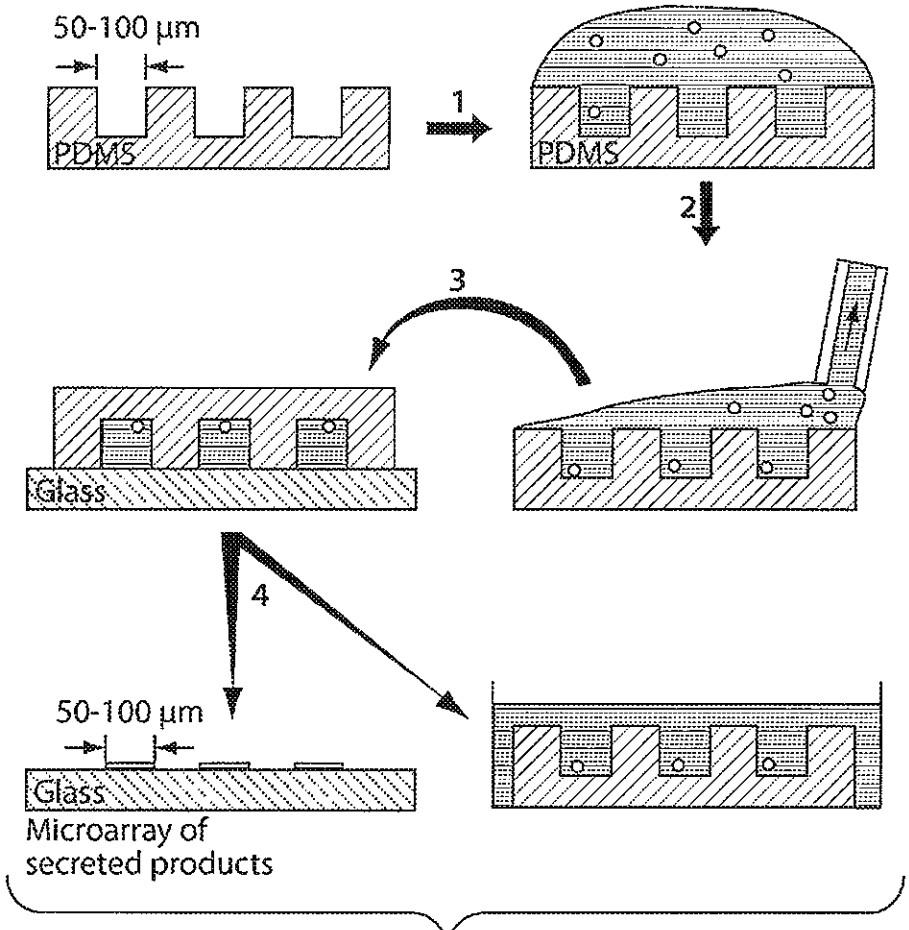

50-100 µm
PDMS
1
PDMS
2
3
4
Glass
4
50-100 µm
Glass
Microarray of
secreted products

