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Bc. Hana Schlorová



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BLIND IMAGE DECONVOLUTION OF ELECTRON MICROSCOPY IMAGES

SLEPÁ DEKONVOLUCE OBRAZŮ KALIBRAČNÍCH VZORKŮ Z ELEKTRONOVÉHO MIKROSKOPU

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AUTHOR

AUTOR PRÁCE

Bc. Hana Schlorová

SUPERVISOR

VEDOUCÍ PRÁCE

Ing. Petr Walek

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Studentka: Bc. Hana Schlorová

ID: 146202

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Slepá dekonvoluce obrazů kalibračních vzorků z elektronového mikroskopu

POKYNY PRO VYPRACOVÁNÍ:

1) Provedte literární rešerši metod slepé dekonvoluce obrazu. Prostudujte vady prozařovacího elektronového mikroskopu a možnosti jejich korekce. 2) Navrhněte postup optimální slepé dekonvoluce pro obrazy z prozařovacích elektronových mikroskopů. 3) Navrhněte způsob vyhodnocení výsledků a závislost akvizičních parametrů elektronového mikroskopu. 4) Provedte nasnímání obrazů ve spolupráci s firmou FEI Česká republika. 5) Otestujte metody na modelových a reálných obrazech a porovnejte s metodou dostupnou v prostředí Matlab – Image processing toolbox. 6) Provedte diskusi získaných výsledků a zhodnoťte účinnost a využitelnost metod. Diplomová práce je tvořena ve spolupráci s firmou FEI Česká republika.

DOPORUČENÁ LITERATURA:

[1] KARLIK M., Úvod do transmisní elektronové mikroskopie. Praha ČVUT, 2011. ISBN 978-80-01-04729-3.

[2] CAMPISI, P., Egiazarian, K., Blind Image Deconvolution“ Theory and Applications. CRC Press: Boca Raton: 2007, 114 s. ISBN 978-1-4200-0729-9.

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Vedoucí práce: Ing. Petr Walek

Konzultant: Ing. Miloš Malinský, Ph.D.

prof. Ing. Ivo Provazník, Ph.D.
předseda oborové rady

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ABSTRACT

Blind deconvolution has spread around multiple technical fields in recent years. Problems with computational demands are no more its limitations. Blind deconvolution signal processing techniques are promising solution for enhancement of electron microscope performance. The aim of this work is the problem formulation and proposition of appropriate solution for blind deconvolution of electron microscope images. The final goal is to develop Matlab algorithm correcting aberrations arising from imperfections of image formation and its comparison with built-in Matlab approach implemented in Image Processing Toolbox. Proposed approach is given by regularization techniques of blind deconvolution.

KEYWORDS

Blind deconvolution, image restoration, degradation process, blur, electron microscopy, Bayesian framework, regularization, deconvblind

ABSTRAKT

V posledních letech se metody slepé dekonvoluce rozšířily do celé řady technických a vědních oborů zejména, když nejsou již limitovány výpočetně. Techniky zpracování signálu založené na slepé dekonvoluci slibují možnosti zlepšení kvality výsledků dosažených zobrazením pomocí elektronového mikroskopu. Hlavním úkolem této práce je formulování problému slepé dekonvoluce obrazů z elektronového mikroskopu a hledání vhodného řešení s jeho následnou implementací a porovnáním s dostupnou funkcí Matlab Image Processing Toolboxu. Úplným cílem je tedy vytvoření algoritmu korigujícího vady vzniklé v procesu zobrazení v programovém prostředí Matlabu. Navržený přístup je založen na regularizačních technikách slepé dekonvoluce.

KLÍČOVÁ SLOVA

Slepá dekonvoluce, restaurace obrazu, process degradace, zkreslení, elektronová mikroskopie, Bayesovský rámec, regularizace, deconvblind

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DECLARATION

I declare that I have written the Master's Thesis titled "Blind deconvolution of electron microscope sample images" independently, under the guidance of the advisor and using exclusively the technical references and other sources of information cited in the thesis and listed in the comprehensive bibliography at the end of the thesis.

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Brno

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INTRODUCTION

Digitized images are essential in science and for everyday life. No matter the field, there is always the true scene we want to observe with its ideal representation by original (true) image. Since the observation process of true image is never perfect, output digitized image is degraded by blur and noise. Image restoration techniques provide approximation of original non-degraded image. Particular approach is chosen with respect to *a priori* known information. In case of known blur and noise properties, the classical restoration techniques are used. While the properties of degradation are not known properly, techniques of blind deconvolution can solve the problem better.

Theoretically, the better observation procedure would increase the quality of received image. But there is always some physical limit or suboptimal conditions that determines quality of obtained images. Moreover, high quality optics and sensing equipment are expensive, therefore software solution becomes valuable tool for image improvement. Extensive processing power and trend of complex “one button” solution without complicated calibrations also represents the reason of blind deconvolution popularity nowadays.

Blind deconvolution methods are commonly used in various technical areas such as astronomical imaging ([24],[51]), remote sensing [3], microscopy [16], medical imaging [29], optics ([44],[36]), photography ([55],[65]), super resolution applications [52] and motion tracking applications [11], among others . The earliest algorithms identifying known patterns in blur occur in mid-1970s ([6], [54]) with its renaissance in 1990s (reviews [25],[26]) . Prior image characterization by natural image characteristics (such as gradient) is the key idea of new algorithms converging to a correct solution in less time.

Purpose of this thesis is to study the ability of blind deconvolution methods to restore degraded image from electron microscope, chose and implement proposed technique and to study its advantages and disadvantages in comparison with Matlab built-in implemented method. Application on real data taken upon commonly used conditions should reveal the benefits for use in practice.

1 THEORY OF TRANSMISSION ELECTRON MICROSCOPY

1.1 General construction of TEM microscope

Common transmission electron microscope system consists of illumination part, part focusing rays and magnifying observable image of sample placed in a microscope vacuum chamber and imaging system representing the phosphor screen observed by camera or camera sensor with solid-state scintillator included.

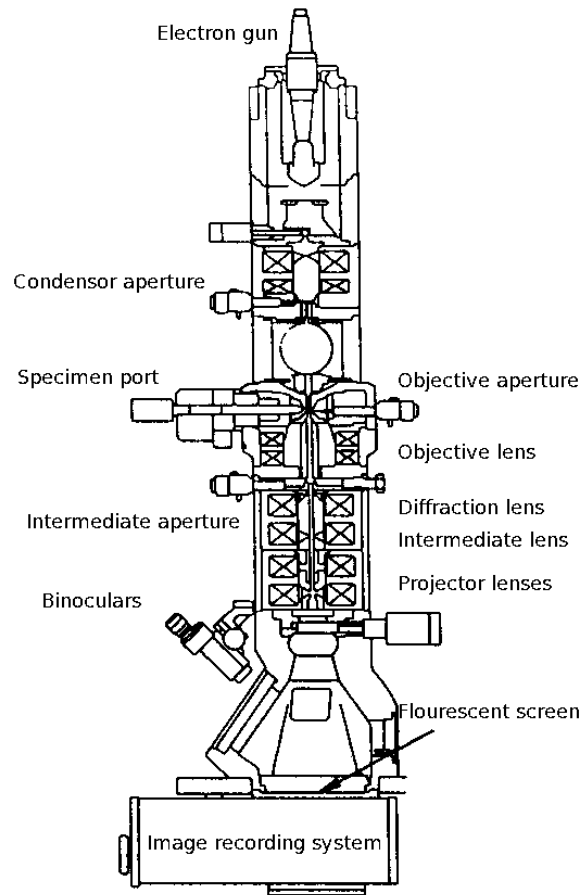


Fig. 1.1: General construction of TEM microscope [13]

Illumination part represented by **gun** and **condenser electromagnetic lenses** is responsible for beam formation, coherence and beam size and intensity on illuminated area of observed sample. Quality of electron gun sources working upon thermionic or field emission principal determines the achievable resolution by energy spread of cathode emission.

Primary role of strong **objective electromagnetic lens** with sample in between

its upper and lower part is to focus beam onto specimen, form the images and diffraction patterns that are magnified by other lenses.

Projection system consisting of lenses magnifying and transmitting image or diffraction to the fluorescent screen or directly to camera sensor. Final magnification is given by strong projection lens forming the observable image.

Except main lenses, beam is formed and tilted by multiple **deflectors** and **stigmators** coils. Role of perpendicularly to the beam positioned deflectors according to its excitation is shifting and tilting the beam to appropriate position. Position of beam is controlled by gun, beam or image deflectors in the microscope column. Despite effort to form the ideal shape of the beam, lenses imperfections destroy the shape therefore it must be corrected several times. Correction factors, stigmators, are position next to the main lenses compensating astigmatic shape of the beam.

Optimization of electron beam properties is given by fixed or variable circular shape **apertures**. They are located along the optical axis of microscope changing the beam diameter. In special cases, additional image contrast is determined by aperture position in focal plane.

Contrast of output image created by interactions along the microscope column is stored in the energy and phase of interacting electrons. To be shown on the display electrons must be transformed into visible light (by **fluorescent screen** or integrated solid-state scintillator next to the camera chip) and detected by **photon-sensitive sensor**. Charge coupled device (CCD) and complementary metal oxide semiconductor (CMOS) image sensors are two different technologies for capturing images digitally.

Brief introduction of TEM construction is based on study of [23].

1.2 Aberrations of TEM

In general, there is nothing like a perfectly corrected microscope. The more complicated systems with better performance you have, the more can go wrong. Coherency of electron beam, electron lenses imperfections and axial dependency of ray position together with used magnification determine the real observable image (the expression observable will be used for a formed image with full information about amplitude and phase before entering the camera). The image is consequently transformed by camera into a digitalized output image with incorporated blur given by this process.

The dependency of parasitic aberrations of the physical nature on magnification can be illustrated in the following way (Fig. 1.2):

The simplified graph illustrates the general dependency of the aberration magnitude on distance from optical axis (commonly known as off-axis aberrations) and on the convergence angle of rays entering the aperture of lens (on-axis aberrations).

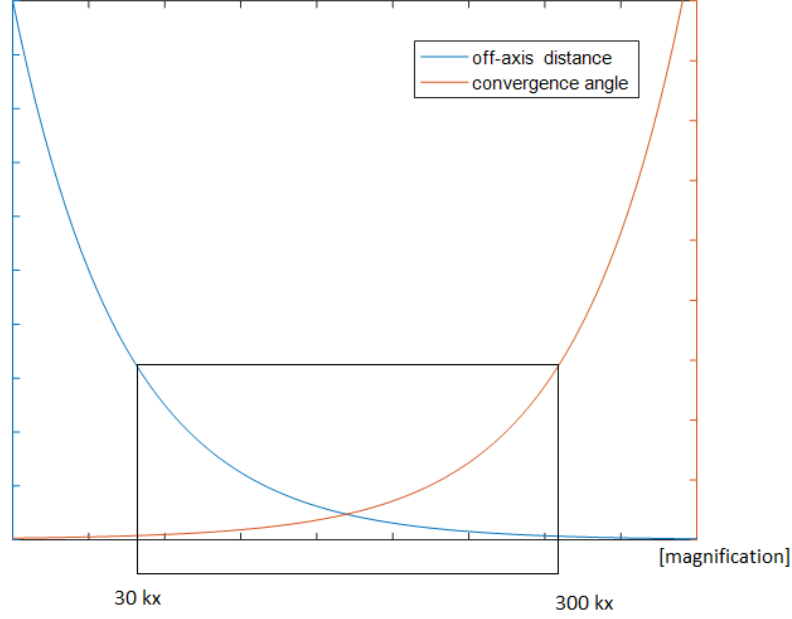


Fig. 1.2: Dependency of parasitic aberrations on magnifications

With respect to the used magnification it is possible to neglect the contribution of insignificant errors caused by parasitic aberrations with small influence.

In case of small magnifications (approximately up to 30kx) the observable image is made largely by non-paraxial (further from optical axis) rays. The main contribution to each point element aberration is dependent on distance from the axis and the convergence angle is usually very low. The influence of axial aberrations can therefore be neglected. On the other side, working in high magnification (typically more than 300kx) means the observed area becomes smaller and the convergence angle increases. This means the observable image will be constructed mostly from paraxial rays and the error source comes mainly from the convergence angle of the lens. In this case, off-axis aberrations can be neglected when compared to axial ones.

Obviously, there is an interval of magnifications where both contributions have to be taken in an account. Therefore, a formal general expansion of wave aberrations during imaging is presented. Using Krivanek's notation [37], the difference between an ideal and aberrated phase of the wave χ can be expressed by complex general formula :

$$\chi(\theta, \phi, r) = \sum_p \sum_q \sum_n \sum_m \frac{r^p \theta^{(n+1)}}{n+1} \left\{ C_{n,m,a}^{p,q,a} \cos(q\omega) \cos(m\phi) + \right. \\ \left. + C_{n,m,a}^{p,q,b} \sin(q\omega) \cos(m\phi) + C_{n,m,b}^{p,q,a} \cos(q\omega) \sin(m\phi) + C_{n,m,b}^{p,q,b} \sin(q\omega) \sin(m\phi) \right\}. \quad (1.1)$$

where $r = |w|$ is a distance from optical axis, ϕ complex slope coordinate, $C_{n,m,b}^{p,q,a}$

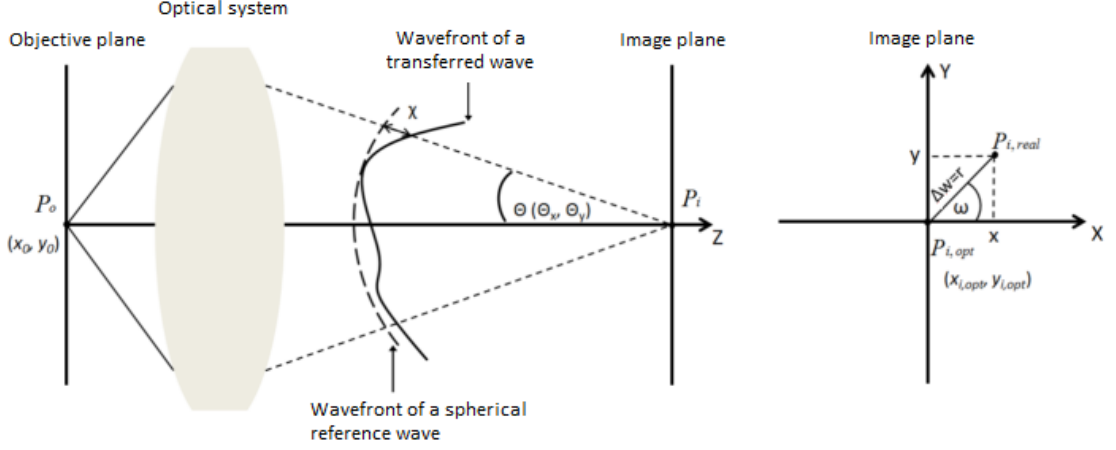


Fig. 1.3: Graphical illustration of eq. 1.1 [53]

represents an aberration coefficient of order $n + p$ and multiplicity m defining the number of maximums of distorted wave with range of ϕ in $0 - 2\pi$. q stands for field multiplicity index in range of values from 0 to p , a, b separates orthogonal contributions to the aberrations. Overall theory above the equation exceeds the scope of the thesis. Graphical illustration is given by Fig. 1.3 or more to be studied in [37].

The axial aberrations (dependency on convergence angle) are associated with magnetic field intrinsic properties (e.g. spherical aberration), mechanical imperfections of the lens construction (causing asymmetrical parasitic field contributions – e.g. astigmatism) and also tilt if the beam entering the lens (e.g. coma). The off-axis aberrations are associated mainly with magnetic field decrease far from the axis and the lens geometry (e.g. distortion).

$$\sum_{l,k} C_{n,m}^{l,k} \cdot r^l \cdot \bar{r}^{-k} \sum_{n,m} C_{n,m} \cdot \varphi^n = \sum_{l,k,n,m} C_{n,m}^{l,k} \cdot r^l \cdot \bar{r}^{-k} \cdot \varphi^n \quad (1.2)$$

In the used notation $C_{n,m}$ is an axial aberration coefficient (generally a complex number), where n is the aberration order (dependency on convergence angle φ^n) and m is the aberration symmetry. This number represents the number of axes which the aberrated shape can be folded on itself, provided the input shape was a circular beam. Zero means full symmetry with infinite number of axes. An example of $m = 0$ is the spherical aberration ($C_3, 0$) and defocus ($C_1, 0$). One axis symmetry shape is created by coma, two by 2-fold astigmatism and three 3-fold astigmatism. Explanation illustrated by images (1.5, 1.6, 1.7, 1.8). Aberrations of higher orders are neglected due to their small influence when compared to lower-order aberrations. Also in used notation, $C_{n,m}^{l,k}$ is an off-axis aberration coefficient where $(l + k)$ is the

order of aberration and the individual indices l, k do not have a simple interpretation like the axial ones do. These are the main contributions in non-paraxial (low magnification) imaging. Shift, rotation, linear distortion (1.10) and barrel and pincushion distortion (1.9) and more have the nature of such geometrical errors, shifting the projective point from its ideal position by vector r . In this mathematical description, even magnification itself (both isotropic and anisotropic due to the field imperfections) is regarded as “aberration” as it is also a form of geometrical transformation of image. Even though for our purposes, this is a desired transformation. Another type of aberration is the one associated with energy dependency of the lens focusing power. Lower energy electrons with lower velocity will be affected by the magnetic field in a different way – they will be bent more by the field. Therefore the lens’ focal length for such electrons will be shorter. Cumulative imaging of more rays created by different energy electrons will cause the output blur in the image plane. In an ideal system, the beam extracted from the cathode is monochromatic (i.e. has zero energy spread), the stability of high tension is infinite and the excitation current of lenses does not fluctuate. This is not true in a real system. The contributions from these areas in a real system can be approximated by the following equation:

$$\Delta H = \sqrt{\left(\frac{\Delta U}{U}\right)^2 + \left(\frac{\Delta E}{U}\right)^2 + \left(\frac{\Delta I}{I}\right)^2} \quad (1.3)$$

This causes a point blur dependent on ΔH and the convergence angle. Its characteristic magnitude can be expressed as $C_c.H.\varphi$, where C_c is the chromatic aberration coefficient.

Diffraction, Chromatic aberration

Diffraction error occurs due to the wave nature of electrons and the aperture size of the final lens.

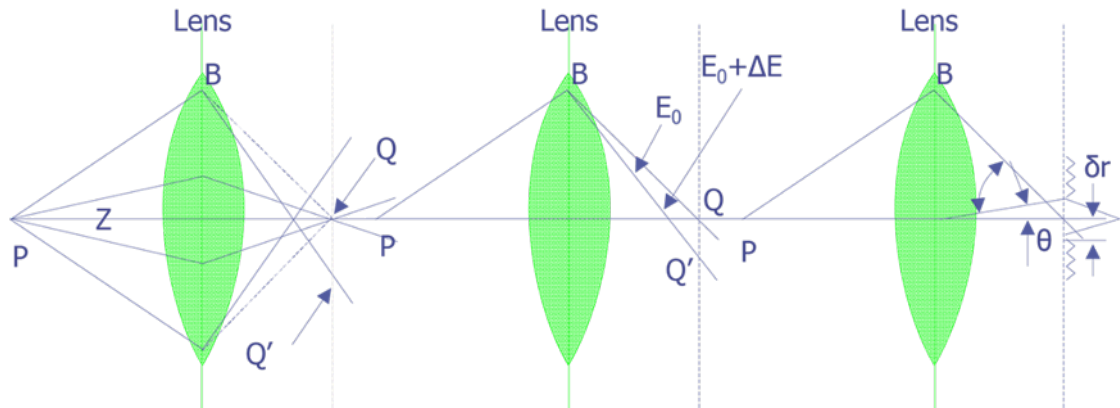


Fig. 1.4: Comparison of nature of parasitic aberrations

Astigmatism $C_{1,2}, C_{2,3}, C_{3,4}, C_{4,5}, C_{5,6}$

Astigmatism occurs when electrons sense a non-uniform magnetic field and starts to twist around the optic axis, the effect arises according to the imperfections of symmetricity of polepieces or of the microstructural inhomogeneity which causes local variation in magnetic field strength. Even without mechanical imperfections the problem arises also with introduction of apertures, that may not be perfectly circular. Astigmatism can be corrected by stigmators (small octupoles lenses) that compensate the inhomogeneities.

