
CDReview Manual (v0.1.0)

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1. Introduction

CDReview is a software plugin for the image analysis software ImageJ (<http://imagej.nih.gov/ij>).

It is designed for testing and comparison of different mathematical colour deconvolution methods and for the creation of benchmark images.

The software is a supplement to the publication:

Haub, P. and Meckel, T. A Model based Survey of Colour Deconvolution in Diagnostic Brightfield Microscopy: Error Estimation and Spectral Consideration. Sci. Rep. 5, 12096; doi: 10.1038/srep12096 (2015).

2. Installation

2.1 Requirements

- Microsoft Windows (currently tested on Windows 7 32/64 bit)
- ImageJ for MS Windows >= 1.49 (JRE 1.6.0)
- CDReview.jar (Plugin file)
- Spectra files

2.2 Installation procedure

- Unpack the CDReview_Installation_xxx.zip file to any folder
- From the unpacked data copy the folder \CDReview into the folder `$ImageJ-Path$\plugins` in your ImageJ directory
- (optional: From the unpacked data copy the folder \CDReview_spectra to an arbitrary folder)
- Restart ImageJ

2.3 Source Code

The source code is provided in the _CDReview.jar archive. Use a zip software to unpack the jar file.

3. Usage

Open one of the provided example images and start **CDR_run** - the main plugin function.

3.1 Processing modes

CDR_run automatically selects the processing mode (**PMode**) depending on the image type. Grayscale image stacks are interpreted as concentration images and processing mode I or II is used. Single channel RGB colour images are processed in mode III.

PMode I: Creation of multiple stain benchmark RGB image and direct deconvolution

Open a 2- or 3-channel concentration image (grey channel image stack) and start **CDR_run** from the ImageJ menu 'Plugins\CDReview'

Select the spectra folder (\CDReview_spectra)

Select spectra and additional options in the user dialog

(2- and 3-channel 32bit test images are provided with the plugin)

PMode II: Creation of single stain benchmark RGB image

Open a concentration image (single grey channel image or grey channel image stack) and start **CDR_run** from the ImageJ menu 'Plugins\CDReview'

Selected spectra folder (\CDReview_spectra)

Select spectra for Stain 1 in the user dialog (Stain 2 and 3 must be empty)

(First channel from grey channel image stack is used for image creation.)

PMode III: Deconvolve RGB image

Open a single RGB colour image and start **CDR_run** from the ImageJ menu 'Plugins\CDReview'

Selected spectra folder (\CDReview_spectra)

Select spectra and additional options in the user dialog

The image currently selected in ImageJ will be used for processing.

If the plugin is used repeatedly, e.g. for testing different parameter settings, make sure that the correct image is selected BEFORE starting the plugin!

3.2 Working principle

In PMode I and II an image formation is simulated according to Lambert-Beer law (see publication), a benchmark image is created and in mode I the intermediate 32bit absorbance values are directly deconvolved.

In PMode III absorbance values are calculated from the 8bit RGB information and subsequently deconvolved.

The processing (simulation of image formation and deconvolution) is based on the selected spectra. The stain vectors are determined for the specified stains based on the selected illumination and sensor spectra.

For all PModes all 3 spectra must be selected for the illumination and sensor channels.

Depending on the number of selected stains the processing modes are:

Image Type \ Number of stains	Single grey channel image	Grey channel image stack	Single RGB image
1	PMode II		PMode III
2	PMode I		
3			

In any case Stain 1 must be specified.

The colour deconvolution can be performed based on an algebraic solution (mode 'DET'), based on a direct solver per LU decomposition (mode 'LU') or based on a QR decomposition solver (mode 'QR').

In case of 2-stain processing the calculations can be based on absorbance projection onto the absorbance planes 'GB', 'GR' or 'RB'. Alternatively a third stain vector perpendicular the two stain vectors of the selected stains can be used by activating the option 'Use pseudo3Stain'.

3.3 User dialog

After starting **CDR_run** and selecting the spectra folder a parameter dialog (Fig. 1) is displayed. In this dialog the spectra and additional parameters can be specified.

