

Chapter 1 A Complete Guide to Peripheral Blood Film Examination & Hematopoiesis Basics

Mindray Morphology Corner | Series Course-I Common

Hi there, Readers!

Welcome to Mindray's Morphology Corner! We're thrilled to have you join us on this journey into the fascinating world of blood cells. In this series, we'll bridge the gap between what you see under the microscope and what the analyzer shows on screen, making your learning more efficient, systematic, and complete. Let's dive into Chapter 1!

Part 1: Your Step-by-Step Guide to Peripheral Blood Film Examination

A perfect blood film is the cornerstone of morphological analysis. This section guides you through the entire process from preparation to microscopic review.

1.1 The Toolkit for the "Art" of Film Making (Figure 1-4)

- ① The Sample: Fresh venous blood (with EDTA) or from a finger/heel prick.
- ② The Canvas: A clean glass slide (75x25 mm).
- ③ The Spreader: Another slide to smoothly spread the blood drop.
- ④ The Dropper: To place about 4 μ L of blood drop.
- ⑤ You, The Artist: A skilled technician to make the smear.
- ⑥ The Colors: Stains like Wright or Wright-Giemsa.
- ⑦ The Magnifying Glass: A good microscope.
- ⑧ The Interpreter: A morphologist with a sharp eye for cell details.



Figure1

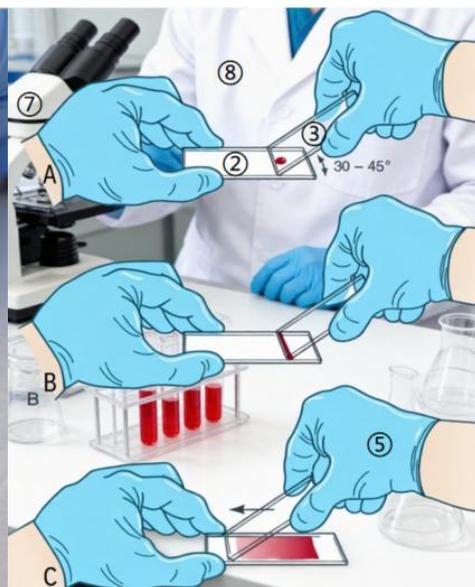


Figure2



Figure3



Figure4

1.2 Crafting the Perfect Blood Film: A Three-Step Process

Schematic of Blood Film Preparation (Figure 2)

- A. **Drop:** Place a small drop of blood approximately 1 cm from the end of the slide.
- B. **Attach:** Hold the spreader slide at a **30-45° angle**. Gently touch the drop, allowing the blood to spread along the edge of the spreader.
- C. **Spread:** Maintaining the angle, push the spreader forward rapidly, smoothly, and evenly to create a film with a thick head and a thin, feathered tail.

1.3 Staining: Bringing Cellular Details to Life (Wright-Giemsa Stain Example, Figure 5-6)

- A. **Dry:** Let the film air-dry completely.
- B. **Fix:** Immerse in absolute methanol for approximately 1 minute.
- C. **Stain:** Transfer to Stain A for approximately 3 minutes.
- D. **Buffer/Rinse:** Transfer to a mixture of Stain B and buffer for approximately 5 minutes.
- E. **Rinse:** Rinse gently by directing a stream of water to the back of the slide. Stand vertically to dry.

