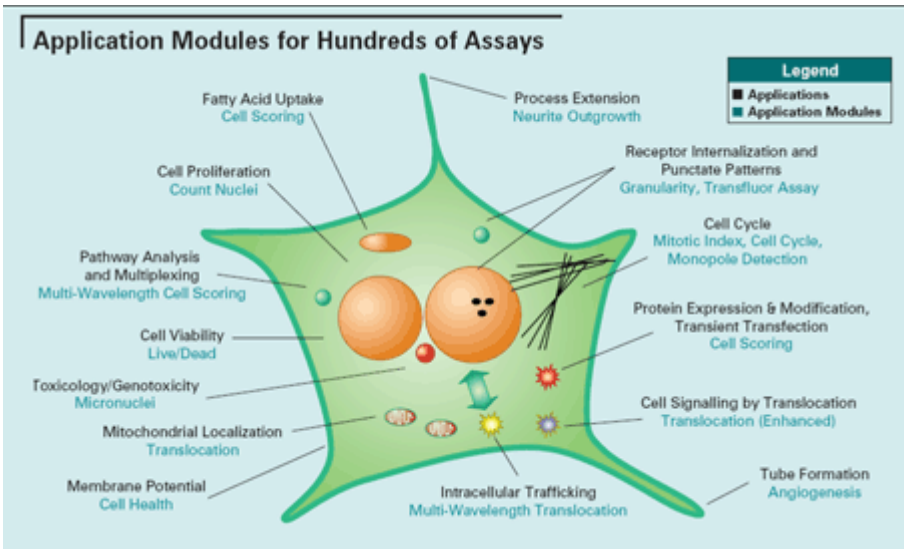
Documentation MetaMorph Software Application Modules

Combine ease-of-use & flexibility to accommodate automated image analysis for hundreds of applications in both research and high throughput environments.

A modular toolbox for image analysis, the suite of MetaMorph® Software Application Modules enable both novice and expert users to set up hundreds of assays in minutes without compromising the mature image processing algorithms required to address complex applications. The Application Modules provide an interactive environment to determine features of interest based on size, intensities, and distances. Some modules handle very specific and challenging assays (e.g., neurite outgrowth) while others (e.g., multi-wavelength cell scoring) can be configured to quantify dozen of different cellular events. To further tailor analysis to your research-oriented goals, journals can be amended to Application Modules, allowing to customize any analysis.



***12 Application Modules** allow MetaMorph Software users to automate common analysis routines and accommodate 100's of applications.*

The Application Modules don't sacrifice quality for throughput and ease of use because they are designed to automate microscopy in either High Content Screening (HCS) or research imaging settings and use superior segmentation and feature extraction. All application modules are equipped with a proprietary adaptive background correction algorithm, adapting image segmentation to the local intensity ranges and shape features within and between cells. This feature helps mitigate poor sample quality and provides the most robust segmentation available in an image-based system. The newest developments in image analysis and a large quantity of outputs (extracted features) are available. For example, the micronuclei Application Module provides more than 90 outputs and a proprietary algorithm to easily discriminate mono-, bi-, and multi-nucleated cells in genotoxicity experiments. Analysis results are saved at the cell-by-cell and image-by-image level with segmentation overlays and can be reviewed interactively by selecting a cell and visualizing the corresponding analysis data or vice-versa.

