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INTRODUCTION

Over the past few years photothermal infrared (IR) nanoscopy attracted great scientific interest due to the possibility of nondestructive and label free analysis of biological samples at the nanoscale. [1]

The working principle of this technique is based on the detection of the local thermal expansion of a sample induced by absorption of pulsed IR radiation. By tuning the laser at different wavelengths and measuring the cantilever deflection the absorption spectrum of the sample is determined.

Recently, important advances in photothermal IR nanoscopy with regard to high spatial resolution (better than 25 nm) and monolayer sensitivity were developed. [2] However, so far, only static systems have been investigated. In the current work a dynamic system using photothermal IR nanoscopy was investigated for the first time.

Investigations of conformational transitions from β -sheet to α -helix in poly-L-lysine (PLL) thin film initiated by change in relative humidity are presented.

PLL THIN FILM

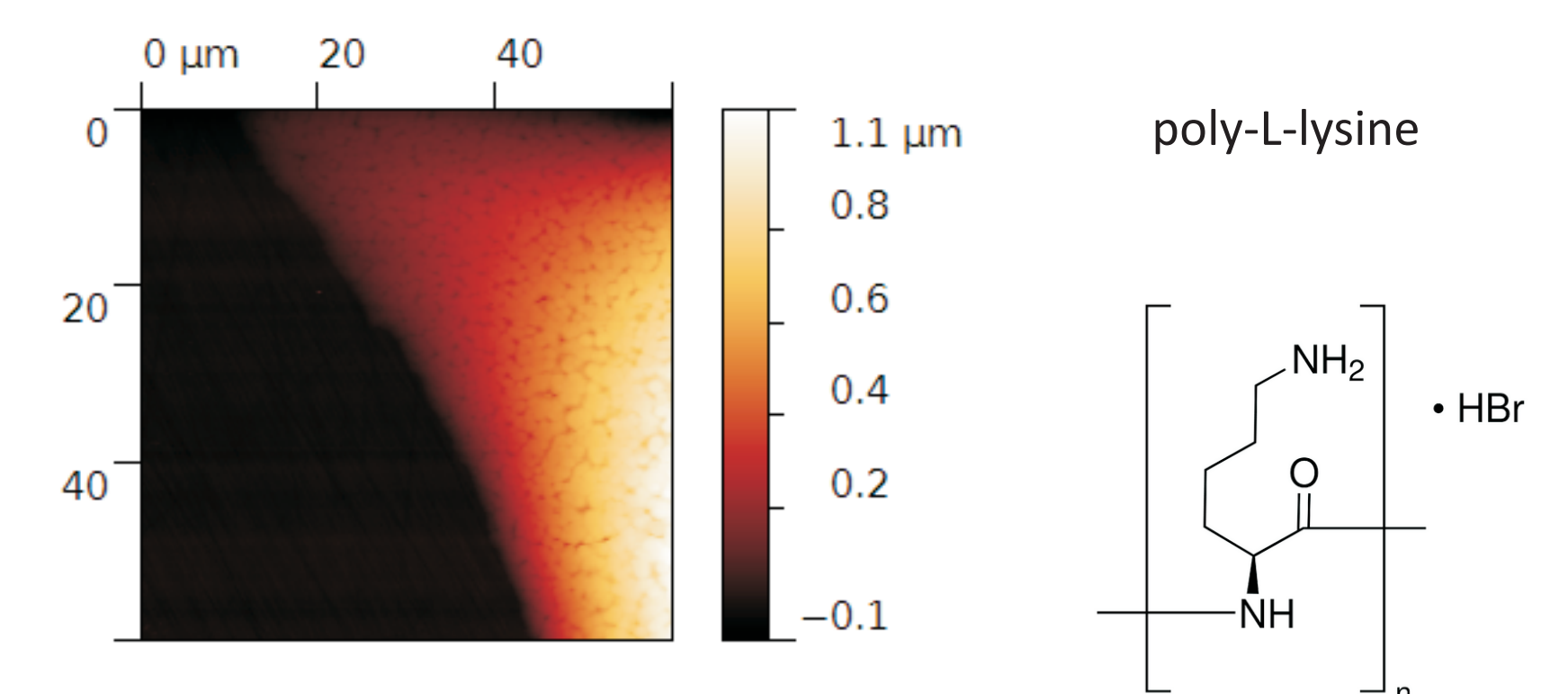


Figure 1. Topography image of the PLL thin film spin coated on a CaF_2 substrate.

