PhD PROJECT PROPOSAL

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Probing apical ECM–Cytoskeleton Interactions In Vivo Using Advanced Polarization and SHG Microscopy in the *C. elegans* skin

Supervisor 1 **Nathalie Pujol**
Centre d'Immunologie de Marseille-Luminy (CIML), pujol@ciml.univ-mrs.fr
Affiliated Doctoral School: ED62 – Sciences de la Vie et de la Santé

Supervisor 2 **Sophie Brasselet**
Institut Fresnel, sophie.brasselet@fresnel.fr
Affiliated Doctoral School: ED352 – Physique et Sciences de la Matière

Abstract (10 lines)*

Mechanical forces shape tissue morphogenesis, and emerging evidence suggests that the extracellular matrix (ECM) can actively direct tissue shaping rather than serving only as a passive scaffold. In the *C. elegans* skin, specific collagens of the apical ECM (aECM) form periodic structures that allow transient alignment of cytoskeletal components during morphogenesis. This suggests a direct mechanical interplay between aECM organization and cytoskeletal dynamics. This Centuri PhD project aims to analyze the dynamic molecular organization of the cytoskeleton and aECM using and developing advanced polarization fluorescence and second harmonic generation (SHG) microscopy in the genetic model *C. elegans*. By integrating these optical and biological approaches, we aim to uncover how cytoskeletal order emerges from the mechanical and structural properties of the aECM during morphogenesis.

Keywords*
C. elegans, morphogenesis, cytoskeleton, extracellular matrix, SHG microscopy, fluorescence polarization, live imaging, mechanobiology

Scientific question and Objectives (10 lines)*
We recently showed that specific, periodically organized collagens in the aECM guide and instruct cytoskeletal alignment during molting (Aggad et al., 2023). To determine how these aECM structures influence cytoskeletal dynamics, we will:

Aim 1 –Use 3D fluorescence polarization microscopy to monitor actin and microtubule organization in *C. elegans* larvae during aECM morphogenesis.

Aim 2 –Develop 3D polarized two-photon SHG microscopy to simultaneously and directly visualize native collagens, actin, and microtubule assemblies to quantify filament orientation, anisotropy.

Aim 3 –Investigate how aECM and cytoskeletal organization respond to genetic and mechanical changes during morphogenesis.

*: Mandatory

By integrating fluorescence polarization and SHG microscopy, this project will uncover in vivo collagen–cytoskeleton interactions and drive methodological advances in 3D polarized imaging of living tissues.

Proposed approach (experimental / theoretical / computational) and research plan (20 lines)*

Live imaging of *C. elegans* larvae expressing genetically encoded actin and microtubule filament reporters will be performed using fluorescence polarization microscopy to define their alignment during aECM morphogenesis using technology recently developed at the Fresnel institute. For actin, we will use available, validated strains (Martins et al. 2025). Microtubule markers will be first based on standard existing fluorescent strains. These will be tested for their orientational flexibility using polarized microscopy. If more rigid labels are needed, constructs will be developed based on our prior experience (Martins et al. 2025).

Development of polarized two-photon SHG microscopy will then enable simultaneously imaging of collagen (SHG) and the cytoskeleton, providing a key technological advance. For this, the polarized fluorescence labels validated in the previous step will be transposed on a microscope capable of measuring polarized signatures on both two-photon fluorescence (actin, microtubule) and SHG (collagen).

To account for the curved geometry of larvae, we will benefit from the ongoing development of 3D orientation analysis tools (Rimoli et al., 2022), extending the existing optical strategies to two-photon/SHG imaging, thanks to a dedicated postdoc at the Fresnel Institute.

Quantitative image analysis in R and Python will finally extract filament orientation, anisotropy, and correlations with tissue-scale deformations. Appropriate data analysis tools (correlative alignment analysis, time-lapse analyses of multi-color organization maps) will help us construct 3D organization models.

Targeted genetic perturbations, guided by our previous work (Sonntag et al.), will test the roles and epistatic relationships of key molecular actors linking the ECM and cytoskeleton. These models will then be challenged by controlled mechanical perturbations, such as changes in hydrostatic pressure, to evaluate how well they predict the effects of stress on collagen and cytoskeletal organization, building on our recent AFM and Brillouin imaging results in collaboration with Claire Valotteau (Dynamo) and Carlo Bevilacqua (EMBL).

*: Mandatory

Interdisciplinarity and Implication of the two labs (15 lines)*
(In this section the collaboration of the two laboratories will be explained in details to explain why the project cannot be conducted by one team alone)

This project sits at the interface of cell and developmental biology (CIML) and biophotonics and optical physics (Fresnel Institute). The collaboration is essential: CIML brings expertise in *C. elegans* epidermal morphogenesis, while Fresnel contributes advanced imaging and optical instrumentation. Together, the labs will develop multimodal microscopy combining SHG and fluorescence polarization to visualize simultaneously aECM and cytoskeleton organization *in vivo*. Neither lab could achieve these goals independently. The project exemplifies CENTURI's interdisciplinary mission, integrating quantitative physics and developmental biology to uncover how molecular and mechanical architectures are coordinated during tissue morphogenesis.

Specify with whom the person recruited will collaborate and on what aspects *

At Institut Fresnel, the recruited candidate will contribute to the development of a combined polarized fluorescence and polarized SHG microscope, with the focus on the quantification of alignment and organization in 3D in curved tissues. For this, the candidate will interact with a dedicated postdoc working on this topic, with their supervisor S. Brasselet and with the colleagues of the MOSAIC team involved in microscopy developments. They will also interact with the engineer in charge of the imaging microscopes at Institut Fresnel, for all questions related to adaptation of instruments to specific needs (sample manipulation, lasers, etc).

