



## User Guide

### *Count Cells Without Slides*

Version: 31-MAY-2023

<b>1. Introduction</b>	<b>4</b>	
Product Technology .....		4
Key Features .....		4
Optics .....		4
<b>2. Set-up and Safety</b>	<b>5</b>	
<b>3. Instrument Diagram</b>	<b>7</b>	
<b>4. Quick Guide</b>	<b>8</b>	
Cell Count Procedure .....		8
Best Practices .....		9
<b>5. General Operation and Software Information</b>	<b>10</b>	
Top Action Bar .....		10
Software Icons and Buttons .....		11
Chamber Height Options .....		11
Data Storage and Memory Warnings .....		11
Channels .....		12
Count Apps .....		12
Brightfield .....		12
Trypan Blue .....		12
Primary Cell Trypan Blue .....		12
AO .....		12
PI .....		13
AO/PI .....		13
Primary Cell AO/PI .....		13
Nuclei AO/PI .....		13
Nuclei Trypan Blue .....		14
GFP .....		14
Yeast .....		14
Custom Methods .....		14
<b>6. Count Tab View</b>	<b>15</b>	
Sample Name and Sample Information .....		15
Image Controls .....		15
Dilution Calculator .....		17
Count Button .....		17
<b>7. Count Settings</b>	<b>18</b>	
<b>8. Results Tab View</b>	<b>20</b>	
Export Icon .....		20
Next Count .....		20
Common controls .....		20
Export Icon .....		21
Discard. New Count .....		21
Save and e-Sign .....		21
Export and Backup of Signed Record .....		22
Controls After e-Signing Record .....		22
Signed. Next Count .....		23
Add Signature .....		23
Record Security .....		23
<b>9. Result Tab Common Controls</b>	<b>24</b>	
Optimize Settings .....		24
Cell Size Gating .....		24
Intensity Gating (GFP app) .....		24
Action Bar Controls .....		25
Results View Options .....		25
<b>10. Export and Print Options</b>	<b>27</b>	
Screen Capture .....		27
Export .....		27
<b>11. Utility Apps</b>	<b>28</b>	

User Accounts Standard Mode .....	28
User Accounts 21 CFR Mode .....	29
Data.....	31
Count Codes .....	32
Calculator .....	33
Timer .....	33
Settings .....	33
Updater .....	35
ScreenLocker .....	35
Diagnostics.....	35
<b>12. 21 CFR App    36</b>	
21 CFR.....	36
Archiving .....	40
<b>13. Troubleshooting and Maintenance    42</b>	
Quick Help.....	42
Software Errors .....	42
Cleaning.....	42
Solvent Compatibility.....	43
<b>14. Customer Support    44</b>	
Warranty.....	44
Instructions for Disposal of Packaging and Waste Equipment.....	44
Research Use Only .....	44

## 1. Introduction

### Product Technology

DeNovix CellDrop™ Automated Cell Counters are compact instruments with integrated processors and high-resolution touch screens. The software utilizes application specific icons along with a custom operating system to provide an intuitive, easy-to-use instrument for cell count measurements.

The pre-configured EasyApps™ software is pre-installed and factory optimized for ease of use. The software allows the user to customize settings when needed to meet lab specific workflow preferences.

The CellDrop uses three chamber heights to enable cell counts over a wide dynamic concentration range according to specifications. This innovative, patented technology (US 10,302,568) along with user-defined protocols allows users the flexibility required for today's cell culture workflows and allows users to *Count Cells Without Slides*.

This User Guide is designed for both CellDrop BF (Brightfield) and CellDrop FL (Dual Fluorescence plus Brightfield) owners. Several applications and software features are only applicable for instruments with Fluorescence mode (CellDrop FL) and do not apply to the CellDrop BF.

### Key Features

- No consumable slides required
- Stand-alone (no PC required) with integrated processor and Hi-Definition 7-inch touchscreen
- Live preview of sample and immediate, on-screen analysis of count result
- Custom software interface with intuitive EasyApps software
- Variable chamber height to enable an accurate count for a broad range of cell population densities

### Optics

#### Brightfield

- Illumination = LED 530 nm

#### Fluorescence

- Excitation: 470 nm +/- 20 nm
- Green Fluorescence Emission: 525 nm +/- 25 nm
- Red Fluorescence Emission 645 nm +/- 37 nm

### Upgrade Options

CellDrop instruments may be upgraded at any time. Contact DeNovix or your distributor if you are interested in the following:

- Convert Pay-As-You-Go to Unlimited
- Convert CellDrop BF (Brightfield only) to CellDrop FL (Dual Fluorescence plus Brightfield)
- Activate 21 CFR Part 11 Compliance Ready Software

## 2. Set-up and Safety

1. Remove all packing materials including materials protecting the arm and sample surfaces.
2. Place the instrument on a flat bench and avoid vibrations to ensure optimal performance.
3. Plug the unit into a 100-240 VAC, 50-60/Hz receptacle using the grounded power supply (GlobTek, Inc. part number TR9CE3000C95CP-NR6B, 12V / 3A / 36W) included with each unit.
4. Toggle the power switch to the On position to turn on the instrument. To complete initialization, ensure that the arm is down and no materials are between the sample surfaces. The operating system will boot up in less than a minute. Toggle the power switch to Off to initiate shutdown of the instrument.

### Biological Hazard Safety

Trypan Blue, Acridine Orange, Propidium Iodide and other reagents are known hazardous materials. Consult Safety Datasheets (SDS) for these assays prior to use.

Biological samples such as tissues and bodily fluids of humans and other animals have the potential to transmit infectious diseases. Follow all applicable local regulations. Wear appropriate personal protective equipment (PPE). Read and follow the guidelines in this User Guide, in the SDS for any chemicals in use and additional biosafety publications applicable for the types of sample used and institution/region of use.

### Instrument Connectivity

The CellDrop comes equipped with Wi-Fi, Ethernet, HDMI and three USB ports. The USB ports are used to facilitate data transfer to a FAT32 formatted USB storage device and support the use of an Opticon OPI 3601 USB barcode reader. USB mouse and keyboard devices may also be used with CellDrop Series instruments. The CellDrop does not support printers connected directly to the device via USB.

### Cautions

**DO NOT REMOVE COVER:** No operator serviceable components inside. Refer servicing to qualified personnel. CellDrop Cell Counters are designed for indoor use under the following conditions:  
Temperature: 15° to 35° C | Humidity: 35 to 65 % | Elevation: 2000m or less | Pollution: Pollution degree 2

Use only the power supply provided with the instrument. Use of the instrument in any manner not specified by the manufacturer may impair the protection provided by the supplied power cord and power supply.

**NE PAS OUVRIR L'APPAREIL.**

Aucun composant réparable par un utilisateur est inclus. Confiez l'entretien à du personnel qualifié. Le CellDrop est conçu pour une utilisation en intérieur dans les conditions suivantes :  
Température : 15° à 35°C | Humidité : .....35 à 65% | Élévation : 2000m ou moins  
| Pollution: Degré de pollution 2

Utilisez uniquement le bloc d'alimentation inclus avec l'instrument. Utiliser l'appareil dans des conditions non spécifiées par le fabricant peut compromettre la protection offerte par le câble d'alimentation et le bloc d'alimentation fournis avec l'appareil.

VCCI (Class B) compliance statement for users in Japan:

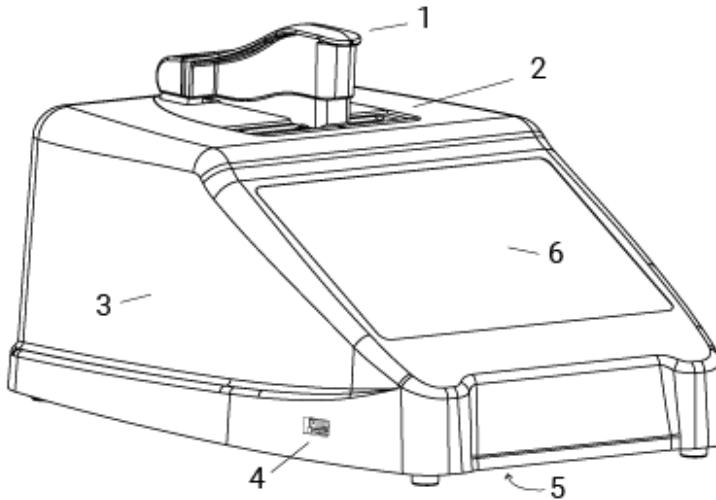


この製品は、技術基準適合証明に関する条例等特定無線設備(MPT省令第37号または1981)のタイプによって認定に準拠しています。この機器のカテゴリ：第2条、第1項のアイテム(19) 第2条第1項、項目(19)-2

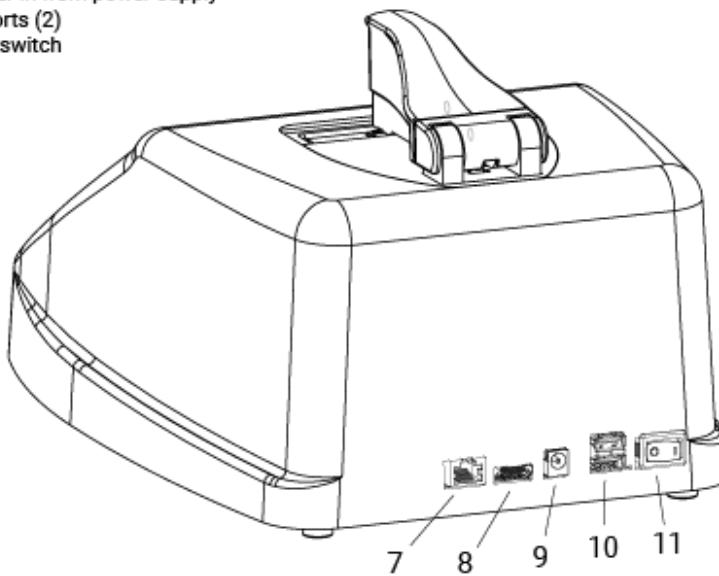
This is a Class B product based on the standard of the Voluntary Control Council for Interference (VCCI) from Information Technology Equipment. If this is used near a radio or television receiver in a domestic environment, it may cause radio interference. Install and use the equipment according to the instruction manual. This equipment has been tested for radio frequency emissions and has been verified to meet CISPR 22.2 Class B.

