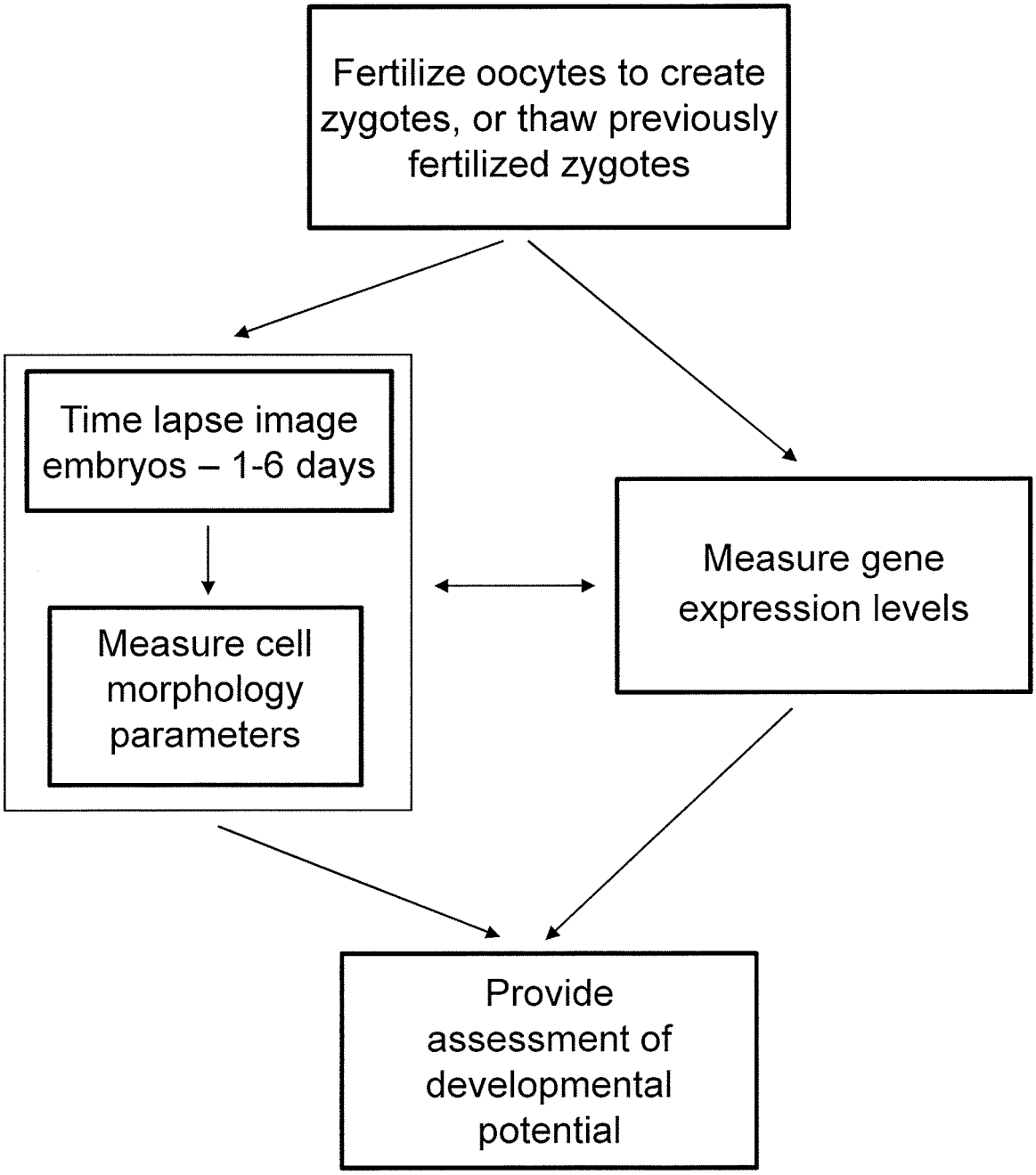

Fertilize oocytes to create zygotes, or thaw previously fertilized zygotes
Time lapse image embryos – 1-6 days
Measure cell morphology parameters
Measure gene expression levels
Provide assessment of developmental potential

Fig. 1

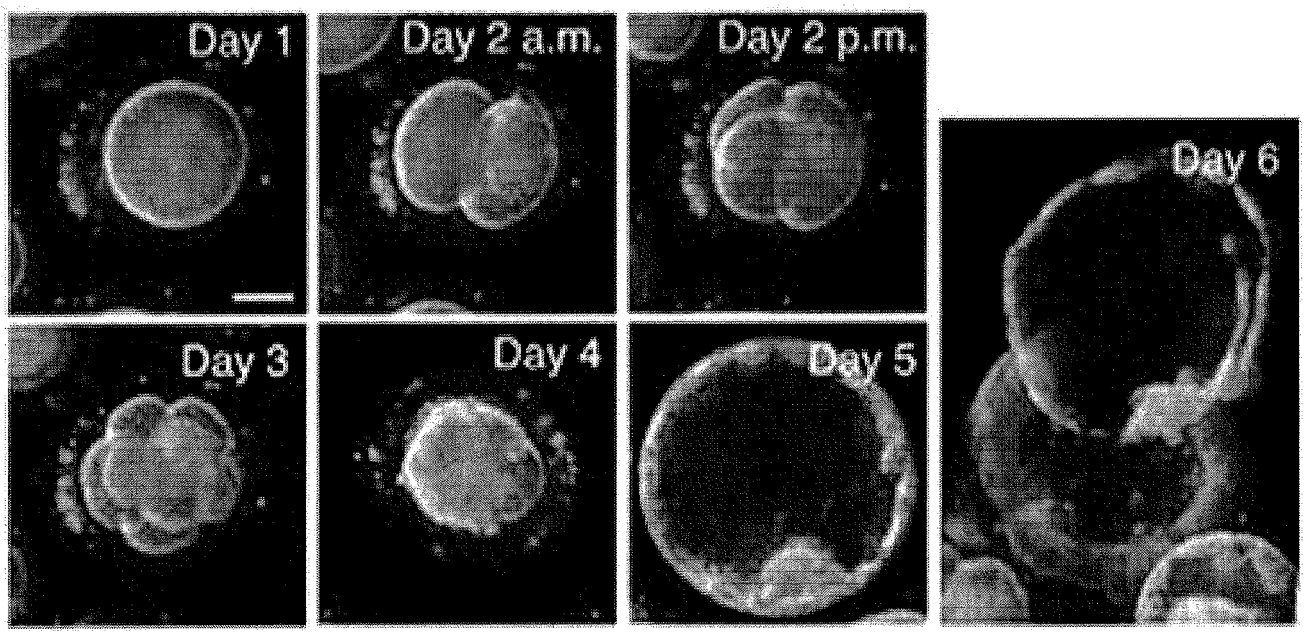


Fig. 2

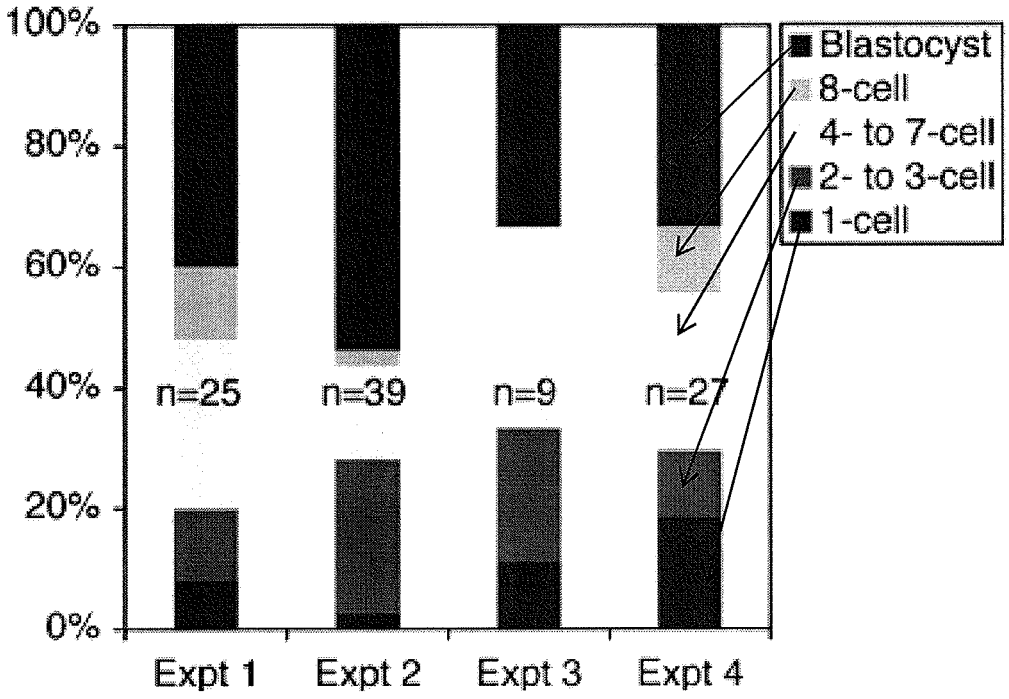

100%
80%
60%
40%
20%
0%
n=25
n=39
n=9
n=27
Expt 1
Expt 2
Expt 3
Expt 4
Blastocyst
8-cell
4- to 7-cell
2- to 3-cell
1-cell

Fig. 3

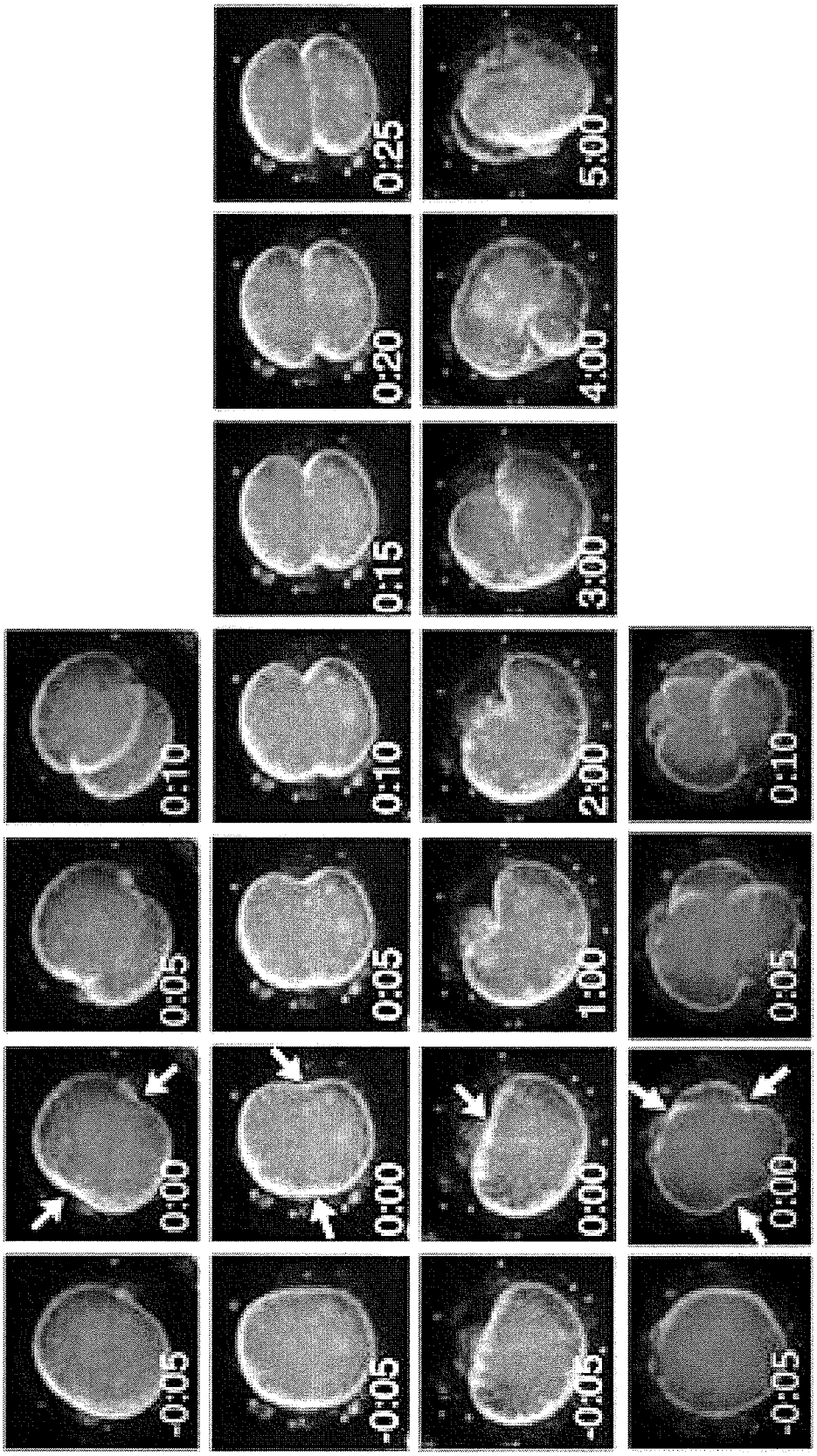

-0:05
0:00
0:05
0:10
0:15
0:20
0:25
-0:05
0:00
0:05
0:10
1:00
2:00
3:00
4:00
5:00
-0:05
0:00
0:05
0:10