Defocus $C_{1,2}$

Optically defocus refers to the distance (Δz) away from the plane or surface with best sharpness and contrast (in focus).

Coma $C_{2,1}, C_{4,1}$

Coma is caused by a cone of rays passing through the specimen at an angle. Therefore the centre of imaged circles is shifted up to the angle of illumination.

Spherical aberration $C_{3,0}$

Occurring when the rays are bent more strongly away from the axis with respect to ones closer to the axis. It can be corrected by set of multipole and cylindrical lenses which introduce a well-defined asymmetry to the beam, spread it, rotate and then reconverge, so that it compensates the spherical aberration.

Magnification and rotation $C^{1,0}$

In the specification given by position from optical axis upon the used magnification initial point to be imaged is shifted away from optical axis with given enlargement/reduction of image size. Together with magnification the rotation of optical system is defined therefore rotation free system does not change the direction of the coordinates with respect to the initial plane. Practically with rotation of pi radians the element on the left from axis will be transferred into point in the image on the right while the rotation-free system preserves rotation.

Linear distortion $C^{0,1}$

Linear distortion means the image preserves parallelism but not the proportional distances in perpendicular directions.

Pincushion and barrel distortion $C^{2,1}$

The easiest explanation of the effect of geometrical barrel and pincushion distortion with its graphical representation.

For individual aberration effects, the graphical illustration is given below. Instead of one point, the input object represents a set of five concentric circles and the aberrations deform their shape. The simulation have been done by Mgr. Jan Jíša (System Engineer in FEI company responsible for optics system properties) in the Excel environment.

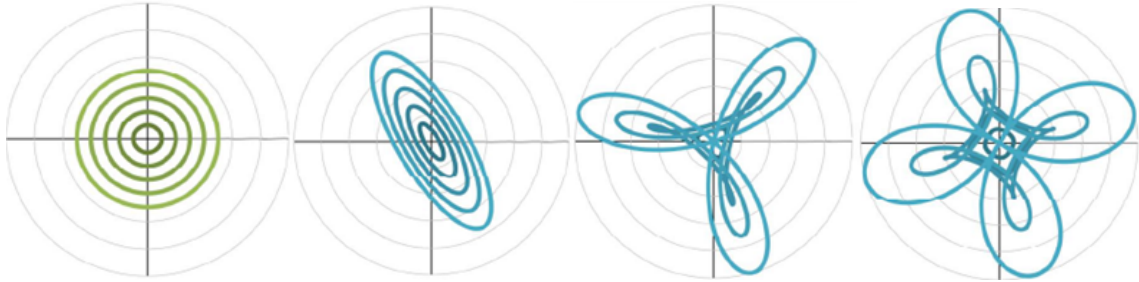


Fig. 1.5: Graphical illustration of aberration (2-fold ast.,3-fold. ast.,4-fold ast.,blue) influence on ideal circular beam (green)



Fig. 1.6: Graphical illustration of aberration (defocus;blue) influence on ideal circular beam (green)



Fig. 1.7: Graphical illustration of aberration (coma;blue) influence on ideal circular beam (green)



Fig. 1.8: Graphical illustration of aberration influence (spherical aberration; blue) influence on ideal circular beam (green)

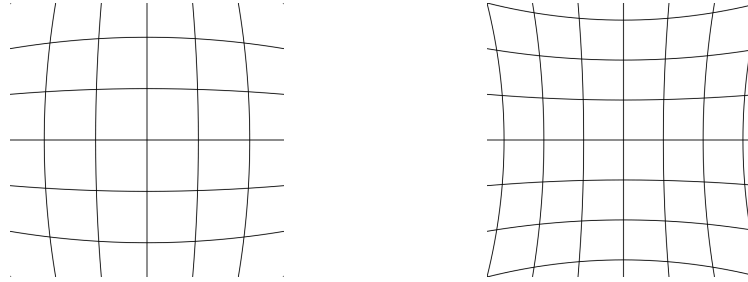


Fig. 1.9: Graphical illustration of barrel and pincushion distortion

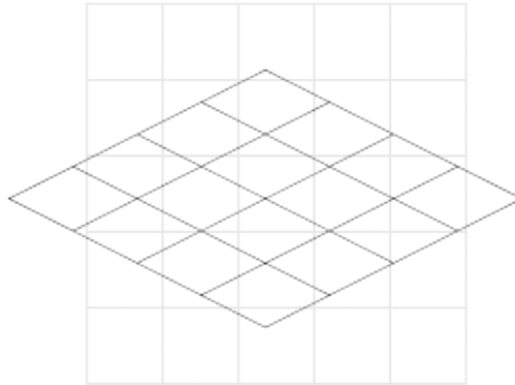


Fig. 1.10: Graphical illustration of linear distortion

1.3 Theoretical possibility of correction of aberrations by Blind Deconvolution

It is known that the process of electron imaging using electromagnetic lenses is not ideal, but multiple alignment techniques can suppress its influence into final image. The aim of this work is to reconstruct images taken by differently aligned system, therefore it should be mentioned how strong image degradation caused by

aberrations in the final digitized image.

Concerning about image processing part of system according to the ability of blind deconvolution techniques to correct microscope aberrations we would have to look for specifications that gives information about detectors and cameras performance. The most important technical specifications that should be taken in an account are **pixel size**, **effective pixel size**, **full well capacity**, **antiblooming** ability of camera, its **binning** and **exposure time**. Some of will be considered as acquisition parameters for image to be reconstructed.

The **pixel** is the smallest element in a visual display and behaves as a bucket (well) collecting electrons to convert them into photons and electrical signal afterwards. Each CCD or active pixel sensor CMOS has a vertical and horizontal array of pixels that determines the overall resolution achievable [38]. **Pixel size** value and **effective pixel size** tells us an information about sampling of detector area. For given size of chip, how big will be single one area associated for signal collection [38]. Size of pixel element also determines how can be our points of interest spatially close to each other to be recognised as two elements (point resolution). While the overall influence of aberrations is smaller in diameter than the camera pixel size (magnification must be included in the assumption), the blur given by this process would not affect the output image, Output response of Dirack distribution would be theoretically just one integrated pixel-value in digital image, assuming the blur would be given just by lenses aberrations in real but it is not fully true.

Specific values of pixel size by various distributors of TEM camera imaging systems are in range of 6 – 14 μm (Emsis [20], Gatan [21], FEI [22], AMT [19]) and evaluated diameter of spherical aberration for some of the microscopic would be in order of tenth of nm . The meaning is that for given pixel size and evaluated aberrations diameter together with best resolution available, considering approximately in general orders of variables, our initial point in imaged scene would be blurred by the process and despite that detected afterwards as one pixel value up to magnification in range of 25 000-120 000. Information above this boundary will be collected from more than one pixel element even that ideal image has just single value equivalent to Dirack distribution).

Pixel binning used for an advantage of decreased acquisition time needed is technique of reading pixel elements information not one-by-one (1x1 binning) but in integrated groups of pixels, „super-pixels “ [38]. For example, 2x2 pixel binning integrates 4 pixel elements to be treated as one to increase sensitivity and signal to noise ratio (SNR), decrease the time for image acquisition, while resolution decreases by factor 4 [38]. Using the same assumption as above binning increase by factor 4 also the magnification required to the aberration influence necessary to be taken in an account. For pixel binning 2x2 is then the range 100k-480k, for binning 3x3

225k-1080k limit that would not be affected by point spread blur caused by lenses aberrations.

Explanation of higher SNR illustrates the example of 4x4 binning („on-chip” sum) in contrast with „off-chip” summation as an cumulation technique approach for increasing SNR. Summation could theoretically have been done digitally at the camera output, the improvement of SNR is more dramatic if all operations are done within the sensor itself. If a 4×4 neighbourhood of pixels were summed together off-chip, the signal level would increase by $4 \times 4 = 16$, but the CCD’s inherent noise floor would simultaneously go up by $\sqrt{16} = 4$. Thus, the SNR increase obtained by off-chip summation would be $16/4 = 4x$ increase. In contrast, if a 4×4 neighbourhood of pixels is summed on-chip, the signal increases by 16x and the noise stays as it was, resulting in an increased SNR of 16x. In case we think about the practical use of blind deconvolution techniques, considering our approaches ideals with best results available, we can think about practical side of the problem from different point of view. As has been mentioned mechanical imperfections of round symmetry of lenses apertures, homogeneity of material or inhomogeneity of emission together with not perfectly aligned sequence of lenses are responsible for distortion. Parasitical aberrations called in general. According to physical properties of process chromatic and spherical aberration and diffraction incorporates other errors and everything together makes observable blurry image.

The assumption of possible correction is based on the spatial in/dependency of each aberration. Because the blind deconvolution techniques in general suppose the PSF to be spatially invariant therefore it does not count with imperfections depending on the spatial coordinate and aberration with the spatial dependency can not be suppressed by blind deconvolution unless the variant PSF algorithms are used .

2 THEORY OF BLIND DECONVOLUTION

2.1 Problem formulation

General model for a linear space invariant degradation caused by blurring and additive noise is given by linear degradation system (LDS) as follows:

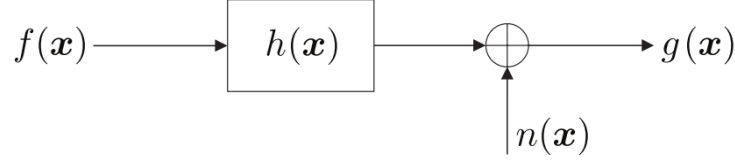


Fig. 2.1: General model of degradation

Where $f(x), g(x), h(x)$ and $n(x)$ denotes true image, output image, operator of blur (PSF) and additive noise originated in image acquisition process. In equation form

$$f * h + n = g \quad (2.1)$$

Purpose of image registration techniques is to reconstruct the original image from $g(x)$ with no or some prior knowledge about $f(x), h(x)$ or $n(x)$ therefore to deal with solving the inverse problem. Issue of inverse problem suffers from extreme sensitivity of inputs, if one changes slightly, output can be completely different.

Blind deconvolution is considered to be an ill-posed problem, that means the solution is not unique, is not continuously changing with inputs or may not exists.

2.2 Origin of blur

Blur originated in image formation process given by whole microscope system (from signal emission to digitalization into image) can be expressed by

$$h = h_{system} * h_{scintillator} * h_{cameralens} * h_{sensor} \quad (2.2)$$

Origin of blur can be divided into two separate sources, first covering entire process after entering the sensor of camera (with appropriate light optics), and second caused by the process of observable image formation. Parasitical aberrations resulting from imperfections of system are discussed above (1.2) representing h_{sys} in (2.2). Errors occurring due to the nature of image formation process are described in section 1.3.

Camera $h_{scintillator} * h_{cameralens} * h_{sensor}$ with sensor and light optics represent the second group of blur origins. Specific parameter pixel well capacity says how

many electrons can be accumulated in one pixel element, above this capacity the excess electrons spill out into neighbouring pixels and cause blooming artefact [7]. Prevention of blooming artefact can be realised by additive electronics controlling the spill. Additional components improve the image quality, otherwise the point in initial scene can generate signal into multiple pixels, the blur.

2.2.1 Origin of noise degradation

Due to the nature of signal and image generation and in electron microscopy Poisson noise or its approximation by the Gaussian distribution and Gaussian noise itself are incorporated in the process of image formation. Theoretical occurrence of impulse noise is also possible but thanks to the camera's ability to detect error (dead) pixels we do not have to concern about. Therefore the influence of impulse noise will not be included in the general degradation model.

While we have the measurement based on the detection of photons we have to deal with the uncertainty associated with quantized nature of light and independence of photon detection, Photon noise, known as Poison, Shot or Schott noise [18]. Photon noise is not considered to be additive nor multiplicative, rather applied on the input signal (image) depending on signal amplitude. Poison distribution with given parameter λ assume that each pixel of an image is drawn from Poison distribution with $\lambda = f_0(x)$, where $f_0(x)$ is the original noise-free image to undergo reconstruction [12].

$$P(f(x)) = \frac{\lambda^k e^{-\lambda}}{k!} \quad (2.3)$$

Process of image formation as electrons hitting the scintillator could be considered as the Poison distribution of events too.

Generated photons in scintillator proportionally to amount of the electrons hitting its edge are relatively to the position of the camera and detection process directed by extra support or surface layer with low atomic number (smaller diffraction) or by fiberoptics to appropriate side of the scintillator for more accurate and efficient detection. Low atomic number of layer is required for small ability of generation of redundant backscattered electrons. That decreases with increasing atomic number as illustrated where η means backscatter coefficient as a function of atomic number (Z) given as ratio of generated backscattered electrons to overall beam electrons incident, in general almost independent of beam energy. In case of camera mounted below the column and detection based on the scintillator-next to-sensor system is needed to deliver photons from the scintillator by fibre optics perpendicular to the surface and aluminium layer covers the top of scintillator. The second approach based on the reflection of photons by mirror onto sensor mounted on side,

the closer to the edge photons detected the better and aluminium layer is then in form of support under scintillator.

No matter chosen approach to electron inversion and directioning afterwards both options handle the generated photons in same way. Photons directed onto chip (CCD or CMOS) with optional micro lens technology for improvement of quantum efficiency [38] hits the pixels, interacts and afterwards captured electrons in the potential traps are assigned to be read. Proportional generation of electrons to number of incident photons on pixel represents the useful signal for conversion, related to previously mentioned, well capacity, pixel size and chip characteristics determines the quality of output image.

In contrast with use of CMOS chip, working with CCD we have to deal with the read noise that determines effective dynamic range of the useful signal given as ratio of maximum well capacity to read noise of sensor (ex. 128:1 as signal: noise) or by level of signal to noise ratio in dB (ex. 42 dB for 128:1 ratio) [38]. CMOS camera has virtually zero level of read noise originated in the technology of charge read without no shift and direct analogue to digital (AD) conversion.

In both cases of on-chip or off-chip digitization the very low voltages are treated, consequently the amplification before entering analogue to digital converter (ADC) is needed as another not noiseless process [?]. Output signal from AD converter is determined prior to pre-set of linear working range of the converter, therefore even the big input range of the signal, narrow linear range of the ADC determines the output.

Moreover, dark noise is also taken in an account. Dark current measured as the response for dark frame with no illumination and no signal expected corresponds to thermal generated electrons of working sensor [7]. To decrease dark current, camera sensors are cooled, robbing electrons of the thermal energy required to reach an intermediate state [?].

Noise types mentioned above are responsible for degradation of an image as much as the blur given by process of generation of image physically in the microscope column. Processes of noise based on the fluctuation of events occurred (hitting the pixel by photons) will be modelled by Poisson noise applied to the initial image, other types of noise follow the Gaussian distribution with given mean value and standard deviation. With appropriate assumption (explanation, source) Poisson distribution can be expressed by Gaussian. Noise degradation in general can be modelled by white Gaussian additive noise. Its influence on the ability of blind deconvolution to reconstruct initial image despite noise degradation will be evaluated. Possible practical assumptions from the results can be discussed as the required properties of detection electronics for reliable results of the blind deconvolution.

2.3 Methods of blind deconvolution

According to the broad scope of blind deconvolution techniques across all technical disciplines it is quite complicated to classify the methods. Several divisions into groups can be made depending on application, noise statistics, blur kernel parametrization or proposed estimation method [5].

There are two main approaches o blind deconvolution of images:

1. **PSF is identified separately from true image** and one of the known classical images restoration methods is used.
2. **PSF identification is done jointly with restoration** with incorporated prior knowledge about image into deconvolution process.

Considering the **group of methods treating the blur estimation and the image restoration independently**, there are two approaches, experimental and theoretical. Determination of PSF can be made directly from the image with known scenes (stars supposed to be points in astronomy) or weaker prior knowledge of contours of observed scene (x-ray imaging).

While the property of blur is known correctly (motion blur, out-of-focus lens) methods expecting the zeros in spectrum corresponding to zeros of transfer function can be used, therefore the problem is reduced on zeros estimation limited by noise occurrence. Survey on parametric methods can be found in [5] . Real PSF differs slightly from its parametric model therefore the finding the solution often fails. Despite the helpful concept, algorithms are highly sensitive to noise with computational requirements.

When **PSF identification is done jointly with restoration**, because no prior knowledge of PSF is available or theoretical form is changing while process, the prior knowledge about image and blur is incorporated in the deconvolution model. Commonly used models will be introduced. According to the amount of methods , based on its potential to solve the problem of blind deconvolution of microscope images, different complexity of description is given.

2.4 Zero sheet separation

Zero sheet explanation of deconvolution is based on the Z-transform, assuming no additive noise in the process. Therefore the formulation of degradation is reformulated

$$G(z_1, z_2) = H(z_1, z_2)F(z_1, z_2) \quad (2.4)$$

And the task is to find convolution factors of blurred image G . While the $G(z_1, z_2)$ is polynomial in z_1, z_2 the aim is to identify the zeroes belonging to components H and F .

The method of zero sheet is more helpful in understanding the deconvolution problem. According to the assumption of no additive noise present and high computational demands, practical applicability is limited.

2.5 ARMA parameter estimation

ARMA parameter estimation is based on the modelling the output blurred image as autoregressive moving average process (ARMA) while assuming the PSF to be moving average (MA) process and true image autoregressive (AR).

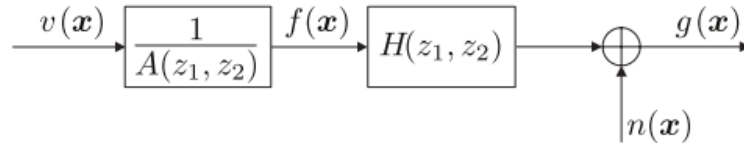


Fig. 2.2: ARMA parameter estimation degradation model

Finding the solution is based on parameters estimation of degradation and image representing filters. Estimation can be done in many ways by techniques of second order statistics, such as maximum likelihood estimation [28], generalised cross-validation (CGV) [42] or neural networks [10]. Concerning about non-Gaussian models, the techniques of Higher-order spectra (HOS) [41] can be used. Maximum likelihood and GCV methods are most successful for image processing [26].

2.6 Nonparametric Deterministic Constraints methods

Class of the methods that do not explicitly represent the PSF and image by model is represented by methods constraining the image and PSF by deterministic principles [5]. Methods has limited scope of applications. Including Iterative Blind Deconvolution (IBD) ([35] , [59]), simulated annealing algorithm (SAA) [31], Nonnegativity And Support constraints with with recursive Image Filtering (NAS-RIF) as most common among others.

NAS-RIF [27] method minimizing the cost function by updating the FIR restoration filter that is convolved with degraded image, giving the estimation of true image [5]. potential of methods is limited by need of finite support. Practically the uniform

black background is needed therefore applications in medical imaging and astronomy are possible.

IBD early proposed algorithm by Ayers and Dainty [1] is based on same principle. With addition to nonnegativity and finite support it uses constraint to image and blur in Fourier domain incorporated in each iteration of algorithm. The application of method follows the NAS-RIF.

Nonparametric Algorithms based on Higher -order Statistics using non-linear Gaussian representation are typically applied to images with „spike-like“ nature, for example deblurring the images of star fields [5].

2.7 Bayesian framework

Standard formulation of image restoration problem can be express by stochastic approach assuming that images are random vector fields with known prior probability distribution functions. Bayesian logic describes deconvolution problem as probabilistic problem. It is a from of statistical analysis that quantifies input by probability of its occurrence If there is no prior information available, each entry has the same probability, because of equal possibility. In case of blind deconvolution, observed image corresponds to the apriori information.

General Bayes formula is based on multiplication of rules for conditional and unconditional probabilities of occurrence of phenomena A (hypothesis) and B (expected):

$$p(A|B) = \frac{p(B|A)p(A)}{p(B)} \quad (2.5)$$

where $p(A|B)$ represents the conditional probability event A occurs, assuming occurrence of B . Conversely, $p(B|A)$ represents the probability of event B if the phenomenon occurred A . $p(A)$ and $P(B)$ are the unconditional probability of the occurrence of event A and B . The probability hypotheses before performing random experiment $p(B)$ is called a priori probability and the probability similarity hypotheses after the random experiment $p(B|A)$ is termed the likelihood A posteriori.

