

DOSSIER TECHNIQUE

SPECTROPHOTOMÈTRE BECKMAN DU 640

Comporte les documents suivants :

- Caractéristiques filtre interférentiel Ealing :
- Caractéristiques filtres de densité Melles Griot:
- Généralités sur les spectrophotomètres :
- Configuration du Beckmann
- Schéma optique DU640 :
- Spécifications techniques DU640 :
- Résumé notice :
- Principe du "blanc" :
- Modes d'acquisition des données :
- Spectrophotomètre DU64 :

page 2 page 3 page 4 page 5 page 5 page 6 page 7 et 8 page 9 page 10 à 15 page 13 à 15



cadre 1 : Transmission du filtre interférentiel Ealing réf. 03FIM006.





cadre 2 : Filtre de densité optique Melles Griot.



cadre 3.

cadre 5

Un spectrophotomètre comprend 4 parties essentielles :

A.1. Source lumineuse

Elle est constituée par :

- Une lampe à décharge au deutérium utilisée dans le domaine de 190 à 400 nm avec un maximum d'émission à 652.1 nm (voir cadre 15).
- Une lampe à filament de tungstène pour la région allant de 350 à 800 nm (cadre 16).
- Parfois une lampe à décharge au xénon utilisée dans le domaine UV et visible. Ce type de lampe est très énergétique. Elle fonctionne sous forme de flash, juste au moment de faire une mesure.

A.2. Monochromateur

L'élément de base est un prisme, un réseau ou un filtre coloré. Le rôle du monochromateur est d'isoler le rayonnement sur lequel on fait la mesure. Il est composé principalement d'un système dispersif, d'une fente d'entrée et d'une fente de sortie (cadre 17).

A.3. Cuve

Elle contient soit l'échantillon, soit la référence. La longueur de la cuve est définie (1, 2, 4 ou 5 cm de trajet optique). Elle doit être transparente aux radiations d'étude. Par exemple en UV, les cuves sont en quartz, elles ne peuvent ni être en verre, ni en plastique.

A.4. Détecteur

A.4.1. Photodiode (semi-conducteur)

Lorsqu'un photon rencontre un semi-conducteur, il peut transférer un électron de la bande de valence (niveau énergétique bas) vers la bande de conduction (niveau énergétique haut) en créant une paire électron-trou. Le nombre de paires électron-trou est fonction de la quantité de lumière reçue par le semi-conducteur qui peut donc être utilisé en tant que détecteur optique.

A.4.2. Barette CCD linéaire

L'emploi d'une barette de diodes permet une mesure simultanée sur toute l'étendue du spectre. Une barette CCD est un alignement de photodiodes de petites dimensions (14 x 14 µm) qui fonctionnent en intégrateur de lumière. La charge qui apparaît dans une photodiode est proportionnelle à l'exposition, c'est à dire au produit de l'éclairement par le remps de pose et elle dépend de la longueur d'onde. A la fin de la pose, le contenu des capteurs est transféré dans un registre analogique à décalage et une nouvelle pose commence. Ce registre transmet les données mémorisées en mode série, c'est à dire l'une après l'autre à un rythme fixé par l'électronique de commande de la barette CCD. Ces données apparaissent sous forme

de grandeur électrique. Couplé à un ordinateur, le spectrophotomètre permet de tracer très rapidement des spectres d'absorption ou transmission. Le logiciel gère le temps de pose du capteur CCD.

A.4.3. Photomultiplicateur

Une radiation (voir cadre 19) incidente arrache un électron de la cathode par effet photoélectrique. Cet électron est alors accéléré vers une seconde électrode appelée dynode portée à un potentiel supérieur. L'énergie de l'électron incident est suffisante pour arracher plusieurs autres électrons et ainsi de suite, d'où l'effet multiplicatif. Pour un électron arraché sur la cathode on peut récupérer une centaine d'électrons sur l'anode.



cadre 6.











cadre 4.





