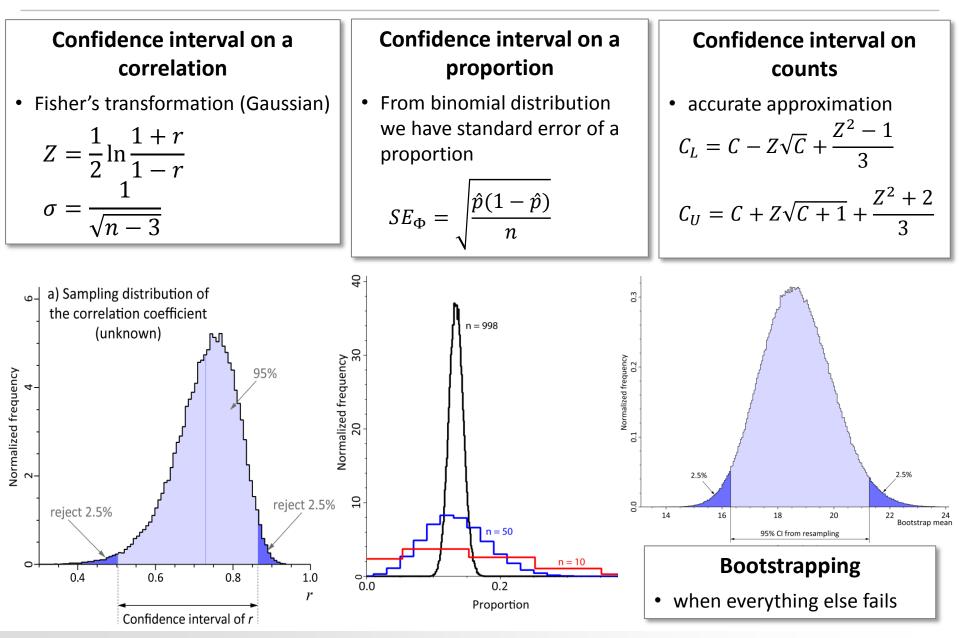
Error analysis in biology

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Hand-outs available at http://is.gd/statlec

Errors, like straws, upon the surface flow; He who would search for pearls must dive below *John Dryden (1631-1700)*

Previously on Errors...



5. Error bars

"Errors using inadequate data are much less than those using no data at all"

Charles Babbage

A good plot

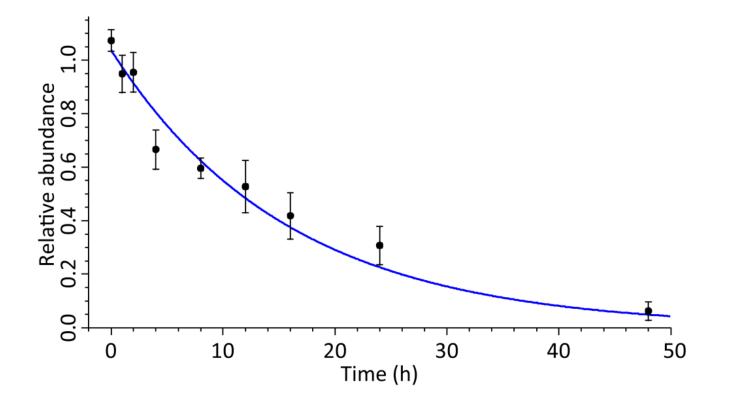
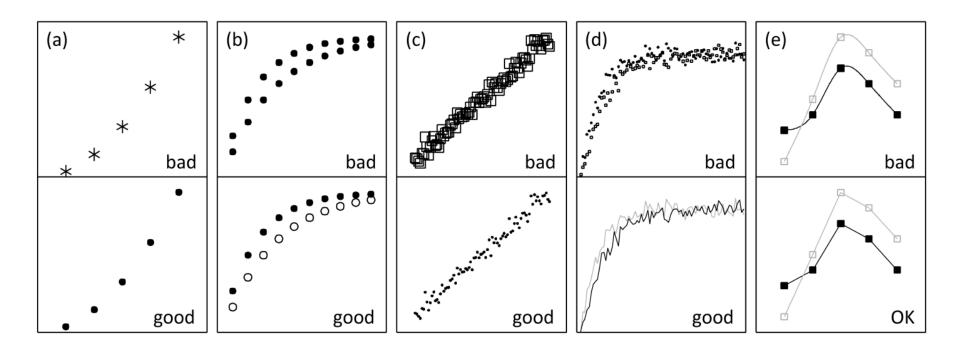


Figure 6-1. Exponential decay of a protein in a simulated experiment. Error bars represent propagated standard errors from individual peptides. The curve shows the best-fitting exponential decay model, $y(t) = Ae^{-t/\tau}$, with $A = 1.04 \pm 0.05$ and $\tau = 16 \pm 3$ h (95% confidence intervals).

