Conditions for the Alexa Fluor 555 channel

Experimental conditions of imaging acquisition

Sensitivity (HV)	370
Gain	0
Offset	1
Experimental conditions of laser intensity (%)	Intensity in the upper layer (depth = 15 μm) = 8.0 - 0.0066 × (total thickness/2 - 15 μm)
	Intensity in the middle layer (total thickness /2) = 8.0
	Intensity in the lower layer (latest depth = 15 μm) = 8.0 + 0.0166 × (total thickness/2 + 15 μm)

Conditions for the DiD channel

Experimental conditions of imaging acquisition

Sensitivity (HV)	370
Gain	0
Offset	1
Experimental conditions of laser intensity (%)	Intensity in the upper layer (depth = 15 μm) = 0.4 - 0.005 × (total thickness/2 - 15 μm)
	Intensity in the middle layer (total thickness /2) = 0.4
	Intensity in the lower layer (latest depth = 15 μm) = 0.4 + 0.0266 × (total thickness/2 + 15 μm)

Conditions for the SYTO16 channel

Experimental conditions of imaging acquisition

Sensitivity (HV)	370
Gain	0
Offset	1
Experimental conditions of laser intensity (%)	Intensity in the upper layer (depth = 15 μm) = 0.8 - 0.0083 × (total thickness - 15 μm)
	Intensity in the middle layer (total thickness /2) = 0.8
	Intensity in the lower layer (latest depth =15 μm) = 0.8 + 0.0333 × (total thickness + 15 μm)

*Laser intensity changed according to depth: the modification followed a fixed slope between the upper and the middle layers of the middle and lower layer; adjustments were performed using the Bright Z mode of the FV-3000 software.

*Unit of measure for thickness = μm