Quasi-spectral characterization of intracellular regions in bright-field light microscopy images

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Investigation of cell structure is hardly imaginable without bright-field microscopy. Numerous modifications such as depth-wise scanning or videoenhancement makes this method being state-of-the-art. This raises a question what maximal information content can be extracted from ordinary (but well acquired) bright-field images in nearly model-free way. Here we introduce a method of a physically correct extraction of features for each pixel when these features resemble a transparency spectrum. The method is compatible with existent, ordinary bright-field microscopes and requires mathematically sophisticated data processing. Dimensionality reduction and unsupervised clustering of the spectra yield reasonable semantic segmentation of cells without any a priori information about their structures. Despite the lack of reference data (to strictly prove that the proposed feature vectors coincide with transparency) we believe that this method is the right approach to an intracellular (semi)quantitative and qualitative chemical analysis.

INTRODUCTION

Bright-field microscopy in videoenhancement mode shows an unprecedented success as a method of living object investigation since it is cheap and non-intrusive in preparation of samples, and, in its innovative set-up, has an excellent spatial and temporal resolution,¹ which opens many possibilities for automation. Classical image-processing techniques such as feature extraction or convolution neural networks do not work so well due to huge variability in micro-world data. It calls for image pre-processing techniques that would utilize all available information to supply rich, physically relevant feature vectors in the subsequent methods of analysis.

Indeed, classical bright-field microscopy measures properties of incoming light affected by a sample. If multi-photon processes are negligible and, then, intensities are reasonable, a linear response model can be used. Then, a medium observed in such a model can be fully characterized by a transparency spectrum $T(\vec{r})$ defined for each pixel. Such spectra can give ultimate information about the medium and significantly boost subsequent machine learning
methods.

The most convenient, classical way of obtaining such spectra is to modify a measuring
device (microscope). It is mostly done using single scanning interferometers,\textsuperscript{2} matrices of
them\textsuperscript{3} or matrices of color filter arrays\textsuperscript{4} or other adjustable media.\textsuperscript{5,6} Such technical arrange-
ments can be further successfully coupled with machine learning methods as well.\textsuperscript{7} Purely
instrumental methods are certainly the most correct but require sophisticated equipment
and are not fully compatible with typical bright-field techniques like depth-wise z-scanning.
Due to both hardware and algorithms, this makes these methods rather a separated group
than a subtype of the bright-field methods.

For classical bright-field microscopy, the most approaches rely on trained (or fitted)
models based on a set of reference images with known properties.\textsuperscript{8} Most mature methods rely
on the principal component analysis\textsuperscript{9} or sparse spatial features.\textsuperscript{10} Some of such techniques
does not aim to full-spectral reconstruction but rather to a more effective colour resolution
(which has been very useful in distinguishing fluorescence peaks).\textsuperscript{11} The main disadvantages
of such methods is the global approach, which is feasible only for homogeneous images. Most
'local' methods include different artificial neural networks\textsuperscript{12} and can work well if they are
trained with a reference dataset that is similar to the observed system. Although the data of
this kind almost never occurs in microscopy due to bigger variability of objects in microworld
(for the reason that, e.g., known objects are artificial, an investigated system is living, or
the in-focus position can be ambiguous). This gives a cutting edge to physically inspired
methods which make no assumption about type of observed object and does not use special
equipment except of a classical bright-field microscope.

Theoretical model

For most biologically relevant objects multi-photon interactions can be neglected.\textsuperscript{13} Thus, a
linear response model can be used for description of the measurement process. The model
consists of four entities (Fig. 1) which are physically characterized as follows:

1. **Light source** gives a light spectrum $S(\lambda)$, which is assumed to be constant and spa-
tially homogeneous.

2. **Medium** is, in each point of the projection onto a camera sensor plane, characterized
by an unknown transparency spectrum $T(x, y, \lambda)$.

3. **Camera filter**, where each camera channel $c$ is characterized by a quantum efficiency
curve $F_c(\lambda)$.

4. **Camera sensor** is described (by purely phenomenological approach) by exposure time
$t_e$ and energy load curve $I_c = f(E)$, where $I_c$ is the pixel sensor output (intensity) and
$E$ is energy absorbed by the pixel sensor during the exposure time. We assume that the image is not saturated and, thus, $f(E)$ can be approximated linearly.

