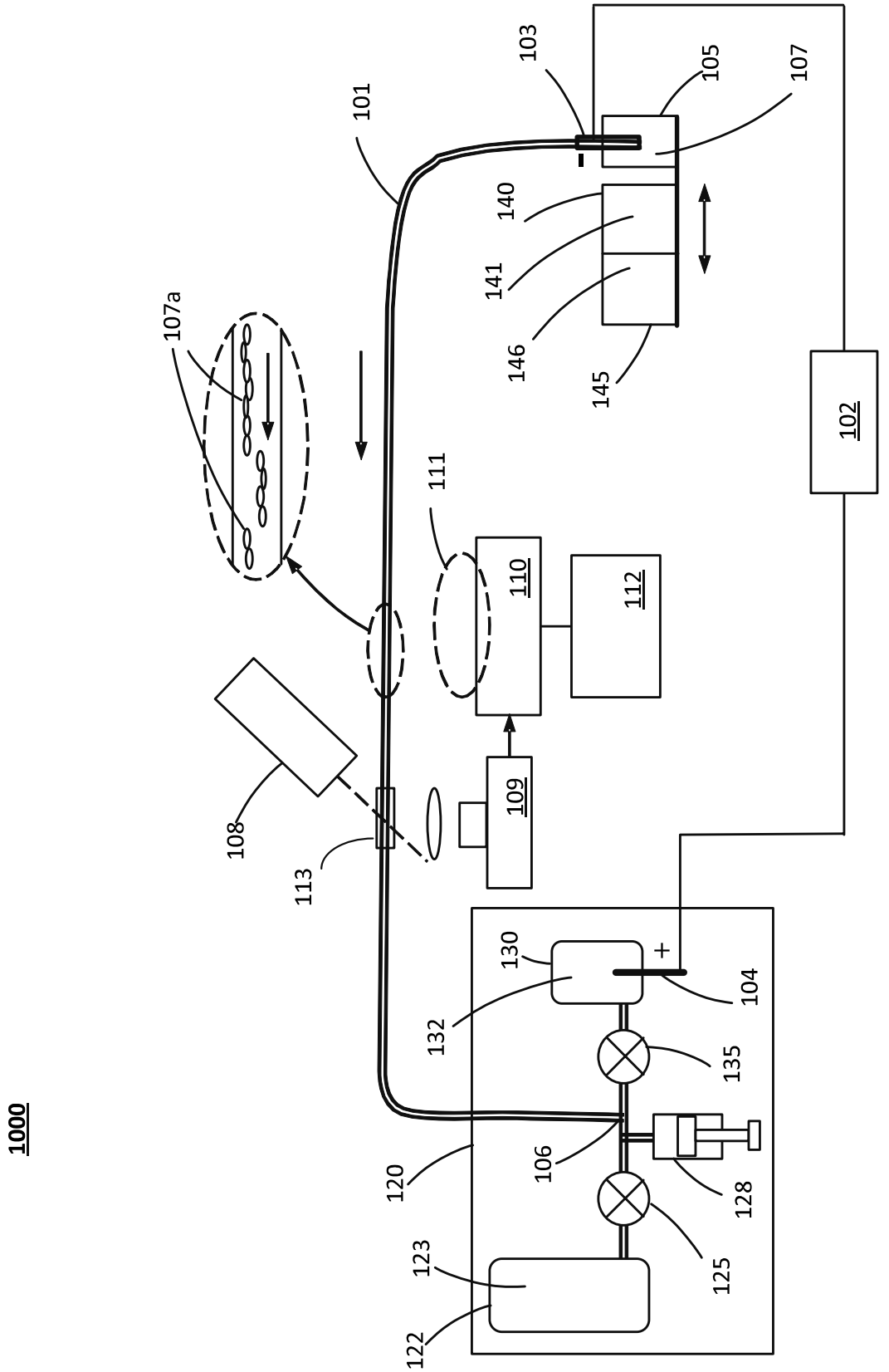
1000
101
103
105
107
140
141
146
145
107a
102
111
110
112
108
113
109
130
132
104
135
106
120
128
123
125
122

FIG. 1

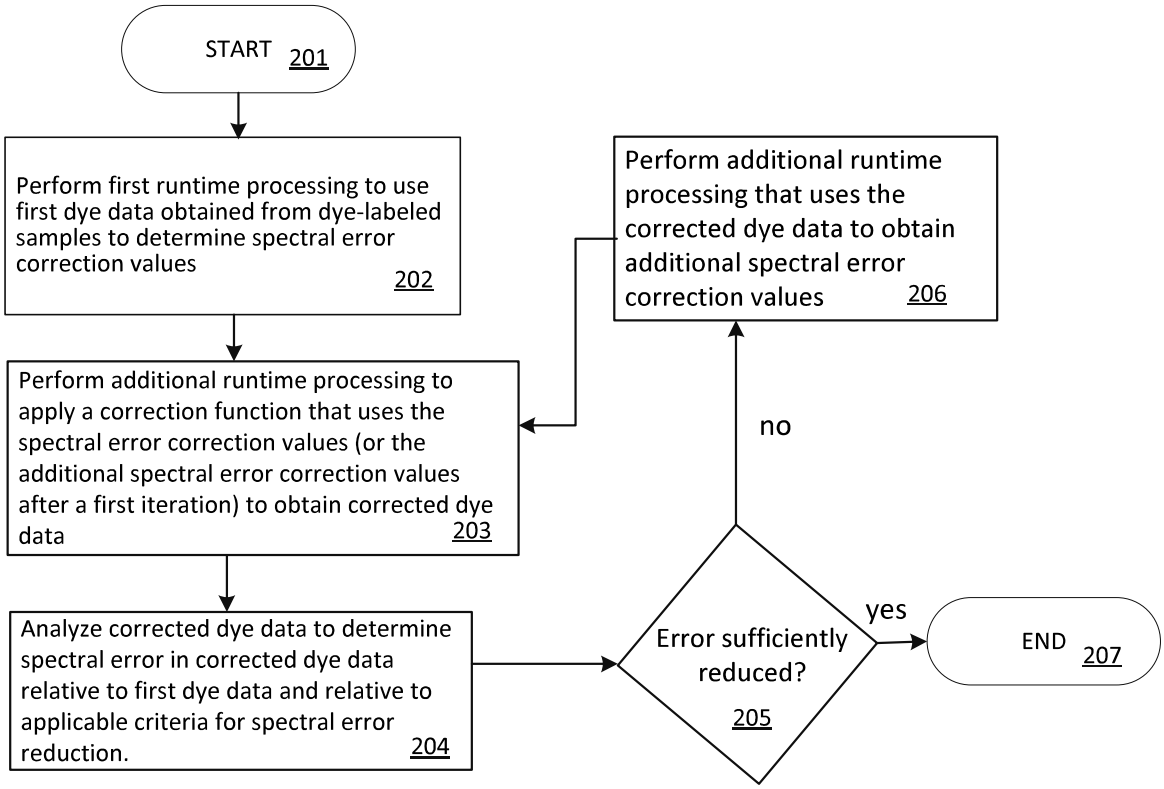
START 201
Perform first runtime processing to use first dye data obtained from dye-labeled samples to determine spectral error correction values 202
Perform additional runtime processing to apply a correction function that uses the spectral error correction values (or the additional spectral error correction values after a first iteration) to obtain corrected dye data 203
Analyze corrected dye data to determine spectral error in corrected dye data relative to first dye data and relative to applicable criteria for spectral error reduction. 204
Error sufficiently reduced? 205
no
Perform additional runtime processing that uses the corrected dye data to obtain additional spectral error correction values 206
yes
END 207

