

**Marine Scotland**

# The D2 Standard and Sample Analysis



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# Background

- **Assume samples have been collected in relation to the D2 standard size ranges**
- **Overview of methods**
- **Sources of error**
- **Discussion of variation in analysis**
- **Brief statistical overview**



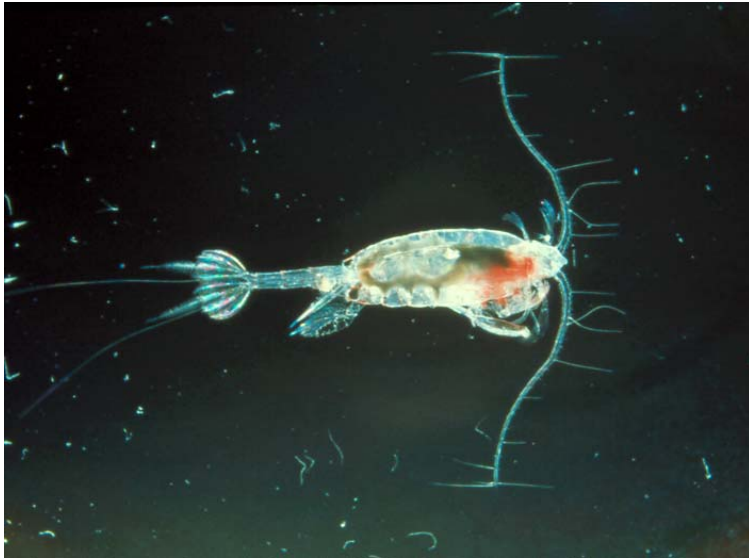
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## “Traditional Methods”

- **Based on preserved samples**
- **Identifying and counting species**
- **Usually carried out over a long time period**
- **Well established methods and statistics**





## Methods for the D2 Standards

- **Adaptation of traditional methods**
- **Assessment of viability**
- **Measurements of minimum dimension**
- **Proof of compliance with standard**
- **Different methods of sample handling**

# Sample analysis stages

- **Measurements**
- **Viability**
- **Counts**
- **Statistical considerations**



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[www.mdsg.umd.edu/issues/chesapeake/food\\_web/](http://www.mdsg.umd.edu/issues/chesapeake/food_web/)

## Measurements

- Should be based on body size
- Need to account for three dimensional structures
- Rely on mesh to separate into size categories?

