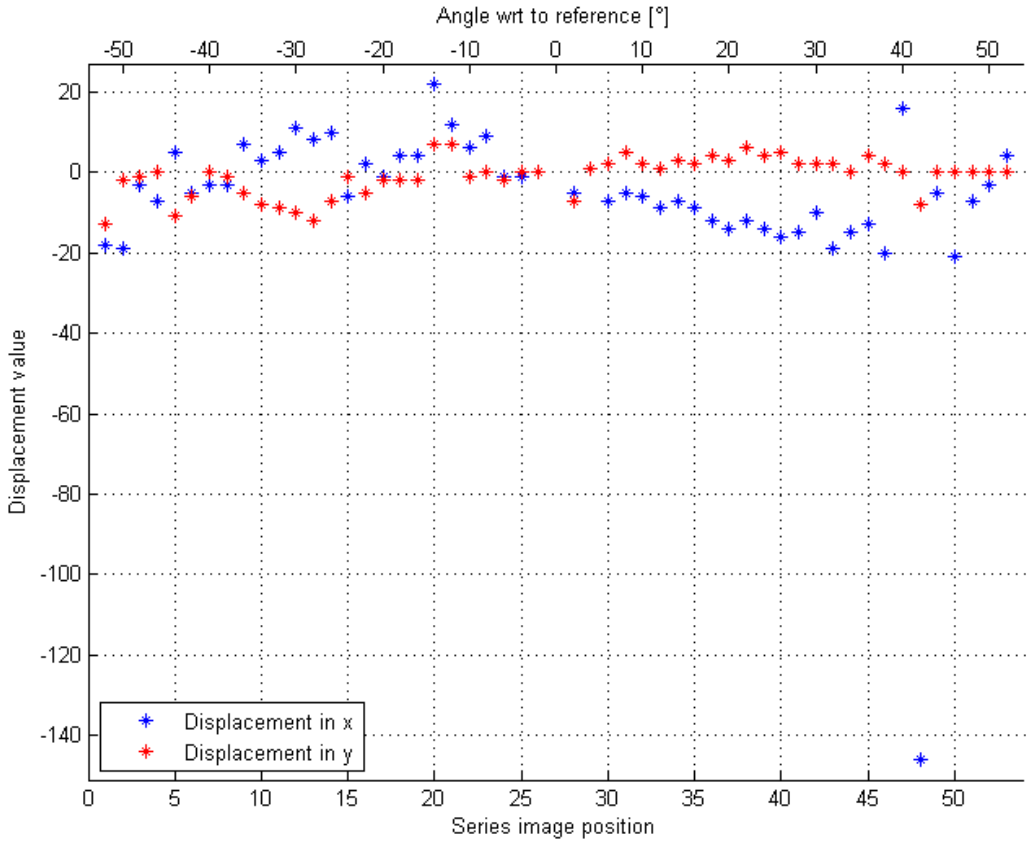


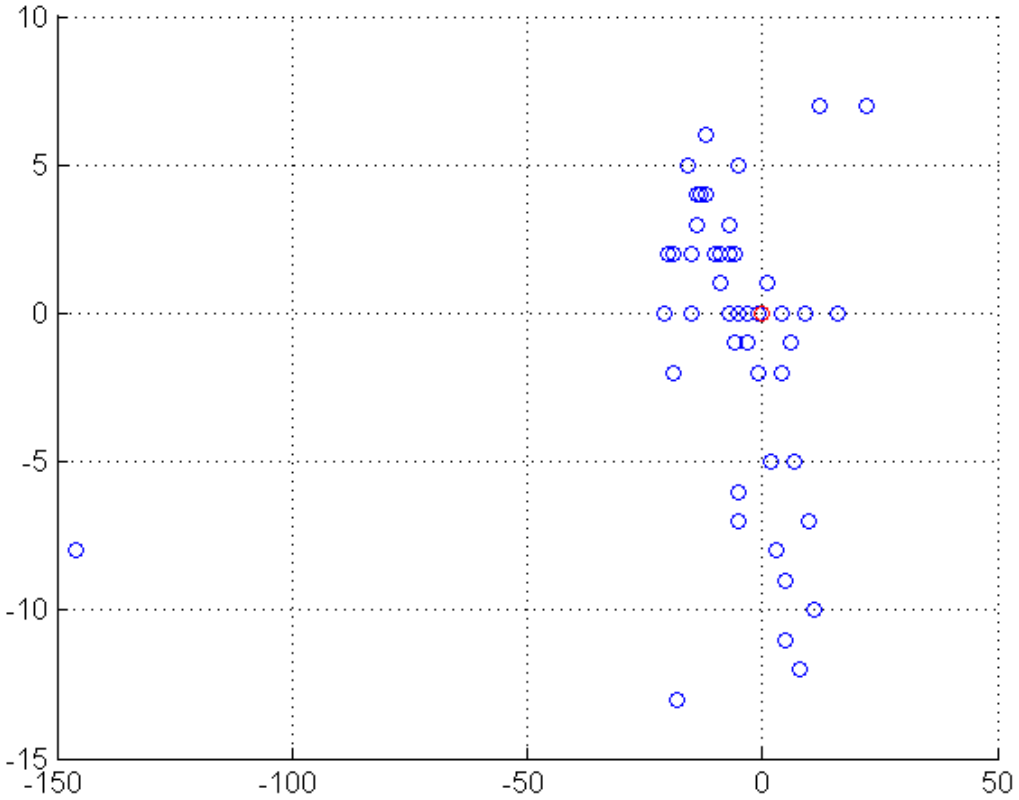
# Cobalt particles in carbon tube

- 53 images
- Global correlation coefficient: 0.876694



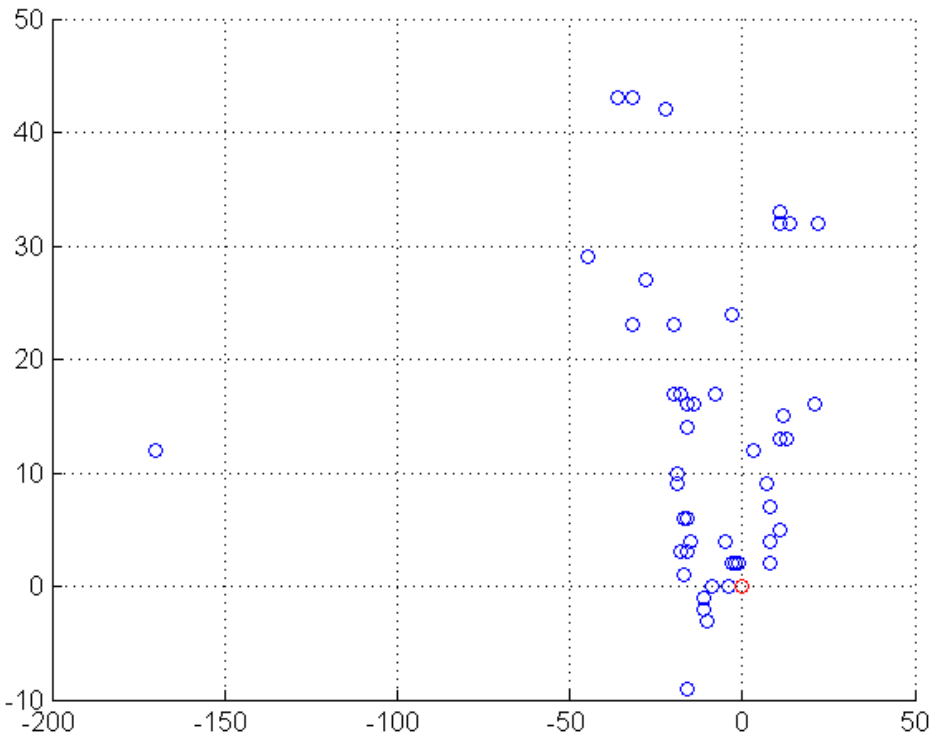
# Misalignment distribution – Fixed reference



