High Content Image Analysis using a Laser Scanning Microplate Cytometer

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Abstract
Microscope-based high-content instruments offer high optical resolution, however, the limited field of view afforded by their objective lenses can mean lengthy read times for some assays, especially where multiple image capture per well is required. Wherever possible, only a small percentage of the total number of cells present in a test well are analysed to keep plate read times at a minimum, which may not always be ideal. Microplate laser-scanning cytometers, such as TTP LabTech's Acumen® eX3, are an alternative platform. This technology offers higher throughput than imaging based systems processing 300,000 wells of data in 24 hours in 1536 plates. The Acumen eX3 is capable of generating cytometric data and TIFF images (8 or 16-bit) simultaneously. The images represent the whole well and correlate with those captured using a 20x microscope objective.

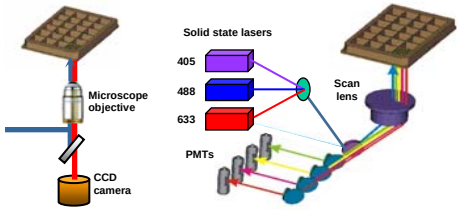
The images produced can be subjected to the full range of algorithms contained in commercially available image analysis software. In most cases where image analysis and cytometric analysis have been validated, both methods were comparable where single cells were represent. For a limited number of applications, however, secondary information provided by image analysis may increase understanding. Such assays include angiogenesis, cell colony formation and tissue scanning.

Microplate cytometry offers whole well, high throughput capability with the increased flexibility of simultaneous cytometric and TIFF image outputs and presents a comprehensive solution for high content analysis studies.

Conclusion

- TIFF image export feature extends the application of the Acumen eX3 into more complex high content assays requiring image processing
- Images generated are equivalent to those from a 20X microscope objective
- Whole well imaging is ideal for rare event detection assays
- No requirement for image stitching steps prior to image analysis on large objects such as cell colonies, tubules, *C.elegans* and tissue sections.
- The speed to capture whole well images is not reduced with higher density plates.
- Exported TIFF image are compatible with many commercial imaging packages.

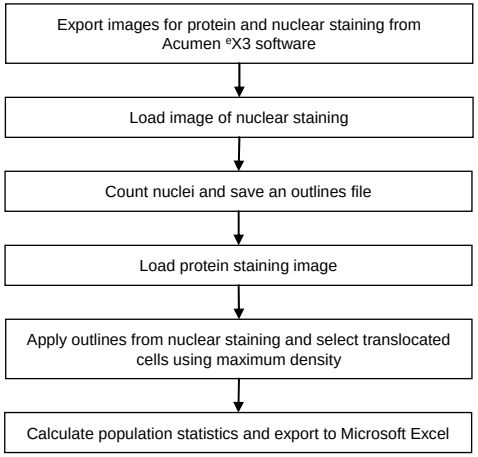
1 Comparison of the Optics of High Content Instrumentation



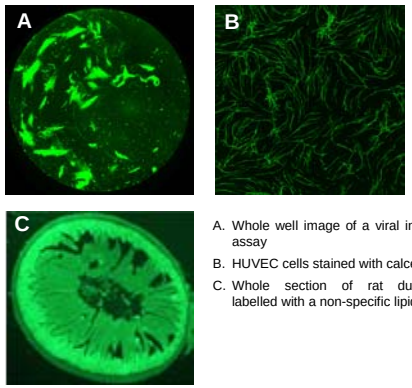
1 mm² (x10)	Field of View	400 mm²
1	Colours / Scan	4

The large field of view (400 mm² (20 x 20 mm)) is far greater than that offered by microscope-based CCD imagers (~1 mm² for a x10 objective). The small field of view on microscope-based CCD imagers is only sufficient to obtain resolved images of around 100 cells at once. The application of laser scanning over a large area means that analysis is performed on an area, not a well basis. This equates to the simultaneous scanning of 4, 16 and 64 wells in 96, 384 and 1536 well format, respectively. Reconfiguration of assays into higher density plate formats results in a concomitant increase in throughput up to 300,000 samples per day in 1536 well microplates.

