

# Speed: Opera Phenix Plus high-content screening system technical performance.

As shown by an analysis of HCS-related publications, the number of high-content screens being run each year continues to increase, however a large number of screens are not as “high-content” as they could be (Singh et al. 2014). The majority of high-content screens described in published papers rely on only 1-2 image-based features rather than multi-parametric results for single cells. This is indicative of more target-based approaches being used in drug screening. In very recent times, there is evidence of a reversal towards more phenotypic based screens. Prior to this, reasons for the limited adoption of phenotypic screening approaches might have included insufficient hardware but also the lack of suitable software to analyze complex phenotypes (see also Roberts and Tesdorpf 2015). The Opera Phenix® Plus high-content screening system has been specifically developed to enable users to perform highly multi-parametric phenotypic screens effectively.

The Opera Phenix Plus combines state of the art hardware to allow simultaneous acquisition of up to four fluorescent channels, with Harmony® high-content imaging and analysis software for convenient experimental set-up and analysis. The following table summarizes the new features and main improvements of the Opera Phenix Plus system and explains how they benefit acquisition and analysis speed.

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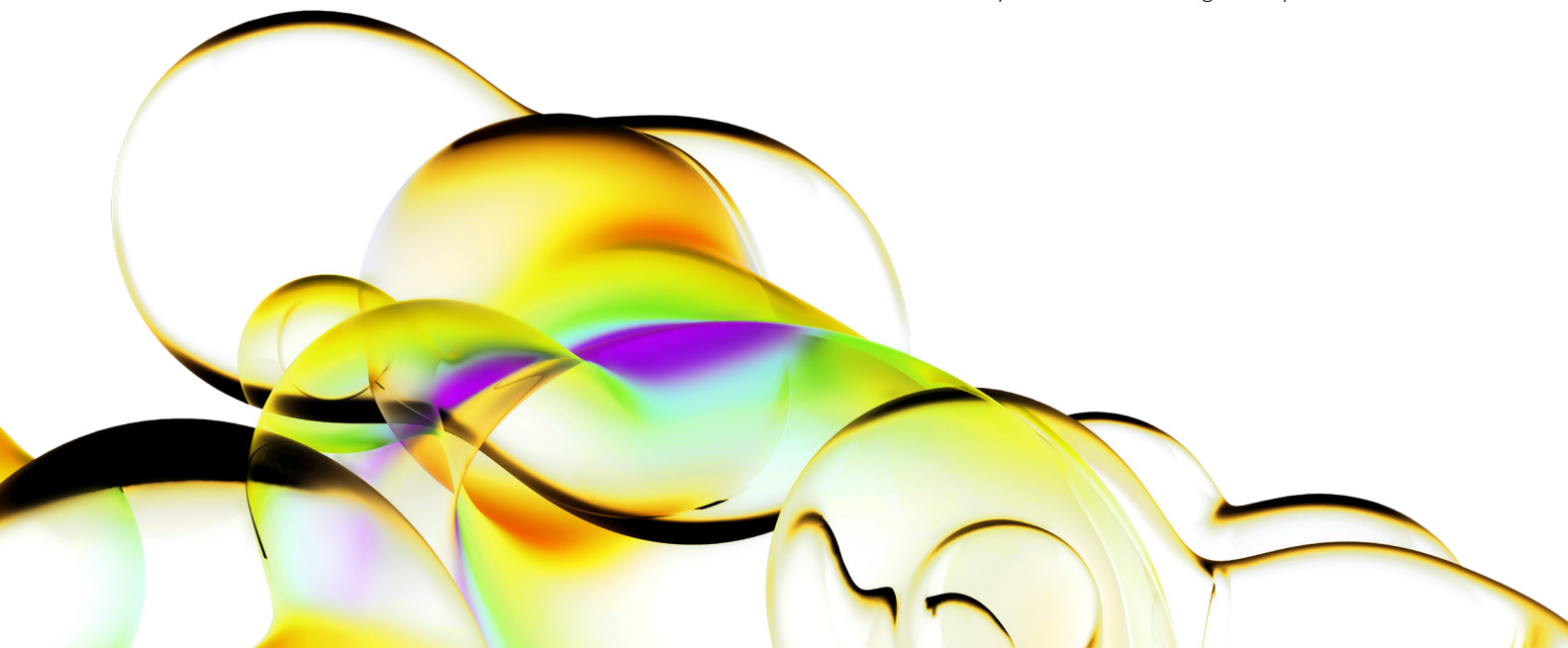


