Counting Platelets

21st ITP Annual Convention

And

International Global Alliance Meeting

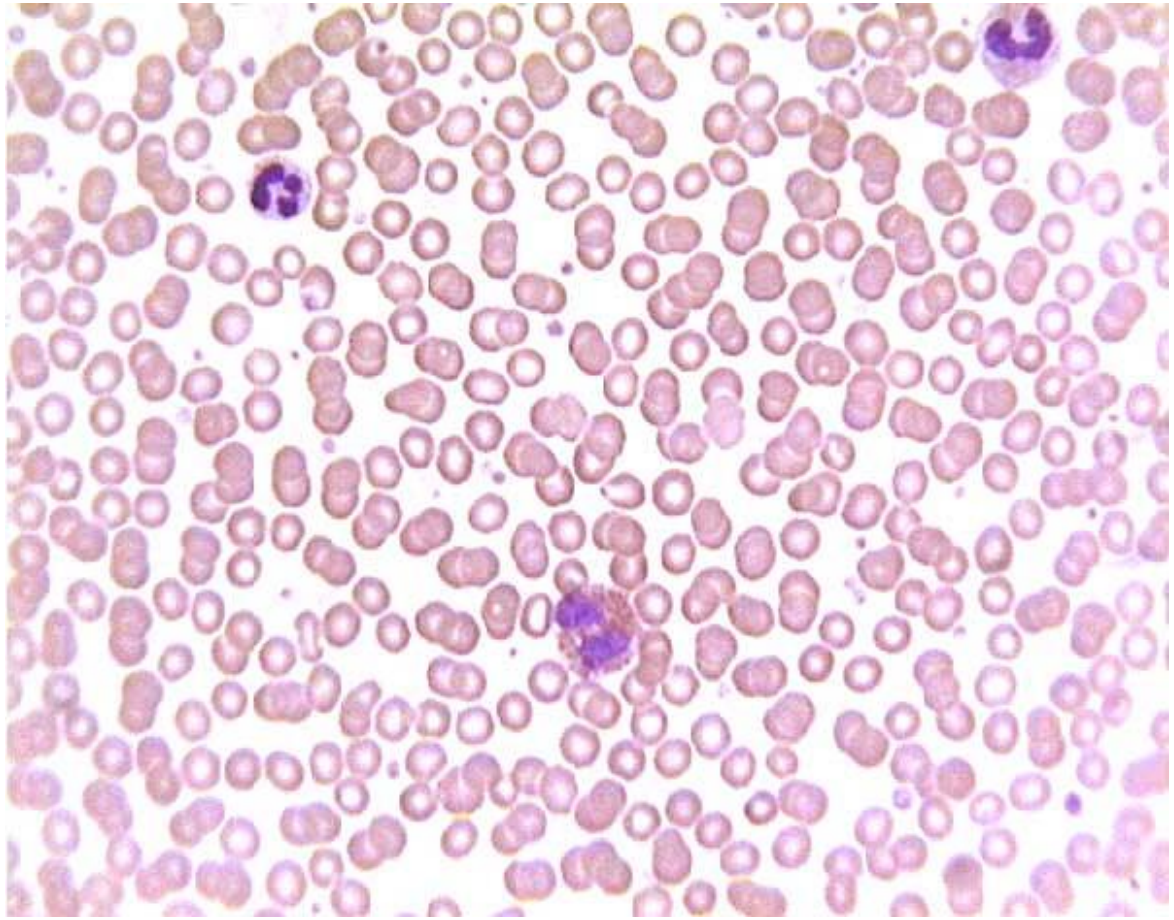
Roodee Racecourse Chester

Saturday 27th October 2018

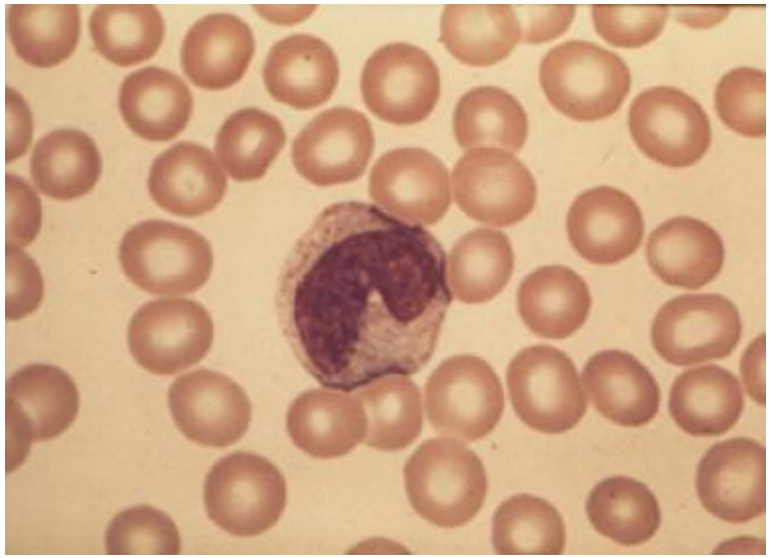
Is this good enough?

- Before we were able to easily, accurately and reliably count platelets it was usual to make comments on the platelet appearance on a blood film. Such as platelets appear normal in size and number.

