COMPUTER ASSISTED 3D-ANALYSIS OF PHOTODYNAMIC EFFECTS IN LIVING CANCER CELLS

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ABSTRACT

Photodynamic Therapy (PDT) [4] is a promising new procedure to treat tumors in a less invasive form. PDT has the potential to extirpate malignant cells by way of photo-activation of a photo-sensitizer located within a tumor cell. Upon activation, the initially inert sensitizer agent becomes toxic by producing singlet oxygen as well as free radicals which then destroy the cell. A crucial aspect for the success of this therapy is a better understanding of the basic mechanisms involved in PDT. In this study we have investigated dark exposure toxicity and the spatial density distribution of the sensitizer in living tumor-spheroids. The selected approach makes use of a in vitro model for the spheroids, a set of photo-sensitizers, confocal laser scanning microscopy (CLSM), and dedicated 3D-image processing and visualisation techniques. The results obtained with this approach document its capabilities to localize photo-sensitizers within living tumor cells prior and after light exposure in a time saving manner.

Key Words: photodynamic therapy, photo-sensitizer, 3D-image processing

1. INTRODUCTION

Localisation and eradication of tumor cells without affecting or damaging normal tissue is a challenging target in today's cancer therapy. This objective may soon become reality thanks to a new method of cancer treatment named Photodynamic Therapy (PDT), which has the potential to selectively destroy malignant cells. With PDT the patient is treated with a photo-sensitizing substance which should accumulate in tumor cells only. This substance is nontoxic until it is exposed to light irradiation with the appropriate wavelength. As such it induces photoactivation and production of phototoxins like singlet oxygen and free radicals which initialize tumor necrosis by destroying the cell membrane and/or structures within the cytoplasm.

In the current study our main interest was focused on the investigation of basic mechanisms involved in photodynamic processes such as the spatial distribution of the photo-sensitizer in normal and malignant cell populations and the inherent toxicity of the agent in the dark. For this purpose a system has been devised consisting of a set of photo-sensitizers based on derivatives of porphyrin (PD) as well as hematoporphyrin (HPD), a confocal laser scanning microscope and a suitable image workstation with appropriately devised 3D-image processing software. Detailed information about the spatial density distribution of the photo-sensitizer contributes to an improved understanding of the mechanisms involved in PDT and to the optimization of its clinical treatment modalities.

2. CELLS AND PREPARATION

For these investigations a human tumor cell line (mamma carcinoma - derived, MCF-7) was cultivated in soft agar-medium in the form of multi-cellular tumor-spheroids. These spheroids were incubated with photo-sensitizers (Photosan (HPD) and TPPS** (PS), for up to 24 hours. The location and distribution of the sensitizers in the cell population was then analysed with a confocal laser scanning microscope (Leica CLSM, F.R.G.) equipped with an Argon ion laser (excitation wavelength of 488nm or 514nm, respectively). The stack of CLSM image data stem from experiments performed during a medical thesis and for a poster presentation [1].

** Tetraphenylporphyrin sulfonate. This was a generous gift from Dr. A. Rück, Institut für Lasertechnologien in der Medizin an der Universität Ulm, Ulm, F.R.G.

3. IMAGE PROCESSING HARDWARE

In general the processing of 3D-image data sets and their analysis within reasonable time frames call for extensive on-line computing power and large memory capacity. This is particularly true for the processing of stacks of medical images which are generated by confocal laser scanning microscopy and which often have a very low signal-to-noise ratio. To achive a cost-effective yet flexible way of processing and analysis, we have made use of a combination of commercially available workstations and of a custom

made PC-AT-based image workstation with a scaleable-performance architecture.

An overview of the image processing system in use is given in Figure 1. A scaleable image processor (SIP), consisting of a network of processing elements (PE), is surrounded by a host system providing local high resolution display and graphics capabilities as well as basic I/O-services. Selected PE's are connected to a dedicated Ethernet-PE which links the image processing system to a network of RS/6000 3D-graphics workstations.

A detailed description of the SIP hardware can be found in [2]. In summary, our SIP consists of 8 custom transputer modules (PE, T805, 25MHz, 8MB RAM [3]) arranged in a cube-shaped network which is controlled by the 'Root'-module (RPE, T805, 25MHz, 12MB RAM). A fast DMA-link (4.5MB/s peak data throughput) allows the RPE to work directly into the host's memory system, thus providing fast image and graphics output to the high resolution display hardware. The SIP-network is closely coupled to a dedicated Ethernet-module (EPE, T805, 25MHz, 8MB RAM) which establishes a fast connection to the powerful RS/6000 workstation network of the IBTZ. This data link becomes crucial for the ever-increasing storage and processing requirements in the field of 3D-image analysis. The raw computing power of the SIP-network equals 120 MIPS and 19 MFLOPS.

4. IMAGE PROCESSING SOFTWARE

The stack of up to 100 confocal laser microscope image slices with a resolution of 512 by 512 pixels is mapped onto the cube-shaped SIP in a way which minimizes communication pathlength. In order to be able to quantify the spatial density distribution of the photo-sensitive agents in the cell population, we use a multi-level, parallel and true 3D-processing scheme. First, a spatial nonlinear filter process usually referred to as Inisotropic Diffusion (ID) [5], is applied in order to eliminate noise artefacts and to simplify edge detection within the image volume in preparation of the subsequent step. ID is a numerical heat equation solver where temperature is substituted by voxel intensity. This diffusion process minimizes intensity fluctuations whereas sharp edges are preserved by reducing the heat conductivity along them. Subsequent binarisation splits the volume into 3D-objects and background. Parametrisation converts the objects into data structures which describe their geometry, topology and texture. The main challenge of this step is to generate a connectivity graph that leads to a closed surface or 'skin' of the object. The resulting surface-descriptors can be fed into todays standard solid modellers. Our own modeller features an additional smoothing step prior to raster conversion. In either case any arbitrary three-dimensional view of the cell population obtained with the CLSM becomes available for detailed analysis and quantification of the structures of interest.