3 rules for making good plots

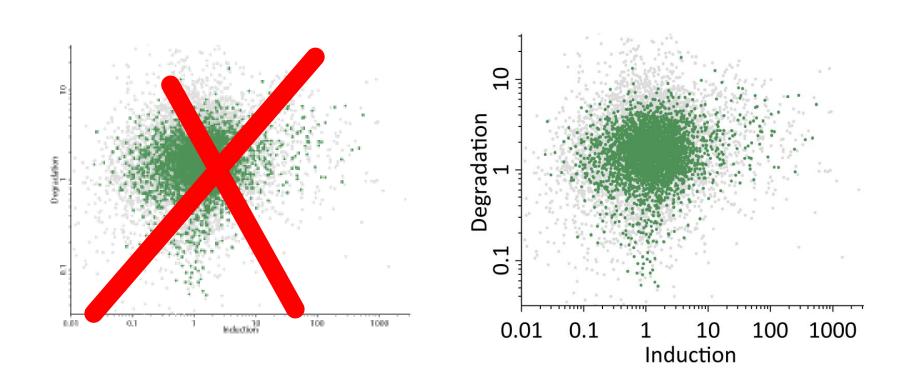
- 1. Clarity of presentation
- 2. Clarity of presentation
- 3. Clarity of presentation

Lines and symbols

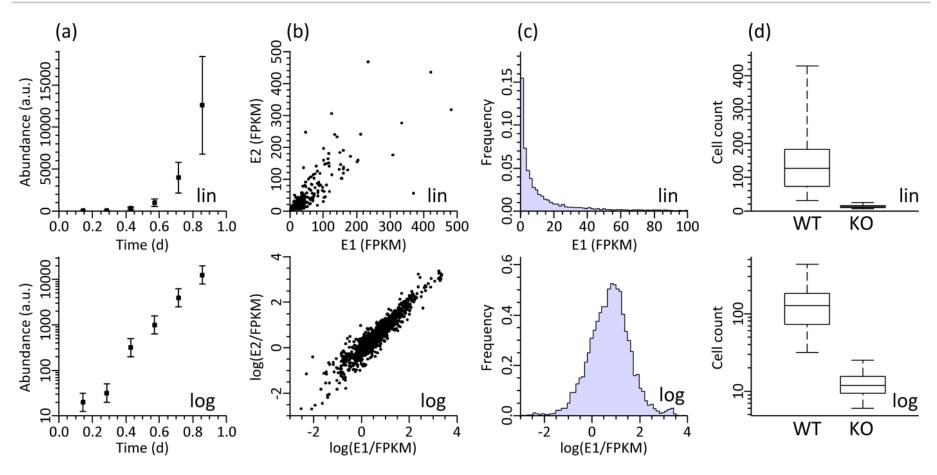


- Clarity!
- Symbols shall be easy to distinguish
- It is OK to join data points with lines for guidance

Labels!

