

The Application of High Content Analysis to Modern Cell Biology Challenges

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Introduction

In any biological experiment there are two classes of variables. Those controlled by the experimenter (*Independent variables*) and those that are measured (*dependent variables*). Most assay techniques in cell biology either allow the exploration of a large independent variable space, but return only one measurement, or return many dependent variables but require lots of sample and so are limited to few independent variable manipulations (*Table I*).

Table I: Comparison of information content and throughput of common cell biology assays

Assay	Throughput	Information Content
Westerns	Low	Low
ELISA	Medium	Low
Fluorescence Microscopy	Low	Medium
Flow Cytometry	Low - Medium	Medium
Binding Assays	High	Low
High Content Analysis	Medium-High	High

The beauty of automated fluorescence microscopy (*High Content Analysis: HCA*) is the workflow is simple enough to allow medium to high throughput while the computer-based analysis of images preserves the rich information content of fluorescence microscopy. This means that an HCA assay allows both the exploration of a large independent variable space while also returning many dependent variable measurements, making the data return from these assays arguably the largest in the cell-based assay class.

Too much information running through my brain!

There are, however, several challenges to face when dealing with large multidimensional data sets. Firstly, one has to reliably map all the dependent variable measurements to the independent variable values that were set up within the experiment. Secondly, this information then needs to be processed into data visualisations that allow the researcher to understand the overall experiment.

Our solution to these challenges was to create an automated program (*The PlateMaker Wizard*) that builds a single table where any number of independent variable dimensions are captured in columns while the dependent variables are in rows. This format allows the rapid visualisation of the data using Pivot tables and is highly compatible with most statistical packages and Spotfire.

Table II: General table solution to multiparametric independent and dependent variable association data.

Idep 1	Idep 2	Idep 3	Idep 4	Dep Name	Dep Values
Value 7	Value X	Value L	Value 11	Cell Count	1000
Value 7	Value X	Value L	Value 12	Cell Count	1200
Value 7	Value X	Value L	Value 13	Cell Count	1500
Value 1	Value A	Value B	Value 1	pERK	20000
Value 1	Value A	Value B	Value 2	pERK	15000

Uses of HCA

HCA is ideally suited to any research that requires a thorough investigation of cell biology through numerous treatment conditions and/or cell lines. Areas of maximum impact include:

- System Biology
- siRNA
- Stem Cell Characterisation
- Therapeutic Antibodies
- Signal transduction pathways (especially translocation data)

