**Objective function section**

The objective function plays an important role in finding the optimal design using simulated annealing algorithm. A sensible objective function will allow us to find a design that able to conduct a valid test for the treatment differences.

The main differences of finding the optimal two-phase experiment design to general optima design is that we need to allocate the random factors from the first phase experiment to the random factors from the second phase experiment.

This section discussed three issues as follows

1. Compare MS and A-optimal designs based on the animal effects

2. Add the treatment effects into the A-optimal using the weighted sum and how much weight

3. Add another component which monitors the degrees of freedom associated with the treatment effects.

The construction of the objective function that is applied here is shown, based on these three points, for finding the optimal two-phase designs.

**Explain MS and A-optimality criteria**

Finding an MS-optimal design is a two-stage process. The first stage is to find the design with the highest trace of the information matrix. The second stage is to find the design with the lowest trace of the square of the information matrix out of the designs that were found in the first stage. This method is fast and easier to implement, because this method does not require computing of the eigienvalues. The objection function of finding the MS-optimal design is by adding the trace of the information matrix to the inverse of the trace of the square of information matrix.

The A-optimal design is the design with the highest average efficiency factor. The objection function of finding the A-optimal design is first compute the canonical efficiency factors from the eigenvalues divided by the replication number. The average efficiency factor is then calculated by the harmonic mean of the canonical efficiency factors.

**The information matrix**

It is important to define the information matrix, because it is where the optimality criteria are computed. This is also the initial step of the constructing the objective function.

For the case of iTRAQ experiments, the aim of the animals and treatments allocations to runs and tags is to minimise the level of confounding between animal and treatment effects with both runs and tag effects. Instead, we can find the design that has the most animal and treatment information within the runs and tags stratum. Hence, the second phase design can be consider as the row-column design. The orthogonal projector for the within runs and tags stratum can be written as

where *nr* and *nt* denotes the total number of runs and tags are used in the second phase experiment. The information matrix of animal within runs and tags stratum can be written as

where denotes the animal design matrix.

The information matrix of the treatments in the within runs and tags stratum can be written as

where denotes the treatment design matrix.

The aim is to find matrices and X that generate the MS or A-optimal design.

**1. Compare MS and A-optimal designs based on the animal effects**

The first comparison to constructing the objective is to decide whether the MS or A-optimal criterion is used for the assignments of animals of Phase 1 experiment to the runs and tags of Phase 2 experiment. For the purpose of comparing these two criteria, only the maximisation of the animal information is considered for this part.

The MS and A-optimal designs are compared using an experiment with following design parameters:

Phase 1 experiment - 2 treatments, 3 biological replicates,

Phase 2 experiment – 2 technical replicates, 3 runs and 4 tags.

Animals A, C and E are assigned to Treatment 1 and animals B, D and F are assigned to Treatment 2. Since there are 2 technical replicates, which mean there are total of 12 samples to be measured in the second phase experiment. Using the 4-plex experiment, three runs are required.

The theoretical ANOVA for this first phase experiment is as follows,

$ANOVA

DF Ani

Between Ani

Trt 1 1

Residual 4 1

$EF

Trt eff.Trt

Between Ani

Trt 3 1

All the treatment information is in the between animals stratum.

Using MS-optimality criterion in the objective function, the animal allocation of this design is shown as follows

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | E | F | C | D |
| 2 | B | C | A | E |
| 3 | A | D | F | B |

The animals is confounded with both runs and tags. The incidence matrix of animals and runs is as follow,

Run

Ani 1 2 3

A 0 1 1

B 0 1 1

C 1 1 0

D 1 0 1

E 1 1 0

F 1 0 1

which shows that this design is a binary design, where no treatments, in this case animals, occurs more than once in any block, in this case run. The concurrence matrix of animals and runs is

Ani

Ani A B C D E F

A 2 2 1 1 1 1

B 2 2 1 1 1 1

C 1 1 2 1 2 1

D 1 1 1 2 1 2

E 1 1 2 1 2 1

F 1 1 1 2 1 2

From this concurrence matrix, we cannot identify any grouping for the animals. Hence, the design of assigning the animals to the runs is connected. This design generated 5 canonical efficiency factors, of animals in the in the within runs stratum, which are 1, 1, 1, 0.75 and 0.75. The average efficiency factor is 0.8823529. This means all 5 DF for animals are all in the within runs stratum for this design, which means this design of assigning animals to runs is connected. However, 2 out of 5 DF for animal only have 0.75 of the information in the within runs stratum, this means 2 out 5 DF for animals have 0.25 of information in the between runs stratum.

