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Technical note

Visualizing ultrastructural details of placental tissue with super-resolution structured illumination microscopy

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ABSTRACT

Super-resolution fluorescence microscopy is a widely employed technique in cell biology research, yet remains relatively unexplored in the field of histopathology. Here, we describe the sample preparation steps and acquisition parameters necessary to obtain fluorescent multicolor super-resolution structured illumination microscopy (SIM) images of both formalin-fixed paraffin-embedded and cryo-preserved placental tissue sections. We compare super-resolved images of chorionic villi against diffraction-limited deconvolution microscopy and show the significant contrast and resolution enhancement attainable with SIM, demonstrating the applicability of this imaging technique for both clinical diagnosis and biological research.

1. Introduction

Traditionally, fluorescent optical microscopy (OM) served as a tool to observe cellular dynamics with high specificity at low resolution, whereas electron microscopy (EM) helped to visualize ultrastructural details but at the cost of specificity. About two decades ago, the development of fluorescence-based super-resolution fluorescence microscopy (SRM), also known as optical nanoscopy, bridged the gap between OM and EM, enabling high specificity studies at the sub-cellular level [1]. Among the existing super-resolution methods, structured illumination microscopy (SIM) stands out as the fastest and most suitable technique for histological examination, enabling high-resolution and high-throughput imaging for decision support in clinical settings [2,3].

In placental biology, SRM studies have focused primarily on trophoblast cell cultures [4,5], leaving placental tissues comparatively unexplored. Here we describe the sample preparation steps and imaging parameters necessary to obtain super-resolved multicolor micrographs of both formalin-fixed paraffin-embedded (FFPE) and cryo-preserved placental sections with SIM. We report our observations of imaging chorionic villi using optical nanoscopy and compare them with deconvolution microscopy (DV), a state-of-the-art diffraction-limited fluorescent microscopy technique [10] widely used in life sciences. To the best of our knowledge, this is the first report of SIM on placental tissue.

2. Materials and methods

Full-term placentae from 10 different Caucasian healthy patients were collected anonymously immediately after delivery at the University Hospital of North Norway. Written consent was obtained from the participants according to the protocol approved by the Regional Committee for Medical and Health Research Ethics of North Norway (REK Nord reference no. 2010/2058–4).

The samples were prepared in two ways according to the preservation technique, as outlined below and in Fig. 1 A,B.

2.1. Preparation of FFPE-sections

Placental tissue samples were fixed in formalin and embedded in

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paraffin, according to standard histological procedures [6]. Sections of 4 μm thickness were cut from the paraffin blocks (HM 355S Automatic Microtome, Thermo Fisher Scientific, Waltham, Massachusetts, USA), placed on poly-L-lysine coated #1.5 coverslips, and deparaffinized in xylene (3 × 5 min), followed by rehydration in descendent series of ethanol: 100% (2 × 10 min), 96% (2 × 10 min) and 70% (10 min). To reduce the autofluorescence, the rehydrated samples were immersed in bleaching solution (30 min) and then washed with MilliQ water (5 min), before incubation with CellMask Orange (CMO) membrane staining (10 min). Thereafter, the samples were washed with PBS (2 × 5 min) and incubated with DAPI nuclear staining (5 min), followed by a wash with MilliQ water (2 × 5 min). The stained samples were mounted in the center of standard microscope glass slides with Prolong Gold and sealed with nail varnish after the mounting medium hardened. All incubations were performed at room temperature. For autofluorescence characterization, the samples were prepared according to the above-described steps, excluding fluorescent dyes. Supplementary information S1 provides a detailed list of the materials used in the study.

2.2. Preparation of cryosections

Chorionic tissue was collected, dissected and rinsed in 9 mg/mL sodium chloride as described elsewhere [7]. Collected tissue samples were transferred to 5 mL of 1 × PHEM-buffer. Placental samples of 1 mm^3 were immersed in 5 mL 8% formaldehyde in PHEM buffer and incubated at 4 °C overnight. Tissue samples were immersed in 0.12% glycine at 37 °C (1 h), infiltrated with 2.3 M sucrose at 4 °C overnight and transferred to specimen pins before storage in liquid Nitrogen. The samples were cut into 1 μm thick cryosections (EMUC6 ultramicrotome, Leica Microsystems, Vienna, Austria), collected with a wire loop filled with a 1:1 mixture of 2% methylcellulose and 2.3 M sucrose per Tokuyasu method [8], and placed on poly-L-lysine coated #1.5 coverslips. The samples were washed with PBS (3 × 7 min) at 4 °C to dissolve the methylcellulose. Thereafter, the tissues were incubated in phalloidin-Atto 647 N for F-actin staining (15 min) and washed with PBS (2 × 5 min). The CMO membrane staining and successive steps were performed identically as described for the FFPE sections, at room temperature.

