The Difference Between Repeats and Replicates

There are many attendees today! *To avoid disrupting the Voice over Internet Protocol (VoIP) system, I will mute all. Please use the Questions feature on GotoWebinar which Shari Kraber will answer during the presentation.*
-- Pat

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Agenda
Replicates versus Repeats

1. **Define Replicates and Repeats**
2. Why use Replicates
   - Brief Power Discussion
   - Replication Example
3. Why use Repeats
   - Repeat Measures example
4. Recap

Replicates and Repeats

- **Replicates:**

  The same settings are run and measured multiple times.

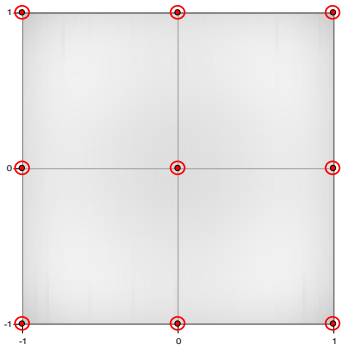
- **Repeats:**

  Measurements are done multiple times on a single run and averaged.

Replicates
Two Kinds

- **Design Replicates**
  - increase power
  - provide an estimate for pure error
  - reduce the prediction error
- **Point Replicate**
  - provide an estimate for pure error
  - to a lesser extent increase power
  - chosen to reduce over all prediction errors
  - are often…
    - center points to detect curvature
    - standard operating conditions

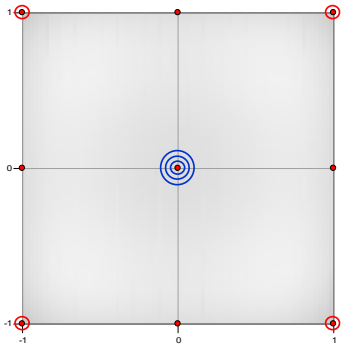
Replicates
Design Replicates



Design Replicates multiply the number of runs.

Every run is done again.

Replicates
Point Replicates



Point Replicates are more selective.

Center points are replicated to test for curvature.

Extreme combinations (high leverage) replicates improve prediction errors.

Replicates

- A Replicate is a new run.
  - Ideally, the process is reset after each run
    - Calibrating the equipment
    - Cleaning the equipment
    - Changing factor settings, etc.
  - All runs should have the same setup process.

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What is Power?

No Factor Effect; H_0: $\Delta = 0$

Power = (1−β)*100%

Power is the probability of revealing an active effect of size delta (Δ) relative to the noise (σ) as measured by signal to noise ratio (Δ/σ).

It should be high (at least 80%!) for the effect size of interest.

Effect?		ANOVA says:	
		Retain H_0	***Reject H_0***
Truth:	***No***	OK☺	Type I Error (alpha) *False Alarm*
	Yes	Type II Error (beta) *Failure to detect*	OK☺

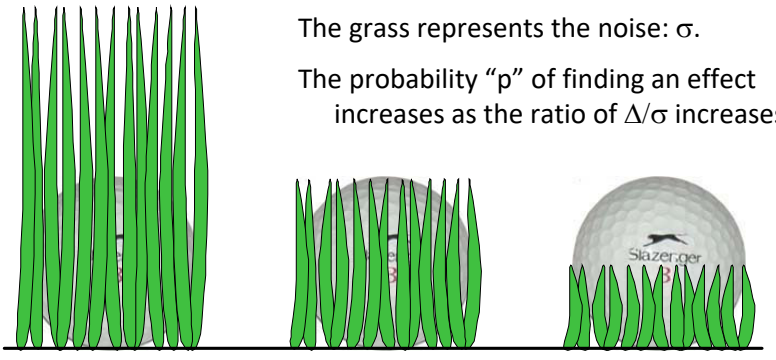
Power

Depends on Signal to Noise Ratio

The golf ball represents the effect: Δ.

The grass represents the noise: σ.

