Since about 20 years Abbe’s resolution limit has been overcome in fluorescence microscopy by the first demonstration of STED microscopy (1). Later, methods based on single-molecule localization joined the field, such as PALM (2) and STORM (3). These methods use principles that operate beyond the diffraction of light. Increased spatial resolution can also be achieved by a class of methods exploiting excitation and detection modalities still bound to light diffraction like structured illumination microscopy (SIM) (4) and image scanning microscopy (ISM) (5). While not reaching the resolution of STED, PALM, and STORM, they do not require any special labels, sample conditions, or excitation power, and may be applied to any sample at any wavelength.

ISM was presented in a theoretical study by Sheppard (6) finding that it is able to double the resolution of a scanning confocal microscope. The first implementation needed several minutes to record one image. York et al. (7) had overcome this limitation by using a multifocal excitation scheme. We demonstrated an acquisition time of less than 1 s per image using a spinning-disk confocal microscope (CSD-ISM) (8). The drawback of these systems is that the images have to be processed after recording, so a huge amount of data has to be processed.

Here, we present an all-optical implementation of ISM based on a resonant confocal laser-scanning microscope using two-photon excitation. The all-optical implementation means that the images are recorded directly with the full resolution and no post-processing is necessary. By this one can take the full advantage of the speed of the resonant laser-scanner providing a frame-rate of about 30 fps and a live view to the sample.