Table 1. Technical advancements of the Opera Phenix Plus high-content screening system compared to one of its predecessors, the Opera QEHS system

Feature	Benefits
Synchrony Optics™ for simultaneous confocal acquisition of up to 4 channels with minimal spectral crosstalk	<ul style="list-style-type: none"> <li>Reduces acquisition time in proportion to the number of simultaneously acquired channels</li> <li>Maintains assay quality in simultaneous acquisitions</li> </ul>
Up to 4 sensitive, large field of view sCMOS cameras with high dynamic range	<ul style="list-style-type: none"> <li>Fewer fields per well needed to image sufficient cell numbers for robust statistics</li> <li>Low noise for shorter exposure times as images with few counts can be analyzed reliably</li> </ul>
High power laser based excitation with efficient excitation path (e.g. microlens enhanced spinning disk)	<ul style="list-style-type: none"> <li>Reduces exposure time, by delivering more energy to the sample</li> </ul>
High NA water immersion lenses with proprietary automated water supply system	<ul style="list-style-type: none"> <li>Capture maximum amount of photons emitted from the sample to minimize exposure time</li> </ul>
Fast laser-based autofocus system with low moveable mass z-drive	<ul style="list-style-type: none"> <li>Reduces focusing time and focus failures</li> </ul>
Encoded linear drive - fast moving x-y table	<ul style="list-style-type: none"> <li>Reduces movement time, improved accuracy</li> </ul>
Speed-optimized filter- and dichroic-movements	<ul style="list-style-type: none"> <li>Reduces measurement time</li> </ul>
Easy to use building block based image analysis with speed optimized algorithms	<ul style="list-style-type: none"> <li>Analysis results available faster</li> </ul>

## Improving speed through Synchrony Optics

At the heart of the Opera Phenix Plus system is its Synchrony Optics (details can be found in the Technical Note: Crosstalk Suppression, shown in the reference list), an innovative optical concept which reduces spectral crosstalk during simultaneous confocal acquisition to an absolute minimum. Synchrony Optics enable simultaneous multi-color acquisition and increase speed immensely, without compromising image quality and assay statistics. This is particularly true for crosstalk-sensitive assays.

To show the increase in speed of the Opera Phenix Plus compared to a classical spinning disk confocal system with multiple cameras, we recorded the time required to measure a full 384-well plate with a 2-channel epigenetic assay.

The assay relies on detecting the signal of Alexa Fluor® 488 labeled antibodies bound to varying levels of acetylated histones inside Hoechst stained nuclei and is therefore highly sensitive to spectral crosstalk. Acquisition was performed either sequentially or simultaneously on the Opera Phenix Plus or one of the previous versions of the Opera system, the Opera QEHS. As can be seen in Fig. 1, Opera Phenix Plus delivers the same assay quality (Z') in simultaneous mode as the conventional spinning disc system in sequential mode and is up to 6 times faster. This is mainly due to three major technical improvements:

1. Synchrony Optics: Confocal images can be acquired simultaneously on the Opera Phenix Plus due to the effective suppression of the strong spectral crosstalk from Hoechst into the green channel.
2. sCMOS Cameras: The three times larger field of view of the Opera Phenix Plus requires only four fields of view to be imaged in order to analyze the same number of cells per well as from ten fields on the Opera QEHS.
3. Efficient excitation path and high NA water immersion lenses: Effectively deliver and capture more photons to and from the sample.

## Improving speed through water immersion lenses and binning

Revvity was the first company to introduce fully automated water immersion lenses on a high-content screening instrument, the Opera QEHS. The Opera Phenix Plus system features an improved version which combines customized high NA Zeiss objectives with an optimized water supply and removal system. In combination with dedicated multiwell imaging plates, such as Revvity PhenoPlate™ microplates, water immersion objectives allow

for exceptional image quality at high speed. In particular, the high numerical aperture of water immersion objectives increases the acquisition speed dramatically. Using a water immersion objective lens instead of an air lens reduces the exposure time about 5-fold, due to its better light collecting

efficiency. This leads to shorter exposure times, higher resolution, less photo bleaching and improved 3D imaging. A further increase in acquisition speed can be achieved by binning (BIN),

