On a Generalized Stochastic Model for Estimating the Sizes of Spheres from Profiles in Thin Slices and an Associated Problem of Non-Identifiability

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1. Introduction.

The results presented here are based on work inspired by a live problem about which the authors were consulted by Dr. Shirley Bayer of the Biology Department at Purdue University. The problem she was concerned with is the estimation of the total number of cells of certain type in a specified region of the rat brain based on experimental data obtained from microtome sections of the region (Bayer (1982), Bayer *et al* (1982)). More specifically, the data were based on the counts and individual areas of thin nuclei slices obtained by projecting microtome sections onto the plane of observation. A commonly used method for estimating the total number of cells is based on volumetric analysis which in turn necessitates estimation of the cell nuclear-size distribution from the observed areas of nuclei slices. This naturally leads to the question: Is it possible to identify the underlying nuclei-size distribution *uniquely* from the distribution of the sizes of the planer projection of the nuclei sections? It is this question that we will primarily address ourselves to in this paper.

The above problem is an old problem which has arisen in many areas of scientific interest such as metallurgy (see Nicholson 1970) stereology (see Coleman (1982), Cruz-Orive (1983), Cruz-Orive and Weibel (1981), Tallis (1970) and Weibel (1979)), crystalography as well as biology. The underlying stochastic modeling typically involves some aspects of geometric probability (see Kendall and Moran (1963), Solomon (1978)). A commonly occurring complication is observed in most of the experimental areas mentioned above, namely a noticeable lack of sections of small sizes in the observed data. Some early workers used models which failed to explain this complication. More recent workers however have adapted models which postulate the existance of thresholds so that only those sections with sizes falling within the threshold boundaries are observable while others are lost,(see Coleman (1982), Cruz-Orive (1982), Greeley and Crapo (1978), Hendry (1976), Nicolson (1970) and Weibel (1979)). The threshold type models, clearly imply that a nuclear section with size barely within the threshold boundaries is observable with certainty whereas another section with size barely outside the boundaries is *not* observable, again with certainty. Such an extreme behavior seems unrealistic from a practical viewpoint. In fact, even if the two sections are of exactly the same size there is no reason to believe that they will be observed (or not observed) with *certainty* because of factors other than size which are either unknown or cannot be experimentally determined. For instance, in the biological context microtome slices are stained to enhance observability with different nuclear sections absorbing differing amounts of stain and thus introducing an element of

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chance in their observability. Another possibility might be that some (not all) of the small sections are lost due to the cutting process itself. These and other similar possibilities cannot be explained by the threshold type models where the chance of observing any section of a certain size is either 0 or 1 and not in between. A more realistic model is the one which postulates the existence of a function $p(x, y)$ representing the probability that a nuclear section with projected size y obtained from a nucleus of size x will be observed. This function, as a probability, may take any value over the interval 0 to 1. The threshold models are simply the special cases where the function $p(x, y)$ takes only the values 0 and 1. Consequently, the models based on the $p(x, y)$ function will be referred to as generalized stochastic models for the phenomenon under consideration.

In the next section we specify in detail the assumptions that underlie the class of generalized stochastic models to be studied in this paper. In particular, we derive a useful relation between the unobserved nuclei (three dimensional) size distribution and the observed projected (planar) section size distribution. Throughout this paper, the nuclei are assumed to be spherical bodies. Depending upon the area of application these bodies may be referred to as particles, nuclei, spheres, etc., nevertheless we shall continue referring to them as nuclei here for convenience. Section 2.2 considers various expressions for the function $p(x, y)$, some of which correspond to the threshold type and other models dealt with in the literature. Sections 3 and 4 deal with the question of identifying the underlying sphere size distribution uniquely from the distribution of the sizes of the planar projections of their sections. The paper ends with a discussion of some open problems.