Figure 4

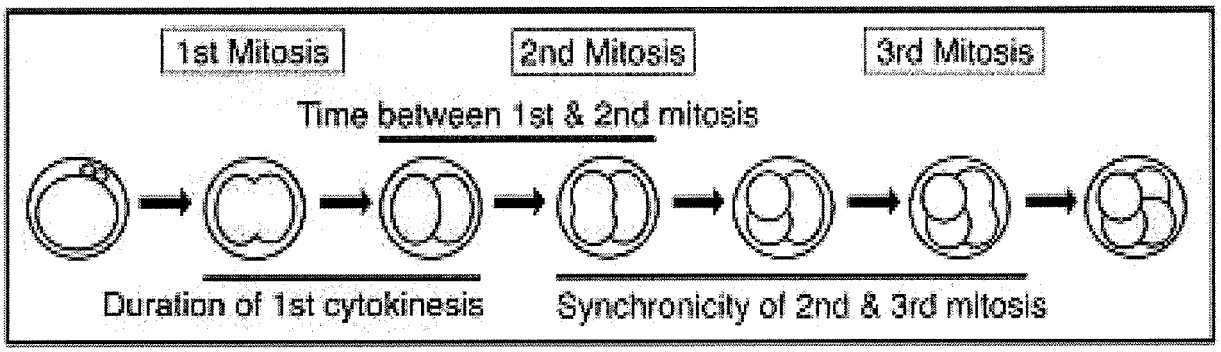

1st Mitosis
2nd Mitosis
3rd Mitosis
Time between 1st & 2nd mitosis
Duration of 1st cytokinesis
Synchronicity of 2nd & 3rd mitosis

Fig. 5

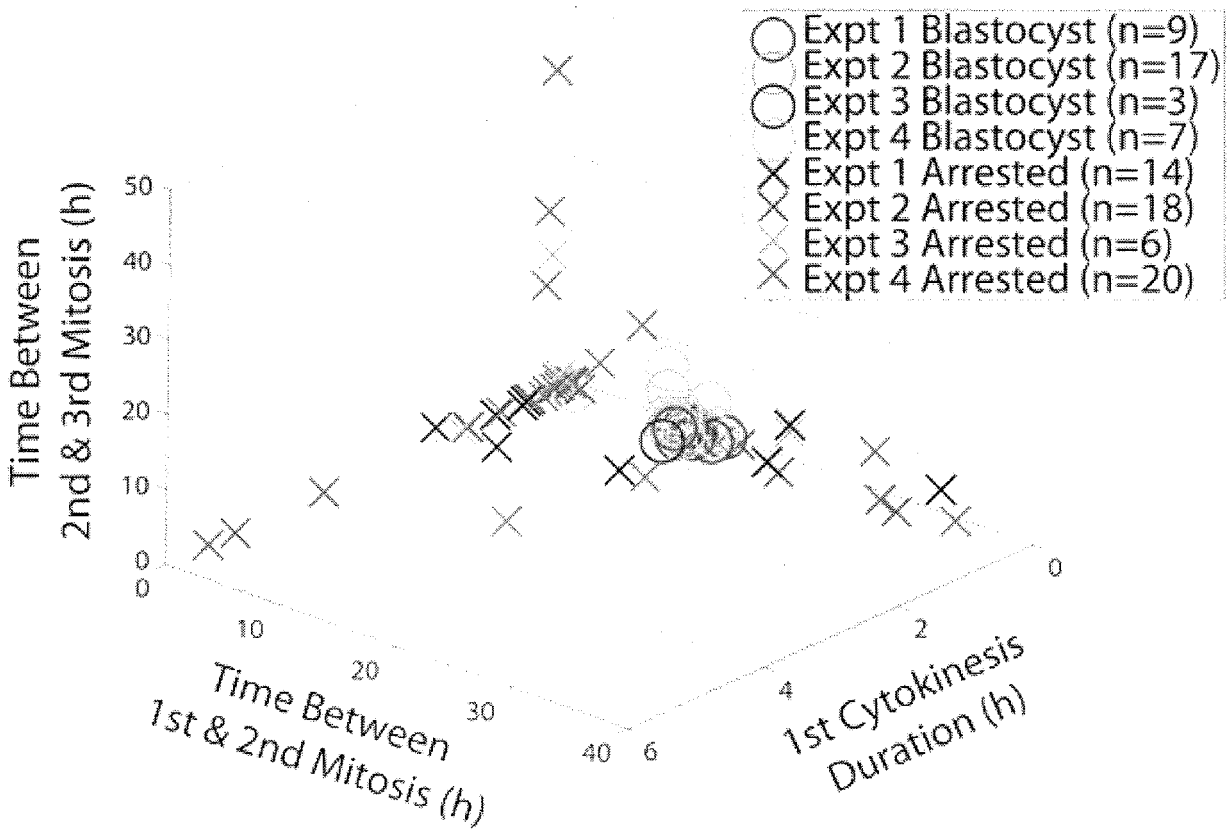

Expt 1 Blastocyst (n=9)
Expt 2 Blastocyst (n=17)
Expt 3 Blastocyst (n=3)
Expt 4 Blastocyst (n=7)
Expt 1 Arrested (n=14)
Expt 2 Arrested (n=18)
Expt 3 Arrested (n=6)
Expt 4 Arrested (n=20)
Time Between 2nd & 3rd Mitosis (h)
0
10
20
30
40
50
Time Between 1st & 2nd Mitosis (h)
10
20
30
40
1st Cytokinesis Duration (h)
0
2
4
6

Fig. 6

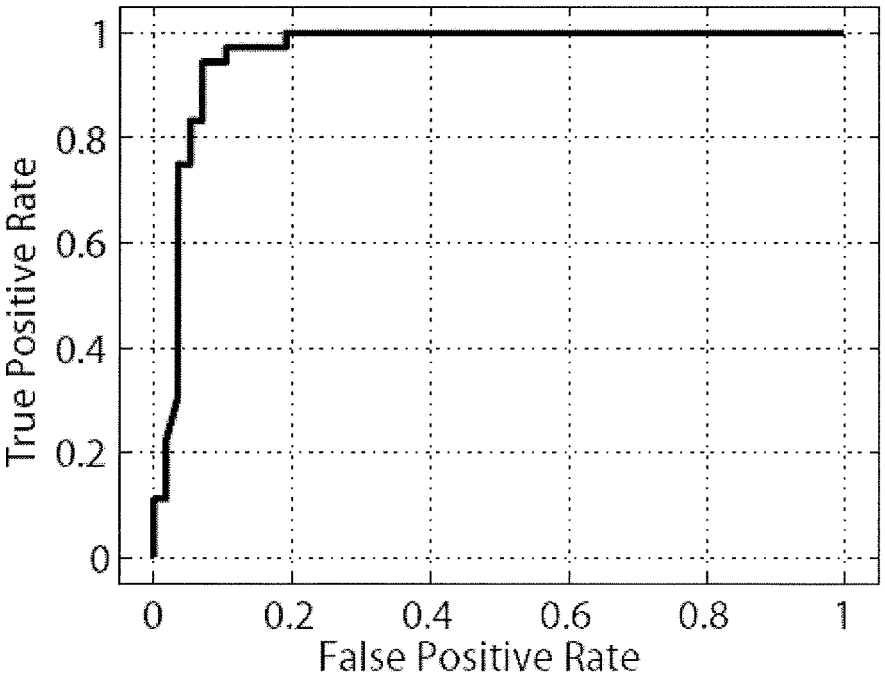


Fig. 7

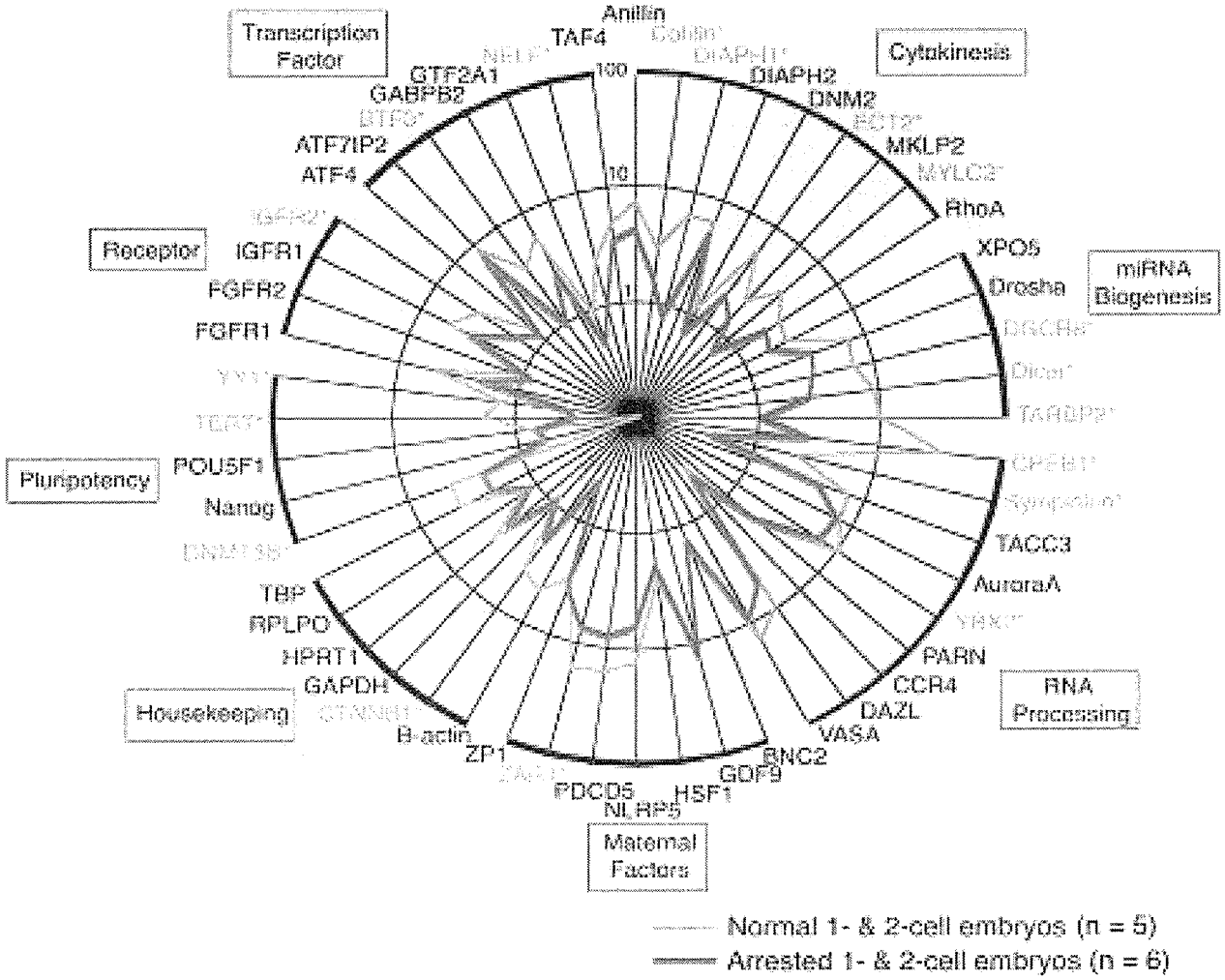

Transcription Factor
TAF4
GTF2A1
GABPB2
ATF7IP2
ATF4
Receptor
IGFR1
FGFR2
FGFR1
Pluripotency
POU5F1
Nanog
Housekeeping
TBP
RPLPO
HPRT1
GAPDH
B-actin
Maternal Factors
ZP1
PDCD5
NLRP5
GDF9
BNC2
Cytokinesis
Anillin
DIAPH2
DNM2
MKLP2
RhoA
miRNA Biogenesis
XPO5
Drosha
Dicer
TACC3
AuroraA
RNA Processing
PARN
CCR4
DAZL
VASA
100
10
1
Normal 1- & 2-cell embryos (n = 5)
Arrested 1- & 2-cell embryos (n = 6)

