A4Cell-Promega GloMax® Galaxy

INTEGRATION OF A4CELL'S SPACHIP TECHNOLOGY WITH PROMEGA'S GLOMAX® GALAXY SYSTEM: ADVANCED FLUORESCENCE IMAGING FOR SINGLE-PARTICLE ANALYSIS

INTRODUCTION

SPAchip® technology developed by A4cell enables long-term and non-invasive monitoring as well as quantitative analysis of diverse cell types. In this study, conducted in collaboration with Promega, we evaluated the compatibility and performance of the CytoCHECK SPAchip® pH Green Kit in cultured fibroblast cells. Measurements were performed using the Promega GloMax® Galaxy Bioluminescence Imager System, a multimodal imaging platform capable of detecting both fluorescence and bioluminescence signals.

MATERIALS AND METHODS

Fibroblast cell cultures were incubated with SPAchip® pH Green, a pH-sensitive fluorophore, and phalloidin, a marker of filamentous actin (F-actin) to visualize cytoskeletal structures. Imaging was performed using the GloMax® Galaxy, employing its high-NA fluorescence optics. Fluorescent signals were acquired in the green channel corresponding to SPAchip® emission and subsequently analyzed for single-particle segmentation and quantitative measurements of fluorescence intensity.

- Proof of Concept:
  - Sample: **CCD-1095Sk fibroblasts** with internalized **CytoCHECK SPAchip® pH Green Kit.**
  - Instrument: **GloMax® Galaxy.**

RESULTS

In a representative field of view, 198 individual SPAchips® were successfully identified and segmented based on green fluorescence intensity and morphology.