### 3. Instrument Diagram

#### CellDrop Automated Cell Counter Component Details



1. Arm
2. Sample Surfaces
3. Cover
4. USB port
5. Serial number & regulatory info (underside)
6. Touchscreen
7. Ethernet port
8. HDMI
9. DC power in from power supply
10. USB ports (2)
11. Power switch

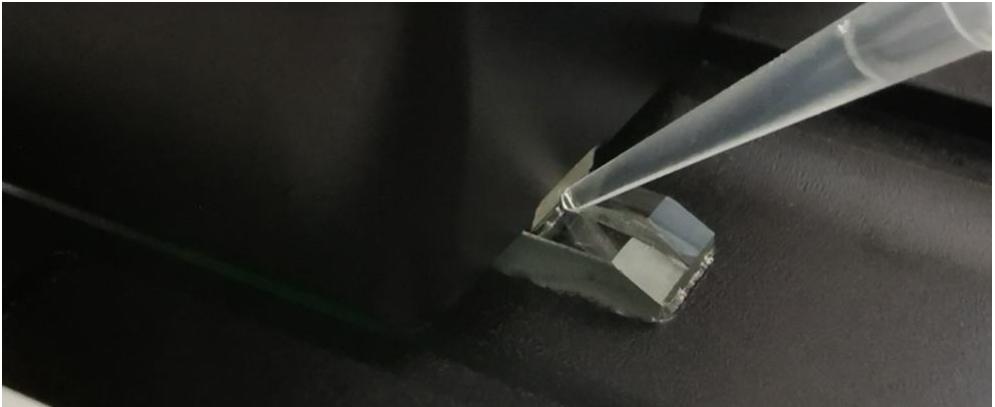


DeNovix<sup>®</sup>

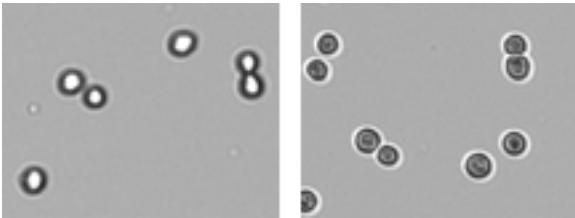
## 4. Quick Guide

### Cell Count Procedure

1. Launch any EasyApps Count app.
2. Lower arm to start live preview. Check to ensure that the field of view is clear.
3. Select a protocol.  
Optional: Enter sample name and additional sample info.  
Optional: Edit the Protocol Count Settings for the sample being measured. Check that the protocol includes the correct Dilution Factor.
4. Mix the sample well. Load the sample volume indicated on the Count button. Slide the pipette tip along the alignment groove until it rests against the top surface. Dispense the sample into the counting chamber formed between the upper and lower sample surfaces.



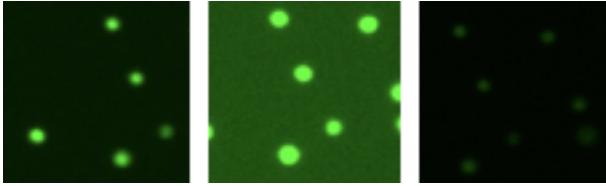
5. Allow the cells to settle.
6. Touch the focus icon and then adjust while viewing the Brightfield channel. Live cells should have a bright center and a dark outline. The image below on the left is correctly focused. The image on the right is out of focus. Verify focus in green channel for AO, AO/PI and Yeast apps.



7. Adjust Trypan Exposure: Touch the exposure icon and select Normal or Low exposure. Live cells should have a white center with a dark ring like the image below on the left. The middle image is overexposed and the right image is underexposed.



8. Fluorescence Exposure: Select the green or red fluorescence channel. Touch the exposure icon and then adjust the setting. The fluorescence signal should not extend outside of the boundaries of the cell when viewed in brightfield. The image on the left is correct. The middle image is overexposed and the right image is underexposed. Adjust the exposure for each channel independently.



9. Press **Count**.
10. Review the result.  
Optional: Adjust gating to exclude debris or alternative cell populations from analysis.  
Optional: Press **Optimize Settings** to adjust count settings and reanalyze the image(s).
11. Once a measurement is complete, lift arm and wipe away sample with a clean, dry laboratory wipe and close the arm. When the arm is lifted, the result is immediately saved. Pressing **Count** again prior to lifting the arm will overwrite the previous result for that sample.
12. Results from the active measurement session may be viewed while the app is open or viewed later using the Data app. Saved results may be reanalyzed if desired.
13. Export results as desired.

### Best Practices

**Note:** See *Technical Note 186 – CellDrop Best Practices* for additional details.

- Ensure that surfaces are clean prior to loading a sample into the sample chamber.
- If a previous sample has dried on the surfaces or if the sample is not loading easily, clean the sample surfaces. Lower arm and pipette 15  $\mu$ L of 70% ethanol into the alignment groove and sample chamber. Wait 10 seconds then firmly wipe both upper and lower surfaces with a dry laboratory wipe.
- Vortex samples before loading.
- Ensure that all sample concentrations fall within concentration limits of the instrument for accurate results.
- Use the appropriate protocol settings for Chamber Height when measuring high or low concentration samples or when using slides.
- Avoid introducing air bubbles into the sample solution when mixing or dispensing samples.
- Avoid drawing sample back into pipette tip after dispensing.
- Refer to DeNovix Assay protocols or other manufacturer guidelines for assay specific instructions.

## 5. General Operation and Software Information

CellDrop Automated Cell Counters include pre-installed EasyApps software for rapid counting and viability assessment. Each app is designed for a common cell counting technique such as Brightfield, Trypan Blue, AO/PI, etc.

Activation of **21 CFR Part 11 Compliance Ready Software** affects several aspects of the user interface. Where applicable in this guide, additional instructions for **21 CFR Mode** operation are provided. A license and activation code must be purchased to activate these features. Contact DeNovix or your local Distributor. 21 CFR Mode requires users to login to the device.

Apps include a common **Top Action Bar** that allows a user to easily navigate between the live preview on the Count tab and the Result tab. Controls available from the action bar are described below.

### Top Action Bar



- **Count Tab:** Select to view a live preview on screen. View the loaded sample, select saved protocols, edit protocol settings, define dilution guidance parameters and initiate a cell count.
- **Results Tab:** This tab enables the user to review the data for all samples measured in the current measurement session.
- **Alert:** The alert icon (bell) is displayed to notify the user of system alerts such as timer expiration or low count alerts (Pay-As-You-Go models).
- **Account:** The current account in use is displayed on the top action bar. The General account is the default account for CellDrop applications. Tap the account name to display a list of available accounts. Changing an account will automatically use the protocol indicated as the default option selected in the User Accounts app.
  - **21 CFR Mode:** The account that is actively logged in is displayed. The default accounts “Primary Admin” and “General” are disabled and may not be used.
- **Home Icon:** Tapping the Home icon will navigate back to the Home screen and close the app and current measurement session. All previous results are saved and accessible from the Data app.
- **Overflow:** The Overflow icon is used to access a menu of screen specific options:
  - **Screen Capture:** Screen shots may be taken of most but not all views. Refer to the Export and Print Options chapter for more information.
  - **Count Code Log (PAYG models only):** Displays a log of count code use.
  - **Tech Support:** Sends an email with diagnostic information to DeNovix. Email must be preconfigured from the Settings app for this function to work correctly.
  - **Set Focus:** Allows the user to save a global focal position for DirectPipette™ mode or for slide mode. This position is the initial position used when opening a count application and essentially eliminates the need to adjust focus once set for CellDrop.
  - **Reset Auto Focus:** Used to clear the saved auto focus starting position allowing the user to rerun the algorithm to define a best starting position.
  - **About:** The About feature brings up an information box displaying the current app version number.

## Software Icons and Buttons

CellDrop applications include a set of standard icons and buttons used throughout the software. The following table defines each button.

	Home		Add		Select All
	Overflow Menu		Edit		Increase / Decrease
	Count		Copy		Export
	Exposure		Delete		Clear Selected
	Focus		Expand / Collapse		Information
	Calculator		Notifications		Favorite

## Chamber Height Options

CellDrop Cell Counters use three chamber heights to enable measurements over a wide cell density range. The 100 um chamber height is equivalent to the 0.1 mm depth used on standard hemocytometers.

The default chamber height is 100 um and is suitable for most counts. Users may choose a different chamber height based on the anticipated density. The upper surface position is automatically moved to the position defined in the protocol. The table below is a guideline of the best chamber height to choose based on the density of the sample.

Gap Height	Required Volume	Minimum Density (cells/mL)	Maximum Density (cells/mL)
400 um	40 µL	$7 \times 10^2$	$1 \times 10^5$
100 um	10 µL	$5 \times 10^4$	$1 \times 10^7$
50 um	5 µL	$1 \times 10^7$	$2.5 \times 10^7$

## Data Storage and Memory Warnings

CellDrop models include a solid-state drive with 120 GB or more of storage capacity, sufficient for several thousand results. The user will be alerted if the memory approaches capacity. Data can be deleted from the CellDrop at any time from the Data App (Standard Mode) or Archived (21 CFR mode). If the **Memory Full** warning is activated, CellDrop will not save any more results. The user or System Administrator must make sufficient memory available to make additional counts. Memory upgrade options are available. Contact DeNovix or your DeNovix Distributor for details.

## Display Sleep

The backlight for the screen will automatically turn off after a user-defined period of inactivity. Touch the screen to turn it back on. Adjust the Sleep Time from Display section in the Settings App.

## Preview Suspend

The CellDrop live preview on the Count screen is suspended if the arm is left in the down position for longer than two minutes. The user may resume the preview when desired.

## Count Apps

Count apps are designed for a common cell counting techniques such as Brightfield, Trypan Blue, AO/PI, etc. To properly count a sample and optimize a result, the researcher must first capture the best image(s) possible. Examples of proper and improper adjustments are included in this guide in Section 6, Count Tab.

## Protocols and Optimizing Settings

Each Count app includes the relevant group of count settings saved as a Protocol. Once a sample has been counted, protocol parameters can be adjusted using the **Optimize Settings** button.

## Channels

Count apps use an application specific set of optical channels. The CellDrop FL model includes Brightfield, Green Fluorescence and Red Fluorescence channels. The CellDrop BF model includes Brightfield only.

Channel	Brightfield	Trypan Blue	Primary Cell Trypan Blue	Nuclei Trypan Blue	AO/PI	Primary Cell AO/PI	Nuclei AO/PI	AO	PI	GFP	Yeast	Custom Methods
 Brightfield	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y	Y
 Green Fluorescence					Y	Y	Y	Y		Y	Y	Y
 Red Fluorescence					Y	Y	Y		Y		Y	Y

## Count Apps

### Brightfield



The Brightfield app is used for quick, label-free total cell or particle counts. A brightfield count does not determine cell viability. This app will count cells with a bright, white center and a defined border. The application is not designed to count dark, solid objects.