Mathematically, it can be expressed as

$$I_c = f \cdot \int_0^{t_e} \int_{\lambda_{min}}^{\lambda_{max}} S(\lambda) \cdot T(\lambda) \cdot F_c(\lambda) \cdot d\lambda \cdot dt,$$

where $I_c$ is the image intensity at a given pixel. All observable, biologically relevant, processes are slow compared with the camera exposure time (usually in a few ms) and, therefore, the outer integral can be eliminated. More importantly, let variable $f$, which reflects the dependence between the spectral energy and the sensor response, be 1. The multiplication inside the internal integral is commutative, which allows us to introduce an effective incoming light $L_c(\lambda) = S(\lambda) \cdot F_c(\lambda)$. These all mathematical treatments give the reduced equation for the measurement process as

$$I_c = \int_{\lambda_{min}}^{\lambda_{max}} L_c(\lambda) \cdot T(\lambda) \cdot d\lambda.$$  

(2)

Intentionally, this simple model does not include any properties of optics, sophisticated models of light-matter interactions, and spatial components (focus, sample surface, etc.). The aim of the method is to describe an observed object in the best way, with minimal assumptions on its nature or features.

**Model extension for continuous media**

In order to extract a transparency profile from the proposed model, one has to solve an inverse problem for a system of 3 integral equations (in case of a 3-channel, RGB, camera). This cannot be solved directly, since the model is heavily underdetermined. (In this text, by terms "transparency" and "spectrum" we mean "quasi-transparency" and "quasi-spectrum" since this method determines only the properties of a microscopy image which are similar to the transparency spectra but not the transparency itself.)

Additional information can be squeezed from the physical meaning of the observed image – neighbouring pixels are not fully independent. The observed object usually has no purely vertical parts (which is quite typical for cell-like structures) and other Z-axis related changes are not fast. If this holds, the image can be treated as a continuous projection of the object’s surface (in optical meaning) onto the camera sensor. In this case, the neighbouring pixels correspond to neighbouring points in the object.

In addition, let us assume that the object’s volume can be divided into subvolumes in a way that the transparency spectra inside a subvolume will be spatially continuous (in L2
meaning). This assumption is quite weak, because it can be satisfied only if the volumetric image has a subvolume of the size which is equal to the voxel size.

For biological samples which show almost no strong gradients of structural changes holds that the pixel demarcates the projected image. Formally, this criterion can be expressed as

$$\int_{\lambda_{\text{min}}}^{\lambda_{\text{max}}} |T(\vec{r}, \lambda) - T(\vec{r} + \vec{u}, \lambda)|^2 d\lambda < q, \quad \forall |\vec{u}| < \epsilon,$$

(3)

where \(\vec{u}\) is a random vector and \(q, \epsilon\) are small numbers. This equation closely resembles the Lyapunov stability criterion. The \(\epsilon\) reflects the neighbourhood size and \(q\) is related to the degree of discontinuousness. It can be violated, if \(\vec{u}\) crosses a border between objects, but not inside a single object.

**Optimization procedure**

For pixel \(m\), the combination of optimization criteria in Eqs. 2–3 gives (in discrete form)

$$F_m = \sum_{c=1}^{C} e^{\int_{\lambda_0}^{\lambda_w} L_c(\lambda) \cdot T_m(\lambda) d\lambda - I_m} - C + \frac{1}{N} \sum_{n \in N_m} G_{mn} \sum_{i=1}^{w} [T_m(\lambda_i) - T_n(\lambda_i)]^2,$$

(4)

where \(C\) is the number of channels, \(w\) is the number of discrete wavelengths, \(G_{mn}\) is a measure of discontinuousness between pixels \(m\) and \(n\). The \(N_m\) is a set of points, which have the Euclidean distance to the pixel \(m\) equal or less than \(T_{ED}\). Authors used \(T_{ED} = 1\), but a larger neighbourhood may improve convergence speed. The integral in the first part of Eq. 4 is supposed to be solved numerically. Authors used the Simpson integration method\(^{15}\) with discretization \(||\lambda_i|| = 48\).

The trickiest issue in Eq. 4 is calculation of discontinuousness measure \(G_{mn}\). We defined it as

$$G_{mn} = \frac{1}{L_{mn}} \prod_{k \in B_{mn}} \{[E_k = 0] + [E_k \neq 0] \cdot (1 - T_b) \cdot (1 - D_k)\},$$

(5)

where \(D_k\) is a central gradient in pixel \(k\), \(T_b\) is a bias parameter (authors used \(T_b = 0.9\)), and \(B_{mn}\) is a set of points, which form lines between pixels \(m\) and \(n\). The set of such points is calculated using the Bresenham algorithm.\(^{16}\) The \(E_k\) indicates whether pixel \(k\) is classified as an edge. For this we used the Canny edge detection algorithm\(^{17}\) applied to a gradient matrix smoothed by a 2D Gaussian filter with the standard deviation equal\(^{18}\) to 0.5.

