# Technical Note:
# Microscopic imaging of biological tissue ex vivo using the Linea HS Multifield line scan camera (Teledyne DALSA, Boston, MA, USA)

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This technical note addresses microscopic imaging of biological tissue ex vivo using the Linea HS Multifield line scan camera (Teledyne DALSA, Boston, MA, USA). A specific advantage of this camera is the possibiliy to simultaneously capture multiple image channels (i.e. different lasers and different fluorescent proteins and/or fluorescent dyes) in a single scan without need to use excitation and emission filters as well as related filter wheels. Here we present suitable combinations of lasers, fluorescent proteins and fluorescent dyes for (i) transgenic expression of two fluorescent proteins, (ii) transgenic expression of two fluorescent proteins and ex vivo immunofluorescence or fluorescent labeling, or (ii) ex vivo triple-immunofluorescence for this purpose.

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For certain microscopic imaging modalities in modern basic biomedical research the use of a line scan camera may be superior to the use of a camera with two-dimensional (2D) sensor, as line scan cameras can reduce scattering effects and enhance image contrast at depth (e.g. [1-3]). In this regard the Linea HS Multifield line scan camera (Teledyne DALSA, Boston, MA, USA) [4] is of particular interest as this camera enables to simultaneously capture multiple image channels (i.e. different lasers and different fluorescent proteins and/or fluorescent dyes) in a single scan without need to use excitation and emission filters as well as related filter wheels. This may offer higher speed of image acquisition when imaging multiple fluorescent channels, combined with improved precision in colocalization analysis. However, due to the specific responsitivity of the three channels MF3-R, MF3-G and MF3-B of the Linea HS Multifield line camera (shown in Fig. 1) not every combination of fluorescent proteins and/or fluorescent dyes is suitable for this purpose.

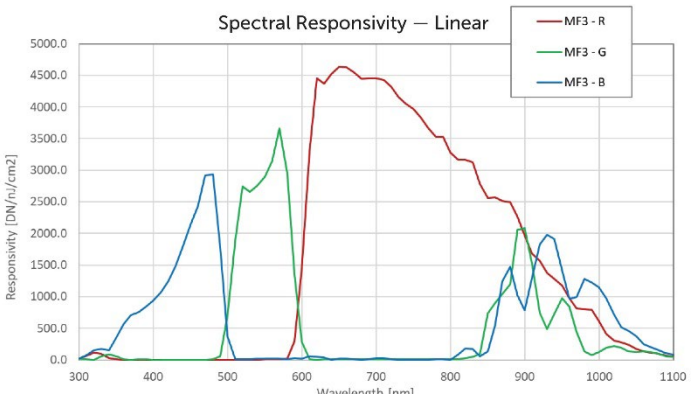


**Fig. 1** | Specific responsitivity of the three channels MF3-R, MF3-G and MF3-B of the Linea HS Multifield line camera (Teledyne DALSA, Boston, MA, USA).

Specifically, in Fig. 2 a combination of two fluorescent proteins (eGFP; tdTomato) is shown that was applied in studies in the literature (e.g. [5]) and is not suitable for simultaneous capturing using the Linea HS Multifield line scan camera; in Fig. 3 the same situation is shown for two fluorescent dyes (Alexa 568 and Alexa 647) that were applied in studies in the literature (e.g. [6]).

In contrast, Fig. 4 shows a suitable combination of two fluorescent proteins (eGFP and mCherry) and a fluorescent dye (Alexa 405) for simultaneous capturing using the Linea HS Multifield line scan camera, and Fig. 5 a suitable combination for three fluorescent dyes (Alexa 405, Alexa 488 and Alexa 568).

