全自動活細胞影像分析測定系統 (IN Cell Analyzer)
細胞水平功能測定及高內涵藥物篩選

1. 緣由

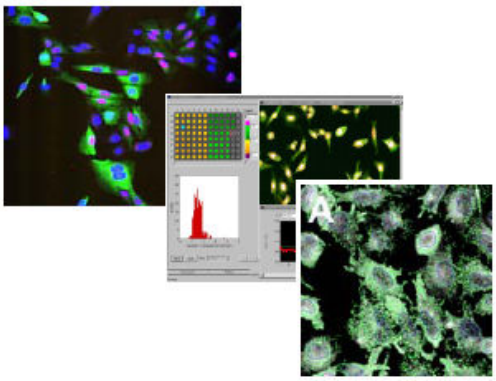
基於基因組學及蛋白質組學的發展及藥物篩選的需要，近一年來高內涵細胞及亞細胞水平的分析技術（High Content Screen, or HCS）發展神速。大量的未知功能的基因及蛋白質的出現急需能有快速，定量定性的功能測定的技術，並且從ＤＮＡ及蛋白質分子的研究轉入細胞中基因及蛋白質的功能研究。藥物開發中也急需細胞水平的篩選來得到更多信息從而加快及降低藥物開發的成本。全自動活細胞影像分析系統就是這一技術的代表。

從基因組及蛋白質組的研究將得到一系列與疾病有關的特定基因或蛋白，利用全自動活細胞影像分析測定系統，可快速定量的觀察到它們在細胞內的功能及作用機制，從而為疾病的產生，預防及治療提供依據，完成從基因，蛋白到細胞生物體內的完整資訊。

全自動活細胞影像分析測定系統結合高內涵細胞水平分析技術是近年來在藥物篩選中應用最廣的技術。例如：治療SARS 中藥篩選，可透過全自動活細胞影像分析測定系統結合高內涵細胞水平分析技術篩選出對非典型肺炎不同病理環節和改善其臨床症狀有效的中成藥。

藥物篩選急需有新的方法來降低成本，提高效率，讓無效的候選藥物儘快在臨床測定前就被剔除，而在細胞水平層面的篩選完全符合這些要求。觀察候選藥物在單個細胞中的活動情景能清晰的告訴我們藥物能否進入細胞，在細胞內如何分佈，候選藥物對細胞的作用如何，對細胞的毒性如何等多重資訊。

2. IN Cell Analyzer 活細胞圖像分析系統



技術包括儀器，試劑及分析軟體三部分。

(1) 儀器

儀器包括一個自動升降平台，高解析度CCD 相機，鐳射自動聚焦系統以滿足多螢光成像。對於一個常規雙色成像，可在5 分鐘內或15 分鐘內完成96 孔板或384 孔板樣品分析。IN Cell Analyzer 1000 提供多種圖像軟體後處理應用功能。該系統也是一個開放平台，可容納多種實驗室自動系統，同時也可升級，增加如溫度控制或線上加樣等功能。

系統可提供多種圖像分析功能軟體。通過調整用戶自定義參數，操作者可採用專一分析途經追蹤多種細胞變化過程。包括胞內關鍵信號分子轉位元、轉移因數、活化、受體內源化、鈣離子通道、酶活化、細胞凋亡和細胞週期，以及神經突生長。先進的圖像分析技術可採用由多通道採集的圖像彙集為四色圖像。逐一細胞分析可用於觀察細胞群的整體特性，從而避免由於使用不同細胞群的平均反應值而帶來的信號衰減。

- 實驗台儀器 安裝簡便，對實驗室環境無特殊要求
- 有4×，10×，20×，40×物鏡
- 100 瓦氙燈，亮度高，產熱低，壽命長
- 可冷卻到-30℃高質量數碼相機高靈敏度，背景噪音低
- 高解析度 12bit，圖像解析度1392×1040
- 自動位移裝置，快速並精確地移動細胞培養板或載玻片
- 鐳射主導的自動聚焦快速，小於150 毫秒
- 自動濾光片轉換盤，自由轉換各種波長的激發及散射濾光片
- 可安裝溫度控制系統，研究活細胞，動力學測定。溫度控制範圍25℃～42℃
- 可加裝自動加樣系統，可加藥品或化合物1～100ml，並有自動清洗裝置
- 可加白光系統，無需標記，高解析度的細胞形態測定
- 先進的圖像分析，高效且使用簡易的分析軟體，多視野圖像動力學分析模式

