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Designing the Future

The technology behind understanding cells and diseases

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A system that integrates pulsed laser microbeam irradiation and polymer microdevices is expected to lead to a greater understanding of cellular behavior and disease progression.

The technology, known as iCell, was developed by a research and development team at LightWorks Optics, Inc, a maker of advanced optics systems for biomedical companies. Collaborating with the University of California Irvine, LightWorks was awarded a grant through the National Institute of Health's (NIH) Small Business Innovation Research (SBIR) "Lab to Marketplace" program.

At the core of this cutting-edge technology are biocompatible polymer micropallets that are spin-coated on a glass slide. These pallets are designed such that cultured live cells remain on the top surface of individual pallets, which also can be coated with collagen or fibronectin in order to enhance cell attachment and growth [1]. The use of photolithography allows the pallets to be formed with sizes ranging from tens to hundreds of micrometers. This provides an adequate growth area for single cells or large colonies.

The plasma formation results in the emission of a shock wave and ablation of material within the focal volume, which produces a concurrent release of the micropallet from the glass slide. The use of these micropallets offers many advantages over other techniques such as LCM/LPC [3]:

- No UV laser microdissection step is involved, thereby eliminating potential UV damage to living cells.
- Micropallets are ~50-100 µm in thickness, ~ 10-20 x thicker than polymer foils used in LPC. The increased thickness combined with the inherent rigidity of the pallet polymer provides a mechanically stable substrate for living cells to withstand the mechanical stresses of the pallet release process. In addition, this provides a greater insulation of cells against damaging laser thermal effects.
- The release is carried out via mi-

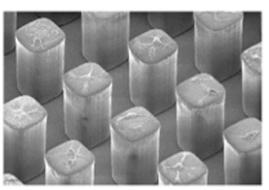

Figure 1

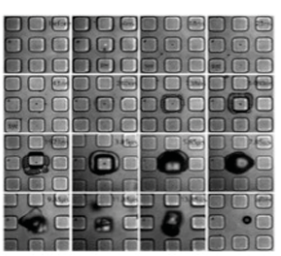

Figure 2

Figure 1. Magnified image of micro-pallets. The laser microbeam irradiation results in plasma formation at the interface between the glass slide and the polymer micropallet (see Figure 2).

Figure 2. The process of micro-pallet release. The center micropallet is released as an optimized low energy laser pulse is applied at the micropallet base [2].

Figure 3. Preview of the LightWorks Optics iCell system

Figure 4. The iCell system in operation.

cropallets that are immersed in growth media at all times, wherein living cells are best nourished.

• The micropallet arrays, with over 20k micropallets (for 100µm micropallet size) on a slide, facilitates process automation since a particular cellular sample can be released by addressing the coordinates of a specific pallet (similar to Tissue Micro-Array or TMA technology).

The development team's primary focus was to design a system that delivers the optimized micropallet release features in a compact, automated, affordable, and easy-to-use package that provides excellent cell viability, without causing any damage to the samples. Further, the iCell system can be mounted on top of any standard industry microscope, independent of the camera, while handling up to eight micropallet slides, or four micropallet Petri dishes containing thousands of micropallets.

The operator selects the cell(s) of interest, then releases the micropallet using a laser pulse, and collects the cell(s) for further expansion and analysis.

To date, excellent progress has been made in the development of the micropallet laser release system prototype. Our focus will be on testing various commercial applications of the system to ensure that the medical research community can achieve optimal identification and selection of adherent cells.

Looking ahead

Since the NIH's introduction of Laser Microdissection technology in the mid-1990s, the market has evolved at a rate of about 10-15% per year. Currently, other companies have viable Laser Microdissection systems that use somewhat similar techniques for cell selection, but produce the characteristic residual damage to collected cells. Additionally, these systems are priced from about $100,000 up to $500,000, making them cost prohibitive in some laboratory settings.

In addition to a healthy live-cell selection capability, the main driving force for higher penetration in this market in the years ahead will be simplicity and cost efficiency of systems. Industry statistics report approximately 5,000 system placements worldwide. With about 50,000 labs, academic sites and institutes worldwide, the global cell selection market size and potential can be roughly estimated at $5 billion.

This is a market at its early growth stage. The cell selection market is also part of a huge global

Current methods of cell selection

The selection, separation, and collection of specific single cells or small cell groupings from a mixed cell population is an important and common process in biomedical research and in biotechnology and pharmaceutical industries. For example, the development of cell lines derived from primary patient cells, stem cells, or genetically engineered cells, requires the isolation of specific single cells that are subsequently cloned or cultured to form a homogeneous cell population.

While established methods for selection exist to identify and select non-adherent cells, such as flow cytometry, chromatography, limiting dilution, and magnetic sorting, techniques for selecting adherent cells remain limited. This has produced an emergent universal need for positive selection of adherent mammalian cells [4]. The techniques for selection of adherent cells such as enzymatic digestion or mechanical release have drawbacks that include loss of cell morphology, removal of cell surface markers, damage to cell membranes, alterations in cellular physiology and loss of viability [5-8]. The methods to sort the cells after disaggregation, such as FACS (Fluorescence Activated Cell Sorter/ Flow Cytometry) can also lead to loss of membrane integrity and induction of apoptosis [9-11].

