

REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-00-

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1. REPORT DATE (DD-MM-YYYY) 08-03-2000		2. REPORT TYPE final		3. DATES COVERED (From - To) 2/1/1996 - 1/31/1999	
4. TITLE AND SUBTITLE THE ROLE OF MITOCHONDRIA IN THE DETECTION OF INFRARED LIGHT SOURCES BY MAMMALIAN CELLS.				5a. CONTRACT NUMBER	
6. AUTHOR(S) Guenter Albrecht-Buehler, Ph.D, Robert Laughlin Rea Professor of Cell Biology, Department of Cell and Molecular Biology				5b. GRANT NUMBER F49620-96-1-0051	
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(S) Northwestern University Medical School 303 E. Chicago Ave., CHICAGO, IL 60611T Tel.: (312)-503-4261, Fax: 312-503-7912, e-mail: g-buehler@nwu.edu				5c. PROGRAM ELEMENT NUMBER	
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(S)				5d. PROJECT NUMBER	
12. DISTRIBUTION/AVAILABILITY STATEMENT public availability				5e. TASK NUMBER	
13. SUPPLEMENTARY NOTES				5f. WORK UNIT NUMBER	
				8. PERFORMING ORGANIZATION REPORT NUMBER	
				10. SPONSOR/MONITOR'S ACRONYMS)	
				11. SPONSOR/MONITOR'S REPORT NUMBERS)	
14. ABSTRACT If biological materials absorb specifically in the near infrared it is likely that they use some of the quantum energy for structural and other changes of the absorbing molecules and, thus, re-emit light of lesser quantum energy. In other words, one may expect that they fluoresce in the near infrared if exposed to near infrared light. The fluorescence, in turn, is likely to reflect functionally important structural properties of the light sensing and processing materials. 1. We tested this conjecture by developing a near infrared fluorescence microscope. As a first application of this microscope we report that Rhodospirillum rubrum, a species of purple bacteria, autofluoresce in the near infrared and offer evidence that the autofluorescent material is bacteriochlorophyll. By comparing the autofluorescence of live and fixed bacteria we suggest that the bacteria regenerate their chlorophyll with a time constant of approximately 20 s. In the future we will apply the new microscope to study the autofluorescence of mitochondria. 2. The movements of mitochondria have been linked to microtubules. We tested whether we can detect signals that travel along microtubules in cells irradiated with pulsating near-infrared light. We found, indeed, that under these conditions an unknown type of signals travels to the plus end of microtubules in cells and destabilizes them temporarily.					
15. SUBJECT TERMS					
16. SECURITY CLASSIFICATION OF:					
a. REPORT	b. ABSTRACT	c. THIS PAGE	17. LIMITATION OF ABSTRACTS	18 NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
U	U	U	UU		19b. TELEPHONE NUMBER (Include area code)

LIST OF MANUSCRIPTS SUBMITTED OR PUBLISHED:

1. Albrecht-Buehler, G., Autofluorescence of live purple bacteria in the near infrared. (1997) Experimental Cell Research 236:43-50
2. Albrecht-Buehler, G., Altered Drug Resistance of Microtubules in Cells Exposed to Infrared Light Pulses: Are Microtubules the "Nerves" of Cells? Cell Motility and the Cytoskeleton (1998) 40:183-192
3. Albrecht-Buehler, G. Phagokinetic Track Assay of Cell locomotion in Tissue Culture, In 'Cells: A Laboratory Manual' Vol. 2, Cold Spring Harbor, NY (1997) 77.1-77.10
4. Albrecht-Buehler, G. The conceptual challenge of cellular gravi-sensing, In 'Frontiers of Biological Science in Space' Taiyo Ltd. Tokyo, Japan (ed. A. Sato) 54-65

SCIENTIFIC PERSONNEL SUPPORTED BY THIS GRANT

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BRIEF OUTLINE OF RESEARCH FINDINGS

1. "Autofluorescence of live purple bacteria in the near infrared "

We have developed a novel microscope to study the fluorescence of cells in the near infrared region ($\lambda = 750 \text{ nm} - 2500 \text{ nm}$). As one of its first application we report the autofluorescence of live purple bacteria, *Rhodospirillum rubrum*, and suggest that the autofluorescent component is bacteriochlorophyll. The rapid fading of the autofluorescence of fixed bacteria and of purified bacteriochlorophyll suggests that the live bacteria are able to regenerate their pigment with a time constant of appr. 20 s.

2. "Altered Drug Resistance of Microtubules in Cells Exposed to Infrared Light Pulses: Are Microtubules the 'Nerves' of Cells? "

A most important required step in the project of the past 9 years was to identify the specific structures and mechanisms which mediated between the light detection at the cell center on one hand and the extension of specific pseudopodia at the peripheral cellular cortex on the other. The most promising candidate for this function seemed to be the microtubules. This led to the following question. Are any signals, indeed, propagated along the microtubules to the cell cortex in response to pulsating near-infrared light? If so, how can they be detected?

In order to approach it experimentally, I hypothesized that the signals might enhance or diminish the effects of some other agent that was known to change the structure of microtubules. For example, it seemed possible that the traveling signals were strong enough to alter the speed of disassembly of microtubules which were exposed to an anti-microtubular drug. Therefore, I measured the stability of cytoplasmic microtubules in the presence of nocodazole while exposing the entire cell culture to pulsating near-infrared signals.

In order to erase any possible imprints of past irradiation of the cells and also to increase the degree of uniformity of microtubule stability in the cells, I used cells that contained only freshly assembled microtubules. Therefore, I first disassembled the cytoplasmic microtubules of the cells by an anti-microtubular drug and subsequently allowed them to re-grow in the absence of the drug. This step in the procedure required to use the most readily reversible anti-microtubular drug, nocodazole, for the study.

