

## **Nanobody labeling and super resolution gSTED nanoscopy of the bacterial cell division machinery**

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Bacterial cells are critically dependent on their ability to divide in order to proliferate. In many bacteria this is achieved by binary fission, by dividing a mother cell into two identical daughter cells. The constriction and subsequent separation of the cell envelope is orchestrated by a large and highly dynamic protein assembly known as 'the divisome'. More than 30 different proteins are involved in the division process. In fast growing cells with division times around 20 minutes these 30 or so proteins assemble, constrict the membranes and subsequently disassemble. Using fluorescence microscopy this phenomenon is quite well understood on a micron scale, however, the mechanisms by which this occurs on a molecular scale are poorly understood.

To get a better overall understanding of this process at a nanometer scale we have recently started to employ super resolution fluorescence nanoscopy in combination with a novel labeling approach involving fluorescently labeled nanobodies, and image the cells trapped in a vertical 'standing' position allowing the whole machinery to be captured in one image without the need for 3D reconstruction of the raw data.

Our results indicate that the divisome proteins are organized in different rings, which may partly function independently. This suggests that rather than being treated as one large macromolecular super complex the cell division proteins should be treated as an assembly of different smaller complexes that only partly and transiently are constituents of the same complex.