Capturing rapid cellular events using simultaneous image acquisition

Image-based studies of biological processes in living creatures allow researchers to follow kinetic and time-dependent processes in real-time with minimal intervention. In these studies it is often required to follow rapid cellular events with multiple readouts. Furthermore, in many cases these features or events should be measured simultaneously.

Simultaneous image acquisition refers to imaging of more than one fluorescent probe at the same time (exciting and detecting the signal through all filters at the same time). This technique has two major advantages for live cell imaging: (1) avoiding displacement of the object between the acquired images, and (2) faster image acquisition.

The need in biological research

Simultaneous image acquisition is used both for whole-organism imaging and for cellular imaging.

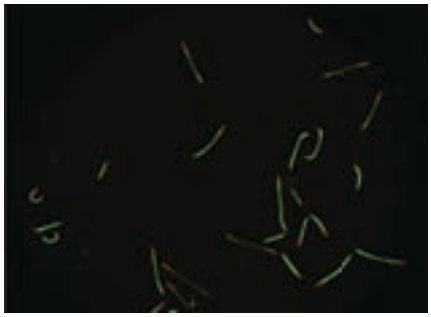

Figure 1
C-elegance imaged with two fluorescence wavelength for whole body labelling (green) and specific head labelling (red)

A common example for a whole-organism imaging is that of *c-elegance* (**Figure 1**). Measurements of co-localised events in living and moving *c-elegance* are practically impossible in a sequential imaging mode. The limiting factor is the delay between the acquisition of an image in each wavelength. The living worm moves during the time gap between image acquisition, and consequentially conclusions about co-localisation are unfeasible.

Measuring Calcium flux and subsequent events in neurons is another common example where simultaneous acquisition of images in different wavelengths is necessary in order to accurately follow simultaneous, rapidly occurring events.

Technical requirements for simultaneous image acquisition

The Hermes WiScan® imaging system provides a built-in solution for simultaneous imaging. It features image acquisition in four separate imaging channels, which can be operated in parallel or sequentially, according to the user's requirements. This configuration allows for simultaneous measurements in live cell experiments on the one hand, and for extremely rapid scanning rates on the other hand.

Successful acquisition of several fluorescence images with different wavelengths simultaneously depends on adequate experimental design, in which fluorophores are spectrally separated in order to avoid (or at least minimise) spectral bleed-through, which otherwise occur as a result of overlap between the emission spectra of probes.

The Hermes WiScan® system offers a variety of optional fluorophore combinations for simultaneous acquisition to choose from, according to specific research requirements (see **Table 1**).

Simultaneous image acquisition is an essential feature in many live experiments, which enhances the capabilities of high content screening experiments.

Table 1: Possible Fluorophore combinations for simultaneous acquisition using Hermes WiScan®

FLUOROPHORE COMBINATION	EXCITATION (NM)	EMISSION (NM)
DAPI/HOECHST TRITC/Cy3	390/22 560/32	440/40 607/36
DAPI/HOECHST CY5	390/22 648/20	440/40 694/44
GFP/FITC CY5	485/25 648/20	525/30 694/44
CFP mCherry	485/25 575/35	525/30 624/40

IDEA Bio-Medical's Hermes WiSCAN® is a genuine breakthrough in cell-imaging systems. Hermes WiScan® allows researchers to achieve high content screening at high throughput rates, while maintaining unparalleled simplicity.