Toward single particle reconstruction without particle picking: Breaking the detection limit

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Abstract

Here comes the abstract

1 Introduction

[Revise–Cryo–electron microscopy (cryo–EM) is an innovative technology for single particle reconstruction (SPR) of macromolecules.] In a cryo–EM experiment, biological samples are rapidly frozen in a thin layer of vitreous ice. Within the ice, the molecules are randomly oriented and positioned. The microscope produces a 2-D image of the samples embedded in the ice called a *micrograph*. Each micrograph contains tomographic projections of the samples at unknown locations and under unknown viewing directions. The goal is to construct 3-D models of the molecules from the micrographs.

The signal to noise ratio (SNR) of the projections in the micrographs is a function of two dominating factors. On the one hand, the SNR is a function of the electron dose. To keep radiation damage within acceptable bounds, the dose must be kept low, which leads to high noise levels. On the other hand, the SNR is a function of the molecule size. The smaller the molecules, the fewer detected electrons carry information about them.

All contemporary methods in the field split the reconstruction procedure in several stages. The first stage consists in extracting the various particle projections from the micrographs. This is called *particle picking*. Later stages aim to construct a 3-D model of the molecule from these projections. The quality of the reconstruction eventually hinges on the quality of the particle picking stage. As can be seen from Figure 1, locating the particles becomes increasingly challenging as the SNR degrades.

Crucially, it can be shown that reliable detection of individual particles is impossible below a certain critical SNR. This fact has been recognized early on by the cryo-EM community. In particular, in a highly cited paper from 1995, Henderson [28] investigates the following questions:

> *For the purposes of this review, I would like to ask the question: what is the smallest size of free-standing molecule whose structure can in principle be determined by phase-contrast electron microscopy? Given what has already been demonstrated in published work, this reduces to the question: what is the smallest size of molecule for which it is possible to determine from images of unstained molecules the five*

parameters needed to define accurately its orientation (three parameters) and position (two parameters) so that averaging can be performed?

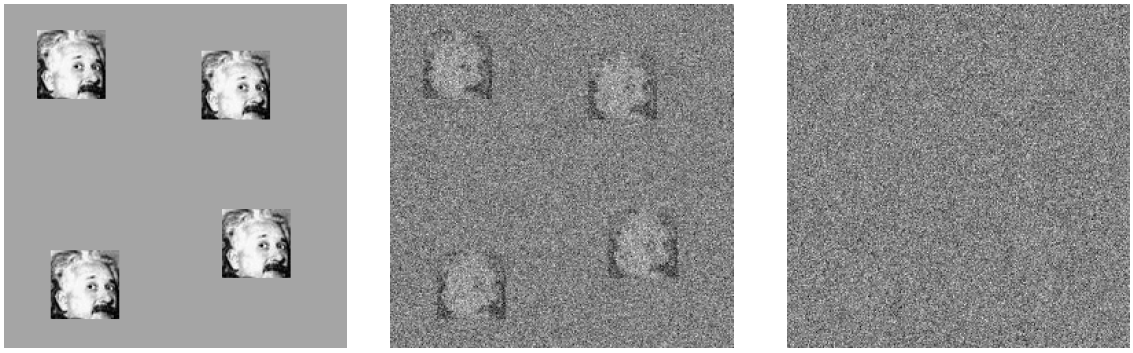
In that paper and in others that followed (e.g., [22]), it was established that particle picking is impossible for molecules below a certain weight. As a result, it is impossible to reconstruct such small molecules by any of the existing computational pipelines for single particle analysis, as the particles themselves cannot be picked from the micrographs. This has motivated recent technical advances in the field, including the use of Volta phase plates [31, 35] and scaffolding cages [37]. Despite this progress, detecting small molecules (below 50 kDa) in the micrographs remains a challenge. We note that nuclear magnetic resonance (NMR) spectroscopy and X-ray crystallography are well suited to reconstruct small molecules. Yet, cryo–EM has a lot to offer even for molecules with already known structures obtained via NMR spectroscopy or X-ray crystallography, because these methods have limited ability to distinguish conformational variability. [Need a ref for this claim.]

In this paper, we argue that there is a gap between the two questions in the quoted excerpt above, and that one may be able to exploit it to design better reconstruction algorithms. Specifically, the impossibility of particle picking does not necessarily imply impossibility of particle reconstruction. Indeed, the aim is only to reconstruct the molecule: estimating the locations of the particles in the micrograph is merely a helpful intermediate stage when it can be done. Our main message is that the limits particle picking imposes on molecule size do not translate into limits on particle reconstruction.

As a proof of concept, we study a simplified model where an unknown image appears numerous times at unknown locations in several micrographs, each affected by additive Gaussian noise—see Figure 1 for an illustration. The goal is to estimate the planted image. The task is interesting in particular when the SNR is low enough that particle picking cannot be done reliably. A precise mathematical formulation of the model, including an extension where more than one planted images are to be recovered, is provided in Section 4. To be clear, we do not consider here many prominent features of real SPR experiments and do not aim to reconstruct any 3-D structure. Instead, we solve a simpler problem that we believe captures key elements of the SPR problem. We note that similar models emerge in spike sorting [34], passive radar [23] and system identification [38].

In order to recover the planted image, we use autocorrelation analysis. In a nutshell, we relate the autocorrelation functions of the micrographs to the autocorrelation functions of the planted image. For any noise level, these autocorrelations can be estimated to any desired accuracy, provided individual occurrences of the image are well separated and the image appears sufficiently many times in the micrographs. Importantly, there is no need to detect individual occurrences. The autocorrelations of the micrographs are straightforward to compute and require only one pass over the data. These directly yield estimates for the autocorrelations of the image. To estimate the image itself from its estimated autocorrelations, we solve a nonlinear inverse problem via least-squares or a phase retrieval algorithm; see Figure 2 for an illustration and Section 4 for details. As a side note, we mention that expectation-maximization (EM)—a popular framework in SPR—is intractable for this problem; see Appendix C for a discussion.

Another interesting feature of the described approach pertains to model bias, whose importance in cryo-EM was stressed by a number of authors [47, 44, 29, 46]. In the classical "Einstein from noise" experiment, multiple realizations of pure noise are aligned to a picture of Einstein using cross-correlation and then averaged. In [44], it was shown that the averaged



(a) $\sigma = 0$ (b) $\sigma = 0.5$ (c) $\sigma = 3$

Figure 1: Example of micrographs of size 250×250 with additive white Gaussian noise of variance σ^2 for increasing values of σ. Each micrograph contain the same four occurrences of a 50×50 image of Einstein. In panel (c), the noise level is such that it is very challenging to locate the occurrences of the planted image. In fact, it can be shown that at low SNR, reliable detection of individual image occurrences is impossible, even if the true image is known. By analogy to cryo-EM, this depicts a scenario where particle picking cannot be done.

noise rapidly becomes remarkably similar to the Einstein template. In the context of cryo–EM, this experiment exemplifies how prior assumptions about the particles may influence the reconstructed structure. This model bias is common to all particle picking methods based on template matching. In our approach, no templates are required, thus significantly reducing concerns about model bias.

