ON RANDOM TOMOGRAPHY IN STRUCTURAL BIOLOGY

by

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May 2008

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Technical Reports No. 2008-3
May 2008

This research was supported in part by grants from National Science Foundation, DMS 0502385 and NSF – Graduate Research Fellowship

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On Random Tomography in Structural Biology

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May 8, 2008

Abstract

We consider a statistical inverse problem of a random tomographic nature, where a probability density function is to be recovered from observation of finitely many of its marginals (line integrals), in random and unobservable directions. This problem arises in single particle electron microscopy, a powerful method that biophysicists employ to learn the structure of biological macromolecules. In contrast to the more traditional crystallographic methods, this method images unconstrained particles, providing two-dimensional particle profiles at arbitrary and unknown orientations. The aim is to reconstruct the particle in three dimensions. It is seen that the problem is unidentifiable and a formulation based on finite mixtures is suggested that enables the use of ideas the theory of Euclidean shape.

Keywords: Deconvolution; Mixture Density; Modular Inference; Projection; Radon Transform; Shape Theory; Single Particle Electron Microscopy; Statistical Inverse Problem.

1 Introduction

1.1 Structural Biology via Single Particle Cryo-Electron Microscopy

A core problem of molecular biology is the study and interpretation of the structural characteristics of biological macromolecules. It is the structure of biological macromolecules that is at the heart of the quest to understand life in purely physical terms, and thus is fundamental to any biophysical project. Resolving the structure of a biological particle is an undertaking that involves piecing together numerous facets of a complex investigation. It may be said that, in general, one has to fuse together background knowledge, scientific speculation and experimental evidence. The evidential component plays a particularly important role, as it may be both exploratory as well as confirmatory. A large part of what we call experimental evidence in the case of structure determination comes from our attempts to see biological particles. Such attempts are hindered by the microscopic scale of the structures we wish to access – sometimes reaching Angstrom dimensions, $1 \, \text{Å} = 10^{-10} \, \text{m}$. The mechanisms which enable us to gain structural information

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will typically provide indirect knowledge (posing inverse problems), which will have to be translated into initial structural terms in a mathematically sound way. Such mechanisms include X-ray crystallography and electron microscopy, among others. While X-rays have traditionally been associated with structure determination, as in the case of DNA, electron microscopy has arisen as a powerful tool in these endeavours, since it possesses important advantages such as retaining phase information and high scattering power.

The structure of a biological particle is typically described by the relative positioning of its atoms in space. Every atom is comprised of a nucleus of positive charge, which is surrounded by electrons of equal negative charge. The electrons create a shield of potential around the atom. The ensemble of these potentials gives rise to a potential distribution in three-dimensional space, the shielded Coulomb potential distribution. It is always assumed that this distribution is absolutely continuous, so that it admits a potential density. We usually think of a particle as being one and the same as its potential density.

This potential density provides the means of interaction with the electron beam. We may conceptualise the way the electron microscope functions by drawing an analogy to the light microscope, where, instead of photons, we now imagine electrons. An approximate mathematical description is as follows. Let \( \rho(x, y, z) \) be the potential density describing the particle. When an electron beam passes through the specimen (particle) at the \( z \)-direction, there is a reduction to the beam intensity caused by the scattering of electrons due to the interaction with the specimen. According to the Abbe image formation theory (Glaeser et al [10]), the intensity recorded on the film under the specimen is approximately linear in the projection of the particle in the \( z \)-direction

\[
\int_{-\infty}^{+\infty} \rho(x, y, z) \, dz,
\]

Essentially, knowledge of the optical density recorded on the film corresponds to knowledge of the two-dimensional marginal density of \( \rho \) in the \( z \)-direction. Therefore, the information provided to us via the imaging mode of the electron microscope is the projection of the particle potential density, in the sense of a line integral along a specific direction. If this information can be obtained for many different orientations of the potential density

\[
I_n(x, y) = \int_{-\infty}^{+\infty} \rho[(x, y, z)A_n] \, dz, \quad \{A_n\}_{n=1}^{N} \subset SO(3),
\]

then the inverse problem posed is: can we recover the particle structure given integral information over a wide range of directions? In statistical terms, can we recover a probability density function given marginals at a wide range of directions? This is the classic problem of the inversion of the Radon transform (or X-ray transform) that is well understood (see section 1.2). Informally, under certain assumptions on the unknown density, one may approximately reconstruct the original density if integrals are available over a large and relatively exhaustive number of directions.

In practice, things are not as straightforward, and this is mainly due to the problem of radiation damage. Extended exposure to the electron beam will cause chemical bonds of the particle to break, and thus will alter the structure of the particle. It follows that it is impossible to image the same particle under many different orientations: the electron dose required in order to obtain well-defined projection images would destroy the specimen, leading to images of an altered structure.

The traditional way to surpass this problem was via the imaging of a two-dimensional crystal of identical particles. When particles are crystallised, so as to form a regular array, one is able to distribute the required
Figure 1: (a) Sample of 8 random projections of the potential density corresponding to pyruvate-ferredoxin oxidoreductase (PFOR) from a data set obtained via single-particle cryo-electron microscopy at the Lawrence Berkeley National Laboratory (Courtesy of F. Garzanrek and R.M. Glaeser). (b) Reconstruction of the 3D potential density after the projection angles have been estimated (Garzanrek et al. [8]).

electron dose among many identical particles, and then use the Fourier transform to obtain a well-defined projection image of the particle. To obtain angular variety, one may image crystals under different Eulerian angles.