C. Configuration Beckmann

cadre 8 : Schéma optique du DU640.



cadre 9 : Configuration de la partie communication du Beckmann

D. Caractéristiques techniques du DU640

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<u>.</u>	TECHNICAL SPECIFICATIONS
13.1 Performance Specifications	3
Scan Speeds	120, 240, 600, 1200, 2400 nm/min
Data Collection Rate	20 samplings/second
Response Time	0.05 second
Wavelength Range	190 to 1100 nm
Wavelength Accuracy at 656.1 nm Full Range	±0.2 nm ±0.5 nm
Wavelength Repeatability at 656.1 nm Full Range	±0.1 nm ±0.2 nm
Spectral Bandwidth (from 200 to 680 nm)	≤1.8 nm
Photometric Readout	-0.300 to 3.000 A or 0.0 to 200.0 %T
Photometric Accuracy (at 1 A with NIST 930D solid filter at 546 nm)	±0.005 A
RMS Noise (0A, average of 10 standard deviations of 10 readings at 0.05 sec intervals at 500 nm)	≤0.0002 A rms ¹ / ≤0.0005 A rms

Stray Light (measured using NaI at 220 nm per ASTM E387-84)	<0.05%
Stability (0A, constant ambient conditions, measured for 1 hour at 340 nm)	<0.003 A
Baseline Flatness (from 200 to 900 nm, at 0 A)	±0.001 A rms ² / ±0.003 A rms
13.2 Physical and Environmental Sp	pecifications
Width	69 cm (27 inches)
Height	58 cm (23 inches)
Depth	53 cm (21 inches)
Weight	33 kg (73 lbs)
Line Voltage	100/120V±10% or 220/240V±10%
Frequency	50/60 Hz
Power	200 watts typical
Ambient Temperature Operating Range	+15 to 40°C (59 to 104°F)
Humidity	<85% maximum relative humidity, not to exceed 32.5°C WBT

cadre 10 : Spécifications techniques.

cadre 11 : Spécifications techniques.



E WAVELENGTH SCAN	Use the Wavelength Scan mode to scan a sample in absorbance from 350 to 700 nm at 600 nm/min. Automatically scale the data and find the peaks. To set up the parameters:	1. Verify that the Main window is displayed.	2. Verify that the visible source is on.	 Click on "WAVELENGTH SCAN" to display the wave- length Scan window. 	 4. The following parameters should be listed near the top of the Wavelength Scan window. To change any parameter, the Wavelength Scan window. To change any parameter, 	lick on the alshayed value to display an input window. Input the desired value, then click on [OK] to remove the window.	Scan directory: VIEW Start wl: 330 nm	End wl: 700 nm to Overlay scans: [No]	ck Autoprint: [No] ay Autosave: [No]	d, Scans per sample: 1 Interval: 10.0 [sec]	Method name: A:\UEFAULI Autosave name: [A:\]SCANS	of Sampling device: None r, Scan speed: 1200 nm/min	W. 5. Click on <function></function> to display the Function window.	Click on to darken the poixes in front of solar and reak Pick". Click on $\langle Exit>$ to remove the Function window.	To analyze the samples:	Click on Explanation	Insert blank or clear path (air blank).	< BLANK>> Read and store the blank scan.	Insert sample.	ReadSamples> Read and display the sample scan.	<autoscale> Automatically scale the data. (absorbance limits) Change the absorbance limits, if desired.</autoscale>	s. <print> Print the window.</print>	ay <saveclear> Display the Save Clear window. To</saveclear>	store the scard, citox, on the fire name to display the apphanumeric keypad. Input de accessed fire access displayed.	[OK], [OK], to store the data.	To <quit> Display the Quit window. Click on</quit>		10
FIXED WAVELENGT	Use the Fixed Wavelength Scan to make absorbance readin at 230, 260, 280 and 320 nm. To set up the parameters:	 Verify that the Main window is displayed. Verify that the UV source is on. 	3. Click on "FIXED WAVELENGTH" to display the Fix	Wavelength window.	 cuted on transmeters > to display une transmeters windo To set up the desired parameters, the first four rows in t table should be the following: 	Wavelength Factor Units Use 230 1.000 ms/ml (Yes)	260 1.000 mg/ml Yes 280 1.000 mg/ml Yes 320 1.000 mg/ml Yes	To change a parameter, click on the displayed value	display an input window. Input the desired value, then cli on [OK] to remove the window. (Any information m	appear in the other rows, except that [No] should be in t "Use" column.) When the desired information is displaye	click $\omega < Fxit > to remove the Parameters window.$	5. The following parameters should be listed near the top the Fixed Wuvelength window. To change a parameter	click on the displayed value to display an input windo Input the desired value, then click on [OK] to remove t	window.	Results file: A:\WORK RES Read average filme: 0.50 sec Read mode: [Abs]	Method name: A:\DEFAULT Sampling device: None	To analyze the sumples:	Click on Explanation	Insert blank or clear path (air blank).	< <biank>> Read and store the blank readings.</biank>	Insert sample.	<pre><kendsamples> Rcad and display the sample reading</kendsamples></pre>	↔. ← Slide the display horizontally to displ	data at all wavelengths.	<pre><pre>Print the window.</pre></pre>	SaveClear> Display the Save Clear window. store the data, click on the file name	display the applantments keypar. In the desired file name, then click [OK] to store the data	<quit> Display the Quit window. Click [OK] to exit the mode.</quit>
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cadre 12 : Résumé notice DU640.

