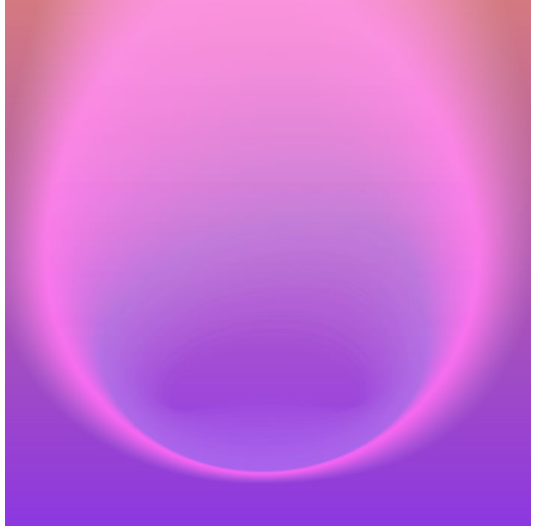
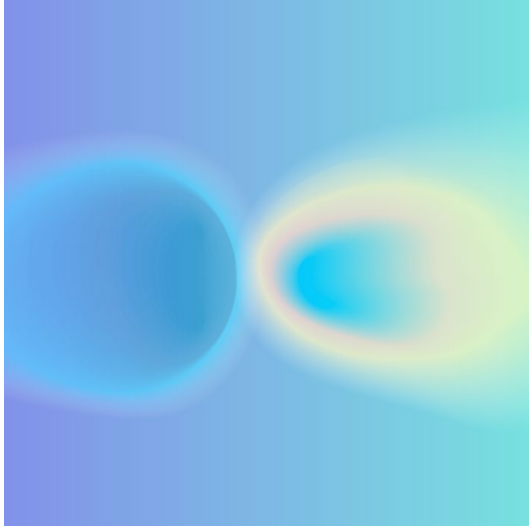
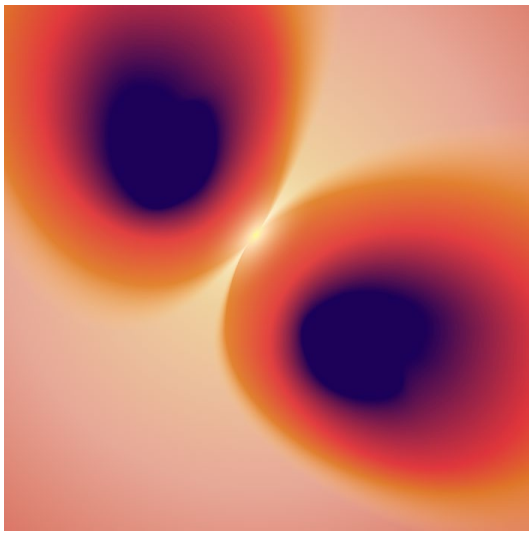




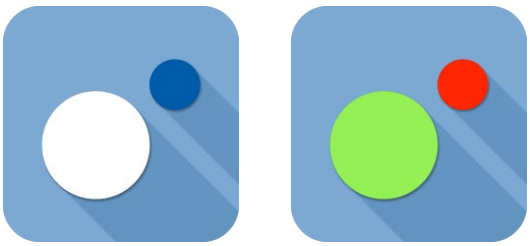


Automated Counting of Isolated Nuclei

Advances in Quality Control Methods for Single Nuclei Sample Prep

DeNovix®

Contents



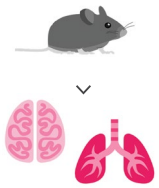
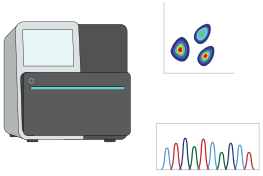
The Nuclei Trypan Blue (left) and Nuclei AO/PI (right) apps on the CellDrop™ Automated Cell Counter are optimized with protocols for isolated nuclei counts.

Keep reading to learn how to count isolated nuclei on the CellDrop.

Counting Isolated Nuclei

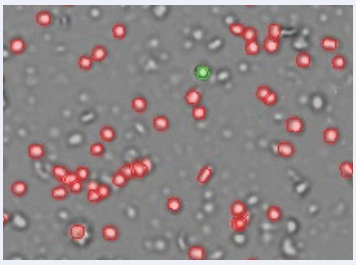
Isolating nuclei is critical for single nuclear workflows such as RNA-sequencing and ATAC-sequencing. Ensuring that non-clustered, debris-free samples are obtained is crucial to successful library preparation. Even when debris does not affect library preparation itself, it is important to count nuclei accurately for single-cell analysis.