However, reliance on crystals has several drawbacks. The most important is that few types of particles occur naturally as crystals, and while it is possible to induce a crystal artificially, the process is usually cumbersome, time-consuming, and unpredictably varying for different types of particles. Single particle cryo-electron microscopy is a technique of electron microscopy, that aims at obtaining a 3D representation of the particle without crystallising the sample. In this approach, a large number of structurally identical particles are imbedded unconstrained (i.e. uncrystallised) in an aqueous solution, then frozen and finally imaged via the electron microscope. Since the particles are unconstrained, they move and rotate freely within the aqueous solution, assuming varying haphazard orientations at the moment they are frozen. After preliminary processing, the data yielded are essentially noisy versions of the projected potential densities, at a number of random and unknown orientations. Figures 1 and 2 present characteristic examples of such data in the presence of noise (Figure 1), and in the ideal – and practically impossible– noiseless case (Figure 2), for two different types of particles.

Hence, there arises a Radon transform problem that is qualitatively different from the standard one, in the sense that the imaging angles are both random and unobservable. When the projection angles are unknown, the methods available in order to produce a reconstruction break down, since inversion of a Radon transform requires knowledge of the projection angles. Biophysicists typically proceed via estimating the unobservable orientations, in order to then be able to use the standard tomographic techniques. However, the resulting estimates are usually unsatisfactory and often rely on a priori knowledge on the structure of the particle. This leads one to ask the following question: is there anything interesting that can be said (statistically) about
the unknown density without attempting to estimate the unobservable directions? The purpose of this paper is to describe a parametric framework within which such a question admits an answer in the affirmative.

1.2 A Stochastic Radon Transform

The Radon transform is an integral transform named after Johann Radon, who first introduced it in 1917 (Radon [29]). It relates a (suitable) function to its integrals over all possible hyperplanes in its domain. Specifically, let \( f : \mathbb{R}^n \to \mathbb{R} \) be a real function, integrable over all hyperplanes of its domain. If \( \mathcal{H}^d \) is the space of all \( d \)-dimensional hyperplanes \( (1 \leq d < n) \) in \( \mathbb{R}^n \), we define the \( d \)-dimensional Radon transform of \( f \) as the mapping \( f \mapsto \tilde{f} \) given by

\[
\tilde{f}(H) = \int_{H} f(x) \Lambda_H(dx), \quad H \in \mathcal{H}^d,
\]

where \( \Lambda_H \) is Lebesgue measure on \( H \). Under certain regularity conditions on \( f \), the Radon transform can be seen to be invertible (Helgason [14]; Jensen [15]), and the function \( f \) can be recovered by means of explicit formulas which we omit. We will be interested in the special case \( d = 1 \). The 1-dimensional Radon transform of \( f \) – sometimes referred to as the X-ray transform – is usefully parameterised as a function on the tangent bundle \( \{(\xi, x) : \xi \in S^{n-1}, x \in \xi^\perp\} \),

\[
\tilde{f}(\xi, x) = \int_{-\infty}^{+\infty} f(x + \tau \xi) d\tau, \quad \xi \in S^{n-1} \text{ and } x \in \xi^\perp
\]
and is seen to map a suitable real function on $\mathbb{R}^n$ to its projections onto every possible $(n - 1)$-dimensional subspace. If one holds $\xi$ fixed and considers $\hat{f}$ as a function of $x \in \xi^\perp$, then one obtains the profile of $f$ at orientation $\xi$.

In the special cases $n = 3$ and $n = 2$ the Radon transform has found an important application in the problem of tomographic reconstruction. In this problem, an object that is described by a distribution in space (or on the plane) is to be reconstructed by knowledge of its projected distributions (profiles) from a wide range of different directions. Problems of this nature arise in a variety of disciplines including medicine, astronomy, optics, geophysics and electron microscopy. In all these problems one seeks to invert a Radon transform, sampled at finitely many directions $\xi$. Several algorithms have been proposed, and these are often problem-specific, although one may single out broad classes, such as Fourier methods (based on the projection-slice theorem) and back-projection methods. For a detailed discussion, the reader is referred to Deans [6] and Natterer [23]. See also O'Sullivan & Pawitan [24] for an application of such inversion methods to multidimensional density estimation.

We may distinguish three main differences from the standard tomographic problem in the case of single particle electron microscopy:

(I) The samples of the Radon transform are obtained at haphazard orientations $\xi$. This perturbation adds a stochastic aspect to the problem, where the projection orientations are uncontrollable and can be thought as random.

(II) The physics of the data collection process allow for the possibility of variations within the same orientation: a profile could be observed at all possible within-plane rotations. This is because instead of keeping the original density fixed and taking projections onto varying planes, we rotate the particle and then project it on a fixed plane.

(III) Most importantly, in single particle electron microscopy, one does not observe $\xi$, so that none of the traditional techniques are applicable if no other information is provided.

These aspects become clear once we have a precise working definition, and for this reason we define a random analogue to the Radon transform. Let $\rho : \mathbb{R}^3 \to [0, \infty)$ be a continuous probability density function with compact support, which we assume without loss of generality to be the ball $\Delta_3 := \{x \in \mathbb{R}^3 : \|x\| \leq \pi\}$. Let $A$ be a random element of the special orthogonal group $\text{SO}(3)$, distributed according to normalised Haar measure. Finally, write $A\rho(x) := \rho(A^T x)$ for $x \in \mathbb{R}^3$ and $A \in \text{SO}(3)$. We define a random profile (or random projection) of $\rho$ as

$$\Pi\{\rho\}(A)(x, y) = \int_{-\infty}^{+\infty} A\rho(x, y, z)dz. \quad (5)$$

Given a collection $\{A_n\}_{n=1}^N$ of independent random elements of $\text{SO}(3)$, identically distributed according to normalised Haar measure we may define a stochastic Radon transform as the collection of random projections $\{\Pi\{\rho\}(A_n)\}_{n=1}^N$.

