

The Simple Method for Immunohistochemistry (IHC) Analyzing of Intensity using Corel Photo-Paint® with CMYK Split-channel

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Abstract: Immunohistochemistry (IHC) is a popular method to detect protein in the tissue. But, determining protein level in tissue is using ELISA which needs more cost. Although, the intensity of IHC color relates to an amount in tissue. Therefore, image processing to measure the intensity of IHC quantitatively was developed using much software. The common software used in Indonesia is Corel Photo-Paint® (CCP). This article described the simple method to measure the IHC intensity using CCP based on Cyan-Magenta-Yellow-Black (CMYK) Split channel. The conclusions were CCP could be used for image processing software to measure the IHC intensity. The higher intensity has fewer grey level, the lower intensity has a higher grey level

1 INTRODUCTION

Immunohistochemistry (IHC) is a popular technique to detect a specific protein in the tissue. The IHC was conducted by pathologists or histologist to determine the expression of the protein (Kaliyappan et al. 2012). The disadvantage of IHC is that can not measure the protein in the tissue so that it has to use another technique such as Enzyme-linked Immunosorbent Assay (ELISA) (Drijvers et al. 2017). It will make more cost due to the use of ELISA. However, if there has more contained protein in the tissue will have more intensity in IHC expression. Therefore, the amount of protein inside the tissue relates to increasing the intensity of IHC expression. Many software such as Image J (Jensen 2013), QuPath (Bankhead et al. 2017), ImunoRatio (Tuominen et al. 2010), and IHC profiler (Varghese et al. 2014) is developed to be image processing of tissue pictures to quantitative measurements. These software result in the number based on IHC pictures then continue to statistic analysis. The quantitative of IHC expression could be area percentages, number of expressed cells, and intensity level. The image processing of IHC expression would be reduced more cost to check other parameter associated protein in tissue (Kaczmarek, Górna, and Majewski 2004). Goldy chocolate of IHC expression in the picture is composed of Magenta and Yellow color.

Yellow and Magenta color could be split from a picture using CMYK split-channel (Pham et al. 2007) into a grey mode. The intensity is measured based on the grey level using a histogram. Grey level is described from 0 (black) until 255 (white) (Padmavathi and Thangadurai 2016).

Corel Photo Paint® is a popular software for image processing in Indonesia accompanied by Corel Draw®. Corel Photo Paint® has the ability to Cyan-Magenta-Yellow-Black (CMYK) split-channel and measuring grey level with Histogram. Thus, this article explained the simple method using Corel Photo Paint® with CMYK split-channel for intensity measurement of IHC expression in tissue pictures.

2 MATERIALS AND METHODS

2.1 Material

Materials for this research were Corel Photo-Paint (CCP) ® Software version X7 and one photo of IHC result of Positive Dopamine expression in neurons of the brain.

2.2 Method

1. Open the Corel Photo Paint® Software (Figure 1)



Figure 1: Opening Corel Photo Paint®

2. Click Open in Menu, please choose the file of the figure 2

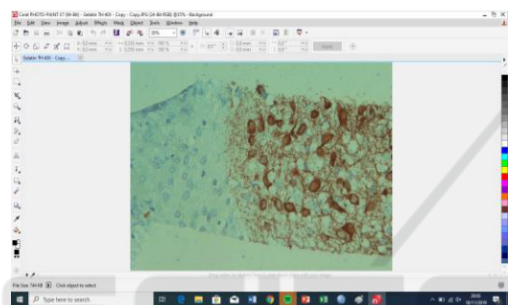


Figure 2: Opening the picture file

3. Click Image in Menu > Split Channel > CMYK. The result would be as extraction into 4 pictures in Cyan, Magenta, Yellow and Black picture in grey mode (Figure 3-6).

