The spectroscopic study of abnormal cells for the infrared femtosecond laser cell processing

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Abstract: This study is about the spectroscopic characterization of abnormal cells in a macro to micro approach. In the first step a commercial UV-Vis apparatus is used, which is ultimately altered to the limits to decrease detection volume. In the ultimate stage an infrared femtosecond laser setup is used to measure on individual cells.

Current life sciences research in spectroscopy focuses on the investigation of tissues, rather than individual cells. One advantage of spectroscopy on individual cells is the reduction of required biological material. Another advantage is a reduction in convolution of information [1]. Spectroscopy at the cellular can therefore be very powerful for the study of abnormal cells. The goal of this study is to investigate abnormalities at the cellular level, such as dysplasia and neoplasia, with spectroscopy using ultimately infrared femtosecond laser.

The path to individual cell measurements will be taken through a macro to micro approach. The first step is using a regular commercial UV-Vis spectrophotometer (Shimadzu® UV-2450) on single cell layer samples of different cell lines. The second step is altering the commercial apparatus to reduce the detection volume and within the limits of the device. The ultimate step is using an infrared femtosecond laser to investigate individual cells.

To make the commercial spectrophotometer suitable for single layer cell measurements, it had to be altered. A microscope slide holder, similar to the cuvette holder, was designed with the intention to easily change between cuvette and microscope slide system. To keep in mind the second stage of research, the design was incorporated with the possibility to use standard optical table equipment to alter the beam shape within the detection compartment of the apparatus. See Figure 1 for picture of the design.

To use vertical samples a simple microtiter slide design is used. It consists of two fused silica glass slides with a spacer of silicone (Dow Corning 7-9800) sandwiched in between. In this spacer a hole is created in which fluid can be preserved. The adhering nature of silicone to glass makes it easy to remove and attach to the slides and prevents leakage. See Figure 2 for the design.

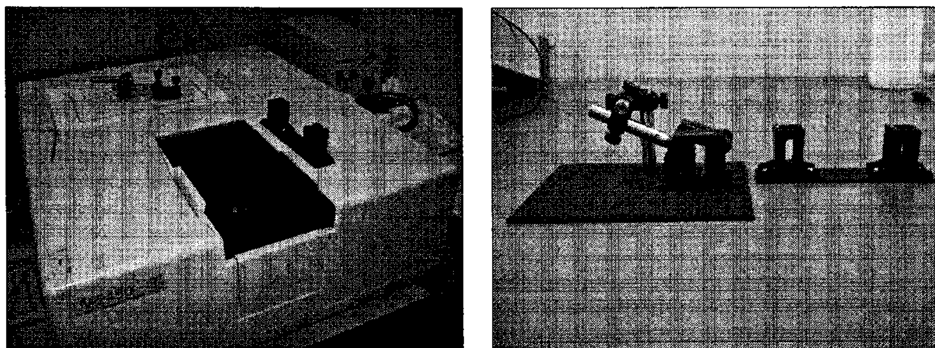

Figure 1 The Shimdazo® UV-2450 with the microscope slide holder design with standard optical mount and posts next to the original cuvette design

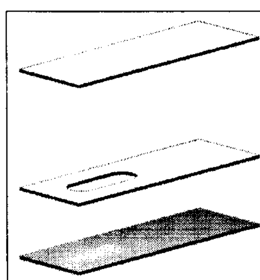

Figure 2 3D model of microtiter slide design with in the middle the spacer made of silicone

The distribution of absorption levels in tissues and cells as a function of wavelength depends on size, shape and optical properties. The optical properties depend on the chemical composition and the activity of natural chromophores [1]. Dysplastic cells are liable to structural changes, such as change in shape, nuclear to cytoplasm ratio and chromatin structure. These affect the elastic scattering properties of the cells. The metabolic activity increases in dyplastic cells, which affects mitochondrial chromophores and therefore changes autofluorescence [2].

Current research is at the premature stage and the first measurements with fixated single cell layers on fused silica microscope slides with cover slips are conducted. Cell lines MCF10A, MCF7 and MDA-MB-231 are used to investigate normal, dysplasia and neoplasia of epithelial cells from the mammary gland [3]. From these cell lines spectra were taken from 200 to 450 nm. Currently the data is being processed for interpretation.

Further research with the commercial apparatus implies reaching the limits of this device by means of reducing the detection volume on the sample without compromise of sensitivity and keeping in mind the potential damaging effect of UV light on cell samples. Future research implies the use of femtosecond laser setup to narrow down to the cellular level and reduce the risk of damage to cell samples.

References

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