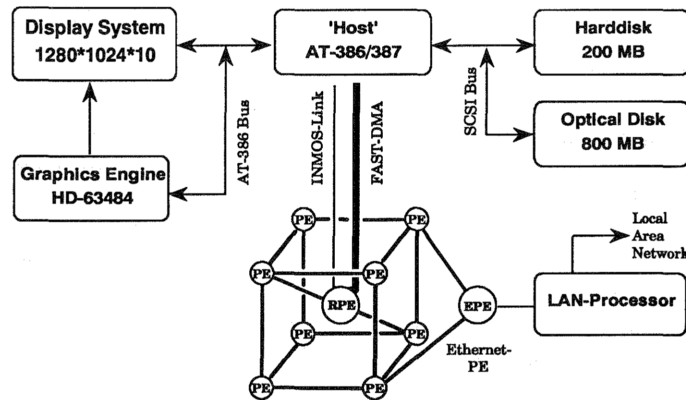

Figure 1:
Overview of the image processing hardware consisting of the host system and the transputer based image processor. ***PE*** *= Processing Element.* ***RPE*** *= Root Processing Element with dedicated high speed DMA-channel to host system.* ***EPE*** *= Ethernet Processing Element connected to LAN-module.*

5. RESULTS

Tumor spheroids present strikingly fluorescent nuclei after TPPS incubation and subsequent illumination. Fig. 2a and Fig. 2b show identical parts of a slice of a tumor spheroid after incubation of the photo-sensitizer prior to and after exposure to light irradiation. A view of a three-dimensional reconstruction of the fluorescent cells of Fig. 2b is given in Fig. 3.

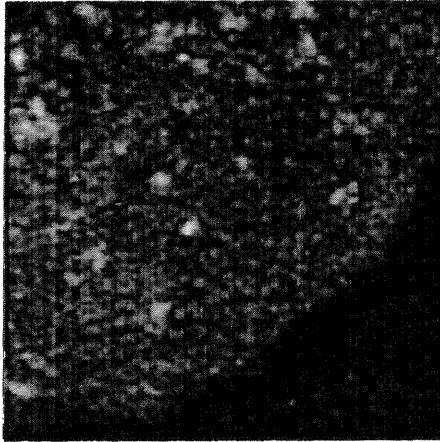

Figure 2a:
Partial view of an image slice from a tumor spheroid with photo-sensitizer (TPPS) prior to light activation (Magnification: 25x).

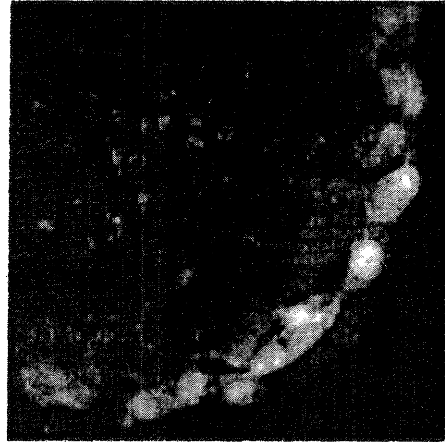

Figure 2b:
Partial view of the same tumor spheroid after light activation. Reacting cells present intense fluorescence (Magnification: 40x).

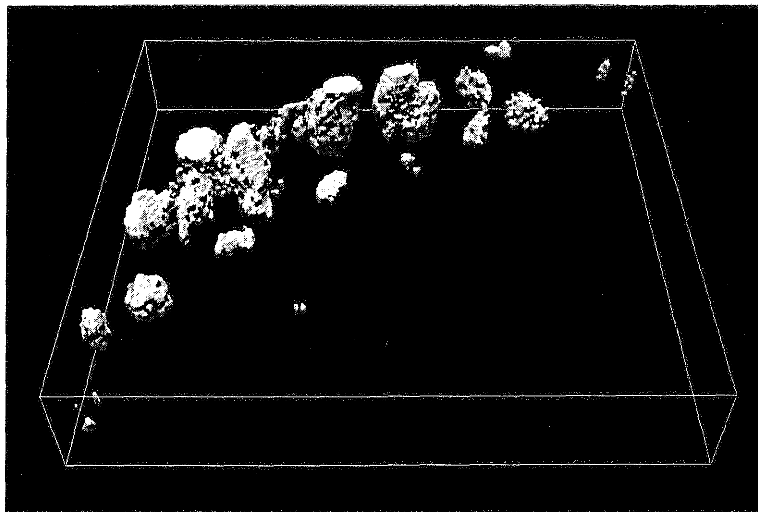

Figure3:
*Partial view of 3D-reconstructed fluorescent part of the tumor spheroid of Fig. 2b (20 slices 512 * 512 pixels).*

6. CONCLUSIONS

3D-image analysis by use of a confocal laser scanning microscope and dedicated computer hard- and software, as described in this paper, proves to be very useful for the fast localization of photo-sensitizers within living tumor cells prior and after exposure to light of appropriate wavelength. Further investigations on living tumor cells from patients to be treated are necessary in order to systematically improve this kind of cancer treatment.

7. REFERENCES

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