## Motivation:

Mathematical modeling is a powerful tool for solving complex problems in many areas of science and engineering. One area where mathematical modeling has proven particularly useful is in the field of image processing. Image deconvolution is an important problem in image processing, and mathematical modeling can provide a powerful framework for addressing this problem.

Natural image statistics is a powerful tool in image processing, computer vision, and computational photography. Denoising, deblurring, transparency separation, and super-resolution, are all tasks that are inherently ill-posed. Priors based on natural image statistics can regularize these problems to yield high-quality results. However, digital cameras now have sensors that record images with tens of megapixels. Fast image deconvolution is one of the many image processing techniques to solve the above tasks for such images in a reasonable time frame and is what we will be focusing on for this project. In a nutshell, deconvolution is a post-processing step that makes images appear "sharper," or more in focus, and helps you resolve the finer details of your image. Deconvolution is particularly and extensively utilized in microscopy imaging and fluorescent imaging. What we find interesting is how images become convoluted has to do with the limits of microscopy imaging systems. As light passes from your sample through the optical path of the microscope, it spreads out or becomes diffracted. This creates blur, which causes the final image to appear less sharp.

Image deconvolution is the process of removing the blurring effects of an imaging system from an observed image. In many imaging systems, such as cameras or microscopes, the image is blurred due to various factors such as lens aberrations, motion blur, or atmospheric turbulence. Image deconvolution aims to recover the original, sharp image from the observed, blurred image.

Fast image deconvolution is a particularly challenging problem because it requires processing large amounts of data in a short amount of time. One approach to solving this problem is to use mathematical modeling to develop efficient algorithms for image deconvolution.

One popular mathematical model for image deconvolution is based on the concept of convolution. Convolution is a mathematical operation that describes how two functions interact with each other. In image processing, convolution is used to describe how an image is blurred by an imaging system. The blurred image can be modeled as the convolution of the original, sharp image with a blurring function.

To perform fast image deconvolution, the convolution model can be inverted using a process known as deconvolution. However, deconvolution is an ill-posed problem, meaning that there are many possible solutions that could fit the observed data. This makes deconvolution difficult, as it is not possible to simply invert the blurring process to recover the original image.

To overcome this challenge, various regularization techniques can be applied to constrain the solution space and improve the accuracy of the deconvolution process. For example, total variation regularization can be used to impose smoothness on the solution, while sparse regularization can be used to encourage sparsity in the solution.

In addition to regularization techniques, optimization algorithms can also be used to efficiently solve the deconvolution problem. Gradient-based optimization algorithms, such as

gradient descent or conjugate gradient methods, can be used to find the optimal solution to the deconvolution problem.

## Mathematical Modeling:

Deconvolution is a computationally intensive image processing technique that is being increasingly utilized for improving the contrast and resolution of digital images captured in the microscope. The foundations are based upon a suite of methods that are designed to remove or reverse the blurring present in microscope images induced by the limited aperture of the objective.

