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FULL-LENGTH RESEARCH ARTICLE

Row–Column Designs for 2^n factorial 2-Colour Microarray Experiments for Estimation of Main Effects and Two-Factor Interactions with Orthogonal Parameterization

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Abstract A method of construction of row–column designs for estimation of main effects and two factor interaction effects in 2^n factorial microarray experiments based on orthogonal parameterization has been developed in minimum number of replications. A catalogue of designs for $2 \leq n \leq 9$ has been prepared. The catalogue also gives the main effects and two-factor interactions confounded in different replications and the factorial effects that are not confounded in a replication. The efficiency factor of estimable main effects and two-factor interactions has been given. For each 2^n factorial, two designs have been given, one in which main effects are estimated with more efficiency and another in which two-factor interactions are estimated with more efficiency. A procedure of construction of row–column designs for estimation of all factorial effects with odd number of factors has been given. Row–column designs with unequal replication of different treatment combinations have also been obtained for estimation of all main effects and two-factor interactions.

Keywords Confounded row–column design · 2-Colour microarray experiments · Factorial experiments · Orthogonal parameterization · Confounding

Introduction

2-Colour cDNA microarray experiments are used to study the expression levels of thousands of genes simultaneously. In these experiments, there are four basic experimental factor viz., array (A), dye (D), variety (V) {variety may be a treatment or crop variety} and gene (G). These four factors give rise to 15 effects that include 4 main effects, 6 two-factor interactions, 4 three-factor interactions and one four factor interaction. But all the four main effects and selected two-factor interactions viz., array–gene interaction (AG), dye–gene interaction (DG), variety–gene interaction (VG) are the seven effects of primary interest to the experimenter. In most 2-colour microarray experiments, same set of genes is spotted on each array and as a consequence genes/gene specific effects (G, AG, DG, VG) are orthogonal to main effects of the other three factors (A, D, V). Therefore, a design obtained for above three factors and efficient for these main effects, is also efficient for gene specific effects when gene is also considered as one of the factors. In a 2-colour microarray experiment, only two varieties can be accommodated on a single array as these are labelled with two dyes, red and green. Efficient row–column designs for 2-colour microarray experiments have been obtained by taking arrays as columns, dyes as rows (two only) and varieties as treatments. A lot of literature is available on efficient designs for single factor microarray experiments. For details on these a reference may be made to [8], [10], [11] and references cited therein.

In 2-colour microarray experiments, there also arise many experimental situations wherein it is desired to study the effect of more than one factor (different types of tissues, drug treatments or time points of a biological process) simultaneously. Consider an example given by Glonek and

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Solomon [5] wherein it is desired to study and compare the two mutants at times zero hour and 24 hours. The interest is in measuring the changes over time. Therefore, there are two factors viz., varieties (two mutants) and times of exposure. The varieties could be diverse genotypes of crops and times of exposure could be two crop growth stages. Another example was reported by Churchill [3] in which the experimenter is interested to compare gene expression in liver tissues of mice from a gallstone-susceptible strain (pera) and two gallstone-resistant strains (DBA and I) on low-fat and high-fat diets. In this experiment, there are two factors viz., live tissues (three levels) and diets (two levels). Jin et al. [7] studied expression pattern in two strains of Drosophila, using both sexes and two ages. Several hundred flies representing each of eight ($2 \times 2 \times 2$) combinations of these factors were used to create pooled RNA samples. Twenty-four microarrays were used to compare 48 independent labelling reactions, six per pool, obtained from these RNA samples.

For obtaining efficient designs for multi-factor 2-colour microarray experiments, it is required to obtain confounded row–column designs in two rows as only two treatment combinations can be arranged in one array i.e. column. If interest is in the estimation of all factorial effects, then balanced factorial designs may be useful. Gupta [6] gave balanced factorial experiments for 2^2, 2^3 and 3×2 factorial microarray experiments.

In factorial experiments, generally, the lower order effects are of more interest than the higher order interactions. Therefore, we assume that interest is in orthogonal estimation of main effects or orthogonal estimation of main effects and two-factor interactions. In the literature, approaches to obtain efficient block designs for 2^n factorial experiments in blocks of size 2 have been given and reviewed in the sequel.

Yang and Draper [13] gave an approach to obtain block designs for 2^n ($n \leq 5$) factorial experiments with blocks of size 2, which provide orthogonal estimates of main effects and two-factor interactions by searching from all confounding patterns, which is a tedious process. It becomes difficult to find all confounding patterns as the number of factors increases.

Wang [12] studied designing 2^{n-p} fractional factorial plans in blocks of size two and suggested that the number of runs to estimate all the available effects, as is possible in experiments without blocking, is $(n-p)2^{n-\mathrm{p}}$ for 2^{n-p} fractional factorial plans.

