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Optical-sectioning microscopy by patterned illumination

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Abstract.
We propose a very simple method for the flexible production of 1D structured illumination for high resolution 3D microscopy. Specifically, we propose the insertion of a Fresnel biprism after a monochromatic point source for producing a pair of twin, fully coherent, virtual point sources. The resulting interference fringes are projected into the 3D sample and, by simply varying the distance between the biprism and the point source, one can tune the period of the fringes, while keeping their contrast, in a very versatile and efficient way.

1. Variable-frequency structured illumination

Non-uniform illumination techniques are between the most promising proposals to overcome the inherent limitations of conventional light microscopes regarding both lateral spatial resolution and optical sectioning capability[1]. In structured illumination (SI) microscopy a transverse periodic 1D pattern is projected onto the specimen and a stack of 2D images is recorded after scanning the object axially [2, 3, 4]. This illumination structure acts as a *carrier* periodic pattern, which generates several spectrally-shifted replicas of the original spatial-frequency content of the object. Through a phase-shift method, an extended spectrum of the object can be recovered over the image. This completion not only extends the lateral spatial resolution but also provides the system with optical sectioning capabilities.

From a practical point of view, in the classic SI microscopy an illumination plane wave is splitted into two tilted ones by means of a 1D diffraction grating. Their interference generates the desired periodic pattern inside the 3D sample under issue. It is worth to note, however, that different goals in SI microscopy —optimum optical sectioning or maximum lateral resolution— cannot be achieved simultaneously, and require different values of the frequency of the illumination pattern. Moreover, this frequency depends not only on the period of the *splitting* grating, but also on the optical power of the microscope objective. Thus, a change in this inspection objective would require a readjustment of the period of the grating to achieve the proposed optimization of the features of the SI microscope. This desirable tunability is not easily reached in the classic SI scheme, unless sophisticated variable-frequency gratings are used.

Note that a pair of interfering plane waves can be also obtained from two fully coherent point sources *collimated* by an spherical lens. In this case, the angle between the exiting waves is controlled by the focal length of the lens and, remarkably, by the distance between the point sources. Our proposal is to generate these fully coherent twin sources by use of a Fresnel biprism. When this element is illuminated by a point source, two virtual point sources are generated on

the same transverse plane as the original one, symmetrically located around it, with a separation proportional to the distance between the biprism and the real source. Thus, by simply shifting the biprism respect to the real point source one can change the separation between the virtual sources. If we consider now an spherical lens with its back focal plane laying on the virtual twin sources location, a pair of interfering plane waves is generated, whose relative angle can be varied simply by changing the gap between the sources. This tunability can be added to the classic SI microscope by replacing the grating and the plane-wave illumination by a point source, a Fresnel biprism and a collimating lens. The axial displacement of the biprism in this new setup generates a continuous variation in the frequency of the 1D periodic illumination pattern into the sample. Note that our proposal implies very small changes in the classic scheme of the SI microscope, in such a way that its implementation is suitable even into commercially available SI instruments.

As a test for our proposal, we use implement a tunable-frequency SI microscope by use of a Fresnel biprism with refringence angle $\delta = 0.5^\circ$ and refraction index $n = 1.51$. We generate the real point source from the output of an optical fiber coupled with a standard HeNe laser ($\lambda = 632.8$ nm). For the collimation of the twin spherical wavefronts we use an achromat with focal length $f = 200$ mm. This setup was followed by a standard microscope with a $f = 150$ mm tubelens and a low-NA (0.10) low-magnification ($5\times$) objective.

We present in Fig. 1(a) and Fig. 1(b) two images of the illuminated sample plane for two different axial positions of the biprism. As a target we use a *USAF 1951* test, that allows a direct comparison of the periodic illumination and the callibrated spatial-frequency sections of the test. A variation by a factor of 5 in the illumination spatial-frequency is clearly shown in this two pictures. We use also the highest of these *carrier* spatial-frequencies to obtain a superresolved synthetic image of the target under issue. Figures 1(c) and (d) show, respectively, the original and synthetic images obtained in this case, showing clearly the improvement in the lateral resolution predicted by the SI technique.

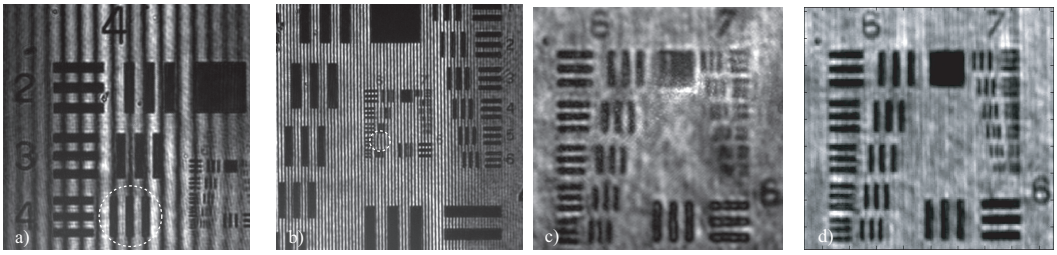

Figure 1. Experimental results for the the tunable-frequency SI microscope: (i) Illuminated target for two different axial locations of the biprism; the dashed circles correspond to the groups in the target with: (a) 22 lp/mm; (b) 102 lp/mm. (ii) Images of the central region of the target: (c) Original brightfield setup; (d) SI synthetic image with the illumination pattern in (b).

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