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Cryo-EM Image Alignment Based on Nonuniform Fast Fourier Transform

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Abstract

In single particle analysis, two-dimensional (2-D) alignment is a fundamental step intended to put into register various particle projections of biological macromolecules collected at the electron microscope. The efficiency and quality of three-dimensional (3-D) structure reconstruction largely depends on the computational speed and alignment accuracy of this crucial step. In order to improve the performance of alignment, we introduce a new method that takes advantage of the highly accurate interpolation scheme based on the gridding method, a version of the nonuniform Fast Fourier Transform, and utilizes a multi-dimensional optimization algorithm for the refinement of the orientation parameters. Using simulated data, we demonstrate that by using less than half of the sample points and taking twice the runtime, our new 2-D alignment method achieves dramatically better alignment accuracy than that based on quadratic interpolation. We also apply our method to image to volume registration, the key step in the single particle EM structure refinement protocol. We find that in this case the accuracy of the method not only surpasses the accuracy of the commonly used real-space implementation, but results are achieved in much shorter time, making gridding-based alignment a perfect candidate for efficient structure determination in single particle analysis.

Keywords

single particle analysis; alignment; image processing; gridding

I. INTRODUCTION

The goal of single particle analysis is to obtain a high-resolution three-dimensional (3-D) reconstruction of the mass density distribution of a biological macromolecule. For that purpose, multiple 2-D electron microscope (EM) images are collected that constitute projections of the macromolecule in different orientations. These observed particle projections may have a few preferential orientations, or their orientations may be uniformly distributed; the exact distribution depends on the specimen, the preparation technique, and some other difficult to define factors. In any case, one must align and classify the collected particle projections for further processing [1]. This is achieved using 2-D alignment procedures, which determine two translations and one rotation angle between two 2-D images and brings them into register [2].

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Due to the physical limitation of the cryo-EM, the collected images are usually extremely noisy and the typical value of Signal-to-Noise Ratio (SNR) ranges from 0.1 to 10. In order to reduce the effect of noise, a large number of particle projections (from 10^4 to 10^6) must be collected and analyzed, which means the computational load is very heavy even for a modern multi-processor computer cluster. Therefore, it is of great interest to develop accurate and efficient 2-D alignment algorithms.

In order to achieve high efficiency of data processing, currently available single particle software packages use low order interpolation schemes for image manipulations. In most cases it is linear interpolation, although some operations in SPIDER system are implemented using quadratic interpolation [3]. The deterioration of results due to interpolation errors is customarily minimized by oversampling of EM micrographs with respect to the target resolution. For example, to determine the 7.3 Å structure of the 70S ribosome, it was necessary to use a pixel size of 1.22 Å [4]. If we consider that the usable information in this structure extends to ~4.8 Å, as estimated by the Fourier Shell Correlation method [5], according to the Shannon sampling theorem the necessary pixel size should be 2.4 Å. Thus, the data has been oversampled nearly two times, in agreement with [6], where a 1.5 ratio was recommended. Nevertheless, in high-end applications, a ratio of 3.0 is recommended if the adverse effects of linear interpolation are to be minimized. In effect, the size of the 2-D data set is increased 9 times, while 3-D maps are increased 27 times in size! This seriously increases the amount of time necessary for processing, the amount of disk storage occupied, and the size of computer memory necessary to hold the data.

The need for oversampling can be eliminated or greatly reduced by the use of more advanced interpolation schemes and 3-D reconstruction algorithms [7]. To some extent, this was demonstrated in our recent work on a 3-D reconstruction algorithm that is capable of recovering a virtually perfect map almost to the Nyquist frequency [8]. It was made possible by taking advantage of a novel interpolation scheme based on the nonuniform fast Fourier transform (NUFFT) [9,10]. This interpolation method, also known as gridding method, is known for its accuracy [11]. Here we apply the NUFFT-based image processing method to 2-D and 3-D alignment applications and compare its performance with that of linear and quadratic interpolation schemes in terms of both accuracy and efficiency.

We focus our comparison of 2-D alignment methods on the approach based on resampling of the image to polar coordinates, as we have previously demonstrated its superior properties [2]. In this approach, one conducts an exhaustive search of the alignment parameters with the translation limited to a few pixels conducted in steps set to integer values. This necessarily limits the resulting accuracy of the alignment. In order to achieve sub-pixel accuracy, one needs to refine the results using finer grid, interpolate cross-correlation around the location of the maximum, take advantage of more accurate interpolation methods, or all of the above. If the interpolation is sufficiently accurate, the problem can be considered quasi-continuous and it becomes possible to use well established optimization methods to determine alignment parameters.

After 2-D alignment of all particle projections and after obtaining a preliminary, low resolution 3-D map of the structure, one usually employs a version of a 3-D projection alignment method in order to improve the resolution of 3-D results [12]. Briefly, the initial reference structure is used to calculate its 2-D projections and, using a correlation technique, the original data is compared with template projections yielding the necessary orientation parameters: three Eulerian angles and two translations. This procedure is also referred to as image to volume (or 2-D to 3-D) registration [13]. The procedure was conceived to perform exhaustive searches. This is achieved by generating template projections such that their projection directions cover the entire range of possibilities (in case of a symmetric structure, those within asymmetric unit

suffice). Necessarily, only a finite set of such projections can be generated, so a pre-selected angular step is used. On one hand, this makes the procedure very efficient, on the other hand, it sets a limit on the accuracy that can be achieved [12]. As the concern is usually computational efficiency, simple interpolation schemes are used throughout. Recently, an improvement of the computational efficiency of the 3-D projection alignment method was reported using a multi-resolution approach, in which the data was initially decimated in order to accelerate searches [14]. Improved accuracy of the method was achieved by interpolating cross-correlation functions computed during the searches [15].

The step that logically follows the 3-D projection alignment incorporates 'local' optimization of determined alignment parameters. The exhaustive search is replaced by a minimization of a goal function (a form of discrepancy between projection data and what can be derived from the current approximation of the structure), which is parameterized by Eulerian angles and translations, this time treated as continuous. In their early work, Provencher and Vogel represented both the data and the structure in the basis of spherical harmonics, which afforded efficient manipulation of Eulerian angles [16]. The main target application was *ab initio* structure determination [17]. In the popular package FREALIGN, the searches are performed in Fourier space and the transformation between the arbitrarily oriented Fourier 2-D central section and the 3-D Fourier Euclidean grid is implemented using trilinear interpolation [18]. The program uses a gradient-based Powell optimization algorithm in order to systematically refine the orientation parameters [19]. Jonic and colleagues [13] used B-spline interpolation in order to extract 2-D central sections from the 3-D Fourier transform of the structure and compare them with Fourier transforms of the data. The optimization was done using the Levenberg-Marquardt optimization algorithm with gradients calculated directly from the B-splines-interpolated volume. Finally, a method that unifies optimization of alignment parameters with updates of the density distribution of the structure was proposed by Yang and co-workers [20]. The projections of the structures are calculated in real space using trilinear interpolation and free parameters of the method (orientation parameters and voxel values of the structure) are determined using the limited-memory Broyden, Fletcher, Goldfarb, and Shannon bound (LBFGSB) optimization algorithm [21].

