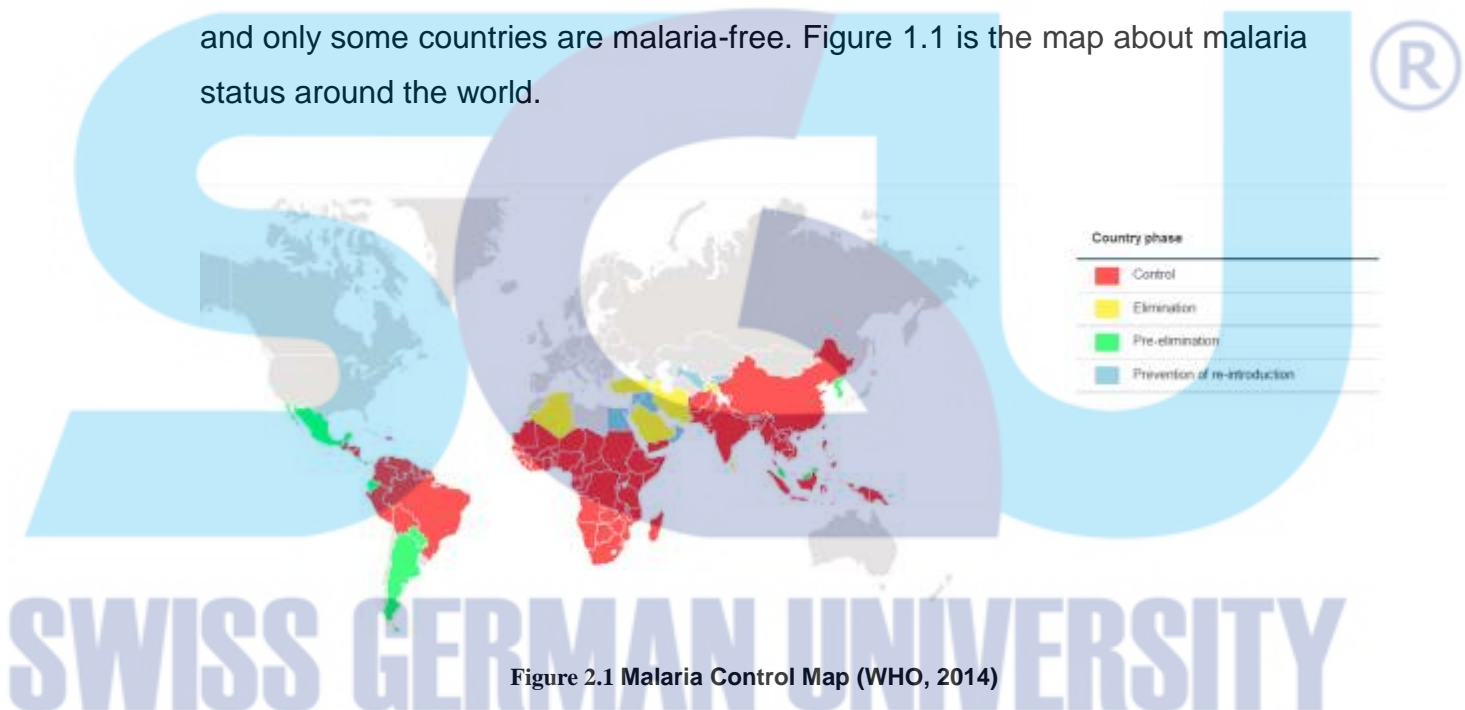


CHAPTER 2 - LITERATURE REVIEW

2.1 Malaria

Countries all around the world concern about malaria control. In 2012, International funding for malaria control was rocketed to almost two billion USD. The funding was allocated for 70 million new bed nets for malaria-endemic countries in 2012. 331 million of treatment courses were delivered in 2012 (WHO, 2013). Up to now, malaria cases still reported around the world and only some countries are malaria-free. Figure 1.1 is the map about malaria status around the world.



Malaria is an infectious disease caused by protozoan parasite *Plasmodium*. The malaria parasite is transmitted to human blood by female *Anopheles* bites. Five species of *Plasmodium* that have been discovered to infect human, namely: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium ovale*, *Plasmodium malariae*, and *Plasmodium knowlesi*. *Plasmodium vivax* cases have been reported by people living in (sub)tropical countries or area at risk (WHO, 2014). *Plasmodium vivax* is transmitted in 95 countries in tropical, sub-tropical and temperate regions (Kakkilaya , 2012). *Plasmodium vivax* can remain dormant in human liver. Similar characteristic also happen at

Plasmodium ovale (WHO, 2014). However, *Plasmodium ovale* usually appears in tropical Africa. Among these species, *Plasmodium falciparum* is the most deadly specie. It also contributes to the largest number of malaria-associated deaths and is very common in tropical and subtropical area. In 2007, 2.37 billion people were being estimated as being at risk of *Plasmodium falciparum* (Kakkilaya, 2012).

Same with the other protozoans, plasmodia undergo some particular form of life cycle. All infecting species share common three phases of development that includes exo- erythrocytic stages (hepatic stage), erythrocytic schizogony, and the sexual process. Exo-erythrocytic phase literally means phase of development outside erythrocyte of the host and it usually happened in the liver while during erythrocytic schizogony phase or protozoan asexual reproduction stage, the parasite develops inside red blood cell (RBC) of the host organism (K.Murray and Bennett, 2009). The sexual process takes place both in the host i.e. human and in the mosquito.

Malaria infection starts when a female Anopheles mosquito bites humans. As it bites, it delivers several hundred sporozoites, which are the protozoan cells that develop in the salivary gland of the mosquito, into the blood stream (Tortora et al., 2007). Human's anti-body can usually kill the malaria parasites, but it is possible that some parasites enter the liver cells where exo-erythrocytic schizogony happen. exo- erythrocytic schizogony enables the sporozoites to multiply rapidly (Jenkins et al., 2007).

Exo- erythrocytic schizogony stage produces cells called merozoites. After hepatic stage complete, merozoites are released and possible to invade erythrocytes. As for *Plasmodium vivax* and *Plasmodium ovale* which can stay at liver, some of the sporozoites do not turn into merozoites; instead they turn into hypnozoites that remain latent in the liver cells for some period of time before undergoing schizogony.

