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BioApplication summary

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BioApplications: pieces of algorithms dedicated to your biology

- BioApplications are Image Analysis Modules that run on a Cellomics instrument or vHCS Discovery Toolbox

- They are validated algorithms that measure and report information about your cellular images

- BioApplications analyze the images 'on-the-fly' as soon as they are acquired by the reader. IQ allows a real-time phenotyping to target populations like transfected cells or rare events (mitosis). This feed-back loop control the acquisition

- Cellomics BioApplications allow you to easily customize the algorithm to measure your biological target of interest, without script writing

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Huge Range of Biologies Cellomics can address

- Receptor Activation
- Cell membrane receptor binding
- GPCR internalization
- Labeled Ligand internalization
- Cell proliferation
- Cell morphology
- Cell survival signaling
- Cell migration signaling
- Toxicity
- Cell viability
- Apoptosis/necrosis
- Nuclear count
- Fluorescent protein localization
- Transcription factors
- Reporter gene expression
- Cell cycle status
- DNA replication studies
- Wound healing
- Stem cell differentiation
- Colocalization

- Nuclear-cytoplasmic translocation
- Plasma membrane translocation
- Neurite outgrowth
- Synaptogenesis
- Tube formation
- Microtubule arrangement
- Cytoskeletal reorganization
- Micronuclei formation
- Genotoxicity
- Hepatotoxicity
- Oxidative stress
- PhospholipidosisCholeostasis
- Calcium homeostasis
- Neurotoxicity
- Stress response
- Cell classification
- Viral clearing
- Gene function
- Model organisms
- Tissues…

Cellomics Bioapplications

- **Flexible Developer Tools**
  - Compartmental Analysis
  - Target Activation
  - Morphology Explorer
  - Spot Detector

- **Compartment Changes**
  - Nuclear Translocation
  - Molecular Translocation
  - GPCR Signaling
  - Cytoplasm to Membrane
  - Colocalisation

- **Physiology & Phenotype**
  - Cell Cycle
  - Cell Motility
  - Neuronal Profiling3.5
 - Cell Spreading
  - Tube Formation

- **Toxicology**
  - Micronucleus
  - Cell Health Profiling
  - Multiparameter Cytotoxicity
  - Comet
  - ZebraTox

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"Do I have to know all the BioApps by heart ???"

- Your job is science and biology

- FASes job is to know Cellomics system and help you in developing your HCS assays

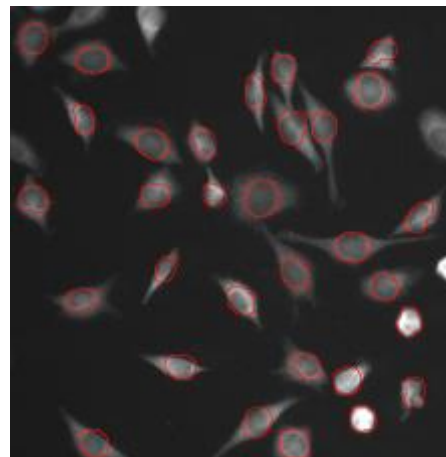- If you have questions about new assays, bioapplications, software, contact your FAS or HCSTechnicalsupport@thermofisher.com (also available for technical issues)

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Target Activation BioApplication
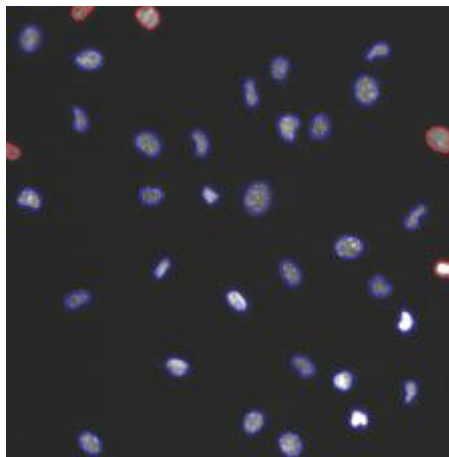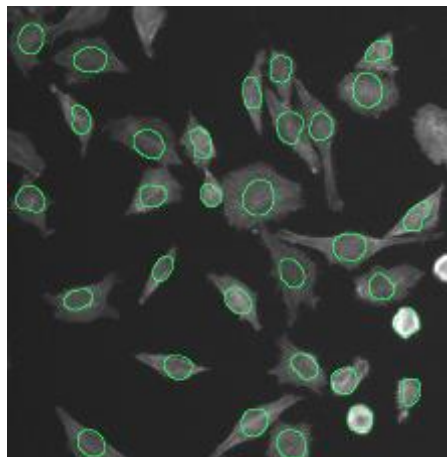
| Target 1 | Target 2 | Target 3 | Target 4 |
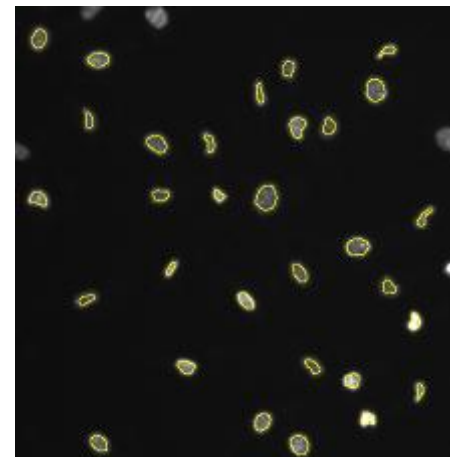
Function: General purpose assay for measurement of average and total intensity within a user-defined cellular domain that can be individually defined in each of 6 channels.

Uses: Flexible application for a wide range of biological assays, including rare event analysis, population analysis, gene expression or knockdown.

Research Applications: Broad application across multiple disciplines

Ouptput Features: nuclei number, intensity, size and shape, target intensities from Ch2 to Ch6 (Average, Total, Variance)

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Cytoplasm-to-Nucleus Translocation
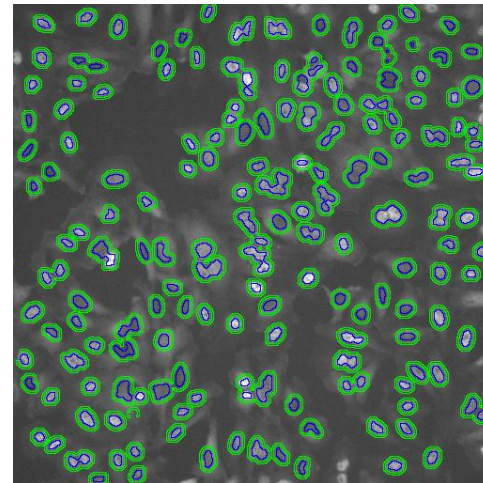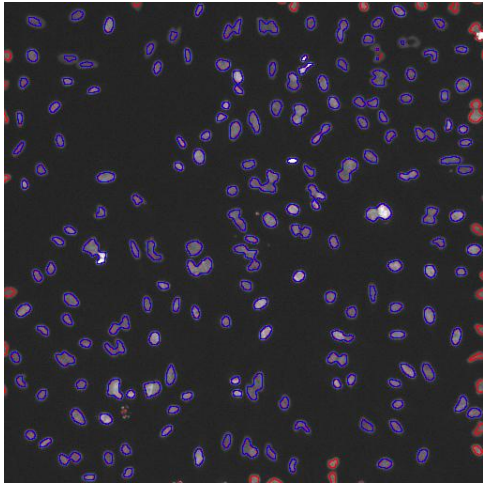
Function: Patented method to monitor target activation and translocation between the cytoplasm and nucleus

Uses: Analysis of nuclear translocation for kinases and transcription factors, simple intensity measurements within the cell, and analysis of nuclear or cytoplasmic protein levels.

