Supplementary Material for
A fast fiducial marker tracking model for fully automatic alignment in electron tomography

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S1 A general proof for affine transformation relationship between two micrographs

Part 1:

Typically, the projection is modeled as an affine or orthogonal projection. A classic orthogonal model is described as follows:

$$\begin{pmatrix} u \\ v \end{pmatrix} = s\mathbf{R}_\gamma \mathbf{P}\mathbf{R}_\beta \mathbf{R}_\alpha \begin{pmatrix} X \\ Y \\ Z \end{pmatrix} + \mathbf{t}, \tag{1}$$

where $(X, Y, Z)^T$ is the spatial location of the ultrastructure or fiducial markers; s is the image scale change, γ is the inplane rotation angle; α is the pitch angle of the tilt axis of the projection; β is the tilt angle of the sample; $\mathbf{t} = (t_0, t_1)^T$ is the translation of the view; $(u, v)^T$ is the measured projection point; and $\mathbf{P}$ denotes the orthogonal projection matrix. The details of $\mathbf{R}_\alpha$,$\mathbf{R}_\beta$,$\mathbf{P}$ and $\mathbf{R}_\gamma$ are defined as follows:

$$\begin{aligned}
\mathbf{R}_\alpha &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & \cos\alpha & \sin\alpha \\ 0 & -\sin\alpha & \cos\alpha \end{pmatrix}, \\
\mathbf{R}_\beta &= \begin{pmatrix} \cos\beta & 0 & -\sin\beta \\ 0 & 1 & 0 \\ \sin\beta & 0 & \cos\beta \end{pmatrix}, \\
\mathbf{P} &= \begin{pmatrix} 1 & 0 & 0 \\ 0 & 1 & 0 \end{pmatrix}, \\
\mathbf{R}_\gamma &= \begin{pmatrix} \cos\gamma & \sin\gamma \\ -\sin\gamma & \cos\gamma \end{pmatrix}.
\end{aligned}$$

For two arbitrary views (micrographs), we can always construct a single transformation that serves for all fiducial markers to align the corresponding fiducial marker projections within a limited deviation. We will first prove a Lemma that theoretically guarantees the upper bound of deviation on any arbitrary fiducial marker, and then apply this Lemma to prove a Theorem (in the main text) which guarantees the upper bound over all the fiducial markers.

Lemma: Suppose the pitch angle is fixed during tilt, for any arbitrary fiducial marker $(X_j, Y_j, Z_j)^T$ and its arbitrary two projections (denoted as $\mathbf{p}_{ij}$ and $\mathbf{p}_{i'j}$), there is always a transformation $\mathbf{A}$ and $\mathbf{t}$ (Eq.(11) and Eq.(12)) that can be applied to this fiducial marker ($\mathbf{p}'_{ij} = \mathbf{A}\mathbf{p}_{ij} + \mathbf{t}$) to make the deviation $\|\Delta_j\| = \|\mathbf{p}'_{ij} - \mathbf{p}_{i'j}\| \leqslant s_{i'}|\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i}(Z_j - Z_{\boldsymbol{\mu}})|$, where α is the fixed pitch angle; $s_{i'}$ is the scale change of the i'th micrograph; β_i and $\beta_{i'}$ are the tilt angles of the corresponding projections; $\Delta\beta = \beta_{i'} - \beta_i$; and $Z_{\boldsymbol{\mu}} = \frac{1}{N}\sum_{j=1}^{N} Z_j$.

Proof The main idea is to construct such a transformation and prove that the deviation of this transformation is exactly $s_{i'}|\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i}(Z_j - Z_{\boldsymbol{\mu}})|$ as given in the Lemma. Therefore, the optimal transformation will always be upper bounded by this value.