EXPERIMENTAL SETUP

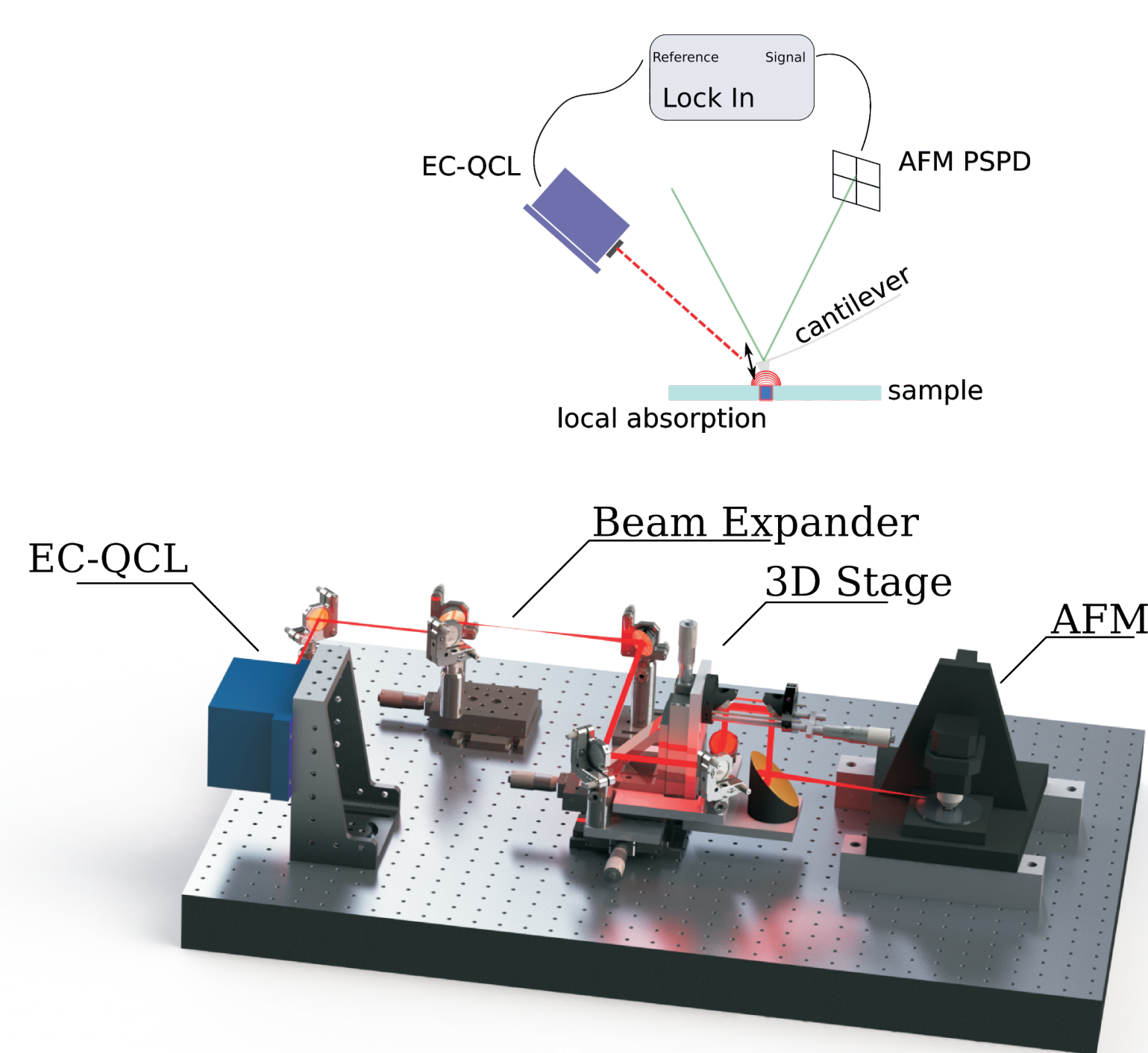


Fig. 3a. The scheme of experimental setup utilized for photothermal IR nanoscopy.