The spectra folder and the selected spectra are stored as preferences and will be reloaded when **CDR_run** is used repeatedly.

Available parameters

// Spectra

Stain 1/2/3

Stain spectra

(1 stain mode (PMode I): Stain1 must be specified.)
(2 stain mode (PMode I or III): Stain1 and second stain must be specified.)
(3 stain mode (PMode I or III): All 3 stains must be specified.)

Illumination 1/2/3

Illumination spectra

(All 3 channels must be specified.)

Sensor 1/2/3

Sensor channel spectra

(All 3 channels must be specified.)

// Processing options

Use normalized vectors

Stain vectors are normalized to unit length before processing

Use pseudo3Stain

Create third stain vector perpendicular to vectors of selected stains and use 3-Colour deconvolution
(Operative only if two stains.)

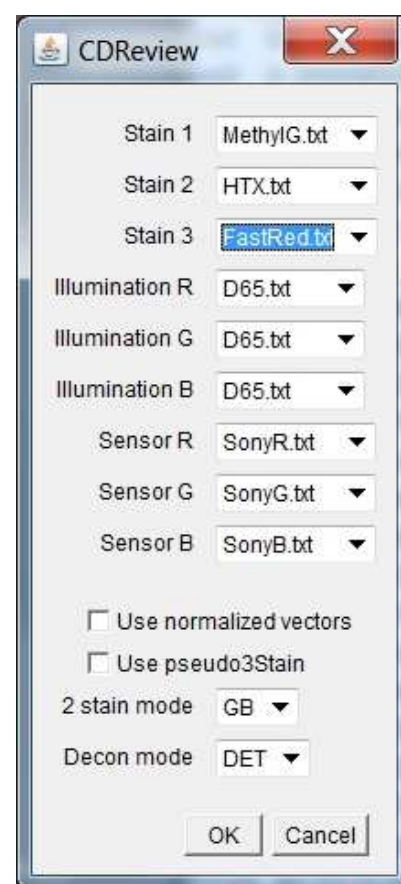


Fig. 1: User Dialog

2 stain mode

Selection of the absorbance planes (GB, GR or RB) for 2-stain deconvolution
(Operative only if two stains are selected.)
(Operative only in Decon mode 'DET' and 'LU'.)

Decon mode

Mathematical deconvolution methods
(Algebraic solution : **DET**)
(LU decomposition : **LU**)
(QR decomposition : **QR**)

3.4 Typical settings

The settings shown in Fig. 2 and 3 are the typical settings used for polychromatic D65 illumination and quasi-monochromatic RGB LED illumination. (For more information see publication.)

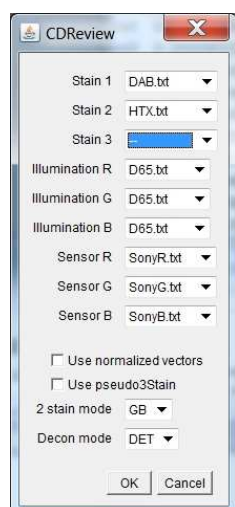


Fig. 2: Spectra selection for D65 illumination with Sony Color CCD sensor (2 stain mode)

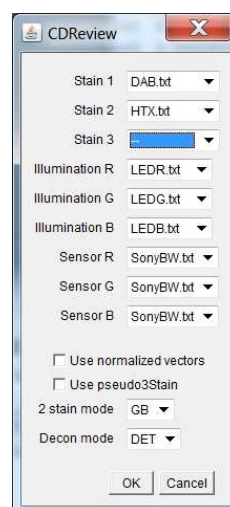


Fig. 3: Spectra selection for RGB LED illumination with Sony B/W CCD sensor (2 stain mode)

3.5 Examples

As an example, the 3-stain test pattern image provided with the plugin is shown in Fig. 4. The 32bit 3channel image stack is displayed by applying a colour lookup table (LUT). The stain channels are displayed row by row.

