CO-ORIENTATION: SIMULTANEOUS COLOCALIZATION AND ORIENTATIONAL ALIGNMENT OF FILAMENTS IN NANOSCOPIC IMAGES

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The cellular cytoskeleton is comprised of networks of protein filaments in the cell’s cytoplasm, which play important roles in determining the spatial organization of the cell and in many cellular processes. To understand how different types of cytoskeletal filaments together perform these functions, it is important to obtain insight into the nanoscale spatial arrangements of these networks. Until recently the analysis of these dense networks was hindered by the diffraction limit to the resolution of optical microscopy. Superresolution microscopy techniques circumvent this limit, thus making it possible to resolve these networks and characterize their spatial arrangements.

We introduce a rigorous quantitative framework for characterizing the spatial relationships between filament networks in nanoscopic images. In this framework we determine the orientations of filaments using an orientation space representation [1]. We then quantify how the orientational alignment of filaments affects their colocalization (as measured by the pair cross-correlation function [2]); the simultaneous orientational alignment and colocalization of filaments will be referred to as co-orientation. Because the orientation depends on the length scale at which it is evaluated, we include this scale as a separate informative dimension for analysis.

Moreover, we show how the strength of the co-orientation can be quantified locally by an anisotropic generalization of Ripley’s K-coefficient and we propose a test for its statistical significance. We demonstrate our methods on simulated localization microscopy data of filament structures, as well as experimental multi-color images of filamentous structures.

![Figure 1. Left: Two filament networks with an overlay indicating the local orientation strength (blue). Right: co-orientation plot.](image)

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