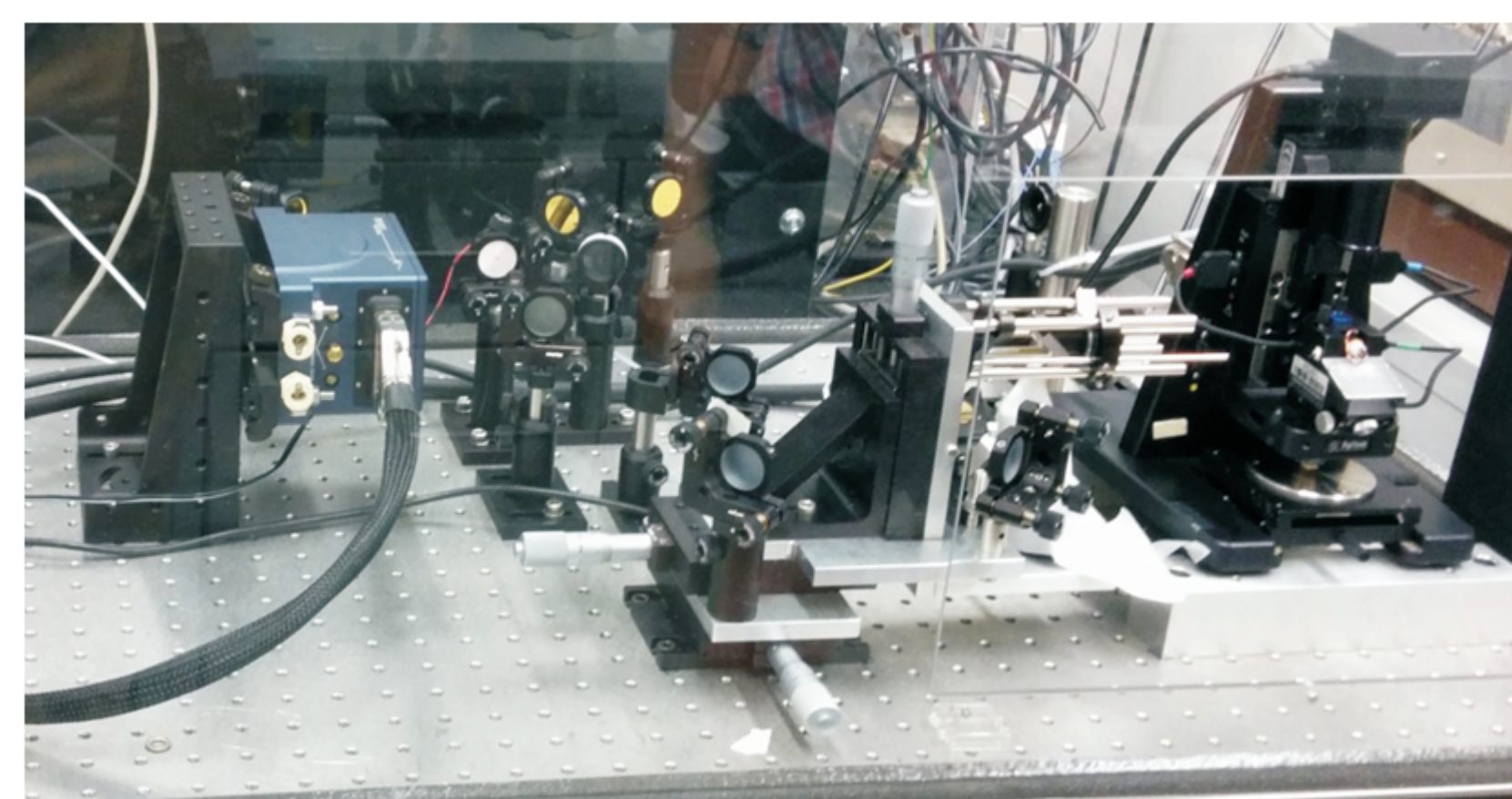


Fig. 3b. The photo of experimental setup utilized for photothermal IR nanoscopy.

