3-2-2006

2^k Factorials in Blocks of Size 2, with Application to Two-Color Microarray Experiments

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1. Introduction

This paper investigates the problem of arranging 2^k factorials in blocks of size 2. We adopt a conventional assumption of factorial design, that lower-order factorial effects are of greater interest than higher-order effects. Specifically, we seek minimal designs to achieve (i) independent estimates of all main effects or (ii) independent estimates of all main effects and 2-factor interactions. However, while we assume that higher-order factorial effects are not of primary interest, we do not assume such effects are negligible.

This research was directly motivated by the problem of designing experiments for two-color microarrays, which are an important tool in modern molecular biology. Microarrays are used to quantify levels of gene transcription, which can loosely be considered the level of "activity" of a gene. Microarrays can make these measurements for thousands of genes at a time. Two-color microarrays are small slides containing thousands of spots. Each spot contains single-stranded DNA molecules corresponding to a particular gene in the genome of an organism under study. In a microarray assay, purified messenger RNA from cell populations under study are reverse-transcribed into cDNA and labeled with one of two fluorescent dyes, "red" or "green." Two pools of oppositely labeled cDNA are combined and applied to a microarray. Each dye-labeled strand of cDNA has the opportunity to hybridize to its complementary strand, which is spotted on the microarray. After the hybridization period, unhybridized cDNA is washed off the array. The microarray is then scanned, and "red" and "green" intensity measurements are acquired for each spot. Properly normalized, the relative intensity of the red and green signals from a spot measures the relative abundance of the corresponding transcript in the two cell populations.

For more information on microarrays, Nguyen, Arpat, Wang and Carroll (2002) give an excellent review of the technology for a statistical audience. Previous work on experimental design for microarrays includes Kerr and Churchill (2001a), (2001b), who argue that microarray designs can be considered as incomplete block designs for block size 2. This paper is directly applicable to microarray studies with multiple binary factors.

There is a large literature on designs for multiple binary factors, including several papers that discuss blocking of full factorials. An introduction to full factorial experiments at two levels and fractional factorials can be found in many textbooks, so we review these topics only briefly. Wu and Hamada (2000) is an excellent reference. Three papers closely related to this one are Sun, Wu and Chen (1997), Draper and Guttman (1997), and Wang (2004). Sitter, Chen and Feder (1997) is a related paper on blocked fractional factorial designs, but fractional factorials are not appropriate in our circumstances because we do not assume higher-order factorial effects are negligible.

This paper is organized as follows. We begin with background and notation, followed by an example to illustrate the important concepts. We then establish some basic results about the kinds of designs that arise under the circumstances we consider. Next, we consider designs for $2 \leq k \leq 8$ factors, and then give a construction that produces economical designs for arbitrary k. Finally, we consider issues that arise in the application of the general results to the design of two-color microarray experiments, and give an example of a specific design problem in this setting. Much of the material in the first few sections is not really new. However, these earlier sections synthesize what is already known, develop our formulation of the problem, and define some useful terms ("blocked factorial," "estimability").

2. Background and Notation

In this section we briefly review important concepts and established results. The reader should consult Sun, Wu and Chen (1997) or Wu and Hamada (2000) for more background. Notation generally follows Mitchell, Morris and Ylvisaker (1995) or Sun, Wu and Chen (1997).

The number of binary factors is k, represented by the first k letters of the alphabet, upper-case. Denote the two levels of each factor with "1" and "-1" or "+" and "$-$." The set of experimental *runs* T is all k-dimensional vectors with entries "1" and "-1." The set of runs T can be visualized as the vertices of a square for $k = 2$ and the vertices of a cube for $k = 3$. T is a metric space under Hamming distance, where $d(\mathbf{t}, \mathbf{s})$ is the number of factors for which the runs $\mathbf{t}$ and $\mathbf{s}$ differ. Define $|\mathbf{t}| = d(\mathbf{1}, \mathbf{t})$. Also note that T forms a group via component-wise multiplication with identity $\mathbf{1}$ and every element self-inverse.

