Emerging technologies and PGD

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Challenges of PGD

Sample sufficient genetic material from oocytes or early embryos without adversely affecting their viability and development

Perform rapid and reliable genetic diagnosis on single or very few cells

Embryo biopsy

Cleavage stage still most widely used

- ~90% of cycles reported to ESHRE PGD Consortium

Techniques have changed little in past 20 years

- Based on holding embryo stationary with micromanipulators
- Breach of the zona
- Aspiration of genetic material

All methods of embryo biopsy:

- Are labour intensive
- Require a high degree of skill
- Can take months for even experienced staff to learn
- Take a significant proportion of time available to perform genetic test

Lasers and optical tweezers

Lasers now commonly used to open zona pellucida

Optical tweezers used to move and manipulate cells

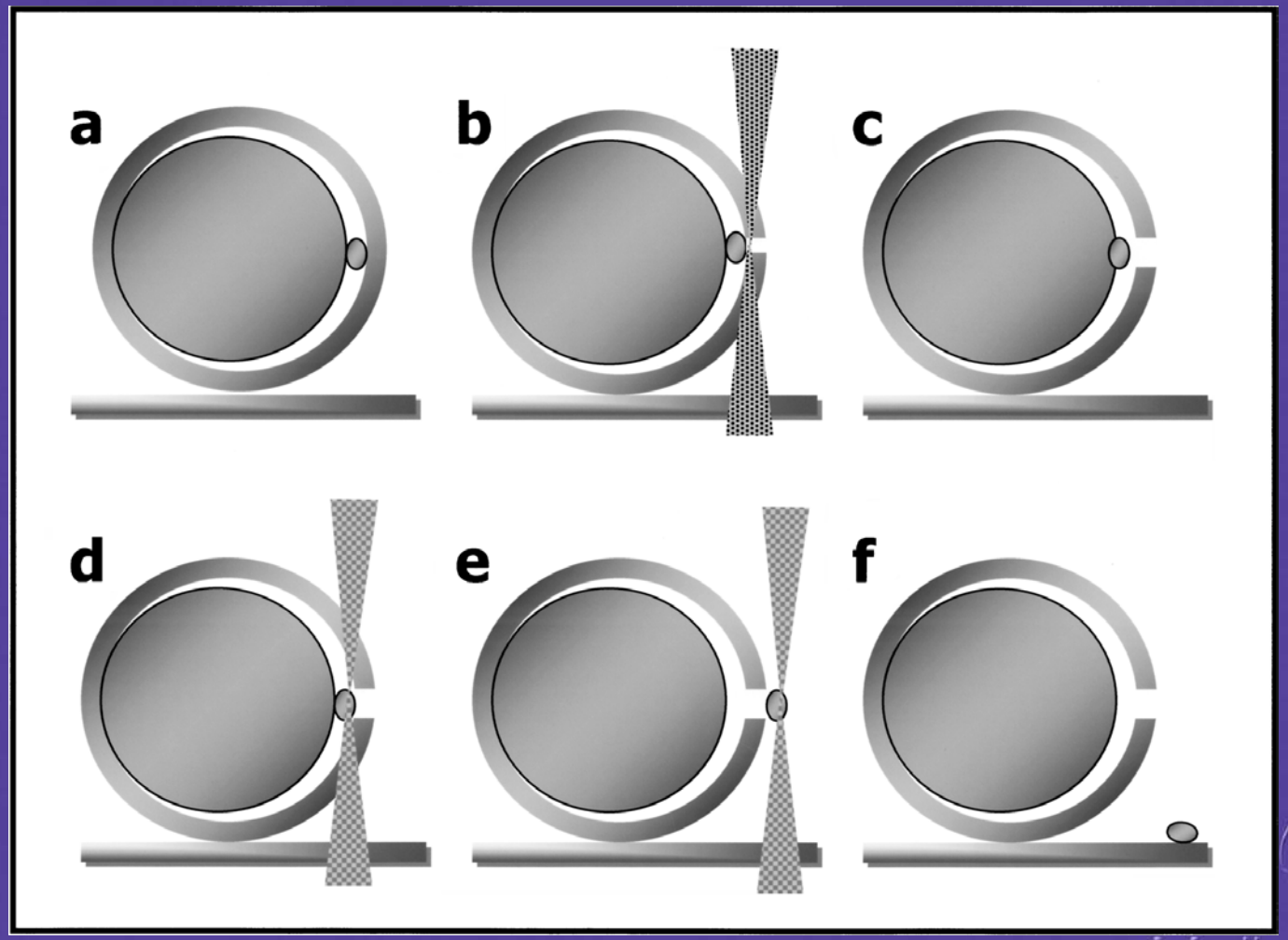
- Cell sorting (Ashkin et al., 1987)
- Sperm manipulation (Clement-Sengewald et al., 1996)

