Three-dimensional image reconstruction of micrographs from a laser scanning confocal microscope of CdSe-core quantum dots in human umbilical vein endothelial cells

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Abstract

Usage of Quantum Dots (QDs) for fluorescent labelling on biological samples has some favorable properties over traditional organic molecules. The intensity of the fluorescence from QDs is greater than that from some organic molecules, allowing lower excitation intensities which results in lower phototoxicity in the biological sample. QDs usually have a diameter in the the scale of a few nanometers, which is smaller than the resolution limit of confocal light microscopes. The aim of this thesis is to try to resolve more contrast and detail in micrographs of human embilical vein endothelial cells stained with QDs. The method of choice here is deconvolution with $L_1$-norm regularization combined with Arnoldi iterations for computational optimization. The resulting deconvolutions of fluorescence micrographs have higher contrast constrained to smaller spatial regions than in the original micrographs. We conclude that the regularization implemented here could possibly be a viable alternative to commercially available deconvolution methods, but more research is needed to optimize computation speed and accuracy.

Zusammenfassung


Sammanfattning

Acknowledgments

We would like to thank our supervisor Ying Fu and the rest of the team on SciLifeLab 3 for supporting us during our work.

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List of Acronyms

LSCM  Laser scanning confocal microscope
LSM780 Laser scanning microscope 780, Carl Zeiss, Germany
QD  Quantum dot
3MPA-QD 3-mercaptopropionic acid coated quantum dot
HUVEC  Human umbilical vein endothelial cell
PSF  Point spread function
Chapter 1

Introduction

Advancements in microscopy and cellular biology have gone hand in hand since the invention of the microscope in the 17th century, allowing us to see life in greater detail than visible to the naked eye. In particular, fluorescence microscopy has been a prominent method for intracellular studies. The technology has seen great improvement in the last decades. The laser scanning confocal microscope (LSCM), introduced in the 1980’s, allows for clear, high resolution fluorescence images by illuminating and scanning the sample in sections [1].

Fluorescent beads are widely used for fluorescent labelling in both in vitro and in vivo settings. They are particles of varying size consisting of organic molecules with fluorescent dyes in a range of colours. Quantum dots (QDs) have proven to be a relevant alternative to the well established organic molecules for fluorescent labelling. QDs are clusters of semiconductor particles that emit light after excitation from a light source such as a laser. Their optical properties vary with the size of the core. A smaller size increases the electron excitation energies which results in the QD emitting photons with shorter wave lengths after excitation. For CdSe-core QDs, which were used here, the emission can be tuned from deep red to green by reducing the core size [2]. QDs have some preferable optical features such as a broad absorption spectrum, and a tunable emission spectrum as mentioned. Their fluorescence also has high intensity in comparison with the beads, so strong signal amplification is not needed [3].

The use of QDs in biological applications is however complicated by the fact that they behave as nanocolloids and not molecules, so standard protocols for organic fluorescent molecules do not apply [3]. When using QDs in live cells, the toxicity also has to be considered. 3MPA-QDs have been studied in human embilical vein endothelial cells (HUVECs), which is the type of cell that was used here, and found to be nontoxic in terms of having no effect on in vitro wound healing and not triggering apoptotic cell death [4].

Cells have different endocytic mechanisms for uptake of different particles, molecules and bacteria. The pathway depends on factors such as particle size, charge and sur-
face coating [5]. Studying the uptake of QDs is of great importance and interest because of the increasing usage in biomedical imaging. Both fluorescent beads and QDs are small particles. The beads used here were 20 nm and the QDs less than 6 nm, both smaller than the maximum resolution of the confocal microscope used. Their small size combined with properties of the microscope and nonideal experimental setups results in blurred and noisy micrographs. Here, an attempt at a three-dimensional image reconstruction was made by implementing $L_1$-norm regularization and the results were compared to those obtained by using an already available method. The ambition was to improve image quality to facilitate future studies of cellular processes involving QDs as fluorescent labels.
Chapter 2

Background

2.1 Laser scanning confocal microscopy

The technique of laser scanning microscopy (LSCM) typically involves exciting a fluorescent sample with a laser through a high numerical aperture objective and collecting the fluorescence through the same objective. The fluorescence is then filtered out through a dichroic mirror and passed through an interference filter in order to obtain an image of the sample. This is illustrated in figure 2.1 [6]. A three dimensional image of the sample can be captured by scanning the sample from different heights, thus capturing micrographs at different depths of the sample.

