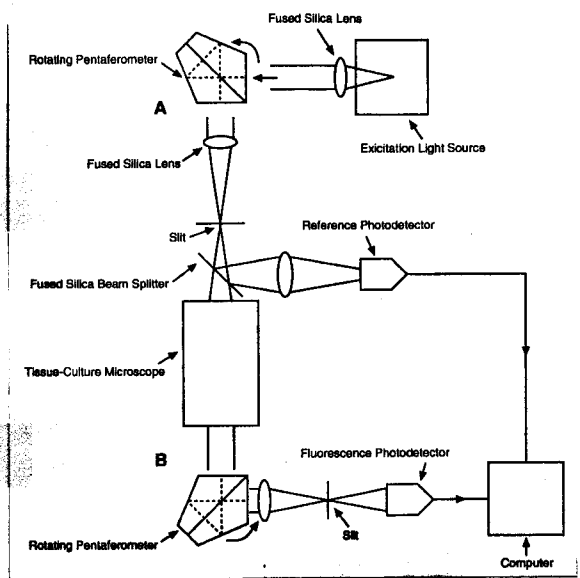


**FOURIER INTERFEROMETRY/SPECTRAL IMAGING OF RESPONSE TO METABOLIC CHALLENGES IN PERINUCLEAR MITOCHONDRIA OF NORMAL AND MALIGNANT CELLS.** Elli Kohen<sup>1</sup>, Joseph G. Hirschberg<sup>2</sup>, Ceren Örnek<sup>1</sup>, John Berry<sup>3</sup>, Marco Monti<sup>2</sup>, Dietrich O. Schachtschabel<sup>4</sup>.

<sup>1</sup> Department of Biology, <sup>2</sup> Department of Physics, <sup>3</sup> Department of Chemistry, University of Miami, Coral Gables, FL, USA; <sup>4</sup> Gerontology, Medizinische Fakultät der Philipps Universität, Marburg/Lahn, Germany.

**INTRODUCTION.** The problem of mitochondrial responses in sequence to metabolic challenges continues to be a puzzling one. While cytoplasmic and nuclear (*i.e.* extra-mitochondrial) responses to glucose and glycolytic intermediates are rather regular and reproducible, notably in ATP-depleted cells, mitochondrial responses remain erratic and irregular. There are, however, striking changes in mitochondrial distribution, intracellular organization and morphology which are associated with cell growth conditions, differentiation and transformation. Thus, a study of mitochondrial morphology and distribution is first attempted with vital probes such as DASPMI, Mitotracker Green™ and TMRE. The acquisition of structural and functional information on mitochondria of living normal and malignant cells can be enhanced by introduction of new methods using Fourier interferometry and spectral imaging.

**Instrumental Design.** The studies reported here were carried out by microspectrofluorometry and fluorescence imaging using tricolor and black & white CCD cameras, the latter supplemented by an integrator for image accumulation [1-2]. A Fourier interferometry design is being implemented to measure the excitation and emission spectra simultaneously; the exciting and emission beams are encoded by modulating the light according to wavelength [3]. To achieve Fourier coding by double-beam interferometry, a "Pentaferometer" has been constructed [4]. The long sides of two prisms are provided with a beam-splitting coating over half of their length and opposite side is aluminized. They are cemented together with an offset that determines the angular separation of fringes. For applications with the tissue culture microscope two pentaferometers are used, one on the excitation and one on the emission side; both are rotated at constant speed (Fig. 1).



**Fig. 1.** Excitation-emission design for fluorescence microscope.

## Report Documentation Page

<b>Report Date</b> 25OCT2001	<b>Report Type</b> N/A	<b>Dates Covered (from... to)</b> -
<b>Title and Subtitle</b> Fourier Interferometry/Spectral Imaging of Response to Metabolic Challenges in Perinuclear Mitochondria of Normal and Malignant Cells	<b>Contract Number</b>	
	<b>Grant Number</b>	
	<b>Program Element Number</b>	
<b>Author(s)</b>	<b>Project Number</b>	
	<b>Task Number</b>	
	<b>Work Unit Number</b>	
<b>Performing Organization Name(s) and Address(es)</b> Department of Biology University of Miami Coral Gables, FL	<b>Performing Organization Report Number</b>	
<b>Sponsoring/Monitoring Agency Name(s) and Address(es)</b> US Army Research, Development and Standardization Group (UK) PSC 802 Box 15 FPO AE 09499-1500	<b>Sponsor/Monitor's Acronym(s)</b>	
	<b>Sponsor/Monitor's Report Number(s)</b>	
<b>Distribution/Availability Statement</b> Approved for public release, distribution unlimited		
<b>Supplementary Notes</b> Papers from the 23rd Annual International Conference of the IEEE Engineering in Medicine and Biology Society, October 25-28, 2001 held in Istanbul, Turkey. See also ADM001351 for entire conference on cd-rom.		
<b>Abstract</b>		
<b>Subject Terms</b>		
<b>Report Classification</b> unclassified	<b>Classification of this page</b> unclassified	
<b>Classification of Abstract</b> unclassified	<b>Limitation of Abstract</b> UU	
<b>Number of Pages</b> 4		

