GC – Mt analysis

Matov Oct 1 2013

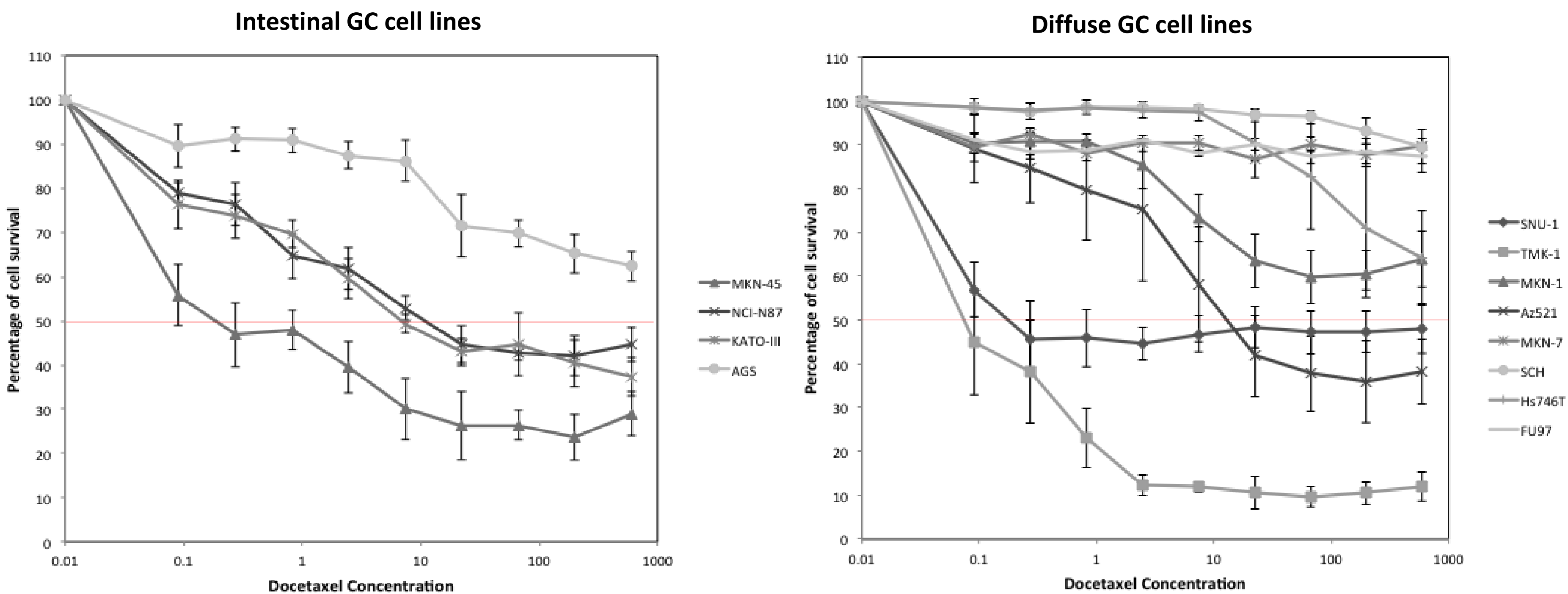
Cell Lines and Treatment

- **Cell Line : SCH – resistant (30 cells) – imaged by Peppe**
- **Cell Line : HS746T – resistant (30 cells) – imaged by Peppe**
- **Cell Line : MKN7 – resistant (30 cells) – imaged by Guang**
- **Cell Line : TMK1 – sensitive (50 cells) – imaged by Guang**
- **Cell Line : SNU1 – sensitive (30 cells) – imaged by Guang**
- **Cell Line : AZ521 – sensitive (30 cells) – imaged by Jesus**
- **Cell Line : MKN45 – sensitive, <u>intestinal</u> (30 cells) – imaged by Jesus and re-imaged by Peppe**

Imaging MTs before and after treatment with 100nM DTX for each cell line

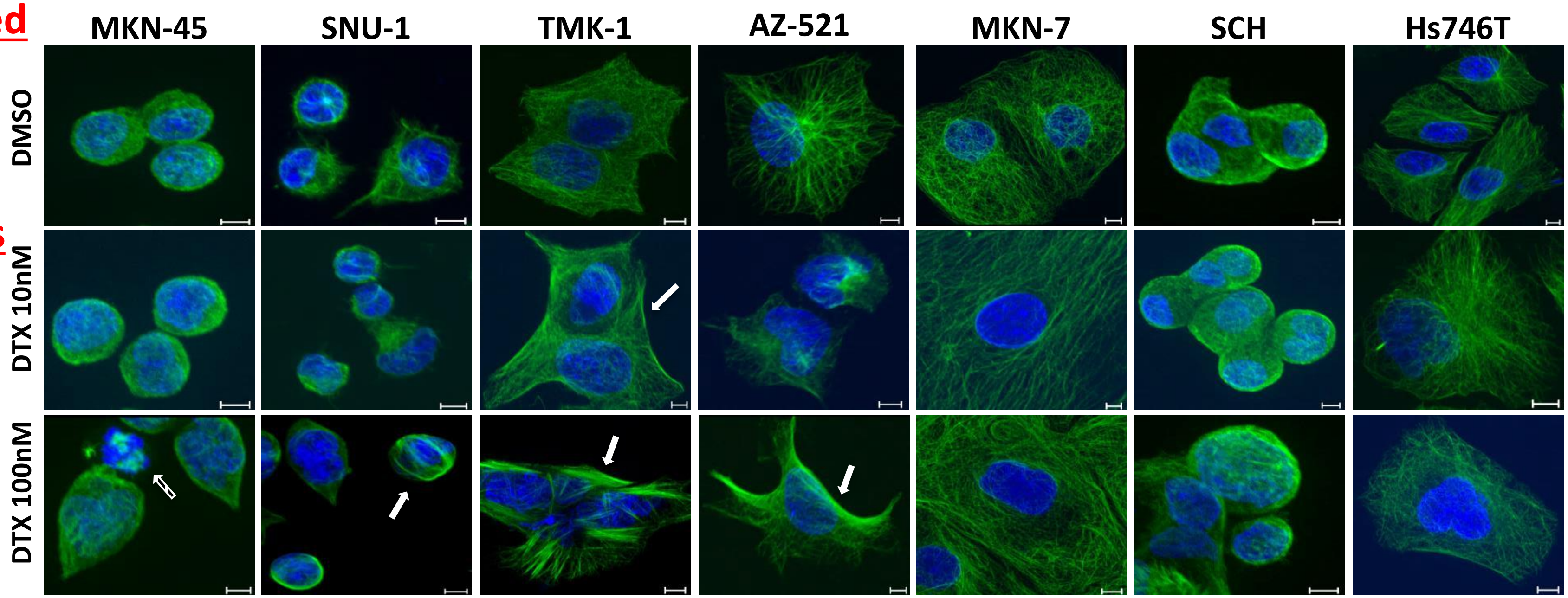
920 high magnification (63x) images in total for MT analysis (together with DAPI)

Figure 2



Intestinal GC cell lines
Percentage of cell survival
Docetaxel Concentration
110
100
90
80
70
60
50
40
30
20
10
0
0.01
0.1
1
10
100
1000
MKN-45
NCI-N87
KATO-III
AGS
Diffuse GC cell lines
Percentage of cell survival
Docetaxel Concentration
SNU-1
TMK-1
MKN-1
Az521
MKN-7
SCH
Hs746T
FU97

Corrected Test 15 of 21 Features Show changes



Feature		3	9	3	1	2	2
1 obj:number			1				
2 obj:EulerNmbr			1				
3 obj_size:aver			1				
4 obj_size:var			1				
6 obj_dist:aver					1		
7 obj_dist:var			1				
9 Edge area fract		1		1			1
10 Edge homog			1				
11 E dir maxmin		1					1
12 E dir maxnex		1					
14 Skel length			1				
15 S hul area rat				1			
19 Hull overlap			1	1			
20 Hull shape						1	
21 Hull eccentric			1			1	

TMK1ch =

-1 -1 1 1 1 1 1 1 -1 1 1 1 -1 1 -1 -1 -1 1 1 1 1 (pos means down)

MKN7ch =

-1 -1 1 1 1 1 -1 1 1 1 -1 1 1 1 -1 1 1 -1 -1 1 1 (neg means up)

AZ521ch =

1 -1 -1 -1 1 1 1 -1 1 -1 -1 -1 -1 -1 1 1 1 -1 -1 1 1

SNU1ch =

-1 -1 1 -1 -1 -1 1 -1 1 1 1 1 1 1 -1 -1 -1 -1 -1 1 1

SCHch =

1 -1 1 1 1 1 1 1 1 1 1 -1 -1 1 1 1 1 -1 -1 1 1

HS746Tch =

1 1 -1 -1 -1 -1 -1 -1 1 1 1 -1 1 -1 -1 -1 -1 1 -1 1 1

21 MT CHANNEL FEATURES in 4 CATEGORIES

CELL MORPHOLOGY 1-8

1) object: number – number of fluorescent objects in image

2) object: EurlerNumber - number of objects in the image minus the total number of holes in those objects – **distinguishes reticular or mesh-like patterns vs more uniformly distributed patterns**

3) object_size:average - The average number of above-threshold pixels per object – captures information about the **size of objects in cell MT area**

4) object_size:variance - The variance of the number of above-threshold pixels per object – quantifies the homogeneity of fluorescent objects in cells

5) object_size:ratio - The ratio of the size of the largest object to the smallest within the cell – assessing the distribution of florescent object sizes

6) object_distance: average – average object distance to cell center of fluorescence (COF) – provides information about how individual objects are distributed throughout the cell

7) object_distance: variance – variance of object distance to cell center of fl (COF) – captures information about the distribution of objects around a central point

8) Object_distance: ratio – ratio of largest to smallest distance to cell center of fl (COF – center of fluorescence)

CELLULAR EDGES 1-5

9) edges:area_fraction - fraction of the non-zero pixels in a cell that are along an edge – **distinguishes protein that localizes along the edges**

10) edges:homogeneity - Measure of edge intensity homogeneity - captures homogeneity of edge gradients, or '**are the edges primarily steep or more gradually slopin**g?'

11) edges:direction_maxmin_ratio - Measure of edge direction homogeneity 1 – captures homogeneity of edge direction, or are the edges primarily in one direction or are they more evenly distributed? images with **patterns containing edges oriented predominantly along a particular direction** result in edge gradient histograms

12) edges:direction_maxnextmax_ratio - Measure of edge direction homogeneity 2 – ratio of the largest to the next largest value in the histogram from above feature

13) edges:direction_difference - Measure of edge direction difference - this feature **distinguish MT patterns in which there are parallel edges**

CELLULAR MOPHOLOGICAL SKELETON 1-5

14) obj_skel_len - The average length of the morphological skeleton of objects

15) obj_skel_hull_area_ratio - The ratio of object skeleton length to the area of the convex hull of the skeleton, averaged over all objects

16) obj_skel obj area ratio - The fraction of object pixels contained within the skeleton

17) obj_skel obj fluor ratio - The fraction of object fluorescence contained within the skeleton

18) obj_skel_branch_per_len - The ratio of the number of branch points in skeleton to length of skeleton

CELLULAR CONVEX HULL 1-3

19) convex_hull: fraction of overlap - fraction of convex hull occupied by protein fluorescence (above-threshold pixels)

20) convex_hull: shape_factor- roundness of convex hull

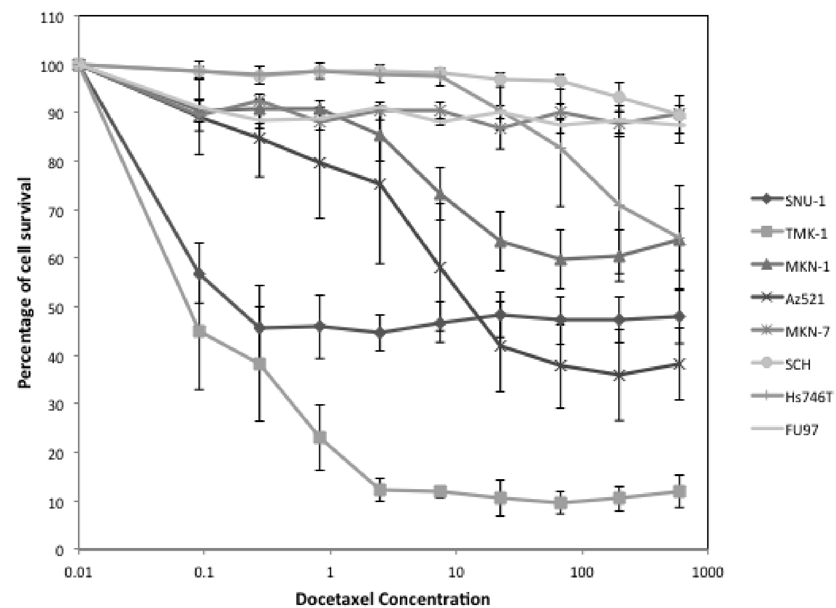
21) convex_hull: eccentricity - eccentricity (elongation) of convex hull

Features

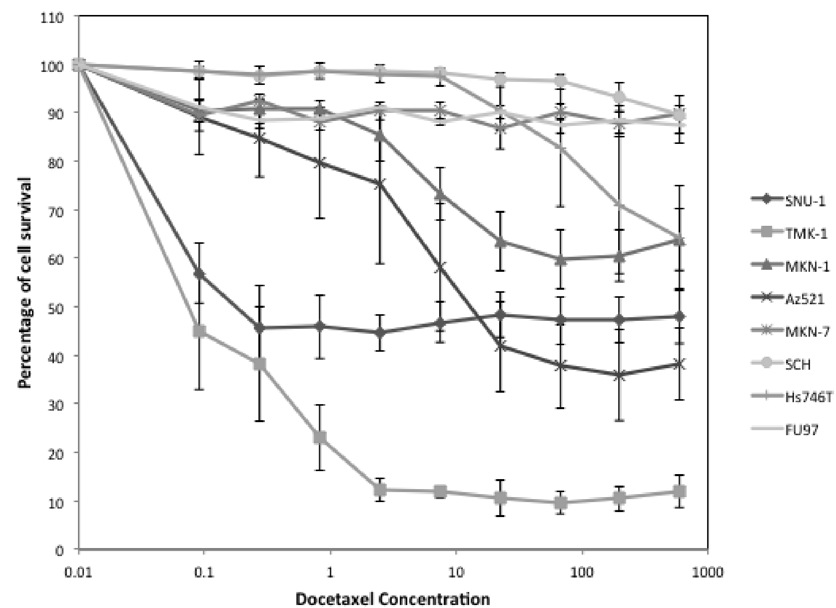
Comparison of subcellular location feature sets. All features that measure length or area are calculated in pixels that are 0.23 μm square in the sample plane.

Feature description	SLF3	SLF7
Morphological features: Number of fluorescent objects in image, Euler number of image, average object size, variance of object size, ratio of largest to smallest object size, average object distance to cell center of fluorescence, variance of object distance to cell center, ratio of largest to smallest object distance to cell center	SLF1.1 through SLF1.8	SLF1.1 through SLF1.8
Edge-related features: Fraction of above-threshold pixels along edge, measure of edge gradient intensity homogeneity, measure of edge direction homogeneity 1, measure of edge direction homogeneity 2, measure of edge direction difference	SLF1.9 through SLF1.13	SLF7.9 through SLF7.13 (minor error corrections)
Convex hull features: Fraction of convex hull occupied by above-threshold pixels, roundess of convex hull, eccentricity of convex hull	SLF1.14 through SLF1.16	SLF1.14 through SLF1.16
Zernike moment features through order 12, calculated for a unit circle with radius equal to the average radius of the cell type being analyzed (150 pixels or 34.5 μm for HeLa)	SLF3.17 through SLF3.65	SLF3.17 through SLF3.65
Haralick texture features: angular second moment, contrast, correlation, sum of squares variation, inverse difference moment, sum average, sum variance, sum entropy, entropy, difference variance, difference entropy, info. measure of correlation 1, info. measure of correlation 2	SLF3.66 through SLF3.78	SLF7.66 through SLF7.78 (after downsampling to 1.15 μm/pixel and 256 gray levels)
Fraction of non-object fluorescence	–	SLF7.79
Skeleton features (see text)	–	SLF7.80 through SLF7.84

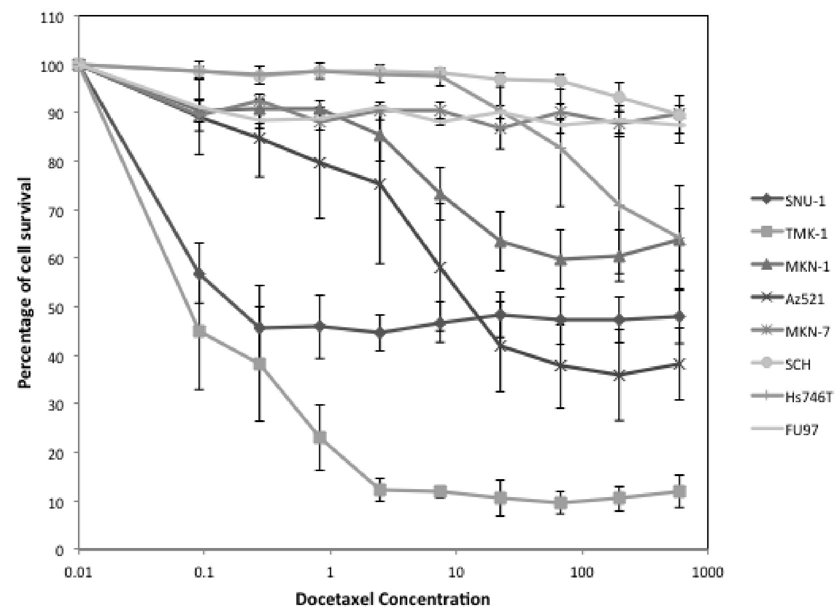
- Cell Line:SCH
- FeatureName p<0.0024 p
- object:number 0 0.3666
- object:EulerNumber 0 0.0468
- object_size:average 0 0.9385
- object_size:variance 0 0.1852
- object_size:ratio 0 0.0530
- object_distance:average 0 0.1785
- object_distance:variance 0 0.0292
- object_distance:ratio 0 0.7446
- edges:area_fraction 0 0.0177
- edges:homogeneity 0 0.9207
- edges:direction_maxmin_ratio 0 0.1135
- edges:direction_maxnextmax_ratio 0 0.0073
- edges:direction_difference 0 0.2082
- obj_skel_len 0 0.5825
- obj_skel_hull_area_ratio 0 0.1697
- obj_skel_obj_area_ratio 0 0.1122
- obj_skel_obj_fluor_ratio 0 0.0993
- obj_skel_branch_per_len 0 0.2128
- convex_hull:fraction_of_overlap 0 0.0164
- convex_hull:shape_factor 1 0.0015
- convex_hull:eccentricity 1 0.0004



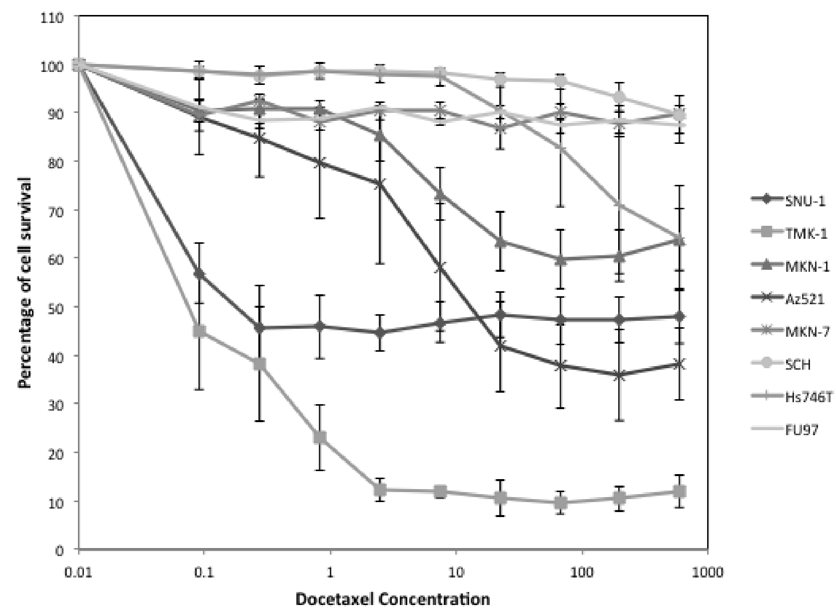
- Cell Line:HS746T
- FeatureName p<0.0024 p
- object:number 0 0.4485
- object:EulerNumber 0 0.0646
- object_size:average 0 0.6876
- object_size:variance 0 0.5237
- object_size:ratio 0 0.0279
- object_distance:average 0 0.3173
- object_distance:variance 0 0.2418
- object_distance:ratio 0 0.8296
- edges:area_fraction 1 0.0011
- edges:homogeneity 0 0.7999
- edges:direction_maxmin_ratio 1 0.0004
- edges:direction_maxnextmax_ratio 0 0.4087
- edges:direction_difference 0 0.1352
- obj_skel_len 0 0.5460
- obj_skel_hull_area_ratio 0 0.3784
- obj_skel_obj_area_ratio 0 0.9464
- obj_skel_obj_fluor_ratio 0 0.9765
- obj_skel_branch_per_len 0 0.4570
- convex_hull:fraction_of_overlap 0 0.3399
- convex_hull:shape_factor 0 0.3004
- convex_hull:eccentricity 0 0.8351



