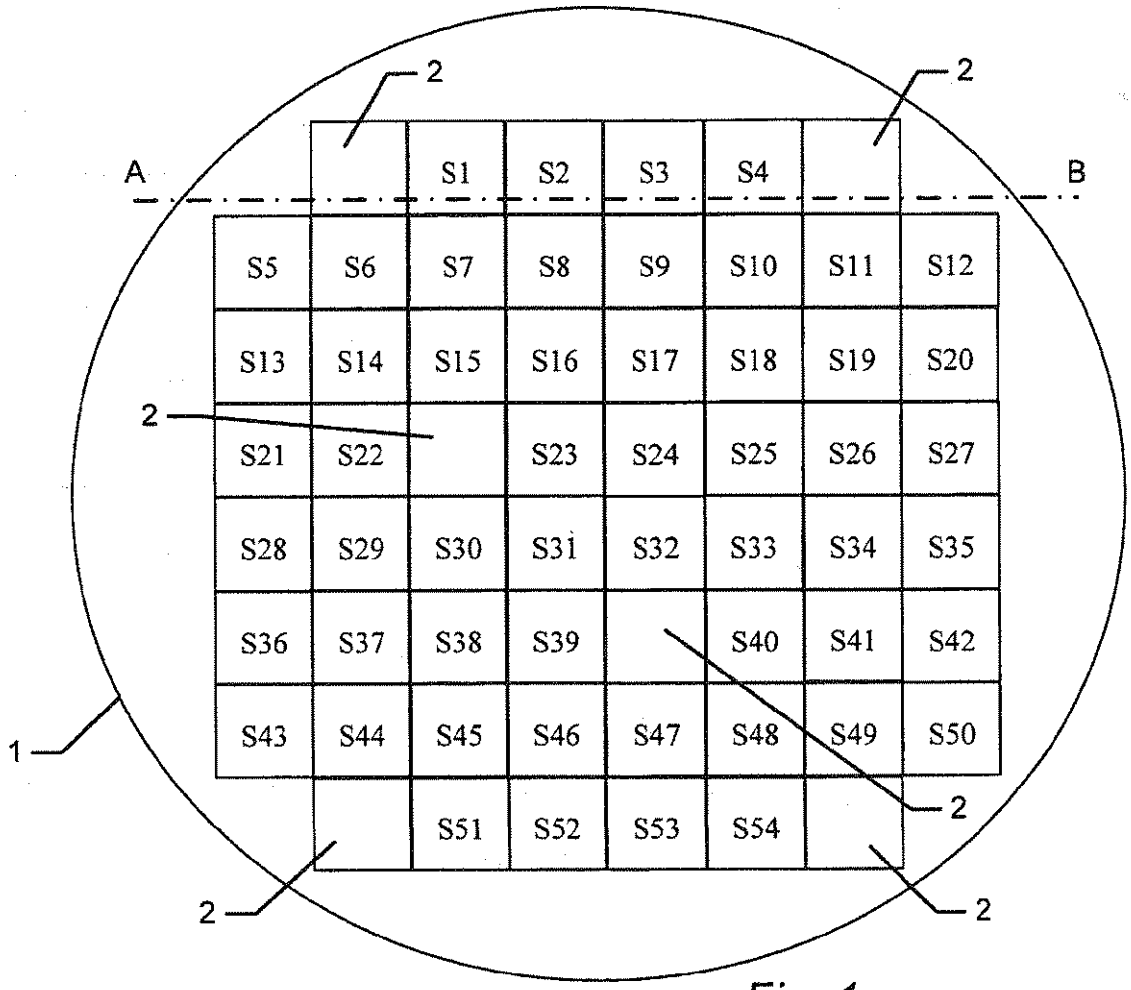

A
B
1
2
2
2
2
2
2
S1
S2
S3
S4
S5
S6
S7
S8
S9
S10
S11
S12
S13
S14
S15
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S20
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S44
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S47
S48
S49
S50
S51
S52
S53
S54

Fig. 1

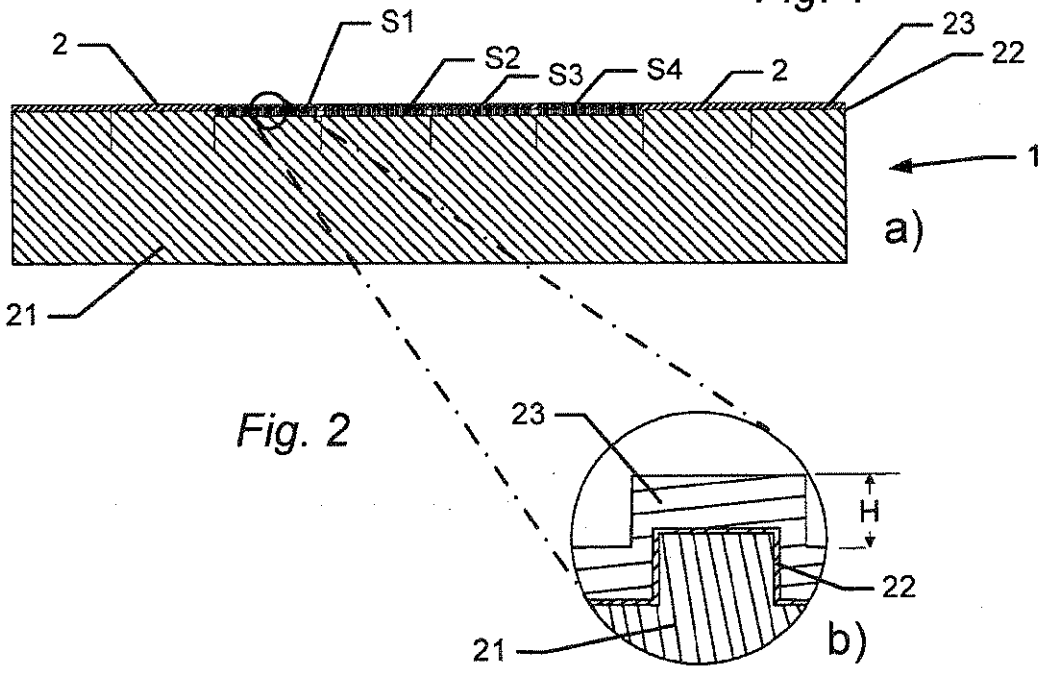

2
S1
S2
S3
S4
2
23
22
1
a)
21
23
H
22
21
b)

Fig. 2

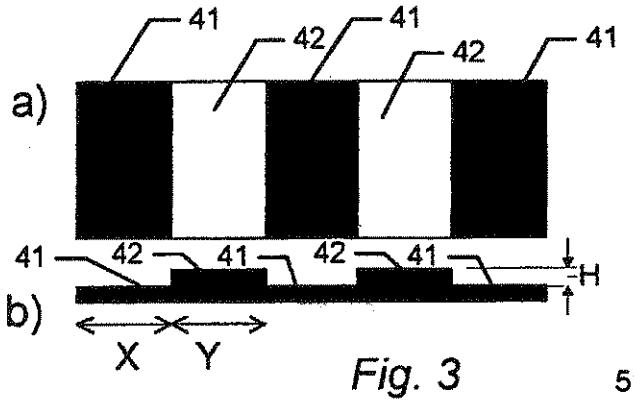


Fig. 3

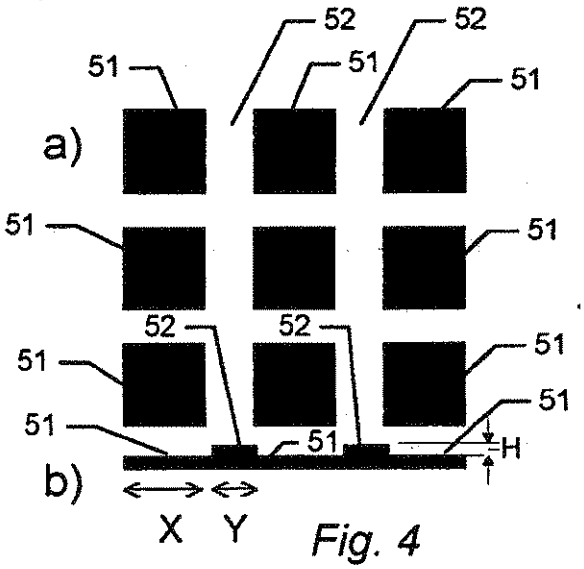


Fig. 4

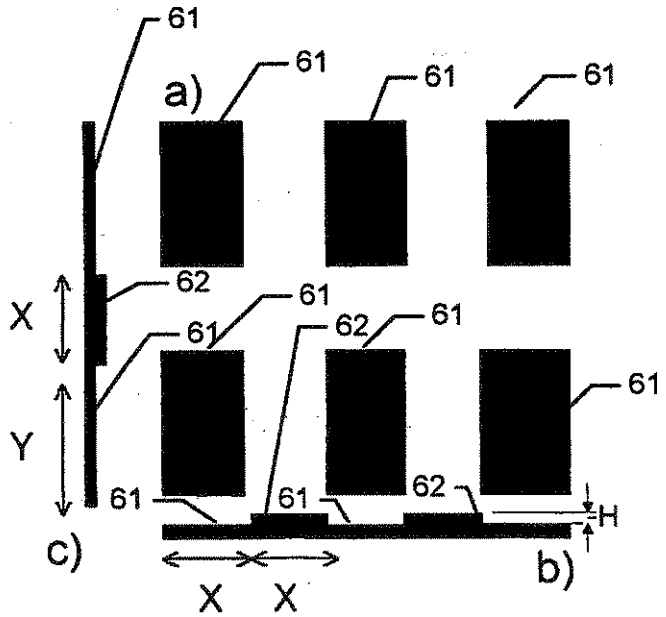


Fig. 5

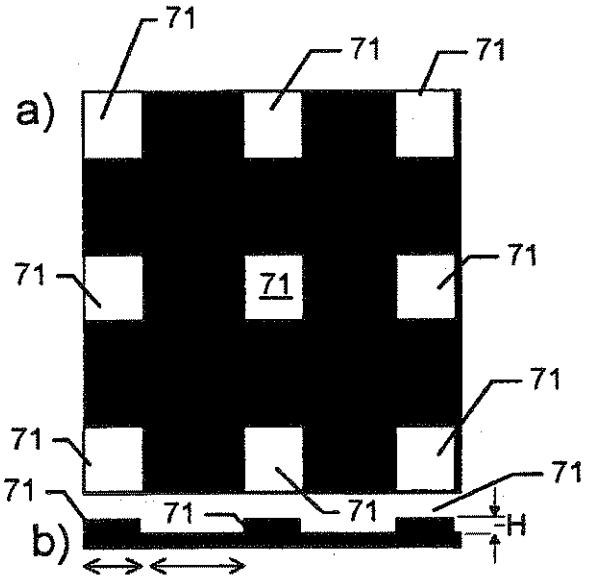


Fig. 6

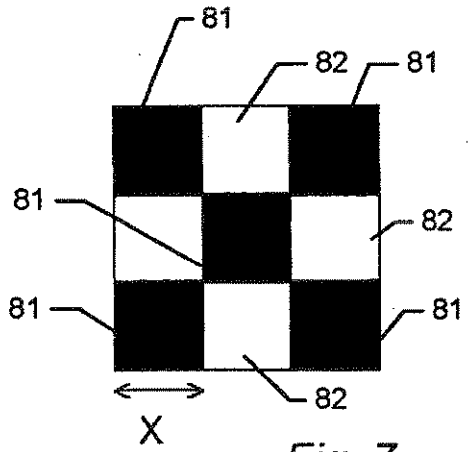


Fig. 7

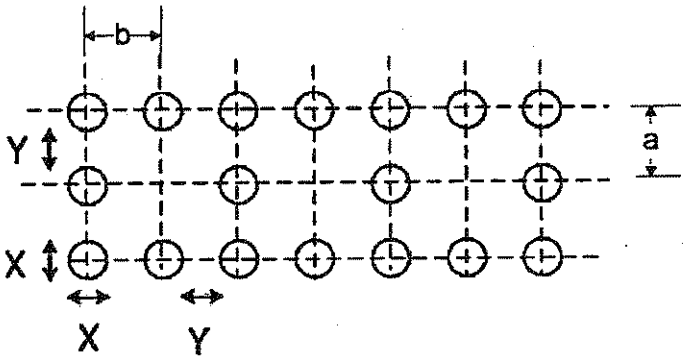


Fig. 8a

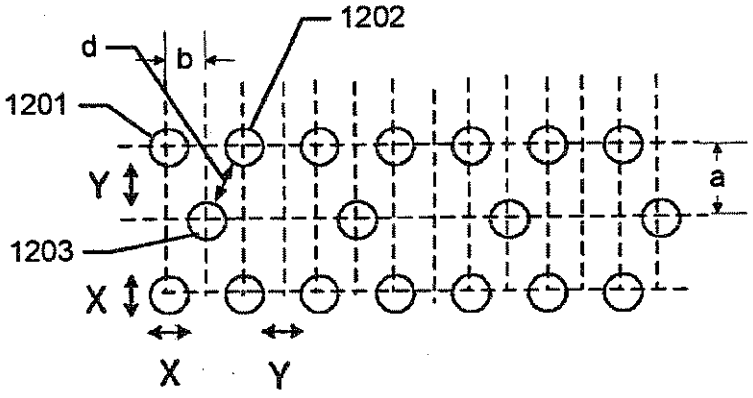


Fig. 8b

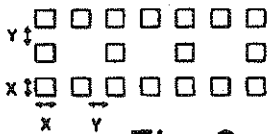


Fig. 8c

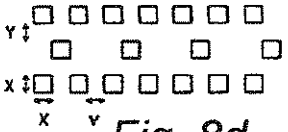


Fig. 8d

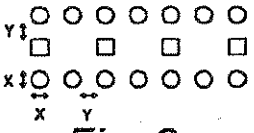


Fig. 8e



Fig. 8g

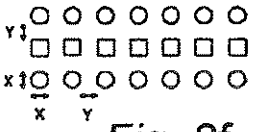


Fig. 8f

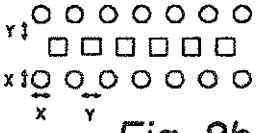


Fig. 8h

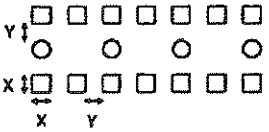


Fig. 8i

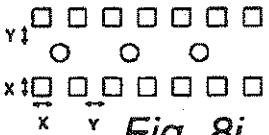


Fig. 8j

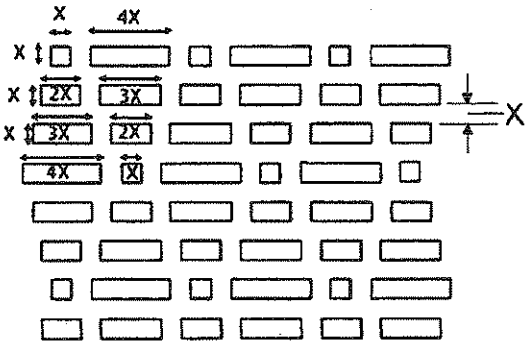


Fig. 8k

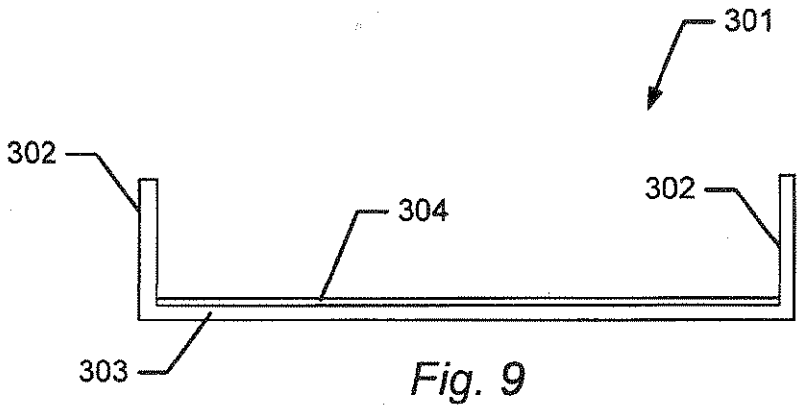
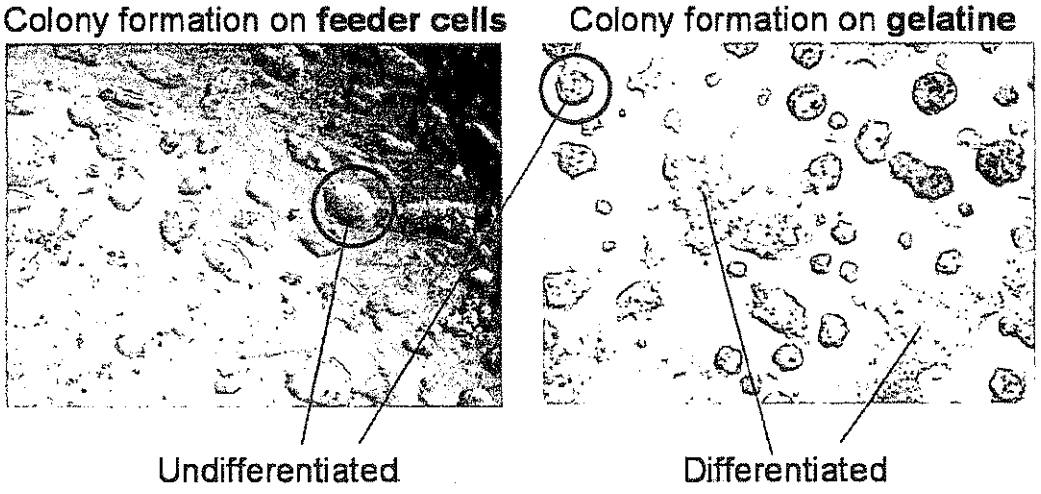