Polar body biopsy with optical tweezers

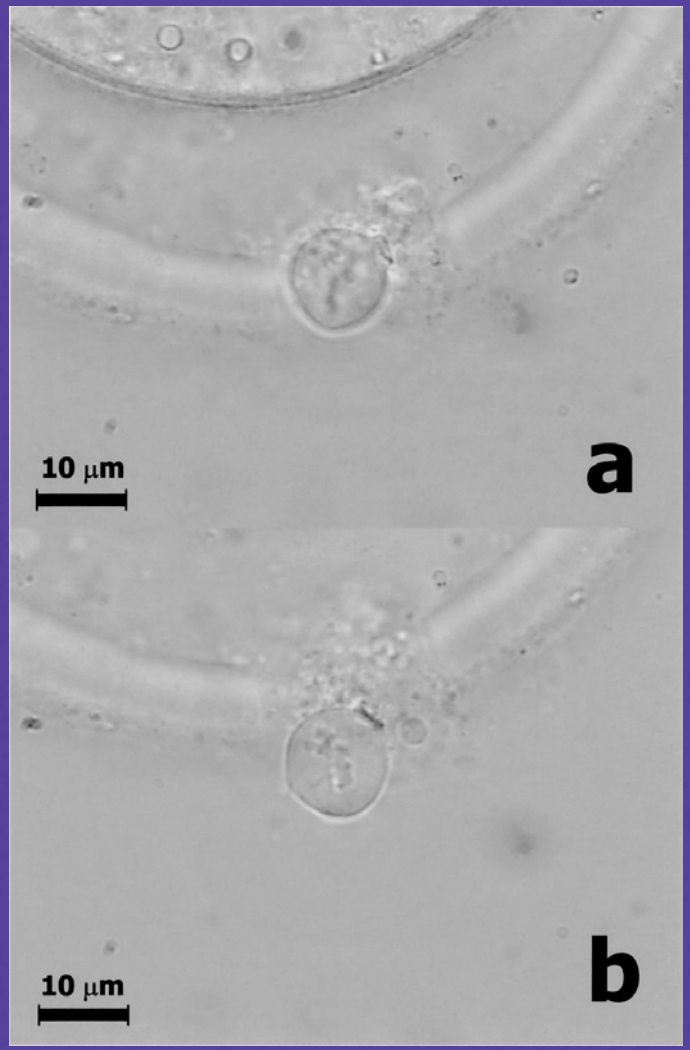
Clement-Sengewald et al.,2002

- Cutting laser
  - Breach zona pellucida
- Optical tweezers
  - Trap polar body
  - Drag polar body through zona pellucida
  - Polar body placed on polyethylene naphthalene membrane
- Laser pressure catapulting
  - Propel polar body into lid of PCR tube

Clement-Sengwald et al., 2002



Polar body biopsy with optical tweezers



Clement-Sengwald et al., 2002

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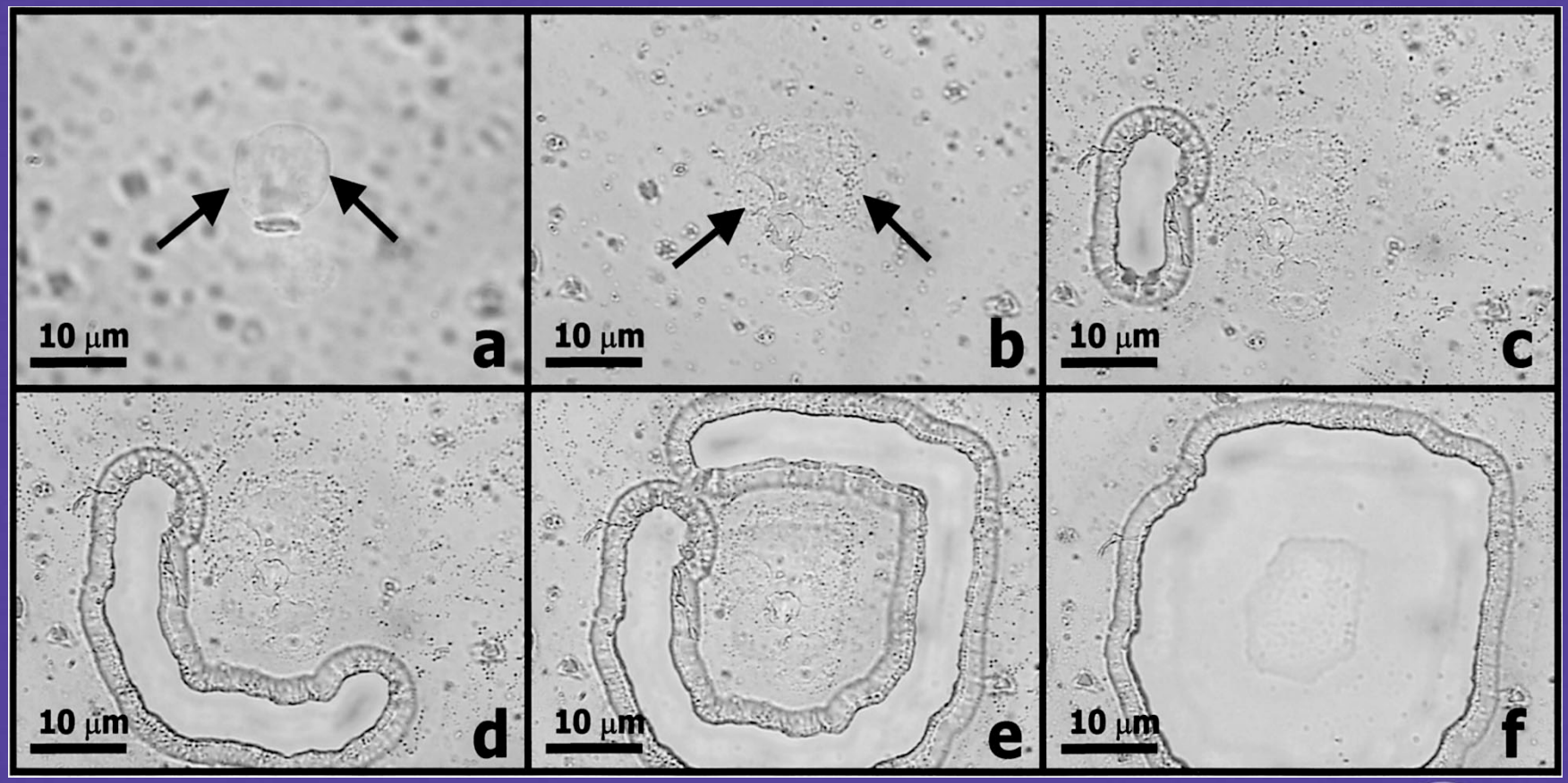
Laser pressure catapulting



Clement-Sengwald et al., 2002

Optical tweezers for PB/embryo biopsy

Lasers are tangential to oocyte

Micromanipulation not required

- No micromanipulators
- No glass pipettes
- Minimal staff training
- Rapid (~ 40 secs)

Human contact minimised

- Reduce contamination risk in PCR based cases

Can laser tweezers trap/manipulate blastomeres?

- Not tested clinically => unknown impact on embryo development
- Approach holds promise but further research needed

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Non-invasive genetic analysis?