## RESULTS.

**Yeast mitochondria.** Yeast studies are used as a model for studies in cultured mammalian cells. To show the mitochondria, the cells have been treated with DASPMI, Mitotracker Green <sup>TM</sup> and TMRE. The mitochondria first exhibit a perinuclear ring-like distribution (Fig. 2). Upon continuing irradiation with the exciting light, at 436 nm, the mitochondria were observed to burst suddenly, like undergoing tiny explosions and the fluorescent probe then spread almost instantly throughout the cells (Fig. 3).

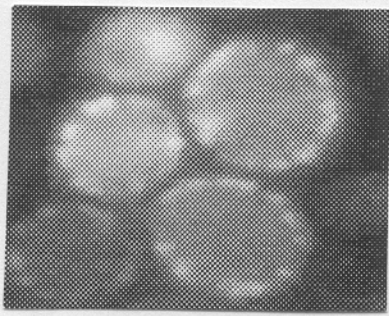


Fig. 2A. Yeast DASPMI

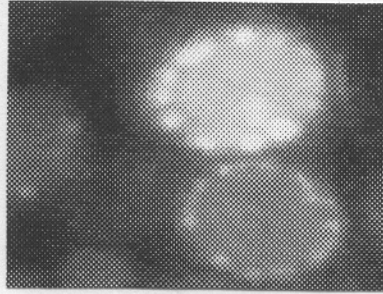


Fig. 2B. Yeast DASPMI

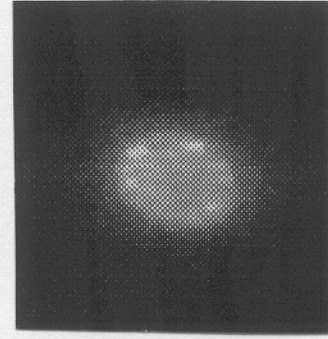


Fig. 2C. Yeast TMRE

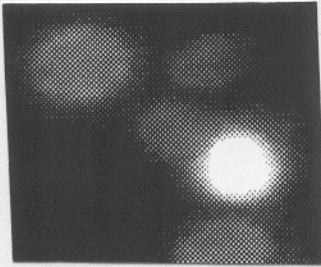


Fig. 2D. TMRE microexplosion (stage 1)

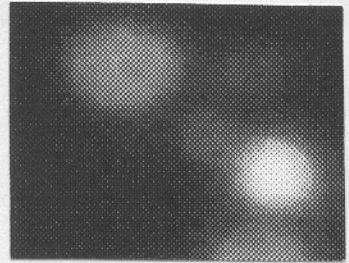


Fig. 2E. TMRE microexplosion (stage 2)

**Hepatocyte mitochondria.** The changes in culture of murine hepatic cells (NMuLi) are crucial for diagnosis of cell transformation. In normal hepatocytes, mitochondria are clearly globoid. After a few to several generations in culture changes in mitochondrial morphology parallel those in cellular morphology; as the cells transform from "epitheloid polygonal" to "fibroblastoid elongated", the mitochondria become filamentous in form, and no longer resemble the normal globoid shape (Fig. 3).

