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Common Problems and Solutions of Leica Laser Scanning Confocal Microscope SP8 in Biomedical Applications

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Abstract

Laser scanning confocal microscopy is a cellular biomedical analysis instrument used for fluorescence observation, and it has been widely used in many fields of biomedical research. Article in Guangdong medical university scientific research platform management service center of the existing German leica TCS-SP8 laser confocal microscope (hereinafter referred to as SP8), for example, combined with the entire school teachers and students in the actual situation of the daily training and operation process, summarizes the instrument in the use of common problems and solutions, provide standardized for the instrument users operation ideas.

Keywords

Laser Scanning Confocal Microscope; SP8; Common Problems and Solutions; Standardized Operations.

1. Introduction

Laser confocal microscopy is a vital scientific research instrument in cell biology and medical research. Compared to conventional fluorescence microscopes, it offers higher resolution and achieves clearer imaging results. Widely used for fluorescence imaging of cells, tissues, and three-dimensional materials, laser confocal microscopy provides indispensable and powerful technical support for in-depth studies of cell structure, tissue function, and material characteristics [1].

In 2016, the Leica TCS SP8 laser confocal microscope (SP8) was acquired by the Scientific Research Platform Management and Service Center of Guangdong Medical University for the construction of a university-level large-scale instrument sharing platform. This microscope features an intuitive software interface and precise imaging capabilities, currently logging over 2,000 operational hours annually. The SP8 is shared and open to all faculty and students, with daily management following a structured approach: regular basic operation training, a qualification examination, independent computer operation by trained users, and personalized imaging guidance. To date, 105 research teams have applied to use the shared instrument.

Given the diverse research directions of these teams, faculty and students have varying demands for the instrument's functionalities. Insufficient attention to operational details can lead to misoperation. Based on the author's experience in managing the SP8, this paper summarizes common issues encountered during daily use, proposes solutions, and provides standardized operational guidelines for users to ensure efficient and accurate utilization of the instrument.

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2. Common Applications of Laser Scanning Confocal Microscope

2.1. Fluorescence Imaging of Cells

Confocal microscopy, combined with fluorescence labeling technology, not only can present the fine structure inside the cell, such as nucleus, mitochondria, endoplasmic reticulum, with high resolution and high contrast, but also its high speed and high sensitivity makes it particularly suitable for real-time three-dimensional imaging of living cells. For example, 3i's rotary confocal microscope not only improves imaging speed and resolution, but also significantly reduces phototoxicity, allowing researchers to go deeper into the fine structural changes in cells, as demonstrated in studying responses to TFH cell and humoral immunity. By labeling target molecules with fluorescent proteins (e. g., GFP, RFP) or specific dyes, researchers can precisely investigate the cellular localization, interaction, and function of these molecules in cells. In addition, confocal microscopy also supports real-time dynamic imaging, which can track the movement of intracellular molecules, vesicle transport, signal transduction and other processes, providing a powerful tool to reveal the mechanism of cellular life activities [2,3,4].

2.2. Fluorescence Imaging of the Tissues

Laser confocal microscopy (CLSM) has unique advantages in the field of tissue fluorescence imaging, enabling high-resolution 3D imaging of fluorescently labeled tissue samples. It can not only observe the deep internal subtle structure of cells and tissues, but also conduct the measurement and analysis of cell morphological parameters and fluorescence intensity, which is widely used in the cell structure analysis of cytoskeleton, cell apoptosis, cell organelles and so on. For example, laser confocal microscopy has extensive applications in the field of neurobiology, enabling clearly observing the morphological structure of neurons, including the details of cell bodies, dendrites, axons, as well as synaptic connections. Moreover, this technique monitors the dynamic changes in calcium ion concentration within neurons through calcium imaging technology in real time, which contributes to understanding the neurobiological processes such as excitatory, inhibitory, and synaptic transmission of neurons. In addition, this technique can also be used to study the structure and function of other organs and tissues, such as angiogenesis, immune cell distribution, etc., providing an important means to reveal the operation mechanism of complex biological systems [5,6,7].

