

CERTIFICATION OF CALIBRATION

The Weight(s) listed below have been compared with known standards of mass. The estimated uncertainties listed are expressed at 95% confidence level.

The value(s) stated is the hypothetical weight of density 8000 kg/m³ which balances the weight when compared in air density of 1.2kg/m³.

The measured values are derived via United Kingdom National Standards. Ref: NAMAS Laboratory 0134 Certified Standards, Certificate Numbers T08243 and T11002.

The weight(s) tested satisfy the accuracy requirements of OIML class F1 standards at the time of testing.

Calibration carried out in accordance with a quality system registered to BS EN ISO 9002:1994 Registration No.FS13125.

TEST REF:	NOMINAL MASS g	MEASURED VALUE g	UNCERT +/- MG
181320295	1	1.0000197	0.022

Signed



Approved Signatory



BDH Laboratory Supplies
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COULTER

COULTER - Latex Size: 3µm (Page 1)

COULTER CALIBRATION STANDARD

Assay Sheet

INTENDED USE:

Polymer latex particles intended for the calibration of COULTER COUNTER instruments. Other than those to be used for the red and white blood cell sizing or counting.

NUMBER MODE (Figure A)	3.10 µm DIAMETER 15.60 DIVOLUME
(MULTISIZER and CHANNELYZER Instruments)	
SIZE DISTRIBUTION (ZM/C CHANNELYZER 25C)	See overleaf

BATCH	Z.12
MATERIAL	POLYSTYRENE LATEX
DO NOT USE AFTER	31ST MARCH 1998

Other parameters:

SINGLET NUMBER MEDIAN DIAMETER (Non-multichannel instruments; Figure B).

3.24 µm DIAMETER

WEIGHT PEAK SPLIT (Models TA/TA II; Figure C).

3.26 µm DIAMETER

1:2 WEIGHT PEAK SPLIT (Models TA/TA II; Figure D).

3.18 µm DIAMETER

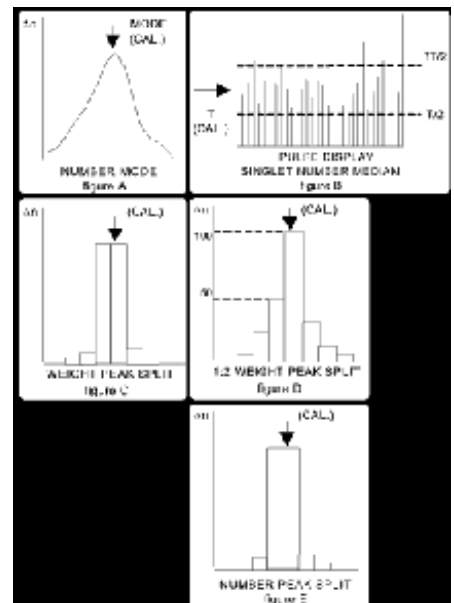
NUMBER PEAK SPLIT (Models TA II/PCA (PCA 1); Figure E).

3.22 µm DIAMETER

TYPICAL USAGE RATE:

30µm aperture, 2 drops per 50ml

50µm aperture, 1 drop per 100ml



CALIBRATION TRACEABILITY - QUALITY ASSURANCE:

ASSAY VALUES have been determined using COULTER COUNTER models MULTISIZER, TA II/PCA, ZM and CHANNELYZER 256 which are verified for performance and calibrated and checked with primary reference standard particles from the National Institute of Standards and Technology, N.I.S.T., (formerly National Bureau of Standards, N.B.S.), and the Community Bureau of Reference, B.C.R. (1).

Properly documented records of the calibrations performed with this Coulter Calibration Standard, when used as recommended in the relevant Instrument Instruction Manual, will allow traceability of those calibrations to the N.I.S.T. (N.B.S.) and B.C.R. particle size Reference Materials, as required by some National authorities, such as BS 5750 and NAMAS in the United Kingdom.

CALIBRATION STANDARD SUSPENSION:

The suggested concentration for both accuracy and speed of calibration is at a concentration giving around 5% coincidence loss. Particles are suspended at a concentration in aqueous dispersant plus preservative such that the typical usage rate gives approximately 5% coincidence for calibration of an aperture ten times the latex's nominal diameter. Poly (styrene-divinyl benzene) (P.D.V.B) lattices are durable and will not change size upon immersion in most electrolyte solutions used with COULTER COUNTER instruments. Lattices may swell or dissolve in some organic liquids, e.g. ketones, chlorinated hydrocarbons and some higher alcohols. With constant current COULTER COUNTER models, calibration may be performed in an electrolyte solution different from that used for sample analysis. There is no evidence of instability with time of Coulter Calibration Standards, as packed, but for maximum security it is recommended that the product is discarded at the expiry date given above.

SIZE CALIBRATION:

COULTER COUNTER instruments can be self calibrated by measuring a suspension of a narrow size range of the material under investigation at a known concentration. From the immersed relative density (g/ml) of the material, and from the total volume measured, the calibration factor can be calculated for the COULTER COUNTER instrument and sensing aperture in use (2). This procedure will remove any doubt concerning accurate calibration with very irregularly shaped or conducting particles. In practice, it is more convenient to calibrate using narrow range particles which have already been sized by other techniques. The most common method is to calibrate with spherical polymer latex particles; Secondary Calibration procedure of BS 3406: Part: 1983 (3).

Using a series of sensing apertures and latex particles. Harfield, Wharton and Lines (4) verified that the response of a COULTER COUNTER Model ZM was linear with particle volume to some 80% of the aperture diameter. By using a series of apertures and verified COULTER COUNTER models, each calibrated to the mode of the size distribution of the relevant N.I.S.T. (N.B.S.) Standard or B.C.R. Certified latex reference materials (1), this Coulter Calibration Standard has been assigned the values given above.

Instruments of high definition size distribution (e.g. MULTISIZER, CHANNELYZER models) are most conveniently calibrated using the number mode size (diameter or volume), see figure A above.

Single or double threshold instruments (e.g. Models A, B, D Industrial, ZB, and ZM without CHANNELYZER accessory) are more conveniently calibrated using the singlet number median diameter, see Figure B above. The 'weight peak split' method (Figure C) is intended for calibration of TA and TAI Models without a population Count Accessory, whereby the calibration point is the junction of two consecutive channels containing equal weight (volume). Earlier calibration methods for the TA or TAI used the 1:2 weight peak split method (Figure D) whereby the calibration point is the junction of two consecutive channels containing twice the weight (volume) in the right hand channel as in the left. The 'number peak split' method (Figure E) is intended for the Model TAI with PCA or PCA 1 population count accessory.

