STUDY OF THE BIOLOGICAL SPECIMEN BY SCINTILLATION BSE AND GASEOUS SE DETECTOR AT VARIABLE PRESSURES IN ESEM

Ing. Jan LINHART, Doctoral Degree Programme (1)
Dept. of Electrical and Electronic Technology, FEEC, BUT
E-mail: xlinha02@stud.feec.vutbr.cz

Ing. Vilém NEDĚLA, Doctoral Degree Programme (4)
Dept. of Electrical and Electronic Technology, FEEC, BUT
E-mail: vilem@isibrno.cz

Supervised by: Prof. Rudolf Autrata

ABSTRACT

This paper deals with the study of the biological specimen imaging in environmental scanning electron microscopy (ESEM). Two detector systems are used: gaseous and scintillation detector. Direct comparative images of biological specimen at variable pressures are confronted with the dependence signal to the pressure.

1 INTRODUCTION

As it is showed in [1], the principle of environmental scanning electron microscopy is in the possibility to investigate specimens in higher pressures in the specimen chamber. There is no negative charge on the surface of nonconductive samples thanks to ionization collisions between electrons and gaseous molecules. For this reason it is possible to study electrical nonconductive specimens in their natural form, without any preparation. The higher pressure in the chamber (up to 611 Pa at 0°C) allows to study specimens containing water.

The main difference between scanning electron microscopy (SEM) and environmental scanning electron microscopy (ESEM) is consequently the size of the pressure in the chamber. While the electron gun is placed in an area with a pressure at least of 10^{-3} Pa, the pressure in the specimen chamber in ESEM can be in orders of units of thousands of Pa. Pressure limiting apertures (PLA) are used to separate the areas with these different pressures in ESEM. These apertures effectively reduce flow of gases between the separated areas in the microscope, arrange separated pumping of the areas with different pressures and, at the some time, allow passage of the primary electron beam to the specimen.

2 MATERIAL AND METHODS

There are many signals generated by the interaction between the primary electron beam and the surface of the sample. Secondary (SE) and backscattered electrons (BSE) are the most important in surface imagining in ESEM.

For the detection of BSE, the scintillation detector in configuration by Fig. 1a can be used. The detector consists of scintillation monocrystal (YAG), light pipe, photomultiplier and preamplifier.

Gaseous ionization detector (fig. 1b) can be used for the SE detection in ESEM. This detector exploits the impact ionization in the gases [1], [2].

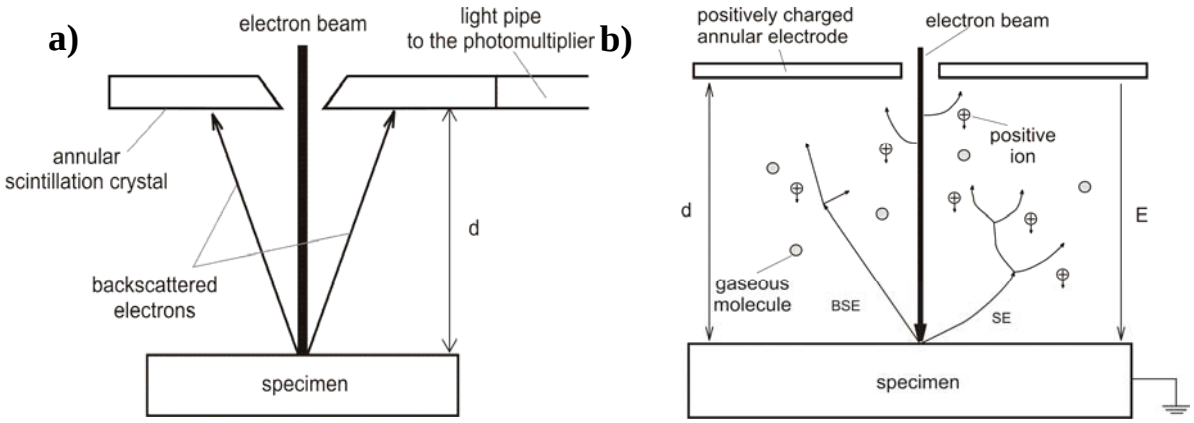

Fig. 1: *a) Schematic diagram of the scintillation detector for BSE detection in ESEM b) Schematic diagram of the gaseous ionization detector for SE detection in ESEM [1]*

3 RESULTS

3.1 COMPARATIVE IMAGES FROM SCINTILLATION AND IONIZATION DETECTOR

For experimental verification of the difference between SE and BSE imaging at a changing pressure (from 50 to 1100 Pa) a biological specimen (a leg of spider) was used. The experiment was carried out using the environmental scanning electron microscope AQUASEM – VEGA [3] in conditions of high pressure in air conditions. The used accelerating voltage of primary electrons was 30 kV, the working distance 3 mm, the current of the beam 280 pA. The gaseous ionization detector electrode voltage was 350 V.