Subsets of factors are called *words*. Words can be "multiplied" as illustrated by the following examples: the product of any word with itself is the "null" word, usually denoted I; $A \cdot A = I$; $AD \cdot BC = ABCD$; $ABC \cdot CD = ABC^2D = ABD$. This algebra leads to a notion of independence for words: ABC, CD, and ABD are not independent since $ABC \cdot CD = ABD$.

Any word W partitions the set of runs in T into two sets of equal size depending on whether $\prod_{i \in W} t_i = 1$ or $= -1$. The *factorial effect* corresponding to a word W is the expected contrast between the experimental outcome between these two sets. Thus a factorial effect is estimated by a linear combination of the experimental runs. It is important to note that we can also write each experimental run as a linear combination of the factorial effects (see Mitchell, Morris and Ylvisaker (1995)).

We write factorial effects with lower-case letters to distinguish them from words, e.g., *bcd* is the three-way factorial effect, or *interaction*, between factors B, C, and D. *Main effects* are factorial effects corresponding to a single factor. For convenience, abbreviate "main effect" as ME and "two-factor interaction" as 2fi. A conventional assumption in factorial design, adopted here, is that lower-order effects are of greater interest than higher-order effects. However, as noted, we do not assume that higher-order effects are negligible.

2.1 *Fractional Factorials and Blocked Factorials*

One can arrange a 2^k factorial in 2^p blocks of size 2^{k-p} by identifying p independent words. These p independent words generate a set of 2^p words when we consider all products. The 2^p factorial effects corresponding to these words will be confounded with block effects in the blocked design. The remaining factorial effects are estimable, and in fact they are also orthogonal so that their estimates are statistically independent.

A regular *fractional factorial* design is specified by p independent words W_i, $i = 1, ..., p$, and the "defining relation" $I = W_1 = \cdots = W_p$. There are 2^{k-p} runs in the fractional factorial design that satisfy the defining relations. That is, there are 2^{k-p} runs $\mathbf{t}$ such that $\prod_{i \in W} t_i = 1$ for all words W in the defining relation. Sun, Wu and Chen (1997) describe the strong correspondence between 2^{k-p} fractional factorials and full factorials arranged in blocks of size 2^{k-p}. However, despite the strong correspondence, the latter are not actually fractional factorials, and we refer to these designs as *blocked factorials*. (Wang (2004) simply used the word "group" instead.) A blocked factorial is all 2^k runs arranged into blocks, whereas a fractional factorial is a $\frac{1}{2^p}$ fraction of these runs, unblocked.

This paper considers the special case of block size 2, so that $p = k - 1$ throughout.

3. Example: 3 Factors in Blocks of Size 2

This section gives an example for the case of $k = 3$ binary factors to illustrate the important background concepts.

For three factors, the eight runs in T can be represented as the vertices of the 3-dimensional cube (Figure 1). The vertices of the cube are identified by a triple (A, B, C), indicating whether a run is level "−" or "+" for factors A, B, and C, respectively.

Consider the runs $(+, +, +)$ and $(-, -, -)$. In terms of the factorial effects, these runs are represented as:

$$\begin{aligned} &\mu + a + b + c + ab + ac + bc + abc \\ &\mu - a - b - c + ab + ac + bc - abc. \end{aligned} \tag{1}$$

Suppose these two runs are paired in a block of size 2 in the experimental design. Analysis of data from this block will use only "within-block" differences in observations. This block therefore provides information on the difference between the expressions (1). Taking half that difference, the quantity $a + b + c + abc$ is estimable from this block.

This block can be represented by a diagonal line through the cube in

Figure 1. Consider another such diagonal "block," say the diagonal between run $(-,-,+)$ and run $(+,+,-)$. In terms of the factorial effects, the expected difference between these runs is proportional to the difference between

$$\mu + a + b - c + ab - ac - bc - abc$$

and

$$\mu - a - b + c + ab - ac - bc + abc,$$

or $a + b - c - abc$. The other two diagonals of the cube allow estimation of $a - b + c - abc$ and $a - b - c + abc$. We see that data from any one, two, or three of these blocks would not allow estimation of any factorial effect (without further assumptions that some factorial effects are zero). On the other hand, data from all four blocks (the full "blocked factorial") allow independent estimates of all four factorial effects a, b, c, and abc. The four diagonal lines through the cube in Figure 2 represent the blocked factorial.

