

DeNovix[®]



CellDrop[™]

User Guide

December 2019

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1. Introduction

Product Technology

DeNovix CellDrop™ 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 app settings are 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.

This User Guide is designed for both CellDrop BF (Brightfield only) 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®
- 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 converting from:

- Pay-As-You-Go to Unlimited
- CellDrop BF (Brightfield only) to CellDrop FL (Dual Fluorescence plus Brightfield)

2. Set-up and Safety

1. Remove all packing materials including materials protecting the arm, sample surfaces and the protective cover on the screen.
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 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 %

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%

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:



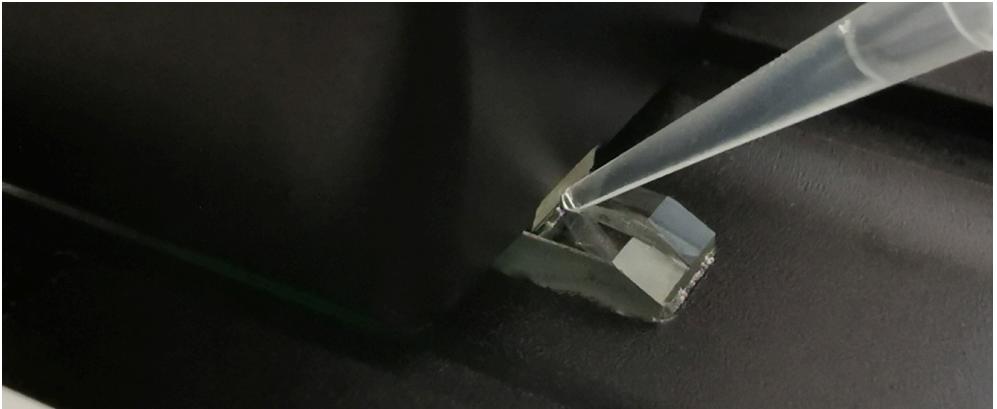
この製品は、技術基準適合証明に関する条例等特定無線設備(MPT省令第37号または1981)のタイプによって認定に準拠しています。この機器のカテゴリ：第2条、第1項のアイテム(19) 第2条第1項、項目(19)-2

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.

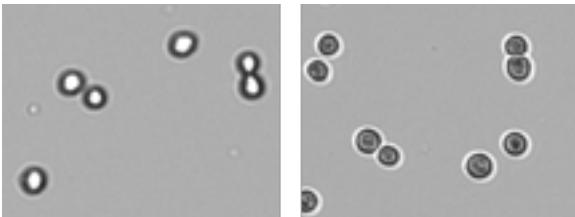
3. Quick Guide

Cell Count Procedure

1. Launch a 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.



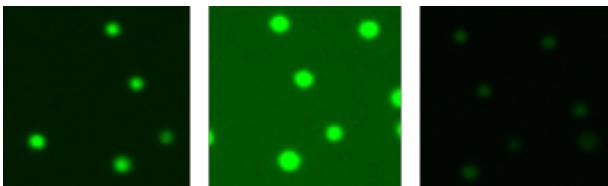
5. Allow the cells to settle.
6. Touch the focus icon and then adjust while viewing the Brightfield channel. Live cells should have a bright center and a dark outline. The image below on the left is correctly focused. The image on the right is out of focus.



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.

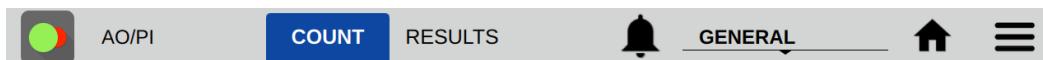
- Ensure that surfaces are clean prior to loading a sample into the sample chamber.
- If a previous sample has dried on the surfaces or if the sample is not loading easily, clean the sample surfaces. Lower arm and pipette 15 uL of 70% ethanol into the alignment groove and sample chamber. Wait 10 seconds 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.

4. 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.

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.
- **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.
 - 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 μm chamber height is equivalent to the 0.1 mm depth used on standard hemocytometers.

The default chamber height is 100 μm 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 μm	40 μL	7×10^2	1×10^5
100 μm	10 μL	5×10^4	1×10^7
50 μm	5 μL	1×10^7	2.5×10^7

Data Storage

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 may be deleted from the CellDrop at any time from the Data App. If the limits are exceeded, old data will be automatically deleted on a first-in, first-out basis.

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.

5. 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	AO/PI	AO	PI	GFP	Yeast	Custom Methods
 Brightfield	✓	✓	✓	✓	✓	✓	✓	✓
 Green Fluorescence			✓	✓		✓	✓	✓
 Red Fluorescence			✓		✓		✓	✓

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.

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

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 fluoresce 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).