2. A Generalized Stochastic Model.

Consider a three dimensional medium of a certain volume V containing a certain number of distinguishable spherical bodies randomly located throughout V. In this connection we shall adopt the standard hypothesis involving a spatial Poisson process for the number and locations of the spheres. Moreover, we visualize a probe B consisting of two parallel planar surfaces with 2ϵ distance separating them. The intersection of probe B with the medium will contain a random number of sections of spheres with (random) radii greater than ϵ. These sections, when B is projected on a planar field (slide) of observation, lead to circles. We now make this more precise with the following postulates.

A_1 (Poisson-Postulate): Sphere centers are distributed over the volume V according to a homogeneous spatial Poisson process with rate per unit volume equal to $\lambda > 0$, so that

i) $N(R)$, representing the number of sphere centers falling in a given region $R \subset V$, is a Poisson random variable with mean $\lambda V(R)$, where $V(R)$ is the volume of region R.

ii) The random variable $N(R_1), N(R_2), \ldots, N(R_k)$ corresponding to arbitrary non-overlapping regions $R_1, R_2, \ldots, R_k$ within the medium are mutually independent, for any k.

Remark 1. The Poisson point process assumption is commonly made in modeling stereological problems and is considered a reasonable approximation to the live situation (see

Nicholson 1970, Coleman 1982 and Cruz-Orive 1983).

$\mathbf{A_2}$ (Postulate for sphere radii): Associated with each center is a positive random variable X, representing the radius of a sphere located at that center. The random variables, X's, associated with various centers are assumed to be mutually independent with a common distribution function $G(\cdot)$.

$\mathbf{A_3}$ (Postulate for probability function $p(x, y)$): Given the location of the probe B, in the medium, in the event a sphere intersects B, let Y denote the radius of the corresponding circle formed by the planar projection of that intersection upon the field of observation. We postulate the existence of a function $p(x, y)$ representing the conditional probability that a projected circle will be observed given that $X = x$ and $Y = y$. In the event a center falls within B, we will have $Y = X$ and $p(x, x)$ will be the probability that we observe the corresponding projection. On the other hand if a center falls outside B we will have $Y < X$.

Remark 2. In the following development we shall ignore the so called *edge effect* caused by a sphere center which is located at a distance less than X from the boundary of V. The edge effect arises because sphere centers are distributed randomly in V and a sphere of random radius is associated to each center. There is no assurance that the sphere will not extend beyond the boundary of V and if it does we propose to extend V to include such a sphere. Note however that the sphere centers are distributed only in the original V and are not allowed to fall in the extension of V. (This is achieved through the function $A(z)$ in equation (2) below.) Furthermore, B may intersect a sphere which is partially contained in an extension of V and such an intersection will be treated as a part of B. This treatment of the edge effect is commonly done and is a reasonable approximation to the real situation (see Coleman (1982)). Similarly we shall ignore the problem caused by overlapping of spheres. This is not unreasonable when the spheres are sparsely located in the medium (ie, the particle distribution in the medium is sufficiently dilute) so that the parameter λ is small making the overlappings unlikely (see Nicholson (1970) for a related discussion).

We shall now derive a useful relation between the distribution of the radii of the spheres and the distribution of the radii of the projected circles. Without loss of generality, let the origin of the three dimensional space lie in the central plane (CP) parallel to the faces of the probe so that each of the two faces are at a distance ϵ from the origin. Let the Z-axis be perpendicular to these faces (see figure 1). Let $A(s) \geq 0$ be the area of the intersection of the plane $Z = s$ with the medium V defined for all $-\infty < s < +\infty$, so that $V = \int_{-\infty}^{\infty} A(s)ds$.