FIG. 2 **2000**

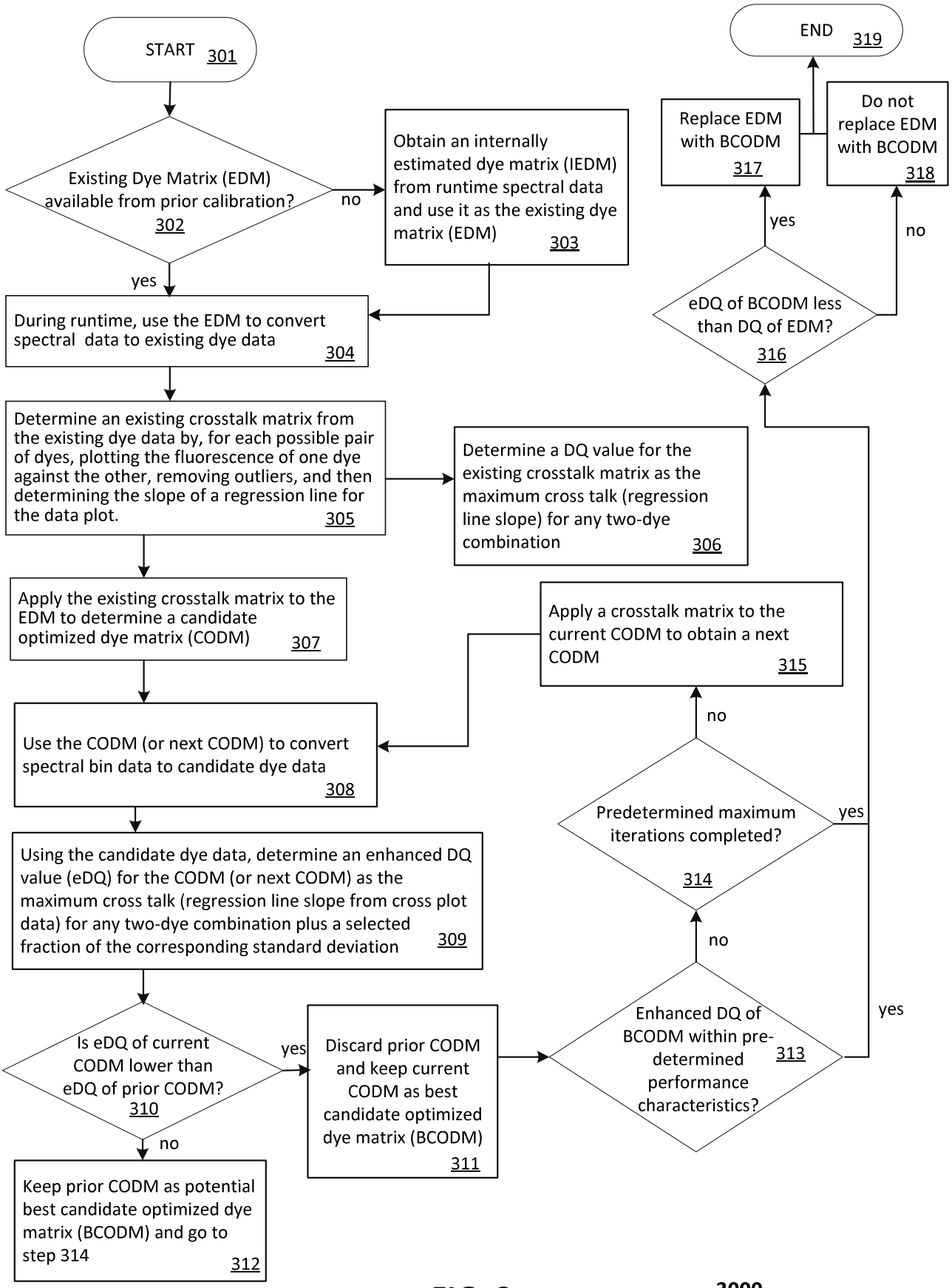
START 301
Existing Dye Matrix (EDM) available from prior calibration? 302
no
Obtain an internally estimated dye matrix (IEDM) from runtime spectral data and use it as the existing dye matrix (EDM) 303
yes
During runtime, use the EDM to convert spectral data to existing dye data 304
Determine an existing crosstalk matrix from the existing dye data by, for each possible pair of dyes, plotting the fluorescence of one dye against the other, removing outliers, and then determining the slope of a regression line for the data plot. 305
Determine a DQ value for the existing crosstalk matrix as the maximum cross talk (regression line slope) for any two-dye combination 306
Apply the existing crosstalk matrix to the EDM to determine a candidate optimized dye matrix (CODM) 307
Use the CODM (or next CODM) to convert spectral bin data to candidate dye data 308
Using the candidate dye data, determine an enhanced DQ value (eDQ) for the CODM (or next CODM) as the maximum cross talk (regression line slope from cross plot data) for any two-dye combination plus a selected fraction of the corresponding standard deviation 309
Is eDQ of current CODM lower than eDQ of prior CODM? 310
yes
no
Discard prior CODM and keep current CODM as best candidate optimized dye matrix (BCODM) 311
Keep prior CODM as potential best candidate optimized dye matrix (BCODM) and go to step 314 312
Enhanced DQ of BCODM within pre-determined performance characteristics? 313
yes
no
Predetermined maximum iterations completed? 314
yes
no
Apply a crosstalk matrix to the current CODM to obtain a next CODM 315
eDQ of BCODM less than DQ of EDM? 316
yes
no
Replace EDM with BCODM 317
Do not replace EDM with BCODM 318
END 319

FIG. 3

3000

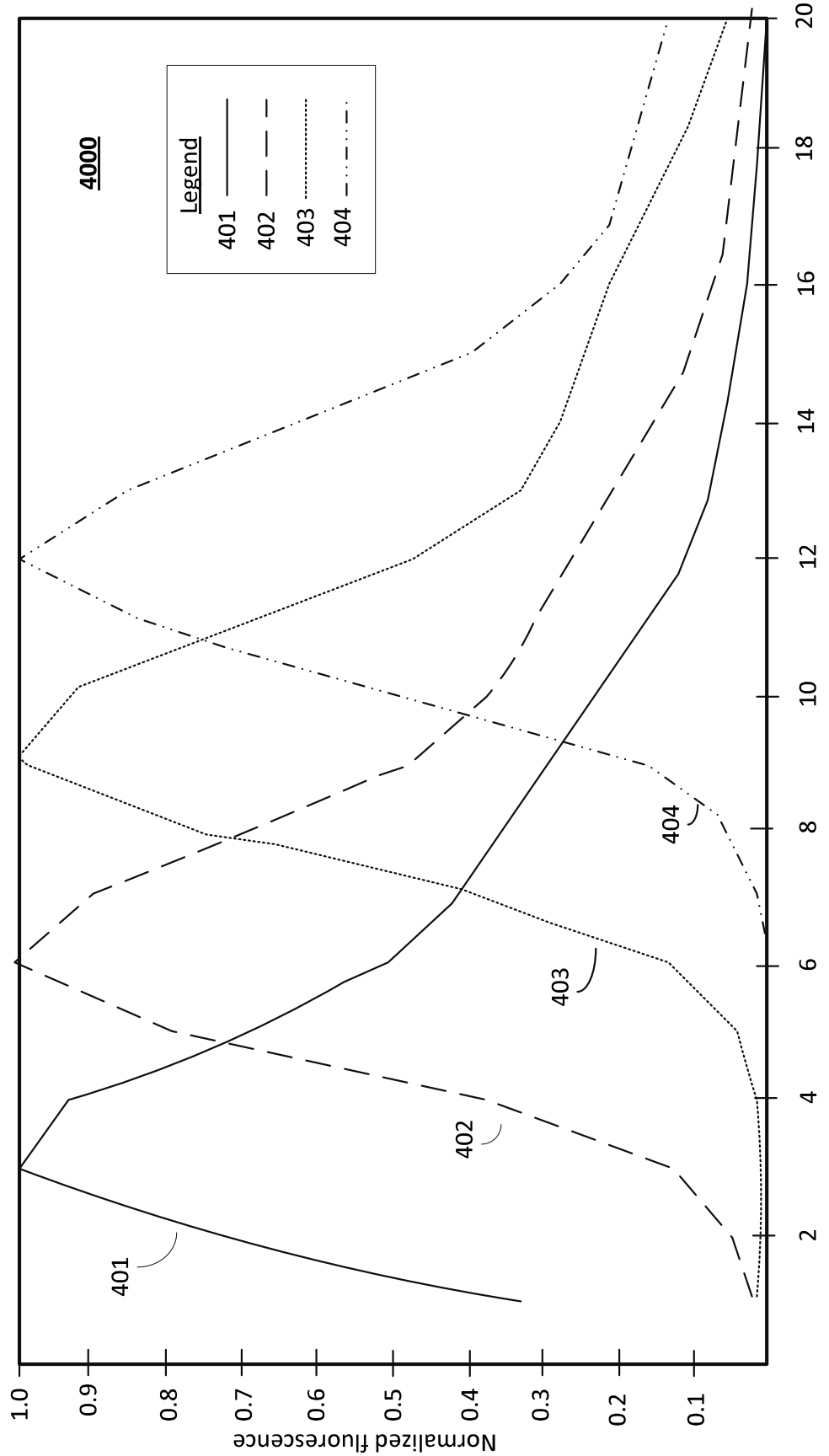
4000
Legend
401
402
403
404
Normalized fluorescence
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
2
4
6
8
10
12
14
16
18
20
Bins

