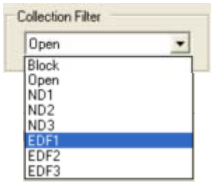
Quick Start Guide: EDF

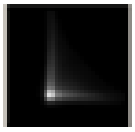
# Extended Depth of Field Imaging

*Extended depth of field (EDF) is a novel technique used in a variety of applications including FISH spot counting where having the entire cell in optimal focus is critical to obtaining accurate results. There are two steps to utilizing the 16um EDF; first images must be acquired with the EDF element in place, and second the data must be deconvolved using the EDF kernel prior to analysis. This guide describes how to collect and analyze data using the EDF element.*

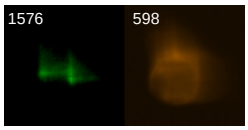
### *To collect a data file using the EDF element:*

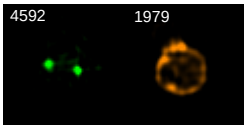
1. Follow the normal setup and data collection routine as described in the ImageStream Quick Start Guide.
2. Choose **EDF1** from the Collection Filter menu on the setup page.
3. Change the cell classification parameters to accommodate using EDF.
4. The calibration kernels saved during the last EDF calibration will be appended to the file and the file name will be appended with –EDF.



EDF image of a small bead showing characteristic L-shaped pattern




Cell images as they appear on the instrument with (left pair) or without (right pair) EDF element in place. Raw Max Pixel values indicated in upper left

### *General characteristics of using EDF:*

- The EDF element spreads all points of light within a cellular image into consistent L-shaped patterns. When EDF images are opened in ideas, the data is deconvolved to create an image of the entire cell projected simultaneously in focus.
- During acquisition and before deconvolution, images will appear blurred into characteristic L-shaped patterns and raw max pixel values will be lower with EDF than with standard mode collection.
- Compensation controls for EDF data can be collected with or without the EDF element in place.
- When analyzing data in IDEAS, after the deconvolution process there will be more light per pixel than in non-deconvolved imagery. Therefore, raw max pixel values may exceed 1023 (for the IS100 instrument) or 4095 (for the ISX). As long as the images did not saturate the camera during acquisition, these pixel values are valid.
- Object, Morphology and System Masks will be smaller in EDF mode.
- Focus gating is not required. However if there are blurred events due to streaking, these can be removed from the analysis using a focus gate.
- EDF images exhibit increased texture due to higher resolution.
- Brightfield imagery is not as crisp in EDF mode as in standard mode.
- An in-depth discussion of EDF can be found in the following reference:
  *Cytometry Part A (2007) 71A:215-231*

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