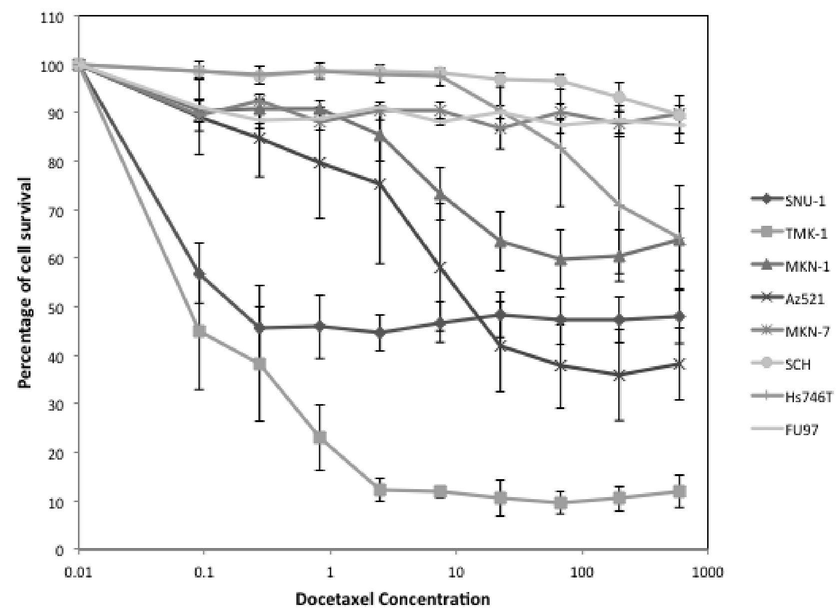
- Cell Line:MKN7
- FeatureName p<0.0024 p
- object:number 0 0.5177
- object:EulerNumber 0 0.1326
- object_size:average 0 0.0890
- object_size:variance 0 0.0159
- object_size:ratio 0 0.0283
- object_distance:average 1 0.0000
- object_distance:variance 0 0.3068
- object_distance:ratio 0 0.1857
- edges:area_fraction 0 0.2718
- edges:homogeneity 0 0.0659
- edges:direction_maxmin_ratio 0 0.2596
- edges:direction_maxnextmax_ratio 0 0.0596
- edges:direction_difference 0 0.1094
- obj_skel_len 0 0.0373
- obj_skel_hull_area_ratio 0 0.7156
- obj_skel_obj_area_ratio 0 0.1170
- obj_skel_obj_fluor_ratio 0 0.1162
- obj_skel_branch_per_len 0 0.0145
- convex_hull:fraction_of_overlap 0 0.2667
- convex_hull:shape_factor 0 0.0687
- convex_hull:eccentricity 0 0.6528



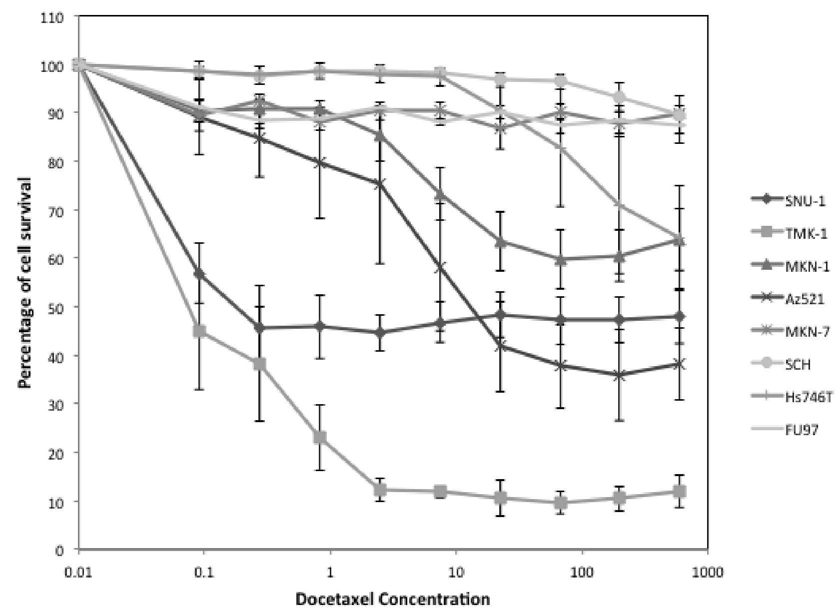
- Cell Line:TMK1
- FeatureName p<0.0024 p
- object:number 1 0.0004
- object:EulerNumber 1 0.0000
- object_size:average 1 0.0000
- object_size:variance 1 0.0000
- object_size:ratio 0 0.0181
- object_distance:average 0 0.0489
- object_distance:variance 1 0.0001
- object_distance:ratio 0 0.0528
- edges:area_fraction 0 0.0135
- edges:homogeneity 1 0.0000
- edges:direction_maxmin_ratio 0 0.0090
- edges:direction_maxnextmax_ratio 0 0.1082
- edges:direction_difference 0 0.9562
- obj_skel_len 1 0.0000
- obj_skel_hull_area_ratio 0 0.2486
- obj_skel_obj_area_ratio 0 0.4571
- obj_skel_obj_fluor_ratio 0 0.4648
- obj_skel_branch_per_len 0 0.6773
- convex_hull:fraction_of_overlap 1 0.0000
- convex_hull:shape_factor 0 0.0081
- convex_hull:eccentricity 1 0.0004



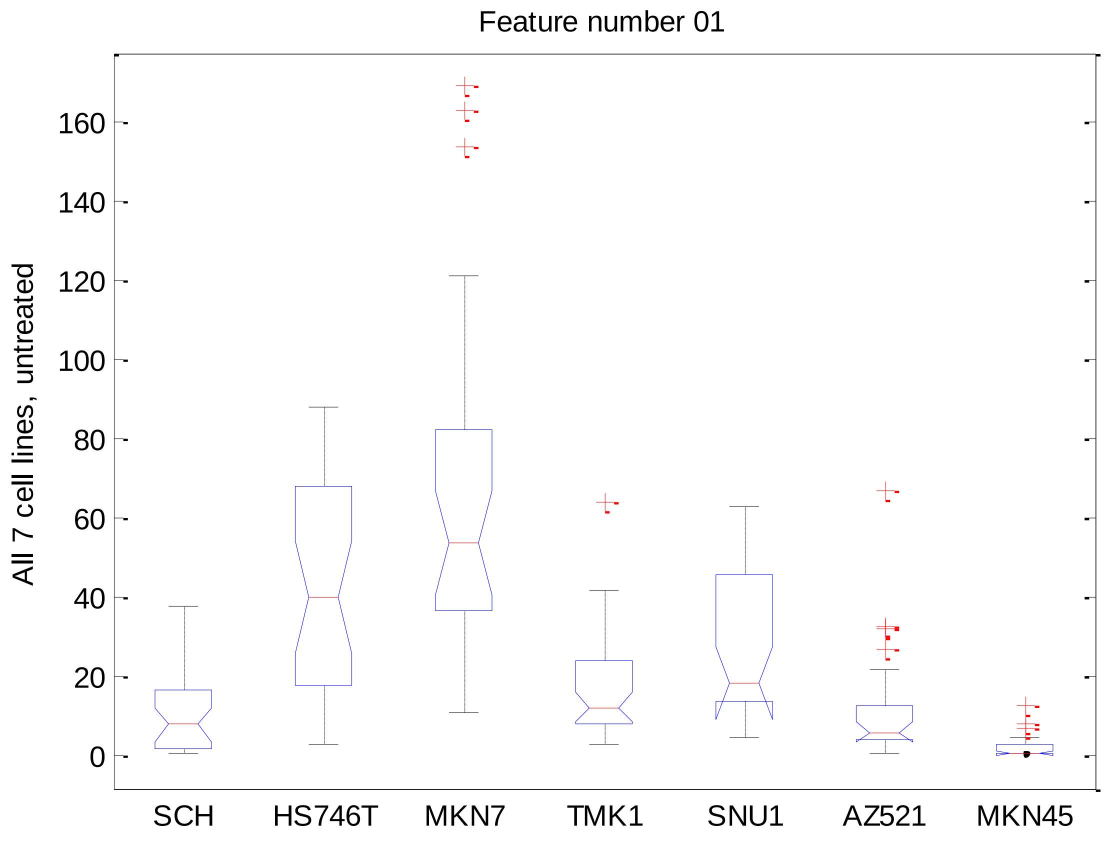
- Cell Line:SNU1
- FeatureName p<0.0024 p
- object:number 0 0.2043
- object:EulerNumber 0 0.0279
- object_size:average 0 0.0390
- object_size:variance 0 0.1368
- object_size:ratio 0 0.3181
- object_distance:average 0 0.2248
- object_distance:variance 0 0.9796
- object_distance:ratio 0 0.6974
- edges:area_fraction 1 0.0000
- edges:homogeneity 0 0.0435
- edges:direction_maxmin_ratio 1 0.0021
- edges:direction_maxnextmax_ratio 1 0.0003
- edges:direction_difference 0 0.0080
- obj_skel_len 0 0.1965
- obj_skel_hull_area_ratio 0 0.2808
- obj_skel_obj_area_ratio 0 0.1603
- obj_skel_obj_fluor_ratio 0 0.1551
- obj_skel_branch_per_len 0 0.8024
- convex_hull:fraction_of_overlap 0 0.2285
- convex_hull:shape_factor 0 0.5757
- convex_hull:eccentricity 0 0.6879



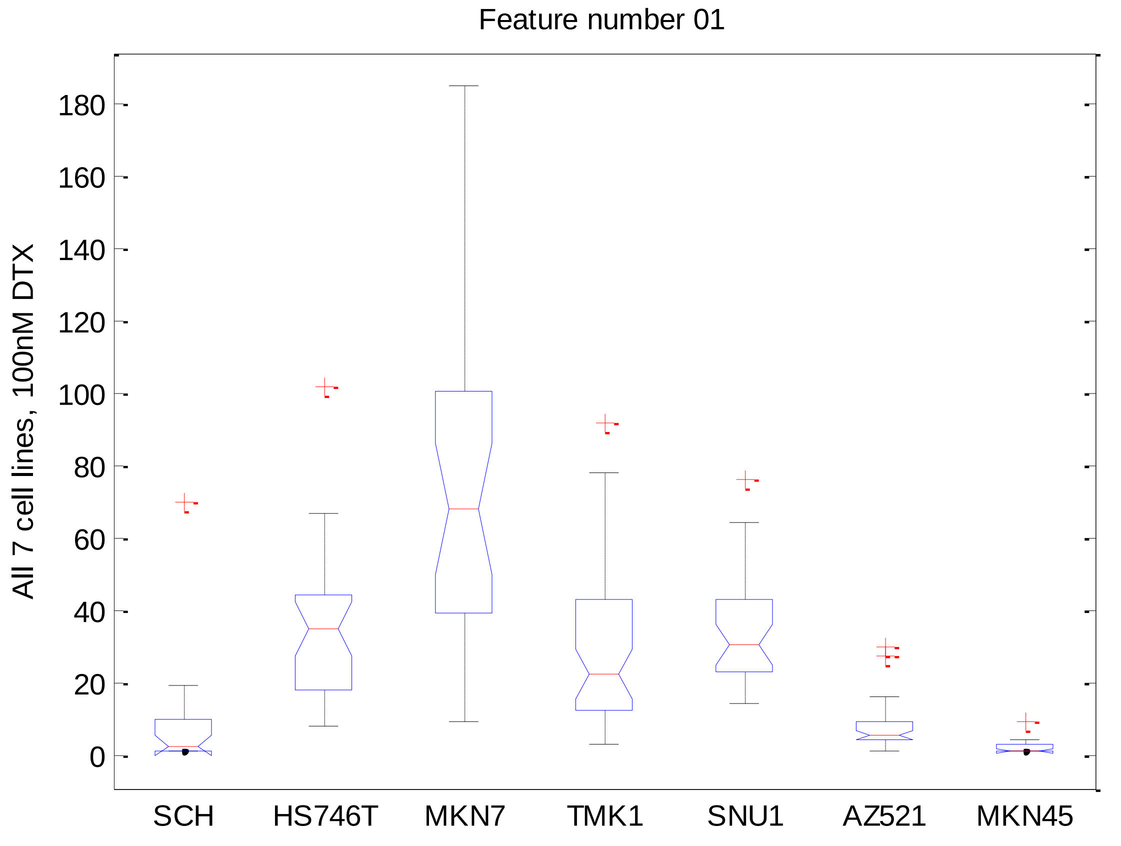
- Cell Line:AZ521
- FeatureName p<0.0024 p
- object:number 0 0.1103
- object:EulerNumber 0 0.0785
- object_size:average 0 0.0254
- object_size:variance 0 0.1188
- object_size:ratio 0 0.0263
- object_distance:average 0 0.0692
- object_distance:variance 0 0.5256
- object_distance:ratio 0 0.4200
- edges:area_fraction 1 0.0000
- edges:homogeneity 0 0.1146
- edges:direction_maxmin_ratio 0 0.0136
- edges:direction_maxnextmax_ratio 0 0.0723
- edges:direction_difference 0 0.1317
- obj_skel_len 0 0.0038
- obj_skel_hull_area_ratio 1 0.0003
- obj_skel_obj_area_ratio 0 0.6782
- obj_skel_obj_fluor_ratio 0 0.8323
- obj_skel_branch_per_len 0 0.9507
- convex_hull:fraction_of_overlap 1 0.0001
- convex_hull:shape_factor 0 0.0063
- convex_hull:eccentricity 0 0.1111



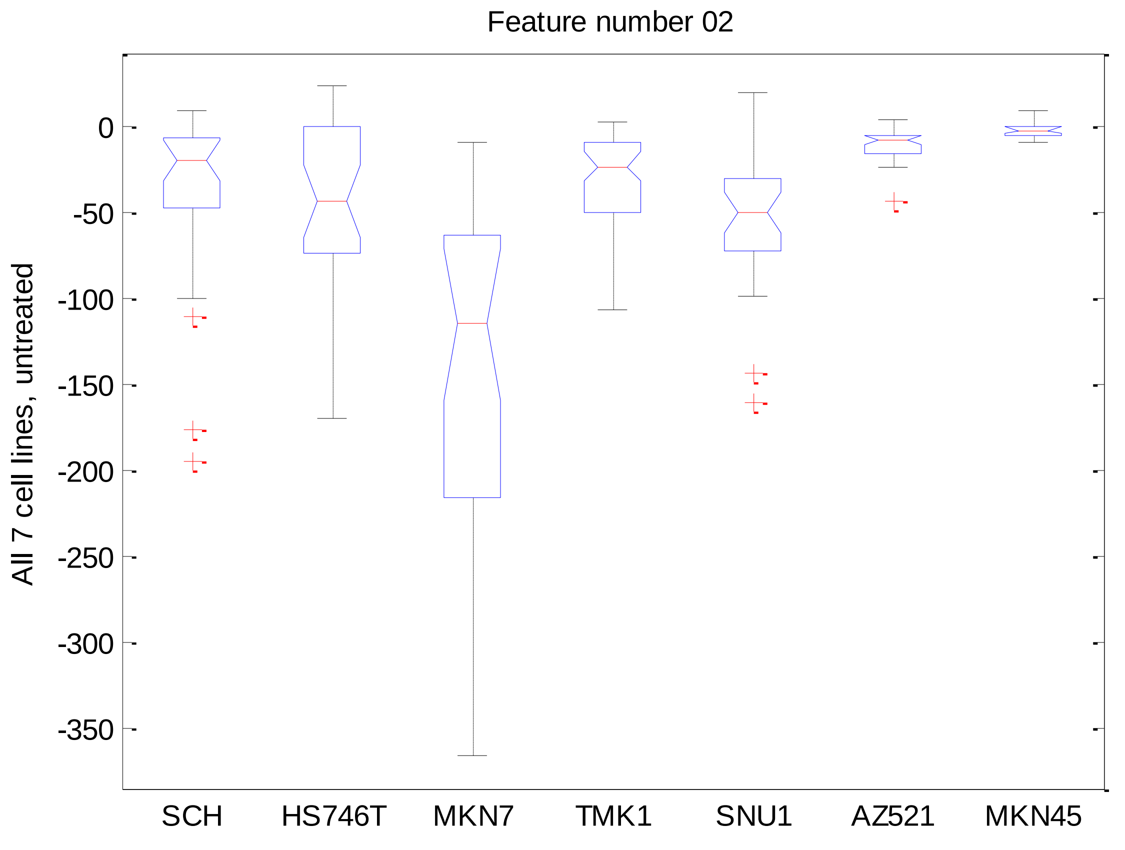
- Cell Line:MKN45
- FeatureName p<0.0024 p
- object:number 0 0.3860
- object:EulerNumber 0 0.0151
- object_size:average 1 0.0004
- object_size:variance 0 0.0234
- object_size:ratio 0 0.5540
- object_distance:average 0 0.6923
- object_distance:variance 0 0.4520
- object_distance:ratio 0 0.4829
- edges:area_fraction 0 0.0285
- edges:homogeneity 0 0.0036
- edges:direction_maxmin_ratio 0 0.6929
- edges:direction_maxnextmax_ratio 0 0.2949
- edges:direction_difference 0 0.5692
- obj_skel_len 1 0.0023
- obj_skel_hull_area_ratio 0 0.7300
- obj_skel_obj_area_ratio 0 0.7947
- obj_skel_obj_fluor_ratio 0 0.7844
- obj_skel_branch_per_len 0 0.2782
- convex_hull:fraction_of_overlap 0 0.1549
- convex_hull:shape_factor 0 0.7487
- convex_hull:eccentricity 0 0.3172



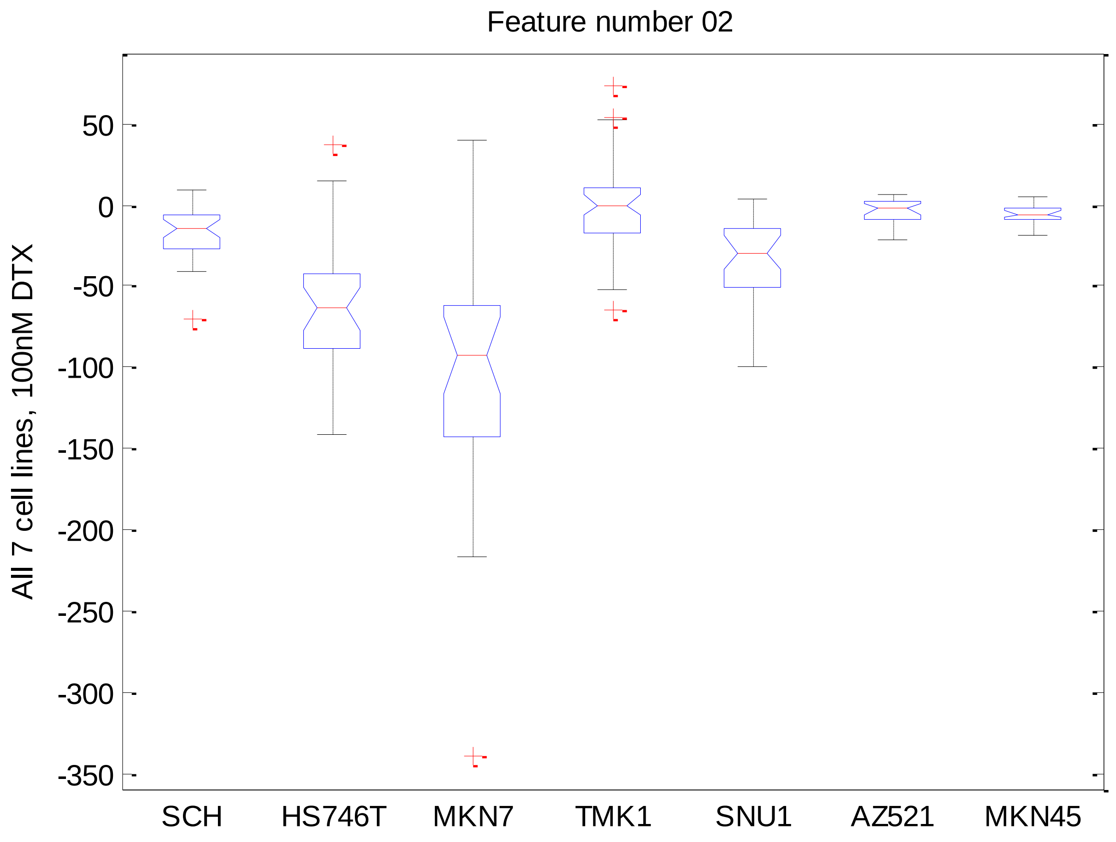
Feature number 01
All 7 cell lines, untreated
160
140
120
100
80
60
40
20
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