Measurement of Protein Translocation

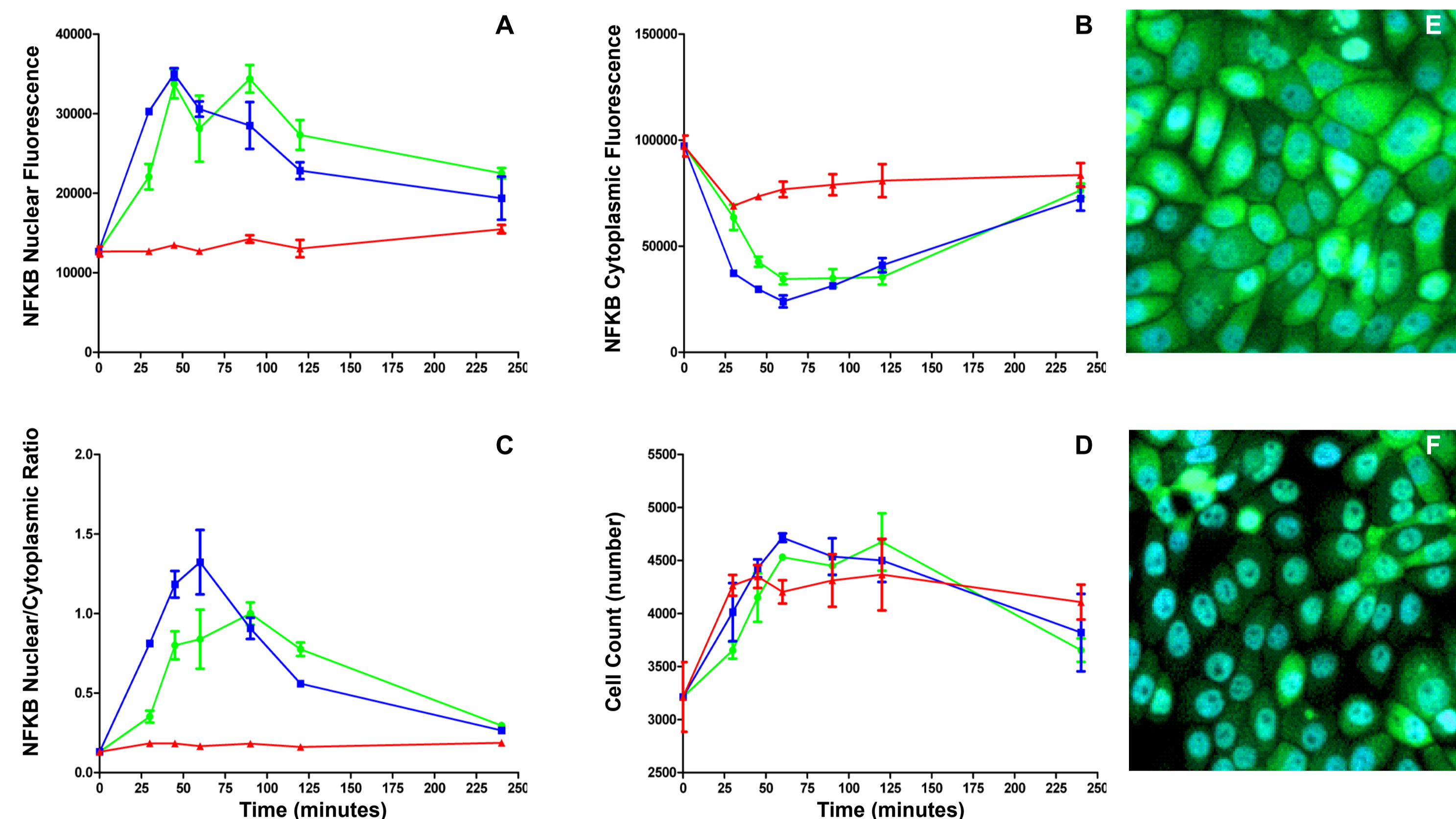


Figure 1: Translocation of NFKB in oral epithelial cells treated with either agent A (green circles), agent B (blue squares) or untreated (red triangles) for up to 250 minutes. Cells were dual stained with Hoechst 33342 and an NFKB antibody and scanned on a Cellomics Arrayscan. The green fluorescence in the nuclear (panel A) and cytoplasm (panel B) was measured using nuclear and cytoplasmic gates. The ratio of these values was also calculated (panel C). Cell counts were determined from the nuclear channel (panel D). An image of control (panel E) and cells treated with agent B for 100 minutes (panel F) gives visual conformation of the translocation event. Data is Mean \pm SEM of 2 experiments.