In view of postulate $A_1, N(V)$ has a Poisson distribution with mean λV. According to a well known property (see Karlin and Taylor 1975) of a homogeneous spatial Poisson process the conditional distribution of the locations of the centers of the spheres, given that $N(V) = n$, is the same as that of n points which are distributed independently according to a uniform distribution over the region V. Consider one such point, say d, which is distributed over V in the manner indicated above. The sphere probability density function (p.d.f.) of the Z-coordinate of location of the point d is given by $A(s)/V, -\infty < s < +\infty$. Let X be the radius of the sphere associated with the point d (see postulate A_2). Let s be the z coordinate of the point d. Consider first the case with $|s| > \epsilon$. In order that the sphere at d intersects B and that the radius, Y, of the circle formed by the planar projection of the corresponding intersection as defined in postulate A_3, be no more than y, note that the radius X of the sphere at d must lie between $|s| - \epsilon$ and $\sqrt{y^2 + (|s| - \epsilon)^2}$ (see figure 1). Also, given that X is within these limits, the corresponding $Y = \sqrt{x^2 - (|s| - \epsilon)^2}$, so that $p(x, \sqrt{x^2 - (|s| - \epsilon)^2})$ is the conditional probability that the corresponding circle of projection will be observed (see postulate A_3). If on the other hand $|s| \leq \epsilon$, so that point d lies in B, then $X = Y$.

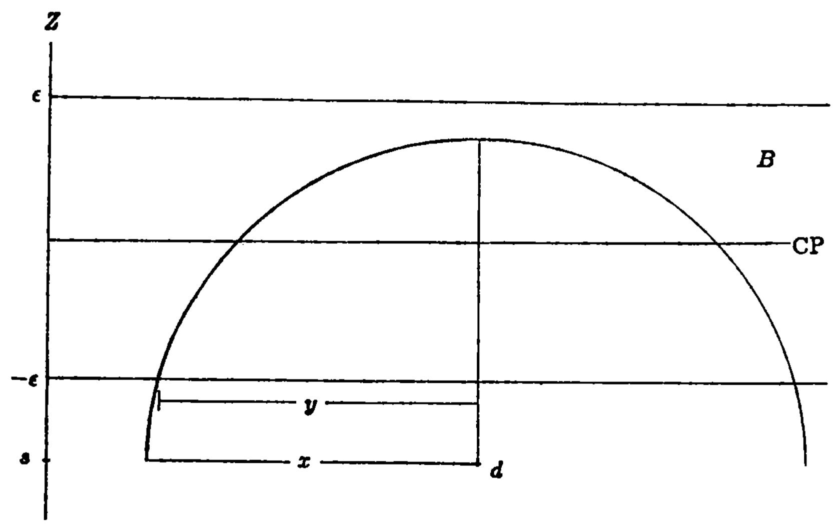

Figure 1. The sphere at d intersects the probe B. In this example $|s| > \epsilon$

Let E denote the event "the sphere associated with d intersects the probe and the corresponding circle of projection is observed". Also let

$$\psi(E, y) = P(E \cap [Y \leq y]). \tag{1}$$

Then from the above considerations we are led to

$$\psi(E, y) = \left[\frac{1}{V} \int_{(-\infty,-\epsilon)\cup(\epsilon,\infty)} A(z) \int_{|z|-\epsilon}^{\sqrt{y^2+(|z|-\epsilon)^2}} p(x, \sqrt{x^2 - (|z| - \epsilon)^2}) dG(x) dz + \frac{V(B)}{V} \int_0^y p(x, y) dG(x) \right] \tag{2}$$

where $\frac{V(B)}{V}$ is the probability that the point d falls in B with

$$V(B) = \int_{-\epsilon}^{\epsilon} A(z)dz \tag{3}$$

After splitting the first integral into two parts, one over $(-\infty, -\epsilon)$ and the other over (ϵ, ∞), changing the order of integration in each part followed by a change of variable, $t = \sqrt{x^2 - (|z| - \epsilon)^2}$ for fixed x and then combining the resulting two parts into one, we obtain

$$\psi(E,y) = \frac{1}{V}\{\int_0^\infty dG(x)\int_0^{\min(x,y)} \frac{t}{\sqrt{x^2-t^2}}\left[A^*(\epsilon + \sqrt{x^2-t^2}\right].p(x,t)dt$$
$$+ V(B)\int_0^y p(x,y)dG(x)\} \tag{2a}$$

where $A^*(z) = A(z) + A(-z)$.

