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PRELIMINARY CONCEPT OF A CONFOCAL MICROSCOPE ROTOR WITHIN THE MODULAR CULTIVATION SYSTEM FOR THE SPACE STATION

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RÉSUMÉ - La microscopie confocale est une solution présentant de nombreux attraits pour l'étude d'échantillons en microgravité, car elle permet l'obtention de coupes et de vues tridimensionnelles de la morphologie de spécimens biologiques ou autres avec une résolution submicronique.

Cet article décrit la conception préliminaire d'un microscope confocal à cellules d'observation multiples implanté sur un rotor extractible au sein du Système Modulaire de Culture que l'Agence Spatiale Européenne envisage pour la Station Spatiale Internationale Alpha.

ABSTRACT - *The confocal microscopy is a very attractive solution for the study of specimen morphologies under microgravity as it provides sections and 3D-images of biological and other materials with sub-micron resolution.*

This paper describes the preliminary concept of a multi-channel confocal microscope to be accommodated as an extractable microscope rotor within the Modular Cultivation System (MCS) proposed by ESA for the International Space Station Alpha.

1. INTRODUCTION

The confocal microscopy has proven since many years its interest in biological light microscopy as it enables to acquire precise optical sections of biological and other materials free from out-of-focus blur. Furthermore, the acquisition of sections at regular focus steps almost in real time allows by combining them to build up 3D-images.

The implementation of such a microscope within a space borne experiment will allow the in-situ study of various species and to analyse the gravity effect on their behaviour, the final aim being the identification of the so-called g-sensors within biological specimens. The accommodation of the experiment chambers and of the microscope imaging unit on an extractable centrifuge rotor allows to follow the modifications induced by varying in flight the acceleration which is seen by the samples.

First, the basic design principles of the confocal microscope are recalled. Further on, a modular concept of the MCS confocal microscope is proposed, based on the bilateral scan concept using a 2D CCD detector. The design and layout to allow for multiple observation cells to be implemented is then described. Special emphasis is given to the centrifuge rotor induced microvibrations and their impact on image stability, which could be detrimental to confocal microscope observation.

2. BASIC DESIGN PRINCIPLES OF THE CONFOCAL MICROSCOPY

The confocal microscope is a light microscope with the ability to produce sections and 3-D images of observed samples with sub-micron resolution. The basic principle of operation, based on the spatial filtering of the received light is sketched in figure 1. The sample is illuminated locally by imaging a pinhole acting as a point source. Then, the reflected or transmitted light is detected through another pinhole, which is image of the illumination pinhole. A 2-D section is then obtained by a transverse scanning of this illumination / detection point over the sample. This is usually done by scanning mirrors. A 3-D image is obtained by storing and processing of several 2-D sections at regular focus steps.

Fig.1 : Only light originating from the illuminated point in the specimen will contribute optimally to the confocal signal recorded by the detector located behind the detection pinhole

Most of the existing on-ground confocal microscopes work in fluorescence mode, either based on natural fluorescence of samples, either using specific markers with excitation wavelength in the 480 nm-550 nm range. The wavelengths of the emitted fluorescence light are typically from about 550 nm to 650 nm and sometimes up to 900 nm. So, the emitted light can easily be discriminated from the illumination beam by transmission through a dichroic window. Considering the above wavelength ranges, the illumination source is sometimes a filtered white light source but more commonly a frequency doubled Nd-Yag laser. The detector is either a photomultiplier or a Silicon device, depending on the expected received light level.

3. COMMERCIAL CONFOCAL MICROSCOPE SYSTEMS

The first confocal microscope was conceived more than 30 years ago and since that time, many concepts have been developed. Today, most of the equipments are based on a very limited number of concepts which are presented here.

The existing commercial confocal microscopes are based on fluorescence microscopy in the so-called epi mode as depicted in figure 1. Only one objective is used for the illumination of the specimen and the collection of the generated fluorescence radiation. The design principles of most of the existing commercial systems is depicted in figure 2.

