High content screening applications

In a multi-user, multi-disciplinary biomedical imaging research unit

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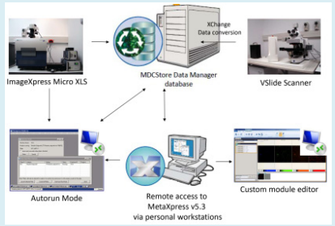
Introduction

High content screening and image analysis is an essential tool in hypothesis-driven research today. Advanced imaging systems such as the ImageXpress Micro XLS (Molecular Devices) and VSlide scanner (MetaSystems) in conjunction with image analysis software MetaXpress and data management solution MDCStore (Molecular Devices), provide a powerful analysis package for users. Analysis can be tailored to unique biogical experiments with speed and accuracy.

Virtual environment overview



▲ Images acquired with ImageXpress are saved directly to the MDCStore database. Users sitting at their personal work stations can remote in to MetaXpress software (from anywhere in the world!) and send analysis jobs to an Autorun queue. The Autorun instance accesses images directly from the database, completing jobs one-by-one in the queue and saves analysis automatically to the database also. Images from other imaging modalities can be imported into the database upon conversion using XChange.

Conclusion

Here we demonstrate the utility of high content screening technologies to a diverse range of biological experiments. ImageXpress high content screening platform was used for fluorescence image capture of primary cells differentiated in glass chamber slides and for transmitted light imaging of spheroids cultured in plastic micro-well plates. VSlide scanner was used for imaging of immuno-labelled tissue micro-array slides. Images acquired with these modalities were subsequently analysed in an automated fashion using either built-in custom modules or customised journals, tailored to more complex data sets using MetaMorph or MetaXpress image analysis software. The marriage of these technologies offers users fast, standardised, reproducible and accurate data generation potential which is free of human bias and error.

Methods and Results

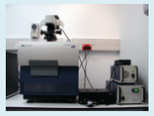
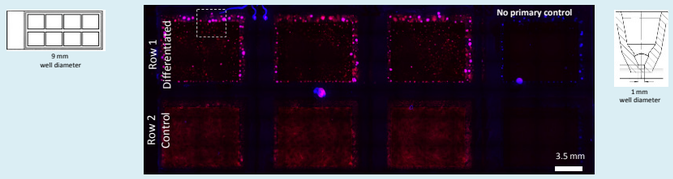


Image acquisition of chamber glass slides or plastic micro-well plates is performed using high content screening modality: ImageXpress Micro XLS (Molecular Devices)

Chamber Slides

▼ Example 1: Differentiation of primary human adipocyte precursor cells into chondrocyte nodules in 8-well chamber slides (immuno-labelled for Hoechst and a chondrocyte marker, 12 sites/well acquired using 10x objective and Dapi and TritC wavelengths)



Micro-well plates

▼ Example 2: Spheroids grown in GravityTRAP™ (inSphero) 96 micro-well plates, used for cancer drug screening (unlabelled, 1 site/well acquired using 4x objective and Transmitted light imaging)

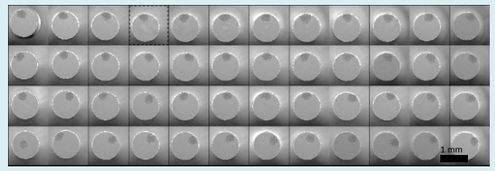
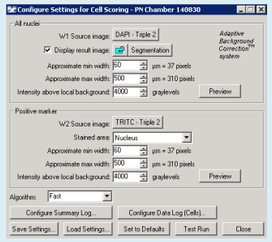
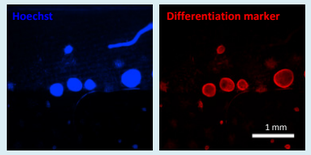


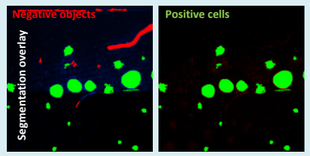
Image analysis is performed using: MetaXpress software (Molecular Devices)



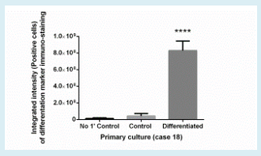
▲ Custom modules are user friendly methods for analysis of a range of different biological samples. Users can specify parameters such as minimum and maximum width of an object and staining intensity of label, for up to 7 wavelengths simultaneously. A range of different semi-quantitative measurements can be logged directly to excel spreadsheets for further statistical analysis in GraphPad Prism



▲ Debris in original images could be a source of false positives, use segmentation to identify positive nodules

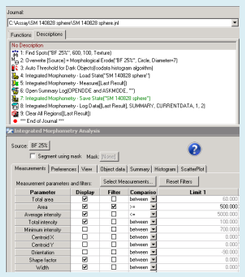


▲ Custom module 'Cell Scoring' can identify positive nodules accurately. Segmentation masks are overlaid on original images, green marks positive objects and red marks negative (excluded) objects