In the single particle problem, the realisations of the random projections are not coupled with the corresponding orientations, that is, we observe $\Pi\{\rho\}(A_n)$ as functions on the plane, but not $A_n$. For this reason,
we will suppress the dependence on $A_n$ whenever this does not cause confusion, and write

$$\hat{\rho}_n(x, y) = \Pi\{\rho\}(A_n)(x, y).$$

(6)

With practical situations in mind, one may also consider the case where projections are contaminated by noise,

$$I_n(x, y) = \hat{\rho}_n(x, y) + \varepsilon_n(x, y),$$

(7)

with $\{\varepsilon_n(x, y)\}$ a collection of independent and identically distributed stationary and isotropic noise fields, independent of the $\{A_n\}$.

Two important assumptions are inherent in the above formulations. First, we assume that the projections occur over "uniformly" distributed rotations, so that the stochastic Radon transform provides representative information from all possible profiles, without being biased towards any particular direction. Next, it is assumed that that different profiles are independent, both in terms of the orientations that produced them, as well as in terms of their noise components.

When one observes samples of the deterministic Radon transform, there are several approaches that lead to an estimate of the original marginal, as discussed earlier. On the other hand, when one observes a realisation of $N$ profiles of the stochastic Radon transform, the statistical question that is immediate from this definition is: what can we infer about the original density?

2 Invariance and Parametrisation

2.1 Invariance and Shape

We wish to consider the recovery of a density given its stochastic Radon transform, i.e. to investigate the possibility of a statistical inversion of the stochastic transform. When seen as an estimation problem, the recovery problem has certain special characteristics due to the Haar measure assumption and the unobservability of the projection orientations. These characteristics arise in terms of invariance under the action of an orthogonal group. Group invariance, in fact, manifests itself both in the parameter space as well as in the sample space, as unidentifiability and sufficiency, respectively.

First, we focus on the parameter space. Recall that a parametric family of models (distributions) $\{P_\theta\}$ with parameter space $\Theta$ is identifiable if the map $\theta \mapsto P_\theta$ is a bijection. Non-identifiability is the breakdown of the injective requirement, that is when one has

$$\exists \theta_1 \neq \theta_2 : P_{\theta_1} = P_{\theta_2}. \quad (8)$$

When a parametrisation is unidentifiable, the corresponding model is ill-defined, since we may be unable to distinguish between two distinct parameters regardless of the amount of data available. For the time being, let us think of the parameter space $\Theta = \mathcal{F}$ as being a function space containing continuous probability density functions supported on the ball $\Delta_3$ with zero first moment (to remove location effects),

$$\mathcal{F} := \left\{ f \in C(\Delta_3) : f \geq 0, \int_{\Delta_3} f(x)dx = 1, \int_{\Delta_3} x f(x)dx = 0 \right\}.\n$$

6
The probability model parameterised is the distribution of a random profile $\Pi\{\theta\}$ with $\theta \in \mathcal{F}$. Such a parametrisation, however, leads to uniqueness problems (unidentifiability) since one readily observes that for all $B \in O(3)$, the orthogonal group on $\mathbb{R}^3$, and for $A, M \sim \text{Haar}[SO(3)]$

$$
\Pi\{B\theta\}(x, y) := \int_{-\infty}^{+\infty} AB\theta(x, y, z)dz = \int_{-\infty}^{+\infty} AB \cdot \begin{pmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & \det(B)
\end{pmatrix} \cdot \begin{pmatrix}
1 & 0 & 0 \\
0 & 1 & 0 \\
0 & 0 & \det(B)
\end{pmatrix} \theta(x, y, z)dz
\overset{d}{=} \int_{-\infty}^{+\infty} M\theta(x, y, \det(B) \cdot z)dz \overset{d}{=} \Pi\{\theta\}(x, y).
$$

Therefore, the model is unidentifiable, except perhaps up to orthogonal transformations. This suggests that ideally, we could recover the original function modulo $O(3)$ and leads to the need for a parametrisation of the model in terms of those characteristics of the functions of $\mathcal{F}$ that are invariant under orthogonal transformations. Formally, let $O(3)$ be the group of orthogonal transformations in $\mathbb{R}^3$. Define a group of transformations $G(\mathcal{F})$ on the function class $\mathcal{F}$ whose action is defined as

$$
(\gamma f)(u) := f(\gamma^{-1} u), \quad \gamma \in O(3), f \in \mathcal{F}.
$$

(9)

We call the group $G(\mathcal{F})$ the group of rotations and reflections on the function space $\mathcal{F}$, or simply the group of orthogonal transformations on $\mathcal{F}$. For our purposes, we define the shape of a function $f \in \mathcal{F}$ as its orbit under the action of the group $G(\mathcal{F})$. The shape of a function $f \in \mathcal{F}$ will be denoted as $[f]$ so that

$$
[f] = \{h \in \mathcal{F} \mid \exists \gamma \in G(\mathcal{F}) : \gamma h = f\}.
$$

(10)

Consequently, we will call the quotient space $\mathcal{F}/G(\mathcal{F})$, the shape space of $\mathcal{F}$, and denote it by $\Sigma\mathcal{F}$. It follows from our discussion that we cannot recover "more than $[\rho]$" from the stochastic Radon transform of $\rho$, that is we cannot recover more than invariants with respect to orthogonal transformations. It is thus crucial to be able to obtain an identifiable parametrization in terms of shape.

