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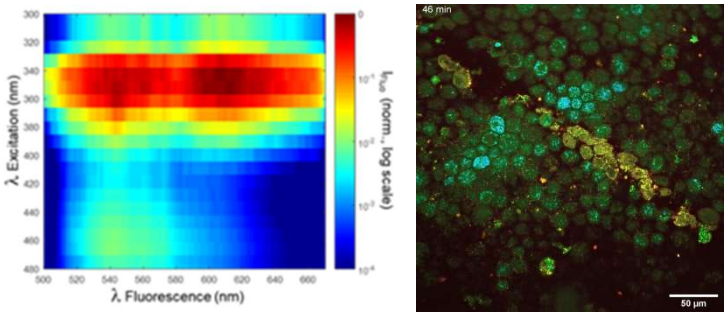
One and Two-photon spectroscopy and dynamic microscopy to study the Retinal Pigmented Epithelium (RPE) and its diseases

Fluorescence Excitation-Emission Matrix (EEM) micro-spectroscopy is an emerging technique which yields much richer information than classical fluorescence spectroscopy by recording emission spectra while varying the excitation wavelength. The resulting 2Dmatrix $I_{fluorescence}=f(\lambda_{excitation}, \lambda_{fluorescence})$ acquired at each location is sufficiently molecule-specific to allow the identification of many autofluorescent proteins and is increasingly used in its classical single photon, single point spectroscopy implementation in clinical applications (Leavesley et al., 2016). Recently, this technique allowed us to identify flavins and porphyrins, which are strong sources of autofluorescence, in pork and non-human primate retinas, possibly playing a major role in AMD (Figure 3, [1]).

In a first step (9 months), the postdoc will design and implement a system combining fast wavelength-scanning single photon excitation to fluorescence spectroscopy and scan-based imaging to obtain 5D ($\lambda_{excitation}$, $\lambda_{fluorescence}$, x, y, z) autofluorescence imaging.

In order to increase the penetration depth and reach RPE cells, but also to increase specificity, infrared excitation 2-photon excitation spectroscopy will be implemented.

In a second step (9 months extension), the postdoc will collaborate to the development of Dynamic Full Field OCT (DFFOCT) [2]. Within the team of K. Grieve and M. Paques at the 15-20 hospital, this technique which detects fast, metabolism-related changes will be applied to study RPE and organoids.



Left : 1-Photon Fluorescence excitation emission spectra in the outer segment of fixed pig retinas, showing peaks characteristic of Flavins and porphyrins [1]. Right : DFFOCT [2] in RPE culture 46 minutes after an injury, during wound healing

Location : Institut de la Vision / 15-20 Hospital, Paris (Bastille)
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[1] M. Marie, et al., *Blue light damage of cone photoreceptors is correlated to porphyrin absorption,* Cell Death & Disease 11, 711 (2020), https://doi.org/10.1038/s41419-020-02918-8
[2] Scholler et al., Dynamic full-field optical coherence tomography: 3D live-imaging of retinal organoids, Light: Science & Applications 9(1):140 (2020)