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cadre 13 : Résumé notice DU640.





F. Réalisation d'un "blanc"

A blank is always required before data collection; any reading taken without a blank is invalid. A blank reading is taken when **< BLANK> >** (located in the permanent menu bar on the bottom of the window) is clicked on.

NOTICE

In the RediRead Mode the blank command is **<ReadBlank>**. In the RediScan Mode, the blank command is **<ScanBlank>**.

When the instrument blanks, the following steps are performed:

- 1. The monochromator is moved to the proper wavelength. This is the specified wavelength for a single wavelength reading.
- 2. The proper detector gain value is selected automatically. This minimizes the noise level and maximizes photometrie accuracy.
- 3. Dark current is measured and corrected. This compensation assures accurate readings at high absorbance.
- 4. In the Wavelength Scan mode, only, a background scan is made. The blank (or reference) is automatically scanned over the same range at the same speed that the samples will be scanned, so that the background correction is optimal.

This calibration assures repeatable readings every time the instrument is used.

In all modes, a blank solution should be in the sample compartment during the blank. It is suggested that the solvent used to prepare the samples be used for the blank. However, air (no sample) may be used. A new blank reading should be taken each time the solvent is changed.

NOTICE

Plastic cuvettes, glass (Pyrex) cuvettes, and some solvents have significant absorption in the UV region. Verify that they transmit UV light by scanning them versus air before using them in the UV region.

To re-zero the instrument at any time between samples, insert the same blank solution and click on < <BLANK> >.

The instrument stores the blank and uses it until either the sources are turned off or another blank reading is taken. For best results, the instrument should be blanked frequently, allowing the blank reading to be taken shortly before the sample measurement is taken. A new blank should be read if the instrument has not been used for an hour.



G. Modes d'acquisition des données

The DU Series 600 Spectrophotometer has five data collection modes: RediRead Mode, RediScan Mode, Fixed Wavelength, Wavelength Scan and Kinetics/Time.

G.1. RediRead Mode

The RediRead Window is used to take fixed wavelengths readings at one or more wavelengths quickly and easily. This window can be displayed whenever the instrument is not collecting data, regardless of the operating mode of the instrument. Data collected in this mode cannot be stored.

- Click on < <RediRead> >, located in the permanent menu bar at the bottom of the display, to display the RediRead window, *figure 1*.
- 2) Sets the parameters :
 - a) Click on the wavelength value displayed and input the desired wavelength.
 - b) Click on "**Read avg time**" and input the desired read average time.
 - c) Verify that the desired reading mode is displayed, [Abs] or [%T]. Click on the mode to change it.
- Place a cuvette of solvent in the cell holder and click on <ReadBlank>. (If the instrument has previously been blanked at the selected wavelength using <
 <BLANK> >, it is not necessary to blank in the Redi-Read mode. <ReadBlank> in the RediRead mode does not affect the blank stored using < <BLANK> >.)

RediRead ReadSample	ReadBlank	Print	HELP Exit
500.0nm 0.12	78 A	-	
Read avg time Sample Wave	e: 0.50 length	Read Mode: Reading	[Abs]
1 500.1 2 500.1 3 500.1 4 500.1 5	90m 90m 90m 90n 90n	0.0109 A 0.0481 A 0.1091 A 0.1278 A	
	1.		

- 4) Place a cuvette of sample solution in the cell holder and click on **<ReadSample>**. The reading is displayed figure 1 : RediRead Window.
- 5) Repeat step 4 for all samples. The parameter input in step 2 can be changed at any time.

