Factorial and time course designs for cDNA microarray experiments

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Microarray Analysis Group

- University of Adelaide
  - Patty Solomon
  - Gary Glonek*
  - Jonas Lloyd (Ph.D.)
  - Michael Calvert (Grad. Dip.)
  - Adam Kister (Grad. Dip.)
- Hanson Institute/IMVS
  - Anna Tsykin

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- *Acknowledgement:* Terry Speed, UC Berkeley and WEHI.

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  - ⋆ Short oligonucleotide arrays (Affymetrix).
  - ⋆ *Spotted cDNA arrays: Brown & Botstein (1999).
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- *There are common themes to all these technologies.*

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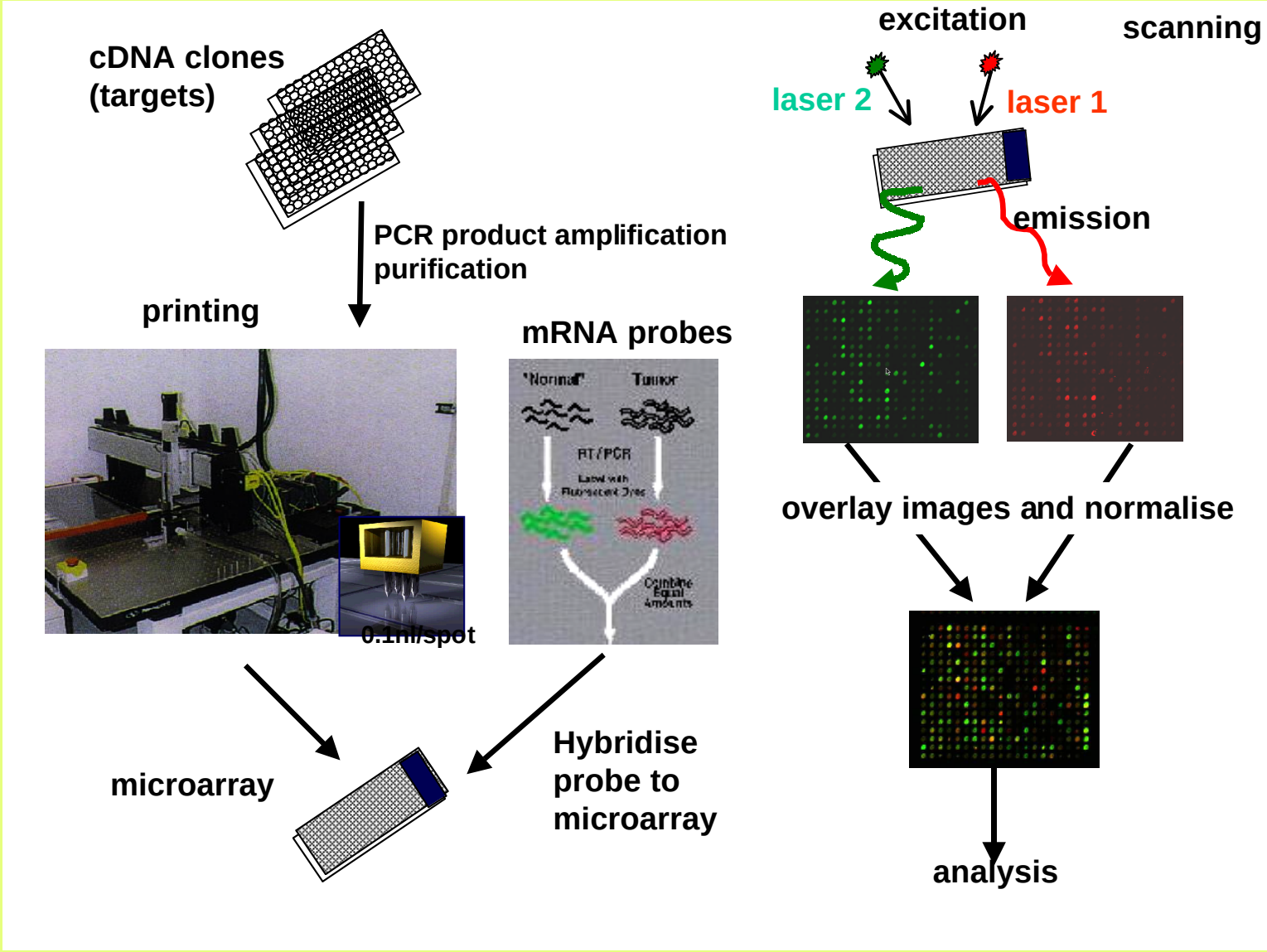
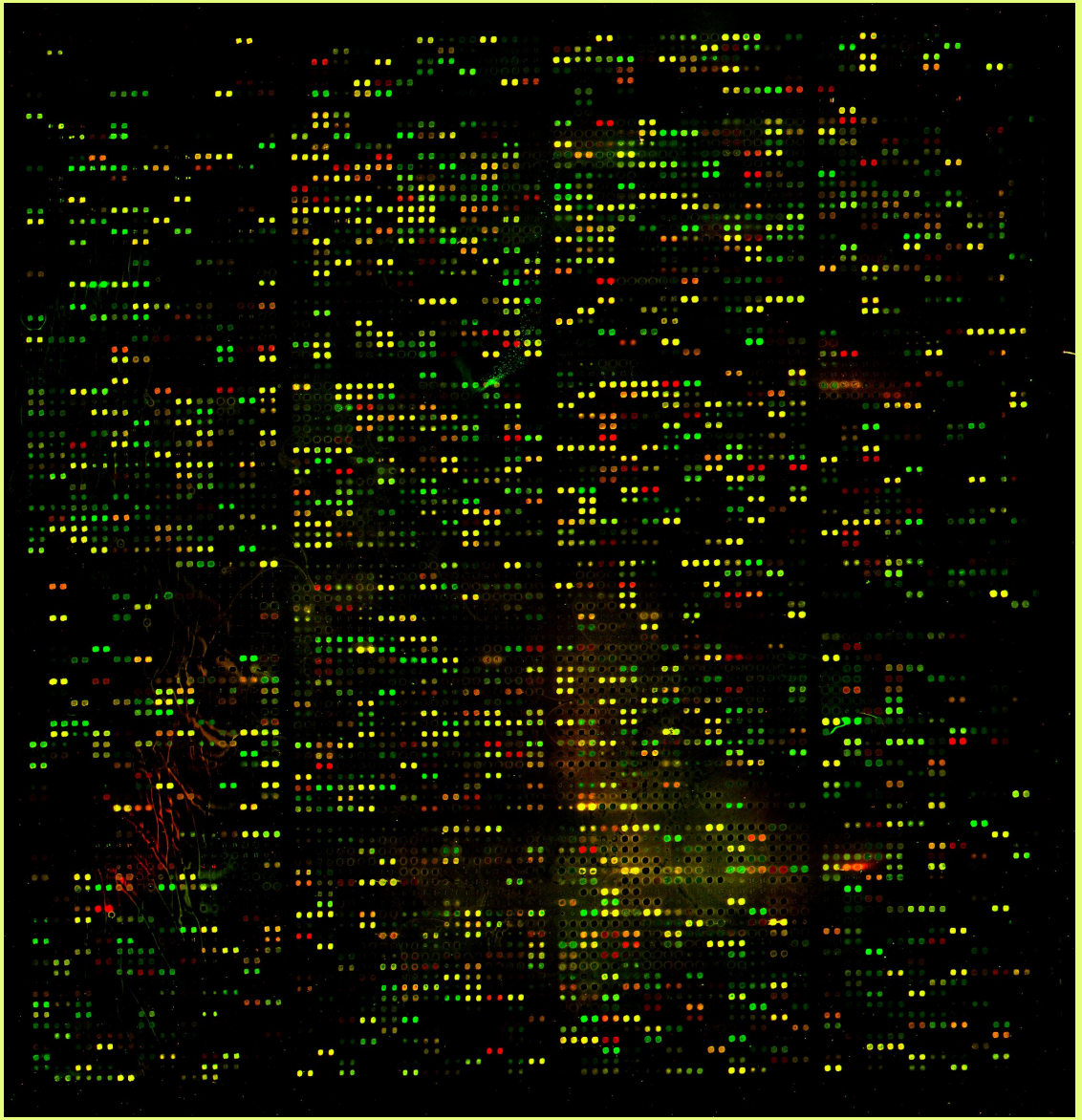
- *More complex experiments* seek to identify *patterns in groups of genes* or monitor *expression profiles* over time.
- A single experiment can involve 1 to 100's of slides.

