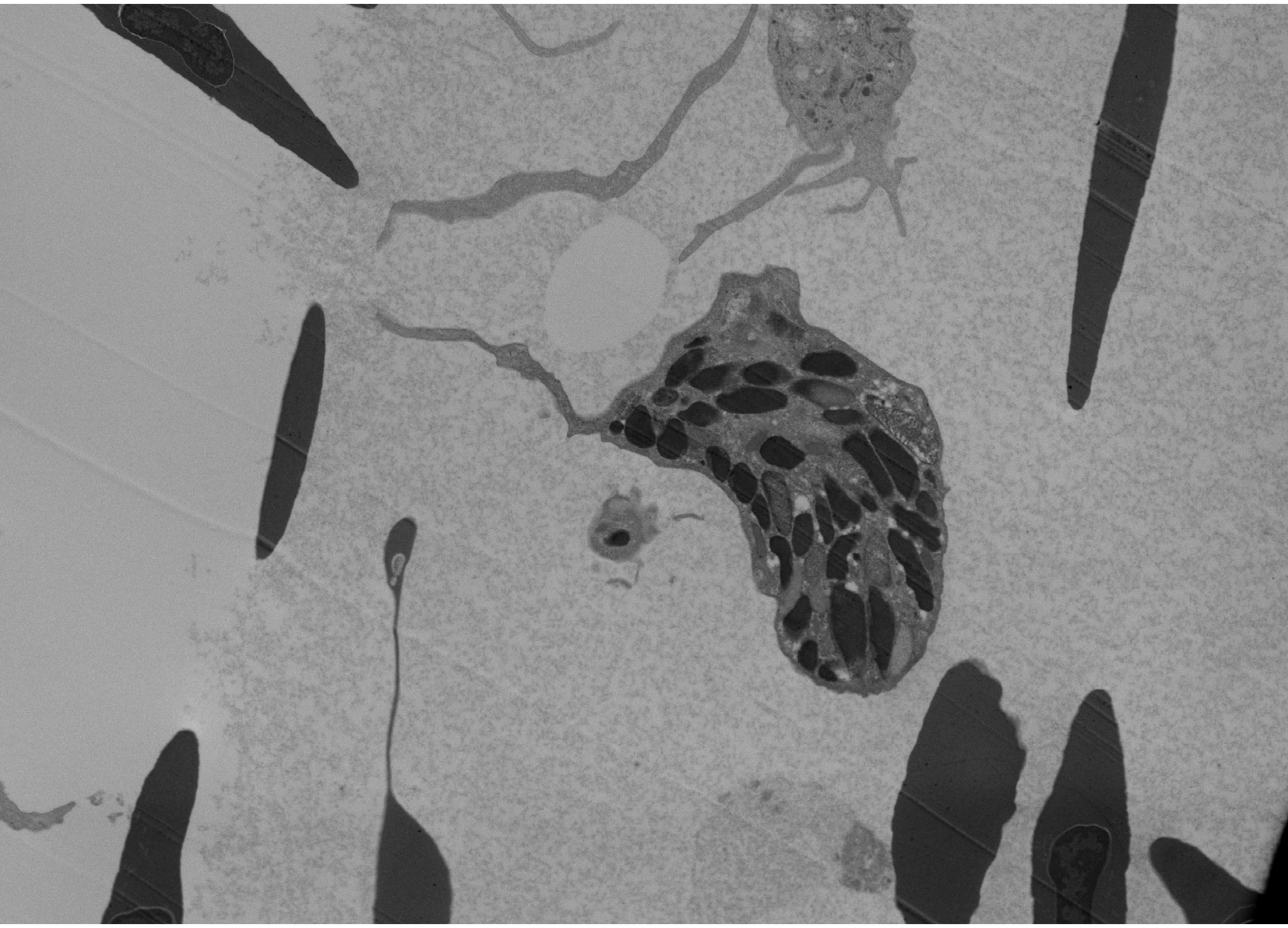
Application Note



Python Blood Analysis by STEM

We make it visible.