When the LSCM technique is employed to capture a three dimensional image of a sample, the recorded image will not accurately represent the samples’ true spatial distribution. While one depth of the sample is in focus, fluorescence from surrounding depths will still be visible and be included. This creates a diffused cone of light co-axial to the direction towards the sensor from the sample, as is illustrated in figure 2.2. To obtain a better representation of the true positions of the point light sources, an image reconstruction technique called deconvolution can be used [7].
CHAPTER 2. BACKGROUND

Figure 2.1: Schematic figure of a simplified Laser Scanning Confocal Microscope setup.

Figure 2.2: Simplified microscope.
2.2 Deconvolution

In order to understand how images from a LSCM can be treated to find the accurate depiction of the sample, it is helpful to first look at the inverse of the problem. That is, we are given the solution and a way to convert the solution to the untreated micrograph. Let $L$ be a linear convolution operator $L : \mathbb{R}^m \to \mathbb{R}^n$ such that for any $x_1, x_2 \in \mathbb{R}^m$,

$$L(x_1) + L(x_2) = L(x_1 + x_2) \quad (2.1)$$

If for instance $x_1$ is a point light source, then $L(x_1)$ is the original untreated image of the point light source. We call this untreated image the point spread function (PSF) of the microscope, since it is the result of how the optical setup spreads the light of a point source. The property of $L$ is illustrated in figure 2.3. One could argue that the operator $L$ describes how the ”solution space” maps onto the ”blurred space”. Because of the linear property of $L$, if an untreated image $b$ contains $k$ arbitrary point sources $x_i$, it invites us to write:

$$\sum_{i=1}^{k} L(x_i) = L \left( \sum_{i=1}^{k} x_i \right) = b \quad (2.2)$$

2.2.1 $L_1$ and $L_2$-norm regularization

Because an image is split up into discrete pixels, $L$ can be represented as convolution matrix $A$ of size $m \times n$. Then equation (2.2) becomes a system of linear equations

$$Ax = b. \quad (2.3)$$

The problem in equation (2.3) is unfortunately often ill-posed because of $A$ having singular values near zero in its singular value decomposition $A = U \Sigma U^T$, which

![Figure 2.3: Illustrated example of an operator $L$ that takes point sources in $\mathbb{R}^m$ and diffuse them in $\mathbb{R}^n$.](image-url)
makes a least squares approximation of \( x \) (equation 2.4) unsatisfactory \[8\].

\[
\min_{x \in \mathbb{R}^m} \|Ax - b\|_2^2. \tag{2.4}
\]

The sensitivity to perturbations in \( b \) is exemplified in figure 2.4, where a PSF with and without noise has been deconvoluted using a least squares approximation.

The sensitivity to errors or noise in \( b \) can be overcome by changing to the less sensitive system in equation 2.5. This is a common regularization method called Tikhonov regularization or \( L_2 \)-norm regularization \[9\]. The solution \( x \) is

\[
\min_{x \in \mathbb{R}^m} \left\{ \|Ax - b\|_2^2 + \frac{1}{\mu} \|x\|_2^2 \right\}. \tag{2.5}
\]

where \( \mu \) is a parameter that regulates the weight of the penalty term \( \|x\|_2^2 \).

An alternative minimization that yields higher constrast in the final image is achieved by changing the penalty term to the \( L_1 \) norm of \( x \) \[10\]:

\[
\min_{x \in \mathbb{R}^m} \left\{ \|Ax - b\|_2^2 + \frac{1}{\mu} \|x\|_1 \right\}. \tag{2.6}
\]

2.2.2 Arnoldi iterations

The convolution matrix \( A \) that describes the relationship between the PSF \( b \) and the solution \( x \) is large for a three dimensional micrograph with a side of a few hundred pixels. The method of Arnoldi iterations can be used for large deconvolutional problems on the form of equations 2.5 and 2.6 to reduce computational costs \[9\].
2.2. DECONVOLUTION