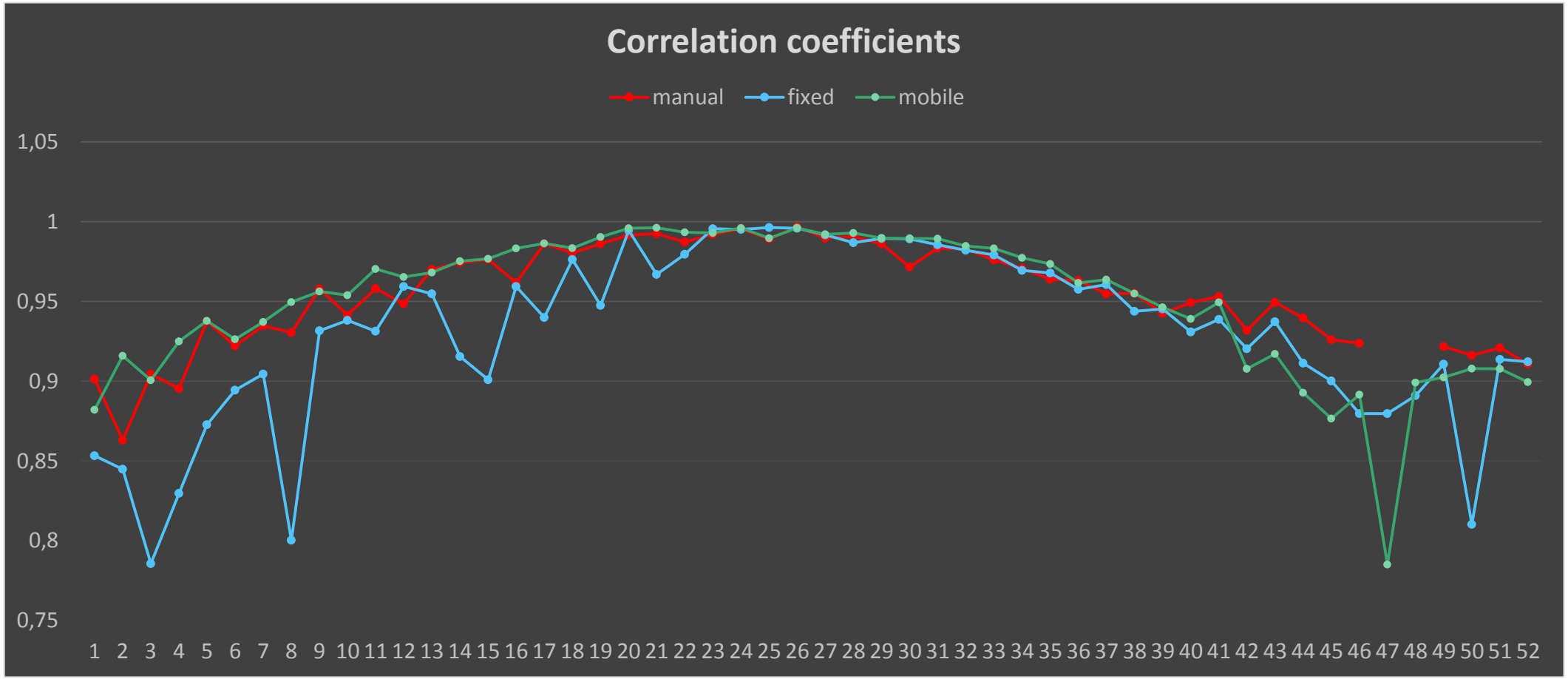



# Misalignment distribution – Mobile reference

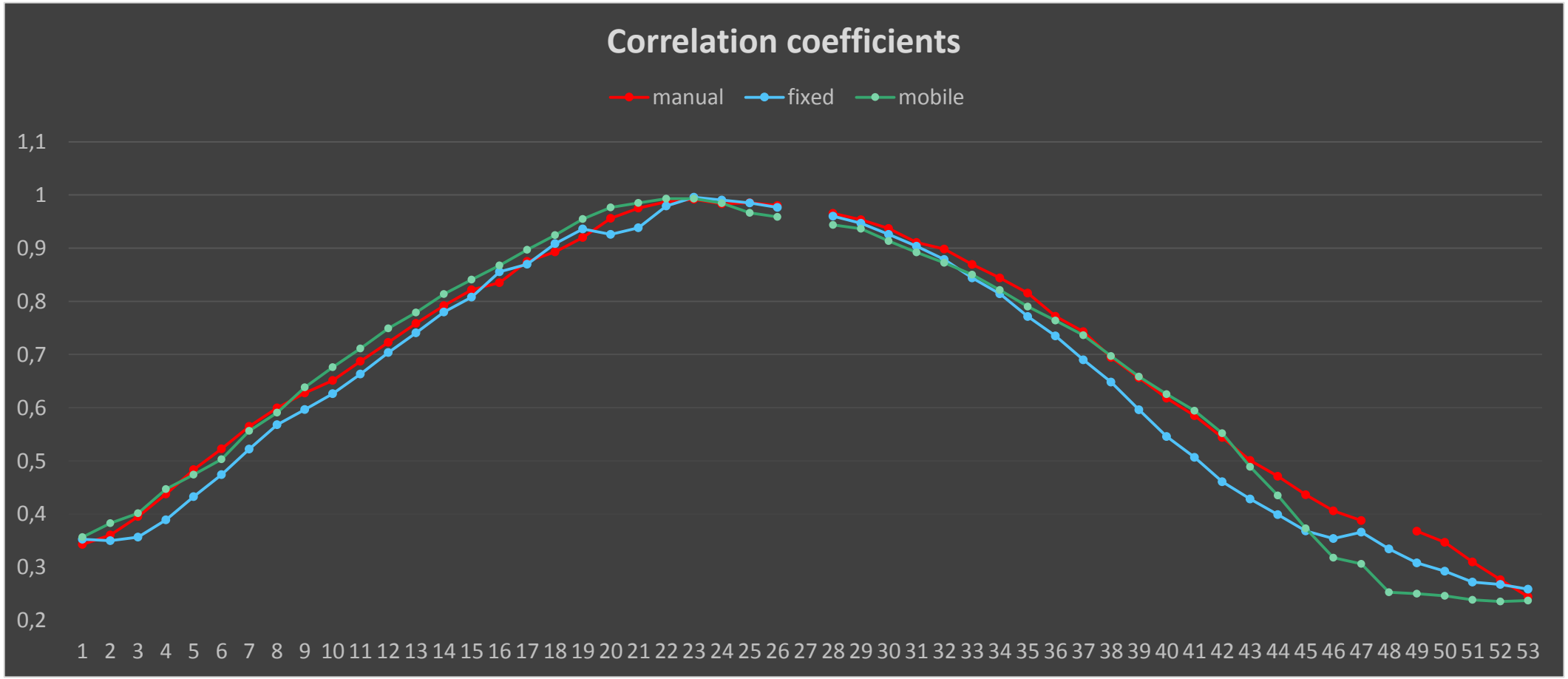
- The image shows the distribution of the displacements of images wrt reference image