Research Applications: Broad applications in Cancer, Cardiovascular, Inflammation, Neurology, Cell Signaling, and Infectious Disease

Output Features: **nuclei number, intensity, size and shape,** intensities of cytoplasmic and nuclear regions (Ch2), intensity ratio and difference between cell regions (Ch2), intensity of Targets Ch3 to Ch6
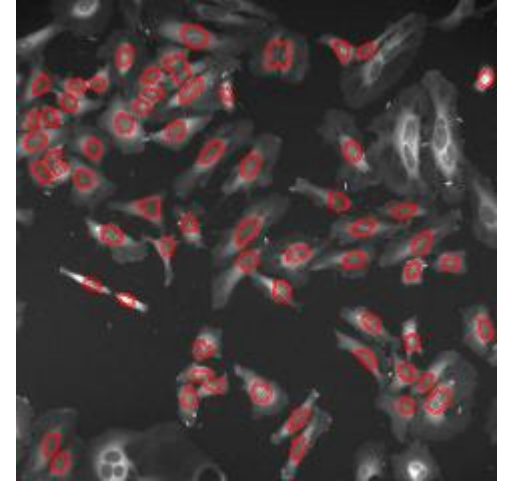
Molecular Translocation BioApplication
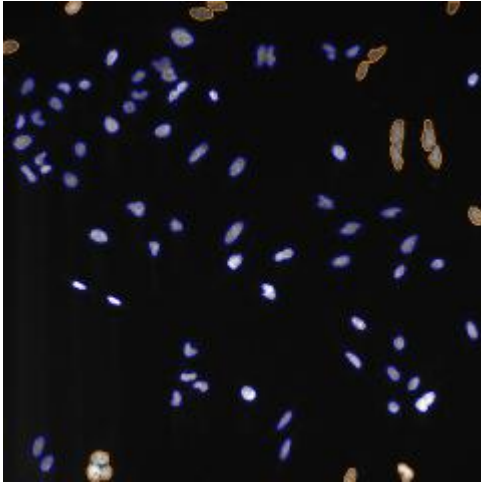
Nuclei
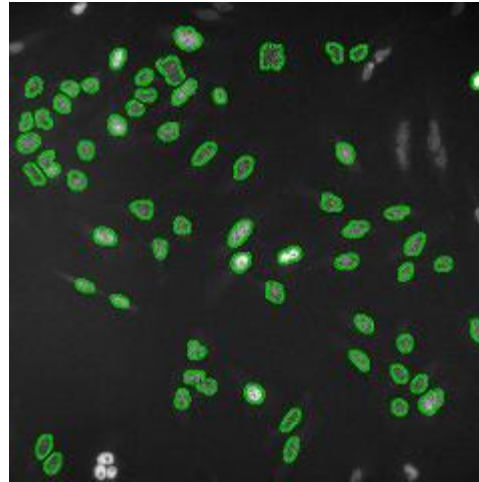
Translocation Target 1

Translocation Target 2

Function: Multiparameter assay to measure and correlate multiple translocation events at the single cell level.

Uses: Pathway analysis, kinase specificity studies, transcription factor activity profiling

Research Applications: Cell signaling, cancer, RNAi, functional genomics

Output Features: nuclei number, intensity, size and shape, intensities of cytoplasmic and nuclear regions (Ch3 to Ch6), intensity ratio and difference between cell regions (Ch3 to Ch6)

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# Spot Detector BioApplication
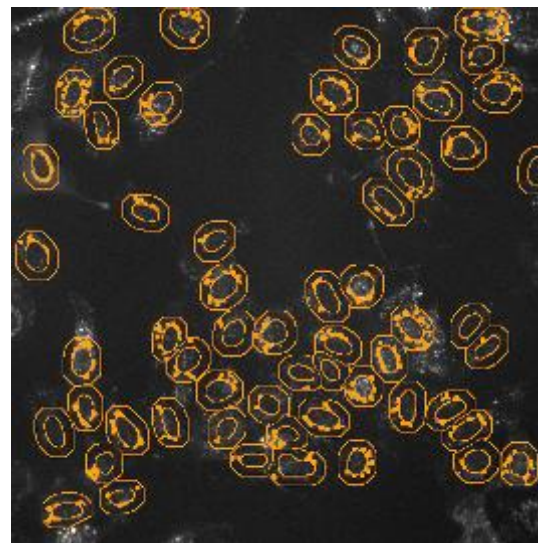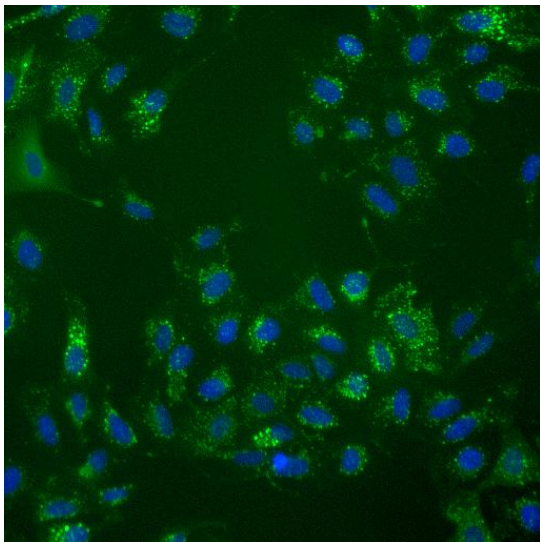
<u>Function:</u> Rapid yet flexible assay that measures "spots" representing internalized receptors or other punctate targets in up to four channels.

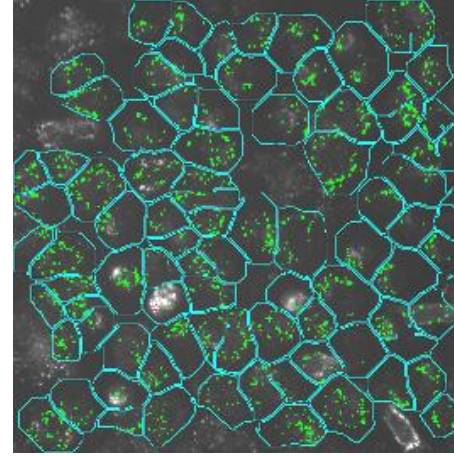<u>Uses:</u>   Designed for rapid detection of peaks and spots of intensity.