The idea behind Arnoldi iterations is to find approximations of the eigenvalues of large matrices. It has been shown that Krylov spaces are good approximations for the eigenvalues of symmetric matrices [11]. The Arnoldi process involves producing orthogonal basis vectors \( \{ q_1, q_2, \ldots, q_l \} \) such that \( \text{Span} \{ x, A x, A^2 x, \ldots, A^{l-1} x \} \), where \( A \in \mathbb{R}^{n \times n}, x \in \mathbb{R}^n \) and the Krylov space \( K_l(A, x) \) is defined as

\[
K_l(A, x) := \text{Span} \{ x, A x, A^2 x, \ldots, A^{l-1} x \}. \tag{2.7}
\]

A matrix consisting of these orthogonal vectors \( \{ q_1, q_2, \ldots, q_l \} = Q_l \) can be shown to transform a large matrix \( A \) into its Hessenberg form if \( \text{Span} \{ x, A x, A^2 x, \ldots, A^{l-1} x \} \) is invariant under \( A \). That is,

\[
AQ_l = Q_l H_l \tag{2.8}
\]

where \( H_l \in \mathbb{R}^{l \times l} \) is an upper Hessenberg matrix [11].

It has been suggested to perform a few iterations of the Arnoldi process, with an initial vector \( q_1 = \frac{A b}{\| A b \|_2} \), and then perform an \( L_2 \)-norm regularization on vectors in the resulting Krylov space [9]. However, since \( L_1 \)-norm is said to generate higher contrast in the final processed images [10], it was used instead of the \( L_2 \)-norm in this implementation.

It is highly unlikely that the Arnoldi process creates an invariant subspace under \( A \) with just a few iterations \( l \) of a large matrix. If \( K_l(A, q_1) \) is not invariant under \( A \) the relation in equation 2.8 becomes

\[
AQ_l = Q_{l+1} H_l, \tag{2.9}
\]

where \( H_l \in \mathbb{R}^{l+1 \times l} \) is an upper Hessenberg matrix. This is called range-restricted Arnoldi decomposition, since \( \mathcal{R}(Q_l) \subset \mathcal{R}(A) \) [9]. Combining equation 2.9 and the initial convolution in equation 2.3 and using the substitution \( x = Q_l y, \ y \in K_l(A, q_1) \) gives

\[
Q_{l+1} H_l Q_l^H x = b \\
H_l Q_l^H x = Q_{l+1}^H b \\
H_l y = Q_{l+1}^H b, \tag{2.10}
\]

where a superscript \( H \) denotes the conjugate transpose of a matrix. \( L_1 \)-norm regularization can now be applied to equation 2.10 which results in the minimization problem

\[
\min_{y \in K_l(A, q_1)} \left\{ \| H_l y - Q_{l+1}^H b \|_2^2 + \frac{1}{\mu} \| y \|_1 \right\}. \tag{2.11}
\]

When \( y \) is found, the solution to the deconvolution is simply \( x = Q_l y \).

2.2.3 Ritz pairs

In order to determine the "quality" of a Krylov space \( K_l(A, q_1) \) that is not invariant under \( A \), one can introduce Ritz pairs \((\lambda, v)\). Ritz pairs can be thought of as
approximations to the eigenvalues and eigenvectors of $A$. If the norm of the residual $\|Av - \lambda v\|$ is small, then the Ritz pair is a good approximation of one eigenvalue and eigenvector of $A$. Ritz values are calculated as the eigenvalues of $H_l = Q_l^H A Q_l$, but to calculate the norm of the residual to a Ritz pair it is enough to know the vector $h_{l+1,l}$ from $H_l$. A proof of this property is shown below. [11]

Lemma 2.2.1. The norm of the residual can be expressed using $\|Av - \lambda v\| = \|h_{l+1,l}\| \|z_l\|$, where $(\lambda, v)$ is the Ritz-pair to $K_l(A, q_1)$ and $v = Q_l z$ [11].

