Comparison of Specificity of Confocal and Super-Resolution Approaches to Detecting Mitochondria-ER Contact Sites

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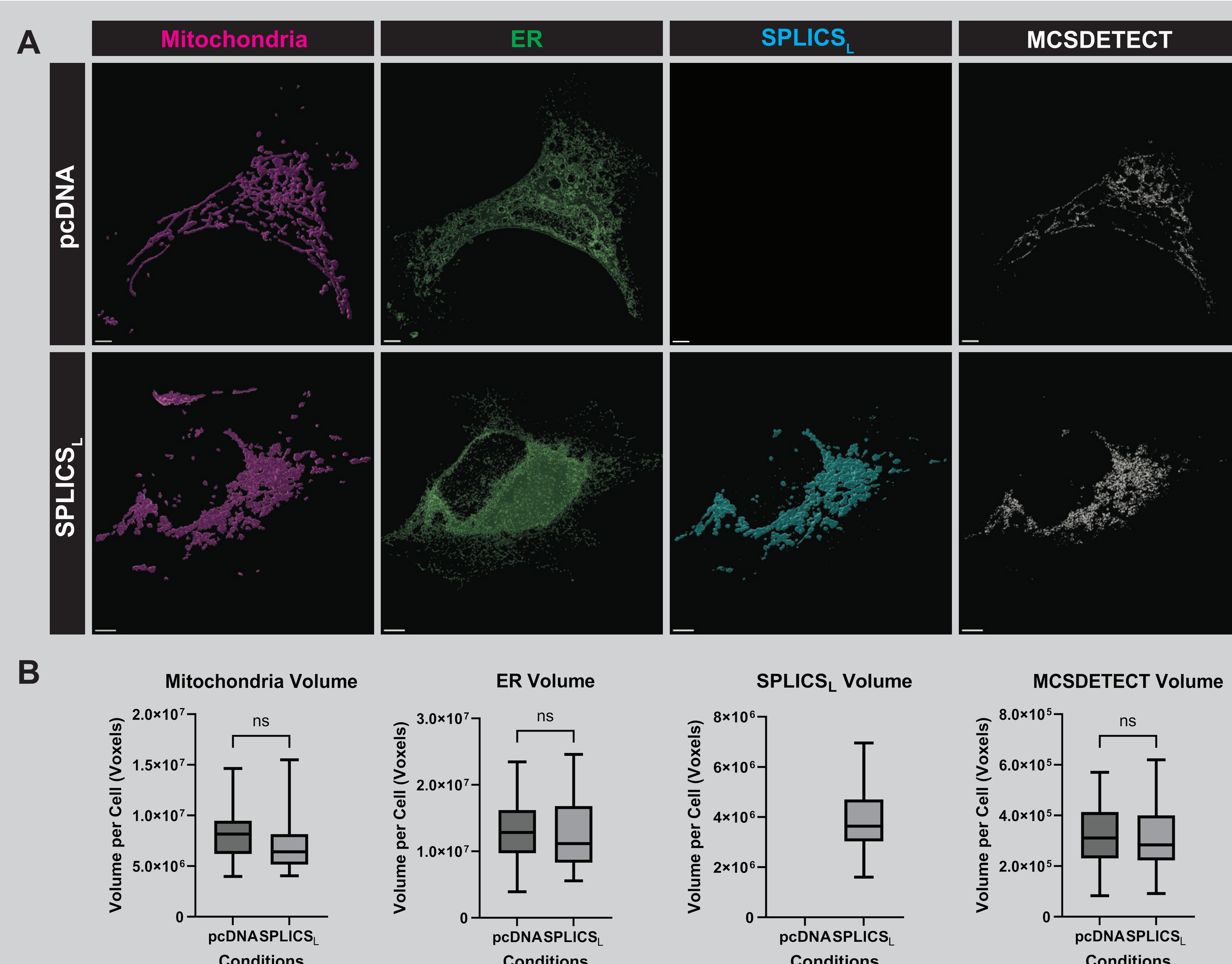
Abstract