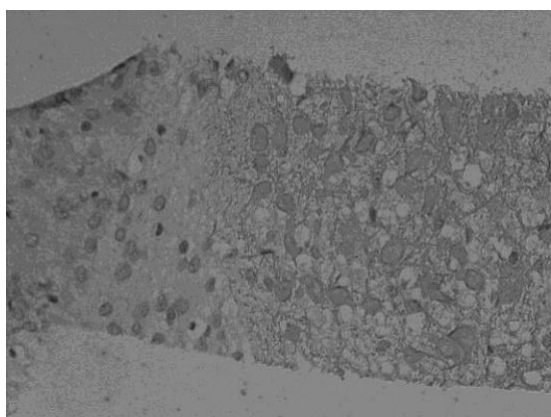


Figure 3. The cyan channel result in grey mode

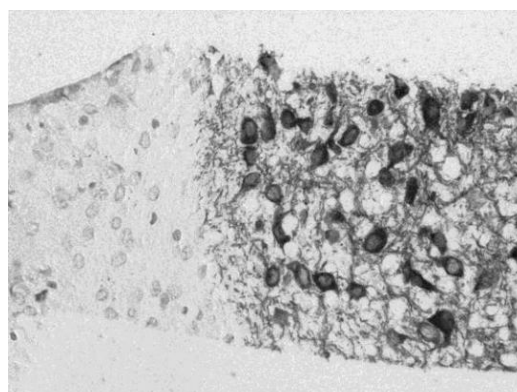


Figure 4. The magenta channel result in grey mode

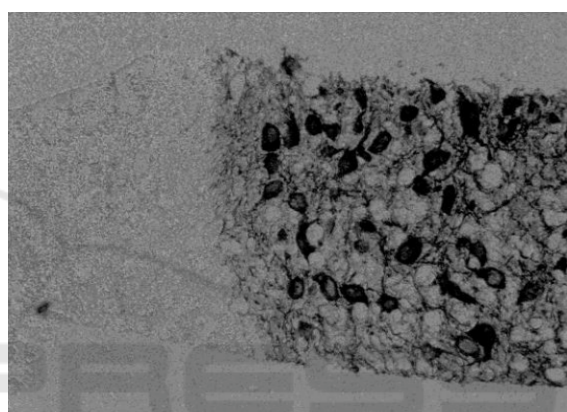


Figure 5. The yellow channel result in grey mode

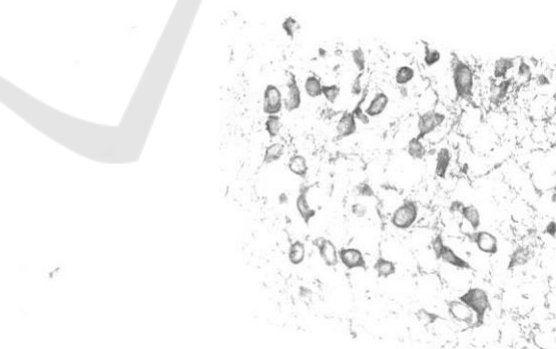


Figure 6. The black channel result in grey mode

4. Combining the Magenta channel and Yellow channel with click Image > Calculation (Fig 7). Choose the magenta channel in source 1 and the yellow channel in source 2 and then click OK.

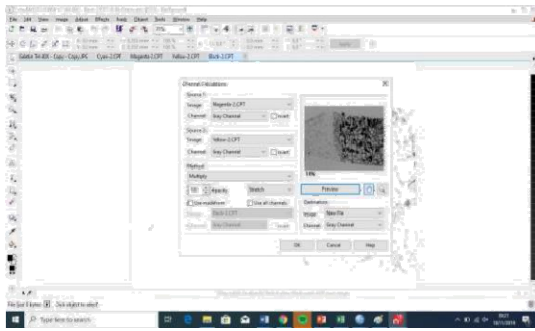


Figure 7. Calculation image as combining the Yellow channel and the magenta channel