The development of Laser Capture Microdissection (LCM) followed by Laser Pressure Catapulting (LPC) in the 1990s provided an improved technology for the selection and separation of cellular samples. In these methods, cellular or tissue samples are grown or mounted on a thin (~5 µm) polymer film that is subsequently placed on a microscope cover glass. The periphery of the cellular/ tissue samples to be captured is first dissected using a pulsed UV laser. The dissected sample is then catapulted into a collection vial through a single visible laser pulse. However, LCM/LPC is typically used to collect cells for direct genetic analysis rather than continued culture. Expansion of cells using LCM/LPC often results in cellular and tissue injuries due to direct UV photo-damage associated with the dissection step, or injuries associated with the catapulting process. These damages typically include:

A. Nonspecific heating or perforation of the thin polymer film
B. Exposure of cellular samples to harsh extensional and shear stresses

Unfortunately, no instrument currently exists within the life sciences market for the sorting and subsequent expansion of adherent-type cells with high post-sort viability.

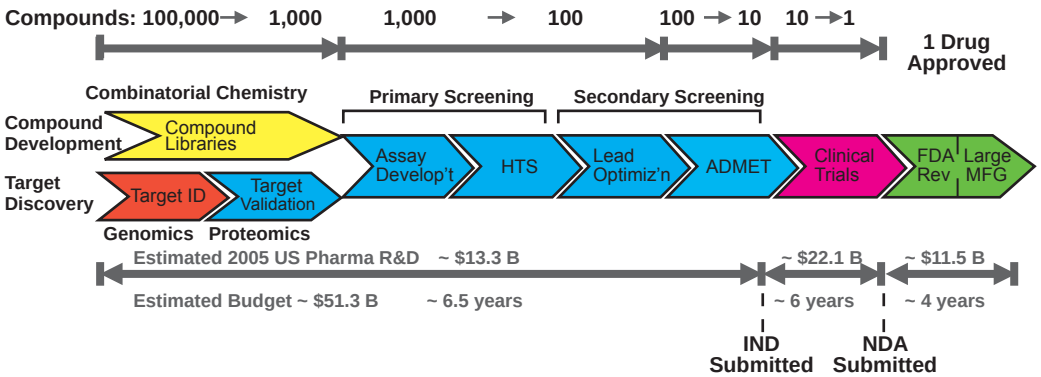

Figure 5. Process of drug discovery and development.

drug discovery and development research valued at more than $100 billion. Just one new drug introduction can take about 15 years to develop and cost about $500,000 to $1 billion.

In addition to improving accuracy and boosting throughput, the introduction of automated systems such as iCell will significantly speed the drug discovery and development process and help reduce health care costs over time.

Selection, separation, and collection of specific cell(s) will continue to be an important process in biomedical research and in biotechnology and pharmaceutical industries. While technology is firmly in place for selecting non-adherent cells, researchers are still in need of the new and effective methods of adherent cell selection and collection. With an emphasis on simplicity, automation, functionality, compactness, and cost, LightWorks Optics' iCell technology will provide the medical research industry with superior capabilities for live cell selection of adherent-type cells now, and well into the future.

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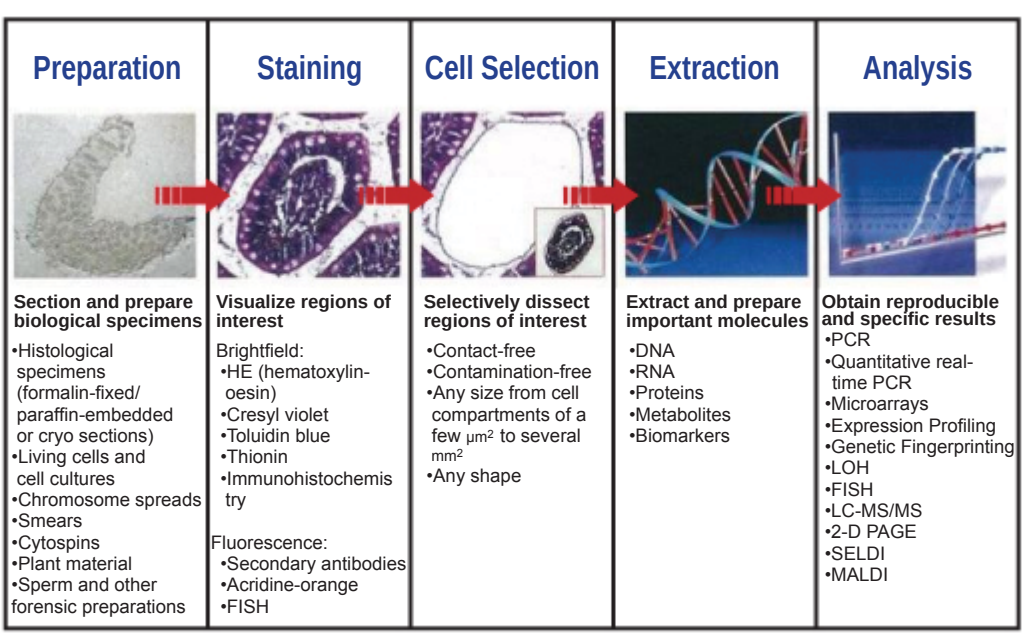

Figure 6. Typical workflow in the cell selection process. (Courtesy of Leica Microsystems)

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