

Simulator of a Confocal in FIJI (SCiF), a teaching tool for laser scanning confocal microscopy

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Abstract

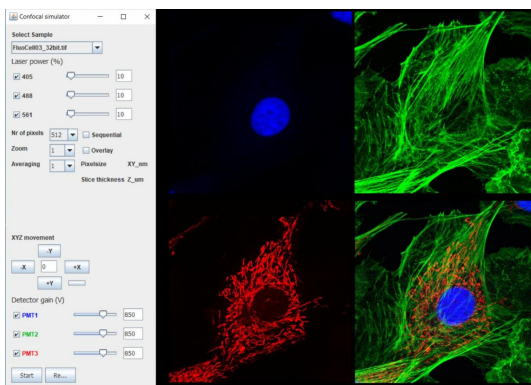
Laser scanning confocal fluorescence microscopy is a cornerstone imaging technique in life sciences. Acquiring confocal images can be relative straightforward, once the instrument parameters are setup. However, understanding the effect of changing these parameters is more demanding.

Confocals are relatively widespread, these microscopes are in high demand and therefore often busy. Training new user can be time consuming and poses a burden on the available operating hours. This burden could be significantly reduced, if part of the hands-on training could be done on-line and away from the confocal.

Simulator of a Confocal in FIJI (SCiF) is a digital learning tool for new users to get familiar with the confocal principles, before actually using the confocal microscope itself.

SCiF simulates a laser scanning confocal equipped with three lasers and three PMT detectors. It simulates many important confocal scanning principles, including signal-to-noise, bleaching, crosstalk and pinhole effects. SCiF uses the measured AOTF responds and voltage-dependent PMT gain and noise characteristics of a real confocal.

SCiF contains a set of samples, consisting of digitally generated images or high quality multicolor 3D confocal datasets. Images can be collected with or without sequential imaging to demonstrate the effect of crosstalk. In addition, fluorescence bleaching due to laser power, zoom factor and averaging is taken into account.



SCiF simulates a laser scanning confocal equipped with 3 lasers, 3 PMT detectors and emission filters for DAPI, AlexaFluor488 and AlexaFluor555