Fig. 9

ES cell colony morphology

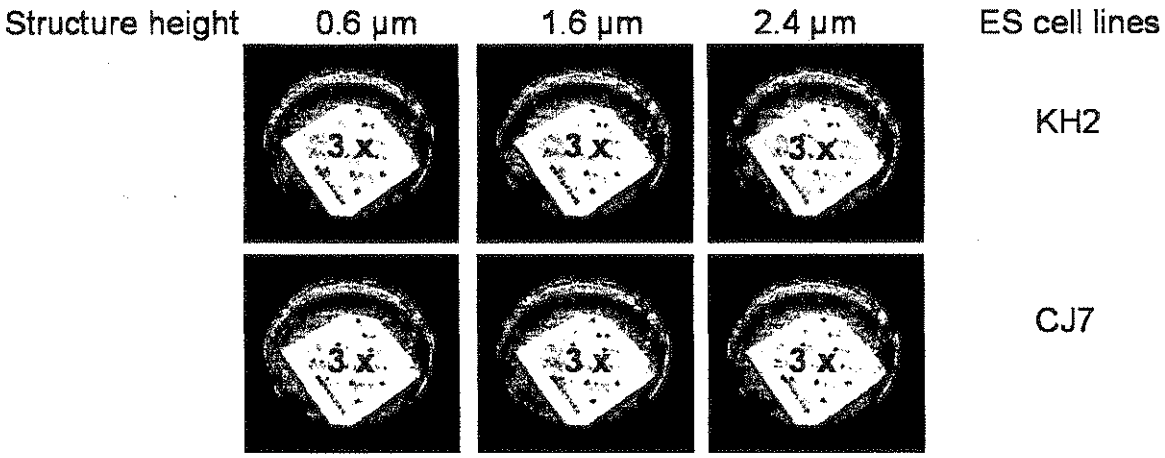
KH2 cells



Intracellular markers of pluripotency:	Oct4 Nanog Alkaline phosphatase (AP)

Fig. 10

Eksperimental setup



Triple assay with tantalum wafers of varying structure heights

How does the topographical tantalum structures affect ES cell differentiation state?

Fig. 11

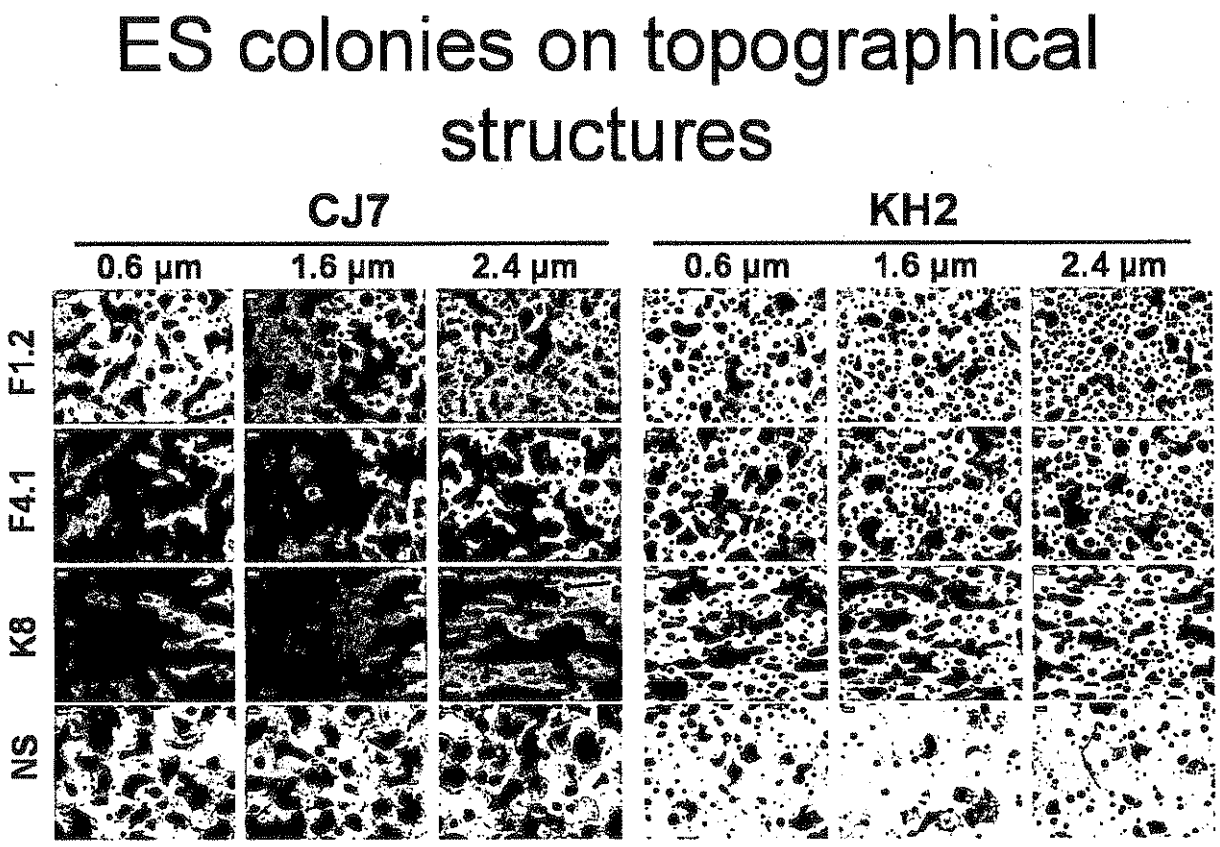

ES colonies on topographical structures
CJ7
KH2
0.6 µm
1.6 µm
2.4 µm
0.6 µm
1.6 µm
2.4 µm
F1.2
F4.1
K8
NS

