Footprint
43 cm W x 45 H cm x 63 depth

Coulter Counter

Instructor and Student Protocol

(Preliminary)

Link to Beckman Coulter Counter Z series Manual – pdf

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Coulter Counter: Basic Controls

Waste canister

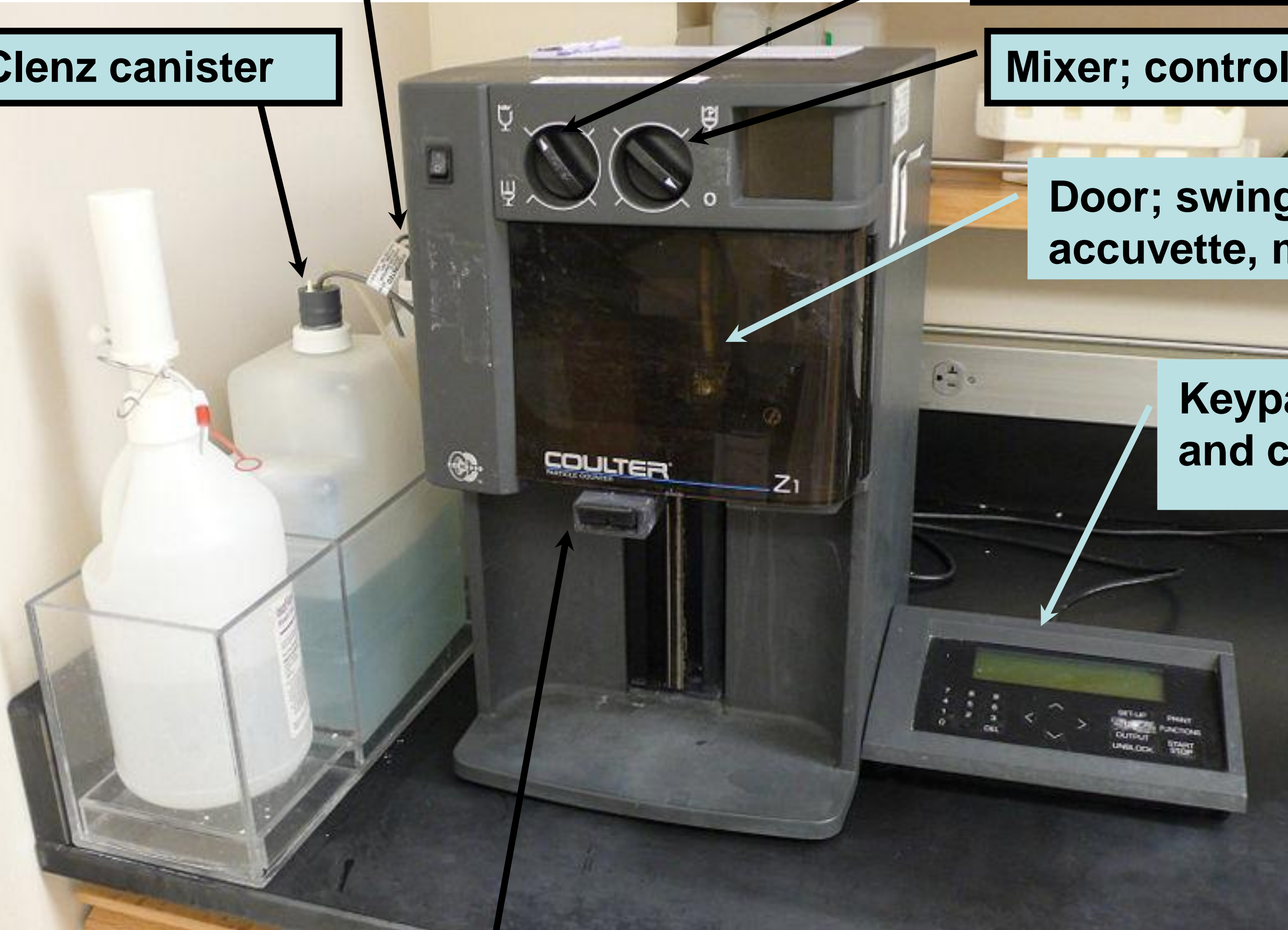
Raise/lower mixer in accuvette

Clenz canister

Mixer; control speed

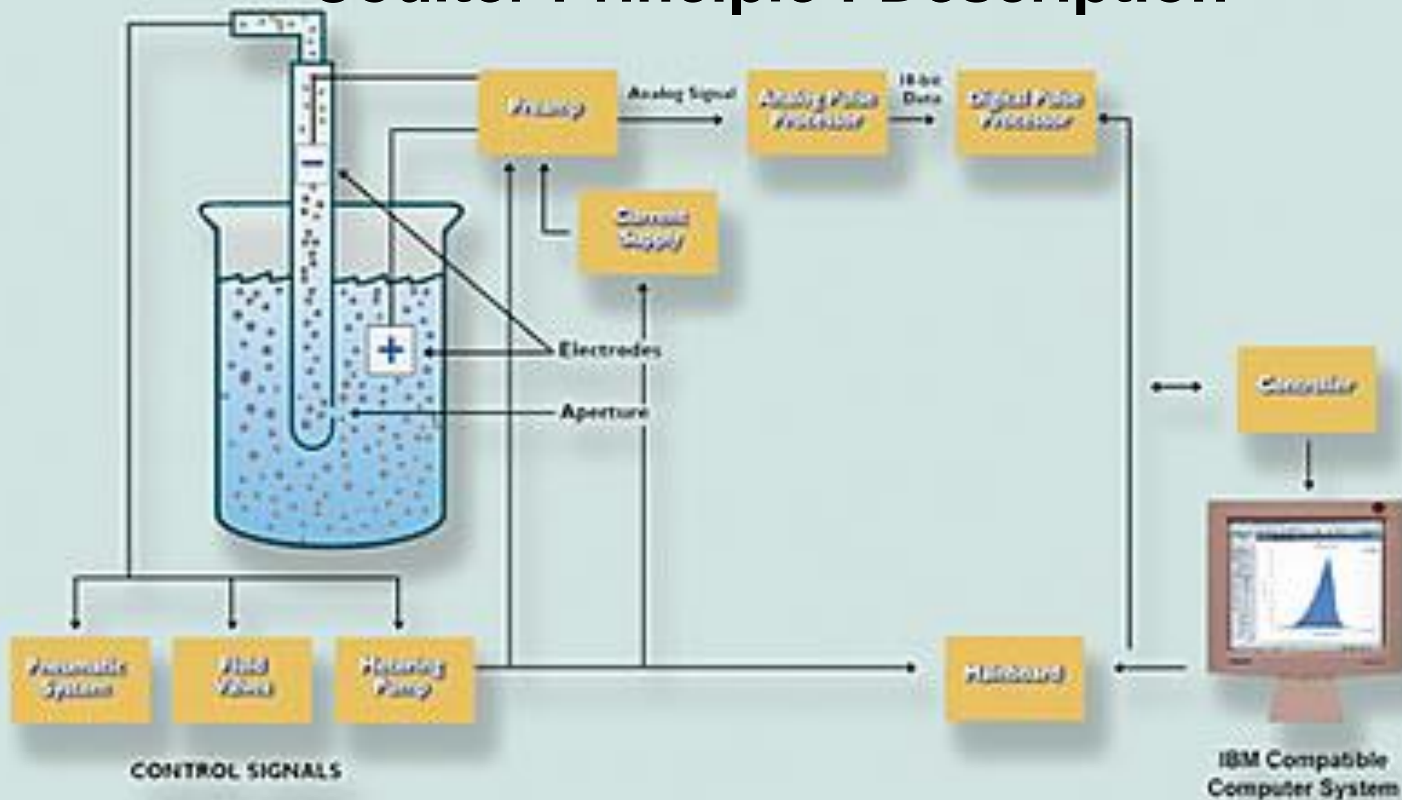
Door; swing open to access accuvette, mixer, and aperture