Nearly any image acquired on a digital fluorescence microscope can be deconvolved, and several new applications are being developed that apply deconvolution techniques to transmitted light images collected under a variety of contrast enhancing strategies. Among the most suitable subjects for improvement by deconvolution are three-dimensional montages constructed from a series of optical sections.
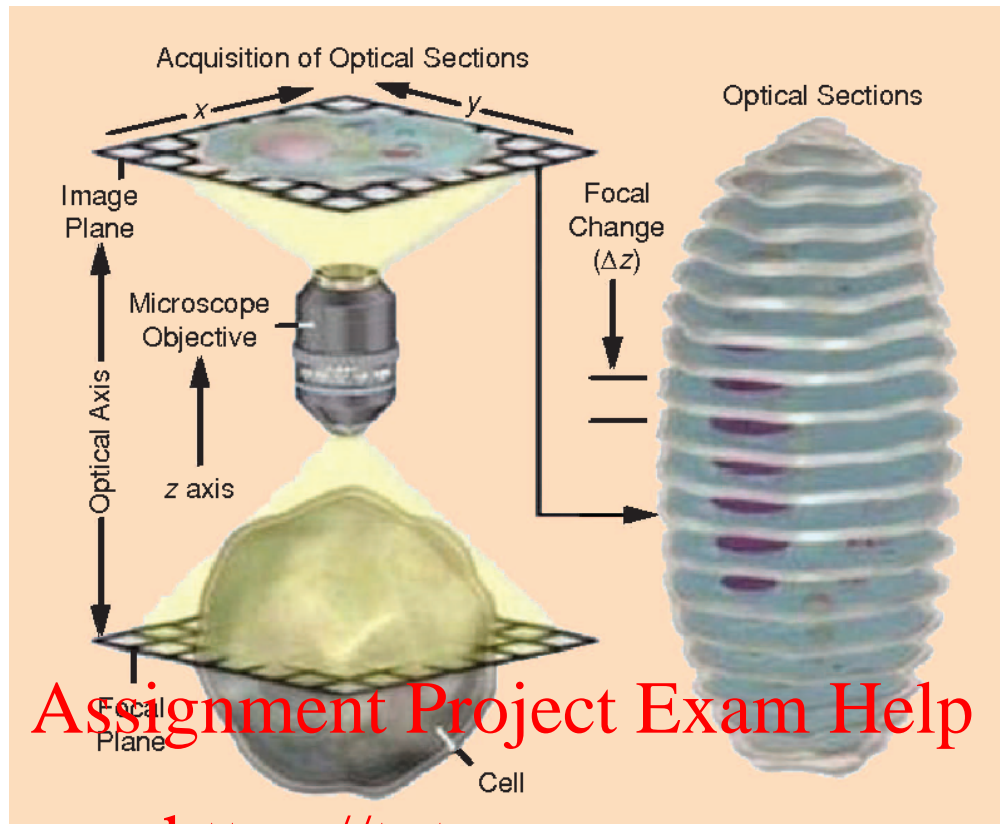
**[FIG1]** An example of the acquired 3-D image of a cell, captured by a

The basic concepts surrounding the acquisition of z-series images for deconvolution analysis are presented with a schematic diagram in Figure 1. A series of images are recorded of the sample, each shifted slightly from one another along the z-axis. This change in the focal plane results in a slightly different image, with subtle changes caused by out-of-focus light coming from above and below the current z-plane. During deconvolution analysis, the entire z-series is analyzed to create a clearer, higher-resolution data set that is not convoluted by out-of-focus fluorescence.

Deconvolution is often suggested as a good alternative to the confocal microscope, as both techniques seek to minimize the effect of out-of-focus fluorescence on your final image. This is not strictly true because images acquired using a pinhole aperture in a confocal

microscope benefit from deconvolution processing. Confocal microscopy prevents out-of-focus light from being detected by placing a pinhole aperture between the objective and the detector, through which only in-focus light rays can pass. In contrast, widefield microscopes allow out-of-focus light to pass directly to the detector. Deconvolution is then applied to the resulting images to either subtract the out-of-focus light or reassign it back to its source. Confocal microscopy is especially well suited for examining thick specimens such as embryos or tussues, while widefield deconvolution processing has proven to be a powerful tool for imaging specimens requiring extremely low light levels. These tools can even be combined to reduce the noise in images acquired on a confocal microscope. However, a majority of the deconvolution experiments reported in the literature apply to images recorded on a standard widefield fluorescence microscope.