Kerr [9] obtained block designs for 2^n factorial experiments in blocks of size 2 for estimation of all main effects and two-factor interactions. The upper bound on minimum number of replications required for orthogonal estimation of all main effects and two-factor interactions is $[\log_2 n] + 1$, here [.] denotes greatest integer function. The upper bound on minimum replications for orthogonal estimation of all main effects and two-factor interactions for 2^n factorial experiments with $n = 2, 3, 4, 5, 6, 7, 8$ are, respectively, 2, 2, 3, 3, 3, 3, 4. Kerr [9] has also given the procedure of obtaining block designs for 2, 3, 4 and 8 factors. For obtaining a design for 5, 6 or 7 factors, it has been suggested that by making a computer-aided search of all possible blocked factorials in 3 replications, solution can be attained that may provide orthogonal estimation of all main effects and two-factor interactions.

The computer-aided search is quite time consuming. Therefore, the question is $\sim$ can we obtain designs that provide orthogonal estimation of all main effects and two-factor interactions as a ready reckoner solution to the users? Further, it is required to develop a procedure of getting row–column designs in two rows from these block designs with block size two and provide a ready reckoner of row–column designs. Kerr [9] suggested finding out a factorial effect that is not confounded with blocks (represented as columns in a row–column set up) and then confound it with rows. Kerr, however, did not provide any list of factorial effects that are not confounded with column effects. Therefore, in "Method of Construction of Confounded Row–Column Designs" section we propose a method of construction of row–column designs with two rows for orthogonal estimation of main effects and two factor interaction effects in 2^n ($2 \leq n \leq 9$) factorial microarray experiments in minimum number of replications. Let the n factors be denoted as $1, 2, 3, \ldots, n$ and the levels by 0, 1. A catalogue of designs for $2 \leq n \leq 9$ has been prepared along with main effects and two-factor interactions confounded in different replications and some of the factorial effects that are not confounded in a replication and given in Table 3. For each 2^n factorial, two designs have been given one in which main effects are estimated with more efficiency and another in which two-factor interactions are estimated with more efficiency. The efficiency factor of estimable main effects and two-factor interactions has also been given in Table 4. The procedure of obtaining a block design in blocks of size 2 for 2^n factorial experiments has been given by Box et al. [1] that provides orthogonal estimation of all main effects. In the present investigation, we extend this to obtain row–column designs in two rows that provide orthogonal estimation of all main effects and present in "Method of Construction of Confounded Row–Column Designs" section. In fact, we shall show that the design obtained through proposed approach provides orthogonal estimation of all odd order factorial effects.

In some experimental situations, due to cost or time considerations, it may not be possible to run a design even in the minimum number of replications required for estimation of all main effects and all two-factor interactions. Therefore, it is desired to develop a procedure of obtaining row–column designs for estimation of all main effects and two-factor interactions for the situations having unequal replication of different treatment combinations in smaller number of columns than the number of columns required for minimum number of replications for orthogonal estimation of all main effects and two-factor interactions. An approach to obtain designs for such situations is given in "Row–Column Designs with Unequal Replications" section.

Method of Construction of Confounded Row–Column Designs

In this section, we propose a method of construction of row–column designs with two rows for orthogonal estimation of main effects and two factor interaction effects in 2^n $(2 \leq n \leq 9)$ factorial microarray experiments in minimum number of replications. An approach to obtain row–column designs in two rows that provide orthogonal estimation of all odd order factorial effects is also given in this section.

Method 1

We shall describe a procedure of obtaining confounded row–column designs in two rows for estimation of all main effects and two-factor interactions.

Step 1: First we obtain a block design with block size 2. A block design of block size 2 for a 2^n factorial experiment is represented as $(2^n, 2)$. For obtaining this design, 2^n treatment combinations are arranged in blocks of size 2. Here, total number of treatment combinations $= 2^n$, number of blocks of size two per replication $= 2^{n-1}$, total number of factorial effects confounded $= 2^{n-1} - 1$, number of independent factorial effects confounded $= n - 1$, number of generalized factorial effects confounded $= (2^{n-1} - 1) - (n - 1)$. Write all possible 2^{n-1} different combinations of $n - 1$ independent factorial effects to be confounded in a given replication, as described in the sequel.

These 2^{n-1} different combinations of $n - 1$ independent factorial effects may have all main effects, all two-factor interactions or some main effects and some two-factor interactions confounded. Depending upon the number of main effects and two-factor interactions in $n - 1$ independent factorial effects to be confounded gives rise to n different blocking types. Different blocking types are given in Table 1. In this table S represents the main effect and D represents two-factor interaction.

Step 2: For obtaining a design in r replications, select r blocking types in $\binom{2^n - 1}{r}$ different ways. Out of these $\binom{2^n - 1}{r}$ different blocking types, select those combinations that estimate the desired factorial effects (all main effects and two-factor interactions) in maximum number of the r replications. Let s be the number of blocking arrangements or replications in which a given factorial effect is not confounded. Then the efficiency factor of a factorial effect is s/r.

