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Automated Detection of Congested Central Vein Liver Histology of Mice Infected with *Plasmodium berghei* Using CellProfiler 2.0

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***Abstract*— Malaria is initiated by *Plasmodium* sporozoites infections, which are inoculated by mosquitoes. Histopathologic lesions often described in the liver of rodent with malaria are congested central vein with neutrophils and eusinophils within the lumen. Detection of congested central vein has a possibility to do automatically using image analysis software. Here the used of CellProfiler an open access cell image analysis software for automated detection congested central vein liver histology of mice infected with *Plasmodium berghei* is reported. The results are compared to the manual detection. Wilcoxon rank test was used for statistical analysis with H_0 hypothesis that means there was no significant difference between manual analysis and those with CellProfiler. Totally 10 images were analysed for both manually and using CellProfiler. Results showed that there were no significant difference between manual and automatic counting (p>0,05). Overall it appears that in our research analyzes with CellProfiler are comparable but not better than manual.**

Keywords* — CellProfiler, Central Vein, Congested, Pipelines, *Plasmodium berghei

I. INTRODUCTION

Malaria is the most serious and widespread parasitic disease of humans. It affects at least 200 to 300 million people every year and causes an estimated 3 million deaths per year. Malaria is initiated by Plasmodium sporozoites, which are inoculated by mosquitoes. The disease is characterized by a range of clinical features from asymptomatic infection to a fatal disease [1]. There are four species of Plasmodium that infect man and result in four kinds of malaria fever: P. falciparum, P. vivax, P. ovale, and P. malariae [2].

Malarial involvement of liver is now a known entity with it is specific histopathological lesions. Histopathologic lesions often described in the liver of rodent with malaria is congested central vein with neutrophils and eusinophils within the lumen (Fig. 1) [2,3]. Detection of congested central vein commonly done manually under microscope. This process has a possibility to do automatically using image analysis software. With the availability of digital photography, the congested central vein detection process can be done on the image by marking the central vein first using an image analysis performed with image analysis software then detection the congested area inside central vein. The results can be documented by saving the overlay image with the marked target cells.

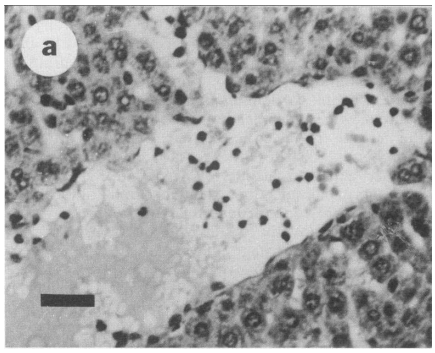

Fig 1. Congested central vein [3]

CellProfiler is freely available modular image analysis software that is capable of handling hundreds of thousands of images. The software contains already-developed methods for many cell types and assays and is also an open-source, flexible platform for the sharing, testing, and development of new methods by image analysis experts. CellProfiler uses the concept of a 'pipeline' of individual modules. Each module processes the images in some manner, and the modules are placed in sequential order to create a pipeline: usually image processing, then object identification, then measurement. Most modules are automatic, but CellProfiler also allows interactive modules (for example, the user clicks to outline a region of interest in each image). Modules are mixed and matched for a specific project and each module's settings are adjusted appropriately. Upon starting the analysis, each image (or group of images if multiple wavelengths are available) travels through the pipeline and is processed by each module in order [5].

Here the used of CellProfiler an open access cell image analysis software for automated detection congested central vein liver histology of mice infected with *Plasmodium berghei* is reported. The results are

compared to the manual detection of congested central vein in liver histology. The advantages using Cellprofiler is it could be instructed to process images in batches of several hundred to automatically generate parasitemia values without the need for supervision. This also eliminates factors such as user fatigue and lack of standardization that are often associated with manual enumeration.

II. MATERIALS AND METHODS

2.1. Mice

Male Swiss mice ages 8 to 12 weeks were purchased from Pusat Penelitian dan Pengembangan Gizi dan Makanan, Kementerian Kesehatan Indonesia.