Fig. 8

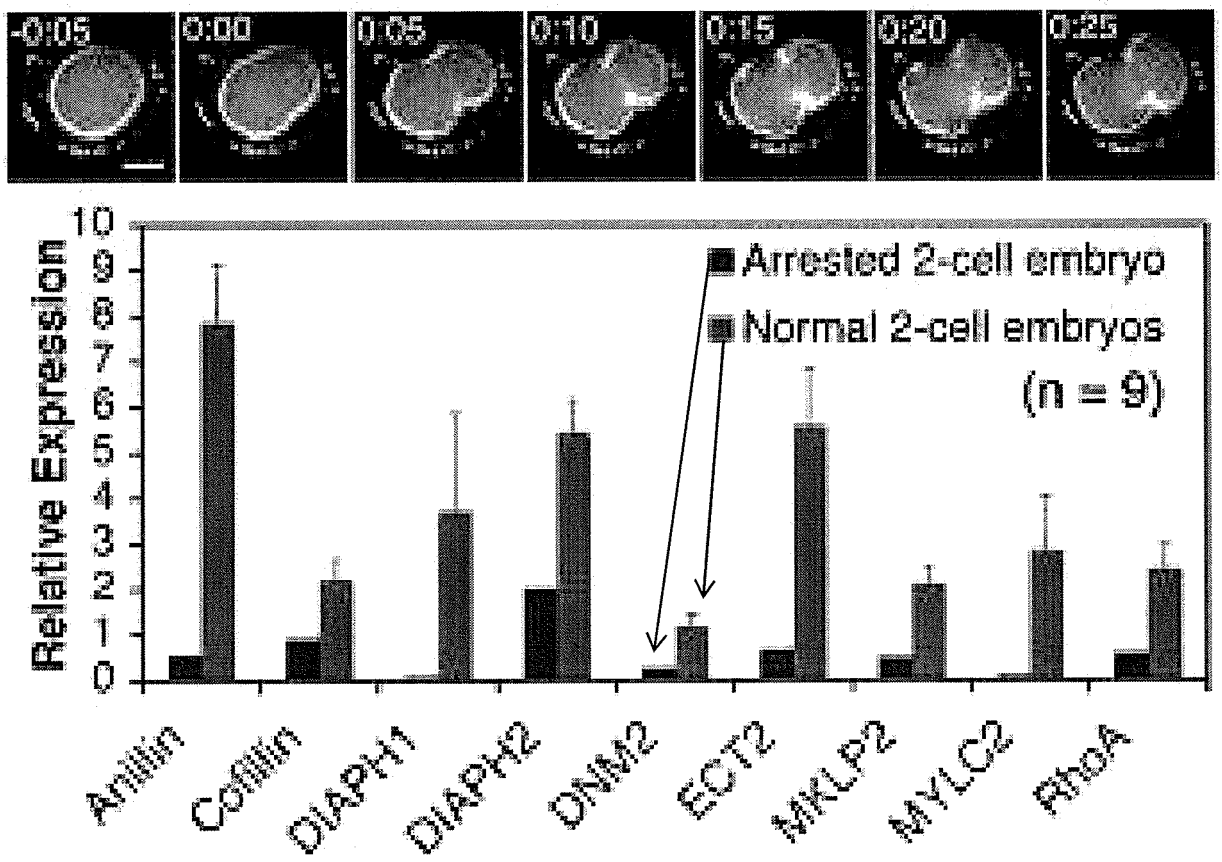

-0:05
0:00
0:05
0:10
0:15
0:20
0:25
Relative Expression
0
1
2
3
4
5
6
7
8
9
10
Arrested 2-cell embryo
Normal 2-cell embryos
(n = 9)
Anillin
Cofilin
DIAPH1
DIAPH2
DNM2
ECT2
MKLP2
MYLC2
RhoA

Fig. 9

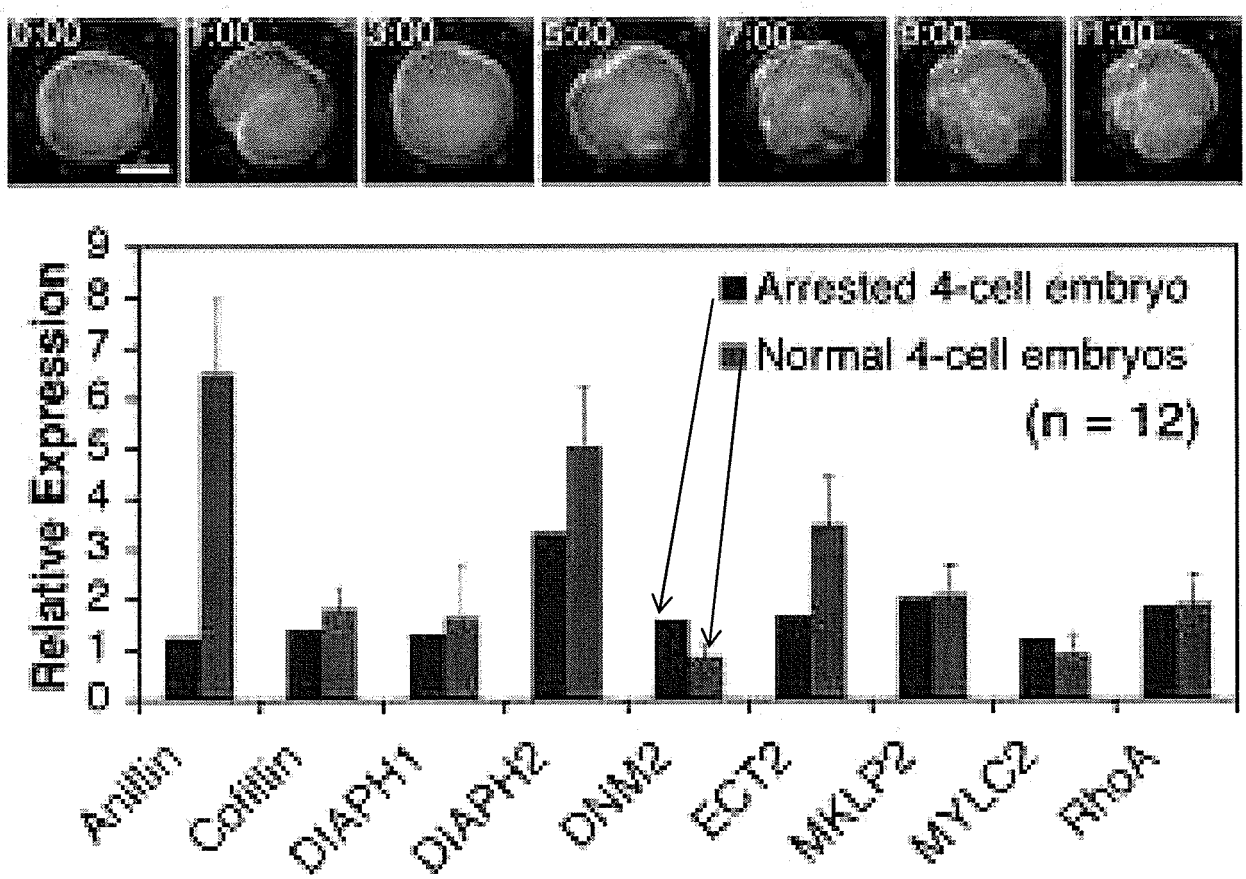

Relative Expression
0
1
2
3
4
5
6
7
8
9
Arrested 4-cell embryo
Normal 4-cell embryos
(n = 12)
Anillin
Cofilin
DIAPH1
DIAPH2
DNM2
ECT2
MKLP2
MYLC2
RhoA

Fig. 10

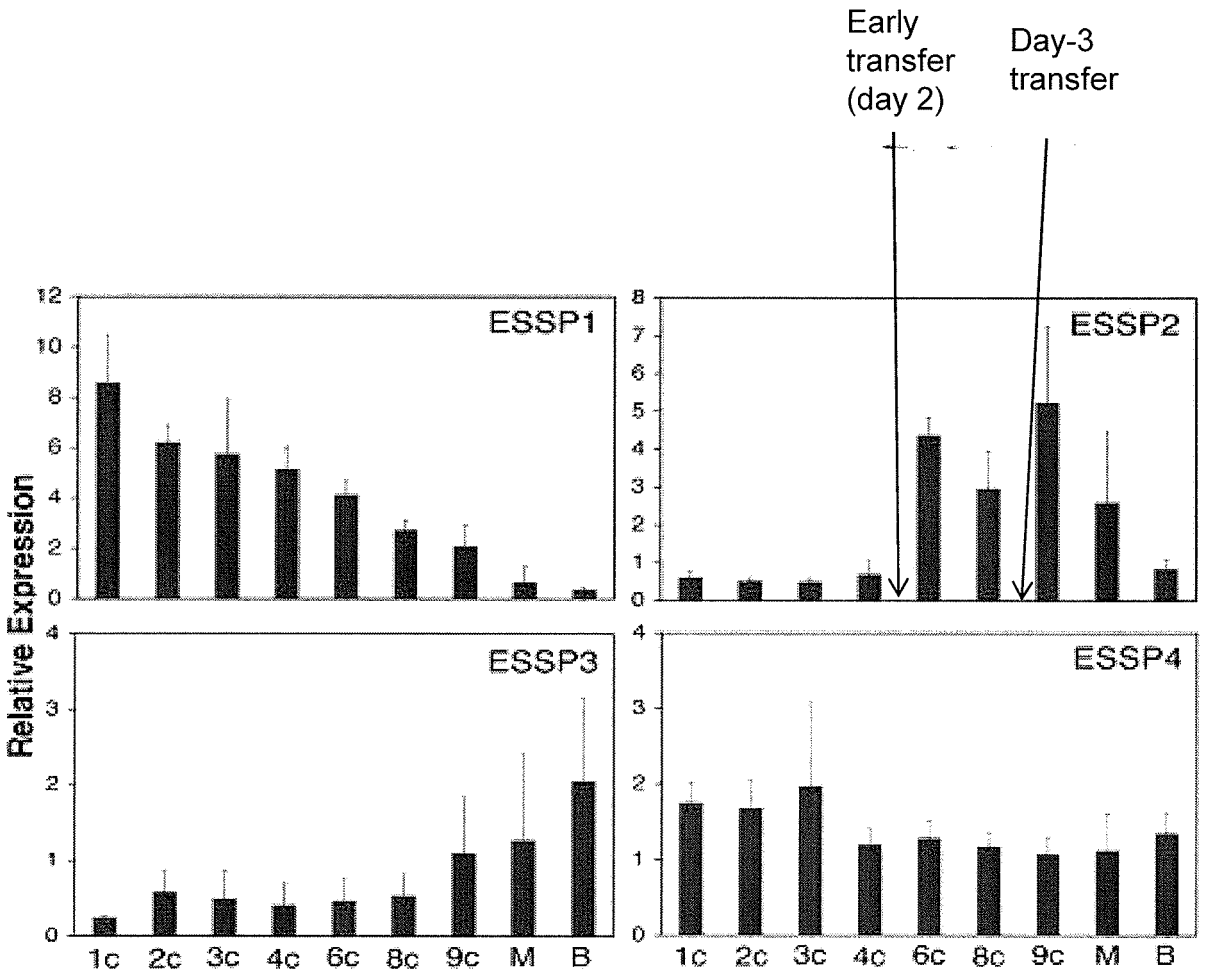
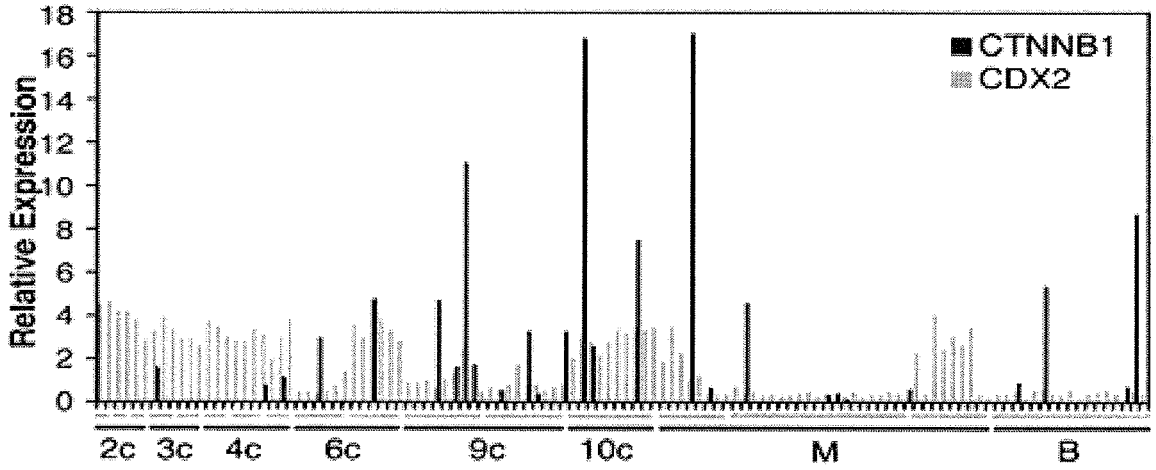

Early transfer (day 2)
Day-3 transfer
ESSP1
ESSP2
ESSP3
ESSP4
Relative Expression
1c
2c
3c
4c
6c
8c
9c
M
B

Fig. 11

a.


Relative Expression
18
16
14
12
10
8
6
4
2
0
CTNNB1
CDX2
2c
3c
4c
6c
9c
10c
M
B

b.

