Quality assessment for high-dimensional data:

- Dead pixels in CT scans for 3D printed objects
- Molecular biology in cancer treatment decisions

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Application of statistical genomics: Cancer prognosis

Afirma Thyroid Analysis May Help Patients Avoid Surgery
Nodules No Longer Classified as Inconclusive or Indeterminate

By Mary Shomon – Reviewed by a board-certified physician.
Updated July 16, 2016

Thyroid cancer is the fastest-growing cancer in the United States. There were an estimated 44,670 new cases in 2010, according to the American Cancer Society. Along with the increased awareness of thyroid cancer comes increased scrutiny of thyroid nodules. Thanks to more vigilant monitoring, ultrasounds, and x-rays, more thyroid nodules are being detected and evaluated.

When a thyroid nodule is considered suspicious -- meaning that it has characteristics that may suggest thyroid cancer -- the key evaluation is a fine needle aspiration (FNA) biopsy.

The FNA biopsy helps determine whether the nodule is malignant (thyroid cancer) or benign.
Application of spatial statistics: Dead pixels

Nintendo Switch owners complain about dead pixels

By Jane Wakefield
Technology reporter

7 March 2017

Some users have reported "annoying" screen glitches

Thousands of owners of Nintendo's new console, Switch, have complained about dead or stuck pixels creating distracting and annoying dark squares on their screens.
Application of spatial statistics:

Dead pixels in detectors of computed tomography machines

Part of quality control for 3D printed objects

joint project with Warwick Manufacturing Group
Dead pixels

- Occur on detectors of LCD screens, digital cameras, CT scanners...
- Quantify damage
- Describe characteristics
- Reasons for damage
- Speed of decay
X-ray detectors and bad pixel maps

Perkin Elmer
XRD 1621

- "Underperforming" (sensitivity, noise, uniformity)
- Bad pixel map with coordinates
Local defects: Dead lines

- Lines on bad pixel images
- From centre horizontal line outwards
- Clusters at the end
Local defects: Isolated dead pixels

Singles, doubles, small clusters

A_0: Grey image [R]
A_0: bp binary image [R]
A_0: Black image [R]
Local defects: Corners

B_0: Binary bad pixel image [R]
Local defects: Patches

- Areas with high density area of bad pixels
Point processes and spatial statistics

Mathematical model:
Interpret dead pixels a spatial point process

What is its distribution?
E.g. are there clusters?
Point processes and spatial statistics

Mathematical model:
Interpret dead pixels a spatial point process

What is its distribution? Clusters? Repulsion?

K-function: for $h > 0$, $K(h)$ is the expected number of extra points in circle of radius $h$, rescaled by density

$$K(h) = \frac{1}{\lambda} E[N(C_h - \{s\}) | N(s) = 1] \quad \text{(s location with point)}$$

For stationary processes: Proportional to its area $K(h) = \lambda a(C_h)$

Under CSR: $K(h) = \frac{1}{\lambda} (\lambda \pi h^2) = \pi h^2$
Higher level defect model (Step 1)

Conversion of point process to event process

Defect pixels

Defect events
Density based thresholding (Step 2)

Remove areas with local density above threshold (medial +1.5 IQR)
Point pattern and K-function modified

Dead pixels

Pixel process K function

Event process K function

Completely spatially at random
Spatial statistics for detector QA

- Identify special causes of poor quality
- Remaining area CSR means general cause of poor quality
- Density in remaining area gives global quality score for the detector
Application of statistical genomics:

Data quality for cancer prognosis and treatment decision

Collaboration with Terry Speed’s group in University of Berkeley, California

Results used by Bay Area Biotech companies
Decisions about invasive medical treatments

- Uncertainty, complex information (clinical tests e.g. OncotypeDX)
- Emotions interfering with judgement, multiple decision makers
Afirma (Veracyte) in practice:

- Traditional diagnosis in thyroid cancer 30% inconclusive and lead to surgery (plus life long treatment), but 80% turn out to be benign tutors
- Afirma avoid half of these surgeries (plus morbidity)
- Potential economics impact for US: $122 million savings
Traditional diagnosis in thyroid cancer 30% inconclusive and lead to surgery (plus life long treatment), but 80% turn out to be benign tutors.

Afirma (Veracyte) in practice:

- Crucial step for commercial success: control error rates of test
- Essential: Data quality assessment for custom made measurement instruments
- Traditional diagnosis in thyroid cancer 30% inconclusive and lead to surgery (plus life long treatment), but 80% turn out to be benign tutors
- Afirma avoid half of these surgeries (plus morbidity)
- Potential economics impact for US: $122 million savings

Statistical requirements:
Genomics 101: From DNA to cells
Metaphor: Architecture

Textual description

..., stone walls, roof, divided into room, glass windows, wooden frames, hardwood doors, ...

Plan

Design team

Construction

Product

Casa Loma, Toronto
Process and product are not deterministic

- **Textual description (same)**
  
  ..., stone walls, roof, divided into room, glass windows, wooden frames, hardwood doors,...

- **Plan (different)**

- **Product (different)**

  Hackesche Höfe Berlin (built 1906/07)

- **Design team**

  Construction
Central Dogma of Molecular Biology

Textual description
- DNA
- RNA

Plan
- RNA

Construction
- Protein Translation
- Chromosome
- RNA Transcription
- RNA Polymerase

Product
- Cell

Design

2 microns
**Gene expression**

Gene expression =

the gene’s degree of biochemical activity

(here: amount of RNA produced by the gene)

Depends on **factors** such as:

- Type of the cell
- State of cell
- Developmental stage

Use to gene expression to **detect genes** involved in cellular processes, diseases, development etc.
High throughput gene expression measurement with microarrays

- Assesses expression levels of tens of thousands of genes
- Simultaneously in one experiment

Workflow

1. RNA extraction
2. cDNA reaction, purification and labeling by IVT
3. fragmentation (heat + Mg²⁺)
4. hybridization
5. washing
6. labeling with streptavidin-PE
7. laser scanning
8. computer analyses
9. "absolute" gene expression levels

http://www.nature.com/leu/journal/v17/n7/images/2402974f1.jpg
How do we measure gene expression data quality?

