## Recent results on stereology

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Part I

1. Classical construction of motion invariant test probes. Crofton formulae. Applications to stereology. Exercise 1.
2. Motion invariant test lines in $\mathbb{R}^{3}$. Invariator construction. Applications to stereology. Exercise 2.
3. Case of a convex particle. Surface area in terms of the flower set.

## Classical construction of motion invariant test probes.

 Crofton formulae. Applications to stereology.Invariant test probes in $\mathbb{R}^{2}$ : Buffon's needle problem


George Louis Leclerc, Comte de Buffon (1707-1788)

Buffon's needle problem (1777)



Invariant density for straight lines in $\mathbb{R}^{2}$ :

$$
\mathrm{d} L_{1}^{2}=\mathrm{d} z \wedge \mathrm{~d} t, z \in \mathbb{R}, t \in \mathbb{S}_{+}^{1}:=[0, \pi) .
$$

For straight lines hitting a disk of diameter $h$, the joint probability element of $(z, t)$ is,

$$
\mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{\mathrm{d} z}{h} \cdot \frac{\mathrm{~d} t}{\pi}, z \in[-h / 2, h / 2], t \in[0, \pi)
$$

## Buffon-Steinhaus estimation of curve length in $\mathbb{R}^{2}$. Preliminaries



- Rectifiable curve: $Y \in \mathbb{R}^{2}$
- Target: $B:=\operatorname{length}(Y)$

$$
\begin{aligned}
& Y_{n}:=\bigcup_{i=1}^{n} y_{i}, \quad y_{i}:=\text { straight line segment, length }\left(y_{i}\right)=b_{i} \\
& B_{n}:=\operatorname{length}\left(Y_{n}\right)=\sum_{i=1}^{n} b_{i} \\
& B=\sup _{n \in \mathbb{N}} B_{n}
\end{aligned}
$$



$$
\begin{aligned}
& \mathbb{E} I\left(y \cap L_{1}^{2}\right)=\mathbb{P}\left(L_{1}^{2} \uparrow y\right)=\int_{L_{1}^{2} \uparrow y} \mathbb{P}(\mathrm{~d} z, \mathrm{~d} t)=\frac{1}{\pi h} \int_{0}^{\pi} \mathrm{d} t \int_{0}^{b|\cos t|} \mathrm{d} z=\frac{b}{\pi h} \int_{0}^{\pi}|\cos t| \mathrm{d} t=\frac{2 b}{\pi h}, \text { Buffon's answer, } \\
& \mathbb{E} I\left(Y_{n} \cap L_{1}^{2}\right)=\mathbb{E} I\left(\left(\cup_{1}^{n} y_{i}\right) \cap L_{1}^{2}\right)=\mathbb{E} \sum_{i=1}^{n} I\left(y_{i} \cap L_{1}^{2}\right)=\sum_{i=1}^{n} \mathbb{E} I\left(y_{i} \cap L_{1}^{2}\right)=\frac{2}{\pi h} \sum_{i=1}^{n} b_{i}=\frac{2 B_{n}}{\pi h}
\end{aligned}
$$

Apply the Monotone Convergence Theorem (Beppo Levi) (e.g. Bogachev, V.l. (2007) Measure Theory, Vol.1):

$$
\mathbb{E} I\left(Y \cap L_{1}^{2}\right)=\mathbb{E}\left\{\sup _{n \in \mathbb{N}} I\left(Y_{n} \cap L_{1}^{2}\right)\right\}=\sup _{n \in \mathbb{N}} \mathbb{E} I\left(Y_{n} \cap L_{1}^{2}\right)=\frac{2}{\pi h} \cdot \sup _{n \in \mathbb{N}} B_{n}=\frac{2 B}{\pi h}
$$

(Voss, F. (2005) Diplomarbeit, Univ. D-Ulm).