Following the result of [9], the minimum number of replications (r) required for estimation of all main effects and two factor interaction in two-level factorials with $n = 2, 3, 4, 5, 6, 7, 8, 9$ are, respectively, 2, 2, 3, 3, 3, 3, 4, 4. One can search all possible $\binom{2^n - 1}{r}$ combinations of

Table 1 Blocking types for obtaining block design of block size 2 for a 2^n factorial experiment

Blocking type	Independent factorial effects to be confounded	Number of arrangements	Remarks
1	S SSS… S {$(n - 1)$ times}	$\binom{n}{n-1} = n$	All possible cases of $n - 1$ main effects to be confounded out of n main effects in a single replication.
2	S SSS… S {$(n - 2)$ times} D	$\binom{n}{n-2} = \frac{n(n-1)}{2}$	All possible cases of selecting $n - 2$ main effects out of all n main effects in a single replication and combining them with a two factor interaction involving remaining 2 factors
⋮	⋮	⋮	⋮
n-1	S D DD … D ($(n - 2)$ times)	$\binom{n}{1} = n$	All possible cases of selecting one main effect out of all n main effects in a single replication and combining them with $n - 2$ two-factor interactions of remaining $n - 1$ main effects obtained in such a way the factor numbered at last among the factors not appearing the main effect S is a part of all two-factor interactions with $n - 2$ other factors
n	D DD D…D ($(n - 1)$ times)	$\binom{n-1}{n-1} = 1$	All two-factor interactions having fixed factor with each of $n - 1$ other factors

The total number of blocking arrangements is $2^n - 1$

blocking arrangements and select the combination which gives the design for orthogonal estimation of all main effects and two-factor interactions. When the number of combinations becomes large, then it becomes tedious, computer intensive and time prohibitive to search a design. Therefore, we have attempted to reduce the search for estimation of all main effects and two-factor interactions for two-level factorials with $n = 2, 3, 4$ and 5. For $n = 2$, 3, 4, one can obtain a design by taking any 2 combinations of blocking arrangements of the type SD, SDD and SDDD, respectively, and for $n = 5$, by taking any 3 combinations of blocking arrangements of the type SDDDD. This yields $\binom{n}{2}$ {for $n = 2, 3, 4$} and $\binom{n}{3}$ {for $n = 5$} block design in blocks of size 2 which are all isomorphic solutions and one can be obtained from another by just renumbering of factors.

For $n \geq 6$, it is not possible to obtain a block design with block size 2 for orthogonal estimation of all main effects and two-factor interactions in minimum number of replications by only taking r combinations of the blocking arrangement of the type SDDDD…D {$(n - 2)$ times}. Therefore, to obtain block design in minimum number of replications one has to search from $\binom{2^n - 1}{r}$ combinations.

We have made a search of these combinations and identified the combination of block arrangements for $2 \leq n \leq 9$ factors that would yield block design in minimum number of replications. These block arrangements are given in Table 3 as design D1. All the designs given as D1 provide orthogonal estimation of all main effects and two-factor interactions. In all these designs main effects are estimated with more precision in comparison to two-factor interactions.

If one is interested in more precision for two-factor interactions in comparison to main effects, then some other combination of blocking arrangements out of $\binom{2^n - 1}{r}$ combinations need to be selected. We also identified the combination of block arrangements for $2 \leq n \leq 9$ factors that would yield block design in minimum number of replications with two-factor interactions being estimated with more precision than the main effects. These are presented as design D2 in Table 3. Therefore, one can choose design D1 or design D2 depending upon the requirement of experimental situation.

Step 3: Once a block design in block size 2 is obtained, the next step is to convert it into a row–column design in such a way that the treatment combinations become most balanced with respect to rows. For achieving this, we make use of Lemma 2.1 of [2]. Consider a symmetrical factorial experiment conducted using a row–column design with row and column sizes less than the number of treatment combinations. Let $D_R[D_C]$, respectively, denote the block designs obtained ignoring column [row] classification and the confounding done in such a way that the factorial effect which is confounded in D_R is unconfounded in D_C and vice versa. Then, the factorial effects which are unconfounded in both D_R and D_C remain unconfounded in row–column design as well. Further, the factorial effects which are confounded separately for D_R and D_C, are also confounded in row–column design.