Fig. 12

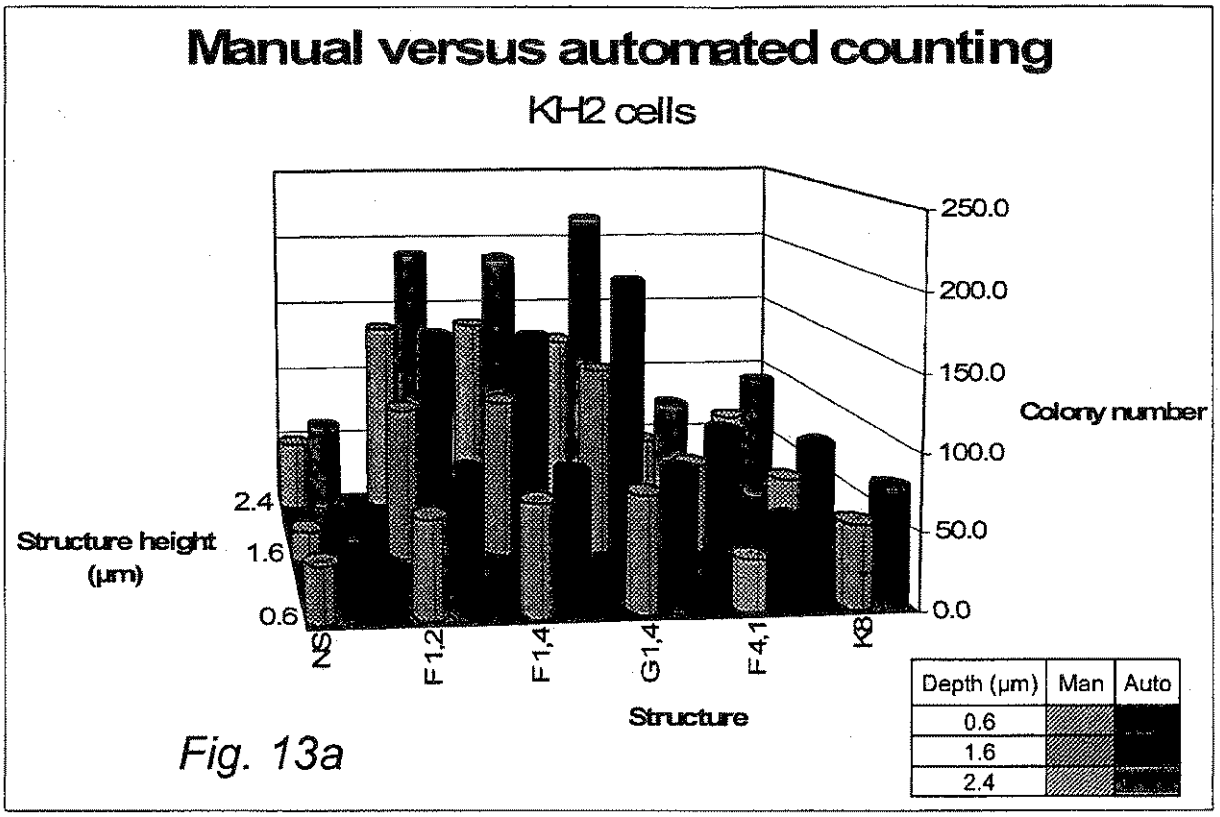


Fig. 13a

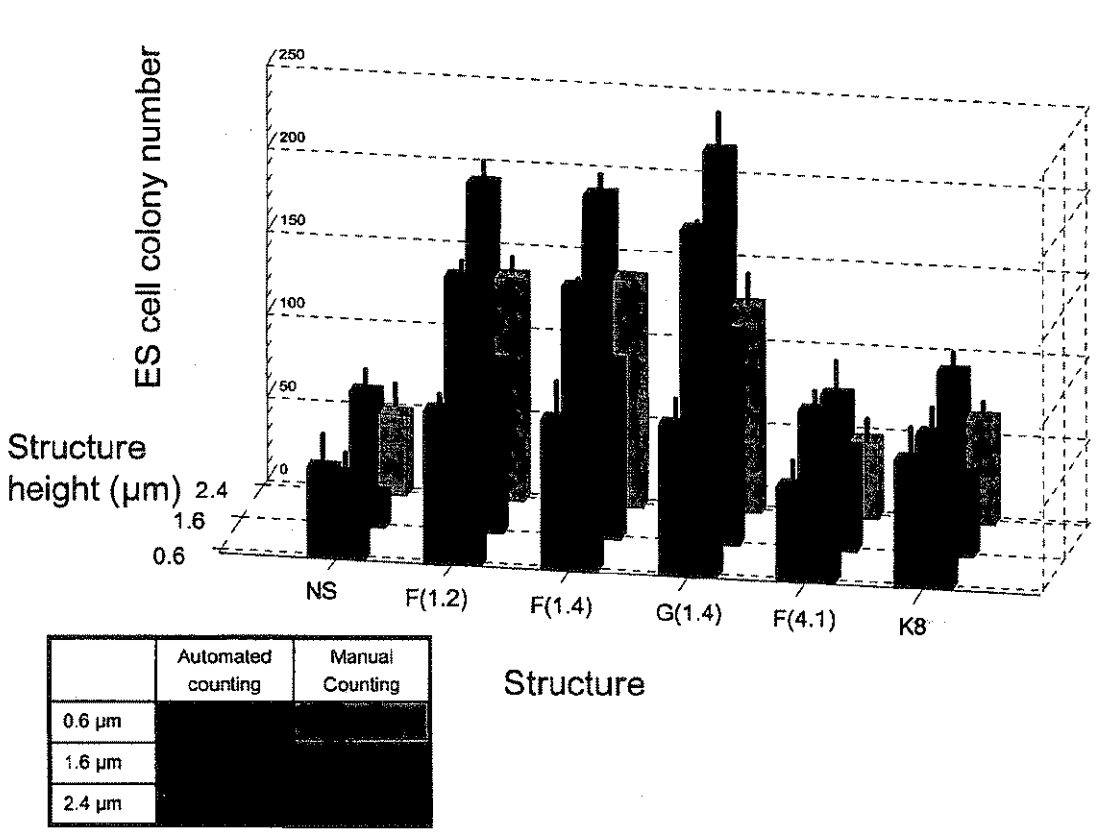


Figure 13b: Automated versus manual counting with standard deviations

CJ7

	X	1				2				4				6				
	Y	1	2	4	6	1	2	4	6	1	2	4	6	1	2	4	6	
0.6 µm	A	14.1	10.3	10.6	12.7	5.5	3.1	10.7	8.8	2.7	4.8	16.5	15.6	7.9	8.7	16.6	13.2	
	C	23.6	17.1	22.5	21.3	22.9	19.2	23.3	8.3	6.9	19.9	20.3	15.3	8.9	14.3	21.7	23.9	
	E	14.7	5.6	19.2	17.0	23.3	22.1	23.3	24.1	21.9	11.2	14.0	10.8	5.5	3.7	4.9	9.3	
	I	8.1	12.9	22.1	17.1	10.7	20.1	22.6	16.5	14.4	16.8	15.6	22.7	7.5	2.5	10.6	17.6	
	B	9.6	14.9	12.8	17.3	8.6	12.5	13.1	17.9	8.3	12.1	17.5	17.7	11.7	11.2	16.0	12.8	
	D	33.8	35.0	18.0	14.7	7.3	10.8	20.2	24.2	13.0	14.6	21.8	25.6	19.6	12.2	14.3	24.0	
	G	28.6	36.2	30.5	23.2	10.0	7.2	11.8	7.2	8.8	11.5	15.5	15.5	10.9	18.1	30.5	33.8	
	J	14.2	16.9	32.5	19.0	10.6	10.5	17.3	20.1	14.8	4.6	7.8	11.8	7.1	6.0	14.2	8.8	
	F	16.6	23.6	26.3	17.5	17.1	15.3	20.3	15.7	8.2	1.7	5.5	9.4	16.0	12.7	19.3	25.8	NS
	H	30.3	14.9	10.1	9.5	8.2	9.7	24.4	25.1	9.9	15.7	19.6	21.4	18.8	19.6	10.3	17.5	20.1
1.6 µm	A	49.3	67.9	55.1	17.7	24.6	31.9	32.2	18.8	14.8	12.4	23.3	24.1	12.2	14.0	30.0	24.6	
	C	37.1	72.9	51.4	39.8	30.5	45.6	42.5	22.8	18.6	28.3	60.1	42.7	20.7	19.7	24.7	26.1	
	E	47.8	52.9	56.8	32.3	27.7	58.7	60.5	25.7	15.9	32.4	33.2	36.6	13.3	11.3	16.0	24.7	
	I	30.5	55.7	54.8	53.8	32.7	48.9	44.1	39.6	20.3	29.6	53.0	49.3	15.1	14.9	19.6	34.7	
	B	58.5	64.9	56.3	28.3	19.3	28.0	48.6	39.0	19.6	27.9	25.0	29.9	26.5	27.1	24.8	23.0	
	D	46.1	94.7	68.4	26.0	12.5	47.7	36.0	52	19.0	26.2	43.4	29.8	16.0	24.4	31.7	26.2	
	G	57.7	84.5	79.0	47.6	34.7	31.4	37.7	18.2	11.9	19.7	25.8	39.2	15.4	29.7	33.1	32.0	
	J	52.7	83.9	51.8	39.1	24.7	40.3	38.9	33.9	14.0	31.4	33.2	29.7	9.8	15.0	19.1	18.8	
	F	30.5	57.4	80.3	61.7	19.4	43.4	59.5	43.6	9.2	10.5	34.2	28.7	19.0	17.9	19.8	38.1	NS
	H	42.1	73.8	58.0	29.2	11.4	35.0	62.5	41.8	16.6	19.9	53.2	42.3	15.4	30.7	28.7	25.1	19.6
2.4 µm	A	66.9	109.4	90.9	38.8	41.0	63.7	63.8	34.4	30.0	45.2	38.1	32.1	29.3	42.0	45.0	47.6	
	C	80.2	109.7	79.0	54.4	57.5	71.1	72.6	38.2	29.9	60.2	66.5	46.6	25.6	36.3	35.9	33.7	
	E	60.2	103.3	91.5	63.0	74.9	90.6	74.7	65.8	36.1	50.6	54.3	50.7	26.8	30.1	48.6	54.4	
	I	70.7	110.4	102.7	73.4	60.8	80.7	97.9	83.3	46.5	54.0	72.7	71.8	40.6	54.5	69.8	69.2	
	B	89.1	117.1	79.3	43.6	52.7	64.8	79.7	44.3	40.1	49.4	39.7	45.8	50.5	55.2	44.8	27.7	
	D	101.8	121.3	72.0	40.7	49.2	79.0	103.3	56.3	43.0	54.1	59.3	49.4	38.1	39.6	44.1	44.1	
	G	116.1	118.4	107.5	80.8	68.7	92.1	67.5	33.8	19.7	78.8	68.3	63.0	39.6	60.4	70.0	69.1	
	J	80.8	122.4	116.4	70.8	60.6	78.4	90.6	45.6	33.4	53.7	54.2	42.5	21.9	33.7	41.4	39.1	
	F	79.2	139.3	126.7	79.0	46.0	72.4	104.8	67.9	20.8	35.2	48.7	52.5	23.9	39.5	67.2	58.1	NS
	H	105.5	125.4	119.3	63.5	39.8	92.1	110.6	88.1	25.3	48.7	61.8	60.9	26.2	44.6	60.6	58.3	18.8

T	1	2	3	4	5	6	7	8	NS
K (0.6 µm)	8.3	7.5	5.8	6.1	7.5	10.2	11.3	4.9	20.1
K (1.6 µm)	14.6	13.5	7.9	4.0	9.5	14.9	15.0	16.9	19.6
K (2.4 µm)	32.3	28.1	20.0	13.0	18.0	18.4	14.9	20.4	18.8

CJ7	0.6	1.6	2.4
cn<10			
10≤cn<15			
15≤cn<20			
20≤cn<25			
25≤cn<30			
30≤cn<35			
35≤cn<40			
40≤cn<45			
45≤cn<50			
50≤cn<55			
55≤cn<60			
60≤cn<65			
65≤cn<70			
70≤cn<75			
75≤cn<80			
80≤cn<85			
85≤cn<90			
90≤cn<95			
95≤cn<100			
100≤cn			

Fig. 14a

KH2

	X	1				2				4				6				
	Y	1	2	4	6	1	2	4	6	1	2	4	6	1	2	4	6	
0.6 µm	A	81.5	92.7	97.7	77.0	44.8	59.2	93.7	82.4	41.3	70.2	68.7	95.0	72.8	56.0	82.3	86.8	
	C	81.6	75.8	75.6	76.1	70.9	64.2	109.2	84.8	75.6	55.8	86.9	79.9	58.1	51.2	60.3	104.1	
	E	83.3	87.9	106.1	90.3	56.4	63.6	93.5	70.4	31.7	55.0	71.6	79.3	45.5	66.5	80.6	79.4	
	I	87.1	81.2	86.3	78.9	58.2	62.4	74.7	68.1	42.2	61.7	60.9	103.0	69.0	61.4	84.4	102.4	
	B	81.4	91.5	87.7	89.2	58.3	66.4	110.1	101.5	66.8	66.1	81.2	87.5	57.4	62.6	81.0	85.7	
	D	77.3	100.4	84.7	93.5	72.5	81.2	87.2	87.8	44.1	48.2	46.6	78.0	33.3	48.3	65.7	77.6	
	F	98.7	98.3	89.8	72.4	61.8	76.8	92.1	59.6	42.5	53.9	100.0	84.9	40.2	55.7	93.4	104.1	
	H	81.8	70.1	78.6	68.8	41.1	35.5	89.9	85.8	44.3	87.4	90.0	79.9	41.5	51.8	70.2	74.6	
	J	78.9	91.8	90.8	76.2	84.3	59.6	91.9	101.8	57.2	53.0	81.5	81.4	40.3	48.8	54.3	77.8	NS
	L	98.9	99.3	108.3	114.9	59.4	55.1	71.4	115.8	76.8	28.	76.8	93.6	53.2	61.2	84.4	85.2	62.9
1.6 µm	A	114.7	137.0	130.0	104.8	91.3	94.3	126.0	120.6	83.6	94.4	111.5	129.5	80.0	106.7	116.6	117.1	
	C	118.0	151.8	117.2	125.5	93.8	132.1	125.3	122.2	87.1	101.9	137.2	125.1	98.7	103.6	126.0	121.5	
	E	100.1	121.9	132.2	129.3	107.7	139.5	158.7	144.6	108.8	140.7	141.1	123.8	78.3	81.4	101.5	100.8	
	I	120.9	127.6	150.1	95.7	104.7	152.7	135.7	135.0	110.3	121.5	130.0	128.0	95.4	93.1	102.8	115.5	
	B	145.6	154.6	148.6	116.8	93.4	118.4	142.6	124.6	106.8	104.9	122.8	127.6	89.9	122.7	113.7	94.1	
	D	131.1	159.5	136.6	110.2	85.7	131.0	147.9	147.7	103.8	124.7	129.2	128.3	88.0	103.4	128.3	122.9	
	F	146.8	166.4	188.5	142.4	104.4	148.4	129.0	93.5	88.9	102.6	95.6	130.1	92.8	103.9	131.3	110.3	
	H	142.7	150.4	169.9	131.4	111.8	132.8	133.0	127.1	85.3	125.3	123.9	79.8	82.3	83.6	92.3	113.4	
	J	110.7	152.5	150.5	139.8	96.9	134.6	152.7	139.6	83.4	100.1	102.7	99.6	94.1	105.0	117.1	120.5	NS
	L	134.6	155.3	142.9	107.5	61.5	122.4	140.1	116.8	77.4	121.7	149.0	128.7	84.5	101.9	111.3	104.7	30.3
2.4 µm	A	149.0	167.4	154.4	162.9	134.0	149.3	171.1	141.6	134.8	157.6	134.4	136.9	122.6	136.3	152.5	152.1	
	C	156.7	161.2	171.6	163.4	158.1	159.1	141.7	136.5	101.6	159.5	164.0	161.7	117.1	134.0	158.4	155.3	
	E	114.7	144.8	176.6	154.8	158.6	172.1	171.7	144.6	129.4	167.7	185.8	138.2	101.7	128.7	155.5	139.4	
	I	100.2	136.0	168.5	163.6	146.2	156.6	162.8	173.4	118.7	146.4	171.4	149.6	114.4	115.9	117.3	167.2	
	B	174.2	185.9	191.4	152.4	140.6	160.0	162.7	167.0	141.5	152.7	144.1	139.3	129.3	164.9	163.0	164.8	
	D	157.5	189.8	151.0	118.0	106.1	164.3	159.6	168.3	110.8	163.0	167.5	144.2	110.4	151.3	162.8	161.7	
	F	172.0	195.9	216.0	145.5	119.8	163.4	174.1	148.2	117.0	142.3	176.6	168.0	132.1	156.6	153.1	165.7	
	H	157.4	174.3	152.2	149.4	111.9	146.2	156.3	118.9	110.8	135.0	153.5	122.5	115.5	129.0	117.6	119.7	
	J	139.2	190.2	186.1	196.4	113.5	148.4	166.3	147.1	72.7	103.7	129.4	147.5	98.4	143.9	151.1	162.1	NS
	L	148.0	119.7	155.3	134.7	78.5	140.2	162.2	186.5	97.2	147.0	149.9	119.2	76.7	64.9	82.7	124.6	61.0

T	1	2	3	4	5	6	7	8	NS
K (0.6 µm)	52.6	33.9	43.8	40.8	47.9	68.6	65.1	77.9	52.9
K (1.6 µm)	69.1	65.0	68.6	67.3	64.6	63.9	72.4	72.4	30.3
K (2.4 µm)	88.2	74.4	77.4	82.9	81.4	71.3	88.3	90.4	61.0

KH2	0.6	1.6	2.4
cn<50			
50≤cn<60			
60≤cn<70			
70≤cn<80			
80≤cn<90			
90≤cn<100			
100≤cn<110			
110≤cn<120			
120≤cn<130			
130≤cn<140			
140≤cn<150			
150≤cn<160			
160≤cn<170			
170≤cn<180			
180≤cn			