The runs in the block $\{(-,-,-),(+,+,+)\}$ are the runs in the 2^{3-2} fractional factorial design with defining relation $I = AB = AC = BC$. The other three blocks described above are simply the variants of this fractional factorial, e.g., the block $\{(-,-,+),(+,+,-)\}$ corresponds to the defining relation $I = AB = -AC = -BC$. Considering the group structure of the design space T, the block $\{(-,-,-),(+,+,+)\}$ is a subgroup of T and the other three "diagonal" blocks are the cosets of this subgroup. With the block size fixed at 2, it is more convenient to think of the blocks directly, in terms of a subgroup of T and its cosets, rather than considering the set of defining relations. However, notice that the words in the defining relations correspond exactly to the factorial effects that are *not* estimable from the complete blocked factorial (comprised of all four "diagonal" blocks).

4. k Factors in Blocks of Size 2

The example in the previous section illustrates the key features of arranging a 2^k factorial experiments in blocks of size 2. Representing the runs in terms of the factorial effects, any two runs have the same sign for exactly half the factorial effects and have opposite sign for the other half. Therefore, the difference between two runs in a block estimates a linear combination of the effects for which the two runs have opposite sign. It follows, then, that estimating these 2^{k-1} effects requires 2^{k-1} blocks. A design with fewer blocks is not useful without further assumptions about negligible effects. A blocked factorial contains 2^{k-1} blocks of size 2 and allows unbiased, independent estimates of half of the factorial effects.

The following observations follow from these preliminary results. Recall that we consider only block size 2.

Observation 1. We can concisely represent a blocked factorial by a single *generator*. If we say $\mathbf{t}$ is the generator of a blocked factorial, we mean that $\{\mathbf{1}, \mathbf{t}\}$ is one block in the design. The other blocks in the design are cosets, i.e., any other block can be written $\{\mathbf{s}, \mathbf{st}\}$ for some run $\mathbf{s}$. There are $2^k - 1$ possible generators, so there are $2^k - 1$ blocked factorials.

Observation 2. Let $\mathbf{t} = (t_1, \ldots, t_k)$ be the generator of a blocked factorial. The factorial effect corresponding to a word W is estimable in this blocked factorial if and only if $\prod_{i \in W} t_i = -1$. Otherwise, the effect is confounded with block effects. Consequently, the main effect for the i^{th} factor is estimable if and only if $t_i = -1$. The two-way interaction between the i^{th} and j^{th} factors is estimable if and only if $t_i t_j = -1$.

Observation 3. As a consequence of Observation 2, we see that the design generated by $-\mathbf{1} = (-, -, \ldots, -)$ allows estimation of all main effects. Further, we see that this is the unique blocked factorial that allows estimation of all main effects. If the goal of an experiment is limited to estimating main effects, this can therefore be achieved with 2^{k-1} blocks and one full replication (Box et al. (1978), Draper and Guttman (1997)). The example at the beginning of the paper illustrated this design for three factors. In that design, the three-way interaction was also estimable but none of the two-factor interactions were estimable. For general k, all odd-order factorial effects will be estimable with this design but none of the even-order effects.

Observation 4. Two blocked factorials are *isomorphic* if they are isomorphic as fractional factorials. That is, the defining relations of design 1 can be gotten from design 2 simply by re-labeling factors. For block size 2, consider two blocked factorials generated by $\mathbf{t_1}$ and $\mathbf{t_2}$ respectively. If $d(\mathbf{1}, \mathbf{t_1}) = d(\mathbf{1}, \mathbf{t_2})$, then the blocked factorials are isomorphic. Therefore, there are $k - 1$ non-isomorphic blocked factorials.