After removal of the nocodazole the cells formed new radial arrays of cytoplasmic microtubules with the centrosome at their center. In the following I shall call them 'cytoplasmic asters' in order to distinguish them from 'mitotic asters'. With the exception of a few drug-insensitive microtubules, the cytoplasmic asters contained microtubules no older than 30 [min]. Their degree of stability could be assumed to be considerably more uniform, and to carry no imprints of past illumination periods.

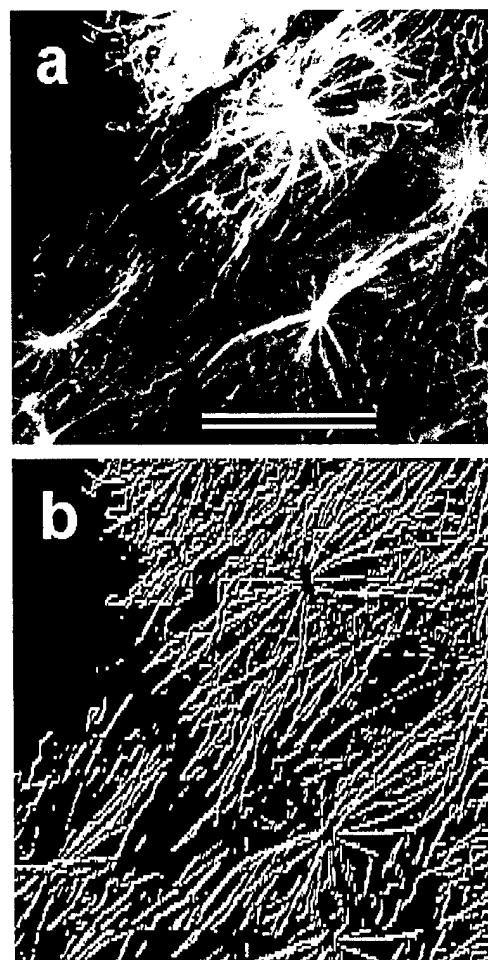


Figure 1. Computerized measurement of the total amount of stained microtubules.

a. Immunofluorescent micrograph of DCAs showing various levels of background staining. (Bar indicates 30 [mm]).

b. The same image field after image processing by the computer program 'hicount.exe' (see Methods) showing only the outlines of the microtubules.

b. Quantitative description of reconstituted cytoplasmic asters.

In order to measure the number of reconstituted microtubules I developed for a digital camera special software that processed the immuno-fluorescent images of cytoplasmic asters stained with anti- α -tubulin. It calculated the number N of highlighted pixels per cell and thus the amount of microtubular stain per cell.

$$(1) \quad A_m = \frac{N}{C} [\text{pixels} / \text{cell}]$$

A copy of the program can be downloaded from the website

"<http://www.basic.nwu.edu/g-buehler/>".:

Fig. 1 shown an example of the evaluation method

In order to quantify the change of microtubule stability, CMS, I measured the amounts of microtubules per cell in irradiated and control cells which had been exposed a second time to nocodazole as described above in equ. (1) and calculated the relative difference.

$$(2) \quad CMS = \frac{\overline{A_m^{control}} - \overline{A_m^{irradiated}}}{\overline{A_m^{control}}}$$

Fig 2 shows the result in form of the spectral sensitivity of the destabilisation of the microtubules by the infrared signals.

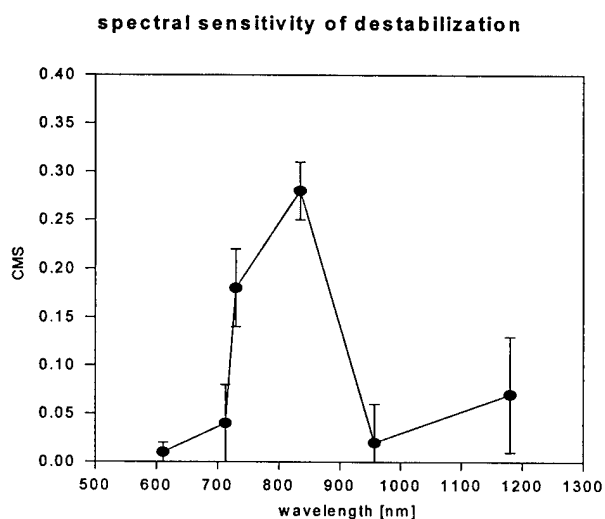


Figure 2: Wavelength dependence of the destabilization of the microtubules of CV1 cells (ordinate CMS (see equ. 2); rectangular pulses; pulse length = 1 [s]; intensity = 4 [mW]; average sample size: 2340 cells/ data point (range 1270 - 5060 cells/data point). The error bars show the errors of the mean.

INTERACTIONS/TRANSITIONS

a. Participation at meetings where the work was presented

1. " AFOSR Workshop on DALM (Diazoluminmelanin)", March 3-5, 1996, North Redington Beach, FL
2. "The Cytoskeleton", Workshop Center for Advanced Studies in the Space Life Sciences, Marine Biol. Laboratory, Woods Hole, November 15-17,1996
3. AFOSR Workshop: "Infrared reception in nature and its practical application" December 7-8,1996, Yokohama, Japan
4. "Frontiers of Biological Science in Space" National Space Development Agency of Japan, January 27-28, 1997, Tokyo, Japan

b. Transitions

Infrared fluorescent microscopes are entirely new. There is a possibility for a new technological application.