k- Microscopy – resolution beyond the diffraction limit

Matthias Geissbuehler[1], Theo Lasser[1], Rainer A. Leitgeb[1,2]

1) Laboratoire d'Optique Biomedicale, Ecole Polytechnique Federale de Lausanne, CH-1015 Lausanne, Switzerland;
2) Center of Biomedical Engineering and Physics, Medical University of Vienna, A-1090 Vienna, Austria;

Abstract

We present a novel Fourier domain method for microscopic imaging – so-called k-microscopy– with lateral resolution independent of the detection numerical aperture. The concept is based on sample illumination by a lateral fringe-pattern of varying spatial frequency, which probes the lateral spatial frequency or k- spectrum of the sample structure. The illumination pattern is realized by interference of two collimated coherent beams. Wavelength tuning is employed for modulation of the fringe spacing. The uniqueness of the proposed system is that a single point detector is sufficient to collect the total light corresponding to a particular position in the sample k-space. By shifting the phase of the interference pattern, we get full access to the complex frequencies. An inverse Fourier transformation of the acquired band in the frequency- or k-space will reconstruct the sample. The resulting lateral resolution will be defined by the temporal coherence length associated with the detected light source spectrum as well as by the illumination angle. The feasibility of the concept has been demonstrated in 1D.

Keywords: k-microscopy, sub-diffraction limit, microscopy, wavelength tuning, biomedical imaging, Fourier optics;

1. Introduction

There have been different strategies to increase the lateral resolution in microscopy beyond the actual diffraction limit as stated by Abbe[1-3]. Structured illumination has been applied yielding a two-fold increase in lateral resolution. The idea of enhancing the lateral resolution is related with the extension of the associated Fourier or k-space of the structure. Structured illumination shifts higher spatial frequencies into the range covered by the detection transfer function. Recent papers showed how to enhance the k-space by illuminating the sample with a set of fringe patterns with different spacing, and detecting the modulated sample with a sensitive CCD array.
We propose illuminating again the sample with a set of fringes, but instead of detecting the full transverse structure with an array detector we show that it is sufficient to use a single point detector.

*) corresponding author: rainer.leitgeb@meduniwien.ac.at tel. +43 1 4277 60727, fax. +43 1 4277 9607

2. Theory

The central idea is to collect information in the k-space and reconstruct the actual transverse sample structure via Fourier transform similar to Fourier domain OCT.
Let us first describe the effect of probing the sample with a set of fringes corresponding to different optical frequencies shown in Fig. 1.

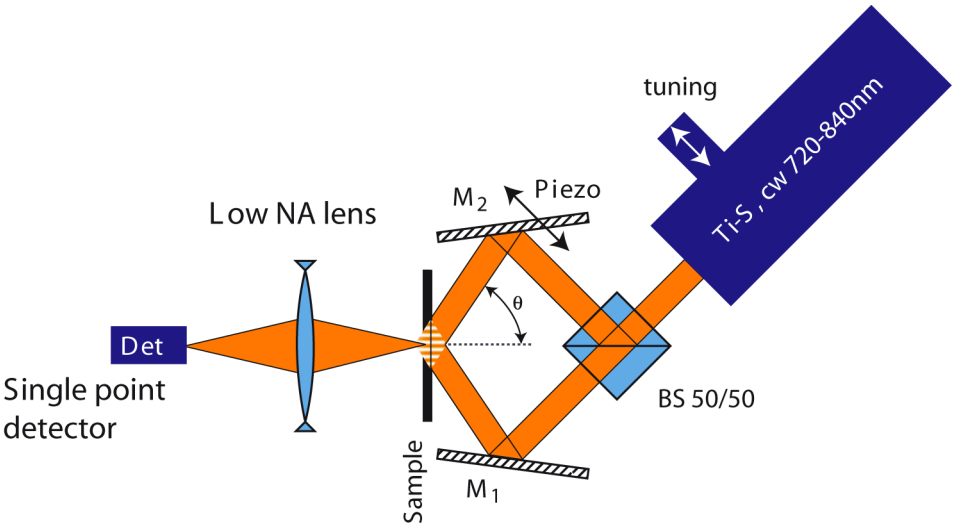

Fig. 1: Interference of two beams creates a fringe pattern. A single point detector is sufficient with low NA optics to collect the whole light emitted from the illuminated structure at the surface sample.

