Millipore®

User Guide

Scepter™ 3.0 Handheld Automated Cell Counter



PHCC30000

DOMINIQUE DUTSCHER SAS

The life science business of
Merck KGaA, Darmstadt, Germany
operates as MilliporeSigma
in the US and Canada.

MERCK

Introduction

The Scepter™ 3.0 Handheld Automated Cell Counter ("Scepter™ 3.0") provides a fast and convenient method for counting cells or particles using a disposable sensor. Using the Coulter Principle, the Scepter™ 3.0 does all the work and rapidly delivers accurate and reliable cell counts.

The Scepter™ 3.0 quantifies cells based on size and discriminates larger cells from smaller debris, unlike vision-based techniques, which rely on object recognition software and cannot reliably detect small cells.

A sample of interest is diluted, then acquired using a precision-engineered, microfluidic Scepter™ 3.0 disposable sensor. In seconds, Scepter™ 3.0 displays cell concentration, cell diameter or volume measurements, along with a histogram of cell distribution.

Up to 999 histograms can be stored in the instrument. The Scepter™ 3.0 has a wireless network interface which allows you to send histograms and data files to your Wi-Fi® printer or computer easily. No more cables.

The new Charging Station stores Scepter™ 3.0 out-of-the-way when not in use, freeing up valuable hood or lab bench space.

This system is intended for research use only.

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Symbols and Safety Precautions

This system is intended for research use. Go to SigmaAldrich.com/techservice for assistance.

IT IS IMPORTANT TO READ THESE INSTRUCTIONS BEFORE USING THIS INSTRUMENT.

⚠	• Please read these safety instructions and user manual carefully before using the cell counter and/or any accessory supplied with this product. • Keep these instructions for future reference. • If there is any contradiction between the information in the manual and the information in this notice, the information in this notice takes precedence. • The protection provided by this equipment may be compromised if it is used incorrectly. • Obey the instructions that are given to you. Do not perform any work on the inside of the instrument.
⚠	This symbol indicates that there is a component inside the cell counter that might represent a particular hazard (refer to the operating manual) (HAZARD).

For Global Registration Marks of Conformity, p.26.

CE	Affixed in accordance with the most recent European Council Directives which apply to this instrument. Please refer to the Declaration of Conformity for further detail.
FC	Federal Communications Commission (FCC) conformity marking TFB-1003.
	This symbol represents the products compliance to Chinese RoHS. For more information on RoHS compliance please see the products Declaration Of Conformity.

This product has been evaluated by an OSHA certified Nationally Recognized Testing laboratory (NRTL).

⚠ WARNINGS

- To avoid danger of electric shock, do not install the instrument in an area with a high humidity level, such as a greenhouse or an incubator. Refer to **Operating Environmental Conditions, p.24**.
- Do not touch the charger station and plug with wet hands.
- To avoid potential shock hazard, choose the correct plug configuration and make sure that the USB cable/power adapter is plugged securely into a properly grounded AC power outlet.
- Always ensure that the power supply input voltage matches the voltage available in your location.
- Do not use with flammable or explosive liquids.
- Do not immerse instrument body in liquid, or allow liquid to enter any part of the instrument.
- Do not expose instrument to vibrations. Vibrations may cause instrument malfunction or damage.
- Do not autoclave or expose to high temperature.
- To avoid damage to internal electronic components, do not spray instrument with sanitizing agents. Refer to **Cleaning/Sanitizing, p.18** for sanitizing information.
- Use only authorized accessories (Charging Station).
- If the instrument is broken or dropped, Do not try to disassemble the instrument. Go to SigmaAldrich.com/techservice for assistance.

BATTERIES

Battery is not user accessible or user replaceable. For more information, go to SigmaAldrich.com/techservice.
Do not use instrument in a humid and/or corrosive environment.

- Do not disassemble, pierce or modify the batteries or subject them to unnecessary shocks that would risk damaging them.
- Do not leave near or in direct contact with a heat source (risk of leakage and/or explosion which may cause injuries and/or damage).
- In the event of leakage and direct contact with the fluid, rinse the exposed area with plenty of water and seek medical advice immediately.
- Do not put the batteries in your mouth. If swallowed, seek medical advice or contact the nearest poison control center immediately.

THE LITHIUM-ION BATTERY OF YOUR INSTRUMENT CONTAINS SUBSTANCES THAT MUST BE RECYCLED. DO NOT DISCARD WITH COMMON SOLID WASTE AT END OF LIFE. PLEASE DISPOSE OF IT IN A RECYCLING BIN SUITABLE FOR THIS TYPE OF PRODUCT. SEGREGATE WITH OTHER WASTE ELECTRICAL AND ELECTRONIC EQUIPMENT (WEEE) IN THE EUROPEAN UNION, PLEASE VISIT SigmaAldrich.com/weee.

SYSTEM DISMANTLING – WEEE directive

In accordance with European Union directive on the management of waste electrical and electronic equipment (WEEE), this product must not be disposed of in unsorted municipal waste at the end of its life. It must be taken to a collection and recycling center. For further information, go to SigmaAldrich.com/weee.

ANTENNA

Unauthorized modification of the built-in antenna or use of unauthorized accessories might damage the system and render it non-compliant with directives such as the EU RED directive and FCC regulations. This instrument complies with part 15 of the FCC Rules. Operation is subject to the following two conditions: (1) This instrument may not cause harmful interference, and (2) this instrument must accept any interference received, including interference that may cause undesired operation.

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Parts and Functions

Features

Display Screen

- Displays all information needed for operation
- Displays histograms with with cell concentration, and diameter or volume

Eject Button

Ejects Sensor

Sensor Port

Insert sensor

Disposable Sensor

- Required for sampling cells
- 40 µm or 60 µm
- See **Attach the Sensor, p.11** before inserting





Tech Service Port

For Service use only

USB Port

Connects instrument to USB thumb drive

Ergonomic Hook

- Allows comfortable handling of the instrument
- Secures the instrument to charger

Instrument Schematic

Control Buttons and Screen Icons



MENU Button

- TURN ON / OFF: press and hold
- Return to the menu screen
- Abort any process immediately



ARROW Buttons

- Scroll through menus: right = down, left = up
- Scroll to next/previous histogram



OK Button

- Accept selected fields
- Advance to continue



EJECT Button

Ejects the sensor



Bluetooth® icon connected if visible

Wi-Fi® icon connected if visible

Battery life indicator icon

V Continue Arrow appears in the lower right corner of the screen, only when there are additional menu options. Scroll to see more options

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Battery

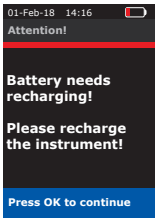
The Scepter™ 3.0 is equipped with an internal 3.7 V lithium ion battery that recharges in the Charging Station. Install the Charging Station (see page 6) and plug into AC wall outlet. Place Scepter™ 3.0 onto the Charging Station. **RECHARGING** will be displayed. Allow battery to charge for 6 hours prior to use (including first use).

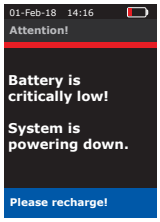
FIRST USE: Fully charge to 100% (4 indicator bars on display; about 6 hours).

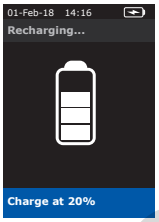
The overall battery life is approximately 2 to 5 years, depending on use.

When not in use, the instrument should be stored on the Charging Station so that the battery maintains charge. It is not necessary to deplete the battery before recharging Scepter™ 3.0. For optimal performance, operate when battery levels are greater than 20%.

If "WARNING - Low Battery" appears on the display screen, stop using the instrument and charge the battery.







If the battery is **completely dead** and does not show the Recharging screen when placed in the Charging Station, the following steps must be followed to start charging the Scepter™ 3.0 battery:

1. Place Scepter™ 3.0 into the Charging Station for 15 minutes.
2. While on the station, press and hold the MENU button for 5-10 seconds, until the the vibrant M appears.
3. Release the MENU button and allow Scepter™ 3.0 to remain in the charger for approximately 6 hours or until fully charged.

CAUTION: To prevent battery damage, use ONLY the specified Charging Station (Cat. No. PHCC3CHARG). Charging cables from previous Scepter™ products are not suitable for charging this instrument.

DO NOT try to use the instrument while on the Charging Station. The Scepter™ 3.0 Handheld Automated Cell Counter is designed to operate on battery power only. Electrical interference with sensor function may produce inaccurate readings.

WARNING: Lithium-ion batteries can easily rupture, ignite, or explode when exposed to high temperatures, or direct sunlight. Short-circuiting a Lithium-ion battery can also cause it to ignite or explode. ONLY use Charging Station (Cat. No. PHCC3CHARG). Never open the instrument or battery's casing. Contact Technical Service for more information. Battery is not accessible or replaceable by the user.



