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Cell Painting

A high-content, image-based assay for morphological profiling using multiplexed fluorescent dyes

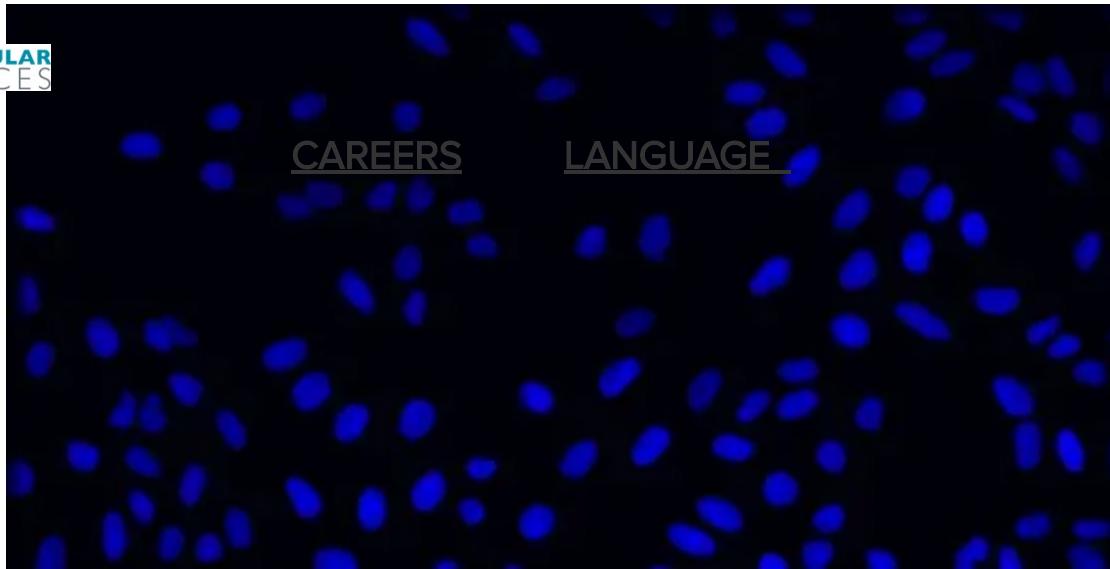
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What is Cell Painting?

Cell Painting is a high-content, multiplexed image-based assay used for cytological profiling. In a Cell Painting assay, up to six fluorescent dyes are used to label different components of the cell including the nucleus, endoplasmic reticulum, mitochondria, cytoskeleton, Golgi apparatus, and RNA. The goal is to “paint” as much of the cell as possible to capture a representative image of the whole cell.

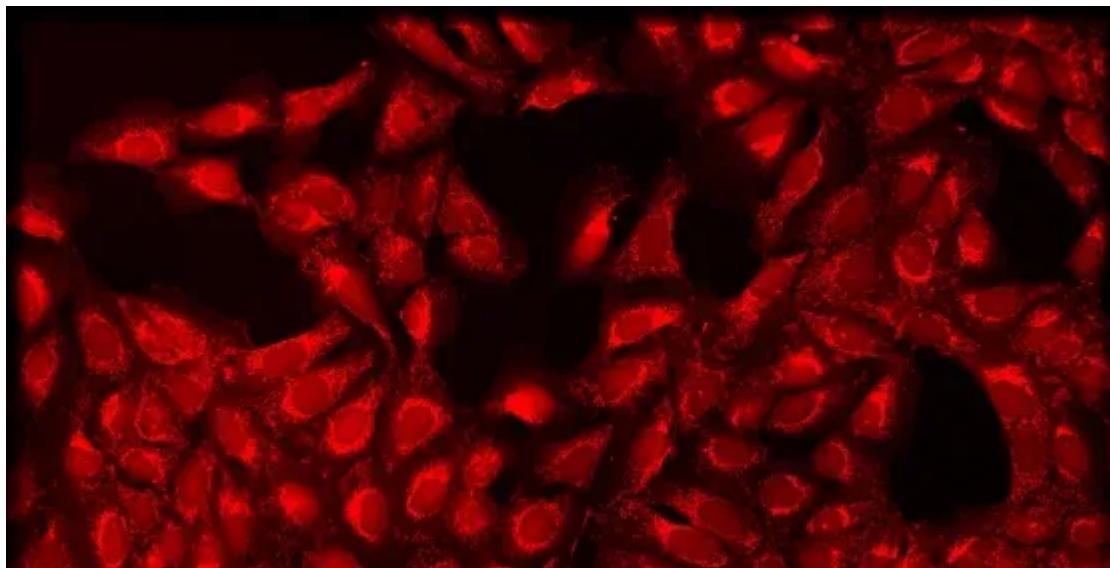
Automated image analysis software is used to extract feature measurements from each cell. The number of unique measurements is usually in the range of 100 to 1000 per cell. These measurements typically include intensity, texture shape, size as well as the proximity of an object to its neighboring structure, which provides an indication of the spatial relationship between organelles. Together, these measurements form the phenotypic profile.