Dual fluorescence counting using the CellDrop™ FL Automated Cell Counter can clearly distinguish nuclei, unlysed intact cells, and debris by acridine orange/propidium iodide (AO/PI) counting.

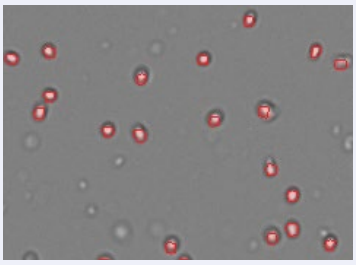
Tissue	Nuclei Isolation	Count with CellDrop	Downstream Applications
			
• Fresh Tissue • Frozen Tissue • Fixed Tissue	User-Formulated Buffer	• Nuclei concentration • Nuclei extraction efficiency • Cluster reports • Debris visualization	• RNA-seq • ATAC-seq • Flow Cytometry

Example Images: Isolated nuclei (red) & intact cells (green) counted with CellDrop + AO/PI dye

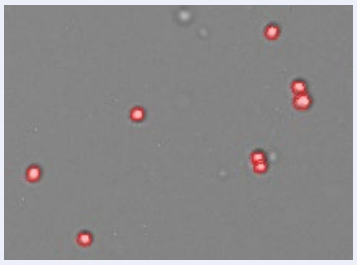
Nuclei extractions have been successfully tested on Frozen Mouse Kidney, Frozen Mouse Prostate, Frozen Rat Lung, Fresh Mouse Kidney, Fresh Mouse Lymph Node, Fresh Mouse Lung, and Fresh Mouse Brain. This list is not intended to be exhaustive, but simply to highlight testing completed in our lab. Many other tissues and methods are possible and have been successfully measured by our customers.



10X Genomics™ Nuclei Isolation Kit
Sample from Mouse Kidney



Miltenyi Biotec Nuclei Isolation Buffer
Sample from Rat Lung



User-Formulated Nuclei Isolation Buffer
Sample from Mouse Lymph Node

Nuclei Isolation Procedure & Considerations

Nuclei were isolated from HEK293T cell cultures according to the 10X Genomics® protocol for the "Isolation of Nuclei from Single Cell Suspensions. CG000124 Rev D." Before lysis, cell density and viability were assessed using the standard CellDrop AO/PI protocol to confirm a minimum of 2.5 million cells/mL at >90% viability.

Minimizing debris and large clusters is important for the downstream workflow of single-cell sequencing, since these can clog the fluidic chips resulting in low quality libraries or failed sequencing experiments. It is therefore critical for nuclei suspensions to be filtered to remove cellular debris post-lysis. Refer to the manufacturer's protocol if large clusters of nuclei are observed.

Similarly, removing intact cells that did not lyse during the procedure is also critical. Single-cell sequencing procedures such as those employed by 10x Genomics® rely on isolated nuclei for the technology to appropriately detect expression differences in a cellular population.

Many applications, such as ATAC-seq, require intact nuclei for the technique to work properly. Typically, the sample volume for such methods is a limiting factor, so using a single analysis volume for multiple quality control purposes can be beneficial.

CellDrop Automated Cell Counter

The unique DirectPipette™ technology of CellDrop Automated Cell Counters enables counting without disposable slides, and the variable chamber volume allows counting volumes of between 5 and 40 µL of sample.

The CellDrop is also compatible with common disposable plastic or reusable slides. This can allow the user to quantify the nuclear isolation on the CellDrop and transfer the same slide to a microscope with a higher magnification for nuclear integrity analysis.

The CellDrop has saved millions of plastic slides from disposal.

Watch Video

Counting Nuclei with AO/PI Stain

Acridine Orange and Propidium Iodide (AO/PI) are used to determine the success of a nuclei isolation. In traditional cell viability testing, the AO/PI dye combination stains live cells so they fluoresce green and dead cells fluoresce red.

However, the stain will also label successfully isolated nuclei red and any remaining intact cells green. This allows the user to calculate the residual intact cells that carryover as a percent of total counted and determine if the experimental workflow can proceed.