Firstly, by substituting $\mathbf{P}$,$\mathbf{R}_\beta$,and $\mathbf{R}_\alpha$ into Eq.(1), the orthogonal projection can be rewritten as:

$$\begin{pmatrix} u \\ v \end{pmatrix} = s\mathbf{R}_\gamma \begin{pmatrix} \cos\beta & \sin\alpha\sin\beta \\ 0 & \cos\alpha \end{pmatrix} \begin{pmatrix} X \\ Y \end{pmatrix} + s\mathbf{R}_\gamma \begin{pmatrix} -\sin\beta\cos\alpha \\ \sin\alpha \end{pmatrix} Z + \begin{pmatrix} t_0 \\ t_1 \end{pmatrix}, \tag{2}$$

Now we let $\{(X,Y,Z)^T\}$ be the fiducial markers embedded in the specimen. Considering the jth fiducial marker $(X_j,Y_j,Z_j)^T$ $(j=1,2,...,N)$, its projections in the ith and i'th views ($\mathbf{p}_{ij}=(u_{ij},v_{ij})^T$ and ($\mathbf{p}_{i'j}=u_{i'j},v_{i'j})^T$) can be written as:

$$\begin{aligned}
\begin{pmatrix} u_{ij} \\ v_{ij} \end{pmatrix} &= s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} \cos\beta_i & \sin\alpha_i\sin\beta_i \\ 0 & \cos\alpha_i \end{pmatrix}\begin{pmatrix} X_j \\ Y_j \end{pmatrix} + s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} -\sin\beta_i\cos\alpha_i \\ \sin\alpha_i \end{pmatrix} Z_j + \mathbf{t}_i, \\
\begin{pmatrix} u_{i'j} \\ v_{i'j} \end{pmatrix} &= s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'} \\ 0 & \cos\alpha_{i'} \end{pmatrix}\begin{pmatrix} X_j \\ Y_j \end{pmatrix} + s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} -\sin\beta_{i'}\cos\alpha_{i'} \\ \sin\alpha_{i'} \end{pmatrix} Z_j + \mathbf{t}_{i'}.
\end{aligned} \tag{3}$$

Considering a transformation $\mathcal{T}(\cdot;\mathbf{A}_{ii'j},\mathbf{t}_{ii'j})$ that makes $\mathbf{p}'_{ij}=\mathbf{A}_{ii'j}\mathbf{p}_{ij}+\mathbf{t}_{ii'j}$, the deviation between $\mathbf{p}'_{ij}$ and $\mathbf{p}_{i'j}$ can be derived as following:

$$\begin{aligned}
\Delta_j &= \mathbf{p}'_{ij}-\mathbf{p}_{i'j} \\
&= \left[\mathbf{A}_{ii'j}\left[s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} \cos\beta_i & \sin\alpha_i\sin\beta_i \\ 0 & \cos\alpha_i \end{pmatrix}\begin{pmatrix} X_j \\ Y_j \end{pmatrix} + s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} -\sin\beta_i\cos\alpha_i \\ \sin\alpha_i \end{pmatrix} Z_j + \mathbf{t}_i\right] + \mathbf{t}_{ii'j}\right] \\
&- \left[s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'} \\ 0 & \cos\alpha_{i'} \end{pmatrix}\begin{pmatrix} X_j \\ Y_j \end{pmatrix} + s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} -\sin\beta_{i'}\cos\alpha_{i'} \\ \sin\alpha_{i'} \end{pmatrix} Z_j + \mathbf{t}_{i'}\right] \\
&= \left[\mathbf{A}_{ii'j}s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} \cos\beta_i & \sin\alpha_i\sin\beta_i \\ 0 & \cos\alpha_i \end{pmatrix} - s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'} \\ 0 & \cos\alpha_{i'} \end{pmatrix}\right]\begin{pmatrix} X_j \\ Y_j \end{pmatrix} \\
&+ \left[\mathbf{A}_{ii'j}s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} -\sin\beta_i\cos\alpha_i \\ \sin\alpha_i \end{pmatrix} - s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} -\sin\beta_{i'}\cos\alpha_{i'} \\ \sin\alpha_{i'} \end{pmatrix}\right] Z_j + \mathbf{A}_{ii'j}\mathbf{t}_i + \mathbf{t}_{ii'j} - \mathbf{t}_{i'}.
\end{aligned}$$