Python Blood Analysis by STEM

Authors: William Cooley
AHVLA, UK

Ken Robinson
Carl Zeiss Microscopy Ltd., UK

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Introduction

Conservation of animals in zoological collections is a regular activity for veterinary organisations around the world. An 8 year old male Diamond python developed lower respiratory tract disease and systemic bacterial sepsis which led to the clinical demise of the snake. Prior to the death of the snake a blood sample was taken for hematologic analysis. This revealed a marked leukocytosis which is a condition characterized by an elevated number of white cells in the blood.

Some of the taken blood was centrifuged to concentrate the blood cells. The pellet of cells was prepared conventionally for electron microscopy by fixing with gluteraldehyde, treatment with osmium and embedding in resin. Sections were prepared using an ultramicrotome and contrasted with uranyl acetate and Pb citrate.

In this application note the utility of STEM in imaging thin sections is demonstrated.

Instrumentation
An EVO15 HD SEM fitted with the transmission electron microscope (STEM) attachment was used for these studies of the python blood cells. EVO HD features a very high brightness electron source that provides the benefit of high spatial resolution whilst retaining good signal to noise images.

One specimen thin section was imaged at 30 keV and short working distances using OptiBeam resolution mode in high vacuum.

Figure 1
Diamond python. Photograph courtesy of Pavel German.

The STEM technique is shown in Figure 2. The electron beam is focused onto the thin section and transmitted electrons detected using a Si diode detector. Contrast is developed when different parts of the specimen scatter electrons away from the optical axis. A region of high transmissivity appears white in the image and regions of low transmissivity (high scattering) appear dark.

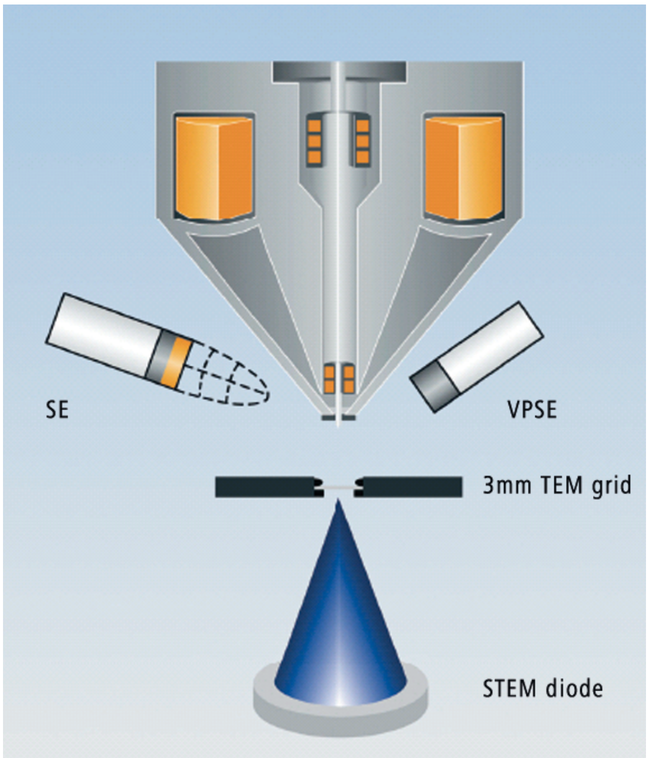

Figure 2
EVO detector positioning. Showing ET, VPSE and STEM detectors.

Biology

STEM was used to examine the leukocytes (white) and erythrocytes (red) blood cells. The red blood cells are responsible for oxygen transport around the body and are the numerous elongated cells with a central nucleus visible in the thin section images.

The white blood cells attack invading organisms. The complex shapes seen in the micrographs reflect their activity at the time the blood was fixed. Many white blood cells cannot divide and reproduce on their own, but instead are produced in the bone marrow.

Image Interpretation

The overview image in Figure 3 shows the supporting Cu grid in black. The thin section itself is mid grey with the numerous red blood cells being slightly darker than the blood plasma.