The gradient calculation is different for the first and the further iterations. In the first iteration, there is no valid spectral guess and the gradients and the edge detection are calculated for the original image. The used edge detection algorithm requires a single-channel
(grayscale) image, however, the input image is RGB. We used the principal component analysis (PCA)\textsuperscript{19,20} and retained only the first principal component in order to obtain the maximal information on the grayscale representation of data.

In the non-first iterations, there is a spectral guess and, instead of the gradient, we used the cross-correlation with zero lag: $D_k = T_{k-1}(\lambda) \ast T_{k+1}(\lambda)$. The vertical and horizontal gradient were merged by the Euclidean norm.

For numerical optimization of Eq. 4, the covariance matrix adaptation evolution strategy (CMA-ES)\textsuperscript{21} was proved to be a suitable robust global optimization method.\textsuperscript{22} Due to the mean-field nature of the second part of Eq. 4, the method is iterative with, usually, 20–40 iterations to converge. In each iteration step and for each pixel, the minimization is conducted until a predefined value of loss function is achieved. Different schedules of tolerance changes can be applied, authors used the simplest one – linear decrease. The algorithm flow chart is presented in Fig. 2.

**RESULTS**

The method essentially required only 3 specific inputs: an image, incoming light spectra, and camera filter profiles. The sample has to obey 3 assumptions: localized gradients, reasonable flatness, and linear response. If it holds, the obtained results will be in agreement with physical properties of the medium. In order to show the capacity of the method, we applied it to images of L929 mouse fibroblasts recorded using video-enhanced bright-field wide-field light microscope with through-focusing. For determination of the best focal position, we used the graylevel local variance.\textsuperscript{23} The effective light spectrum as the result of multiplication of the light source spectrum by the camera filter transparency curves is shown in Fig. 3b. The original raw image is shown in Fig. 3a, and looks greenish due to the prevalence of green color in incoming light spectrum.

As clearly seen in Fig. 3d, the method has a non-trivial convergence behaviour with the local minimum at iteration 6 and the global maximum at iteration 13. This behaviour is not related to the tolerance change schedule, which is linearly decreasing until iteration 10, and then is kept constant at value 0.01. We have not investigated the reason for this course deeply, but it is definitely repeatable for all the tested measurements. A natural way of visual verification of an image of transparency spectra is artificial illumination. We used a spectrum of the black body at $T = 5800$ K according to the Planck Law, see Fig. 3c. The transformed image is quite similar to the raw data, which supports the method validity. To obtain such an image, we multiplied each pixel’s transparency spectra by the illumination spectrum and the CIE standard matching curves. The integrals of the corresponding curves gave coordinates in the CIE 1931 color space.
In order to evaluate information content of the reconstructed spectrum, we have to reduce the dimensionality. Amount of data per image is quite big, which makes most advanced methods infeasible. Therefore, we picked the basic methods: PCA, Factor Analysis (FA) and Non-Negative Matrix Factorization (NNMF). The visualization based on these methods is shown in Fig. 4.

Visually, the PCA yields more information, whereas the FA and the NNMF rather yield cluster-like solutions, which can be helpful in cell objects segmentation. To get some other practical use of the spectra, another additional step was required – clustering. We used Self-Organizing Map with 9 classes, see Fig. 4d. The classes are reordered according to their similarity in a way that the classes with similar numbers correspond to the similar spectra. As measure of similarity we used the Euclidean norm of the vector difference in the PCA space. The mean spectra of the classes are plotted in Fig. 4e.

At the ends of spectral intervals, the method does not work well due to the lack of color resolution in the input data, see Fig. 3b. It supports the trivial criterion of spectral reconstruction in a certain interval of the wavelength: at least two channels of effective light have to be non-zero.

To show the method stability, we applied the method independently to two images of different cells of the same species (Fig. 5). The cell in Fig. 5a is better focused which is slightly reflected in its spectra: magenta and black classes are sharper in case of an in-focus image. Overall, the classes (and their spectra) are similar, which supports applicability of this approach. Visually, classes of cell borders, interiors, and steep parts correspond to the same part of the cell. However, we have not identified the detected cell parts yet due to the absence of a reference segmentation.

