Enhancement of weak signals by dynamic stochastic resonance in dark-field microscopy

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Enhancing weak signal acquisition is pivotal for bolstering analysis and detection capabilities. Herein a dynamic stochastic resonance (DSR) algorithm for weak signal amplification is presented, which was identified to significantly improve the imaging visibility of dark-field microscopy (DFM) nanoparticle under low illumination conditions. By combining with composite filed microscopy (CFM) further, DSR displays much more effectively signal amplification, showing high promise for detection purpose.

Keywords: Dynamic stochastic resonance, dark field microscopy, weak signal amplification, composite filed microscopy.

Weak signals pose a challenge to analysis and detection techniques owing to their own signal-to-noise ratio (SNR) and extremely low detectability fluctuating around the detection threshold of the instrument. Dark field microscopy (DFM) technology, which has high sensitivity and strong real-time performance, has been widely used in chemical analysis of single nanoparticle, monitoring cellular life activities, live cell imaging, and molecular detection. However, the weak scattering performance caused by certain particles in real case of DFM, or reactions results in low signal intensity in high background interference, greatly hinders effective analysis and detection.

The amplification of weak signals that enhances the SNR directly or indirectly is thus a strategy so as to enable improved performance, and one effective amplification is by updating microscope in dark-field microscopy through side-illumination, or construction of composite field microscopy (CFM). It has identified that microscope modification is one important strategy to enhance detection sensitivity, and both the side-illumination and use of CFM significantly are good ways to enhance weak signals.

Artificial intelligence (AI) techniques, particularly deep learning, have been effectively employed to enhance the accuracy of DFM imaging, extract scattering signals from the complex background of live cells, analyze single particles and clusters. Digital image processing techniques, by accurately modeling the signal acquisition process, aim to improve the quality of various signals and imaging have been reported, which are not required to have hardware modification and avoid massive data collection involved, allowing for real-time and fast amplification of weak signals during the detection process.

Stochastic resonance (SR) is a phenomenon that exists in nonlinear systems, involving the interaction between weak signals and noise. Stochastic resonance occurs when the SNR and input/output correlation exhibit a notable maximum at a specific noise level. In contrast to conventional understanding, noise in the system does not degrade the performance of the system signal output, but amplify the input signal of the system by modulating the noise to an optimal level. Therefore, SR has been applied in the fields such as magnetic resonance imaging, physical circuits, neurobiology, and night vision imaging for signal enhancement in strong background. In the enhancement of weak signals, methods such as stochastic resonance, neural networks, and multi-objective optimization have been employed in the development of early diagnosis and recognition of mechanical faults. Therefore, integrating SR principles with computer algorithms to enhance weak signals holds considerable potential for applications.

Scheme 1. DFM image processed via the DSR algorithm to enhance the brightness.

Here, we develop a digital image processing dynamic stochastic resonance (DSR) algorithm based on the SR principle, which enhances the weak signals in DFM imaging by increasing the V value in the HSV (hue, saturation, value) mode. As Scheme 1 shows, after obtaining DFM images, for the convenience of calculation and to ensure that the implicit colour of the image are preserved, we convert the RGB image to the HSV domain. Subsequently, we extract the inherent noise of the images and employ the distribution information of the noise intensity for iterative resonance with the images. This process effectively enhances weak scattering signals in imaging. As a proof of concept, DSR applied to DFM images of AgNPs. It was found that the DFM images processed by the algorithm with images obtained under increased illumination power is greatly proved without distortion. In addition, we also explored the application of DSR algorithm to CFM to enhance the weak scattering signal of AgNPs, demonstrating that the DSR algorithm is effective for high universality of scattering images with broad application prospects of.

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Considering that SR displays the interaction between randomly perturbed noise and weak signals in certain nonlinear systems, which actually enhances the system's response to this weak signal,\(^1\) we use the Langevin equation of motion to model a classical one-dimensional nonlinear dynamic system with SR, and the two stable states of the double well potential system represent the signals before and after resonance:

\[
\frac{dx(t)}{dt} = [ax - bx^3] + B\sin(\omega t) + \sqrt{D}\epsilon(t)
\]

(1)

wherein \(a\) and \(b\) are the parameters of the bistable systems, \(\frac{dx}{dt}\) is the rate of change of the particle position, \(B\sin(\omega t)\) is the system input of periodic signal, and \(\sqrt{D}\epsilon(t)\) is noise.

Different from DFM imaging, which has poor visibility generally,\(^2\) the particles are difficult to be distinguished owing to insufficient illumination, and their scattering light signals might be concealed by inherent noise, inherent noise in SR is a type of special noise, which exerts SR effects without the need for collecting a large number of frames for processing and with low computational complexity. It can be considered as the optimal noise under specific conditions to induce SR. When each pixel in a low contrast image SR with inherent noise, the overall grayscale distribution of the image is improved, thereby improving the visibility of scattering signals in dark field imaging.

We regard the pixels of an image as physical dynamical particles in a nonlinear system. The grayscale values of image pixels correspond to the one-dimensional state information parameters of particles. In such case, the image processing steps of DSR are roughly as follows:

Firstly, convert the input RGB image to an HSV color model; Secondly, adjust the parameters of the bistable system to enhance the intensity value of pixels. By solving the function at the optimal SNR, we obtain that the double well system has the best stability when \(a = 2\sigma_0^2\) and \(b < 4a^2/27\), where \(\sigma_0\) is the standard deviation of noise distribution at the optimal SNR. In specific experiments, we set \(\Delta t = 0.01\), \(a = 4\), \(b = 0.0001 \times \left(4a^2/27\right)\), ensuring that the input signal can effectively enhance the weak signal in DFM through SR.