In order to synthesize the k-space we need the full complex signal. This is obtained via phase shifting of one illumination beam in Fig. 1. Four phase shifted copies of the interference pattern on the sample allow for reconstructing the complex contribution from the interference part, suppressing the DC offset.
If we scan the spectrum we will of course have a limited coverage of the k-space. In fact we confine ourselves to a ring with a diameter given by the wavenumber of the spectrum and corrected by the illumination angle. The fringe spacing is given by $d= \lambda/(2\ n\ \sin(\theta))$, where n is refractive index of the substrate, λ the wavelength and θ the illumination angle at the surface (Fig.1). In principle one could also scan the angle, but this will take more time than employing fast wavelength tuning sources.
Assume now a spectral bandwidth spanning over $\Delta\lambda$ and centered at λ_0. The corresponding k values are related via $k=2\pi/\lambda$. The lateral spatial frequency associated to this k value is $k_s=k.n.\sin(\theta)$.
The lateral resolution obtained with this k-sampling is given by observing at which distance there is a phase delay of the sinusoidal illumination pattern of π between the associated spectral components much like as for the definition of temporal coherence. The lateral coherence envelope or lateral resolution is found by superposition of the interference patterns of the different k-values of the spectrum and will be for a Gaussian spectrum $l_c= (4\ln2/\pi)\ (\lambda_0)^{2}/\ (\Delta\lambda.\ n.\sin(\theta))$. Let us assume a spectral width of 300nm centered at 500nm an angle of 70deg and n=2. This results in a lateral resolution of

882nm (see also Fig. 2). We immediately observe that it is important to keep the central wavelength low as the resolution increases quadratically as well as to use large refractive index substrate materials. Note as well that the resolution is independent of the detection optics. It is only limited by the illumination spectrum and geometry. Hence the resolution properties of the proposed microscope have been shifted from the detection to the illumination part.

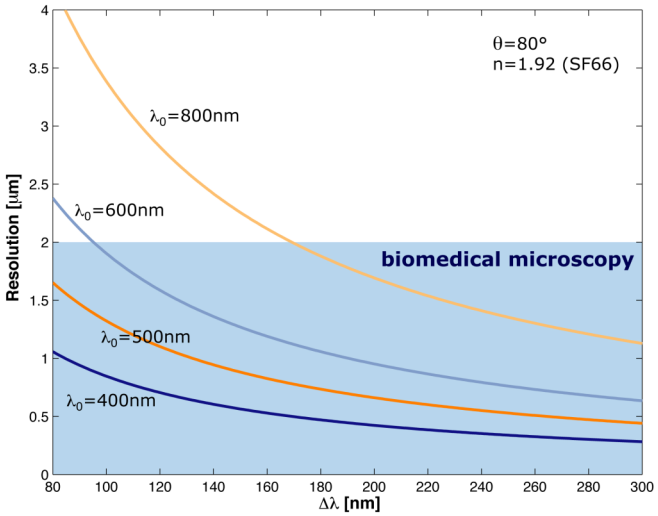

Figure 2: Theoretical resolution of k-microscopy

We did not yet discuss the property of the sample itself. If we use amplitude objects on the substrate surface, we would at the detector observe a coherent superposition of the transmitted sample light. In the reconstruction we need then to multiply the actual excitation spectrum, that defines the k-spectrum, with the spectral envelope of the structure emission spectrum. This will of course have a reduction effect on the achievable transverse resolution.

If we look back to the setup in Fig. 1 we observe the low NA detection optics. In fact since we detect the whole emitted light at once our resolution is not limited by the diffraction limit of this optics. It is only dependent as already stated by the ability to produce an as large number as possible of fringe patterns with different spacings and different directions across the sample. Hence the optical detection system has a naturally large signal-to-noise ratio being an equivalent to the Felgett advantage of Fourier transform spectroscopy.

We have for a given spatial frequency k_s the intensity distribution across x

$I_{S,0}(k_s,x)=I_e(k)\ I_0(x,k_s).S(x)$,

Where S(x) is the sample structure, Ie(k) is the spectral emission characteristic of the sample, and I_0 is the source intensity. The total measured intensity is the integral across the x direction. If we add an additional 90deg phase shifted copy of the illumination pattern we have for the complex signal entry $I_S(k)=I_{S,0}(k_x)-jI_{S,90}(k_x)$. The structure is finally obtained via inverse Fourier transform of the complex k-domain

$I_S(x) = \{FFT\ (I_S(k_s)\}$.

The Fourier relation defines also the achievable transverse field of view. It is given by the sampling density of the recorded spectrum. Assuming N spectral sampling points, and a

covered full spectral width of Λ, we finally end up with a lateral Nyquist-limited range of $Z_{max}= N.(\lambda_0)^2/ (2.\Lambda. n.\sin(\theta))$.
Fig. 3 displays schematically the reconstruction process with an illustrative sample. We indicated in the Fourier plane of the image the actual finite spectral support, which is ring shaped. The width and position of the ring is defined by the swept spectrum of the light source. The final image will then resemble a high-pass filtered version of a standard transmission microscopy image much like with dark field contrast.

