A WAVELET APPROACH FOR CELLULAR OPTICAL PHASE SHIFT DATA ANALYSIS

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ABSTRACT

The project focuses on applying the continuous complex Morlet wavelet transform to analyzing the phase shift data of a HeLa cell sample obtained by the Fourier phase microscopy technique. The result of the analysis shows that there is some rhythmic processes happened steadily at some certain frequencies over periods of time. They are supposed to be related to some characteristics of the cell and the mutual effects between cells.

Index Terms— Cell dynamics, Fourier phase microscopy, wavelet analysis

1. INTRODUCTION

In the recent decades, quantitative retrieval of the phase delay techniques became very useful in cell dynamics to discover the features associates with the multitude of regulatory processes which provide much information about the cells and tissues[1]. These techniques provide us with the phase imaging data in the format of intensity images of phase shifted caused by optical properties of different parts of cells. Supervising the change of this feature over time, we can extract intracellular information which is useful for both cell dynamics understanding and various applications such as disease diagnosis.

Continuous wavelet analysis is considered to be a good method to explore the dynamical properties of intracellular regulatory mechanism operating at different scale [2]. Among various wavelet mother functions, Morlet wavelet is typically considered in analysis of rhythmic components of endogenous modes of the cell and possible interactions between the processes inside the cells.

In this project, at first, we will implement the data analysis introduced in [3] on the data obtained from quantifying the optical phase shifts associated with cells with the continuous Morlet wavelet transform. Based on the result of this analysis, we will find the relation from phase shift features with the intracellular properties.

2. PHASE SHIFT DATA OF CELL DYNAMICS

There are various kinds of processes happen inside living cells: Cell division, protein synthesis, movement or motility These processes present biological conditions of cells and provide the clues to detect the abnormal phenomena which can be related to diseases. Optical phase shift associated with cells quantifying is a well-known method to have information about morphology and dynamics of the cells at the nanometer scale.

Among recent methods used to investigate the cellular optical phase shift, Fourier phase microscopy (FPM) is a new full-field phase imaging techniques developed by the Spectroscopy Laboratory at Massachusetts Institute of Technology. FPM method combines the principles of phase contrast microscopy and phase shifting interferometry, such that the scattered and unscattered light from a sample are used as the object and reference fields of an interferometer. The experiment setup of FPM is addressed in detail in [1].

At the experiment at MIT's Spectroscopy Laboratory, Fourier phase microscopy was used to quantify the cellular dry mass through the refractive index and thickness of HeLa cell samples. The result is the set of 400 phase shift images of size 512×768 pixels which are captured at the sampling period 15s in time and $0.12 \times 0.12m^2$ in spatial sampling resolution. These images were stored in raw format and can be loaded as two dimensional matrices of double real numbers ranged from -420 to 420 which correspond to the range of dry mass content rate from 0 to $35pg/mm^2$.One of these images is plotted in Fig.1.

Quantitative information obtained about the dry mass of the cells is known to be able to allow investigation of cell movement, growth or shape change in a totally non-invasive manner through some analyzing means. One of them is wavelet analysis which will be introduced in the next paragraph.

3. CONTINUOUS WAVELET DATA ANALYSIS

The analyzing method chosen for the cellular phase shift data obtained is wavelet analysis which is becoming a common tool for analyzing localized variations of power within a time series [4]. By decomposing a time series into time-frequency

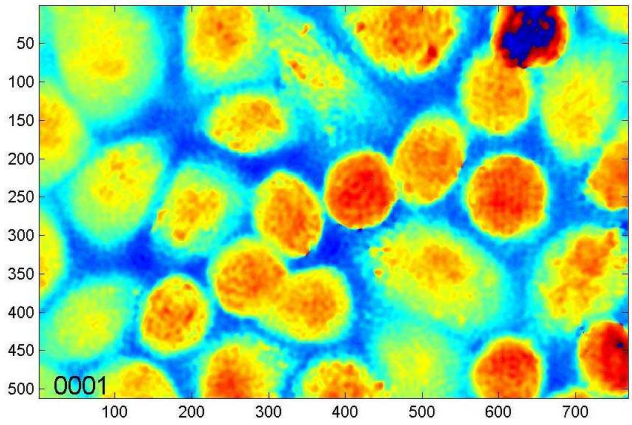


Fig. 1. A phase shift data image obtained by FPM .

space by wavelet transform, we can determine both the dominant modes of variability and how those modes vary in time [5]. The continuous wavelet transform of a signal $x(t)$ is defined as follows:

$$T_x(a,t) = \frac{1}{\sqrt{a}} \int_{-\infty}^{+\infty} x(u)\psi^*(\frac{u-t}{a})\, du.$$

In above formula, ψ is a mother function,$T_x(a,t)$ are the wavelet coefficients and a is a time scale parameter. The choice of ψ depends on the problem to be solved.