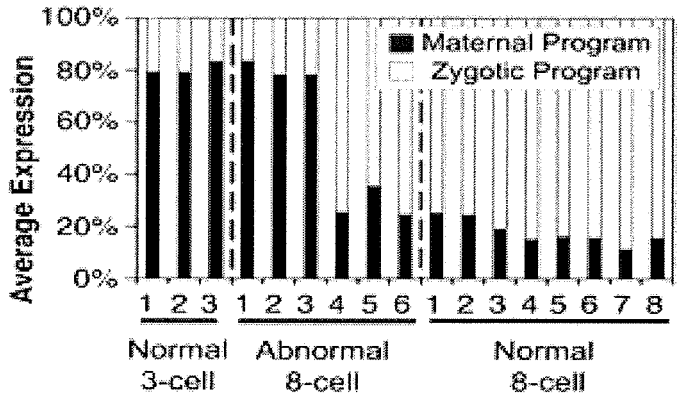
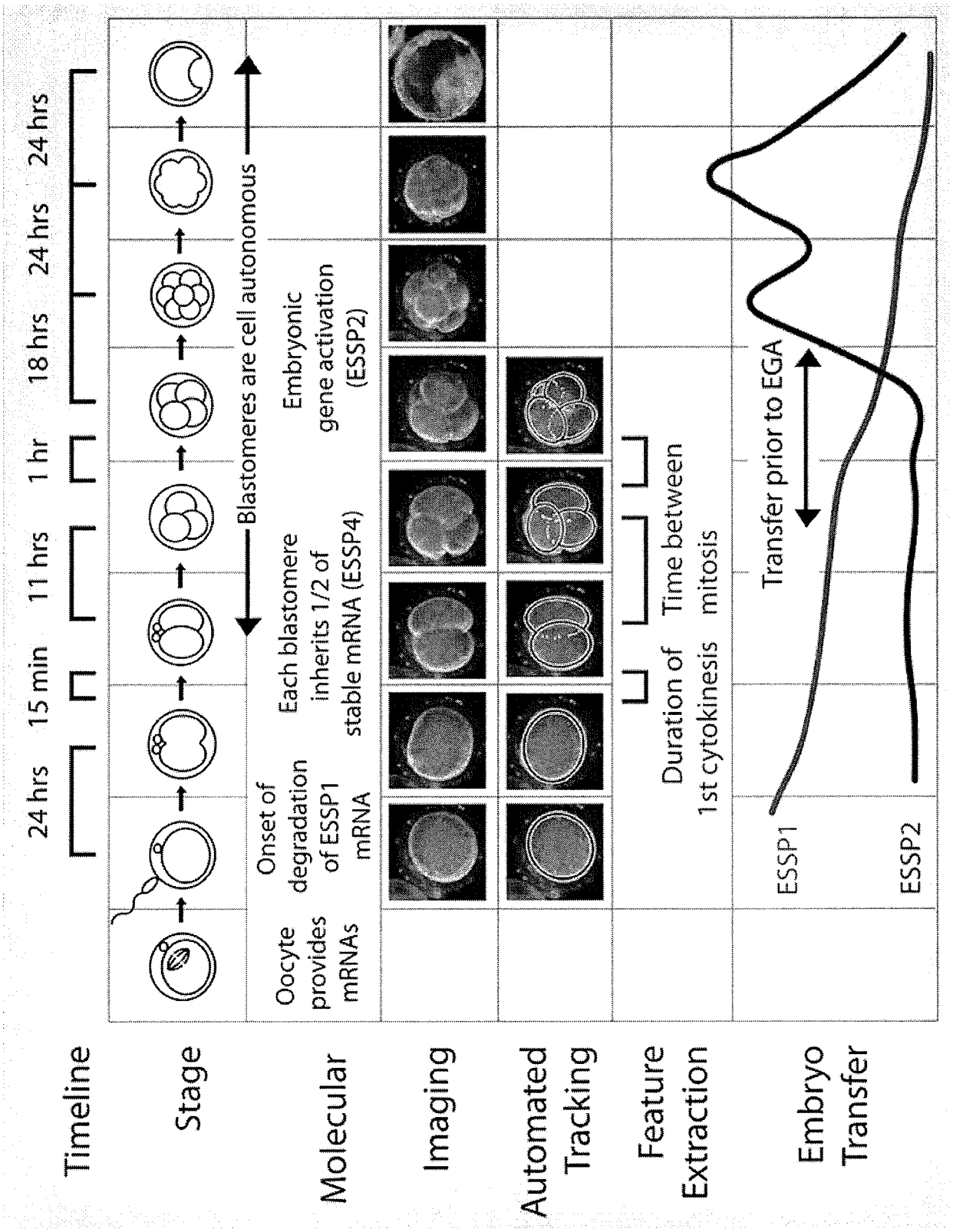

Average Expression
100%
80%
60%
40%
20%
0%
Maternal Program
Zygotic Program
1 2 3
1 2 3 4 5 6
1 2 3 4 5 6 7 8
Normal
3-cell
Abnormal
8-cell
Normal
8-cell

Fig. 12

Figure 13


Timeline
24 hrs
15 min
11 hrs
1 hr
18 hrs
24 hrs
24 hrs
Stage
Molecular
Oocyte provides mRNAs
Onset of degradation of ESSP1 mRNA
Each blastomere inherits 1/2 of stable mRNA (ESSP4)
Blastomeres are cell autonomous
Embryonic gene activation (ESSP2)
Imaging
Automated Tracking
Feature Extraction
Duration of 1st cytokinesis
Time between mitosis
Embryo Transfer
ESSP1
ESSP2
Transfer prior to EGA

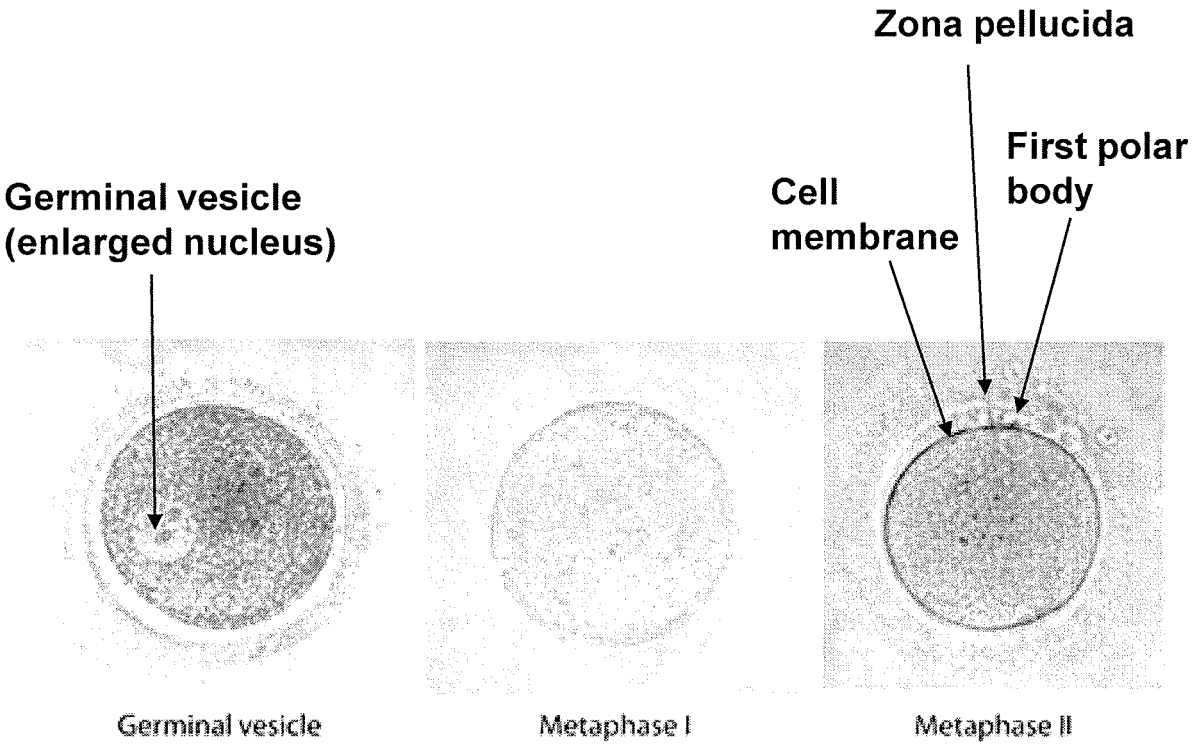

Zona pellucida
First polar body
Cell membrane
Germinal vesicle (enlarged nucleus)
Germinal vesicle
Metaphase I
Metaphase II

Fig. 14

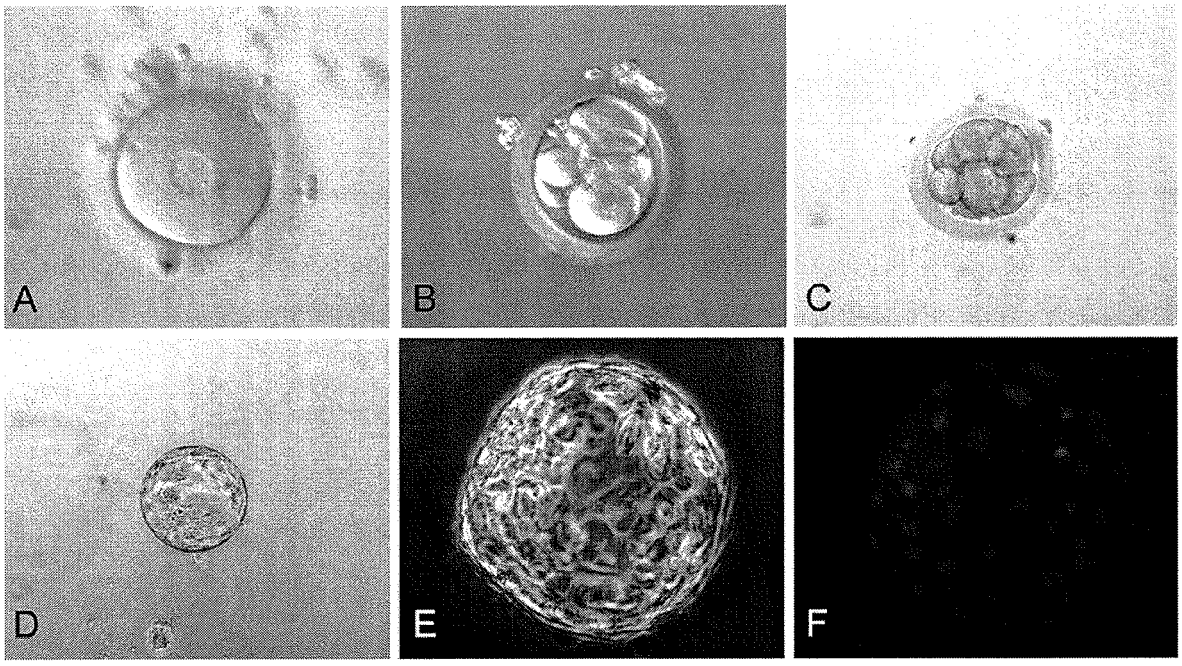


Fig. 15

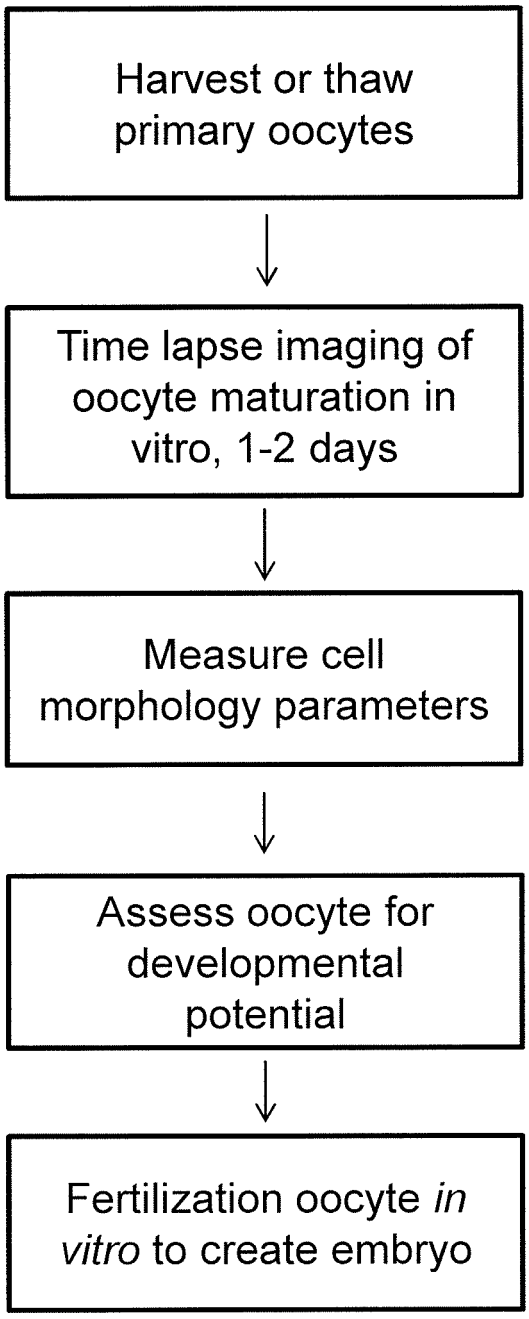

Harvest or thaw primary oocytes
Time lapse imaging of oocyte maturation in vitro, 1-2 days
Measure cell morphology parameters
Assess oocyte for developmental potential
Fertilization oocyte *in vitro* to create embryo

