

**Supplementary Material:**

Automated correlation of single particle tilt pairs for random conical tilt and orthogonal tilt reconstructions

Florian Hauer<sup>1</sup>, Christoph Gerle<sup>2</sup>, Jan-Martin Kirves<sup>1</sup>, Holger Stark<sup>1†</sup>.

<sup>1</sup>Max-Planck Institute for Biophysical Chemistry, Am Fassberg 11, 37077 Göttingen, Germany

<sup>2</sup>Career Path Promotion Unit for Young Life Scientists, Kyoto University, Bldg. E, Yoshida Konoe Cho, Sakkyo-Ku, 606-8501 Kyoto, Japan

<sup>†</sup>Corresponding author, email: [hstark1@gwdg.de](mailto:hstark1@gwdg.de), Phone: +49-551-201-1302, Fax: +49-551-201-1197

**Supplementary Information:**

Supplementary Figure 1

Supplementary Table 1

Represent each coordinate  $P$  in tilted and untilted micrograph by affine invariant homogeneous barycentric coordinates (HBCs) calculated using surrounding coordinates  $P_1, P_2, P_3$  where

$$P = \alpha P_1 + \beta P_2 + \gamma P_3$$

and

$$\alpha + \beta + \gamma = 1$$

Sort  $P_1, P_2, P_3$  according to numerical value of  $\alpha, \beta, \gamma$  and calculate barycentric error  $error_{bary}$  for each combination of untilted coordinate  $P_{ut}$  and tilted coordinate  $P_t$ :

$$error_{bary} = (\alpha_{ut} - \alpha_t)^2 + (\beta_{ut} - \beta_t)^2 + (\gamma_{ut} - \gamma_t)^2$$

For combinations of every untilted coordinate  $P_{ut}$  with three surrounding coordinates and up to 20 tilted coordinates  $P_t$  with surrounding coordinates for the lowest  $error_{bary}$  is calculated, the underlying affine transformation is estimated as

$$P_t = AP_{ut} + t$$

Subsequently,  $A$  and  $t$  are used to predict a coordinate set in the tilted micrograph  $V$  from  $P_{ut}$  and up to 70 surrounding points. From this coordinate set predicted from  $U$  and all coordinates in the tilted micrograph  $V$ , the enhanced Hausdorff distance (EHD) (Gope and Kehtarnavaz, 2007) is calculated:

$$H_{EH}(U, V) = \max(h_{EH}(U, V), h_{EH}(V, U))$$

where

$$h_{EH}(U, V) = \frac{1}{m - \psi} \sum_{u \in U} d(u, V)$$

and

$$d(u, V) = \min_{v \in V} \|u - v\|$$

For the set of up to 40 coordinate pairs  $P_{ut}, P_t$  and surrounding coordinates which were used to calculate the lowest EHDs, estimated transformation parameters  $A$  and  $t$  are used to predict coordinates in the tilted micrograph from coordinates in the untilted micrograph. The error of prediction is calculated as the sum of all EHDs from the predicted coordinate sets and all coordinates in the tilted micrograph  $V$ . The combination of pairs  $P_{ut}, P_t$  with the lowest summed EHD is selected as initial correlating point set.

For each coordinate in the untilted micrograph for which the tilt mate is not known:

- select three closest coordinates to which tilt mates are known
- calculate HBCs using these coordinates
- estimate  $A$  and  $t$  using these coordinates
- predict tilt mates using both HBCs and  $A$  and  $t$

If the coordinates in the tilted micrograph closest to the predicted coordinates are the same from both predictions and the distance to the second best prediction is two times larger than then the distance to the best prediction: accept new tilt mates

Yes

New tilt mates identified?

No

End program

**Supplementary Figure 1** Flow Chart of the *MaverickTilt* Software.