Ideal approach would be to avoid cellular sampling of embryos

- Very high resolution
- No impact on embryo viability

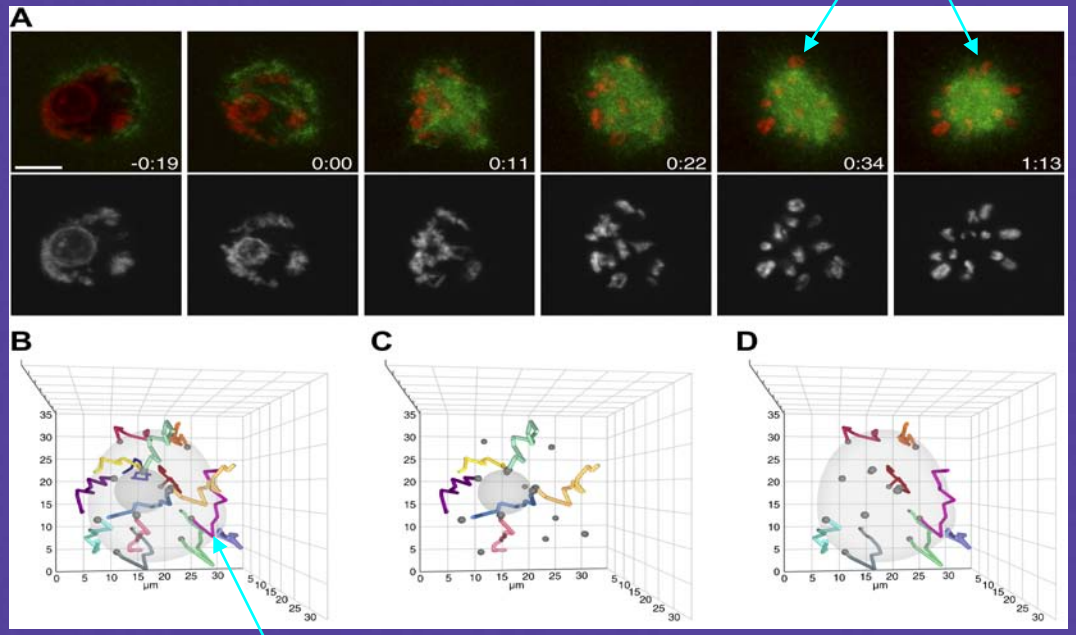
There have been significant advances in microscopy and live cell imaging in recent years

- 4D confocal fluorescence microscopy
- 3D tomography of single cells
- STED nanoscopy
- CLASS microscopy

4D confocal fluorescence microscopy

Schuh and Ellenberg, Cell, 2007

Individual chromosomes (red)



Chromosome movement tracks

Time lapse high resolution confocal imaging in live cells

Analysed spindle assembly and chromosome movement in maturing mouse oocytes

Not able to identify individual chromosomes

Requires fluorescent staining/UV exposure so not suitable for in situ analysis of blastomeres

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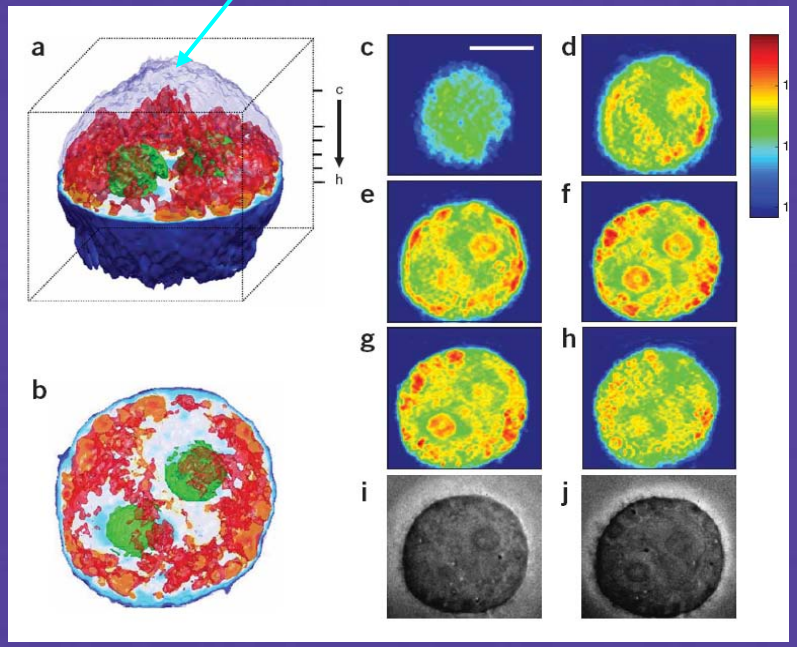
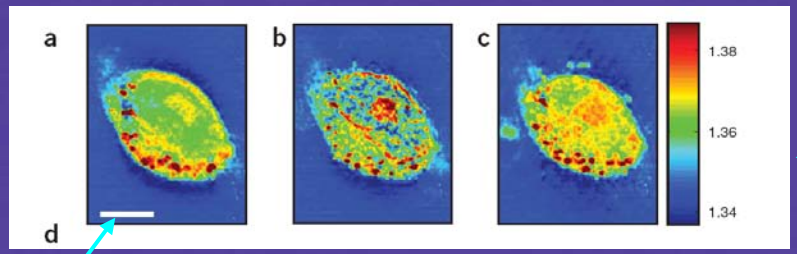
Tomographic phase microscopy