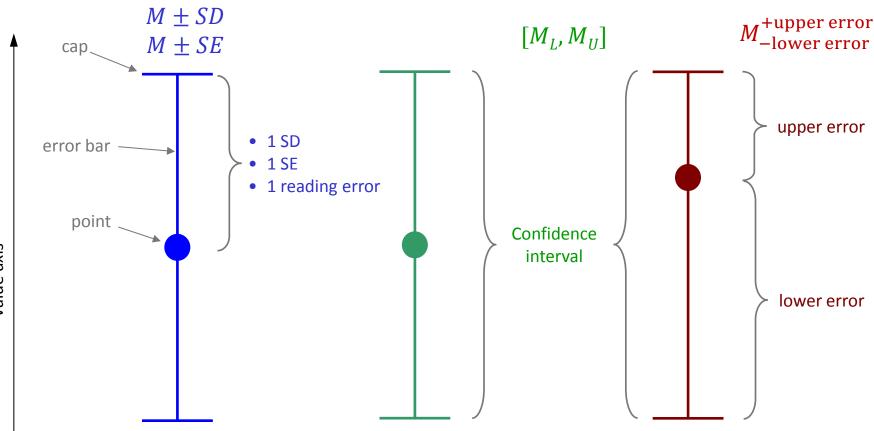


Logarithmic plots



- Clarity!
- Use logarithmic axes to show data spanning many orders of magnitude

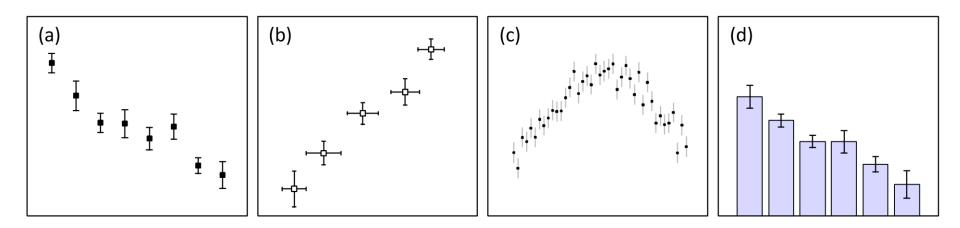
How to plot error bars



The point represents

- statistical estimator (e.g. sample mean)
- best-fitting value
- direct measurement

How to plot error bars



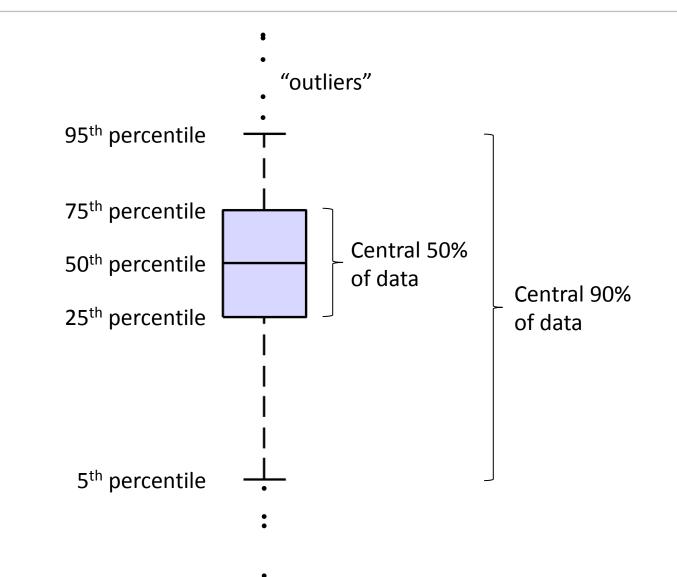
- Clarity!
- Make sure error bars are visible

Types of errors

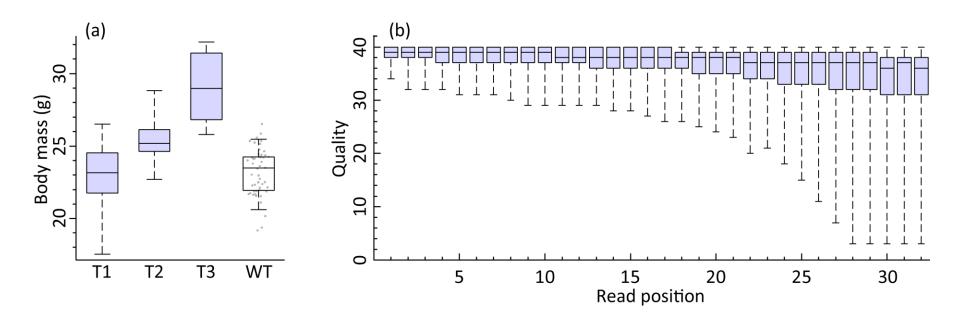
Error bar	What it represents	When to use
Standard deviation	Scatter in the sample	Comparing two or more samples, though box plots make a good alternative
Standard error	Error of the mean	Most commonly used error bar, though confidence intervals have better statistical intuition
Confidence interval	Confidence in the result	The best representation of uncertainty; can be used in almost any case