<u>Research Applications:</u> Cell Signaling, Cancer, GPCRs Metabolic Disease, general research

<u>Output Features:</u> nuclei number, intensity, size and shape, spots count, area and intensity (Average and Total) from Ch1 to Ch4

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GPCR Signaling BioApplication
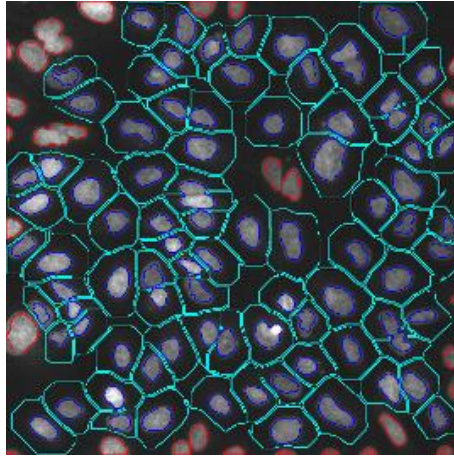
Nuclei and cell borders


Internalized receptors spots

Function: Monitors complex intracellular target re-distribution during cell signaling events. Classifies cells based on pattern of target distribution within the cell.

Uses: Validated for analysis of b-arrestin trafficking during GPCR signaling. Can also be used for analysis of receptor trafficking and internalization, and functional genomics analysis of novel expressed proteins.
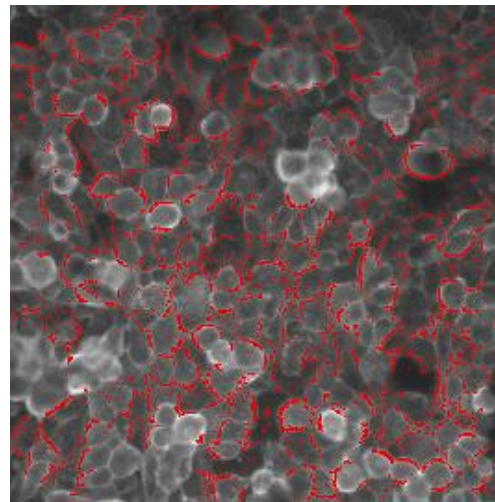
Research Applications: Cell signaling, Cancer, Cardiovascular, Functional Genomics

Output Features: nuclei number, intensity, texture, size and shape, cytoplasm, spots and membrane intensity (Ch3), GPCR colocalization with membrane (staining Ch2), sorting cells in different phases of GPCR recycling.

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Cyto-Cell Membrane Translocation
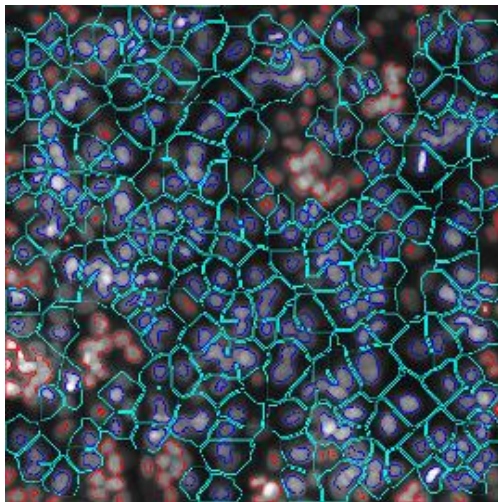
Nuclei and cell borders

**Membrane staining**

Function: Specifically measures target translocation between the cytoplasm and cell membrane.

Uses: Unique application for analysis of novel targets that translocate between the cytoplasm and cell membrane, such as PKC, GLUT-4, and ras. Also useful for comparison of receptor ratio between the cell surface and interior.
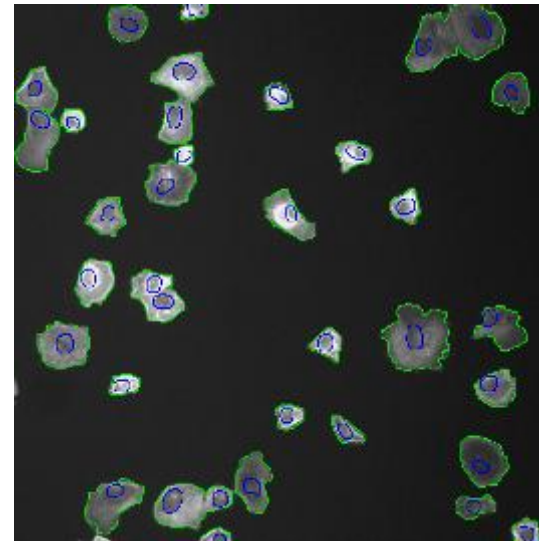
Research Applications: Cancer, Cell Signaling, and Metabolic Disease

Output Features: nuclei number, intensity, size and shape, cytoplasm and membrane intensity (Ch3 to Ch6), receptor (Ch3 to Ch6) colocalization with membrane (membrane staining Ch2)

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Cell Spreading BioApplication
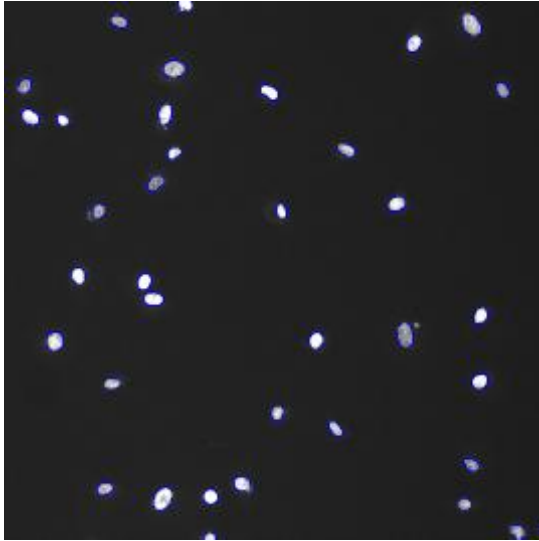

Nuclei

F-actin staining

Function: Makes basic shape and size measurements of cells and colonies

Uses: Analysis of cellular shape change during attachment, motility or differentiation. Successfully used to analyze differential adhesion properties on various matrices.
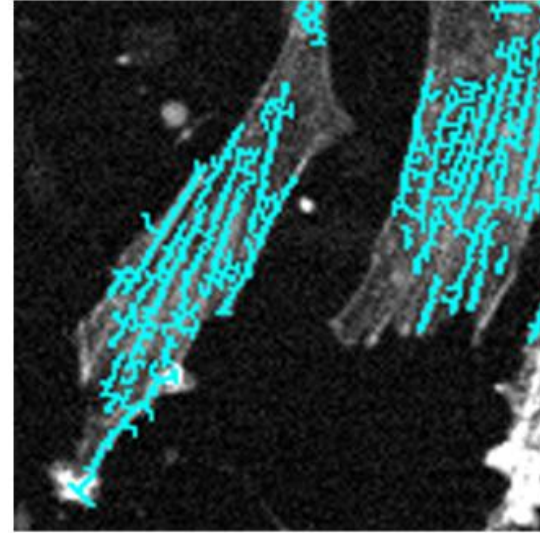
Research Applications: Cancer, Cell Signaling, and Cellular Profiling