Readings from 11 samples are displayed on the window. When the sample 12 is read, the data is written over the data for sample 1.

- 6) To print the window, click on **<Print>**. Only the data that are displayed are printed.
- 7) To remove the RediRead window, click on **<Exit>**.

G.2. RediScan Mode

The RediScan window is used to make a wavelength scan at 1200 nm/min on a sample with minimum parameter setup. <u>Data collected using this window cannot be stored</u>; the Wavelength Scan Mode must be used for data storage.

- 1. Click on < <**RediScan>** >, located in the permanent menu bar at the bottom of the display, to display the RediScan window, *figure 2*.
- 2. Verify that the proper ordinate label is displayed, [Abs] or [%T]. Click on the label to change it.
- 3. Verify that the desired wavelength limits are displayed. To change them, click on the displayed value and input the desired value. The sample will be scanned over the displayed wavelength range, only.
- Place a cuvette of solvent in the cell holder and click <ScanBlank>. (If the instrument has previously been blanked in the Wavelength Scan mode at 1200 nm/min over the selected range, it is not necessary to blank in the RediScan mode).
- 5. Place a cuvette of sample solution in the cell holder and click on **<ScanSample>**. The scan data is displayed.
- 6. The following functions are available to reformat the data :
 - a) The data can be autoscaled by clicking on <AutoScale>.
 - b) Individual axis limit values can be changed by clocking on them and inputting the desired value.
- 7. To display the wavelength and ordinate readings at any point i the spectrum, click on **<Trace>**. Then move the mouse to the point of interest in the spectrum and click on the center mouse button to place a vertical line on the spectrum. The values at the place where the vertical line is placed are displayed in the lower right-hand



side of the window. To move the vertical line to eighter the right or left, click on the right or left mouse button, respectively.

- 8. To annotate the data, click on <Annotate>. Then click on the graph to position a cross and input information from the alphanumeric keypad or keyboard. Up to four annotations can be placed on the graph. The annotations are printed with the window, but are not stored with the data.
- 9. To print the wavelength scan in the window, click on <Print>.
- 10. Repeat steps 5 to 8 for all the samples.
- 11.To remove the RediScan window, click on <Exit>.

G.3. Fixed Wavelength

The Fixed Wavelength mode is used to collect data from a series of samples at up to 12 wave- figure 2 : RediScan Window. lengths. The data can be multiplied by user-input fac-

tor(s) to calculate a result at each wavelength. Any of the sampling devices can be used to simplify sample handling. Data can be stored for later recall.

To select the analysis parameters :

- 1. With the Main window displayed, click on "FIXED WAVELENGTH" to display the Fixed Wavelength window, figure 3.
- 2. Click on **<Parametres>** to display the Parameters window, figure 4.
 - a) Listed in the Parameters window are 12 wavelength values, with a factor and units that correspond to each wavelength. To change any of these values, click on the displayed value to display a keypad. Input the desired value on the keypad, then click on [OK] to accept the input and remove the keypad.
 - b) The fourth column in the Parameters window is the "Use" column. Each wavelength that is to be used

in the analysis must have a "Yes" displayed. If a "No" is displayed for a desired wavelength, click on the "No" to disply a "Yes".

- c) When all the desired values are displayed, click on the <Exit> to remove the Parameters window. The input values are immediately displayed on the Fixed Wavelength window.
- 3. Readings can be taken either absorbance or transmittance. The selection is displayed following "Read mode" in the parameter listing near the top of the window. To change the read mode, click on the displayed option.



eadSamples	Method	Param	eters	Sa	veClear	Print	Qu
Results file: Read average t	A:\FIXED1 ine: 0.50	Read mode	: [Abs]	Method n Sampling	ane: A:\FI) device: No	(ED)ne	٠
Sample ID	λ Factor	350.0 56.00	λ Factor	440.0 230.0	λ 52 Factor	8.0 6.500	
	Abs	Result mg/ml	Abs	Result ng/ml	Abs F	Result ng/nl	
12	0.2790	15.6221 0 20.4213 0	.1535	35.2955	0.3152	2.0490	
43F 43T 46J	0.6413	35.9108 0 58.5056 0	.2421	55.6869	0.6864	4.4613	
48K 7	8.9504	53.2240 0	.3767	86.6482	1.3800 8	3.9698	

figure 3 : Fixed Wavelength Window.