2.2. Parasites and infections

Mice were inoculated intraperitoneally with 10^6 erythrocytes infected by *P. berghei*. Mice were subjected to euthanasia at one week after inoculation. Fragments of the liver were fixed by immersion in 10% buffered formalin during 24 hours. These samples were then dehydrated, and processed for paraffin embedding. Five µm sections were cut and stained with hematoxylin-eosin (H&E).

2.3. Image acquisition

A Nikon Biophot microscope attached with Nikon D3000 digital single lens reflects (DSLR) camera system was used to capture images of the smears. The slides were examined under 10× objective lens. Images were captured at a resolution of 1936×1296 and saved as JPEG files.

2.4. Manual detection congested central vein

Ten images of liver histology section were analyzed under personal computer using Microsoft Windows XP SP 2 32-bit platform as operating system. Processor type used inside the computer is AMD Athlon(tm) 64 X2 Dual Core 5000+ with memory (RAM) is 1.87 GB. .

2.5. Automated detection congested central vein

An open access cell image analysis software CellProfiler 2.0 r10997 that developed by Broad Institute was used for an automated detection congested central vein. CellProfiler (CP) runs on Microsoft Windows XP SP 2 32-bit platform. Processor type used inside the computer is AMD Athlon(tm) 64 X2 Dual Core 5000+ with memory (RAM) is 1.87 GB. A pipelines was developed to doing automatic detection congested central vein (Fig. 2).

2.6. Statistical analysis

Totals congested central vein obtained by manually or with CellProfiler were compared using Wilcoxon test with H_0 hypothesis mean there are no significant difference variation between was no significant difference between manually analysis and with CellProfiler. H_1 hypothesis mean there was a significant difference between manually analysis and with CellProfiler. Significant level used in this research is 0.05 (5%).

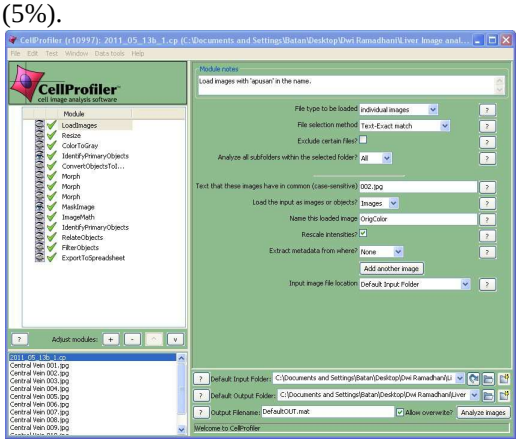

Fig 2. Pipelines for detected congested central vein.

III. RESULTS

3.1. Automated and Manual Detection

Ten images were collected and subjected to the automated, as well as being analyzed manually by pathologies. Scatter plots graph show linear relation (r = 0.55; Fig 3) between analyzes using CellProfiler and with manual counting. In our pipelines the time needed for process one single image is approximately 17 seconds.

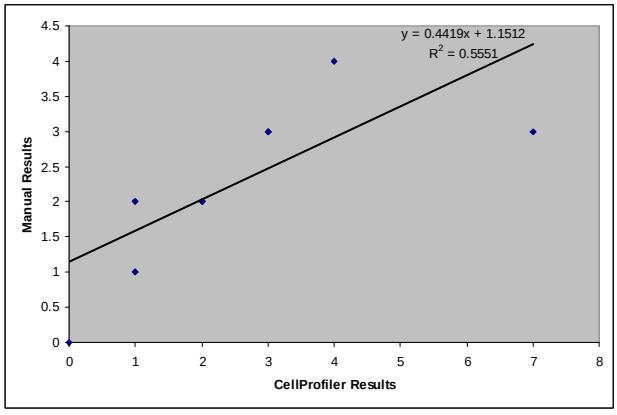

Fig 3. Scatter plots comparing congested central vein defined by manually and with CellProfiler.

3.2. Statistical analysis compare between automated and manual results

Statistical analysis using Wilcoxon Rank test show that there are no significant different between manual counting and automated counting (P = 1), because the p-value is bigger than 0.05 it is mean H_0 hypothesis is not rejected.