The treatment allocation to runs and tags follows the assignment of treatments to the animals in the Phase 1 experiments which is shown as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 1 | 2 |
| 2 | 2 | 1 | 1 | 1 |
| 3 | 1 | 2 | 2 | 2 |

The theoretical ANOVA table that is generated from this design is as follows

DF e Ani Run

Between Run

Between Ani

Trt 1 1 1/2 4

Residual 1 1 1/2 4

Within

Between Ani

Tag 2 1 9/5 0

Trt 1 1 811/490 0

Residual 2 1 367/196 0

Residual

Tag 3 1 0 0

Residual 1 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 1 1/6

Within

Between Ani

Tag 3/2 26/15 1/2 13/45

Trt 49/15 49/90

Residual

Tag 27/19 9/19

The theoretical ANOVA table shows that this design does not provide a valid F-test for the treatment differences, because the coefficients of between animals variance components, , are not identical. A valid F-test can be conducted by adjusted the coefficients of between animals variance components from the linear combination of the residual in the within animals within runs stratum. The DF for the newly estimated EMS is approximated using the mean squares from the experimental results. Hence, the variances of the treatment effects cannot be estimated directly for this design. This design is connected design where all five DF associated with the animal effects are in the within runs stratum. However, two of five DF have 0.75 of animal information in the within runs stratum. In additional, treatment is also confounded with tag, from the theoretical ANOVA table, there is 0.5444 of pure treatment information.

Using A-optimality criterion in the objective function, the animal allocation for this design is shown below

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | C | F | F | C |
| 2 | E | A | B | D |
| 3 | D | B | A | E |

Two groups of animals can be observed different set of runs can be observed. Run 1 comprised of Animals C and F. Run 2 and 3 comprised of Animals A, B, D and E.

The incidence matrix of animal and run is

Run

Ani 1 2 3

A 0 1 1

B 0 1 1

C 2 0 0

D 0 1 1

E 0 1 1

F 2 0 0

This matrix shows that this design is a non-binary design. The binary design is a design where no treatment, in this case animal, occurs more than once in any block, or in this case runs. The non-binary design can be observed for Animal A, B, D and E. The binary design can be observed for Animal C and F. Hence, animals can be split into two groups, which mean this design is disconnected.

The disconnectedness can also be observed in the concurrence matrix of animals and run which is

Ani

Ani A B C D E F

A 2 2 0 2 2 0

B 2 2 0 2 2 0

C 0 0 4 0 0 4

D 2 2 0 2 2 0

E 2 2 0 2 2 0

F 0 0 4 0 0 4

where the grouping of Animals A, B, D and E and Animals C and F can be seen.

This design generated 4 canonical efficiency factors all unity, for the animals in the within runs stratum. The average efficiency factor is also 1. This also means 1 out of 5 DF for animals are in the between runs stratum.

The treatment allocation to runs and tags follows the assignment of treatments to the animals in the Phase 1 experiments which is shown as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 2 | 2 | 1 |
| 2 | 1 | 1 | 2 | 2 |
| 3 | 2 | 2 | 1 | 2 |

The theoretical ANOVA table that is generated from this design is as follows

$ANOVA

DF e Ani Run

Between Run

Between Ani 1 1 2 4

Residual 1 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 2 1 2 0

Residual

Tag 2 1 0 0

Residual 3 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Residual

Within

Between Ani

Tag 3 2/3 1 1/9

Trt 16/3 8/9

Residual

Tag 3 1

The theoretical ANOVA table shows that this design provides a valid F-test for the treatment differences, because both the treatment and residual EMS, in the between animals within runs stratum, contains variance components of . The variances of the treatment effects can also be estimated directly from the residual mean squares divided by the treatment replication number. However, the treatment effects are still confounded with tag effects, but this shows that there is still 88.89% of pure treatment information remaining. In addition, this design is disconnected, because 1 DF of animals are in the between runs stratum which leaves 4 DF of animals in the with runs stratum.

Therefore, despite the MS-optimal design is connected, for the purpose of conducting an experiment and testing for the treatment differences, the A-optimal design is more preferable. In addition, the variance component estimates of the animals in the between runs stratum can be recovered using the restricted maximum likelihood method.

