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Micro- to sub-microscale investigation of fossilization in amber using IR spectro-imaging

1. Introduction

Amber is an exceptional medium for fossilising fine anatomical structures of past organisms in 3D. Fossil inclusions exhibit varying states of preservation, and while the taphonomic processes behind this are not well understood, preservation fidelity has been linked to the resin type [1]. The chemical composition of fossil-bearing amber classes is well known, but the structural and chemical interactions between the matrix and the fossilized organism remain unexplored. The use of novel super high resolution techniques such as AFM-IR, allows for the **study of the interface between a fossil inclusion and its amber matrix at a sub-micro scale** to characterise the interactions between both components.

[1] McCoy et al., 2016

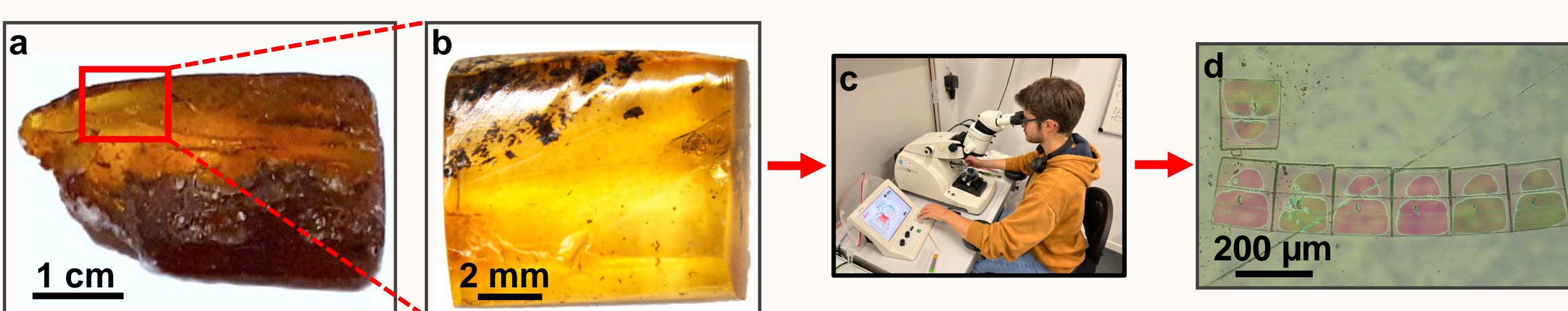
2. Objective and sample preparation

Study of the amber - fossil interface

Local characterisation of the chemical and/or structural interactions at a nanometric resolution with **AFM-IR**.

→ **Constraints on sample preparation**

Sample preparation



(a) original amber nodule, (b) 5-8 mm amber cube with isolated fossil, (c) ultramicrotome, (d) thin section - 500 nm thickness