Mitochondria-endoplasmic reticulum contact sites (MERCs) are a subclass of membrane contact sites (MCS), where the membranes of two different organelles are in close proximity, tethered together by proteins, yet not fusing. MERCs play important roles in a variety of cellular processes, including calcium signaling, lipid biosynthesis, mitochondrial dynamics, and more. Fluorescent colocalization analysis of mitochondria and ER as well as split fluorescent probes are commonly used to detect MERCs from diffraction limited confocal images. However, inter-organelle distances (~ 10-60 nm) for MERCs are far lower than the 200-250 nm diffraction limited resolution obtained by standard confocal microscopy. Super-resolution microscopy of 3D volume analysis provides only a two-fold resolution improvement (~ 120 nm XY; 250 nm Z), that remains unable to resolve MERCs.

Previous work from the lab has developed a Membrane Contact Site (MCS) detection algorithm, MCS-DETECT, that when applied to 3D STED super-resolution image volumes, faithfully detects elongated ribosome-studded riboMERCs. Here, HeLa cells have been transfected with the Split-GFP-Based Contact Site Sensor (SPLICS) together with the ER reporter KDEL-RFP and labelled for mitochondrial protein TOM20. Contact sites identified by MCS-DETECT analysis of 3D STED volumes was compared to contacts determined by co-occurrence colocalization analysis of mitochondria and ER, as well as the SPLICS probe. Contact site volumes obtained by MCS-DETECT were significantly smaller than those obtained for colocalization analysis or SPLICS, and more closely matches contact site metrics obtained by 3D EM.

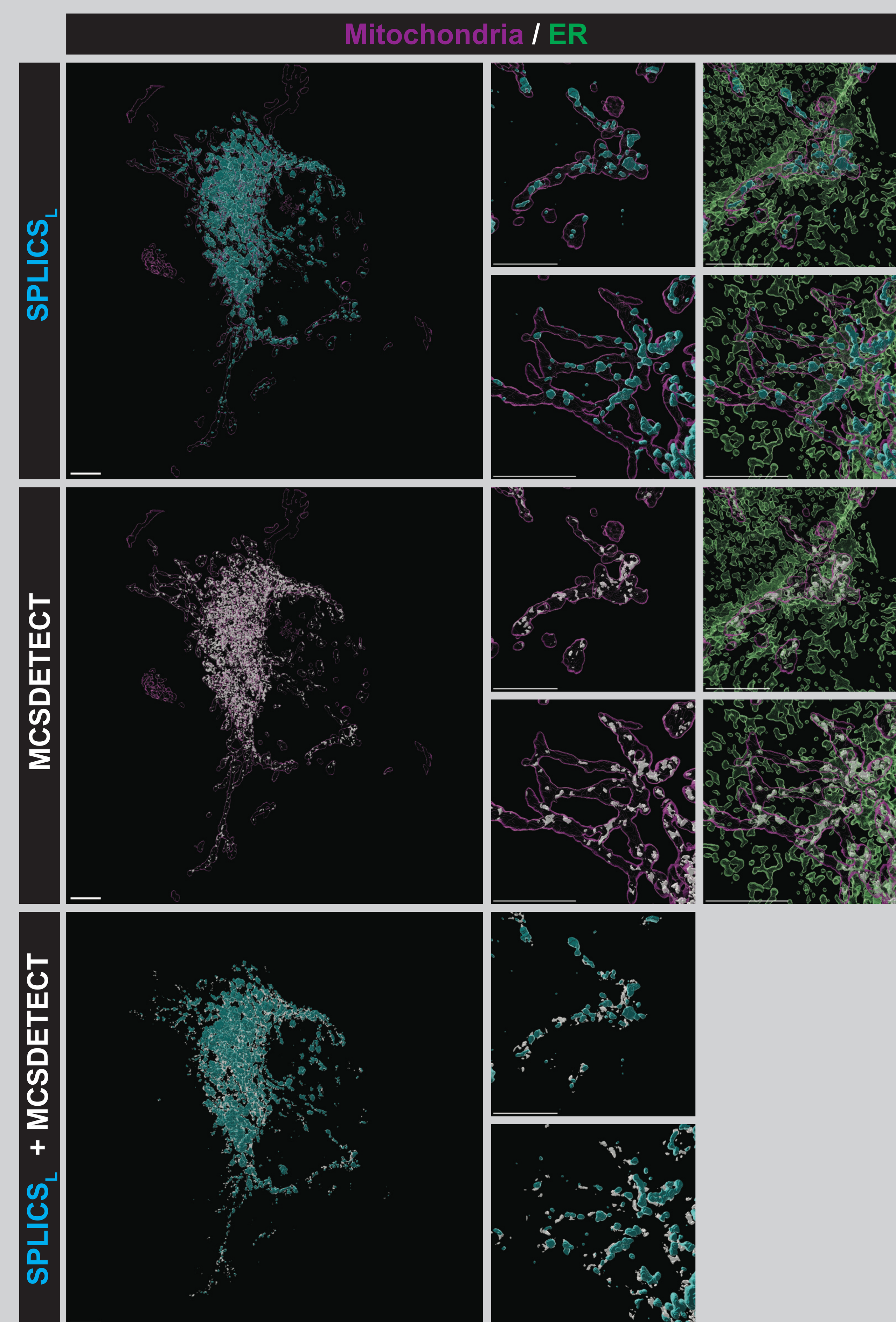
Interestingly, STED analysis localized a subset of the SPLICS label exclusively to mitochondria, which was not as apparent in confocal analysis. Future work aims to better characterize the mitochondria localization of SPLICS and determine effectors of this phenotype in order to examine the functional role of MERCs in the mechanism and regulation of mitochondrial import.