Keypad: Menu display and control activation



Push to raise/lower accuvette platform

Coulter Principle : Description



The Coulter Principle applied in the Beckman Coulter Multisizer™ 3

In a Coulter counter, a tube with a small aperture on the wall is immersed into a beaker that contains particles suspended in a low concentration electrolyte. Two electrodes, one inside the aperture tube and one outside the aperture tube but inside the beaker, are placed and a current path is provided by the electrolyte when an electric field is applied (Figure 1). The impedance between the electrodes is then measured. The aperture creates what is called a “sensing zone." Particles in low concentration, suspended in the electrolyte, can be counted by passing them through the aperture. As a particle passes through the aperture, a volume of electrolyte equivalent to the immersed volume of the particle is displaced from the sensing zone. This causes a short-term change in the impedance across the aperture. This change can be measured as a voltage pulse or a current pulse. The pulse height is proportional to the volume of the sensed particle. If constant particle density is assumed, the pulse height is also proportional to the particle mass. This technology thus is also called aperture technology.

Coulter Counter (CC) : Instructor Setup and Testing

1. The Aperture Tube should be immersed in an accuvette (AV) of Coulter Clenz solution (recommended short term storage method). Open the swing door for access to the AV. Lower the platform by depressing on the black button on the front and pulling down on the platform
2. Remove (lift) and throw away the Clenz AV solution and prepare another AV with 10uL of Coulter Isoton solution as a Blank. Gently mix this solution by inversion and place the AV on the platform in the slotted square hole.
3. Depress the black button and raise the platform so that the aperture tube and metal electrode are immersed in the AV solution. A light should come on when the platform is in the fully raised and locked position.
4. Observe the aperture opening in the small display in the upper right corner of the device. If necessary, open the swing door and adjust the focus knob.
5. Run the “Fill System” protocol using the Isoton solution.
6. Run the Isoton AV as a blank before student testing to confirm that particle count is low (<50).
7. During Student testing, periodically inspect the aperture hole to make sure it is not clogged. Rinse the aperture with DI water between student samples to avoid contamination. (Use a spray bottle and catch spill in extra AV).
8. Run the Isoton blank AV after every 5 samples to ensure that particle count is low.

Coulter Counter (CC) : Instructor Maintenance and Shutdown

NEVER LEAVE the Aperture exposed to air or in a sample with Cells for more than a few hours.

1. Prepare an AV with the Isoton solution of 20ml and place this on the platform.
2. Run a standard measurement using the Isoton solution.
3. For Maintenance of short term non-use (days) , periodically check the level of the solution in the AV and make sure the level is always above the actual aperture opening, replenish if necessary.
4. Run the Flush System protocol at least once per week.
5. For Maintenance during long-term non-use (weeks), prepare an AV with the Clenz AV solution and repeat steps 1-3 above.
6. Periodically replenish the AV to keep solution levels covering the aperture at all times.

Coulter Counter (CC) : Instructor Calibration Setup

1. Each Aperture tube has its own characteristic calibration constant, Kd. In general once this is determined and set within the device, it does not have to be redone unless the tube is improperly stored or contaminated with solutions that leave residue on the aperture.
2. To calibrate the aperture tube, use a CC Calibration latex particle solution selected within 5% to 20% of the aperture opening (found printed on the side of the aperture tube). The tube we currently have at FVCC is 100um (Part#9912786). A 10um Calibration Standard Particle solution (Part#6602796) is available as of Aug 2008 (RGM).
3. Prepare the calibration accuvette (AV) by extracting 10mL of the Isoton diluent from the storage bottle tap.
4. Add one drop of the Calibration Index solution to this AV, close the cap on the AV, and gently mix by inversion.
5. Open the CC swing door, push the black button, lower the platform, and place the Calibration solution AV into the platform hole. Return the platform to the top position and close the glass door.
6. A light should come on inside and a lens display of the aperture opening will appear in the upper right corner of the device. If the aperture opening is not clearly visible, open the swing door and use the focus knob to make adjustments.

