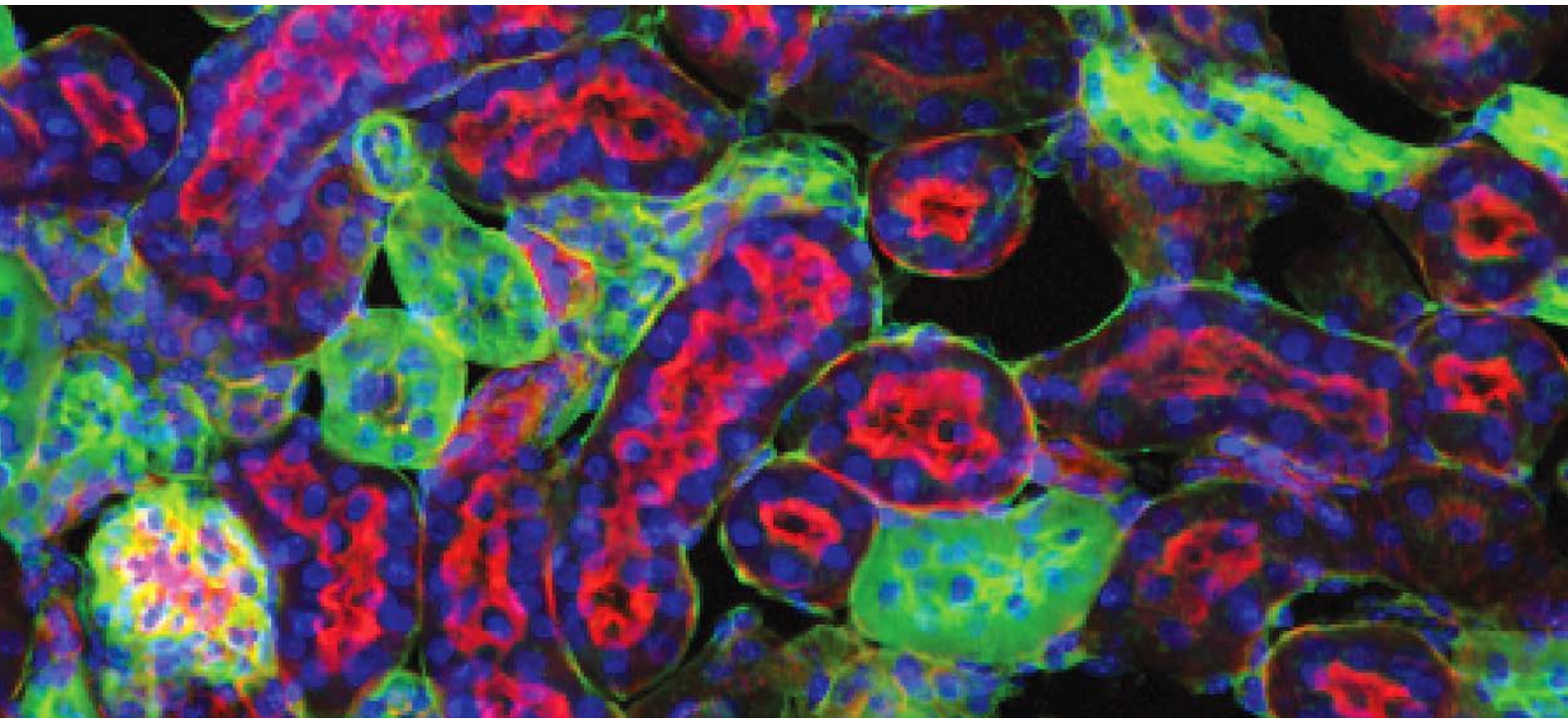

Fueling discovery with high-content imaging and analysis

Thermo Scientific HCA platforms for automated, quantitative analyses.

A wealth of information about a physiological process or pathological condition can be collected by monitoring the localization and abundance of specific proteins using fluorescence imaging (Figure 1). The use of fluorescence imaging in conjunction with biosensors that incorporate synthetic organic dyes or fluorescent proteins allows researchers to visualize protein and organelle functioning during cellular processes in real time. However, quantifying these essential processes through the capture and analysis of fluorescence imagery is both time consuming and laborious. With the advent of high-content analysis platforms that automate image capture and data analysis, these obstacles have been removed, affording researchers both the sample sizes and precise quantitation tools required for truly robust and reproducible research.

Figure 1. Confocal image of a fluorescently stained mouse kidney. See Figure 2 caption for experimental details.