Measurement of Protein co-localisation

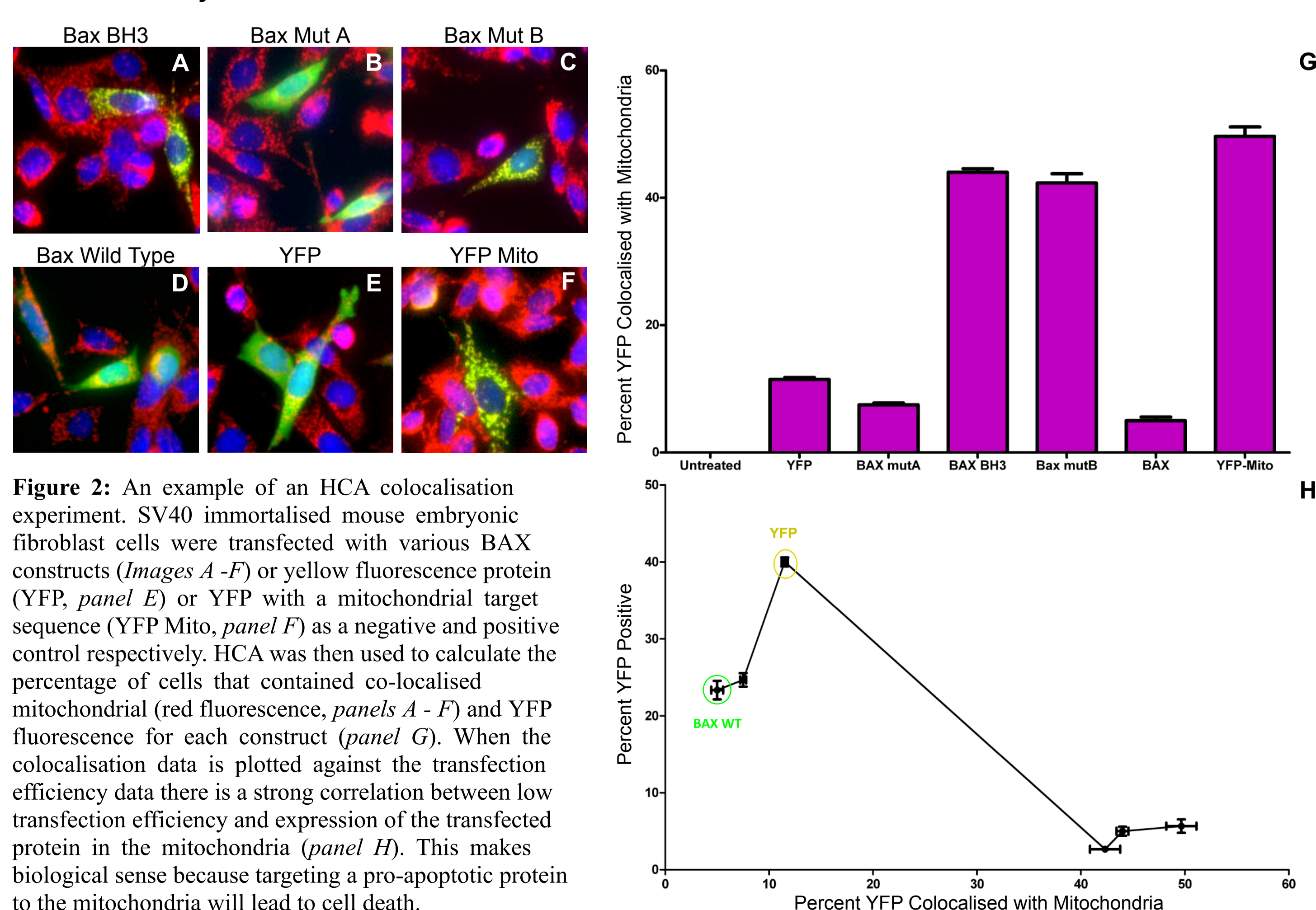


Figure 2: An example of an HCA colocalisation experiment. SV40 immortalised mouse embryonic fibroblast cells were transfected with various BAX constructs (Images A-F) or yellow fluorescence protein (YFP, panel E) or YFP with a mitochondrial target sequence (YFP Mito, panel F) as a negative and positive control respectively. HCA was then used to calculate the percentage of cells that contained co-localised mitochondrial (red fluorescence, panels A-F) and YFP fluorescence for each construct (panel G). When the colocalisation data is plotted against the transfection efficiency data there is a strong correlation between low transfection efficiency and expression of the transfected protein in the mitochondria (panel H). This makes biological sense because targeting a pro-apoptotic protein to the mitochondria will lead to cell death.