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Nucleus

Dye: Hoechst 33342



Mitochondria

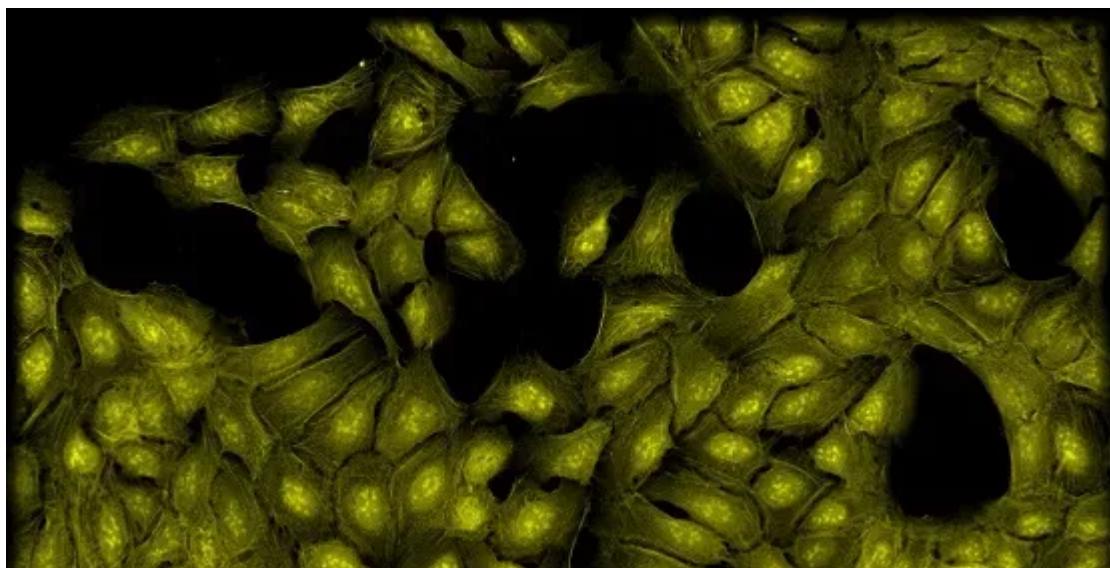
Dye: MitoTracker Deep Red

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Endoplasmic reticulum

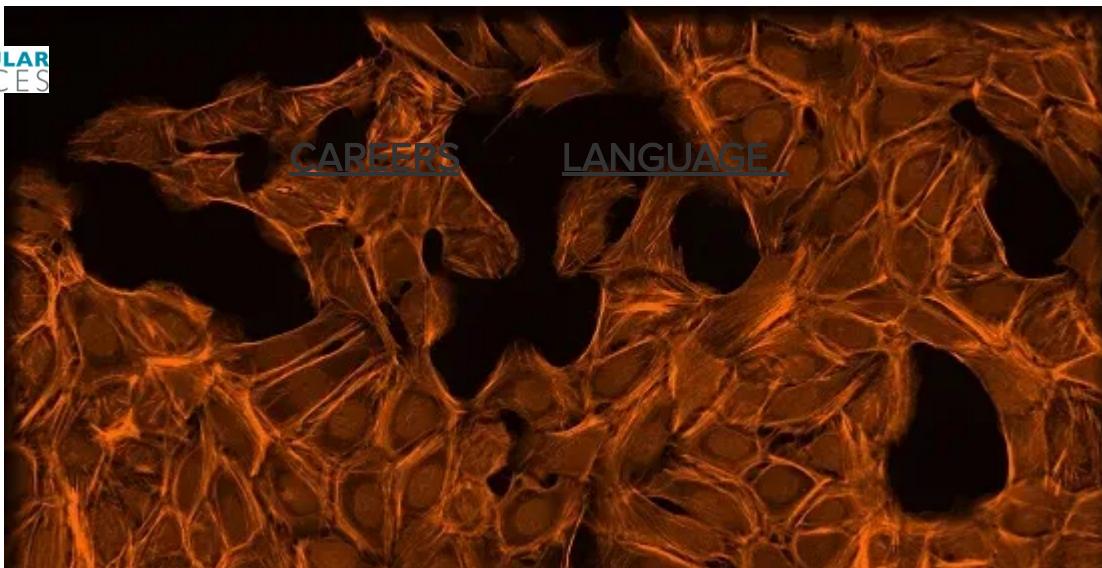
Dye: Concanavalin A/Alexa Fluor 488 conjugate



