Introduction
This application note presents a brief study of the mechanical properties of red blood cells (RBC) using the Agilent NanoVision nanomechanical microscopy option. NanoVision delivers quantitative imaging by coupling a linear electromagnetic actuation-based indentation head with a closed-loop nanopositioning stage on the Agilent G200 Nano Indenter.

Sample Preparation and Imaging
A blood smear was prepared on a microscope slide and was dried for one hour before testing. The microscope slide was then mounted as a regular sample in an Agilent Nano Indenter G200.

Next, the sample is translated in X and Y under a diamond tip. A small load is applied to the tip in order to keep it in contact with the surface. The height variations recorded by the tip create the topographic image of the sample’s surface. The piezo-actuated sample stage used in the NanoVision option provides high accuracy in lateral positioning (2 nm) and imaging of the samples. A cube-corner diamond tip is utilized to image the surface profile of the sample.

Indentation
Indentation experiments were performed using Continuous Stiffness option (CSM). With the option elastic modulus and hardness are measured as a continuous function of penetration. (Without this option measurement of elastic modulus and hardness can only be achieved at the maximum penetration depth).

Two series of indentations were performed on the red blood cell sample. The first group of four indents was performed on a single red blood cell (Figure 3). The four indents were made at a constant strain rate of 0.2/sec and with a maximum penetration depth of 1000nm. The hardness and modulus measurements

To provide a high degree of accuracy as well as excellent control for the application of the load during imaging, the Agilent Dynamic Contact Module (DCM) indentation head option is also used with the G200.

The image in Figure 1 shows the well-known biconcave disk shape of the red blood cells. A single cell (Figure 2) was selected for the first study of mechanical properties.
(Figures 4 and 5, respectively) present very good repeatability. The substrate effect is detected around 100 nm on the modulus.

The second group of indents was made on several cells. One indent was made at the center of each cell. The eight indents were made at a constant strain rate of 0.2/sec and with a maximum penetration depth of 500 nm. The results for the measurements of hardness and modulus are consistent from cell to cell (Figures 6 and 7, respectively). The indents were imaged via the same technique used previously.

Conclusions
The use of NanoVision in conjunction with a Nano Indenter G200 allows both indentation and imaging of biological materials, such as red blood cells. Measurement of mechanical properties of such materials using this instrumentation yields highly accurate and repeatable data.

Technology and Applications
The Nano Indenter G200 is powered by electromagnetic actuation to achieve unparalleled dynamic range in force and displacement. The instrument’s unique design avoids lateral displacement artifacts, while software compensates fully for any drift in force. The DCMII option offers impressive performance and advantages including 3x higher loading capability (30 mN max load), easy tip exchange for quick removal and installation of application-specific tips, and a full 70 µm range of indenter travel. Using the G200, researchers can measure Young’s modulus and hardness in compliance with ISO 14577. Deformation can be measured over six orders of magnitude (from nanometers to millimeters).

Applications include semiconductor, thin films, and MEMs (wafer applications); hard coatings and DLC films; composite materials, fibers, and polymers; metals and ceramics; and biomaterials and biology.