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BIOLOGICAL AND BIOMEDICAL APPLICATIONS OF SUPER-RESOLUTION MICROSCOPY

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AIM

To review the biological and biomedical applications of super-resolution microscopy.

INTRODUCTION

- Optical microscopy is limited by its optical resolution, especially concerned with the diffraction limit.
- Super-resolution microscopy (SRM) holds new promise in a wide range of applications.
- SRM improves resolution limit by a factor of two over diffraction by temporally or spatially modulating excitation or activation of light.
- Development of SRM techniques like stimulated emission depletion (STED), photoactivation localization microscopy (PALM), stochastic optical reconstruction microscopy (STORM), and structured illumination microscopy (SIM) has paved new avenues in the field of biological and biomedical research.
- SRM enables imaging of cellular structures with a level of detail that was not possible with optical microscopy.
- The extraction of quantitative data on spatial distributions is also made possible by SRM.
- SRM also offers details on genetic material modifications and the precise quantities of proteins or other macromolecules in subcellular compartments
- SRM techniques delivers experimental input for various complicated biological interaction models and show three-dimensional (3D) structural features.

EXPERIMENTAL SETUP

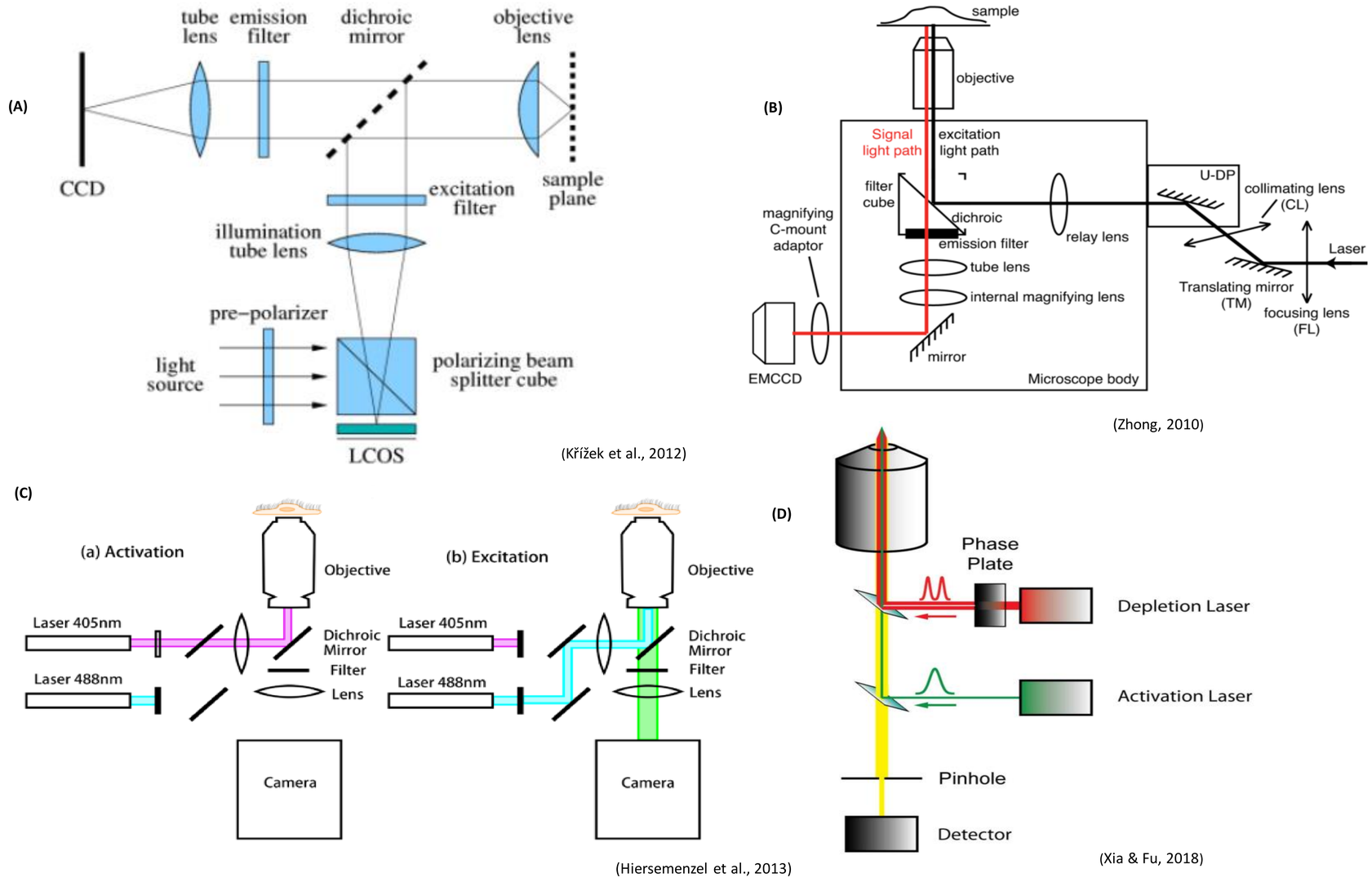

Fig 1 : Figures representing Optical setup of **(A) Structured Illumination Microscopy (SIM) (B) Photoactivation localization microscopy (PALM) (C) Stochastic optical reconstruction microscopy (STORM) (D) Stimulated emission depletion (STED)**

RESULTS

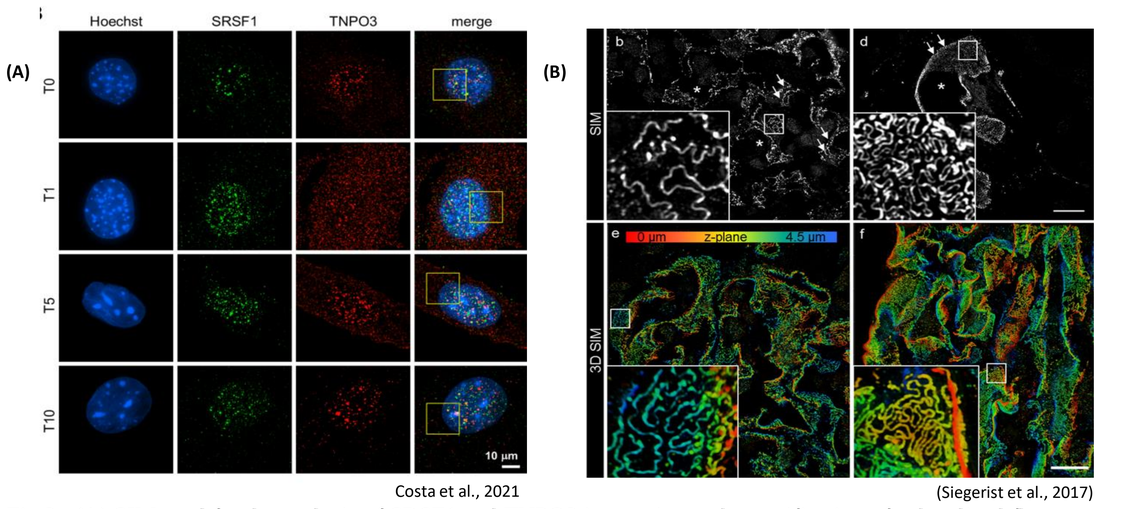

Fig 2 : (A) SIM used for the analysis of SRSF1 and TNPO3 interaction and quantification of colocalized fluorescent signal.) Immunofluorescence double staining for TNPO3 (in red) and SRSF1 (in green) was observed through a structured illumination microscope (SIM). Nuclei are counterstained with Hoechst (in blue) . In the fourth column merge of TNPO3 and SRSF1 fluorescent signals (B) Micrographs of nephrin-stained glomeruli obtained after SIM reconstruction.