Nucleoli, cytoplasmic RNA

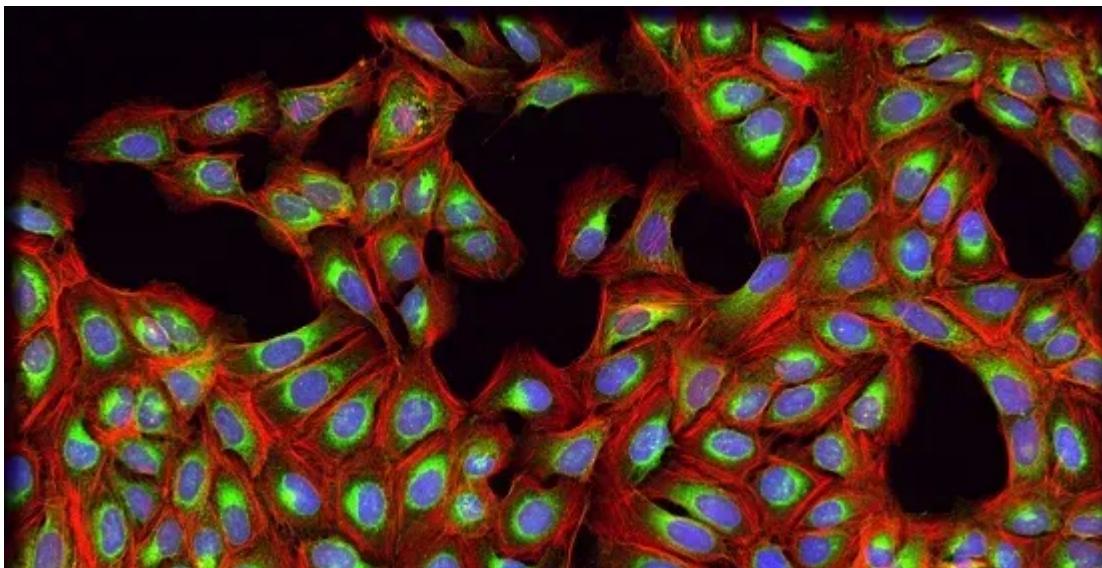
Dye: SYTO 14 green fluorescent nucleic acid stain

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F-actin cytoskeleton, Golgi, plasma membrane

Dye: Phalloidin/Alexa Fluor 568 conjugate, wheatgerm agglutinin/Alexa Fluor 555 conjugate



Composite image consisting of actin, ER (endoplasmic reticulum), and the nuclei.

Cell Painting for phenotypic profiling

The phenotypic profile of a cell reveals the specific biological state of a cell. More specifically, it can be used to interrogate biological perturbations because the cellular morphology, genetic and epigenetic state of the cell, and environmental cues can all be assessed simultaneously. This allows researchers to distinguish healthy cells from diseased cells. Because a phenotypic profile is an average of many cells, it is more sensitive to deviations or changes to those features extending beyond the individual cell. A phenotypic profile can capture certain characteristics of the cell that may not be obvious to the naked eye.

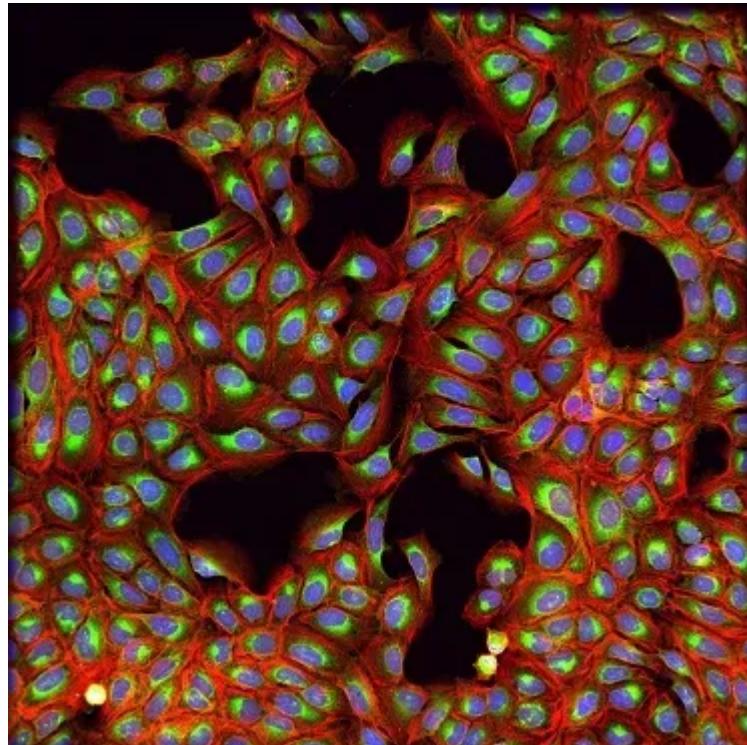
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metabolism, terize healthy rens wo