### Trypan Blue



Trypan blue is a cell membrane impermeable dye that only enters cells with compromised membranes. Upon entry, the dye binds to intracellular proteins resulting in bluish colored cells. The trypan blue exclusion assay enables a direct counting of live (unstained) and dead (stained) cells.

Published literature suggests that using trypan blue for samples with viabilities less than 70% may result in higher reported cell viability values when compared to fluorescent-based detection methods.

$$\% \text{ Viability} = (\text{Live Cell Count} / (\text{Live Cell Count} + \text{Dead Cell Count})) * 100.$$

### Primary Cell Trypan Blue



The Primary Cell Trypan Blue app is configured in a similar manner as the Trypan Blue app. The application includes optimized protocol settings for counts and viability assessment of Trypan Blue stained primary cells such as PBMCs.

### AO



The AO app uses green fluorescence images to count live nucleated cells.

Acridine orange is a nuclear staining cationic fluorescent dye that enters live and dead cells and interacts with DNA and RNA by intercalation or electrostatic attractions. When bound to DNA, it is spectrally very similar to fluorescein, with an excitation maximum at 502 nm and an emission maximum at 525 nm.

Although AO enters dead cells, the intensity of the green fluorescence is very low for these cells compared to the green fluorescence emitted from live cells. This app may be useful when counting solutions containing either red blood cells and/or significant cellular debris.

The % cell viability is not provided when using this app.

## PI



The PI app uses brightfield images to capture live cell counts and red fluorescence images for counting dead cells.

Propidium iodide (PI) is a red-fluorescent nuclear and chromosome stain that only enters dead cells. Upon entry, the PI stain binds to DNA by intercalating between the bases with little or no sequence preference. In aqueous solution, the dye has excitation/emission maxima of 493/636 nm. When bound, the excitation/emission maxima shift to 535/617 nm respectively.

Calculation:

$$\% \text{ Viability} = (\text{Brightfield count} / (\text{Brightfield count} + \text{PI count})) * 100.$$

## AO/PI



The AO/PI App uses green fluorescent images to capture live nucleated cell counts and red fluorescent images for counting dead cells. The application is suited for counting cell culture samples or primary samples.

Acridine orange (AO) and propidium iodide (PI) are both cell permeable nuclear dyes that in combination are used to count live and dead cells within a cell solution. The AO enters both live and dead cells and results in green fluorescing cells. PI enters only dead, nucleated cells with compromised membranes to generate red fluorescence. Cells stained with both AO and PI fluoresce red due to fluorescence resonance energy transfer (FRET).

$$\% \text{ Viability} = (\text{AO count} / (\text{AO count} + \text{PI count})) * 100$$

## Primary Cell AO/PI



The Primary Cell AO/PI app is configured in the same manner as the AO/PI app. The application includes optimized protocol settings for counts and viability assessment of AO/PI stained primary cells such as PBMCs.

## Nuclei AO/PI



The Nuclei AO/PI app uses red fluorescence to capture images of isolated nuclei and green fluorescence to capture images of unlysed or intact cells. Acridine orange (AO) and propidium iodide (PI) are cell permeable nuclear dyes that in combination are used to count live and dead cells or nuclei within a cell sample. The AO enters both unlysed cells and isolated nuclei and fluoresces green. PI is only able to permeate the membrane of dead cells or isolated nuclei and once inside, fluoresces red. The nuclei are stained with both AO and PI but overwhelmingly fluoresce red due to fluorescence resonance energy transfer (FRET) from the AO molecule to PI.

$$\text{Extraction Efficiency (\%)} = (\text{PI count} / (\text{AO} + \text{PI count})) * 100$$

## Nuclei Trypan Blue



The Nuclei Trypan Blue app uses the colorimetric membrane impermeable dye trypan blue to distinguish unlysed or intact cells from isolated nuclei or dead cells. The trypan blue dye is able to permeate the membranes of isolated nuclei resulting in a bluish colored stain and allowing their correct identification. Trypan is not able to enter the membranes of living cells which can then be counted as unlysed or intact cells.

Extraction Efficiency (%) = (Stained count / (Stained + Unstained count)) \*100

## GFP



The GFP App uses green fluorescent images to count GFP transfected cells and brightfield images to count non-transfected cells.

As GFP intensity can be highly variable it is recommended that the fluorescent threshold is set for each sample *after* the count using the intensity slider in the results screen.

The green fluorescent protein (GFP) isolated from the *Aequorea victoria* jellyfish is a useful reporter molecule for monitoring gene expression. GFP emits green light (lambda max = 509 nm) when excited with UV or blue light resulting in cells that fluoresce green.

The GFP app is used to determine transfection efficiency of transient transfections.

% Efficiency =(GFP+ count / (GFP+ count+ Brightfield count)) \*100

## Yeast



The Yeast App uses green fluorescent images to capture live yeast cell counts and red fluorescent images for counting dead yeast cells.

Yeast cell live/dead staining is performed using fluorescein diacetate (FDA) and propidium iodide (PI). FDA freely moves in and out of the plasma membrane of diverse organisms from bacteria to mammalian cells. The non-fluorescent FDA taken up by cells is converted into the green fluorescent metabolite fluorescein. The green fluorescence is used as an indicator of live cells. PI enters only nucleated cells with compromised membranes to generate red fluorescence indicating dead cells.

**Note:** Yeast cells are smaller than typical mammalian cells and may take a little longer to settle into position.

% Viability =(FDA count / (FDA count+ PI count)) \*100

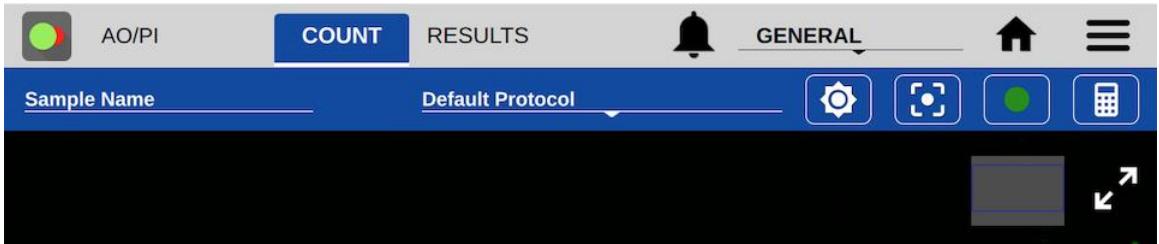
## Custom Methods



The Custom Methods app allows users to count cells using brightfield, green fluorescence or red fluorescence techniques. The application is intended to provide users with the tools to conduct experiments using any of the available measurement channels.

## 6. Count Tab View

From the Count tab, users can preview the live image, input sample information, select the protocol for counting, adjust the image and initiate the cell count.



### Sample Name and Sample Information

- Tapping the Sample Name field will bring up the Sample Information dialogue. An optional sample name, short description and a passage number may be entered. This information is saved with the count result.

### Protocol Selector

- Protocols allow the user to adjust and save count settings within each application. Count settings are the screening criteria used by the counting algorithms such as diameter, roundness and threshold. See Section 7 - Count Settings for details
- Each app opens to the Default Protocol, suitable for counting most mammalian cell lines within this application. Users may set a custom protocol as their default in the User Accounts app.

### Image Controls

- **Channel Selector:** For the CellDrop FL model, use the Channel Selector to switch between brightfield and fluorescence preview channels. Within fluorescence apps, the icon indicates the active channel. Tap the icon to select a channel. CellDrop BF units and brightfield applications (Brightfield, Trypan Blue) only include the brightfield channel.



Brightfield

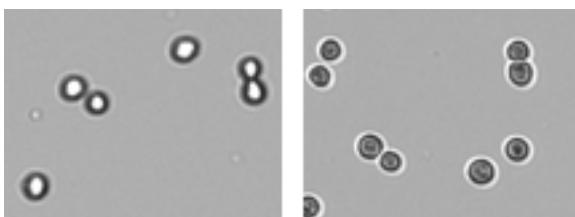


Green Fluorescence



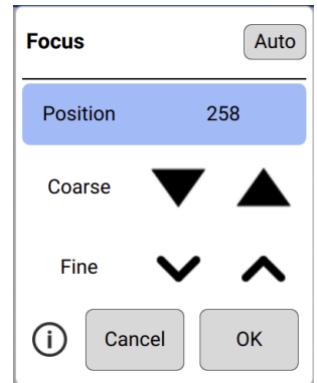
Red Fluorescence

- **Focus** : The focus controls may be used when previewing any Count screen channel (brightfield, green or red fluorescence). Changing the focus when viewing one channel will automatically change the focus of the other channels. Use the arrows to change the focus.
- **Manual focus:** Adjust focus while viewing the brightfield channel. Live cells should have a bright center and a dark outline. The image below on the left is correctly focused. The image on the right is out of focus.



- For the **AO/PI, AO, Primary AO/PI and Yeast Apps** – check the focus in the **green** channel prior to counting. In some cases, the ideal fluorescence focal plane is different than the brightfield focal plane. A sharp focus in the green channel will deliver the best results.
- Auto focus: Autofocus finds the best focal position for a count. Load cells and ensure cells are roughly in focus. Press the “Auto” button.

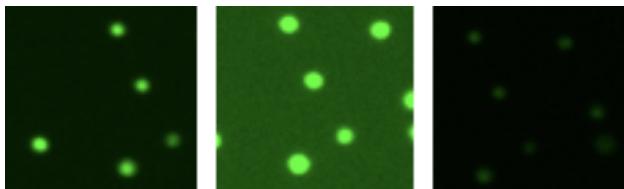
If auto focus does not regularly find a best focal position, select “Reset Auto Focus” from the overflow menu. Follow the onscreen instructions. Load cells, roughly set the focus and rerun the algorithm.



- Exposure : Touch the exposure icon to display the exposure control in the Trypan app or for fluorescence channels. The control is not required for brightfield counts which use a preset value.
- Trypan Blue and Primary Trypan apps: Choose the correct exposure based on the concentration of trypan dye used or set a **Custom Exposure**.
  - If the assay is 0.4% trypan dye mixed 1/1 with cell suspension, select **Normal**
  - If the assay is 0.2% trypan dye mixed 1/1 with cell suspension, select **Low**
  - Live cells should have a white center with a dark ring like the image below on the left. The middle image is overexposed and the right image is underexposed.