Shape is not only crucial as a notion in the context of the parameter space. Since the projection orientations are Haar distributed, any orthogonal transformation of the two-dimensional projection data yields the same information on the function-valued parameter: the projections contain no more information for the original density than their shapes do; the shape of an element in the sample space, a space of functions of two arguments, being defined analogously with the shape of an element of the parameter space $\mathcal{F}$, with the two-dimensional orthogonal group replacing the three-dimensional orthogonal group. In statistical terms, the functional that maps a projection to its shape (the quotient mapping) defines a statistic and this statistic can be seen to be sufficient for the shape of the original density (for details, see Panaretos [25]).

The concept of shape was introduced by D.G. Kendall [16] in the case of finitely many labelled points in Euclidean space. In Kendall’s approach, shape was the collection of those characteristics of a labelled point pattern that are invariant under rotation, translation and scaling. The shape spaces induced have a manifold structure, and their geometry depends both on the number of points and the dimension of the ambient space (Le & Kendall [19]). Carne [5] generalised the concept of shape in the case that a group is acting on an ensemble of points on a manifold. Generally speaking, the concept of shape admits many different mathematical definitions, according to the setting one is interested in. In the setting Kendall was
initially interested in (alignments of megalithic stone monuments; see Kendall & Kendall [18]) size was not of importance, but reflections were, so that one would quotient out rotations, translations and dilatations but not reflections. The same concept of shape, although with a different “representation theory”, was independently proposed by Bookstein [1], who was interested in biological applications and morphometrics, and for whom labelled points represented sites of biological interest. Table 1 contains several versions of “shape”, depending on the characteristics one is interested in. In Kendall’s terminology, our version of shape would be called “unoriented shape-and-size”, to stress the fact that rotoinversions (elements of $O(3) \backslash SO(3)$) are quotiented out while scalings are not.

<table>
<thead>
<tr>
<th>Shape Type</th>
<th>Transformations</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Similarity Shape</td>
<td>special similarity group</td>
<td>Kendall [17], Bookstein [2]</td>
</tr>
<tr>
<td>Unoriented Shape</td>
<td>similarity group</td>
<td>Goodall [11]</td>
</tr>
<tr>
<td>Shape-and-Size</td>
<td>Euclidean group</td>
<td>Dryden &amp; Mardia [7]</td>
</tr>
<tr>
<td>Unoriented Shape-and-Size</td>
<td>rigid motion group</td>
<td>Lele [20]</td>
</tr>
<tr>
<td>Affine Shape</td>
<td>linear transformations</td>
<td>Rohlf &amp; Slice [30]</td>
</tr>
<tr>
<td>Projective Shape</td>
<td>projective transformations</td>
<td>Goodall &amp; Mardia [13]</td>
</tr>
<tr>
<td>Scale Shape</td>
<td>scaling to the sphere</td>
<td>Mardia [22], Watson [33]</td>
</tr>
<tr>
<td>Translation Shape</td>
<td>translations</td>
<td>randomised block designs</td>
</tr>
</tbody>
</table>

In the stochastic Radon transform setting, there is no special need to discriminate between rotoinversions for two reasons. The first is the identifiability issue, which leads us to naturally consider the whole orthogonal group. On the other hand, it can be seen (Panaretos [27]) that reflections in the profiles can only occur as projections of the original density at opposite angles, so that discriminating between reflection yields no additional information. (in fact the second reason is closely linked to the first one). Therefore, in the present scenario, shape will be connected with orthogonal transformations (if the original density is centred, then the effect of translations is a priori removed).

Of course, the common characteristic of all these situations is that shape is defined for finite arrangements of points. Apparently, there is no practical formulation of the shape of a function, though there is considerable work on practical parameterisations of the shape of closed curves on the plane and in space (e.g. Younes [34], Small & Le [32]). This is motivated principally from the problem of object recognition and classification in computer vision. This does not appear useful, though, when attempting to find connections between the shape of a function and the shape of its transform. For this reason, we will hinge on Euclidean shape-theoretic ideas that will enable us to establish such connections, via an appropriate parametrisation (Panaretos [26, 27]).

It is important to emphasise that at least three ingredients come into play when considering a parametrisation for the shape of a density in this particular context. First, it is important that the parametrisation allows for the problem to be posed as one of parameter estimation. In addition, one may ask for a parametrisation that makes it feasible to always explicitly be able to pick out a member from a particular shape class.
(an instance of shape), that is, always be able to choose a representative element from a shape orbit. Most important, though, is the need to be able to find a connection between original shape and projected shape, i.e. be able to invert the Radon transform “in the shape domain”.

2.2 Parametrisation: Radial Expansions and the Gram Matrix

Following the discussion in the previous section, we choose to model the unknown density as being a member of a parametric class depending on a finite-dimensional Euclidean parameter. The problem of recovery then becomes one of statistical inference for the unknown parameter. A possible such class is that of finite mixtures of radial location densities:

\[ \rho(x) = \sum_{k=1}^{K} q_k \varphi(x|\vec{\mu}_k), \quad \vec{\mu}_k \in \mathbb{R}^3, \quad q_k > 0, \quad \sum_{k=1}^{K} q_k = 1, \tag{11} \]

where \( \varphi(\cdot|\vec{\xi}) \) is a spherically symmetric probability density with expectation \( \vec{\xi} \), that is

\[ \varphi(x|\vec{\xi}) = f(||x - \vec{\xi}||), \tag{12} \]

for some appropriate \( f \). The choice of this particular type of expansion appears useful both from the applied and mathematical points of view. The two-dimensional densities recorded via the electron microscope are typically smooth due to the smoothing effects resulting from the optics of the imaging procedure. As a result, although biological particles are “rough” in the sense that they present intricate details at the most fundamental level, the images recorded appear very smooth. In a number of cases, they do appear as an ensemble of roughly circular “blobs” (see e.g. Figures 1, 2, 3).