We should remember that our aim is to find a transformation to minimize the total deviation of the corresponding fiducial marker projections in the ith and i'th views, i.e., a single $\mathcal{T}(\cdot;\mathbf{A},\mathbf{t})$ that is applied to $\{\Delta_j|j=1,...N\}$ to minimize $\sum_{j=1}^{N}\|\Delta_j\|$.

In practice, the specimens always have a relatively small thickness z but large $x-y$ dimensions. For example, commonly seen values for x and y are 1024, 2048 and 4096, whereas that for z is often 50, 100 and 150. Therefore, we will construct a transformation $\mathbf{A}_{ii'j}$ to make Δ_j independent of X_j and Y_j:

$$\mathbf{A}_{ii'j}s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} \cos\beta_i & \sin\alpha_i\sin\beta_i \\ 0 & \cos\alpha_i \end{pmatrix} - s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'} \\ 0 & \cos\alpha_{i'} \end{pmatrix} = 0, \tag{4}$$

from which $\mathbf{A}_{ii'j}$ can be solved as

$$\mathbf{A}_{ii'j} = \frac{s_{i'}}{s_i}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'} \\ 0 & \cos\alpha_{i'} \end{pmatrix}\begin{pmatrix} \frac{1}{\cos\beta_i} & \frac{-\sin\alpha_i\sin\beta_i}{\cos\alpha_i\cos\beta_i} \\ 0 & \frac{1}{\cos\alpha_i} \end{pmatrix}\mathbf{R}_{-\gamma_i}. \tag{5}$$

Furthermore, if we represent $\mathbf{A}_{ii'j}\mathbf{t}_i+\mathbf{t}_{ii'j}-\mathbf{t}_{i'}$ as $\mathbf{T}_j$, i.e.

$$\mathbf{t}_{ii'j} = \mathbf{t}_{i'} - \mathbf{A}_{ii'j}\mathbf{t}_i + \mathbf{T}_j, \tag{6}$$

the expression of Δ_j can be reduced to

$$\begin{aligned}
\Delta_j &= \left[\mathbf{A}_{ii'j}s_i\mathbf{R}_{\gamma_i}\begin{pmatrix} -\sin\beta_i\cos\alpha_i \\ \sin\alpha_i \end{pmatrix} - s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} -\sin\beta_{i'}\cos\alpha_{i'} \\ \sin\alpha_{i'} \end{pmatrix}\right] Z_j + \mathbf{T}_j \\
&= s_{i'}\mathbf{R}_{\gamma_{i'}}\left[\begin{pmatrix} \cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'} \\ 0 & \cos\alpha_{i'} \end{pmatrix}\begin{pmatrix} \frac{1}{\cos\beta_i} & \frac{-\sin\alpha_i\sin\beta_i}{\cos\alpha_i\cos\beta_i} \\ 0 & \frac{1}{\cos\alpha_i} \end{pmatrix}\begin{pmatrix} -\sin\beta_i\cos\alpha_i \\ \sin\alpha_i \end{pmatrix} - \begin{pmatrix} -\sin\beta_{i'}\cos\alpha_{i'} \\ \sin\alpha_{i'} \end{pmatrix}\right] Z_j + \mathbf{T}_j \\
&= s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \Delta u \\ \Delta v \end{pmatrix} Z_j + \mathbf{T}_j,
\end{aligned} \tag{7}$$