Use the Euler Maruyama iterative discretization method to solve the stochastic differential equation given in eq (1), as follows:

\[
x(n + 1) = x(n) + \Delta t[ax(n) - bx(n)^3 + V]
\]

(2)

Wherein the value of \(V\) is the given image intensity. Finally, based on the parameters set in the previous stage, modify the pixel intensity value and implement the DSR algorithm using MATLAB.

Both AuNPs and AgNPs were prepared and characterized according to commonly accepted protocols.\(^3\) \(^4\) SEM images showed that both particles have spherical morphology and sizes around 50nm (Supplementary S1a,b), while dark field imaging exhibits scattered light of green and blue. The UV spectrum shows that the absorption peaks of the two particles are at 532nm and 430nm respectively (Supplementary S1c,d).

We established the DSR algorithm in MATLAB based on the proposed principle. To validate the algorithm's effectiveness in enhancing weak signals, we chose the Fenton reaction of hydroxyl radicals (•OH) etching AgNPs. The etching AgNPs through the Fenton reaction in solution was at first identified (Supplementary S2), and then real-time DFM monitoring of the etching process of AgNPs was made (Supplementary S3).

DFM images revealed that as the reaction progressed, scattering signal of particles was weak, and the visibility of particles gradually decreases, during which some particles even disappear (Supplementary S3). After 10 minutes of reaction, the overall scattered intensity in the imaging decreased to 28% of the pre-reaction level (Supplementary S4a). Statistical analysis was conducted separately for particles exhibiting strong and weak scattering.

It was observed that during the reaction, the signal intensity of weakly scattering particles decreased more rapidly. This posed a challenge to our analysis and monitoring of the weak signals and their changes during the reaction. Furthermore, maintaining the integrity of monitoring in some reactions can improve our understanding of the reactions.

Figure 1. Dark field imaging before and after Fenton reaction etching of AgNPs, as well as dark field imaging enhanced by DSR after etching.

Figure 1 demonstrates that the scattering signal of AgNPs was enhanced with DSR processing, improved visibility of single particle image was obviously obtained. After DSR processing, the overall scattering signal intensity in the region increased to 73% of the pre-reaction level (Supplementary S4b). The number of particles identifiable by the software increased from 19 to 23 (Supplementary S5). Statistical analysis was conducted separately for particles exhibiting strong and weak scattering, revealing intensity increases of 201% and 193% (Supplementary S4c,d).

Noticeably, some particles still cannot be detected even if through SR process, which is due to the complete etching of small AgNPs in the reaction, indirectly confirming enhancement of weak scattering signals during the reaction is critical for us to clearly distinguish between particles that are completely etched or have weak scattering signals. That is, imaging of etching AgNPs particles...
Figure 2. DFM images of AuNPs with increasing illumination power (upper row), and images under low illumination after DSR processing (down row).

Figure 3. RGB value distribution of selected particles (1,2,3 in Figure 2), and contrast of RGB value changes with increasing illumination power and DSR treatment.

Figure 4. Imaging and intensity distribution of AgNPs in different modes. (a) DFM. (b) CFM (c) DSR processing (d) CFM-DSR hybrid mode. (e-h) 3D intensity distribution maps of DFM, CFM, DFM-DSR, CFM-DSR imaging modes.

These results showed that our algorithm not only improves the visibility of the particle scattering signal but also ensures accuracy to a certain extent. The "distortion" observed in larger particles with initially stronger scattering intensity after SR does not affect the algorithm's ability to enhance particle signal visibility. In addition, we compared the weak signal enhancement effect of DSR with other...
commonly used image enhancement methods (Multi-Scale Retinex, Gamma correction and Homomorphic filtering etc.) on DFM imaging, and the results showed that DSR is more suitable for enhancing weak scattering signals in DFM (Supplementary S7).

Composite Field Microscopy introduces a direct single-channel illumination source into a dark-field oblique illumination setup, utilizing the Plasma-Induced Light Concentration (PILC) effect to enhance weak signals. We contrasted the enhancement effects using DSR and CFM methods and attempted the synergistic application of DSR-CFM in enhancing weak scattering signals in DFM. Initially, we conducted low illumination intensity DFM imaging of AgNPs (Fig. 4a). Subsequently, we employed both CFM and the DSR algorithm to enhance particle visibility (Fig. 4b, c). Both methods exhibited a significant improvement in signal visibility.

Since the CFM necessitates the introduction of a direct single-channel illumination source, it also introduced significant background noise while enhancing signal visibility. From the 3D signal intensity plot (Fig. 4e-g), it is evident that, compared to CFM, our established DSR strategy avoids introducing additional background noise and greatly increases the SNR.

Due to the fact that the underlying logic of the two signal enhancement strategies does not conflict, it can be seen from the Figure 4d that in the CFM-DSR hybrid mode, the intensity of weak signals is further enhanced. Comparing the 3D signal intensity plots between CFM and CFM-DSR hybrid mode, it is evident that our proposed DSR algorithm exhibits a significant enhancement effect on faint scattering signals enhanced by CFM. This validates the effectiveness of our proposed DSR weak signal enhancement strategy. Furthermore, experimental results from the CFM-DSR hybrid mode.

In summary, we have developed a DSR algorithm to enhance weak signals. Experimental validation of signal enhancement for weak scattering signals of nanoparticles under DFM demonstrates that this algorithm effectively improves signal visibility and SNR while ensuring the signal is not distorted. Furthermore, in experiments combining our CFM-DSR signal enhancement strategy with hardware modifications, our approach exhibits a synergistic enhancement effect, further boosting the detection capability of weak signals by data acquisition equipment. This method not only directly increases the SNR in detection but also opens up new avenues for utilizing digital technology to enhance the performance of instrument modifications.

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References and Notes
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