Projet de Recherche Doctoral Concours IPV 2021

Intitulé du Projet de Recherche Doctoral : Diffuser-based wavefront imaging for organoid cell sorting and flow cytometry

Directeur de Thèse porteur du projet (titulaire d'une HDR):
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(site, adresse, bât., bureau)
Unité de Recherche :
Intitulé : Institut de la Vision
Code *(ex. UMR xxxx)* : CNRS UMR7210- INSERM UMRS968
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Departement Photonique - Equipe Microscopies 3D**
Thématique de recherche : Microscopie, Holographie, imagerie de phase, photothermique
Responsable d'équipe :
NOM : Tessier Prénom : Gilles
Ecole Doctorale de rattachement de l'équipe & d'inscription du doctorant : **ED564-Physique en IdF**

Doctorants actuellement encadrés par le directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :

3 doctorants, pour un total de 80% :
- Chang Liu / 2017 / 50 % (co-direction, avec V. Emiliani) Soutenance prévue le 15/3/2021 (prolongation/Covid)
- Clémence Gentner / 2018 / 20% (encadrant principal R. Kuszelewicz)
- Anwesh Bhattacharya / 2020 / 20% (encadrant principal Y. De Wilde)

CO-DIRECTION *(obligatoire)*
Co-Directeur de Thèse (titulaire d'une HDR) :
NOM : **Reichman** Prénom : **Sacha**
Titre : Chargé de Recherche ou HDR ☐
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Unité de Recherche :
Intitulé : Institut de la Vision
Code *(ex. UMR xxxx)* : CNRS UMR7210- INSERM UMRS968
Equipe de Recherche *(au sein de l'unité)* **:**
Intitulé : **Développement et régénération de la rétine : apport des cellules souches pluripotentes**
Thématique de recherche :
Responsable d'équipe :
NOM : GOUREAU Prénom : Olivier
Ecole Doctorale de rattachement : **ED394-Physiologie,Physiopathologie Thérapeutique**
Ou si ED non SU :

Doctorants actuellement encadrés par le co-directeur de thèse (préciser le nombre de doctorants, leur année de 1ere inscription et la quotité d'encadrement) :

Aucun. Veuillez noter que Sacha Reichman n'est pas encore titulaire de l'HDR, mais la présentera d'ici fin 2020. D'autres membres du laboratoire ont donné leur accord pour diriger cette thèse si ce point s'avérait bloquant.
Cotutelle internationale : ☒ Non ☐ Oui, précisez Pays et Université :

Précisez ici les éventuels co-encadrants (non HDR)

Co-encadrant :
NOM : **BERTO** Prénom : **Pascal**
Titre : Maître de Conférences des Université HDR ☐
ou
e-mail : pascal.berto@parisdescartes.fr
Unité de Recherche :
Intitulé : Institut de la Vision
Code *(ex. UMR xxxx)* : CNRS UMR7210- INSERM UMRS968
Equipe de Recherche *(au sein de l'unité)* **:**
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NOM : Tessier Prénom : Gilles
Ecole Doctorale de rattachement : **ED564-Physique en IdF**
Ou si ED non SU :

Résumé (2 000 caractères maximum) :

This PhD project proposes to contribute to the development of a novel type of Phase imaging device that enables **single-shot measurement of wavelength-multiplexed phase information**. This **low-cost Phase imager (or Wavefront Sensor) based on a diffuser will provide** high sensitivity (≈nm), resolution (few camera pixels per phase pixel), dynamic range (tens of λ), accuracy (typ. λ/100), for fast Quantitative Phase Imaging of intracellular morphology. Cells will be illuminated at various angles, each with a different wavelength. From a single acquisition of multiplexed speckle images, phase images at each wavelength will be separated, yielding multiple angles of observation and therefore 3D imaging. After validation on labelled cells, this **multiplexed tomographic device** will allow **label-free fast 3D tomographic imaging for flow cytometry** of cells with high throughput in order to separate cell types in retinal organoids.