After preparation, both FFPE and cryosections were protected from light and stored at 4 °C before imaging.

2.3. Optical nanoscopy

The samples were imaged in SIM mode using a commercial microscope DeltaVision OMX V4 Blaze imaging system (GE Healthcare, Chicago, USA) equipped with a 60X/NA1.42 oil-immersion objective lens (Olympus, Tokyo, Japan). The acquisition parameters were optimized following the steps depicted in Fig. 1 C. These include (1) refractive index matching of the immersion oil, (2) choice of appropriate excitation intensity and exposure time, (3) selection of sampling steps along the optical axis of the microscope, (4) sequential image acquisition from long to short wavelengths, and (5) overlap between adjacent fields of view to enable mosaic stitching of the data. After the acquisition, the super-resolved SIM images were reconstructed using the software package SoftWoRx provided by the microscope's manufacturer, and post-processed with the open-source software Fiji [9]. For comparative analysis, diffraction-limited deconvolution microscopy (DV) images were acquired with the same microscope. Supplementary information S2 provides a detailed description of the acquisition parameters used for both SIM and DV, as well as the working principle of these two microscopy techniques.

3. Results and discussion

The advantages of SIM include rejection of out-of-focus light and a two-fold resolution enhancement in all three axes (x, y, z) which gives an eight-fold contrast improvement as compared to diffraction-limited microscopy techniques such as DV. In the case of FFPE placental sections (Fig. 2 A,E,I), SIM provides a sharp visualization of biological structures such as the syncytiotrophoblast (SYN), individual cytotrophoblast (CT), fetal capillaries (FC), fetal red blood cells (fRBC) and maternal red blood cells (mRBC), as well as subcellular details such as