The experimental setup consisted of an AFM (Agilent 5400 scanning probe microscope) coupled to an external cavity quantum cascade laser (EC-QCL, Daylight Solutions, California). The EC-QCL covered the wavelength region $1730\text{--}1565\text{ cm}^{-1}$. Measurements were performed in the resonant photothermal nano spectroscopy mode as pioneered by

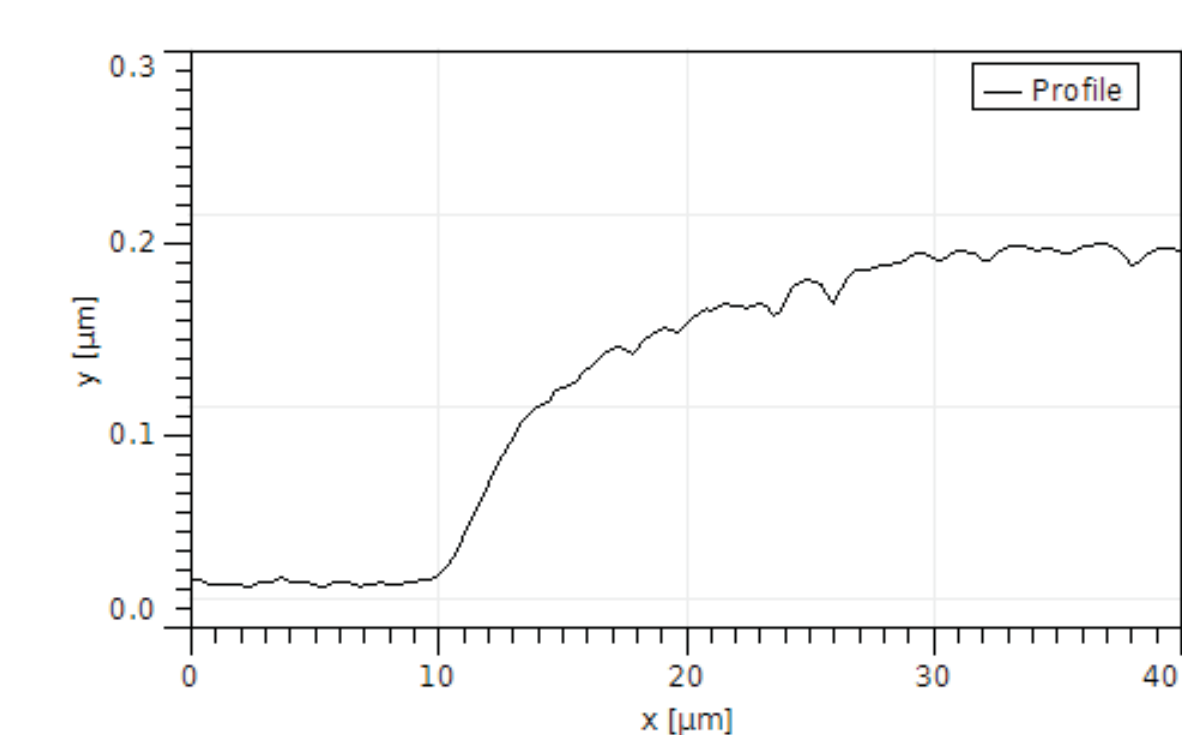


Figure 2. Sample profile

The film was cast from an aqueous solution of PLL. The solution was dropped onto a spinning CaF_2 substrate with a rotation speed of 1500 rpm. Afterwards the substrate was rotated for 1 minute to remove the residual solution. In the area of measurement the film thickness was $200 \pm 20\text{ nm}$.