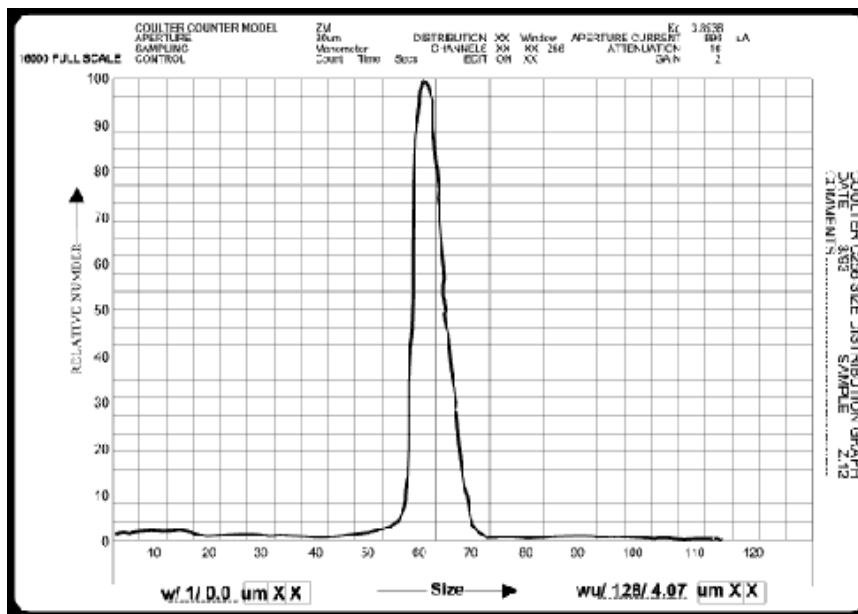
CHOICE OF CALIBRATION STANDARD:

COULTER COUNTER instruments need only to be calibrated at one size level for each aperture, preferably between 5 and 20% of the aperture diameter, optimally 10 %, unless a specific calibration point is required by the user.

REDISPERSION OF CALIBRATION STANDARDS:

Coulter Calibration Standards contain both a preservative and a surface active agent specifically chosen to reduce any tendency of the particles to agglomerate or cake upon storage. Before use, shake, and then roll vial between the palm of the hands until any sediment is resuspended. Prepare calibration suspension by adding the appropriate number of drops (see Typical Usage Rate) to the electrolyte solution. Further dispersion by ultrasonic agitation may help, but should not be necessary. The calibration procedures recommended by Coulter Electronics Ltd. are independent of minor changes in dispersion quality, as doublets, triplets etc. are excluded from the measurements. Stir the calibration suspension during the calibration procedure.

Dry calibration standards must be dispersed by first spatulating with a surfactant, such as Coulter Dispersant. Further dispersion with ultrasonic energy is recommended.



FREQUENCY OF CALIBRATION:

It is expected that the COULTER COUNTER instrument and aperture combination in use will not require calibration at intervals of less than one week so long as the aperture is kept clean. It is recommended that the calibration constant recorded to monitor any long term drift in the electronic components, and that recalibration is performed immediately after any servicing.

INDICATION OF PRODUCT INSTABILITY OR DETERIORATION:

Inability to obtain expected values may indicate product instability or deterioration. Particle suspensions should not be allowed to dry out; calibration powders must be kept dry until use. Discard diluted calibration standard after use. If three or more consecutive calibrations fall outside your expected range, it may mean that the instrument is not operating properly or that the calibration standard is changing in one or more ways. The calibration of the COULTER COUNTER instrument SHOULD NOT BE CHANGED unless one or more of the following steps make it necessary:

- (a) Check that the aperture is clear, and that the wafer and the aperture tube are clean and free from bubbles.
- (b) Check the background count of electrolyte solution.
- (c) Check the performance of your COULTER COUNTER instrument by using another batch of calibration standard suitable for that aperture tube.
- (d) Consult your Coulter Service Representative.

HEALTH AND SAFETY:

COULTER CALIBRATION STANDARDS in suspension contain 0.1% sodium azide (Na N3) as preservative. Contact with heavy metals may produce explosive deposits.

Avoid inhaling dry calibration standards.

REFERENCES:

1. (a) SRM 1960, nominal 10µm latex; SRM 1961, Nominal 30µm latex; (National Institute of Standards and Technology; formally National Bureau of Standards), Gaithersburg, Maryland 20889, U.S.A.).
(b) CRM 167, nominal 9.6µm latex; CRM 166, nominal 4.8µm latex; CRM 165, nominal 2µm latex (Community Bureau of Reference- BCR, 200 Rue de la Loi, BrusselsB-1049, Belgium).
2. Theory of the COULTER COUNTER - Bulletin T-1; Coulter Electronics. Inc. 1957. (reprinted in most COULTER COUNTER instruction Manuals from Coulter Electronics Ltd.)
3. BS 3406: Part 5:1983. British Standard Methods for Determination of particle size distribution, Part 5, recommendations for electrical sensing zone method (the Coulter principle), 34pp
4. Harfield J. G., Wharton R. T., and lines R. W., Part. Charact., 1, 32-34, 1984.

WARRANTY:

This Coulter Calibration Standard is intended for Laboratory use only, as described above, and by trained scientific or technical personnel. It is not intended for use in food, drugs or cosmetics, or for haematology. The sizes quoted are the best obtainable according to the state of the art known to Coulter Electronics Ltd. at the time of assaying.

COULTER - Latex Size: 5µm (Page 2)

COULTER CALIBRATION STANDARD

Assay Sheet

INTENDED USE:

Polymer latex particles intended for the calibration of COULTER COUNTER instruments. Other than those to be used for the red and white blood cell sizing or counting.

NUMBER MODE (Figure A)	5.06 µm DIAMETER 67.0 f.VOLUME
MULTISIZER and CHANNELYZER INSTRUMENTS	
SIZE DISTRIBUTION (210 CHANNELYZER 256)	See overleaf

BATCH	C.19
MATERIAL	P.D.V.B. LATEX
DO NOT USE AFTER	30th June 1998

Other parameters:

SINGLET NUMBER MEDIAN DIAMETER
(Non-multichannel instruments; Figure B).
5.24 µm DIAMETER

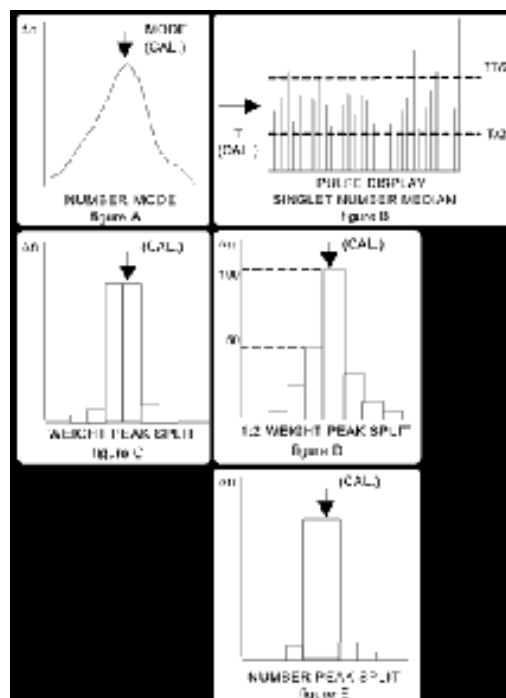
WEIGHT PEAK SPLIT
(Models TA/TA II; Figure C).
5.22 µm DIAMETER

1:2 WEIGHT PEAK SPLIT
(Models TA/TA II; Figure D).
5.12 µm DIAMETER

NUMBER PEAK SPLIT
(Models TA II/PCA (PCA 1); Figure E).
5.19 µm DIAMETER

TYPICAL USAGE RATE:

70µm aperture, 3 drops per 50ml
100µm aperture, 1 drop per 50ml



CALIBRATION TRACEABILITY - QUALITY ASSURANCE:

ASSAY VALUES have been determined using COULTER COUNTER models MULTISIZER, TA II/PCA, ZM and CHANNELYZER 256 which are verified for performance and calibrated and checked with primary reference standard particles from the National Institute of Standards and Technology, N.I.S.T., (formerly National Bureau of Standards, N.B.S.), and the Community Bureau of Reference, B.C.R. (1).

