In a healthy organism, the process of mineralization and the deposition of calcium salts occur naturally in places like bones and teeth. However, in some cases, these compounds accumulate abnormally in tissues that normally do not undergo mineralization, known as pathological microcalcifications. This issue has significant medical relevance, as microcalcifications play a key role in many diseases, such as atherosclerosis and inflammatory conditions. Despite their clinical importance, the mechanisms underlying the formation of these deposits at the subcellular level remain poorly understood, and their early detection continues to pose a challenge.

Until now, the analysis of microcalcifications has relied mainly on FT-IR spectroscopy techniques, which, while effective, have limited spatial resolution, making it impossible to observe mineralization processes at the level of single cells and their internal structures. Yet it is precisely at this microscale that the key steps of nucleation and growth of pathological deposits occur, which determine the further progression of disease. To date, we have lacked technology that would allow us to observe these changes in real time with such high precision.

This project aims to leverage a modern spectroscopic technique called **Optical Photothermal Infrared (O-PTIR) spectroscopy**, which enables the acquisition of chemical images and spectroscopic spectra with a resolution on the order of hundreds of nanometers.[1] Our goal is therefore not only to investigate, in real time, the nucleation and growth processes of both physiological and pathological mineral deposits in human stem cells cultured in vitro, but also to significantly expand our knowledge of the application of vibrational spectroscopy in biological imaging. By developing new spectroscopic markers, our project will allow us to distinguish pathological mineralization processes from physiological ones at a very early stage.

The O-PTIR technique enables the simultaneous study of both the chemical composition of mineral deposits and the surrounding biomolecules (such as proteins or lipids), providing more complete data of mineralization processes within cells. This is a significant advantage over Raman spectroscopy, which has limitations in detecting certain biomolecules due to autofluorescence and weak light scattering. On the other hand, Raman is excellent at identifying the mineral composition. Combining O-PTIR with Raman allows for the simultaneous collection of IR and Raman spectra from the same location, facilitating the correlation of chemical and structural information and greatly expanding our research capabilities.[2]

This, in turn, will pave the way for developing modern diagnostic methods for diseases associated with abnormal calcium deposition in tissues. This field remains underexplored, offering broad opportunities for investigation. Thus, the project is pioneering in nature, combining innovative technology with an important medical issue. The results of this research could, in the future, contribute to the development of early diagnostic methods for diseases related to abnormal calcium deposition in tissues, as well as to a better understanding of their pathogenesis, through the application of the innovative O-PTIR technique in biomedical sciences.

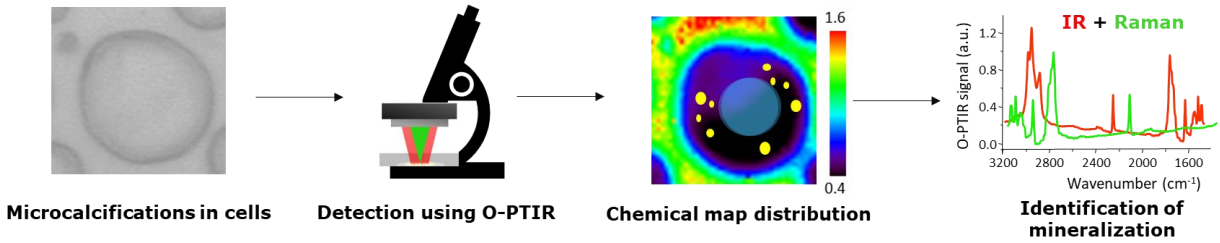

Figure 1. The combination of vibrational spectroscopy with spatial information enables the identification and distribution of different chemical compounds in the area under study.

[1] Paulus, A., *et al.*, Correlative imaging to resolve molecular structures in individual cells: Substrate validation study for super-resolution infrared microspectroscopy, *Nanomedicine: Nanotechnology, Biology, and Medicine* 43 (**2022**) 102563
[2] Bazin, D. *et al.* Using mid infrared to perform investigations beyond the diffraction limits of microcristalline pathologies: advantages and limitation of Optical PhotoThermal IR spectroscopy; Comptes Rendus Chimie (**2022**), Vol. 25, Special Issue S1, p. 105-131