Technology Offer

High-Resolution Scanning Luminescence Light Microscope

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Abstract

This advanced scanning luminescence light microscope enables spatial, high-resolution imaging of luminescence-labeled structures. Utilizing innovative periodic patterns created by crossed line gratings of luminescence inhibition and excitation light, the microscope surpasses traditional diffraction limits. These patterns precisely confine the luminescent marker's activation and emission regions, delivering imaging clarity. The device is ideal for applications requiring nanoscale resolution, such as cellular imaging and material analysis, offering enhanced detection accuracy and operational flexibility.

Background

Advancements in super-resolution microscopy, such as Stimulated Emission Depletion (STED) and Ground State Depletion (GSD) microscopy, have significantly enhances imaging resolution beyond the diffraction limit. STED utilizes focused excitation light and a depletion beam to selectively deactivate luminescence markers, leaving only signals from regions of minimal light intensity. Similarly, GSD employs luminescence prevention light to switch markers to no-emissive states, restricting luminescence to defined intensity minima. Both methods, categorizes under RESOLFT (Reversible Saturable Optical Fluorescence Transitions), are pivotal in fields like nanoschience and biology, yet further refinements in creating efficient and precise light intensity distributions are addressed by innovative technologies like the present invention.

Technology

The patented system employs two non-coherent beams of luminescence inhibition light, passed through optical grids to form coherent partial beams (Fig. 1a). These beams are focused to generate intersecting line gratings with one-dimensional loca intensity minima, resulting in a two-dimension field of uniform, at least two-dimensionally confined local intensity minima in the sample (Fig. 1b). At least one beam of excitation light is similarly manipulated, ensuring its intensity distribution superposes with the luminescence inhibition light. The microscope's scanning system shifts these patterns across the sample, allowing comprehensive imaging. The detector captures luminescence signals from localized markers, translating them into high-resolution images.

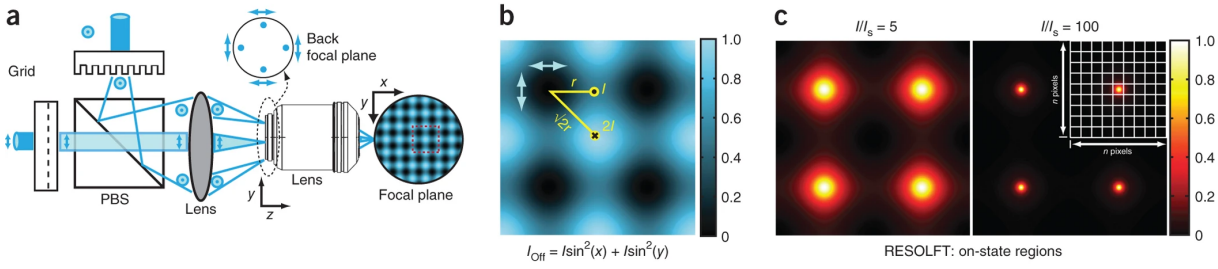

Figure 1: This figure illustrates the optical setup and functionality of the invented high-resolution scanning luminescence microscope. Panel (a) shows the schematic setup of the microscope, where structured light patterns are generated using optical grids, polarizing beam splitters (PBS), and lenses to create achromatic periodic patterns in the focal plane. Panel (b) depicts the intensity distribution at the focal plane (I_{Off}=I $\sin^2$(x) + I $\sin^2$(y)), highlighting the critical intensity minima where fluorescence is inhibited, allowing precise spatial control of luminescence activation. Panel (c) demonstrates the resulting fluorescence activation regions under varying light intensities (I/I_S=5; I/I_S=100), showcasing the microscope's ability to confine fluorescence to nanoscale regions, enabling super-resolution imaging. This visualization highlights the technology's innovative use of structured light for achieving diffraction-unlimited resolution (Chmyrov et al., 2013).

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Advantages

- **Enhanced Spatial Resolution:** Achieves imaging beyond the diffraction limit by utilizing innovative periodic light patterns for precise localization of luminescence markers (Fig. 1c, Fig. 2).
- **Reduced Photobleaching:** Minimizes photodamage by confining activation to targeted regions.
- **Efficient Scanning:** Parallelized scanning reduces acquisition times while maintaining precision.
- **Versatile Marker Compatibility:** Supports various luminescent markers, including fluorescent proteins and dyes.
- **Robust and Scalable Design:** Optical grating setup with achromatic periodic patterns ensures consistent performance and allows system scaling.

Potential applications

- **Biological research:** Imaging cellular structures, such as filaments and organelles, at nanoscale resolution.
- **Medical diagnostics:** High-precision visualization of pathological samples.
- **Material sciences:** Structural analysis of nanomaterials and surfaces.
- **Pharmaceutical development:** Monitoring molecular interactions in drug discovery.

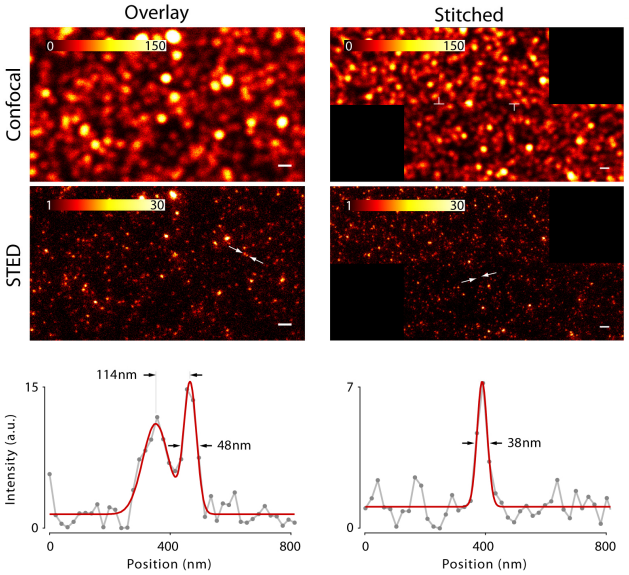

Figure 2: This figure compares the imaging performance of confocal and STED microscopy. The top panels show fluorescence images, where STED reveals significantly enhanced resolution, resolving individual nanoscale features that appear blurred in confocal images. The "Overlay" and "Stitched" approaches demonstrate how multiple scans improve the field of view without compromising detail. The bottom panels provide intensity profiles, highlighting the superior resolution of STED, reducing feature sizes from 114 nm in confocal to 48 nm or even 38 nm in stitched STED images. This demonstrates the microscope's ability to achieve nanoscale imaging with unmatched clarity (Bingen et al., 2011).

Patent Information

- DE102015109305B9 (Application 11.06.2015)
- US9835838B2 (Application 13.06.2016)

Publications

- Bingen, Pit, et al. "Parallelized STED fluorescence nanoscopy." *Optics Express* 19.24 (2011): 23716-23726.
- Chmyrov, Andriy, et al. "Nanoscopy with more than 100,000 'doughnuts'." *Nature methods* 10.8 (2013): 737-740.
- Hell, Stefan W., and Andriy Chmyrov. "Scanning luminescence light microscope with gratings of luminescence inhibition light and further light." U.S. Patent No. 9,835,838. 5 Dec. 2017.
- Hell, Stefan W., and Andriy Chmyrov. "Raster luminescence light microscope with luminescence-preventing light and other light lattices." DE Patent No. 102015109305B9. 11 Aug. 2016.

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