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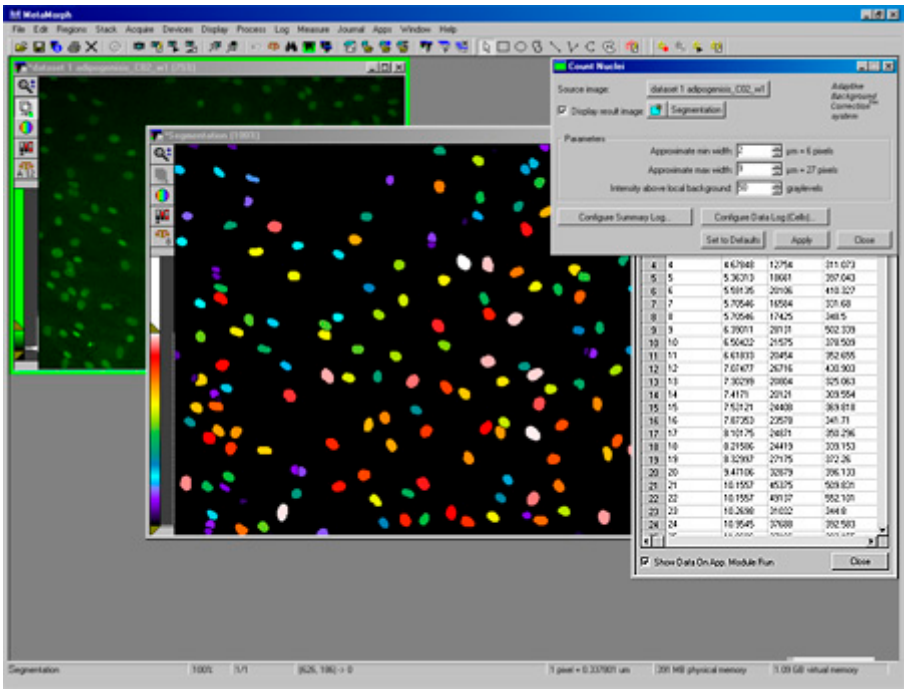
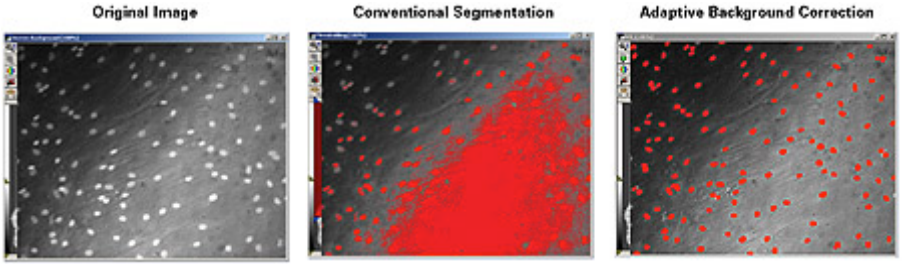


Count Nuclei Applciation module guides user through selection of segmentation parameters and output measurements yeilding accurate results even in the presence of background shading and closely spaced cells.



All MetaXpress Software Application Modules are equipped with an adaptive background correction algorithm to mitigate poor sample quality while improving image segmentation. (Left) Unevenly stained sample before segmentation (Center) after conventional segmentation, some of the features are not identified (Right) with Adaptive Background Correction, all the features are identified.

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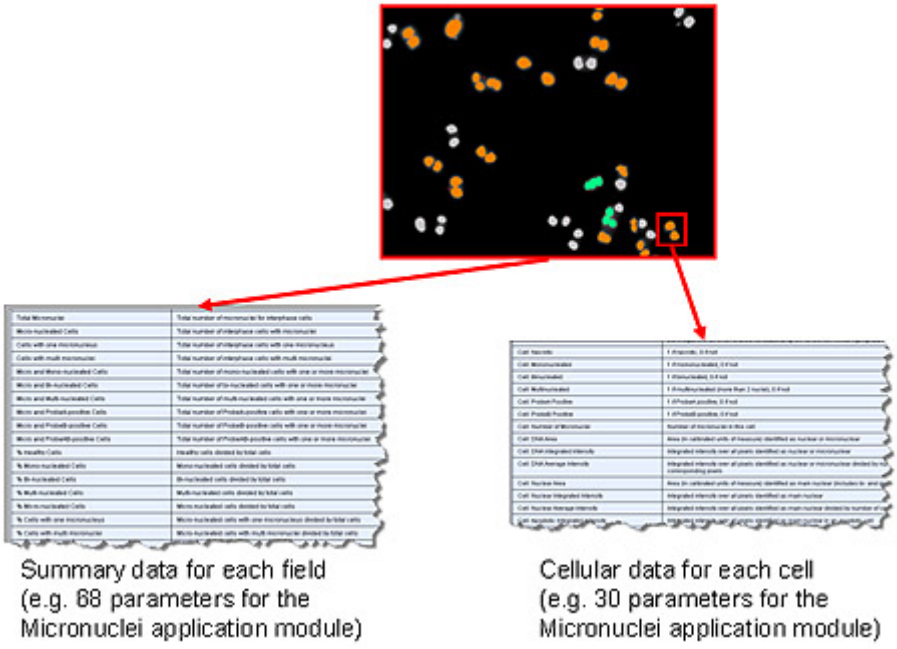

Field-by-field (summary) data, cell-by-cell (cellular) data, and segmentation overlays are made available to ensure in-depth downstream analysis.