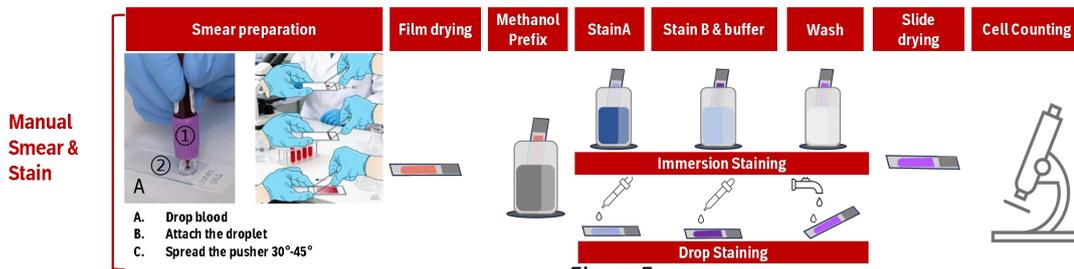


Figure5

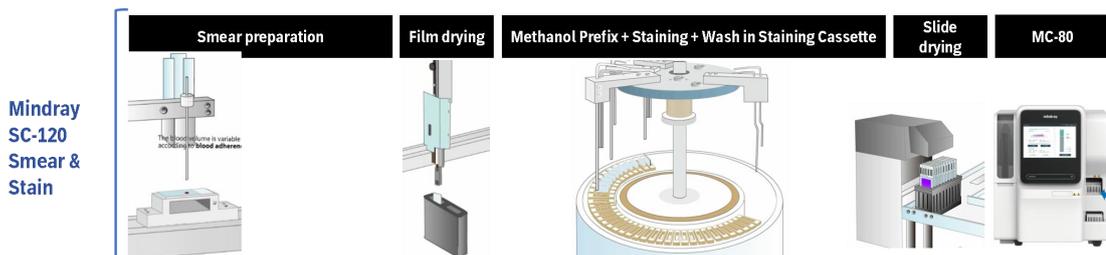


Figure6

1.4 Quality Assessment: Is Your Film and Stain Optimal?

Assessment Area	Ideal Criteria
Film Appearance (Figure 7)	<ol style="list-style-type: none"> 1. The film should cover 2/3 to 3/4 of the slide length. 2. It should end in a smooth "feathered" edge, not a pointy tail. 3. It should have clearly defined lateral edges. 4. It should be smooth, free of streaks, waves, or holes. 5. A rainbow-like sheen should be visible in the thin area when held to light.
Staining Quality (Figure 8)	<ol style="list-style-type: none"> 1. Red Blood Cells (RBCs): Pink to salmon-pink. 2. Nuclei: Dark blue to purple. 3. Neutrophil Granules: Lavender to lilac. 4. Basophil Granules: Dark blue to black. 5. Eosinophil Granules: Bright red to orange. 6. Background: Clean and colorless, free of stain precipitate.

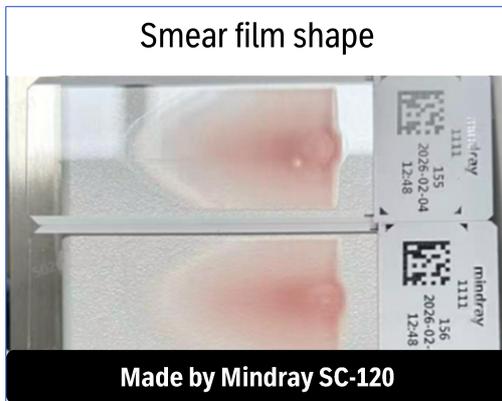


Figure7

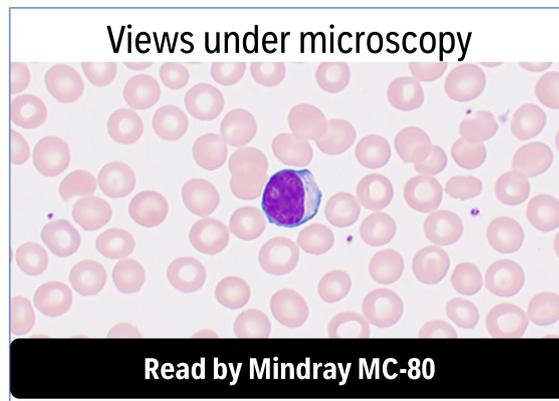


Figure8

1.5 Systematic Microscopic Examination Protocol

Step	Objective	Primary Tasks & Observations
A. Initial Scan	Low Power (10x)	Scan the entire film. Assess RBC distribution, check for Rouleaux formation , and look for any large abnormal cells (often at the edges).
B. Preliminary Assessment	High Power (40x/50x)	In an area where RBCs are just touching, count WBCs in 8-10 fields. Average the count to estimate the total WBC count. Compare with the analyzer result. Investigate significant discrepancies.
C. Detailed Differential	Oil Immersion (100x)	<p>Area Selection: Start in an area where approximately 50% of RBCs overlap and scan systematically to the feathered edge.</p> <p>Differential Count: Identify and classify at least 100¹ (or ideally 200²) white blood cells (e.g., segs, bands, lym, mon)</p> <p>Reporting: Report results as percentages.</p> <p>Other Observations: Note the number of nucleated RBCs per 100 WBCs.</p> <p>Platelet Estimate: Assess platelet number and morphology in 5-10 fields.</p>

