Immersion oil for high-resolution live-cell imaging at 37°C: optical and physical characteristics

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Although frequently overlooked normal immersion oils rated to be used at 23°C are not suited for imaging at 37°C, because the oil refractive index (RI) changes significantly as a function of temperature. Over the distance between the objective-oil and oil–cover slip interface, the RI mismatch may generate spherical aberrations. Spherical aberrations greatly deteriorate the axial point spread function (PSF), especially when high NA lenses are used [1, 2]. This ‘spread’ in PSF results in both loss of spatial resolution and peak intensity and holds for all forms of fluorescence microscopy. However, in the case of confocal microscopy in which for each point in the focal plane the intensity is the product of the illumination and detection PSF, the spreading of both causes the recorded intensity to decrease greatly [1]. Here we address one of the main causes of deteriorated image formation in live-cell imaging at temperatures higher than room temperature, i.e. the mismatch between designed and effective refractive index of the oil used in immersion microscopy. We describe the development and characterization of 37DF, an immersion oil meeting the goals of a correct RI at 37°C and good other optical and physical characteristics. We show that as compared to standard 23°C immersion oils, 37DF provides significant improvements in both resolution and recorded intensity at 37°C. For both aspects the improvement obtained amounts to a factor of two or even higher. The huge improvement in intensity is of great importance in live-cell imaging because it improves detectability of dim signals and also allows use of reduced excitation intensities, resulting in less phototoxicity. In conclusion, we consider the 37DF oil to be a valuable asset for high resolution live-cell imaging at 37°C, bringing clear benefits in resolution and image brightness.

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