Joindre en annexe un descriptif du PRD avec références au format pdf
(« NOM_2_IPV_2020 » / 3 pages maximum, taille police 11)

AVIS et VALIDATION de l'ECOLE DOCTORALE :

Avis favorable

Ecole Doctorale
Physique en Ile de France
ED n° 564
Pr Marla Chamarro
Directeur-adjoint SU

à envoyer simultanément par e-mail à l'ED de rattachement et au programme : interfaces_pour_le_vivant@listes.upmc.fr avant le lundi 15 Février minuit.

Diffuser-based wavefront imaging for organoid cell sorting and flow cytometry

Light radiations not only carry information about their emission sources but also about the properties of the materials they travelled across. Due to the high oscillation frequency of light (~10^{14}Hz), conventional detectors are only sensitive to intensity, making direct phase measurements impossible. However, phase information is crucial for all applications where *in-situ*, nanometric-sensitivity, non-contact phase sensing is required. In biological imaging, it allows the label-free, non-invasive quantitative characterization of transparent samples. Quantitative phase imaging of biological samples[1] also gives access to their local *dry mass* and, as such, can monitor their metabolism. Current progresses in biomedecine demand the development of specific instruments to allow high-speed measurement of highly complex information with high throughput and at a reasonable price, and label-free detection is crucial in clinical applications where chemical labels cannot be used.

Flow cytometry is particularly critical and promising in the context of organoid tissues, where cell-sorting is essential to isolate specific cell types for downstream analysis, cryopreservation or generation of pure cell libraries.

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Retinal organoids and cell sorting

The production of specialized cells from pluripotent stem cells provides a powerful tool to develop new approaches for regenerative medicine. The use of human-induced pluripotent stem cells (iPSCs) is particularly attractive for neurodegenerative disease studies, including retinal dystrophies, where iPSC-derived retinal cell models mark a major step forward to understand and fight blindness.

At the Institut de la Vision, Sacha Reichman and his colleagues have developed protocols mimicking the early phases of retinal development to generate iPSC-derived retinal organoids analogues to human foetal neuroretinal tissue[2]. These organoids can be maturated in floating culture conditions, enabling the differentiation of retinal progenitor cells (RPCs) into the seven retinal cell types present in the adult human retina following successive waves of cell emergence (Fig. 1). Owing to this developmental characteristic, the dissociation of retinal structures at a specific stage allows to recover the retinal cell type of interest (i.e. photoreceptors) for both basic and clinical research[3].

From organoids, **efficient cell-sorting is essential in order to obtain pure ensembles of a given cell type**, but all retinal cell types don't have a specific (and innocuous) surface marker to target them by immunochemistry approaches. Although label-free sorting is essential to purify certain cell types, it remains a seldom explored option.

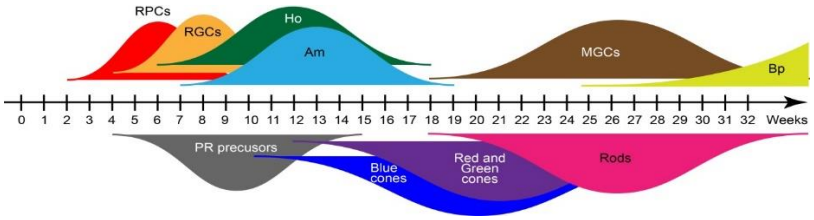


Figure 1: Waves of human iPSC-derived retinal cell generation obtained at different stages of organoid development. Cell sampling and disassociation conducted at a given time yields a mix of cells, which will be sorted using multiplexed phase imaging. Abbreviations: Retinal progenitor cells (RPCs), retinal ganglion cells (RGCs), horizontal cells (Ho), amacrine cells (Am), Müller glial cells (MGCs), bipolar cells (Bp), and photoreceptors (PR). From [2].