On the most basic level, most of the local optimization methods incorporate a version of 2-D alignment between experimental image and a template, be it in reciprocal space, as in FREALIGN or the method of Jonic, or in real space, as in the unified approach. However, little is known about accuracy-limiting factors such as interpolation errors, errors in approximating gradients, limitations of optimization methods employed, and overall strategies of performing the refinement. Curiously, with the possible exception of work by Jonic *et al.* [13], no test results are given that would justify treating orientation parameters as continuous. Here we seek to fill this void, but focus exclusively on 2-D to 3-D registration of single 2-D projection data. We present results obtained by incorporating the newly developed NUFFT-based 2-D alignment into the 2-D to 3-D registration method and compare it with results obtained using linear interpolation. We also point out the ultimate limits of the approach, with regard to cryo-EM structure determination.

II. NONUNIFORM FAST FOURIER TRANSFORM

In this section, we briefly introduce the nonuniform fast Fourier transform (NUFFT). The conventional FFT requires that both input and output grids are equally spaced grid. This requirement is met in many scientific and technical applications, but it is not unusual that the input or the output grid is nonuniform. The NUFFT allows an efficient calculation of the discrete Fourier transform in such cases [22]. There are various forms of NUFFT, depending on whether the input grid or the output grid is nonuniform. Besides, NUFFT are known under various other names, such as nonequispaced FFT [23], unequally spaced FFT [24], or gridding

method [9,10,25]. We tend to use the term gridding method here. Nonuniform input or output grids are often encountered in the field of image reconstruction. The gridding method has been used in Computed Tomography [10] and is widely used in Magnetic Resonance Imaging, see e.g. [9,25]. Matey and Fessler used NUFFTs in an iterative Fourier-based 2-D tomographic image reconstruction algorithm [26]. Penczek and co-workers [8] used the gridding method to devise a fast reconstruction algorithm to be used in single particle analysis. Gridding method has also been applied to the resampling of discrete data [11].

All boldface letters in this section are d -dimensional (d -D) vectors. The components of a vector $\mathbf{a}$ are denoted by $a_1, \dots, a_d$. Operations such as $\mathbf{ka}$, $\mathbf{K}/2$, $\mathbf{a} > 0$, $\mathbf{N} = \mathbf{K}$ are understood component-wise. In the classical d -D FFT, both input grid and output grid are equidistant Cartesian grids:

$$\begin{aligned} \{\mathbf{ka} : -\mathbf{K}/2 \leq \mathbf{k} < \mathbf{K}/2\} & \text{ with } \mathbf{a} \in \mathbb{R}^d, \mathbf{a} > 0, \mathbf{K}/2 \in \mathbb{Z}^d \\ \{\mathbf{nb} : -\mathbf{N}/2 \leq \mathbf{n} < \mathbf{N}/2\} & \text{ with } \mathbf{b} \in \mathbb{R}^d, \mathbf{b} > 0, \mathbf{N}/2 \in \mathbb{Z}^d, \end{aligned} \quad (1)$$

where $\mathbf{a}$, $\mathbf{b}$ are the grid spacings and $\mathbf{N}$, $\mathbf{K}$ are the grid sizes. The two grids are said to be conjugates of each other if $\mathbf{N} = \mathbf{K}$ and $\mathbf{b} = 1 / (\mathbf{Ka})$

The gridding method comprises three steps. Given nonuniformly distributed samples of $g^\wedge$ ($g^\wedge$ is a d -D Fourier transform of g), the steps of the gridding method are:

1. calculate the convolution $h^\wedge = w^\wedge \otimes g^\wedge$ on an appropriate uniform grid, using the available samples of $g^\wedge$ and a suitable window function $w^\wedge$;
2. calculate the inverse discrete Fourier transform of $h^\wedge$ to obtain $h = wg$;
3. remove the so-called weighting function w and window out the relevant area of h to obtain the function $\tilde{g} = h / w$ on the uniform output grid.

Both the accuracy and efficiency of the method are largely determined by the choice of the window function $w^\wedge$. $w^\wedge$ should be selected such that it is real, even-sized, and vanishes rapidly outside of compact support. If fulfilled, the latter requirement makes it possible to truncate $w^\wedge$ outside of a small region (few pixels) without compromising the accuracy while maintaining acceptable computational time of the convolution operation in step 1. Also, its Fourier pair w (called weighting function) must be positive in $[-\mathbf{Ka}/2, \mathbf{Ka}/2]$. We choose the d -D separable *Kaiser-Bessel* window function as $w^\wedge$, as recommended in [10]:

$$\widehat{w}(\mathbf{y}) = \begin{cases} \prod_{v=1}^d \frac{I_0\left(2\pi\alpha_v r_v s_v \sqrt{1 - (y_v/s_v)^2}\right)}{2s_v} & \mathbf{y} \in [-\mathbf{s}, \mathbf{s}] \\ 0 & \mathbf{y} \notin [-\mathbf{s}, \mathbf{s}] \end{cases} \quad (2)$$

where I_0 is the zero-order modified Bessel function and $\alpha = 1.25$, $\mathbf{s} = \mathbf{Lb}/2$, $\mathbf{L} = 6$ and $\mathbf{r} = \mathbf{Ka}/2$. The Fourier pair of (2), the associated weighting function is:

$$w(\mathbf{x}) = \prod_{v=1}^d \frac{\sinh\left(2\pi\alpha_v r_v s_v \sqrt{1 - (x_v/(\alpha_v r_v))^2}\right)}{2\pi\alpha_v r_v s_v \sqrt{1 - (x_v/(\alpha_v r_v))^2}}. \quad (3)$$

The application of gridding method to 3-D reconstruction from projections is described in [8], where data g is a 2-D projection available on a Cartesian grid, but arbitrarily oriented in 3-D space. Thus, there is a preparatory step required, in which g is padded with zeroes to twice the size and its FFT computed to yield $g^\wedge$. The convolution in step 1 recovers from $g^\wedge$ samples of the target 3-D function on a Cartesian (uniform) 3-D grid. The implementation of gridding method described in [8] is significantly more complex than it would follow from the simple schematic given above. In application to 3-D reconstruction from projections nonuniform

distribution of the input samples has to be accounted for by additional geometrical weighting. Thus, generally the efficiency and accuracy of the gridding method will depend on a particular application and geometrical nonuniformity of the data.