Coulter Counter (CC) : Instructor Calibration Execution

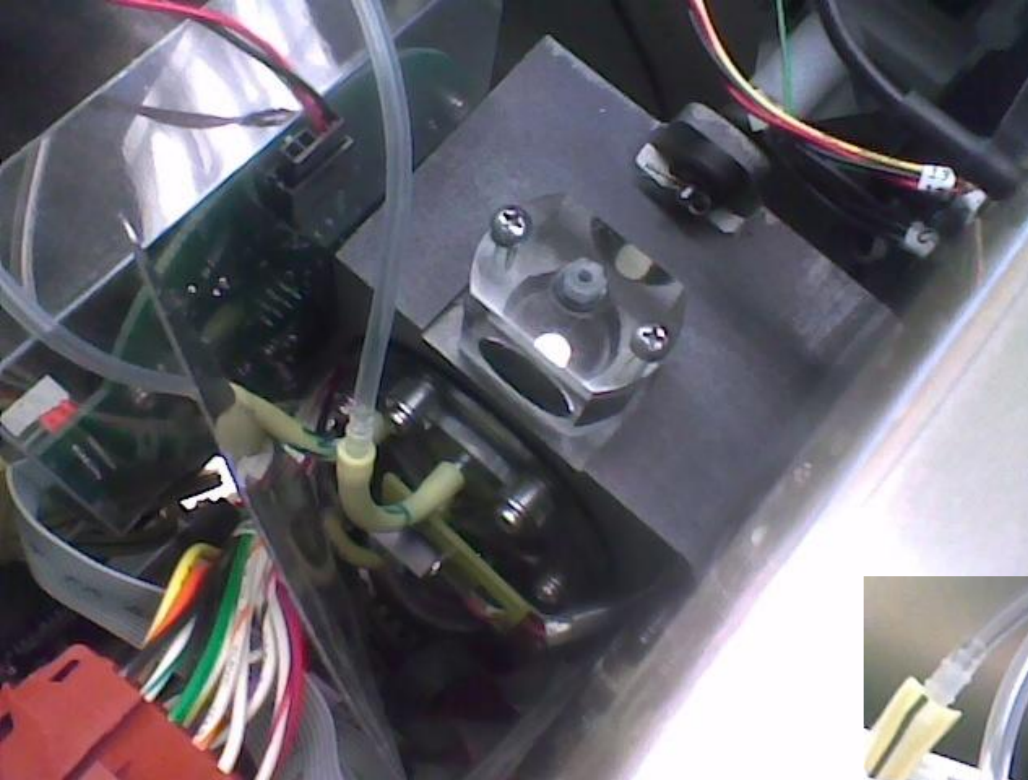
1. Press the [Setup] membrane button on the control panel. An S1: message will appear called [Enter size Data] showing the aperture diameter, current Kd, and lines for selecting units and setting the upper and lower size expected particle sizes. Use the ^ and v arrows to move to these lines and set new values if necessary. Since the NIST standard Calibration solution is non-biological, set the units as “um”.
2. Press [Setup} again and an menu S2: [Enter Analysis Data] appears. If a Kd already exist in the device for this tube diameter, it will be displayed. Make sure the “Metered Volume” is set to .5 for a 100um aperture tube.
3. Press [Setup] to return to the S1 screen, then press [CAL] and the C1 screen will display. Use the arrow keys to set the “Calibrator Size” value to that of the provided calibrated solution , 10 um. Make sure the “Measure Aperture” line is set to “YES” to force a remeasurement of the aperture paramenter.
4. Press [Start] and a C4: Calibration screen will appear with various messages ending in a C3: “Instrument settings” menu.
5. Press [Start] and a c6: Calibrate screen appears while the calibration is being accomplished. Follow subsequent instructions and/or refer to the device manual Section 3.
6. If the Calibration is successful, and C7: Calibration Factor screen will display with the new calculated Kd value. Press the [Start] button to overwrite and store this value. Press [CAL] if you do not wish to store the new Kd value.