PLL SECONDARY STRUCTURE FTIR

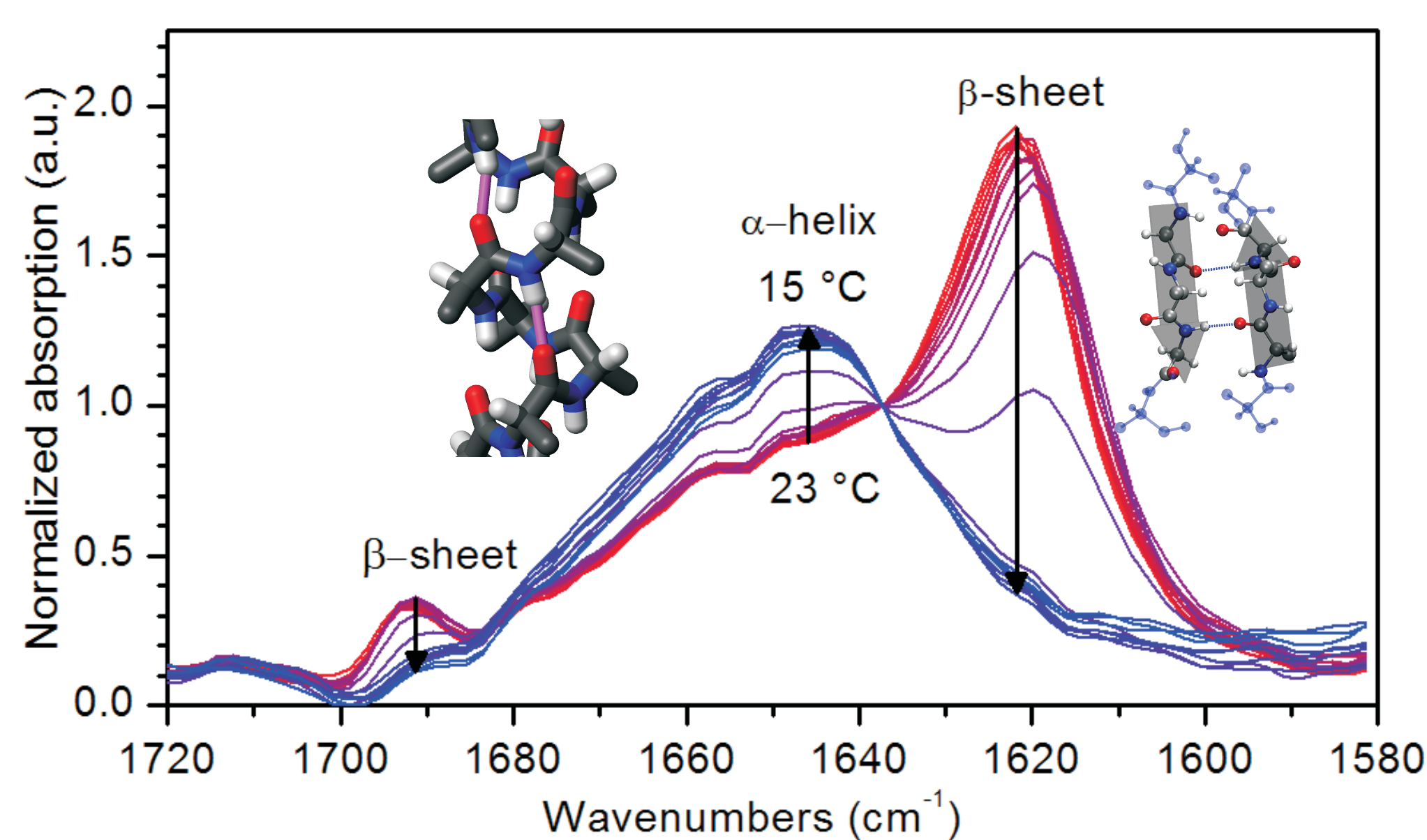


Figure 4. Time resolved FTIR spectra of secondary structure change of the PLL thin film deposited on CaF_2 substrate during the HD exchange.

Reference measurements were performed by means of FTIR transmission spectroscopy using a Bruker FTIR microscope (Hyperion 3000). During the measurement the film was flushed with air containing deuterium oxide vapor in order to perform an HD exchange. The transition from α -helix to β -sheet was initiated by increasing the relative content of deuterium oxide in PLL film [3,4]. The degree of the film hydration was changed by cooling the substrate from $23\text{ }^\circ\text{C}$ to $15\text{ }^\circ\text{C}$ during 22 minutes. The sample was mounted onto Peltier element.