- **Custom Exposure** – Some dyes used in brightfield applications have a different opacity than common grades of Trypan Blue. The Custom Exposure Wizard instructs the User on the steps to follow to establish proper exposure values for the 50, 100 and 400 micron chamber heights. Follow the on-screen instructions.
- Fluorescence Apps: Select a fluorescence channel. Touch the exposure icon  to open the exposure settings. Tap the arrows to adjust. The fluorescence signal should not extend outside of the boundaries of the cell when viewed in brightfield. The image on the left is correct. The middle image is overexposed and the right image is underexposed. Adjust the exposure for each channel independently.



- **Zoom Controls:** All preview and result images on the CellDrop support zoom and pan capabilities. Pinch and zoom using two fingers to zoom in or out. Tap and drag within the image to move the preview location.
- **Image map:** A small position map in the top right is an indicator of what portion of the full image is currently displayed on the screen.

- **Expand/Collapse**   : Tapping the expand or collapse icon for a preview or result will toggle between a full screen view and the standard view.

### Dilution Calculator

The dilution calculator enables the user to define target cell number or density and culture volume. The counted result will automatically provide instructions to correctly dilute cultures to reach the desired target.

- Tap the calculator icon to set dilution guidance parameters for the next sample count.
- Setting targets is optional for any specific count. Inputs may be added or modified post measurement from the Results tab Calculator screen.
- Once targets are set, they will stay in effect for all subsequent sample measurements within a session until changed.
- When applied, the inputs are used to generate the dilution guidance presented under the Single Sample view as well as the Results tab Results List view when the Show Dilution Guidance box is checked.

### Count Button

- The Count button initiates a measurement.
- The required volume of sample is displayed on the Count button.



## 7. Count Settings

Each Count app includes an applicable group of count settings saved within protocols. Count settings may be adjusted prior to a count. A subset of the parameters can be also adjusted after a count to optimize a result.

- **Cell Outline**
  - Enhanced Outline - Draws an outline around the identified cell boundary (recommended).
  - Circle Outline - Marks each counted cell with a circle. The outlined image will be rendered more quickly.
- **Sample Mode**
  - DirectPipette - This is the default mode and is used when samples are loaded directly into the CellDrop sample chamber.
  - Slide - Choose this option when applications require cells to be contained in a slide while counting.
- **Chamber Height:** A unique feature of CellDrop Cell Counters is an adjustable chamber height that allows for a greater dynamic range than any slide-based counter on the market. The default chamber height is 100  $\mu\text{m}$  and is recommended for most counts. For very low density samples, choose 400  $\mu\text{m}$ . For very dense samples, select 50  $\mu\text{m}$ . The table below includes guidelines.

Gap Height	Required Volume	Minimum Density (cells/mL)	Maximum Density (cells/mL)
400 $\mu\text{m}$	40 $\mu\text{L}$	$7 \times 10^2$	$1 \times 10^5$
100 $\mu\text{m}$	10 $\mu\text{L}$	$5 \times 10^4$	$1 \times 10^7$
50 $\mu\text{m}$	5 $\mu\text{L}$	$1 \times 10^7$	$2.5 \times 10^7$

- **Small Cell Mode:** Small Cell Mode can improve brightfield channel counts of small cells near the 4  $\mu\text{m}$  limit of detection. The additional algorithm complexity increases the length of the analysis.
- **Irregular Cell Mode:** Enabling irregular cell mode improves counts of cells that are elliptical, elongated or irregular in shape.
- **Dilution Factor:** This value is applied to the cell count when calculating cells/mL to account for any dilutions or reagents added to the sample. The Dilution Factor is the ratio of the total assay volume to the volume of cell suspension in the assay. Enter the proper dilution factor to calculate the correct cell density.

Example Dilution Factor calculations:

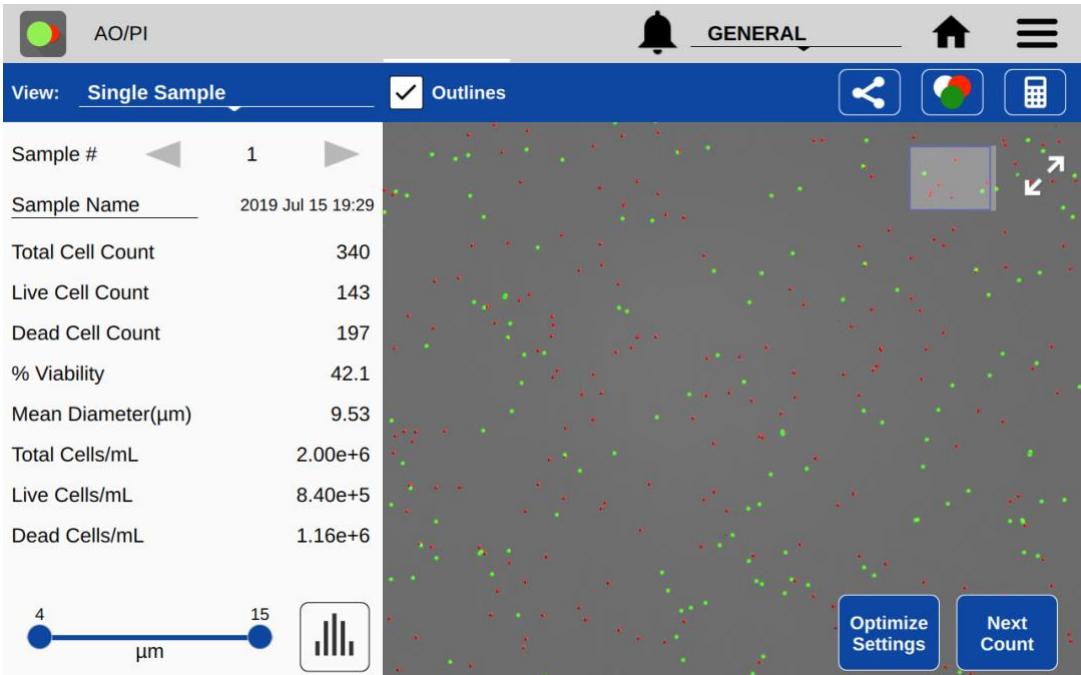
- For pure cell suspensions, the Protocol Dilution Factor = 1.
- For a mixture of 5  $\mu\text{L}$  of assay working solution to 5  $\mu\text{L}$  of cell suspension, set Dilution Factor = 2.
- For a mixture of 8  $\mu\text{L}$  of assay working solution to 2  $\mu\text{L}$  of cell suspension, set Dilution Factor = 5.

- **Diameter (um):** The Diameter settings specify the size range of objects to seek during a count. The algorithm rejects and does not process objects below the minimum size or above the maximum limits. The limits define the boundaries of the data set, graphs and histograms. The total allowable range is 2-400 um.
  - Minimum Diameter - This setting defines the diameter of the smallest objects to be counted. The proper value ensures that cellular debris will not be counted. Increase this setting when counting larger cells if they are being counted as multiple smaller cells. Decrease if small cells are not being counted.
  - Maximum Diameter - Objects above the maximum diameter will not be counted. Adjust the maximum diameter to provide a realistic size band to analyze during counts. If clusters of cells are being counted as a large individual cell, reducing the maximum diameter to reflect the true cell size will improve the results.
  
- **Roundness:** CellDrop uses Roundness settings to identify cells based on morphology. The higher the roundness, the more circular and completely outlined a cell must be to be included in the count. Reducing the roundness setting allows cells that are not perfectly circular or cells with incomplete structures to be counted. If single cells are counted as multiple cells or artifacts are counted, increase the roundness setting. If cells are not counted, decrease the roundness.
  - Brightfield Roundness - Used for Brightfield counts.
  - Live Roundness - Used in apps counting live cells.
  - Dead Roundness - Used in apps counting dead cells.
  - Green Roundness - Used in the Custom Methods app for the green fluorescence channel.
  - Red Roundness - Used in the Custom Methods app for the red fluorescence channel.
  - FL Roundness - Used in the GFP app to count fluorescing cells.
  
- **Threshold:** The CellDrop Series uses Threshold settings to evaluate each counted cell to see if it is above the intensity criteria set in the protocol. The higher the threshold is, the greater the intensity must be to be included in the count.
  - Stained Threshold - Used in the Trypan Blue app to determine if a cell is dead or alive. The higher the setting, the darker a cell must be to be counted as dead. If dead cells are not being counted, decrease the threshold. If live cells are being counted as dead, increase the threshold value.
  - Green Fluorescence Threshold - Used in apps that count cells using the green fluorescence channel.
  - Red Fluorescence Threshold - Used in apps that count cells using the red fluorescence channel.
  - Decrease the fluorescence threshold if cells that are positively stained are not being counted in the analysis and vice versa.

## 8. Results Tab View

This section details the Results Tab user interface and some differences seen when an instrument is in Standard Mode or 21 CFR Mode.

### STANDARD MODE VIEW



### Export Icon

The export  button is active immediately after a count in Standard Mode.

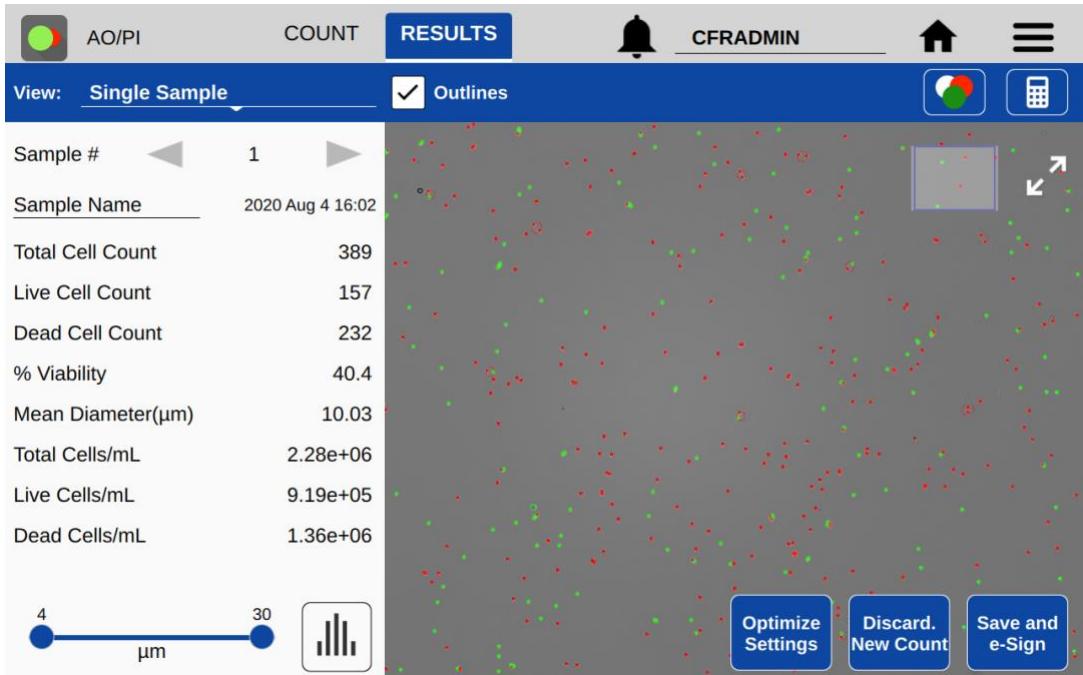
### Next Count

This button changes the view back to the Count tab and starts a live preview. The previous result is not saved until the user raises the arm. If the Count button is pressed without raising the arm, the previous count will be overwritten. To capture a new result of a new sample, raise the arm, clean the upper and lower sample surfaces, lower the arm then load the next sample.