Property documented records of the calibrations performed with this Coulter Calibration Standard, when used as recommended in the relevant Instrument Instruction Manual, will allow traceability of those calibrations to the N.I.S.T. (N.B.S.) and B.C.R. particle size Reference Materials, as required by some National authorities, such as BS 5750 and NAMAS in the United Kingdom.

CALIBRATION STANDARD SUSPENSION:

The suggested concentration for both accuracy and speed of calibration is at a concentration giving around 5% coincidence loss. Particles are suspended at a concentration in aqueous dispersant plus preservative such that the typical usage rate gives approximately 5% coincidence for calibration of an aperture ten times the latex's nominal diameter. Poly (styrene-divinyl benzene) (P.D.V.B) lattices are durable and will not change size upon immersion in most electrolyte solutions used with COULTER COUNTER instruments. Lattices may swell or dissolve in some organic liquids, e.g. ketones, chlorinated hydrocarbons and some higher alcohols. With constant current COULTER COUNTER models, calibration may be performed in an electrolyte solution different from that used for sample analysis. There is no evidence of instability with time of Coulter Calibration Standards, as packed, but for maximum security it is recommended that the product is discarded at the expiry date given above.

SIZE CALIBRATION:

COULTER COUNTER instruments can be self calibrated by measuring a suspension of a narrow size range of the material under investigation at a known concentration. From the immersed relative density (g/ml) of the material, and from the total volume measured, the calibration factor can be calculated for the COULTER COUNTER instrument and sensing aperture in use (2). This procedure will remove any doubt concerning accurate calibration with very irregularly shaped or conducting particles. In practice, it is more convenient to calibrate using narrow range particles which have already been sized by other techniques. The most common method is to calibrate with spherical polymer latex particles; Secondary Calibration procedure of BS 3406: Part: 1983 (3).

Using a series of sensing apertures and latex particles. Harfield, Wharton and Lines (4) verified that the response of a COULTER COUNTER Model ZM was linear with particle volume to some 80% of the aperture diameter. By using a series of apertures and verified COULTER COUNTER models, each calibrated to the mode of the size distribution of the relevant N.I.S.T. (N.B.S.) Standard or B.C.R. Certified latex reference materials (1), this Coulter Calibration Standard has been assigned the values given above.

Instruments of high definition size distribution (e.g. MULTISIZER, CHANNELYZER models) are most conveniently calibrated using the number mode size (diameter or volume), see figure A above. Single or double threshold instruments (e.g. Models A, B, D Industrial, ZB, and ZM

without CHANNELYZER accessory) are more conveniently calibrated using the singlet number median diameter, see Figure B above. The 'weight peak split' method (Figure C) is intended for calibration of TA and TAI Models without a population Count Accessory, whereby the calibration point is the junction of two consecutive channels containing equal weight (volume).

Earlier calibration methods for the TA or TAI used the 1:2 weight peak split method (Figure D) whereby the calibration point is the junction of two consecutive channels containing twice the weight (volume) in the right hand channel as in the left. The 'number peak split' method (Figure E) is intended for the Model TAI with PCA or PCA 1 population count accessory.

CHOICE OF CALIBRATION STANDARD:

COULTER COUNTER instruments need only to be calibrated at one size level for each aperture, preferably between 5 and 20% of the aperture diameter, optimally 10 %, unless a specific calibration point is required by the user.

REDISPERSION OF CALIBRATION STANDARDS:

Coulter Calibration Standards contain both a preservative and a surface active agent specifically chosen to reduce any tendency of the particles to agglomerate or cake upon storage. Before use, shake, and then roll vial between the palm of the hands until any sediment is resuspended. Prepare calibration suspension by adding the appropriate number of drops (see Typical Usage Rate) to the electrolyte solution. Further dispersion by ultrasonic agitation may help, but should not be necessary. The calibration procedures recommended by Coulter Electronics Ltd. are independent of minor changes in dispersion quality, as doublets, triplets etc. are excluded from the measurements. Stir the calibration suspension during the calibration procedure.

Dry calibration standards must be dispersed by first spatulating with a surfactant, such as Coulter Dispersant. Further dispersion with ultrasonic energy is recommended.

FREQUENCY OF CALIBRATION:

It is expected that the COULTER COUNTER instrument and aperture combination in use will not require calibration at intervals of less than one week so long as the aperture is kept clean. It is recommended that the calibration constant recorded to monitor any long term drift in the electronic components, and that recalibration is performed immediately after any servicing.

INDICATION OF PRODUCT INSTABILITY OR DETERIORATION:

Inability to obtain expected values may indicate product instability or deterioration. Particle suspensions should not be allowed to dry out; calibration powders must be kept dry until use. Discard diluted calibration standard after use. If three or more consecutive calibrations fall outside your expected range, it may mean that the instrument is not operating properly or that the calibration standard is changing in one or more ways. The calibration of the COULTER COUNTER instrument SHOULD NOT BE CHANGED unless one or more of the following steps make it necessary:

- (a) Check that the aperture is clear, and that the wafer and the aperture tube are clean and free from bubbles.
- (b) Check the background count of electrolyte solution.
- (c) Check the performance of your COULTER COUNTER instrument by using another batch of calibration standard suitable for that aperture tube.
- (d) Consult your Coulter Service Representative.

HEALTH AND SAFETY:

COULTER CALIBRATION STANDARDS in suspension contain 0.1% sodium azide (Na N₃) as preservative. Contact with heavy metals may produce explosive deposits.

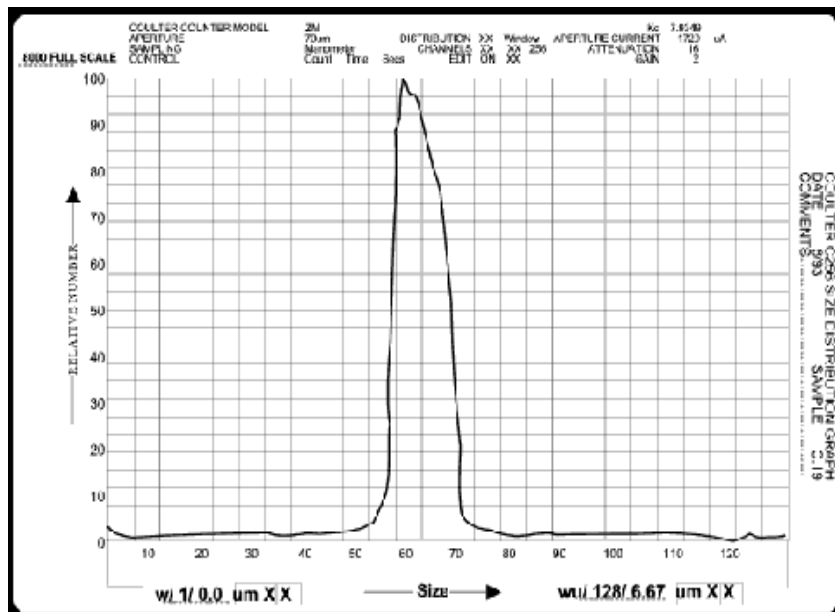
Avoid inhaling dry calibration standards.

REFERENCES:

1. (a) SRM 1960, nominal 10µm latex; SRM 1961, Nominal 30µm latex; (National Institute of Standards and Technology; formally National Bureau of Standards), Gaithersburg, Maryland 20889, U.S.A.).
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COULTER CALIBRATION STANDARD

Assay Sheet

INTENDED USE:

Polymer latex particles intended for the calibration of COULTER COUNTER instruments. Other than those to be used for the red and white blood cell sizing or counting.