The gridding method was originally introduced to calculate FFT accurately and efficiently when the input grid is nonuniform and output grid is uniform. If we reverse the three steps of gridding method, we can compute nonuniformly distributed samples given uniform distributed samples of $g^\wedge$ (in d -D Fourier space). We call this method the reverse gridding method. Note the weighting function is now applied in Fourier space.

The required steps of reverse gridding method are (Fig. 1):

1. calculate $(g^\sim)^\wedge = g^\wedge/w^\wedge$ and zero-pad it in Fourier space; here, $w^\wedge$ is a weighting function;
2. calculate the inverse FFT of $(g^\sim)^\wedge$ to obtain $g^\sim$;
3. obtain samples of g at arbitrary locations (the polar coordinates shown in Fig. 1 are a suitable example) by evaluating $g = w \otimes g^\sim$ at the desired locations.

It is possible to use the reverse gridding method to conduct interpolation. This requires addition of a preparation step in which we calculate FFT of g to obtain $g^\wedge$. We call this method a *gridding-based interpolation* and it has been shown to have excellent interpolation properties [11], as due to the smoothing effect of the convolution, evaluation of $w^\wedge \otimes g^\sim$ is much less error-prone than merely interpolating g , and in effect it yields sub-pixel accuracy.

We implemented the gridding-based form of interpolation in SPARX system to discrete data onto arbitrary grids and to perform all basic image manipulation operations used for processing of EM images: rotation, translation, change of scale (i.e, resampling of image in order to change 'physical' pixel size), and resampling to polar coordinates. In addition, it is possible to use reverse gridding method to recover only selected samples of g with sub-pixel accuracy. Indeed, the convolution in step 3 can be performed only at needed locations. An obvious application is high-accuracy determination of a maximum of a cross-correlation function in the vicinity of a known peak location, as described in Section III.

We use a simple example to demonstrate the accuracy of the gridding-based interpolation method. A 128×128 image containing Gaussian noise is rotated 33° clockwise and then 33° counterclockwise using quadratic, and gridding-based interpolation schemes. The resulting image is compared with the original image using the Fourier Ring Correlation (FRC) technique [5] (Fig. 2). The FRC curve of the gridding-based interpolation method is virtually one for all spatial frequencies, which means all frequency components are nearly ideally preserved. On the other hand, the performance of quadratic interpolation resembles that of a lowpass filter in that the information in the high-frequency region is suppressed. If the image is 1.5x oversampled before quadratic interpolation, the lowpass filtering effect can be greatly reduced but it is still evident as compared to the outcome of the gridding-based interpolation.

III. METHODS

3.1. 2-D alignment

The problem of 2-D alignment is defined as: given two images f and g , we wish to find two translation parameters x and y and one rotation angle θ which minimize the least square discrepancy:

$$\int_{\mathbf{u} \in D} |f(\mathbf{u}) - g(\mathbf{T}\mathbf{u})|^2 d\mathbf{u}, \quad (4)$$

where D is the region of interest (for example, a disk with diameter d_0), $\mathbf{u}$ is vector $[u_x \ u_y \ 1]^T$ containing the coordinates, and $\mathbf{T}$ is the transformation matrix defining rotation and translations of image [2]. We designate f the reference (or template) image and g the subject (aligned) image.

As a measure of alignment accuracy we use the maximum pixel error of alignment [2]:

$$e_{\max}(\mathbf{T}_1, \mathbf{T}_2) = \max_{\mathbf{u} \in D} \|\varepsilon_{\mathbf{T}_1, \mathbf{T}_2}(\mathbf{u})\|, \quad (5)$$

where $\mathbf{T}_1(\theta_1, x_1, y_1)$ and $\mathbf{T}_2(\theta_2, x_2, y_2)$ are the two transformation matrices we wish to compare and

$$\varepsilon_{\mathbf{T}_1, \mathbf{T}_2}(\mathbf{u}) = \mathbf{T}_2^{-1} \mathbf{T}_1 \mathbf{u} - \mathbf{u} = (\mathbf{T}_2^{-1} \mathbf{T}_1 - \mathbf{I}) \mathbf{u}, \quad (6)$$

which yields the following closed-form formula:

$$e_{\max}(\mathbf{T}_1, \mathbf{T}_2) = d_0 \left| \sin \frac{\Delta\theta}{2} \right| + \sqrt{\Delta x^2 + \Delta y^2}, \quad (7)$$

where $\Delta\theta = \theta_1 - \theta_2$ is the difference between respective rotation angles, and $\Delta x = x_1 - x_2$ and $\Delta y = y_1 - y_2$ are differences between respective translations.

We implement the procedure of 2-D alignment in three separate steps: (1) preparation of the reference image, (2) coarse search, (3) fine search. In the preparation step, we resample the reference image to polar coordinates using one of two interpolation method (either quadratic or gridding-based interpolation). In EM applications, the number of subject images is usually much larger than the number of reference images, so this step is done only once in the preparation phase, thus eliminating unnecessary repetitions.

In the second step, the coarse search, we conduct an exhaustive search of possible translations within specified limits and determine the rotation angle *via* 1-D rotational cross-correlation. If we choose the possible translations to span an equidistant grid, then their number is determined by two factors: (1) the possible range of translation $[-r, r]$ and (2) the desired step size of the coarse search t (which can be a non-integer number of pixels). When we obtain the EM particle projections of the macromolecule, the images are already roughly centered due to the particle windowing process, but rotation angles are uniformly distributed in $[0^\circ, 360^\circ]$ range (possible nonuniformity can be introduced if an automated particle picking method is used, but this is of no consequence in what follows). Therefore, it is reasonable to assume the translation is within the range of several pixels (e.g., $r=4$ for the image size of 128×128 pixels). The determination of t is related to the properties of fine search, which will be discussed in details in the next paragraph. As determined in our tests, for typical, properly sampled cryo-EM single particle data, $t=2.0$ is a good choice, i.e., initial search is performed using a step of two pixels. Given r and t , the total number of possible translations is:

$$n_t = (\text{int } [2r/t] + 1)^2. \quad (8)$$

For each possible translation, the subject image is resampled to polar coordinates (either quadratic or gridding-based interpolation) with respect to appropriately translated origin of the system of coordinates. The 1-D rotational cross-correlation function between the reference image and the subject image is then calculated and location of its maximum determines the rotation angle. The value at the maximum is recorded and compared among all possible translations. The translation corresponding to the overall maximum is the result of the coarse search (Fig. 3). The possibility to consider a mirrored version of reference image is easily and efficiently incorporated into the alignment described by taking advantage of the fact that the 1-D Fourier-polar transform of the mirrored image is the complex conjugate of the 1-D Fourier-polar transform of the original image [2].