5. The outcome was grey mode picture combination the yellow and the magenta channel (Figure 8)

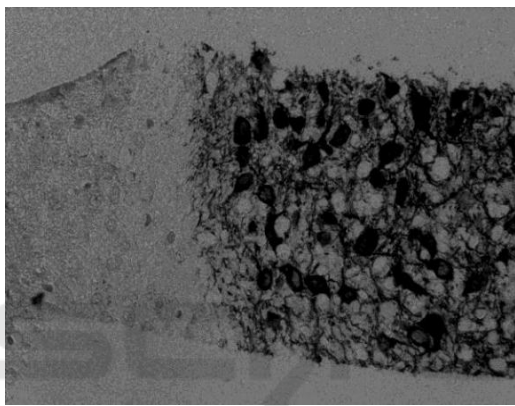


Figure 8. Picture as the result of calculation processing combining the Yellow and the Magenta channel

6. Click Rectangle Mask (in the Left Side) > Normal (upper side) and change to Fixed Sized (Figure 9)

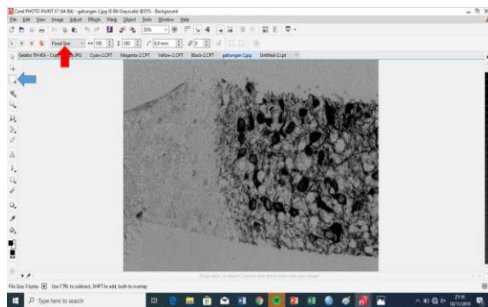


Figure 9. Rectangle mask (blue arrow) and Fixed sized (red arrow)

7. Please determine the size example 100x100, and please choose the area or cell which want to be calculated its intensity (Figure 10)

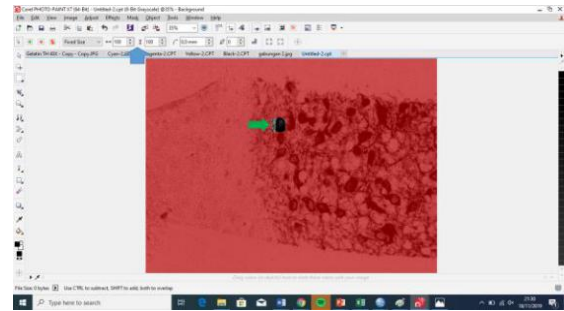


Figure 10. Determining the size 100x100 (blue arrow), and choose the area or the cell which want to be measured the intensity (green arrow)

8. Please click Image > Histogram to know the mean grey level (Figure 11). Please record the mean grey level into the data

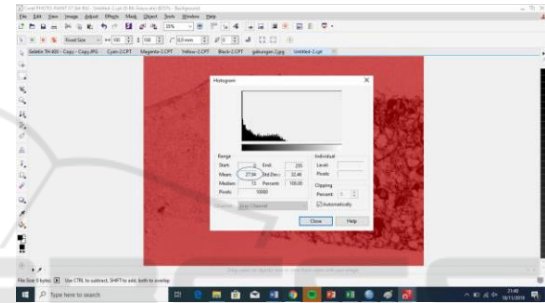


Figure 11. The calculation of IHC intensity of cells based on the mean grey level (blue circle).

2.2.1 Measurement the Neuron and the Dendrit Intensity of IHC expression

As many as 10 areas randomly were calculated either of neuron and dendrite by the method for intensity measurement.

2.2.2 Data Analysis

Data were analyzed using an independent T-test with significance 95% ($p < 0.05$) comparing the intensity IHC expression of the neuron and that of dendrites.

3 RESULTS AND DISCUSSIONS

3.1 Results

3.1.1 Comparison of the Intensity of IHC Expression between the Neuron and the Dendrites

Based on the measurement, there was significantly different ($p < 0.05$) of intensity between neuron and

dendrite associated IHC expression (Table 1, Figure 12).