(2) 試劑

細胞圖像分析的工具---細胞測定細胞株，報告基因系統和試劑
多種GFP 信號傳導細胞株，利用GFP(源於Aequorea Victoria) 建構確證的穩定細胞株和表達載體。

- GFP-MAKAP-k2 Assay P38 途徑
- GFP-Rac-1 Assay 胞質分裂，遷移，轉換
- GFP-PLCδPH domain Assay PI(4,5)P2 信號傳導途徑
- EGFP-2×FYVE Assay 生長因數RTK 細胞因數信號途徑
- AKT1-EGFP Assay 細胞存活、增殖
- EGFP-STAT3 Assay 細胞因數轉導
- EGFP-NFAT c1 Assay 免疫反應，前列腺素信號傳導途徑
- EGFP-SMAD Assay TGF-b 信號傳導
- G2/M cell cycle phase marker assay 細胞週期標記

CypHer: pH 敏感螢光表達試劑和方法
CypHer 可用來研究細胞的表面受體在受到配體啟動後發生內源化進入細胞內的變化過程。

硝基還原酶報告基因系統 (Nitroreductase Reporter Gene System)
已確證的既可在活細胞中表達也可作為轉染方法，將紅移螢光底物CytoCy5STM 由無色轉化為紅色產物。該系統同時可與其他報告基因系統，如GFP 在同一細胞中同時使用。

siRNA 文庫篩選
採用siRNA 基因剔除，同時以GFP 為指針，將涉及細胞週期過程的約100 個基因進行功能研究。

腺病毒載體轉導細胞檢測 (Adenoviral Vector Transduction Assay)使用腺病毒載體轉導，將多靶點基因轉入關鍵細胞信號傳導途徑，在多種細胞中得到直接高水準轉化基因表達。

(3) 分析軟體

資料分析軟體模組
彈性的分析軟體模組可用於多種應用，並提供全自動，快速分析，以適應定量分析所需的大量原始資料。

- 細胞內空間位移（Nuclear Trafficking）
- 核位移動力學（Kinetic Nuclear Trafficking）
- 神經突生長（Neurite Outgrowth）
- 受體顆粒形成（Granularity）
- 目標蛋白強度（Object Intensity）
- 細胞週期（Cell Cycle Trafficking）
- 細胞程式死亡（Apoptosis）
- 細胞形態（PM Trafficking Morphology）

3. 相關應用

- 核運輸（nuclear trafficking）
- 神經生長（neurite extension）
- 受體啟動（receptor activation）
- 內體運輸（endosomal trafficking）
- 線粒體運輸（mitochondrial translocation）
- 鈣離子通道（calcium flux）
- 細胞週期研究（cell cycle analysis）
- 動力學分析（kinetic options）
- 報導基因表達（reporter gene expression）
- 蛋白表達（protein expression）
- 存活率（viability）
- 配體結合（ligand binding）
- 多重凋亡探針（multiplexed apoptotic probes）
- 細胞表面位移（cell surface translocations）
- 細胞形態變化（shape changes）
- 研發客戶自主分析模式（develop new analysis routines）

4. 參考文獻

IN Cell Analyzer reference list
Updated 15 July 2004
The cellular distribution of fluorescently labeled arrestins provides a robust, sensitive, and universal assay for screening G protein-coupled receptors.
AUTHOR: Oakley Robert H; Hudson Christine C; Cruickshank Rachael D; Meyers Diane M; Payne Richard E; Rhem Shay M; Loomis Carson R
JOURNAL: ASSAY and Drug Development Technologies 1 (1): p21-30 November 2002
ABSTRACT: G protein-coupled receptors (GPCRs) have proven to be a rich source of therapeutic targets; therefore, finding compounds that regulate these receptors is a critical goal in drug discovery. The TransfluorTM technology utilizes the redistribution of fluorescently labeled arrestins from the cytoplasm to agonist-occupied receptors at the plasma membrane to monitor quantitatively the activation or inactivation of GPCRs. Here,we show that the Transfluor technology can be quantitated on the IN Cell Analyzer system (INCAS) using the vasopressin V2 receptor (V2R), which binds arrestin with high affinity, and the beta2-adrenergic receptor (beta2AR), which binds arrestin with low affinity. U2OS cells stably expressing an arrestin-green fluorescent protein conjugate and either the V2R or the beta2AR were plated in 96-well plastic plates and analyzed by the INCAS at a screening rate of 5 min per plate. Agonist dose-response and antagonist dose-inhibition curves revealed signal-to-background ratios of approximately 25:1 and 8:1 for the V2R and beta2AR, respectively. EC50 values agreed closely with Kd values reported in the literature for the different receptor agonists. In addition, small amounts of arrestin translocation induced by sub-EC50 doses of agonist were distinguished from the background noise of untreated cells.
Furthermore, differences in the magnitude of arrestin translocation distinguished partial agonists from full agonists, and Z' values for these ligands were >0.5. These data show that the Transfluor technology, combined with an automated image analysis system, provides a direct,robust, and universal assay for high throughput screening of known and orphan GPCRs.