Based on this logic the problem of deconvolution can be described probabilistically. Substituting the observed image $f(m, n)$, and distorted and noisy an image $g(m, n)$, solution is given in form

$$p(f|g) = \frac{p(g|f)p(f)}{p(f)}. \quad (2.6)$$

The probability $p(f)$ is called the prior probability of hypothesis (true image f) and represents the probability of hypothesis before signal observation. If no

information is available, each hypothesis is equally possible. Conditional probability density $p(g|f)$ represents the similarity measure of a test results on data g assuming hypothesis f . $p(g)$ is considered as evidence (complete) probability because degraded image is known and conditional probability density $p(f|g)$ is determined by degraded image and apriori information. Formula (rov) indicates how probable is hypothesis f (one of all theoretically possible) after observed image g . Blind deconvolution with unknown PSF and true image requires modification of formula as follows

$$p(f, h|g) = \frac{p(g|f)p(f)p(h)}{p(g)}. \quad (2.7)$$

Where $p(h)$ denotes apriori probability density of PSF hypothesis.

2.7.1 Maximum aposterior estimation (MAP)

MAP deconvolution method uses the Bayesian approach for solution of blind image deconvolution maximizing the formula

$$f_{MAP} = \arg \{ \max p(f|h, g) \} \quad (2.8)$$

That maximizes posterior probability density with respect to unknown values of f and h . Many deconvolution methods can fit into this Bayesian formulation. The main differences among these algorithms come from the form of the likelihood, the particular choice of priors on the image, blur and the optimization methods used to find the solutions [5].

2.7.2 Maximum likelihood estimation (MLE)

Method based on the statistical model of noise restores the image deciding which of the hypothesis of image f is the most likely according to known distribution of observed image g . Like MAP method maximizes the probability

$$f_{MAP} = \arg \{ \max p(g|f, h) \} \quad (2.9)$$

The right side of formula (rov) $p(g|f, h)$ is distribution of g for given f and h . Assuming $n = g - h * f$, it is exactly the distribution of noise measurement, while the noise follows zero-mean Gaussian distribution (2.10), probability density function (PDF) can be rewritten

$$p(n) = p(g|f, h) \sim e^{\frac{1}{2\pi} \sum_i (g_i - (h*f)_i)^2}, \quad (2.10)$$

Therefore the maximization problem becomes minimization as follows

$$f_{ML} = \arg \{ \min \|g - f * h\|_2^2 \} \quad (2.11)$$

The above maximization of the likelihood is typically seen as a non-Bayesian method, although it is identical to the MAP solution with uninformative (flat) prior distributions [5].

2.8 Regularization

The linear image restoration problem is recovering the original brightness distribution from blurred and noisy observation. Typically ill-posed inverse problem. Conventional method to stabilize the problem introduce the constraints (regularization term) into designed cost function (E), the reconstruction is then the image minimizing the cost (energy) function [14].

Solving the ill-posed problem by introduction additional information in the form of extra constraints on image overcome the problem of sensitivity to noise degradation. Regularization terms also determine the preservation of image properties as sharpness of edges [29].

Problem of deconvolution can be express by finding the minimization of energy [2] representing the technique of data fitting with the form

$$E(f) = \|Hf - g\|_p^2, \quad (2.12)$$

where $\|\cdot\|_p^2$ denotes the L_p norm of the vector, H degradation operator, u and f represent observed and real image. In case of $p = 2$ the problem reduces to finding the estimate of f satisfying $\hat{u} = \arg \min_u E(f)$ equivalent to least-square fit. Solving the minimization problem by regularization is given by additive terms. Regularization terms conveys additional prior knowledge of the image in the energy function. Prior formulation and rule is highly application dependant. Image statistic studies reveal the image gradient to be a useful feature that can distinguish between edges and smooth regions. Therefore the regularization terms are often functions of Δf while the regularized energy function becomes

$$E(f) = \|Hf - g\|_2^2 + \lambda^n \|\nabla f\|_p^p \quad (2.13)$$

2.8.1 Tikhonov-like regularization

Tikhonov regularization proposed by Russian mathematician Andrey Tikhonov, fixes the issue arising from least square method by adding the constraints in form

$$E(f) = \|Hf - g\|_2^2 + \lambda^n \|\nabla f\|_p^p \quad (2.14)$$

λ is regularization parameter, constant representing non-negative weighting factor to control the importance of the regulation term. While the first part of equation forces the solution to be close to observed data, gradient norm guarantees the smoothness of regions but sharpness of edges.

Weighting factor in mathematical sense multiply each coefficient of image in frequency domain according to the value of corresponding blur degradation operator (PSF) coefficients [29]. Therefore, small values in PSF are introduced as weighting by value close to zero to prevent amplification of noise.

2.8.2 Total variation (TV)

Another class of regularization terms is based on total variation (TV) that was first presented for techniques of image denoising. TV can better preserve sharp edges and object boundaries that are in general features of image to concern about the most. Added constraints are in the form

$$E(f) = \|Hf - g\|_2^2 + \lambda^n \|\nabla f\|. \quad (2.15)$$

3 PROPOSED METHOD

Practical aim of electron microscopy are images with high resolution and low noise level. From the nature of real images, the robust method insensitive to additive noise preserving the edges is required. Most methods require to constrain both image and blur in different ways. However, such constraints are often application-specific and could not be used across multiple fields. Any general approach to solve this double ill-posed problem can not be found. Therefore, it is required to develop specific method to process microscope image effectively.

Therefore regularization methods using the total variation (2.15) will be implemented with model function (3.1) to be minimized. Equation describes discrete form evaluating discrete gradient D for each pixel i for $n \times n$ image size.

$$\min \left\{ \|Hf - g\|_2^2 + \sum_{i=1}^{n^2} \|D_i f\| \right\} \quad (3.1)$$

Simultaneous estimation of variables H and f is a major problem. Solving the optimization problem minimizing both variables simultaneously is not effective. Minimizing problem over the one variable while keeping the other fixed (constant) if analytically possible, is considered to be better approach. Commonly used approach is alternating minimization (AM). AM algorithm with respect to variables p, q then proceeds in two steps:

1. $\min \{f(p, g^n)\}$ over p to get $p = p^{n+1}$
2. $\min \{f(p^{n+1}, g)\}$ over q to get $q = q^{n+1}$

Various numerical methods are developed to solve the (3.1) via AM. Including the classical Euler, Newton or Runge Kutta or recently developed time-marching [45], primal-dual methods [9], lagged diffusivity fixed point schemes [60], and half-quadratic regularization ([13], [64]). Reviews on different approaches revealed the pros and cons of its performance. Methods excellent in image deblurring suffers the bad contrast preservation. Time marching method equivalent to the steepest descent is easy to implement but with poor convergence rate. Others yields to highly accurate solutions able to become golden standard for solution qualities but requires excessive memory.

Alternating minimization algorithm for total variation image reconstruction with half-quadratic regularization in case of image deblurring has been proposed in [64]. Potential fast and robust algorithm is described with strong convergence. Algorithm for deblurring is defined for given PSF form, therefore the iterative procedure based on alternating minimization coupled with single channel blind deconvolution based on [56] could fulfil desired conditions. Both methods show good functionality on

model and photography images. **Since the blind deconvolution techniques in general are image-specific methods neither of this assumption do not guarantee the functionality in case of microscope images.**

3.1 Solution structure

Defining the working image deconvolution algorithm consists of model images testing and processing of sample images acquired by TEM. Each subsolution has it specifics that will be introduced in following sections.

General structure :

1. Model images
 - Degradation of model image
 - Blind deconvolution algorithm
2. Real images
 - Definition of initial PSF guess
 - Blind deconvolution algorithm

3.1.1 Simulation of degradation model (MATLAB© implementation)

Blur origin degradation of image is modelled by Gaussian function. Although the function is one-parameter-dependent, with parameter of standard deviation (σ) the case of using the Gaussian as the PSF to model the process and verify the function of proposed algorithm is the property of reducibility:

$$G_{\sigma} = G_{\sigma_1} \otimes G_{\sigma_2} = \sigma_1^2 + \sigma_2^2 \quad (3.2)$$

Even without noise, the problem is well known as the ill-posed problem to reconstruct the reducible function. Assuming ill-posed problem means the solution either does not exist, is not unique nor the solution's behaviour does not change continuously with the inputs, therefore the Gaussian simulated PSF's are the most common models for reconstruction. Blurr of the image is obtaine by convolution with Gaussian function dependant on two parameters, area size and standart deviation. General Gaussian process is of the form:

$$f(x) = ae^{-\frac{(x-b)^2}{2c^2}} \quad (3.3)$$

While a donates the height of symmetric "bell" curve, b specifies the position of center of the peak and c controls the width. For normalized Gaussian curve

with mean value and expected variance $a = 1/(\delta\sqrt{2\pi})$, $b = \mu$ and $c = \sigma$. For our purpose, Gaussian matrix convoled with image works as the smoothing filter, removing higher frequencies from the original image. The bigger kernel the more smoothening is done by the filter 3.2.

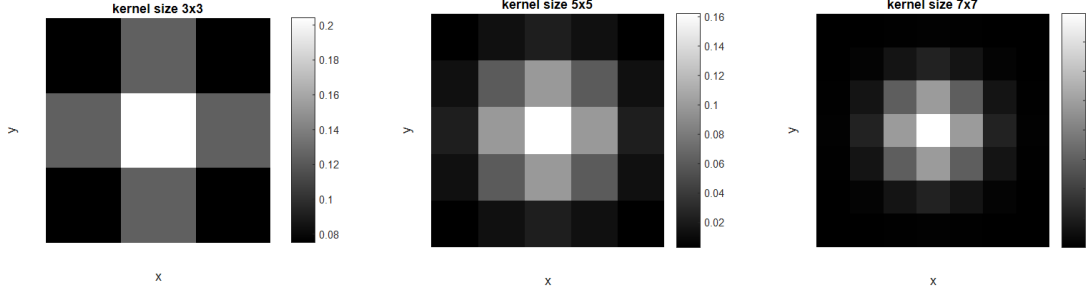


Fig. 3.1: Simulation of degradation kernel size

According to the property of Gaussian matrix to be summed up to unit ($\Sigma = 1$) to preserve image energy, the size of the kernel gives the property of discretization of Gaussian distribution in each direction. For practical size of kernel 3x3, 5x5 or 7x7 is given by quantization into 3, 5 or 7 discrete values of blur matrix in x or y direction (3.1).

The blurring factor (PSF) is convoled with the original (model) image in frequency domain, while Fourier transform of blurring kernel represents frequency response of Gaussians smoothing filter modelled by this mask and its product in frequency domain the spectrum of output image formed by convolution. Therefore *ifft2* function is used to transform back to time domain.

Blurred image is afterwards donated by additive Gaussian and applied Poisson noise.

The level of noise degradation of additive noise is given by formula for evaluation of noise standard deviation (variance) from given value of standard deviation (variance) of image and wanted SNR.

$$SNR = 10 \log_{10} \left(\frac{\sigma_s^2}{\sigma_n^2} \right) \quad (3.4)$$

$$\sigma_n^2 = \frac{\sigma_s^2}{10^{\frac{SNR}{10}}} \quad (3.5)$$

For purpose of evaluation the degradation level (combination of noise level, blurring function size/standard deviation) and effectivity of applied method of blind deconvolution to reconstruct original image from the degraded variant, mean square

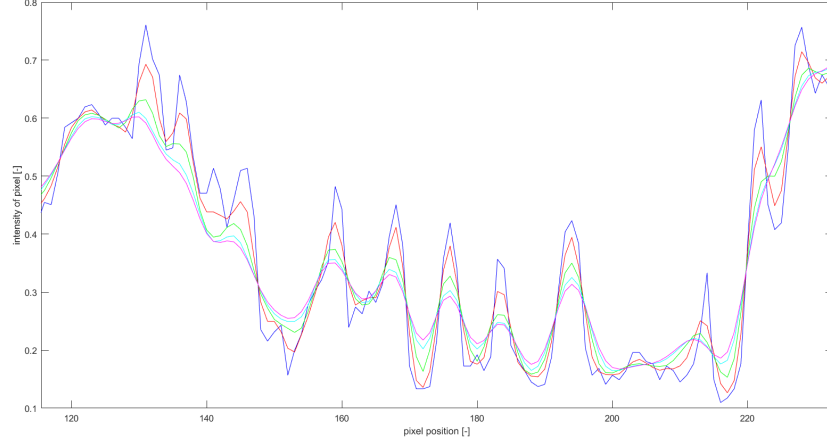


Fig. 3.2: Comparison of **original** and blurred image amplitude;
Gaussian kernel **3x3**,**5x5**,**7x7**,**9x9** ($\mu = 0, \sigma = 2$)

error (MSE) is evaluated as factor of success of algorithm. Expecting the MSE decreases with increasing efficiency of algorithm with comparable degradation of initial images. From the definition, it is an average of the squares of the difference between the actual observations and those predicted. The squaring of the errors tends to heavily weight statistical outliers, affecting the accuracy of the results.

3.1.2 Definiton of initial PSF guess

Initial guess of blurring function allows the algorithm to proceed more accurate results. Optimizing from zero position (Dirack distribution) comparing to estimated initial guess should clearly demonstrate the importance of accurate approximation of PSF. Even the partial information about the system can provide visible improvement. In this case the blind deconvolution framework is necessary, the obtained guess defines proximity of desired minimum but needs to be optimized.

Mentioned before, blur is originated in camera part of microscope or image formation parts of system in general (lenses,apertures,...). Lenses caused distortion for given aberration coefficients can be calculated and simulated but hardly converted into PSF form moreover in case of multiple combinations of lenses (stigmator,deflectors,objective lens...). Therefore the blur originated in camera part offers better options to be estimated.

Indirect electron detection is performed by using the camera coupled to scintillator screen, converting electron signal to photons. Characterization of electron detectors can be performed qualitatively by observation of Thone rings [58] or more quantitatively by detective quantum efficiency (DQE) estimation. Estimation of

DQE is subject to modulation transfer function (MTF) definition, that measures the response of an imaging system in the frequency domain. While the PSF measure the system response in image domain, defining the Dirack distribution transformation by system for each point, PSF can be easily obtained by MTF inverse Fourier transform

Determination of MTF approaches are based on single electron events observation [15] if detectors are sufficiently sensitive. Holographic fringes from crystallic structures providing the periodic pattern to be measured [33] can be evaluated if experimental complexity is not limiting or knife-edge method can be used to transform line spread function (LSF) into MTF[49]. Disadvantage of the method is the requirement of sharp straight-edged object to be placed into microscope.

Since all of the mentioned methods has limitations preventing its implementation, different approach to PSF definition has been performed using silhouette of a beam stop [4] . The silhouette method is based on estimating the input image before detection by converting the detected image into a black-and-white by thresholding. In order to simulate the effects of aliasing, the output image is up-sampled four times using bilinear interpolation and then thresholded to generate the black-and-white beam stop image [47]. A blurring kernel is applied to this image to simulate the MTF before pixel integration, and pixel integration is then performed by replacing each group of 4 x 4 by average value. Procedure expressed by equation:

$$Image_{sim}(r) = DOWN(FT^{-1}[kernel.FT(TH[UP(Image_{out}(r))])]) \quad (3.6)$$

Therefore the simulated image is obtained and blurring kernel (PSF) optimized to minimize squared difference between simulated and detected image 3.6. Blurring kernel is of the sum of five Gaussian functions form 3.7, where the sigma and each function weight stands as optimization parameter 3.8.

$$\Delta = (Image_{out} - Image_{sim})^2 \quad (3.7)$$

$$kernel(w) = \sum_{i=1}^5 W_i . e^{-\frac{w^2}{2\sigma_i^2}} \quad (3.8)$$

Estimated kernel profile (Fig. 3.5) simulates the PSF function obtained,ongoing transformation into 2D with given matrix size (7x7,9x9 etc.) afterwards.

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Fig. 3.3: Detected ups. image



Fig. 3.4: Thresholded ups. image

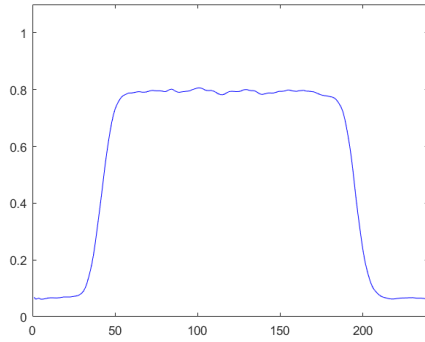


Fig. 3.5: Detected image profile

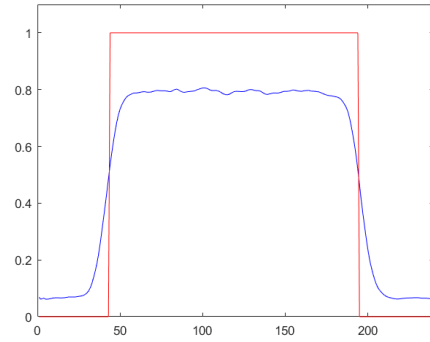


Fig. 3.6: Thresholded image profile

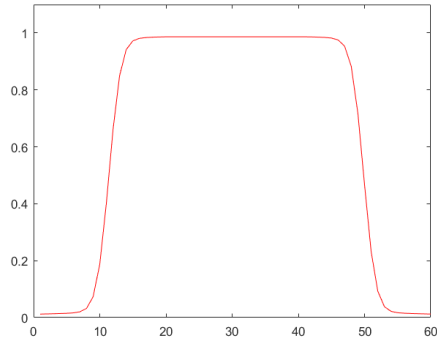


Fig. 3.7: Simulated image profile

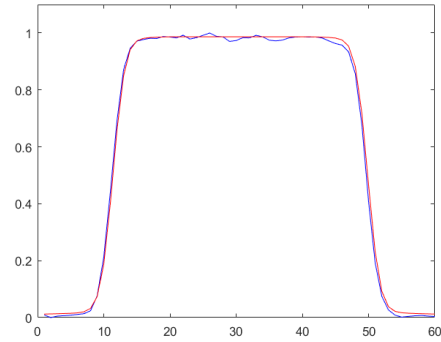


Fig. 3.8: Detected image profile

3.1.3 Blind deconvolution algorithm

A prominent weakness of the regularization approach is the difficulty in recovering the discontinuities. The sharp discontinuities in images are edges and object boundaries, often the most important features to keep. Assuming the prior smoothness constraints (given by regularization term-Tichonov, Total variation etc.) , the important constraint attributes are concavity and its finite asymptotic behaviour. Shortly, the existing maximum of the condition and attribute of convergency. Such lin-

ear constraints permit the recovery of discontinuities without introducing auxiliary variables [14].

Regularization approach of total variation, eventhough more accurate, suffers by introducing non-linearity and non-differentiability in the problem.

Using the TV regularizer with respect to the image writes as :

$$\min \frac{\gamma}{2} \|Hu - g\|^2 + \Phi(D_x u, D_y u) \quad (3.9)$$

So called Rudin-Osher-Fatemi [45] functional with non-differentiable term represented by TV $\|\nabla u\|$ ($\Phi(D_x u, D_y u)$) has proved to be very difficult to minimize despite the simple form.

Penalty function/continuation method represents the algorithmic framework not requiring modification to non-differentiable term $\Phi(D_x u, D_y u)$. The problem of non-linearity of TV (∇u rewritten by $\|\nabla u\|$) has solution by introducing auxiliary variable and converting the non-differentiable form into an unconstrained problem simple to solve as follows:

1. gradient ∇u is replaced by a new variable w
2. the discrepancy between w and ∇u is penalised
by quadratic term $\frac{\beta}{2} \sum_i \|w_i - D_i u\|^2$
3. update of u, w with increasing penalty parameter β until "convergence"

Bregman iteration technique represent a better method solving the variety of constrained optimization without need of continuation scheme with fast convergence attribute [17]. Even better formulation for regularized problems represents split Bregman method. The decoupling of l_1 (TV) and l_2 (quadratic norm) is the key of the method. Then the problem is reduced to a sequence of unconstrained optimization problems and Bregman updates[17].

Minimizing the given formula

Objective function to be minimized becomes half-quadratic term:

$$\min_{w,u} \sum_i \|w_i\|_2 + \frac{\beta}{2} \sum_i \|w_i - D_i u\|_2^2 + \frac{\mu}{2} \|Ku - f\|_2^2 \quad (3.10)$$

Half-quadratic term, quadratic in u , separable in w . Minimizing the function with respect to one of the variables has formula with low computational complexity and high numerical stability [64]. Since the split of components l_1 (TV) and l_2 (quadratic norm), minimization can be performed efficiently. Time consumption of the method is highly dependant on how fast the subproblems are solved.

