Efficient designs for two-colour microarray experiments

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A small microarray experiment

1 + 4 Treatments
96 Genes

8 Slides
2 Dyes
96 Positions

There is 1 'control' treatment (labelled 0) and 4 other treatments. e shows that we need to know a specific (non-orthogonal) design for the allocation of the treatments to the dye-slide combinations, such as slides 1 2 3 4 5 6 7 8 red 0 1 0 2 0 3 0 4 green 1 0 2 0 3 0 4 0
There is 1 ‘control’ treatment (labelled 0) and 4 other treatments.
A small microarray experiment

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<table>
<thead>
<tr>
<th>slides</th>
<th>1 2 3 4 5 6 7 8</th>
</tr>
</thead>
<tbody>
<tr>
<td>red</td>
<td>0 1 0 2 0 3 0 4</td>
</tr>
<tr>
<td>green</td>
<td>1 0 2 0 3 0 4 0</td>
</tr>
</tbody>
</table>
Representation of the design as an oriented graph

Treatments are vertices; slides are edges, oriented from green to red.

<table>
<thead>
<tr>
<th>red</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
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<td>green</td>
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<td>3</td>
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<td>slide</td>
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<td>2</td>
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<td>3</td>
<td>0</td>
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<td>0</td>
</tr>
</tbody>
</table>

double reference

Which is better?
Representation of the design as an oriented graph

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double reference

wheel
Representation of the design as an oriented graph

Treatments are vertices; slides are edges, oriented from green to red.

Which is better?
Model

\[ t \text{ treatments} \quad b \text{ slides (call these “blocks”) \quad 2 dyes} \]
$t$ treatments  $b$ slides (call these “blocks”)  2 dyes

Assume that the logarithm of the intensity of treatment $i$ coloured with dye $l$ in block $k$ has expected value

$$\tau_i + \beta_k + \delta_l$$

and variance $\sigma^2$, independent of all other responses.
Model

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Assume that the logarithm of the intensity of treatment $i$ coloured with dye $l$ in block $k$ has expected value

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and variance $\sigma^2$, independent of all other responses.

To estimate all the $\tau_i - \tau_j$, we need $b \geq t - 1$. 
If there are just 2 treatments, we want $V_{12}$, the variance of the estimator of $\tau_1 - \tau_2$, to be small and we want the confidence interval $I_{12}$ for $\tau_1 - \tau_2$ to be small.
Optimality criteria

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In general, a design is A-optimal if it minimizes the sum of the variances of the estimators of the pairwise differences; a design is D-optimal if it minimizes the volume of the confidence ellipsoid for the vector $(\tau_1, \ldots, \tau_r)$ subject to $\sum \tau_i = 0$. 
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If $t = 2$ then A-optimal = D-optimal.
Temporarily ignore the dyes

We will come back to them later.
Experience with block designs of many sizes

- Designs which are good on the A-criterion are also good on the D-criterion . . .

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Typical behaviour of the optimality criteria

Optimality criteria for all connected equireplicate designs with 8 treatments in 12 blocks of size 2:
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Optimality criteria for all connected equireplicate designs with 8 treatments in 12 blocks of size 2: both criteria are normalized to lie between 0 (worst, for designs where not everything can be estimated) and 1 (best, for designs consisting a single large block)
What happens when $b = t$?

Computer investigation by

- Jones and Eccleston (1980)
- Kerr and Churchill (2001)
- Wit, Nobile and Khanin (2005)
- Ceraudo (2005).
Optimal designs when $b = t$

D-optimal

$t = 6$

$t = 7$

$t = 8$

A-optimal
Optimal designs when $b = t$

$D$-optimal

- $t = 7$
- $t = 8$
- $t = 9$

$A$-optimal
Optimal designs when $b = t$

- **D-optimal**
  - $t = 8$
  - $t = 9$
  - $t = 10$

- **A-optimal**
Optimal designs when $b = t$

D-optimal

$t = 9$

$t = 10$

$t = 11$

A-optimal
D-optimality


\[ E_D = \frac{(t \times \text{number of spanning trees})^{1/(t-1)}}{2\bar{r}} \]