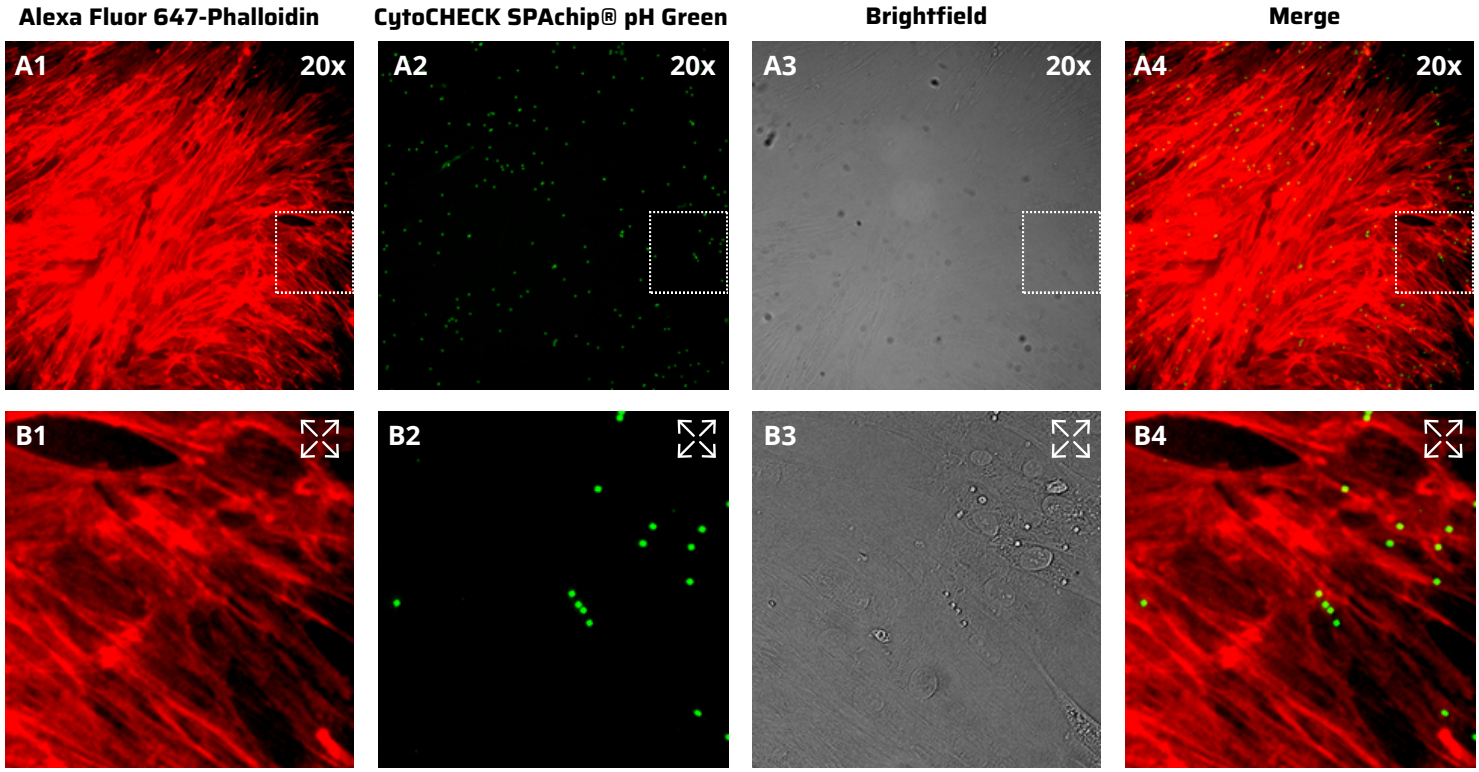

Figure 1: Representative microscopy images of fixed CCD-1095Sk fibroblasts acquired under basal DMEM conditions using the GloMax® Galaxy, demonstrating compatibility with A4cell's SPAchip® technology. Images were captured with a Nikon 20× Plan APO Lambda D objective (NA: 0.75, WD: 1 mm). Emission was detected using a 535/40 nm filter for the SPAchip® pH Green signal and a 600 nm long-pass filter for phalloidin staining. Exposure times: 300 ms (green) and 500 ms (far-red). Bottom panels (B1-B4) show zoomed-in regions from the corresponding top panels (A1-A4). Panels show images of the same field:

- **(A1-B1)** Alexa Fluor 647-Phalloidin staining of F-actin **(Ex/Em: 650/668 nm);**
- **(A2-B2)** CytoCHECK SPAchip® pH Green signal **(Ex/Em: 488/520 nm);**
- **(A3-B3)** Brightfield channel images;
- **(A4-B4)** Merged images combining fluorescent signals.

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Quantitative image analysis of mean fluorescence intensity (MFI) per SPAchip® was performed under standard DMEM culture conditions, revealing uniform signal distribution and reliable single-particle detection. The optical configuration of the GloMax® Galaxy system— including objective lens quality, resolution, and detector sensitivity—proved well-suited for SPAchip®-based cell assays.