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

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.

$$\% \text{ Efficiency} = (\text{GFP+ count} / (\text{GFP+ count} + \text{Brightfield count})) * 100$$

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.

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

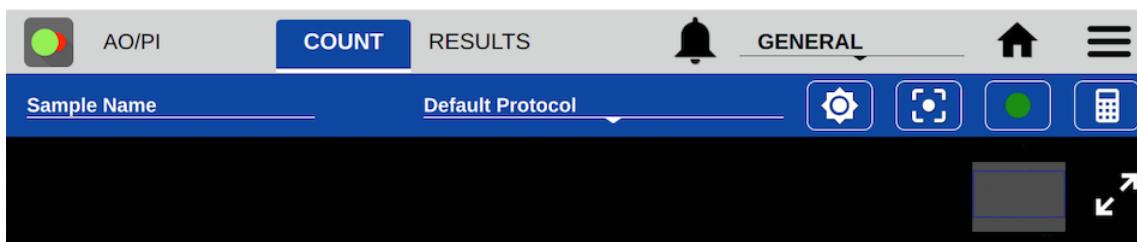
Custom Methods



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 dialog. 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

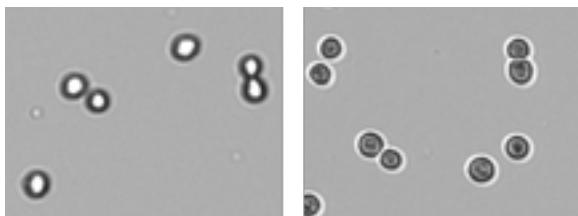


Green Fluorescence



Red Fluorescence

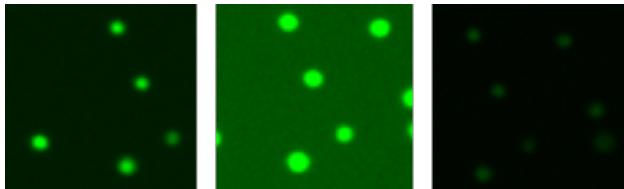
- Focus : The focus controls may be used when previewing any Count screen channel (brightfield, green or red fluorescence). Changing the focus when viewing one channel will automatically changes the focus of the other channels. Use the arrows to change the focus.
 - Adjust focus while viewing the brightfield 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.



- 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 app: Choose the correct exposure based on the concentration of trypan dye used.
 - 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.



- 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 with 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 contract 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 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 μm and is recommended for most counts. For very low density samples, choose 400 μm . For very dense samples, select 50 μm . The table below includes guidelines.

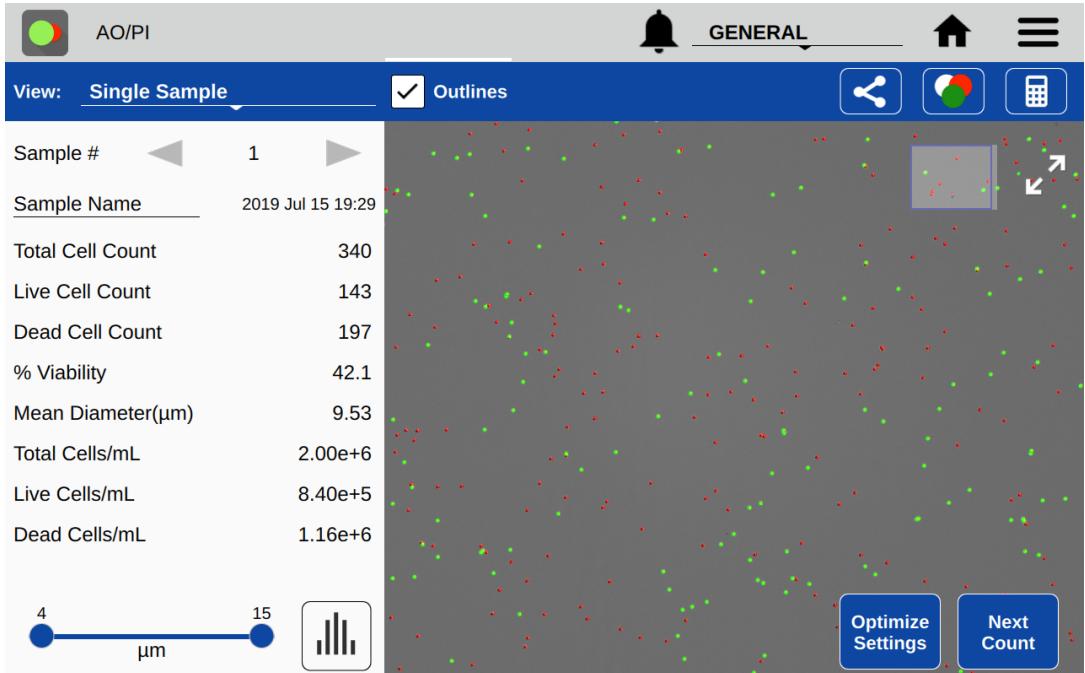
Gap Height	Required Volume	Minimum Density (cells/mL)	Maximum Density (cells/mL)
400 μm	40 μL	7×10^2	1×10^5
100 μm	10 μL	5×10^4	1×10^7
50 μm	5 μL	1×10^7	2.5×10^7

