EDF™ Extended Depth of Field

An upgrade for the ImageStream® system

Think outside the dot.
Break the classic depth of field barrier

- Improve precision
- Enhance discrimination
- Simplify analysis
- Increase resolution
- Reduce acquisition time
- Enable new applications

Images showing the effect of different defocus levels on the resolution and discrimination of fibers.
EDF technology takes your ImageStream to an entirely new level of performance.

**Images the Whole Cell in Focus**
EDF uses a combination of specialized optics and unique image processing algorithms to project all structures within the cell into one crisp plane of focus. **Figure 1** shows the effect of progressive defocus on a test pattern of 2μm bars. **Figure 2** shows how the same pattern imaged with EDF technology maintains excellent image quality over the entire focus range.

**Figure 1**

**Figure 2**

**Enables High Throughput FISH In Suspension (FISHIS®)**
Counting FISH spots accurately requires that all spots be in focus. Since spots may be located anywhere in the nucleus, maintaining focus throughout the cell is critical. EDF technology expands the depth of focus over the entire cell to make FISH in flow a reality.

**Figure 3** Human male PBMC cells were stained with an X chromosome probe and imaged in standard and EDF modes. In standard mode, the FISH spots are detected but can be fuzzy and out of focus, impairing accurate chromosomal counts. In EDF mode, the spots are uniformly in focus throughout the depth of the cell. Automated FISH spot counting accuracy with EDF is dramatically improved. In standard mode, just 72 percent of the in-focus cells were characterized as having the expected single spot, while with EDF mode 98 percent of all cells exhibited a single FISH spot.

Based on Wavefront Coded™ technology developed by CDM Optics, Inc.
EDF technology dramatically extends the depth of field, enabling new applications and improvements in the imaging of cellular features. Here are some key enhancements:

**Improves Nuclear Foci Counting**
Radiation-induced DNA strand breaks can be identified by staining phosphorylated γ-H2AX. The ImageStream with EDF technology enables precise counting of the foci within the nucleus to assess damage on an individual cell level.

**Enhances Discrimination of Nuclear Translocation**
EDF technology enhances the discrimination of cellular features and improves precision in the quantitative analysis of cell imagery. EDF eliminates the need to apply a focus gate to the cell image data. For analyses that are sensitive to focus quality, such as NF-κB translocation, removing this subjective step from the analysis improves the overall reliability of statistical results.

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**Figure 4** Irradiated H1299 cells were labeled with AlexaFluor 488 to stain γ-H2AX and DAPI to stain the nucleus. The standard image panel and the EDF image panel show brightfield (column 1), DAPI (column 2), AF488 (column 3) and a composite of AF488 and DAPI (column 4). The resolution obtained with EDF mode reveals significantly more distinct spots, providing a more precise and accurate spot count measurement.

**Figure 5** The experiment shown here was designed to measure the translocation of NF-κB from cytoplasm to nucleus. A panel showing untreated cells and LPS-stimulated cells is shown as imaged in standard mode and EDF mode. The degree of translocation is quantitated by the degree of similarity between the NF-κB image (FITC stain) and the nuclear image (DRAQ5 stain), which is calculated for each cell. As shown in the histograms and indicated by the Rd values, EDF improves the discrimination between the untreated and LPS-stimulated populations.
**Increases Throughput**

In addition to keeping the whole cell in focus, the EDF extended depth of field upgrade allows the ImageStream to be run with a larger core diameter, thereby increasing throughput.

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**Allows Visual Confirmation of Leukocyte Cell Classification by Nuclear Morphology**

In traditional microscopic analysis, nuclear morphology is the gold standard for leukocyte classification. EDF improves the resolution of nuclear detail, thereby providing the means to confirm immunophenotypic classification.

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**Figure 6** Human whole blood was stained with FITC anti-CD45 (green) and DRAQ5 nuclear dye (red) and run on the ImageStream in standard and EDF imaging modes. The five major cell types are cleanly differentiated, and representative images of each are shown. EDF dramatically improves the imaging of nuclear chromatin structure and nuclear lobicity.

**Figure 7** In standard mode, the ImageStream is run with a 10μm core. EDF enables the use of a 16μm core with virtually no loss of cell focus, effectively increasing the throughput by up to 3X.
EDF Extended Depth of Field Option
The EDF upgrade to the ImageStream system includes all required modifications to the instrument and IDEAS software, installation, testing, documentation and user training. The EDF upgrade can be included with a new ImageStream system or installed as a field upgrade.

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