AFM-STED correlative microscopy reveals an unexpected asymmetry in the aggregation of partially labeled peptides.

Claudio Canale[1], Michela Cosentino[2], Paolo Bianchini[2] and Alberto Diaspro[1,2]

1. Department of Physics, University of Genova, via Dodecaneso 33, 16146, Genova. Italy

2. Department of Nanophysics, Istituto Italiano di Tecnologia, via Morego 30, 16163, Genova, Italy

KEY WORDS: STED, AFM, correlative microscopy, amyloid aggregates

Fluorescence microscopy and spectroscopy are widely employed in the study of molecular processes, both in vitro and in vivo. Except in a few cases, fluorescence techniques are based on the use of an external molecular dye that is bound to particular sample components by using different strategies.

In the last decays, microscopy goes beyond the diffraction limit, thanks to innovative approaches, generally defined as super-resolution microscopy. SR microscopy provides new insights into the biological processes at the, hitherto inaccessible, molecular scale. The majority of the SR techniques are fluorescence microscopy modes.

Correlative nanoscopy is a new term indicating the integrated used of microscopy methods that investigate the sample at the nanoscale. A super-resolution fluorescence microscope combined with an atomic force microscope (AFM) is an example. The two techniques provide different data sets from the sample, defining a new functionality of working and opening new scenarios in biomolecular investigations.

In particular, we employed a stimulated emission depletion (STED) microscope coupled with an AFM to the study of amyloid aggregates formation. We worked in vitro, inducing the aggregation of different peptides, following standard methods, and labeling the samples at different dye to protein ratio, and by using different conventional approaches.

The results obtained for insulin, Aβ1-42, and Aβ1-40 define the same scenario: only a fraction of the fibrillar aggregates is visible in STED images, indicating that the labeled molecules were not participating indistinctly to the aggregation process, and supporting the hypothesis that labeled molecules are able to follow only selected pathways of aggregation. The results obtained by correlative AFM-STED microscopy generate a warning: fluorescence techniques are not able to characterise all the products derived from the aggregation of misfolded proteins. This statement reveals the importance to correlate fluorescence data with other derived from label-free techniques.