From a mathematical point of view, this type of density is well-behaved under orthogonal transformation and projection. By this it is meant that for any orthogonal transformation \( A \in O(3) \), we have

\[ \varphi(A^T x|\vec{\xi}) = f(||A^T x - \vec{\xi}||) = f(||x - A\vec{\xi}||) = \varphi(x|A\vec{\xi}) \tag{13} \]

and letting \( H \) be the projection onto the plane \( z = 0 \),

\[ \int_{-\infty}^{+\infty} \varphi(x, y, z|A\vec{\xi}) dz = \phi(x, y|\mu), \quad \mu = H A\vec{\xi}, \quad \phi(x, y|0) = \int_{-\infty}^{+\infty} \varphi(x, y, z|0) dz \tag{14} \]

so that any rotation of the density can be encoded by a rotation of the location parameters \( \vec{\mu}_k \), while any profile of its Radon transform can be expressed as a mixture of marginals of \( \varphi \), regardless of the projection orientation.

To remove the effects of location, assume that the density is centred with respect to its location vectors, that is assume

\[ \sum_{k=1}^{K} \vec{\mu}_k = 0. \tag{15} \]

Since any rotation of \( \rho \) can be encoded by a rotation of its location vectors, we may use the characteristics of the location vectors to encode the shape of \( \rho \). The Gram matrix generated by the collection \( \{\vec{\mu}_k\} \) is the
Figure 3: Synthetic single-particle projection data from the Klenow fragment of Escherichia coli DNA Polymerase I (Courtesy of A. Leschinger). The projections resemble mixtures of roughly circular components contaminated by noise. The structure has been determined via crystallographic techniques.

A $K \times K$ symmetric non-negative definite matrix, whose $ij$-th element is the inner product $\langle \tilde{\mu}_i, \tilde{\mu}_j \rangle$:

$$\text{Gram}(\{\tilde{\mu}_k\}) = \begin{pmatrix} \|\tilde{\mu}_1\|^2 & \langle \tilde{\mu}_1, \tilde{\mu}_2 \rangle & \cdots & \langle \tilde{\mu}_1, \tilde{\mu}_K \rangle \\ \langle \tilde{\mu}_2, \tilde{\mu}_1 \rangle & \|\tilde{\mu}_2\|^2 & \cdots & \langle \tilde{\mu}_2, \tilde{\mu}_K \rangle \\ \vdots & \vdots & \ddots & \vdots \\ \langle \tilde{\mu}_K, \tilde{\mu}_1 \rangle & \cdots & \cdots & \|\tilde{\mu}_K\|^2 \end{pmatrix}$$ (16)

Furthermore, given a Gram matrix of rank $p$, one can find $K$ vectors in $\mathbb{R}^d$, $d \geq p$, with centroid zero whose pairwise inner products are given by that Gram matrix. In fact, the specification of such an ensemble amounts to merely solving a number of non-degenerate lower triangular linear systems of equations. Therefore, the Gram matrix of a centred ensemble (or, rather, its lower triangular component) encodes all those characteristics of the ensemble that are invariant under orthogonal transformation. We can thus define the shape of a $\phi$-radial mixture as the coupling of its mixing proportions with the Gram matrix generated by its location vectors:

$$[\rho] = \left( \text{Gram}(\{\tilde{\mu}_k\}), \{q_k\} \right).$$ (17)

We call the first component of the parametrisation the Gram component and the second component the mixing component. The shape of a profile of $\rho$, say

$$\tilde{\rho}_0(x, y) = \sum_{k=1}^K q_k \phi(x, y| H A_0 \tilde{\mu}_k),$$

corresponding to a rotation $A_0 \in \text{SO}(3)$ ($H$ is the projection onto the plane $z = 0$) will then be given by,

$$[\tilde{\rho}_0] = \left( \text{Gram}(\{HA_0 \tilde{\mu}_k\}), \{q_k\} \right).$$ (18)
While this parametrisation could be thought of as a coordinate system for the function class under consideration, our interest will not be so much in comparing shapes within a class of given dimension as in establishing a relationship between the shapes of the Radon profiles of a density and the shape of the original density itself. In particular, the next Section describes how this parametrisation can yield a statistical inversion "in the shape domain".

3 Statistical Inversion

3.1 Inversion in the Shape Domain

Since the coefficients involved in the radial mixture expansion are unaffected by projection, the Gram matrix of the location vectors becomes the primary object of interest. Especially in view of sufficiency, we seek a relationship between the Gram components of the projected shape and the original shape. The following theorem provides such a connection and can be seen as an inversion in the shape domain.

**Theorem 1** (Shape Inversion). Let $V$ be a $d \times k$ matrix, $2 \leq d < \infty$, $k \leq \infty$, whose columns encode an ensemble of $k$ elements of $\mathbb{R}^d$ with centroid zero. Let $\Psi$ be normalised Haar measure on $SO(d)$, and $H$ denote the projection onto a subspace of dimension $d-1$. Then,

$$\int_{SO(d)} \text{Gram}(HAV) \Psi(dA) = \frac{d-1}{d} \text{Gram}(V)$$

Informally, we could describe this result as saying that projected shape is, in expectation, proportional to the original shape, when the projection orientations are picked completely at random.