In view of the above description, we suggest identifying factorial effects (possibly higher order interactions) which are unconfounded in all the replications of block design obtained in Step 1 and 2. Now confound this factorial effect, say a g-factor interaction, with row-component design $(1 \leq g \leq n)$. To achieve this, let the design obtained in Steps 1 and 2 be D_{CU}, the column component design before rearranging into row–column set up. Now arrange the treatment combinations in columns of D_{CU} in each replication in such a way that the treatment combinations in two rows represent the two blocks in which the identified g-factor interaction is confounded. Now rearrange the rows in replication number $s[t]$ of D_{CU}, in such a way that in row 1, the sum of the levels of the factors involved in g-factor interaction is 0 (1) and in row 2 this sum is 1 (0) respectively, where $s = 1, 3, \ldots, r$ (if r is odd) and $s = 1, 3, \ldots, r - 1$ (if r is even) and $t = 2, 4, \ldots r - 1$ (if r is odd) and $t = 2, 4, \ldots r$ (if r is even). Now juxtaposing the columns of replications of D_{CU} obtained after the above rearrangement, we get a row–column design D (v, b, k), where $v = 2^n$, b (number of columns) $= r2^{n-1}$ and k (number of rows) $= 2$. In the row−column design so obtained, the factorial effect confounded with rows of each replication of D_{CU} also becomes unconfounded. If r is even, the factorial effect confounded with rows in each replication of D_{CU} can be estimated free from row-effects in D and if r is odd, the factorial effect confounded with rows in each replication of D_{CU} can be estimated after adjustment of row-effects in D. The factorial effects that can be confounded with rows are given in column 6 in Table 3. Using Step 3, it is important to note that the factorial effects confounded with the rows in different replications are estimable in the whole row–column design.

Example A row–column design in two rows for a 2^4 factorial experiment for a two-colour microarray experiments is obtained as given in the sequel. All possible 15 blocking arrangements for obtaining a block design for 2^4 factorial experiments in blocks of size 2 are given in Table 2.

Following step 2, we obtain a block design in block size 2 using any three combinations of the blocking

Table 2 All 15 possible blocking arrangements for 2^4 factorial experiment

Blocking type	Arrangement number	Independent factorial effects to be confounded			Generalized factorial effects confounded			
1	A1	1	2	3	12	13	23	123
	A2	1	2	4	12	14	24	124
	A3	1	3	4	13	14	34	134
	A4	2	3	4	23	24	34	234
2	A5	1	2	34	12	134	234	1234
	A6	1	3	24	13	124	234	1234
	A7	1	4	23	14	123	234	1234
	A8	2	3	14	23	124	134	1234
	A9	2	4	13	24	123	134	1234
	A10	3	4	12	34	123	124	1234
3	A11	1	24	34	124	134	23	123
	A12	2	14	34	124	234	13	123
	A13	3	14	24	134	234	12	123
	A14	4	13	23	134	234	12	124
4	A15	14	24	34	12	13	23	1234

Here 1, 2, 3, 4 represent the factor number

arrangement numbers A11, A12, A13 and A14. One can obtain four block designs in 3 replications each by confounding the factorial effects as per blocking arrangement numbers (A11, A12, A13), (A11, A12, A14), (A11, A13, A14) and (A12, A13, A14), respectively. All these designs would be isomorphic and one can be obtained from another by renumbering the factors. Therefore, without loss of generality, we present the design obtainable from blocking arrangement numbers (A11, A12, A13). The block design is given as

Replication 1								Replication 2							
Blocks→								Blocks→							
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0000	0010	0011	0001	1000	1010	1011	1001	0000	0100	0101	0001	0010	0110	0111	0011
0111	0101	0100	0110	1111	1101	1100	1110	1101	1001	1000	1100	1111	1011	1010	1110

Replication 3							
Blocks→							
17	18	19	20	21	22	23	24
0000	0010	0011	0001	0100	0110	0111	0101
1011	1001	1000	1010	1111	1101	1100	1110

In this design the factorial effect 1234 (4 factor interaction is unconfounded with block effects). Therefore, using step 3, confound 4 factor interaction with rows for obtaining a row–column design. The row–column design obtained after confounding 1234 in each replication of block design and rearranging the column contents in two rows of each replication of the block design is interactions $1n$, $2n$, $3n$, … $(n-1)n$. One can easily see that interaction involving all the n factors is unconfounded with block effects if n is odd and $n-1$ factor interaction involving first $(n-1)$ factors if n is even. Now confound the highest order interaction (when n is odd) and $n-1$ factor interaction when n is even. Now using the rearrangement in step 3 of Method 1, one can get a row–column design in two rows for orthogonal estimation of all main effects.

Replication 1									Replication 2							
Columns→									Column→							
Rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0000	0101	0011	0110	1111	1010	1100	1001	1101	0100	1000	0001	0010	1011	0111	1110
2	0111	0010	0100	0001	1000	1101	1011	1110‘	0000	1001	0101	1100	1111	0110	1010	0011

Replication 3								
Columns→								
Rows	17	18	19	20	21	22	23	24
1	0000	1001	0011	1010	1111	0110	1100	0101
2	1011	0010	1000	0001	0100	1101	0111	1110

In the above design, main effects 1, 2, 3, 4 and two-factor interactions 12, 13, 14, 23, 24, 34 are uncofounded in 2, 2, 2, 3, 2, 2, 1, 2, 1, 1 replications. The above is design D1 in Table 3. The design D2 in Table 3 can also be obtained similarly. In design D2, main effects 1, 2, 3, 4 and two-factor interactions 12, 13, 14, 23, 24, 34 are unconfounded in 1, 1, 1, 3, 2, 2, 2, 2, 2, 2 replications. It is clear that in design D1, the main effects are estimated with more precision than two-factor interactions, and, in design D2, two-factor interactions are estimated with more precision than main effects. The efficiency factors are given in Table 4.