The next two sections address the question of finding designs to estimate all ME's *and* 2fi's. Achieving this must involve combining multiple blocked factorials (another consequence of Observation 3). Toward this end, we make one last definition. In any given blocked factorial, an effect is either estimable or it is confounded with block effects. Therefore, in a design that is the union of m blocked factorials, a given effect is estimable by 0, 1, 2, or … m of the component blocked factorials. We define the *estimability* of an effect to be this integer. Clearly, effects with higher estimability are estimated with greater precision than effects with lower estimability. By definition, "estimable" effects have estimability ≥ 1.

5. Combining Blocked Factorials to Estimate Main Effects and Two-Factor Interactions

There is a unique $2^{k-(k-1)}$ blocked factorial that allows estimation of all main effects (Box et al. (1978), Draper and Guttman (1997)), but this design does not allow estimation of any two-factor interactions. We now consider the problem of identifying designs that allow estimation of all main effects (ME's) and two-factor interactions (2fi's).

In combining blocked factorials to acquire estimability of additional effects, it is clearly pointless to use the same blocked factorial more than once. On the other hand, it is sometimes useful to combine non-identical but isomorphic blocked factorials.

5.1 *Two Factors ($k = 2$)*

A 2^2 factorial can be represented by the vertices of a square; a block is an edge or a diagonal of the square. A 2^{2-1} blocked factorial is either the pair of diagonals of the square, or a pair of parallel edges. It is easy to see by inspection or via a simple counting argument that no single blocked factorial gives estimability of both main effects and the two-factor interaction. Therefore, in order to estimate all three effects, it is necessary to combine two blocked factorials. Using four blocks, the two non-isomorphic options are combining the blocked factorials generated by (Option 1) $(-,-)$ and $(+,-)$ or (Option 2) $(-,+)$ and $(+,-)$, as illustrated in Figure 3 (see also Draper and Guttman (1997)). Option 1 produces a design in which one ME has estimability 2 and the other ME and the 2fi have estimability 1. Option 2 produces a design in which the main effects have estimability 1 and the 2-factor interaction has estimability 2. Option 2 is clearly preferable if the 2fi is of primary interest; otherwise, Option 1 is probably preferable.

5.2 *Three Factors ($k = 3$)*

As illustrated, a 2^3 factorial can be represented by the vertices of a cube. There are $2^3 - 1$ blocked factorials and therefore $\binom{7}{2} = 21$ unions of two different blocked factorials.

One class of blocked factorial is generated by one of $(+,-,-)$, $(-,+,-)$, or $(-,-,+)$. Such a blocked factorial can be represented as a pair of X's on opposing faces of the cube in Figure 1. By inspection, it turns out that any pair of non-identical (although isomorphic) blocked factorials of this type allow estimation of all ME's and 2fi's. With such a design, two 2fi's and one ME have estimability 2 and the other effects have estimability 1.

5.3 *Four Factors ($k = 4$)*

For $k = 4$ binary factors there are 4 ME's and 6 2fi's for a total of ten effects of interest. Since a single blocked factorial allows estimation of 8 factorial effects, one might hope that the union of some pair of blocked factorials would give estimability for all 10 effects of interest. This turns out not to be possible. To achieve estimability of all 10 effects of interest, one must combine three blocked factorials.

A computer search over all 455 triples of blocked factorials reveals 140 triples that allow estimation of all 4 ME's and all 6 2fi's. However, many of these triples are isomorphic. Grouping designs into classes of isomorphic designs, there are twelve such classes.

Table 1 characterizes these twelve classes of designs. Each column in the table represents one class. The entries in the table give the estimability of the corresponding factorial effect for a representative design in the class. Notice that no combination of three blocked factorials can estimate all 15 factorial effects.

The first two designs in Table 1 are noteworthy. Table 2 gives generators of the blocked factorials comprising these designs. Design 1 is the only design in Table 1 for which every main effect has estimability of at least 2. Therefore, when main effects are primarily important this design is a good choice. Design 2 is the only design in Table 1 for which every 2fi has estimability at least 2. When these effects are of primary interest this design is a good choice. Notice, however, that this design is not as good as other designs in Table 1 for estimating main effects.

