Published in final edited form as:

Opt Lett. 2008 October 1; 33(19): 2209–2211.

Measurement of subcellular texture by optical Gabor-like filtering with a digital micromirror device

Robert M. Pasternack¹, Zhen Qian¹, Jing-Yi Zheng¹, Dimitris N. Metaxas², Eileen White³, and Nada N. Boustany^{1,*}

¹ Department of Biomedical Engineering, Rutgers University, Piscataway, New Jersey 08854, USA

² Department of Computer Science, Rutgers University, Piscataway, New Jersey 08854, USA

³ Department of Surgery, Cancer Institute of New Jersey, Robert Wood Johnson Medical School, New Brunswick, New Jersey 08903, USA

Abstract

We demonstrate an optical Fourier processing method to quantify object texture arising from subcellular feature orientation within unstained living cells. Using a digital micromirror device as a Fourier spatial filter, we measured cellular responses to two-dimensional optical Gabor-like filters optimized to sense orientation of nonspherical particles, such as mitochondria, with a width around 0.45 μm . Our method showed significantly rounder structures within apoptosis-defective cells lacking the proapoptotic mitochondrial effectors Bax and Bak, when compared with Bax/Bak expressing cells functional for apoptosis, consistent with reported differences in mitochondrial shape in these cells. By decoupling spatial frequency resolution from image resolution, this method enables rapid analysis of nonspherical submicrometer scatterers in an under-sampled large field of view and yields spatially localized morphometric parameters that improve the quantitative assessment of biological function.

Fundamental biological processes, such as programmed cell death (apoptosis), involve time-dependent dynamic alterations in the morphology and subcellular organization of submicrometer scale organelles. As in any structure-function relationship, these morphological changes are controlled by important molecular pathways and must be quantified objectively to gain a more complete understanding of cellular function. Electron microscopy (EM) can directly image these structures but suffers from low throughput and cannot track dynamic structure changes. Such limitations motivate the development of high throughput optical methods that quantify time-dependent changes in morphology on very small-length scales and over a large field of view.

To assess morphological changes with subwave-length sensitivity in unstained living cells, we developed an optical scatter imaging (OSI) technique based on Fourier spatial filtering using an iris with a variable diameter as a Fourier filter in a dark-field microscope [1]. In this Letter, we demonstrate how the assessment of sample morphology can be greatly extended by utilizing a spatial light modulator (SLM) as a Fourier filter. As a proof of concept, we used the SLM to implement 2D Gabor-like filters that can characterize particle orientation and roundness. Gabor filters have been used extensively in texture analysis of digital images. In the space domain, a Gabor filter corresponds to a sinusoidal wavelet with

*Corresponding author: nboustan@rci.rutgers.edu.

OCIS codes: 070.2615, 070.6120, 170.0180, 170.1530, 170.1420.

a Gaussian envelope and local image texture is described by the Gabor filter that gives a maximum image-filter convolution response [2,3]. Here we extend this approach to the analysis of biological sample textures using analog optical Gaborlike filters. These filters allow us to characterize nonspherical subcellular particles without relying on a detailed scattering model.

The SLM in our setup (Fig. 1) consisted of a digital micromirror device (DMD) (TI 0.7 XGA DMD 1100) and was placed in the conjugate Fourier plane of an inverted microscope (Axiovert 200M, Zeiss, Göttingen, Germany) fitted with a 20× objective an NA of 0.75. Light from a ~5 mW He–Ne laser ($\lambda_o = 632.8$ nm) was passed through a spinning diffuser and coupled into a multimode fiber whose output was collimated and launched into the microscope's condenser aligned in central Köhler illumination (NA < 0.05) to provide a spatially coherent plane wave. Image acquisition consisted of collecting on the CCD a stack of spatially filtered dark-field images using a spatial filter bank generated by the DMD. The DMD is a 1024×768 array of individually addressable $13.7 \mu\text{m} \times 13.7 \mu\text{m}$ mirrors, which can be programmed to deflect the light toward or away from the CCD detector, thus allowing for binary on–off modulation of the field at each mirror. The microscope aperture was projected on a central DMD disc having a radius of 151 mirrors to avoid aberrations originating from the edges of the DMD. In this setup, mirror 151 corresponded to the maximum spatial frequency of $\text{NA}/\lambda_o = 1.185 \text{ cycles}/\mu\text{m}$ passed by the 20× objective, and the frequency increment was $1.185/151 = 0.00785 \text{ [cycles}/\mu\text{m}]/\text{mirror}$.