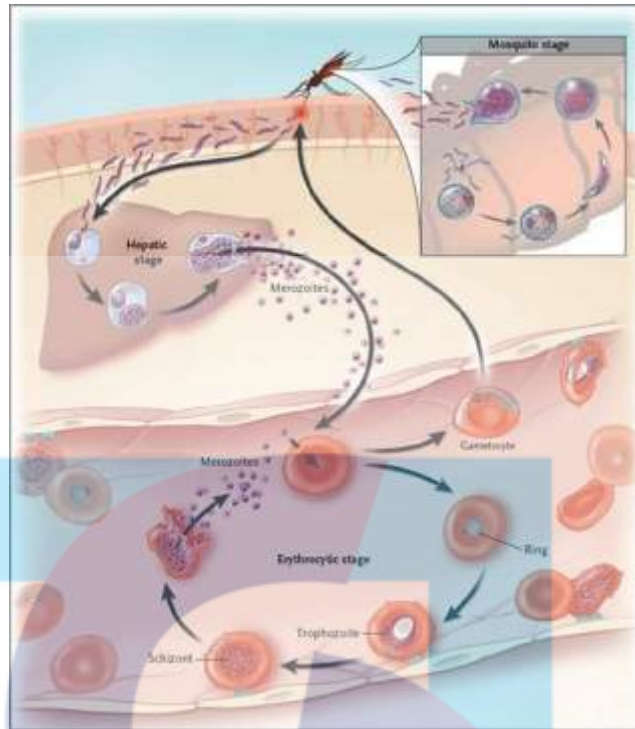


Figure 2.2 Life Cycle of Plasmodia (McW Healthcare, 2008)

The erythrocytic cycle begins after merozoites enter erythrocyte. During erythrocytic schizogony stage, through microscope, the parasite can be first seen as a minute of speck of chromatin surrounded by scanty protoplasm. Then, the parasite changes into a ring-shaped structure and it is referred as ring stage or immature trophozoite as can be clearly observed in Figure 2.2. It then grows and takes form that varies depend on the plasmodium species. However, they share one characteristic of projected nuclei or active pseudopodia (K.Murray and Bennett, 2009). Mature trophozoites are characterized by appearance of pigment granules. The nucleus of parasite will continue divide and the parasite is known as a schizont. The dividing nucleus usually takes up peripheral positions and a small portion of cytoplasm gathers around each. This process leads to rupture of erythrocyte and a number of merozoites that can attack new corpuscles and begin another cycle of erythrocytic schizogony are released. This stage of plasmodia's life cycle is the time when the parasites are observable in blood smears (Chamberlain, 2009).

2.2 Prevalence of Malaria in Indonesia

Aside from being a habitat for its 230 million of population, Indonesia is also one of very few countries that has over 20 anopheline vectors that transmits all four species that infect human (WHO, 2010). Although malaria cases are concentrated on the remote places and tropical forest areas such as Papua, Maluku, Nusa Tenggara, Sulawesi, and Kalimantan, it has been reported that there were 247.3 million of people are at varied degrees of malaria infections. The status of Indonesia toward malaria is still malaria control (WHO, 2013).

Since 2010, nearly 2.000 confirmed cases with more than 200 confirmed malaria deaths have been reported each year. Among those cases, more than 55% of them happened to be caused by *Plasmodium falciparum* (WHO, 2013).

Malaria has become national health problem, the disease is usually occurred in the poor communities and thus affecting national development and takes away major share of health budget. In 2010, global fund spend more than 25 million USD for Indonesia to control malaria problem and rocketing to 40 million USD in 2011. Those were allocated for Management, ITNs, Human resources and the other costs (WHO, 2013).

2.3 Diagnosis of Malaria

Diagnosing malaria parasite in the victim blood is the first step to control the spread of malaria. There are a lot of current researches that focus on performing an accurate diagnosis malaria parasite for clinical and epidemiological has been developed. Base on Anggraini et al. (2011), there are a lot of methods developed to detect malaria in human blood. Clinical, microscopy, nucleic acid-based diagnosis are the methods to diagnose malaria.

2.3.1 Clinical Diagnosis

Based on Johnson and Ineson (2002) paper, the first approach in diagnosing malaria parasite is through the clinical symptoms. The symptoms are including fever, jaundice, hepatomegaly, splenomegaly, and possibility of renal failure. The main problem with clinical diagnosis is the symptoms for mild to moderate malaria are not specific enough. Because of that, if possible it is important to perform chemistry blood examination. The chemistry blood examination consists of WBCs count, RBCs count, hematocrit, hemoglobin level, and platelet count. The high predictive positive value of the panel can be happen in some endemic malaria areas.

Regarding chemistry blood examination, the result of malaria examination can be the same with anemia and this is usually happen in *Plasmodium falciparum* cases since the infection can cause a rupture at the erythrocytes. Based on platelets count, the number of platelets in every malaria case is rarely normal.

Furthermore, the clinical presentation of malaria is even less clear in patients who have had a number of malaria attacks, as they generally show no clear signs or symptoms. Care must also be taken to establish whether the patient has taken antimalarial medicines before going to hospital, as this condition can modify the clinical presentation. Previous treatment with antimalarial medicines, by reducing the parasite density to very low levels, may make microscopic diagnosis more difficult (WHO, 2010).

2.3.2 Microscopy Diagnosis

When there is a suspect of malaria infection on clinical grounds, one technique to obtain laboratory confirmation of the presence of malaria parasites is through direct demonstration of the parasite whole cell inside the

erythrocyte using blood smears observed under light microscope (Johnson and Ineson, 2002).

The basic idea of diagnosing malaria with microscopy is that Malaria is caused by a parasite in the blood; the parasites are very small i.e. microscopic and can be only seen under a microscope with high magnification. Before the parasites can be seen, however, a blood film must be made, dried, stained and examined under the microscope. When the microscopist sees stained parasites, the diagnosis of malaria is confirmed. The stages and species of malaria parasite and the density of the infection can be identified by expert microscopists. Using this information, the physician or health-care worker can treat the patient with the most appropriate antimalarial agents (Anggraini et al, 2011).

2.3.3 Nucleic-acid based Malaria Diagnosis

Conventional microscopy, like the one discussed above, is usually satisfactory for the majority of clinical study. However, this is not the case for low parasitemia i.e. when the number of parasite in the blood is less than 10 parasites/ μ L of blood or when the parasites are drug resistant. This problem is solved by the advancement of molecular biology that enables a nucleic-acid base malaria diagnosis (Samane et al., 2010). Species specific regions of rRNA genes have been exploited in developing a sensitive and specific diagnostic procedure after the success of sequencing two small subunits of rRNA genes from *Plasmodium falciparum* and *Plasmodium vivax*. As a consequence, PCR assays to detect *Plasmodium falciparum* and *Plasmodium vivax* are widely designed with reliable analytical specificity (Laboonchai et al., 2001).