Application	**MetaMorph Software Application Module**
Tube/vessel formation/inhibition in angiogenesis or development	Angiogenesis tube formation
Cell Viability, Membrane potential, Cytotoxicity, Apoptosis	Live/dead, Cell cycle, Multi Wavelength Cell Scoring, Cell Health
Cell Cycle, Mitosis, Apoptosis, Cell proliferation	Mitotic Index, Cell cycle, Count nuclei, Live/dead
Cell migration	Count nuclei
Cell counting	Count nuclei
Determination of cell health and proliferation in populations of mono-, bi- and multi-nucleated cells	Micronuclei
Micronuclei and genotoxicity analysis	Micronuclei
Kinase activation	Cell scoring, Multi Wavelength Cell Scoring

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Fatty acid uptake, Adipogenesis	Cell scoring, Multi Wavelength Cell Scoring
Transfection efficiencies	Cell scoring, Multi Wavelength Cell Scoring
Protein Expression	**Cell scoring, Multi Wavelength Cell Scoring**
Pathway analysis and multiplexing	**Multi Wavelength Cell Scoring**
Studying intracellular structures	Granularity
Receptor internalization	Granularity
Punctate staining	Granularity
Clustering of target molecules	Granularity
Process extension, neurodegenerative or neuroregenerative disease research	Neurite Outgrowth
Protein modification (e.g. phosphorylation)	Cell scoring, Multi Wavelength Cell Scoring
Detect monopolar spindles	Monopole Detection
RNAi (treatment of cancer, neurological disorders, viral infection, hemophilia, and premature aging)	Cell Health, Cell Cycle, Micronuclei, Neurite Outgrowth, Angiogenesis Tube Formation, and Multi-wavelength cell scoring
Embryonic/induced pluripotent stem cells, cell differentiation	Neurite Outgrowth, Cell scoring and Multi Wavelength Cell Scoring
Budding Yeast	Micronuclei

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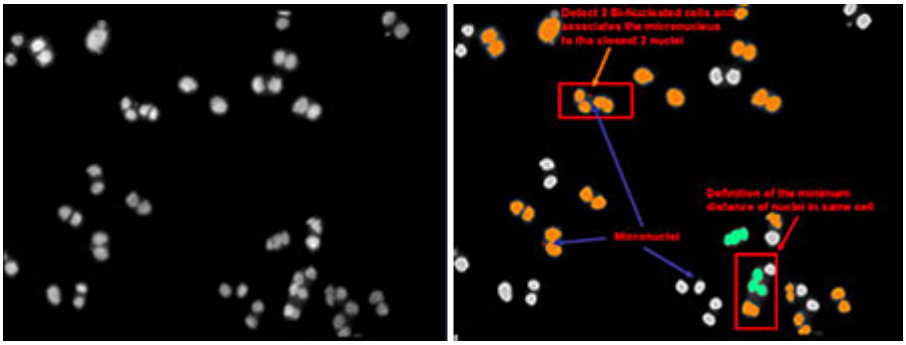
Micronuclei Application Module classifies micro-nucleated cells with great detail for genotoxicity applications but is also ideal for cell health and multi-nucleated cell populations with additional markers for analysis of apoptosis, necrosis or to distinguish any small structure next to a large one such as a yeast bud.

- Highly accurate classification of micro- and multi-nucleated (mono-, bi-, etc.) cells achieved with proprietary algorithm to discriminate phenotypes based on cell morphology, number of nuclei, distance of micronuclei from nucleus, micronuclei vs. "blebs" or "buds".
- Only requires a single fluorophore (nuclear stain) to identify cells in various classifications, eliminating the requirement for a cytoplasm stain, thereby reducing sample preparation, image acquisition and analysis time.
- Use of two additional markers facilitates further phenotypic analysis, such as transfection markers to identify transfected cells or kinetochore markers to differentiate micronuclei originating from clastogens (which create acentric chromosomal fragments) from aneugens (which cause whole chromosomal losses).