2.3. Fluorescence Imaging of 3D Materials

Confocal microscopy performs well in fluorescence imaging of 3D materials, enabling high-precision imaging and analysis of fluorescent markers in 3D space. This technology is widely used in material science, chemistry and other fields, such as: material surface morphology analysis: 3D reconstruction of roughness and morphology of material surface; film and coating research: analysis of thickness, uniformity and defect distribution of film and coating; polymer and nanomaterials research: reveal the structural characteristics and dynamic behavior of materials. In addition, confocal microscopy can also be used to study the microstructure of biomaterials (such as scaffolds, hydrogels) and their interactions with cells, providing an important basis for material design and optimization [8,9,10].

In addition to fluorescence imaging, confocal microscopy can also be used in other types of imaging techniques, such as phase-contrast imaging, polarization imaging, etc. These techniques can provide more information about the sample and help scientists with a more comprehensive understanding of the structure and nature of the sample.

3. Common Problems and Solutions in the Use of Instruments

Laser confocal microscope belongs to the precision large instruments and equipment, Guangdong medical university scientific research platform early for the instrument is

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professional responsible, entrust test mode, with the improvement of instrument utilization, school gradually open to the teachers and students on the independent operation mode, the mode requires after theoretical training, computer training and final assessment, qualified to obtain independent operation authority. Due to the complicated operation steps of the instrument, including sample preparation, parameter setting, shooting mode selection and instrument operation and other technologies, the problems caused by misoperation are also more frequent. At present, the problem of misoperation of laser confocal microscope SP8 is summarized as follows:

3.1. Software Problems

3.1.1. Software Error Reporting

Operation of the laser confocal microscope SP8 software starts by double-clicking on the LASX icon on the computer desktop. After the instrument experiences about 1 minute of self-examination, the dialog box will pop up, and after confirming the parameters are correct, click to the next step. Sometimes the students will find that the laser setting column is blank, click the conventional scan, the software error "no laser light", indicating that there is no laser (Figure 1a). This problem is usually caused by the mismatch between the shooting parameters and the microscope setting when starting the software, which causes the unit to start normally. The solution is: after you restart the software, follow the following steps: First, configure the Configuration to machine mode, so that in this directory, the instrument will start both the microscope and the operation software by default. Next, make sure that the Microscope is set to a DMI 8 that matches your microscope model. For details, please refer to the configuration interface shown in Figure 1b.

(a)Software error interface (b)Correct software interface

Figure 1. Software settings for laser confocal microscopy

3.1.2. False Positive Signal Due to no Sequence Scan Mode

In the experiment of multi-fluorescence imaging, some teachers and students found that the positions of blue, green and red was highly overlapping in the picture analysis, which was far from the theoretical results. At this time, the color series problem of the instrument shooting should be excluded first. Color string refers to the phenomenon that the emission spectra of two or more fluorescent dyes overlap. If the sample is scanned by three lasers, color may occur between different channels (see Figure 2a). The solution is to adopt a sequential scanning mode, in which samples are successively irradiated with laser light at different wavelengths and collected sequentially in the corresponding fluorescence detection channel, followed by confocal images of each fluorescence. For the specific operation process of the double fluorescent staining sample is: first, excite the corresponding first fluorescent material in the first channel, and display the first channel, excite another fluorescent substance, and display the fluorescent image in the second channel. The corresponding sequential scanning software can display and synthesize the different fluorescence images of the two channels