A pH-sensitive fluor, CypHerTM 5, used to monitor agonist-induced G protein-coupled receptor internalization in live cells.
AUTHOR: Adie E J (Reprint); Kalinka S; Smith L; Francis M J; Marenghi A; Cooper M E; Briggs M; Michael N P; Milligan G; Game S
JOURNAL: BioTechniques 33 (5): p1152-1157, 2002
ABSTRACT: G protein-coupled receptors (GPCRs) are the largest family of proteins involved in transmembrane signal transduction and are actively studied because of their suitability as therapeutic small-molecule drug targets. Agonist activation of GPCRs almost invariably results in the receptor being desensitized. One of the key events in receptor desensitization is the sequestration of the receptor from the cell surface into acidic intracellular endosomes. Therefore, a convenient, generic, and noninvasive monitor of this process is desirable. A novel, pH-sensitive, red-excited fluorescent dye, CypHerTM 5, was synthesized. This dye is non-fluorescent at neutral pH and is fluorescent at acidic pH. Anti-epitope antibodies labeled with this dye were internalized in an agonist concentration- and time-dependent manner, following binding on live cells to a range of GPCRs that had been modified to incorporate the epitope tags in their extracellular Nterminal
domain. This resulted in a large signal increase over background. When protonated, the red fluorescence of CypHer 5 provides a generic reagent suitable for monitoring the internalization of GPCRs into acidic vesicles. This approach should be amenable to the study of many other classes of cell surface receptors that also internalize following stimulation.

Title: CypHer 5: A generic approach for measuring the activation and trafficking of G protein-coupled receptors in live cells
Author(s): Adie EJ (REPRINT) ; Francis MJ; Davies J; Smith L; Marenghi A; Hather C; Hadingham K; Michael NP;
Milligan G; Gamel S
Journal: Assay and Drug Development Technologies, V1, N2 (APR), P 251-259, 2003
Abstract: GPCRs are one of the most popular classes of therapeutic drug targets. It is therefore important to design specific assay formats to readily identify ligands at these receptors. CypHer 5 technology utilizes the

general ability of GPCRs to be internalized into the endosomal pathway of a cell in response to agonist ligands. The CypHer 5 dye is fluorescent in acidic environments, but nonfluorescent at neutral pH. When CypHer 5 is bound to a receptor on the extracellular surface of the cell, it is essentially nonfluorescent. On internalization into a cell, it displays a significant increase in fluorescence. Here we demonstrate the detection of agonist activation of two GPCRs in stably transfected live cells using CypHer 5 technology. The G(q)-coupled TRHR-1 and the G(s)-coupled beta(2)-adrenoceptor were both N-terminally tagged with VSV-G. Following addition of CypHer 5-labeled anti-VSV-G antibodies to HEK 293 cells stably expressing the beta(2)-adrenoceptor or CHO-K1 cells stably expressing the TRHR-1, the cells were treated with agonists and then imaged on Amersham Biosciences' IN Cell Analyzer 3000. Data were quantified using a granularity analysis module. Concentration-response curves were obtained with signal-to-background ratios of 7:1 for both receptors. An EC50 of 0.52 nM was observed on TRH stimulation of the TRHR-1, and an EC50 of 30 nM was obtained on isoprenaline stimulation of the beta(2)-adrenoceptor. These results demonstrated that the CypHer technology was capable of measuring high-potency agonist responses. The beta(2)-adrenoceptor antagonist, alprenolol, competed for isoprenaline with an IC50 of 30 nM, indicating that a high-potency antagonist inhibition curve could also be observed using CypHer. CypHer 5 provides a generic tool to measure GPCR activation in a live cell, homogeneous assay format, and may be equally suitable for detecting activation of other classes of cell surface receptors.