**2. Add the treatment effects into the A-optimal using the weighted sum and how much weight**

The objective function is to find A-optimal design instead of the MS-optimal design. This means that the current objective function for the simulated annealing method is to find the design with the highest average efficiency factor when allocating the animals of Phase 1 experiment to the runs and tags of the Phase 2 experiments. The next step is to also maximise the treatment information in the within runs and tags stratum similar to the animals.

The treatment component can be add into the objective function using the weighted sum of the average efficiency factor of animals and treatment in the within runs and tags stratum. Thus, the design criterion is to maximise the average efficiency factor, the aim is to find and that can compute the highest the average efficiency factor from both the information matrix. Note the order of matrix will affect the order of matrix. These two average efficiency factors can be combined as

,

where and are the average efficiency factors of treatments and animals, and are weights of treatments and animals for calculating the combined average efficiency factors.

To comparing different weights, an experiment with following design parameter is used:

Phase 1 experiment - 2 treatments, 2 biological replicates,

Phase 2 experiment – 3 technical replicates, 3 runs and 4 tags.

Animals A and C are assigned to Treatment 1 and animals B and D are assigned to Treatment 2. Since there are 3 technical replicates, which mean there are total of 12 samples to be measured in the second phase experiment. Using the 4-plex experiment, three runs are required.

The theoretical ANOVA table for the first phase experiment is as follows,

$ANOVA

DF Ani

Between Ani

Trt 1 1

Residual 2 1

$EF

Trt eff.Trt

Between Ani

Trt 2 1

The objective function is set to have same weight for both average efficiency factor of animals and treatments in the within run and tags stratum. Using this objective function, the following animal allocation for this design is generated

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | B | A | C | D |
| 2 | C | D | A | B |
| 3 | A | B | D | C |

All three runs contain each of four animals; however, all four tags only have three of four animals.

The incidence matrix of animals and runs is

Run

Ani 1 2 3

A 1 1 1

B 1 1 1

C 1 1 1

D 1 1 1

which shows the assignment of animals to runs is binary, since all elements of matrix are one. This also indicates the assignment of animals to runs is complete block design where each run has each of four animals.

The incidence matrix of animals and tag is

Tag

Ani 1 2 3 4

AA 1 1 1 0

AB 1 1 0 1

AC 1 0 1 1

AD 0 1 1 1

which also shows the assignment of animals to tags is binary, since all elements of matrix are either zero or one. The concurrence matrix of animal and tag as follows

Ani

Ani A B C D

A 3 2 2 2

B 2 3 2 2

C 2 2 3 2

D 2 2 2 3

which shows that any pair of animal occur together in exactly 2 runs. This indicates the assignment of animals to tags is balance incomplete block design, which means all 3 DF associated with tag effects is confounded with the all 3 DF of animals.

The amount of un-confounded tag information can be calculated as follows,

The treatment allocation is based on the phase 1 experiment

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 2 | 1 | 1 | 2 |
| 2 | 1 | 2 | 1 | 2 |
| 3 | 1 | 2 | 2 | 1 |

The canonical efficiency factors of the animal in the within runs stratum are 1, 1 and 1; hence the average efficiency factor of animals in the within runs is also one.

The theoretical ANOVA table is as follows

$ANOVA

DF e Ani Run

Between Run 2 1 0 4

Within

Between Ani

Tag 3 1 3 0

Residual

Tag 3 1 0 0

Residual 3 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Within

Between Ani

Tag 1/3 6 1/9 1

Residual

Tag 8/3 8/9

From the fixed effects table, 1/9 of the tags information for all 3 DF is in the between animals within runs, which means 8/9 of the tags information for all 3 DF is in the within animals within runs stratum. From the random effects table, all three DF associated with tag effects is confounded with 3 DF of between animals within runs stratum. Therefore, the formal test for the treatment differences cannot be conducted. In addition, one of three DF associated with the tag effects is confounded with the treatment effects.

The objective function is set the weight for average efficiency factor of animals to be 0.75 and the weight for average efficiency factor of treatment to be 0.25.The animal allocation for this design is shown below

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | C | A | D | B |
| 2 | A | B | D | C |
| 3 | B | C | D | A |

All three runs again contain each of four animals. Tag 114, 115 and 117 contain animals A, B and C and Tag 116 contains Animal D. In fact, 3-by-3 section of Run 1, 2 and 3 and Tag 114, 115 and 117 is a Latin square design of Animal A, B and C.