FIG. 4

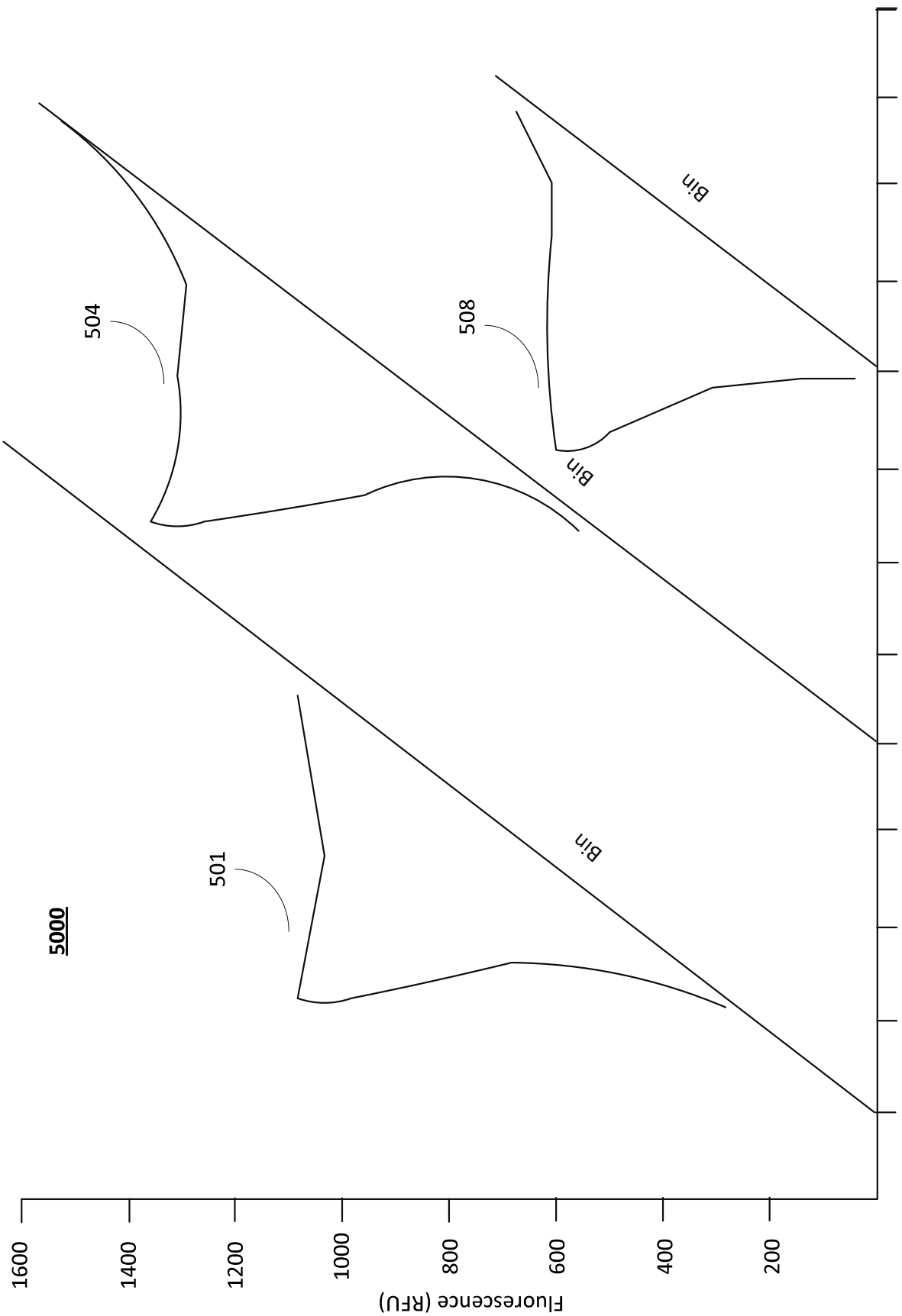
5000
501
504
508
Bin
Bin
Bin
1600
1400
1200
1000
800
600
400
200
Fluorescence (RFU)
3116
3132
3148
Scan Number (over time)

FIG. 5

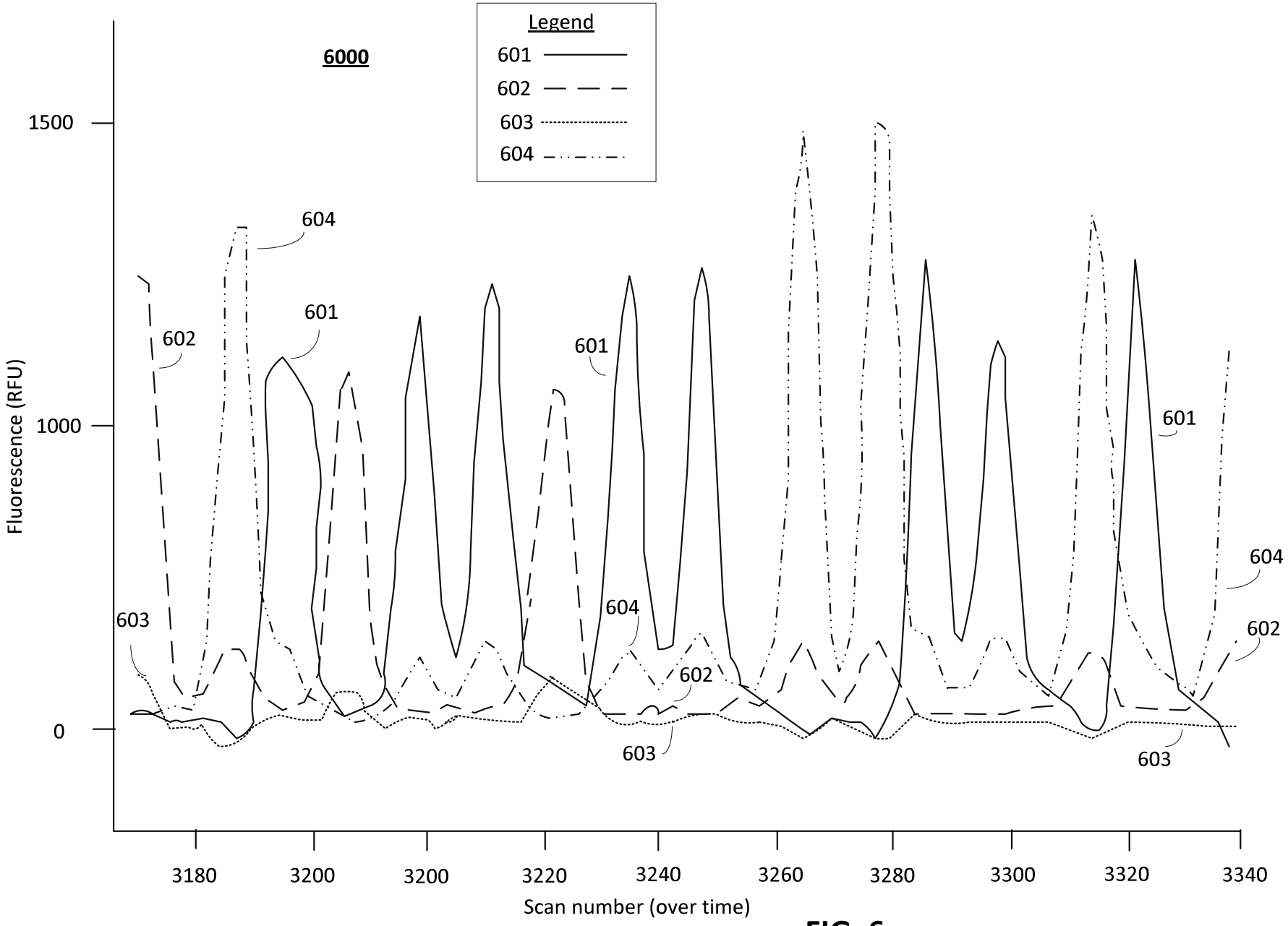
6000
Legend
601
602
603
604
1500
1000
0
Fluorescence (RFU)
3180
3200
3200
3220
3240
3260
3280
3300
3320
3340
Scan number (over time)

