COVARIANCE MATRIX ESTIMATION FOR THE CRYO-EM HETEROGENEITY PROBLEM

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Abstract. In cryo-electron microscopy (cryo-EM), a microscope generates a top view of a sample of randomly-oriented copies of a molecule. The problem of single particle reconstruction (SPR) from cryo-EM problem to use the resulting set of noisy 2D projection images taken at unknown directions to reconstruct the 3D structure of the molecule. In some situations, the molecule under examination exhibits structural variability, which poses a fundamental challenge in SPR. The heterogeneity problem is the task of mapping the space of conformational states of a molecule. It has been previously suggested that the leading eigenvectors of the covariance matrix of the 3D molecules can be used to solve the heterogeneity problem. Estimating the covariance matrix is challenging, since only projections of the molecules are observed, but not the molecules themselves. In this paper, we formulate a general problem of covariance estimation from noisy projections of samples. This problem has intimate connections with matrix completion problems and high-dimensional principal component analysis. We propose an estimator and prove its asymptotic consistency as well as a finite-sample error bound. When there are finitely many heterogeneity classes, the spectrum of the estimated covariance matrix reveals the number of classes. The estimator can be found as the solution to a certain linear system. In the cryo-EM case, the linear operator to be inverted, which we term the projection covariance transform, is an important object in covariance estimation for tomographic problems involving structural variation. Inverting it involves applying a filter akin to the ramp filter in tomography. We design a basis in which this linear operator is sparse and thus can be tractably inverted despite its large size. We demonstrate via numerical experiments on synthetic datasets the robustness of our algorithm to high levels of noise.

Key words. Cryo-electron microscopy, X-ray transform, inverse problems, structural variability, classification, heterogeneity, covariance matrix estimation, principal component analysis, high-dimensional statistics, Fourier projection slice theorem, spherical harmonics

AMS subject classifications. 92C55, 44A12, 92E10, 68U10, 33C55, 62H30, 62J10

1. Introduction.

1.1. Covariance matrix estimation from projected data. Covariance matrix estimation is a fundamental task in statistics. Statisticians have long grappled with the problem of estimating this statistic when the samples are only partially observed. In this paper, we consider this problem in the general setting where “partial observations” are arbitrary linear projections of the samples onto a lower-dimensional space.

PROBLEM 1.1. Let $X$ be a random vector on $\mathbb{C}^p$, with $E[X] = \mu$ and $\text{Var}(X) = \Sigma$ (Var$[X]$ denotes the covariance matrix of $X$). Suppose that $X_1, \ldots, X_n$ are i.i.d. samples from $X$, and $P_1, \ldots, P_n : \mathbb{C}^q \to \mathbb{C}$ are i.i.d. samples (independent of the $X_i$) from a distribution over $\mathbb{C}^{q \times p}$, where $q \leq p$. Let

\begin{equation}
I_s = P_s X_s + \epsilon_s,
\end{equation}

where the $\epsilon_s$ are i.i.d. noises such that $E[\epsilon_s] = 0$, $\text{Var}[\epsilon_s] = \sigma^2 I_q$, independent of $X_s, P_s$. Given $I_s, P_s$, and $\sigma^2$, estimate $\mu$ and $\Sigma$. 

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Many practical applications of covariance matrix estimation relate to principal component analysis (PCA) [40]. If $\Sigma$ has eigenvectors $V_1, \ldots, V_p$ (called principal components) corresponding to eigenvalues $\lambda_1 \geq \cdots \geq \lambda_p$, then PCA states that $V_i$ accounts for a variance of $\lambda_i$ in the data. In modern applications, the dimensionality $p$ is often large, while $X$ typically has much fewer intrinsic degrees of freedom [10]. In this situation, a few leading eigenvalues of $\Sigma$ will be significantly larger than the remaining eigenvalues (thus $\Sigma$ would be approximately low rank), and by projecting the data onto these principal components, significant dimensionality reduction can be achieved.

Problem 1.1 is quite general, and has many practical applications as special cases. The main application this paper addresses is the heterogeneity problem in single particle reconstruction (SPR) from cryo-electron microscopy (cryo-EM). SPR from cryo-EM is an inverse problem where the goal is to reconstruct a 3D molecular structure from its 2D projections [11]. The heterogeneity problem deals with the situation in which the molecule to be reconstructed can exist in several structural classes. In the language of Problem 1.1, $X_s$ represent discretized instances of the molecule, $P_s$ the 3D-to-2D projection matrices, and $I_s$ the noisy projection images. The goal of this paper is to estimate the covariance matrix associated with the variability of the molecule. If there is a small, finite number ($C$) of classes, then $\Sigma$ has low rank ($C-1$). As we demonstrate later, the top principal components can be used in conjunction with the images to reconstruct each of the $C$ classes.

Another class of applications closely related to Problem 1.1 are missing data problems in statistics. In these problems, the statistics of $X$ need to be estimated when certain entries of the samples $X_s$ are missing [31]. This amounts to choosing $P_s$ to be coordinate selection operators, operators which output a certain subset of the entries of a vector. An important problem in this category is PCA with missing data, which is the task of finding the top principal components when some data are missing. Closely related to this is the noisy low rank matrix completion problem [7]. In this problem, only a subset of the entries of a low rank matrix $M$ are known (possibly with some error), and the task is to fill in the missing entries. If we let $X_s$ be the columns of $M$, then the observed variables in each column are $P_s X_s + \epsilon_s$, where $P_s$ acts on $X_s$ by selecting a subset of its coordinates. Note that the matrix completion problem involves filling in the missing entries of $X_s$, while Problem 1.1 requires us only to find the covariance matrix of these columns. However, the two problems are closely related. For example, if the columns are distributed normally, then the missing entries can be found as their expectations conditioned on the known variables [51]. Alternatively, we can find the missing entries by choosing the linear combinations of the principal components that best fit the known matrix entries. A well-known application of matrix completion is in the field of recommender systems (also known as collaborative filtering). In this application, users rate the products they have consumed, and the task is to determine what new products they would rate highly. We obtain this problem by interpreting $M_{i,j}$ as the $j$’th user’s rating of product $i$. In recommender systems, it is assumed that only a few underlying factors determine users’ preferences. Hence, the data matrix $M$ should have low rank. A high profile example of recommender systems is the Netflix prize problem [4].

In both of these classes of problems, $\Sigma$ is large but should have low rank. Despite this, note that Problem 1.1 does not have a low rank assumption. Nevertheless, as our numerical results demonstrate, the spectrum of our (unregularized) covariance matrix estimator reveals low rank structure when it is present in the data. In this
way, our estimator behaves like the sample covariance matrix in the context of the spike model of high-dimensional PCA \[21\]. We elaborate on this in Section 2.3. Thus, we can find the low rank structure without including it in our model. Additionally, the framework we develop in this paper allows regularization to be added in a natural way.

Having introduced Problem 1.1 and its applications, let us delve more deeply into one particular application: SPR from cryo-EM.

1.2. Cryo-electron microscopy. Electron microscopy is an important tool for structural biologists, as it allows them to determine complex 3D macromolecular structures. A general technique in electron microscopy is called single particle reconstruction. In the basic setup of SPR, the data collected are 2D projection images of ideally assumed identical, but randomly oriented, copies of a macromolecule. In particular, one specimen preparation technique used in SPR is called cryo-electron microscopy, in which the sample of molecules is rapidly frozen in a thin ice layer \[11, 63\]. The electron microscope provides a top view of the molecules in the form of a large image called a micrograph. The projections of the individual particles can be picked out from the micrograph, resulting in a set of projection images. Mathematically, we can describe the imaging process as follows. Let \( \phi : \mathbb{R}^3 \to \mathbb{R} \) represent the Coulomb potential induced by the unknown molecule. We scale the problem to be dimension-free in such a way that \( \phi \) is supported in the unit ball \( B \subset \mathbb{R}^3 \), so that \( \phi \in L^2(B) \). To each copy of this molecule corresponds a rotation \( R_s \in SO(3) \), which describes its orientation in the ice layer. The idealized forward projection operator \( P_s : L^2(B) \to L^2(D) \) (where \( D \subset \mathbb{R}^2 \) is the unit disc) applied by the microscope is the X-ray transform

\[
(P_s \phi)(x, y) = \int_{\mathbb{R}} \phi(R_s^T r)dz,
\]

where \( r = (x, y, z)^T \). Hence, \( P_s \) first rotates \( \phi \) by \( R_s \), and then integrates along vertical lines to obtain the projection image. The microscope discretizes \( P_s \phi \) onto an \( N \times N \) Cartesian grid, where each pixel is also corrupted by additive noise. Let there be \( q = \frac{\pi}{4}N^2 \) pixels contained in the inscribed disc of an \( N \times N \) grid (the remaining pixels contain no signal). If \( S : L^2(D) \to \mathbb{R}^q \) is a discretization operator, then the microscope produces images \( I_s \) given by

\[
I_s = SP_s \phi + \epsilon_s,
\]

where \( \epsilon_s \sim \mathcal{N}(0, \sigma^2 I_q) \). While the noise in cryo-EM images is not necessarily white, existing methods can estimate the power spectrum of the noise and then “whiten” it. The microscope has an additional blurring effect on the images, a phenomenon we will discuss shortly, but will leave out of our model. Given the images \( I_s \), the cryo-EM problem is to estimate the orientations \( R_s \) of the underlying volumes and reconstruct \( \phi \). Note that throughout this paper, we will use “cryo-EM” and “cryo-EM problem” as shorthand for the SPR problem from cryo-EM images; we also use “volume” as a synonym for “3D structure”.

The cryo-EM problem is challenging for several reasons. Unlike most other imaging modalities, the rotations \( R_s \) are unknown, so we must estimate them before reconstructing \( \phi \). This challenge is one of the major hurdles to reconstruction in cryo-EM. Since the images are not perfectly centered, they also contain in-plane translations, which must be estimated as well. The main challenge in cryo-EM is that the projection images are corrupted by extreme levels of noise. This problem arises because only
low electron doses can scan the molecule without destroying it. To an extent, this problem is mitigated by the fact that cryo-EM datasets often have tens or even hundreds of thousands of images, which makes the reconstruction process more robust. Another issue with transmission electron microscopy in general is that technically, the detector only registers the magnitude of the electron wave exiting the specimen. Zernike realized in the 1940s that the phase information could also be recovered if the images were taken out of focus [60]. While enabling measurement of the full output of the microscope, this out-of-focus imaging technique produces images representing the convolution of the true image with a point spread function (PSF). The Fourier transform of the PSF is called the contrast transfer function (CTF). Thus the true images are multiplied by the CTF in the Fourier domain to produce the output images. Hence, the $P_s$ operators in practice also include the blurring effect of a CTF. This results in a loss of information at the zero crossings of the (Fourier-domain) CTF and at high frequencies [11]. In order to compensate for the former effect, images are taken with several different defocus values, whose corresponding CTFs have different zero crossings.

Despite its inherent challenges, SPR using cryo-EM is becoming an increasingly attractive alternative to X-ray crystallography, especially for large molecules (of size at least 500 kDa). Cryo-EM is an entirely general imaging method that does not require crystallization, and can capture molecules in their native states. Much work has been done in tackling the challenges of cryo-EM, and the state of the art is approaching resolutions near 0.3nm, which allow tracing of the polypeptide chain and identification of residues in protein molecules [28, 2, 15, 34, 68]. Even lower resolutions (0.9-0.6nm) allow biologists to determine important features of protein molecules [8]. Recently, there has been success in reconstructing membrane proteins using single particle methods [62, 30]. As a testament to the growing importance of cryo-EM, Figure 1.1 shows the quantity of cryo-EM structures uploaded to Protein Data Bank each year (relative to the year 2000), as compared to the corresponding numbers for X-ray crystallography and nuclear magnetic resonance (NMR).

![Fig. 1.1: Structures uploaded to Protein Data Bank each year, relative to 2000. In 2000, 7 EM, 362 NMR, and 2274 X-ray structures were uploaded; in 2013, 236 EM, 517 NMR, and 9305 X-ray structures were uploaded.](image)
The most common method for solving the basic cryo-EM problem is guessing an initial structure and then performing an iterative refinement procedure, where iterations alternate between 1) estimating the rotations of the experimental images by matching them with projections of the current 3D model; and 2) tomographic inversion producing a new 3D model based on the experimental images and their estimated rotations \[ 11, 61, 45 \]. Alternatively, estimating the rotations and reconstructing an accurate initial structure directly from the data avoids the bias possibly incurred by the initial guess and reduces the number of refinement iterations necessary to converge to a high-resolution reconstruction \[ 70 \]. Rotations can be estimated by one of several techniques (see for example \[ 55, 64 \] and references therein).

1.3. Heterogeneity problem. As presented above, a key assumption in the cryo-EM problem is that the sample consists of (rotated versions of) identical molecules. However, in many datasets this assumption does not hold. Some molecules of interest exist in more than one conformational state. For example, a subunit of the molecule might be present or absent, have a few different arrangements, or be able to move continuously from one position to another. These structural variations are of great interest to biologists, as they provide insight into the functioning of the molecule. Unfortunately, standard cryo-EM methods do not account for heterogeneous samples. New techniques must be developed to map the space of molecules in the sample, rather than just reconstruct a single volume. This task is called the heterogeneity problem. A common case of heterogeneity is when the molecule has a finite number of dominant conformational classes. In this discrete case, the goal is to provide biologists with 3D reconstructions of all these structural states. While cases of continuous heterogeneity are possible, in this paper we mainly focus on the discrete heterogeneity scenario.

Fig. 1.2: Classical (left) and hybrid (right) states of 70S E. Coli ribosome (image source: \[ 29 \])

In this publication, we assume that the 3D rotations \( R_s \) (and in-plane translations) have already been estimated. While the rotation estimation problem changes once we allow heterogeneity, existing rotation estimation methods can still be used. One case in which this holds is when the heterogeneity is small compared to the mean volume. That is, if the heterogeneous volumes \( \phi_1, \ldots, \phi_n \) are all not too far from their mean. Since the states of the molecule are defined up to a rotation, by the above condition we imply that the conformational classes can be aligned such that they are all close to their mean. An example of small heterogeneity is localized heterogeneity (see Figure 1.2). Note that if we do have small heterogeneity, then by initially assuming homogeneity, we can still get an accurate estimation of rotations using existing methods. Even if the heterogeneity is large, we can iteratively estimate the rotations and the conformations until convergence.
Note that the finite sampling rate of the images limits the frequencies we can hope to reconstruct. Given an \( N \times N \) grid with a radius of 1, the corresponding Nyquist bandlimit is \( \omega_{\text{max}} = N\pi/2 \). Reconstructing frequencies past \( \omega_{\text{max}} \) in \( \phi \) would constitute aliasing. In practice, the exponentially decaying envelope of the CTF function renders even fewer frequencies possible to reconstruct. Moreover, we shall see in Sections 3.1 and 5.4 that reconstruction of \( \Sigma \) becomes more ill-conditioned as the frequency increases. Hence, it often makes sense to take a cutoff \( \omega_{\text{max}} < N\pi/2 \).

We can choose \( \omega_{\text{max}} \) to correspond to an effective resolution of \( N_{\text{res}} \) pixels, where \( N_{\text{res}} \leq N \). In this case, we would choose \( \omega_{\text{max}} = N_{\text{res}}\pi/2 \). Thus, it is natural to search for \( \phi \) in a space of functions bandlimited in \( \mathcal{B}_{\omega_{\text{max}}} \) (the ball of radius \( \omega_{\text{max}} \)) and with most of their energy contained in the unit ball. The optimal space \( \mathcal{B} \) with respect to these constraints is spanned by a finite set of Slepian functions \[56\]. For a given bandlimit \( \omega_{\text{max}} \), we have

\[
p := \dim(\mathcal{B}) = \frac{2}{9\pi} \omega_{\text{max}}^3.
\]

This dimension is called the Shannon number, and is the trace of the kernel in \[56\], eq. 6. In the following formulation of the heterogeneity problem, we assume the conformational classes of \( \phi \) belong to the space \( \mathcal{B} \).

**PROBLEM 1.2. (Heterogeneity Problem).** Let \( \phi : \Omega \times \mathbb{R}^3 \to \mathbb{R} \) be a random field, where \( (\Omega, \mathcal{F}, \nu) \) is a probability space. Suppose that there exist structures \( \phi^1, \ldots, \phi^C \in \mathcal{B} \) and probabilities \( p_1, \ldots, p_C \) summing to 1 such that

\[
\nu[\phi = \phi^c] = p_c, \quad c = 1, \ldots, C.
\]

Suppose that molecules \( \phi_1, \ldots, \phi_n \) are embedded in the ice layer of a cryo-EM sample, where the \( \phi_s : \mathbb{R}^3 \to \mathbb{R} \) are i.i.d. samples from \( \phi \). Each \( \phi_s \) is embedded at an orientation of \( R_s \in \text{SO}(3) \), where each \( R_s \) is drawn i.i.d. from a distribution over \( \text{SO}(3) \) and is independent of the structure \( \phi_s \). The projection images \( I_1, \ldots, I_n \) are obtained through

\[
I_s = S\mathcal{P}_s \phi_s + \epsilon_s,
\]

where \( \epsilon_s \sim \mathcal{N}(0, \sigma^2 I_q) \) is independent of \( \phi_s \) and \( R_s \), \( S : L^2(D) \to \mathbb{R}^q \) is a discretization operator, and \( \mathcal{P}_s \) is as defined in (1.2). Suppose that the rotations \( R_s \) have already been estimated. Given the images \( I_s \) and the rotations \( R_s \), find the number of classes \( C \), the structures \( \phi^c \) in \( \mathcal{B} \), and the probabilities \( p_c \).

Note that \( S\mathcal{P}_s |_{\mathcal{B}} \) is a linear operator between finite dimensional spaces, and so it has a matrix version \( P_s : \mathbb{R}^p \to \mathbb{R}^q \). If we let \( X \) be the random vector on \( \mathbb{R}^p \) obtained by expanding \( \phi \) in the basis for \( \mathcal{B} \) and define \( X_s \) correspondingly, then we recover the equation \( I_s = P_s X_s + \epsilon_s \) from Problem 1.1. Thus, the main factors distinguishing Problem 1.2 from Problem 1.1 are that the former assumes a specific form for \( P_s \) and posits a discrete distribution on \( X \). As we discuss in Section 4, Problem 1.2 can be solved by first estimating the covariance matrix as in Problem 1.1 followed by a least-squares procedure.

One of the main difficulties of the heterogeneity problem is that, compared to usual SPR, we must deal with an even lower effective signal-to-noise ratio (SNR). Indeed, the signal we seek to reconstruct is the variation of the molecules around their mean, as opposed to the mean volume itself. We propose a precise definition of SNR in the context of the heterogeneity problem in Section 6.4. Another difficulty
is the indirect nature of our problem. Although the heterogeneity problem is an instance of a clustering problem, it differs from usual such problems in that we do not have access to the objects we are trying to cluster – only projections of these objects onto a lower-dimensional space are available. This makes it challenging to apply any standard clustering techniques directly.

The heterogeneity problem is considered one of the most important problems in cryo-EM. In his 2013 Solvay public lecture on cryo-EM, Dr. Joachim Frank emphasized the importance of “the ability to obtain an entire inventory of co-existing states of a macromolecule from a single sample” [12]. Speaking of approaches to the heterogeneity problem in a review article, Frank discussed “the potential these new technologies will have in exploring functionally relevant states of molecular machines” [13]. It is stressed there that much room for improvement remains; current methods cannot automatically identify the number of conformational states and have trouble distinguishing between similar conformations.

1.4. Previous work. Much work related to Problem 1.1 and Problem 1.2 has already been done. There is a rich statistical literature on the covariance estimation problem in the presence of missing data, a special case of Problem 1.1. In addition, work on the low rank matrix sensing problem (a generalization of matrix completion) is also closely related to Problem 1.1. Regarding Problem 1.2, several approaches to the heterogeneity problem have been proposed in the cryo-EM literature.

1.4.1. Work related to Problem 1.1. Many approaches to covariance matrix estimation from missing data have been proposed in the statistics literature [31]. The simplest approach to dealing with missing data is to ignore the samples with any unobserved variables. Another simple approach is called available case analysis, in which the statistics are constructed using all the available values. For example, the \((i,j)\) entry of the covariance matrix is constructed using all samples for which the \(i\)'th and \(j\)'th coordinates are simultaneously observed. These techniques work best under certain assumptions on the pattern of missing entries, and more sophisticated techniques are preferred [31]. One of the most established such approaches is maximum likelihood estimation (MLE). This involves positing a probability distribution on \(X\) (e.g., multivariate normal) and then maximizing the likelihood of the observed partial data with respect to the parameters of the model. Such an approach to fitting models from partial observations was known as early as the 1930s, when Wilks used it for the case of a bivariate normal distribution [66]. Wilks proposed to maximize the likelihood using a gradient-based optimization approach. In 1977, Dempster, Laird, and Rubin introduced the expectation-maximization (EM) algorithm [9] to solve maximum likelihood problems. The EM algorithm is one of the most popular methods for solving missing data problems in statistics. Also, there is a class of approaches to missing data problems called imputation, in which the missing values are filled either by averaging the available values or through more sophisticated regression-based techniques. Finally, see [33] for another approach to covariance estimation in the missing data setup.

Closely related to covariance estimation from missing data is the problem of PCA with missing data. In this problem, the task is to find the principal components, and not necessarily the entire covariance matrix. Not surprisingly, EM-type algorithms are popular for this problem as well. These algorithms often search directly for the low-rank factors. See [13] for a survey of approaches to PCA with missing data. Closely related to PCA with missing data is the low rank matrix completion problem. Many of the statistical methods discussed above are also applicable to matrix completion.
In particular, EM algorithms to solve this problem are popular, e.g., [51, 27].

Note that all the aforementioned problems relate to missing data in one way or another. In this paper, we are also interested in more general kinds of partial observations. For example, [32] considers a general conditional distribution of the observed data on the covariates in the context of high-dimensional sparse regression.

Another more general problem setup related to Problem 1.1 is the **low rank matrix sensing problem**, which generalizes the low rank matrix completion problem. Let \( M \in \mathbb{R}^{p \times n} \) be an unknown rank-\( k \) matrix, and let \( A : \mathbb{R}^{p \times n} \rightarrow \mathbb{R}^d \) be a linear map, called the **sensing matrix**. We would like to find \( M \), but we only have access to the (possibly noisy) data \( A(M) \). Hence, the low rank matrix sensing problem can be formulated as follows [19]:

\[
\text{(1.7) minimize } \|A(M) - b\|, \quad \text{s.t. } \text{rank}(M) \leq k.
\]

Note that Problem 1.1 is a special case of the low rank matrix sensing problem when \( \Sigma \) is low rank. Indeed, consider putting the unknown vectors \( X_1, \ldots, X_n \) together as the columns of a matrix \( M \). The rank of this matrix is the number of degrees of freedom in \( X \) (in the cryo-EM problem, this relates to the number of heterogeneity classes of the molecule). The linear projections \( P_1, \ldots, P_n \) can be combined into one sensing matrix \( A \) acting on \( M \). In this way, our problem falls into the realm of matrix sensing.