MCS-DETECT analysis of cells expressing SPLICS probe



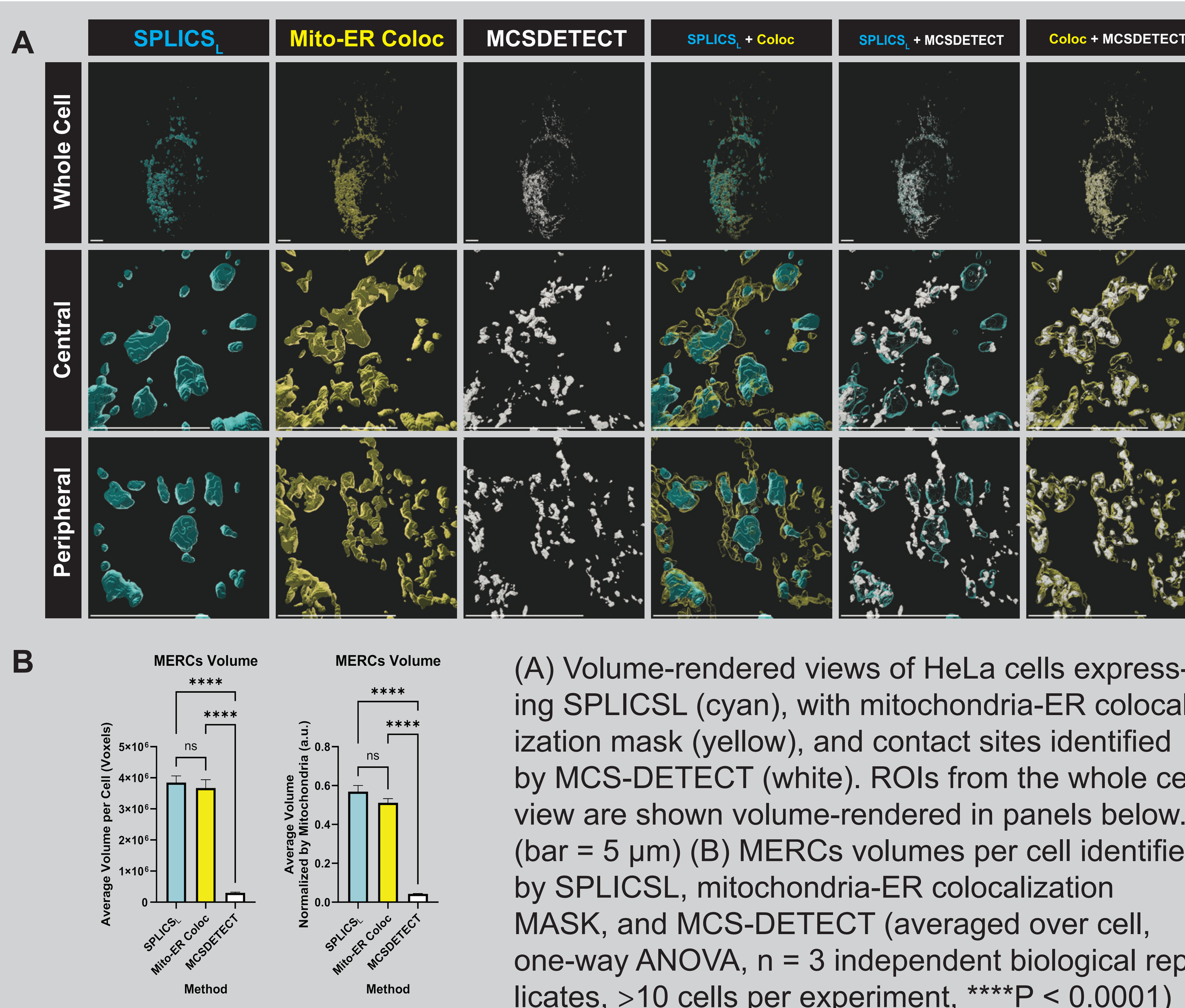
(A) Volume-rendered views of HeLa cells labelled for TOM20 (magenta) and expressing KDEL-RFP (green) together with pcDNA or SPLICSL (cyan), with contact sites (white). (bar = 5 μm) (B) Mitochondria, ER, SPLICSL and contact site volumes per cell are shown for pcDNA and SPLICSL transfected HeLa cells (averaged over cell, Student t test, n = 3 independent biological replicates, >10 cells/condition per experiment)

