Suppose we have 24 experimental units and would like to compare the effects of 4 treatments on gene expression.

Now suppose we are going to measure gene expression using two-color microarrays.

If we have 12 slides, how should experimental units be paired on slides?

This is an example of a Balanced Incomplete Block Design (BIBD). Each treatment is represented by the same number of exp. units. Each pair of treatments appears together in the same block an equal number of times.

Ignore the dye factor for a moment and consider the following pairing.

Use a completely randomized design to assign 6 experimental units to each treatment.
In this example, it is easy to assign dyes so that each treatment will have an equal number of experimental units dyed with each dye.

Balance

• An experiment is balanced if all treatments have the same number of experimental units.
• An experiment is dye balanced if all treatments have an equal number of experimental units dyed with each dye.
• An incomplete block design is a balanced incomplete block design if all treatments have the same number of experimental units and each pair of treatments appears together in the same block an equal number of times.

Some incomplete block designs are not BIBD.

This design is balanced in that each treatment has an equal number of experimental units.

Note, however, that each pair of treatments does not appear together in a block an equal number of times.

Such designs make sense if some treatment comparisons are more interesting than others.

These loop designs are often used when the treatment is a time factor such as hours after inoculation or developmental stage.

This experiment is dye balanced.

It is probably a good idea to loop in opposite directions, but that is not a requirement for dye balance.

This experiment may have been a CRD prior to assignment of experimental units to slides.

The only blocking results from the requirement of two experimental units per slide.
This experiment may have been a RCBD prior to assignment if treatments to experimental units.

The experimental units to the left and right may have formed two blocks, respectively, prior to the assignment of experimental units to slides.

Even if there was no blocking in the original experimental units, we might end up with this structure if all the slides on the left were hybridized on one day and all the slides on the right were hybridized on another.

Suppose all treatment comparisons are of equal interest and that 24 two-color slides are available. How would you assign experimental units to slides?

All possible four-treatment loops appear exactly once in this design. Is this design optimal?