CONCLUSIONS

The proposed method aims at a very challenging problem, which cannot be solved precisely even in theory: some information is irrecoverably lost. The method arises from very general assumptions on the measurement system. The method does not rely on any light-media interaction model or physical properties of the system, which makes this method quite universal. The obtained spectra are applicable in practice for visualization and automatic segmentation task. We intentionally did not consider questions of voxel spectrum, Z-stack spectral behaviour, and meaning of the compromised focus in order to keep the method and its application simple. We pose the described method as an ultimate information squeezing tool, which is a nearly model-free way how to compress the color and spatial information into representation of the physically relevant features. We believe that, in the future, the method will find its use in robust, mainly, qualitative (bio)chemical analysis.
MICROSCOPY DATA ACQUISITION

Sample preparation
A L929 (mouse fibroblast, Sigma-Aldrich, cat. No. 85011425) cell line was grown at low optical density overnight at 37°C, 5% CO₂, and 90% RH. The nutrient solution consisted of DMEM (87.7%) with high glucose (>1 g L⁻¹), fetal bovine serum (10%), antibiotics and antimycotics (1%), L-glutamine (1%), and gentamicin (0.3%; all purchased from Biowest, Nuillé, France).

Cells fixation was conducted in a tissue dish. The nutrient medium was sucked out and the cells were rinsed by PBS. Then, the cells were treated by glutaraldehyde (3%) for 5 min in order to fix cells in a gentle mode (without any substantial modifications in cell morphology) followed by washing in phosphate buffer (0.2 mol L⁻¹, pH 7.2) two times, always for 5 min. The cell fixation was finished by dewatering of the sample in a concentration gradient of ethanol (50%, 60%, and 70%) when each concentration was in contact with the sample for 5 min.

Bright-field wide-field videoenhanced microscopy
The fixed cells were captured using a custom-made inverted high-resolved bright-field wide-field light microscope enabling observation of sub-microscopic objects (ICS FFPW, Nové Hrady, Czech Republic). The optical path starts by two Luminus 360 light emitting diodes charged by the current up to 5000 mA (in the described experiments, the current was 4500 mA) which illuminate the sample by series of light flashes in a gentle mode and enable the videoenhancement.

The microscope optical system was further facilitated by infrared 775 nm short-pass and ultraviolet 450 nm long-pass filters (Edmund Optics). After passing through a sample, light reached an objective Nikon (LWD 40×/0.55, Ph1 ADL, ∞/1.2, WD 2.1). A Mitutoyo tubus lens (5×) and a projective lens (2×) magnify and project the image on a JAI camera with a 12-bpc color Kodak KAI-16000 digital camera chip of 4872×3248 resolution (camera gain 0, offset 300, and exposure 293.6 ms). At this total magnification, the size of the object projected on the camera pixel is 32 nm. The process of capturing the primary signal was controlled by a custom-made control software. The z-scan was performed automatically by a programmable mechanics with the step size of 100 nm.

Microscopy image data correction
The acquired image data were corrected by simultaneous calibration of the microscope optical path and camera chip as described in Suppl. Material 1. In this way, we obtained the most informative images on spectral properties of the observed cells.
LIST OF SYMBOLS