5.4 *Five, Six, Seven, and Eight Factors* ($5 \leq k \leq 8$)

For $k = 5$, 6, or 7 factors, a comprehensive computational search over all possible designs that are the union of two blocked factorials confirms that no such design gives estimability for all ME's and 2fi's. However, for these numbers of factors there are multiple designs that achieve this goal that are unions of three blocked factorials.

For $k = 8$ factors, a computational search reveals that no union of two or three blocked factorials gives estimability for all ME's and 2fi's. The next section demonstrates that four blocked factorials can be used to achieve this goal.

Table 3 summarizes the results of this section.

6. A General Construction and an Upper Bound

A simple construction yields an upper bound on the number of blocked factorials that are necessary to estimate all ME's and 2fi's for k factors. The construction is easy to illustrate by example, so we first show the construction for $k = 8$ factors. We then explain the construction for general k.

For $k = 8$, one of the blocked factorials comprising the design is generated by $-\mathbf{1} = (-1, -1, -1, -1, -1, -1, -1, -1)$. All other generators $\mathbf{t}$ have $|\mathbf{t}| = 4$ and are pairwise orthogonal. The four runs at expression (2) each generate a blocked factorial that is part of the composite design.

$$\begin{array}{l} (-1,-1,-1,-1,-1,-1,-1,-1) \\ (-1,-1,-1,-1,\ \ 1,\ \ 1,\ \ 1,\ \ 1) \\ (-1,-1,\ \ 1,\ \ 1,-1,-1,\ \ 1,\ \ 1) \\ (-1,\ \ 1,-1,\ \ 1,-1,\ \ 1,-1,\ \ 1) \end{array} \tag{2}$$

Recall (Observation 2) that a ME is estimable in a design if and only if at least one generating run is '-1' for the corresponding factor. A 2fi is estimable in a design if and only if at least one generating run is discordant on the two factors (i.e., $t_i t_j = -1$). In the design generated by the runs at expression (2), the last three generators give estimability for all 2fi's among the eight factors. In fact, the last three generators give estimability for all ME's except for the 8th ME. In this sense, the only purpose of the first generator is to provide estimability of the "last" ME.

This construction generalizes naturally to any number of factors k where k is a power of two. When k is not a power of two, we can simply construct the design for the smallest power of two greater than k and then project the design onto any k factors. This construction therefore proves an upper bound for the number of necessary blocked factorials to estimate all ME's and 2fi's:

$$\{\# \text{ required blocked factorials}\} \leq \lceil \log_2 k \rceil + 1. \tag{3}$$

For $k = 8$, this upper bound is 4 and a computer search confirms that there is no combination of three blocked factorials that allows estimation of all ME's and 2fi's.

This upper bound can be tightened for values of k that are not a power of 2. As explained above, there is a sense in which the only purpose of the generator $-\mathbf{1}$ is to achieve estimability of a single ME. When k is not a power of 2, the generator $-\mathbf{1}$ is no longer necessary if we make sure to use a projection of the other generators that eliminates the single non-estimable ME. This proves the tighter upper bound:

$$\{\# \text{ required blocked factorials}\} \leq \lfloor \log_2 k \rfloor + 1. \tag{4}$$

The results in this paper show this bound is sharp for $k \leq 8$.

Wang (2004) gave a construction of designs for estimating all ME's and 2fi's that requires $k-1$ blocked factorials. $\lfloor \log_2 k \rfloor + 1 \leq k - 1$ for $k > 2$ and our construction gives much smaller designs for large k. For example, for seven factors the construction here requires three blocked factorials instead of six as required by Wang's construction.

