

## 4. IMAGE CHARACTERISTICS

- Imaging is the process of *mapping* a certain *physical property* of an object and displaying it in a *visual form* [8].
- Examples of such physical properties include absorption (e.g. light imaging, x-rays), reflectivity (e.g. ultrasound, photography), proton density (e.g. MRI), concentration of radionuclides (e.g. nuclear imaging).
- In microscopy, intrinsic specimen *absorption, emission, and scattering* are the quantities of interest. Using exogenous contrast agents, e.g. stains or fluorescent tags, one can image concentration distributions of certain species.
- We discuss the basic properties of microscope images, irrespective of the method involved in acquiring the images and present simple image enhancement operations allowed by numerical image processing.

## 4.1 Imaging as linear operation

- Often an imaging system, e.g. a microscope, can be approximated by a *linear system*.
- Consider the physical property of the sample under investigation is described by the function  $S(\mathbf{r})$ ,  $\mathbf{r} = (x, y, z)$ . The imaging system outputs an image  $I(\mathbf{r})$  which is related to  $S(\mathbf{r})$ , through a *convolution* operation,

$$I(\mathbf{r}) = S(\mathbf{r}) * h(\mathbf{r}) \quad (4.1)$$

- $h$  defines the *impulse response or Green's function* of the system.  $h$  is commonly called *point spread function (PSF)*. We are not specific as to what quantity  $I$  means. For example,  $I$  can be an intensity distribution, like in incoherent imaging, or complex field like in coherent imaging and QPI.  $h$  can be experimentally retrieved by imaging a very small object, i.e. approaching a delta-function in 3D.

- Thus, replacing the sample distribution  $S(\mathbf{r})$  with  $\delta(\mathbf{r})$ , we obtain

$$\begin{aligned} I_\delta(\mathbf{r}) &= \delta(\mathbf{r}) * h(\mathbf{r}) \\ &= h(\mathbf{r}) \end{aligned} \tag{4.2}$$

- The PSF of the system,  $h(\mathbf{r})$ , defines how an infinitely small object is blurred through the imaging process. The PSF is a measure of the *resolving power* of the system.
- $I(\mathbf{r})$  can be an intensity distribution or a complex field distribution, depending upon whether the imaging uses spatially *incoherent* or *coherent* light, respectively. This distinction is very important because in coherent imaging, the system is linear in complex fields, while in incoherent imaging, the linearity holds in intensities.

## 4.2 Resolution

- The resolution of an imaging system is defined by the minimum distance between two points that are considered “resolved.” What is considered resolved is subject to convention.
- The half-width half maximum of  $h$  in one direction is one possible measure of resolution along that direction. When discussing image formation, we may encounter other conventions for resolution.
- The image can be represented in the spatial frequency domain via a 3D Fourier transform (see Appendix B)

$$\tilde{I}(\mathbf{k}) = \int_V I(\mathbf{r}) \cdot e^{-i\mathbf{k} \cdot \mathbf{r}} d^3\mathbf{r}, \quad (4.3)$$

- $\mathbf{k} = (k_x, k_y, k_z)$  is the (angular) spatial frequency, in units of rad/m.

- Writing  $I$  in separable form,  $I(\mathbf{r}) = I_x(x) \cdot I_y(y) \cdot I_z(z)$ , the 3D Fourier transform in Eq. 3 breaks into a product of three 1D integrals,

$$\begin{aligned} \tilde{I}(\mathbf{k}) &= \left( \int I_x(x) \cdot e^{-ik_x \cdot x} dx \right) \cdot \left( \int I_y(y) \cdot e^{-ik_y \cdot y} dy \right) \cdot \left( \int I_z(z) \cdot e^{-ik_z \cdot z} dz \right) \\ &= \tilde{I}(k_x) \cdot \tilde{I}(k_y) \cdot \tilde{I}(k_z) \end{aligned} \quad (4.4)$$

- To find a relationship between the object and the image in the frequency domain, we Fourier transform Eq. 1 and use the convolution theorem to obtain,

$$\tilde{I}(\mathbf{k}) = \tilde{S}(\mathbf{k}) \cdot \tilde{h}(\mathbf{k}), \quad (4.5)$$

- $\tilde{S}$  and  $\tilde{h}$  the Fourier transforms of  $S$  and  $h$ , respectively.  $\tilde{h}(\mathbf{k})$  is referred to as the *transfer function (TF)*.