TV model approximation and resulting alternating minimization algorithm were first proposed without a convergence analysis [63]. Similar split method using Bregman iterations has been proposed recently. Augmented Lagrangian method (ALM)

equivalent to the split Bregman iterative method applies therefore to solve the sub-problem for our purpose [56].

Optimization process

According to [56] the alternating minimization consists of minimization with respect to image (u-step) and respect to blur(h-step). For our purpose, the blur regularizer is not used (the correctness of the assumption has been consulted with Prof. RNDr. Miloslav Druckmüller, CSc., Faculty of Mechanical Engineering, Institute of Mathematics).PSF matrix is updated in each step with respect to image minimization. The property of positivity and unit energy is preserved while algorithm.

Overall steps shows the diagram Fig. 3.9. Image regularization and minimization step (u-step) given by formula 3.9 applying the variable splitting becomes :

$$\min_{u, v_x, v_y} \frac{\gamma}{2} \|Hu - g\|^2 + \Phi(v_x, v_y) \quad (3.11)$$

$$L(u, v_x, v_y) = \frac{\gamma}{2} \|Hu - g\|^2 + \phi(v_x, v_y) + \frac{\alpha}{2} \|D_x u - v_x - a_x\|^2 + \frac{\alpha}{2} \|D_y u - v_y - a_y\|^2 \quad (3.12)$$

while the ALM (Split Bregman Iterations equivalent) solves this 3.11 constrained problem considering functional 3.12 solved by iterative algorithm explained on 3.10(or more detail explanation found in [56])

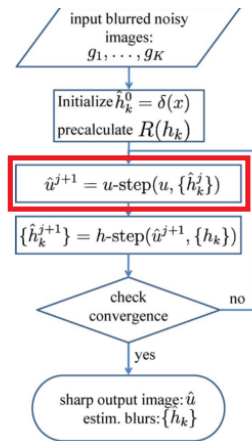


Fig. 3.9: Flowchart of AM algorithm

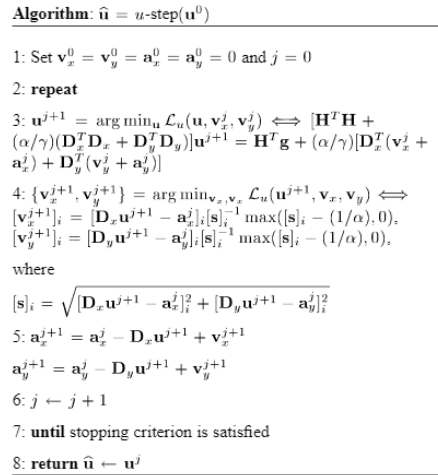


Fig. 3.10: Steps of u optimization

Constants Crucial part of the algorithm represents the constants α and γ settings. According to [56] ideal values corresponds to estimated noise degradation

level ($SNR = 50dB$ then $\gamma = 10^5$, $SNR = 20 dB$ then $\gamma = 10^2$, etc. [56]).

Initial PSF Input to the algorithm is the blurred image (real or model) with initial form of PSF. In case of blind deconvolution for model images the Dirack distribution is created. For purpose of proper approximation odd and even-sized matrices are treated differently.

Iteration steps According to [56] the convergence of the algorithm has been successful in less then 10 steps per step-part (u-step,h-step).The fixed value of 10 has been implemented.

Mentioned algorithm above has been implemented with fixed iteration steps (10), the convergence check is not performed. Equivalent solution, the quality assessment of output images according to used constants combinations has been proposed.

4 EVALUATION OF RESULTS

As a part of semestral thesis, the evaluation of degradation and consequently effectiveness of built in Matlab blind deconvolution algorithm have been tested on model images. Given data from microscope are degraded by degradation model based on Gaussian blur origin process and added (Gaussian) noise.

Theoretically efficiency of blind deconvolution technique in image degradation reconstruction will be evaluated by the results of the algorithm on known images and the estimated mean square root works as simple tool for calculation of blind deconvolution ability to reach the initial original image. This approach works for given model data, but in case of the real images we do not have any information except blurred and noisy image and in ideal case also the initial guess of PSF of the system, that is dependent on used microscope system and works as its “fingerprint”.

But it is still needed evaluate somehow the results in way of quality of reconstruction therefore analogy of modelled image degradation parameters with real acquisition parameters should be explained. For purpose of this study the focus, intensity, magnification, exposure time and binning will be analysed and its analogy with model properties will be explained.

Focus poorly explained as the state of the best sharpness achievable. In words of degradation model parameters focus represents the blur with circular shape of PSF. Focus as the distance in mm from the chosen focal plane upon z-axis is can be set by user of microscope. Moves up to axis by opposite directions (positive and negative direction from 0) represent overfocus and underfocus state. Practically, the exciting current of the strongest main objective lens current adjusts while focusing. Needs to be mentioned with its arising parasitic aberrations (chromatic aberration).

Intensity controlled by weak condenser (C_2) lens together with set spot size represent illuminated area to be imaged. Intensity in terms of degradation model represents the amount of noise incorporated into image. The lower intensity the higher possibility of photon (Poisson) noise to occur. Over-limit high intensity exceeding the well pixel capacity can results in blooming artefact and image blur. For practical purposes, can be intensity limited by used sample. Life science samples are highly sensitive to beam intensity compare to material science samples.

Magnification represents how original point size will be projected on the screen. Magnification is not the microscope optics property only. Camera light optics itself can demagnify formatted image from microscope column to be imaged on sensor. Magnification determines aberration error to be transferred into output image (??). With lower magnification, some errors can be suppressed by the nature of digitalization. Practically exciting currents differs across numerous magnification modes with arising occurrence of aberration.

Exposure time limits achieved SNR of image. Amount of collected signal arises with higher time that lowers the possibility of (photon) noise occurrence. Limitation of duration results from required time resolution and minimization of thermal drift.

Hardware version of cumulative techniques is represented by **Binning**. Determines achieved SNR while higher binning denotes higher SNR but decreases theoretical resolution of system by factor of “superpixel” size quadrate.

Specific techniques of electron microscopy techniques are used among all different technical fields. Therefore different properties of output image are essential for particular application. Considering multiple user purposes used there is no general approach how to objectively compare numerous images.

The efficiency of proposed algorithm is tested on model data with objective evaluation. The similarity criterion (MSE) or noise degradation (SNR) ability assuming the input image noiseless has been proposed and tested in semestral thesis with built-in Matlab function (*deconvblind* 5.1. Evaluation given by formulas:

$$MSE = \frac{1}{n} \sum_{i=1}^n (\hat{Y} - Y_i)^2, \quad (4.1)$$

$$SNR = \frac{P_{signal}}{P_{noise}} \text{ or} \quad (4.2)$$

$$SNR_{DB} = 10 \log_{10} \frac{P_{signal}}{P_{noise}} \text{ where} \quad (4.3)$$

$$P = \frac{1}{N} \sum_{n=0}^{N-1} x_n^2 \quad (4.4)$$

MSE evaluation has revealed the insensitivity to image properties according to image contrast, numerical better values of MSE do not correspond to image quality. MSE represents the simplest implementation of the error sensitivity concept which objectively quantifies the strength of the error signal. Therefore the proper result can not be estimated upon this criterion. New evaluation criterion for diploma thesis is proposed and estimated. Similarity structural index (SSIM) is based on three terms corresponding to image properties. The luminance, contrast and structural information quantified by following equations:

$$SSIM(x, y) = [l(x, y)]^\alpha \cdot [c(x, y)]^\beta \cdot [s(x, y)]^\gamma \quad (4.5)$$

$$l(x, y) = \frac{2\mu_x\mu_y + C_1}{\mu_x^2 + \mu_y^2 + C_2} \quad (4.6)$$

$$c(x, y) = \frac{2\sigma_x\sigma_y + C_2}{\sigma_x^2 + \sigma_y^2 + C_2} \quad (4.7)$$

$$s(x, y) = \frac{\sigma_{xy} + C_3}{\sigma_x \sigma_y + C_3} \quad (4.8)$$

where $\mu_x, \mu_y, \sigma_x, \sigma_y$ and σ_{xy} are local means, standard deviations and cross-covariance for input images x and y computed with circular-symmetric Gaussian weighting function to prevent artefacts of windowing [62]. Constants C_1, C_2, C_3 compensates zero division. $\alpha > 0$, $\beta > 0$ and $\gamma > 0$ are adjustable parameters determining the importance of each from components.

Obtained real reconstructed images will undergo subjective quantification of results quality considering artefacts (ringing artefact, deconvolution artefact), maintaining contrast, sharpness of edges and suppression (amplification) of noticeable noise.

4.1 Evaluation of results from model data reconstruction

Structural similarity index (SSIM)

Blind deconvolution method and algorithm proposed and tested in the thesis is strongly dependant upon initial set up of constants. According to [56] ideal values corresponds to estimated noise degradation level ($SNR = 50dB$ then $\gamma = 10^5$, $SNR = 20 dB$ then $\gamma = 10^2$, etc. [56]). Since the normalizations of images, noise level estimation and edge-taping can differ while implementation, the vector of various constants combinations has been tested. Calculation of chosen quality metrics determines the restoration accomplishment.

The simplest and most widely used full-reference quality metric is the mean squared error (MSE), computed by averaging the squared intensity differences of distorted and reference image pixels, along with the related quantity of peak signal-to-noise ratio (PSNR), useful if images having different dynamic ranges are being compared. These metrics contain the same information, are simple to calculate, have clear physical meanings, and are mathematically convenient in the context of optimization. But they are not very well matched to perceived visual quality, especially in image processing [62]. 4.1 4.2 reveals the failure of MSE approach. Images corresponding to same MSE value affected by different image distortions exhibit strongly different visual quality. Noise degradation becomes quantified by same value as structure change (4.2) or blur degradation of image, therefore different metric reference is needed for evaluation by number not the visual sense.

Image quality calculation suffers the problem of complexity. Definition of quality is not clear, for example some distortions can be visible but not objectionable like

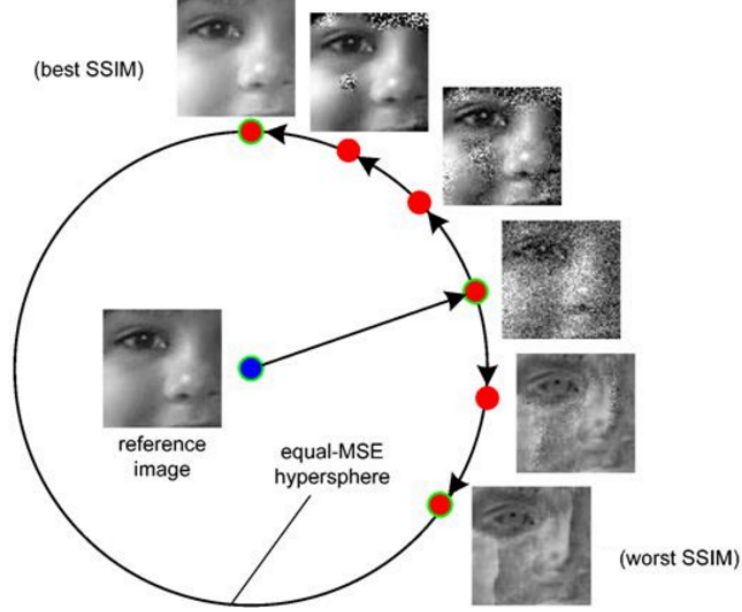


Fig. 4.1: MSE value failure [61]

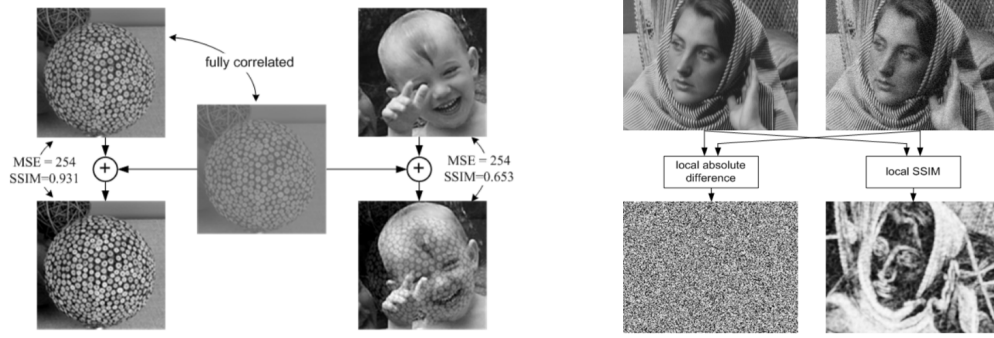


Fig. 4.2: Demonstration of MSE errors [61]

scaling the intensities by global factor, that changes classical MSE quality metric but has no quality importance. PSNR solves the problem of scaling but others remain.

Workflow of SSIM estimation is based on three components and independent comparisons of luminance, contrast and structure. Luminance of each signal is compared first as a function of image μ . After mean intensity removal, an unbiased contrast comparison is made upon image σ dependency. Final step normalizes the image (division by its own standard deviation) therefore the structural comparison is conducted to unit standard deviation images. All components are combined yielding an general similarity measure (eq. 4.5).

Relative importance of components reveals the characteristic features of the compared images (4.4 , 4.5 , 4.7 , 4.9). The brighter the higher similarity according to preferable component. Demonstration examples reveals the SSIM characteristics,

blur degradation (4.4 , 4.5) almost does not affect the intensity (SSIM for $\alpha = 1$) but has the biggest influence on the edges of image, the higher blur deviation σ the higher edges degradation. Scaling the image values by given factor (4.7) strongly affects the luminance component (mean intensity value) while structural information remains the same (4.7 SSIM=1). Noise degradation (4.9) shows the effect on constant areas compare to edges. Constant plane suffers the noise degradation more, while the local characteristics around edges are not that strongly affected by noise(brighter pixels) since local maps are evaluated in 8-neighbourhood by mask with Gaussian weighting function to prevent blocking artefact. Image degraded by gaussian white noise (SNR=26 dB)

Assuming one of images to have perfect quality, the global SSIM can serve as quantitative measurement of reconstruction. SSIM implementation with equal relative importance of each (luminance, contrast, structure) component set by $\alpha, \beta, \gamma = 1$ represents the quality metric available for comparison.



Fig. 4.3: Demonstration of SSIM meaning, global value of 0.8157 with local map (right); Original model image (left) blurred by PSF with $\sigma = 2$, size=15x15 (middle); MSE=19.5051



Fig. 4.4: Demonstration of SSIM components meaning;model image blurred by PSF with $\sigma = 2$,size=15x15 with global SSIM = 0.8157, MSE=19.5051; relative importance of each component set up to 1 for α (left;SSIM=0.998) β (middle; SSIM=0.9031) γ (right;SSIM=0.8969)



Fig. 4.5: Demonstration of SSIM components meaning;model image blurred by PSF with $\sigma = 4$,size=15x15 with global SSIM = 0.7214,MSE=27.8788; relative importance of each component set up to 1 for α (left;SSIM=0.9956) β (middle; SSIM=0.8441) γ (right;SSIM=0.8386)



Fig. 4.6: Demonstration of SSIM meaning, global value of 0.6953 with local map (right);model image (left) scaled by factor 2 (right); MSE=266.9503

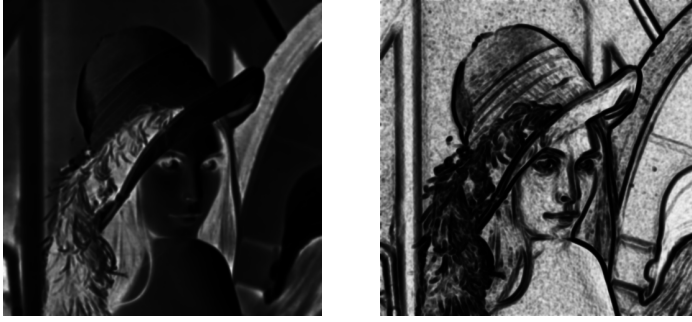


Fig. 4.7: Demonstration of SSIM components meaning;model image intensity scaled by factore of 2 with global SSIM = 0.6953,MSE=266.9503; relative importance of each component set up to 1 for α (left;SSIM=0.8000) β (middle; SSIM=0.8691) γ (right;SSIM=1.000)



Fig. 4.8: Demonstration of SSIM meaning, global value of 0.5115 with local map (right);model image (left) degraded by noise (middle; SNR=26 dB; $\sigma_n = 0.0025^2$) ; MSE=25.5



Fig. 4.9: Demonstration of SSIM components meaning;model image degraded by noise (SNR=26 dB; $\sigma_n = 0.0025^2$) with global SSIM = 0.5115,MSE=25.5; relative importance of each component set up to 1 for α (left;SSIM=0.9997) β (middle; SSIM=0.7485) γ (right;SSIM=0.6607)

4.2 Evaluation of results from real data reconstruction

Subjectively-objective evaluation of image properties after reconstruction Since the SSIM index is referred as the full-reference term, the whole image (reference not degraded) for quantitative comparison is needed. While real image processing, the reference is not available. In general, for applications in which images are ultimately to be viewed by human beings, the “correct” method of quantifying visual image quality is through subjective evaluation [62].

Although the SSIM can not be evaluated, similar philosophy is proposed. Scoring the image by three different values corresponding to image properties. Maintenance of image contrast, artefacts occurrence and sharpness of edges/suppression of noise will be evaluated by reference scale. Constants setting with the highest achieved score can be considered as the best result.

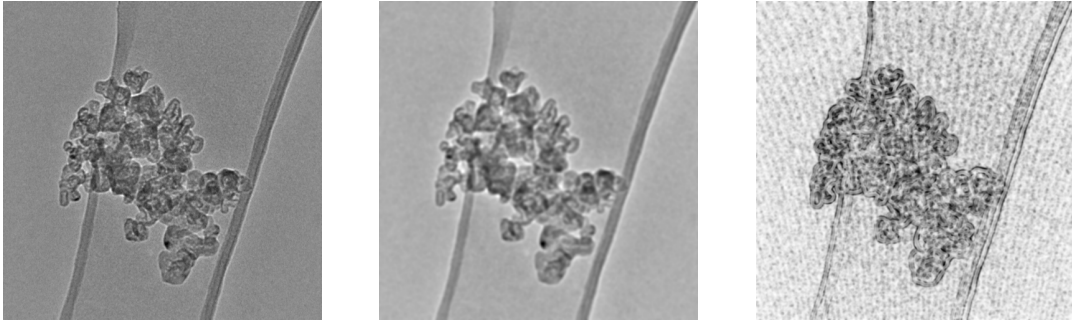


Fig. 4.10: Demonstration of SSIM meaning, global value of 0.8693 with local map (right); Original real image blurred by PSF with $\sigma = 2$, size = 15x15 (middle)

Contrast preservation scale (Fig. 4.11)

Contrast in the meaning of signal processing represents the difference in the values of luminance (grey scale level). According to the particular contrast definition, the image can be represented locally or by overall value. One value representing the image contrast becomes useful in cases of periodic patterns or targets with uniform background. Local expression works better for complex images. Human contrast sensitivity is the function of spatial frequency [40], therefore the achieved spatial resolution (sharpness) of an image goes together and affects the visual perception of image contrast. *The contrast preservation in comparison with initial image is scored by representing one of the restoration quality attribute.*

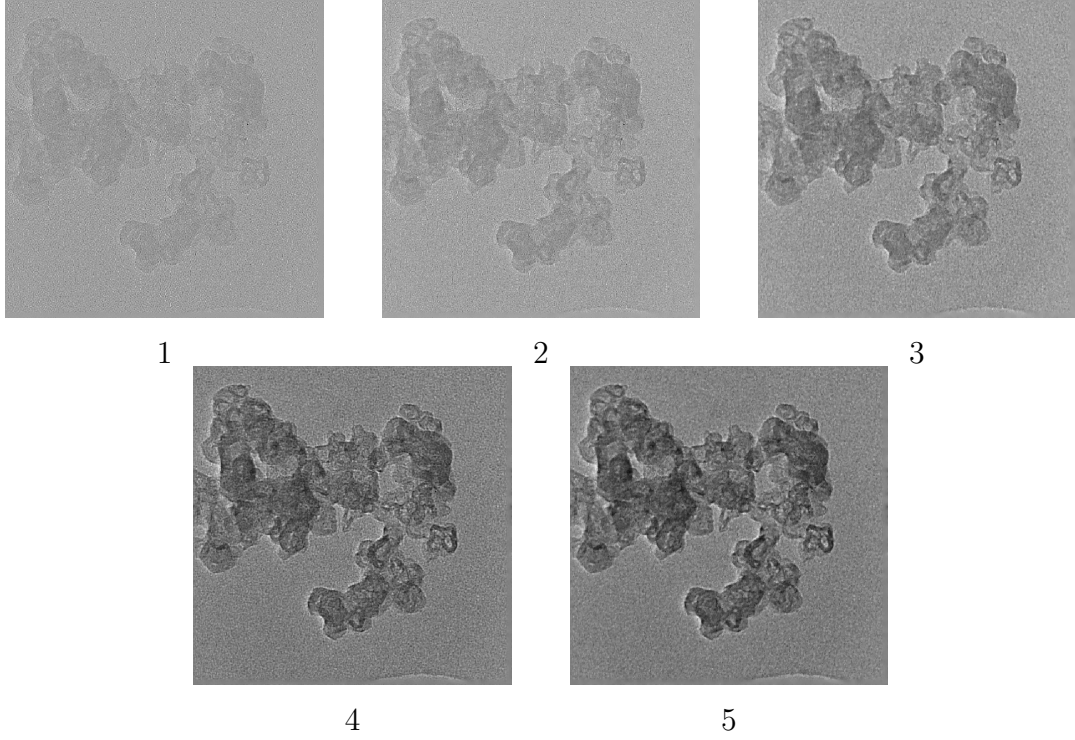


Fig. 4.11: Contrast scale

Sharpness of edges/degradation suppression scale (Fig. 4.12)

Sharpness, the amount of higher spatial frequencies in the image, determines amount of detail an imaging system can produce. The better enhancement of high frequency, the smaller detail becomes visible. Simple estimation while subjective evaluation of results *follows the area boundaries in the image assuming how sharp/blurred the boundary between zones of different contrast is*. Noise suppression associated with image restoration suffers the opposite effect. Since the major energy of an image concentrates on low frequencies, noise removal effects mainly detail-responsible part of spectrum. Sensitivity of the deconvolution to noise is crucial for restoration quality. Preserving edges while blurring the noisy areas is the key attribute of regularization approach with total variation.

