BECKMAN COULTER®
Z Series

User Manual
9914591-D6

Beckman Coulter, Inc.
250 S. Kraemer Blvd.
Brea, CA 92821

Beckman Coulter® Inc makes no representation that, upon furnishing this manual, the holder of the manual will have the necessary technical capabilities and know-how to properly troubleshoot and repair any of the equipment specified in the manual.Beckman Coulter Inc assumes no liability whatsoever, including consequential and incidental damages, resulting from improper operation of Beckman Coulter instruments after maintenance of Coulter instruments has been performed by persons not employed by Beckman Coulter Inc. Furthermore, Beckman Coulter Inc assumes no liability whatsoever for any personal injury or property damage resulting from maintenance and/or repair of Beckman Coulter instruments performed by persons not employed by Beckman Coulter Inc.

READ ALL PRODUCT MANUALS AND CONSULT WITH BECKMAN COULTER-TRAINED PERSONNEL BEFORE ATTEMPTING TO OPERATE INSTRUMENT.

HAZARDS AND OPERATIONAL PRECAUTIONS AND LIMITATIONS

WARNINGS, CAUTIONS, and IMPORTANTS alert you as follows:

WARNING - Might cause injury.
CAUTION - Might cause damage to the instrument.
IMPORTANT - Might cause misleading results.

CAUTION

Use of equipment in a manner other than specified might compromise system integrity and cause operational failures.

Beckman Coulter Inc urges its customers to comply with all national health and safety standards, such as the use of barrier protection. This may include, but is not limited to, protective eye wear, gloves, and suitable laboratory attire when operating or maintaining this or any other automated laboratory analyzer.

BECKMAN COULTER,the stylized logo, COULTER CLENZ and COULTER COUNTER are registered trademarks of Beckman Coulter Inc and are registered in the USPTO.

All trademarks are property of their respective owners.

"This manual contains confidential information of Beckman Coulter Inc and its receipt or possession does not convey any rights to reproduce, disclose its contents, or to manufacture, use, or sell anything it may describe. Reproduction, disclosure, or use without specific written authorization of Beckman Coulter Inc is strictly forbidden."

Copyright © Beckman Coulter Inc 1992-201Ì
All rights reserved.

Revision Status

Initial Issue, January 1997

Introduction of Z Series

Software Levels:

Z Series Data Terminal - 1.11
Z2 - 1.02
Z1 Dual - 2. 2.00
Z1 Single - 1.2.00

Revision B, August 1997

Changes for this revision of the document did not involve hardware or software changes but were only for purposes of information clarity.

Revision C, February 1998

Product Descriptor revised on cover to carry trademark symbolJ
(Policy change by CORPORATE LEGAL COUNSEL).

Revision D, January, 2002

Changes for this revision of the document did not involve hardware or software changes but were Only for purposes of information clarity.

Revision DA, July, 2011

Updates were made to the company corporate address.

Revision DB, April, 2018

Updates to the Section 1.2.3.

This document applies to the latest software listed and higher versions. When a subsequent software version changes the information in this document, a new issue will be released.

List of Contents

Section 1 Introduction

1.1 System Overview 1-1
1.2 Manual Description 1-1
1.2.1 Scope and Organization 1-1
1.2.2 Conventions 1-1
1.2.3 Product Description 1-1

1.3 Limitations 1-2
1.3.1 Chemical 1-2
1.3.1.1 Warnings 1-2
1.3.1.2 Cautions 1-2
1.3.2 Electrical 1-3
1.3.2.1 Warnings 1-3
1.3.2.2 Cautions 1-3
1.3.3 Environmental 1-3
1.3.3.1 Warnings 1-3
1.3.4 Mechanical 1-3
1.3.4.1 Cautions 1-3
1.3.4.2 Sources of Error 1-4
1.3.5 Interfering Substances. 1-4

Section 2 Installation

2.1 Unpacking 2-1
2.2 BECKMAN COULTER Z Series Location Requirements 2-3
2.3 Voltage/Power Configuration 2-3
2.4 Controls and Connectors 2-5
2.4.1 Front Panel (Figure 2-5) 2-5
2.4.2 Data Terminal 2-6
2.4.2.1 Numeric Key Pad 2-6
2.4.2.2 Cursor Keys 2-6
2.4.2.3 Screen Access Keys 2-8
2.4.2.4 Message Area 2-10
2.4.3 Rear Panel 2-10
2.4.3.1 Connectors (Figure 2-6) 2-10
2.4.3.2 Fuses 2-11
2.4.3.3 Power Supply Indicators 2-11
2.5 Start-Up Procedure 2-12
2.5.1 Setting the Time and Date 2-12
2.5.2 Connections 2-12
2.5.3 Fitting the Aperture Tube, Electrode Assembly and Stirrer 2-15
2.5.4 Filling the System 2-16
2.5.5 Adjusting the Optics 2-16

Section 3 Calibration

3.1 Introduction 3-1
3.2 Sample Preparation 3-1
3.3 Instrument Preparation 3-2
3.4 Calibration Procedure 3-3
3.5 Calibration Factor 3-7
3.6 Additional Calibration Information 3-7
3.6.1 If Calibration Stops before Completion 3-7
3.6.2 Calibration with Partial Data 3-8
3.6.3 Temporary Kd 3-8
3.6.4 Editing Kd 3-9

Section 4 Operation

4.1 Introduction 4-1
4.2 Sample Analysis 4-1
4.2.1 S1: Setup - Enter Size Data Screen 4-2
4.2.2 S3: Setup - Instrument Settings Screen 4-4
4.2.3 Threshold Range Too Wide (Dual-Threshold Version Only) 4-5
4.2.4 Aperture Selection Table 4-6
4.2.5 Output Screen 4-7
4.2.6 Printout Configuration 4-8
4.2.7 Counting 4-10
4.2.8 Analysis Result 4-11
4.2.9 Changing Size Settings in the Analysis Screen 4-12
4.2.10 Channelyzer (Z2 Only) 4-13
4.2.10.1 Channelyzer Results 4-13
4.2.10.2 Statistics 4-14
4.3 User Profiles 4-14
4.3.1 P2: Store Profile 4-14
4.3.2 P1: Load Profile 4-15
4.4 Shutdown Procedure 4-16
4.4.1 End of Day 4-16
4.4.2 Long-Term Shutdown 4-16
4.4.3 Storage or Shipment 4-16
4.5 Additional Operating Information 4-17
4.5.1 Draining/Filling the Hydraulics System 4-17
4.5.1.1 Draining the System 4-17
4.5.1.2 Filling the System 4-18
4.5.2 Changing the Aperture Tube 4-19
4.5.2.1 Draining and Removing the Aperture Tube 4-20
4.5.2.2 Storing the Aperture Tube 4-20
4.5.2.3 Fitting and Priming the Aperture Tube 4-21
4.5.2.4 Selecting a Printer 4-23
4.5.3 Setting the Time and Date 4-23

Section 5 Specifications

5.1 Guaranteed Data 5-1
- 5.1.1 Particle Size Range 5-1
- 5.1.2 Particle Count 5-2
- 5.1.3 Thresholds 5-2

5.2 Informative Data 5-2
- 5.2.1 Aperture Tube Sizes 5-2
- 5.2.2 Metering Volumes 5-2
- 5.2.3 Data Output 5-2

5.3 Power, Dimensions and Weight 5-3

Section 6 Principles of Operation

6.1 Basic Principle 6-1
- 6.1.1 Coulter Method of Counting and Sizing 6-1
- 6.1.2 Coincidence Correction 6-2
- 6.1.3 Effect of Diluent (Electrolyte Solution) 6-2

6.2 BECKMAN COULTER Z Series Functional Description (Figure 6-2) 6-2
- 6.2.1 Pulse Generation 6-2
- 6.2.2 Pulse Processing 6-3
- 6.2.3 Control Circuits 6-5

Section 7 Preventative Maintenance

7.1 General 7-1

7.2 User Testing 7-1

7.3 Flushing an Aperture 7-4

Section 8 Status and Error Messages

Section 8 Status and Error Messages 8-1

Appendix 1 Sample Preparation Techniques

A1.1 Introduction A1-1

A1.2 Aperture Tube Selection A1-1

A1.3 Selection of Electrolyte Solution A1-2

A1.4 Filtration of Electrolyte Solutions A1-3

A1.5 Dispersion A1-3

Appendix 2 Aqueous Electrolyte Solutions

A2.1 Description A2-1

Appendix 3 List of Particulate Substances and Suitable Electrolyte Solutions

A3.1 Introduction A3-1
A3.2 Key to Aqueous Electrolyte Solutions A3-2

Appendix 4 Calibration Particles and Aperture Tubes

A4.1 Introduction A4-1
A4.2 Latex Suspensions A4-1
A4.3 Redispersion of Calibration Particles A4-1
A4.4 Determination of Assayed Sizes A4-2

Appendix 5 Salt Water Contaminants

A5.1 Introduction A5-1
A5.2 Sample Analysis A5-1
A5.2.1 Dual Threshold Version A5-1
A5.2.2 Single Threshold Version A5-4

Appendix 6 Parenteral Samples

A6.1 Extracts from British Pharmacopoeia, 1993, Parenteral Preparations A6-1
A6.1.1 Particulate Matter (Reference B.P., 1993, page 748) A6-1
A6.1.2 Limit Test for Particulate Matter (Reference B.P., 1993, Appendix XIII, A163) . . . A6-1
A6.2 Preparation A6-1
A6.2.1 Non-Conducting and Viscous Samples A6-1
A6.2.2 Precautions During Analysis A6-2
A6.3 Calibration A6-3
A6.4 Background Count A6-3
A6.5 Sample Analysis A6-3
A6.5.1 Dual Threshold Version A6-4
A6.5.2 Single Threshold Version A6-5
A6.6 Origin of Contaminants in Parenteral Fluids A6-6

Appendix 7 Disinfecting the COULTER Z Series

A7.1 Introduction A7-1
A7.2 General A7-1
A7.3 Method A7-2
A7.3.1 Hypochlorite Bleach A7-2
A7.3.2 Alternative Disinfectants A7-2
A7.3.2.1 Glutaraldehyde A7-2
A7.3.2.2 Gigasept A7-2
A7.3.2.3 Important Note A7-3
A7.3.2.4 Used (Contaminated) Solution A7-3
A7.4 Disinfecting A7-3
A7.4.1 Draining the System A7-3
A7.4.2 Filling the System with Disinfectant Solution A7-5

A7.4.3 Removing Disinfectant Solution A7-6
A7.4.4 Checking for Residual Bleach A7-6
A7.5 References A7-6

Appendix 8 Blood Cell Counting

A8.1 Installation A8-1
A8.2 Principles of Operation A8-1
A8.3 Performance Characteristics and Specifications A8-1
A8.3.1 Specifications (Blood Cell Counts) A8-1
A8.3.2 Informative Data A8-2
A8.4 Operational Technique for Human Red Cell Counts (RBC) A8-3
A8.4.1 Operational Technique for Human White Cell Counts (WBC) A8-4
A8.4.2 Operational Technique for Human Platelet Counts (PLT) A8-6
A8.4.3 Quality Control A8-8
A8.5 Calibration Procedures A8-8
A8.6 Operational Precautions and Limitations A8-8
A8.7 Hazards A8-8
A8.8 Service and Maintenance A8-9

Appendix 9 Operational Technique for Non-Human Cell Counts A9-1

A9.2 Operational Technique for Non-Human Red Cell Counts (RBC) A9-2
A9.3 Operational Technique for Non-Human White Cell Counts (WBC) A9-5
A9.4 Operational Technique for Non-Human Platelet Cell Counts (PLT) A9-6

Appendix 10 Finding Optimum Count Settings for Counting Cells and Other ‘Monodisperse’ Populations when their Sizes are Known

A10.1 Introduction A10-1
A10.2 Establishing the Correct Current and Gain A10-2
A10.2.1 Procedure A10-2
A10.2.2 Re-Optimizing Automatically in Volume Units A10-4
A10.3 Finding the Optimum Count Settings A10-6
A10.3.1The `Three-Quarter Size' Method of Entering the Optimum Count Setting A10-6
A10.3.1.1 Estimation of Cell or Particle Size A10-6
A10.3.2 Determination of the Optimum Count Setting by ‘Plateau’ or ‘Trough’ Finding A10-11
A10.3.2.1 Restrictions of the ‘Plateau Method’ A10-14
A10.3.3 Count Comparison Method A10-15
A10.4 Updating the Optimum Count Setting Value with Kd Changes A10-15

List of Illustrations

Figure **Page**

2-1 Unpacking 2-1
2-2 Packing Tray Contents 2-2
2-3 Voltage Selector Card/Fuse Housing 2-4
2-4 Local Supply Voltage Orientation 2-5
2-5 Front Panel 2-7
2-6 Rear Panel 2-11
2-7 Connection Diagram 2-13
2-8 Installing the Aperture Tube, Electrode Assembly and Stirrer Paddle 2-14

4-1 Replacing the Aperture Tube 4-22

6-1 Coulter Method of Counting and Sizing 6-1
6-2 BECKMAN COULTER Z Series Functional Diagram 6-4

A9-1 Example of Setting RBC Count Lower Size Setting A9-5
A9-2 Example of Setting Plt Count Size Settings A9-8

A10-1 Size Range of Display Too High A10-4
A10-2 Size Setting Too High A10-4
A10-3 Correctly Positioned Pulses for the "Plateau Method" A10-5
A10-4 Size Range of Display Too Low A10-6
A10-5 Moving the Threshold Line Manually A10-7
A10-6 Correctly Positioned Pulses for Calibration A10-10
A10-7 Calibration Size Range of Display Too Low A10-10
A10-8 Calibration Size Setting Too High A10-11
A10-9 Principle of the "Plateau Method" A10-12
A10-10 Example of Plots for the "Plateau" and "Trough" Method A10-14

List of Tables

Tables **Page**

2-1 COULTER Z Series Connectors 2-10

3-1 Metered Volumes Suitable for Selected Aperture Sizes 3-3

4-1 Minimum 'Set Lower Size Tl' and Suitable Metered Volumes for Selected Aperture Sizes 4-3
4-2 Valid Selections for Printer Configuration Screen 4-9
4-3 'Counting' Screen - Screen Items and Function 4-10

7-1 Preventative Maintenance Check List 7-1

8-1 Status and Error Messages 8-1

A1-1 Useful Standard Aperture Tube Data for the BECKMAN COULTER Z SeriesA1-2

A2-1 Typical Aqueous Electrolyte Solutions A2-1
A2-2 Stability of Dispersion A2-2

A4-1 Calibration Particles and Aperture Tubes for the COULTER Z Series A4-1

A8-1 Within Run Precision in the Normal Blood Range (n = 31) A8-1
A8-2 Linearity Limits A8-2

A9-3 Approximate Normal Range and Mean Values for Various Animal Bloods . . A9-2

A10 Size Settings Sequence A10-5
A10-2A Example of Comparison Matrix for Instrument Counts and Reference Method. A10-16
A10-2B Evaluation A10-16

Introduction

1.1 System Overview

The BECKMAN COULTER® Z Series systems provide the ability to count particles relative to user-defined thresholds. The Z Series consists of three models:

(1) The Z1 (single-threshold model) - Allows the user to ***set one threshold and count all the particles larger than that threshold***.
(2) The Z1 (dual-threshold model) - Allows the user to ***set two thresholds and count three regions***.
(3) The Z2 - Provides the user with on-screen/printed graphs showing various forms of channelyzed data (distribution collected by channelyzer, mean, mode, standard deviation). It also allows all of the counting capabilities of the Z1 (dual-threshold model).

1.2 Manual Description

1.2.1 Scope and Organization

This manual provides the user with the information and procedures needed for operating and maintaining the Z Series instruments.

The material in this manual is organized into eight chapters, nine appendices, and an index.

1.2.2 Conventions

This manual uses the following conventions:

- Bold, Helvetica type indicates text appearing on the Data Terminal's screen. For example: **PRESS 'START' TO CONTINUE** is a message that appears on the Data Terminal's screen.

- [ ] indicates a key on the Data Terminal, such as **[SET-UP]**. You activate that key function by pressing the key.

1.2.3 Product Description

The single and dual threshold models of the BECKMAN COULTER Z Series may be used to determine the human and animal erythrocyte concentration (or red cell count) RBC, and the leukocyte concentration (or white cell count) WBC, from blood collected into a suitable anticoagulant. The Z instrument is a general laboratory use product. For determining thrombocyte concentration (or platelet count) PLT of human or animal species with a discrete RBC and PLT population, a dual threshold Z Series is recommended for simplicity of operation. Most cell types, due to user selectable size settings, may be analyzed on the Z Series instruments.

The recommended anticoagulant is K_2 EDTA or K_3 EDTA, used at a concentration of 1.5mg/mL of blood. Anticoagulated blood may be stored refrigerated (e.g. 4-8 °C) for up to 24 hours before RBC, WBC and/or PLT analysis, if required. Re-mix it thoroughly before analysis; preferably using a rocker-roller mixer.

For analysis, blood must be diluted using a near-physiological saline solution; Beckman Coulter recommends its ISOTON II diluent, used with a BECKMAN COULTER DILUTER. Disposable counting vials (ACCUVETTE vials) are available; or regular laboratory glass beakers may be used to contain the diluted specimen for measurement. To obtain WBC, a lytic agent is used to remove the unwanted red cells.Beckman Coulter recommends its ZAP-OGLOBIN® reagent. To prevent subsequent erroneous RBC and PLT counts due to contaminating lytic agent, WBC containers should be kept separate. Good laboratory practice is to clean any re-usable containers in a non-lytic cleaner, e.g.BECKMAN COULTER CLENZ cleaning agent. In addition, the Z Series should be left with CLENZ cleaning agent around the aperture, overnight, or when not in regular use.

1.3 Limitations

- A **WARNING** indicates a situation or procedure that, if ignored, can cause serious personal injury.
- A **CAUTION** indicates a situation or procedure that, if ignored, can cause damage to the instrument.
- A **Note** contains information that is important to remember or helpful in performing a procedure.

1.3.1 Chemical

1.3.1.1 Warnings

(1) **Do not** use non-aqueous electrolyte solutions. The instrument is designed only for aqueous electrolyte solutions.

(2) Toxicity safety requirements and handling procedures of all reagents should be checked and adhered to (see Merck Index and/or Material Safety Data Sheets).

(3) Care must be taken in mixing some electrolyte solutions. Violent reactions can occur.

(4) Azide **must not** be used in acid solutions.

(5) Flammable electrolyte solutions and organic solvents **must not** be used in the BECKMAN COULTER Z Series instruments.

1.3.1.2 Cautions

(1) If the diluent is ISOTON III®, the system **must be** first drained and then filled with DQ water to remove all traces of bleach, before filling with ISOTON III diluent.

(2) ISOTON III diluent and sodium hypochlorite **must not** be mixed together as they react, releasing gas and inactivating the bleach, possibly making it ineffective in disinfecting the instrument. Gas trapped in the hydraulic system of the Z Series may result sufficient internal pressure rise to cause leaks at tubing connections.

(3) If bleach disinfecting an instrument in which ISOTON III diluent has been used, the Vent Aperture function of the Z Series **must be** invoked before powering the instrument off (see para. 2.4.2.3, Screen Access Keys).

1.3.2 Electrical

1.3.2.1 Warnings

(1) High voltages are present inside the instrument, even when the Power Switch is set to **O**, due to a.c mains supply being routed from the rear connector to the front panel Power Switch.

(2) The instrument **must be** sited on a firm, dry, work bench and **must be** grounded correctly.

1.3.2.2 Cautions

(1) If Mains-borne interference occurs, a supply filter or a constant voltage transformer should be fitted.

(2) **Never** stand container(s) of fluid on top of the instrument. Repair of any instrument damaged by fluid being spilt over it, is not covered by the Warranty or Service Contract.

1.3.3 Environmental

1.3.3.1 Warnings

(1) The Vent Aperture function **must be** selected when an Aperture Tube is being fitted, to prevent the possibility of liquid being sprayed from the aperture.

1.3.4 Mechanical

1.3.4.1 Cautions

(1) If the Diluent tubing is disconnected at the instrument instead of from the lid, diluent will siphon out of the jar.

1.3.4.2 Sources of Error

(1) Only one calibration factor (Kd) can be stored against each letter (A to E). If a letter is chosen that already has a Kd value, it will be overwritten after calibration.

(2) The Aperture Kd value can be temporarily overwritten by the user (a decimal point is necessary), in which case the new value will be used until:

(a) Another aperture is selected.
(b) The aperture Kd menu is revisited.
(c) The instrument is switched off (**O**).

(3) Only one Kd factor can be stored against each letter.

(4) Ultrasonic probes can cause fracture of some types of particles. This is rarely the case with baths (for recommended models contact Beckman Coulter Particle Characterization Group or their authorized Distributors).

1.3.5 Interfering Substances

The presence of interfering substances, as listed in this section, can yield misleading results.

RBC
- Very high WBC count
- High concentration of very large platelets
- Agglutinated RBCs
- RBCs smaller than 25 fL.

WBC
- Certain unusual RBC abnormalities that resist lysing, nucleated RBCs
- fragmented WBCs
- any unlysed particles greater than 35 fL
- very large or aggregated platelets as when anticoagulated with oxalate or heparin.

PLT
- Very small RBCs near the upper size setting
- Cell fragments
- Clumped platelets as with oxalate or heparin
- Platelet fragments or cellular debris near the lower platelet size setting

1.1 **System Overview** 1-1
1.2 **Manual Description** 1-1
1.2.1 **Scope and Organization** 1-1
1.2.2 **Conventions** 1-1
1.2.3 **Product Description** 1-1
1.3 **Operational Warnings and Cautions** 1-2
1.3.1 **Chemical** 1-2
1.3.1.1 **Warnings** 1-2
1.3.1.2 **Cautions** 1-2
1.3.2 **Electrical** 1-3
1.3.2.1 **Warnings** 1-3
1.3.2.2 **Cautions** 1-3
1.3.3 **Environmental** 1-3
1.3.3.1 **Warnings** 1-3
1.3.4 **Mechanical** 1-3
1.3.4.1 **Cautions** 1-3
1.3.4.2 **Sources of Error** 1-3

CAUTION
- definition 1-2

chemical
- cautions 1-2
- warnings 1-2

electrical
- cautions 1-3
- warnings 1-3

environmental
- warnings 1-3

manual
- conventions 1-1
- scope and organization 1-1

mechanical
- cautions 1-3
- sources of error 1-3

Note
- definition 1-2

WARNING
- definition 1-2

Z1 (dual-threshold model)
- description 1-1

Z1 (single-threshold model)
- description 1-1

Z2
- description 1-1

Section 2 Installation

2.1 Unpacking

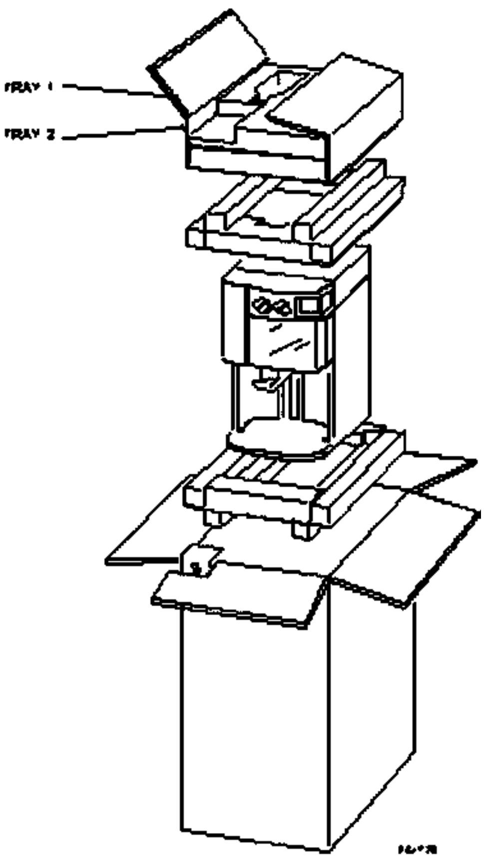


Figure 2-1 Unpacking

(1) Set the transit case the correct way up.

(2) Open and carefully remove accessory Tray 1, Tray 2, and instrument as shown in Figure 2-1. Retain transit case and packing materials.

(3) Check that all parts shown in Figure 2-2 are present, then inspect all items for transit damage.

(4) Any loss or damage should be immediately reported to Beckman Coulter Particle Characterization (using the installation report supplied), or the distributor (if supplied by a Beckman Coulter authorized distributor), and the carrier.

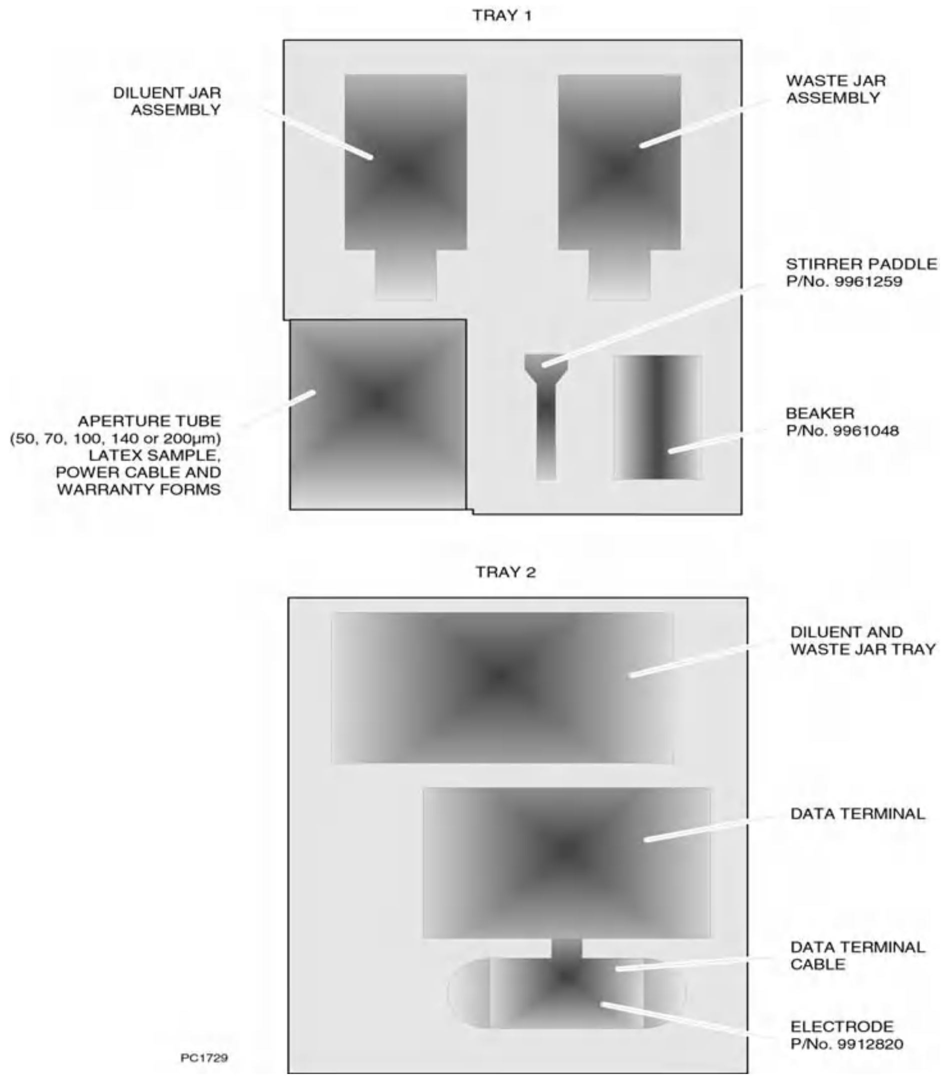


Figure 2-2 Packing Tray Contents

2.2 BECKMAN COULTER Z Series Location Requirements

Warning: The instrument **must be** sited on a firm, dry, work bench and must be earthed (grounded) correctly.

Caution: **Never** stand container(s) of fluid on top of the instrument. Repair of any instrument damaged by fluid being spilt over it, is not covered by the Warranty or Service Contract.

The location environment should be:

(1) Relatively dust free.

(2) Free from electrical interferences such as those caused by brush type motors, flickering fluorescent lights, arcing contacts, water baths, gas chromatographs or bleep paging systems.

Mains-borne interference may require a suitable line conditioner (supply filter or constant voltage transformer).

(3) A surface that is **not** subject to strong vibrations or sounds of high intensity (e.g. ultrasonic baths and probes).

(4) Within the temperature range of 10°C to 35°C and less than 85% relative humidity non-condensing.

2.3 Voltage/Power Configuration

Caution: The BECKMAN COULTER Z Series is dispatched from the factory unconfigured for voltage and power.

The BECKMAN COULTER Z Series can operate on 100, 120, 220, 240V a.c. $\pm$ 10%, at 47 - 63Hz inclusive. Configure the BECKMAN COULTER Z Series for the local power supply as follows:

(1) Locate the voltage configuration kit containing:

(a) One Voltage Selector Card.
(b) Two 0.5A (slow blow) fuses for 230/240V operation.
(c) Two 1.0A (slow blow) fuses for 100/120V operation.
(d) One Voltage Configuration Label.

(2) Disconnect the power cord.

(3) Using a small-blade screwdriver (or similar tool), pry the Voltage Selector card/fuse

housing cover off (refer to Figure 2-3).

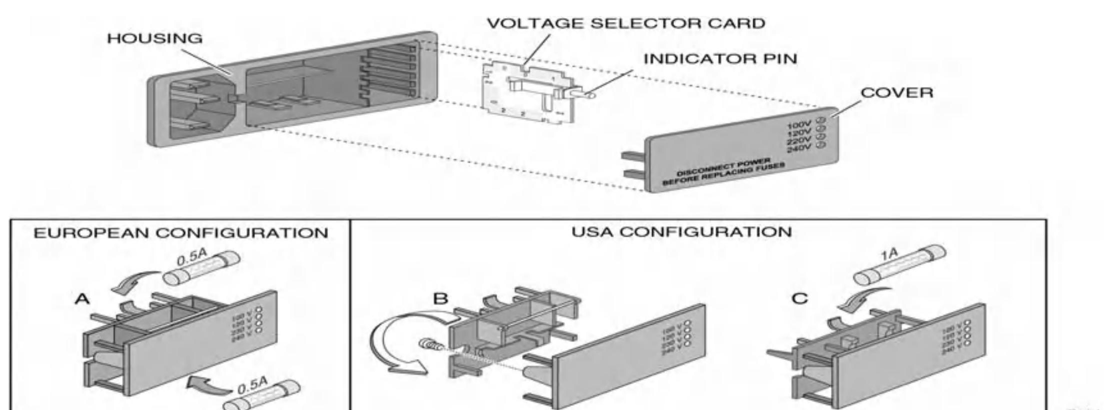


Figure 2-3
Voltage Selector Card/Fuse Housing

(4) The instrument is shipped with the fuse holder configured for European fuses (refer to Figure 2-3A). To reconfigure for USA fuses, refer to Figure 2-3B and do the following:

(a) Remove the Phillips-head screw on the left side of the fuse housing/cover and pull the fuse holder off the cover.

(b) Turn the fuse holder over as indicated by the arrow in Figure 2-3B.

(c) Place the fuse holder on the cover and secure it with the Phillips-head screw.

(5) Select the fuse(s) appropriate to the local supply voltage (para 2.4.3.2).

(6) Fit fuse(s) into the back of the cover (refer to Figure 2-3A and C) and set it aside.

(7) Using the indicator pin, pull the Voltage Selector card straight out of the housing (refer to Figure 2-3).

(8) Orient the Voltage Selector card so that the desired voltage is readable at the bottom of the card (refer to Figure 2-4).

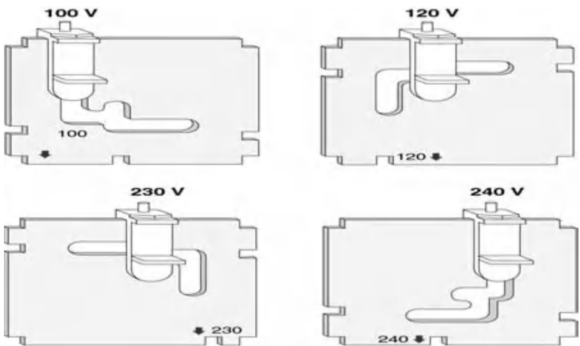


Figure 2-4

Local Supply Voltage Orientation

(7) Orient the indicator pin so that it points up when the desired voltage is readable at the bottom of the card (refer to Figure 2-4)

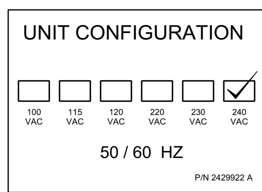
(8) Insert the Voltage Selector Card into the housing with the printed side of the card facing toward the three-prong line cord connector and the edge showing the desired voltage first (refer to Figure 2-3).

(9) Select the fuse appropriate to the local supply voltage (refer to Figure 2-4).

(10) Fit fuse into the back of the cover (refer to Figure 2-3) and replace the cover.

(11) Verify that the indicator pin shows the desired voltage.

(12) Ensure that a suitable plug is fitted to the power cord/mains lead. The connections must be made in accordance with the local safety regulations. The European mains lead color coding is:



BROWN: Live
BLUE: Neutral
GREEN and YELLOW: Earth (Ground).

2.4 Controls and Connectors

2.4.1 Front Panel (Figure 2-5)

Warning: High voltages are present inside the instrument, even when the Power Switch is set to off (**O**), due to a.c mains supply being routed from the rear connector to the front panel Power Switch.

Power Switch

I Power on. A screen appears on the Data Terminal.

O Power off.

Stirrer Position		Adjusts the stirrer paddle height (approximately 28mm adjustment).
Stirrer Speed	O	Stirrer off.
		Rotate clockwise to increase speed. Stirrer operates only when Sample Platform is in raised position.
Platform Release Catch		Adjust sample platform height by pressing and holding the Platform Release Catch.
Focus Control Body		Center image on Aperture Viewing Screen by rotating Focus Control Body or moving it in or out.
Focus Control Knob		Focus the aperture image on the Aperture Viewing screen by rotating the Focus Control Knob.
Aperture Lamp		Illuminates when door is open and during analysis.

2.4.2 Data Terminal

The user area of the Data Terminal is divided into three sections (Figure 2-5). The section on the left houses the numeric key pad. The section in the middle houses the cursor keys. The section on the right houses the screen access keys.

2.4.2.1 Numeric Key Pad

The numeric key pad (Figure 2-5) is composed of the numbers 0 through 9, a decimal point and a ‘Delete’ key. Use these keys to enter numerical values where required.

2.4.2.2 Cursor Keys

There are four keys that control cursor movement (Figure 2-5). In text these keys are referred to as ‘cursor keys.’

Press to move the cursor as follows:

Use [∧] or [∨] to move the cursor up or down one line at a time.

Use [<] and [>] to move the cursor left or right, either one selection (Selection Field) at a time, or one digit (Numeric Entry Field) at a time.