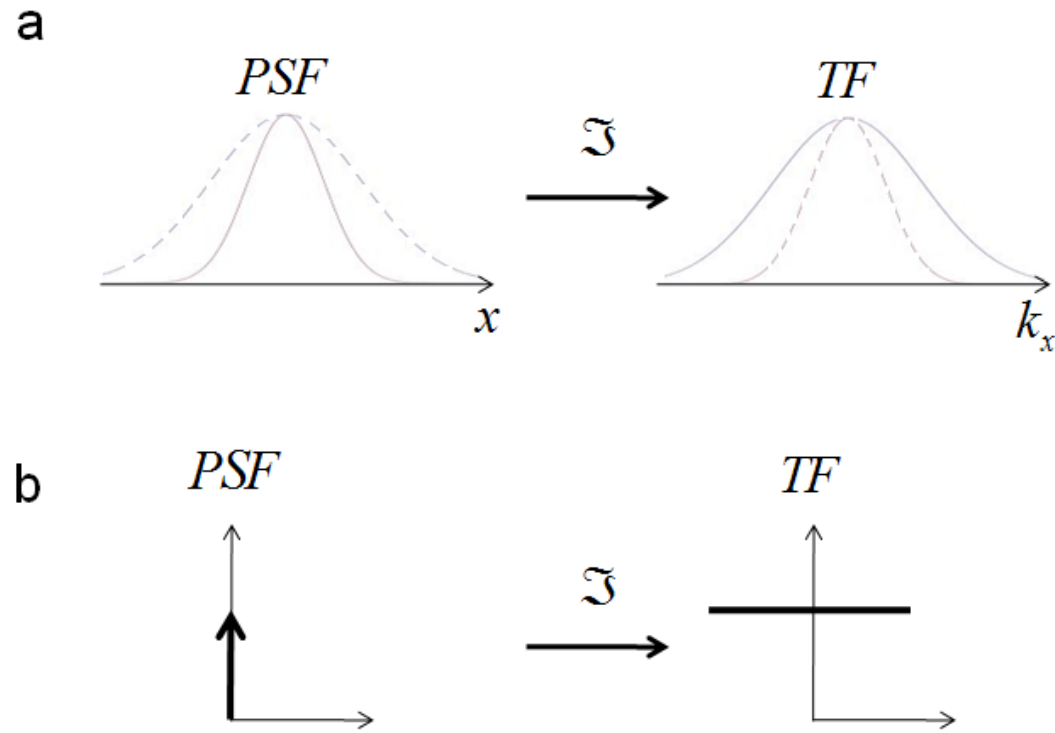


Figure 1. a) Relationship between the modulus of PFS and TF. b) Infinitely narrow PSF requires infinitely broad TF.

- From the Fourier relationship between PSF and TF, it is clear that *high resolution* (i.e. narrow PSF) demands *broad frequency support* of the system (broad TF), as illustrated in Fig. 1.

- The ideal system for which  $h(\mathbf{r}) = \delta(\mathbf{r})$  requires infinite frequency support, which is clearly *unachievable* in practice,

$$\delta(\mathbf{r}) \leftrightarrow 1 \quad (4.6)$$

- The physics of the image formation defines the instrument's PSF and TF. We will derive these expressions for coherent and diffraction-limited microscopy later. For now, we continue the general discussion regarding image characteristics, irrespective of the type of microscope that generated the image, or of whether the image is an intensity or phase distribution.