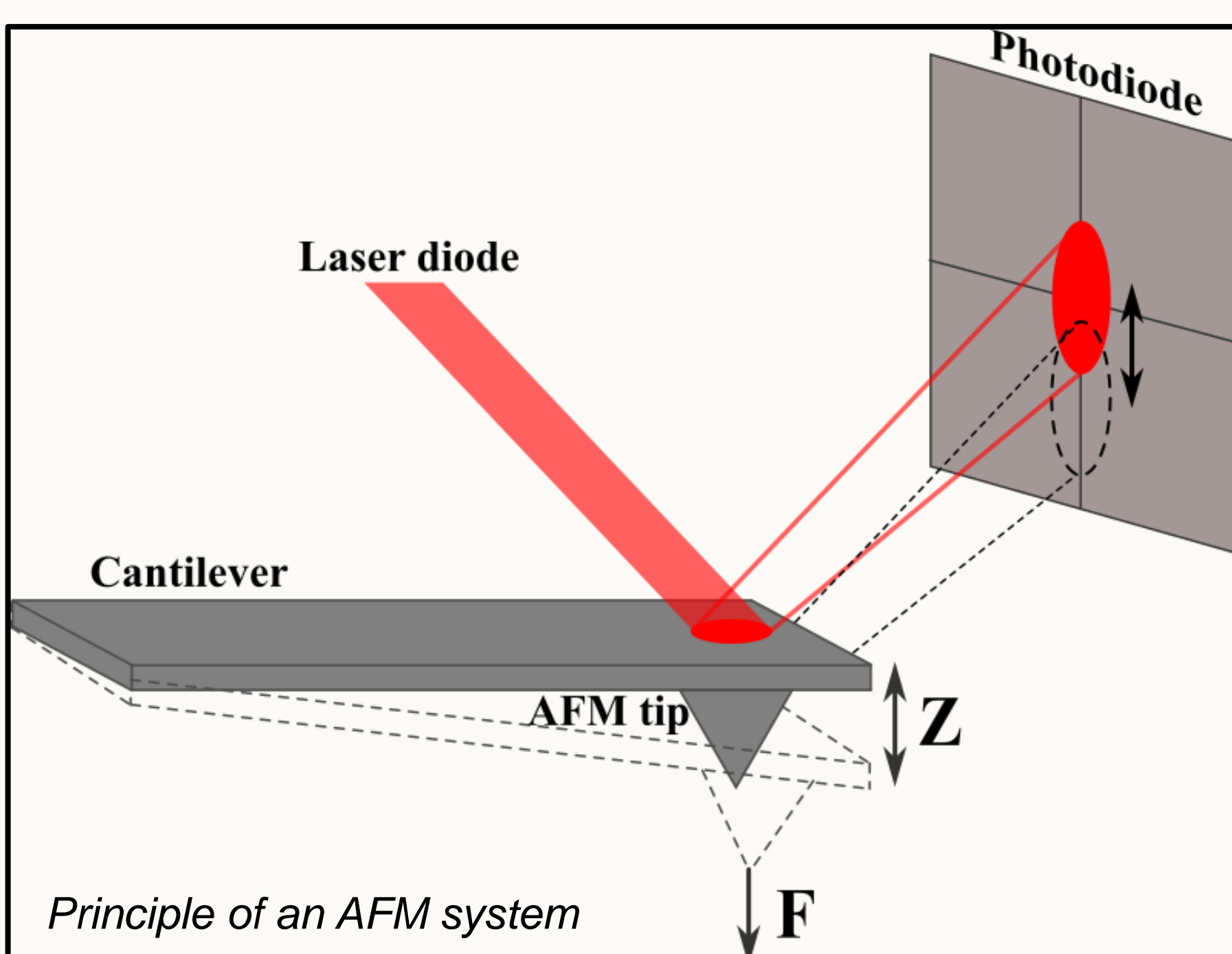
3. AFM-IR principle

Classical IR imaging techniques are limited by the diffraction. To chemically map the interface structures ($< 1\mu\text{m}$) I use novel high resolution techniques like **AFM-IR**.