cDNA microarray and scanner process



Human clones with MCF-7 and Jurkatt probes



Quantifying expression

For each spot on the array, calculate the background-adjusted intensities:

red intensity $R \rightarrow R - Rb$

green intensity $G \rightarrow G - Gb$

and combine them in the log base 2 ratio:

$$M = \log_2(R/G) = \log_2 R - \log_2 G$$

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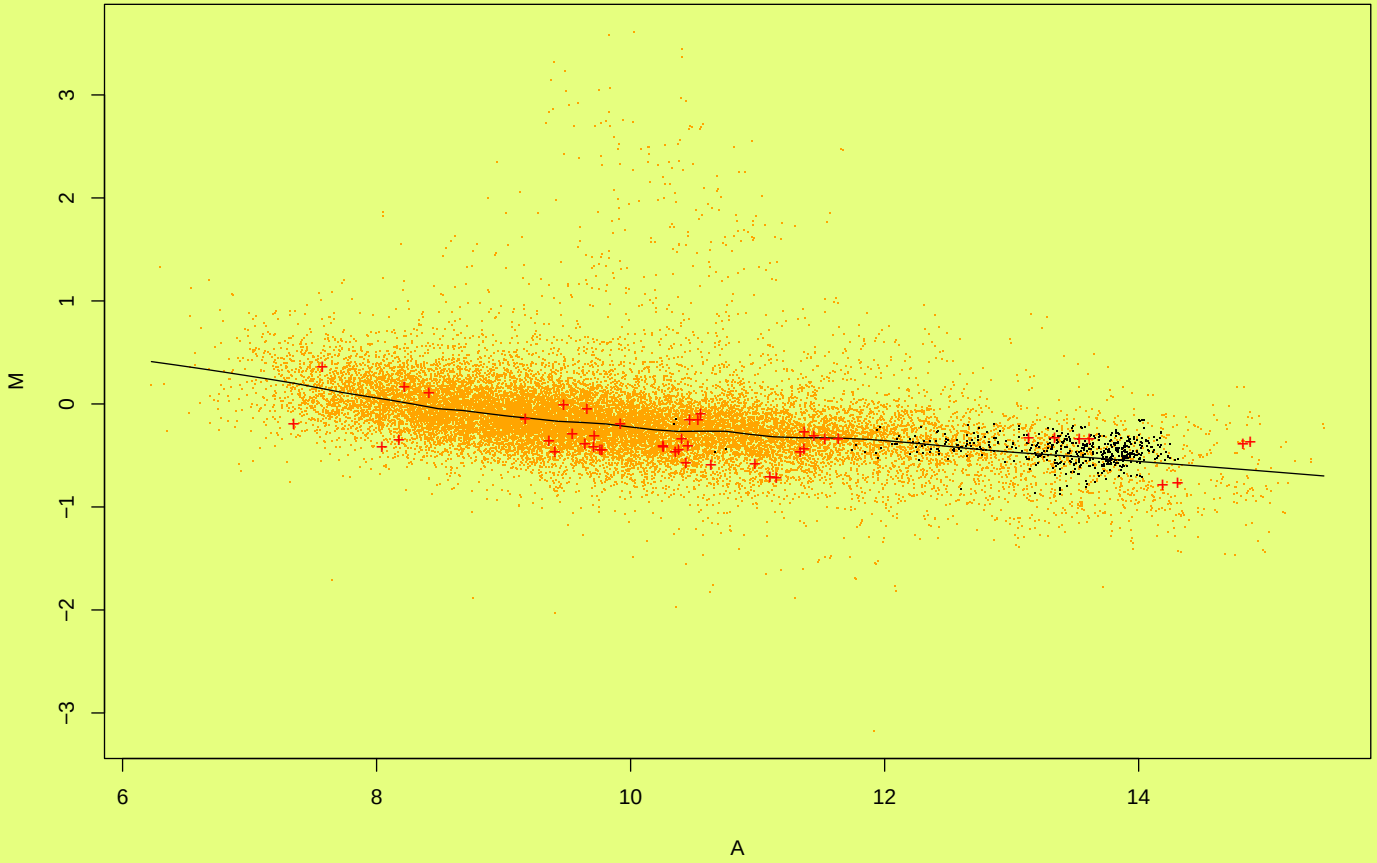
- *Background is a huge issue.*

Angiogenesis: time 0 versus .5 hour



Why is statistics important?

- *The interface between statistics, biology, computer science and medicine has gone from information-poor to information-mega-rich*.
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- *Challenges*: design, analysis and interpretation of data from microarray experiments.

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- Should tissue samples from animals be *pooled* then compared, or should different animals be hybridised to different slides?
  - If pooled samples are to be compared, what is the optimal number of pools?

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  - Kerr & Churchill 2001
  - Speed & Yang 2002, Yang 2002
  - Jin et al. 2001, Wolfinger et al. 2001, Pan et al. 2002

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There are practical constraints

- Limitations on the available mRNA.
- Cost: the number of slides available for experimentation is often fixed in advance.
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- Cost: the number of slides available for experimentation is often fixed in advance.
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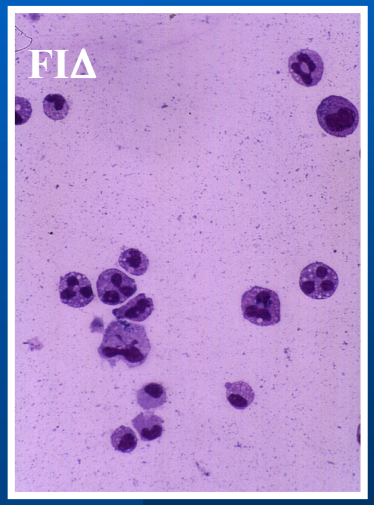
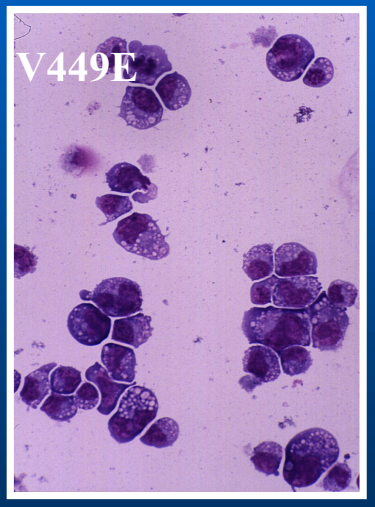
- Risk of failed hybridisations.
- *We contend that the most appropriate way to find differentially expressed genes is to prescribe a design subject to*
  - the key contrasts and parameters of interest
  - and the practical constraints of the problem.