The incidence matrix of animals and runs is

Run

Ani 1 2 3

AA 1 1 1

AB 1 1 1

AC 1 1 1

AD 1 1 1

which also shows the assignment of animals to runs is binary, since all elements of matrix are one. This also indicates the assignment of animals to runs is complete block design where each run has each of four animals which is same as the previous design. However, the incidence matrix of animals and tag is

Tag

Ani 1 2 3 4

AA 1 1 0 1

AB 1 1 0 1

AC 1 1 0 1

AD 0 0 3 0

which is not a binary design anymore, since Animal D only appears in one of four tags. The concurrence matrix of animals and tag is

Ani

Ani AA AB AC AD

AA 3 3 3 0

AB 3 3 3 0

AC 3 3 3 0

AD 0 0 0 9

which confirms the grouping of Animals A, B and C versus Animal D; hence, the allocation of animals to tags is disconnected.

The treatment allocation is based on the phase 1 experiment is follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 1 | 1 | 2 | 2 |
| 2 | 1 | 2 | 2 | 1 |
| 3 | 2 | 1 | 2 | 1 |

Note that Tag 116 contains only Treatment 2.

The canonical efficiency factors of the animal in the within runs stratum are 1, 1 and 1; hence the average efficiency factor of animals in the within runs is also one.

The theoretical ANOVA table is as follows

$ANOVA

DF e Ani Run

Between Run 2 1 0 4

Within

Between Ani

Tag 1 1 3 0

Trt 1 1 3 0

Residual 1 1 3 0

Residual

Tag 2 1 0 0

Residual 4 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Within

Between Ani

Tag 3 2 1 1/3

Trt 4 2/3

Residual

Tag 3 1

From the random effects table, the valid test for the treatment differences can be conducted since the coefficients of the between animal variance components for the treatment and residual mean squares in between animals stratum is identical. In addition, only one DF associated with the tag effects is in the between animals within runs stratum. From the fixed effects table, the tag is confounded with 1/3 of the treatment information, this means there are still 2/3 of pure treatment information remains for conducting the test for treatment difference.

In summary, the weighted sum of the average efficiency factors of treatments and animals is follows

,

If the weights for both are identical, the results from objective function of the first design is

0.5 \* 8/9 + 0.5 \* 8/9 = 8/9.

However, for the second design, results from objective function becomes

0.5 \* 1 + 0.5 \* 2/3 = 5/6,

which is lower than the previous design, even though the second design is more preferable. The objective function is then set the weight for average efficiency factor of animals to be 0.75 and the weight for average efficiency factor of treatment to be 0.25. The results from objective function of the first design is still

0.75 \* 8/9 + 0.25 \* 8/9 = 8/9

However, for the second design, results from objective function becomes

0.75 \* 1 + 0.25 \* 2/3 = 11/12,

which is higher than the previous design. Thus, the second design can only be generated by having to be 0.75 and to be 0.25 for the objective function. What this objective function does is to put more emphasis on the average efficiency factor for assigning the animals to runs and tags to be close to 1 as much as possible while it maximises f both the animal and treatment information in the within runs and tags stratum.

**3. Add another component which monitors the DF associated with the treatment effects**

The current objection function gives a weighted sum of the average efficiency factors of animals and treatments. However, for an experiment consisting of more than 2 treatments, there will be more than 1 DF associated with the treatment effects in some stratum of the ANOVA table.

Using an example with 3 treatments; hence, there will be 2 DF associated with the treatment effects.

Phase 1 experiment - 3 treatments, 2 biological replicates, 2 technical replicates,

Phase 2 experiment – 3 runs and 4 tags.

Animals A and D are assigned to Treatment 1,animals B and E are assigned to Treatment 2 and animals C and F are assigned to treatment 3. Since there are 2 technical replicates, which mean there are total of 12 samples to be measured in the second phase experiment. Using the 4-plex experiment, three runs are required.