Choi et al., Nat. Meth. 2007

Constructed 3D image of HeLa cell





10 µm

Time lapse of HeLa cell responding to acetic acid exposure

Quantitative high resolution 3D-imaging of living cells

Measures refractive index using phase contrast microscopy

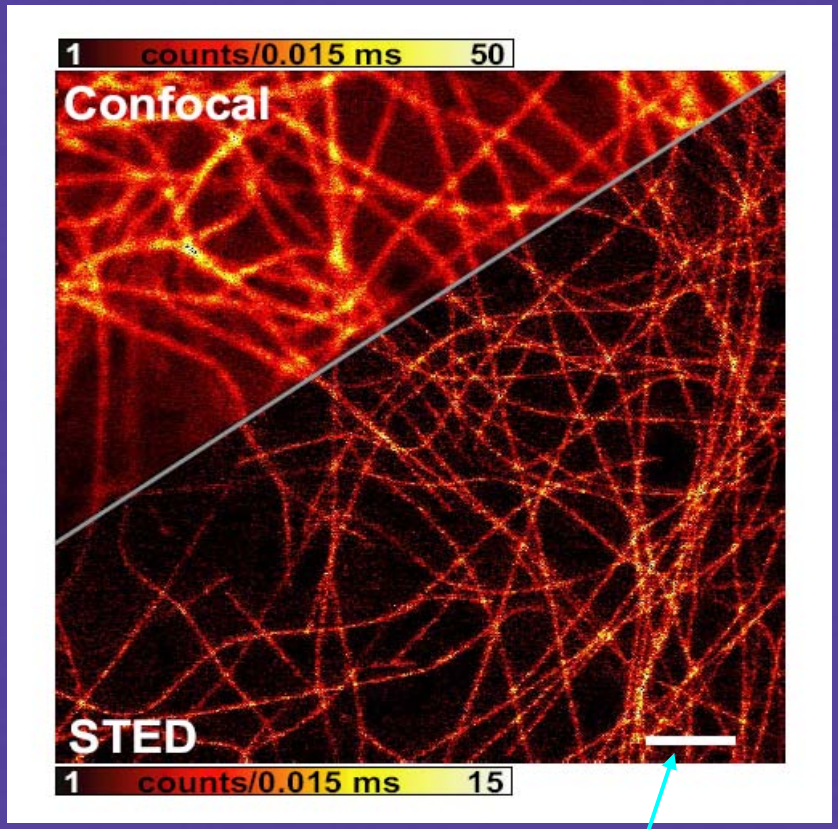
- No fluorescence

Organelles visible but resolution would need to be higher

Stimulated emission depletion (STED) nanoscopy

Hein et al., PNAS, 2008

Endoplasmic reticulum of PtK2 cells



Confocal resolution limited by diffraction

4-fold higher resolution that confocal

- Nanoscopy (not microscopy)

Live cell analysis

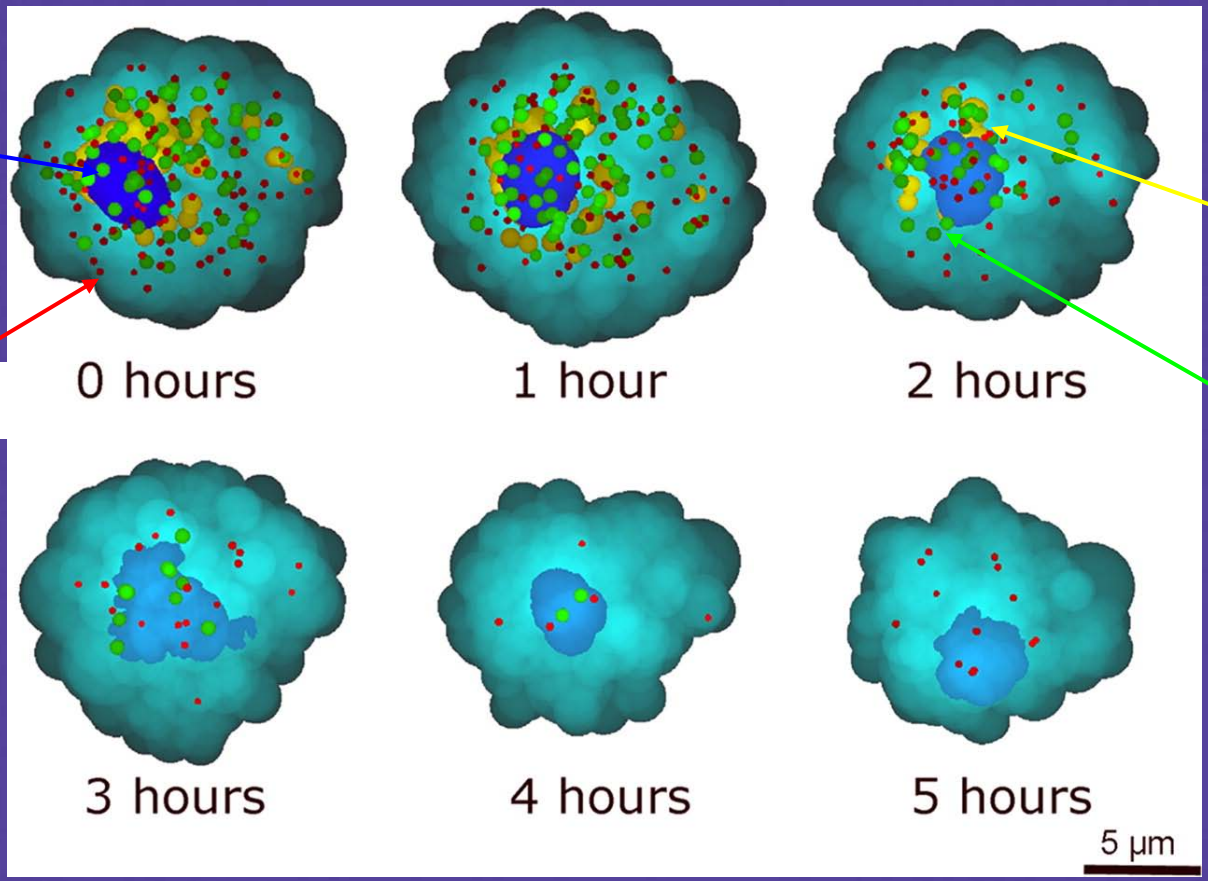
Requires use of fluorescent tags

CLASS microscopy (Itzkan et al., 2007)

- CLASS = Confocal Light Absorption and Scattering Spectroscopic microscopy
- Non invasively determines the dimensions and other physical properties of single sub cellular organelles
- Harnesses the light scattered by small particles and compares properties of this light with refractive index, size and shape
- Measures very small internal cell structures
- No markers or contrast agents required, cell viability maintained
- Does use laser light

