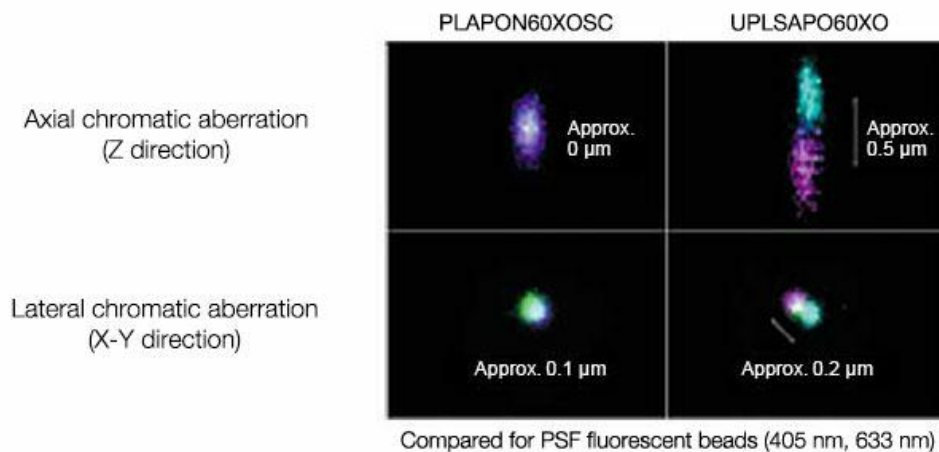# Use of Low Chromatic Aberration Objective PLAPON60XOSC for Quadruple Immunofluorescence of Brain Tissue

Use of Low Chromatic Aberration Objective PLAPON60XOSC for Quadruple Immunofluorescence of Brain Tissue

Introduction

However, accurate visualization of multiple intercellular co-localizations was difficult in a single specimen because color shifts caused by chromatic aberration of the microscopic objective lenses made it difficult to use fluorescent dyes for accurate visualization of the intercellular co-localization in UV and near infrared regions. Olympus' low chromatic aberration objective PLAPON60XOSC lens provides accurate visualization of fluorescence-labelled molecules with few color shifts, even in UV and near infrared regions. The following introduces cases where four types of fluorescent antibody were used to visualize the co-localization of fluorescence-labelled molecules accurately in a single specimen.

Performance Comparison of PLAPON60XOSC and UPLSAPO60XO

Compared for PSF fluorescent beads (405 nm, 633 nm)

An application of the Low Chromatic Aberration Objective - Quadruple Immunofluorescence of Brain Tissue
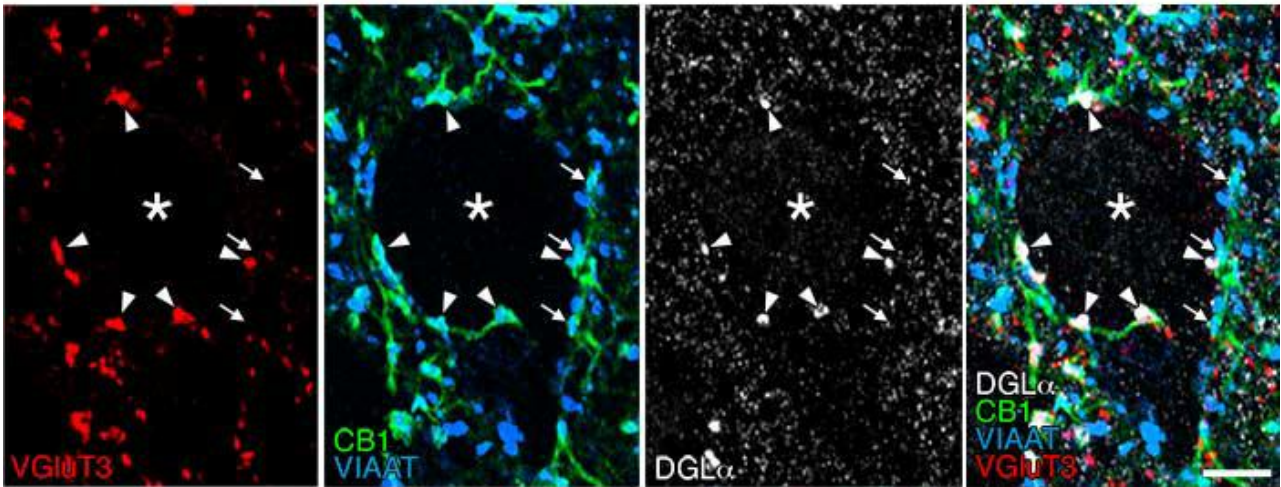
Quadruple immunofluorescence of brain tissue: Picture (1)

VIAAT staining (Alexa Fluor405, blue)

CB1 staining (Alexa Fluor488, green)

VGluT3 staining (Cy3, red)

DGLα staining (Alexa Fluor647, white)