Output Features: colony number, perimeter, cyto-nuc area ratio and difference

Morphology Explorer BioApplication
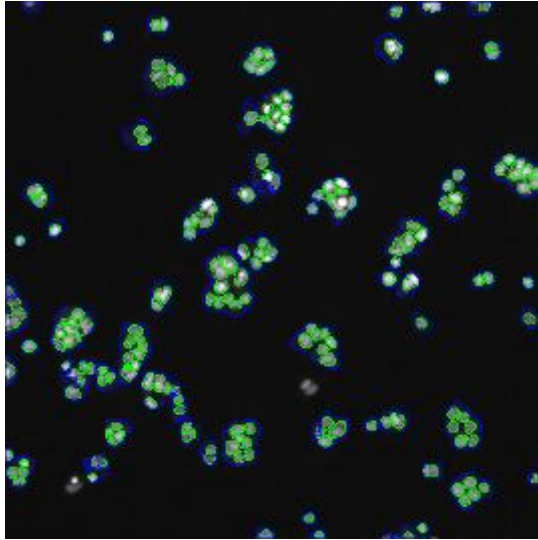
Colony analysis


Cytoskeleton fibers

Function: Analyzes multicellular objects such as colonies and tubes. Reports pattern and distribution of intracellular structures such as F-actin fibers and microtubules.

**Uses:** Extremely flexible for many types of morphological analyses

Research Applications: Too many to count: includes Functional Genomics, cancer research, phenotypic characterization of RNAi knockdowns

Output Features: cell (or colony) number, intensity, size, advanced morphometric and texture measurements, process count and length (Ch1), nuclei count, position, intensity, size and shape, cyto-nuc area ratio and difference (Ch2), spot and fiber count, area and alignement, intensity and advanced texture measurements (Ch3 and Ch4), neighborhood analysis…
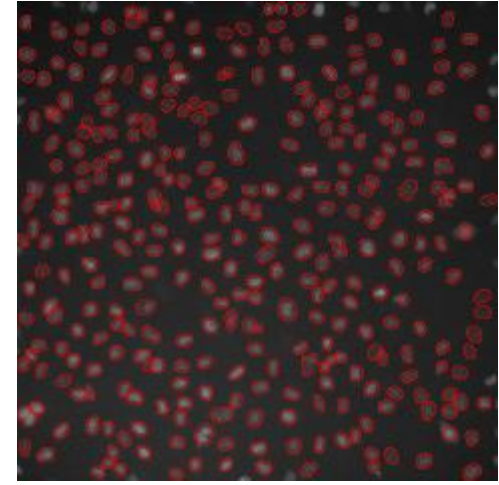
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Cell Cycle BioApplication
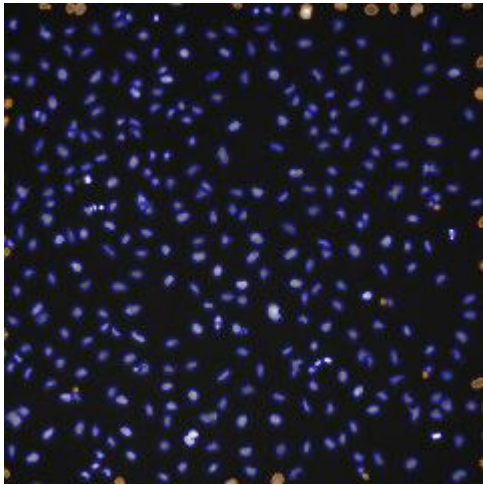

Nuclei
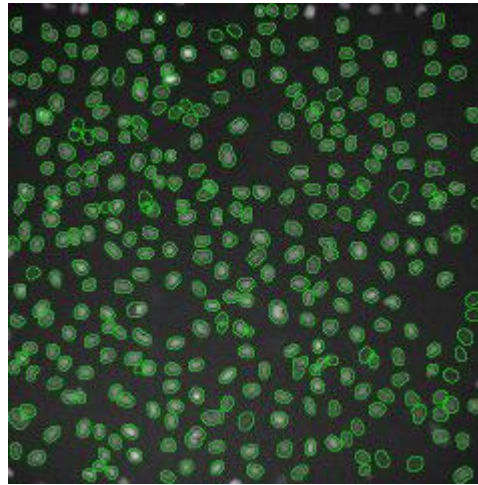
Cell Cycle Target 1

Cell Cycle Target 2

Function: automatically classifies cell cycle phase and correlates levels of up to three related targets at both the single cell and well population levels.

Uses: screening for effectors of cell cycle progression, general life science research, and characterization and quality control of cell types.
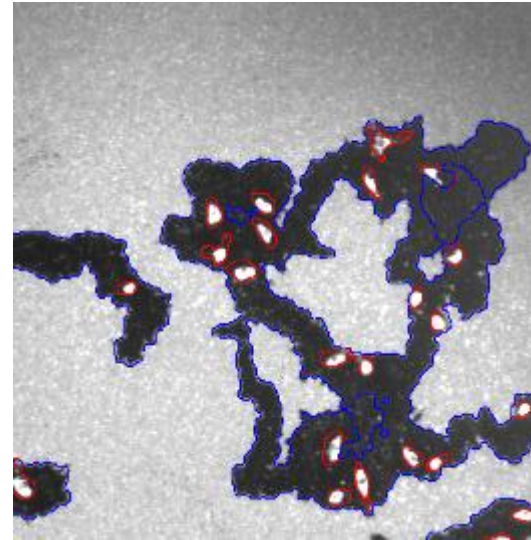
Research Applications: Cancer, Cell Signaling, Basic Research

Output Features: nuclei number, intensity (DNA content), size and shape, % of cells in G1, G2, M, S, and intensities in each sub-population (Ch1 to Ch4) + ratios between targets of Ch2 to Ch4

Cell Motility BioApplication
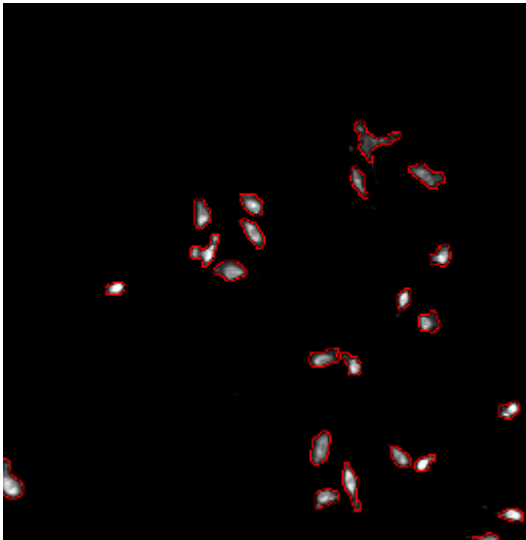
**Cells**

Cells track phagocyting fluorescent beads

<u>Function</u>:   Monitors the track area produced by motile cells over time.

<u>Uses</u>:   Analysis of cellular motility in a fixed endpoint assay. Also useful for profiling cancer cell lines, assessing compounds that inhibit motility or metastasis.