In many experimental situations, due to scarcity of experimental resources, it is required that the experiment be conducted in a single replication and the experimenter is interested in orthogonal estimation of all main effects. A procedure of generating a block design in block size 2 for 2-level factorials for orthogonal estimation of main effects was given by [1], [4], [9]. We propose a simplification and extension of their method to obtain row–column designs in two rows for orthogonal estimation of all main effects in Method 2.

Method 2

Following the procedure given in Step 1 and Step 2 of Method 1, generate a block design in block size 2 in a single replication using the blocking arrangement DDDDD…{$(n-1)$ times}, i.e. by confounding two-factor

It can easily be seen that all odd order factorial effects are orthogonally estimable in this design. For estimation of all odd order interactions, the design would be a saturated design. Therefore, experimental error can be estimated by assuming the higher order odd order interactions as negligible.

Remark 1

A design for estimation of all main effects and two-factor interactions in $n-1$ replications can always be obtained by taking combination of any $n-1$ blocking arrangements of the type SDDD…D{$(n-2)$ times}.

Row–Column Designs with Unequal Replications

In "Method of Construction of Confounded Row–Column Designs" section, we have given a method of construction of row–column designs in 2-rows for orthogonal estimation of all main effects and two-factor interactions in minimum number of replications as given by [9]. In some experimental situations, due to cost or time considerations, it may not be possible to run the design even in the minimum number of replications required for estimation of all main effects and all two-factor interactions. Therefore, we have

Table 3 Factorial Effects to be confounded in different replications to get a partial confounded row–column design for 2^n factorial ($2 \leq n \leq 9$) for orthogonal estimation of main effects and two-factor interactions

Number of factors (1)	Design (2)	Replication number (3)	Independent factorial effects confounded with columns (as blocks) (4)	Generalized factorial effects (only main effects and two factor interaction) cofounded (5)	Factorial Effects confounded with rows for obtaining row–column design (6)
2	D1	R1	1		2
		R2	12		2
	D2	R1	1		12
		R2	2		12
3	D1	R1	1, 23	123	12
		R2	2, 13	123	12
	D2	R1	1, 23	123	13
		R2	3, 12	123	13
4	D1	R1	1, 24, 34	23	1234
		R2	2, 14, 34	13	1234
		R3	3, 14, 24	12	1234
	D2	R1	1, 2, 34	12	123
		R2	1, 3, 24	13	123
		R3	2, 3, 14	23	123
5	D1	R1	1, 5, 24, 34	15, 23	12345
		R2	1, 3, 25, 45	13, 24	12345
		R3	2, 5, 14, 34	25, 13	12345
	D2	R1	1, 2, 35, 45	12, 34	12345
		R2	1, 3, 25, 45	13, 24	12345
		R3	2, 4, 15, 35	24, 13	12345
6	D1	R1	1, 4, 26, 36, 56	14, 23, 25, 35	12456
		R2	3, 4, 5, 16, 26	34, 35, 12	12456
		R3	5, 6, 14, 24, 34	56, 12, 13, 23	12456
	D2	R1	4, 5, 6, 13, 23	45, 46, 56, 12	123456
		R2	2, 3, 6, 15, 45	23, 26, 36, 14	123456
		R3	1, 3, 4, 26, 56	13, 14, 34, 25	123456
7	D1	R1	5, 6, 7, 14, 24, 34	12, 17, 27, 34, 35, 45	123467
		R2	1, 3, 5, 27, 47, 67	35, 57, 37, 12, 14, 24	123467
		R3	2, 3, 7, 16, 46, 56	23, 27, 37, 14, 15, 45	123467
	D2	R1	1, 2, 7, 36, 46, 56	12, 17, 27, 34, 35, 45	123567
		R2	3, 5, 7, 16, 26, 46	35, 37, 57, 12, 14, 24	123567
		R3	2, 4, 5, 17, 37, 67	24, 25, 45, 13, 16, 36	123567
8	D1	R1	18, 28, 38, 48, 58, 68, 78	12, 13, 14, 15, 16, 17, 23, 24, 25, 26, 27, 34, 35, 36, 37, 45, 46, 47, 56, 57, 67	15678
		R2	5, 6, 7, 8, 14, 24, 34	56, 57, 58, 67, 68, 78, 12, 13, 23	15678
		R3	3, 4, 7, 8, 16, 26, 56	34, 37, 38, 47, 48, 78, 12, 15, 25	15678
		R4	2, 4, 6, 8, 17, 37, 57	24, 26, 28, 46, 48, 68, 13, 15, 35	15678
	D2	R1	5, 6, 7, 8, 14, 24, 34	56, 57, 58, 67, 68, 78, 12, 13, 23	123678
		R2	3, 4, 7, 8, 16, 26, 56	34, 37, 38, 47, 48, 78, 12, 15, 25	123678
		R3	2, 4, 6, 8, 17, 37, 57	24, 26, 28, 46, 48, 68, 13, 15, 35	123678
		R4	1, 28, 38, 48, 58, 68, 78	23, 24, 25, 26, 27, 34, 35, 36, 37, 45, 46, 47, 56, 57, 67	123678