**Sources of Image Degradation**

Image degradation cam be divided into four independent sources: noise, scatter, glare, and blur. Figure 2 presents examples of the visual impact of each of these on the same image.
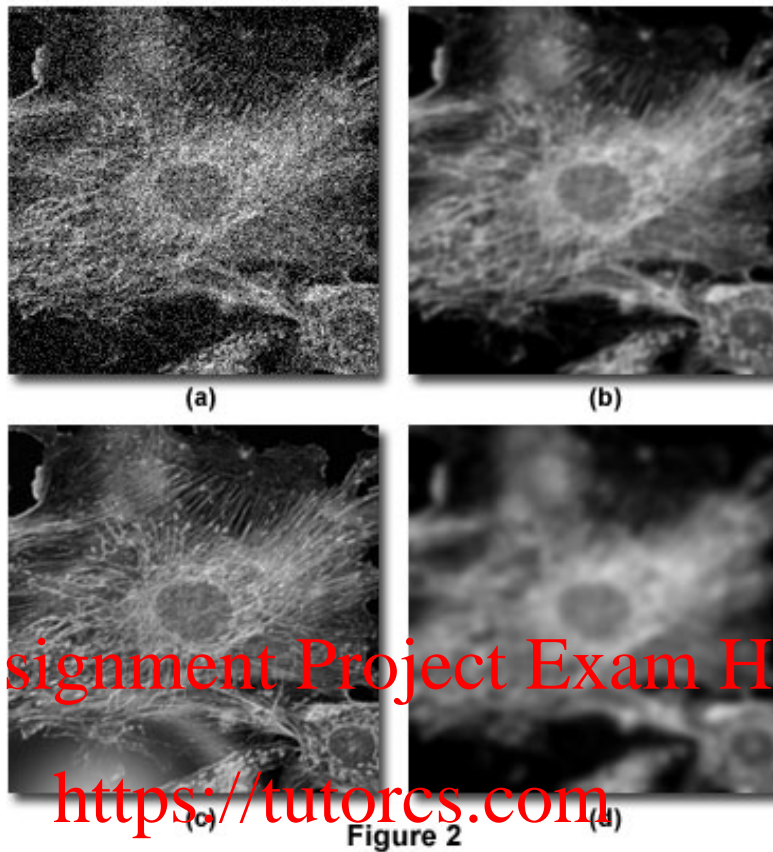
## Noise Sources and Image Degradation



(a)   (b)

(c)   (d)

Figure 2

Noise can be described as a quasi-random disarrangement of detail in an image, which (in its most severe form) has the appearance of white noise or salt-and-pepper noise, similar to what is seen in broadcast television when experiencing bad reception (Figure 2(a)). This type of noise is referred to as "quasi-random" because the statistical distribution can be predicted if the mechanics of the source are known. In digital microscopy, the main source of noise is either the signal itself (often referred to as photon-shot noise) or the digital imaging system. The mechanics of both noise sources are understood and therefore, the statistical distribution of noise is known. Signal-dependent noise can be characterized by a Poisson distribution, while noise arising from the imaging system often follows a Gaussian distribution. Because the source and distribution of common noise in digital mages are so well understood, it can be easily removed by application

of the appropriate image filters, which are usually included in most deconvolution software packages as an optional "pre-processing" routine.

Scatter is usually referred to as a random disturbance of light induced by changes in refractive index throughout a specimen. The net effect of scatter is a truly random disarrangement of image detail, as manifested in Figure 2(b). Although no completely satisfactory method has been developed to predict scatter in a given specimen, it has been demonstrated that the degree of scattering is highly dependent upon the specimen thickness and the optical properties of the specimen and surrounding embedding materials. Scatter increases with both specimen thickness and the heterogeneity of the refractive index presented by internal components within a specimen.

Similar to scatter, glare is a random disturbance of light, but occurs in the optical elements (lenses, filters, prisms, etc.) of the microscope rather than within the specimen. The level of glare in a modern microscope has been minimized by the employment of lenses and filters with antireflective coatings, and the refinement of lens forming techniques, optical cements, and glass formulations. Figure 2(c) illustrates the effect of uncontrolled glare.