<u>Research Applications</u>: Cancer, Cell Signaling, Functional Genomics

<u>Output Features</u>: Full and motion track area, track count, cell count and area, track area and count per cell

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Neuronal Profiling
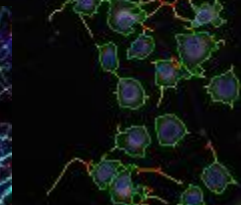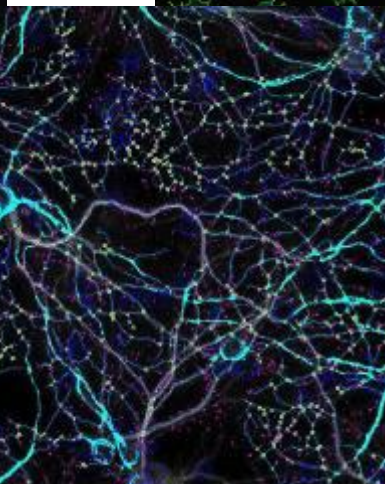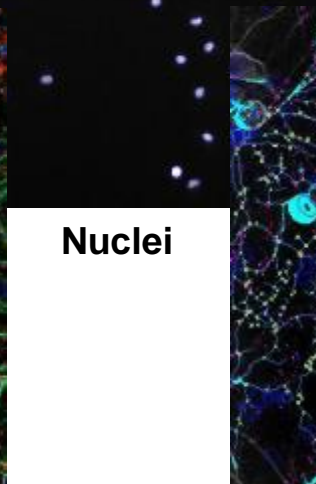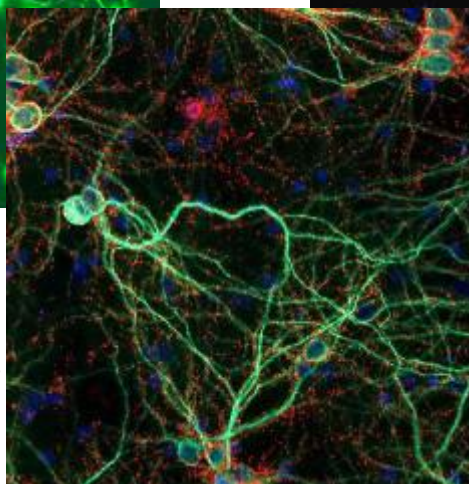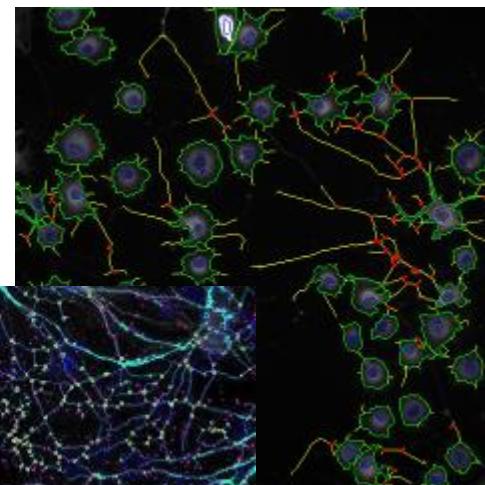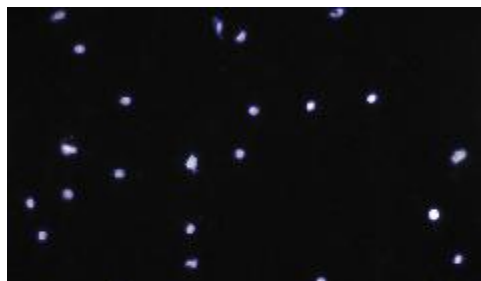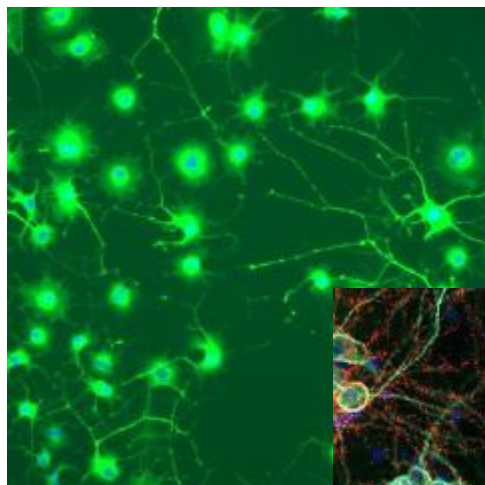
Composite Image

Pre and post synaptic vesicles staining

Nuclei

**Cell bodies and Neurites**

Spot, branch and cross points

Functions: Measure neurite outgrowth and other morphological features including branch points in neuronal and mixed cell populations. Advanced segmentation to analyse dense populations of neurites. Define events to report complex phenotypes. Measure subsets of both neurons and of neurites. Identify pre and post synaptic vesicles and provides colocalization measurements

Uses: Identify subpopulation-specific drug activities, morphology of neurons.

Research Applications: neurological disorders, neurotoxicology, CNS research.

Output Features: nuclei and cell bodies count, intensity, size and shape, neurites count, length, intensity, and morphology, network measurements by branch points, cross points, pre and post synaptical spots count, intensity, area and colocalization

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Multiparameter Cytotoxicity
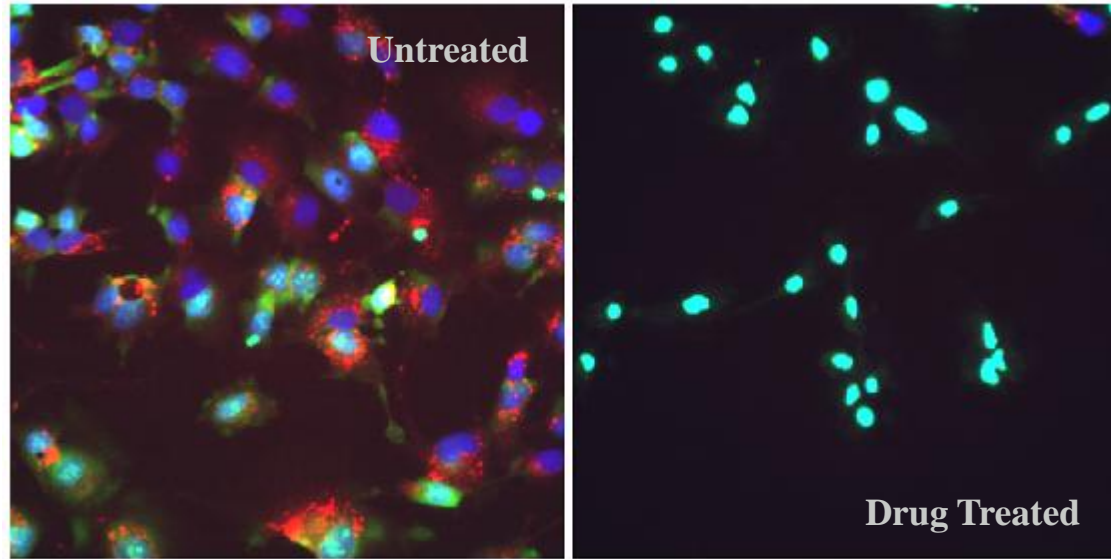
Function: Measures multiple intensity and shape-based outputs in a single assay: nuclear morphology, cell permeability, cell count and lysosomal pH.