FIG. 6

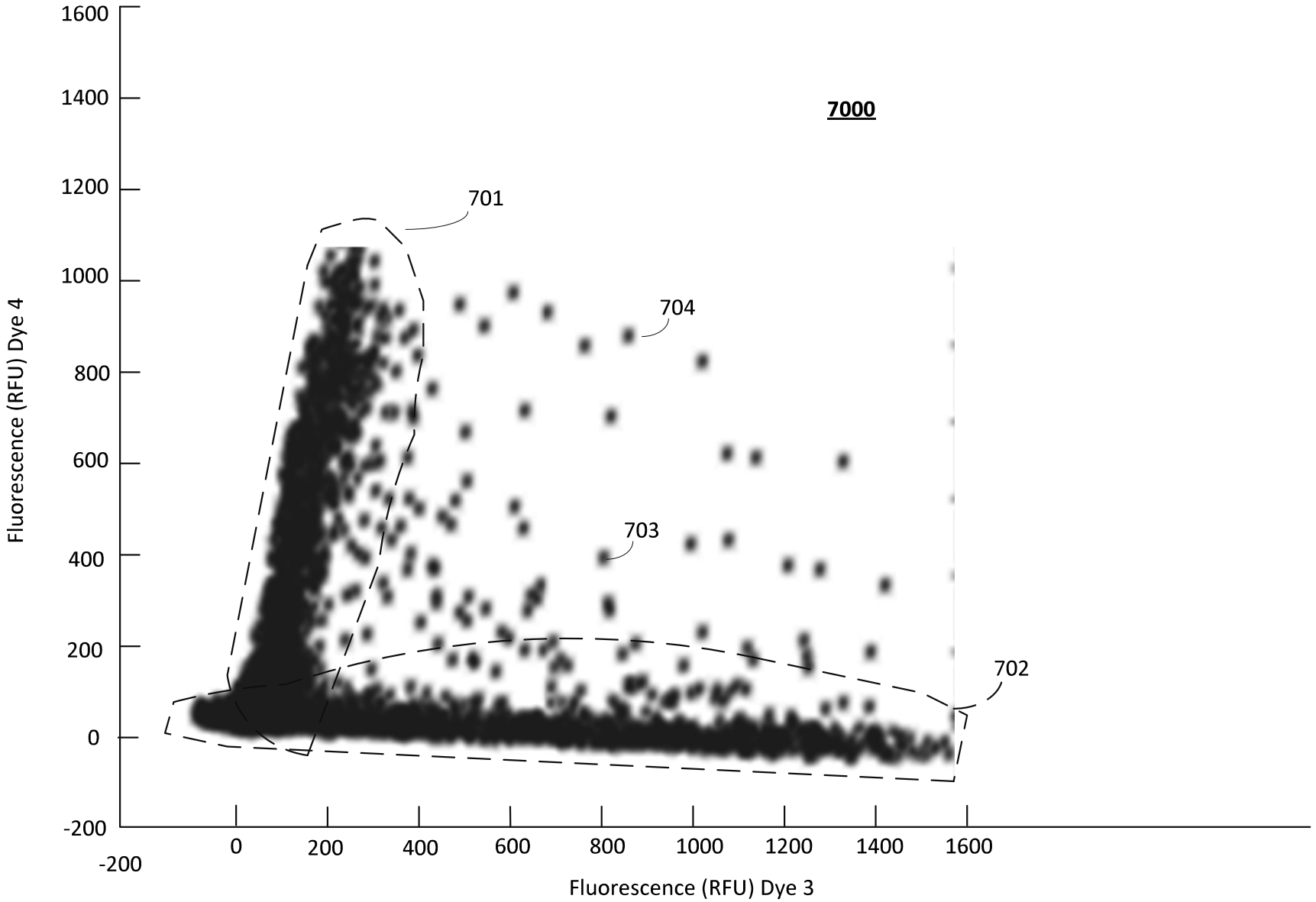
7000
701
704
703
702
1600
1400
1200
1000
800
600
400
200
0
-200
-200
0
200
400
600
800
1000
1200
1400
1600
Fluorescence (RFU) Dye 4
Fluorescence (RFU) Dye 3

FIG. 7

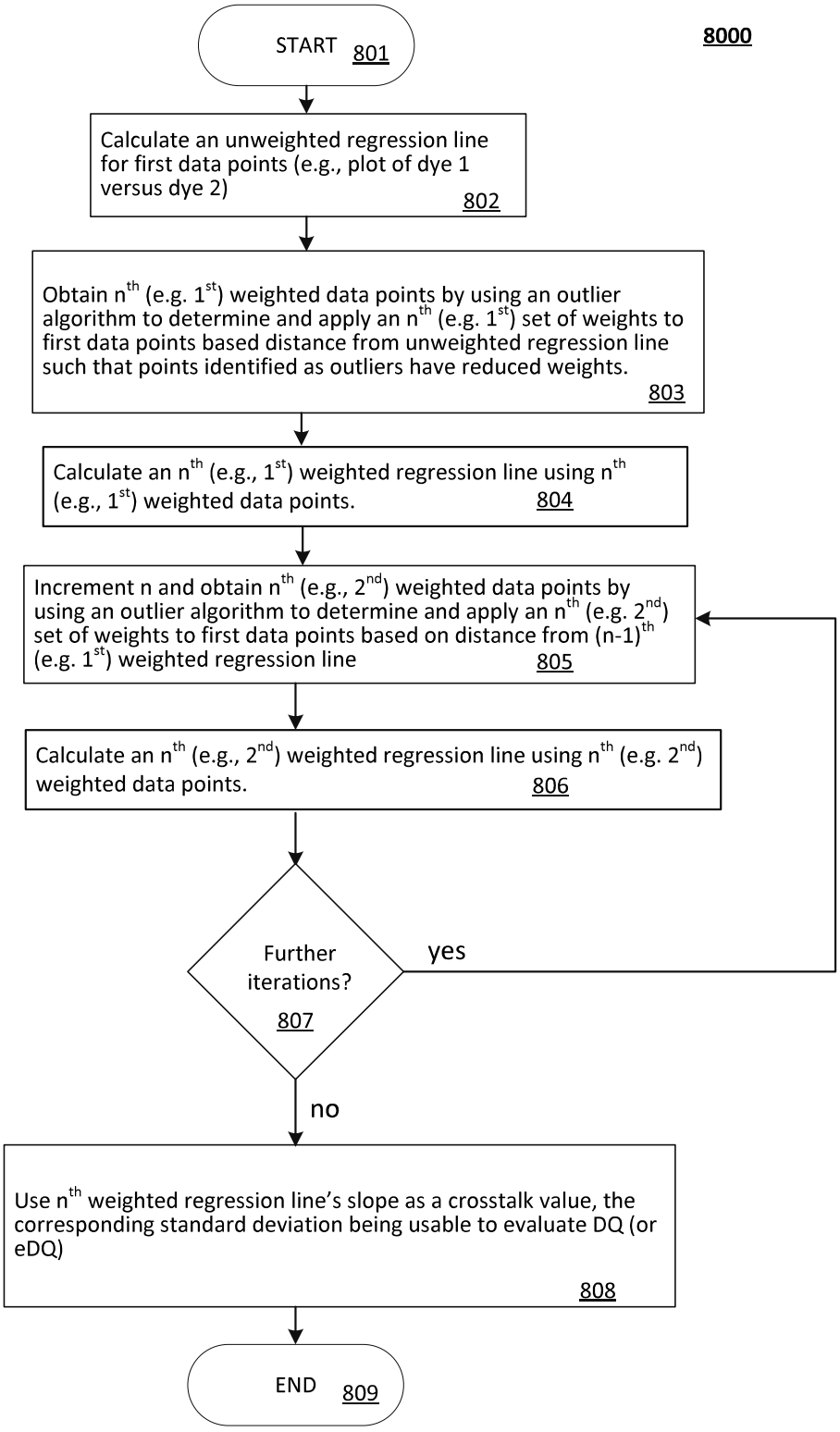
8000
START 801
Calculate an unweighted regression line for first data points (e.g., plot of dye 1 versus dye 2) 802
Obtain n^th (e.g. 1^st) weighted data points by using an outlier algorithm to determine and apply an n^th (e.g. 1^st) set of weights to first data points based distance from unweighted regression line such that points identified as outliers have reduced weights. 803
Calculate an n^th (e.g., 1^st) weighted regression line using n^th (e.g., 1^st) weighted data points. 804
Increment n and obtain n^th (e.g., 2^nd) weighted data points by using an outlier algorithm to determine and apply an n^th (e.g. 2^nd) set of weights to first data points based on distance from (n-1)^th (e.g. 1^st) weighted regression line 805
Calculate an n^th (e.g., 2^nd) weighted regression line using n^th (e.g. 2^nd) weighted data points. 806
Further iterations? 807
yes
no
Use n^th weighted regression line's slope as a crosstalk value, the corresponding standard deviation being usable to evaluate DQ (or eDQ) 808
END 809