In all examples shown in Figs 2-5 activation of the fluorescent proteins and/or fluorescent dyes is demonstrated using an iChrome FLE multi-color laser combiner (TOPTICA Photonics Inc., Pittsford, NY, USA). However, this does not imply that simultaneous capturing of multiple image channels using the Linea HS Multifield line scan camera can only be performed using the iChrome FLE multi-color laser combiner. Rather, e.g. the Cobolt C-Flex C8 multi-color laser combiner (Hübner Photonics Inc., San Jose, CA) and the Celesta VBCTGRN Light Engine (Lumencor, Beaverton, OR, USA) provide almost the same combinations of laser wavelengths.

Fig. 1 and Panels C in Figs 2-5 were taken from the Linea HS 16k Multifield datasheet that is available at [4]; the excitation and emission spectra shown in Panels A and B in Figs 2-5 were created using FPbase [7,8] under the CC BY-SA 4.0 license [9]. The relative excitation values provided in Figs 2-5 were determined also using Fpbase [7,8].

## References


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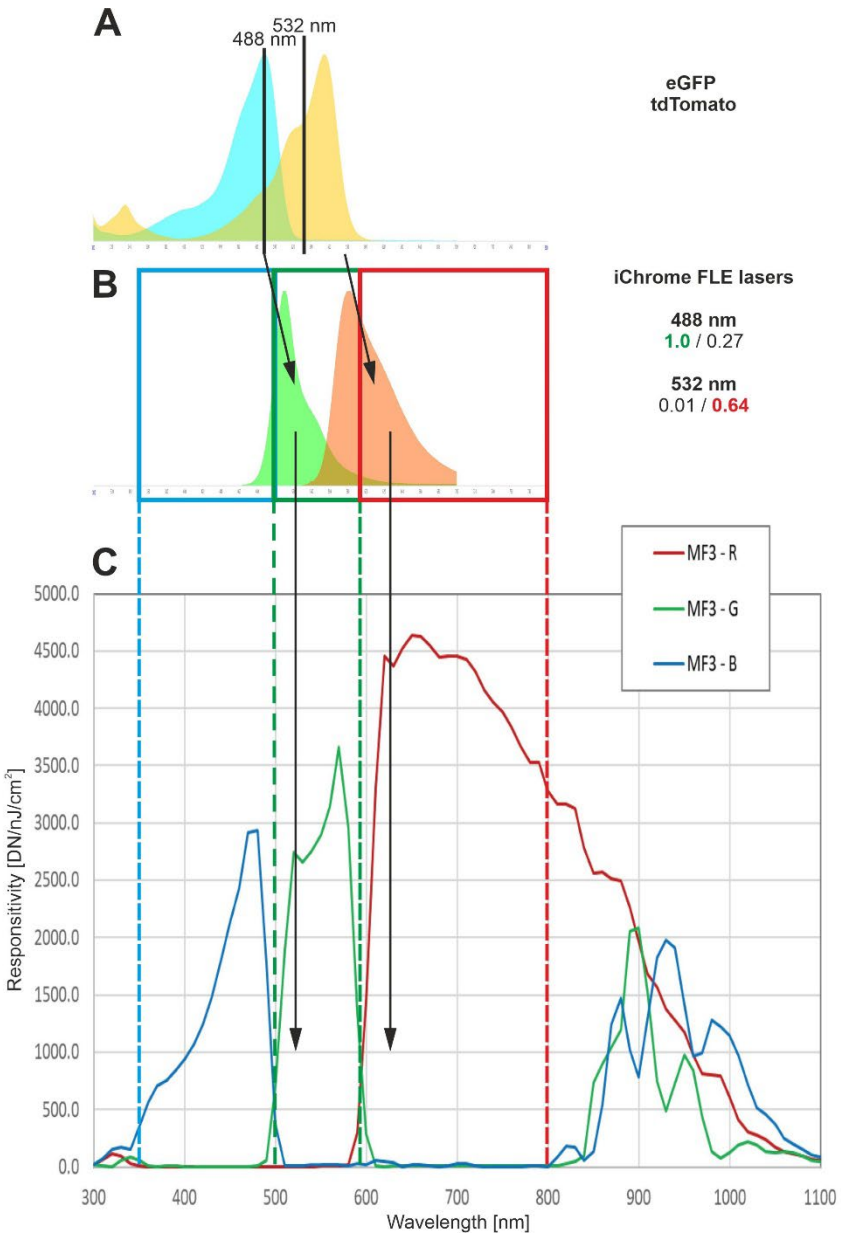