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4 spanning trees
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The loop design is uniquely D-optimal when \( b = t \).
If $b = t$, the graph contains a single circuit.
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\[ V_{67} = V_{61} + V_{10} + V_{07} = V_{10} + 4\sigma^2 \]
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\[
V_{97} = V_{98} + V_{80} + V_{07} = V_{80} + 4\sigma^2
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V_{97} = V_{90} + V_{07} = 4\sigma^2
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For a given size of circuit, the total variance is minimized when everything outside the circuit is attached to the same vertex of the circuit.
Leaves attached to the same vertex of the circuit

Average pairwise variance is a cubic function of the size of the circuit.
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\[
\bar{V} \propto \times 6 \times t = 6
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\[ \bar{V} = t^3 \]
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Average pairwise variance is a cubic function of the size of the circuit.
Optimality criteria for designs for 20 treatments in 20 blocks, using the A-optimal design for each size of circuit.

The two criteria give essentially reverse rankings.
Assigning colours to a circuit with leaves

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More leaves → smaller circuit → larger variance for colour difference.
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Variance between leaves increases unless they all have the same colour.
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Variance between leaves increases unless they all have the same colour.
What happens when $b = t + 1$?

A similar analysis shows that the A-optimality and D-optimality criteria conflict when $t \geq 12$. 
Optimal designs when $b = t + 1$

- **D-optimal**
  - $t = 8$
  - $t = 9$
  - $t = 10$

- **A-optimal**
  - $t = 8$
  - $t = 9$
  - $t = 10$
Optimal designs when $b = t + 1$

D-optimal

$\begin{array}{c}
\text{t = 11} \\
\text{t = 12} \\
\text{t = 13}
\end{array}$

A-optimal

........................................................
........................................................
........................................................
Bad news theorem

*Given any fixed value of $b - t$, there is a threshold $T$ such that when $t \geq T$ the A- and D-optimality criteria conflict.*

When $t \geq T$, the average valency (replication) is much less than 3, so there must be many vertices of valency 2 or many vertices of valency 1 (leaves).
What happens for larger values of $b - t$?

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- Many vertices of valency 2 $\Rightarrow$ long paths $\Rightarrow$ large distances $\Rightarrow$ large pairwise variances $\Rightarrow$ poor design on A-criterion.

- Many leaves $\Rightarrow$ few spanning trees $\Rightarrow$ poor design on D-criterion.
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Many leaves $\implies$ few spanning trees $\implies$ poor design on D-criterion.

A-better designs have many leaves attached to single vertex of some small graph, whereas the D-better designs have no leaves.
How can we construct efficient designs?

Good news theorem
If a given graph has no vertices of valency 1 or 2, then inserting 1 or 2 (or sometimes 3) vertices into the edges of that graph gives a lower average pairwise variance than attaching the extra vertices to a single vertex of that graph.
Strategy for choosing a design when $b \geq 9t/8$

1. Choose the best equireplicate design with replication 3 for $2(b - t)$ treatments in $3(b - t)$ blocks (or with replication 4, for $b - t$ treatments in $2(b - t)$ blocks), including dye allocation.

2. Insert up to 2 treatments in each edge.
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Example
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Example

\[
t = 12 \Rightarrow b - t = 2
\]

\[\Rightarrow 4 \text{ vertices, 6 edges}\]
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1. Ignore the colours.
Choosing a good equireplicate design with replication 4

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2. Find the best graph with all vertices having valency 4 (smaller problem, can use symmetry to speed up the search).
Choosing a good equireplicate design with replication 4

1. Ignore the colours.
2. Find the best graph with all vertices having valency 4 (smaller problem, can use symmetry to speed up the search).
3. Euler’s Theorem (for bridges of Königsberg) says that the arrows can be put on the edges in such a way that every vertex has two edges coming in and two edges going out.
1. Divide the treatments into two halves: “more red” and “more green”.
Choosing a good equireplicate design with replication