A recent development in PCR based technique is the possibility of processing dried blood spots collected on filter paper, without the need for difficult laboratory steps in the field. Consequently, this can reduce the risk of contamination in the laboratory and has yielded satisfactory sensitivity results for *Plasmodium falciparum*. Along with the advantages, a number of drawbacks hamper the use of PCR in detecting malarial infection. One of the most important is about contamination that may result in false positive (Laoboonchai et al., 2001).

2.4 Peripheral Blood Smears for Malaria Diagnosis

As mentioned in preceding chapter, microscopic examination of blood film is the most efficient and reliable malaria diagnosis technique. Furthermore, because each of the four major parasites has distinguishing characteristics, this technique is very sensitive and highly specific and it allows differentiating between species, quantification of parasitemia as well as observation of asexual stages of the parasite (Anggraini et al, 2011).

Peripheral blood smears are prepared from a blood sample that allow microscopical examination of blood cells and typically used for investigation of hematological disorders and for detection of parasites, as with the case of plasmodia. In malaria blood smears investigation two sorts of blood smears are traditionally used: thick and thin blood smears as shown in Figure 2.3. Thick blood smears allow screening of a larger volume of blood and thus provide in high better sensitivity compare to thin slide. Thin blood smears, on the other hand, allow better species identification (WHO, 2010). Anggraini et al. said, in preparing thin blood smears, blood materials were fixed with methanol and thus preventing hemolysis. Consequently, unlike in thick blood smears, there is no loss of parasite during staining; limited artefacts and overlapping cells; parasites are observable in their natural location and shape within the red blood cell since their morphology is conserved.

Principally, blood films or blood slides are prepared by placing a drop of blood for thin smear on one side and 3 drop of fingerprick blood on the other side of a glass slide. For the thin smear, the drop is spread with the corner of other slide or a swab stick to cover an oval area along the slide. The edge of the spreader must remain in even contact with the surface of the other glass slide while the blood is being spread. For the thick slide, the blood is mixed using a swab stick. A circular or rectangular film can be made by three to six quick strokes with the corner of the spreader. The circular thick film should be about 1 cm in diameter (Anggraini et al, 2011).

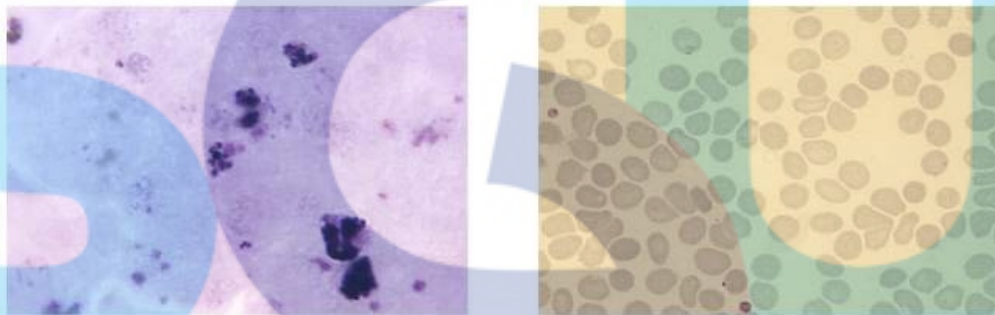


Figure 2.3 Malaria Thick Blood Smear (Center for Biologic Counterterrorism and Emerging Disease, 2009) and Thin Blood Smear

Before drying, the thin slide needs to be fixed with methanol to avoid hemolysis. Following that process, the slide is thoroughly dried. The dry film can be subsequently stained using Giemsa dilution. Giemsa stain is an alcohol-based Romanowsky stain and it enables the differentiation of nuclear and cytoplasmic morphology of platelets, RBCs, White Blood Cells (WBCs), and parasites. The components that contain DNA, i.e. nucleus of WBC and nucleus of parasite are highlighted. This is possible since Giemsa stain is a mixture of eosin and methylene blue. Eosine stains parasite chromatin and stippling shades of red or pink, while methylene blue stains parasite cytoplasm blue. White-cell nuclei stain blue to almost black, depending on the type of white cell (Anggraini et al, 2011).

In order to achieve optimal result, the Giemsa staining solution needs to be prepared with deionized water buffered to pH 7.2 (WHO, 2010). Usually, to stain one slide of malaria sample 5 mL of solution is used. The slide is immersed in staining solution and contact is maintained for 30-40 minutes depending on local climatic conditions. The staining solution is then removed by accurate rising with buffered distilled water and the slide is then drained on filter paper and allowed to dry. In an emergency situation when clinical condition requires immediate action, thick films may be stained with Giemsa accelerated procedure. The thick smear is allowed to dry under a heat of up to 500C and then stained for 8 minutes using a 10% of Giemsa solution before rinsing and drying. However, the qualitative morphological results are far worse than the usual one (Anggraini et al, 2011).

2.5 Image Pre-Processing

In order to reduce image variability and to ease further image processing, several algorithm have been proposed during image pre-processing of malaria thin blood smears. The algorithms are used to enhance the image for further steps.

Savkare et al. (2011) used a 3x3 median filter to reduce the noise in the thin blood smears image. Median filtering is a non-linear digital filtering technique. The median filter also followed by Lapalcian filter to smoothing the image. The Lapalcian filter takes second order derivative of pixel. Abdul Nazir al (2012) enhanced the image contrast using histogram equalization or contrast stretching and then followed by converting the RGB image to HSI colour space.

Makkapati et al. (2009) and Mehrjou et al. (2013), they were not working with grayscale image alone. HSV (Hue, Saturation and Value) colour space is chosen by them to work with. Converting RGB image to grayscale and HSV colour image is the image processing stage. After that, histogram equalization is done to fix the dispersion of pixel and then followed by 3x3x median filtering to remove salt and pepper noise.

2.6 Blood Cell Segmentation

Blood segmentation is needed before feature extraction step. In blood cell segmentation, the cell should be segmented from the background (Mehrjou et al., 2013). Studying cell characteristic without segmented from the background is impossible. To know the characteristic of erythrocyte as object interest of this study, all cells must be separated to single cell and finding the characteristic of each cell.