The probability "p" of finding an effect increases as the ratio of Δ/σ increases.



$\Delta/\sigma = 1/2$ $\Delta/\sigma = 1$ $\Delta/\sigma = 2$

How can you increase the likelihood of finding the golf ball?

What is Power?
Signal, Noise and all that Greek

Δ = (Signal) The minimum amount of change to produce a *Eureka* moment

σ = (Noise) The expected standard deviation

α = The acceptable risk to find false effects; often set to 5%

β = The acceptable risk to not find true effects; kept as low as possible, 20% or less

Confidence = $(1-\alpha)*100\%$

Power = $(1-\beta)*100\%$ -- power to be at least 80%

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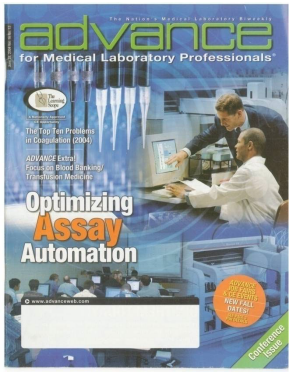
Application of DOE to Mouse Cell Assay*

This case study highlights how a development team applies DOE to study a mouse-cell fluorescent assay performed in a 96-well plate format. They are concerned about the effects of several key factors.



** Detailed in "How Experimental Design Optimizes Assay Automation" by Thomas Erbach & Lisa Fan, Beckman Coulter, Inc., Shari Kraber, Stat-Ease, Inc.,* Advance*, June 28, 2004, Vol. 16, No. 13,pp 18-21.*

Mouse Cell Assay
General background

This assay system is used to detect cell activity.

- Cells are pipetted into a 96-well plate where stimulant is added to induce the mouse cells to express a biomarker.
- The plate is normalized with media and incubated for two hours.
- A fluorescently tagged substrate is added to bind to the biomarker.
- The plate is read on a fluorescent plate reader at a specific wavelength.

The objective of this study is to find the settings of three factors (cell number, stimulant and substrate concentration) that maximize signal (fluorescence).

Mouse Cell Assay

DOE Process *(page 1 of 3)*

1. Identify opportunity and define objective.
   The objective is to maximize signal from the assay.
2. State objective in terms of measurable responses.
   a. Define the change (Δy) that is important to detect.
      A difference of 400 fluorescent units is of interest;
      $\Delta y \approx 400$.
   b. Estimate experimental error (σ) for each response.
      Historical data is used to estimate the standard deviation;
      $\sigma \approx 400$.
   c. Use the signal-to-noise ratio ($\Delta y/\sigma$) to estimate power.
      $\Delta y/\sigma = 400/400 = 1.0$

Mouse Cell Assay

DOE Process *(page 2 of 3)*

3. Select the input factors and ranges to study. (*Choose factor ranges that are likely to change the response by at least Δy.*)

Factor	−1 level	+1 level
A. Cell Number	5000	10000
B. Stimulant	5 µL	10 µL
C. Substrate concentration	0.15 µM	0.30 µM

Mouse Cell Assay
DOE Process *(page 3 of 3)*

4. Select a design (a full 2^3 two-level factorial) and evaluate:
   - Aliases (fractional factorials and/or blocked designs) Not an issue with this design choice (running all combinations).
   - All factor combinations for safety and reasonability (likelihood of producing meaningful information).
     Assume the team knows from subject matter expertise and actual range-finding tests that all runs will be do-able and informative.
   - Power (ideally at least 80% probability for detection).

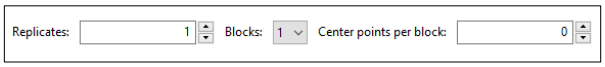
For this study, the experimenter suspects the need to replicate this 2^3 design, but how many replicates are enough?

Evaluating "Power" gives us the answer!