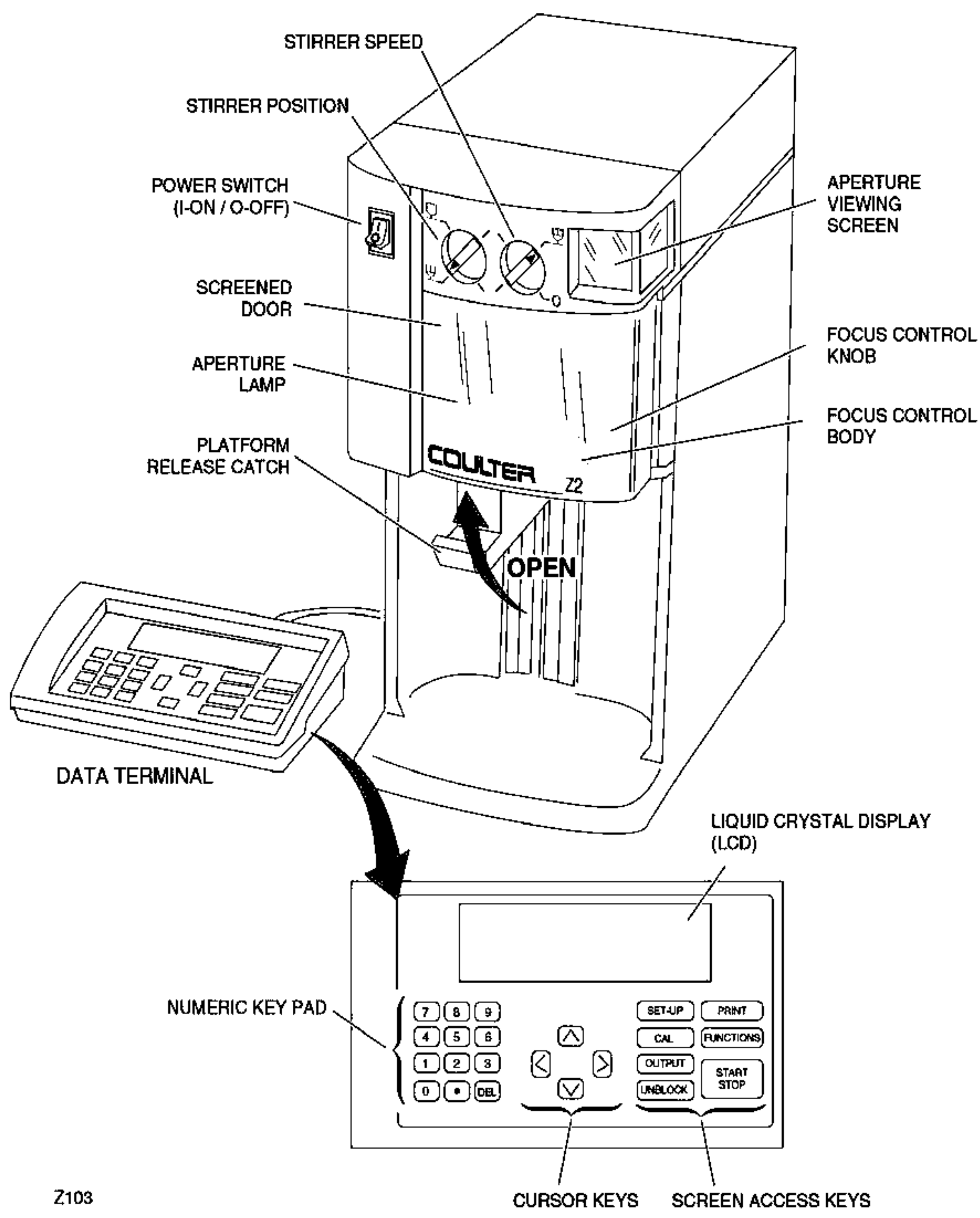


Figure 2-5 Front Panel

2.4.2.3 Screen Access Keys

The following seven screen access keys (Figure 2-5) give the operator access to all the screens in the Z Series environment:

2 SET-UP

Press SET-UP to gain access to the Setup screens. With a Setup screen displayed, pressing SET-UP steps the operator through the following screens:

S1: SETUP - ENTER SIZE DATA
S2: SETUP - ENTER ANALYSIS DATA
S3: SETUP - INSTRUMENT SETTINGS
S10: SETUP - CHANNELYZER SETTINGS
S4: SETUP - APERTURE SELECTION

The Setup screens allows the instrument parameters to be reviewed prior to sample analysis. Refer to Section 4, Operation, for a full explanation.

CAL

Press CAL to gain access to the Calibration screens. With a Calibration Screen displayed, pressing CAL steps the operator through the following screens:

C1: CALIBRATION
C2: CALIBRATION FACTOR
C3: CAL - INSTRUMENT SETTINGS

The Calibration screens allow the instrument parameters to be reviewed prior to calibration. See Section 3, Calibration, for a full explanation.

OUTPUT

Press OUTPUT to gain access to the Analysis screens. With an Analysis screen displayed, pressing OUTPUT steps the operator through the following screens:

A1: ANALYSIS - OUTPUT FORMAT
A10: PRINTOUT CONFIGURATION
A4: ANALYSIS RESULT
A7: CHANNELYSER RESULTS

Analysis screens allow output parameters to be reviewed before and after an analysis. Refer to Section 4, Operation, for a full explanation.

UNBLOCK

Press UNBLOCK to unblock the aperture. This procedure reverses the flow of sample through the aperture, causing any debris to be expelled.

PRINT

Press PRINT to print results or select Automatic printout from an Analysis screen.

FUNCTIONS

Press FUNCTIONS to gain access to the **F1: INSTRUMENT**

FUNCTIONS screen. From there, select any of the following automated procedures:

Note: Press **START** to initiate any selected function.

Prime Aperture Required when an Aperture Tube needs filling or when the rest of the Hydraulic System is full of electrolyte solution (or diluent).
Used to re-establish a low background count at startup,and between sample runs.

Fill System Completely fills the Hydraulic System with diluent and removes air bubbles. Required at installation or when the system has been drained of diluent.

Drain Aperture Empties the Aperture Tube of diluent without draining the Hydraulics System.

Drain System Drains the entire Hydraulics System of diluent. Required when type of diluent is changed. Diluent Tube **must be** placed in a Waste Container during this procedure.

Flush Aperture Flushes particles or air bubbles away from the rear of the aperture.

Vent Aperture Vents interior of the aperture to atmosphere. The Aperture Tube is automatically vented at the end of the Drain Aperture and Drain System functions.

Load Profile Lets you load specific stored profiles.

Store Profile Lets you name and store specific profiles.

Set Clock Lets you set the Z2's real-time clock with the date and time.

User Testing Lets you self-test the following functions:

- Software Versions
- Keyboard
- Display
- Metering Pump
- Control Valve
- Default Settings.

START STOP

Press this key to start or stop a selected procedure. If a procedure is stopped, the instrument electronics and hydraulics systems are automatically reset.

Note: This key is shown in text as either START or STOP depending on whether you want to stop or start a procedure.

2.4.2.4 Message Area

The Z Series interacts with the operator through ‘messages’ at the bottom of the Data Terminal’s LCD display (see Figure 2-5). These messages appear during the analysis and data entry phases of operation. Messages can be status messages or error messages (divided into analysis errors and data entry errors). For a complete listing of messages, type of message, reason for message and action to take, see Section 8, Status and Error Messages.

2.4.3 Rear Panel

2.4.3.1 Connectors (Figure 2-6)

Table 2-1 BECKMAN COULTER Z Series Connectors

Connector	Function
WASTE Stainless steel tubing connector	Connection to Waste Container (Either Jar or Z Pak®)
DILUENT Stainless steel tubing connector	Connection to Diluent Container (Either Jar or Z Pak)
LEVEL SENSE 3-way jack sockets	Connection to level sensors in the Diluent and Waste Jars or Z Pak (connectors are interchangeable). The sensor in the Diluent Jar is activated when the diluent level falls below it. The sensor at the top of the Waste Jar is activated when the waste level reaches it.
PARALLEL PRINTER 25-pin 'D' type socket (IBM® compatible)	Connection to parallel Centronics Printer.
K/BOARD 7-way DIN socket	Connection to Data Terminal.
Power IEC 320/CEE Standard 3-pin plug	Connection to a.c. Power Supply. An integral Voltage Selector Card allows the instrument to be configured over a wide range of line voltages.
External Control Port	For future use.

2.4.3.2 Fuses

External fuses F1 and F2 must have identical values:

either 0.5A for 220/240V **or** 1.0A for 100/120V.

2.4.3.3 Power Supply Indicators

When lit, +15v, -15v, +5v and -225v indicators show the marked d.c. power supply is available.

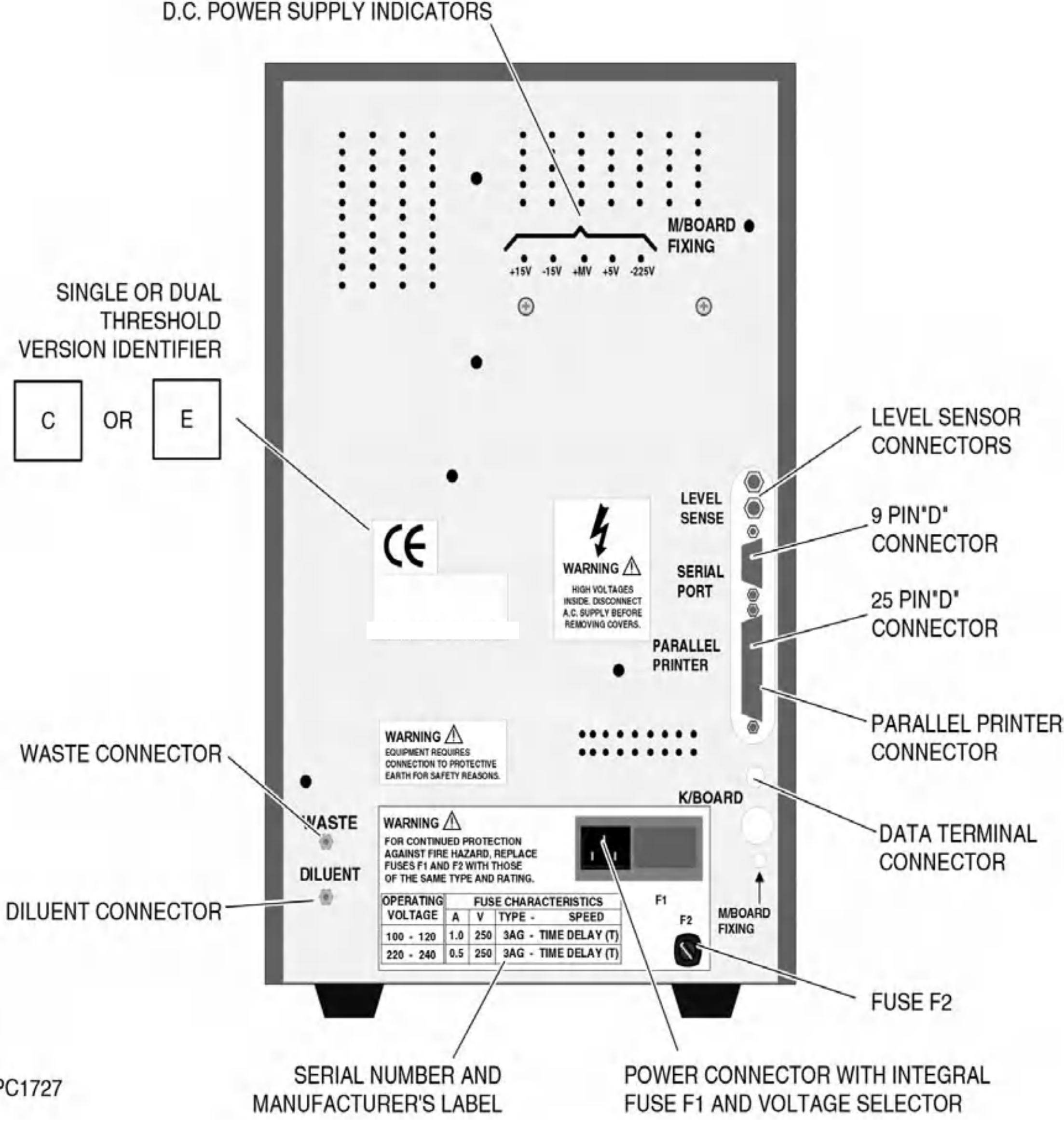


Figure 2-6 Rear Panel

2.5 Start-Up Procedure

2.5.1 Setting the Time and Date

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) Using the cursor keys, move to the line below **Select function, press START** and select **Set Clock**.

F1: INSTRUMENT FUNCTIONS

Prime/Fill/Drain/Flush/Vent
Select function, press START
< Set Clock >
Key beep < On >
Printer: < LaserJet >

(3) Press **START** and screen **F5** displays.

(4) Enter the date and time in the format shown on the screen (**mm/dd/yy hh:mm**).

Note: A 24-hour clock is used.

F5: SET CLOCK

Enter the current time below.
Press START to set the time

mm/dd/yyyy hh:mm
00/00/0000 00:00

(5) Press **START** and the date/time is set and you are returned to the **F1** screen.

2.5.2 Connections

(1) Remove the tubing link between the back panel waste and diluent connectors (see Figure 2-7).

(2) Fill the Diluent Jar with clean electrolyte solution (e.g. ISOTON II diluent).

(3) When using the **Z Pak**, follow (1). Connect the waste and diluent tubing directly to the **Z Pak** . Diluent is already supplied within the **Z Pak** diluent container.

(4) Diluent Dispenser connects to the top of the Diluent Sensor on the **Z Pak**.

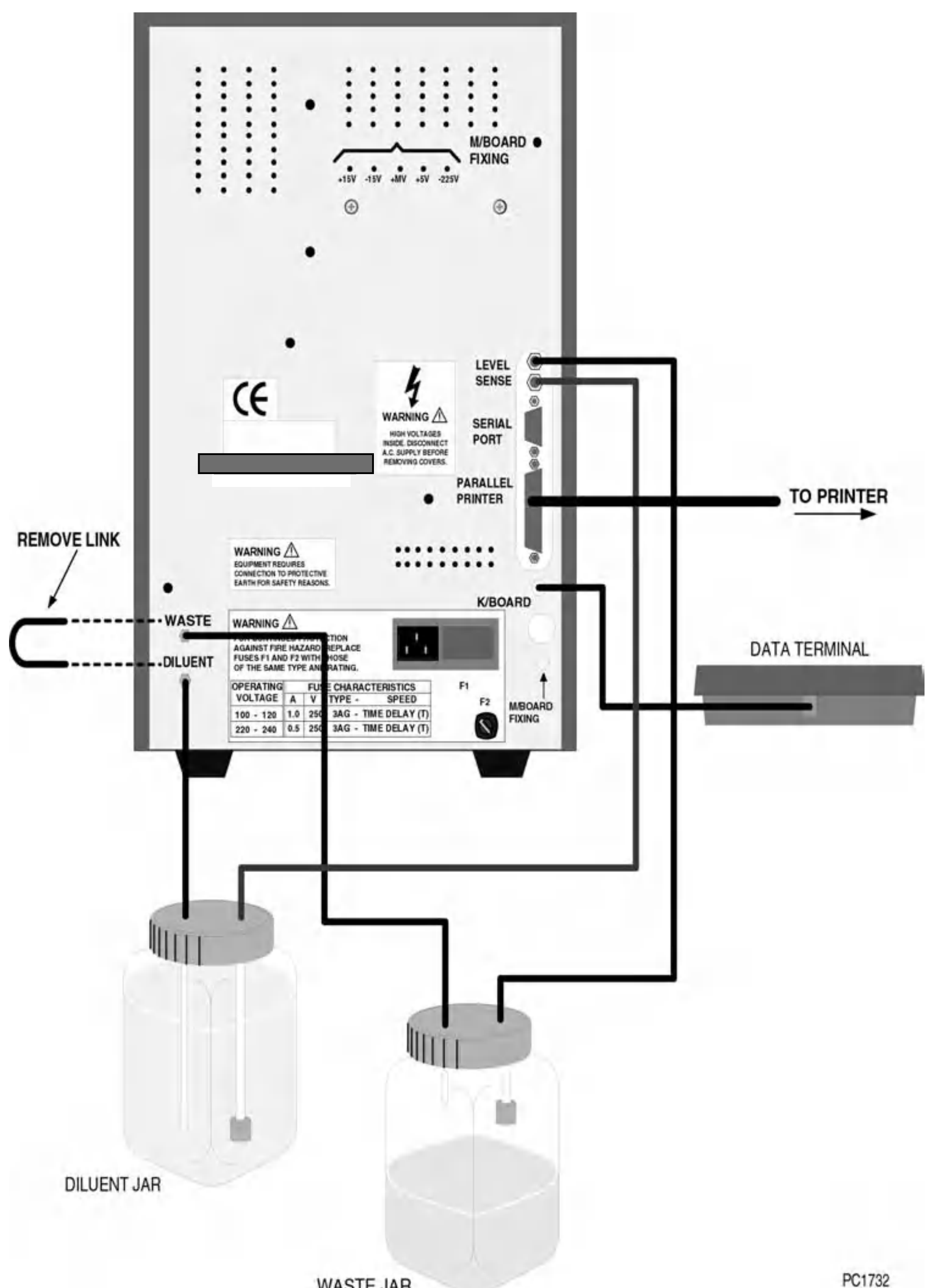


Figure 2-7

Connection Diagram

NOTE: For **Z Pak**: Connect Waste line to Z Pak waste connector. Connect diluent line to the Diluent Connection on the Diluent Sensor of the Z Pak. **Dispenser** connects to the top of the diluent sensor designed for the Z Pak.

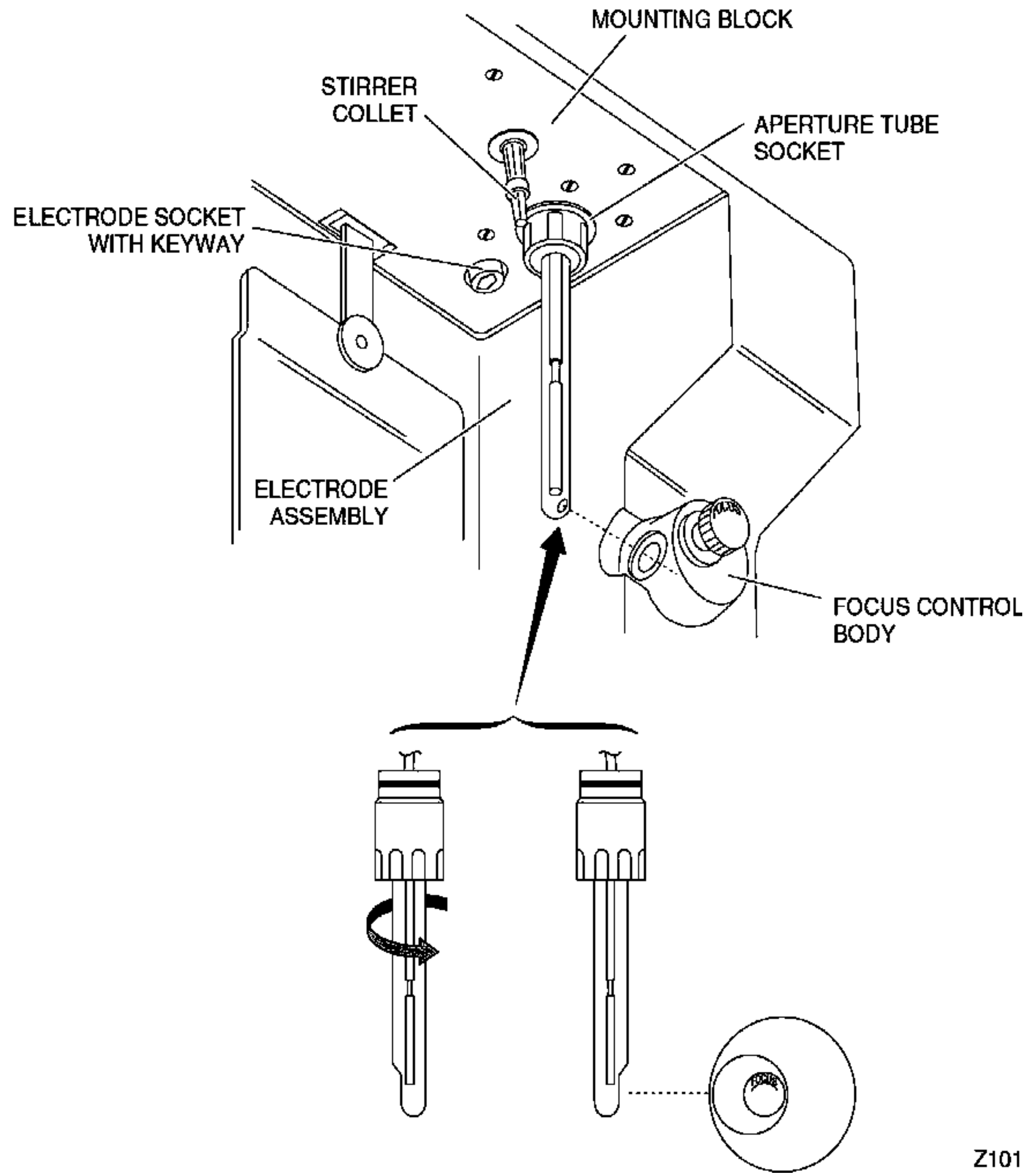


Figure 2-8 Installing the Aperture Tube, Electrode Assembly and Stirrer Paddle

(5) Connect the Waste and Diluent Containers as shown in Figure 2-7.

Note: Level Sense connectors are interchangeable.

(6) Connect the Data Terminal as shown in Figure 2-7.

(7) Connect the Printer (optional extra) to the Parallel Printer connector (see Figure 2-7). Refer to the relevant manufacturer's manual.

(8) Connect the instrument to the mains supply and set the Power Switch (see Figure 2-5) to **I** (on). The Data Terminal first displays the Coulter logo, software version number, and date and time followed by the **S1: SETUP - ENTER SIZE DATA** screen.

2.5.3 Fitting the Aperture Tube, Electrode Assembly and Stirrer

(1) Select an Aperture Tube in the range 50µm to 200µm. Rinse the Aperture Tube, beaker, Electrode Assembly and stirrer in clean diluent. Discard diluent.

(2) Open the Screened Door, the aperture lamp will illuminate. Move the platform to its lowest position.

Caution: If the electrode is pulled/pushed by it glass stem, damage may be caused to the electrode leading to erroneous results. Hold the electrode by the metal fitting only.

(3) Offer the electrode assembly to its socket as shown in Figure 2-8. The Electrode Assembly is keyed to ensure correct location.

(4) Put the Aperture Tube into its socket in the orientation shown in Figure 2-8, with the Beckman Coulter logo facing away from you. Secure by fully rotating in direction shown. In its final position the aperture is oriented towards the FOCUS control but may not directly face it (Figure 2-10). The aperture is slightly angled to produce an optimum image on the aperture viewing screen and this angle **must not** be adjusted.

(5) Turn the Stirrer Position control fully clockwise, hold the stirrer collet with finger and thumb, then press fit the Stirrer Paddle (see Figure 2-8). Ensure Stirrer Speed Control is set to **O**.

Note: The Stirrer Paddle cannot be used with an ACCUVETTE® sample container or a small vial.

(6) Fill the beaker with clean diluent, place it on the platform. Raise the platform until the Aperture Tube, Electrode Assembly and stirrer are fully immersed. Close the Screened Door.

2.5.4 Filling the System

(1) Press FUNCTIONS to select the **F1** screen.

(2) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Fill System**.

```
F1:        INSTRUMENT FUNCTIONS

           Prime/Fill/Drain/Flush/Vent
                < Fill System >
               Key beep  < On >
             Printer: < LaserJet >
```

(3) Using the cursor keys, move to **Key beep** and select either **On** or **Off**.

Note: If **On** is selected, pressing a Data Terminal key produces a beep.

(4) Press START and screen **F3** displays:

```
F3:              FILL SYSTEM

Ensure fill tube is connected
to diluent vessel.
Place clean diluent at sampling
station.
      PRESS ' START ' TO CONTINUE
```

Follow the instructions on screen **F3**. The **PRESS 'START' TO CONTINUE** status message is replaced by **FILLING SYSTEM xx**, where **xx** is the number of cycles required to completion. When the final cycle is completed, screen **F3** is replaced by screen **F1** with the message **SYSTEM FILLED** in the Message area.

2.5.5 Adjusting the Optics

(1) Open the Screened Door, the aperture lamp lights.

(2) Position the aperture image at the center of the Aperture Viewing Screen by rotating and/or moving the FOCUS control body in and out.

(3) To focus the image, rotate the FOCUS control knob clockwise/counterclockwise.

(4) The BECKMAN COULTER Z Series is now ready for use. Before running samples, the instrument should be calibrated using one of the procedures described in Section 3, Calibration.

CONTENTS

2.1 **Unpacking** 2-1
2.2 **BECKMAN COULTER Z Series Location Requirements** 2-3
2.3 **Voltage/Power Configuration** 2-3
2.4 **Controls and Connectors** 2-5
2.4.1 **Front Panel (Figure 2-5)** 2-5
2.4.2 **Data Terminal** 2-6
2.4.2.1 **Numeric Key Pad** 2-6
2.4.2.2 **Cursor Keys** 2-6
2.4.2.3 **Screen Access Keys** 2-8
2.4.2.4 **Message Area** 2-10
2.4.3 **Rear Panel** 2-10
2.4.3.1 **Connectors (Figure 2-6)** 2-10
2.4.3.2 **Fuses** 2-11
2.4.3.3 **Power Supply Indicators** 2-11
2.5 **Start-Up Procedure** 2-12
2.5.1 **Setting the Time and Date** 2-12
2.5.2 **Connections** 2-12
2.5.3 **Fitting the Aperture Tube, Electrode Assembly and Stirrer** 2-14
2.5.5 **Adjusting the Optics** 2-16

ILLUSTRATIONS

Figure 2-1 Unpacking 2-1
Figure 2-2 Packing Tray Contents 2-2
Figure 2-3 Voltage Selector Card/Fuse Housing 2-4
Figure 2-4 Local Supply Voltage Orientation 2-4
Figure 2-5 Front Panel 2-7
Figure 2-6 Rear Panel 2-11
Figure 2-7 Connection Diagram 2-13
Figure 2-8 Installing the Aperture Tube, Electrode Assembly and Stirrer Paddle 2-15

TABLES

Table 2-1 BECKMAN COULTER Z Series Connectors 2-10

INDEX

Aperture Lamp
description 2-6
Aperture Tube
draining 2-9
illustration, installation 2-15
priming 2-9
Aperture Viewing Screen
positioning 2-16
Cal key
function 2-8
clock
setting date and time 2-9
connections
illustration 2-16
connectors
diluent 2-10

external control port 2-10
keyboard 2-10
level sensors 2-10
parallel printer 2-10
power 2-10
table, connectors and function 2-10
waste 2-10
controls and connectors 2-5
cursor keys
moving 2-6
Data Terminal
cursor keys 2-6
description 2-6
message area 2-10
numeric key pad 2-6
screen access keys 2-8
diluent interface 2-10
Drain Aperture
description 2-9
Drain System
description 2-9
Electrode Assembly
illustration, installation 2-15
European mains lead
color coding 2-5
Fill System
description 2-9
Flush Aperture
description 2-9
Focus Control Body
description 2-6
Focus Control Knob
description 2-6
Front Panel 2-5
Functions key
function 2-9
fuses
description 2-3
installation 2-5
keyboard interface 2-10
level sense interfaces 2-10
Load Profile
description 2-9
location requirements 2-3
numeric key pad
function 2-6
Output key
function 2-8
Platform Release Catch
description 2-6
Power Supply indicators
description 2-11
Power Switch
description 2-5
Prime Aperture
description 2-9
Print key

function . . . 2-8
printer interface . . . 2-10
procedures
adjusting the optics . . . 2-16
configuring voltage/power . . . 2-3
connections . . . 2-12
SET CLOCK . . . 2-12
start-up . . . 2-12
unpacking . . . 2-1
profiles
load . . . 2-9
store . . . 2-9
Rear Panel . . . 2-10
screen access keys . . . 2-8
Set Clock
description . . . 2-9
procedure . . . 2-12
Setup key
function . . . 2-8
Start-Up
procedures . . . 2-12
Start/Stop key
function . . . 2-10
Stirrer
position . . . 2-5
speed . . . 2-5
Stirrer Paddle
illustration, installation . . . 2-15
installation . . . 2-16
Store Profile
description . . . 2-9
system
draining . . . 2-9
filling . . . 2-9, 2-16
installation procedures . . . 2-1
Unblock key
function . . . 2-8
unpacking
illustration, components . . . 2-1
procedures . . . 2-1
User Testing
description . . . 2-9
Vent Aperture
description . . . 2-9
voltage/power
configuration procedure . . . 2-3
waste interface . . . 2-10

Section 3 Calibration

3.1 Introduction

Each Aperture Tube has its own characteristic calibration constant. It is recommended that the user checks the size calibration for each Aperture Tube at least once a month to monitor for any system variations. Calibration may be performed using materials of known size, for example Beckman Coulter Calibration Latex for non-biological applications, or a sample of known Mean Cell Volume (MCV) for biological applications. The calibration methods are automatic and take into account the cell fragility of biological calibrators. Calibration is written as the non-biological procedure using the modal size of the calibrator, with the differences from the MCV indicated in the appropriate places. It is also possible to carry out the mass integration calibration as described in BS3406 Part 5.

This procedure assumes:

(1) The instrument is switched on (**I**).
(2) The instrument is fitted with the Aperture Tube to be calibrated.
(3) The instrument is primed with the required diluent.
(4) The operator is familiar with the instrument's controls and displays (refer to Section 2.4, Controls and Connectors).

3.2 Sample Preparation

(1) Select a calibration sample whose assayed size in equivalent spherical diameter is within 5 to 20% of the aperture diameter.

Prepare a calibration suspension as recommended in the assay sheet.

(2) Place a beaker of the calibration suspension on the platform and raise the platform until the Aperture Tube, electrode and stirrer are immersed.

Note: The Screened Door must be closed.

3.3 Instrument Preparation

(1) Press **SET-UP** and screen **S1** displays:

S1: SETUP - ENTER SIZE DATA
100µm C, Kd=Uncal (60)
Select Units: < µm >
Set Upper Size Tu: 8.0 µm
Set Lower Size Tl: 3.0 µm
Count Mode: < above Tl >

(2) At **Select Units** there are two choices, either **µm**, or **fl** (= μm^3). Using the cursor keys, select the required units appropriate to the assay value of the calibrator.

(3) Press **SET-UP** and screen **S2** displays:

S2: SETUP - ENTER ANALYSIS DATA
Select Aperture : <100µm C >
Aperture Kd : Uncal (60)
Metered Volume : < 0.5 >
Measure Aperture : < Yes >
Optimize Settings : < Yes >

(4) At **Select Aperture**, use the cursor keys to select the size of the Aperture Tube that has been fitted.

The **Aperture Kd** field (Diameter calibration constant) changes as you move through the **Select Aperture** choices. There are three types of ‘Aperture Kd’ status:

- **Stored Aperture Kd** - If a calibration constant is already stored in the Aperture Selection Table (screen **S4: SETUP - APERTURE SELECTION**) its value is displayed in the **Aperture Kd** field. The Aperture diameter is followed by a reference letter to identify its position in the Aperture Selection Table.

  Example: **Select Aperture: < 100µm C >**
  Aperture Kd: 60.2

- **Uncalibrated Apertures** - If an aperture exists in the Aperture Selection Table, but has not been calibrated, the **Aperture Kd** field displays **Uncal (xxx)**, where **xxx** is the default constant for the selected aperture size. This default constant may be used for non-critical analysis by entering its value on screen **C2: CALIBRATION FACTOR**.

- **Temporary Kd** - Entering a numeric value into **Aperture Kd** and then leaving screen **S2,** temporarily assigns that value to the **Select Aperture** line. The stored Kd is not however, overwritten. The reference letter selected with that aperture is replaced by an * and the label **Aperture Kd** is replaced by **Temp. Aperture Kd**. Only one aperture size at a time can be assigned a temporary constant and it must appear in the Aperture Selection Table. When power is turned off (**O**), the Z Series retains both the temporary aperture size and its constant in memory.

  Note: The **Temporary Kd** value must be valid for the displayed aperture tube before leaving screen **S2.**

(5) At **Metered Volume**, use the cursor keys to select the sample volume (in mL) for the aperture chosen, using Table 3-1 as a reference.

Table 3-1 Metered Volumes Suitable for Selected Aperture Sizes

Selected Aperture Size (µm)	Suitable Metered Volume (mL)
50	0.1
70	0.5
100	0.5
140	1.0
200	1.0

3.4 Calibration Procedure

(1) At the **S1** screen, press CAL and screen **C1** displays:

```
C1:            CALIBRATION
           Enter calibrator size
              Select Units:  < µm >
           Calibrator Size:  10.0 µm
          Measure Aperture:  < No  >
```

(2) At **Select Units** there are two choices, either **µm**, or **fl** (= µm^3). Using the cursor keys, select the required units appropriate to the assay value of the calibrator.

Note: The automatic calibration procedure assumes a modal value if **µm** is selected and a number average volume if **fl** is chosen.

(3) Move to **Calibrator Size** and use the numeric key pad to enter the assay value of the calibrator (e.g. 10µm for 100µm Aperture Tube). The **Measure Aperture** field automatically changes to **Yes**.

Note: If the calibration is being repeated with the identical calibrator and assay value, it is not necessary to re-enter the assay size at step 3.4..(3). The

Measure Aperture field displays **No**, but can be manually changed to **Yes** if necessary, to force a remeasurement of the aperture parameters, e.g. when there has been a substantial change in diluent properties since the previous calibration.

If the same calibrator is used with more than one aperture size, the **Measure Aperture** field should always be set to **Yes** for each aperture calibration.

(4) Press **START** and screen **C4** displays:

C4: CALIBRATION

Computing calibration settings

The Message area displays several messages including: **FLUSHING** (if just powered on), **MEASURING APERTURE FLOW RATE, MEASURING APERTURE RESISTANCE**.

Note: **FLUSHING** is only displayed if this is the first analysis since power on.

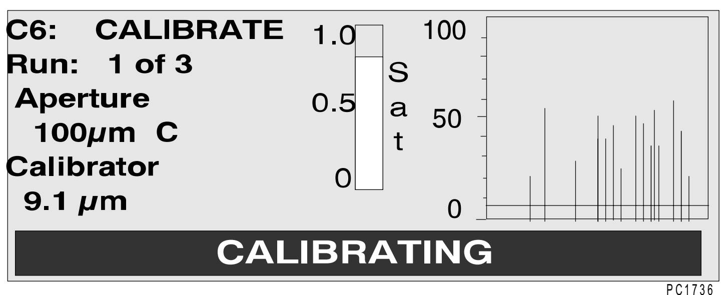
When completed, the **C3** screen displays:

C3: CAL - INSTRUMENT SETTINGS

Gain : 16 < 16>
Current : 0.707 <0.500>ma
Preamp gain : 179.20
Expected mode at 22.3%

Screen **C3** displays the settings calculated by the instrument. In certain circumstances it may be necessary to change the **Gain** and **Current** settings. To make changes, move to the appropriate field and select the required value.

(5) Press START and screen **C6** displays:



The Message area displays several messages including: **FLUSHING** (if just powered on), **BUILDING VACUUM**, **PREPARING TO CALIBRATE** and **CALIBRATING**.

The display has the following features:

Pulse Display Shows the heights of a random selection of analyzed pulses on a scale of **0** to **100** units. The height of each pulse is proportional to the particle's volume. The number of pulses shown on the pulse display represents the concentration.

