

# Complex image-based cell biology endpoint analysis for drug discovery using Definiens software

Elena Di Daniel<sup>1</sup>, Kalpana Patel<sup>1</sup>, Barbara Zenger-Landolt<sup>2</sup>, Jan Schreiber<sup>2</sup>, Colin G. Blackmore<sup>2</sup>, Peter R. Maycox<sup>1</sup>

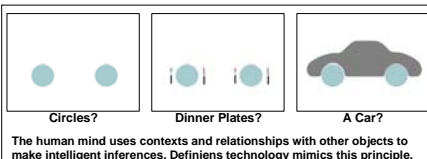
<sup>1</sup>Psychiatry Discovery Technology Group, GlaxoSmithKline, Third Ave., Harlow CM19 5AW, UK,

<sup>2</sup>Definiens AG, TrappentreustraÙe 1, 80339 M¼nchen, Germany



## Introduction

- Microscope-based image acquisition and analysis are used in all phases of the drug discovery process.
- Resource-intensive, subjective measurements, or semi-automated basic image analysis software are often used to address complex analysis problems.
- Definiens Enterprise Image Intelligence™ Suite performs image analysis by identifying “objects” within an image, rather than simply pixels, and then makes inferences about the objects, taking into account *contextual* information available in the whole image.
- Definiens software allows automated *understanding* of images.



- This approach permits quantification of complex cell biological processes.
- An example illustrated here is the neuronal growth cone (GC) assay.

## Growth cone assay

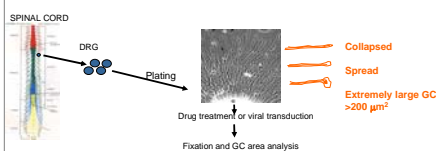
The GC is a specialised structure found at the tip of a neurite, which integrates information from the surrounding environment resulting in GC spreading or collapse.

The GC assay measures the GC area of sensory neurons cultured as explants. This assay seems to be predictive of mood-stabilizing activity, as each of the mood stabilizers, lithium chloride, valproic acid and carbamazepine, increases GC area and decreases the % of collapsed sensory neuron GCs (1, 2). This assay is, therefore, currently used to identify and validate potential novel targets for bipolar disorder.

## Methods

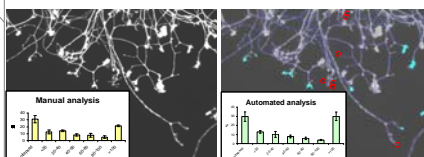
### DRG explants:

Rat or mouse P1-2 explants were plated on poly-D-lysine and laminin coated coverslips in 24-well plates. Compounds or adenoviruses expressing shRNA for targets of interest were added 1-day post-plating. DRGs were fixed 20h post-drug treatment or 2-4 days post-viral transduction with 4% PFA. Cultures were stained with a polyclonal anti-GAP43 antibody followed by anti-rabbit Alexa conjugated secondary antibody. Images were acquired manually using a fluorescence microscope.



## Results

Automation of growth cone area analysis:  
40% time saving

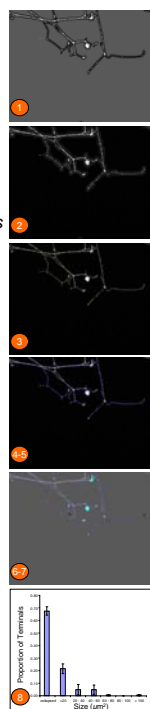


In blue: spread GC. In red circles: collapsed GC. Only well separated GC are identified. Filopodia and collaterals are not measured

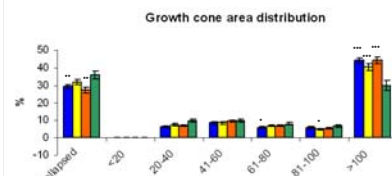
### Manual and automated analysis gave similar results

The rule set was constructed using the following steps:

1. Remove background (dark and homogeneous regions).
2. Grow background towards brighter structures, creating a skeleton where the background elements meet.
3. Identify line segments, nodes (where three or more line segments touch) and terminators (segment ends) within the skeleton.
4. Remove short terminating segments originating far from the neuronal terminal (collateral branches, not to be measured).
5. Grow skeleton in the vicinity of terminators into bright image regions to form candidate GC.
6. Remove or shrink candidates in the dense meshwork, then distinguish valid cones from artefacts (e.g. wide neurites, enlarged branching points) based on size, shape and distance to the terminator.
7. Identify collapsed terminals based on GC width.
8. Calculate the numbers of collapsed terminals, sizes of GC and report the sizes in pre-determined bins for display in histograms.



Example of application of GC rule set for TV of a bipolar disorder target



Three different shRNA were designed and tested for a new potential bipolar disorder target (blue, yellow and orange bars). n=3 independent experiments; ANOVA, followed by LSD test. \*\* comparison to scrambled sh (green bars).

## Conclusions

• Complex cell biology assays are no longer the preserve of low-throughput, qualitative measurements and, instead, are scalable into higher-throughput, information-rich resources for use throughout drug discovery.

• In addition to standard (intensity, shape) output, Definiens Enterprise Image Intelligence™ Suite allows extraction of detailed *relational* information as well as simultaneous extraction of fine detail and gross macrostructure. It can also account for noise and heterogeneity in automated analysis within and between images.

• Definiens allows faster data generation and automated routine image analysis, which lead to better precision and decisions.

• New biological questions, involving extraction of intricate and subtle information, can be addressed by these methods.

• In particular, the GC assay has further potential utility in the identification of novel drug targets for bipolar disorder using compound based as well as RNAi technology.

## References

1. Williams RS, Cheng L, Mudge AW, Harwood AJ, (2002); Nature 417: 292-5.
2. Di Daniel E, Cheng L, Maycox PR, Mudge AW, (2006); Mol. Cell. Neurosci. 32(1-2): 27-36.

## Acknowledgements

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