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Letter to Editor

The Utility of *In Vivo* Imaging in Experimental Ophthalmology

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Experimental ophthalmology is an important and essential research field for the study of retinal pathophysiology as well as for the evaluation of different approaches for the treatment of retinopathies, such as age-related macular degeneration (AMD), diabetic retinopathy (DR) and glaucoma. Since the prevalence of retinal disease dramatically increases over the years, the establishment of new more efficacious treatments is urgent in order to preserve vision and to increase the quality of life for millions of people worldwide. *In vitro* studies using retinal cell cultures and more recently retinal organoids have provided important information about retinal cell interactions and cell behavior under specific conditions and insults [1]. However, since the complexity found in a living organism cannot be mimicked by *in vitro* techniques, the use of animals is inevitable for the translation from basic research to the clinical practice.

The retina is part of the central nervous system and thanks to the optical properties of the eye it can be directly visualized *in vivo*. Imaging modalities, such as spectral domain optical coherence tomography (SD-OCT), fluorescein angiography (FA), optical coherence tomography-angiography (OCT-A) and scanning laser ophthalmoscopy (SLO) are extensively used by ophthalmologists in the daily practice for diagnosis and monitoring of retinal disease [2,3]. More recently, these techniques have been utilized in experimental models of retinal disease and they have already been proven valuable for the investigation of disease mechanisms in small rodents or even in zebrafish [4-7]. These non- or semi-invasive techniques have many advantages compared to histology, which is still the gold standard for retinal disease studies in rodents. Retinal thickness measurements using SD-OCT or detection of vascular abnormalities using FA can be more precise and with less variability compared to histological approaches, where manipulation may alter the actual properties of the tissue. Additionally, important changes in retinal functionality, such as retinal swelling, retinal detachment or sub-retinal fluid accumulation that can be detected by *in vivo* imaging, are not detectable in histological preparations. Another important advantage of *in vivo* imaging is that it can be used for longitudinal studies, providing the researcher with a powerful tool for *in vivo* monitoring of retinal alterations in the same animal overtime, something that cannot be achieved with histology, where animal euthanasia is necessary. Imaging of specific cell types in the retina in real time can also be achieved using semi-invasive imaging techniques, such as SLO. Nowadays, there is a plethora of rodent and zebrafish strains available, where labelling of specific cell types, such as microglia or ganglion cells, with fluorescent probes, enables researchers to visualize the dynamics of these cells *in vivo* [4,8,9]. However despite the advantages of *in vivo* imaging techniques compared to conventional histological studies, there are still certain limitations associated with the use of new technologies, such as OCT-A, where projection artifacts for example may lead to false estimation of vessels location and density.

Since most of the *in vivo* retinal imaging devices are designed for humans, there are several challenges that have to be faced when this equipment is utilized in small animals, mainly due to the differences in the size and curvature between experimental animals and humans. To this end, many efforts have been initiated for the design of devices for rodent retinal imaging, such as the Phoenix Micron *in vivo* imaging system that has been specifically designed for small laboratory animals (Phoenix technology group; Pleasanton, CA, USA). Overall, the

replacement of histology with *in vivo* imaging can potentially reduce the number of animals used in experimental ophthalmology, while it can provide a powerful experimental tool for the monitoring of retinal disease *in vivo* in small animals. More ophthalmology researchers should be encouraged to utilize *in vivo* imaging techniques and additional efforts should be made to design more sophisticated devices based on the geometry and ocular characteristics of small laboratory animals.

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