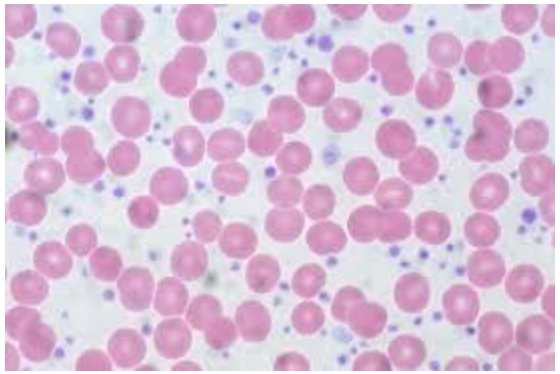
Normal



ITP



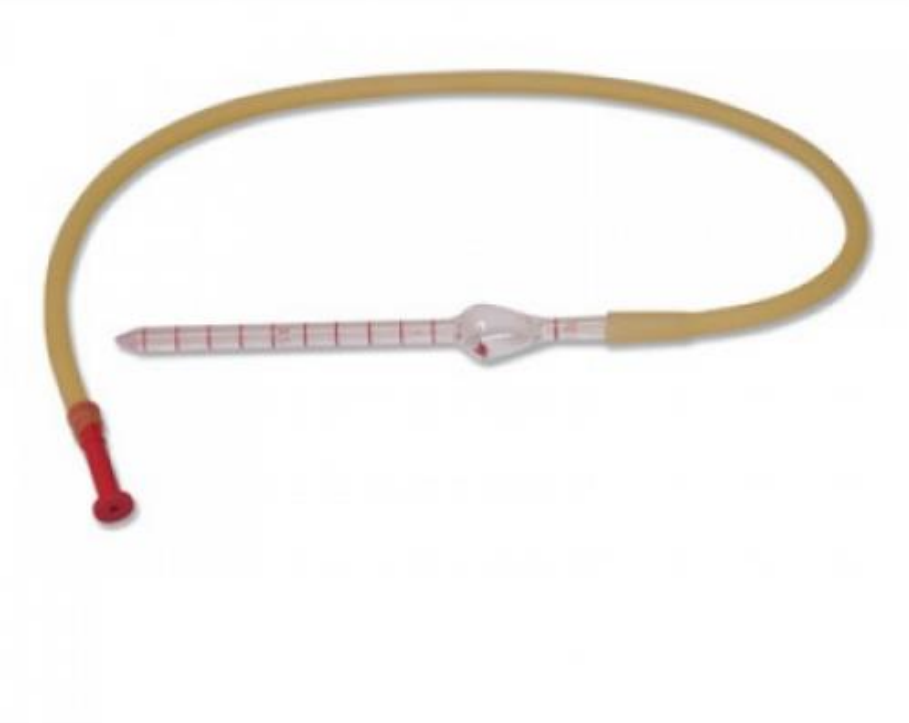
ET (Essential Thrombocythemia)



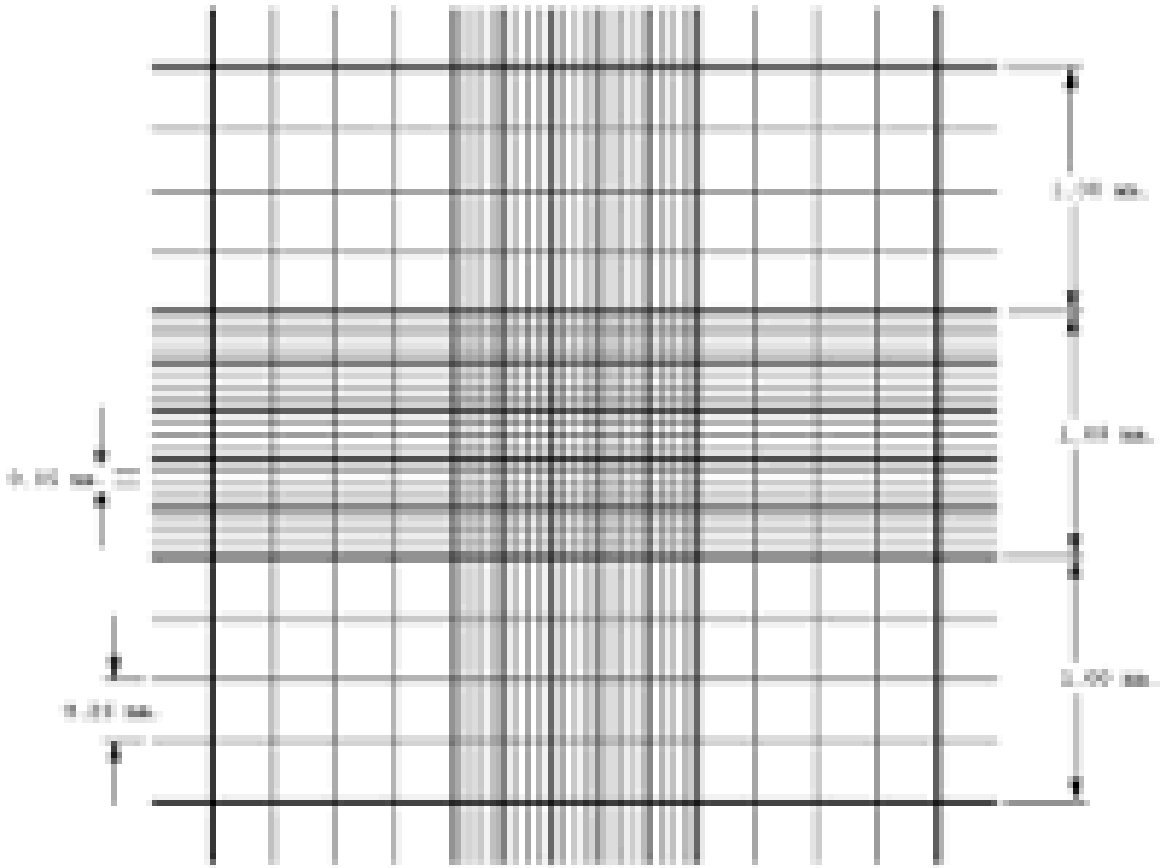
Is this scientific?

