INFRARED SPECTROSCOPY OF LIVE EPITHELIAL CELLS

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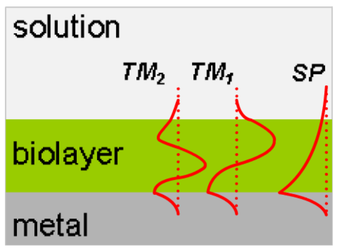
ABSTRACT

We demonstrate a real-time and label-free technique to characterize the live cells on substrate. The technique is based on infrared reflectivity measurements using an FTIR spectrometer with collimated beam. This technique combines information on molecular vibrational modes inherent to Fourier-transform spectroscopy with structural information provided by the angular-dependent reflectivity in the surface plasmon regime.

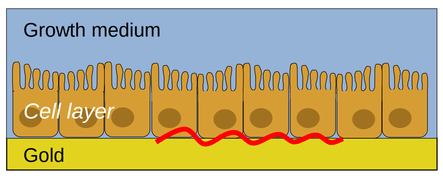
Moreover, we use the infrared light for excitation of waveguide modes inside living cell monolayer. This yields kinetics of cell-cell attachment and cell height during cell spreading, monolayer formation, calcium depletion and replenishment.

PRINCIPLES OF MEASUREMENT

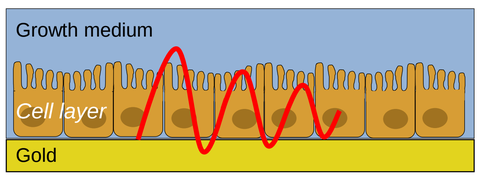


Surface plasmon (SP) senses the part of the layer in contact with substrate.

Waveguide modes (TM_n) sense the whole layer.



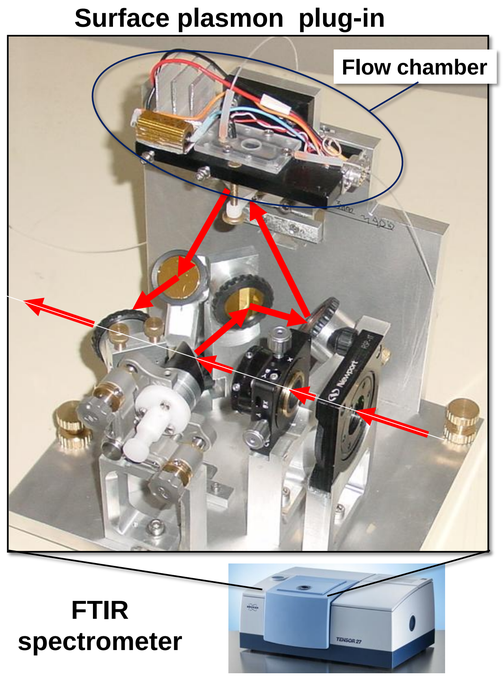
Surface plasmon senses:
- *cell refractive index*
- *cell-substrate adhesion*



Waveguide mode senses:
- *cell-cell adhesion*
- *cell height*

METHODOLOGY

Surface plasmon plug-in



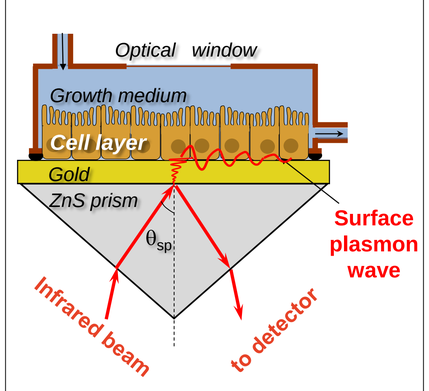
FTIR spectrometer

Cells live in growth medium (mostly water).

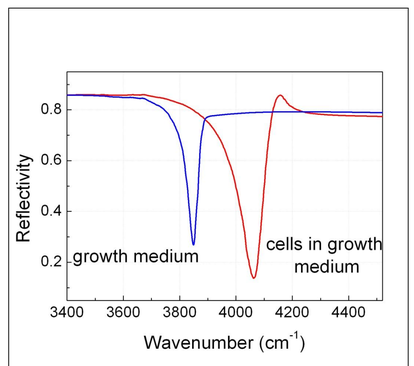
Strong water absorption prevents infrared spectroscopy using *plane waves.*

Surface plasmon wave probes cells directly and is less affected by water absorption.