MERCs identified by MCS-DETECT and SPLICS probe



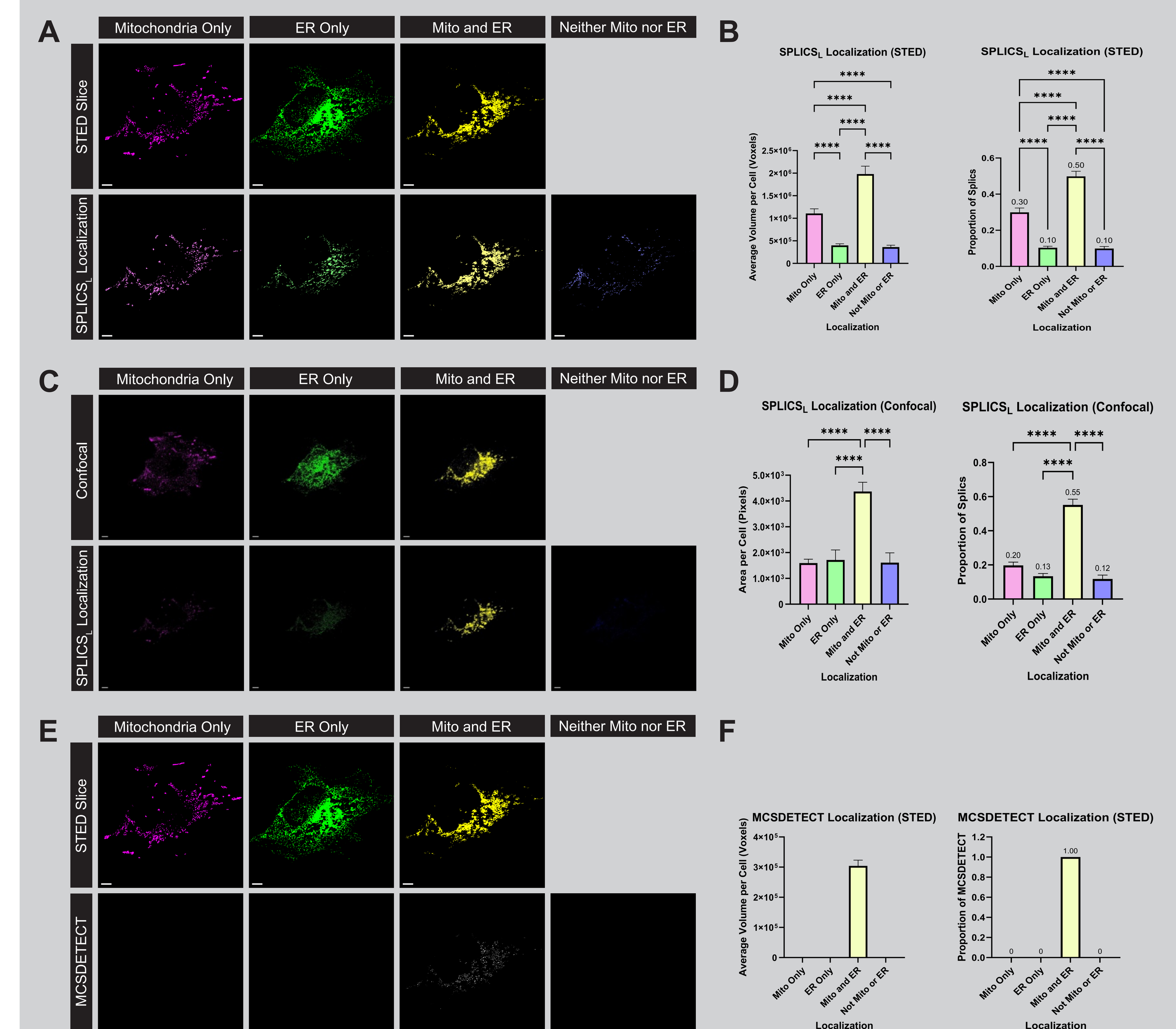
Volume-rendered views of HeLa cells labelled for TOM20 (magenta) and expressing KDEL-RFP (green) together with SPLICSL (cyan) with contact sites overlaid (white). ROIs from the whole cell view are shown volume-rendered in adjacent panels. (bar = 5 μm)