**Fig. 2** | Working principle of imaging biological tissue ex vivo in which the fluorescent proteins eGFP and tdTomato were transgenically expressed (c.f., e.g. [X02]) using an iChrome FLE multi-color laser combiner (TOPTICA Photonics Inc., Pittsford, NY, USA) and a Linea HS Multifield line camera (Teledyne DALSA, Boston, MA, USA). (**A**) Excitation spectra of the fluorophores eGFP (blue) and tdTomato (yellow), and the wavelengths of the corresponding lasers of the iChrome FLE multi-color laser combiner. (**B**) Emission spectra of the fluorophores eGFP (green) and tdTomato (orange), and the relative excitation of these fluorophores using the corresponding lasers of the iChrome FLE multi-color laser combiner. (**C**) Responsitivity of the three channels MF3-R, MF3-G and MF3-B of the Linea HS Multifield line camera that enable capturing multiple images simultaneously using various lighting conditions (here: different lasers and different fluorophores) in a single scan. In the example shown here **the two image channels cannot be simultaneously captured** without additional excitation and emission filters in the light path, as light from both image channels are captured in the MF3-G channel of the camera.

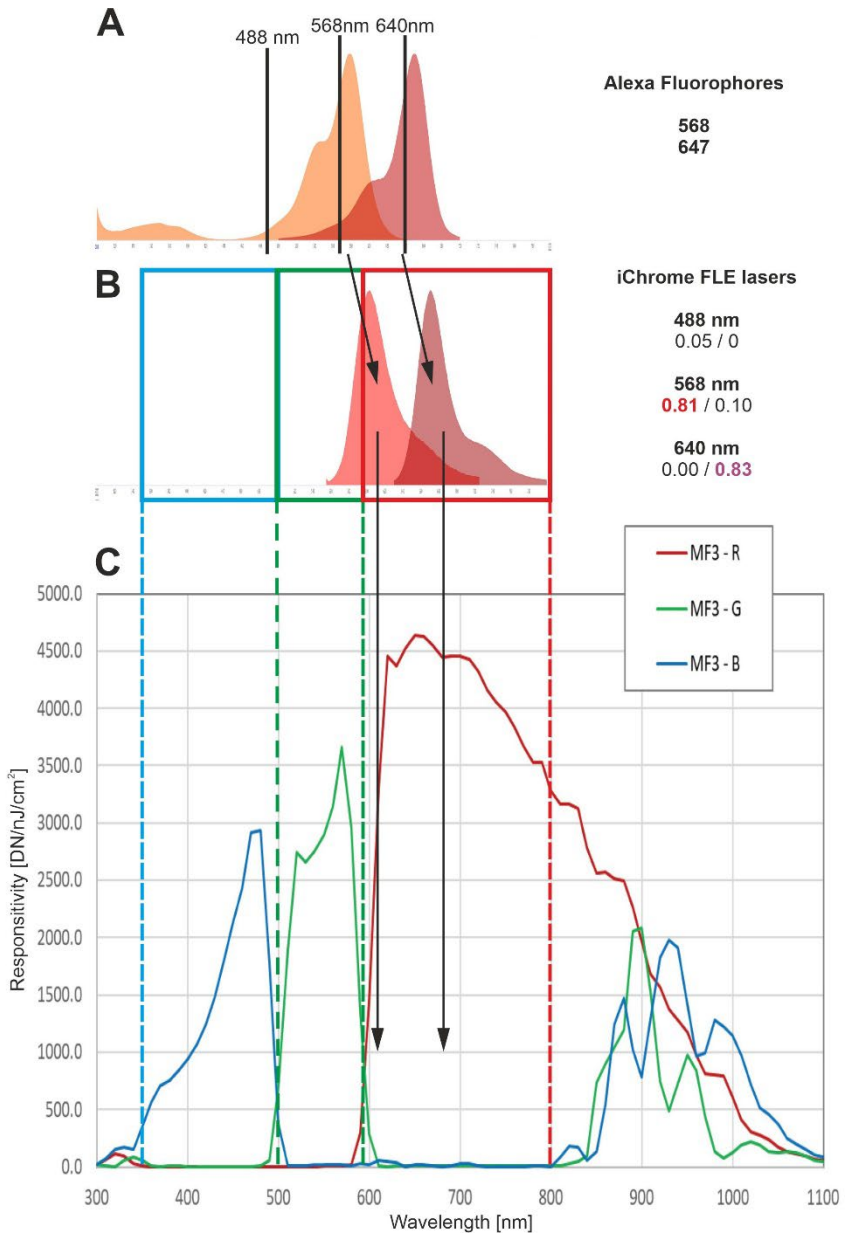


**Fig. 3** | Working principle of imaging biological tissue ex vivo that was immunoprocessed with secondary antibodies labeled with the fluorescent dyes Alexa 568 and Alexa 647 (c.f. [X01]) using an iChrome FLE multi-color laser combiner (TOPTICA Photonics Inc., Pittsford, NY, USA) and a Linea HS Multifield line camera (Teledyne DALSA, Boston, MA, USA). (**A**) Excitation spectra of the fluorophores Alexa 568 (orange) and Alexa 647 (magenta) together with the wavelengths of the corresponding lasers of the iChrome FLE multi-color laser combiner (the wavelength of an additional 488 nm laser for imaging autofluorescence is shown). (**B**) Emission spectra of the fluorophores Alexa 568 (red) and Alexa 647 (magenta), and the relative excitation of these fluorophores using the corresponding lasers of the iChrome FLE multi-color laser combiner. (**C**) Responsitivity of the three channels MF3-R, MF3-G and MF3-B of the Linea HS Multifield line camera that enable capturing multiple images simultaneously using various lighting conditions (here: different lasers and different fluorophores) in a single scan. In the example shown here **the two image channels cannot be simultaneously captured** without additional excitation and emission filters in the light path, as light from both image channels are captured in the MF3-R channel of the camera.

