# A simple method to make identification of aquatic invertebrates much easier


## ABSTRACT

It is proposed that the use of methyl blue will improve routine stereomicroscope identification work, as it allows much greater contrast between important features. We obtain good results with animals with external epidermis (Polychaeta, Echinodermata). Animals with exoskeleton (Crustacea, Bivalvia) give weak or absent contrast effect. Toxicity of methyl blue for humans even it has been swallowed is absent.


## INTRODUCTION

Aniline dyes are often used in polychaete taxonomy to reveal specific structures (such as glandular fields) that are almost invisible without stain (Hofsommer, 1913; Nolte, 1913; Banse, 1970; Green, 1987, 1991; Jirkov, 1989, 2001; Mackie & Gobin, 1993).

The usual method is as follows (as applied to Polychaeta):

(1) place specimen in aniline dye solution (water or alcohol) until it becomes dark blue (in the case of methyl blue);

(2) place specimen in water to remove surplus stain, in the case of poorly preserved specimens it is better to use alcohol rather than water;

(3) place specimen in 70° alcohol: for the first 1015 minutes, stain is lost from different organs at a non-uniform rate, epidermal glandular areas remain much more strongly stained than neighboring areas; this contrast remains for several hours (depending on preservation).

## RESULTS

However, use of aniline dyes in routine analysis makes the process much easier. Researches often find that the contrast between specimen body parts is not optimal for invertebrate identification. Our experience shows that it is possible to obtain much better contrast in many cases. Use of aniline dyes often makes morphological characters more visible. Discussion with other researchers showed the same response each time: whenever somebody began to use aniline dye they always began to use it routinely and commented: "Where were you before? I have been struggling for so long!"

The standard method is as follows:

(1) place specimen in aniline dye water solution; the precise time of exposure depends upon the specimen and the concentration of dye and can easily be determined experimentally; typically, the most appropriate time is a few seconds;

(2) place specimen in water to remove surplus dye;

(3) it is necessary to examine the specimen in water, otherwise the contrast rapidly disappears; only in the case of poorly preserved specimens is there a need to examine them in alcohol, to prevent damage.

If it is necessary to examine part of a specimen, it is possible to add aniline dye by pipette in the required positions and remove any surplus with the same pipette.

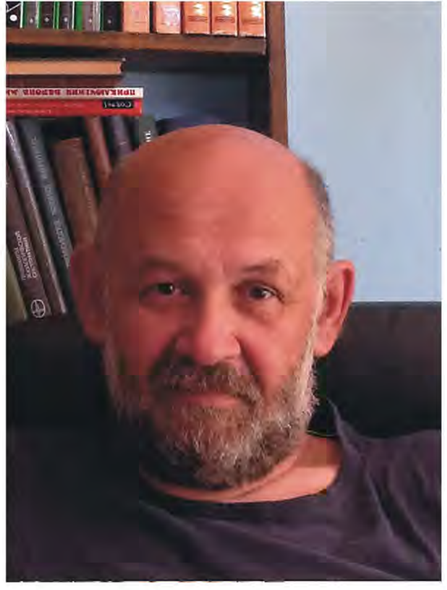
Well-prepared specimens have distinct, but not too strong, colour. In such cases, prominent parts are more strongly stained, with the result that contrast is maximised. Some structures (example: chaetae, glandular pads) are often stained more intensely. We obtain good results with animals without an exoskeleton (Polychaeta) or with exoskeleton covered with epidermis (Echinodermata). With Crustacea and Bivalvia, the contrast effect was weak or absent. Some examples are shown in Fig. 1.

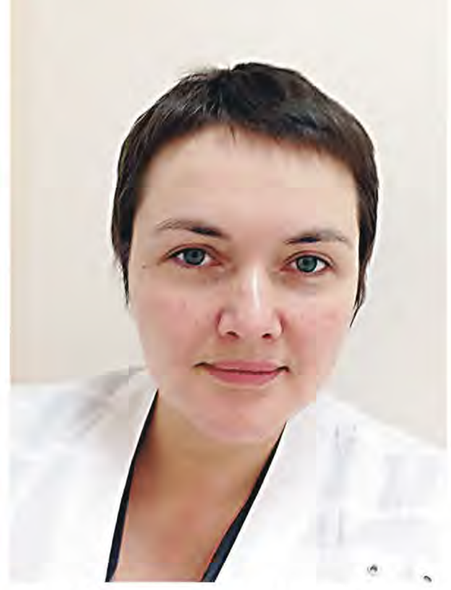
It is necessary to examine the specimen immediately after staining, as the dye quickly diffuses internally and the contrast disappears within a few minutes. However some structures, such as chaetae, remain stained. This allows chaetae that do not rise above the body surface to be seen, which would otherwise be easily overlooked.