- How do we make judgements?

Why is this relevant?

High throughput gene expression measurement quality assessment toolbox
High dim genomic data QA/QC challenges

• Simultaneous measurements of huge numbers of genes
• Missing or partial ‘gold-standards’
• Unknown correlation structure
• No agreement on models for microarray data
• Measurement taken in a multi-step procedure
• Divorcing technical variation and biological variation
• Systematic errors more relevant than random errors
• Platform specific
• Data collections (risk of being swamped with poor quality
<table>
<thead>
<tr>
<th>Gene 1</th>
<th>log probe intensities array 1</th>
<th>log probe intensities array 2</th>
<th>...</th>
</tr>
</thead>
</table>

- Tens of thousands of genes
- 10-1000 arrays
- Various biological conditions (e.g. disease/control, time points)
- With technical replicates
- Note: heterogeneity among probes within the same probe set
<table>
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**Data analysis**

- **Background adjustment**
- **Normalization**
- **Expression estimation**
RMA Model

("Robust Multi Array" (RMA) by Irizarry et al. 2002)

Fix gene (probe set).

\[ Y_{jk} = \log_2 \text{ normalized background corrected PMs} \]

**Probe** effect \( \beta_j \) and **Array** effect \( \alpha_k \),

and error

\[ Y_{jk} = \beta_j + \alpha_k + \varepsilon_{jk} \]

(and sum zero constraint on probe effects)

Fit with iterative reweighed least square algorithm returning weights
1. Relative Log Expression (RLE):

**Median Chip**: median expression over all arrays (gene by gene)

**RLE** (gene A) in array k =
\[ \log \text{ratio gene A's expression in array k and gene A's median expression} \]

**Idea**: use RLE distribution for quality assessment (QA)

**Interpretation on distribution level**, based on two biologic assumptions:

(A) majority of genes similar between different samples
(B) # upregulated genes = # downregulated genes

Then, good quality is indicated by:

\[ \text{Med(RLE)} = 0 \quad \text{small IQR(RLE)} \]
2. Normalized unscaled standard error (NUSE):

\[
NUSE = \frac{1}{\sqrt{W_k}} \frac{1}{\text{med}_{k'} 1/\sqrt{W_{k'}}}
\]

Note:
Normalization because of heterogeneity in # effective probes

Interpretation based on biologic assumptions

(A) majority of genes similar between different samples
(B) # upregulated genes = # downregulated genes

Then, good quality is indicated by:

\[\text{Med}(NUSE)=0 \quad \text{small IQR}(NUSE)\]
3. Quality landscapes

**Weight images:**

Colour a rectangle by probe weights according to their spatial location on array.

- dark green = low weights (poor quality)

**Residual images:**

Same, but with residuals.
- red = positive residuals
- blue = negative residuals

Very helpful for revealing causes for poor quality…
Residual images illustrating poor quality

Fig. J1: “Bubbles”    Fig. J2: “Circle and Stick”    Fig. J3: “Sunset”    Fig. J4: “Pond”    Fig. J5: “Letter S”    Fig. J6: “Compartments”    Fig. J7: “Triangle”    Fig. J8: “Fingerprint”
**NUSE**

**Weights**

Pseudo images of weights: Chip – median(NUSE)
Example for data quality variation between biological conditions

Figure F1. Series of boxplots of log-scaled PM intensities (a), RLE (b), and NUSE (c) for a comparison of nine fruit fly mutants with three to four technical replicates each. The patterns below the plot indicate mutants, and the gray levels of the boxes indicate hybridization dates. Med(RLE), IQR(RLE), Med(NUSE), and IQR(NUSE) all indicate substantially lower quality on the day colored white.
Figure H1. Series of boxplots of log-scaled PM intensities (a), RLE (b), and NUSE (c) for Pritzker gender study brain samples hybridized in two labs (some replicates missing). Gray level indicates lab site (dark for Lab M, light for Lab I). The log-scaled PM intensity distributions are all located around 6 for Lab M, and around 10 for Lab I. These systematic lab site differences are reflected by IQR(RLE), Med(NUSE), and IQR(NUSE), which consistently show substantially lower quality for Lab I hybridizations than for Lab M hybridizations.
Practical uses of our QA toolbox

- Small labs: concrete feedback on design and conduction of experiments
- Core facilities: biases, efficiency, process control
- Controlling error rates in genomic diagnostic tools (Afirma, OncotypeDX etc.)
- Quality benchmarking
Context dependency of quality

Ask: What would be the consequences of poor quality?

Shewhart (1927), Pioneer of industrial QC:

“The applied scientists knows that if he were to act upon the meagre evidence sometimes available to the pure scientist, he would make the same mistakes as the pure scientist makes in estimates of accuracy and precisions. He also knows that through his mistakes someone may lose a lot of money or suffer physical injury or both. [...] He does not consider his job simply that of doing the best he can with the available data; it is his job to get enough data before making this estimate."
Thanks to

Gene expression group at UC Berkeley

Biologists (for really bad microarray data)

R and Bioconductor communities (for packages)

Inside out group at University of Warwick

Perkin Elmer (for lots of dead pixels)