Fig. 16

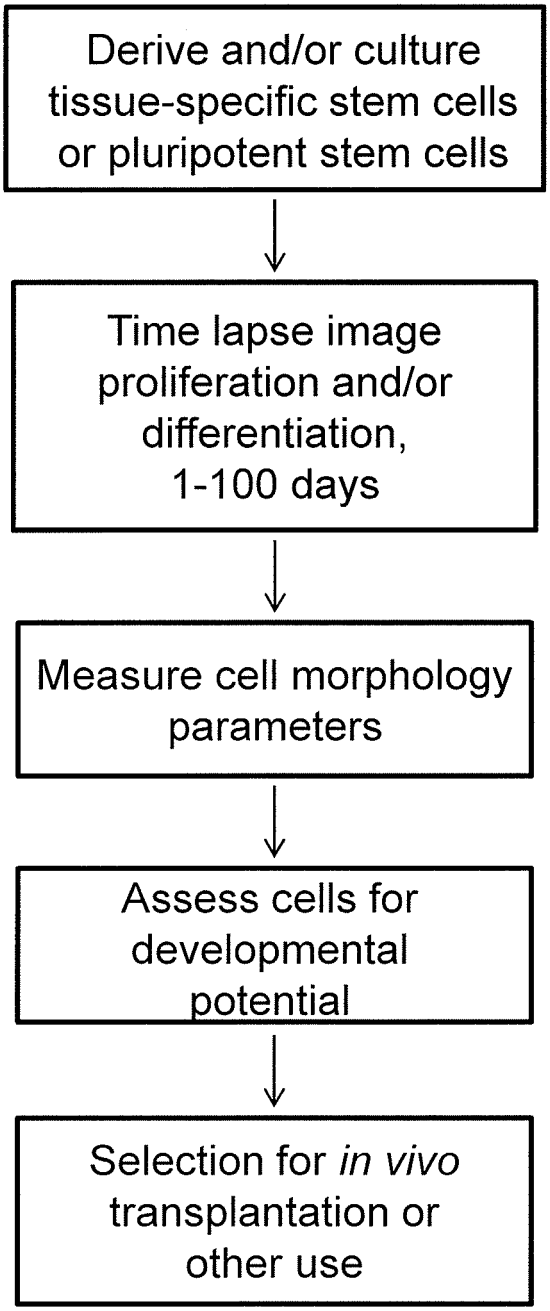

Derive and/or culture tissue-specific stem cells or pluripotent stem cells
Time lapse image proliferation and/or differentiation, 1-100 days
Measure cell morphology parameters
Assess cells for developmental potential
Selection for *in vivo* transplantation or other use

Fig. 17

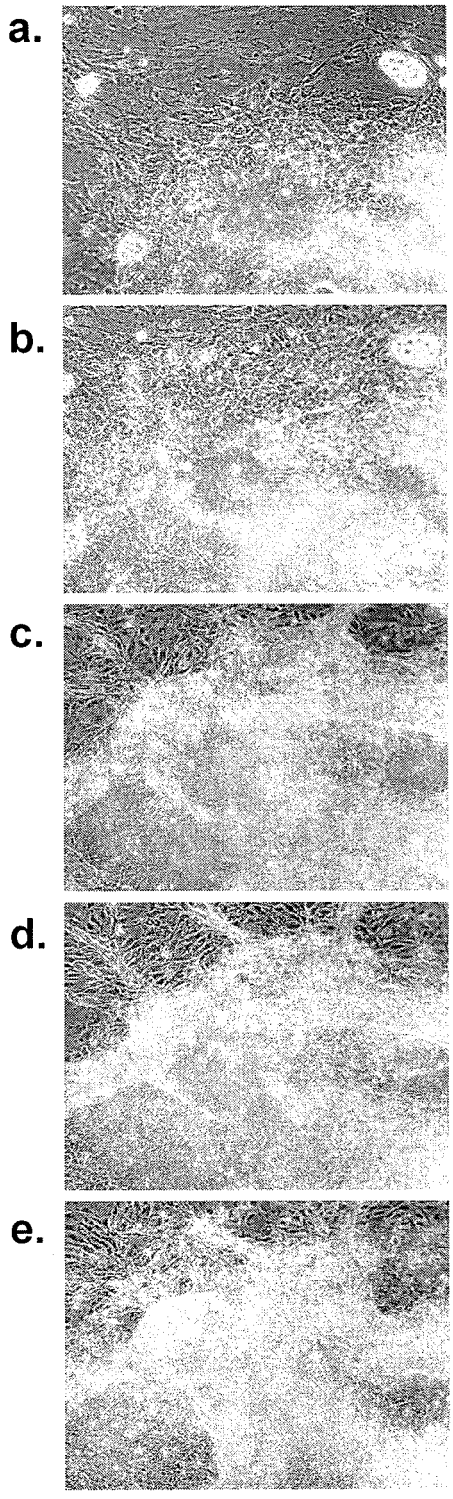


Fig. 18

Figure 19

Table 1

Category	# of genes
Apoptosis	5
Cytokinesis	10
Differentiation	9
Embryonic gene activation	10
Epigenetics	5
Germ cell	9
Housekeeping	10
Ligand / receptor	11
Maternal effect	8
miRNA	5
Pluripotency	7
RNA processing	7
Sex specific	2
TE / ICM	14
Transcription factor	11

Figure 20

Table 2

Pattern 1
Anillin
ATG5
ATP2C1
Aurora A
Bclaf1
Cofilin
CPEB1
DAZL
Dgcr8
DIAPH1
DIAPH2
Dicer
DNAJA4
DNMT3B
Drosha
ECT2
EHMT2
FGFR1
FYN
GABPB2
GDF9
HMOX2
HPRT1
HSF1
IGF1R
IGF2R
LAMB1
MKLP2
MYLC2
NLRP5
PARN
PDCD5
PVR
RhoA
Sycp3
TACC3
TAF4
TBP
TERT
VASA
XPO5
YBX2
Zar1
ZP1

Pattern 2
CCNA1
CCR4
DNM2
EIF1AX
EIF4A3
EOMES
GDF3
GTF2A1
H2AFZ
JARID1B
Nanog
NES
Prdm14
PTMA
RUNX2
SERPINA1
SERTAD1
SOX2
STELLAR
YY1

Pattern 3
B-actin
Bcl2
GCM1
HDAC7A
IFITM1
POU5F1
SALF
TGFBR2

Pattern 4
CTK7
FABP5
FGFR2
GAPDH
HMOX2
Hsp70
IGF2R
LSM3
PABPC1
PRMT5
RPLPO
YY1

Undefined
ATF4
ATF7IP
ATF7IP2
B2M
BNC2
BTF3
CDX2
CTNNB1
Dby
Hsp70.1
PRDM1
Symplekin
TARBP2
Xist

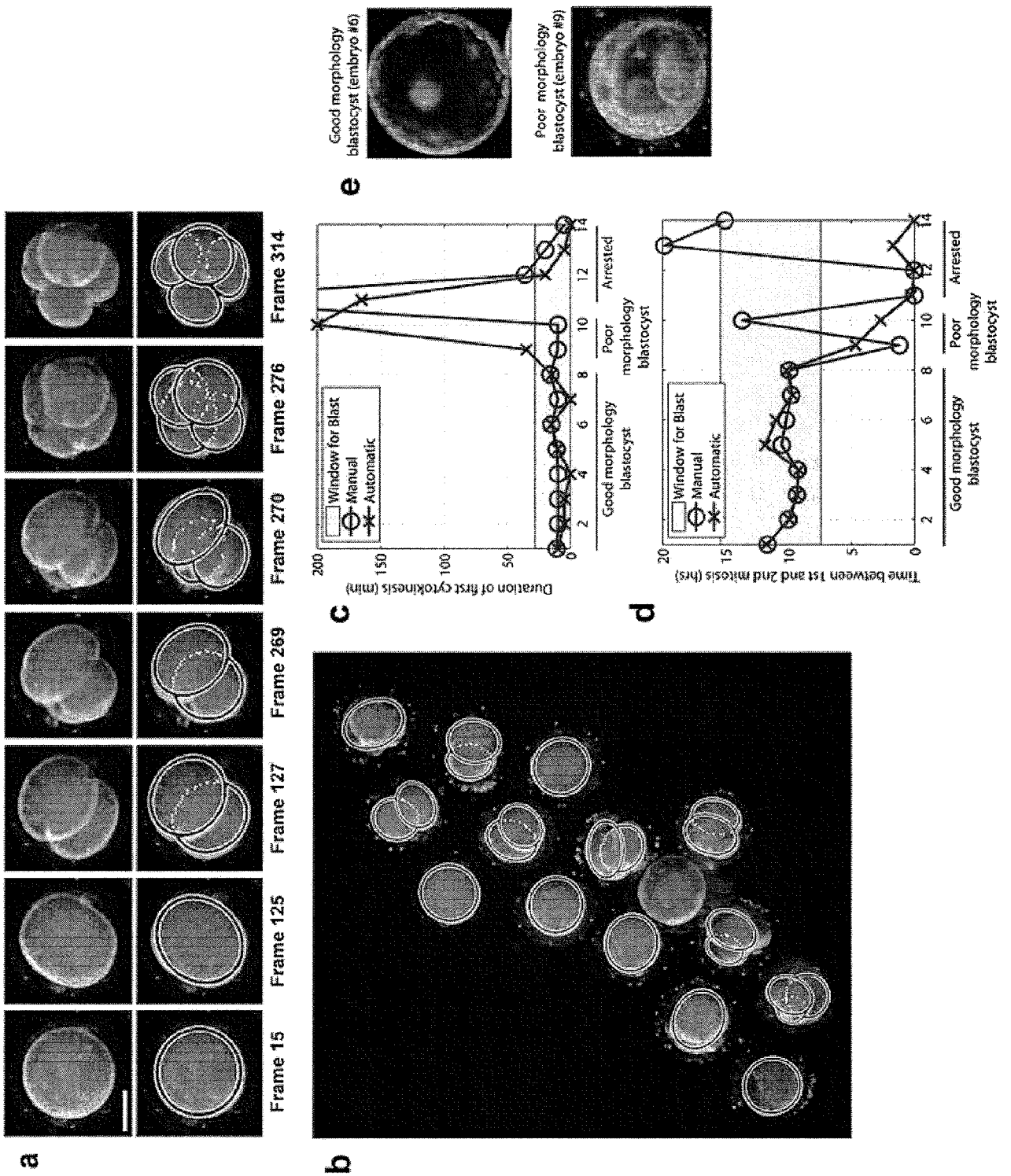

a
Frame 15
Frame 125
Frame 127
Frame 269
Frame 270
Frame 276
Frame 314
b
c
Duration of first cytokinesis (min)
Window for Blast
Manual
Automatic
Good morphology blastocyst
Poor morphology blastocyst
Arrested
d
Time between 1st and 2nd mitosis (hrs)
e
Good morphology blastocyst (embryo #6)
Poor morphology blastocyst (embryo #9)

Figure 21

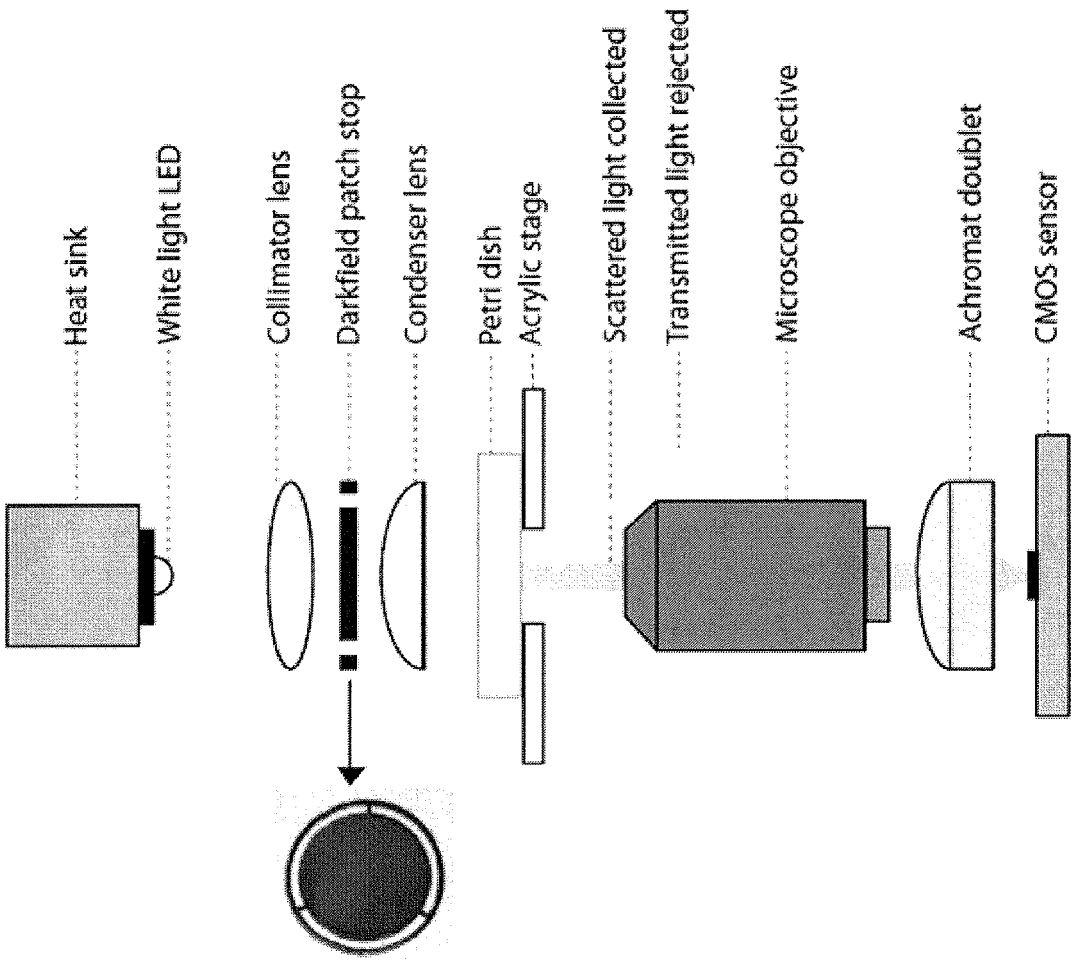
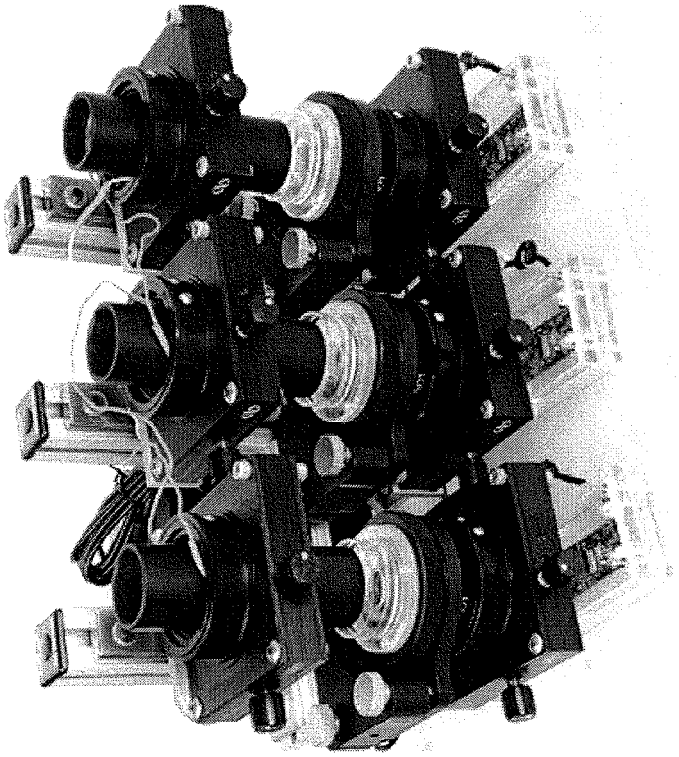
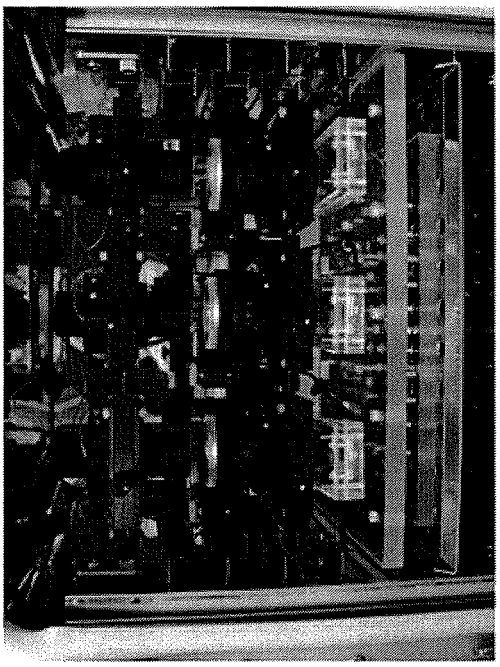