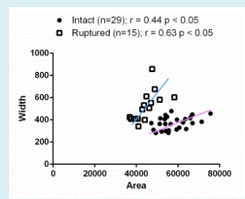


▲ Figure 1. Expression level of chondrocyte marker in differentiated cells

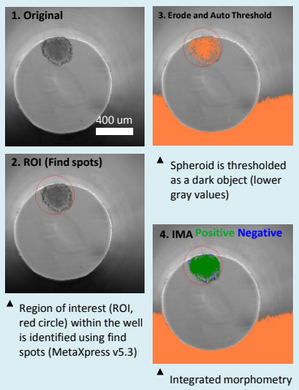
◄ Figure 1 demonstrates the measurement of integrated intensity which is significantly increased in differentiated cells immuno-labelled for a chondrocyte marker compared to controls. Negligible intensity is measured in no primary (1') controls. Data shown is mean +/- SEM. One-way ANOVA with Dunns post hoc test **** indicates P < 0.0001



▲ Customised journals can be written for more complex data sets, using specific commands within 'Journal Editor'. The steps involved in the analysis are shown adjacent. 'Integrated morphometry analysis ' is used to measure objects and define filter criteria. For example in this example any objects less 500um^2 are excluded



▲ Figure 2. Spheroid size evaluation

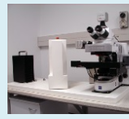


▲ Region of interest (ROI, red circle) within the well is identified using find spots (MetaXpress v5.3)

▲ Spheroid is thresholded as a dark object (lower gray values)

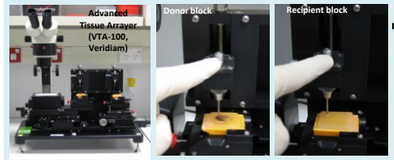
▲ Integrated morphometry analysis is used to measure spheroid size within thresholded ROI. Objects that do not meet filter criteria are excluded (shown in blue). Objects that are counted are shown in green

◄ Figure 2 demonstrates the identification of intact and ruptured spheroid based on the correlation between total area and width of spheroids. Each raw data point is shown, Pearsons correlation was used to find correlation coefficient

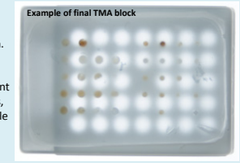


Microscope slides are acquired using the: VSlide Scanner (MetaSystems)

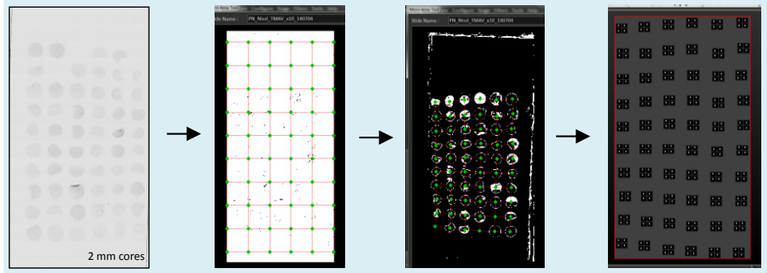
Tissue micro-arrays



▲ Tissue micro-arrays are prepared in-house at the Centre for Brain Research. This enables cores taken from paraffin embedded tissue blocks from different regions, cases or diseases, to be compared on a single slide



▼ Example 3: Work flow for acquisition of Tissue micro-arrays

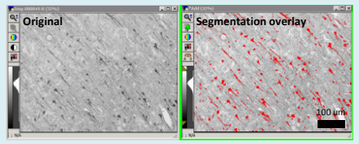


x2.5 objective pre-scan of entire tissue micro-array

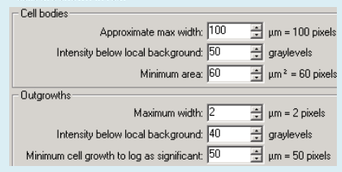
Use pre-scan montage to threshold spots (to identify immuno-labelled cores) and align green dots at the centre of each core

Re-image 4 sites per core at higher power (x10 objective)

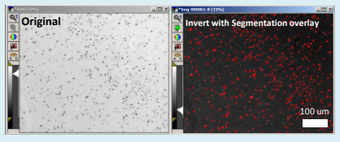
Image analysis is performed using: MetaMorph (Molecular Devices)



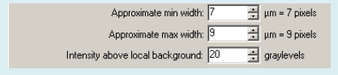
▲ Representative site (x10) of tissue micro-array immuno-labelled for MAP2 and visualised with DAB-nickel is shown; before and after segmentation with custom module 'Neurite outgrowth'. Positive cells are shown in red.



▲ In addition to cell marker width, area and staining intensity, users can also specify parameters for quantifying processes branching from cells of interest.



▲ Representative site (x10) of tissue micro-array immuno-labelled for total histone H3 and visualised with DAB-nickel is shown. The original image is first processed with morphology filter 'invert' to reverse the scale of pixel gray-values within the image. Positive nuclei are then quantified using the custom module 'Count nuclei'. Positive cells are shown in red.



▲ Representative site (x10) of tissue micro-array immuno-labelled for total histone levels and visualised with DAB-nickel is shown; before and after segmentation with custom module 'Count nuclei'.

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