Table 3 continued

Number of factors (1)	Design (2)	Replication number (3)	Independent factorial effects confounded with columns (as blocks) (4)	Generalized factorial effects (only main effects and two factor interaction) cofounded (5)	Factorial Effects confounded with rows for obtaining row–column design (6)
9	D1	R1	1, 2, 3, 9, 48, 58, 68, 78	12, 13, 19, 23, 29, 39, 45, 46, 47, 56, 57, 67	236789
		R2	2, 4, 6, 8, 19, 39, 59, 79	24, 26, 28, 46, 48, 68, 13, 15, 17, 35, 37, 57	236789
		R3	3, 5, 6, 7, 19, 29, 49, 89	35, 36, 37, 56, 57, 67, 12, 14, 18, 24, 28, 48	236789
		R4	1, 5, 6, 8, 9, 27, 37, 47,	15, 16, 18, 19, 56, 58, 59, 68, 69, 89, 23, 24, 34	236789
	D2	R1	5, 6, 7, 8, 9, 14, 24, 34	56, 57, 58, 59, 67, 68, 69, 78, 79, 89, 12, 13, 23	13479
		R2	1, 2, 5, 6, 9, 38, 48, 78	12, 15, 16, 19, 25, 26, 29, 56, 59, 69, 34, 37, 47	13479
		R3	2, 4, 6, 8, 19,39, 59, 79	24, 26, 28, 46, 48, 68, 13, 15, 17, 35, 37, 57	13479
		R4	3, 4, 5, 8, 19, 29, 69, 79	34, 35, 38, 45, 48, 58, 12, 16, 17, 26, 27, 67	13479

Table 4 Efficiency factor of estimable main effects and 2-factor interactions in partial confounded row–column design for 2^n factorial ($2 \leq n \leq 9$)

Factor	r	**1**	**2**	**3**	**4**	**5**	**12**	**13**	**23**	**14**	**24**	**34**	**15**	**25**	**35**	**45**
2^2	2	0.50	1.00				0.50									
	2	0.50	0.50				1.00									
2^3	2	0.50	0.50	1.00			1.00	0.50	0.50							
	2	0.50	1.00	0.50			0.50	1.00	0.50							
2^4	3	0.67	0.67	0.67	1.00		0.67	0.67	0.67	0.33	0.33	0.33				
	3	0.33	0.33	0.33	1.00		0.67	0.67	0.67	0.67	0.67	0.67				
2^5	3	0.33	0.67	0.67	1.00	0.33	0.67	0.33	0.33	0.33	0.33	0.33	0.67	0.67	0.33	0.33
	3	0.33	0.33	0.67	0.67	1.00	0.67	0.33	1.00	1.00	0.67	0.67	0.33	0.67	0.33	0.33
2^6	3	**1**	**2**	**3**	**4**	**5**	**6**	**12**	**13**	**23**	**14**	**24**	**34**			
		0.67	1.00	0.67	0.33	0.67	0.33	0.33	0.67	0.33	0.33	0.67	0.33			
		0.67	0.67	0.33	0.33	0.67	0.33	0.67	0.67	0.33	0.33	1.00	0.67			
		15	**25**	**35**	**45**	**16**	**26**	**36**	**46**	**56**						
		1.00	0.67	0.33	0.67	0.67	0.33	0.67	1.00	0.33						
		0.67	0.67	1.00	0.33	1.00	0.33	0.67	0.67	0.33						
2^7	3	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**12**	**13**	**23**	**14**	**24**	**34**		
		0.67	0.67	0.33	1.00	0.33	0.67	0.33	0.67	0.33	0.33	0.33	0.33	0.67		
		0.67	0.33	0.67	0.67	0.33	1.00	0.33	0.33	0.67	1.00	0.67	0.33	0.67		
		15	**25**	**35**	**45**	**16**	**26**	**36**	**46**	**56**	**17**	**27**	**37**	**47**	**57**	**67**
		0.33	1.00	0.67	0.67	0.67	0.67	1.00	0.33	0.33	1.00	0.33	0.67	0.67	0.67	0.33
		1.00	0.67	0.33	0.33	0.33	0.67	0.33	0.33	0.67	0.33	0.67	0.33	1.00	0.67	0.67