## Buffon-Steinhaus test system



- Choose an isotropic orientation $t \in[0, \pi)$ using $\mathbb{P}(\mathrm{d} t)=\mathrm{d} t / \pi$, e.g. $t=U \pi, U \sim \operatorname{UR}[0,1)$.
- On $L_{1}^{2}(0, t)$, fix a fundamental half open segment ('tile') $J_{0, t}$ of length $h$. Construct the partition $L_{1}^{2}(0, t)=\cup_{k \in \mathbb{Z}} J_{k, t}$, where $J_{k, t}=J_{0, t}+\tau_{k, t}$ and $-\tau_{k, t}$ is a translation of modulus $h$ along $L_{1}^{2}(0, t)$ which brings the tile $J_{k, t}$ to coincide with $J_{0, t}$ leaving the partition invariant for each $k \in \mathbb{Z}$ and $t \in[0, \pi)$.
- Independently of $t$ choose a uniform random point $z \in J_{0, t}$, namely with $\mathbb{P}(\mathrm{d} z)=\mathrm{d} z / h$, e.g. $t=U h, \quad U \sim$ $\mathrm{UR}[0,1)$.
- The system of parallel straight lines

$$
\Lambda_{z, t}=\left\{L_{1}^{2}\left(z+\tau_{k, t}, t\right), k \in \mathbb{Z}\right\}
$$

is a test system of parallel straight lines with motion invariant density.

## Buffon-Steinhaus estimation of curve length in $\mathbb{R}^{2}$

Next, apply a particular version of Santaló's theorem for test systems (Santaló, L.A. (1976) Integral Geometry and Geometric Probability, Ch. 8),

$$
\begin{aligned}
2 B=\int_{0}^{\pi} \mathrm{d} t \int_{\mathbb{R}} I\left(Y \cap L_{1}^{2}(z, t)\right) \mathrm{d} z & =\int_{0}^{\pi} \mathrm{d} t \sum_{k \in \mathbb{Z}} \int_{J_{k, t}} I\left(Y \cap L_{1}^{2}(z, t)\right) \mathrm{d} z \\
& =\int_{0}^{\pi} \mathrm{d} t \sum_{k \in \mathbb{Z}} \int_{J_{0, t}} I\left(Y \cap L_{1}^{2}\left(z+\tau_{k, t}, t\right)\right) \mathrm{d} z \\
& =\int_{0}^{\pi} \mathrm{d} t \int_{J_{0, t}} I\left(Y \cap \Lambda_{z, t}\right) \mathrm{d} z
\end{aligned}
$$

Recalling that $\mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=(\pi h)^{-1} \mathrm{~d} z \mathrm{~d} t$,

$$
B=\frac{\pi}{2} h \mathbb{E} I\left(Y \cap \Lambda_{z, t}\right),
$$

from which we can write an unbiased point estimator of the curve length $B$ in terms of known constants and the observed intersection count,

$$
\widehat{B}=\frac{\pi}{2} h I\left(Y \cap \Lambda_{z, t}\right) .
$$

Invariant test lines and planes in $\mathbb{R}^{3}$


$$
\begin{aligned}
& \mathrm{d} L_{2}^{3}=\mathrm{d} z \wedge \mathrm{~d} t, \quad z \in \mathbb{R}, \quad t \in \mathbb{S}_{+}^{2}, \quad \mathrm{~d} t=\sin \theta \mathrm{d} \phi \mathrm{~d} \theta, \quad \phi \in[0,2 \pi), \quad \theta \in[0, \pi / 2], \\
& \mathrm{d} L_{1}^{3}=\mathrm{d} z \wedge \mathrm{~d} t, \quad z \in \mathbb{R}^{2}, \quad t \in \mathbb{S}_{+}^{2}
\end{aligned}
$$

## Estimation of surface area and volume with test lines in $\mathbb{R}^{3}$. Preliminaries



- $\partial y \in \mathbb{R}^{3}$ : surface element of area $s$
- $y \in \mathbb{R}^{3}$ : volume element of volume $v$,