Flow chamber



Surface plasmon is a surface electromagnetic wave propagating along the metal-dielectric interface

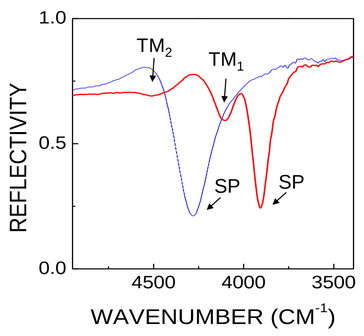


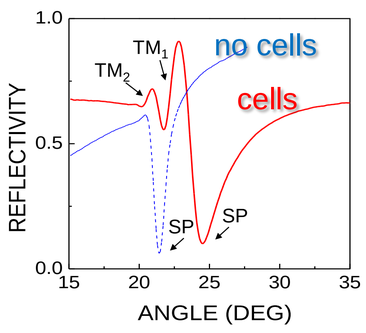
Surface plasmon resonance appears as a sharp dip in the angular – dependent optical reflectivity

Its wavenumber/width yields refractive index/absorption of the analyte

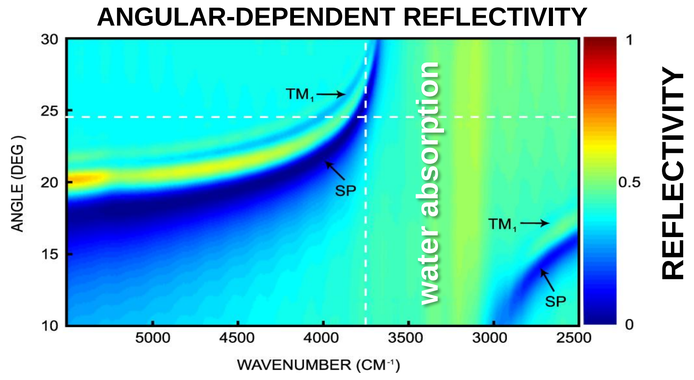
MEASUREMENT EXAMPLES

MDCK CELLS ON GOLD



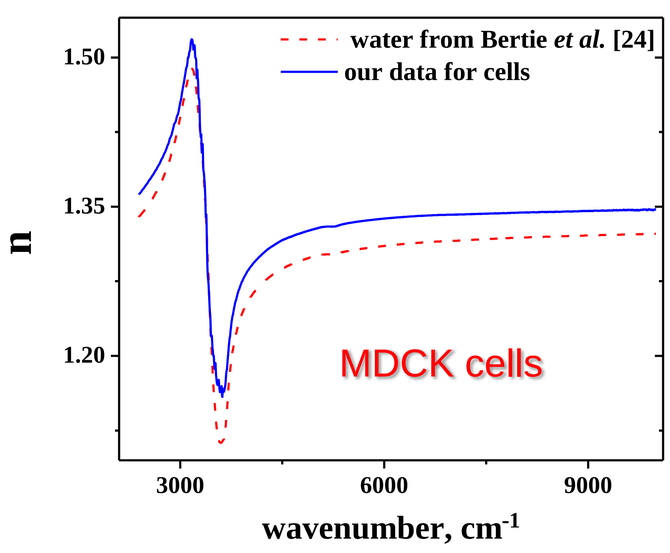


ANGULAR-DEPENDENT REFLECTIVITY

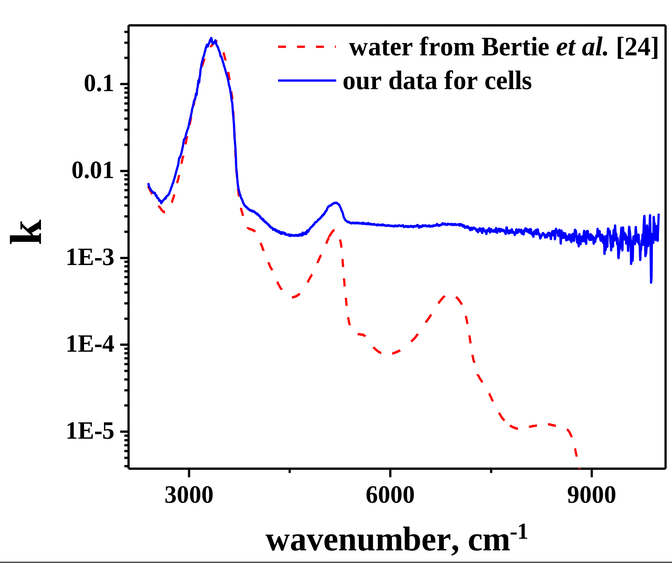


SURFACE PLASMON SPECTROSCOPY YIELDS COMPLEX REFRACTIVE INDEX OF CELLS