Characterisation of anticancer compounds measuring two independent markers of apoptosis and proliferation

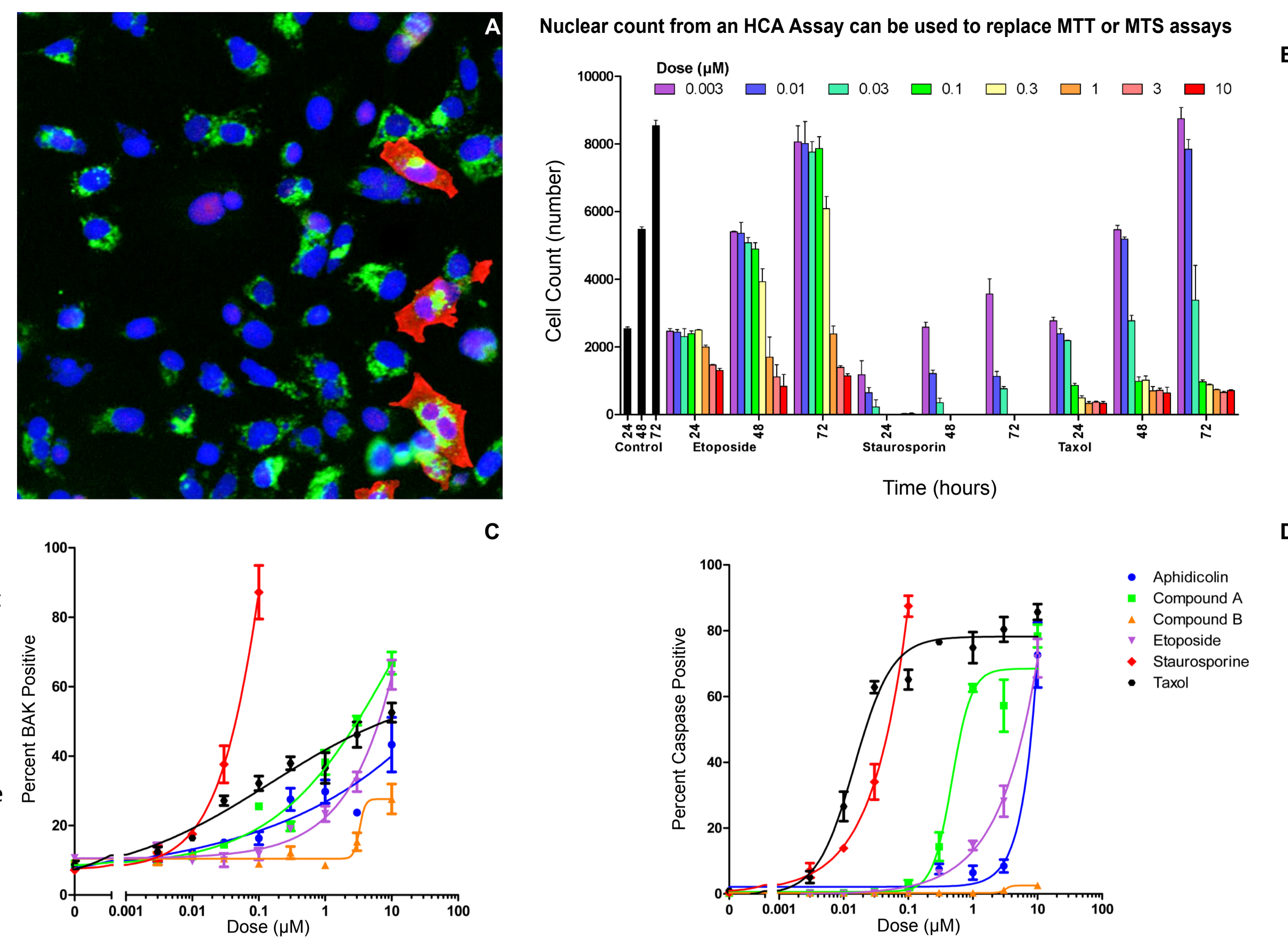


Figure 3: An example of detailed cytotoxic compound fingerprinting using HCA. HCT116 cells were cultured in 96 well plates before treating with several compounds (see figure legend) for 24, 48 and 72 hours. The cells were fixed in 1% formaldehyde for 30 minutes in preparation for immunofluorescence staining. The cells were then dual antibody stained for the N-terminus of the pro-apoptotic protein BAK and the active conformation of caspase 3. The nuclei of the cells were also stained with Hoechst 33342 and cell images captured on a Cellomics Arrayscan. An image from HCT116 cells after 48 hours treatment with 10 nM taxol for 48 hours showing nuclei (blue) active BAK (green) and active caspase (red) is shown in panel A. From the nuclear stain accurate cell counts can be obtained giving detailed information on how each test compound is affecting cell growth (panel B). From the same experiment it is also possible to determine the potency of each compound for its activation of the pro-apoptotic protein BAK (panel C) or caspase (panel D). The time sequence for proliferation, BAK and caspase activation means that each test compound receives its own signature which can be used further in cluster-type analysis. In general, BAK activation proceeds caspase activation so that BAK and caspase are early and late markers of apoptosis respectively. Data point represent the Mean \pm SEM of triplicate readings.