Uses: Profiling multiple aspects of complex cytotoxicity and apoptosis pathways. Monitoring multiple aspects of a pathway in a single assay.

Research Applications: Cytotoxicity Profiling, Cell Survival, Cancer

Output Features: nuclei count, intensity size and shape, circ target average intensity (Ch2), spot average intensity Ch3

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# Cell Health Profiling BioApplication
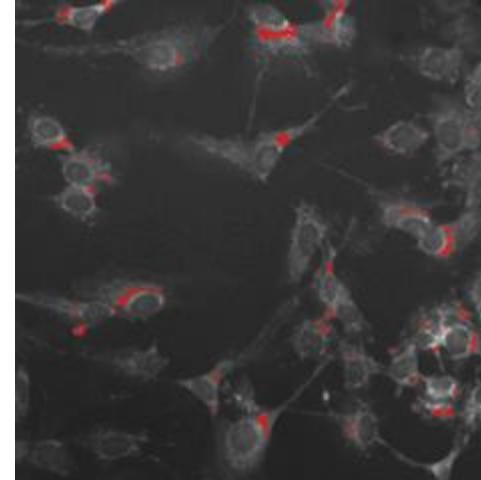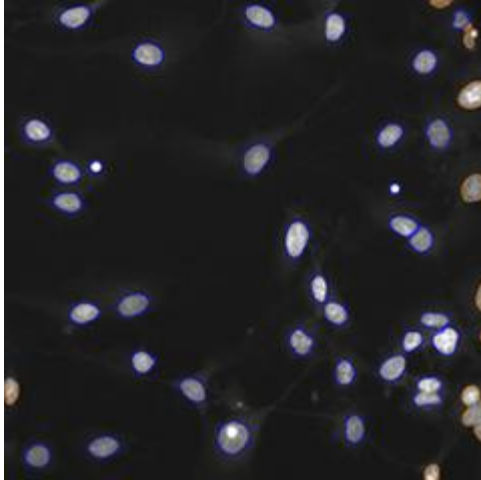
Function: Allows examination of a variety of cell health markers including cell viability, cytotoxicity, and apoptosis. Applies advanced cell-level logic for greater understanding of your high-content data.

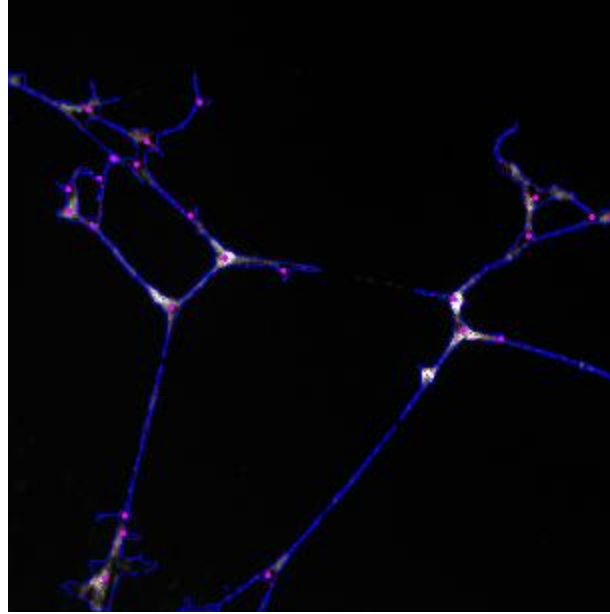**Uses:** Viability assays; apoptosis assays; toxicity profiling; general activity profiling.

Research Applications: cancer, metabolic, cell signaling, toxicology, functional genomics

Output Features: nuclei count, intensity size and shape, targets intensity and area from Ch2 to Ch6
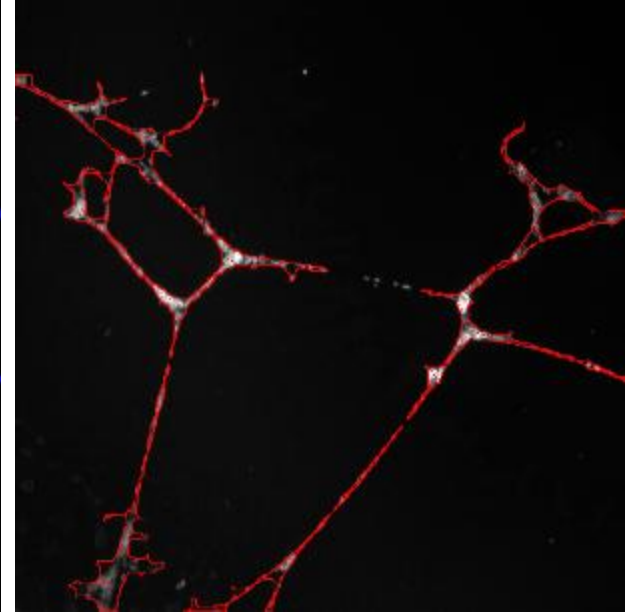
Tube Formation
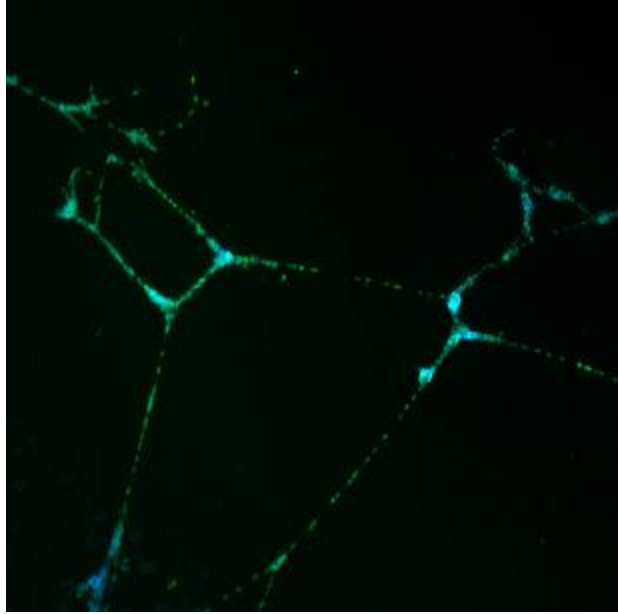
**Composite Image** **Tubes and nodes** **Nuclei**

<u>Function</u>: **Identifies tubes, branch points and nuclei, and reports features including number, size and connectivity of tubes, and intensities of markers in additional channels.**

<u>Uses</u>: **Rapid assessment of angiogenesis inhibitors, direct measurement of the phenotype rather than surrogate marker.**

<u>Research Applications</u>: **Angiogenesis.**

Output Features: Tube count, length, width, intensity, nod count, segment length, classification of tubes in connected and unconnected (Ch1), nuclei count in tubes (Ch2), Tube intensities in downstream channels (Ch2 to Ch6)