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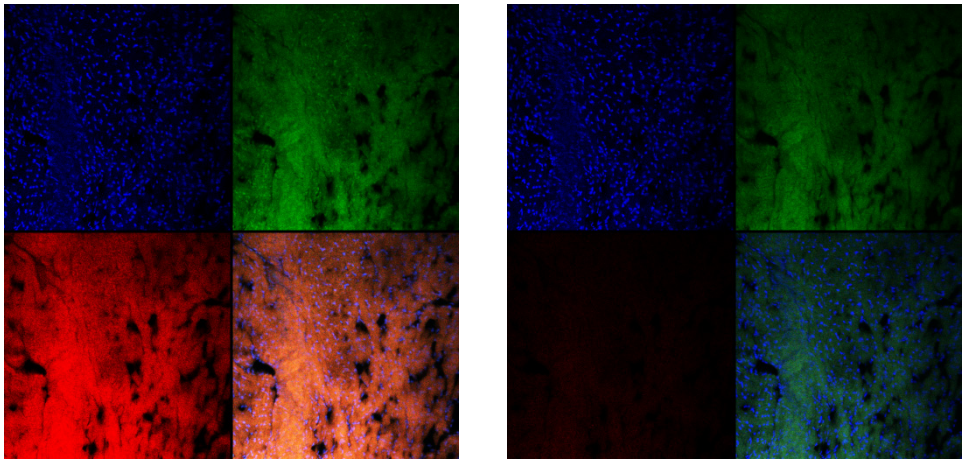
simultaneously and superimposed, showing the spatial localization relationship between the two fluorescence (Figure 2b). By analogy, the sequential scanning method can also collect images of triplex or more multiple fluorescent samples.



(a)Fluorescence cross color false positive imaging (b)Fluorescence positive imaging

Figure 2. Three color fluorescence imaging of organizational samples

3.1.3. The Sample is Large and the Panorama Cannot be Taken

With the development of the school, the research team took more and more samples, such as 3D printed materials, zebrafish, synthetic dressings, etc. Although some teachers and students try to use the 0.75x magnification function of the software, it is still difficult to capture the complete panorama of the subject. At this time, select the puzzle function of the instrument, find the first boundary of the subject through the electric joystick, defined as the starting point, and then find the other boundary as the end point. The picture using Smooth mode will be smoother.

3.2. Sample Observation Problems

3.2.1. No Fluorescence Signal under the Microscope

No fluorescence was visible when the microscope was turned on. In this case, the order of the instrument administrator to find the problem is: exclude the sample problem——exclude the problem that the mercury lamp is not on--exclude the software setting problem. In the case of student A, when he fails to observe the fluorescence under the microscope, the administrator will first replace his sample for standard tablets for testing. If the standard film can clearly show fluorescence under the mirror, it indicates that the problem may be in the sample preparation process. If the standard sheet cannot be imaged, the next step can check whether the power of the mercury lamp is on, the fluorescence intensity is in the second or third position, the light lock of the mercury lamp is open, and the fluorescence intensity knob on the left side of the microscope is open. If all possible problems are excluded, please switch to the lens selection function through the touch screen interface of the microscope to confirm the selected eye observation position. If the camera icon is displayed on the interface, it is currently in shooting mode and must manually switch to the eye icon to observe the fluorescence.

3.2.2. The Blurred Fluorescence of the Sample Cannot Focus

3.2.2.1 Sample is Unqualified

In the case that the sample is fuzzy and unable to focus, whether the bearing plate is a confocal dish and climbing plate can be excluded first. The principle of confocal determines that the sample is suitable for thin bottom observation, and the sample whose thickness is not consistent can not focus. In this case, the confocal dish needs to be replaced and made into climbing plate for observation. In addition, if the preparation takes too long and the sealing

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process is not properly sealed, the sample may be dry. Generally, it is recommended that students do the best imaging effect. If the time is not done, they can prepare the climbing film and thin coat the cover glass with resin or transparent nail polish. In the case of shooting the 3D material, we should also consider whether the sample is too thick and the error caused by the poor light transmittance. In this case, a 3D layer can be swept over the sample to determine the best observation plane.