2 Results

We conducted two experiments in the simplified image formation model described in the introduction:

1. The first experiment aims to recover a 2-D image from an increasing number of micrographs with high noise, similar to the rightmost panel of Figure 1. This is done using moments of second order, as these are sufficient to recover a 2-D image up to elementary symmetries;

2. The second experiment aims to recover three distinct 1-D signals from an increasing number of 1-D micrographs with high noise. For this task, it is necessary to use moments up to third order.

As outlined below, we find that it is indeed possible to recover good estimates of the ground truth signals from the highly corrupted micrographs, without particle picking. Furthermore, we find that the quality of estimation increases with the amount of data collected, despite the fact that particle picking remains challenging. The methods section provides additional details. In the discussion section, we outline how the general approach could be extended to full 3D SPR.

In the first experiment, we estimated Einstein's image of size 50×50 and mean zero from a growing number of micrographs, each of size 4096×4096 pixels. A micrograph contains,

on average, 700 occurrences of the target image at random locations. The latter are chosen so that two occurrences are always separated by at least 49 pixels. Thus, about 10% of each micrograph contains signal. The micrographs are contaminated with additive white Gaussian noise with standard deviation $\sigma = 3$ (this corresponds to $\mathsf{SNR} = 1/20$). This high noise level is illustrated in the right panel of Figure 1. In this first experiment, we assume knowledge of σ and of the total number of signal occurrences across all micrographs.

We compute the average autocorrelation of the micrographs (equivalently, the average of their power spectra). This is a particularly simple computation. In the methods section, we show how, owing to separation of the occurrences, a determined portion of the averaged autocorrelation allows to estimate the power spectrum of the unknown image itself. Mathematically, it is easy to show that the quality of this estimate improves steadily as the amount of data grows, regardless of noise level. Then, to estimate the target image, we resort to a standard phase retrieval algorithm called relaxed-reflect-reflect (RRR) [7, 19].[1] RRR is initialized with a picture of the physicist Isaac Newton and iterates to produce the estimate.[2]

Figure 2 shows several estimated images for a growing number of micrographs. (A movie is available in [supplementary material].) Figure 3 presents the normalized recovery error as a function of the amount of data available. Error is measured as the ratio of the root mean square error (RMSE) to the norm of the ground truth (square root of the sum of squared pixel intensities.) This is computed after fixing elementary symmetries (see methods.) As evidenced by these figures, the ground truth image can be estimated increasingly well from increasingly many micrographs, without particle picking.

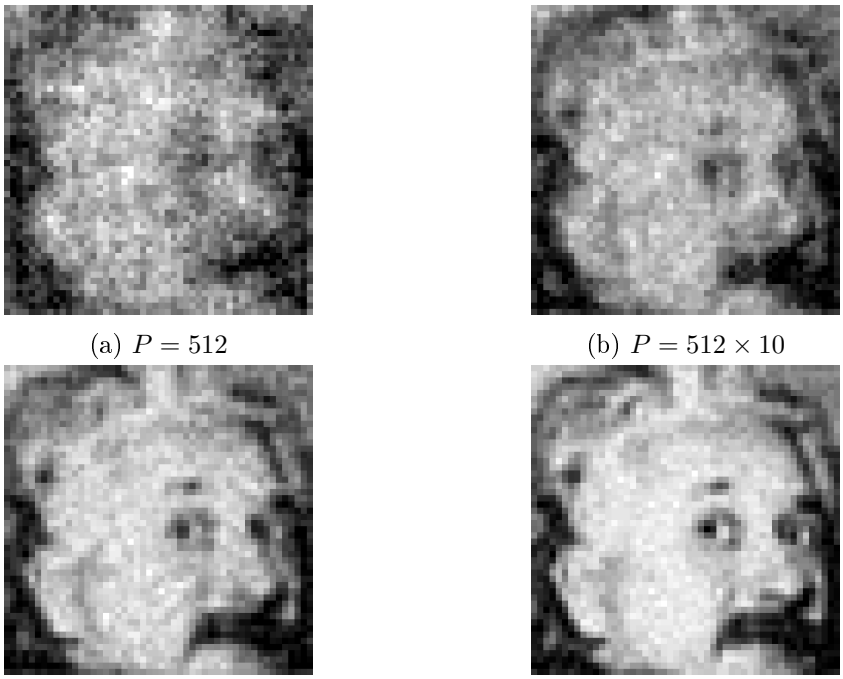
In the second experiment, three 1-D signals, each of length $L = 21$, appear at random locations in one long 1-D signal, which we call a micrograph by analogy. Any two occurrences are separated by at least 20 entries. The signals appear respectively about 30, 20 and 10 million times in a micrograph of length 12.3 billion. The micrograph is then contaminated with additive white Gaussian noise. This results in an SNR of about 1/9, while about 10% of the micrograph contains signal. Neither the number of occurrences nor the noise level σ are known.

In the methods section, we detail how autocorrelations of the micrograph can be used to estimate weighted averages of the autocorrelations of the target signals. The individual signals and their relative densities are then estimated from autocorrelations up to order three by solving a nonlinear least-squares problem.

Figure 4 shows how the estimates improve as we see a larger and larger fraction of the micrograph (that is, as more and more data becomes available.) As is clear from the picture, despite the high noise level which would make it very challenging to locate the signal occurrences, the individual signals can be estimated accurately given enough data. Furthermore, the propensity of each signal can also be estimated.

[1] [Quid of symmetries?]

[2] [Initializing with a face is debatable: it might help the algorithm. On the other hand, I agree that it supports the notion that we don't get trapped too easily.]



(a) $P = 512$ (b) $P = 512 \times 10$

(c) $P = 512 \times 10^2$ (d) $P = 512 \times 10^3$

Figure 2: Recovery of Einstein from micrographs at noise level $\sigma = 3$ (see Figure 1(c)). Averaged autocorrelations of the micrographs allow to estimate the power spectrum of the target image. This does not require particle picking. A phase retrieval algorithm (RRR) produces the estimates here shown, initialized with an image of the physicist Isaac Newton. Estimates are obtained from P micrographs (growing across panels), each containing 700 image occurrences on average.—[Will add image with $P = 512 \times 10^4$ and image of Newton.]

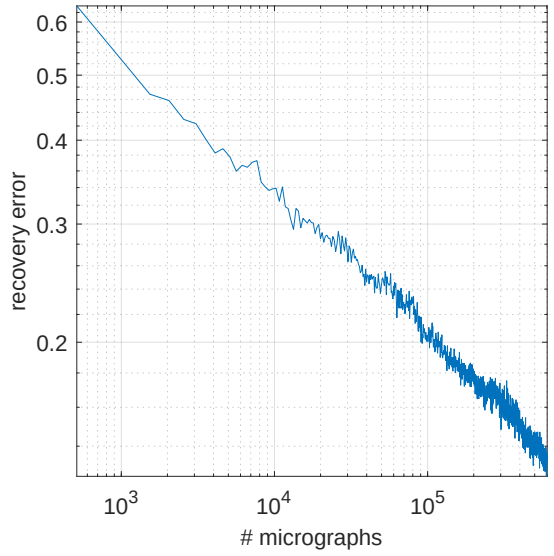


Figure 3: Relative root mean square error of the estimate of Einstein's image as a function of the number of observed micrographs (logarithmic scale along both axes.)