Sat A bar indicator that monitors concentration. To minimize the number of calibration runs and avoid over-concentration of the sample the Sat bar indicator value should be between 0.3 and 0.7

As the value falls, the number of calibration runs to compute a Kd increases and falls below 0.3, the status message **LOW CONCENTRATION** is displayed. Calibration accuracy is not affected by low concentration, although the calibration time and the volume of suspension required may become unacceptably large.

If the Sat bar indicator value is above about 0.7, the calibration material concentration is too high, affecting the accuracy of the calibration.

Run At the end of Run 1, the instrument calculates the number of cycles required to obtain sufficient data to be statistically reliable and displays it as **Run: 1 of N**, where **N** is the number of cycles. **N** is updated at the end of each subsequent cycle and may therefore vary slightly due to the random nature of count data.

Aperture Displays the aperture setting chosen on the **S2** screen. * denotes Temporary Kd and the letters **A** through **E** denote the specific stored profile.

Calibrator Displays the calibrator settings from the **C1** screen.

If more than twenty runs are required, screen **C8: CALIBRATION IS DILUTE** displays.

> **C8: CALIBRATION IS DILUTE**
>
> **Calibration will take 52 cycles, and 26.0 ml of calibration material.**
> **Press 'START' to continue, any other key to Quit.**

The operator may wish to continue with the present concentration or increase it and then continue with the calibration. **N** will not be updated until the end of the next cycle.

The Run number increments at the end of each cycle until **N** runs have been completed, then screen **C7** displays:

> **C7: CALIBRATION FACTOR**
> **For 100µm C**
> **aperture**
> **Stored Kd = 59.32**
> **Kd = 60.51**
> **Press START to store new Kd,**
> **CAL to discard.**

(6) To adjust concentration, press **STOP** and screen **C9** displays:

> **C9: CALIBRATION FACTOR**
>
> **Calibration interrupted.**
> **Press ’ START ’ to resume.**
>
> **STOP KEY PRESSED**

Adjust sample concentration by adding more calibrator or diluting the suspension. Resume calibration by pressing **START**, screen **C6** displays again. Repeat concentration adjustment until Sat bar indicator value is within range.

3.5 Calibration Factor

C7:	CALIBRATION FACTOR
	For 100µm C aperture
	Stored Kd = 59.32
	Kd = 60.51
	Press START to store new Kd,
	CAL to discard.

Any aperture in the Aperture Selection Table can have only one calibration constant at a time. Pressing START overwrites the stored Kd with the new constant. Pressing CAL to discard the new constant, erases it completely from memory and the **C7** status message changes to **Edit Kd if required**.

Note: It is good practice to average a number of calibration constants (e.g. 5) and enter this average as the stored value. Record each calibration constant before discarding.

If START is pressed, **Stored Kd** is updated with the new constant. You are now ready for sample analysis. Press SET-UP to enter size parameters (see Section 4, Operation).

If CAL is pressed, **Stored Kd** is not updated. Press either CAL again to re-enter screen **C1** and review the entire calibration procedure or press START to repeat calibration with the existing parameters.

When the message **Edit Kd if required** displays, it is possible to manually enter a calibration constant (refer to para 3.6.4, Editing Kd).

3.6 Additional Calibration Information

3.6.1 If Calibration Stops before Completion

(1) Before the total number of cycles (**N**) has been reached, screen **C9** displays a status message indicating the probable cause of interruption. See Section 8, Error and Status Messages, for a comprehensive list of these messages.

C9:	CALIBRATION FACTOR
	Calibration interrupted.
	Press ’ START ’ to resume.
	CHECK METERING SYSTEM

(2) After the total number of cycles is complete but before the calibration factor can be calculated, screen **C10** displays:

```
C10:        CALIBRATION FACTOR
        Calibration has been stopped,

the sample mode is 66.0 %
Industrial limits 21 - 42%
                Cannot continue
```

Note: If **fl** are the chosen units, the Biological limits are 25-64% instead.

Screen **C10** gives the modal value as a percent of full scale, plus the limits it must be within to be accepted. The only exit option available is to press **CAL** and go to screen **C3** to review and amend the instrument settings.

In this example the mode is too high, so the **Gain** and/or **Current** settings should be reduced. If the mode is too low, increase the **Gain**, or **Current** settings. In both cases the calibration run ***must*** be repeated.

3.6.2 Calibration with Partial Data

Sometimes, especially with ultra-narrow calibration materials, the concentration of particles is very low but the certainty of calibration is very high due to the high precision with which the mode can be located. Such calibrations may result in a large number of runs being estimated by the Z Series. An experienced operator may wish to interrupt such a long run once sufficient data is deemed to have been collected by the instrument.

If **STOP** is pressed after the Z Series has started Run 2 or greater, screen **C9** displays either the message **CALIBRATION INTERRUPTED, Press 'START' to resume**, or the message **Press CAL to compute Kd from present accumulation**.

3.6.3 Temporary Kd

To accurately calibrate a 'temporary' aperture without overwriting the stored constant in the Aperture Selection Table, follow the steps below:

(1) Ensure that the selected aperture size is marked * on screen **S2** (by entering the default Kd if necessary).

(2) Carry out a complete calibration.

(3) When screen **C7** displays, press **START** to store the Kd and allocate the constant to the 'temporary' aperture.

3.6.4 Editing Kd

It is good practice to average a number of calibration constants (e.g. 5) and enter this average as the stored value. Calibration constants can be entered both before (on screen **C2**) and after (on screen **C7**) calibration. The are no differences in procedures between entering calibration constants before or after calibration just in the screens you access. The following procedure is for screen **C2** (before) and is the same for screen **C7**. The only difference is that screen **C7** is accessible only ***after*** a calibration.

To enter calibration constants before calibration:

(1) At screen **C1** press **CAL** and screen **C2** displays:

C2: CALIBRATION FACTOR
For 100 µm C aperture
Stored Kd = Uncal < 60 >
Kd = Uncal < 60 >
Edit Kd if required.

(2) At **Kd = Uncal**, use the numeric key pad to enter a new Kd value. This changes the **C2** messages as follows:

C2: CALIBRATION FACTOR
For 100 µm C aperture
Stored Kd = Uncal < 60 >
Kd = 60
Press START to store new Kd,
CAL to discard

(3) Store the value entered against the reference letter in the Aperture Selection Table by pressing **START** or discarded the value by pressing **CAL**. Pressing either returns the screen to its 'Edit Kd' version.

3.1 **Introduction** . . . 3-1
3.2 **Sample Preparation** . . . 3-1
3.3 **Instrument Preparation** . . . 3-2
3.4 **Calibration Procedure** . . . 3-3
3.5 **Calibration Factor** . . . 3-7
3.6 **Additional Calibration Information** . . . 3-7
3.6.1 **If Calibration Stops before Completion** . . . 3-7
3.6.2 **Calibration with Partial Data** . . . 3-8
3.6.3 **Temporary Kd** . . . 3-8
3.6.4 **Editing Kd** . . . 3-9

ILLUSTRATIONS

Figure 3-1 Calibration Menu Flow Diagram . . . 3-10

TABLES

Table 3-1 Metered Volumes Suitable for Selected Aperture Sizes . . . 3-3

calibration
adjusting concentration . . . 3-6
description . . . 3-1
editing Kd . . . 3-9
instrument settings . . . 3-4
preparing the instrument . . . 3-2
preparing the sample . . . 3-1
procedure . . . 3-3
run screen description . . . 3-5
stops before completion . . . 3-7
table, suitable metered volumes . . . 3-3
with partial data . . . 3-8
with temporary Kd . . . 3-8

Section 4 Operation

4.1 Introduction

This Section is divided into four parts:

(1) **Sample Analysis** - The procedures for daily operation of the instrument.

(2) **User Profiles** - The procedures to create/store five user setups, once the user is familiar with the instrument and knows the repetitive settings they utilize.

(3) **Shutdown Procedure** - The procedures to 'shutdown' the instrument for both a short time interval (≈ 2 weeks or less) and a long time interval (≈ 2 weeks or more).

(4) **Additional Operating Information** - Procedures for draining/filling the Hydraulic System with diluent and changing the Aperture Tube.

Note: For information concerning Aperture Tube Selection, sample and diluent preparation refer to Appendix 1, Sample Preparation Techniques.

The Z Series is simple to operate and eventually the manual will only be required for occasional reference, for such things as application changes, calibration and preventative maintenance.

This section assumes:

(1) Familiarity with Section 2.4, Controls and Connectors.
(2) That the instrument has been installed as per Section 2, Installation.
(3) That the Aperture Tube has been calibrated using the procedures in Section 3, Calibration.

Warning: **Do not** use non-aqueous electrolyte solutions. The instrument is designed only for aqueous electrolyte solutions.

4.2 Sample Analysis

At the start of each analysis it is good practice to check through this entire procedure, even though no change is required to the previous analysis instrument settings. This section explains the function of each menu in the preferred sequence and each menu is shown with the preset data. Refer to Figure 4-2, Sample Analysis Menu Flow Diagram and Figure 4-3, Output Menu Flow Diagram (at the end of this section) for other allowed sequences.

This procedure assumes:

(1) The system is filled with diluent.
(2) The correct Aperture Tube is fitted.
(3) The Diluent Jar is full.

(4) The Waste Jar is empty.
(5) The instrument is powered on (**I**).

4.2.1 S1: Setup - Enter Size Data Screen

(1) Press **SET-UP** until screen **S1: SETUP - ENTER SIZE DATA** displays:

```
S1:        SETUP - ENTER SIZE DATA
                100µm C,  Kd=60
                  Select Units:  < µm >
          Set Upper Size Tu:   8.0 µm
          Set Lower Size Tl:   3.0 µm
                  Count Mode:  < above Tl >
```

(2) Check that the Aperture Tube and reference letter is the required one (This can be altered on screen **S2**). In this example **100µm C, Kd = 60**.

(3) Move to **Select Units**. Select either **µm** for non-biological or **fl** (= μm^3) for biological analysis.

(4) Move to the appropriate size line(s) and enter numerical value(s) of size(s) above which counts are to be taken. Dual Threshold instruments have two size (Upper and Lower) fields, single thresholds have one (Lower).

Set Upper Size Tu (Dual Threshold only)

The Upper and Lower size levels cannot be set to coincide exactly. If identical sizes are entered in both lines, the Upper size defaults to a value 1% by volume higher, the Lower size stays as entered. The BECKMAN COULTER Z1 does not allow the thresholds to cross. If a Tl value higher than the Tu value is accidentally entered, the Z1 automatically exchanges the thresholds before the count starts. The ratio of Upper to Lower size should not exceed 3:1 by diameter (27:1 by volume). If this ratio is exceeded, screen **S9** displays when the **START** key is pressed (refer to para 4.2.3).

Set Lower Size Tl

The minimum size that can be entered is 2% of the aperture diameter (see Table 4-1). There is no Upper limit to the numerical value of the size that may be entered, but the practical working limit is 60% of the aperture diameter.

Note: When counting, setting the **Set Lower Size Tl** line to the minimum threshold value is not always desirable, because of the possible presence of more than one population. The user would expect a specific population to have a limited range of size values and set the size level(s) accordingly.

Count Mode

At **Count Mode** you have the following choices:

(Dual Threshold only)

(a) **above Tl** - all particles that are equal to, and exceed this setting and pass through the Aperture Tube are counted.

(b) **between** - all particles that are equal to, or exceed the Lower size setting, but do not exceed the Upper size setting are counted.

(c) **above Tu** - all particles that are equal to, and exceed this setting and pass through the Aperture Tube are counted.

Note: All three count results can be accessed at the end of an analysis.

Table 4-1 Minimum 'Set Lower Size Tl' and Suitable Metered Volumes for Selected Aperture Sizes

Selected Aperture (µm)	Minimum 'Set Lower Size Tl' Setting		Suitable Metered Volume (mL)
	µm	fl	
50	1.0	0.52360	0.1
70	1.4	1.43700	*0.5
100	2.0	4.18900	0.5
140	2.8	11.4900	1.0
200	4.0	33.5100	1.0

* For Platelet Counting Use 0.1ml Metered Volume

(5) Press SET-UP and screen **S2** displays. The operator has three situations (a), (b) or (c), depending on the Aperture Tube/electrolyte solution combination:

(a) No change.
(b) Change Aperture.
(c) Change electrolyte.

S2: SETUP - ENTER ANALYSIS DATA
Select Aperture : <100µm C >
Aperture Kd : 60
Metered Volume : < 0.5 >
Measure Aperture : < No >
Optimize Settings : < No >

No Change If the Aperture Tube and Kd do not need altering and the electrolyte solution is the same type, move to **Metered Volume** then select the appropriate volume for the fitted Aperture Tube (see Table 4-1). Press START to initiate an analysis and go to para. 4.2.7.

Change Aperture If the Aperture Tube selected is not the Aperture fitted, move to **Select Aperture** and select the required one. **Measure Aperture** and **Optimize Settings** change to **Yes**. Move to **Metered Volume** and select the appropriate volume for the fitted Aperture Tube (see Table 4-1). Go to step (6).

Change Electrolyte If the electrolyte solution type has changed, move to **Metered Volume** and select the appropriate volume for the fitted Aperture. Then select **Yes** at **Measure Aperture** and **Optimize Settings**. Go to step (6).

(6) Press **START**, the Z Series automatically measures the aperture characteristics and determines the optimum instrument settings for the sizes entered. When complete, screen **S7** displays, showing the recommended instrument settings (screen **S7** functions the same as **S3**, refer to para .4.2.2). Press **START** to accept the Z Series settings and to initiate an analysis. Go to para. 4.2.7.

4.2.2 S3: Setup - Instrument Settings Screen

(1) Press **SET-UP** until screen **S3** displays:

```
S3:     SETUP - INSTRUMENT SETTINGS
          Avail. Range 2.569 - 9.7985 µm
                       Gain  :   128    <128>
                    Current  :  1.00   <1.000> ma
             Preamp gain  :   179.20
              Tl = 13.3%,      Tu = 22.9%
```

Note: Screen **S7** behaves in an identical manner, but is only accessed during an analysis if either of the screen **S2** lines **Measure Aperture** and **Optimize Settings** are set to **Yes** either automatically (changing aperture selected) or by the selection process. The instrument then automatically measures the aperture characteristics and determines the optimum instrument settings for the sizes entered and displays the results on screen **S7**, when **START** is pressed. Screen **S3** displays the ‘Available Range’ of the pulse display (as seen on screen **A2**, see para. 4.2.7) based on the size(s) entered and aperture characteristics. The upper limit is 100% full scale of the pulse display and the lower limit is set by the baseline noise given the instrument settings.

This screen gives the operator the opportunity to change the settings calculated by the instrument. In the unlikely case that changes are required, move to the **Gain** and/or **Current** fields and select the required values.

Note: The left-most values for **Gain** and **Current** are the settings computed by the instrument's internal algorithm. The right-most values are the settings that the Z Series will use. Initially these are set to the same

value.

Gain <1>, <2>, <4>, <8>, <16>, <32>, <64>, <128>, <256>, <512>

Current < √2 moves from 0.0625mA to 4mA >

Note: If **Gain** and/or **Current** are altered, the instrument may not be at its original size setting. If necessary, press [SET-UP] twice to return to screen **S1** to re-enter the required size(s).

Preamp gain This is given for information purposes only.

Tl This gives the Lower Size setting as a percentage of the pulse display range. If **Current** and/or **Gain** are changed to values requiring Tl to be below the **Avail. Range**, Tl appears as **Tl = %Low%**. Conversely, if Tl required is above 100 units, Tl appears as **Tl = %High%**.

Tu (Dual Threshold Only) This gives the Upper Size setting as a percentage of the pulse display range. Tu displays the same out of range messages as Tl.

(2) Press [START] to initiate an analysis with the selected settings. Go to para. 4.2.7.

4.2.3 Threshold Range Too Wide (Dual-Threshold Version Only)

If after pressing [START] in screens **S1** or **S2**, screen **S9** (shown below) displays, the requested size range is too wide. The maximum size available for the instrument settings and the lower size are displayed. This corresponds to 100% full scale of the pulse display.

```
S9:     THRESHOLD RANGE TOO WIDE

   The maximum Tu available
   given the specified Tl is
                      21.99µm
   Press START to accept new Tu
          SETUP to change it
```

There are two ways to change the size range:

(1) Press **START** to **replace the upper size** with the displayed value.

or

(2) Press **SET-UP** to return to screen **S1** and **change either one or both** size settings.

4.2.4 Aperture Selection Table

Screen **S4** lists the stored Aperture Tubes (by reference letter) and Kd factors for selection in screen **S2.**

```
S4:    SETUP - APERTURE SELECTION
 A: < 50>    µm Kd  =  Uncal (30)
 B: < 70>    µm Kd  =  Uncal (42)
 C: <100>    µm Kd  =  Uncal (69)
 D: <140>    µm Kd  =  Uncal (84)
 E: <200>    µm Kd  =  Uncal (120)
```

Note: The reference letter for the Aperture Tube appears throughout the Z Series system menus to inform the operator of the aperture size chosen (i.e. screens **S1**, **S2**, **C1** and the printout data).

Any reference letters (A through E) can be assigned a nominal aperture diameter 50µm to 200µm, together with the calibration constant for that aperture. Each letter can have only one assigned size and one constant stored at a time. Any of the five aperture sizes can be allocated to each letter so that, as in the above example, each letter can represent a different aperture size or, conversely all letters could be given the same nominal size.

Where two or more apertures of the same nominal size (e.g. a conventional aperture and a 'micro-volume' (ampoule insertable style aperture) a separate record must be kept of the aperture tube serial numbers against their assigned reference letters.

Uncalibrated apertures are shown as **Uncal (xxx)**, where **xxx** is the default constant.

To assign an aperture size to a letter:

(1) Press **SET-UP** until screen **S4** displays.

Caution: Only one calibration factor (Kd) can be stored against each letter. If a letter is chosen that already has a Kd value, it is overwritten after calibration.

(2) Move to the appropriate line and select the required value.

4.2.5 Output Screen

(1) Press OUTPUT and screen **A1** displays:

A1: ANALYSIS - OUTPUT FORMAT

Next test: 3
Result type: < Concentration >
Dilution factor: 1 E + 00
Switch units: < µm >
Resolution: < 256 >

This screen allows review of the output parameters before and after an analysis. If any changes are required, move to the required field then enter the options as follows:

Next test A numeric field of up to eight digits, that allows each sample analyzed to be allocated a test number, which automatically increments each time an analysis is completed. In the case of an incomplete analysis, e.g. STOP key pressed, the test number will not increment.

If a decimal point is inserted (one or more decimal points are permitted), only the number to the right of the right-most decimal point is incremented, e.g. 10.12.01 would increment to 10.12.02, where 10.12 could represent the sample type and the incrementing number the test number. If no decimal point is entered, the whole number is incremented, e.g. 1012 increments to 1013, then to a maximum controlled by the number of digits entered e.g. 0 increments to a maximum of 9, 00 to a maximum of 99, 000 to a maximum of 999 etc.

Result type Selection field with choices of either **Count** or **Concentration**.

Note: All results are automatically corrected for coincidence loss.

(1) **Count** is the number of particles counted above, or between (Dual Threshold version only) the selected size(s), in the metered volume of sample.

(2) **Concentration** is the ‘count’ (as defined above) corrected for the metered volume and multiplied by the dilution factor. The result allows the analyses to be expressed in terms of the original sample concentration.

Dilution factor A numeric field for entering the factor conversion of count results to concentration. If a value of **0** is entered or the field is left empty, the factor defaults to 1. A dilution factor of up to four digits including one decimal point can be entered directly. The entered dilution factor can be increased by powers of 10 to 10^{99} by using the cursor keys to move to **Dilution factor** then using the numeric key pad to enter an exponent of 10 from 0 to 99 (E + 00 to E + 99).

e.g. For a 50,000:1 dilution, the figures entered in the dilution factor line in screen **A1** should be:

Dilution factor: 5 E + 04

Switch units A selection field to choose between **µm** and **fL** as the measure of units for the results of an analysis. The units remain switched throughout the Z Series menus, until switched back.

Resolution (Z2 Only) A selection field giving you the option of selecting from three channelyzer resolutions: 64 channels, 128 channels or 256 channels. The default is 256 channels. All printed and displayed graphs and tables use the selected resolution.

The graph displays the full width of the accumulation, 256 channels. If you selected **128** or **64**, the graph still displays the full width of the accumulation but combines (sums) adjacent channels. For example, the first displayed column contains the sum of the first two (if you selected **128**) or four (if you selected **64**) channelyzer channels. The second displayed column contains the sum of the third and fourth (if you selected **128**) or fifth through eighth (if you selected **64**) channelyzer channels.

4.2.6 Printout Configuration

(1) Press OUTPUT until screen **A10** displays:

```
A10:    PRINTOUT CONFIGURATION

          Mode:  < Manual >
          Form:  < Long >
         Graph:  < Yes >
         Table:  < N/A >
```

There are four printout modes available:

- Mode
- Form
- Graph
- Table.

Mode If you select **Automatic**, at the end of the analysis the results are automatically printed. If you select **Manual**, you must initiate printing by pressing PRINT.

Form If you select **Short**, at the start of the first analysis a columnized printout with the instrument settings as headings is produced. Instrument settings are not printed again until one or more are changed.

If you select **Long**, a printout of all the instrument settings with each individual result is produced.

Graph The available selections for this field are controlled by the printer that you select and the value selected in **Form**. Refer to Table 4-2 for valid selections.

Note: If you need to change your printer selection, go to screen **F1: INSTRUMENT FUNCTIONS**.

- If **Yes** is a valid selection and you select it, your analysis results are printed on a graph.
- If either 'Manual/Long' or 'Manual/Short' are selected, printing is only activated when **PRINT** is pressed.
- Pressing **PRINT** sends a copy of the current result to the printer whether in manual or automatic mode.
- Results can be printed in one of two formats, **Short** or **Long**. Refer to previous section for definition of 'short' and 'long' reports.

Table 4-2 Valid Selections for Printer Configuration Screen

		Graph			Table		
Printer	**Form**	**No**	**Yes**	**N/A**	**No**	**Yes**	**N/A**
Dot Matrix	Short			√			√
Dot Matrix	Long			√	√	√	
LaserJet 4L	Short			√			√
LaserJet 4L	Long	√	√		√	√	

√ denotes selection available.

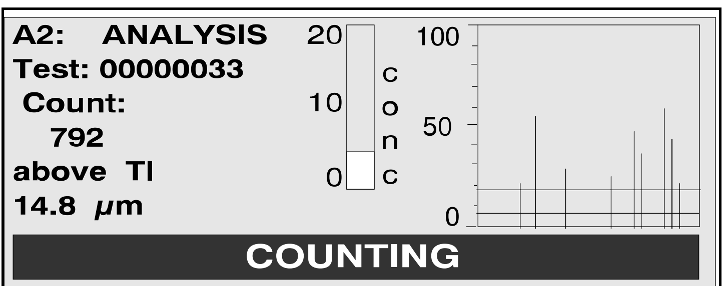
Table The available selections for this field are controlled by the printer that you select and the value selected in **Form**. Refer to Table 4-2 for valid selections.

Note: If you need to change your printer selection, go to screen **F1: INSTRUMENT FUNCTIONS**.

- If **Yes** is a valid selection and you select it, your analysis results are printed on a table.
- If either 'Manual/Long' or 'Manual/Short' are selected, printing is only activated when **PRINT** is pressed.
- Pressing **PRINT** sends a copy of the current result to the printer whether in manual or automatic mode.
- Results can be printed in one of two formats **Short** or **Long**. Refer to previous section for definition of 'short' and 'long' reports.

4.2.7 Counting

(1) After Pressing **START** to initiate an analysis, screen **A2** displays:



Various status messages appear in the Message area of the Data Terminal. Table 4.3 shows the items the screen displays and defines them.

Table 4.3 'Counting' Screen - Screen Items and Function

Screen Item	Function
Test	The test number of the analysis. The start value is set on screen **A1**.
Count	Real time count.
above Tl	The lower size setting.
above Tu or between Tl, Tu (Dual Threshold only)	This is the **Count** mode selected on screen **S1** of the Setup screens (or screen **A3** and **A4** of the Output screens). Tl represents the lower size setting and Tu the upper.
conc	Bar indicator to monitor the particle concentration over the range 0 to 20% coincidence. For optimum analysis results, the concentration should be as low as practicable (e.g. 5%).

Screen Item	Function
Pulse display	Shows the heights of a random selection of pulses on a scale of 0 to 100 units, along with an indication of baseline noise levels, and horizontal line(s) to show the position of the **Lower Size Tl** and the **Upper Size Tu** (Dual Threshold version only). The number of pulses shown on the display represents the sample concentration. The height of each pulse is proportional to the particle's volume.

(2) If STOP is pressed, the analysis is interrupted and screen **A5** displays (the metering system is reset):

```
A5:            ANALYSIS RESULT

           Analysis has been stopped,
              no result available.

               STOP KEY PRESSED
```

Press START to initiate a new analysis.

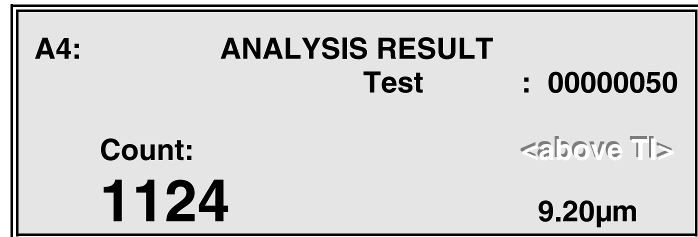
4.2.8 Analysis Result

(1) Once START is pressed and screen **A2** of the Analysis screens displays, the analysis continues until the selected volume of sample has been analyzed. The instrument then resets to its 'start an analysis' state, and either screen **A3** or **A4** displays, depending on whether **Concentration** or **Count** was selected on screen **A1**.

If **Concentration** was selected, screen **A3** displays:

```
A3:            ANALYSIS RESULT
                         Test          :  00000050
               Dilution factor   :  1
            Concentration /ml   :  <above Tl>
5.62  E6                              9.20µm
```

If **Count** was selected, screen **A4** displays:



(2) With the Dual Threshold version, a selection field on both these screens allows access to the three results, **above Tl** (lower size), **above Tu** (upper size), or **between** (between the two sizes).

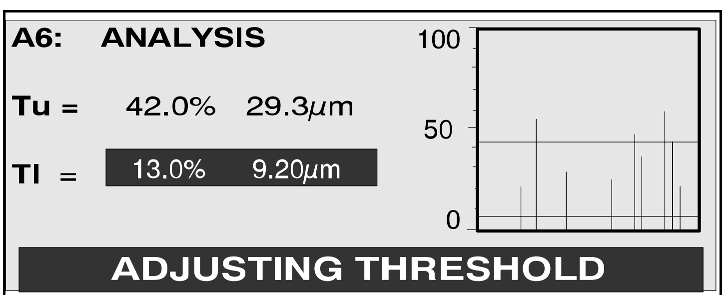
(3) If **Auto** is selected on the **Mode** line of screen **A8**, the results are sent to the printer when screen **A3** or **A4** displays. If **Manual** is selected, **PRINT** must be pressed.

Note: Dual Threshold only - displayed count mode has no effect on printout.

4.2.9 Changing Size Settings in the Analysis Screen

The operator can move the size threshold setting(s) by using the cursor keys, while counting is taking place. This analysis result is invalid and has to be repeated with the new settings. The procedure is as follows:

(1) After pressing **START** to initiate a count, screen **A2: ANALYSIS** displays. When the status message **COUNTING** appears, press either ∨ or ∧ and screen **A6** displays:



Tu = 42.0% 29.3µm (Dual Threshold only) — Tu is shown set to 42.0% of full display scale. The 42.0% represents a size of 29.3µm.

Tl = 13.0% 9.2µm — The Lower Size Threshold is set to 13.0% of full display scale. The 13.0% represents a size of 9.2µm.

(2) The threshold can now be adjusted by using ∨ or ∧. Toggle between the two thresholds using the cursor keys. The selected threshold will be highlighted. The horizontal line(s) on the pulse display, which represent the threshold level(s), move as the threshold(s) are adjusted (a slight delay between pressing the cursor key and the threshold response is

quite normal). The metering system continues to draw the sample through the aperture for as long as possible in order to maintain the pulse display. It will then reset, leaving the pulse display frozen. Once threshold(s) have been set to the required size(s), press START and screen **A2** of the Analysis screens is again displayed. The interrupted count is automatically abandoned and then restarted with the new size(s) settings (which have been copied to the **S1: SETUP - ENTER SIZE DATA** screen) using the same test number.

4.2.10 *Channelyzer (Z2 Only)

The Z2 can channelyze particle data and provide the results in various ways. Through the **A7: CHANNELYZER RESULTS** screen, the Z2 can:

- Provide an on-screen graph of channelyzed results.
- Provide an on-screen display of the numeric values contained in the channelyzer expressed as a percentage of the total number of pulses channelyzed.
- Provide an on-screen display of statistics based on channelyzer results.
- Depending on the mode selected, provide a printed graph or table of the distribution collected by the channelyzer during the most recent analysis.
- When a ‘long’ report is selected, provide the mean, median, mode and standard deviation over a portion of or an entire accumulation.

4.2.10.1 Channelyzer Results

This function provides an on-screen graph of channelyzed data. The resulting graph scales the vertical axis to maximize the data display. No scaling of the horizontal axis is offered. The graph’s resolution is set on the **A1** screen. See para. 4.2.5, Output Screen, for a description of Resolution settings.

The maximum channel is plotted as a column extending from the very bottom of the graph to one pixel from the top of the graph (excluding borders). Cursors are represented by a column of inverted pixels extending from the bottom border to the top border (inclusive).

To the right of the graph, the Z2 displays either a cumulative over and under value or statistics based on the results. This is controlled by a field on the **A7:CHANNELYZER RESULTS** screen labeled **Mode**. When **Cum.Ct** is selected, cumulative data reported as a number of the counts above/below the cursor displays. When **Cum.%** is selected, cumulative data reported as a percentage of the counts above/below the cursor displays. When **Stats** is selected, statistics display.

* Count or concentration results should always be obtained from the A3 or A4 Screens-not Size Distribution Results

4.2.10.2 Statistics

The Z2 displays statistics based on channelyzer results. The range these results cover are defined by the placement of the cursor. As the user moves the cursor, the values displayed show the relative amount of channelyzer data lying above and below the cursor. To obtain statistics:

(1) After pressing **OUTPUT** to initiate channelyzer results, screen **A7** displays.

(2) At the field labeled **Mode**, if **Stats** is not selected, select it now.

4.3 User Profiles

The Z Series instruments give the user the ability to create/store five user setups (Profile A, Profile B, Profile C, etc.). These profiles contain the:

- Aperture Size
- Aperture K_d
- µm/fl Setting
- Thresholds (in µm/fl)
- Cursors Values (in µm/fl)
- Main Amplifier Gain
- Preamp Gain
- Current Setting.

To store information into these profiles, press **FUNCTIONS** to access the **F1: INSTRUMENT FUNCTIONS** screen.

4.3.1 P2: Store Profile

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) Select **Store Profile** using the cursor keys.

(3) Press **START** and screen **P2** displays:

P2: STORE PROFILE

Selected Profile: <Profile x>

Aperture: Tl:
Volume: Th:

Press START to save

(4) Choose a profile name to store your information under by selecting either Profile A, B, C, D, or E, using the cursor keys.

The instrument displays a short description below the selection fields. The description reads **** Blank Profile **** if the profile selected is blank. If the profile selected is not blank, the aperture and threshold settings are displayed.

(5) To store the current settings in the selected profile press **START**.

Note: Pressing any other key aborts the storing of the profile and takes you to the **A1: ANALYSIS - OUTPUT FORMAT** screen.

4.3.2 P1: Load Profile

Note: The **P1: LOAD PROFILE** screen looks and functions the same as the **P2: STORE PROFILE** screen.

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) Select **Load Profile** using the cursor keys.

(3) Press **START** and screen **P1** displays:

P1: LOAD PROFILE

Selected Profile: <Profile x>
**** Blank Profile ****

Aperture: Tl:
Volume: Th:

(4) Choose the profile you want to load by selecting either Profile A, B, C, D, or E, using the cursor keys.

The instrument displays a short description below the selection fields. The description reads **** Blank Profile **** if the profile selected is blank. If the profile selected is not blank, the aperture and threshold settings are displayed.

(5) To load the selected profile press **START**. The instrument compares the current settings to the ones in the selected profile. If they are compatible, the current settings are changed to the ones in the selected profile.

After the profile is loaded, the **S1: SETUP - ENTER SIZE DATA** screen displays showing the new settings.

Note: Pressing any other key aborts the loading of the profile.

4.4 Shutdown Procedure

Note: For **biological applications** it is recommended that the instrument is **regularly decontaminated** to prevent build-up of biological debris which may adversely affect the instrument's performance. For the disinfection procedure and additional maintenance procedures for biological applications refer to Appendix 7.

4.4.1 End of Day

In non-biological applications, if the instrument is to be used the next day or after a short interval, place a beaker of clean diluent on the platform ensuring that the Aperture and the electrode are submerged in diluent, then set the Power Switch to **O** (off) and disconnect from the Mains Supply.

In biological applications (particularly White Blood Cell counting) it is good laboratory practice to Prime the Aperture using a non-lytic cleaner (e.g.BECKMAN COULTER CLENZ® cleaning agent) and leave the cleaning agent around the aperture overnight. Ensure that the Aperture and the electrode are submerged in the cleaning agent before setting the Power Switch to **O** (off).

4.4.2 Long-Term Shutdown

If the instrument is to be left for a long period of time (in excess of 2 weeks) or is in dry ambient conditions, it is advisable to put distilled or de-ionized water in the beaker in place of the diluent, so that salt deposits are not formed as a result of evaporation.

4.4.3 Storage or Shipment

(1) Use the Drain System/Fill System functions (see para 4.5.1) to drain the system of diluent, refill the system with distilled or deionized water containing a small amount of preservative and drain the system again.

(2) Set the Power Switch to **O** (off), and disconnect instrument from Mains Supply.

(3) Remove the Aperture Tube, electrode assembly and Stirrer paddle (see Figure 4-1).

(4) Disconnect the tubing from the Waste and Diluent jars (see Figure 2-7). Disconnect the level sensors from the rear of instrument. Empty the jars and thoroughly wash.

Caution: If the Diluent tubing is disconnected at the instrument, instead of from the lid, diluent will siphon out of the jar.

(5) Disconnect the tubing from the Waste and Diluent connectors at the rear of the instrument. Link the Waste and Diluent connectors with tubing (see Figure 2-7) to prevent the ingress of contamination.

(6) Rinse all glassware and level sensors with distilled or de-ionized water before storage.

(7) Disconnect the Data Terminal (and Printer if connected).

(8) If the instrument is to be moved to another location that requires transportation, place all items in the packing case (see Section 2, Installation).

4.5 Additional Operating Information

4.5.1 Draining/Filling the Hydraulics System

These procedures must be observed when a new type of electrolyte solution is being used, or the existing type renewed, after a period of inactivity, or when the instrument is to be moved.

4.5.1.1 Draining the System

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Drain System**.

F1: INSTRUMENT FUNCTIONS

Prime/Fill/Drain/Flush/Vent
Select Function, press START
< Drain System >
Key beep < On >
Printer: < LaserJet >

(3) Press **START** and screen **F5** displays:

F5: DRAIN SYSTEM

Disconnect tube from diluent vessel and insert in suitable waste container. Place empty vial at sampling station.

