Sysmex® DI-60™ Automated Digital Cell Morphology Analyzer

Principle

The Sysmex DI-60 is an automated digital cell locating device intended to aid morphologists in the location and classification of white blood cells and non-WBC's such as NRBC, the characterization of red cell morphology and estimation of platelets in peripheral blood. Monolayer smears are made and stained on the Sysmex SP-10™, or by manual methods, transferred in cassettes via the Sysmex CF-60™ (cassette feeder) to the DI-60 where slides are scanned one at a time. Using the 10X objective, XY coordinates of potential nucleated cells are located in the optimal monolayer area and the positions are recorded. Oil is applied to the slide and images are obtained through 50X magnification for red cell morphology. Each recorded 10X position is then examined using the 100X oil immersion objective to capture WBC images. The cells are analyzed by the Artificial Neural Network (ANN) and assigned a pre-classification. The operator reviews images to confirm or modify suggested classification of cell types.

For body fluids, cytocentrifuge smears are prepared, stained and placed in cassettes on the CF-60 to be transferred to the DI-60 for analysis. The DI-60 locates and classifies white blood cells and other nucleated cells in the same manner as for peripheral blood. The operator reviews images to confirm or modify suggested classification of cell types. An overview image of the entire sample area is displayed for additional review.

Safety

All specimens, reagents and controls should be handled as though capable of transmitting infectious diseases. Wear appropriate personal protective equipment when running patient samples or performing scheduled maintenance. Refer to: Policy and Procedures Safety Manual Infection Control and Procedures 11-085-01.

Reagents and Supplies

Lens Paper
Isopropyl Alcohol
CellaVision ER barcode labels
CellaVision QC barcode labels
SP-10 slide cassettes designated for re-use on CF-60/DI-60
Immersion Oil Packs (Refractive index 1.5150. Viscosity 300 cSt. PCB free)
Microscope slides, frosted with rounded/clipped corners
(76 x 26 mm; 0.9 – 1.2 mm thick)

Slide Preparation

Peripheral Blood Slides

1. Prepare peripheral blood smears using the Sysmex SP-10, manual wedge technique or mechanical spreader.

   For a good smear:

   - There is no pooling of specimen at the point of application.
   - Both sides of the film are less than 5mm from the edges of the slide.
   - The feathered edge is relatively straight and not pointed.
   - There must not be any streaks, troughs, ridges, holes or bubbles.
   - The blood film must be at least 30 mm in length and terminate 5-15 mm from the end**.**
   - The smear must not be too thick. A thick smear will interfere with the DI-60's ability to find a monolayer and with the Artificial Neural Network which may result in a large number of misclassified WBC's.

   Refer to DI-60 Instructions for Use, Appendix G for examples of good smears.

2. Smears made on the SP-10 will be stained automatically. Smears prepared manually should be stained using the manual mode of the SP-10.
   - For appropriate cell classification, stain must be free of precipitate.
   - Good pre-classification requires that PMN's have dark-stained nucleus and pink cytoplasm.

3. Slides require barcodes to process on the DI-60.

Procedure

A. Start Up

Start up the DI-60

1. Switch on the Slide Scanning Unit (SSU) using the switch located on the front of the unit.
2. Switch on the system computer.
3. Wait until the red status lamp stops flashing and is off.
4. In the Log-on dialog box, type assigned Username and Password.
5. Select the appropriate database from the drop-down menu.
6. Click OK.
7. **System Control** view displays. Make certain the start-up test passes.
   - System self-tests are performed during start-up that detect potential hardware or software problems. The DI-60 will not process slides if the start-up test fails.

Procedure, continued

Start up the CF-60 with the SP-10

1. Place an empty slide storage magazine in the magazine supply unit of the CF-60.
2. Start the SP-10.

   Note: When the SP-10 starts up, the CF-60 also starts. The status indicator lights green when the CF-60 is ready.

Start up the CF-60 without the SP-10

1. Place an empty magazine in the magazine supply unit of the CF-60.
2. Press the startup switch. The status indicator lights green when the CF-60 is ready.

