# PATTERN RECOGNITION FOR LIGHT MICROSCOPES

Martin GUNIA, Master Degree Programme (5) Dept. of Intelligent Systems, FIT, BUT E-mail: xgunia00@stud.fit.vutbr.cz

Supervised by: Martin Drahanský

### ABSTRACT

Focal depth of a light microscope is usually much smaller than the proportions of the specimen and images taken by the microscope consist of sharp and unsharp regions. This problem can be eliminated by taking series of images with different focal adjustment, covering the entire volume of the specimen and combining them into a single sharp image.

#### **1 INTRODUCTION**

An unfavourable limitation of light microscopes is their low depth of field – range along the optical axis in acceptable focus. The specimen often occupies space larger than the attainable depth of field and areas that fall outside appear blurred on the image plane. This makes it impossible to acquire sharp images. According to the Rayleigh criterion [3] the depth of field is rigorously proportional to the numerical aperture of the lens, making the situation worse at higher magnifications.

A common approach to acquire sharp image is to take a series of images with different focal adjustments covering the entire volume of the specimen. Salient regions of each acquired image are extracted and combined together. This technique is commonly called *image fusion* and has applications in many fields such as medicine, robotics, military, security, photography or remote sensing. The data to be fused typically come from multiple sources and have different temporal, spatial, spectral or radiometric characteristics, hence we speak about *multisensor* or *multimodal* image fusion.

#### 2 IMAGE FUSION

Early image fusion methods work directly on the source images. For each pixel in the resulting image, value from the image that maximizes a certain local focus measure is used. The focus measure usually evaluates the amount of high frequency components in the inspected area. The most common measures are the *intensity variance, norm of* 

*image gradient* or *norm of image Laplacian*. Major advantage of these methods is their computational simplicity, as no transformations of the images are needed. Additionally, they can be applied to color images directly.

With the introduction of pyramid transformation [1] sophisticated *multiresolution* approaches began to emerge. Multiresolution analysis then became an important tool in image processing for its capability to provide both spatial and frequency characteristics independent of scale.

Image pyramid is a sequence of copies of low-pass or band-pass filtered copies of the original image. Since the high frequencies are suppressed by the filter, filtered image can be downsampled in the spatial domain without losing information, resulting in a pyramid-like hierarchy. To name few examples, Gaussian pyramid  $g_1 \dots g_n$  is obtained by assigning  $g_1 = I$  and  $g_{i+1} = \downarrow (g_i \star w)$  for each  $i = 1 \dots n - 1$ , where *n* is the number of levels of the pyramid, *I* is the source image, *w* is a symmetric weighting function following the Gaussian probability distribution and  $\downarrow$  denotes subsampling operation. Laplacian Pyramid is a sequence of images  $L_1 \dots L_n$  where each image represents the difference between two levels of a Gaussian pyramid.  $L_i = g_i - \uparrow (g_{i+1})$  for  $i = 1 \dots n - 1$  and  $L_n = g_n$ , where  $\uparrow$  denotes upsampling by interpolation (linear or cubic). Note that the first n - 1 levels carry the detail information and are called *detail levels*. The last level of the decomposition is commonly called *approximation level*. The original image can be reconstructed by upsampling and summing all levels of the pyramid.

Nowadays, the most popular form of image fusion is based on the wavelet transform. In [2] Mallat proposed a fast algorithm for discrete wavelet transform (FDWT) and reconstruction using a pair of low-pass and high-pass conjugate or quadrature mirror filters organized in a filter bank. The input signal is filtered by both the low-pass and high-pass filter and subsampled, yielding two subbands. The process is repeated recursively for the low frequency band. Two dimensional images are analyzed in a similar way. First both filters are applied to the rows of the image, giving a high and a low frequency band. Both bands are subsampled in the spatial domain. Then the same is applied column-wise to the output of the row filtering, yielding four subbands defined by the filters used: low-low, low-high, high-low and high-high. As in the case of one dimensional FDWT the process is repeated for the low-low band. Similarly to the pyramid transform, the last low-low band can be considered as the approximation level of the decomposition while the others represent the detail levels.

Once the source images are transformed using either pyramid or wavelet decomposition, the transform coefficients of each image are fused and transformed back to give the resulting image. The fusion rules can be classified as either pixel based, window based or area based. Pixel based rules perform the fusion on each coefficient separately. For the detail levels, a good fusion rule is to take the maximum of the absolute values of the corresponding coefficients. The approximation level coefficients are usually averaged as they do not carry any detail information. Window based methods use a small window centered at each inspected coefficient. Area based methods aim at locating compact regions in the parametric images and performing fusion of the regions.

## **3 RESULTS**

The algorithms were implemented in C++ and are intended to be merged with a work from last year aimed at classification of diatoms. Our hope is to improve the precision of the classification by providing sharp images with high information contents.

Methods working directly on the source images produce unpleasant artifacts in the fused image (see Figure 1, bottom left image) caused by the lack of the scale sensitivity towards the details. While this can be partially eliminated by increasing size of the inspected window, the computational cost increases proportionally and speaks clearly for the methods working at the transform domain. Both Laplacian Pyramid and Wavelet decomposition yield visually good results.

Further work includes multichannel image fusion, complex wavelets and possibly a GPU implementation of the algorithms for higher speed.



Figure 1: Top: 3 sample images of Surirella Spiralis diatom. Bottom: fused images using (from left to right) intensity variance, Laplacian pyramid and 2D DWT with Daubechies 4 wavelet.

#### REFERENCES

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