Proof.

$$Av - \lambda v = Q_{l+1} \bar{H}_l Q_l^H Q_l z - \lambda Q_l z$$
$$= (Q_l H_l + h_{l+1,l} q_{l+1} e_l^T) Q_l^H Q_l z - \lambda Q_l z$$
$$= (Q_l H_l + h_{l+1,l} q_{l+1} e_l^T) z - \lambda Q_l z$$
$$= Q_l (H_l z - \lambda z) + h_{l+1,l} q_{l+1} z_l$$
$$= Q_l (0) + h_{l+1,l} q_{l+1} z_l$$
$$= h_{l+1,l} q_{l+1} z_l$$

$$\|Av - \lambda v\| = \sqrt{h_{l+1,l} q_{l+1} z_l^* h_{l+1,l}^* q_{l+1}^* z_l^*}$$
$$= \sqrt{h_{l+1,l} h_{l+1,l}^* q_{l+1}^* z_l^* z_l^*}$$
$$= \sqrt{h_{l+1,l} h_{l+1,l}^* z_l^* z_l^*}$$
$$= \sqrt{h_{l+1,l} h_{l+1,l}^*} \sqrt{z_l^* z_l^*}$$
$$= \|h_{l+1,l}\| \|z_l\|$$
2.2.4 Blind deconvolution

Figure 2.5: a) Resulting deconvolution using blind deconvolution with 40 iterations on an example of a point source. b) Resulting deconvolution using blind deconvolution with a varying number of iterations from 5 to 40 on a smoothed point source.

An alternative deconvolution method is blind deconvolution, which is already implemented in Matlab (Mathworks, Massachusetts, U.S.A). The function `deconvblind` takes the micrograph that is to be deconvoluted and an initial guess for the PSF as input elements. It then performs a set number of iterations to find the PSF that maximizes the likelihood that the deconvoluted image, using that PSF, is derived from the original image under the assumption that any noise in the image is from a Poisson distribution \[12\]. It returns both an approximated PSF and the deconvoluted image. As can be seen in figure 2.5, the method is sensitive to perturbations in the reference \(b\) for the PSF. It is also sensitive to the initial guess for the PSF, so a poor guess can give unsatisfactory results. However, because of its advantages of being easily accessible and not needing further implementation, it is used here for comparison with the regularization method.
Chapter 3

Method

3.1 Preparations

Water-dispersible 3-mercaptopropionic acid coated CdSe-CdS/Cd0.5Zn0.5S/ZnS core-multishell QDs (3MPA-QDs) were synthesized by our supervisor Ying Fu in house according to a previously used protocol [13, 14, 15].

Human embilical vein endothelial cells (HUVECs) were cultured in a 35 mm petri dish with a glass bottom and lid. The cells were cultured in 15 mL endothelial growth medium with endothelial cell basal medium, 5 % fetal bovine serum, 1 % penicillin/streptomycin/glutamine and 0.25 µg/mL Gibco amphotericin B. The medium was changed every three days during the culturing.

3.2 Microscopy

A laser scanning LSM780 confocal microscope from Carl Zeiss Microscopy GmbH was used to perform all acquisitions. The "lambda mode" capture setting in the LSM780 microscope captures fluorescence of different wavelengths in separate channels and is used to determine the emission spectrum of the sample. "Channel mode" has one channel for all fluorescence emitted by the sample and one channel for the transmitted light. The "z-stack" setting can be used in combination with any of these modes to get a three-dimensional micrograph of the sample [6]. A C-Apochromat 40x magnification objective with a drop of Immerson W immersion fluid (refractive index 1.334) was used for all acquisitions.

3.2.1 Session one: fluorescent beads

A drop of 20 nm µm FluoSpheres (Life Technologies, Eugene, Oregon, USA) dispersed in water were placed on a glass slide and placed in the incubator in the LSM780 microscope. Z-stack micrographs were taken of single beads using channel mode, with excitation using a 561 nm laser. Fluorescent beads were used rather
than QDs in order to obtain an image of a point light source. Their larger size facilitated isolating a single bead, but they were still smaller than the maximum resolution of the microscope, thus representing a point light source.

