THE USE OF FLUORESCENT PAINTS IN MICROMORPHOMETRIC STUDIES BY AUTOMATIC IMAGE ANALYSIS SYSTEMS

by

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The awareness of empty spaces in the soils is focussed from the standpoint of two theories diametrically opposed to one another. One group led by Swanson, 1941; Kubiena, Beckmann & Geyger, 1961-1962 -1963; Lafeber and Kurbanovic 1965; Guardiola y Delgado, 1969a, 1969b; Jongerius et al. 1972b; tries to differentiate the pores through optical procedure; impregnating the soils with colourless isotropic resin and working almost exclusively on thin sections.

The other group believes that better results can be obtained by introducing within the voids colouring or fluorescent matter and although, on occasions, they work with thin sections, their work is done primarily with polished blocks. It follows that Harper and Volk (1936) introudce within the pores a white casein paste. Day (1948) employs Genciane Violet. Werner (1962) makes use of green fluorochrome (Fluoewazenz-Grünn from FA. Siegle & Co). Lund and Beals (1965) use a red colouring agent described as "Oil Red O". Fitzpatrick (1970) adds a plastic additive resin, the fluorochrome from Ciba FBA K5760. Prevosteau and Regot (1970) use as colouring MACROLEX violet B from Bayer and as fluorochrome TINOPAL PGP from Geiby. Finally, Jongerius et al, (1972 a) employ a green dye BS 1172 from Ferro Company

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Along the above mentioned lines of thought, we include our own study which features as outstanding innovation the proper use of the fluorescences with the aid of electronic apparatus, and the use of "Hipnotic-Exterior" fluorescent paints (1) which may be added to the poliester resin to be used in soils micromorphology.

Table I reproduces the intensity values of the fluorescences given for polished blocks using different fluorochrome. These intensities have been evalued directly according to Microvideomat (Carl Zeiss), using as unit value the fluorescence from powdered Auramine G.

As shown on Table I, best results can be obtained with the use of Hipnotic Exterior paints, and outstanding amongst them is the strong fluorescence of the yellow.

FLUOROCHROME	INTENSITY OF FLUORESCENCE
Rivoflavin	0.2
Rodamine B	0.3
Powdered Auramine G	1
Cyalume (American Cyanamid Co)	3
9-10 Dibromoantracene	3
Rodamine 6 G	3
Tripoflavin	5
Fluorescine	5
Auramine G	7
Rodamine 6 G with auramine G	8
Rivanol	8
Orange Hipnotic paint	8
Red Hipnotic paint	10
Gren Hipnotic paint	13
Yellow Hipnotic paint	22

Table I.- Intensities of polished blocks prepared with various fluorochromes.

(1) Magilux, Rosal, S.A. San Gervasio de Cassolas 75, Barcelona 6.

One of the most important details to keep in mind is the one that corresponds to the enormous influence exerted by the added colouring on the fluorescence of the paint. The manufacture of all these paints is bases on fluorescine plus a colouring additive and as it will be appreciated from Table I, the yellow colouring gives the paints their highest fluorescent values, well above the green, orange and by far higher than the value of the fluorescine itself, product responsible for the fluorescence.

For this reason we have choosen as the most suited fluorochrome to this yellow paint, specifically the "arch yellow" colour, although, the "solar yellow" practically gives as good a result as the one selected.

Various test have been performed in order to establish the dilution ratio between the poliester resin and the paint. The variation in intensity of the fluorescence vis-a vis the proportion of paint is outlined on Table II. Under dilution 1:10 (one part of paint to 10 parts of resin), the fluorescence decreases enormously. In a 2,5:1 concentration the fluorescence is very high, but the viscosity of the resulting mixture opposes itself in practice to its use. Best results are attained with dilutions 1:4 and 1:6 which produce a very satisfactory fluorescence and an acceptable viscosity. Using dilution 1:4 as shown on Table II, the fluorescence of the paint becomes 60 times higher than the auramine.

