

Technical Note: Imaging ChromaLive on the CQ1 (Yokogawa)

1. Introduction

This technical note shows the different steps needed to create custom settings specifically for imaging ChromaLive on the CQ1 (Yokogawa) high content imager. ChromaLive is a novel, non-toxic and multichromatic live-cell dye. To fully benefit from this technology, it is important to image cells stained with this dye at multiple wavelengths. In this technical note, we provide guidance and step-by-step instructions on how to setup these settings for optimal imaging of live samples with ChromaLive.

2. How filters allow the imaging of multiple ChromaLive colors

ChromaLive is a multichromatic dye, revealing distinct cellular staining patterns when excited around 488nm (ChromaLive488, red curve) or around 561nm (ChromaLive561nm, green curve). The graphs below show how the previously selected filters allow us to selectively image each staining pattern.

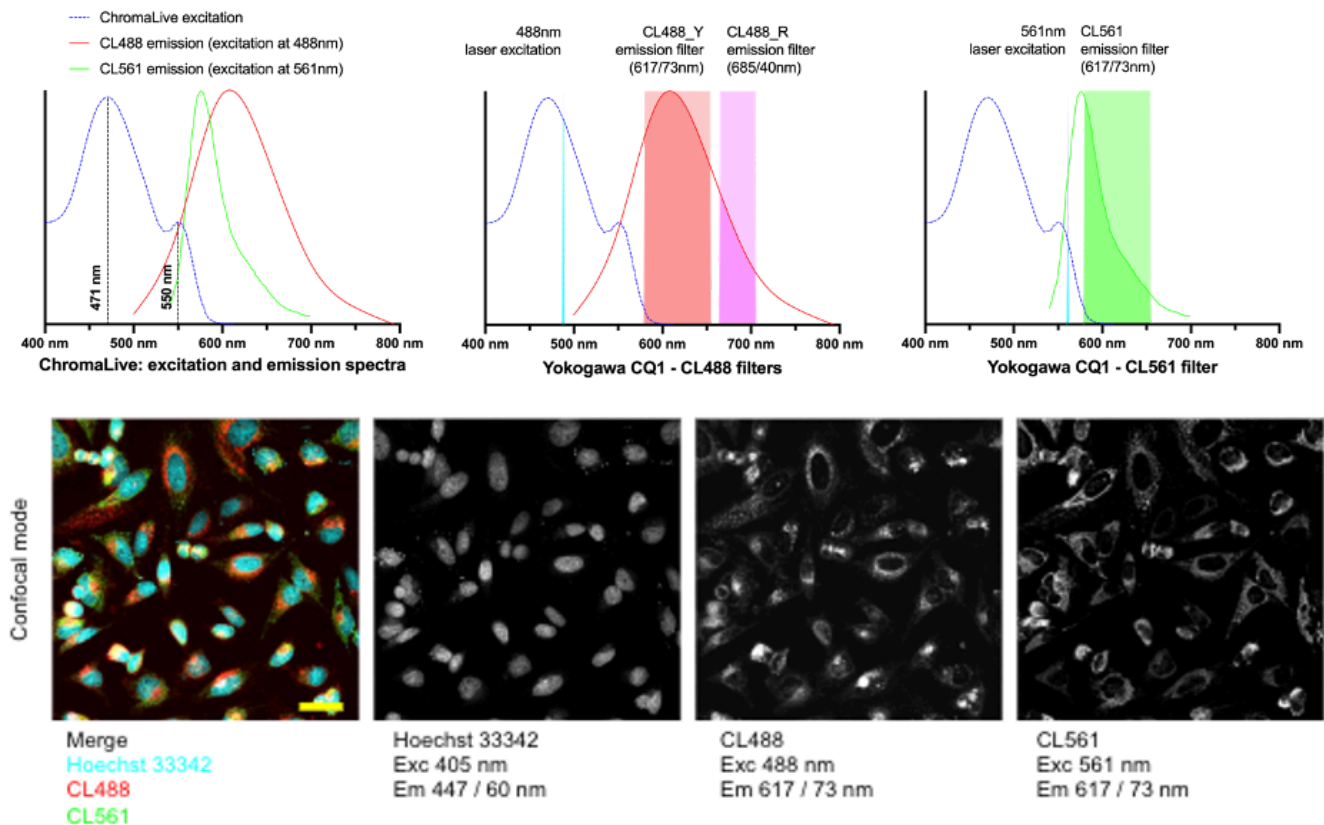


Fig. 1: Top graphs: Fluorescence excitation and emission spectra for ChromaLive, and representation of compatible optical filters. **Bottom:** HeLa cells stained with ChromaLive and Hoechst 33342, imaged on the CQ1 (20x objective, 2x crop). Scale bar = 50µm.