### 4.3 Signal to noise ratio (SNR)

- Any measured image is affected by experimental noise. There are various causes of noise, particular to the light source, propagation medium, and detector, which contribute to the measured signal,

$$\hat{x} = x + \xi. \quad (4.7)$$

- $\hat{x}$  is the measured signal,  $x$  is the true (noise-free) signal, and  $\xi$  the noise.
- The *variance*,  $\sigma^2$ , of the signal describes how much variation around the average there is over N measurements,

$$\sigma^2 = \frac{1}{N} \sum_{i=1}^N \left( \hat{x}_i - \bar{\hat{x}} \right)^2, \quad (4.8)$$

$$\bar{\hat{x}} = \frac{1}{N} \sum_i \hat{x}_i$$

- The N measurements can be taken over N pixels within the same image, which characterizes the noise *spatially*, or over N values of the same pixel in a time-series, which defines the noise *temporally*.
- The *standard deviation*  $\sigma$  is also commonly used and has the benefit of carrying the same units as the signal itself.
- The SNR is defined in terms of the standard deviation as

$$\text{SNR} = \frac{|x|}{\sigma}, \quad (4.9)$$

- The signal is expressed as a modulus, such that  $\text{SNR} > 0$ .
- Let us discuss the effects of averaging on SNR. It can be easily shown that for N *uncorrelated* measurements, the *variance of the sum is the sum of the variance*,

$$\sigma_N^2 = N\sigma^2 \quad (4.10)$$

- Thus, the standard deviation of the averaged signal is

$$\sigma_N = \frac{\sigma}{\sqrt{N}}, \quad (4.11)$$

- and the SNR becomes

$$\text{SNR}_N = \sqrt{N} \cdot \text{SNR} \quad (4.12)$$

- Equation 12 establishes that taking N measurements and averaging the results increases the signal to noise ratio by  $\sqrt{N}$ . This benefit only exists when there is no correlation between the noise present in different measurements.
- For correlated (coherent) noise, Eq. 10 is not valid. To prove this, consider the variance associated with the sum of two correlated noise signals  $a_1$  and  $a_2$  (assume  $\bar{a}_1 = \bar{a}_2 = 0$  for simplicity),

$$\begin{aligned} \overline{(a_1 + a_2)^2} &= \overline{a_1^2} + \overline{a_2^2} + \overline{2a_1a_2} \\ &= \sigma_1^2 + \sigma_2^2 + \sigma_{12}^2 \end{aligned} \quad (4.13)$$

- The total variance is the sum of individual variances,  $\sigma_1^2 + \sigma_2^2$ , only when the cross term vanishes, i.e.  $\sigma_{12} = 0$ .
- If, for example,  $a_1$  and  $a_2$  fluctuate randomly in time, and signal  $a_2$  is measured after a delay time  $\tau$  from the measurement of  $a_1$ , the cross term has the familiar form of a cross-correlation,

$$\sigma_{12}(\tau) = \overline{a_1(t) \cdot a_2(t + \tau)} = \frac{1}{\tau} \int a_1(t) a_2(t + \tau) dt. (4.14)$$

- Equation 14 indicates that if the noise is characterized by some correlation time,  $\tau_c$ , then averaging different measurements increases the signal to noise only if the interval between measurements  $\tau$  is larger than  $\tau_c$ . The noise becomes uncorrelated at temporal scales larger than  $\tau_c$ .

