IncuCyte™ ZOOM System – Time-Lapse Imaging around the clock
IncuCyte™ Live-Cell Imaging System

Real-Time visualization and automated data analysis – directly inside your incubator
IncuCyte™ System – Key Advantages

- Real-time visualization
- Quantitative data
- Multi-vessel compatible including 96 and 384-well
- No disturbance to cells
- Automated measurement from hours to weeks
- Unlimited software licenses
IncuCyte™ System – Key Advantages

HT1080 cell invasion through Matrigel

RWD (%) vs Time (h)
The Power of Long Term Time Lapse Imaging

Different Endpoint = Different Result

- Automated measurement from hours to weeks
- Time based trends support data analysis
Software Advantage - Distributed Access

- Unlimited licenses
- Remote control & access
- Database management
- Integrated analysis

NETWORK
1000s of Users, 100s of Peer Reviewed Publications and Growing

No of Publications (Cumulative)

- Cancer Biology
- Immunology
- Neuroscience
- Virology
- Stem Cell Biology
- 3D Biology

520 as of June 2015
### IncuCyte ZOOM® Key Applications

<table>
<thead>
<tr>
<th>Application</th>
<th>Image</th>
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</thead>
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<tr>
<td>Confluence</td>
<td><img src="image1.png" alt="Confluence Image" /></td>
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<tr>
<td>Cell Migration</td>
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<tr>
<td>Cell Invasion</td>
<td><img src="image3.png" alt="Cell Invasion Image" /></td>
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<tr>
<td>Proliferation &amp; Cell Counting</td>
<td><img src="image4.png" alt="Proliferation &amp; Cell Counting Image" /></td>
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<td>Chemotaxis</td>
<td><img src="image5.png" alt="Chemotaxis Image" /></td>
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<tr>
<td>3D-Spheroids</td>
<td><img src="image6.png" alt="3D-Spheroids Image" /></td>
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<td>Apoptosis</td>
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<td>Cytotoxicity</td>
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<tr>
<td>T-cell killing</td>
<td><img src="image9.png" alt="T-cell killing Image" /></td>
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<tr>
<td>Gene Expression</td>
<td><img src="image10.png" alt="Gene Expression Image" /></td>
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<tr>
<td>Neuronal Co-Culture - Fluorescence</td>
<td><img src="image11.png" alt="Neuronal Co-Culture - Fluorescence Image" /></td>
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<tr>
<td>Neurite Dynamics - Label-free</td>
<td><img src="image12.png" alt="Neurite Dynamics - Label-free Image" /></td>
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<tr>
<td>Clonal Dilution</td>
<td><img src="image13.png" alt="Clonal Dilution Image" /></td>
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<tr>
<td>Angiogenesis</td>
<td><img src="image14.png" alt="Angiogenesis Image" /></td>
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<tr>
<td>Stem Cell Monitoring &amp; Reprogramming</td>
<td><img src="image15.png" alt="Stem Cell Monitoring &amp; Reprogramming Image" /></td>
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</table>
Cell Confluence & Health

- Measure cell proliferation over time & without labels
- Cells remain in your incubator throughout assay
- Applicable to a wide range of cell types
- Validate data and morphology with images & movies
Cell Migration & Invasion

- Create scratch wounds in seconds on 96-well plates
- Automatically analyse full time course of wound healing
- No labels required, amenable to wide range of cells
- Multiplex migration and invasion through ECM
Chemotaxis & Directional Migration

• Visualize cells migrating toward chemo-attractants
• No labels required, low cell usage, surface interactions
• Monitor full time course of chemotaxis
• Set it & forget it – fully automated analysis
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Cell Proliferation and Cell Counting

Single EF-1 alpha promoter used to express both fluorescent label and puromycin resistance marker

Movies

NucLight Red HT1080 and NucLight Green A549 grown in co-culture

Metrics

![Graph showing NucLight Green Count/mm² over time](image)

Throughput

![Time-course data - 384-well plate view](image)

- Create new cell lines or transient labelled primary cultures with red or green nuclei
- Direct, linear quantitation and that is not subject to artefacts
- Can be multiplexed with Apoptosis or other cell health assays
- Cell Lines also available in Green or Red from Essen
3-D Spheroids

- Ultra Low Attachment Round Bottom Plates used
- Spheroids creation easy
- Area and fluorescence intensity metrics used to measure growth, shrinkage and spheroid health
- Validate findings with images and movies

**Vehicle Control**

<table>
<thead>
<tr>
<th>Time</th>
<th>t=0</th>
<th>t=96h</th>
<th>t=198h</th>
<th>t=258h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td><img src="vehicle_control_t0.png" alt="image" /></td>
<td><img src="vehicle_control_t96h.png" alt="image" /></td>
<td><img src="vehicle_control_t198h.png" alt="image" /></td>
<td><img src="vehicle_control_t258h.png" alt="image" /></td>
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<td>SPP 1 µM</td>
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<td><img src="ox_t258h.png" alt="image" /></td>
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<tr>
<td>CHX 10 µM</td>
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<td><img src="chx_t258h.png" alt="image" /></td>
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**Metrics**

