Analyzing the Advantages of the YT-Software® in Stem Cell Research

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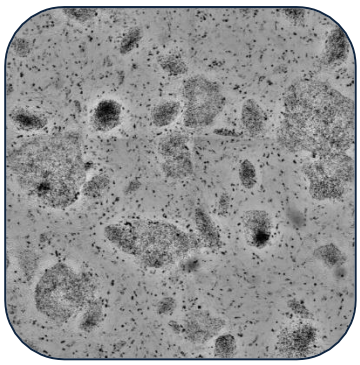

Application Note
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Abstract

The field of stem cell research is extremely diverse. Research projects include experiments on molecular biology, protein biochemistry and on a cellular level.
Whereas analytical methods in molecular biology and protein biochemistry are largely standardized and well-established, image based cell biology often requires laborious, individual solutions, which are carried out manually by the user picture by picture. For this reason, this application note will explore the possibilities and benefits of using automated imaging systems such as Cellavista® or NyONE® in combination with SYNENTEC's YT-software®. For this purpose, both induced pluripotent stem cells (IPSc) and embryonic stem cells (ESc) are taken into account. The analysis of cells cultured in semisolid media as well as on feeder layers is also investigated. Further important approaches in stem cell research at the cell biology level are the detection of alkaline phosphatases (AP), the detection of pluripotency by recombinant lectins and the investigation of differentiation markers as well as the investigation of retroviral expression systems.

KEYWORDS: IPSC, ESC, FEEDER LAYER, AP ASSAY, ALKALINE PHOSPHATASE, COLONY FORMING ASSAY, REPROGRAMMING, RETROVIRAL EXPRESSION SYSTEMS, NON-INVASIVE IPSC-DETECTION, CELL PROLIFERATION

Introduction

Healing of serious diseases in vivo is often limited by the inability of the body to rebuild a sufficient amount of healthy cells. Especially in neurons and chondrocytes (cartilaginous tissue) this is a big challenge. One research goal of many stem cell facilities is to understand the molecular mechanisms of diseases e.g. Parkinson's disease (PD) or to create models for reconstructive medicine and stem cell therapies. For these scientists, induced pluripotent stem (iPS) cells are a highly valuable tool in their research.

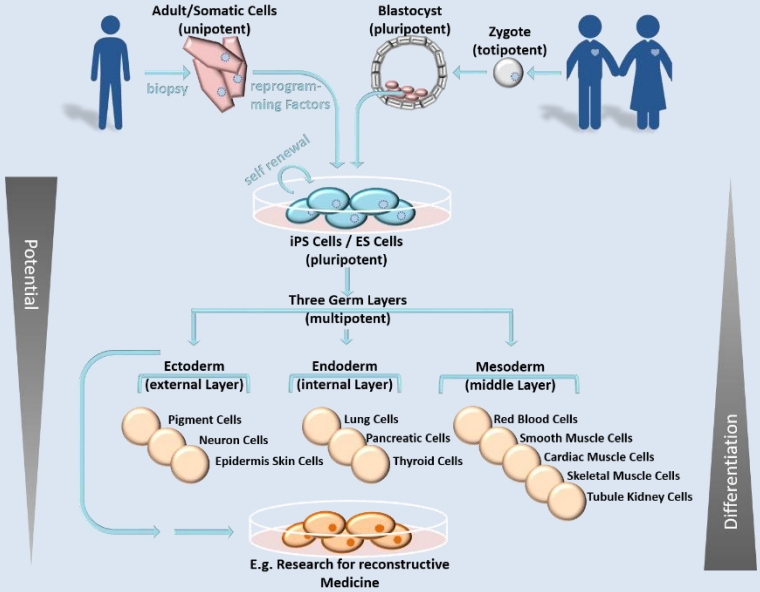


Fig. 1: Overview of cell types in stem cell research
The less differentiated cells are, the greater their potential to differentiate into different cell types.

Stem cells have the ability to differentiate into nearly every cell type of the organism. A distinction is made between embryonic stem cells (ES cells) and induced pluripotent cells (iPS cells). Since a decade, especially iPS cells came into focus of stem cell research more and more.