Fig. 14b

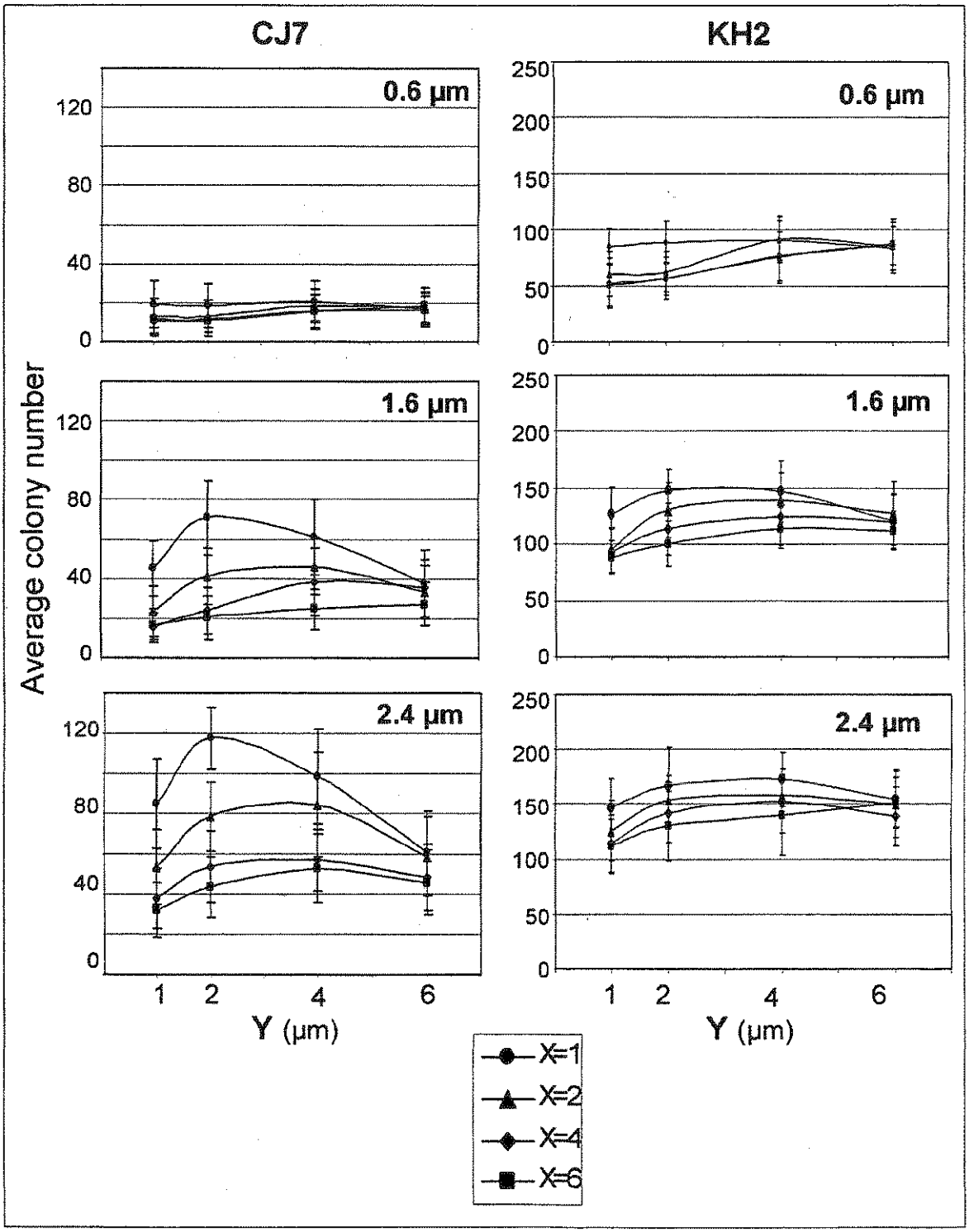


Fig. 15

Structure height influences colony number

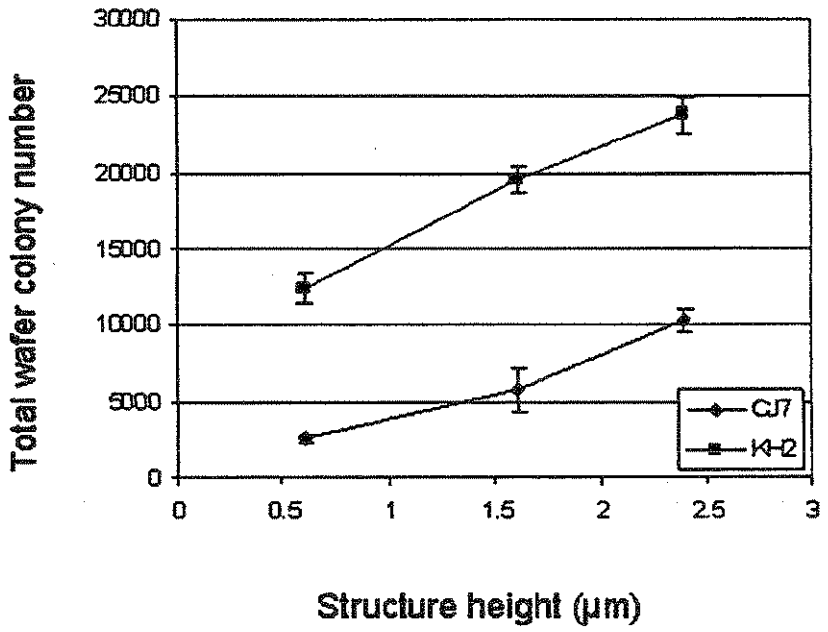


Fig. 16

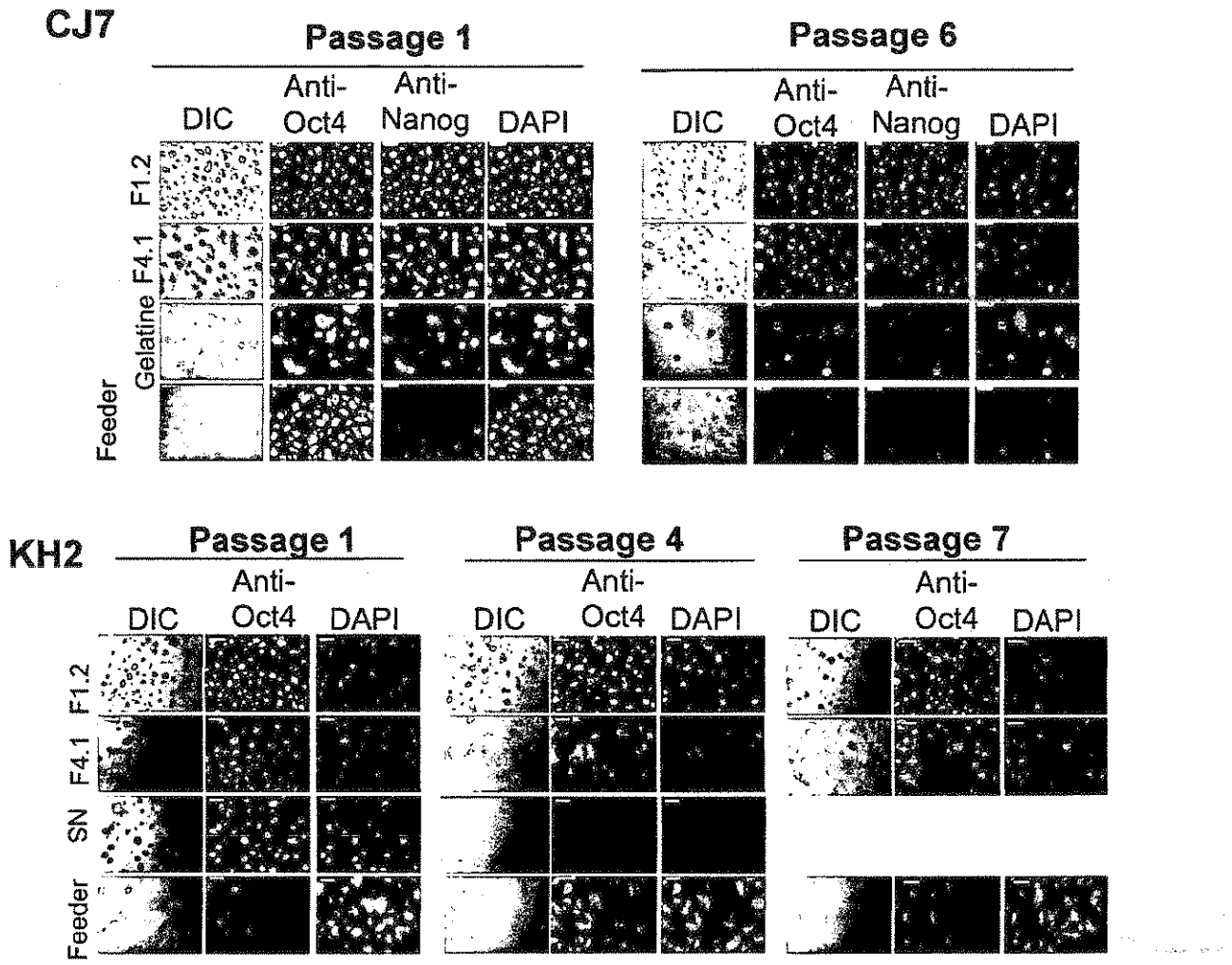

CJ7
Passage 1
Passage 6
DIC
Anti-Oct4
Anti-Nanog
DAPI
F1.2
F4.1
Gelatine
Feeder
KH2
Passage 1
Passage 4
Passage 7
DIC
Anti-Oct4
DAPI
F1.2
F4.1
SN
Feeder

Fig. 17

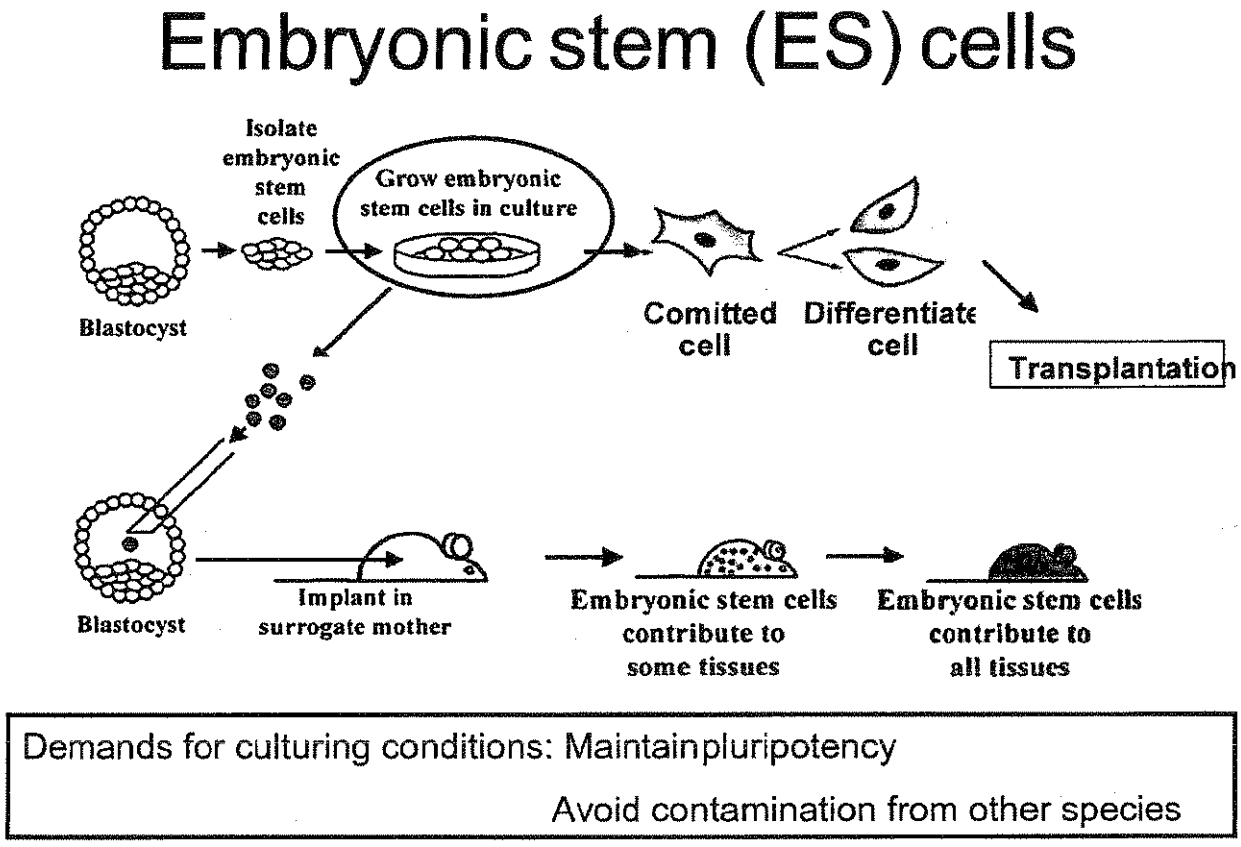

Embryonic stem (ES) cells
Isolate embryonic stem cells
Grow embryonic stem cells in culture
Blastocyst
Comitted cell
Differentiate cell
Transplantation
Blastocyst
Implant in surrogate mother
Embryonic stem cells contribute to some tissues
Embryonic stem cells contribute to all tissues
Demands for culturing conditions: Maintainpluripotency
Avoid contamination from other species

Fig. 18

CJ7 cells passaged on F1.2 have the potential for germline transmission

Growth support	# blastocysts injected	Pups live/total	♂≥50% chimaerism	Mating ♂	Sterile	Germline
F1,2	34	16/20	5	5	4	1
F4,1	41	5/7	3	3	1	0
Gelatine	18	5/5	4	4	3	0
Feeder	31	13/16	3	6	4	1

Fig. 19

CJ7

0.6 µm

X	1				2				4				6				
Y	1	2	4	6	1	2	4	6	1	2	4	6	1	2	4	6	
A	63.3	66.7	68.5	70.5	78.7	78.0	69.4	75.8	83.7	83.1	66.5	64.6	72.7	74.5	58.0	58.5	
C	48.4	50.9	52.9	47.7	46.5	50.4	55.8	71.6	73.8	53.9	46.3	47.8	63.8	54.1	50.1	45.7	
E	58.1	72.9	61.7	52.5	45.5	45.1	46.7	43.3	51.9	57.6	54.0	57.7	72.9	79.1	81.6	69.0	
I	73.0	65.6	54.9	54.5	64.9	51.7	47.2	56.7	59.4	54.0	54.8	52.3	72.1	79.7	71.3	55.5	
B	57.5	60.1	63.4	56.3	70.9	66.7	59.2	52.0	65.9	69.3	65.1	58.4	62.4	62.0	59.4	62.4	
D	43.1	38.2	52.6	55.8	76.3	67.2	45.5	40.0	57.2	54.1	42.4	45.7	53.2	54.5	58.2	49.6	
G	38.6	41.2	38.2	45.4	69.1	75.8	73.2	75.9	66.5	64.6	61.3	51.6	64.3	51.5	44.0	34.1	
J	60.0	58.4	47.5	56.7	65.6	66.8	55.6	51.9	58.9	75.2	75.6	67.3	76.7	80.8	70.8	75.2	
F	48.3	42.9	41.4	45.0	56.1	55.1	49.2	56.4	76.7	85.7	80.6	67.3	60.9	62.3	55.4	41.8	NS
H	38.8	61.4	67.8	70.1	74.4	62.5	48.7	49.2	63.3	61.4	55.8	45.7	45.9	52.5	64.7	67.0	39.9

1.6 µm

A	42.4	36.2	47.9	63.9	63.3	60.6	57.2	65.9	73.0	69.7	63.9	60.6	69.3	63.2	53.0	56.7	
C	51.3	37.8	43.1	45.3	51.0	46.1	45.6	59.2	66.3	50.9	40.4	41.8	60.6	59.1	54.3	53.8	
E	47.3	48.5	44.6	51.3	60.8	41.9	36.5	52.7	63.3	52.5	52.8	50.6	68.3	74.4	66.3	58.8	
I	57.0	49.3	44.3	40.2	55.9	49.1	49.8	48.0	65.8	54.7	43.7	44.6	66.9	70.7	66.6	55.2	
B	39.8	41.4	42.7	51.9	64.9	57.4	47.1	49.4	62.1	56.8	61.4	51.4	54.8	53.7	58.5	53.5	
D	40.8	30.4	36.1	52.0	70.7	51.8	53.3	41.4	58.9	53.1	46.7	50.3	59.8	52.5	51.4	53.1	
G	37.1	33.1	39.2	43.4	61.7	58.9	57.4	63.8	72.2	63.6	55.3	47.2	63.8	53.4	49.4	47.3	
J	45.6	38.6	48.1	49.9	63.1	54.3	49.9	51.7	66.7	55.6	56.2	49.9	75.6	67.0	68.9	70.4	
F	52.4	44.4	34.3	40.2	54.8	53.2	43.5	46.9	71.2	73.0	60.4	56.1	66.8	60.6	56.4	42.4	NS
H	43.7	39.5	45.0	60.5	71.2	56.9	42.0	46.7	63.9	59.6	45.1	47.1	60.6	52.9	54.1	59.1	46.8

2.4 µm

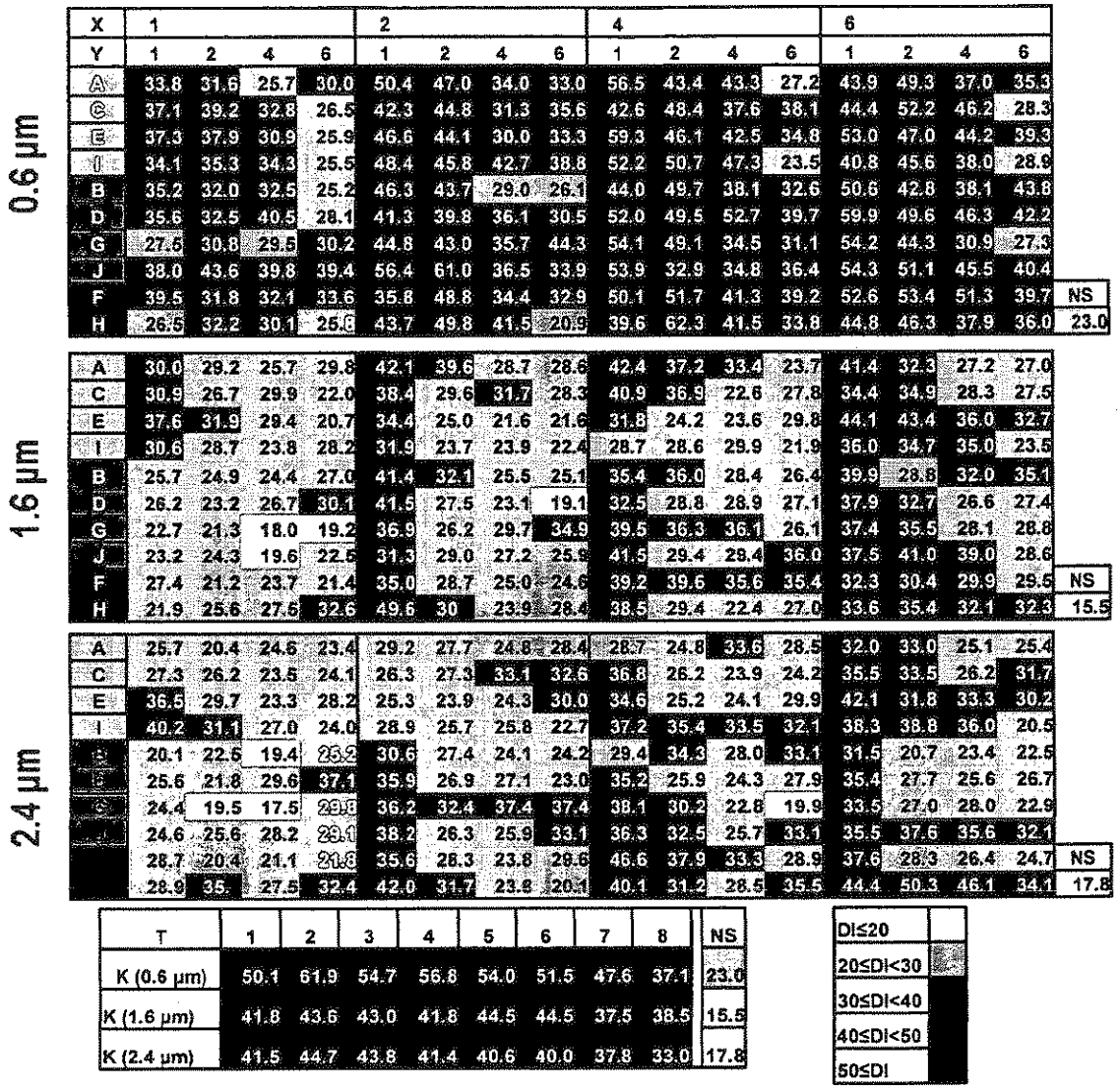
A0	41.5	30.6	31.3	46.6	48.3	38.9	42.8	54.3	57.8	49.3	48.9	48.6	51.6	54.4	46.2	37.5	
C	34.4	33.3	38.9	42.4	43.8	34.4	35.9	43.2	59.4	37.5	39.2	41.4	58.1	49.4	53.6	47.2	
E	34.1	30.0	34.7	38.3	36.9	27.8	33.3	33.5	42.9	44.1	38.4	42.1	59.4	59.4	47.2	48.4	
I	36.7	24.7	24.9	32.2	44.7	36.6	29.1	28.8	46.0	42.7	34.5	44.9	48.5	46.8	39.4	38.8	
B	31.2	24.5	32.7	44.3	47.2	40.5	33.2	39.5	43.0	39.0	47.4	45.1	43.1	39.2	40.8	49.8	
D	29.2	27.6	35.4	44.7	50.5	39.6	30.9	37.3	49.3	45.6	42.3	43.0	52.3	50.1	44.5	45.5	
G	22.2	29.0	28.3	32.2	37.3	39.6	38.7	46.3	47.2	35.4	34.2	34.1	49.6	38.5	35.9	34.0	
J	32.4	26.8	25.0	33.3	42.8	35.2	29.3	38.9	48.6	41.0	40.4	42.4	60.5	48.6	42.6	47.0	
F	29.6	22.4	22.2	35.7	41.9	36.2	28.6	36.9	59.2	53.5	49.1	41.3	55.5	40.7	36.0	38.9	NS
H	22.4	21.6	23.8	38.6	47.9	29.8	22.7	27.8	53.6	44.8	35.8	34.8	49.6	41.6	37.4	36.7	45.3