Time course data – Dose response to Staurosporine

**Throughput**

Staurosporine, Oxaliplatin, Cycloheximide, Media/DMSO

Fluorescence Area (µm²)

Movies

A549 Spheroids

Time (days)
Quantify real time commitment to death by apoptosis
No wash, mix and read assays in 96- and 384-well format
Easily duplex with cell proliferation measures
Validate data with images & movies
Cytotoxicity

- Quantify real time cell death
- No wash, mix and read assays in 96- and 384-well format
- Easily duplex with NucLight Red Proliferation Assay
- Validate data with images and movies

Kinetic Cytotoxicity using YOYO®-1 and Promega CellTox™ Green Dyes

Movies

Metrics

Throughput
T-cell Killing & Immuno-Oncology

- Direct & kinetic measures of target cell death
- No radioactivity, labelling antibodies or cell lifting
- Images/movies validate target/effectector cell interactions
- ADCC, CDC, T-cell clustering & proliferation assays

**Metrics**

- IncuCyte™ Caspase-3/7 Apoptosis Assay Reagent
- NucLight Red

**Time course data**

**Throughput**

- Movie: NucLight Red SK-OV-3 cells + PBMCs + Caspase 3/7 reagent

**BACK**
• Dose response of TNFα added to cells transfected with NFκB-GFP reporter genes is shown in this example.

• The activation of the TNF receptor leads to the activation of NF-κB, a transcription factor that, in turn, stimulates gene expression.

• After the addition of the TNFα, the cells are placed into the IncuCyte ZOOM which kinetically measures GFP expression.

<table>
<thead>
<tr>
<th>Research Area</th>
<th>Reporter Constructs (Transcription Factor)</th>
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<tbody>
<tr>
<td>Cancer</td>
<td>NF-κB, p53, MAPKs, Wnt, HIF-1α, PI3K</td>
</tr>
<tr>
<td>Inflammation</td>
<td>NF-κB, TGF-β, Interferon Response</td>
</tr>
<tr>
<td>Cardiovascular Research</td>
<td>cAMP/PKA, Notch, MEF-2</td>
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<tr>
<td>Stem Cells</td>
<td>KLF4, Oct4, Nanog</td>
</tr>
<tr>
<td>Nuclear Receptors</td>
<td>PPARγ</td>
</tr>
<tr>
<td>Toxicity</td>
<td>AhR (CYPs), Nrf1, Nrf2</td>
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Neurite Dynamics

- Monitor neurite outgrowth over days and weeks
- Label-free automated analysis in mono-culture
- Specialized neuronal fluorescent labels for co-culture
- Validated for primary & iPSC-derived neurons
Clonal Dilution Monitoring

Step 1: Image plates inside IncuCyte for extended periods. Automatic colony identification without cell labels.

Step 2: Inspect colony morphology

Step 3: Confirm clonality by browsing “backward and forward” in time

- Automated image processing to Identify and track clones, confirm clonality
- Mark clones in software, physically mark plates with IncuCyte Marking tool
- Automated image panning of large vessels such as 35mm dishes

Software Demo

Throughput

Marking Tool

ESSEN BioScience
Two well published Angiogenesis co-culture models consisting of cryopreserved cells and supplements available from Essen BioScience:

1) Adipose Derived Stem Cells/Endothelial Colony Forming Cells (model yields rapidly forming (<48h) endothelial cell ‘cord’ structures)

2) Normal Human Dermal Fibroblasts/HUVEC (slowly forming ‘tube-like’ structures appear, grow and branch for 10 days)

3) Different morphological, temporal, and pharmacological profiles
Stem Cell Reprogramming

Day 0
Add Sendai virus containing Yamanaka Factors

Day 13
Colonies begin to emerge

Days 21-27
Transfer iPSC colonies

Day 7
Passage cells (MEFs or Feeder Free)

Cell inspection & refeed

Day -2
Plate cells (Neonatal or Adult fibroblasts)

• Days 0 - 7
  - Evaluate toxicity of reprogramming reagents

• Days 8 – 21
  - Track colony emergence, growth, and morphology
  - Standardize calculation of reprogramming efficiencies

• Days 21+
  - Post-Rep summarization - monitor clone transfer and survival

Movies
Stem cell colony 12 days post passage

Colony Marking and Tracking

IncuCyte Marking Tool
IncuCyte ZOOM® – Key Features

Multiple Imaging Modes

- HD phase-contrast, green & red fluorescence, 4x 10x 20x
- Sample area to whole well image capture, up to 6 plates at once
- 24h scheduling and ‘scan on demand’

Multi-Vessel, Multi-User

- Compatible with over 300 standard plates, dishes & flasks
- Mix & match vessels & assays
  (slides, dishes, flasks, and 6 to 384-well plates)

Remote Viewing & Analysis

- Fully automated, fast on the fly analysis
- Shared access via any networked Windows device
Key Resources

Learn more about IncuCyte™ product offerings:

IncuCyte Instrument

Software Modules

Reagents & Consumables

Chemotaxis

Cell Migration Kit

Cell Invasion Kit

IncuCyte™ Publications

Examples of Technical Data