Image segmentation is an important step in image analysis and image processing. Usually this step is the most difficult step. In this case, blood cell segmentation, segmentation of erythrocyte performed by thresholding algorithm and followed by mask processing. The separation of each object is performed in mask processing. Overlapping cell and artefact are determined as one object in this step. The further process is separate an overlapping cell into two or more cell.

Savkare et al. (2011) began the blood cell segmentation with segmented foreground from background image using Basic Global thresholding and followed by Otsu thresholding. The input image that used was grayscale image and the purpose of doing thresholding is to get the binary image of cells. Then 3x3 median filtering was applied to remove unwanted noise and fill the holes in blood cell.

Using morphological operation, cells that had larger area were identified as overlapping cell. Then distance transform and watersheds algorithm were applied to separate the overlapping cell in two or more single cells. To segmented erythrocyte from the other components, several rules were applied. First leucocyte or White blood cell is bigger than erythrocyte, second thrombocyte and artefact are smaller than erythrocyte. Using morphological operation, bigger and smaller cells were removed from the image. Then with labeling the binary image, total number of erythrocyte is calculated.

Same with Savkare et al., Mehrjou et al. (2013) blood cell segmentation was also began with thresholding. Otsu thresholding was applied to the enhanced grayscale image to get the binary image. The binary image was the input image for next step, which are removing the artefacts and filling holes in the cell. Artefacts are object with an area less than a threshold value and the threshold value was gathered from calculating using a database. So removing the object below the threshold value was done to remove the artefacts. Filling the hole in the blood cell was done by applying a flood-fill operation.

Further process was handling the overlapping cell. Area of the cell and elongation of the cell were used to address the overlapping cell. In this project, elongation is calculated by dividing the length (height) of the cell with breadth (width) of the cell. The values more than 1.5 were potentially to be overlapping cells. The candidates of overlapping cell were applied with distance transform to get the number of cells that linked to single cell.

Different from the previous method, Abdul-Nasir et al. (2012) performed the image segmentation with K-mean Clustering algorithm.

2.7 Feature Extraction

Feature extraction is performed to gather the characteristic of each object. Later the characteristic of each object will be used in the classification step to address each object in the image. The feature or characteristic will show the difference between each object. In previous studies, a lot of sets of features were chosen such as geometrical feature and colour level.

Savkare et al. (2011) chose a set of geometrical features as the parameter. They are Radius of cell which is the average length of radial line, Perimeter which is the total distance between consecutive points of border, Area which is the number of pixels on the interior of the cell, Compactness which is the ratio of perimeter² by area.

2.8 Classification

Classification stage begins with creating a model for each object. A lot of rules are applied to address an object in an image. In this study, the objects will be addressed as healthy erythrocytes, infected erythrocytes, leucocytes, artefacts and thrombocytes. Karnia et al. (2012) gave some rules to address the object. Healthy erythrocyte will be addressed to a component that has normal pixel intensity level. Infected erythrocyte will be addressed to a component that has normal and low pixel intensity level. Leucocytes will be addressed to a component that has normal and low pixel intensity level, the area of object is beneath a threshold value. Artefacts and thrombocytes will be addressed to a component that has normal or low pixel intensity level, the area of object is below a threshold value.

2.9 Related Work

Anggraini et al. (2011) developed CAD system based on Free Open Source System for malaria cases in Indonesia. In her study, she made a system for recognizing infected erythrocyte in the blood component. The dataset for her study is erythrocyte image, healthy and infected erythrocyte, and the system has to recognize the infected erythrocyte.

This study also developing CAD system based on Free Open Source System for malaria cases in Indonesia but the propose of this study is for recognizing erythrocyte from blood images. The dataset for this study is thin blood smears image and the system must identify the erythrocyte in the blood image.

2.10 Algorithm

In this study, a lot of algorithms were used to get the expected result. These algorithms were the basic algorithm in image processing and the purpose of each algorithm is different based on the region that it manipulates.

2.10.1 Image Enhancement (Spatial Filtering)

Spatial Filtering is one of tools that commonly used in image enhancement. Spatial filter consists of a neighborhood and a predefined operation (structured element), like Figure 2.4 that is performed on the image pixels encompassed by the neighborhood. Filtering creates a new pixel with coordinates equal to the coordinates of the center of neighborhood, and whose value is the result of the filtering operation.

1	1	1
1	1	1
1	1	1

Figure 2.4 Structured Element

Figure 2.5 illustrates the formula of linear spatial filtering using a 3 x 3 neighborhood structured elements, Figure 2.4. At any points (x,y) in the image and the response is $g(x,y)$, is the sum of products of the filter coefficients and become the new image pixels.

$$g(x, y) = w(-1, -1)f(x - 1, y - 1) + w(-1, 0)f(x - 1, y) + \dots \\ + w(0, 0)f(x, y) + \dots + w(1, 1)f(x + 1, y + 1)$$

Figure 2.5 Spatial Filtering (Gonzales et al., 2007)

In this study, spatial filtering is used for smoothing and sharpening. Average filtering is used for smoothing. It works by summarize all of the pixel's neighborhoods and then divided by the numbers of neighborhood. Average filtering will give blur effect to the image. Figure 2.6 is the formula for average filtering with 3 x 3 neighborhoods. $Npixel[j][i]$ is the new pixel value where j is the y-axis and i is the x-axis and examples for average filtering can be seen at Figure 2.7.

```
npixel[j][i] = (unsigned char)((pixel[j-1][i-1] + pixel[j-1][i] + pixel[j-1][i+1] +
pixel[j][i-1] + pixel[j][i] + pixel[j][i+1] +
pixel[j+1][i-1] + pixel[j+1][i] + pixel[j+1][i+1])/9);
```