NUMBER MODE (Figure A)	9.7 µm DIAMETER 178 f VOL.UMC
MULTISIZER and CHANNELYZER requirements	
SIZE DISTRIBUTION (7 MULTICHANNELYZER 256)	<i>See manual</i>

BATCH	D.42
MATERIAL	P.D.V.B. LATEX
DO NOT USE AFTER	31 st January 1998

Other parameters:

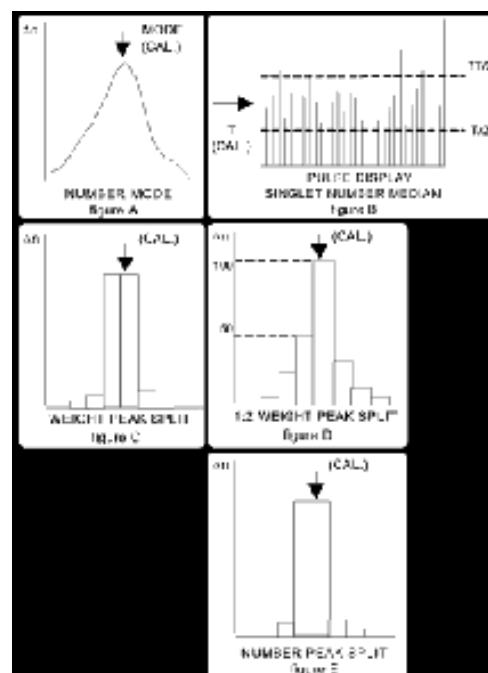
SINGLET NUMBER MEDIAN DIAMETER
(Non-multichannel instruments; Figure B).
10.0 µm DIAMETER

WEIGHT PEAK SPLIT
(Models TA/TA II; Figure C).
10.1 µm DIAMETER

1:2 WEIGHT PEAK SPLIT
(Models TA/TA II; Figure D).
9.8 µm DIAMETER

NUMBER PEAK SPLIT
(Models TA II/PCA (PCA 1); Figure E).
10.0 µm DIAMETER

TYPICAL USAGE RATE:
100µm aperture, 1-2 drops per 100ml
140µm aperture, 1 drop per 150ml



CALIBRATION TRACEABILITY - QUALITY ASSURANCE:

ASSAY VALUES have been determined using COULTER COUNTER models MULTISIZER, TA II/PCA, ZM and CHANNELYZER 256 which are verified for performance and calibrated and checked with primary reference standard particles from the National Institute of Standards and Technology, N.I.S.T., (formerly National Bureau of Standards, N.B.S.), and the Community Bureau of Reference, B.C.R. (1).

Property documented records of the calibrations performed with this Coulter Calibration Standard, when used as recommended in the relevant Instrument Instruction Manual, will allow traceability of those calibrations to the N.I.S.T. (N.B.S.) and B.C.R. particle size Reference Materials, as required by some National authorities, such as BS 5750 and NAMAS in the United Kingdom.

CALIBRATION STANDARD SUSPENSION:

The suggested concentration for both accuracy and speed of calibration is at a concentration giving around 5% coincidence loss. Particles are suspended at a concentration in aqueous dispersant plus preservative such that the typical usage rate gives approximately 5% coincidence for calibration of an aperture ten times the latex's nominal diameter. Poly (styrenedivinyl benzene) (P.D.V.B) lattices are durable and will not change size upon immersion in most electrolyte solutions used with COULTER COUNTER instruments. Latices may swell or dissolve in some organic liquids, e.g. ketones, chlorinated hydrocarbons and some higher alcohols. With constant current COULTER COUNTER models, calibration may be performed in an electrolyte solution different from that used for sample analysis. There is no evidence of instability with time of Coulter Calibration Standards, as packed, but for maximum security it is recommended that the product is discarded at the expiry date given above.

SIZE CALIBRATION:

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figure A above. Single or double threshold instruments (e.g. Models A, B, D Industrial, ZB, and ZM without CHANNELYZER accessory) are more conveniently calibrated using the singlet number median diameter, see Figure B above.

The 'weight peak split' method (Figure C) is intended for calibration of TA and TAI Models without a population Count Accessory, whereby the calibration point is the junction of two consecutive channels containing equal weight (volume). Earlier calibration methods for the TA or TAI used the 1:2 weight peak split method (Figure D) whereby the calibration point is the junction of two consecutive channels containing twice the weight (volume) in the right hand channel as in the left. The 'number peak split' method (Figure E) is intended for the Model TAI with PCA or PCA 1 population count accessory.

CHOICE OF CALIBRATION STANDARD:

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- (d) Consult your Coulter Service Representative.

HEALTH AND SAFETY:

COULTER CALIBRATION STANDARDS in suspension contain 0.1% sodium azide (Na N₃) as preservative. Contact with heavy metals may produce explosive deposits.

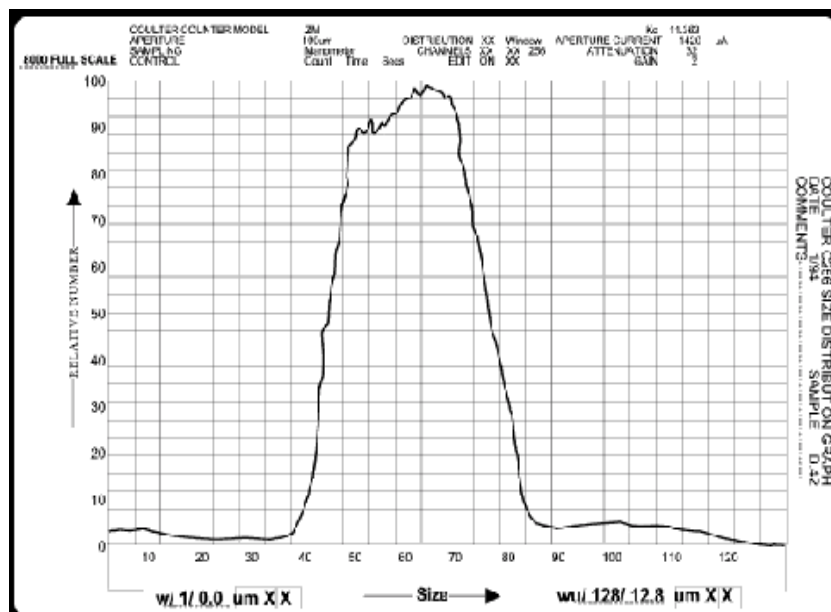
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COULTER CALIBRATION STANDARD

Assay Sheet

INTENDED USE:

Polymer latex particles intended for the calibration of COULTER COUNTER instruments. Other than those to be used for the red and white blood cell sizing or counting.

NUMBER MODE (Figure A)	20.1 μ m DIAMETER 4415 TVTUMF
MULTISIZER and CHANNELYZER Instruments:	
SIEMENSBUCH (ZM CHANNELYZER 256)	See serial#

BATCH	F.32
MATERIAL	P.D.V.B. LATEX
DO NOT USE AFTER	30 th September 1998

Other parameters:

SINGLET NUMBER MEDIAN DIAMETER
(Non-multichannel instruments; Figure B).

21.4 μ m DIAMETER

WEIGHT PEAK SPLIT
(Models TA/TA II; Figure C).

22.3 μ m DIAMETER

1:2 WEIGHT PEAK SPLIT
(Models TA/TA II; Figure D).