Coulter Counter (CC) : Student Operation

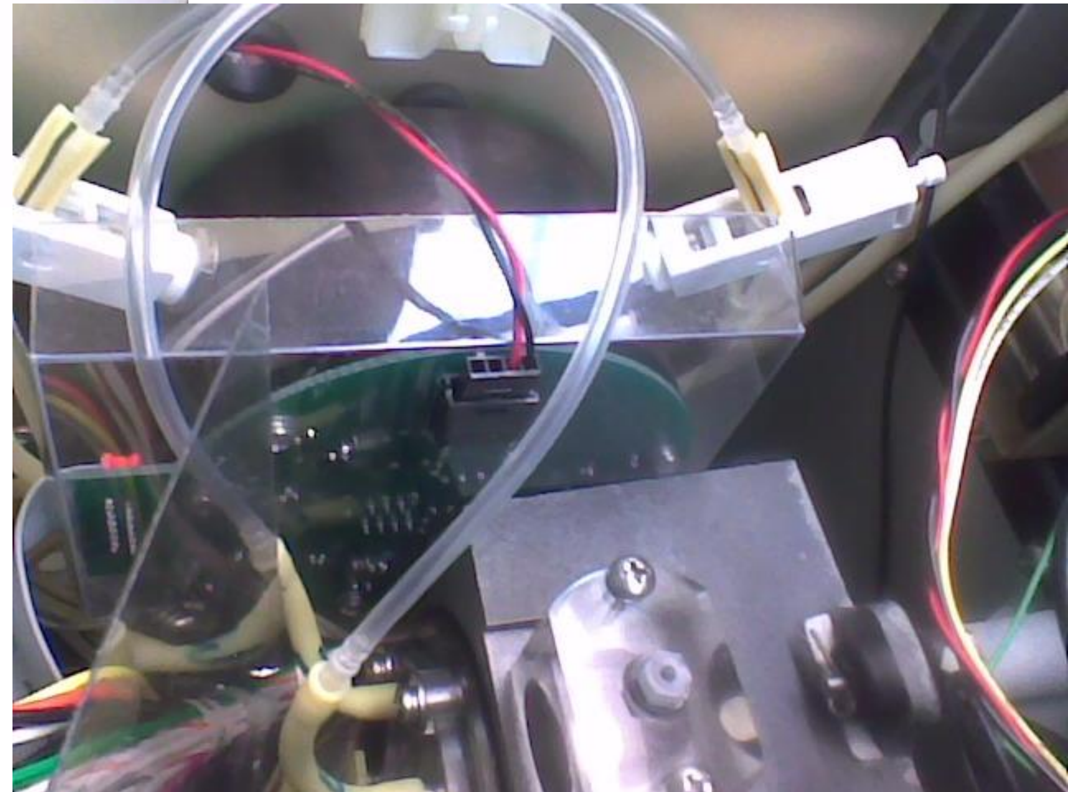
1. Prepare Student samples by diluting 1ml of cells in 9ml of Isoton in AV. Other dilutions may be used, but you need at least 10ml total to trigger aperture operations. Place a cap on the AV until immediately before running a count.
2. Students may access the Isoton from the CC pump dispenser by removing the cap and unscrewing (CCW) the tap. Raise the pump to the desired level and depress. Be sure to replace the cap screw when finished.
3. Gently mix the sample 2-3 times by inversion immediately before placing in on the instrument stand.
4. Depress the SETUP button and verify or set T_{upper} and T_{lower} parameters per your instructors guidance on the S1 screen. Depress the SETUP button again to move to the S2 screen.
5. On the S2 screen, verify or set the Aperture and Metered Volume and inspect to see that the Aperture Measure is set to <no>, and Optimize to <yes>. Do not change the Kd value. It has been previously set by the instructor using a Calibration protocol.
6. Depress the Start/Stop button one or more times to START the cell count.
7. On the A4 Screen, record the count data, and depress the Start/Stop button to run at least 3 measurements on a given sample.
8. Rinse the Aperture with DI before releasing the CC for the next student.