## 4.4. Contrast and contrast to noise ratio

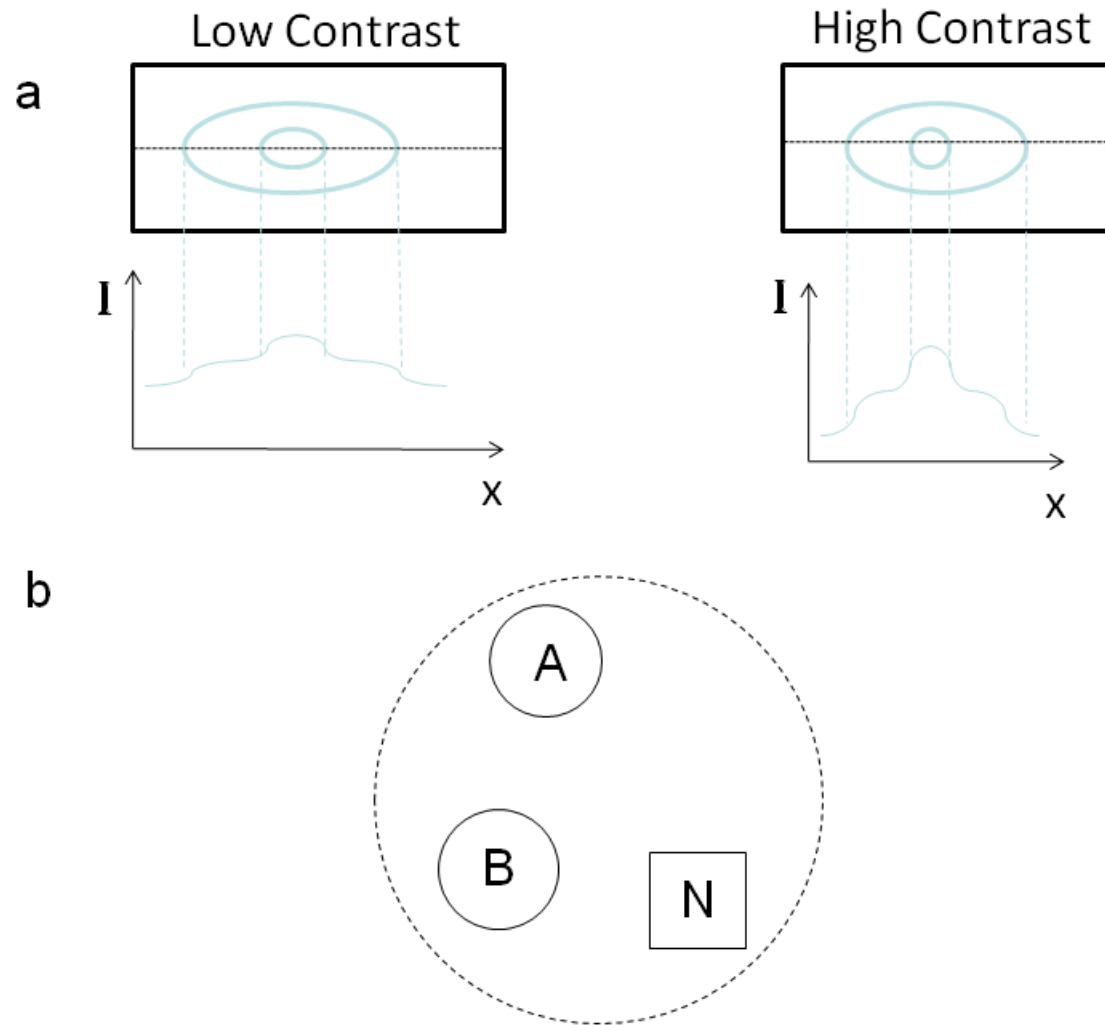


Figure 2. a) Illustration of a low- and high-contrast image. b) Region of interest A, B, and noise region N.

- The *contrast* of an image quantifies the ability to differentiate between different regions of interest within the image (Fig. 2),

$$C_{AB} = |S_A - S_B|, \quad (4.15)$$

- $S_{A,B}$  stands for the signal associated with regions A and B. The modulus in Eq. 15 ensures that the contrast has always positive values.
- Unlike resolution, which is established solely by the microscope itself, contrast is a property of the instrument-sample combination. The same microscope, characterized by the same resolution, renders superior contrast for stained than unstained tissue slices.
- The simple definition of contrast in Eq. 15 is insufficient because it ignores the effects of noise. It is easy to imagine circumstances where the noise itself is of very high contrast across the field of view, which by Eq. 15 generates high values of  $C_{AB}$ .

- A better quantity to use for real, noisy images is the *contrast to noise ratio* (CNR),

$$\begin{aligned} \text{CNR}_{AB} &= \frac{C_{AB}}{\sigma_N} \\ &= \frac{|S_A - S_B|}{\sigma_N} \end{aligned} \tag{4.16}$$

- $\sigma_N$  is the standard deviation associated with the noise in the image.

