Visualizing of migration, interaction, proliferation or differentiation of cells in time lapse exposures using the cell separation robot CellCelector™

Cells are highly active, living objects. Under the microscope just a fixed snap-shot of the cell culture can be visualized. By observation in real-time complex interactions, migration or slowly morphological changes remain invisible. Additional to picking various cell types the CellCelector™ is suitable for exposure of time lapse series to provide an insight into activity, proliferation or morphological changes of cells.

Technology

The CellCelector™ (fig. 1a) is a robot for automated cell harvest. The patented system consists of an inverted microscope (1) equipped with a motorized stage (2) and CCD camera (3), an exchangeable robotic arm (4) as main functional tool and a deck tray for disposable tips (5), capillaries and destination plates (6). The versatile CellCelector™ software (fig. 2) comprises the imaging software CellD (Olympus) and AVISO control and imaging software. Detection of cells is enabled by predefined spectral and morphological parameters. Further applications of the software are long term observations of several objects, measurements of objects or filtering the microscopic images with different functions. Identified target objects can be isolated using one of the harvest tools. After the culture vessel is scanned, picking and documentation can be done automatically, based on the generated particle list. The harvest tools provide the collection of adherent or suspension cells as well as colonies in semi-solid media via mechanically detachment and aspiration. Special polished metal capillaries are used to scrape off adherent cells via a crosswise movement of the motorized stage. The scrape tool is also suitable for picking objects from semi-solid or solid media. For this application the methylcellulose-tool with attached disposable plastic tips works as well. The single cell tool works with a glass capillary attached to a pipetting system filled with mineral oil. Glass capillaries are available in different diameters up to 220 µm. The single cell tool enables precise aspiration of small volumes in microliter range. It is suitable to aspirate semi-adherent cells grown as multilayer. The parameters for cell harvest can be fine-tuned for the users special application.

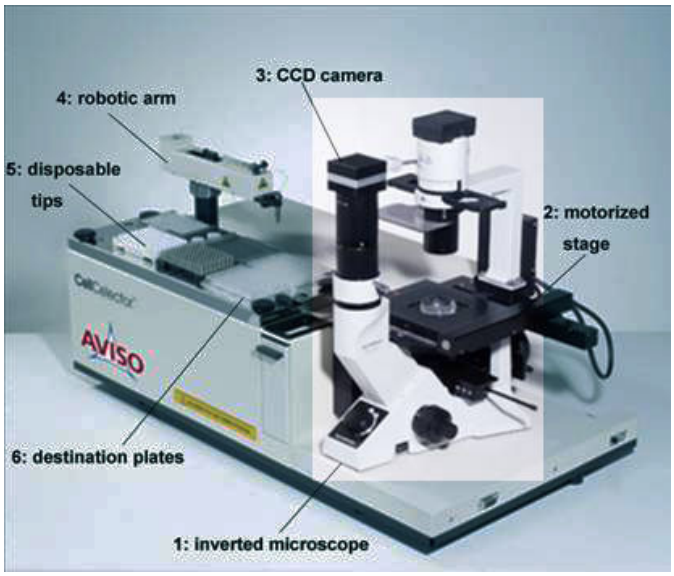

Figure 1: Assembly of AVISO's CellCelector™, robot for automated cell harvest. The microscope (Olympus CKX41) is available in customized solutions for various applications.

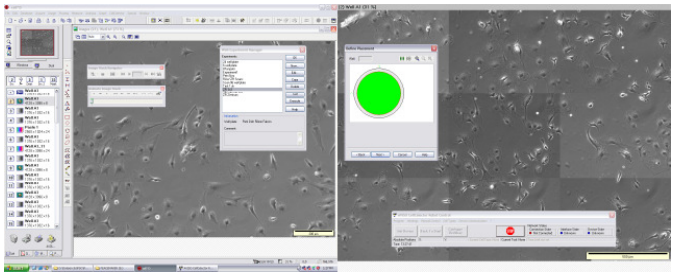

Figure 2: Screenshot of the CellCelector™'s control and imaging software. Microscopic images and scans are displayed on a dual screen. With the "Well Experiment Manager" scans of culture vessels can be defined and executed.