Case Study 1

Biological function of the activated mutants in FDB cells





⇒ Different signals generated by the two mutants

- *Aim: to identify genes that play an important role in receptor signalling and leukaemogenesis in mutant mice.*

A 2x2 factorial design

- It is anticipated that measuring changes over 24 hours will distinguish genes involved in promoting or blocking differentiation, or that suppress or enhance growth, as genes potentially involved in leukaemia.
  - ◇ Compare two cell populations: FIΔ and V449E at two times 0 hours and 24 hours
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- *Sample effects of interest too.*

Notation

- Samples: FIΔ and V449E
- Times: 0 hours and 24 hours

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- Level of expression of each gene is described by four parameters:
  - μ: Overall mean (baseline)
  - α: Main effect for sample
  - β: Main effect for time
  - $\alpha\beta$: Interaction

- On the log scale, the interaction is the difference between the F, V differences at times $0, 24$:

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- The latter is the 'usual' experiment.
- *If replication is possible, do both.*

- Similarly for the main effects:

$$\text{Sample}: \quad (F24 + F0) - (V24 + V0)$$

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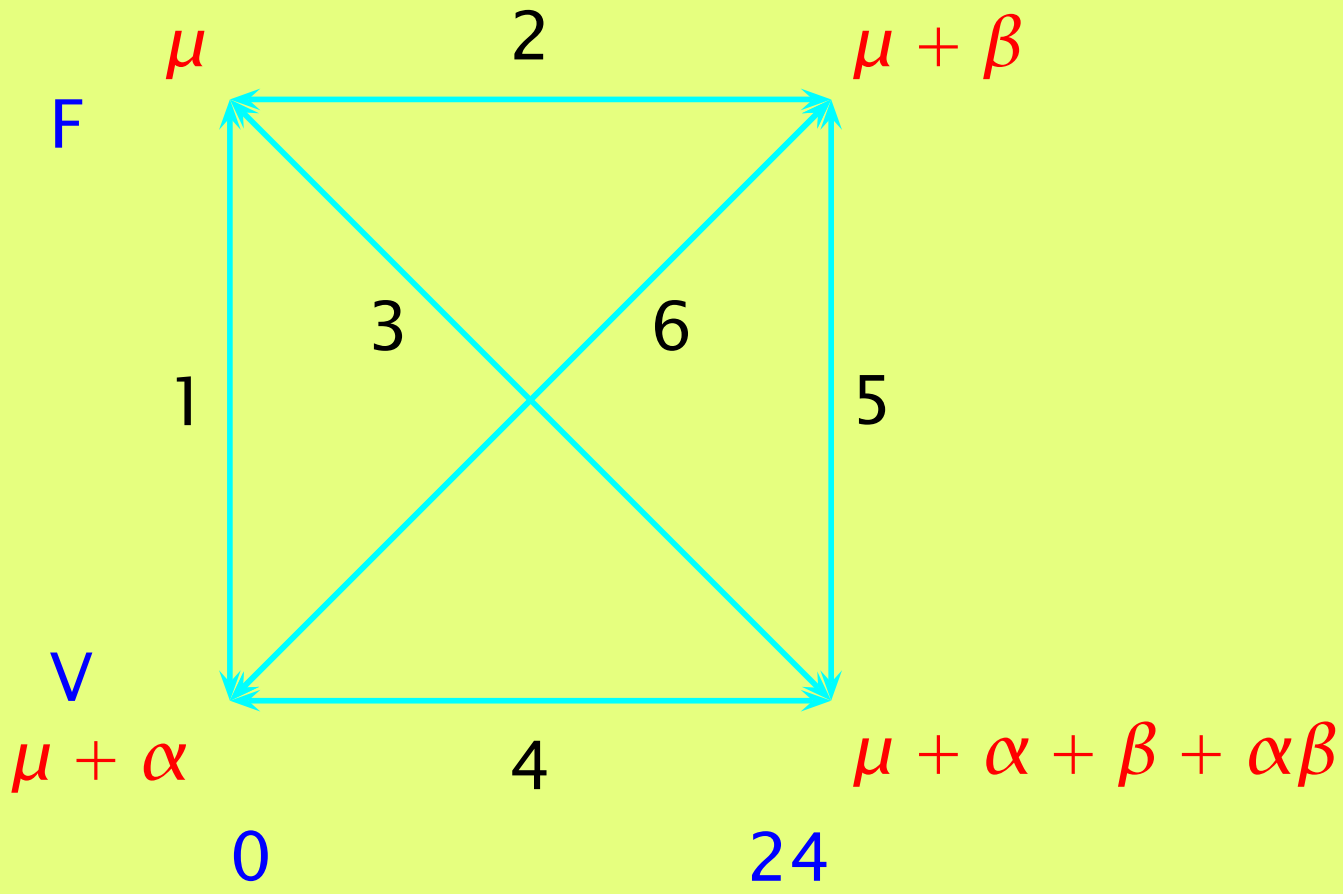
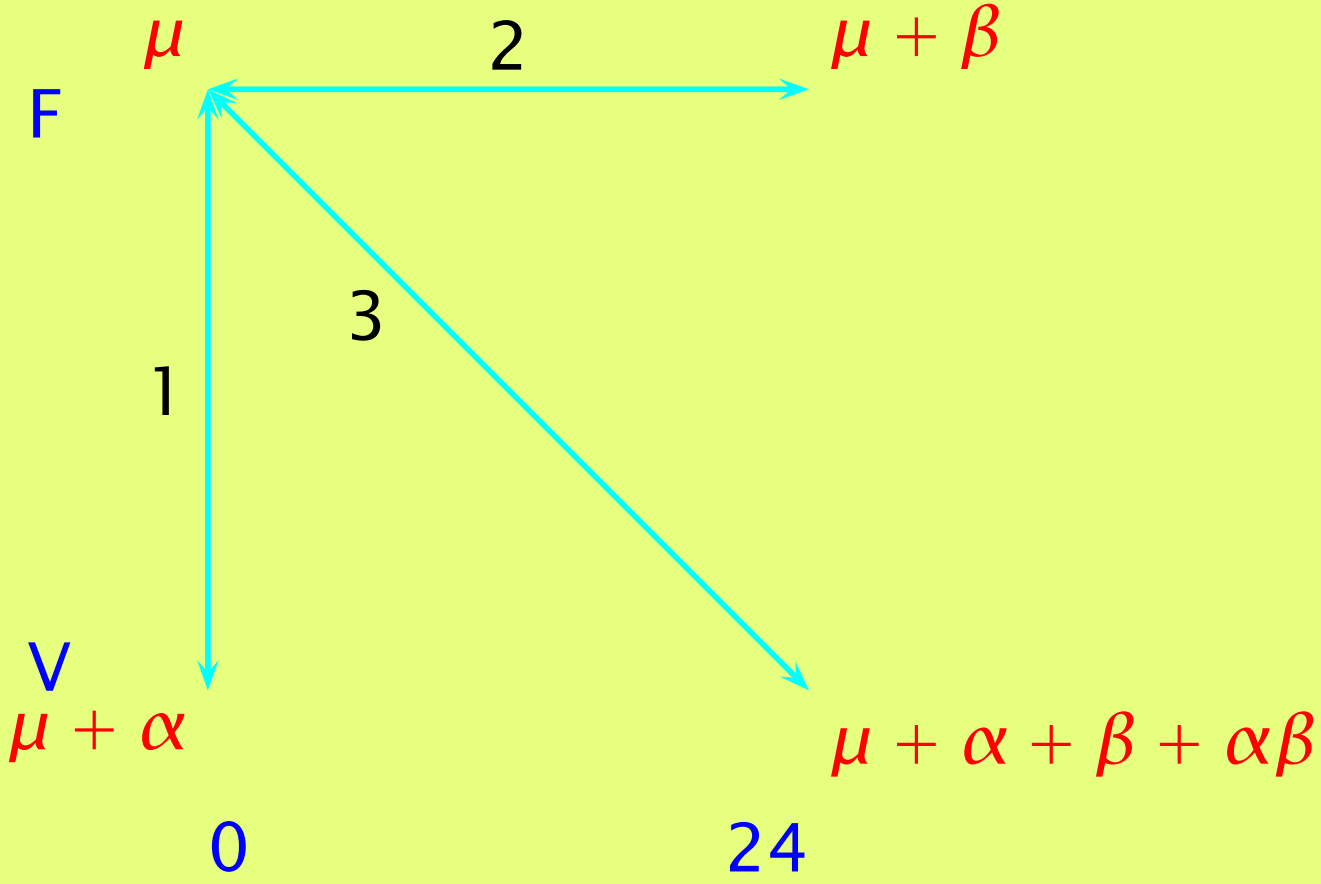
- *Again, if replicates are available, do both.*