Figure 2.6 Average Filtering Formula

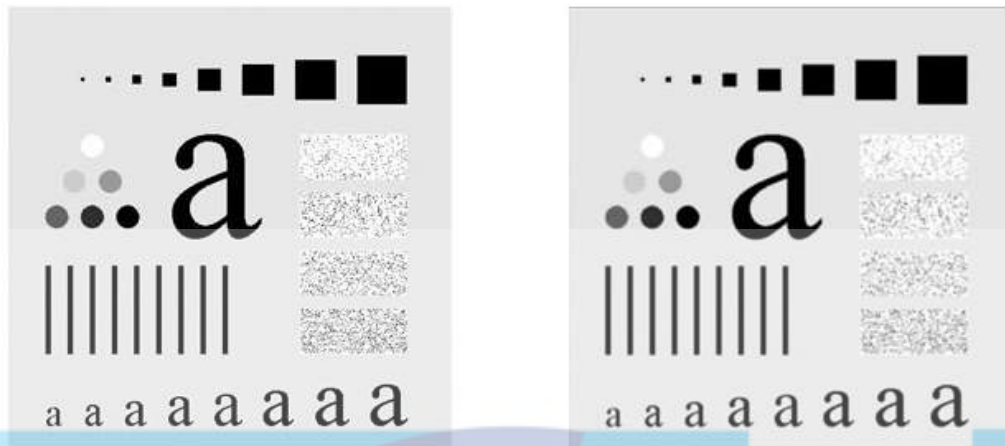


Figure 2.7 Average Filtering (Gonzales et al., 2007)

The other algorithm that used spatial filtering method is median filtering. Median filtering works by getting one pixel point for the center and the neighbors of center pixel as structure element, sorting the structure element and take the middle pixel from the structure elements as the new pixel. Figure 2.8 will show 3x3 median filtering that contain nine pixels in the structure element.



Figure 2.8 Median Filtering

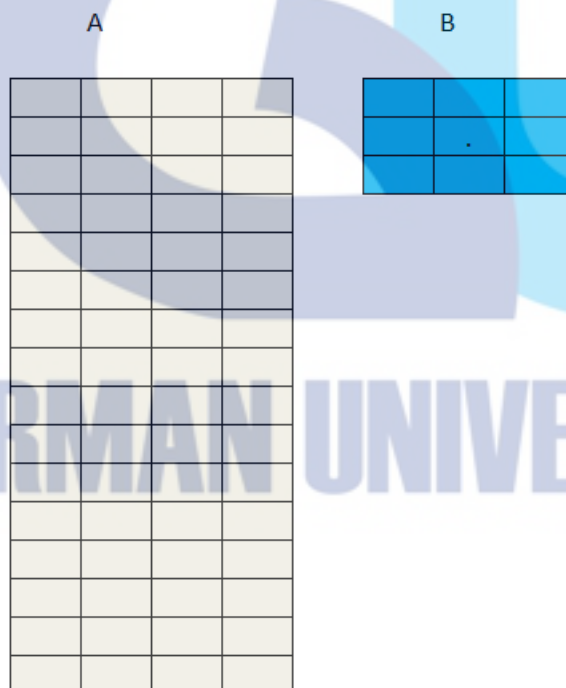
2.10.2 Morphological Image Processing

Morphological image processing is constructed with operations on sets of pixels. The sets of pixel represent the morphology of an image mathematically. The sets of pixel saved in form of vector which use (x,y) as

coordinates. There are a lot of processes that used in morphological image processing for example Erosion and Dilation.

Formula (2.9.2 - 1) is the formula for erosion where A is the input image and B is the structured element. It works by virtually try to match the structure element with every pixel in the input image and put the pixel at the output if the structuring element touch all pixels in original image. This makes the result is smaller than the original input by removing pixels. Figure 2.9 shows how erosion works.

$$A \ominus B = \{ z \mid (B)_z \subseteq A \} \quad (2.9.2 - 1)$$



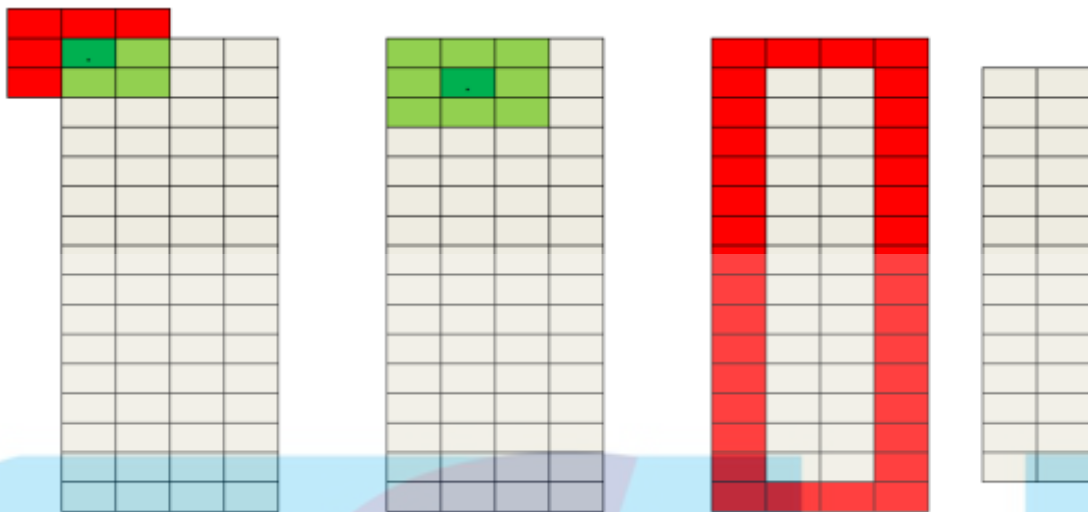


Figure 2.9 Erosion Illustration

The other operation for morphological image processing is dilation. Formula (2.9.2 - 2) is dilation's formula. It works by virtually try to match the structure element with every pixel in the input image and put the pixel at the output if the structuring element touch any pixel in original image. This makes the result is bigger than the original input by adding pixels. Figure 2.10 shows how dilation works.