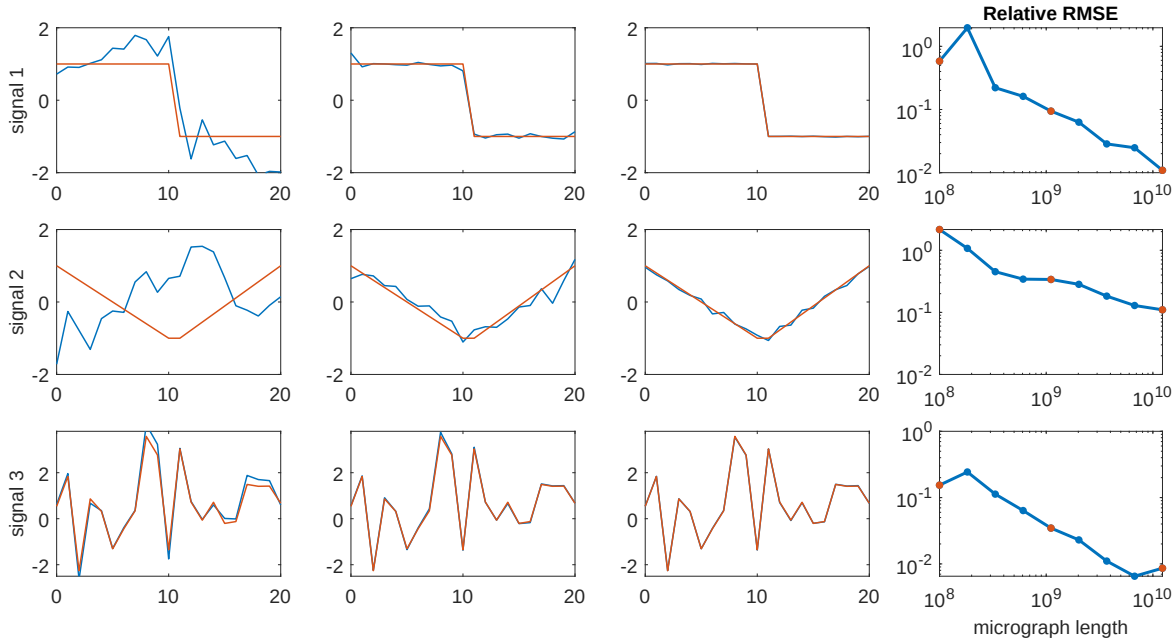


Figure 4: For the second experiment, each row shows, three times, one of the target signals (red), overlaid with an estimate (blue) obtained from a growing portion of the noisy micrograph (about 10^8, 10^9 and 10^{10} entries available to compute autocorrelations.) The last column depicts evolution of the relative root mean square error in estimating each individual target signal. Signals 1 to 3 appear respectively about 30, 20 and 10 million times. With the whole micrograph available, the algorithm estimated those to be 29.8, 21.9 and 10.0 million, respectively.

3 Discussion

[Where and how do we cite Kam? Fred?]

In the simplified model we examined, the aim is to estimate one, or possibly several, images from micrographs. Our strategy is to compute autocorrelation functions of the data and to relate these statistics to the unknown parameters. Recovering the parameters from the statistics reduces to solving a set of polynomial equations. Depending on the scenario, we did so using either a phase retrieval algorithm or a nonlinear least-squares algorithm.

The same general approach can, in principle, be applied directly to SPR from cryo-EM. Here, the micrographs contain numerous tomographic projections of molecules (possibly in different conformations) under unknown viewing directions. The aim is to estimate the 3-D volumes of the different conformations directly from micrographs. Each volume can be expanded linearly in a basis, so that the volume is characterized by its expansion coefficients. Since tomographic projection is a linear operation, autocorrelations of the micrographs (which can be estimated easily) are polynomial functions of the sought coefficients. Thus, autocorrelations of the micrographs provide a system of polynomial equations in the volume parameters, and the question becomes: are these equations sufficient to uniquely identify the volumes, and can we solve the system?

We show in Appendix F that the number of polynomial equations provided by the third-order autocorrelations is of the same order as the number of coefficients required to describe one volume at the same resolution as the particle projections. This hints that it may be possible to reconstruct a constant number of distinct volumes from these equations directly. Crucially, the outlined approach involves no particle picking, and a fortiori no viewing direction estimation. As a result, it may not be limited to large molecules in the same way that particle picking approaches are. Concerns for model bias would also be greatly reduced.

[Do we want to say something about the number of images that we need? Specifically, do we want to address the fact that the numerical experiments suggest we may need a gigantic number of them for cryo, and mention trends in cryo technology that are encouraging in that regard? (Of course, we can't compare to RELION etc. in SNRs so low that one can't particle pick; that's not the point here.)]

As stated, our approach relies on two core assumptions that are not necessarily verified in SPR experiments. First, we assume an additive white noise model, while in practice the noise may be structured or signal dependent. To address this point, it may be necessary to investigate better noise models and to extend the autocorrelation analysis to these models. Second, we assume that any two signal occurrences are sufficiently separated, as required by (4.2). This separation could be induced by careful experimental design [Ref?]. If the signals are not well separated, one can introduce new variables that represent the distribution of the spacing between signal occurrences. Here as well, the relation between the autocorrelation functions of the data and the signals can be derived. These variations lead to different inverse problems. No sufficient conditions for them to be solvable are currently known.

[We recognize that significant challenges lay ahead for the implementation of the proposed approach to 3-D reconstruction directly from the micrographs. Insofar as the computational challenges are concerned, we note that expectation-maximization algorithms (EM) have, over time, become the norm in the cryo-EM community, as exemplified by the popular RELION package. The idea of using EM for cryo-EM can be traced back at least to [Sigworth]. At the time, such approaches also seemed challenging.]

4 Methods

Mathematical model. Let $x_1, \ldots, x_K \in \mathbb{R}^L$ be the sought signals and let $y \in \mathbb{R}^N$ be the data. The forward model can be posed as a mixture of *blind deconvolution* problems between binary signals and the target signals x_i:

$$y = \sum_{i=1}^{K} x_i * s_i + \varepsilon, \quad \varepsilon \sim \mathcal{N}(0, \sigma^2 I). \tag{4.1}$$

The nonzero values of each $s_i \in \{0,1\}^N$ determine the position of x_i's occurrences. We denote the set of these nonzero values by $\mathcal{S}_i$ and its cardinality by $|\mathcal{S}_i| = M_i$. By assuming that all $\mathcal{S}_i$'s are disjoint, we let $s = \sum_{i=1}^K s_i$, $\mathcal{S} = \bigcup_{i=1}^K \mathcal{S}_i$ and $|\mathcal{S}| := M = \sum_{i=1}^K M_i$. Literature survey on blind deconvolution and related problems is given in Appendix B.