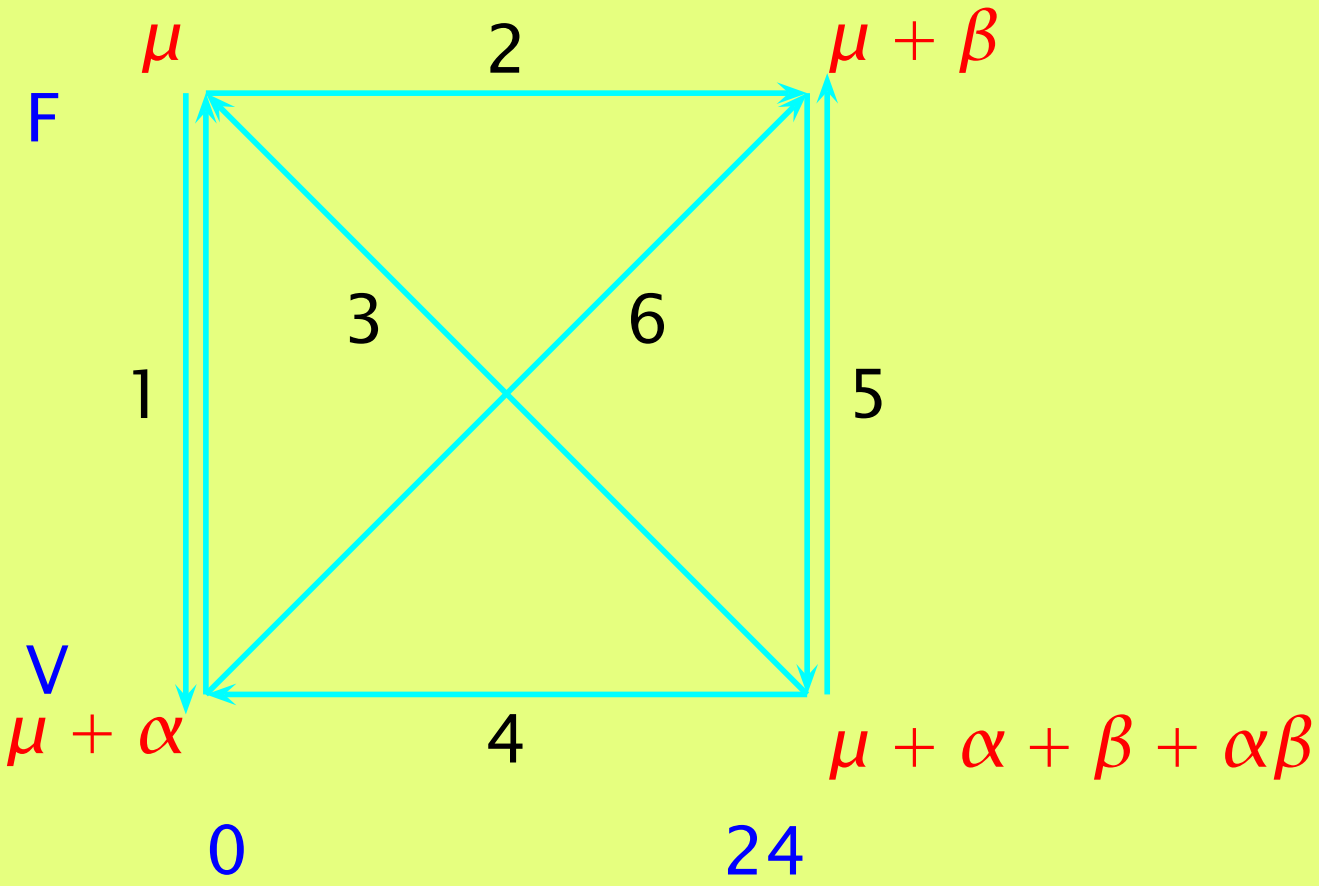
The ‘optimal’ design?



The reference design



Case study: 8 slide limit



- 6 pairwise comparisons with dye-swaps on cell line comparisons at times 0 and 24 hours.

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- It's not quite optimal either.
  - *Designs incorporating all possible multiple direct comparisons rarely, if ever, optimal by the criterion of statistical efficiency.*
- *Reference design*: poor statistical properties.
- Of course, statistical efficiency is not the only criterion for good experimental design.

The design question

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  - *Choose a design such that the standard error for each parameter of interest falls below a certain value.*

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- The standard error of a given parameter estimate is $\sigma\sqrt{c}$, where σ is the standard deviation between slides for a particular gene, and c is derived from the design matrix.
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- However, a single experiment typically involves $> 10,000$ genes in which σ varies greatly from gene to gene, and is usually unknown.
- *The good news: the design with the smallest standard error and thus the highest power will be that which has the smallest value of c and this applies equally to every gene.*

Least squares analysis

- We know the *least squares estimates* are given by

$$(X^T X)^{-1} X^T M$$

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  - ⋆ Unfortunately, this criterion is not straightforward.