Part 2: Hematopoiesis—The "Birth" Journey of Blood Cells

2.1 Hematopoiesis is the process of forming new blood cells in the bone marrow and releasing them into the periphery. Cells undergo predictable changes during maturation. (Figure9)

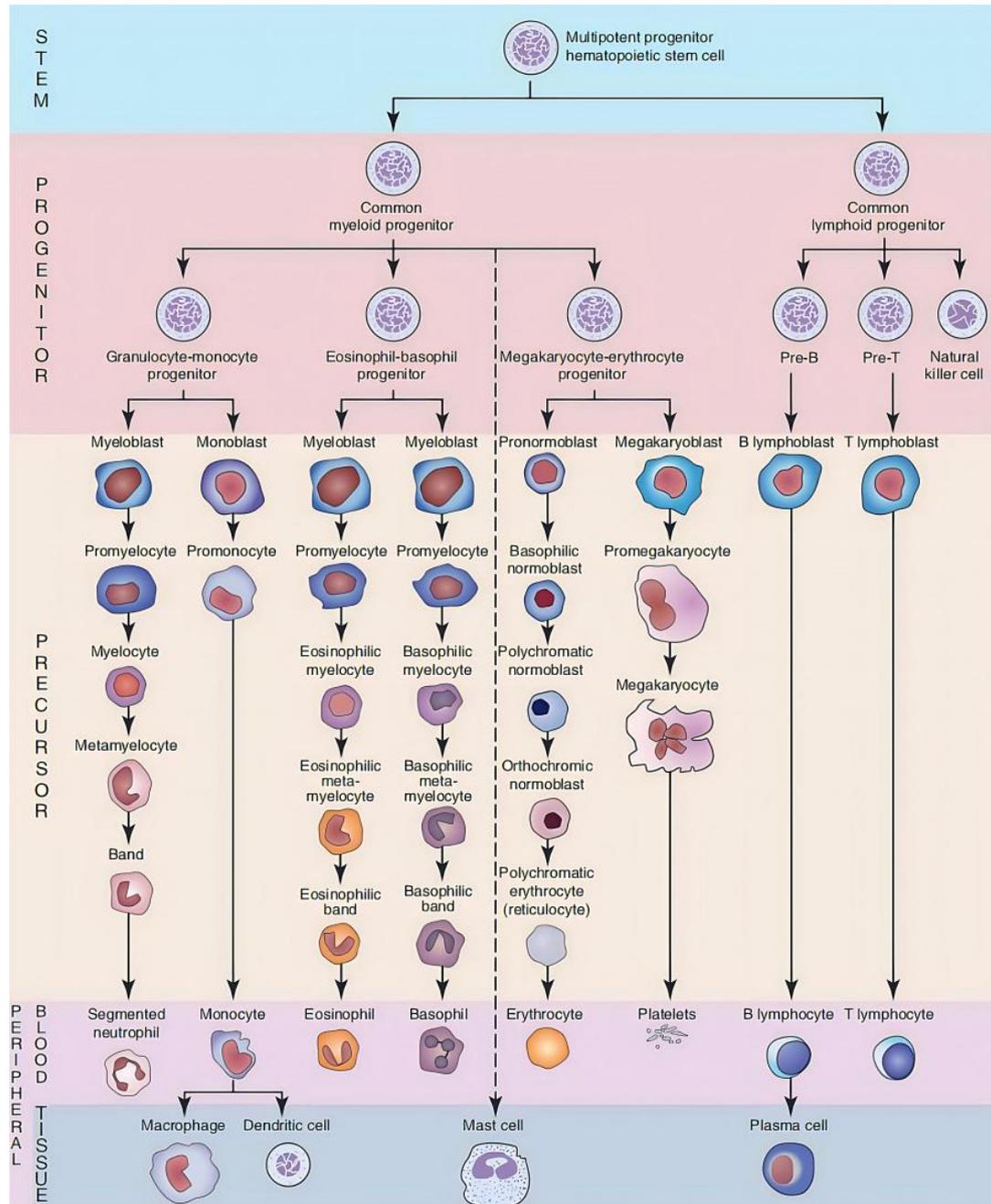


Figure9

Figure 9: The image is from 1. Carr JH. Clinical Hematology Atlas. 6th ed. Elsevier; 2021.

