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OFFICE OF TECHNOLOGY TRANSFER AND ENTERPRISE DEVELOPMENT

Micro-Mirrored Pyramidal Wells

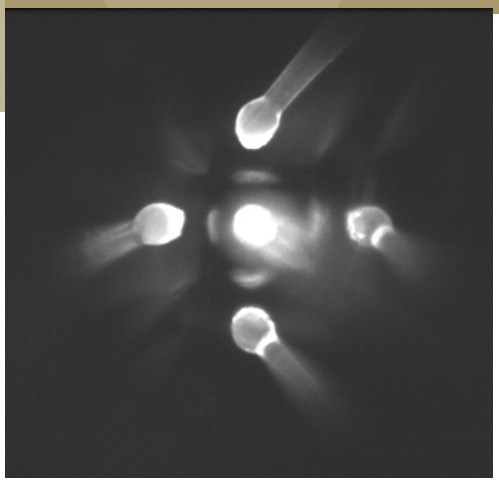

Figure 1.

Summary

Micromachined mirrored pyramidal wells provide simultaneous high-resolution images of cells and groups of cells from multiple vantage points without the need for confocal microscopy. Two classes of wells, introverted and extroverted, are distinguishable by the mirror angle relative to the optical axis of the imaging system and the optimal placement of the sample either physically inside or outside of the well, respectively. The envisioned product is a coverslip or slide that is used in place of the ordinary coverslip or slide and contains an array of mirrored pyramidal wells (either introverted or extroverted) that may be used on an inverted or conventional wide-field microscope with little or no additional effort on the part of the microscopist. In vivo applications are also envisioned for the introverted wells.

Combined with an appropriate microfluidic superstructure and produced in very high dimensional arrays, the micro-mirror device will be useful for cellular studies.

Description

Mirrored Pyramidal Wells (MPWs) are commonly fabricated in silicon and used to obtain simultaneous views of microscopy specimens from multiple, nearly-othogonal vantage points. Figure 1 illustrates a five-perspective simultaneous fluorescent image of a pulled glass pipette tip, coated with rhodamine. Two classes of MPWs exist: introverted and extroverted. Introverted MPWs are those for which the single point being equidistant from all mirrored surfaces (four sides and/or the bottom of the well) is within the well, i.e. beneath the original top surface plane of the silicon wafer. Four sets of introverted MPWs have been made and used to study dictyostelium and other organisms under live-cell conditions and fixed and stained with bright field and fluorescence microscopy. Introverted wells are useful for obtaining and studying three-dimensional images of single cells, cell-cell pairs (in immunology studies) or cell clusters (in a developing embryo). Extroverted wells are not typically fabricated in silicon, but have been constructed in our group by simple embossing. They have the advantage of being used with samples that exist outside of the pyramidal well, as in a cellular monolayer.

In vivo imaging (wide-field and confocal, bright-field and fluorescent) is possible by means of introverted wells being polished until the bottom of the well no longer exists or etched through from the silicon wafer backside. This technique provides a declinated perspective of in vivo tissue when placed (without compression) directly on the tissue in question. In addition to the ordinary (XY) microscope or confo-

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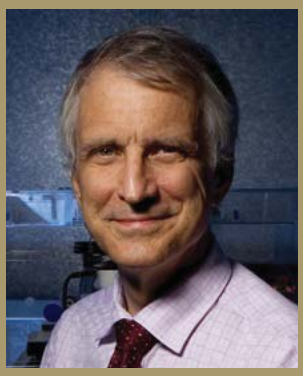

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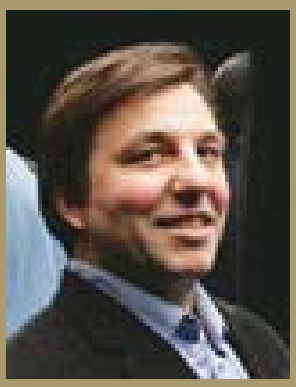

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cal plane ordinarily available, these bottomless MPWs will provide four planes that are nearly orthogonal to the XY plane, giving access to planes that may contain the entire junction between adjacent and connected cells, such as the junction between epithelial cells.

Single molecule imaging, as demonstrated by Eric Betzig's photo-activated light microscopy, may be conducted in MPWs on fixed cells containing photo-activatable moieties, with the result being absolute three-dimensional localization of individual molecules within a cell. Betzig's recent publication is limited to a two-dimensional region accessible by TIRF microscopy.

Combined with an appropriate micro-fluidic superstructure and produced in very high dimensional arrays, the micro-mirror device will be useful for cellular studies.

Advantages

A scanning probe microscope forms images of surfaces using a physical probe that scans the specimen. The resulting three-dimensional topographic image can be analyzed to resolve surface roughness on the nanometer scale. The resolution of the microscope is not limited by diffraction, but by the probe-sample interaction volume. Moreover, the interaction can be used to further modify the sample to create small structures.

Competitive Analysis

The most comparable industry for a sound competitive analysis is the scanning probe microscopes market, which consists of a few major manufacturers in Asia and Europe. Tier 1 includes large multinational organizations, catering to the varied needs of widespread end users, delivering a range of scanning probe microscopes, imaging software and other accessories. The key vendors in this tier include Veeco Instruments and JEOL Ltd. Tier 2 companies are those with significantly lesser revenues when compared with tier one companies. Most of these companies have their manufacturing, sales and services focused on specific geographies. Companies in this tier include SII NanoTechnology Inc., Agilent Technologies, Park Systems, Inc., Nanonics Imaging Ltd. and Nanograph Systems Limited, among others. Key competitive factors in the market include value-based pricing, technology innovation, product features and performance and strong customer relationship management and support.

Adoption

The scanning probe microscope industry has tremendous opportunities for growth in the biosciences industry. With the development of sharper probe tips, there has been a huge improvement in resolution and different imaging methods, and because of this the technique has become highly suitable for the study and analysis of DNA structures. Further development is expected as a result of the increased computing power and the development of systems with high intelligence, which are expected to increase the automation ability of microscopes.

Strategic Plan

Vanderbilt seeks a commercialization partner for the development packaging, manufacture and distribution of the product.

Intellectual Property Status

U.S. patent protection is being pursued.