### Common controls

All remaining controls (i.e. Optimize Settings) are available in both Standard and 21 CFR mode and are detailed in the next section of the guide.

## 21 CFR MODE VIEW



### Export Icon

The export  button is inactive until the record is electronically signed.

### Discard. New Count

This button is visible when the result is un-signed. Tap this button to discard the result and return the user to the live preview in the Count tab. The result is not saved. SOPs should be established related to the use of discard to avoid 'testing into compliance' scenarios. The Discard button may be disabled by an Administrator account using the Permissions tab in the Settings app.

### Save and e-Sign

The user may optimize settings at this time. Once satisfied with the result, the user electronically signs the record and saves the result using the **Save and e-Sign** button. **A result is not saved and may not be exported until the result is electronically signed.** The electronic signature dialogue will open. The logged in user may sign a record with any valid Signature Meaning for his/her User ID. The password is also required for the first signing of a session. 21 CFR Mode configuration details are found in the 21 CFR section of this guide.

**Electronic Signature**

User ID: CFRAAdmin

Signature Meaning: **Choose a signature**

Enter optional comments

Comments:

Result ID: 0426620200804160452

Date and Time: 2020 Aug 4 16:04

Sample ID: Sample Name

Description:

Passage:

Cancel Sign and Save Result

### Export and Backup of Signed Record

After signing a record, the user will be asked to export the signed record to a location of the user's choosing. The choice will be saved during the active Count app session.

**Select Export Destination**

USB FAT32 Formatted CELLDROP

Email

Network Folder

Printer

None

Cancel Next

Exporting the record ensures that the valid electronically signed record as a human readable pdf, as well as any associated files selected, are exported to a secondary location. This creates a backup of the record which should then be further managed according to the organization's policies.

**IMPORTANT** – Organizations should establish SOPs to ensure that signed records from the instrument are exported and backed up on a routine basis. System Administrators can search for results in the Data App that have not been exported.

Records that have not been exported are not backed up and are subject to loss. The end user's organization is solely responsible for ensuring proper procedures are followed for electronic record backup.

### Controls After e-Signing Record

After signing a record, the following buttons are displayed:



### **Signed. Next Count**

This button indicates the record is electronically signed and saved. Pressing the button will take the user back to a live preview on the Count Tab. The data is saved. Pay-As-You-Go systems deduct a count after the arm is raised to remove the previous sample.

### **Add Signature**

This button allows the logged in User to add a signature to a previously signed record.

### **Record Security**

Electronically signed results may not be altered. If the user chooses to optimize settings, change gating or in other ways alter the result, the buttons revert to **Discard. New Count** and **Save and e-Sign**. The user may electronically sign and save the new result. The new result is saved while the original record is unaltered and is retained.

## 9. Result Tab Common Controls

### Optimize Settings

- Once a sample has been counted, a subset of the protocol parameters can be further adjusted to optimize the result. Press the **Optimize Settings** button, adjust settings and then press **Count with Current Settings** to reanalyze the existing image(s) using the new settings. Users have the option to save the adjusted settings to a protocol if desired by using the **Save to Protocol** button that appears when a change is made.

	Current	[Original]	
Dilution Factor	1	[ 1 ]	
Diameter (minimum)	6	[ 6 ]	microns
Diameter (maximum)	30	[ 30 ]	microns
Roundness	50	[ 50 ]	(1-100)
Small Cell Mode	<input type="checkbox"/>	[ Disabled ]	
Irregular Cell Mode	<input type="checkbox"/> ⓘ	[ Disabled ]	

**Outline**

Enhanced     Circle    [ Enhanced ]

Cancel    Restore Original Settings    Count with Current Settings

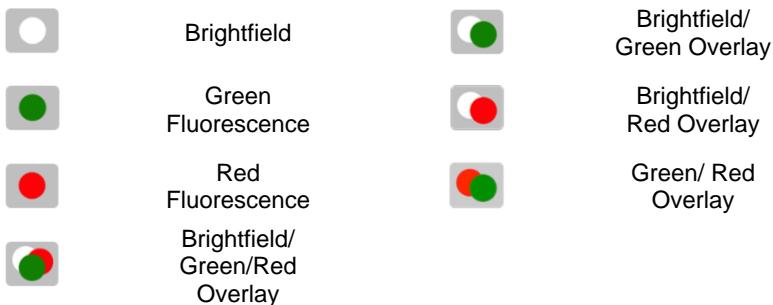
### Cell Size Gating

- Use the on-screen sliders to adjust the size range of interest for the current sample. Cell outlines and count results are updated in real time. Gate to exclude debris or alternative cell populations from analysis. Press the Histogram button to graphically view cell size distribution and apply gating. Press Apply Gating to Result to update and save the result.

### Intensity Gating (GFP app)

- In the GFP app, users may gate cells by size as well as intensity. Use the on-screen slider to adjust the intensity gating from 0-100. The higher the number, the higher the intensity must be for a cell to be included in the counted result. The intensity gating icon will open a graph showing the intensity profile of the cell population.

### Result Overlay Selector





### Action Bar Controls

- View Selector: Use this drop down to access the various Results tab views including viewing a list of multiple results. The Default selection is Single Sample view.
- Outlines: The checkbox is used to toggle on/off the outlines for counted cells for views that include cell images.
- Export : Export results in .csv, .pdf, or .png formats via USB, Email, Network Folder and Network Printer.  
21 CFR Mode: Only results that have been electronically signed may be exported.
- Image Selector: This icon indicates the overlaid images included in the result being viewed. Tap the icon to change the overlay combination for the current sample or after optimizing settings for a previous result.
- Calculator: Access the Dilution Calculator.

### Additional Action Bar Controls



- Select/Deselect All : These icons enable all items in a list to be selected or deselected with one tap of the icon.
- Edit : Highlight a single sample and then tap the edit icon to add or modify sample information. Changes will overwrite the information stored in the database and will be applied in all lists referencing the sample.  
21 CFR Mode: Record may not be edited from this view.
- Delete: Tapping this icon will delete the highlighted samples from all results lists.  
21 CFR Mode: Signed records may not be deleted. Results can only be removed via archiving by a System Administrator. See 21 CFR section for details.

### Results View Options

- Single Sample
  - Sample Number: The arrows on either side of the sample number are used to switch between samples measured in the current session.
  - Sample Name: This is an editable field. Touching will open the Sample Information dialogue for input. Changes will overwrite the information stored in the database and will be applied to all lists referencing the sample.  
21 CFR Mode: Sample information may be edited prior to signing a result. To edit and save the sample information for a previously signed result, a new signature is required.
  - Results Panel: A table of results will be displayed in the Results panel. If dilution parameters were provided at the time the count was initiated, then dilution guidance will also be presented in the panel.
- Results List
  - The list shows all samples counted in the current measurement session.
  - When the Show Dilution Guidance box is checked, the dilution guidance is displayed with each result in the list for which dilution information was entered.
- Image List

- The list displays all samples counted in the current measurement session.
- Cell Count: The sample cell count is presented in the table. This value automatically updates whenever the results are recalculated due to a change in cell size limits or other counting parameters.
- Graph
  - Includes a summary of results to allow the user to select different charts to add.
  - A color legend is included for the various histograms. Select up to 5 samples at a time to chart.
  - **Graph Type Menu:** Use the drop down to select additional graph types.

### Graph Types

- The graph types available are app dependent. Graphs may be expanded to full screen for easier viewing using the expand/collapse button.

#### Graph showing totals only or with a cell size distribution histogram

- Total Cell Count: Available for the Brightfield and GFP apps only.
- Live Cell Count: Available for all apps except Brightfield.
- Dead Cell Count: Available for Trypan Blue, PI, AO/PI and Yeast apps.
- Live and Dead Cell Count: Available for Trypan Blue, PI, AO/PI and Yeast apps.
- **GFP+ Cell Count:** Available for the GFP app only.

#### Additional Views

- Cluster #: Available for all apps. Displays count of cell cluster sizes.
- % Viability: Available for Trypan Blue, PI, AO/PI and Yeast apps.

## 10. Export and Print Options

There are several export and print options available on the CellDrop. Data export options are accessed using the export icon  in the blue action bar. The screen capture option is accessed from the overflow menu. Data available to export includes results, protocol settings, images, and histograms.

### Screen Capture

The screen capture feature in the overflow menu is used to export a .png file. Screen capture is available for most EasyApps screens.

### Export

Tapping the Export icon will bring up a series of two dialogue boxes. The first dialogue is used to select the desired combination of results, images and histograms to include in the export. The second dialogue is used to select the export destination.

### Export Selections

- **CSV:** The Results and Protocol settings will be presented in a .csv format that can be opened in Excel or uploaded to a LIMS system. Additional sample related information not displayed in the Results tab Single Sample view is included when exporting in a .csv format. To open an exported .csv file on a computer, the DeNovix instrument and the computer must both be set to use either period or comma number formats. The CellDrop number format is determined by the language selected using the Settings app.
- **PDF:** A report .pdf file will be generated based on the selected options during export. Report options include Results and Protocol Settings, Images and Histograms.
- **IMAGES:** CellDrop images are exported as .png files. Channel specific images and the current result image may be exported. The available options are App dependent.
- The result image for the active result can be modified to show the channels desired in the overlaid image. The channel selector button shows the combinations available. Cell outlines may also be toggled off if desired. To change the overlaid image of a past result, reprocess the image using the Optimize Settings button. Then select the new overlay desired using the channel selector.
- **Graphs:** Use Screen Capture from the Overflow menu to capture and export histograms or bar charts found in the Graph screens.

### Export Destinations

- Export destinations include: USB, Email, Network Folders and Network Printer. See the Settings app section for Email, Network Folder and Network Printer set up.