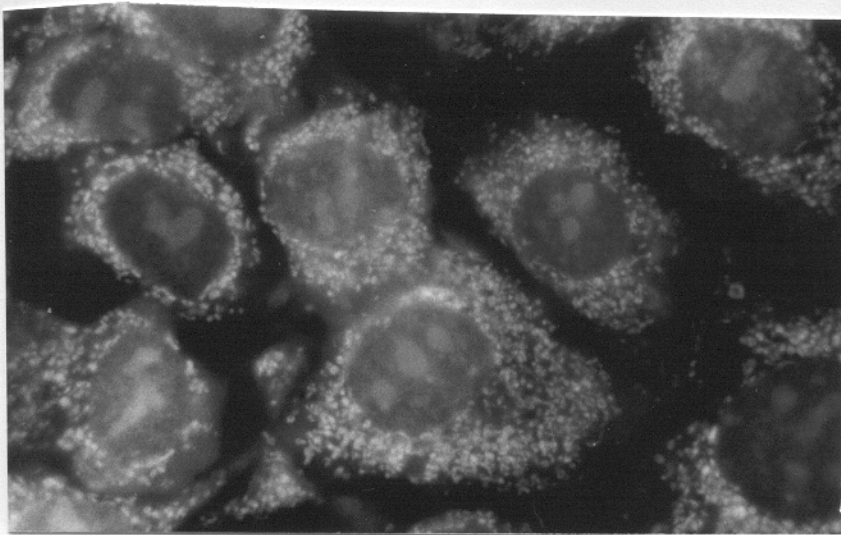
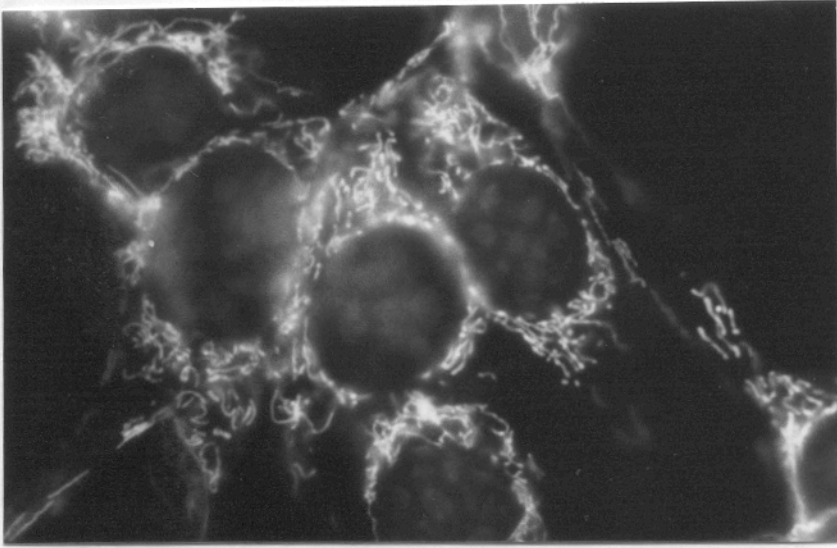


Fig. 3A. Early clone NMuLi DASPMI globoid mitochondria.

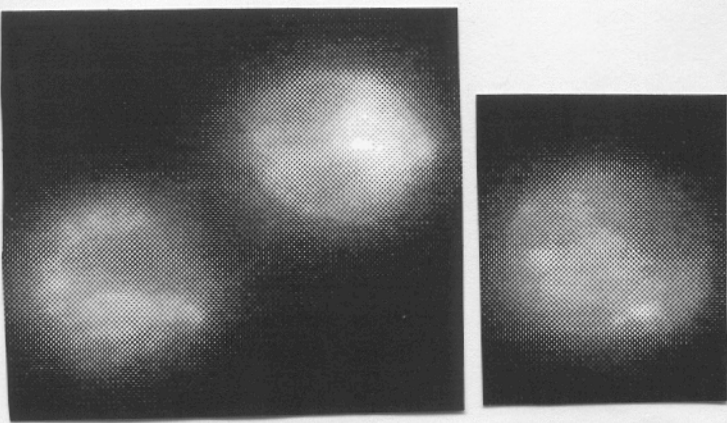


**Fig. 3B.** Later clone (T<sub>4</sub>) NMuLi DASPMI filamentous mitochondria.

**Mitochondria in mouse mastocytoma and human keratinocytes.** With the same probes as in yeast, murine mastocytoma cells exhibit a globular instead of filamentous form (Fig. 4. A,B). In human keratinocytes grown over numerous passages (thus immortalized), by sequentially focusing on different layers of cells, it was possible to identify that the mitochondria were a mixture of globoid and filamentous forms (Fig. 5). This may represent an intermediate stage in the process of keratinocyte transformation.

**Glucose responses in mammalian cells.** Responses to glucose (perfusion), glucose-6-phosphate, malate and isocitrate (microinjection) were followed in a variety of cells including L sarcoma, human melanoma and keratinocyte cultures (Fig. 6). The glucose response starts in the cytosol within seconds, and is characterized by NAD(P) reduction. Mitochondrial response may or may not follow. The response to malate is both extra-mitochondrial and mitochondrial. There is a delay from 1 to 7 seconds between the cytosolic and mitochondrial responses. Response to isocitrate is limited to mitochondria.

