Tabelle 1. Röntgendaten und Dichten von Li_2RhB_2 , $\text{Li}_{1,63}\text{IrB}_2$ und LiPdB

	Li_2RhB_2	LiPdB	$\text{Li}_{1,63}\text{IrB}_2$
Kristallsystem	hexagonal	kubisch	monoklin
Gitterkonstanten [Å]	$a=8,45_5$ $c=4,28_7$	$a=6,75_3$	$a=6,11_6$ $b=10,45_9$ $c=5,50_2$ $\beta=123^\circ 44'$
Raumgruppe			Cc oder $C2/c$
exp. Dichte D_4^{25}	4,34	5,95	7,67
ber. Dichte $D_{\text{R0}}[\text{g}/\text{cm}^3]$	4,33	6,02	7,66
Zahl der Formeleinheiten in der Elementarzelle	5	9	6

feuchtigkeitsempfindlich. Von der Iridium-Verbindung konnten durch 800ständiges Tempern bei 1000°C Einkristalle erhalten werden. In Tabelle 1 sind neben den pyknometrisch bestimmten Dichten die röntgenographischen Daten angegeben, die beim $\text{Li}_{1,63}\text{IrB}_2$ aus Präzessionsaufnahmen, bei den beiden anderen Verbindungen aus Guinier-Aufnahmen ermittelt wurden.

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Staining Autoradiographs with the Fluorochrome "33258"

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Autoradiography, commonly used as a qualitative and quantitative micromethod in cell biology [1], shows several problems in the application of histochemical procedures—fixation and staining—of the cytological specimens. This process is sometimes accompanied by loss of radioactive material [2] or additional artifacts [3].

Recent demonstrations show that staining of lymphocytes or tissue culture cells with a bis-benzimidazole derivative "33258 Hoechst"¹ results in a high specific fluorescence of DNA or of the cell nuclei [4] and offers the possibility of fluorescence photometric DNA measurements in cell nuclei [5].

Because the staining procedure for cell nuclei with "33258" is uncomplicated we tried this method for staining of cells after autoradiography too. We tested the staining effect before stripping film or emulsion was used or through the processed film material. "33258" was used at a concentration of $1\text{--}0.1\text{ }\mu\text{g ml}^{-1}$ at room temperature for 10–20 min in a phosphate-buffered water solution or in tap water. After drying, the autoradiographs could be examined without a coverglass or embedded in

mounting medium in a fluorescence microscope (Zeiss).

Material: Root meristem cells and human lymphocytes.

Stripping film: Kodak AR 10 and Ilford K5 emulsion.

Results: DNA fluorescence of "33258" was shown for plant cells too ([6], Fig. 1). Good results were obtained when "33258" was dissolved at a concentration of $1\text{--}0.5\text{ }\mu\text{g/ml}$ in the final wash water (pH 6.5–7.5) after developing and fixation of the autoradiographs.

There is also a good fluorescence intensity when the specimens were stained before the stripping film or emulsion is used or in a separate staining process through the stripping film after developing and drying the autoradiographs.

Methanol(9%) or ethanol/acetic acid/formaldehyde (89:1:10) fixation results in a higher fluorescence.

For microscopy, embedding in a buffer solution is not necessary. Embedding in immersion oil or mounting medium (Histo-clad) is possible and has a weak influence on fluorescence.

It seemed that a wide range of pH, ionic strength and concentrations of "33258" exist for staining cell nuclei in autoradiographs.

In plant specimens there is a weak fluorescence of the cell walls too (Fig. 1).

Fig. 1. Autoradiograph of a transverse section of plant root meristem (*Triticum*) after ^3H -thymidine treatment ($5\text{ }\mu\text{Ci/ml}$ for 30 min); phase-contrast photomicrograph (a) and in fluorescence microscopy (b) after staining by "33258 Hoechst"; Kodak AR 10 stripping film. As can be seen there is no ^3H -thymidine incorporation in the nucleolus corresponding to an area in the nucleus which shows no fluorescence

These results of a fluorescence technique for staining autoradiographs by "33258 Hoechst" offers some advantages summarized as follows: The procedure is extremely simple in regard to histochemical artifacts; there is no staining of film materials; and there is a very good correlation of DNA-chromatin structure or cell nuclei and silver grains.

For an automation process in quantitative autoradiography [7] it may be possible to find the cells by a scanning process corresponding to their DNA fluorescence followed by a second one in order to measure the silver grains without a background in transmitted light.

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¹ "33258 Hoechst": 2-[2-(4-hydroxyphenyl)-6-benzimidazolyl]-6-(1-methyl-4-piperazolyl)-benzimidazol-trihydrochloride