Micronuclei Application Module: Images of cells before (left) and after (right) image segmentation with the Module. Examples detected by the application module are indicated. In total, more than 60 measurements per image and 30 measurements per cell are available to assist with identification of the wide range of phenotypes associated with micronuclei studies

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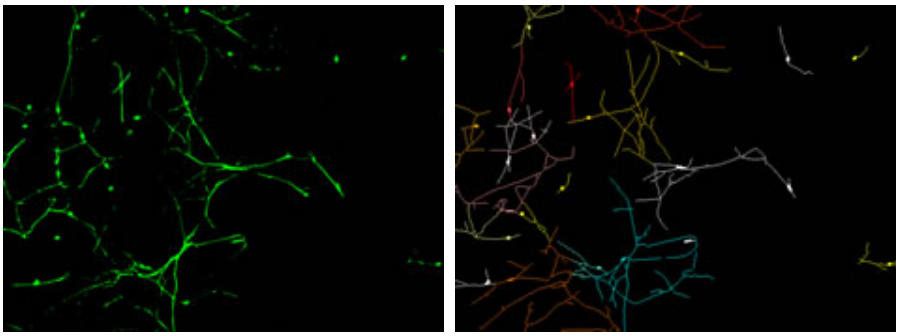
Neurite Outgrowth Application Module is designed to measure and characterize outgrowths (length and branching), the extension of axonal processes from the cell body, which are natural parts of neuronal development. Inhibition or stimulation of neurite outgrowth is implicated in a broad range of CNS disorders or injuries including stroke, Parkinson's disease, Alzheimer's disease, and spinal cord injuries.

- Unique assay that cannot be performed without imaging.
- Provides consistent results faster than manual tracing and counting.



Neurite Outgrowth Application Module: (Left) After image acquisition. (Right) After image segmentation. Each filament is assigned to a cell body. All the filaments and cell bodies are then measured. (Courtesy of Kris Poulsen and Davide Foletti, Rinat Neuroscience Corporation).

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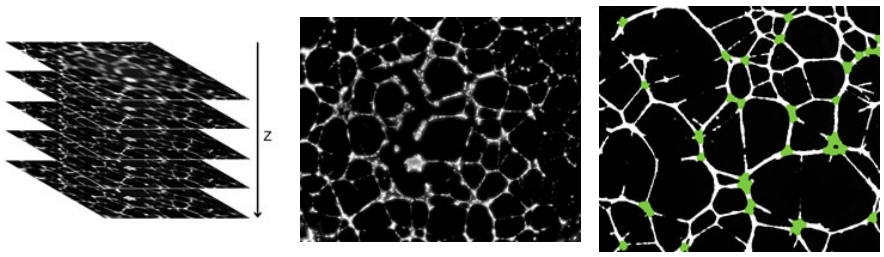
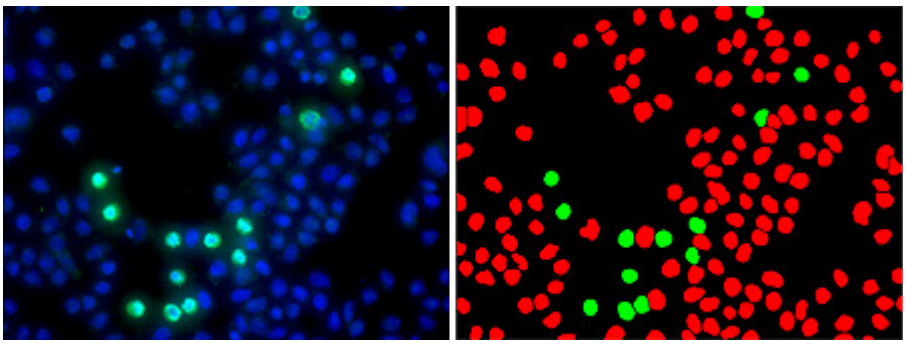
Angiogenesis Tube Formation Application Module facilitates the acquisition and analysis of tube formation experiments, a model system for angiogenesis in research focused on the promotion or inhibition of blood vessel formation (cancer, diabetes and other vascular disease research). Specific kits available from leading manufacturers and this area of research increasing in popularity with success of drugs such as Genentech's Avastin which blocks VEGF activity (Reference and list available of other similar drugs in clinical trials).