The adding of paint to poliester resin will increase, the viscosity of the emulsion, and, conversely, it can be reduced by adding enough dissolver.

The intensity of the fluorescence of the polished block is also modified by the time it takes the poliester resin in poliomerizing. Best results can be obtained during the poliomerization period which runs from 3 to 7 days.

CONCETRATION OF PAINT AND POLIESTER RESIN - RESPECTIVELY	INTENSITY OF THE FLUORESCENCE
1 : 10	15
1 : 4	60
1 : 2	90
2,5 : 1	120

Table II.- Intensity of the fluorescences of the polished blocks mounted on different amounts of "arc yellow Hipnotic Exterior" paint.

To obtain a high fluorescence and a good inclusion we propose the following mixture as the most favourable :

1000 ml	Poliester resin (Cronolita 1.108)
150 - 333 "	Fluorochrome (Hipnotic Exterior yellow arc paint).
400 - 500 "	Dissolver (Monostyrene)
1 - 3 "	Activator (Cobalt Naftenate)
2,5 - 7 "	Catalyzer (Peroxide of Cicloexane)

Two practical examples of this technic are exhibited in Fig. 1 and 2.

As a source of radiation we employ an Osram HBO 200 W. high pressure mercury lamp mounted on a support for Zeiss microscopy, work fitted with exciter filters UG1 and UG5. (Zeiss). As it has become necessary to illuminate large surfaces, long fluorescent tubes (low pressure mercury vapor lamps) has been utilized.

To make full use of the fluorescence of the sample we have fitted the T.V. camera with an additional photographic lens (Micronikkor 55 mm f/3,5).

Having fitted these lens directly into the T.V. camera outlet a 1,6 x to 1,5x magnification is reached (that com - bined to the 25 x on the electronic system make it a total magnification of 37 x - 40 x). If one still wishes to work with larger magnifications, it will be necessary to change to a thelephoto lens (a 135 mm focal lens makes it already possible to get a magnification of 0,6 x ; i.e. 15 x monitor display magnification).

In our opinion, at the present time, this technic offers the best possibilities for differentiation of pores out of all other soils components. A sample of void discrimination with this technic is showed in the fig. 3, 4 and 5.

Its use is synthetized as offering the following advantages:

1) It is the only technic up to this moment, which is capable of differentiating the soils voids out of all the other components without any exception whatsoever. We wish to emphasize in particular that the use of this system allows us to go around this huge problem -and which still remains unresolved in our time by other methods- presented by the isotropic colourless crystals or of the anisotropous crystals in position of isotropic, as well as that of the colourless amorphous materials.

2) Work can be done directly using the sample and without requiring photographs, such as is done with other technics (Lafeber and Kurbanovic, 1965; Jongerius et al. 1972 b) Moreover, measurements can be taken in many cases from polished blocks, which save us the trouble of extending the process for obtention of a thin section.

As disadvantage to our approach we cite:

a) The main limitation constitutes the minimum size of the voids detectable by our technic. This is caused by the fact that in order to detect the fluorescence, it becomes necessary that within the plastic which fills up the pore, there should alreadyexist a sufficient quantity of fluorochrome.

Thus, for a polished block with a 60 unit fluorescence, the large voids of 4 mm (according to the same scale on tables I and II), the 100 microns voids here only present mid-intensities of 10 units.

Consequently the pores with diameters smaller than 30 microns represent the smallest limit of voids that can be detected, as smaller diameters will not allow the entrance of a sufficient quantity of plastic matter with fluorescent paint. In adverse conditions, the presence of highly reflective large crystals, the limit has to be increased to 50 microns in order to obtain satisfactory results.