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Zebratox BioApplication

control

treated
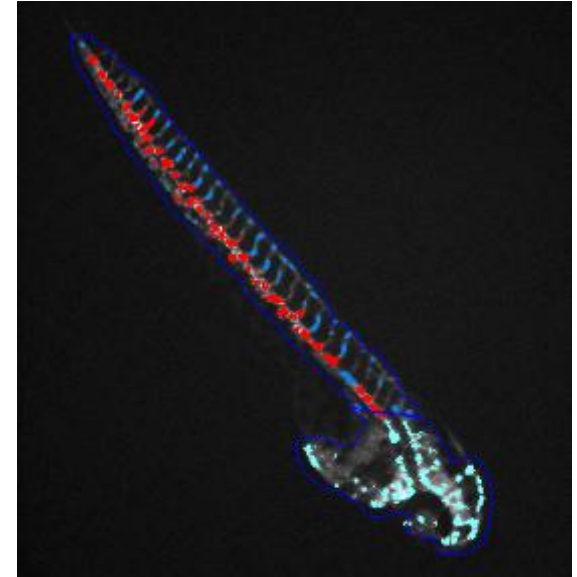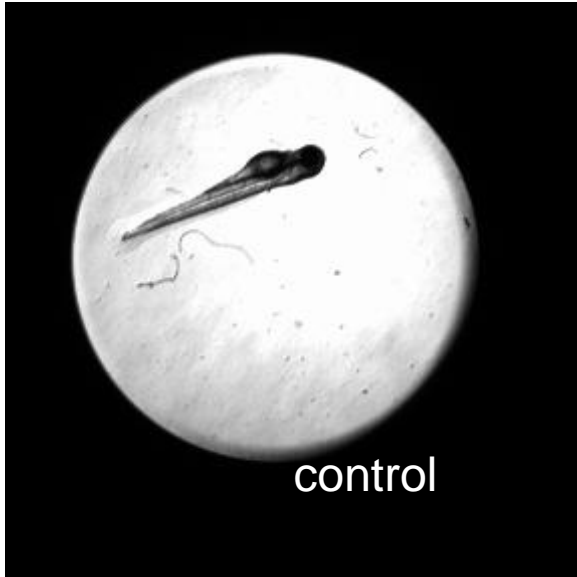
Function: automate a previously manual or semi-automated method of evaluating embryos, image and analyze embryos used for toxicity and angiogenesis work

**Uses**: angiogenesis assay, toxicity, whole organism analysis

Research Applications: cancer, angiogenesis, toxicology, embryology

Output Features: area, shape, intensity, curvature measurements, angio and vasculo vessels area, intensity and count, for fish, head and torso on Ch1, spots count, area and intensity for fish, head and torso on Ch2
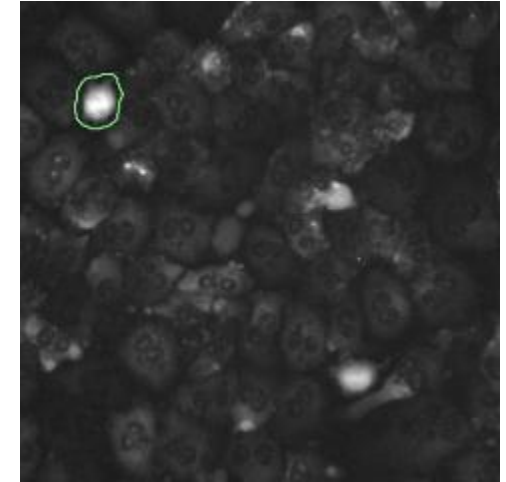
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Micronuclei BioApplication
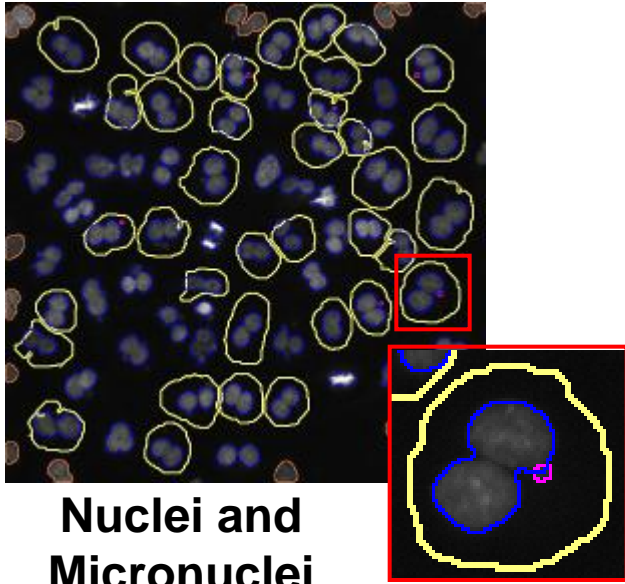
Nuclei and Micronuclei
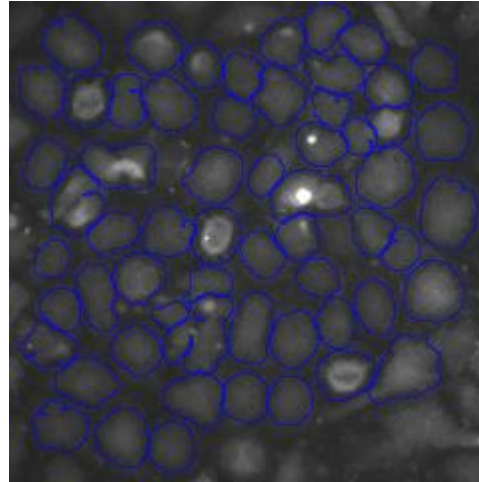

Cell Boundary

Permeability

<u>Function</u>: detects and quantifies micronuclei as an indicator of clastogenicity, reports early cytotoxicity and late cytotoxicity, integrated sample preparation and analysis.

<u>Uses</u>: Micronuclei assays

<u>Research Applications</u>: genotoxicity, toxicology

<u>Output Features:</u> mono, bi and multi nucleated cell count, intensity, size and shape, proliferation index, micronuclei count (Ch1), cell area, intensity and shape (Ch2), cell nuclei and micro nuclei intensity on Ch3

