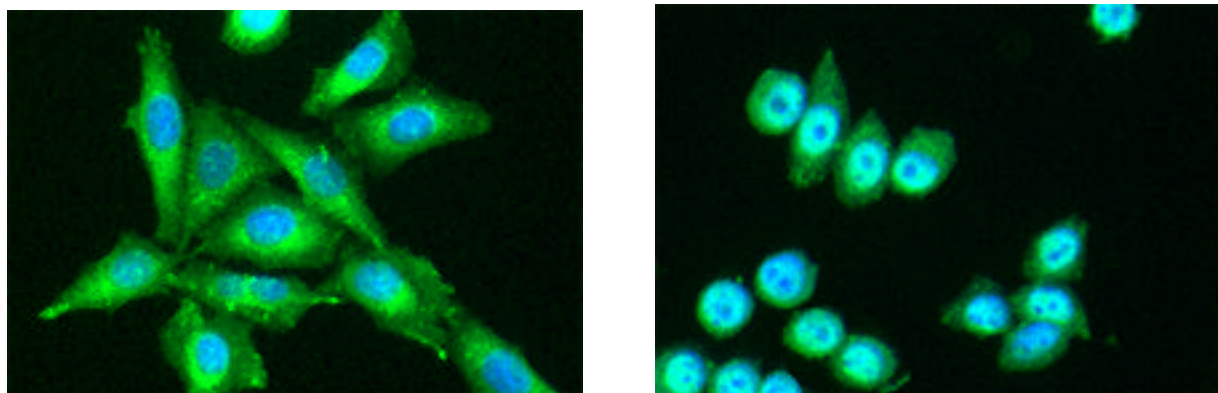


## Analysis of Nuclear-Cytoplasmic Translocation on the EIDAQ™ 100 High Throughput Microscopy System

*A quantifiable measurement of translocation for the transcription factor NF- $\kappa$ B is described, employing the EIDAQ™ 100 High Throughput Microscopy system from Q3DM. The movement of fluorescently labeled proteins between the cytoplasm and nucleus of the cell is monitored by measuring the distribution of fluorescence in defined sub-cellular compartments. For accurate reading, the EIDAQ™ 100 automatically images populations of cells, defines nucleus and cytoplasm, measures the amount of fluorescent signal in each, and then quantifies the proportion of labeled protein in each cell compartment as a measure of cellular response. Validated results of nuclear-cytoplasmic translocation using the EIDAQ™ 100 HTM system are presented with representative fluorescent images and dose response curves.*

### Background and Significance

Cell signal transduction pathways frequently activate the transcription of specific genes that co-ordinate programmed responses such as the initiation of cell division, exocytosis, differentiation, and apoptosis. Activation of certain transcription factors leads to the translocation of molecules from the cytoplasm of a cell to its nucleus. Once in the nucleus, these molecules bind to regulatory sequences in nuclear DNA. These molecules may be fluorescently labeled using antibodies, or by expression as a GFP fusion protein. Activation can be measured by the relative movement of the fluorescent label within the cell, from the cytoplasm to nucleus.



**Figure 1.** Example 20X 0.5 N.A. fluorescent micrographs of HeLa cells using the EIDAQ™ 100 High Throughput Microscopy (HTM) system. NF- $\kappa$ B is detected with Alexa 488 (green) and the nucleus is defined by Hoechst 33243 staining (blue), visualized before (left) and after (right) stimulation of NF- $\kappa$ B nuclear translocation. Note the clear but incomplete shift of green fluorescence from the cytoplasm to the nucleus.

Members of the nuclear factor kappa B (NF- $\kappa$ B) family of transcription factors are activated by multiple signal transduction pathways. Activation of a cytoplasm-sequestered complex called NF- $\kappa$ B /IKB results in the dissociation of the protein subunit NF- $\kappa$ B p65 from the complex. What follows is the translocation of NF- $\kappa$ B p65 from the cytoplasm into the nucleus, where it binds to specific regulatory sequences that initiate transcription.

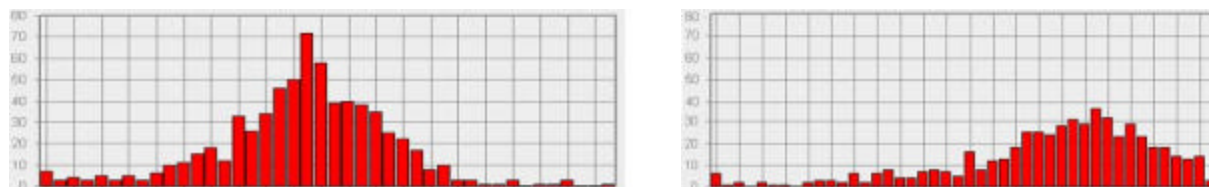
Key proteins in the NF- $\kappa$ B pathway are well recognized targets for the development of anti-inflammatory drugs. In addition, NF- $\kappa$ B has been found to promote tumorigenesis through the suppression of apoptosis and the stimulation of cell proliferation. Consequently, inhibitors of NF- $\kappa$ B translocation are considered to have important therapeutic benefits for the treatment of certain cancers.