Image artefacts scale (Fig. 4.13)

A challenging problem in image restoration is the *presence of wavelike artefacts called ringing that appear near strong edges/high frequencies*. There is more than one origin of this periodical pattern while process of restoration. Assuming the Image blurring modelled by convolution of original latent image with blurring operator. Field of view of convolution mask hence to observe the image above its boundaries. Missing information coupled with FFT propagate the effect of boundary pixels throughout entire image. The problem known as boundary value problem

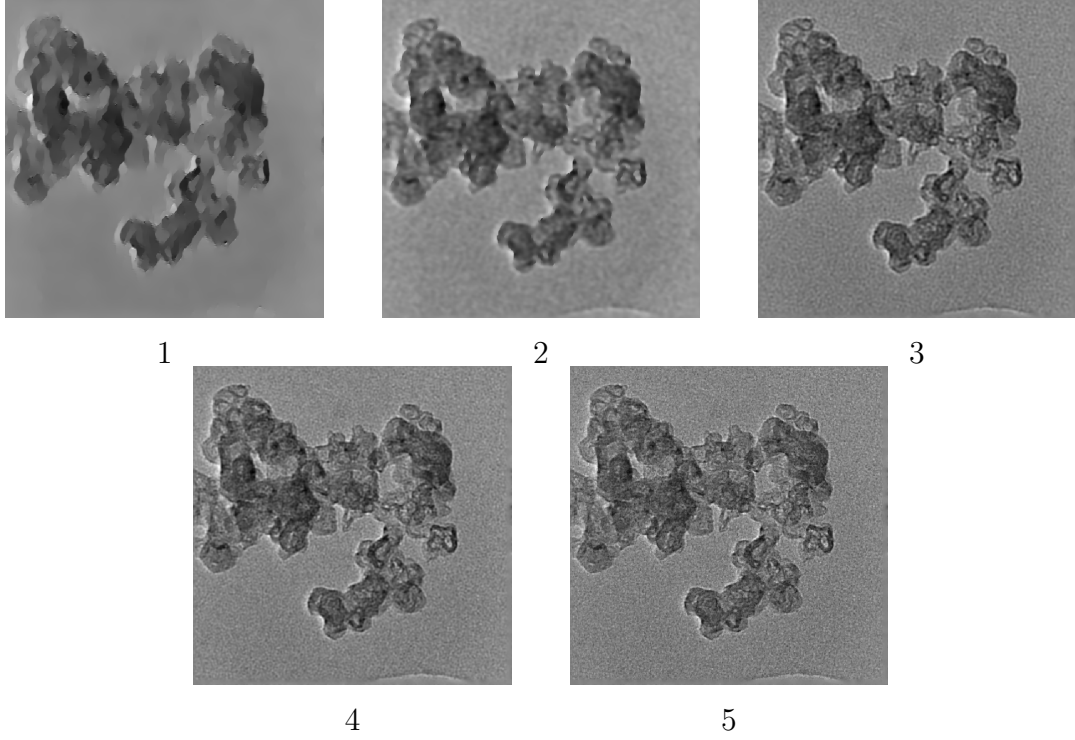


Fig. 4.12: Sharpeness scale

causing the ringing artefact.

Two possible ways of solving the problem exists, depending whatever the spatial or Fourier domain restoration filter is used. The missing boundary pixels can be replaced or extrapolated from image pixels upon Boundary Conditions ([8],[30]) removing discontinuities by remain of continuity of image or image gradient. Assuming periodic boundary conditions in spatial domain DFT can be performed, under the Neumann boundary condition two-dimensional Discrete Cosine Transform alternatively. Fourier domain filters adopt the DFT with precondition of data periodicity, otherwise the high-frequency drop-off on the image edge creates the effect of boundary related ringing. Under the periodicity conditions, missing pixels at the left-side boundary of image will be taken from right-side and vica versa [30]. Simple implementation of problem solution performs the Matlab built-in function *edgetaper*. Removal of high-frequency drop-off is performed by blurring the entire image and replacing the centre pixels by original image data. The procedure is performed in the algorithm part responsible for deconvolution as much as in SSIM index calculation to not to affect the resulting value of similarity.

Mainly neglecton of noise in models of deconvolution techniques is responsible for another ringing artefact origin. Since the deconvolution is the inverse problem, inevitable presence of zero values is problematic. The solution depends on chosen restoration filter technique (review [39]). Incorporation of noise models, extra filters

or increase of weight of regularizer term can suppress the effect. **The particular combination of constants α and γ leads to various image restoration quality of proposed algorithm.**

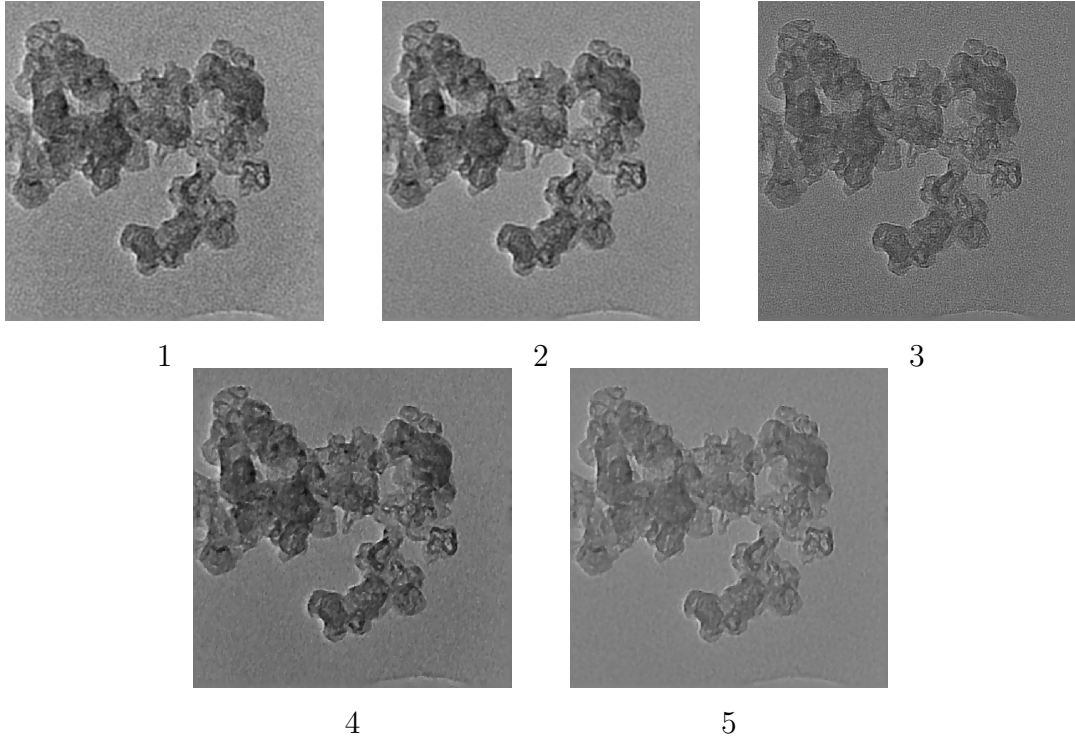


Fig. 4.13: Artefacts scale

5 RESULTS

5.1 Results of model data

Functional test of MATLAB built-in function *deconvblind* has been part of the previous semestral thesis therefore bigger amount of simulated images proceeded the restoration process. Model images are constructed by the implemented degradation model (explanation in section 3.1.1). Overall results of quality assesment of model data can be found in an attachment A.9.

5.1.1 Maximum Likelihood

Simulation of results have been done with 16 000 combinations settings of variables representing number of iterations of algorithm (range 1-20), standard deviation of Gaussian smoothing filter (range 1-5), achieved SNR (range 1-40) dependant on variance of input image and size of smoothing filter kernel (3,5,7,9) with evaluation similarity indexes. Explanation of similarity graph axes is done on example of relative MSE obtained values (figures 5.2 , 5.3).

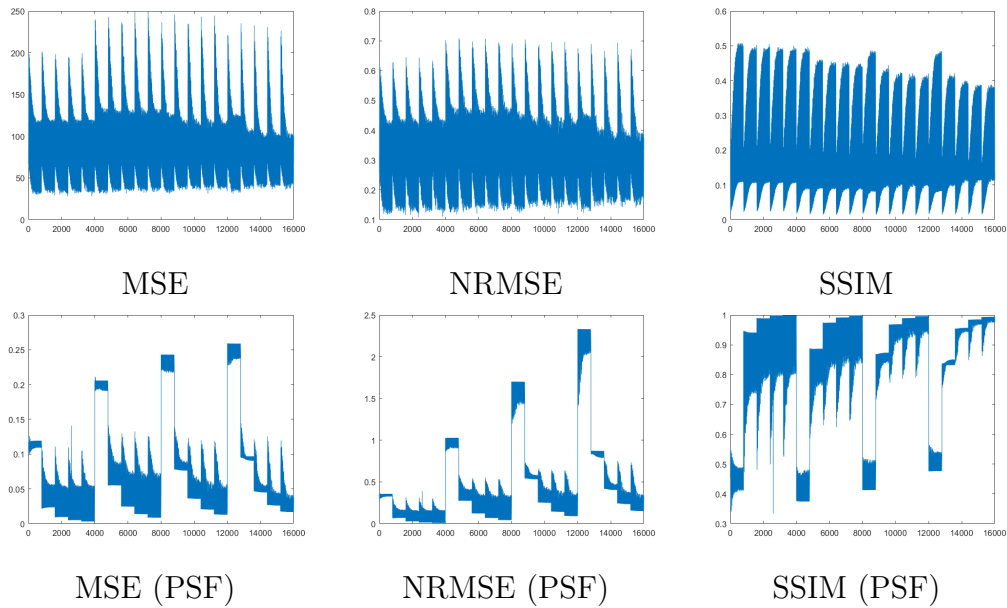


Fig. 5.1: Different similarity evaluations

Results of simulation reveals the influence of each parameter on reconstruction success coupled with specific similarity index (Fig. 5.1). MSE absolute values correspond to the exact values of reconstructed images and its differences. The higher the MSE value is the smaller the similarity of images. Normalised root mean

square (NRMSE) of similarity reveals the same dependency. Relative values of MSE (Fig. 5.2) better represents image quality, converting values into range from 0 to 1. The meaning of SSIM values is the opposite, the higher the similarity, the higher the value. Upon the x axis meaning (Fig. 5.2), best results correspond to the smallest PSF kernel sizes and the highest SNR values, so does the relative MSE.

Considering SNR the parameter of the biggest effect, noise corruption will be discussed with obvious outcomes, the lower noise degradation the higher quality of reconstruction. Closer look to iterative approach (Fig. 5.4) reveals different behaviour of similarity of image and PSF to its original form. Ideal number of iteration with respect to achieved quality differs and revealed best settings corresponds to MATLAB default value of iterations (10).

Interesting fact arises from values dependant upon σ of blurring function. With increasing size of the blur kernel (7,9) the significantly different results comes from smaller σ (sd=1,2). Effect on similarity indexes also differs, smaller σ with bigger kernel size represents higher SSIM between images (higher similarity) but higher value of relative MSE (lower similarity). Same comparison for PSF matrices follows same value trend, for $\sigma = 1$ are significantly different. However, the similarity indexes values interpretation is not same like in case of image. Relative MSE and SSIM evaluated for PSF obtained by the algorithm reveals in both cases less similarity in favour of small σ (lower SSIM, higher MSE). Demonstration of the difference on Fig. 5.5 , 5.6 and 5.7 . Subjective visual inspection better confirms the meaning of lower similarity although the structure of image is proper.

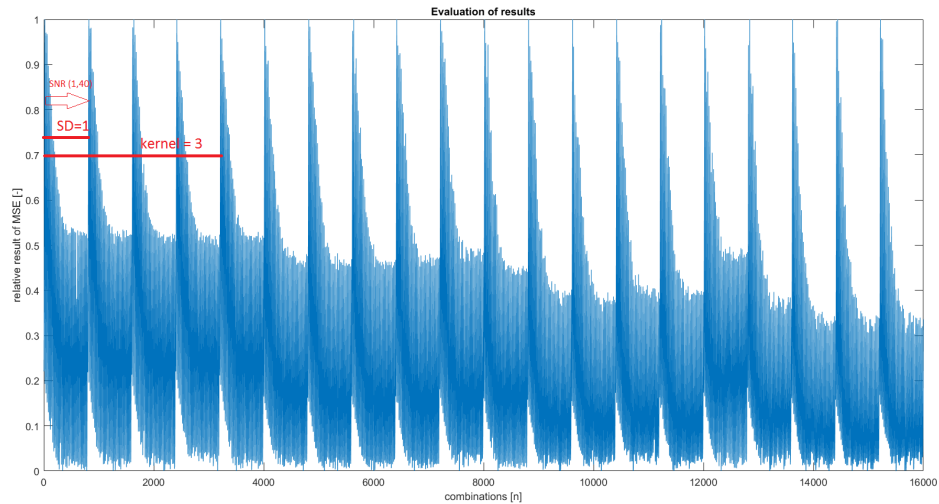


Fig. 5.2: Evaluation of similarity between original and reconstructed image for different combination of parameters settings (relative MSE example)

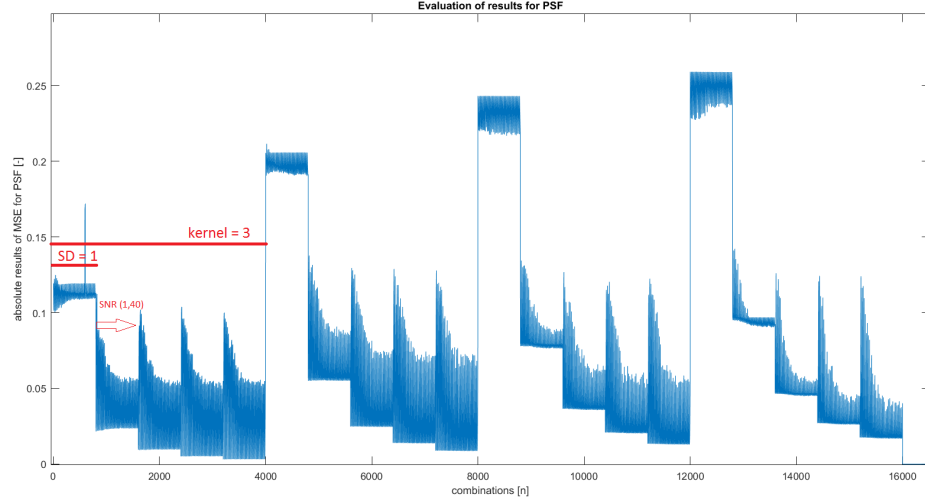


Fig. 5.3: Evaluation of similarity between original and reconstructed PSF for different combination of parameters settings (relative MSE example)

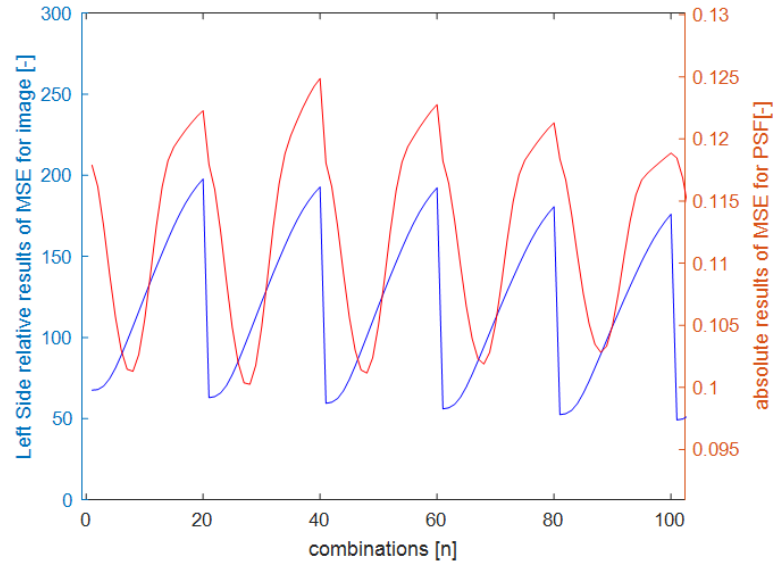


Fig. 5.4: Comparison of MSE values for image and PSF for given parameters combination

Gaussian filter kernel size in combination with standard deviation of this process dramatically affects reconstruction quality. The higher kernel size, the better sensitivity of reconstruction to noise. Ability to preserve real intensity and contrast of image is presented on images below (5.5 , 5.6 , 5.7.) while degradation is determined by Gaussian filter differing in kernel size with no additional noise.

