CAMERA-BASED SOLUTIONS FOR HIGH-CONTENT IMAGING

The evaluation and testing of new imaging technologies for high-throughput imaging equipment and systems is a critical – and, at times, aggravating or confusing — process.
Finite organizational resources make efficiency, reliability, and price performance key metrics when implementing, replacing, or upgrading such technologies. Organizations face constant pressure to rationally allocate talent, time, and money, so there is inherent resistance to altering the price-performance ratio of any given system.
Consider that the life cycle of a high-throughput biological/biomedical imaging system may be two to four years, so you have short windows to test/evaluate new components. Still, updates are a necessary burden. By providing higher sensitivity, a higher frame rate, and/or an upgraded interface, a new camera can help the optical train in a high-content system do a job cheaper, better (i.e., locating more targets because the camera is more sensitive, collecting more information, viewing in better resolution), or faster – demonstrably improving price performance.
So, you've decided to upgrade an existing system or you are considering cameras for a new one. To test a new camera, you get your hands on everything you can, demo everyone's offering, pick one, and then spend a year or two actually developing, marketing, and maintaining that instrument before you have to do it all over again.
During this process, your talent is sidetracked — testing typically is done by optical engineers or people that do more of the actual integration — and the risk always exists that a poor decision may be made. This high-stakes environment means product analysis must look not only at commonsense performance and dependability metrics but the team also must consider whether the product's support service is robust. Can the vendor deliver in time? Will it provide integration guidance?
This article seeks to help readers identify both their needs and their options when implementing a new camera into a high-content system, empowering individuals who evaluate and test new imaging technology to make informed decisions.

Defining high-content, high-throughput imaging

High-content screening involves a biological investigation of a large volume of different samples, in many cases using fluorescent emission as an indicator, completed at a maximum throughput. The process has applications in pharmaceutical and medical research, from the investigation of different cell variances to cells' reaction to different antibiotics — anything that needs to be investigated in more than one sample. High-content/high-throughput imaging is subject to varying degrees of automation, from sample prep to data analysis. Ideally, a system removes as much user interaction as possible, reducing user error and delays (e.g., you may be trying to enable the scanning of slides at a higher speed but, if you have to manually inject or extract the slides, it costs significant time). A popular example might be a pharmaceutical application, where screening machines are running 24/7 trying to process (i.e., image and analyze) thousands or hundreds of thousands of molecules at a time. However, each application has its own requirements to be examined, defined, and met.

Defining your application`s requirements

First, system engineers must define the performance parameters and costs factoring into their equipment acquisition decision. When looking at your performance-based line, what do you need in terms of image quality, in terms of imaging speed and utility for different applications? Each application imposes different demands: some high-content applications seek ultimately to maximize throughput. They have plenty of light, plenty of signal, and sensitivity doesn't matter as much.
Regardless of the application, though, high-throughput imaging requires high reliability — a system shouldn't require any recalibration. These systems are generating data around the clock, so if a camera or light source goes down, you're missing valuable time to collect information (e.g., pharma companies are under incredible pressure to run through as many molecules as possible).
In addition to robust hardware, reliability demands a stable software development kit because if there are any changes to the product, manufacturers will not always change the firmware controlling the instruments.
Still, in most cases, two or three factors emerge as the most important (admittedly, overall cost almost always resides in this top three) to system designers.
The weight of particular performance metrics depends greatly on the method by which you're creating a signal that you need to capture. For example, if you use fluorescence, sensitivity becomes the most important factor. Simply put, it's just hard to generate large amounts of fluorescence. So sensitivity and noise reduction rise to the top of the list. Conversely, if you're using light microscopy — perhaps using a high-speed flow chamber, looking at the morphology of a cell — you can flood it with light, and noise and sensitivity become less important than high frame rate, high speed, and high resolution.

