

# Autofluorescence imaging of tissue samples using super-high sensitivity fluorescence microscopy

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Single molecule fluorescence microscopy is a technique for super-high sensitive detection of fluorescence allowing visualization of single molecule fluorescent dyes or proteins on a coverslip [1]. The technique has been artfully used in modern biosciences for a variety of purposes such as super resolution imaging beyond the diffraction limit of light [2,3] and number counting of mRNA or protein molecules [4,5] often in cultured cells on a dish. In this work, I used this microscopy to obtain autofluorescence images of a tissue that is not fluorescently labeled. It is expected that the high sensitivity detection by the microscope allows non-invasive imaging of clear cellular autofluorescence that cannot be detected in conventional fluorescence microscopy, and allows analysis of amounts of specific autofluorescence molecules. In human, it is known that NAD(P)H, riboflavin and lipofuscin causes blue, yellow and red autofluorescence, respectively [6-8].

Here, I used a wide-field single molecule fluorescence microscopy, in which a widely expanded laser light is exposed onto the sample, and the emitted fluorescence is detected using a high-sensitivity electron multiplying CCD camera (iXon897, Andor). To obtain a color spectrum of autofluorescence, I performed imaging respectively using four different lasers at 405 (Cube 405, Coherent), 488 (Innova 70C, Coherent), 560 (2RU-VFL-P-2000-560, MPB Communications) and 640 (MRL-III-640-200mW, CNI Laser) nm wavelengths. I used two objective lens with different magnifications: 4x (UPLFLN 4X, NA = 0.13, Olympus) and 30x (UPLSAPO 30XS, NA = 1.05, Olympus).

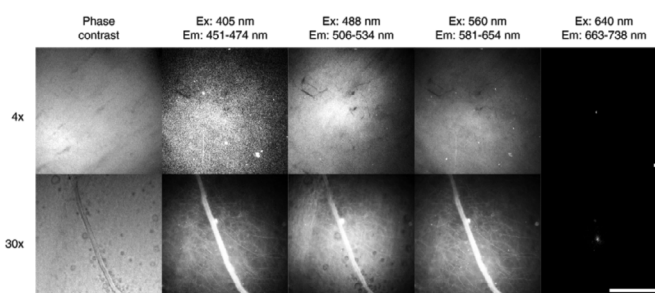
I imaged tissue from liver (Figure 1), breast (Figure 2), skin of thigh (Figure 3) and foot (Figure 4) of chicken. In liver, I could visualize blood vessels and capillaries at 405, 488 and 560 nm excitation. The appearance among these wavelengths except for 640 nm is not much changed, indicating that the autofluorescent molecules have a broad spectrum. In contrast, the image at 640 nm did not show significant signals compared to the background. This suggests that this wavelength causes less artifacts when we perform imaging of fluorescently labeled molecules targeting on specific proteins or molecules.

Meanwhile, in breast, I could visualize blood vessels as a bright signal and cleavage of muscles as a dark signal (Figure 2). In thigh and foot skin, I could observe patterns of lipid accumulations (Figures 3 and 4). I observed similar fluorescence spectrum tendency to liver, where the images at 405, 488 and 560 nm excitation are similar, but the image at 640 nm is not. This imaging itself cannot specify identity of the autofluorescent molecules, and further analyses using mass spectroscopy or immunostaining will provide this kind of information. Accumulation of imaging data with macroscopic symptoms will allow diagnostic analyses of physiological conditions of tissues.

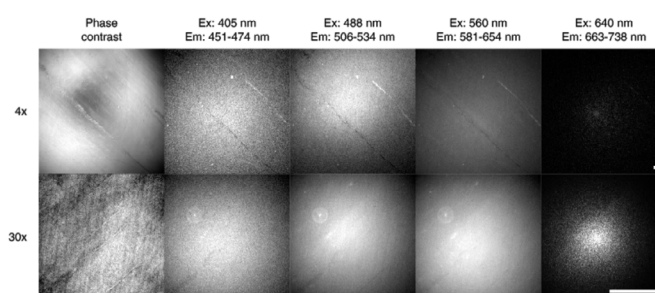
These attempts and results will open the door to the use of ultra-high sensitive fluorescence microscopy for non-invasive and spectroscopic diagnosis in medical tissue samples.

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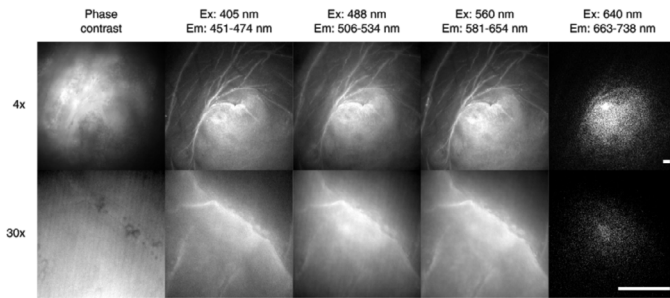
**Figure 1.** Autofluorescence images of chicken liver. Scale bar: 100  $\mu$ m. Ex: excitation wavelength. Em: emission wavelength.



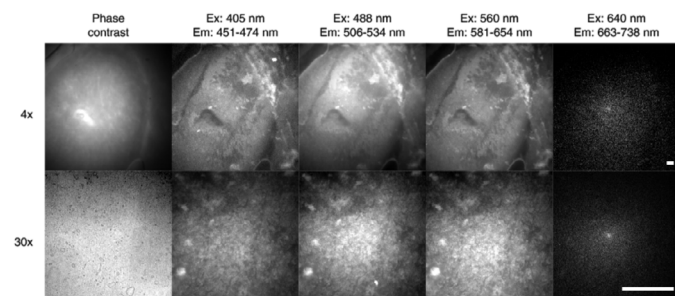
**Figure 2.** Autofluorescence images of chicken breast. Scale bar: 100  $\mu$ m.

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**Figure 3.** Autofluorescence images of chicken thigh skin. Scale bar: 100  $\mu$ m.



**Figure 4.** Autofluorescence images of chicken foot skin. Scale bar: 100  $\mu$ m.

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