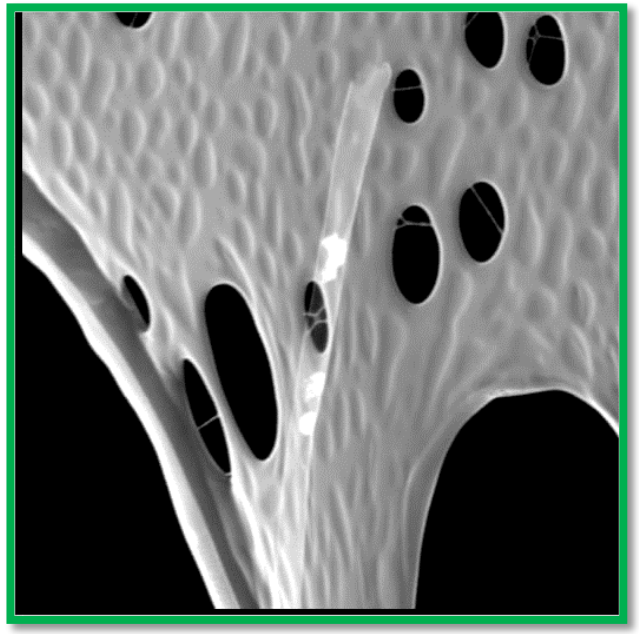
# Correlation coefficients – Consecutive images



# Correlation coefficients – reference image

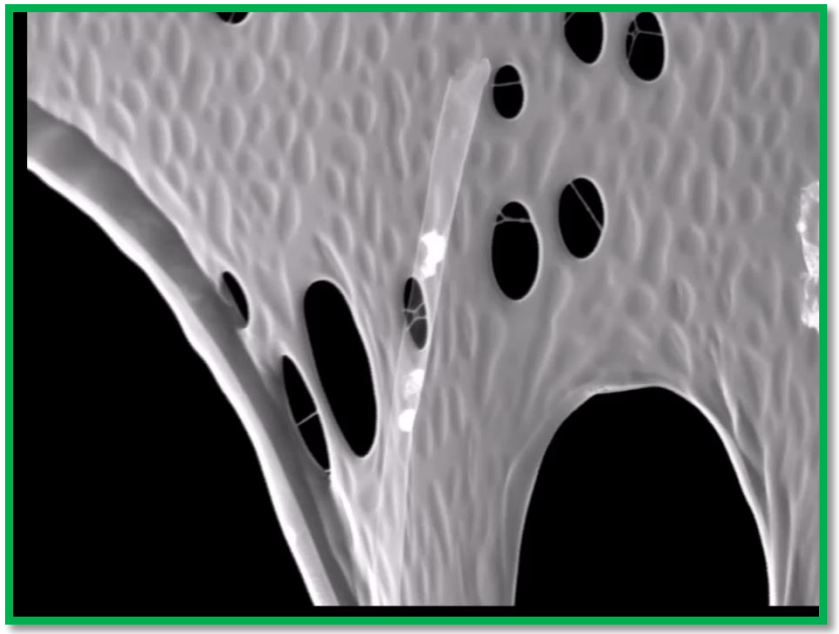


# Results comparison

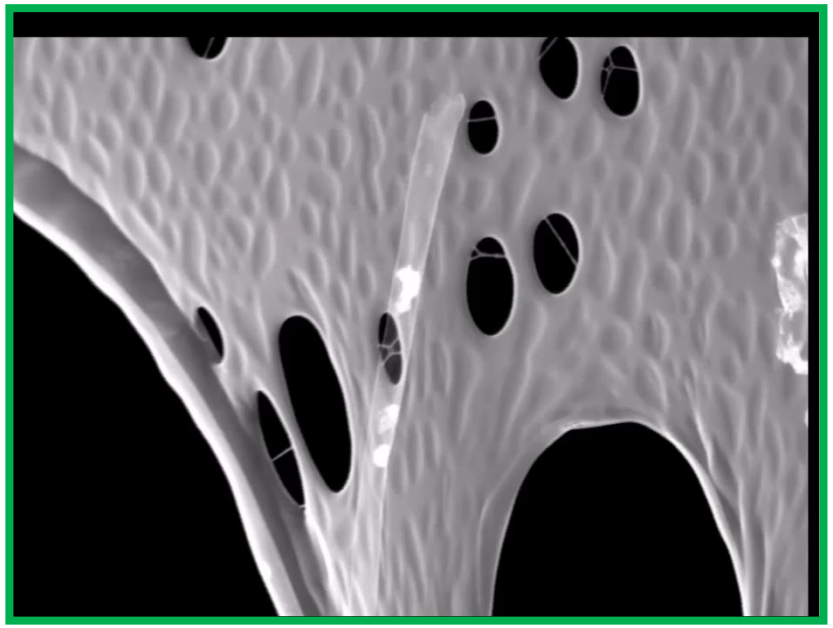


Manual alignment

Global correlation coefficient: 0.9462

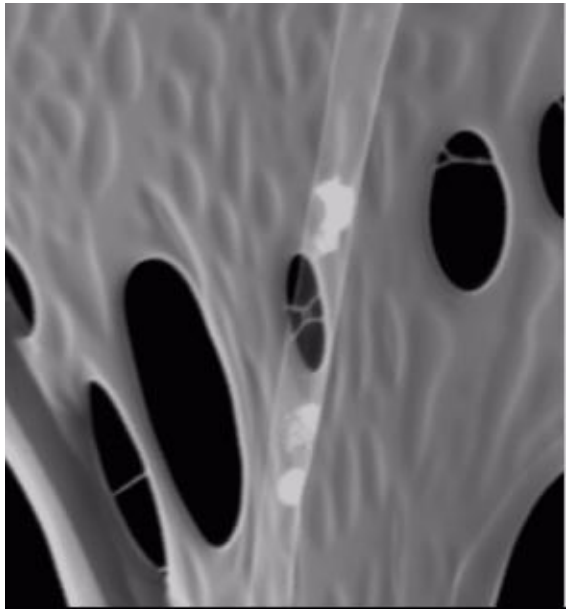


Automatic alignment with fixed reference
Global correlation coefficient: 0.9280



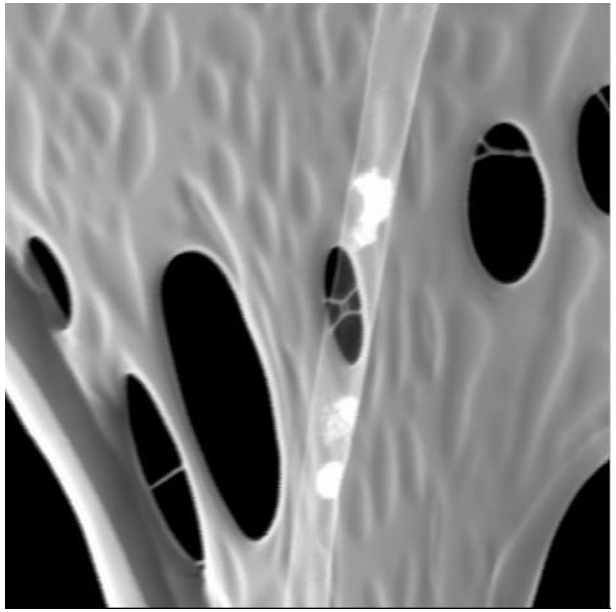
Automatic alignment with mobile reference
Global correlation coefficient: 0.9398