2.2 The Trilogy of Maturation

2.2.1 Cells "Shrink" (Figure 10): Overall cell size generally decreases with maturation. (Note: promyelocyte may be larger than its precursor)

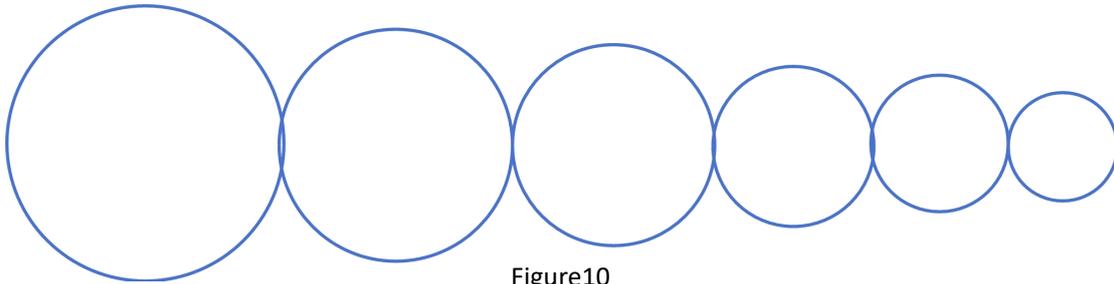


Figure10

2.2.2 Nucleus "Transforms" (Figure 11): The nuclear-to-cytoplasmic (N: C) ratio decreases. Chromatin becomes coarser and more clumped. Nucleoli disappear.

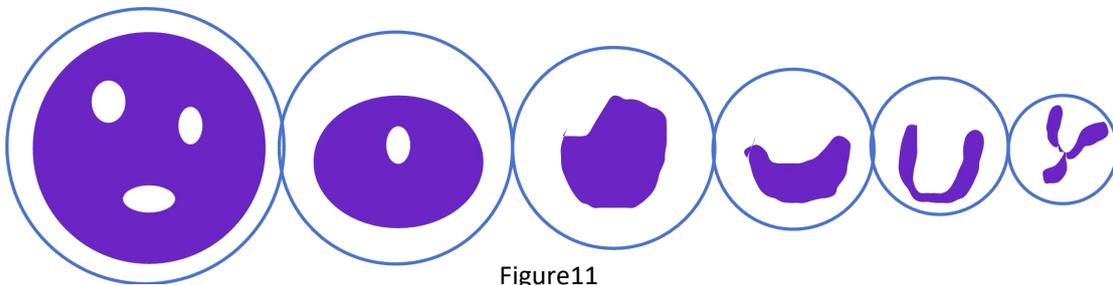


Figure11

2.2.3 Cytoplasm "Gains Color" (Figure 12- Wright-Giemsa stain): Erythroid precursors synthesize hemoglobin (cytoplasm becomes pink/red). Granulocyte precursors develop specific secondary granules.

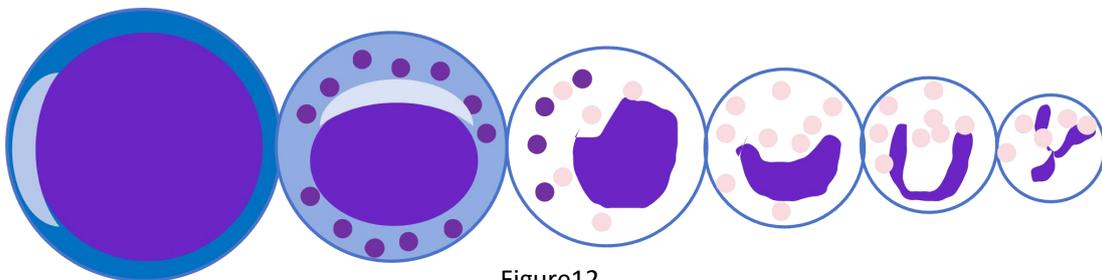


Figure12

2.3 The "Workshops" in the Cellular "Factory" (Organelles)

2.3.1 Under routine microscopy, we primarily observe:

2.3.1.1 Nucleus: The "command center" containing DNA.

2.3.1.2 Nucleolus: Inside the nucleus; the site of ribosomal RNA synthesis ("parts production").

2.3.1.3 Golgi Apparatus: Often appears as a perinuclear clear area (hof) on Wright-Giemsa stains, functioning as the "packaging and shipping department."