Fig. 1

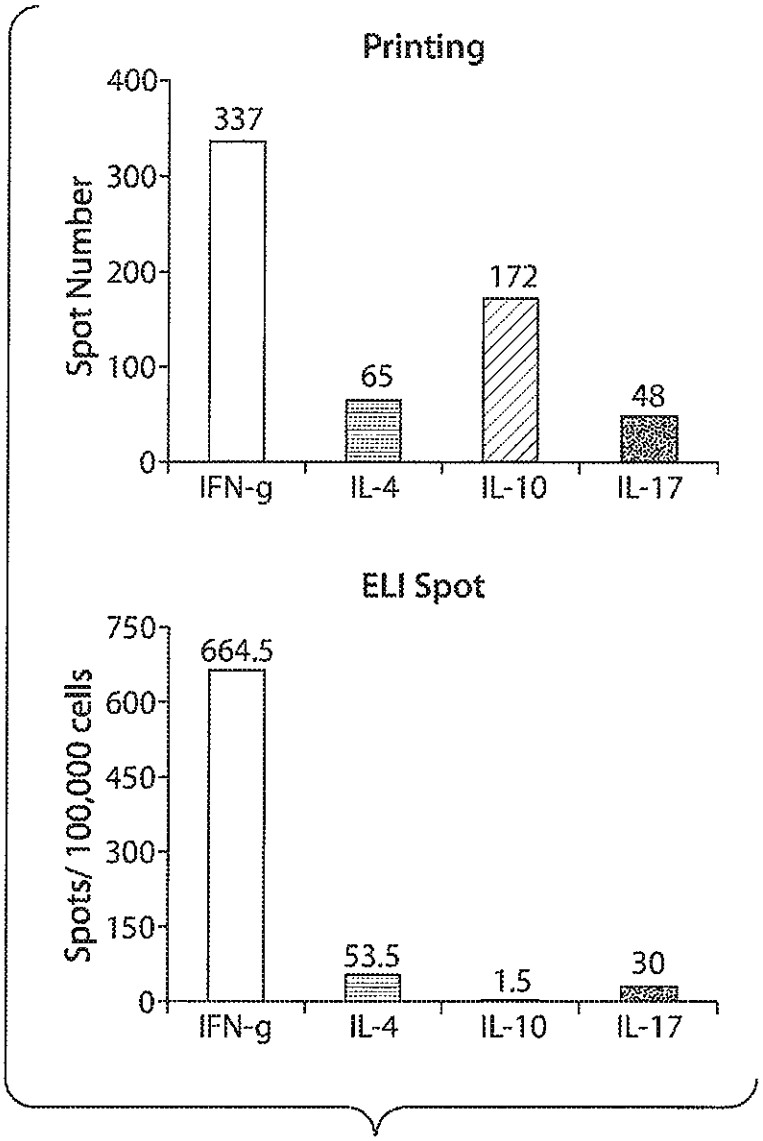

Printing
Spot Number
400
300
200
100
0
337
65
172
48
IFN-g
IL-4
IL-10
IL-17
ELI Spot
Spots/ 100,000 cells
750
600
450
300
150
0
664.5
53.5
1.5
30
IFN-g
IL-4
IL-10
IL-17