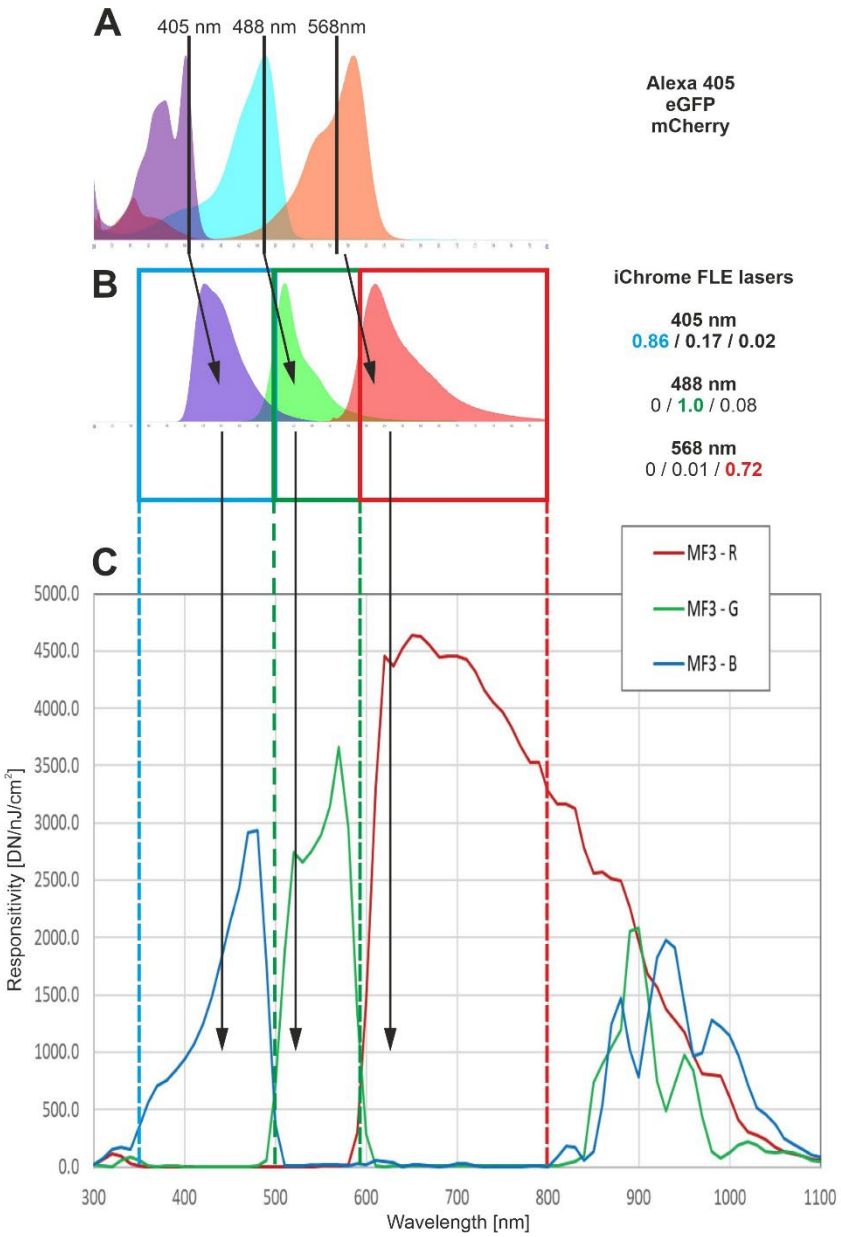


**Fig. 4** | Working principle of imaging biological tissue ex vivo in which the fluorescent proteins eGFP and mCherry were transgenically expressed and that was (optionally) immunoprocessed with a secondary antibody labeled with the fluorescent dye Alexa 405 using an iChrome FLE multi-color laser combiner (TOPTICA Photonics Inc., Pittsford, NY, USA) and a Linea HS Multifield line camera (Teledyne DALSA, Boston, MA, USA). (**A**) Excitation spectra of the fluorophore Alexa 405 (magenta), eGFP (blue) and mCherry (orange), and the wavelengths of the corresponding lasers of the iChrome FLE multi-color laser combiner. (**B**) Emission spectra of the fluorophores Alexa 405 (dark blue), eGFP (green) and mCherry (red), and the relative excitation of these fluorophores using the corresponding lasers of the iChrome FLE multi-color laser combiner. (**C**) Responsitivity of the three channels MF3-R, MF3-G and MF3-B of the Linea HS Multifield line camera that enable capturing multiple images simultaneously using various lighting conditions (here: different lasers and different fluorophores) in a single scan. In the example shown here **the three image channels can be simultaneously captured** without additional excitation and emission filters in the light path.