A novel Quantitive Phase Sensing approach: speckle as a multiplexed phase information carrier.

Among the few techniques able to retrieve optical phase, **Wave Front Sensing** (**WFS**) has many advantages as compared to digital holography: it is simpler to implement, does not require laser illumination prone to random interference, and is less sensitive to vibrations and air convection. Shack-Hartmann WFS and lateral shearing interferometry are the most common WFS techniques. Both are based on a phase-encoding element placed before a camera (microlens array and grid phase-mask respectively), creating a grid of spots which is distorted by gradients in the incident WF. While the use of Shack-Hartmann in imaging is limited by the number of microlenses and therefore phase pixels (typically from 10^2 to 10^4), lateral shearing yields higher resolution, but its commercial price is still high, due to demanding mask fabrication and alignment processes (~ 50k€).

Taking advantage of specific properties of scattering media, we recently proposed a simple, low-cost, and original implementation of high-resolution WFS[5] based on the *memory effect* of a thin diffuser[6] (N.B.: the development of this approach is currently supported by a SATT maturation project). When passing through a diffuser, a WF creates on a camera a speckle pattern which carries high-spatial-density information. Distortions in this pattern are proportional to the WF gradient. The PhD student will *develop and implement a diffuser-based high-resolution wavefront sensor and imager* taking advantage of **two tunable speckle properties** *:* **P1**: thin diffusers exhibit a *memory effect* resulting in a simple shift of the speckle pattern when inducing a tilt on the wavefront (see Fig 2(a)(b)). **P2**: Since speckle patterns result from the interference of many waves with random phases, they strongly depend on the wavelength (see Fig 3(b)). For these reasons, speckle patterns intrinsically offer **spectrally resolved** and **high resolution** phase imaging capabilities[7].

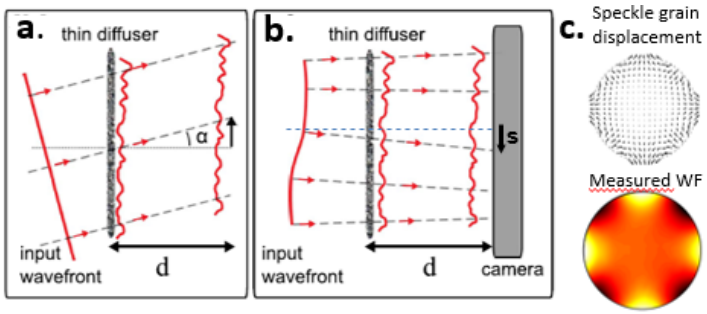


Figure 2 - WFS based on the memory effect of a thin diffuser (P1). *(a) a tilt results in a shift of the speckle pattern (P1). (b) Speckle grains are locally shifted for a distorted wavefront. (c) Estimation of the speckle grain displacement and reconstructed WF.*

Current WFS can only measure spectral channels sequentially: whenever several WFs at different wavelengths are superimposed, they yield a meaningless wavelength-averaged measurement. We recently demonstrated *(*and *patented*[8]*)* that using **P2**, a random speckle patterns is an efficient wavelength-compressed sensing approach allowing **simultaneous phase imaging at several wavelengths**.

Single-shot Tomographic flow cytometry: Towards label-free cell classification and sorting-

Flow cytometry consists in optically analyzing cells flowing at high speed in a microfluidic channel in order to ultimately sort them on-the-fly. It typically makes use of fluorescent dyes, which require invasive labelling that interferes with natural biological functions. New *label-free* techniques are thus of key interest, especially in the context of organoids, where cells need to be efficiently sorted before subsequent culture. **T*omographic WFS*** recently demonstrated its ability to classify different cell types, including stem cells and lymphocytes exhibiting similar morphology[9]. Indeed, while classical phase imaging provides a projection of the refractive index ($\varphi \propto \int n(z)dz$), tomographic phase imaging gives access to a **3D reconstruction** of the refractive index $n(z)$ from which quantitative and structural features can be better extracted for robust classification. Here, 3D derives from the presence of several angles of incidence (as in e.g. binocular vision or holography). However, tomographic imaging currently demands many sequential measurements under different angles[10] (see *Nanolive* or *Tomocube*), or different cell orientations[11], which are not compatible with high throughput flow cytometry.