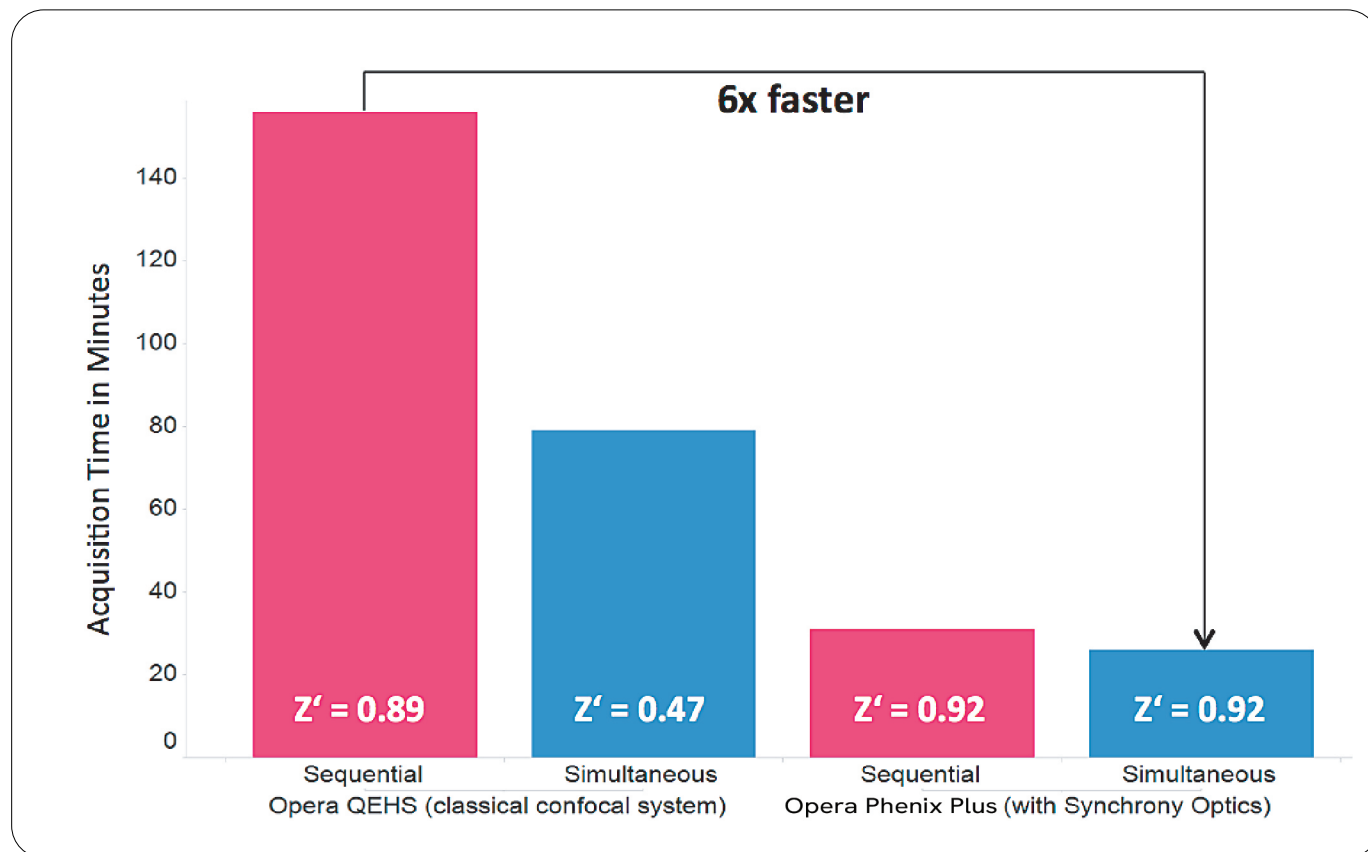


Figure 1. Speed improvement in a crosstalk-sensitive epigenetic assay. Compared to the Opera QEHS, the two channel measurement of a full 384-well plate on the Opera Phenix Plus is up to six times faster while still providing an optimal z' value. This is due to the crosstalk suppression during simultaneous confocal acquisition enabled by Synchrony Optics. Values for sequential measurements are in red, simultaneous in blue.