- **Small Cell Mode:** Small Cell Mode can improve brightfield channel counts of small cells near the 4 μm limit of detection. The additional algorithm complexity increases the length of the analysis.
- **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 μL of assay working solution to 5 μL of cell suspension, set Dilution Factor = 2.
 - For a mixture of 8 μL of assay working solution to 2 μL of cell suspension, set Dilution Factor = 5.
- **Diameter (μm):** 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 μm .
 - 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 round and completely outlined a cell must be to be included in the count. Reducing the roundness setting allows more elliptically shaped cells, irregularly shaped cells 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



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.

Optimize Settings			
	Current	[Original]	
Dilution Factor	2	[2]	
Diameter (minimum)	4	[4]	microns
Diameter (maximum)	30	[30]	microns
Live Roundness	1	[1]	(1-20)
Dead Roundness	1	[1]	(1-20)
Green Fluorescence Threshold	1	[1]	(1-100)
Red Fluorescence Threshold	1	[1]	(1-100)
Outline			
<input checked="" type="radio"/> Enhanced	<input type="radio"/> Circle		[Enhanced]

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.

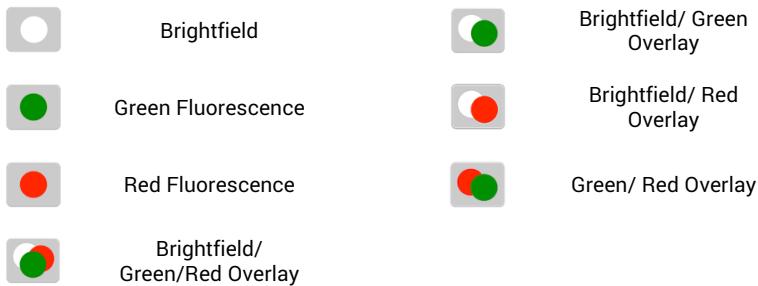
Cell Size Gating

- 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 only)

- 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 and Network Printer.
- 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.

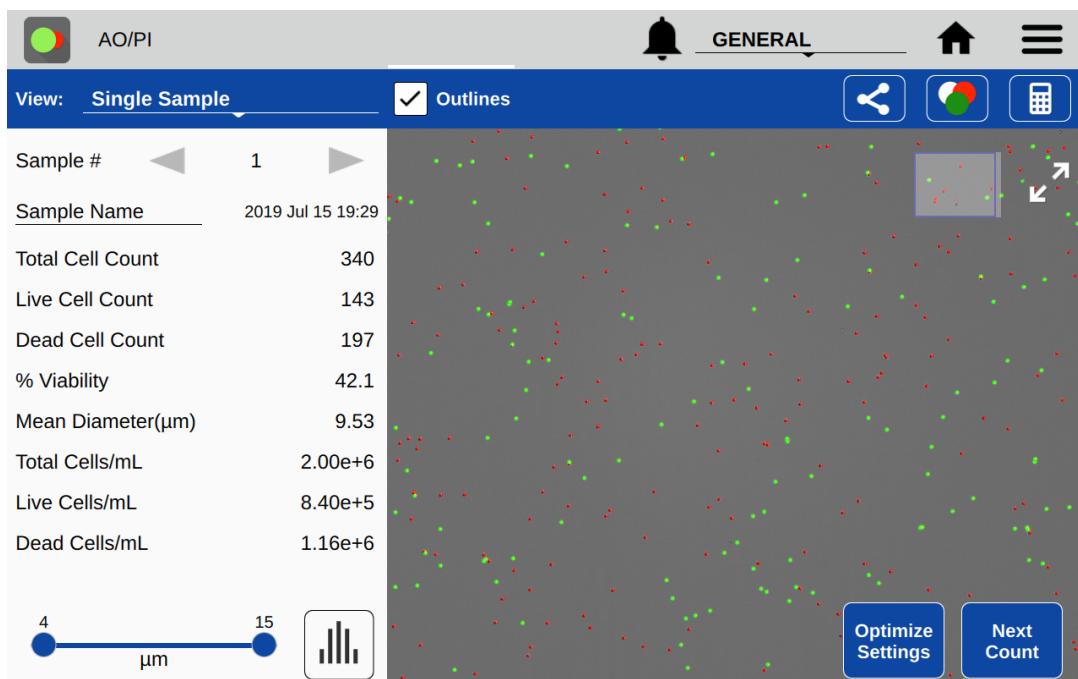
Additional Action Bar Controls



- 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.
- Delete: Tapping this icon will delete the highlighted samples from all results lists.