B. Slide Processing - Peripheral Blood

1. Slides prepared on the SP-10 are sent to the DI-60 for processing.
   a. Slide cassettes with stained slides are transferred from the SP-10 into the cassette supply conveyor of the CF-60.
   b. A slide is removed from a cassette by the gripper and inserted into the shuttle.
   c. The shuttle moves the slide to the DI-60 where the gripper removes it and the system checks for a readable barcode.
      - If the barcode cannot be read, it is displayed as “ERR + date and time” (ERRYYYYMMDDhhmmss), in the *System Control Log* and *Order List* of the ***Database*** view.
      - An image is taken of the barcode and displayed in *Order Data*.
   d. The slide is analyzed on the DI-60.
      - ***System Control*** view displays ongoing slide processing.
      - The *System Control Log*, located in the upper left of the ***System Control*** view, shows the processing status for each batch and slide.
      - In ***Database*** view, “Analyzing” displays at the top of the screen.
   e. Following analysis, the slide is inserted into the shuttle then into a slide storage magazine.

2. Process manually prepared slides.
   a. Insert the stained slide into a slide cassette with the barcode facing the Sysmex logo.
   b. Place the cassette with the Sysmex logo facing forward into the CF-60 cassette supply unit.
      - Processing of the slide takes place the same as for automatic process.

Procedure, continued

3. Reanalyze a slide on DI-60.
   a. Prior to reanalyzing a slide on the DI-60, wipe the oil off.
   b. Place the slide in a cassette designated for re-use.
   c. Place the cassette in the cassette supply unit of the CF-60.
   NOTE: A cassette that has been re-used on the DI-60 should never be used on the SP-10. Oil in the cassette may contaminate the SP-10.

C. Slide Review – Peripheral Blood

- Slides that are ready for review display in the ***Database*** view.
- An unopened order is in black text. Open orders display in blue text. Slides being reviewed by another user at a Remote Review Station display in red.
- Double-click on a slide/order to open the ***Verification*** view screen to review the images. WBC, RBC and PLT images are divided by tabs at the top of the screen.

WBC REVIEW

- Cells can be viewed in different formats.
  - The “Full Screen” view displays all cells grouped by pre-classification.
  - The galleries display 1, 2, or 3 classes of cells in side-by-side format. In the gallery fields, select the cell type to view using a drop-down at the top of the field. When viewing in the gallery format, a WBC panel displays to the left with a list of all WBC and Non-WBC parameters. Check marks beside a parameter indicate that required review was performed.
  - A library of reference cells is available for different cell classes. To view in gallery 2 or 3, select the checkbox “Reference cells”. Use the drop-down to select the reference cell type.
- Double clicking on a cell enlarges it. Use the mouse wheel to zoom in and out.
- All cell classes must be viewed prior to signing a slide.
- All “unidentified” cells must be classified.
- Demographic information, hemogram, auto differential and flags display on the far lower left of the screen.

1. Reclassification of WBC’s:
   a. Left click on the cell and drag it to the correct classification in a gallery or to the cell name in the WBC or Non-WBC panel to the left of the gallery.
   b. Right click on a cell and select the appropriate classification from a drop-down menu.
   c. To reclassify a grouping of cells, click on the first cell in the group, hold down the **shift** key and click on the last cell of the group; this marks the entire group. Click on the group and drag it to a classification or right click to reclassify with the drop-down menu.

Procedure, continued

d. To reclassify non-consecutive multiple cells, hold down the **ctrl** key while clicking on each cell. Once all cells are marked, click on a cell to drag all cells to the appropriate classification or right click to display the drop-down menu, and select the appropriate classification.

e. To split cells: If more than one cell appears in an image, click on the **Cell Marker** button to display a green box around the cells in each image. Right click on the image and select **Split Cell** from the drop-down menu. Click on the unmarked cell in the box. Two pictures of the same image display. For each image, classify the cell that is marked by the red X.

2. Confirm Cell Counter:

   a. Review cell classifications in full screen view for abnormal cells.

   b. If no abnormal cells are found, review cell counter (automated) differential displayed in the Patient Data field on the left side of the screen.

   c. Click **Confirm Cell Counter** to accept the automated differential results.

3. Adding Comments:

   a. WBC comments can be added for each slide. Click on the Comment icon next to the comment box to open the field.

      - Enter a free-text comment in the comment field **OR**
      - Select from the list of standard comments. To add a standard comment, highlight the comment and double click, or click "**Append**".

   b. Comments can be added to a specific cell.

      - Right clicking on a cell opens a field that allows a free-text cell-specific comment to be added.
      - Cell-specific comments will not display on the report.

RBC REVIEW

- The RBC panel is composed of 8 - 100X fields.

