Label-free imaging of virus-cell interactions using 200 Hz ROCS microscopy

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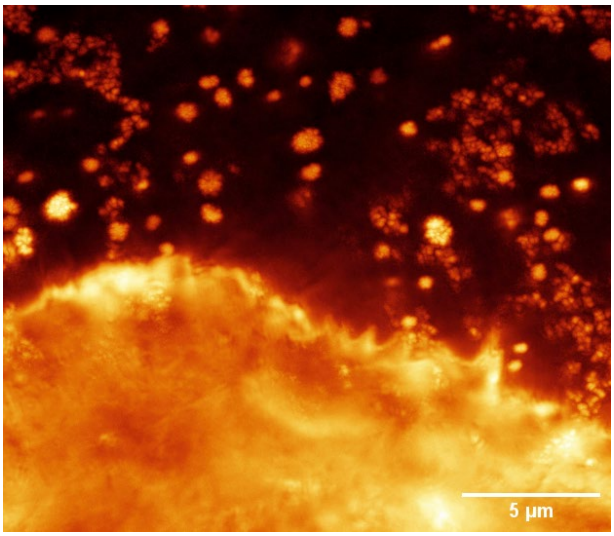
Background incl. aims
The emergence of SARS-CoV-2 has shown the profound impact that viruses can have on human health and society. Due to their small size and remarkable speed it is very challenging to observe cell-virus interactions in life-cell imaging. Techniques like fluorescence microscopy, often struggle to visualize these interactions, especially due to their susceptibility to bleaching and the difficulty to label different structures without altering their function. This underscores the ongoing need for innovative approaches to unravel the complexities of virus-cell interactions and advance our understanding of infectious diseases.

Methods
In our research we use Rotating Coherent Scattering (ROCS) microscopy in order to visualize the diffusion of 100 nm sized virus-mimicking particles and their interactions with macrophages or epithelial cells. Rotating Coherent Scattering (ROCS) microscopy, a label-free imaging technique, harnesses the coherent backscattering of a rotating laser beam for super-resolution (150nm). The method involves oblique illumination of the sample from all azimuthal directions, producing nearly artifact-free images by integrating all scattered light during a single laser rotation. Currently operating at a frame rate of 200 Hz, ROCS minimizes motion blur, without any need for extensive post-processing. With its capability to adjust the illumination angle up to 70° and to utilize 4 distinct illumination wavelengths in the range of 405 nm to 561 nm, ROCS proves highly versatile in brightfield or darkfield mode, transitioning between total internal reflection (TIR) and non-TIR.

Results
By tracking particle trajectories and analyzing their fluctuations we are able to analyze the speed of our diffusing virus-mimicking particles and are able to follow binding events of single particles to cells. After binding of particles to filopodia or lamellipodia the observed fluctuations decrease over time and Additionally, by using multiple illumination angles and wavelengths in ROCS microscopy we observe different appearances of differently absorbing structures, enabling the analysis of subtle z-motions of particles and the visualization of distinct cellular structures.

Conclusion
Our research highlights Rotating Coherent Scattering (ROCS) microscopy, as a powerful tool to observe virus-cell interactions. Through ROCS, we successfully image the diffusion of virus-mimicking particles and visualize discrete binding events to cells, enabling detailed analysis of particle trajectories and binding properties. Furthermore by capturing scattered light from multiple wavelengths at once, we extend the capability of ROCS towards obtaining specificity for different structures.

Graphic:



Keywords:

Rotating-Coherent-Scattering-Microscopy (ROCS), single-virus tracking, label-free

Reference:

Jünger, F., D. Ruh, D. Strobel, R. Michiels, D. Huber, … A. Rohrbach (2022). "100 Hz ROCS microscopy correlated with fluorescence reveals cellular dynamics on different spatiotemporal scales." Nature Communications 13(1): 1758.