3.2.2.2 The Interface Oil is Misapplied to the Air Mirror

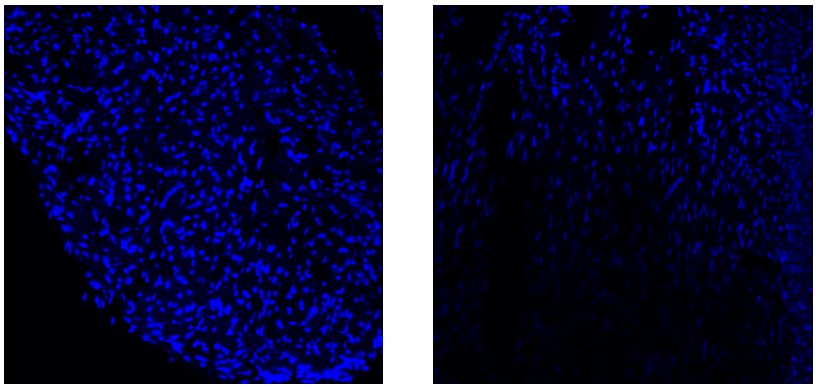
The failure of the operator to follow the correct rules will cause the interface oil to touch the air lens, causing the lens to be blurred. It is suggested that if teachers and students need to use the sample, they should first locate the shooting area through the low power air lens, then switch to the oil lens and add the interface oil for observation. After use, avoid switching back to the air mirror to prevent lens contamination. In case of special circumstances, if you need to switch to the air mirror for observation, please make sure that the bottom of the oil mirror and the dish (the glass bottom) are clean.

3.2.2.3 Oil Lens Lock

In the process of cleaning the oil lens, if it presses down when wiping, the lens will lock and fail to focus. The exclusion method is: after gently pressing down, gently rotate counterclockwise to make the lens reset.

3.2.2.4 The Glass Slide is not Set Flat

In the process of shooting fluorescence samples, some feedback pictures from teachers and students showed clear positions and fuzzy positions, so a flat fluorescence map cannot be presented (Figure 3-1). In this case the first consider is the sample is not flat, can stand on the microscope side, the first case is the confocal dish (slide) placed not put flat card in place, the second common case for the adapter is not flat, the solution is, please adapter red point and carrier red point, and then the adapter flat into the carrier (Figure 3-2). If it is a tissue section sample, we should also consider the problem of uncut and flat sample.



(a) Confocal dish not placed flat (b) Confocal dish placed flat

Figure 3. Imaging of cell nuclei in confocal dishes

3.3. Data Problems

After taking sample pictures using a Leica TCS SP8 laser confocal microscope, the operator must save the original document in LIF format. The LIF file contains detailed parameters in all filming sessions, which is crucial for later analysis because it allows the investigator to compare the shooting settings across different sample groups. Moreover, if you need to adjust the exported experimental images, such as modifying the ruler or pseudo-color parameters, it can be achieved by reopening the LIF file.

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3.3.1. Color Pictures are Saved into Black and White Pictures

The sample was set as a color picture, but found that the data was saved in black and white, because the RAW data option was selected when exporting the data. The solution is to call up the raw data and uncheck the RAW data option upon reexport.

3.3.2. Reduce One Channel in Fig

If the open field of view or multi-fluorescent channel is selected when shooting and saving, if need to remove one of the channels later, teachers and students can open the software, enter the process interface, use the drop function to remove a channel, and export new data.

4. Conclusion

Laser confocal microscopy is powerful and plays a key role in the qualitative and quantitative detection of fluorescence in cells, tissues, real-time observation of live cells, 3D materials, and tracking observation of zebrafish. The operation process of the instrument is relatively complex. When researchers encounter problems in the operation process, they should first check whether the connection of the instrument is normal, whether the parameter setting and so on are correct. If the problem persists, eliminate the causes one by one based on the solutions mentioned in this article. Every step in the process of scientific research experiment is very important, and the negligence of any link may lead to the deviation of the experimental results. Every researcher needs to treat every experiment with rigorous operation and learn to identify problems.

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