CLASS microscopy – reconstructed image

Itzkan et al., 2007



Single cell exposed to docosahexaenioc acid

Organelles represented as spheroids

Itzkan et al., 2007

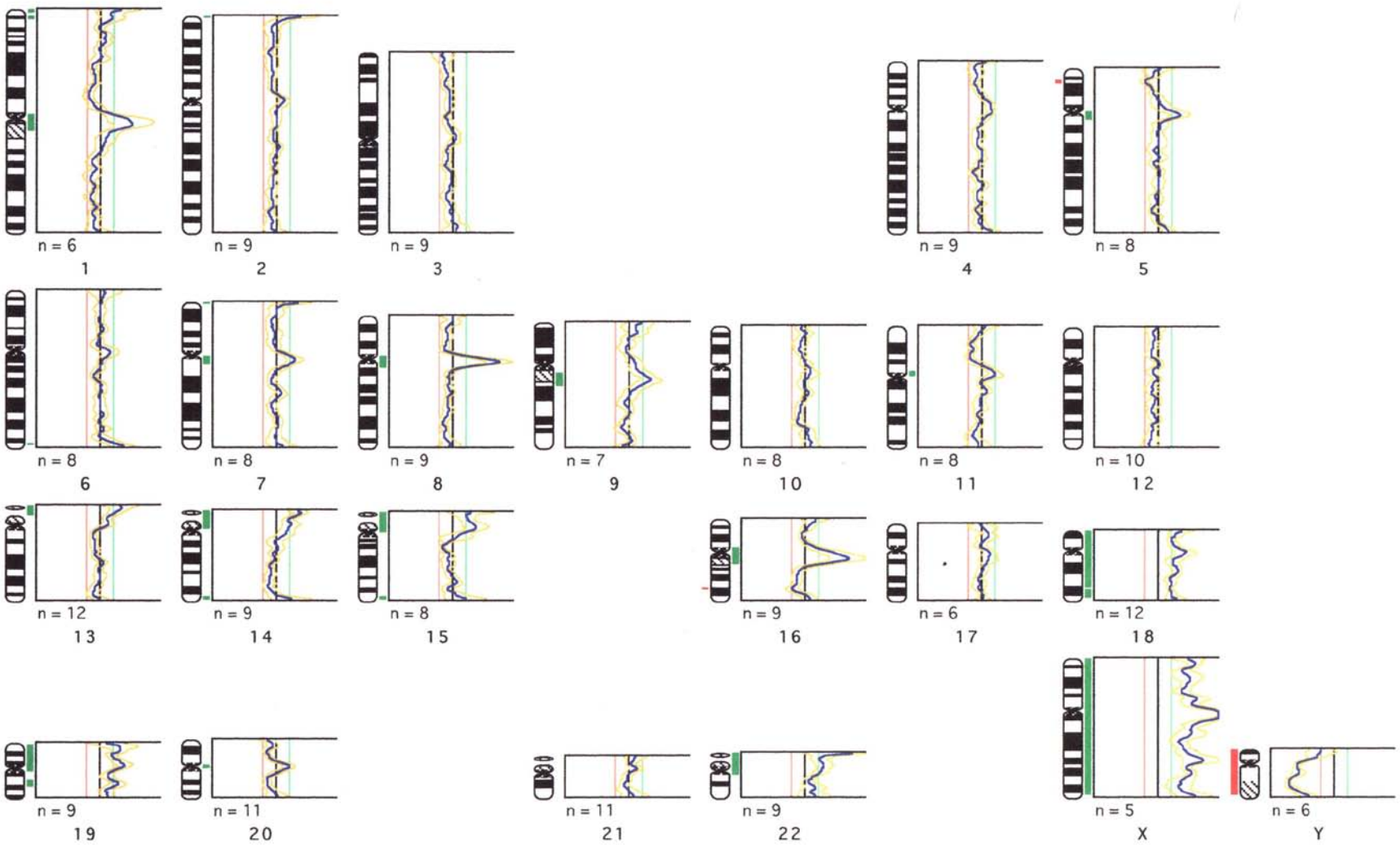
Advances in genetic testing

Many advances in single cell genetic testing since the advent of PGD

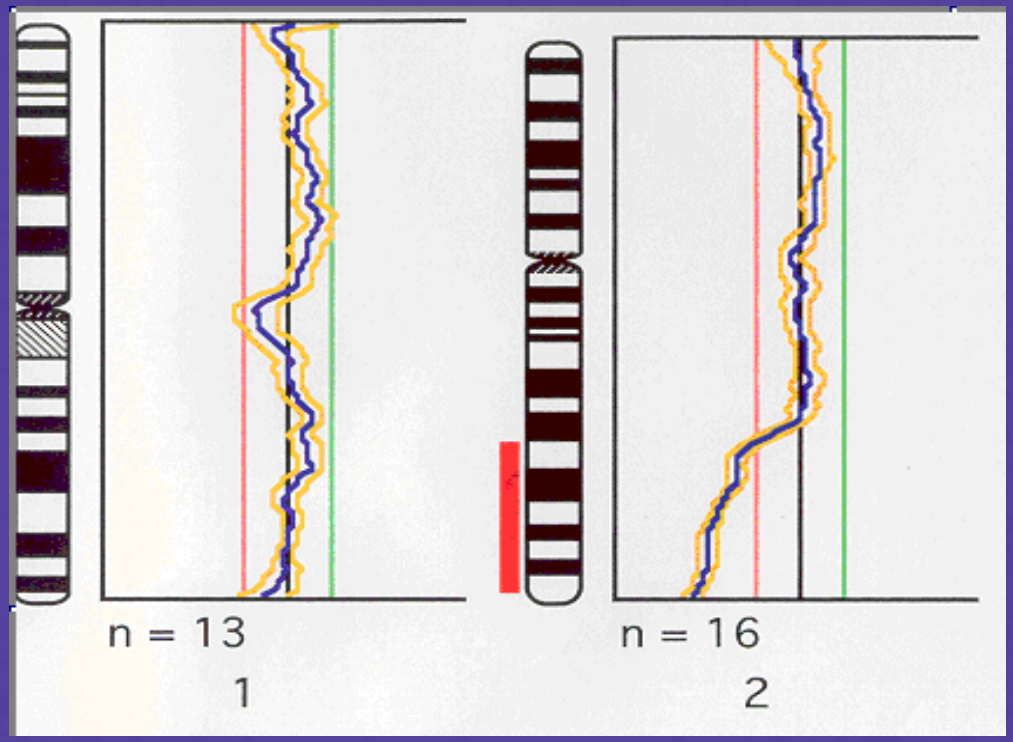
- Expansion of available FISH probes and fluorochromes
- mf-PCR
- Rapid tests
- Etc, etc

Metaphase CGH

- Full molecular karyotyping on single cells



Partial aneuploidy detected by CGH



CGH vs. FISH

CGH analysis of over 400 cells from approx. 200 human embryos

- Errors of all chromosomes occur in early embryos at measurable frequencies
- Full chromosome analysis of blastomeres is optimal