Measurement of neurite outgrowth in Stem Cell Research

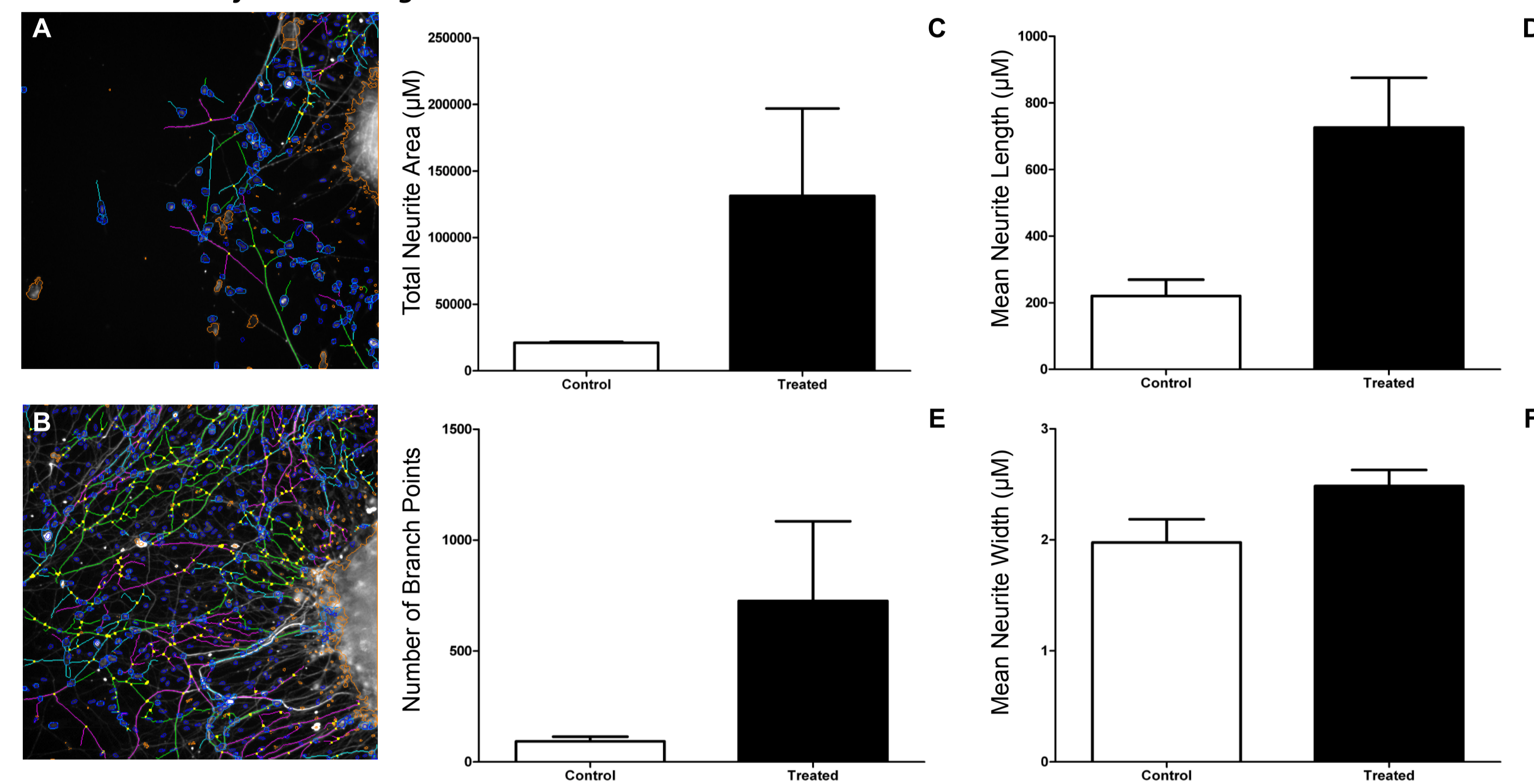


Figure 4: The effect of human mesenchymal stem cell-conditioned medium (MSC-CM) on neurite outgrowth from explants of chick dorsal root ganglia (DRG) was measured using a neuronal analysis algorithm. Images of neurite outgrowth along with computer analysis overlays are shown for untreated (panel A) and MSC-CM (panel B) respectively. The algorithm was optimised to measure a number of parameters including total neurite area (panel C), mean neurite length (panel D), number of branch points (panel E) and mean neurite width (panel F). Data is the mean \pm SEM of 4 donor experiments.