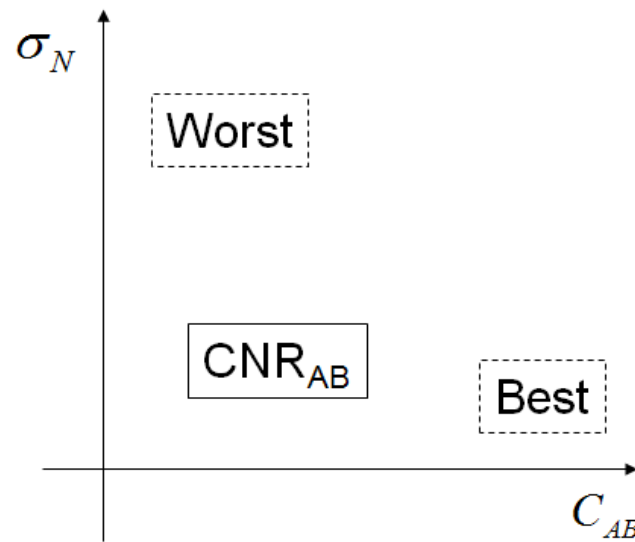


Figure 3. Contrast to noise ration vs. contrast  $C_{AB}$  and noise standard deviation  $\sigma_N$ .

- Of course, the best case scenario from a measurement point of view happens at low noise and high contrast (Fig. 3).

## 4.5 Image filtering

- *Filtering* is a generic term that relates to any operation taking place in the frequency domain of the image,

$$\tilde{I}(\mathbf{k}) = \tilde{I}_0(\mathbf{k}) \cdot \tilde{H}(\mathbf{k}), \quad (4.17)$$

- $\tilde{I}_0$  is the Fourier transform of the image and  $H$  is the *filter* function.
- The *filtered image*  $I(\mathbf{r})$  is obtained by Fourier transforming  $\tilde{I}(\mathbf{k})$  into the space domain,

$$I(\mathbf{r}) = I_0(\mathbf{r}) * H(\mathbf{r}). \quad (4.18)$$

- The filtered image  $I$  is the convolution between the *unfiltered image*  $I_0$  and the Fourier transform of the filter function  $H$ . Any measured image is a filtered version of the object (the transfer function, TF, of the instrument is the filter), as stated in Eq. 5.
- Filtering is used to enhance or diminish certain features in the image. Depending on the frequency range that they allow to pass, we distinguish three types of filters: *low-pass*, *band-pass*, and *high-pass*.

### Low-pass filtering

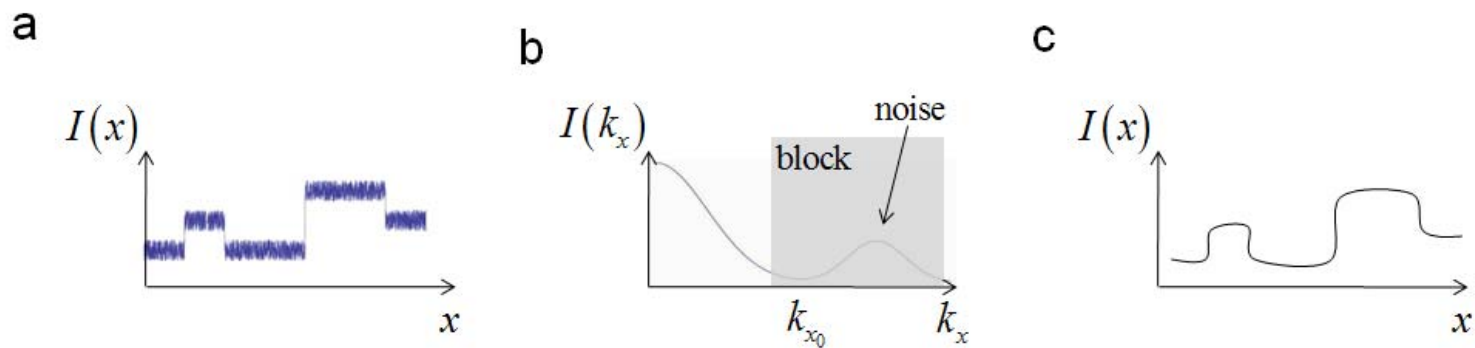


Figure 4. Low-pass filtering: a) noisy signal, b) filtering high frequencies; c) low-passed signal.