Table 4 continued

Factor	r	**1**	**2**	**3**	**4**	**5**	**12**	**13**	**23**	**14**	**24**	**34**	**15**	**25**	**35**	**45**
2^8	4	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**12**	**13**	**23**	**14**	**24**	**34**	
		1.00	0.75	0.75	0.50	0.75	0.50	0.50	0.25	0.25	0.25	0.50	0.50	0.25	0.75	
		0.75	0.75	0.75	0.50	0.75	0.50	0.50	0.25	0.50	0.50	0.50	0.75	0.25	0.25	
		15	**25**	**35**	**45**	**16**	**26**	**36**	**46**	**56**	**17**	**27**	**37**	**47**	**57**	**67**
		0.25	0.50	0.50	0.75	0.50	0.25	0.75	0.50	0.25	0.50	0.75	0.75	0.50	0.25	0.50
		0.50	0.50	0.50	0.75	0.75	0.50	0.75	0.50	0.25	0.75	0.75	0.25	0.75	0.25	0.50
		18	**28**	**38**	**48**	**58**	**68**	**78**	**19**	**29**	**39**	**49**	**59**	**69**	**79**	**89**
		0.75	0.50	0.50	0.25	0.50	0.25	0.25								
		1.00	0.50	0.50	0.25	0.50	0.50	0.25								
2^9	4	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**12**	**13**	**23**	**14**	**24**	**34**
		0.75	0.50	0.50	0.75	0.50	0.25	0.50	0.50	0.75	0.25	0.25	0.50	0.50	0.25	0.25
		0.75	0.50	0.50	0.50	0.50	0.25	0.75	0.25	0.50	0.50	0.50	0.75	0.75	0.50	0.25
		15	**25**	**35**	**45**	**16**	**26**	**36**	**46**	**56**	**17**	**27**	**37**	**47**	**57**	**67**
		0.75	1.00	0.50	0.25	1.00	0.75	0.75	0.50	0.25	0.75	1.00	0.50	0.75	0.25	0.25
		0.50	0.75	0.50	0.75	0.50	0.25	1.00	0.75	0.50	0.50	0.75	0.50	0.75	0.50	0.50
		18	**28**	**38**	**48**	**58**	**68**	**78**	**19**	**29**	**39**	**49**	**59**	**69**	**79**	**89**
		0.75	0.50	1.00	0.25	0.50	0.25	0.50	0.25	0.50	0.50	0.75	0.50	0.75	0.50	0.50
		1.00	0.75	0.50	0.25	0.50	0.50	0.50	0.25	0.50	0.75	1.00	0.25	0.25	0.25	0.75

proposed a procedure of obtaining row–column designs for estimation of all main effects and two-factor interactions for the situations having unequal replication of different treatment combinations in smaller number of columns or arrays than the number of columns or arrays required for minimum number of replications for orthogonal estimation of all main effects and two-factor interactions in the sequel.

Consider an experimental situation in which 4 factors each at two levels are to be studied in a 2-colour micro-array experiment. The experimenter is interested in studying all main effects and two-factor interactions. For orthogonal estimation of all main effects and two-factor interactions in blocks of size 2 or a row–column design in two rows, the minimum number of replications required is three and number of arrays (columns) required is 24. As per availability of resources, at most 20 arrays (columns) can be used for the experiment. For this experimental situation, a row–column design in two rows can be obtained as given in the sequel.

Step 1 Find out a combination of 2 blocking arrangements for a 2^4 factorial experiment for generating a block design with block size 2 in two replications such that 3 main effects and all 6 two-factor interactions are estimable. One such arrangement is

Blocking arrangement 1: 1, 2, 34
Blocking arrangement 2: 1, 3, 24

Using this blocking arrangement, the block design in two replications is

Replication 1								Replication 2							
Blocks→								Blocks→							
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
0000	0001	0100	0101	1000	1001	1100	1101	0000	0001	0010	0011	1000	1001	1010	1011
0011	0010	0111	0110	1011	1010	1111	1110	0101	0100	0111	0110	1101	1100	1111	1110

Step 2 Identify the factorial effect that is unconfounded in both the replications. In this example 123 is the factorial effect which is unconfounded in both the replications. Confound this factorial effect and obtain rows in each of the two replications above as per procedure given in "Method of Construction of Confounded Row–Column Designs" section, Method 1. The row–column design in two rows obtained is

Columns→									Column→							
Rows	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	0000	0001	0111	0110	1011	1010	1100	1101	0101	0100	0010	0011	1000	1001	1111	1110
2	0011	0010	0100	0101	1000	1001	1111	1110	0000	0001	0111	0110	1101	1100	1010	1011

In the above design, main effect of factor 1 is confounded in column effects, so it is not estimable. We can go up to 20 arrays, and therefore, we have the possibility of adding four arrays. The arrays/columns added should be distinct from the above 16 columns. To have the balance with respect to rows, we have to swap the pairs of treatment combination in a column. Therefore, we can add two new arrays/columns. Now the question is which two new columns/arrays to be added? Generate a block design in block size 2, using blocking arrangement of the type DDD. Select two blocks of this design which contain the treatment combinations where keeping the level of factor 1 constant, there is a change in levels of maximum number of factors.