Continuous Wavelet transformation shown above has been widely considered as a powerful tool for spectral analyses of biological time series [3]. The obvious advantages of this approach in comparison with the classical Fourier transform are the zooming property, which allows us to characterize signals locally in time or spatial domain and the shift invariant property which give us stable result with different data configurations.

Especially, in the case of cell dynamics, the advantage of continuous wavelet method is underlined by its excellent ability to deal with the nonstationarity which may be associated with some different frequency regions [6]. Specifically, the Fourier analysis work well in the case the nonstationarity is associated only with the low-frequency region of the power spectrum relative to the rhythms of interest. Such nonstationarity is treated as a slow trend and may simply be filtered out from the data. However, in some experimental recordings, this condition is not true. In many situations, the nonstationarity may be also associated with higher frequencies. Analysis of such time series using Fourier transform can lead to misinterpretation of the obtained results. As example, two peaks coexisting in the power spectrum can correspond to two independent modes or only a single mode whose instantaneous frequency changes in time from one value to another. As stated earlier, the choice of the mother function needs to be based on the essence of the data. With the strength of working with rhythmic components, the Complex Morlet function [7] is chosen to be the mother function in our project. A simplified expression of the Morlet function has the below form [2]:

$$\psi(\tau) = \pi^{-1/4} exp(j2\pi f_0 \tau) exp[-\frac{\tau^2}{2}])$$

This kind of wavelet has a disadvantage of some possible spurious effects, especially for time series with nonzero mean [3]. To avoid such effects we need to transform the data series in time into zero mean value before applying the wavelet technique. With this adjustment, there is practically no difference between the results obtained with the Morlet wavelet and with other mother functions which are proposed to prevent mentioned type of error [3].

4. EXPERIMENT AND RESULT

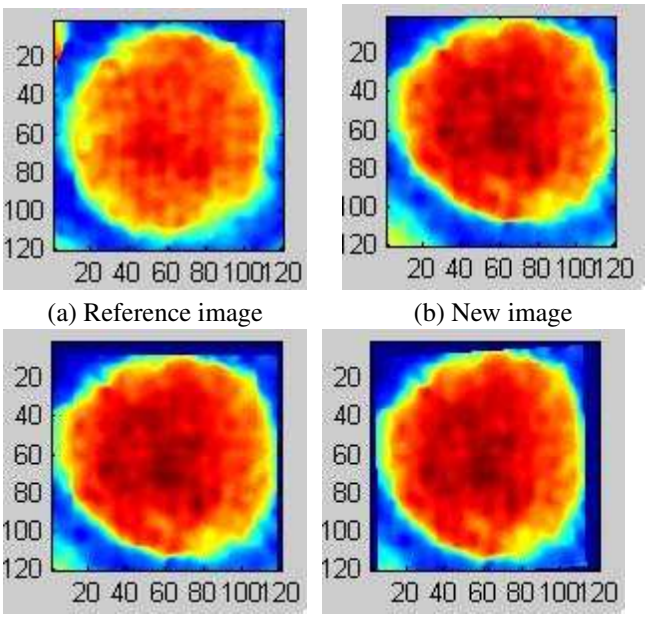
The phase images obtained from Fourier phase microscopy by the Spectroscopy Laboratory at MIT are the input data of our experiment. In order to create the good condition for the analysis work, the preliminary stage is held to prepare the input data to have the convenient form.

Firstly, since these images have the very big size and high resolution which can slow down the program, we need to select a region of interest and downsample it into a small subset of original data. The 120×120 regions are downsampled by 4 to produced subimages which contains one complete cell are created as shown in fig. 2(a).

Secondly, after having a concise data set, we need to register the images to prevent the aberrance made by the movement and shape changes of the cells in the sample. The new subimage (fig 2(b)) is compared with a reference (fig. 2(a)) to find the suitable parameter of translation (fig. 2(c)) and warping (fig. 2(d)) which produce the best match with the reference image. The registration module used in the project is made by Yaser Sheikh from Robotics Institute Carnegie Mellon University.

With the standardized data, we applied the wavelet transform on the time series at some points at instant locations and obtained the sequence of wavelet coefficient $T_x(a,t)$ at each point. From these sequences, we calculate the energy density of the coefficient as $E_x(a,t) = Ca^{-1}|T_x(a,t)|$ where C is a constant which can be chosen arbitrarily.