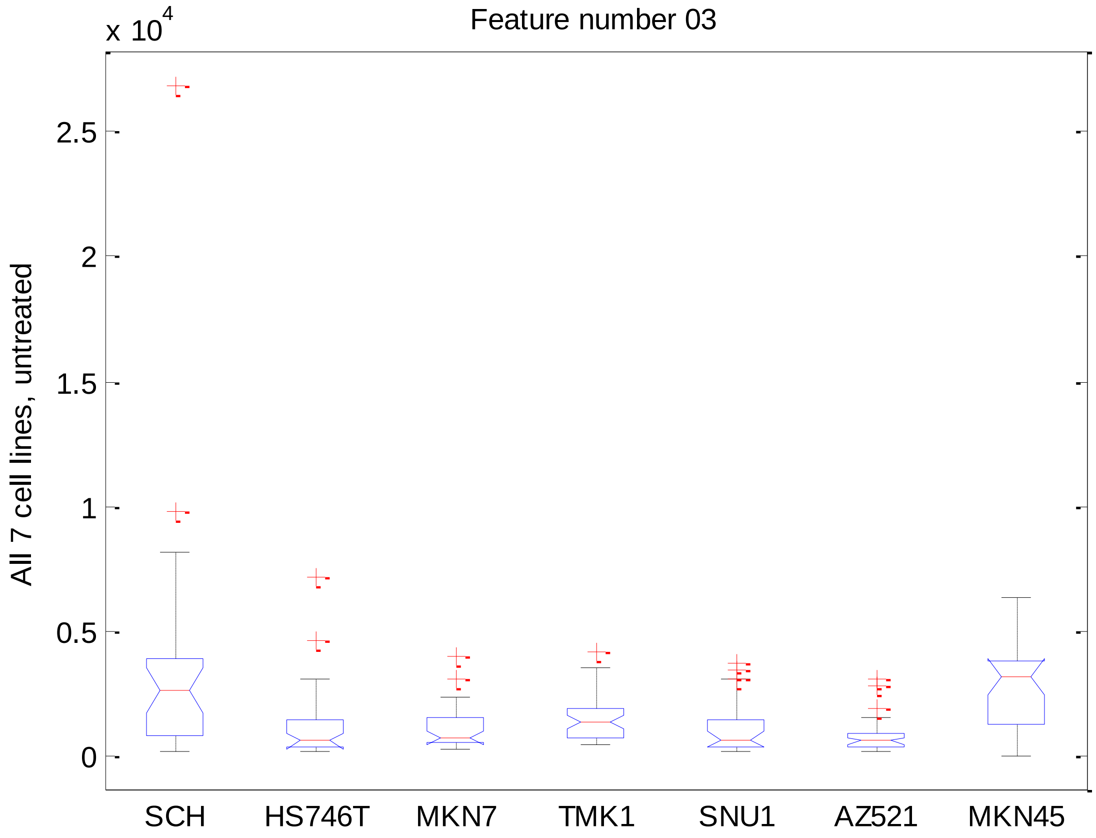
Feature number 01
All 7 cell lines, 100nM DTX
0
20
40
60
80
100
120
140
160
180
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

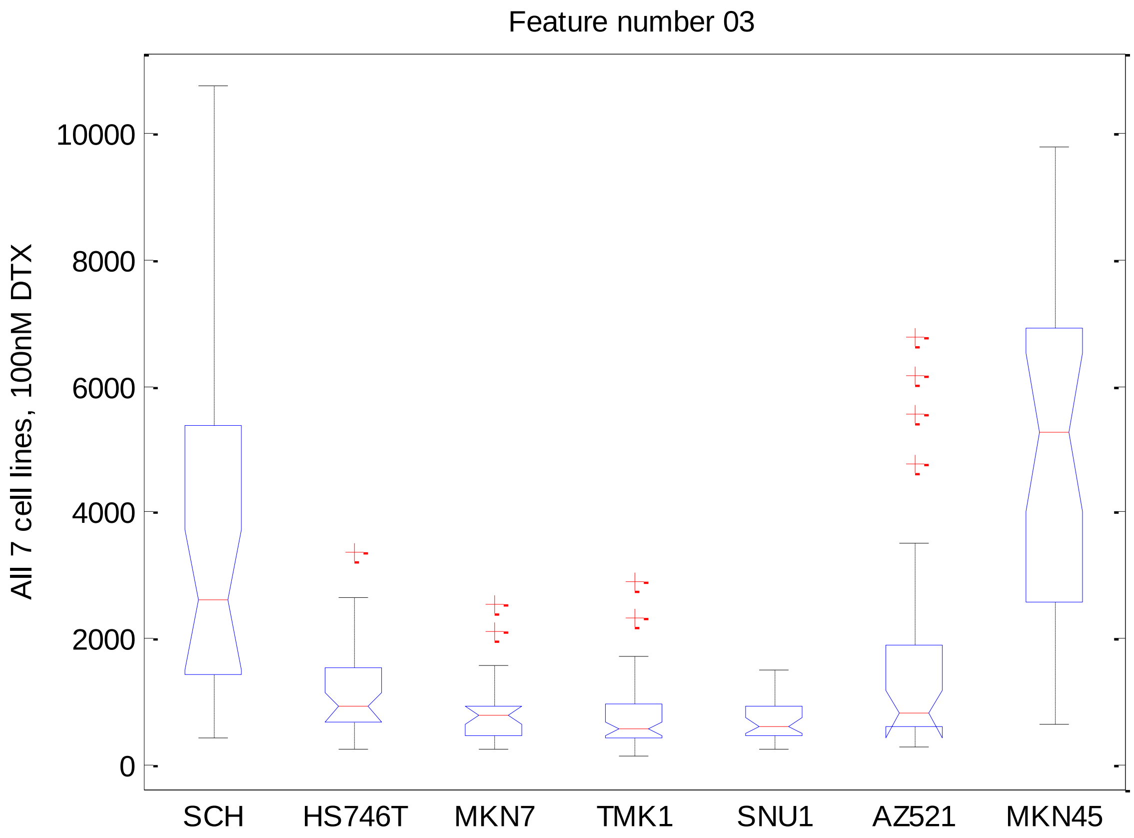


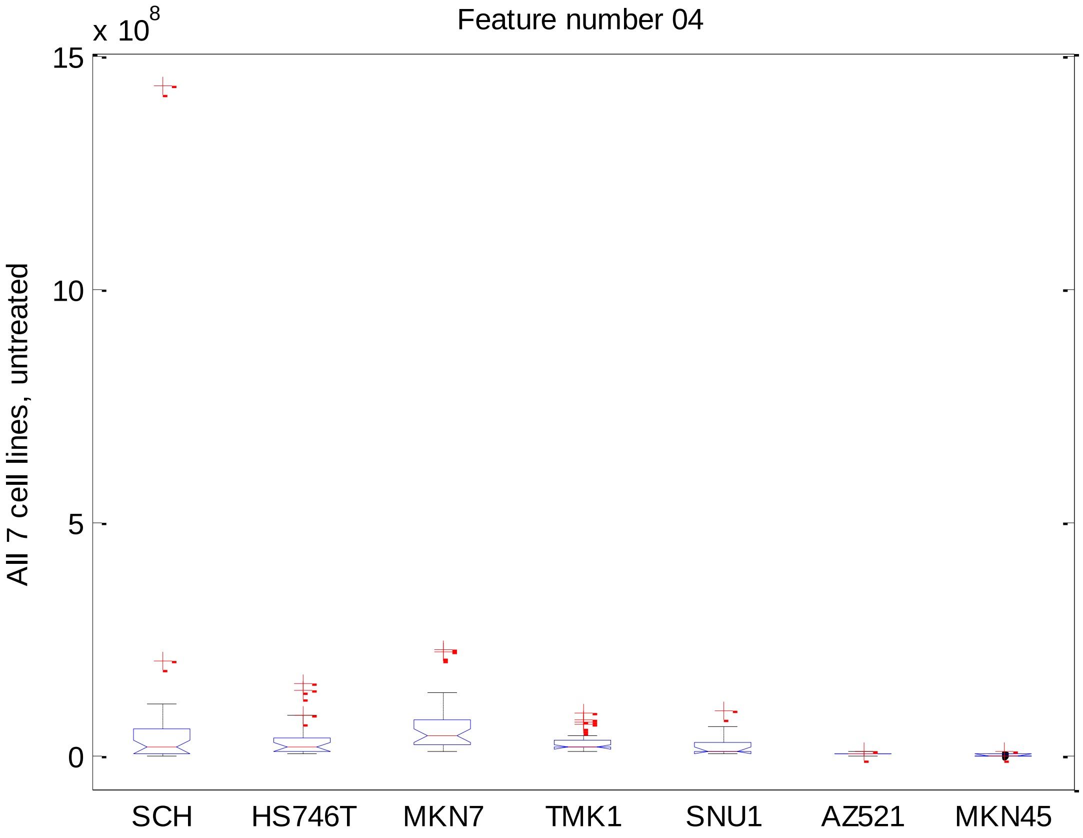
Feature number 02
All 7 cell lines, untreated
0
-50
-100
-150
-200
-250
-300
-350
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



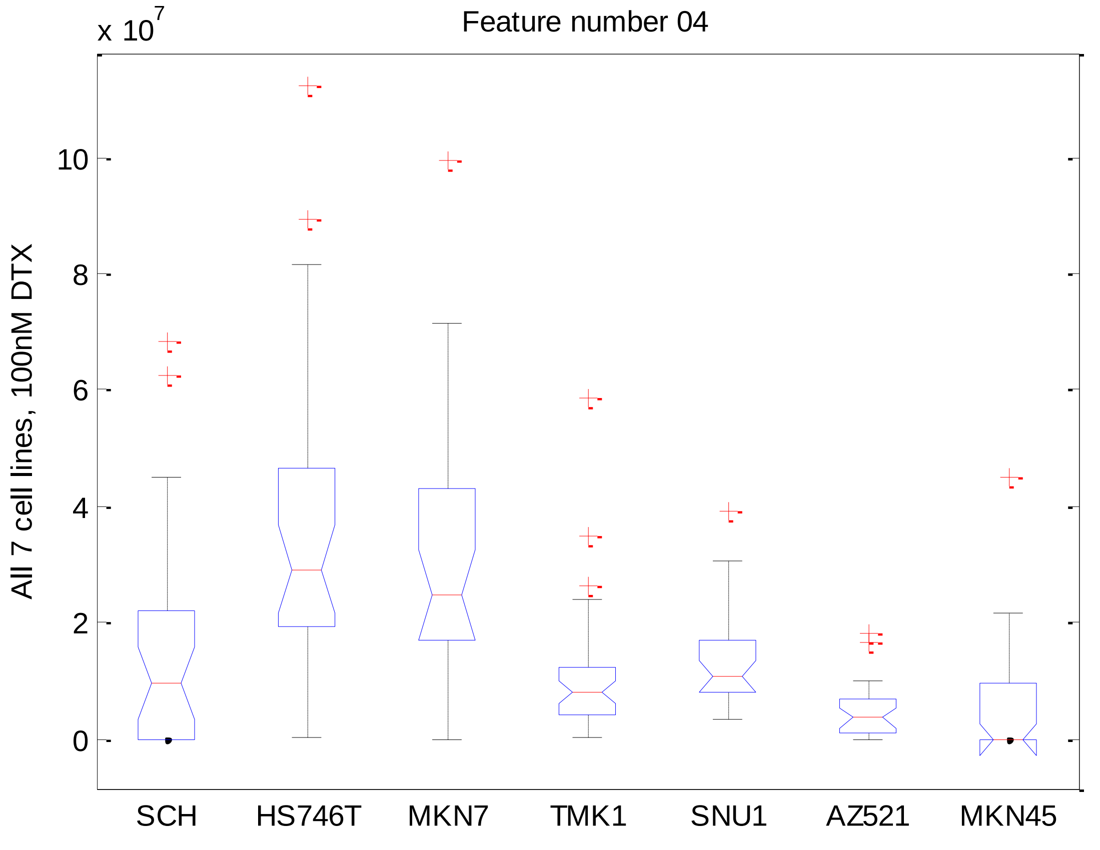
Feature number 02
All 7 cell lines, 100nM DTX
50
0
-50
-100
-150
-200
-250
-300
-350
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

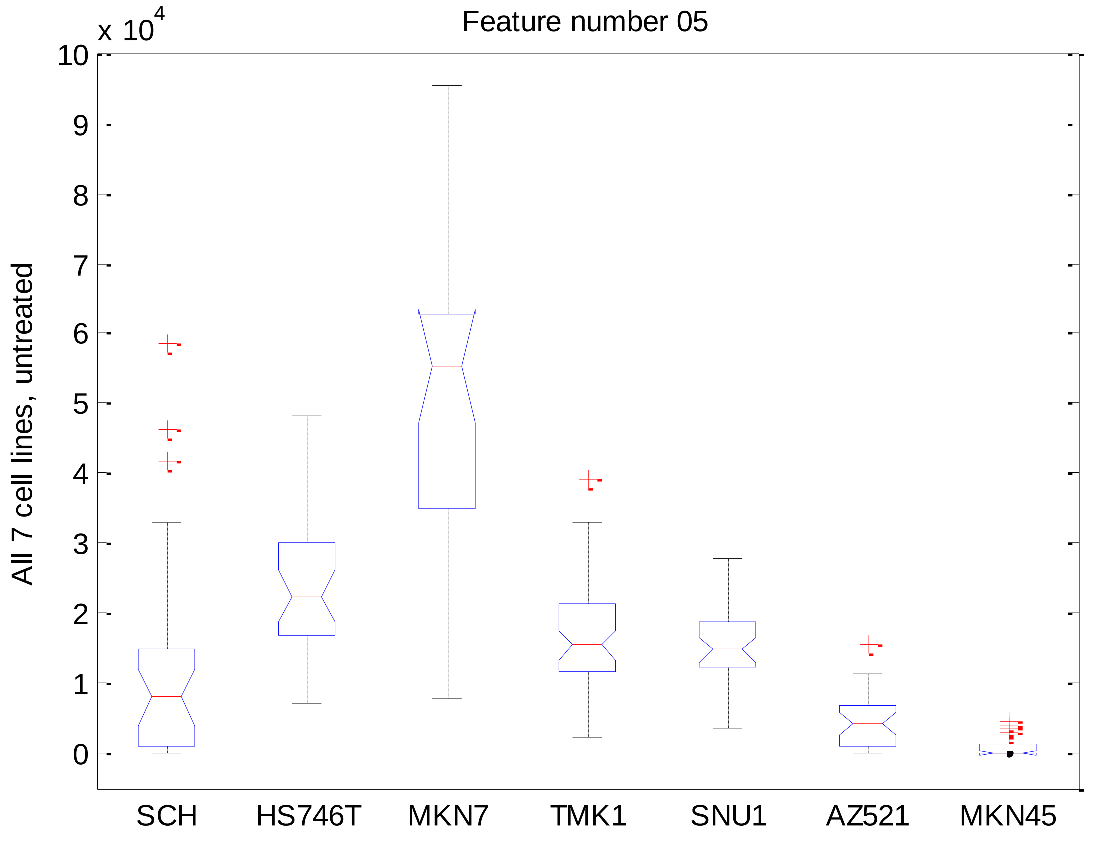

Feature number 03
x 10^4
2.5
2
1.5
1
0.5
0
All 7 cell lines, untreated
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

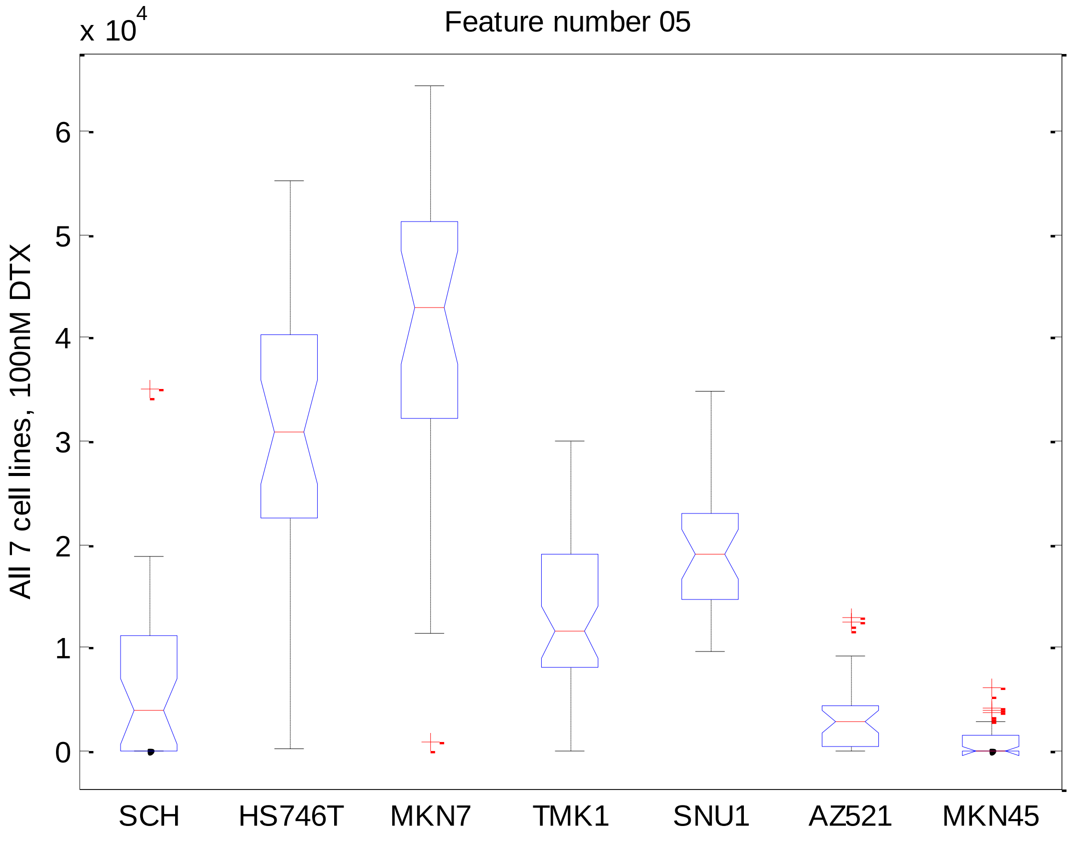

Feature number 03
All 7 cell lines, 100nM DTX
10000
8000
6000
4000
2000
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