It follows from the Poisson process property (see Karlin and Taylor 1975) that for every $y > 0$, the number $N_E(y)$ of those spheres which satisfy the event E and have $Y \leq y$ has a Poisson distribution with mean $\lambda V\psi(E,y)$. Furthermore for arbitrary $k \geq 1$ and $0 = y_0 < y_1 < y_2 < \ldots < y_k < \infty$, the numbers $N_E(y_i) - N_E(y_{i-1}), i = 1, 2, \ldots, k$, are independent Poisson with means $\lambda V[\psi(E,y_i) - \psi(E,y_{i-1})]$. From these it easily follows that the observed radii $Y_1, Y_2, \ldots, Y_m$ of the projected circles, given that the total number of spheres satisfying the event E namely $N_E(\infty)$ is equal to m, are conditionally mutually independent with a common cumulative distribution function (c.d.f) given by

$$F(y) = \psi(E,y)/\psi(E,\infty), \qquad 0 < y < \infty, \tag{4}$$

which is the theoretical analog of what is actually observed in practice.

2.1 A Possible Further Generalization.

Following Keiding (1972) we now introduce a modification of our postulate A_2, namely that the distribution of the radius X of a sphere is dependent upon its location vector $\mathbf{W}$ in the medium. Thus conditionally given $N(V) = n$. The vectors $(\mathbf{W}_i, X_i), i = 1, \ldots, n$, corresponding to the n spheres are independently and identically distributed (i.i.d), with the marginal distribution of vector $\mathbf{W}$ being uniform over the volume V and the conditional distribution of X given $\mathbf{W} = \mathbf{w}$ is given by c.d.f. $G(\cdot|\mathbf{w})$, for all $\mathbf{w}\epsilon V$. With this formulation the modified version of $\psi(E,y)$ takes the following form.

$$\psi(E,y) = \frac{1}{V}\Big[\int_{B^c\cap V}\Big\{\int_{|w_3|-\epsilon}^{\sqrt{y^2+(|w_3|-\epsilon^2}} p(x, \sqrt{x^2-(|w_3|-\epsilon^2)})dG(x|\mathbf{w})\Big\}d\mathbf{w}$$
$$+ \int_{B\cap V}\int_0^y p(x,x)dG(x|\mathbf{w})d\mathbf{w}\Big] \tag{5}$$

Remark 3. Note that here the function $G(x|\mathrm{w})$ takes into account the so called edge effect which was ignored in the previous formulation (see remark 2 in the preceding section). However identifying the family of distribution functions $G(\cdot|\mathrm{w})$ for various values of w based on the observed section radii is impossible. For this reason we shall address the identifiability problem only under the formulation of equation (2).

Remark 4. In (5), the function $A(w_3)$ which occurs in (2) is absorbed into the limits of integration by the intersection of B^c with V. However, in all of the references that we have reviewed the function $A(w_3)$ is not taken into account either directly, as in (2), or indirectly, as in (5). It is evident from (2) that $\psi(E, y)$ depends on the function $A(w_3)$ through the even function $A(w_3) + A(-w_3)$. Thus, previous work which ignores $A(\cdot)$ is justified only if some severe condition on $A(\cdot)$ is imposed such as $A(w_3) + A(-w_3)$ being a constant.

2.2 Special cases of $p(x, y)$.

In some of the earlier models, $p(x, y)$ was taken to be identically one. More recently, when it became known that small pieces were missing among the observed circles, several researchers modified the model by using truncation approaches to adjust for the missing circles. This, in terms of our notation, leads to a function $p(x, y)$ taking only the values 0 or 1. Some of those cases are considered below.