In the third step of 2-D alignment, the fine search, we use the result of the coarse search as the starting point and refine the results to the accuracy limited only by the accuracy of the interpolation method used. We select the least-square discrepancy (4) between the two images as the goal function with three alignment parameters to be determined given their initial values obtained from the coarse search. The problem is solved by a selected multi-dimensional optimization algorithm.

The simplest multi-dimensional optimization algorithm is the steepest descent method. It uses the derivative of the goal function with respect to the free parameters in order to determine the search direction and it can be shown to converge to a local extremum in a finite number of steps. Unfortunately, it is also known for its inefficiency [27].

The second optimization algorithm we have tested is the LBFGSB algorithm, which is designed to tackle large-scale optimization problems [28]. In addition to the gradient, it uses an approximation to the Hessian of the goal function in order to accelerate the convergence. Thus, in order to use this algorithm, one needs to evaluate the derivatives as well as the value of the goal function. In our case, the derivatives cannot be derived analytically and have to be approximated *via* finite differences, which means calculating the goal function one more time for each parameter. The computational burden is hence very high, although it might be mitigated by the excellent convergence properties of the LBFGSB algorithm.

In order to avoid possible problems caused by inaccuracies of the approximation of the derivatives, we selected the downhill simplex method [27]. This method uses the geometrical concept of a simplex and takes advantage of several operations on the simplex, such as expansion and contraction in order to determine the search pattern. A proper combination of these operations will converge to the extremum of the goal function. This method only requires calculation of the goal function, not the derivatives; this eliminates potential errors caused by the finite difference approximation of derivatives. Given reasonable guesses of the initial values of the parameters, the method is also more likely to converge to global extremum of the goal function than the gradient-based algorithms.

We studied two different strategies of determining the three alignment parameters in the fine search phase. The first strategy we call *concurrent*, as we refine two translation and one rotation alignment parameters simultaneously minimizing the goal function (4). This strategy is straightforward and easy to implement. However, for each set of orientation parameters considered by the optimization procedure, it is necessary to rotate/translate (thus resample) the subject image, which is computationally costly. To alleviate this problem, we introduce a second strategy that we call *cascaded*. Here, we refine translation parameters assuming that for each set of their values the optimum rotation angle is known. The angle is determined independently for each translation using the 1-D cross-correlation technique described below; thus, the angle is found using an exhaustive search. This requires resampling of the subject image to polar coordinates, but it has to be done only once for each set of translation parameters tried by the optimization algorithm and while the angle is always optimally determined given current translation parameters. In terms of computational efficiency, the strategy compares favorably with the concurrent strategy, in which numerous resampling of the subject image are required to find the correct angle, even if its approximate value is known.

The remaining problem to solve is the accurate determination of the rotation angle within the framework of 1-D cross-correlation approach used in cascaded strategy. The 1-D cross-correlation search for the rotation angle is implemented using FFT technique with image

discretized in polar coordinates (as $c = (\hat{f} \hat{g}^*)$). In effect, it essentially yields discrete values of angles, which sets a limit on the accuracy of the method [2]. Although some improvement can be achieved by interpolating a 1-D position of the maximum by fitting a parabola or a Gaussian

function at pixel locations of the cross-correlation function in the vicinity of the maximum, the gain is often illusory and it is difficult to demonstrate that the improvement is guaranteed under broad range of circumstances. In order to rectify this problem, we applied reverse gridding method to locate the maximum of the 1-D discrete cross-correlation function with increased accuracy. The steps are:

- 0 calculate the FFTs of f and g ;
- 1 calculate the Fourier product $(\tilde{c})^\wedge = f^\wedge g^{\wedge*} / w^\wedge$;
- 2 calculate the inverse Fourier transform to obtain an 'upweighted' cross-correlation functions $\tilde{c}$;
- 3 obtain values of the cross-correlation function at arbitrary locations using convolution to remove the weights, $c = w \otimes \tilde{c}$.

Note that as the goal is to calculate a circulant 1-D cross-correlation function, no padding with zeroes is included in step 1. In addition, to improve computational efficiency, we 'concatenated' logically linked operations. More precisely, we eliminated redundant Fourier transform by incorporating upweighting of the cross-correlation function into step 1.

A comparison of the accuracy of the reverse gridding localization of the maximum of a discrete cross-correlation function with localization obtained by fitting a 7-point parabola is given in Figure 4. The error of the parabola fit localization is much larger than that of the reverse gridding method; in the latter case, the error is virtually on the level of numerical accuracy. Moreover, in the former case the error is systematic and discontinuous forming a curious sawtooth pattern around correct value, while the error of the reverse gridding method is almost uniformly random.

3.2. 2-D to 3-D registration

The problem of 2-D to 3-D registration is defined as: given a 3-D object V and its 2-D projection image f , we need to determine five projection parameters: three Eulerian angles (ϕ, θ, ψ) and two translation parameters (s_x, s_y) (see [29] for convention used), such that the following least square discrepancy is minimized:

$$\int_{(u_x, u_y) \in D} |f(u_x, u_y) - (P(V(\mathbf{R}\mathbf{v} + \mathbf{S}))) (u_x, u_y)|^2 du_x du_y, \quad (9)$$

where $\mathbf{R}$ is the 3-D rotation matrix, $\mathbf{v}$ is the coordinate vector $[v_x \ v_y \ v_z]^T$, $\mathbf{S}$ is translation vector $[s_x \ s_y \ 0]^T$, P is the projection operator whose direction coincides with z -axis and D is the region of interest. We designate V the reference volume, PV the reference projection, and f the subject image.

As in 2-D alignment, we evaluate the accuracy of 2-D to 3-D registration in terms of maximum pixel error. If $\mathbf{T}_1 = \mathbf{R}_1 \mathbf{v} + \mathbf{S}_1$ and $\mathbf{T}_2 = \mathbf{R}_2 \mathbf{v} + \mathbf{S}_2$ are two different transformations of coordinate vector $\mathbf{v} = [v_x \ v_y \ v_z]^T$ we wish to compare, then we define the maximum pixel error as:

$$e_{\max}(\mathbf{T}_1, \mathbf{T}_2) = \max_{\mathbf{v} \in V_r} \|\varepsilon_{\mathbf{T}_1, \mathbf{T}_2}(\mathbf{v})\|, \quad (10)$$

where V_r is a sphere with radius r and error $\varepsilon_{\mathbf{T}_1, \mathbf{T}_2}(\mathbf{v})$ is given by

$$\varepsilon_{\mathbf{T}_1, \mathbf{T}_2}(\mathbf{v}) = \mathbf{T}_2^{-1} \mathbf{T}_1 \mathbf{v} - \mathbf{v} = \mathbf{R}_2^{-1} (\mathbf{R}_1 \mathbf{v} + \mathbf{S}_1 - \mathbf{S}_2) - \mathbf{v}. \quad (11)$$

Unlike in the 2-D case, in which the maximum pixel error can be conveniently expressed by a closed-form formula, in the 3-D case this formula would be rather complicated and not particularly informative. Therefore, we evaluate (11) numerically.