Always state what type of uncertainty is represented by your error bars

Box plots



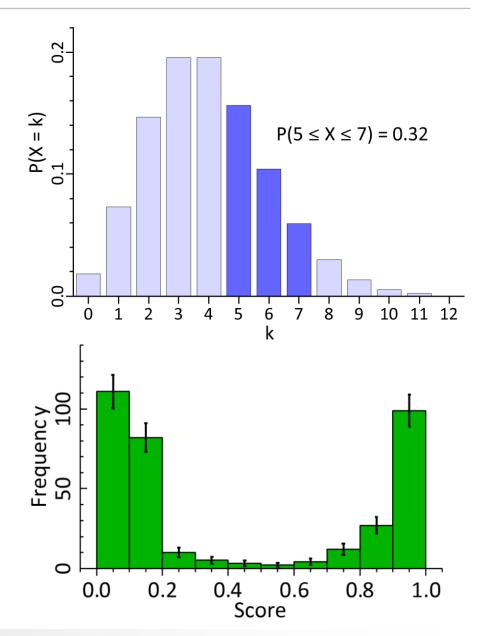
Box plots



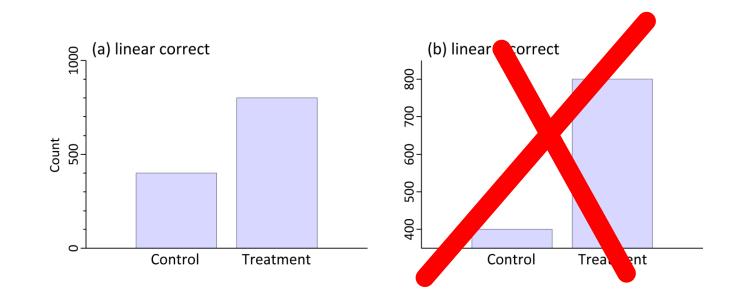
- Box plots are a good alternative to standard deviation error bars
- They are non-parametric and show pure data

Bar plots

- Area of a bar is proportional to the value presented
- Summed area of several bars represents the total value
- Bar plots should **only** be used to present additive quantities: counts, fractions, proportions, probabilities, etc.
- Against a continuous variable data are integrated over the bar width
- Each bar is two-dimensional
- Bar width matters!

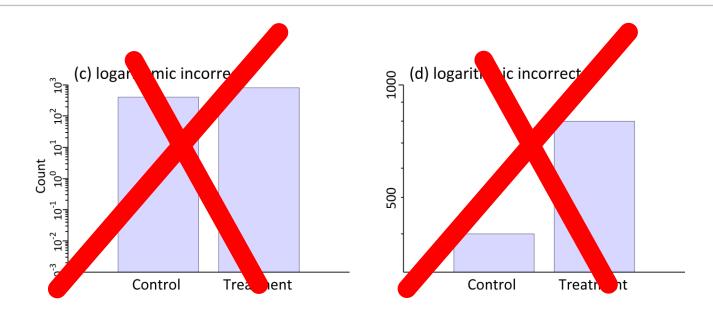


Bar plots start at zero



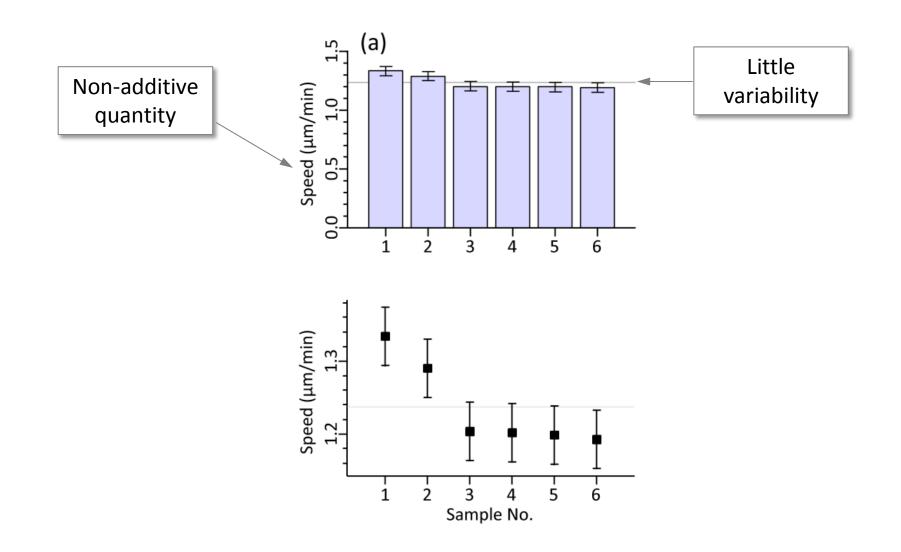
- Bar area represents its value
- Hence, baseline must be at zero
- If not, the plot is very misleading
- Don't do it!