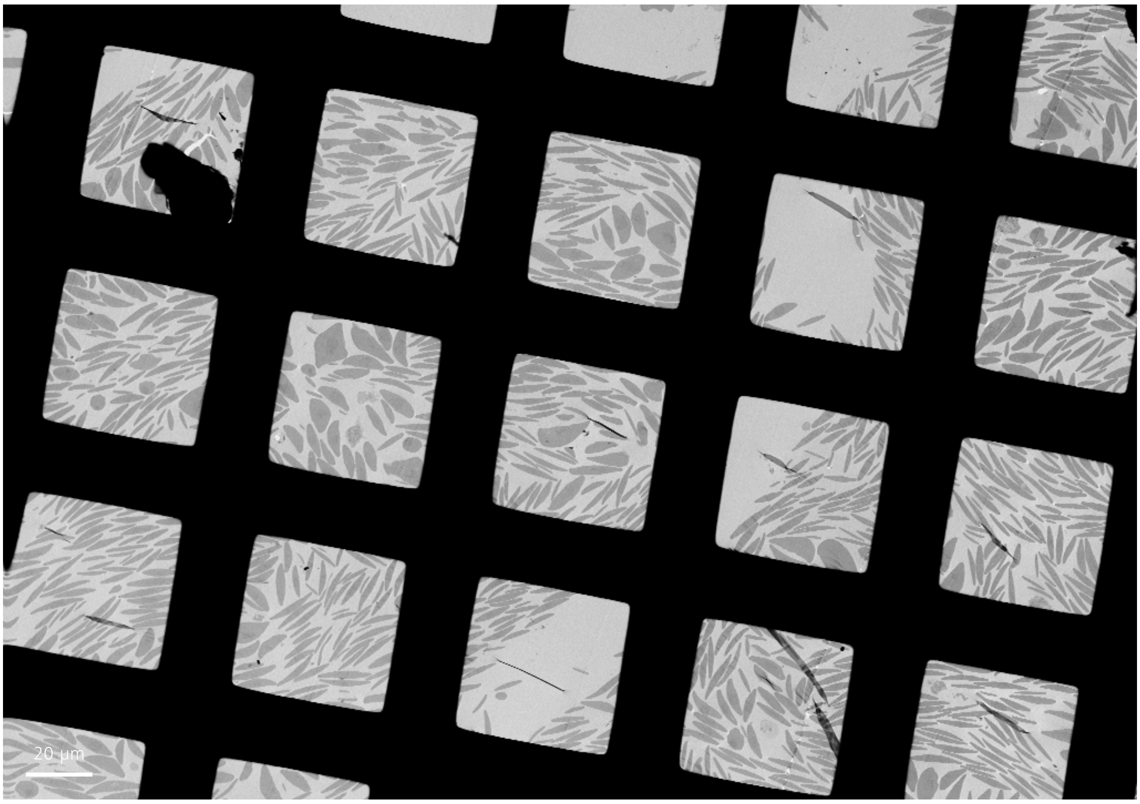

Figure 3
General view of grid with ultra-thin sections of python blood preparation. Beam energy is 30 keV.

