CellDrop™ Best Practices

Technical Note 186

Introduction

This document provides best practices for obtaining the highest quality images for counting cells using CellDrop™ Automated Cell Counters. Techniques for proper sample loading, focus, exposure and surface cleaning are reviewed.

Sample Preparation

Cells should be trypsinized to completion to minimize clumps. Ensure that a sample is homogenous by vortexing or mixing immediately prior to loading. CellDrop EasyApps® Software contains sophisticated algorithms to identify individual cells within clumps. However, taking care to correctly prepare cells prior to loading will ensure that results are representative of the stock sample.

Sample Loading

1. Open the desired Count app and select a protocol.
2. Lower the arm. CellDrop requires the arm to be in the down position while loading.
3. Clean the sample surfaces if there is visible debris in the preview image. See "Clean Sample Surfaces".
4. Load pipette with the sample volume indicated on the Count button. Always use a fresh pipette tip.
5. Place the pipette tip in the alignment groove on the lower surface. Slide the tip against the upper sample surface and dispense the sample (Figure 1).
6. Avoid drawing sample back into the tip.
7. Let cells settle prior to adjusting focus and exposure or pressing the Count button.

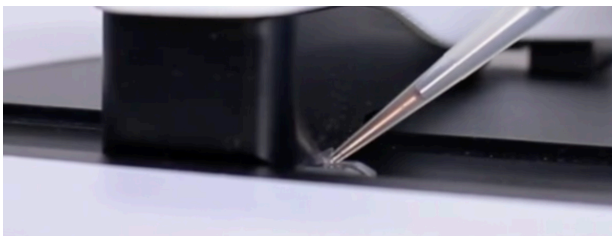

Figure 1: Loading. Rest pipette tip against upper surface while dispensing sample.

Focus

Correct focus is necessary for proper cell counts. Adjust focus of live cells while viewing the brightfield channel. Touch the focus icon to open the focus control panel. The software includes both coarse and fine focus controls. With optimal focus, the membranes of live cells will appear as a dark ring around a bright white center (Figure 2).

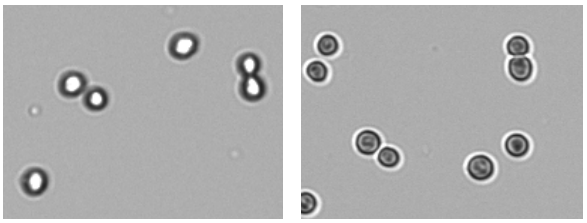

Figure 2: Optimal Focus. Correct focus (left) and poorly focused cell image (right).

Exposure

Trypan Blue App

To count cells using the Trypan app, first properly focus on the **live** cells in the sample, as shown in Figure 2. The Trypan Exposure settings are calibrated to work with commonly used trypan concentrations.

Use Normal exposure for a 1-to-1 solution of 0.4% trypan blue and cell suspension. Use Low exposure for a 1-to-1 solution of 0.2% trypan blue and cell suspension. Touch the exposure icon to select. Examples of proper and improperly exposed samples are shown in Figure 3.

Figure 3: Trypan Blue Exposure Examples. Correct exposure (left) with proper contrast. Over-exposed (center) with an image that is too light and has insufficient contrast. Under-exposed (right) with an image that is too dark to be properly counted.

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Fluorescence Exposure

After setting proper focus in the brightfield channel, use the channel selector to choose the green or red fluorescence live preview. Touch the exposure icon to open the exposure control panel. Adjust the exposure so that the intensity is maximized without allowing the signal to exceed the cell boundary (Figure 4).

Switch between the brightfield and fluorescence channels during this process. The fluorescence signal should not appear larger in diameter than the boundaries of the cell when viewed in brightfield. For dual fluorescence applications, exposure in each channel should be set independently.

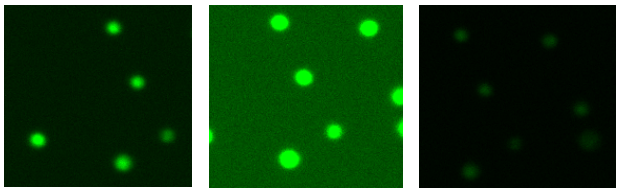

Figure 4: Fluorescent Exposure. Correct (left), over-exposed (center), under-exposed (right).

Count Settings, Protocols and Optimizing Results

The Default Protocol in each Count app includes count settings that are an appropriate starting point for counting most mammalian cells. Within any app, users may optimize settings for a result and save those settings in a user defined protocol.

While viewing a results, press the **Optimize Settings** button to display a list of parameters that can be edited. Technical Note 189 – CellDrop Count Settings includes detailed information about all count settings and how each impact the cell count. Press **Count with Current Settings** to reanalyze the image(s) using the new settings. Review the result and make additional changes as desired. Save the changes to a protocol using the "Save to Protocol" button. Use the Data app to review results and optimize settings of previous results.

Cleaning Sample Surfaces

CellDrop sample surfaces are constructed of optical grade sapphire and are highly resistant to lab chemicals and scratches. In normal operation, wiping with a dry laboratory wipe is sufficient for cleaning between counts.

If the cell suspension is not drawn into the sample chamber, clean the chamber surfaces and alignment grooves by flushing the chamber and groove with 70% ethanol as described below.

Improper loading technique, dried samples on the sample surfaces or routinely counting cell suspensions in media may require cleaning of CellDrop sample surfaces.

Procedure (video available at denovix.com)

1. Load 15 µL of 70% ethanol into a pipette.
2. Open the Brightfield app.
3. Position the pipette in the alignment groove so that ethanol will flush out any debris in the groove (Figure 5).
4. Dispense ethanol and wait 15 seconds.
5. Raise arm.
6. Firmly wipe both the upper and lower sample surfaces with a clean, dry laboratory wipe. For best results wipe two to three times in the same direction to prevent lint deposit.

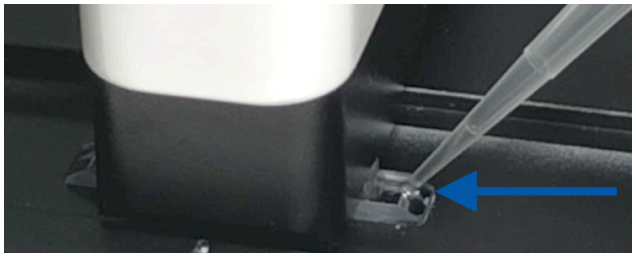

Figure 5: Pipette position for cleaning. Load 70% ethanol into the alignment groove.

See Technical Note 190 – CellDrop Cleaning for additional details related to general cleaning and disinfection.

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