



Cytometer on a Chip

Analyses could be performed rapidly in compact instruments using disposable chips.

Lyndon B. Johnson Space Center, Houston, TX

A cytometer now under development exploits spatial sorting of sampled cells on a microarray chip followed by use of grating-coupled surface-plasmon-resonance imaging (GCSPRI) to detect the sorted cells. This cytometer on a chip is a prototype of contemplated future miniature cytometers that would be suitable for rapidly identifying pathogens and other cells of interest in both field and laboratory applications and that would be attractive as alternatives to conventional flow cytometers.

The basic principle of operation of a conventional flow cytometer requires fluorescent labeling of sampled cells, stringent optical alignment of a laser beam with a narrow orifice, and flow of the cells through the orifice, which is subject to clogging. In contrast, the principle of operation of the present cytometer on a chip does not require fluorescent labeling of cells, stringent optical alignment, or flow through a narrow orifice. The basic principle of operation of the cytometer on a chip also reduces the complexity, mass, and power of the associated laser and detection systems, relative to those needed in conventional flow cytometry.

Instead of making cells flow in single file through a narrow flow orifice for sequential interrogation as in conventional flow cytometry, a liquid containing suspended sampled cells is made to flow over the front surface of a microarray chip on which there are many capture spots. Each capture spot is coated with a thin (≈ 50 -nm) layer of gold that is, in turn, coated with antibodies that bind

to cell-surface molecules characteristic of the cell species of interest. The multiplicity of capture spots makes it possible to perform rapid, massively parallel analysis of a large cell population.

The binding of cells to each capture spot gives rise to a minute change in the index of refraction at the surface of the chip. This change in the index of refraction is what is sensed in GCSPRI, as described briefly below. The identities of the various species in a sample of cells is spatially encoded in the chip by the pattern of capture spots. The number of cells of a particular species is determined from the magnitude of the GCSPRI signal from that spot.

GCSPRI as used here can be summarized as follows: The cytometer chip is fabricated with a diffraction grating on its front surface. The chip is illuminated with a light emitting diode (LED) from the front. By proper choice of grating parameters and of the wavelength and the angle of incidence of a laser beam, laser light can be made to be coupled into an electromagnetic mode that resonates with surface plasmons and thus couples light into surface plasmons. Coupling of light into a surface plasmon at a given location reduces the amount of incident light reflected from that location. A change in the index of refraction at the surface of a capture spot gives rise to a change in the resonance condition. Depending on the specific design, the change in the index of refraction could manifest itself as a brightening or darkening, a change in the wavelength needed to ex-

cite the plasmon at a given angle of incidence, or a change in the angle of incidence needed to excite the plasmon at a given wavelength.

Whereas a multiwavelength laser system with multichannel detection would be needed to detect multiple species in conventional flow cytometry, it suffices to use an LED and a single detector channel in the GCSPRI approach: this contributes significantly to reductions in cost, complexity, size, mass, and power. GCSPRI cytometer chips could be made of plastic and could be mass-produced cheaply by use of molding and other methods adopted from the manufacture of digital video disks. These methods are amenable to a high degree of miniaturization: such additional features as fluidic channels, reaction chambers, and fluid-coupling ports could readily be incorporated into the chips, without incurring substantial additional costs.

This work was done by Salvador M. Fernandez of Ciencia, Inc., for Johnson Space Center. Further information is contained in a TSP (see page 1).

In accordance with Public Law 96-517, the contractor has elected to retain title to this invention. Inquiries concerning rights for its commercial use should be addressed to:

*Dr. Salvador M. Fernandez, President
Ciencia, Inc.*

*111 Roberts Street, Suite K
East Hartford, CT. 06108*

Phone No.:(860) 528-9737

Fax No.:(860) 528-5658

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Principles, Techniques, and Applications of Tissue Microfluidics

This technique can be used in the diagnosis of diseases such as cancer.

NASA's Jet Propulsion Laboratory, Pasadena, CA

The principle of tissue microfluidics and its resultant techniques has been applied to cell analysis. Building microfluidics to suit a particular tissue sample

would allow the rapid, reliable, inexpensive, highly parallelized, selective extraction of chosen regions of tissue for purposes of further biochemical analysis.

Furthermore, the applicability of the techniques ranges beyond the described pathology application. For example, they would also allow the posing and success-

ful answering of new sets of questions in many areas of fundamental research.

The proposed integration of microfluidic techniques and tissue slice samples is called “tissue microfluidics” because it molds the microfluidic architectures in accordance with each particular structure of each specific tissue sample. Thus, microfluidics can be built around the tissues, following the tissue structure, or alternatively, the microfluidics can be adapted to the specific geometry of particular tissues. By contrast, the traditional approach is that microfluidic devices are structured in accordance with engineering considerations, while the biological components in applied devices are forced to comply with these engineering presets.

The proposed principles represent a paradigm shift in microfluidic technology in three important ways:

- Microfluidic devices are to be directly integrated with, onto, or around tissue samples, in contrast to the conventional method of off-chip sample extraction followed by sample insertion in microfluidic devices.
- Architectural and operational principles of microfluidic devices are to be subordinated to suit specific tissue structure and needs, in contrast to the conventional method of building devices according to fluidic function alone and without regard to tissue structure.
- Sample acquisition from tissue is to be performed on-chip and is to be integrated with the diagnostic measurement within the same device, in contrast to the conventional method of off-chip

sample prep and subsequent insertion into a diagnostic device. A more advanced form of tissue integration with microfluidics is tissue encapsulation, wherein the sample is completely encapsulated within a microfluidic device, to allow for full surface access.

The immediate applications of these approaches lie with diagnostics of tissue slices and biopsy samples — e.g. for cancer — but the approaches would also be very useful in comparative genomics and other areas of fundamental research involving heterogeneous tissue samples.

The approach advocates and utilizes the bottom-up customization of microfluidic architectures to biosamples, in contrast to the traditional top-down approach of building the architectures first and then putting the biosamples inside. Further, as particular embodiments of the above principle, novel techniques of sub-sample selection and isolation are presented. These techniques would have wide applicability in fundamental research and biomedical diagnostics.

In particular, an *in situ* microfluidic technique of single-cell isolation, or multiple single-cell isolations, is described, and is performed upon tissue sections attached to pathology glass slides. The technique combines the advantages of preserving the architectural integrity of the tissue section while allowing flexibility and precision of cell selection, rapid prototyping, and enhanced sample purity, while benefiting from the experience of the pathologist in the selection process. The result is a system that would allow the rapid and reliable biochemical analysis and diagnosis of

pathologic processes with sensitivity extended down to the level of even a single cell, with high levels of confidence in the diagnostic determination.

These techniques would allow the extraction of cells and cell nuclei chosen for their potentially pathologic origin, e.g. cancer. The chief advantages of the proposed methods are their speed, ease, reliability, and capacity to select individual cells from a tissue slice population with a higher degree of purity and specificity. The result is the extracted DNA can be biochemically analyzed with a higher degree of diagnostic accuracy, as the isolated sub-sample would not be diluted by unwanted material from the ambient tissue. In comparison to other techniques, the proposed methods would combine high specificity with high speed.

This work was done by Lawrence A. Wade of Caltech and Emil P. Kartalov, Darryl Shibata, and Clive Taylor of the University of Southern California for NASA's Jet Propulsion Laboratory. Further information is contained in a TSP (see page 1).

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*Innovative Technology Assets Management
JPL*

Mail Stop 202-233

4800 Oak Grove Drive

Pasadena, CA 91109-8099

E-mail: iaoffice@jpl.nasa.gov

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