Fig. 2

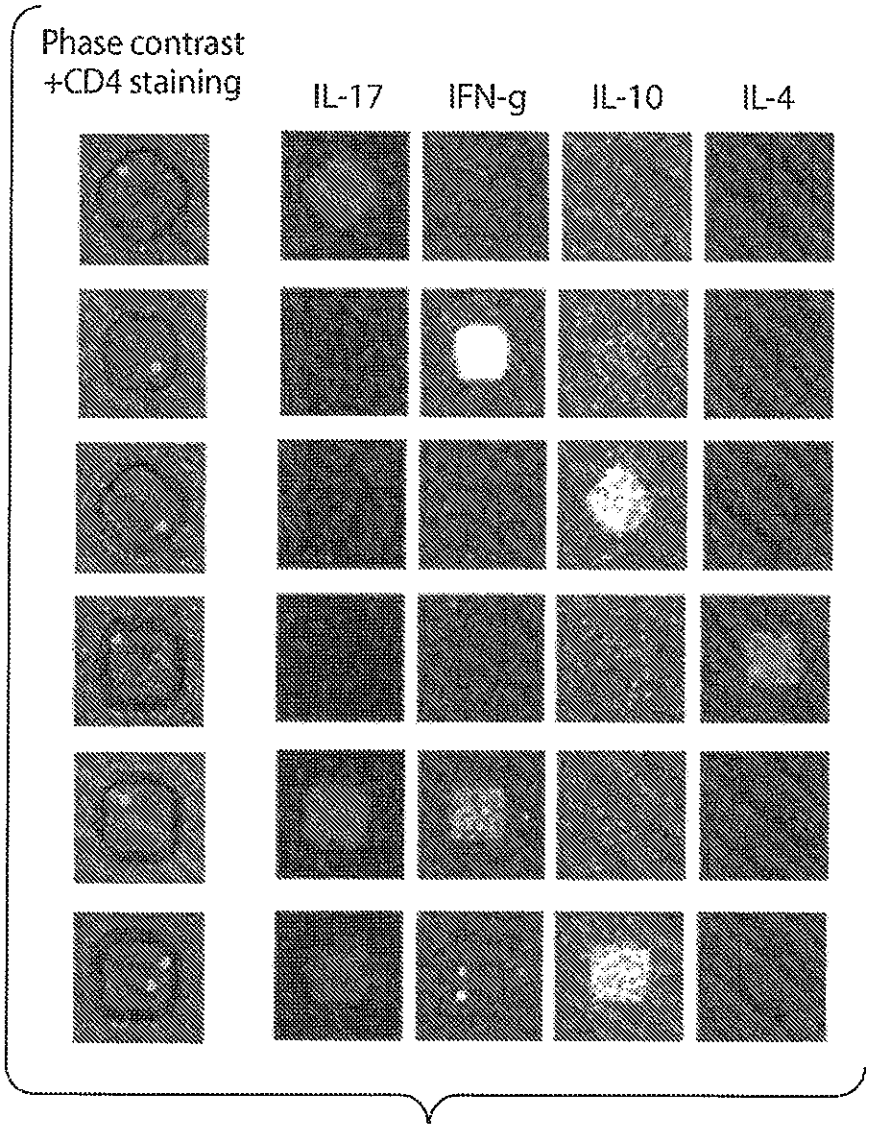


Fig. 3

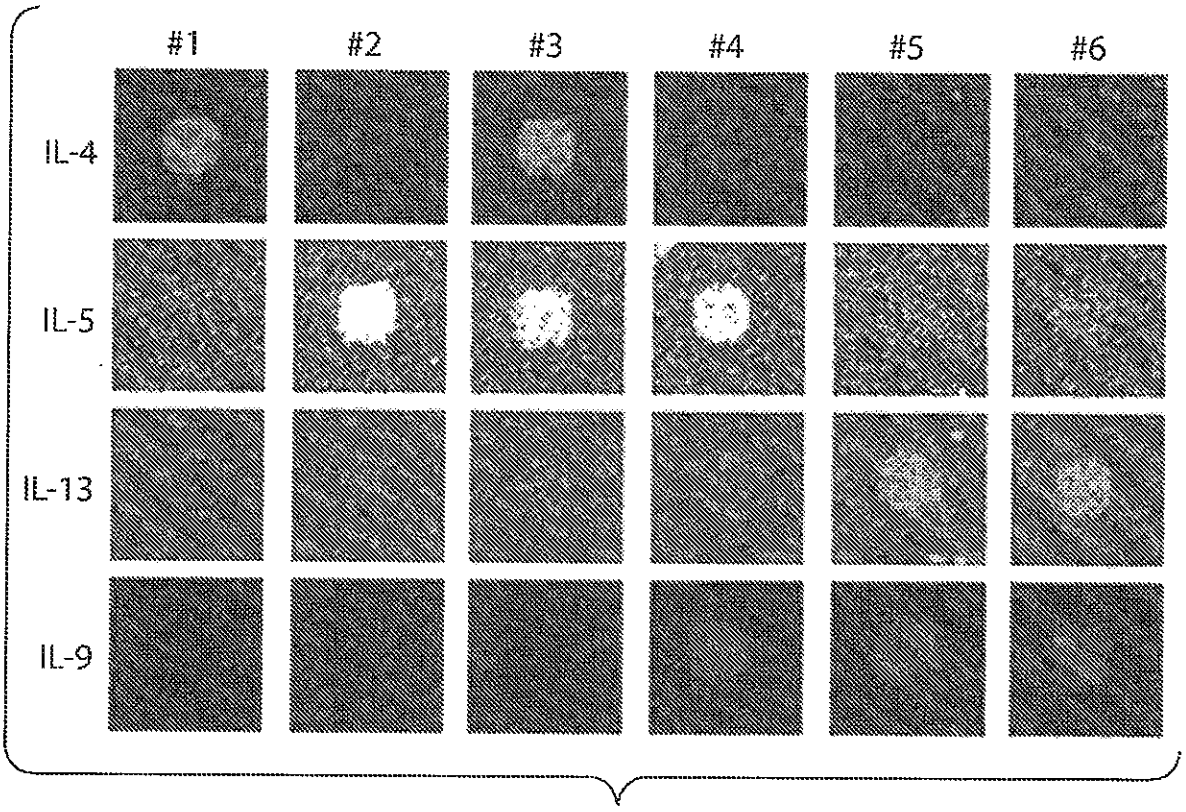


Fig. 4

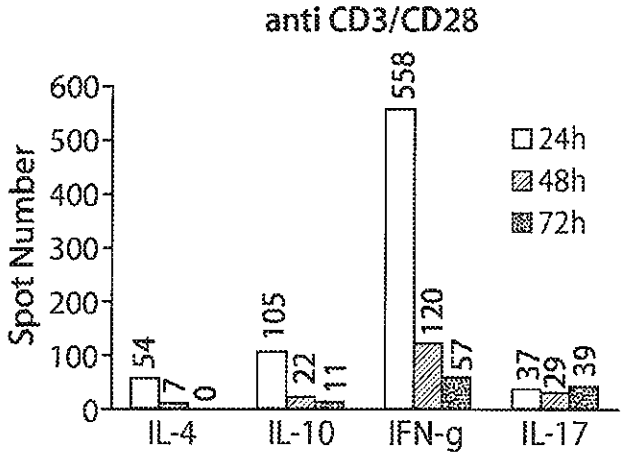


Fig. 5A

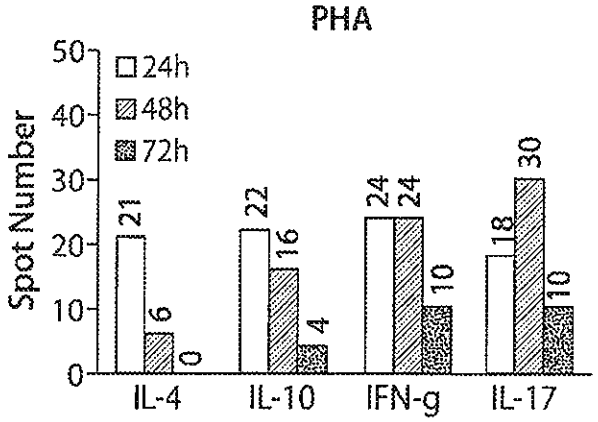


Fig. 5B

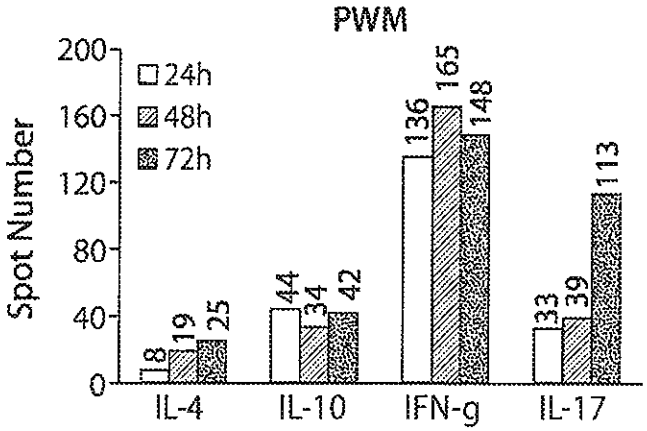


Fig. 5C

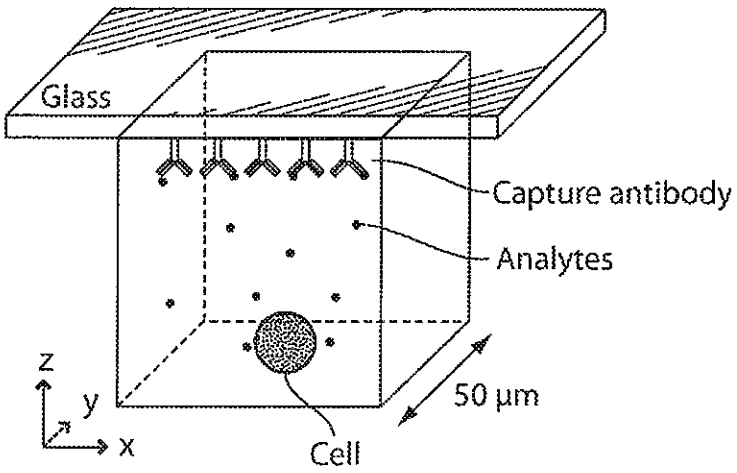