The 30-50 microns, limit is practically in consonance with the resolution limit established by the electronic equipment, under our own working conditions (31 x monitor display magnification). On the other hand it also corresponds to the minimum size of the pores which can be measured by any other optic means working on thin sections.

b) With the magnification of work (31 x) the limit between the fluorescent plastic and the soil matrix usually does not present itself very clear, causing errors of discrimination, if not properly done.

c) The mixture of poliester resin and paint does not constitute a disolution, but forms an emulsion. In spite of this, with the held of a dissolver both blend perfectly well offering very good results on solidifying.

d) We know only too well that certain crystals and organic matter can present luminiscence phenomena. Regarding the fluorescence of these materials we should like to clarify that in cases of size parity between the material and the pores, the plastic with the fluorochrome presents a fluorescence higher by far to their own. After inspecting well over 700 thin sections and polished blocks, covering about 150 different soils, we have been unable to find any crystals nor any remnant of organic matter which presented fluorescence of an intensity higher than 0,6 under the working conditions used by us (1). Therefore the fluorescence of the crystals will not affect the micromorphometry of the pores. Although we recognize that the fluorescence of the crystals by itself is not a cause for any problem, the crystals due to their transparency can at times interfere when working on polished blocks having a normal thickness. In effect, in those cases where colourless crystals exist, separated from the soil plasma and surrounded by fluorescent plastic, the mineral grain can present an anomalous luminosity, which proceeds out of the fluorescence of the plastic surrounding it. When this situation develops it can be solved satisfactorily, with as little trouble as cutting the block, paralel to its surface, in such a manner that only a fine sheet will remain of approximately 0,5mm (or thin section if it were necessary), with which the source of radiation will dissappear and which illuminated the grain from below and there will only be a faint transmision form the crystal edges.

In Fig. 6 and 11 we show some examples as to how the discrimination of voids is to be accomplished with the Microvideomat for the cases under revue (fields with abundant and large crystals).

e) The blocks and thin sections may lose part of their fluorescence after a certain period of years, o perhaps even after weeks if they are continuously exposed

(1). It is important to stress the fact that in order to favour the transmition of the fluorescence from our plastics and especially eliminate the intense reflection on the part of the crystals of the ultraviolet radiation, before the objective fitted into a TV camera was placed an barried filters nº 2B and nº 12 of Kodak, serie Wratten.

to the direct action of the sunshine for which they must be carefully handles (2).

SUMMARY

The use of fluorescent paints added to poliester resin impregnation is proposed for soil micromorphometric investigations, with the use of automatic image analysis systems.

The paint most suited for the task is "Hipnotic-Exterior" arc yellow in colour. We also point out in this paper the proportion paint/poliester resin required to produce the best results as well as the pros and cons in the proposed technic.

(2) The fluorescent block was exposed to the action of the open air and of the sunshine for 7 days (about 42 hours of sunshine) and the intensity os its fluorescence fell to exactly one half.

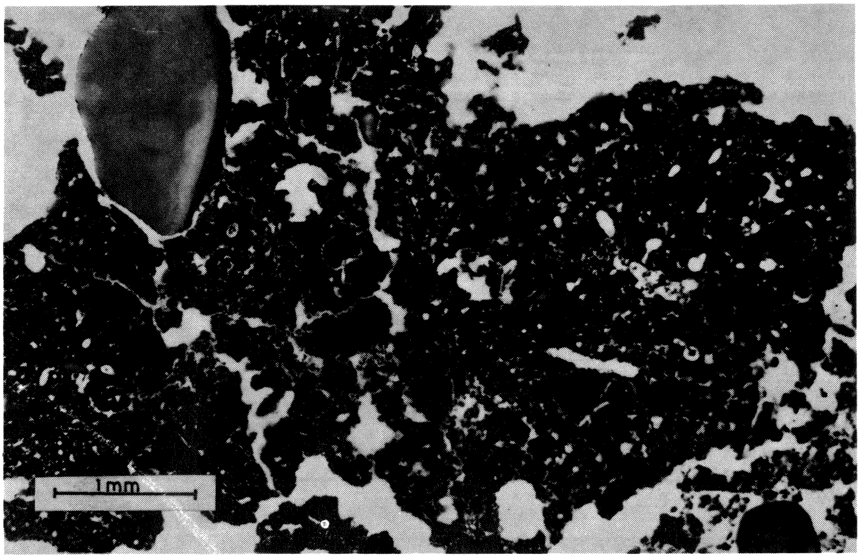

Fig. 1. Fluorescent illumination. Polished block .