Coulter Counter: Reference Information

Information on Cell Size, Consumables, and
Use of the Coulter for Blood Analysis is presented
In the following slides. (RGM)



Coulter Counter
Internal Pump and
Tubing Configuration
(taken by RGM 4/7/09 investigating
"metering pump failure"

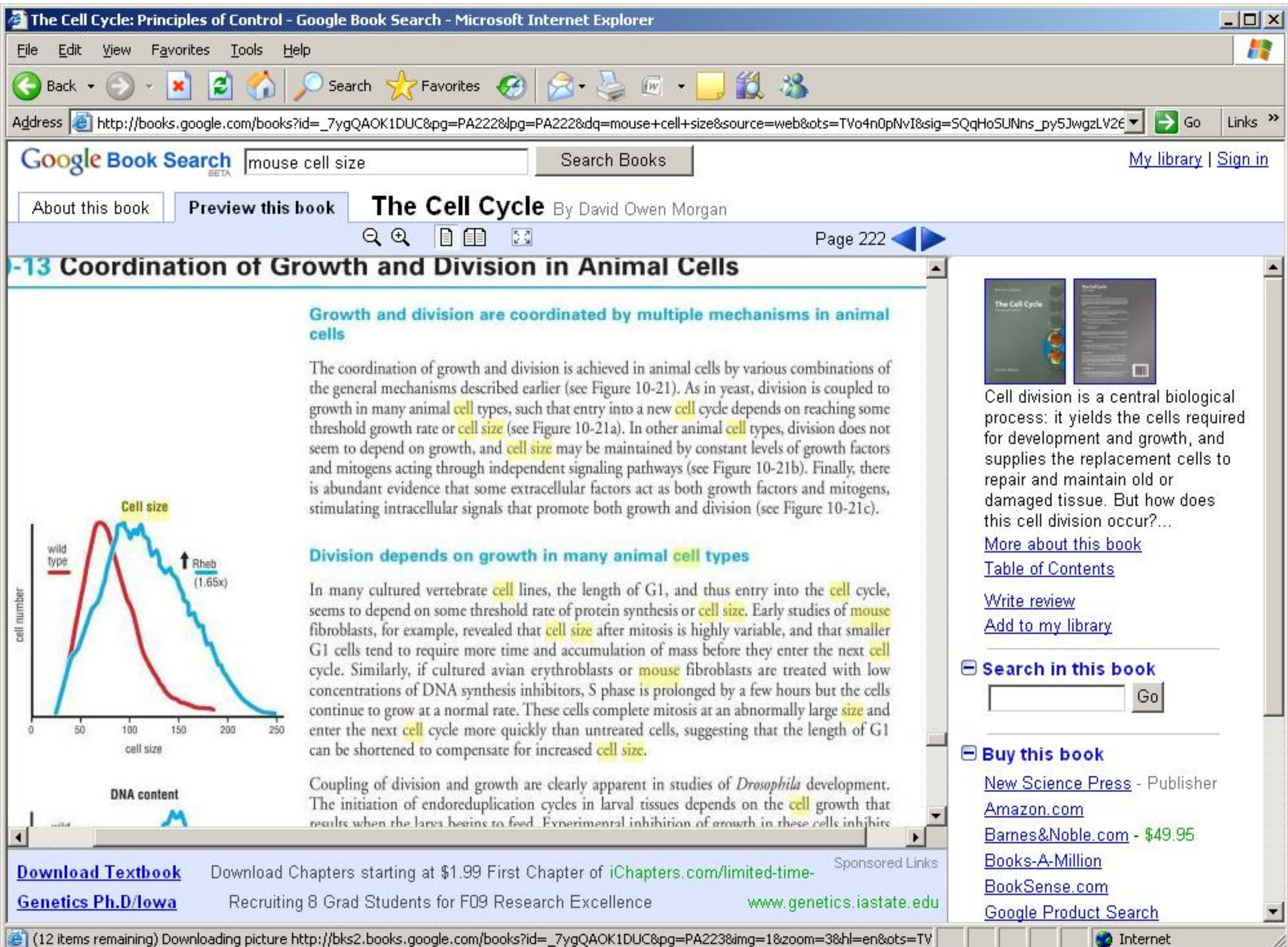


Coulter Counter: Reagents and Controls

Part No.	Description
	Z Series Starter Kit ***(Necessary for setup and installation)***
	•1 pk.: Z Series Tri-Pak
	•200 ea: Accuvettes
	•2 ea: Sensors
	•1 ea: Calibrator
	•1 ea: COULTER CLENZ®
	•1 ea: Dispenser
8320312	Z Series Tri-Pak (3 Z Paks) (1 pk.)
8320319	Z Series Z Pak™ Conversion Kit (1 ea.))
8546719	ISOTON® II Diluent (20 L)
8546929	COULTER CLENZ (500 ml)
8546930	COULTER CLENZ (5 L)
8546931	COULTER CLENZ (10 L)
8320307	Diluent Sensor Assembly (for use with Z Series Z Pak)
8320308	Waste Sensor Assembly (for use with Z Series Z Pak)
8320309	10ml Dispenser (Adjustable Volume Pipette) for use with Z
177495	Aperture Instrument Concentration Control (1 x 20 ml)
6600703	Dispersant IA Nonionic (5 x 15 ml)
6600704	Dispersant IB Nonionic (5 x 15 ml)
6600707	Dispersant IIIA Cationic (5 x 15 ml)
6601329	Standards Mixed Kit: 1 ea: 2µm, 5µm, 10µm, 20µm, 43µm (5 x 15 ml)
6602790	L1 Standard, nominal 1µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602792	L2 Standard, nominal 2µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602793	L3 Standard, nominal 3µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602794	L5 Standard, nominal 5µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602796	L10 Standard, nominal 10µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602797	L15 Standard, nominal 15µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602798	L20 Standard, nominal 20µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602799	L30 Standard, nominal 30µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602800	L43 Standard, nominal 43µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602801	L65 Standard, nominal 65µm Latex Particle (NIST Traceable) (1 x 15 ml)