In an interview with the Science Explorer, Angeline Lim, PhD., explained, “I like to think of Cell Painting or **MOLECULAR**ling as analogous to facial recognition. It's a little bit like how Facebook or iPhoto tags people. Once you have a photograph and you tag it, the software in the background extracts information about the photo and calculates a profile. When another photo pops up, the software compares its profile to that of the previous photo to see if they are the same or different. **CAREERS** **LANGUAGE** This is basically how Cell Painting works. With Cell Painting, we expect the image analysis part to cluster like cells from unlike cells, or diseased cells from healthy cells. This potentially has many applications, especially in drug discovery.”

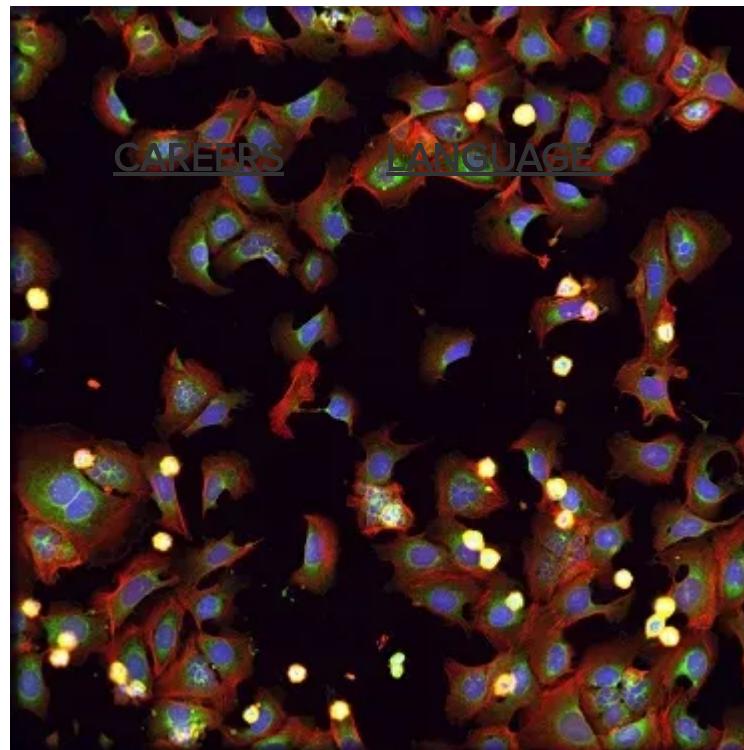
Examples of treated cells and phenotypic changes

Here is an example in which treated cells show obvious changes in their phenotypes—only three of the five wavelengths are shown in this composite: nuclei in blue, endoplasmic reticulum in green, actin & Golgi in red.

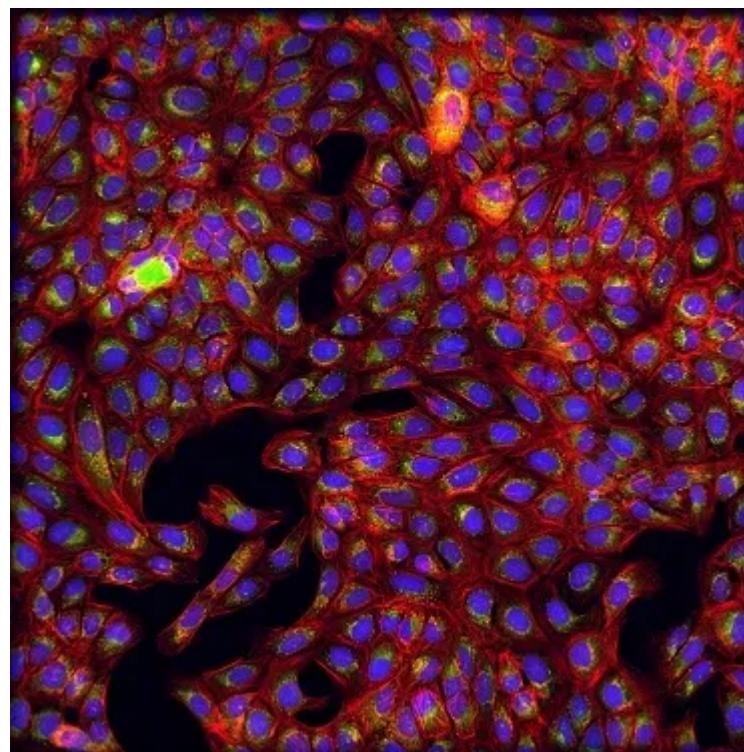


Control, untreated cells.