# Results comparison: cropped images



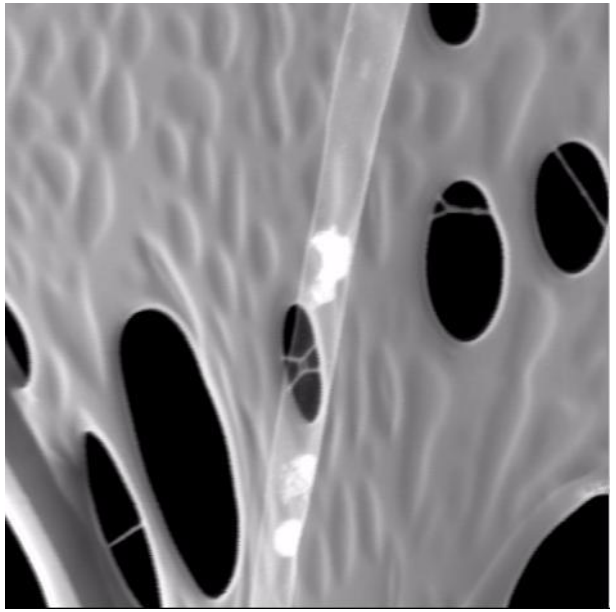
Manual alignment

Global correlation coefficient: 0.9549



Automatic alignment with fixed reference

Global correlation coefficient: 0.9316

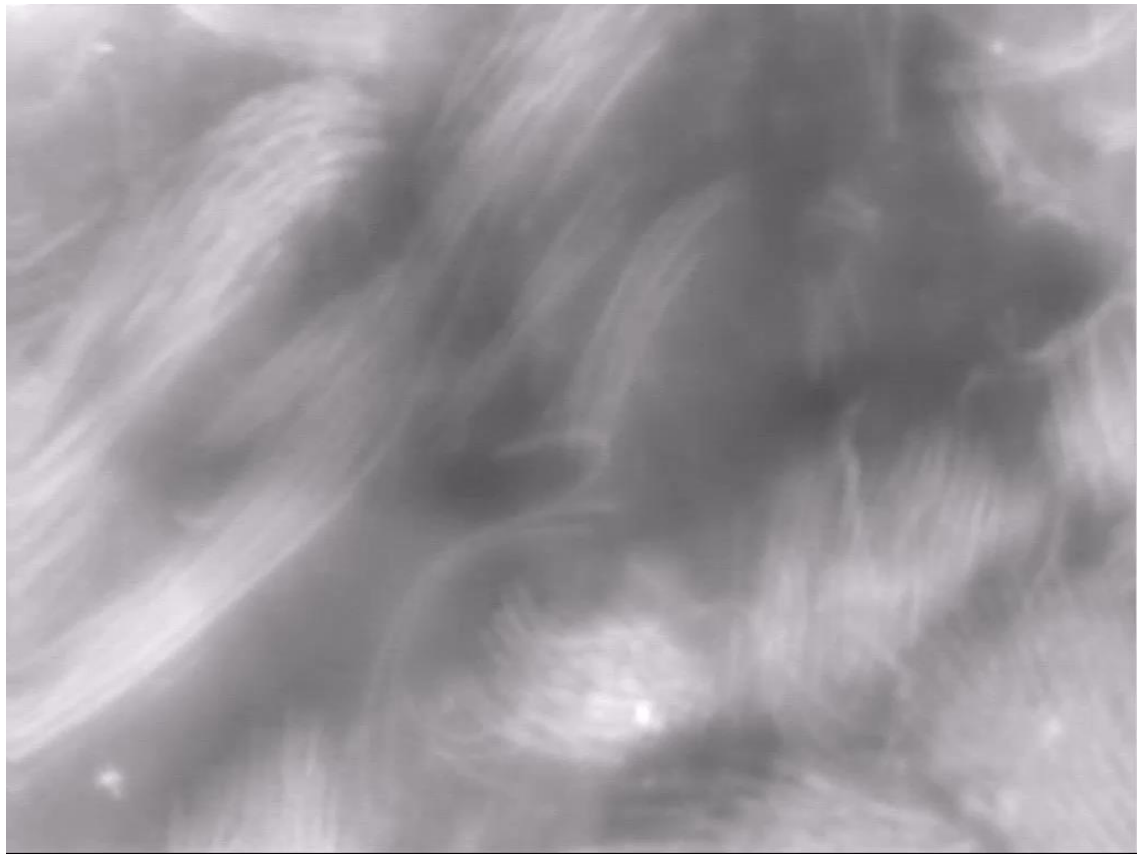


Automatic alignment with mobile reference