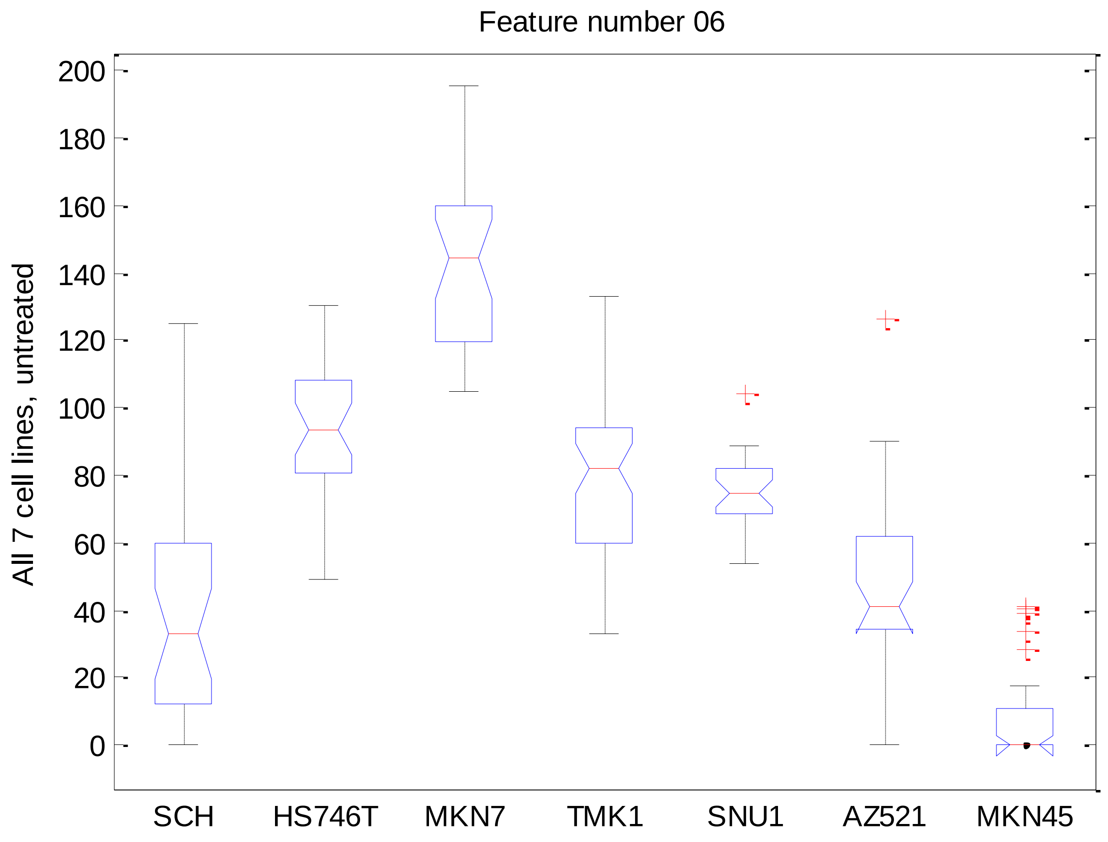
Feature number 04
x 10^8
15
10
5
0
All 7 cell lines, untreated
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

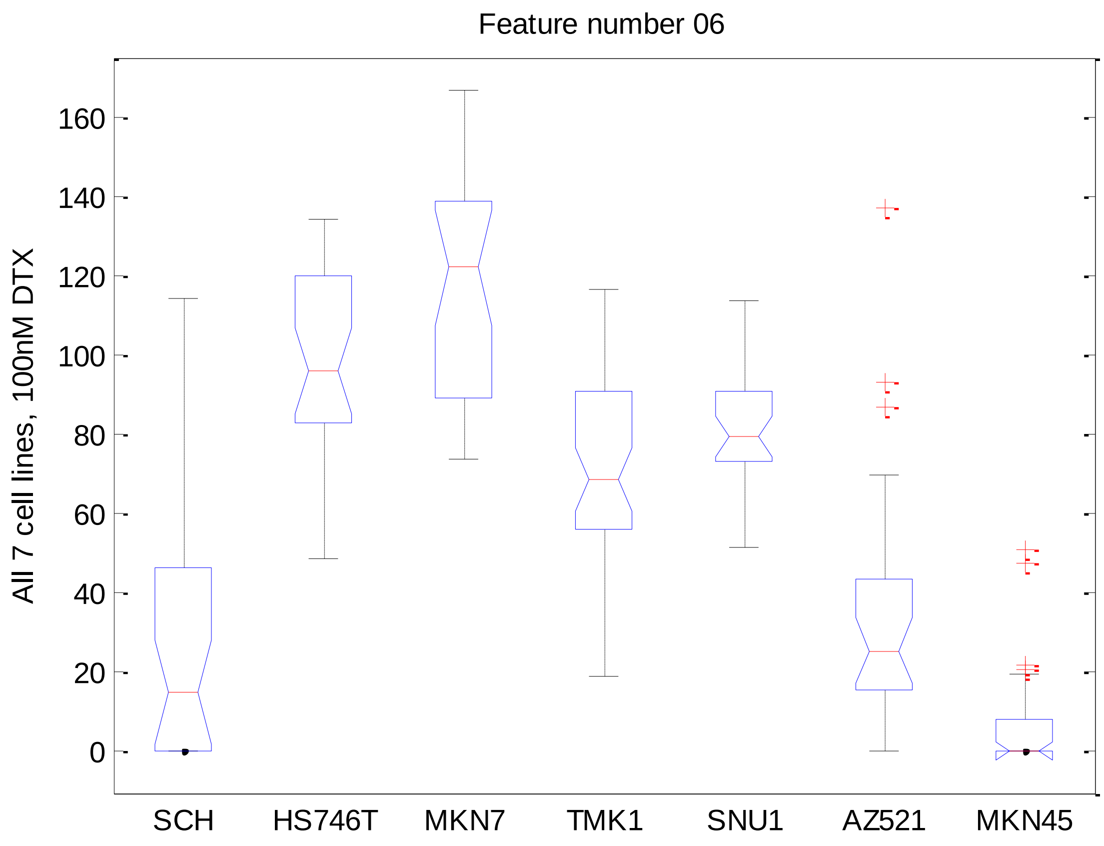

Feature number 04
x 10^7
All 7 cell lines, 100nM DTX
0
2
4
6
8
10
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

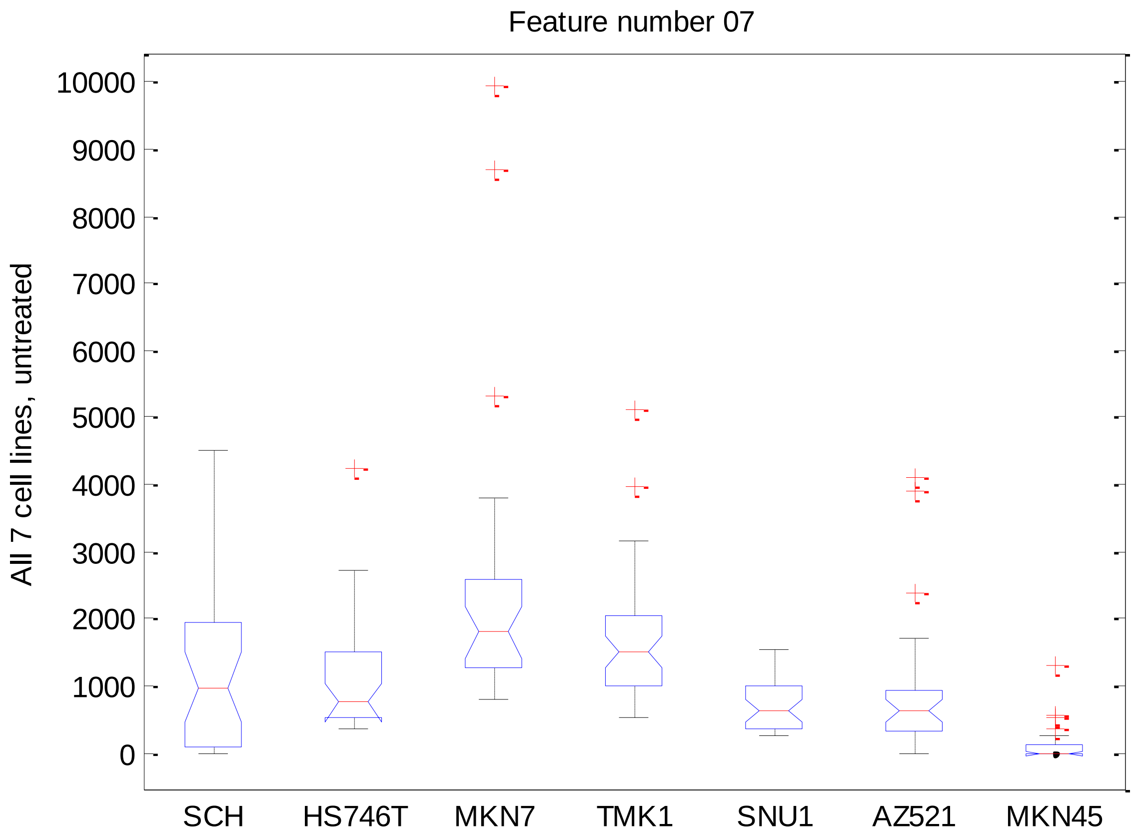

Feature number 05
x 10^4
10
9
8
7
6
5
4
3
2
1
0
All 7 cell lines, untreated
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

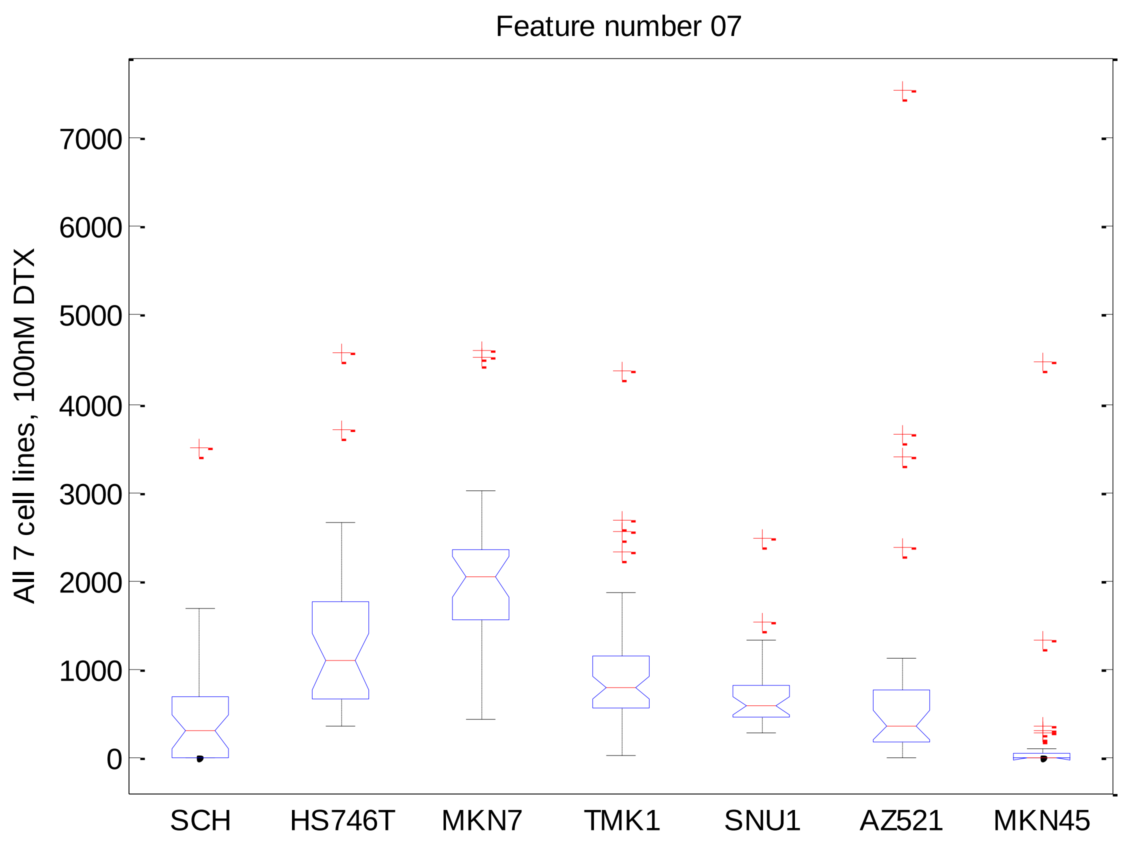


Feature number 05
x 10^4
All 7 cell lines, 100nM DTX
0
1
2
3
4
5
6
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

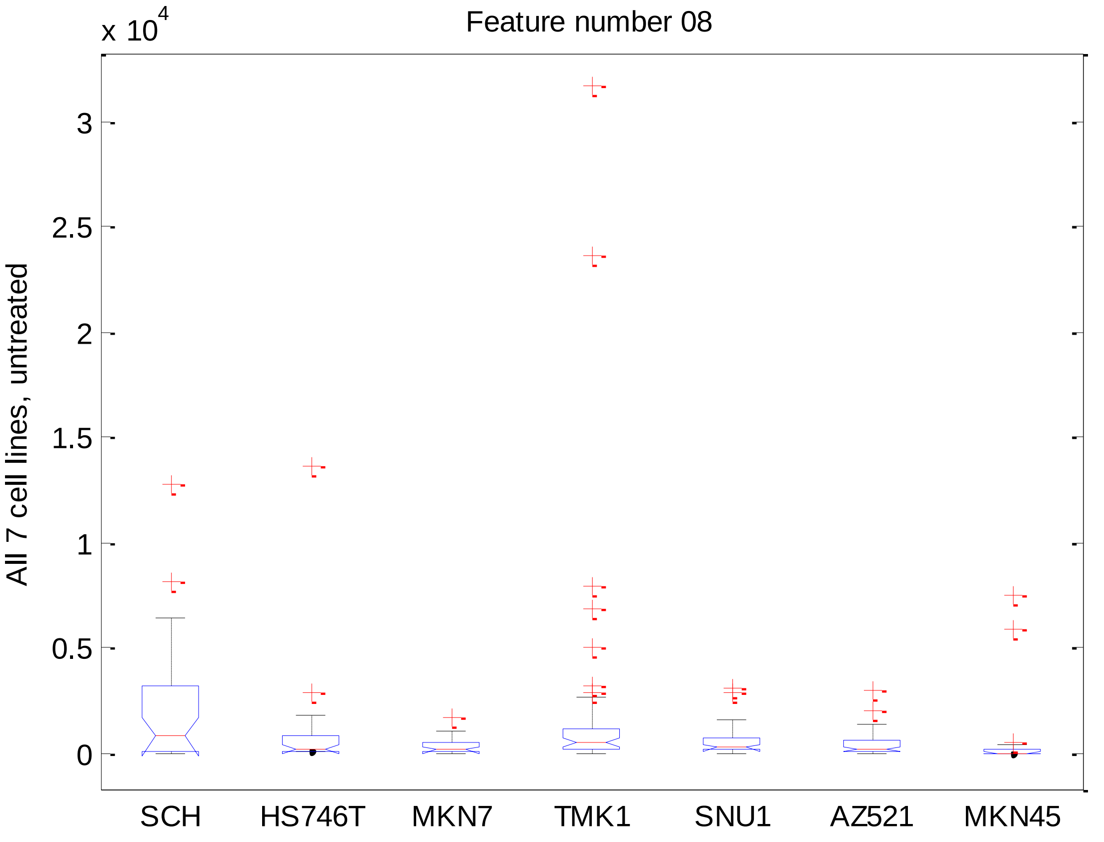

Feature number 06
All 7 cell lines, untreated
200
180
160
140
120
100
80
60
40
20
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 06
All 7 cell lines, 100nM DTX
0
20
40
60
80
100
120
140
160
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

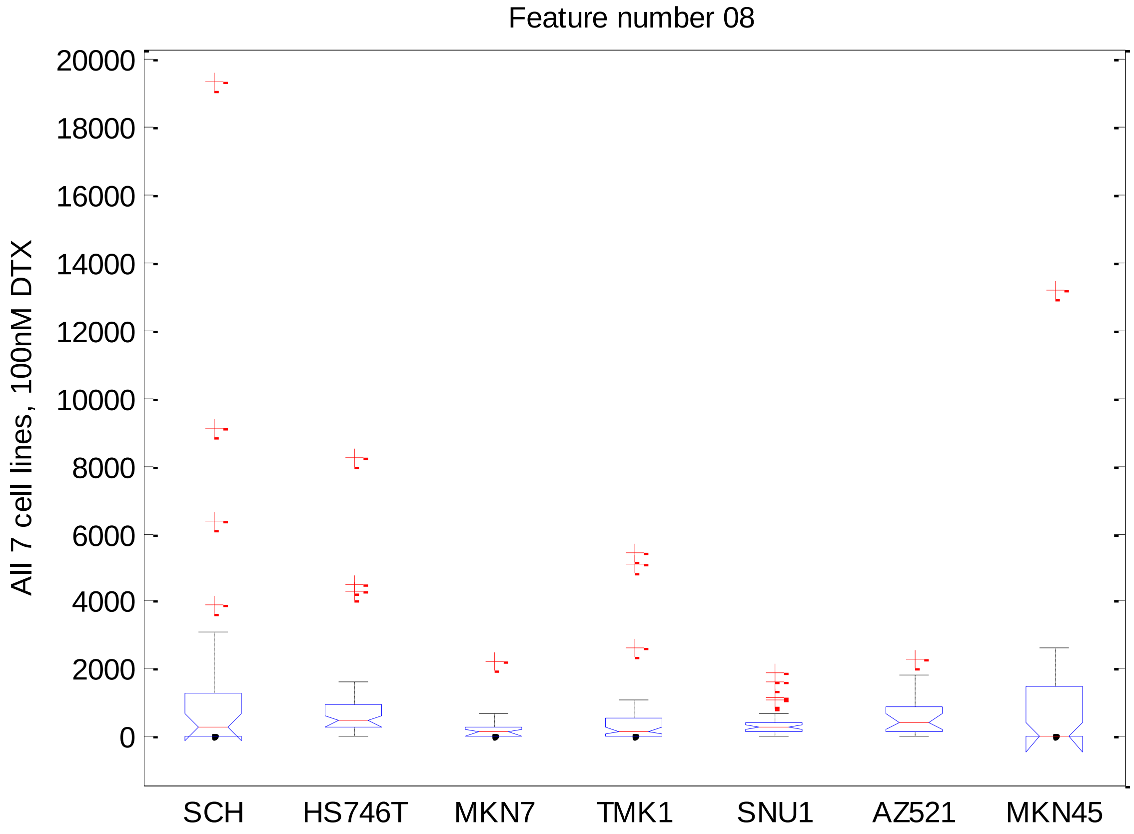

Feature number 07
All 7 cell lines, untreated
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

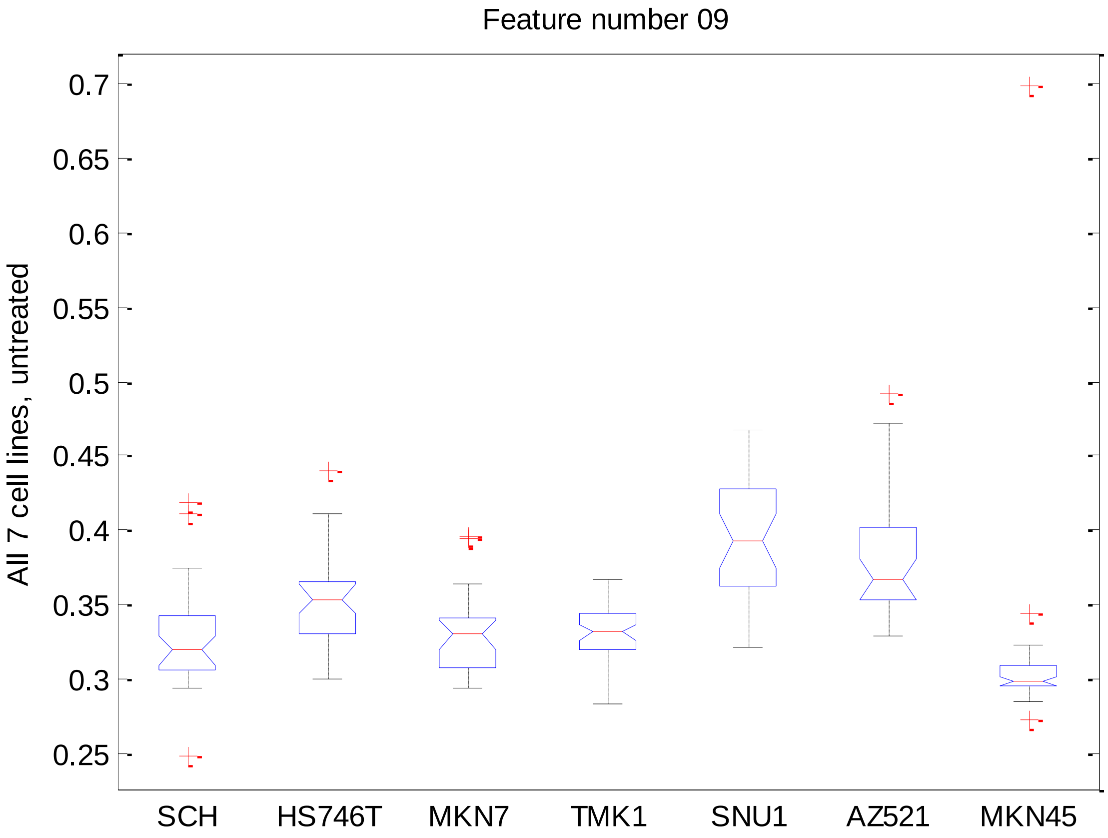


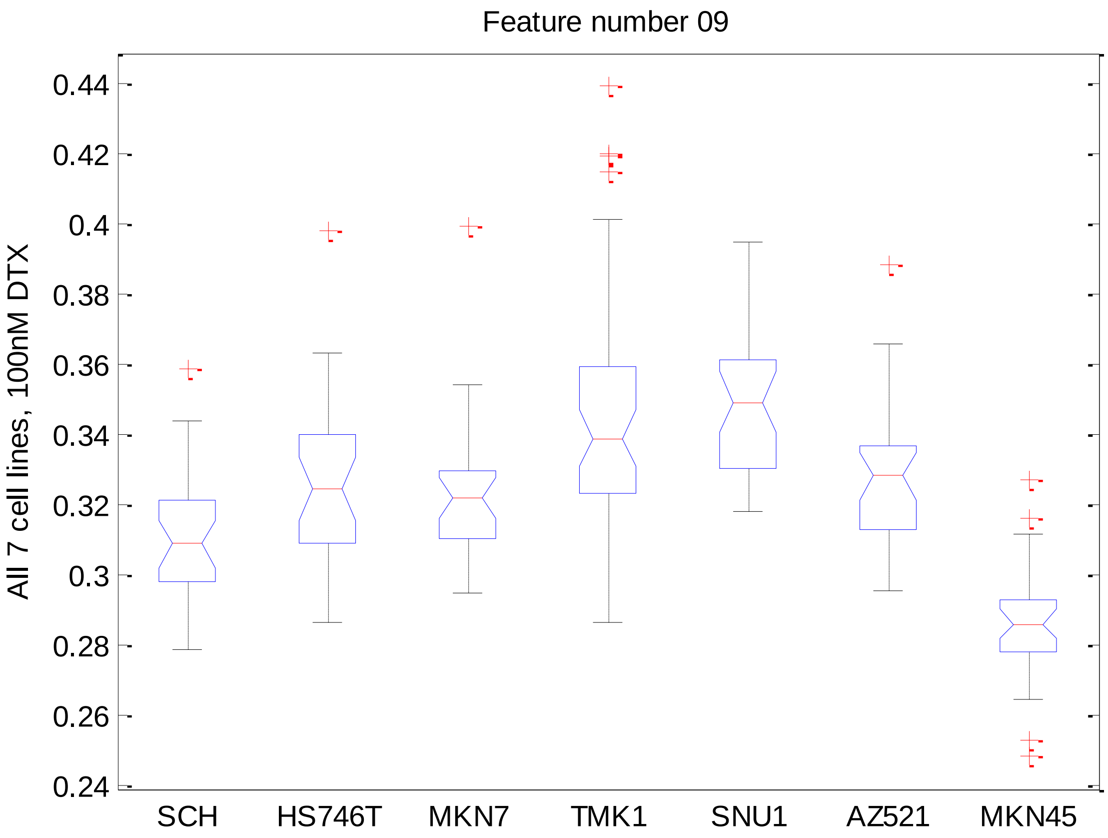
Feature number 07
All 7 cell lines, 100nM DTX
7000
6000
5000
4000
3000
2000
1000
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