In order to estimate the mixture of autocorrelations, we assume that the support of s is not clustered. In particular, we assume that

$$|i - j| \geq 2L - 1, \quad \text{for all } i, j \in \mathcal{S} \text{ such that } i \neq j. \tag{4.2}$$

The goal of the problem is to estimate $x_1, \ldots, x_K$ from y.

Aperiodic autocorrelation functions For $z \in \mathbb{R}^L$ and $k \geq 2$, the autocorrelation of order k is defined for any integer shifts $\ell_1, \ldots, \ell_{k-1}$ by

$$a_z^k[\ell_1, \ldots, \ell_{k-1}] = \sum_{i=-\infty}^{+\infty} z[i] z[i+\ell_1] \ldots z[i+\ell_{k-1}], \tag{4.3}$$

where indexing of z out of the bounds $0, \ldots, L-1$ is zero-padded, as usual. Explicitly, the mean (which can be thought of as a first-order autocorrelation) and the second and third autocorrelations are given by

$$\begin{aligned} a_z^1 &= \sum_{i=0}^{L-1} z[i], \\ a_z^2[\ell] &= \sum_{i=\max\{0,-\ell\}}^{L-1+\min\{0,-\ell\}} z[i] z[i+\ell], \\ a_z^3[\ell_1, \ell_2] &= \sum_{i=\max\{0,-\ell_1,-\ell_2\}}^{L-1+\min\{0,-\ell_1,-\ell_2\}} z[i] z[i+\ell_1] z[i+\ell_2]. \end{aligned} \tag{4.4}$$

Note that the autocorrelation functions are symmetric so that $a_z^2[\ell] = a_z^2[-\ell]$ and also

$$a_z^3[\ell_1, \ell_2] = a_z^3[\ell_2, \ell_1] = a_z^3[-\ell_1, \ell_2 - \ell_1].$$

Estimating the autocorrelation function of a single signal. We first consider the problem of estimating the autocorrelations of a single signal from the data. The main principles carry through for $K > 1$ as will be shown next.

For the purpose of the analysis, we consider the asymptotic regime where $M, N \to \infty$, while preserving fixed ratio. Specifically, we define the ratio of the measurement occupied by the signal as

$$\gamma = \frac{ML}{N}. \tag{4.5}$$

Under the spacing constraint (4.2), we have $\gamma \leq \frac{L}{2L-1} \approx 1/2$.

The main pillar of this work is the following simple observation. If the support signal s satisfies the spacing constraint (4.2), then the first L entries of the data autocorrelations converge to a scaled, biased, version of the signal's autocorrelation:

$$\begin{aligned}
\lim_{N\to\infty} a_y^1 &= \gamma a_x^1, \\
\lim_{N\to\infty} a_y^2[\ell] &= \gamma a_x^2[\ell] + \sigma^2\delta[\ell], \\
\lim_{N\to\infty} a_y^3[\ell_1,\ell_2] &= \gamma a_x^3[\ell_1,\ell_2] + \sigma^2\gamma a_x^1\big(\delta[\ell_1,0] + \delta[0,\ell_2] + \delta[\ell_1,\ell_2]\big),
\end{aligned} \tag{4.6}$$

for $\ell, \ell_1, \ell_2 = 0, \dots L-1$, and where δ denotes the Kronecker delta function.

Estimating the autocorrelation function of multiple signals. As before, we consider the asymptomatic regime where $M_1, \dots, M_K, N \to \infty$, while preserving fixed ratios

$$\gamma_k = \frac{M_k L}{N}, \quad \gamma = \sum_{k=1}^{K} \gamma_k. \tag{4.7}$$

If the support s satisfies the spacing constraint (4.2), then similarly to (4.6) one can estimate the mixture of the K signals' autocorrelations:

$$\begin{aligned}
\lim_{N\to\infty} a_y^1 &= \sum_{k=1}^{K} \gamma_k a_{x_k}^1, \\
\lim_{N\to\infty} a_y^2[\ell] &= \sum_{k=1}^{K} \gamma_k a_{x_k}^2[\ell] + \sigma^2\delta[\ell], \\
\lim_{N\to\infty} a_y^3[\ell_1,\ell_2] &= \sum_{k=1}^{K} \gamma_k a_{x_k}^3[\ell_1,\ell_2] + \sigma^2 \left(\sum_{k=1}^{K} \gamma_k a_{x_k}^1\right)(\delta[\ell_1,0] + \delta[0,\ell_2] + \delta[\ell_1,\ell_2]),
\end{aligned} \tag{4.8}$$

This relation is proven in Appendix A.

Numerical experiments details. For the 2-D experiment shown in Figures 2 and 3, we generated P micrographs of size 4096×4096 pixels. In each micrograph, we placed Einstein's image of size 50×50 in random locations, while keeping the separation condition (4.2). This is done by randomly selecting 4000 placements in the micrograph, one at a time with an accept/reject rule based on the separation constraint and locations picked so far. In average, 700 images were placed in each micrograph. Then, an i.i.d. Gaussian noise with standard deviation $\sigma = 3$ was added inducing SNR of approximately $1/20$. An example of a micrograph's excerpt is presented in the right panel of Figure 1.

In this experiment, we assume to know the number of Einstein's occurrences and the noise level. In this situation, the second-order autocorrelation (or, equivalently, the Fourier magnitudes of the signal) determines almost all images uniquely up to reflection through the origin [25, 10]. Therefore, we estimate the signal's Fourier magnitude from the Fourier magnitude of the micrographs, in the cost of one FFT per micrograph. To estimate the signal, we use a phase retrieval algorithm called relaxed-reflect-reflect (RRR) that iterates by

$$z \to z + \beta(P_2(2P_1(z) - z) - P_1(z)), \tag{4.9}$$

where we set $\beta = 1$. RRR estimates a $2L \times 2L$ image. Exact solution contains Einstein's image in the upper-left corner and zero else where. The operator $P_2(z)$ combines the Fourier phases of the current estimation z with the Fourier magnitudes of the signal (estimated from the data). The operator $P_1(z)$ zeros out all entries of z outside the $L \times L$ upper-left corner. In order to compare the performance in multiple cases and different noise levels, the algorithm stopped after a fixed number of 1000 iterations and the iteration with the smallest error compared to the ground truth (also taking into account the reflection ambiguity) was chosen as the solution. While this cannot be done in practice since we do not have access to the ground truth, this procedure enables us to compare a large number of instances in different noisy environments [Note the last two sentences!].

For the 1-D experiment, we worked with three signals of length $L = 21$ and generated the data in the same way as in the 2-D example. The only difference is that here, for each placement, one of the three signals is picked at random proportionally to the desired number of occurrences of each. In this experiment, we computed the first three autocorrelation functions. We do not assume to know the number of occurrences of each signal γ_i ahead and we removed the biased terms (see (4.6)) so we do not need to know σ either. In Appendix C we provide an argument on the number of equations we get from the first three autocorrelation functions.

