Type of presentation: Poster

LS-3-P-2756 The "blinking" FISH: Localization microscopy of chromatin nanostructure using combinatorial oligonucleotide fluorescence in situ hybridization (COMBO-FISH)

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Expansions of DNA repeats are responsible for many human hereditary disorders. The fragile X syndrome (FXS), a neuro-developmental disorder, is the most prominent cause of heritable mental retardation. In the last years it became evident that unusual structural features of the chromatin and the nuclear dynamics in these expandable repeats causes instability of the gene region and lead to their further expansion. In this study the quantification of the repeat expansion and the resulting nanostructural changes were examined by a novel interdisciplinary approach that is based on combinatorial oligonucleotide fluorescence in situ hybridization (COMBO-FISH) and spectrally assigned localization microscopy. For this, fluorescence labelled oligo-nucleotides were used to label the repeats uniquely as well as the FMR1 gene regionin 3D intact cell nuclei. The localization of the hybridized and labelled oligo-positions with nanometer precision was performed using Spectral Precision Distance/Position Determination Microscopy (SPDM) in the ‘blinking’ mode. The correlation of the obtained data on the single cell level with standard human genetic diagnostic methods with bulk DNA may contribute to understand the impact of chromatin nano-architecture on repeat instability and expansion. Furthermore, a new diagnostic approach based on optical sequencing is envisaged to count repeats in cell nuclei especially in cases of premutated alleles that are very unstable with a strong tendency to expand to the full mutation upon transmission to the next generation.

C. Cremer et al. (2011)Superresolution Imaging of Biological Nanostructures by Spectral Precision Distance Microscopy (SPDM), Biotechnology Journal 6: 1037 – 1051

Figure legend

Left: Dual colour COMBO-FISH experiment on human fibroblast cells. (1) conventional wide-field fluorescence image of the fragile X gene region labelled with Alexa488 and Alexa568. (2) SPDM image λex: 488nm. (4) SPDM image λex: 568nm. (3) Overlay of (2) + (4). Right: (A) Reconstruction of the SPDM data, the intensity values of the individual signals are proportional to the number of adjacent signals. (B) The positions of the signals are shown in green and the determined area of the clusters in red. (C) Visualization of the detected structure

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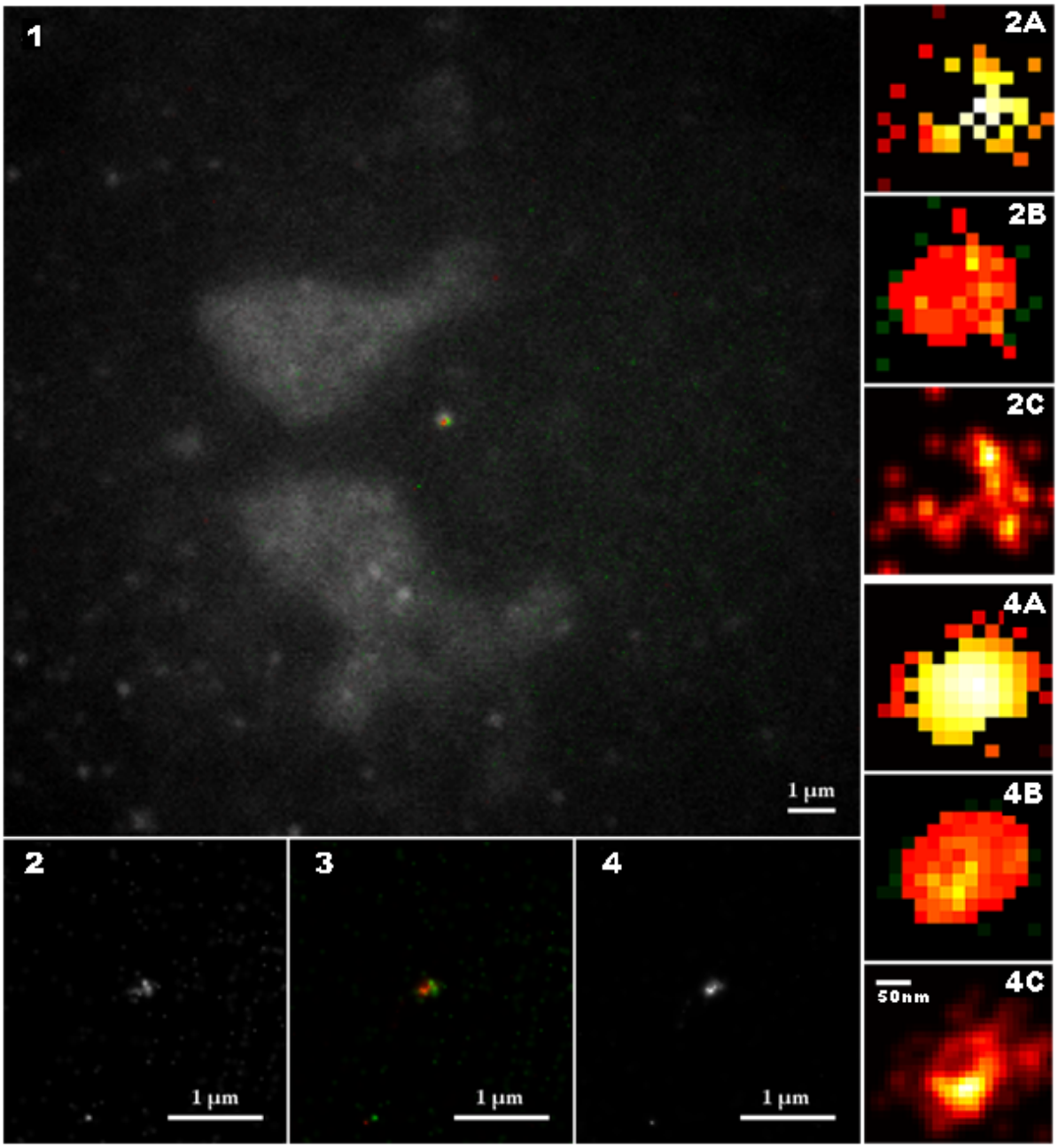

Fig. 1: see Text