\( B_{mn} \)  set of pixels that form lines between pixels \( m \) and \( n \)  
\( c \)  colour of a camera filter or an image channel; for colour camera \( c = \{ \text{red, green, blue} \} \)  
\( C \)  number of image channels  
\( D_k \)  central intensity gradient in pixel \( k \in B_{mn} \) in calculation of \( G_{mn} \)  
\( E \)  energy absorbed by a camera sensor during an exposure time \( t_e \)  
\( E_k \)  parameter in computation of \( G_{mn} \) which indicates if the pixel \( k \) is classified as an region edge  
\( f \)  variable which reflects a dependence between the spectral energy and the sensor response; \( f = 1 \)  
\( F_c(\lambda) \)  spectral quantum efficiency of a camera filter \( c \)  
\( F_m \)  spectral quantum efficiency of a pixel \( m \)  
\( G_{mn} \)  measure of discontinuousness between pixels \( m \) and \( n \)  
\( i \)  label of a discrete wavelength; \( i = \{1, 2, \ldots, w\} \)  
\( \text{iter} \)  iteration  
\( \text{it\_max} \)  maximal iteration (predetermined)  
\( I_c \)  pixel intensity at colour channel \( c \)  
\( k \)  pixel in the set \( B_{mn} \)  
\( L_c \)  light effectively incoming onto a camera sensor, i.e. onto a camera filter  
\( m, n \)  pixel labels  
\( N \)  number of pixels in the set \( \mathbb{N}_m \)  
\( \mathbb{N}_m \)  set of pixels with the Euclidean distance to the pixel \( m \) equal or less than \( T_{ED} \)  
\( q \)  parameter related to the degree of discontinuousness in spectral regions  
\( \vec{r} \)  position vector for a pixel at coordinates \( (x, y) \)  
\( S(\lambda) \)  light spectrum of a light source  
\( t_e \)  camera exposure time  
\( T \)  thermodynamic temperature; kelvin [K]  
\( T_m(\lambda_i) \)  transparency spectrum of pixel \( m \) at wavelength \( \lambda_i \)  
\( T_n(\lambda_i) \)  transparency spectrum of pixel \( n \) at wavelength \( \lambda_i \)  
\( T(x, y, \lambda) \)  transparency spectrum of a medium at each pixel in general  
\( T_b \)  bias parameter in computation of \( G_{mn} \); \( T_b = 0.9 \)  
\( T_{ED} \)  threshold for the selection of the neighbourhood of pixel \( m \), i.e., the Euclidean distance between pixels \( m \) and \( n \); \( T_{ED} = 1 \)  
\( \vec{u} \)  change of a pixel position vector  
\( w \)  number of discrete wavelengths  
\( x, y \)  vertical and horizontal pixel coordinates  
\( \epsilon \)  parameter which reflects the studied pixel’s neighbourhood size in general  
\( \lambda \)  light wavelength; nanometer [nm]
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COMPETING FINANCIAL INTERESTS  The authors declare that they have no competing financial interests.

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Supplemental Data

Data Availability Statement
Supplementary Material:
Simultaneous Colorimetric Calibration of Digital Camera and Optical Path

The calibration of the optical path and camera chip together with image (raw files) correction was performed in the following steps:

1. Experimental Part:
   a. Using a microscope photocamera, images (raw files) of the set of gray layers covering a 2 mm thick glass (type Step ND Filter NDL–10S–4) on a microscope stage were captured successively. The image of zero and the highest intensity was acquired in dark and without any layer, respectively.¹ The microscope and microscope camera was arranged and set up the same as in the biological experiment.
   b. The microscope objective was replaced for a fibre spectrophotometer Ocean Optics USB 4000 VIS-NIR-ES by which the spectra (Fig. S1a) of the series of the gray layers (including the zero and the highest spectrum) relevant to the gray images were measured successively.²

2. Computational Part:
   a. Construction of a calibration curve for each pixel
      (1) Red, green, and blue camera Bayer filter profiles (supplied by a camera producer; Fig. S1b) were transformed into the numeric format. The results are saved in the spectrum.txt file of the calibration data.
      (2) The light spectra captured by each pixel of the colour camera filter were obtained by multiplication of the measured incident spectra by the digitalized camera filter.
      (3) For each gray layer, a total number of photons (i.e., counts) captured by each pixel was calculated as an integral (trapezoidal rule) of the area below the respective incident spectrum (Fig. S1c).
      (4) For each pixel of the mean calibration image (see item 1a), a calibration point was constructed (Fig. S1d) as a dependency of the total number of photons reaching the pixel on the pixel intensity. Each pair of two consecutive calibration points was fitted by linear interpolation.
   b. Intensity correction of the series raw file
      (1) Using the calibration relation of the relevant section of the calibration curve, the intensity of each pixel of the testing image was converted to values that, after the calibration of the fiber spectrophotometer, correspond to the total number of photons (in double precision numbers).
      (2) For further image operations, the resulted matrix was transferred into a 14-bit PNG format.

The preparation of the calibration curve (item 2a) is the content of Algorithm 1. The series raw files were then corrected using Algorithm 2 described in item 2b. Both algorithms are implemented in the VerCa software (Institute of Complex Systems, Nové Hrady, CZ).