To estimate the signal, we employ an optimization algorithm on the following nonlinear least-squares problem that estimate the signals and their number of occurrences simultaneously:

$$\min_{\substack{\hat{x}_1,\ldots,\hat{x}_K \in \mathbb{R}^W \\ \hat{\gamma}_1,\ldots,\hat{\gamma}_K > 0}} w_1 \left(a_y^1 - \sum_{k=1}^{K} \hat{\gamma}_k a_{\hat{x}_k}^1 \right)^2 + w_2 \sum_{\ell=1}^{L-1} \left(a_y^2[\ell] - \sum_{k=1}^{K} \hat{\gamma}_k a_{\hat{x}_k}^2[\ell] \right)^2 + w_3 \sum_{\substack{2 \le \ell_1 \le L-1 \\ 1 \le \ell_2 \le \ell_1 - 1}} \left(a_y^3[\ell_1, \ell_2] - \sum_{k=1}^{K} \hat{\gamma}_k a_{\hat{x}_k}^3[\ell_1, \ell_2] \right)^2. \tag{4.10}$$

where $W \ge L$ is the length of the sought signals and $w_1 = \frac{1}{2}, w_2 = \frac{1}{2}(L-1), w_3 = \frac{1}{(L-1)(L-2)}$; see [16] see for discussion on how to choose the weights properly.

Setting $W = L$ (as is a priori desired) is problematic because the above optimization problems appears to have numerous poor local optimizers. Thus, we first run the optimization with $W = 2L - 1$. This problem appears to have fewer poor local optima, perhaps because the additional degrees of freedom allow for more escape directions. Since we hope the signals estimated this way correspond to the true signals zero-padded to length W, we extract from each one a subsignal of length L whose autocorrelation functions are the closest the measured ones in the sense of (4.10). This estimator is then used as initial iterate for (4.10), this

time with $W = L$. We find that this procedure is reliable for a wide range of experimental parameters. To solve (4.10), we run the trust-region method implemented in Manopt [15], which allows to treat the positivity constraints [reference] on coefficients $\hat{\gamma}_k$. Notice that the cost function is a polynomial in the variables, so that it is straightforward to compute it and its derivatives.

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A Proof of (4.8)

Throughout the proof, we consider the case of one signal $K = 1$. The extension to $K > 1$ is straightforward by averaging the contributions of all signal with appropriate weights; see [16].

We will let the number of instances of the signal M grow with N, and write $M = M_N$ to emphasize this. We assume M_N grows proportionally with N, and define:

$$\gamma = \lim_{N\to\infty} \frac{M_N L}{N} < 1. \tag{A.1}$$

We will assume that $M_N = \Omega(N)$, so that $\gamma > 0$. In the sequel, we will suppress the explicit dependence of M on N for notational convenience.

We start by considering the mean of the data:

$$a_y^1 = \frac{1}{N}\sum_{i=0}^{N-1} y[i] = \frac{1}{N/L}\sum_{j=0}^{M-1}\frac{1}{L}\sum_{i=0}^{L-1} x[i] + \underbrace{\frac{1}{N}\sum_{i=0}^{N-1}\varepsilon[i]}_{\text{noise term}} \xrightarrow{a.s.} \gamma a_x^1, \tag{A.2}$$

where the noise term converges to zero almost surely (a.s.) by the strong law of large numbers.

We proceed with the (second-order) autocorrelation for fixed $\ell \in [0, \ldots, L-1]$. We can compute:

$$\begin{aligned} a_y^2[\ell] &= \frac{1}{N} \sum_{i=0}^{N-1-\ell} y[i]y[i+\ell] \\ &= \underbrace{\frac{1}{N} \sum_{j=1}^{M} \sum_{i=0}^{L-\ell-1} x[i]x[i+\ell]}_{\text{signal term}} + \underbrace{\frac{1}{N} \sum_{i=0}^{N-1-\ell} \varepsilon[i]\varepsilon[i+\ell]}_{\text{noise term}} + \underbrace{\frac{1}{N} \sum_{j=1}^{M} \sum_{i=0}^{L-1} x[i]\varepsilon[s_j+i+\ell]}_{\text{cross-term}}. \end{aligned} \tag{A.3}$$

The cross-terms are linear in the noise, and are easily shown to vanish almost surely in the limit $N \to \infty$, by the strong law of large numbers. As for the signal term, we break it into M different sums, each containing one copy of the signal. This gives:

$$\frac{1}{N} \sum_{j=1}^{M} \sum_{i=0}^{L-\ell-1} x[i]x[i+\ell] = \frac{ML}{N} \frac{1}{L} \sum_{i=0}^{L-\ell-1} x[i]x[i+\ell] \xrightarrow{N\to\infty} \gamma a_x^2[\ell]. \tag{A.4}$$

We next analyze the pure noise term. When $\ell \neq 0$, we can break the noise term into a sum of independent terms:

$$\frac{1}{N} \sum_{i=0}^{N-1-\ell} \varepsilon[i]\varepsilon[i+\ell] = \frac{1}{\ell} \sum_{i=0}^{\ell-1} \frac{1}{N/\ell} \sum_{j=0}^{N/\ell-1} \varepsilon[j\ell+i]\varepsilon[(j+1)\ell+i]. \tag{A.5}$$

Each sum $\frac{1}{N/\ell} \sum_{j=0}^{N/\ell-1} \varepsilon[j\ell+i]\varepsilon[(j+1)\ell+i]$ is an average of N/ℓ independent terms with expectation zero, and thus converges to zero almost surely as $N \to \infty$. If $\ell = 0$, then we have:

$$\frac{1}{N} \sum_{i=0}^{N-1} \varepsilon^2[i] \xrightarrow{a.s.} \sigma^2. \tag{A.6}$$

We now analyze the third-order autocorrelation. Let us fix $\ell_1 \geq \ell_2 \geq 0$. We have:

$$\begin{aligned}
a_y^3[\ell_1,\ell_2] &= \frac{1}{N}\sum_{i=0}^{N-1-\ell_1} y[i]y[i+\ell_1]y[i+\ell_2] \\
&= \underbrace{\frac{ML}{N}\frac{1}{M}\sum_{j=1}^{M}\frac{1}{L}\sum_{i=0}^{L-1-\ell_1} x[i]x[i+\ell_1]x[i+\ell_2]}_{(1)} + \underbrace{\frac{1}{N}\sum_{i=0}^{N-1-\ell_1}\varepsilon[i]\varepsilon[i+\ell_1]\varepsilon[i+\ell_2]}_{(2)} \\
&+ \underbrace{\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-1} x[i]\varepsilon[s_j+i+\ell_1]\varepsilon[s_j+i+\ell_2]}_{(3)} + \underbrace{\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-1}\varepsilon[s_j+i-\ell_1]x[i]\varepsilon[s_j+i+\ell_2-\ell_1]}_{(4)} \\
&+ \underbrace{\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-1}\varepsilon[s_j+i-\ell_2]\varepsilon[s_j+i+\ell_1-\ell_2]x[i]}_{(5)} + \underbrace{\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-\ell_1+\ell_2-1}\varepsilon[s_j+i]x[i+\ell_1-\ell_2]x[i]}_{(6)} \\
&+ \underbrace{\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-\ell_2-1} x[i]\varepsilon[s_j+i+\ell_1]x[s_j+i+\ell_2]}_{(7)} + \underbrace{\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-\ell_1-1} x[i]x[i+\ell_1]\varepsilon[s_j+i+\ell_2]}_{(8)}.
\end{aligned} \tag{A.7}$$