Plug into electrical wall outlet

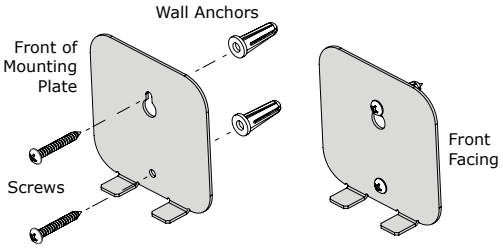
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Installing the Charging Station

Scepter™ 3.0 can be placed on the benchtop horizontally or vertically wall-mounted, within 5 feet (1.5 meters) from an electrical outlet. The mounting kit is required to mount the Charging Station. See **Product Ordering, p.25** for accessory and kit purchasing options.
Figure 1 shows the wall area required.

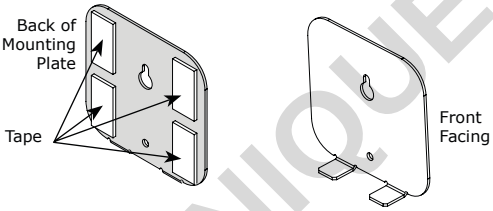
For mounting on drywall, wood or similar:

1. Use screws and anchors.
2. Use 0.25 inch diameter drillbit for anchor holes. Seat anchors gently with a hammer.
3. Install screw in top anchor first, hook plate into place using screw keyhole. Install 2nd screw tight, then tighten 1st screw. (Continue to steps 4 and 5 below.)



For mounting on glass, metal or smooth stickable surface:

1. Use double-sided mounting tape.
2. Remove tape liner from one side of tape and affix to back of mounting plate as shown below.
3. Remove remaining tape liner and press firmly onto glass, metal or smooth stickable surface.



4. Position the Charging Station with mounting plate bottom tabs seated in mounting tab recesses. The mounting plate magnets hold the Charging Station in place.

   NOTE: Do not use the magnets without mounting plate. Doing so may cause the charger to rotate slightly, compromizing the charging contact.

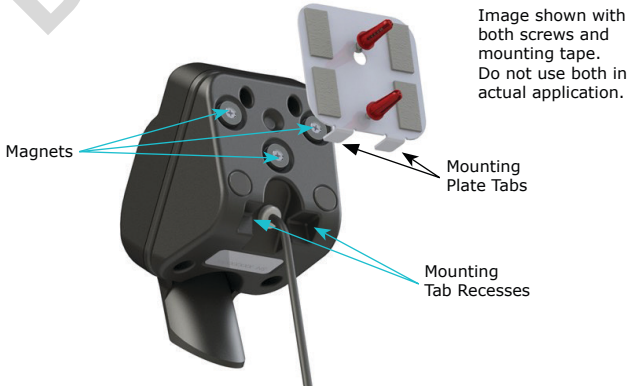
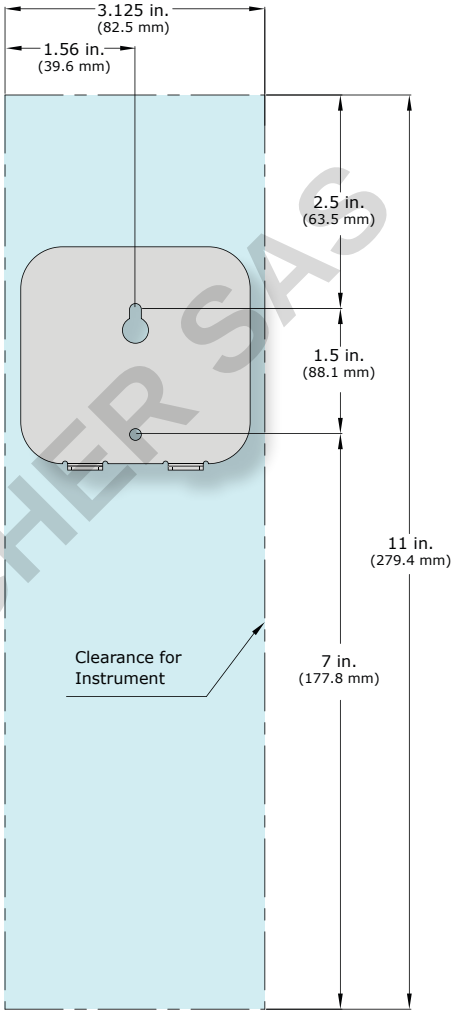


Figure 1: Wall area required for mounting Charging Station.



5. Hang the Scepter™ 3.0 on the Charging Station. See **Battery, p.5** before use.



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Getting Started

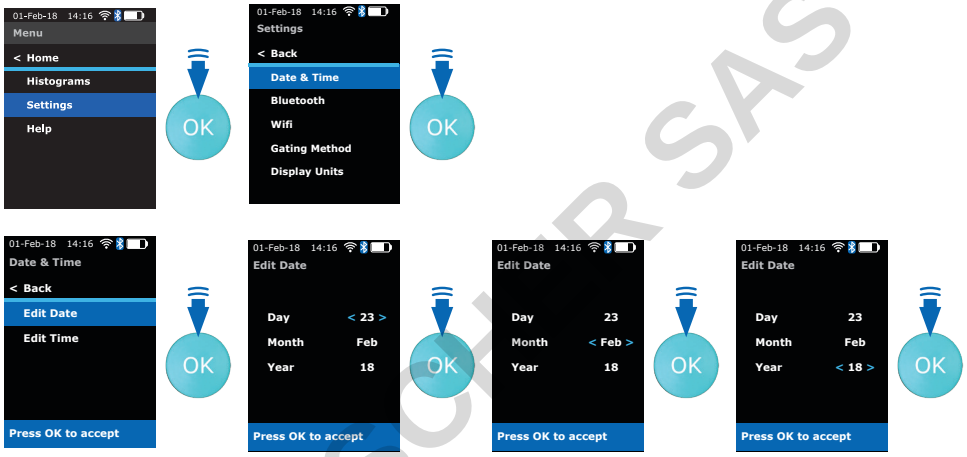
To turn on, make sure Scepter™ 3.0 is fully charged and press/hold the MENU button (5-10 seconds). Release when the vibrant M appears on screen. For setup, the sensor is not needed, press MENU to advance to **Settings**. Use the ARROW keys to scroll to **Settings**, press OK. It is recommended to set up all aspects of Scepter™ 3.0 before use.

Date and Time

The date and time are used in the file name when the histogram is uploaded to a computer.

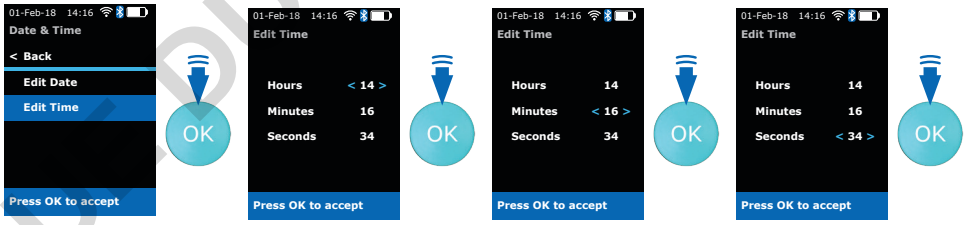
SET UP DATE

1. From the **Menu** screen scroll to **Settings**, press the OK button.
2. Scroll to **Date & Time**, press the OK button.
3. Select **Edit Date,** and press the OK button.
4. Scroll to enter the desired day/month/year. Press the OK button after each selection.



SET UP TIME

1. Scroll to **Edit Time,** and press the OK button.
2. Scroll to enter the desired hour/minute/seconds. Press the OK button after each selection.
3. Press the MENU button to return to Menu Screen.

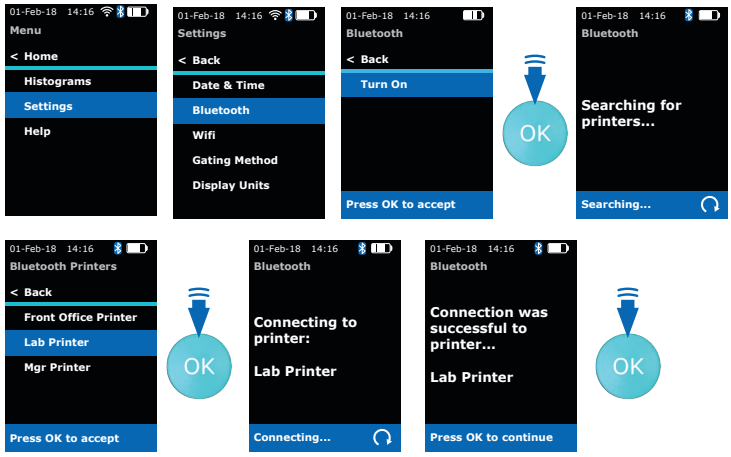


Bluetooth® Enabled Printer

The only Bluetooth® printers supported by Scepter™ 3.0 are Cannon® IVY, Polaroid ZIP™ Instant Photoprinter, and HP® Sprocket Photo Printer. No other printers currently supported.

From the **Settings** Menu, scroll to **Bluetooth** and press the OK button.

