A novel sub-precision detection method (MCS-DETECT) identifies shape complexity of mitochondria-ER contacts (MERCs) in 3D STED super-resolution microscopy


1 Simon Fraser University, British Columbia, Canada
2 University of British Columbia, British Columbia, Canada
*,# Equal contribution

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With the inter-organelle distance of mitochondria-ER contacts (MERCs) below the resolution of 3D fluorescence and super-resolution microscopy, identification and morphological analysis of MERCs is restricted. We illustrate how a novel Membrane Contact Site detection algorithm (MCS-DETECT) is able to accurately reconstruct sub-precision MERCs from 3D STED super-resolution microscopy and describe their morphological diversity. Our approach reconstructs the sub-precision MERC interface using a windowed Spearman correlation of the 2nd intensity differential, making the approach robust against inherent fluctuations in fluorescence markers across channels and datasets. To enable quantitative analysis, we compute shape features of the produced contacts, as well as a confidence map to report on reliability of contact detection. We validate MCS-DETECT by a parallel electron microscopy (EM) study of elongated ribosome-studded MERCs (riboMERCs), present in HT-1080 but not COS-7 cells. MCS-DETECT reconstructs large, tubular riboMERCs selectively in HT-1080 cells and identifies morphological differences between riboMERCs and large contacts induced by expression of an ER-mitochondria linker in COS-7 cells. MCS-DETECT registers decreased large riboMERCs in Gp78 knockout HT-1080 cells, and increased riboMERCs, that retain the elongated, tubular morphology, upon overexpression in COS-7 cells of wild-type Gp78 but not a Ring finger mutant Gp78 lacking ubiquitin ligase activity. Gp78-dependent riboMERCs present complex tubular shapes that intercalate between and contact multiple mitochondria. MCS-DETECT applied to whole cell 3D super-resolution microscopy therefore shows that Gp78 ubiquitin ligase activity regulates the formation of novel tubular shaped riboMERCs.

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Novel Sub-Precision Detection Method (MCS-DETECT) Identifies Shape Complexity of Mitochondria-ER contacts (MERCS) in 3D STED Super-resolution Microscopy

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Ben Cardoen†, #, Kurt Vandevoorde‡, #, Guang Gao‡, #, William Liu‡, Ellia Tiliakou‡, A. Wayne Vogl‡, Ghassan Hamarneh†*, Ivan Robert Nabi ‡, §*

† Medical Image Analysis Laboratory, School of Computing Science, Simon Fraser University
‡ Department of Cellular and Physiological Sciences, Life Sciences Institute, University of British Columbia
§ School of Biomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada
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EM: MERCs in HT-1080 and COS-7 cells shows distinct differences
MCS: Reconstructing sub-precision contacts

A

Spearman correlation of negative Laplacian detects sub-precision contacts

- Intensity
- Laplacian
- Spearman

Distance between objects (% of system precision)

0 25 50 75 100

B

Input

X x Y x Z
Channel 1

X x Y x Z
Channel 2

Logical AND of masks

Bleedthrough (Z) filter
Parameter z

Binary mask X x Y x Z

Remove non-mitochondrial targets

Vesicle filter
Parameter size, intensity

Sampled contact features
Parameter window size

Filtered Contacts

Full contact features

MERC detection

Differential correlation

Spearman

Confidence filter
Parameter a (Type I), b (Type II)

Remove pixelation artifacts

Gradient filter

Post-processing
MCS-Detect recovers distinct MERC signatures in HT-1080 and COS-7 in STED and shows they are regulated by Gp78
Gp78 regulates contact profiles in HT-1080 and COS-7 (STED)
RRBP1 knockdown reduces riboMERCS independent of Gp78
MCS-DETECT captures changes induced by RRBP1 knockdown

A

HT-1080
siCTL

HT-1080
Gp78 KO
siCTL

HT-1080
siRRBP1

HT-1080
Gp78 KO
siRRBP1

B

Contact coverage/microtubuli (%)