1. If there is no significant morphology, select "**Report all as O-Normal"**.
2. Red cell morphology can be graded 1+ to 3+ by selecting **"Use Characterization"** and selecting the appropriate radio buttons.
3. The Zoom feature can be used to enlarge the image by one of the following methods:

   a. Click on the magnifying glass icon (**Zoom Mode**) with +/- signs. Hold down the left mouse and move up or down on the image. Moving up zooms in; moving down zooms out.

Procedure, continued

b. "Zoom In" by clicking on the magnifying glass icon with a "+" sign. By clicking on the icon 5 times, an image equal to a 100X field displays. "Zoom Out" is identified by a magnifying glass with a "-" sign. Return to full view by clicking on "**Entire RBC Image**" icon.

4. Navigation within an image can be performed by using the **Scroll** (Hand) icon or by using the scroll bars on the bottom and right side of the screen. To use the Scroll mode, click on the Hand icon. Place the hand on the image and hold down the left side of the mouse to move the image from side to side and up/down.

5. Adding Comments:

   a. Click on the Comment icon.
   b. Enter a free-text comment or select from the list of standard comments.

6. Select **Exclude RBC Analysis** on the bottom of the screen if no RBC review is required.

PLATELET REVIEW

- The PLT image corresponds to 8 – 100X fields.
- Gridlines can be added to aid in estimation by clicking **Help Lines** icon. These do not correspond to the grid squares used for PLT estimate entry.

There are two (2) methods for PLT estimation. A PLT estimate factor is determined during validation and is entered in the PLT settings.

Counting Platelets in the Overview Image:

a. Count PLTs per grid square:

   1. Select the "**Count PLTs per grid square**" radio button.
   2. Select each field individually and enter the number of platelets counted in that field. Press **tab** to access each entry field until all grids have been viewed.

b. Enter approximate PLT count per grid square.

   1. Select the "**Approximate PLTs per grid square**:" radio button.
   2. Tab between entry fields to view grid squares.
   3. Estimate the average PLT count per grid square and enter the value in the field.

c. Click "**Calculate PLT Result**" to obtain results following action "a" or "b" above.

Procedure, continued

Two (2) options are available to report the PLT results calculated from the number of PLTs per HPF:

1. Select "**Calculated estimate**" to report a number. Average PLTs/HPF are multiplied by the PLT estimate factor determined during the validation of the DI-60 and entered in settings. **OR**
2. Select "**Calculated level**" to report "significantly decreased", "decreased", "normal" or "increased". The level is determined by multiplying the estimate by the estimate factor.

NOTE: The User may override the calculated PLT results by selecting **Manual Level** and selecting one of the four levels.

Estimating the Platelet concentration level (Manual):

a. Use the entry fields in the PLT image overview to estimate the PLT's in each grid.
b. Select the concentration level from the drop-down menu

Adding Comments:

a. Click on the Comment icon.
b. Enter a free-text comment or select from the list of Standard Comments.

Quality Control

Perform QC Cell Location – Peripheral Blood.

1. Select a blood sample with a WBC count greater than $7x10^3/\mu L$ to reduce the processing time.
2. Prepare a slide in the manual mode of the SP-10.
   a. Select **Manua**l from the Menu screen.
   b. Enter the sample ID
   c. For **Op. Mode** select "SmSt/SP" to send the stained slide to the cassette output area of the SP-10.
3. Remove the cassette from the output area and label the slide with a QC label.
4. Place the cassette on the Cassette Supply conveyor of CF-60 for processing. The slide is scanned using the same method used to collect images for patient samples.
5. Once processing is complete, open the **Tools** menu and select **Cell Location**. Select the new slide at the top of the list. (Cell location results automatically delete after 5 days.)
6. Review each image for any missed nucleated cells. Double-click an area for magnification if necessary

Quality Control, continued

- Green boxes mark nucleated cells. The cell does **NOT** have to be completely inside the box. As long as there is a box associated with a cell, it indicates the system found the cell.
- Blue boxes mark artifacts or other objects. The number of these objects must not exceed 30%.
- Missed cells are those not marked with ANY box.
- Black boxes mark cells not needed in the 200 cell process for cell location.

7. Review all images by clicking the right arrow. For each image, enter the number of missed cells in the input field. When all images have been examined, the result will appear as a '%'.
8. Record results on the appropriate log. Results may also be printed by clicking "**Print Result**".