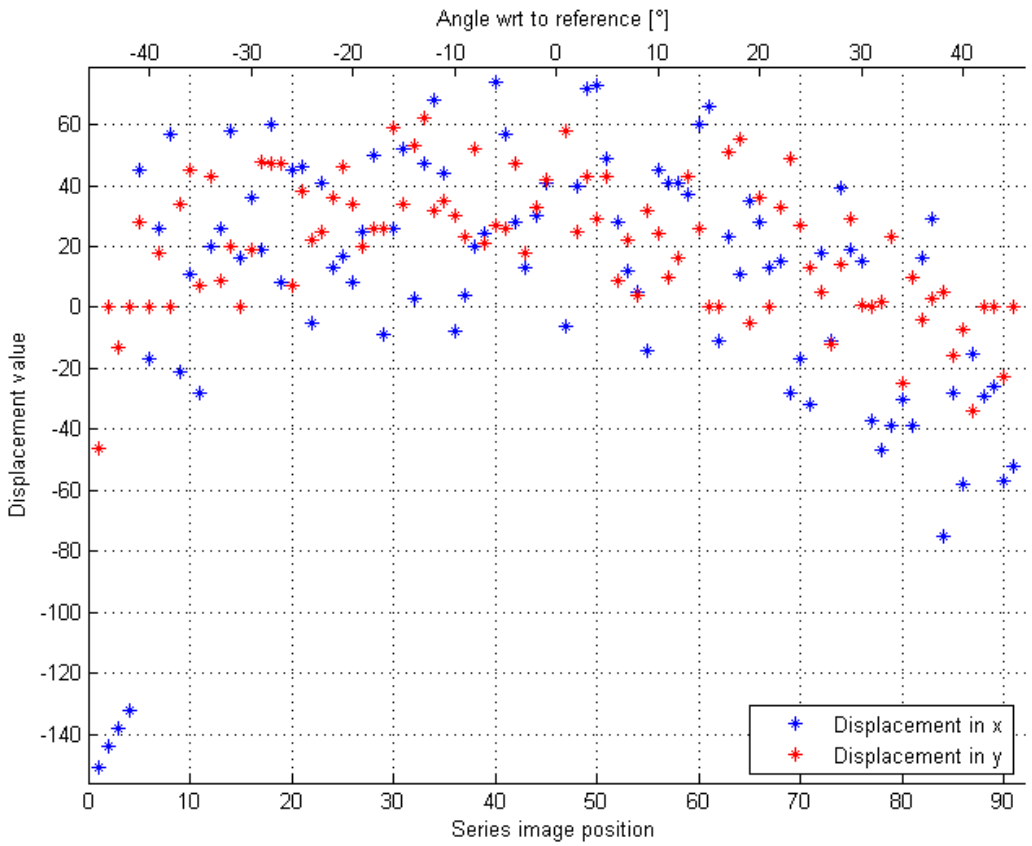
Global correlation coefficient: 0.9502

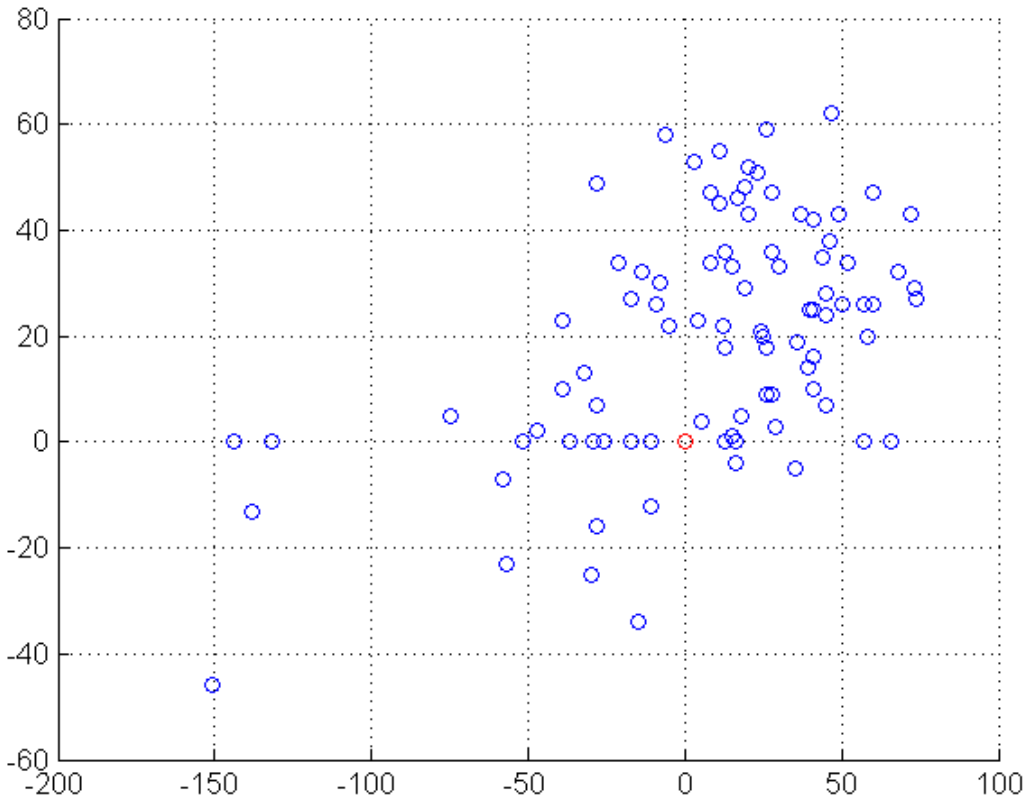
# Collagen Fibers

- 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



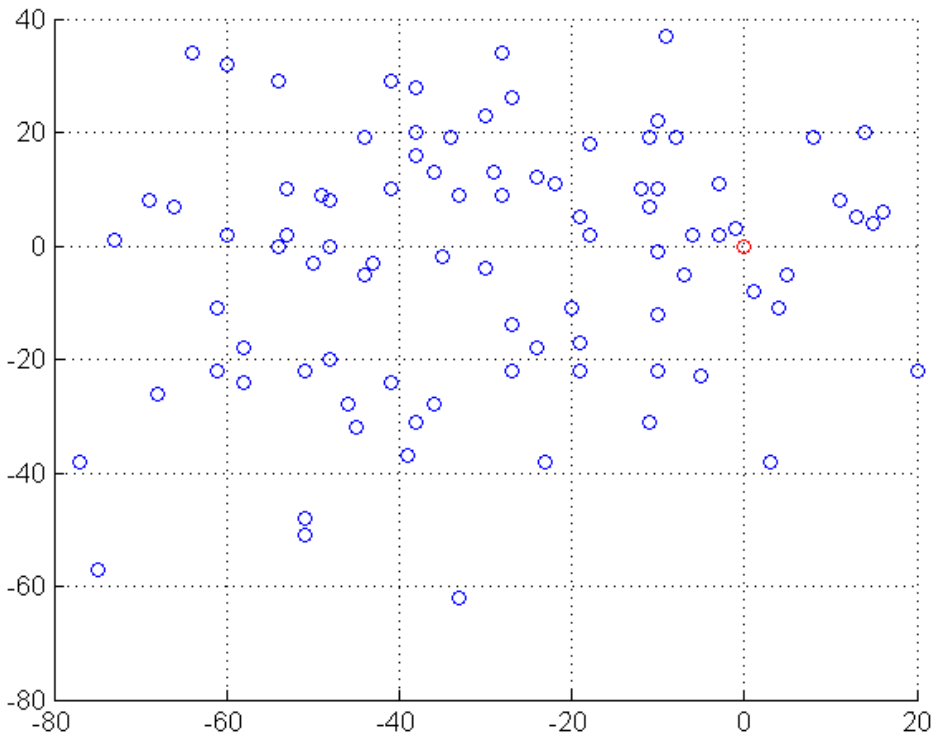
# Misalignment distribution – Fixed reference