1. Scroll to **Turn On** (or **Turn Off**), and press the OK button. The display shows *Searching for printers*. All the printers within range will be displayed.
2. Using the ARROW buttons, scroll to select the desired printer, press the OK button to start the connection. Wait for confirmation screen.
3. Press the OK button to return to **Settings**.



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Wi-Fi® Network

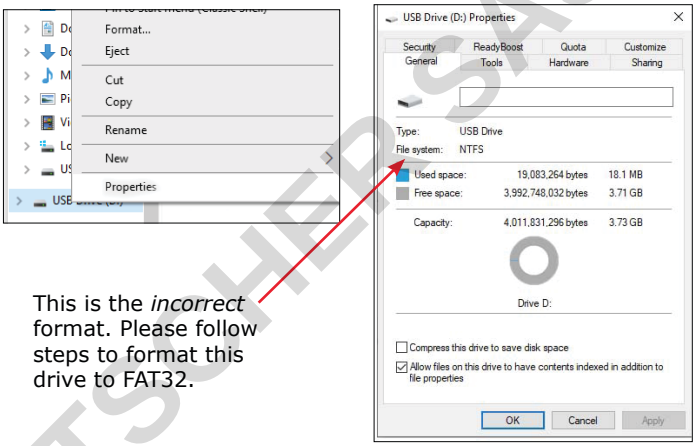
Materials required to link Scepter™ 3.0 to your Wi-Fi® network:

- Wireless Network broadcasting at 2.4 Ghz
- USB thumb drive formatted to FAT32 (FAT32 is a common filesystem format compatible with Windows, Mac® OS X, and Linux)
- PC, Mac® or UNIX® Computer with text editing program (Microsoft® Windows® Notepad, TexEd or similar)
- Scepter™ 3.0 Instrument

USB Thumb Drive Formatting

Scepter™ 3.0 is compatible with USB thumb drives formated to FAT32. No other format is compatible.

To check your USB thumb drive format, insert it into a USB port on your computer. From your computer, open File Explorer or similar, and locate the USB thumb drive. Right click on the USB thumb drive icon and select **Properties**.



This is the *incorrect* format. Please follow steps to format this drive to FAT32.

- If the File system is identified as "FAT32", no formatting is needed. Continue to *Creating a Wi-Fi® Configuration File.*

  NOTE: This view will also show how much space is available on your USB thumb drive.

- If the File system is identified as any other format, the following steps should be followed.

  NOTE: Formatting a USB thumb drive will erase all contents of the drive. Please back up any data you wish to retain prior to formatting.

  1. After identifying the existing format on the USB thumb drive, right click again. Select Format.
  2. The Formatting dialog window will open. Select FAT32. Select the Quick Format option and press enter.

     NOTE: Removing the USB thumb drive during formating may irreparably damage the USB thumb drive.
  3. Once formating has completed your computer should indicate Format Complete. Continue to *Creating a Wi-Fi® Configuration File.*

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TIP Having Trouble? See **Troubleshooting**

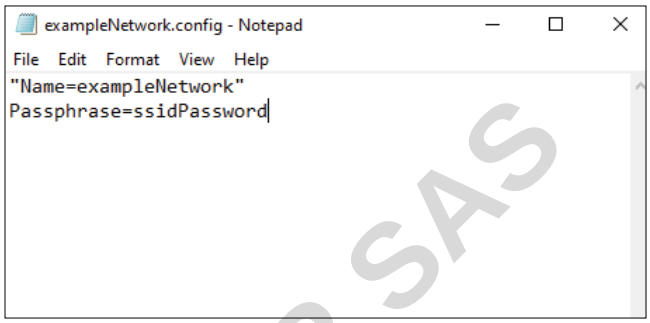
Creating a Wi-Fi® Configuration File

A "Wi-Fi® configuration file" is needed for set up or to change Wi-Fi® networks.

On a computer, open up the text editing program. The examples below are using Microsoft® Windows® Notepad.

1. Type following parameters with no spaces, as shown below: Replace *exampleNetwork* with the name of the desired wireless network. Replace *ssidPassword* with the password to the desired wireless network:

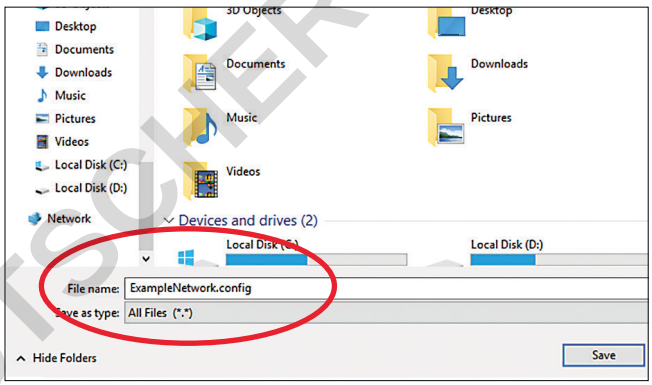
   Name=*exampleNetwork*
   Passphrase=*ssidPassword*
   Type=wifi
   Favorite=true
   AutoConnect=true



2. Save and name the file the same name of the desired wireless network with ".config" file extension.

   exampleNetwork.config

   Note: If you're using Notepad included with Microsoft® Windows® software, it will try to save the file as a .txt file by default. For this file to be saved properly, ensure it is being saved as All Files type like the example (right).

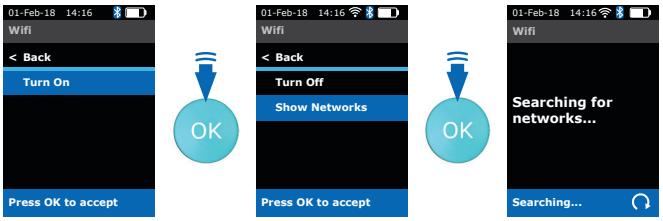


3. Drag the file from the computer onto the USB thumb drive. Once the file has loaded, remove the USB thumb drive from the computer port.

Connect to Wi-Fi® Network

1. From the Main Menu, select **Settings** and press the OK button. Select **Wi-Fi** and press the OK button. Insert the USB thumb drive containing the credential file into the Scepter™ 3.0 USB port.

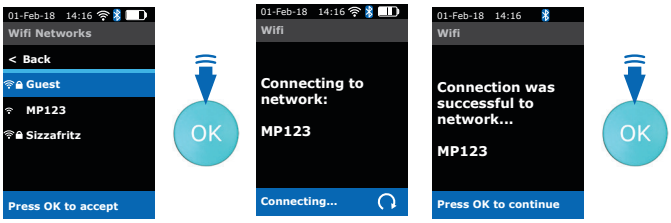
2. Scroll to **Turn On**, and press the OK button. Scroll to **Show Networks**, press the OK button. The instrument will begin *Searching for networks...*.



3. The instrument will list networks within range. Scroll to the desired network and press the OK button. When connection is complete, the screen will confirm successful connection.

4. Press the OK button to return to Settings Menu.

5. Remove USB thumb drive.

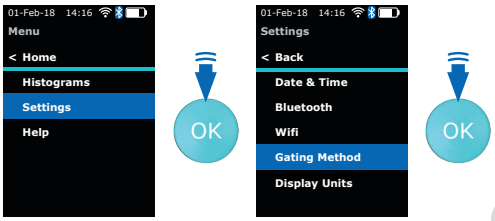


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TIP Having Trouble? See **Troubleshooting**

Gating Method

There are two options for setting the default gates (upper and lower diameter or volume limits of the histogram) prior to counting. One option is to choose "Use Last" and the other is "Auto" which automatically sets the gates based on the histogram profile. The gates can also be changed manually after the count has been performed. Manual gating is recommended when using the 40 µm sensor or when optimizing counting parameters for a new sample.



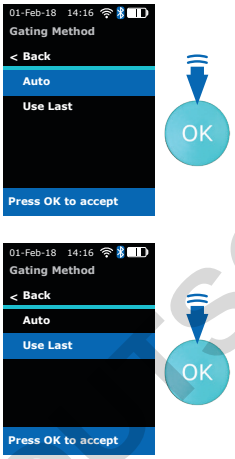
1. From the **Settings** menu, select **Gating Method** and press the OK button.
2. Scroll to select desired setting, and press the OK button.

Auto

This setting will set the gates automatically based on the histogram profile. Use this setting when sampling Scepter™ 3.0 Test Beads.

Use Last

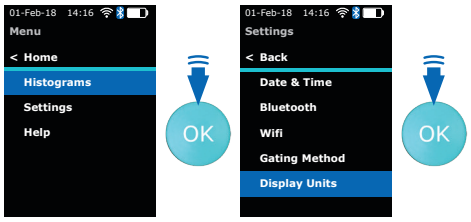
This setting maintains on whatever gating parameters were last set by the user, either during a count or during post count data management.



Display Units

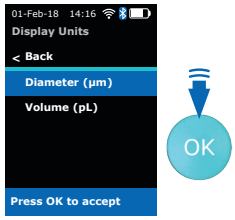
Set the histogram default display to either cell (or particle) volume in picoliters (pL) or in cell (or particle) diameter in microns (µm). Individual histograms can be switched in the **Histogram Explorer, p.13**.