IV. DISCUSSION

Our pipelines consist several steps to detected the congested central vein in liver histology. First we rescale the image to speed up the time required for detected congested central vein in the image, and then we convert the images to grayscale images. Grayscale images show bright color on objects and a dark color in background. Because the central vein area is cover by a bright color then it can be easy to identify using the thresholding methods in Identify Primary Objects module. Unfortunately because sometimes the material inside central vein is attached to the border line of the central vein, then the central vein area that detected by thresholding method became very tight.

In order to refine the central vein area results we apply several images morph processing to get better central vein detection. Based on our experiment dilate the images, fill holes after dilate the images and last erode the images can be used for getting a better detection central vein area. Interestingly CellProfiler provided all image morph processing in Morph module. To define which one is the congested central liver we must detect is there any materials inside the central vein. Because thresholding method is detected the bright area and the materials are in gray color, first we must invert the images so the material inside the central vein became bright and can be detected by thresholding methods. In our pipelines, the congested central vein detection is based on "parent-child relationship" between the central vein and the materials inside the central vein. The central vein (parent) should define as the congested central vein if it has at least one or more material (child) in it. It will not define as congested central vein if the material inside the central vein is not overlapping with the central vein. Details of all steps of our pipelines are shown in Fig 4.

A significant variation between manually and automatically detection of congested central vein observed in one image. This happened because there are large sinusoids areas and because the inside the sinusoid areas there are many Kupffer cells then the pipelines also detected as a congested central vein. A large sinusoid area is also commonly histological lesions because infection of Plasmodium, this phenomenon usually calls a sinusoid dilatation. Baheti et al research show that seventy five percent histological lesion in liver cause by Plasmodium is sinusoid dilatation [7]. We are now in the process of developing a better pipelines than can be used for detected the congested central vein and can differentiate between the congested central vein and sinusoid dilatation.

V. CONCLUSION

We have developed pipelines for CellProfiler software that can be used to detect the congested central vein in liver histology section of mice infected with *Plasmodium berghei*. Overall, our pipelines worked very well to detection of congested central vein.

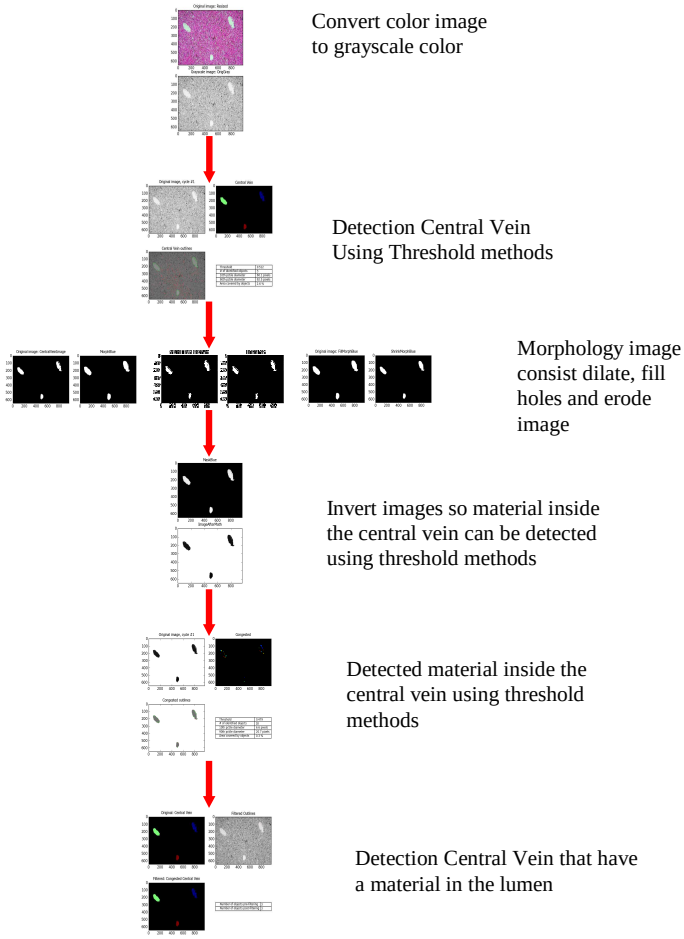

Fig 4. Flowchart automatic detection of congested central vein defined by CellProfiler.

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