- This was not thought scientific, so methods were developed for the visual counting of platelets.
- This process involved:-
- Collecting the blood sample
- Make a manual dilution in the pipette and leave to stand for 10 minutes.
- Mix the sample and put into the haemocytometer (counting chamber) place it into a petri dish with moist cotton wool and leave to settle for 10 minutes
- Count by eye all 25 squares of the grating

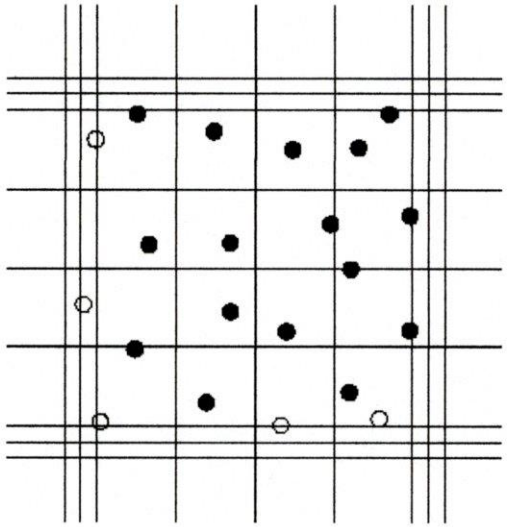
Platelet Pipette



Haemocytometer Grating



What’s in and what’s out



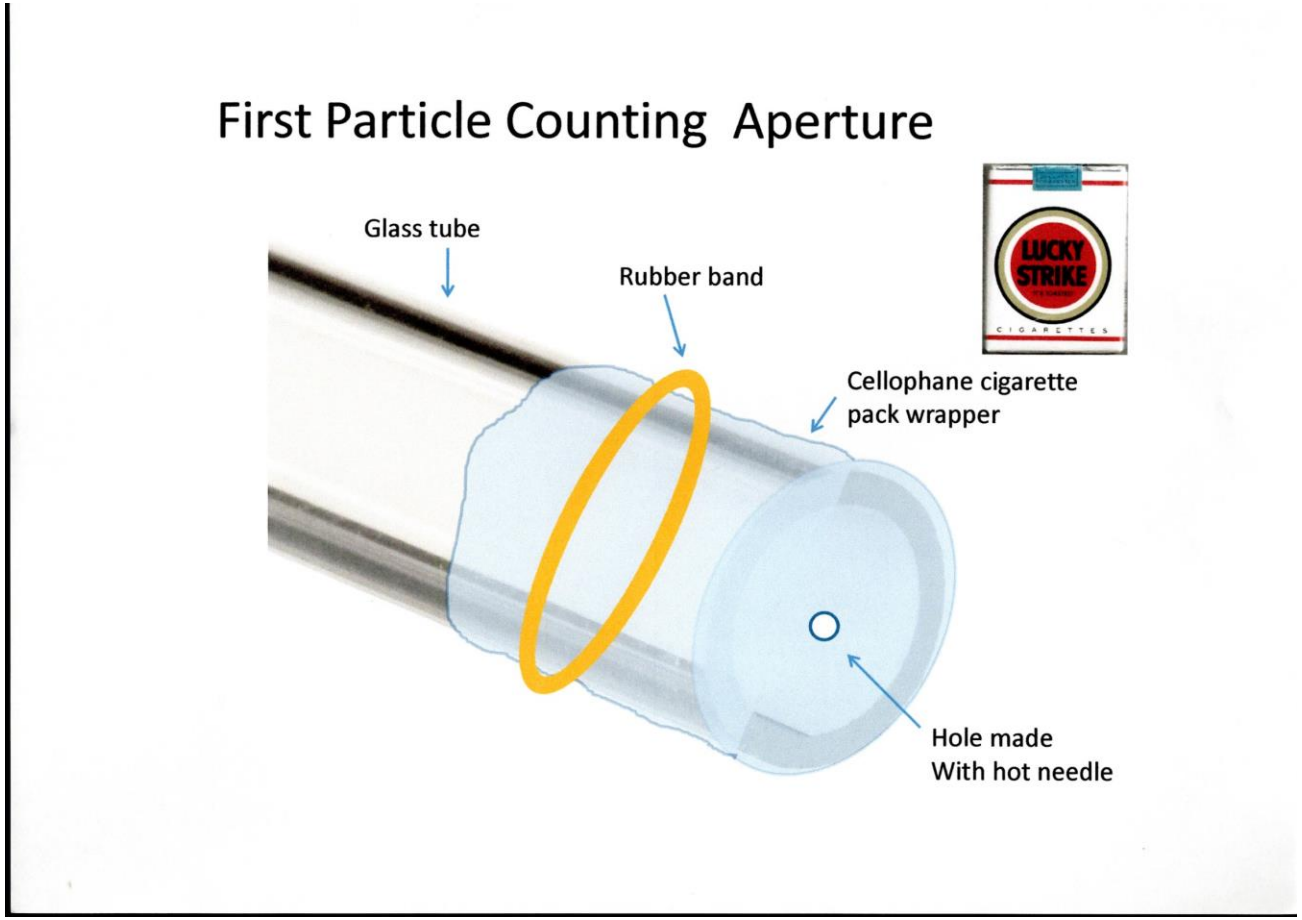
How bad can it be?

- As you may now appreciate manual platelet counting is (or can be) inaccurate, imprecise, time consuming and unreliable.
- So to try and improve on this we could count both sides of the counting chamber
- 2 people would do the count
- More cells = better accuracy and precision
- What is the difference between accuracy and precision?

Meanwhile, back in the bat cave …….

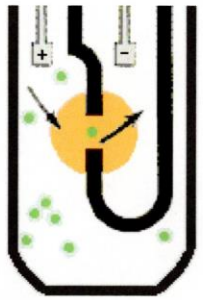
- Or in Wallace Coulters' garage with the help of his younger brother Joseph.
- Wallace was an electronics enthusiast who worked for General Electric, Joseph worked for Motorola.
- He was working on a problem of paint adhesion to the hulls of battleships thought to be due to the paint particle size/variation in size.
- He then created or invented this ……

What the!?


First Particle Counting Aperture
Glass tube
Rubber band
Cellophane cigarette
pack wrapper
LUCKY STRIKE
CIGARETTES
Hole made
With hot needle

How does this work?