Fig. 2 :Confocal mirror scanning point mode imaging

In the point mode confocal imaging, the light from the laser point source is scanned in mutually perpendicular directions by the two mirrors M1 and M2. The longer wavelength fluorescence light is detected by the detector located behind the confocal pinhole D, after transmission through the dichroic beamsplitter. Using 1 kHz fast scanning mechanisms, the image collection time in this mode is in the order of one second for one section plane (500 lines), and at least 10 seconds for a 3-D image (10 sections).

In the slit mode confocal imaging, the point source and the confocal detection pinhole are replaced by slits, as depicted in figure 3. Scanning of the specimen requires only one mirror to be rotated. The detector located behind the slit is either a line of photodiodes, with the drawback of a quite low sensitivity or a linear CCD. In this mode, the image collection is achieved at twice the frequency of the scan mechanism, say up to 2 kHz. Indeed, the collection of a 3-D image at video rate would only require 125 Hz scan mirror frequency.

The most elegant and convenient design solution for confocal imaging, known as the direct field / bilateral scanning imaging, is depicted in figure 3. It can make use of either a two-axis scan mirror in point mode or a one-axis scan in slit mode.

Fig.3 :Confocal imaging with the bilateral scan principle.

The fluorescence light from the specimen, after passing the confocal detector aperture is driven back to the other side of the non-transparent bilateral mirror M1 to a CCD on which the confocal image is collected. This results in a direct relationship between the specimen geometry and the CCD pixel position. The requirements on the scan mirror positioning accuracy and on the synchronisation of the image acquisition with the scan mechanism are relaxed. Such a design, using preferably the slit imaging mode, appears then to be the most appropriate to a space based equipment.

4. THE MODULAR CONFOCAL MICROSCOPE AND ROTOR CONFIGURATION

The proposed baseline configuration for the confocal microscope to be implemented on the rotor of the MCS rack is based on the following:

- limited number of observation chambers on the rotor: a number of 3 appears reasonable
==> this leaves sufficient place on the rotor for routing cables, life support connections, etc....
- bilateral scanning using slit illumination and non-cooled MPP technology CCD
==> 2D images at video rate/ nearly real time 3-D imaging
==> simple one axis scan mirror to be implemented, with relaxed scanning performances required
- compact solid state frequency doubled Nd-Yag laser ($\lambda=532\text{nm}$ / @ 10mW CW) on the rotor
==> simple arrangement / no need for an optical feedthrough to be implemented
- low and high magnification objectives (no immersion) on a remotely controlled turret with XYZ translation capabilities inside the observation chamber
==> Easy identification of the field of interest using low magnification objective and XY translations
==> Z scanning and coarse axial positioning achieved at objective level using the same actuator.

The implementation of a confocal microscope onto a space borne centrifuge rotor requires a modular concept to be considered, as conceptually represented in figure 4.

Fig. 4: The modular confocal microscope concept

The modular confocal microscope concept in which the output from several observation chambers are coupled with an optical router to the confocal imaging unit allows sequential imaging of various specimen.

Each channel of the optical router must simultaneously conjugate the image (located at infinity) and the pupil from the microscope objective respectively to the image plane and the input pupil of the confocal imaging unit (located respectively at infinity and on the scan mirror surface), while authorising the implementation of switching mirrors. This is achieved by a telecentric optical configuration of the router as sketched hereafter in figure 5.

Fig.5 : Basic design rules for the optical router.

In such a configuration, the microscope objective must be corrected at infinite image distance. A dedicated mirrors arrangement, within the observation chambers, maintain the output beam invariant in direction and pupil position, whatever the objective axial and lateral displacements are, as shown in figure 6.

Fig. 6 : objective holder optical design principles

The mirror M1 is moved in X and Y with the objective whereas the mirror M2 is moved in X only, to maintain the beam direction unchanged. The M3-M4 diedre arrangement maintain the exit pupil fixed in axial position if moved by

$$x = -05 (X+Y+Z)$$

A preliminary implementation of the confocal microscope on the 600 mm diameter centrifuge platform has been derived from the above considerations. It has been also assumed that:

- the observation chambers dimensions are 100 mm x 100 mm x 200 mm, one half dedicated to the cell containing the biological system and its life support system, the other half housing the microscope objective lens holder unit
- the confocal imaging unit dimensions are 120 mm x 150 mm x 300 mm, including the confocal scan unit, the laser head and the CCD detector head. Indeed, a miniaturised model of bilateral microscope scan unit has already been developed and fits within 40 mm x 100 mm x 150 mm
- the central area of the platform must be kept free over a diameter of 70 mm to allow the implementation of a combitrans transducer used for data/ electrical power transmission with the fixed part of the MCS.