$$
\begin{aligned}
& \mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{\mathrm{d} z}{a} \cdot \frac{\mathrm{~d} t}{2 \pi}, z \in B_{2, t}, t \in \mathbb{S}_{+}^{2}, \\
& \mathbb{E} I\left(\partial y \cap L_{1}^{3}\right)=\int_{L_{1}^{3} \uparrow \partial y} I\left(y \cap L_{1}^{3}(z, t)\right) \mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{1}{2 \pi a} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{\mathbb{R}^{2}} I\left(y \cap L_{1}^{3}(z, t)\right) \mathrm{d} z=\frac{1}{2 \pi a} \int_{\mathbb{S}_{+}^{2}} s \cdot \cos \theta \mathrm{~d} t=\frac{s}{2 a}, \\
& \mathbb{E} L\left(y \cap L_{1}^{3}\right)=\int_{L_{1}^{3} \uparrow y} L\left(y \cap L_{2}^{3}(z, t)\right) \mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{1}{2 \pi a} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{\mathbb{R}^{2}} L\left(y \cap L_{2}^{3}(z, t)\right) \mathrm{d} z=\frac{1}{2 \pi a} \int_{\mathbb{S}_{+}^{2}} v \mathrm{~d} t=\frac{v}{a} .
\end{aligned}
$$

## Estimation of surface area and volume with the Fakir Probe



Fakir probe $\Lambda_{z, t}$


Making successive use of the Monotone Convergence Theorem and of Santaló's Theorem, the surface area and the volume of a bounded subset $Y \subset \mathbb{R}^{3}$ can be expressed as follows,

$$
S(\partial Y)=2 a \mathbb{E} I\left(\partial Y \cap \Lambda_{z, t}\right), \quad V(Y)=a \mathbb{E} L\left(Y \cap \Lambda_{z, t}\right),
$$

from which the corresponding unbiased point estimators are straightforward.

## An equivalence



By virtue of the Petkantschin-Santaló identity, a motion invariant straight line in $\mathbb{R}^{3}$ is equivalent to a motion invariant straight line within a motion invariant plane:

$$
\begin{aligned}
\mathrm{d} L_{1}^{3}(z, t) \wedge \mathrm{d} L_{2[1]}^{3}(\alpha) & =[\mathrm{d} z \wedge \mathrm{~d} t] \wedge \mathrm{d} \alpha \\
& =[\mathrm{d} p \wedge \mathrm{~d} q \wedge \mathrm{~d} t] \wedge \mathrm{d} \alpha \\
& =[\mathrm{d} p \wedge \mathrm{~d} t] \wedge[\mathrm{d} q \wedge \mathrm{~d} \alpha] \\
& =\mathrm{d} L_{2}^{3}(p, t) \wedge \mathrm{d} L_{1}^{2}(q, \alpha) .
\end{aligned}
$$

## Application: IUR test lines on isotropic Cavalieri sections



With the preceding results, application of Santaló's Theorem leads to the following representations,

$$
S(\partial Y)=2 T d \cdot \mathbb{E}(I), \quad V(Y)=T d \cdot \mathbb{E}(L) .
$$

## Estimation of volume and surface area with test planes in $\mathbb{R}^{3}$. Preliminaries



- $y \in \mathbb{R}^{3}$ : volume element of volume $v$,
- $\partial y \in \mathbb{R}^{3}$ : surface element of area $s$

$$
\begin{aligned}
& \mathbb{E} A\left(y \cap L_{2}^{3}\right)=\int_{L_{2}^{3} \uparrow y} A\left(y \cap L_{2}^{3}(z, t)\right) \mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{1}{2 \pi h} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{\mathbb{R}} A\left(y \cap L_{2}^{3}(z, t)\right) \mathrm{d} z=\frac{1}{2 \pi h} \int_{\mathbb{S}_{+}^{2}} v \mathrm{~d} t=\frac{v}{h}, \\
& \mathbb{E} B\left(\partial y \cap L_{2}^{3}\right)=\int_{L_{2}^{3} \uparrow \partial y} B\left(\partial y \cap L_{2}^{3}(z, t)\right) \mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{1}{2 \pi h} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{\mathbb{R}} B\left(\partial y \cap L_{2}^{3}(z, t)\right) \mathrm{d} z=\frac{1}{2 \pi h} \int_{\mathbb{S}_{+}^{2}} s \cdot \sin \theta \mathrm{~d} t=\frac{\pi s}{4 h} .
\end{aligned}
$$