Particle Counting Diagram

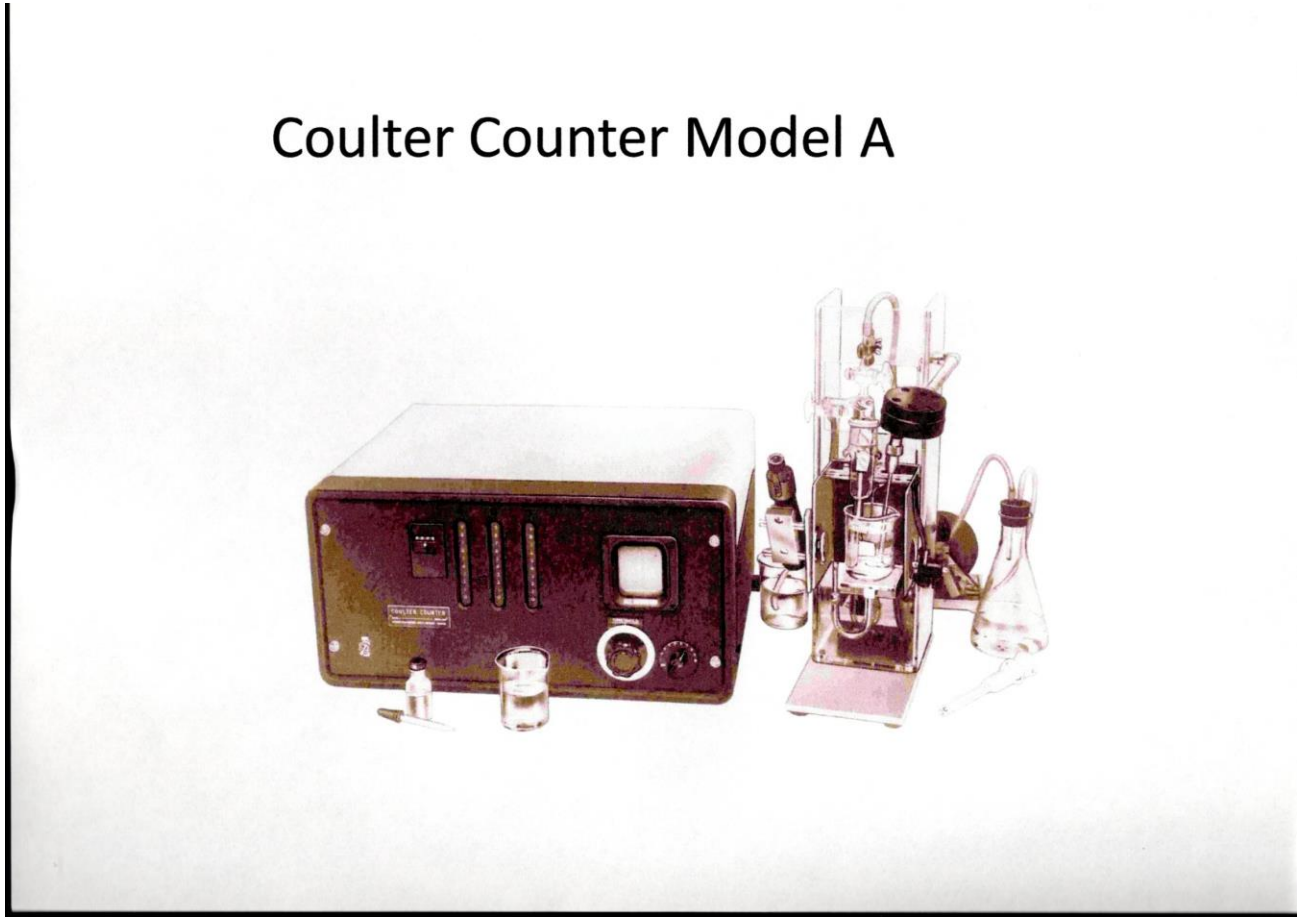


Particles passing through the aperture interrupt electricity flow through the liquid between electrodes, producing electrical pulses

Patent it Wallace

- Having created his particle counter he then went to patent it.
- Often told “you can’t patent a hole”
- But, he managed to find a lawyer to take it on and in August 1949 he filed his patent.
- The patent was issued October 20th 1953 (65 years and one week ago today)
- This has since become known universally as **The Coulter Principle**

Model A



A Coulter Model A on display at the Beckman Coulter Headquarters High Wycombe

Formal Announcement of the Coulter Principle

- At the National Electronics Conference, Chicago October 3rd 1956
- Wallace presented his paper “High speed automatic blood cell counter and cell size analyser”
- Intro: “it is difficult to overstate the need for instrumentation to reduce the tedium of visual cell counting and to increase the accuracy of the counts”
- He described the operating principles and the advantages of his new system

Faster

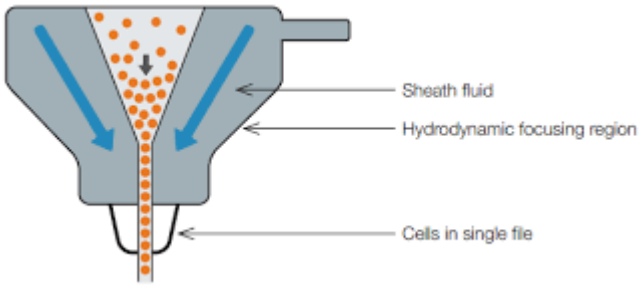
- Counting rate of in excess of 6,000 cells per second
- Counts individual cells and provides a size distribution
- Reducing statistical error by a factor of 10
- Counting time reduced from 30 minutes to 15 seconds

