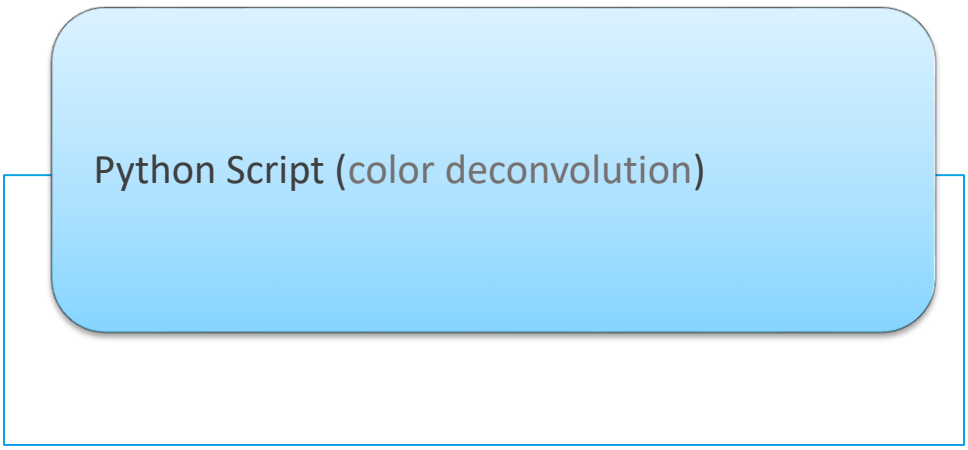
Application Note #31

How to perform : «Colour deconvolution: stain unmixing in histological imaging»

The application-note purpose is to guide the user in applying the stain unmixing technique to IHC color images.
The colour deconvolution algorithm enables to decompose an RGB image into channels representing the optical absorbance and transmittance of the dyes when their RGB representation is known. The algorithm was developed to deconvolve the color information acquired with red-green-blue (RGB) cameras and to calculate the contribution of each of the applied stains based on stain-specific RGB absorption. The algorithm was tested using different combinations of diaminobenzidine, hematoxylin and eosin at different staining levels. The colour deconvolution is useful to unmix dyes in images where colours mix subtractively (bright field microscopy using histological stains, water colours or printed material using transparent inks). It is not suitable for fluorescence microscopy (fluorophores mix additively) or reflectance images.

Application Flowchart



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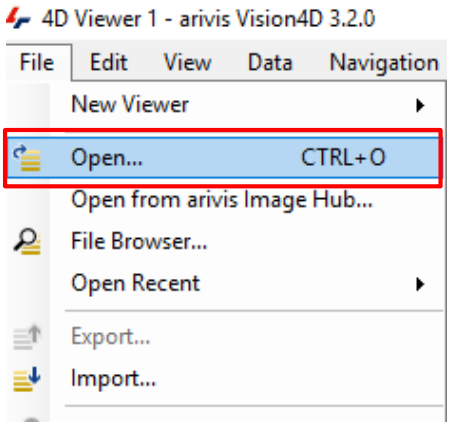
NOTE:
This script implements the method described in the following paper: Ruifrok, A.C. & Johnston, D.A. (2001), "Quantification of histochemical staining by color deconvolution", *Anal. Quant. Cytol. Histol.* 23: 291-299, PMID 11531144

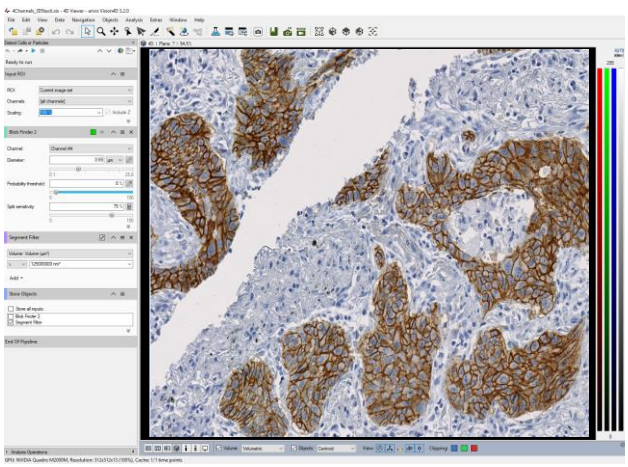
1. Open the working dataset on Vision4D

Step 1.1
Select the *Open..* item from the file menu.