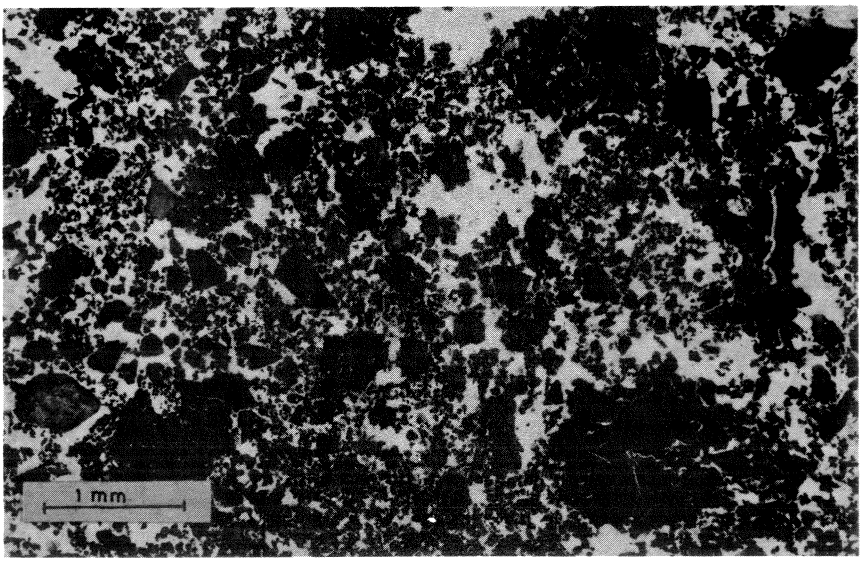

Fig. 2. Fluorescent illumination. Polished block .

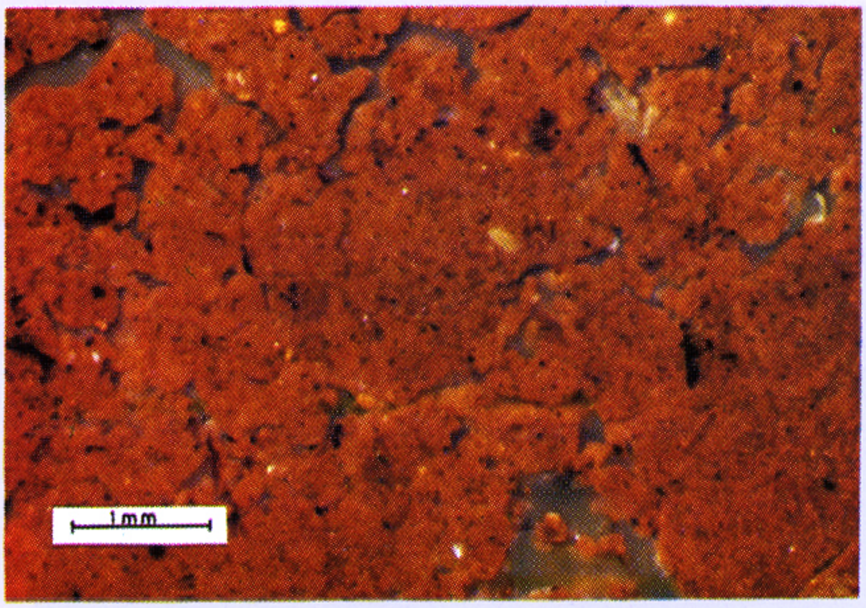

Fig. 3. Polished block. Natural reflected illumination.