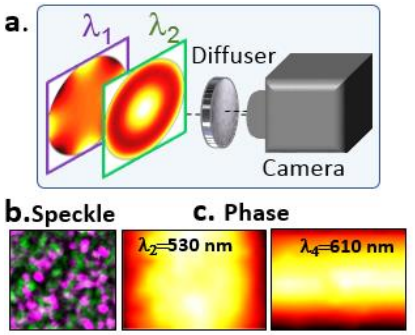
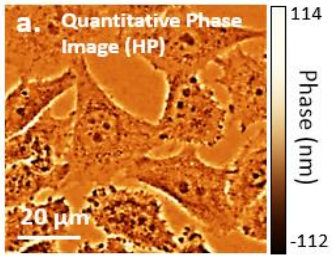


*Figure 1 - (a) Single-shot hyperspectral WFS (b) Each color generates a speckle pattern uncorrelated with the other ones allowing multiplexing (**P2**) (c). Reconstruction of 2 multiplexed WFs at 530nm and 610nm with vertical and horizontal cylindrical wavefront profiles, respectively (preliminary; collab. M. Guillon, U. Paris Descartes).*



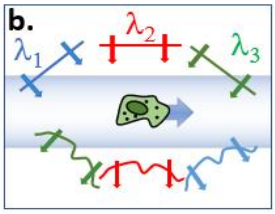


Figure 3 – (a) WFS Image of CHO cells acquired with our system. (b) Principle of Single-shot Tomography based on polychromatic multiplexing.

In this project, we propose to **encode various illumination angles using different wavelengths** (see **fig. 3 b**). The PhD student will design and demonstrate a single-shot, compact tomographic system where **(P1)** will provide imaging abilities, while (**P2**) will yield wavelength, and therefore angular discrimination, to reconstruct 3D images of cells. Experiments will be carried out in the NIR to minimize scattering and dispersion (see Fig 3b). This compressed sensing system will allow reduced data flow and fast 3D characterization, thus allowing higher cell throughput (limited only by the camera and computing speeds).

Main tasks :

- Development of wavelength- and angle-multiplexed illumination adapted to microfluidic environments,
- Optimization of the WFS and calibration procedures for wavelength discrimination and imaging,
- Development of 3D reconstruction and cell sorting algorithms and validation on static cells,. Optionally, and depending on the PhD candidate's wishes and skills (the PhD advisors are not specialists of the field), we will explore the use of deep learning techniques to discriminate cell types,
- Sampling and culture of various cell-types with fluorescent labelling to train and validate the sorting-process,
- Validation of the imaging system on label-free cell lines.

[1] Park et aL. Nat. Photonics 12, 578–589 (2018).
[2] Reichman et al. PNAS 2014 et Stem Cells 2017
[3] Gagliardi et al. stem cell research 2018
[4] Slembrouck-Brec, et al., Reichman, JoVE, e57795 (2018)
[5] Berto, Rigneault and Guillon. Opt. Lett. 42, (2017)
[6] Freund et al.. Phys. Rev. Lett. 61, 2328–2331 (1988).
[7] Redding et al. Nat. Photonics 7, 746–751 (2013).
[8] Guillon, Berto, Papadopoulos, Tessier. Patent EP19305382, 26/3/2019
[9] Yoon et al. Sci. Rep. 7, 6654 (2017).
[10] Simon, Debailleul, Houkal, et al. Optica 4, 460 (2017).
[11] Merola et al. Light Sci. Appl. 6, e16241–e16241 (2017).