Blur is described by a nonrandom spreading of light that occurs by passage through the optical path of the imaging system (Figure 2(d)). The most significant source of blur is diffraction, and an image whose resolution is limited only by blur is considered to be diffraction-limited. This represents an intrinsic limit of any imaging system and is the determining factor in assessing the resolution limit of an optical system. Fortunately, sophisticated models of blur in an optical microscope are available which can be utilized to determine the source of out-of-focus photons. This is the basis for deconvolution. Because of its

fundamental importance in deconvolution, the theoretical model for blur will be discussed in much greater detail in other parts of this section. However, it should be emphasized that all imaging systems produce blur independently of the other forms of image degradation induced by the specimen or accompanying instrumental electronics. It is precisely this independence of optical blur from other types of degradation that enables the possibility of blur removal by deconvolution techniques.

The interaction of light with matter is the primary physical origin of scatter, glare, and blur. However, the composition and arrangement of molecules in a given material (whether it be glass, water, or protein) bestows upon each material its own particular set of optical properties. For the purposes of deconvolution, what distinguishes scatter, glare, and blur are the location where they occur and the possibility of generating a mathematical model for these phenomena. Because scatter is a localized, irregular phenomenon occurring in the specimen, it has proven difficult to model. By contrast, because blur is a function of the microscope optical system (principally the objective), it can be modeled with relative simplicity. Such a model renders it possible to reverse the blurring process mathematically, and deconvolution employs this model to reverse or remove blur.
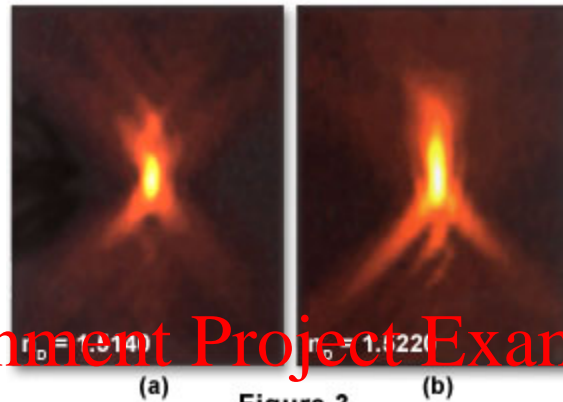
**The Point Spread Function:**

The model for blur that has evolved in theoretical optics is based on the concept of a three-dimensional point spread function (PSF). This concept is of fundamental importance to deconvolution and should be clearly understood in order to avoid imaging artifacts. The point spread function is based on an infinitely small point source of light originating in the specimen (object) space. Because the microscope imaging system collects only a fraction of the light

emitted by this point, it cannot focus the light into a perfect three-dimensional image of the point. Instead, the point appears widened and spread into a three-dimensional diffraction pattern. Thus, the point spread function is formally defined as the three-dimensional diffraction pattern generated by an ideal point source of light.



**Effect of Spherical Aberration on the PSF**

(a)    (b)

Figure 3

Depending upon the imaging mode being utilized (widefield, confocal, transmitted light), the point spread function has a different and unique shape and contour. In a widefield fluorescence microscope, the shape of the point spread function resembles that of an oblong "football" of light surrounded by a flare of widening rings. To describe the point spread function in three dimensions, it is common to apply a coordinate system of three axes (x, y, and z) where × and y are parallel to the focal plane of the specimen and z is parallel to the optical axis of the microscope. In this case, the point spread function appears as a set of concentric rings in the x-y plane and resembles an hourglass in the x-z and y-z planes (as illustrated in Figure 3). An x-y slice through the center of the widefield point spread function reveals a set of concentric rings: the so-called Airy disk that is commonly referenced in texts on classical optical microscopy.

Two x-z projections of point spread functions showing different degrees of spherical aberration are presented in Figure 3. The optical axis is parallel to the vertical axis of the image. The point spread function on the left displays minimal spherical aberration, while that on the right shows a significant degree of aberration. Note that the axial asymmetry and widening of the central node along the optical axis in the right-hand image leads to degraded axial resolution and blurring of a signal. In theory, the size of the point spread function is infinite, and the total summed intensity of light in planes far from focus is equal to the summed intensity at focus. However, the light intensity falls off quickly and eventually becomes indistinguishable from noise. In an unaberrated point spread function recorded with a high numerical aperture (1.40) oil immersion objective, light occupying 0.2 square micrometers at the plane of focus is spread over 90 times that area at 1 micrometer above and below focus. The specimen utilized to record these point spread function images was a 0.1 micrometer-diameter fluorescent bead mounted in glycerol (refractive index equal 1.47), with immersion oils having the refractive indices noted in the figure.