PHOTOTHERMAL IR NANOSCOPY

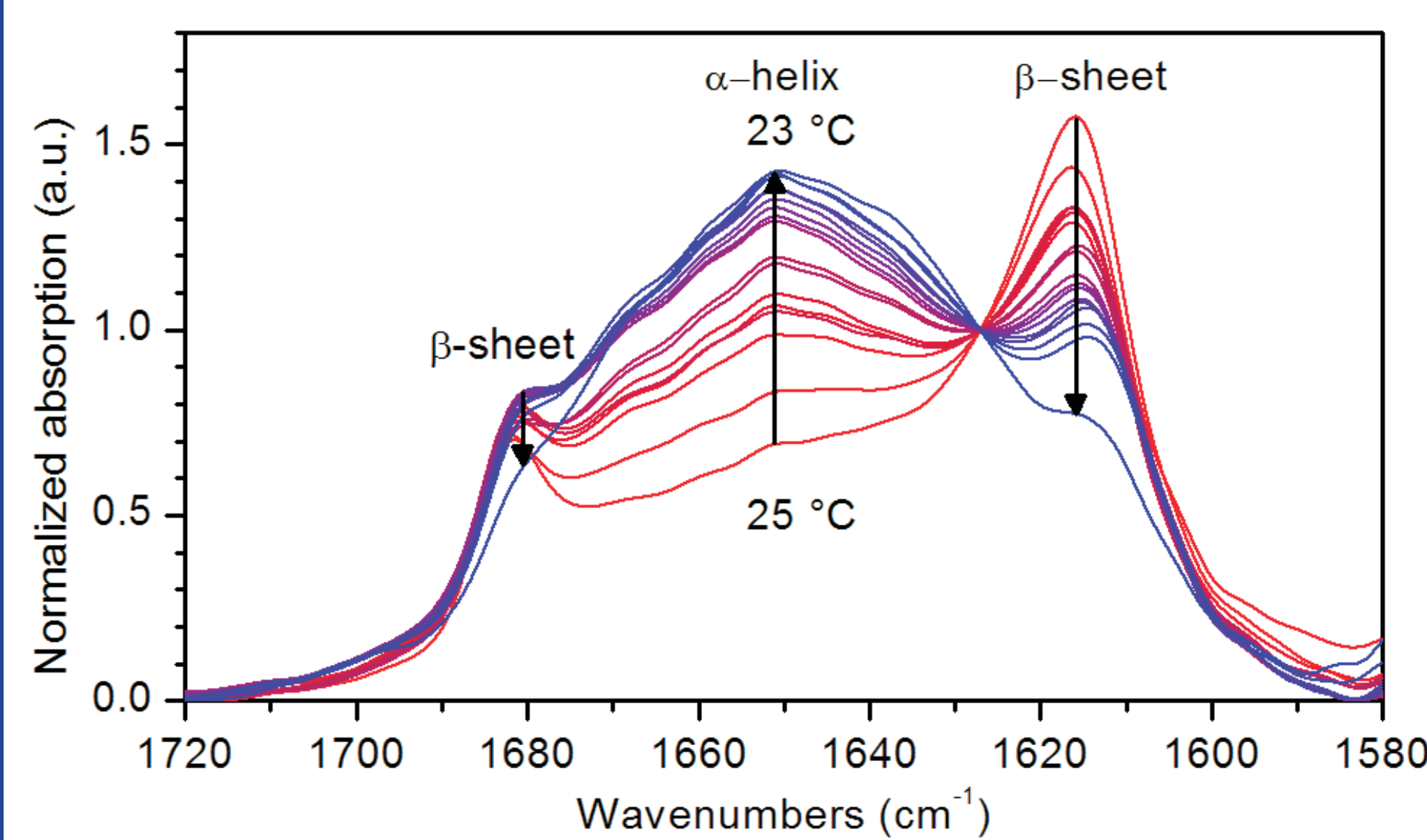


Figure 5. Time resolved photothermal IR nanoscopic measurements of secondary structure change of the PLL thin film during the HD exchange.

Similarly to the FTIR experiments change of secondary structure under the photothermal IR nanoscopy measurements was performed under a stream of air saturated with deuterium oxide. The transfer from β -sheet to α -helix was initiated by increasing the hydration level of the film by slowly decreasing the temperature of the sample from $23\text{ }^\circ\text{C}$ to $15\text{ }^\circ\text{C}$ over a time of 19 minutes. Figures 5 and 6 demonstrate the consistent results.