Use the ARROW buttons to scroll through options.



1. Scroll to **Histograms**, press the OK button.
2. Select **Display Units** and press the OK button.
3. Scroll to choose **Diameter (µm)** or **Volume (pL).**

   Note: Scepter™ 3.0 shows your last choice first.
4. Press the OK button to return to the **Settings** menu.



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TIP Pressing the **Menu** button will end any procedure menu

Sensors

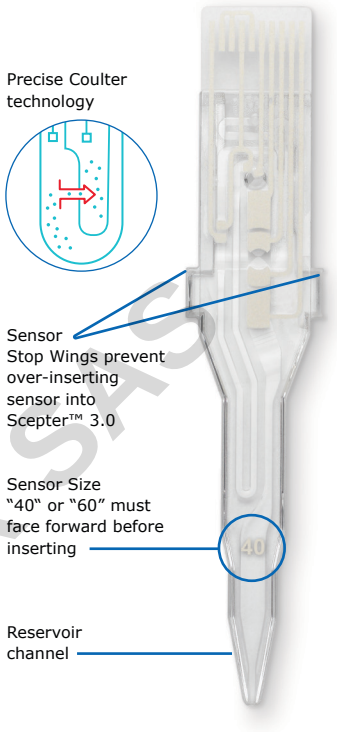
Technology

The Scepter™ 3.0 Sensors are microfluidic channels designed to replicate the Coulter Principle. Cell suspension is drawn into the reservoir channel and through the aperture sensing region. Increase in resistance causes an increase in voltage proportional to cell size (diameter). For each passing cell, voltage changes are recorded providing sample count and concentration. The sample is contained in the sensor and disposed of after each sample reading.

The Scepter™ 3.0 can be used with either a 60 µm or a 40 µm sensor. Depending on the sample being counted, some user optimization may be required relating to cell size and concentration.

Sensor size	Operating Range	Sensor X-Axis Scale (µm)	Working Diameter Range (µm)
40 µm	50,000–1,500,000 cells/mL	4-20	5-15
60 µm	10,000–500,000 cells/mL	6-36	8-25

Sensors are not reusable. Attempting to reuse the sensor will result in an error message on the instrument. However, an unused sensor may be removed from the Scepter™ 3.0 and reinserted. Only Scepter™ 3.0 Sensors will fit into the Scepter™ 3.0 instrument. Do not use sensor if visibly damaged.



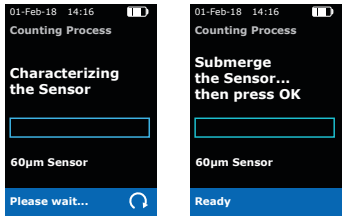
Attach the Sensor

1. When the Scepter™ 3.0 is powered on, it will show **Attach the Sensor to begin**.
2. Holding the sensor with **the sensor size number facing you, and the Scepter™ 3.0 with the screen facing you**, firmly insert the sensor into the sensor port. The sensor stop wings prevent over insertion.

   IMPORTANT: To avoid severe instrument damage and costly repair, orient the sensor correctly before inserting it into the attachment port. **Extreme force is not required** to insert the sensor. Check the orientation of the sensor if having difficulty.



3. When the sensor is properly inserted, Scepter™ 3.0 will begin *Characterizing the Sensor*.
4. The screen will indicate *Submerge the Sensor...then press OK*. You are ready to begin **Cell Counting.**



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TIP Pressing the **Arrow** buttons to scroll through options

Cell Counting

- Prepare dilutions in 1.5 mL microcentrifuge tubes. Other tubes may not be able to accommodate the width of the sensor, or provide sufficient sample depth for the instrument to function properly.
- Use a diluting solution compatible with the characteristics of cells. The diluting solution used should not cause changes in the cell size and should have sufficient conductivity to enable operation of the instrument. Recommended diluting solutions include:
    - Dulbecco's Phosphate buffered saline, D-PBS, with or without calcium and magnesium (Embryomax® D-PBS Cat. No. BSS-1006)
    - 10% Fetal Bovine Serum, FBS (Embryomax® serum Cat. No. ES-009-B) in D-PBS
    - Dulbecco's Modified Eagle's Medium, DMEM (Embryomax® solution DMEM Cat. No. SLM-220B)
    - Complete media (10% FBS in DMEM), and diluted in D-PBS
    - 1% dimethyl sulfoxide (DMSO) cell freezing media (Hybri-Max™ DMSO Cat. No. D2650) in D-PBS
    - Cell detachment solution (Accutase® Cat. No. SCR005)
- **Water, hypotonic, or hypertonic solutions are not acceptable diluting solutions.**

  Note: Detergents may interfere with counting; 10% DMSO is not an acceptable diluting solution, but 1% DMSO may be used. Serum-enriched media >10% may also interfere with counting. Dilute samples with D-PBS, diluted serum in D-PBS (≤10% FBS), DMEM basal medium, DMEM-based complete medium with ≤10% FBS (undilute or diluted in D-PBS), and Accutase® cell detachment solution.
- Some suspensions may require a pre-filtration step to remove larger than rated cell clumps, particle populations, or debris that would clog the sensor's aperture. A Steriflip® filter with 20 µm nylon net is recommend (use low vacuum ≤5"Hg).

Cell, Bead, and Particle Suspensions

Materials Required

- ◇ Cell sample, Scepter™ 3.0 Test Beads (Cat. No. PHCC3BEADS), or particle suspensions

  Note: Some particle suspensions may have a broad diameter range. Debris and/or particles near or over sensor rating may clog aperture resulting in an **Aperture block error, see p. 22**.
- ◇ 1.5 mL microcentrifuge tubes
- ◇ Scepter™ 3.0 Sensors

- The sample volume must be at least 100 µL to draw into the sensor's microfluidic channels.
- Perform **Installing the Sensor** steps prior to proceeding.

In a 1.5 mL microcentrifuge tube, dilute the single-cell suspension with an appropriate diluting solution so the cell concentration is within the operating range of the instrument for the sensor being used. The appropriate dilution will depend on cell type, seeding density, and suspension volume. The volume required for an accurate count is 100 µL.

Sensor size	Operating Range	Sensor X-Axis Scale (µm)	Working Diameter Range (µm)
40 µm	50,000–1,500,000 cells/mL	4-20	5-15
60 µm	10,000–500,000 cells/mL	6-36	8-25

Scepter™ 3.0 Test Beads

To ensure proper system operation, the Scepter™ 3.0 can be tested periodically with Scepter™ 3.0 Test Beads. The beads can be used to test the system when first received, as well as for practice and troubleshooting. Refer to the Scepter™ 3.0 Test Bead container label for expected bead concentration and diameter. The procedure for testing with Scepter™ 3.0 Test Beads and counting cells is the same, except that the beads are ready to use and require no dilution. The Scepter™ 3.0 Test Beads are compatible with both 40 and 60 µm sensors.

- Allow Scepter™ 3.0 Test Beads to come to room temperature before use.
- Mix Scepter™ 3.0 Test Beads gently by shaking bead vial for 30 seconds. If using vortex, mix at low speed. Excessive mixing can lead to inaccurate counts.

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TIP Pressing the **Menu** button will end any procedure menu

Counting Process

Perform **Attach the Sensor, p.11** before continuing with this section.

1. Immediately after mixing, submerge the tip of the sensor into the middle of the sample tube solution and press the OK button to begin counting. Resting the sensor against the tube bottom may result in inaccurate results. It is normal to hear the pump clicking.

   Note: It is important to press the OK button only after the sensor is submerged, and keep it submerged as long as the screen displays *Keep Sensor submerged*. Do not to rest your thumb on the EJECT button during sampling, as it can cause inaccurate results.



2. Scepter™ 3.0 will beep when sample loading is completed and the sensor can be lifted out of the solution. Pause briefly, while Scepter™ 3.0 creates the histogram. Press the EJECT button to eject the sensor into an appropriate cast off container when directed to do so on the screen. Discard used sensors appropriately. Scepter™ 3.0 Sensors are not reuseable.
3. When the count is complete, the histogram is automatically created. The concentration and diameter or volume will be displayed on the screen. Press the OK button to save (up to 999 histograms in the Scepter™ 3.0 memory), and open the **Histogram Explorer.** Press the OK button to return to Home screen.

   Changing the histogram gating method and other features are done in **Histogram Explorer**.

   Note: Files are automatically named: *ScepterID.Sample#.Date_time.png (or .csv)*.

   Note: When using Scepter™ 3.0 Test Beads, set gating to Auto for best results.

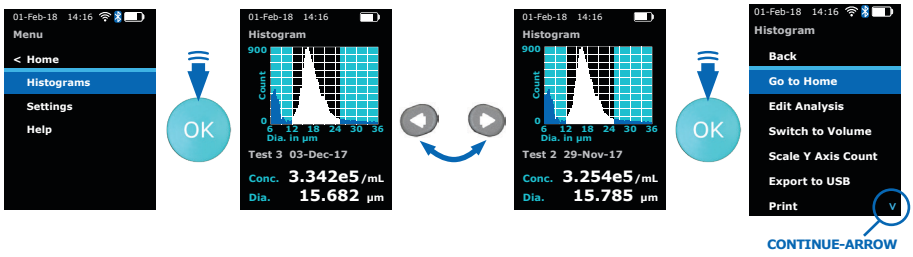
Histogram Explorer

Up to 999 histograms can be saved for future review. When the display screen shows Disk Full, the acquired data can no longer be saved. To continue, histograms must either be deleted or uploaded to a computer.