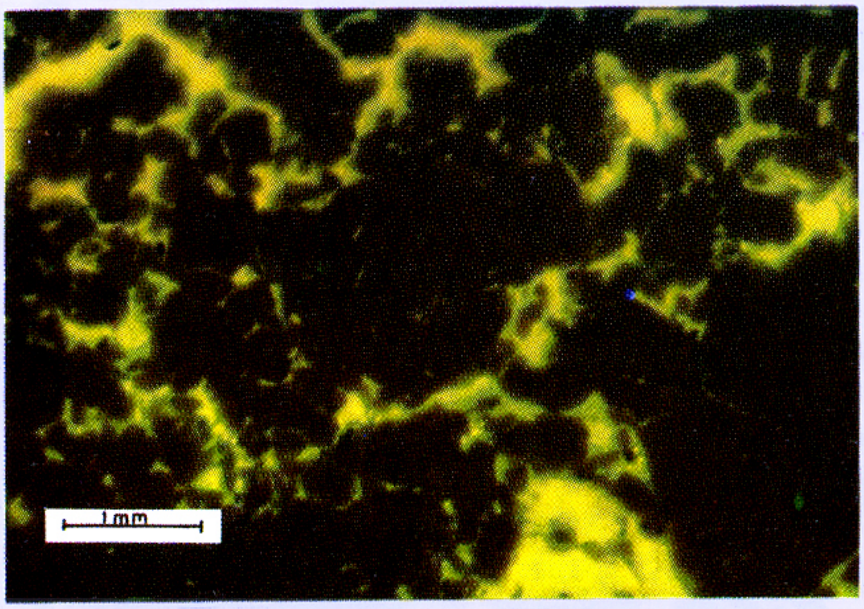

Fig. 4. Fluorescent illumination of the same field.

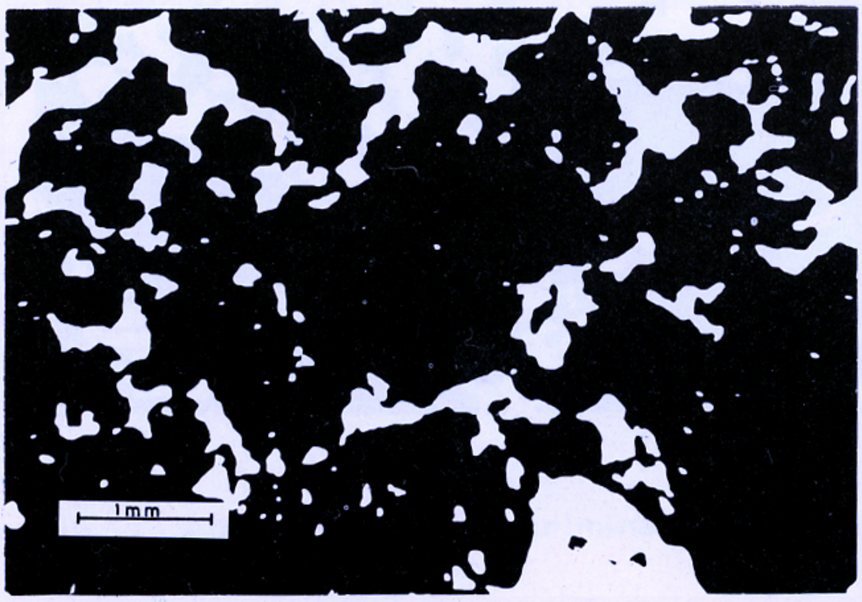

Fig. 5. Image of the anterior soil discriminated in the Microvideomat.