PRESS ' START ' TO CONTINUE

Follow the instructions on the screen, ensuring that the platform is fully raised and the door is closed. A spare connection is provided on the Waste Jar cap to accept the disconnected tube. Press START and; the **PRESS ' START ' TO CONTINUE** status message is replaced by the sequence **DRAINING APERTURE** and then **DRAINING SYSTEM**. The number of cycles to completion is shown in the status message. The total procedure takes approximately ten minutes. When the final cycle is completed, screen **F5** is replaced by screen **F1** with the message **SYSTEM DRAINED/APERTURE VENTED** in the Message area.

Note: The procedure can be aborted by pressing STOP. This causes the Drain System procedure to stop when the current cycle has completed. Screen **F1** displays with the status message **STOP KEY PRESSED**. If procedure is then resumed (by pressing START again), the Drain System procedure starts from the beginning.

(4) If the instrument is not to be filled immediately, set the Power Switch to **O** (off).

4.5.1.2 Filling the System

If the instrument and power supply have not been switched off, since the system has been drained, ignore steps (1) to (2) and start at step (3).

(1) Set the Power Switch to **I** (on). The Data Terminal's LCD Display illuminates and screen **S1**: **SETUP - ENTER SIZE DATA** displays.

(2) Press FUNCTIONS and screen **F1** displays.

(3) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Fill System**.

```
F1:          INSTRUMENT FUNCTIONS
             Prime/Fill/Drain/Flush/Vent
                  < Fill System >
                  Key beep  < On >
               Printer: < LaserJet >
```

(4) Press **START** and screen **F3** displays:

```
F3:                 FILL SYSTEM

Ensure fill tube is connected
to diluent vessel.
Place clean diluent at sampling
station.
        PRESS ' START ' TO CONTINUE
```

(5) Follow the instructions on the screen, ensuring that the Diluent Jar is at least a quarter full with clean diluent, the platform is raised and the door is closed.

(6) Press **START**. The **PRESS 'START' TO CONTINUE** status message is replaced by **FILLING SYSTEM xx** (where **xx** is the number of cycles to completion), and the system starts to fill up with diluent. When the final cycle is completed, screen **F3** is replaced by screen **F1** with the message **SYSTEM FILLED** in the Message area.

4.5.2 Changing the Aperture Tube

This procedure **must be** observed if an Aperture is to be replaced without draining the entire system.

4.5.2.1 Draining and Removing the Aperture Tube

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) Using the cursor keys, move to the line under **Prime/Fill/Drain/Flush/Vent**, and select **Drain Aperture**.

F1: INSTRUMENT FUNCTIONS
Prime/Fill/Drain/Flush/Vent
< Drain Aperture >
Key beep < On >
Printer: < LaserJet >

(3) Press **START** and screen **F4** displays:

F4: DRAIN APERTURE

Disconnect tube from diluent
vessel and insert in suitable
waste container. Place empty
vial at sampling station.
PRESS ' START ' TO CONTINUE

Follow the instructions on the screen, ensuring that the platform is fully raised and the door is closed. A spare connection is provided on the Waste Jar cap to accept the disconnected tube. Press **START**. The **PRESS ' START ' TO CONTINUE** status message is replaced by **DRAINING APERTURE**. The number of cycles to completion is shown in the status message. When the final cycle is completed, screen **F4** is replaced by screen **F1** with the message **APERTURE DRAINED AND VENTED** in the Message area.

(4) Turn the Stirrer Position Control fully clockwise, hold the stirrer collet with finger and thumb and remove the stirrer paddle (push fit) if fitted and then carefully unscrew the Aperture Tube from its socket in the Mounting Block, as shown in Figure 4-1.

4.5.2.2 Storing the Aperture Tube

(1) For short term storage, aperture tubes should be capped and stored wet e.g. in a beaker of distilled water.

(2) For long term storage, aperture tubes should be thoroughly rinsed inside and out with clean distilled water and allowed to dry. Then capped and stored in its original packaging.

4.5.2.3 Fitting and Priming the Aperture Tube

If the instrument and Power Supply have not been switched off since an Aperture Tube was removed, ignore steps (1) to (4) and start at step (5).

(1) Set the Power Switch to **I** (on). The Data Terminal LCD illuminates and screen **S1: SETUP - ENTER SIZE DATA** displays.

(2) Press **FUNCTIONS** and screen **F1** displays:

Warning: The Vent function must be selected when an Aperture Tube is being fitted, to prevent the possibility of liquid being sprayed from the aperture.

(3) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Vent Aperture**.

```
F1:     INSTRUMENT FUNCTIONS
        Prime/Fill/Drain/Flush/Vent
             < Vent Aperture >
             Key beep  < On >
           Printer: < LaserJet >
```

(4) Press **START** to initiate the Vent Aperture function. On completion the status message **APERTURE VENTED** displays at the bottom of screen **F1**.

(5) Offer the Aperture Tube to the socket in the Mounting Block with the orientation shown in Figure 4-1, Coulter logo to rear. Then secure it by fully rotating in the direction shown. The aperture will be oriented towards the FOCUS control as shown in Figure 4-1, but may not directly face it. When in place with the Coulter logo to the front, the aperture is slightly angled to produce an optimum image on the aperture viewing screen. **Do not** attempt to adjust the angle of the aperture.

(6) Turn the Stirrer Position Control fully clockwise, hold the stirrer Collet with finger and thumb, then press fit the Stirrer Paddle (see Figure 4-1).

(7) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Prime Aperture**.

```
F1:     INSTRUMENT FUNCTIONS
        Prime/Fill/Drain/Flush/Vent
             < Prime Aperture >
             Key beep  < On >
           Printer: < LaserJet >
```

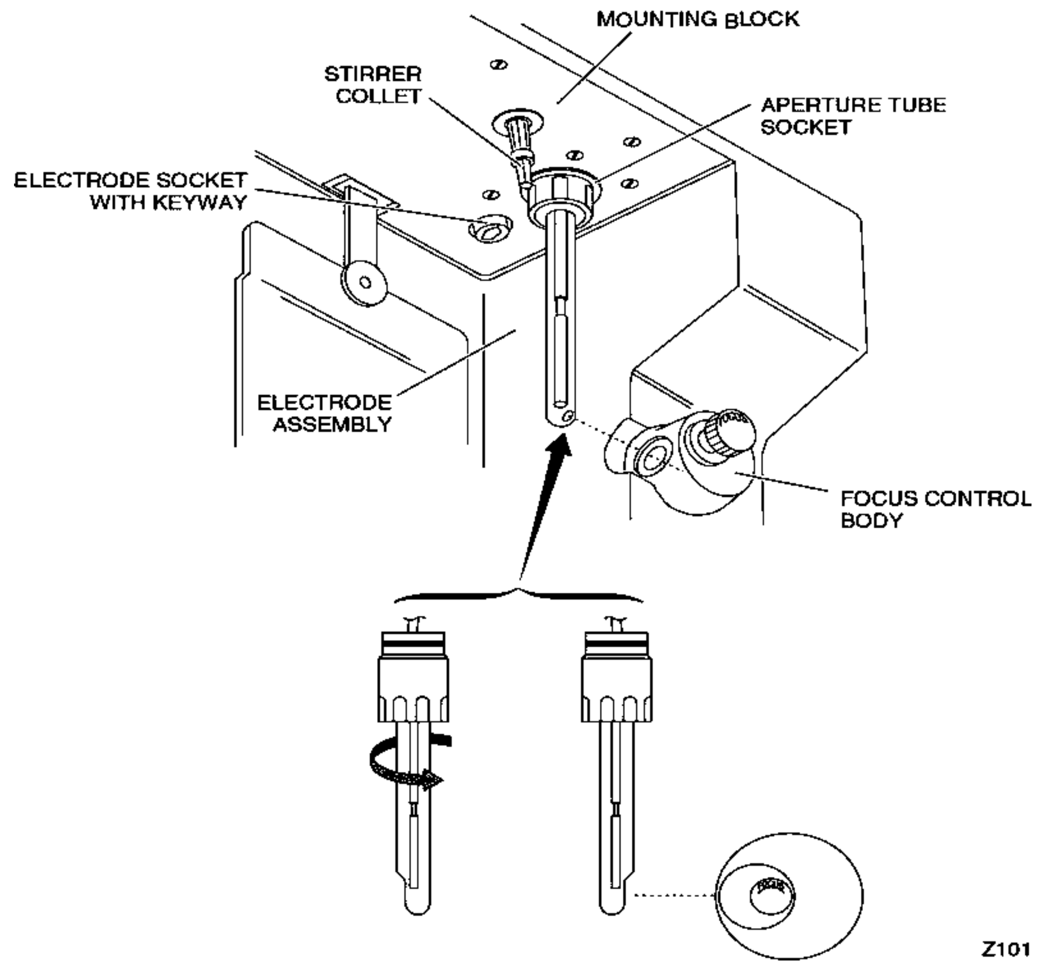


Figure 4-1
Repacing the Aperture Tube

(8) Press **START** and screen **F2** displays:

> **F2: PRIME APERTURE**
>
> **Ensure fill tube is connected**
> **to diluent vessel.**
> **Place clean diluent at sampling**
> **station.**
> **PRESS ' START ' TO CONTINUE**

(9) Follow the instructions on the screen, ensuring that the Diluent Jar is at least a quarter full with clean diluent, the platform is raised and the door is closed.

(10) Press **START**. The **PRESS ' START ' TO CONTINUE** status message will be replaced by **PRIMING APERTURE**, and the aperture starts to fill up with diluent. The number of

cycles to completion is shown in the status message. On completion, screen **F1** is again displayed with the status message **APERTURE PRIMED**.

4.5.2.4 Selecting a Printer

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) Using the cursor keys, move to **Printer** and select **LaserJet** or **Dot Matrix**.

```
F1:        INSTRUMENT FUNCTIONS
           Prime/Fill/Drain/Flush/Vent
           Select Function, press START
           <  Prime Aperture  >
           Key beep:  < Off >
           Printer: < LaserJet >
```

4.5.3 Setting the Date and Time

It is important that the results you print have the correct date and time displayed on them. Upon power-up initialization, the Z2 checks to see if its real time clock (RTC) has been set. If it has, the process proceeds. If the instrument finds that the RTC has never been set, it displays the message **Please initialize the time and date** and the **F5: SET CLOCK** screen appears. If you receive this message, the Z2 will not allow you to continue until date and time have been entered and **START** has been pressed.

Note: The Z2 does **not** perform automatic daylight savings time adjustments due to the differences in daylight savings worldwide. It is a user responsibility to change the time manually through the **F5: SET CLOCK** screen.

To set/change the date and time:

(1) Press **FUNCTIONS** and screen **F1** displays.

(2) Using the cursor keys, move to the line below **Select function, press START** and select **Set Clock**.

(3) Press **START** and screen **F5** displays.

(4) Enter the date and time in the format shown on the screen (**mm/dd/yyyy hh:mm**).

Note: A 24-hour clock is used.

```
F5:                    SET CLOCK

Enter the current time below.
Press START to set the time

               mm/dd/yyyy   hh:mm
               00/00/0000   00:00
```

(5) Press START and the date/time is set and you are returned to the **F1** screen.

4.1 **Introduction** 4-1
4.2 **Sample Analysis** 4-1
4.2.1 S1: Setup - Enter Size Data Screen 4-2
4.2.2 S3: Setup - Instrument Settings Screen 4-4
4.2.3 Threshold Range Too Wide (Dual-Threshold Version Only) 4-5
4.2.4 Aperture Selection Table 4-6
4.2.5 Output Screen 4-7
4.2.6 Printout Configuration 4-8
4.2.7 Counting 4-10
4.2.8 Analysis Result 4-11
4.2.9 Changing Size Settings in the Analysis Screen 4-12
4.2.10 Channelyzer (Z2 Only) 4-13
4.2.10.1 **Channelyzer Results** 4-13
4.2.10.2 **Statistics** 4-14
4.3 **User Profiles** 4-14
4.3.1 P2: Store Profile 4-14
4.3.2 P1: Load Profile 4-15
4.4 **Shutdown Procedure** 4-16
4.4.1 End of Day 4-16
4.4.2 Long-Term Shutdown 4-16
4.4.3 Storage or Shipment 4-16
4.5 **Additional Operating Information** 4-17
4.5.1 Draining/Filling the Hydraulics System 4-17
4.5.1.1 **Draining the System** 4-17
4.5.1.2 **Filling the System** 4-18
4.5.2 Changing the Aperture Tube 4-19
4.5.2.1 **Draining and Removing the Aperture Tube** 4-20
4.5.2.2 **Storing the Aperture Tube** 4-20
4.5.2.3 **Fitting and Priming the Aperture Tube** 4-21
4.5.2.4 **Selecting a Printer** 4-23
4.5.3 Setting the Time and Date 4-23

ILLUSTRATIONS

Figure 4-1 Replacing the Aperture Tube 4-22

TABLES

Table 4-1 Minimum ‘Set Lower Size Tl’ and Suitable Metered Volumes for Selected Aperture Sizes 4-3
Table 4-2 Valid Selections for Printer Configuration Screen 4-9
Table 4.3 ‘Counting’ Screen - Screen Items and Function 4-10
Table 4-4 Z2 Cursor Actions while Obtaining Statistics 4-14

INDEX

aperture
table, suitable metered volumes for selected aperture sizes 4-3
Aperture Tube
draining 4-20
illustration, replacing 4-22
installation 4-21
replacing 4-21
selection 4-21
storage 4-20
channelyzer
description 4-13
results 4-13
statistics 4-14
table, using the cursor 4-14
operation 4-1
output
screen 4-7
profiles
Load Profile (P1) 4-15
description 4-14
loading 4-15
Store Profile (P2) 4-15
storing 4-14
sample analysis
Analysis (A6) 4-12
Analysis - Output Format (A1) 4-7
Analysis Result (A3) 4-11
Analysis Result (A4) 4-12
Analysis Result (A5) 4-11
changing threshold settings 4-12
interrupted 4-11
Setup - Aperture Selection (S4) 4-6
Setup - Enter Analysis Data (S2) 4-3
Setup - Enter Size Data (S1) 4-2
Setup - Instrument Settings (S3) 4-4
Threshold Range Too Wide (S9) 4-5
shutdown procedure
decontamination 4-16
end of day 4-16
for storage/shipment 4-16
long term 4-16
system
Drain System (F5) 4-18
draining 4-17
Fill System (F3) 4-19
filling 4-18
thresholds
Threshold Range Too Wide (S9) 4-5
Vent Aperture
procedure 4-21

Section 5 Specifications

5.1 Guaranteed Data

The following data is guaranteed when the instrument is operating within the environment now specified:

Input Voltage within Set Range : 100V, 120V, 230V, 240V a.c ±10%, single phase.

Supply Frequency : 47 to 63 Hz inclusive.

Operating Temperature : 10°C to 35° C.

Relative Humidity : 10% to 85% without condensation.

Electrolyte Solution : Aqueous: 1% to 5% sodium chloride, 2% to 5% sodium phosphates, ISOTON II, and ISOTON III

Particle Size : Each size of aperture allows the measurement of particles in the approximate diameter range of 2% to 60% of the aperture diameter.

Location : The instrument is placed on a bench that is **not subject to**:

(1) Strong vibrations or sounds of high intensity (e.g. ultrasonic baths and probes).

(2) Excessive airborne dust.

(3) Electrical interferences such as that caused by brush type motors, flickering fluorescent lights or arcing contacts, water baths, gas chromatographs or bleep paging systems.

(4) Spillage or splashing.

5.1.1 Particle Size Range

1µm to 120µm equivalent spherical diameter (depending on the size of the aperture).

5.1.2 Particle Count

At 10,000 count level (average of at least twenty replicate determinations), at concentrations below 20% coincidence.

Accuracy : Within ± 1% of a reference counter system.

Precision : Better than 1% CV at metered volumes 0.5mL and 1.0ml.

Better than 2% CV at metered volume of 0.1ml.

5.1.3 Thresholds

The displayed pulse height range is divided into 100 Threshold Units (100% equals full scale). Each threshold (i.e. Lower and Upper) is resolvable and selectable in 0.1 unit increments.

Note: The Dual Threshold is an optional extra.

Accuracy : Lower Threshold is better than ± 0.2% full scale (i.e. ± 0.2 Threshold Units) across selectable range.

Upper Threshold relative to the lower threshold, is better than ± 0.2% full scale i.e. ± 0.2 Threshold Units.

5.2 Informative Data

5.2.1 Aperture Tube Sizes

Standard Aspect Ratio : 50µm, 70µm, 100µm, 140µm and 200µm.

Ampoule Insertable Style : 50µm, 70µm, and 100µm.

5.2.2 Metering Volumes

0.1ml, 0.5mL and 1.0mL.

5.2.3 Data Output

Liquid Crystal Display : Displays the menus required for setting up the operation of the instrument.

During accumulation, displays the incremental count.

On completion of analysis, displays the count, size and count/ml.

Printer Interface	: Code format: Centronics Parallel, IBM compatible, 25-way ‘D’ connector. Instrument can either automatically or manually (press PRINT) output data after each accumulation. The data output can either be results only or results with instrument settings.
External Control Port	: For future use.

5.3 Power, Dimensions and Weight

Power (VxI)	: Less than 55 watts.	
Main Unit	: Height	445mm (17.5in)
	Width	265mm (10.4in)
	Depth	355mm (14.0in)
	Weight	13.5 kg (1.5lb).
Data Terminal	: Height	64.0mm (2.5in)
	Width	155mm (6.1in)
	Depth	151mm (5.9in)
	Weight	0.7kg (1.5lb).
Diluent & Waste Station	: Height	190mm (7.5in)
	Width	285mm (11.2in)
	Depth	165mm (6.5in)
	Weight	1.3kg (2.9lb).

5.1 **Guaranteed Data** . . . 5-1
5.1.1 Particle Size Range . . . 5-1
5.1.2 Particle Count . . . 5-2
5.1.3 Thresholds . . . 5-2
5.2 **Informative Data** . . . 5-2
5.2.1 Aperture Tube Sizes . . . 5-2
5.2.2 Metering Volumes . . . 5-2
5.2.3 Data Output . . . 5-2
5.3 **Power, Dimensions and Weight** . . . 5-3

INDEX

Aperture Tube
- sizes . . . 5-2

guaranteed data
- coincidence corrected count . . . 5-2
- particle size range . . . 5-1
- thresholds . . . 5-2

informative data
- Aperture Tube sizes . . . 5-2
- data output . . . 5-2
- metering volumes . . . 5-2
- power, dimensions, weight . . . 5-3

location requirements . . . 5-1
metering volumes . . . 5-2
operating temperature . . . 5-1
particle count . . . 5-2
particle size range . . . 5-1
power, dimensions, weight . . . 5-3
printer interface . . . 5-3
relative humidity . . . 5-1
specifications . . . 5-1

system
- specifications . . . 5-1

thresholds
- description . . . 5-2

Section 6 Principles of Operation

6.1 Basic Principle

6.1.1 Beckman Coulter Method of Counting and Sizing

The Beckman Coulter method of counting and sizing is based on the detection and measurement of changes in electrical resistance produced by a particle or cell suspended in a conductive liquid (diluent) traversing through a small aperture.

When particles or cells are suspended in a conductive liquid, they function as discrete insulators. When a dilute suspension of particles is drawn through a small cylindrical aperture, the passage of each individual particle momentarily modulates the impedance of the electrical path between two submerged electrodes located on each side of the aperture. Figure 6-1 illustrates the passage of a particle through an aperture. An electrical pulse, suitable for counting and sizing, results from the passage of each particle through the aperture.

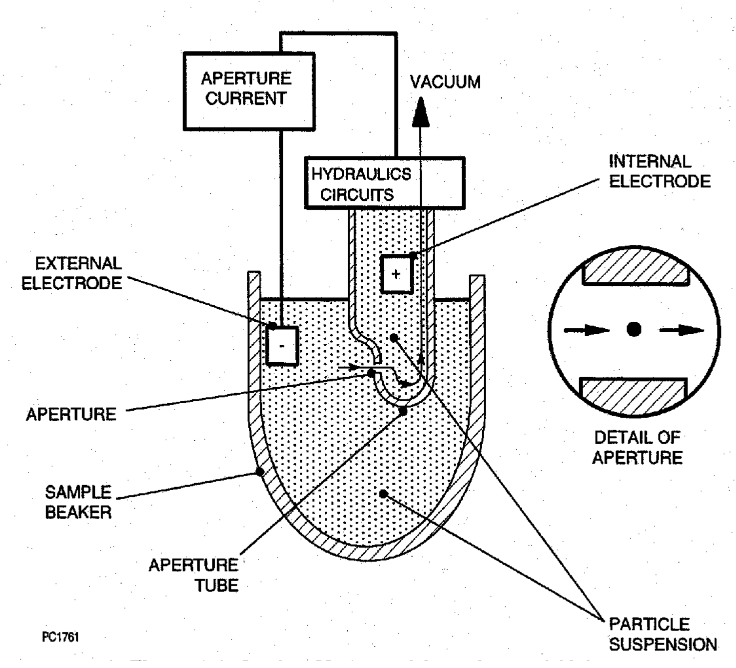


Figure 6-1 Coulter Method of Counting and Sizing

While the number of pulses indicates particle count, the amplitude of the electrical pulse produced depends on the particle's volume. The effective resistance between the electrodes is due to the resistance of the conductive liquid within the boundaries of the aperture. The presence within the aperture boundaries of a particle or cell, raises the resistance of the conductive pathway by an amount that depends on the particle's volume. Theoretical and practical analysis of the behaviour of particles within an aperture shows that the height of the electrical pulse produced by the particle is the characteristic that most nearly exhibits proportionality to the cell volume (Eckhoff 1967, Grover et al. 1972, Waterman et al. 1975, Kachel et al. 1976, Harfield et al. 1984). This method permits the selective counting of particles and cells within very narrow size-distribution ranges by electronic selection of the pulses they generate.

6.1.2 Coincidence Correction

Occasionally, more than one particle is within the boundaries of an aperture at the same time (coincidence). When this occurs, only one larger pulse is generated. This results in low particle count and high particle volume measurements. However, the frequency of coincidence is a statistically predictable function of particle concentration, and is corrected by the instrument.

6.1.3 Effect of Diluent (Electrolyte Solution)

In a counting system highly sensitive to the volume of the individual particles being counted, the conductive liquid in which the particles or cells are suspended must have a minimum influence on their integrity, and, hence, on their size.

6.2 COULTER Z Series Functional Description (Figure 6-2)

The primary purpose of the BECKMAN COULTER Z Series is to produce an electrical pulse each time a particle within the size range of the Aperture Tube passes through the aperture. It then counts the number of pulses, as a known volume of sample passes through the aperture. The count is then corrected for coincidence errors (e.g. the error caused by two particles entering the aperture together and being counted as only one particle).

To achieve this purpose, the instrument contains circuitry that, on command from the software, controls the Hydraulics System, automatic calibration facilities, and an output interface to an optional printer.

The following description is based on the simplified block diagram in Figure 6-2.

6.2.1 Pulse Generation

A constant aperture current, with a value that is either set automatically or chosen from the Setup Menu is generated by the Aperture Current Generator and routed via the safety circuits to the external electrode. The safety circuits are controlled by the Screened Door and Beaker platform micro-switches. These switches are closed only when the Screened Door is closed and the Beaker Platform is fully raised. The safety circuits prevent the external electrode from being touched

while current is flowing.

With aperture current flowing between the external and internal electrodes, a known volume of sample is drawn through the aperture. As each particle passes through the aperture, the impedance between the electrodes changes, causing a pulse whose size is essentially proportional to the volume of the particle. This pulse is routed, via the isolation capacitor, to the preamplifier.

6.2.2 Pulse Processing

Note: The Dual Threshold facility is an optional extra, therefore all text references to the Upper Threshold, Difference Circuit and Difference Counter only apply if that option is fitted.

After amplification, the resultant voltage pulses are sent to threshold comparators, which only allow pulses through that are equal to, or exceed, the size setting. One comparator is controlled by Count Above (Lower Threshold} and the other by the Upper Threshold. The pulses from the Lower Threshold comparator are routed to:

(1) A 'Set Lower Size Tl' counter.

(2) A 'Difference Circuit' which subtracts the pulses that have passed through the upper threshold comparator. The output of the 'Difference Circuit' is the number of pulses that have equalled or exceeded the Lower Threshold, but not reached the Upper Threshold. This output is then routed to the Difference Counter.

Both counters accept only pulses when the 'Start/Stop' signal is present. The duration of this signal is determined by the selected volume of sample passing through the aperture. The counts are routed to the Data Terminal's LCD Display, which during the count shows:

(1) The incrementing 'Count' total.

(2) A display that gives an indication of pulse height (and thus size) relative to the setting of the Lower Threshold. A sudden absence of pulses before the end of a count would give an immediate indication of a blocked aperture.

When the count is completed, coincidence correction is applied to the counts within the CPU and the coincidence corrected results are displayed on the Data Terminal's LCD Display and routed to the Printer (if connected).

In addition to the two counters found in the Z1, the Z2 adds the ability to channelyze pulses. After a pulse has been amplified, it is measured and the channelyzer bin corresponding to the pulse's height is incremented. This process continues until the metered volume has been drawn through the aperture. The resulting histogram shows the distribution of particle sizes. Statistics such as mean and standard deviation are then calculated by the CPU and are available for viewing on either the Z2 Data Terminal's LCD Display or from a printed report.

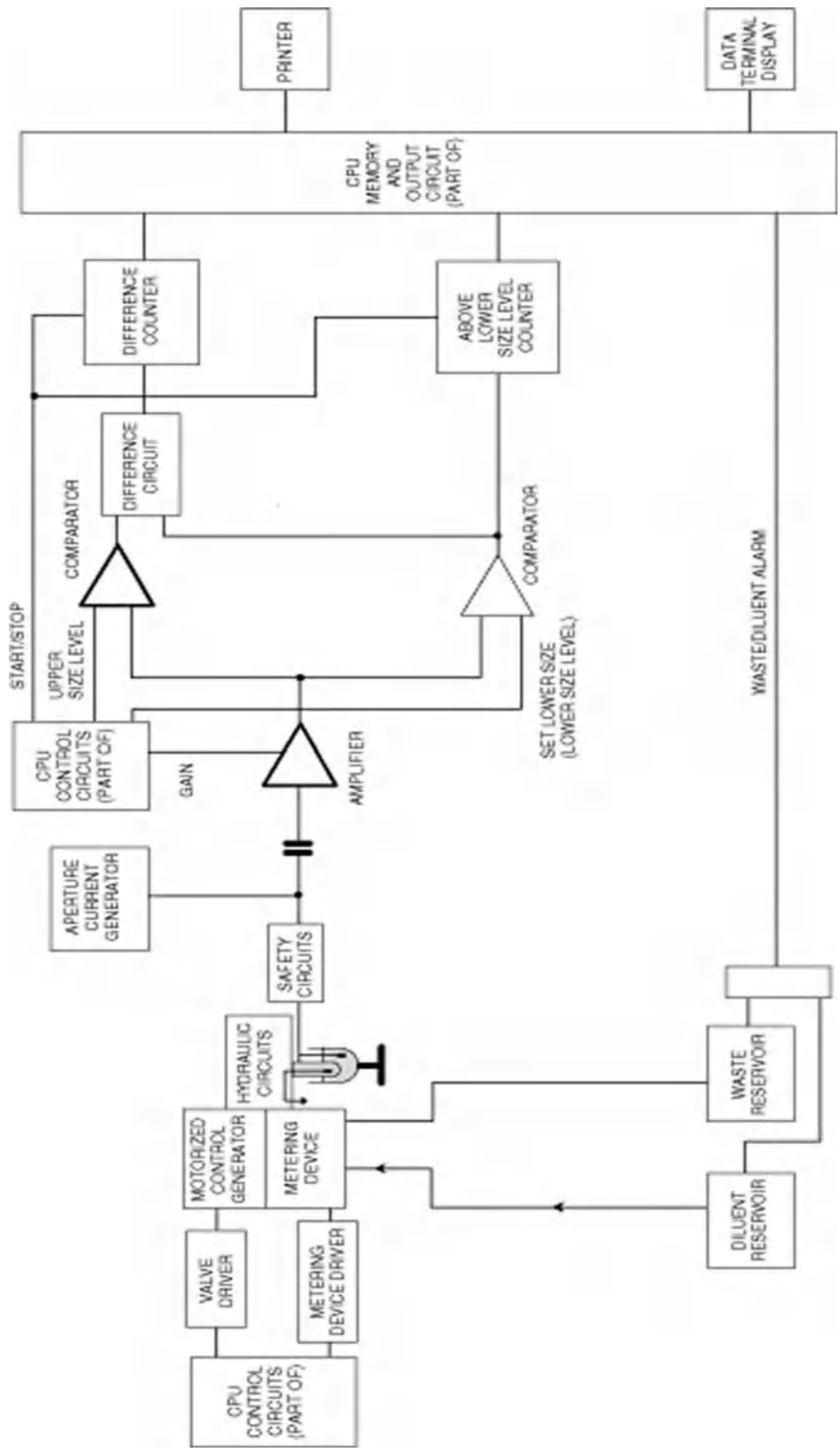


Figure 6-2 BECKMAN COULTER Z Series Functional Diagram

6.2.3 Control Circuits

The Central Processing Unit (CPU) controls, via various dedicated circuits, the complete operation of the Hydraulic System. Figure 6-2 shows the relationship of these circuits to the Hydraulic System in a very simplified manner.

6.1 **Basic Principle** 6-1
6.1.1 Beckman Coulter Method of Counting and Sizing 6-1
6.1.2 Coincidence Correction 6-2
6.1.3 Effect of Diluent (Electrolyte Solution) 6-2
6.2 **BECKMAN COULTER Z Series Functional Description (Figure 6-2)** 6-2
6.2.1 Pulse Generation 6-2
6.2.2 Pulse Processing 6-3
6.2.3 Control Circuits 6-5

ILLUSTRATIONS

Figure 6-1 Beckman Coulter Method of Counting and Sizing 6-1
Figure 6-2 BECKMAN COULTER Z Series Functional Diagram 6-4

INDEX

basic principle 6-1
channelyzer
  description 6-3
coincidence correction
  description 6-2
control circuits
  description 6-5
Coulter Method of counting and sizing 6-1
functional description
  control circuits 6-5
  pulse generation 6-2
  pulse processing 6-3
operation
  principles of 6-1
principles of operation 6-1
pulse generation
  description 6-2
pulse processing
  description 6-3
system
  functional description 6-2
  principles of operation 6-1

Section 7 Preventative Maintenance

Warning: **Mains voltages and d.c. voltages exceeding 50V are used within the instrument. The instrument must be disconnected from the mains supply before removing the covers. If the mains lead requires attention, have it repaired by a qualified electrician.**

7.1 General

Very little maintenance is required other than standard laboratory good housekeeping procedures. The maintenance required is given in Table 7-1.

Daily use of a non-lytic cleaner (BECKMAN COULTER CLENZ cleaning agent,PN(8546929) can alleviate protein build-up from biological samples on aperture and electrode surfaces.

Table 7-1 Preventative Maintenance Check List

Time Period	Subject	Description
Weekly	Sample Compartment	Remove any visible deposits of salt or dust.
	User Testing	Operator selection of the following self tests; keyboard, display, metering pump, control valve. Display of current software version and ability to reset default settings.
Bi-Monthly	Biological Applications	Bleach the aperture tube by filling an Accuvette 50/50 with hot water and household bleach. Run 5 counts. Let sit for 5 min. Run 5 counts. Pergorm prime cycle with Isoton II. Very background count. System Flush-Perform a drain system function. Fill system with 80% hot tap water, 20% household bleach. Sit for 10 minutes. Drain system. Fill instrument with Isoton II.
	Electrode Assembly	Inspect for cleanliness and security

Note: For biological applications it is recommended that the instrument is regularly decontaminated to prevent build-up of biological debris which may adversely affect the instrument's performance. For the disinfection procedure refer to Appendix 7.

In biological applications such as tissue culture, blood, etc., the unit should be completely Drained with a “DRAIN SYSTEM” Function, then filled with a “FILL SYSTEM” with 80% hot tap water and 20% household bleach and left to sit for 5 minutes. Then “DRAIN SYSTEM” followed by a “FILL SYSTEM” with Isoton II. This procedure should be performed **twice monthly**

.

7.2 User Testing

(1) Press FUNCTIONS and screen **F1** displays.

(2) Select **User Testing** using the cursor control keys.

F1: INSTRUMENT FUNCTIONS

Prime/Fill/Drain/Flush/Vent
Select function, press START
< User Testing >
Key beep < On >
Printer: <LaserJet>

(3) Press START and screen **T1** displays:

T1: USER TESTING

Select a test and press START
< Software Versions >

(4) Using the cursor keys, select and initiate the following:

Software Versions

Press START and the installed software version is displayed. For example:

Dual	**Z1 Terminal - - Version 1.00** **07/19/96 14:38:39**
Single	**Z1 Terminal - - Version 1.00** **07/19/96 14:38:39**

Press START to return to screen **T1.**

Keyboard

Press START and follow the instructions displayed at the bottom of the screen to check the keyboard. When no further instructions occur, the keyboard functionality has been satisfactorily checked.

Display

Press START and the status message **Press START to continue** displays. Press START. When the display returns to screen **T1** the display has been successfully checked.

Metering Pump

Press START and the instrument initiates a series of automatic test routines. During each test, one of the following status messages displays:

Valve to fill position

Wait for away sensor

Resetting Valve

Wait for home sensor

Control Valve

Press START and the instrument initiates a series of automatic test routines. During each test, one of the following status messages displays:

Going to Count Position

Going to Flush Position

Going to Discharge Position

Going to Reset Position

Going to Discharge Position

Going to Flush Position

Going to Count Position

Going to Reset Position

Default Settings

Note: Do not reset default settings unless advised by qualified Beckman Coulter personnel as all user settings will be lost.

Press START and the screen displays:

Loading the default values
will overwrite all stored
information including Kd 's.

Press START to accept

Press **START** and the screen displays:

> **Power the unit off for the**
> **new defaults to take effect.**
>
> **POWER OFF NOW**

7.3 Flushing an Aperture

To flush particles or air bubbles away from the rear of the aperture proceed as follows:

(1) Press **FUNCTIONS** until screen **F1** displays.

(2) Select **Flush Aperture** using the cursor keys.

> **F1: INSTRUMENT FUNCTIONS**
>
> **Prime/Fill/Drain/Flush/Vent**
> **Select function, press START**
> **< Flush Aperture >**
> **Key beep < On >**
> **Printer: < LaserJet >**

(3) Press **START** to initiate the flush operation. On completion, the status message **FLUSHING** is replaced by **FLUSH COMPLETED**.

7.1 **General** 7-1
7.2 **User Testing** 7-1
7.3 **Flushing an Aperture** 7-4

TABLES

Table 7-1 Preventative Maintenance Check List 7-1

INDEX

Flush Aperture
- procedure 7-4

preventative maintenance
- Flush Aperture 7-4
- table, check list 7-1
- User Testing (T1) 7-1

User Testing
- list of tests 7-2
- procedure 7-1

Section 8 Status and Error Messages

Various messages appear at the bottom of the Data Terminal's LCD Display (Message area) during analysis and data entry. These messages are divided into the following categories:

Status Messages These are informative messages that tell the operator what the instrument is doing and are part of normal operation. They appear at the bottom of the display, in inverse video.

Error Messages These warn the operator of any abnormal conditions requiring intervention. These appear at the bottom of the display, in inverse video. The error message is continuously displayed, and the sounder is activated. The operator is allowed to exit the menu screen.