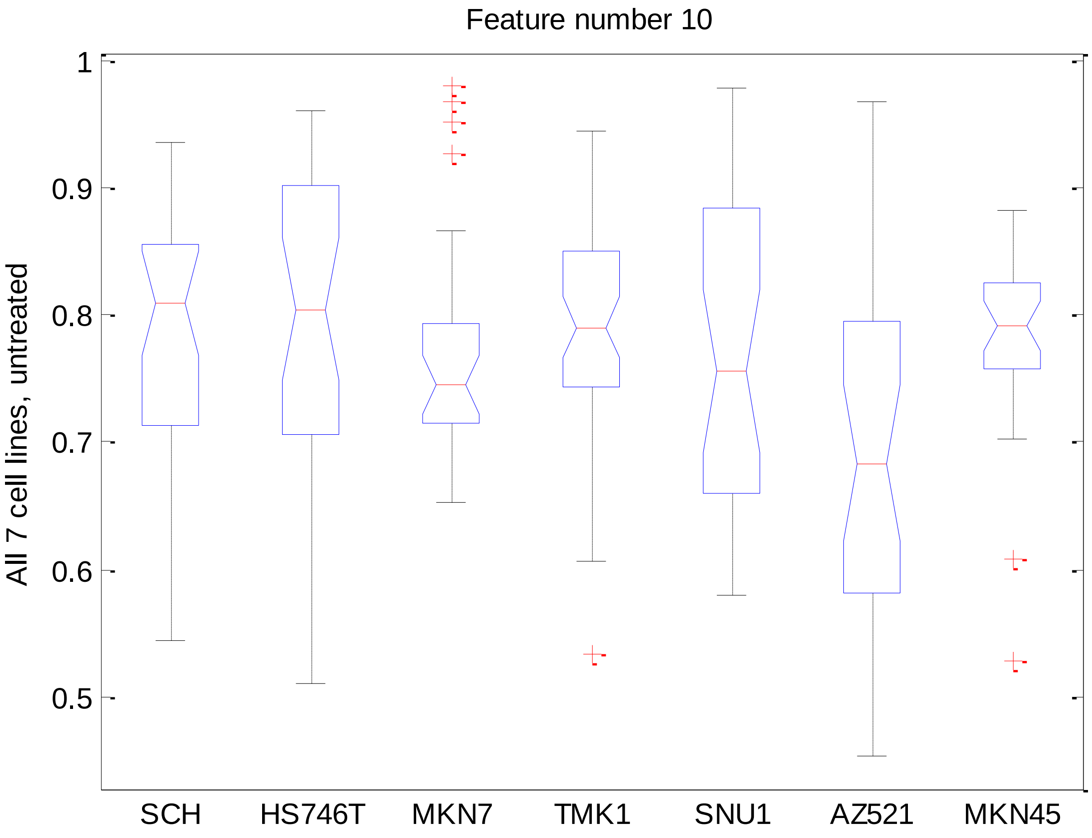


Feature number 08
x 10^4
All 7 cell lines, untreated
3
2.5
2
1.5
1
0.5
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

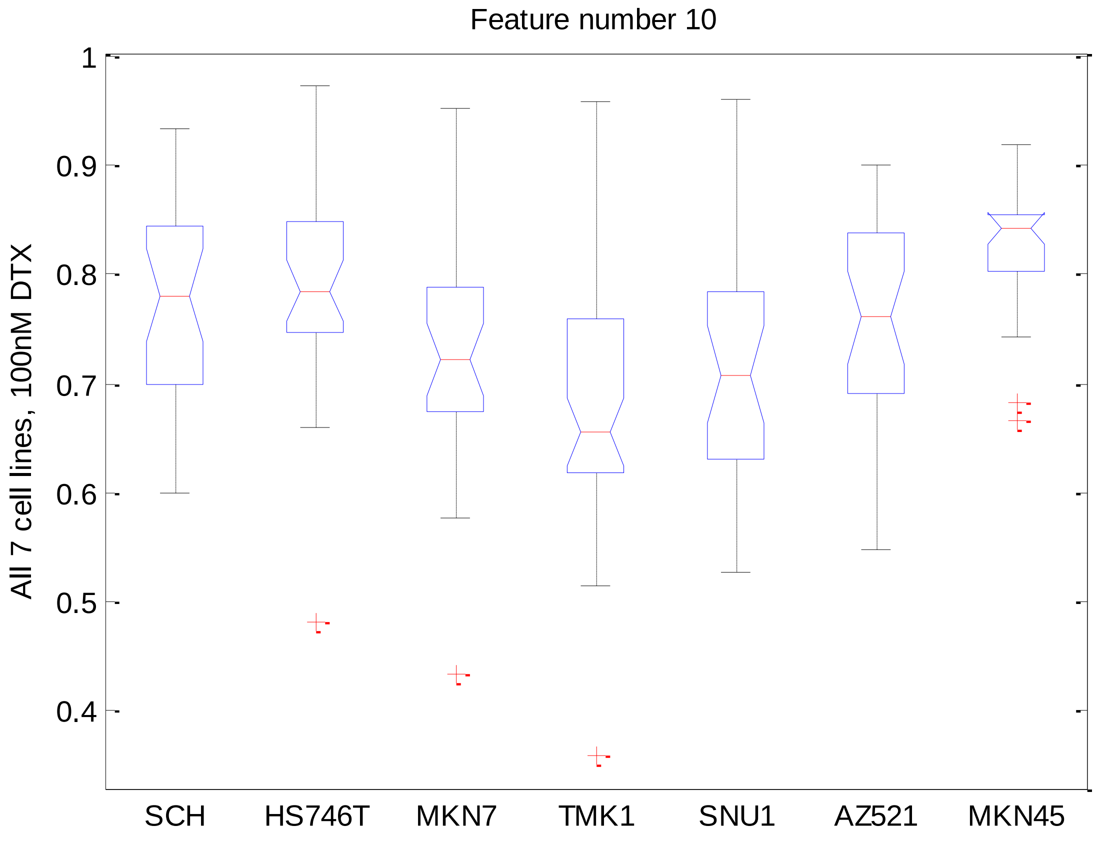

Feature number 08
All 7 cell lines, 100nM DTX
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 09
All 7 cell lines, untreated
0.7
0.65
0.6
0.55
0.5
0.45
0.4
0.35
0.3
0.25
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

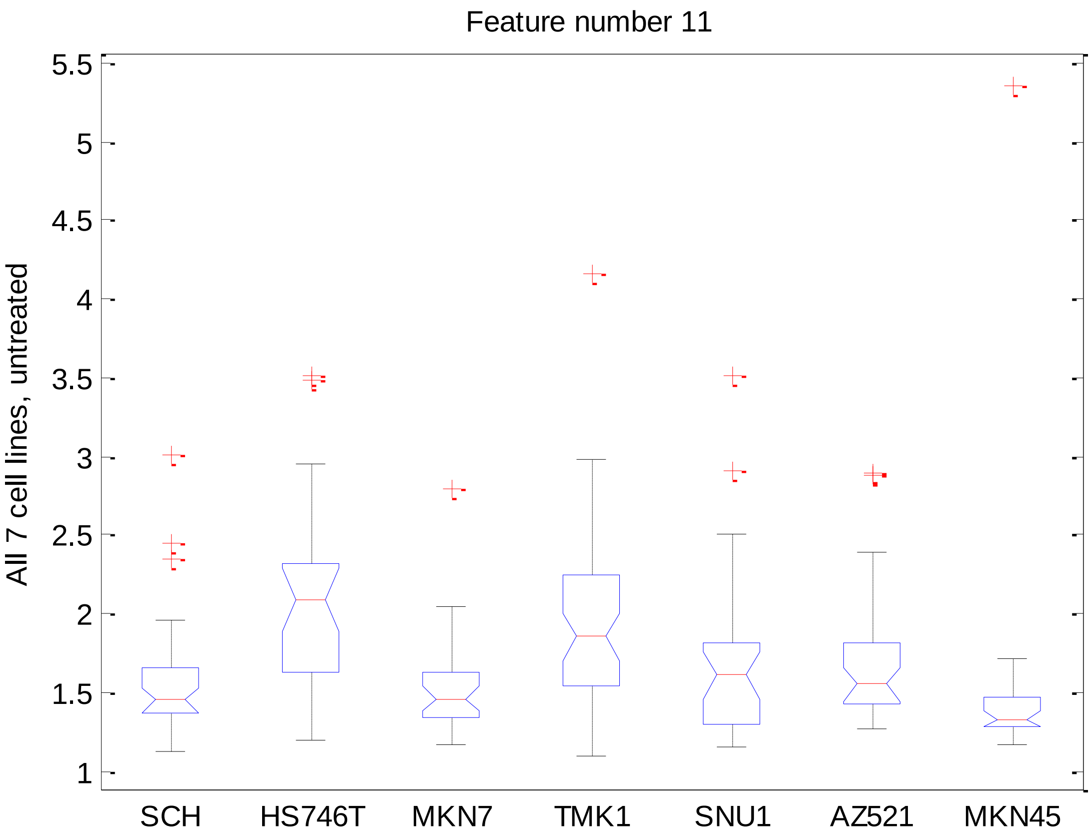

Feature number 09
All 7 cell lines, 100nM DTX
0.44
0.42
0.4
0.38
0.36
0.34
0.32
0.3
0.28
0.26
0.24
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



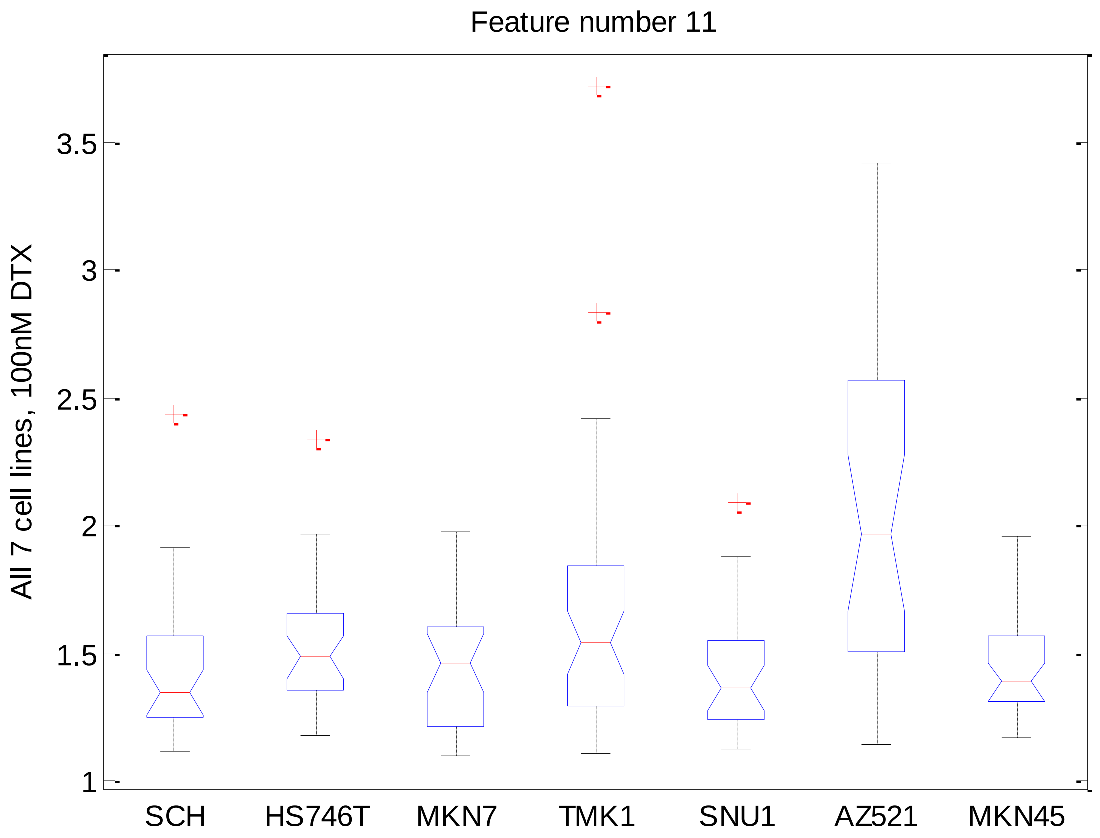
Feature number 10
All 7 cell lines, untreated
1
0.9
0.8
0.7
0.6
0.5
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



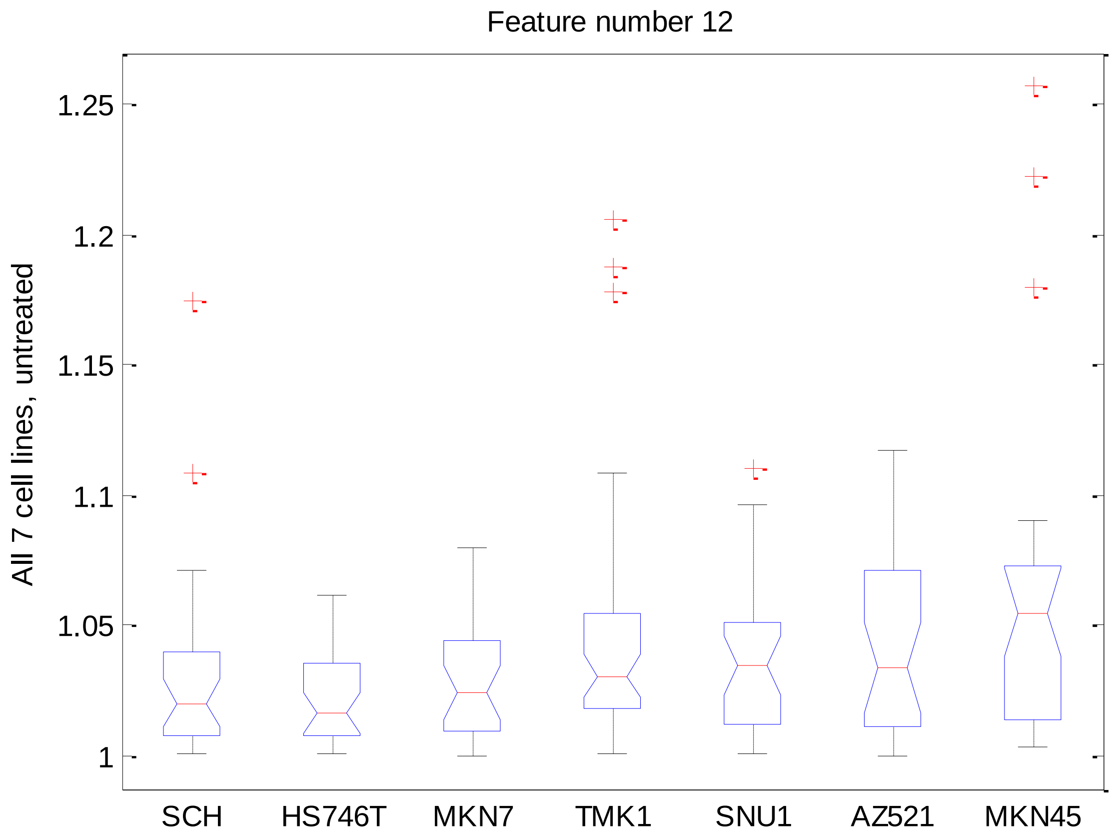
Feature number 10
All 7 cell lines, 100nM DTX
1
0.9
0.8
0.7
0.6
0.5
0.4
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