21.3 μ m DIAMETER

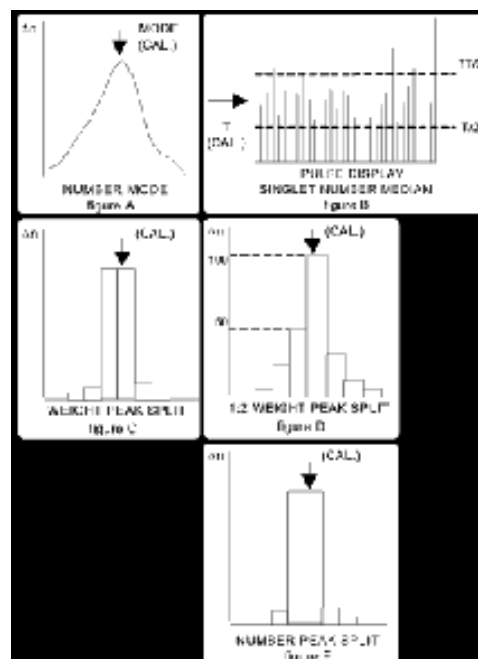
NUMBER PEAK SPLIT
(Models TA II/PCA (PCA 1); Figure E).

21.7 μ m DIAMETER

TYPICAL USAGE RATE:

200 μ m aperture, 1-2 drops per 100ml

280 μ m aperture, 1 drop per 100ml



CALIBRATION TRACEABILITY - QUALITY ASSURANCE:

ASSAY VALUES have been determined using COULTER COUNTER models MULTISIZER, TA II/PCA, ZM and CHANNELYZER 256 which are verified for performance and calibrated and checked with primary reference standard particles from the National Institute of Standards and Technology, N.I.S.T., (formerly National Bureau of Standards, N.B.S.), and the Community Bureau of Reference, B.C.R. (1).

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CALIBRATION STANDARD SUSPENSION:

The suggested concentration for both accuracy and speed of calibration is at a concentration giving around 5% coincidence loss. Particles are suspended at a concentration in aqueous dispersant plus preservative such that the typical usage rate gives approximately 5% coincidence for calibration of an aperture ten times the latex's nominal diameter. Poly (styrenedivinyl benzene) (P.D.V.B) latices are durable and will not change size upon immersion in most electrolyte solutions used with COULTER COUNTER instruments. Latices may swell or dissolve in some organic liquids, e.g. ketones, chlorinated hydrocarbons and some higher alcohols. With constant current COULTER COUNTER models, calibration may be performed in an electrolyte solution different from that used for sample analysis. There is no evidence of instability with time of Coulter Calibration Standards, as packed, but for maximum security it is recommended that the product is discarded at the expiry date given above.

SIZE CALIBRATION:

COULTER COUNTER instruments can be self calibrated by measuring a suspension of a narrow size range of the material under investigation at a known concentration. From the immersed relative density (g/ml) of the material, and from the total volume measured, the calibration factor can be calculated for the COULTER COUNTER instrument and sensing aperture in use (2). This procedure will remove any doubt concerning accurate calibration with very irregularly shaped or conducting particles.

In practice, it is more convenient to calibrate using narrow range particles which have already been sized by other techniques. The most common method is to calibrate with spherical polymer latex particles; Secondary Calibration procedure of BS 3406: Part: 1983 (3). Using a series of sensing apertures and latex particles. Harfield, Wharton and Lines (4) verified that the response of a COULTER COUNTER Model ZM was linear with particle volume to some 80% of the aperture diameter. By using a series of apertures and verified COULTER COUNTER models, each calibrated to the mode of the size distribution of the relevant N.I.S.T. (N.B.S.) Standard or B.C.R. Certified latex reference materials (1), this Coulter Calibration Standard has been assigned the values given above.

Instruments of high definition size distribution (e.g. MULTISIZER, CHANNELYZER models) are most conveniently calibrated using the number mode size (diameter or volume), see figure A above. Single or double threshold instruments (e.g. Models A, B, D Industrial, ZB, and ZM without CHANNELYZER accessory) are more conveniently calibrated using the singlet number median diameter, see Figure B above. The

'weight peak split' method (Figure C) is intended for calibration of TA and TAI Models without a population Count Accessory, whereby the calibration point is the junction of two consecutive channels containing equal weight (volume).

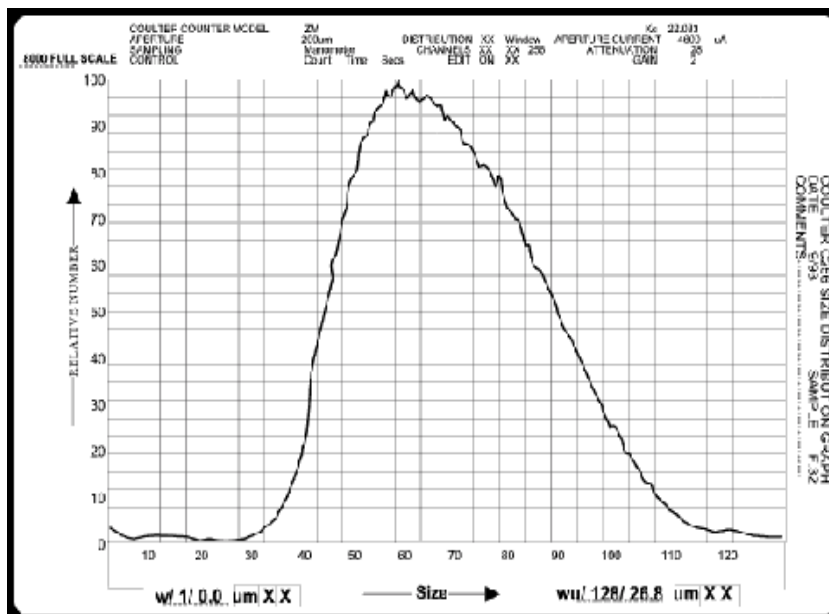
Earlier calibration methods for the TA or TAI used the 1:2 weight peak split method (Figure D) whereby the calibration point is the junction of two consecutive channels containing twice the weight (volume) in the right hand channel as in the left. The 'number peak split' method (Figure E) is intended for the Model TAI with PCA or PCA 1 population count accessory.

CHOICE OF CALIBRATION STANDARD:

COULTER COUNTER instruments need only to be calibrated at one size level for each aperture, preferably between 5 and 20% of the aperture diameter, optimally 10 %, unless a specific calibration point is required by the user.

REDISPERSION OF CALIBRATION STANDARDS:

Coulter Calibration Standards contain both a preservative and a surface active agent specifically chosen to reduce any tendency of the particles to agglomerate or cake upon storage. Before use, shake, and then roll vial between the palm of the hands until any sediment is resuspended. Prepare calibration suspension by adding the appropriate number of drops (see Typical Usage Rate) to the electrolyte solution. Further dispersion by ultrasonic agitation may help, but should not be necessary. The calibration procedures recommended by Coulter Electronics Ltd. are independent of minor changes in dispersion quality, as doublets, triplets etc. are excluded from the measurements. Stir the calibration suspension during the calibration procedure. Dry calibration standards must be dispersed by first spatulating with a surfactant, such as Coulter Dispersant. Further dispersion with ultrasonic energy is recommended.



FREQUENCY OF CALIBRATION:

It is expected that the COULTER COUNTER instrument and aperture combination in use will not require calibration at intervals of less than one week so long as the aperture is kept clean. It is recommended that the calibration constant recorded to monitor any long term drift in the electronic components, and that recalibration is performed immediately after any servicing.