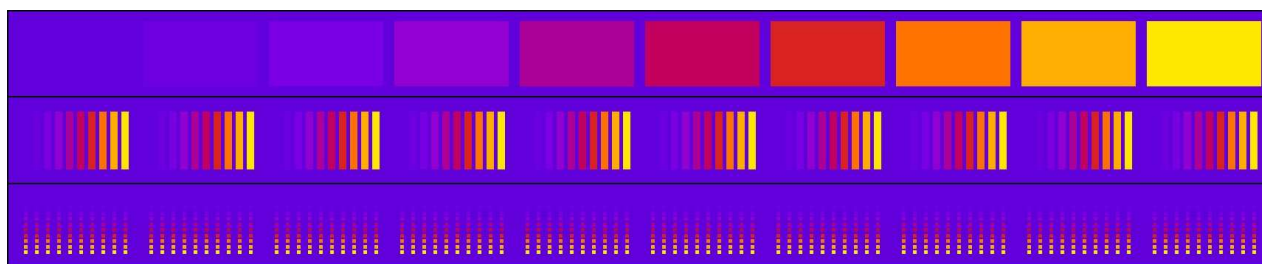


Fig. 4: 3-stain test image displayed with colour LUT 'Fire' (stain channels row by row)

This 3-stain test pattern image was deconvolved with the settings: MethylG/HTX/FastRed; D65; SonyR/SonyG/SonyG; DET. The result is shown in Fig. 5 with the LUT and display range from Fig. 4.

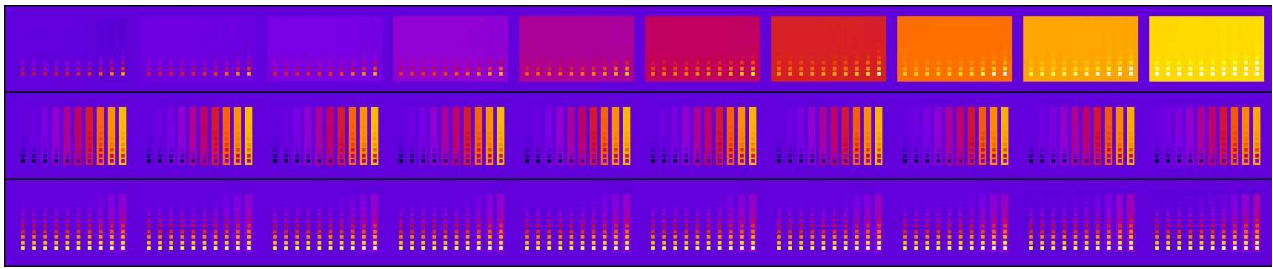


Fig. 5: Processed (deconvolved) 3-stain test image (stain channels row by row)

The deconvolved image differs from the original image due to a deconvolution error, as can be seen by visual comparison of Fig. 4 and 5.

To ensure correct visual evaluation results the display range of both images has to be set to the minimum and maximum grey values of both of the images. The display range can be adjusted with the 'Set' button in ImageJ 'Image%Adjust%Brightness/Contrast' function. The adequate display range can be propagated to both images.

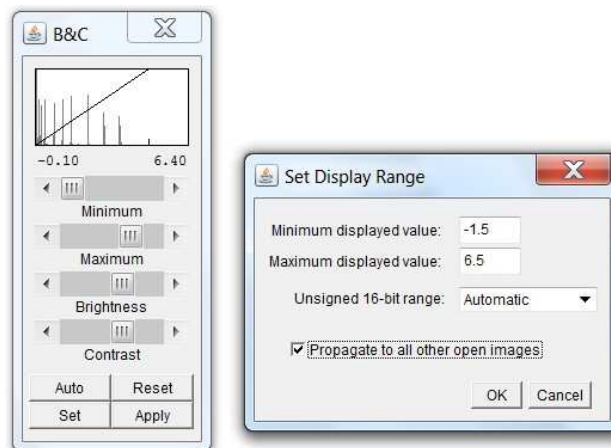


Fig. 6: Set Display Range / Propagate to all open images

The original and the deconvolution image can also be compared by calculating the intensity differences with the ImageJ Image Calculator.