which is the combination of several pixels into one larger pixel containing the sum of the intensities of the un-binned pixels. In BIN2, for example, two by two pixels are combined into one single larger pixel and hence exposure times can be reduced by a factor of four. Additionally, binning also decreases image size, which speeds up image analysis. Owing to the combination of pixels, binning reduces image resolution; however, this is usually only relevant for very fine cellular structures and has limited impact on the cellular structures as shown in Fig. 2, for example. In our experience, 90% of all assays perform well with BIN2. Overall, the use of high NA water immersion objectives with BIN2 decreases the exposure time almost 20-fold (Fig. 2). Whether an assay should be acquired at full resolution or BIN2 should be established during assay development by comparing Z' values under both conditions.

### Opera Phenix Plus speed performance under standardized conditions

The Opera Phenix Plus system is not only designed for speed, image resolution and sensitivity, it is also designed to deliver accurately focused images over a whole plate. Especially in confocal high-content screens, unfocused images will falsify any intensity-, texture- or morphology-based read-out. Therefore, the Opera Phenix Plus is equipped with a fast laser-based autofocus system, allowing an autofocus at each position without compromising acquisition speed. To show this, we measured how fast different plate types (96-, 384- and 1536- well plates) can be imaged using a defined test set-up with the following parameters: 20x water immersion objective, 100 ms

exposure time per channel, one field per well and confocal mode (Synchrony Optics active).

The different plates were measured either with one, two or four camera systems to show the power of Synchrony Optics in combination with multiple cameras. Measured acquisition times for all three plate types are summarized in Fig. 3. The acquisition time for a single channel is the same on all systems. However, whenever the number of acquired channels exceeds the number of installed cameras, the acquisition time will increase due to sequential imaging.

Hence, the acquisition time for a given plate type is the same on a four camera system, regardless of the number of acquired channels. The most pronounced time savings can be seen when comparing simultaneous 4-channel acquisition on an Opera Phenix Plus with four cameras with sequential acquisition of 4 channels on a one camera system (Fig. 3): here the total acquisition time can be reduced by a factor of two for 384-well and 1536-well plates. A whole 96-well plate can be imaged in less than two minutes, up to 1.5 times faster than on a single camera system.