Bar plots in logarithmic scale

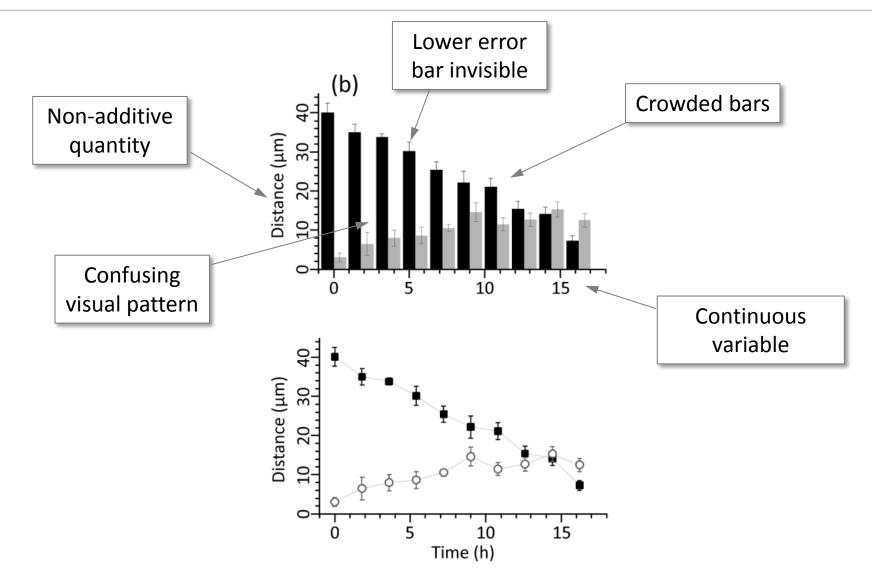


- There is no zero in a logarithmic scale!
- Bar size depends on an arbitrary lower limit of the vertical axis
- Don't do it!