**Proof of Theorem 1.** Without loss of generality, we may assume that $H = \text{diag}\{1, 1, 0\}$. We begin by noticing that

$$\text{Gram}(HAV) = V^T A^T HAV,$$

(19)

since $H$ is symmetric idempotent. We recognise that $A^T HA$ is the spectral decomposition of a projection onto the plane $\{A^T x : x \in \text{Im}(H)\}$, where $\text{Im}(H)$ is the image of $H$. As such, we should be able to encode the same projection relying solely on a unit sphere parametrisation, as opposed to using the special orthogonal group. Indeed,

$$B^T HB \overset{d}{=} I - uu^T$$

(20)

for $B \sim \text{Haar}[SO(d)]$ and $u$ a uniformly random unit vector, $u \sim \mathcal{U}(S^{d-1})$. The right hand side of equation (20) is the projection onto the orthogonal complement of a uniformly random unit vector. Hence, the proof of the theorem reduces to verifying that, for $u \sim \mathcal{U}(S^{d-1}),$

$$\mathbb{E}[uu^T] = d^{-1}I.$$

(21)

The uniform distribution on the hypersphere is invariant under orthogonal transformations,

$$Wu \overset{d}{=} u, \quad \forall W \in O(d).$$

Therefore $\mathbb{E}[uu^T] = W\mathbb{E}[uu^T]W^T$ for all $W \in O(d)$, implying that $\mathbb{E}[uu^T] = cI$ for some constant $c \in \mathbb{R}$. Finally, note that $\text{trace}(\mathbb{E}[uu^T]) = \text{trace}(\mathbb{E}[u^Tu]) = 1$, so that it must be that $c = d^{-1}$. \qed
Theorem 1 says that the expectation of the distribution of the projected Gram matrix is proportional to the original Gram matrix, reducing the recovery problem to one of coupling an estimator of the mixing parameters with an estimator for the mean value. Supposing that we can estimate \( \{q_k\} \) consistently by some estimator \( \{\hat{q}_k\} \), then an obvious consistent estimator is given by

\[
\left( \frac{d}{(d-1)N} \sum_{n=1}^{N} \text{Gram}(\hat{\rho}_n), \{\hat{q}_k\} \right),
\]

(22)

which is essentially a method of moments estimator coupled with \( \{\hat{q}_k\} \). Unfortunately, things are not so straightforward. Given a finite realisation of the stochastic Radon transform of \( \rho \), the expansion

\[
\hat{p}_n(x, y) = \sum_{k=1}^{K} q_k \phi(x, y | A_n \hat{\mu}_k)
\]

(23)

is unobservable. Contrary to the case of orthogonal expansions in Hilbert space, there is no transform yielding the above expansion, so that the unobservable elements \( \{q_k\}_{k=1}^{K} \) and \( \{A_n \hat{\mu}_k\}_{1 \leq k \leq K, 1 \leq n \leq N} \) must be estimated from the data. This estimation problem involves “parallel deconvolutions” with some shared parameters (the mixing proportions) thus posing a further subtle problem. Since the expansion is unobservable, the indices are also unobservable. When looking at a projection, regardless of how we arrange the location vectors to build the Gram matrix and coefficient vector, the information encoded is the same. However, we must be able to choose this arrangement consistently across all projections, since we will be averaging the Gram matrices across projections, so that it is imperative that we average the appropriate quantities! If the indices are unobservable, then guaranteeing this consistent construction of the Gram matrices is non-trivial. This is yet a further unidentifiability issue. We must use the data at hand as a guide to this construction, and this requires that we impose a further assumption on the density expansion:

**Assumption 1.** The components of the density are distinguishable, that is, in the setup given in (11), we further assume that

\[ q_i \neq q_j, \forall i \neq j. \]

Under Assumption 1, once we have obtained a reasonable deconvolution estimate of the unobservable expansion, we can use auxiliary parameters (the mixing and perhaps the scaling coefficients, if these are included) to assign labels to the location vectors for each projection in a way that is consistent across projections. This enables the definition of a hybrid maximum likelihood/method of moments estimator.

### 3.2 A Hybrid Estimator

For simplicity and tidiness, we will treat the planar case. The treatment of the three-dimensional case, as well as higher dimensions, is directly analogous. Let \( \rho : \mathbb{R}^2 \to [0, \infty) \) be a continuous density function with compact support, which we assume without loss of generality to be the disc of radius \( \pi \), \( \Delta_2 = \{ x \in \mathbb{R}^2 : \|x\| \leq \pi \} \). We let \( N \) be a positive integer and \( \{A_n\}_{n=1}^{N} \) be independent and identically distributed random elements of the special orthogonal group \( \text{SO}(2) \) drawn according to the corresponding normalised Haar
measure. Finally, we write \( A \rho(x) := \rho(A^{-1}x) \) for \( x \in \mathbb{R}^2 \) and \( A \in \text{SO}(2) \). The corresponding stochastic Radon transform is the collection of projections (profiles)

\[
\tilde{\rho}_n(x) := \Pi\{\rho\}(A_n)(x) = \int_{-\infty}^{+\infty} A_n \rho(x, y) dy, \quad x \in [-\pi, \pi].
\] (24)

Let \( \varphi(\cdot|\tilde{\xi}) \) be a planar radial density function centred at \( \tilde{\xi} \), and let \( \phi(x|0) = \int_{-\infty}^{+\infty} \varphi(x, y|0) dy \) be a symmetric 1-dimensional location density, centered at the origin. Our model is

\[
\rho(x, y) = \sum_{k=1}^{K} q_k \varphi(x, y|\mu_k), \quad q_i \neq q_j \forall i \neq j
\] (25)

so that the \( n \)-th profile is \( \tilde{\rho}_n(x) = \sum_{k=1}^{K} q_k \phi(x|\mu_k^{(n)}) \). Here, \( \mu_k^{(n)} \in [-\pi, \pi] \) denotes the projection of the \( k \)-th location vector in the \( n \)-th profile of the stochastic Radon transform: \( \mu_k^{(n)} = HA_n \mu_k, H = (1, 0) \). Since we assume that \( \rho \) is supported on the disc \( \Delta_2 \), it must be that \( \text{diam} \{\text{supp}\varphi\} < 2\pi \).