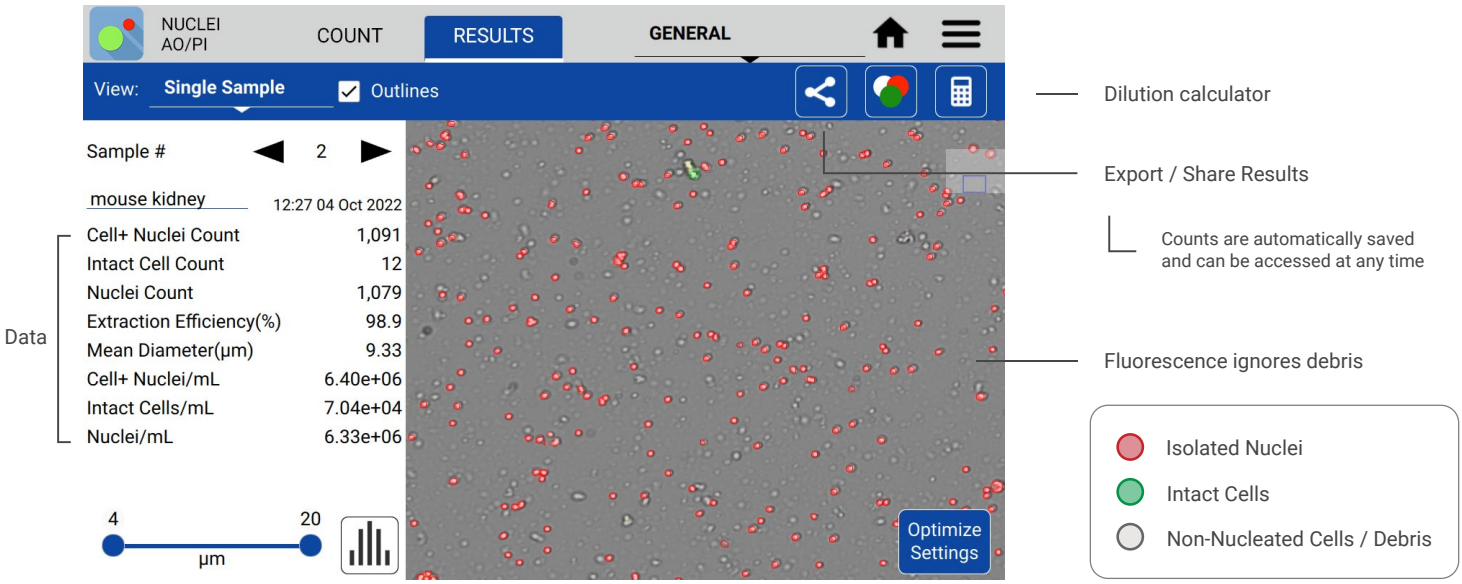
As the nuclear pore complex will allow passive diffusion up to 30-60 kDa, both AO and PI (~0.6 kDa) freely pass into the nucleus and will display a red signal due to a FRET interaction between the two fluorophores.

Minimizing the number of intact cells in the isolation is important, so accurately enumerating the intact cells with AO/PI can improve quality control and improve consistency in the results of downstream workflows.

Nuclei Isolation AO/PI Count

The following count (Figure 1) was made using the CellDrop Nuclei AO/PI App to analyze nuclei isolated from a mouse kidney sample. The CellDrop quantifies isolated nuclei (stained red) and the intact cells (stained green) to calculate the nuclei extraction efficiency for each sample.

This data is readily presented to the user on the results screen with other relevant sample data. The CellDrop fluorescence mode offers advantages, such as easily distinguishing background debris from nuclei, to increase the accuracy of results.



Nuclei were isolated using the Miltenyi Biotec gentleMACS™ Tissue Dissociator and Nuclei Extraction buffer.

Figure 1

Isolated nuclei result image from a mouse kidney sample counted on the CellDrop. Nuclei are stained red while leftover intact cells are stained as green.

Nuclei Count and Cluster Size Reporting

CellDrop data provides the user with a count of intact cells vs. nuclei for quality control purposes. All images are automatically saved to the large onboard hard drive and can be inspected for debris using the large HD touchscreen or exported using Wi-Fi, Ethernet or USB. Comprehensive reports can also be generated for printing to network printers or saving as PDF.

Single-cell sequencing technologies recommend lysing as a part of the sample prep to ensure size limits for microfluidics systems are not exceeded. CellDrop reports cluster size (Figure 2) enabling an additional quality control step. Clusters can be excluded from analysis using size gating options available in the software.