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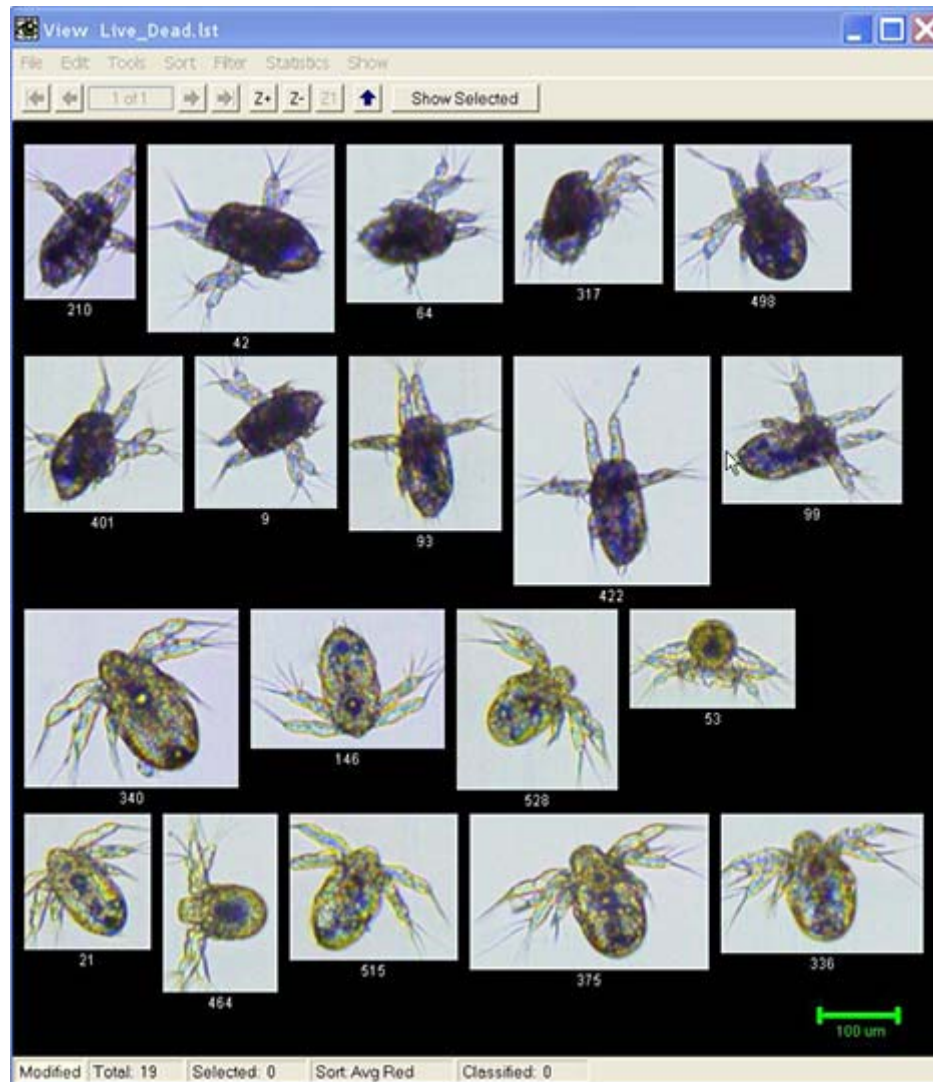
## Methods of measuring

- **Manually using an eyepiece graticule**
- **Automatically using image capture and specially designed software**
- **Automatically as part of the method  
e.g. flow cytometer**



## Measuring Problems

- **Some cells are tricky to measure when moving**
- **Manual measuring is time consuming and can affect sample analysis**
- **Automatic measuring would need to be carefully calibrated**



Zooplankton stained with Neutral Red (dead on top, live on bottom)

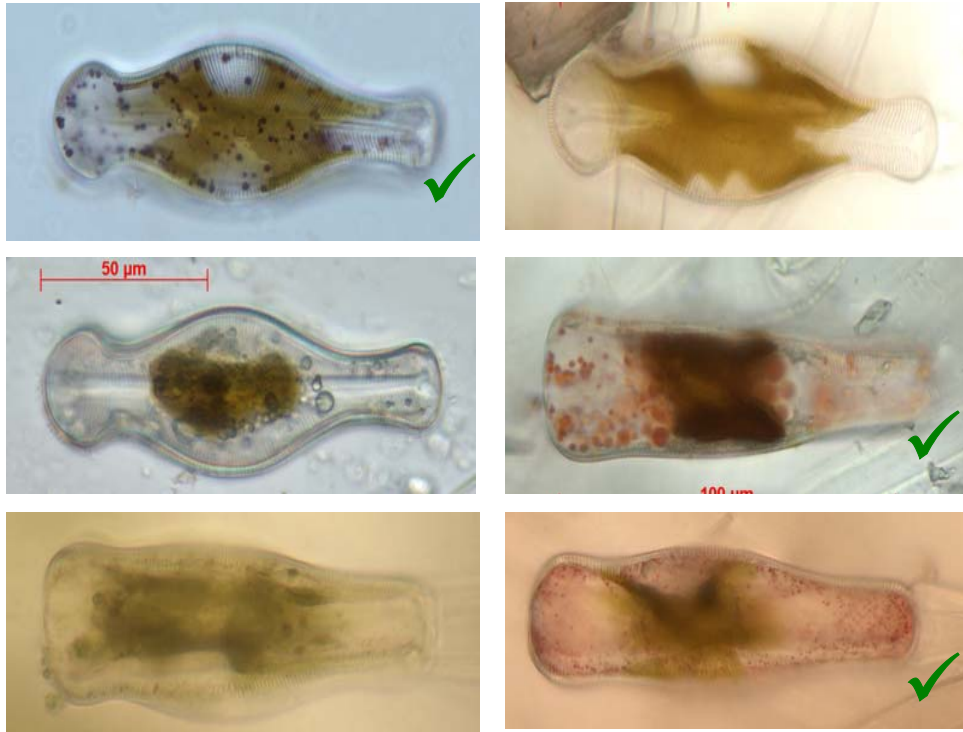
[www.fluidimaging.com/image-gallery.aspx](http://www.fluidimaging.com/image-gallery.aspx)