	40xA	40xW
BIN1	1900 msec	410 msec
BIN2	480 msec	100 msec

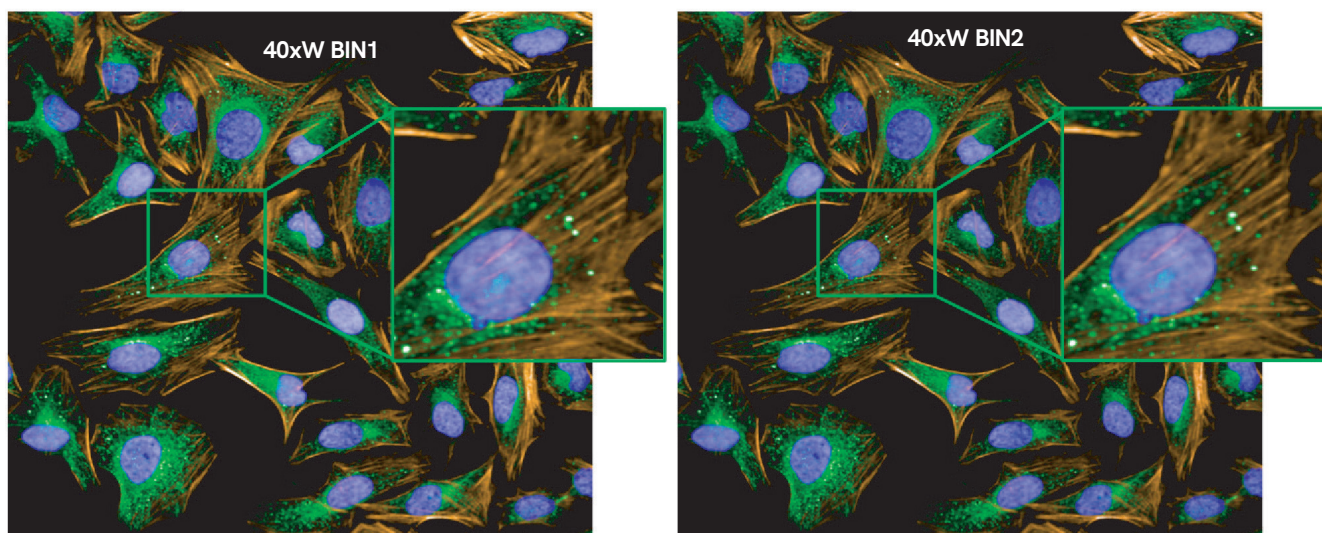


Figure 2. **Comparison of triple stained HeLa cells recorded either with or without binning using a 40x NA 1.1 water immersion objective.** Nuclei of HeLa cells were stained with Hoechst in blue, tubulin with anti- $\alpha$ -tubulin-Alexa Fluor<sup>®</sup> 488 in green and actin fibers with TRITC-phalloidin in orange. While for these object structures the difference in resolution resulting from binned pixels is hardly visible, the exposure time is reduced by a factor of four. The use of water immersion objectives decreases the exposure time by an additional factor of five resulting in total in almost 20-fold shorter exposure times. In our experience, 90% of all imaging tasks perform equally well with BIN2. 40xA = 40x air objective, 40xW = 40x water immersion objective..