3. Creating and naming new filter settings

- In the "Imaging" tab, double-click on the "Channels" window to open the "Channel Setting" window
- By click the "Add" button, a first channel can be created with the "Excitation" set to 488nm (drop-down menu), and the "Emission" set to "BP617/73" (drop-down menu)
- Repeat these steps to create a second channel, with "Excitation" set to 488nm, and "Emission" set to "BP685/40"
- A third channel can be also created, with "Excitation" at 561nm, and "Emission" set to "BP617/73"
- Additional channels can also be added to image Hoechst staining of nuclei, for example.
- The information for these different channels is summarized in a table at the end of this technical note.

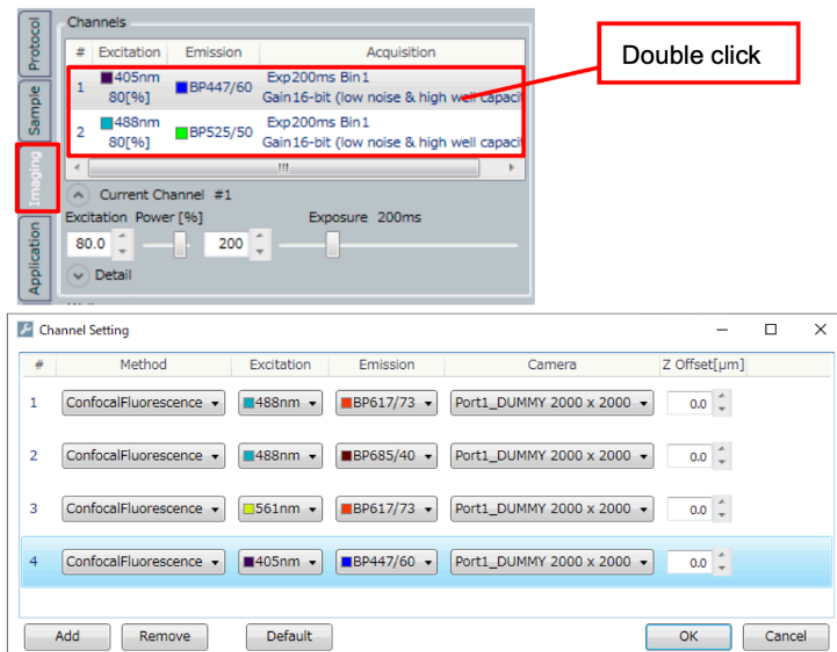


Fig. 2: "Imaging" and "Channel Setting" windows in the CQ1 software (Yokogawa).

4. Table summarizing filters settings to image ChromaLive

	<i>ChromaLive488_Yellow</i>	<i>ChromaLive488_Red</i>	<i>ChromaLive561</i>
<i>Laser excitation: 488nm Emission filter: 617/73 nm</i>	+		
<i>Laser excitation: 488nm Emission filter: 685/40nm</i>		+	
<i>Laser excitation: 561nm Emission filter: 617/73nm</i>			+

Table 1: Recommended filter settings for the CQ1 (Yokogawa).