in which

$$\begin{aligned}
\Delta u &= -\frac{\cos\beta_{i'}\sin\beta_i\cos\alpha_i}{\cos\beta_i} - \frac{(\sin\alpha_i)^2\sin\beta_i\cos\beta_{i'}}{\cos\alpha_i\cos\beta_i} + \frac{\sin\alpha_{i'}\sin\beta_{i'}\sin\alpha_i}{\cos\alpha_i} + \sin\beta_{i'}\cos\alpha_{i'}, \\
\Delta v &= \frac{\cos\alpha_{i'}\sin\alpha_i - \sin\alpha_{i'}\cos\alpha_i}{\cos\alpha_i} = \frac{\sin(\alpha_i-\alpha_{i'})}{\cos\alpha_i}.
\end{aligned} \tag{8}$$

Note that the construction of $\mathbf{A}_{ii'j}$ and $\mathbf{t}_{ii'j}$ should all be independent of j. Therefore, for all the fiducial markers, their $\mathbf{A}_{ii'j}$ and $\mathbf{t}_{ii'j}$ are identical. By further denoting $\mathbf{A}_{ii'j}$ as $\mathbf{A}$ and denoting $\mathbf{t}_{ii'j}$ as $\mathbf{t}$ (assuming the freedom parameter $\mathbf{T}_j$ are identical for all the j and the value is $\mathbf{T}$), we would come back to our original problem and our aim becomes to find such $\mathbf{T}$ to make the transformation $\mathcal{T}(\cdot;\mathbf{A},\mathbf{t})$ minimize $\sum_{j=1}^{N}\|\Delta_j\|$ for $\{(X,Y,Z)^T\}$:

$$COST \quad = \quad \sum_{j=1}^{N}\|\Delta_j\| = \sum_{j=1}^{N}\|s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix}\Delta u\\ \Delta v\end{pmatrix}Z_j+\mathbf{T}\|. \tag{9}$$

Let $\mathbf{w}=s_{i'}\mathbf{R}_{\gamma_{i'}}(\Delta u,\Delta v)^T$, and we can find that all the Z_j have the coefficient $\mathbf{w}$. By writing $\mathbf{T}$ as $\mathbf{T}=\mathbf{w}l$, the cost function is derived as:

$$COST \quad = \quad \|\mathbf{w}\|\sum_{j=1}^{N}\|Z_j+l\| \tag{10}$$

It becomes a 1-dimensional cluster problem and the optimal solution is $l=-Z_{\boldsymbol{\mu}}=-\frac{1}{N}\sum_{j=1}^{N}Z_j$. Therefore, the optimal solution for $\mathcal{T}(\cdot;\mathbf{A},\mathbf{t})$ becomes

$$\mathbf{A}=\frac{s_{i'}}{s_i}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix}\cos\beta_{i'} & \sin\alpha_{i'}\sin\beta_{i'}\\ 0 & \cos\alpha_{i'}\end{pmatrix}\begin{pmatrix}\frac{1}{\cos\beta_i} & \frac{-\sin\alpha_i\sin\beta_i}{\cos\alpha_i\cos\beta_i}\\ 0 & \frac{1}{\cos\alpha_i}\end{pmatrix}\mathbf{R}_{-\gamma_i} \tag{11}$$

and

$$\mathbf{t}=\mathbf{t}_{i'}-\mathbf{A}\mathbf{t}_i-\mathbf{w}Z_{\boldsymbol{\mu}}, \tag{12}$$

where $\mathbf{w}=s_{i'}\mathbf{R}_{\gamma_{i'}}(\Delta u,\Delta v)^T$, $Z_{\boldsymbol{\mu}}=\frac{1}{N}\sum_{j=1}^{N}Z_j$. In most electron tomography systems, the micrographs are taken with the pitch angle fixed, which means that $\alpha_i\approx\alpha_{i'}$. Suppose $\alpha_i=\alpha_{i'}=\alpha$, and let $\beta_{i'}-\beta_i=\Delta\beta$, Δu and Δv can be rewritten as:

