Technical Note: Imaging ChromaLive on the Opera Phenix and Opera Phenix Plus

1. Introduction

This technical note shows the different steps needed to create custom settings specifically for imaging ChromaLive on the Opera Phenix (PerkinElmer / Revvity) high content imagers. These include the Phenix and Phenix Plus instruments. 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 set up 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.

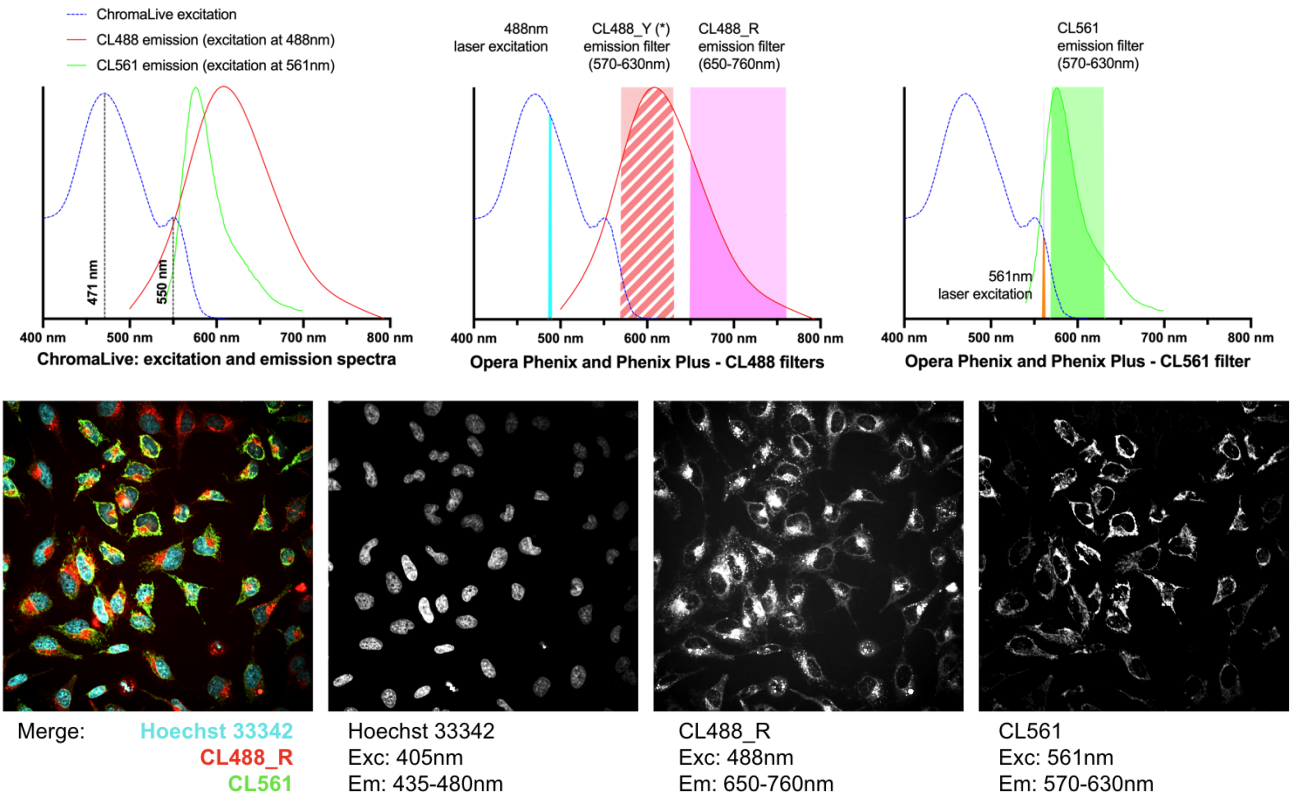
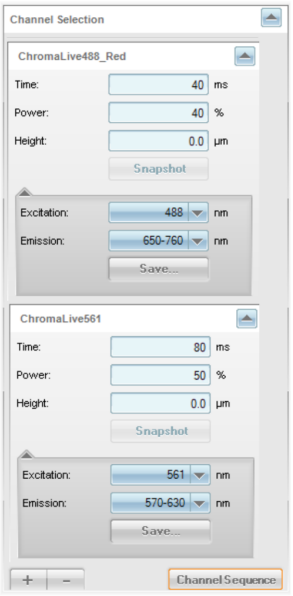


Fig. 2: Top graphs: *Fluorescence excitation and emission spectra for ChromaLive, and representation of compatible optical filters. (*) The ChromaLive488_Yellow channel (emission filter: 570-630nm) is not available when imaging in confocal mode. Bottom: HeLa cells stained with ChromaLive and Hoechst 33342, imaged in confocal mode on the Opera Phenix (40x objective).*

3. Creating and naming new filter settings

a. In the "Channel Selection" panel in the Harmony software, click the "+" button to add channels
b. A first channel can be created with the "Excitation" set to 488nm (drop-down menu), and the "Emission" set at 650-760nm (drop-down menu)
c. This channel can be saved and named "ChromaLive488_Red"
d. Repeat these steps to create a second channel, with "Excitation" set to 561nm, and "Emission" set to 570-630nm. Save this channel to "ChromaLive561"
e. A third channel can be also created, with "Excitation" at 488nm, and "Emission" at 570-630nm, named "ChromaLive488_Yellow". Of note, this channel is not compatible with the confocal imaging mode
f. Additional channels can also be added to image Hoechst staining of nuclei, for example
g. As summarized in the table at the end of this technical note, other filters may also be compatible with imaging ChromaLive -stained samples, we simply present here the standard
h. The "Channel Sequence" button allows users to decouple certain channels. In the case of ChromaLive, it is important to decouple ChromaLive488 and ChromaLive561 channels, to avoid bleed-through between these channels



4. Table summarizing filters settings to image ChromaLive

	ChromaLive488_Yellow	*ChromaLive488_Red*	*ChromaLive561*
Laser excitation: 488nm Emission filter: 570-630nm	***++ (Not available in confocal mode)***		
Laser excitation: 488nm Emission filter: 571-596nm	***+ (Not available in confocal mode)***		
Laser excitation: 488nm Emission filter: 605-630nm	***+ (Not available in confocal mode)***		
Laser excitation: 488nm Emission filter: 650-680nm		***+***	
Laser excitation: 488nm Emission filter: 650-760nm		***++***	
Laser excitation: 488nm Emission filter: 690-720nm		***+***	
Laser excitation: 561nm Emission filter: 570-630nm			***++***
Laser excitation: 561nm Emission filter: 571-596nm			***+***
Laser excitation: 561nm Emission filter: 605-630nm			***+***

Table 1: *Recommended filter settings for the Opera Phenix and Opera Phenix Plus (PerkinElmer / Revvity)*