Table 8.1 lists these messages in alphabetical order, with possible reasons and the action to take.

Table 8.1 Status and Error Messages

Message	Category	Reason	Action
ABNORMAL APERTURE FLOW RATE	Analysis Error	Aperture blocked.	Press UNBLOCK to clear.
ABNORMAL APERTURE RESISTANCE	Analysis Error	Aperture not submerged in diluent, or aperture blocked, or improperly made up electrolyte.	If aperture submerged in diluent, press UNBLOCK to clear, check electrolyte solution preparation.
ADJUSTING THRESHOLD	Status	Sample Analysis stopped to change Upper (Tu), or Lower (Tl) Thresholds.	None
APERTURE IS NOT PRIMED	Analysis Error	No diluent in Aperture Tube, or aperture not submerged in sample.	Check sufficient sample in beaker. Press FUNCTIONS, select **Prime Aperture**. Press START.
APERTURE Kd IS INVALID	Data Entry Error	Kd > aperture diameter, or < 0.25 x aperture diameter.	Check correct entry for **Select Aperture** and **Aperture Kd** in Setup Menu.
APERTURE DRAINED AND VENTED	Status	Completion of Drain Aperture function on screen **F1** of Functions Menu.	None
APERTURE PRIMED	Status	Completion of Prime Aperture function on screen **F1** of Functions Menu.	None
APERTURE VENTED	Status	Completion of Vent Prime Aperture function on screen **F1** of Functions Menu.	None
BUILDING VACUUM	Status	START is pressed. Shows instrument preparing to carry out an analysis or a procedure on screen **F1** of Functions Menu.	None

Message	Category	Reason	Action
CALIBRATING	Status	Calibration underway.	None
CALIBRATOR TOO DILUTE	Analysis Error	Not enough particles counted during calibration for Kd factor to be calculated.	Increase analysis volume or add more calibrator to sample beaker.
CALIBRATOR TOO LARGE	Data Entry Error	Calibrator Diameter > 20% of selected aperture.	Calibration inhibited until calibrator diameter < 20% entered.
CALIBRATOR TOO SMALL	Data Entry Error	Calibrator Diameter < 5% of selected aperture.	Calibration inhibited until calibrator diameter > 5% entered.
CHECK DILUENT VESSEL	Analysis Error	Diluent vessel empty.	Fill Diluent Jar with diluent.
CHECK METERING SYSTEM	Analysis Error	Metering pump reached end of travel.	Call Coulter Service.
CHECK SAMPLE LEVEL	Analysis Error	No current between electrodes.	Check aperture filled/sample present.
CHECK VACUUM SYSTEM	Analysis Error	Unable to achieve stable vacuum for sample analysis.	Call Coulter Service.
CHECK WASTE LEVEL	Analysis error	Waste Jar Full.	Empty Waste Jar.
CONTROL VALVE FAILED	Analysis Error	**Control Valve** selected on screen **T1** of User Testing Menu.	Call Coulter Service.
COUNTING	Status	Analysis underway, results being gathered.	None
DOOR IS OPEN	Analysis Error	If **START** pressed when Screened Door is open, procedure is inhibited and instrument reset.	Ensure Screened Door closed. Press **START**.
DRAINING APERTURE	Status	Drain Aperture procedure underway.	None
DRAINING SYSTEM	Status	Drain System procedure underway.	None
FILLING SYSTEM	Status	Filling System procedure underway.	None
FLUSHING	Status	Flushing procedure underway.	None
FLUSH COMPLETED	Status	Flushing procedure completed satisfactorily.	None
HIGH CONCENTRATION	Analysis Error	Number of particles too high to establish proper base line, analysis may be affected.	Prepare new sample at higher dilution. Press **START**, enter new dilution factor. Repeat analysis.
HIGH MASKING	Analysis Error	Too many pulses with large pulse widths at Lower Threshold setting.	Check aperture size (too small), lower threshold setting.
LOW CONCENTRATION	Analysis Error	Number of particles too low for accurate results. Message only occurs during calibration. Can not occur during sample analysis.	Add more calibrator, repeat calibration procedure.

Message	Category	Reason	Action
MEASURING APERTURE FLOW RATE	Status	Informs operator flow rate is being measured. Only occurs if **Measure Aperture** selected on screen **S2** of Setup Menu.	None
MEASURING APERTURE RESISTANCE	Status	Informs operator aperture resistance is being measured. Only occurs if **Measure Aperture** selected on screen **S2** of Setup Menu.	None
METERING PUMP FAILED	Analysis Error	**Metering Pump** selected on Screen **T1** of User Testing Menu.	Call Coulter Service.
NOISE MISMATCH	Analysis Error	Aperture impedance and preamplifier impedance not matched. Occurs when conductivity of electrolyte solution significantly changed without going back through Setup Menus.	Press SET-UP, check recommended settings before repeating analysis.
PERFORMING CALCULATION	Status	Occurs during calibration calculations.	None
PICKING BEST SETTINGS	Status	Occurs briefly when **Optimize Settings** set to **YES** and START pressed.	None
PLATFORM IS DOWN	Analysis Error	START pressed when sample platform is down. Procedure inhibited, instrument reset.	Raise platform until aperture submerged in sample beaker diluent.
PREPARING TO COUNT	Status	Resetting instrument prior to counting.	None
PREPARING TO CALIBRATE	Status	Resetting instrument prior to calibrating.	None
PRESS 'START ' TO CONTINUE	Status	Operator decision.	Press START.
PRIMING APERTURE	Status	Prime Aperture procedure underway.	None
RESETTING METERING SYSTEM	Status	Resetting Hydraulics System.	None
SELECTED CURRENT TOO HIGH	Data Entry Error	Aperture current selected requires aperture supply voltage exceeding maximum available.	Reduce selected aperture current, check gain to obtain better match.
STOP KEY PRESSED	Status	START/STOP pressed during instrument procedure.	Press START again to remove inhibit.
SYSTEM DRAINED/APERTURE VENTED	Status	Completion of System Drain function.	Operator decision to refill with fresh diluent or leave empty for storage or transportation.
SYSTEM DRAINED: NEED TO FILL	Analysis Error	Any key pressed when system drained of diluent.	Refer to Para 4.5.1.2, Filling the System, in Section 4, Operation.
SYSTEM FILLED	Status	Procedure to fill system with diluent completed satisfactory.	None

Message	Category	Reason	Action
THRESHOLD NEAR NOISE: Raise Tl	Analysis Error	’Count above’ (Lower Threshold) low enough to begin to count noise. Is within noise level of 5 and 2.5 Standard Deviations (SD). The rms (root mean square) value of electronic noise is measured independently of count. This is a warning. Sample analysis not inhibited as the resultant increase in count. Still makes count useful in some cases for comparison data.	Raise ’Count above’ (Lower Threshold) if possible. Alternately change to an Aperture Tube with smaller aperture.
THRESHOLD IN NOISE	Analysis Error	’Count above’ (Lower Threshold) < 2.5 SD noise level, Sample Analysis inhibited. So much noise being counted that results are unreliable.	Review all Setup Menu settings, do not just raise ’Count above’ (Lower Threshold). Consider using an Aperture Tube with smaller aperture and raising conductivity of electrolyte solution.
TIMEOUT: CHECK METERING SYSTEM	Analysis Error	Sample analysis or calibration run > 2 minutes.	(1) Check aperture for partial blockage. Press UNBLOCK. (2) If no obvious cause found, call Coulter Service.
VENTING APERTURE	Status	Vent Aperture function underway.	None

Section 8 Status and Error Messages 8-1

TABLES

Table 8.1 Status and Error Messages 8-1

INDEX

error messages
definition 8-1
table 8-1
status messages
definition 8-1
table 8-1

Appendix 1 Sample Preparation Techniques

A1.1 Introduction

To prepare the BECKMAN COULTER Z Series instrument for Particle Characterization Industrial Applications, various specialized operating techniques are required. The information contained in this appendix is designed to help operators who are unfamiliar with these techniques. The relevance of each of the following procedures is dependent on the type of analysis to be performed.

A1.2 Aperture Tube Selection

(1) The equivalent spherical diameter of most of the particles or cells to be counted must be between 2 and 60% of the nominal aperture diameter. If a significant amount of sample lies outside the size range of one aperture diameter, more than one size of Aperture Tube may be required for a complete analysis. In such a case, the determination of the largest and smallest particles present in the sample for analysis can be found by one of the following methods:

(a) Microscopic inspection.

(b) Trial and error procedure with various aperture sizes.

(c) An alternative test method, perhaps allied with knowledge of the method of sample manufacture.

(2) Ideally, monodispersed samples should lie at around 10% of the nominal aperture diameter for optimum aperture response. If however, two or more apertures are suitable for an application, the larger size will complete the measurements more rapidly. A smaller aperture is more suitable for high concentration suspensions.

(3) The presence of particles larger than 60 to 80% of the aperture diameter is likely to lead to aperture blockages.

(4) The following two types of Aperture Tubes can be used on the Z Series:

(a) Standard aspect ratio Aperture Tubes in the range; 50µm, 70µm, 100µm, 140µm and 200µm. Standard Aperture Tubes are defined as Aperture Tubes which have a 0.75:1 ratio between wafer thickness (L) and aperture diameter (D), the exception being the 50µm (1:1 approximately). Refer to Table A1-1 for useful information on Standard Aperture Tubes.

(b) Ampoule insertable style Aperture Tubes in the range; 50µm, 70µm and 100µm.

Table A1-1 Useful Standard Aperture Tube Data for the BECKMAN COULTER Z Series

Nominal Aperture Diameter	Nominal Particle Diameter Range	Typical Background Count > 2% of Aperture Diameter	Suitable Calibration Particles	Metered Volume (Conventionally used in bold)	Approx. Flow Time Seconds
50µm	1.0 to 30.0µm	500 at 1.0µm per 0.1ml	3 to 5µm	**0.1ml** 0.5ml 1.0ml	10.0 50.0 100.0
*70µm	1.4 to 42.0µm	1,200 at 1.4µm per 0.5ml	5 to 10µm	0.1ml **0.5ml** 1.0ml	5.0 25.0 50.0
100µm	2.0 to 60.0µm	400 at 2.0µm per 0.5ml	10 to 15µm	0.1ml **0.5ml** 1.0ml	2.5 12.5 25.0
140µm	2.8 to 84.0µm	300 at 2.8µm per 1.0ml	15 to 20µm	0.1ml 0.5ml **1.0ml**	1.5 6.5 12.5
200µm	4.0 to120.0µm	100 at 4.0µm per 1.0ml	15 to 20µm	0.1ml 0.5ml **1.0ml**	0.5 3.0 6.5

*** NOTE: FOR PLATELETS USE 0.1mls METERED VOLUME**

A1.3 Selection of Electrolyte Solution

Caution: Before mixing electrolyte solutions consider **any** possible risk. Some electrolytes are mutually incompatible and may generate gas or cause precipitation, if mixed. **Always** drain the Hydraulics System of old electrolyte solution before using a new or different one.

It is essential to use an electrolyte solution compatible with the characteristics of the material being analyzed. An electrolyte solution **must not** change the size or dispersed state of the sample but must have sufficient conductivity to make the operation of the Z Series possible. Typical diluent resistivities will be around 50 Ωcm (1% sodium chloride solution). Higher salt concentrations (5% sodium chloride) increase conductivity and can increase the noise characteristics of small apertures.

It is essential that the blank count of a diluent is statistically insignificant relative to the sample count. Typical values based on ISOTON II diluent are given in Table A1-1. Also refer to Para A1.4, Filtration of Electrolyte Solutions.

Caution: Damage may occur if solvent-based solutions are used. The BECKMAN COULTER Z Series should be used **only with** water-based solvents.

ISOTON II diluent is a filtered, phosphate-buffered saline solution compatible with human blood cells, and may be used for the suspension of most biological cells and many industrial samples.

The composition of a diluent can be modified to take into account particular sample characteristics (e.g., by the addition of common ions to suppress sparingly soluble particles). For biological samples a main consideration is the avoidance of the lytic properties in the diluent.

To help select a suitable electrolyte solution, Appendix 2 lists aqueous electrolytes, and Appendix 3 lists many of the more common particulate substances, together with most suitable electrolyte solution(s).

A1.4 Filtration of Electrolyte Solutions

It is essential that the background count from the electrolyte solution be as low as practicable (refer to Table A1-1). This is achieved by careful filtration. The following procedures are satisfactory for most laboratory-made diluents:

Note: ISOTON II diluent needs no further filtration.

(1) Add suitable preservatives to aqueous electrolyte solutions (e.g., 0.1% of either formaldehyde for acid solutions) or sodium azide for alkaline solutions.

(2) Pass the solution through either a ceramic No. 5 sinter or 0.8-µm glass fibre filter to remove most particles greater than 1-2µm diameter.

(3) Pass the filtrate through a 0.8µm pore size or smaller membrane filter and repeat if necessary.

A1.5 Dispersion

The BECKMAN COULTER Z Series instrument gives a count analysis of the particulate material presented to the aperture. If this material is presented in an agglomerated or flocculated form then an untrue count results. In some instances however, especially with airborne dusts, it is important to count the particles in an agglomerated state and dispersion to the ultimate particle size is undesirable.

In almost all cases, dispersion will be aided by the addition of a few drops of non-ionic dispersant such as Beckman Coulter Dispersant, obtainable from Beckman Coulter Particle Characterization, or its distributors. There are four usual methods of dispersion, which are as follows.

Gentle Mixing Gentle mixing of the powder with a drop or two of glycerol, alcohol, or suitable dispersant (e.g., Beckman Coulter Dispersant). This is done on a watch glass, using a spatula.

Caution: Ultrasonic probes can cause fracture of some types of particles. This is rarely the case with baths (for recommended models contact Beckman Coulter Particle Characterization or their authorized Distributors).

Ultrasonics After gentle mixing as described above, a sub-sample of the material is placed in a clean beaker and diluted by stirring with a few drops of electrolyte solution. More electrolyte solution is added and the beaker placed in a small ultrasonic bath for 15 to 60 seconds, or until dispersion is complete. Simultaneous stirring with a clean glass rod reduces the agitation time required. This method is the one generally found most satisfactory, but should not be used with fragile particles or biological cells.

Mortar and Pestle When the particle surface is hard (e.g., metal powders) work the sample in an agate mortar and pestle with a dispersant.

High Speed Mixing By violent stirring or shaking after the addition of several volumes of liquid (using, for example, a Waring Blender®). Allow air bubbles to disperse before starting the count. High-speed mixing can cause unwanted fracture of some particulate materials.

A1.1 **Introduction** A1-1
A1.2 **Aperture Tube Selection** A1-1
A1.3 **Selection of Electrolyte Solution** A1-2
A1.4 **Filtration of Electrolyte Solutions** A1-3
A1.5 **Dispersion** A1-3

Table A1-1 Useful Standard Aperture Tube Data for the BECKMAN COULTER Z Series A1-2

INDEX

Aperture Tube
- selection A1-1
- table of useful, standard data A1-2

diluent (electrolyte solution)
- filtration A1-3
- selection A1-2

dispersion
- description A1-3

electrolyte solution (diluent)
- filtration A1-3
- selection A1-2

Appendix 2 Aqueous Electrolyte Solutions

A2.1 Description

A wide range of electrolyte solutions may be required, dependent on the nature of the material and its solubility in water.

Warnings: (1) **Toxicity safety requirements and handling procedures of all reagents should be checked and adhered to (see Merck Index and/or Material Safety Data Sheets).**

(2) **Care must be taken in mixing some electrolyte solutions. Violent reactions can occur.**

Table A2-1 Typical Aqueous Electrolyte Solutions

Electrolyte Solution	Solvent	Uses
ISOTON II diluent (Coulter filtered electrolyte solution based on 0.9% saline)	Water	General water-insoluble materials.
0.9 to 4% Sodium chloride (NaCl)	Water (distilled or deionized)	General water-insoluble materials including drugs.
5% Tri-sodium orthophosphate (Na_3PO_4)	Water	General water-insoluble materials including negatively charged samples such as clays.
2% Sodium acetate ($NaOOC.CH_3$)	Water	Metals such as zinc and others which react with chlorides.

Notes: (1) Glycerol (CH_2OH. CHOH. CH_2OH) or sucrose (common sugar) may be added to most aqueous electrolyte solutions to increase viscosity (and density) and to assist suspension of large or dense particles (up to 70% by volume or l00% by weight, respectively).

(2) A preservative, for example, a 5ml/liter formaldehyde solution ('Formalin')*, or 0.l% w/v **sodium azide, NaN_3, should be added to aqueous electrolyte solutions before filtration to prevent micro-organism growth (the former in acid solutions, the latter in alkaline) if the electrolyte solution is to be stored.

*Formalin in alkaline solutions (e.g., sodium phosphates) tends to precipitate out of solution, causing small particle counts upon storage, and may interact with dispersant.

****Warning: Azide must not be used in acid solutions.**

(3) Electrolyte solutions **must be** filtered before use.

(4) Disperse dry particles in glycerol or Beckman Coulter Dispersant, then dilute out with electrolyte solution.

Beckman Coulter Particle Characterization and its distributors maintain a detailed list of electrolyte solutions found suitable for several hundred materials and will be pleased to recommend electrolyte solutions for any given material, or assist in developing a suitable technique for new materials. A partial list is given in Appendix 3.

Before using electrolyte solutions, check the stability of dispersion of the material. After dispersion and mixing for one minute with the stirrer on the beaker platform, take several counts at the coarse end (d max) and the fine end (d min) of the size range. After the completion of the full analysis, or after l5 minutes further stirring, recount at the same levels. Table A2-2 shows what a comparison of the counts will indicate.

Table A2-2 Stability of Dispersion

d max	d min	Symptom
Increasing	Increasing	Precipitation/Crystallization
Increasing	Decreasing	Flocculation
Decreasing	Decreasing	Dissolution
Decreasing	Increasing	Deflocculation
Decreasing	Steady	Settling of large particles
Steady	Steady	Stable dispersion

Flocculation: Try different electrolyte solutions (e.g., change to phosphate from chloride), dispersants, and pH.

Dissolution: Presaturate electrolyte solution with sample before filtration. Use common-ion effect to depress solubility (e.g., 5% sodium sulfate solution for barium sulfate, 2% calcium chloride for chalk or whiting).

Deflocculation: Wait until stable. Use different electrolyte solution, dispersant, pH. Use ultrasonics (low power bath).

Settling: Increase viscosity and/or density of electrolyte solution.

A2.1 **Description** 1

Table A2-1 Typical Aqueous Electrolyte Solutions A2-1
Table A2-2 Stability of Dispersion A2-2

aqueous electrolyte solutions
  recommendations A2-1
  table, typical aqueous electrolyte solutions A2-1
  Warnings A2-1
diluent (electrolyte solution)
  selection A2-1
electrolyte solution (diluent)
  selection A2-1

Appendix 3 List of Particulate Substances and Suitable Electrolyte Solutions

A3.1 Introduction

This section contains, alphabetically, a range of typical particulate substances and systems which can be analyzed using a BECKMAN COULTER Z Series instrument, together with the aqueous electrolyte solution(s) found to be suitable. Other specific notes are included where necessary.

In general, water-insoluble materials can be dispersed using appropriate surfactants in aqueous electrolyte solutions such as 1% W/V sodium chloride or 5% trisodium orthophosphate solution. Also, a non-sterile but bacteriostatted phosphate-buffered saline solution (ISOTON II diluent) can be purchased from Beckman Coulter Particle Characterization Group or its distributors (20 liter packs).

Warning: Flammable electrolyte solutions and organic solvents must not be used in a BECKMAN COULTER Z Series instrument.

An electrolyte solution list cannot guarantee a perfect result; this also depends on sample preparation, dispersion, and even particle size - the finer the material the more reactive (soluble, undispersible) it will usually be. This list, however, provides a starting point.

There are very few materials which cannot be analyzed using the Beckman Coulter technique. Occasionally some organic compounds (pesticides, drugs) are too soluble in any electrolyte solution, and particles dispersed in mineral oils may require prior separation (see list under Membrane Filters) before size analysis. Contact Beckman Coulter Inc. for instruments that can be used with non-aqueous electrolyte solutions.

Large particles, or those of high-specific gravity, may be difficult to suspend for analysis, and up to 75% V/V glycerol may be added to most electrolyte solutions to increase the viscosity. Alternatively, common sugar at up to 100% W/V may be added to aqueous electrolyte solutions to increase both viscosity and density.

Always observe the usual chemical precautions; for example, do not mix strong oxidizing agents such as chromium trioxide, nitric acid, chlorates, perchlorates, or permanganates with glycerol.

When using a 50µm aperture, an increase of salt concentration by a factor of two can reduce background count due to ‘heating noise’ in the aperture. Always check however, that this does not cause flocculation or poor dispersion.

An increased salt concentration may also be necessary with conducting particles (e.g., silicon, metals) to keep the voltage across the electrodes below some maximum limit, in order to allow

correct sizing. The surface of conductive particles can be rendered less conducting with quaternary ammonium compounds, see METAL POWDERS (Page A3-8).

Usually, non-ionic dispersants are best for use with electrolyte solutions, e.g., BeckmanCoulter Dispersant (Beckman Coulter Particle Characterization) or Triton X-100 (Rohm and Haas) although specific anionic or cationic surfactants can have advantages for some materials. The optimum choice is largely a matter of trial and error. The amount of surfactant used should be small (e.g., 2 to 3 drops per l00ml electrolyte solution) to keep below the critical micelle concentration.

A dispersant can dissolve some organic materials (e.g., some drugs) and glycerol or polyethylene glycol may be used instead. Glycerol reacts slowly with phosphate electrolyte solutions. This may not be significant if only large particle sizes, e.g., more than 5µm, are to be examined.

Dispersion is best aided by low power ultrasonics (baths - not probes, which can fracture some particles), and in some cases (e.g., clays) high-speed mixers.

The following list of electrolyte solutions has been provided by Beckman Coulter Particle Characterization laboratories from their more comprehensive records. Beckman Coulter Particle Characterization provides an advisory service for customers with problems regarding materials not listed here.

A3.2 Key to Aqueous Electrolyte Solutions

(a) 1% W/V sodium chloride (NaCl) (often interchangeable with b).

(b) ISOTON II diluent (Beckman Coulter Particle Characterization).

(c) N/10 sodium hydroxide (NaOH).

(d) N/10 hydrochloric acid (+ 0.1% W/V cetrimide).

(e) 2-5% W/V trisodium orthophosphate (Na_3P0_4); 2-5% W/V sodium pyrophosphate ($Na_4P_20_7$ $10H_20$).

(f) 20% W/V sodium chloride (NaCl).

(g) 1% W/V sodium nitrate ($NaNO_3$).

(h) 4% W/V zinc chloride ($ZnCl_2$).

(i) 2-5% W/V sodium sulfate (Na_2S0_4).

(j) 1% W/V potassium silicate (K_2Si0_3).

+G Glycerol ($CH_2OH.CHOH.CH_2OH$). Often added to help suspend large particles, and to increase the viscosity of the electrolyte solution when using large apertures.

+S Sugar (sucrose). Often added to help suspend large particles.

Satd. Electrolyte solution must be pre-saturated with sample.

ACETYL SALICYLIC ACID	-	See ASPIRIN.
ACRYLIC EMULSION or POWDER	a,b,e	See chemical name.
ALDACTONE®A	a,b	(Spironolactone).
ALUMINA	a,b e	Coarse powders only. Any aluminas.
ALUMINIUM	a	Alkaline (phosphatic) electrolyte solutions react. See metal powders.
ALUMINIUM OXIDE	-	See ALUMINA.
ALUMINIUM SILICATE (ANDALUSITE)	e	
AMPHOTERICIN (B)	a satd. b satd.	
ANIONIC BITUMEN EMULSION	c	Beckman Coulter Dispersant.
ASPHALT EMULSION	-	See ANIONIC or CATIONIC BITUMEN EMULSION.
ASPIRIN	a satd.	Beckman Coulter Dispersant or Aerosol 0.T. dispersant.
(Acetyl salicylic acid)	b satd.	
ATTAPULGITE	e	
AVICEL	a	(Food additive to control calories).
BALL CLAY	-	See CLAY.
BARIUM SULFATE	i	Common-ion effect depresses solubility.
BARK	a	
BARYTES	e	
BAUXITE (HYDRATED ALUMINA)	a	
BEER	a	Can also be counted ‘Direct’ with no extra

		electrolyte solution added. De-gas first; ultrasonic agitation 2 minutes very suitable. 50 to 100µm aperture covers particle range well. Beta-glucan haze is too fine in particle size to measure using the BECKMAN COULTER Z Series instrument.
BENTONITE	e	
BENZOIC ACID	b	
BENZYL PROCAINE PENICILLIN	a satd.	
BERYLLIA (Be0)	e,a,b	Daxad has been used as dispersant.
BITUMEN EMULSION	-	See ANIONIC or CATIONIC.
BLOOD CELLS	b	100µm aperture for red and white cells. 50µm or 70µm for platelets. See Appendix 8.
BONE	e	
BORON CARBIDE	a,b	Sulframin has been used as dispersant; electrolyte solution has been presaturated with Boric Acid.
BRONZE	-	5% v/v sodium silicate (S.G. 1.57) solution.
	-	1% sodium carbonate (Na_2CO_3); cannot increase electrolyte solution concentration because of chemical attack. Sodium chloride reacts. Difficult to wet mechanically, but ultrasonics with Coulter Dispersant very successful. In flake form it gives the characteristic streaming patterns in the stirred suspension, looking unhomogeneous, but this does not affect results.
CADMIUM & ZINC SULFIDE	-	See ZINC CADMIUM SULFIDE.
CALAMINE	e	
CALCINED MAGNESIA	e (+G)	For coarse calcined magnesia. (See also MAGNESIA.)
CALCITE AND CALCIUM CARBONATE (chalk, whiting)	a satd.	Teepol XL successful dispersant.
CALCIUM STEARATE	a,b,d,	Difficult to wet; *alcohols or Beckman Coulter

		Dispersant with ultrasonics and spatulation very successful.
CARBON (and CARBON BLACK)	e,a	Activated carbon will release adsorbed gases on passage through aperture giving spurious results. Disperse powder in a little warm glycerol with spatula, add a little electrolyte solution and **boil** for a few minutes. Cool and add remainder of electrolyte solution; count.
CARBONYL IRON	a (+G),b(+G)	
CARBORUNDUM	-	See ALUMINA and EMERY.
CATIONIC BITUMEN	d	See EMULSION.
CERAMIC POWDERS	e	
CERIUM OXIDE	e	
CHALK	-	See CALCIUM CARBONATE.
CHINA CLAY (KAOLIN)	-	See CLAY.
CHLORAMPHENICOL	a satd.	Up to 5% NaCl. Add 0.05% P.V.P. (poly vinyl pyrrolidone) to reduce sample solubility.
CHROMIUM POWDER	e	
CLAY	e	Usually very fine, much may be below the limit of the 50µm aperture. Weight integration (i.e., calculation of weight % measured) must be done from the relative density measured in the same medium at the time of immersion because of water present in the lattice structure, esp. of China Clay (kaolin). Correlation of results with other techniques (e.g., sedimentation) usually poor because of extreme shape factors. 80% < 2µm (Stokes) may relate to 20% < 2µm (Coulter). Disperse mechanically, (e.g., Waring Blender 30 mins) leave wetted for 1 to 2 days before analysis for clays absorbing water in their structure (e.g., montmorillonite).
COAL	e,a,	
COKE	a,b,e	

COPPER	a,b	No reaction during time for analysis.
CORN STARCH	a,b	
D.D.T	a satd. e satd.	
DIAMOND (POWDER)	e(+S),b(+S)	At 500 g/l sugar, can suspend 100 to 120 mesh fraction (125 to 250µm), in extreme case.
DIATOMITE		See CLAY.
DUST (FLUE, COAL, etc.)	e,a,b	e is the best choice if metal particles are suspected in the sample, e.g., gas mains dust.
ELECTROLYTE SOLUTIONS	-	To measure particles in electrolyte solutions, e.g., plating or parenteral solutions, measure directly, or if particle size range is reduced because the aperture resistance is too low, dilute with filtered distilled water; or to high, dilute with filtered salt solution.
EMERY (Al_2O_3)	a(+G) b (+G)	Usually coarse and in very narrow size range, e.g., 75 to 85µm.
EMULSIONS (including lubrication and coolant emulsions for Rolling Mills).	a,b	Solids or oil in water can be measured. Two-stage dilution of e.g. 1:100 each stage is often preferable to reduce coagulation. Usually very fine particle size, try 50µm aperture. Stability of emulsion over analytical period must be checked and maintained.
*EXPLOSIVES:		
-HMX	a satd	Beckman Coulter Dispersant.
-PETN	a satd	Beckman Coulter Dispersant.
-RDX	a satd	Beckman Coulter Dispersant.
FELSPAR (FELDSPAR)	a,b	
FERRITES	e	+50% glycerol reduces re-agglomeration rate. Heat treated ferrites are magnetic and cannot be dispersed stably to unit crystals; the agglomerate size will be fairly stable and measurable. Range 1 to 20µm. Preferably measure before heat-treatment.
FIBERS (paper pulp, wool top, etc.)	a	Fibers cut to constant known length can be measured for diameter (the denier distribution). Those of constant known diameter can be measured for length distribution, if they do not exceed 2 x diameter of aperture used.

FLINT	a,e	
FULLERS EARTH	e	See CLAY.
GARNET	e	
GLASS POWDER	a,b	Almost any electrolyte solution may be used.
GOLD	a,b,e,g, (+G)	
GRAPHITE	a,e	Satisfactory for coarse graphites; Very slow flocculation at 1µm level. 0.2N NaOH + 0.3% Teepol XL for fine graphite.
GRISEOFULVIN	a satd.	Add 0.01% GOULAC for dispersion.
HERBICIDES	a,b	Usually insoluble.
INJECTION FLUIDS	-	Usually 'Direct.' Most can be diluted in filtered 'a.' Iron dextran apparently has never been analyzed successfully, possibly because of complex formation, giving unstable counts.
ION EXCHANGE RESIN	a,f	See also ELECTROLYTE SOLUTIONS. Coulter Dispersant has been used as dispersant. Saturate sample in electrolyte solution (high concentration) before analyzing.
IRIDIUM	e(+S)	Extremely dense (22g/cm^3). With up to 500 g/l added sugar, analyses can be performed up to 60 to 80µm equivalent spherical diameter (e.s.d.).
IRON	a(+G) e	Reacts fairly quickly. Better, no reaction, but will not suspend particles above 30 to 40µm e.s.d.
	-	0.3% NaCl generally most suitable, + 50% glycerol if necessary.
		See also METAL POWDERS.
IRON OXIDE	a(+G), b(+G)	
KAOLINITE ($Al_2O_3.2SiO_2.2H_2O$)	e	See CLAY.
KETCHUP (CATSUP)	a,b	Has been reported that BECKMAN COULTER COUNTER® instrument may not respond to envelope volume for tomato cells. (Unconfirmed). This may be an effect of using too much aperture current.

LATEX (Rubber)	a,b,e	
LATEX (Synthetic)	a,b,e	For most latices. Slow flocculation according to Smoluchowsky equation. Useful for flocculation studies.
LEAD	a(+S), f (+S)	*Methyl alcohol and sulframin have been used as dispersants.
LEAD OXIDE (yellow and red)	a(+G) i	Up to 25% glycerol has been used. Lead oxide is soluble in alkaline solutions.
LEAD (RED)	e	
LIGNITE DUST	e	
LIME	a saturated	
LYCOPODIUM POWDER	a,b	Disperse by spatulation (e.g., in Beckman Coulter Dispersant) before dilution with electrolyte solution.
MAGNESIA, CALCINED	e (+G)	Suitable for coarse calcined magnesia.
MAGNESIUM	e (+S)	Very slow reaction; will not affect accuracy of results in time available.
MAGNESIUM HYDROXIDE	e saturated	
METAL POWDERS	a,b,f	Keep aperture voltage below some 6V to 12V. Conductivity effects may be minimized by coating the surface of the particles with a quaternary ammonium compound, e.g., Cetrimide. (Disperse powder in a few drops of a 1% solution in water, then dilute out with the usual electrolyte solution.)
MICA	e,a,b	Flaky shape, use largest suitable aperture.
MILK:		
- POWDER	a	For water-insoluble fraction. Disperse with a few drops of 5N sodium hydroxide.
MOLYBDENUM DISULPHIDE	- e	0.02N (8g/l) sodium hydroxide. Widely used.
MUD	a,b,e	

NICKEL	a,e	Some reaction may occur with e. See also RANEY NICKEL and METAL POWDERS.
OCEAN SEDIMENT	-	See SEDIMENT.
OIL, CUTTING	a,b	Some may lie below the limit of the 50µm Aperture Tube.
ORANGE EXTRACT	a,b	High particle concentration; weight integration analysis method may be required. May require 50µm Aperture Tube.
PAPER PULP	a,b	See FIBERS.
PENICILLIN	a satd.	
PHENACETIN	a satd.	
PHENOTHIAZINE	a satd.	
PHOSPHORS	a,b,e	See also under chemical name.
PIGMENTS	e,a,b	See under chemical name. May be too fine for 50µm Aperture Tube, weight integration or extrapolation may be required.
PLASTICS	a,b,e	See also under chemical name.
PLATING SOLUTIONS	-	Direct; or diluted with filtered distilled water.
POLLENS	a,b	Disperse by spatulation (e.g., in Beckman Coulter Dispersant) before dilution with electrolyte solution.
POLYETHYLENE (POLYTHENE)	a,b	
POLYPROPYLENE	a,b	
POLYSTYRENE	a,b	
POLY (STYRENE DIVINYL BENZENE)	a,b,e,	Often the 'monosized' particles used for calibration with most electrolyte solutions (Beckman Coulter Particle Characterization, calibration materials).
POLYTETRAFLUOROETHYLENE	a,b,e,etc	Almost any electrolyte solution is suitable. Finer powders may be difficult to wet (disperse).
POLYVINYL ACETATE (P.V.A.)	a	

POLYVINYL CHLORIDE (P.V.C.)	a,b	
POLYVINYL PROPYLODONE (P.V.P.)	a	
POLYVINYL TOLUENE (P.V.T.)	a,b	
PORCELAIN	e	
POTATO STARCH	a,b	
QUARTZ	e	See SILICA.
RIVER SEDIMENT	-	See SEDIMENT.
RIVER WATER	a,b,e	Phosphate electrolyte solution will precipitate any dissolved calcium salts present.
ROUGE	a	
RUST IN GASOLINE	-	Filter out and analyze in e.
RUTILE	-	See TITANIUM DIOXIDE.
SAND	a,b,e(+G)	
SEDIMENT	a,b,e	See RIVER SEDIMENT, OCEAN SEDIMENT.
SHALE	e	Sulframin has been used as dispersant.
SILICA (sand, quartz)	a e	 Slight reaction with fine particles (smaller than 1µm).
SILICATES	e	
SILICON CARBIDE	a,b,e	
SILVER HALIDE	-	4% potassium nitrate. 0.9% sodium chloride for AgBr.
SILVER OXIDE	a,g	
SLAG (BASIC)	a,e	
SOY FLOUR	b	
SPIRONOLACTONE	-	See ALDACTONE A.
STARCH	a,b,e	Corn, potato.