## Estimation of surface area and volume with the isotropic Cavalieri design



Making successive use of the Monotone Convergence Theorem and of Santaló's Theorem,

$$
\widehat{S}=\frac{4}{\pi} \cdot T \cdot\left(B_{1}+B_{2}+\cdots+B_{n}\right) \text { and } \widehat{V}=T \cdot\left(A_{1}+A_{2}+\cdots+A_{n}\right),
$$

are unbiased estimators of the surface area and the volume of a bounded subset $Y \subset \mathbb{R}^{3}$, respectively. Further, if the sections are analysed with an IUR square grid test system of size $d$, then the corresponding two-stage unbiased estimators read,

$$
\widetilde{S}=T d \cdot\left(I_{1}+I_{2}+\cdots+I_{n}\right) \text { and } \widetilde{V}=T d^{2} \cdot\left(P_{1}+P_{2}+\cdots+P_{n}\right) .
$$

## An aid to the isotropic Cavalieri design: The antithetic isector



## The invariator principle and its applications

## Invariator construction of a motion invariant test line in $\mathbb{R}^{3}$



$$
\begin{aligned}
\mathrm{d} L_{1}^{3} & =\mathrm{d} z \wedge \mathrm{~d} t \\
& =r[\mathrm{~d} r \wedge \mathrm{~d} \alpha] \wedge \mathrm{d} t \\
& =r \cdot \mathrm{~d} L_{1}^{2} \wedge \mathrm{~d} t
\end{aligned}
$$

Thus, the invariator principle states that a point-weighted test line ( $=$ a $p$-line) $L_{1}^{2}(z ; t)$ on a pivotal plane $L_{2}^{3}(0, t)$ is equivalent to a test line $L_{1}^{3}(z, t)$ with a motion invariant density in three dimensional space.

## Probability element for a $p$-line



Isotropic orientation


Classical construction


Invariator alternative

$$
\begin{aligned}
& \mathrm{d} L_{1}^{3}(z, t)=\mathrm{d} z \wedge \mathrm{~d} t, \quad \text { classical, } \\
& \mathrm{d} L_{1}^{3}(z, t)=\mathrm{d} t \wedge \mathrm{~d} L_{1}^{2}(z ; t), \quad \text { invariator, } \\
& \mathbb{P}(\mathrm{d} z, \mathrm{~d} t)=\frac{\mathrm{d} z}{a} \cdot \frac{\mathrm{~d} t}{2 \pi}, \quad z \in B_{2, t}, t \in \mathbb{S}_{+}^{2}
\end{aligned}
$$

## Surface area and volume with a $p$-line



Crofton formulae:

$$
\begin{aligned}
& S(\partial Y)=\frac{1}{\pi} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{B_{2, t}} I(z, t) \mathrm{d} z=2 a \mathbb{E} I(z, t), \text { where } I(z, t):=\text { number of intersections, } \\
& V(Y)=\frac{1}{2 \pi} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{B_{2, t}} L(z, t) \mathrm{d} z=a \mathbb{E} L(z, t), \text { where } L(z, t):=\text { intercept length } .
\end{aligned}
$$

## Surface area and volume with the Invariator Test Grid



Fakir probe (classical motion invariant test lines)


$p$-line (invariator principle)


Implementation of the invariator grid

$$
S(\partial Y)=2 a \mathbb{E} I(z, t), \quad V(Y)=a \mathbb{E} L(z, t) .
$$

## Application: rat brain surface area and volume with the Invariator

(Cruz-Orive LM, Ramos-Herrera ML \& Artacho-Pérula E (2010) J. Microscopy)

Magnetic Resonance Imaging


Unbiased estimators (with square grid generator of tile area $a$ ) for each brain:

$$
\widehat{S}=2 a I, \quad \widehat{V}=a L
$$

## Classical isotropic Cavalieri design with antithetic arrangement



One stage UE's:

$$
\widehat{S}=\frac{4}{\pi} T \sum B_{i}, \quad \widehat{V}=T \sum A_{i} .
$$

Two stage UE's (with square grid of size $d$ to measure the sections):

$$
\widetilde{S}=T d \sum I_{i}, \quad \widetilde{V}=T d^{2} \sum P_{i} .
$$

## Application to rat brain (contd.)

Exhaustive MRI isotropic Cavalieri series of brain \#5 with antithetic arrangement of the two brain hemispheres. Slice thickness $=1.2 \mathrm{~mm}$.