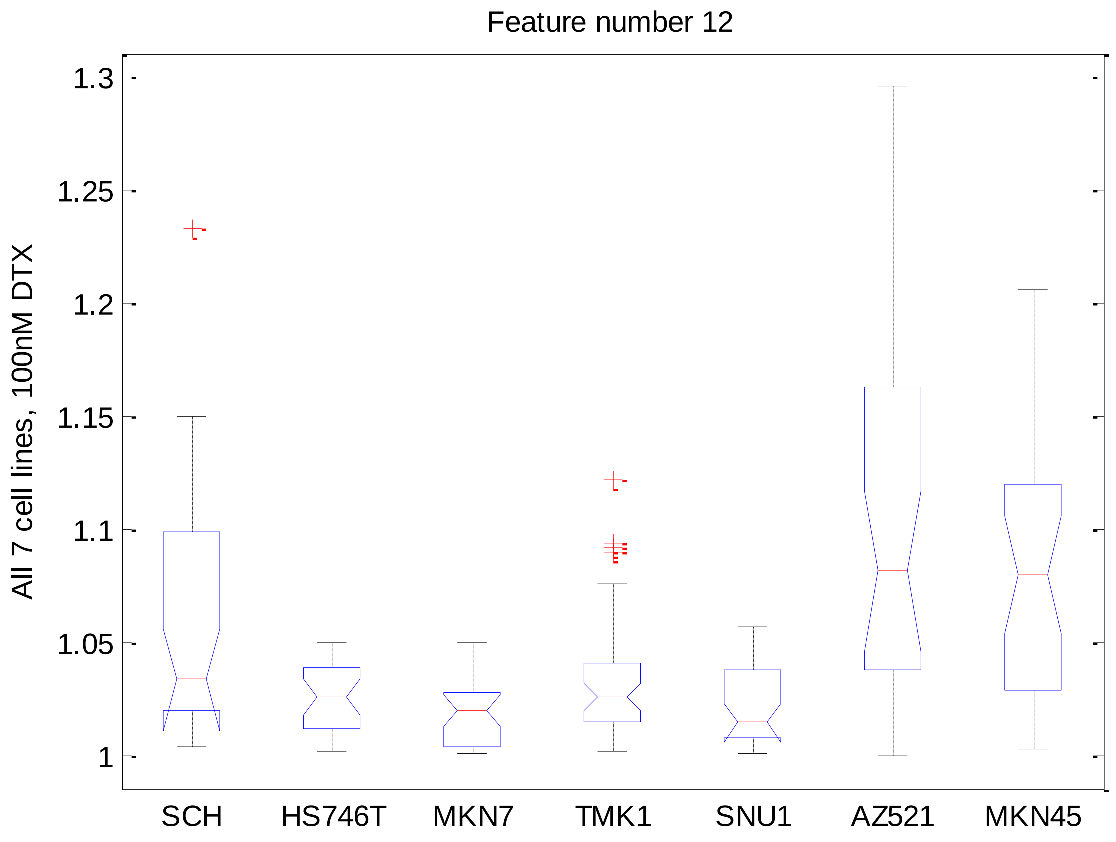


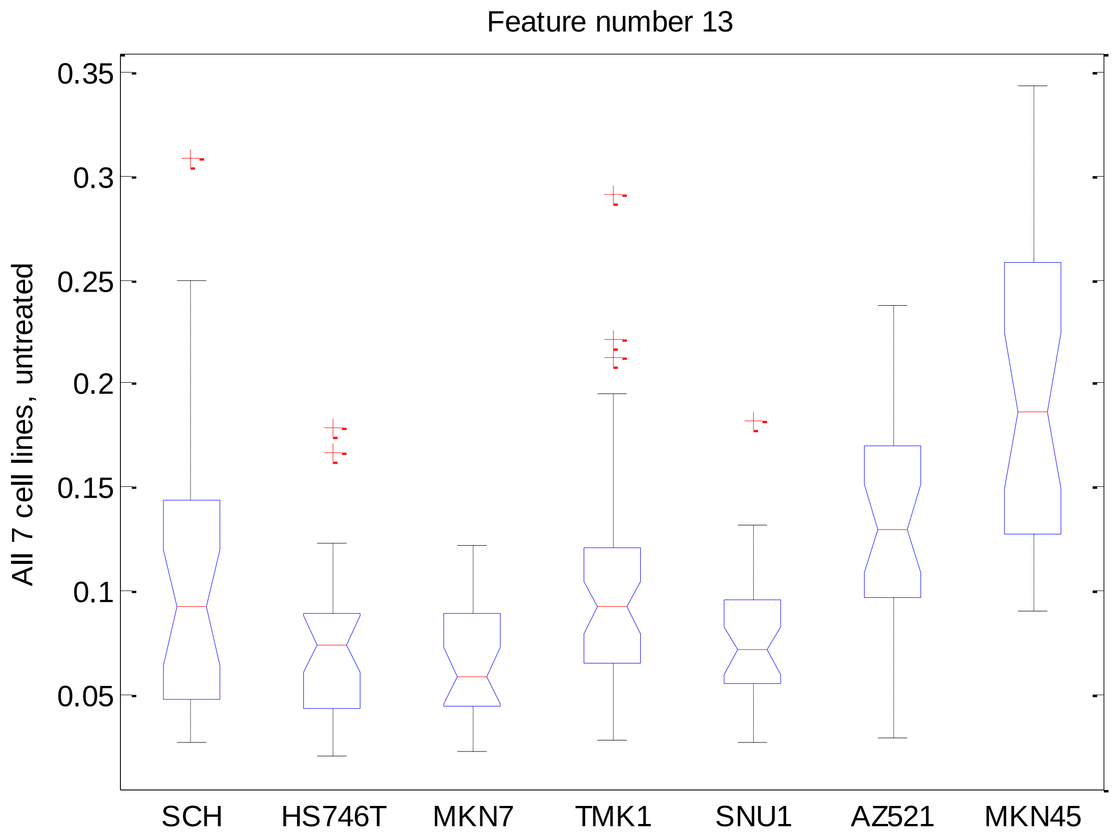
Feature number 11
All 7 cell lines, untreated
5.5
5
4.5
4
3.5
3
2.5
2
1.5
1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

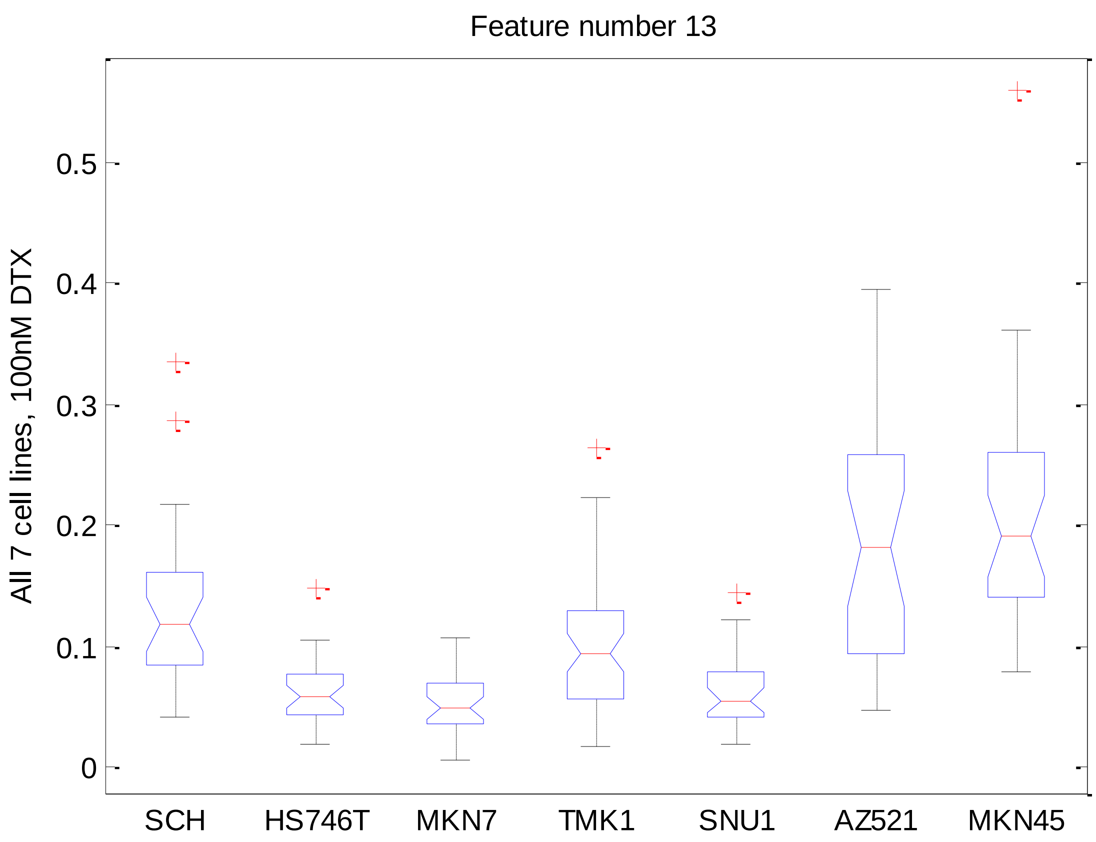


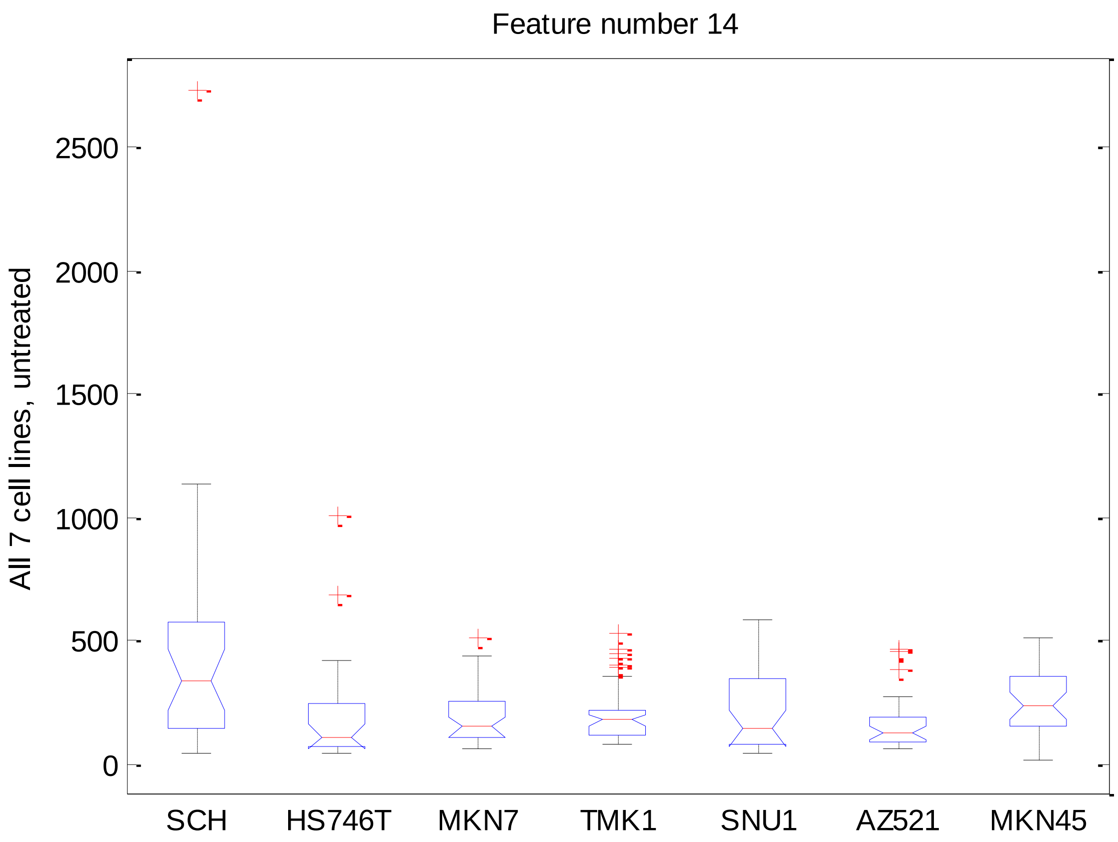
Feature number 11
All 7 cell lines, 100nM DTX
3.5
3
2.5
2
1.5
1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 12
All 7 cell lines, untreated
1.25
1.2
1.15
1.1
1.05
1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

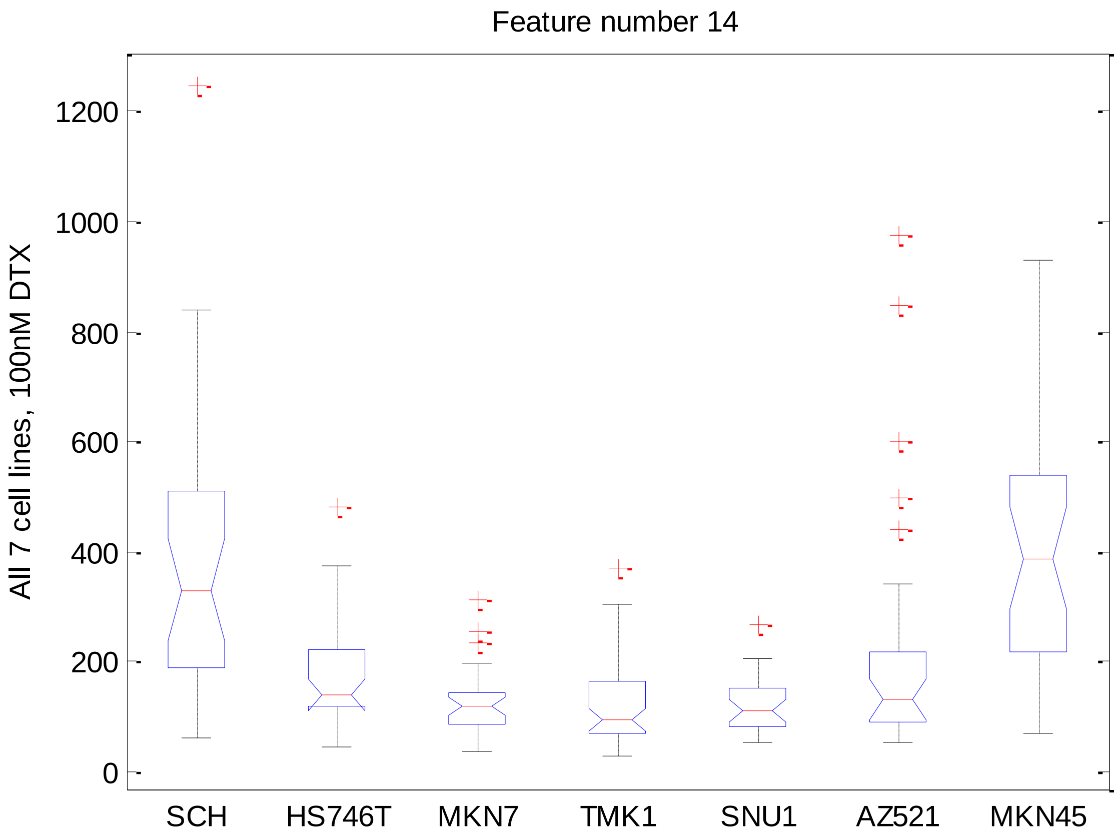

Feature number 12
All 7 cell lines, 100nM DTX
1.3
1.25
1.2
1.15
1.1
1.05
1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

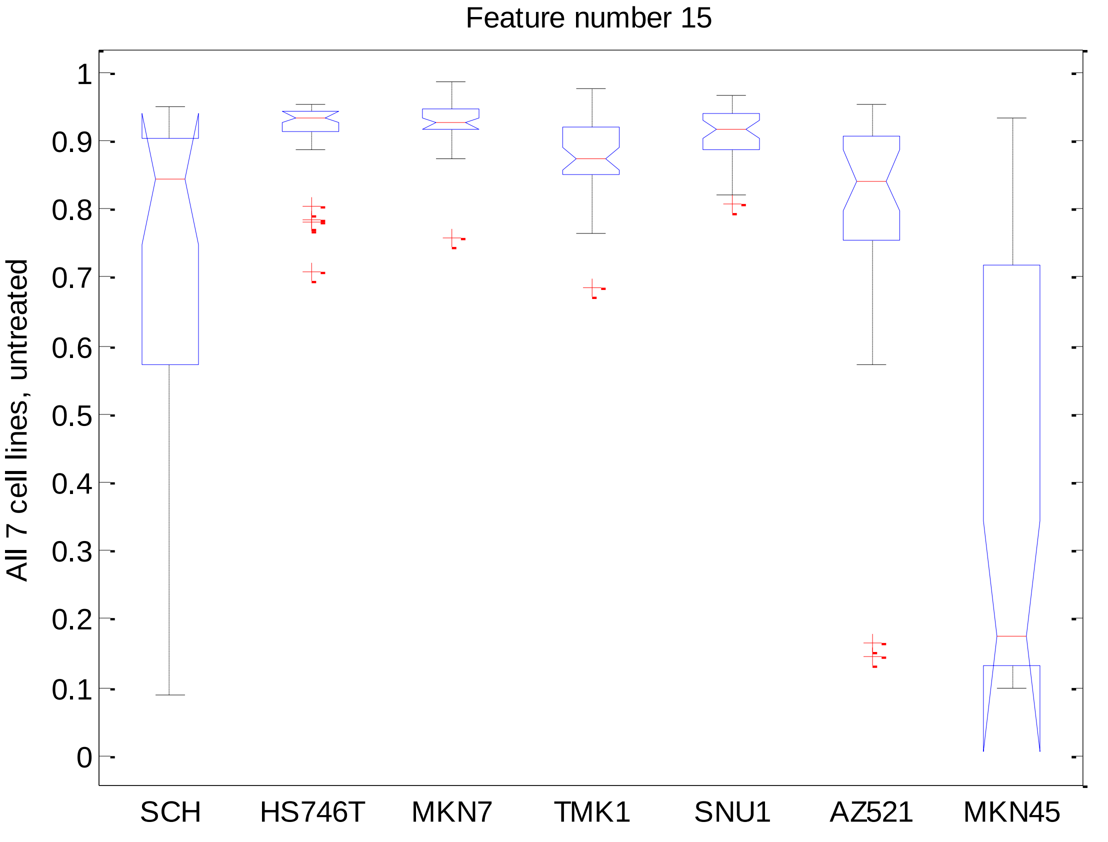

Feature number 13
All 7 cell lines, untreated
0.35
0.3
0.25
0.2
0.15
0.1
0.05
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

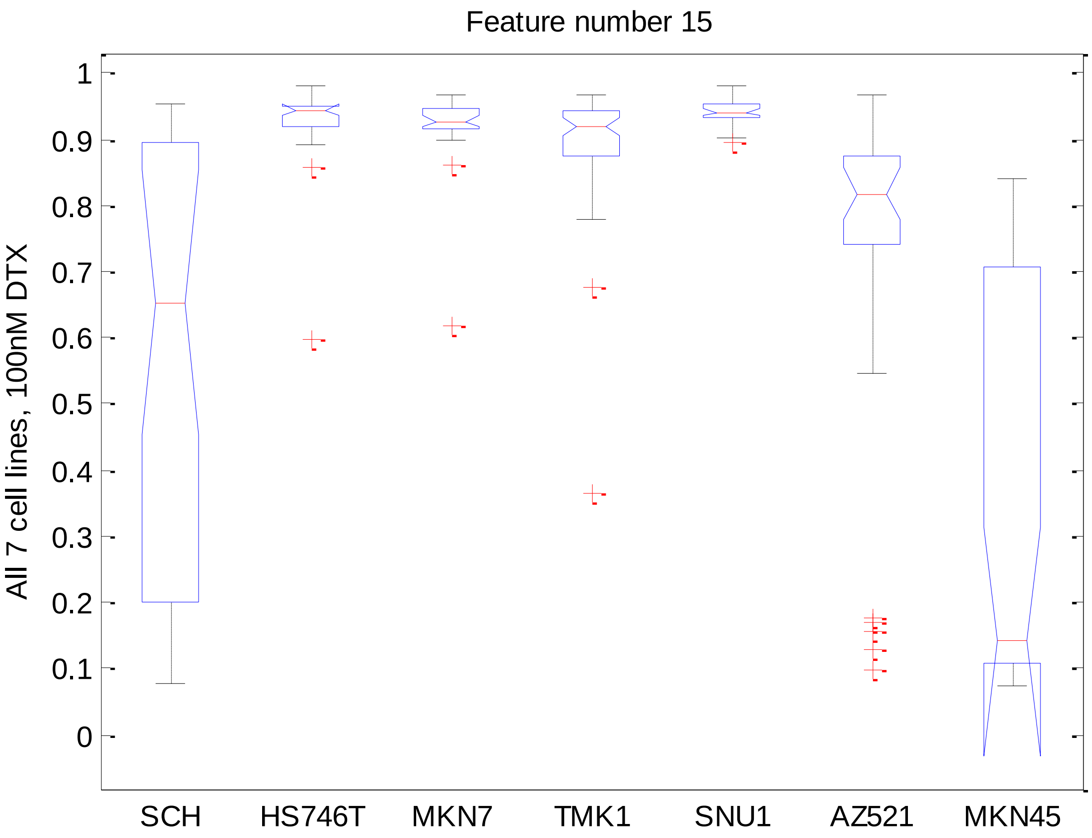

Feature number 13
All 7 cell lines, 100nM DTX
0.5
0.4
0.3
0.2
0.1
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