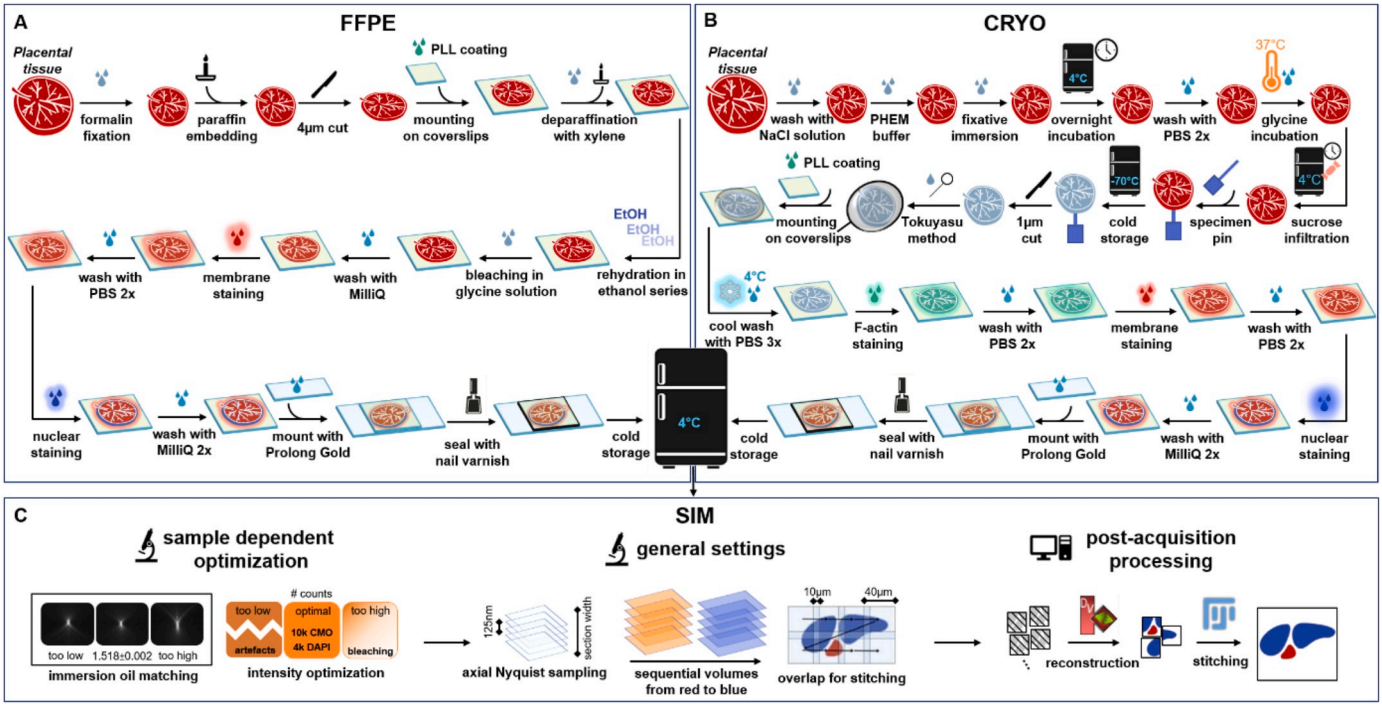

Fig. 1. Sample preparation protocols and imaging acquisition steps for successful super-resolution structured illumination microscopy (SIM) of placental tissue sections. (A) Sample preparation protocol for FFPE tissue sections. (B) Sample preparation protocol for cryopreserved tissue sections. (C) Imaging steps for SIM.