Step 1.2
Select the dataset from the file browser.

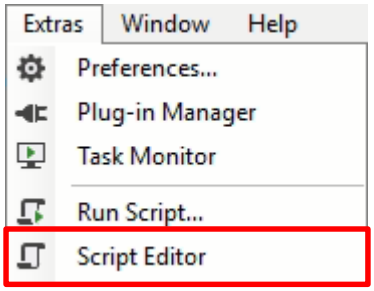
TIPS :
If the dataset is a 2D image, please visualized it using the 2D view mode. Please refer to the (arivis Vision4D Help) for more details



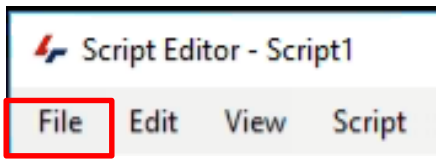
DETAILS:
The dataset is a multi dimensional, discrete, representation of your real sample volume. It can be structured as a Z series of planes (Optical sectioning) of multiple channels (dyes) in a temporal sequence of time points (located in several spatial positions).
Usually the dataset shows a single experimental situation (a complete experiment can be composed by several datasets). The datasets are available as graphic files saved in plenty of file formats (standard formats as well as proprietary formats)

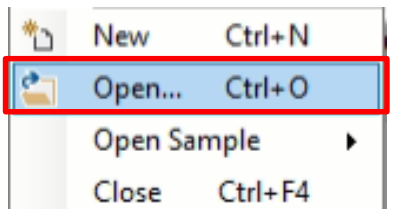
2. Load the Python Script

Step 2.1
Open Python Script Editor.
From the «***Extra***» menu, select the «***Script Editor***» item



Step 2.2
Load the "***ColorDec***" Python Script.
Browse the folder on which the file has been saved





Python script code usage rights.

```
# coding: utf-8
# NAME: Color Deconvolution conversion
# FILE: ColorDec_Rev2
# REVISION : 1.1.0 - 01/07/2021
# AUTHOR : Maurizio Abbate
# Copyright(c) 2021 arivis AG, Germany. All Rights Reserved.
#
# Permission is granted to use, modify and distribute this code,
# as long as this copyright notice remains part of the code.
#
# PURPOSE : Convert a RGB image in  Stain colors
#
# Tested for V4d Release : 3.4
#
# NOTE:
#   This script implements the method described in the following paper:
#   Ruifrok, A.C. & Johnston, D.A. (2001),
#   "Quantification of histochemical staining by color deconvolution",
#   Anal. Quant. Cytol. Histol. 23: 291-299, PMID 11531144
#
#   Landini G, Martinelli G, Piccinini F. Colour Deconvolution
#    - stain unmixing in histological imaging. Bioinformatics 2020,
#    https://doi.org/10.1093/bioinformatics/btaa847
# ------------------------------ External Package Import ----------------
```

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3. Set the Script features

Step 3.1

Set the RGB input channels. The channels count starts from 0.
The default RGB channels ordering is the following:
Red = Channel#1 -> (0)
Green = Channel#2 -> (1)
Blue = Channel#3 -> (2)

```
# ------------------------------------------------------------
# Skeleton section - Script
# INPUT_CHANNEL channel with the binary image
#       is only used to read/write the voxels
# ---> imageset.get_channeldata / imageset.set_channeldata
# ------------------------------------------------------------
INPUT_CHANNEL_R = 0
INPUT_CHANNEL_G = 1
INPUT_CHANNEL_B = 2
```