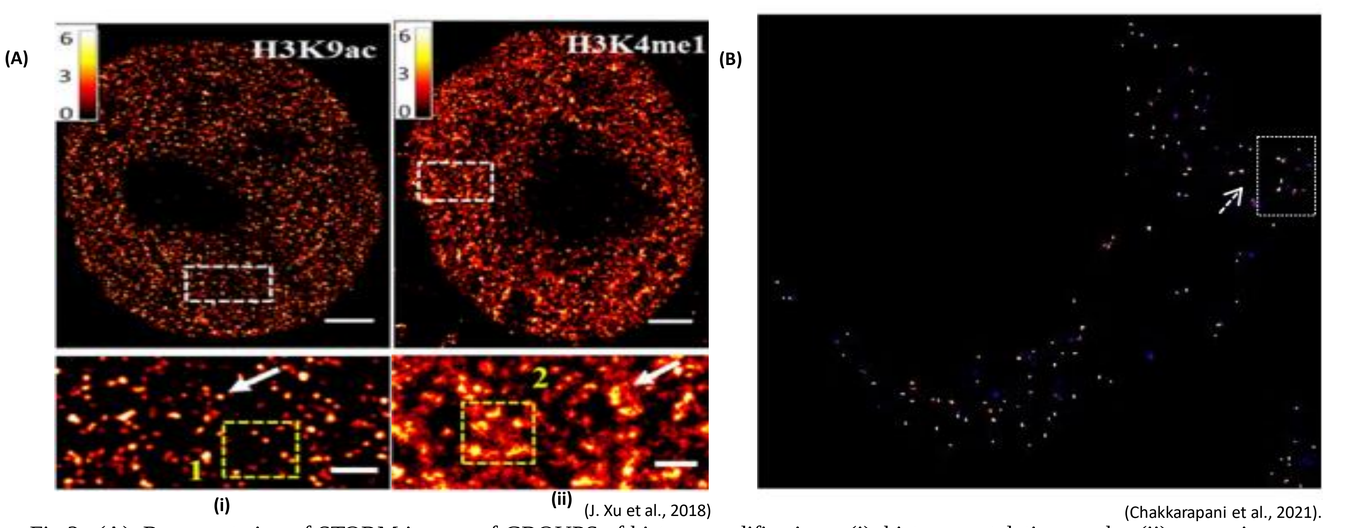

Fig 2 : (A) Representation of STORM images of GROUPS of histone modifications: (i) histone acetylation marks, (ii) repressive marks. (B) dSTORM images of an HEK293 cell internalized with 50-nm-sized f silica-coated magnetic nanoparticles containing rho-damine B isothiocyanate dye MNPs@SiO2(RITC).

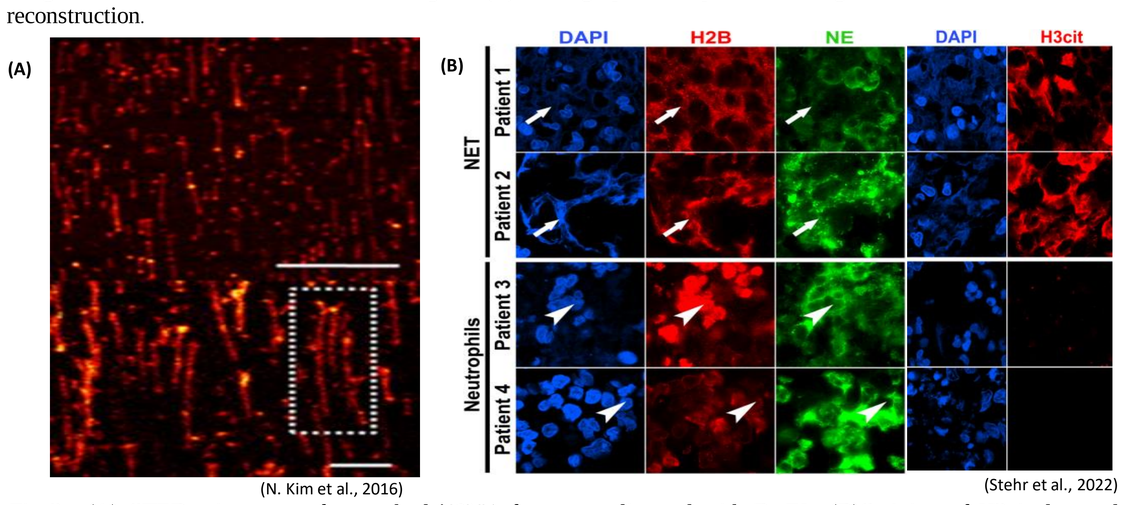

Fig 3 : (A) STED microscopy for combed λDNA fragments digested with BstEI. .(B)Staining of H3cit detected NETs in colon cancer tissue and allowed their differentiation from intact neutrophils. STED images representing H3cit-positive tissues (patients 1 and 2) showed co-localisation of fibrously and granularly spread NE, H2B and DNA (indicated by DAPI staining; white arrows). In contrast, in H3cit-negative tissues (patients 3 and 4), nuclear DAPI and H2B staining co-localised and NE staining showed cytoplasmic localisation (arrowheads).