$$A \oplus B = \{z | (B)_z \cap A \neq \emptyset\} \quad (2.9.2 - 2)$$

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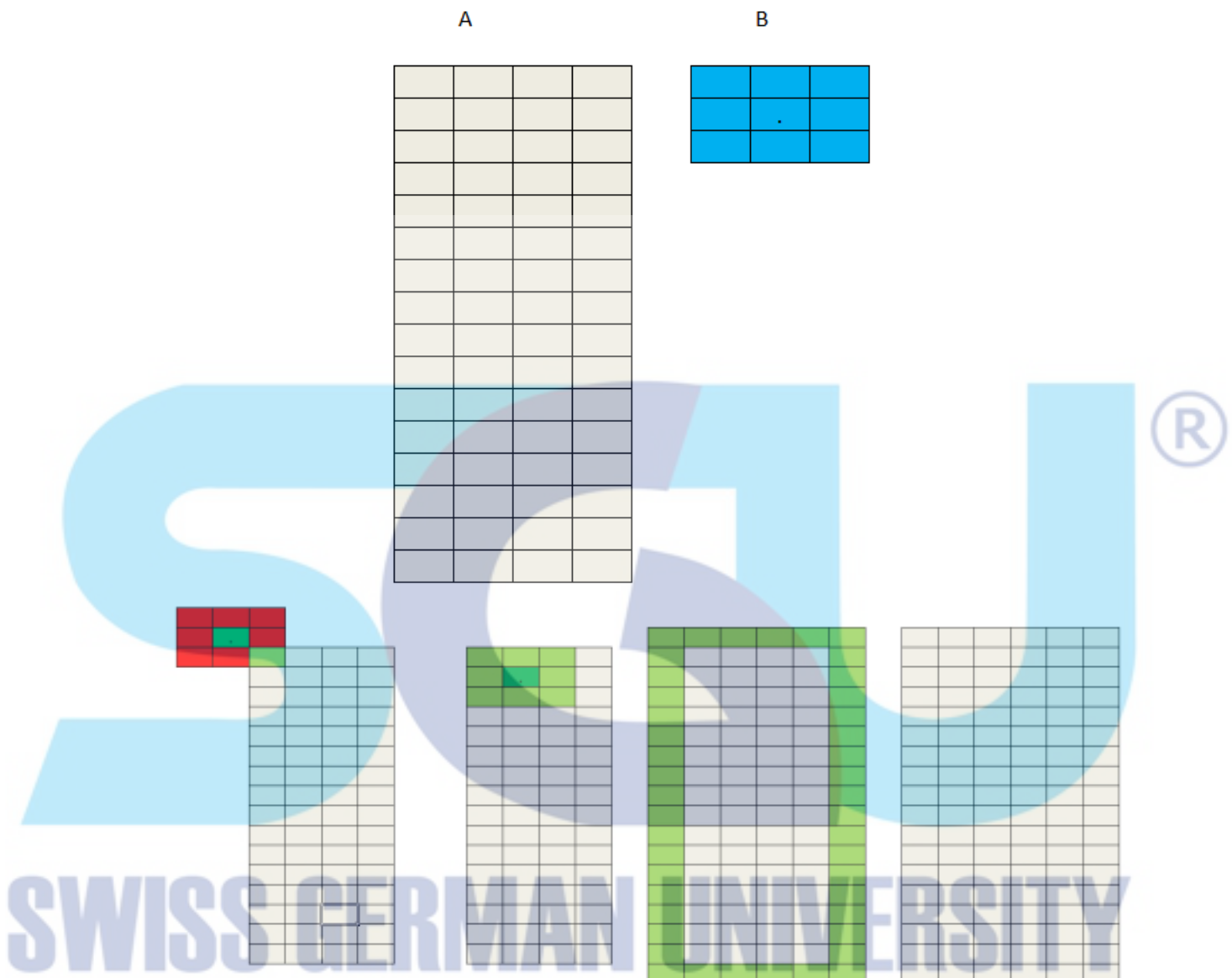


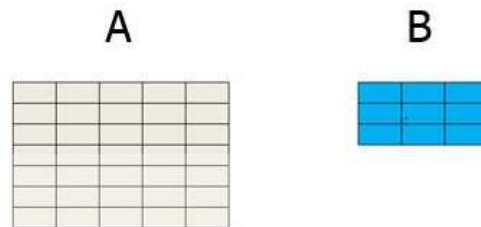
Figure 2.10 Dilation Illustration

2.10.3 Boundary Extraction

Boundary extraction is performed to get the boundary of an object. Boundary of a set A , addressed as $\beta(A)$, can be gathered by eroding A by B and then performing the set difference between A and its erosion. Mathematically, boundary extraction can be formulated as Formula (2.9.3 - 1)

$$\beta(A) = A - (A \ominus B) \quad (2.9.3 - 1)$$

From that formula, boundary extraction can be illustrated like Figure 2.11



$$(A \ominus B)$$



$$\beta(A) = A - (A \ominus B)$$

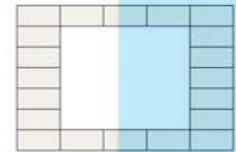
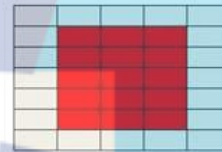
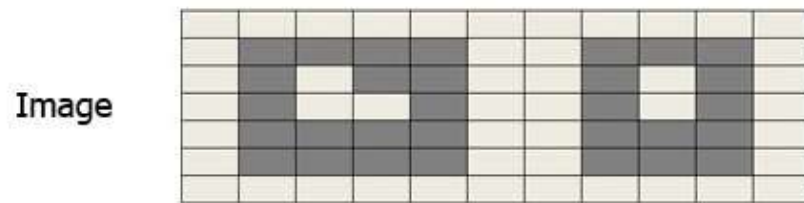


Figure 2.11 Boundary Extraction Illustration

2.10.4 Hole Filling

After thresholding, an object may have a hole inside of it. In order to get the right mask of object, Mehrjou et al (2013) applied a flood fill operation. Usually the background of an object is white, the object is black and the color of the hole is the same as the background (white). Since all the image in background is connected to the edge of the image, filling the background is the first thing that can be done. Then filling the white color with black. Hole filling can be illustrated like Figure 2.12.



Filling the background



Filling the rest white pixel



Result



Figure 2.12 Hole Filling Illustration

2.10.5 Connected Component Extraction

Extraction of connected components from a binary image is the core to many automated image analysis systems. The purpose of connected component extraction is to get each connected component (object) as a single object. To illustrate connected component extraction, let A be a set containing one or more connected components, and form an array X_k with the same size as array A that contains A . Then the connected component extraction starts by searching for the first pixel of the object and labeling it as one (1). Do conditionals

dilations continuously and label the object pixel to one. It stops when the dilation do not find any pixel object. The next step is searching for another object and labels it into two (2). Connected component extraction can be formulated as

$$X_k = (X_{k-1} \oplus B) \cap A \quad k = 1, 2, 3 \dots\dots \quad (2.9.5 - 1)$$

Where B is a suitable structuring element and k is the number of connected component. Connected component extraction is illustrated at Figure 2.13.