STEARATES	a	Difficult to wet. Spatulate with alcohol and use ultrasonics (low power).
STEEL	a(+G),f(+G)	
SULFADIMIDINE	a satd.	
SULFUR	a,e	Wet by spatulation with dispersant (alcohol) before dilution with electrolyte solution.
TALC	e	
TANTALUM	a,e(+G), f(+G)	
TIN	a(+G), f (+G)	
TIN OXIDE	e	Disperse in 5% Calgon, ultrasonics.
TITANIUM DIOXIDE	e	May be too fine for 50µm Aperture Tube; weight integration or extrapolation may be required.
TOMATO JUICE	e	Up to 4% salt.
TONER, XEROGRAPHIC	a	100µm aperture.
TUNGSTEN	a,e(+G/S) f (+G)	Spatulate in glycerol or dispersant before dilution with electrolyte solution.
TUNGSTEN CARBIDE	e,a(+G)	Spatulate in glycerol or dispersant before dilution with electrolyte solution. Can cold-sinter within hours of milling to cause permanent agglomeration.
URANIUM	e(+G),f(+G)	
URANIUM DIOXIDE	e	Can cold-sinter (see TUNGSTEN CARBIDE).
VISCOSE	c	Or dilute with filtered distilled water.
WATER, CONTAMINANTS IN	a	Dilute as required into filtered electrolyte solution.
WHITING	-	See CALCIUM CARBONATE.
XEROGRAPHIC TONER	-	See TONER, XEROGRAPHIC.
YEAST	a,b	Adjustment for isotonicity may be needed.
YTTRIUM IRON GARNET	e (+G)	Magnetic and needs frequent redispersing by ultrasonics.

ZEOLITE	e	See CLAYS.
ZINC CADMIUM SULPHIDE	e	A phosphor. Range usually 15 to 1µm.
ZINC STEARATE	a,b,e,h	Wet with Sulframin AB. (See CALCIUM STEARATE).
ZIRCONIUM OXIDE	a	Wet with 4% sodium pyrophosphate solution.

Comments on, or additions to this list will always be welcomed by the Customer Support Laboratory,Beckman Coulter Particle Characterization Group.

A3.1 **Introduction** A3-1

A3.2 **Key to Aqueous Electrolyte Solutions** A3-2

aqueous electrolyte solutions
list of suitable A3-3
recommendations A3-1
diluent (electrolyte solution)
selection A3-1
electrolyte solution (diluent)
selection A3-1
particulate substances and suitable electrolyte solutions A3-1

Appendix 4 Calibration Particles and Aperture Tubes

A4.1 Introduction

Calibration of the BECKMAN COULTER Z Series instrument (in absolute terms) for non-biological materials is done by the mass integration procedure on narrow distributions of smooth particles of known density. Quick, accurate and satisfactory calibrations may be made with essentially monosized particles available from various sources. The following materials (Beckman Coulter calibration materials) can be obtained from Beckman Coulter Particle Characterization Group for calibration by using the modal sizes. Table A4-1 lists the recommended calibration material with Part Number and the available Aperture Tubes with Part Number.

A4.2 Latex Suspensions

These particles are suspended at a concentration in distilled water (containing surfactant and preservative) such that one to five drops are required in approx. 200ml of electrolyte solution to calibrate an aperture tube approximately ten times the nominal latex diameter. Recommended concentrations are given with each vial, as are the various assayed sizes under different measurement conditions.

Polystyrene Divinyl Benzene latexes are particularly recommended as they will not readily change size upon immersion in alcohols, ketones or any aqueous electrolyte solution.

Table A4-1 Calibration Particles and Aperture Tubes for the Z Series

Calibration Standard				Aperture Tube	
Nominal size µm	Part Number	Quantity Supplied	Approximate Number per Vial	Aperture Size µm	Part Number
3	6602793	10ml	8.0×10^9	50	9912784
5	6602794	10ml	4.4×10^8	70	9912785
10	6602796	10ml	8.0×10^8	100	9912786
15	6602797	10ml	2.8×10^8	140	9912787
20	6602798	10ml	1.0×10^8	200	9912788

A4.3 Redispersion of Calibration Particles

Beckman Coulter calibration suspensions contain both a preservative and a surface active agent especially chosen to reduce any tendency of the particles to agglomerate or cake upon storage.

Before use, shake the vial vigorously for several seconds, and ensure that no sediment is left in the vial. Add one or more drops to the electrolyte solution as required. Further dispersion by ultrasonic agitation should not be necessary.

A4.4 Determination of Assayed Sizes

Note: Each pack of Beckman Coulter Particle Characterization Calibration Standards carries an assay sheet expressing median and modal diameters (etc.) of the particles, determined in ISOTON II diluent.

The basic procedures used by Beckman Coulter Particle Characterization to assay these sizes are detailed by Harfield and Wood[1].

When used as recommended, these Particle Characterization Calibration Standards allow direct traceability of calibration back to international Certified Reference Materials, for instance those of BCR (Community Bureau of Reference) and NIST (National Institute of Standards and Technology).

Reference:

[1]. Harfield J. G. and Wood W. M. Proc. Particle Size Analysis Conf., Bradford, Sept. 1970; Society for Analytical Chemistry 1971, p. 293-300.

A4.1 **Introduction** A4-1

A4.2 **Latex Suspensions** A4-1

A4.3 **Redispersion of Calibration Particles** A4-1

A4.4 **Determination of Assayed Sizes** A4-2

Table A4-1 Calibration Particles and Aperture Tubes for the Z Series A4-1

Aperture Tubes
- and calibration particles A4-1

calibration particles and Aperture Tubes
- description A4-1
- determination of assayed sizes A4-2
- latex suspensions A4-1
- redispersion of calibration particles A4-1
- table of recommendations A4-1

Appendix 5 Salt Water Contaminants

A5.1 Introduction

The purpose of this Appendix is to provide a rapid procedure for monitoring salt water contamination using the BECKMAN COULTER Z Series instrument. This procedure assumes that the Aperture Tube selected has been calibrated using the procedures in Section 3, Calibration.

The requirement for analyzing salt water contaminants is to count the number of particles larger than either of the following two groups of specific diameters:

(1) 2, 4, 8, and 16µm - choose 50 or 70µm Aperture Tube.
(2) 5, 10, 15, and 20 µm - choose 100 or 140 µm Aperture Tube.

The procedure for (1) and (2) are identical except for the size-level value entered.

This procedure assumes that a Dual Threshold version of the BECKMAN COULTER Z Series is being used. The information to adapt this procedure to a Single Threshold version (taking at least twice as long) is provided at the end of this Appendix.

A5.2 Sample Analysis

Ensure that the BECKMAN COULTER Z Series Hydraulics System is filled and primed with the sea water sample by filling the Diluent Jar with the sample and using the **Fill System** function. Fill the beaker with the sample, place on platform and raise it until the aperture is submerged.

A5.2.1 Dual Threshold Version

Note: In this example we are considering 5, 10, 15, and 20 µm size settings with a 100µm Aperture Tube.

(1) Press **SET-UP** and screen **S1** displays:

```
S1:        SETUP - ENTER SIZE DATA
                 100µm C,  Kd=60
                    Select Units:  < µm >
             Set Upper Size Tu:  10.0 µm
             Set Lower Size Tl:   5.0 µm
                    Count Mode:  < above Tl >
```

(2) Check that **Select Units** is set to µm.

(3) Move to **Set Upper Size Tu** using the cursor keys. Enter 10.0 using the numeric key pad.

(4) Move to **Set Lower Size Tl** using the cursor keys. Enter 5.0 using the numeric key pad.

(5) Move to **Count Mode** using the cursor keys then select **above Tl**.

(6) Press **SET-UP** and screen **S2** displays:

```
S2:     SETUP - ENTER ANALYSIS DATA
            Select Aperture : <100µm C >
                Aperture Kd :  60
           Metered Volume : < 0.5 >
         Measure Aperture : < No >
         Optimize Settings : < No >
```

(7) Check that **Select Aperture** and **Metered Volume** are set to the correct values.

(8) Press **OUTPUT** and screen **A1** displays:

```
A1:       ANALYSIS - OUTPUT FORMAT
              Next test:    33
           Result type:  < Count >
           Dilution factor:    1E + 00
             Switch units:  < µm >
               Resolution:  < 256 >
```

This menu allows review of the output parameters before and after an analysis. Move to **Result type** and select **Count**. Move to **Switch units** and select **µm**. Alter **Resolution** as required.

(9) Press **START** and screen **A2** displays:

(10) At the end of the analysis, screen **A4** displays:

```
A4:              ANALYSIS RESULT
                          Test    :  33

     Count:                          <above Tl>

     1124                              5.0µm
```

Record the **Count: above Tl**. Using the cursor keys, select **above Tu** to read/ record the result. Repeat the analysis five times to give greater statistical accuracy and record each Tl and Tu result. Calculate the average above Tl and Tu counts using these results.

(11) Press **SET-UP** and screen **S1** displays:

```
S1:        SETUP - ENTER SIZE DATA
                100µm  C,  Kd=60
                  Select Units:  < µm >
            Set Upper Size Tu:  20.0 µm
            Set Lower Size Tl:  15.0 µm
                  Count Mode:  < above Tl >
```

(12) Move to **Set Upper Size Tu** using the cursor keys. Enter 20.0 using the numeric key pad.

(13) Move to **Set Lower Size Tl** using the cursor keys. Enter 15.0 using the numeric key pad.

(14) Restart the analysis by pressing **START** and screen **A2** is again displayed followed at the end of the analysis by screen **A4.**

(15) Perform five analyses, recording the above Tl and above Tu counts. Calculate the average above Tl and Tu counts.

The number of particles larger than 5, 10, 15, and 20 µm have now been counted. Analyses can be repeated as long as the type of electrolyte remains unchanged.

A5.2.2 Single Threshold Version

The differences between the Dual and Single Threshold procedures are as follows:

(1) Set the size level to 5µm and initiate a count. Repeat the count five times and record the result.

(2) Repeat step (1) with the size level set to 10µm, then 15µm, then 20µm. Record the results.

A5.1 **Introduction** A5.1

A5.2 **Sample Analysis** A5.1
A5.2.1 **Dual Threshold Version** A5.1
A5.2.2 **Single Threshold Version** A5.3

Aperture Tube
in salt water contamination monitoring A5-1
salt water contamination
and sample analysis A5-1
monitoring A5-1
sample analysis
Analysis - Output Format (A1) A5-2
Analysis Display (A2) A5-2
Analysis Result (A4) A5-3
and salt water A5-1
Setup - Enter Analysis Data (S2) A5-2
Setup - Enter Size Data (S1) A5-1, A5-3

Appendix 6 Parenteral Samples

A6.1 Extracts from British Pharmacopoeia, 1993, Parenteral Preparations

A6.1.1 Particulate Matter (Reference B.P., 1993, page 748)

The following extract is taken from *Parenteral Preparations of the British Pharmacopoeia*.

"Solutions to be injected that are supplied in containers with a nominal content of 100 ml or more comply with the *limit test for particulate matter*, Appendix XIII."

A6.1.2 Limit Test for Particulate Matter (Reference B.P., 1993, Appendix XIII, A163)

The following extract is taken from *Limit Test for Particulate Matter*.

"The test should be carried out using suitable apparatus, that operates on the electrical zone principle.

Determine the numbers of particles per ml of the preparation being examined the effective diameters of which are greater than that of a sphere of diameter 2.0µm and those the effective diameters of which are greater than that of a sphere of diameter 5.0µm.

The average count, as determined by the sampling procedure recommended by the instrument manufacture determined on each container examined, for the undiluted preparation, does not exceed 1,000 per ml greater than 2.0µm and does not exceed 100 per ml greater than 5.0µm."

A6.2 Preparation

A6.2.1 Non-Conducting and Viscous Samples

Some samples encountered, such as purified water for injection are so non-conducting as not to give any response other than electronic noise on the BECKMANCOULTER Z Series. Electrolyte solution must be added to make the water more conductive; for instance a strong saline solution (10 to 20% NaCl) is to be added 5 parts to 95 parts of the sample.

The saline needs to be specially prepared and membrane filtered at 0.22µm to ensure almost perfect cleanliness. It should be stored in siliconized glass or preferably plastic containers. Although sterilized by filtration, it will be necessary to add preservatives to salines below 20% strength. Experience will show whether stable blank counts can be obtained from day-to-day or whether it will be necessary to perform a background count on the diluent each day.

Where 50% or even 25% dextrose or samples of equivalent viscosity are counted, the time taken for 0.5 ml to pass through a 70µm aperture is prohibitive. These samples should be diluted with a physiological saline filtered through a 0.22µm membrane.

It has been reported that changes in molecular structure of dextrose can occur during autoclaving leading ultimately to caramelising of the sugar. Such changes make it difficult to mix efficiently with the physiological saline. Therefore to ensure proper mixing the following procedure should be observed:

(1) Add 50 ml sample and then 50 ml saline diluent to a beaker.

(2) Place the beaker containing the sample on the platform and move the platform up until the Aperture Tube, electrode and stirrer are fully immersed. Close the Screened Door.

(3) Set the Z Series stirrer to on. Stir until all visible 'ropiness' and density fluctuation have disappeared and then continue for a further 30 seconds (total time approximately 2 minutes).

A6.2.2 Precautions During Analysis

(1) The following extract is taken from *Limit Test for Particulate Matter* (Reference B.P., 1993, Appendix XIII, page A163).

"It is important to avoid the introduction of extraneous particulate contamination into preparations being examined during the testing procedure. Contamination can be reduced by carrying out all manipulative procedures in filtered air complying with the standard included in British Standard 5295:1976 (Environmental cleanliness in enclosed spaces) for a Class 1 environment, such as that in a suitable laminar airflow cabinet or hood.

Gently invert the container twice. Care is required to avoid the introduction of air bubbles into the preparation being examined and special attention should be paid to the transfer of portions of the preparation being examined to the vessel in which the determination of the particle count is to be performed."

(2) All glassware must be thoroughly rinsed and maintained in a particulate-free condition. Reserve a set of beakers and volumetric equipment for parenteral sampling only. After final daily use, rinse all glassware with filtered distilled water, and store in a drawer, upside down on glazed paper or under a Bell jar. Leave the aperture on the instrument immersed in clean electrolyte solution.

(3) Because filtered rinses are frequently called for, the user will find it convenient to filter distilled water and physiological saline in reasonable quantity and to maintain the plastic wash bottles with each of these freshly filled each day, or to use commercially available pressure-rinse bottles fitted with terminal filters of 0.45µm porosity.

(4) Diluents such as 20% saline and rinse liquids **must not** be autoclaved.

(5) The Diluent Jar **must be** filled with a sample filtered to 0.45µm. The internal glassware of the Z Series **must be** primed with sample.

A6.3 Calibration

Note: This is a rapid procedure. If a more detailed explanation of calibration procedures is required, refer to Section 3, Calibration.

(1) Check that a 70µm Aperture Tube is fitted.

(2) Rinse and fill a beaker with filtered sample, as the blank electrolyte.

(3) Ensure that the Diluent Jar is filled with filtered sample and the system has been primed with filtered sample.

(4) Prepare the calibration sample (mean size between 2 to 5µm) as recommended in its assay sheet, noting the assayed size value (diameter).

(5) Calibrate the Aperture Tube (see Section 3, Calibration).

A6.4 Background Count

(1) Remove the beaker containing the calibration sample. Thoroughly rinse the Aperture Tube, electrode and stirrer paddle with filtrated diluent.

(2) Place a clean beaker of filtrated sample electrolyte on the beaker platform and repeat counts until the count drops to a constant level.

A6.5 Sample Analysis

This procedure assumes that a Dual Threshold version of the BECKMAN COULTER Z Series is being used. The information to adapt this procedure to a Single Threshold version (taking at least twice as long) is provided in para A6.5.2.

A6.5.1 Dual Threshold Version

(1) Press **SET-UP** and screen **S1** displays:

```
S1:        SETUP -  ENTER SIZE DATA
                 70µm C,  Kd=42.51
                    Select Units:  < µm >
              Set Upper Size Tu:   5.0 µm
              Set Lower Size Tl:   2.0 µm
                    Count Mode:  < above Tl >
```

(2) Check that **Select Units** is set to **µm**.

(3) Move to **Set Upper Size Tu** using the cursor keys. Enter 5.0 using the numeric key pad.

(4) Move to **Set Lower Size Tl** using the cursor keys. Enter 2.0 using the numeric keypad.

(5) Move to **Count Mode** using the cursor keys and select **above Tl**.

(6) Press **SET-UP** and screen **S2** displays:

```
S2:    SETUP -  ENTER ANALYSIS DATA
              Select Aperture : < 70µm C >
                   Aperture Kd :  42.51
             Metered Volume : < 0.5 >
           Measure Aperture : < No >
               Optimize Setup : < No >
```

(7) Check that **Select Aperture** is set to 70µm and **Metered Volume** is set to 0.5.

(8) Press **OUTPUT** and screen **A1** displays:

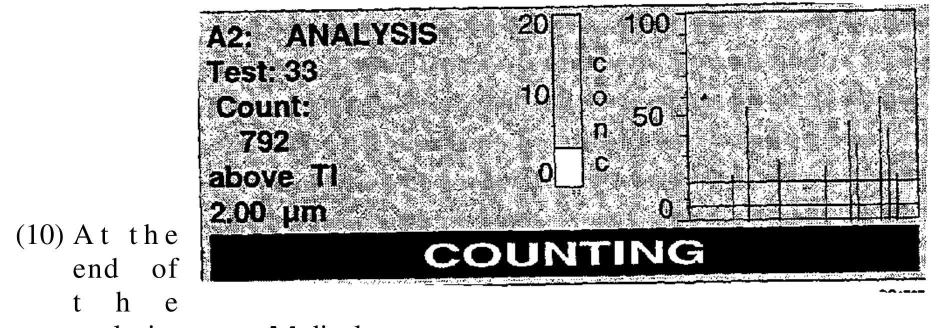
```
A1:       ANALYSIS - OUTPUT FORMAT

                Next test:    33
      Result type:  < Concentration >
        Dilution factor:    5        E + 00
           Switch units:  < µm >
             Resolution:  < 256 >
```

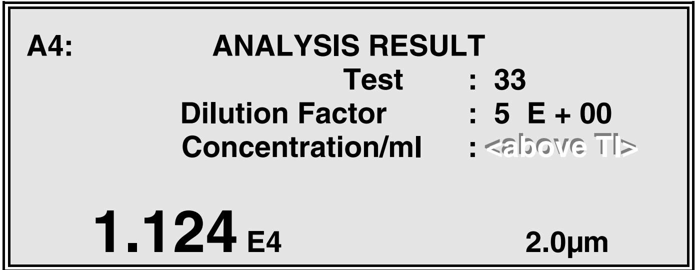
Screen **A1** allows review of the output parameters before/after an analysis. Move to **Result type** and select **Concentration**. Move to **Dilution factor**, enter the Dilution factor of the sample from the original concentration (BECKMAN COULTER Z Series takes into account the selected metered volume). Move to **Switch units** and select **µm**. Alter

Resolution if required.

(9) Press **START** and screen **A2** displays:



(10) At the end of the analysis, screen **A4** displays:



Record the concentration above Tl. Press the cursor keys to read concentration above Tu and record the result. Repeat the analysis five times to give greater statistical accuracy and record each Tl and Tu result. Calculate the average above Tl and Tu concentrations using these results.

A6.5.2 Single Threshold Version

The only difference in the procedure is that separate analyses have to be taken with size settings set to 2.0µm and 5µm.

A6.6 Origin of Contaminants in Parenteral Fluids

The origin of the contaminants in parenteral fluids is discussed fully by Dr. Groves in his book Parenteral Products, Heinemann, London, 1973. There are four basic sources of particle contamination:

(1) Introduction of particles into saline during filtration due to:

(a) Particles passing through the filter.
(b) Particles shed from the filter.
(c) From the surrounding environment.

(2) Introduction of particles from the container and closure surfaces. These may be present as debris before or during filling, or they may be generated physico-chemically by contact with the contents during or after autoclaving.

It is well known that untreated glass sheds particles into salines, citrates and particularly into alkaline solutions. Also the shedding of particles from closures, especially un-lacquered ones, has been well attested to.

(3) Introduction of particles after filling. Particles may enter around the closure during spray cooling, or for the purpose of testing may enter when the closure is penetrated or removed.

(4) Formation of particles (including air bubbles) by agglomeration, polymerization, precipitation or nucleation during storage.

Since there is such a low level of nuclei for air bubbles to form upon, air dissolved during autoclaving may take a long time to come out. Users have reported a high count after one or two weeks from physiological saline that passed specification before, and two days, after autoclaving. The same sample again passed specification after a month's storage.

It has been suggested that this could be an aggregation of sub-micron particles passing the filter that subsequently redisperse. Air bubbles seem a more likely explanation. Whatever the explanation, users should check for themselves to see if there is a 'forbidden' period of storage during which salines should neither be tested or used.

A6.1 **Extracts from British Pharmacopoeia, 1993, Parenteral Preparations** . . . A6-1
A6.1.1 **Particulate Matter (Reference B.P., 1993, page 748)** . . . A6-1
A6.1.2 **Limit Test for Particulate Matter (Reference B.P., 1993, Appendix XIII, A163)** . . A6-1

A6.2 **Preparation** . . . A6-1
A6.2.1 **Non-Conducting and Viscous Samples** . . . A6-1
A6.2.2 **Precautions During Analysis** . . . A6-2

A6.3 **Calibration** . . . A6-3

A6.4 **Background Count** . . . A6-3

A6.5 **Sample Analysis** . . . A6-3
A6.5.1 **Dual Threshold Version** . . . A6-4
A6.5.2 **Single Threshold Version** . . . A6-5

A6.6 **Origin of Contaminants in Parenteral Fluids** . . . A6-6

parenteral sample
background count . . . A6-3
calibration . . . A6-3
Extracts from British Pharmacopoeia, 1993, Parenteral Preparatio . . . A6-1
origin of the contaminants in parenteral fluids . . . A6-6
precautions during analysis . . . A6-2
preparation, non-conducting and viscous samples . . . A6-1
sample analysis . . . A6-3
sample analysis
Analysis - Output Format (A1) . . . A6-4
Analysis Display (A2) . . . A6-5
Analysis Result (A4) . . . A6-5
Setup - Enter Analysis Data (S2) . . . A6-4
Setup - Enter Size Data (S1) . . . A6-4

Appendix 7 Disinfecting the BECKMAN COULTER Z Series

A7.1 Introduction

Staff can be exposed to the risk of contracting diseases such as viral hepatitis and AIDS (HIV) when handling specimens of blood, or equipment contaminated with blood, from patients with these diseases or from patients who are carriers of them.

The most reliable means of disinfection is by autoclaving, but in cases where this is not possible, the World Health Organization[1] specifies the use of hypochlorite bleach against viral hepatitis, as does the procedure laid down by the DHSS[2]. For such cleaning and disinfecting of the BECKMAN COULTER Z Series, a solution of laboratory grade bleach may be used (not a commercial grade such as Domestos, or other domestic bleaches - they contain lytic agents difficult to remove).

The manufactures of the proprietary viricide 'Gigasept' claim that it is active at 5% strength against hepatitis B virus.

The Centers for Disease Control in Atlanta, Ga., U.S.A., has approved the use of hypochlorite bleach against AIDS[3], as has the College of American Pathologists[4]. The D.H.S.S. and the Health and Safety Executive[5] recommend the use of hypochlorite bleach of up to 10,000 ppm (1.0%) available chlorine or 2% glutaraldehyde for use in blood spills and gross contamination where AIDS is suspected. The Beckman Coulter procedures detailed in this Appendix exceed these recommended concentrations.

A7.2 General

This procedure requires at least 200ml of working disinfectant solution. Larger amounts can be made up by diluting raw stock pro rata.

Water for dilution should be of distilled or de-ionized quality, referred to here as DQ water.

Always use freshly made working disinfectant solution. Used (contaminated) solutions should be discarded immediately.

Warning: **ISOTON II diluent and sodium hypochlorite must not be mixed together as they react, releasing gas and inactivating the bleach, possibly making it ineffective in disinfecting the instrument. Gas trapped in the hydraulic system of the Z Series may result in sufficient internal pressure rise to cause leaks at tubing connections.**

If bleach disinfecting an instrument using ISOTON II diluent, the VENT function of the Z Series must be invoked before powering the instrument Off (See Section 2, para. 2.4, Controls and Connectors).

A7.3 Method

A7.3.1 Hypochlorite Bleach

A fresh solution of hypochlorite is required containing at least 2% available (or active) chlorine.

Caution: Hypochlorite solutions, particularly those > 5% total available chlorine, lose available chlorine upon storage at ambient temperature; therefore unless a guaranteed product is used, source material should be assayed for total available chlorine content to ensure a correct working concentration[8].

(1) add one volume of 8% laboratory grade sodium hypochlorite solution (Fisons[6]) to three volumes of DQ water, e.g., make up 50ml hypochlorite solution to 200ml total.

or,

(2) add one volume of 14% sodium hypochlorite (BDH[7]) to six volumes of DQ water, e.g. make up 34ml solution to 200ml total.

A7.3.2 Alternative Disinfectants

A7.3.2.1 Glutaraldehyde

Prepare 200ml of a 2% working solution by activating Cidex® [9] according to the manufacturer's instructions. The solution remains active for 14 days. An equivalent solution prepared from Cidex® Long Life remains active for up to 28 days.

A7.3.2.2 Gigasept

Prepare a working disinfectant solution according to the manufacturer's instructions (typically by adding one volume of Gigasept[10] to nine volumes of DQ water, e.g., make up 20ml Gigasept to 200ml total). The working solution remains active for up to 28 days.

A7.3.2.3 Important Note

With the current emphasis on disinfection, several manufacturers offer ‘Disinfection Tablets,’ usually based on sodium dichloroisocyanurate. While these may be reasonable products for general use, they **cannot** be recommended for use with the BECKMAN COULTER Z Series. They will be found to be both inconvenient and expensive and may attack some of the instrument constructive materials.

Preparation of a suitable solution with 2% available chlorine may require as many as 72 tablets per litre of water. A precipitate is often formed with blood products which then has to be removed.

Beckman Coulter Particle Characterization, however, continues to recommend the use of hypochlorite bleach.

A7.3.2.4 Used (Contaminated) Solution

Used (contaminated) solutions should be discarded daily.

A7.4 Disinfecting

Disinfection should be carried out following the analysis of any high risk samples, (e.g., at the end of the day shutdown, or before work is to be carried out by service personnel). It may also be used for low risk samples where there is a possibility of microbial or fungal growth within the system.

A7.4.1 Draining the System

(1) Press FUNCTIONS and screen **F1** displays.

(2) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Drain System**.

F1: INSTRUMENT FUNCTIONS

Prime/Fill/Drain/Flush/Vent
Select Function, press START
< Drain System >
Key beep < On >
Printer: < LaserJet >

(3) Press START and screen **F5** displays:

> **F5: DRAIN SYSTEM**
>
> **Disconnect tube from diluent vessel and insert in suitable waste container. Place empty vial at sampling station.**
>
> **PRESS ' START ' TO CONTINUE**

Follow the instructions on the screen, ensuring that the platform is fully raised and the door is closed. A spare connection is provided on the Waste Jar cap to accept the disconnected tube. Press START and; the **PRESS ' START ' TO CONTINUE** status message is replaced by the sequence **DRAINING APERTURE** and then **DRAINING SYSTEM**. The number of cycles to completion is shown in the status message. The total procedure takes approximately 10 minutes. When the final cycle is completed, screen **F5** is replaced by screen **F1** with the message **SYSTEM DRAINED/APERTURE VENTED** in the Message area.

Note: The procedure can be aborted by pressing STOP. This causes the Drain System procedure to stop when the current cycle has completed. Screen **F1** displays with the status message **STOP KEY PRESSED**. If procedure is then resumed (by pressing START again), the Drain System procedure starts from the beginning.

(4) If the instrument is not to be filled immediately, set the Power Switch to **O** (off).

(5) Remove the Diluent tube from the connection on the Waste Jar and place it in a vessel containing working disinfectant solution. Empty the sample vial and Waste Jar and dispose using correct laboratory standards. Rinse Waste Jar and sample vial with working disinfecting solution.

Note: It is inadvisable to put disinfectant solution into the Diluent Jar. Considerable rinsing may be needed to remove all traces before the Diluent Jar can be refilled with diluent.

(6) Rinse the outside surfaces of the electrode and Aperture Tube thoroughly into beaker using a wash beaker containing a working disinfectant solution. A 250ml standard laboratory beaker ('squat' form) makes an ideal rinse receiver.

(7) Remove the stirrer (if fitted), rinse it thoroughly with the rinse bottle and store.

A7.4.2 Filling the System with Disinfectant Solution

(1) Set the Power Switch to **I** (on). The Data Terminal's LCD Didplay illuminates and screen **S1**: **SETUP - ENTER SIZE DATA** displays.

(2) Fill a sample vial with sufficient working disinfectant solution, place it on the platform and move the platform up until Aperture Tube and electrode are fully immersed. Close the Screened Door.

(3) Press **FUNCTIONS** and screen **F1** displays.

(4) At the line under **Prime/Fill/Drain/Flush/Vent**, use the cursor keys to select **Fill System**.

F1: INSTRUMENT FUNCTIONS
Prime/Fill/Drain/Flush/Vent
< Fill System >
Key beep < On >
Printer: < LaserJet >

(5) Press **START** and screen **F3** displays:

F3: FILL SYSTEM

Ensure fill tube is connected to diluent vessel.
Place clean diluent at sampling station.
PRESS ' START ' TO CONTINUE

Ignore the on-screen instructions and press **START** again, the **PRESS 'START' TO CONTINUE** status messages will be replaced by **FILLING SYSTEM**, and the system starts to fill up with disinfectant solution. The number of cycles to completion is shown in the status message. On completion, the screen **F1** status message is replaced with **SYSTEM FILLED**.

(6) If the system is to be vented (ISOTON II was the diluent in use), select **Vent Aperture** by using the cursor keys and press **START**. At completion, the screen **F1** status message is replaced with **APERTURE VENTED**.

(7) Leave to stand for a minimum of one hour. While waiting, empty the Waste Jar and wash its interior surfaces thoroughly with working disinfectant solution. Leave the solution in place.

Thoroughly wipe the exterior surfaces of the instrument and the sample compartment with tissues or swabs moistened with the working disinfectant solution, paying

particular attention to the platform and its release mechanism. Wipe the Data Terminal's key pad and the Z Series control knobs. After a few minutes repeat the disinfectant wipe with a distilled water wipe (to remove disinfectant).

A7.4.3 Removing Disinfectant Solution

(1) After the working disinfectant solution has been in situ for at least an hour, remove the sample vial containing the disinfectant solution and wash the Aperture Tube and electrode with DQ water.

Caution: If the diluent is ISOTON II, the system **must be** first drained of disinfectant and then filled with DQ water to remove all traces of bleach, before filling with ISOTON III diluent.

(2) Repeat the Drain procedure in this appendix. After draining the system, clean the outside of the fill tube with DQ water and reconnect to the Diluent Jar.

(3) Empty the Waste Jar and thoroughly rinse.

(4) To refill the Z Series repeat the Fill procedure in this appendix using diluent (or DQ water) instead of disinfectant.

A7.4.4 Checking for Residual Bleach

Once samples have passed through the aperture, substances affecting size and number have no effect on Z Series results. It is possible however, to leave traces of bleach or other disinfectants on the Aperture Tube and/or electrode and stirrer. In the same way, liquid inside the Aperture Tube cannot, in theory, pass back through the aperture to affect fresh samples. In practice however, unblocking or diffusion can allow traces to pass to the outside and cause difficulties with fragile cells.

It is strongly recommended that, where fragile cells are being analyzed, tests are carried out (e.g., with trial samples) following disinfection, to demonstrate that no residual bleach remains.

A7.5 References

(1) World Health Organization Technical Report Series, No. 512, Geneva, 1973, p. 47 et seq.

(2) Safety in Pathology Laboratories, Department of Health and Social Security, Alexander Fleming House, Elephant and Castle, London, SE1 6TE; suppliers of relevant documentation.

(3) Centers for Disease Control 1983. Private communication, to Coulter Electronics Inc..

(4) J. Histotechnology, 6, No. 4, p. 197, December 1983.

(5) Acquired Immune Deficiency Syndrome (AIDS) - Interim Guidelines, Advisory Committee on Dangerous Pathogens, HSE/DHSS, December 1984,
and,
LAV/HTLV III - the causative agent of AIDS and related conditions - revised guidelines. Advisory Committee on Dangerous Pathogens, HSE/DHSS, June 1986, p. 13.

(6) Fisons Scientific Apparatus Ltd., Bishop Meadow Road, Loughborough, Leics; suppliers of sodium hypochlorite solution, technical, cat. S/5040, (minimum 8% available chlorine when fresh).

(7) British Drug Houses Ltd., Poole, Dorset; suppliers of sodium hypochlorite solution, cat. 30169 6S, (10 to 14% available chlorine when fresh).

(8) The instability of sodium hypochlorite solutions, Bradshaw A. and Lines R.W., Laboratory Practice, 36, No. 4, p. 94, April 1987.

(9) Arbrook Products, Kirkton Campus, Livingstone, West Lothian EH54 7AT, suppliers of Cidex® for glutaraldehyde. (Telephone 01506-413441; contact for local distributor).

(10) Sterling-Winthrop Group Ltd., Onslow Street, Guildford Surrey GU1 4YS; suppliers of 'Gigasept' viricide (available in 2 litre or 500ml bottles). (Telephone 01483-505515).

A7.1 **Introduction** A7-1

A7.2 **General** A7-1

A7.3 **Method** A7-2
A7.3.1 **Hypochlorite Bleach** A7-2
A7.3.2 **Alternative Disinfectants** A7-2
A7.3.2.1 **Glutaraldehyde** A7-2
A7.3.2.2 **Gigasept** A7-2
A7.3.2.3 **Important Note** A7-3
A7.3.2.4 **Used (Contaminated) Solution** A7-3

A7.4 **Disinfecting** A7-3
A7.4.1 **Draining the System** A7-3
A7.4.2 **Filling the System with Disinfectant Solution** A7-5
A7.4.3 **Removing Disinfectant Solution** A7-6
A7.4.4 **Checking for Residual Bleach** A7-6

A7.5 **References** A7-6

checking for residual bleach
recommendation A7-6
disinfecting the instrument
"Disinfection Tablets" A7-3
checking for residual bleach A7-6
description A7-1
draining the system A7-3
filling the system with disinfectant A7-5
Gigasept method A7-2
glutaraldehyde method A7-2
hypochlorite bleach method A7-2
references A7-6
removing disinfectant solution A7-6
used (‘contaminated') solutions A7-3
when to A7-3
Drain System
Drain System (F5) A7-4
Instrument Functions (F1) A7-3
procedures A7-3
Fill System
Fill System (F3) A7-5
Instrument Functions (F1) A7-5
procedures A7-5
removing disinfectant solution
procedures A7-6

Appendix 8 Blood Cell Analyses

A8.1 Installation

Refer to Section 2, paras, 2-1, 2.2, 2.3, and 2.5, of this manual for instructions regarding unpacking, location, voltage/power configuration, and initial start-up procedures.

A8.2 Principles of Operation

Refer to Section 6 of this manual for the basic principles of operation and the Z Series functional descriptions.

A8.3 Performance Characteristics and Specifications

A8.3.1 Specifications (Blood Cell Counts)

The following performance specifications were derived at 20-25°C, 60-70% relative humidity and about 1000 mbar atmospheric pressure, for human blood.