Comparison of Contact Site Detection Methods



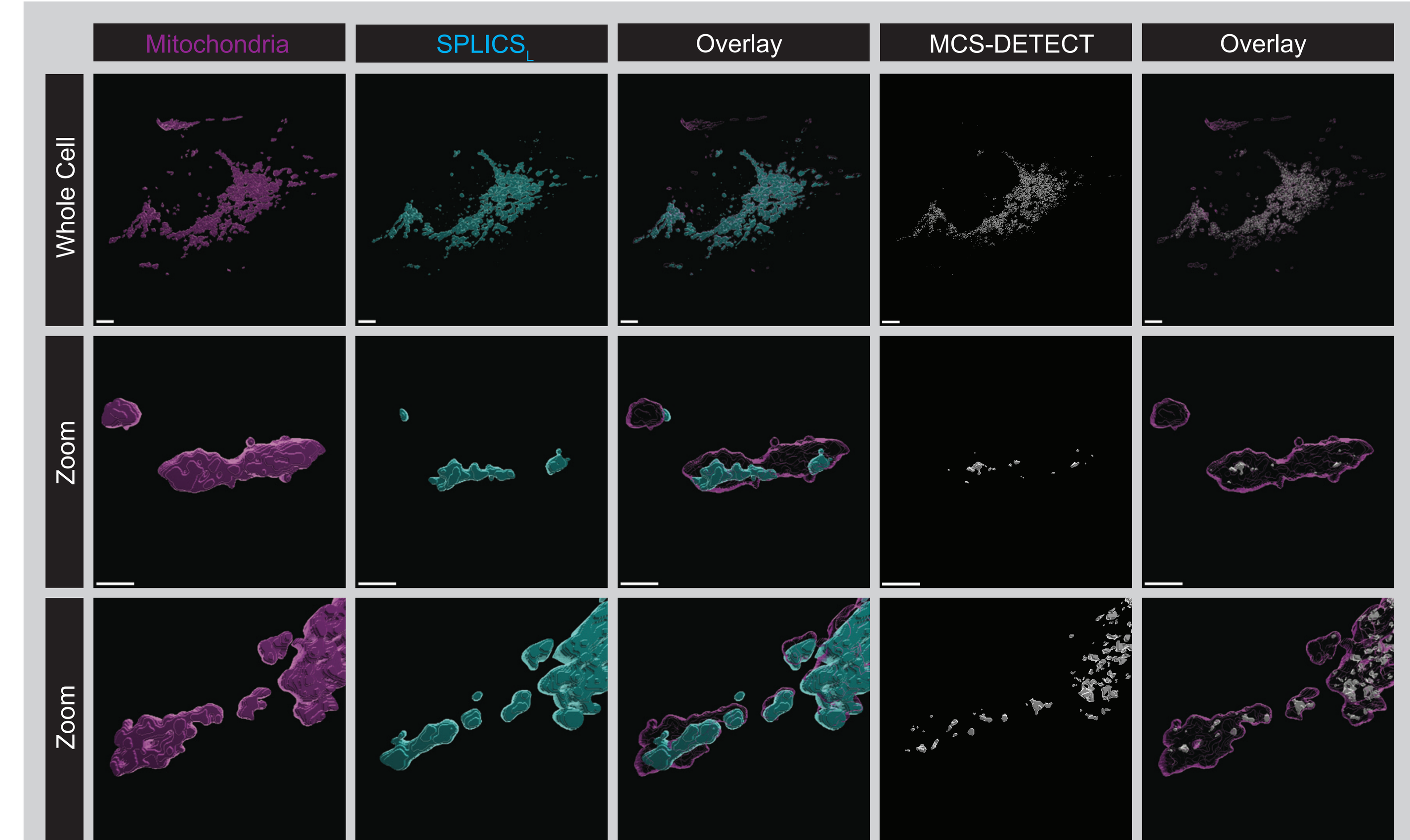
(A) Volume-rendered views of HeLa cells expressing SPLICSL (cyan), with mitochondria-ER colocalization mask (yellow), and contact sites identified by MCS-DETECT (white). ROIs from the whole cell view are shown volume-rendered in panels below. (bar = 5 μm) (B) MERCs volumes per cell identified by SPLICSL, mitochondria-ER colocalization MASK, and MCS-DETECT (averaged over cell, one-way ANOVA, n = 3 independent biological replicates, >10 cells per experiment, ****P < 0.0001)

Subcellular Localizations of SPLICS and MCS-DETECT Contact Sites



3D STED and confocal images of HeLa cells labelled for TOM20 (magenta) and expressing KDEL-RFP (green) together with SPLICSL, with mitochondria-ER colocalization mask (yellow). Contact site localization subgroups: exclusive to mitochondria (light magenta), exclusive to ER (light green), colocalized with mitochondria-ER colocalization mask (light yellow), localized with neither mitochondria nor ER (blue). (bar = 5 μm). (A) Slices from 3D STED images showing SPLICS probe localization subgroups. (B) Quantification from 3D STED volumes of SPLICS localization subgroups. (averaged over cell, one-way ANOVA, n = 3 independent biological replicates, >10 cells per experiment, ****P < 0.0001). (C) Confocal images showing SPLICS probe localization subgroups. (D) Quantification from confocal images areas of SPLICS localization subgroups. (averaged over cell, one-way ANOVA, n = 3 independent biological replicates, >10 cells per experiment, ****P < 0.0001). (E) Slices from 3D STED images showing localization subgroups of contact sites identified by MCS-DETET. (F) Quantification from 3D STED volumes of localization subgroups of contact sites identified by MCS-DETECT (averaged over cell, one-way ANOVA, n = 3 independent biological replicates, >10 cells per experiment).

3D STED Reveals Mitochondrial Localization of SPLICS probe



(A) Volume-rendered views of HeLa cells labelled for TOM20 (magenta), expressing KDEL-RFP and SPLICSL (cyan), with contact sites identified by MCS-DETECT (white). ROIs from the whole cell view are shown volume-rendered in panels below. (bar = 5 μm whole cell, 1 μm insets)

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