INDICATION OF PRODUCT INSTABILITY OR DETERIORATION:

Inability to obtain expected values may indicate product instability or deterioration. Particle suspensions should not be allowed to dry out; calibration powders must be kept dry until use. Discard diluted calibration standard after use. If three or more consecutive calibrations fall outside your expected range, it may mean that the instrument is not operating properly or that the calibration standard is changing in one or more ways. The calibration of the COULTER COUNTER instrument SHOULD NOT BE CHANGED unless one or more of the following steps make it necessary:

- Check that the aperture is clear, and that the wafer and the aperture tube are clean and free from bubbles.
- Check the background count of electrolyte solution.
- Check the performance of your COULTER COUNTER instrument by using another batch of calibration standard suitable for that aperture tube.
- Consult your Coulter Service Representative.

HEALTH AND SAFETY:

COULTER CALIBRATION STANDARDS in suspension contain 0.1% sodium azide (Na N3) as preservative. Contact with heavy metals may produce explosive deposits.

Avoid inhaling dry calibration standards.

REFERENCES:

- SRM 1960, nominal 10µm latex; SRM 1961, Nominal 30µm latex; (National Institute of Standards and Technology; formerly National Bureau of Standards), Gaithersburg, Maryland 20889, U.S.A.).
 - CRM 167, nominal 9.6µm latex; CRM 166, nominal 4.8µm latex; CRM 165, nominal 2µm latex (Community Bureau of Reference- BCR, 200 Rue de la Loi, Brussels B-1049, Belgium).
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- BS 3406: Part 5:1983. British Standard Methods for Determination of particle size distribution, Part 5, recommendations for electrical sensing zone method (the Coulter principle), 34pp
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WARRANTY:

This Coulter Calibration Standard is intended for Laboratory use only, as described above, and by trained scientific or technical personnel. It is not intended for use in food, drugs or cosmetics, or for haematology. The sizes quoted are the best obtainable according to the state of the art known to Coulter Electronics Ltd. at the time of assaying.

COULTER CALIBRATION STANDARD

Assay Sheet

INTENDED USE:

Polymer latex particles intended for the calibration of COULTER COUNTER instruments. Other than those to be used for the red and white blood cell sizing or counting.

NUMBER MODE (Figure A)	39.2 µm DIAMETER 3.15x10 ¹ ml volume
MULTISIZER and CHANNELYZER Instruments	
SEEDSTEINBUCH (ZIMM CHANNELYZER 256)	See manual

BATCH	G.18
MATERIAL	P.D.V.B. LATEX
DO NOT USE AFTER	

Other parameters:

SINGLET NUMBER MEDIAN DIAMETER
(Non-multichannel instruments; Figure B).

41.2 µm DIAMETER

WEIGHT PEAK SPLIT
(Models TA/TA II; Figure C).

43.3 µm DIAMETER

1:2 WEIGHT PEAK SPLIT
(Models TA/TA II; Figure D).

41.0 µm DIAMETER

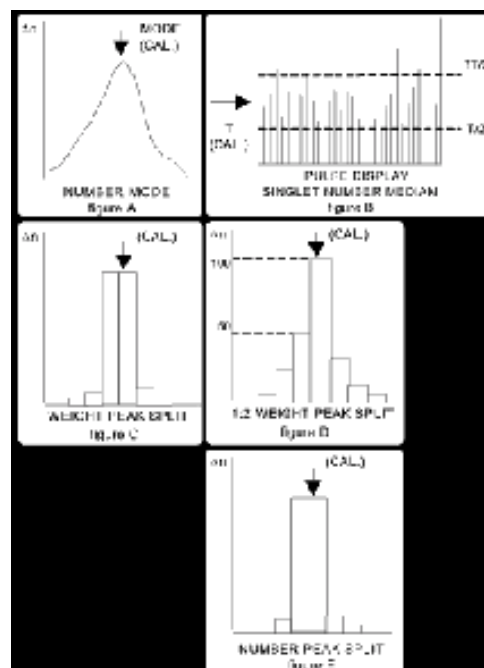
NUMBER PEAK SPLIT
(Models TA II/PCA (PCA 1); Figure E).

41.9 µm DIAMETER

TYPICAL USAGE RATE:

400µm aperture, 1-2 drops per 100ml

560µm aperture, 1 drop per 200ml



CALIBRATION TRACEABILITY - QUALITY ASSURANCE:

ASSAY VALUES have been determined using COULTER COUNTER models MULTISIZER, TA II/PCA, ZM and CHANNELYZER 256 which are verified for performance and calibrated and checked with primary reference standard particles from the National Institute of Standards and Technology, N.I.S.T., (formerly National Bureau of Standards, N.B.S.), and the Community Bureau of Reference, B.C.R. (1). Property documented records of the calibrations performed with this Coulter Calibration Standard, when used as recommended in the relevant Instrument Instruction Manual, will allow traceability of those calibrations to the N.I.S.T. (N.B.S.) and B.C.R. particle size Reference Materials, as required by some National authorities, such as BS 5750 and NAMAS in the United Kingdom.

CALIBRATION STANDARD SUSPENSION:

The suggested concentration for both accuracy and speed of calibration is at a concentration giving around 5% coincidence loss. Particles are suspended at a concentration in aqueous dispersant plus preservative such that the typical usage rate gives approximately 5% coincidence for calibration of an aperture ten times the latex's nominal diameter. Poly (styrenediviny benzene) (P.D.V.B) lattices are durable and will not change size upon immersion in most electrolyte solutions used with COULTER COUNTER instruments. Lattices may swell or dissolve in some organic liquids, e.g. ketones, chlorinated hydrocarbons and some higher alcohols. With constant current COULTER COUNTER models, calibration may be performed in an electrolyte solution different from that used for sample analysis. There is no evidence of instability with time of Coulter Calibration Standards, as packed, but for maximum security it is recommended that the product is discarded at the expiry date given above.

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figure A above. Single or double threshold instruments (e.g. Models A, B, D Industrial, ZB, and ZM without CHANNELYZER accessory) are more conveniently calibrated using the singlet number median diameter, see Figure B above.

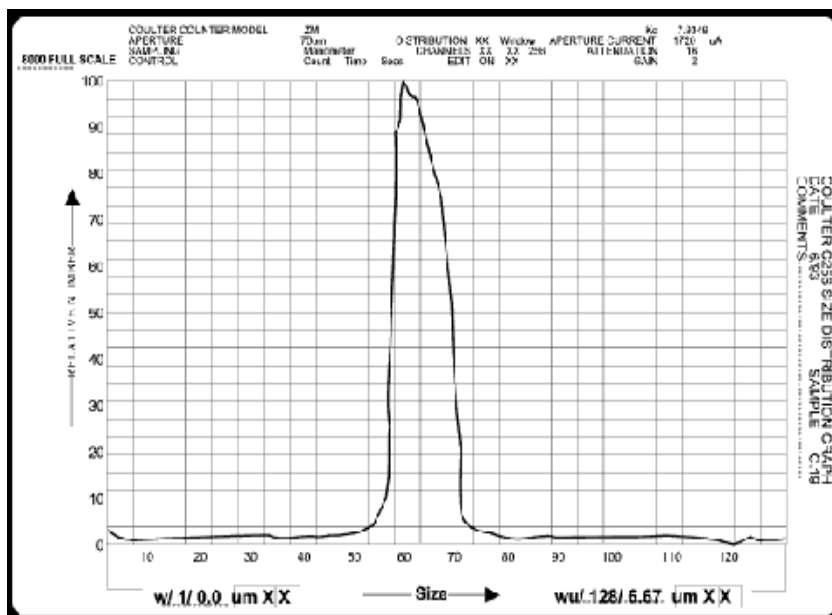
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REFERENCES:

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NIST



UNITED STATES DEPARTMENT OF COMMERCE
National Institute of Standards and Technology
Gaithersburg, Maryland 20899-

REPORT OF CALIBRATION NIST Test No. 821/263573-00

For: Whitehouse Scientific Ltd.
Whitchurch Road, Waverton
Chester, CH3 7PB, England

Item: 10 MM Stage Micrometer
Graticules, Ltd.,
NIST #5594

This stage micrometer was calibrated with the NIST Line Scale Interferometer (LSI). The LSI consists of a scanning electro-optical line detector, a high precision one-axis motion system, and a high accuracy heterodyne interferometer for determining the displacement of the test artifact beneath the line detector. The wavelength of a stabilized helium-neon laser corrected for temperature, humidity and atmospheric pressure is used as the length standard. The instrument is housed in an environmental chamber in which all environmental properties are carefully monitored. The complete description of the design and operation of the NIST LSI is given in the Journal of Research of the National Institute of Standards and Technology Volume 104, Number 3, May-June 1999, "The NIST Length Scale Interferometer".

Results of the calibration are given on the following pages of this report. Each length value is the mean of 4 measurements and the expanded uncertainty in each value is

$$U=ku_c$$

Where the combined standard uncertainty

$$u_c = \sqrt{(u_i^2 + u_j^2)}$$

Where u_i is the standard uncertainty arising from random effects and u_j is the standard uncertainty arising from systematic effects in the measurement process. The coverage factor $k=2$ was used which gives for each reported value a level of confidence of approximately 95 percent.

Measurements were made from line center to line center using a 0.16 mm segment of each.

NIST Test No. 821/263573-00
10 MM Stage Micrometer
Graticules, Ltd., NIST #5594

Graduation line midway between the line tips. During measurement the stage micrometer was placed on a flat surface.

During the measurement the environmental chamber and the artifact temperature were held within $\pm 0.005^\circ\text{C}$ of 20°Celsius . All lengths are reported at a temperature of 20°Celsius ($68^\circ\text{Fahrenheit}$). A coefficient of linear thermal expansion of $8.5 \times 10^{-6} / ^\circ\text{Celsius}$ was used in normalizing the lengths to 20°Celsius .

Certified correct by

A handwritten signature in black ink that reads "William B Penzes". The signature is written in a cursive style and is underlined.

William B Penzes

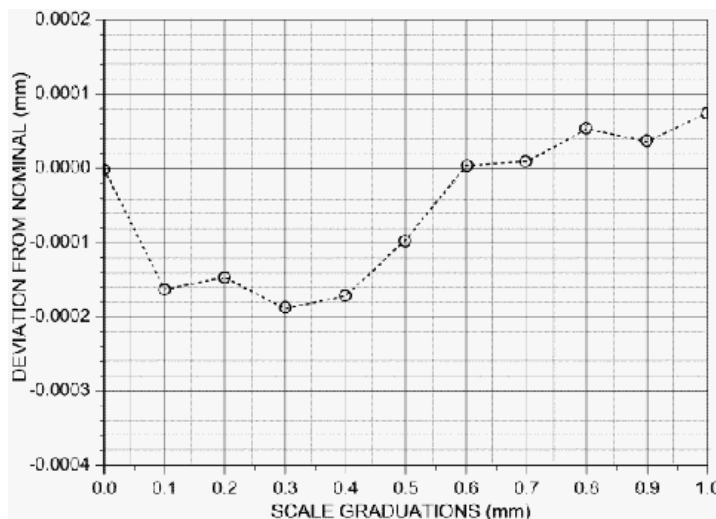
For the Director

A handwritten signature in blue ink that reads "Michael T. Postek". The signature is written in a cursive style and includes the letters "Dr." and "NIST" at the end.

Dr. Michael T. Postek, Group Leader
Nano-Scale Metrology Group
Precision Engineering Division
Manufacturing Engineering Laboratory

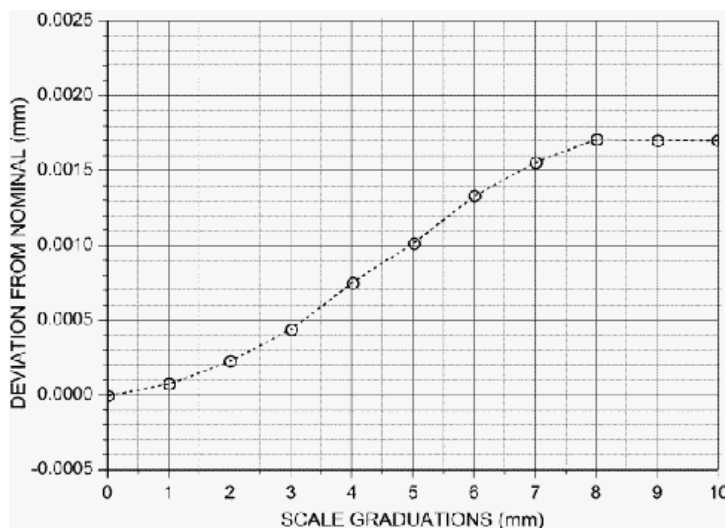
NIST Test No. 821/263573-00
 10 MM Stage Micrometer
 Graticules, Ltd., NIST #5594

INTERVAL (MILLIMETER)	LENGTH (mm)	CORRECTION (mm)	u_i (mm)	u_j (mm)	U (mm)
0.000 TO 0.100	0.099837	-0.000163	0.000002	0.000000	0.000004
0.000 TO 0.200	0.199853	-0.000147	0.000003	0.000000	0.000006
0.000 TO 0.300	0.299811	-0.000189	0.000003	0.000000	0.000006
0.000 TO 0.400	0.399828	-0.000172	0.000003	0.000000	0.000007
0.000 TO 0.500	0.499904	-0.000096	0.000005	0.000000	0.000009
0.000 TO 0.600	0.600004	0.000004	0.000005	0.000000	0.000011
0.000 TO 0.700	0.700009	0.000009	0.000005	0.000000	0.000010
0.000 TO 0.800	0.800053	0.000053	0.000003	0.000000	0.000006
0.000 TO 0.900	0.900036	0.000036	0.000003	0.000000	0.000006
0.000 TO 1.000	1.000075	0.000075	0.000002	0.000000	0.000005



NIST Test No. 821/263573-00
 10 MM Stage Micrometer
 Graticules, Ltd., NIST #5594

INTERVAL (MILLIMETER)	LENGTH (mm)	CORRECTION (mm)	u_i (mm)	u_j (mm)	U (mm)
0.000 TO 1.00	1.000080	0.000080	0.000002	0.000000	0.000004
0.000 TO 2.00	2.000233	0.000233	0.000008	0.000000	0.000015
0.000 TO 3.00	3.000448	0.000448	0.000006	0.000000	0.000012
0.000 TO 4.00	4.000759	0.000759	0.000006	0.000000	0.000012
0.000 TO 5.00	5.001022	0.001022	0.000006	0.000000	0.000013
0.000 TO 6.00	6.001336	0.001336	0.000004	0.000000	0.000009
0.000 TO 7.00	7.001562	0.001562	0.000005	0.000000	0.000010
0.000 TO 8.00	8.001715	0.001715	0.000001	0.000000	0.000002
0.000 TO 9.00	9.001707	0.001707	0.000003	0.000000	0.000006
0.000 TO 10.00	10.001704	0.001704	0.000003	0.000001	0.000005





NATIONAL PHYSICAL LABORATORY

Teddington Middlesex UK TW11 0LW Switchboard 0181-977 3222

CENTRE FOR MECHANICAL AND OPTICAL TECHNOLOGY

Certificate of Calibration

REFERENCE STAGE GRATICULE

FOR: Whitehouse Scientific Ltd
Whitchurch Road
Waverton
Chester
CH3 7PB

For the attention of Dr Graham Rideal,

DESCRIPTION

The NPL reference stage graticule consists of a 25 mm x 75 mm glass slide bearing a design, in chromium, produced by direct copying of a master drawn using electron-beam lithography. The design consists of a central area, in the middle of which is the symbol "NPL", surrounded by the following four test areas.