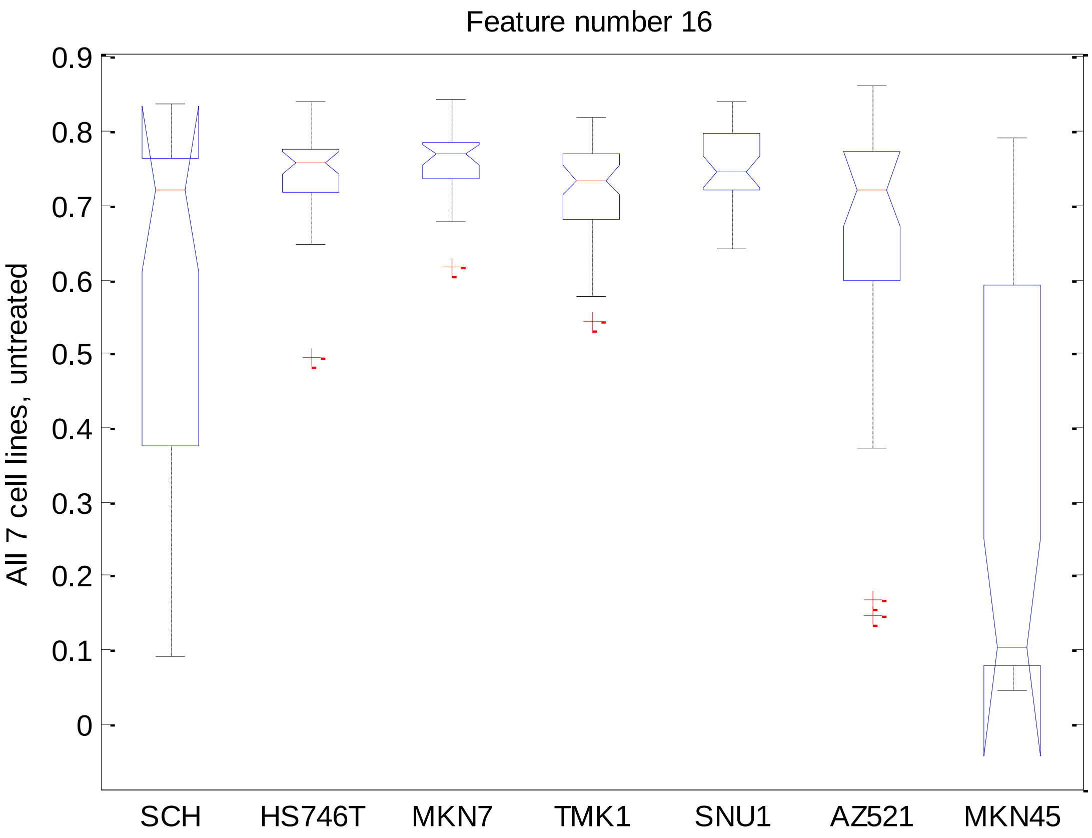


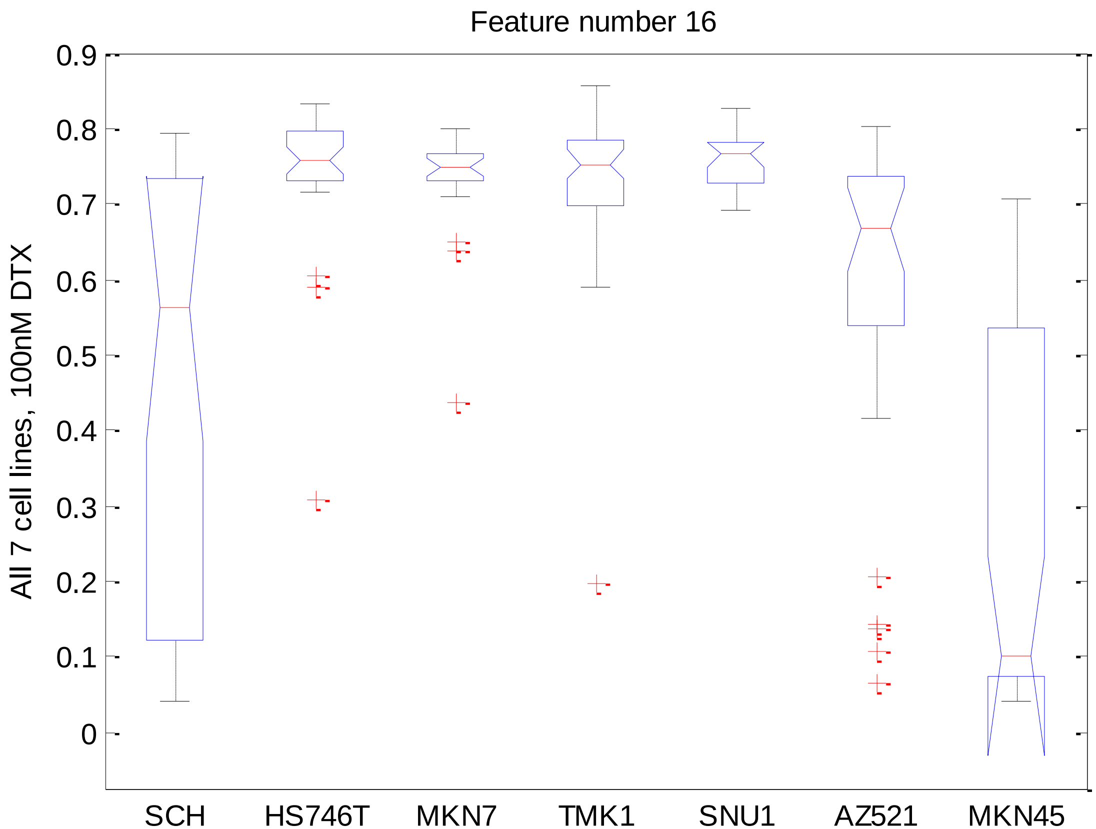
Feature number 14
All 7 cell lines, untreated
0
500
1000
1500
2000
2500
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

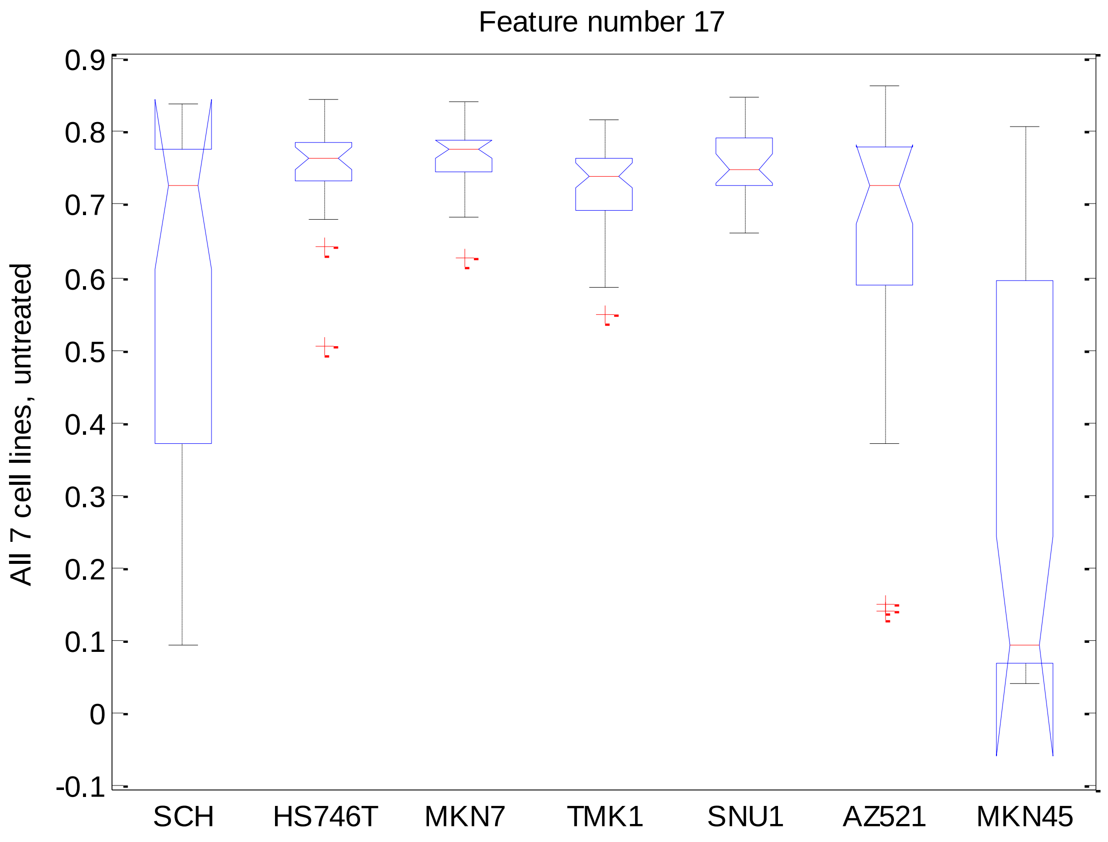

Feature number 14
All 7 cell lines, 100nM DTX
1200
1000
800
600
400
200
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

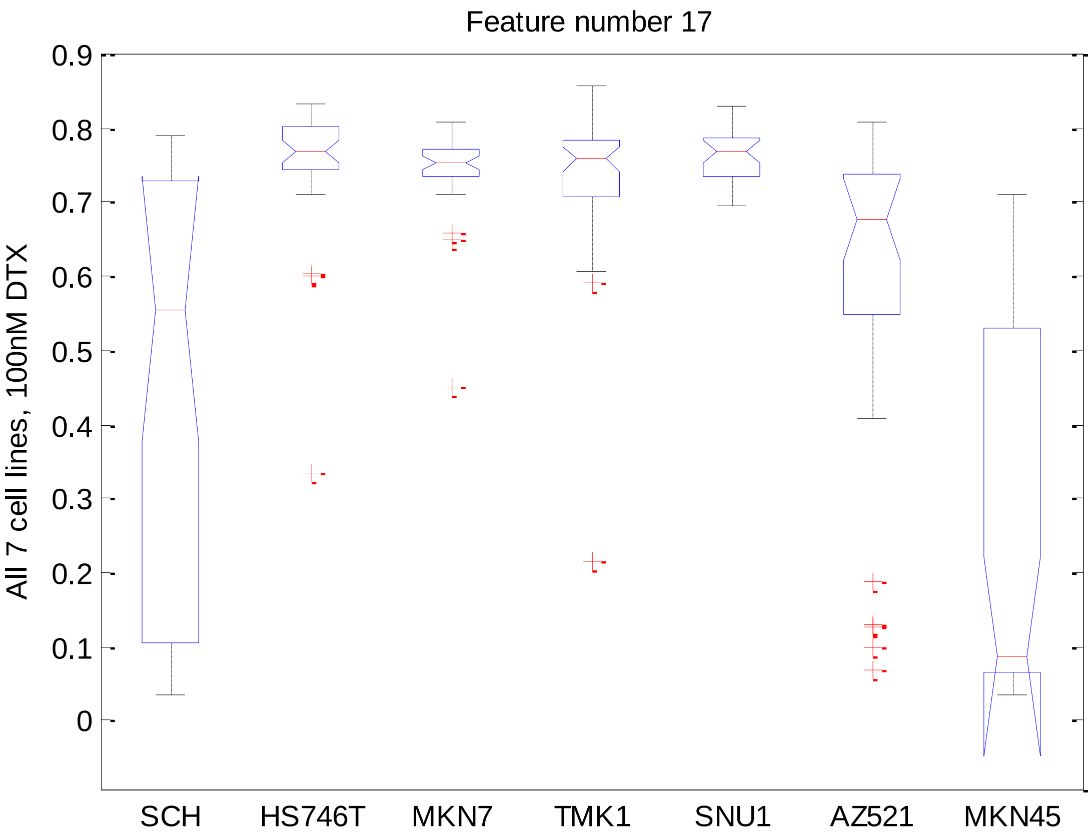

Feature number 15
All 7 cell lines, untreated
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 15
All 7 cell lines, 100nM DTX
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

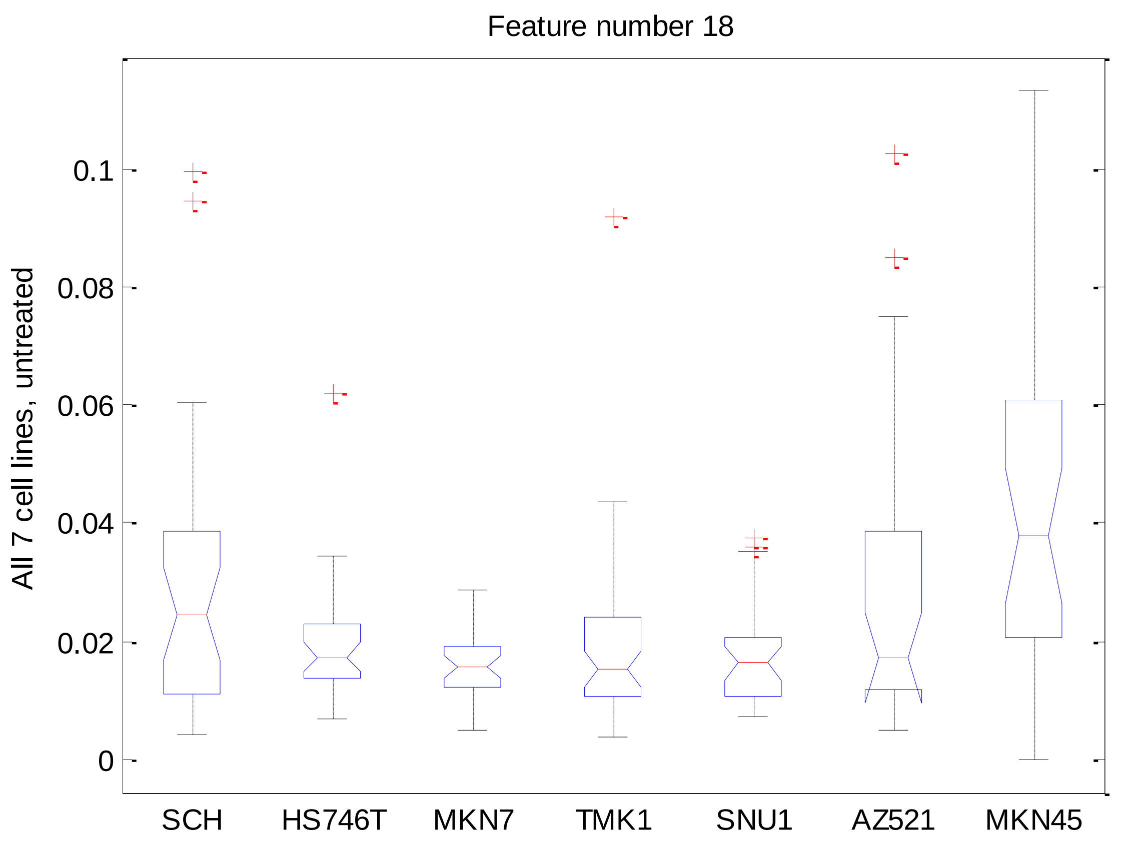

Feature number 16
All 7 cell lines, untreated
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

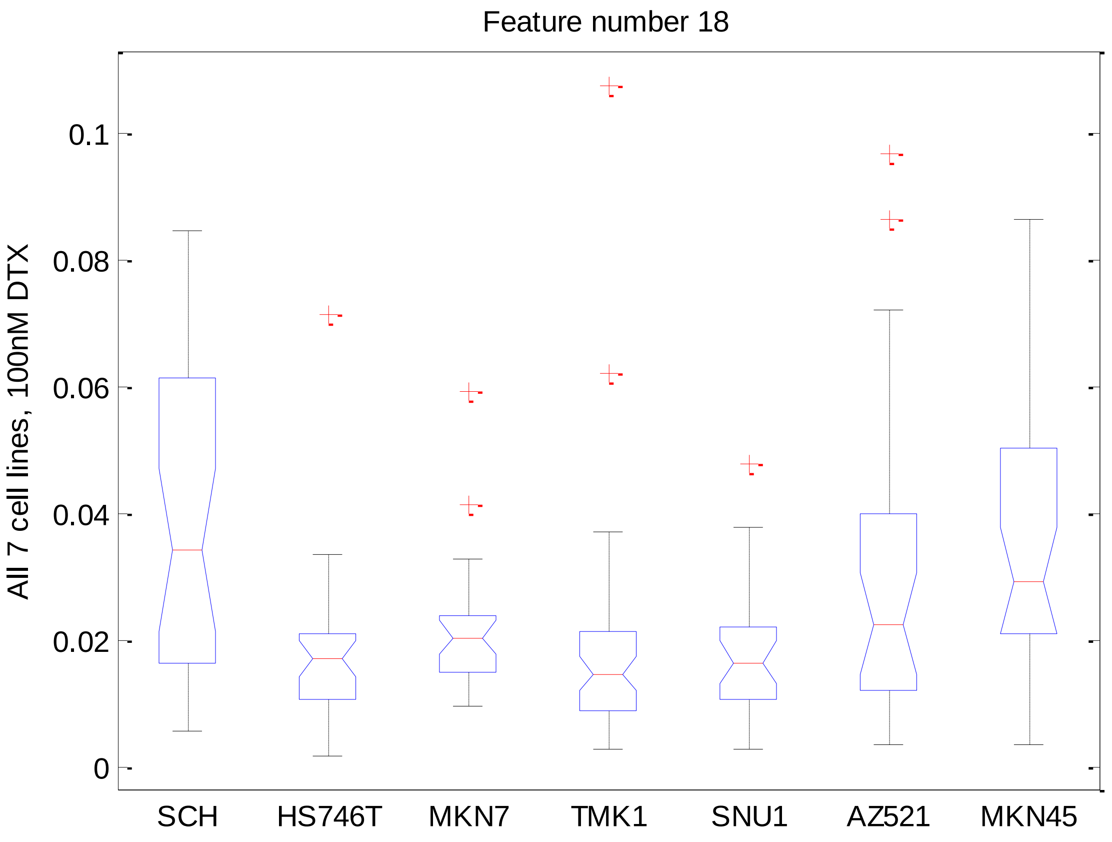

Feature number 16
All 7 cell lines, 100nM DTX
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

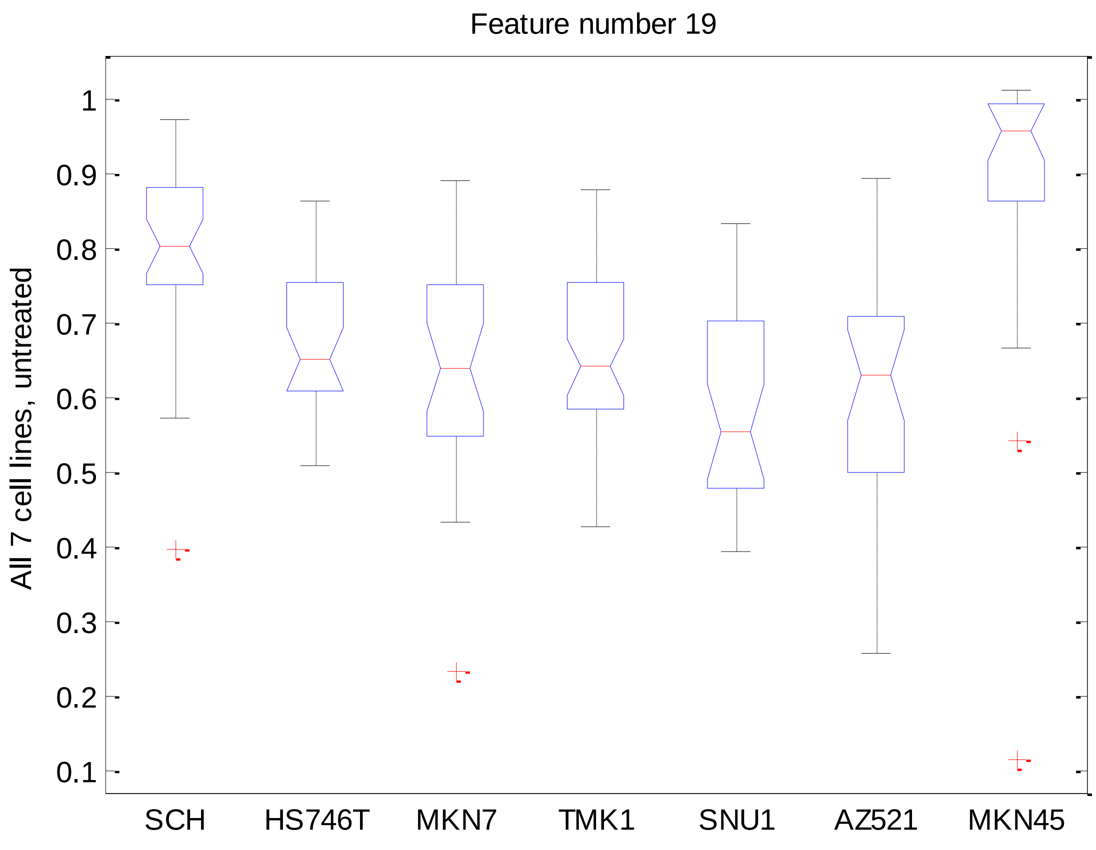

Feature number 17
All 7 cell lines, untreated
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
-0.1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