# contacts/sliding window

0.02

0.04

0.06

0.08

0.10

0.12

0.14

3.0

2.5

2.0

1.5
Gp78 induces convoluted tubular MERCs
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- Parsa Alan
- William Liu
- Ellie Tiliakou
- Prof. A. Wayne Vogl

Preprint (under revision JCB Tools) ↓
https://www.biorxiv.org/content/10.1101/2022.06.23.497346v2
Poster ↓
Code ↓
https://github.com/bencardoen/SubPrecisionContactDetection.jl
Contact ↓
bcardoen@sfu.ca
**Abstract**

With the inter-organellar distance of mitochondria-ER contacts (MERCs) below the resolution of 3D fluorescence and super-resolution microscopy, identification and morphological analysis of MERCs is restricted. We illustrate how a novel Membrane Contact Site detection algorithm (MCS-DETECT) is able to accurately reconstruct sub-precision MERCs from 3D STED super-resolution microscopy and describe their morphological diversity. Our approach reconstructs the sub-precision MERC interface using a windowed Spectral correlation of the 2nd intensity derivative, making the approach robust against inherent fluctuations in fluorescence markers across channels and datasets. To enable quantitative analysis, we compute shape features of the produced contacts, as well as a compliance map to report on reliability of contact detection. We validate MCS-DETECT by a parallel electron microscopy (EM) study of elongated ribosome-studded MERCs (riboMERCs), present in HT-1080 but not COS-7 cells. MCS-DETECT reconstructs large tubular riboMERCs selectively in HT-1080 cells and identifies morphological differences between riboMERCs and large contacts induced by expression of an ER-mitochondria linker in COS-7 cells. MCS-DETECT regions decreased large riboMERCs in Gp78 knockout HT-1080 cells, and increased riboMERCs, that retain the elongated, tubular morphology, upon overexpression in COS-7 cells of wild-type Gp78 but not a RING-finger mutant Gp78 lacking ubiquitin ligase activity. Gp78-dependent riboMERCs present complex tubular shapes that interconnect between and contact multiple mitochondria. MCS-DETECT applied to whole cell 3D super-resolution microscopy therefore shows that Gp78 ubiquitin ligase activity regulates the formation of novel tubular shaped riboMERCs.

**NOVEL SUB-PRECISION DETECTION METHOD (MCS-DETECT) IDENTIFIES SHAPE COMPLEXITY OF MITOCHONDRIA-ER CONTACTS (MERCs) IN 3D STED SUPER-RESOLUTION MICROSCOPY**

Ben Cardoen	extsuperscript{†,}‡, Kurt Vandevenooi	extsuperscript{†,}§, Guang Gao	extsuperscript{†,}§, Parsa Alan	extsuperscript{†,}§, William Liu	extsuperscript{*}, Ellia Tiliakou	extsuperscript{†,}‡, A. Wayne Vogl	extsuperscript{†}, Chahsan Hamarneh	extsuperscript{†,}‡, and Ivan R. Nabi	extsuperscript{†,}*  

†School of Computing Science, Simon Fraser University, Burnaby, British Columbia, Canada  
§Life Sciences Institute, University of British Columbia, Vancouver, British Columbia, Canada  
‡School of Bioomedical Engineering, University of British Columbia, Vancouver, British Columbia, Canada

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**MCS-DETECT analysis of sub-precision contacts**

**Gp78 regulation of riboMERCs**

**MCS-DETECT captures changes induced by RRBP1 knockout**

**RRBP1 knockdown reduces riboMERCs independent of Gp78**

**Gp78 induces convoluted tubular MERCs**

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**Quantitative EM analysis of ER-mitochondria contacts in HT-1080 and COS-7 cells**

**Cells expressing RiboMERCs and labelled for TOMM20 (red) and MERCs are visualized in white, with quantification (B-C). (mean/cell, 2-sided MWU, n=30; Bar = 10 μm whole cell; 1 μm insets).**

**RRBP1 knockdown reduces riboMERCs independent of Gp78**

**Gp78 induces convoluted tubular MERCs**

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