FIG. 8

9000

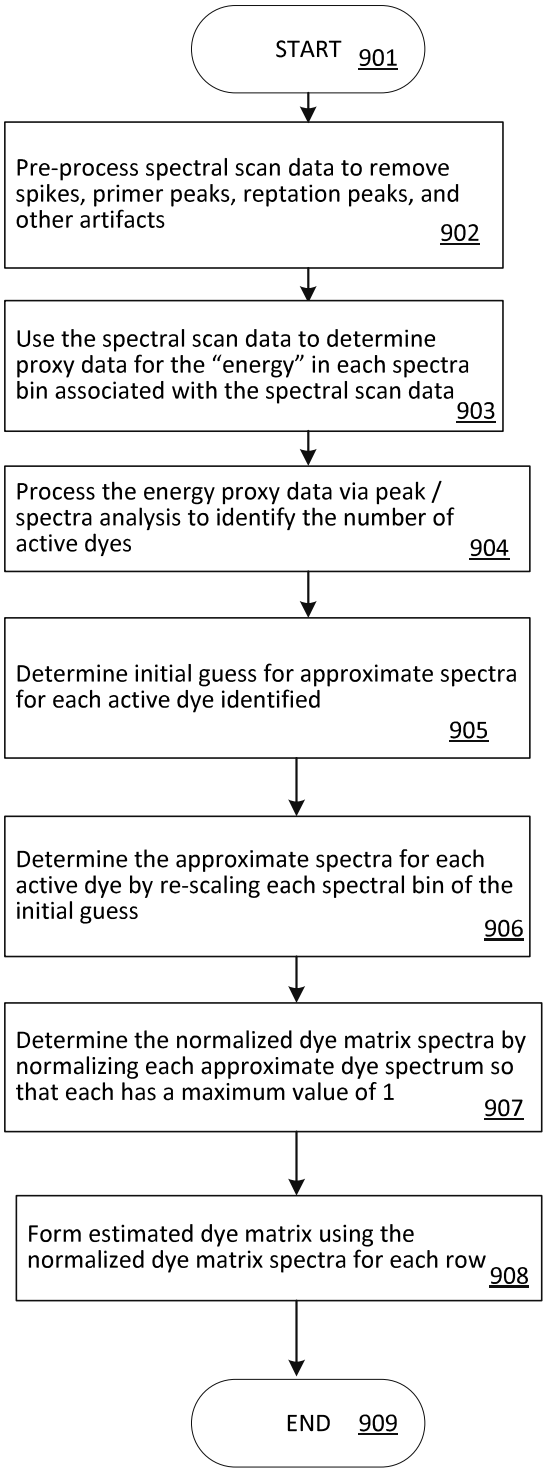
START 901
Pre-process spectral scan data to remove spikes, primer peaks, reptation peaks, and other artifacts 902
Use the spectral scan data to determine proxy data for the "energy" in each spectra bin associated with the spectral scan data 903
Process the energy proxy data via peak / spectra analysis to identify the number of active dyes 904
Determine initial guess for approximate spectra for each active dye identified 905
Determine the approximate spectra for each active dye by re-scaling each spectral bin of the initial guess 906
Determine the normalized dye matrix spectra by normalizing each approximate dye spectrum so that each has a maximum value of 1 907
Form estimated dye matrix using the normalized dye matrix spectra for each row 908
END 909

FIG. 9

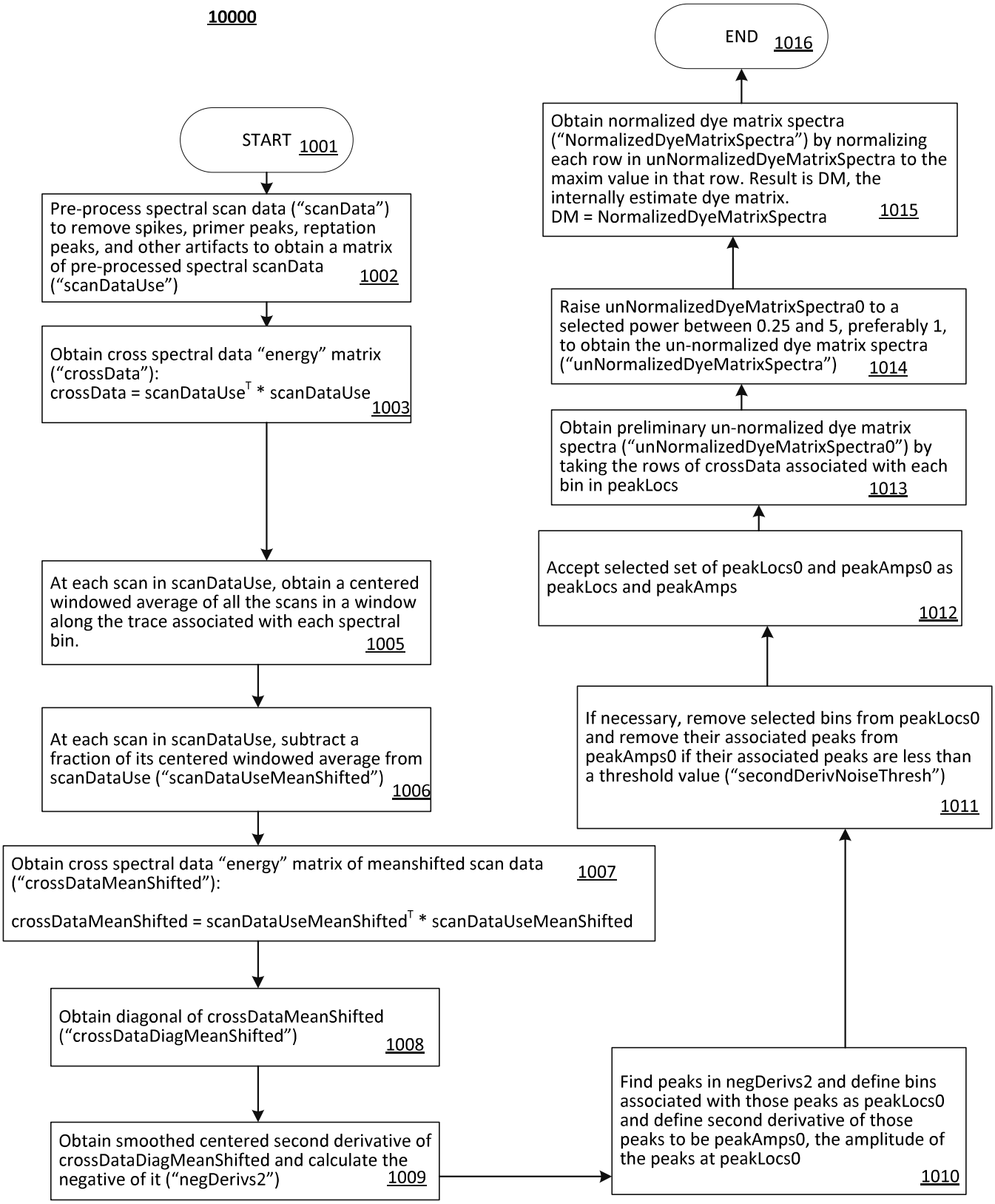
10000
START 1001
Pre-process spectral scan data ("scanData") to remove spikes, primer peaks, reptation peaks, and other artifacts to obtain a matrix of pre-processed spectral scanData ("scanDataUse") 1002
Obtain cross spectral data "energy" matrix ("crossData"):
crossData = scanDataUse^T * scanDataUse 1003
At each scan in scanDataUse, obtain a centered windowed average of all the scans in a window along the trace associated with each spectral bin. 1005
At each scan in scanDataUse, subtract a fraction of its centered windowed average from scanDataUse ("scanDataUseMeanShifted") 1006
Obtain cross spectral data "energy" matrix of meanshifted scan data ("crossDataMeanShifted"): 1007
crossDataMeanShifted = scanDataUseMeanShifted^T * scanDataUseMeanShifted
Obtain diagonal of crossDataMeanShifted ("crossDataDiagMeanShifted") 1008
Obtain smoothed centered second derivative of crossDataDiagMeanShifted and calculate the negative of it ("negDerivs2") 1009
Find peaks in negDerivs2 and define bins associated with those peaks as peakLocs0 and define second derivative of those peaks to be peakAmps0, the amplitude of the peaks at peakLocs0 1010
If necessary, remove selected bins from peakLocs0 and remove their associated peaks from peakAmps0 if their associated peaks are less than a threshold value ("secondDerivNoiseThresh") 1011
Accept selected set of peakLocs0 and peakAmps0 as peakLocs and peakAmps 1012
Obtain preliminary un-normalized dye matrix spectra ("unNormalizedDyeMatrixSpectra0") by taking the rows of crossData associated with each bin in peakLocs 1013
Raise unNormalizedDyeMatrixSpectra0 to a selected power between 0.25 and 5, preferably 1, to obtain the un-normalized dye matrix spectra ("unNormalizedDyeMatrixSpectra") 1014
Obtain normalized dye matrix spectra ("NormalizedDyeMatrixSpectra") by normalizing each row in unNormalizedDyeMatrixSpectra to the maxim value in that row. Result is DM, the internally estimate dye matrix.
DM = NormalizedDyeMatrixSpectra 1015
END 1016