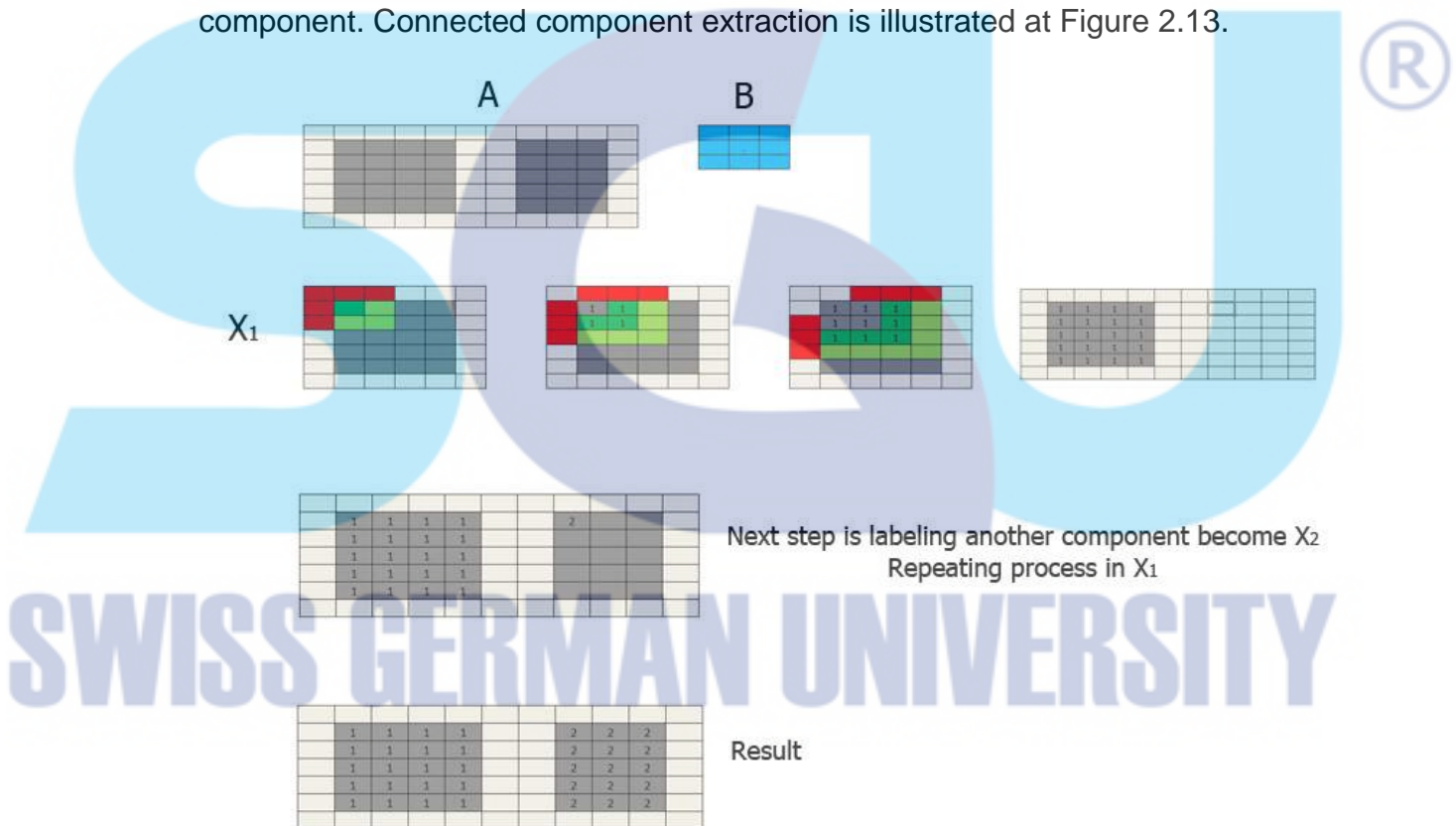


Figure 2.13 Connected Component Extraction Illustration

2.10.6 Thresholding

Thresholding is the core algorithm for image segmentation. The purpose of this step is to differentiate between background and foreground. The most common algorithms have been used are Basic global thresholding and Otsu thresholding.

Basic global thresholding is used when there is clear valley between the regions of the histogram related to objects and background. First, select an initial global threshold T . Then segment the pixel image into two groups of pixels. G_1 consist all pixels beneath T and G_2 consist all pixels below T . After G_1 and G_2 have been collected, compute the average (mean) intensity values m_1 and m_2 for the pixels in G_1 and G_2 . Formula (2.9.6 – 1) is used to calculate the new threshold.

$$T = \frac{1}{2} (m_1 + m_2) \quad (2.9.6 - 1)$$

T is the new threshold value. Steps 2 until 4 are repeated until it produces the same T value sequentially or the ΔT is zero (0).

The other thresholding method is Otsu method. Otsu method calculates the new threshold value by looking the largest *between-class variance*. Otsu method performs the computation based on the histogram of an image. First, prepare an array N to store the image pixel. Then calculate the probability (P_i) of each image value. P_i is calculated by dividing number of pixel i (N_i) with total pixel ($\sum_{i=0}^{L-1} N_i$) where L the range of pixel intensity level (256).

$$P_i = \frac{N_i}{\sum_{i=0}^{L-1} N_i} \quad (2.9.6 - 2)$$

Suppose $T(k) = k$, $0 < k < L - 1$. $T(k)$ used to threshold the input into two classes, C_1 and C_2 , where C_1 consist of all pixels in image with intensity value in range of $[0, k]$ and C_2 consist of the pixels with values in range of $[k+1, L-1]$. By using this threshold, the probability ($P_1(k)$) that a cumulative sum from P_i where $0 < i < k$ which is pixels that assigned to C_1 , can be calculated. Same with $P_1(k)$, $P_2(k)$ value can be calculated. $P_2(k)$ is the cumulative of P_i where $k+1 < i < L-1$ which is assigned to C_2 .

$$P_1(k) = \sum_{i=0}^k P_i \quad (2.9.6 - 3)$$

$$P_2(k) = \sum_{i=k+1}^{L-1} P_i \quad (2.9.6 - 4)$$

The other thing that needed to calculate *between-class variance* is the mean intensity value of the pixels assigned to C_1 and C_2 which is m_1 and m_2 . The mean of all pixel value, m_G is needed to.

$$m_1(k) = \frac{1}{P_1(k)} \sum_{i=0}^k iP_i \quad (2.9.6 - 4)$$

$$m_2(k) = \frac{1}{P_2(k)} \sum_{i=k+1}^{L-1} iP_i \quad (2.9.6 - 5)$$

$$m_G = \sum_{i=0}^{L-1} iP_i \quad (2.9.6 - 6)$$

After calculate P_1 , m_1 , P_2 , m_2 and m_G , the *between-class variance* can be calculated. *Between-class variance* is assigned to σ_B^2 .

$$\sigma_B^2 = P_1(m_1 - m_G)^2 + P_2(m_2 - m_G)^2 \quad (2.9.6 - 7)$$

2.10.7 Distance Transform

In image processing, usually it is useful to be able to calculate the distance between two points in an image. Distance transform reconstruct the pixel image to the distance of pixel point to the background.