7. Design of Two-Color Microarray Experiments

Here we consider the application of our results to the problem of the design of two-color microarray experiments, described in the Introduction. Early papers on experimental design for microarrays include Kerr and Churchill (2001a) and (2001b), but these do not consider multifactorial experiments. Yang and Speed (2002) and Glonek and Solomon (2004) specifically consider studies of 2×2 factorials with two-color microarrays. However, these papers take interest in a different set of contrasts than the classical factorial effects considered in this paper. We return to this issue shortly.

There is great importance in finding economical designs for microarray studies. The high cost of the "blocks" (the arrays), is a primary limitation for many scientists conducting these experiments. Note also that the common assumption that higher-order interactions are negligible is not generally reasonable for microarray experiments due to the complexity of biology.

7.1 *Dye balance*

In assigning the runs in a microarray experiment to blocks, there is the additional issue of dye assignment. More specifically, the block of size two – the microarray – has two different channels, "red" and "green." That is, one red-labeled RNA and one green-labeled RNA are assayed on an array. In any experiment one prefers the effects of interest to be orthogonal to ancillary sources of variation. As noted by Kerr and Churchill (2001a), one prefers a microarray experiment to be balanced such that the effects of interest are orthogonal to dye effects.

One way to achieve dye-balance is to replicate the design, swapping the dye-orientation in the replicate (dye-swap). While this strategy guarantees dye-balance, it is very expensive because it doubles the number of arrays. It would be preferable to identify a suitable dye assignment without adding to the cost of the experiment.

The proposed methodology is as follows. For each blocked factorial, choose a single factorial effect that is not confounded with blocks and confound this factorial effect with the dye effect. This strategy is effective because any factorial effect that is estimable in a blocked factorial is orthogonal to the other estimable factorial effects and to the blocks. Therefore, confounding one estimable factorial effect with the dye effect ensures that the dye effect is orthogonal to the remaining estimable factorial effects.

We illustrate this method on our early example of a blocked factorial for $k = 3$ factors. This design has generator $(-,-,-)$. As discussed with the example for 3 factors in blocks of size 2, the design can be pictured as the four diagonals of the 3-dimensional cube (Figure 1). The factorial effects confounded with blocks are *ab*, *ac*, and *bc*. The main effects and the three-factor interaction *abc* are orthogonal to block effects and to each other. We choose to confound *abc* with the dye-effect, which produces the design illustrated in Figure 2. With this dye assignment, the estimability of the main effects is not affected.

7.2 *Example*

This example is a fictionalized version of an actual microarray experiment. An interesting type of mutant mice has increased lifespan compared to the non-mutant, or wild-type, mice. Investigators are interested in studying the effects of the genetic mutation in young and old mice and male and female mice. Mice will be studied from two different founder lines. Therefore, there are four binary factors: MUTATION, AGE, SEX, and FOUNDER. From the results presented here, the investigators must combine three blocked factorials to study all ME's and 2fi's of their factors. Because 2fi's are of particular interest, the investigators choose to use the second design in Table 1, whose generators are given in Table 2. They note from Table 1 that one main effect (D) can be estimated with better precision than the others, and they choose to assign MUTATION to this factor. AGE is then assigned to factor C for best precision in estimating the interaction between AGE and MUTATION. SEX is assigned to factor A and FOUNDER is assigned to factor B. In each blocked factorial, the thee-way interaction between SEX, FOUNDER, and AGE is used to determine the dye-assignment, as described above. Therefore, in the final design every factorial effect is estimable except for this three-factor interaction and the four-factor interaction.

7.3 *Parameterization*

Glonek and Solomon (2004) examine microarray designs in the 2×2 factorial case. The authors use a different definition for the factorial effects than used here or in the general literature on factorial design. Specifically, these authors use "baseline" constraints to define the factorial effects. For example, the main effect of factor A is defined to be the contrast between $(-,-)$ and $(+,-)$, i.e. the contrast between runs differing in 'A' within a single level of the factor B.