FIG. 10

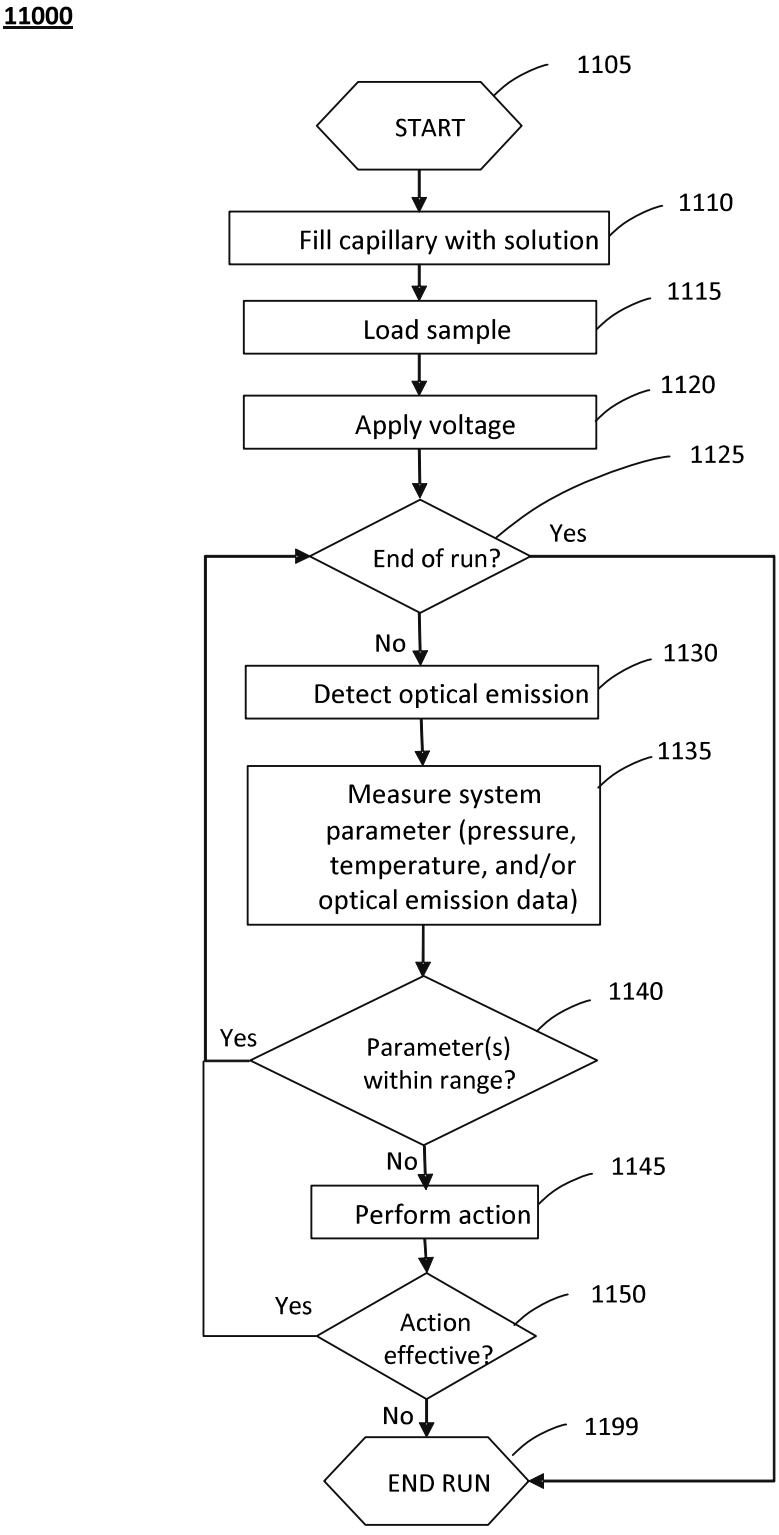
11000
1105
START
1110
Fill capillary with solution
1115
Load sample
1120
Apply voltage
1125
End of run?
Yes
No
1130
Detect optical emission
1135
Measure system parameter (pressure, temperature, and/or optical emission data)
1140
Parameter(s) within range?
Yes
No
1145
Perform action
1150
Action effective?
Yes
No
1199
END RUN

FIG. 11

12000

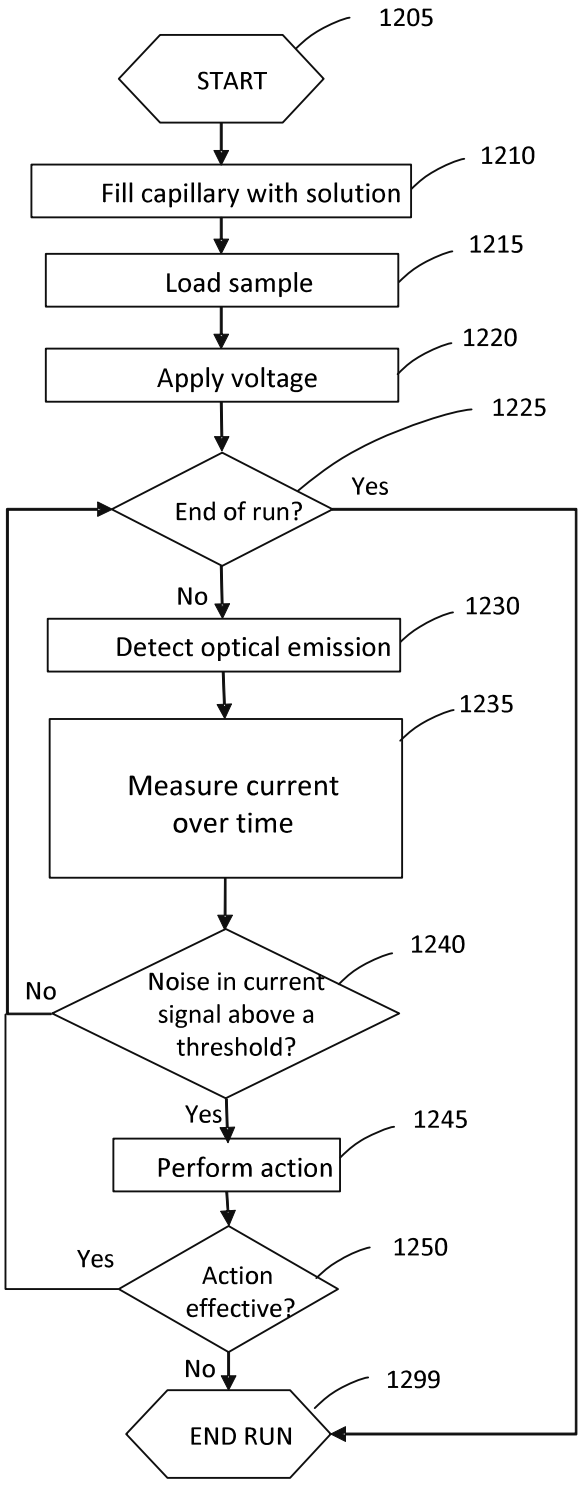
1205
START
1210
Fill capillary with solution
1215
Load sample
1220
Apply voltage
1225
End of run?
Yes
No
1230
Detect optical emission
1235
Measure current over time
1240
Noise in current signal above a threshold?
No
Yes
1245
Perform action
1250
Action effective?
Yes
No
1299
END RUN