The Phase 1 theoretical ANOVA table is as follows

$ANOVA

DF Ani

Between Ani

Trt 2 1

Residual 3 1

$EF

Trt eff.Trt

Between Ani

Trt 2 1

Using the objective function mentioned with the weighted sum but does not monitor the DF of the treatment effects, the allocation of animals to runs and tags is found as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | B | B | D | D |
| 2 | C | C | F | F |
| 3 | A | A | E | E |

Three groups of animals to runs can be observed, where Run 1 contains Animals B and D, Run 2 contains Animals C and F and Run 3 contain Animal A and E. Two groups of animals to tags can also be observed, where Tag 114 and 115 contains only Animals A, B and C and Tag 116 and 117 contain Animals D, E and F.

The treatment allocation is based on the Phase 1 experiment.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 2 | 2 | 1 | 1 |
| 2 | 3 | 3 | 3 | 3 |
| 3 | 1 | 1 | 2 | 2 |

Two groups of treatments to runs Run 1 and 3 contain Treatment 1 and 2 and Run 2 has only treatment 3. All four tags have one of each 3 treatments.

The concurrence matrix of treatment to runs

Trt

Trt 1 2 3

1 8 8 0

2 8 8 0

3 0 0 16

which confirms the two groups of treatments.

The theoretical ANOVA table is as follows

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 2 4

Residual 1 1 2 4

Within

Between Ani

Tag 1 1 2 0

Trt 1 1 2 0

Residual 1 1 2 0

Residual

Tag 2 1 0 0

Residual 4 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 4 1

Within

Between Ani

Tag 3 1

Trt 4 1

Residual

Tag 3 1

From the random effects table, the valid test for the treatment differences can be conducted. However, the test is only based on one DF associated with the treatment effects in the between animals within runs stratum. Another one DF associated with the treatment effects is in the between animals between runs stratum. The treatment information for each of these two DF is 100%.

This means the given design found from the current objective function is disconnected in the assignment of treatments to runs. To avoid generating such the disconnected designs when searching for the optimal design using the simulated annealing algorithm, an additional component of the objective function is to add another term that monitors the DF of the treatment effects. Therefore, the modified objection function gives a weighted sum of the average efficiency factors of animals and treatments and the proportion of the DF of treatment of current design found during the simulated annealing algorithm to the total DF of treatment, i.e.

The weights for the average efficiency factors of animals and treatments, i.e. and , are set to 2/3 and 1/9, respectively, and the weight for the proportion of treatment DF, i.e. , is set to 2/9.

Using the newly modified objective function, the following design was found. The allocation of animals to runs and tag can be shown as follows,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | C | C | D | D |
| 2 | B | A | E | F |
| 3 | A | B | F | E |

The allocation of animals to runs can be divided into two groups, where Run 1 contains Animals C and D. Run 2 and 3 contains Animal A, B, E and F.

Treatment allocation is again based on the Phase 1 experiment,

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Run | Tag | | | |
| 114 | 115 | 116 | 117 |
| 1 | 3 | 3 | 1 | 1 |
| 2 | 2 | 1 | 2 | 3 |
| 3 | 1 | 2 | 3 | 2 |

The concurrence matrix of treatment to runs

Trt

Trt 1 2 3

1 6 4 6

2 4 8 4

3 6 4 6

There is no obvious grouping of the treatment from observing this concurrence matrix, which suggests that the allocation of the treatments to runs may not be disconnected.

A different theoretical ANOVA table is shown as follows,

$ANOVA

DF e Ani Run

Between Run

Between Ani

Trt 1 1 2 4

Residual 1 1 0 4

Within

Between Ani

Tag 1 1 2 0

Trt 2 1 2 0

Residual 1 1 2 0

Residual

Tag 2 1 0 0

Residual 3 1 0 0

$EF

Tag Trt eff.Tag eff.Trt

Between Run

Between Ani

Trt 1 1/4

Residual

Within

Between Ani

Tag 3 1

Trt 24/7 6/7

Residual

Tag 3 1

From the random effects table, all 2 DF of treatment effects is in the between animals within runs stratum for conducting the test of the treatment group differences. However, one of DF of treatment effects has its 1/4 of information in the between animals between runs which remains 3/4 of the treatment information. The canonical efficiency factors for 2 DF are 1 and 3/4; hence the average efficiency factor which is the harmonic mean of the canonical efficiency factors gives 6/7.

The main idea of this objective function is to allow the animal to be disconnected to Runs and tags; hence the valid test for the treatment differences in the between animals within runs stratum can be conducted. In the meantime, this objective function will also preventing the treatment effects to be disconnected as much as possible, as most of the treatment information remains in the within runs stratum.