3.2.2 Session two: live cells and 3MPA-QDs

The cultured HUVECs in their dish were placed in the incubator. The temperature was set to 37.5°C and the atmospheric CO$_2$ level was set to 5.2 %. The cells were then exposed to 5 $\mu$l of 2 $\mu$M QDs dispersed in water. A single cell was localized and an acquisition of a z-series was then started 5 min after adding the QDs. The excitation of the QDs was achieved using a 405 nm laser. After 10 min, a time-series of z-stacks was started. Each time-series covered 10 mins with a z-stack acquisition made every 1 min automatically.

3.3 Image processing

The original micrographs were exported as TIFF files without compression using the ZEN 2.6 software (Zeiss, Germany) and loaded into memory using importdata in Matlab R2018a (Mathworks, Massachusetts, U.S.A). The image processing steps described in the next section were implemented and performed in Matlab and C++.

3.3.1 The convolution matrix $A$

To solve for the approximation of the true depiction of the sample $x$, the convolution matrix $A$ is needed. $A$ was constructed by using a micrograph $b_0$ of a 20 nm Fluosphere as a reference for a convoluted point light source. To remove some noise from $b_0$ a new image $\hat{b}_0$ was constructed by using the moving mean average in three dimensions of $b_0$ with sliding window of length 10 pixels and then blurring the resulting averaged image with Gaussian blur with a standard deviation of 2. An index $k$ was then calculated as the center of intensity in $\hat{b}_0$, and was assumed to be the true location of the point light source that generated $\hat{b}_0$. By shifting the index $i$ in $x_i$ in equation 3.1 all possible positions of a point source were covered by shifting $\hat{b}_0$ in the corresponding way. The shifting of $\hat{b}_0$ is refered to as $\hat{b}_{i-k}$.

$$[\cdots \hat{b}_{i-k} \cdots]_A = \hat{b}_{i-k}$$

(3.1)
The shifting method described by equation 3.1 makes it possible to approximate $A$ without explicitly capturing all images of all possible positions of the point source.

### 3.3.2 Computational optimizations

An issue that arises from representing the images as vectors is that when processing a two dimensional square image of size $n \times n$, then $b \in \mathbb{R}^{n^2} \Rightarrow A \in \mathbb{R}^{n^2 \times n^2}$. With larger images this becomes a computational issue, and even more so if a third dimension is introduced as it was here. For example, an image of dimensions $512 \times 512 \times 15$ pixels will require a deconvolutional matrix the size of $(512 \times 512 \times 15)^2 \times 8$ bytes $\approx 136$ TB using double precision [16].

In order to reduce the size of $A$ two techniques are applied. Firstly, the fact that $A$ can be defined according to equation 3.1 makes it possible to calculate $A$ column wise from $\hat{b}_{i-k}$ when needed and not be stored in entirety. This reduces the space complexity from $O(n^2)$ to $O(n)$, where $n$ is the size of $\hat{b}_{i-k}$. Secondly, the fact that most elements of $\hat{b}_{i-k}$ are near zero is exploited by making $\hat{b}_{i-k}$ sparse.

### 3.3.3 Normalization and contrast

In order to compare the result of a deconvolution to the original image, the intensity is normalized such that $\|b\|_\infty$ corresponds to the highest RGB value. A fair way to normalize the result $x$ is to multiply by a factor $\alpha \in \mathbb{R}$ such that the integrated intensity is the same in both images [17]:

$$\int_V b \, d\tau = \int_V \alpha x \, d\tau,$$

where $\hat{x} = \alpha x$ is the normalized result.

Contrast in a point $p$ of a picture $b$ is calculated as the Euclidian norm of the gradient of the picture, formulated as

$$\text{Contrast}(b, p) = \sqrt{\nabla b \cdot (\nabla b)^*}_p$$

where superscript $*$ denotes complex conjugate [18].
Chapter 4

Result

Deconvolution of the fluorescence from a single 20 nm FlouSphere obtained by the method of section 3.2.1 was performed in one dimension. The method of Arnoldi iterations described in section 2.2.2 followed by solving the minimization problem in equation 2.11 resulted in figure 4.1. The convolution matrix $A$ referred to in equation 2.11 was obtained by using equation 3.1 on the same single 20 nm FlouSphere bead micrograph. Deconvolution was also performed on $\hat{b}_0$ as referenced in section 3.3.1 to attempt a “self-deconvolution”, as can be seen in figure 4.2. The original micrographs and the deconvolutions were normalized according to equation 3.2. A larger peak in intensity was obtained in both deconvolutions, as can be seen in figure 4.1. A larger $\mu$ appear to increase the peak intensity, but to a cost of large oscillations around the peak which can be seen most clearly in figure 4.2b.