The most straightforward way of carrying out 2-D to 3-D registration is to generate projections of the 3-D structure (*via* either linear interpolation with summation of pixels to approximate integration or NUFFT which, as follows from Fourier slice theorem, yields 2-D projection) and compare them with the subject image. This approach is similar to 2-D alignment in the sense that for each considered projection direction we try to minimize the least-square discrepancy (9) between a computationally generated projection of the 3-D structure and the subject image. The main difference is that one has to consider multiple reference images. In addition, now the goal function has five free parameters: three Eulerian angles defining projection direction and two translations.

We divide 2-D to 3-D registration into two steps. The first step is the coarse search, as described earlier [12]. We generate a set of quasi-evenly distributed 2-D reference projections and we conduct a 2-D alignment between the subject image and each of them. For each 2-D alignment, we obtain a peak value of the cross-correlation function as well as the corresponding translation and rotation parameters. We then select as a new projection direction Eulerian angles corresponding to the largest cross-correlation value among all reference projections. These Eulerian angles as well as the translation and rotation parameters associated with this projection constitute the results of the coarse search. The accuracy of this search is determined by the angular step set to generate reference projections [29].

Here we introduce the second step of 2-D to 3-D registration, the fine search. We use the result of the coarse search as the starting point in either the LBFGSB algorithm or the downhill simplex method. For the same reason as in 2-D alignment, we choose the goal function to be the least-square discrepancy (9) between the reference projection and the subject image.

Five projection parameters ($\phi, \theta, \psi, s_x, s_y$) are simultaneously refined by maximizing the goal function (Fig. 5). We call this refinement strategy *concurrent strategy*. As in 2-D alignment, we also developed a *cascaded strategy* for 2-D to 3-D registration. Here, we refine three Eulerian angles assuming that for each set of their values the optimum translation of the projection is known. The two in-plane translations of projection are determined independently from determination of the angles using the 2-D cross-correlation technique described below. Although this calls for an additional 2-D FFT, the calculation has to be done only once for each set of Eulerian angles tried by the optimization algorithm and yields optimum translations given current projection direction, in effect comparing favorably with the concurrent strategy in terms of computational efficiency. As the concurrent strategy calls for sub-pixel accuracy determination of a peak location in the 2-D cross-correlation function, we only implemented it within the context of NUFFT-based methods.

We compared two implementations of 2-D to 3-D registration. The first uses linear interpolation and summation in the spatial domain and generally corresponds to the way 3-D operations are conducted in many popular EM software packages. The second implementation exploits the Fourier slice theorem and uses NUFFTs. The main computational burden of the problem is in generation of 2-D projections of a 3-D reference structure.

In the first implementation, projections are generated using a form of numerical integration, i.e., we approximate, using a trilinear interpolation, between-grid voxel values in a 3-D volume and add them to a 2-D projection in locations given by the transformation resulting from current five orientation parameters (three Eulerian angles and two translations). For the volume size n^3 voxels, the computational complexity of this approach is $O(n^3)$ with a small constant (<10).

For the second implementation, we used the central section theorem [30] and NUFFTs to compute projections. The method was introduced [8] and it corresponds to the reverse gridding method described in Section II of this work (also see Fig.1), although now the real and Fourier

space of the Section II schematic are reversed. Given uniformly distributed samples of g , the steps are:

1. divide the volume by the weights $\tilde{g} = g/w$ and pad the results with zeros;
2. calculate the FFT of $\tilde{g}$, thus obtain $(\tilde{g})^\wedge = \widehat{\tilde{g}} \otimes \left(1/w\right)^\wedge$;
3. calculate the convolution $h^\wedge = w^\wedge \otimes (\tilde{g})^\wedge$ on an appropriate grid to remove the weights w and to obtain a 2-D central section of the 3-D object;
4. if necessary, calculate inverse 2-D FFT and window out the area of interest to obtain 2-D projection h .

If the goal were to generate a single projection of a 3-D structure, the Fourier-space implementation could not possibly compete in terms of computational efficiency with real-space implementation, as in step 2 it calls for a 3-D FFT at a cost of $O(n_3 \log_2 n)$. However, in our application it is necessary to generate large number of projections (the exact number depends on the optimization algorithm used, but it is in the order of 10^2). In this case, first two steps have to be performed only once and the computational cost of remaining steps is $O((L+1)^3 n_2)$ for step 3 (recall L is the size of window function (2) set to 6 irrespective of the image size) and $O(n^2 \log_2 n)$ for step 4; thus, it is $O(n^2)$ with a large constant, which however for large volumes is less than n of the real space implementation. Finally, we have to note that the Fourier-space-based technique yields an FT of the projection. This suggests that the evaluation of the goal function (9) can be done in Fourier space, which requires the calculation of a 2-D FFT of the subject image in the preparation step, but eliminates step 4 with its repetitive 2-D FFT.

Similarly as in 2-D alignment, we can ‘concatenate’ logically linked operations in the cascaded approach to generate the 2-D cross-correlation function. Indeed, the reduction of the computational load is even larger here, as the step 3 of the reverse gridding method above yields Fourier transform of the projection padded with zeroes. Thus, it is sufficient to multiply

it, in Fourier space, by the Fourier transform of the subject image upweighted by $\left(1/w\right)^\wedge$ (both latter operations have to be done only once in the preparation phase), compute inverse FFT, and, using convolution in real space with the window function w , locate the maximum with sub-pixel accuracy. This is done rapidly using the simplex method, as the initial values of the translation parameters are known to a good accuracy from the coarse search.

IV. RESULTS

We conducted our tests using simulated data within the environment of the SPARX single particle package (<http://macro-em.org/sparxwiki>) [31]. All methods were tested on a Linux workstation equipped with two AMD 2.4 GHz CPUs and 8GB of memory. We selected the test object to be the X-ray crystallographic structure of a 70S ribosome-tRNA complex converted from the combination of PDB files 1VS9 and 2IIC to discretized electron densities using the pixel size of 3.0 Å and window size of 128 pixels. Although for simplicity we did not model the entire image formation process of the electron microscope, we emulated limited low-frequency content of EM data by suppressing Fourier amplitudes of test data with a Butterworth homomorphic filter (stop-band frequency 0.01, pass-band frequency 0.1, the value of the filter at zero-frequency 0.1) [32].