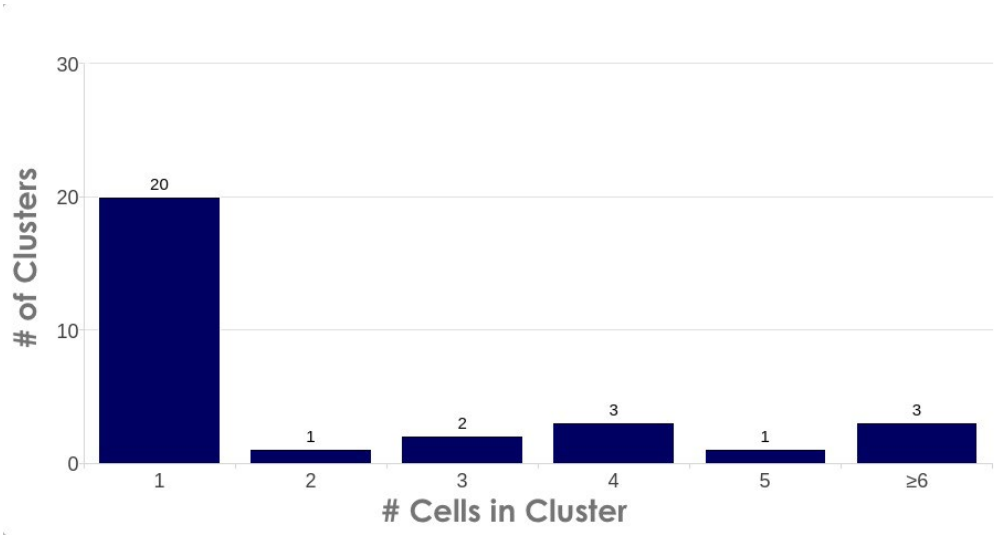


Figure 2

Count cluster data from the CellDrop. When CellDrop counts cells/nuclei it also analyzes the result for clusters of objects. The software allows the user to visualize the cluster size and the frequency of up to six different cluster sizes for each count on the device.

Counting Nuclei with Trypan Blue Stain

Where dual fluorescence instrumentation is not available it is also possible to analyze the success of a nuclear isolation using Trypan Blue (Figure 3, page 9).

DeNovix recommends the use of fluorescent assays for quantifying isolated nuclei where possible, due to the increased accuracy ensured by the clear differences in green and red signals.

It should be noted that counting debris laden samples using trypan blue can increase the number of erroneous counts either with an automated cell counter or by manual count.

Nuclei Isolation Trypan Blue Count

The following image (Figure 3) was taken in the CellDrop Trypan Blue Nuclei App during a measurement of HEK293T cells. The trypan blue dye enters all of the successfully extracted nuclei (circled in red) and stains them, giving them a dark appearance. The intact cells (circled in green) that were not successfully lysed exclude the trypan blue dye and appear white.

The numbers of nuclei and intact cells are used to quantify the extraction efficiency for a sample to ensure that enough nuclei are available for the subsequent experiments.

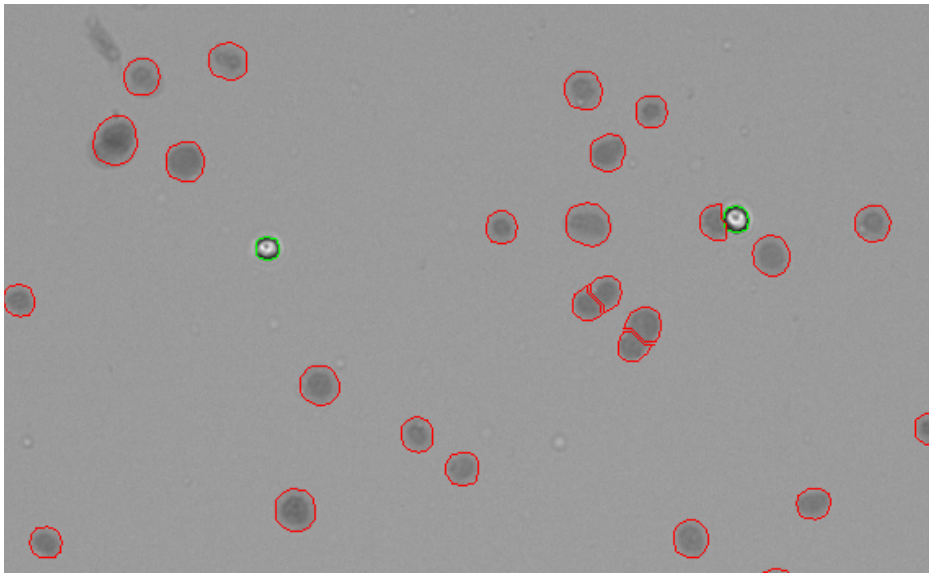


Figure 3

Isolated nuclei counted with trypan blue on the CellDrop. The CellDrop does an excellent job of recognizing isolated nuclei in a carefully purified isolation of HEK293T cells. The nuclei are stained dark, indicating uptake of the trypan blue dye (circled red), while intact cells exclude the dye and remain bright (circled green).

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- TN 215: Counting Isolated Nuclei from Mouse Brain
- TN 184: AO/PI Viability Assay Protocol
- CellDrop Automated Cell Counter Page
- Webinar: Comparing Fluorescence & Trypan Blue Automated Cell Counting

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