TIME RESOLVED MEASUREMENTS

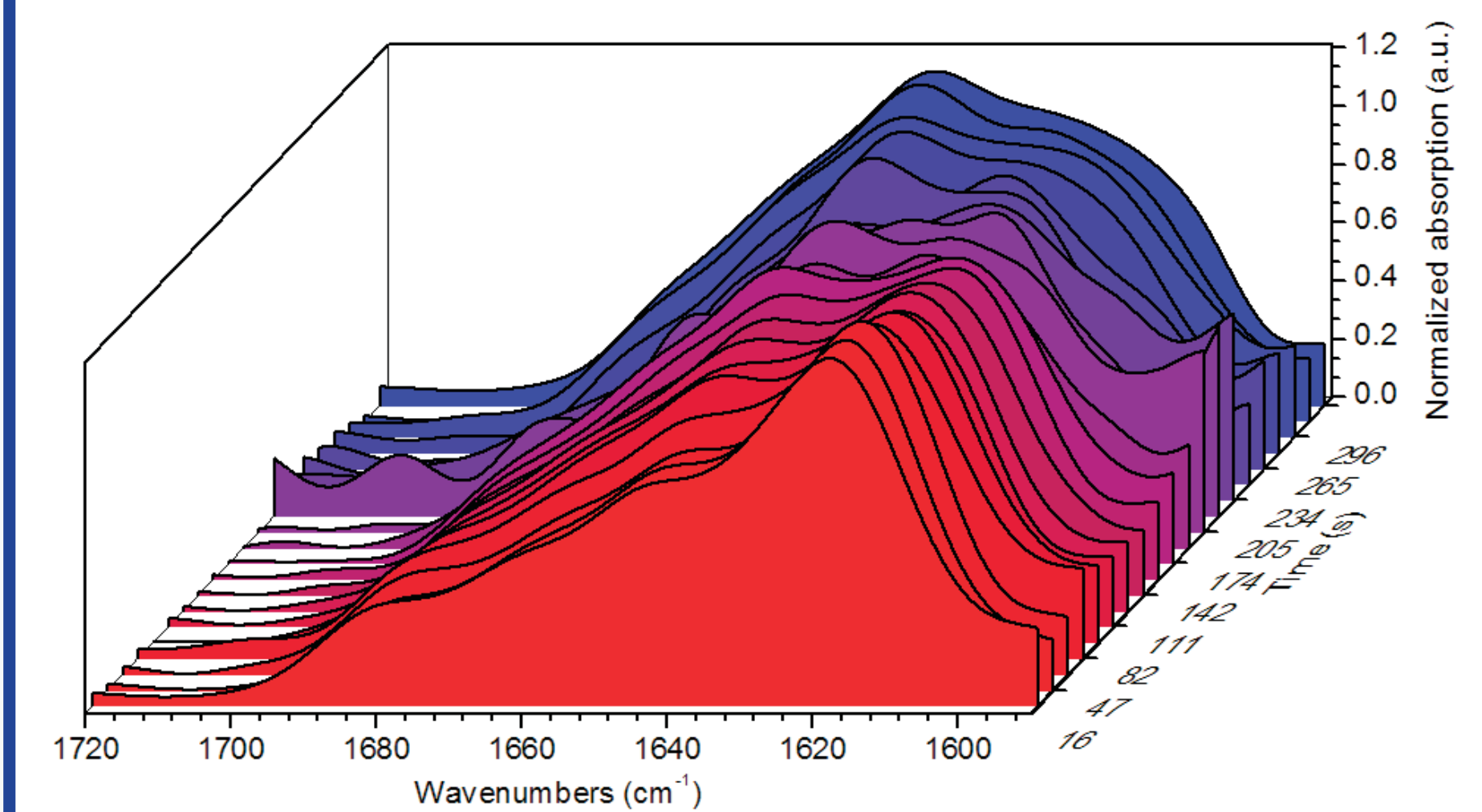


Figure 6. Fast time resolved photothermal IR nanoscopic measurements of secondary structure change of the PLL thin film taken every 15 seconds.

Photothermal IR nanoscopy based on broadly tunable EC-QCL source coupled with AFM allows fast time-resolved monitoring of spectral changes in PLL thin film as a protein model substance.

RESULTS

In this study the dynamic changes of protein secondary structure were detected by photothermal IR nanoscopy. The achieved spectra were found to consistent with analogous data obtained using a common FTIR technique. Taking into account the spatial resolution of photothermal IR nanoscopy this technique opens promising perspectives for the dynamic investigation of biological membranes. In the future we plan to improve upon our current results by increasing the spectral range of our instrument and by improving the stability and the temporal resolution of our system.

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