- Captures the three-dimensional behavior of tubes utilizing Z-stack acquisition available through the MetaMorph Software. A best focus image is calculated from the stack and tubes and nodes are identified and quantified from that image.



Angiogenesis Tube Formation Application Module: Human Mammary Epithelial Cells (HMEC-1) tube formation. (a) 3D Acquisition (b) Best focus algorithm (c) image analysis with the module (Data courtesy of BD Biosciences)

Mitotic Index Application Module is designed for the quantitative discrimination of mitotic and interphase cells, a critical tool for oncology drug discovery programs, providing insight into potential anti-cancer therapeutics that rely on arresting mitosis in cancerous cells to prevent uncontrolled proliferation.



Mitotic Index Application Module: (Left) CHO-K1 cells treated with Nocodazole for 18 hours before staining with anti-phospho-Histone H3 (Ser28). 50 ng/mL Nocodazole. (Right) green: mitotic, red: interphase.

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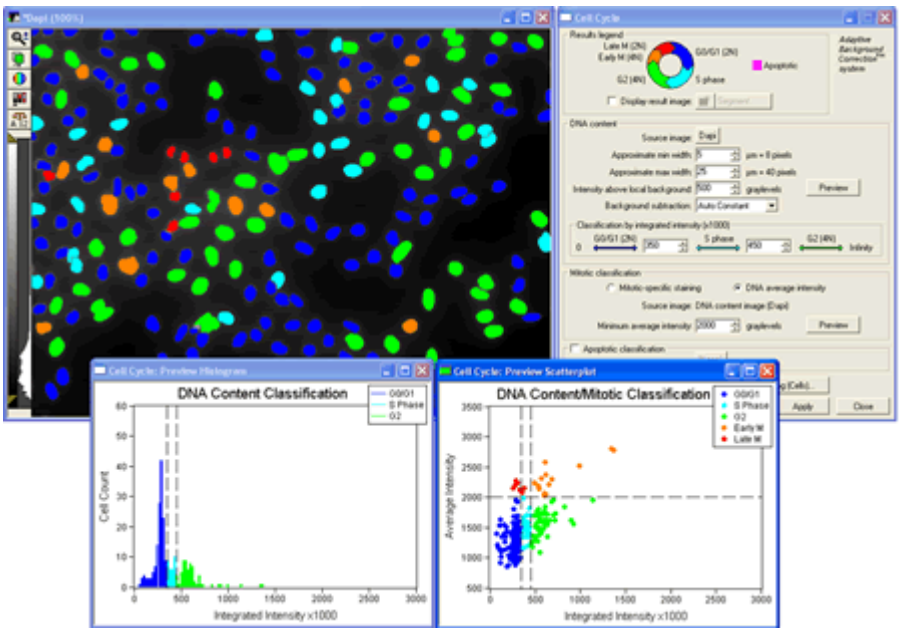
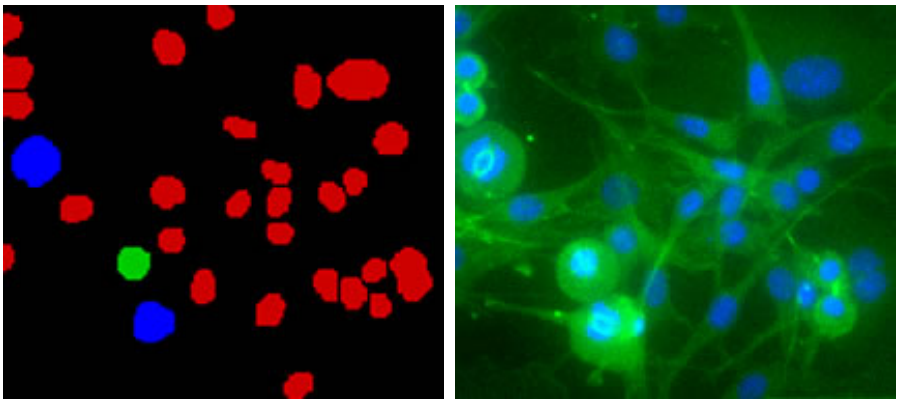
Cell Cycle Application Module classifies and quantifies cells in various stages of the cell cycle to investigate cell cycle progression. In healthy non-cancerous cells, challenges with DNA damage, hypoxia, metabolic changes or spindle disruption result in the triggering of checkpoints and cell cycle arrest. Cancerous cells commonly lose checkpoints and divide uncontrollably, even in challenging conditions. With the appropriate tools, researchers can screen for drugs that cause cell cycle arrest or cell death in cancerous cells.