$$\begin{aligned}\Delta u \quad &= \quad \frac{\sin\alpha^2}{\cos\alpha}\cdot\left(\frac{\sin\beta_{i'}\cdot\cos\beta_i-\cos\beta_{i'}\cdot\sin\beta_i}{\cos\beta_i}\right)\\ &+ \quad \frac{\cos\alpha^2}{\cos\alpha}\cdot\left(\frac{\sin\beta_{i'}\cdot\cos\beta_i-\cos\beta_{i'}\cdot\sin\beta_i}{\cos\beta_i}\right)\\ &= \quad \frac{\sin\alpha^2+\cos\alpha^2}{\cos\alpha}\cdot\left(\frac{\sin(\beta_{i'}-\beta_i)}{\cos\beta_i}\right)=\frac{\sin\Delta\beta}{\cos\alpha\cdot\cos\beta_i},\\ \Delta v \quad &= \quad \frac{\sin(\alpha-\alpha)}{\cos\alpha}=0.\end{aligned} \tag{13}$$

The deviation Δ_j can be denoted as

$$\Delta_j=s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix}\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i}\\ 0\end{pmatrix}(Z_j-Z_{\boldsymbol{\mu}}). \tag{14}$$

By calculating the norm of $\|\Delta_j\|$, we have $\|\Delta_j\|=s_{i'}\|\mathbf{R}_{\gamma_{i'}}\||\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i}(Z_j-Z_{\boldsymbol{\mu}})|=s_{i'}|\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i}(Z_j-Z_{\boldsymbol{\mu}})|$. Since the transformation we constructed in Eq.(11) and Eq.(12) always exists, and is just one of all the possible transformations, the deviation of the optimal transformation will thus always be upper bounded by $s_{i'}|\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i}(Z_j-Z_{\boldsymbol{\mu}})|$. ■

Part 2:

A more general case is to consider the geometric model, in which the pitch angle and offset are both considered (Eq.(1) only considers the pitch angle, which implicitly assumes that the tomogram is flat and horizontal to the x-y plane). Fig. S1 illustrates an example of geometric parameters. The introduction of tilt angle offset (φ) will enlarge the deviation of $Z_j-Z_{\boldsymbol{\mu}}$. If we take the tilt angle offset into consideration, we will not need the horizontal assumption. In this condition, the projection model can be generalized to:

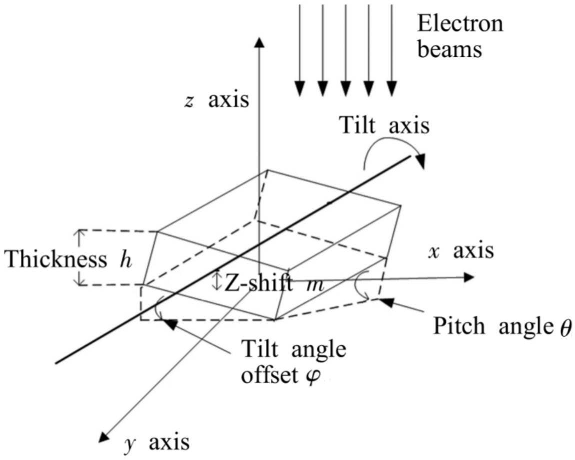


Figure S1: Geometric parameters of the specimen.

$$\begin{pmatrix} u \\ v \end{pmatrix} = s\mathbf{R}_\gamma \mathbf{P}\mathbf{R}_\beta \mathbf{R}_\alpha \mathbf{R}_\varphi \begin{pmatrix} X \\ Y \\ Z \end{pmatrix} + \mathbf{t}, \tag{15}$$

where all the attributes are the sample as in Eq. (1) except for $\mathbf{R}_\varphi$

$$\mathbf{R}_\varphi = \begin{pmatrix} \cos\varphi & 0 & -\sin\varphi \\ 0 & 1 & 0 \\ \sin\varphi & 0 & \cos\varphi \end{pmatrix}. \tag{16}$$