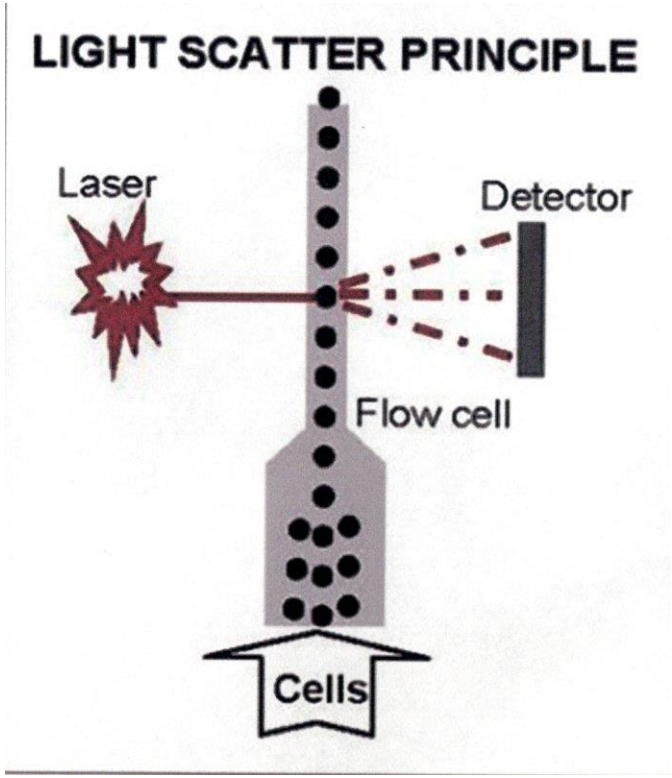
Platelets late to arrive at the party

- It was not until the late 1970's that platelet counting became automated with the Coulter model S Plus (semi Automated)
- This analyser now has 3 counting apertures
- Improved accuracy and precision

The other way to count particles

- Was by using light scatter





Old light scatter method

- The first light scatter methods used ‘pure white’ light. Laser was an eighties development
- As you may notice the principle is to count shadows
- Early analysers using this method could not differentiate platelets from white blood cells and would report Platelets (including wbcs)
- Platelet reference range 150-450
- White blood cell reference range 4.0-11.0
- So could have very little impact on the ‘real’ result

Improving competition

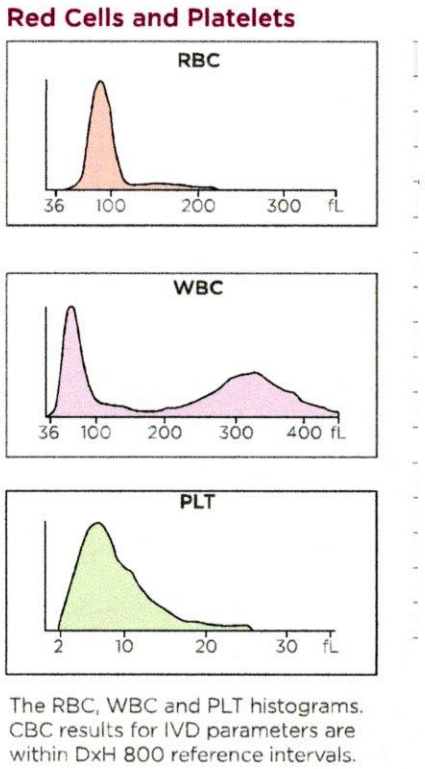
- Nowadays light scatter methods employ the use of laser light
- This now allows the measurement of the particle size, we can now separate platelets from white cells, giving us a more accurate platelet count

UniCel DxH 800Coulter Cellular Analysis System

- Warning serious techy speak

- Platelet technology has evolved over the last 30 years, utilizing the speed of modern microprocessors to drive dynamic and flexible algorithms capable of performing pattern-specific analysis on data from multiple sources. The UniCel DxH Coulter Cellular Analysis System employs these advanced algorithms to increase both accuracy and flagging efficiency.

Platelet Histograms



The RBC, WBC and PLT histograms. CBC results for IVD parameters are within DxH 800 reference intervals.

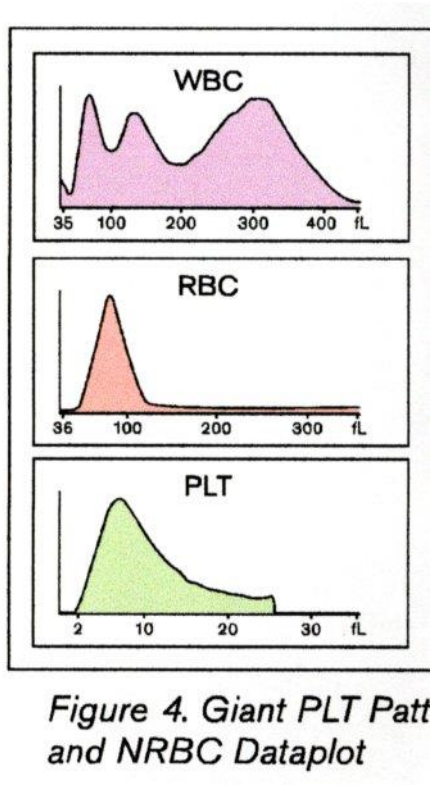


Figure 4. Giant PLT Patt and NRBC Dataplot

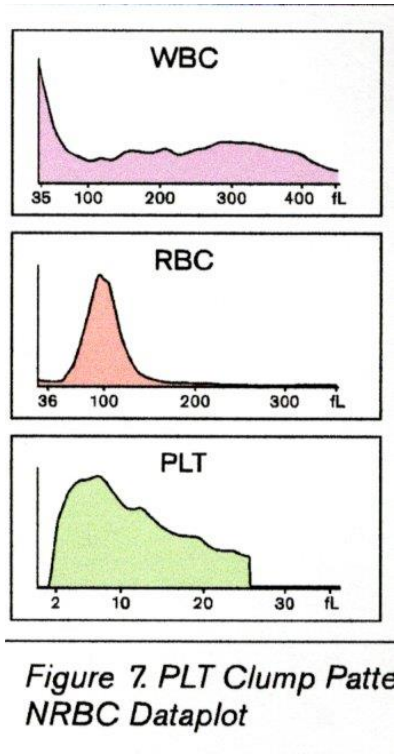


Figure 7. PLT Clump Patte NRBC Dataplot

Are you happy with the results?

