# Laser-induced photobleaching of endogenous fluorescence

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Naturally occurring endogenous fluorescence of flavins and NAD(P)H, arising in response to excitation by visible or ultraviolet light, offers broad possibility of investigation of mitochondrial metabolic state directly in living cells and tissues[1]. However, prolonged exposure to light lead to phenomenon of photobleaching, the loss of (auto)fluorescence intensity. This phenomenon is inherent, occurs during the fluorescence acquisition and can thus have a negative impact on the recorded data, particularly in the context of measurement of metabolic modulations in pathophysiological conditions. In the presented contribution we discuss analysis of endogenous fluorescence and repercussions of photobleaching arising in living cells during spectrally- and/or time-resolved studies[2]. We compare photobleaching induced by different approaches on individual spectral components, resolved by linear unmixing of the recorded signals. Induced photodamage, despite being without effect on the cell morphology, often leads to significant modifications of the cell functioning, suggesting functional metabolic alterations of the recorded cells. These findings point to the necessity of taking the phenomenon of photobleaching into consideration when designing recording protocols and aim at inducing minimal photodamage during metabolic screening in all studies involving visible and ultraviolet light excitation and fluorescence acquisition in living cells[3].

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[1] A. Chorvatova, D. Chorvat D., Jr, "Tissue fluorophores and their spectroscopic characteristics" in Marcu L, French PMW, Elson DS V (Eds). Fluorescence Lifetime Spectroscopy and Imaging for Tissue Biomedical Diagnostic, (CRC Press Publ., 2014)

[2] Chorvatova A, Mateasik A, Chorvat D. Laser-induced photobleaching of NAD(P)H fluorescence components in cardiac cells resolved by linear unmixing of TCSPC signals. Proceeding of SPIE 790326-1-790326-9 (2011).

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