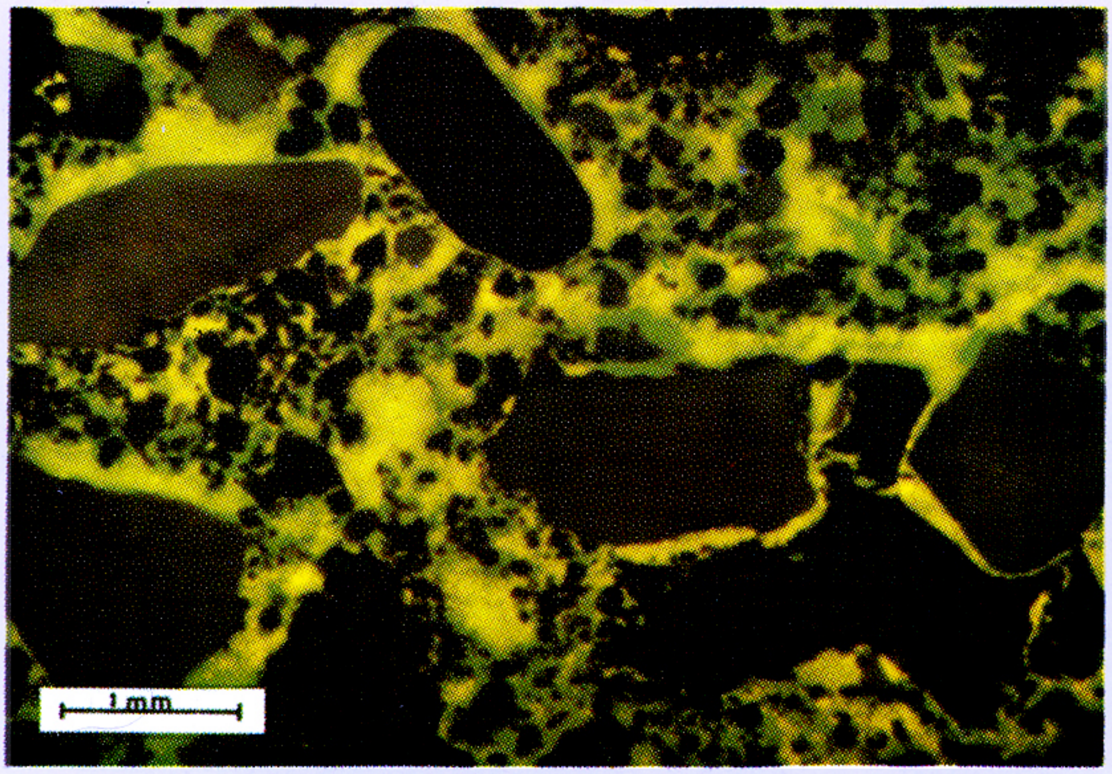

Fig. 6. Thin section. Fluorescent reflected illumination. Field with numerous quartz and carbonate crystals of sand size.