A control measurement of the fluorescence of the FlouSphere beads and 3MPA-coated QDs, along side with the autofluorescence of a HUVEC obtained by the

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure41.png}
\caption{Deconvolution using Arnoldi iterations and solving the minimization problem 2.11 in one dimension on a convoluted image $b$ of a 20 nm FlouSphere bead with (a) $l = 4$ and $L_1$-norm, (b) $l = 4$ and $L_2$-norm.}
\end{figure}
method of section 3.2.2 was made in order to determine their spectra by using lambda-mode as referred to in section 3.2. The total wavelength interval of the acquisition was from $\lambda_1 = 411$ nm to $\lambda_3 = 694$ nm. As can be seen in figure 4.3a, the QDs and the FluoSpheres have similar fluorescence spectra. This is beneficial, since the wavelength influences how light behaves in an optical system. Their spectra being similar justifies using a micrograph of a FluoSphere as a reference for the PSF that was used for deconvoluting micrographs of QDs.

The autofluorescence of the HUVECs is of significantly lower intensity than the 3MPA-coated QD fluorescence, as can be seen in figure 4.3. This means that the intensity in the fluorescence channel in channel mode is dominated by the fluorescence of the QDs and can therefore be approximated as to only being the QD’s.

The fluorescent channel of the micrographs of a HUVEC were deconvoluted in three dimensions. The convolution matrix $A$ referred to in equation 2.11 was again obtained by using the single 20 nm FlouSphere bead micrograph as a reference. Ritz values of $K_l(A, q_1)$ were calculated for different dimensions $l$ and residuals were calculated using the simplified expression in lemma 2.2.1. The values of $\|\lambda\|$ versus $l$ were plotted in figure 4.4. The original micrographs and the deconvolutions of the fluorescent channel are normalized according to equation 3.2. Using equation 3.3 on one dimensional slices of the deconvolution and original fluorescence the contrast can be visualized. Contrast is contained to smaller spatial regions in the deconvolutions, which can be seen in figure 4.5 and 4.6. Higher dimensional Krylov spaces yields more noise in the deconvoluted contrast plots, as can be seen in figure 4.7.
Figure 4.3: (a) Control measurements of the fluorescence spectra for the Fluospheres and the 3MPA-QDs. (b) Control measurements of the fluorescence spectra for the 3MPA-QDs and HU-VECs, as well as their combined spectrum from experimentation. Intensities are not in the same scale, due to different settings during the acquisitions.

Figure 4.4: Ritz-values of $K_{l}(A,q_{l})$, red colors indicate large residuals $\|Av - \lambda v\|$ where $v$ is the ritz eigenvector and $\lambda$ the associated ritz value. Blue colors indicate small residuals.
Figure 4.5: x-y cross section of deconvolution results on QDs in a HUVEC in three dimensions using equation 2.11 with \( l = 4 \), \( \mu = 0.125 \). Left column: original micrograph, right column: result of the deconvolution. Top row: contrast profile of fluorescence along the yellow line of x-y cross section. Middle row: fluorescence only. Lower row: overlap of fluorescence and transmitted light.
Figure 4.6: y-z cross section of deconvolution of QDs in a HUVEC in three dimensions using equation 2.11 with $l = 4$, $\mu = 0.125$. Left column: original micrograph, right column: result of the deconvolution. Top row: contrast profile of fluorescence along the yellow line of y-z cross section. Middle row: fluorescence only. Lower row: overlap of fluorescence and transmitted light.
CHAPTER 4. RESULT

Figure 4.7: Cross sections of deconvolution of QDs in a HUVEC in three dimensions using equation 2.11 with \( l = 10 \). Left column: \( \mu = 0.125 \), right column: \( \mu = 15 \). Top row: contrast profile of fluorescence along the same coordinates as the yellow line as in figure 4.6, middle row: y-z cross section of deconvolution, bottom row: x-y cross section of deconvolution.
4.1 Blind deconvolution results

