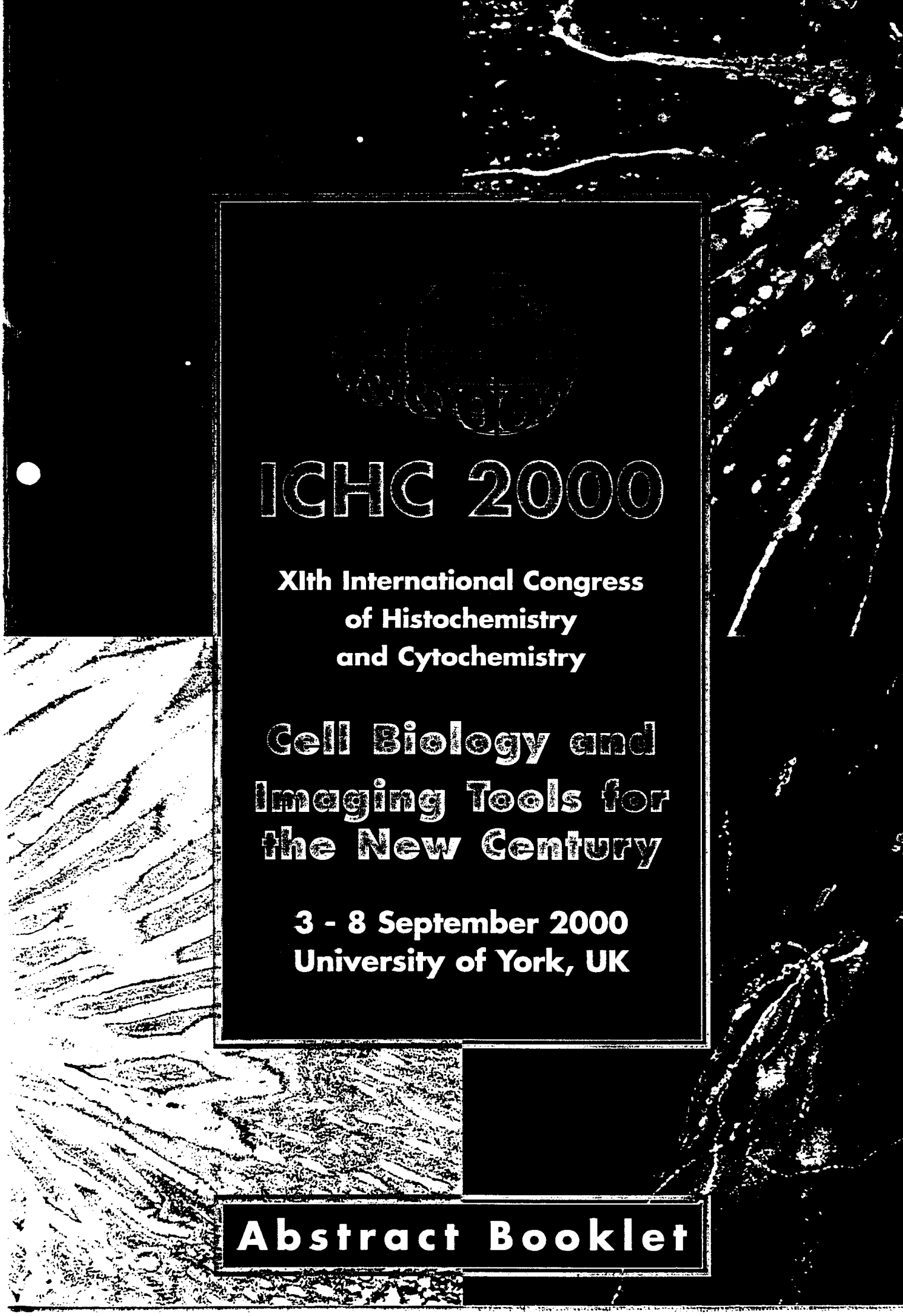

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Abstract Booklet

B1 - Assessment of cytotoxicity in *Tetrahymena pyriformis* using fluorescent probes

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Ciliated protozoa have a wide distribution in aquatic environment and play an essential role in the purification process of either aerobic and anaerobic biological wastewater treatment systems. Their sensitivity to environmental changes suggested their use as biological indicators of water pollution. Furthermore, the simplicity of laboratory handling and culturing is an essential requirement in order to make these unicellular eukaryotes suitable as test organisms for environmental toxicity assessment.

Cytotoxicity and cell death have been evaluated by the use of markers either retained by living cells or released from lysed ones. Fluorogenic esterase derivatives substrates stain live cells, being widely used as cytoplasmic markers and viability probes.

The compound 4'5'-bis (*N'N'*-bis (carboxymethyl) aminomethyl fluorescein acetoxymethyl ester) - calcein/AM, is a non-fluorescent lipophilic ester that diffuses passively into cells and is converted by intracellular esterases to calcein, a fluorescent hydrolysed salt. Non-lipophilic calcein is impermeant to the membrane, being retained inside cells with intact membrane, witch stain green at 530 nm. A second dye is added to stain dead cells. Ethidium homodimer-1 is a cell impermeant fluorochrome that passes through compromised membranes, strongly binds to nucleic acids and emits red fluorescence at 617 nm.

The ciliated *Tetrahymena pyriformis* is able to grow on anexic conditions cultivated in a standard medium, free from any other organism. It has been used in this work as a model organism for the assessment of protozoa citotoxicity exposed to different concentrations of toxicants (e.g. Triton X-100). Viable green cells *versus* red dead cells can be easily detected by fluorescence microscopy. Quantification and data analysis should be then performed by advanced techniques such as flow cytometry or image analysis. It is expected that this assay will represent a simple, low-cost, as well as a sensitive method for cytotoxicity evaluation.