At the CIML, the recruited candidate will interact with S. Mazzoli, a highly skilled engineer in the group, with extensive expertise in imaging, genetics, and cell biology and with their supervisor N. Pujol on all biological and mechanical aspect on *C. elegans*.

PhD student's expected profile*

This is an interdisciplinary PhD project. The selected candidate should enjoy working in two interactive teams- one on *C. elegans* epidermal morphogenesis and cytoskeletal dynamics at the CIML, and the other developing SHG polarized fluorescence for quantifying molecular organization at Institut Fresnel. A biology background with microscopy experience is expected, and the candidate should be open to contributing to instrumentation development, for instance for imaging tests on reference samples, or for tests on existing or new labels to quantify their orientational mobility. An interest in programming and FAIR data management would be a plus. Experimental experience with *C. elegans* is not required, but basic knowledge of cell biology and genetics is expected.

Is this project the continuation of an existing project or an entirely new one?
This is a new project that was not yet funded by Centuri.
In the case of an existing project, please explain the links between the two projects (5 lines)*

*: Mandatory

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Two to five references related to the project*

The extracellular matrix in tissue morphogenesis: No longer a backseat driver.
Cells Dev. 2024

The *Caenorhabditis elegans* cuticle and precuticle: a model for studying dynamic apical extracellular matrices in vivo. Genetics. 2024

Genetically encoded reporters of actin filament organization in living cells and tissues.
Cell. 2025

Illuminating cellular architecture and dynamics with fluorescence polarization microscopy.
J Cell Sci. 2024

Two main publications from each PI over the last 5 years*

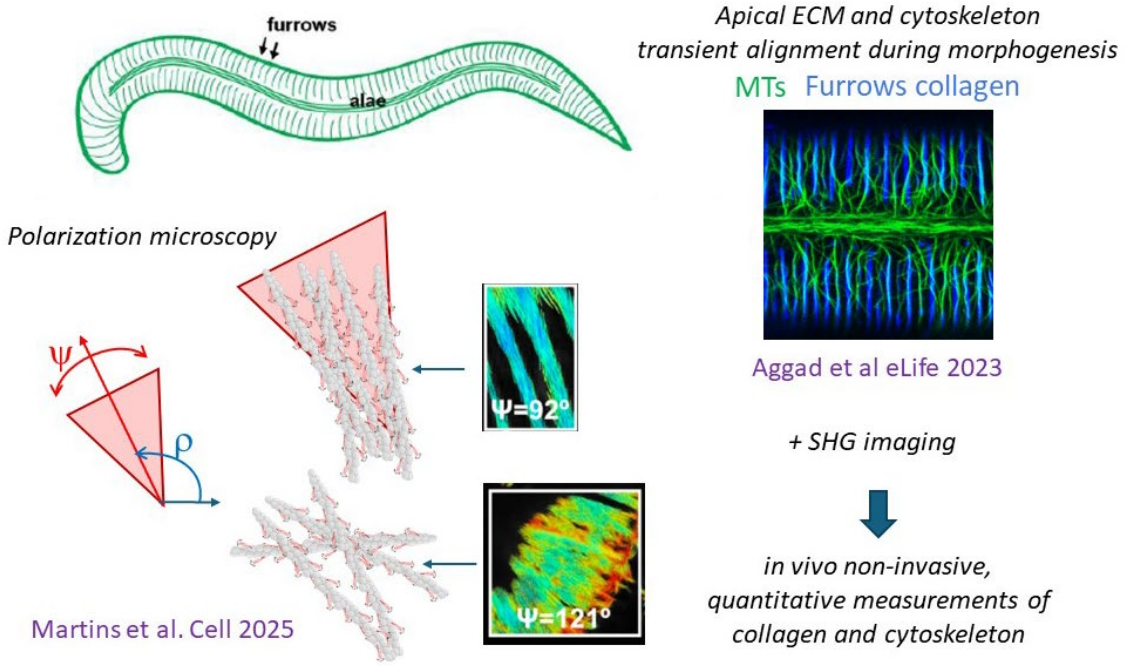
Genetically encoded reporters of actin filament organization in living cells and tissues.
Martins CS, Iv F, Suman SK, Panagiotou TC, Sidor C, Ruso-López M, Plancke CN, Omi S, Pagès R, Gomes M, Llewellyn A, Bandi SR, Ramond L, Arbizzani F, Rimoli CV, Schnorrer F, Robin F, Wilde A, LeGoff L, Pedelacq JD, Jégou A, Cabantous S, Rincon SA, Chandre C, **Brasselet S***, Mavrakis M*. Cell. 2025

4polar-STORM polarized super-resolution imaging of actin filament organization in cells.
Rimoli CV, Valades-Cruz CA, Curcio V, Mavrakis M, **Brasselet S.** Nat Commun. 2022

A defining member of the new cysteine-cradle family is an aECM protein signalling skin damage in *C. **elegans***. Sonntag T, Omi S, Andreeva A, Valotteau C, Eichelbrenner J, Chisholm AD, Ward JD, **Pujol N.**
PLoS Genet. 2025

Meisosomes, folded membrane microdomains between the apical extracellular matrix and epidermis.
Aggad D, Brouilly N, Omi S, Essmann CL, Dehapiot B, Savage-Dunn C, Richard F, Cazevieille C, Politi KA, Hall DH, Pujol R, **Pujol N.** Elife. 2023

Project's illustrating image



*: Mandatory