In experiments in which there is clearly a "null" state of all the factors, the "baseline" parameterization is clearly more natural. For example, in a toxicological study each binary factor may be the presence or absence of a particular toxin. Scientists may consider the absence of all toxins to be the natural reference group for all comparisons, which leads naturally to the "baseline" parameterization. In contrast, in situations in which at least one experimental factor does not have a natural "null" or "baseline" level, this parameterization is unappealing because one factor level must be arbitrarily singled out. Examples of such factors are sex, genetic strain, and age group (see Rocke (2004)). A statistical disadvantage of the baseline parameterization is that none of the effects are orthogonal (in contrast to the traditional parameterization wherein all factorial effects are orthogonal). Wu and Hamada (2000) describe further disadvantages of the "one-factor-at-a-time" approach to factorial studies. One argument is that results have a more general interpretation if effects are defined in terms of averages over other factor combinations. In any case, Glonek and Solomon (2004) make the incontrovertible point that experimental design problems should be formulated to correspond as closely as possible to the underlying scientific questions of interest, and that one cannot expect any single design to be optimal for answering all formulations of all questions.

7.4 *Reference Designs*

An alternative experimental strategy used in many microarray experiments is the so-called "reference design" (Kerr and Churchill (2001a)). In this design, every sample is compared to a reference sample. Employing this strategy for the 2^k full factorial requires 2^k microarrays (blocks), the same number as a design that is the union of 2 blocked factorials. However, we have seen that for $3 \leq k \leq 8$ at least three blocked factorials are required to estimate all ME's and 2fi's. In contrast, the reference design strategy gives estimability for *all* factorial effects. From this perspective, the reference design strategy is a practical choice when it is crucial to minimize the number of blocks.

However, if more than the minimum number of blocks is affordable, there are severe disadvantages to the reference design in terms of efficiency. For $k = 4$ factors, compare the reference design with a design from Table 1 that is the union of three blocked factorials. In the reference design, every factorial effect is estimated with the same precision. Let σ^2 be the variance of a within-block difference. If data come from a reference design, then the variance of any estimated factorial effect is $\frac{1}{4}\sigma^2$. Next, consider a design that is the union of three blocked factorials. A factorial effect with estimability 1 has variance $\frac{1}{8}\sigma^2$, a factorial effect with estimability 2 has variance $\frac{1}{16}\sigma^2$, and a factorial effect with estimability 3 has variance $\frac{1}{24}\sigma^2$. Suppose an experimentalist chose Design 1 from Table 1 instead of a reference design. This achieves twice the precision of the reference design for three 2fi's, four times the precision of the reference design for 3 ME's and 3 2fi's, and six times the precision of the reference design for 1 ME. These are large gains in precision relative to the additional resources used (50% more blocks).

In summary, the reference design is a good choice if array resources must be kept to an absolute minimum. However, a relatively large gain in efficiency can be achieved by using unions of blocked factorials as described in this paper.

7.5 *Biological replication*

As noted by many authors (Dobbin and Simon (2002), Yang and Speed (2002), Kerr (2003)), it is important to distinguish biological and technical replicates in microarray experiments. Only biological replication can reduce the uncertainty associated with biological variability. As presented, the results in this paper pertain to experiments with a single replicate of any given factorial combination. However, if there are n biological replicates of each factorial combination, then one replicate of each type can be used in multiple implementations of the chosen design.

8. Discussion

This paper considered designs to organize the runs of a full 2^k factorial into blocks of size 2. It is well-established that a unique single blocked factorial gives estimability of all ME's. We sought combinations of blocked factorials to achieve estimability for all ME's and 2fi's.

Table 3 can be considered a compilation of many of the findings of this paper, including both theoretical results and results from computational searches. The results, as given in Table 3, show the upper bound at expression (4) for the number of blocked factorials required to estimate all ME's and 2fi's is sharp for $k \leq 8$. This upper bound is better than the one given by Wang (2004).

The concrete results we have presented consider up to 8 binary factors. The number of blocks required for $k = 8$ factors is 512. (see Table 3). In many applications, this number is already prohibitively large, indicating that there are practical limits on the number of experimental factors that can be considered simultaneously. Table 3 can additionally guide investigators in deciding the number of experimental factors they should consider given the resources at their disposal.