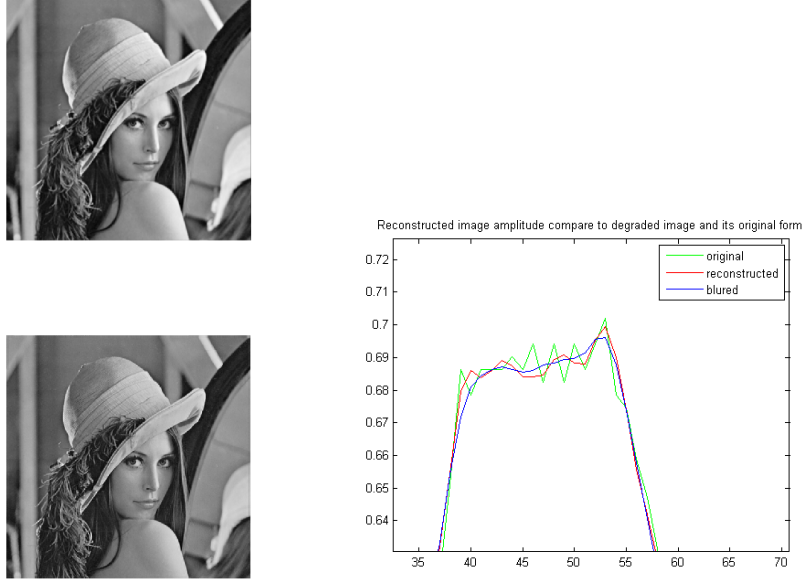


Fig. 5.5: Reconstructed image amplitude for blur kernel $3 \times 3, \sigma = 1$

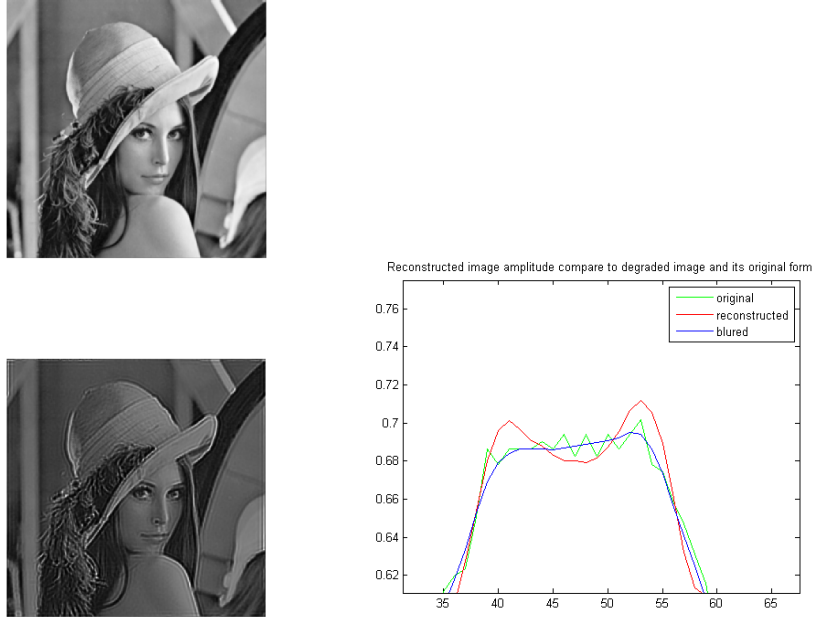


Fig. 5.6: Reconstructed image amplitude for blur kernel $7 \times 7, \sigma = 1$

Comparison illustrates the difference in objective and subjective evaluation. Images also reveal contrast preserving inability of reconstruction for particular blur functions ($\sigma = 1, 2$). Magnified intensity profiles of images describe the edge ef-

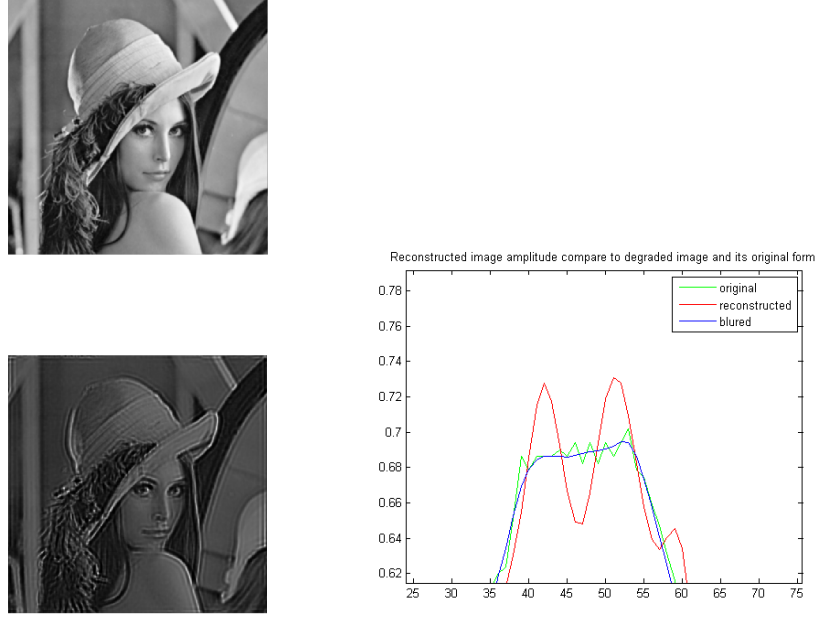


Fig. 5.7: Reconstructed image amplitude for blur kernel $9 \times 9, \sigma = 1$

fect caused by the algorithm. The origin rises from optimization function used in algorithm, not following the original intensity profile.

Noise degradation is essential problem associated with deconvolution of image therefore performance of an algorithm needs to be tested on noise degraded images. Increasing SNR representing higher level of useful signal affects the reconstruction quality as follows (Fig. 5.8 , 5.9 , 5.10).

. . .

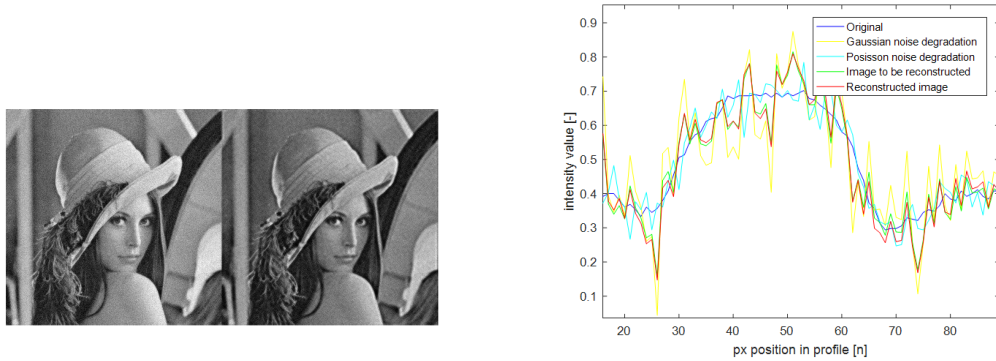


Fig. 5.8: Degradation of image with SNR=5 dB

Increasing noise degradation represented by higher SNR leads to expected better results. Images degraded by 5, 15 and 20dB of additive noise and model parameters as follows: $\nu=0$, $\sigma=2$, kernel size=9, iterations=1.

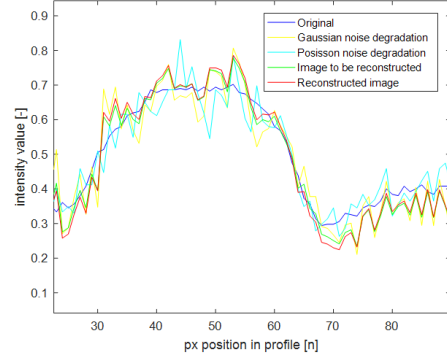


Fig. 5.9: Degradation of image with SNR=10 dB

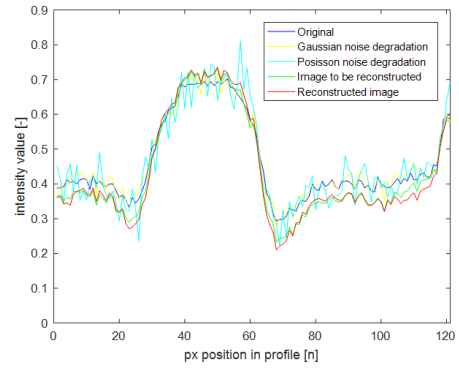


Fig. 5.10: Degradation of image with SNR=20 dB

5.1.2 Alternating Minimization

According to the test approach proposed for model images, implemented algorithm of AM has been tested on degraded images with given kernel size, σ and achieved SNR. Overall numerical results can be found in an attachment A.9.

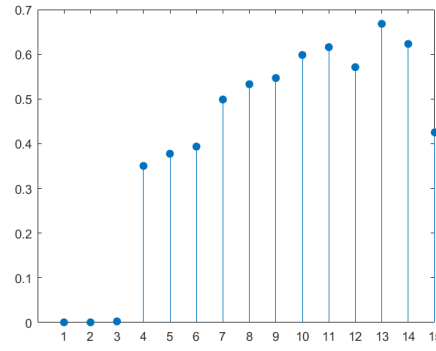
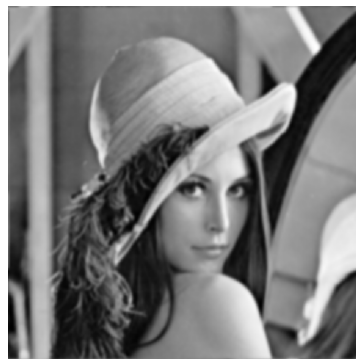
Quality of AM algorithm results depends on the constants value. For this purpose the tested combinations are indexed by numbers from 1 to 15 (5.1). As mentioned ideal values correspond to estimated noise degradation level ($SNR = 50dB$ then $\gamma = 10^5$, $SNR = 20 dB$ then $\gamma = 10^2$, etc. [56]) assuming the same normalizations, iterations settings etc. as implemented by [56]. Since the noise level of real images and concrete algorithm parameters are unknown, **models testing range is 10-50 dB achieved SNR** (according to formula $SNR = 10 \log \frac{\sigma_s^2}{\sigma_n^2}$) **with 3 values for α and 5 for γ inspired by article.**

Example results are represented by model degradation of image with blur kernel of 21x21 samples and $\sigma = 2$ and no additional noise (Fig. 5.11). Comparison of α and γ meaning is illustrative Particular combinations of constants responsible for image constraints weight can generate additional artefacts or blurring the image

Tab. 5.1: Constants combinations indexes

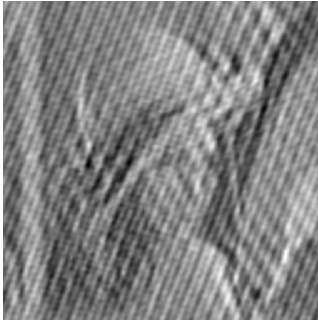
α	0.01	0.1	0.5	0.01	0.1	0.5	0.01	0.1	0.5	0.01	0.1	0.5	0.01	0.1	0.5
γ	1	1	1	10	10	10	100	100	100	1000	1000	1000	10000	10000	10000
i	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15

completely. Plot of SSIM index between reconstructed and original latent image corresponds with visual inspection of images and its quality. Higher SSIM values are coupled with better image properties, less blurred, without artefacts and preserved contrast.



Degraded image

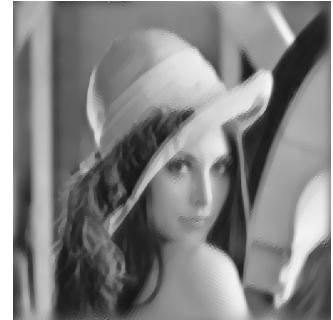
SSIM index (y axis) vs constants comb. (x)



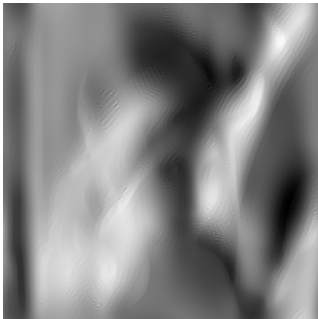
$\alpha = 0.01 \gamma = 1$ (i. 1)



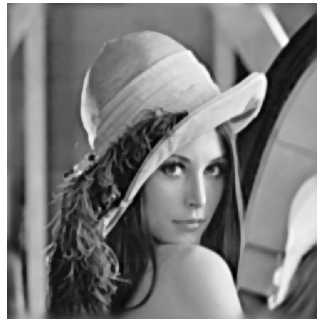
$\alpha = 0.01 \gamma = 10000$ (i. 13)



$\alpha = 0.1 \gamma = 10$ (i. 5)



$\alpha = 0.5 \gamma = 1$ (i. 3)



$\alpha = 0.5 \gamma = 100$ (i. 9)



$\alpha = 0.5 \gamma = 1000$ (i. 12)

Fig. 5.11: Demonstration of AM functionality, depending on constant values

5.1.3 Results discussion

Discussion on results summarizes the conclusions from restoration by both discussed algorithms, Matlab implemented based on Maximum Likelihood (2.7.2) and alternative regularization-based Alternating Minimization with Total Variation regularizer(2.8.2) and its function on model data.

Considered degradation of model images combines only the blur ($\sigma = 1-5$, $k_a = 7, 9, 21$) or SNR (7-50dB) and also its mutual combinations. Experimental results has revealed the logical pattern in behaviour of algorithms in most cases (A.9). The higher the blur size, the lower quality (similarity of reconstructed to original) of image. Bigger size of degradation kernel corresponds to higher suppression of frequencies responsible for detail. Wider convolution mask operator couples more pixels to produce one output value, causing higher level of blurring and restoration becoming more difficult.

Comparison of similarity evaluations techniques (5.1) discovered the inability of ML algorithm to properly reconstruct images with bigger PSF coupled with smaller σ . Inspection on real PSF obtained from beam-stopper of microscope system compared to simulated form illustrates the exact similarity of the problematic matrices to real blurring kernels (5.12). Middle and the right column are real, left corresponds to simulated kernels with increasing value of σ . Eventhough the obtained PSF serves as the initial guess and partial estimate of system degradation, the analogy of inability can explain worse results.

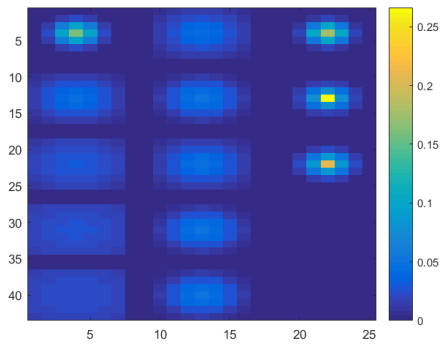


Fig. 5.12: PSF matrices 7x7 size, simulated (left) vs. real

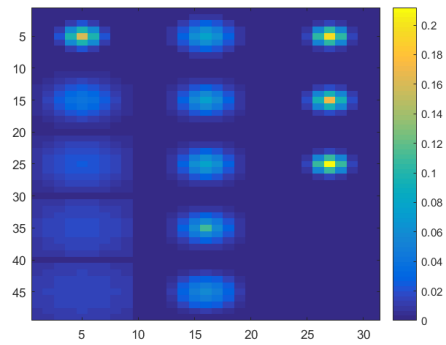


Fig. 5.13: PSF matrices 9x9 size, simulated (left) vs. real

Images corrupted by noise effects fulfils logical assumption on quality of reconstruction . The higher SNR (less noise) the higher similarity index value. Even stronger effect performs additive noise coupled with blur kernel, the nearest simulation of real images. An example of combined degradation show best images

according to similarity values for both used algorithms (5.14,5.15 ML on the left, AM on the right side). Pixel intensity profile in the middle reveals the better contrast preservation ability of AM by decreased grey-level range especially on the flat areas. Images reconstructed by ML suffers by ringing artefact and the noise amplification, that arises from optimization function to be minimized. On the other hand preservation of strong edges and image sharpness performs better for all images reconstructed by ML. The assumption is supported by higher values of similarity for all degraded images. The subjective visual inspection is required to reveal the artefacts and confirm the quality.

Determination of suitable settings of algorithm parameters for real images represents one of the aims of the model images assessment, despite the general assumption of particular-image-dependency of deconvolution techniques. Quality assessment according to constant setting does not show definite result. Combination indexed by 15 represents often the best choice for noisy images without blur. So does the combination 13 for just blurred images, however in case of combined degradation non of the constant values repeats often.

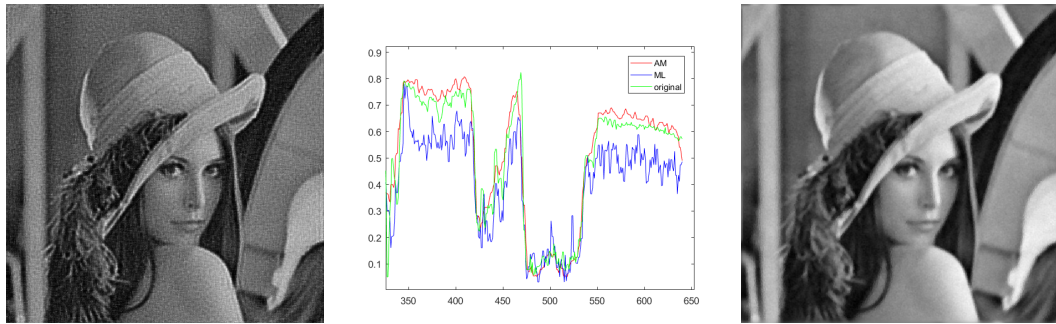


Fig. 5.14: SNR 10dB, $k_a = 7$, $sd=3$; ML-left, AM-right

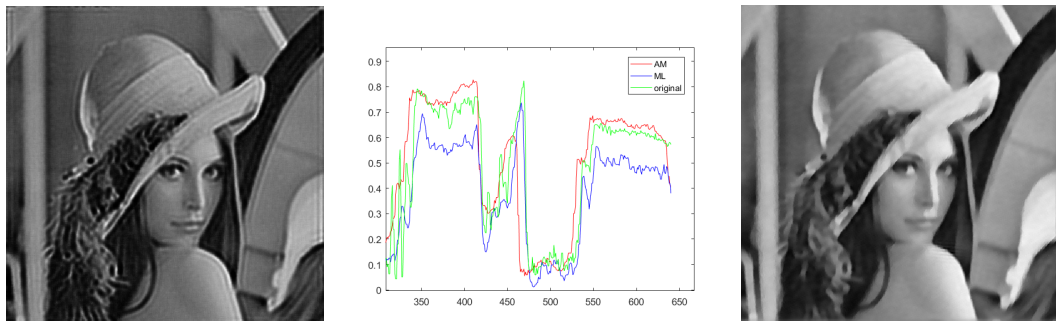


Fig. 5.15: SNR 20dB, $k_a = 9$, $sd=3$; ML-left, AM-right

5.2 Results of real data

For purpose of functionality test on microscope images several real images have been taken with variable acquisition parameters. PSF determination from calibration sample representing beam-stop needle has been implemented according to [47] and tested. Overall approximately 60 images of graphitised carbon samples with various intensity (nA), magnification (N x) and exposure time (ms) simulated by level of blur and noise degradation in case of model images.

Results illustrates dependency of proposed reconstruction technique quality on known authentic PSF of camera system, acquisition parameters and parameters of reconstruction algorithm. Default MATLAB function (*deconvblind*, based on Maximum Likelihood - ML) with same properties was applied to sample images. Reconstructions has been performed with or without obtained initial guess of PSF from beam-stop needle. Constant-value matrix (proposed in *deconvblind* help) or energy preserving matrix summed up to unit (Dirack distribution proposed for AM) has been tested as initial guess.

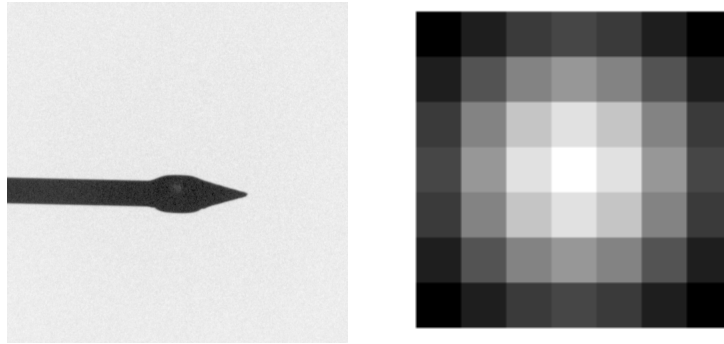


Fig. 5.16: Illustration of beam-stop needle and obtained PSF (9x9)

Theoretical results correspond with meaning of initial guess. Individual microscope system suffers from multiple imperfections and misalignments. Its performance is determined by achieved combination of corrected and uncorrected errors. While properties of system changes during multiple different acquisition so do the PSF therefore initial guess does not correspond to exact blur specification of individual image but should support the algorithm to obtain better results.

5.2.1 Alternating Minimization

Summarization of proposed quality assessment results (5.17) combines the constants values (x axis) with given indices by 5.1) corresponding quality dependency (y axis) with acquisition parameters effect comparison, detailed value inspection can

be found in an attachment (A.3). Three best marked indexes and corresponding α and γ values represents the best average result on group of images with shared parameter. Results examination reveals the commonly repeated combinations 2,7, and 12 through various acquisition parameters data sets.