In this case, the projection model can be rewritten as:

$$\begin{pmatrix} u \\ v \end{pmatrix} = s\mathbf{R}_\gamma \begin{pmatrix} \cos\beta\cos\varphi - \cos\alpha\sin\beta\sin\varphi & \sin\alpha\sin\beta \\ \sin\alpha\sin\varphi & \cos\alpha \end{pmatrix} \begin{pmatrix} X \\ Y \end{pmatrix} + s\mathbf{R}_\gamma \begin{pmatrix} -\cos\beta\sin\varphi - \sin\beta\cos\alpha\cos\varphi \\ \sin\alpha\cos\varphi \end{pmatrix} Z + \begin{pmatrix} t_0 \\ t_1 \end{pmatrix}. \tag{17}$$

Here we denote

$$\mathbf{T} = \begin{pmatrix} \cos\beta\cos\varphi - \cos\alpha\sin\beta\sin\varphi & \sin\alpha\sin\beta \\ \sin\alpha\sin\varphi & \cos\alpha \end{pmatrix}, \tag{18}$$

and

$$\mathbf{V} = \begin{pmatrix} -\cos\beta\sin\varphi - \sin\beta\cos\alpha\cos\varphi \\ \sin\alpha\cos\varphi \end{pmatrix}. \tag{19}$$

Now we let $\{(X, Y, Z)^T\}$ be the fiducial markers embedded in the specimen. Considering the jth fiducial marker $(X_j, Y_j, Z_j)^T$ $(j = 1, 2, ..., N)$, its projections in the ith and i'th views ($\mathbf{p}_{ij} = (u_{ij}, v_{ij})^T$ and ($\mathbf{p}_{i'j} = u_{i'j}, v_{i'j})^T$) can be written as:

$$\begin{aligned} \begin{pmatrix} u_{ij} \\ v_{ij} \end{pmatrix} &= s_i \mathbf{R}_{\gamma_i} \mathbf{T}_i \begin{pmatrix} X_j \\ Y_j \end{pmatrix} + s_i \mathbf{R}_{\gamma_i} \mathbf{V}_i Z_j + \mathbf{t}_i, \\ \begin{pmatrix} u_{i'j} \\ v_{i'j} \end{pmatrix} &= s_{i'} \mathbf{R}_{\gamma_{i'}} \mathbf{T}_{i'} \begin{pmatrix} X_j \\ Y_j \end{pmatrix} + s_{i'} \mathbf{R}_{\gamma_{i'}} \mathbf{V}_{i'} Z_j + \mathbf{t}_{i'}. \end{aligned} \tag{20}$$

Just like what we have done in Part 1, we can find such a transformation $\mathcal{T}(\cdot; \mathbf{A}_{ii'j}, \mathbf{t}_{ii'j})$, where

$$\mathbf{A}_{ii'j} = \frac{s_{i'}}{s_i} \mathbf{R}_{\gamma_{i'}} \mathbf{T}_{i'} \mathbf{T}_i^{-1} \mathbf{R}_{-\gamma_i}, \mathbf{t}_{ii'j} = \mathbf{t}_{i'} - \mathbf{A}_{ii'j}\mathbf{t}_i + \mathbf{T}_j, \tag{21}$$

that satisfies $\mathbf{p}'_{ij} = \mathbf{A}_{ii'j}\mathbf{p}_{ij} + \mathbf{t}_{ii'j}$ and makes

$$\Delta_j = \mathbf{w} Z_j + \mathbf{T}_j; \mathbf{w} = s_{i'} \mathbf{R}_{\gamma_{i'}} \left(\mathbf{T}_{i'} \mathbf{T}_i^{-1} \mathbf{V}_i - \mathbf{V}_{i'} \right). \tag{22}$$