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2. Strategy: make every block contain one treatment from each half.
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3. RAB theorem: the best way to do this is to use the Levi graph of the best design for \( t/2 \) treatments equally replicated in \( t/2 \) blocks of size 3. (Smaller problem.)
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4. Using the algorithm from Hall’s Marriage Theorem, (also König’s Theorem) orient the edges so that each lower vertex has 2 out-edges and 1 in-edge and each upper vertex has 1 out-edge and 2 in-edges.
Example for 14 treatments with replication 3
Example for 14 treatments with replication 3
Example for 14 treatments with replication 3

1
2
3
4
5
6
7

124
235
346
457
156
267
137
Example for 14 treatments with replication 3
Example for 14 treatments with replication 3
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Example for 14 treatments with replication 3
Generic designs with bounded pairwise variance

... $s$ triangles glued at one vertex

$t = 2s + 1 \quad b = 3s \quad b/t \approx 1.5$

$V_{ij} = 1.33\sigma^2$ (same triangle) or $2.67\sigma^2$ (otherwise)
Generic designs with bounded pairwise variance

- **s triangles glued at one vertex**
  
  \[ t = 2s + 1 \quad b = 3s \quad b/t \approx 1.5 \]
  
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- **double reference design**
  
  \[ t = s + 1 \quad b = 2s \quad b/t \approx 2 \]
  
  \[ V_{ij} = \sigma^2 \text{ (control) or } 2\sigma^2 \text{ (otherwise)} \]
Generic designs with bounded pairwise variance

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\[ \text{double reference design}\]
\[ t = s + 1 \quad b = 2s \quad b/t \approx 2\]
\[ V_{ij} = \sigma^2 \text{ (control) or } 2\sigma^2 \text{ (otherwise)}\]

\[ s \text{ copies of } K_5 \text{ glued at one vertex}\]
\[ t = 4s + 1 \quad b = 10s \quad b/t \approx 2.5\]
\[ V_{ij} = 0.8\sigma^2 \text{ (same } K_5) \text{ or } 1.6\sigma^2 \text{ (otherwise)}\]
Generic designs with bounded pairwise variance

\[ \text{s triangles glued at one vertex} \]
\[ t = 2s + 1 \quad b = 3s \quad b/t \approx 1.5 \]
\[ V_{ij} = 1.33\sigma^2 \text{ (same triangle)} \text{ or } 2.67\sigma^2 \text{ (otherwise)} \]

\[ \text{double reference design} \]
\[ t = s + 1 \quad b = 2s \quad b/t \approx 2 \]
\[ V_{ij} = \sigma^2 \text{ (control)} \text{ or } 2\sigma^2 \text{ (otherwise)} \]

\[ \text{wheel with 2s spokes} \]
\[ t = 2s + 1 \quad b = 4s \quad b/t \approx 2 \]
\[ V_{ij} \leq 0.9\sigma^2 \text{ (control)} , \leq 1.8\sigma^2 \text{ (otherwise)} \]

\[ \text{s copies of } K_5 \text{ glued at one vertex} \]
\[ t = 4s + 1 \quad b = 10s \quad b/t \approx 2.5 \]
\[ V_{ij} = 0.8\sigma^2 \text{ (same } K_5) \text{ or } 1.6\sigma^2 \text{ (otherwise)} \]
Comparing the wheel design with the double-reference design

- $\times \text{ average}
- \bigcirc \text{ with control}

- $0.5\sigma^2$
- $\sigma^2$
- $1.5\sigma^2$
- $2\sigma^2$

number of non-control tmts

- \text{variance}
Comparing the wheel design with the double-reference design

- $\times$ average
- o with control

- $\sigma^2$
- $1.5\sigma^2$
- $2\sigma^2$

number of non-control tmts

- average
- double reference
- wheel
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   ▶ Needs $1 \leq \frac{b}{t} \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   ▶ Needs $2 \leq \frac{b}{t} \leq 2$.
   ▶ Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   ▶ In RAB's experience, never beats previous method.