Keiding et al., (1972) consider a model where it is assumed that the (projected) circle of intersection is not observed whenever the angle ϕ of intersection, the so called capping angle, of the corresponding nucleus with the microtome is less than some fixed threshold. This, in terms of our notation, amounts to taking the probability (see figure 2)

$$p(x, y) = \begin{cases} 1 & \text{if } y \geq x \sin\phi \\ 0 & \text{otherwise} \end{cases} \tag{6}$$

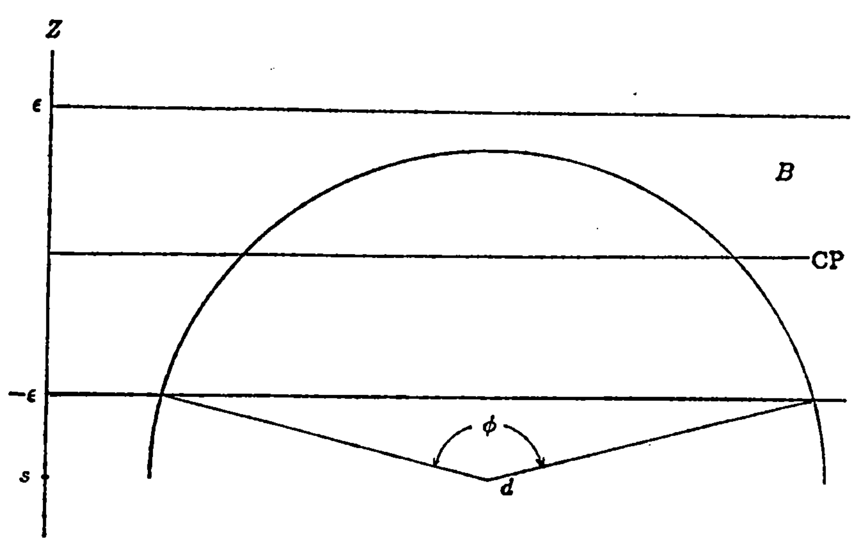

Figure 2. The capping angle ϕ.

Cruz-Orive (1983) introduces another truncation mechanism in addition to that of Keiding et al., both acting simultaneously. This additional mechanism is the introduction of a positive resolution threshold Q on the diameter of the observed circle so that if $y < Q/2$ it is not observed. In this case, then

$$p(x,y) = \begin{cases} 1 & \text{if } y \geq \max(\frac{Q}{2}, x \sin \phi) \\ 0 & \text{otherwise} \end{cases} \tag{7}$$

The above cases are based on lower truncation bounds for y which depend on X. Coleman (1982) considers a more general case where he postulates the existence of a resolution interval $(\xi_0(X), \xi_1(X))$ for every X such that if the profile radius y falls outside the resolution interval it will not be observed even though it intersects the probe B. Thus in our notation it is equivalent to taking the probability function

$$p(x,y) = \begin{cases} 1 & \text{if } \xi_0(x) < y < \xi_1(x) \\ 0 & \text{otherwise} \end{cases} \tag{8}$$

The reader may refer to Coleman (1982) for several special cases of the threshold functions $\xi_0(x), \xi_1(x)$ considered there.

Puri and Yackel (1983) consider an alternative truncation mechanism where the projected circle is not observed whenever the maximum depth (of the corresponding nucleus) contained in the probe is less than a threshold ρ. When a sphere of radius x intersects the probe yielding a projected circle of radius y, the maximum depth of the corresponding nucleus contained in the probe is given by $x - \sqrt{x^2 - y^2}$. The probability $p(x,y)$ in this case is given by

$$p(x,y) = \begin{cases} 1 & \text{if } x - \sqrt{x^2 - y^2} \geq \rho \\ 0 & \text{otherwise.} \end{cases} \tag{9}$$

In spite of the above examples, it may be 4emarked that in most of the practical situations one would not expect the probability $p(x,y)$ to abruptly change values from 1 to 0 thereby justifying our consideration here of the more general case. In particular, for the case considered by Puri and Yackel above, a better form of (9) would be to take

$$p(x,y) = h(x - \sqrt{x^2 - y^2}) \tag{10}$$

where h is a non-decreasing function lying between 0 and 1.

