

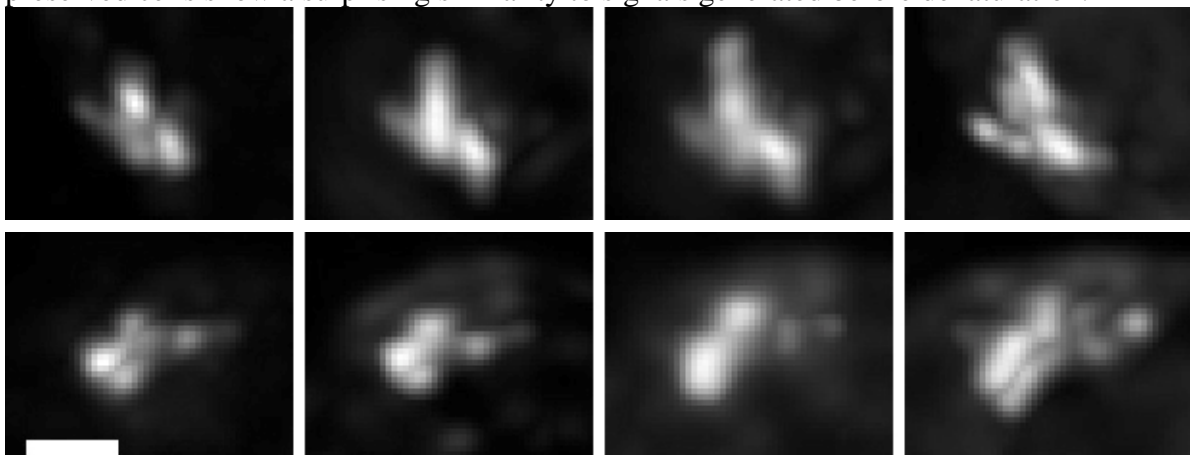
QUANTITATIVE COMPARISON OF FLUORESCENT SIGNALS CREATED BY GFP-TAGGING, IMMUNOSTAINING AND FLUORESCENCE IN SITU HYBRIDIZATION FROM THE SAME STRUCTURE

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KEY WORDS: green fluorescent protein, immunostaining, fluorescence in situ hybridization; detection, cell biology.

Green fluorescent protein (GFP)-fusion proteins and immunostaining are methods broadly applied in modern cell biology to generate fluorescent signals for high resolution microscopy. This allows investigation of the three-dimensional organization of cells and cell nuclei, the latter often studied in addition by fluorescence in situ hybridization (FISH). Direct comparisons of these detection methods are scarce, however. We performed a quantitative comparison of all three approaches, calculating correlation coefficients. We made use of a cell line that contains a transgene array of lac operator repeats which are detected by GFP-lac repressor fusion proteins. Thus we could detect the same structure in individual cells by GFP fluorescence, by antibodies against GFP and by FISH with a probe against the transgene array. Anti-GFP antibody detection was repeated after FISH. Our results show that while all four signals obtained from a transgene array generally showed qualitative and quantitative similarity, they also differed in details. Each of the tested methods revealed particular strengths and weaknesses, which should be considered when interpreting respective experimental results. Despite the required denaturation step, FISH signals in structurally preserved cells show a surprising similarity to signals generated before denaturation.



Fluorescent signals from two structures generated by (from left to right): GFP, anti-GFP immunofluorescence, anti-GFP immunofluorescence after FISH, FISH. Projections of widefield-deconvolution images, scale bar: 1 μ m.

Reference: I.H. Kim, J. Nagel, S. Otten, B. Knerr, R. Eils, K. Rohr, and S. Dietzel, "Quantitative comparison of DNA detection by GFP-lac repressor tagging, fluorescence in situ hybridization and immunostaining" *BMC Biotechnol.* **7**, 92 (2007).