Platelet Related Flags, Codes and Messages

Flags/Codes	
[xx.x] -	Result < instrument-defined measuring range
[xx.x] +	Result > instrument-defined measuring range
[xx.x] a	Result < or > user-defined action limits
[xx.x] c	Result < or > user-defined critical limits
[xx.x] D	Result < or > user-defined delta check limits
[xx.x] E	Edited result
[xx.x] e	Result recalculated from edited result
[xx.x] H	Result > user-defined reference range
[xx.x] L	Result < user-defined reference range
[xx.x] N	Non-blood specimen
[xx.x] P	Partial aspiration
[xx.x] R	Result requires review
[.....]	Result unavailable due to insufficient or unreliable data
[-----]	Result unavailable due to significant differences between the three apertures
[+++++]	Result > instrument-defined operating range
[xxxxx]	Result disabled

Definitive Messages	
Thrombocytopenia	PLT < user-defined lower limit
Thrombocytosis	PLT > user-defined upper limit
Small Platelets	MPV < user-defined lower limit
Large Platelets	MPV > user-defined upper limit

Suspect Messages	
Giant Platelets	Data pattern consistent with giant platelets

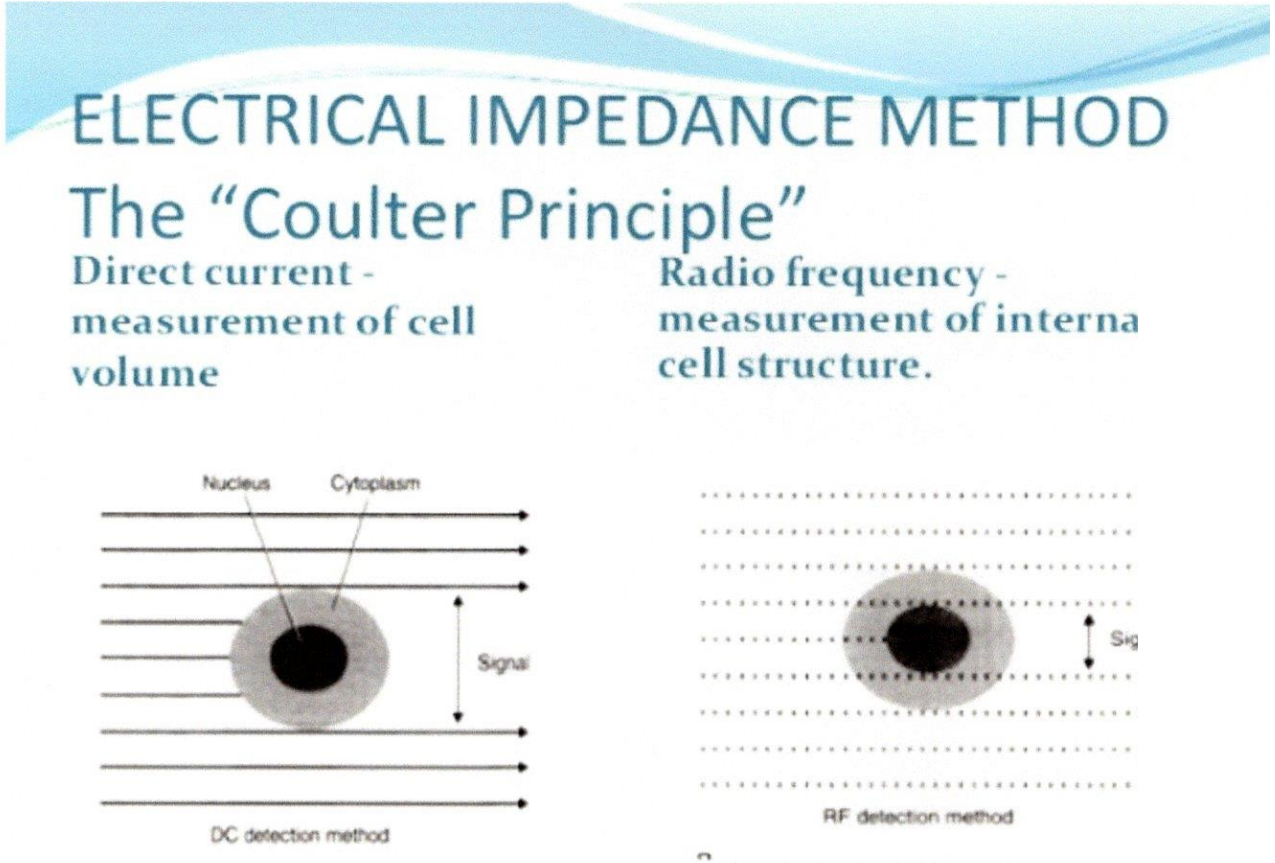
System Messages	
Low Events: PLT	Low confidence in platelet related parameters due to decreased platelet
Platelet Clumps	Data pattern consistent with platelet clumps
PLT Carryover	Potential for high to low platelet carryover
PLT Inter (Debris)	Data pattern consistent with low end interference
RBC-PLT Overlap	Data pattern consistent with high end interference
PLT Interference	Data pattern consistent with smaller and larger platelets
System Event: PLT	Hardware function exceeds limit

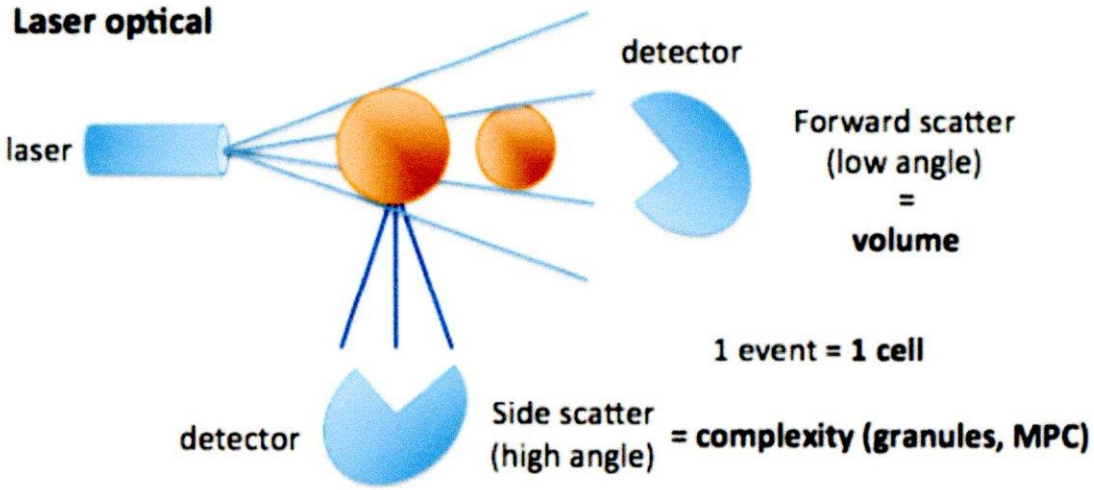
**PDW and Pct are for Research Use Only. Not for use in diagnostic procedures. They are printed and displayed with an ®.*

How long to do a platelet count?