T	1	2	3	4	5	6	7	8	NS
K (0.6 µm)	72.0	75.0	75.4	78.0	71.4	65.5	70.6	75.4	39.9
K(1.6 µm)	73.8	73.6	77.4	80.8	73.3	65.6	66.4	60.4	46.8
K (2.4 µm)	52.2	55.1	59.5	63.4	59.5	57.7	63.2	56.4	45.3

DI<30	
30≤DI<40	
40≤DI<50	
50≤DI<60	
60≤DI<70	
70≤DI	

Fig. 20a

KH2



	X	1				2				4				6				
	Y	1	2	4	6	1	2	4	6	1	2	4	6	1	2	4	6	
0.6 µm	A	33.8	31.6	25.7	30.0	50.4	47.0	34.0	33.0	56.5	43.4	43.3	27.2	43.9	49.3	37.0	35.3	
	C	37.1	39.2	32.8	26.5	42.3	44.8	31.3	35.6	42.6	48.4	37.6	38.1	44.4	52.2	46.2	28.3	
	E	37.3	37.9	30.9	25.9	46.6	44.1	30.0	33.3	59.3	46.1	42.5	34.8	53.0	47.0	44.2	39.3	
	I	34.1	35.3	34.3	25.5	48.4	45.8	42.7	38.8	52.2	50.7	47.3	23.5	40.8	45.6	38.0	28.9	
	B	35.2	32.0	32.5	25.2	46.3	43.7	29.0	26.1	44.0	49.7	38.1	32.6	50.6	42.8	38.1	43.8	
	D	35.6	32.5	40.5	28.1	41.3	39.8	36.1	30.5	52.0	49.5	52.7	39.7	59.9	49.6	46.3	42.2	
	G	27.6	30.8	29.5	30.2	44.8	43.0	35.7	44.3	54.1	49.1	34.5	31.1	54.2	44.3	30.9	27.3	
	J	38.0	43.6	39.8	39.4	56.4	61.0	36.5	33.9	53.9	32.9	34.8	36.4	54.3	51.1	45.5	40.4	
	F	39.5	31.8	32.1	33.6	35.8	48.8	34.4	32.9	50.1	51.7	41.3	39.2	52.6	53.4	51.3	39.7	NS
	H	26.5	32.2	30.1	25.8	43.7	49.8	41.5	20.5	39.6	62.3	41.5	33.8	44.8	46.3	37.9	36.0	23.0
1.6 µm	A	30.0	29.2	25.7	29.8	42.1	39.6	28.7	28.6	42.4	37.2	33.4	23.7	41.4	32.3	27.2	27.0	
	C	30.9	26.7	29.9	22.0	38.4	29.6	31.7	28.3	40.9	36.9	22.6	27.8	34.4	34.9	28.3	27.5	
	E	37.6	31.9	29.4	20.7	34.4	25.0	21.6	21.6	31.8	24.2	23.6	29.8	44.1	43.4	36.0	32.7	
	I	30.6	28.7	23.8	28.2	31.9	23.7	23.9	22.4	28.7	28.6	29.9	21.9	36.0	34.7	35.0	23.5	
	B	25.7	24.9	24.4	27.0	41.4	32.1	25.5	25.1	35.4	36.0	28.4	26.4	39.9	28.8	32.0	35.1	
	D	26.2	23.2	26.7	30.1	41.5	27.5	23.1	19.1	32.5	28.8	28.9	27.1	37.9	32.7	26.6	27.4	
	G	22.7	21.3	18.0	19.2	36.9	26.2	29.7	34.9	39.5	36.3	36.1	26.1	37.4	35.5	28.1	28.8	
	J	23.2	24.3	19.6	22.5	31.3	29.0	27.2	25.9	41.5	29.4	29.4	36.0	37.5	41.0	39.0	28.6	
	F	27.4	21.2	23.7	21.4	35.0	28.7	25.0	24.6	39.2	39.6	35.6	35.4	32.3	30.4	29.9	25.5	NS
	H	21.9	25.6	27.6	32.6	49.6	30	23.9	28.4	38.5	29.4	22.4	27.0	33.6	35.4	32.1	32.3	15.5
2.4 µm	A	25.7	20.4	24.6	23.4	29.2	27.7	24.8	28.4	28.7	24.8	33.6	28.5	32.0	33.0	25.1	25.4	
	C	27.3	26.2	23.5	24.1	26.3	27.3	33.1	32.6	36.8	26.2	23.9	24.2	35.5	33.5	26.2	31.7	
	E	36.5	29.7	23.3	28.2	25.3	23.9	24.3	30.0	34.6	25.2	24.1	29.9	42.1	31.8	33.3	30.2	
	I	40.2	31.1	27.0	24.0	28.9	25.7	25.8	22.7	37.2	35.4	33.5	32.1	38.3	38.8	36.0	20.5	
	B	20.1	22.5	19.4	25.2	30.6	27.4	24.1	24.2	29.4	34.3	28.0	33.1	31.5	20.7	23.4	22.5	
	D	25.6	21.8	29.6	37.1	35.9	26.9	27.1	23.0	35.2	25.9	24.3	27.9	35.4	27.7	25.6	26.7	
	G	24.4	19.5	17.5	20.8	36.2	32.4	37.4	37.4	38.1	30.2	22.8	19.9	33.5	27.0	28.0	22.9	
	J	24.6	25.6	28.2	28.1	38.2	26.3	25.9	33.1	36.3	32.5	25.7	33.1	35.5	37.6	35.6	32.1	
		28.7	20.4	21.1	21.8	35.6	28.3	23.8	29.6	46.6	37.9	33.3	28.9	37.6	28.3	26.4	24.7	NS
		28.9	35.	27.5	32.4	42.0	31.7	23.8	20.1	40.1	31.2	28.5	35.5	44.4	50.3	46.1	34.1	17.8

T	1	2	3	4	5	6	7	8	NS
K (0.6 µm)	50.1	61.9	54.7	56.8	54.0	51.5	47.6	37.1	23.0
K (1.6 µm)	41.8	43.6	43.0	41.8	44.5	44.5	37.5	38.5	15.5
K (2.4 µm)	41.5	44.7	43.8	41.4	40.6	40.0	37.8	33.0	17.8

DI≤20	
20≤DI<30	
30≤DI<40	
40≤DI<50	
50≤DI	

Fig. 20b

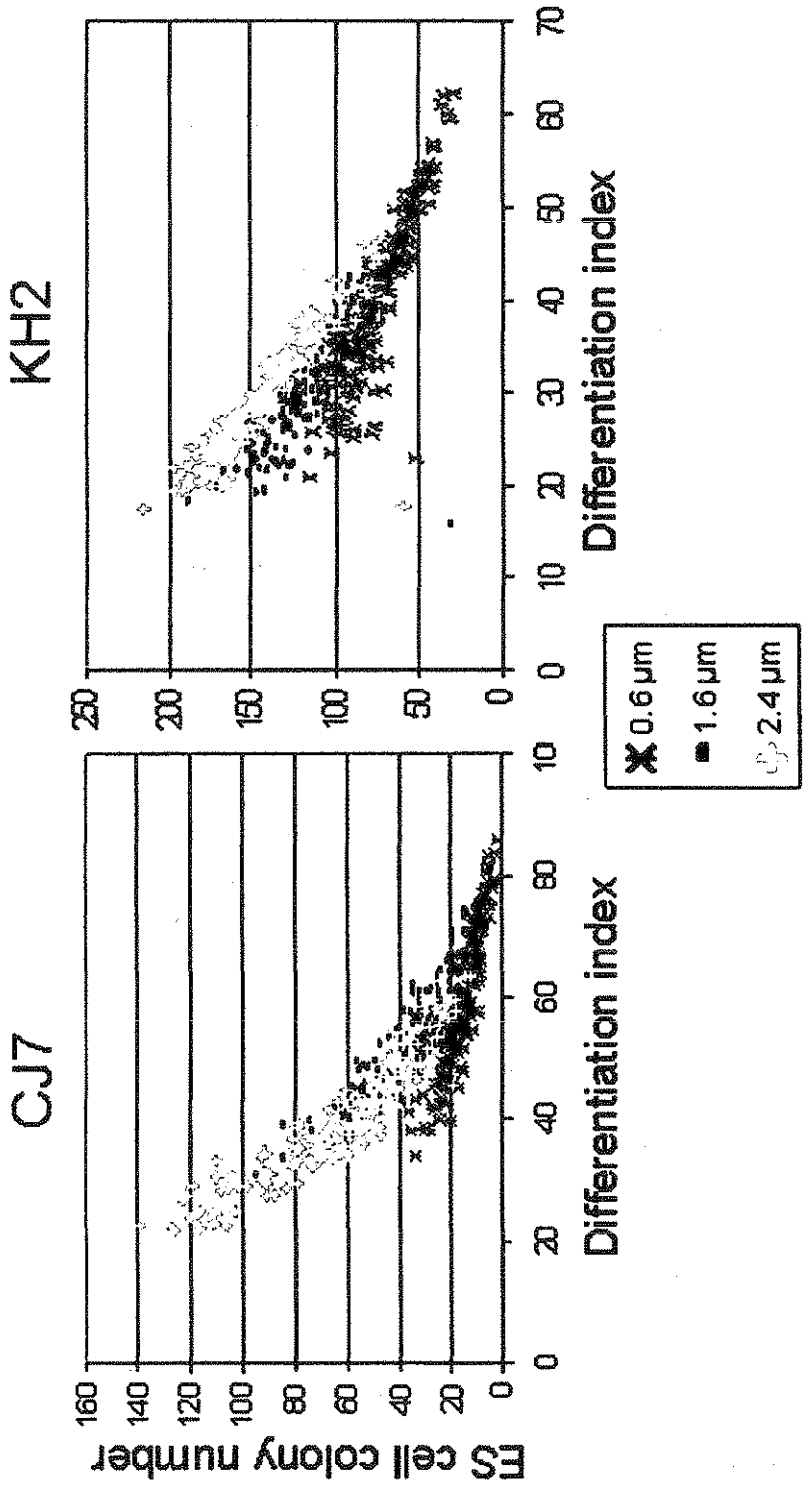

CJ7
KH2
ES cell colony number
Differentiation index
Differentiation index
0.6 μm
1.6 μm
2.4 μm

Fig. 21

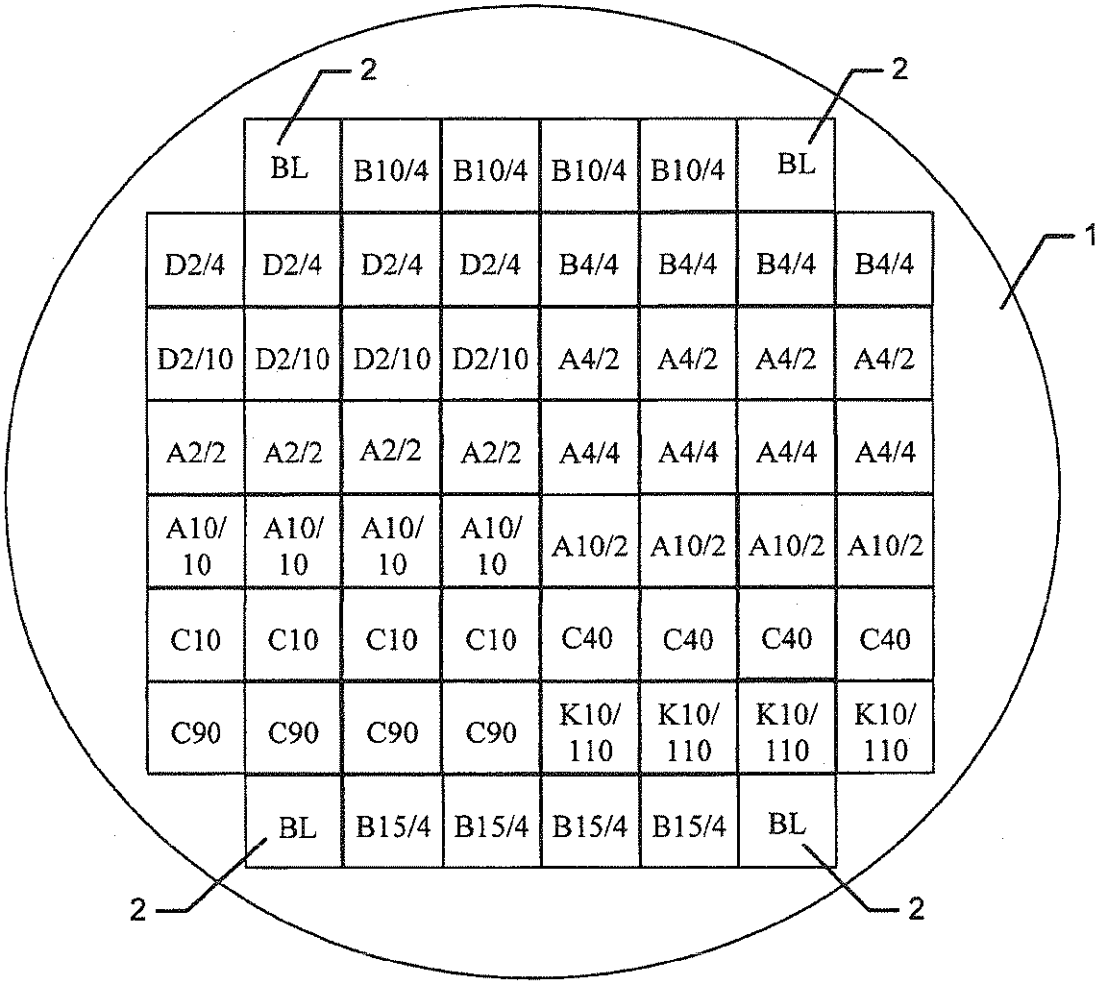

2
2
1
BL
B10/4
B10/4
B10/4
B10/4
BL
D2/4
D2/4
D2/4
D2/4
B4/4
B4/4
B4/4
B4/4
D2/10
D2/10
D2/10
D2/10
A4/2
A4/2
A4/2
A4/2
A2/2
A2/2
A2/2
A2/2
A4/4
A4/4
A4/4
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A10/2
A10/2
A10/2
A10/2
C10
C10
C10
C10
C40
C40
C40
C40
C90
C90
C90
C90
K10/110
K10/110
K10/110
K10/110
BL
B15/4
B15/4
B15/4
B15/4
BL
2
2