- Consider the situation where the noise in the measured image exhibits high-frequency fluctuations (Fig. 4a).
- This noise contribution is effectively diminished if a filter that blocks the high frequencies (passes the low frequencies) is used (Fig. 4b).
- The resulting filtered image, has better SNR (Fig. 4c). However, in the process, resolution is diminished.

### *Band-pass filter*

- In this case the filter function  $H$  passes a certain range (band) of frequencies, from the image. This type of filtering is useful when structures of particular range of sizes are to be enhanced in the image.

### *High-pass filter*

- Using a filter that selectively passes the high frequencies reveals finer details within the image.

- One particular application of high-pass filtering is *edge detection*. This application is particularly useful in selecting (*segmenting*) an object of interest (e.g. a cell) from the image. The gradient of the image along one dimension can significantly enhance the edges in an image (Fig. 5).

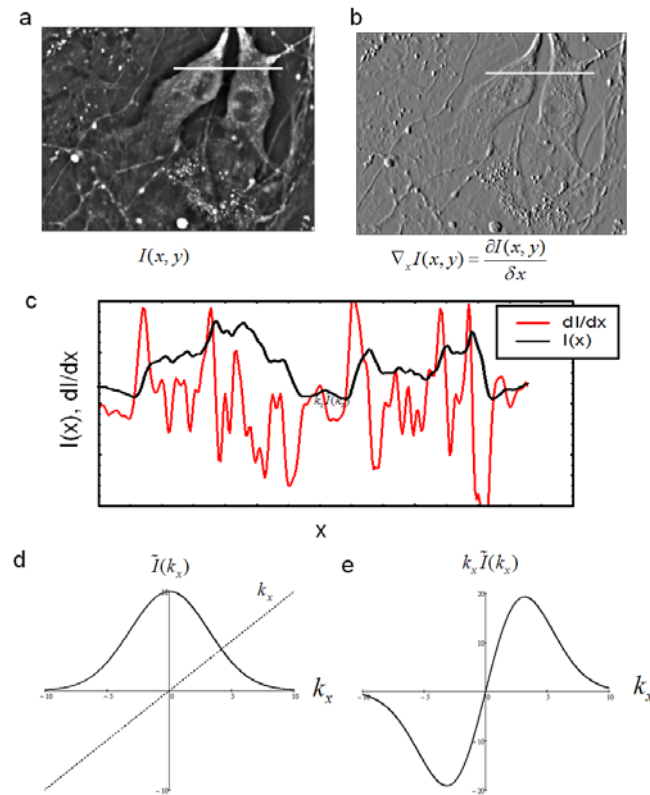


Figure 5. Edge enhancement: a) original image; b) gradient along one direction; c) Profiles along the lines shown in a and b. d) Frequency domain profile associated with a. e) Frequency domain profile associated by b, i.e.  $k_x \tilde{I}(k_x)$ .