Note: The counting procedure can still be performed when the disc is full, but histograms and data will not be saved. To delete histograms, refer to **Deleting Saved Data, p.16**.

Histogram Explorer can be found two ways:

From the Main Menu, select Histograms, and the last saved or acquired histogram will be shown. Scroll to see the next histogram. Press the OK button to select.

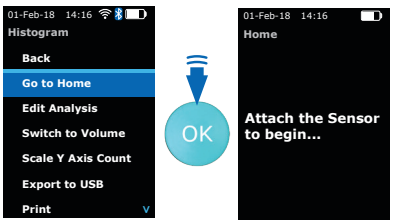


After Counting Process, the histogram will be displayed.

Press the OK button to select. The Histogram Explorer Menu will appear. The blue "continue-arrow" is in the lower right corner, indicating additional option(s) can be viewed by scrolling down.

Go to Home

Prompts to start new sample count.

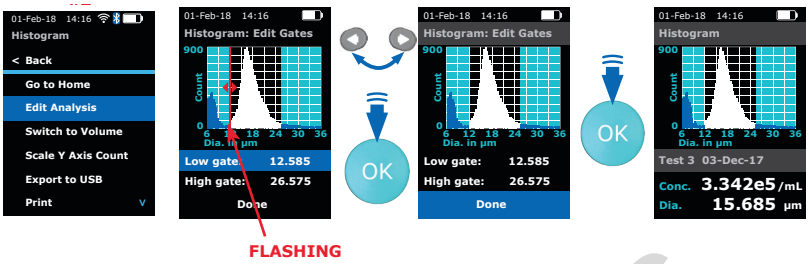


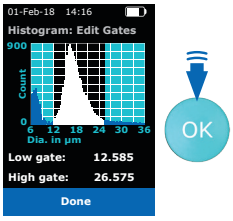
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TIP The continue-arrow in the lower right screen indicates more menu options

Edit Analysis

1. Select **Histogram** from the main menu and scroll to **Edit Analysis**, press OK. **Low gate** will be highlighted and a flashing red line flanked by red arrows shows on the histogram. Press the OK button.
2. Increase or decrease the Low gate number using the ARROW buttons. Press the OK button to select. The low gate will flash red on the histogram again.
3. Scroll to advance to **High Gate**, press the OK button.
4. Adjust the **High Gate** similarly by using the ARROW buttons, press the OK button.
5. Scroll down to **Done** and press the OK button to return to the histogram.

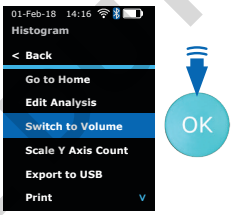




Switch to Volume / Diameter

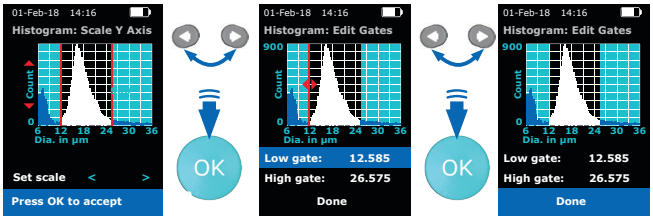
This option switches the view between volume and diameter (X-Axis) for the current histogram only. Scroll to **Switch to _____.**

From here, Scepter™ 3.0 automatically takes you to **Edit Analysis, p.14** to adjust the gating.



Scale Y-Axis Count

1. Scroll to **Scale Y-Axis Count**, press the OK button.
2. The count will appear as a red line flanked by red arrows on the histogram. Adjust by pressing the ARROW buttons. Press the OK button.



When using the 40 µm sensor, the Y-Axis may need to be scaled and the gates may need to be adjusted manually to see the desired peak.

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TIP Pressing the **Arrow** buttons to scroll through options

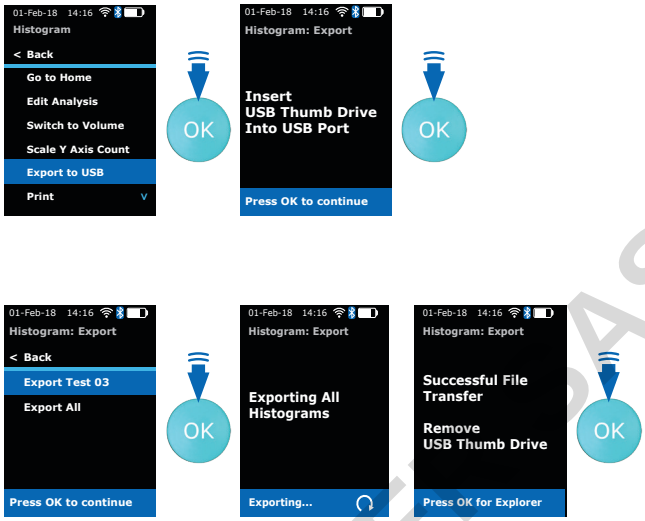
Exporting Data

Export to USB thumb drive

1. Select **Histogram** from the main menu and scroll to **Export to USB**, press the OK button.
2. Insert USB thumb drive into Scepter™ 3.0 USB port when prompted. Drive must be formated to FAT32. Press the OK button.
3. Select histogram(s):

   TO EXPORT ONE, Scroll to the desired histogram and press the OK button.

   TO EXPORT ALL, Scroll to **Export All** and press the OK button.
4. *Exporting (All) Histograms* will be displayed on the screen. Data will export as an image (PNG) and data (CSV).



Export is complete when *Successful File Transfer Remove USB thumb drive* shows on screen. Removing the USB thumb drive early may result in corrupted data. It is safe to remove the USB thumb drive now.

5. Press the OK button to return to the main Histogram Explorer screen.

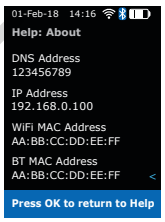
Export to computer over Wi-Fi® Network

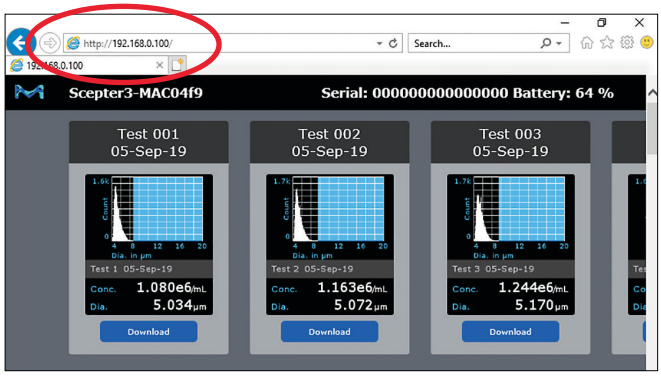
The Scepter™ 3.0 hosts its own web page allowing users to download test data wirelessly.

Materials Required

- Wireless Network broadcasting at 2.4 Ghz.
- PC, Mac® or UNIX® Computer ***connected to the same Wi-Fi® Network as Scepter™ 3.0***.
- Scepter™ 3.0 Instrument. Follow **Wi-Fi® Network, p.8** directions first.

1. Obtain Scepter™ 3.0 IP address. (See **Help Screens, p.17**.)
2. Confirm Scepter™ 3.0 is connected to the same Wi-Fi® network as the computer. (Follow **Wi-Fi® Network, p.8** again if needed.)
3. From the computer, open the internet browser. Type the Scepter™ 3.0 IP address in the address bar (see red circle on image right). Press ENTER. The current data on Scepter™ 3.0 will be displayed.





4. Scroll to the desired histogram, click Download. Data will download as an image (PNG) and data (CSV). The CSV file contains sample histogram data and summary results that is best opened through a spreadsheet program, such as Microsoft® Excel, OpenOffice® Calc, or Google Docs™ web-based word-processing program. Log files or other device-maintenance formats may also be downloaded. These files are used by Tech Service if troubleshooting is needed.
5. To disconnect the communication, close the browser on the computer or power off the Scepter™ 3.0.

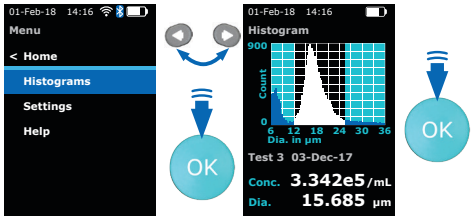
Note: Scepter™ 3.0 must remain powered on during this process, and can be actively in-use. Data can be downloaded from Scepter™ 3.0 multiple times, as long as the histogram remains in Scepter™ 3.0 memory. Scepter™ 3.0 can store a maximum of 999 histograms at one time. Files can only be deleted from Scepter™ menus, not from the web, see **Deleting Saved Data**.