Admissible designs

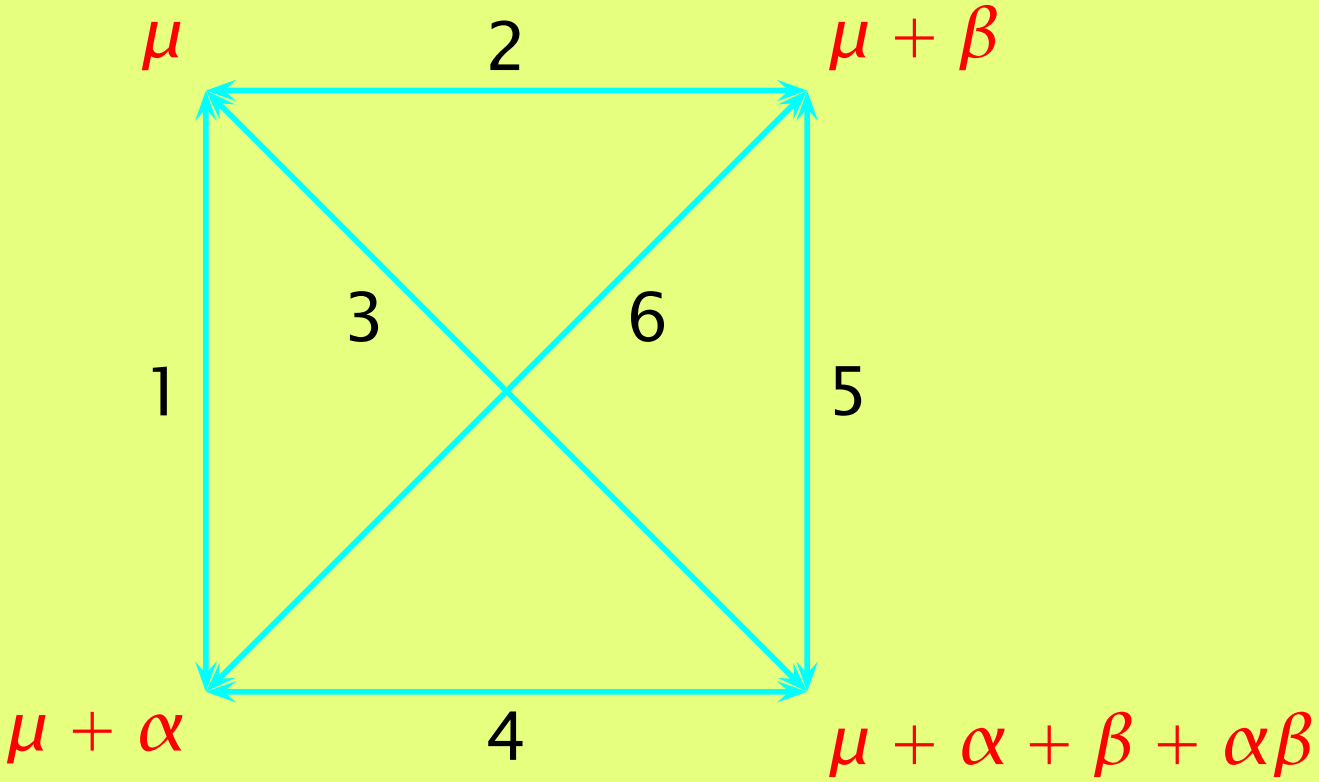
A design with a total of n slides and design matrix X is said to be *admissible* if there exists no other design with n slides and design matrix X_* such that

$$c_i \geq c_i^*$$

for all i *with strict inequality for at least one* i, where c_i, c_i^* are respectively the diagonal elements of $(X^T X)^{-1}$ and $(X_*^T X_*)^{-1}$. A design that is not admissible is said to be *inadmissible*.

- *There is no simple way to identify the set of admissible designs.*
  - e.g. for 24 slides, there are 118,755 possible ways to allocate them to the 6 slide types.
- *However, for small problems, we can simply enumerate the possibilities.*