2.3.1.4 Other organelles like the endoplasmic reticulum, mitochondria, and lysosomes typically require electron microscopy for clear visualization.

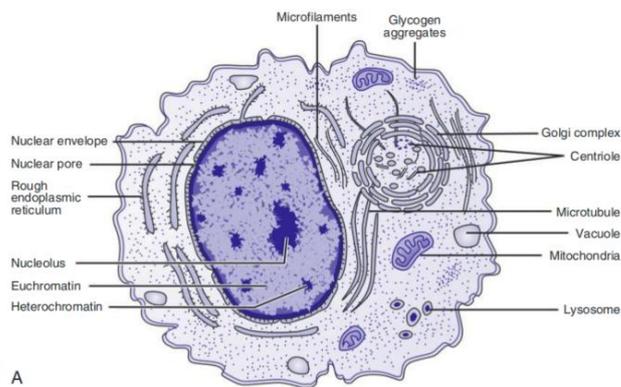


Figure13

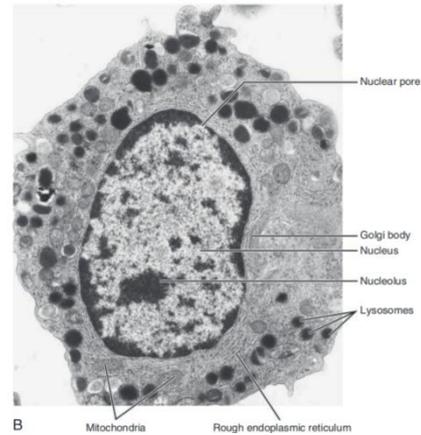


Figure14

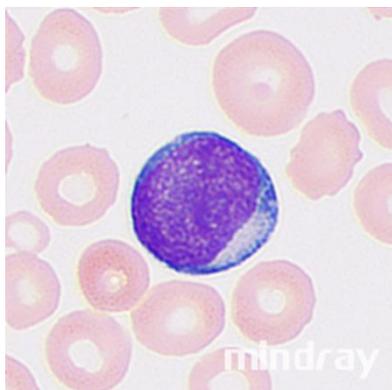


Figure15-1

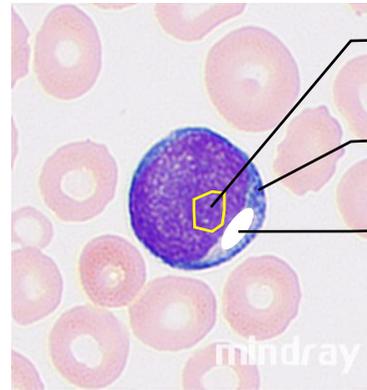


Figure15-2

- ① Nucleolus
- ② Rough endoplasmic reticulum
- ③ Golgi body

2.3.2 Schematic (Figure 13)

[A: Organelle diagram]

2.3.3 Electron Micrograph (Figure 14)

[B: Actual EM image]

2.3.4 Light Micrograph (Wright-Giemsa) (Figure 15)

[Figure 15-1: Original Blast cell image]

[Figure 15-2: Copied Blast cell image]

[Tips]:

- ① Nucleolus;
 - ② Dark blue areas represent rough endoplasmic reticulum (with ribosomes);
 - ③ Perinuclear hof represents the Golgi area;
- Other organelles are not clearly visible with routine stains.

Part 3: Interactive Discussion—How Does Your Lab Scan the Film?

Did you know? Laboratories around the world follow different scanning patterns. There is no single "right" way, often based on convention or standard.