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Cells treated with rotenone, a toxin frequently used in insecticides. It is also known to inhibit mitochondrial ATP production and has been shown to have anticancer activity in various cancer cells.



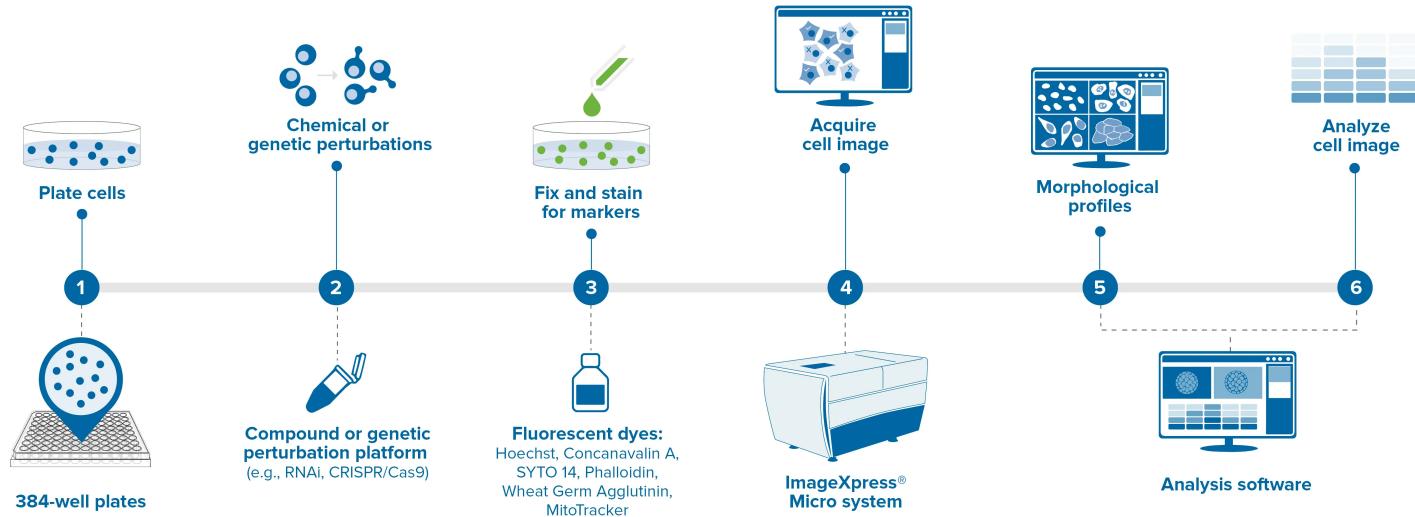
Cells treated with chloroquine. Chloroquine was first developed for malaria treatment.

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General workflow for Ce

One of the advantages in the Cell Painting assay is that the overall workflow is one that is familiar to many you plate the cells. Next, you introduce a type of perturbation; it can be chemical or genetic (e.g., adding of compounds, small molecules, or an RNAi library). After a suitable incubation period, the cells are stained with a set of Cell Painting dyes. It is also possible to use other dye combinations that are more suited to your [CAREERS](#) [LANGUAGE](#).

Once the cells are “painted,” cell images are acquired with a high-content imager such as our [ImageXpress® Confocal HT.ai](#), the latest addition to our portfolio of [high-content imaging system](#). An automated [image analysis software](#) like our [MetaXpress®](#) or our [IN Carta image analysis software](#) is used for feature extraction where cells and their components are identified and measured. Finally, the measurements are further processed using various data analysis tools to create and compare phenotypic profiles, perform clustering analysis, and identify targets to derive the morphological profiles.