One of the first algorithms for matrix sensing was inspired by the compressed sensing theory [47]. This approach uses a matrix version of \( \ell_1 \) regularization called nuclear norm regularization. The nuclear norm is the sum of the singular values of a matrix, and is a convex proxy for its rank. Another approach to this problem is alternating minimization, which decomposes \( M \) into a product of the form \( UV^T \) and iteratively alternates between optimizing with respect to \( U \) and \( V \). The first proof of convergence for this approach was given in [19]. Both the nuclear norm and alternating minimization approaches to the low rank matrix sensing problem require a **restricted isometry property** on \( A \) for theoretical guarantees.

While the aforementioned algorithms are widely used, we believe they have limitations as well. EM algorithms require postulating a distribution over the data and are susceptible to getting trapped in local optima. Regarding the former point, Problem 1.1 avoids any assumptions on the distribution of \( X \), so our estimator should have the same property. Matrix sensing algorithms (especially alternating minimization) often assume that the rank is known in advance. However, there is no satisfactory statistical theory for choosing the rank. By contrast, the estimator we propose for Problem 1.1 allows automatic rank estimation.

**1.4.2. Work related to Problem 1.2.** Several approaches to the heterogeneity problem have been proposed. Here we give a brief overview of some of these approaches.

One approach is based on the notion of common lines. By the Fourier projection slice theorem (see Theorem 3.1), the Fourier transforms of any two projection images of an object will coincide on a line through the origin, called a common line. The idea of Shatsky et al. [52] was to use common lines as a measure of how likely it is that two projection images correspond to the same conformational class. Specifically, given two projection images and their corresponding rotations, we can take their Fourier transforms and correlate them on their common line. From there, a weighted graph of the images is constructed, with edges weighted based on this common line measure.
Then spectral clustering is applied to this graph to classify the images. An earlier common lines approach to the heterogeneity problem is described in [16].

Another approach is based on MLE. It involves positing a probability distribution over the space of underlying volumes, and then maximizing the likelihood of the images with respect to the parameters of the distribution. For example, Wang et al [65] model the heterogeneous molecules as a mixture of Gaussians. A challenge with MLE approaches is that the resulting objective functions are nonconvex and have a complicated structure. [65] overcomes this challenge by employing the EM algorithm. For more discussion of the theory and practice of maximum likelihood methods, see [53] and [50], respectively. Also see [49] for a description of a software package which uses maximum likelihood to solve the heterogeneity problem.

A third approach to the heterogeneity problem is to use the covariance matrix of the set of original molecules. Penczek outlines a bootstrapping approach in [44] (see also [41, 43, 67, 29]). In this approach, one repeatedly takes random subsets of the projection images and reconstructs 3D volumes from these samples. Then, one can perform principal component analysis on this set of reconstructed volumes, which yields a few dominant “eigenvolumes”. [44] proposes to then produce mean-subtracted images by subtracting projections of the mean volume from the images. The next step is to project each of the dominant eigenvolumes in the directions of the images, and then obtain a set of coordinates for each image based on its similarity with each of the eigenvolume projections. Finally, using these coordinates, this resampling approach proceeds by applying a standard clustering algorithm such as $K$-means to classify the images into classes.

While existing methods for the heterogeneity problem have their success stories, each suffers from its own shortcomings: the common line approach does not exploit all the available information in the images, the maximum likelihood approach requires explicit a-priori distributions and is susceptible to local optima, and the bootstrapping approach based on covariance matrix estimation is a heuristic sampling method that lacks in theoretical guarantees.

Note that the above overview of the literature on the heterogeneity problem is not comprehensive. For example, very recently, an approach to the heterogeneity problem based on normal mode analysis was proposed [20].

1.5. Our contribution. In this paper, we propose and analyze a covariance matrix estimator $\Sigma_n$ to solve the general statistical problem (Problem 1.1), and then apply this estimator to the heterogeneity problem (Problem 1.2).

Our covariance matrix estimator has several desirable properties. First, we prove that the estimator is asymptotically consistent as $n \to \infty$ for fixed $p$, $q$, with the variance decaying as $1/n$. Second, we prove a finite sample error bound. Third, our estimator does not require a prior distribution on the data, unlike MLE methods. Fourth, when the data have low intrinsic dimension, our method does not require knowing the rank of $\Sigma$ in advance. The rank can be estimated from the spectrum of the estimated covariance matrix. This sets our method apart from alternating minimization algorithms that search for the low rank matrix factors themselves. Fifth, our estimator is given in closed-form and its computation requires only a single linear inversion.

To implement our covariance matrix estimator in the cryo-EM case, we must invert a high-dimensional matrix $L_n$ (see definition (2.8)). The size of this matrix is so large that it cannot even be stored on a computer; thus, inverting $L_n$ is the greatest practical challenge we face. In order to address this challenge, we first replace the $L_n$
by its limiting operator $L$, which does not depend on the rotations $R_s$ and is a good approximation of $L_n$ as long as these rotations are distributed uniformly enough. We then carefully construct new bases for images and volumes to make $L$ a sparse, block diagonal matrix. While a dense matrix of the same size as $\hat{L}$ would have dimensions on the order of $N_{\text{res}}^6 \times N_{\text{res}}^6$, in the bases we construct, $\hat{L}$ has only $O(N_{\text{res}}^9)$ total nonzero entries. These innovations lead to a practical algorithm to estimate the covariance matrix in the heterogeneity problem.

The limiting operator $L$ is a fundamental object in tomographic problems involving variability, and we call it the projection covariance transform. The projection covariance transform relates the covariance matrix of the imaged object to data that can be acquired from the projection images. Standard tomographic reconstruction algorithms involve application of the ramp filter to the data [28], and we find that the inversion of $L$ entails applying a similar filter, which we call the triangular area filter. The triangular area filter has many of the same properties as the ramp filter, but reflects the slightly more intricate geometry of the covariance estimation problem. The projection covariance transform is an interesting mathematical object in its own right, and we begin studying it in this paper. We make the rudimentary observations that $L$ is self-adjoint and positive semidefinite. Practically, the condition number of (the finite-dimensional version of) this operator is important. In this paper, we bound the smallest eigenvalue of $L$ from below, and make a conjecture about the growth of the largest eigenvalue as we increase the bandlimit $\omega_{\text{max}}$. We also observe a rotational invariance property of $L$ induced by its underlying geometry.

Finally, we validate our method numerically. We demonstrate our method’s robustness to noise on synthetic datasets by obtaining a meaningful reconstruction of the covariance matrix and molecular volumes even at low SNR levels. Excluding precomputations, reconstructions for 10000 projection images of size $65 \times 65$ pixels takes fewer than five minutes on a standard laptop computer.

The paper is organized as follows. In Section 2, we construct an estimator for Problem 1.1, state theoretical results about this estimator, and connect our problem to high-dimensional PCA. In Section 3, we specialize the covariance estimator to the heterogeneity problem and investigate its geometry. In Section 4, we discuss how to reconstruct the conformations once we have estimated the mean and covariance matrix. In Section 5, we present the construction of a basis in which $L$ is block diagonal and sparse. In Section 6, we present numerical results for the heterogeneity problem. We conclude with a discussion of future research directions in Section 7. Appendices A, B, and C contain calculations and proofs.

2. An estimator for Problem 1.1

2.1. Constructing an estimator. We define estimators $\mu_n$ and $\Sigma_n$ through a general optimization framework based on the model (1.1). As a first step, let us calculate the first- and second-order statistics of $I_s$. It is important to note that throughout this paper, all the expectations taken are implicitly conditioned on the (observed) operators $P_s$. Our assumption that $X_s$ and $\epsilon_s$ are independent of $P_s$ makes these conditional expectations equal to their unconditional counterparts, e.g., $E[X_s|P_s] = E[X_s] = \mu$. Keeping this in mind and using the linearity of $P_s$, we find that

\begin{equation}
\text{E}[I_s] = \text{E}[P_s X_s + \epsilon_s] = \text{E}[P_s X_s] = P_s \mu.
\end{equation}
and

\[(2.2) \quad \text{Var}[I_s] = \text{Var}[P_s X_s + \epsilon_s] = \text{Var}[P_s X_s] + \text{Var}[\epsilon_s] = P_s \Sigma P_s^H + \sigma^2 I_q.\]

Note that $P^H_s$ denotes the conjugate transpose of $P$. Based on (2.1) and (2.2), we devise least-squares optimization problems for $\mu_n$ and $\Sigma_n$:

\[(2.3) \quad \mu_n = \arg\min_{\mu} \frac{1}{n} \sum_{s=1}^{n} \|I_s - P_s \mu\|_F^2 ;\]

\[(2.4) \quad \Sigma_n = \arg\min_{\Sigma} \frac{1}{n} \sum_{s=1}^{n} \| (I_s - P_s \mu_n)(I_s - P_s \mu_n)^H - (P_s \Sigma P_s^H + \sigma^2 I_p) \|_{F}^2 .\]

Here we use the Frobenius norm, which is defined by $\|A\|_F^2 = \sum_{i,j} |A_{ij}|^2$.

Note that these optimization problems do not encode any prior knowledge about $\mu$ or $\Sigma$. However, since $\Sigma$ is a covariance matrix, it must be positive semidefinite (PSD). Also, as discussed above, in many applications $\Sigma$ is also low rank. The estimator $\Sigma_n$ need not satisfy either of these properties. Also, if the quadratic form in (2.4) is not positive definite in all the entries of $\Sigma$, some entries might not be recoverable from this unregularized optimization problem. To remedy these problems, note that (2.4) can be modified by adding a positive semidefinite constraint or a nuclear norm regularization term. Alternatively, without increasing the complexity of the optimization, we could add a Tikhonov regularization term $\lambda \| \Sigma \|_F^2$. This would have the effect of setting any unattainable entries of $\Sigma_n$ to zero. If there are not too many such entries, then the error incurred by this procedure would not be too large. Such an argument is made more precise in [24]. Finally, regularization may be applied to promote the sparsity of $\Sigma_n$ in a suitable basis. For example, in the case of localized variability in the discrete heterogeneity problem, $\Sigma$ is sparse in the real domain Cartesian basis.

Despite these possibilities for regularization, in this paper we will only explore the unregularized estimator $\Sigma_n$. Note that in most practical problems, we only are interested in the leading eigenvectors of $\Sigma_n$, and if these are estimated accurately, then it does not matter if $\Sigma_n$ is not PSD or low rank. Our numerical experiments show that in practice, the top eigenvectors of $\Sigma_n$ are indeed good estimates of the true principal components for high enough SNR.

Note that we first solve (2.3) for $\mu_n$, and then use this result in (2.4). This makes these optimization problems quadratic in the elements of $\mu$ and $\Sigma$, and hence they can be solved by setting the derivatives with respect to $\mu$ and $\Sigma$ to zero. This leads to the following equations for $\mu_n$ and $\Sigma_n$ (see Appendix A for the derivative calculations):

\[(2.5) \quad \frac{1}{n} \left( \sum_{s=1}^{n} P_s^H P_s \right) \mu_n = \frac{1}{n} \sum_{s=1}^{n} P_s^H I_s ;\]

\[(2.6) \quad \frac{1}{n} \sum_{s=1}^{n} P_s^H P_s \Sigma_n P_s^H P_s = \frac{1}{n} \sum_{s=1}^{n} P_s^H (I_s - P_s \mu_n)(I_s - P_s \mu_n)^H P_s - \sigma^2 \frac{1}{n} \sum_{s=1}^{n} P_s^H P_s .\]

Observe that (2.5) requires inversion of the matrix

\[(2.7) \quad A_n = \frac{1}{n} \sum_{s=1}^{n} P_s^H P_s ,\]
and (2.6) requires inversion of the linear operator $L_n : \mathbb{C}^{p \times p} \to \mathbb{C}^{p \times p}$ defined by

$$L_n(\Sigma) = \frac{1}{n} \sum_{s=1}^{n} P_s^H P_s \Sigma P_s^H P_s^T.$$

(2.8)

We assumed that $P_s$ are drawn independently from a probability distribution, so by the law of large numbers, there must be an $A \in \mathbb{C}^{p \times p}$ and an operator $L : \mathbb{C}^{p \times p} \to \mathbb{C}^{p \times p}$ for which

$$A_n \to A \quad \text{and} \quad L_n \to L \quad \text{almost surely},$$

(2.9)

where the convergence is in the operator norm. The invertibilities of $A$ and $L$ depend on the distribution of $P_s$. Intuitively, if $P_s$ has a nonzero probability of “selecting” any coordinate of $X_s$, then $A$ will be invertible. If $P_s$ has a nonzero probability of “selecting” any pair of coordinates of $X_s$, then $L$ will be invertible. For the asymptotic theoretical results in Section 2.2, we will assume that $A$ and $L$ are invertible. Under this assumption, $A_n$ and $L_n$ will be invertible for sufficiently large $n$, and thus (2.5) and (2.6) define the estimators $\mu_n$ and $\Sigma_n$. Otherwise, our unregularized approach will not be able to fully reconstruct $\Sigma$, and additional information about $\Sigma$ would need to be imposed. We will find that in the cryo-EM case, $A$ and $L$ are invertible if, for example, the rotations are sampled uniformly from $SO(3)$.

Let us make a few observations about $A_n$ and $L_n$. By inspection, $A_n$ is symmetric and positive semidefinite. We claim that $L_n$ satisfies the same properties, with respect to the Hilbert space $\mathbb{C}^{p \times p}$ equipped with the inner product $\langle A, B \rangle = tr(A^H B)$. Using the property $tr(AB) = tr(BA)$, we find that

$$\langle L_n(\Sigma_1), \Sigma_2 \rangle = tr(L_n(\Sigma_1)^H \Sigma_2) = tr \left[ \frac{1}{n} \sum_{s} P_s^H P_s \Sigma_1^H P_s^H P_s \Sigma_2 \right]$$

(2.10)

$$= tr \left[ \frac{1}{n} \sum_{s} P_s^H P_s \Sigma_2 \Sigma_1^H P_s^H P_s \Sigma_2 \right] = \langle \Sigma_1, L(\Sigma_2) \rangle.$$

Thus, $L_n$ is self-adjoint. Next, we claim that $L_n$ is positive semidefinite. Indeed,

$$\langle L_n(\Sigma), \Sigma \rangle = tr(L(\Sigma)^H \Sigma) = tr \left[ \frac{1}{n} \sum_{s} P_s^H P_s \Sigma^H \Sigma P_s^H P_s \Sigma \right]$$

(2.11)

$$= \frac{1}{n} \sum_{s} tr \left[ (P_s^H \Sigma P_s^H)(P_s^H \Sigma P_s^H) \right] = \frac{1}{n} \| P_s \Sigma P_s^H \|_F^2 \geq 0.$$

We discussed in the introduction that when the $P_s$ are coordinate-selection operators, then our problem reduces to a standard missing data problem in statistics. Let us interpret (2.5) and (2.6) in this case. The first thing to note is that in the coordinate selection case, $P_s^H P_s$ is a diagonal matrix. The $i$’th diagonal entry is a $1$ if the observation of the $i$’th coordinate of $X_s$ is present in $P_s X_s$, and a $0$ if it is missing. Modulo noise, the quantities $P_s^H I_s$ are vectors in $\mathbb{C}^p$ that coincide with $X_s$ in the observed entries and have zeros in the missing entries. From here, it is easy to see that (2.5) estimates the mean by averaging all the available observations for each coordinate, and (2.6) estimates the covariance matrix by averaging over all samples for which both coordinates are observed. Note that these are exactly the available-case estimators discussed in [31, Section 3.4]. Available-case estimators are known to
be consistent when the data is missing completely at random (MCAR). This means that the pattern of missingness is independent of the (observed and unobserved) data. Accordingly, in Problem 1.1, we assume that $P_s$ and $X_s$ are independent, a generalization of the MCAR condition. In sum, $\mu_n$ and $\Sigma_n$ reduce to well-known estimators in the case when the $P_s$ are coordinate-selection operators. We shall see in the next section that the consistency of $\mu_n$ and $\Sigma_n$ carries over Problem 1.1.

2.2. Theoretical results about $\mu_n$ and $\Sigma_n$. In this section, we state asymptotic and finite-sample results about the estimators $\mu_n$ and $\Sigma_n$. We prove these results in Appendix C. Under some assumptions on the operators $P_s$, we will show that the estimator $\Sigma_n$ is asymptotically consistent as $n \to \infty$. Moreover, for fixed $n$ we will bound $\|\Sigma - \Sigma_n\|$ with high probability. Note that here, and throughout this section, $\|\cdot\|$ will denote the operator norm. Moreover, $\lambda_{\min}(M)$ and $\lambda_{\max}(M)$ denote the smallest and largest eigenvalues of a matrix $M$, respectively.

**Proposition 2.1.** As soon as $A_n$ is invertible, the estimator $\mu_n$ is unbiased. Moreover, if the limiting operators $A$ and $L$ from (2.9) are invertible, then for fixed $p, q$,

$$\text{Var}[\mu_n] = O\left(\frac{1}{n}\right).$$

**Proposition 2.2.** If $A$ and $L$ are invertible and $\|P_s\|_2$ is uniformly bounded over all $s$, then for fixed $p, q$,

$$\|\mathbb{E}[\Sigma_n] - \Sigma\| = O\left(\frac{1}{n}\right).$$

If in addition the random variable $X$ has bounded fourth moments, then for all indices $i, j, k, \ell$,

$$\text{Cov}[(\Sigma_n)_{ij}, (\Sigma_n)_{kl}] = O\left(\frac{1}{n}\right).$$

Next, we will state a finite-sample result about $\Sigma_n$. For this result, we require additionally that $X$ and each $\epsilon_s$ are bounded almost surely. Note that this is not the strongest possible result; we can weaken this boundedness assumption and replace it by a bound on the tails of the distributions of $X$ and $\epsilon_s$. As in Proposition 2.2, we also require a uniform bound on the norms of $P_s$. Finally, we will prove the following result assuming that $\mu = 0$. While we do not verify it rigorously in this paper, the bias incurred by an incorrectly estimated $\mu$ is usually negligible.

Our bound will depend on $\lambda_{\min}(L_n)$. Since $L_n$ is self-adjoint and positive semidefinite, we know that $L_n$ admits an eigenvalue decomposition and satisfies $\lambda_{\min}(L_n) \geq 0$.

**Proposition 2.3.** In the statistical model (1.1), suppose additionally that $\|X\| \leq C_1$, $\|\epsilon_s\| \leq C_2$, $\|P_s\| \leq C_3$ a.s. Also, assume that $X$ is centered. If $\lambda = \lambda_{\min}(L_n)$ and $B = C_3^2C_1 + C_3C_2$, then

$$\mathbb{P}\{|\Sigma - \Sigma_n| \geq t\} \leq p \exp\left(-\frac{3n\lambda^2t^2}{2B^4 + 2B^2M}\right), \quad t \geq 0.$$  

This shows that if the smallest eigenvalue of $L_n$ is separated from zero, then the estimator’s bias is small with high probability for appropriate settings of $p$ and $n$. 


2.3. Connection to high-dimensional PCA. To fully understand the estimator \( \Sigma_n \) in the case when we only want the leading principal components, we must analyze it from the point of view of high-dimensional PCA. In this section, we first discuss the existing high-dimensional PCA literature, and then raise some open questions about \( \Sigma_n \) in the context of this field.

2.3.1. Overview of high-dimensional PCA. Given i.i.d. samples \( Y_1, \ldots, Y_n \in \mathbb{R}^p \) from a centered distribution with covariance matrix \( \Sigma \) (called the population covariance matrix), the sample covariance matrix \( \hat{\Sigma}_n \) is defined by

\[
\hat{\Sigma}_n = \frac{1}{n} \sum_{s=1}^{n} Y_s Y_s^H.
\]

We use the new tilde notation because in the context of Problem 1.1, \( \hat{\Sigma} \) is the signal plus noise covariance matrix, as opposed to the covariance of the signal itself. High-dimensional PCA is the study of the spectrum of \( \hat{\Sigma}_n \) for various distributions of \( Y_s \) in the regime where \( n, p \to \infty \) with \( p/n \to \gamma \).

The first case to consider is \( Y_s \sim \mathcal{N}(0, \sigma^2 I_p) \). In a landmark paper, Marčenko and Pastur [35] proved that the spectrum of \( \hat{\Sigma}_n \) converges to the Marčenko-Pastur (MP) distribution, which is parameterized by \( \gamma \) and \( \sigma^2 \):

\[
MP(x) = \frac{1}{2\pi \sigma^2} \sqrt{(\gamma_+ - x)(x - \gamma_-)} \frac{1}{\gamma x} 1_{[\gamma_- , \gamma_+]}, \quad \gamma_{\pm} = \sigma^2 (1 \pm \sqrt{\gamma})^2.
\]

The above formula assumes \( \gamma \leq 1 \); a similar formula governs the case \( \gamma > 1 \). Note that there are much more general statements about classes of \( Y_s \) for which this convergence holds; see e.g., [54]. See Figure 2.1 for MP distributions with a few different parameter settings.

![MP Distributions](image)

Fig. 2.1: MP distributions \((2.17)\) for \( \sigma^2 = 1 \)

Johnstone [21] took this analysis a step further and considered the limiting distribution of the largest eigenvalue of \( \hat{\Sigma}_n \). He showed that the distribution of this eigenvalue converges to the Tracy-Widom distribution centered on the right edge of
the MP spectrum. In the same paper, Johnstone considered the spiked covariance model, in which the population covariance matrix is \( \Sigma = \text{diag}(\tau_1, \ldots, \tau_r, 1, \ldots, 1) \), with \( \tau_1, \ldots, \tau_r > 1 \). In this model, the top \( r \) eigenvalues correspond to “signal” while the rest are viewed as “noise.” In this view, the goal is to accurately recover the top \( r \) eigenvectors. The question then is the following: for what values of \( \tau_1, \ldots, \tau_r \) will the top \( r \) eigenvectors of the sample covariance matrix be good approximations for the top eigenvectors of the population covariance? Since we might not know the value of \( r \) a-priori, it is important to first determine for what values of \( \tau_1, \ldots, \tau_r \) we can detect the presence of nonunit population eigenvalues. In [3], the spectrum of the sample covariance matrix in the spiked model was investigated. It was found that the bulk of the distribution still obeys the MP law, whereas for each \( \tau_k \geq 1 + \sqrt{\gamma} \), the sample covariance matrix will have an eigenvalue tending to \( \tau_k + \frac{\tau_k \gamma}{n} \). The sample eigenvalues corresponding to nonunit population eigenvalues below this threshold tend to the right edge of the noise distribution. Hence, nonunit eigenvalues can be detected from the sample covariance matrix if and only if they exceed the threshold \( 1 + \sqrt{\gamma} \).