3.1 "Y-Direction" Scan (Vertical): As shown in some classic atlases, starting at the tail and moving vertically. (Figure 16: Indiana Atlas scanning path diagram)

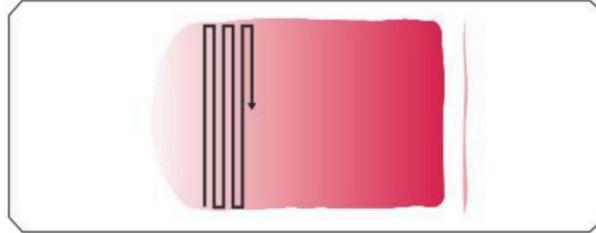


Figure16

3.2 "X-Direction" Scan (Horizontal): As recommended by the CLSI H20-A2 standard, starting at the tail and moving horizontally. (Figure 17)

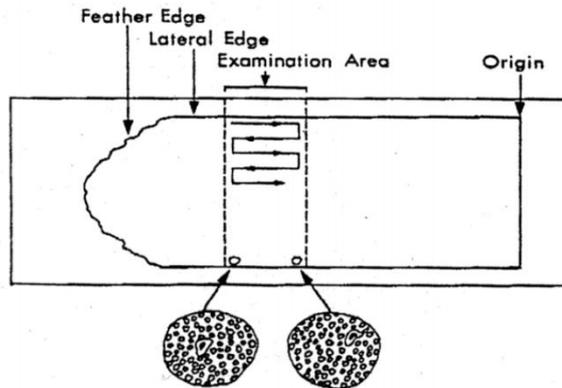


Figure17

3.3 "Y-Direction" Scan (Body to Tail): As described in the Chinese industry standard WS/T 246-2005, starting from the "body" (area with ~50% RBC overlap) towards the tail³. (Figure 18)

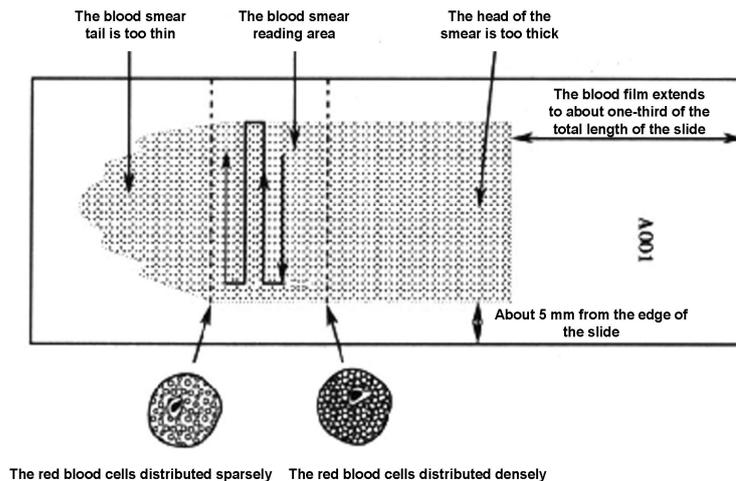


Figure18

We'd love to know: Which scanning method is commonly used in your laboratory or country? Share your practical experience!

Key Summary

- (1) A qualified blood film is fundamental for morphological diagnosis. Its preparation, staining, and review must follow a standardized protocol.
- (2) During maturation, blood cells follow the basic pattern of "nuclear condensation, increased cytoplasm, and decreased cell size."
- (3) A systematic and consistent microscopic scanning path is crucial for the accuracy and reproducibility of differential counts.

Think About It

- (1) If you observe a large number of abnormally clustered cells at the edge of the film under low power, what might this indicate? What should be the next step?
- (2) During hematopoiesis, what biological processes do the changes in the nucleus and cytoplasm primarily reflect?

References & Image Sources**References:**

1. Carr JH. Clinical Hematology Atlas. 6th ed. Elsevier; 2021.
2. Clinical and Laboratory Standards Institute (CLSI). Reference Leukocyte (WBC) Differential Count (Proportional) and Evaluation of Instrumental Methods; Approved Standard-Second Edition (H20-A2). CLSI; 2007.
3. Chinese Health Industry Standard. WS/T 246-2005: Reference Method for Leukocyte Differential Count. National Health Commission of the People's Republic of China; 2005.

Images & Technical Support: All morphology images in this course are derived from analysis by the Mindray automated digital morphology analyzer **MC-80**. Blood films were prepared by the Mindray automated slide maker and stainer **SC-120**.

— *Coming Next: **Normal Myeloid Development and Morphology*** —