**In situ miRNA Expression Profiling using Multispectral Imaging to Elucidate Pathways Involved in Neurodegenerative Disease**

**Introduction**

Non-coding RNAs are emerging as important mediators of epigenetic regulation. Significant effort has been devoted to screening for miRNA signatures and identifying functional and clinical links between non-coding RNA expression patterns and various normal and disturbed physiological states. miRNAs constitute one class of post-transcriptional gene regulators that have been shown to be differentially expressed during development and in many clinical disorders including cancer. Understanding the roles of epigenetic regulators and potentially linking these to their tissue-specific expression patterns may open up new possibilities for diagnosing and staging cancer, and ultimately contribute towards the development of more effective therapeutics.

Until recently, the study of miRNA expression patterns has been limited to the estimation of their levels using high-throughput biochemical techniques such as quantitative polymerase chain reaction (qPCR) or using highly multiplex-able array-based techniques that offer exquisite sensitivity but suffer from a key drawback of many of the high-throughput biochemical assays face – they are, in essence, measurements sampled from an average over a heterogeneous collection of cells, whereas much of the revealing detail can only emerge through studies at the individual cell level. Several studies have shown that the numbers of mRNA molecules expressed by individual cells of an identical genotype correlate poorly with the averages reported by qPCR, and one would expect, based on the pleiotropic nature of miRNAs, that they would fare worse in such assays. Moreover, it would be advantageous to examine additional multiple molecular signals, such as proteins and other nucleic acid species, simultaneously, at the same spatial scale, thus requiring generalizable in situ methods.

**miRNA detection in a control vs. labeled human hippocampus specimen**

*Figure 1A.* An unstained control showing spectral separation of non-specific autofluorescent species using the Nuance multispectral imaging system.
Microscopy-based multi-analyte immunofluorescence (IF) and immunohistochemistry (IHC) methods offer the benefit of visualizing miRNAs within the context of disease-specific molecular anatomy. However, accurate imaging of two or more co-localized antigens, especially chromogenically labeled ones, has been hindered by difficulty in discriminating and quantifying overlaying signals. Immunofluorescence is well suited for multiplexed imaging approaches but can be confounded by the prominent autofluorescence signals present in many tissue samples.

These challenges can be addressed using commercially available multispectral imaging (MSI) technologies. Caliper’s microscope-based Nuance systems allow the simultaneous imaging and quantitation of multiple analytes, even in the presence of spatial and spectral overlap. Multispectral imaging methodologies can spectrally characterize and computationally eliminate autofluorescence, revealing otherwise invisible molecular targets.

**MultiSpectral Imaging of miRNAs in Parkinson’s and Alzheimer’s Disease**

Great strides have been taken in the study of miRNA expression profiling in an effort to elucidate pathways involved in neurodegenerative diseases. We provide a specific example demonstrating how multispectral imaging can be utilized to visualize and quantitate miRNA expression in the hippocampus of diseased brain tissue (Figure 1).

Multispectral imaging (MSI) is an approach that optimizes the opportunities for multiplexing and at the same time can overcome the effects of autofluorescence on detectability and reliable quantitation. The Caliper Nuance multispectral imaging system combines a unique liquid-crystal tunable filter coupled with a scientific-grade CCD camera along with sophisticated but simple-to-use unmixing algorithms, providing molecularly specific information with unmatched spatial and spectral precision.