Reporting Results

A. Once all tabs have been viewed, select the **Sign Slide** tab.

- All cell classes on the WBC tab must be viewed in order to sign the slide.
- All "Unidentified" images on the WBC tab must be reclassified.
  1. The "**Sign Slide**" dialog displays if all ordered analyses have been viewed.
  2. Enter Username and Password.
  3. If not already selected (as default Settings), select "Sign order when signing slide", "Send to LIS" and/or "Print order".
     NOTE: The option to "Sign order when signing slide" is not available if the slide is part of a multi-slide order; all slides must be signed before the order can be signed.

B. To merge multiple slides from an order, click the "Slide Merge" tab in "Report View". Results from all slides in the order display.

- Click in the checkbox next to a slide ID to include it in the results of the order. If a slide is excluded, a dialog displays to enter explanation of exclusion.

C. Barcode errors on slides can be edited in *Order Data* and reported to the LIS.

1. Prior to reviewing the slide, click on the *Order Data* icon. An image of the barcode is displayed.
2. Edit the Order ID (at the top left of the dialog box) with the correct number.
3. When the order/slide is signed, the results will be sent to the LIS.

Maintenance

A. Daily Maintenance
   1. Shut down the CF-60 with the SP-10
      a. Shut down the SP-10.
      b. The CF-60 shuts down after the magazine in the tower is ejected.

      NOTE: If the CF-60 or DI-60 is in operation when the SP is shut down, the CF-60 will not shut down automatically.

   2. Shut down the CF-60 independently.
      a. Ensure that the CF-60 status light is green.
      b. Hold down the startup switch on the front of the CF-60 for 2 seconds.
      c. The CF-60 shuts down after the magazine in the tower is ejected.

B. Weekly Maintenance
   1. Clean the Objectives and LED table.
      a. Open the hood of the DI-60.
      b. Gently clean the objectives and LED table with lens paper.

         NOTE: Take care not to get oil on the 10x objective. Use a different piece of lens paper to clean each objective.
      c. Use isopropyl alcohol when needed.

         NOTE: Bubbles, which can affect image quality, may form on the objectives when cleaned with alcohol. It is suggested to run 2 slides after the maintenance and then delete those slides from the database.
   2. Delete Unsigned Orders.
      a. In the ***Database*** view, select orders/slides for which the “Order Status” field is empty or there is a “Failed” indicator in the “Process Status” column.
      b. Click “Delete” at the bottom of the Database View for the selected orders/slides.
   3. Clean Bottom Plate.
      a. Pull out bottom plate from lower rear of DI-60 and wipe clean of any immersion oil. Perform only when the DI-60 is not in operation.
   4. Shut down the DI-60.
      a. Select Exit in the File menu on the computer.
      b. Press ctrl/alt/delete.
      c. Select Shutdown.
      d. Switch off the Slide Scanning Unit (SSU) using the switch on the front of the unit.

Maintenance

C. As Needed Maintenance
   1. Change Immersion Oil Pack
      a. Open the DI-60 hood.
      b. Place a blue clip on the oil hose.
      c. Push down on the oil hose connection and pull out the hose.
      d. Change the oil pack and connect the hose.
      e. Remove the clip from the hose.
      f. Go to *Maintenance/Oil*.
      g. Click **Reset Oil Drop Counter.**

   2. Clean Slide Storage Magazines
      a. Clean slide storage magazines with a neutral detergent (dish soap) when they become dirty with oil.

   3. Remove slide cassettes from the CF-60 storage conveyor. A maximum of 90 cassettes can be stored on the conveyor.
      a. Cassettes will be cleaned for re-use on the SP-10.
      b. Cassettes designated for re-use on DI-60, should never be used on the SP-10.

Trouble-shooting

For comprehensive information on troubleshooting, refer to the Troubleshooting sections of the DI-60 Instructions for Use and/or CF-60 Instructions for Use.

References:

1. Sysmex DI-60 *Instructions for Use*, Sysmex Corporation, Kobe, Japan
2. Sysmex CF-60 *Instructions for Use*, Sysmex Corporation, Kobe, Japan
3. Sysmex SP-10 *Instructions for Use*, Sysmex Corporation, Kobe, Japan

Document History Page

Change type: New, Major, Minor etc.	Changes Made to SOP – describe	Name of responsible person/date	Med. Dir. Reviewed/ Date	Lab Manager reviewed/ date	Date change Implemented
New	Procedure for new Cellavision DI-60.	Julius Salomon, 7/1/17			