Terms (6), (7) and (8) are linear in ε, and can easily be shown to converge to 0 almost surely by the law of large numbers, by similar arguments as used previously. Term (1) converges to $\gamma a_x^3[\ell_1,\ell_2]$ almost surely, for the same reasons as (A.4). To deal with terms (2)–(5), we must distinguish between different values of ℓ_1 and ℓ_1.

Case 1: $0 < \ell_2 < \ell_1$. Here, all summands with elements of ε involve products of distinct entries, which have expected value 0. Consequently, the usual argument shows that terms (2)–(5) all converge to 0 almost surely as $N \to \infty$.

Case 2: $0 = \ell_2 < \ell_1$. Term (2) is an average of products of the form $\varepsilon[i]^2\varepsilon[i+\ell_1]$, which have mean zero; consequently, term (2) converges to 0 almost surely. The same argument as for Case 1 shows that (3) and (5) also converge to 0. For term (4), we write:

$$\begin{aligned}
&\frac{1}{N}\sum_{j=1}^{M}\sum_{i=0}^{L-1}\varepsilon[s_j+i-\ell_1]x[i]\varepsilon[s_j+i+\ell_2-\ell_1] \\
&= \frac{ML}{N}\frac{1}{L}\sum_{i=0}^{L-1} x[i]\frac{1}{M}\sum_{j=1}^{M}\varepsilon[s_j+i-\ell_1]^2 \xrightarrow{N\to\infty} \gamma\frac{1}{L}\sum_{i=0}^{L-1} x[i]\sigma^2 = \gamma a_x^1\sigma^2.
\end{aligned} \tag{A.8}$$

Case 3: $0 < \ell_2 = \ell_1$. An argument nearly identical to that for Case 2 shows that terms (2), (4) and (5) converge to 0, while term (3) converges to $\gamma a_x^1\sigma^2$.

Case 4: $0 = \ell_2 = \ell_1$. The same argument as for term (4) in Case 2 shows that terms (3), (4) and (5) all converge to $\gamma a_x^1\sigma^2$. Term (2) is an average of $\varepsilon[i]^3$, which is mean zero; consequently, it converges to 0.

This completes the proof of (4.8).

B Related work

System identification. For $K = 1$, our problem can be interpreted as a special case of the system identification problem. Similarly to (4.1), the system identification forward model takes the form $y = x * w + \varepsilon$, where x is the unknown signal (the "system"), w is an unknown, random, input sequence, and ε is an additive noise. The goal of this problem is to estimate x, usually referred to as "identifying the system." The question of identifiability of x under this observation model is addressed for certain Gaussian and non-Gaussian w in [13, 32]. In the special case where $w \in \{0,1\}^N$, satisfying the spacing requirement (4.2), we obtain our model in the case of a single signal ($K = 1$). The same observation model is used for blind deconvolution, a longstanding problem arising in a variety of engineering and scientific applications such as astronomy, communication, image deblurring, system identification and optics; see [30, 43, 5, 2], just to name a few. [ref this by Giannakis: [21]]

Likelihood-based methods. Likelihood-based methods estimate x as the maximizer of some function $f(x|y)$, where f is derived from the likelihood function of x given the observed signal y. For example, f may be the likelihood itself, or a related function with a similar form (leading to the class of "quasi-likelihood" methods). If some prior is assumed on x, then $f(x|y)$ can be taken to be the posterior distribution of x given the data; this is the simplest form of Bayesian inference.

Optimizing the function $f(x|y)$ exactly is often intractable, and thus heuristic methods are used instead. One proposed technique is to use Markov Chain Monte Carlo (MCMC) [17]. Another paper considers parameterized models for multiple distinct signals, as in our framework ($K > 1$) [4]. Their proposed solution is an MCMC algorithm tailored for their specific parametrized problem.

In special cases, including the case where w is binary, expectation maximization (EM) has been used [17]. The EM method for discrete w is based upon a certain "forward-backward" procedure used in hidden Markov models [41]. However, the complexity of this procedure is nonlinear in N, and therefore its usage is limited for big data sets. Indeed, on each iteration of EM, a probability must be assigned to any feasible combination of positions for the current signal estimate in M locations on the grid $\{1, \ldots, N\}$. In total, even when excluding forbidden combinations due to the spacing constraint, there are $O(N^M)$ such combinations, and the problem becomes computationally intractable when M grows with N and N is large.

Because likelihood methods are computationally expensive, methods based on recovery from moments, which are akin to our method, have also been previously used for system identification. Methods based on the third- and fourth-order moments are described and analyzed in [36, 21, 45].

C Theory

The impossibility of detection in low SNR. If x is known and $K = 1$, then the locations s_i can be estimated via linear programming in the high SNR regime [6, 18, 12, 9, 14]. However, in the low SNR regime, estimating the binary sparse signal s is impossible. To see this, suppose that an oracle provides us M windows of length $W > L$, each containing exactly one copy of x. Suppose too that the oracle provides us with x itself. That is to say, we get a series of

windows of length W, each one containing a signal x at an unknown location; and our only task is to estimate the locations. This is an easier problem than detecting the support of s. Nevertheless, even this simpler problem is impossible in the low SNR regime [3]. Consequently, detecting the nonzero values of s is impossible in low SNR.

Estimating a signal from its third-order autocorrelation. A one-dimensional signal is determined uniquely by its second- and third-order autocorrelations. Indeed, since $z[0]$ and $z[L-1]$ are non-zero by definition, we have the formula:

$$z[k] = \frac{z[0]z[k]z[L-1]}{z[0]z[L-1]} = \frac{a_z^3[k, L-1]}{a_z^2[L-1]}. \tag{C.1}$$

In particular, we have proven the following proposition:

Proposition C.1. *Let $z \in \mathbb{R}^L$ and suppose that $z[0]$ and $z[L-1]$ are nonzero. Then z is determined uniquely from a_z^2 and a_z^3.*

Some remarks are in order. First, formula (C.1) is not numerically stable if $z[0]$ and/or $z[L-1]$ are close to 0. In practice, we recover z by fitting it to its autocorrelations using a nonconvex least-squares procedures, which is empirically more robust to additive noise; we have seen similar phenomena for related problems [11, 16].