AFM-IR = Atomic Force Microscopy + IR spectroscopy

Atomic Force Microscope

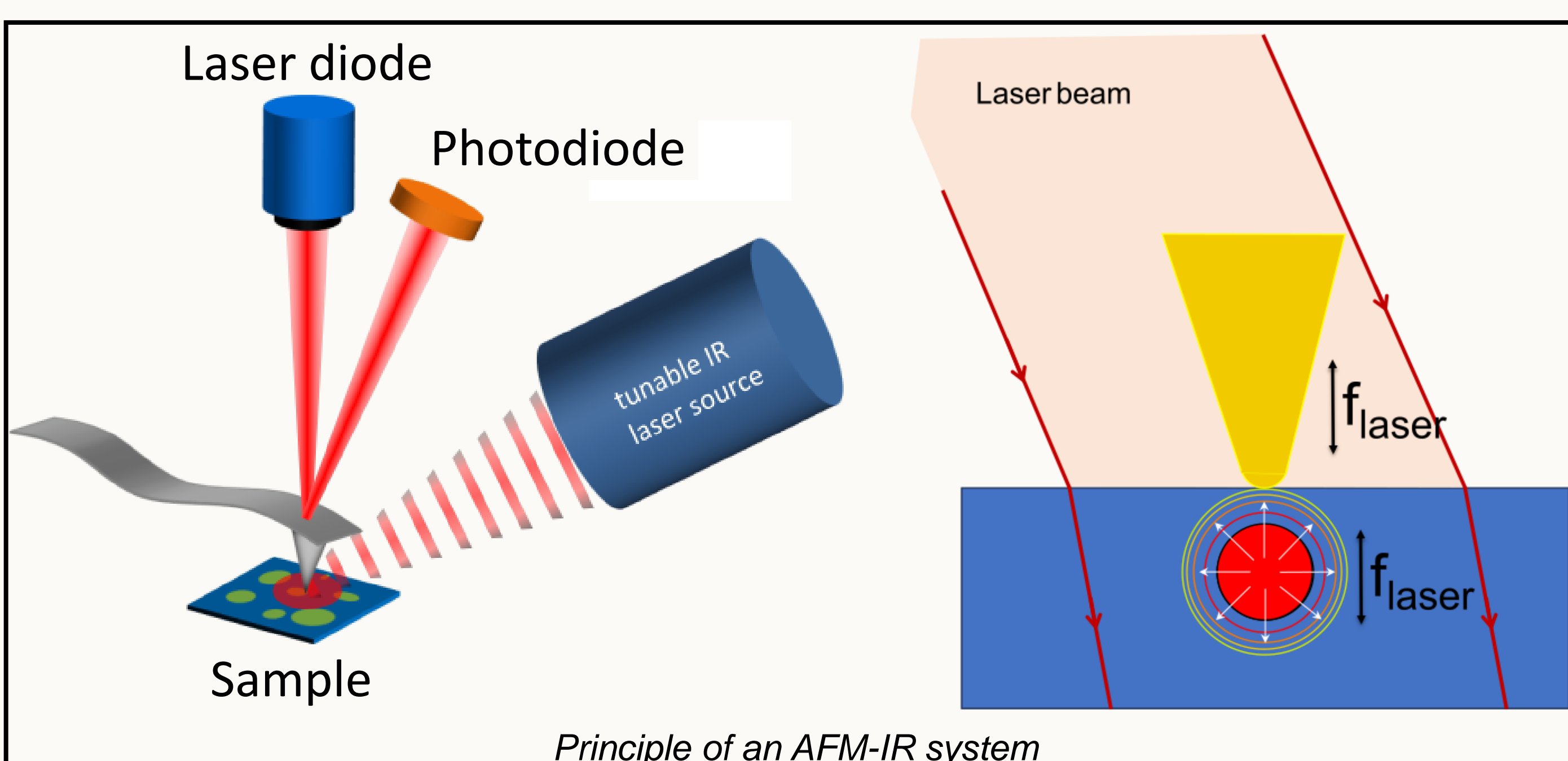
AFM for **surface analyses** → topography and mechanical properties