Fig. 22

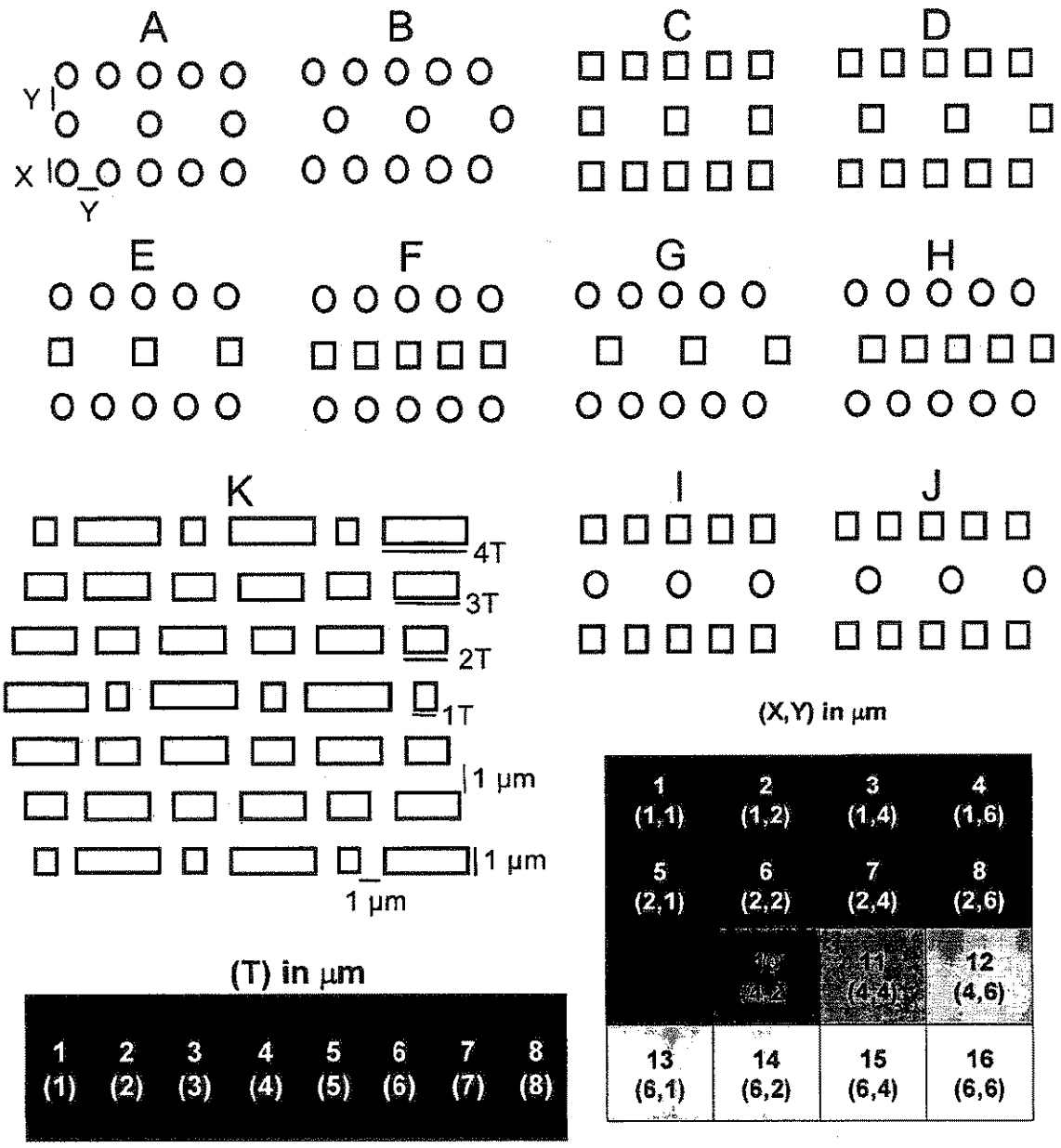

A
B
C
D
Y
X
Y
E
F
G
H
K
I
J
4T
3T
2T
1T
1 µm
1 µm
1 µm
(X,Y) in µm
1 (1,1)
2 (1,2)
3 (1,4)
4 (1,6)
5 (2,1)
6 (2,2)
7 (2,4)
8 (2,6)
11 (4,4)
12 (4,6)
13 (6,1)
14 (6,2)
15 (6,4)
16 (6,6)
(T) in µm
1 (1)
2 (2)
3 (3)
4 (4)
5 (5)
6 (6)
7 (7)
8 (8)

Fig. 23a

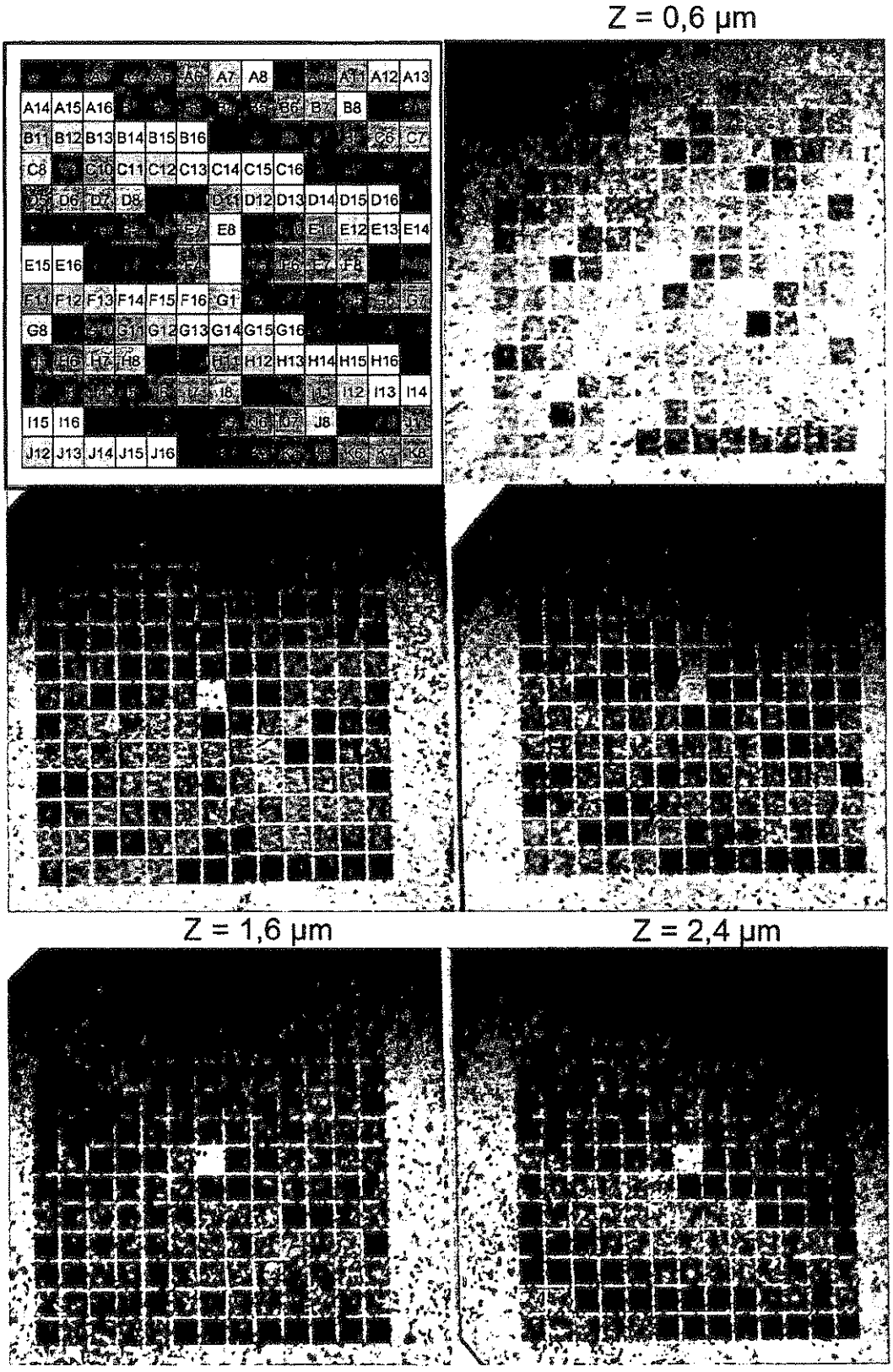
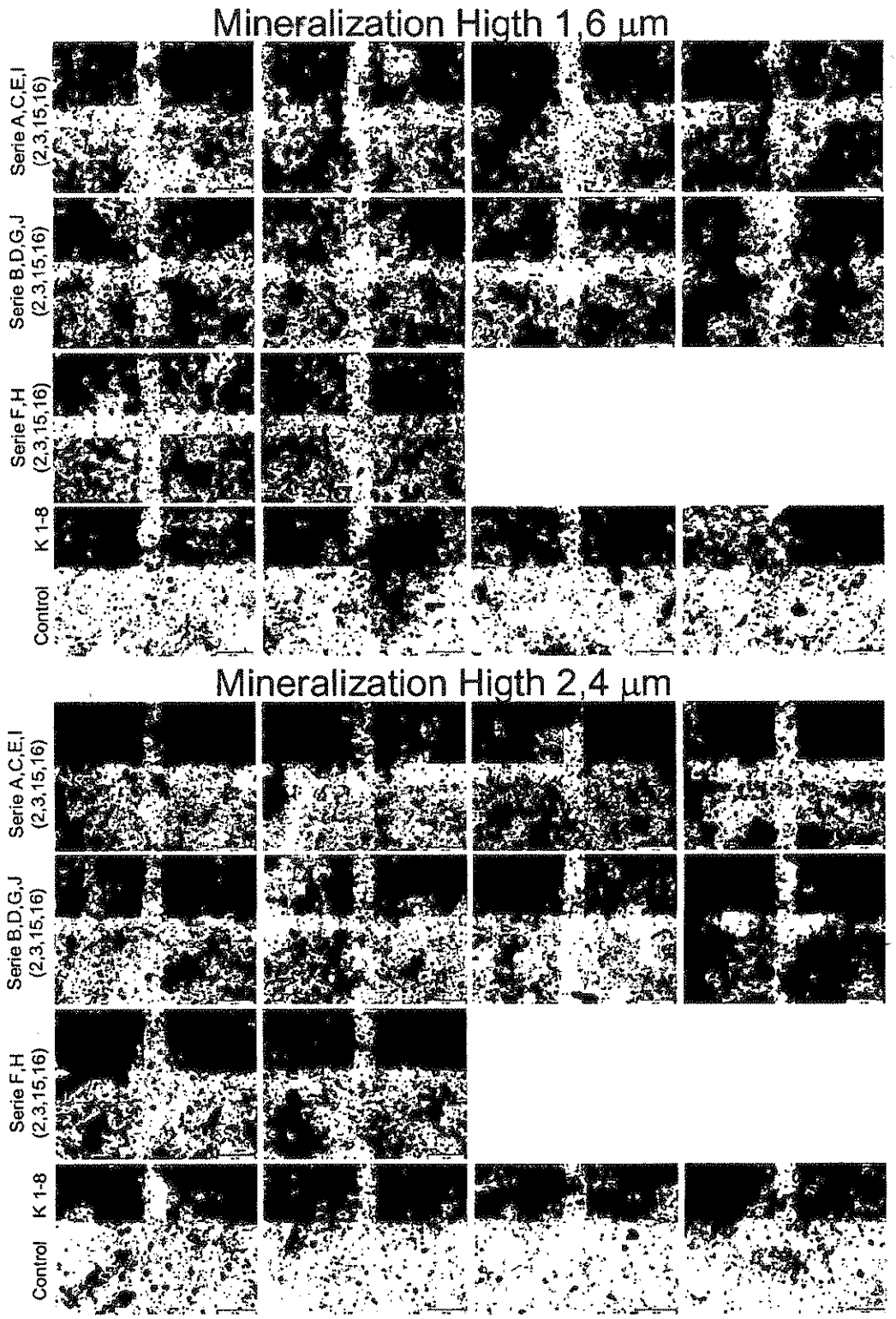

Z = 0,6 µm
A14 A15 A16
B11 B12 B13 B14 B15 B16
C8 C10 C11 C12 C13 C14 C15 C16
D11 D12 D13 D14 D15 D16
E8 E12 E13 E14
E15 E16
F11 F12 F13 F14 F15 F16 G1
G8 G11 G12 G13 G14 G15 G16
H12 H13 H14 H15 H16
I12 I13 I14
I15 I16
J8
J12 J13 J14 J15 J16
Z = 1,6 µm
Z = 2,4 µm

Fig. 23b



Alizarin red: Upper three rows; Serie A to J (2,3 and 15,16) Last row; K 1-8 and control surfaces

Fig. 23c

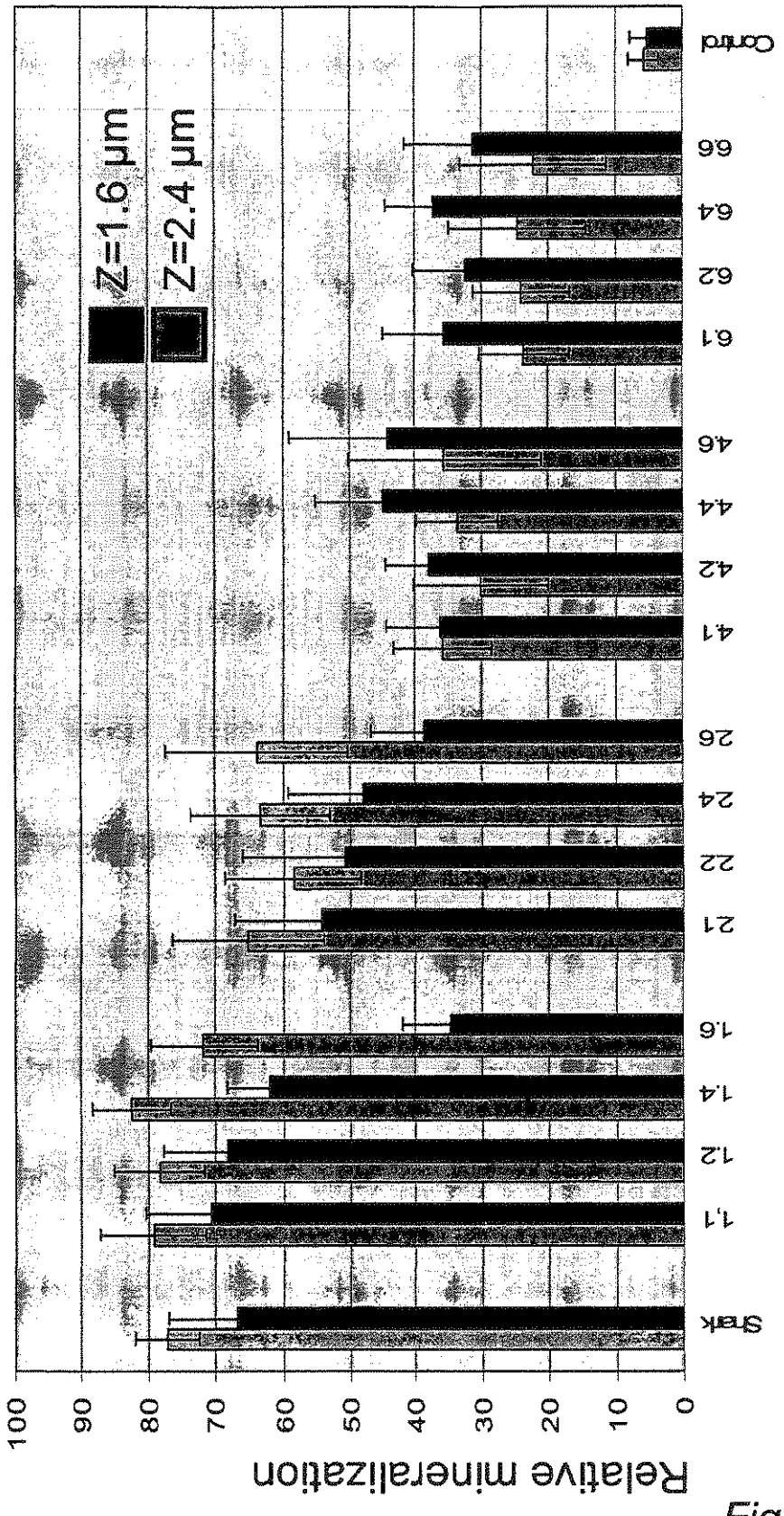

Relative mineralization
0
10
20
30
40
50
60
70
80
90
100
Z=1.6 µm
Z=2.4 µm
Control
6.6
6.4
6.2
6.1
4.6
4.4
4.2
4.1
2.6
2.4
2.2
2.1
1.6
1.4
1.2
1.1
Stark