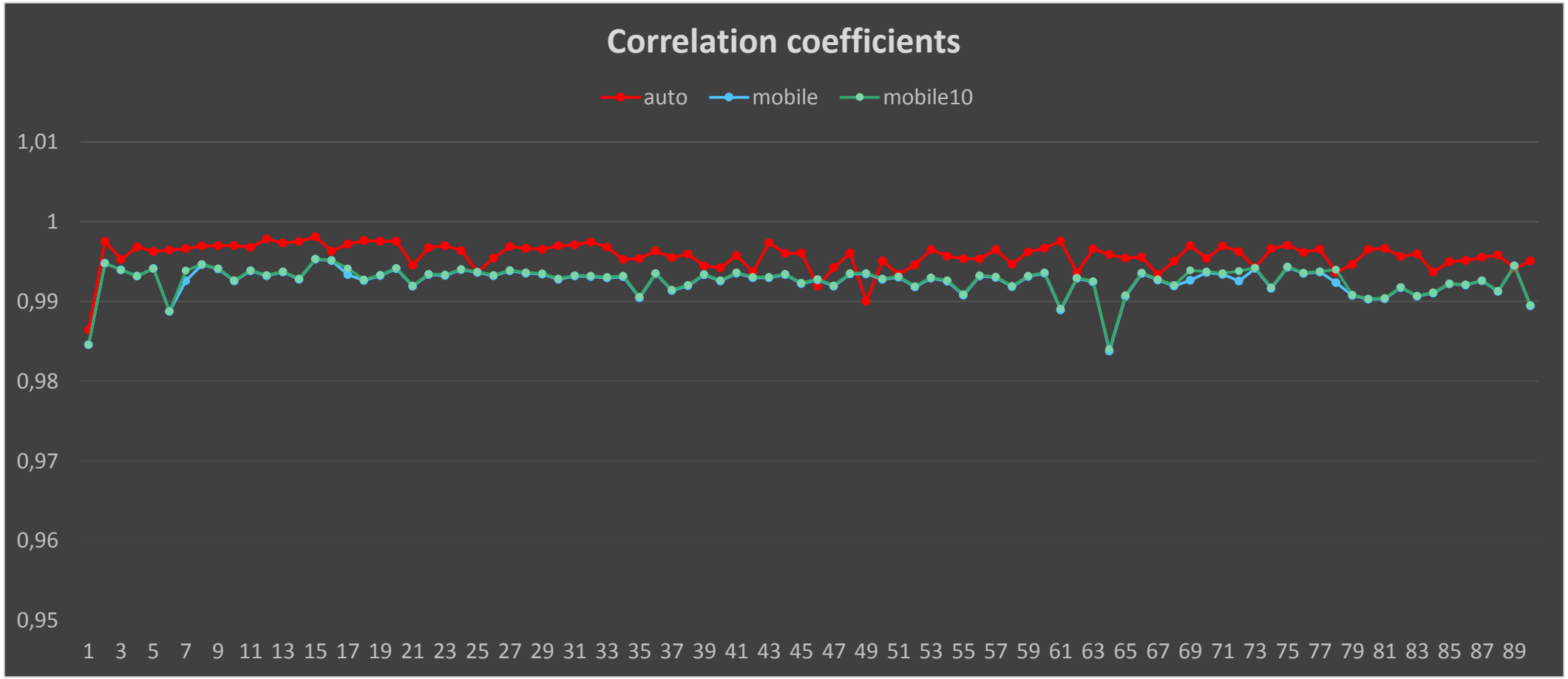



# Misalignment distribution – Mobile reference

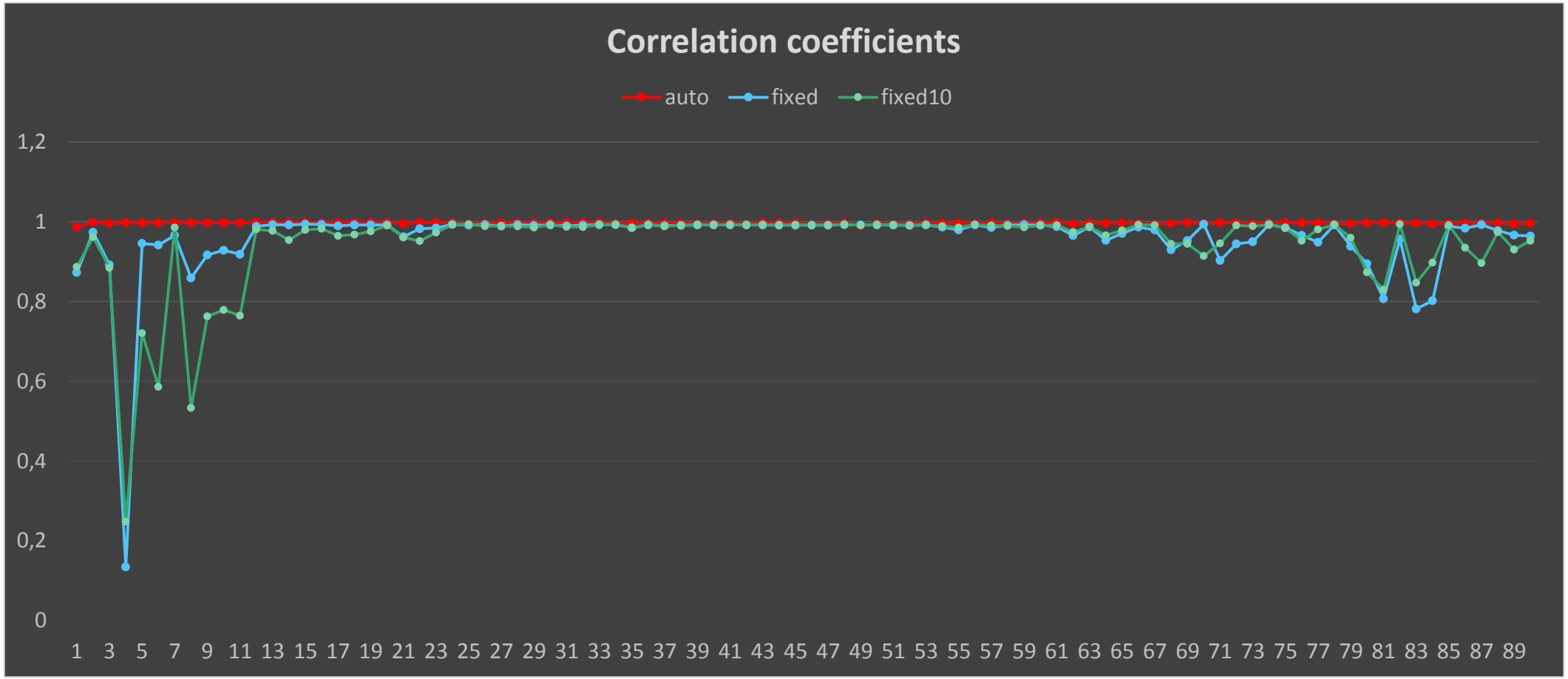
- The image shows the distribution of the displacements of images wrt reference image