Cannabinoid synthetase DGLα (white) and cannabinoid receptors CB1 (green) are clustered around the pyramidal cell somata (asterisk) in mouse basal amygdala, forming invaginating synapses (arrowhead) with a unique invagination structure. These invaginating synapses express vesicular inhibitory amino acid transporter (VIAAT) (blue) and vesicular glutamate transporter-3 (VGluT3) (red), which suggests that they have the neurochemical properties of both inhibitory and excitatory transmitters—GABA and glutamine acid, respectively. In contrast, the CB1-positive, VIAAT-positive common type of inhibitory synapses did not express VGluT3 if DGLα clusters were not formed around them, showing that they have the neurochemical properties of GABA only. Scale bar: 5 μm.
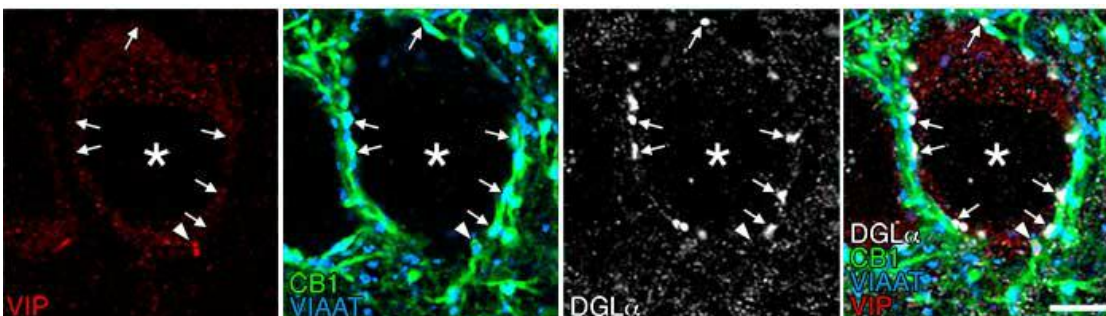
Quadruple immunofluorescence of brain tissue: Picture (2)

VIAAT staining (Alexa Fluor405, blue)

CB1 staining (Alexa Fluor488, green)

VIP staining (Cy3, red)

DGLα staining (Alexa Fluor647, white)

There are two subtypes of GABAergic basket cells that express cannabinoid receptors CB1 and govern the pyramidal cell somata one subtype expresses cholecystokinin (CCK), and the other type expresses vasoactive intestinal peptide (VIP). This quadruple immunofluorescence staining shows the lack of DGLα (white) around the -positive nerve terminals (red) that strongly express CB1 (green) and VIAAT (blue), indicating that the VIP-positive nerve terminals are not involved in the formation of invaginating synapses. Scale bar: 5 μm.

Imaging system:

Microscope: Biological Confocal Laser Scanning Microscope FV1200

Objective: PLAPON60XOSC (60X, NA: 1.4)

Diode laser: 405 nm, 473 nm, 559 nm, 647 nm

Image data courtesy of:

Masahiko Watanabe, M.D., Ph.D.

Departments of Anatomy, Hokkaido University Graduate School of Medicine

Reference:

J Neurosci. 2015 Mar 11;35(10):4215-28. doi: 10.1523/JNEUROSCI.4681-14.2015.

Conclusion

Quadruple immunofluorescence for multiple functional molecules and cell markers as shown in Picture (1) and (2) can provide detailed information on cell expression and subcellular localization, which includes the codependent or independent relationship between related functional cells and intercellular spatial distances. Significantly, the detection sensitivity and the resolution of microscopes as well as the optical performance of objective lenses that can minimize chromatic aberrations are important factors in achieving the aims of immunofluorescence.

PLAPON60×OSC   UPLSAPO60×O

Related Products

Confocal Laser Scanning Microscope

FV4000

- Game-changing dynamic range for imaging from the macro scale to subcellular structures
- Multiplex up to six channels simultaneously with TruSpectral technology
- Redesigned high-speed, high-resolution scanners for fixed and live cell imaging
- Improved depth and photosensitivity with pioneering NIR capabilities and renowned optics

- Peace of mind with the reliable, repeatable SilVIR detector
- Industry leading[*] ten laser lines with a broader spectral range from 405 nm to 785 nm

*As of October 2023.

Learn More ▸ https://www.olympus-lifescience.com/laser-scanning/fv4000/

Super-Corrected Apochromat Objectives

PLAPON-SC

This super-corrected apochromat objective corrects a broad range of color aberrations to provide images that capture fluorescence in the proper location. Delivering a high degree of correction for lateral and axial chromatic aberration in 2D and 3D images, it offers reliability and accuracy for colocalization analysis.

- Chromatic aberration is compensated to the utmost limit
- Every single objective is delivered with measured chromatic aberration data and less than 0.1 μm on-axis chromatic aberration between 405 nm and 650 nm
- Suitable for strict colocalization analysis or super resolution

Learn More ▸ https://www.olympus-lifescience.com/objectives/plapon-sc/