Heat sink
White light LED
Collimator lens
Darkfield patch stop
Condenser lens
Petri dish
Acrylic stage
Scattered light collected
Transmitted light rejected
Microscope objective
Achromat doublet
CMOS sensor

Fig. 22



23A



23B

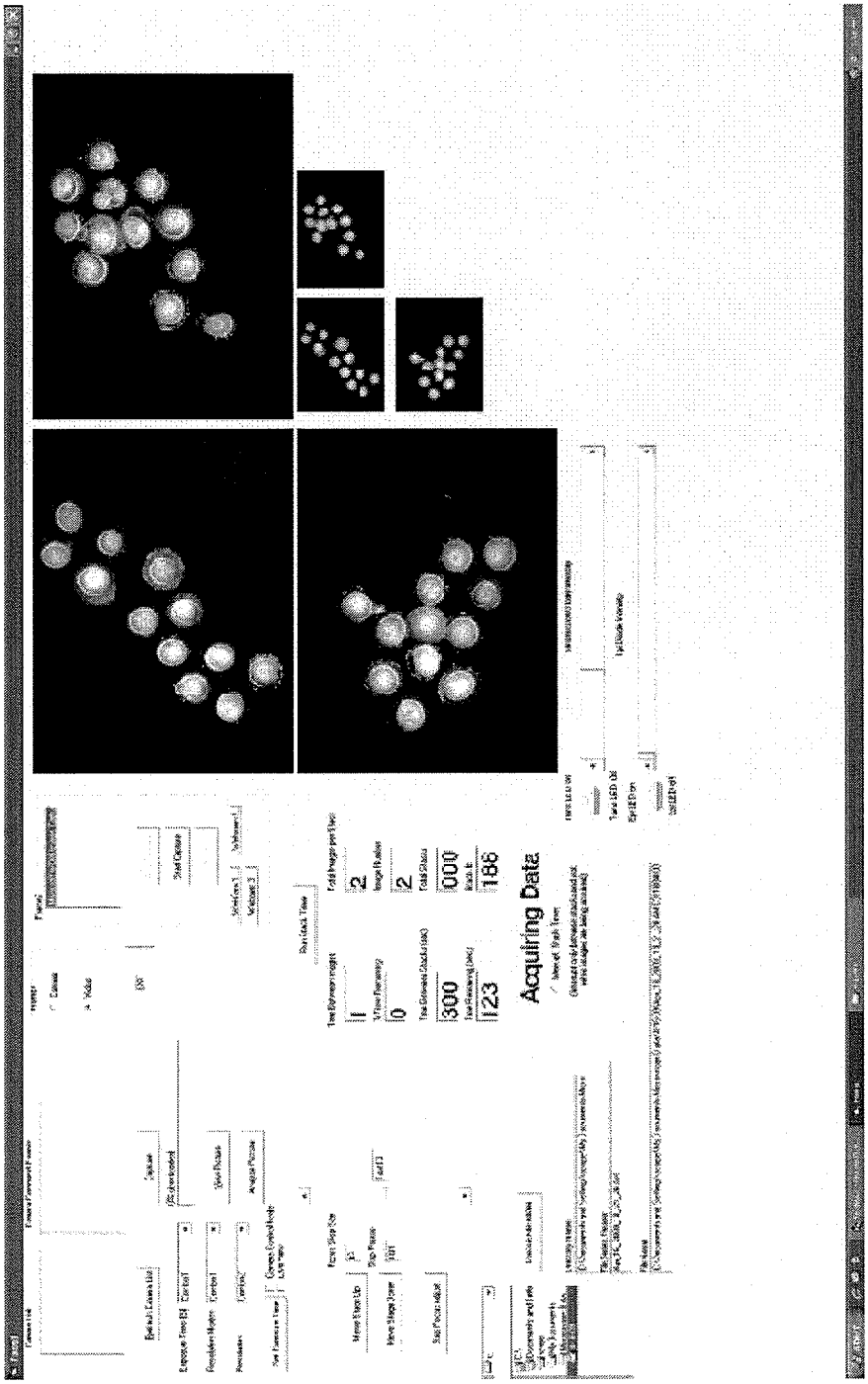


Fig. 24

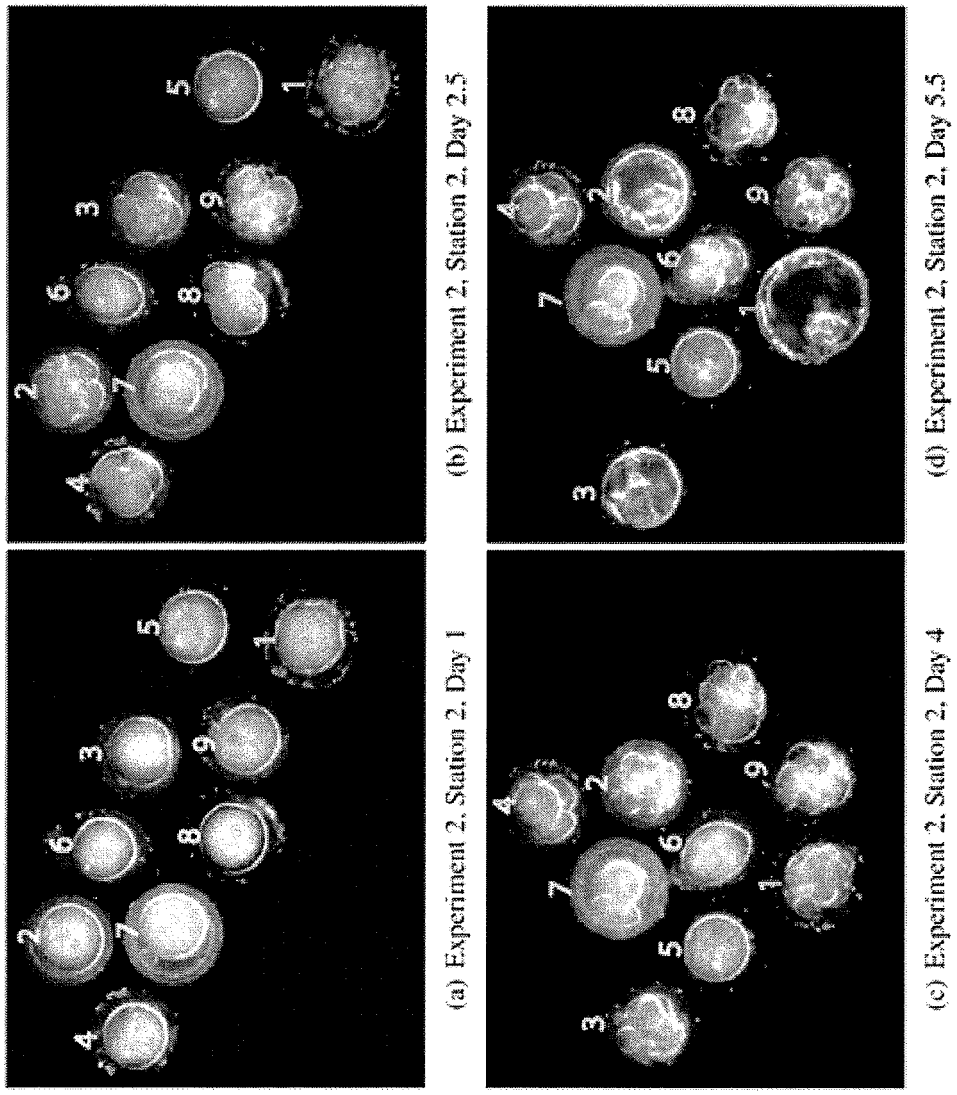

(a) Experiment 2, Station 2, Day 1
(b) Experiment 2, Station 2, Day 2.5
(c) Experiment 2, Station 2, Day 4
(d) Experiment 2, Station 2, Day 5.5