Accuracy

Using the built-in metering system, a correctly set up instrument will provide RBC, WBC and PLT results to within ±5% of reference methodology, when using the recommended reagents and macrodilutions. See Table A8-1.

A higher degree of accuracy may be obtained by calibrating the RBC, WBC and PLT results to results obtained from reference methodology, or to a reference blood cell preparation, e.g.BECKMAN COULTER S-CAL® Calibrator.

Within Run Precision

Within run precision is specified as a Coefficient of Variation (CV) based on a minimum of 31 determinations of the same stirred pre-diluted sample, within an acid-washed glass container.

Table A8-1 Within Run Precision in the Normal Blood Range (n = 31)

Parameter	CV	Maximum 2 SD Range - 95%
RBC at 5.0 x 10^{12} Cells/L	≤2%	4.8 - 5.2
WBC at 10.0 x 10^{9} Cells/L	≤3%	9.4 - 10.6
PLT at 250 x 10^{9} Cells/L	≤5%	225 - 275

Linearity

When tested using dilutions made from a stable sample having no interfering substances, the instrument value will be equal to the expected value within the limits given in Table A8-2. To obtain the same results, background counts must be subtracted from the instrument values. In addition, multiple readings must be taken at each point in order to reduce any statistical imprecision.

Table A8-2 Linearity Limits

Parameter	Range	Linearity (Whichever is Greater)
RBC x 10^{12} Cells/L	0 to 8	±0.10 or 5%
WBC x 10^{9} Cells/L	0 to 45	±0.5 or 3%
PLT x 10^{9} Cells/L	0 to 900	±15 or 3%

Carryover

The effect of sample (A) on the values obtained for the sample (B) shall be less than 3.0% when calculated as follows:

$$\% \text{ carryover} = (\Delta B/A - B) * 100$$

Where ΔB = error in B due to carryover.

A8.3.2 Informative Data

Dilutions Required

RBC 1:50,000 (whole blood plus diluent)
WBC 1:500 (whole blood plus diluent plus lysing agent)
PLT 1:5000 (whole blood plus diluent)

Sample Volume Recommended

Up to 1 mL of whole blood (for RBC, WBC and PLT)

Typical Aperture Size

100-µm diameter (RBC and WBC); 50-µm or 70-µm diameter (PLT)

Measurement Conditions

See Operator Instructions (para. 8.5)

Typical Counting Time

13 seconds for RBC and WBC
10 seconds for PLT

Controls	Cell Control (for RBC, WBC) Platelet-rich Plasma Cell Control
Recommended Reagents:	
Diluent	ISOTON II diluent
Lysing Agent	ZAP-OGLOBIN reagent
Cleaning Agent	COULTER CLENZ cleaning agent

A8.4 Operational Technique for Human Red Cell Counts (RBC)

a) INSTRUMENT SETUP

Note: For a single threshold Z Series model ignore the **Set Upper Size Tu** and **Count Mode** instructions.

1. Calibrate a suitable aperture tube (generally a 100-µm aperture is used) for its cell size response, employing the automatic calibration procedure with fL entered in the **Select Units** field. Use a suitable dilution of either COULTER S-CAL Calibrator or a whole blood sample with a known Mean Cell Volume (MCV). A 1:100,000 dilution is preferred.

2. Press SET-UP until screen **S1** displays:

```
S1:        SETUP - ENTER SIZE DATA
                100µm C,  Kd=60
                   Select Units:  < µm >
           Set Upper Size Tu:   25 fL
           Set Lower Size Tl:   25 fL
                   Count Mode:  < above Tl >
```

3. Set the **Set Upper Size Tu** and **Set Lower Size Ti** fields to 25 fL and **Count Mode** to **above Tl**.

4. Press SET-UP and screen **S2** displays:

```
S2:    SETUP - ENTER ANALYSIS DATA
              Select Aperture : <100µm  C >
                  Aperture Kd :  60
             Metered Volume : < 0.5 >
           Measure Aperture : < No >
            Optimize Settings : < Yes >
```

5. Set **Metered Volume** to 0.5 and **Optimize Settings** to **Yes**.

6. Press [START]. If the resulting main gain is 512, then change this value to 256 and raise the aperture current to compensate for the reduction in gain.

b) OBTAINING THE RBC RESULT

1. Prepare a 1:50,000 dilution of the blood specimen. 200μL of a previously made WBC dilution(prior to adding ZAP-OGLOBIN II lytic reagent) into 19.8 mls of Isoton II diluent. Carefully mix well.

2. Obtain the RBC count with the BECKMAN COULTER Z Series set as above. (Typically, it may be in the region of 38,000-65,000 for a 'Normal' sample using the above settings).

3. Divide the count by 10,000 to obtain the RBC result (expressed as 10^6 cells/μL).

   For example, a particle count of 45,512 gives a RBC of 4.55 x $10^6/\mu$L.

   Alternatively, a result expressed as **count/ml** may be obtained by setting the **Result type** to **Concentration** and the **Dilution factor** to 5 E+04, which will generate a result already corrected for dilution and metered volume.

c) OBTAINING THE MCV RESULT(Z2 Instrument Only)

1. When the count is displayed, press OUTPUT key to obtain the Size Distribution.

2. Position the lower cursor to a position at 25fL. Position the upper cursor to the position nearest to 200fL. Select Mean from the list of statistics. MCV is displayed. Press PRINT to obtain a report.

A8.4.1 Operational Technique for Human White Cell Counts (WBC)

a) INSTRUMENT SETUP

Note: For a single threshold Z Series model ignore the **Set Upper Size Tu** and **Count Mode** instructions.

1. Calibrate a suitable aperture tube (generally a 100µm aperture is used) for its cell size response. Use the automatic calibration procedure and enter **fL** in the **Select Units** field. Use a suitable dilution of either BECKMAN COULTER S-CAL Calibrator or a whole blood sample with a known Mean Cell Volume (MCV). A 1:100,000 dilution is preferred.

2. Press [SET-UP] until screen **S1** displays:

S1: SETUP - ENTER SIZE DATA
100µm C, Kd=60
Select Units: < µm >
Set Upper Size Tu: 35 fL
Set Lower Size Tl: 35 fL
Count Mode: < above Tl >

3. Set the **Set Upper Size Tu** and **Set Lower Size Ti** fields to 35 fL and **Count Mode** to **above Tl**.

4. Press **SET-UP** and screen **S2** displays:

S2: SETUP - ENTER ANALYSIS DATA
Select Aperture : <100µm C >
Aperture Kd : 60
Metered Volume : < 0.5 >
Measure Aperture : < No >
Optimize Settings : < Yes >

5. Set **Metered Volume** to 0.5 and **Optimize Settings** to **Yes**.

6. Press **START**. If the resulting main gain is 512, then change this value to 256 and raise the aperture current to compensate for the reduction in gain.

b) OBTAINING THE WBC RESULT

1. Prepare a 1:500 dilution by mixing 40µL of blood into 20mls of Isoton II diluent. Carefully mix well.(If a RBC count is to be obtained, remove 200µL required for the RBC dilution now).

2. Add 6 drops of lysing agent(approx. 200µL). Obtain the WBC count with the BECKMAN COULTER Z Series set up as above. [Re-mix the suspension if analysis is delayed by more than a few minutes]. (Typically, it may be in the region of 4,000 to 11,000 for a 'Normal' sample, using the above settings).

3. Divide the count by 1,000 to obtain the WBC result (expressed as 10^3 cells/µL).

   For example, a particle count of 4,709 gives a WBC of 4.7 x 10^3/µL.

   Alternatively, a result expressed as **count/ml** may be obtained by setting the **Result type** to **Concentration** and the **Dilution factor** to 5 E+02. This generates a result corrected for dilution and metered volume.

Where other manual dilutions have been made, adjust the **Dilution factor** accordingly.

A8.4.2 Operational Technique for Human Platelet Counts (PLT)

Note: A Dual Threshold Z Series is recommended for this analysis.

a) INSTRUMENT SETUP

1. Calibrate a 50µm aperture tube (preferred) for its cell size response. Use the automatic calibration procedure and enter **µm** in the **Select Units** field. Use a suspension of latex particles with a known mean diameter in the region of 5µm.

2. If using BECKMAN COULTER Calibration Standard, (P/N 6602794), prepare a suitable dilution following the procedure described on the assay sheet enclosed with the Standard.

3. Press **SET-UP** until screen **S1** displays:

```
S1:        SETUP - ENTER SIZE DATA
                100µm C,  Kd=60
              Select Units:  < µm >
          Set Upper Size Tu:   30 fL
          Set Lower Size Tl:    3 fL
              Count Mode:  < between >
```

4. Set the **Set Upper Size Tu** field to 30 fL, the **Set Lower Size Ti** field to 3 fL, and **Count Mode** to **between**.

5. Press **SET-UP** and screen **S2** displays:

```
S2:    SETUP - ENTER ANALYSIS DATA
            Select Aperture : <100µm  C >
               Aperture Kd :  60
          Metered Volume : < 0.1 >
        Measure Aperture : < No >
         Optimize Settings : < Yes >
```

6. Set **Metered Volume** to 0.1 and **Optimize Settings** to **Yes**.

b) OBTAINING THE PLT RESULT

1. Prepare a platelet-rich-plasma (PRP) specimen from each whole blood sample. The method of Eastham (J. Clin. Path. (1963), 16, 168) is recommended, as follows.

2. Prepare a 1:5,000 dilution of PRP into ISOTON II diluent by diluting 200µL of whole blood into 4.8mL of ISOTON II diluent and centrifuge for 90 seconds at 600g. (Exact time and force may need to be established for optimum separation).
3. Remove 500µL of the platelet-rich supernatant and dilute into 100mL of ISOTON II diluent.

4. Obtain the PLT count with the BECKMAN COULTER Z Series set as above. Typically, it may be in the region of 3,000 to 9,000 for a 'Normal' sample.

5. Multiply the PLT result by 5 then divide by 100; expressed as (x 10^9/L).

   For example, a particle count of 5,612 gives a PLT of 281 x 10^9/L.

   Alternatively, a result expressed as **count/ml** may be obtained by setting the **Result type** to **Concentration** and the **Dilution factor** to 5 E+03. This generates a result corrected for dilution and metered volume.

Note: Single threshold instruments may be used to obtain a PLT result. Record the count result at a size setting of 3fL, repeat the analysis at 30fL and subtract this from the count at 3fL to give the PLT result. This value can be used in step **5** above to calculate the platelet concentration.

c) OBTAINING THE MPV RESULT(Z2 Instruments Only)

1. When the count is complete, press OUTPUT key to display the size Distribution.

2. Position the lower cursor to a position below 3fL. Position the upper Cursor to the position nearest to 30fL. Select Mean from the list of Statistics. MPV is Displayed. Press PRINT key to obtain report.

A8.4.3 Quality Control

Beckman Coulter recommends performing quality control checks using in-house or commercially available controls (for RBC and WBC) and a Platelet-rich Plasma Cell Control (for PLT). Investigate any failure to recover control values within your laboratory's expected limits, or the presence of unexplained shifts or trends. Part of any quality control programm should start with verifying instrument functions on a periodic basis. See para 7.2, User Testing, for instrument functional testing.

Acceptable functional performance should be demonstrated with quality control materials before testing is performed.

Procedural steps to be followed for testing quality control material must be the same as described in para. A8.5.1. a) and b) for RBC counts; para. A8.5.2. a) and b) for WBC counts; and para. A8.5.3. a) and b) for PLT counts.

A8.5 Calibration Procedures

Refer to:
- Section 3, Calibration, for information concerning sample preparation, instrument preparation, calibration procedure and calibration factor.
- Appendix 4, Calibration Particles and Aperture Tubes, for information concerning calibration particles and aperture tubes.
- Para. A8.5, Operating Instructions for In Vitro Diagnostic Use, for specific calibration techniques for red cell counts, white cell counts, and platelet counts.

A8.6 Operational Precautions and Limitations

Refer to para. 1.3 for information on operational Warnings and Cautions.

A8.7 Hazards

Beckman Coulter urges its customers to comply with all national health and safety standards, such as the use of barrier protection. This may include, but is not limited to, protective eye wear, gloves, and suitable laboratory attire when operating or maintaining this or any other laboratory analyser. Hazards associated with the use of the BECKMAN COULTER Z Series, as well as warnings and precautions, are provided in para. 1.3 of this manual. Wherever a particular warning or hazard may be encountered, appropriate instructions are included throughout the manual.

A8.8 Service and Maintenance

Refer to Section 7 for information on preventive maintenance and servicing.

Appendix 9 Operational Technique for Non-Human Cell Counts

A9.1 The single and dual threshold models of the BECKMAN COULTER Z Series may be used to determine the non-human erythrocyte concentration (or red cell count) RBC, and the leukocyte concentration (or white cell count) WBC. For determining thrombocyte concentration (or platelet count) PLT of animal species with a discrete RBC and PLT population, a dual threshold BECKMAN COULTER Z Series is recommended for simplicity of operation.

The general procedures for non-human RBC, WBC and PLT are somewhat similar to those for human blood. However, as the red cells, white cells and platelets are often of different sizes to those in human bloods, and the cell concentrations are often different, small variations in the method will be needed.

Table A9.3 gives typical blood parameters for a range of animals. [Adapted from Veterinary Haematology, by Oscar W. Schalm, publ. Balliere, Tindall and Cassell Ltd., London 1965].

For species with smaller (red cell) mean cell volumes (MCVs) than man, the RBC is usually higher and the RBC size setting of course will accordingly be different. To count at such higher RBCs, a greater dilution will be required, for instance 1:100,000.

RBC size settings may be set by either referring to Table A9-3 for the mean MCV of the relevant species and entering a third of this value into the **Set Lower Size Tl** and **Set Upper Size Tu** fields or, for a more accurate determination of the optimum size setting, perform a cell size distribution analysis ('Plateau' technique) for RBC or WBC counting, or a frequency size distribution analysis for PLT counting, as described below. Further information on the 'Plateau' technique, if needed, is given in Appendix 10.

Aperture tube selection will depend on the species being analysed. Animals with an MCV above 50fL may be counted for RBC and WBC using a 100µm aperture tube. Below this volume, a smaller aperture may be required. A 70µm or 50µm aperture tube will be required for platelet counting.

Table A9-3 Approximate Normal Range and Mean Values for Various Animal Bloods

SPECIES	RANGE/ MEAN	WBC 10^9/L	RBC x 10^{12}/L	MCV fL	Hct L/L	PLATELETS x 10^9/L
MAN	Range Mean	5-10 7.4	4.1-6.2 5.1	80-97 88	0.37-0.54 0.445	125-690 409

Table A9-3 Approximate Normal Range and Mean Values for Various Animal Bloods

SPECIES	RANGE/ MEAN	WBC 10^9/L	RBC x 10^{12}/L	MCV fL	Hct L/L	PLATELETS x 10^9/L
CAT	Range Mean	5-19 12.5	5-10 7.5	39-55 45	0.24-0.45 0.37	300-700 450
CATTLE	Range Mean	4-12 8	5-10 7.5	40-60 52	0.24-0.46 0.35	100-800 500
CHICK	Range Mean	4-45 20	1.6-4.5 3	120-135 127	0.28-0.36 0.32	25-35 27
DOG	Range Mean	6-17 11.5	5.5-8.5 6.8	60-77 70	0.37-0.55 0.45	200-900 550
GOAT	Range Mean	4-13 9	8-18 13	15-30 22.8	0.195- 0.385 0.285	25-150 50
GUINEA PIG	Range Mean	6-16 10.8	4.0-7.0 5.7	71-91 83	0.35-0.48 0.43	250-500 350
HORSE	Range Mean	5.5-12.5 9	6.5-12.5 9.5	34-58 46	0.32-0.52 0.42	100-600 330
MONKEY (Macaca)	Range Mean	9-21 15	4.8-6.3 5.6	50-90 66	0.30-0.44 0.37	200-900 375
PIG	Range Mean	11-22 16	5-8 6.5	50-69 63	0.32-0.50 0.42	320-720 520
RABBIT	Range Mean	4-20 9	4-8 6	60-69 65	0.36-0.50 0.42	-- 500
RAT	Range Mean	6.4-26.2 15	7-10 8.6	57-65 61	0.35-0.51 0.45	190-1000 800
SHEEP	Range Mean	4-12 8	8-16 12	23-48 33	0.25-0.50 0.38	250-750 400

A9.2

Operational Technique for Non-Human Red Cell Counts (RBC)

a) INSTRUMENT SET-UP

Non-Plateau technique: Refer to para. A8.5.1 a), modifying the size setting entered into Tl and Tu.

Plateau technique:

1. With a 100µm Aperture tube fitted to the Z Series, prepare about 50mL of an approximate 1:100,000 dilution of blood in ISOTON II diluent, and place it in the Z Series, stirring continuously.

2. Press **SET-UP** until screen **S1** displays:

```
S1:        SETUP - ENTER SIZE DATA
                100µm C,  Kd=60
                  Select Units:  < µm >
            Set Upper Size Tu:   35 fL
            Set Lower Size Tl:   35 fL
                  Count Mode:  < above Tl >
```

3. Set the **Set Upper Size Tu** and the **Set Lower Size Tl** fields to 35 fL and **Count Mode** to **above Tl**.

4. Press **SET-UP** and screen **S2** displays:

```
S2:    SETUP - ENTER ANALYSIS DATA
                Select Aperture : <100µm  C >
                   Aperture Kd :  60
                Metered Volume : < 0.1 >
              Measure Aperture : < Yes >
              Optimize Settings : < Yes >
```

5. Set **Select Aperture** to the appropriate value (eg.100µm) and **Measure Aperture** to **Yes**.

6. Press **START** to begin an analysis. Record the instrument settings the Z Series computes.

7. Press **START** again and observe the red cell pulses. They should lie clearly on the display screen with most pulse tips reaching to about 30-50% of the pulse display.

   a. ***If most pulse tips lie below about 30%***:

   1) Press **STOP**.

   2) Press **SET-UP** until screen **S3** displays.

```
S3:      SETUP - INSTRUMENT SETTINGS
           Avail. Range 2.569 - 9.7985 µm
                       Gain :        128       <128>
                    Current :      1.00      <1.000>
             Preamp gain:        179.20
             Tl = 13.3%,       Tu = 22.9%
```

   3) Increase the value of the **Gain** or **Current** setting.

b. ***If most pulse tips lie above 50%***:

1) Press [STOP].

2) Press [SET-UP] until screen **S3** displays.

3) Decrease the value of the **Gain** or **Current** setting. Repeat this step until pulse tips are between 30-50% of the pulse display.

8. Press [START] and screen **A2** displays. When a status message appears, press [∧] and screen **A6** displays.

9. Set **Tu** to 99.9% and **Tl** to 5.0%, using [∨] and [∧].

10. Press [START] and record the resulting particle count obtained above that lower size setting of 5.0%.

11. Press [START]. Return to screen **A6** by pressing [∧].

12. Set **Tl** to 10.0% using [∨] and [∧].

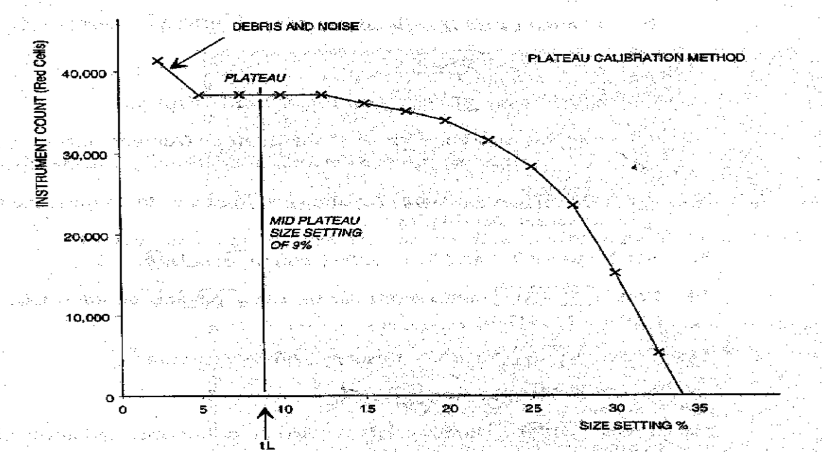
13. Press [START] and record the particle count obtained above that size setting.

14. Repeat at different size settings in order to accumulate data for a cumulative size distribution plot (e.g. as in Figure A8-1).

15. To obtain a suitable size setting for subsequent RBCs from that species, select the mid-point (or slightly to the left of the mid-point) of the ‘plateau’ (horizontal) region; in the case of this example (Figure A8-1), a **Tl** of 9%.

Note: Instrument settings will be lost when the instrument is turned off. Repeat the procedure for future analyses.

Figure A9-1 Example of Setting RBC Count Lower Size Setting



b) OBTAINING THE RBC RESULT

1. Prepare a suitable RBC dilution and refer to para. A8.5.1 b), entering the appropriate size level as determined from the above RBC plateau procedure in step **15** above.

A9.3 Operational Technique for Non-Human White Cell Counts (WBC)

Note: Some species (birds) have nucleated red cells and a WBC count is not possible using this instrument. In addition, RBCs from some animals are resistant to lysis and may require either a longer lysis time or an increased volume of ZAP-OGLOBIN reagent. In severe cases, (e.g. bovine and feline species) add ZAP-OGLOBIN reagent directly to the RBCs before the addition of ISOTON II diluent.

a) INSTRUMENT SETUP

Plateau technique:

1. Prepare about 50mL of an approximate 1:495 dilution of blood into ISOTON II diluent. Add about 0.5mL (15 drops) of ZAP-OGLOBIN reagent. Mix and leave for two minutes in order to clear fully. Place it in the Z Series, and stir continuously.

2. In the way described for RBC [para. A8.10.1 a) Plateau technique] obtain data for a cumulative size distribution plot of the white cells, and select the appropriate size level for subsequent WBC counts from that species.

b) OBTAINING THE WBC RESULT

1. Prepare a suitable WBC dilution and refer to para. A8.5.2 b), entering the

appropriate size level as determined from the above WBC plateau procedure.

A9.4 Operational Technique for Non-Human Platelet Cell Counts (PLT)

Note: Some species' platelets cannot be separated well enough from whole blood to allow their enumeration by this instrument.

a) INSTRUMENT SETUP

Dual threshold size distribution technique:

1. With a 70µm aperture tube fitted to the Z Series, prepare a suitable PLT dilution, as in para. A8.5.3 b), but note that the speed and duration of centrifugation will require modification depending on the species of animal. Place it in the Z Series, and stir continuously.

2. Press SET-UP until screen **S1** displays:

```
S1:        SETUP - ENTER SIZE DATA
                100µm C,  Kd=60
                    Select Units:  < µm >
             Set Upper Size Tu:   10 fL
              Set Lower Size Tl:   10 fL
                    Count Mode:  < between >
```

3. Set the **Set Upper Size Tu** and **Set Lower Size Ti** fields to 10 fL and **Count Mode** to **between**.

4. Press SET-UP and screen **S2** displays:

```
S2:     SETUP - ENTER ANALYSIS DATA
                Select Aperture : <70µm  C >
                    Aperture Kd :  60
              Metered Volume : < 0.5 >
            Measure Aperture : <Yes >
             Optimize Settings : < Yes >
```

5. Set **Select Aperture** to **70µm** and **Measure Aperture** to **Yes**.

6. Press START to take a count. Record the instrument settings the Z Series computes.

7. Press START again and observe the platelet pulses, they should lie clearly on the display screen with most pulse tips reaching to about 20-70% of the pulse display.

a. ***If most pulse tips lie below about 20%***:

1) Press [STOP].

2) Press [SET-UP] three times to get to screen **S3.**

```
S3:     SETUP - INSTRUMENT SETTINGS
          Avail. Range 2.569 - 9.7985 µm
                  Gain :       128      <128>
               Current :      1.00     <1.000>
            Preamp gain:        179.20
            Tl = 13.3%,      Tu = 22.9%
```

3) Increase the value of the **Gain** or **Current** setting or use a 50µm aperture instead.

b. ***If most pulse tips lie above 70%***:

1) Press [STOP].

2) Press [SET-UP] three times to get to screen **S3.**

3) Decrease the value of the **Gain** or **Current** setting. Repeat this step until pulse tips are between 20-70% of the pulse display.

8. Press [START]. Press [∧] to get to screen **A6**. Use [∨] and [∧] to adjust **Tu** to 7.5% and **Tl** to 5.0%.

9. Press [START] and record the resulting particle count obtained between that lower threshold setting of 5.0% and that upper size setting of 7.5%.

10. Press [START]. Return to screen **A6** by pressing [∧]. Set **Tu** and **Tl** to 10.0 and 7.5%, respectively.

11. Press [START] and record the particle count obtained between those size levels.

12. Repeat at different size levels in order to accumulate data for a frequency size distribution plot (e.g. as in Figure A8-2). The points will carry a statistical scatter and so producing a smoothed curve through them will help to define the minima more clearly.

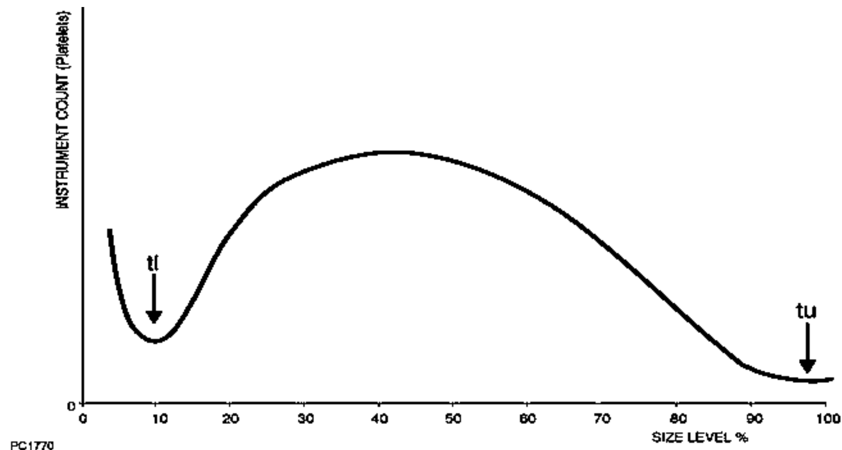


Figure A9-2 Example of Setting Plt Count

13. From this plot, select two minima (tu and tl), at which to set the instrument for subsequent platelet counts. If two minima (and especially the lower size setting minimum) are not apparent, the platelets may be too small to count using this instrument. Try a 50μm aperture, and different settings for **Current** and **Gain**.

Note: Instrument settings will be lost when the instrument is turned off. Repeat the procedure for future analyses.

b) OBTAINING THE PLT RESULT

1. Prepare a suitable Plt dilution as in A8.10.3 a and refer to para. A8.5.3 b), entering the appropriate size levels as determined in the above Plt frequency size distribution procedure.

Appendix 10

Finding Optimum Count Settings for Counting Cells and Other ‘Monodisperse’ Populations when their Sizes are Unknown

A10.1 Introduction

Most samples to be counted have sizes that are known, at least approximately. Settings above which counts are to be taken can often be derived from these or are determined by the nature of the sample and established practice. For example, human red blood cells are normally counted above 25fl regardless of their exact cell size. Narrow size distributions of, say, latex particles can be adequately counted at half the height of the pulses they produce which is 50% of their volume (pulse height is proportional to volume) and therefore approximately 75% of their diameter. (A convenient definition of a narrow distribution is one with a c.v. <10%).

When the size at which cells or particles should be counted is unknown, there are three commonly employed ways of choosing appropriate instrument settings. The most robust is the so-called ‘plateau’ method. Though often referred to as a calibration, this procedure does not yield a calibration constant.

A second method is to estimate the size of the particle population then take the optimum count setting as the diameter corresponding to 75% of that particle size. This is referred to for simplicity elsewhere in this appendix as the ‘three-quarter size’ method.

The third method, usually employed only where population distributions are ill-defined, eg. when cells counts are low and debris counts are high, compares the instrumental count to a reference count via some statistical procedure. Optimum settings are those that give best agreement with the reference(s).

Each type of particle or cell may have its own characteristic optimum setting determined individually by one of the above methods. The setting is always entered as a ‘size setting’ on Setup screen **S1**, even if the size scale on which it is based is entirely arbitrary. So, it is not recommended that counts be taken on uncalibrated apertures. In the absence of proper calibrations with reference materials, the default Kd value for an aperture should be entered. All ‘sizes’ derived or entered will then be reasonable approximations to true values.

Although the Z Series retains an optimum setting when power is turned off, where more than one is needed, an external record of each should be made so that it can be re-entered as required. Counts recorded by the optional printer automatically include the size setting at which they were taken. All three methods require the particle pulses to be ‘on screen’ while counts are made.

A10.2 Establishing the Correct Current and Gain

Since the BECKMAN COULTER Z Series cannot know what size an unknown cell or particle population is, optimum instrument settings cannot be automatically computed. The procedure below enters a sequence of sizes until pulse heights are at an acceptable height for determining the optimum count setting and optimizes each size in turn automatically.

A10.2.1 Procedure

(1) Assuming that the Z Series has been fitted with an appropriate aperture tube, use Table A10-1 to select a suitable metering volume. If there has been no prior calibration of the aperture then allocate the default aperture Kd either permanently using CAL (as described in Section 3, Calibration) or as a temporary constant (see para. 3.6.3, Temporary Kd values). Default Kd values are given in Table A10-1 as the first line of the primary size steps (bold type) and correspond to 60% of the aperture diameter, D_A.

(2) Press **SET-UP** until screen **S1: SETUP - ENTER SIZE DATA** displays.

(3) Enter the largest size setting (this is also the default Kd) from the primary size steps (bold type) given in Table A10-1 appropriate to the aperture diameter D_A. If your Z Series has two thresholds, enter the value in both fields.

Time can be saved if you have some idea of the size of particles or cells, by picking a value from Table A10-1 that is a little larger and entering this instead.

(4) Offer a suspension of the cells or particles to the aperture. If the sample appears turbid, the concentration may be too high or there may be excessive debris.

(5) Press **SET-UP** and screen **S2** displays:

```
S2:   SETUP - ENTER ANALYSIS DATA
            Select Aperture : <100µm C >
               Aperture Kd :  60
          Metered Volume : < 0.5 >
        Measure Aperture : < Yes >
         Optimize Settings : < No >
```

(6) Ensure that the **Optimize Settings** field displays **Yes**. (If not toggle it with the **<** and **>** keys). If the aperture has only just been fitted, set **Measure Aperture** to **Yes**. The Z Series's automatic setting up algorithm will position the instrument's pulse height sensitivity close to the top of the aperture range where only the largest particles will register.

(7) Press **START**. The Z Series computes optimum settings for the size you have entered.

(A Flushing message is perfectly normal: The instrument always flushes behind the aperture before making any measurement for the first time after being switched on).

(8) Press **START** again to accept the settings and start a count. Observe the pulse representation on screen A2 while the count is in progress. There should be virtually no pulses visible on the display. If a significant number are present, your sample may be too large for the aperture. Particles and cells above 60% of aperture diameter are likely to cause frequent aperture blockage.

If the display appears more like Figure A10-3 or A10-4 than Figure A10-1 when first set to 60% of aperture diameter, the particles or cells are definitely too large. Check that the size displayed genuinely is 60%. If it is, fit a larger diameter aperture tube and begin again.

(9) Allow the count to complete, or press **STOP**. Press **SET-UP** to access screen **S1**. Enter the next size down in the appropriate primary size step (bold type) in Table A10-1, e.g. for 100µm aperture enter 47.62µm. (For two threshold instruments, enter the value in both size fields).

(10) Press **SET-UP** to access screen **S2**, ensure the **Optimize Settings** flag is set to **Yes**, then press **START** to optimize. Press **START** again to begin another count. If pulses do not appear on the display of screen **A2**, then repeat steps 9 and 10.

Continue entering decreasing sizes (using the values in bold type) from Table A10-1 until pulses appear on the display. Figures A10-1, A10-2, and A10-3, show how a sequence of pulse representations can be expected to appear as the sample pulses are brought within the range of the display by decreasing the entered size. Figure A10-4 shows a display with pulses generated by an entered diameter that is too small.

(11) If pulses are above the count discriminator line (threshold) but less than half the display height as in Figure A10-2, then select the next lower intermediate diameter (italic type) from Table A10-1. Enter this in screen **S1**. If, after decreasing the size by one or more steps, the pulses clearly approach or exceed full scale as in Figure A10-4, increase the diameter by one or more intermediate steps to bring the display back to that of Figure A10-3.

(12) Once the display resembles that of Figure A10-3, the Z Series current and gain can be considered optimised. Fragile cells may benefit from current and gain being optimised with volume units selected, since the Z Series will then calculate the electrical stress applied to the particles and attempt to limit it to less than the maximum applicable to human red blood cell membranes.

(13) Counts taken without any further adjustment of the size setting, simply by pressing **START**, may well be adequate for many purposes where the most accurate knowledge of concentration is not required. Alternatively, go on to paragraph A9.3 and continue with finding the optimum count size setting.

A10.2.2 Re-Optimizing Automatically in Volume Units

Press SET-UP to access screen **S1**. If your Z Series has two thresholds, make the upper size setting equal to the lower size setting. Move to **Size Units** and set it to **fl**.

Press SET-UP to enter screen **S2**. Move down to **Optimize Settings** and toggle the flag to **Yes**. Press START. The Z Series re-optimizes automatically to limit the electrical stress on the particles. No substantial change should be evident in the pulse display, although a small increase or decrease (e.g. by a factor of $\sqrt{2}$) in pulse height would be perfectly normal owing to the stepwise nature of the gain and current selection.

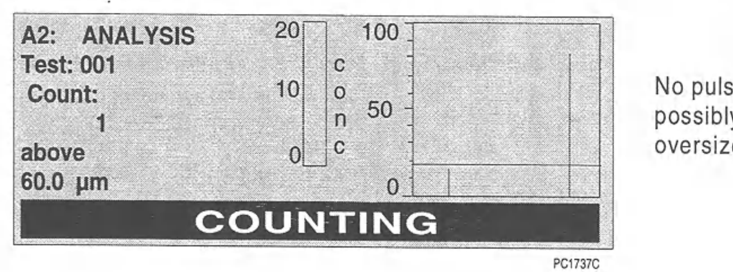


Figure A10-1 Size Range of Display Too High

*The size range of the display is **too high**, so the size setting of the Z Series must be decreased. **Decreasing** the size setting increases pulse height.*

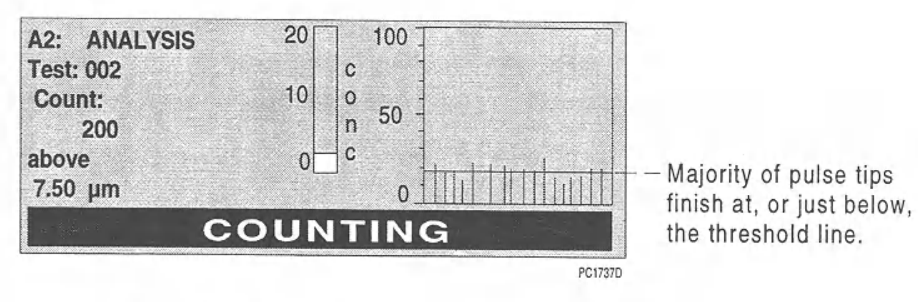


Figure A10-2 Size Setting Too High

*The size setting is **too high**. Pulses are on scale but not high enough to resolve the 'plateau' properly. If particles are small relative to the aperture diameter, this may be as high as it is possible to get with the available current and gain. Otherwise, the size setting needs to be **lowered** probably by not more than one full step in Table A10-1, (doubling the pulse height). One intermediate step lower in size may be enough (increasing the pulse height by $\sqrt{2}$).*

Table A10-1 Size Settings Sequence

Diameter Size Sequence for Optimizing Settings Sequence starts at 60% of aperture diameter (also the default Kd) and doubles pulse heights with each primary step (bold type). Intermediate steps (italic figures) increase pulse height by √2.