**Fig. 4A&B.** Mastocytoma



**Fig. 4C.** Keratinocyte

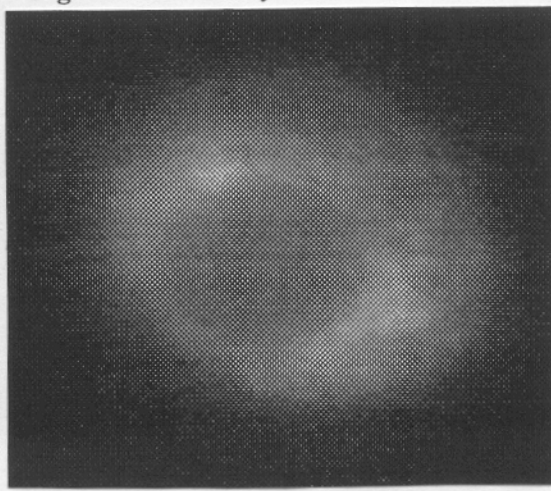


Fig. 5A. Keratinocyte before glucose

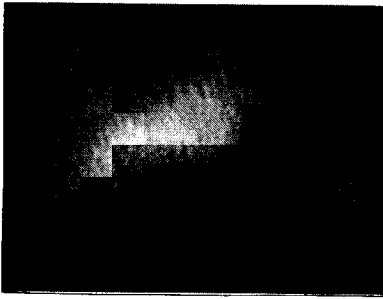
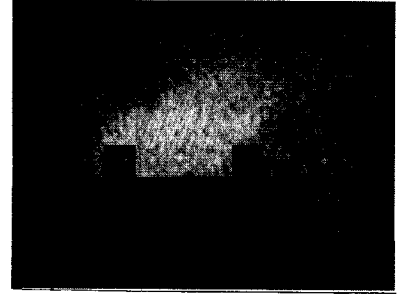


Fig. 5B. Keratinocyte after glucose

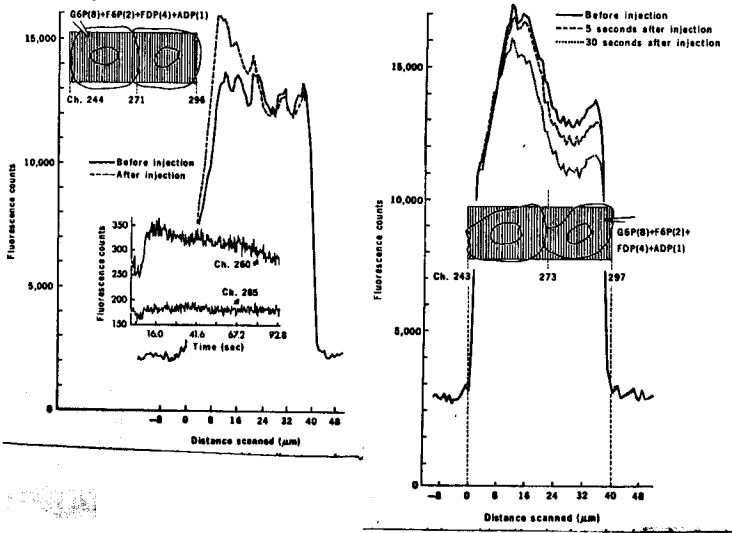


**Glucose response in pancreatic islet cells.** In cultures of dispersed and reaggregated islet cells the NAD(P)H response to glucose reveals metabolic coupling between interconnected islet cells. Such coupling may extend to interconnections between A, B and D cells. Glucose responses are bistable (reductive or oxidative) depending upon antecedent metabolic state.

Fig. 6. Glucose response across two cells.

A. Uncoupled

B. Coupled



CONCLUSION.

Mitochondrial responses continue to be erratic but may exceed in intensity cytoplasmic ones. Malate responses recorded at nuclear sites (which seems to suggest the presence of a nuclear dehydrogenase pathway) are particularly enhanced in cells treated with mitochondria-toxic agents e.g. anthralin and azelaic acid. Reported studies have implications: (1) in terms of cell metabolism, with eventual substrate dosimetry within cell compartments; (2) in terms of biomedical applications to cell diagnostics, prognostics, and therapeutics.

( From: Kohen et al, Science 204 :p. 864 )

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This work was supported by NSF BIR-9521478 and research funds from the University of Miami.