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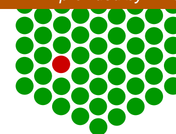

Synchrotron IR spectroscopy of single living human cells at ANKA-IR

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INTRODUCTION

The edge radiation beamline ANKA-IR promises particularly high brilliance compared to conventional synchrotron sources. This should allow us to record infrared spectra of biological materials at a spatial resolution sufficient for the resolution of single cells or even sub-cellular details, with an unprecendently high data quality.

In our five-day allocation of beamtime in August 2003, we carried out the first measurements of biological materials at ANKA-IR. Around 2000 spectra of single living human fibroblasts and adenocarcinoma cells (SW480 cell line) were recorded. These included spatially resolved spectra of single cells or of nucleus/cytoplasm regions, as well as time-resolved monitoring of spectral changes under various treatments. Analysis and evaluation of these spectra will take much longer than the time to record them, but in this poster we will present a preliminary view of our data.

EXPERIMENTAL SETUP

Cells were grown at 37 °C on 32 x 3 mm CaF_2 windows, and were assembled in a Spectra-Tech demountable IR cuvette with a pathlength of 10 microns immediately before each experiment. The cuvette was mounted on the microscope stage in a thermostatted cuvette holder (Graseby-Specac) connected with a circulating water bath set to 40 °C. The temperature in the cuvette during measurements was approx. 35 °C. The cuvette was supplied with a constant slow flow of medium from a reservoir in the same water bath.

Overview of the experimental setup

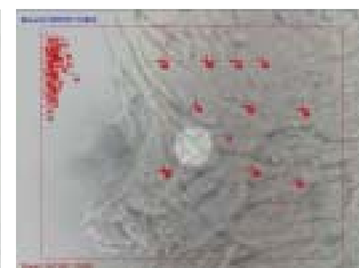
Detail of the microscope stage

RESULTS

Some snapshots of the cells

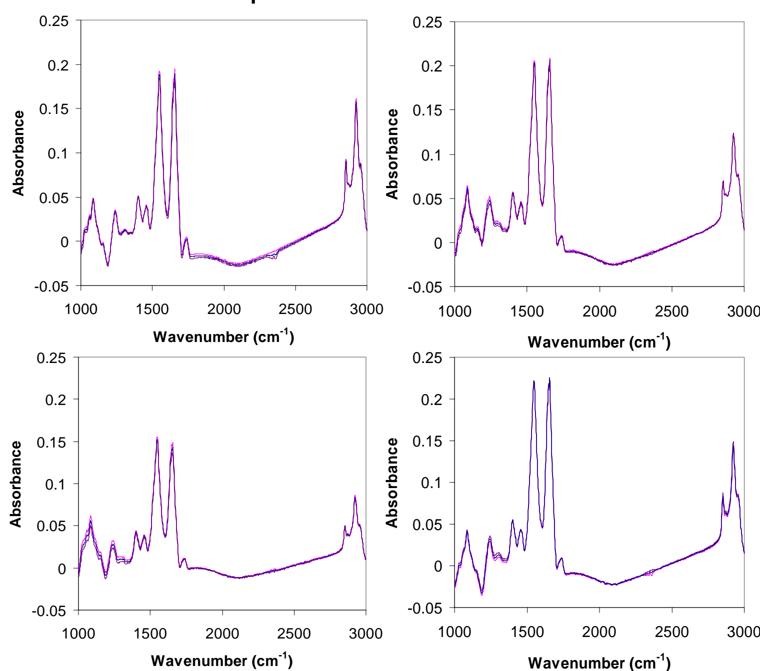
These are superimposed images with and without the aperture defining the area to be measured. The red crosses show the selected measuring positions, the software centers each of these in the aperture before the measurement.

Individual SW480 cells, 20 micron aperture



Confluent fibroblasts, 30 micron aperture

Some FTIR spectra



Mean and standard deviation IR spectra of four individual living cells from a confluent human fibroblast culture. Individual spectra were recorded through a 30 µm aperture at 512 scans/67 secs per spectrum, at intervals of 24 mins over a period of 2 hours. Reference for the absorbance calculation was an adjacent cell-free region of the culture plate. The spectra have not been scaled, normalized, smoothed or baseline corrected in any way.

CONCLUSIONS

Ask us after we finish analyzing and evaluating the 2000 spectra!