	FISH 13,14,15,16,21,22,X/Y
Cells “misdiagnosed”	40%

e.g. -1q,+5,+6,+8,+12

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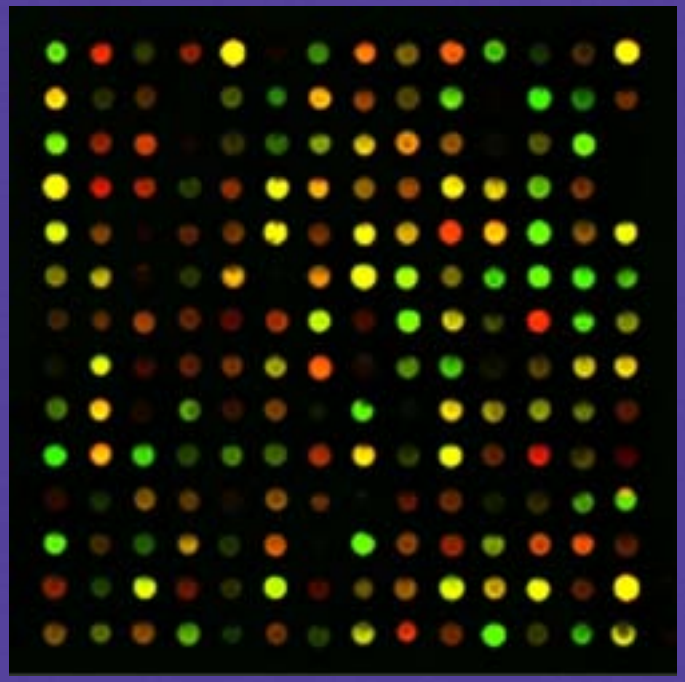
Array-CGH

Same principle as metaphase CGH

- Template is solid support
- Spotted with known short sequences of DNA
- Sequences specific to different chromosomal regions
- Chromosomal loss or gain identified by relative fluorescence ratio
- Rapid analysis, easily automated
- Successfully applied to single cells



Types of microarray platform

BAC
- Few thousand clones of large pieces of chromosome (~150kb)

Oligonucleotide
- Probes synthesized in situ, ~ 50 nucleotides long

Chromosome libraries
- Each spot represents whole chromosome

SNP
- Target single nucleotide polymorphisms

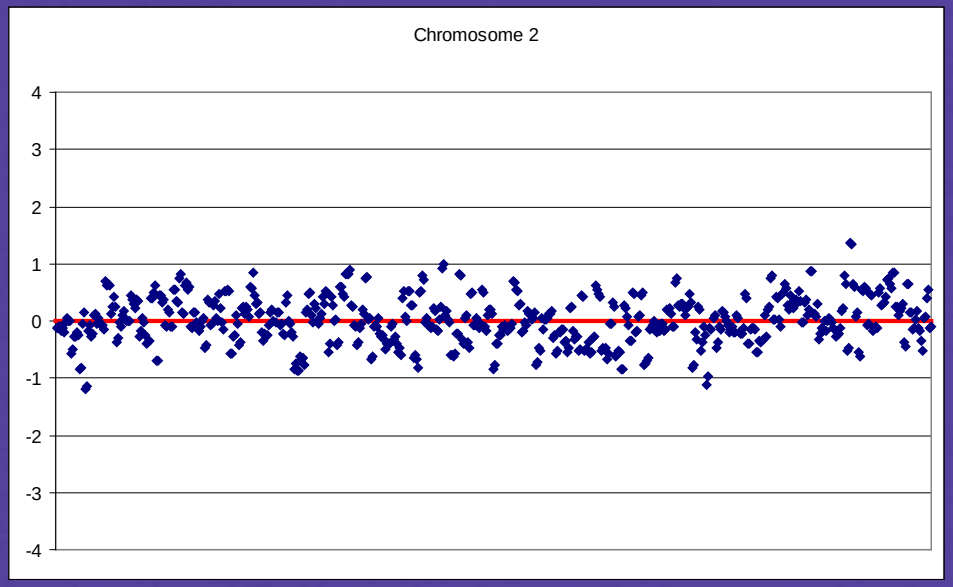
Pros and cons of each type of array related to resolution, reliability and expense

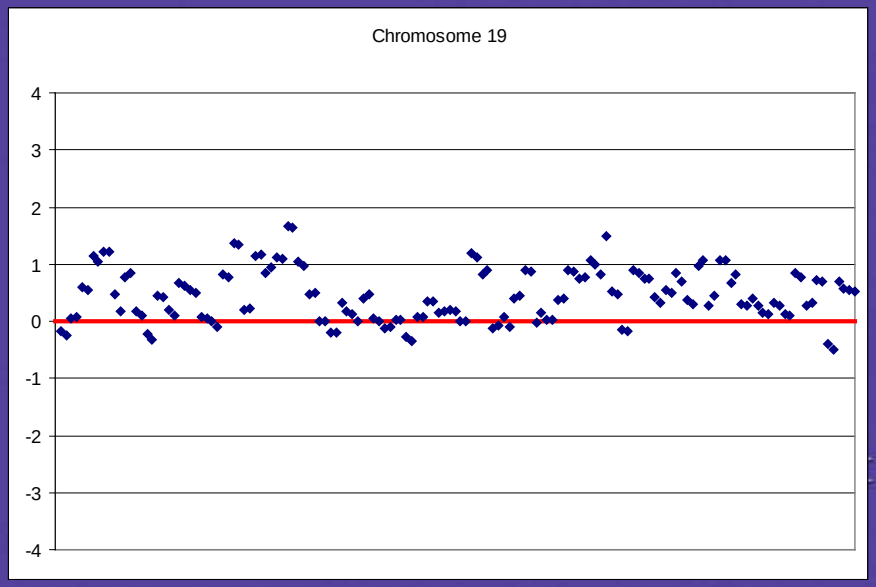
Many groups are in validation phase at the moment

Hellani et al., 2008
- Clinical application of oligo-array
- Patients $\geq$ 7 consecutive IVF failures (mean female age=36)
- 6/8 patients with ET, 5/6 pregnant

VUB Brussels/Melbourne IVF microarray study

- Blinded study
- Embryos donated for research separated into single blastomeres
- Whole genome amplification by MDA
- Array-CGH using BAC array, 1MB resolution
- 3000 clones in duplicate