‘Simplest’ case

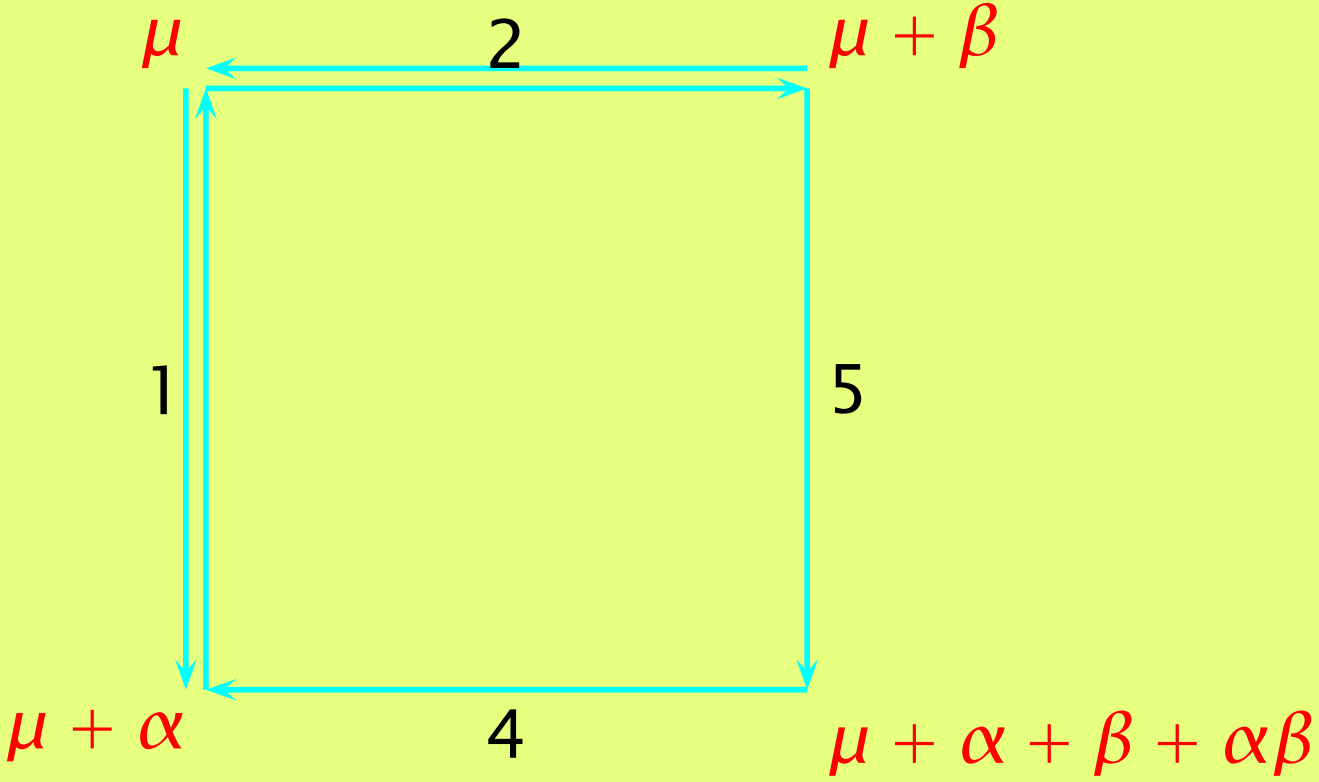


Forward

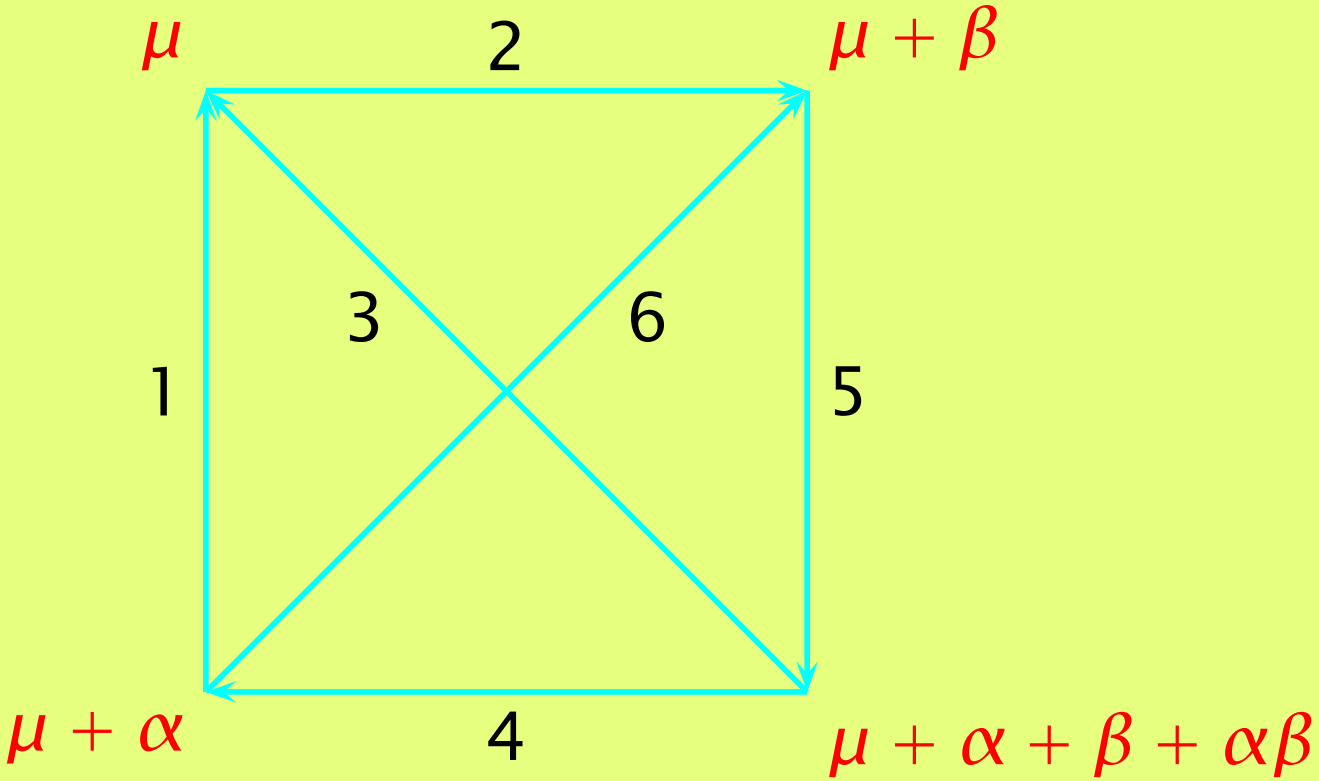
```
macsolomon2[psolomon]% ./a.out

% parameters a b ab;
% comparisons a vs baseline; b vs baseline;
Comparison % a+b+ab vs baseline;
Comparison % a+b+ab vs a;
Comparison % a+b+ab vs b;
Comparison % b vs a;;
% slides 6
% show
1 0 0
0 1 0
1 1 1
0 1 1
1 0 1
-1 1 0
```

```
% generate
Total: 462
Admissible: 21

% equal 1 2;
Admissible: 3
0.4167 0.4167 0.7500 |2 2 0 1 1 0
0.3750 0.3750 1.3750 |2 2 0 1 0 1
0.3750 0.3750 1.3750 |2 2 0 0 1 1

% better 1 1 1 1 1 1
Your Design:
0.5000 0.5000 1.0000 |1 1 1 1 1 1
Better Designs:
0.4167 0.4167 0.7500 |2 2 0 1 1 0
```

6 slides: admissible design 1

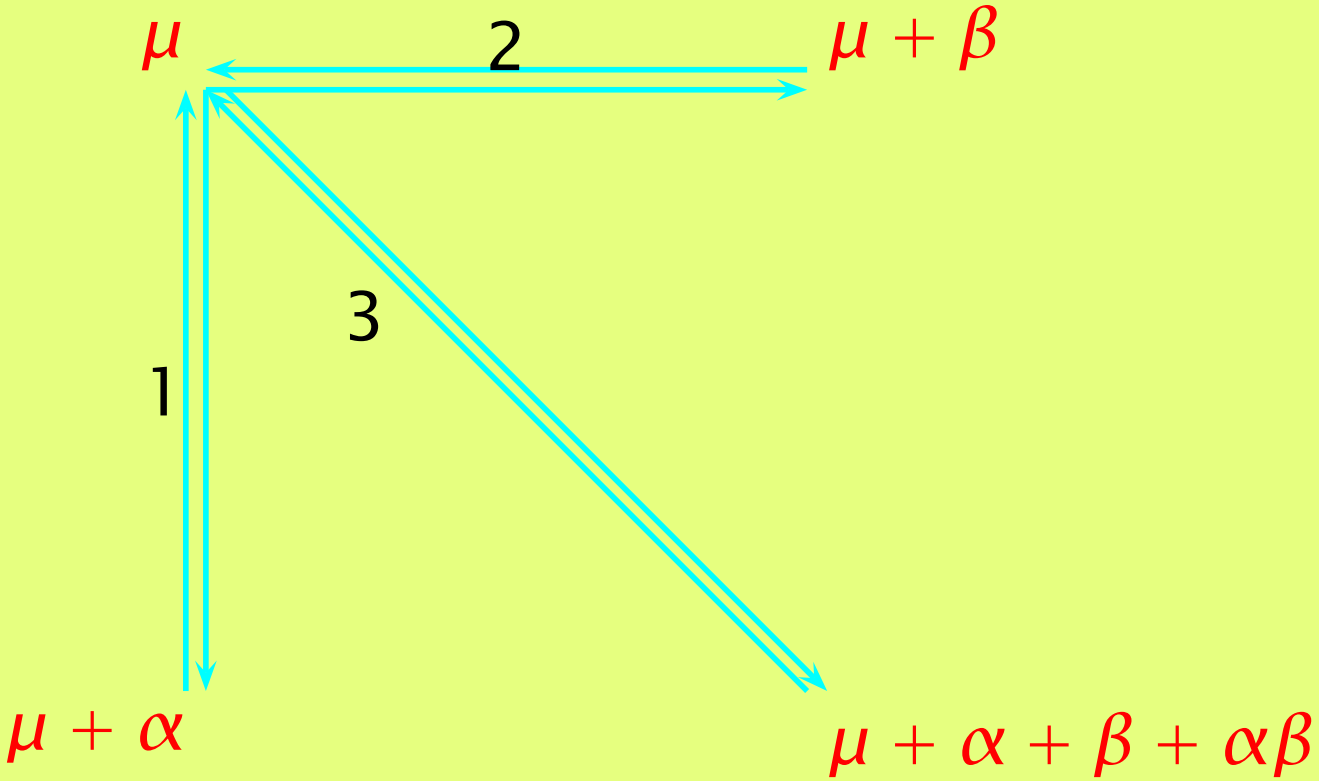


6 slides: inadmissible design



All pairwise comparisons.

6 slides: inadmissible design



The common reference design.

Designs with 6 slides

Des	Replication Configuration 1	2	3	4	5	6	C_α	C_β	$C_{(\alpha\beta)}$
A 1	2	2	0	1	1	0	0.4167	0.4167	0.7500
A 2	2	2	0	1	0	1	0.3750	0.3750	1.3750
A 3	2	2	0	0	1	1	0.3750	0.3750	1.3750
Ref	2	2	2	0	0	0	0.5000	0.5000	1.5000
All	1	1	1	1	1	1	0.5000	0.5000	1.0000

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A 3	2	2	0	0	1	1	0.3750	0.3750	1.3750
Ref	2	2	2	0	0	0	0.5000	0.5000	1.5000
All	1	1	1	1	1	1	0.5000	0.5000	1.0000

- *Large gains in efficiency can be obtained using admissible designs.*

Case study 2: a 2x3 factorial design

- Aim: to study the role in human disease of cell-surface receptors for a group of signalling molecules.