- Differentiates 5 phases of the cell cycle using only a nuclear stain (G0/G1, S, G2, Early or Late M).
- Optional detection of mitosis with specific fluorophores to better distinguish M-phase cells when using low magnification.
- Optional detection of apoptotic markers to detect conditions that trigger apoptosis.
- Interactive color-coded graphs to easily set classification cutoffs.



Cell Cycle Application Module: classification and quantification of cells in 5 phases of the cell cycle; additional use of an apoptotic marker to determine the apoptotic status of cells. Interactive color coded graphs allow to easily set classification cutoffs.

Monopole Detection Application Module monitors the disruption of the formation of bipolar spindles, a successful mechanism used by drugs such a Monastol to stop the progression of cancerous cells through mitosis. The module quantifies mitotic cells with monopolar or bipolar spindles in cells stained with a DNA probe and a microtubule probe.



Monopole Detection Application Module: (Left) 3T3-L1 mouse fibroblast cells treated with monastrol and stained with mouse anti-beta tubulin primary antibody detected with a FITC conjugated goat anti-mouse secondary antibody. Nuclei are stained with Hoechst 33342. (Right) the module identifies interphase cells (red), bipolar spindles (blue) and monopoles (green).

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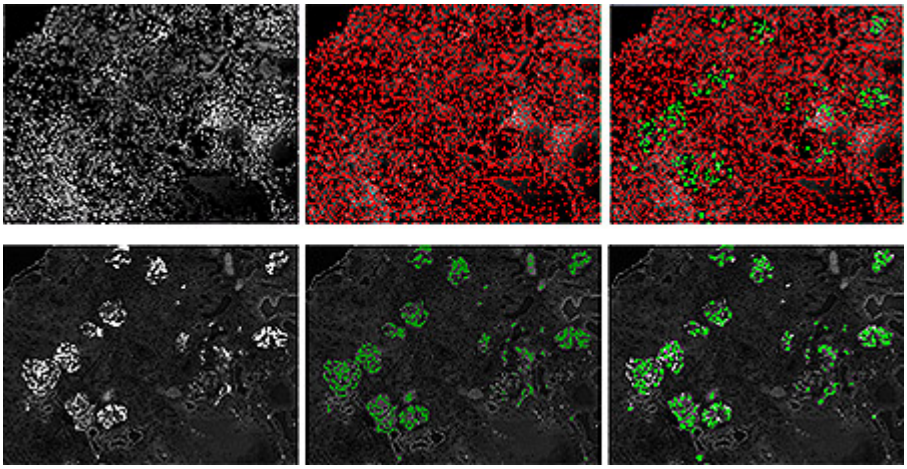
Cell Scoring Application Module is a general and flexible solution to identify subpopulations of cells tagged with a second fluorescent probe and is ideal to examine transfection efficiencies, kinase activation (pathway analysis) or adipogenesis.



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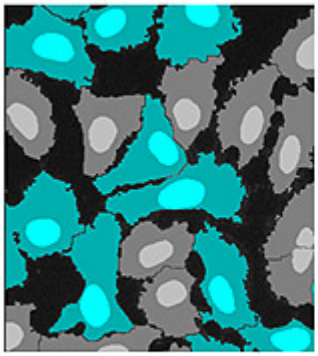
Multi Wavelength Cell Scoring Application Module enhances the functionality of the Cell Scoring Application Module to include configuration of your own custom module to score populations of cells.

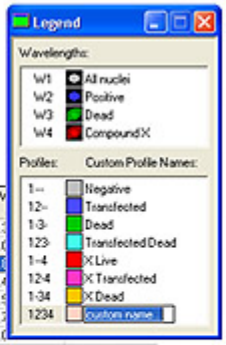
- Customize wavelength to match your research.
- Interactive graphics link scoring data between individual wavelengths and full multi-wavelength profiles to help optimize settings
- Up to 7 probes (including Nuclear probe) to customize your analysis of 7 markers (or analyze the same marker with different settings)

Segmentation: robust algorithms and easy setup.