Fig. 25

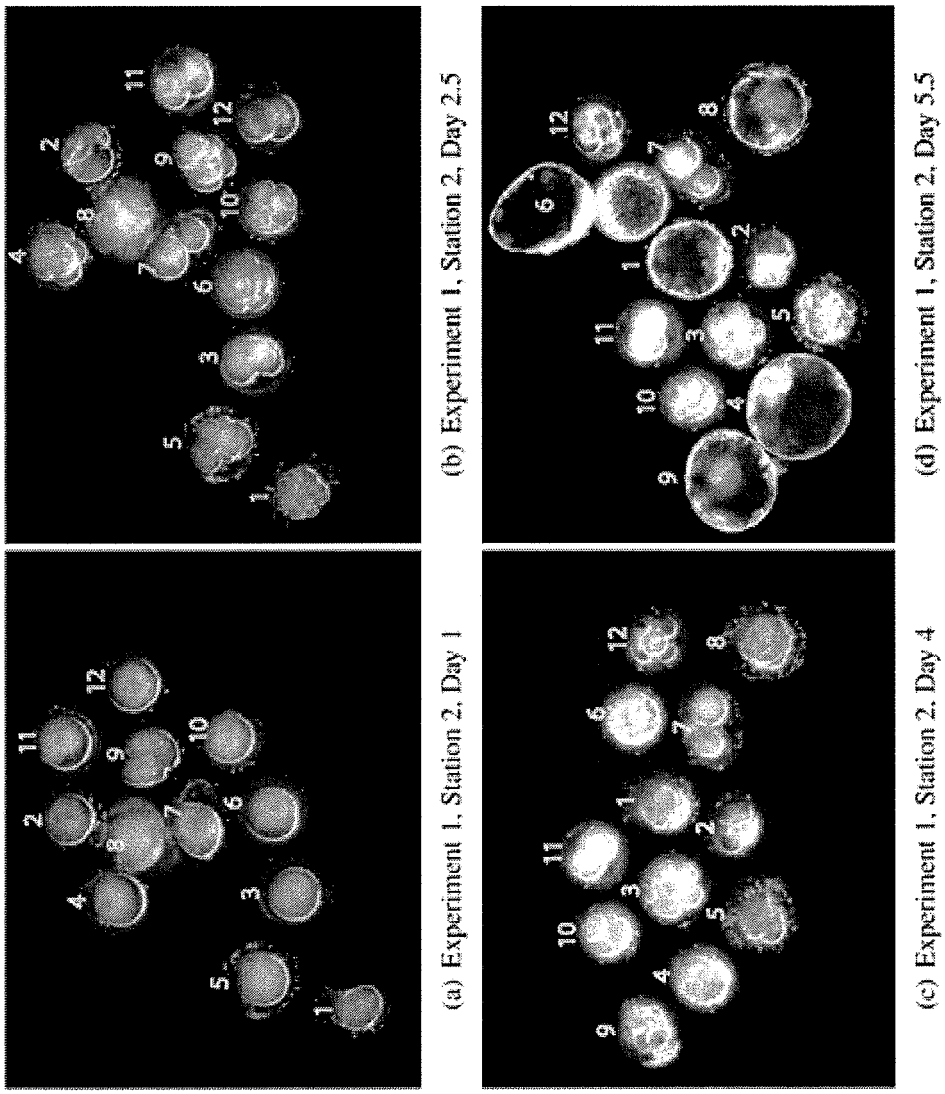
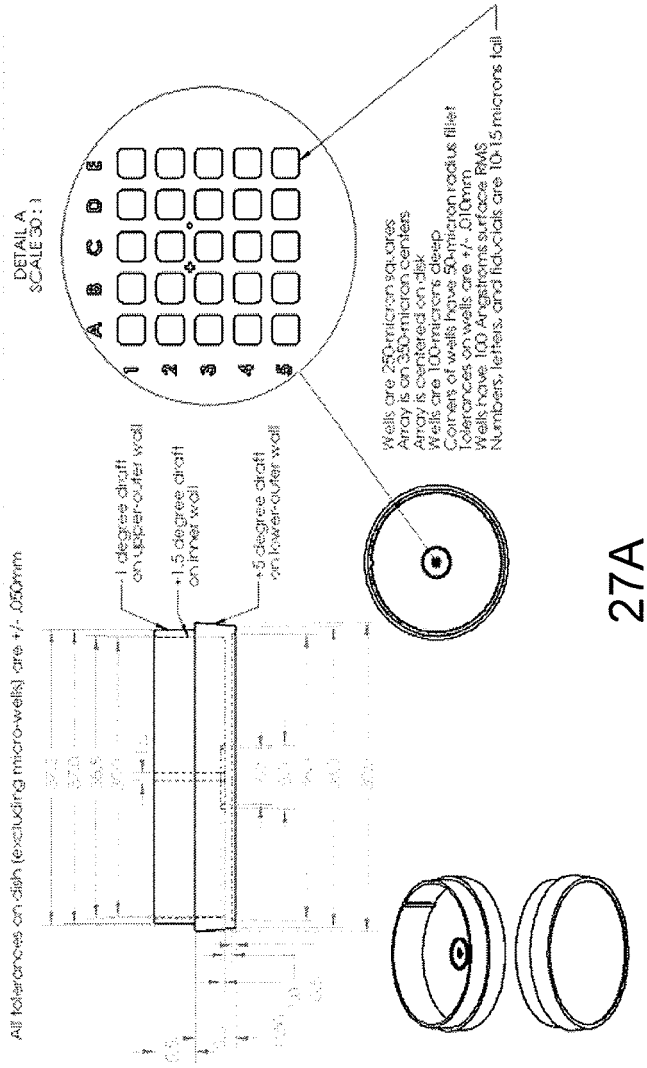
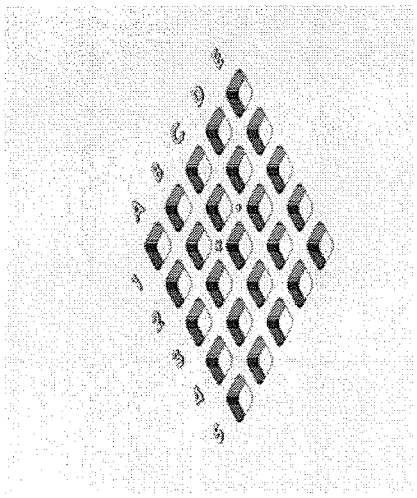

(a) Experiment 1, Station 2, Day 1
(b) Experiment 1, Station 2, Day 2.5
(c) Experiment 1, Station 2, Day 4
(d) Experiment 1, Station 2, Day 5.5

Fig. 26



27A



27B

Fig. 27

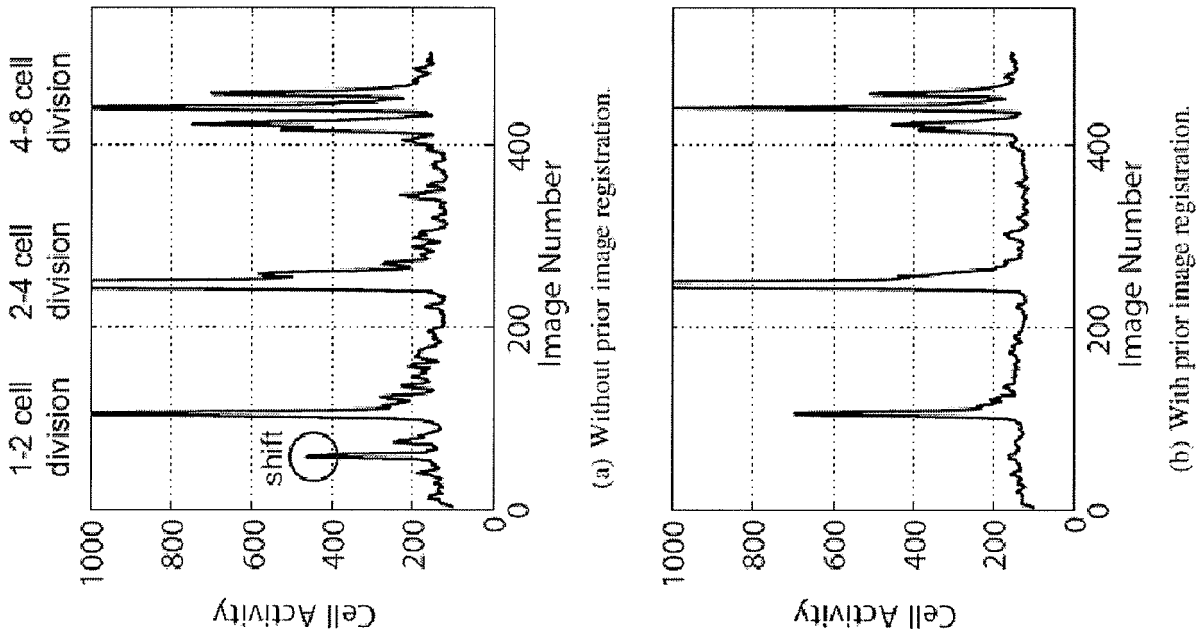
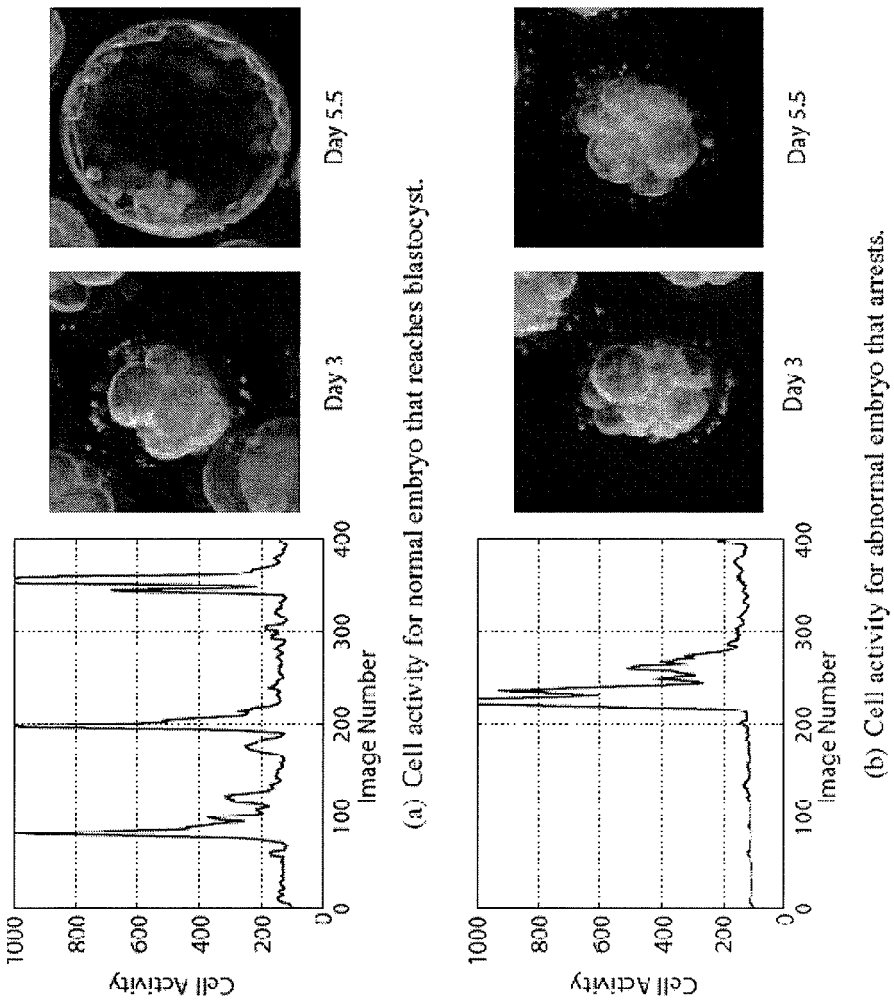

1-2 cell division
2-4 cell division
4-8 cell division
shift
Cell Activity
Image Number
0
200
400
600
800
1000
(a) Without prior image registration.
(b) With prior image registration.

Fig. 28



(a) Cell activity for normal embryo that reaches blastocyst.

(b) Cell activity for abnormal embryo that arrests.

Fig. 29

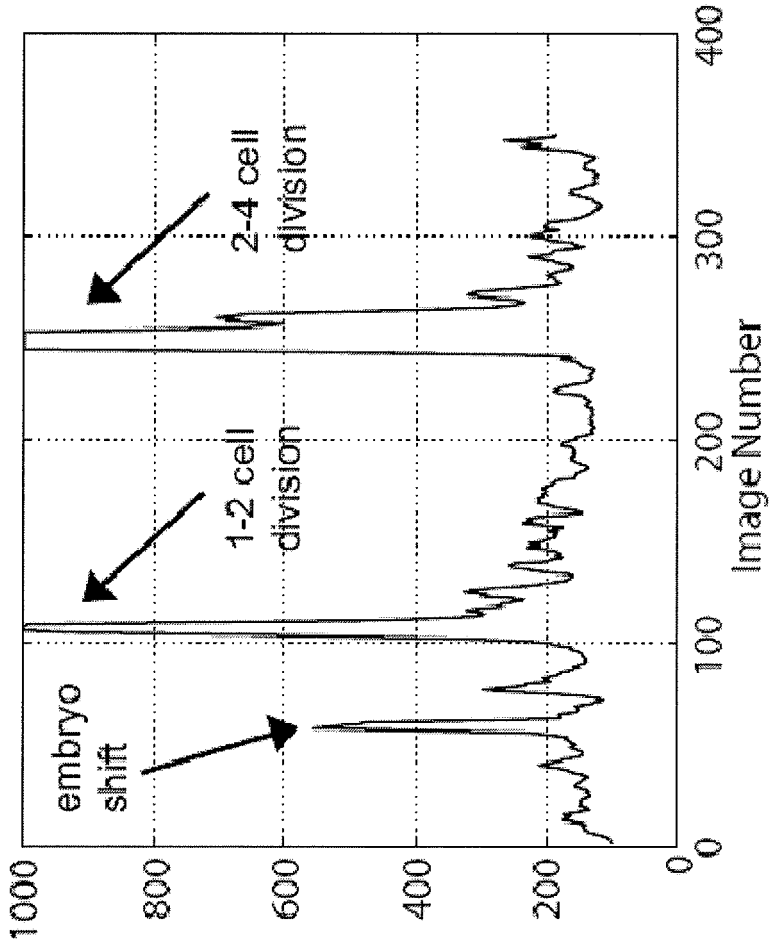

embryo shift
1-2 cell division
2-4 cell division
Image Number
0
100
200
300
400
200
400
600
800
1000

