STED MICROSCOPY OF INFLUENZA VIRUS ASSEMBLY

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Influenza A viruses cause seasonal epidemics and occasionally lead to pandemics. Therefore, it is of great interest to understand their infection and replication. Each virus contains a genome composed of 8 single stranded RNA segments, which exist as ribonucleoprotein complexes (vRNPs). How the genome is assorted and selectively packaged into newly forming virus particles is an area of ongoing research [1].

Multicolor STED microscopy is emerging as a new tool to study the interaction of several molecular species simultaneously at a high spatial resolution in living or fixed cells [2]. Imaging with diffraction unlimited resolution is very beneficial when multiple targets are present at a high density. It enables the discrimination of otherwise overlapping signals from inside a confocal volume, allowing for example an accurate colocalization analysis.

The feasibility of using multicolor STED microscopy to image several viral RNA segments at once in infected cells, concurrently with other cellular structures of interest, was demonstrated. Viral RNA was stained with FISH using fluorescently labeled oligonucleotides. Other targets which may play a role in the viral replication and assembly process, such as tubulin or stress granules, were labeled with antibodies. Multicolor STED imaging of fixed infected cells in 2D and 3D was performed on a specialized, custom-built microscope setup featuring four intrinsically coaligned detection channels [3]. The acquired raw images were unmixed using a non-negative matrix factorization algorithm first introduced by [4].

We identified coupling of the oligonucleotide probes with STED-compatible organic dyes, the ability to repeatedly localize a region of interest in the sample after sequential staining and to image it with diffraction unlimited resolution in 3D as key technical challenges. Once the labeling challenge is mastered and a suitable microscope combining all available technology is at hand, multicolor STED imaging can be established as a valuable tool to study virus replication.

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