Mouse Cell Assay
Evaluating Power *(Instructor Led)*

1. Launch Design-Expert software.
2. Set up a three-factor full factorial design (**2^3**, **8** runs).

   Leave defaults:



3. Enter the factor names, levels and units.

	Name	Units	Type	Low	High
A [Numeric]	Cell Number	count	Numeric	5000	10000
B [Numeric]	Stimulant	uL	Numeric	5	10
C [Numeric]	Substrate	uM	Numeric	0.15	0.3

Mouse Cell Assay
Evaluating Power *(Instructor Led)*

4. Enter the response name, delta and sigma:

Name	Units	Diff. to detect Delta ("Signal")	Est. Std. Dev. Sigma ("Noise")	Delta/Sigma (Signal/Noise Ratio)
Fluorescent Units		400	400	1

5. Check out the power report>>

Name	Units	Delta (Signal)	Sigma (Noise)	Signal/Noise	Power for A	Power for B	Power for C
Fluorescent Units		400	400	1	19.5%	19.5%	19.5%

Power too low: Best be 80% or more for effects of interest.
More runs needed!

Mouse Cell Assay
Evaluating Power *(Instructor Led)*

6. Click on the <<Back button to the factorial design matrix menu and build a 2^3 factorial with **2** replicates (16 runs):

Name	Units	Delta (Signal)	Sigma (Noise)	Signal/Noise	Power for A	Power for B	Power for C
Fluorescent Units		400	400	1	45.2%	45.2%	45.2%

7. Click on the <<Back button to the factorial design matrix menu and build a 2^3 factorial with **5** replicates (16 runs):

Name	Units	Delta (Signal)	Sigma (Noise)	Signal/Noise	Power for A	Power for B	Power for C
Fluorescent Units		400	400	1	86.8%	86.8%	86.8%

Mouse Cell Assay
Evaluating Power

Power to detect main effects at 5% alpha:

- 8 unique design runs: 19.5% ☹
- 16 runs – 2nd replicate of original 2^3: 45.2% ☹
- 24 runs – 3rd replicate: 64.5% ☹
- 32 runs – 4th replicate : 77.9% 😐
- 40 runs – 5th replicate : 86.8% ☺

Less than half of the 96 wells suffice for adequate power (80%). Further runs provide greatly diminishing returns.

Power
The probability of finding an effect!

Power depends on:

- The size of the difference Δ:
  the larger the difference the higher the power.
- The size of the experimental error σ:
  the smaller σ the higher the power.
- The α risk chosen:
  the larger α the higher the power.
- The design:
  more orthogonal and larger designs have more power.
- **The number of replicates:**
  replicates give better estimates - increasing power.

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Increasing Power
by Reducing Variation

Replication reduces all sources of variation by a factor of **r**. Where **r** is the number of times the design is completed.

Repeating Measurements reduces the variation from only the measurement source by a factor of **m**. Where **m** is the number of measurements taken.

Reducing the total variation by any amount increases the signal to noise ratio. Increasing Signal/Noise ratio increases power.

Repeats
What's the Budget

Question: If **Repeating Measurements** m times only decreases measurement variation, why not always **replicate**?

Answer 1: It often costs less to re-measure than it does to replicate.

Answer 2: If most of the variation is coming from the measurement system – the biggest bang for the buck comes from reducing measurement variation.

Replicates vs. Repeats

- A Repeat is a measurement done again without resetting the system.
  - Repeat measurements are averaged and reported as a single response.
  - Averaged measurements reduce the effect of measurement variability.
  - If the test is destructive, multiple parts are created during each run to be measured later.

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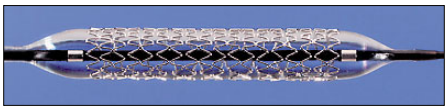
MR5 Design
Stent Delivery System

A stent is a wire mesh tube used to prop open an artery that's recently been cleared using angioplasty. The stent is collapsed to a small diameter over a balloon catheter. It's then moved into the area of the blockage.