The corresponding overall configuration is shown in figure 7.

5. PRELIMINARY PERFORMANCES BUDGETS

The performances of the above configuration have been evaluated and lead to the following budgets:

- Power dissipation : about 25 W with non cooled CCD (up to 20 W additional for CCD cooling)
- Data handling : 1 Mbytes/ sec. data stream in real time imaging
data storage capacity around 4-5 Gbytes required
- Mass : around 15 kg (confocal imaging unit + router + 3 observation cells)

• Optical performances

Gy	N.A.	f. length (mm)	Ø pupil (mm)	Working distance (mm)	Lateral resolution (µm)	Axial resolution (µm)	Section capability (µm)
4	0.10	50	10	15	3.36	110	78
10	0.25	25	10	7	1.34	17.6	12.6
16	0.35	12.5	9	3	0.96	8.98	6.41
40	0.75	5	8	0.6	0.45	1.96	1.40
80	0.90	2.5	5	0.3	0.37	1.36	0.97

Only air objectives are considered here. Immersion objectives which could give even better resolution have been discarded considering the microscope will be remotely controlled.

Fig. 7 : Confocal microscope implementation on the centrifuge platform

The implementation of three observation chambers ($10 \times 10 \times 20 \text{ cm}^3$) together with the confocal imaging unit ($12 \times 15 \times 30 \text{ cm}^3$) is made possible by a dedicated arrangement of three telecentric relays on each channel.

6. MICROVIBRATIONS ASPECTS

The centrifuge rotor induced microvibrations could be detrimental to confocal microscope observations where details within the micron range or even lower will have to be imaged in good conditions. Indeed, the modular design is favourable with respect to microvibrations.

The confocal imaging properties being generated within the confocal imaging unit, a stiff and compact design of this unit and the mounting to the rotor with flexure blades will permit to achieve the required decoupling.

The optical router is almost not affected as the required alignment stability remain in the range of 0.1 mm.

The optical imaging being generated at observation chamber level, with the specimen and the objective in the same unit, a stiff and compact design of this unit and its mounting to the rotor with flexure blades will also permit to achieve required decoupling.

The main critical parameter is in fact the alignment stability of the specimen with the microscope objective focus point, to be kept within 0.1 μm with high NA objectives, knowing the sample is in general in a liquid medium which provides the life support.

The identified perturbations are the ball bearings geometrical defaults and the rotor rotational speed irregularities.

The ball bearings generate harmonic perturbations with a pulsation $k\Omega$, Ω being the rotor angular speed. With $\Omega = 1 \text{ Hz}$, which generates 1g at 25 cm radius, the major effects will be:

- the 1Hz dynamic unbalance (misalignment between rotation axis and principal axis of inertia)
- the 1Hz axial unbalance parallel to the axis of rotation
- the 2Hz ovalization effects

This may induce a few tenths of a micron perturbation on a specimen floating in a liquid medium.

The rotation speed irregularities $\delta\Omega$ may also generate radial and tangential microvibrations

To maintain an image stability of 0.1 μm , These irregularities have to be such that:

$$\delta\Omega < 10^{-7} * \min (\omega/r, \omega^2/2 r \Omega_0)$$

as illustrated in figure 8.

Fig. 8: allowable vibrations speed irregularities

7. CONCLUSIONS

Although the confocal microscope is no more considered in the baseline configuration of the MCS, this study has allowed MMS-F to set up the basic design rules, to define the preliminary configuration and to assess the feasibility of a space borne confocal microscope to be implemented on an extractable centrifuge rotor compatible of the Modular Cultivation System, to be installed in the Space Station Alpha.

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