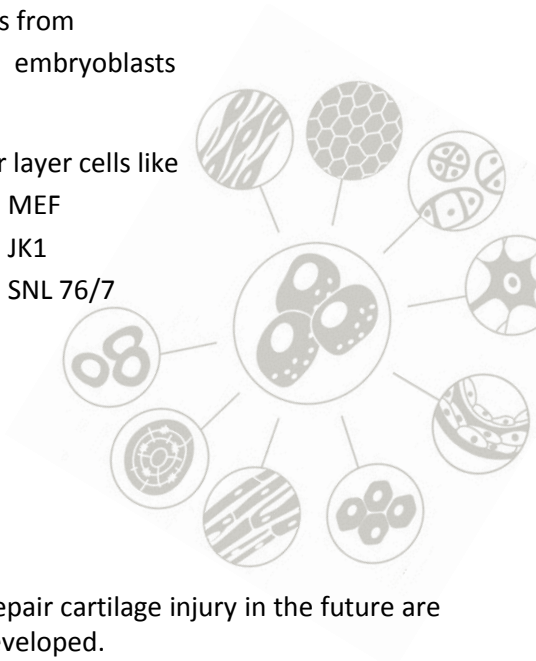
The main work in this field of research consists of iPS cell

derivation, cultivation, genetic modification, characterization of pluripotency of ES and iPS cells and finally the differentiation of this cell types. For this reason, this application note will explore the possibilities and benefits of using automated imaging systems such as Cellavista® or NyONE® in combination with SYNENTEC's YT-software®.

Supported Cell Types & Assays

Cell Types:

- iPS cells from
  - all species and all origins
  - adherent and suspension cells
  - independent from transcription faktor combinations (Oct-3/4, Sox-family…)
- Mesenchymal stem cells (MSCs) from
  - bone marrow
  - adipocytes
  - dental pulp tissues
- ES cells from
  - embryoblasts
- Feeder layer cells like
  - MEF
  - JK1
  - SNL 76/7



Assays:

1. Non-Invasive iPS Cell Detection

Since iPS cells are very rare and of high value, a reliable and highly sensitive quantification method for the success of the studies is essential.

Cellavista® and NyONE® in combination with YT-image analysis software® are capable of detecting iPS colonies grown on a feeder layer. The challenge is to identify these colonies which appear in low contrast brightfield images during early stages of growth.

To differentiate human iPS cells, these cells were sown on a special feeder cell layer. After a defined growth period, the size and number of the in the 2nd dimension grown iPS colonies can quickly be determined by brightfield measurement with 4x magnification.

2. Fluorescent Modification Of Stem Cell Proteins

The investigation of induced pluripotent stem (iPS) cells is moving more and more into the focus of research! Different systems to generate cell sources which produce hyaline cartilage with the aim to repair cartilage injury in the future are still being developed.

Different reprogramming methods to generate chondrocytes using fibroblasts are under investigation: e.g. direct reprogramming of fibroblasts into chondrocytes (iChon cells) and the generation of chondrocytes from reprogramming adult dermal fibroblasts to an undifferentiated state (Hiramatsu et. al 2011).

During their studies the Cellavista® with its high resolution, strong detection capabilities and advanced autofocus functionality has strongly supported their need to identify cells expressing low GFP signals as marker for induced cell properties.

3. Stem Cell Characterization

The genetic modification of e.g. human embryonic stem cells (HESCs) or human induced pluripotent stem cells (hiPSCs) is becoming an essential tool in studying stem cell biology and developing potential clinical approaches.

Despite the standard use of this method, it is associated with many difficulties. It is well

known how challenging and time consuming it may be to establish an ideal transfection protocol. Even if the desired vector is designed and cloned, the way to achieving reliable transfection is still far!

A widely applied method is the co-expression of selection markers in combination with fluorescent markers (e.g. eGFP, DsRED, mCherry…) additionally to the gene of interest. The read out for the qualitative and quantitative analysis of transfection will be usually performed by user-dependent and time-consuming manual microscopy or, respectively, precise but invasive flow cytometry.