The stain usually completely disappears in alcohol within one day. With strongly stained specimens, replacement of alcohol may be necessary. In either case, the dye is completely removed form the specimen. This allows use of the method with very valuable specimens (types, material from loan etc.).

We tested the suitability of four aniline dyes: methyl blue, methyl green, malachite green and brilliant green. The last, despite being much easier to source (an alcohol solution that can be readily bought in a pharmacy) gives the worse results. Malachite green gives insufficient contrast. So we would not recommend use of these.

Methyl blue and methyl green give similar results but, as the former is much easier to source and half the price of methyl green, we recommend the use of methyl blue.






I.A. Jirkov N.Yu. Dnestrovskaya M.K. Leontovich

Department of Hydrobiology, Biological faculty, Moscow Lomonosov State University, Russia,

ampharete@yandex.ru ndnestro@mail.ru axionice@mail.ru


### Stained and unstained fixed animals

*Glycera maculata*

*Spio filicornis*

*Ophiura albida*

*Poecilochaetus serpens*

*Pontoporeia* sp.

## TOXICITY OF METHYL BLUE FOR LIVING ORGANISMS

### data from literature

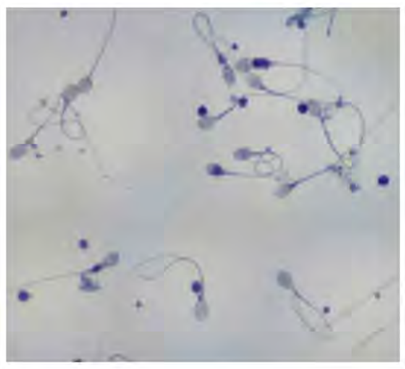
As we recommend use methyl blue in everyday work, it is necessary to evaluate its toxicity. Methyl blue is a vital dye and can be used to stain living organisms. "In a weak solution of methyl blue, dye penetrates across ciliary cells and stains the base of the cilia of ciliary bands in living *Dinophilus*. Then the cilia of the nephridia of the intestinal epithelium become stained. After a long time… the dermal muscular system and some parts of the peripheral nervous system become stained» (Ivanov *et al*, 1941: 345).

Methyl blue is also used as a remedy in veterinary practice for the treatment of demodicosis and other parasitic illnesses from fish to mammals (e.g. for pyroplasmosis in dogs). Methyl blue is used in medicine as anti-infective agent: externally on the skin for the irrigation of impetiginous illness and internally for inflammation of the bladder. Methyl blue is used as antidote (even intravenously) to poisoning by cyanide, carbon monoxide and hydrogen sulphide. In the early 20th century it was used as malaria remedy. There are data on the effectiveness of methyl blue as a remedy for cancer and Alzheimer's disease (Wikipedia).