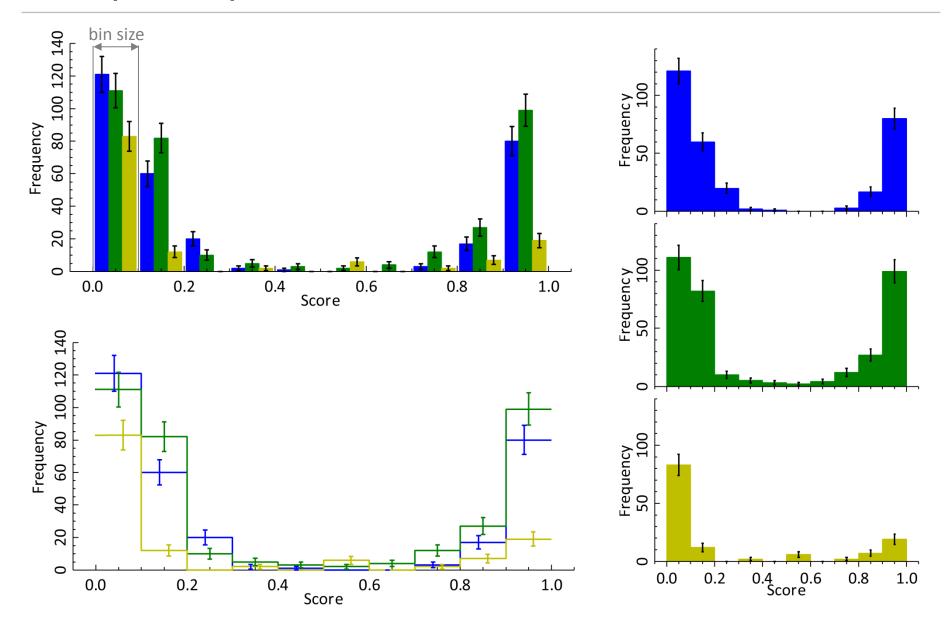
Bar plot problems



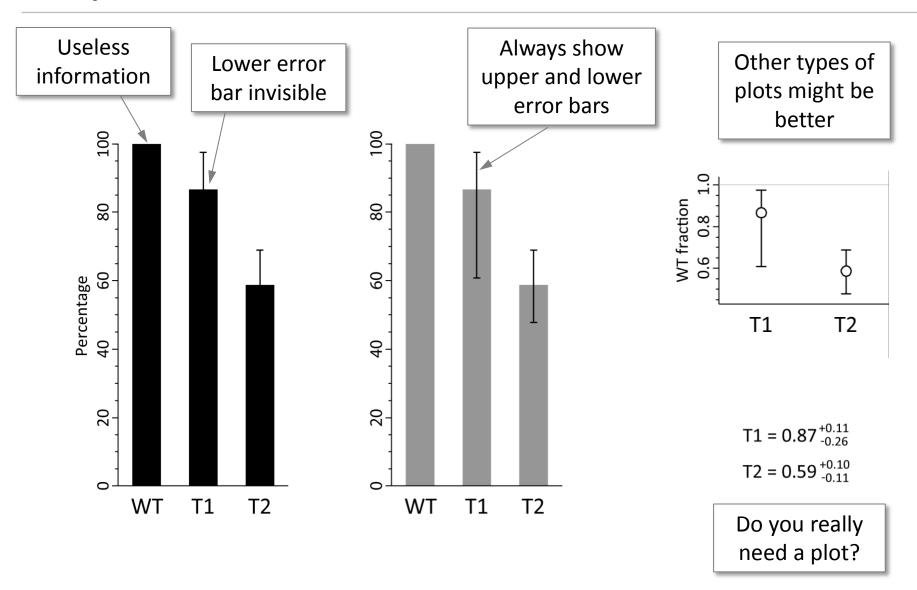
Bar plot problems



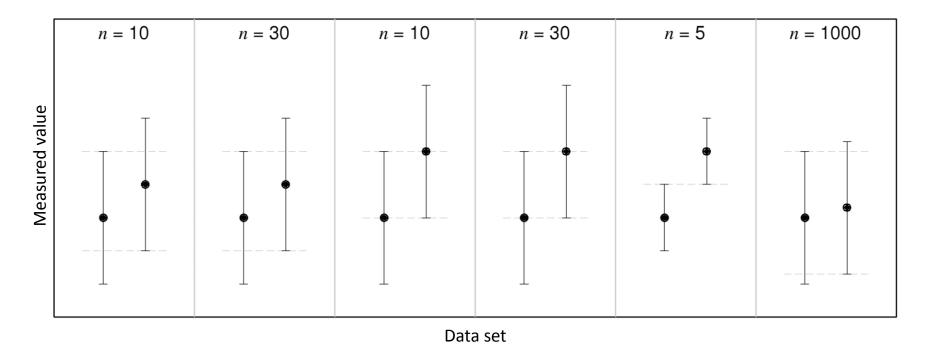
Multiple bar plots and a continuous variable



Bar plots with error bars



Exercise: overlapping error bars



- Each panel shows results from a pair of samples of the same size
- Mean and standard deviation are shown
- Which of these pairs of means are significantly different?

Rules of making good graphs

- 1. Always keep **clarity of presentation** in mind
- 2. You shall use axes with scales and labels
- 3. Use logarithmic scale to show data spanning over many orders of magnitude
- 4. All labels and numbers should be easy to read
- 5. Symbols shall be easy to distinguish
- 6. Add error bars were possible
- 7. Always state what type of uncertainty is represented by your error bars
- 8. Use model lines, where appropriate
- 9. It is OK to join data points with lines for guidance
- 10. You shall not use bar plots unless necessary

Bar plots: recommendations

- 1. Bar plots should only be used to present additive quantities: counts, proportions and probabilities
- 2. Each bar has to start at zero
- 3. Don't even think of making a bar plot in the logarithmic scale
- 4. Bar plots are not useful for presenting data with small variability
- 5. Multiple data bar plots are not suited for plots where the horizontal axis represents a continuous variable
- 6. Multiple data bar plots can be cluttered and unreadable
- 7. Make sure both upper and lower errors in a bar plot are clearly visible