## Viability

- Movement
- Vital or mortal stains
  - Neutral Red
  - Sytox Green
- Flow cytometry
  - Automated
  - Can be combined with a camera
- All have limitations

# Measurements of movement

- **Can be subjective**
- **Is very time consuming**
- **Could be used in combination with a stain**
- **Can require trained analyst**



Kilroy et al. 2006 NIWA Project MAF 06506

## Staining

- Has to be targeted
- Can be variable
- Analysis has to be carried out rapidly
- Can be used in combination with automated systems

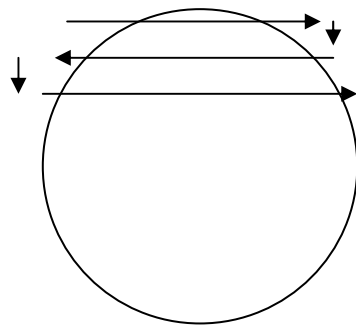
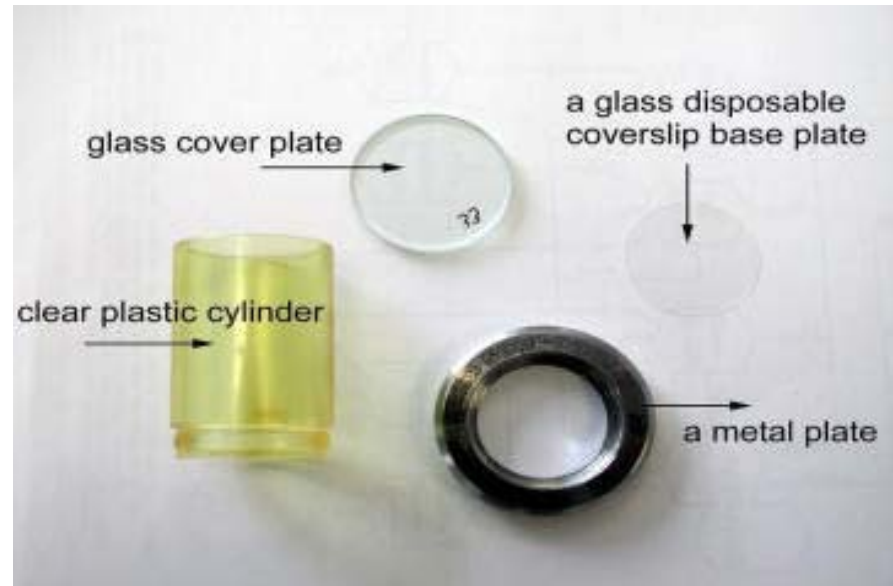
# Example of one method

- **Utermöhl settling method**
  - Widely used
  - Well established counting procedure
  - Intercalibration tests carried out
  - Provides example of level of variability