- AFM tip is in **direct contact** with the sample surface.
- When the surface topography changes the cantilever is deflected.
- Deflection is measured by a laser diode reflected from the cantilever to a photodiode.

AFM-IR

AFM-IR is a **photothermal imaging technique** developed by Alexandre Dazzi [2]. The **photothermal effect** is the emission of heat by an object that has been excited with an IR radiation.



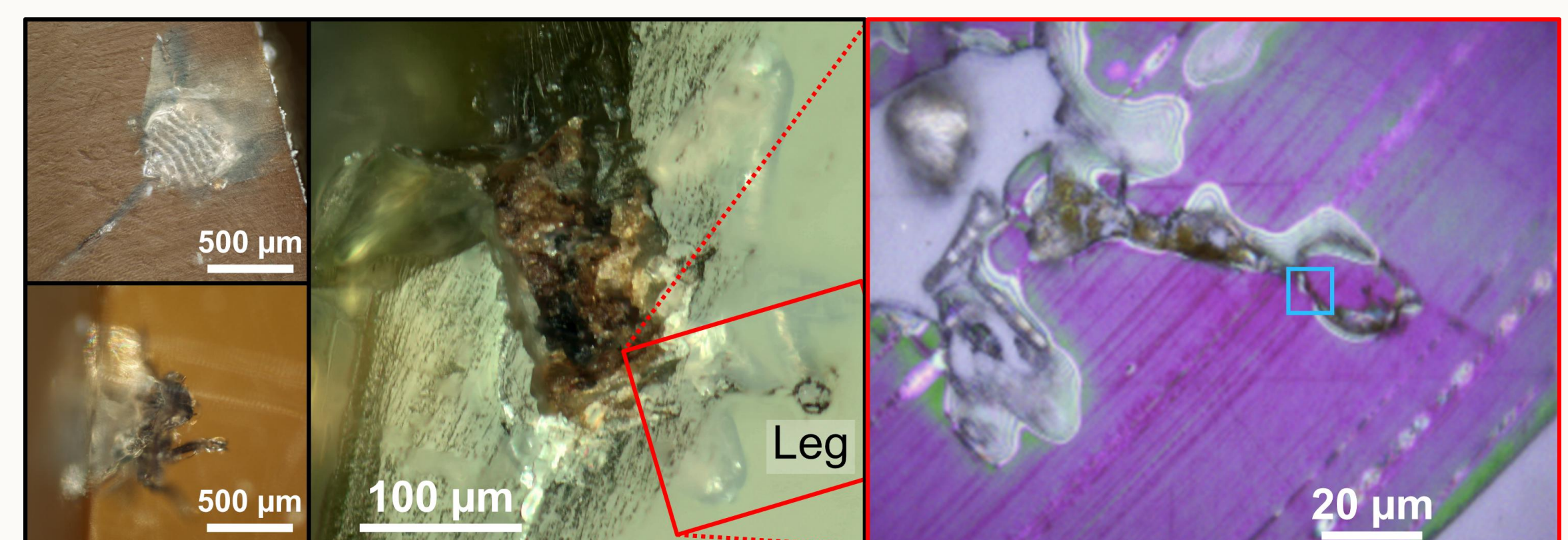
Lateral resolution ~25 nm

- IR laser source is **pulsed** and **tunable** in wavenumber and repetition rate (f_{laser}).
- Photothermal effect causes a **thermal expansion**, the resulting impulsion is felt by the tip and transmitted to the cantilever, which then oscillates.
- **The oscillation amplitude is proportionnal to the IR absorption.**

[2] Dazzi et al., 2004

4. Amber – fossil interface at nanoscale

We probe the amber – fossil interface to observe the structural and chemical interactions between the amber and the fossil at nanoscale using AFM-IR.