Thermo Scientific HCA platforms and software

Among the most cited in scientific articles, high-content analysis (HCA) instruments from Thermo Fisher Scientific are the go-to choice for researchers requiring the resolution of microscopy with the statistical power inherent in a highly quantitative analysis of a large number of cells. These instruments include the Thermo Scientific™ ArrayScan™ High-Content Platforms, the Thermo Scientific™ CellInsight™ CX5 High-Content Screening (HCS) Platform, and the recently introduced Thermo Scientific™ CellInsight™ CX7 High-Content Analysis (HCA) Platform. The CellInsight CX7 HCA Platform provides an integrated benchtop instrument that interrogates multiple sample types with a wide range of techniques, taking advantage of next-level image acquisition and analysis software (Figure 2).

In the first half of this year, our HCA platforms have been used in nearly one hundred peer-reviewed publications. Many of these high-content imaging and analysis studies take advantage of the Thermo Scientific™ HCS Studio™ software provided with our HCA instruments (Figures 2 and 3). This intuitive, icon-driven tool helps to manage the experimental design and workflow, starting with plate maps and protocol setup, all the way through image acquisition and data analysis. Once images are acquired, users can leverage the software's suite of available bioapplications, purpose-built for specific biological areas such as proliferation, translocation, neurite outgrowth, and autophagy. The following sections contain short summaries of key publications in which Thermo Scientific HCA platforms and software have been used to make critical discoveries across multiple research areas.

Investigating infectious diseases

Love and coworkers recently published work on the infection of human intestinal epithelial cells by the parasite *Cryptosporidium parvum*, which causes a life-threatening diarrheal disease in children [1]. Using the CellInsight CX5 High-Content Screening (HCS) Platform, Love et al. screened a library of existing FDA-approved molecules in search of inhibitors of *Cryptosporidium* spp. proliferation. This research highlights the use of automated image acquisition and analysis to detect events within a cell that can lead to potential novel therapeutic applications, in this case the identification of the drug clofazimine as an inhibitor of *C. parvum* infection. →

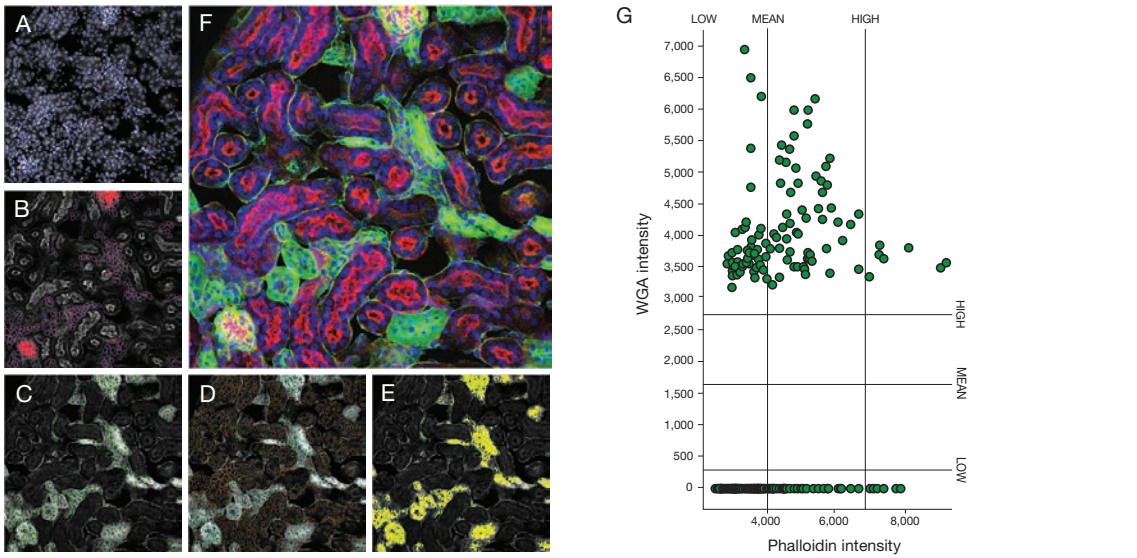

Figure 2. Confocal image analysis of a fluorescently stained mouse kidney. A 16 µm cryostat section of mouse kidney was stained with Invitrogen™ Alexa Fluor™ 488 Wheat Germ Agglutinin (WGA) (green, Cat. No. W11261), Invitrogen™ Alexa Fluor™ 568 Phalloidin (red, Cat. No. A12380), and DAPI nucleic acid stain (blue, Cat. No. D1306) and imaged on the Thermo Scientific™ CellInsight™ CX7 High-Content Analysis Platform using laser autofocus and confocal acquisition at 20x magnification. Thermo Scientific™ HCS Studio™ Cell Analysis Software was used for analysis: **(A)** nuclear identification and segmentation (blue); **(B)** phalloidin detection in WGA+ cells (red); **(C)** WGA+ cell selection (green); **(D)** WGA mask modification (green); **(E)** spot detection for quantifying WGA signal; **(F)** composite confocal image. **(G)** Scatter plot shows relative labeling intensity of the WGA vs. phalloidin conjugates in order to characterize phenotypes using intensity cutoffs.