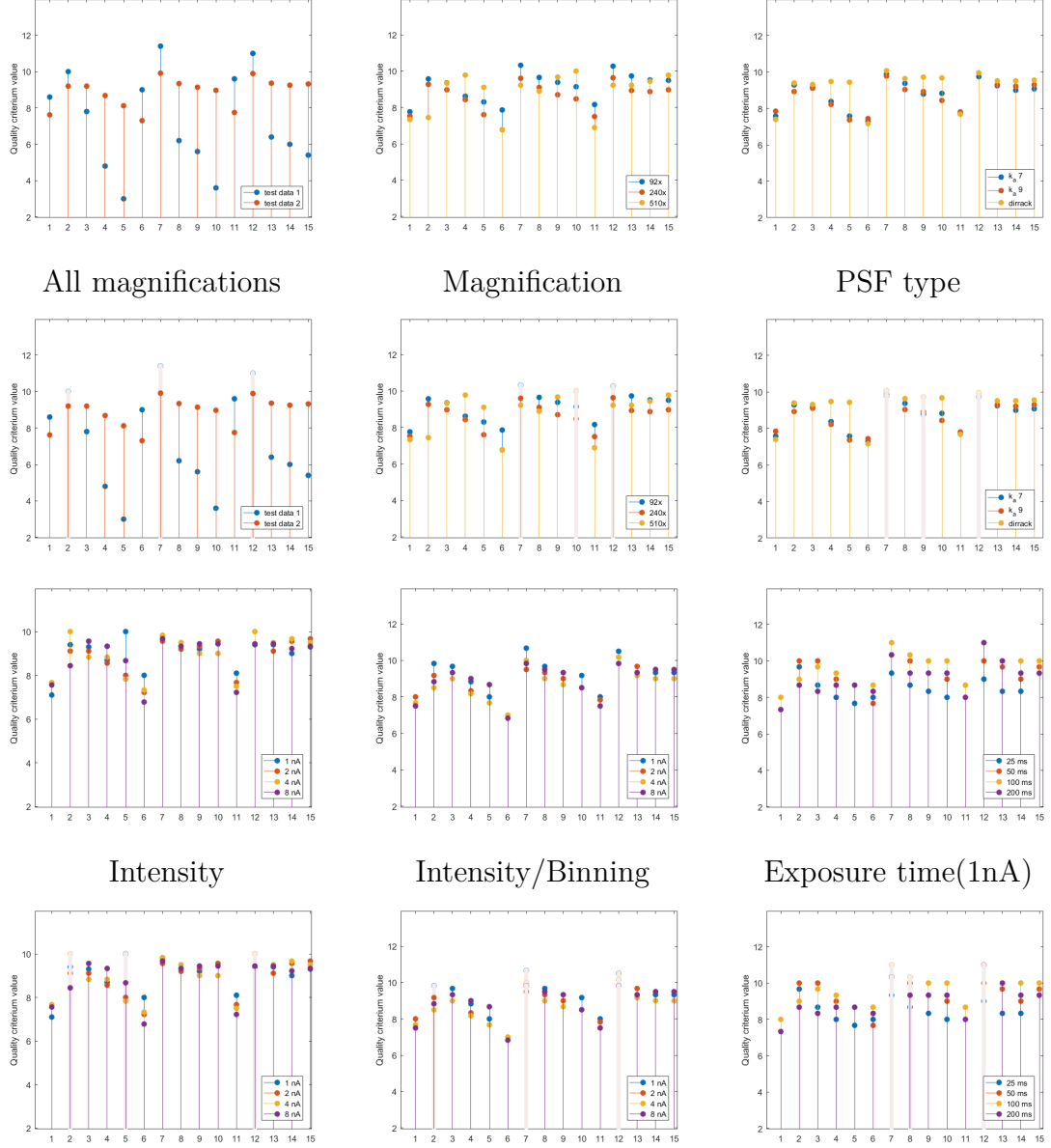


Fig. 5.17: Results of real images quality assessment after reconstruction with three best constants combination marked

General outcomes, no significant difference of **magnification** on quality, average best results are coupled with the smallest magnification value (92x), where the

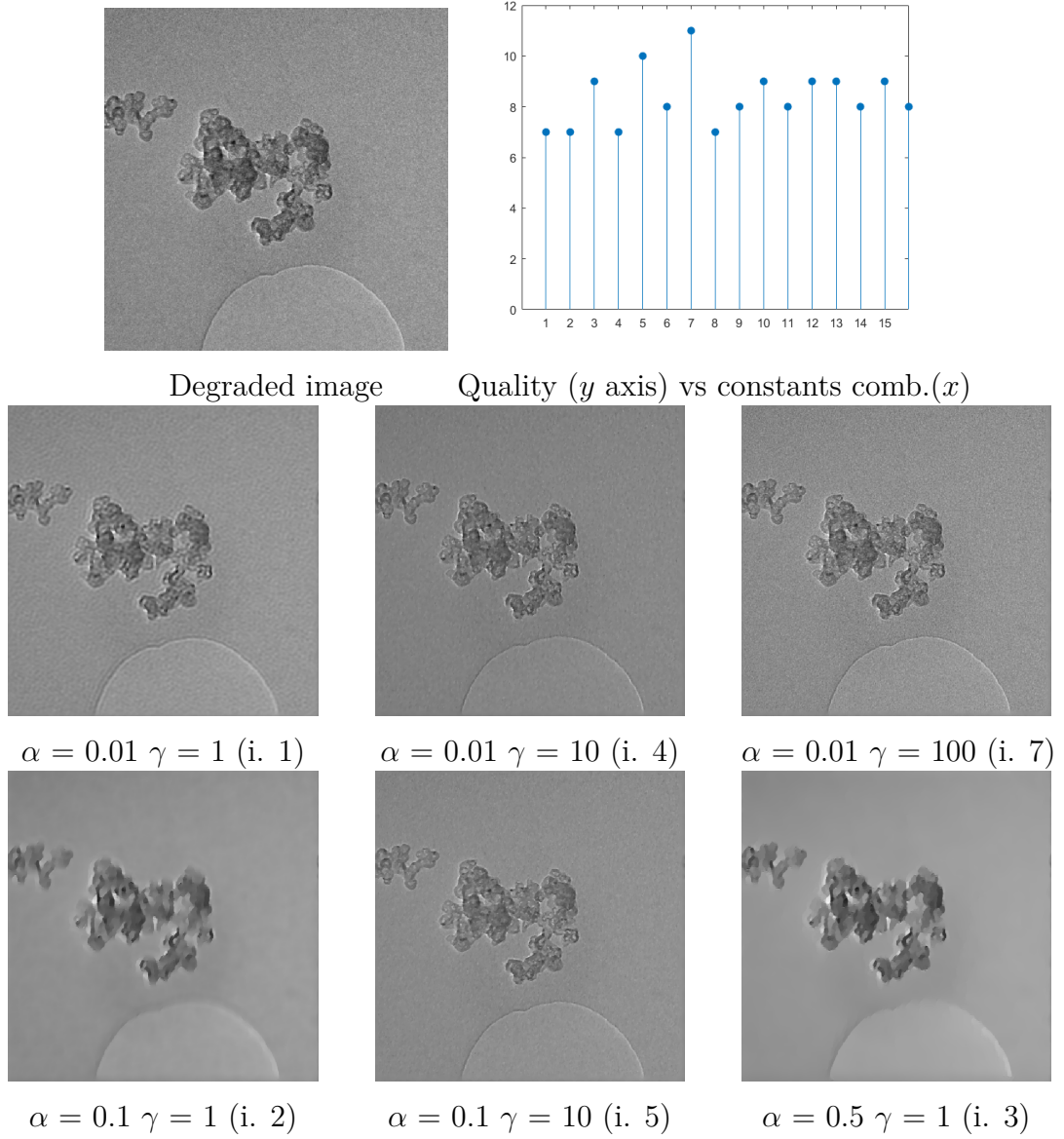


Fig. 5.18: Demonstration of AM functionality on real data, depending on constant values

loss of details is theoretically less visible (assuming the subjective visual inspection, details are easier to overlook). Different choice of **binning** parameter, where general 'intensity' represents binning 2, therefore 4 pixels creates one super-pixel to be visualised while 'high res' corresponds to binning 1, affects the quality result. Average value is significantly higher in case of 'high res' images supporting the theoretical assumption. Higher binning used arises in better resolution achievable. Use of best algorithm settings can overcome the disadvantage of higher noise level coupled with lower binning value. Increasing **exposure time** physically means the higher amount of collected signal, therefore higher achieved SNR but with the risk

of motion of scene arising in the blurring. Theoretically the higher the value, the better the result if the limits of detection chip are not exceeded. Real results confirm the assumption since the 25ms, 50 ms, 200 ms and 100 ms represents average best results sequence. **Initial guess of PSF** should support better quality of result images. However, results are different. Deconvolution supported by 7x7 or 9x9 initial known represents lower average quality of results. In detail, the overall quality of results is on same level no matter the constants combination (5.17 'PSF type') unlike the similarity character for used initial guess where are significant differences and resulting average evaluation is worse.

Finally, **two different datasets**, one with known acquisition (dataset 1) and different sample measurement without the knowledge (data set 2) were compared (5.17 'all magnification'). Character of the quality assessment values differs, however the best results are achieved by same set up of constants.

Images with various constants and AM algorithm represent the support to mentioned conclusions (5.18).

5.2.2 Maximum Likelihood

Quality assessment by proposed subjectively-objective evaluation has been implemented for real images in case of Matlab built-in function *deconvblind*. Summary of results can be found in 5.2 , 5.3 .

Overview results are mainly corresponding with ML conclusions and acquisition parameters dependency. **Magnification** represents the exception, quality outputs increase with magnification rate increasing, therefore best results in case of 510x. **Binning** parameter affects the quality equivalently, higher binning value (2 'high res') produces better quality results as assumed. The sequence of **exposure time** best results follows the 25 ms, 50 ms, 200 ms and 100 ms as in case of AM. Available initial guess of 7x7 and 9x9 matrix is not improving reconstruction quality by result evaluation. Data set dependency of average quality of outputs has also revealed the significant difference (5.2).

5.2.3 Results discussion

Final demonstration of best results on real data sets comparing both examined techniques is given before conclusion on Fig. 5.19, 5.20. Properties of images are given by various acquisition parameters. Intensity mainly affects the amount of incorporated noise, so do the binning and exposure time with possibility of influence on image blur too. Size of the PSF matrix primary affects the sharpness of image and achieved contrast. .

Tab. 5.2: Quality results for different conditions (ML)

deconvblind		deconvblind			deconvblind		
data set		magnification			blur matrix size		
set 1	set 2	92	240	510	7	9	dirr
mean	mean	mean	mean	mean	mean	mean	mean
7.89	8.63	8.56	8.63	8.89	8.16	8.57	9
min	min	min	min	min	min	min	min
7	7	7	7	7	7	7	9
max	max	max	max	max	max	max	max
9	10	10	10	10	10	10	10

Tab. 5.3: Quality results for different conditions (ML)

deconvblind				deconvblind				deconvblind			
intensity high res [nA]				intensity [nA]				exposure time [ms]			
1	2	4	8	1	2	4	8	25	50	100	200
men	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean
9.11	9.11	9.33	8.78	8	8.5	8.17	8.6	7.33	8.67	9.67	9
min	min	min	min	min	min	min	min	min	min	min	min
9	8	9	8	7	8	7	8	7	8	9	9
max	max	max	max	max	max	max	max	max	max	max	max
10	10	10	9	9	9	9	10	8	9	10	9

Demonstrative comparison should illustrates the potential of both approaches. As examined before (5.12), ML method suffers the problem with particular PSF matrices type. Assuming revealed similarity between real and those problematic PSFs, visually confirmed lower ability of ML to contrast preservation can be explained. Sharpness of obtained images is comparable, but the level of noise amplification related to deconvolution artefact is higher in case of ML. Noise reduction sensitivity is not implemented in optimization function. Based on Lucy-Richardson model assumes Photon probabilistic occurrence like the random distribution.

Needs to be mentioned that i swell known, Matlab built in function *deconvblind* has variable input arguments (*NUMIT*, *DAMPAR*, *WEIGHT*, *READOUT*) that serves to improve the algorithm performance while noise is present and preservation of image structures needed. *NUMIT* can change number of iterations of algorithm, *DAMPAR* specifies standard deviation of Poisson noise while iterations pixels with lower are suppressed . Created array of *WEIGHTS* would specify the level of how corresponding pixels are considered while optimization.*READOUT* given by an array of additive noise noise of camera represents another variable input. Optional settings of method have not been tested since the robustness and possible simplicity of use has been prioritized.

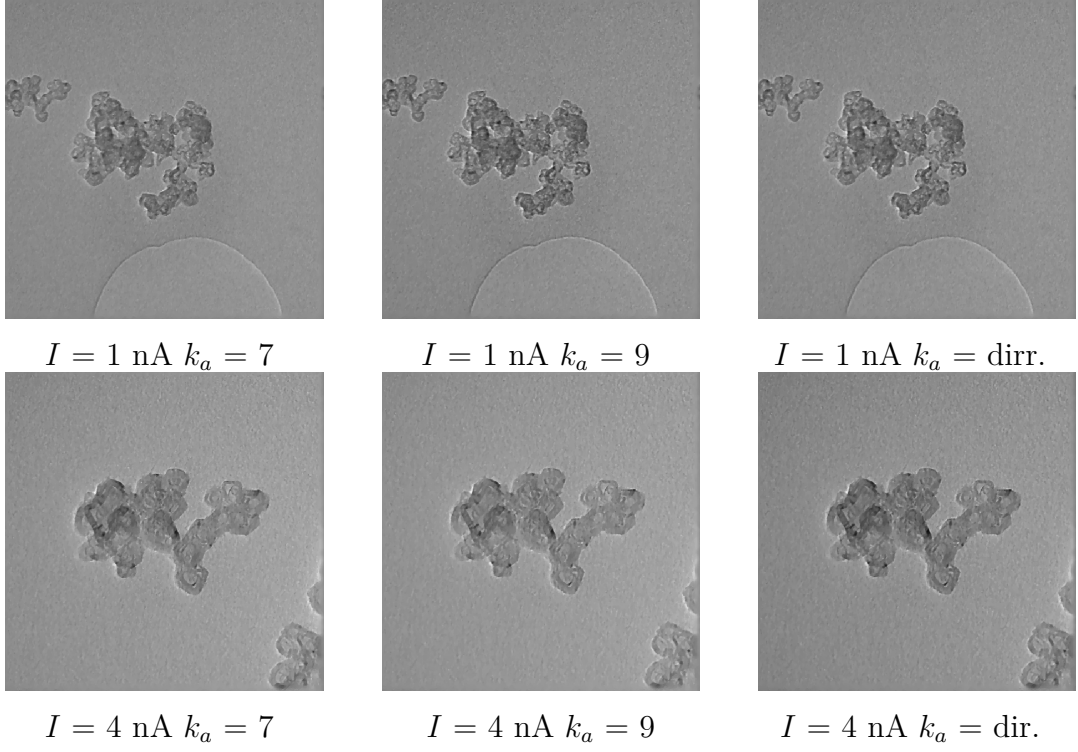


Fig. 5.19: Demonstration of AM functionality on real data, acquisition and algorithm parameters dependency (magnif.=92x UP, 240x DOWN)

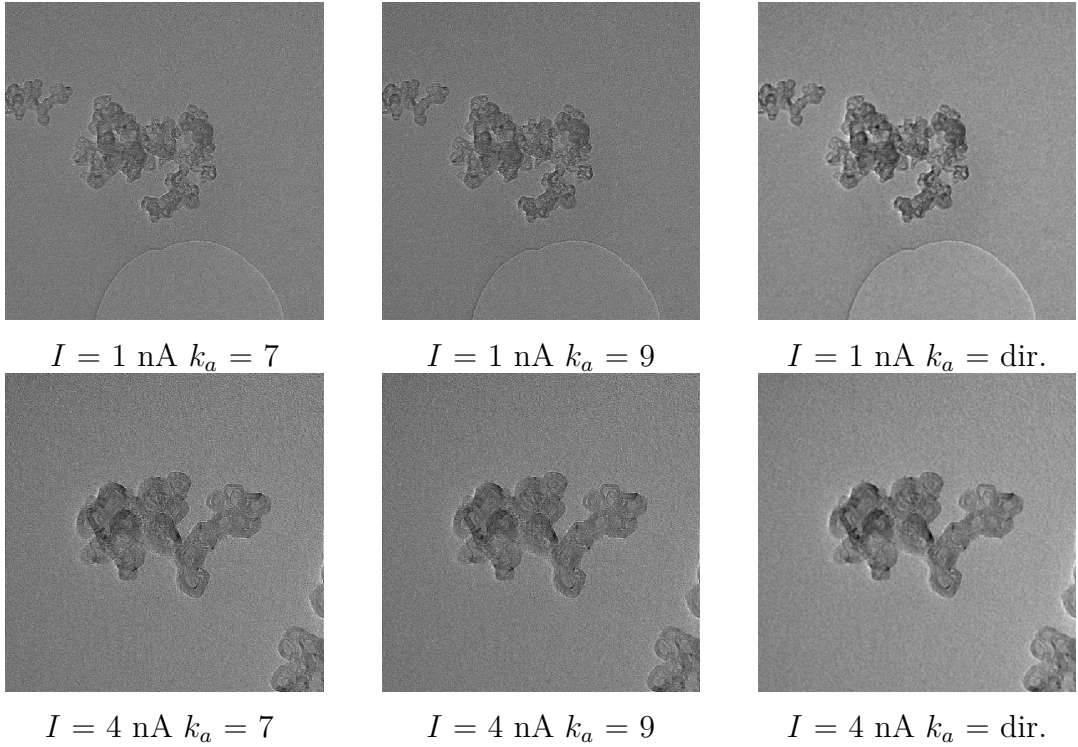


Fig. 5.20: Demonstration of ML functionality on real data, acquisition and algorithm parameters dependency (magnif.=92x UP, 240x DOWN)

6 CONCLUSION

Blind deconvolution has spread around multiple technical fields in recent years. Problems with computational demands are no more the limitations. Therefore blind deconvolution signal processing techniques are considered to be promising solution for enhancement of electron microscope performance. Multiple techniques have been studied, by the complexity of range of used methods and its assumptions, the framework of blind deconvolution has been partitioned. Main model groups are based on parametric, Bayesian or regularization approach.

Imperfections of mechanical alignment of construction parts, lens aberrations and properties of sensor determines the achievable quality of output image. Illustrative description of lens aberrations, blur and noise corruption has been given. Theoretical part also discuss practical influence of aberrations on output image.

The simulation of degradation process has been designed representing real situation. The analogy of model parameters with acquisition parameters such as magnification, beam intensity, exposure time, focus or binning has been discussed.

Crucial part of the work represents the assessment of appropriate method to be implemented. High sensitivity of noise and computational complexity restricts the parametric methods to be used as solution of the task. Models based on the Bayesian probability becomes considered approach. Commonly used and Matlab implemented maximum likelihood method has been tested in comparison with proposed algorithm of alternating minimization. Incorporation of initial guess into algorithm has been formulated and implemented representing prior restriction of optional solution. An empirically obtained PSF reflects system specific aberrations, both the optical and sensor parts. Slightly visible improvement has been revealed in case of Matlab function. Since, the maximum likelihood does not include the image characteristics preservation demands, the improvement is not significant. Theoretically better results should be obtained by proposed method of alternating algorithm for total variation regularization.

According to chance of simple variability of an AM algorithm in case of combinations set up, general potential of this technique to be used for the electron microscope images is higher despite the worse results in model part. Considering the use of AM algorithm on different data sets of electron microscope images, examination of one or three best constants combination, according to previous results, should prove comparable good functionality. High SNR images (photographies) are better to be restored by Matlab algorithm, like revealed model data. The implemented function assumes the Photon probabilistic noise occurrence and proved to be sufficient. Data collected by different technique would need to undergo examination to discover best parameters combination for group of same type images.