Glass
Capture antibody
Analytes
z
y
x
50 µm
Cell

Fig. 6A

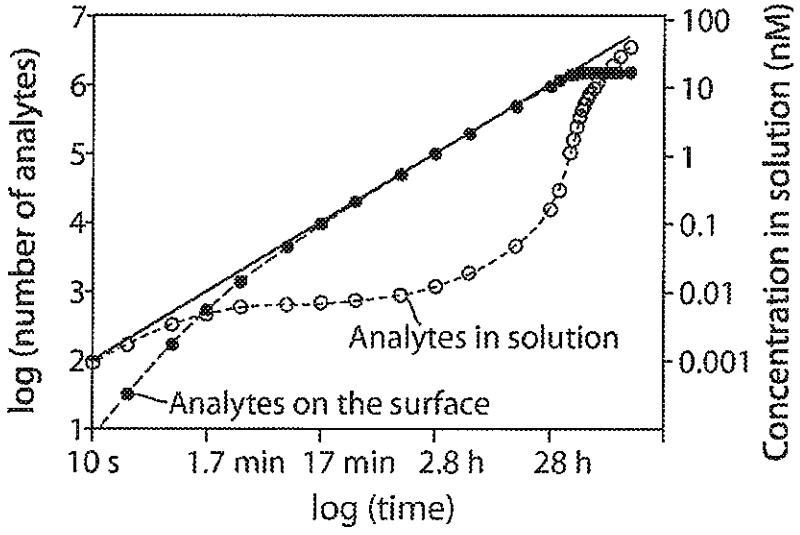

log (number of analytes)
7
6
5
4
3
2
1
100
10
1
0.1
0.01
0.001
Concentration in solution (nM)
Analytes in solution
Analytes on the surface
10 s
1.7 min
17 min
2.8 h
28 h
log (time)

Fig. 6B