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Understanding high-content imaging camera performance

Acknowledging this “stat weight” approach, it must be noted that the resolution, speed, and sensitivity “triangle” always will tilt one way or the other. If you strive for high frame rate, high resolution, low noise, and high sensitivity, and associate this with a high dynamic range, positive changes to some parameters will negatively affect other parameters.

The typical parameter for evaluating dynamic range is the fullwell capacity of one pixel at maximum capacity of electrons, divided by the minimum signal level that you need to come out of the noise (i.e., the readout noise). Fullwell capacity depends on the size of the pixel.

To achieve higher resolution, you shrink the pixel, thus reducing maximum full-well capacity. Therefore, the only way to keep a high dynamic range would be further reduce the readout noise further, to which theoretical limits exist. To wit, reduction of readout noise has not technically reached a limit, but the threshold has become quite small. You may have a readout noise as low as one electron, and with multisampling effective read noise lower than that is possible, but future advancements in this direction are incremental.

Now, a small pixel may be necessary to properly sample optically in some applications, such as working with a low magnification. Otherwise, you are easily undersampled, and you don’t get the image’s sharpness and the required resolution.

For example, if you’re at a microscope, a certain magnification defines the area of the sample that you’re able to investigate. If you go for larger samples, and you want to have a larger overview, you need small pixels to get proper sampled images. A lower magnification means you get a larger size or a larger field of view for your sample, and you might accomplish your task in one shot rather than, say, four or five different samples. Consider that a pathologist may want to have an overview, while metabolic researchers would like to see the single cell.

As stated before, you don’t get a big dynamic range when you have a small pixel. But even with a small pixel, that dynamic range can be adequate for the applications described here, where a 10-bit and 12-bit dynamic range is likely to suffice.

That said, advances like the pco.edge 26 — a cooled, high-content/high-throughput camera — prove that small-pixel/dynamic range trade-offs do not have to suffer the same severity as previous cameras. The pco.edge 26 is a global shutter camera featuring 2.5 x 2.5 µm² pixels with a full-well capacity of around 4,500 electrons, yet still boasts 10-bit and 12-bit dynamic range modes. Thus, it produces sensitivity and noise characteristics that, to this point, have usually been associated with much larger, 6.5 µm or 11 µm, pixels. This application example shows the types of systems the pco.edge 26 can empower and improve.

PCO’s expertise guides you

PCO understands the pressures faced by system designers, as well as the need to show proof of performance, quality, and ROI when justifying purchase decisions. For each camera it creates, PCO has compiled an extensive file of quality measurements, following compliance to the EMVA 1288 Standard for Objective Camera Characterization, defined by the European Machine Vision Association (EMVA).

EMVA 1288 lays out the current standards relevant to the measurement and display of quality data in cameras. PCO customers automatically receive basic EMVA 1288 info about their camera and can request the full EMVA 1288 data. These data files are stored in easily accessible HDF formats. Thus, you know for a fact that you’re getting a quality product before you even open the packaging — rather than risking an off-the-shelf device that may be a lemon of an instrument.

Finally, PCO has hands-on engineers available to address technical issues, as well as experience to inform their guidance. The company’s commitment to quality ensures that even PCO sales engineers are prohibited from heading out into the field until they can demonstrate a deep knowledge of the cameras’ technology.

Nonetheless, whatever camera you choose, seek a product that is well-documented: the quality of each camera, the quality of measurements, the quality of data, the quality of the camera’s manufacture — if a camera has to run all day, you cannot live with a product that inexplicably allows humidity inside because it’s not tight enough, and it ceases to function properly.

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Conclusions

Camera-based high-content/high-throughput imaging, bolstered by advances in technology, is a viable solution for a number of applications and an ideal solution for many. Misconceptions about the cost and utility of this technology may be preventing you from investing in it to enhance your overall operation, be it quality control or research. However, PCO can help you find the information you need to build or improve your system, lifting both your company's position and reputation in the market — all for less cost than you may realize. For more information on high-content/high-throughput cameras, visit www.pco.de and direct any inquiries to info@pco.de

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