  - Two cell lines: mutant (M) versus wildtype (W).
  - Three time points: 0, 6 and 12 hours.
  - 9 slides.

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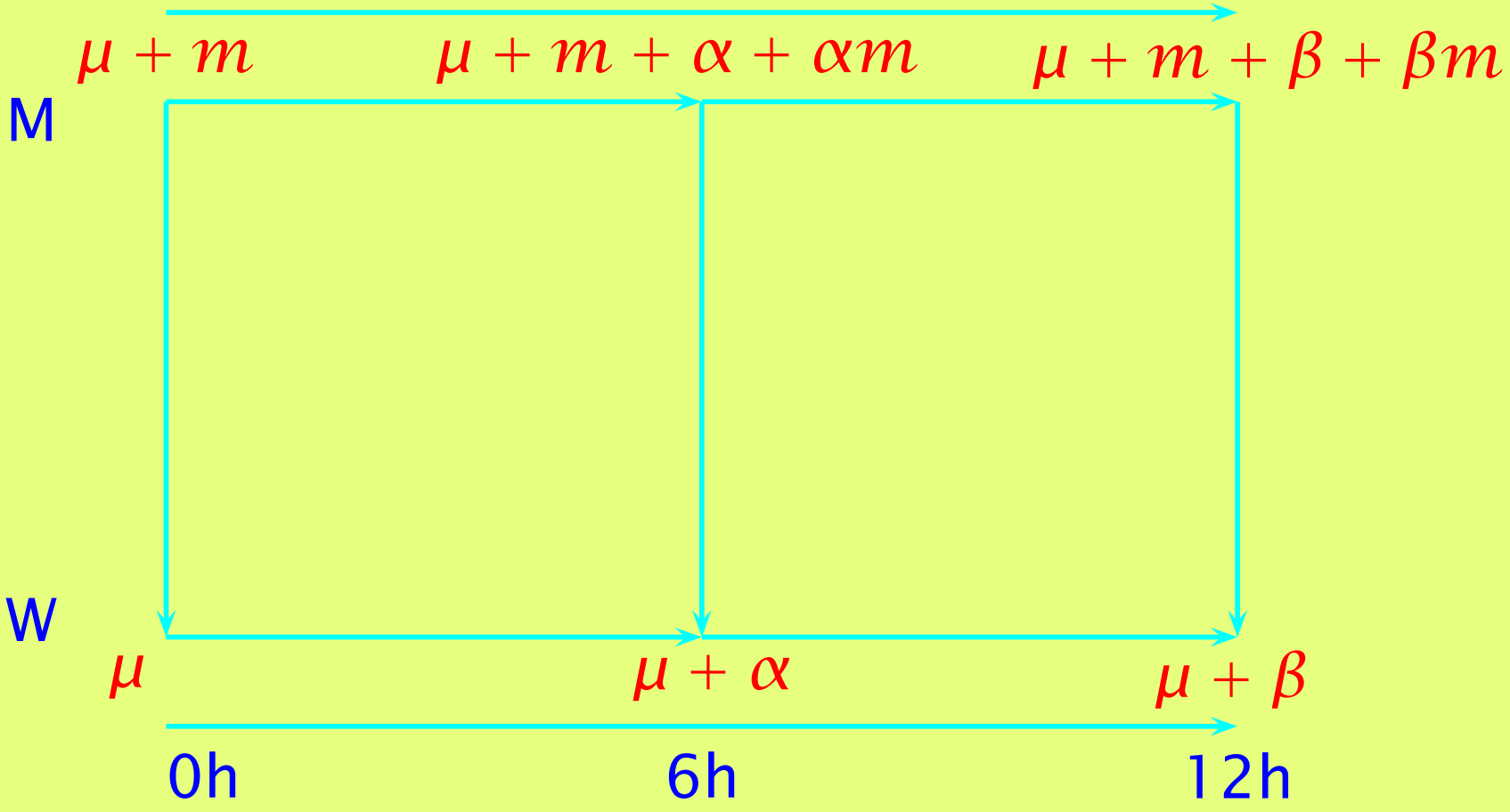
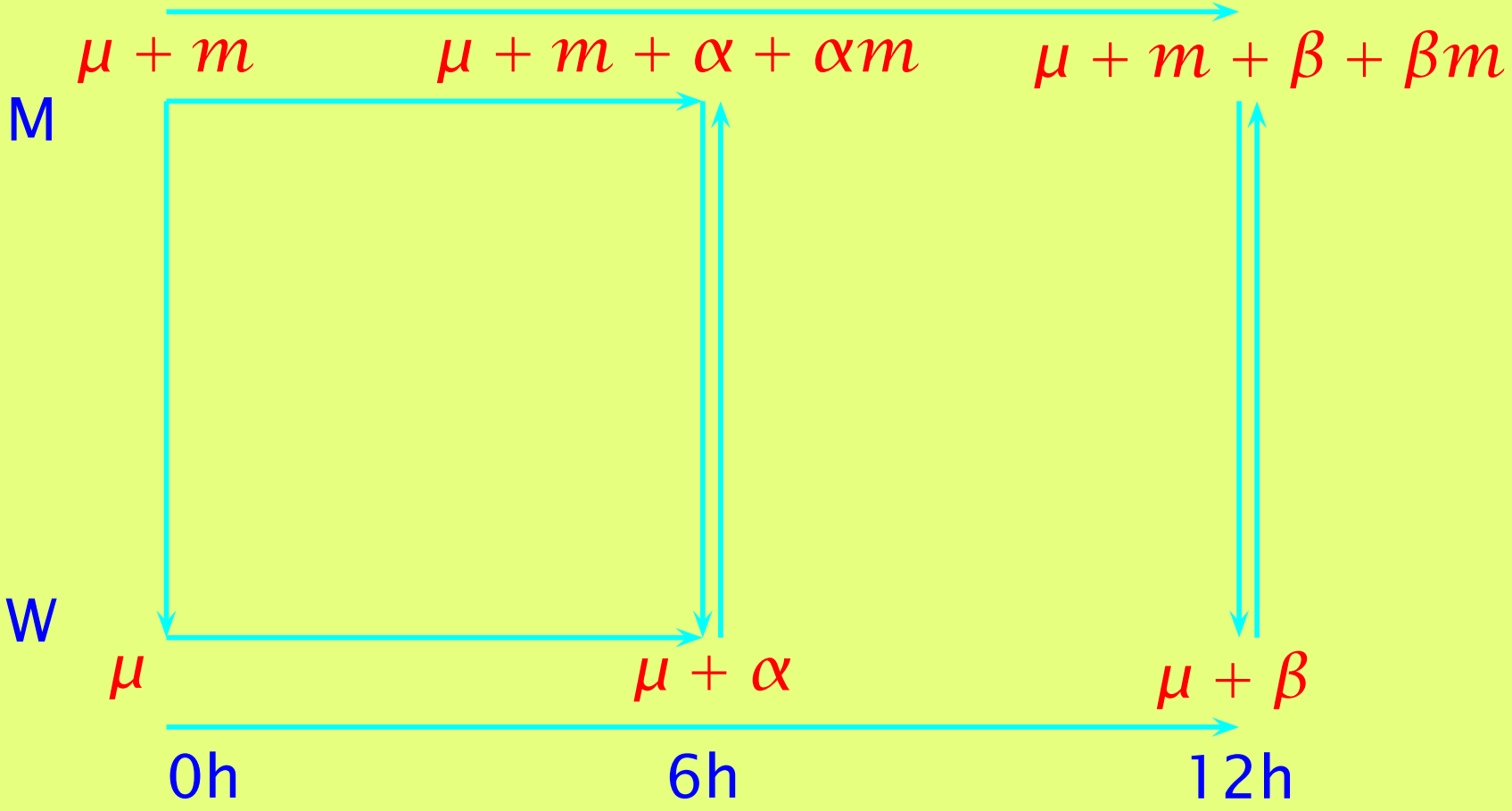
- *Researchers are particularly interested in the cell line comparisons at 6 and 12 hours.*