In Figure 2.2, we illustrate these results with a numerical example. We choose \( p = 800 \), \( n = 4000 \), and a spectrum with two nonunit eigenvalues above and one nonunit eigenvalue below the threshold corresponding to \( \gamma = p/n = 0.2 \). Figure 2.2 is a normalized histogram of the eigenvalues of the sample covariance matrix. The predicted MP distribution for the bulk is superimposed. We see that indeed we have two eigenvalues separated from this bulk, at locations close to what [3] predicts. Moreover, the eigenvalue of \( \Sigma_n \) corresponding to \( \tau_3 \) does not pop out of the noise distribution.

![Spectrum of Sample Covariance](image)

Fig. 2.2: Empirical spectrum for spiked model, \( \tau_1 = 1.8^2, \tau_2 = 1.5^2, \tau_3 = 1.1^2, p = 800, n = 4000 \).

The limiting empirical distribution of eigenvalues now being fairly well understood, the next task is to compare the top eigenvectors of the sample and population covariance matrices. Considering the simpler case of a spiked model with \( r = 1 \), [37] showed a “phase transition” effect: as long as the top eigenvalue is above the threshold, the correlation of the top eigenvector with the true principal component tends to a limit (depending on the SNR, \( n \), and \( p \)) between 0 and 1; otherwise, the limiting correlation is zero. Thus, high-dimensional PCA is in general inconsistent. However, if the SNR is sufficiently high, then the top eigenvector of the sample covariance matrix is still a useful approximation.
An important note is that these results are asymptotic with \( n,p \to \infty \). As \([37]\) shows, these results have (slightly more complicated) finite sample analogues with the same spirit.

\[2.3.2. \text{High-dimensional PCA perspective on covariance estimation from data projections.}\] Next, let us connect the spiked model with Problem \([1.1]\). We already mentioned that the spiked model has a “signal plus noise” interpretation. Coming back to our statistical model \((1.1)\), let us consider the case when the \(P_s\) are all identity matrices (and thus \( p = q \)). We get

\[I_s = X_s + \epsilon_s.\]  

Suppose for simplicity that \( X \) has only one degree of freedom: \( X = \xi V \), where \( V \in \mathbb{R}^p \) is fixed and \( \xi \) is a zero mean, unit variance random variable. Suppose that \( \epsilon_s \sim \mathcal{N}(0, I_p) \), so \( \sigma = 1 \) (as in the spiked model above). Then, the eigenvalues of \( \Sigma = \text{Var}[I_s] \) are \( \{\|V\|^2 + 1, 1, \ldots, 1\} \). Note that here, \( \|V\|^2 \) is the signal power and 1 is the noise power. Let us fix \( n,p \). Given these values, let us see what signal power we need so that this signal can be detected from the sample covariance matrix. The condition for the top eigenvalue to be separate from the bulk MP distribution is

\[\|V\|^2 + 1 \geq 1 + \sqrt{\gamma} = 1 + \sqrt{\frac{p}{n}} \iff \|V\|^2 \geq \sqrt{\frac{p}{n}}.\]  

Generalizing to arbitrary noise power \( \sigma^2 \), the top eigenvalue will pop out if

\[\text{SNR} = \frac{\|V\|^2}{\sigma^2} \geq \sqrt{\frac{p}{n}}.\]  

Moreover, in this setup, the squared correlation between the estimated eigenvector and the population eigenvector is

\[\langle V_{\text{PCA}}, V \rangle^2 \approx \frac{(n\|V\|^4)/(p\sigma^4) - 1}{(n\|V\|^4)/(p\sigma^4) + \|V\|^2/\sigma^2}\]  

if \((2.20)\) holds (see, e.g., \([37]\)). The order of approximation above is made precise in \([37]\).

Note that \((2.20)\) has a practical interpretation. When faced with a problem of the form \((2.18)\) with a given \( p \) and SNR, one can determine how many samples one needs in order to detect the signal. If \( V \) represents a spatial object as in the cryo-EM case, then \( p \) can reflect the resolution to which we reconstruct \( V \). Hence, if we have a dataset with a certain number of images \( n \) and a certain estimated SNR, then \((2.20)\) determines the resolution to which \( V \) can be reconstructed from the data.

Given the more general framework of Problem \((1.1)\) we seek a condition of the type \((2.20)\). For cases of low rank \( \Sigma \), such a condition would quantify the relationship between \( n, p, q \), and the SNR needed for the top eigenvectors of \( \Sigma_n \) to be good approximations of the top eigenvectors of \( \Sigma \). Our problem is a generalization of the spiked model \((2.18)\), and in particular, the estimator \( \Sigma_n \) arising from \((2.6)\) reduces to the sample covariance matrix. Indeed, when \( P_s = I_p \) and \( \mu_n = 0 \), \((2.6)\) becomes

\[\Sigma_n = \frac{1}{n} \sum_{s=1}^{n} I_s I_s^H - \sigma^2 I_p.\]
The subtraction of the diagonal matrix $\sigma^2 I_p$ accounts for the fact that the sample covariance matrix $\tilde{\Sigma}_n$ has contributions from both the noise and the signal. It was not necessary to subtract the noise contribution in the case (2.18) because the additive term $\sigma^2 I_p$ simply shifts the eigenvalues and does not change the eigenvectors. In the general case of (2.6), however, note that the contribution of the noise is not necessarily a multiple of the identity matrix. Thus, its effects on the spectrum of $\Sigma_n$ are nontrivial, and it must be subtracted. Hence, the spectrum of interest is not the signal-plus-noise spectrum as in the usual spiked model, but the spectrum of the signal itself.

Having seen that our construction $\Sigma_n$ is a generalization of the sample covariance matrix, we raise open questions about whether and how the high-dimensional PCA properties of the sample covariance matrix generalize to $\Sigma_n$:

1. What is the most natural parameter regime in which to consider $\Sigma_n$? With the addition of the parameter $q$, the traditional regime $p \approx n$ might no longer be appropriate. For example, in the random coordinate selection case with the (extreme) parameter setting $q = 2$, it is expected that $n = p^2 \log p$ samples are needed for $L_n$ to be invertible (by the coupon collector problem).
2. In the case when there is no signal ($X = 0$), we have $I_s = \epsilon_s$. In this case, what is the limiting eigenvalue distribution of $\Sigma_n$ (in an appropriate high-dimensional parameter regime of $n, p, q$)? Is it still the MP law? How does the distribution depend on $P_s$?
3. In the no-signal case, what is the limiting distribution of the largest eigenvalue of $\Sigma_n$? Is it still Tracy-Widom? How does this depend on $n$, $p$, $q$, and $P_s$?
4. When $X$ takes values in a low-dimensional subspace of $\mathbb{R}^p$, we have a generalized spiked model. Is there still a phenomenon in which the limiting distribution is a bulk distribution with a few separated eigenvalues? If so, what is the generalization of the SNR condition (2.20) that would guarantee separation of the top eigenvalues? What would these top eigenvalues be, in terms of the population eigenvalues? Would there still be a phase-transition phenomenon in which the top eigenvectors of $\Sigma_n$ are correlated with the principal components as long as the corresponding eigenvalues are separated from the bulk distribution?

Answering these questions theoretically would require tools from random matrix theory such as the ones used by [21, 3, 37]. We do not attempt to address these issues in this paper, but remark that such results would be very useful theoretical guides for practical applications of our estimator $\Sigma_n$. Our numerical results show that the spectrum of the cryo-EM estimator $\Sigma_n$ has qualitative behavior similar to that of the sample covariance matrix.

At this point, we have concluded the part of our paper focused on the general properties of the estimator $\Sigma_n$. Next, we move on to the cryo-EM heterogeneity problem.

3. Covariance estimation in cryo-EM heterogeneity problem. Now that we have examined the general covariance matrix estimation problem, let us specialize to the cryo-EM case. In this case, the matrices $P_s$ have a specific form: they are a finite-dimensional version of $P_s$ (we defined the latter in (1.2)). We begin by describing the Fourier-domain counterpart of $P_s$, which will be crucial in analyzing the cryo-EM covariance estimation problem. Our Fourier transform convention is

$$\hat{f}(\xi) = \int_{\mathbb{R}^d} f(x)e^{-ix\cdot\xi}dx; \quad f(x) = \frac{1}{(2\pi)^d} \int_{\mathbb{R}^d} \hat{f}(\xi)e^{ix\cdot\xi}d\xi.$$
The following classical theorem in tomography shows that the operator $\mathcal{P}_s$ takes on a simpler form in the Fourier domain.

**Theorem 3.1. (Fourier Projection Slice Theorem).** Suppose $\hat{\phi}_s \in L^2(\mathbb{R}^3) \cap L^1(\mathbb{R}^3)$ and $I_s : \mathbb{R}^2 \to \mathbb{R}$. Then

\[
(3.2) \quad \mathcal{P}_s \hat{\phi}_s = I_s \iff \hat{\mathcal{P}}_s \hat{\phi}_s = \hat{I}_s,
\]

where $\hat{\mathcal{P}}_s : C(\mathbb{R}^3) \to C(\mathbb{R}^2)$ is defined by

\[
(3.3) \quad (\hat{\mathcal{P}}_s \hat{\phi}_s)(\hat{x}, \hat{y}) = \hat{\phi}_s(R_s^T(\hat{x}, \hat{y}, 0)) = \hat{\phi}_s(\hat{x}R_s^1 + \hat{y}R_s^2).
\]

Here, $R_s^i$ is the $i$'th row of $R_s$.

Hence, $\hat{\mathcal{P}}_s$ rotates a function by $R_s$ and then restricts it to the horizontal plane $\hat{z} = 0$. If we let $\rho = (\hat{x}, \hat{y}, \hat{z})$, then another way of viewing $\hat{\mathcal{P}}_s$ is that it restricts a function to the plane $\rho \cdot R_s^3 = 0$.

### 3.1. Infinite-dimensional heterogeneity problem.

Using the Fourier slice theorem, we perform most of our calculations for the heterogeneity problem in the Fourier domain. As a first step in exploring the Fourier-domain geometry of the heterogeneity problem, we shall interpret formula (2.6) in an (unrealistic) continuous, noise-free setting in Fourier space. Thus, all the molecules and images introduced in this example exist in the Fourier domain. The following setup arises if we assume that detector technology improves to the point that images can be measured continuously and that we have access to projection images from all viewing directions.

**Problem 3.2.** Let $\hat{\phi} : \Omega_1 \times \mathbb{R}^3 \to \mathbb{C}$ be a mean-zero random field, where $(\Omega_1, \mathcal{F}_1, \nu_1)$ is a probability space. Here $\hat{\phi}_{\omega_1} = \hat{\phi}(\omega_1, \cdot)$ is a Fourier volume for each $\omega_1 \in \Omega_1$. Let $R : \Omega_2 \to SO(3)$ be a random rotation, where $(\Omega_2, \mathcal{F}_2, \nu_2)$ is another probability space, with $R$ having the uniform distribution over $SO(3)$. Let $\hat{\mathcal{P}}(R_0)$ be the projection operator defined by (3.3) corresponding to a given $R_0 \in SO(3)$. Define the random field $\hat{I} : \Omega_1 \times \Omega_2 \times \mathbb{R}^2 \to \mathbb{C}$ by

\[
(3.4) \quad \hat{I}_{\omega_1, \omega_2} = \hat{\mathcal{P}}(R_{\omega_2})\hat{\phi}_{\omega_1}.
\]

Given the random field $\hat{I}$ and the random rotation $R$, the task is to estimate the covariance function $\hat{\Sigma} : \mathbb{R}^3 \times \mathbb{R}^3 \to \mathbb{C}$ defined

\[
(3.5) \quad \hat{\Sigma}(\rho_1, \rho_2) = \mathbb{E}_{\omega_1}[\hat{\phi}_{\omega_1}(\rho_1)\overline{\hat{\phi}_{\omega_1}(\rho_2)}].
\]

This expectation is defined if $\hat{\phi}$ is a second-order random field, i.e., $\mathbb{E}_{\omega_1}[|\hat{\phi}_{\omega_1}(\rho)|^2] \leq C$ for all $\rho \in \mathbb{R}^3$.

We seek to extend formulas (2.5) and (2.6) to apply to Problem 3.2 by replacing the matrices $P_s$ with their infinite-dimensional counterparts $\hat{P}_s$, and by taking expectations over the spaces of rotations and over the space of conformations. As a first step, note that $\hat{P}_s^H$ should be replaced by the adjoint $\hat{P}_s^*$. Note that for $\hat{I} : \mathbb{R}^2 \to \mathbb{C}, \hat{\psi} : \mathbb{R}^3 \to \mathbb{C}$, we have

\[
\left< \hat{P}_s^* \hat{I}, \hat{\psi} \right> = \left< \hat{I}, \hat{P}_s \hat{\psi} \right> = \int_{\mathbb{R}^2} \hat{I}(\hat{x}, \hat{y})\overline{\hat{\psi}(\hat{x}R_s^1 + \hat{y}R_s^2)}d\hat{x}d\hat{y} = \int_{\mathbb{R}^3} \hat{I}(\rho \cdot R_s^1, \rho \cdot R_s^2)\overline{\hat{\psi}(\rho)}\delta(\rho \cdot R_s^3)d\rho,
\]

from which it follows that in the sense of distributions,

\[
(3.7) \quad (\hat{P}_s^* \hat{I})(\rho) = \hat{I}(\rho \cdot R_s^1, \rho \cdot R_s^2)\delta(\rho \cdot R_s^3).
\]
It follows that

\[(3.8) \quad (\hat{P}_s^* \hat{P}_s \hat{\psi})(\rho) = \hat{\psi}(\rho \cdot R_1) R_1^3 + (\rho \cdot R_2) R_2^3 \delta(\rho \cdot R_3) = \hat{\psi}(\rho) \delta(\rho \cdot R_3).\]

We arrive at the infinite-dimensional interpretation \(\hat{A}\) of the operator \(A_n\) (2.5) by taking the expectation of the LHS of this equation with respect to the space of rotations:

\[
\hat{A} \hat{\psi} = \hat{\psi}(\rho) \int_{\Omega_2} \delta(\rho \cdot R_3) d\nu(\omega_2) = \hat{\psi}(\rho) \frac{1}{4\pi} \int_{S^2} \delta(\rho \cdot \theta) d\theta = \hat{\psi}(\rho) \frac{1}{2|\rho|}. 
\]

(3.9)

Here, \(d\theta\) is the surface measure on \(S^2\) (hence the normalization by \(4\pi\)). We used the homogeneity property of \(\delta\) in going from the first to the second line. The last step holds because the integral over \(S^2\) is equal to the circumference of a great circle on \(S^2\), so it is \(2\pi\). Taking the expectation of the RHS of (2.5) over \(\Omega_1\) and \(\Omega_2\), we arrive at the formula for the recovery of \(\hat{\mu}\) in the fully continuous case:

\[
(3.10) \quad \hat{A} \hat{\mu}(\rho) = \hat{\mu}(\rho) \frac{1}{2|\rho|} = \int_{\Omega_1} \int_{\Omega_2} \hat{P}^*(R_{\omega_2}) \hat{L}_{\omega_1, \omega_2}(\rho) d\nu_2(\omega_2) d\nu_1(\omega_1).
\]

Note that inverting \(\hat{A}\) involves multiplying by the radial factor \(|\rho|\). In tomography, this factor is called the ramp filter \([38]\). Traditional tomographic algorithms proceed by applying the ramp filter to the projection data and then backprojecting. Note that (3.10) has these operations in the reverse order; however, backprojection and application of the ramp filter commute.

Let us now translate (2.6). Note that in the context of this equation, \(\Sigma\) should be interpreted as the operator \(\hat{\psi} \mapsto \int_{\mathbb{R}^3} \hat{\Sigma}(\rho_1, \rho_2) \hat{\psi}(\rho_2) d\rho_2\). Using (3.8), we find that

\[
(3.11) \quad (\hat{P}_s^* \hat{P}_s \hat{\Sigma} \hat{P}_s^* \hat{P}_s \hat{\psi})(\rho_1) = \int_{\mathbb{R}^3} \hat{\Sigma}(\rho_1, \rho_2) \delta(\rho_1 \cdot R_3) \delta(\rho_2 \cdot R_3) \hat{\psi}(\rho_2) d\rho_2
\]

From this it follows that

\[
(3.12) \quad (\hat{P}_s^* \hat{P}_s \hat{\Sigma} \hat{P}_s^* \hat{P}_s)(\rho_1, \rho_2) = \hat{\Sigma}(\rho_1, \rho_2) \delta(\rho_1 \cdot R_3) \delta(\rho_2 \cdot R_3)
\]

Let us interpret this result geometrically. The data we have about \(\hat{\phi}_s\) is the central slice \(\hat{P}_s \hat{\phi}_s\), perpendicular to \(R_3\). The above formula tells us that in terms of the covariance matrix, this information contributes to our knowledge of \(\hat{\Sigma}(\rho_1, \rho_2)\), where \(\rho_1\) and \(\rho_2\) both lie on this central slice.

Taking the expectation over \(\Omega_2\) in the LHS of (2.6), we arrive at an expression for the infinite-dimensional operator \(\hat{L}\) corresponding to \(L_n\):

\[
(\hat{L} \hat{\Sigma})(\rho_1, \rho_2) = \int_{\Omega_2} (\hat{P}^*(R_{\omega_2}) \hat{P}(R_{\omega_2}) \hat{\Sigma} \hat{P}^*(R_{\omega_2}) \hat{P}(R_{\omega_2}))(\rho_1, \rho_2) d\nu_2(\omega_2)
\]

(3.13)

\[
= \hat{\Sigma}(\rho_1, \rho_2) \int_{\Omega_2} \delta(\rho_1 \cdot R_3) \delta(\rho_2 \cdot R_3) d\nu_2(\omega_2)
\]

\[
=: \hat{\Sigma}(\rho_1, \rho_2) K(\rho_1, \rho_2).
\]

Note that the operator \(\hat{L}\) is diagonal; it operates independently on each entry of \(\hat{\Sigma}\). \(\hat{L}\) is a fundamental operator in tomographic inverse problems involving variability;
we term it the projection covariance transform. We replace the RHS of \((2.6)\) by its expectation over the spaces of conformations \(\Omega_1\) and rotations \(\Omega_2\), yielding the equation

\[
(\hat{L}\hat{\Sigma})(\rho_1, \rho_2) = \hat{\Sigma}(\rho_1, \rho_2)K(\rho_1, \rho_2)
\]

\[
= \int_{\Omega_1} \int_{\Omega_2} \hat{\theta}^*\{R_{\omega_2}\}I_{\omega_1,\omega_2}(\rho_1)\hat{\theta}^*\{R_{\omega_2}\}I_{\omega_1,\omega_2}(\rho_2)d\nu_2(\omega_2)d\nu_1(\omega_1).
\]

Thus, \((3.14)\) is the infinite-dimensional version of \((2.6)\).

At this point, let us pause to compute the kernel \(K(\rho_1, \rho_2)\). We have

\[
K(\rho_1, \rho_2) = \frac{1}{4\pi} \int_{S_2} \delta(\rho_1 \cdot \theta)\delta(\rho_2 \cdot \theta)d\theta.
\]

We compute first \(K(\alpha, \beta)\), where \(\alpha, \beta \in S^2\). For fixed \(\alpha\), note that \(\delta(\alpha \cdot \theta)\) induces the uniform measure on the great circle of \(S^2\) perpendicular to \(\alpha\). Similarly, \(\delta(\beta \cdot \theta)\) corresponds to a great circle perpendicular to \(\beta\). Choose \(\alpha \neq \pm \beta\). Then, note that these two great circles intersect in two antipodal points \(\theta = \pm (\alpha \times \beta)/|\alpha \times \beta|\), and the RHS of \((3.15)\) corresponds to the total measure of \(\delta(\alpha \cdot \theta)\delta(\beta \cdot \theta)\) at those two points.

To calculate this measure explicitly, let us define the approximation to the identity \(\delta_\epsilon(t) = \frac{1}{2\pi} \chi_{[-\epsilon, \epsilon]}(t)\). Fix \(\epsilon_1, \epsilon_2 > 0\). Note that \(\delta_{\epsilon_1}(\alpha \cdot \theta)\) is a strip of width \(\epsilon_1\) centered at the great circle perpendicular to \(\alpha\). \(\delta_{\epsilon_2}(\beta \cdot \theta)\) is a strip of width \(\epsilon_2\) intersecting the first strip transversely. For small \(\epsilon_1, \epsilon_2\), the intersection of the two strips consists of two approximately parallelogram-shaped regions, \(S_1\) and \(S_2\). The sine of the angle between the diagonals of each of these regions is \(|\alpha \times \beta|\), and a simple calculation shows that the area of one of these regions is \(2\epsilon_1\epsilon_2/|\alpha \times \beta|\). It follows that

\[
K(\alpha_1, \alpha_2) = \frac{1}{4\pi} \int_{S_2} \delta(\alpha \cdot \theta)\delta(\beta \cdot \theta)d\theta = \lim_{\epsilon_1, \epsilon_2 \to 0} \frac{1}{4\pi} \int_{S_2} \delta_{\epsilon_1}(\alpha \cdot \theta)\delta_{\epsilon_2}(\beta \cdot \theta)d\theta
\]

\[
= \lim_{\epsilon_1, \epsilon_2 \to 0} \frac{1}{4\pi} \int_{S_1 \cup S_2} \frac{1}{2\epsilon_1} \frac{1}{2\epsilon_2}d\theta = \lim_{\epsilon_1, \epsilon_2 \to 0} \frac{1}{4\pi} \frac{2\epsilon_1\epsilon_2}{|\alpha \times \beta|} \frac{1}{2\epsilon_1} \frac{1}{2\epsilon_2}
\]

\[
= \frac{1}{4\pi} \frac{2}{|\alpha \times \beta|}.
\]

Finally, by the homogeneity property of the delta function, we have

\[
K(\rho_1, \rho_2) = \frac{\mathcal{K}\left(\frac{\mu_1}{|\mu_1|}, \frac{\mu_2}{|\mu_2|}\right)}{|\mu_1||\mu_2|} = \frac{1}{4\pi} \frac{2}{|\mu_1 \times \mu_2|}.
\]

Since \(\hat{L}\hat{\Sigma}(\rho_1, \rho_2)\) is aggregated from the values of \(\hat{I}\) on central planes passing through \(\rho_1, \rho_2\) (often there is only one such plane), it follows that the multiplicative kernel \(\mathcal{K}\) in \(\hat{L}\) is proportional to the density of central planes passing through \(\rho_1\) and \(\rho_2\) under the uniform distribution of rotations. Note that this density is nonzero everywhere, which reflects the fact that there is a central plane passing through each pair of points in \(\mathbb{R}^3\). The denominator in \(\mathcal{K}\) is proportional to the magnitudes \(|\rho_1|\) and \(|\rho_2|\), which indicates that there is a greater density of planes passing through pairs of points nearer the origin. Finally, note that \(\mathcal{K}\) varies inversely with the sine of the angle between \(\rho_1\) and \(\rho_2\); indeed, a greater density of central planes pass through a pair of points nearly collinear with the origin. In fact, there is a singularity in \(\mathcal{K}\) when
\(\rho_1, \rho_2\) are linearly dependent, reflecting the fact that infinitely many central planes pass through collinear points. As a way to sum up the geometry encoded in \(K\), note that except for the factor of \(1/4\pi\), \(1/K\) is the area of the triangle spanned by the vectors \(\rho_1\) and \(\rho_2\). For this reason, we call \(1/K\) the triangular area filter.