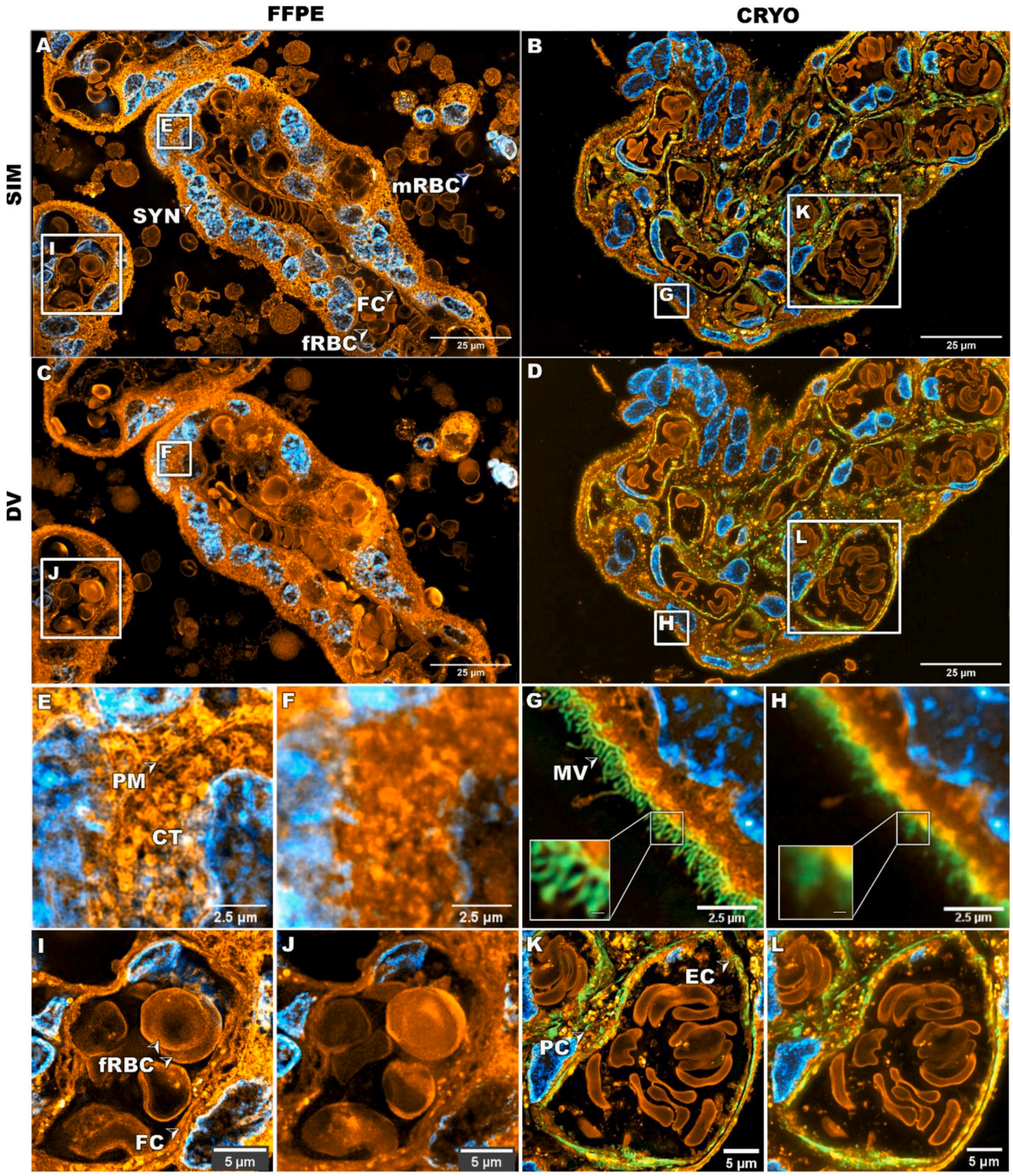

Fig. 2. Fluorescent micrographs of 4 µm thick FFPE and 1 µm thick cryo-preserved tissue sections of human chorionic villi imaged with both SIM and DV microscopy for comparison. F-actin stained with phalloidin-Atto 647 N (displayed in green), membranes stained with CMO (displayed in orange), and nuclei stained with DAPI (displayed in blue). (A,B,E,G,I,K) SIM images. (C,D,F,H,J,L) DV images. (A,C) The enhanced contrast of SIM compared to DV allows for a clearer visualization of relevant structures in the FFPE tissue section such as the syncytiotrophoblast «SYN», fetal capillaries «FC», fetal red blood cells «fRBC» and maternal red blood cells «mRBC». (E,F) Magnified views of the FFPE tissue section show the enhanced resolution of SIM over DV, revealing the plasma membrane «PM» of an individual cytotrophoblast «CT». (I,J) SIM enables the distinction of two adjacent «fRBCs» on a «FC» that are not discernible by DV. (B,D) The SIM image exhibits superior contrast as compared to the DV image of the cryo-preserved tissue section. (G,H) The enhanced resolution provided by SIM reveals the syncytiotrophoblast microvilli «MV» brush border, a morphological structure not resolvable by diffraction-limited OM techniques such as DV. Inlays have digitally enhanced contrast and scale bars of 200 nm. (K,L) SIM enables a clearer distinction of morphological features, such as a pericyte «PC» along with the underlying endothelial cell «EC» lining of a fetal capillary demostrated here, as compared to DV. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

the plasma membrane border (PM) between adjacent cells. In comparison, results from diffraction-limited DV microscopy offer significantly fewer details and contrast (Fig. 2 C,F,J). Notably, the resolution attainable with SIM on FFPE sections was largely limited by the level of ultrastructural preservation of the sample during the preparation steps and not by the SIM method per se. The main challenges with FFPE sections were the autofluorescence signal and the refractive index mismatch between the specimen and the imaging medium, which often led to reconstruction artifacts on the SIM images. We successfully minimized the artifacts by introducing a bleaching step in the preparation protocol and a series of iterative oil changes to match the refractive index, as illustrated in supplementary information S2 and S3.

The SIM images obtained from placental cryosections were significantly sharper and richer in detail than their FFPE counterparts (Fig. 2 B, G,K). The ultrastructural preservation attainable with the Tokuyasu method allowed the visualization of ultrastructural details such as the syncytiotrophoblast's microvilli brush border (MV), a morphological feature not resolvable by diffraction-limited OM techniques. Moreover, the relatively low autofluorescent signal and the thin section thickness of these samples further aided the contrast enhancement of SIM by reducing the out-of-focus information, enabling the distinction of morphological features, such as a pericyte (PC) along with the underlying endothelial cell (EC) lining of a fetal capillary (Fig. 2 K, L).

A further advantage of imaging cryosections is the ease of tissue preparation, which allowed for sectioning, staining, and imaging within the same day. This positions SIM very favorably over traditional high-resolution techniques such as EM, where the sample preparation alone can take several days to weeks. The main drawback of imaging cryosections is the dependence of high precision cutting instruments together with highly skilled operators to obtain sections with minimal morphological damage.

Taken together, the availability of optical super-resolution SIM microscopes in conjunction with optimized tissue preparation and imaging parameters accelerate the imaging of placental tissue samples, aiding the identification of subcellular and ultrastructural features at high-throughput, hence improving the quality of histopathological diagnosis of placental disorders and image-based research in placental biology.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2020.06.007.

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