An important consideration is how the point spread function affects image formation in the microscope. The theoretical model of image formation treats the point spread function as the basic unit of an image. In other words, the point spread function is to the image of what the brick is to the house. The best an image can ever be is an assembly of point spread functions, and increasing the magnification will not change this fact. As a noted theoretical optics textbook (Born and Wolf: Principles of Optics) explains, "It is impossible to bring out detail not present in the primary image by increasing the power of the eyepiece, for each element of the primary image is a small diffraction pattern, and the actual image, as seen by the eyepiece, is only the ensemble of the magnified images of these patterns".

As an example, consider a population of subresolution fluorescent beads sandwiched between a coverslip and microscope slide. An in-focus image of this specimen reveals a cloud of dots, which is actually a disk surrounded by a tiny set of rings (in effect, an Airy disk; see Figure 4(a)). If this specimen is removed from focus slightly, a larger set of concentric rings will appear where each dot was in the focused image (Figure 4(b)). When a three-dimensional image of this specimen is collected, then a complete point spread function is recorded for each bead. The point spread function describes what happens to each point source of light after it passes through the imaging system.



Fluorescent Beads Focused and Defocused

(a)　(b)
Figure 4

The blurring process just described is mathematically modeled as a convolution. The convolution operation describes the application of the point spread function to every point in the specimen: light emitted from each point in the object is convolved with the point spread function to produce the final image. Unfortunately, this convolution causes points in the specimen to become blurred regions in the image. The brightness of every point in the image is linearly related by the convolution operation to the fluorescence of each point in the specimen. Because the point spread function is three-dimensional, blurring from the point spread function is an inherently three-dimensional phenomenon. The image from any focal plane contains blurred

light from points located in that plane mixed together with blurred light from points originating in other focal planes.

The situation can be summarized with the idea that the image is formed by a convolution of the specimen with the point spread function. Deconvolution reverses this process and attempts to reconstruct the specimen from a blurred image.

**Aberrations in the Point-spread Function:**

The point-spread function can be defined either theoretically by utilizing a mathematical model of diffraction, or empirically by acquiring a three-dimensional image of a fluorescent bead (in Figure 3). A theoretical point-spread function generally has axial and radial symmetry. In effect, the point spread function is symmetric above and below the x-y plane (axial symmetry) and rotationally about the z-axis (radial symmetry). An empirical point spread function can deviate significantly from perfect symmetry (as presented in Figure 3). This deviation, more commonly referred to as aberration, is produced by irregularities or misalignments in any component of the imaging system optical train, especially the objective, but can also occur with other components such as mirrors, beamsplitters, tube lenses, filters, diaphragms, and apertures. The higher the quality of the optical components and the better the microscope alignment, the closer the empirical point spread function comes to its ideal symmetrical shape. Both confocal and deconvolution microscopy depends on the point spread function being as close to the ideal case as possible.

The most common aberration encountered in optical microscopy, well known to any experienced and professional microscopist, is spherical aberration. The manifestation of this aberration is an axial asymmetry in the shape of the point spread function, with a corresponding

increase in size, particularly along the z-axis (in Figure 3). The result is a considerable loss of resolution and signal intensity. In practice, the typical source of spherical aberration is a mismatch between the refractive indices of the objective front lens immersion medium and the mounting medium in which the specimen is bathed. A tremendous emphasis should be placed on the importance of minimizing this aberration. Although deconvolution can partially restore lost resolution, no amount of image processing can restore lost signal.

https://stanford.edu/class/ee367/reading/lecture6_notes.pdf

https://haesleinhuepf.github.io/BioImageAnalysisNotebooks/18a_deconvolution/introduction_deconvolution.html