The form (10) is appropriate in applications where a dye is used to stain the nuclear material and thereby rendering it opaque facilitating its observation. The greater the depth of nuclear material at a point, the greater will be the density of the absorbed dye at that point, hence making it more opaque.

Finally, another case which we have considered primarily because of the mathematical simplification that it allows in equation (2) is,

$$p(x,y) = p_1(x)p_2(y), \qquad 0 \leq y \leq x. \tag{11}$$

While it is understandable that the probability $p(x, y)$ should depend on y in a monotone non-decreasing manner yet in some situations, independently of this, it may be conceivable that this probability also depends on the occurrence of some "event" regulated by the cell size x only. This then allows some practical considerations for this case.

3. On the identifiability of G based on equation (2) with known $p(x, y)$.

For many practical purposes often one needs to study certain properties of the underlying nuclear radii distribution function $G(\cdot)$ (see for example Nicholson 1970). For this it is essential that the distribution function $G(\cdot)$ be uniquely determined by the distribution of the observed sizes of the nuclear sections. The lack of this uniqueness is often referred to as the problem of non-identifiability. As pointed out in Puri (1979, 1985), this problem of non-identifiability in the context of stochastic modeling is often more acute than is usually thought of or looked into or even reported. In general it is important to investigate this question first before the model is put to any practical use for the purposes of inferences. Otherwise, in the presence of nonidentifiability, as indicated by Clifford (1977) through numerical examples in his case, one may arrive at quite conflicting predictions by using such models.

In the following subsections, in the context of equation (2), we shall investigate this problem for several special cases under the condition that the function $p(\cdot, \cdot)$ is known.

Since the considerations from here on will be based on equation (2) we wish to remind the reader about our standing assumption (see remark 2) which takes care of the edge effect.

3.1 The case of discrete G.

With a minor condition on the given function $p(\cdot, \cdot)$ the following theorem demonstrates that G is completely identifiable whenever it is discrete.

Theorem 1. *Subject to the relation, (2) if G is purely discrete with $p(x, x) > 0$ for every $x > 0$ with $g(x) = G(x) - G(x-) > 0$, then based on the distribution of observed section radii, G is uniquely determined (see 15 below), where $G(x-)$ denotes the left hand limit of G at the point x.*

Proof: Let the support of G be $\{0 < r_i, i = 1, 2, \ldots\}$. It follows from (2) and (4) that

$$F(y) = \frac{1}{KV} \int_{(-\infty,-\epsilon)\cup(\epsilon,\infty)} A(z)dz \sum_{i=1}^{\infty} I_{r_i}((|z| - \epsilon, \sqrt{y^2 + (|z| - \epsilon)^2}))p(r_i, \sqrt{r_i^2 - (|z| - \epsilon)^2})g(r_i)$$
$$+ \frac{V(B)}{KV} \sum_{r_i \le y} p(r_i, r_i)g(r_i) \qquad (12)$$

where $K = \psi(E, \infty)$ and $I_x(A)$ is 1 if $x \in A$ and is 0 otherwise.

It is easy to verify that the first term on the right side of (12) is a continuous function of y. Furthermore, the second term is purely discontinuous with discontinuity points coinciding with the points of support of $G, \{0 < r_i, i = 1, 2, \ldots\}$. Therefore it follows that the discontinuity points of F coincide with those of G.

In particular we have

$$F(r_i) - F(r_i-) = \frac{V(B)}{KV} p(r_i, r_i) g(r_i) \tag{13}$$

and hence

$$g(r_i) = \frac{KV[F(r_i) - F(r_i-)]}{V(B)p(r_i, r_i)}, \quad i = 1, 2, \ldots \tag{14}$$

Since $\Sigma g(r_i) = 1$, this leads to

$$g(r_i) = \frac{[F(r_i) - F(r_i)]/p(r_i, r_i)}{\sum_{j=1}^{\infty} [F(r_j) - F(r_j-)]/p(r_j, r_j)}, \quad i = 1, 2, \ldots \tag{15}$$

Remark 5. The above proof demonstrates that even if G is not purely discrete, any discontinuity point of G coincides with that of F and conversely. Furthermore (14) gives the size of the jump of G at such a point.