4.1. 2-D alignment

We first studied the accuracy and efficiency of 2-D alignment algorithms for noise-free images. The reference image was a projection of the test object described above. For each 2-D alignment

test, the subject image was copied from the reference image and then randomly rotated and shifted using gridding-based interpolation. The rotation angle θ was evenly distributed in $[0^\circ, 360^\circ)$ and the two translations (x, y) were independently and evenly distributed in $[-4, 4]$. The reference image remained unchanged. We then aligned these two images beginning with the coarse search (see Section III). We set the range of the coarse search to $[-4, 4]$. (thus $r=4$), and the step size t to 2. The result of the coarse search was used as the starting point of the fine search, which generated the final alignment parameters. We compared these parameters with the known values using formula (7) (Fig. 6).

In Section 3.1, we have shown that there were three major factors that could affect the performance of the 2-D alignment algorithm: (1) optimization algorithm, (2) interpolation method, and (3) optimization strategy. We present our test results first in terms of the optimization algorithm. For each optimization algorithm, we compared the performance of three different interpolation methods. The first interpolation scheme was quadratic interpolation. In the second interpolation scheme, in order to reduce the adverse impact of quadratic interpolation on high frequency content of the image data, we first oversampled both images 1.5 times by zero-padding in the Fourier space before applying the 2-D alignment procedure using quadratic interpolation. This roughly corresponds to a situation common in EM data processing, where data is collected at the microscope at the magnification too high for the target resolution and subsequently low-passed filtered to reduce excessive noise. In the third scheme, we used gridding-based interpolation (Section II). For the simplex optimization algorithm we also present the results of both concurrent and cascaded strategy for each interpolation method used.

The first refinement algorithm we employed was steepest descent method [27]. We set the maximum number of iterations to 200 and repeated the simulation 1,000 times. In our tests the algorithm often failed to converge within the preset number of iterations. For quadratic interpolation, the failure rate was 28%, it was reduced to 26% for quadratic interpolation with image 1.5 times oversampled, and was only 23% for the griddingbased interpolation method. We concluded the steepest descent method was insufficiently reliable for practical applications. In addition, even if it converged to the correct solution, due to the number of iterations required the algorithm was not competitive with respect to remaining optimization algorithms tested.

The second optimization method tested was LBFGSB algorithm [28]. We repeated the simulation 1,000 times and calculated the average alignment error (Table 1) for three different interpolation schemes. It can be seen from the results that the 2-D alignment based on gridding-based interpolation method is most accurate. It is about forty times more accurate than the quadratic interpolation with 1.5 times oversampling while only taking 80% more runtime.

We next show the average alignment error for three different interpolation schemes with the downhill simplex method [27] as the optimization algorithm (Table 2). For each interpolation scheme, we used both strategies discussed in Section 3.1 for the refinement of the parameters. Each result shown is an average over 1,000 trials. It can be seen that when using simplex algorithm, gridding-based interpolation is significantly more accurate than quadratic interpolation with image 1.5x oversampled and only takes approximately twice the runtime. In particular, the concurrent strategy yields almost perfect alignment results although it take more time than cascaded strategy.

Compared with the LBFGSB algorithm, the simplex algorithm requires ~20–50% less runtime, depending on the interpolation method. This is due to the fact that LBFGSB algorithm is designed for large-scale optimization program and is excessively complicated for our application, which requires determination of only three parameters. The fact that in our problem it uses approximate derivatives also makes it less accurate.

From the comparison above, we conclude that combination of the downhill simplex method and the concurrent strategy constitutes the best optimization algorithm and strategy for 2-D alignment problem. Gridding-based interpolation is the best interpolation method if alignment accuracy is our primary concern.

Next, we consider 2-D alignment of a Gaussian noise-corrupted image with a perfect template, which is the same as the reference image used in the noise-free case. The simulation is conducted in the same manner as the 2-D alignment of noise-free images. For the sake of brevity we only show the results using simplex algorithm and cascaded strategy (Fig. 7), as our study revealed that this was the best combination in the presence of noise. Here, SNR is defined as the ratio of variance of signal and variance of noise. It can be found that in the presence of noise, gridding-based interpolation achieved better results than quadratic interpolation with images oversampled 1.5 times, although this advantage gradually narrowed for low SNR values. We also found that the average runtime were the same as in noise-free case.

4.2. 2-D to 3-D registration

The test object was a 3-D X-ray crystallographic structure of 70S ribosome placed in a $128 \times 128 \times 128$ voxels volume, as described above. In order to have results comparable to those of 2-D alignment, we applied additional high-pass filtration of the test structure. For each simulation, we generated its projection with orientation parameters $(\varphi + \Delta\varphi, \theta + \Delta\theta, \psi + \Delta\psi, s_x + \Delta s_x, s_y + \Delta s_y)$, where $(\Delta\varphi, \Delta\theta, \Delta\psi, \Delta s_x, \Delta s_y)$ were assumed to be 'small'. Since we studied only fine searches, we assumed that the approximate orientation parameters $(\varphi, \theta, \psi, s_x, s_y)$ had already been determined by the coarse search process of 2-D to 3-D registration and we used them as the starting point of the fine search. We also let $(\Delta\varphi, \Delta\theta, \Delta\psi)$ be independently and evenly distributed in $[-2^\circ, 2^\circ]$ and $(\Delta s_x, \Delta s_y)$ be independently and evenly distributed in $[-4, 4]$.

We first studied the accuracy and efficiency of 2-D to 3-D registration algorithms for noise-free data. We compared the 2-D to 3-D registration results for different combinations of interpolation schemes, optimization algorithms and optimization strategies (Table 3). Since 2-D to 2-D registration takes much longer time to run than 2-D alignment, each result shown here is an average over only 200 simulations. To avoid bias by a particular choice of the Eulerian angles, we used different sets of projection orientations (φ, θ, ψ) for each simulation. The 200 sets of projection orientations were quasi-uniformly distributed using Saff's method [33].