Note that the triangular area filter is analogous to the ramp filter: it grows linearly with the frequencies \(|\rho_1|\) and \(|\rho_2|\) to compensate for the loss of high frequency information incurred by the geometry of the problem. So, this filter is a generalization of the ramp filter appearing in the estimation of the mean to the covariance estimation problem. The latter has a somewhat more intricate geometry, which is reflected in \(K\).

The properties of \(K\) translate into the robustness of inverting \(\hat{L}\) (supposing we added noise to our model). In particular, the robustness of recovering \(\hat{\Sigma}(\rho_1, \rho_2)\) grows with \(K(\rho_1, \rho_2)\). For example, recovering higher frequencies in \(\hat{\Sigma}\) is more difficult. However, the fact that \(K\) is everywhere positive means that \(\hat{L}\) is at least invertible. This statement is important in proving theoretical results about our estimators, as we saw in Section 2.2. Note that an analogous problem of estimating the covariance matrix of 2D objects from their 1D line projections would not satisfy this condition, because for most pairs of points in \(\mathbb{R}^2\), there is not a line passing through both points as well as the origin.

### 3.2. The discrete covariance estimation problem.

The calculation in the preceding section shows that if we could sample images continuously and if we had access to projection images from all viewing angles, then \(\hat{L}\) becomes a diagonal operator. In this section, we explore the modifications necessary for the realistic case where we must work with finite dimensional representations of volumes and images.

Our idea is to follow what we did in the fully continuous case treated above and estimate the covariance matrix in the Fourier domain. One possibility is to choose a Cartesian basis in the Fourier domain. With this basis, a tempting way to define \(\hat{P}_s\) would be to restrict the Fourier 3D grid to the pixels of a 2D central slice by nearest-neighbor interpolation. This would make \(\hat{P}_s\) a coordinate-selection operator, making \(\hat{L}_a\) diagonal. However, this computational simplicity comes at a great cost in accuracy; numerical experiments show that the interpolation errors are unacceptably large. See also [RS] for a discussion of the deficiencies of this interpolation scheme. Hence, we must choose other bases for the Fourier volumes and images.

For the purposes of this section, let us work abstractly with the finite-dimensional spaces \(\hat{\mathcal{V}} \subset L^2(\mathbb{R}^3)\) and \(\hat{\mathcal{I}} \subset L^2(\mathbb{R}^2)\), which represent Fourier volumes and Fourier images, respectively. For example, \(\hat{\mathcal{V}}\) could be spanned by the Fourier transforms of the 3D Slepian functions. Let

\[
(3.18) \quad \hat{\mathcal{I}} = \text{span}\{\hat{g}_i\}, \quad \hat{\mathcal{V}} = \text{span}\{\hat{h}_j\},
\]

with \(\dim(\hat{\mathcal{I}}) = \hat{q}\) and \(\dim(\hat{\mathcal{V}}) = \hat{p}\). Assume that \(\hat{P}_s(\hat{\mathcal{V}}) \subset \hat{\mathcal{I}}\) (i.e., we do not need to worry about interpolation). Denote by \(\hat{P}_s\) the matrix expression of \(\hat{P}_s\mid_{\hat{\mathcal{V}}}\). Thus, \(\hat{P}_s \in \mathbb{C}^{\hat{q} \times \hat{p}}\). Let \(\hat{X}_1, \ldots, \hat{X}_n\) be the representations of \(\hat{\phi}_1, \ldots, \hat{\phi}_n\) in the basis for \(\hat{\mathcal{V}}\).

Since we are given the images \(I_s\) in the pixel basis \(\mathbb{R}^q\), let us consider how to map these images into \(\hat{\mathcal{I}}\). Let \(Q_1 : \mathbb{R}^q \rightarrow \hat{\mathcal{I}}\) be the mapping which fits (in the least-squares sense) an element of \(\hat{\mathcal{I}}\) to the pixel values defined by a vector in \(\mathbb{R}^q\). It is easiest to express \(Q_1\) in terms of the reverse mapping \(Q_2 : \hat{\mathcal{I}} \rightarrow \mathbb{R}^q\). The \(i\)th column of \(Q_2\) consists of the evaluations of \(g_i\) at the real-domain gridpoints inside the unit disc. It is easy to see that the least-squares method of defining \(Q_1\) is \(Q_1 = Q_2^+ = (Q_2^H Q_2)^{-1} Q_2\). Note that if \(\epsilon_s\) is the noise in \(I_s\), then \(\hat{\epsilon}_s = Q_1 \epsilon_s\) is the noise in \(\hat{I}_s = Q_1 I_s\). We have
We would like the latter matrix to be a multiple of the identity matrix so that the noise in the images remains white. Let us calculate the entries of \( Q_2^H Q_2 \) in terms of the basis functions \( g_i \). Given the fact that we are working with volumes \( h_i \) which have most of their energy concentrated in the unit ball, it follows that \( g_i \) have most of their energy concentrated in the unit disc. If \( x_1, \ldots, x_q \) are the real-domain image gridpoints, it follows that

\[
(Q_2^H Q_2)_{ij} = \sum_{r=1}^{q} \frac{g_i(x_r)g_j(x_r)}{\pi} \int_{|x| \leq 1} \frac{g_i(x)g_j(x)}{x} \, dx \\
\approx \frac{q}{\pi} \langle g_i, g_j \rangle_{L^2(\mathbb{R}^2)} = \frac{q}{\pi} \frac{1}{(2\pi)^2} \langle \hat{g}_i, \hat{g}_j \rangle_{L^2(\mathbb{R}^2)}.
\]

It follows that in order for \( Q_2^H Q_2 \) to be (approximately) a multiple of the identity matrix, we should require \( \{g_i\} \) to be an orthonormal set in \( L^2(\mathbb{R}^2) \). If we let \( c_q = 4\pi^3/q \), then we find that

\[
Q_1 Q_1^H \approx c_q I_q.
\]

It follows that

\[
\hat{I}_s = \hat{P}_s \hat{X}_s + \hat{\epsilon}_s,
\]

where \( \text{Var}[\hat{\epsilon}_s] = \sigma^2 c_q I_q \). Thus, we are in the situation of Problem \[1.1\], with \( \sigma^2 \) replaced by \( \sigma^2 c_q \). We seek \( \hat{\mu} = \mathbb{E}[\hat{X}] \) and \( \hat{\Sigma} = \text{Var}[\hat{X}] \), where \( \hat{X} \) is a random vector on \( C^p \) obtained by expanding the random field \( \phi \) in the basis for \( \hat{V} \). \[2.5\] and \[2.6\] become

\[
\hat{A}_n \hat{\mu}_n := \left( \frac{1}{n} \sum_{s=1}^{n} \hat{P}_s^H \hat{P}_s \right) \hat{\mu}_n = \frac{1}{n} \sum_{s=1}^{n} \hat{P}_s^H \hat{I}_s.
\]

and

\[
\hat{L}_n \hat{\Sigma}_n := \frac{1}{n} \sum_{s=1}^{n} \hat{P}_s^H \hat{P}_s \hat{\Sigma}_n \hat{P}_s^H \hat{P}_s \\
= \frac{1}{n} \sum_{s=1}^{n} \hat{P}_s^H (\hat{I}_s - \hat{P}_s \hat{\mu}_n) (\hat{I}_s - \hat{P}_s \hat{\mu}_n)^H \hat{P}_s - \sigma^2 c_q \frac{1}{n} \sum_{s=1}^{n} \hat{P}_s^H \hat{P}_s = : \hat{B}_n.
\]

### 3.3. Exploring \( \hat{A}_n \) and \( \hat{L}_n \)

In this section, we seek to find expressions for \( \hat{A}_n \) and \( \hat{L}_n \) like those in \[3.1\]. We assumed in Problem \[1.2\] that the rotations \( R_s \) are drawn from a certain distribution over \( SO(3) \); here, we assume that this is the uniform distribution (as we did in \[3.1\]). Our plan is to use the law of large numbers to change averages over \( s \) in the formulas for \( \hat{A}_n \) and \( \hat{L}_n \) to expectations over \( SO(3) \). These will take the form of integrals over \( S^2 \) (instead of over \( SO(3) \), because each term only depends on the viewing angle \( R_s^i \in S^2 \)). The reason for finding these limiting operators is two-fold. First of all, recall that the theoretical results in \[2.2\] depend on the invertibility of these limiting operators. Secondly, we will see later that the limiting operator for \( \hat{L}_n \) is sparse and block diagonal for an appropriate choice of \( \hat{V} \), and thus can be inverted tractably.

In \[3.1\], we worked with functions \( \psi : \mathbb{R}^3 \rightarrow \mathbb{C} \) and \( \Sigma : \mathbb{R}^3 \times \mathbb{R}^3 \rightarrow \mathbb{C} \). Now, we are in a finite-dimensional setup, and we have formulated \[3.22\] and \[3.23\]
in terms of vectors and matrices. Nevertheless, in the finite-dimensional case we can still work with functions as we did in Section 3.1 via the dual representation

\[(3.24) \quad \hat{\psi} \in \mathbb{C}^\hat{p} \leftrightarrow \sum_{i=1}^{\hat{p}} \hat{\psi}_i \hat{h}_i \in \hat{\mathcal{V}}, \quad \hat{\Sigma} \in \mathbb{C}^{\hat{p} \times \hat{p}} \leftrightarrow \sum_{i,j=1}^{\hat{p}} \hat{\Sigma}_{i,j} \hat{h}_i \otimes \hat{h}_j \in \hat{\mathcal{V}} \otimes \hat{\mathcal{V}}, \]

where we define

\[(3.25) \quad (\hat{h}_i \otimes \hat{h}_j)(\rho_1,\rho_2) = \hat{h}_i(\rho_1)\hat{h}_j(\rho_2). \]

Thus, we identify \(\mathbb{C}^\hat{p}\) and \(\mathbb{C}^{\hat{p} \times \hat{p}}\) with spaces of bandlimited functions. For these identifications to be isometries, we must endow \(\hat{\mathcal{V}}\) with an inner product for which \(\hat{h}_i\) are orthonormal. We consider a family of inner products, weighted by radial functions \(w(|\rho|)\):

\[(3.26) \quad \langle \hat{h}_i, \hat{h}_j \rangle_{L^2_w(\mathbb{R}^3)} = \int_{\mathbb{R}^3} \overline{\hat{h}_i(\rho)} \hat{h}_j(\rho) w(|\rho|) d\rho = \delta_{ij}. \]

The inner product on \(L^2_w(\mathbb{R}^3 \times \mathbb{R}^3)\) is inherited from that of \(L^2_w(\mathbb{R}^3)\).

Note that \(A_n\) and \(L_n\) both involve the projection-backprojection operator \(\hat{P}_s^H \hat{P}_s\). Let us see how to express \(\hat{P}_s^H \hat{P}_s\) as an operator on \(\hat{\mathcal{V}}\). The \(i\)'th column of \(\hat{P}_s\) is the representation of \(\hat{P}_s \hat{h}_i\) in the orthonormal basis for \(\hat{\mathcal{I}}\). Hence, using the isomorphism \(\hat{\mathcal{C}}^q \leftrightarrow \hat{\mathcal{I}}\) and reasoning along the lines of (3.6), we find that

\[(3.27) \quad \langle \hat{P}_s^H \hat{P}_s \rangle_{i,j} = \langle \hat{P}_s \hat{h}_i, \hat{P}_s \hat{h}_j \rangle_{L^2(\mathbb{R}^3)} = \int_{\mathbb{R}^3} \overline{\hat{h}_i(\rho)} \hat{h}_j(\rho) \delta(\rho \cdot R^3_s) d\rho. \]

Hence, if \(\hat{\psi} = \sum_{j=1}^{\hat{p}} \hat{\psi}_j \hat{h}_j \in \hat{\mathcal{V}},\) then

\[(3.28) \quad (\hat{P}_s^H \hat{P}_s) \hat{\psi} = (\hat{P}_s^H \hat{P}_s) \sum_{j=1}^{\hat{p}} \hat{\psi}_j \hat{h}_j = \sum_{i,j=1}^{\hat{p}} \hat{h}_i \hat{\psi}_j \int_{\mathbb{R}^3} \overline{\hat{h}_i(\rho)} \hat{h}_j(\rho) \delta(\rho \cdot R^3_s) d\rho \]

To interpret \(\hat{P}_s^H \hat{P}_s\) as an operator on \(\hat{\mathcal{V}}\) with a weighted \(L^2\) norm, note that

\[(3.29) \quad (\hat{P}_s^H \hat{P}_s) \hat{\psi} = \sum_{i=1}^{\hat{p}} \int_{\mathbb{R}^3} \overline{\hat{h}_i(\rho)} \left( \hat{\psi}(\rho) \frac{1}{w(|\rho|)} \delta(\rho \cdot R^3_s) \right) w(|\rho|) d\rho. \]

Hence, we find that

\[(3.30) \quad (\hat{P}_s^H \hat{P}_s) \hat{\psi}(\rho) = \pi^w_{\mathcal{V}} \left( \hat{\psi}(\rho) \frac{\delta(\rho \cdot R^3_s)}{w(|\rho|)} \right), \]

where \(\pi^w_{\mathcal{V}} : L^2_w(\mathbb{R}^3) \rightarrow \hat{\mathcal{V}}\) is the orthogonal projection onto the finite-dimensional subspace \(\hat{\mathcal{V}}\) (with respect to the weight \(w\)).

In analogy with (3.9), the law of large numbers implies that \(A_n \rightarrow \hat{A}\), where

\[(3.31) \quad (\hat{A} \hat{\psi})(\rho) = \pi^w_{\mathcal{V}} \left( \hat{\psi}(\rho) \frac{1}{w(|\rho|)} \frac{1}{4\pi} \int_{S^2} \delta(\rho \cdot \theta) d\theta \right) = \pi^w_{\mathcal{V}} \left( \hat{\psi}(\rho) \frac{1}{2|\rho|w(|\rho|)} \right) \]
Note that with \( w = 1 \), \( \hat{A} \) is similar to the operator \( \hat{A} \) obtained in (3.9), with the addition of the “low-pass filter” \( \pi_\gamma^w \). As an alternative to \( w = 1 \), consider \( w(|\rho|) = 1/|\rho| \). This weight has the effect of canceling the ramp filter. We obtain

\[
(3.32) \quad (\hat{A}\psi)(\rho) = \pi_\gamma^w \left( \frac{1}{2} \psi(\rho) \right) = \frac{1}{2} \psi(\rho), \quad w(|\rho|) = 1/|\rho|.
\]

Thus, for this weight we find that \( \hat{A} = \frac{1}{2} \hat{\psi} \).

Using (3.28), we obtain that for \( \hat{\Sigma} \in \mathcal{V} \otimes \hat{\mathcal{V}} \),

\[
(3.33) \quad (\hat{P}_s^H \hat{P}_s \hat{\Sigma} \hat{P}_s \hat{P}_s)(\rho_1, \rho_2)
= \sum_{i_1, i_2 = 1}^\hat{p} \hat{h}_{i_1} \otimes \hat{h}_{i_2} \int_{R^3 \times R^3} \hat{h}_{i_1} \otimes \hat{h}_{i_2}(\rho_1, \rho_2) \hat{\Sigma}(\rho_1, \rho_2) \delta(\rho_1 \cdot R_3) \delta(\rho_2 \cdot R_3) d\rho_1 d\rho_2
= \sum_{i_1, i_2 = 1}^\hat{p} \hat{h}_{i_1} \otimes \hat{h}_{i_2} \int_{R^3 \times R^3} \hat{h}_{i_1} \otimes \hat{h}_{i_2}(\rho_1, \rho_2) \hat{\Sigma}(\rho_1, \rho_2) \delta(\rho_1 \cdot R_3^2) \delta(\rho_2 \cdot R_3^2) w(|\rho_1|) w(|\rho_2|) d\rho_1 d\rho_2
= \pi_\gamma^w \left( \hat{\Sigma}(\rho_1, \rho_2) \frac{1}{w(|\rho_1|) w(|\rho_2|)} \delta(\rho_1 \cdot R_3^2) \delta(\rho_2 \cdot R_3^2) \right).
\]

Then, the law of large numbers implies that \( \hat{L}_n \to \hat{L} \), where

\[
(3.34) \quad (\hat{L}\hat{\Sigma})(\rho_1, \rho_2) = \pi_\gamma^w \left( \hat{\Sigma}(\rho_1, \rho_2) \frac{1}{w(|\rho_1|) w(|\rho_2|)} \frac{1}{4\pi} \int_{S^2} \delta(\rho_1 \cdot \theta) \delta(\rho_2 \cdot \theta) d\theta \right)
= \pi_\gamma^w \left( \hat{\Sigma}(\rho_1, \rho_2) \frac{K(\rho_1, \rho_2)}{w(|\rho_1|) w(|\rho_2|)} \right)
\]

Thus, \( \hat{L} \) is a discrete version of \( \hat{L} \) (recall (3.13)), which we call the discrete projection covariance transform. In contrast to its infinite-dimensional counterpart, note that this operator is not necessarily diagonal due to the addition of the low-pass filter. Note that for \( w(|\rho|) = 1/|\rho| \), the multiplicative factor above becomes (a constant multiple of) \( |\rho_1|/|\rho_2|/|\rho_1 \times \rho_2| \), which is \( 1/\sin(\gamma) \), where \( \gamma \) is the angle between the vectors \( \rho_1 \) and \( \rho_2 \). The factor remains singular for \( \rho_1, \rho_2 \) collinear, but the radial dependency is eliminated. Thus, inverting \( \hat{L} \) becomes better conditioned, because it requires multiplication by \( \sin \gamma \), a bounded quantity. By contrast, \( w = 1 \) requires multiplication by an increasing function of frequency.

**3.4. Properties of \( \hat{A} \) and \( \hat{L} \).** In this section, we will prove several results about \( \hat{A} \) and \( \hat{L} \), defined in (3.31) and (3.34).

First of all, note that \( \hat{A} \) is self-adjoint and positive semidefinite because each \( \hat{A}_n \) satisfies this property. In the next proposition, we bound the minimum eigenvalue of \( \hat{A} \) from below.

**Proposition 3.3.** Let \( M_{\omega_{\max}}(w) = \max_{|\rho| \leq \omega_{\max}} |\rho| w(|\rho|) \). Then,

\[
(3.35) \quad \lambda_{\min}(\hat{A}) \geq \frac{1}{2M_{\omega_{\max}}(w)}.
\]
\[ \langle \hat{A}\hat{\psi}, \hat{\psi} \rangle = \langle \pi^w_\hat{V} \left( \hat{\psi} \frac{1}{2|\rho|w(|\rho|)} \right), \hat{\psi} \rangle = \langle \hat{\psi} \frac{1}{2|\rho|w(|\rho|)}, \hat{\psi} \rangle = \int_{B_{\omega_{\max}}} |\hat{\psi}(\rho)|^2 \frac{1}{2|\rho|w(|\rho|)} d\rho \geq \frac{1}{2M_{\omega_{\max}}(w)} \|\hat{\psi}\|^2. \]

All the inner products above are in \( L^2_w(\mathbb{R}^3) \). The second step holds because \( \hat{\psi} \in \hat{V} \).

The third holds because \( \hat{\psi} \) is supported in \( B_{\omega_{\max}} \). \( \square \)

In particular, if \( M_{\omega_{\max}}(w) < \infty \), Proposition 3.3 implies that \( \hat{A} \) is invertible.

Next, we prove a similar proposition about \( \hat{L} \).

**PROPOSITION 3.4.** \( \hat{L} \) is self-adjoint. Moreover,

\[ \lambda_{\min}(\hat{L}) \geq \frac{1}{2\pi M^2_{\omega_{\max}}(w)}, \]

where \( M_{\omega_{\max}}(w) \) is as defined in Proposition 3.3.

**Proof.** Let \( \hat{\Sigma}_1, \hat{\Sigma}_2 \in \hat{V} \otimes \hat{V} \), and define \( K_w(\rho_1, \rho_2) = K(\rho_1, \rho_2)/w(|\rho_1|)w(|\rho_2|) \).

Then, we have

\[ \langle \hat{L}\hat{\Sigma}_1, \hat{\Sigma}_2 \rangle = \langle \pi^w_\hat{V}((\hat{\Sigma}_1 K_w)) \hat{\Sigma}_2 \rangle = \langle \hat{\Sigma}_1 K_w, \hat{\Sigma}_2 \rangle = \langle \hat{\Sigma}_1, \hat{\Sigma}_2 K_w \rangle = \langle \hat{\Sigma}_1, \pi^w_\hat{V}(\hat{\Sigma}_2 K_w) \rangle = \langle \hat{\Sigma}_1, \hat{L}\hat{\Sigma}_2 \rangle. \]

All inner products above are in \( L^2_w(\mathbb{R}^3 \times \mathbb{R}^3) \). The second and fourth equalities follow from the fact that \( \hat{\Sigma}_1, \hat{\Sigma}_2 \in \hat{V} \otimes \hat{V} \), while the third follows because \( K_w \) is real. Hence, \( \hat{L} \) is self-adjoint.

To bound the minimum eigenvalue of \( \hat{L} \), note first that

\[ \min_{\rho_1, \rho_2 \in B_{\omega_{\max}}} K_w(\rho_1, \rho_2) = \min_{\rho_1, \rho_2 \in B_{\omega_{\max}}} \frac{1}{2\pi|\rho_1 \times \rho_2|w(|\rho_1|)w(|\rho_2|)} \geq \frac{1}{2\pi M^2_{\omega_{\max}}(w)}. \]

It follows that if \( \hat{\Sigma} \in \hat{V} \otimes \hat{V} \), then

\[ \langle \hat{L}\hat{\Sigma}, \hat{\Sigma} \rangle = \langle \hat{\Sigma} K_w, \hat{\Sigma} \rangle = \int_{B_{\omega_{\max}} \times B_{\omega_{\max}}} |\hat{\Sigma}(\rho_1, \rho_2)|^2 K_w(\rho_1, \rho_2) d\rho_1 d\rho_2 \geq \frac{1}{2\pi M^2_{\omega_{\max}}(w)} \|\hat{\Sigma}\|^2, \]

All inner products are taken in \( L^2_w(\mathbb{R}^3 \times \mathbb{R}^3) \). In the second step, we use the fact that \( \hat{\Sigma} \) is supported on \( B_{\omega_{\max}} \times B_{\omega_{\max}} \). This proves (3.37). \( \square \)

Thus, \( \hat{L} \) is invertible if \( M_{\omega_{\max}}(w) < \infty \). Note the maximum eigenvalue of \( \hat{L} \) cannot be bounded as easily, since \( K_w \) is not bounded from above. A bound on \( \lambda_{\max}(\hat{L}) \) can be obtained by using the fact that the bandlimited \( \hat{\Sigma} \) can have only be concentrated to a limited extent around the singular set \( \rho_1 = \pm \rho_2 \).