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Reviewing Saved Data

Although Scepter™ 3.0 has the capacity to hold 999 histograms, we recommend exporting data for easier file maintenance. See **Exporting Data, p.15**.

Select Histograms, press the OK button. The Histogram that was last edited or acquired will be displayed. Using the ARROW keys, scroll to the desired histogram. When the desired histogram is on screen, press the OK button to go to the Histogram Explorer.

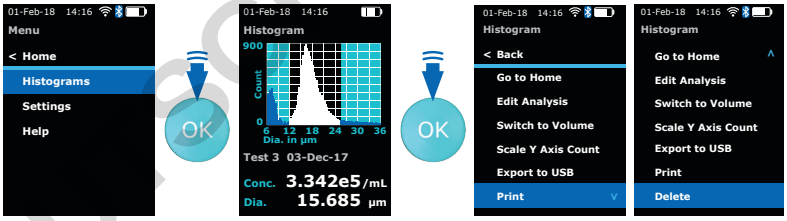


Deleting Saved Data

After exporting data, it is recommended to delete it from Scepter™ 3.0. Please confirm export was successful before deleting. ***Once a file is deleted from Scepter™ 3.0, it cannot be recovered.***

1. Select Histograms, press the OK button. The last histogram that was created or viewed is shown.
2. **TO DELETE ONE HISTOGRAM:** Scroll to the desired histogram, press the OK button to open the Histogram Explorer menu.

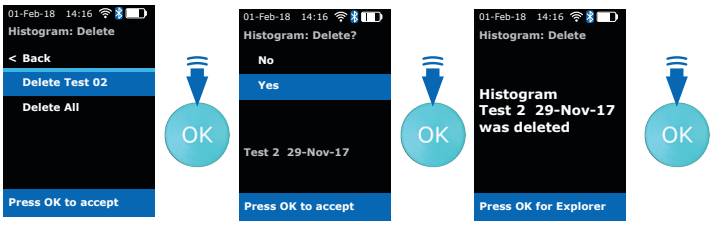
   TO DELETE ALL HISTOGRAMS: Press the OK button from any histogram on screen to open the Histogram Explorer menu.



3. The Delete option is on the second screen of the Histogram Explorer menu. Scroll past **Print** to advance to the next screen. Select **Delete**, press the OK button.

   Note: A blue arrow in the lower right corner of any screen indicates there are more options to that menu.

4. Scroll to select *Delete Test _name_* OR *Delete All*, press the OK button.
5. Scroll to Yes, and press OK to proceed with deleting.



If you do not wish to delete histograms, scroll to **No** and press the OK button. The system will return to **Menu**.

Note: Once the Delete process is initiated, the histograms cannot be recovered.

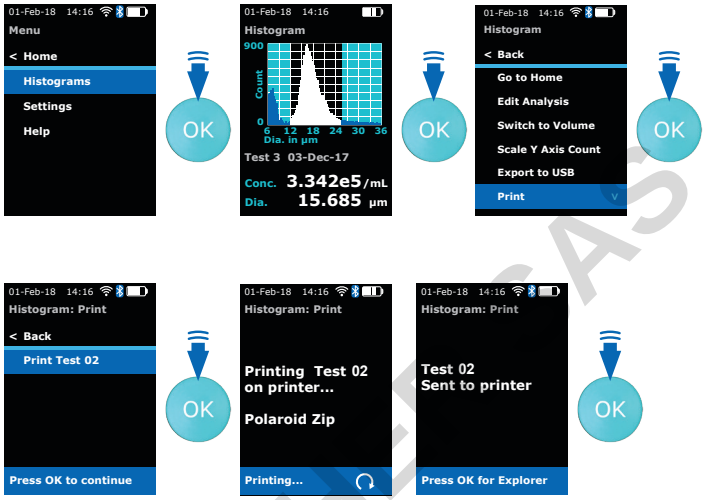
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TIP The continue-arrow in the lower right screen indicates more menu options ∨

Printing

Before printing, the Scepter™ 3.0 must be paired to a printer with Bluetooth® capablities (see **Bluetooth® Enabled Printer, p.7**). Confirm the printer is ON and within signal range.

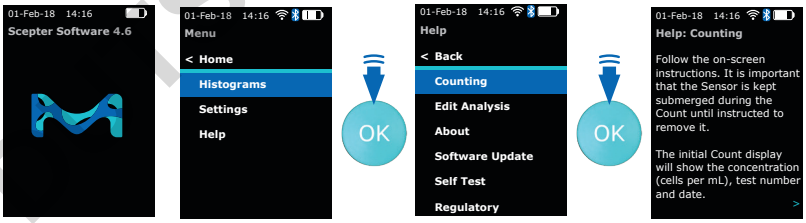
1. From the Main Menu, scroll to Histograms, and press the OK button.
2. Once a histogram is displayed, press the OK button to open the Histogram Explorer.
3. Scroll to Print then press the OK button to advance to **Histogram: Print** screen. Scepter™ 3.0 will display the histogram name, press the OK button to confirm print.
4. The name of the file will be displayed on the screen while printing. After **Successfully Printed** is displayed, press the OK button to return to Histogram Explorer.



Help Screens

Tutorials

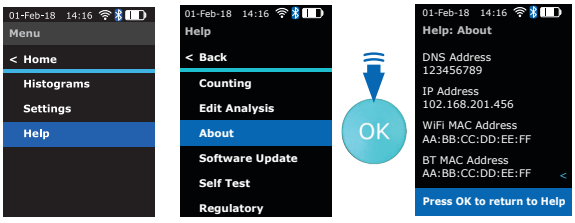
1. From the Menu screen, scroll to **Help**, press the OK button.
2. Scroll to the topic of interest, press the OK button.
3. A small blue arrow in the lower right corner of any screen indicates there is more information or options, use ARROW keys to scroll down to next screen.



DNS, IP, Mac® Address

This information will be needed to upload data from Scepter™ 3.0 to your computer.

Under the Help options, scroll to **About**, press the OK button. This will bring up the Wireless information screen.



Software Updates

Scepter™ 3.0 software may be updated periodically. For information on the most up-to-date software, go to SigmaAldrich.com/Scepter. Register your instrument in order to receive notification about relevant software updates.

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TIP Pressing the **Menu** button will end any procedure menu

Storage and Maintenance

Storage

- Store the Scepter™ 3.0 Cell Counter and Scepter™ 3.0 Sensors in a clean, dry environment at 15-30 °C.
- Do not expose the instrument, sensors or charger to UV light.
- Store Scepter™ 3.0 Test Beads in refrigerator after opening. Do not freeze.

Cleaning/Sanitizing

- The Scepter™ 3.0 is **NOT** autoclavable. Extreme heat will damage the display screen and other electronic components.
- Keep the Service Test and USB port covers closed so no liquid enters any part of the instrument during cleaning.
- The Scepter™ 3.0 body and instrument control buttons can be sanitized by wiping with a soft cloth moistened with 70% ethanol, ≤10% bleach solution, phenol-based solution (Sporicidin® Disinfectant), pre-saturated wipes with bleach or alkyl with detergents (Hype-Wipe® disinfecting towels with bleach, Lysol® and Clorox® wipes). Do not clean the display screen with sanitizing agents or other aggressive solutions. Wipe the screen with a soft, dry, non-abrasive cloth.
  CAUTION: When sanitizing, make sure that no liquid enters any part of the instrument.
- The Scepter™ 3.0 Sensors are **NOT** reuseable.

Maintenance

Instrument repairs must be carried out by authorized personnel only. Contact tech service at SigmaAldrich.com/techservice.

Self Test

Conduct **Self Test** regularly to ensure Scepter™ 3.0 functionality and pump conditions are working properly.

Process

1. From the Menu screen scroll to Help, press the OK button.
2. Scroll to Self Test press the OK button.
3. Press the OK button.
4. Insert unused sensor into Scepter™ 3.0.
5. Block sensor tip (reservoir channel) with fingertip throughout Self Test (approximately 1 minute).
6. Press the OK button to start the sequence of tests or press Menu to Exit.



Ambient Test starts.

If Passed, the next test begins automatically (not pictured).

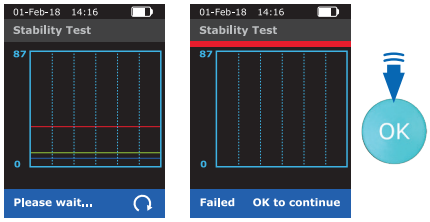
If Failed, press the OK button to start next test.

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Stability Test starts.

If Passed, the next test begins automatically (not pictured).

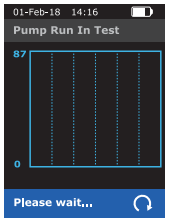
If Failed, press the OK button to start next test.



Pump Run In Test starts.

(Listen for the pump noise at this step.)