Fixed Waveleng ClearAll	ith: Para Print	meters	Exit
Wavelength	Factor	Units	Use
350.0 440.0 520.0 200.0 400.0 250.0 300.0 550.0 600.0 650.0 700.0 750.0	56.00 230.0 6.500 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000 1.000	ng/n} ng/n] ng/n] ng/n] ng/n] ng/n] ng/n] ng/n] ng/n]	[Yes] [Yes] [No] [No] [No] [No] [No] [No] [No] [No

figure 4 : Parameters Window.



To take readings :

- 1. Place a cuvette of solvent in the instrument. Click on < <BLANK> >.
- 2. If desired, click on the next displayed sample number and input up to an 11-gigit alphanumeric sample identification. If a sample identification is not input, the instrument numbers the samples consecutively.
- 3. Place a cuvette of sample solution in the cell holder and click on <ReadSamples>.
- 4. Data from up to 3 wavelengths are displayed at one time. To display data at other selected wavelengths, click on the right and left arrows, located on the right-hand side of the analysis parameters.
- 5. Repeat steps 2 to 4 until all samples have been read.
- 6. To print the sample data, click on **<Print>**.
- When the analysis is complete, click on <Quit>. To store the method and/or results, click on the displayed file name(s) and input the desired file name(s). Then click on [OK] to store the data and return to the Main window.

G.4. Wavelength Scan

The Wavelength Scan mode is used to collect, manipulate and sore scan data.

To select the analysis parameters :

- 1. With the Main window displayed, click on "WAVELENGTH SCAN" to display the Wavelength Scan window, *figure 5*.
- 2. Twelve parameters are listed near the top of the window.
 - a) Locate the "Start wl" and "End wl" parameters. To change the values, click on the displayed value and input the desired value.
 - b) Verify that the following parameters are as follow : Overlay scans : [No] Autoprint : [No] Autosave : [No] Scans per sample : 1 Sampling device : None Scan speed : 1200 nm/min



If any of the parameters are different than those listed, click on the displayed value and input the listed value.

3. The ordinate label and lilits are displayed on the graphic portion of the window. To change any of these values, click on the displayed value and input the desired value.

To take readings :

- 1. Place a cuvette of solvent in the cell holder. Click on < <BLANK> >.
- 2. Place a cuvette of sample solution in the cell holder and click on <ReadSamples>.
- 3. The following functions are available to reformat the data :

Autoscale : Automaticaly scales the ordinate axis.

Limit changes : The limits on either axis can be changed by clicking on the displayed value and inputing the desired value.

Zoom : The "Zoom" feature is used to expand any portion of the graph. Click on **<Zoom>**, then click two points on the graph to place crosses at the opposite corners of the area to be elarged. When the second cross is clicked on,the graph is replotted. This can be repeated as often as desired. To return to the original plot, click on **<ZoomOut>**.

4. To smooth the data, click on **"Smoothing"** and select the desired number of points of calculation. If too many poinys are used, real peaks can be lost.





- To display a derivative or log scan, peak pick, valley pick and/or point pick, click on <Function> to display the Function Selection window. To select a function, click on to darken the box preceding the selection. Then click on <Edit> to remove the Function selection window from the display. The data are replotted using the selected function(s).
- 6. To display the wavelength and ordinate readings at any point in the spectrum, click on **<Trace>**. Then move the mouse to the point of interest in the spectrum and click on the center mouse button to place a vertical line on the spectrum. The values at the place where the vertical line is placed are displayed in the lower right-hand side of the window. To move the vertical line to eighter the right or left, click on the right or left mouse button, respectively.
- 7. To annotate, click on **<Annotate>**. Then, click on the graph to position a cross and input information from the alphanumeric keypad or keyboard. The annotation is printed with the window, but is not stored with the data.
- 8. To print the sample data, click on <Print>.
- 9. To store data before scanning another example, click on **<SaveClear>**. Click on the displayed file name, input the desired file name, then click on **[OK]**. The data are stored and the graphic area is cleared.
- 10.To scan additional samples, repeat steps 2 to 9, above.
- 11. When all the samples have been scanned, click on **<Quit>**. To store the method and/or displayed scan, click on the displayed file name(s) and input the desired file name(s). Then click on **[OK]** to store the data and return to the Main window.





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