Comparing the accuracy of gridding-based interpolation (scenario 1) with the accuracy of linear interpolation with image 1.5x oversampled (scenario 2), both using simplex optimization, concurrent strategy, and real space evaluation of goal function (9), we found that reverse gridding method was about 80 times more accurate than linear interpolation. In addition, the average runtime of the gridding method was five times shorter than the method with linear interpolation. This result is in agreement with our assessment of computational complexity of two respective methods given in Section 3.2 and it virtually disqualifies methods that generate projections in real space as competitive for the fine search in 2-D to 3-D registration approach considered here. Moreover, the real-space-based method is expected to be progressively slower relative to Fourier-space-based method with increased volume size, although the former may have some merit for small volume data, particularly given the ease of implementation and use.

We then studied the utility of the LBFGSB optimization method (scenario 3 of Table 3) to 2-D to 3-D registration by comparing its performance with that of the simplex method (scenario 1), using the gridding method and real space evaluation of goal function (9) in both cases. Here, the simplex method outperformed the LBFGSB algorithm in terms of accuracy and also runtime, similarly as was the case in 2-D alignment.

Next, we compared four different refinement strategies of 2-D to 3-D registration that all used gridding-based interpolation and the simplex optimization algorithm, but differed in the search strategy (concurrent *versus* cascaded) and evaluate the goal function (9) either in real or in Fourier space (scenarios 1, 4, 5 and 6 in Table 3). Here, unlike in the 2-D case, the overall best performance was achieved for the concurrent strategy, which resulted in 1–2 orders of magnitude better accuracy than the cascaded strategy. This is due to stronger correlations between angular and translational parameters than is the case in the 2-D problem. The higher accuracy was achieved at a significantly higher computational time. Although the difference may appear insignificant (13.0s for the concurrent strategy *versus* 7.9s for the cascaded strategy), given the massive amount of data processed in EM ($\sim 10^5$ – 10^6 EM projection images), it translates into savings of two days of calculations for one week of runtime. Small gain in computational time was achieved by evaluating the goal function in Fourier space, which is understandable as most time was spent on generating reference projections. Again, argument can be made that 5% acceleration reduces by an hour for 24 hours of the EM structure refinement time.

In order to test 2-D to 3-D registration in the presence of noise, we corrupted the subject image by additive Gaussian noise while we retained the test structure (thus its reference projections) noise-free. We computed average maximum pixel error (10) by repeating the registration 200 times using different realizations of noise and we plotted the results as a function of SNR of the subject image (Fig. 8). The results shown correspond to the six scenarios of the noise-free case given in Table 3. Although gridding-based interpolation is more accurate than linear interpolation with the data 1.5x oversampled, this advantage gradually disappears for low SNR value (<1.0), more typical for EM data; this is also consistent with our observations in the 2-D alignment case. When SNR is in the range of 0.01 and 1.0, the differences between interpolation methods tested remain indistinguishable and the average maximum pixel errors continue to increase linearly in the double logarithmic scale of the plot (results not shown). In addition, for SNR values lower than 5.0, the cascaded strategy yielded almost the same accuracy as concurrent strategy and the accuracy was the same irrespective whether the goal function was evaluated in real or in Fourier space. Finally, we also determined that the average runtime remained the same as in noise-free case, irrespective of the SNR of the subject image (results not shown). Taken together, for SNR values commonly encountered in EM (~ 1.0), the test results indicate that gridding-based interpolation with cascaded strategy, simplex optimization method, and evaluation of the goal function in Fourier space should be the method of choice, as it provides the best tradeoff between accuracy and time of calculations.

V. CONCLUSIONS

We introduced an interpolation scheme based on the nonuniform fast Fourier transform and presented its application to EM data processing in 2-D alignment and in 2-D to 3-D registration. Our simulation results show that in the noise-free case the average maximum pixel error was $\sim 10^{-4}$ pixels in both 2-D and 3-D cases, i.e., the alignment is nearly perfect and far more accurate than that achieved by any alignment method that relies on discretization of searches [34]. The much more accurate results reported here are due to introduction of semi-continuous fine search alignment strategies and due to excellent interpolation qualities of gridding-based interpolation. The speed of the gridding-based 2-D alignment method is also excellent; its runtime is only about two times longer than of that based on quadratic interpolation with images oversampled 1.5 times. We also demonstrated that the simplex algorithm performs very well in our application and outperforms gradient-based optimization algorithms used by others [13,20]. This result may however simply reflect the fact that our optimization problem was relatively simple, as it sought to optimize at most five orientation parameters, while in the work of others orientation parameters of large number of subject images were being determined simultaneously.

In the presence of noise, gridding-based alignment yields better results than that based on quadratic interpolation with reference image oversampled 1.5x, but this advantage gradually reduces as SNR value decreases. Most importantly, in case of 2-D to 3-D registration, gridding method required only 20% of runtime of the real-space method, which is mainly thanks to the way reference projections were generated. While in real space calculation of a projection requires $\sim n^3$ operations, reverse gridding requires only $\sim n^2$. Additional speed up of the gridding-based method is achieved if the number of FFT operations is reduced and the goal function is evaluated in Fourier space. We conclude that the very high computational cost of real-space-based method limits its applicability to fine searches in 2-D to 3-D registration to small volume data.

In application to alignment of EM data, i.e., images that have SNR no larger than one, we recommend combination of gridding-based interpolation with the simplex algorithm and the cascaded strategy as a method that yields optimum combination of speed and accuracy. In this strategy, the search for the translations is separated from the search for the rotation angle. In 2-D case, the translations are determined on a higher level while assuming that the rotation angle is known to sufficient accuracy while it is being determined on a lower level. In 3-D case, the strategy is reversed: the Eulerian angles are determined on a higher level while the translations are found with very high accuracy on the lower level using 2-D cross-correlation and reverse gridding techniques. Although such a separation of parameter determination can cause the optimization algorithm to be trapped in a local minimum, as illustrated by inferior performance in noise-free cases, the very high noise level in EM data makes this potential problem tolerable.

Image alignment methods used in EM are not only computationally intensive, but also require frequent alternation between operations performed in real space and in Fourier space. For example, monitoring of alignment quality by FRC and correction for the transfer function effects of electron microscope are performed in Fourier space. Similarly, in order to reduce the time of calculations, cross-correlations are often computed using the FFT technique. As the gridding method requires calculation of the Fourier transform of the image, it opens up a possibility to fuse logically connected steps and omit redundant FFT operations. We took advantage of this option and merged extraction of a central section of a 3-D Fourier transform with reverse gridding-based search for translation parameters with sub-pixel accuracy. We anticipate that more significant savings of runtime will be achieved when NUFFT techniques are applied to alignment of large sets of EM images instead of pairs of images, as used in this work.

