

## APPLICATION NOTE

# Detection of autophagy using automated imaging

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## Introduction

Autophagy is a highly regulated process of degrading and recycling damaged proteins and organelles in response to cellular stress.<sup>1,2</sup> It is mediated by a unique vesicle called the autophagosome, which is assembled by the formation of a double membrane around the cellular component marked for destruction.<sup>1</sup> The autophagosome vesicle then fuses with the lysosome to deliver its contents for degradation by lysosomal hydrolases.

Autophagy has a variety of complex physiological and pathophysiological roles, such as adaptation to nutrient starvation, clearance of damaged intracellular proteins and organelles, cell development, anti-aging, elimination of microorganisms, cell death, tumor suppression, and antigen presentation.<sup>2</sup> Mitophagy is the selective degradation of mitochondria by autophagy.<sup>3</sup> This process often eliminates defective mitochondria following cellular damage or stress. Recently, the autophagy process has been linked to neurodegenerative disorders including Parkinson disease. The pathogenesis of Parkinson disease partially involves neuronal cell death as a result of dysregulation of autophagy and disruption of normal recycling of damaged mitochondria. Several genetic mutations implicated in the disease, including the loss of function of PINK1 and Parkin, can lead to the accumulation of damaged mitochondria and protein aggregates, promoting cellular degeneration.

Since dysregulation of autophagy has been established to play a role in various neurodegenerative diseases and cancers, the discovery of novel therapeutic agents targeting various stages along this process has emerged as a promising new approach for drug therapies.

## Materials

- Cyto-ID® Autophagy Detection Kit (Enzo Life Sciences)
- MitoTracker Orange dye (ThermoFisher Scientific)
- LysoTracker Red dye (ThermoFisher Scientific)
- Hoechst (ThermoFisher Scientific)
- ImageXpress Nano Automated Imaging System with CellReporterXpress software (Molecular Devices)

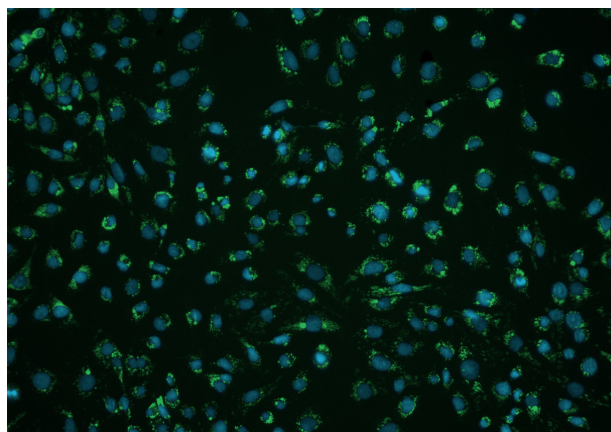
## Detecting compound effects on autophagy

We evaluated the efficiency of the ImageXpress® Nano system for screening assays exploring compound effects on autophagy. PC12 human neuroblastoma cell line was used as a model for assay development. Cells were plated into 384-well plates at 6,000 cells/well and incubated for 48h. To evaluate effects on autophagy, cells were treated with various compounds for 24-48h. Then, live cells were stained with the Cyto-ID® Autophagy Detection Kit for tracking autophagosomes, MitoTracker Orange dye for detection of mitochondria, LysoTracker Red dye for labeling lysosomes, and Hoechst to identify nuclei (0.2  $\mu$ M, 0.2  $\mu$ M, and 1  $\mu$ M, respectively). Images were taken using ImageXpress Nano system with 20x or 40x magnification and four detection channels for the appropriate dyes (FITC, TRITC, Cy5, and DAPI, respectively). One image per well was captured at 20x magnification, while 2-4 images were acquired per well at 40x magnification to ensure better statistical results.

## Benefits

- Detect compound effects on autophagy
- Identify new therapeutic agents that target autophagy
- Run efficient autophagy assays using automated imaging

Images were analyzed using the Granularity application module in CellReporterXpress™ Automated Image Acquisition and Analysis Software. The Granularity algorithm detects and characterizes small objects, such as autophagosomes, lysosomes, and mitochondria in the cytoplasm of cells while using the nuclear marker to segment cells. Readouts included total numbers and total area of “granules” (subcellular objects). Representative images and analysis traces for cells treated with chloroquine (30 μM) are shown in Figure 1.