1. Plate cells into labware (384-well plate)
2. Treat cells with chemical or genetic perturbation (e.g., RNAi, CRISPR/Cas9) or viruses.
3. Stain with fluorescent dyes (e.g., Hoechst, Phalloidin, MitoTracker)
4. Acquire cell images with [high-content imaging system](#)
5. Analyze cell images to extract features and measurements using [automated image analysis software](#)
6. Derive morphological profiles from measurements

Learn more about Cell Painting

Cell Painting is emerging as a valuable tool with its many ...p discovery. Learn how to optimize high-content imaging capab preconfigured or custom modules from [MetaXpress software](#)

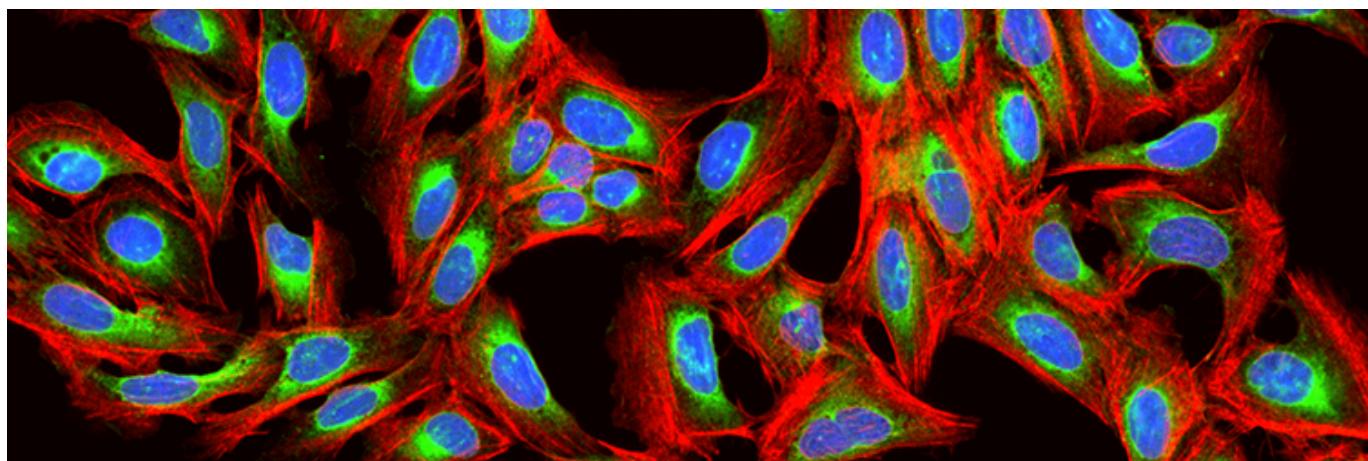
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Tips for running a successful live cell imaging experiment

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How Cell Painting is making its mark on drug discovery

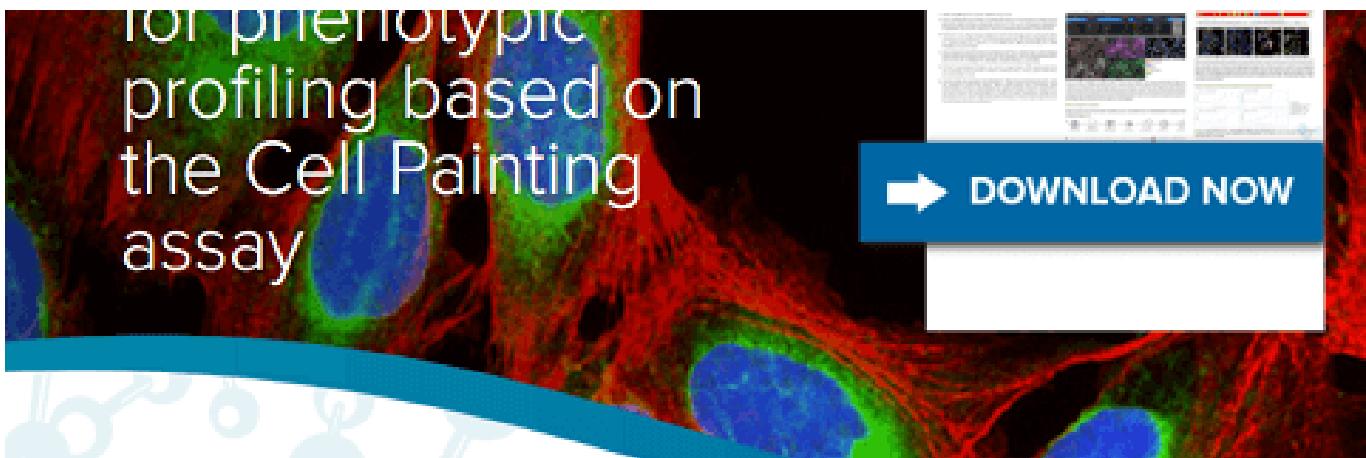
Have you ever heard the old adage, "A picture is worth a thousand words?" When it comes to Cell Painting, this saying is...

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In this study, we demonstrate improvement in assay sensitivity, precision, and speed of acquisition with a new configuration...