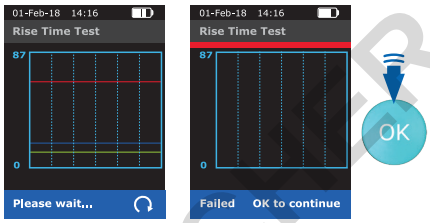
After pump cycle has stopped, the next test begins automatically.



Rise Time Test starts.

If Passed, the next test begins automatically (not pictured).

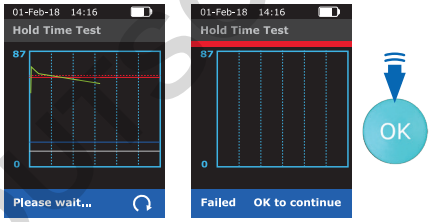
If Failed, press the OK button to start next test.



Hold Time Test starts.

If Passed, the next test begins automatically (not pictured).

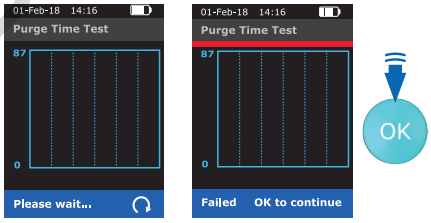
If Failed, press the OK button to start next test.



Purge Time Test starts.

If Passed, the next test begins automatically (not pictured).

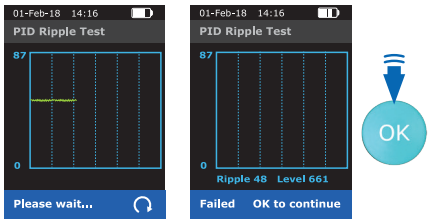
If Failed, press the OK button to start next test.



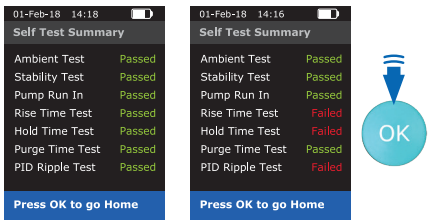
PID Ripple Test starts.

If Passed, the next test begins automatically (not pictured).

If Failed, press the OK button to start next test.



7. Remove finger once Passed or Failed **Self Test** Summary results are displayed. Press the OK button or eject sensor to return to **Home** for file error file downloads (e.g. LOG, S3R files) and all other functions.



Results

- If all tests have Passed results, instrument is functioning properly. Start sample test with currently inserted sensor by pressing the OK button, or Eject the current sensor and start from **Home** with new sensor.
- If any test has a Failed result, retry with another unused sensor. If failure(s) still detected, contact Tech Service at SigmaAldrich.com/techservice.

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Troubleshooting

Symptom	General Cause	Corrective Action
Questionable concentration	Sensor not fully immersed in solution while sample is loading	Keep sensor fully immersed while screen displays **Keep Sensor Submerged**.
	Concentration of cell sample is too high or too low	Make sure concentration of cell sample is within recommended operating range. See **Cell, Bead, and Particle Suspensions.**
		Test sample using Scepter™ 3.0 Test Beads (Cat. No. PHCC3BEADS) to ensure unit operating properly for concentration.
	Wrong diluting solution	Use a diluting solution that is compatible with cells being counted.
	Cell clumping	Ensure that cells are in a single-cell suspension. Break clumps by pipetting up and down with a standard pipettor or Pre-filter using Steriflip® filter unit with 20 µm nylon net.
Questionable cell diameter	Diameter of cell sample is too high or too low	Perform **Cell Counting, p.12** procedure using the Scepter™ 3.0 Test Beads to ensure it is operating properly for size.
		Review histogram, Y-axis scale, and or reset gates manually if possible. Refer to **Gating Method, p.10** for instructions on adjusting the gates manually.
	Wrong diluting solution	Use a diluting solution that is compatible with cells being counted. See **Cell, Bead, and Particle Suspensions, p.12.**
	Cell clumping	Ensure that cells are in a single-cell suspension. Break clumps by pipetting up and down with a standard pipettor or pre-filter using Steriflip® filter unit with 20 µm nylon net.
Failure to advance to next display screen	Sensor is not inserted correctly	Make sure the sensor size number and laminated circuit are facing the front of the instrument.
Peak of interest indistinct	Y-Axis not optimized for peak of interest	Refer to **Scale Y-Axis Count, p.14** for instructions on adjusting the Y-Axis.
	Cell clumping	Sample has high level of debris or death.
		Incorrect diluting solution.
Peak of interest not selected by gates	Use of auto-gating feature	After counting, reset gates manually. Refer to **Gating Method, p.10** for instructions on adjusting the gates manually.
Sensor does not fit into sensor port	Incorrect sensor orientation or Sensor 2.0 stock.	Make sure the sensor size number and laminated circuit are facing the front of the instrument. See **Sensors, p.11.**
		Only Scepter™ 3.0 Sensors can be used.
Aperture block	Wrong diluent, clogging, air bubble, debris	Refer to list of acceptable diluents in the **Cell, Bead, and Particle Suspensions, p.12** and more detail in Error Messages: **Aperture block, p.22**.
Test timeout	Sample may be clumpy	Re-insert sensor. Try new sensor or pre-filter using Steriflip® filter unit with 20 µm nylon net. If problem persists, contact Tech Service.
	Sensor issue	
No response on display when pressing control buttons	Internal software issue	Power off by pressing menu botton 3-5 seconds, until it shuts down. Restart after 10 seconds, retry previous action.
Unable to reach website using the Scepter™ 3.0 IP address	Not on the same network or network firewall setting blocking	Ensure the computer is on the same network as the Scepter™ 3.0. If problem persists, check with your network administrator to ensure LAN to LAN connections are not blocked.

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Error Messages

Message	General Cause	Corrective Action
Battery needs recharging! Please recharge the instrument! Battery is critically low! System is powering down.	Low battery/no battery	Battery needs to be re-charged. Place Scepter™ 3.0 on charging stand to recharge the instrument. See **Battery, p.5**.
	Battery is not charging	Ensure that the unit is correctly engaged in charging stand. Try horizontal mounting of charge stand if vertical is not charging. An animated ascending power bar should be looping if connected properly. Ensure the charging station is plugged into a working AC wall outlet. • If instrument is on or asleep, display will flash when on charging stand and beep. • If instrument is fully off, it will recharge but will not display on screen until turned on.
		If battery is completely drained: • Place the Scepter™ 3.0 into the Charging Station for 15 min. • While on the station, press and hold the MENU button for 5-10 seconds, until the vibrant M appears. • Release the MENU button and allow Scepter™ 3.0 to remain in the charger for approximately 6 hours or until fully charged. If no vibrant M appears, contact Tech Service.
Memory Almost Full Delete Histograms or Export to USB thumb drive	Nearly 999 histograms have been saved	Move saved histograms to USB thumb drive or upload to website. Delete historgrams once the transfer has been confirmed. Deleted histograms cannot be restored.
Memory Full	Instrument has exceeded maximum storage capacity of 999 saved histograms	
Unhandled exception	Internal software issue due to the instrument malfunction or high level of external interference	Internal software issue due to the instrument malfunction or high level of external interference.
Lost Sensor	Sensor was not properly inserted Sensor removed or ejected prematurely	Follow steps in **Sensors.**
Histogram data not displayed on screen	Sensor tip still installed in unit	Eject sensor to continue.
Please eject the Sensor to continue	Used sensor is in Scepter™ 3.0	Eject sensor to continue.
Warning High concentration	Concentration of sample is too high	Dilute sample so that it is no higher than recommended maximum operating range guideline. See **Specifications, p.24**.
Lost Start	Sample volume too small, sensor not fully immersed in solution while sample is loading, air bubble in sensor, or sensor ejected.	Make sure sample volume is ≥ 100 µL. Keep sensor fully immersed while screen displays. Keep the Sensor Submerged. Do not press the Eject button, OK or MENU buttons during test.
False start False stop	Sensor malfunction	Replace with unused sensor.
Start/Stop short	Previously used sensor	Sensors are not reuseable, replace with unused sensor.
Electrode short	Sensor malfunction	Replace sensor.