When the balloon is inflated, the stent expands, locks in place and forms a scaffold. This holds the artery open.
The stent stays in the artery permanently, holding it open to improve blood flow to the heart muscle.

Stent Delivery System
MR5 Design *(page 1 of 4)*

1. Identify opportunity and define objective.
   Relate stent safety and deliverability to process factors.
2. State objective in terms of measurable responses.
   Safety is quantified by Burst pressure. Deliverability is quantified by Pushability and Trackability. Want to estimate 2FI model, this requires a res V design.
   a. Define the change (Δy) that is important to detect for each response. Δ_{Burst} = 6 psig, Δ_{Push} = 15 g/cm and Δ_{Track} = 10 g*cm.
   b. Estimate error (σ):
      σ_{Burst} = 7 psig;
      σ_{Push} = 28 g/cm;
      σ_{Track} = 6 g*cm;
   c. Calculate signal to noise:
      Δ/σ = 6/7 = 0.857
      Δ/σ = 15/28 = 0.536
      Δ/σ = 10/6 = 1.67

Stent Delivery System
MR5 Design *(page 2 of 4)*

3. Select the input factors* to study (there are ten):

	Name	Units	Type	Low	High
A [Numeric]	Balloon wall	in	Numeric	0.0011	0.0015
B [Numeric]	Waist length	mm	Numeric	2	3
C [Numeric]	Waist wall	in	Numeric	0.001	0.002
D [Categoric]	Wing fold	#	Categoric	3	5
E [Numeric]	Inner ID	in	Numeric	0.016	0.018
F [Numeric]	Inner wall	in	Numeric	0.002	0.003
G [Numeric]	Inner weld	cm	Numeric	3	9
H [Numeric]	Inner length	mm	Numeric	2	3
J [Numeric]	Tip length	mm	Numeric	2	4.2
K [Numeric]	Tip matl	durometer	Numeric	43	69

** Do not enter – a file will be provided to save on typing.*

Stent Delivery System
MR5 Design *(page 3 of 4)*

4a. Select a design:

- Evaluate aliases (fractional factorials and/or blocked designs) *During build*
- Evaluate power (desire power > 80% for effects of interest) *During build (default order is main effects)*
- Examine the design layout to ensure all the factor combinations are safe to run and are likely to result in meaningful information (no disasters)

Stent Delivery System
MR5 Design *(page 4 of 4)*

4b. Select a design:

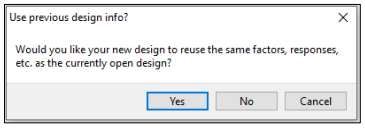
- A regular fraction requires a 2^{10-3} or 128 runs.
- A Minimum-Run Res V (MR5) fraction requires 56 runs.

The winner is: **Min-Run Characterize** (MR5)

Stent Delivery System
Build the MR5 Design

1. To save time (and typing) open "**Stent starter.dxpx**" (do not worry about grey columns) and rebuild the design (File, New Design).

   "**Yes**" to "Use previous design info?"



2. There are three responses (**enter missing info**):

Name	Units	Diff. to detect Delta ("Signal")	Est. Std. Dev. Sigma ("Noise")	Delta/Sigma (Signal/Noise Ratio)
Burst	psig	6	7	0.857143
Push	g/cm	15	28	0.535714
Track	g*cm	10	6	1.66667

MR5 Design: Power
Stent Delivery System

Design Power

Recommended power is at least 80%.