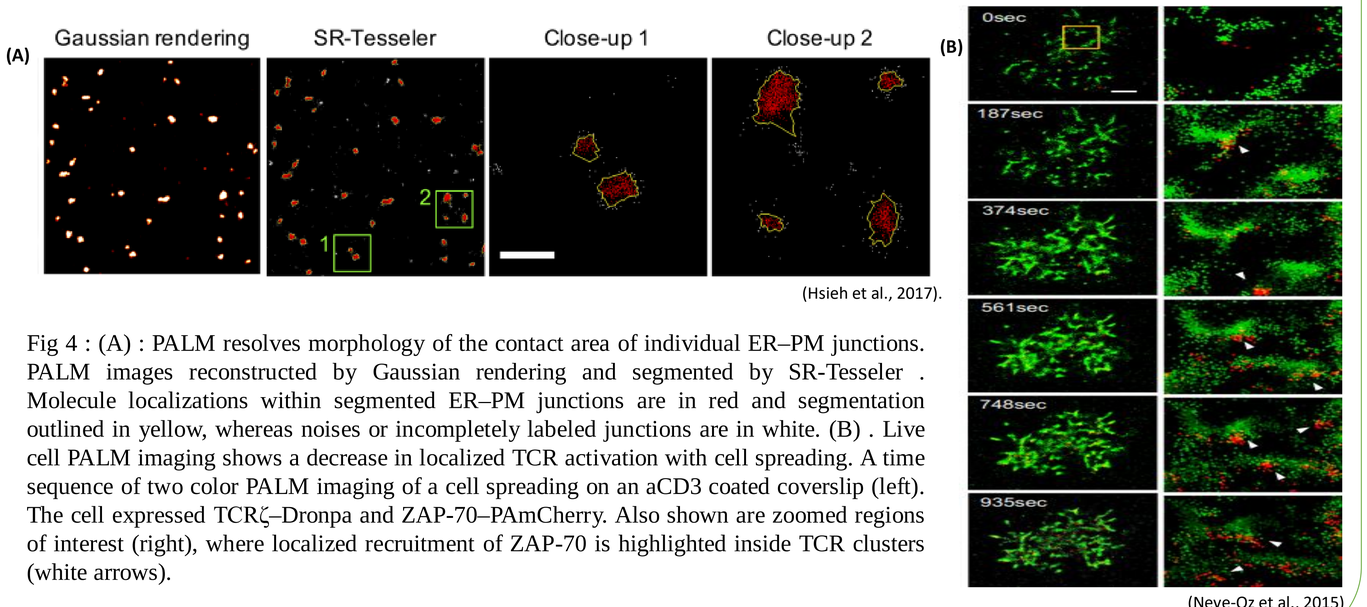

Fig 4 : (A) : PALM resolves morphology of the contact area of individual ER–PM junctions. PALM images reconstructed by Gaussian rendering and segmented by SR-Tesseler . Molecule localizations within segmented ER–PM junctions are in red and segmentation outlined in yellow, whereas noises or incompletely labeled junctions are in white. (B) . Live cell PALM imaging shows a decrease in localized TCR activation with cell spreading. A time sequence of two color PALM imaging of a cell spreading on an aCD3 coated coverslip (left). The cell expressed TCRζ–Dronpa and ZAP-70–PAmCherry. Also shown are zoomed regions of interest (right), where localized recruitment of ZAP-70 is highlighted inside TCR clusters (white arrows).

CONCLUSION

In this review , we discuss the applications of different types of SRM techniques in the field of biomedical and biological research at a molecular, cellular and tissue level.

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