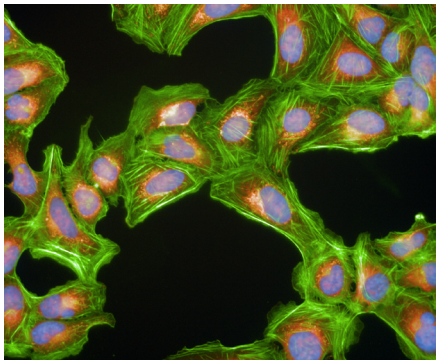
cyprotex
AN EVOTEC COMPANY

Cell Painting

Drug-induced toxicity is a major unwanted side effect of any newly developed drug. Although drug-induced toxicity is usually evaluated by looking at different cellular pathways, this toxicity also affects cellular and organellar structures.

Cell painting is a powerful and cost-effective method for screening multiple compounds for phenotypic effects on cells of interest. This method has become a useful tool for screening large libraries of compounds for use in the drug discovery process. At Cyprotex, we have optimized this method so it can be used to assess potential safety and toxicity risks.

- Simple, quick and automated workflow providing thousands of different features based on intensity, texture and granularity of each dye
- Comparison of phenotypic changes with a database of compounds with known toxic effects helping to accelerate safety in the drug discovery pipeline
- Flexible and capability to build a cell painting assay to meet your safety and toxicity needs.



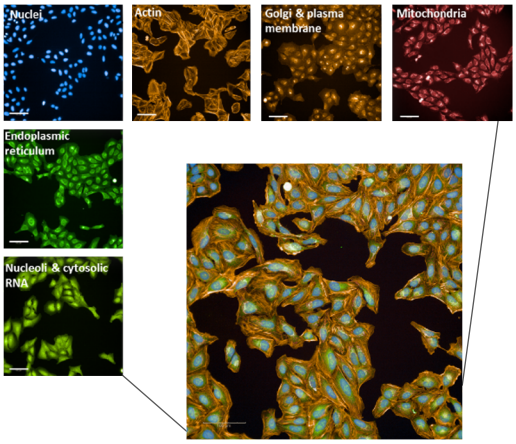
Workflow of a cell painting assay

1. **Seed:** Cells of interest are seeded into multi-well screening plates at desired density.
2. **Dose:** After 24 hours, cells are dosed with a set of control compounds and test compounds of interest.
3. **Label:** Following a 24-hour treatment, cells are labelled with fluorescent dyes specific to different cellular features.
4. **Image:** Cell are imaged using a high content screening platform.
5. **Analysis:** Fluorescently labelled cells are segmented using image analysis software to extract features from each of the labelled cellular components.
6. **Data processing:** Analysis of the extracted features using machine learning and high-performance computing to interrogate this multi-dimensional data set.

Cyprotex offer two different Cell Painting protocols

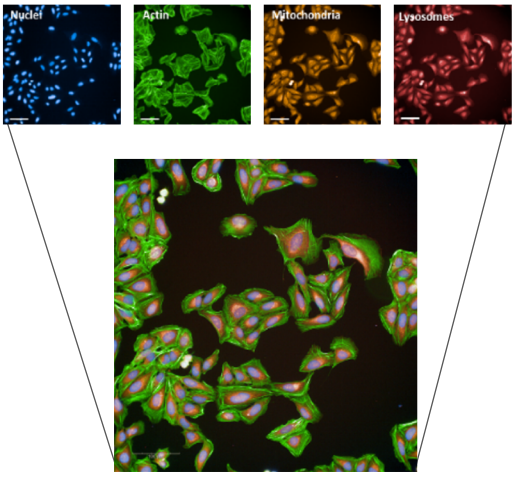
6 dye (following JUMP-CP protocol)

- **Nuclei:** Hoechst 33342 (nuclei)
- **Endoplasmic reticulum:** concanavalin A
- **Nucleoli and cytosolic RNA:** Syto-14
- **Cytoskeleton (F-actin):** phalloidin (F-actin)
- **Golgi and plasma membrane:** wheat germ agglutinin
- **Mitochondria:** MitoTracker deep red



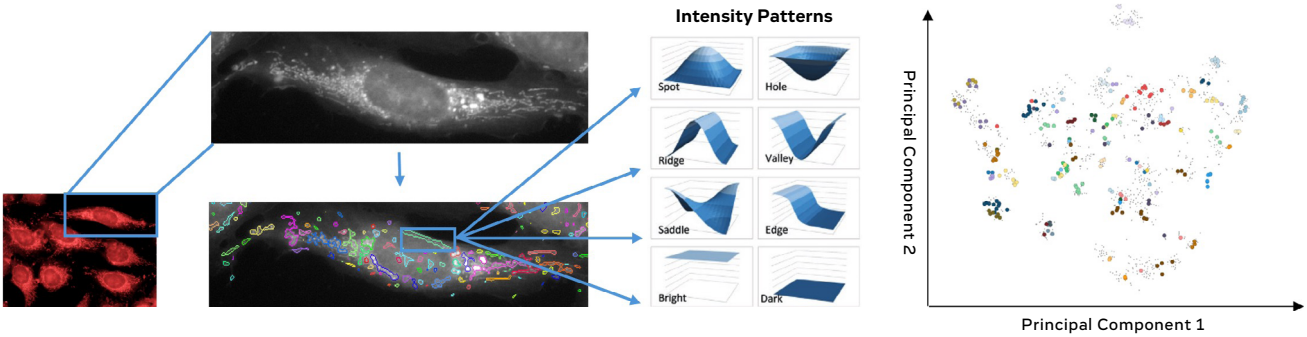
4 dye (developed by US EPA)

- **Nuclei:** Hoechst 33342 (nuclei)
- **Cytoskeleton (F-actin):** phalloidin (F-actin)
- **Lysosomes:** LysoTracker deep red
- **Mitochondria:** MitoTracker orange



Feature extraction and cluster analysis

Both methods use the same principle of extracting multi-feature data from images of the fluorescently labelled cells allowing interrogation of cellular responses to compound exposure. Using previously established automated analysis methods (e.g. cluster and principal component analysis), compound libraries can be quickly and efficiently screened to effectively identify individual compounds with potential safety and toxicity risks.



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