Second, if the spacing condition (4.2) holds, then the length of the signal can be determined from the autocorrelations. In particular, if (4.2) holds for some spacing $W \geq L$, then $a_z^2[i] = 0$ for all $i > L-1$.

Note too that the second-order autocorrelation is not by itself sufficient to determine the signal uniquely [8, 10]. However, for dimensions greater than one, almost all signals are determined uniquely up to sign (phase for the complex signals) and reflection through the origin (with conjugation in the complex case) [25, 26]. The sign ambiguity can be resolved by the mean of the signal if it is not zero. However, determining the reflection symmetry still requires additional information, beyond the second-order autocorrelation.

Identifiability of parameters from the moments of y. The observed moments a_y^1, a_y^2 and a_y^3 of y do not immediately give the moments of the signal x, as seen by formula (4.6); rather, the two are related by the noise level σ and the ratio $\gamma = \lim_{N\to\infty} ML/N$, where $M = M_N$ grows with N. We will show, however, that x is still identifiable from the observed moments of y. In general, we say a parameter is "identifiable" if its value is uniquely determined in the limit $N \to \infty$.

First, we observe that if the noise level σ is known, one can estimate γ from the first two moments of the observed vector y.

Proposition C.2. *Let $K = 1$ and $\sigma > 0$ be fixed. If the mean of x is nonzero, then*

$$\gamma = \lim_{N\to\infty} \frac{(a_y^1)^2}{\sum_{j=0}^{L-1} a_y^2[j] - \sigma^2} \quad a.s.$$

Proof. The proof follows from plugging the explicit expressions of (4.6) into the right hand side of the equality. □

Using third-order autocorrelation information of y, both the ratio γ and the noise σ are identifiable. For the following results, when we say that a result holds for a "generic" signal x, we mean that it holds for all x inside a set $\Omega \subset \mathbb{R}^L$, whose complement $\mathbb{R}^L \setminus \Omega$ has Lebesgue measure zero.

Proposition C.3. *Let $K = 1$, and $\sigma > 0$ be fixed. Then, a_y^1, a_y^2 and a_y^3 determine the ratio γ and noise level σ uniquely for a generic signal x. If $\gamma \geq \frac{1}{4L(L-1)}$, then this holds for any signal x with nonzero mean.*

Proof. See Appendix D. □

From Propositions C.1 and C.3 we can directly deduce the following:

Corollary C.4. *Let $K = 1$ and $\sigma > 0$ be fixed. Then the signal x, the ratio γ, and the noise level σ are identifiable from the first three autocorrelation functions of y if:*

- *Either the signal x is generic; or*
- *Both $x[0]$ and $x[L-1]$ are nonzero, x has nonzero mean, and $\gamma \geq \frac{1}{4L(L-1)}$.*

Open theoretical questions. Our method of estimating x uses the third-order moments of the observations. These empirical moments are used to obtain consistent estimators of population parameters related to the the mean and second- and third-order autocorrelations of x, to which we fit the signal x. Consequently, the number of signal occurrences M should grow at least as fast as $1/\mathsf{SNR}^3$ to achieve a constant estimation error. In the related problem of multireference alignment [40, 1], this is optimal in the low SNR regime; we conjecture that the same is true for our problem.

Another interesting question is how many signals $x_1, \ldots, x_K$ can be demixed from their mixed autocorrelation functions. In [16], we empirically observed that $K \sim \sqrt{L}$ signals can be estimated simultaneously from their mixed second- and third-order autocorrelations, using the least-squares procedure. In [49] [TKTK: add reference to Alex Wein's thesis, or put personal correspondence], this result is shown theoretically for a different, and much less efficient, algorithm. In our current setting, the additional parameters γ and σ make the problem more challenging; however, we conjecture that the number of estimable signals still grows like $\sqrt{L}$.

D Proof of Proposition C.3

We will prove that both σ and γ are identifiable from the observed first three moments of y. For convenience, we will work with $\beta = \gamma/L$ rather than γ itself. We will construct two quadratic equations satisfied by β from observed quantities, independent of σ. Then, we will show that these equations are independent, and hence that β is uniquely defined. Given β, we can estimate σ using Proposition C.2.

Throughout the proof, it is important to distinguish between observed and unobserved values. We denote the observed values by E_i or a_y^1, a_y^2, a_y^3, while using F_i for functions of the signal's autocorrelations.

Recall that $a_y^1 = \beta(\mathbf{1}^T x)$ and and $a_y^2[0] = \beta\|x\|^2 + \sigma^2$, where $\mathbf{1} \in \mathbb{R}^L$ stands for vector of ones. Taking the product:

$$\begin{aligned} E_1 &:= a_y^1 a_y^2[0] = (\beta(\mathbf{1}^T x))(\beta\|x\|^2 + \sigma^2) \\ &= \sigma^2 a_y^1 + \beta^2 F_1, \end{aligned} \tag{D.1}$$

where $F_1 := a_x^3[0,0] + \sum_{j=1}^{L-1}(a_x^3[j,j] + a_x^3[0,j])$. The terms of F_1 can be also estimated from a_y^3, while taking the scaling and bias terms into account:

$$E_2 := \beta F_1 + (2L+1)\sigma^2 a_y^1. \tag{D.2}$$

Therefore, from (D.1) and (D.2) we get

$$E_2\beta - (2L+1)\sigma^2\beta a_y^1 = E_1 - \sigma^2 a_y^1. \tag{D.3}$$

Let $a_y^2 := \sum_{j=0}^{L-1} a_y^2[j]$ and recall from Proposition C.2:

$$\sigma^2 = a_y^2 - (a_y^1)^2/(\beta L). \tag{D.4}$$

Plugging into (D.3) and rearranging we get

$$\mathcal{A}\beta^2 + \mathcal{B}\beta + \mathcal{C} = 0, \tag{D.5}$$

where

$$\begin{aligned} \mathcal{A} &= E_2 - (2L+1)a_y^1 a_y^2, \\ \mathcal{B} &= -E_1 + \frac{2L+1}{L}(a_y^1)^3 + a_y^1 a_y^2, \\ \mathcal{C} &= -(a_y^1)^3/L. \end{aligned}$$

Importantly, these coefficients are observable quantities.

