Light yellow (Ednalite K2), light green (G1) and light blue (No. 80) photographic filters have proved more satisfactory than deep red (R2), but this may be a personal matter and possibly a function of one's color vision. An example of a stereopair for which this approach has proven successful is given in the Figure. This method has been found equally useful for sectioned material.

Résumé. On propose l'emploi d'un écran coloré, disposé devant un des oculaires, comme aide à la superposition d'images paires pour l'observation stéréoscopique d'objets examinés en microscopie à balayage. Le filtre permet un alignement facile et rapide, ce qui réduit appréciablement la fatigue et les maux de tête que beaucoup ressentent au cours de ces manipulations.

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A New Method in Cytospectrophotometry

Classical cytospectrophotometry measures quantitatively the various chemical components existing in a given part of the cell. Unlike the classical methods, the method used here reveals the changes occurring in the proportions in which these different chemical components exist within the cell. As an example, the changes shown are induced by X-rays in the rat lymphocyte nucleus. One starts from the premises: 1. The lymphocytes of the same age, from the peripheral circulation, have the same chemical nuclear composition, i.e. the proportions of the nucleic acids, nucleoproteins, are the same in the all nuclei. 2. These proportions determine the colour of the nucleus in a panchromatic staining made with a complex stain, like Giemsa, in which the colouring substances (Eosine, Azur and Methylblau), have different affinities for the nuclear components. 3. By any chemical, physical or biological aggression (here a physical one), which provokes defense reactions, the proportions of different nuclear components must change, and these changes are revealed by changes in colour.

Thus, by determining with the 'Tristimuli' method[1,2] the coordinates of 1 lot of lymphocyte nuclei panchromatically stained, one obtains in the colours triangle a rectangular-shaped confidence area, with the basis: $x_{med} \pm n\varepsilon$ and the height: $y_{med} \pm n\varepsilon$, in which reside, statistically significant, the great majority of the nuclei.

The measurements were made with a microspectrophotometer built by an adequate assembling of a Reichert Zetopan microscope with a Beckman B spectrophotometer, a Leitz Panphot microphotodevice and a Graphispot Sefram recorder. A group of 24 white mice, unselected as concerns age, sex or pathological antecedentes, was utilized. The programm of blood drawing from the tail vein and irradiation of the mice is presented in the Table.

In the unirradiated stage, 2 smears were made to check whether the injuries provoked to the tail do not induce unspecific chromatic changes; this was not the case. To avoid staining artefacts, the samples were chemically fixed immediately after smearing, and were stained, all together, in the same bath up to the 10th day. Microspectrophotometric curves were plotted from $\lambda = 400$ nm to $\lambda = 760$ nm for 2 different lymphocytes on each sample. The area explored in the nucleus was round with a diameter of about 3.5 µm. When the peaks differed with more than $\lambda = 5$ nm, 2 supplementary curves for 2 other elements were plotted and introduced in the mean value. The transmission was calculated as function of the blank plotted in a free area in the neighborhood of the measured elements. The chromatic coordinates were calculated in conformity with the method of 10 selected ordinates.

Results. 1. The colour of Giemsa stained mouse lymphocytes occupies in the colour triangle a rectangular-shaped confidence area with the coordinates:

$$x \pm 3\varepsilon = 0.2684 \pm 0.0102$$
$$y \pm 3\varepsilon = 0.1782 \pm 0.0192$$

2. Irradiation with X-rays, 55 KV and 250 R, provokes its shift to the coordinates:

$$x \pm 3\varepsilon = 0.2583 \pm 0.0105$$
$$y \pm 3\varepsilon = 0.1563 \pm 0.0147$$

3. A new irradiation with the same dose maintains the size, direction and sense of the shift, but reduces the dispersion

$$x \pm 3\varepsilon = 0.2583 \pm 0.0099$$
$$y \pm 3\varepsilon = 0.1530 \pm 0.0087$$

4. The visual efficiency (the green stimuli value Y) also suffers a shift:

before irradiation:	$Y \pm 3\varepsilon = 0.256 \pm 0.0054$
after 250 R:	$Y \pm 3\varepsilon = 0.222 \pm 0.0048$
after 500 R:	$Y \pm 3\varepsilon = 0.200 \pm 0.0021$

The animals being unselected, the chromatic shift is induced only by irradiation and is specific to it. In our experiment the type of cell and of aggression, the stain, and the time delay between aggression and measurement have been taken without any optimization study; such a study could have determined: 1. the cell with the most significant reaction for every kind of aggression, 2. the stain with the most sensitive colour variation for every type of change occurring in the nuclear composition, and 3. the delay for which the chromatic shift has its maximal value.