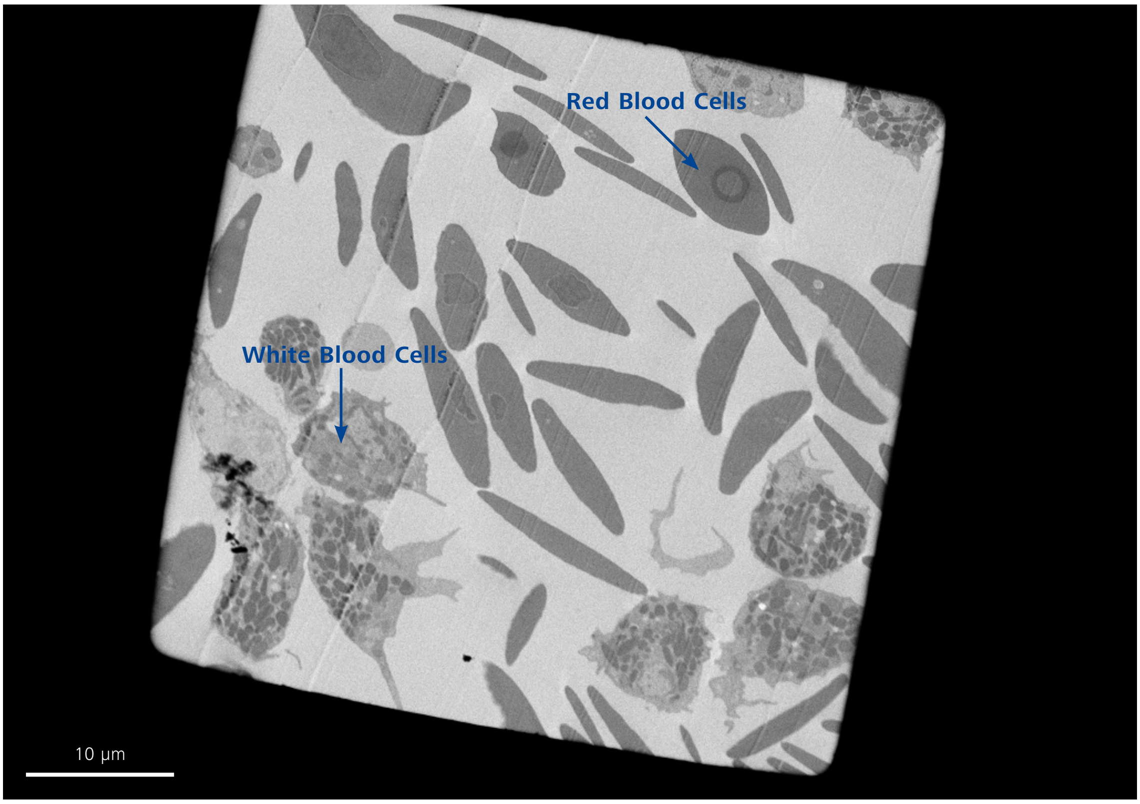

Figure 4
Single grid square, red and white blood cells indicated. Beam energy is 20 keV.