We are now proceeding to derive the second quadratic equation. We notice that

$$E_3 = \frac{1}{L}(a_y^1)^3 = \frac{1}{L}\beta^3(\mathbf{1}^T x)^3 = \frac{1}{L}\beta^3 F_2, \tag{D.6}$$

where

$$F_2 = a_x^3[0,0] + 3\sum_{j=1}^{L-1} a_x^3[j,j] + 3\sum_{j=1}^{L-1} a_x^3[0,j] + 6\sum_{1\le i<j\le L-1} a_x^3[i,j].$$

On the other hand, from a_y^3 we can directly estimate F_2 up to scale and bias

$$E_4 = \beta F_2 + (6L-3)\sigma^2 a_y^1. \tag{D.7}$$

Taking the ratio:

$$\frac{E_4}{E_3} = \frac{L}{\beta^2} + \frac{(6L-3)L\sigma^2 a_y^1}{E_3},$$

we conclude:

$$\sigma^2 = \frac{E_4}{a_y^1 L(6L-3)} - \frac{E_3}{\beta^2 a_y^1(6L-3)}.$$

Using (D.4) and rearranging we get the second quadratic:

$$\mathcal{D}\beta^2 + \mathcal{E}\beta + \mathcal{F} = 0, \tag{D.8}$$

where

$$\begin{aligned}
\mathcal{D} &= a_y^2 - \frac{E_4}{a_y^1 L(6L-3)}, \\
\mathcal{E} &= -(a_y^1)^2/L, \\
\mathcal{F} &= \frac{E_3}{a_y^1(6L-3)}.
\end{aligned}$$

To complete the proof, we need to show that the two quadratic equations (D.5) and (D.8) are independent. To this end, it is enough to show that the ratio between the coefficients is not the same. From (D.5) and (D.1), we have

$$\begin{aligned}
\frac{\mathcal{B}}{\mathcal{C}} &= \frac{LE_1 - (2L+1)(a_y^1)^3 - La_y^1 a_y^2}{(a_y^1)^3} \\
&= \frac{La_y^2[0] - (2L+1)(a_y^1)^2 - La_y^2}{(a_y^1)^2}.
\end{aligned}$$

In addition, using (D.6)

$$\frac{\mathcal{E}}{\mathcal{F}} = \frac{(3-6L)(a_y^1)^3}{LE_3} = 3 - 6L.$$

Now, suppose that the quadratics are dependent. Then, $\frac{\mathcal{B}}{\mathcal{C}} = \frac{\mathcal{E}}{\mathcal{F}}$, or,

$$La_y^2[0] - (2L+1)(a_y^1)^2 - La_y^2 = (a_y^1)^2(3-6L)$$

Rearranging the equation and writing in terms of x we get

$$4(L-1)\beta(a_x^1)^2 - \sum_{i=1}^{L-1} a_x^2[i] = 0. \tag{D.9}$$

For generic x, this polynomial equation is not satisfied. Therefore, the equations are independent. More than that, for any nonzero x, $(a_x^1)^2 > \sum_{i=1}^{L-1} a_x^2[i]$. Therefore, if $4(L-1)\beta \geq 1$, or,

$$\beta \geq \frac{1}{4(L-1)},$$

the condition (D.9) cannot be satisfied for any signal.

E Stuff

Many automatic and semi-automatic methods for particle picking have been proposed, based on edge detection, template matching and deep learning; see for instance [24, 39, 50, 20, 42, 27]. However, most of these procedures are prone to *model bias*. For instance, in the popular framework of RELION [42], the user manually marks hundreds of spots on the micrograph,

believed to contain projections. Therefore, the algorithm's performance depends on the prior assumptions of the users about the particle's structure; the same holds true for deep learning based approaches which require constructing labeled sets of data. Other methods use disks or differences of Gaussians as templates [33, 48]. Nowadays, it also still popular to pick particles manually. This method, while it exploits the researcher's experience, is both tedious and subject to model bias.

F The autocorrelation functions in cryo–EM

The 3-D Fourier transform of an L-bandlimited 3-D volume (e.g., particle) can be expanded by spherical harmonics:

$$\hat{V}(k,\theta,\phi) = \sum_{\ell=0}^{L} \sum_{m=-\ell}^{\ell} A_{\ell,m}(k) Y_\ell^m(\theta,\phi), \tag{F.1}$$

where (θ,ϕ) are two angles on the sphere, k is the radial coordinate, $Y_\ell^m(\theta,\phi)$ is the spherical harmonic of degree ℓ and order m and $A_{\ell,m}(k)$ are the associated spherical harmonics coefficients, to be estimated. A rotation of the volume by $\omega \in SO(3)$ can be described using the Wigner D-function $D_{m,m'}^\ell$:

$$\begin{aligned}(R_\omega \hat{V})(k,\theta,\phi) &= \sum_{\ell=0}^{L} \sum_{m=-\ell}^{\ell} A_{\ell,m}(k) (R_\omega Y_\ell^m)(\theta,\phi) \\ &= \sum_{\ell=0}^{L} \sum_{m=-\ell}^{\ell} A_{\ell,m}(k) \sum_{m'=-\ell}^{m=\ell} D_{m,m'}^\ell(\omega) Y_\ell^{m'}(\theta,\phi).\end{aligned} \tag{F.2}$$

By the Fourier slice theorem, each cryo–EM measurement (projection) is equivalent (through 2-D Fourier transform) to the slice of $\hat{V}$, associated with $\theta = \pi/2$, after $\hat{V}$ was rotated by $\omega \in SO(3)$. Explicitly, the Fourier transform of a projection from the viewing direction ω is related to the spherical harmonic coefficients of the object through:

$$P_\omega(k,\phi) = \sum_{\ell=0}^{L} \sum_{m=-\ell}^{\ell} A_{\ell,m}(k) \sum_{m'=-\ell}^{m=\ell} D_{m,m'}^\ell(\omega) Y_\ell^{m'}(\pi/2,\phi). \tag{F.3}$$

Next, we want to relate the projections P_ω with the autocorrelation functions computed from the micrograph. We assume the projections are sufficiently separated so that the (Δ_x,Δ_y) entry of the second-order autocorrelation of the micrograph is proportional to:

$$M_2(\Delta_x,\Delta_y) \propto \sum_{n=1}^{N} \sum_{x,y} P_n(x,y) P_n(x+\Delta_x, y+\Delta_y) + \text{bias}, \tag{F.4}$$

where P_n denotes the nth projection. The assumption here is that (Δ_x,Δ_y) are small enough so that, in computing the auto-correlation, points (x,y) and $(x+\Delta_x, y+\Delta_y)$ do not touch distinct particles. By taking $n \to \infty$ and assuming uniform distribution of viewing directions, we get

$$M_2(\Delta_x,\Delta_y) \propto \sum_{x,y} \int_\omega P_\omega(x,y) P_\omega(x+\Delta_x, y+\Delta_y) d\omega. \tag{F.5}$$

In the same way and under the same conditions, the third moment is given by

$$M_3(\Delta_x^1, \Delta_y^1; \Delta_x^2, \Delta_y^2) \propto \sum_{x,y} \int_\omega P_\omega(x,y) P_\omega(x+\Delta_x^1, y+\Delta_y^1) P_\omega(x+\Delta_x^2, y+\Delta_y^2) d\omega + \text{bias.} \quad \text{(F.6)}$$

In order to determine the particle, by (F.1) one needs to estimate on the order of L^3 spherical harmonics coefficients. If the pixel size is proportional to $1/L$ (to match the volume's resolution), then M_3 provides on the order of L^4 equations involving triple products of P_ω. Since P_ω depends (after coordinate transformation) linearly in the spherical harmonic coefficients through (F.3), this means we have a system of $\sim L^4$ cubic equations in the $\sim L^3$ sought parameters. Importantly, the coefficients of these equations can be estimated from the micrographs directly, without particle picking stage.