### Experimental Methods

Human cervical carcinoma cells (HeLa) were grown in a monolayer and treated with tumor necrosis factor alpha (TNF- $\alpha$ ). NF- $\kappa$ B distribution in HeLa cells was detected using polyclonal p65 antibodies and Alexa 488 (green emission) following manufacturer's recommendations and nuclei stained with 1 $\mu$ g/ml Hoechst 33243 (blue emission). In another experimental system, human synovial fibroblasts were treated with a combination of cytokines in a dose response experiment as shown in Figure 3. Q3DM's proprietary metric FLIN (fractional localized intensity in nucleus) was used to automatically quantify the degree of translocation directly from images acquired using the EIDAQ™ 100 system.

### Experimental Results

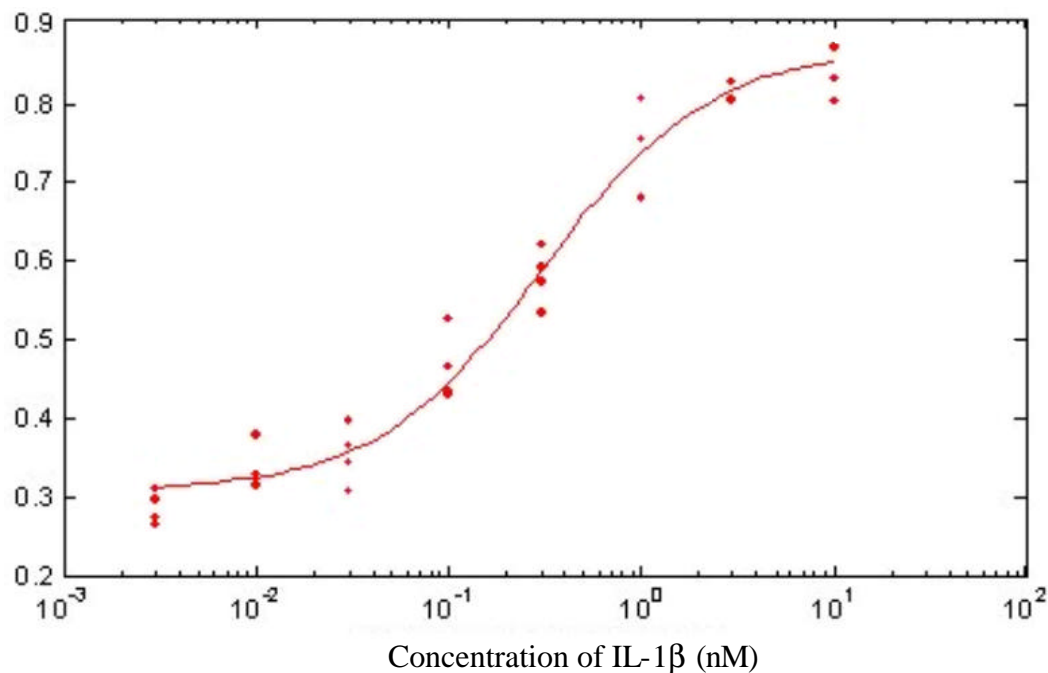
The combination of automated sub-micron imaging, proprietary image processing and computational geometric methods for cell compartment definition enabled the accurate measurement of transcription factor activation. Figure 2 shows two representative histograms of FLIN measurements taken from distinct populations of cells before and after activation.



**Figure 2.** *Distribution of the metric FLIN (X axis) among cells in the well. FLIN is defined as the fractional localized intensity in the nucleus, as a ratio measurement it is independent of cell to cell variations in signal intensity. Stimulation causes a shift to higher values of FLIN.*

The mean FLIN response is generally reported on a well-by-well basis to measure dose response.

Representative data are shown in Figure 3. In this experiment, the NF- $\kappa$ B activation by interleukin 1 beta (IL-1 $\beta$ ) in synovial fibroblasts is determined using FLIN. The resulting EC50 value of 0.3 nM is consistent with values reported in the literature.



**Figure 3.** Four replicate wells are shown for each of an 8-point dose response curve demonstrating nuclear translocation of NF- $\kappa$ B induced by interleukin 1 beta in synovial fibroblasts. EC50 0.3nM (mean std error 0.0002)

### Conclusion

Q3DM's proprietary metric FLIN (fractional localized intensity in nucleus) provides a sensitive and accurate measurement of protein translocation from cytoplasm to nucleus. The EIDAQ™ 100 is used to generate data that measures translocation of the transcription factor NF- $\kappa$ B in both human cervical carcinoma cells (HeLa) and human synovial fibroblasts. Q3DM's proprietary technologies enable researchers to monitor fluorescence signal variations across multiple sub-cellular compartments.

Though data shown here are specific to NF- $\kappa$ B response, the same techniques are used to observe many other proteins that translocate between nucleus and cytoplasm. These data are clear examples of the powerful combination of robust autofocus, rapid high-resolution imaging and advanced image processing working together to generate valuable datasets for drug discovery, clinical diagnostics, and basic research.

**The EIDAQ™ 100 automated High Throughput Microscopy (HTM) system from Q3DM Inc. delivers an unmatched combination of speed, accuracy and precision to quantitative imaging and analysis of cell populations. The EIDAQ™ 100 is used to accelerate drug discovery, for clinical diagnostics, and in basic research.**