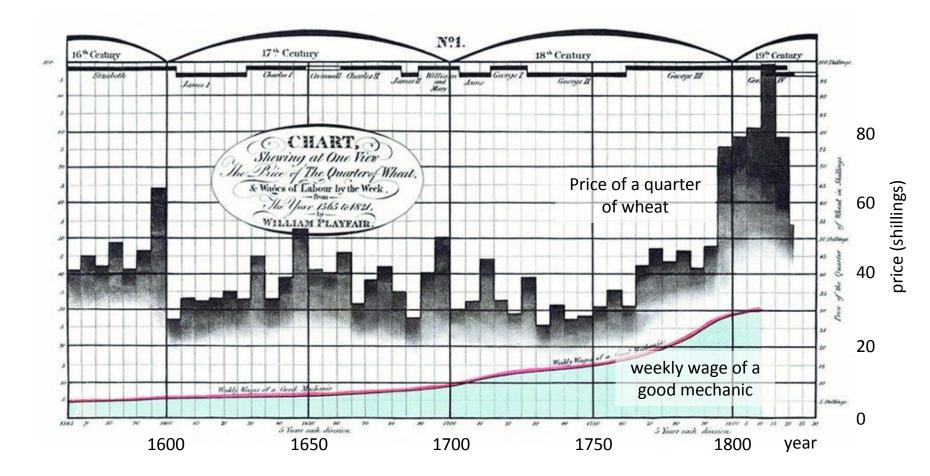
William Playfair

- Born in Liff near Dundee
- Man of many careers (millwright, engineer, draftsman, accountant, inventor, silversmith, merchant, investment broker, economist, statistician, pamphleteer, translator, publicist, land speculator, blackmailer, swindler, convict, banker, editor and journalist)
- He invented
 - \Box line graph (1786)
 - □ bar plot (1786)
 - pie chart (1801)



William Playfair (1759-1823)

William Playfair



"Chart showing at one view the price of the quarter of wheat & wages of labour by the week" (1821)

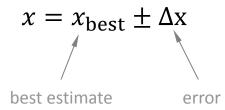
6. Quoting numbers and errors

"46.345% of all statistics are made up"

Anonymous

What is used to quantify errors

In a publication you typically quote:



- Error can be:
 - Standard deviation
 - Standard error of the mean
 - Confidence interval
 - Derived error
- Make sure you tell the reader what type of errors you use

Significant figures (digits)

- Significant figures (or digits) are those that carry meaningful information
- More s.f. more information
- The rest is meaningless junk!
- Quote only significant digits

Example

A microtubule has grown 4.1 µm in 2.6 minutes; what is the speed of growth of this microtubule?

 $\frac{4.1 \ \mu m}{2.6 \ min} = \ 1.576923077 \ \mu m \ min^{-1}$

- There are only two significant figures (s.f.) in length and speed
- Therefore, only about two figures of the result are meaningful: 1.6 μm min⁻¹

Which figures are significant?

Non-zero figures are significant	Number	Significant figures
Leading zeroes are not significant	365	3
□ 34, 0.34 and 0.00034 carry the same	1.893	4
amount of information	4 000	1 or 4
	4 ×10 ³	1
 Watch out for trailing zeroes 	4.000 ×10 ³	4
before the decimal dot: not significant	4000.00	6
after the decimal dot: significant	0.000 34	2
	0.000 3400	4

Rounding

- Remove non-significant figures by rounding
- Round the last s.f. according to the value of the next digit

 \square 0-4: round down (**1.3**42 \rightarrow 1.3)

- \square 5-9: round up (**1.3**56 \rightarrow 1.4)
- So, how many figures are significant?