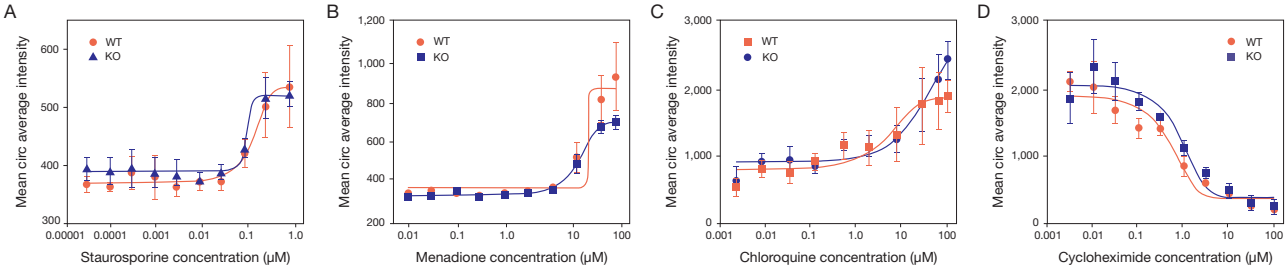

Figure 3. Rapid analysis of various cell health parameters using a high-content analysis (HCA) platform. Wild-type (WT) and CRISPR-edited (functional knockout or KO) HAP1 cells were analyzed using the Thermo Scientific™ CellInsight™ CX5 High-Content Screening Platform for **(A)** apoptosis, using Invitrogen™ CellEvent™ Caspase-3/7 Green ReadyProbes™ Reagent (Cat. No. R37111), **(B)** oxidative stress, using Invitrogen™ CellROX™ Green Reagent (Cat. No. C10444), **(C)** protein degradation, with the Invitrogen™ Click-iT™ HPG Alexa Fluor™ 488 Protein Synthesis Assay Kit (Cat. No. C10428), and **(D)** protein synthesis, using the Invitrogen™ Click-iT™ Plus OPP Alexa Fluor™ 488 Protein Synthesis Assay Kit (Cat. No. C10456). Thermo Scientific™ HCS Studio™ Cell Analysis Software was used for nuclear segmentation and fluorescence analysis (mean circ average intensity).

Assaying cell function and oxidative stress

High-content imaging and analysis can also be applied to the study of cell–cell interactions and more comprehensive tissue functions. Using the CellInsight CX5 High-Content Screening Platform, Summermatter and colleagues investigated the role of metallothioneins in regulating skeletal muscle mass [2]. Metallothioneins, a family of cysteine-rich metal-binding proteins, are involved in zinc storage and transport and have been implicated in muscle atrophy. Automated image capture of stained myofibers and subsequent analysis of their width showed that myofiber width increases following siRNA-mediated knockdown of metallothioneins, suggesting a new strategy for increasing muscle mass and strength.

Zhang et al. employed the ArrayScan VTI HCS Reader to study a different muscle cell type—cardiomyocytes. These authors demonstrated that, in neonatal mouse cardiomyocytes, mitochondria are the main source of the reactive oxygen species (ROS) generated following β-adrenergic receptor stimulation [3]. They further characterized the properties of these ROS bursts using the fluorogenic probes dihydroethidium and Invitrogen™ MitoSOX™ Red Mitochondrial Superoxide Indicator to show that two discrete pathways regulate the temporal profile of ROS generation. Faster mitochondrial ROS bursts are controlled by the cAMP/PKA pathway, whereas slower mitochondrial ROS production is regulated by the β-arrestin1 pathway.

Another important regulator of oxidative stress is the Nrf2 pathway, which was investigated by Pistollato and coworkers using the ArrayScan XTI High-Content Platform. These researchers measured the nuclear translocation of Nrf2 in response to rotenone treatment of neural stem cells derived from human induced pluripotent stem cells (iPSCs) [4]. They showed that Nrf2 translocation increases during differentiation and is associated with cytotoxicity. Using the neurite outgrowth bioapplication built into the HCS Studio software, they also found that Nrf2 activity was correlated with neurite retraction.