3.2 The case of absolutely continuous G with bounded support.

The following theorem establishes identifiability of G from among the class of G's which have bounded support and which are absolutely continuous with respect to lebesgue measure μ with bounded densities.

Theorem 2. *Let the common distribution of sphere radii have bounded support with a bounded p.d.f. g. Let each of the functions $p(x, y)$, $A(z)$ and the p.d.f. g, be an almost everywhere (with respect to μ) continuous function of its arguments. Furthermore let there exist a constant $\delta \geq 0$ such that $p(x, x) > 0$ for all $x > \delta$ with*

$$p(x, y) \leq p(x, x), \qquad \text{for all } \; y \leq x. \tag{16}$$

Then subject to (2) and (4), for the given functions $p(x, y)$ and $A(z)$ assumed to be bounded, knowledge of $F(\cdot)$ determines g uniquely provided $g(x) = 0$ for all $x \leq \delta$.

Proof: The expression for $\psi(E, y)$, given by (2), can be equivalently written as

$$\begin{aligned} \psi_g(\epsilon, y) = & \int_y^{\infty} \int_0^y A^*(\epsilon + \sqrt{x^2 - u^2}) p(x, u) u (x^2 - u^2)^{-1/2} \, g(x) du dx \\ & + \int_0^y \int_0^x A^*(\epsilon + \sqrt{x^2 - u^2}) p(x, u) u (x^2 - u^2)^{-1/2} g(x) du dx \\ & + V(B) \int_0^y p(x, x) g(x) dx, \end{aligned} \tag{17}$$

where the subscript g is added to ψ to indicate its dependence upon g. Let there be two distinct p.d.f.'s g_1 and g_2 satisfying the conditions of the theorem and be such that the corresponding F_1 and F_2 as determined by (17) and (4) coincide. In order to prove the theorem it suffices to show that for $x > \delta$, $g_1(x) = g_2(x)$, a.e. (μ). Let

$$g^*(x) = \frac{g_1(x)}{K_1} - \frac{g_2(x)}{K_2} \quad \text{for all } x > \delta, \qquad \text{where } K_i = \psi_{g_i}(E,\infty), i = 1,2.$$

Since each g_i integrates over (δ,∞) to one, given $F_1(y) = F_2(y)$ or equivalently

$$\frac{1}{K_1}\psi_{g_1}(E,y) - \frac{1}{K_2}\psi_{g_2}(E,y) \equiv 0, \quad \text{for all } y > \delta, \tag{18}$$

it is sufficient to show that $g^*(x) = 0$, a.e. (μ) for all $x > \delta$. From (17) and (18), it follows that, for $y > \delta$

$$\begin{aligned}\int_y^\infty \int_0^y & A^*(\epsilon + \sqrt{x^2-u^2})p(x,u)u(x^2-u^2)^{-\frac{1}{2}}g^*(x)\,du\,dx \\ &+ \int_0^y \int_0^x A^*(\epsilon + \sqrt{x^2-u^2})p(x,u)u(x^2-u^2)^{-\frac{1}{2}}g^*(x)\,du\,dx \\ &+ V(B)\int_0^y p(x,x)g^*(x)\,dx \equiv 0.\end{aligned} \tag{19}$$

Differentiating (19) with respect to y, we have

$$V(B)p(y,y)g^*(y) = -\int_y^\infty g^*(x)A^*(\epsilon + \sqrt{x^2-y^2})p(x,y)y(x^2-y^2)^{-\frac{1}{2}}\,dx, \tag{20}$$

holding for all $y \in N^c$, where N is a μ-null set. Since supports of g_1 and g_2 are bounded, let U be the least upper bound such that $g^*(x) = 0$, a.e.(μ), for all $x > U$. If $U = \delta$, the result trivially holds. Thus we let $U > \delta$. Also we define

$$\theta = \min(U-\delta, V^2(B)(8u\alpha^2)^{-1}), \tag{21}$$

$$M = \sup_y [|p(y,y)g^*(y)|:\ y \in [U-\theta, U] \cap N^c], \tag{22}$$

and $\alpha = \sup_x A^*(x)$.