In contrast to these methods SYNENTEC's cell imagers quantify the transfection efficiency and subsequently the quantification of the expression of the gene of interest within minutes via software assisted image analysis within a wide range of fluorescence spectra. The YT-Software® can analyze a whole microplate non-invasively and even track your culture over days to determine growth-curves (fig. 2) and thereby the health of your stem cells.

4. Recombinant Lectins As Probes For Undifferentiated hES and hiPS Cells

New developed recombinant BC2LCN can be used for research of human induced pluripotent stem (iPS) and embryonic stem (ES) cells. Those recombinant lectins (e.g. rBC2LCN lectin) can be coupled to fluorescent markers, such as fluoroisothiocyanate (FITC) or rhodamine derivatives and are suitable as novel probes for the localization of glycoconjugates on cell surfaces.

rBC2LCN is expected to be applicable not only to stem cell research but also to regenerative medicine since it is reportedly a useful marker for the detection of undifferentiated cells. rBC2LCN reportedly recognized exclusively an intramolecular sugar chain of Podocalyxin which exposes on the cell surface of undifferentiated hiPS and human ES.

Cellavista® and NyONE® in combination with the YT-image analysis software® are capable to detect and evaluate the fluorescent labeled r-lectin-probes which specifically mark undifferentiated stem cells.

5. Cell Proliferation

Confluence monitoring is a useful tool to determine various properties of your cell line. SYNENTEC's confluence image processing analysis is capable to solve a vast range of different questions – e.g. to monitor the growth rate under selective conditions or to prove effectiveness by adding a differentiation factor and monitor the growth rate over time as a first step of proving the influence on the cell type of interest.

With SYNENTEC's automated cell culture microscopes NyONE® and Cellavista® and the included YT-image analysis software® the proliferation analysis is possible for a variety of cells. Adherent lines can be detected as well as suspension cells. In addition, it is very easy to quantify additional fluorescent labels and their ratio to the total growth area.

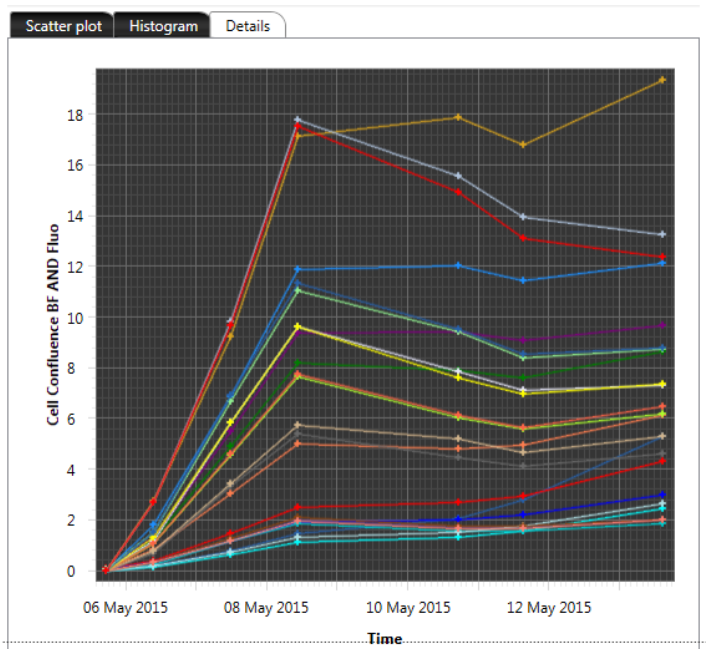
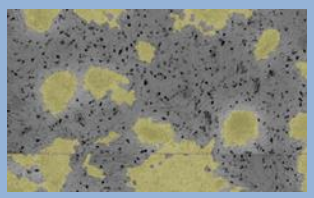
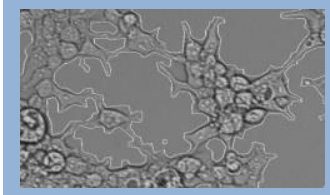
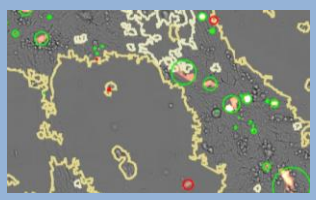
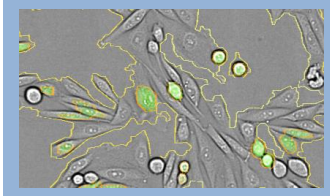
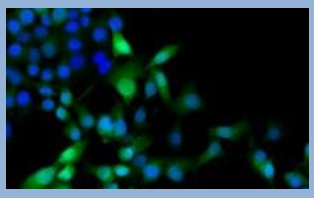
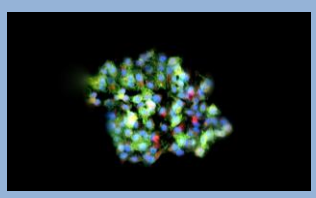