- Thus, the new, edge-enhanced image is

$$\begin{aligned}
 I_x(\mathbf{r}) &= \nabla_x [I(\mathbf{r})] \\
 &= \frac{\partial I(\mathbf{r})}{\partial x}
 \end{aligned}
 \tag{4.19}$$

- Taking the Fourier transform of Eq. 19, we find

$$\tilde{I}_x(\mathbf{k}) = ik_x \cdot \tilde{I}(\mathbf{k})
 \tag{4.20}$$

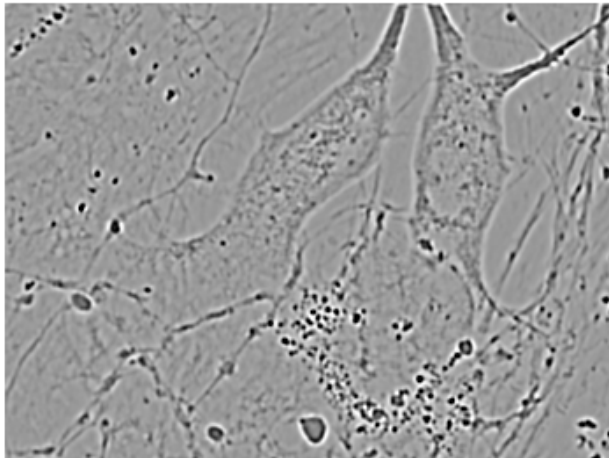
- $i$  indicates a  $\pi/2$  shift that occurs upon differentiation (i.e. sines become cosines). The new frequency content of the gradient image  $\tilde{I}_x$  is enhanced at high frequencies due to the multiplication by  $k_x$  (Fig. 5).
- The gradient image suffers from “shadow” artifacts due to the change in sign of the first order derivative across an edge (Fig. 5b). This artifact is commonly known in DIM microscopy, where the gradient is computed “optically” [9].

- Taking the Laplacian of the image removes these anisotropic artifacts related to the change in sign of the first order derivative.

$$\nabla^2 I(\mathbf{r}) = \frac{\partial^2 I}{\partial x^2} + \frac{\partial^2 I}{\partial y^2} \quad (4.21)$$

$$\nabla^2 I(\mathbf{r}) \rightarrow -k^2 \cdot I(\mathbf{k})$$

a



$$\nabla^2 I(x, y) = \frac{\partial^2 I(x, y)}{\delta x^2} + \frac{\partial^2 I(x, y)}{\delta y^2}$$

b

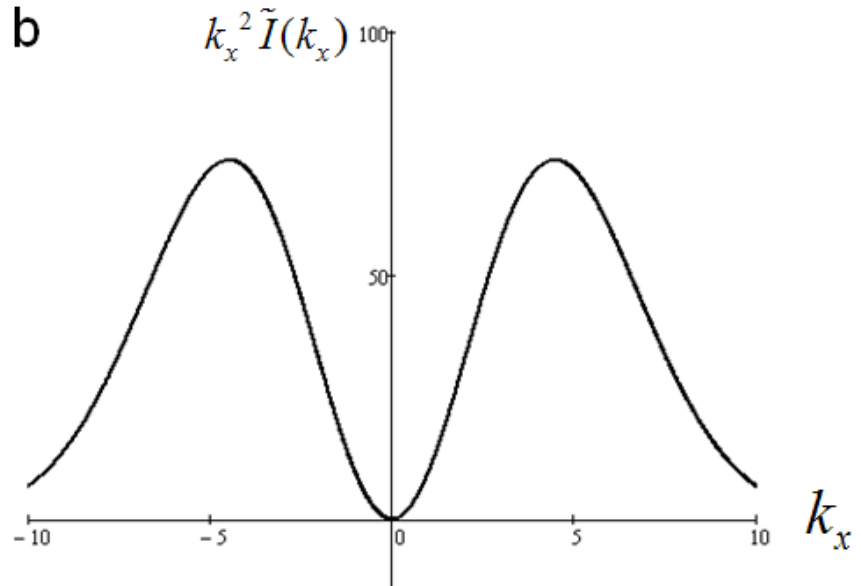


Figure 6. Laplacian (a) and frequency domain profile (b) associated with the image in Fig 5a.

- Figure 6a shows the results of taking the Laplacian of the same image. Even finer details are now visible in the image, as the high-pass filter is stronger due to the  $k^2$  multiplication of  $I(k)$ . The shadow artifacts are less disturbing because they do not change sign across each edge.
- There are many, much more sophisticated filters and algorithms for image enhancement and restoration that are beyond the scope of this discussion.