Finally, we prove another property of \( \hat{L} \): that it commutes with rotations. Let us define a rotation by \( R \in SO(3) \) of a function \( \Sigma : \mathbb{R}^3 \times \mathbb{R}^3 \rightarrow \mathbb{C} \) as \( \Sigma_R(\rho_1, \rho_2) = \Sigma(R\rho_1, R\rho_2) \), where \( Rp \) is the point in \( \mathbb{R}^3 \) obtained by rotating \( \rho \in \mathbb{R}^3 \) by \( R \in SO(3) \).

**PROPOSITION 3.5.** Suppose that the subspace \( \hat{V} \) is closed under rotations. Then, for any \( \hat{\Sigma} \in \hat{V} \otimes \hat{V} \) and \( R \in SO(3) \), we have

\[ (\hat{L}\hat{\Sigma})_R = \hat{L}\hat{\Sigma}_R. \]
Proof. First, note that \((K_w)_R = K_w\). Second, note that for any \(\hat{M} : \mathbb{R}^3 \times \mathbb{R}^3 \to \mathbb{C}\), we have \(\pi_{\hat{V} \otimes \hat{V}}(\hat{M}_R) = (\pi_{\hat{V} \otimes \hat{V}}(M))_R\). This follows because \(\hat{V}\) is closed under rotations. Putting these facts together, we find

\[
(3.42) \quad (\hat{L}_R) = \pi_{\hat{V} \otimes \hat{V}}((\hat{\Sigma})_R) = \pi_{\hat{V} \otimes \hat{V}}(\hat{\Sigma} R K_w) = \hat{L}_R.
\]

\[\]

This property of \(\hat{L}\) is to be expected, given the rotationally symmetric nature of this operator. This suggests that \(\hat{L}\) can be studied further using the representation theory of \(SO(3)\).

Finally, let us check that the assumptions of Propositions 2.1 and 2.2 hold in the cryo-EM case. Through the above propositions, we have proved that as long as \(M_{\text{max}}(w) < \infty\), the limiting operators \(\hat{A}\) and \(\hat{L}\) are invertible. Of course, it is always possible to choose such a weight \(w\). In particular the weights already considered, \(w = 1, 1/|\rho|\) satisfy this property. Moreover, by rotational symmetry, \(\|\hat{P}_s\|_2\) is independent of \(R_s\), and so of course this quantity is uniformly bounded. Thus, we have checked all the necessary assumptions to arrive at the following conclusion:

**Proposition 3.6.** If we neglect the errors incurred in moving to the Fourier domain and assume that the rotations are drawn uniformly from \(SO(3)\), then the estimators \(\hat{\mu}_n\) and \(\hat{\Sigma}_n\) obtained from (3.22) and (3.23) are asymptotically consistent.

Finally, note that all the assumptions of Proposition 2.3 hold in the cryo-EM case, except the almost sure boundedness of the noise \(\epsilon_s\), which we have modeled as Gaussian. As we mentioned in Section 2.2, the boundedness assumption can be replaced by a sub-gaussian assumption, so a version of the finite sample result will hold for the cryo-EM case as well. However, we will not provide a precise statement of such a result here.

**4. Using \(\hat{\mu}_n, \hat{\Sigma}_n\) to determine the conformations.** To solve Problem 1.2, we must do more than just estimate \(\hat{\mu}\) and \(\hat{\Sigma}\). We must also estimate \(C, \hat{X}_C\), and \(p_c\). Note that \(\hat{X}_c\) is the coefficient vector of \(\phi_c\) in the basis for \(\hat{V}\). Once we solve (3.22) and (3.23) for \(\hat{\mu}_n\) and \(\hat{\Sigma}_n\), we perform the following steps.

From the discussion on high-dimensional PCA in Section 2.3, we expect to determine the number of structural states by inspecting the spectrum of \(\hat{\Sigma}_n\). We expect the spectrum of \(\hat{\Sigma}_n\) to consist of a bulk distribution along with \(C - 1\) separate eigenvalues (assuming the SNR is sufficiently high), a fact confirmed by our numerical results. Hence, given \(\Sigma_n\), we can estimate \(C\).

Next, we discuss how to reconstruct \(\hat{X}_1, \ldots, \hat{X}_C\) and \(p_1, \ldots, p_C\). Our approach is similar to Penczek’s [44]. By the principle of PCA, the leading eigenvectors of \(\hat{\Sigma}\) span the space of mean subtracted volumes \(\hat{X}_1 - \hat{\mu}, \ldots, \hat{X}_C - \hat{\mu}\). If \(V_1, \ldots, V_{C-1}\) are the leading eigenvectors of \(\Sigma_n\), we can write

\[
(4.1) \quad \hat{X}_s \approx \hat{\mu}_n + \sum_{c'=1}^{C-1} \alpha_{s,c'} V_{c'}.
\]

Note that there is only approximate equality because we have replaced the mean \(\hat{\mu}\) by the estimated mean \(\hat{\mu}_n\), and the eigenvectors of \(\hat{\Sigma}\) by those of \(\Sigma_n\). We would like to recover the coefficients \(\alpha_s = (\alpha_{s,1}, \ldots, \alpha_{s,C-1})\), but the \(\hat{X}_s\) are unknown.
Nevertheless, if we project the above equation by \( \hat{P}_s \), then we get

\[
\sum_{c'=1}^{C-1} \alpha_{s,c'} \hat{P}_s \hat{V}^{c'} \approx \hat{P}_s \hat{X}_s - \hat{P}_s \hat{\mu}_n \approx \hat{I}_s - \hat{P}_s \hat{\mu}_n.
\]

Note that we have approximated \( \hat{P}_s \hat{X}_s \) by \( \hat{I}_s \), neglecting the noise term. For each \( s \), we can now solve this equation for the coefficient vector \( \alpha_s \) in the least-squares sense. This gives us \( n \) vectors in \( \mathbb{C}^{C-1} \). These should be clustered around \( C \) points \( \alpha^c = (\alpha^c_1, \ldots, \alpha^c_{C-1}) \) for \( c = 1, \ldots, C \), corresponding to the \( C \) underlying volumes.

At this point, Penczek proposes to perform \( K \)-means clustering on \( \alpha_s \) in order to deduce which image corresponds to which class. However, if the images are too noisy, then it would be impossible to separate the classes via clustering. Note that in order to reconstruct the original volumes, all we need are the means of the \( C \) clusters of coordinates. If the mean and eigenvectors are approximately correct, then the main source of noise in the coordinates is the Gaussian noise in the images. It follows that the distribution of the coordinates in \( \mathbb{C}^{C-1} \) is a mixture of Gaussians. Hence, we can find the means \( \alpha^c \) of each cluster using either an EM algorithm (of which the \( K \)-means algorithm used by Penczek is a limiting case [6]) or the method of moments, e.g. [23]. In the current implementation, we use an EM algorithm. Once we have the \( C \) mean vectors, we can reconstruct the original volumes using (4.1). Putting these steps together, we arrive at a high-level algorithm to solve the heterogeneity problem (see Algorithm 1).

**Algorithm 1** High-level algorithm for heterogeneity problem (Problem 1.2).

1: **Input:** \( n \) images \( I_s \) and the corresponding rotations \( R_s \)
2: Estimate the noise level \( \sigma^2 \) from the corner regions of the images.
3: Choose bases for \( \hat{I} \) and \( \hat{V} \).
4: Map the images \( I_s \) into \( \hat{I}_s \in \hat{I} \).
5: Estimate \( \hat{\mu}_n, \hat{\Sigma}_n \) by solving (3.22) and (3.23).
6: Compute the eigendecomposition of \( \hat{\Sigma}_n \) and estimate its rank \( C \).
7: Estimate each \( \alpha_s \in \mathbb{C}^{C-1} \) by solving (4.2) using least squares.
8: Find \( \alpha^c \) and \( p_c \) by applying either EM or a method of moments algorithm to \( \alpha_s \).
9: Using \( \alpha^c \), find the \( \phi^1, \ldots, \phi^C \) molecular volumes from (4.1). Map volumes back to real domain for visualization.
10: **Output:** \( C, \phi^1, \ldots, \phi^C, p_1, \ldots, p_C \).

5. **Practical approach to inverting \( \hat{L}_n \).** Recall that \( \hat{\Sigma} \in \mathbb{C}^{p \times p} \), where \( p \) is defined in (1.4). Thus, for a bandlimit \( \omega_{\text{max}} \), \( \hat{\Sigma} \) has size on the order of \( \omega_{\text{max}}^6 \times \omega_{\text{max}}^6 \), from which it follows that \( \hat{L}_n \) has size \( \omega_{\text{max}}^6 \times \omega_{\text{max}}^6 \). Even for a small \( N_{\text{res}} \) value such as \( 17 \), \( \hat{L}_n \) is a square matrix with each dimension \( 1.8 \times 10^6 \). Just storing such a large \( \hat{L}_n \) requires over 23 terabytes, and inverting this matrix na"ively is completely intractable. This is the main practical challenge we face in implementing Algorithm 1. We overcome this challenge by approximating \( \hat{L}_n \) with \( L \); we show that the latter operator has a sparse block diagonal structure in a basis orthogonal with respect to an appropriately chosen weight \( w \).

5.1. **Making \( \hat{L} \) sparse and block diagonal.** In this section, we write down an expression for an individual element of \( \hat{L} \), and see what basis functions \( h_i \) we need to make \( \hat{L} \) sparse and block diagonal.
First, let us fix a functional form for the basis elements \( \hat{h}_i \): let

\[
\hat{h}_i(r, \alpha) = f_i(r) a_i(\alpha),
\]

where \( f_i : [0, \infty) \to \mathbb{R} \) are radial functions and \( a_i : S^2 \to \mathbb{C} \) are spherical harmonics. Note, for example, that the 3D Slepian functions have this form \[56\] eq. 110. If \( \hat{h}_i \) are orthogonal with respect to the weight \( w \), then

\[
\langle f_i, f_j \rangle_{L^2_w(\mathbb{R})} (a_i, a_j)_{L^2(S^2)} = \delta_{ij},
\]

where we use \( L^2_w \) as a shorthand for \( L^2_w([0, \infty)) \). The 3D Slepian functions satisfy the above condition with \( w = 1 \), because they are orthogonal in \( L^2(\mathbb{R}^3) \).

Next, we write down the formula for an element \( \hat{L}_{i_1, i_2, j_1, j_2} \) (here, \( j_1, j_2 \) are the indices of the input matrix, and \( i_1, i_2 \) are the indices of the output matrix). From \[3.34\], we find

\[
\hat{L}_{i_1, i_2, j_1, j_2} = \left\langle \hat{L}(\hat{h}_{j_1} \otimes \hat{h}_{j_2}, \hat{h}_{i_1} \otimes \hat{h}_{i_2}) \right\rangle_{L^2_w(\mathbb{R}^3 \times \mathbb{R}^3)}
\]

\[
= \int_{\mathbb{R}^3 \times \mathbb{R}^3} \frac{K(\rho_1, \rho_2)}{w(\rho_1)w(\rho_2)} w(|\rho_1|)w(|\rho_2|) d\rho_1 d\rho_2
\]

\[
= \frac{1}{2\pi r_1 r_2 |\alpha \times \beta|} \int_{S^2 \times S^2} \int_{[0, \infty)^2} (h_{i_1} \otimes h_{i_2})(\rho_1, \rho_2) (h_{j_1} \otimes h_{j_2})(\rho_1, \rho_2) \frac{1}{r_1^2 r_2^2} dr_1 dr_2 d\alpha d\beta
\]

\[
= \langle f_{i_1}, f_{i_2} \rangle_{L^2_w} \langle f_{j_1}, f_{j_2} \rangle_{L^2_w} \int_{S^2 \times S^2} \frac{a_{i_1} \otimes a_{i_2}}{a_{j_1} \otimes a_{j_2}} (\alpha, \beta) (\alpha, \beta) \frac{1}{2\pi |\alpha \times \beta|} d\alpha d\beta.
\]

Thus, we see that to make many of the radial inner products in \( \hat{L} \) vanish, we see from \[5.2\] that the correct weight is

\[
w(r) = \frac{1}{r}.
\]

Recall that this is also the weight needed to cancel the ramp filter in \( \hat{A} \) (see \[3.32\]). From this point on, \( w \) will represent the weight above, and we will work in the corresponding weighted \( L^2 \) space.

What are sets of functions of the form \(5.1\) that are orthonormal in \( L^2_w(\mathbb{R}^3) \)? Consider first \( \mathcal{V} \) obtained by taking the Fourier transforms of the functions in \( \mathcal{B} \) (the space to which we originally assumed our real-domain functions belonged). Then, we would have

\[
\hat{h}_{k, \ell, m}(r, \alpha) = f_{k, \ell}(r) Y_{\ell}^m(\alpha).
\]

These functions are orthonormal, but with the weight \( w = 1 \). Consider modifying this construction by replacing the \( f_{k, \ell}(r) \) by the radial functions arising in the 2D Slepian functions. These satisfy the property

\[
\langle f_{k_1, \ell_1}, f_{k_2, \ell_2} \rangle_{L^2_w} = 0 \quad \text{if} \quad \ell_1 = \ell_2, k_1 \neq k_2.
\]

With this property \(5.5\) becomes orthonormal in \( L^2_w(\mathbb{R}^3) \). This gives \( \hat{L} \) a certain degree of sparsity. However, note that the construction \(5.5\) has different families of \( L^2 \)-orthogonal radial functions corresponding to each angular function. Thus, we
only have orthogonality of the radial functions \( f_{k_1, \ell_1} \) and \( f_{k_2, \ell_2} \) when \( \ell_1 = \ell_2 \). Thus, many of the terms \( \langle f_i, f_j \rangle_{L^2} \) in (5.3) are still nonzero.

A drastic improvement on (5.5) would be to devise an orthogonal basis in \( L^2_{w_r} \) that used one set of \( r \)-weighted orthogonal functions \( f_k \) for all the angular functions, rather than a separate set for each angular function. Namely, suppose we chose

\[
\hat{h}_{k, \ell, m}(r, \alpha) = f_k(r)Y^m_\ell(\alpha), \quad (k, \ell, m) \in J,
\]

where \( J \) is some indexing set. Note that \( f_k \) and \( J \) need to be carefully constructed so that the above set of basis functions approximately span the space of 3D Slepian functions (see Section 5.2). Note that

\[
f_k(r)Y^m_\ell(\alpha) = \hat{h}_{k, \ell, m}(r, -\alpha) = f_k(-r)Y^{m}_{\ell, m}(-\alpha) = (-1)^\ell f_k(-r)Y^m_{\ell, m}(\alpha).
\]

Here, we assume that each \( f_k \) is either even or odd at the origin, and we extend \( f_k(r) \) to \( r \in \mathbb{R} \) according to this parity. The above calculation implies that \( f_k \) should have the same parity as \( \ell \). Let us suppose that \( f_k \) has parity according to the parity of \( k \). Then, it follows that \( (k, \ell, m) \in J \) only if \( k = \ell \mod 2 \). Thus, \( h_{k, \ell, m} \) will be orthonormal in \( L^2_w \) if

\[
\{ f_k : k = 0 \mod 2 \} \quad \text{and} \quad \{ f_k : k = 1 \mod 2 \}
\]

are orthonormal in \( L^2_w \).

If we let \( (k_i, \ell_i, m_i) \) be the indices corresponding to \( i \), then we claim that the above construction implies

\[
\hat{L}_{i_1, i_2, j_1, j_2} = \delta_{k_{i_1} k_{i_2}} \delta_{k_{j_1} k_{j_2}} \int_{S^2 \times S^2} \frac{(a_{i_1} \otimes a_{j_1})(\alpha, \beta)(a_{i_2} \otimes a_{j_2})(\alpha, \beta)}{2\pi|\alpha \times \beta|} d\alpha d\beta.
\]

This statement does not follow immediately from (5.9), because we still need to check the case when \( k_{i_1} \neq k_{j_2} \mod 2 \). Note that in this case, the dependence on \( \alpha \) in the integral over \( S^2 \times S^2 \) is odd, and so indeed \( \hat{L}_{i_1, i_2, j_1, j_2} = 0 \) in that case as well. If \( \hat{V}_k \) is the space spanned by \( f_k(r)Y^m_\ell(\alpha) \) for all \( \ell, m \), then the above implies that \( \hat{L} \) operates separately on each \( \hat{V}_k \oplus \hat{V}_{-k} \). In the language of matrices, this means that if we divide \( \Sigma_n \) into blocks \( \Sigma_{n_{k_1, k_2}} \) based on radial indices, \( \hat{L} \) operates on these blocks separately. We denote each of the corresponding “blocks” of \( \hat{L} \) by \( \hat{L}^{k_1, k_2} \). Let us re-index the angular functions so that \( a_{i_1}^k \) denotes the \( i \)th angular basis function paired with \( f_k \). From (5.4), we have

\[
\hat{L}^{k_1, k_2}_{i_1, i_2, j_1, j_2} = \int_{S^2 \times S^2} \frac{(a_{i_1}^k \otimes a_{j_1}^k)(\alpha, \beta)(a_{i_2}^k \otimes a_{j_2}^k)(\alpha, \beta)}{2\pi|\alpha \times \beta|} d\alpha d\beta.
\]

This block diagonal structure of \( \hat{L} \) makes it much easier to invert. Nevertheless, the blocks \( \hat{L}^{k_1, k_2} \) are square matrices whose dimensions grow quartically in \( k_1 \) and \( k_2 \). Hence, even inverting each block can be difficult. Remarkably, it turns out that each block of \( \hat{L} \) is sparse. In Appendix B, we simplify the above integral over \( S^2 \times S^2 \). Then, (5.11) becomes

\[
\hat{L}^{k_1, k_2}_{i_1, i_2, j_1, j_2} = \sum_{\ell, m} c(\ell) C_{\ell, m}(a_{i_1}^{k_1} a_{j_1}^{k_1}) C_{\ell, m}(a_{i_2}^{k_2} a_{j_2}^{k_2}),
\]
where the constants $c(\ell)$ are defined in (B.8) and $C_{\ell,m} \hat{\psi}$ is the $\ell, m$ coefficient in the spherical harmonic expansion of $\hat{\psi} : S^2 \to \mathbb{C}$. It turns out that the above expression is zero for most sets of indices. To see why, recall that the functions $a_i^k$ are spherical harmonics. It is known that the product $Y^m_\ell Y^m_{\ell'}$ can be expressed as a linear combination of harmonics $Y^M_L$, where $M = m + m'$ and $|\ell - \ell'| \leq L \leq \ell + \ell'$. Thus, $C^m_\ell(a_i^k)$ are sparse vectors, which shows that each block $\hat{L}_{k_1,k_2}$ is sparse. For example, $\hat{L}_{15,15}$ has each dimension approximately $2 \times 10^4$. However, only about $10^7$ elements of this block are nonzero, which is only about 3% of its the total number of entries. This is about the same number of elements as a $3000 \times 3000$ full matrix, which is still a reasonable size to work with on a computer. In general, numerical experiments confirm the following conjecture:

**Conjecture 5.1.**

\[ \text{nnz}(\hat{L}_{k_1,k_2}) \leq \frac{1}{k_1 + k_2 + 1} \left( \frac{(k_1 + 1)(k_1 + 2)(k_2 + 1)(k_2 + 2)}{4} \right)^2 , \]

where $\text{nnz}(M)$ is the number of nonzero elements in $M$, and the term involving the square is the total number of elements in $\hat{L}_{k_1,k_2}$.

Thus, we have found a way to tractably solve the covariance matrix estimation problem: reconstruct $\hat{\Sigma}_n$ (approximately) by solving the (sparse) linear systems

\[ \hat{L}_{k_1,k_2} \hat{\Sigma}_{k_1,k_2} = \hat{B}_{k_1,k_2}, \]

where we recall that $\hat{B}_n$ is the RHS of (3.23). Also, using the fact that $\hat{A}_n \approx A = \frac{1}{2} I_q$, we can estimate $\hat{\mu}_n$ from

\[ \hat{\mu}_n = \frac{2}{n} \sum_{s=1}^{n} \hat{P}_s^H \hat{I}_s. \]

In the next two sections, we discuss how to choose the radial components $f_k(r)$ and define $\hat{\mathcal{V}}$ more precisely.

5.2. Constructing $f_k(r)$ and the space $\hat{\mathcal{V}}$. We have discussed so far that

\[ \hat{\mathcal{V}} = \text{span}\{(f_k(r)Y^m_\ell(\theta, \phi) : (k, \ell, m) \in J}\}, \]

with $(k, \ell, m) \in J$ only if $k = \ell \mod 2$. Moreover, we have required the orthonormality condition (5.9). However, recall that we initially assumed that our real-domain functions $\phi_s$ belonged to the space of Slepian functions $\mathcal{B}$. Thus, we must choose $\hat{\mathcal{V}}$ to approximate the image of $\mathcal{B}$ under the Fourier transform. Hence, the basis functions $f_k(r)Y^m_\ell(\theta, \varphi)$ should be supported in the ball of radius $\omega_{\text{max}}$ and have their inverse Fourier transforms concentrated in the unit ball. Moreover, we must have $\dim(\hat{\mathcal{V}}) \approx \dim(\mathcal{B})$. Finally, since the Slepian functions are eigenfunctions of the bandlimit-timelimit-bandlimit operator, it follows that their Fourier transforms are smooth. Thus, we would like $f_k(r)Y^m_\ell(\alpha)$ to be smooth for each $(k, \ell, m) \in J$. We begin by examining this condition.