Interactive data: image graphics, spreadsheet view, setup dialog and legend.

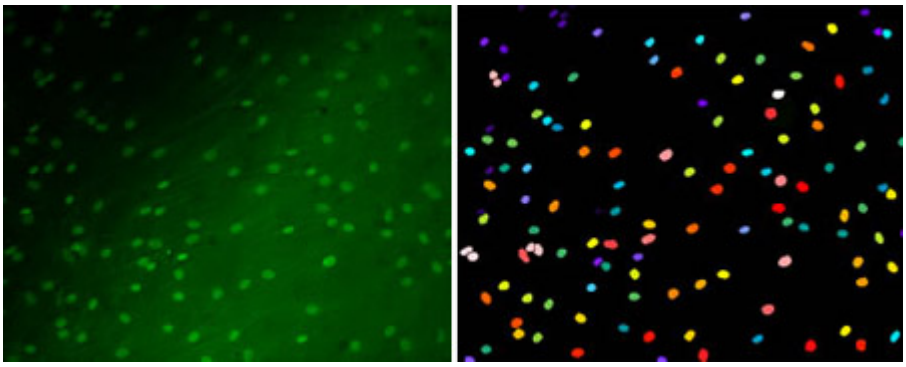
Customization: Configure wavelengths and scoring profiles with your terminology.



Cell: Custom Profile Name	Cell: Total Area	Cell: W1 Stained Area	Cell: V	
Negative	1685.37	180.549	1685.	
Transfected	3627.01	426.621	3627.0	
Transfected	2920.82	433.173	2920.8	
Transfected	4011.4	769.519	4011.4	
Transfected	2992.9	208.942	2992.9	
Transfected	3627.73	535.096	3627.7	
Transfected	3418.06	540.92	3418.0	
Negative	2323.85	546.016	2323.85	466.242
Negative	1708.67	475.398	1708.67	449.467
Transfected	3033.67	584.602	3033.67	369.587
Negative	1887.03	394.588	1887.03	422.841
Negative	1815.69	422.253	1815.69	401.123
Negative	2476.73	421.525	2476.73	377.003
Negative	2363.89	428.077	2363.89	389.5

Count Nuclei automate accurate counting of nuclei for most types of cells and are ideal for the study of cell proliferation, cell counting or cell migration. These advanced modules count nuclei even when the background is uneven, providing superior segmentation compared to simple thresholding.

- All modules identify nuclei, taking into consideration nuclear size and varying background and splitting touching objects.
    - More consistent between users and faster than manual counting.
    - Cheaper than radioactive counting
    - Easier and faster than flow cytometry, which requires trypsinization of cells and is a low throughput method.



Count Nuclei Application Module: (Left) Image has uneven illumination and touching cells (Right) the module identifies touching cells as separate objects (as indicated by the red arrow). Adaptive Background Correction™

compensates for the variable background intensity - even when background intensity in one area of the image is greater than nuclei intensity in another area.

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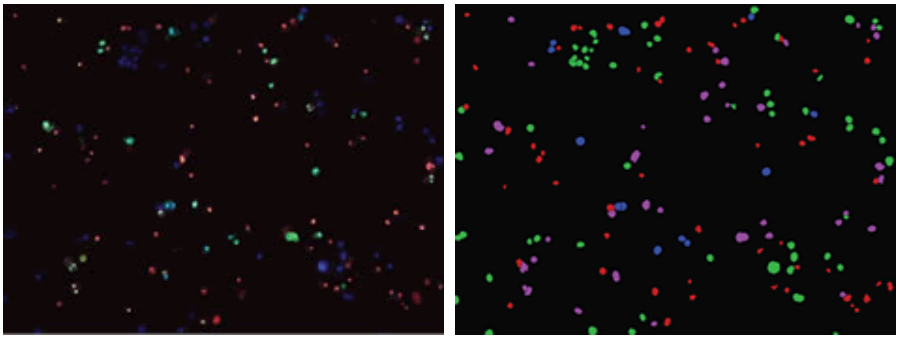
Cell Health Application Module classifies cells in viable, early apoptosis, late apoptosis or necrosis.