**Figure 1. Autophagy detection assay.** Image of PC12 neuroblastoma cells treated with chloroquine for 24h and stained with Cyto-ID autophagy detection kit. Images were taken by ImageXpress Nano system, 20x magnification. Autophagy particles indicated in green, nuclei shown in blue.

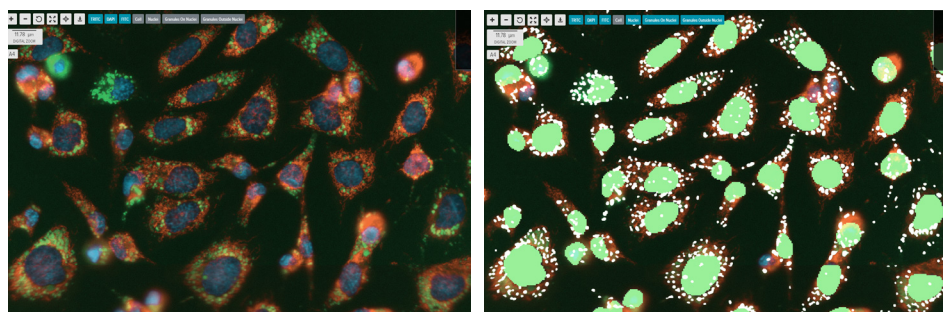
Concentration responses for three known inducers of autophagy, chloroquine, verapamil, and amiodarone, were examined and EC<sub>50</sub> values were evaluated. Chloroquine, Verapamil and Amiodarone treatment increased autophagy levels by 6.2, 4.3, and 3.4-fold, respectively, as determined by total autophagosome counts (EC<sub>50</sub> values were 3.05, 3.1 and 14.7 μm, respectively).

## Conclusion

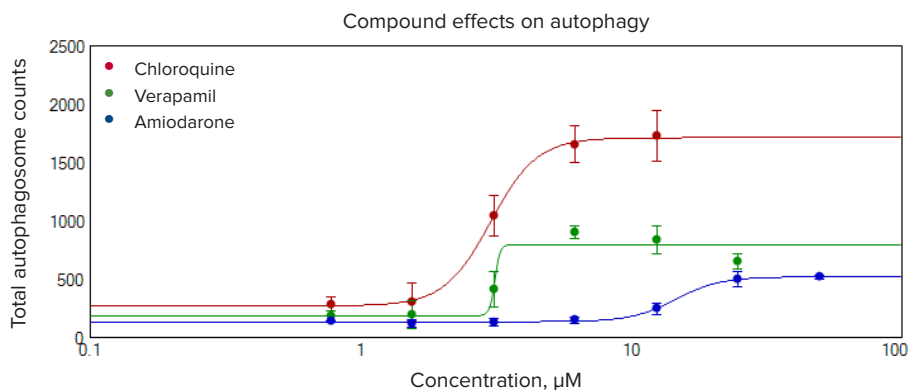
This assay demonstrates the reliability of the ImageXpress Nano system and CellReporterXpress software for high-content screening for inducers or inhibitors of autophagy, and allows the identification of new therapeutic agents that target autophagy early in drug development.

## References

1. Mizushima, N; Komatsu, M (2011). Autophagy: renovation of cells and tissues. *Cell*. 147: 728–41
2. Mizushima, N; (2007) Autophagy: process and function, *Genes & Dev*. 21: 2861-2873
3. Lemasters, J (2005). Selective mitochondrial autophagy, or mitophagy, as a targeted defense against oxidative stress, mitochondrial dysfunction, and aging. *Rejuvenation Research*. 8: 3–5



**Figure 2. Autophagy image analysis.** **Left:** Image of PC12 neuroblastoma cells treated with chloroquine for 24h and stained with Cyto-ID reagent, MitoTracker Orange, and Hoechst dyes. Images were taken by ImageXpress Nano system, 40x magnification. Autophagy particles (green), mitochondria (red), and nuclei (blue). **Right:** Analysis masks shown for autophagy particles and nuclei after using Granularity application module in CellReporterXpress software. Nuclei (green) and autophagosomes (white).



**Figure 3. Compound effects on autophagy.** Concentration-dependent responses for autophagy indicated for three compounds using “total granules” (total autophagosome count) as a read-out. Chloroquine (red), verapamil (green), and amiodarone (blue)

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