It is well-known that spherical harmonics are restrictions to the sphere of homogeneous polynomials. Thus, for each $\ell, m$, there is a homogeneous polynomial $R^m_\ell$ of degree $\ell$ for which

\[ R^m_\ell(\rho) = r^\ell Y^m_\ell(\alpha), \quad \rho = (r, \alpha). \]
Using this, we find that
\begin{equation}
(5.18) \quad f_k(r)Y^m_\ell(\alpha) = \frac{f_k(r)}{r^\ell} R^m_\ell(\rho).
\end{equation}
If near the origin, we have \( f_k(r) \sim r^{i_k} \), then
\begin{equation}
(5.19) \quad f_k(r)Y^m_\ell(\alpha) \sim |\rho|^{i_k-\ell} R^m_\ell(\rho).
\end{equation}
Note that the RHS expression is smooth at the origin if an only if \( i_k = \ell, \ell + 2, \ldots \).
This shows that if \( (k, \ell, m) \in J \), then \( f_k(r) = a_\ell r^\ell + a_{\ell+2} r^{\ell+2} + \cdots \), where some coefficients can be zero. One possible way to satisfy this requirement is to construct \( f_0, f_1, \ldots \) so that
\begin{equation}
(5.20) \quad f_k(r) = a_{k,k} r^k + a_{k,k+2} r^{k+2} + \cdots
\end{equation}
for small \( r \) with \( a_{k,k} \neq 0 \), and only combine \( f_k \) with \( Y^m_\ell \) if \( k = \ell \) mod 2 and \( \ell \leq k \).
This leads to the following set of 3D basis functions:
\begin{equation}
(5.21) \quad \{ \hat{h}_k \} = \{ f_0 Y^0_0, f_1 Y^{-1}_1, f_1 Y^1_1, f_2 Y^0_0, f_2 Y^2_2, \ldots \}.
\end{equation}
Written another way, we define
\begin{equation}
(5.22) \quad \mathcal{V} = \text{span} \left( \{ f_k(r)Y^m_\ell(\theta, \varphi) : 0 \leq k \leq K, \ \ell = k \text{ (mod 2)}, \ 0 \leq \ell \leq k, \ |m| \leq \ell \} \right).
\end{equation}

Now, we return to the real and Fourier-domain content of \( \hat{h}_i \). The bandlimitedness requirement on \( \phi_x \) is satisfied if and only if the functions \( f_k \) are supported in the interval \([0, \omega_{\text{max}}]\). To deal with the real domain requirement, we need the inverse Fourier transform of \( f_k(r)Y^m_\ell(\theta, \varphi) \). With our Fourier convention \((5.1)\), it follows from \([1]\) that
\begin{equation}
(5.23) \quad \mathcal{F}^{-1} \left( f_k(r)Y^m_\ell(\theta, \varphi) (r, \theta, \varphi) \right) = \frac{1}{2\pi^2} \ell^\ell \left( \int_0^\infty f_k(r) j_\ell(r r_x) r^2 dr \right) Y^m_\ell(\theta_x, \varphi_x) = \frac{1}{2\pi^2} \ell^\ell (S_\ell f_k)(r_x) Y^m_\ell(\theta_x, \varphi_x).
\end{equation}
Here, \( j_\ell \) is the spherical Bessel function of order \( \ell \), and \( S_\ell \) is the spherical Hankel transform. Also note that \( (r, \theta, \varphi) \) are Fourier-domain spherical coordinates, while \( (r_x, \theta_x, \varphi_x) \) are their real-domain counterparts. Thus, satisfying the real-domain concentration requirement amounts to maximizing the percentage of the energy of \( S_\ell f_k \) that is contained in \([0, 1]\) for \( 0 \leq k \leq K \), \( 0 \leq \ell \leq k \), \( \ell = k \text{ mod 2} \).

Finally, we have arrived at the criteria we would like \( f_k(r) \) to satisfy:
1. \( \text{supp} f_k \subset [0, \omega_{\text{max}}] \)
2. \( \{ f_k : k \text{ even} \} \) and \( \{ f_k : k \text{ odd} \} \) orthonormal in \( L^2([0, \infty), r) \)
3. \( f_k(r) = a_{k,k} r^k + a_{k,k+2} r^{k+2} + \cdots \) near \( r = 0 \).
4. Under the above conditions, maximize the percentage of the energy of \( S_\ell f_k \) in \([0, 1]\), for \( 0 \leq k \leq K \), \( 0 \leq \ell \leq k \), \( \ell = k \text{ mod 2} \).

While it might be possible to find an optimal set of such functions \( \{ f_k \} \) by solving an optimization problem, we can directly construct a set of functions that satisfactorily satisfies the above criteria.

Note that since \( \ell \) ranges in \([0, k]\), it follows that for larger \( k \), we need to have higher-order spherical Bessel transforms \( S_\ell f_k \) remain concentrated in \([0, 1]\). Since
higher-order spherical Bessel transforms tend to be less concentrated for oscillatory functions, it makes sense to choose \( f_k \) to be less and less oscillatory as \( k \) increases. Note that the functions \( f_k \) cannot all have only few oscillations because the even and odd functions must form orthonormal sets. Using this intuition, we construct \( f_k \) as follows. Since the even and odd \( f_k \) can be constructed independently, we will illustrate the idea by constructing the even \( f_k \). For simplicity, let us assume that \( K \) is odd, with \( K = 2K_0 + 1 \). Define the cutoff \( \chi = \chi([0, \omega_{\text{max}}]) \). First, consider the sequence

\[
J_0(z_{0,K_0+1}^m r/\omega_{\text{max}})\chi, J_2(z_{2,K_0} r/\omega_{\text{max}})\chi, \ldots, J_{2K_0}(z_{2K_0,1} r/\omega_{\text{max}})\chi,
\]

where \( z_{k,m} \) is the \( m \)th positive zero of \( J_k \) (the \( k \)th order Bessel function). Note that the functions in this list satisfy criteria 1 (by construction) and 3 (due to the asymptotics of the Bessel function at the origin). Also note that we have chosen the scaling of the arguments of the Bessel functions so that the number of zero crossings decreases as the list goes on. Thus, the functions become less and less oscillatory, which is the pattern that might lead to satisfying criterion 4. However, since these functions might not be orthogonal with respect to the weight \( r \), we need to orthonormalize them with respect to this weight (via Gram-Schmidt). However, we need to be careful to orthonormalize them in such a way as to preserve the properties that they already satisfy. This can be achieved by running the \( (r\text{-weighted}) \) Gram-Schmidt algorithm from higher \( k \) towards lower \( k \). This preserves the supports of the functions, their asymptotics at the origin, and the oscillation pattern. Moreover, the orthogonality property now holds as well. See Figure 5.1 for the first several even radial basis functions. Constructing the odd radial functions requires following an analogous procedure. Also, changing the parity of \( K \) requires the obvious modifications.

It remains to choose \( K \). We do this based on how well criterion 4 is satisfied. For example, we can calculate how much energy of \( S_\ell f_k \) is contained in the unit
Recall from (1.4) that $p$ choice led to interval for all $0 \leq \ell, k \leq K$ that (5.28)

$$f(I)$$

where the constants $\hat{p}$.

Recall from (1.4) that $p = \dim(B) = \frac{2}{\pi} \omega_{\text{max}}^3$. Hence, we have $\hat{p}/p = 6/\pi^2 \approx 0.6$.

Hence, the dimension of the space $\hat{V}$ we have constructed is within a constant factor of the dimension of Slepian functions. This factor is the price we pay for the computational simplicity $\hat{V}$ facilitates.

Note that a different construction of $f_k$ might have even better results. Choosing better radial functions can be the topic of further research. In any case, the specific choice of $f_k$ does not affect the structure of our algorithm at all because $\hat{L}$ is independent of these functions, as can be seen from (5.11). Thus, the selection of the radial basis functions can be viewed as an independent module in our algorithm. In any case, the radial functions we choose here work well in numerical experiments; see Section 6.

5.3. Constructing $\hat{L}$. Finally, the remaining piece in our construction is the finite-dimensional space of Fourier images, $\hat{L}$. Recall that we required $\hat{L}$ to satisfy the property that $\hat{P}_s(\hat{V}) \subset \hat{L}$. By interpolating if necessary, we can even drop this condition as long as $\hat{L}$ represents the Fourier transforms of functions which are concentrated in the unit disk and bandlimited by $\omega_{\text{max}}$. In particular, a valid choice for $\hat{L}$ would be the space of 2D Slepian functions corresponding to $\omega_{\text{max}}$.

However, to avoid interpolation, we chose $\hat{L}$ to exactly satisfy $\hat{P}_s(\hat{V}) \subset \hat{L}$. To motivate our construction, consider applying $\hat{P}_s$ to a basis element of $\hat{V}$. The first observation to make is that the radial components $f_k(r)$ factor through $\hat{P}_s$ completely:

(5.26) $$\hat{P}_s(f_k(r)Y_{\ell}^m(\theta, \varphi)) = f_k(r)\hat{P}_s(Y_{\ell}^m(\theta, \varphi)).$$

Note that the $\hat{P}_s$ on the LHS should be interpreted as $C(\mathbb{R}^3) \to C(\mathbb{R}^2)$, whereas the one on the RHS is the restricted map $C(S^2) \to C(S^1)$, which we also call $\hat{P}_s$.

The correct interpretation should be clear in each case. Viewed in this new way, $P_s : C(S^2) \to C(S^1)$ rotates a function on the sphere by $r_s \in SO(3)$, and then restricts the result to the equator. This shows that $\hat{P}_s$ operates on each $\hat{V}_k$ separately, and so it has a block-diagonal structure, as depicted in Figure 5.2.

By the rotational properties of spherical harmonics, a short calculation shows that

(5.27) $$\hat{P}_s(Y_{\ell}^m(\theta, \varphi)) = \sum_{\substack{|m| \leq \ell \mod 2}} c_{\ell, m, m'}(r_s) \frac{1}{\sqrt{2\pi}} e^{im'\varphi},$$

where the constants $c_{\ell, m, m'}$ depend on the Wigner D matrices $D^\ell$ [30]. Hence, $\hat{P}_s(\hat{V}) \subset \hat{L}$ if

(5.28) $$f_k(r)Y_{\ell}^m(\theta, \varphi) \in \hat{V} \Rightarrow \frac{1}{\sqrt{2\pi}} f_k(r)e^{im\varphi} \in \hat{L}, \quad m = -\ell, -\ell + 2, \ldots, \ell - 2, \ell.$$
Fig. 5.2: Block diagonal structure of $\hat{P}_s$. The shaded rectangles represent the nonzero entries. For an explanation of the specific pairing of angular and radial functions, see (5.29) and (5.21) and the preceding discussion. A short calculation shows that the $k$th block of $\hat{P}_s$ has size $(k+1) \times \frac{(k+1)(k+2)}{2}$.

Thus, we construct $\hat{I}$ by pairing $f_k$ with $\frac{1}{\sqrt{2\pi}}e^{im\phi}$ if $k = m \mod 2$ and $m \leq k$. This leads to the 2D basis functions

$$\{\hat{g}_i\} = \left\{ \frac{1}{\sqrt{2\pi}} f_0(r), \frac{1}{\sqrt{2\pi}} f_1(r)e^{-i\phi}, \frac{1}{\sqrt{2\pi}} f_1(r)e^{i\phi}, \frac{1}{\sqrt{2\pi}} f_2(r)e^{-2i\phi}, \frac{1}{\sqrt{2\pi}} f_2(r)e^{-i\phi}, \frac{1}{\sqrt{2\pi}} f_2(r)e^{i\phi}, \frac{1}{\sqrt{2\pi}} f_2(r)e^{2i\phi}, \ldots \right\}.$$ (5.29)

Written another way, we construct

$$\tilde{I} = \text{span} \left( \left\{ \frac{1}{\sqrt{2\pi}} f_k(r)e^{im\phi} : 0 \leq k \leq K, m = k \mod 2, |m| \leq k \right\} \right).$$ (5.30)

Let us now compare the dimension of $\tilde{I}$ to the corresponding space of Slepian functions, as we did the previous section. We have

$$\hat{q} = \dim(\tilde{I}) = \sum_{k=0}^{K} (k+1) = \frac{(K+1)(K+2)}{2} \approx \frac{N_{\text{res}}^2}{2} = \frac{2\omega_{\text{max}}^2}{\pi^2}. $$ (5.31)

The Shannon number in 2D corresponding to the bandlimit $\omega_{\text{max}}$ is $\frac{\omega_{\text{max}}^2}{4}$. Thus, we are short of this dimension by a constant factor of $8/\pi^2 \approx 0.8$. Another comparison to make is that the number grid points in the disc inscribed in the $N_{\text{res}} \times N_{\text{res}}$ grid is $\pi N_{\text{res}}^2 = \omega_{\text{max}}^2/\pi$. Thus, $\dim(\tilde{I})$ is short of this number by a factor of $\frac{2}{\pi}$. Note that this is exactly the same factor that was obtained in a similar situation in [69].

Thus, by this point we have fully specified our algorithm for the heterogeneity problem. After finding $\hat{\Sigma}_n$ numerically via (5.14), we can proceed as in steps 6-9 of Algorithm 1 to solve Problem 1.2.

5.4. Condition number of $\hat{L}$. Now that we have fixed the bases in our problem, it is practically important to investigate the condition number of the matrix $\hat{L}$ to be inverted. We already proved in Proposition 3.4 that $\lambda_{\text{min}}(\hat{L}) \geq 1/2\pi$. This theory
is confirmed by a numerical experiment: in Figure 5.3(a) are plotted the minimum eigenvalues of \( \hat{L}^{k,k} \) for \( 0 \leq k \leq 15 \). Note how the eigenvalues actually approach the value \( 1/2\pi \) (marked with a horizontal line) as \( k \) increases. We remarked in Section 3.4 that an upper bound on the maximum eigenvalue is harder to find. Nevertheless, numerical experiments have led us to the following conjecture:

**Conjecture 5.2.** The maximal eigenvalue of \( \hat{L}^{k_1,k_2} \) grows linearly with \( \min(k_1,k_2) \).

We found empirically that the maximal eigenvalue of \( \hat{L}^{k_1,k_2} \) only depends on \( \min(k_1,k_2) \). Secondly, a plot of the maximal eigenvalue of \( \hat{L}^{k,k} \) shows a clear linear dependence on \( k \). See Figure 5.3(b). The line of best fit is approximately

\[
(5.32) \quad \text{maximum eigenvalue of } \hat{L}^{k,k} = 0.2358 + 0.1357k.
\]

Taken together, Proposition 3.4 and Conjecture 5.2 imply the following conjecture about the condition number of \( \hat{L}^{k_1,k_2} \), which we denote by \( \kappa(\hat{L}^{k_1,k_2}) \):

**Conjecture 5.3.**

\[
(5.33) \quad \kappa(\hat{L}^{k_1,k_2}) \leq 1.4818 + 0.8524 \min(k_1,k_2).
\]

**5.5. Algorithm complexity.** Here, we estimate the computational complexity of Algorithm 1. We proceed step by step through the algorithm and estimate the complexity at each stage. Before we do so, note that due to the block-diagonal structure of \( \hat{P}_s \) (depicted in Figure 5.2), it can be easily shown that an application of \( \hat{P}_s \) or \( \hat{P}_s^H \) costs \( O(K^4) \).

Sending the images from the pixel domain into \( \hat{I} \) requires \( n \) applications of the matrix \( Q_1 \in \mathbb{C}^{\hat{q} \times q} \), which costs \( O(nq\hat{q}) = O(nN^2N_{res}^2) \). Note that this complexity can be improved using an algorithm of the type [39], but in this paper we do not delve into the details of this alternative.

Solving for (5.15) for \( \hat{\mu} \) requires \( n \) applications of the matrix \( \hat{P}_s^H \), and so has complexity \( O(nK^4) = O(nN_{res}^4) \).
Next, we must compute the matrix $\hat{B}_n$. Note that the second term in $\hat{B}_n$ can be replaced by a multiple of the identity matrix by (3.32), so only the first term of $\hat{B}_n$ must be computed. Note that $\hat{B}_n$ is a sum of $n$ matrices, and each matrix can be found as the outer product of $\hat{P}_s (\hat{I}_s - \hat{P}_s \hat{\mu}_n) \in \mathbb{C}^p$ with itself. Calculating this vector has complexity $O(K^4)$, from which it follows that calculating $\hat{B}_n$ costs $O(nK^4) = O(nN_{\text{res}}^4)$.

Next, we must invert $\hat{L}$. It is well-known (see, e.g., [57]) that inversion of a sparse matrix $M$ via the conjugate gradient algorithm has complexity $\sqrt{\kappa(M)} \text{nnz}(M)$: $\sqrt{\kappa(M)}$ iterations, each of complexity $\text{nnz}(M)$ (recall the definition of $\text{nnz}$ from Conjecture 5.1). Conjectures 5.1 and 5.3 imply that complexity of inverting $\hat{L} \lesssim K \sum_{k_1, k_2=0}^{K} \sqrt{\kappa(\hat{L}^{k_1, k_2})} \text{nnz}(\hat{L}^{k_1, k_2})$.

(5.34)

\[
\text{complexity of inverting } \hat{L} \lesssim \sum_{k_1, k_2=0}^{K} \sqrt{\kappa(\hat{L}^{k_1, k_2})} \text{nnz}(\hat{L}^{k_1, k_2}) \\
\lesssim \sum_{k_1, k_2=0}^{K} \sqrt{\min(k_1, k_2)} \frac{1}{k_1 + k_2 + 1} \left( \frac{(k_1 + 1)(k_1 + 2)(k_2 + 1)(k_2 + 2)}{4} \right)^2 \\
\lesssim \sum_{k_1=0}^{K} k_1^{1/4} k_1^{1/2} \sum_{k_2=0}^{K} k_2^{1/4} k_2^{1/2} \lesssim K^{4.75} K^{4.75} = K^{9.5}.
\]

Since $\hat{L}$ has size of the order $K^6 \times K^6$, note that the complexity of inverting a full matrix of this size would be $K^{18}$. Thus, our efforts to make $\hat{L}$ sparse have saved us a $K^{8.5}$ complexity factor. Moreover, the fact that $\hat{L}$ is block diagonal makes its inversion highly parallelizable.

Assuming that $C = O(1)$, solving each of the $n$ least-squares problems (4.2) is dominated by a constant number of applications of $\hat{P}_s$ to a vector. Thus, finding $\alpha_s$ for $s = 1, \ldots, n$ costs $O(nN_{\text{res}}^2)$.

Next, we must fit a mixture of multinomials to $\alpha_s$ to find $\alpha^c$. An EM approach to this problem to this problem requires $O(n)$ operations per iteration. Assuming that the number of iterations is constant, finding $\alpha^c$ has complexity $O(n)$.

Finally, reconstructing $\hat{X}^c$ via (4.1) is a constant-time operation.

Hence, neglecting lower-order terms, we find that the total complexity of our algorithm is

(5.35)

\[O(nN^2N_{\text{res}}^2 + N_{\text{res}}^{9.5}).\]

6. Numerical results. Here, we provide numerical results illustrating Algorithm 1 with the bases $I$ and $V$ chosen so as to make $\hat{L}$ sparse, as discussed in Section 5. The results presented below are intended for proof-of-concept purposes, and they demonstrate the qualitative behavior of the algorithm. They are not, however, biologically significant results. We have considered an idealized setup in which there is no CTF effect, and have assumed that the rotations $R_s$ (and translations) have been estimated perfectly. In this way, we do not perform a “full-cycle” experiment, starting from only the noisy images. Therefore, we cannot gauge the overall effect of noise on our algorithm because we do not account for its contribution to the misspecification of rotations; we investigate the effect of noise on the algorithm only after the rotation estimation step. Moreover, we use simulated data instead of
experimental data. The application of our algorithm to experimental datasets is left for a separate publication.

6.1. Definitions of SNR and Fourier Shell Correlation. Before we discuss our experiments, we define two important concepts: SNR and Fourier Shell Correlation (FSC).

The definition of SNR is

\[ \text{SNR} = \frac{P(\text{signal})}{P(\text{noise})}, \]

where \( P \) denotes power. In our setup, we will find appropriate definitions for both \( P(\text{signal}) \) and \( P(\text{noise}) \). Let us consider first the noise power. The standard definition is \( P(\text{noise}) = \sigma^2 \). However, note that in our case, our noise has a power of \( \sigma^2 \) in each pixel of an \( N \times N \) grid, but we reconstruct the volumes to a resolution \( \omega_{\text{max}} \), corresponding to \( N_{\text{res}} \). Hence, if we downsamled the \( N \times N \) images to size \( N_{\text{res}} \times N_{\text{res}} \), then we would still obey the Nyquist criterion (assuming the volumes actually are bandlimited by \( \omega_{\text{max}} \)). This would have the effect of reducing the noise power by a factor of \( N_{\text{res}}^2 / N^2 \). Hence, in the context of our problem, we define

\[ P(\text{noise}) = \frac{\sigma^2}{N^2 / N_{\text{res}}^2}. \]

Now, consider \( P(\text{signal}) \). In standard SPR, a good definition of signal power is

\[ P(\text{signal}) = \frac{1}{n} \sum_{s=1}^{n} \frac{1}{q} \| P_s X_s \|_2^2, \]

where \( \| \cdot \| \) denotes the norm of a vector of pixel values. However, in the case of the heterogeneity problem the object we are trying to reconstruct is not the volume itself, but rather the deviation from the average volume, due to heterogeneity. Thus, the relevant signal to us is not the images themselves, but the parts of the images that correspond to projections of the deviations of \( X_s \) from \( \mu \). Hence, a natural definition of signal power in our case is

\[ P(\text{signal}_{\text{het}}) = \frac{1}{n} \sum_{s=1}^{n} \frac{1}{q} \| P_s (X_s - \mu) \|_2^2. \]

Using the above definitions, let us define \( \text{SNR}_{\text{het}} \) in our problem by

\[ \text{SNR}_{\text{het}} = \frac{P(\text{signal}_{\text{het}})}{P(\text{noise})} = \frac{\frac{1}{n} \sum_{s=1}^{n} \| P_s (X_s - \mu) \|_2^2}{\sigma^2 N_{\text{res}}^2 / N^2}. \]

Even with the correction factor \( N_{\text{res}}^2 / N^2 \), \( \text{SNR}_{\text{het}} \) values are lower than the SNR values usually encountered in structural biology. Hence, we also define

\[ \text{SNR} = \frac{P(\text{signal})}{P(\text{noise})} = \frac{\frac{1}{n} \sum_{s=1}^{n} \frac{1}{q} \| P_s X_s \|_2^2}{\sigma^2 N_{\text{res}}^2 / N^2}. \]

We will present our numerical results primarily using \( \text{SNR}_{\text{het}} \), but we will also provide the corresponding SNR values in parentheses.
Fig. 6.1: The top row shows a clean 65 × 65 projection image and noisy projection images with two different SNRs (using phantom 2 from the two class case). The bottom row contains the corresponding images, except with the projection of the mean volume subtracted. Observe that the signal is much weaker in the bottom row than in the top row. Note: here, we list the SNR_{het} and SNR values without normalizing by $N^2/N_{res}^2$ in order to illustrate the signal present in a projection image.

To get a sense of the difference between this definition of SNR and the conventional one, compare the signal strength in a projection image to that in a mean-subtracted projection image in Figure 6.1.

To quantify the resolution of our reconstructions, we use the FSC [48]. For two volumes $V_1$ and $V_2$, we can calculate their correlation on each spherical shell in Fourier space. The FSC is given by

$$FSC(i) = \frac{\sum_{j \in \text{Shell}_i} F(V_1)(j) \overline{F(V_2)(j)}}{\sqrt{\sum_{j \in \text{Shell}_i} |F(V_1)(j)|^2} \sqrt{\sum_{j \in \text{Shell}_i} |F(V_2)(j)|^2}}.$$ (6.7)

We define $\text{Shell}_i = \{ j : i - 0.5 + \epsilon \leq \|j\| < i + 0.5 + \epsilon \}$ for $\epsilon = 10^{-4}$ and $i = 1, \ldots, \lfloor \omega_{\text{max}} - 1 \rfloor$. Thus, the FSC of two volumes is a 1D array of shell correlations. Since we know the ground truth mean, eigenvectors, and conformations, we can calculate the FSC between these computed and true volumes. When used to compare reconstructed against ground truth volumes, FSC is also called Fourier Cross-Resolution (FCR) [42].