Aperture	50		70		100		140		200	
Metered Volume, ml	0.1		0.5		0.5		1.0		1.0	
	30.0	*26.7*	**42.0**	*37.4*	**60.0**	*53.4*	**84.0**	*74.8*	**120.0**	*108.9*
	23.8	*21.2*	**33.3**	*29.7*	**47.6**	*42.4*	**66.7**	*59.4*	**95.2**	*84.8*
	18.9	*16.8*	**26.5**	*23.6*	**37.8**	*33.7*	**52.9**	*47.1*	**75.6**	*67.3*
	15.0	*13.4*	**21.0**	*18.7*	**30.0**	*26.7*	**42.0**	*37.4*	**60.0**	*53.4*
	11.9	*10.6*	**16.7**	*14.8*	**23.8**	*21.2*	**33.3**	*29.7*	**47.6**	*42.4*
	9.45	*8.41*	**13.2**	*11.8*	**18.9**	*16.8*	**26.5**	*23.6*	**37.8**	*33.7*
	7.50	*6.68*	**10.5**	*9.35*	**15.0**	*13.4*	**21.0**	*18.7*	**30.0**	*27.7*
	5.95	*5.30*	**8.33**	*7.42*	**11.9**	*10.6*	**16.7**	*14.8*	**23.8**	*21.2*
	4.72	*4.21*	**6.61**	*5.89*	**9.44**	*8.41*	**13.2**	*11.8*	**18.9**	*16.8*
	3.75	*3.34*	**5.25**	*4.67*	**7.50**	*6.68*	**10.5**	*9.35*	**15.0**	*13.4*
	2.97	*2.65*	**4.16**	*3.71*	**5.95**	*5.30*	**8.33**	*7.42*	**11.9**	*10.6*
	2.36	*2.10*	**3.31**	*2.94*	**4.72**	*4.21*	**6.61**	*5.89*	**9.44**	*8.41*
	1.87	*1.67*	**2.62**	*2.34*	**3.75**	*3.34*	**5.25**	*4.67*	**7.50**	*6.68*
	1.49	*1.32*	**2.08**	*1.85*	**2.97**	*2.65*	**4.16**	*3.71*	**5.95**	*5.30*
	1.18		**1.65**		**2.36**		**3.30**		**4.72**	

PRIMARY STEPS

INTERMEDIATE STEPS

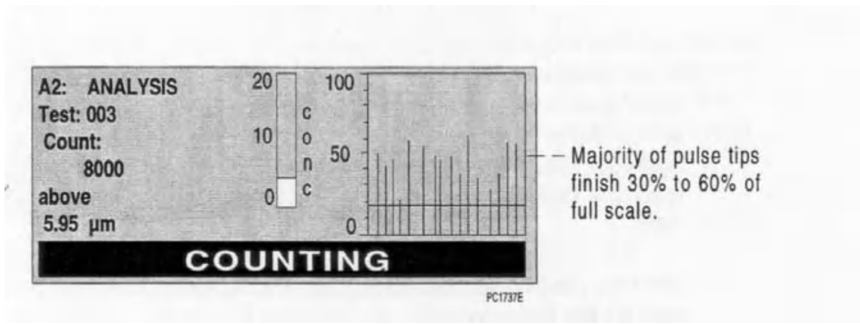


Figure A10-3 Correctly Positioned Pulses for the 'Plateau' Method

*Correctly positioned pulses for the 'plateau method'. The size range of the display is **exactly right**. Continue with the procedure from paragraph A10.3 onwards.*

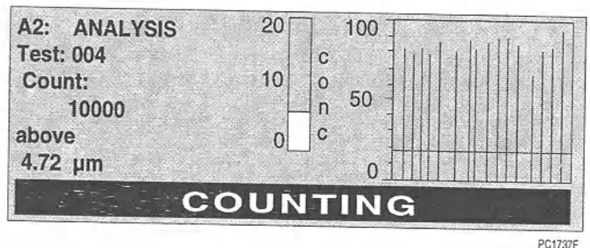


Figure A10-4 Size Range of Display Too Low

The size range of the display is ***too low****. The Z Series size setting must be* ***increased*** *probably by one primary step from Table A10-1, (halving the pulse height). Increasing the size setting reduces pulse height. If pulse tips are only just above 60% then increasing the size setting by one intermediate step (a factor of* $\sqrt{2}$*) should be sufficient.*

A10.3 Finding the Optimum Count Settings

A10.3.1 The 'Three-Quarter Size' Method of Entering the Optimum Count Setting

A simple way of setting the Z Series to the optimum count setting (OCS) when only one population of particles is present and little baseline debris or noise is evident, is to enter a value 75% of the diameter of the modal cell or particle diameter (50% of the modal cell or particle volume). This method works well for samples such as 'monosized' latex when a size estimate is easily obtained and the diluent is virtually particle free by comparison.

A10.3.1.1 Estimation of Cell or Particle Size

The modal diameter or volume can often be estimated by eye or the size setting returned by the optimization procedure detailed in para. A102, above, but this usually gives only a coarse guide to the OCS unless the size distribution is very narrow. For a first approximation, assuming that the particle pulse display appears similar to that shown in Figure A10-3, the cell or particle size is roughly 1.5 times the diameter (3.3 times the volume) of the lower size setting that results from the optimization procedure in A10.2 above.

This value can be considerably improved for most reasonably narrow distributions by using the size setting indication line as a guide to locating the pulse peak, method 1 below.

The median particle size may be a useful substitute for the modal size since a median is relatively simple to determine by counting, method 2 below.
If a size distribution is reasonably narrow, use may be made of the self

calibration algorithm of the Z Series to locate the mean or modal pulse height, method 3 below. Size is determined on a scale based on the default calibration constant. Where some other calibration constant is in use, the size can be correspondingly adjusted, see Figure A10.5.

Broader distributions such as polydisperse latexes and actively budding yeasts may not have a modal pulse height distinctive enough for the calibration algorithm to function properly. In such cases, the result should always be confirmed by inspection of the pulse representation on screen **A2**.

Method 1 - Using the 'threshold adjustment' screen to estimate peak pulse heights.

(1) While the Z Series is counting with screen **A2** displayed, press the [∨] or [∧] key to enter the adjustment screen, screen **A6.** Move the size setting indication line (threshold) with the [∧] key to intersect the majority of the pulse tips, see Figure A10-5. If the Z Series has two thresholds move the upper threshold by using [<] and [>] to highlight it and then moving it with [∨] or [∧].

(2) Once positioned, the threshold line will show both its percentage full scale value and the size setting equivalent to the modal cell or particle size. The value can be quoted either in **µm** or **fl** by switching between units before counting either on screen **S1** (Setup) or on screen **A1** (Output. Return to counting particles, **A2**, by pressing [**START**]. The count can then be interrupted by pressing [**STOP**].

(3) Press [**SET-UP**] to return to screen **S1**, compute and record 75% of the appropriate diameter size setting (50% of the volume size setting). Enter the result into the lower size setting field. Re-optimize again automatically if necessary.
Counts may now be routinely taken above this size.

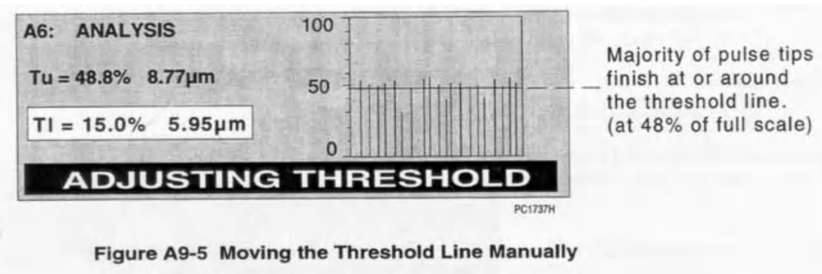


Figure A9-5 Moving the Threshold Line Manually

Threshold line moved to the peak of the sample pulses by means of the [∨] and [∧] keys. Pressing and holding the keys produces faster line movement. Pressing either [<] and [>] key switches between upper threshold and lower threshold lines for individual movement. The corresponding screen text is highlighted.

Method 2 - Substituting the median size.

(1) Retaining the settings from the optimization procedures, A10.2.1 or A10.2.2 above, press START to take and complete a count. Repeat, to take several taking counts, and average. Divide the average by two and record the result.

(2) Press **SET-UP** to access screen **S1**. Enter 1.5 times the diameter or 3 times the volume in the lower size setting field. Press **START** to take another count. Compare this count with step 1. If the step 1 result is higher, reduce the size on screen **S1** by a small amount and count again. If the step 1 result is lower, increase the size by a small amount and count again.

(3) Repeat step 2 until counts are reasonably close (within a few percent of step 1's result).
Confirm by taking several counts and averaging. The final setting giving agreement is the median size.

(4) Compute 0.75 times the diameter of this final setting (0.5 times the volume) and enter it in the 'size setting'on screen **S1**. Counts may now be routinely taken above this size.

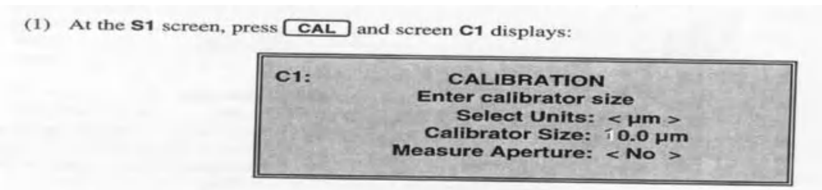
Note: Method 1 is often used to provide a starting point for step 2 in method 2 and reduce the number of repetitions.

Method 3 - Using the Z Series Self calibration algorithm to determine a mode or mean.

When the Z Series optimizes automatically, it takes into account the different requirements of the algorithm currently running. Calibration optimum settings are not identical to those needed for counting and require an initial estimate of the size setting to establish pulse heights at roughly the correct level. Calibration optimum settings are always based on the default calibration constant of 60% of the aperture diameter regardless of the actual constant displayed in a Setup screen.

To obtain a Mode

(1) At the **S1** screen, press **CAL** and screen **C1** displays:



(2) At **Select Units** there are two choices, either µm, or fl (=μm^3). Using the cursor keys, select the required units appropriate to the assay value of the calibrator.

Note: The automatic calibration procedure assumes a modal value if **µm** is slected and a number average volume if **fl** is chosen.

(2) Compute the first approximation of diameter as in para.. A10.3.2 (1.5 times the size setting returned from the optimization procedure) and enter the value in **Calibrator Size**. Call this diameter $d_{1.}$ Set **Measure aperture** to **Yes.**

(3) Offer a suspension of the cells or particles to the aperture.

(4) Press **START** to compute the optimum calibration settings for size d_1. Screen **C4** displays the message **Computing calibration settings**.

(5) Press **START** to accept the new settings and begin a calibration.

(6) Observe the pulse representation while calibration is in progress. The display should resemble Figure A10-6. If pulses are the correct height (about 30% of the scale) but a **HIGH CONCENTRATION** message displays, dilute the sample, present it again and repeat step 7. If the display resembles Figure A10-7 or A10-8 press **STOP** to interrupt calibration, press **CAL** to access screen **C1** and enter a larger or smaller value for d_1 as appropriate. Repeat steps 1 through 4. (A larger value decreases pulse height and vice-versa. For convenience, values can be chosen from the appropriate column of Table A 9-1 selecting a diameter one step above or below the size nearest to the original value for d_1).

(7) Once the display resembles that of figure A10-6, allow the calibration to go to completion. (Follow on-screen instructions or refer to section 3, Calibration, step 5 onwards). Record the new constant presented on screen C7, and calculate the effective modal size from:

$$\mathbf{D_{mode} D_1 {}^{*} (Default\ Kd/New\ Kd)}$$

Where New Kd is the value returned from screen **C7** and d_1 is the diameter value finally entered into screen **C1** at the end of step 7, above. The default Kd values are given in Table A10-1 (60% of aperture diameter).

(9) Press CAL to exit screen C7 without accepting the new Kd into memory.

<u>To obtain a Mean</u>

Complete step 1 but switch to **fl** units on screen **C1** before going to step 2. If an adjustment To pulse heights is needed at step 3, switch back to **µm** units to enter a new diameter then return to **fl** to complete the remainder of the steps. Record volume size equivalent to d_1 instead of d_1 at the end of step 7 (call this V1).

Meanvolume is given by:

$$\mathbf{V_{mean} = V^1 * (Default\ Kd/New\ Kd)^3} \qquad \textbf{..........Equation (2)}$$

The mean diameter can be obtained by aplying Equation (1) to the new Kd resulting from a volume calibration.

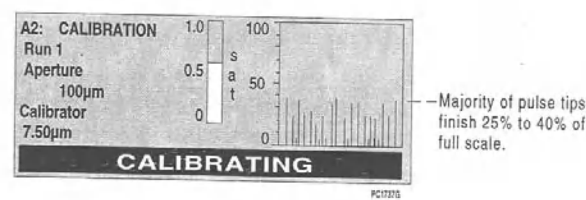
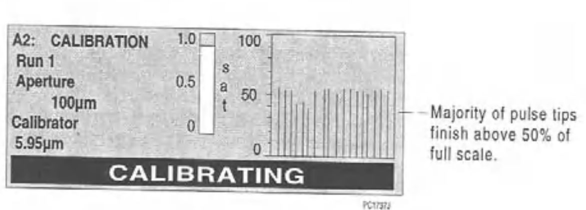


Figure A9-6 Correctly Positioned Pulses for Calibration



Figure

A10-7 Size Range of Calibration Display Too Low

The size range of the display is just too low. The Z Series size setting must be raised by increasing the value of d_1, typically by one primary step from Table A10-1. If pulse tips are only just above 50% then increasing d_1 by one intermediate step should be sufficient.

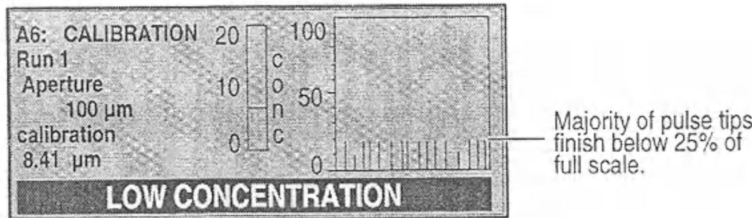


Figure A10-8 Calibration size Setting Too High

The value of d_1 is just too high. One intermediate step lower from Table A10-1 may be enough (Increasing the pulse height by √2).

A10.3.2 Determination of the Optimum Count Setting by 'Plateau' or 'Trough' Finding

Figure A10-9 shows the essence of the whole procedure. Counts are taken at a sequence of size levels shown as a 'grid' placed over the pulse representation. Spacing is achieved by considering the size level where pulses start to merge with debris or unwanted fines, an approximate size for the mode/mean of the pulses, (a size estimated as in para. A10.3.2 above) and dividing this into sufficient intervals. At least ten are recommended.

Usually the range for the 'plateau' is established from experience or observation of the pulse representation during optimization, e.g. for human red blood cells, the 'plateau' is typically from 20fl to 60fl but, in the absence of any prior information or guidance, a default upper limit of the range can be taken as equal to the first approximation of cell or particle size from para. A10.2 above, i.e. 1.5 times the size setting resulting from optimization. The lower limit can be taken as half the first approximation.

For example, in figure A10-3, a modal pulse height of 50% would correspond to a size of 8.93µm (= 1.5 x 5.95µm). The lower limit would then be 4.46µm, (=0.5x.83µm) and each interval would be in 0.45µm steps. Clearly, adjusting values to avoid awkward size entries simplifies the method, so that an obvious 'plateau' sequence of ten steps would be start at 4.45µm then proceed 4.50µm, 4.50µm, etc, finish at 8.95µm. Counts need not be taken in strict size order, so that additional sizes can be added to either end if the chosen range is found to be too narrow, or inserted between levels to improve resolution provided that compensation is applied for unequal size interval widths (see Figure A10-9).

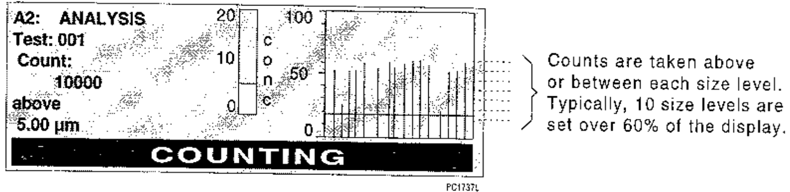


Figure A10-9 Principle of the 'Plateau' Method

Counts are obtained above and/or between successive sizes. The sequence portrays a sizing 'grid'with a region of constant cumulative count, or a minimum in differential count, to be determined. Optimum size for counting corresponds to the center region and minimum.

Procedure

For single threshold Z Series instruments:

(1) Press **SET-UP** until Screen **S1: SETUP - ENTER SIZE DATA** displays.

(2) Enter the lower limit size level (in this example 5µm) into the size setting field of screen **S1.**

(3) If the setting shave not already been optimized automatically, press **SET-UP** and screen **S2** displays. Set **Optimize Settings** to **Yes**. Accept the Z Series setting s and initiate a count by pressing **START**. Record the count **above TI.** Repeat and record a second count at this size.

(4) Enter the next higher size level on screen **S1** and take and record two more counts (there is noo need to optimize again).

For dual threshold Z Series instruments:

(1) Press **SET-UP** until screen **S1: SETUP - ENTER SIZE DATA** displays.

(2) Enter the lowest size level and the next higher (in the above example 4.45µm and 4.9µm) in the **Set Lower Size Tu** and **Set Upper size Tu** fields respectively.

(3) If the setting shave not already been optimized automatically, press **SET-UP** and screen **S2** displays. Set **Optimize Settings** to **Yes**. Accept the Z Series setting and initiate counting. Repeat and record counts both **between** and **above TI.**

(4) Enter the next size interval on screen **S1**. The upper size from the previous interval becomes the lower size for the next, so that intervals are adjacent. In the above example, this would be 4.9µm and 5.35µm. Take and record two more pairs of **between** and **above TI** counts (there is no need to optimize again).

(5) For both single and dual threshold instruments, continue entering each next higher size or size interval and counting until the upper limit, e.g. above 8.95µm, is reached.

Plot the cumulative counts against the size at which they were taken, as shown by the Counts **above** line in figure A10-9, and/or the frequency counts (differences between cumulatives) against centers of size intervals, the Counts **between** line in the same figure.

- For **single threshold** instruments, the count results are only cumulative i.e. Count **above**. To obtain the frequency counts required for the counts **between**, subtract the average count at each size level from the average count at the next lower size. Plot any negative values as zero (these can arise from statistical scatter that are essentially identical but at successive size levels).

- The **dual threshold** results, **above TI** and between provide both plots directly.

The distinction between particle pulses and 'noise' or debris, generates a minimum in the frequency or counts **between** plot. This can be read from the plot and entered into screen **S1**. The example in Figure A10.10 shows two minima. The small difference in counts between adjacent size levels in the 'plateau' region, inevitably leads to statistical variation in these counts being as large as that at each individual size level. Multiple minima are therefore a common phenomenon. The cumulative plot in figure A10-10 shows that closely similar counts would be obtained whichever minimum is chosen. Selecting the lower minimum, however, would set the counting threshold unnecessarily close to the debris level. Fluctuations in the latter could affect count accuracy.

Choosing the upper minimum sets the counting level rather closely to the smallest particles (particularly if the sample is a cell population with natural size variability). Fluctuations in size could affect count accuracy.

The optimum count setting would be size level corresponding to a point between the two minima, probably nearer to the lower than the upper (an optimum setting of 5.7µm is indicated in Figure A10-10 by reference to the cumulative plot).

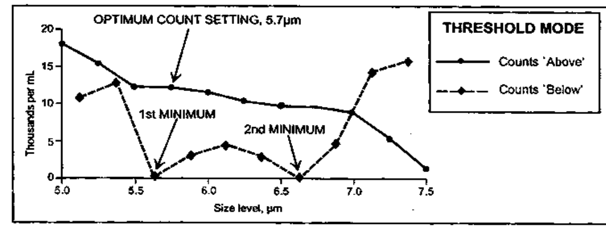


Figure A10-10 Examples of Plots for the 'Plateau' and 'Trough' Methods

Example of plots for the 'plateau' and 'trough' method to derive the best size level at which to count cells of particles of unknown size. It is good practice to divide counts 'between' by the size interval to express results as true frequency, e.g., particles per micrometer, to correct for any inequalities in size steps. In this example, the size steps are all equal but particles per micrometer has still been calculated to illustrate best practice.

A10.3.2.1 **Restrictions of the 'Plateau Method'**

Certain assumptions are made about the sample.

(1) Particle concentration in the 'calibrating' sample must be sufficiently high to enable statistically reliable counts. Counts in excess of 10,000 are ideal. This can be achieved with low concentrations by several times and adding the results, or using a 1.0ml metering volume. As the procedure involves, typically, counts at ten different sizes, multiple counts greatly extend the time taken to complete it, considerably reducing its practicality.

(2) A clear distinction must exist between the cell or particle population and baseline noise, debris, or background particles, that is, there must be some size within the range of the aperture tube above which it is possible to say that all sample particles can be counted. The method cannot be used for populations that extend below the minimum resolution of the aperture, such as some oil in water emulsions.

(3) Mixed populations can be counted individually, provided that the size separation between components considerably exceeds the sum of the widths of their size. A rough guide is to divide the sum of the product of component size and standard deviation by the difference between the component sizes. It becomes increasingly more difficult to count each component individually as the result exceeds3. (If coefficients of variation are used instead of the standard deviations, the comparison value is 0.03 instead of 3).

A10.3.3 Count Comparison Method

Although the correct instrument count is generally of greater importance than aligning counts with another method, occasionally, sample distributions are not well defined enough to determine the optimum counting point solely from instrument count data. An example might be a low concentration of cells in a high debris environment, such as somatic cell counting in stored bovine milk samples.

Where a reference method exists, counts from the Z Series can be compared with reference results. It should always be borne in mind, however, that most reference methods rely on counting by eye using a microscope, and that the uncertainty in this is very high.

Instrument counts plotted as in Figure A10-2A. A simple test is normally all that is required, e.g., a Chi^2 test. Table A9-2B shows this applied to the individual members of the matrix by separately comparing the average count each size level in each sample (column in Table A9-2A) with the appropriate reference value for that sample. Alternatively, by summing all Z Series counts regardless of sample at each size level and comparing the sum with the sum of references for each sample. The result of the former test is given in the $(\sum E - \sum O)^2$ column. The two can be combined together by taking the square root of the product, final column.

A10.4 Updating the Optimum Count Setting Value with Kd Changes

At some point, after a procedure is applied to anaperture calibrated only with a default Kd, a proper size calibration may be carried out using a calibrator material. Since the Optimum count Setting (OCS) relates to the Kd value in use at the time, you avoid repeating an entire OCS procedure by updating the OCS to correspond to a new Kd.

Updated OCSum = Previous OCSum * (New Kd/Old Kd) .....Equation (3)

Updated OCSfl = Previous OCSfl * (New Kd/Old Kd)^3 .....Equation (4)

Sample No. =	1	2	3	4	Sum 1 - 4
	Counts Expected, E (thousands / 0.5ml)				$\sum E$
Reference Results --→	10.2	5.8	3.2	2.5	21.7
Results Size, µm	Counts Observed, O (thousands / 0.5ml)				$\sum O$
7.50	1.15	0.63	0.38	0.267	2.42
7.25	4.85	2.70	1.63	1.14	10.32
7.00	8.14	4.63	2.66	2.05	17.48
6.75	9.25	5.25	2.96	2.28	19.75
6.50	8.92	5.25	2.89	2.24	19.38
6.25	9.69	5.47	3.12	2.43	20.79
6.00	10.69	6.11	3.46	2.70	22.95
5.75	11.43	6.44	3.69	2.89	24.45
5.50	11.51	6.44	3.72	2.89	24.56
5.25	14.47	8.21	4.67	3.65	31.00
5.00	16.98	9.69	5.47	4.29	36.44

Table A10-2A Example of Comparison Matrix for Instrument Counts and Reference Method

Method = Size, µm	$(\sum E - \sum O)^2$	$\sum (E - O)^2$	$[(\sum E - \sum O)^2 \times \sum (E - O)^2]$
7.50	371.64	121.64	212.64
7.25	129.46	42.56	74.23
7.00	17.83	6.12	10.44
6.75	3.81	1.30	2.23
6.50	5.76	2.11	3.48
6.25	0.96	0.37	0.60
6.00	1.57	0.44	0.83
5.75	7.54	2.31	4.10
5.50	8.16	2.54	4.55
5.25	86.55	27.53	48.81
5.00	217.36	69.55	122.95

'Plateau' region (6.75–5.50) — Optimum counting point (6.25)

Table A10-2B Evaluation

The cont values shown are purely illustrative, but have essentially the same distribution as in Figure A10-9.

Alphabetical Index

A

Page

aperture
  table, suitable metered volumes for selected aperture sizes 4-3
Aperture Lamp
  description 2-6
Aperture Tube
  and calibration particles A4-1
  draining 2-9, 4-20
  illustration, replacing 2-15, 4-22
  in salt water contamination monitoring A5-1
  installation 4-21
  priming 2-9
  replacing 4-21
  selection 4-21, A1-1
  sizes 5-2
  storage 4-20
  table of useful, standard data A1-2
Aperture Viewing Screen
  positioning 2-16
aqueous electrolyte solutions
  list of suitable A3-3
  recommendations A2-1, A3-1
  table, typical aqueous electrolyte solutions A2-1
  Warnings A2-1

B

basic principle 6-1
blood cell counting
  calibration A8-5
  description A8-1
  hazards A8-8
  informative data A8-3
  operational precautions and limitations A8-8
  procedures for human red cell counts (RBC) A8-3
  procedures for non-human platelet cell counts (PLT) A9-6
  specifications A8-1
  table, linearity limits A8-2
  table, within run precision in the normal blood range A8-1

C

Cal key
  function . . . 2-8
calibration . . . A8-9
  adjusting concentration . . . 3-6
  description . . . 3-1
  editing Kd . . . 3-9
  instrument settings . . . 3-4
  preparing the instrument . . . 3-2
  preparing the sample . . . 3-1
  procedure . . . 3-3
  run screen description . . . 3-5
  stops before completion . . . 3-7
  table, suitable metered volumes . . . 3-3
  with partial data . . . 3-8
  with temporary Kd . . . 3-8
calibration particles and Aperture Tubes
  description . . . A4-1
  determination of assayed sizes . . . A4-2
  latex suspensions . . . A4-1
  redispersion of calibration particles . . . A4-1
  table of recommendations . . . A4-1
cell size
  estimating . . . A10-6
cell/particle size
  estimating . . . A10-6
CAUTION
  definition . . . 1-2
channelyzer
  description . . . 4-13, 6-3
  results . . . 4-13
  statistics . . . 4-14
checking for residual bleach
  recommendation . . . A7-6
chemical
  cautions . . . 1-2
  warnings . . . 1-3
clock
  setting date and time . . . 2-12
coincidence correction
  description . . . 6-2
connections
  illustration . . . 2-13

connectors
  diluent . . . 2-10
  external control port . . . 2-10
  keyboard . . . 2-10
  level sensors . . . 2-10
  parallel printer . . . 2-10
  power . . . 2-10
  table, connectors and function . . . 2-10
  waste . . . 2-10
control circuits
  description . . . 6-5
controls and connectors . . . 2-5
Coulter Method of counting and sizing . . . 6-1
cursor keys
  moving . . . 2-6
count
  comparison method . . . A10-15
count settings
  finding optimum . . . A9-1
current/gain
  establishing correct . . . A9-2

D

Data Terminal
  cursor keys . . . 2-6
  description . . . 2-6
  message area . . . 2-10
  numeric key pad . . . 2-6
  screen access keys . . . 2-8
diluent (electrolyte solution)
  filtration . . . A1-3
  selection . . . A1-2, A2-1, A3-1
diluent interface . . . 2-10
disinfecting the instrument
  "Disinfection Tablets" . . . A7-3
  checking for residual bleach . . . A7-6
  description . . . A7-1
  draining the system . . . A7-3
  filling the system with disinfectant . . . A7-5
  Gigasept method . . . A7-2
  glutaraldehyde method . . . A7-2
  hypochlorite bleach method . . . A7-2
  references . . . A7-6
  removing disinfectant solution . . . A7-6
  used (‘contaminated') solutions . . . A7-3
  when to . . . A7-3
dispersion
  description . . . A1-3

Drain Aperture
description 2-9
Drain System
description 2-10
Drain System (F5) A7-4
Instrument Functions (F1) A7-3
procedures A7-3

E

electrical
cautions 1-3
warnings 1-3
Electrode Assembly
illustration, installation 2-15
electrolyte solution (diluent)
filtration A1-3
selection A1-2, A2-1, A3-1
environmental
warnings 1-3
error messages
definition 8-1
table 8-1
European mains lead
color coding 2-5

F

Fill System
description 2-9
Fill System (F3) A7-5
Instrument Functions (F1) A7-5
procedures A7-5
Flush Aperture
description 2-9
procedure 7-4
Focus Control Body
description 2-6
Focus Control Knob
description 2-6
Front Panel 2-5

functional description
- control circuits 6-5
- pulse generation 6-2
- pulse processing 6-3

Functions key
- function 2-9

fuses
- description 2-3
- installation 2-4

G

guaranteed data
- coincidence corrected count 5-2
- particle size range 5-1
- thresholds 5-2

I

informative data
- Aperture Tube sizes 5-2
- data output 5-2
- metering volumes 5-2
- power, dimensions, weight 5-3

K

keyboard interface 2-10

L

level sense interfaces 2-10

Load Profile
- description 2-9

location requirements 2-3, 5-1

M

manual
- conventions 1-1
- scope and organization 1-1

mechanical

cautions 1-3
sources of error 1-3
metering volumes 5-2

N

Note
definition 1-2
numeric key pad
function 2-6

O

operating temperature 5-1
operation
description 4-1
principles of 6-1
principles of operation 6-1
Output key
function 2-8
operation 4-1
output
screen 4-7

P

parenteral sample
background count A6-3
calibration A6-3
Extracts from British Pharmacopoeia, 1993, Parenteral Preparation A6-1
origin of the contaminants in parenteral fluids A6-6
precautions during analysis A6-2
preparation, non-conducting and viscous samples A6-1
sample analysis A6-3
particle count 5-2
particle size range 5-1
particulate substances and suitable electrolyte solutions A3-1
plateau method
procedures A10-11
restrictions A10-14
Platform Release Catch
description 2-6
Power Supply indicators
description 2-11
Power Switch
description 2-5

power, dimensions, weight 5-3
preventative maintenance
  Flush Aperture 7-4
  table, check list 7-1
  User Testing (T1) 7-1
Prime Aperture
  description 2-9
Print key
  function 2-8
printer interface 2-10, 5-3
procedures
  adjusting the optics 2-17
  configuring voltage/power 2-3
  connections 2-12
  SET CLOCK 2-12
  start-up 2-12
  unpacking 2-1
procedures for Human Platelet Counts (PLT)
  instrument setup A8-6
  obtaining the Plt result A8-7
  Setup - Enter Analysis Data (S2) A8-7
  Setup - Enter Size Data (S1) A8-6
procedures for human red cell counts (RBC)
  instrument setup A8-3
  obtaining the RBC result A8-4
  Setup - Enter Analysis Data (S2) A8-4
  Setup - Enter Size Data (S1) A8-3
procedures for human white cell counts (WBC)
  instrument setup A8-4
  obtaining the WBC result A8-5
  Setup - Enter Analysis Data (S2) A8-5
  Setup - Enter Size Data (S1) A8-5
  interfering substances 1-4
  quality control A8-8
procedures for non-human cell counts
  procedures for non-human red cell counts (RBC) A9-2
  table, typical blood parameters for a range of animals A9-2
procedures for non-human platelet cell counts (PLT)
  illustration, setting count size settings for Plt A9-8
  instrument setup A9-15
  obtaining the Plt result A9-9
  Setup - Enter Analysis Data (S2) A9-3
  Setup - Enter Size Data (S1) A9-3
  Setup - Instrument Settings (S3) A9-4

procedures for non-human red cell counts (RBC)
  illustration, setting count lower size setting for RBC A9-3
  obtaining the RBC result A9-5
  Setup - Enter Analysis Data (S2) A9-3
  Setup - Enter Size Data (S1) A9-3

Setup - Instrument Settings (S3) A9-4
procedures for non-human white cell counts (WBC)
instrument setup A9-5
obtaining the WBC result A9-6
profiles
description 4-14
load 2-9, 4-15
Load Profile (P1) 4-15
store 2-9, 4-14
Store Profile (P2) 4-15
pulse generation
description 6-2
pulse processing
description 6-3

R

re-optimizing
automatically A10-4
Rear Panel 2-11
relative humidity 5-1
removing disinfectant solution
procedures A7-6

S

salt water contamination
and sample analysis A5-1
monitoring A5-1

sample analysis
Analysis (A6) 4-12
Analysis - Output Format (A1) 4-7, A5-2, A6-4
Analysis Display (A2) A5-2, A6-5
Analysis Result (A3) 4-11
Analysis Result (A4) 4-12, A5-3, A6-5
Analysis Result (A5) 4-11
and salt water A5-1
changing threshold settings 4-12
interrupted 4-11
Setup - Aperture Selection (S4) 4-6
Setup - Enter Analysis Data (S2) 4-3, A5-2, A6-4
Setup - Enter Size Data (S1) 4-2, A5-1, A5-3, A6-4
Setup - Instrument Settings (S3) 4-4
Threshold Range Too Wide (S9) 4-5
screen access keys 2-8
Set Clock
description 2-9
procedure 2-12
Setup key
function 2-8
shutdown procedure
decontamination 4-16
end of day 4-16
for storage/shipment 4-16
long term 4-16
specifications 5-1
Start-Up
procedures 2-12
Start/Stop key
function 2-10
status messages
definition 8-1
table 8-1
Stirrer
position 2-6
speed 2-6
Stirrer Paddle
illustration, installation 2-15
installation 2-16
Store Profile
description 2-9

system
- Drain System (F5) . . . 4-18
- draining . . . 2-10, 4-17
- Fill System (F3) . . . 4-19
- filling . . . 2-9, 2-16, 4-18
- functional description . . . 6-2
- installation procedures . . . 2-1
- principles of operation . . . 6-1
- specifications . . . 5-1

T

three-quarter size method
- description . . . A10-6

thresholds
- description . . . 5-2
- Threshold Range Too Wide (S9) . . . 4-5

U

Unblock key
- function . . . 2-8

unpacking
- illustration, components . . . 2-1
- procedures . . . 2-1

User Testing
- description . . . 2-9
- list of tests . . . 7-2
- procedure . . . 7-1

V

Vent Aperture
- description . . . 2-9
- procedure . . . 4-21

Voltage Selector card
- illustration, orientation . . . 2-5

voltage/power
- configuration procedure . . . 2-3

W

WARNING
- definition . . . 1-2

waste interface . . . 2-10

Z

Z1 (dual-threshold model)
description 1-1
Z1 (single-threshold model)
description 1-1
Z2
description 1-1
Z Pak. 2-13