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To Whom It May Concern:

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The dissertation "Computational methods in single molecule localization microscopy" by Martin Ovesný presents Martin's work in single molecule super-resolution microscopy. Super-resolution microscopy is one of the most important research areas today, and Martin has made several important contributions to the field.

After an introduction and literature review in chapters one and two, chapter three of the dissertation details Martin's software for super-resolution single molecule localization microscopy. Named "ThunderSTORM," Martin's publication in the high-impact journal *Bioinformatics* has been heavily cited, and has become one of the standard software packages used in the field. The software is used by researchers from all over the world to analyze and quantify super-resolution data. The chapter also describes Martin's participation in the international *Single Molecule Challenge*, hosted by the École polytechnique fédérale de Lausanne (EPFL, Lausanne, Switzerland), where Martin's software was chosen as the overall winner. Today Martin is continuing his involvement in the ongoing challenge as a member of the organizing committee. Martin also continually updates the software, making improvements and adding the newest methods.

Chapter four of the dissertation describes a new super-resolution microscope which we constructed in our lab. The new microscope further improves resolution compared to previously used setups. So far as we know, the microscope is completely unique in the Czech Republic, with only a handful of similar designs having been realized worldwide. After assembly and taking initial data, Martin created many new methods for analyzing the data. One of the critical steps involves estimating the 3D position of molecules based on the images of a single molecule at two different focal positions as acquired by two detectors. Here Martin developed several new statistical approaches.

Chapter five describes Martin's work in high-density algorithms for single molecule microscopy. Martin developed a new approach, drawing on concepts from compressed sensing methods. Martin's publication in the journal *Optics Express* details not only the newly developed theoretical work, but also presents 3D super-resolution imaging of real cells, and the software Martin created to analyze the data. The dissertation contains a considerable amount of new, unpublished work including numerous derivations, simulations, and analysis thereof.

Martin's work as a PhD student has been exemplary and exceeded my expectations. Martin is now one of the top experts in this area and his knowledge and technical ability in his field far exceeds my own. I fully recommend that his dissertation be accepted by the committee.

Sincerely,

Dr. Guy M. Hagen