To analyze subcellular texture due to particle orientation, we programmed the DMD to display 2D Gaborlike filters with a circularly symmetric Gaussian envelope. In the space domain, the Gabor filter is composed of a 2D Gaussian multiplied by a complex 2D sinusoid [2]. Thus, in the Fourier domain, our Gabor filter's impulse response is a single Gaussian-shaped bandpass filter,

$$H(u, v) = (\pi S^2 / 2) \cdot e^{-(\pi^2 S^2 / 2) [(u-U)^2 + (v-V)^2]},$$

whose center is located at the frequencies (U, V) in k space. In the space domain, the period and orientation of the sinusoid are $S = 1 / \sqrt{U^2 + V^2}$ and $\varphi = \arctan(V/U)$, respectively, and the extent of the Gaussian envelope is $\sigma = S/2$. As a result, the rotation of the Gabor filter is determined only by the 2D frequencies of the complex sinusoid, and two periods of the wavelet are included in four standard deviations of each Gaussian kernel in the space domain. In the present analysis, $S = 0.9 \mu\text{m}$ and $0^\circ \leq \varphi < 180^\circ$ in 20° increments. To map the angle of maximum filter response to actual object orientation, the object's field amplitude or phase profile in space may be approximated by a rectangular function with width $2s$ and orientation θ . The maximum Gabor response given our filter definition will be achieved when $S \sim 4s$ and $\theta = \varphi + \pi/2$. Thus, our filter design emphasizes the signal from oriented objects $\sim 0.45 \mu\text{m}$ in width, and one can retrieve to within 20° the object orientation at each pixel based on the filter response. In cells, our filter will emphasize elongated organelles such as mitochondria, which are oriented and have widths between 0.3 and $0.6 \mu\text{m}$ [4]. Although the DMD filtering is binary, we approximated the Gaussian Gabor filter by generating four binary concentric discs located at a radial distance of 142 mirrors from the DMD center in the Fourier plane corresponding to $1.11 \text{ cycles}/\mu\text{m}$. Because the four discs are centered at the same position in the Fourier plane, the phase component of each associated filtered image is not significantly altered thereby allowing for the approximation of each Gabor-filtered image simply by summing the four filtered images with equal exposure time. The Gaussian envelope is thus reconstructed by four binary filter steps (Fig. 1, upper left) centered at mirror 142 with step radii 65, 45, 31, and 17 in DMD mirrors.

We first applied our orientation sensitive filter bank to a marine diatom sample (Carolina Biological Supply Company) with oriented features that were clearly visible in dark-field imaging [Fig. 2(a)]. The set of nine Gabor-filtered images of the diatom were processed pixel by pixel for object orientation and roundness. Processing consisted of (1) summing measured responses of all nine Gabor-filtered images at each pixel to determine the overall magnitude of the signal response thereby encoding response significance and (2) finding the Gabor filter orientation, ϕ , at which the response is maximized and taking the ratio of this maximal response to the average response for all angles thereby encoding the extent to which objects at each pixel have a preferred orientation. The degree of orientation is closely related to the geometric aspect ratio of the particle. In Fig. 2(b), the overall response of the pixel to the filter bank (parameter 1) and the degree of orientation or aspect ratio (parameter 2) are encoded in the color saturation and hue, respectively. Substructure particle orientation is encoded in a quiver plot [Fig. 2(c)], where each line closely agreed with the underlying local object orientation visible in the dark field.

As a biological proof of principle, we applied the same method to measure orientational texture in a small number of immortalized baby mouse kidneys (iBMK) cells [5] (Fig. 3). The cells were mounted onto the microscope as described previously [1]. We used apoptosis competent cells (W2, $n=20$), and apoptosis-defective cells (D3, $n=22$) lacking the genes encoding for Bax and Bak, two proteins required for apoptosis effected by mitochondria [6]. Histograms of the orientation image pixels show that there are significantly more round features in the D3 compared with the W2 cells [Fig. 3(g)]. The mean aspect ratio per cell was 1.58 for W2, 1.38 for D3 ($p<0.04$ by student t test). Our histograms could be consistent with recent data showing that mitochondria in cells lacking Bax and Bak are shorter and have lower fusion rates compared with cells expressing Bax and Bak [7] to the extent that shorter mitochondria in our D3 cells will result in a larger number of particles with a lower mean aspect ratio.

The current technique is an extension of OSI [1] and exhibits the same trade-off between frequency sensitivity in the Fourier domain and image resolution in the space domain. Owing to their Gaussian envelopes, Gabor filters confine the bandpass filtering to local spatial areas. They also simultaneously achieve the optimal localizations in both the spatial and the spatial frequency domains [3]. Given the choice of the filtering parameters used in this Letter, the Gabor filter responses should have been confined to object areas on the order of $0.9\mu\text{m}\times 0.9\mu\text{m}$. However, by positioning the Gabor filter near the NA passed by the microscope, the Gaussian envelope of our filters was effectively truncated on the side facing the NA in the Fourier plane. Objects, such as mitochondria with a signal at high frequencies, require analysis near the edge of the NA in many optical settings, and in such situations, this caveat may be unavoidable but does not fundamentally compromise the ability of the filtering technique to detect object orientation as indicated in the diatom and cell data sets. Eliminating the filter truncation in the Fourier plane by shifting S to $1\mu\text{m}$ and halving the standard deviation of the Gaussian in the frequency plane resulted in similar differences between the aspect ratio histograms of the W2 and D3 cells (not shown), but the sensitivity trade-off manifests as lower resolution orientation data images in the image plane. Ultimately, histogram differences became less pronounced once the frequency confinement of the filter was further decreased to correspond to an expanded object area of $\sim 3\mu\text{m}\times 3\mu\text{m}$. The degree of particle orientation is thus decreasingly detectable as the subcellular regions being probed begin to include several particles with different orientations and is averaged out.