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Here, we demonstrate improvement in assay sensitivity, quality, and speed of acquisition with the ImageXpress® Confocal HT....

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High-content phenotypic profiling using the Cell Painting assay

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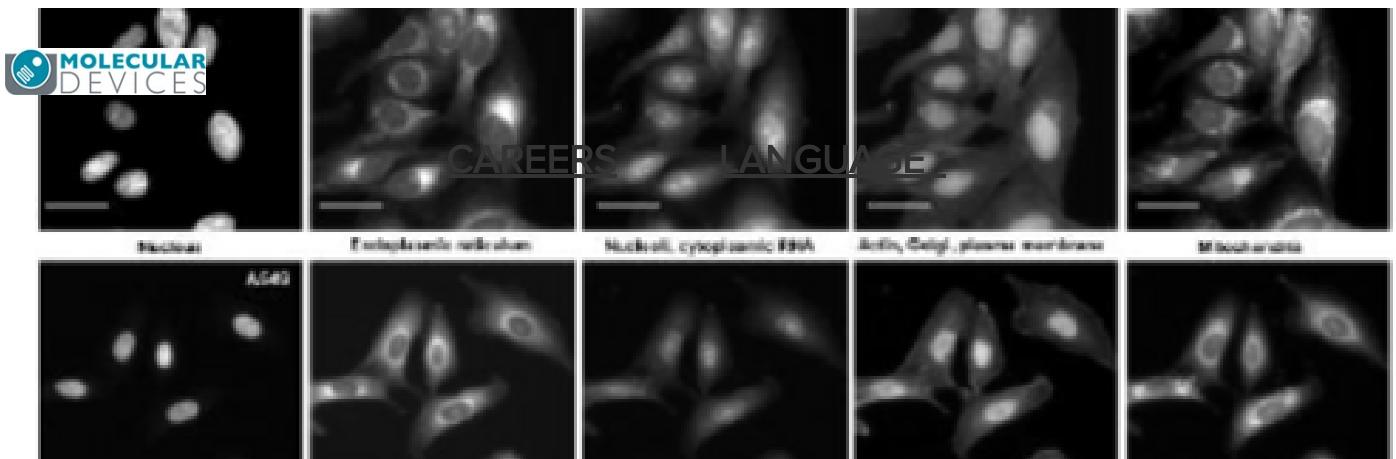
References

APPLICATION NOTEHigh-content phenotypic profiling using the Cell Painting assay

Here, we present a complete workflow for a cell painting assay that can be easily implemented using the ImageXpress Micro...

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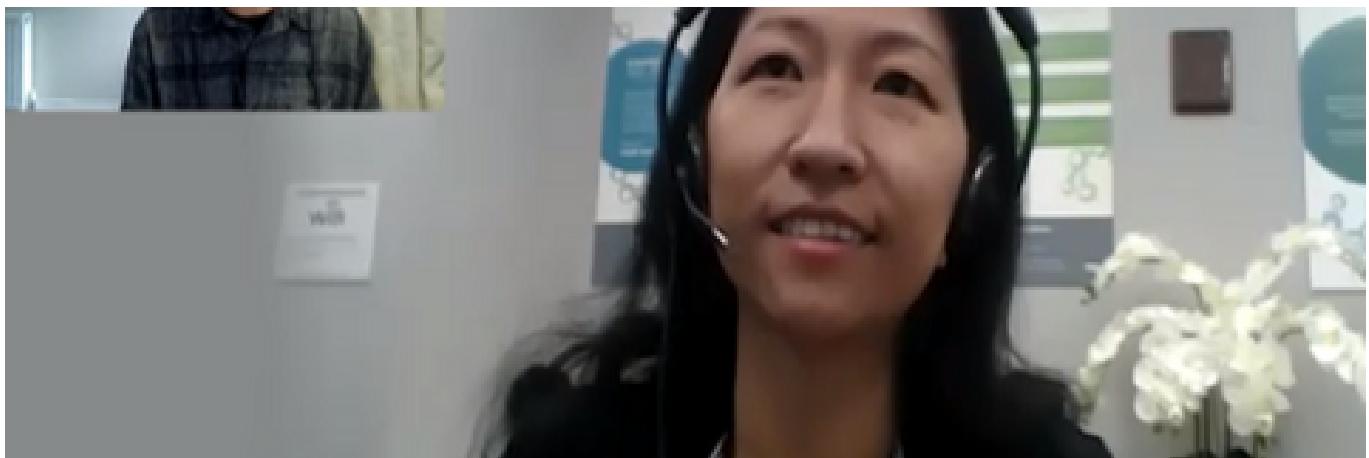


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Cell Painting, a high-content image-based assay for morphological profiling using multiplexed fluorescent dyes

In morphological profiling, quantitative data are extracted from microscopy images of cells to identify biologically...