- 1) A square grid comprising boxes varying in size from 25 μm to 400 μm . The numbers in the centre of the squares represent the nominal length of the sides of the square in which they appear.
- 2) A 20 x 17 array of spots of nominally the same 15 μm diameter.
- 3) A row of nine spots forming a log-normal distribution with diameters varying from 4.5 μm - 27 μm .

The uncertainties are for a confidence probability of not less than 95%.

DATE OF CALIBRATION 18 June 1997

IDENTIFICATION NPL Reference Stage Graticule 126

Reference: 08A038/970127/106-66

Date of issue: 18 June 1997 Signed: (Authorised Signatory)

Checked by: Name: Nicholas P Turner For Managing Director

This certificate provides traceability of measurement to recognised national standards, and to the units of measurement realised at the NPL or other recognised national standards laboratories. This certificate may only be published in full, unless permission for publication of an approved extract has been obtained in writing from the Managing Director. It does not itself impute to the subject of calibration any attributes beyond those shown by the data contained herein.

NATIONAL PHYSICAL LABORATORY

Continuation Sheet

MEASUREMENTS

The grid was measured along all four of the 400 μ m sides with a length measuring microscope, using a 50x objective and a refractive index compensated laser interferometer fitted to the stage. The lengths were measured between the centres of the two parallel lines forming opposite sides of the grid.

The spots were measured using a microscope with a 100x objective and an image analyser. They were compared with spots measured using an image-shearing microscope, calibrated against an interferometrically measured stage micrometer. The diameters are calculated from the area of the spots and thus represent an average spot diameter.

RESULTS:

- 1) The lengths of the sides of the grid are 399.99 μ m.
- 2) The monosize array has a mean diameter of 14.70 μ m. The largest single spot is 14.84 μ m and the smallest is 14.58 μ m.
- 3) The diameters of the spots forming the root-2 array are:

Spot Number	Diameter (μ m)
1	2.53
2	3.75
3	5.57
4	8.09
5	11.63
6	16.61
7	23.64
8	33.5
9	47.67

- 4) The log-normal distribution has a mean of 10.58 μ m and a standard deviation of 1.51 μ m.

Reference: 08A038/970127/106-66

Checked by:

NATIONAL PHYSICAL LABORATORY

Continuation Sheet

UNCERTAINTIES:

The uncertainty in the grid measurement is +/- 0.50 μ m.

The uncertainty in the diameters of the spots forming the monosize array and the log-normal array is +/- 0.30 μ m.

The uncertainty in the diameters of the spots forming the root-2 array is +/- 0.30 μ m.

The uncertainty in the mean of the log-normal distribution is +/- 0.30 μ m.

NOTES:

A guide to the practical use of the reference stage graticule and the formulae used to calculate the log-normal parameters are given in the enclosed sheets.

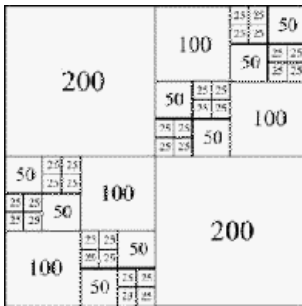
Reference: 08A038/970127/106-66

Checked by:

NPL

USE OF THE NPL REFERENCE STAGE GRATICULE

The reference stage graticule comprises four test areas. They are as follows:

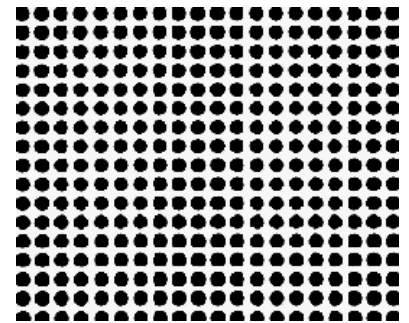


1. THE GRID

The grid is used to calculate the image analyser and to detect any gross image distortion. An appropriate sized square should be imaged on the screen and the analyser calibrated across the square in the normal way. The squareness of the calibration can be checked by using the other two sides of the square (on some analysers calibration in a second direction is not possible and a variable frame can be used). Many image analysers can produce a software generated grid. If this grid is superimposed on the image of the graticule grid, any large-scale image distortions will become evident.

2. THE MONOSIZE ARRAY

The monosize array is used to check for localised distortions of the image. The distortions are quite common at the edge of the field of view and a knowledge of where and by how much the scaling breaks down allows determination of the usable measuring area of the image. The slide is positioned so that the 20x17 array of spots fill the screen. The height and width of the spots (usually feret 90 and feret 0 respectively) are then measured and either by printing out these sizes with the spot position or by labelling the image on the screen if this is possible, the deviation in the sizes of the spots at the edges can be seen by comparison with spots in the middle of the screen. Measurements must be made rather than direct visual observations because screen distortions may affect judgement.

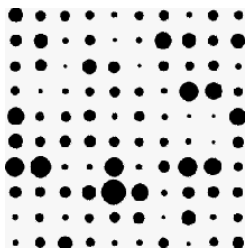


3. THE ROOT-2 ARRAY



The root-2 array is used to determine the threshold level required to measure the spots correctly. The image analyser works by the user choosing a grey level at which anything darker is spot and anything lighter is background. As the edge of a spot is not a clean edge but a blurred one due to the limited resolution of the camera and the physical limits of optical microscopy, the specified threshold level affects the measured size of the spots. This array of spots provides a useful research tool for investigating the effects of background lighting, detect level, focus etc. on measured spot size.

4. THE LOG-NORMAL ARRAY



The final test area is the log-normally distributed array. This is an idealised distribution of maximum dynamic range for a full screen and is used as a final check on the analyser when all other variables have been corrected for evaluated. The 100 spots should be arranged to fill the screen and are measured. Using the software that may be provided with the image analyser, the mean and standard deviation of the log-normal distribution can be determined and compared with the certified values. The mean and standard deviations have been calculated using the following equations.

The equations that define the mean and standard deviation of the log-normal distribution are:

$$\text{MEAN} = \exp$$

$$\left(\sum_{i=1}^{100} \frac{\log d_i}{100} \right)$$

$$\text{STANDARD DEVIATION} = \exp$$

$$\left(\sum_{i=1}^{100} \frac{(\log \text{MEAN} - \log d_i)^2}{100} \right)^{1/2}$$

Where d_i is the diameter of spot number i .

Please contact NPL if you wish the log-normal parameters to be calculated using alternative formulae.

Always ensure the graticule is clean before use.