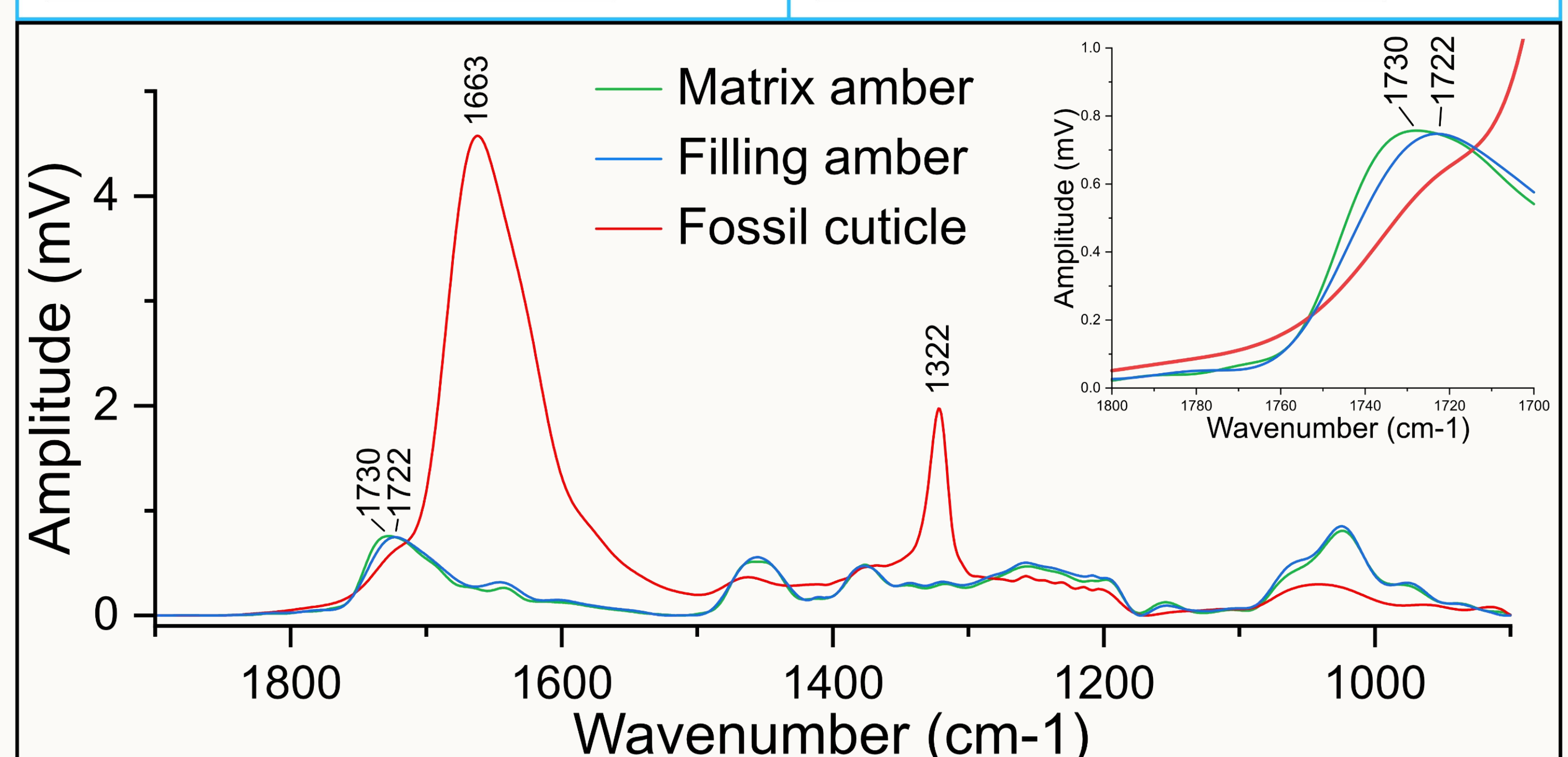
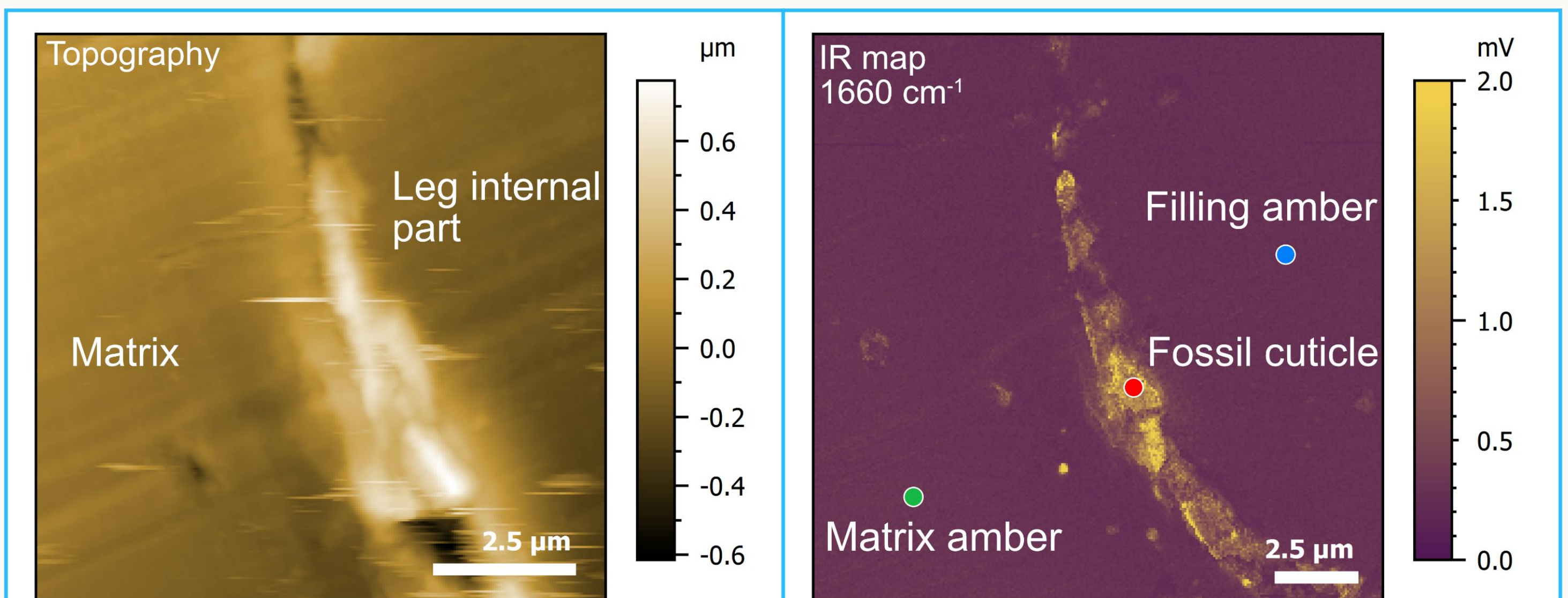


Optical images of the fossil arthropod (left), the surfaced sample (center), and of the fossil's leg in thin section (right)

Methodology

- AFM-IR measurements: ICON-IR system
- Amber/fossil cuticle interaction: IR maps
- Local chemical description of the interface: local spectra

Results: Chemical mapping and IR spectra of the fossil leg



Observations:

- **Fossil cuticle shows a distinct signal with bands at 1322 and 1663 cm^{-1} .**
- Amber penetrates behind the cuticle.
- IR signals of the matrix and filling ambers show varying band ratios in the C=O region (1730-1700 cm^{-1}), potentially due to different oxydation states.

5. Perspectives

- Identify the chemical composition of the fossil material with complementary analyses (mass spectrometry, EDX ...).
- Compare the interfaces of each sample in regard to their preservational state.
- Degradation experiments on cuticle components like chitin.