Unlike digital processing, analog filtering generally allows for direct manipulation of the object's field transform rather than the intensity of the discretized object convolved with the system's point-spread function. While the DMD only allowed for binary filtering, a liquid-

crystal device (LCD) could be used for graded amplitude modulation with one Gaussian-shaped filter rather than the sum of four intensity-based step filters. In addition, LCD-enabled phase modulation of specific frequency components could allow for better sensitivity to specific biological activity involving motion or deformation around these frequency components. Analog processing also confers the advantage that frequency resolution is decoupled from image quality and depends only on the properties of the SLM filter. For example, digital processing of optically resolved objects that are $0.5\ \mu\text{m}$ in size requires a sampling rate of at least one pixel every 250 nm. In analog processing, one pixel could correspond to the object region being probed by the filter or an area as large as $1\ \mu\text{m} \times 1\ \mu\text{m}$ for detection of orientational texture based on the present Gabor filter bank. With 512×512 camera pixels, this $4\times$ demagnification translates into analyzing subcellular textures simultaneously in a few hundred cells instead of 20–30 at comparable seeding densities. To simulate this situation, we show our cell data after block processing the initial Gabor-filtered images such that every adjacent 4×4 pixel region is averaged into one pixel before orientation processing, effectively demagnifying the filtered images from 512×512 to 128×128 pixels, or approximately $1\ \mu\text{m}^2/\text{pixel}$. Although the pixels in the resulting images are large [Figs. 3(e) and 3(f)], pixel histograms after demagnification [Fig. 3(h)] give an aspect ratio mean of 1.54 for W2 and 1.35 for D3 ($p < 0.05$) and still demonstrate orientation detection substantially similar to that of the higher magnification [Fig. 3(g)].

In conclusion, we have demonstrated the use of a DMD as a spatial Fourier filter that can be used to quantify the orientation and geometrical aspect ratio of subcellular organelles. Furthermore, although this technique essentially measures angular scatter information, it does not rely on a scattering model such as Mie theory. The morphometric features of subcellular organelles are directly characterized by their differential response to Gabor filters with different dimensions and orientations. This technique is therefore applicable to nonspherical organelles for which a precise theoretical scatter description is not easily given and provides distinctive morphometric parameters that can be obtained within unstained living cells to assess their function.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank B. Beaudoin and K. Degenhardt for iBMK cells and protocols. This work was supported by Whitaker Foundation grant RG02-0682 to N. Boustany, grant NCI R37CA53370 to E. White, and a Rutgers Presidential Fellowship to R. Pasternack.

References

1. Boustany NN, Kuo SC, Thakor NV. *Opt Lett* 2001;26:1063. [PubMed: 18049520]
2. Dunn D, Higgins WE, Wakeley J. *IEEE Trans Pattern Anal Mach Intell* 1994;16:130.
3. Daugman JG. *J Opt Soc Am* 1985;A2:1160.
4. Loud AV. *J Cell Biol* 1968;37:27. [PubMed: 5645844]
5. Degenhardt K, White E. *Clin Cancer Res* 2006;12:5298. [PubMed: 17000662]
6. Degenhardt K, Chen G, Lindsten T, White E. *Cancer Cells* 2002;2:193.
7. Karbowski M, Norris KL, Cleland MM, Jeong SY, Youle RJ. *Nature* 2006;443:658. [PubMed: 17035996]

Fig. 1.

Object's transform is focused at F_1 and F_2 ; its filtered image is at I_{CCD} . The zeroth order (dotted rays) is blocked at F_2 and F_1 to minimize the dark-field background. The imaging beam (solid rays) incident on the DMD is collimated, and Gabor filtering is applied by the DMD at F_2 . Inset, Gabor filter frequency response.

Fig. 2. (Color online) (a) Dark field image of diatom. (b) Object orientation image. Colorscale encodes degree of orientation (aspect ratio) while brightness encodes significance of the total Gabor filter response. (c) Orientation of objects with response intensity $\geq 10\%$ of maximum. Line segment indicates the corresponding structure's long axis.

Fig. 3.

(Color online) W2 and D3 images. (a) Differential interference contrast. (b) Dark-field. (c) Object orientation as in Fig. 2(b). (d) Orientation of objects with response intensity $\geq 15\%$ of maximum. Line segment indicates the corresponding structure's long axis. (e), (f) W2 and D3 orientation images after block-processing the initial Gabor-filtered images to simulate a 4× demagnification. (g), (h) Pixel histograms of all W2 and D3 cells (g) before and (h) after demagnification. Color-bar values in panels (c), (e), and (f) are taken as “aspect ratio.”