## 11. Utility Apps

### User Accounts Standard Mode



This section describes the User Accounts app in **Standard Mode**. See the next section for 21 CFR Mode.

The User Accounts app allows users to create and manage individual accounts on CellDrop. Users can adjust their default settings. Accounts are also used as a means of segregating data when performing searches in the Data app. For Pay-As-You-Go models, users may also define how count codes are shared amongst user groups.

#### App Structure

Accounts are listed in the left pane of the screen. A menu of app function options is presented in the action bar, where the selected option is highlighted with a blue background.

- Select a user account from the dropdown list in the top grey bar and select an option to edit: Profile, Report Emails, File Share, and Defaults.

#### App Functions

There are five basic functions that are controlled using the Account app.

- Adding and deleting accounts
- Managing passwords for accounts
- Managing email addresses associated with an account
- Setting account specific app protocol preferences

#### Adding or Deleting Accounts

There are two preconfigured system accounts built into the software - the General Account and the Primary Administrator Account. These accounts may not be deleted.

##### Primary Administrator

- This account is used to manage instrument settings. It is not used within the count applications.
- The primary administrator account is not initially password protected. A password may be applied at any time.

##### General Account

- This account is used as the default selection for all apps each time an app is opened. This account may not be password protected or deleted.

There are two types of accounts that may be added, edited or deleted as described below.

##### User Added Standard Accounts

- Any user may add a standard level account. It is not necessary to select an account before adding a new standard account.
- Standard accounts may only modify or delete protocols or emails created from within their account.

##### User Added Administrator Accounts

- An administrator level account may only be created by another administrator. The primary administrator or another existing administrator account must be highlighted in order to enable the adding of a new administrator.

- Administrator accounts may modify or delete user entries of any sort created from within any account. A drop-down menu will be used to select the account of interest.
- Administrators may change the password for any account as well as delete any account.

### Account Passwords

- Passwords are optional for all added administrator or standard user accounts.
- Contact the holder of an administrator account if a standard user account password is forgotten.
- Contact DeNovix customer support or your local distributor if the Primary Administrator password is forgotten. Please have the serial number of the instrument available when requesting help for the Primary Administrator password reset.

### Deleting an Account

- When an account is deleted, all protocols and custom report formats created by that account are reassigned to the General Account.
- The account specific saved data will also be permanently removed from the instrument at the time the account is deleted.

### Tab - Email Addresses

Multiple “to” email addresses for exporting results may be set up for each account. The nickname is used in the drop-down list presented on an export destination dialog box.

### Tab - Defaults

Set the default protocol you would like to have at start up for this user account. Select the app and then choose any established protocol as the default.

## User Accounts 21 CFR Mode



This section describes the User Accounts app in **21 CFR Mode**. See the previous section for Standard Mode. Account management features must follow rigid guidelines in 21 CFR mode.

### Account Management for 21 CFR Part 11 Compliance

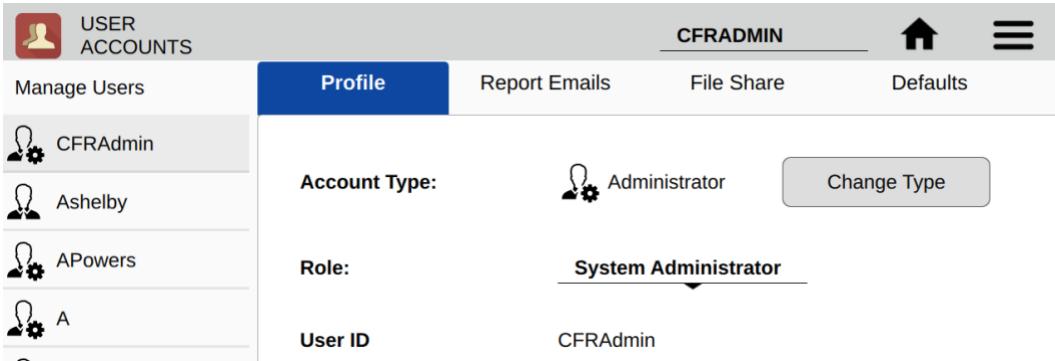
System Administrators use the User Accounts app to create and manage user accounts on the device. The app is also used for selecting roles, deactivating users, setting temporary passwords and changing account information. Each user is required to have a unique User ID and password that meets the organization’s password rules set in the 21 CFR App. All User Account changes are captured in the 21 CFR Audit Trail data.

Accounts also serve as a convenient means of segregating data when performing searches. For Pay-As-You-Go systems, users may also define how count codes are shared amongst user groups.

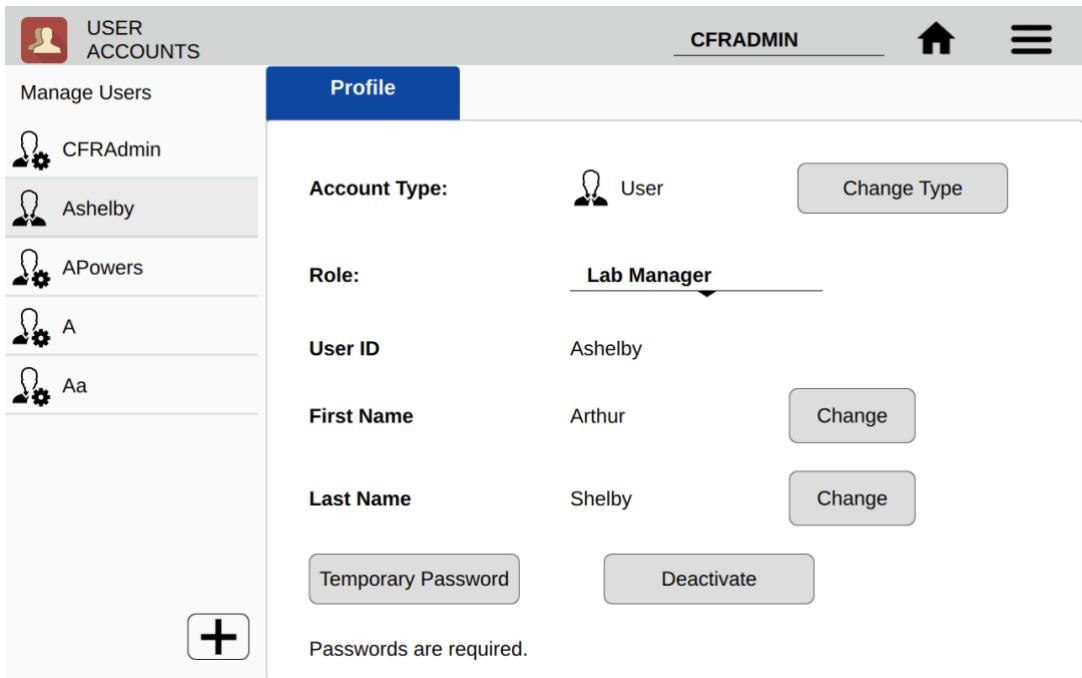
**IMPORTANT: Organizations are responsible for following the strict security requirements of 21 CFR Part 11 regulations. DeNovix software enables a method of compliance, but organizations are responsible for establishing and following procedures to ensure compliance.**

### App Structure

User accounts are listed in the left pane of the screen. Within a user’s own account, tabs in the right pane allow access to User Profile, Emails, Network Folders (pending) and Default settings.



Administrators can access the Profile tab of other users by selecting that user from the list in the left pane.



### App Functions

Administrators have the following functions within the User Accounts app.

- Add accounts
- Set Account Type
- Assign Role
- Change first name or last name
- Set Temporary Passwords
- Deactivate / Reactivate accounts

### Adding Accounts

Choose the + symbol from the left column to add a new user. Assign a User ID and the user's information. Create a temporary password for the new user. All users must have a unique User ID.

## Account Type

When a new user is added to the system, the Administrator must define the Account Type. Administrators can change the Account Type of any user.

- User – This account type has full access to count apps and permitted utility apps. User account types do not have any access to Administrator level system settings. A user may change first name, last name or password of his/her own account, but the user cannot change anything related to other user accounts.
- Administrator – This user type has full access and control over all system settings. At least one Administrator account must always be active in 21 CFR Mode. All Administrator accounts have the same administrative controls on the device.

## Role

Each user must be assigned to an available Role as defined in the 21 CFR App. See 21 CFR App section for configuration details.

## Account Passwords

- Passwords are required for all Users.
- Password must follow the organization rules set in the 21 CFR App (special characters, length, etc.)
- A user may not reuse a password they have previously used.
- If a user is unsuccessful logging in over a set number of times (set in 21 CFR app), the account is locked. An administrator can set a temporary password to unlock the account.
- Organizations are responsible for establishing password security procedures to ensure compliance with 21 CFR Part 11 requirements.
- Contact DeNovix customer support or your local distributor if the sole Administrator password is forgotten. Please have the serial number of the instrument available when requesting help for the reset.

## Deactivate / Reactivate an Account

User accounts may be deactivated by an Administrator. Per 21 CFR Part 11 guidelines, a user account may not be deleted. User activation/deactivation is tracked in the Audit Trail.

## Tab - Email Addresses

Multiple “to” email addresses for exporting results may be set up for each account. The nickname is used in the drop-down list presented on an export destination dialog box.

## Tab - Defaults

Set the default protocol you would like to have at start up for this user account. Select the app and then choose any established protocol as the default.

## Data



This app allows the user to search for all saved data.

- Data may be filtered in the following ways:
  - Standard mode: App, date, protocol name sample, Account name
  - 21 CFR Mode: App, export status, User ID, Signature Type, date
- Once the filter criteria have been selected, tap the **Search** button at the bottom of the screen. Tap any column header to sort the results list.
- Highlight the sample(s) of interest and tap the **Add to Report** button to include the samples on Results tab pages.

- All data must be from the same app type for the samples to be added to the Report tab.
- Features and functions previously described for the Result screens apply to the corresponding Data App Report screens.

## Export

- All data is automatically saved to the CellDrop onboard memory at the time of measurement and may be exported as described in the previous section.
- When the export function is used, the data is not deleted.

## Delete

### 21 CFR Mode

- The Delete function is not active for instruments in 21 CFR Mode. Data can only be archived from the system by an Administrator through the 21 CFR app.
- The storage capability of the CellDrop is a minimum of 120 GB of data. If the storage is approaching capacity a warning message will prompt the need to free space for continued operation.