In practice, we observe a discrete version of the profiles, on certain lattice points \( \{x_t\}_{t=1}^{T} \in [-\pi, \pi] \), for \( T \) a positive integer. In particular, we assume the lattice to be regular, i.e. the \( x_t \) being equally spaced. Furthermore, the digital images of the profiles will be contaminated by additive noise, which is assumed to be Gaussian and white. Therefore, the data we obtain are described by

\[
I_n(x_t) = \tilde{\rho}_n(x_t) + \epsilon_n(t),
\]

\[
= \sum_{k=1}^{K} q_k \phi(x_t|\mu_k^{(n)}) + \epsilon_n(t), \quad n \in \{1, ..., N\}, t \in \{1, ..., T\},
\]

for \( \{\epsilon_n(t)\} \) a collection of \( N \) independent Gaussian white noise processes on \( \{1, ..., T\} \). By independence, both between and within the white noise processes and the random rotations in equation (24), we may write down the following log-likelihood expression for the parameters of the unobservable mixture expansion,

\[
\ell(\mu, q) \propto -\frac{2\pi}{NT} \sum_{n=1}^{N} \sum_{t=1}^{T} \left\| I_n(x_t) - \sum_{k=1}^{K} q_k \phi(x_t|\mu_k^{(n)}) \right\|^2.
\] (26)

Maximisation of this loglikelihood requires that we choose \( NK \) location parameters \( (K \) for each profile), as well as a unique set of \( K \) mixing proportions to be shared across the profiles, so as to minimise the residual sum of squares between the observed and stipulated profiles (note that \( K \) is assumed to be known; see the discussion in Section 5).

Our hybrid estimator for the shape of the two-dimensional density \( \rho \) is then formally written as:

\[
\hat{\rho} = \left( \frac{2}{N} \sum_{n=1}^{N} \text{Gram}(\{\mu_k^{(n)}\}_{k=1}^{K}, \{\tilde{q}_k\}_{k=1}^{K}) \right) \hat{\mu}, \quad \hat{\mu} = \text{arg max}_{(\mu, q)} \ell(\mu, q).
\] (27)

We will call \( \hat{\mu} \) the estimator of the Gram component of the shape \( [\rho] \) and \( \hat{q} \) the estimator of the mixing component. To consider the “large sample” properties of the hybrid estimator, we need to consider both
the resolution \(T\) and the number of profiles \(N\). The \(T \to \infty\) asymptotics relate to the MLE step, while the \(N \to \infty\) asymptotics relate to the inversion stage. If the growth of \(T\) is "fast" enough compared to the growth of \(N\), the hybrid estimator can be seen to be consistent and asymptotically Gaussian in an appropriate sense (Panaretos [25]). This dependence arises because, when performing the MLE step, we ask that the mixing proportions are the same for all profiles. As a result, the dimension of the parameter describing the locations grows as \(N\) grows, leading to a need for \(\kappa\). If the MLE step is performed independently for each profile, i.e. we separately obtain

\[
\arg\min_{\left(q_k^{(n)}\right)_{k=1}^K, \left(\mu_k^{(n)}\right)_{k=1}^K} \frac{2\pi}{T} \sum_{t=1}^T \left\| I_n(x_t) - \sum_{k=1}^K q_k^{(n)} \phi \left( x_t | \mu_k^{(n)} \right) \right\|^2, \quad n = 1, 2, ..., N, \tag{28}
\]

then exchangeability implies a consistency result of the form

\[
\forall \epsilon > 0 \exists T_0, N_0 : \mathbb{E} \left\| \hat{G}(T, N) - \text{Gram}(\rho) \right\|_2 < \epsilon, \quad \forall N \geq N_0 \& T \geq T_0. \tag{29}
\]

While in the case of overall optimisation, more projection data may require better resolution \(T\), in the case of separate (or grouped) optimisations, the projection asymptotics do not directly influence the resolution asymptotics, so that for a fixed high resolution, it makes sense to independently send \(N\) to infinity. In biological practice, of course, the instruments will give a certain — hopefully high — resolution. This depends on the current state of technology and can be thought of as being inflexible. On the other hand, the number of projections can become arbitrarily large, the only constraint being computing time (Glaeser [9]).

If we carry out the MLE step separately for each image, then a standard \(\sqrt{n}\)-type central limit theorem for the maximum likelihood estimates of the projected location parameters applies for the MLE deconvolution within a single profile. A delta method argument subsequently implies that picking \(\tau_N = O(N^{\kappa})\) for \(\kappa > 1\) is necessary and sufficient in order to guarantee consistency and asymptotic normality.

4 An Example

The objective function given by the loglikelihood (26) has to be minimised over a high-dimensional (the more the projections, the higher the dimension of the unknown parameter). Furthermore, the form of the objective function is expected to lead to a large number of local optima. This renders the practical solution of the optimisation problem challenging. In order to provide a simple example we consider a simulated two-dimensional mixture. The mixtures are purposely chosen to be somewhat sparse and there is no noise contamination, so that deterministic spike deconvolution methods apply (e.g. Pisarenko's [28] method and variants [21]).

Assume that we observe a finite sample of \(N = 150\) profiles from the stochastic Radon transform of the density function

\[
\rho(u) = \sum_{j=1}^5 \frac{j}{2\pi \sigma^2} \exp \left\{ -\frac{1}{2\sigma^2} (u - \mu_j)^T (u - \mu_j) \right\}, \quad u = (u_x, u_y) \in \mathbb{R}^2 \tag{30}
\]

with \(\sigma = 0.3\) and \(\{\mu_j\}\) given in the following table:

<table>
<thead>
<tr>
<th>(j)</th>
<th>(\mu_x)</th>
<th>(\mu_y)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.1</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.2</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>0.4</td>
</tr>
<tr>
<td>5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
</tbody>
</table>
Figure 4: (a) Contour plot of the density $\rho$ with dots indicating the locations of the means. (b) Intensity plot of the density $\rho$. (c) Superimposition of the two plots.