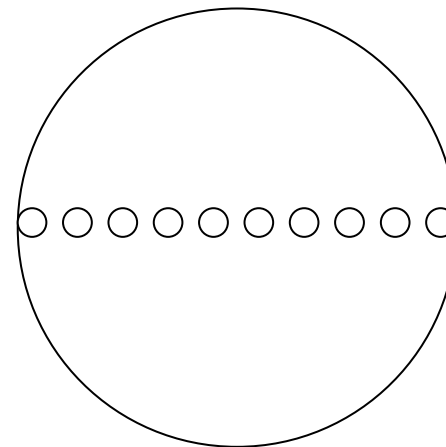
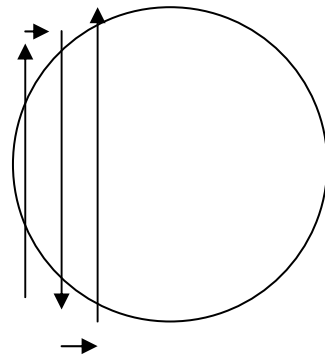




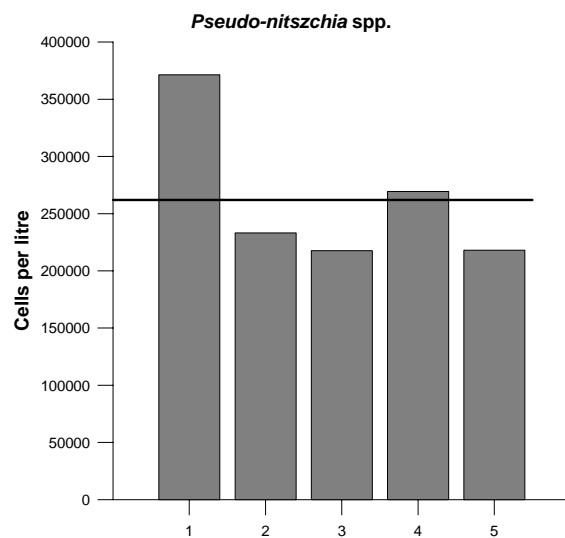
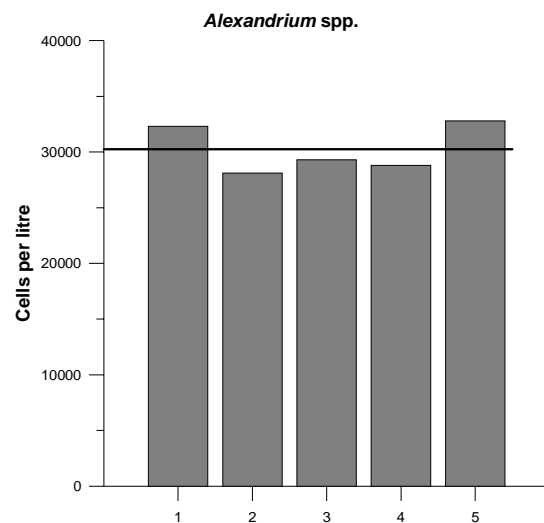
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Counting a whole base plate