There are three algorithms that commonly used to calculate the distance between two points at the image. The first one is Euclidean distance or straight line distance. Euclidean distance can be used to calculate distance between two points horizontally, vertically or even diagonally. The second one is City Block distance or Manhattan distance. Manhattan distance is used to calculate distance between two points horizontal or vertically, diagonal is now allowed. The last is Chessboard Distance. Chess board distance assumes that you can make moves on pixel grid as if you are a King in chess.

$$D_{Euclidean} = \sqrt{(x_2 - x_1)^2 + (y_2 - y_1)^2} \quad (2.9.7 - 1)$$

$$D_{City} = |x_2 - x_1| + |y_2 - y_1| \quad (2.9.7 - 2)$$

$$D_{Chess} = \max(|x_2 - x_1|, |y_2 - y_1|) \quad (2.9.7 - 3)$$

2.10.8 Watersheds

The concept of watersheds is based on visualizing an image in three dimensions, two special coordinates versus intensity. It can be illustrated like there are three types of points, points belonging to regional minimum, points which a place to drop of water or catchment basin, and points at which water would be equally fall or divide lines of watershed. Suppose there is a hole in each regional minimum as catchment basin and let the catchment basin is flooded from below by letting the water rise through the hole. The flood will reach a stage when only the surface of the basin is visible and the boundary of the basin is the divide lines of watershed.

In image processing, watershed is used to find the divide lines of the watershed. The regional minimum is the center of the object and then the catchment basin is the region for searching the divide lines. The center and the searching region can be calculated by applying the distance transform algorithm. First, locate center of the each object and give it a label, $L = 1, 2, 3 \dots$, for each object. Then do a conditional dilation and change the pixel into the center of the object. The dilations stop when it meets the background or the other object. Then put a dot at the meeting points so finally the dot will form the divide lines of watershed.

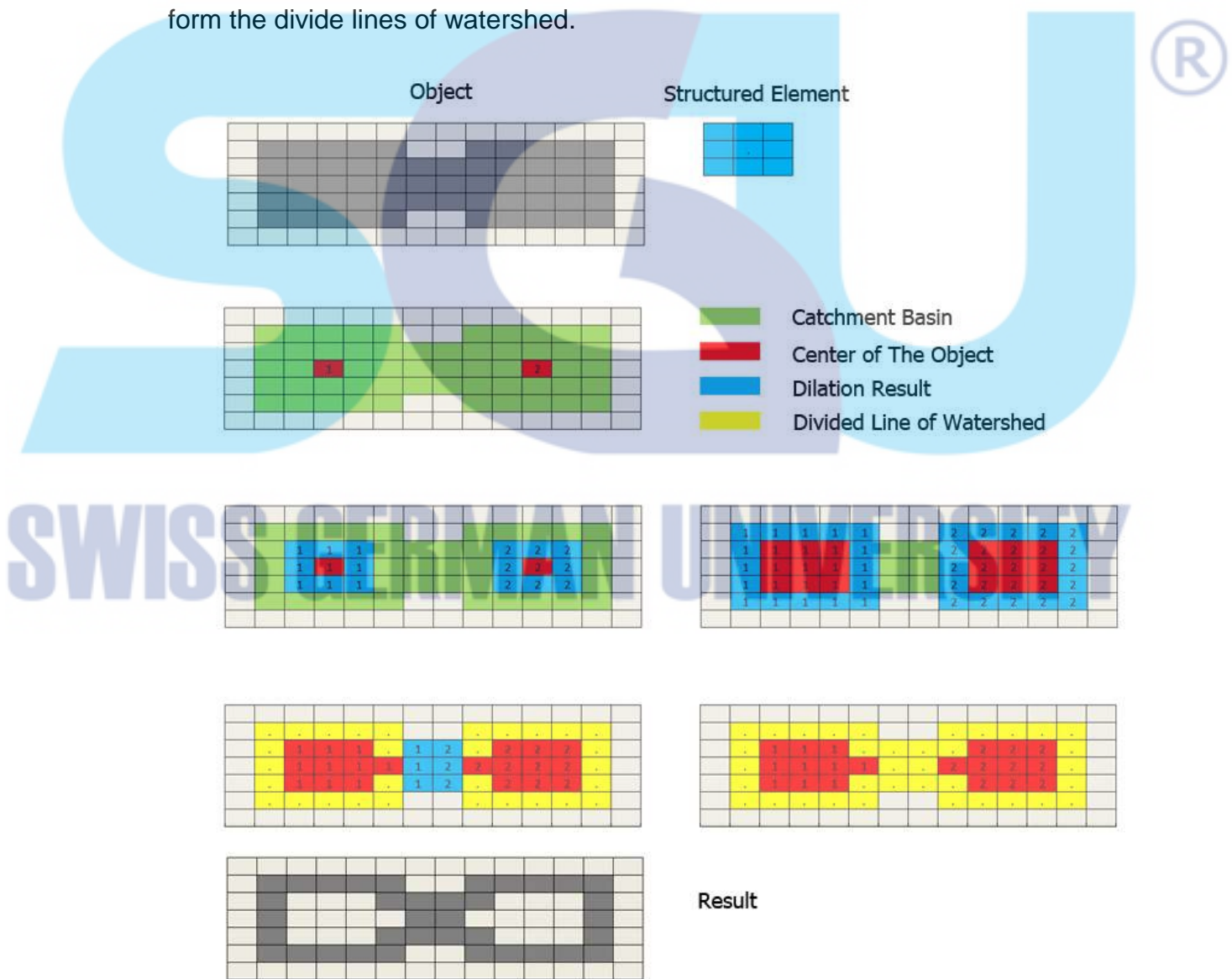


Figure 2.14 Watershed Illustration