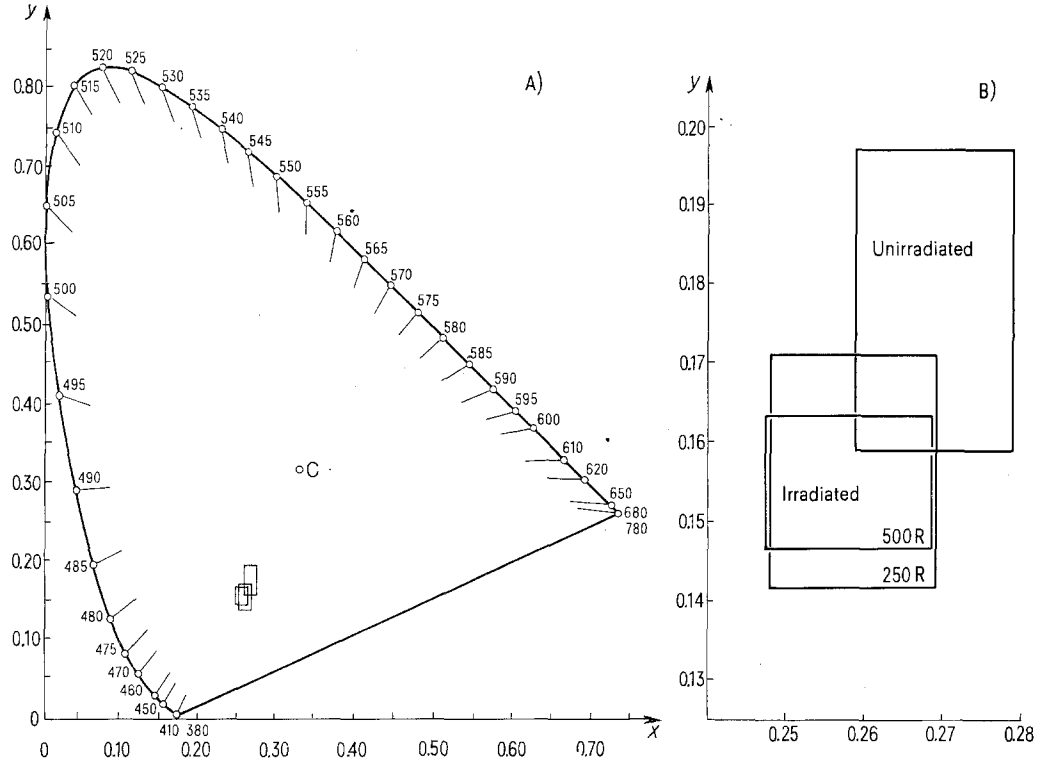
Discussion. The capital letters stand for the nuclear components of a well-defined kind of cell, e.g. A_1 = the amount of DNA in the nucleus of lymphocyte No. 1. B_2 = the amount of B type protein in the nucleus of lymphocyte No. 2, we have:

$$A_1 + B_1 + C_1 + \ldots\ldots\ldots + N_1 = \emptyset_1$$
$$A_2 + B_2 + C_2 + \ldots\ldots\ldots + N_2 = \emptyset_2$$
$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$
$$A_n + B_n + C_n + \ldots\ldots\ldots + N_n = \emptyset_n$$

[1] *The Science of Colour* (The Commitee on Colourimetry of the Optical Society of America; Thomas Crowel Co., New York 1953).

[2] A. C. HARDY, *Handbook of Colorimetry* (The Technology Press M.I.T., Cambridge, Mass. 1936).

	1st day	4th day	7th day	10th day
First operation	Blood draw.	Blood draw.	Blood draw.	Blood draw.
Second operation	—	1st irradiation 250 R-55 KV	2nd irradiation 250 R-55 KV	—.



A) The location of confidence areas in the colours triangle. B) The same as A) at a larger scale.

in which $\varnothing_1$ represents the weight of the nucleus of cell No. 1. The cells being of different sizes

$$\varnothing_i = \varnothing_j \quad \text{then} \quad A_i \neq A_j,\ B_i \neq B_j,\ \ldots\ldots\ N_i \neq N_j$$

but for cells of the same kind and age, we can consider

$$\frac{A_1}{\varnothing_1} = \frac{A_2}{\varnothing_2} = \ldots\ldots = \frac{A_n}{\varnothing_n} = \alpha$$

$$\frac{B_1}{\varnothing_1} = \frac{B_2}{\varnothing_2} = \ldots\ldots = \frac{B_n}{\varnothing_n} = \beta$$

$$\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots$$

$$\frac{N_1}{\varnothing_1} = \frac{N_2}{\varnothing_2} = \ldots\ldots = \frac{N_n}{\varnothing_n} = \psi$$

and $\alpha + \beta + \ldots\ldots\ldots + \psi = 1$ for all cells.

The classical cytospectrophotometry measures A_i, B_i, $..N_i$. These quantities are f(Ø, S), i.e. function of 2 variables in which Ø represents the size or weight of the cell and S represents the chemical structure of the nucleus, required by the immuno-pathologicals state of the animal. Acordingly, the interpretation of differences $\Delta A_i .. \Delta N_i$ is equivocal, because we do not know $\Delta N_i = f(\varnothing)$ or $\Delta N_i = f(S)$ or $\Delta N_i = f(\varnothing, S)$.

The method here described points out the variations of chemical components which being independent of cell size, are a function only of nucleus structure. By adequate measurements the shift in the colour triangle may be calibrated and the chromatical coordinates can be standardized as regards chemical composition.

Zusammenfassung. Versuch der Anwendung einer colorimetrischen Methode für die Zytospektrophotometrische Untersuchung biologischer Systeme. Als geeignetes Untersuchungsobjekt erwies sich der Lymphozytenkern mit seiner besonderen Empfindlichkeit gegenüber mutagenen Agentien. Die Methode erlaubt ein präzises Verfolgen der verursachten Veränderungen.

A. CIURES

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