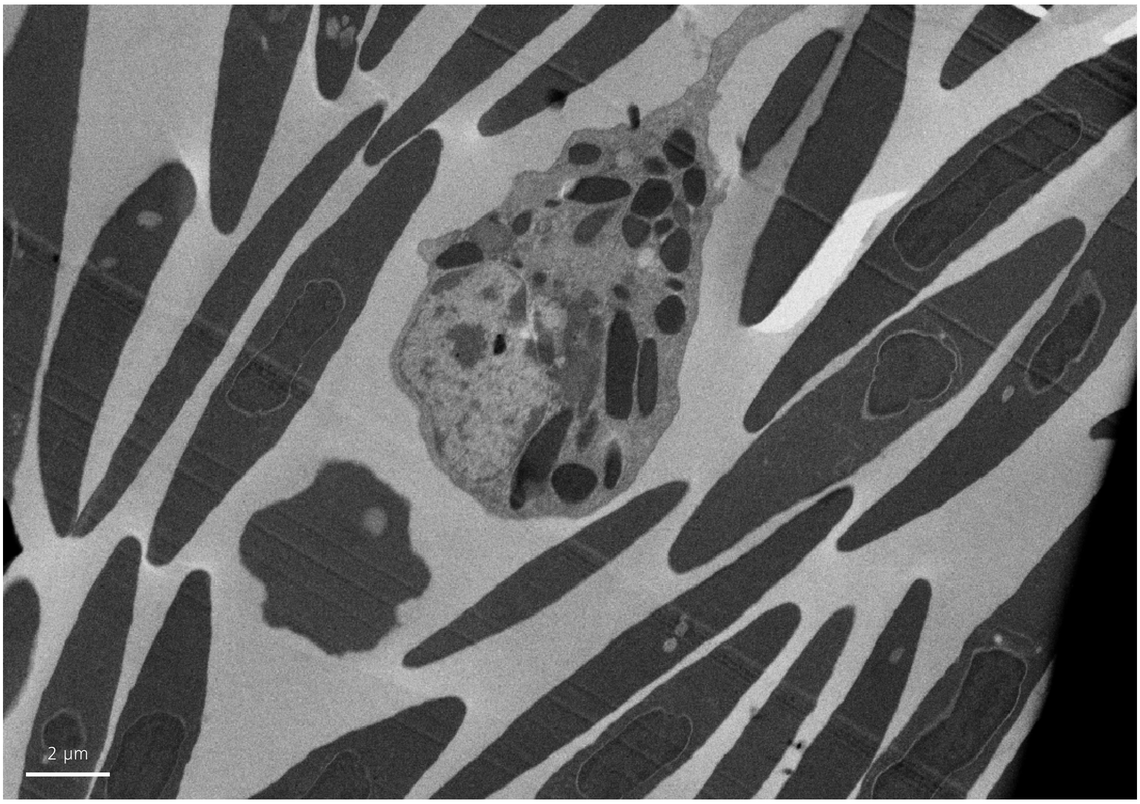

Figure 5
Higher magnification showing cells. Beam energy is 20 keV.

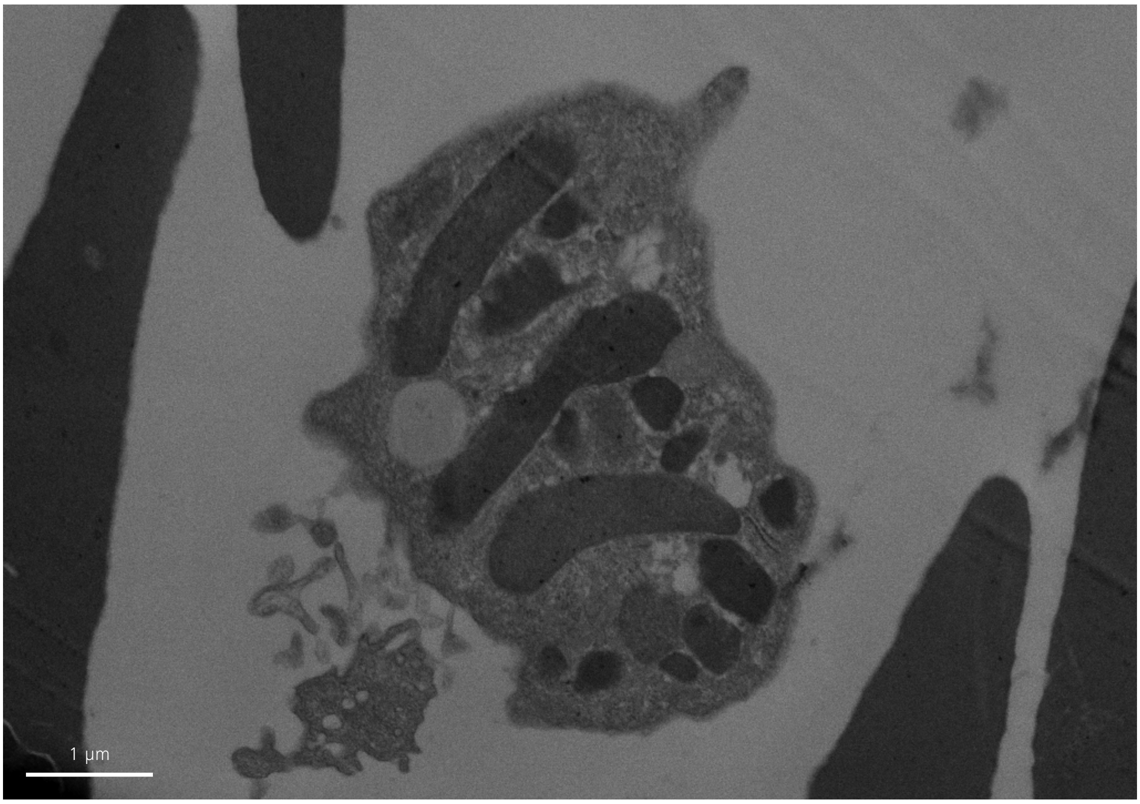

Figure 6
Granulocyte cell. Beam energy is 30 keV.