Zebrafish Angiogenesis Assay

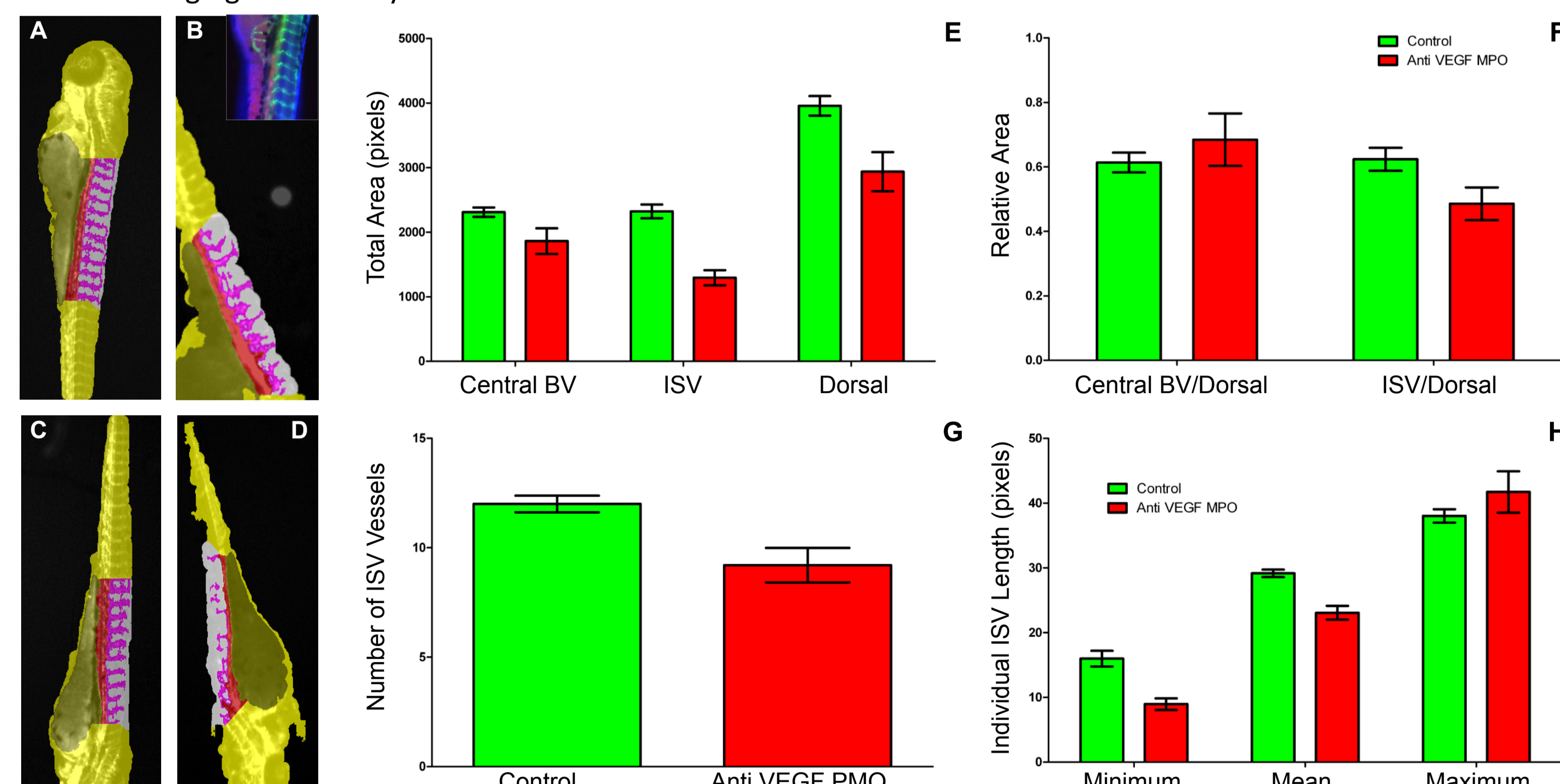


Figure 5: Automated analysis of zebrafish transgenic, with GFP-labelled vasculature, using Definiens recognition image analysis software. **Panels A-D:** Cygel (Biostatus) was placed in 96 well microtitre plates to help position fish and stop yolk sack autofluorescence. Images were then acquired on an Arrayscan but transferred into Definiens recognition development suite. The fish was identified in yellow and then the yolk sack was delineated (brown region). The program creates a dorsal region of interest immediately adjacent to the yolk sack (white area) and the central blood vessel inside this region was then defined (red region). Finally the individual intersegmental vessels (ISV) were delineated in such a way that each individual ISV is treated as a separate object allowing detailed statistical analysis on each individual vessel (panel H). Control fish images analysed by Definiens are shown in Panels A & C with a typical fluorescence image obtained from the arrayscan shown in the inset of panel B. Abrogation of angiogenesis was achieved by blocking the expression of the VEGF receptor using a morpholino oligonucleotide targeting mRNA translation. The morpholino was injected directly into the oocyte before it reached the 8 cell stage and typical morpholino fish images (with Definiens analysis) are shown in panels B & D. The effect of the morpholino treatment on the blood vessels and dorsal area is shown in panel E. It is clear that there is a concomitant decrease in both the ISV and dorsal areas. When the blood vessel measurements are normalised against the dorsal area, the changes in blood vessel area do not reach significance (panel F). However, the number of ISV blood vessels are significantly decreased in the morpholino treated animals (panel G). It is also possible to get the values for the minimum, maximum and mean length of ISVs in an individual fish. These data are plotted in panel H. It is interesting to note that in morpholino treated fish the maximum length vessels are equivalent to untreated fish (panel G, maximum) indicating that the treatment does not completely block all blood vessels from developing normally. Each data point is the mean \pm SEM of 41 control and 15 morpholino treated fish respectively.

Conclusion

The above data demonstrate just a few of uses of HCA in modern biological research. Given the simple workflow and amount of data that can be returned from this type of experiment, HCA offers a very powerful research tool for modern drug discovery and makes it ideal for investigating complex biological processes where many different treatment conditions are required to fully elucidate mechanisms of action.