We shall show by contradiction that $M = 0$. Thus we assume that $M > 0$. Choose $v \in N^c \cap [U-\theta, U]$ such that $|p(v,v)g^*(v)| > M/2$. From (20) it follows that

$$V(B)|p(v,v)g^*(v)| = \left|\int_v^u A^*(\epsilon + \sqrt{x^2-v^2})p(x,v)v(x^2-v^2)^{-\frac{1}{2}}g^*(x)\,dx\right|$$

$$
\begin{aligned}
&\le M\alpha \int_v^U v(x^2 - v^2)^{-\frac{1}{2}} dx \\
&= M\alpha(U^2 - v^2)^{\frac{1}{2}} \\
&\le M\alpha(2U\theta)^{\frac{1}{2}} \\
&\le M\alpha(2UV^2(B)(8U\alpha^2)^{-1})^{\frac{1}{2}} \\
&\le V(B)\frac{M}{2},
\end{aligned}
\tag{23}
$$

which is a contradiction. Thus we must have $M = 0$. This in turn implies that $g^*(y) = 0$, a.e.(μ), for all $y \ge U - \theta$, which contradicts the fact that U is the least upper bound such that $g^*(y) = 0$, for all $y > U$. This proves that U must be equal to δ.

4. The case where $p(x, y)$ is unknown.

In our experience, $p(x, y)$ is generally unknown. This as exhibited through the following example will in general prevent our identifying the unknown G.

Consider the case where G is discrete and puts probability masses β at x, and $1-\beta$ at x_2, $(0 < \beta < 1, \beta \ne 1/2)$, whereas G^* is discrete and puts probability masses $1-\beta$ at x_1 and β at x_2. Associated with G and G^*, let the corresponding functions p and p^* be related as follows:

$$
\begin{aligned}
p(x_1, y) &\equiv p^*(x_1, y)\left(\frac{1-\beta}{\beta}\right) \\
p(x_2, y) &\equiv p^*(x_2, y)\left(\frac{\beta}{1-\beta}\right).
\end{aligned}
\tag{24}
$$

From (2a) it follows that the corresponding ψ and ψ^* are identical which precludes distinguishing G from G^*. Hence, we cannot identify the underlying G.

Note that the problem of non-identifiability was demonstrated in the above even for the simple class of distributions G concentrated on two points. In the more general case, the problem is even more acute because of the unknown nature of the probability function $p(x, y)$. This brings out the practical importance of how the function $p(x, y)$ influences our ability to estimate the unknown distribution G. Thus, without precise knowledge of the function $p(x, y)$, the question of estimation of G based on this model is not meaningful.

5. Concluding Remarks.

(a) In Theorem 2 the identifiability of the p.d.f. g was shown to hold within the class of bounded p.d.f.s. However, we believe that g is identifiable without the boundedness condition.

(b) The problem of identifiability of g for the special case where $p(x, y) = p_1(x)p_2(y)$, as mentioned at the end of section 2, was considered by the authors in great detail.

For the case where $A(z)$ was assumed to be constant one can show identifiability of g under mild regularity conditions by obtaining an explicit expression for g. The case of non-constant unspecified $A(z)$ appears too involved to yield an explicit expression for g.

(c) The problem of an edge effect was highlighted earlier in our Remarks 2 and 3. To deal honestly with the edge effect we proposed in section 2.1 a further generalization of our model. As is evident from equation (5), the practical problem here is not to identify a single $G(\cdot)$ but a single mixture of $G(\cdot|z)$ as z varies. This problem poses interesting theoretical questions, viz. even for the case of known function $p(x,y)$, it is not clear under what meaningful conditions on the family of functions $G(\cdot|z)$ a solution to this problem will result.

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