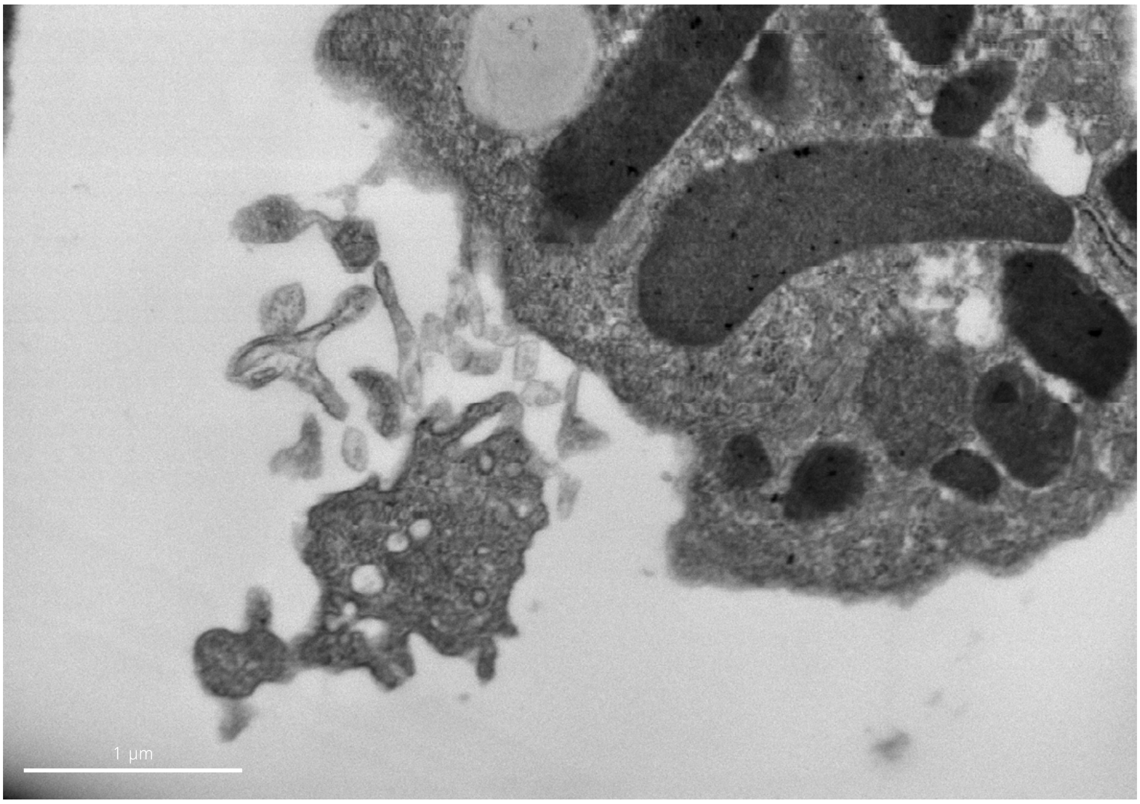

Figure 7
Ultrastructure of Granulocyte cell. Beam energy is 30 keV.

White blood cells are divided into three main classes:

- Granulocytes – which make up 50 % to 60 % of all leukocytes (and are themselves divided into three classes: neutrophils, eosinophils and basophils).
  Granulocytes get their name because they contain granules, and these granules contain different chemicals depending on the type of cell.
- Lymphocytes – which make up 30 % to 40 % of all leukocytes come in two classes: B cells (those that mature in bone marrow) and T cells (those that mature in the thymus).
- Monocytes – which make up 7 % or so of all leukocytes and evolve into macrophages.

The white blood cells, featured in Figures 6 and 7, are of different forms (pleomorphic) containing numerous granules, dark staining inclusions and vacuoles visible in the thin section.