Fig. 23d

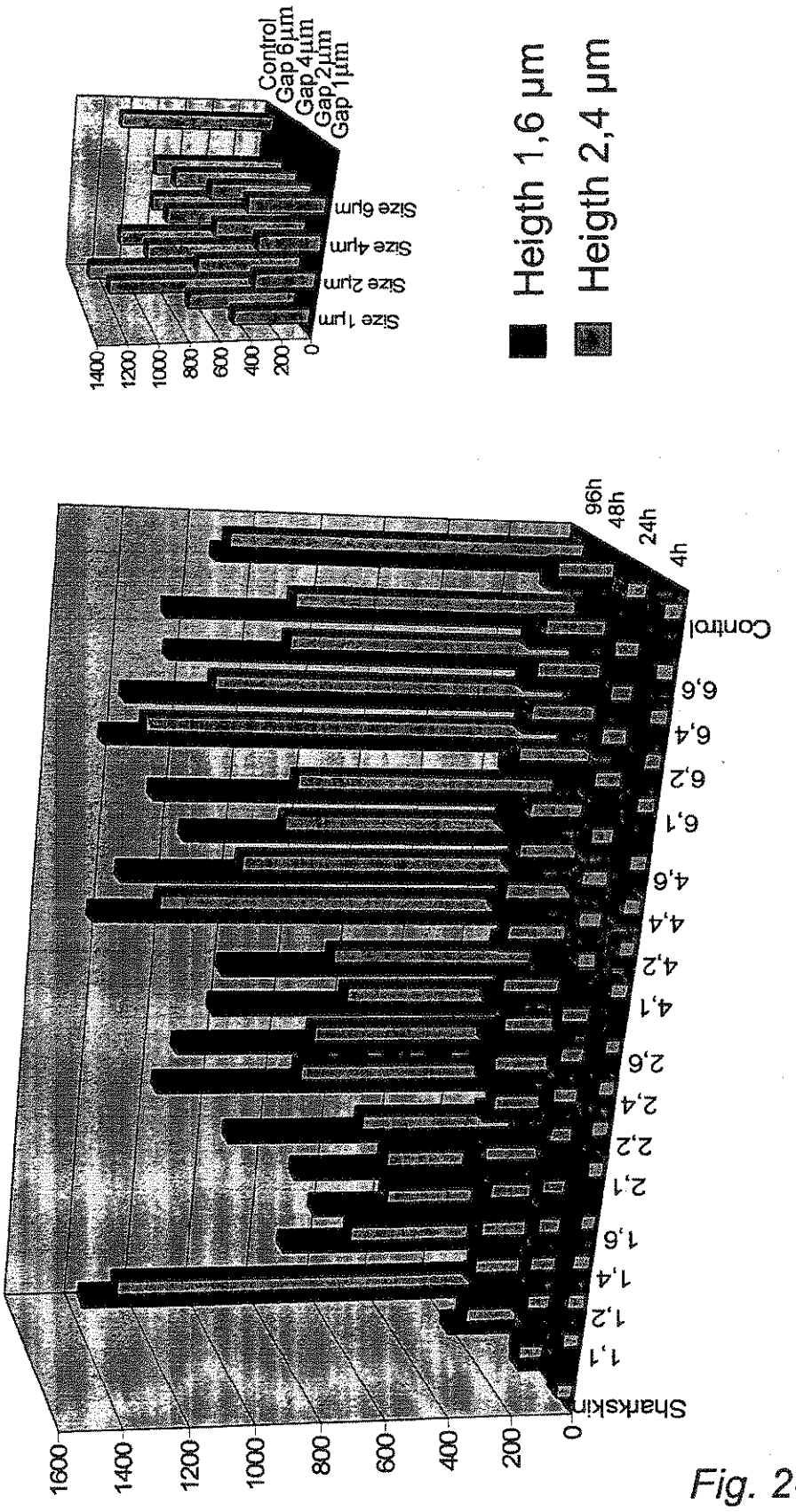

Heigth 1,6 µm
Heigth 2,4 µm
Control
Gap 6µm
Gap 4µm
Gap 2µm
Gap 1µm
Size 1µm
Size 2µm
Size 4µm
Size 6µm
0
200
400
600
800
1000
1200
1400
Sharkskin
1,1
1,2
1,4
1,6
2,1
2,2
2,4
2,6
4,1
4,2
4,4
4,6
6,1
6,2
6,4
6,6
Control
4h
24h
48h
96h
0
200
400
600
800
1000
1200
1400
1600

Fig. 24

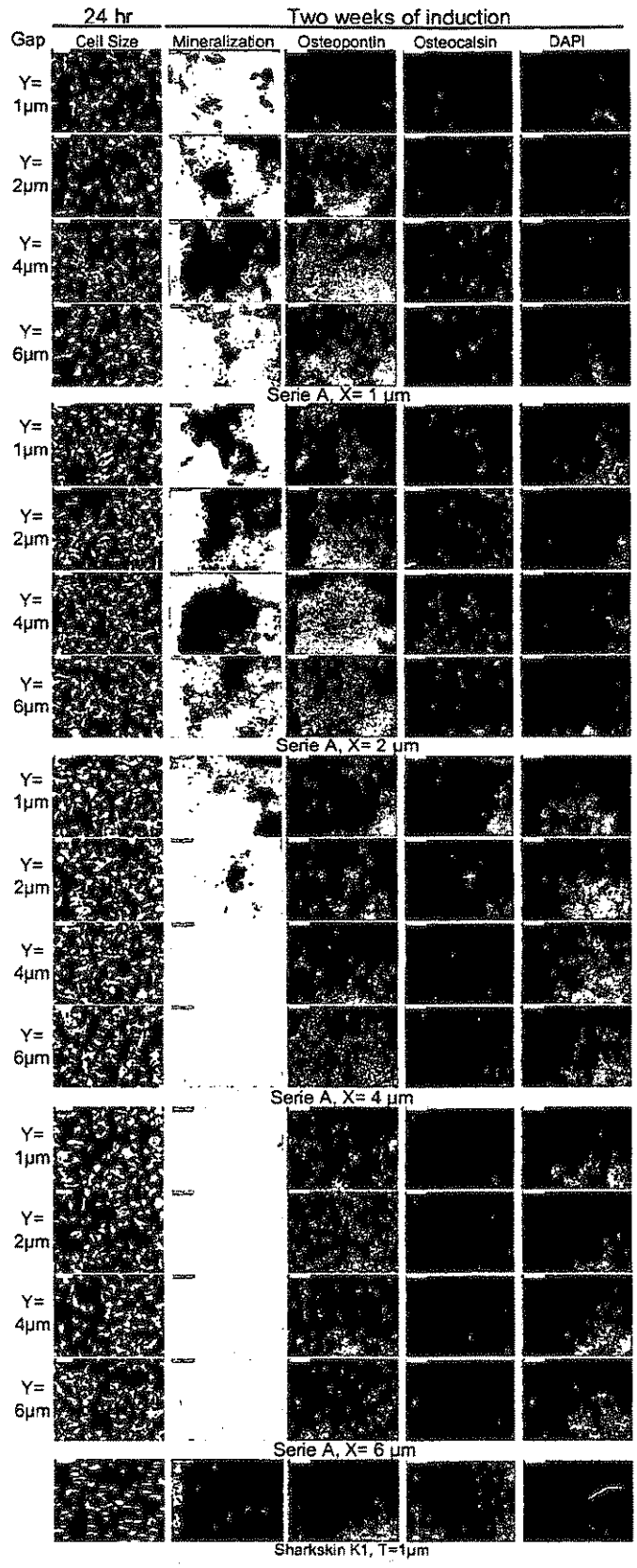

24 hr
Two weeks of induction
Gap
Cell Size
Mineralization
Osteopontin
Osteocalsin
DAPI
Y= 1µm
Y= 2µm
Y= 4µm
Y= 6µm
Serie A, X= 1 µm
Serie A, X= 2 µm
Serie A, X= 4 µm
Serie A, X= 6 µm
Sharkskin K1, T=1µm

Fig. 25

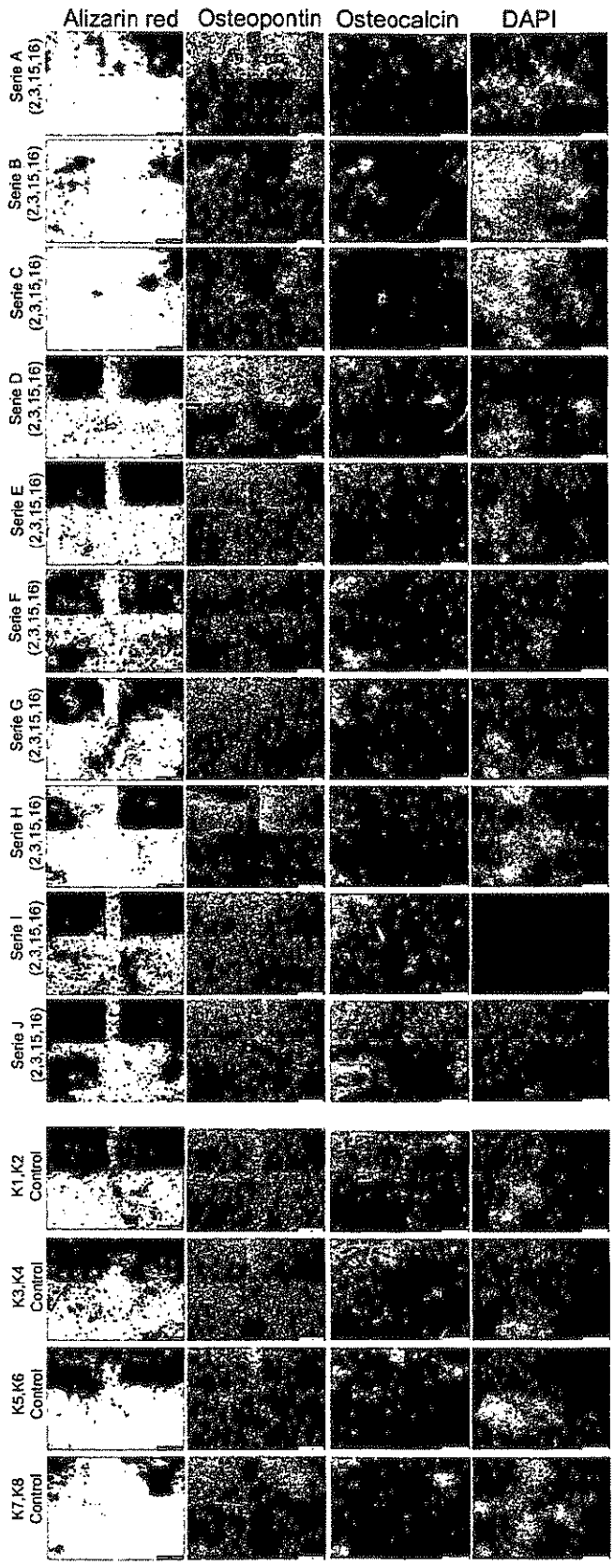

Alizarin red
Osteopontin
Osteocalcin
DAPI
Serie A (2,3,15,16)
Serie B (2,3,15,16)
Serie C (2,3,15,16)
Serie D (2,3,15,16)
Serie E (2,3,15,16)
Serie F (2,3,15,16)
Serie G (2,3,15,16)
Serie H (2,3,15,16)
Serie I (2,3,15,16)
Serie J (2,3,15,16)
K1,K2 Control
K3,K4 Control
K5,K6 Control
K7,K8 Control

Fig. 26

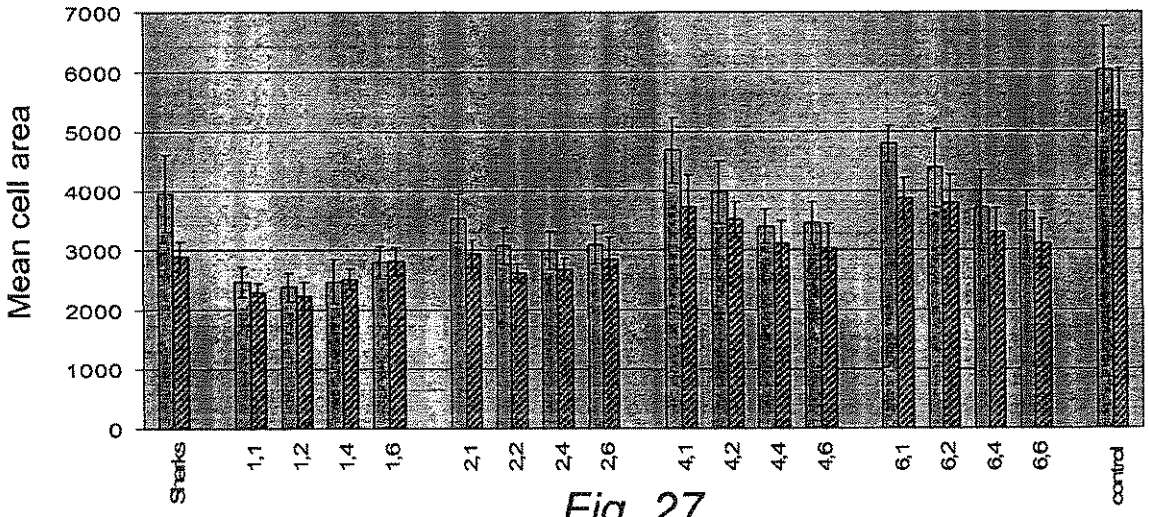

Mean cell area
7000
6000
5000
4000
3000
2000
1000
0
Sharks
1,1
1,2
1,4
1,6
2,1
2,2
2,4
2,6
4,1
4,2
4,4
4,6
6,1
6,2
6,4
6,6
control

Fig. 27

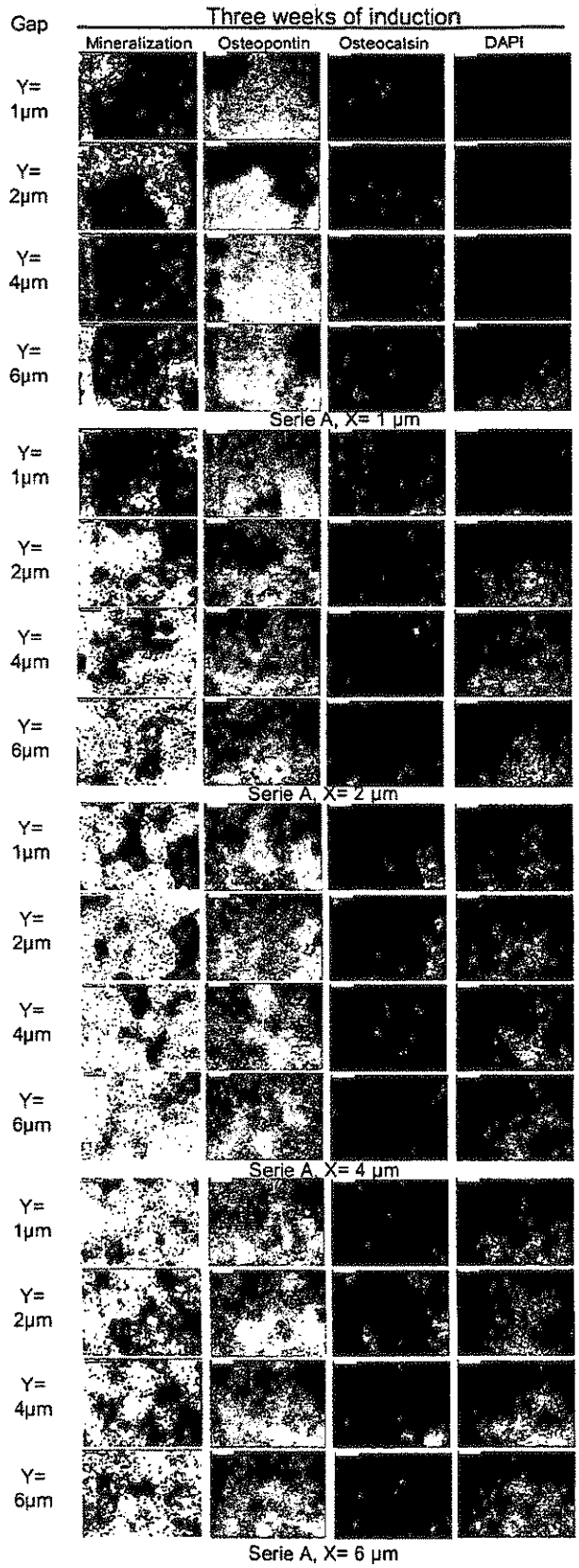

Gap
Three weeks of induction
Mineralization
Osteopontin
Osteocalsin
DAPI
Y= 1µm
Y= 2µm
Y= 4µm
Y= 6µm
Serie A, X= 1 µm
Serie A, X= 2 µm
Serie A, X= 4 µm
Serie A, X= 6 µm

