

## FAQ's



**Q. What additional steps (other than FSE/CPS visit) are necessary to set up the Cytation/XFe/Controller system?**

- a. When BioTek's Gen5 is set up, the image library must be located on either an external hard drive or a network location. XF Imaging and Cell Counting software (ICC) and Wave will use a database that is automatically generated upon installation to link images to cell plates – the database location should not require attention, but can be changed using the help menus in both applications.

**Q. Can I use ICC and Wave at the same time? How about Gen5 and Wave?**

- a. The ICC software utilizes Gen5 to run image collection protocols. Gen5 will be running while ICC is open, but without an open window and as a background process. Wave can be used at the same time. Gen5 cannot be opened in addition to ICC software. Additionally, you may need to navigate to the task manager and close the background instance of Gen5 after closing ICC, before you can open Gen5 separately.

**Q. How can I ensure best image quality?**

- a. It is highly recommended that users wipe the bottom of their cell plates with isopropyl/ethanol before imaging. Debris will affect autofocus and image quality.

**Q. Can I adjust the images that have been collected by ICC?**

- a. Zoom, brightness and contrast can be adjusted during image review both in ICC and in Wave. These adjustments will not change the raw pixel information.

**Q. Can I adjust the image collection protocols for ICC?**

- a. No, not at this time – the image protocols have been optimized for XF plates and assays. Custom protocols and cell counts can be created and run in Gen5 software. If a custom cell counting protocol is created in Gen5, the results can be copied and pasted into Wave's normalization mode. The imagery will only be imported in Wave when ICC is used to acquire them.

**Q. Well View is not available for my Wave results, but I collected images. How can I import them?**

- a. The images must be collected using the ICC software (not Gen5). Additionally, double check that Wave is using the same image database as ICC. If it needs to be changed, you may need to restart the software. If problems persist, contact Agilent Technical Support.

**Q. How are cell counts generated from only the center field of view?**

- a. Using only the center image field of view, the cell count per well is calculated based on the area of the well upon importing into the Wave Normalization tab.

**Q. Can I use a different fluorescent dye for cell counting?**

a. In ICC, only dyes compatible with the DAPI filter set may be used at this time.

**Q. How can I confirm cell count accuracy?**

a. Upon completion of the fluorescence image acquisition, you will be able to review the images. At this time, there is a checkbox for viewing the cell counting “mask.” The mask highlights all counted objects within the image. The count is only for the field of view shown. Upon importing cell counts into Wave, an area factor is applied to generate a cell count for the entire well.

**Q. My cells are not consistently stained with Hoechst dye. What can I do?**

a. We recommend optimizing the concentration of Hoechst dye – just like you would do for cell density or FCCP concentration. We have used a 20 µM dilution successfully in all validation testing.

**Q. Which versions of Cytation are compatible?**

a. At this time, only the Cytation 1 and Cytation 5 are compatible with the XF Imaging and Cell Counting software.

**Q. How can I export the full resolution images?**

a. Full resolution images can be exported from the XF Imaging and Cell Counting software interface. Navigate to the plate menu and either export an entire plate of images, or open to the review screen and select specific wells to export. Additionally, “masked” images may only be exported when they are being reviewed – upon completion of fluorescence image acquisition.

**Q. Where do I find the User Manual?**

a. The XF Imaging and Cell Counting Software User Manual can be found on the Agilent website, here: [https://www.agilent.com/cs/library/usermanuals/public/S7810AA\\_Seahorse\\_XF\\_Imaging\\_and\\_Cell\\_Counting\\_User\\_Manual.pdf](https://www.agilent.com/cs/library/usermanuals/public/S7810AA_Seahorse_XF_Imaging_and_Cell_Counting_User_Manual.pdf)

**Q. Do I need to set up my cell plate in a particular manner?**

a. ICC software requires two specific wells to be seeded with cells in order to accurately perform initialization; this includes preliminary auto-focus, auto-exposure, and white balance steps. Ensure wells D5 and E8 are seeded in XF96 plates and wells B3 and C4 in XF24 plates.

**Q. How many image sets can I import into Wave?**

a. When importing into Wave, you can import a maximum of four image sets.

**Q. Who do I contact when**

a. I have a question about XF Imaging and Cell Counting software, Wave, importing cell counts and images, normalizing XF data, or my XF Analyzer?

i. Contact Agilent Technical Support (seahorse.support@agilent.com)

b. I have a question about the Cytation 1, Cytation 5, Gen5 Image+ software, cell counting, or imaging?

i. Contact BioTek Technical Support (tac@biotek.com)

[www.agilent.com/chem/normalization](http://www.agilent.com/chem/normalization)

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**Not for use in diagnostic procedures.**

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