6602802	L90 Standard, nominal 90µm Latex Particle (NIST Traceable) (1 x 15 ml)
7546138	Zap-Oglobin II

Part No	
8320515	20 µm, Aperture Tube (1 ea)
8320516	30 µm, Aperture Tube (1 ea)
8320517	50 µm, Aperture Tube (1 ea)
8320518	70 µm, Aperture Tube (1 ea)
8320519	100 µm, Aperture Tube (1 ea)
8320520	140 µm, Aperture Tube (1 ea)
8320521	200 µm, Aperture Tube (1 ea)
8320522	280 µm, Aperture Tube (1 ea)
8320523	400 µm, Aperture Tube (1 ea)
8320932	560 µm, Aperture Tube (1 ea)
8320525	1000 µm, Aperture Tube (1 ea)
8320527	2000 µm, Aperture Tube (1 ea)
8321693	50 µm, Aperture Tube, High Resolution (1 ea)
8321694	70 µm, Aperture Tube, High Resolution (1 ea)
8321695	100 µm, Aperture Tube, High Resolution (1 ea)
8321696	140 µm, Aperture Tube, High Resolution (1 ea)
383480	200 µm, Aperture Tube, High Resolution (1 ea)
8320592	Accuvettes (200/pk)

Cell Size; Mouse Fibroblast



-13 Coordination of Growth and Division in Animal Cells

Growth and division are coordinated by multiple mechanisms in animal cells

The coordination of growth and division is achieved in animal cells by various combinations of the general mechanisms described earlier (see Figure 10-21). As in yeast, division is coupled to growth in many animal cell types, such that entry into a new cell cycle depends on reaching some threshold growth rate or cell size (see Figure 10-21a). In other animal cell types, division does not seem to depend on growth, and cell size may be maintained by constant levels of growth factors and mitogens acting through independent signaling pathways (see Figure 10-21b). Finally, there is abundant evidence that some extracellular factors act as both growth factors and mitogens, stimulating intracellular signals that promote both growth and division (see Figure 10-21c).