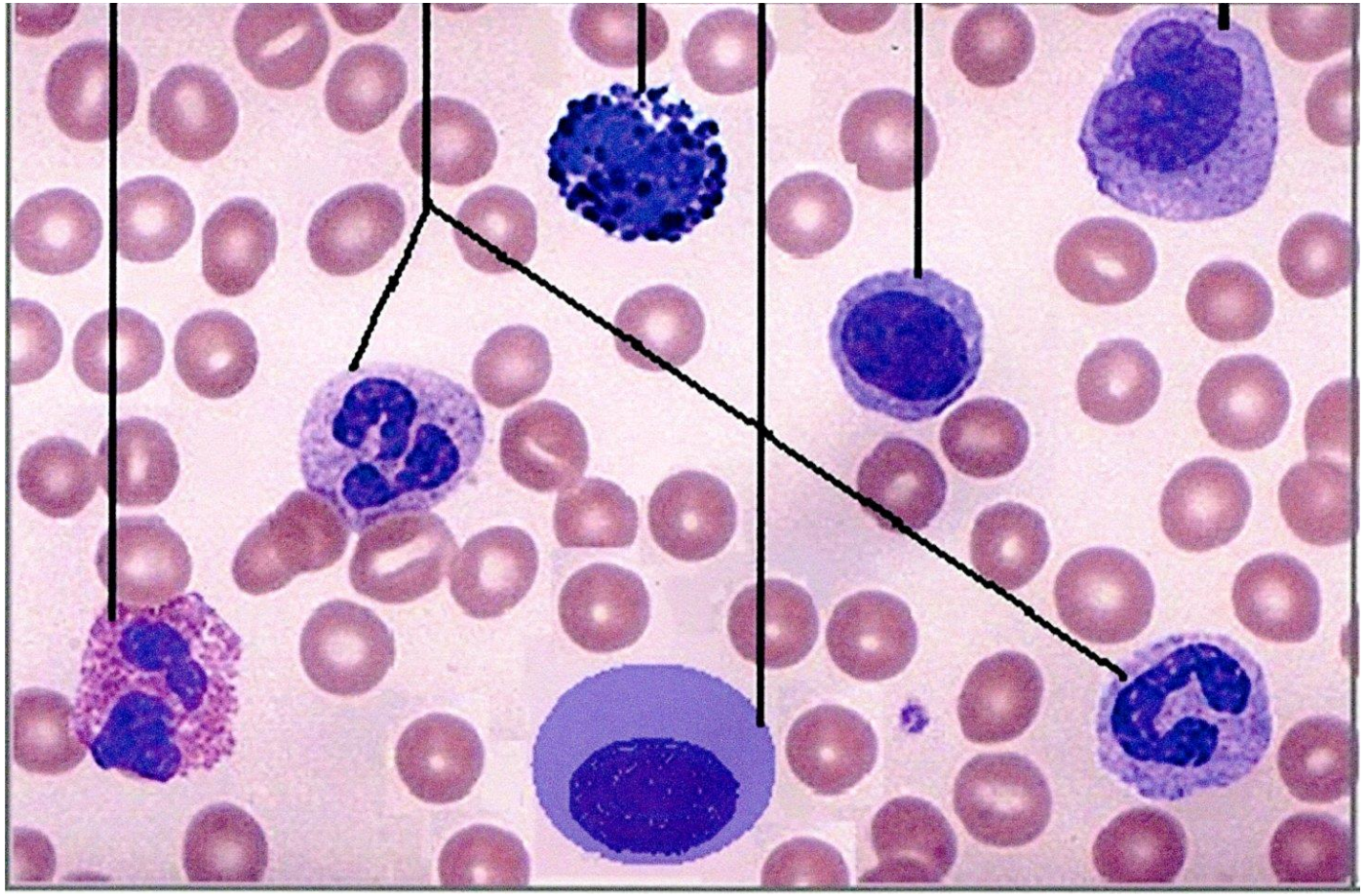
- For platelets there is a target minimum of 1800 cells in the platelet channel
- Low platelet counts however, the analyser will automatically extend the counting time in 1 second increments (up to 20 seconds) to meet this number.

Capable of performing pattern-specific analysis on data from multiple sources VCS.