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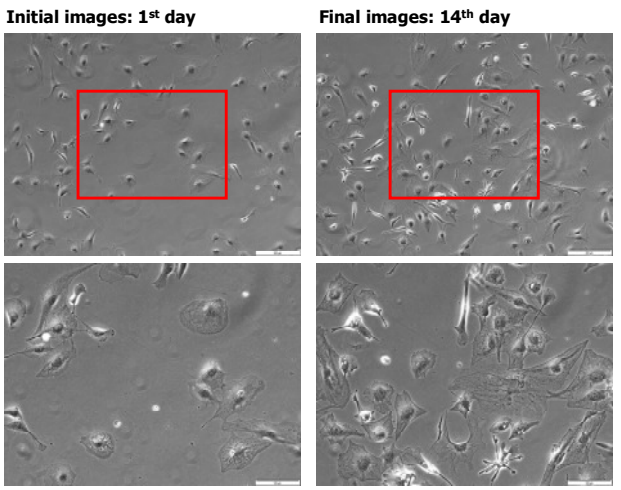
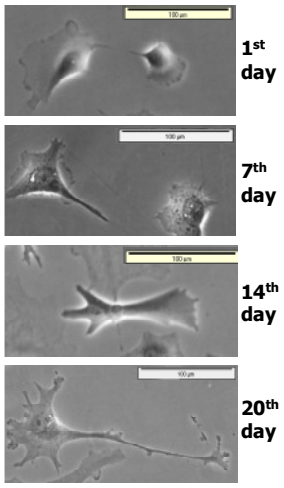


Figure 3 (left): Long term observation of stem cell differentiation and proliferation in neuronal differentiation medium over 14 days. Cells lost their stem cell properties and either became senescent or underwent morphological changes to neuronal cells.

Figure 4 (right): Typical examples for cells at different points of time during differentiation to neurons. In the first days cells formed dendritic extensions. Differentiated cells showed the characteristic morphology of neurons. Scale bars = 100 µm



During the cell cycle or differentiation cells undergo morphological changes. Also changes of cell lines over several passages or effects of various chemical or biochemical influences might be interesting to observe. Time lapse exposures are a useful tool for visualization of changing or migrating cells to get information that is not captured with a snap-shot. CellCelector™ microscope and software were applied for long term and short term observations in time lapse exposures of different adherent cell types in pure or co-cultures. To achieve a detailed visualization several objectives are mounted. Customized solutions for microscope assembly are available.

Neuronal differentiation of human induced pluripotent stem cells could be observed in long term observation of the cell culture over 14 days. The culture vessel was inserted in a special tray in which it could be incubated. To capture single images for the time lapse series every 12 h the tray was placed on the motorized stage. Calibrated positions were scanned with user-defined scan experiments. Thus it was ensured that the same area in the vessel was scanned at every point of time. Morphological changes (fig. 3 and 4) and increasing cell density (fig. 3) were captured in several time lapse movies.

In short term observations over 1 h interesting interactions between murine RAW264.7 macrophages and human fibroblasts isolated from a joint of a rheumatoid arthritis (RA) patient could be seen. For this application fast image sequences were acquired automatically. In pure culture cells showed their characteristic morphology and activity. Co-culturing the cell lines resulted in activation of macrophages and cellular stress of fibroblasts (fig. 5). Macrophages had formed dendritic extensions, migrated faster and were located close to or on fibroblasts. The fibroblasts surface became frazzled and cells slowly detached. These interactions might be caused by intercellular mediators (cytokines, chemokines, growth factors) secreted by the RA-fibroblasts [1,2,3] that activated the macrophages. It has also been shown that murine macrophages interact with human RA-fibroblasts *in vitro* by measurement of human and murine cytokines [4].

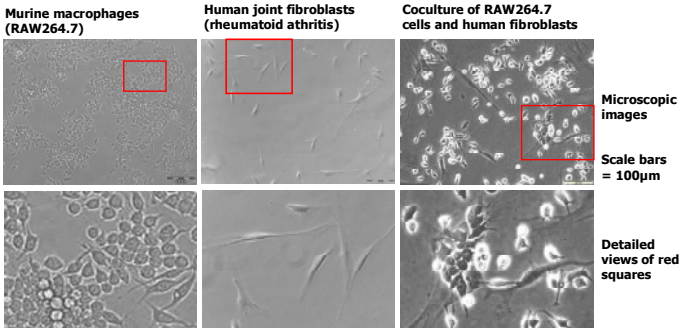


Figure 5: Short term observations of murine macrophages and human RA-fibroblasts. In co-culture the RAW264.7 macrophages became activated. The macrophages migrated faster and were located close to or on fibroblast cells. Morphological changes in both cell lines could be observed.

Conclusion

Results of cell culture observations show that time lapse movies can be an important tool for cell line characterization. Using the CellCelector™ system it was possible to obtain microscopic images in excellent quality and high resolution. Interesting cells can be identified and picked. In the CellCelector™'s flow cabinet observation, detection and cell harvest can take place sterile and under culture conditions (37 °C, 5 % CO_2) which reduce the cellular stress and increase the vitality of observed or harvested cells.

The CellCelector™ shows broad applicability for visualization and detection of target objects and for picking of different cell types or other objects from liquid, semi-solid or solid media.

References

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4. Lehmann J, Jüngel A, Lehmann I, Busse F, Biskop M, Saalbach A *et al.*: **Grafting of fibroblasts isolated from the synovial membrane of rheumatoid arthritis (RA) patients induces chronic arthritis in SCID mice - A novel model for studying the arthritogenic role of RA fibroblasts in vivo.** *Journal of Autoimmunity* 2000, **15:** 301-313.