¹ The image of each gray layer was taken at least in 6 parallels. A mean calibration image was computed as an intensity average for each pixel through all parallel measurement.
² The spectrum of each gray layer was taken at least in 6 parallels from which a mean spectrum was obtained.
Figure S1. (a) Light spectra of grayscale layers measured by a fiber spectrophotometer, (b) declared spectra of RGB camera filters, (c) calculated spectra of incoming light reaching the blue camera channel. Integral under the curve (c) was used as a calibration value for the construction of the calibration curve. (d) Calibration curves for selected blue camera pixels lying in the same column (pixel indices are depicted).
Input: sQE as a quantum efficiency spectrum of one colour camera channel; sFt as a spectrum of a stack of gray layers (N-item folder); iFt as a relevant colour channel of the stack of gray layers (N-item folder);

Output: k as a matrix of the slopes of the linear sections of the calibration curve; s as a matrix of the shifts of the linear sections of the calibration curve; int as a matrix of the intensities which demarcates the ranges of the linear sections of the calibration curve;

\[ A = \text{zeros}(N,1); \] % create an empty (zero) N-element vector

for \( i = 1 \) to \( N \) do

\[ \text{sFt} = \text{readSp}(i); \] % read a spectrum sFt for (i) gray layers

\[ \text{wSp} = \text{sFlt} .* \text{sQE}; \] % for each wavelength, weight the spectrum sFt by the spectrum sQE

\[ A(i) = \text{integrateSpectrum}(\text{wSp}); \] % integrate the area under the weighted spectrum to obtain a total number of photons reaching the colour channel of the camera chip

end

\[ \text{int} = \text{zeros}(N, nPx); \]
\[ k = \text{zeros}(N, nPx); \]
\[ s = \text{zeros}(N, nPx); \]  % create empty (zero) matrices of the output calibration files (i.e., of the calibration parameters)

for \( i = 1 \) to \( N - 1 \) do

\[ \text{iFt1} = \text{readIm}(i); \]
\[ \text{iFt2} = \text{readIm}(i + 1); \]  % read raw image files of relevant colour channel for (i) and (i+1) gray layers

for \( j = 1 \) to \( nPx \) do

\[ \text{int}(i, j) = \text{iFt1}(j); \] % read and save the first edge point of the section of the calibration curve

\[ k(i, j) = (A(i + 1) - A(i))/(\text{iFt2}(j) - \text{iFt1}(j)); \]
\[ s(i, j) = A(i) - k(j) .* \text{iFt1}(j); \] % calculate and save a slope and a shift of the relevant section of the calibration curve

end

end

Algorithm 1: Construction of the calibration curve and creation of the calibration file for one colour channel.
Input: $I$ as an original $(nPx)$-resolved uncorrected raw image file; $\text{int, } k, \text{ and } s$ as a content of the calibration file (see Algorithm 1)

Output: $cI$ as a corrected image

$cI = I .* 0; \quad % create an empty (zero) matrix of the size of the I$

for $j = 1$ to $nPx$ do
    if $I(j) < \text{int}(2,j)$ then
        $cI(j) = k(1,j) .* I(1,j) + s(1,j);$
    else if $I(j) \in \langle \text{int}(2,j), \text{int}(3,j) \rangle$ then
        $cI(j) = k(2,j) .* I(2,j) + s(2,j);$
    else if $I(j) \in \langle \text{int}(3,j), \text{int}(4,j) \rangle$ then
        $cI(j) = k(3,j) .* I(3,j) + s(3,j);$
    else
        $cI(j) = k(N,j) .* I(N,j) + s(N,j);$
    end
end

% for intensity of each pixel of the image I, find the relevant linear section of the calibration curve and use its mathematical relation to recalculate this intensity to the total number of photons

Algorithm 2: Image correction of one colour channel.
Figure 1: Measurement process model.

Figure 2: Flow chart of the method. The magenta lines denote the routes for the 1st iteration. The red and blue lines show the direct and indirect feedback between iterations, respectively.
Figure 3: A raw image from the bright-field light microscope (c) combined with the effective light spectra (b) was used for optimization, convergence curve (e). The variation coefficient (d) shows self-organization of the model. After the transparency spectra reconstruction, the image can be viewed under arbitrary illumination such as the absolute black body with $T = 5800$ K, see (a).
Figure 4: The visualization of the transparency spectra by the PCA (a), FA (b), and NNMF (c). For all these reconstruction techniques, the 1st, 2nd, and 3rd component was treated as green, red, and blue channel, respectively. The FA was used for the SOM classification (d), which mean spectra are plotted in (e).

Figure 5: Independent SOM-based clusterization for two cells (a, b) and their corresponding spectra (d, e). Image c is original to spectral image b.