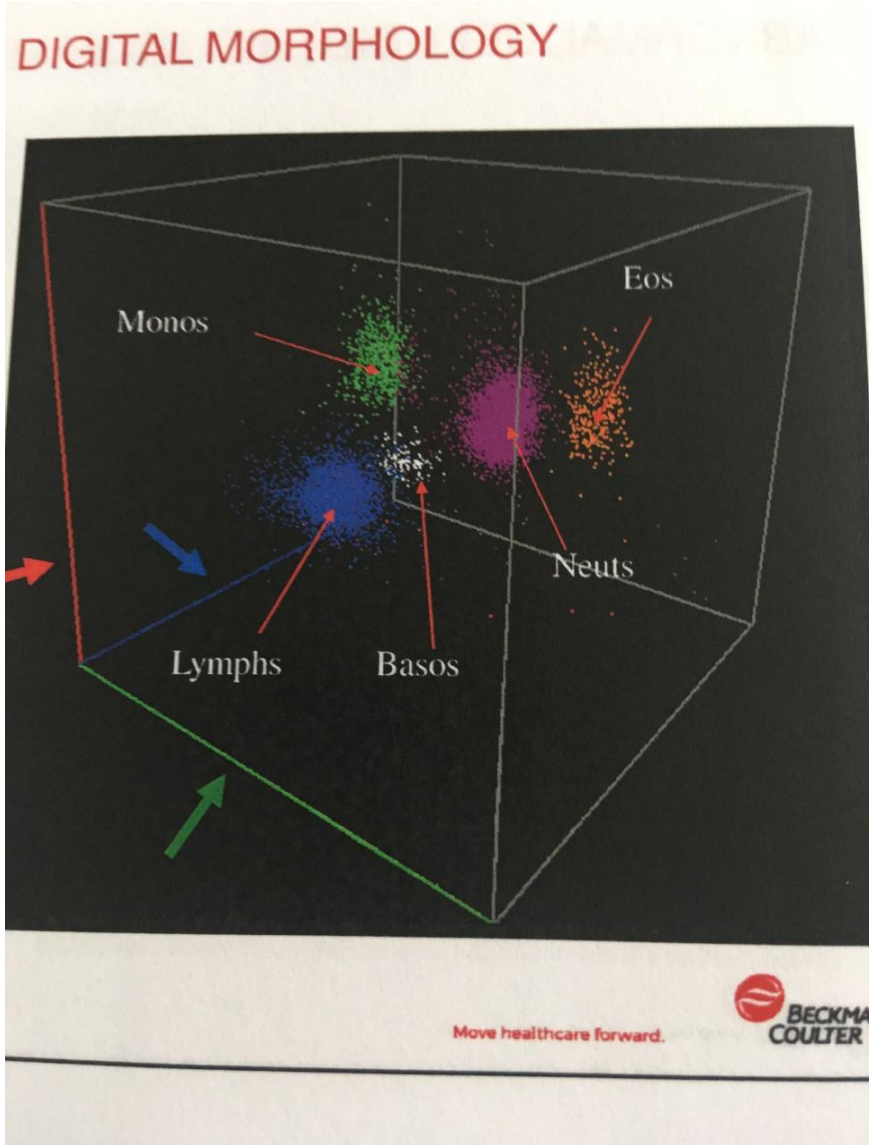




Why do we need all this information and what can we do with it?



Digital white cell differential using Volume, Scatter and Conductivity



What is it like in a pathology laboratory of a DGH?

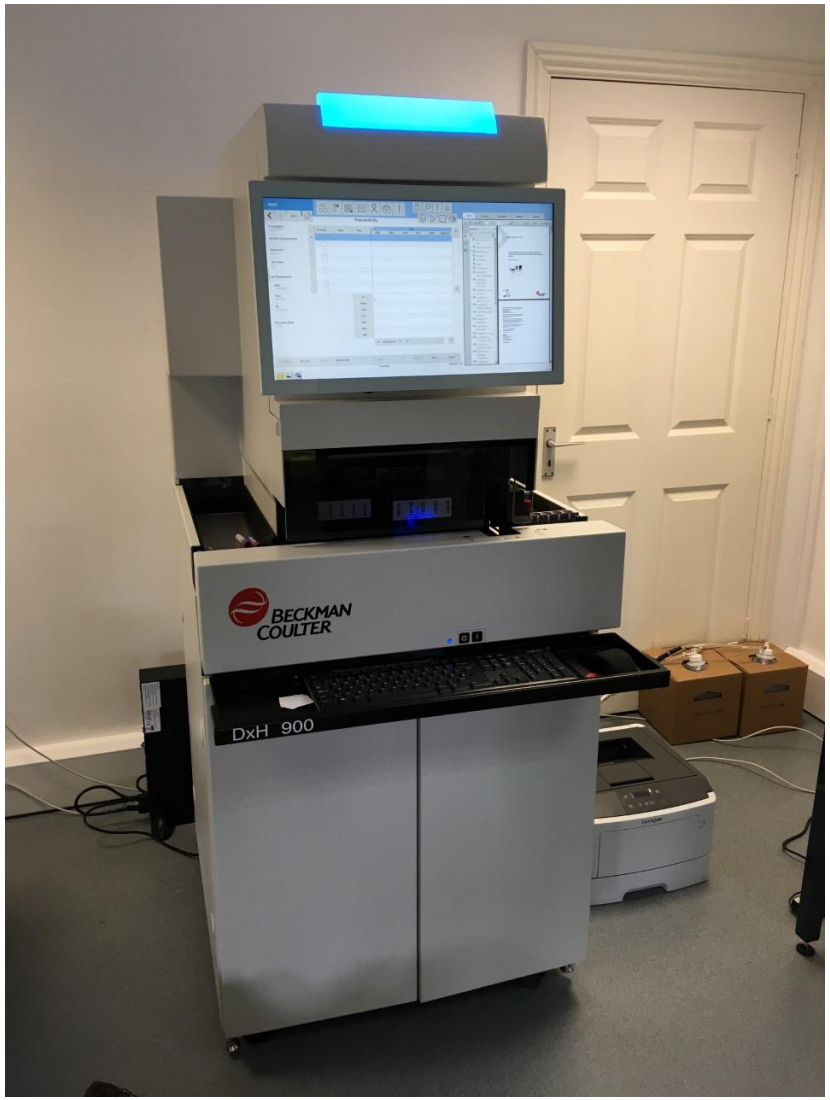
- What are your laboratory's opening hours?
- 09.00-17.30hrs Monday to Friday
- No 24/7 365 the lab is always staffed
- How many samples a day do we analyse?
- 900 - 1000 samples, most arrive after 16.00hrs from GP and outlying clinics
- How fast does the analyser work?
- Up to 100 samples per hour
- 1 hour = 3600 seconds divided by 100 = 36 seconds per sample

What is it like in a pathology laboratory of a DGH?

- Wait a minute …… 1000 samples 100 per hour = 10 hours work!
- And most of these come in after 16.00hrs
- How do you manage?
- More then one analyser and we are working after 17.30hrs
- Very often work load is not completed until 21-22.00hrs
- We are still looking after you after your clinic has closed and you've gone home

That is why we need two of these

The Future?



Immediate Future

- Turn your clocks and watches **back 1 hour** tonight