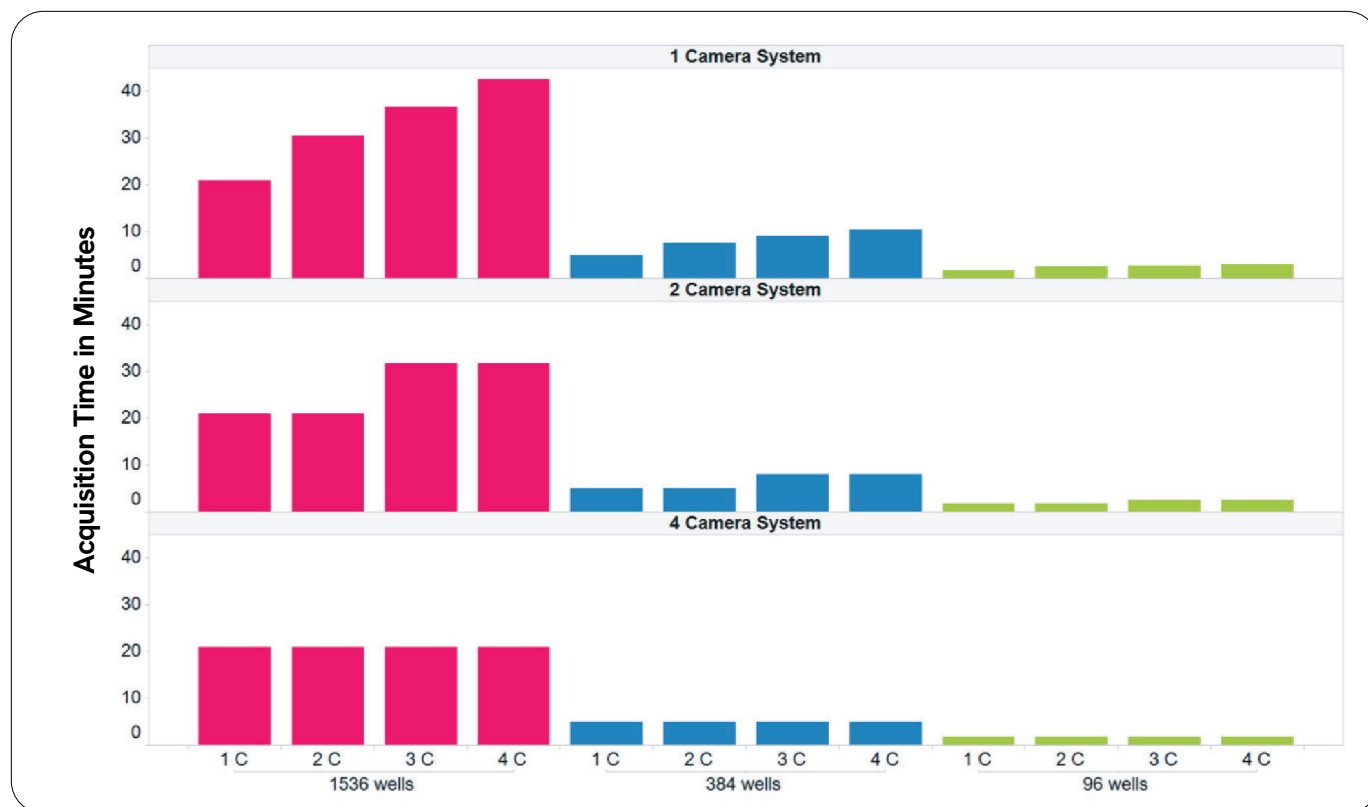


Figure 3. **Summary of acquisition times for different plate types on 1-, 2- or 4-camera systems.** The time in min to record a complete plate on a 1-, 2- and 4-camera system with different numbers of channels (1C = one channel, 2C = two channels, 3C = three channels, 4C = four channels) under standardized conditions (100 ms exposure time per channel, 1 center field per well, 20x water immersion lens, BIN2, confocal acquisition mode) is shown. On a 4-camera system the acquisition time for one plate type is the same regardless of the number of acquired channels, on a 2-camera system the time increases once if more than two channels are acquired and on the 1-camera system the time increases with every additional channel. The most pronounced time saving effect can be seen when comparing simultaneous 4-channel acquisition on a 4-camera Opera Phenix Plus with sequential acquisition of 4 channels on a 1-camera system.

## Comparative study of acquisition and analysis speed

Improving acquisition time is only one aspect of improving overall time-to-results: other aspects include improving software to enable extraction of complex phenotypic fingerprints whilst being easy-to-use, and, additionally, the interpretation of phenotypic data. To show the speed improvements of the analysis tools, i.e. the Harmony software, that are an integral part of the Opera Phenix Plus system, an in-depth study compared the former Opera QEHS system's performance with the performance of the Opera Phenix Plus. For this study, a micronucleus assay was measured and analyzed. This assay combines a straightforward 2-color acquisition (Hoechst stained nuclei and CellMask™ Deep Red stained cytoplasm) acquired with a 20x water immersion objective in confocal simultaneous acquisition mode, with a complex micronuclei image analysis. As a result of the three times larger field of view, the Opera Phenix Plus required only eight fields per well

in contrast to 24 fields acquired on the Opera QEHS. The analysis of the Opera QEHS data used an Acapella®\* script while the Opera Phenix Plus data was analyzed using a building block based approach in the Harmony software. The analysis was either performed parallel to acquisition or post acquisition. To keep the hardware and analysis protocol constant, both data sets were also analyzed using the Columbus™ image data storage and analysis system. The results are summarized in Fig. 4. The Opera Phenix Plus provides a significant time saving, for both acquisition and analysis. In addition to saving 66% time during acquisition the newly improved analysis tools give another 85% saving when the analysis is done post acquisition. This leads to a combined time saving of 77%. If the analysis is done in parallel to acquisition (online analysis) there is a 79% time-saving. Part of this difference can be attributed to improved computing hardware provided with the Opera Phenix Plus. To exclude this and the differences between Acapella and Harmony software, the same analysis was performed in the Columbus system. Under the same analysis

conditions, the Opera Phenix Plus data set was analyzed in a third of the time. This can be explained by the larger field of view of the sCMOS camera, leading to a data set that only

contains a third of the number of images. This requires less overhead for loading and decompressing of images during the analysis.

\* Acapella is the high-content imaging and analysis software for the Opera QEHS high-content screening system, which is no longer commercially available.