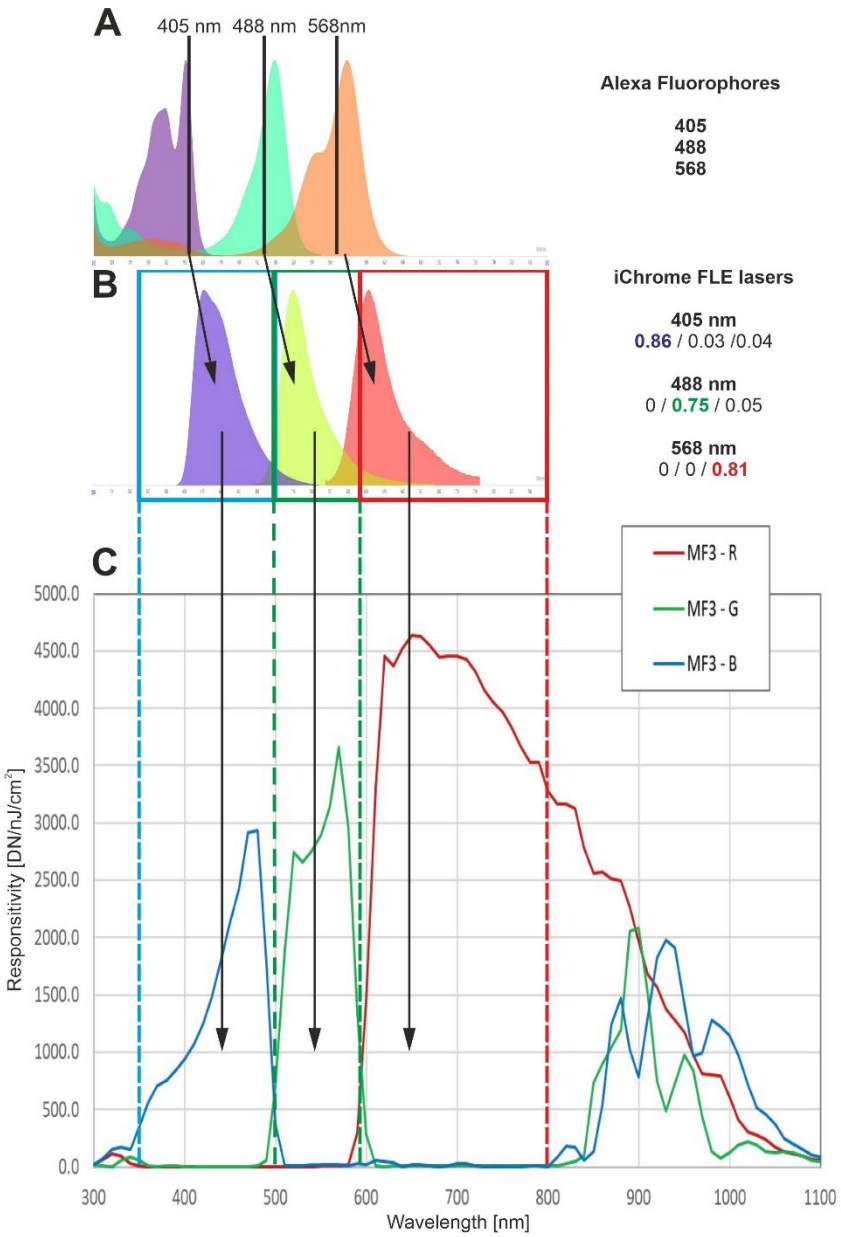


**Fig. 5** | Working principle of imaging biological tissue ex vivo that was immunoprocessed with secondary antibodies labeled with the fluorescent dyes Alexa 405, Alexa 488 and Alexa 568 using an iChrome FLE multi-color laser combiner (TOPTICA Photonics Inc., Pittsford, NY, USA) and a Linea HS Multifield line camera (Teledyne DALSA, Boston, MA, USA). (**A**) Excitation spectra of the fluorophores Alexa 405 (magenta), Alexa 488 (green) and Alexa 568 (orange), and the wavelengths of the corresponding lasers of the iChrome FLE multi-color laser combiner. (**B**) Emission spectra of the fluorophores Alexa 405 (blue), Alexa 488 (yellow) and Alexa 568 (red), and the relative excitation of these fluorophores using the corresponding lasers of the iChrome FLE multi-color laser combiner. (**C**) Responsitivity of the three channels MF3-R, MF3-G and MF3-B of the Linea HS Multifield line camera that enable capturing multiple images simultaneously using various lighting conditions (here: different lasers and different fluorophores) in a single scan. In the example shown here **the three image channels can be simultaneously captured** without additional excitation and emission filters in the light path.