FIG. 12

13000

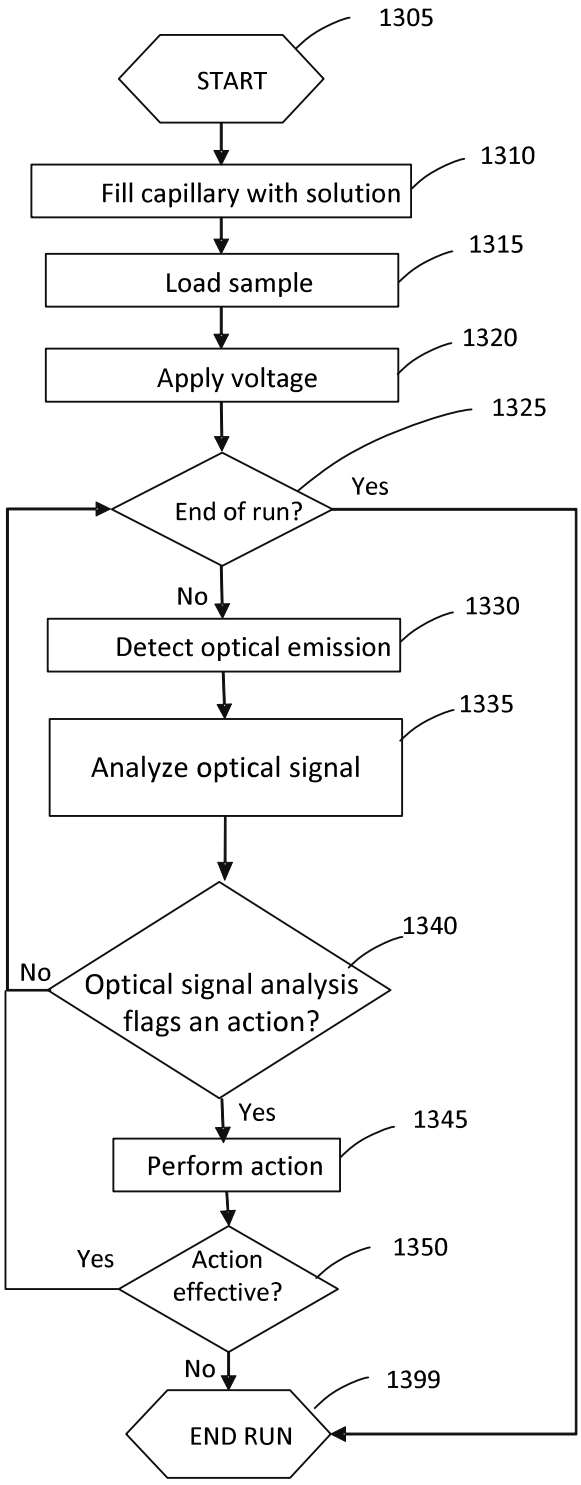
1305
START
1310
Fill capillary with solution
1315
Load sample
1320
Apply voltage
1325
End of run?
Yes
No
1330
Detect optical emission
1335
Analyze optical signal
1340
No
Optical signal analysis flags an action?
Yes
1345
Perform action
1350
Yes
Action effective?
No
1399
END RUN

FIG. 13

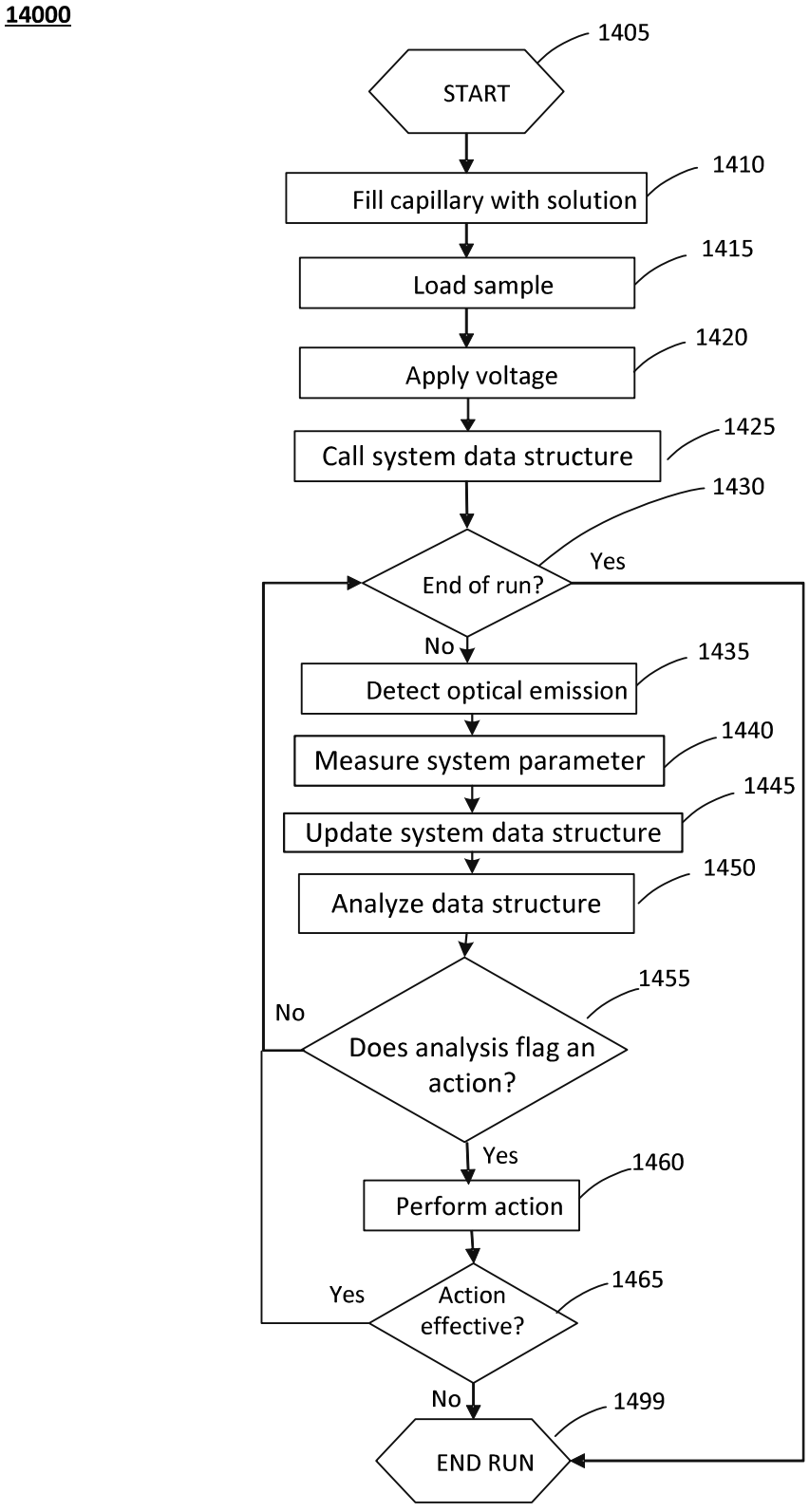
14000
1405
START
1410
Fill capillary with solution
1415
Load sample
1420
Apply voltage
1425
Call system data structure
1430
End of run?
Yes
No
1435
Detect optical emission
1440
Measure system parameter
1445
Update system data structure
1450
Analyze data structure
1455
No
Does analysis flag an action?
Yes
1460
Perform action
1465
Yes
Action effective?
No
1499
END RUN