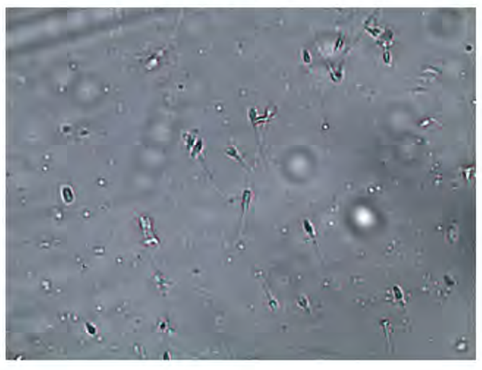
### our data: sperm of *Homo sapiens*

Human fertility often depends on semen. It is important to investigate not only the quantity of sperms, but their shape and ability to move normally. However sperms are the smallest human cells. Their investigation require very high magnification: objective at least 150x and well contrast but sperms by itself are low contrast cells. Investigation of sperm morphology according to recommendation of World Health Organization based on Kruger Sperm Function Test. This test requires Georgios Papanikolaou staining of cytological smears. This complicate process includes thirteen stages of staining only and fixations between these stages. Unfortunately all of used solutions have strong smell and it is absolutely impossible to make staining in the same room where human embryos are cultivated. So it should be done in special room well separated from embryological laboratory, but not all clinic are big enough to have two well separated rooms. Additionally fixation of sperms modify their morphology (head become inflated and tail curved). At the same time addition a drop of methyl blue to ejaculate allows to investigate sperm characteristics (quantity of sperms, their shape and ability to move normally) simultaneously and for low price. Sperms after addition of methyl blue are live and their morphology do not changed. As methyl blue has no smell at all such investigation can be done in the same room where human embryos are cultivated.

Papanikolaou staining



methyl blue staining



### our data: usage per os

Most convincing are observations of direct effects on humans in situations unconnected with medicine (i.e. in everyday life). We are lucky to have such data.

N worked as a school teacher. Once he came home late at night and decided to have some drink. He remembered that there was a bottle on the shelf. In the dark (he did not switch on the light, as he was afraid to wake his wife) he took the bottle and drank it all. He then realised that it was not a port. So N switched on the light and asked his wife what it was. She told him that it was methyl blue water solution for dying linen blue. As N felt nothing bothering him, he went to bed but, in the morning while he shaved, he discovered that his skin had become blue. Thinking the situation through, N realised that it would be impossible to conduct a lesson in such a condition. This event took place in Stalin's time, when absence from work was absolutely impossible: if somebody was late he would, at best, lose his job. So N covered his face and rushed to a doctor to get a sick-leave certificate. The doctor examined him and found nothing wrong except the color of his skin and refused to give him a medical certificate, even though N was a teacher. However N, probably having a better understanding of what would happen when a blue-skinned teacher tried to conduct a lesson, went home instead of to school. Being in a frustrating situation, as he could not go to school due to his blue skin and absence from work could lead to the prison, N made an attempt to hang himself. Luckily, he was immediately removed from the noose and a doctor was called. A psychiatrist examined N and concluded that he was completely healthy, with exception of the color of his skin, which disappeared after a couple of days.

This case gives us assurance that methyl blue, even when taken orally in large doses causes no physical harm.


## ACKNOWLEDGEMENTS

The authors thank Moscow ambulance psychiatrist K.F. Leontovich for valuable information on the influence of methyl blue on human health. Animal photographs were prepared at MES Ltd (Bath, UK); we give special thanks to the staff at MES. Also we thank Tim Worsfold (APEM Limited) who made edit to the English.


## REFERENCES


Banse K. 1970. The small species of *Euchone* Malmgren Sabellidae, Polychaeta. // Proceedings of The Biological Society of Washington Vol.83. P.387408.

Green K. 1987. Revision of the genus *Sonatsa* Polychaeta: Maldanidae. // Bulletin of the Biological Society of Washington Vol.7. P.8996.

Green K. 1991. *Maldane californiensis*, a new species Polychaeta: Maldanidae. and a review of its relations. In Reish D.J. ed. // Proceedings of the 3rd International Polychaete Conference, Long Beach 611 August 1989. Bulletin of Marine Science Vol.48. P.214226.

Hofsommer A. 1913. Die Sabelliden-Ausbeute der 'Poseidon'-Fahrten und die Sabelliden dcr Kieler Bucht // Wiss. Meeresunters. Kiel., Bd.15. S. 305364.

Ivanov A.V., Petrushevsky G.K., Pojansky Ju.I., Strelkov A.A. 1941. [The greater practicum on invertebrate zoology. Pt. I. Leningrad: State education-pedagogical publisher of the Ministry of education of RSFSR] [In Russian].

Jirkov I.A. 1989. [Bottom fauna of the USSR]. Polychaeta. Moscow: Moscow State Univiversity Press 141 p. [in Russian].

Jirkov I.A. 2001. [Polychaeta of the Arctic Ocean]. Moscow: Yanus-K Press 632p. [in Russian].

Mackie A.S.Y., Gobin J. 1993. A review of the genus.*Johnstonia* Quatrefages, 1866 Polychaeta, Maldanidae., with a description of a new species from Trinidad, West Indies. // *Zoologica Scripta* Vol.22. P.229241.