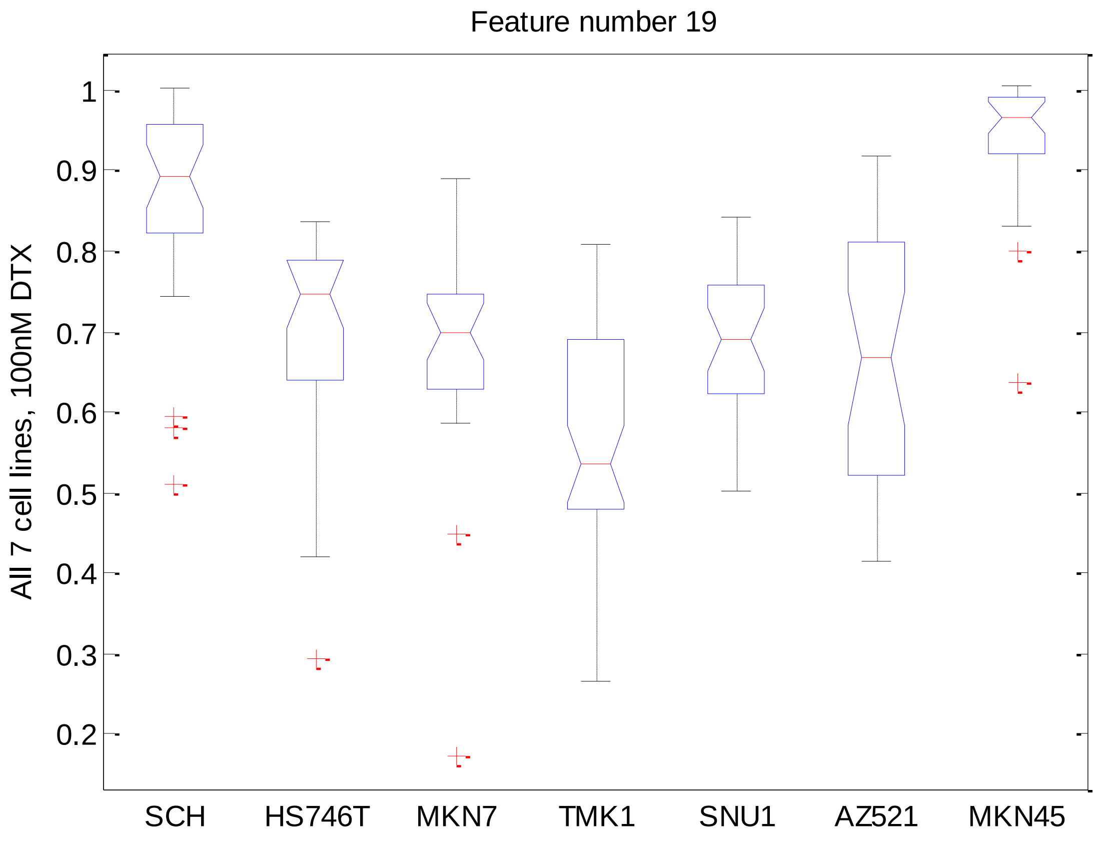


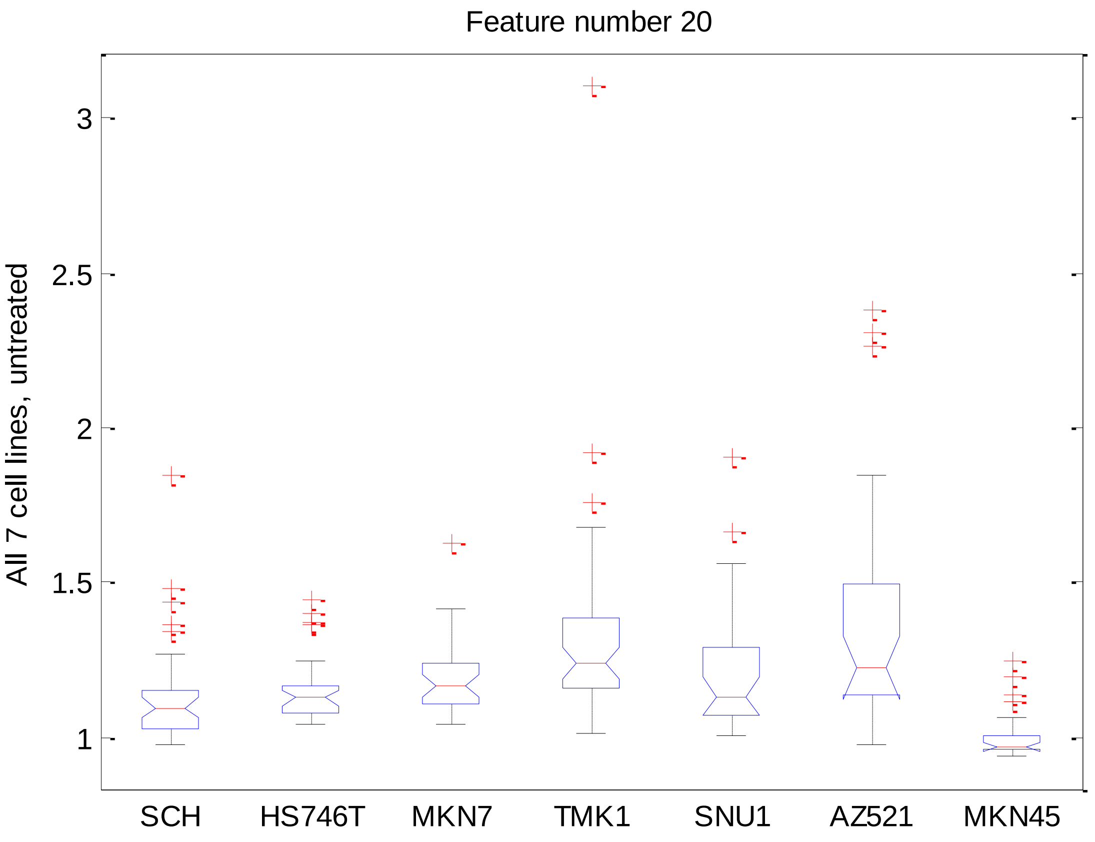
Feature number 17
All 7 cell lines, 100nM DTX
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 18
All 7 cell lines, untreated
0.1
0.08
0.06
0.04
0.02
0
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

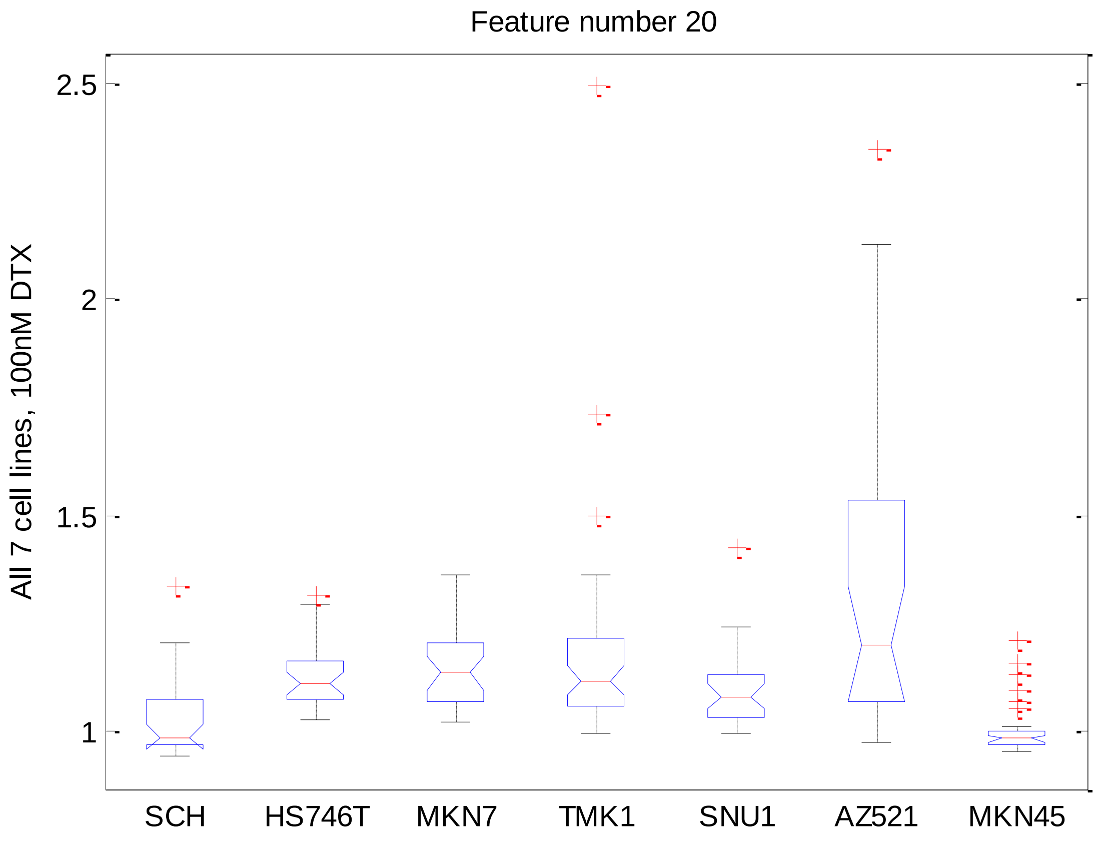

Feature number 18
All 7 cell lines, 100nM DTX
0
0.02
0.04
0.06
0.08
0.1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 19
All 7 cell lines, untreated
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

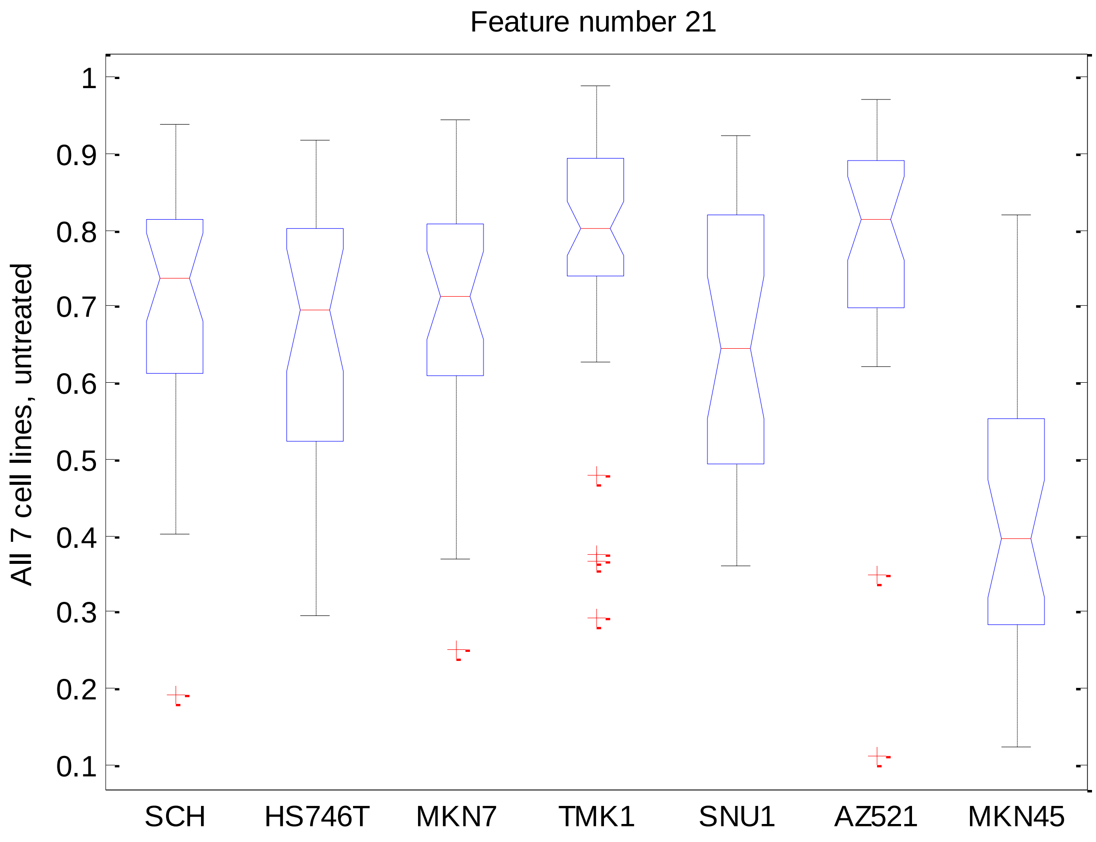

Feature number 19
All 7 cell lines, 100nM DTX
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

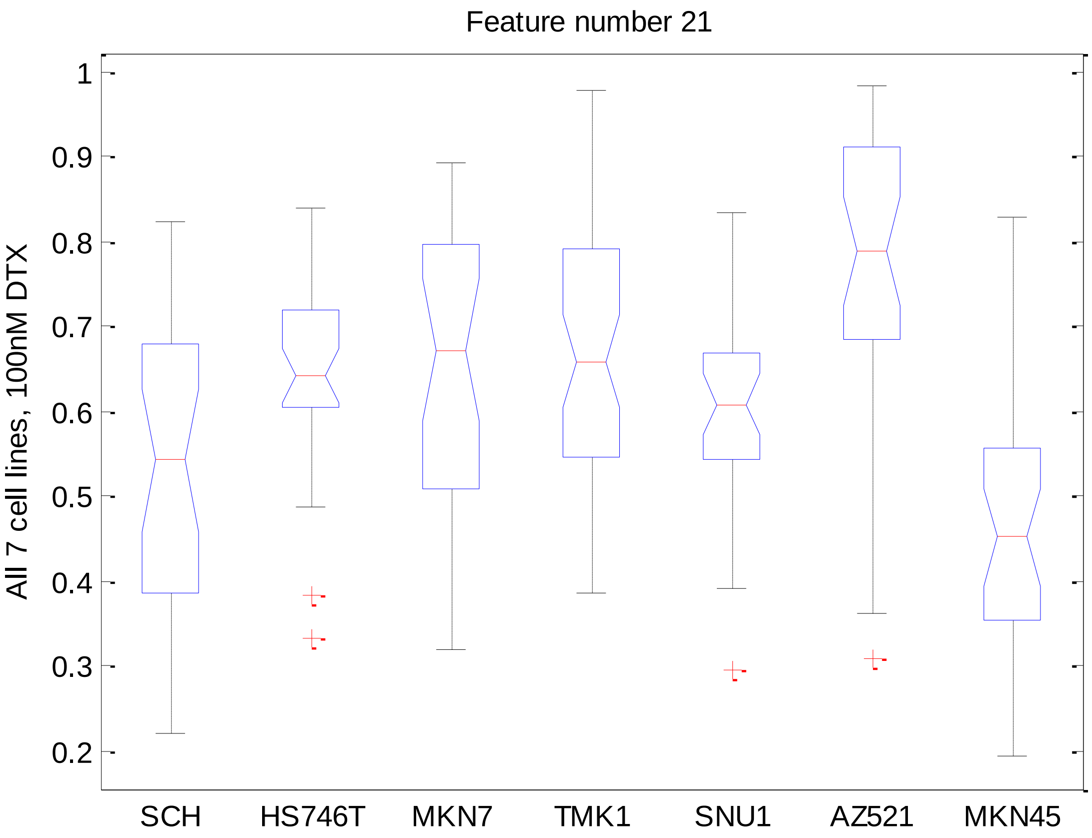


Feature number 20
All 7 cell lines, untreated
3
2.5
2
1.5
1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



Feature number 20
All 7 cell lines, 100nM DTX
2.5
2
1.5
1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45


Feature number 21
All 7 cell lines, untreated
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45



Feature number 21
All 7 cell lines, 100nM DTX
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
SCH
HS746T
MKN7
TMK1
SNU1
AZ521
MKN45

Balanced histogram thresholding

From Wikipedia, the free encyclopedia

In image processing, the **balanced histogram thresholding method** (BHT),[1] is a very simple method used for automatic image thresholding. Like Otsu's Method[2] and the **Iterative Selection Thesholding Method**,[3] this is a histogram based thresholding method. This approach assumes that the image is divided in two main classes: The **background** and the **foreground**. The **BHT** method tries to find the optimum threshold level that divides the histogram in two classes.

This method *weighs* the histogram, checks which of the two sides is heavier, and removes weight from the heavier side until it becomes the lighter. It repeats the same operation until the edges of the weighing scale meet.

Given its simplicity, this method is a good choice as a first approach when presenting the subject of *automatic image thresholding*.

Original image.

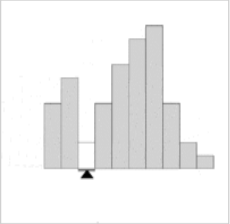
Thresholded image.

Algorithm [edit source | edit beta]

The following listing, in C notation, is a simplified version of the **Balanced Histogram Thresholding** method:

```
int BHThreshold(int[] histogram) {
    i_m = (int)((i_s + i_e) / 2.0f); // center of the weighing scale I_m
    w_l = get_weight(i_s, i_m + 1, histogram); // weight on the left W_l
    w_r = get_weight(i_m + 1, i_e + 1, histogram); // weight on the right W_r
    while (i_s <= i_e) {
        if (w_r > w_l) { // right side is heavier
            w_r -= histogram[i_e--];
            if (((i_s + i_e) / 2) < i_m) {
                w_r += histogram[i_m];
                w_l -= histogram[i_m--];
            }
        } else if (w_l >= w_r) { // left side is heavier
            w_l -= histogram[i_s++];
            if (((i_s + i_e) / 2) > i_m) {
                w_l += histogram[i_m + 1];
                w_r -= histogram[i_m + 1];
                i_m++;
            }
        }
    }
    return i_m;
}
```

This method may have problems when dealing with very noisy images, because the *weighing scale* may be misplaced. The problem can be minimized by ignoring the extremities of the histogram.[4]



Evolution of the method.

References [edit source | edit beta]

1. ^ A. Anjos and H. Shahbazkia. Bi-Level Image Thresholding - A Fast Method. BIOSIGNALS 2008. Vol:2. P:70-76.
2. ^ Nobuyuki Otsu (1979). "A threshold selection method from gray-level histograms". IEEE Trans. Sys., Man., Cyber. 9: 62–66.
3. ^ Ridler TW, Calvard S. (1978) Picture thresholding using an iterative selection method, IEEE Trans. System, Man and Cybernetics, SMC-8: 630-632.
4. ^ A. Anjos, R. Leite, M. L. Cancela, H. Shahbazkia. MAQ – A Bioinformatics Tool for Automatic Macroarray Analysis. International Journal of Computer Applications. 2010. Number 7 - Article 1.

External links [edit source | edit beta]

Correspondence

Picture Thresholding Using an Iterative Selection Method

T. W. RIDLER AND S. CALVARD

Abstract—**An object may be extracted from its background in a picture by threshold selection. Ideally, if the object has a different average gray level from that of its surrounding, the effect of thresholding will produce a white object with a black background or vice versa. In practice, it is often difficult, however, to select an appropriate threshold, and a technique is described whereby an optimum threshold may be chosen automatically as a result of an iterative process, successive iterations providing increasingly cleaner extractions of the object region. An application to low contrast images of handwritten text is discussed.**

I. Introduction

Features of interest in an image may often be extracted from their surroundings using a thresholding technique [1] in which all gray levels below the threshold are mapped into black, those levels above are mapped into white, or vice versa. The success of the technique depends on the object that is desired to be extracted occupying a range of gray levels distinct from that of the background. In practice it is difficult to select the optimum threshold, especially if a range of image scenes with widely differing properties is being considered, and some automatic means of threshold selection is required in each case. Thresholding at too high a level results in a loss of information, while thresholding at low levels can give rise to objectionable background clutter [2].

A common method of automatically deriving a threshold at which to segment a given picture is to examine its gray level

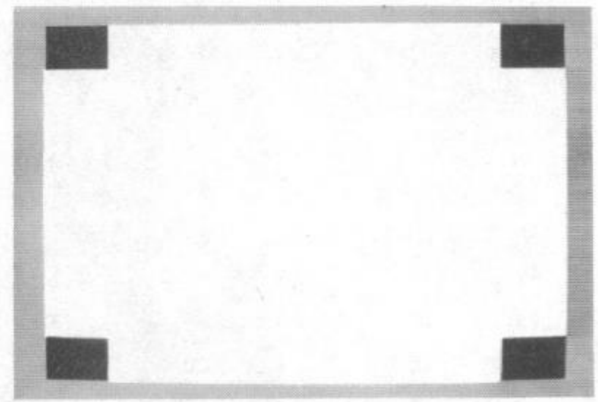


Fig. 1. Initial object-background estimation.

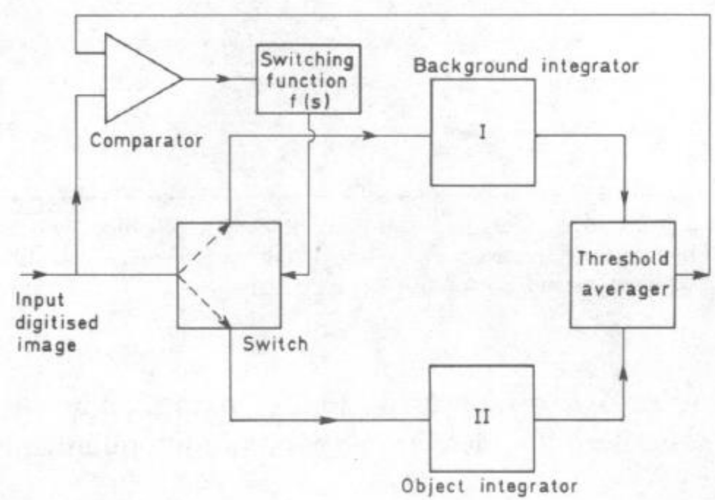


Fig. 2. Schematic image processor for iterative threshold selection.