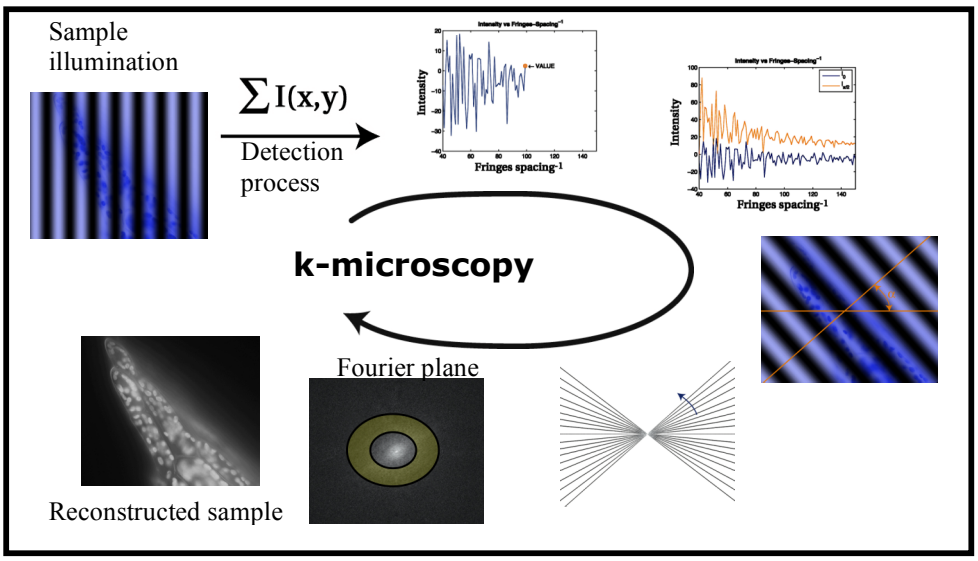

Figure 3: illustration of the image reconstruction process

Like in OCT we can however directly profit of the fact that we have a complex signal detection method. This also means that we have a high detection sensitivity of lateral structural changes of fractions of the wavelength. Such possibility is not provided by standard microscopy techniques.
Complex signal retrieval can be either achieved by phase stepping methods or by continuously scanning the phase such as with an electro-optic modulator. The latter could be used for heterodyne signal detection, which would help to eliminate DC offsets and further increase the detection sensitivity.

3. Results and Discussion

For demonstrating the feasibility of the proposed concept we measured a resolution test target in one dimension as shown in Fig.4. In order to countercheck our results, we used a CCD camera at the detection side and used pixel binning to simulate point detection. Fig. 4(a) displays the sub-region of the resolution target that was measured. The measured spectrum and reconstructed structure is given in Fig. 4(b) and (c), respectively. Note that we observe only the edges of the actual structure due to the finite spectral support.
For the measurement we covered a spectral bandwidth of 120nm centered at 780nm resulting in a theoretical transverse resolution of 3.3µm. We recorded 1024 spectral

sampling points. The relatively long measurement time of 2h is currently limited by the slow wavelength tuning mechanics.

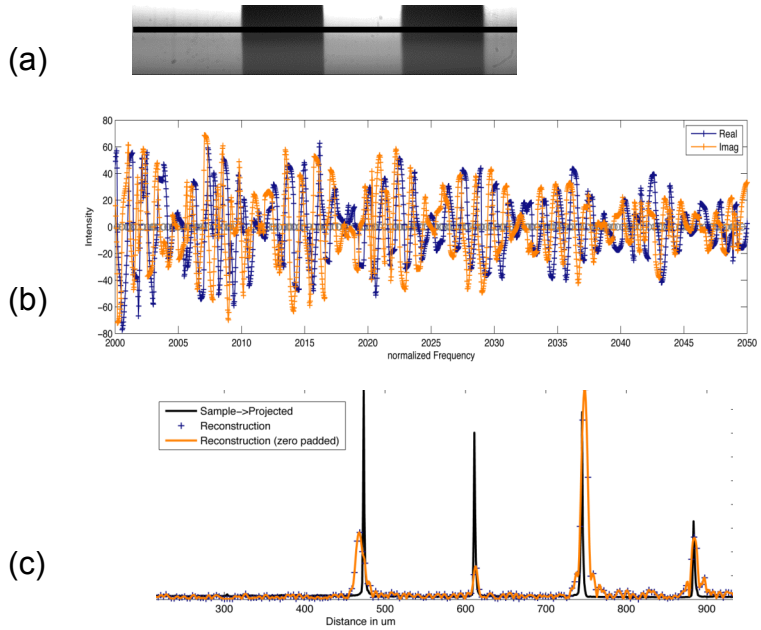

Figure 4: (a) resolution test target (group 1 (7)) measured along indicated line;(b) recorded complex spectrum (sine and cosine-component shown);(c) reconstructed 1D sample. Due to the finite bandwidth we observe only the edges of the actual sample structure.

In conclusion we present a method together with a first proof of concept that has the advantage of large throughput, high resolution independent of the detection diffraction limit, and the availability of lateral phase. The technique is in the line of current technological development for fast tunable wavelength sources [4]. Future efforts are aimed at full 2D image reconstruction as well as at speeding up the wavelength tuning process.

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