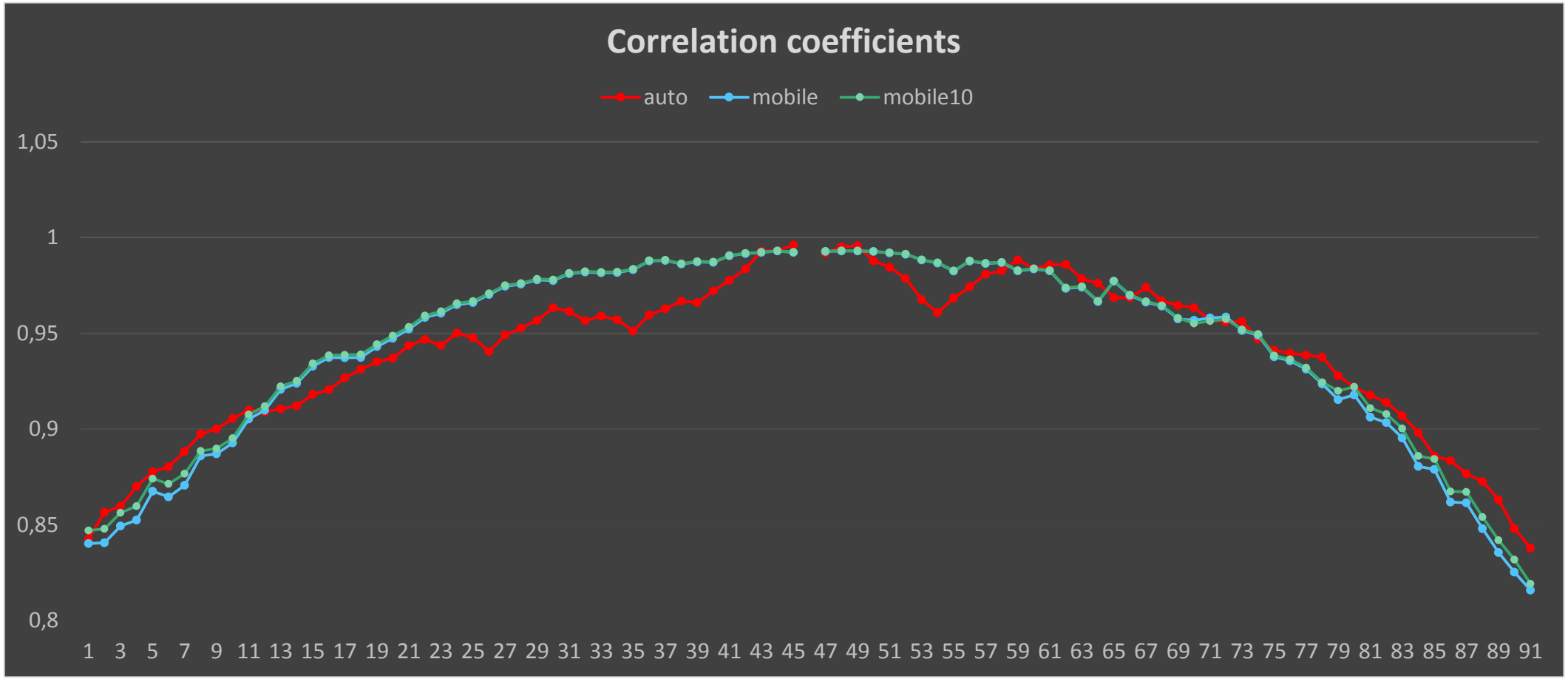
# Correlation coefficients – Consecutive images



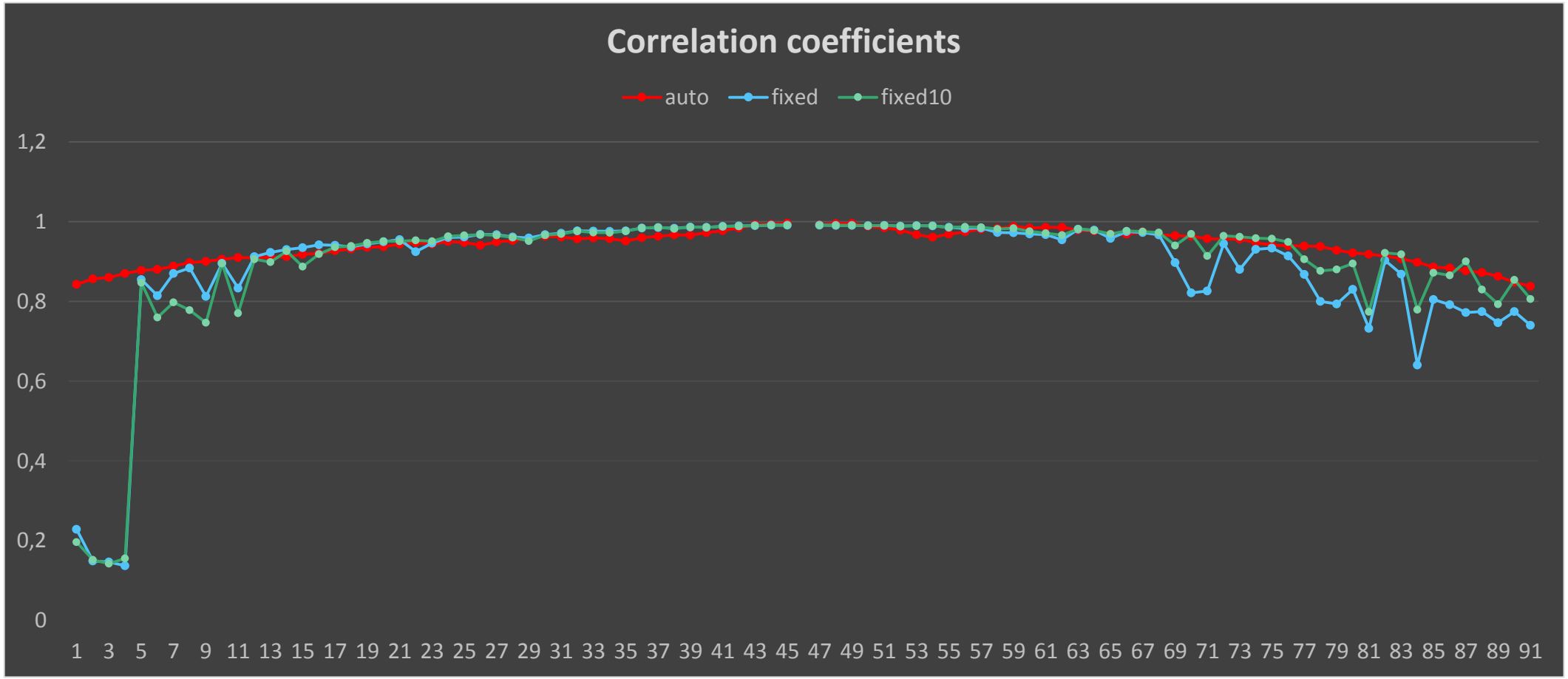
# Correlation coefficients – Consecutive images



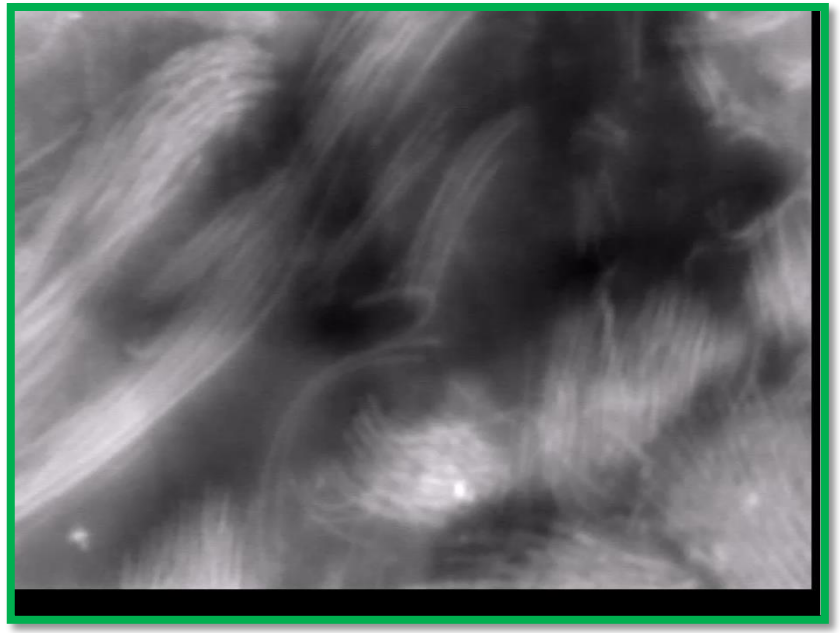
# Correlation coefficients – reference image