Table 1: The comparison of the intensity of IHC expression between neuron and dendrite

Group	Grey level	p-value T-test
Neuron	26,13±9,05	0.000
Dendrite	52,96±8,09	

Notes: p-value showed under 0.05 indicate a significant difference between group

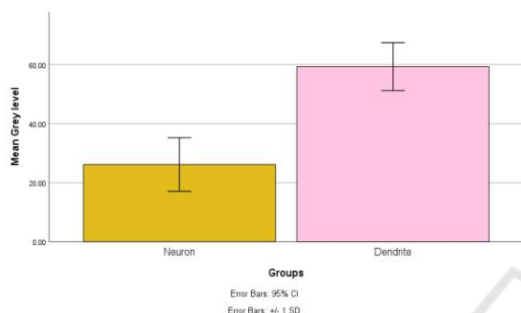


Figure 12: The graph of the mean of grey level between neuron and dendrite for IHC expression of intensity.

3.2 Discussions

The software for image processing of the IHC picture was developed in many forms. CPP® is the popular software used in Indonesia for photo editing besides Adobe Photoshop®. This article was showed another function of CPP® to image processing relate to medical or biological aspects for examining IHC pictures.

Analyzing IHC picture give benefits such as reduce the cost of other technique to tissue protein measurement. This article explained the simple way to measure the intensity of IHC expression (Kaczmarek, Górna, and Majewski 2004).

This method was similar to Pham et al. 2007, using the CMYK method but this method was simpler to apply. The differences between expression in the neuron (high expression) and the dendrite (less expression) were significantly different ($p < 0.05$, Table 1). The higher expression showed the lower grey level and the less expression showed the higher grey level. It was indicated that the CPP® could be used as a software to IHC expression analysis for intensity. This article was also be a module to operate CPP® to analyze IHC picture to intensify expression.

4 CONCLUSIONS

The conclusions of this method were :

- Corel Photo Paint® could be used easily to measure the intensity of IHC expression using CMYK Split channel
- higher intensity is the fewer grey level, the less intensity is the higher grey level

REFERENCES

Bankhead, Peter, Maurice B. Loughrey, José A. Fernández, Yvonne Dombrowski, Darragh G. McArt, Philip D. Dunne, Stephen McQuaid, et al. 2017. "QuPath: Open Source Software for Digital Pathology Image Analysis." *Scientific Reports* 7 (1): 16878. <https://doi.org/10.1038/s41598-017-17204-5>.

Drijvers, Jefte M., Imad M. Awan, Cory A. Perugino, Ian M. Rosenberg, and Shiv Pillai. 2017. "The Enzyme-Linked Immunosorbent Assay." In *Basic Science Methods for Clinical Researchers*, 119–33. Elsevier. <https://doi.org/10.1016/B978-0-12-803077-6.00007-2>.

Jensen, Ellen C. 2013. "Quantitative Analysis of Histological Staining and Fluorescence Using ImageJ: Histological Staining/Fluorescence Using ImageJ." *The Anatomical Record* 296 (3): 378–81. <https://doi.org/10.1002/ar.22641>.

Kaczmarek, E., A. Górna, and P. Majewski. 2004. "Techniques of Image Analysis for Quantitative Immunohistochemistry." *Roczniki Akademii Medycznej W Białymstoku (1995)* 49 Suppl 1: 155–58.

Kaliyappan, Karunakaran, Murugesan Palanisamy, Jeyapradha Duraiyan, and Rajeshwar Govindarajan. 2012. "Applications of Immunohistochemistry." *Journal of Pharmacy and Bioallied Sciences* 4 (6): 307. <https://doi.org/10.4103/0975-7406.100281>.

Padmavathi, K., and K. Thangadurai. 2016. "Implementation of RGB and Grayscale Images in Plant Leaves Disease Detection – Comparative Study." *Indian Journal of Science and Technology* 9 (6). <https://doi.org/10.17485/ijst/2016/v9i6/77739>.