Fig. 30

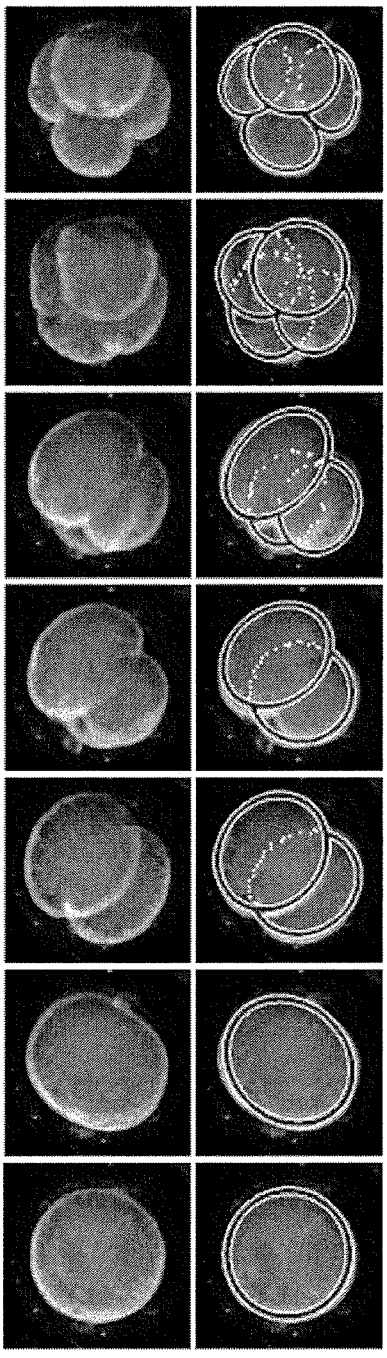
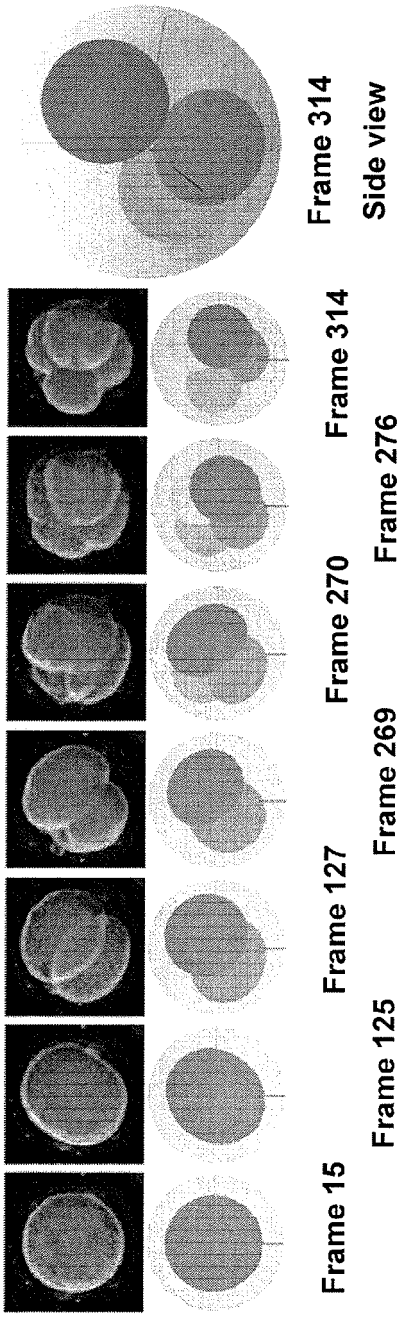
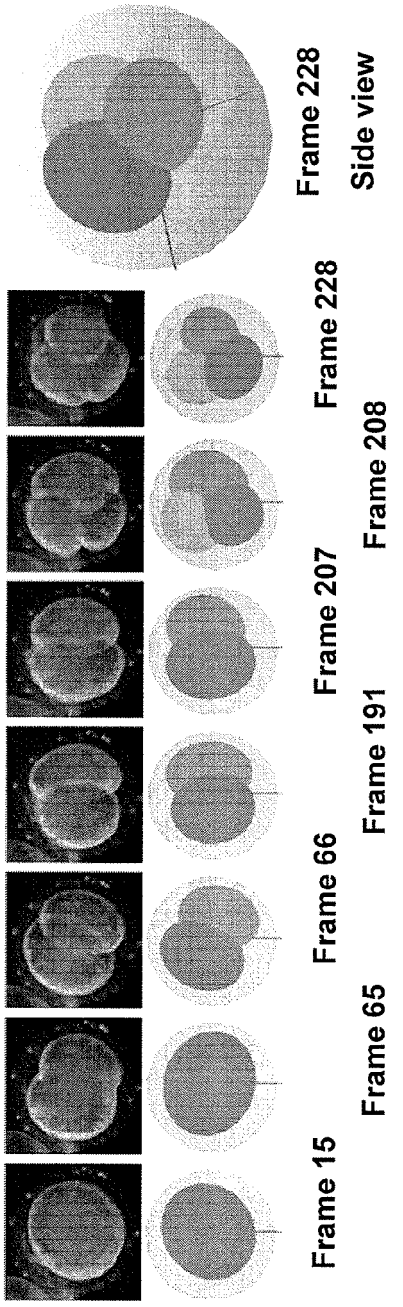

Frame 15
Frame 45
Frame 48
Frame 189
Frame 190
Frame 196
Frame 234
31A
31B
31C
31D
31E
31F
31G

Fig. 31



31A



32B

Fig. 32

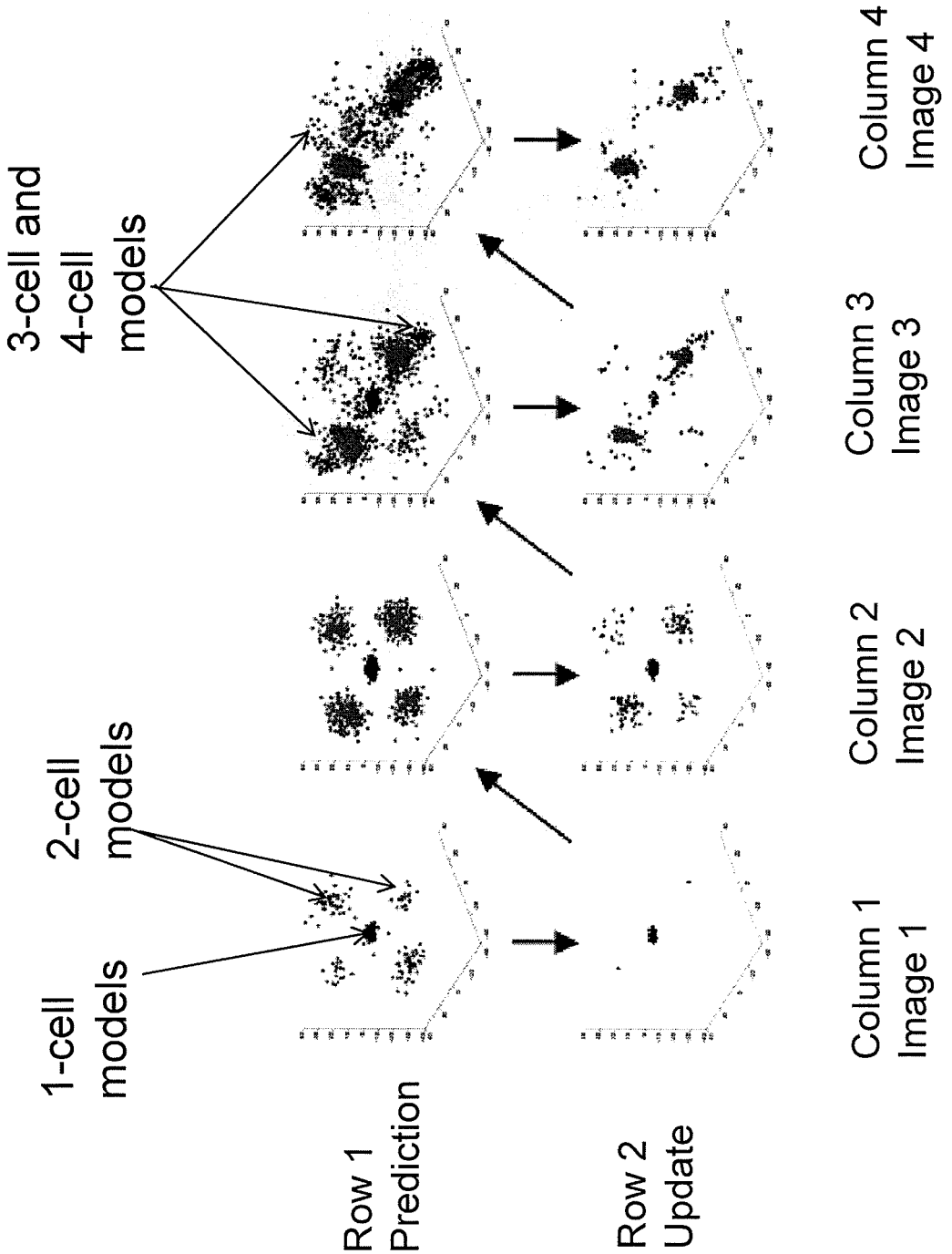
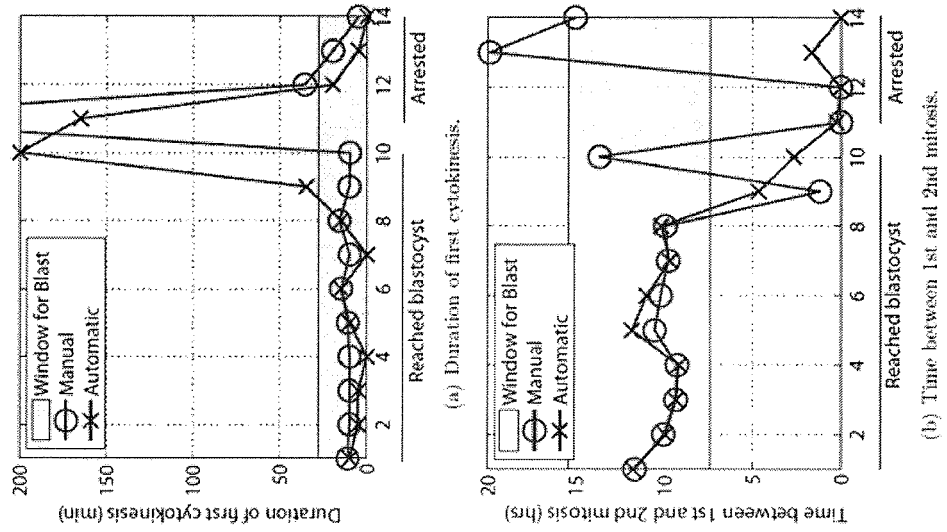

1-cell models
2-cell models
3-cell and 4-cell models
Row 1 Prediction
Row 2 Update
Column 1 Image 1
Column 2 Image 2
Column 3 Image 3
Column 4 Image 4

Fig. 33



34A

34B

Fig. 34

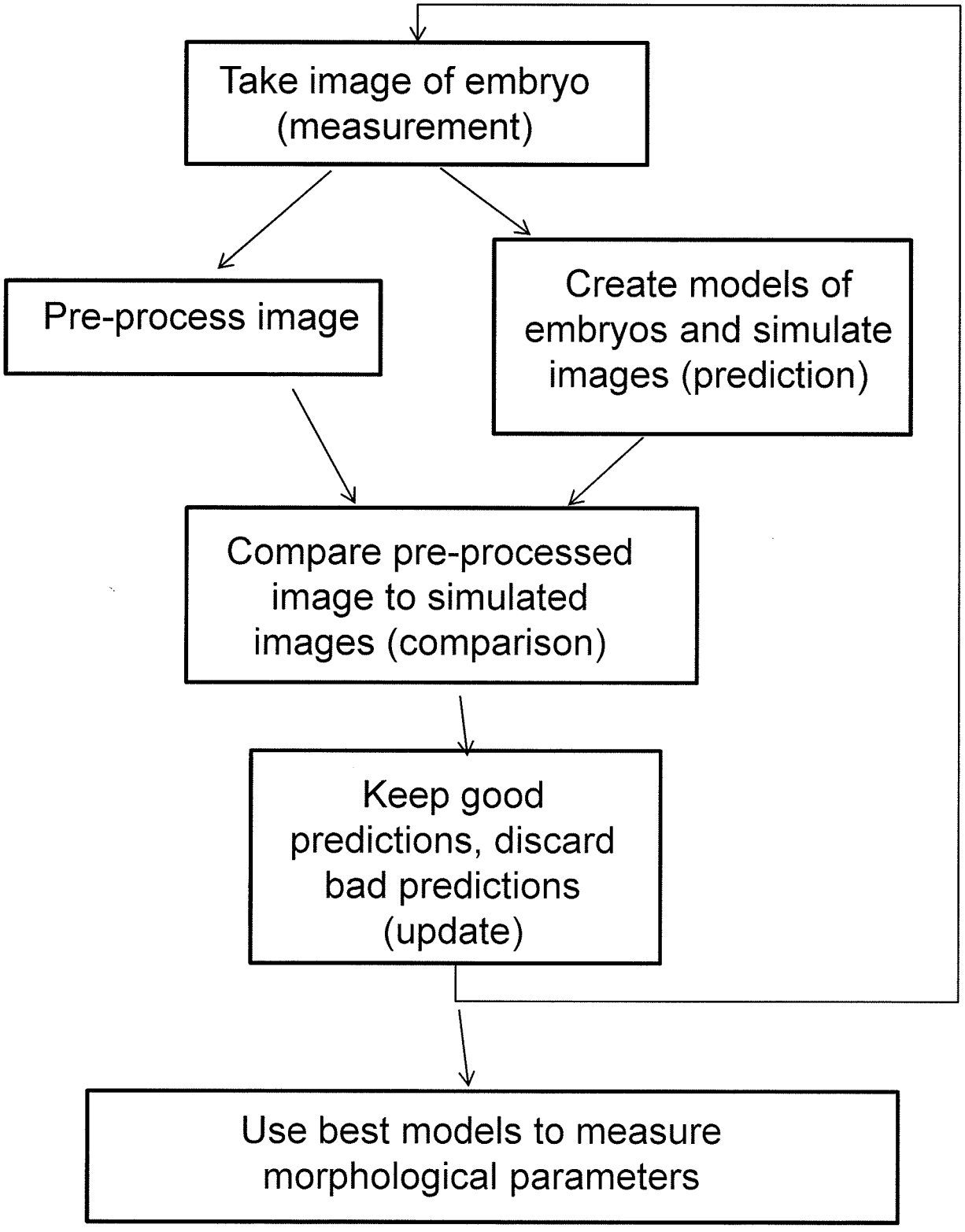

Take image of embryo (measurement)
Pre-process image
Create models of embryos and simulate images (prediction)
Compare pre-processed image to simulated images (comparison)
Keep good predictions, discard bad predictions (update)
Use best models to measure morphological parameters

Fig. 35