Blind deconvolution was performed on the same using Matlab’s function `deconvblind` with the treated micrograph $\hat{b}_0$ of a single 20 nm Fluosphere as an initial guess for the PSF. The result of 500 iterations is shown in figure 4.8. The blind deconvolution increased intensity slightly and somewhat improved contrast but did not result in any remarkable differences. The most noticeable difference between the blind deconvolution and the regularization deconvolution was that the blind deconvolution did not visually improve the noise in the images.
Figure 4.8: Cross sections of blind deconvolution of QDs in a HUVEC in three dimensions using Matlab’s `deconvblind` with 500 iterations. Left column: original micrograph, right column: result of the deconvolution. Top row: contrast profile of fluorescence along the yellow line in the image below, middle row: x-y cross section of fluorescence images, bottom row: y-z cross section of fluorescence images.
Chapter 5

Discussion and Conclusion

The implemented Arnoldi algorithm for finding orthonormal vectors in the $l$-th Krylov-space to transform the $L_1$-norm minimization problem in equation 2.6 into the range restricted system in equation 2.11 appears to be working as intended. The minimization then gives satisfactory results in our case, by concentrating the contrast to smaller spatial regions and simultaneously removing noise. What was not determined in this thesis is how to choose the dimension $l$, and then what value of $\mu$ will yield the best result for the chosen $l$. Concerning figure 4.7, large differences in $\mu$ do not appear to change the deconvolution result significantly in three dimensions. However, changes in the one dimensional deconvolution in figure 4.1 are significantly dependent on $\mu$. It would be interesting to further investigate the relationship between $l$, $\mu$, and the resulting deconvolution. The choice of values for $l$ and $\mu$ have previously been studied by looking at the residual $b - Ax_\mu$, where $x_\mu$ is the solution of the minimization problem after changing basis to the original space by $x_\mu = Q_1 y$.

The choice of $l$ has large implications on the final result. Figure 4.4 indicate that as the dimension $l$ increases, the residuals of the Ritz-pairs decrease. In theory this would indicate that a higher dimensional Krylov space would be a better approximation of $A$. Hence, a deconvolution made with a Krylov space of a higher dimension would in theory produce a deconvolution more accurate than on a smaller Krylov space. Figure 4.5, 4.6 and 4.7 contradict this, showing that higher a higher dimension instead appears to give a larger amount of wave-patterns. Deconvolution was also performed on a Krylov space with $l = 20$ but was omitted in the result as the resulting image contained even more noisy patterns.

A parallel can be drawn between the range restricted Arnoldi algorithm and the singular value decomposition $x = U\Sigma V^H$ of an image. Since the matrix $\Sigma$ contains the largest singular values in decreasing order, one can construct an approximate image by omitting the smaller singular values. When looking at figure 4.4, this parallel becomes obvious. As $l$ increases, smaller Ritz pairs are introduced.
5.1 Microscopy

There are possible discrepancies between the two lab sessions described in section 3.2.2 and 3.2.1 regarding the setup of the LSM780 microscope. There might be a difference in acquisition depths of the point source in section 3.2.1 and the HUVEC. A different acquisition depth or a different pinhole size would affect the theoretical point spread function \([20]\). If the empirical point spread function used for deconvolution of the fluorescent channel of the micrograph of the HUVEC does not accurately represent the actual point spread function it might explain the wave-patterns seen in figure 4.7.

5.2 Future development

It would be interesting to investigate the convergence of the norm of the residuals in figure 4.4 for larger values of \(l\) (\(10^2, 10^3\) etc.), but the computational power we had access to limited us to \(l = 20\). The solving of the minimization in equation 2.11 also scales badly, as the \(l = 20\) case took approximately 5 hours while the \(l = 4\) case is almost instantaneous.

Fields of interest for further applications of QDs are for example biofluorescent tagging and traceable drug delivery. In all cases imaging is involved, which is why improvement of image quality is of the utmost importance.
Bibliography