a 2x3 factorial design



Best admissible design: 9 slides



Time course experiments

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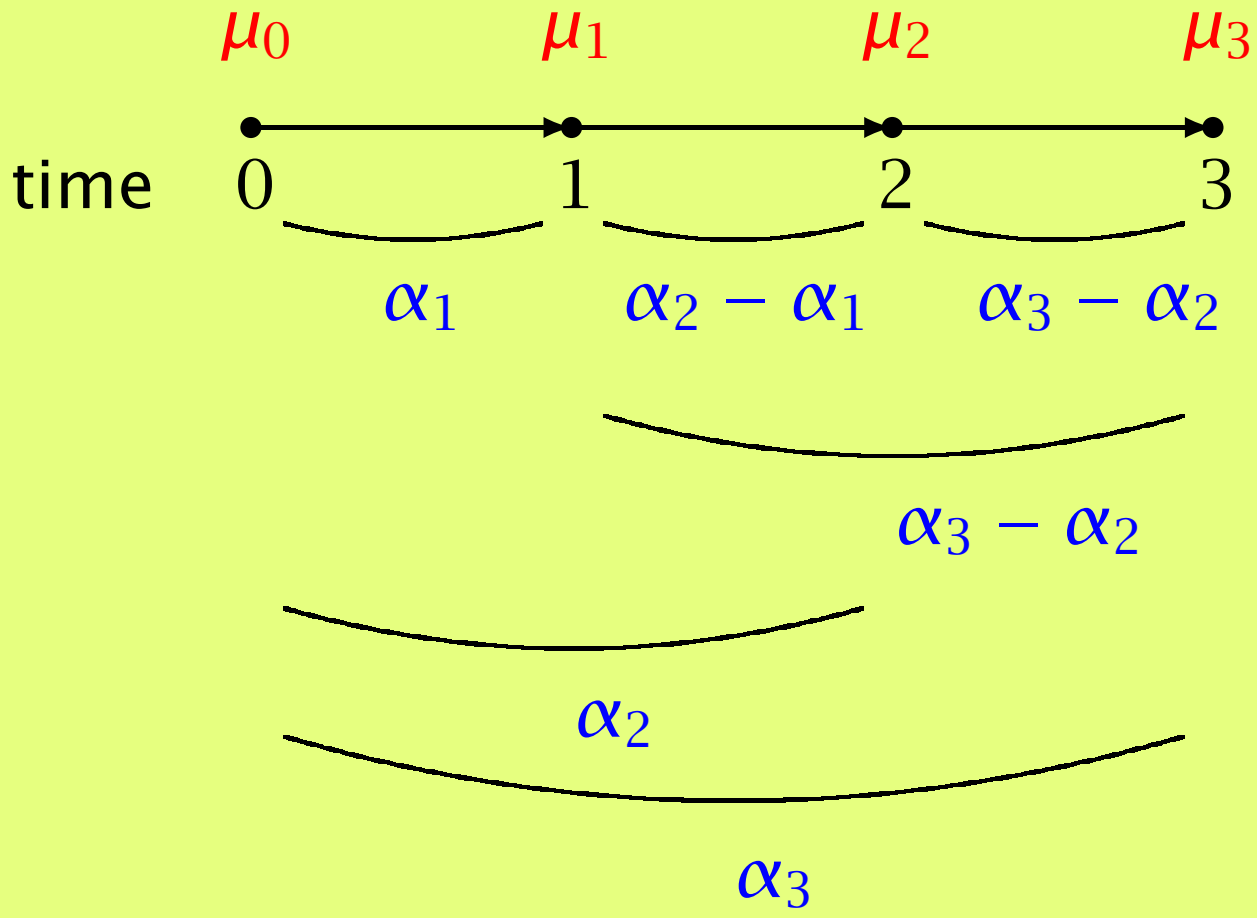
- *But 'best' design will be experiment dependent.*
  - There are $r(r+1)/2$ possible slides, so, how many of *each type* should be made?
- *Key step is to identify the parameters of interest.*
  - e.g. monitoring smooth changes, or
  - e.g. detecting sudden changes or unusual patterns in expression.

- One approach is to take the *differences* relative to time 0

$$\alpha_t = \mu_t - \mu_0 \quad \text{for} \quad t = 1, 2, \ldots, r$$

as the parameters of interest.
In the case $r = 3$, there are six possible slide types:

	Configuration		Expected Log Ratio
	Red	Green	
1	Time 0	Time 1	α_1
2	Time 0	Time 2	α_2
3	Time 0	Time 3	α_3
4	Time 1	Time 2	$\alpha_2 - \alpha_1$
5	Time 1	Time 3	$\alpha_3 - \alpha_1$
6	Time 2	Time 3	$\alpha_3 - \alpha_2$


μ_0
μ_1
μ_2
μ_3
time
0
1
2
3
α_1
$\alpha_2 - \alpha_1$
$\alpha_3 - \alpha_2$
$\alpha_3 - \alpha_2$
α_2
α_3

6 slides: admissible designs

Replication								
Configuration								
1	2	3	4	5	6	c_{α_1}	c_{α_2}	c_{α_3}
2	2	2	0	0	0	0.500	0.500	0.500
1	1	1	1	1	1	0.500	0.500	0.500

6 slides: admissible designs

Replication								
Configuration								
1	2	3	4	5	6	c_{α_1}	c_{α_2}	c_{α_3}
2	2	2	0	0	0	0.500	0.500	0.500
1	1	1	1	1	1	0.500	0.500	0.500

- Covariance matrices:

$$\begin{pmatrix} 0.5 & 0 & 0 \\ 0 & 0.5 & 0 \\ 0 & 0 & 0.5 \end{pmatrix} \quad \text{and} \quad \begin{pmatrix} 0.5 & 0.25 & 0.25 \\ 0.25 & 0.5 & 0.25 \\ 0.25 & 0.25 & 0.5 \end{pmatrix}$$

A useful tool

- Use admissible designs when time course patterns pre-specified and parameterised in advance
- $\longrightarrow$ *choose best design for allocating a given number of slides to the possible slide types to optimise detection of patterns or profiles of interest.*

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- *Biology looks set to dominate statistics at the beginning of this century, just as it did at the beginning of the last one.*

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[1] Ad hoc Committee on Resources for the Mathematical Sciences, US National Research Council, 1981.

Further reading and web sites

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THE END