### Standard Mode

- The **Delete** function *permanently* removes the selected data from the instrument database and cannot be restored to the instrument.
- The deleting privilege for standard accounts is enabled or disabled using the Settings app. When disabled, only administrative accounts may delete or archive data.
- The storage capability of the CellDrop is a minimum of 120 GB of data. When the storage capacity has been reached, the software uses a first in/first out paradigm for remaining sample data. The **Delete** function should be used as an infrequent method of removing a large amount of data from the CellDrop database.
- The delete process continues to work even when the interface goes to sleep after 10 minutes of no user activity. Touch the screen to wake-up the user interface and update the progress message.

## Count Codes



The purpose of the Count Codes app is to manage pre-paid counts using codes on a per account basis. This app will appear on instruments purchased on a pay-as-you-go basis.

The Primary Administrator account is only used to add, review or transfer codes for other accounts. This account cannot be used to make cell measurements.

For Admin accounts and Standard accounts- Tab menu options include:

- Enter New Code
- Review Code History
- Transfer Counts
- Share Counts

Administrators may transfer or share counts between all accounts on the instrument. Standard accounts may transfer or share counts from only their account.

Please include the serial number of your instrument when contacting DeNovix by email to purchase additional count codes.

Outside of the US, please contact your local distributor for assistance.

## Calculator



The Calculator app includes a scientific calculator as well as convenient dilution calculators.

## Timer



The Timer app enables the setting of up to two timers at one time. An alert will display when a timer has finished its set runtime.

## Settings



The Settings app may be found on the second Home screen page. Only administrator accounts may access and change settings. The app provides access to the following functions and information:

### System Info

- View software and firmware versions, IP addresses, current language and hostname.

### Date/Time

- Select manual or automatic date and time options.

### Language

- Select the language of operation for the device. Not all languages are fully translated. Setting the language also determines whether a period or a comma is used for the decimal place.

### Network Information

- The Wi-Fi and Ethernet MAC addresses and IP addresses can be found under this setting.

### Permissions

- Use Permissions to set access to the Settings app tabs for non-Administrator accounts.

<input checked="" type="checkbox"/>		System Info	<input checked="" type="checkbox"/>		Date/Time
<input checked="" type="checkbox"/>		Language	<input checked="" type="checkbox"/>		Network Information
<input type="checkbox"/>		Permissions	<input checked="" type="checkbox"/>		Sound
<input checked="" type="checkbox"/>		Storage	<input checked="" type="checkbox"/>		WiFi
<input checked="" type="checkbox"/>		Ethernet	<input checked="" type="checkbox"/>		Display
<input checked="" type="checkbox"/>		Network Folders	<input checked="" type="checkbox"/>		Network Printing
<input checked="" type="checkbox"/>		Email			

21 CFR Mode: Administrators can disable the “Discard” button from this screen if desired.

### Sound (enabled for all models in future release)

- Play a test sound.

## Storage

- Displays current storage statistics.

## Wi-Fi

- Turn Wi-Fi on or off and connect/disconnect from a network.
- Connect CellDrop to any 2.4 GHz network. SN-07409 and higher may also connect to 5 GHz networks.

## Country

- Select the country where the unit is installed in order to ensure the Wi-Fi radio uses the correct frequency bands.

## Ethernet

- Ensure that the Ethernet cable is connected.
- Tap the Edit button to configure Ethernet connection.

Contact your local IT department if additional help setting up Wi-Fi or Ethernet is needed.

## Display

- Includes controls for screen brightness and when the screen sleeps.

## Network Printing

- Search and add compatible network printers for easy export of hard copy reports.

## Email

### Email Set-up

- It is recommended that a free, dedicated Gmail or Outlook account be used for outgoing email from the instrument. Follow instructions to create a two factor authentication (2FA) “app passwords” for the account in order to ensure emails can use current SMTP and TLS protocols.
- Ensure that the instrument is connected to Wi-Fi or Ethernet.
- Launch the Settings app and navigate to the Email section on the left scroll menu.
- Enable the **POP3** option on your Email configuration. Do not use an IMAP account as messages from IMAP accounts could occupy a significant amount of the instrument's memory.

See Technical Note 205 on [denovix.com](http://denovix.com) for a step by step walkthrough of email setup.

Refer to your IT support group and CellDrop Technical Notes on [denovix.com](http://denovix.com) if additional assistance is required.

## Network Folders

CellDrop can export to SMB network folders within the same network the instrument is connected to. For best results connect CellDrop to the network through an ethernet connection, especially if exporting pdfs and images.

- Create a name for your network connection, then enter the network information required to connect to the network folder. Please consult your IT support group for server, domain, path and login details required.
- Press **Connect** to the connect to the network folder. Press **Send Test File** to transfer the text file “denovix.txt” to the network folder. The txt file content will include a time stamp.

A screenshot of a network configuration dialog box. It features six input fields labeled 'Name:', 'Server:', 'Domain:', 'Path:', 'Username:', and 'Password:'. Below these fields are three buttons: 'Connect', 'Send Test File', and 'Cancel'. At the bottom right, there are two buttons: 'Cancel' and 'OK'.

### Updater



This app can be opened by Administrator level user accounts. Updater is used to update CellDrop software and firmware to the most current available versions. Updates may be downloaded using the Updater app when connected to Ethernet or Wi-Fi. An Ethernet connection is recommended for software updates.

Updates may be downloaded on line or downloaded to a FAT32 formatted USB drive from [denovix.com](http://denovix.com).

### ScreenLocker



Launch the ScreenLocker app from the Home screen prior to cleaning the touch screen. This will disable swiping motions from activating other software activities. Tap the home button to exit.

### Diagnostics



*This app is not yet implemented and may be included in a future software release.*

## 12. 21 CFR App

### 21 CFR



DeNovix 21 CFR Part 11 Compliance Ready Software is available for activation on CellDrop instruments. An activation code is required and can be purchased from DeNovix or a DeNovix distributor. Once activated, 21 CFR Mode provides a suite of seamless, integrated controls for electronically signing records, managing user accounts, configuring the organization and accessing the audit trail. The software enables the following:

- User login with unique User ID and password for each user.
- Operation as a closed system with a single, continuous period of controlled system access.
- Users review results and electronically sign records while logged in. Records can be electronically signed with a Signature Meaning that is associated with the user's Role.
- Automatic export (back up) of electronically signed records to a second location when signing.
- Export of human readable pdf reports and optional raw data such as images and csv files. The pdf report includes details of each electronic signature associated with the record.
- A user interface for viewing stored information but no ability to directly access and alter electronically stored data.
- Administrator tools to customize and manage the organization's Roles, Signature Levels and Signature Meanings.
- Administrator tools for User Account management including Role assignment, activation, deactivation, name changes and password resets.
- An Audit Trail function to automatically log system events and user activities in a database. If audited, System Administrators can easily search the Audit Trail and export a human readable .csv report as desired.
- A convenient configuration wizard for easy set up and customization during activation.

### Activation Wizard Overview

The activation wizard is a step-by-step procedure for setting up CellDrop to comply with your organization's needs. Default values are included but are fully configurable during activation. 21 CFR Mode:

- Each user is assigned to Role
- Each Role has a Signature Level which establishes a rank in the organization
- A set of Signature Meanings must be defined (tested, rejected, approved, etc.)
- Roles are linked to one or more Signature Meanings, meaning that any User who is assigned to a Role can electronically sign a record with one of the available signature meanings.
- Once the organization rules are configured, each user is assigned to a Role. This establishes the signature privileges of each user.

### Activation Wizard 1/7 - Administrator Account

To begin, open the 21 CFR app and select an Administrator level account from the Account selector at the top of the screen. If a user created Administrator account isn't selected or none exists, the user is prompted to create a new Administrator account.

When 21 CFR Mode is activated, the open access accounts "General" and "Primary Admin" are deactivated. For PAYG systems, any existing count balance in the General account is automatically

transferred to the Administrator activating 21 CFR Mode. The Administrator can use the Count Code app to redistribute counts.

### Activation Wizard 2/7 – Enter Activation Code

Enter the twenty-digit activation code and press Apply Code.

#### Activation Code 2/7

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21 CFR Part 11 compliance ready software can be activated on this instrument. Please enter your activation code. To purchase an activation license, US customers should contact DeNovix and international customers should contact their local DeNovix Distributor. Please include the serial number in your request.

Enter Code:                    -                    -                    -                    -

### Activation Wizard 3/7 - Roles

Users will be assigned to a Role in the final step of activation. In this step set up the Roles for the organization. Each Role includes a value for Signature level. The Signature Level value establishes a hierarchy in the organization. Users can view data for users at their Signature Level and below. But they cannot view data for Roles with higher Signature Level number.

#### Configure Organization (Roles) 3/7

---

Define the Roles for the organization. These may be changed later by a System Administrator

Role	Signature Level
System Administrator	1000
Principle Investigator	900
Lab Manager	700
Sr. Scientist	500
Scientist	400
Sr. Technician	300



Next

### Activation Wizard 4/7 – Signature Meaning

Each User, through their assigned Role, will be able to electronically sign records with the Signature Meanings as defined in this table. Configure the Signature Meanings as required for the organization but using the add, edit and delete icons. At least one Signature Meaning must exist.

### Configure Organization (Signature Meaning) 4/7

Define the signature Meanings for the organization. Permitted Signature Meanings will be assigned to Roles in the next step of configuration. These may be changed later by a System Administrator.

Signature Meaning
<input type="checkbox"/> Tested
<input type="checkbox"/> Reviewed
<input type="checkbox"/> Approved
<input type="checkbox"/> Released
<input type="checkbox"/> Rejected

Previous

Next

### Activation Wizard 5/7 – Role-Signature Meaning Linking

In this step, set the Signature Meanings for each Role. Select a Role from the table and then check Include to allow that Role to electronically sign records with that particular Signature Meaning. In the example shown, the Sr. Technician Role can e-Sign records with Tested, Reviewed and Edit sample name/description Signature Meanings. Any Role must be linked to at least one Signature Meaning.

### Configure Organization (Role - Signature Meaning) 5/7

Select a Role and then check boxes to assign the Signature Meanings permitted for each Role. These may be changed later by a System Administrator.

Role	Signature Meaning	Include?
System Administrator	Tested	<input checked="" type="checkbox"/>
Principle Investigator	Reviewed	<input checked="" type="checkbox"/>
Lab Manager	Approved	<input type="checkbox"/>
Sr. Scientist	Released	<input type="checkbox"/>
Scientist	Rejected	<input type="checkbox"/>
Sr. Technician	Edit sample name/description	<input checked="" type="checkbox"/>

Previous

Next

### Activation Wizard 6/7 – Password Rules

Set password rules as desired for the organization. Some requirements are optional. Minimum password length and account locking after failed attempts to login are requirements of 21 CFR Part 11 compliant software.