Mertzanidou et al., 2008

Sample	aCGH results
1	49, XX, +13, +21, +22
2	46,XX
3	44, X0, dup(13)(q31qter), -18, -20
4	47, XY, +1, del(2)(pter2q31),del(17)(17p12q24),dup(X)(q26qter)
5	44, XY, -3, -11, +13, -14
6	46,XX
7	44, XY, -1, del(13)(q31qter), -16
8	42, XX, -16, -19, -20, -22
9	48, XXY, dup(1)(pterp34), dup(9)(q22qter), +22
10	49, XXY, +13, +21
11	Not arrayed
12	Not arrayed
13	45, XY, -6
14	48, XXY, +13
15	50, XXY, dup(1) (pterp34), +13, +19, +22
16	Not arrayed
17	46, XX
18	46, XX
19	Not arrayed
20	46, XX

Mertzanidou et al., 2008

Sample	aCGH results	DECODE
1	49, XX, +13, +21, +22	2
4	47, XY, +1, del(2)(pter2q31),del(17)(17p12q24),dup(X)(q26qter)	2
5	44, XY, -3, -11, +13, -14	2
9	48, XXY, dup(1)(pterp34), dup(9)(q22qter), +22	2
13	45, XY, -6	2
15	50, XXY, dup(1) (pterp34), +13, +19, +22	2
3	44, X0, dup(13)(q31qter), -18, -20	3
7	44, XY, -1, del(13)(q31qter), -16	3
2	46,XX	5
6	46,XX	5
17	46, XX	5
18	46, XX	5
20	46, XX	5
8	42, XX, -16, -19, -20, -22	6
10	49, XXY, +13, +21	6
14	48, XXY, +13	6

SNP arrays

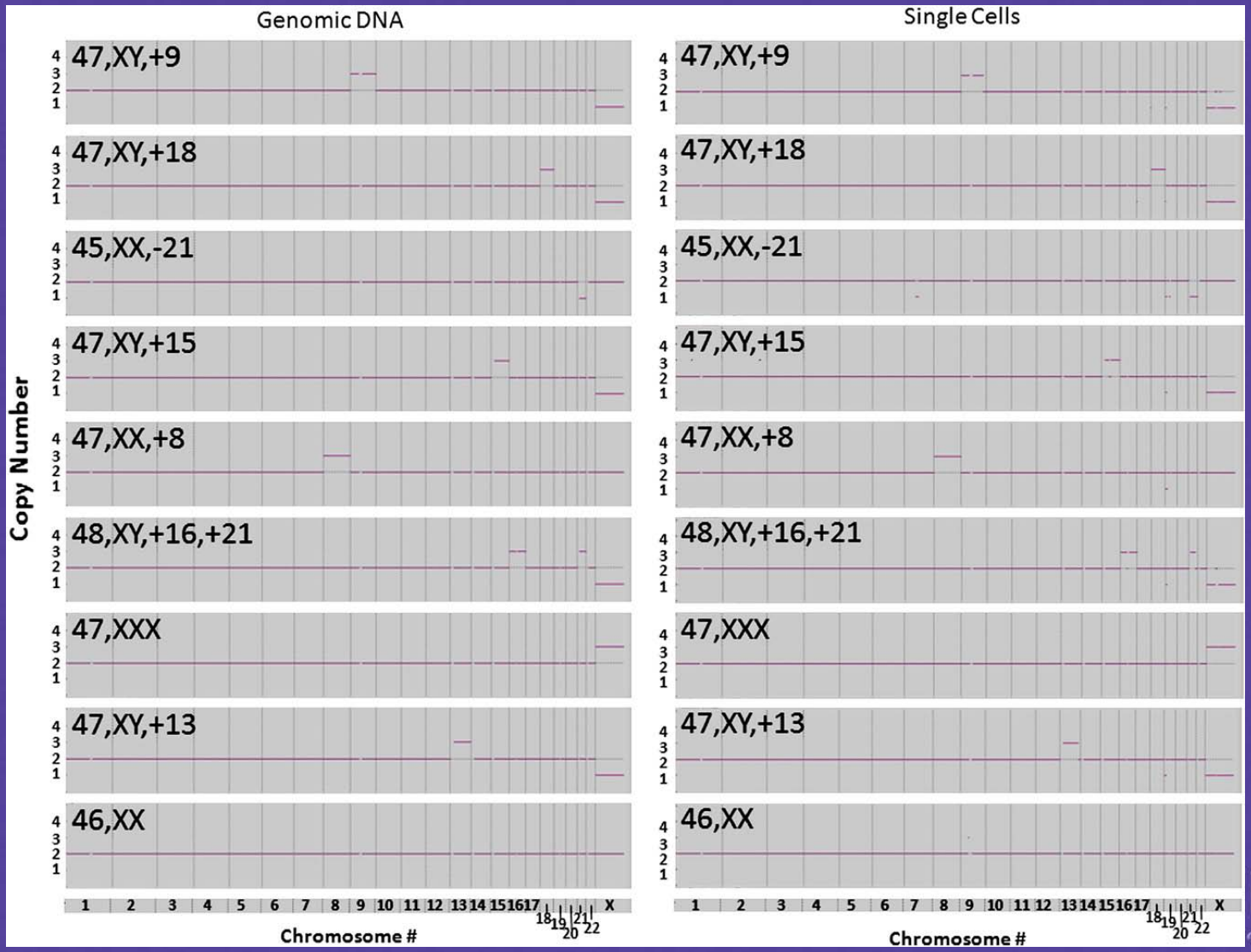
- SNP = single nucleotide polymorphism
  - Genome positions where there are two distinct alleles in a significant proportion of population i.e. highly polymorphic
- Estimated >10 million SNPs in human genome
  - >600 for every BAC
- SNP arrays
  - More than 1 million SNPs
  - High resolution
- Copy number variation and genotyping
  - Aneuploidy, deletions, duplications,
  - Uniparental disomy
  - Mutations by linkage
- Several groups in validation stage
  - Data analysis is challenging

Treff et al., 2010 (Fertil. Steril)