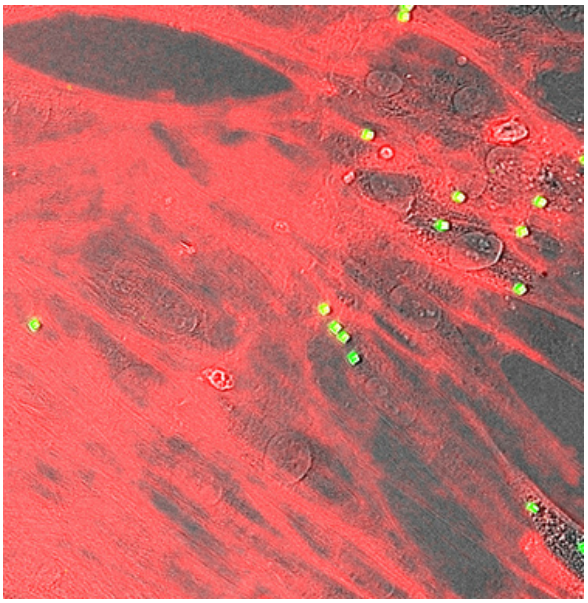

Figure 2: Merged image of fixed fibroblast cells showing fluorescent signals from far red F-actin staining (Ex/Em: 650/668 nm) and CytoCHECK SPAchip® pH Green (Ex/Em: 488/520 nm), displayed alongside the corresponding brightfield image. The composite enables simultaneous visualization of cytoskeletal architecture and intracellular pH-related signal. The fluorescence channels reveal both SPAchip® localization and F-actin structures, while the brightfield image allows identification of fibroblast nuclei, facilitating the distinction between extracellular and intracellular SPAchip® signals.

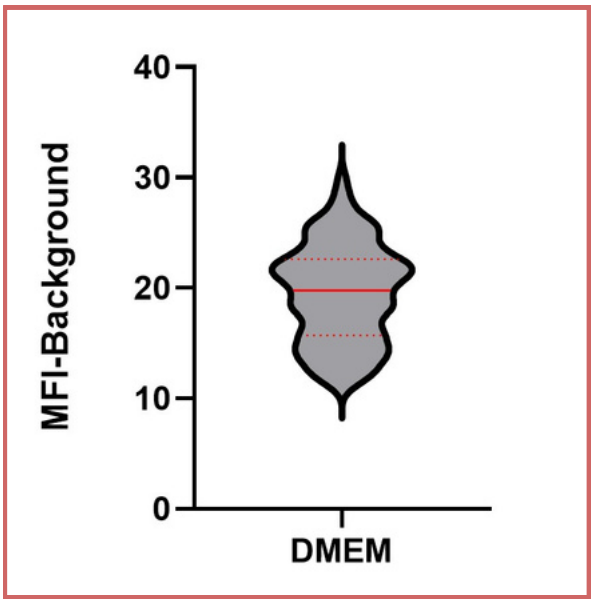

Figure 3: Quantitative fluorescence analysis of 198 individual SPAchip® segmented from a single field of view acquired under standard DMEM culture conditions. Mean fluorescence intensity (MFI) values, expressed in arbitrary units (a.u.), are plotted on the Y-axis, with corresponding basal condition (DMEM) indicated on the X-axis. Single-particle segmentation and intensity quantification were performed using image analysis software. The results reflect high signal uniformity and robust SPAchip® detection, highlighting the sensitivity and reliability of the GloMax® Galaxy for single-particle fluorescence assays. N=198.

DISCUSSION

The successful segmentation and quantification of SPAchip® confirms the compatibility of A4cell's technology with Promega's GloMax® Galaxy. While this study focused on fluorescence-based analysis, the system's integrated bioluminescence detection offers a promising avenue for future applications involving bioluminescent reporters, such as luciferase-tagged proteins or signaling pathways, in conjunction with SPAchip® technology assays.

This dual-imaging capability enables potential co-localization and functional readout from both fluorescence and bioluminescence channels, making the platform highly versatile for advanced cell-based assays, particularly in fields like drug screening, cellular metabolism, intracellular trafficking, and real-time monitoring of dynamic cellular responses.

CONCLUSION - *Promega GloMax® Galaxy Platform — Optimized for SPAchip® Technology*

- Seamless integration for multimodal imaging: A4cell's SPAchip® technology is fully compatible with the GloMax Galaxy, which uniquely integrates fluorescence and bioluminescence detection in a single system.
- Ideal for users already working with bioluminescence: Researchers conducting bioluminescent assays can now easily integrate SPAchip® technology into their workflows, enabling simultaneous or sequential multimodal analysis.
- High-resolution, quantitative imaging: The platform provides efficient, high-quality imaging of SPAchip® technology, enabling detailed cell-based studies.
- Enhanced analytical flexibility: The dual-mode imaging capabilities open new possibilities for integrated assays, offering deeper insights into cellular processes through complementary readouts.

ACKNOWLEDGMENTS

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