### 6.2. Experimental procedure.

We performed three experiments: one with two heterogeneity classes, one with three heterogeneity classes, and one with continuous variation along the perimeter of a triangle defined by three volumes. The first two demonstrate our algorithm in the setup of Problem 1.2, and the third shows that we can estimate the covariance matrix and discover a low-dimensional structure in $X$ in more general setups than the discrete heterogeneity case.

As a first step in each of the experiments, we created two or three phantoms analytically. We chose the phantoms to be linear combinations of Gaussian densities:

$$\phi^c(r) = \sum_{i=1}^{M_c} a_{i,c} \exp \left( -\frac{||r - r_{i,c}||^2}{2\sigma_{i,c}^2} \right), \quad r_{i,c} \in \mathbb{R}^3, a_{i,c}, \sigma_{i,c} \in \mathbb{R}_+, \quad c = 1, \ldots, C.$$ (6.8)
For the discrete heterogeneity cases, we chose probabilities \( p_1, \ldots, p_C \) and generated \( \phi_1, \ldots, \phi_n \) by sampling from \( \phi_1, \ldots, \phi_C \) accordingly. For the continuous heterogeneity case, we generated each \( \phi \) by choosing a point uniformly at random from the perimeter of the triangle defined by \( \phi^1, \phi^2, \phi^3 \).

For all of our experiments, we chose \( n = 10000, N = 65, N_{\text{res}} = 17, K = 15, \) and selected the set of rotations \( R_s \) to be approximately uniformly distributed on \( SO(3) \). For each \( R_s \), we calculated the clean continuous projection image \( P_s \phi_s \) analytically, and then sampled the result on an \( N \times N \) grid. Then, for each SNR level, we used \( (6.5) \) to find the noise power \( \sigma^2 \) to add to the images.

After simulating the data, we ran Algorithm 1 on the images \( I_s \) and rotations \( R_s \) on an Intel i7-3615QM CPU with 8 cores, and 8 GB of RAM. The runtime for the entire algorithm with the above parameter values (excluding precomputations) is 257 seconds. For the continuous heterogeneity case, we stopped the algorithm after computing the coordinates \( \alpha_s \) (we did not attempt to reconstruct individual volumes in this case). For the discrete cases, we calculated FSC curves for the mean, the top eigenvectors, and the mean-subtracted reconstructed volumes. We also plotted the correlations of the mean, top eigenvectors, and mean-subtracted volumes with the corresponding ground truths for a range of SNR values. Finally, we plotted the coordinates \( \alpha_s \) and the estimated values of \( p_c \). For the continuous heterogeneity case, we tested the algorithm on only a few different SNR values. By plotting \( \alpha_s \) in this case, we recover the triangle used in constructing \( \phi_s \).

6.3. Experiment: two classes. In this experiment, we constructed two phantoms \( \phi^1 \) and \( \phi^2 \) of the form \( (6.8) \), with \( M_1 = 1, M_2 = 2, \) and

\[
\begin{align*}
\phi^1 &= a_{1,1} = 1, r_{1,1} = (0.1, 0.2, 0.2), \sigma_{1,1} = 0.26, \\
\phi^2 &= a_{2,2} = 1, r_{2,1} = (0.1, 0.2, 0.2), r_{2,2} = (-0.3, -0.3, 0.2), \sigma_{2,1} = 0.26, \sigma_{2,2} = 0.19.
\end{align*}
\]

The cross-sections of \( \phi^1, \phi^2 \) are depicted in Figure 6.2. We chose the two heterogeneity classes to be equiprobable: \( p_1 = p_2 = 1/2 \). Note that the theoretical covariance matrix in the two-class heterogeneity problem has rank 1, with dominant eigenvector proportional to the difference between the two volumes.

![Fig. 6.2: Slices of the two phantoms, given by (6.9)](image)

Figure 6.3 shows the reconstructions of the mean, top eigenvector, and two volumes for \( \text{SNR}_{\text{het}} = 0.013, 0.003, 0.0013 (0.25, 0.056, 0.025) \). In Figure 6.4, we display eigenvalue histograms of the reconstructed covariance matrix for the above SNR values. Figure 6.5 shows the FSC curves for these reconstructions. Figure 6.6 shows
the correlations of the computed means, top eigenvectors, and (mean-subtracted) volumes with their true values for a broader range of SNR values. In Figure 6.7 we plot a histogram of the coordinates $\alpha_s$ from step 7 of Algorithm 1. Finally, Figure 6.8 shows the reconstructed $p_1$ and $p_2$ for a range of SNR values, with the shaded area representing one standard deviation across ten experiments.

![Image of reconstructed images]

Fig. 6.3: Reconstructions of the mean, top eigenvector, and two volumes for three different SNR values (these values are given in the captions). The top row contains clean and reconstructed slices of the mean, the next row corresponds to the top eigenvector, and the last two rows to the two volumes.

Our algorithm was able to meaningfully reconstruct the two volumes for SNR$_{\text{het}}$ as low as about 0.005 (0.1). Note that the means were always reconstructed with at least a 94% correlation to their true values. On the other hand, the eigenvector reconstruction shows a phase-transition behavior, with the transition occurring between SNR$_{\text{het}}$ values of 0.001 (0.002) and 0.003 (0.006). Note that this behavior is tied to the spectral gap (separation of top eigenvalues from the bulk) of $\hat{\Sigma}_n$. Indeed, the disappearance of the spectral gap going from panel (b) to panel (c) of Figure 6.4 coincides with the estimated top eigenvector becoming uncorrelated with the truth, as reflected in Figures 6.5(b) and 6.6(a). This phase transition behavior is very similar to that observed in the usual high-dimensional PCA setup, described in Section 2.3.
Fig. 6.4: Eigenvalue histograms of reconstructed covariance matrix in two volume case for three SNR values. Note that as the SNR decreases, the distribution of eigenvalues associated with noise comes increasingly closer to the top eigenvalue that corresponds to the structural variability, and eventually the latter is no longer distinguishable.

(a) $\text{SNR}_{\text{het}} = 0.013 \ (0.25)$  
(b) $\text{SNR}_{\text{het}} = 0.003 \ (0.056)$  
(c) $\text{SNR}_{\text{het}} = 0.0013 \ (0.025)$

Fig. 6.5: FSC curves for the mean volume, top eigenvector, and one mean-subtracted volume at the same three SNRs as in Figure 6.3. Note that the mean volume is reconstructed successfully for all three SNR levels. On the other hand, the top eigenvector and volume are recovered at the highest two SNR levels but not at the lowest SNR.

(a) Mean  
(b) Top eigenvector  
(c) Volume 1

Fig. 6.6: Correlations of computed quantities with their true values for different SNRs (averaged over 10 experiments) for the two volume case. Note that in the two volumes case, the mean-subtracted volume correlations are essentially the same as the eigenvector correlation (the only small discrepancy is that we subtract the true mean rather than the computed mean to obtain the former).

(a) Mean and eigenvector correlations  
(b) Volume correlations
Regarding the coefficients $\alpha_s$ depicted in Figure 6.7, note that in the noiseless case, there should be distribution composed of two spikes. By adding noise to the images, the two spikes start blurring together. For SNR values up to a certain point, the distribution is still bimodal. However, after a threshold the two spikes coalesce into a unimodal distribution. Inspecting Figure 6.8 we see that the $p_c$ are also reliably estimated until a certain threshold, around SNR$_{\text{het}} = 0.006$ (0.11).

(a) SNR$_{\text{het}} = 0.013$ (0.25)  (b) SNR$_{\text{het}} = 0.0058$ (0.11)  (c) SNR$_{\text{het}} = 0.003$ (0.056)

Fig. 6.7: Histograms of $\alpha_s$ for two class case. Note that (a) has a bimodal distribution corresponding to two heterogeneity classes, but these two distributions merge as SNR decreases.

Fig. 6.8: Estimated $p_1$ (left) and $p_2$ (right) across SNR values. The shaded area represents one standard deviation from ten trials. The horizontal lines indicate the true value for each $p_c$.

6.4. Experiment: three classes. In this experiment, we constructed three phantoms $\phi^1, \phi^2, \phi^3$ of the form (6.8), with $M_1 = 2, M_2 = 2, M_3 = 1$, and

(6.10)

$a_{1,1} = a_{1,2} = 1, \quad r_{1,1} = (0.15, 0.15, 0.15), \quad r_{1,2} = (-0.15, -0.15, -0.15), \quad \sigma_{1,1} = 0.2, \quad \sigma_{1,2} = 0.2,$

$a_{1,2} = a_{2,2} = 1, \quad r_{2,1} = (0.15, 0.15, 0.15), \quad r_{2,2} = (0.2, -0.2, -0.2), \quad \sigma_{2,1} = 0.2, \quad \sigma_{2,2} = 0.19,$

$a_{3,1} = 1, \quad r_{3,1} = (0.15, 0.15, 0.15), \quad \sigma_{3,1} = 0.2.$

The cross-sections of $\phi^1, \phi^2, \phi^3$ are depicted in Figure 6.9. We chose the three classes to be equiprobable: $p_1 = p_2 = p_3 = 1/3$. Note that the theoretical covariance matrix in the three-class heterogeneity problem has rank 2.
Figures 6.9, 6.10, 6.11, 6.12, 6.13, 6.14, 6.15 are the three-class analogues of Figures 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8 in the two-class case.

Qualitatively, we observe behavior similar to that in the two-class case. The mean is reconstructed with at least 90% accuracy for all SNR values considered (Figure 6.13(a)), while both top eigenvectors experience a phase-transition phenomenon (Figure 6.13(b)). As with the two-class case, we see that the disappearance of the eigengap coincides with the phase-transition behavior in the reconstruction of the top eigenvectors. However, in the three-class case we have two eigenvectors, and we see that the accuracy of the second eigenvector decays more quickly than that of the first eigenvector. This reflects the fact that the top eigenvalue of the true covariance $\Sigma$ is $2.1 \times 10^5$, while the second eigenvalue is $1.5 \times 10^3$. These two eigenvalues differ because $\phi^1 - \phi^3$ has greater norm than $\phi^2 - \phi^3$, which means that the two directions of variation have different associated variances. Hence, recovering the second eigenvector is less robust to noise. In particular, there are SNR values for which the top eigenvector can be recovered, but the second eigenvector cannot. $\text{SNR}_{\text{het}} = 0.0044 (0.03)$ is such an example. We see in Figure 6.11 that for this SNR value, only the top eigenvector pops out of the bulk distribution. In this case, we would incorrectly estimate the rank of the true covariance as 1, and conclude that $C = 2$.

The coefficients $\alpha_s$ follow a similar trend to those in the two-class case. For high SNRs, there is a clearly defined clustering of the coordinates around three points, as in Figure 6.14(a). As the noise is increased, the three clusters become increasingly less defined. From Figure 6.15, the probabilities $p_c$ are reconstructed reliably until a threshold SNR near 0.02 (0.13), at which point the reconstructions start fluctuating much more. In Figure 6.14(b), we see that in this threshold case, the three clusters begin merging into one. By the time $\text{SNR} = 0.0044 (0.03)$, there is a unimodal distribution of the $\alpha_s$, just as we observed in the two-class case. Note that although the SNR threshold for finding $p_c$ from the $\alpha_s$ coefficients comes earlier than the one for the eigengap, the quality of volume reconstruction roughly tracks the quality of the eigenvector reconstruction. This suggests that the estimation of cluster means is more robust than that of the probabilities $p_c$. 

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Fig. 6.10: Cross-sections of clean and reconstructed objects for the three class experiment (captions indicate SNR$_{het}$ and SNR). The top row contains clean and reconstructed slices of the mean, the next two rows represent the first and second eigenvectors, and the last three rows correspond to the three volumes.
(a) $\text{SNR}_{\text{het}} = 0.044 (0.3)$  (b) $\text{SNR}_{\text{het}} = 0.0044 (0.03)$  (c) $\text{SNR}_{\text{het}} = 0.0015 (0.01)$

Fig. 6.11: Eigenvalue histograms of reconstructed covariance matrix in the three class case for three SNR values. Note that the noise distribution first engulfs the second eigenvalue, and eventually the top eigenvalue as well.

(a) Mean  (b) Eigenvector 1  (c) Eigenvector 2  (d) Volume 1

Fig. 6.12: FSC curves for the mean volume, top eigenvector, and one mean-subtracted volume at the same three SNRs as in Figure 6.10. Note that the mean volume is reconstructed successfully for all three SNR levels. Note also that the second eigenvector is recovered less accurately than the first.

(a) Mean and eigenvector correlations  (b) Volume correlations

Fig. 6.13: Correlations of computed means, eigenvectors, and mean-subtracted volumes with their true values for different SNRs (averaged over 30 experiments). Note that the mean volume is consistently recovered well, whereas recovery of the eigenvectors and volumes exhibits a phase-transition behavior.
Fig. 6.14: The coordinates $\alpha_s$ for the three class case, colored according to true class. The middle scatter plot is near the transition at which the three clusters coalesce.

Fig. 6.15: Estimated $p_1, p_2, p_3$ (from left to right). The shaded area represents one standard deviation from thirty trials. The horizontal lines indicate the true value for each $p_c$.

6.5. Experiment: continuous variation. In this experiment, we sampled $\phi_s$ uniformly from the perimeter of the triangle determined by volumes $\phi^1, \phi^2, \phi^3$ (from the three class discrete heterogeneity experiment). This setup is more suitable to model the case when the molecule can vary continuously between each pair $\phi^i$ and $\phi^j$. Despite the fact this experiment does not fall under Problem 1.2, Figure 6.16 shows that we still recover the rank two structure. Indeed, it is clear that all the clean volumes still belong to a subspace of dimension 2. Moreover, we can see the triangular pattern of heterogeneity in the scatter plots of $\alpha_s$ (Figure 6.17). However, note that once the images get moderately noisy, the triangular structure starts getting drowned out. Thus in practice, without any prior assumptions, just looking at the scatter plots of $\alpha_s$ will not necessarily reveal the heterogeneity structure in the dataset. To detect continuous variation, a new algorithmic step must be designed to follow covariance matrix estimation. Nevertheless, this experiment shows that by solving the general Problem 1.1 we can estimate covariance matrices beyond those considered in the discrete case of the heterogeneity problem.
7. Discussion. In this paper, we proposed a covariance matrix estimator from linearly projected data. We proved a finite-sample error bound and asymptotic convergence results. The covariance matrix approach to the cryo-EM heterogeneity problem is essentially a special case of the general statistical problem under consideration, but has its own practical challenges. We overcame these challenges and proposed a methodology to tractably estimate the covariance matrix and reconstruct the molecular volumes. We proved the consistency of our estimator in the cryo-EM case and also began the mathematical investigation of the projection covariance transform. We discovered that inverting the projection covariance transform involves applying the triangular area filter, a generalization of the ramp filter arising in tomography. Finally, we validated our methodology on simulated data, producing accurate reconstructions at low SNR levels. Our implementation of this algorithm is now part of the ASPIRE package at spr.math.princeton.edu In what follows, we discuss several directions for future research.

As discussed in Section 2.3, our statistical framework and estimators have opened many new open questions in high-dimensional statistics. While a suite of results are already available for the traditional high-dimensional PCA problem, generalizing these results to the projected data case would require new random matrix analysis. Our numerical experiments in the cryo-EM case have shown many qualitative similarities between the estimated covariance matrix in the cryo-EM case and the sample
covariance matrix. There is again a bulk distribution with eigenvalues separated from it. Moreover, there is a phase-transition phenomenon in the cryo-EM case, in which the top eigenvectors of the estimated covariance lose correlation with those of the population covariance once the corresponding eigenvalues are absorbed by the bulk distribution. Answering the questions posed in Section 2.3 would be very useful in quantifying the theoretical limitations of our approach.

As an additional line of further inquiry, note that the our optimization problem (2.4) for the covariance matrix is amenable to regularization. If \( n \approx f(p, q) \) is the high-dimensional statistical regime in which the unregularized estimator still carries signal, then of course we need regularization when \( n \ll f(p, q) \). Here, \( f \) is a function depending on the geometry of the operators \( P_s \). Moreover, regularization increases robustness to noise, so in applications like cryo-EM, this is very useful. As we mentioned in Section 2.1, Tikhonov regularization does not increase the complexity of our algorithm, but has the potential to make \( \hat{L}_n \) invertible. Under what conditions can we still achieve accurate recovery in a regularized setting? Other regularization schemes can take advantage of our knowledge of \( \Sigma \), such as using nuclear norm regularization in the case when \( \Sigma \) is known to be low rank. See [25] for an application of nuclear norm minimization in the context of dealing with heterogeneity in cryo-electron tomography. Another special structure \( \Sigma \) might have is that it is sparse in a certain basis. For example, the localized variability assumption in the case of the heterogeneity problem is such an example; in this case, the covariance matrix is sparse in the real Cartesian basis. This sparsity can be encouraged using a matrix 1-norm regularization term. Other methods, such as sparse PCA [22] or covariance thresholding [5] might be applicable in certain cases when we have sparsity in a given basis.

We developed our algorithm in an idealized environment, assuming that the rotations \( R_s \) (and in-plane translations) are known exactly and correspond to approximately uniformly distributed viewing directions, and that the molecules belong to \( B \). Moreover, we abstracted from the CTF effect of the electron microscope. In practice, of course rotations and translations are estimated with some error. Also, certain molecules might exhibit a preference for a certain orientation, invalidating the uniform rotations assumption. Note that as long as \( \hat{L}_n \) is invertible, our framework produces a valid estimator, but without the uniform rotations assumption, the computationally tractable approach to inverting this matrix proposed in Section 5 no longer holds. Moreover, molecules might have higher frequencies that those we reconstruct, which could potentially lead to artifacts. Thus, an important direction of future research is to investigate the stability of our algorithm to perturbations from the idealized assumptions we have made.

We began investigating the properties of \( \hat{L} \) in Section 3.4. However, there is still much to be done in studying this operator. Compelling numerical evidence led us to conjecture that the maximum eigenvalue of \( \hat{L}^{k,k} \) grows linearly with \( k \). Proving this conjecture would be a good first step in analyzing \( \hat{L} \). Going further, it would be interesting to study the eigenvalues and “eigenmatrices” of \( \hat{L} \). The rotational invariance property stated in Proposition 3.5 shows that if \( \hat{M} \) is an eigenmatrix of \( \hat{L} \), then so is \( \hat{M}R \). Thus, the spectrum of \( \hat{L} \) should have some special properties. Moreover, finding the eigendecomposition of \( \hat{L} \) would have the practical benefit of making it easier to invert. It would also be interesting to see what other symmetries or geometric properties this operator has.

As we discussed in the introduction, our statistical problem (1.1) is actually a special case of the matrix sensing problem. In future work, it would be interesting
to test matrix sensing algorithms on our problem. In the cryo-EM case, it would be useful to compare our approach with matrix sensing algorithms. It would also be interesting to explore the applications of our methodology to other tomographic problems involving variability. For example, the field of 4D electron tomography focuses on reconstructing a 3D structure that is a function of time [20]. This 4D reconstruction is essentially a movie of the molecule in action. The methods developed in this paper can in principle be used to estimate the covariance matrix of a molecule varying with time. This is another kind of “heterogeneity” that is amenable to the same analysis we used to investigate structural variability in cryo-EM.

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Appendix A. Matrix derivative calculations. The goal of this appendix is to differentiate the objective functions of (2.3) and (2.4) to verify formulas (2.5) and (2.6). In order to differentiate with respect to vectors and matrices, we appeal to a few results from [17]. The results are as follows:

\[
\begin{align*}
D_z(z^H a) &= a \\
D_z(z^H A z) &= A z \\
D_z(tr(AZ)) &= A \\
D_z(tr(ZAZ^H A)) &= AZ^H A.
\end{align*}
\]

(A.1)

Here, the lowercase letters represent vectors and the uppercase letters represent matrices. Also note that \( z^* \) denotes the complex conjugate of \( z \). The general term of (2.3) is

\[
\|I_s - P_s \mu \|^2 = (I_s^H - \mu^H P_s^H)(I_s - P_s \mu) = \mu^H P_s^H P_s \mu - \mu^H P_s^H I_s - I_s^H P_s \mu + \text{const}.
\]

(A.2)

We can differentiate this with respect to \( \mu^* \) by using the first two formulas of (A.1). We get

\[
D_{\mu^*} \|I_s - P_s \mu \|^2 = P_s^H P_s \mu - P_s^H I_s.
\]

(A.3)

Summing in \( s \) gives us (2.5).

If we let \( A_s = (I_s - P_s \mu_n)(I_s - P_s \mu_n)^H - \sigma^2 I \), then the general term of (2.4) is

\[
\begin{align*}
\| & (I_s - P_s \mu_n)(I_s - P_s \mu_n)^H - (P_s \Sigma P_s^H + \sigma^2 I) \|^2 \\
& = \| A_s - P_s \Sigma P_s^H \|^2 \\
& = tr(A_s^H - P_s \Sigma P_s^H)(A_s - P_s \Sigma P_s^H) \\
& = tr(P_s \Sigma P_s^H A_s - P_s \Sigma P_s^H A_s) - tr(A_s^H P_s \Sigma P_s^H) + \text{const}, \\
& = tr(P_s \Sigma P_s^H A_s - P_s \Sigma P_s^H A_s) - tr(A_s^H P_s \Sigma P_s^H) + \text{const}.
\end{align*}
\]

Using the last two formulas of (A.1), we find that the derivative of this expression with respect to \( \Sigma \) is

\[
P_s^H P_s \Sigma P_s^H P_s^H A_s - P_s^H A_s P_s.
\]

Taking a Hermitian and summing in \( s \) gives us (2.6).