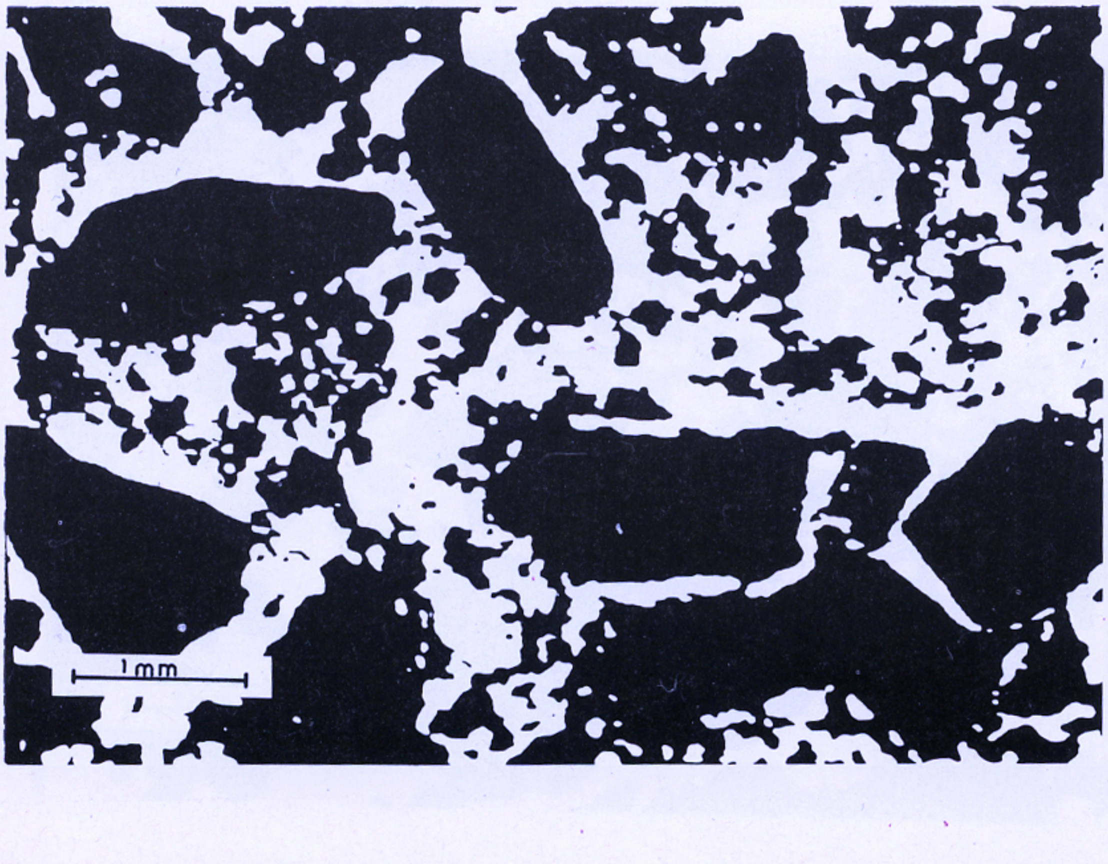

Fig. 7. Anterior image discriminated with the microvideomat

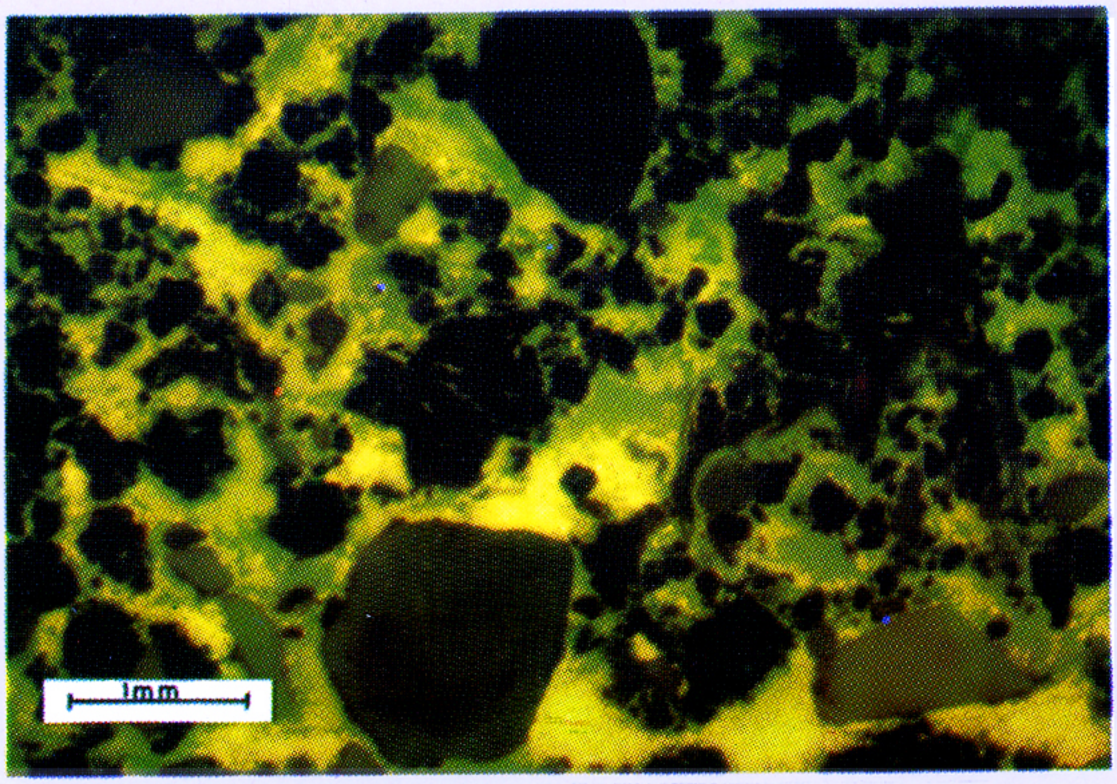

Fig. 8. Thin section. Fluorescent reflected illumination. Field with numerous quartz and carbonate