Suppose we have 2 s.f. in each number

Raw number	Quote
12 34	1200
12 87	1300
1.4 91123	1.5
1.4 49999	1.4

Error in the error

- To find how many s.f. are in a number, you need to look at its error
- Use sampling distribution of the standard error
- Error in the error is

 $\Delta SE = \frac{SE}{\sqrt{2(n-1)}}$

This formula can be applied to SD and CI

Example

n = 12

$$SE = 23.77345$$

 $\Delta SE = 5.1$

- True SE is somewhere between 18.7 and 28.8 (with ~68% confidence)
- We can trust only one figure in the error
- Round *SE* to one s.f.:

SE = 20

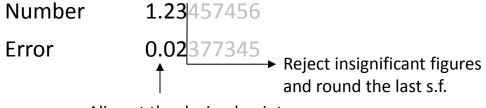
Error in the error

n	$\frac{\Delta SE}{SE}$	s.f. to quote
10	0.24	1
100	0.07	2
1,000	0.02	2
10,000	0.007	3
100,000	0.002	3

 An error quoted with 3 s.f. (2.567±0.165) implicitly states you have 10,000 replicates

Quote number and error

- Get a number and its error
- Find how many significant figures you have in the error (typically 1 or 2)
- Quote the number with the same decimal precision as error



```
Align at the decimal point
```

Correct	Incorrect
1.23 ± 0.02	1.2 ± 0.02
1.2 ± 0.5	1.23423 ± 0.5
6.0 ± 3.0	6 ± 3.0
75000 ± 12000	75156 <u>+</u> 12223
$(3.5 \pm 0.3) \times 10^{-5}$	$3.5 \pm 0.3 \times 10^{-5}$

Error with no error

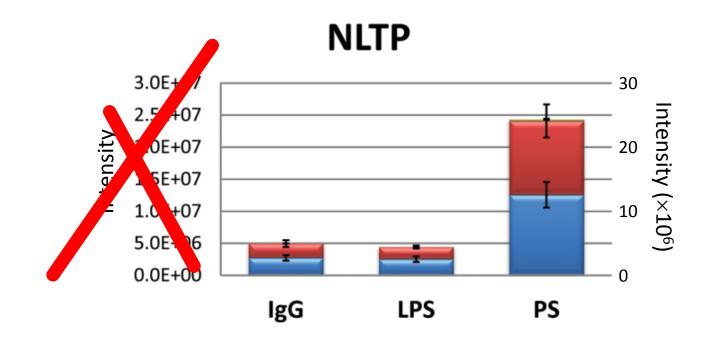
- Suppose you have a number without error
- Go back to your lab and do more experiments)
- For example
 - \square Centromeres are transported by microtubules at an average speed of 1.5 μ m/min
 - $\hfill\square$ The new calibration method reduces error rates by ~5%
 - $\hfill\square$ Transcription increases during the first 30 min
 - Cells were incubated at 22°C
- There is an implicit error in the last significant figure
- All quoted figures are presumed significant

Avoid computer notation

Example from a random paper off my shelf "p-value = 5.51E-14"

I'd rather put it down as

p-value = 6×10^{-14}



Fixed decimal places

- Another example, sometimes seen in papers
- Numbers with fixed decimal places, copied from Excel
- Typically fractional errors are similar and we have the same number of s.f.

raw data	1 decimal
	place
14524.21	14524.2
2234.242	2234.2
122.1948	122.2
12.60092	12.6
2.218293	2.2
0.120024	0.1
0.021746	0.0

Assume there are only 2 s.f. in these measurements

How to quote numbers (and errors)

WHEN YOU KNOW ERROR

- First, calculate the error and estimate its uncertainty
- This will tell you how many significant figures of the error to quote
- Typically you quote 1-2 s.f. of the error
- Quote the number with the same precision as the error
 - $\square 1.23 \pm 0.02$
 - □ 1.23423 ± 0.00005 (rather unlikely in biological experiments)
 - $\square 6 \pm 3$
 - □ 75 ± 12
 - \Box (3.2 ± 0.3) × 10⁻⁵

WHEN YOU DON'T KNOW ERROR

- You still need to guesstimate your error!
- Quote only figures that are significant, e.g.
 p = 0.03, not p = 0.0327365
- Use common sense!
- Try estimating order of magnitude of your uncertainty
- Example: measure distance between two spots in a microscope
 - □ Get 416.23 nm from computer software
 - Resolution of the microscope is 100 nm
 - 🗆 Quote 400 nm

Rounding numbers 0-4: down (6.64 \rightarrow 6.6) 5-9: up (6.65 \rightarrow 6.7)



Hand-outs available at http://is.gd/statlec

Please leave your feedback forms on the table by the door