Results View



- 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. Changes will overwrite the information stored in the database and will be applied to all lists referencing the sample.
 - 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.

9. 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. Options include:

- Screen Captures
- Data Export for results and protocol settings, images, and histograms

Note: 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.

Screen Capture

The screen capture feature accessed via the overflow menu and 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 dialog boxes. The first dialog is used to select the desired combination of results, images and histograms to include in the export. The second dialog is used to select the export destination.

Export Selections

- Results and Protocol Settings - The calculated results and protocol settings may be exported either as .csv, .pdf, or .png files.
 - .csv: The results and protocol settings will be presented in a 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.
 - .pdf: The results and protocol settings will be presented in a table format. The results table will include only the fields included on the Results tab Single Sample View.
 - .png: The selected images will be exported as .png files.
- When results are not included in the export selection, images and histograms will be exported as .png files.
- Images: Both raw and annotated (showing the counted cells) may be exported. When the results and protocol settings are exported in the .pdf format, the images will be included in the .pdf document. When the results and settings are exported in the .csv format, the images will be exported as .png files.
- Histograms: The cell size distribution and/or the cell cluster histogram may be selected for export. When included with results and setting tables, they will be included in a .pdf file. When selected alone or in combination with images only, histograms will be exported as .png files.
 - Note: When exporting histograms for multiple samples using the Export function, the graphs will be single sample cell distribution or single sample cell cluster graphs. To export histograms with multiple samples overlaid as is available on the Results Tab Graph screen, use the Overflow menu Screen Capture option.

Export Destinations

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

10. Utility Apps

User Accounts



The User Accounts app allows users to create and manage individual accounts on CellDrop. Users can create user-specific protocols and 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.

Email Addresses

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

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.

Data



This app allows the user to search for all saved data filtering by date, app name, protocol name and/or sample names. Data can then be added to the Result tab screens for review and export. In addition, data may be archived from the Search Screen.

- 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 Results** 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 Result tab.
- Features and functions previously described for the Results tab screens apply to the corresponding Data app 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 Data

- 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.

If the Cancel button is tapped during the archiving process, samples already copied up to the stop point may be deleted from the instrument at the user's discretion.

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- the drop-down menu options include:

- Enter New Code
- Review Code History
- Transfer Counts

Administrators may transfer unused counts between accounts on the instrument.

For Standard accounts- the drop-down menu options include:

- Enter New Code
- Review Code History

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



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

Timer



The Timer app enables the setting of up to two timers at one time. An alarm will sound 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:

System Info

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

Date/Time

- Select manual or automatic date and time options.

Language

- Although the custom operating system provides translations for some routine functions in many languages, the majority of the app specific text will be available in a limited number of language options.

Network Information

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

Permissions

This selection is used to enable the following options:

- Automatic Archive: Use this selection to enable or disable all standard accounts, including the General Account, to archive data from the instrument. Refer to the Delete Data section for additional information.
- Network Folders: Use this selection to enable or disable the general account and/or standard user accounts to set-up network folders for data export.

Sound

- Volume controls are available for media and timer alarms.

Storage

- Displays current storage statistics.

WiFi

- Turn Wifi on or off and connect/disconnect from a network.

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 WiFi or Ethernet is needed.

Display

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

Network Printing

This feature is not yet implemented and will be included in a future software release.

Email

Email Set-up

1. It is recommended that a new, dedicated POP3 **Gmail** account be used for all outgoing email from the CellDrop. This type of account works very well with the instrument's operating system and prevents personal incoming email from being stored on the unit.
2. Ensure that the instrument is connected to WiFi or Ethernet.
3. Launch the Settings app and navigate to the Email section on the left scroll menu.
4. 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.
5. Input Outgoing Server Information:

Email Configuration	Example Input for Gmail
SMTP Server	smtp.gmail.com
Security	SSL
Port	465
Email Username	example@gmail.com
Email Password	Examplegmail123
Test Email To	info@denovix.com

Refer to your local IT support group if additional assistance is required.

Updater



This app 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 Wifi. An Ethernet connection is recommended for software updates.

Updates maybe downloaded on line or downloaded to a FAT32 formatted USB drive from 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 will be included in a future software release.

11. 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.

- Clean sample surfaces prior to dispensing a sample onto the measurement surface.
- 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 before loading into the count chamber.
- Ensure that cells are in focus before tapping the Count button.

- 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 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 uL 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.

12. Customer Support

DeNovix Inc.
3411 Silverside Road, Hanby Building
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.

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