Fig. 28

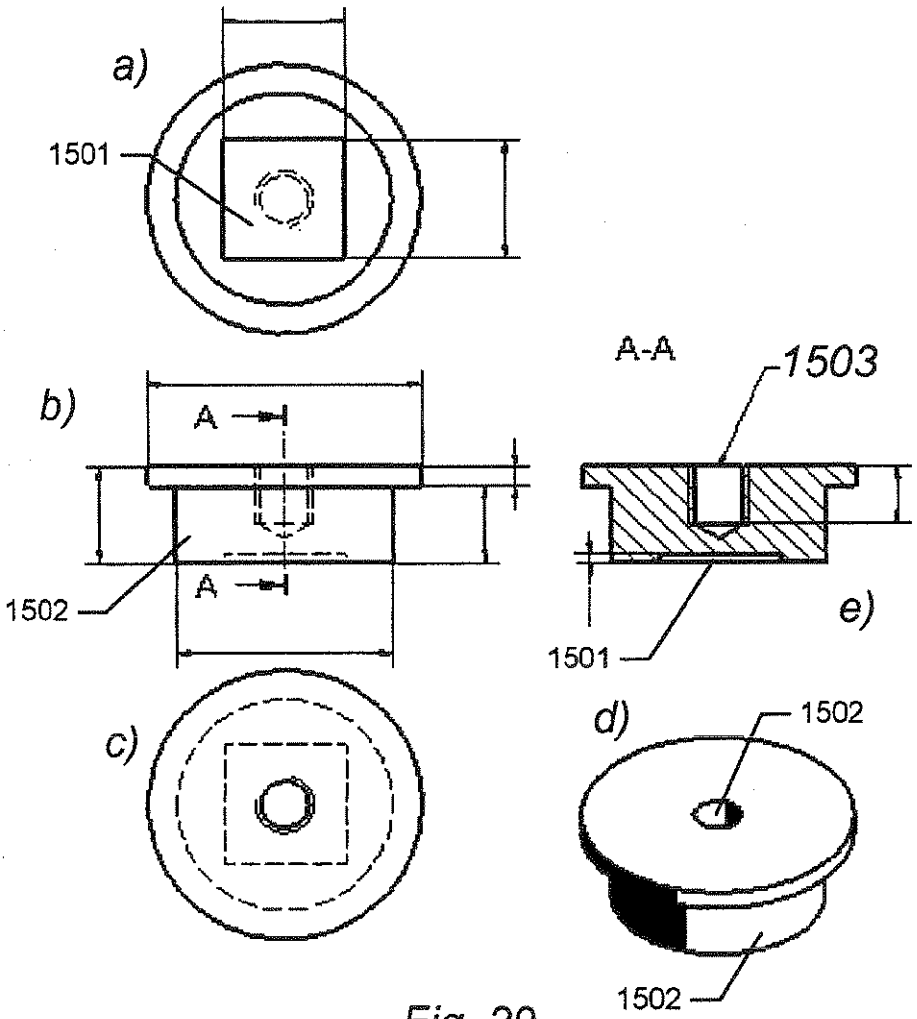
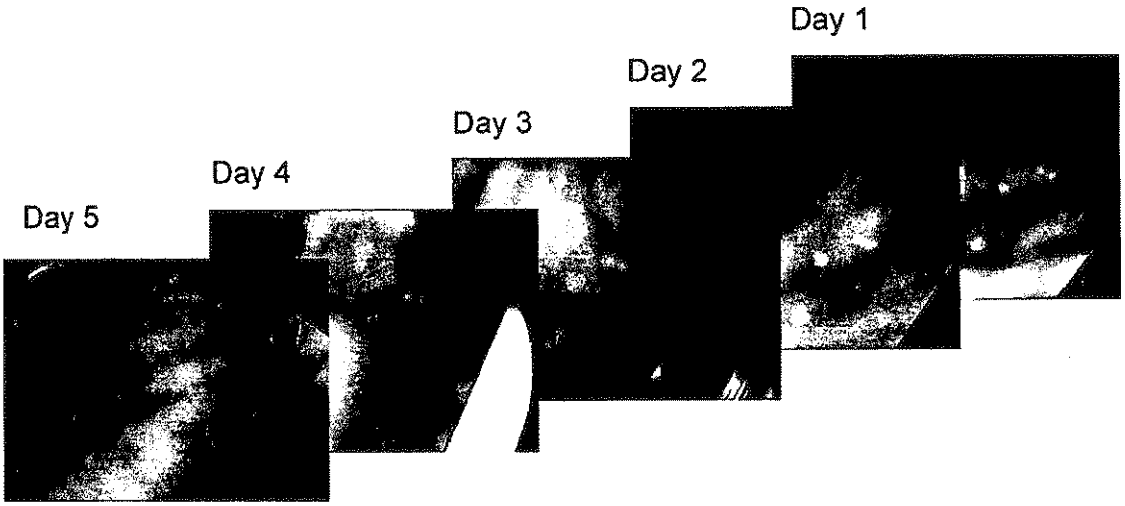


Fig. 29

a.



b.

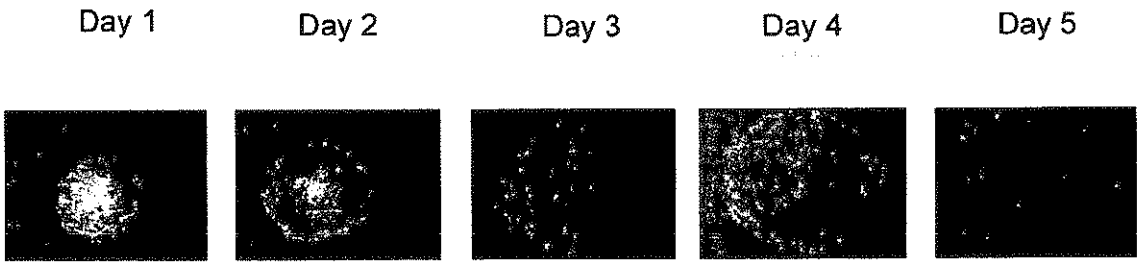
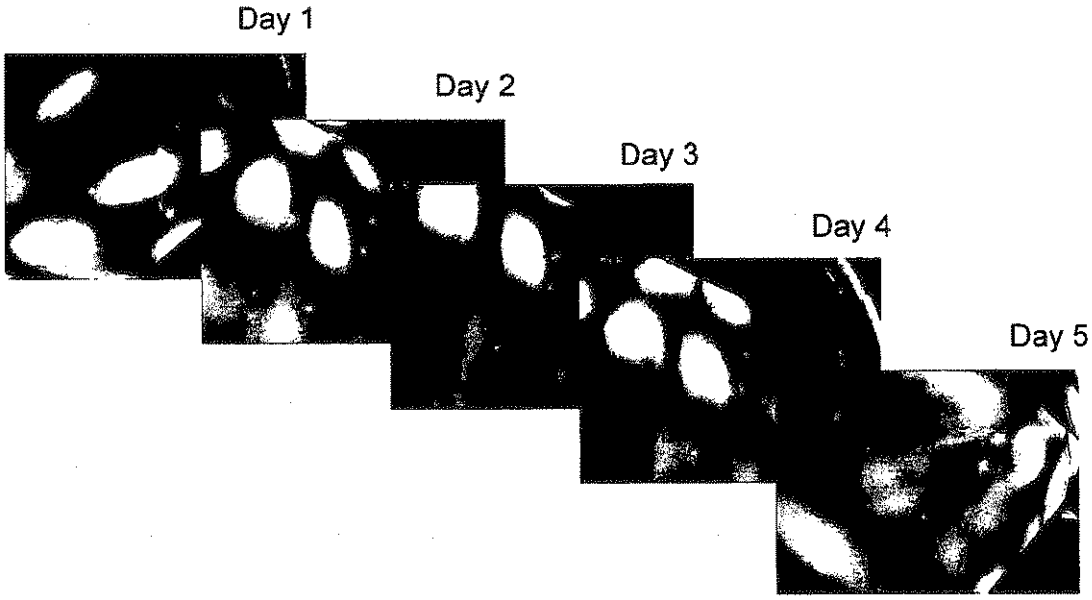


Fig. 30

a.



b.

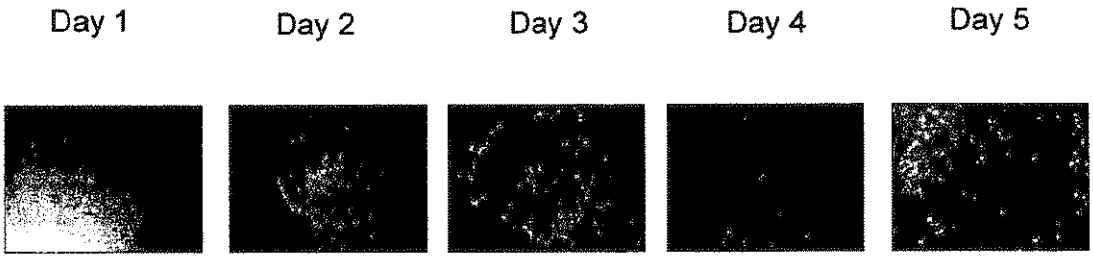


Fig. 31

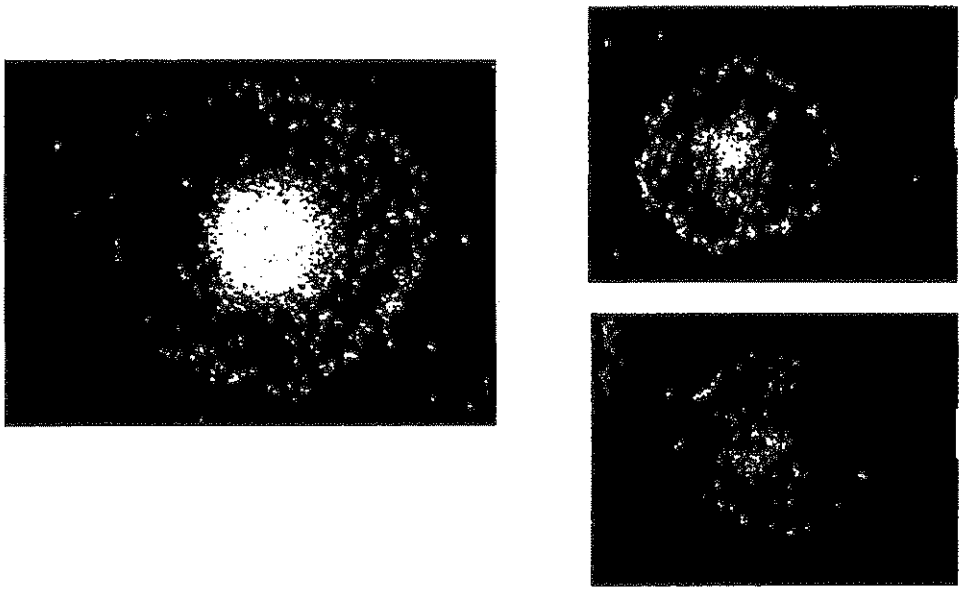


Fig. 32

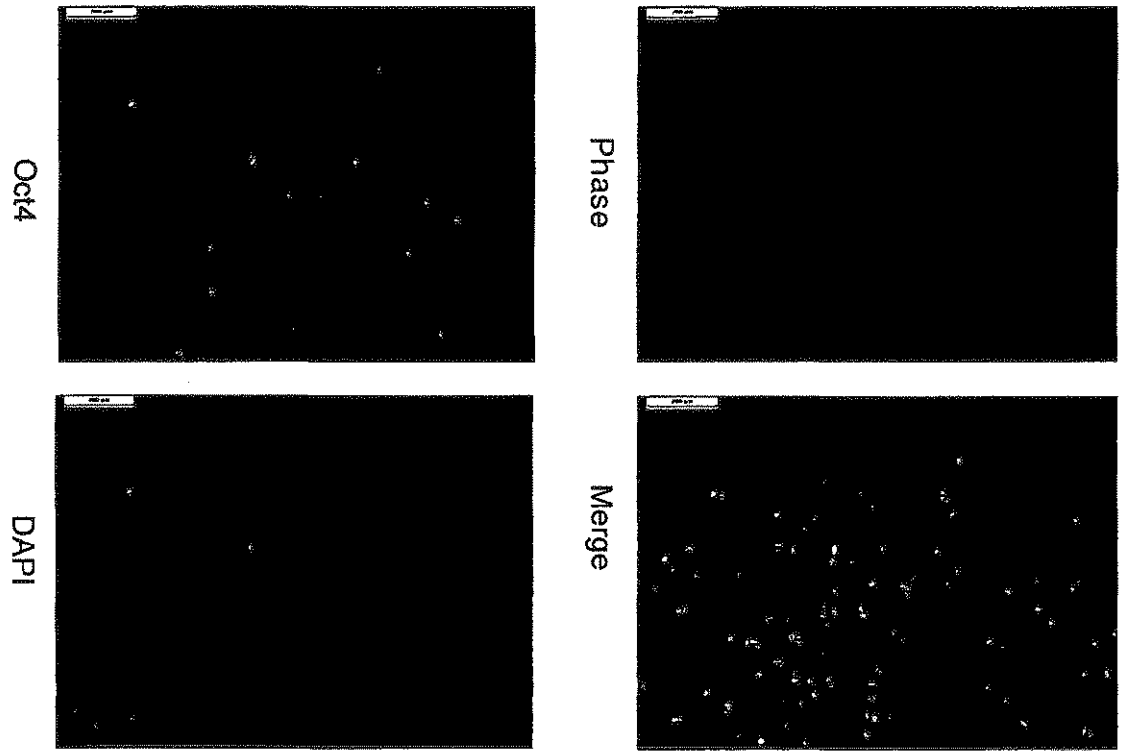


Fig. 33

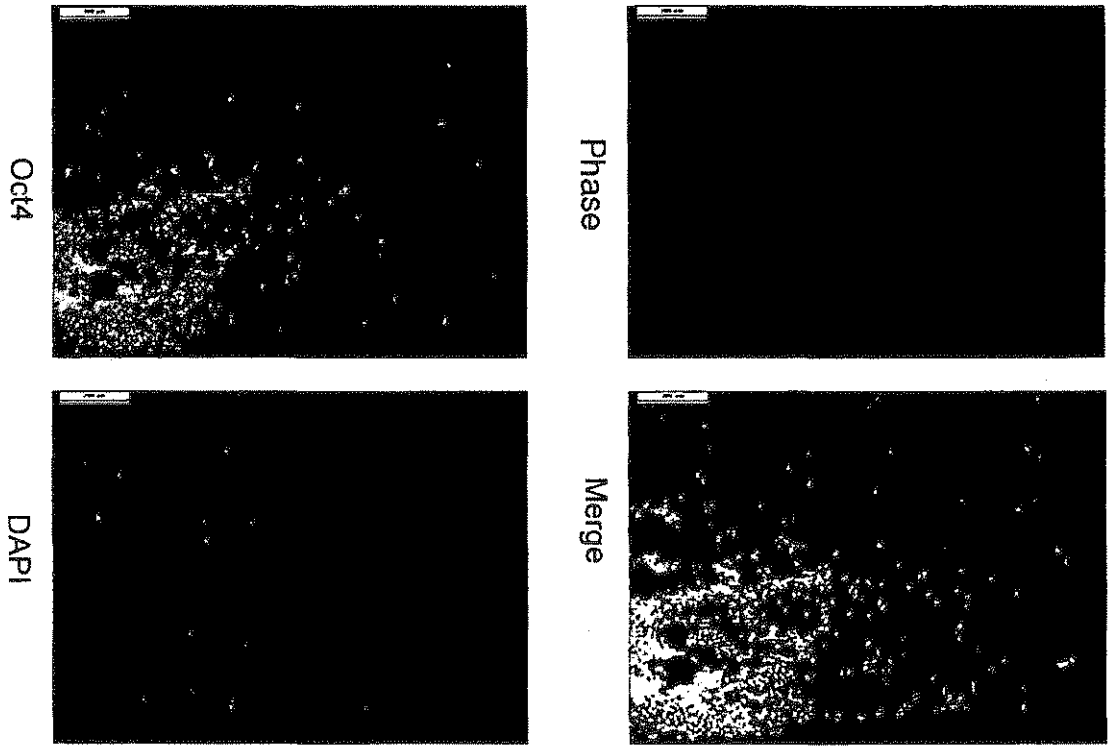


Fig. 34