Superresolved Holographic Microscopy

Superresolution Imaging Beyond Abbe’s Diffraction Limit

Superresolution methods in digital holographic microscopy provide a useful tool to overcome the Abbe’s diffraction limit when using modest microscope lenses. The process improves the cutoff frequency of the microscope lens by means of the generation of a synthetic aperture based on time multiplexing and using 3 main stages: optical coding, optical decoding, and digital post-processing. After the whole process, a superresolved image is obtained by Fourier transformation of the synthetic aperture.

The Limited Resolving Power of Imaging Systems

One of the most important issues regarding optical imaging is concerning resolution. Filter theory applied to the spatial-frequency domain establish that optical imaging systems act as a low band pass filter that selects the low spatial-frequencies in the object’s spectrum. Then, the imaging system introduces a physical limitation given by its cutoff frequency or, in other words, the imaging system can be represented in the spatial-frequency domain by a limited aperture that limits its resolution.

As shown by Abbe more than a century ago [1], given the illumination wavelength (\(\lambda\)), the maximum achievable resolution for an imaging system is bounded by its numerical aperture (NA), reaching at best the \(\lambda/2\) value for air immersion optical systems. In that sense, the aim of superresolution techniques is to produce an improvement in the resolution limit without changes in the physical properties of the optical imaging system. This fact can be understood as the generation of a synthetic aperture that expands the frequency coverage of the optical system beyond the limit defined by its cutoff frequency, that is, beyond its physical limited aperture. This procedure is based on information capacity theory and a priori knowledge about the input object. The main concept relies in encoding additional information regarding a given degree of freedom into another one that is unused due to the object restrictions [2-4]. In such a way, time multiplexing achieves the passage of additional spatial-frequency information of the object’s spectrum through the limited system aperture by encoding it as temporal-frequency
information using that the object is temporally restricted or static over the imaging time.

Obviously, this temporal-frequency information needs to be decoded to obtain a high-resolution image.

**The Goal of Time Multiplexing Superresolution**

Over the last years, time multiplexing has been combined with digital holography in a wide variety of ways with the motivation of recovering both the amplitude and the phase distribution of the sample under test. In particular, interferometric imaging is yielded in microscopy field [5-10] where synthetic aperture generation permits high-resolution images using low NA microscope lenses and thus taking the highly attractive advantages provided by such objectives, that is, large depth of field, long working distance, large field of view and relatively low cost. These techniques are based on off-axis illumination onto the object to downshift its high-frequency content and enable its transmission through the limited system aperture (encoding stage). After that and by means of holographic recording at the image plane (decoding stage), a synthetic aperture is generated by digital relocation of each transmitted frequency band to its original position in the object's spectrum (digital post-processing stage). And finally, a superresolved image is obtained by simple Fourier transformation of the generated synthetic aperture. Moreover, as the involved process for each frequency band is coherently performed, a flat transfer function with no attenuation of the higher frequencies is achieved. In the encoding stage, off-axis illumination impinges the input plane where the sample is placed. Off-axis illumination can be done in sequential mode, by shifting a single point source or by lighting on sequentially different sources in a two-dimensional (2-D) array [such as Vertical Cavity Surface Emitting Laser (VCSEL) array], or can be produced in one-shot using a 2-D source array. In any case, this is one of the main advantages of the proposed methods: the possibility to synthesize any shape for the expanded aperture. Since the generated synthetic aperture using the suggested approaches is the convolution of the source array and the coherent transfer function of the imaging system, by light on and off the different single elements in the 2-D array, any synthetic transfer function may be realized at will. Moreover, by temporally varying the relative amplitudes of each source in the array, not only flat synthetic apertures can be generated but different types of optical filtering, such as low-pass filtering, border enhancement, etc, can be produced [5]. Figure 1 depicts two different cases (cross-shape and octagonal) of generated synthetic apertures in different experimental configurations [8,10]. In
both cases, a negative high-resolution USAF test target is used as sample to show the method capabilities. A resolution improvement factor 2.5 and 3 is achieved in the first case [(a)-(b)-(c)] and the second one [(d)-(e)-(f)] respectively. The decoding stage is characterized by interferometric image plane recording either by off-axis holographic recording or by using phase-shifting method. Different architectures of interferometers can be used in the digital holographic recording [7-10] to recover the complex amplitude distribution regarding each transmitted frequency band. The elementary pupils depicted in figure 1(b) and (e) were recovered using off-axis recording while that ones presented in figure 2(e) were obtained using phase-shifting procedure. Figure 2 depicts another example of superresolution microscopy by synthetic aperture generation where human red blood cells are used as biosample. In this case, octagonal synthetic aperture allows full 2-D frequency space coverage at the Fourier domain and the cutoff frequency of the conventional system is nearly tripled.

As a final stage, digital processing consisting on a filtering process of the -1 hologram diffraction order over each transmitted frequency band due to the different off-axis illuminations allows the recovery of each elementary pupil. After that and by properly assembly of the different recovered single pupils to its original position in the object's spectrum, the enlarged synthetic aperture is defined in terms of time multiplexing in the Fourier domain. Then, a Fourier transformation of the synthetic aperture yields in the final superresolved image.

**Synthetic Aperture Microscopy as a Way to Quantitative Phase Imaging**

The previous methods are not only useful to improve the spatial resolution of imaging systems. Since they allow access to the complex amplitude distribution of the sample, quantitative phase superresolved imaging can also be obtained. This means that three-dimensional information of the sample under test is also achievable because it is inherent to the phase information map. Figure 3 depicts the unwrapped phase distribution corresponding with the solid white rectangles drawn in fig. 2(b) and (d). Aside the superresolution effect, a quantitative way for high-resolution phase imaging is obtained. Although the results reported here are for a microscope lens with low NA value, the methods can be applied to medium NA lenses and the generated synthetic aperture can reach the maximum air immersion value. Moreover the use of small aperture greatly simplifies the interferometric setup needed for the phase retrieval, while the synthetic aperture permits achieving useful resolution in many biological microscopic applications.

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