Appendix B. Simplifying (5.11). Here, we simplify the expression for an element of \( \hat{L}_{k_1,k_2} \):

\[
\begin{align*}
\hat{L}_{k_1,k_2} &= \int_{S^2 \times S^2} (a_{i_1}^{k_1} \otimes a_{j_2}^{k_2})(\alpha,\beta)(a_{j_1}^{k_1} \otimes a_{j_2}^{k_2})(\alpha,\beta)K(\alpha,\beta)d\alpha d\beta.
\end{align*}
\]

(B.1)

Let \( A_{i,j}^k = a_{i}^k a_{j}^k \). Then, (B.1) becomes

\[
\begin{align*}
\hat{L}_{k_1,k_2} &= \int_{S^2 \times S^2} A_{i_1,j_1}^{k_1}(\alpha)A_{i_2,j_2}^{k_2}(\beta)K(\alpha,\beta)d\alpha d\beta.
\end{align*}
\]

(B.2)
Recall from Section 5.2 that \( a_k \) is a spherical harmonic of order up to \( k \). It follows that \( A_{i_1 j_1}^{k_1} \) has a spherical harmonic expansion up to order \( 2k_1 \) (using the formula for the product of two spherical harmonics, which involves the Clebsch-Gordan coefficients). The same holds for \( A_{i_2 j_2}^{k_2} \), where the order goes up to \( 2k_2 \). Let us write \( C_{\ell}^m \) for the \( \ell, m \) coefficient of the spherical harmonic expansion of \( A_{i_j}^{k} \). Thus, we have

\[
A_{i_1 j_1}^{k_1} (\alpha) = \sum_{\ell=0}^{2k_1} \sum_{|m| \leq \ell} C_{\ell, m}(A_{i_1 j_1}^{k_1}) Y_{\ell}^m(\alpha), \quad A_{i_2 j_2}^{k_2} (\beta) = \sum_{\ell'=0}^{2k_2} \sum_{|m'| \leq \ell'} C_{\ell', m'}(A_{i_2 j_2}^{k_2}) Y_{\ell'}^m(\beta)
\]

It follows that

\[
\hat{L}_{i_1 j_1, i_2 j_2}^{k_1, k_2} = \sum_{\ell, m, \ell', m'} C_{\ell, m}(A_{i_1 j_1}^{k_1}) C_{\ell', m'}(A_{i_2 j_2}^{k_2}) \int_{S^2} \int_{S^2} Y_{\ell}^m(\alpha) K(\alpha, \beta) Y_{\ell'}^m(\beta) d\alpha d\beta.
\]

Since \( K(\alpha, \beta) \) depends only on \( \alpha \cdot \beta \), by an abuse of notation we can write \( K(\alpha, \beta) = K(\alpha \cdot \beta) \). Thus, the Funk-Hecke theorem applies \[35\], so we may write

\[
\int_{S^2} Y_{\ell}^m(\alpha) K(\alpha, \beta) d\alpha = c(\ell) Y_{\ell}^m(\beta),
\]

where

\[
c(\ell) = \frac{2\pi}{P_\ell(1)} \int_{-1}^{1} K(t) P_\ell(t) dt.
\]

Note that \( P_\ell \) are the Legendre polynomials. Since \( K \) is an even function of \( t \) and \( P_\ell \) has the same parity as \( \ell \), it follows that \( c(\ell) = 0 \) for odd \( \ell \). For even \( \ell \), we have

\[
c(\ell) = 2 \int_{0}^{1} \frac{1}{\sqrt{1-t^2}} P_\ell(t) dt.
\]

It follows from formula 3 on p. 423 of [40] that

\[
c(\ell) = 2 \int_{0}^{1} \frac{1}{\sqrt{1-t^2}} P_\ell(t) dt = \pi \left( \frac{\ell!}{2^\ell (\ell/2)!} \right)^2.
\]

Using Stirling’s formula, we can find that \( c(\ell) \sim \ell^{-1/2} \) for large \( \ell \).

Finally, plugging the result of Funk-Hecke into (B.4), we obtain

\[
\hat{L}_{i_1 j_1, i_2 j_2}^{k_1, k_2} = \sum_{\ell, m, \ell', m'} c(\ell) C_{\ell, m}(A_{i_1 j_1}^{k_1}) C_{\ell', m'}(A_{i_2 j_2}^{k_2}) \int_{S^2} Y_{\ell}^m(\beta) Y_{\ell'}^m(\beta) d\beta
\]

\[
\quad = \sum_{\ell, m} c(\ell) C_{\ell, m}(A_{i_1 j_1}^{k_1}) C_{\ell, m}(A_{i_2 j_2}^{k_2}).
\]

Thus, we have verified (5.12).

**Appendix C. Analysis of \( \mu_n \) and \( \Sigma_n \).**

In this appendix, we will prove the theoretical results about \( \mu_n \) and \( \Sigma_n \) stated in Section 2.2. We will adopt the notation \( Q_s = P_s^H P_s \). For convenience, we will also restate these results here.
C.1. Asymptotic statements. In this section, we prove Propositions 2.1 and 2.2.

**Proposition C.1.** As soon as $A_n$ is invertible, the estimator $\mu_n$ is unbiased. Moreover, if the limiting operators $A$ and $L$ from (2.9) are invertible, then for fixed $p,q$,

(C.1) \[ \text{Var}[\mu_n] = O\left(\frac{1}{n}\right). \]

**Proof.** Note that $E[I_s] = P_s \mu$. From this and from linearity it follows that

(C.2) \[ E[\mu_n] = A_n^{-1} \frac{1}{n} \sum_{s=1}^{n} P_s^H E[I_s] = A_n^{-1} \frac{1}{n} \sum_{s=1}^{n} P_s^H P_s \mu = A_n^{-1} A_n \mu = \mu. \]

This proves the first part of the proposition.

Next, we introduce the notation

(C.3) \[ \delta_s = I_s - P_s \mu \quad \text{and} \quad \epsilon_n = \mu_n - \mu. \]

Note that $E[\delta_s] = 0$ and $\text{Var}[\delta_s] = P_s \Sigma P_s^H + \sigma^2 I$, with $\delta_1, \ldots, \delta_n$ independent. Moreover, note that

(C.4) \[ \epsilon_n = \mu_n - \mu = A_n^{-1} \frac{1}{n} \sum_{s=1}^{n} P_s^H (I_s - P_s \mu) = A_n^{-1} \frac{1}{n} \sum_{s=1}^{n} P_s^H \delta_s. \]

A brief calculation shows that

(C.5) \[ \text{Var}[\mu_n] = \frac{1}{n^2} A_n^{-1} \left( \sum_{s=1}^{n} P_s^H P_s \Sigma P_s^H P_s \right) A_n^{-1} + \sigma^2 \frac{1}{n} A_n^{-1} \]

\[ = \frac{1}{n} \left[ A_n^{-1} (L_n \Sigma) A_n^{-1} + \sigma^2 A_n^{-1} \right]. \]

By the convergences $A_n \rightarrow A$ and $L_n \rightarrow L$, the term inside the brackets tends to a limit, so the conclusion follows. \[ \square \]

**Proposition C.2.** If $A$ and $L$ from (2.9) are invertible and $\|P_s\|_2$ is uniformly bounded for all $s$, then for fixed $p,q$,

(C.6) \[ \|E[\Sigma_n] - \Sigma\| = O\left(\frac{1}{n}\right). \]

Also, if the random variable $X$ has bounded fourth moments, then for all indices $i,j,k,\ell$

(C.7) \[ \text{Cov}[(\Sigma_n)_{ij}, (\Sigma_n)_{k\ell}] = O\left(\frac{1}{n}\right). \]

**Proof.** First we prove (C.6). Let $B_n$ denote the RHS of (2.6). We first find $E[B_n]$.

To do this, let us investigate the quantity

(C.8) \[ (I_s - P_s \mu_n)(I_s - P_s \mu_n)^H = (\delta_s - P_s \epsilon_n)(\delta_s^H - \epsilon_n^H P_s^H) = \delta_s \delta_s^H - P_s \epsilon_n \delta_s^H - P_s \epsilon_n^H P_s^H + P_s \epsilon_n \epsilon_n^H P_s^H. \]
Taking the expectation of this quantity, we get

\[(C.9)\]
\[\mathbb{E}[(I_s - P_s \mu_n)(I_s - P_s \mu_n)^H] = \mathbb{E}[\delta_s \delta^H_s] - \mathbb{E}[\delta_s \epsilon_n^H]P_s^H + P_s \mathbb{E}[\epsilon_n \epsilon_n^H]P_s^H\]
\[= P_s \sum P_s^H + \sigma^2 I - \frac{1}{n} P_s A_n^{-1} P_s^H (P_s \sum P_s^H + \sigma^2 I)\]
\[= \frac{1}{n} (P_s \sum P_s^H + \sigma^2 I) P_s A_n^{-1} P_s^H + \frac{1}{n^2} P_s A_n^{-1} \left( \sum_{s=1}^n P_s^H P_s \sum P_s^H P_s \right) A_n^{-1} P_s^H + \sigma^2 \frac{1}{n} P_s A_n^{-1} P_s^H.\]

To find \(\mathbb{E}[B_n]\), we need to conjugate the above expression by \(P_s\), sum over \(s\), and then subtract \(\sigma^2 A_n\). Recalling that \(Q_s = P_s \sum P_s \), we find that

\[\mathbb{E}[B_n] = \mathbb{E}\left[ \frac{1}{n} \sum_{s=1}^n P_s^H (I_s - P_s \mu_n)(I_s - P_s \mu_n)^H P_s - \sigma^2 A_n \right]\]
\[= \frac{1}{n} \sum_{s=1}^n Q_s \sum Q_s - \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s \sum Q_s - \frac{1}{n^2} \sum_{s=1}^n Q_s \sum Q_s A_n^{-1} Q_s\]
\[+ \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} (L_n \Sigma) A_n^{-1} Q_s - \sigma^2 \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s\]
\[= L_n \Sigma - \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s \sum Q_s - \frac{1}{n^2} \sum_{s=1}^n Q_s \sum Q_s A_n^{-1} Q_s\]
\[+ \frac{1}{n} L_n (A_n^{-1} (L_n \Sigma) A_n^{-1}) - \frac{1}{n} \sigma^2 L_n (A_n^{-1})\]

It follows that

\[(C.11)\]
\[\mathbb{E}[\Sigma_n] - \Sigma = L_n^{-1} (\mathbb{E}[L_n \Sigma_n] - L_n \Sigma)\]
\[= L_n^{-1} (\mathbb{E}[B_n] - L_n \Sigma)\]
\[= -L_n^{-1} \left( \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s \sum Q_s \right) - L_n^{-1} \left( \frac{1}{n^2} \sum_{s=1}^n Q_s \sum Q_s A_n^{-1} Q_s \right)\]
\[+ \frac{1}{n} A_n^{-1} (L_n \Sigma) A_n^{-1} - \sigma^2 \frac{1}{n} A_n^{-1}\]

Using the uniform bound on \(\|P_s\|_2\), we find that \(\|Q_s\|_F\) is uniformly bounded by some constant \(C\). Thus, we have

\[(C.12)\]
\[\left\| \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s \sum Q_s \right\|_F \leq \frac{1}{n} \|A_n\|^{-1} \|\Sigma\|_F \leq \frac{C}{n} \left( \frac{1}{n} \right).\]

Thus, we have

\[(C.13)\]
\[\left\| L_n^{-1} \left( \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s \sum Q_s \right) \right\|_F \leq \left\| L_n^{-1} \right\|_2 \left\| \frac{1}{n^2} \sum_{s=1}^n Q_s A_n^{-1} Q_s \sum Q_s \right\|_F = \left( \frac{1}{n} \right).\]
Moreover, note that

\[(C.14) \quad \left\| \frac{1}{n} A_n^{-1} (L_n \Sigma) A_n^{-1} \right\|_F = O \left( \frac{1}{n} \right) \]

and

\[(C.15) \quad \left\| \sigma^2 \frac{1}{n} A_n^{-1} \right\|_F = O \left( \frac{1}{n} \right).\]

Putting everything together, it follows from \[(C.11)\] that the bias of \(\Sigma_n\) decays like \(1/n\), which proves \[(C.6).\]

Next, we move on to proving \[(C.7).\] Recall that \(\Sigma_n = L_n^{-1} B_n\). Since \(L_n^{-1} = O(1)\), it suffices to prove that \(\text{Var}[B_n] = O(1/n)\).

Let us introduce the new notation

\[(C.16) \quad \gamma_s = P_s^H (I_s - P_s \mu).\]

Then, up to an additive non-stochastic term, we have

\[(C.17) \quad B_n = \frac{1}{n} \sum_{s=1}^n \left( \gamma_s - P_s^H P_s \frac{1}{n} A_n^{-1} \sum_{s'} \gamma_{s'} \right) \left( \gamma_s - P_s^H P_s \frac{1}{n} A_n^{-1} \sum_{s'} \gamma_{s'} \right)^H.\]

Let us denote

\[(C.18) \quad C_{s,s'} = I_p - P_s^H P_s \frac{1}{n} A_n^{-1}, \quad C_{s,s'} = -P_s^H P_s \frac{1}{n} A_n^{-1}, \quad s \neq s'.\]

Note that \(C_{s,s} = I_p + O(1/n)\) and \(C_{s,s'} = O(1/n)\) for \(s \neq s'\). Using this notation, we have

\[(C.19) \quad B_n = \frac{1}{n} \sum_{s=1}^n \left( \sum_{s'} C_{s,s'} \gamma_{s'} \right) \left( \sum_{s''} \gamma_{s''} C_{s,s''}^H \right) \]

\[= \frac{1}{n} \sum_{s=1}^n \left( C_{s,s} \gamma_s + \sum_{s' \neq s} C_{s,s'} \gamma_{s'} \right) \left( \gamma_s C_{s,s}^H + \sum_{s'' \neq s} \gamma_{s''} C_{s,s''}^H \right) \]

\[= \frac{1}{n} \sum_{s=1}^n C_{s,s} \gamma_s \gamma_s C_{s,s}^H + \frac{1}{n} \sum_{s=1}^n \sum_{s' \neq s} C_{s,s'} \gamma_{s'} \gamma_{s'} C_{s,s}^H \gamma_s C_{s,s}^H \]

\[+ \frac{1}{n} \sum_{s=1}^n \sum_{s'' \neq s} C_{s,s''} \gamma_{s''} C_{s,s''}^H + \frac{1}{n} \sum_{s=1}^n \sum_{s' \neq s} C_{s,s'} \gamma_{s'} \gamma_{s'} C_{s,s}^H \gamma_s C_{s,s}^H \]

\[+ \frac{1}{n} \sum_{s=1}^n \sum_{s'' \neq s} C_{s,s''} \gamma_{s''} C_{s,s''}^H \gamma_s C_{s,s}^H \gamma_s C_{s,s}^H \gamma_s C_{s,s}^H \]

Let \(\Sigma_{s,s'} = \mathbb{E}[\gamma_s \gamma_{s'}^H]\). Note that \(\Sigma_{s,s'}\) is nonzero only for \(s = s'\). It follows that

\[(C.20) \quad B_n - \mathbb{E}[B_n] = \frac{1}{n} \sum_{s=1}^n C_{s,s} (\gamma_s \gamma_s^H - \Sigma_{s,s}) C_{s,s}^H + \frac{1}{n} \sum_{s=1}^n \sum_{s' \neq s} C_{s,s'} \gamma_{s'} \gamma_s^H C_{s,s} \gamma_s C_{s,s}^H \gamma_s C_{s,s}^H \]

\[+ \frac{1}{n} \sum_{s=1}^n \sum_{s'' \neq s} C_{s,s''} \gamma_{s''} \gamma_s^H C_{s,s''} \gamma_s C_{s,s}^H \gamma_s C_{s,s}^H \gamma_s C_{s,s}^H \gamma_s C_{s,s}^H \]

\[= A + B + C + D.\]
Hence we need to prove that the terms $\mathbb{E}[A_{ij}A_{kl}]$, $\mathbb{E}[A_{ij}B_{kl}]$, ... are all $O(1/n)$. First, due to the independence of $\gamma_1, \ldots, \gamma_n$, note that
\begin{equation}
\mathbb{E}[A_{ij}A_{kl}] = \frac{1}{n^2} \sum_{s=1}^{n} \mathbb{E} \left[ \left( C_{s,s}(\gamma_s \gamma_s^H - \Sigma_{s,s})C_{s,s}^H \right)_{ij} \left( C_{s,s}(\gamma_s \gamma_s^H - \Sigma_{s,s})C_{s,s}^H \right)_{kl} \right].
\end{equation}

We defined $\gamma_s = P_s^H(I_s - P_s \mu) = P_s^H(P_s X_s + \epsilon_s - P_s \mu)$. Hence, each summand in the above expression will depend on the fourth moments of $X$ (which we assumed are bounded), as well as the fourth moments of $\epsilon_s$ (which are bounded by $\sigma^4$). Since $C_{s,s} = O(1)$, it follows that the above expression is $O(1/n)$.

Next we claim that $\mathbb{E}[A_{ij}B_{kl}] = \mathbb{E}[A_{ij}C_{kl}] = 0$. Indeed, this follows because all terms in these products have the form $\gamma_s \gamma_s \gamma_{s_1} \gamma_{s_2}$, where $s_1 \neq s_2$ (we have abused notation here: in reality these four terms to multiply will be individual elements of each of the $\gamma_s$ vectors, as opposed to the vectors themselves). Hence, by the independence of $\gamma_s$ for different $s$, we will get zero when we take the expectation of each such term.

Now consider $\mathbb{E}[A_{ij}D_{kl}]$. By the preceding argument, the only terms left will be those for which $s' = s''$. Therefore, for the purposes of evaluating this term, we can write $D$ as
\begin{equation}
D = \frac{1}{n} \sum_{s=1}^{n} \sum_{s' \neq s} C_{s,s'}(\gamma_{s'} \gamma_{s'}^H - \Sigma_{s',s'})C_{s,s'}^H + \cdots,
\end{equation}

where the ellipsis denotes terms whose expectation will be zero when multiplied with $A$. Note that when we multiply $A$ with each inner sum, using the independence of the $\gamma_s$, the product will only have $n$ terms, but since each $C_{s,s'} = O(1/n)$, it follows that each of these products is $O(1/n)$. It follows that the interaction term between $A$ and $D$ is $O(1/n^2)$.

We can “count” the powers of $n$ for the interaction terms between $B, C, D$ to see that they are all $O(1/n^2)$. The calculations are all of the same type as the ones above, so we omit them for brevity. This proves (C.7), and so we are done. \hspace{1cm} \Box

**C.2. Finite sample bound.** Next we will prove our finite sample bound. Our bound will rely on the matrix Bernstein inequality [58], which we reproduce here for the reader’s convenience as a lemma.

**Lemma C.3.** (Matrix Bernstein’s Inequality). Consider a finite sequence $Y_s$ of independent, random, self-adjoint matrices with dimension $p$. Assume that each random matrix satisfies
\begin{equation}
\mathbb{E}[Y_s] = 0 \quad \text{and} \quad \lambda_{\max}(Y_s) \leq R \quad \text{a.s.}
\end{equation}

Then, for all $t \geq 0$,
\begin{equation}
\mathbb{P} \left\{ \lambda_{\max} \left( \sum_s Y_s \right) \geq t \right\} \leq p \cdot \exp \left( \frac{-t^2/2}{\sigma^2 + Rt/3} \right), \quad \text{where} \quad \sigma^2 := \left\| \sum_s \mathbb{E}(Y_s^2) \right\|.
\end{equation}

**Proposition C.4.** In the statistical model (1.1), suppose additionally that $\|X\| \leq C_1$, $\|\epsilon_s\| \leq C_2$ a.s. Also, suppose we have the uniform bound $\|P_s\| \leq C_3$ for all $s$. Finally, assume that $X$ is centered. If $\lambda = \lambda_{\min}(L_n)$, then for all $t \geq 0$ we have
\begin{equation}
\mathbb{P} \{ \|\Sigma - \Sigma_n\| \geq t \} \leq p \exp \left( \frac{-3n\lambda^2t^2/2}{3B^4 + 2B^2\lambda t} \right),
\end{equation}

which completes the proof of Proposition C.4.
where

\[(C.26)\]
\[B = C_3^2 C_1 + C_3 C_2.\]

**Proof.** We will mostly follow the argument of Example 1.6.3 of [59], which proves a similar result. Let us define the random vector

\[(C.27)\]
\[y_s = P_s^H I_s = P_s^H P_s X_s + P_s^H \epsilon_s.\]

Note that the \(y_s\) are independent, zero-mean, and satisfy

\[(C.28)\]
\[E[y_s y_s^H] = P_s^H P_s \Sigma_p^2 P_s + \sigma^2 P_s^H P_s =: M_s.\]

We would like to apply Lemma C.3 for

\[(C.29)\]
\[Y_s = \frac{1}{n}(y_s y_s^H - E[y_s y_s^H]) = \frac{1}{n}(y_s y_s^H - M_s).\]

Note that

\[(C.30)\]
\[\sum_{s=1}^n Y_s = \frac{1}{n} \sum_{s=1}^n (y_s y_s^H - E[y_s y_s^H])\]
\[= \frac{1}{n} \sum_{s=1}^n P_s^H I_s^H P_s - \frac{1}{n} \sum_{s=1}^n P_s^H P_s \Sigma_p^2 P_s - \frac{\sigma^2}{n} \sum_{s=1}^n P_s^H P_s\]
\[= L_n \Sigma_n - L_n \Sigma.\]

Note that almost surely,

\[(C.31)\]
\[\|y_s\| \leq \|P_s\| \|X_s\| + \|P_s^H \| \|\epsilon_s\| \leq C_3^2 C_1 + C_3 C_2 =: B.\]

It follows that almost surely,

\[(C.32)\]
\[\|y_s y_s^H\| \leq \|y_s\|^2 = B^2,\]

and so by Jensen's inequality,

\[(C.33)\]
\[\|M_s\| = \|E[y_s y_s^H]\| \leq \|E[\|y_s\|^2]\| \leq B^2.\]

Putting these two together and using the triangle inequality, we find that

\[(C.34)\]
\[\|Y_s\| \leq \frac{2B^2}{n}.\]

Next, let us bound the variance of \(Y_s\). We have

\[(C.35)\]
\[E[Y_s^2] = \frac{1}{n^2} E[(y_s y_s^H - M_s)^2] = \frac{1}{n^2} E \left[ \|y_s\|^2 y_s y_s^H - (y_s y_s^H)M_s - M_s(y_s y_s^H) + M_s^2 \right]\]
\[\leq \frac{1}{n^2} \left( B^2 E[y_s y_s^H] - M_s^2 - M_s^2 + M_s^2 \right) \leq \frac{B^2}{n^2} M_s.\]

It follows that

\[(C.36)\]
\[\sigma^2 = \sum_{s=1}^n E[Y_s^2] \leq \sum_{s=1}^n \|E[Y_s^2]\| \leq \sum_{s=1}^n \frac{B^2}{n^2} \|M_s\| \leq \frac{B^4}{n}.\]
Plugging the results of (C.34) and (C.36) into the tail bound (C.24), we obtain

\[ P[\|L_n \Sigma - L_n \Sigma_n\| \geq t] = P \left\{ \left\| \sum_s Y_s \right\| \geq t \right\} \leq p \cdot \exp \left( \frac{-t^2/2}{B^4/n + 2B^2t/3n} \right) = p \exp \left( \frac{-3nt^2/2}{3B^4 + 2B^2t} \right). \]

(C.37)

Now, note that

\( ||\Sigma - \Sigma_n|| = ||L_n^{-1}(L_n \Sigma - L_n \Sigma_n)|| \leq ||L_n^{-1}|| ||L_n \Sigma - L_n \Sigma_n|| = \lambda^{-1} ||L_n \Sigma - L_n \Sigma_n||. \)

(C.38)

It follows that

\[ P[||\Sigma - \Sigma_n|| \geq t] \leq P[||L_n \Sigma - L_n \Sigma_n|| \geq \lambda t] \leq p \exp \left( \frac{-3n\lambda^2 t^2/2}{3B^4 + 2B^2\lambda t} \right), \]

(C.39)

so we are done. ∎