Title: Automated screening of neurite outgrowth
Author(s): Ramm P; Alexandrov Y; Cholewinski A; Cybuch Y; Nadon R; Soltys
Journal: Journal of Biomolecular Screening, V8, N1 (FEB), P7-18 , 2003
Abstract: Outgrowth of neurites in culture is used for assessing neurotrophic activity. Neurite measurements have been performed very slowly using manual methods or more efficiently with interactive image analysis systems. In contrast, medium-throughput and noninteractive image analysis of neurite screens has not been well described. The authors report the performance of an automated image acquisition and analysis system (IN Cell Analyzer 1000) in the neurite assay. Neuro-2a (N2a) cells were plated in 96-well plates and were exposed to 6 conditions of retinoic acid. Immunofluorescence labeling of the cytoskeleton was used to detect neurites and cell bodies. Acquisition of the images was automatic. The image set was then analyzed by both manual tracing and automated algorithms. On 5 relevant parameters (number of neurites, neurite length, total cell area, number of cells, neurite length per cell). the authors did not observe a difference between the automated analysis and the manual analysis done by tracing. These data suggest that the automated system addresses the same biology as human scorers and with the same measurement precision for treatment effects. However, throughput of the automated system is orders of magnitude higher than with manual methods.

Title: pH-sensitive cyanine dyes for biological applications
Author(s): Cooper ME (REPRINT) ; Gregory S; Adie E; Kalinka S
Journal: Journal of Fluorescence V12, N3-4 (DEC), P425-429, 2002
Abstract: A series of functionalized, water-soluble, pH-sensitive pentamethine cyanine (Cy(TM)5) dyes has been designed and synthesized. These probes are fluorescent in acidic media but are non-fluorescent in an alkaline environment. Subtle changes to the structure of these probes can lead to pronounced changes in the pKa of these probes. These probes have been utilized in a cellular environment to detect localized changes in PH using the IN Cell Analyzer, a confocal imager formatted for imaging of cell-based assays.

Title: Confocal optics microscopy for biochemical and cellular high-throughput screening
Author(s): Zemanova L.; Schenk A.; Valler M.J.; Nienhaus G.U.; Heilker R. L.
Journal: Drug Discovery Today (DRUG DISCOV. TODAY) (United Kingdom) 01 DEC
Abstract: In recent years, both academia and pharmaceutical industry have produced significant advances in confocal detection and spectroscopy by laser-induced fluorescence. Confocal fluorescence studies provide information on identity, size, diffusion coefficient and concentration of the fluorescently labeled entity. This enables the establishment of sophisticated biochemical drug screening assays using the multitude of fluorescence parameters that can be observed (e.g. molecular brightness, fluorescence lifetime, anisotropy, resonance energy transfer). In cellular screening assays, confocality introduces spatial resolution in the vertical direction and reduces background fluorescence from outside the focal plane. Confocal HTS systems focusing on femtoliter-sized observation volumes allow for assay volumes far beyond current limits.

Title: Systematic genome-wide screens of gene function
Author(s): Carpenter A.E.; Sabatini D.M.
Journal: Nature Reviews Genetics (NAT. REV. GEN.) (United Kingdom), 5/1, P 11-22, 2004
Abstract: By using genome information to create tools for perturbing gene function, it is now possible to undertake systematic genome-wide functional screens that examine the contribution of every gene to a biological process. The directed nature of these experiments contrasts with traditional methods, in which random mutations are induced and the resulting mutants are screened for various phenotypes. The first genome-wide functional screens in Caenorhabditis elegans and Drosophila melanogaster have recently been published, and screens in human cells will soon follow. These high-throughput techniques promise the rapid annotation of genomes with high-quality information about the biological function of each gene.

Title: The first automated high content screening system
Author(s): Allison K.; Adamson V.
Journal: JALA - Journal of the Association for Laboratory Automation (JALA J. ASSOC. LAB. AUTOM.) (United
States), 8/3, P 27-29, 2003
Abstract: With biological discovery and technology advancing in parallel, AstraZeneca (AZ) announced that it would soon take delivery of what is thought to be the first fully automated high-throughput high-content screening system. This custom designed assay platform from RTS Life Science
International (RTS) automates the IN Cell Analyzer 3000 sub cellular analysis system from Amersham Biosciences. RTS has integrated its advanced scheduling system with the imaging tool to enable AZ to evaluate the effect of drug compounds on cellular processes.