Step 3.2

STAINING : Set the active staining code.
Refer to the following list:

```
#  0 == HEMA_DAB
#  1 == HEMA_EOSIN
#  2 == HEMA_EOSIN2
#  3 == FEULGEN_LGRENN
#  4 == HEMA_EOSIN_DAB
#  5 == GIEMSA
#  6 == Fast Red, Fast Blue and DAB
#  7 == Methyl green and DAB
#  8 == Haematoxylin, Eosin and DAB (H&E DAB)
#  9 == Haematoxylin and AEC (H AEC)
# 10 == Azan-Mallory
# 11 == Alcian blue & Haematoxylin
# 12 == MASSON THRICROME
# 13 == Haematoxylin and Periodic Acid of Schiff (PAS)
```

```
STAINING = 0
```

NOTE :
Only the parameters located in the "*USER SETTING*" area can be modified. Don't change any other number, definition or text in the code outside this dedicated area.

Step 3.3

Set the INVERT_IMAGE == True, the staining channels are inverted.

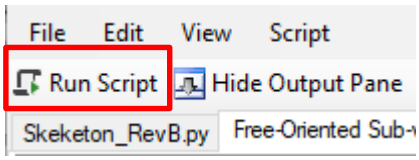
```
INVERT_IMAGE = True
```

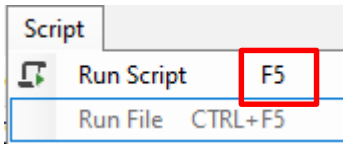
NOTE :
3 channels are always created. If a single stain is selected, only one channel is valid, the others are empty. The same if 2 stains are selected. One channel if left empty.

4. Run the Python Script

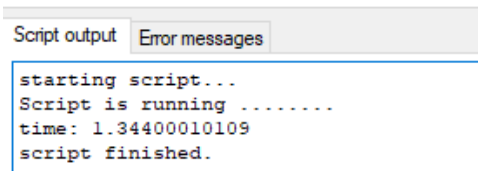
Step 4.1

Run the "***ColorDec***" Python Script pressing the "Run Script" button or pressing the F5 key.

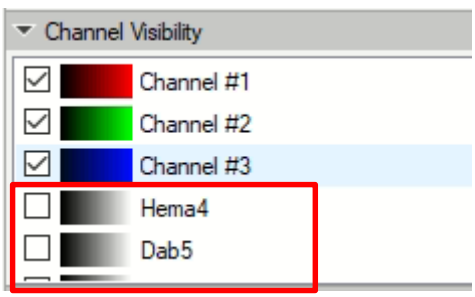




TIPS :
Activate, if not already displayed, the "Output Panel". The status of the script execution (errors including) will be visualized here



Three new channels are created. Depending on the staining combination selected, one or two channels can be empty. See here below



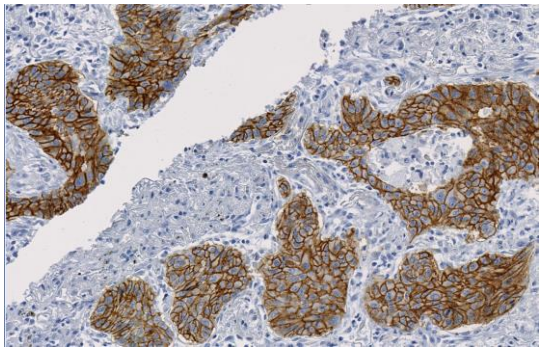
0 = Hematoxylin-diaminobenzidine (Dab)	***-> 1 channel empty***
1 = Hematoxylin- eosin	***-> 1 channel empty***
2 = Hematoxylin- eosin (alternative setting)	***-> 1 channel empty***
3 = Feulgen-Light Green	***-> 1 channel empty***
4 = Hematoxylin-eosin-diaminobenzidine (Dab)	***-> 0 channels empty***
# 5 == GIEMSA	***-> 2 channels empty***
# 6 == Fast Red, Fast Blue and DAB	***-> 0 channels empty***
# 7 == Methyl green and DAB	***-> 1 channel empty***
# 8 == Haematoxylin, Eosin and DAB (H&E DAB)	***-> 0 channels empty***
# 9 == Haematoxylin and AEC (H AEC)	***-> 1 channel empty***
# 10 == Azan-Mallory	***-> 1 channel empty***
# 11 == Alcian blue & Haematoxylin	***-> 1 channel empty***
# 12 == MASSON THRICROME	***-> 0 channels empty***
# 13 == Haematoxylin and Periodic Acid of Schiff (PAS)	***-> 1 channel empty***