Fig. 2: Time chart generated with SYNENTEC's YT-software®
After measuring and processing all conducted images and measurements, the *Time Chart* function at the *Analyst*-Tab can be selected. By marking the desired wells a detailed presentation of the confluence development over time is obtained as shown in this diagram.

Software Capabilities

Tab. 1: Overview of different automated image analysis operators of SYNETEC's YT-Software®

Application	Information	Result-output of YT-software®
iPS on Feeder Layer 	• Ultra fast non-invasive Brightfield measurement • 4x lens • *'iPS Colony Count'* image analysis operator	• # of iPS Colonies • Cell Confluence [%] • iPS Colony Area [%] • average Colony Size [µm²] • …
Cell Proliferation 	• Non-invasive brightfield measurement • 4x lens • *'Cell Confluence'* image analysis operator	• covered Area [µm²] • evaluated Area [µm²] • Confluence [%] • …
Stem Cell Characterization 	• Multichannel fluorescence and brightfield measurement • 10x lens • *''Confluence (dots 1F)' or 'Confluence (dots 2F)'* image analysis operator	• % Confluence BF • % Confluence FL • FL Objects on BF Area/ BF Area • Avg. Fluorescence Intensity • …
Transfection Efficiency 	• Multichannel fluorescence and brightfield measurement • 10x lens • *''Confluence 1F' or 'Confluence 2F'* image analysis operator	• % Confluence BF • % Confluence FL • % Ratio of Confluence BF/FL • …
Genetic Modification 	• Multichannel fluorescence measure-ment • 10x lens • Hoechst 33342 as counter stain • *'Virtual Cytoplasm 1F'* image analysis operator	• total # of Cells • # of F1 Marker expressing Cells • % of F1 marker expressing Cells • Average Fluorescence Intensity • …
Recombinant Lectins 	• Multichannel fluorescence measure-ment • 10x lens • Hoechst 33342 as counter stain • *'Virtual Cytoplasm 2F'* image analysis operator	• total # of Cells • % of Hoechst33342 and rBC2LCN-Cy3 stained Cells or: • % of Hoechst33342 and rBC2LCN-FITC stained Cells • …

Conclusion

SYNENTEC's automated imaging systems like Cellavista® and NyONE® in combination with the YT-image analysis software® lead to reliable results and speed up your work regarding e.g. conventional fluorescently labeled marker antibodies against pluripotent cell surface biomarkers, fluorescently labeled recombinant lectins to discriminate pluripotent cells from differentiated cells as well as challenging and non-invasive brightfield imaging for detection of stem cell colonies on feeder layer and cell proliferation.
Due to the very flexible and comprehensive image analysis capabilities of the YT-software®, it is also possible to automatically evaluate further assays in the field of stem cell research in a standardized and reproducible manner.
Thus, e.g. cells from a colony Forming assay in semi-solid media after media lysis can be evaluated. Also possible is the detection of the alkaline phosphatase (AP), as marker for mouse and human embryonic stem cells (ES) and embryonic germ cells (EG).

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