Counting fields of view

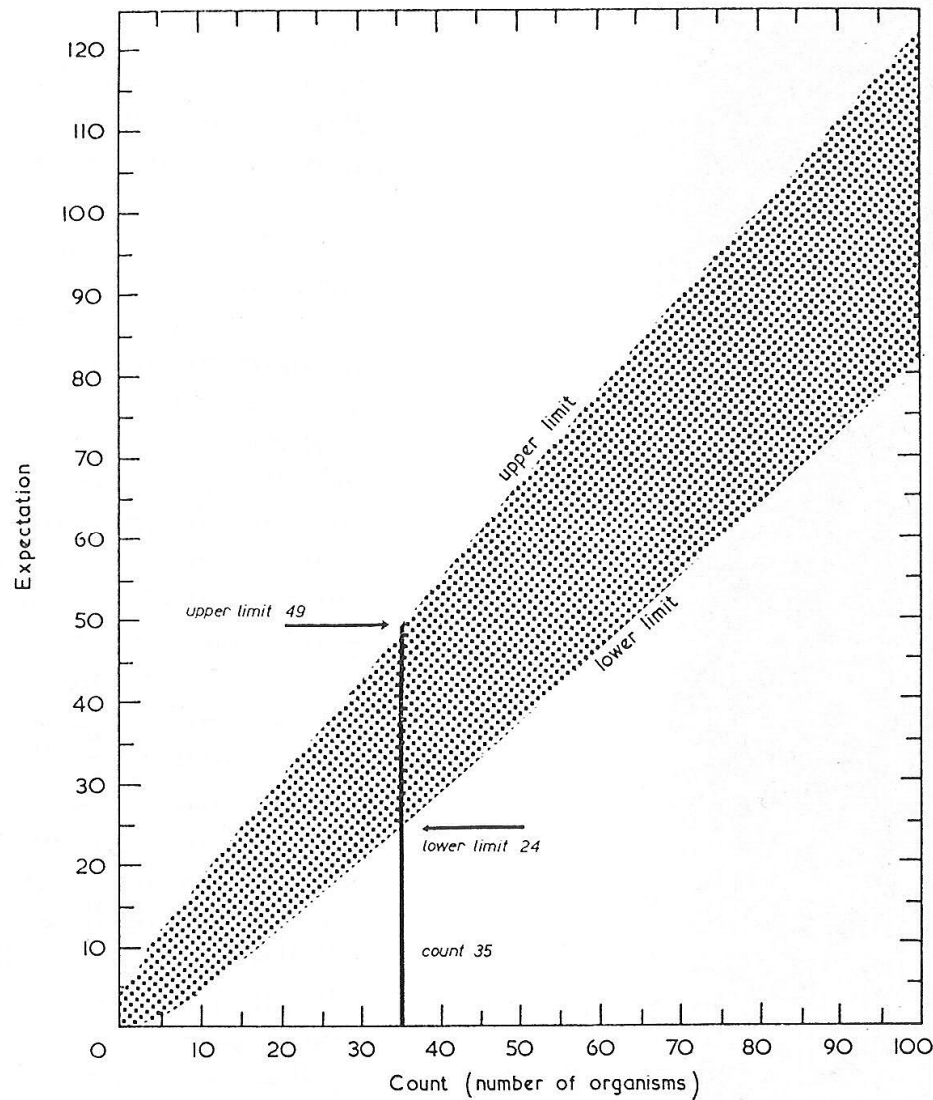


## Counting variability

- Between samples
- Between analysts
- Uptake of stains
- Limit of detection

# How many cells to count?

- Need  $< 10$  per  $m^3$  or  $< 10$  per ml
- How many replicates to ensure results are statistically robust?
- Has to be realistic and practical
- Automated methods would help



Lund (1958)

## How many cells to count?

- Standard statistical tests
- Will always be variability
- Balance between time spent counting and improvement in accuracy of result



**The effect of sample volume on 95% confidence limits of observed counts, assuming a Poisson distribution with mean equal to 10**

Sample (ml)	Observed Count	Lower 95% CI	Upper 95% CI	Scaled Volume (ml)	Lower 95% CI	Upper 95% CI
1	10	4.8	18.4	1	4.8	18.4
5	50	37.1	66.0	1	7.4	13.2
10	100	81.8	122.0	1	8.2	12.2
25	250	220.0	284.0	1	8.8	11.4
50	500	458.0	546.0	1	9.2	10.9

**Probability of observing counts less than or equal to 10% below the mean,  
as the sample size increases**

<b>Volume</b>	<b>Count</b>	<b>Probability of Observing</b>	<b>Count</b>	<b>Probability of Observing</b>
1	$\leq 9$	0.458	$\geq 10$	0.542
5	$\leq 45$	0.267	$\geq 46$	0.733
10	$\leq 90$	0.171	$\geq 91$	0.829
25	$\leq 225$	0.059	$\geq 226$	0.941
50	$\leq 450$	0.012	$\geq 451$	0.988

# Summary

- **Constant improvements in analysis methods**
- **Proof of non compliance close to the discharge standard will be challenging**
- **Experience from land based tests will inform the process**

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Questions?



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