9. Acknowledgment

We thank Chip Lawrence for discussions of microarray design that motivated this research. This work was supported in part by the UW Center for Ecogenetics and Environmental Health, NIEHS P30ES07033; the FHCRC/UW Toxicogenomics Consortium, NIEHS U19ES11387; and the UW NHLBI shared mircroarray facility, NHLBI HL072370.

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	Design											
Effect	1	2	3	4	5	6	7	8	9	10	11	12
a	2	1	1	1	1	1	1	1	1	1	2	2
b	2	1	1	1	1	1	1	2	2	2	2	2
c	2	1	2	2	2	2	2	2	2	2	1	1
d	3	3	1	2	3	2	3	2	3	3	1	3
ab	2	2	2	2	2	2	2	1	1	1	2	2
ac	2	2	1	1	1	1	1	1	1	3	1	1
ad	1	2	2	1	2	3	2	3	2	2	3	1
bc	2	2	1	1	1	3	3	2	2	2	3	3
bd	1	2	2	3	2	1	2	2	1	1	1	1
cd	1	2	3	2	1	2	1	2	1	1	2	2
abc	0	3	0	0	0	2	2	3	3	1	1	1
abd	1	1	3	2	1	2	1	1	2	2	1	1
acd	1	1	2	3	2	1	2	1	2	0	2	2
bcd	1	1	2	1	2	1	0	0	1	1	2	0
abcd	3	0	1	2	3	0	1	1	0	2	0	2

Table 1

Estimability of factorial effects for triples of blocked factorials, $k = 4$. Each design represented in the table is the union of three blocked factorials for block size 2 and has the property that all ME's and 2fi's are estimable. Pairs of designs in the table are not isomorphic. The number in each row indicates the estimability of the corresponding factorial effect for the given design. The table shows that no combination of three blocked factorials for $k = 4$ gives estimability for all factorial effects. Table 2 gives the generators of the blocked factorials comprising Designs 1 and 2.

Design 1				Design 2			
(1,	-1,	-1,	-1)	(1,	1,	-1,	-1)
(-1,	1,	-1,	-1)	(1,	-1,	1,	-1)
(-1,	-1,	1,	-1)	(-1,	1,	1,	-1)

Table 2

The designs in Table 1 are each comprised of three blocked factorials. The generating runs for the first two designs are given here.

Number of factors	k	2	3	4	5	6	7	8
# blocked factorials	$2^k - 1$	3	7	15	31	63	127	255
# pairs of blocked factorials	$\binom{2^k-1}{2}$	3	21	105	465	1953	8001	32385
# triples of blocked factorials	$\binom{2^k-1}{3}$	1	35	455	4495	3971	333375	2731135
Min # blocked factorials to estimate all ME's and 2fi's	m	2	2	3	3	3	3	4
Min # blocks to estimate all ME's and 2fi's	$m2^{k-1}$	4	8	24	48	96	192	512

Table 3

Summary of design requirements for 2^k factorials in blocks of size 2, $2 \leq k \leq 8$. The number of factors is k and $m = m_k$ denotes the minimum number of blocked factorials to estimate all main effects and two-factor interactions.

Figure Captions.

Figure 1. The design space T for $k = 3$ binary factors can be represented as the vertices of the cube. Blocks of size 2 can be represented by lines connecting vertices, as in Figure 2.

Figure 2. Pictorial representation of the design for estimating all main effects with 3 binary factors and a single blocked factorial. The eight runs are paired into blocks represented by the diagonal lines through the cube. For a microarray experiment, the dye assignment depicted in the figure produces a design in which the main effects are orthogonal to block effects and the dye effect, the two-factor interactions are confounded with blocks, and the three-factor interaction is confounded with the dye effect (but orthogonal to blocks).

Figure 3. Pictorial representation of the two designs for estimating all main effects and two-factor interactions for $k = 2$ factors. Option 1 favors estimability of the main effect for factor B. This can be seen because every hybridization is "across" factor B. Option 2 favors estimability of the two-factor interaction.