The resultant images are presented in fig. 2. There is always an image from the gaseous ionization detector on the left side and an image from the scintillation detector on the right side.

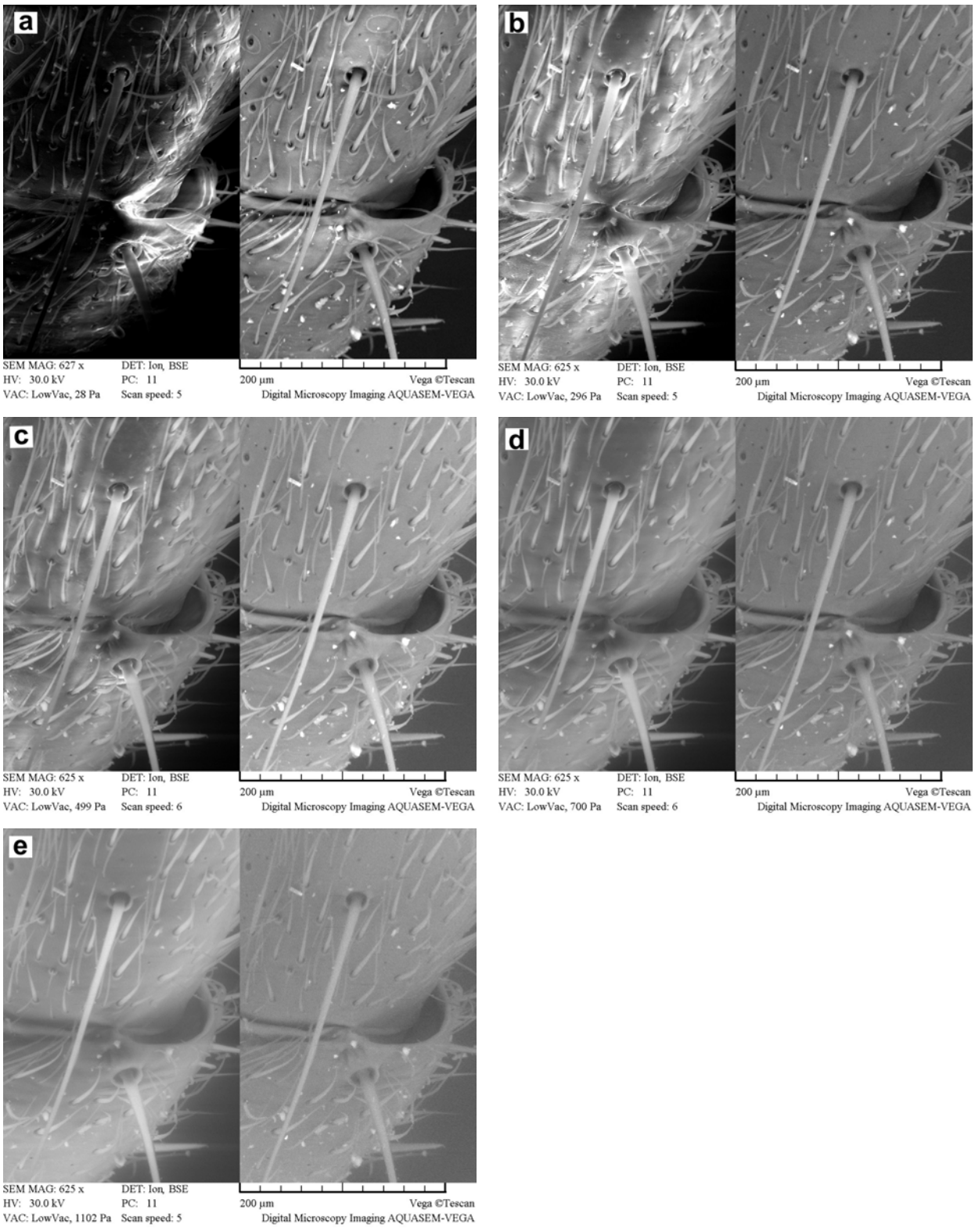

Fig. 2: *Images of biological specimen (leg of the spider) at a changing pressure: a - 30Pa, b - 300 Pa, c – 500 Pa, d - 700 Pa, e – 1100 Pa. There is always image from the gaseous ionization detector on the left side and image from the scintillation detector on the right side*

Predominating influence of SE is very well visible in the image from the gaseous ionization detector. The topography of the specimen surface and typically intensive brightness of the edges can be observed.

On the contrary, the topography of the specimen surface is practically not visible in the images from the scintillation detector. Material contrast (between the leg, hairs and deposited dirt) is included in these images. Influence of BSE electrons is hence outstanding in this case.

The best image of the scintillation detector is at the pressure of 30 Pa (fig. 3a), because there occurs the least number of collisions between BSE and gaseous molecules. Image successive degradation (rise of the noise) is visible at higher pressures (fig. 3b, c, d, and e).