<table>
<thead>
<tr>
<th></th>
<th>$\mu_1$</th>
<th>$\mu_2$</th>
<th>$\mu_3$</th>
<th>$\mu_4$</th>
<th>$\mu_5$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$x$</td>
<td>0.6</td>
<td>0.6</td>
<td>-0.1</td>
<td>-1</td>
<td>-0.2</td>
</tr>
<tr>
<td>$y$</td>
<td>0</td>
<td>0.8</td>
<td>0.1</td>
<td>-0.3</td>
<td>-0.6</td>
</tr>
</tbody>
</table>

The choice of the location parameters as well as the standard deviation was made so as to ensure a certain sparsity: the pairwise distance between means is such that given the specific standard deviation, any two of the mixture components are not significantly overlapping. In terms of the biological framework, a Gaussian mixture appears strange, since it has unbounded support. This can be corrected through truncation at an appropriate level. Figure 4 gives a contour and intensity plots of the mixture density.

The 150 profiles are sampled on a grid of $T = 256$ regular lattice points. A sample of six profiles from this stochastic Radon transform is presented in Figure 5. It is unknown to which angles these correspond.

The mixing proportions and projected location parameters are recovered through Pisarenko's method [28], which essentially translates the problem to one of frequency detection in a harmonic signal. Figure 6 contains the contours of the estimated density, and its superimposition on the contour plot of the true density and a "coincidence plot" for the estimated mixing proportions.

A challenging problem is uncertainty estimation and presentation. It is straightforward to calculate covariance matrices and use normal approximations to construct high-dimensional confidence regions for the parameters of the expansion, but this does not seem useful to the data analyst and the scientist, who need an intuitive feel for the variation in terms of the structure itself. For this reason we resample the 150 profiles and construct bootstrap replicates of the estimated density. We then superimpose the contour plots (see Figure 7) in order to obtain a visual appreciation of the uncertainty involved in the estimation procedure: the superimposition reveals uncertainties through the tangling of the contour lines. The rule of thumb is that the more tangled the contours appear the more uncertainty is associated with that particular region. The important aspect of these figures is that the overall shape (used here in its non-mathematical sense) is seen to be preserved and not to be highly variable. Our presentation is motivated by Brillinger et al. [3], where, in
Figure 5: Six sample profiles from the 150 profiles of the realisation of the stochastic Radon transform of the density given in equation 30.

Figure 6: Contour plot of the estimated density (left), superimposition of the contour plots of the true (red) and estimated (blue) densities (middle), and straight line fit Plot for the true ad estimated mixing coefficients.
Figure 7: Superimposed bootstrap replicates as a means of assessment of uncertainty. Panels (a)-(d) contain the superimposition of 15, 40, 75 and 100 replications, respectively.
order to assess the reconstruction of the planar density of a crystalline structure, the rotational symmetries of the structure were taken advantage of, by superimposing rotations that ought to leave the structure invariant.

5 Concluding Remarks

Obtaining a reconstruction from a stochastic Radon transform with no angular is an ambitious task. However, it seems that it should be possible to at least obtain an initial rough estimate (a low resolution model in biophysical terminology) that may provide the basis for further work, or perhaps even play the role of an a priori model originating strictly from the data at hand, without dependence on angular estimates. A careful statistical formulation of the problem, involving a statistically simple yet flexible model, enables us to achieve a "statistical inversion" without resorting to a priori models or estimation of the projection directions. While we focused on a mixture approach, one can consider more general radial basis expansions (e.g. Buhmann [4]), or include scale parameters, as long as the expansion remains identifiable. However, the next step of implementation poses a number of practical challenges.

The first task is the determination of an algorithm for the global optimisation of the loglikelihood function (26). The optimisation problem is non-linear and is quite likely to present multiple local optima; indeed the search space is high-dimensional. A crucial aspect here, of course, is that of suitably taking into account the specific characteristics of the problem, since each profile contains useful information for each other profile.

Other issues that should be taken into account are: the choice of spherically symmetric density, and the choice of the number of mixture components. The input of the scientist is crucial for these matters, as it is likely that he/she will have an approximate idea about what might be more appropriate. However, this should perhaps be supplemented by more formal statistical procedures.

Complications may arise that depend on the nature of the specific biological particle. While a number of interesting particles do not tend to have a preferred orientation within the aqueous solution, there are numerous exceptions where the Haar measure assumption on the rotation group is violated. Finally, in Section 3.2 we took advantage of Assumption (1) which states that the mixing proportions are distinct. There is no physical reason why the mixing proportions for a particle should be distinct. Consider, for example, any particle with a non-trivial symmetry group, such as PF0R seen in Figure 1—this is not to say that there do not exist particles for which it it can be expected that this assumption be reasonable, e.g. the eIF3 factor or the Klenow fragment, Figures 2 and 3. In interesting question is that of finding approaches to attempt to recover (indeed estimate) the unknown labels, through some sort of overall consistency criterion: which labels give the "most consistent" set of shapes.

Acknowledgements

I wish to warmly thank Professor David Brillinger for motivating me to work on this problem and for many stimulating discussions, and Professor Robert Glaeser (Molecular and Cell Biology, UC Berkeley & LBNL) for our most helpful interactions on the structural biology side. I also wish to thank Professor Sir David Cox for a number of thought-provoking conversations on the topic and Professor Anthony Davison for useful comments.
References