We may select any of the two blocks from the following 4 set of blocks.

$$\begin{aligned}&\text{Set 1} : \{0000, 1111\}, \{0111, 1000\};\\&\text{Set 2} : \{0001, 1110\}, \{0110, 1001\};\\&\text{Set 3} : \{0010, 1101\}, \{0101, 1010\};\\&\text{Set 4} : \{0011, 1100\}, \{0100, 1011\};\end{aligned}$$

Now using Set 1, and swapping the pairs of treatment combinations, in blocks, we get four new columns as

Rows	1	2	3	4
1	0000	1111	0111	1000
2	1111	0000	1000	0111

Renumber 16 treatment combinations written in lexicographic order 0000, 0001, 0010, 0011, 0100, 0101, 0110, 0111, 1000, 1001, 1010, 1011, 1100, 1101, 1110, 1111 as 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15,16, respectively. Using the coefficient matrix of reduced normal equations of the above row–column design under a fixed effects model, say C, it can easily be seen that all main effects and two-factor interactions are orthogonally estimable with variances of estimated main effects (1, 2, 3, 4) and estimated two-factor interactions 12, 13, 14, 23, 24, 34 as 4.5, 1, 1, 0.5, 1, 1, 0.5, 0.5, 1, 1 assuming error variance as one.

Remark 2

The designs obtained in Step 1 have a limitation that the main effect of one factor is non-estimable. However, it is always possible to select such a factor as the factor of least interest. It may be noted that the new columns added would contain those treatment combinations for which keeping the level of factor, main effect of which is not estimable in the original design, as constant there is a change in levels of maximum number of factors. If n is even and change is only with respect to levels of odd number of factors (involving any of less than $n-1$ factors), the main effect can be estimated but is non-orthogonal with respect to some of other main effects. If n is even and the change is only with respect to levels of even number of factors, estimation of desired main effects is not possible.

Discussion

In the present investigation, the designs obtained and catalogued are for 2-colour microarray experiments. This, however, is an application of row–column designs in two rows for 2^n factorial microarray experiments. The designs obtained and catalogued have a wider scope of applications in all those experimental situations in which a confounded row–column design in two rows is required. Further, it would be of importance to develop a software module for generation of row–column designs in two rows. The whole discussion revolves around two-level factorial experiments. The methods of construction/computer algorithms for generation of confounded row–column designs in two rows for asymmetrical factorial experiments for estimation of all main effects and two factor interaction also needs attention.

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References

1. Box GEP, Hunter WG, Hunter JS (1978) Statistics for experimenters: an introduction in design, data analysis and model building. Wiley, New York
2. Choi KC, Gupta S (2008) Confounded row-column designs. J Stat Planning Inf 138:196–202
3. Churchill GA (2002) Fundamentals of experimental design for cDNA microarrays. Nat Genet (Suppl.) 3:490–495
4. Draper NR, Guttman I (1997) Two-level factorial and fractional factorial designs in blocks of size two. J Qual Technol 29:71–75

5. Glonek GFV, Solomon PJ (2004) Factorial and time course designs for cDNA microarray experiments. Biostatistics 5:89–111
6. Gupta S (2006) Balance factorial design for cDNA microarrray experiments. Commun Stat Theory Methods 35:1469–1476
7. Jin W, Riley RM, Wolfinger RD, White KP, Gurgel GP, Gibson G (2001) The contributions of sex, genotype and age to transcriptional variance in Drosophila melanogaster. Nat Genet 29:389–395
8. Kerr MK (2003) Design considerations for efficient and effective microarray studies. Biometrics 59:822–828
9. Kerr KF (2006) Efficient 2^k factorial designs for blocks of size two with microarray applications. J Qual Technol 38(4):309–318
10. Nguyen NK, Williams ER (2006) Experimental designs for 2-colour cDNA microarray experiments. Appl Stoch Models Bus Ind 22:631–638
11. Sarkar A, Parsad R, Gupta VK, Chatterjee K, Rathore A (2010) Efficient row-column designs for microarray experiments. J Indian Soc Agric Stat 64(1):89–117
12. Wang PC (2004) Designing two-level fractional factorial experiments in blocks of size two. Sankhya 66:327–342
13. Yang YJ, Draper NR (2003) Two-level factorial and fractional factorial designs in blocks of size two. J Qual Technol 35(3):294–305