Parallel illumination for depletion microscopy through acousto-optic spatial light modulation

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Abstract. Several types of super-resolution microscopy, such as Stimulated Emission Depletion (STED), Reversible Saturable Optical Fluorescence Transitions (RESOLFT) or Switching Laser Mode (SLAM) microscopies, employ Laguerre-Gaussian beams (also called vortex or doughnut beams) to obtain fluorescence information within a sub-wavelength region of the specimen under observation, thus breaking the diffraction limit and producing images of greatly improved quality. However, in general, these techniques operate on a point-by-point basis, so we need to raster scan the sample in order to build a full, meaningful image, which takes time. Parallelization of the illumination is the only way to make these microscopes more suitable for live cell imaging applications. Here, we demonstrate the parallel production of arbitrary arrays of Gaussian and Laguerre-Gaussian laser foci suitable for super-resolution microscopy, together with the possibility to fast scan through the sample, by means of acousto-optic spatial light modulation, a technique that we have pioneered in the past in several other fields.

1 Introduction

Resolution is a major limitation of fluorescence microscopy, even for the sophisticated confocal instruments that are the workhorse for visualization in many applications in the Life Sciences. It is then of not much surprise that the development of optical super-resolution, roughly at the turn of the century [1, 2], has completely changed the landscape of modern microscopy. Among the new super-resolution techniques, Stimulated Emission Depletion (STED) and its derivatives stand out for their resolution capacity (down to 1-2 nm for MINFLUX [2]), use of standard fluorophores and living cells friendliness (e.g. RESOLFT [2]). Depletion microscopies such as STED are based on the confinement of the fluorescent emission within a subwavelength region by means of the action of two lasers in tandem. The first laser beam features a common TEM, Gaussian intensity profile and is responsible for the excitation (or switch on, in RESOLFT) of the fluorophores present in the sample. A second laser beam, with a "doughnut" intensity profile (i.e. a Laguerre-Gaussian beam), quenches the fluorescence (or switches off the fluorophores) around the central singularity, leaving the molecules closer to the dark core unaffected. The end result is a fluorescent emission that comes from a diminished, subdiffractional area of the sample. The subsequent image composed by scanning the sample while collecting the emitted photons has an effective point spread function (PSF) that is super-resolving in the range of 30-70 nm [1, 2]. However, one of the several drawbacks of STED and STED-derivatives is the relatively slow operation arising from its sequential image acquisition.

Although highly parallel implementations of depletion microscopy (essentially of RESOLFT due to its benign properties) that considerably speed up the scanning process have been described in the literature [3], these rely on the periodic minima of a standing wave illumination to confine the fluorescence at many spots simultaneously. Here, we demonstrate an alternative: the production of large arrays of switchable Gaussian and Laguerre-Gaussian lasers foci by means of acousto-optic holography.

Acousto-optic deflectors (AODs) are beam-steering devices made with a TeO₂ crystal, whose index of refraction is modulated by an acoustic wave produced by a vibrating piezo-electric transducer driven by an electrical signal. We [4], and others, have shown that AODs can be used too as general-purpose spatial light modulators, by computing the driving signals with techniques of digital holography and experimentally feeding the transducer with these by means of a programmable radiofrequency generator. The effect within the crystal is the appearance of a designated refractive index variation that enables the spatial control of a laser beam travelling through the device. We show in the next sections that properly designed acousto-optic holograms can be the basis for the parallelization of the paired excitation/de-excitation beams required in STED-like super-resolution microscopy. Further, acousto-optic holography enables a convenient way to fast scan the sample with these illumination arrays, thus making up a promising technology to develop future live-cell, optical super-resolution microscopes working at video rates.

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In this contribution we aim to produce, assess, and validate the optical quality of a TEM01 Laguerre-Gauss mode, also known as an optical vortex or doughnut beam, for parallel super-resolution microscopy. To accomplish this goal, we will utilize the experimental setup depicted in Fig. 1, which employs a two-path scheme illuminated with a TEM00 Gaussian beam. This setup provides flexibility in controlling the light's path by adjusting the input polarization using both a half-wave plate and a rotation mount. The setup offers flexibility in controlling the active light path by adjusting the input polarization using both a half-wave plate and a rotation mount which allows for precise tuning at the entrance port of the polarizing beam splitter. In one of the paths, the TEM01 L-G mode is generated when a Gaussian beam passes through the vortex phase plate. In the other path, the input TEM00 Gaussian mode is propagated freely. Although the proposed setup is currently not fast enough to perform real-time microscopy due to the slow rotation mount, we anticipate achieving sufficient time resolution by replacing the rotation mount with Pockels cells.

To begin, we will evaluate the quality of the Gaussian and TEM01 L-G mode at the exit of the two-path scheme and ensure the proper alignment between the two paths. Following this, we will analyse the spot array produced at the output of the acousto-optic deflectors (AODs), which consists of an NxN array of either TEM01 L-G or Gaussian modes (as shown in Fig. 2). Additionally, we will investigate how the high numerical aperture objective microscope objective affects the quality of the TEM01 L-G mode.

Finally, we will employ the parallelized spot array to generate super-resolution images using the intensity-weighted subtraction algorithm presented in [5]. As a preliminary result, we present in Fig. 3 a comparison between numerically simulated images with and without the weighted subtraction algorithm. The obtained results demonstrate a clear enhancement using the proposed approach in agreement with the improvement up to a factor of two stated in [5]. The proposed approach not only improves image contrast and resolution but also mitigates over-subtraction and image distortions.

Fig. 1. Experimental setup for production of large arrays of switchable Gaussian and Laguerre-Gaussian beams. RM: Rotation mount, PBS: Polarizing beam splitter, AOD: Acousto-optical deflector, l/2: Half wave-plate, l/4: Quarter wave-plate, O: high numerical aperture microscope objective.

Fig. 2. Experimental parallelized 5x5 gaussian and TEM01 spot array.

Fig. 3. Numerically simulated super resolution image obtained using weighted subtraction algorithm.