	Name	Units	Delta (Signal)	Sigma (Noise)	Signal/Noise	Power for A	Power for B	Power for C	Power for D	Power for E	Power for F	Power for G	Power for H
	Burst	psig	6	7	0.857143	87.9%	87.4%	87.3%	87.6%	87.7%	87.4%	87.6%	87.7%
	Push	g/cm	15	28	0.535714	49.9%	49.2%	49.1%	49.5%	49.7%	49.3%	49.5%	49.7%
	Track	g*cm	10	6	1.66667	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%	99.9%

*Stay on this screen – do NOT click **Finish**.*

MR5 Design: Power
Stent Delivery System

Power is low (~50%) for Push; to increase power:

1. Increase design size: replicating the design 56x2=112 runs gives adequate power (~82%), but there are too many runs to be practical.
2. Increase Δ_{Push} = 15 g/cm: No – we are interested in a difference of 15 g/cm.
3. Decrease σ_{Push}= 28 g/cm: By partitioning the variance, we determine that the push measurement contributes most (75%) of the variation. Repeating the test (not the experimental run) to reduce σ is the answer.

See next slide. ☞

MR5 Design: Power
Stent Delivery System

$$\sigma_{Push} = 28 \quad \& \quad \sigma^2_{Push} = \sigma^2_{Process} + \sigma^2_{Measurement}$$

$$784 = 200 + 584 \quad \therefore \quad \%Contribution = 584/784 \approx 75\%$$

Make three independent push measurements for each run.
Enter the average of the measurements as the response:

Then by the central limit theorem $\left(\sigma^2_{Average} = \frac{\sigma^2_{Measurement}}{m} \right)$:

$$\sigma^2_{Push} = 200 + \frac{584}{3} = 395 \qquad \%Contribution \approx 50\%$$

$$\sigma_{Push} = \sqrt{395} \approx 19.9 \qquad \frac{\Delta}{\sigma} = \frac{15}{19.9} = 0.75$$

MR5 Design: Power
Stent Delivery System

Go back to the responses and enter σ_{Push}= **19.9**:

Design Power

Recommended power is at least 80%.

	Name	Units	Delta (Signal)	Sigma (Noise)	Signal/Noise	Power for A	Power for B	Power for C	Power for D	Power for E	Power for F	Power for G	Power fo H
	Burst	psiq	6	7	0.857143	**87.9%**	**87.4%**	**87.3%**	**87.6%**	**87.7%**	**87.4%**	**87.6%**	**87.7**
	Push	q/cm	15	19.9	0.753769	**78.6%**	**77.9%**	**77.8%**	**78.2%**	**78.4%**	**78.0%**	**78.2%**	**78.4**
	Track	q*cm	10	6	1.66667	**99.9%**	**99.9%**	**99.9%**	**99.9%**	**99.9%**	**99.9%**	**99.9%**	**99.9**

Close enough to 80%. Finish

MR5 Design: Power
Stent Delivery System

Summary:

- Replicating runs will reduce the system error; from both process and measurement.
- Repeating the measurement reduces only the measurement error.
- The magnitude of each of these errors and the relative cost of replicating runs versus repeating measurements dictates which will give the most “bang” for your buck.

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Increasing Power
It's all about the Power

As shown in the Mouse Assay Case Study, power was increased by **replication** (adding more runs).

In the Stent Case Study **repeating measurements** proved to be a more cost-effective method to reduce the noise variation.

The two techniques can be combined to reduce costs and variation.

Increasing Power
Or Maybe its About Reducing Variation

Replication reduces the total variation by a factor of r the number of times the design is completed.

$$\sigma^2_{total} = \frac{\sigma^2_{process} + \sigma^2_{measurement}}{r},$$

where r is the number of design replicates

Reducing the total variation increases the signal to noise ratio, thus increasing power.

Increasing Power
Or Maybe Just Some of the Variation

Repeating Measurements m times only reduces the variation from the measurement source.

$$\sigma^2_{total} = \sigma^2_{process} + \frac{\sigma^2_{measurement}}{m},$$

where r is the number of design iterations and
m is the number of measurements

Unless the measurement variation makes up a large percentage of the total, repeating measurements may not help very much.

The Difference Between Repeats and Replicates

Reminder, this presentation is posted at:
www.statease.com/webinar.html

If you have additional questions email them to:
stathelp@statease.com

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