

Label-free Analysis of Tissue Sections and Cells using Laser Scanning Reflectance Imaging

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Abstract

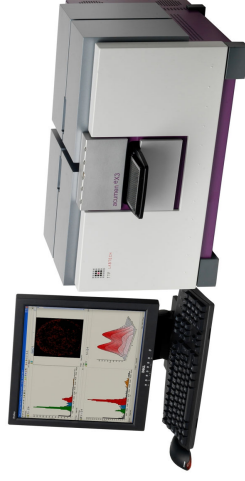
Brightfield transmission microscopy is the most common method for viewing tissue sections and cells in scientific and clinical diagnostic laboratories. Simple staining procedures and ease of use make it a readily accessible technology, however, there are drawbacks for some applications. For example, when examining large tissue sections the limited field-of-view afforded by microscope objective lenses can make analysis of large areas a laborious and prolonged procedure. In addition, when automated microscopes are employed individual images need to be stitched together. The small field-of-view also limits the number of cells that can be examined when they are cultured in microplates for cell-based screening assays.

Laser-scanning microplate cytometers, such as TTP LabTech's Acumen® eX3, represent an alternative platform for certain applications. Such technology is routinely used for the high content screening of fluorescent cells since it offers rapid analysis over a large field-of-view (20 mm x 20 mm – approximately 400 times greater than a 10X microscope objective). The Acumen eX3 is capable of generating TIFF images (8 or 16-bit) of the raw data received from the sample which can be subjected to the full range of algorithms contained in commercially available image analysis software.

In this study, modifications were made to the collection optics of an Acumen eX3 so that light reflected by the sample could be detected as the laser spot rastered across the sample. This technique called Laser Scanning Reflectance Imaging (LSRI) was used successfully to scan a range of unstained tissue samples mounted on glass microscope slides using a 488nm laser. The resultant images correlated well with observations made using traditional phase contrast brightfield microscopy. In microplates, LSRI required the removal of culture medium to increase the contrast of cells. In all experiments, there was little evidence of background laser reflections from labware, routinely observed with laser scatter detection.

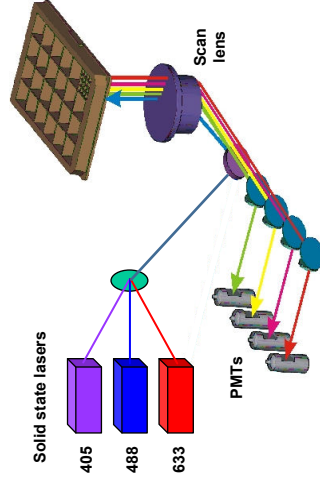
In summary, LSRI using microplate cytometers offers rapid label-free imaging of tissue sections and cells in an automated workflow for more efficient analysis. In addition, LSRI has the potential for multiplexing with other measurements such as fluorescence and fluorescence anisotropy.

1 Acumen eX3 Microplate Cytometer



The Acumen eX3 laser scanning fluorescence cytometer (TTP LabTech) offers triple laser excitation (405, 488 & 633nm) in a compact bench top unit. This design enables a wide range of high content assays to be performed at high throughput, especially when the instrument is fully integrated. Patented signal thresholding methods enable 'on-the-fly' cytometric analysis and dramatically reduces file sizes to around 50Kb in HTS screening mode.

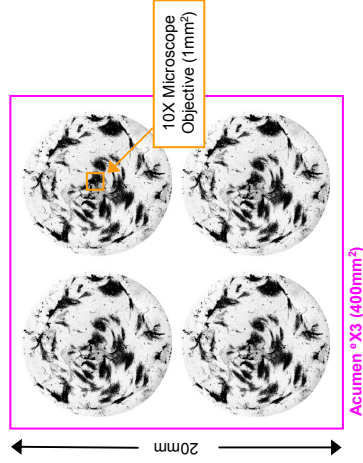
2 Laser Scanning Reflectance Imaging (LSRI)



Microplate cytometers, such as the Acumen eX3, utilise a specialised F-theta scan lens to focus scanning laser onto the bottom of a microplate. For LSRI the same lens is used to focus light reflected by the sample back down the excitation optical path is detected by PMT detectors. Reflected light is thus directly correlated with the object being scanned and not subject to the inherent randomness associated with the detection of scattered light. The latter can result in scanning artifacts in microplates from reflective surfaces such as plate mouldings, lid and the media meniscus. LSRI on an Acumen eX3 is thus from a confined detection region providing high resolution images for cytometric analysis using Acumen eX3's patented thresholding procedures or exported as a TIFF image file for processing by third party image processing software.