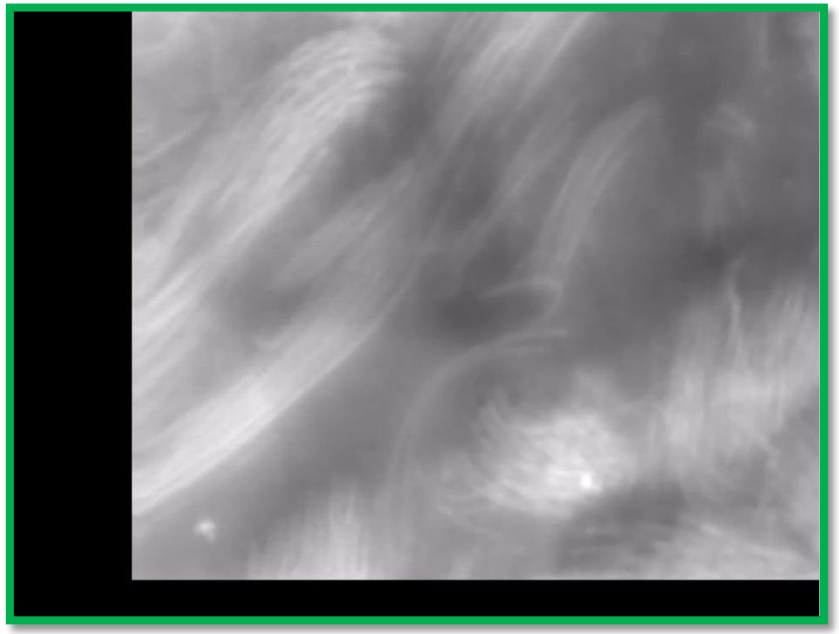
# Correlation coefficients – reference image



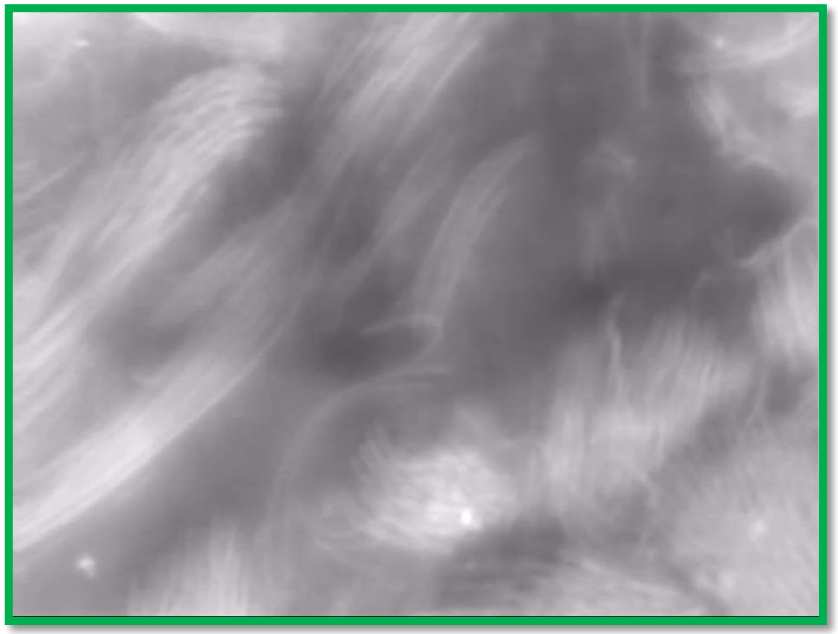
# Results comparison



Automatic alignment using dedicated software
Global correlation coefficient: 0.8747

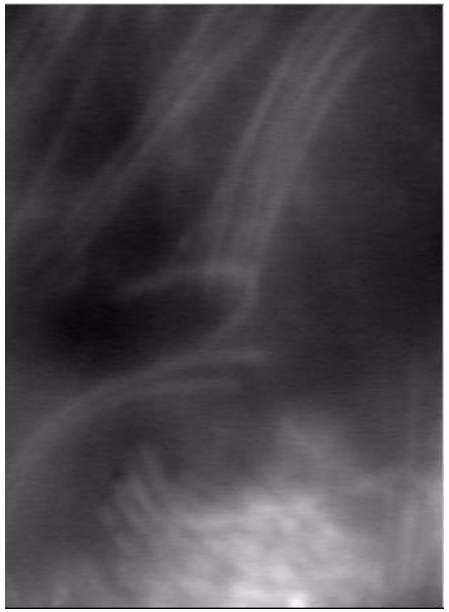


Automatic alignment with fixed reference
Global correlation coefficient: 0.8471

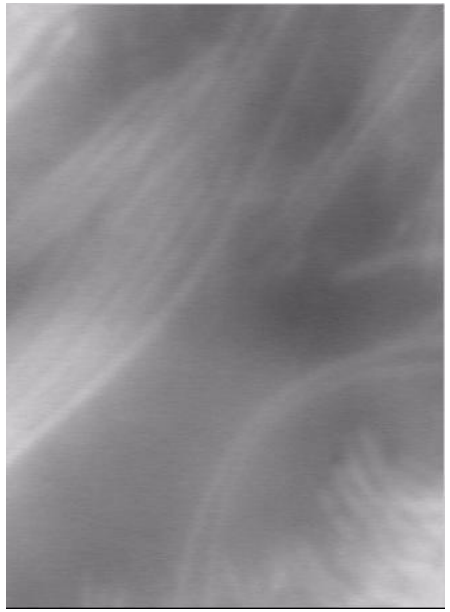


Automatic alignment with mobile reference
Global correlation coefficient:0.8751

# Results comparison (central axis): cropped images



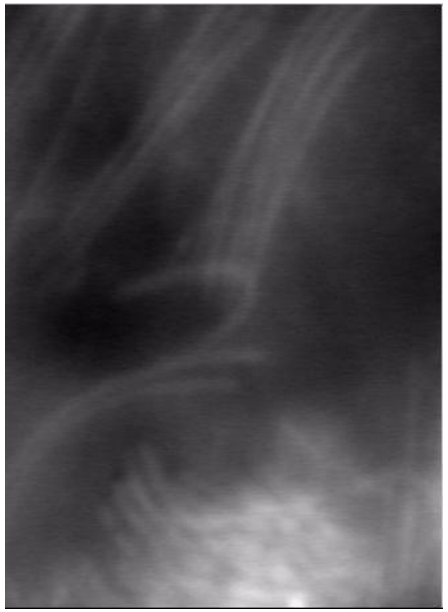
Automatic alignment using dedicated software
Global correlation coefficient: 0.9957



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