It is difficult to put results described here in a broader perspective, as there are relatively few published test results of alignment methods used in EM single particle software packages. For example, Fourier harmonic-based alignment [35] is not integrated into any EM software package and splines-based [13] alignment is only used in Xmipp package [36]. Most authors focus on accuracy of the structure refinement protocol [13,15,34]; however, such protocols contain various elements that may influence the result, the overall refinement strategy is certainly a major factor. Therefore, here we focused on simple 2-D and 3-D image registration tasks and on commonly used in EM linear and quadratic interpolations and compared them with gridding-based interpolation. Future work on optimum alignment methods should be facilitated by the release of the SPARX system [31]. By promoting seamless access to multiple software packages it encourages standardization of test data within the community and simplifies testing. By implementing our gridding-based alignment method in the SPARX system and providing access to the source code as well as test scripts we hope to initiate this process.

The accuracy and efficiency of the gridding-based alignment algorithms introduced here provide a good foundation for the development of higher level steps of single particle analysis, such as 2-D alignment of multiple images and 3-D structure refinement. Our future work includes incorporation of the principles of our algorithms into these steps and development of more reliable and efficient single particle analysis methods.

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Figure 1.

Schematic of resampling of an image to polar coordinates using reverse gridding method. A preparation step is required to transform the image from real space to Fourier space and then resample it to an FFT-friendly auxiliary grid. In the first step of reverse gridding method, the image is divided by the weighting function. The second step is a conventional inverse FFT. In the third step, we use convolution in the real space to resample the image from the auxiliary grid to the output grid. The two insets show the shape of the weighting function (3) and the window function (2) for the parameter values used and for image size 512 pixels.

Figure 2.

Fidelity of 2-D image interpolation methods evaluated in Fourier space. A 128×128 test image is rotated 33° clockwise and then 33° counterclockwise using three different interpolation schemes. The resulting image is compared with the original image using the FRC technique. Solid curve: quadratic interpolation; dashed curve: quadratic interpolation with image 1.5 times oversampled; dotted curve: reverse gridding method.

Figure 3. Schematic of coarse search in 2-D alignment. The possible translations (s_x, s_y) form an equidistant grid and are within several pixels of the image center.

Figure 4. Accuracy of the localization of the maximum of a discrete 1-D rotational cross-correlation function. A 128×128 test image is rotated by a specified angle and a 1-D rotational cross-correlation function is computed using original image as a reference. The alignment error is the deviation of the angle found from the correct angle. Localization obtained by: fitting a 7-point parabola to discrete values of the cross-correlation function (solid line) and by reverse gridding method (dashed line).

Figure 5.

Schematic of 2-D to 3-D registration. $(\varphi_i, \theta_i, \psi_i)$ are the Eulerian angles of projection i . (α_i, x_i, y_i) are the 2-D alignment results between projection i and the subject image, p_i is the peak value of the cross-correlation function. $(\varphi_j, \theta_j, \psi_j, x_j, y_j)$ are the results of the coarse search of 2-D to 3-D registration.

Figure 6.

Simulation of 2-D alignment process. Subject image is a copy of reference image and is randomly rotated and shifted before 2-D alignment with reference image. The alignment results are compared with known values in order to evaluate the alignment error (7).

Figure 7.

Average 2-D alignment maximum pixel error (7) for three interpolation schemes as a function of the SNR value of the subject image. The optimization algorithm is the downhill simplex method and the optimization strategy is cascaded. Interpolation method: quadratic (solid line), quadratic with data 1.5x oversampled (dashed line), reverse gridding (dotted line). Each result shown is an average of 1,000 trials. Vertical bars represent the standard deviation.

Figure 8.

Average 2-D to 3-D registration maximum pixel error (10) as a function of the SNR value of the subject image for two interpolation schemes. Scenario 1 (See Table 3), solid line; Scenario 2, dashed line; Scenario 3, dotted line; Scenario 4, densely dotted line; Scenario 5, densely punctuated line; Scenario 6, loosely punctuated line. Each result shown is an average over 200 trials. Vertical bars represent the standard deviation.

Average 2-D alignment error $e_{\max}$ (7) and average runtime for three interpolation schemes with LBFGSB algorithm, simulated noise-free data used. Each result is an average over 1,000 trials.

Interpolation method	$\Delta\theta$ (°)	Δx	Δy	$e_{\max}$	Runtime (s)
Quadratic interpolation, no oversampling	0.0110	0.0771	0.0787	0.1342 ± 0.0136	0.4 ± 0.1
Quadratic interpolation, oversample 1.5 times	0.0028	0.0305	0.0311	0.0517 ± 0.0046	0.8 ± 0.2
Reverse gridding	0.0005	0.0005	0.0005	0.0012 ± 0.0003	1.4 ± 0.3

Average 2-D alignment error $e_{\max}$ (7) and average runtime for three interpolation schemes and two optimization strategies with downhill simplex algorithm, simulated noise-free data used. Each result is an average over 1,000 trials.

Interpolation method	Search for alignment parameters	Table 2			$\Delta\theta$ (°)	Δx	Δy	$e_{\max}$	Runtime (s)
Quadratic interpolation, no oversampling	concurrent				0.0148	0.0786	0.0775	0.1379 ± 0.0190	0.19 ± 0.03
	cascaded				0.0149	0.0902	0.0880	0.1570 ± 0.0649	0.15 ± 0.01
Quadratic interpolation, 1.5x oversampled	concurrent				0.0026	0.0313	0.0308	0.0514 ± 0.0039	0.52 ± 0.05
	cascaded				0.0036	0.0364	0.0383	0.0633 ± 0.0281	0.40 ± 0.02
Reverse gridding	concurrent				0.0001	4.7e-5	4.7e-5	0.0002 ± 0.0001	1.19 ± 0.07
	cascaded				0.0015	0.0012	0.0012	0.0034 ± 0.0017	0.73 ± 0.03

Table 3 Average 2-D to 3-D registration error $e_{\max}$ (10) and average runtime for different combinations of interpolation method, optimization algorithm and refinement strategy, noise-free data used. Each result is an average over 200 trials.

Scenario	Interpolation method	Optimization algorithm	Search for alignment parameters	Evaluation of goal function (9) in	$e_{\max}$	Run-time (s)
1	Reverse gridding	simplex	concurrent	real space	0.0004 ± 0.0031	13.0
2	Linear, 1.5x oversampled	simplex	concurrent	real space	0.0317 ± 0.0171	66.7
3	Reverse gridding	LBFGSB	concurrent	real space	0.0018 ± 0.0022	21.2
4	Reverse gridding	simplex	concurrent	Fourier space	0.0024 ± 0.0036	12.6
5	Reverse gridding	simplex	cascaded	real space	0.0133 ± 0.0139	7.9
6	Reverse gridding	simplex	cascaded	Fourier space	0.0151 ± 0.0133	7.1