crystals of sand sizes and remmants of organic matter very little transformed.

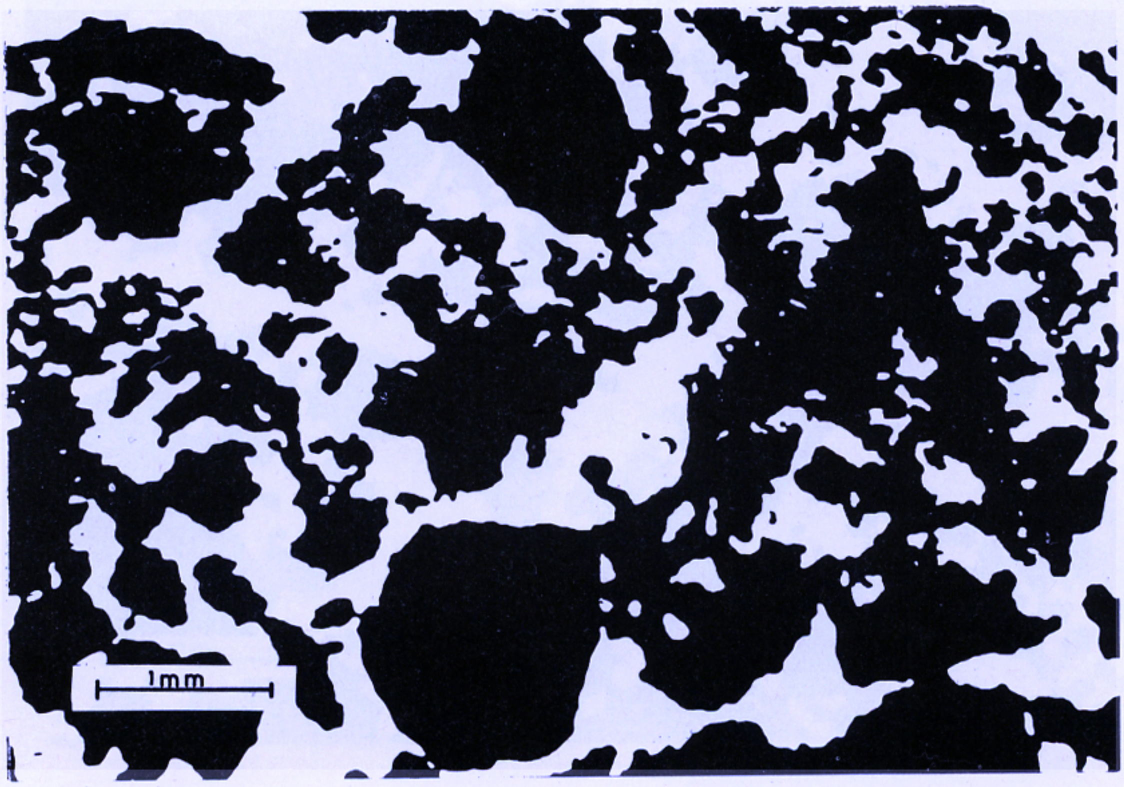

Fig. 9. Anterior image discriminated with the microvideomat.

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