Similarly, since $\mathbf{A}_{ii'j}$ and $\mathbf{t}_{ii'j}$ are all independent of specific fiducial markers, for all the fiducial markers, they are identical. By further denoting $\mathbf{A}_{ii'j}$ as $\mathbf{A}$ and denoting $\mathbf{t}_{ii'j}$ as $\mathbf{t}$ (assuming the freedom parameter $\mathbf{T}_j$ for all the markers are identical and the value is $\mathbf{T}$), we can find such $\mathbf{T}$ to make the transformation $\mathcal{T}(\cdot;\mathbf{A},\mathbf{t})$ minimize $\sum_{j=1}^{N}\|\Delta_j\|$ for $\{(X,Y,Z)^T\}$:

$$COST \quad = \quad \sum_{j=1}^{N}\|\Delta_j\| = \sum_{j=1}^{N}\|\mathbf{w}Z_j + \mathbf{T}\|. \tag{23}$$

Here, the optimal solution is $\mathbf{T} = -\mathbf{w}Z_{\boldsymbol{\mu}}, Z_{\boldsymbol{\mu}} = \frac{1}{N}\sum_{j=1}^{N} Z_j$.

Now we can find that the offset-considered solution has the same form with that in Part 1, in which the optimal transformation $\mathcal{T}(\cdot;\mathbf{A},\mathbf{t})$ are

$$\begin{aligned}\mathbf{A} \quad &= \quad \frac{s_{i'}}{s_i}\mathbf{R}_{\gamma_{i'}}\mathbf{T}_{i'}\mathbf{T}_i^{-1}\mathbf{R}_{-\gamma_i},\\ \mathbf{t} \quad &= \quad \mathbf{t}_{i'} - \mathbf{A}_{ii'j}\mathbf{t}_i - \mathbf{w}Z_{\boldsymbol{\mu}},\end{aligned} \tag{24}$$

where $\mathbf{w} = s_{i'}\mathbf{R}_{\gamma_{i'}}\left(\mathbf{T}_{i'}\mathbf{T}_i^{-1}\mathbf{V}_i - \mathbf{V}_{i'}\right), Z_{\boldsymbol{\mu}} = \frac{1}{N}\sum_{j=1}^{N} Z_j$. Here we also assume the pitch angle is stable (i.e., $\alpha_i = \alpha_{i'}$). Considering the expression of $\mathbf{T}$ and $\mathbf{V}$ (Eq.(18) and Eq.(19)) and let $\beta_{i'} - \beta_i = \Delta\beta$, we will have the algebraic simplified result of

$$\mathbf{T}_{i'}\mathbf{T}_i^{-1}\mathbf{V}_i - \mathbf{V}_{i'} = \begin{pmatrix} \frac{\sin(\Delta\beta)}{\cos\alpha\cos\beta_i\cos(\varphi) - \sin\beta_i\sin\varphi} \\ 0 \end{pmatrix}. \tag{25}$$

Consequently, for arbitrary $\{(X_j,Y_j,Z_j)^T\}$ and its two projection $\mathbf{p}_{ij}$ and $\mathbf{p}_{i'j}$, the transformed deviation Δ_j can be denoted as

$$\Delta_j = s_{i'}\mathbf{R}_{\gamma_{i'}}\begin{pmatrix} \frac{\sin(\Delta\beta)}{\cos\alpha\cos\beta_i\cos(\varphi) - \sin\beta_i\sin\varphi} \\ 0 \end{pmatrix}(Z_j - Z_{\boldsymbol{\mu}}). \tag{26}$$

By calculating the norm of $\|\Delta_j\|$, we have
$\|\Delta_j\| \leqslant s_{i'}\|\mathbf{R}_{\gamma_{i'}}\||\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i\cos\varphi - \sin\beta_i\sin\varphi}(Z_j - Z_{\boldsymbol{\mu}})| = s_{i'}|\frac{\sin\Delta\beta}{\cos\alpha\cos\beta_i\cos\varphi - \sin\beta_i\sin\varphi}(Z_j - Z_{\boldsymbol{\mu}})|$. If $\varphi = 0$, we will have the same bound in Part 1.