Division depends on growth in many animal cell types

In many cultured vertebrate cell lines, the length of G1, and thus entry into the cell cycle, seems to depend on some threshold rate of protein synthesis or cell size. Early studies of mouse fibroblasts, for example, revealed that cell size after mitosis is highly variable, and that smaller G1 cells tend to require more time and accumulation of mass before they enter the next cell cycle. Similarly, if cultured avian erythroblasts or mouse fibroblasts are treated with low concentrations of DNA synthesis inhibitors, S phase is prolonged by a few hours but the cells continue to grow at a normal rate. These cells complete mitosis at an abnormally large size and enter the next cell cycle more quickly than untreated cells, suggesting that the length of G1 can be shortened to compensate for increased cell size.

Coupling of division and growth are clearly apparent in studies of *Drosophila* development. The initiation of endoreduplication cycles in larval tissues depends on the cell growth that

Cell, Bacteria, Virus, Particle Size; Examples

How Big is a ... ? - Microsoft Internet Explorer

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about 2mm in diameter. Use this animation to compare
cells and organisms sitting on a pinhead. Nearly
nification, dust mites dwarf pollen grains and human
and viruses are even smaller.

Get...
Stock
the CD
Downloa

Galleries...
Cell Gallery
Crystal Gallery

Tools...
Translate
Homework

Search cellsalive

Search

Google™ Custom Search

ive! HowBig Interactive

Human hair — Thickness 200um
Dust mite
Ragweed pollen
Lymphocyte
Red blood cells — 15um
Baker's yeast
E. coli — 2um
Staphylococcus
Ebola virus — 200nm
Rhinovirus

20 micrometers

Magnification

1 10 100 1,000 10,000 100,000 1,000,000

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Done Internet

Complete Blood Count

http://www.medicinenet.com/complete_blood_count/article.htm

- **White blood cell count (WBC). The number of white blood cells in a volume of blood. Normal range varies slightly between laboratories but is generally between 4,300 and 10,800 cells per cubic millimeter (cmm). This can also be referred to as the leukocyte count and can be expressed in international units as 4.3 - 10.8 x 109 cells per liter.**

  Automated white cell differential. A machine generated percentage of the different types of white blood cells, usually split into granulocytes, lymphocytes, monocytes, eosinophils, and basophils.

  Red cell count (RBC). The number of red blood cells in a volume of blood. Normal range varies slightly between laboratories but is generally between 4.2 - 5.9 million cells/cmm. This can also be referred to as the erythrocyte count and can be expressed in international units as 4.2 - 5.9 x 1012 cells per liter.

- **Hemoglobin (Hb). The amount of hemoglobin in a volume of blood. Hemoglobin is the protein molecule within red blood cells that carries oxygen and gives blood its red color. Normal range for hemoglobin is different between the sexes and is approximately 13 - 18 grams per deciliter for men and 12 - 16 for women (international units 8.1 - 11.2 millimoles/liter for men, 7.4 - 9.9 for women).**

  Hematocrit (Hct). The ratio of the volume of red cells to the volume of whole blood. Normal range for hematocrit is different between the sexes and is approximately 45 - 52% for men and 37 - 48% for women.

Complete Blood Count (continued)

http://www.medicinenet.com/complete_blood_count/article.htm

Mean cell volume (MCV). The average volume of a red cell. This is a calculated value derived from the hematocrit and red cell count. Normal range is 86 - 98 femtoliters.

Mean cell hemoglobin (MCH). The average amount of hemoglobin in the average red cell. This is a calculated value derived from the measurement of hemoglobin and the red cell count. Normal range is 27 - 32 picograms.

Mean cell hemoglobin concentration (MCHC). The average concentration of hemoglobin in a given volume of red cells. This is a calculated volume derived from the hemoglobin measurement and the hematocrit. Normal range is 32 - 36%.

Red cell distribution width (RDW). A measurement of the variability of red cell size. Higher numbers indicate greater variation in size. Normal range is 11 - 15.

Platelet count. The number of platelets in a volume blood. Platelets are not complete cells, but actually fragments of cytoplasm from a cell found in the bone marrow called a megakaryocyte. Platelets play a vital role in blood clotting. Normal range varies slightly between laboratories but is in the range of 150,000 - 400,000/ cmm (150 - 400 x 109/liter).