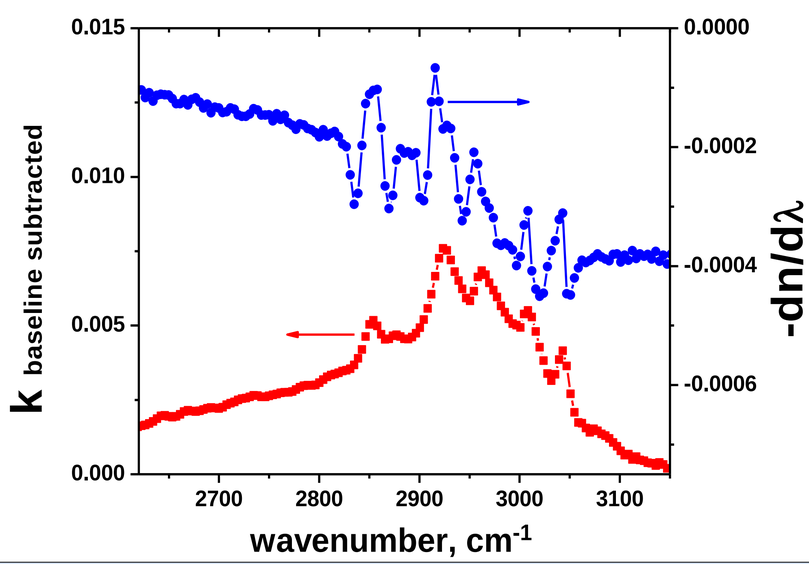
cell refractive index, n



cell absorption, k

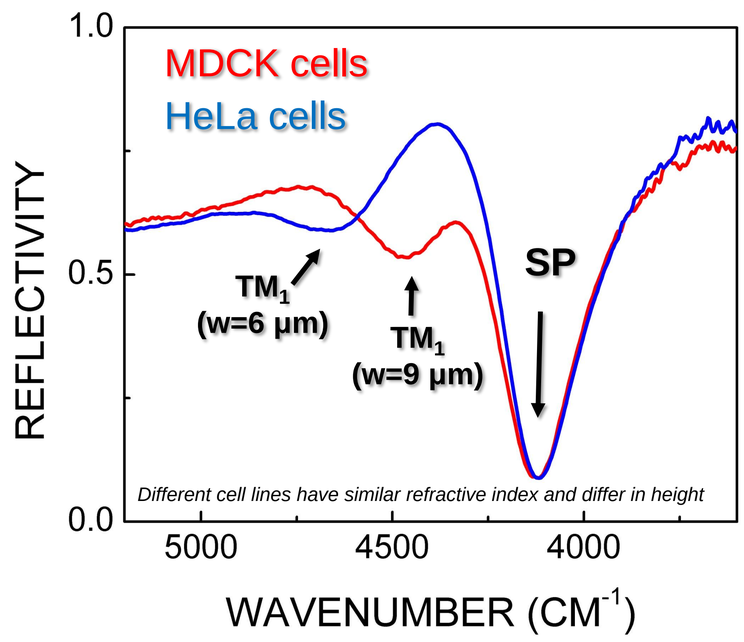


Specific absorption bands of live cells



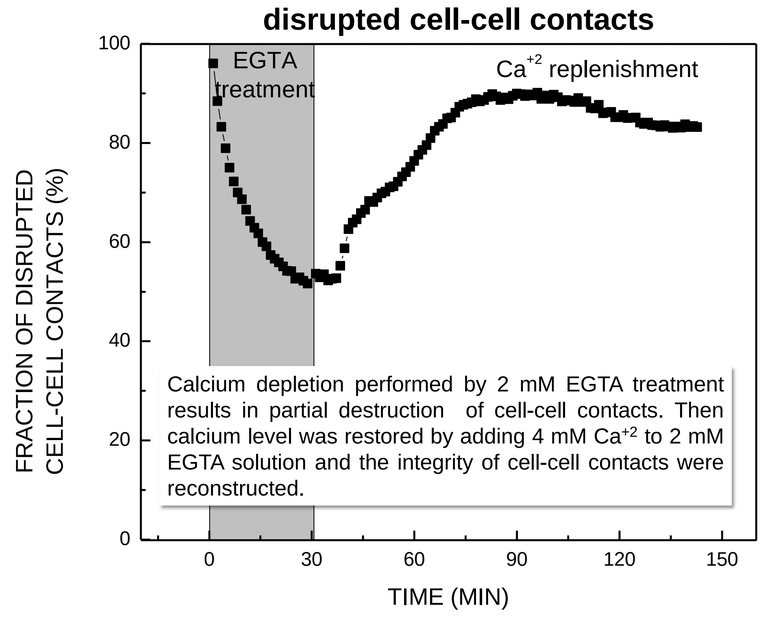
WAVEGUIDE MODE SPECTROSCOPY YIELDS STRUCTURAL INFORMATION ON CELLS

Measurement of *cell monolayer height*



Kinetic measurement of *cell-cell adhesion*

disrupted cell-cell contacts



Calcium depletion performed by 2 mM EGTA treatment results in partial destruction of cell-cell contacts. Then calcium level was restored by adding 4 mM Ca^{+2} to 2 mM EGTA solution and the integrity of cell-cell contacts were reconstructed.

cell layer height