

# Theory Training Workbook Digital Morphology (DI-60/DM-Series)



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#### Disclaimer

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# **Revision History**

Revised section	Alteration	Name	Date
New Document	New document to replace the following documents: - DM-96 Onsite Training Workbook - DM1200 Onsite Training Workbook - DI-60 Onsite Training Workbook	J Hammersley	September 2020

#### **Reference Documents**

Document title	Version	Date
CellaVision Review Software – Instructions for use 7.0	PM-10892-01	27 May 2019
CellaVision DM1200 – Instructions for Use Software 7.0	PM-10835-01	01 June 2018



# Digital Morphology Analyser Overview

Digital morphology analysers are used for the precalssification/reclassification of cells in peripheral blood films and body fluid cytospin smears. DM-Series analysers are standalone analysers whereas the DI-60 can be standalone or connected to a Sysmex automated slide maker and stainer such as SP-10 or SP-50 using a CF-60 or CF-70 unit respectively, allowing full automation of smear workflow. Through the 'Remote Review Software' there is the ability for collaboration and remote viewing by users from other locations. Databases on the analyser allow permanent electronic images of the slides to be stored.

Facts and Figures			
Analysers	DI-60	DM9600	DM1200
Modes	Peripheral Blood Mode (F Body Fluid Mode (BF)	PB)	
<ul><li>Throughput</li><li>Peripheral Blood Application Processing Database</li></ul>	30 slides per hour (RBC/	PLT/100-WBC)	20 slides per hour (RBC/PLT/100-WBC)
Slide storage capacity	CF-60 - 120 CF-70 - 50	96	12
Objectives	10x and 100x		
Intermediate optics	0.5x and 1.0x		
Magnifications	5x, 10x, 50x and 100x		
Quality control	Cell location (PB and BF mode) Smear checker (PB mode only)		
Databases	Processing database Export Database Scan Database		
Slide requirements	75.7 - 76.3 x 25.7 - 26.3 x Ground edge slides with Cut corners	x 0.9 - 1.2 frosted end	
Optional Applications	Advanced RBC Application Body Fluid Application Veterinary Software	on	



# **Principles of Analysis**

### Step 1: Determination of Analysis Area

Slide analysis starts with the determination of the analysis area using the 10x magnification (10x objective and 1x intermediate optics). The 10x objective moves from a fixed start point, 33 mm from the non-frosted end of the slide, down the central line of the slide towards the tail end of the smear. The motorised microscope takes continuous images, analysing the number of RBC contours and the average size of the cell. Once set criteria have been fulfilled the analyser knows where to perform WBC analysis (WBC monolayer). The RBC/PLT analysis area has a predetermined start point set from within the WBC analysis area and is where at a later stage the RBC/PLT monolayer is created and RBC precharacterisation is performed.



Because of the fixed start point 33 mm from the end of the slide the quality of slide analysed is critical. Slides that are too short, too long or have the wrong start point will cause total failure of analysis or incomplete analysis, respectively, due to insufficient monolayer. The quality of the slide produced can be examined using SmearChecker software.





# Step 2: Locating Nucleated Cell Coordinates

Following the identification of the WBC monolayer the coordinates of the nucleated cells are obtained at 10x magnification (10x objective and 1x intermediate optics).



Nucleated cells are located by battlement tracking using 10x objective in the pre-defined monolayer. The coordinates of 3 times as many nucleated cells than required are stored. For example, for a 100 cell WBC differential, 300 nucleated cells coordinates are located and stored.



# Step 3: Determination of RBC/PLT Monolayer

Following determination of the nucleated cells coordinates the RBC/PLT monolayer is created.



The RBC/PLT monolayer is created using 50x magnification (100x objective and 0.5x intermediate optics). The 100x objective moves using battlement tracking, towards the thinner part of the film, continuously taking images. The RBC/PLT monolayer is created by stitching together 35 images taken at 50x magnification giving an overview image of the entire area.

#### Step 4: Pre-classification of RBC



Cells within the RBC/PLT monolayer are evaluated for colour, size and shape. They are then classified into 6 RBC characteristics according to mathematical algorithms:

- Polychromasia
- Hypochromia
- Anisocytosis
- Microcytosis
- Macrocytosis
- Poikilocytosis



### Step 5: WBC Image Capture

Following analysis of the RBC/PLT monolayer the analyser performs WBC image capture using a magnification of 100x (100x objective and 1x intermediate optics).



The digital microscope obtains the WBC images using 100x magnification by going back to the nucleated cell coordinates stored following identification of the WBC monolayer, during which continuous focus is used to maintain image quality. Once images are captured, they are automatically enhanced and compressed to bring out the maximum detail.

### Step 6: WBC Pre-classification

As soon as individual WBC images are captured cell pre-classification is performed. Cell preclassification is achieved using an Artificial Neural Network (ANN). Microscope images are transformed into electrical signals using the red, green, and blue light signals produced by the stain used. These signals are used to identify over more than 300 different features. The cytoplasmic and nuclear features for each cell are then processed through the ANN, where algorithms compare the features identified to the standard values determine for each cell type. Once the desired number cells have been preclassified no further WBC images will be obtained and the smear results will be ready for review.





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