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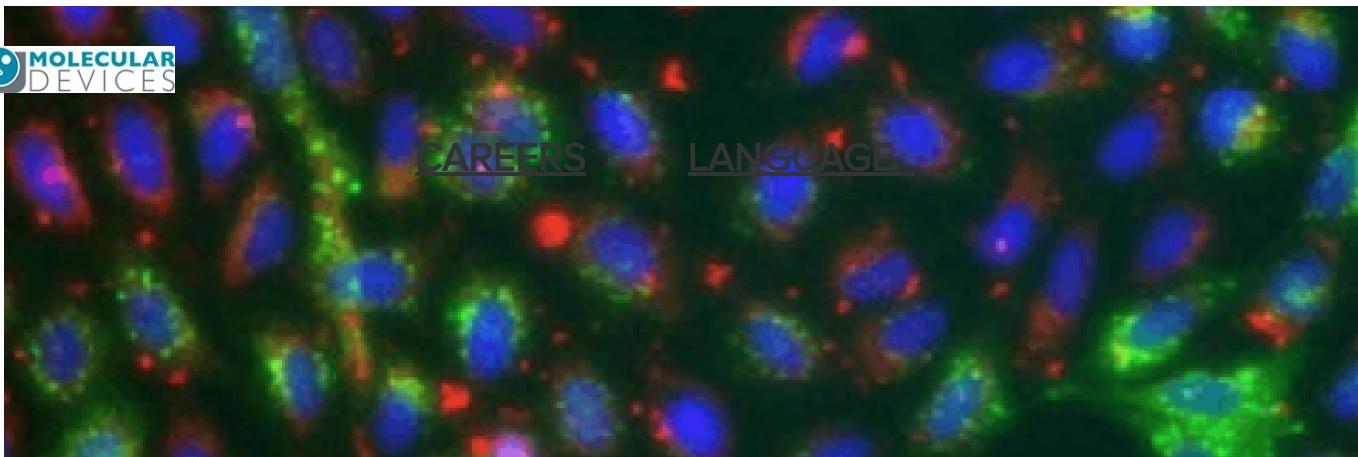
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Unleash the power of Cell Painting

The Science Explorer interviews Angeline Lim, an Applications Scientist at Molecular Devices, to learn more about Cell...

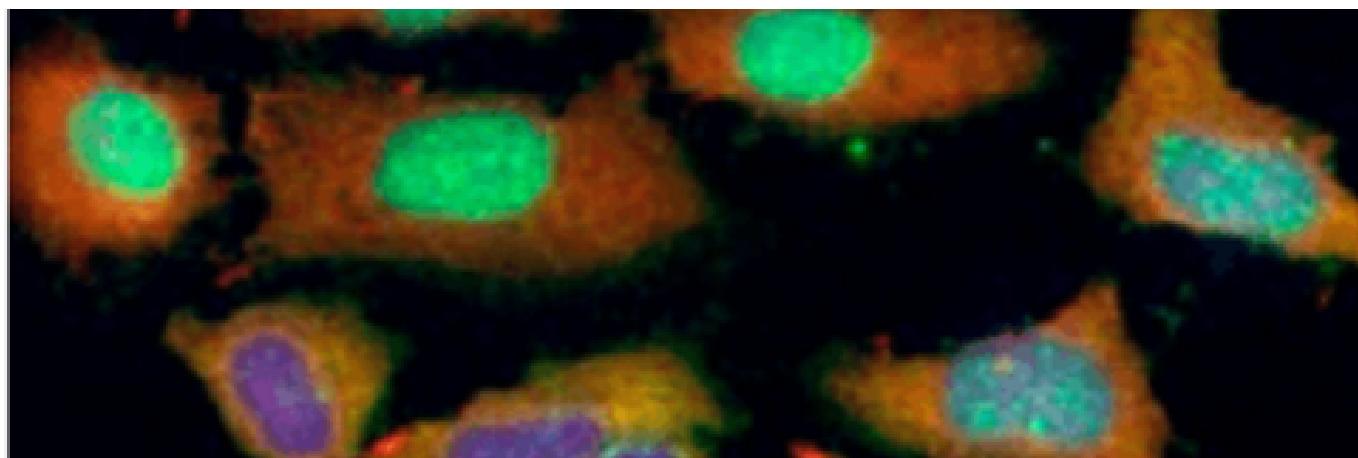
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