FIG. 14

15000

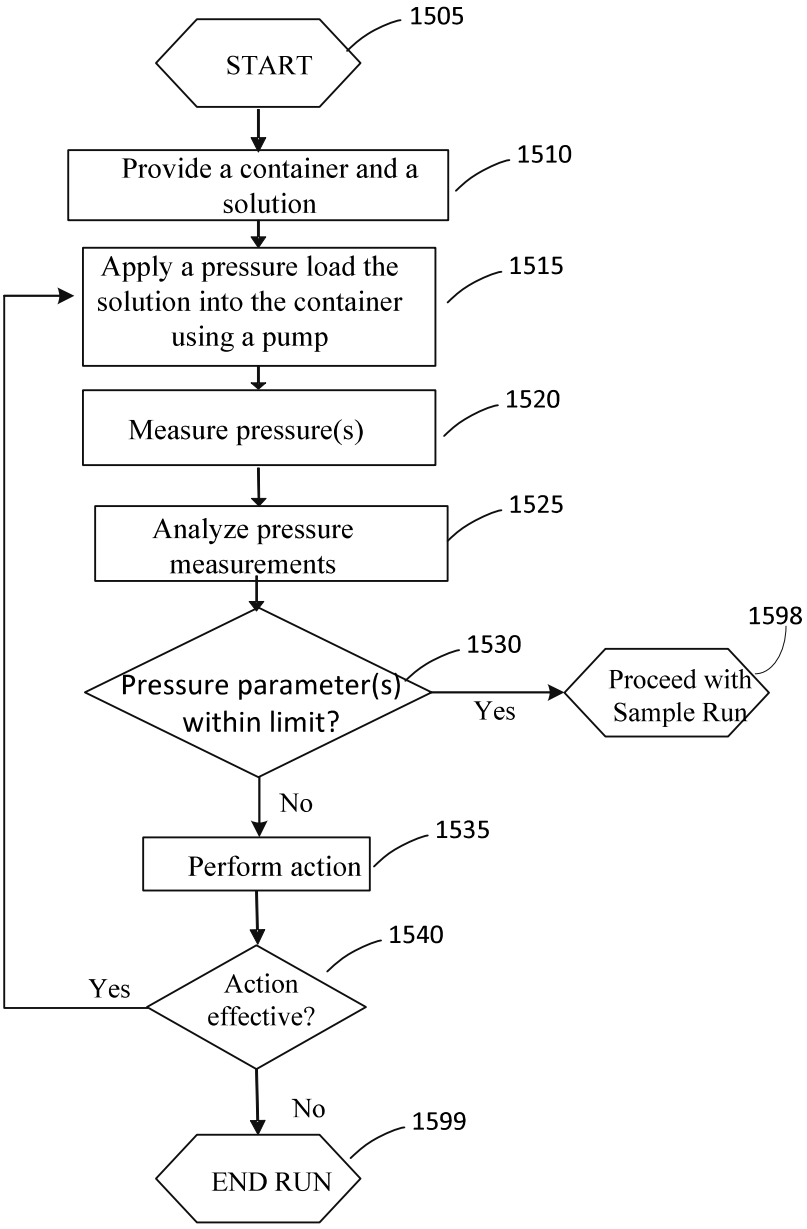
1505
START
Provide a container and a solution
1510
Apply a pressure load the solution into the container using a pump
1515
Measure pressure(s)
1520
Analyze pressure measurements
1525
Pressure parameter(s) within limit?
1530
Yes
Proceed with Sample Run
1598
No
Perform action
1535
Action effective?
1540
Yes
No
END RUN
1599

FIG. 15

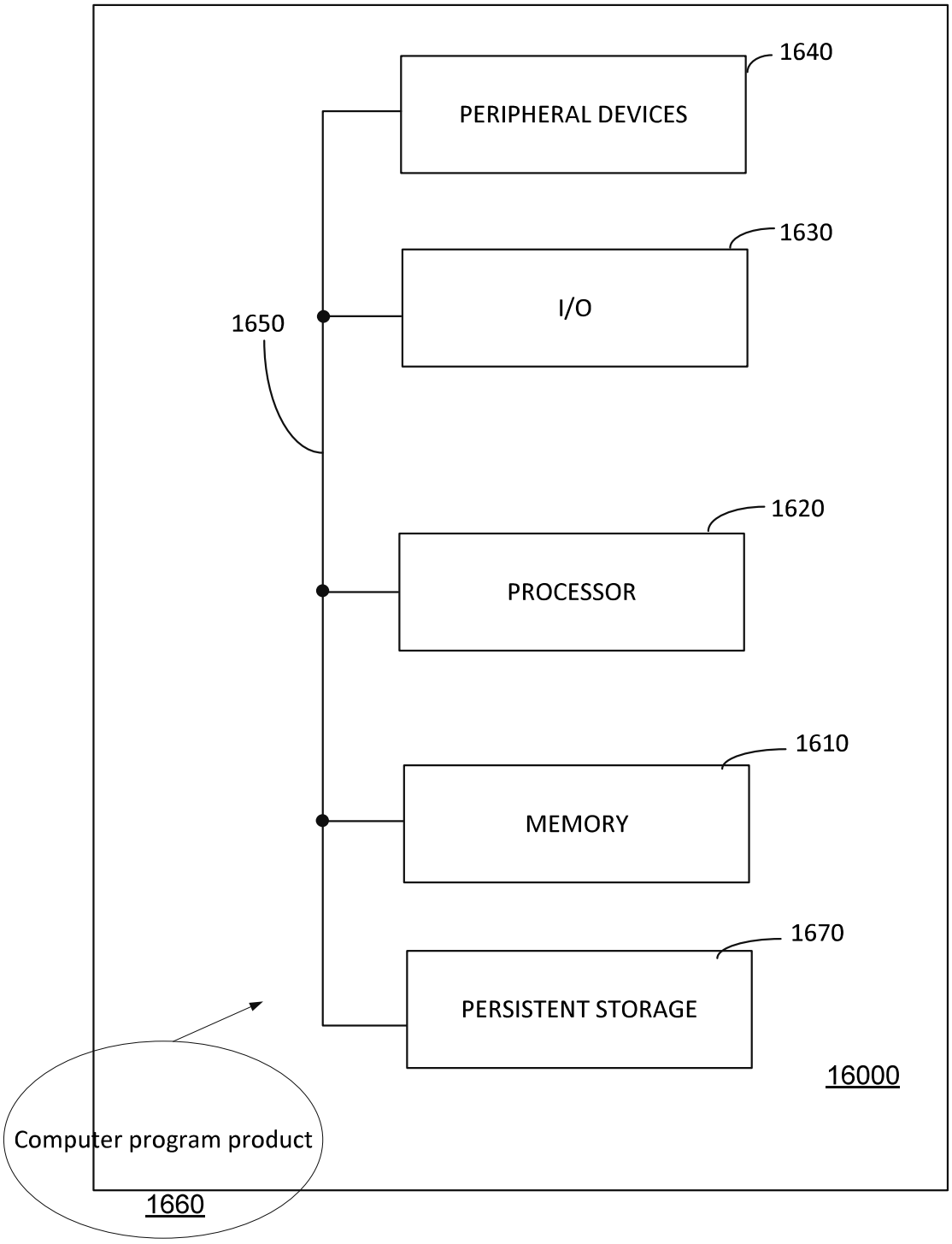
1640
PERIPHERAL DEVICES
1630
I/O
1650
1620
PROCESSOR
1610
MEMORY
1670
PERSISTENT STORAGE
16000
Computer program product
1660

FIG. 16