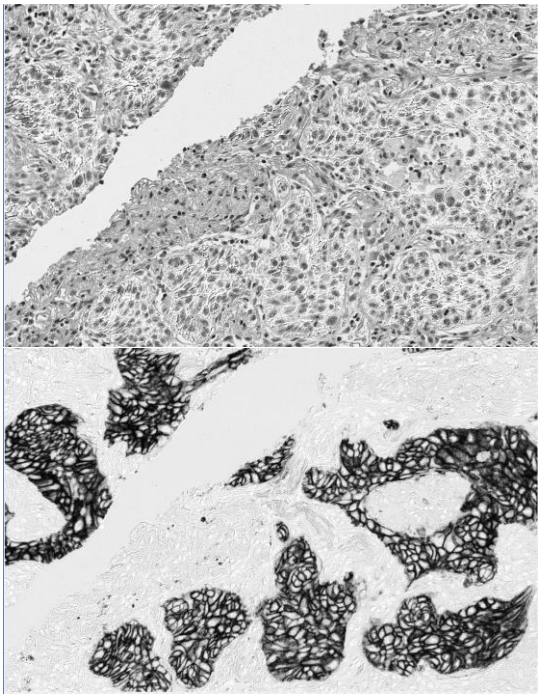
5. Results

Here some example of IHC color deconvolution.
On the left, the source RGB images, the staining channels on the right