4 Flow Chart for Image Analysis of Protein Translocation Assay

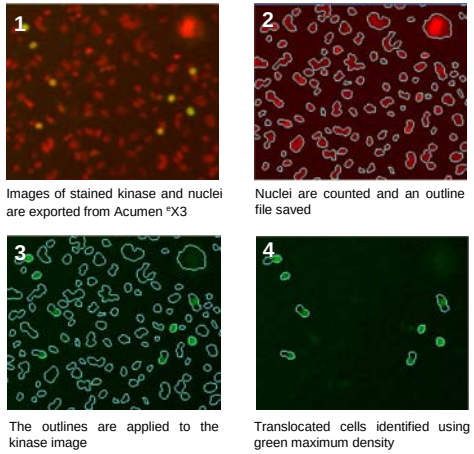


2 Large Field of View Images from an Acumen eX3



A. Whole well image of a viral infectivity assay
B. HUVEC cells stained with calcein-AM
C. Whole section of rat duodenum labelled with a non-specific lipid stain

5 Image Analysis of a Kinase Nuclear Translocation Assay



Images of stained kinase and nuclei are exported from Acumen eX3

Nuclei are counted and an outline file saved

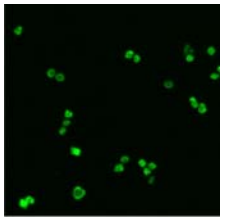
The outlines are applied to the kinase image

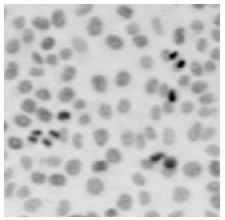
Translocated cells identified using green maximum density

The assay was run in a 96 well plate and an image of the whole well area was obtained. The scan time was 25 minutes per plate. The results showed that 25% of cells were found to have translocated kinase. This compared with the data obtained using standard Acumen eX3 software with a scan time of 10 minutes per plate. (Data was analysed using Image-Pro Plus version 6.1)

3 Demonstration of the Resolution of Images from an Acumen eX3

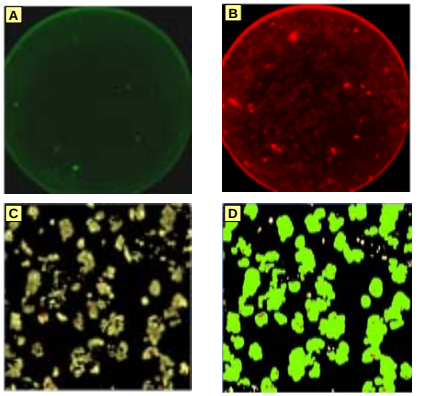
Mitotic Index: cells were stained with anti-phosho-histone H3 (FITC) and the plate scanned on an Acumen eX3 to generate 8-bit images. The cells were pseudo coloured green. The circular arrangement of DNA within the nucleus is clearly visible upon compound treatment demonstrating that the images generated are approximately equivalent to a 20X microscope objective.





HeLa cells were stained with Hoechst and the plate scanned on an Acumen eX3 microplate cytometer. The image shown is an 8-bit image of PMT measurements. The resolution is sufficient to distinguish daughter cells and dividing cells within the cell population.

6 Application for Rare Event Detection: Stem Cell Research



Stem cells were scanned using an Acumen eX3 microplate cytometer. **A & B**, cell proliferation using anti-BrdU antibody (Alexa 488 secondary) and PI nuclear counterstain. **C & D**, stem cell differentiation using calcein-AM (green) and a selective reporter gene (red). Cytometric data were exported as 8-bit images and image-processed to identify and count differentiated cells within each cluster. *(Media Cybernetics, Berkshire, UK). Data were supplied by Epistem Ltd, Manchester, UK.*