- Affymetrix 262K SNP array
- Three phase study
  - 72 single cells from 9 known aneuploid cell lines
  - Blind analysis of 27 single cells from known aneuploid cell lines
  - 335 single blastomeres from 235 cleavage stage embryos
    - Multiple cells from 16 arrested embryos

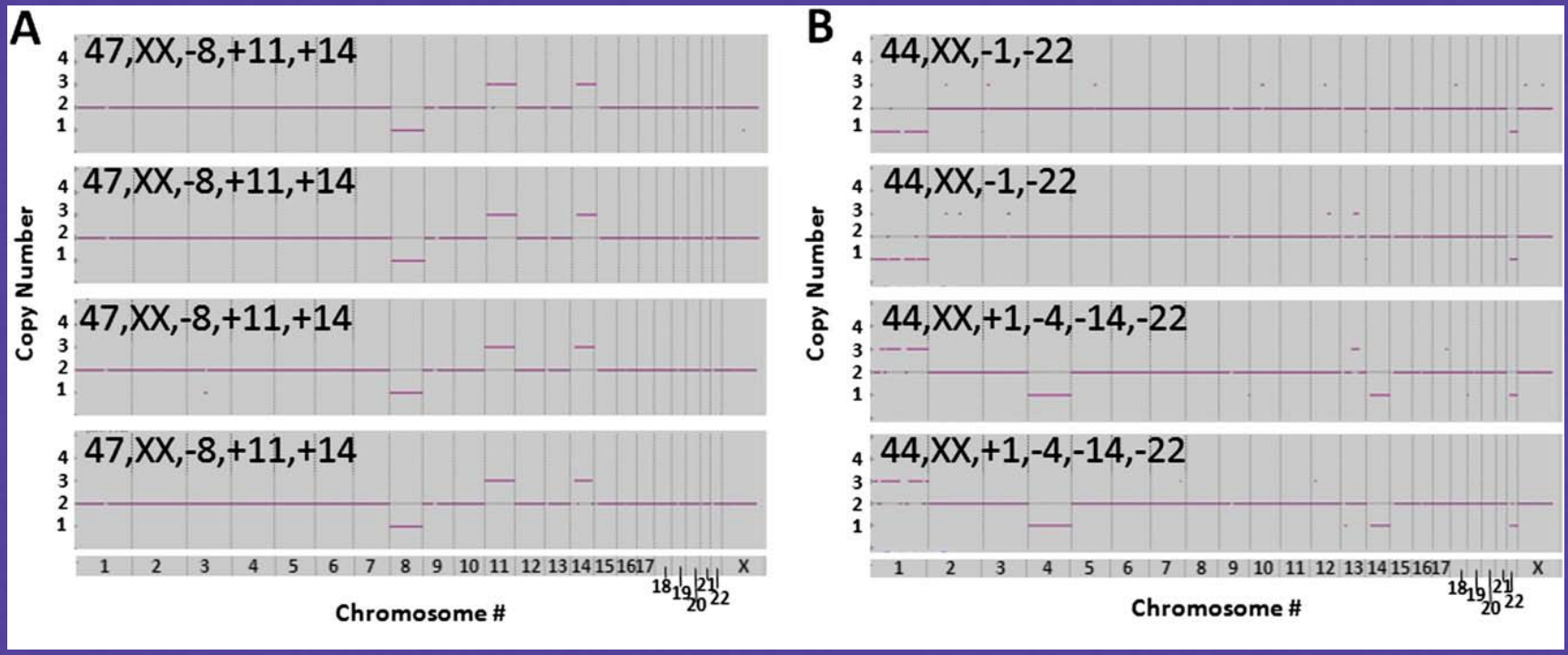
SNP analysis of known trisomic single cells

(Treff et al., 2010)



SNP analysis of single blastomeres

(Treff et al., 2010)



Amenable to higher resolution analysis

- Partial aneuploidies
- Imbalance caused by translocations

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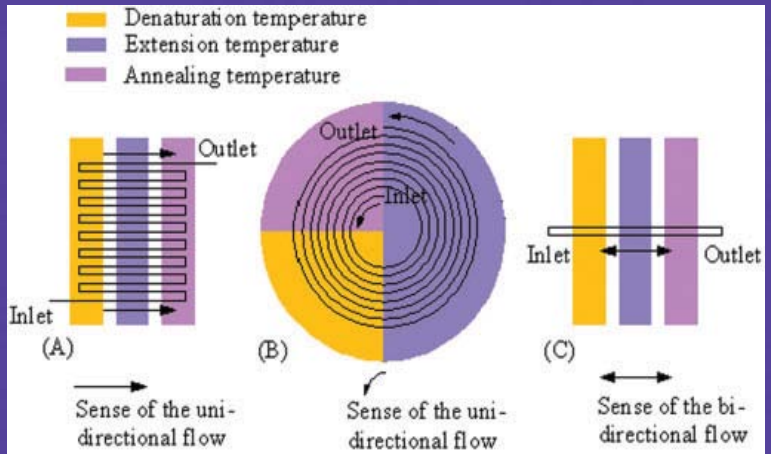
“Lab on a chip” - microfluidic PCR (Zhang and Xing, 2007)

- Small chips
  - Fast reaction times
  - Rapid heating and cooling times
- Small reaction volumes
  - Use less reagents
- Used to amplify multiple genes from single bacteria (Ottesen et al., 2006)
- Can be coupled to analysis and information chips
  - Closed system
  - Minimise sample handling



Next generation sequencing

- DNA sequencing first described ~30 years ago (Sanger, Maxam-Gilbert)
  - E.coli ~1000 years, human ~1 million years
- Current routine sequencing technology enables up to 96 sequences to be read simultaneously
- “Next generation” or “massively parallel” sequencing enables entire human genome to be sequenced in days to hours
  - Short fragments of DNA
  - Processes millions of sequence reads in parallel
  - Simultaneous screening for mutations in hundreds of loci
  - Detection of novel mutations
  - Copy number variation
  - Detection of balanced translocations
  - Inexpensive
- Next-next generation or 3rd generation sequencing
  - Single molecule analysis
  - ?? 15 minute, $1000 human genome sequence by 2013

Future of genetic diagnosis of the embryo

- Has been rapid progress in genetic analysis
- Genetic testing strategies continue to develop
- Cell sampling techniques need to be improved
- Non-invasive analysis would be ideal

Acknowledgements

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Thank you

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