Pham, Nhu-An, Andrew Morrison, Joerg Schwock, Sarit Aviel-Ronen, Vladimir Iakovlev, Ming-Sound Tsao, James Ho, and David W. Hedley. 2007. "Quantitative Image Analysis of Immunohistochemical Stains Using a CMYK Color Model." *Diagnostic Pathology* 2 (February): 8. <https://doi.org/10.1186/1746-1596-2-8>.

Tuominen, Vilppu J., Sanna Ruotoistenmäki, Arttu Viitanen, Mervi Jumppanen, and Jorma Isola. 2010. "ImmunoRatio: A Publicly Available Web Application for Quantitative Image Analysis of Estrogen Receptor (ER), Progesterone Receptor (PR), and Ki-67." *Breast Cancer Research: BCR* 12 (4): R56. <https://doi.org/10.1186/bcr2615>.

Varghese, Frency, Amiral B. Bukhari, Renu Malhotra, and Abhijit De. 2014. "IHC Profiler: An Open Source Plugin for the Quantitative Evaluation and Automated

- Scoring of Immunohistochemistry Images of Human Tissue Samples.” Edited by Syed A. Aziz. *PLoS ONE* 9 (5): e96801. <https://doi.org/10.1371/journal.pone.0096801>.
- Bankhead, P., Loughrey, M.B., Fernández, J.A., Dombrowski, Y., McArt, D.G., Dunne, P.D., McQuaid, S., Gray, R.T., Murray, L.J., Coleman, H.G., James, J.A., Salto-Tellez, M., Hamilton, P.W., 2017. QuPath: Open source software for digital pathology image analysis. *Sci Rep* 7, 16878. <https://doi.org/10.1038/s41598-017-17204-5>
- Drijvers, J.M., Awan, I.M., Perugino, C.A., Rosenberg, I.M., Pillai, S., 2017. The Enzyme-Linked Immunosorbent Assay, in: *Basic Science Methods for Clinical Researchers*. Elsevier, pp. 119–133. <https://doi.org/10.1016/B978-0-12-803077-6.00007-2>
- Jensen, E.C., 2013. Quantitative Analysis of Histological Staining and Fluorescence Using ImageJ: Histological Staining/Fluorescence Using ImageJ. *Anat. Rec.* 296, 378–381. <https://doi.org/10.1002/ar.22641>
- Kaczmarek, E., Górna, A., Majewski, P., 2004. Techniques of image analysis for quantitative immunohistochemistry. *Rocz. Akad. Med. Białymst.* 49 Suppl 1, 155–158.
- Kaliyappan, K., Palanisamy, M., Duraiyan, J., Govindarajan, R., 2012. Applications of immunohistochemistry. *J Pharm Bioall Sci* 4, 307. <https://doi.org/10.4103/0975-7406.100281>
- Padmavathi, K., Thangadurai, K., 2016. Implementation of RGB and Grayscale Images in Plant Leaves Disease Detection – Comparative Study. *Indian Journal of Science and Technology* 9. <https://doi.org/10.17485/ijst/2016/v9i6/77739>
- Pham, N.-A., Morrison, A., Schwock, J., Aviel-Ronen, S., Iakovlev, V., Tsao, M.-S., Ho, J., Hedley, D.W., 2007. Quantitative image analysis of immunohistochemical stains using a CMYK color model. *Diagn Pathol* 2, 8. <https://doi.org/10.1186/1746-1596-2-8>
- Tuominen, V.J., Ruotoistenmäki, S., Viitanen, A., Jumppanen, M., Isola, J., 2010. ImmunoRatio: a publicly available web application for quantitative image analysis of estrogen receptor (ER), progesterone receptor (PR), and Ki-67. *Breast Cancer Res.* 12, R56. <https://doi.org/10.1186/bcr2615>
- Varghese, F., Bukhari, A.B., Malhotra, R., De, A., 2014. IHC Profiler: An Open Source Plugin for the Quantitative Evaluation and Automated Scoring of Immunohistochemistry Images of Human Tissue Samples. *PLoS ONE* 9, e96801. <https://doi.org/10.1371/journal.pone.0096801>