### Configure Password Rules 6/7

Set the password rules for the organization

Rule	Required	Quantity
Minimum password length	<input checked="" type="checkbox"/>	6
Require special character	<input type="checkbox"/>	0
Require capital letter	<input checked="" type="checkbox"/>	1
Require numeric character	<input checked="" type="checkbox"/>	1
passwords automatically expire (days)	<input checked="" type="checkbox"/>	90
maximum number of login failures before account is locked	<input checked="" type="checkbox"/>	5

Previous

Next

### Activation Wizard 7/7 – Existing Users

The final step of the Activation Wizard is to complete the user records for any existing user accounts that are on the system. Each user must have a User ID, First Name, Last Name, Role and a temporary password set in order to complete the wizard. Edit user accounts by selecting them from the list and then using the edit icon.

Note: This is last opportunity to delete existing user accounts. After 21 CFR activation is completed, user accounts may be deactivated, but they may not be deleted.

### Existing Users 7/7

Select a Role and then check boxes to assign the Signature Meanings permitted for each Role. These may be changed later by a System Administrator.

User ID	First Name	Last Name	Role	Password	Complete?
CFRAdmin	James	Smith	System Administrator	Done	<input checked="" type="checkbox"/>
Ashelby	Arthur	Shelby	Lab Manager	Done	<input checked="" type="checkbox"/>
APowers	Austin	Powers	System Administrator	Done	<input checked="" type="checkbox"/>



Previous

Finish

### Audit Trail

DeNovix 21 CFR software continually creates an Audit Trail which is a time-stamped log of events and changes that take place in the system throughout the life of the instrument. Administrators can search the audit trail to filter the results by date, Event Type, User ID and Signature Meaning.

21 CFR SEARCH REPORT CFRADMIN

**Audit Trail Search** Add To Report

Start Date End Date

Event Type - All

User ID - All

Signature Type - All

Search

Account	Event	Signature	Date
CFRAdmin	Signature	Released	2020 Aug 4 16:12
CFRAdmin	Record Management		2020 Aug 4 16:11
CFRAdmin	Signature	Approved	2020 Aug 4 16:11
CFRAdmin	Record Management		2020 Aug 4 16:11
CFRAdmin	Signature	Approved	2020 Aug 4 16:10
CFRAdmin	Signature	Approved	2020 Aug 4 16:10
CFRAdmin	Record Management		2020 Aug 4 16:05
CFRAdmin	Record Management		2020 Aug 4 16:03

Event types tracked include Signature, System, User Account, Record Management and Application/Machine errors. Some examples of logged events are the electronic signature of a result, deactivating a user, a failed login attempt, exporting a result and archiving a record. This list is only representative and is not a complete list of all actions logged.

Set the filters as desired and press Search to update the result list. Select records and press “Add to Report”. Move to the Report Tab to see additional information about each audit trail entry. To export a copy of the Audit Trail as a .csv file, select records, press the export icon and select the destination.

21 CFR SEARCH REPORT CFRADMIN

**Audit Trail List** Clear

Account	Result ID	Event	Signature	Comment	Date
CFRAdmin		System		Update user information CFRAdmin, John, Smith, System Administrator Exported	2020 Aug 4 15:43
CFRAdmin	0426620200804160249	Record Management			2020 Aug 4 16:03
CFRAdmin	0426620200804160452	Record Management		Exported	2020 Aug 4 16:05
CFRAdmin	0426620200804160452	Signature	Approved		2020 Aug 4 16:10
CFRAdmin	0426620200804160452	Signature	Approved		2020 Aug 4 16:10
CFRAdmin	0426620200804160452	Record Management		Exported	2020 Aug 4 16:11
CFRAdmin	0426620200804160452	Signature	Approved		2020 Aug 4 16:11
CFRAdmin	0426620200804160452	Record Management		Exported	2020 Aug 4 16:11

**Archiving**

Results that have been previously exported from the instrument may be permanently deleted through use of the Archiving function in the 21 CFR app. This is the only mechanism for removing data from the instrument. Prior to removal, the Administrator should confirm records that will be removed have been properly backed up to another location. Use the Data app to export additional copies of the electronically signed results prior to archiving. **Archiving immediately and permanently deletes all data and images associated with the signed record. Do not archive a record before confirming a secure copy of the record exists in a secondary location or has been properly managed according to the organization’s records retention policies.** All Archiving events are captured in the Audit Trail database.

Filter the results by date, App, User ID and Signature Type as desired. Touch Search to update the search results. Select desired results and then Add to Report.

Signature/UserID	Sample	Date
Tested CFRAdmin, Reviewed CFRAdmin	Sample Name	2020 Aug 5 17:22
Rejected APowers	Sample Name	2020 Aug 4 17:21
Tested APowers	Sample Name	2020 Aug 4 17:20
Tested APowers, Released CFRAdmin	Sample Name	2020 Aug 4 17:19
Tested APowers	CHO	2020 Aug 4 17:16

The Report tab view shows if a result has been previously exported from the system. Only results that have been exported can be selected for archiving. Touch the delete icon to initiate archiving. The system Administrator must enter their password to complete archiving. **The archived records are immediately deleted. Since the record has been previously exported, an additional backup copy is not created at the time of archiving.**

Exported	Date&Time	Signature/UserID	App	Sample ID	Count	Sample Name
<input checked="" type="checkbox"/>	2020 Aug 4 17:16	Tested APowers	AO/PI	0426620200804171653	139	CHO
<input checked="" type="checkbox"/>	2020 Aug 4 17:19	Tested APowers, Released CFRAdmin	AO/PI	0426620200804171901	153	Sample Name
<input checked="" type="checkbox"/>	2020 Aug 4 17:20	Tested APowers	AO/PI	0426620200804172034	183	Sample Name
<input checked="" type="checkbox"/>	2020 Aug 4 17:21	Rejected APowers	AO/PI	0426620200804172118	207	Sample Name
<input checked="" type="checkbox"/>	2020 Aug 5 17:22	Tested CFRAdmin, Reviewed CFRAdmin	AO/PI	0426620200805172205	367	Sample Name

## 13. Troubleshooting and Maintenance

### Quick Help

Most issues concerning accuracy or reproducibility are sample, technique or protocol related and may be resolved by following the suggestions below. See Technical Note 186 – CellDrop Best Practices for additional details.

- Follow initialization instructions when powering on unit. Do not leave any materials between the sample surfaces. Clean with ethanol and press OK to complete the initialization.
- Clean sample surfaces between samples using a dry, folded laboratory wipe. Wipe both samples surfaces firmly in one direction.
- Do not over or under fill the counting chamber. Follow the Count button dispense volume directions. Volume is dependent on the chamber height specified in the protocol.
- Ensure that samples are monodispersions of cells in solution. Vortex or thoroughly mix with a pipette before loading into the count chamber.
- Ensure that cells are in focus before tapping the Count button. Live cells should have a sharp dark ring and a bright white center. Tap the (i) button in the focus adjustment control for example images of properly and improperly focused cells.
- Ensure the proper exposure is set for Trypan Blue and Fluorescence counts. Tap the (i) button in the focus adjustment control for example images of proper exposure. For fluorescence applications, the exposure of the green and red channels are adjusted independently.
- Ensure that all sample concentrations fall within concentration limits of the instrument for accurate results. When measuring samples with very low or very high cell densities, it is best to use an alternative chamber height setting and sample volume.
- Review the protocol settings used to make the sample count. Some parameters such as cell size limits, fluorescence intensity thresholds and cell roundness values may need to be empirically determined for the cell type and culture solution in question.
- Ensure that cells have stopped moving before tapping the Count button. The time for cells to settle may vary by cell type and should be determined empirically.
- Avoid introducing air bubbles into the sample solution when mixing or dispensing samples.
- Refer to the manufacturer of cell dyes such as acridine orange, propidium iodine or FDA for assay specific troubleshooting guidance.

### Software Errors

Pop-up messages will provide guidance for software-based errors or invalid user actions. In most cases, simply re-launching the app will allow the user to continue to make measurements. Update the software using the Updater app if experiencing a reoccurring software error.

Contact DeNovix Customer Support at 302-442-6911 or [info@denovix.com](mailto:info@denovix.com) if additional help is required. Please include the serial number of your instrument and all pertinent data when contacting Customer Support by email. The serial number may be found on the bottom of the instrument. Outside of the US, please contact your local distributor for assistance.

### Cleaning

Between Measurements:

- Use only a dry, lint-free lab wipe to clean top and bottom sample surfaces. Check the live preview to verify the sample has been removed.
- Optional: For additional disinfection or cleaning if sample residue has dried on the surface: Lower the arm and pipette 15  $\mu$ L of 70% ethanol or 10% bleach into the sample chamber. Wait approximately 10 seconds. Lift the arm and wipe both the top and bottom sample surfaces with a dry, lint free wipe.

Full Instrument Cleaning:

- To clean the entire instrument, spray 70% ethanol or 10% bleach onto a paper towel first and wipe down the surface of the instrument.
- **Warning! Do not use a spray bottle to douse the entire instrument.** Excessive liquid may damage internal components.

**Solvent Compatibility**

CellDrop Series instruments are compatible with most solvents typically used in life science laboratories.

**Exception: Do not use Hydrofluoric Acid (HF) as the fluoride ion will etch the surfaces of the measurement surfaces.**

## 14. Customer Support

DeNovix Inc.  
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Wilmington, DE USA 19810  
302-442-6911  
info@denovix.com  
www.denovix.com

Please include the serial number of your instrument when contacting Customer Support by email. If sending Customer Support a screen capture or a report file using the CellDrop email feature include your name, institute/company, phone number and return email address. **Please note responses will not be sent directly to the email account used by the instrument.**

Outside of the US, please contact your local distributor for assistance.

### Warranty

All product(s) and accessories sold by DeNovix Inc. are under warranty against manufacturing defects.

### Disclaimer

All information in this document is for reference purposes only. DeNovix Inc. makes no claims that this document is complete or error-free and assumes no responsibility and will not be liable for any errors, omissions, damage or loss that might result from any use of this document.

### Instructions for Disposal of Packaging and Waste Equipment

DeNovix encourages customers to locally recycle shipping and packaging materials. Corrugated cardboard and paper can be recycled wherever it is accepted locally (e.g., curbside bins, recycling facilities, etc.). For foam and plastics, please contact your local recycling office for information on how to recycle.

When an instrument has reached its end of life, DeNovix recommends that customers responsibly dispose of the equipment by following in line with local regulations. Material construction is available upon request. Visit [denovix.com/sustainability](http://denovix.com/sustainability) for more information.

### Research Use Only

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