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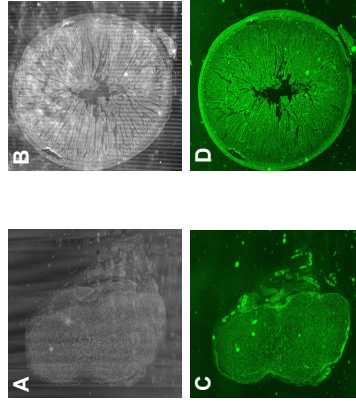
Field-of-View Comparison for High Content Instrumentation



Due to its large field-of-view of 400mm², Acumen eX3 enables scanning of whole wells rather than imaging small well areas like the microscope based systems. In fact an Acumen eX3 scans an area the size of four 96 wells per field-of-view. Whereas, a typical image from a 10X microscope objective only report data from around 2% of a single well containing around 100 cells.

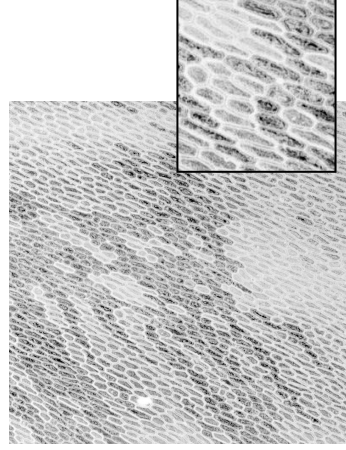
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Simultaneous LSRI and Fluorescence Imaging of Tissue Sections



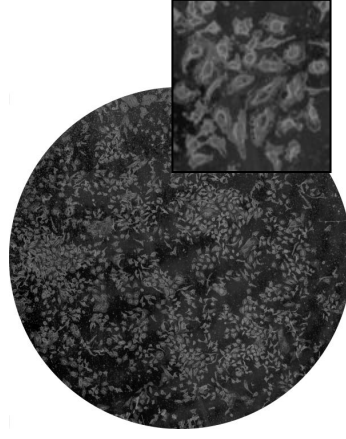
Tissue sections were fixed, dehydrated and mounted onto microscope slides. Images of reflected laser light (A & B) and tissue autofluorescence (C & D) were obtained simultaneously by scanning with a 488nm laser and detection with two PMTs. A high degree of correlation was observed between LSRI and autofluorescence images in both solid tumours (A & C) and intestine (B & D). In tumours, LSRI appeared to resolve nuclei that were not evident with autofluorescence.

4 LSRI of Unstained Onion Skin



A thin layer of onion skin has been routinely used for basic histological practicals due to its simple preparation and characteristic cellular structure. A 20 x 20 mm section of skin was mounted on a phase microscope slide, covered with a coverslip, subjected to LSRI using a 488nm solid state laser on an Acumen eX3. Raw TIFF images of the reflected laser light exhibits good resolution of the cellular arrangement within the onion skin. It was possible to differentiate groups of cells with the sample due to variations in the reflective properties of the cell contents. No background reflections were observed.

6 LSRI of HeLa Cells Cultured in Clear Plastic Microplates



HeLa cells were cultured in standard clear-bottom microplates and allowed to adhere overnight. The medium was removed and plates subjected to LSRI using a 488nm laser. Clear images of the adhered cells were produced that could be used to estimate cell confluency. Closer examination revealed fine cell processes and annular reflectance within the cell that may represent the nucleus or a vertical contour of the cell body. No background reflections were observed suggesting a confined region of detection from the patented Acumen eX3 optics.



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