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Message	General Cause	Corrective Action
Aperture block	Sample population size exceeds aperture size	Dilute to reduce incidences, but may not eliminate. Use D-PBS for diluting solution. See **Cell, Bead, and Particle Suspensions, p.12.**
	Viscous sample (e.g. serum-enriched media)	Keep sensor submerged in sample until Count Complete appears on the display. Use D-PBS for diluting sample.
	Cell Counter sensor is blocked	Sample concentration is too high; dilute cell sample more. Ensure that the cells are in a single-cell suspension. Break clumps by pipetting up and down with a standard pipettor or pre-filter using Steriflip® filter unit with 20 µm nylon net.
	Air bubble in sensor due to: Pre-mature pressing of OK button	Fully submerge sensor in sample before pressing OK button.
	Air bubble in sample	Mix sample gently to avoid air bubbles.
	Sensor lifted from sample before screen displays **Sample Loaded**	Keep sensor submerged in sample while screen displays Keep the Sensor Submerged.
	Wrong diluting solution	Refer to list of acceptable diluting solutions in **Cell, Bead, and Particle Suspensions, p.12**.
	Debris from diluting solution	Diluents can be filtered to remove debris using ≥0.2 µm using Stericup®, Steritop® or Steriflip® filters. See **Product Ordering** for catalogue numbers.
Open Start	Issues detected upon sensor insertion	Re-insert sensor. Try new sensor; if problem persists, contact Tech Service.
Open stop	Previously used sensor detected	Re-insert sensor. Try new sensor; if problem persists, contact Tech Service.
System stopped	Error occurred or Menu bottom pressed during counting to abort test	Replace sensor and retest.
No histograms currently stored	No stored data	The instrument is new or all histograms have been deleted.
Unsuccessful file transfer Some histograms failed to export	Low battery	Charge battery and re-try file transfer.
Can not mount USB thumb drive	or USB thumb drive not inserted or USB thumb drive not fully inserted or USB thumb drive is malfunctioning or full or USB thumb drive is not formatted to FAT32	Push USB thumb drive into the Scepter™ 3.0 USB port firmly. Check the USB thumb drive to ensure it is formatted FAT32, has sufficient space available and is functioning properly. See **USB Thumb Drive Formatting.**
Can not find file or credentials are incorrect	Does not connect to Wi-Fi® network	Check or insert the "credential file" on USB thumb drive. See **Creating a Wi-Fi® Configuration File, p.9.**
Network scan timeout	No 2.4 GHz network(s) within range	Go within range of a 2.4 GHz wireless network and try again.
Cannot find file (networkname.config)	No USB thumb drive inserted or USB thumb drive is not properly formatted, or proper config file not present on drive	Ensure proper config file is located on FAT32 formatted USB thumb drive and try again. See **Wi-Fi® Network, p.8** to create a config file.

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Message	General Cause	Corrective Action
Invalid Config (networkname.config line #)	Config file not correct	See **Creating a Wi-Fi® Configuration File, p.9.**
Network scan timeout	No 2.4 GHz network(s) within range	Go within range of a 2.4 GHz wireless network and try again.
WIFI Config (networkname. config) missing paraphrase	Config file not correct	Recreate config file. See **Creating a Wi-Fi® Configuration File, p.9.**
Wrong or missing update file on USB thumb drive	No USB thumb drive inserted, drive is not properly formatted, or corrupt update file on drive	Ensure proper update file is located on FAT32 formatted USB thumb drive and try again. See **USB Thumb Drive Formatting, p.8.**
Software update failed	Corrupt update file on drive	Ensure proper update file is located on FAT32 formatted USB thumb drive and try again. See **USB Thumb Drive Formatting, p.8.**
Software update blocked on battery low	Insufficient battery to perform update	Recharge Scepter™ 3.0 and try again.
File(s) did not print	Printer malfunction or not connecting to printer or Low Battery	Confirm printer is on, and there is no paper jam. See **Bluetooth® Enabled Printer, p.7** to establish new printer connection. Confirm WiFi® signal is on, WiFi® icon should be visible on screen. See **Wi-Fi® Network, p.8** to establish a new network connection. Recharge Scepter™ 3.0 and try again.
Connection was unsuccessful		
Printer not ready		
Printer spooling unsuccessful		
Printer Scan timeout		
Printing unsuccessful... Make sure printer is on and within range		
Self Test failed results	Scepter™ 3.0 malfunction	Replace sensor and re-run the Self Test. If failed results continue, contact Tech Service.
	Low battery	Ensure battery is charged to ≥ 20%.
Rise, Hold and/or Ripple failed result tests	Vacuum issue	Replace sensor and re-run the Self Test. Ensure sensor tip is fully covered throughout the entire test. If failed results continue, contact Tech Service.
	Low battery	Ensure battery is charged to ≥ 20%.

Notice

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Technical Assistance

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Standard Warranty

The applicable warranty for the products listed in this publication may be found at SigmaAldrich.com/terms.

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Specifications

Scepter™ 3.0 Instrument Body

Body Material	Acrylonitrile butadiene styrene (ABS) body, polycarbonate digital screen, silicone control buttons
Height	28.321 cm (11.15 in.)
Width	6.350 cm (2.5 in.)
Depth	10.922 cm (4.3 in.)
Weight (approximate)	229.5 g (0.5 lb)

Wireless Connection Specifications

Wireless (Wi-Fi®, WLAN) Network	IEEE 802.11 b/g/n, 1MByte/s, up to 25m line-of-sight 2.4 Ghz
Bluetooth® Network	IEEE 802.15.1, support FTP, OPP and Bluetooth® 2.4 Ghz Low Energy features, 8m line-of-sight

Electrical Specifications

Handheld Instrument	Internal rechargeable 3.7 V⎓ lithium ion battery
Charging Station	Input and Output 5 or 6 V⎓*, 1 A
External Power Supply	Input 100–240 V~ (50/60 Hz), 0.2 A; Output 5 or 6 V⎓*, 1 A
	* Kits may include a 5 or 6 V⎓ power supply. The difference in V⎓ will not affect Scepter™ 3.0 performance.

Operating Environmental Conditions

Temperature	15 °C to 25 °C
Maximum Relative Humidity	20–80% (non-condensing)

Storage Conditions

Temperature	15 °C to 30 °C
Maximum Relative Humidity	20–80% (non-condensing)

Scepter™ 3.0 Sensor

Material	Polycarbonate with Mylar® brand polyester film
Height	8.6 cm (3.4 in.)
Width	1.6 cm (0.6 in.)
Weight	2.3 g (0.1 oz)
Aspiration Volume	50 µL
Sensor Orifice/Aperture	40 µm or 60 µm
Sensor Scale 40 µm	4–20 µm
Sensor Scale 60 µm	6–36 µm

Sensor Performance	**40 µm sensor**	**60 µm sensor**
Sample Volume Required	≥ 100 µL	≥ 100 µL
Operating Range		
Cell Size	5 µm–15 µm	8 µm–25 µm
Cell Concentration	50,000–1,500,000 cells/mL	10,000–500,000 cells/mL
Processing Time	< 40 seconds	< 30 seconds

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Product Ordering

Purchase products online at SigmaAldrich.com.

Product Description	Qty.	Catalogue No.
Scepter™ 3.0 Handheld Automated Cell Counter Kit		
Includes Scepter™ 3.0 Handheld Automated Cell Counter, 50/pk of Scepter™ 3.0 Sensors (40 µm or 60 µm), Scepter™ 3.0 Test Beads, Charging Station and Mounting Kit, Quick Start Guide, and Safety information.		
with 40 µm sensors	1	PHCC340KIT
with 60 µm sensors	1	PHCC360KIT
Scepter™ 3.0 Handheld Automated Cell Counter Only	1	PHCC30000
Scepter™ 3.0 Sensors only, 40 µm	50 250	PHCC340050 PHCC340250
Scepter™ 3.0 Sensors only, 60 µm	50 250	PHCC360050 PHCC360250
Scepter™ 3.0 Test Beads only, 5 mL	1	PHCC3BEADS
Scepter™ 3.0 Charging Station only	1	PHCC3CHARG
Scepter™ 3.0 Charging Station Mounting Kit only	1	PHCC3WKIT

Related Products

Description	Membrane	Pore Size (µm)	Media Bottle Capacity (mL)	Thread Size (mm)	Qty.	Catalogue No.
Stericup® E-GP Sterile Vacuum Filtration System	Millipore Express® PLUS PES	0.22	500	38	12	SEGPU0538
		0.22	500	45	12	SEGPU0545
		0.22	1000	38	12	SEGPU1138
		0.22	1000	45	12	SEGPU1145
Steritop® E-GP Sterile Funnel-less Vacuum Filtration System	Millipore Express® PLUS PES	0.22	--	38	12	SEGPT0038
		0.22	--	45	12	SEGPT0045

Description	Membrane	Pore Size (µm)	Funnel Capacity (mL)	Reciever Bottle (mL)	Qty.	Catalogue No.
Stericup® E Quick Release Filter	Millipore Express® PLUS PES	0.22	150	150	12	S2GPU01RE
		0.22	250	250	12	S2GPU02RE
		0.22	500	500	12	S2GPU05RE
		0.22	500	1000	12	S2GPU10RE
		0.22	1000	1000	12	S2GPU11RE
Steriflip® Filter (sterile)	Millipore Express® PLUS PES	0.22	fits 50 mL tube	50 mL tube	50	SCGP00525
	nylon net, 20.0 µm				25	SCNY00020
Steriflip® Funnel Attachment (non-sterile)	--	--	50 mL, fits Steriflip® filter	--	25	SC50FL025
Embryomax® D-PBS (Dulbecco's Phosphate buffered saline) with or without calcium and magnesium					1 L	BSS-1006
Embryomax® Fetal Bovine Serum					500 mL	ES-009-B
Embryomax® DMEM (Dulbecco's Modified Eagle's Medium)					500 mL	SLM-220B
Hybri-Max™ DMSO cell freezing media					5 x 5 mL 5 x 10 mL 100 mL	D2650-5x5ML D2650-1x10ML D2650-100ML

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注意!

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