The use of STEM in this application demonstrated that no viral agent was present in the blood cells. STEM was also able to identify the various types of white blood cells present in the blood (Figs 4-7) and confirmed that many of these cells contained dark staining organelles and inclusions of various size. More detailed examination confirmed that some of these dark staining granules contained melanin in the form of melanosomes. These were mainly present in several macrophages (melanomacrophages), and the increased number of these cells may be indicative to the response to infectious disease.

Application Area
Biological STEM imaging

Recommended Instrument Type
EVO Series
4QBSD
STEM detector

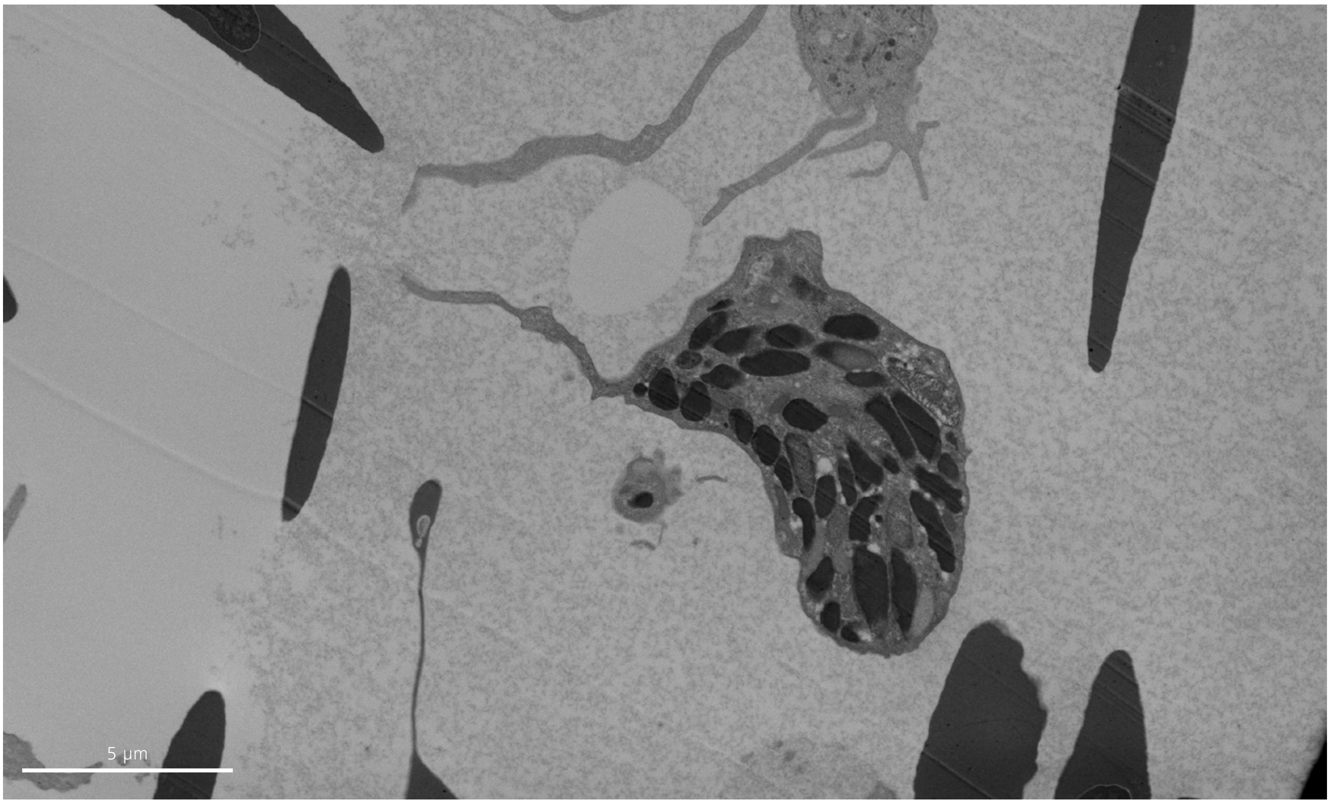

Figure 8
Granulocyte and red blood cells. Beam energy is 30 keV.

Carl Zeiss Microscopy GmbH
BioSciences
microscopy@zeiss.com
www.zeiss.com/microscopy

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