- Validated with most common flow cytometry kits such as Vybrant #7 from Molecular Probes, Annexin V detection, or JC-1 combined with Hoechst to study loss of mitochondrial potential.



Cell Health Application Module: (Left) Cells were treated with 1 µM Staurosporine for 12 hours and stained with Hoechst 33342, YO-PRO-1 and PI (Right) Image analysis results are shown as a colored overlay on the source image. Viable nuclei are shown in green. Early apoptotic nuclei are shown in blue. Late apoptotic nuclei are shown in purple. Necrotic nuclei are shown in red.

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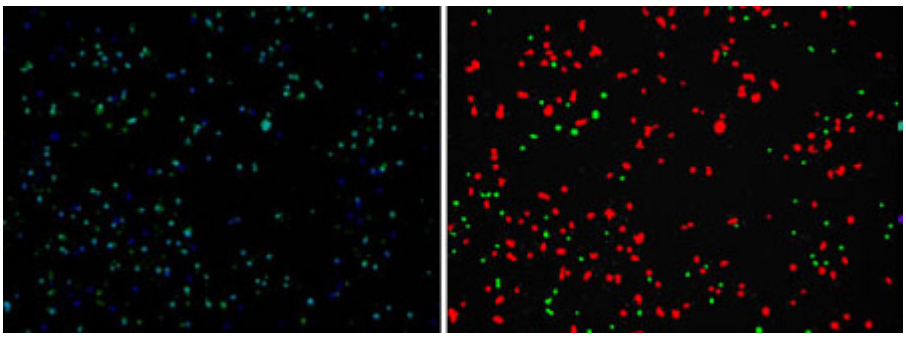
Live Dead Application Module is compatible with commercially available Live/Dead assay kits designed to study cell proliferation or death. Common applications monitor cell proliferation associated with cancer, premature cell death involved in neuromuscular diseases such as Alzheimer's and Parkinson's, in addition to cytotoxicity and apoptotic events.

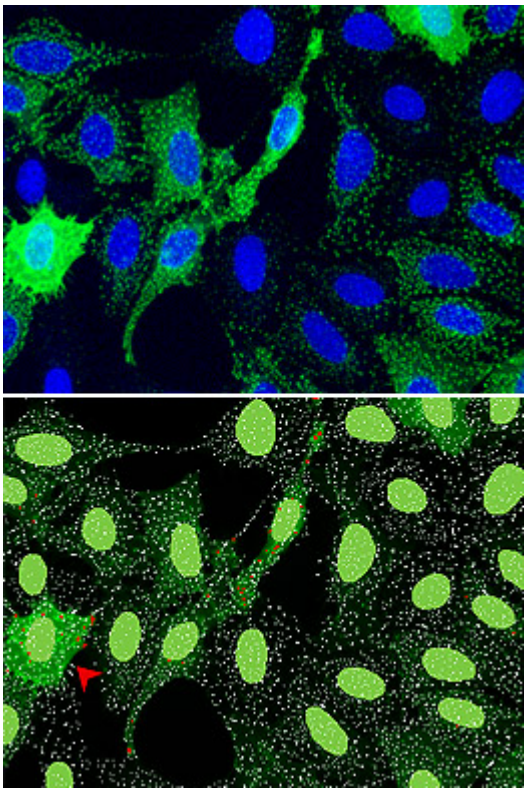
- Probes can target any part of cell, does not require a nuclear marker



Live Dead Application Module: (Left) 1 µM Staurosporine. CHO cells in one 96-well plate at ~10,000 cells per well. Cells were incubated for ~24 hours and apoptosis was induced by incubating different concentrations of Staurosporine for 6 hours. Assay kit used: Molecular Probes, Vybrant Assay Kit #7. (Right) Live/dead cells are identified simultaneously.

Granularity, facilitate the analysis of punctate structures such as the one observed during clustering of target molecules for receptor internalization or within the nucleus or even the punctate patterns of mitochondria.

- Granularity Application Module detects punctate structures



U2OS cells, top: overlay, bottom: the module identifies granules and nuclei, even in cells with high background (red arrow).