In Fig. 7 the resulting RGB benchmark image is shown. Those benchmark images will be created automatically in PMode I by transformation and white balancing of the simulated sensor signals into linear RGB colour values.

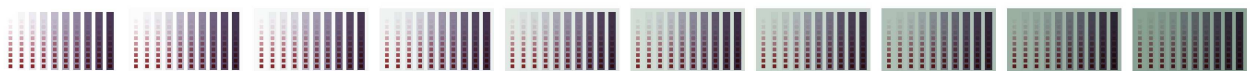


Fig. 7: RGB benchmark image (concentration channels from Fig. 4 are used for creation)

The 2-stain example discussed in the publication can be reproduced by opening and processing the provided 2- stain test image.

3.6 Spectral files

The spectral files provided with the plugin contain optical density characteristics of different stains, relative radiation power of different illuminations and the relative response of different CCD sensors. (For detailed information see publication.)

The provided spectra are represented as 60 spectral values in 5nm steps ranging from 405nm to 700nm.

The plugin is not limited to the provided example spectra. Own spectra with higher spectral resolution can be used for processing. Spectra used in combination must contain identical number of spectral values!

The spectra files are organized in the subfolders \stain, \illumination and \sensor. Added spectra for stains, illuminations and sensors have to be stored in these subfolders accordingly.

3.7 Create concentration pattern image

A test pattern image, as shown in Fig. 4, can be created with the command `CDR_CreatePatternImage` from ImageJ menu 'Plugins\CDReview. The concentration variations can be adapted to user conditions in the source code. For more details refer to the source code.

4. License

```

/*****
 *
 * License
 *
 * PlugIn:           CDReview
 *
 * Description:      CDReview - Software for testing of colour deconvolution methods
 *                  and for creation of benchmark RGB colour images
 *
 *                  This software is provided as a supplement to:
 *
 *                  Haub, P. and Meckel, T. A Model based Survey of Colour Deconvolution
 *                  in Diagnostic Brightfield Microscopy: Error Estimation and Spectral
 *                  Consideration. Sci. Rep. 5, 12096; doi: 10.1038/srep12096 (2015).
 *
 * @author:          Peter Haub, 2015
 *
 * Copyright(C)      2015 Peter Haub
 *                  phaub@dipsystems.de
 *                  www.dipsystems.de
 *
 * Version:          0.1.0 - July 2015
 *
 * License:          This program is free software; you can redistribute it and/or modify it
 *                  under the terms of the GNU General Public License as published by the
 *                  Free Software Foundation; either version 2 of the License, or (at your
 *                  option) any later version.
 *
 *                  This program is distributed in the hope that it will be useful, but
 *                  WITHOUT ANY WARRANTY; without even the implied warranty of
 *****/

```

```
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*      with this program; if not, write to the Free Software Foundation, Inc.,
*      51 Franklin St, Fifth Floor, Boston, MA 02110-1301 USA.
*
* ***** /
```

4.1 Spectral Data

The spectral data distributed together with CDReview software are derived from following sources:

CIE spectral norm values (Commission International d'Éclairage). Available at <https://law.resource.org/pub/us/cfr/ibr/003/cie.15.2004.tables.xls> (Accessed: 22nd May 2014)

Cree® XLamp® XP-E LED data sheet. Available at <http://www.cree.com/~media/Files/Cree/LED%20Components%20and%20Modules/XLamp/Data%20and%20Binning/XLampXPE.pdf> (Accessed: 06th June 2014)

McNamara, G. 2002. Chromogen Spectra. Los Angeles: CHLA Image Core. Available at http://home.earthlink.net/~geomcnamara/spectra_links.htm (Accessed: 28th May 2014)

Sony ICX285AQ Color CCD sensor data sheet. Available at <http://www.sony.net/Products/SC-HP/datasheet/01/data/E01420B3Z.pdf> (Accessed: 26th March 2014)