Two-photon multifocal microscopy for in vivo single-molecule and single-particle imaging

Vlieg, R.C.

Stellingen

Behorend bij het proefschrift

Two-photon multifocal microscopy for *in vivo* singe-molecule and single-particle imaging

1. The efficient near-infrared light absorption of gold nanorods is highly advantageous for accurate single-particle tracking but impedes single-molecule sensing.
   Chapter 2 and 3 of this thesis.

2. Linear absorption of light is the main restriction for prolonged two-photon multifocal microscopy.
   Chapter 3 and 4 of this thesis.

3. The success of a microscopy technique is determined by its adoption by researchers who were not responsible for its development.
   Chapter 5 and 6 of this thesis.

4. Because of their excellent photostability, barrel-shaped fluorescent proteins should be the first labels to be considered for two-photon microscopy.
   Chapter 3 and 4 of this thesis.

5. Two-photon light-sheet microscopy should allow for faster and more stable single-molecule experiments than two-photon multifocal microscopy.
   *T. Truong et al.: Nature Methods **9**, 757 (2011)*.

6. If Graham et al. had been aware of previous literature stating that two-photon photobleaching of eGFP is independent of oxygen concentration, the conclusion of their photobleaching study should have been that multiphoton absorption of excited eGFP molecules is the sole bleaching mechanism.
   *D. Graham et al.: Journal of Fluorescence **25**, 1775 (2015)*

7. The reason for the reduction in photobleaching when switching to higher repetition rate Ti:Sa lasers, as reported by Niu and co-workers, is the lower peak power.
   *F. Niu et al.: Laser Physics **29**, 805 (2019)*

8. The flexibility to generate any number of beamlets with a spatial light modulator allows a two-photon multifocal microscope to be used for both single-molecule and deep-tissue imaging.
   *N. Matsumoto et al.: Optics Express **25**, 7055 (2017)*

9. The time one spends on scientific literature research can be drastically reduced when every article includes the three major advantages and shortcomings of the presented results as determined by the peer-reviewers.

10. The major advantage of single-molecule experiments is also its major challenge: one does not directly visualize the environment and context of the molecule.