The capabilities of digital multifocus microscopy for different spectral ranges use

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Abstract. Digital multifocus microscopy is successfully used by microscopists when the image received with a microscope and a digital camera is subjected to digital processing. One of possibility of multifocus microscopy can be the integration into a single whole of several images obtained by focusing the microscope in different spectral ranges or at various fixed wavelengths.

1. Introduction. To build an optical system for digital multifocus microscopy, the principle of modular construction is typical. In this case, a set of opto-mechanical assemblies is used, from which it is possible to assemble a device that functionally most fully meets the specified technical parameters. One of the design features is that the projection optical system must be constructed taking into account the coordination of the linear dimensions of the object and the digital image receiver. In this case, it is necessary to achieve a diffraction limit in the aberration correction of the optical system. Similar relationships in the calculation of the projection optical system are also valid for the realization of the required resolving power, the value of which depends on the numerical aperture between the value of the input (on the object) and the output (in the image) there is a dependence through the value of the linear magnification of the projection system.
However, for different wavelengths with the same numerical aperture, the resolution and depth of focus will be different, which determines the need for optical calculation of systems that have achromatic or apochromatic aberration correction in a certain spectral range.

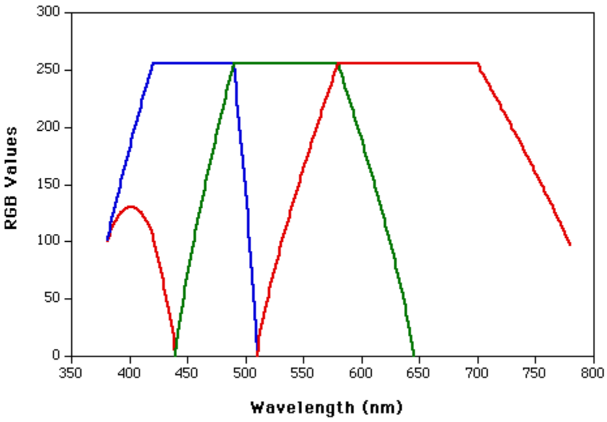

Figure 1. Approximate RGB values for visible wavelengths range.

2. Use of standard achromatic and apochromatic objectives for microscope.
A system is known which allows the integration of several images obtained by several iterations of focusing a microscope into one unit. In this case, we can use objectives for microscopes that do not have an ideal correction for such aberrations, for example, the curvature of the image and some others. This is achieved, including, with the help of special software for example, described in source. In this case, during the work, the researcher makes a consistent focusing of the objective for the microscope – first, for example, to the central part of the object under investigation, then on some of its peripheral parts, which, when focused on the central part, look blurred. The software allows all the resulting images to be converted into a single image that looks sharp throughout the entire linear field.

Consider the features of the objective work for a microscope when using it to obtain an image in a certain spectral range. It is known that objectives that have achromatic correction of aberrations are corrected in the spectral range from 480 to 650 nm, for objectives having apochromatic correction of aberrations, the working spectral range is expanded, it ranges from 435 to 650 nm. It is believed that in the working spectral range, a single focusing plane is achieved for all wavelengths of the specified range.

However, studies show that this is not the case. Consider an example of the optical design of an objective with a linear magnification of 20x and a numerical aperture of 0.65, having a planapochromatic correction of aberrations. Figure 2 shows aberration graphs for the case of focusing in polychromatic light (in the range from 435.83 to 643.8 nm) and graphs for focusing cases in monochromatic light at wavelengths of 435 and 650 nm separately. Obviously, the quality of aberration correction is significantly reduced when switching from work in polychromatic light to work in monochromatic light. Moreover, the image quality deteriorates, even if you use not only the general plane of focusing, but also your focusing plane for each wavelength separately. This deterioration is more noticeable for wavelengths for a shorter spectral range, for example, 435 or 480 nm, but it is also noticeable for wavelengths of a “longer” spectral range.

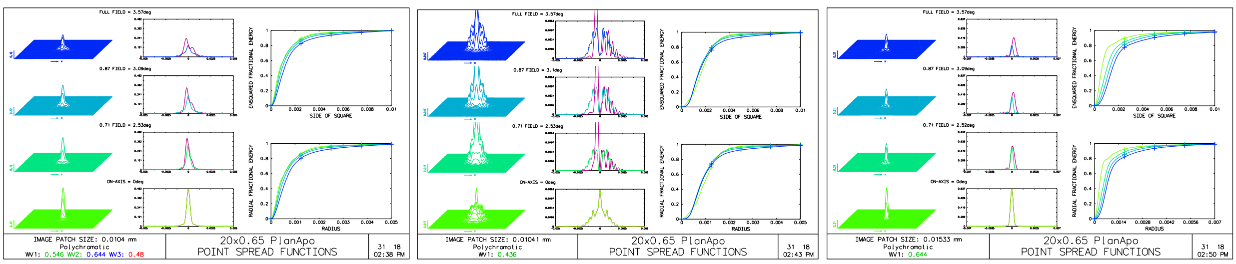

Figure 2. Aberration graphs for objective 20x/0.65 use in a different wavelengths.

3. Use of special monochromatic objectives.

We propose to use instead of one objective that has an extremely complex optical construction – three different objectives, each of which has a very simple optical design. There should be three such objectives, each of which has a monochromatic correction of aberrations at the corresponding (RGB) wavelengths. Figure 1 shows graphs of approximate RGB values for wavelengths of the visible spectral range, according to which optical designs of the three objectives can be made.

4. Conclusion. We presented a model for use in digital multifocus microscopy in different spectral ranges or for different wavelengths. The microscope objective, which has a monochromatic type of aberration correction, is the basis of such a model. We can use three lenses to get a full-color image. In this case, the lighting system (can be constructed, for example, using monochrome or tunable LED sources) should also be tuned to the same wavelengths. We can use photometric methods for three images obtained at different wavelengths. Then we can use special software to reconstruct a full-color image.