The pressure of 30 Pa is insufficient for the beginning of the impact ionization and signal amplification (fig. 3a). The best image of the gaseous ionization detector is at the pressure of 300 Pa (fig. 3b). There are optimal conditions for the impact ionization and for the sufficient signal amplification for this specimen. The quality of images decreases (the noise rises and contrast gets worse) with increasing pressure (fig. 3c, d, and e).

3.2 BEHAVIOUR OF THE SIGNAL FROM SCINTILLATION AND IONIZATION DETECTOR

In some previous work [3] there was measured (by use of LINESCAN function) the signal from the BSE scintillation and the SE ionization detector behavior depended on the pressure. The testing specimen (gold on the carbon) was used.

It is evidently that the signal from the BSE scintillation detector rapidly degreases with the rising pressure (fig. 3).

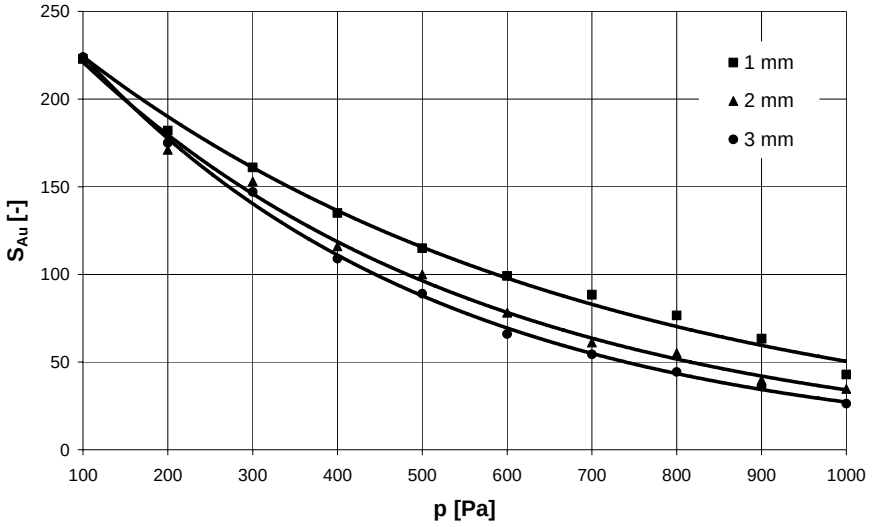

Fig. 3: *Behaviors of the signal from the BSE scintillation detector depended on the pressure in the air condition for working distances 1, 2 and 3mm [3]*

Behavior of the signal from the SE ionization detector is not so simple (fig. 4). The value of the signal rise with rising pressure to the value approximately 200 - 300 Pa and than already degreases. The position of the maximum is given by used condition in the specimen chamber, the working distance and by the voltage on the detector electrode.

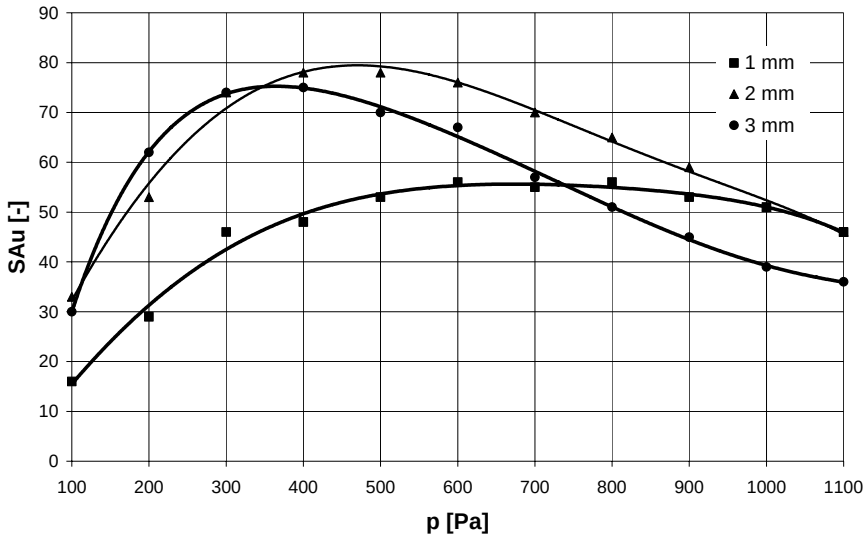

Fig. 4: *Behaviors of the signal from the SE ionization detector depended on the pressure in the air condition, by the working distance 1, 2 and 3 mm and by voltage on the detector electrode 350 V [3]*

4 CONCLUSION

In this work there is compared signal contrast of images of biological specimen from BSE scintillation and SE gaseous ionization detector at variable pressure. It is demonstrated that the quality of this images respond with the previously measured signal to the pressure dependence.

ACKNOWLEDGEMENTS

The paper has been prepared as a part of the solution of GAČR project No. 102/05/0886.

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