Haematoxylin channel

Haematoxylin / Dab staining

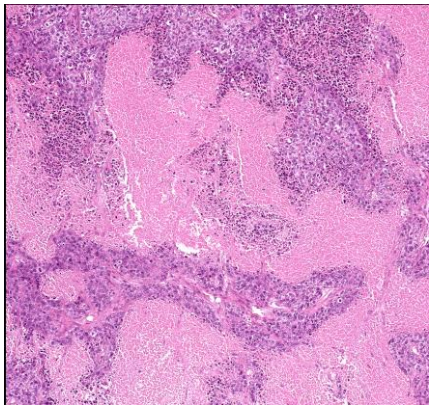


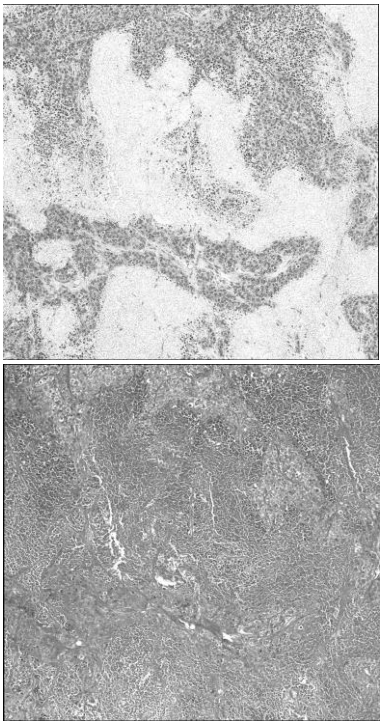


Dab channel

Haematoxylin channel

Haematoxylin / Eosin staining

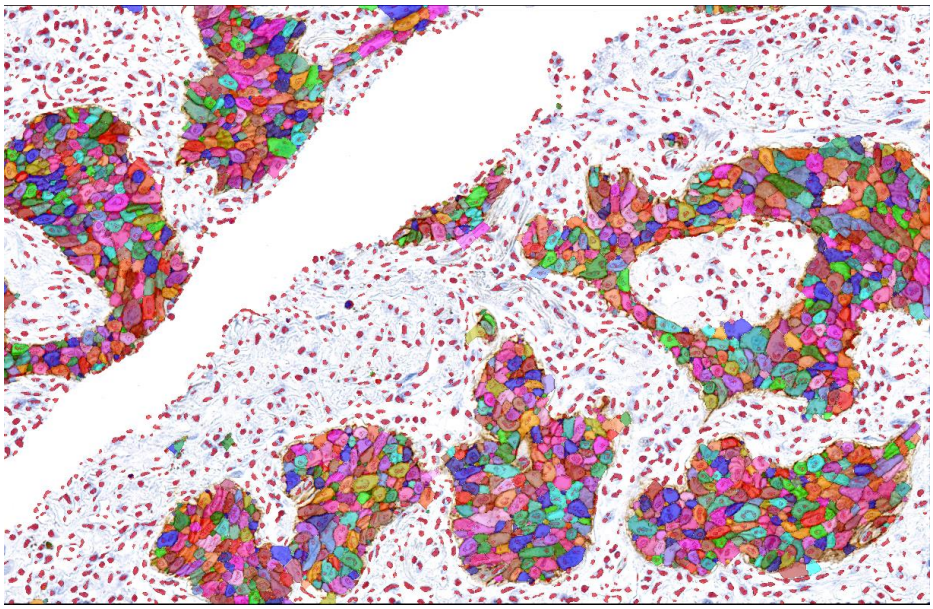


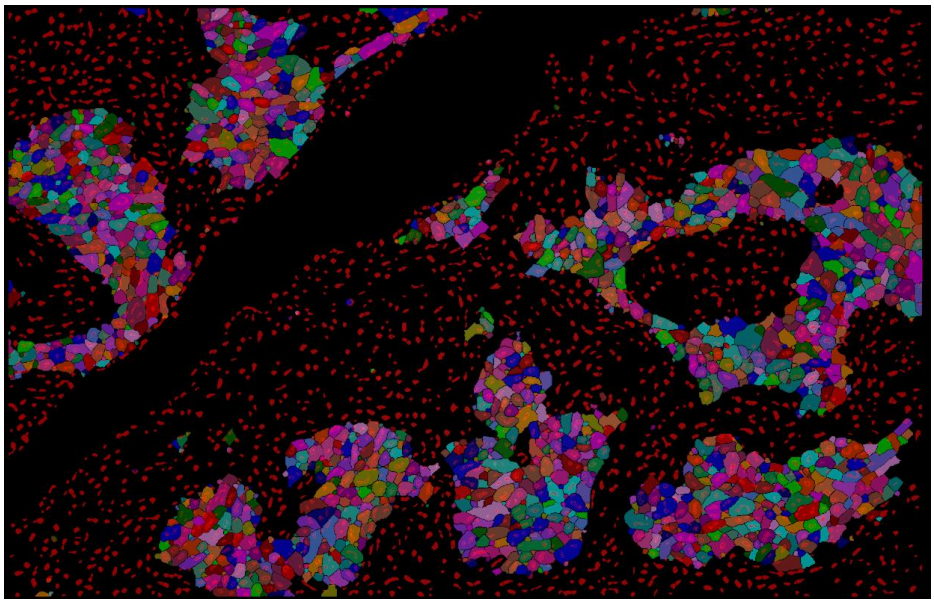


Eosin channel

5. Results

Analysis overview





NOTE :
The analysis has been executed using a dedicated pipeline applied on the converted staining channels.

Contact the arivis local area sales manager to get more information about how to get the python script mentioned here.

Contact the arivis application support to receive additional technical details about the topic described in the application note, or how to adapt the application workflow to your requirements.

"The quantitative analysis of the images represents the art of transforming a visual sensation into its schematic and discrete form allowing its univocal description, classification and mathematical and logical interpretation of its spatial and temporal components"

arivis AG, Am Kabutzenhof 21,
18057 Rostock, Germany

Email : support@arivis.com