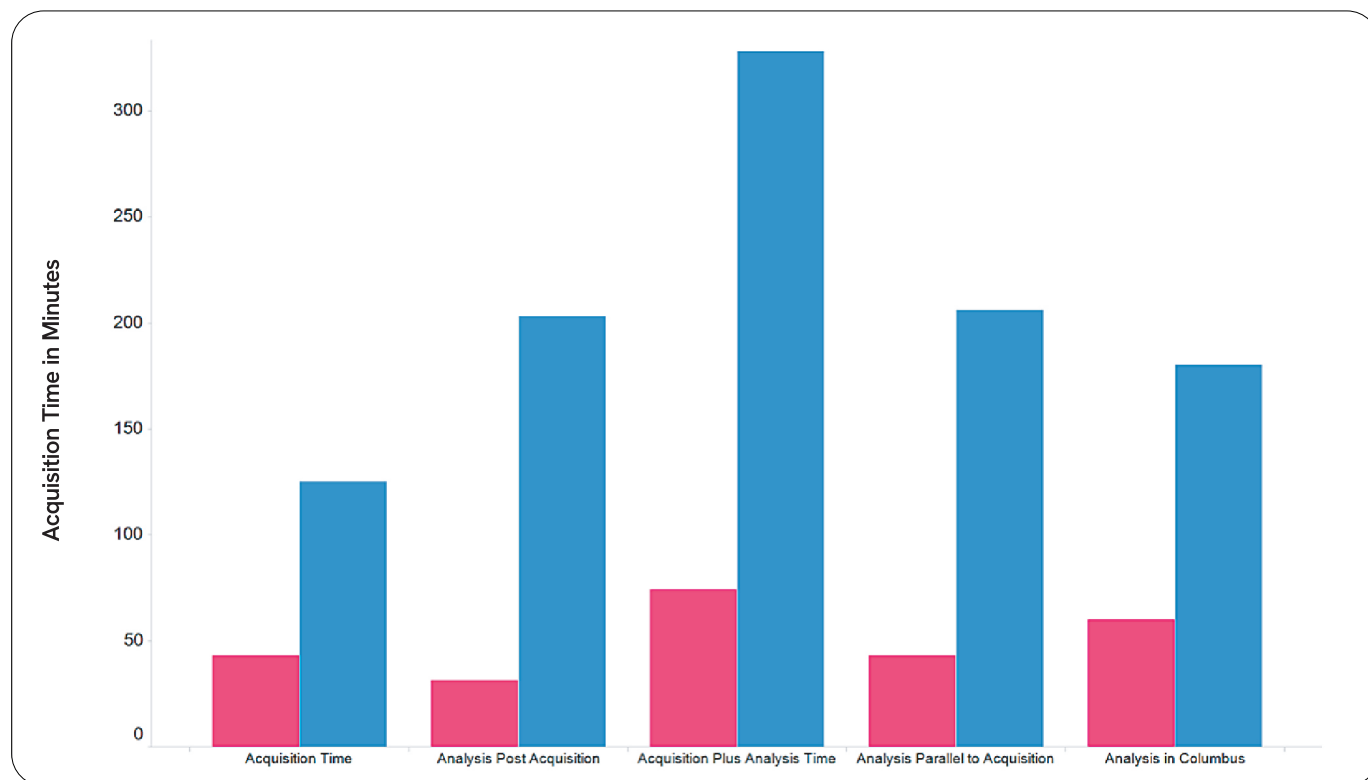


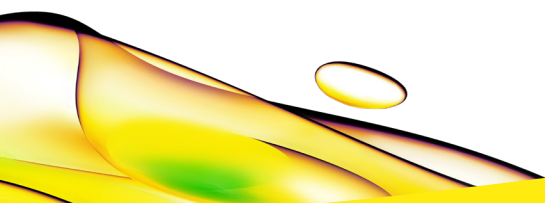
Figure 4. **Opera Phenix Plus delivers results faster due to improved acquisition and analysis speed.** A micronucleus assay was performed on the Opera Phenix Plus and the Opera QEHS systems to compare acquisition and analysis times of a complex phenotypic assay. To allow comparable conditions, both systems were equipped with four cameras and acquisition was done in confocal mode using water immersion objectives. Acquisition and analysis were either done sequentially or in parallel (online analysis). To exclude analysis software and hardware differences the data sets were also imported onto the Columbus server and analyzed using the same analysis protocol. Opera Phenix Plus values are shown in red and Opera QEHS values in blue.

## Conclusions

The Opera Phenix Plus high-content screening system is specifically designed to tackle even the most demanding assays, whether the demanding part of the campaign results from the image acquisition side or the analysis side. The system can acquire four channel image sets from 100,000 wells per day and even larger number of images when running z-stacks, which easily adds up to several terabytes of data depending on whether binning is used or not. Together with the powerful analysis tools of Harmony, provided with the instrument, this data can be analyzed conveniently in a timely manner. The Opera Phenix Plus is also fully compatible with the Columbus image data storage and analysis system. Combined with the Opera Phenix Plus's improved 3D imaging capabilities (as described in separate Technical Notes, as shown in the reference list) the system is also the ideal instrument to analyze complex cellular models including 3D microtissues of different origin in a high-content/high throughput assay.

## References

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