3. Glue many leaves to a single vertex of some small graph.
   ▶ Few spanning trees, but no pairwise variance is bigger than $4 \sigma^2$.

4. Glue many triangles to a single vertex of some small graph.
   ▶ Needs $\frac{b}{t} \approx 1$.
   ▶ Few spanning trees, but no pairwise variance bigger than $2 \sigma^2$.

5. Use a wheel design.
   ▶ Needs $\frac{b}{t} \approx 2$.
   ▶ No pairwise variance bigger than $1 \sigma^2$. 
   ▶ $87 \sigma^2$. 


Proposed strategy

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2. Insert vertices with valency 2 into the best graph with valency 4.
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Compare the following.

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2. Insert vertices with valency 2 into the best graph with valency 4.

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Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.

2. Insert vertices with valency 2 into the best graph with valency 4.

3. Glue many leaves to a single vertex of some small graph.

4. Glue many triangles to a single vertex of some small graph.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.

2. Insert vertices with valency 2 into the best graph with valency 4.

3. Glue many leaves to a single vertex of some small graph.

4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq b/t \leq 1.5$.
2. Insert vertices with valency 2 into the best graph with valency 4.

3. Glue many leaves to a single vertex of some small graph.

4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq b/t \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   - Needs $1.2 \leq b/t \leq 2$.

3. Glue many leaves to a single vertex of some small graph.

4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   ▶ Needs $1.125 \leq b/t \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   ▶ Needs $1.2 \leq b/t \leq 2$.
   ▶ Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.

3. Glue many leaves to a single vertex of some small graph.

4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   ▶ Needs $1.125 \leq b/t \leq 1.5$.
2. Insert vertices with valency 2 into the best graph with valency 4.
   ▶ Needs $1.2 \leq b/t \leq 2$.
   ▶ Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   ▶ In RAB’s experience, never beats previous method.
3. Glue many leaves to a single vertex of some small graph.

4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq \frac{b}{t} \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   - Needs $1.2 \leq \frac{b}{t} \leq 2$.
   - Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   - In RAB’s experience, never beats previous method.

3. Glue many leaves to a single vertex of some small graph.
   - Few spanning trees, but no pairwise variance is bigger than $4\sigma^2$.

4. Glue many triangles to a single vertex of some small graph.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   ▶ Needs $1.125 \leq b/t \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   ▶ Needs $1.2 \leq b/t \leq 2$.
   ▶ Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   ▶ In RAB’s experience, never beats previous method.

3. Glue many leaves to a single vertex of some small graph.
   ▶ Few spanning trees, but no pairwise variance is bigger than $4\sigma^2$.

4. Glue many triangles to a single vertex of some small graph.
   ▶ Needs $b/t \approx 1.5$.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq b/t \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   - Needs $1.2 \leq b/t \leq 2$.
   - Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   - In RAB’s experience, never beats previous method.

3. Glue many leaves to a single vertex of some small graph.
   - Few spanning trees, but no pairwise variance is bigger than $4\sigma^2$.

4. Glue many triangles to a single vertex of some small graph.
   - Needs $b/t \approx 1.5$.
   - Few spanning trees, but no pairwise variance bigger than $2.67\sigma^2$.

5. Use a wheel design.
Proposed strategy

Compare the following.

1. Insert vertices with valency 2 into the best graph with valency 3.
   - Needs $1.125 \leq b/t \leq 1.5$.

2. Insert vertices with valency 2 into the best graph with valency 4.
   - Needs $1.2 \leq b/t \leq 2$.
   - Contrast between dyes does not interfere with comparisons between treatments, but there are more vertices of valency 2.
   - In RAB’s experience, never beats previous method.

3. Glue many leaves to a single vertex of some small graph.
   - Few spanning trees, but no pairwise variance is bigger than $4\sigma^2$.

4. Glue many triangles to a single vertex of some small graph.
   - Needs $b/t \approx 1.5$.
   - Few spanning trees, but no pairwise variance bigger than $2.67\sigma^2$.

5. Use a wheel design.
   - Needs $b/t \approx 2$.
   - No pairwise variance bigger than $1.8\sigma^2$. 