In this brain the approximately equatorial section \#14 was used for the invariator.
Sections $\{7,10,13,16,19,22\}$ constitute a systematic subsample of period $T=3.6 \mathrm{~mm}$.

## Rat brain surface area and volume: results


'Exact' := One stage estimators from isotropic Cavalieri series.

In this way the biological variance among brains could be estimated fairly accurately. Subtraction of the biological variance from the observed variance of the invariator data yielded a mean individual invariator ce $(\cdot)$ of about $30 \%$ for both $\widehat{S}$ and $\widehat{V}$.

Conclusion: Use the invariator for cell populations, say, rather than individual organs.

The Pivotal Tessellation


## The Poisson Pivotal Tessellation



For a connected region from the intersection of a Poisson pivotal tessellation with a disk of radius $R$ we have,

$$
\begin{aligned}
& \mathbb{E}\{N(R)\}=\text { Mean number of vertices, or of sides }=4+O\left(R^{-2}\right), \\
& \mathbb{E}\{B(R)\}=\text { Mean boundary length }=O\left(R^{-1}\right), \\
& \mathbb{E}\{A(R)\}=\text { Mean area }=O\left(R^{-1}\right) .
\end{aligned}
$$

## INTERPRETATION ?

(Cruz-Orive (2009) IAS 28, 63-67.)

## Case of a convex particle: Surface area from the 'FLOWER' of a pivotal section



Flower of the convex pivotal section $Y \cap L_{1}^{2}(z ; t): \quad H_{t}:=\left\{z \in \mathbb{R}^{2}: Y \cap L_{1}^{2}(z ; t) \neq \emptyset\right\}$.

$$
\begin{aligned}
\therefore I(z, t)= & \begin{cases}2 & \text { with probability } A\left(H_{t}\right) / a, \\
0 & \text { with probability } 1-A\left(H_{t}\right) / a,\end{cases} \\
& \therefore S(\partial Y)=4 \mathbb{E} A\left(H_{t}\right) .
\end{aligned}
$$

## Interlude: A duality



```
Y convex
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$$
S(\partial Y)=4 \mathbb{E} A\left(H_{t}\right)
$$

Flower formula

## Special case: Surface area of a triaxial ellipsoid



Pivotal section


Flowers of identical areas if $l$ is fixed

$$
\begin{aligned}
S(\partial Y)= & 4 \mathbb{E}\left\{A\left(H_{t}\right)\right\}, \quad A\left(H_{t}\right)=\frac{\pi}{2}\left(M^{2}+m^{2}+l^{2}\right) \\
& \therefore S(\partial Y)=2 \pi \mathbb{E}\left(M^{2}+m^{2}+l^{2}\right) .
\end{aligned}
$$

Remark:

$$
A\left(H_{K}\right)=\frac{1}{2} \int_{0}^{2 \pi} h_{K}^{2}(\omega) \mathrm{d} \omega, \quad O \in K \subset \mathbb{R}^{2}, \text { convex. }
$$

## Volume of an arbitrary particle via the mean WEDGE volume




Pivotal section


$$
V(Y)=2 \pi \mathbb{E} V\left(W_{t}\right)
$$

Hint of proof. Use Crofton's formula with $\mathrm{d} z=r \mathrm{~d} r \mathrm{~d} \omega$,

$$
V(Y)=\frac{1}{2 \pi} \int_{\mathbb{S}_{+}^{2}} \mathrm{~d} t \int_{0}^{2 \pi} \mathrm{~d} \omega \int_{0}^{h(\omega ; t)} L(r, \omega ; t) r \mathrm{~d} r
$$

But the integral

$$
\int_{0}^{h(\omega ; t)} L(r, \omega ; t) r \mathrm{~d} r
$$

can be interpreted as the volume of a well defined $45^{\circ}$ ' WEDGE SET' $W(\omega ; t)$. The result follows if, or each pivotal section, i.e. for each $t \in \mathbb{S}_{+}^{2}$, we set $V\left(W_{t}\right):=\mathbb{E} V\{W(\omega ; t)\}$.

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