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LIST OF SYMBOLS, PHYSICAL CONSTANTS AND ABBREVIATIONS

ADC	Analog to digital convertor
AM	Alternating minimization
AR	auto-regressive
ARMA	Auto-regressive moving average
CCD	Charged couple device
CMOS	Complementary metal oxide semiconductor
DFT	Dicscrete Fourier transform
DP	Diffraction pattern
DQE	Detective quatnum efficiency
EM	Electron microscope
FEG	Field emission gun
FT	Fourier transform
FFT	Fast Fourier transform
GCV	Geeralised cross-validation
HOS	Higher-order spectra
IBD	Iterative blind deconvolution
LSD	Linear degradation system
LSE	Least square error
LSF	Line spread function
MA	Moving average
MAP	Maximum A posterior
ML	Maximum likelihood
MSE	Mean square error
MTF	Modulation transfer function
NAS-RIF	Nonnegativity and support constraints with with recursive Image Filtering
NRMSE	Normalized root mean square error
PSF	Point spread function
PSNR	Peak signal-to-noise ratio
SAA	Simulated annealing algorithm
SNR	Signal-to-noise ratio
SSIM	Structural similarity index
TEM	Transmission electron microscope
TV	Total variation

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A RESULTS

Tab. A.1: Quality results for ML 1/2

deconvblind (ML)		deconvblind (ML)			deconvblind (ML)		
data set		magnification			blur matrix size		
set 1	set 2	92	240	510	7	9	dirr
mean	mean	mean	mean	mean	mean	mean	mean
7.89	8.63	8.56	8.63	8.89	8.16	8.57	9
min	min	min	min	min	min	min	min
7	7	7	7	7	7	7	9
max	max	max	max	max	max	max	max
9	10	10	10	10	10	10	10

Tab. A.2: Quality results for ML 2/2

deconvblind (ML)				deconvblind (ML)				deconvblind (ML)			
intensity [nA]				intensity high res [nA]				exposure time [ms]			
1	2	4	8	1	2	4	8	25	50	100	200
men	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean	mean
9.11	9.11	9.33	8.78	8	8.5	8.17	8.6	7.33	8.67	9.67	9
min	min	min	min	min	min	min	min	min	min	min	min
9	8	9	8	7	8	7	8	7	8	9	9
max	max	max	max	max	max	max	max	max	max	max	max
10	10	10	9	9	9	9	10	8	9	10	9

Tab. A.3: Quality results for different **data sets**(AM)

	i	γ	α	$mean$		i	γ	α	$mean$
test_data	1	1	0.01	8.60	test_data	1	1	0.01	7.62
1	2	1	0.1	10.00	2	2	1	0.1	9.20
	3	1	0.5	7.80		3	1	0.5	9.20
	4	10	0.01	4.80		4	10	0.01	8.68
	5	10	0.1	3.00		5	10	0.1	8.12
	6	10	0.5	9.00		6	10	0.5	7.30
	7	100	0.01	11.40		7	100	0.01	9.91
	8	100	0.1	6.20		8	100	0.1	9.34
	9	100	0.5	5.60		9	100	0.5	9.14
	10	1000	0.01	3.60		10	1000	0.01	8.97
	11	1000	0.1	9.60		11	1000	0.1	7.75
	12	1000	0.5	11.00		12	1000	0.5	9.89
	13	10000	0.01	6.40		13	10000	0.01	9.36
	14	10000	0.1	6.00		14	10000	0.1	9.25
	15	10000	0.5	5.40		15	10000	0.5	9.32

Tab. A.4: Quality results for different **magnification**(AM)

	i	γ	α	$mean$		i	γ	α	$mean$
Magnification	1	1	0.01	7.76	Magnification	1	1	0.01	7.53
92x	2	1	0.1	9.57	240x	2	1	0.1	9.27
	3	1	0.5	9.35		3	1	0.5	8.97
	4	10	0.01	8.62		4	10	0.01	8.43
	5	10	0.1	8.30		5	10	0.1	7.60
	6	10	0.5	7.86		6	10	0.5	6.77
	7	100	0.01	10.32		7	100	0.01	9.60
	8	100	0.1	9.65		8	100	0.1	9.10
	9	100	0.5	9.38		9	100	0.5	8.70
	10	1000	0.01	9.14		10	1000	0.01	8.47
	11	1000	0.1	8.16		11	1000	0.1	7.50
	12	1000	0.5	10.27		12	1000	0.5	9.63
	13	10000	0.01	9.73		13	10000	0.01	8.93
	14	10000	0.1	9.51		14	10000	0.1	8.87
	15	10000	0.5	9.49		15	10000	0.5	8.97
	i	γ	α	$mean$		i	γ	α	$mean$
Magnification	1	1	0.1	7.33					
510x	2	1	0.01	7.44					
	3	1	0.5	9.33					
	4	10	0.01	9.78					
	5	10	0.1	9.11					
	6	10	0.5	6.78					
	7	100	0.01	9.22					
	8	100	0.1	8.89					
	9	100	0.5	9.67					
	10	1000	0.01	10.00					
	11	1000	0.1	6.89					
	12	1000	0.5	9.22					
	13	10000	0.01	9.22					
	14	10000	0.1	9.44					
	15	10000	0.5	9.78					

Tab. A.5: Quality results for different **intensity**(AM)

	i	γ	α	$mean$		i	γ	α	$mean$
Intensity	1	1	0.01	7.10	Intensity	1	1	0.01	7.67
1nA	2	1	0.1	9.40	2nA	2	1	0.1	9.11
	3	1	0.5	9.30		3	1	0.5	9.11
	4	10	0.01	8.70		4	10	0.01	8.56
	5	10	0.1	10.00		5	10	0.1	8.00
	6	10	0.5	8.00		6	10	0.5	7.22
	7	100	0.01	9.80		7	100	0.01	9.56
	8	100	0.1	9.20		8	100	0.1	9.22
	9	100	0.5	9.20		9	100	0.5	9.33
	10	1000	0.01	9.00		10	1000	0.01	9.56
	11	1000	0.1	8.10		11	1000	0.1	7.67
	12	1000	0.5	9.40		12	1000	0.5	9.44
	13	10000	0.01	9.40		13	10000	0.01	9.11
	14	10000	0.1	9.00		14	10000	0.1	9.56
	15	10000	0.5	9.30		15	10000	0.5	9.67
	i	γ	α	$mean$		i	$gamma$	α	$mean$
Intensity	1	1	0.01	7.67	Intensity	1	1	0.01	7.56
4nA	2	1	0.1	10.00	8nA	2	1	0.1	8.44
	3	1	0.5	8.83		3	1	0.5	9.56
	4	10	0.01	8.83		4	10	0.01	9.33
	5	10	0.1	7.83		5	10	0.1	8.67
	6	10	0.5	7.33		6	10	0.5	6.78
	7	100	0.01	9.83		7	100	0.01	9.67
	8	100	0.1	9.50		8	100	0.1	9.33
	9	100	0.5	9.00		9	100	0.5	9.44
	10	1000	0.01	9.00		10	1000	0.01	9.44
	11	1000	0.1	7.50		11	1000	0.1	7.22
	12	1000	0.5	10.00		12	1000	0.5	9.44
	13	10000	0.01	9.50		13	10000	0.01	9.44
	14	10000	0.1	9.67		14	10000	0.1	9.22
	15	10000	0.5	9.50		15	10000	0.5	9.33

Tab. A.6: Quality results for different **binning** (AM)

	i	γ	α	$mean$		i	γ	α	$mean$
Intensity 1nA	1	1	0.01	7.67	Intensity 2nA	1	1	0.01	8.00
	2	1	0.1	9.83		2	1	0.1	9.17
	3	1	0.5	9.67		3	1	0.5	9.00
	4	10	0.01	8.83		4	10	0.01	8.33
	5	10	0.1	8.00		5	10	0.1	7.67
	6	10	0.5	6.83		6	10	0.5	7.00
	7	100	0.01	10.67		7	100	0.01	9.50
	8	100	0.1	9.67		8	100	0.1	9.33
	9	100	0.5	9.33		9	100	0.5	9.00
	10	1000	0.01	9.17		10	1000	0.01	8.50
	11	1000	0.1	8.00		11	1000	0.1	7.83
	12	1000	0.5	10.50		12	1000	0.5	10.17
	13	10000	0.01	9.33		13	10000	0.01	9.67
	14	10000	0.1	9.33		14	10000	0.1	9.00
	15	10000	0.5	9.33		15	10000	0.5	9.00
	i	γ	α	$mean$		i	γ	α	$mean$
Intensity 4nA	1	1	0.01	7.67	Intensity 8nA	1	1	0.01	7.50
	2	1	0.1	8.50		2	1	0.1	8.83
	3	1	0.5	9.00		3	1	0.5	9.33
	4	10	0.01	8.17		4	10	0.01	9.00
	5	10	0.1	7.67		5	10	0.1	8.67
	6	10	0.5	7.00		6	10	0.5	6.83
	7	100	0.01	10.00		7	100	0.01	9.83
	8	100	0.1	9.00		8	100	0.1	9.50
	9	100	0.5	8.67		9	100	0.5	9.33
	10	1000	0.01	8.50		10	1000	0.01	8.50
	11	1000	0.1	7.50		11	1000	0.1	7.50
	12	1000	0.5	10.17		12	1000	0.5	9.83
	13	10000	0.01	9.17		13	10000	0.01	9.33
	14	10000	0.1	9.00		14	10000	0.1	9.50
	15	10000	0.5	9.00		15	10000	0.5	9.50

Tab. A.7: Quality results for different **exposure time(AM)**

	i	γ	α	$mean$		i	γ	α	$mean$
Exposure 25ms	1	1	0.01	8.00	Exposure 50ms	1	1	0.01	8.00
	2	1	0.1	9.67		2	1	0.1	10.00
	3	1	0.5	8.67		3	1	0.5	10.00
	4	10	0.01	8.00		4	10	0.01	9.00
	5	10	0.1	7.67		5	10	0.1	8.67
	6	10	0.5	8.00		6	10	0.5	7.67
	7	100	0.01	9.33		7	100	0.01	11.00
	8	100	0.1	8.67		8	100	0.1	10.00
	9	100	0.5	8.33		9	100	0.5	9.33
	10	1000	0.01	8.00		10	1000	0.01	9.00
	11	1000	0.1	8.00		11	1000	0.1	8.00
	12	1000	0.5	9.00		12	1000	0.5	10.00
	13	10000	0.01	8.33		13	10000	0.01	9.67
	14	10000	0.1	8.33		14	10000	0.1	9.00
	15	10000	0.5	9.33		15	10000	0.5	9.67
	i	γ	α	$mean$		i	γ	α	$mean$
Exposure 100ms	1	1	0.01	8.00	Exposure 200ms	1	1	0.01	7.33
	2	1	0.1	9.00		2	1	0.1	8.67
	3	1	0.5	9.67		3	1	0.5	8.33
	4	10	0.01	9.33		4	10	0.01	8.67
	5	10	0.1	8.67		5	10	0.1	8.67
	6	10	0.5	8.67		6	10	0.5	8.33
	7	100	0.01	11.00		7	100	0.01	10.33
	8	100	0.1	10.33		8	100	0.1	9.33
	9	100	0.5	10.00		9	100	0.5	9.33
	10	1000	0.01	10.00		10	1000	0.01	9.33
	11	1000	0.1	8.67		11	1000	0.1	8.00
	12	1000	0.5	11.00		12	1000	0.5	11.00
	13	10000	0.01	10.00		13	10000	0.01	10.00
	14	10000	0.1	10.00		14	10000	0.1	9.33
	15	10000	0.5	10.00		15	10000	0.5	9.33

Tab. A.8: Quality results for different **PSF type(AM)**

	i	γ	α	$mean$		i	γ	α	$mean$
PSF type	1	1	0.01	7.58	PSF type	1	1	0.01	7.85
k_a=7	2	1	0.1	9.29	k_a=9	2	1	0.1	8.93
	3	1	0.5	9.17		3	1	0.5	9.11
	4	10	0.01	8.38		4	10	0.01	8.22
	5	10	0.1	7.58		5	10	0.1	7.37
	6	10	0.5	7.29		6	10	0.5	7.44
	7	100	0.01	9.88		7	100	0.01	9.78
	8	100	0.1	9.38		8	100	0.1	9.04
	9	100	0.5	8.79		9	100	0.5	8.93
	10	1000	0.01	8.83		10	1000	0.01	8.44
	11	1000	0.1	7.75		11	1000	0.1	7.81
	12	1000	0.5	9.75		12	1000	0.5	9.96
	13	10000	0.01	9.25		13	10000	0.01	9.30
	14	10000	0.1	9.00		14	10000	0.1	9.22
	15	10000	0.5	9.08		15	10000	0.5	9.30
	i	γ	α	$mean$		i	γ	α	$mean$
PSF type	1	1	0.01	7.40					
Dirrack	2	1	0.1	9.40					
	3	1	0.5	9.32					
	4	10	0.01	9.48					
	5	10	0.1	9.44					
	6	10	0.5	7.16					
	7	100	0.01	10.08					
	8	100	0.1	9.64					
	9	100	0.5	9.72					
	10	1000	0.01	9.68					
	11	1000	0.1	7.68					
	12	1000	0.5	9.96					
	13	10000	0.01	9.52					
	14	10000	0.1	9.52					
	15	10000	0.5	9.56					

Tab. A.9: Quality assesment of results of model data AM,ML

			AM						SSIM	
k_a	sd	SNR	Alpha		Gamma		Index		AM	ML
			image	h	image	h	image	h		
		5	0.5	0.5	100	10	9	3	0.5331	
		7	0.5	0.5	10000	1	15	3	0.5657	0.5493
		10	0.5	0.1	100	1	9	2	0.6048	0.6898
		15	0.5	0.1	10000	1	15	1	0.6456	0.8507
		20	0.5	0.5	10000	1	15	3	0.7078	0.9338
		30	0.5	0.1	10000	1	15	2	0.7442	0.9647
		40	0.5	0.01	10000	1	15	1	0.7488	0.9700
		50	0.5	0.01	1000	1	12	1	0.7492	0.9698
k_a	sd	SNR								
7	1	0	0.01		10000		13		0.8353	0.8918
7	2	0	0.01		10000		13		0.6905	0.8099
7	3	0	0.01		10000		13		0.7046	0.7819
7	4	0	0.01		10000		13		0.7112	0.7713
7	5	0	0.01		10000		13		0.7152	0.7663
9	1	0	0.01		10000		13		0.8348	0.8917
9	2	0	0.01		10000		13		0.6683	0.7945
9	3	0	0.01		10000		13		0.6412	0.7513
9	4	0	0.01		10000		13		0.6530	0.7340
9	5	0	0.01		10000		13		0.6587	0.7257
21	1	0	0.01		10000		13		0.8312	0.8917
21	2	0	0.01		10000		13		0.6723	0.7881
21	3	0	0.01		10000		13		0.5724	0.7199
21	4	0	0.01		10000		13		0.5077	0.6732
21	5	0	0.01		10000		13		0.4353	0.6477
k_a	sd	0								
7	2	10	0.5	0.5	10000	1	15	3	0.4678	0.5041
9	2	10	0.01	0.1	10	1	4	2	0.2033	0.4796
21	2	10	0.5	0.1	10000	1	15	1	0.3945	0.4499
7	2	20	0.1	0.1	1000	1	11	2	0.5035	0.7314
9	2	20	0.01	0.1	10	10	4	5	0.2053	0.7017
21	2	20	0.1	0.01	10	1	5	1	0.0783	0.6679
7	2	30	0.1	0.5	1000	1	14	3	0.5266	0.7797
9	2	30	0.01	0.5	100	100	7	9	0.2051	0.7466
21	2	30	0.5	0.1	1	1	3	2	0.0783	0.7144
7	2	40	0.01	0.1	1000	1	10	2	0.5323	0.7820
9	2	40	0.01	0.01	10	10	4	4	0.2052	0.7509
21	2	40	0.5	0.5	1	10000	3	15	0.0784	0.7216
7	2	50	0.01	0.1	1000	1	13	2	0.5331	0.7821
9	2	50	0.01	0.5	10	1	4	3	0.2052	0.7513
21	2	50	0.5	0.5	1	10000	3	15	0.0783	0.7199

B PROGRAMMING PART

Data sets coupled with implemented AM algorithm can be found on attached CD. Files to be tested are stored in method-related folders. Depending on if model or real images are tested, two separate groups of files are created (method 1,2 vs. method 3,4). Division in groups represents the choice of pre-modeled data and pre-estimated PSFs or the method of modelling and testing coupled to the user choice. The meaning of all possible choices is described in following section.

Whole code is based on work with configuration structure (*cfg*), which consists of default and determined settings. The workflow is designed for results examination therefore functions to save images and results into .mat and .tiff files are also used.

```
1 %% DEFAULT settings of configuration structure
2 % constants
3 cfg.alfa_con=[ 0.01 0.1 0.5 ];
4 cfg.gama_con=[1 10 100 1000 10000];
5
6 % constants combinations to get best results
7 cfg.constants=combvec(cfg.alfa_con,cfg.gama_con);
8
9 % algorithm properties
10 cfg.integr_step=10;
11 cfg.u_step=10;
12 cfg.h_init=[];
13 cfg.blur_sd=9;
14 cfg.blur_size=2;
15 cfg.I_orig_lena=
16 im2double(rgb2gray(imread('lena512color.tiff')));
```

Method 1 models the image blur with user given parameter and default initial PSF .

```
1 %% INPUT SETTINGS
2 %1 method ('model')
3 cfg.blur_sd=3;
4 cfg.blur_size=21;
```

Method 2 uses pre-defined files (.mat) of degraded model (lena) images with given degradation parameters (kernel size, σ and SNR). These parameters are estimated from file name to be used while algorithm (function *degr_properties_define*) with default initial PSF.

```

1 %% INPUT SETTINGS
2 % 2 method ('model degradation')
3 [FILENAME, PATHNAME]=uigetfile('.mat');
4 load([PATHNAME,FILENAME])
5 cfg.I_degr=I_final_degr;
6 % degr_blur_size,degr_sd_definition from file name
7 [cfg.blur_size,cfg.blur_sd,cfg.degr_SNR]
8 =degr_properties_define(FILENAME);

```

Method 3 works with real microscope images stored in .tiff format. Examination of all constants combinations defined in default settings in *cfg* will be done. Initial PSF is to be chosen from pre-estimated (.mat) files.

```

1 %% INPUT SETTINGS
2 % 3 method ('real with PSF')
3 % Degraded image
4 [FILENAME_im, PATHNAME_im]=uigetfile('.tiff')
5 cfg.I_degr=im2double(imread([PATHNAME_im,FILENAME_im]));
6 % initial PSF
7 [FILENAME_h, PATHNAME_h]=uigetfile('.mat')
8 load([PATHNAME_h,FILENAME_h]);
9 % dirrack distributin incaseof no initial guess of PSF
10 cfg.h_init=zeros(cfg.blur_size,cfg.blur_size);
11 h_init(round(cfg.blur_size/2),round(cfg.blur_size/2))=1;
12 cfg.h_init=h_init;

```

Method 4 works with real microscope images stored in .tiff format. Examination of all constants combinations defined in default settings in *cfg* will be done. Initial PSF is not chosen but estimated by algorithm described in (3.1.2) with given kernel size.

```

1 %% INPUT SETTINGS
2 % 4 method ('real, PSF estimation')
3 % Degraded image
4 [FILENAME_im, PATHNAME_im]=uigetfile('.tiff')
5 cfg.I_degr=im2double(imread([PATHNAME_im,FILENAME_im]));
6 % initial PSF with given size
7 cfg.blur_size=7;
8 PSF=PSF_estimation (cfg.blur_size)

```

Following steps are then common for all variants of methods. Blind deconvolution via implemented AM is performed for all combinations of given constant parameters, if model image is present, SSIM index is evaluated, otherwise just the images are saved to undergo subjectively-objective evaluation process.

```

1  for ii=1:length(cfg.constants)
2  cfg.alfa=cfg.constants(1,ii)
3  cfg.gama=cfg.constants(2,ii)
4  cfg = BD_ssim (cfg,method);
5  [cfg.ssim_index_image{ii}]=ssim_index_calc(cfg.u,cfg.
    u_step,cfg.I_orig_lena);
6  [cfg.ssim_index_h{ii}]=ssim_index_calc(cfg.h,cfg.u_step,
    cfg.I_orig_lena);
7  cfg.out{ii}=cfg.u;
8  end
9
10 [cfg.alfa_best_image,cfg.gama_best_image,cfg.
    ind_best_image,cfg.ssim_best_image]
11 =ssim_index_eval(cfg.ssim_index_image,cfg.constants,cfg.
    u_step);
12
13 [cfg.alfa_best_h,cfg.gama_best_h,cfg.ind_best_h,cfg.
    ssim_best_h]
14 =ssim_index_eval(cfg.ssim_index_h,cfg.constants,cfg.
    u_step);

```