Studying neuronal development and disease

Neurite outgrowth is a key step in neuronal development. Once a critical cell mass is reached, a neural progenitor cell line derived from cortical neuroepithelium can extend neurites and form synaptic connections. Hill et al. studied how mutations in the transcription factor gene *TCF4* affect human cortical cell progenitor proliferation using the Thermo Scientific™ CellInsight™ NXT High-Content Screening Platform [5], and discuss the implications for cognitive deficits found in individuals with Pitt-Hopkins syndrome.

Lorenz et al. also characterized the cellular phenotype arising from gene mutations, in this case in the mitochondrial gene *MT-ATP6* [6]. *MT-ATP6* mutations have been shown to be associated with several neurological diseases, including Leigh syndrome, retinitis pigmentosa, and episodic paralysis with spinal neuropathy. These researchers produced neural progenitor cells from patient-derived iPSCs carrying mutations in the *MT-ATP6* gene, and showed that these cells exhibited abnormal mitochondrial membrane potential using the ArrayScan XTI Infinity High-Content Platform. They then performed a phenotypic screen of existing FDA-approved drugs using mitochondrial membrane potential as a phenotype, and found that one compound, avanafil, was able to alleviate the phenotype in mutation-carrying cells.

Table 1. Which HCA system is right for you?

	Compact screening system to scale up your throughput	Integrated performance modes for screening and analysis	Modular solutions to address specialized applications
	 CellInsight CX5 Platform	 CellInsight CX7 Platform	 ArrayScan Systems
Illumination	5 channels	7 channels	7 channels
Camera	Photometrics X1		
Widefield	5 channels	7 channels	7 channels
Brightfield	White	White + 4 colors	White (optional)
Confocal	NA	7 channels	7 channels (optional)
Objectives	1-position turret 2x to 20x	3-position turret 2x to 40x	4-position turret 1.25x to 63x
Focus	Software	Laser and software	Laser and software
Live cell	NA	NA	Live cell (optional)
Software	HCS Studio Cell Analysis Client Software		
Thermo Fisher Cloud	Save files to Cloud through computer		
Database	Store Image and Database Management Software (optional)		

Mitochondrial and lysosomal dysfunctions often coexist in neuronal diseases. Using primary neurons, Jinn and colleagues produced knockout mutations in *TMEM175*, a known risk-factor gene for Parkinson's disease [7]. Following gene knockout, they used the ArrayScan XTI Live High-Content Platform to show that cells lacking *TMEM175* exhibited unstable lysosomal pH, reduced autophagosome clearance, and decreased lysosomal enzyme activity, as well as impaired mitochondrial respiration.

Find the HCA system that fits your laboratory

High-content screening and analysis instruments from Thermo Fisher Scientific—including CellInsight CX5, CellInsight CX7, and ArrayScan platforms (Table 1)—have directly impacted every segment of biological research, from cellular and systems biology to drug discovery. These technology platforms build on a 20-year legacy of HCA instrument and software development and over 40 years of fluorescence imaging and probe development in our cell and protein analysis laboratories. To learn more about our high-content instrument platforms, software, applications, and analysis reagents, or to request an in-lab demonstration of one of our HCA instruments, visit **thermofisher.com/hcabp76**. ■

References

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3. Zhang J, Xiao H, Shen J et al. (2017) *Biochem Biophys Res Commun* 489:393–398.
4. Pistollato F, Canovas-Jorda D, Zagoura D et al. (2017) *Neurochem Int* 108:457–471.
5. Hill MJ, Killick R, Navarrete K et al. (2017) *J Psychiatry Neurosci* 42:181–188.
6. Lorenz C, Lesimple P, Bukowiecki R et al. (2017) *Cell Stem Cell* 20:659–674.
7. Jinn S, Drolet RE, Cramer PE et al. (2017) *Proc Natl Acad Sci U S A* 114:2389–2394.

Product	Quantity	Cat. No.
CellInsight™ CX5 High-Content Screening Platform	1 each	CX51110
CellInsight™ CX7 High-Content Analysis Platform	1 each	CX7A1110
CellInsight™ CX7 High-Content Analysis Platform and Store Standard Edition	1 each	CX7B1112
CellInsight™ CX7 High-Content Analysis Platform and Store Standard Edition plus Robotic Plate Handling	1 each	CX7C1115