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COMET BioApplication
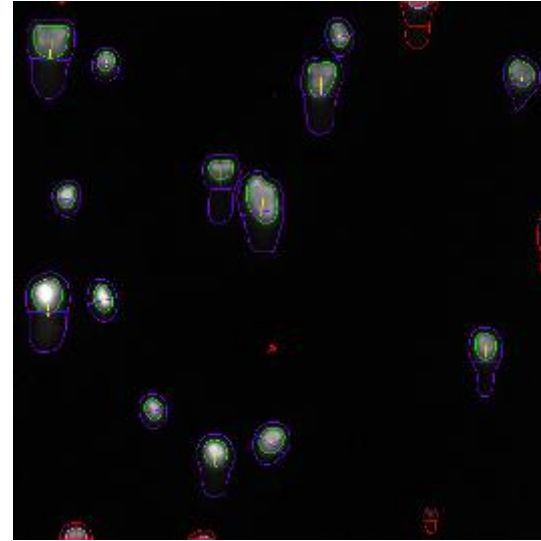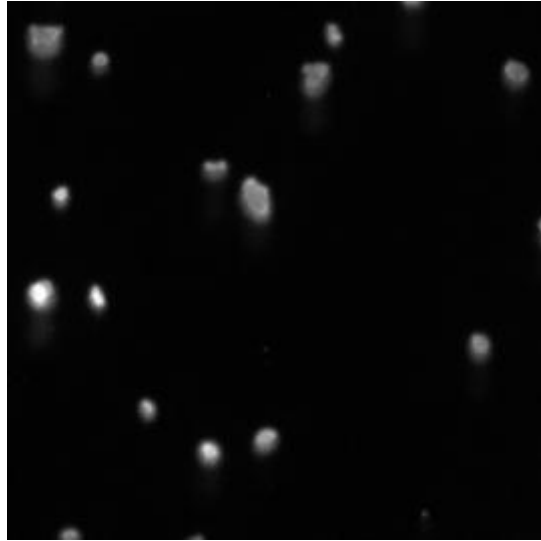
Function: Allows automated and robust analysis of COMET assays to determine the extent of DNA migration. Possible combination with FISH. Provides most commonly reported features in DNA damage studies.

Uses: COMET assays ; COMET + FISH assays ; FISH assays.

Research Applications: genotoxicity, DNA damage, cytotoxicity.

Output Features: normal and abnormal comets, head and tail count, size, intensity and shape, tail extent moment, tail olive moment (Ch1), fish spot count, area and intensity in comet, head and tail (Ch2 and Ch3).

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Compartmental Analysis
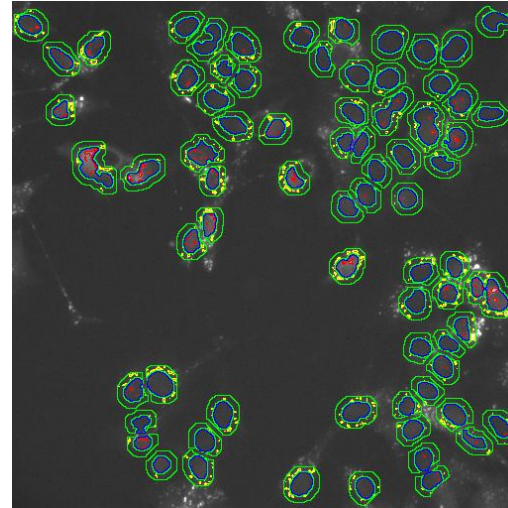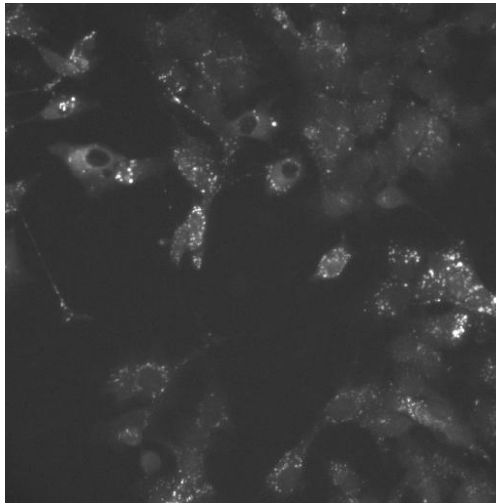
Function: Allows examination of intensity and spots expression in several cellular compartments

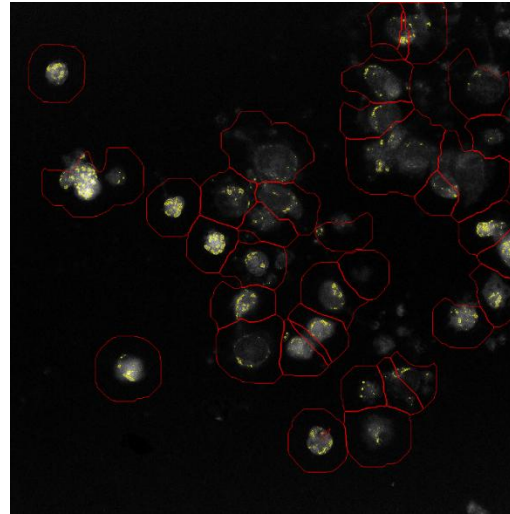**Uses:** Addresses a very large and flexible range of biologies

Research Applications: cancer, metabolic, cell signaling, toxicology, functional genomics…

Output Features: nuclei count, intensity size and shape, targets (Circ and Ring) intensity, spots intensity count and area from Ch2 to Ch6, cross-Ch and cross-target intensity ratios
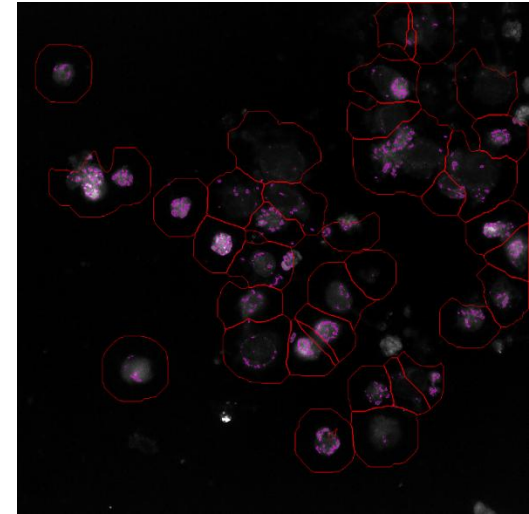
Colocalisation Bioapplication
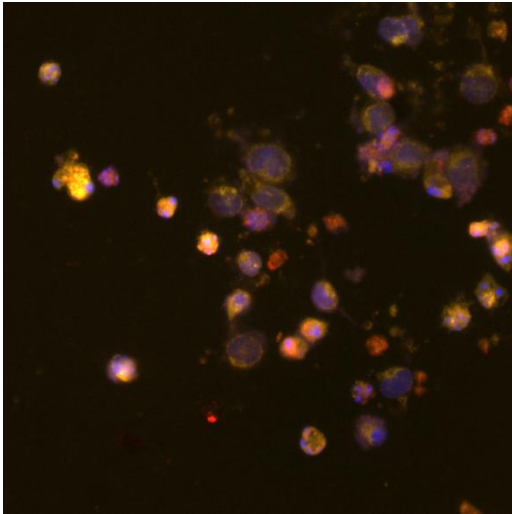
Composite   ROI_A_Target_I   ROI_A_Target_II

Function: Allows examination of a vast amount of colocalisation measurements of two targets in different sub cellular regions of interest

Uses: Colocalisation of proteins, Translocation, membranes study, overlap of regions…

Research Applications: cancer, metabolic, cell signaling, toxicology, functional genomics, fundamental research, protein localisation

Output Features: nuclei count, intensity size and shape - Intensity, translocation, colocalisation and overlap features for Targets and ROIs- spots count, morphology and intensity

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