Each of these energy density sequences represents a surface in three-dimensional space whose sections at fixed time moments correspond to the local energy spectrum. This surface can be simplified and visualized by the dynamics of the local maxima of $E_x(a,t)$. Each of these points is found as the peaks through the frequency range at corresponding time period. Fig. 3a demonstrates all maxima of $E_x(a,t)$ detected in the original signal at time moment t. Supplementing to the local maxima plot, we investigate the dominant spectral components by calculating the time averaged power spectrum, which is an analogue to the Fourier power spectrum. The plot



(a) Reference image (b) New image

(c) Translated image (c) Warped image

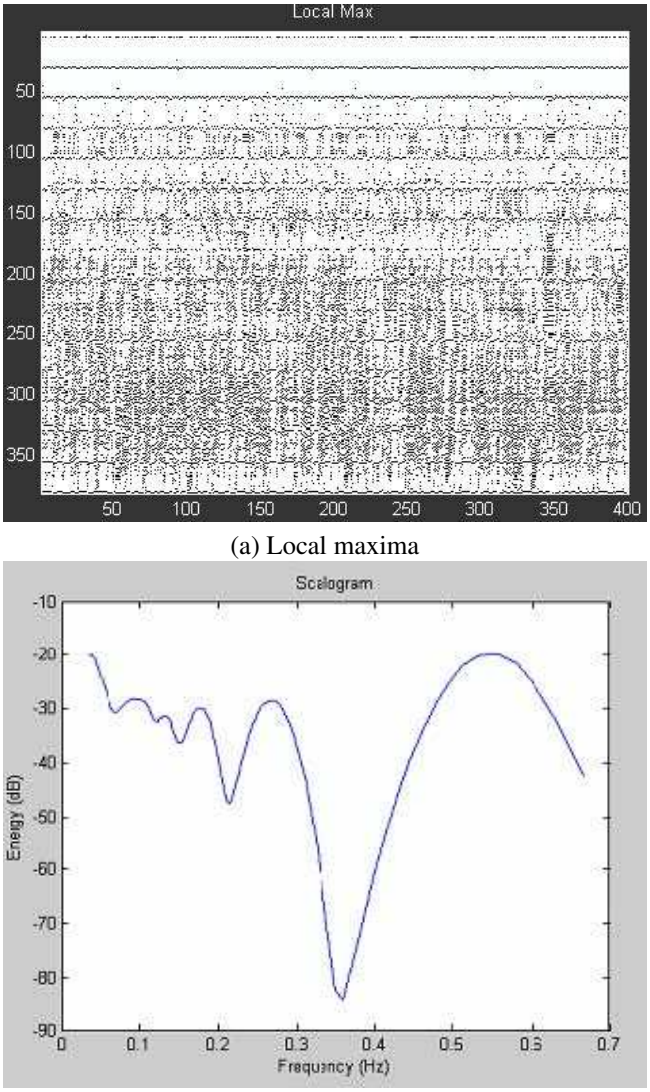
Fig. 2. Image registration stage

of this power spectrum is shown in Fig. 3b.

In the local maxima plot and power spectrum, we can observe some stable peaks, i.e, it virtually does not change over the time, at certain frequencies . These stable positions seem to be very clear in the high frequencies and go blurry in lower ones. This phenomenon is similar to some previous works shown in [3] and [2]. In these papers, the stable frequencies are supposed to be made by some particular rhythmic processes in the cell. Similarly, in our case, t(f_{fast}) and the other recognizable peak at 0.033 Hz (f_{slow})derives from the slow myogenic dynamics. This result suggests that, the HeLa cells could have the common characteristics as neurons, another kind of cell, in some rhythmic processes of both slow and fast modes with another range of frequency. Moreover, supervising the distribution of stable peaks in frequency axis, we found out that these common local maxima appear periodically. This periodicity represents the relation between different rhythmic processes which need to be explored in the next stage of study.

5. DISCUSSION

In this project, we have studied the fundamental mechanism of interference microscopy technique used in recording the complicated features produced in cell dynamics. One instance of wavelet transformation - continuous complex Morlet wavelet - has been used in analyzing data obtained by FPM. The result of the analysis is compared with other works which showed the relation of computational data with



(a) Local maxima

(b) Averaged power spectrum

Fig. 3. Analysis result plots

dynamical properties of intracellular regulatory mechanisms operating at different time scales. In the next step, the project would concentrate on matching each peak in energy density to some particular process in the cell. On the other hand, we will try to apply the double wavelet technique which suggests to extract the time dependence of instantaneous frequencies f_{slow} and f_{fast} and applying a second wavelet transform on that data.

6. REFERENCES

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