S2 Robustness of the algorithm

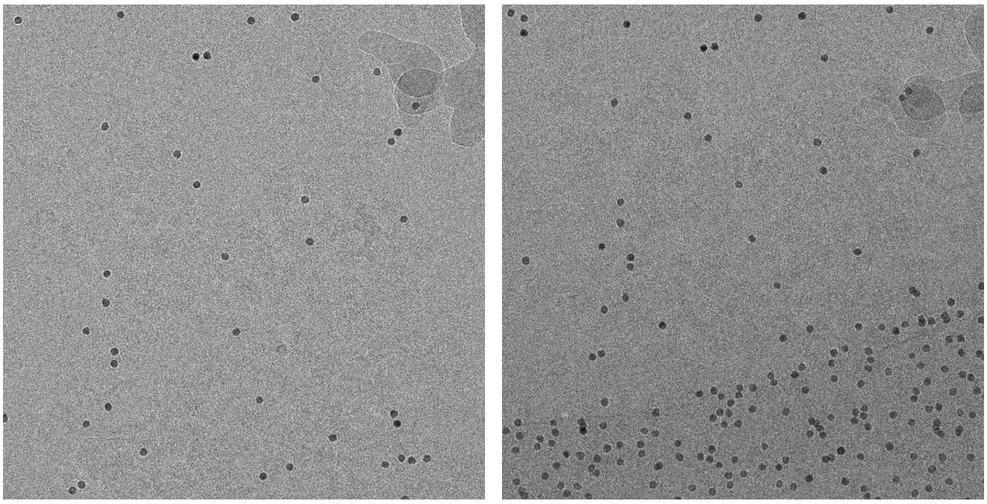
We sampled random subsets of markers of different sizes to measure the robustness of the proposed algorithm. There are two ways to sample the subsets: 1) randomly sample a number of fiducial markers and their corresponding projections, so that each fiducial marker has a corresponding projection; and 2) randomly sample a number of fiducial marker projections from each micrograph, which means some markers may not have corresponding projects.

We conducted our experiments following both ways. The first situation is simple, because all the sampled fiducial markers have their corresponding projections, which means the dataset is noise-free (we call fiducial markers that do not have corresponding markers in previous views as outliers). Our theorem guarantees the small deviation for the noise-free case. Experimental results also support our theory. The proposed method almost always achieved 100% accuracy in such cases.

The second situation is much more difficult as there are outliers (markers without corresponding ones in previous views). According to our experiments, if the outlier ratio is controlled between 10% to 20% (e.g., if there are 100 fiducial markers in the $(i+1)$th view with 90 of them having corresponding markers in the

ith view, the outlier ratio is 10%), and the fiducial markers are well distributed (not degenerated), there is almost no influence on the performance of our proposed method.

Even though our method is quite robust with respect to the outlier ratio, it cannot handle the cases where the outlier ratio is extremely high. Here we give such an example. In our recent work Han *et al.* (2017), we tested the random sampling method against several public datasets and achieved very good accuracy. Those datasets contain about 50-150 fiducial markers. Here we also tested the proposed method on those datasets. When there are not too many outilers, the proposed method had high accuracy and short runtime. When there are too many outliers, the proposed method might fail. The main reason for failure is not due to the missing markers but the introduction of markers that do not appear in previous views. Fig. S2 below shows such an example, where Fig. S2(a) looks normal but Fig. S2(b) introduces a large number of fiducial markers that do not appear in Fig. S2(a), which causes the total change of the probability distribution and thus the failure of the proposed method. How to further improve the robustness of the proposed method is our ongoing work.



(a) (b)

Figure S2: An example dataset that contains a high number of outliers.

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