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# ACCEPTED MANUSCRIPT

# Quantification of Fenestrations in Liver Sinusoidal Endothelial Cells by Atomic Force Microscopy

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#### **Highlights**

- High-resolution AFM imaging of fixed LSECs resolving single fenestrations is performed.
- Contact mode and force-curve based imaging mode results are compared.
- A protocol for the calculation of porosity of LSECs using AFM data is proposed.
- AFM-based characterization of fenestration diameter and porosity of LSEC is presented.

## Abstract

Liver sinusoidal endothelial cells present unique morphology characterized by the presence of transmembrane pores called fenestrations. The size and number of fenestrations in live cells change dynamically in response to variety of chemical and physical factors. Although scanning electron microscopy is a well-established method for investigation of fixed liver sinusoidal endothelial cells morphology, atomic force microscopy is the interesting alternative providing detailed 3D topographical information. Moreover, simple sample preparation, only by wet-fixation, minimizing sample preparation artifacts enable high-resolution atomic force microscopy-based measurements. In this work, we apply imaging methods based on atomic force microscopy, to describe characteristic features of glutaraldehyde-fixed primary murine liver sinusoidal endothelial cells, namely: mean fenestration diameter, porosity, and fenestrations frequency. We also investigate the effect of different tip apex radius on evaluation of single fenestration diameter.

By quantitative description of fenestrations, we demonstrate that atomic force microscopy became a well competing tool for nondestructive quantitative investigation of the liver sinusoidal endothelial cell morphology.

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