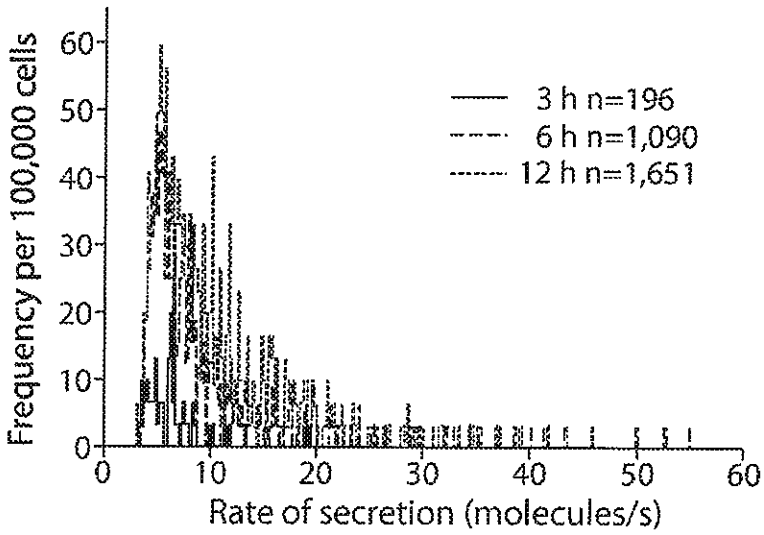

Frequency per 100,000 cells
60
50
40
30
20
10
0
0
10
20
30
40
50
60
Rate of secretion (molecules/s)
3 h n=196
6 h n=1,090
12 h n=1,651

Fig. 6C

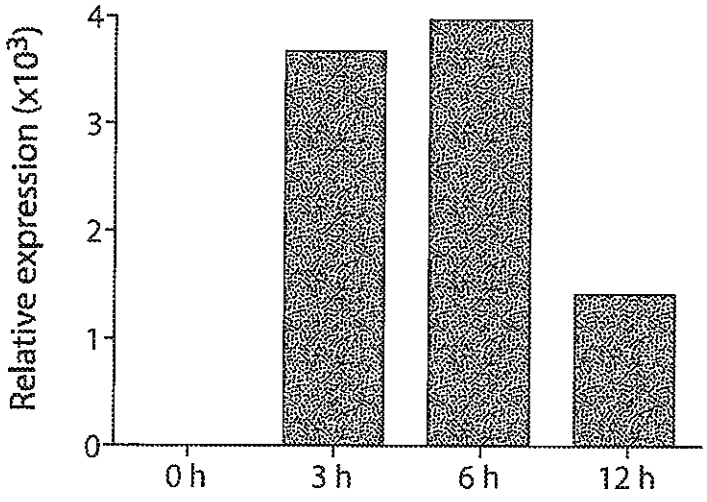

Relative expression (x10^3)
4
3
2
1
0
0 h
3 h
6 h
12 h

Fig. 6D

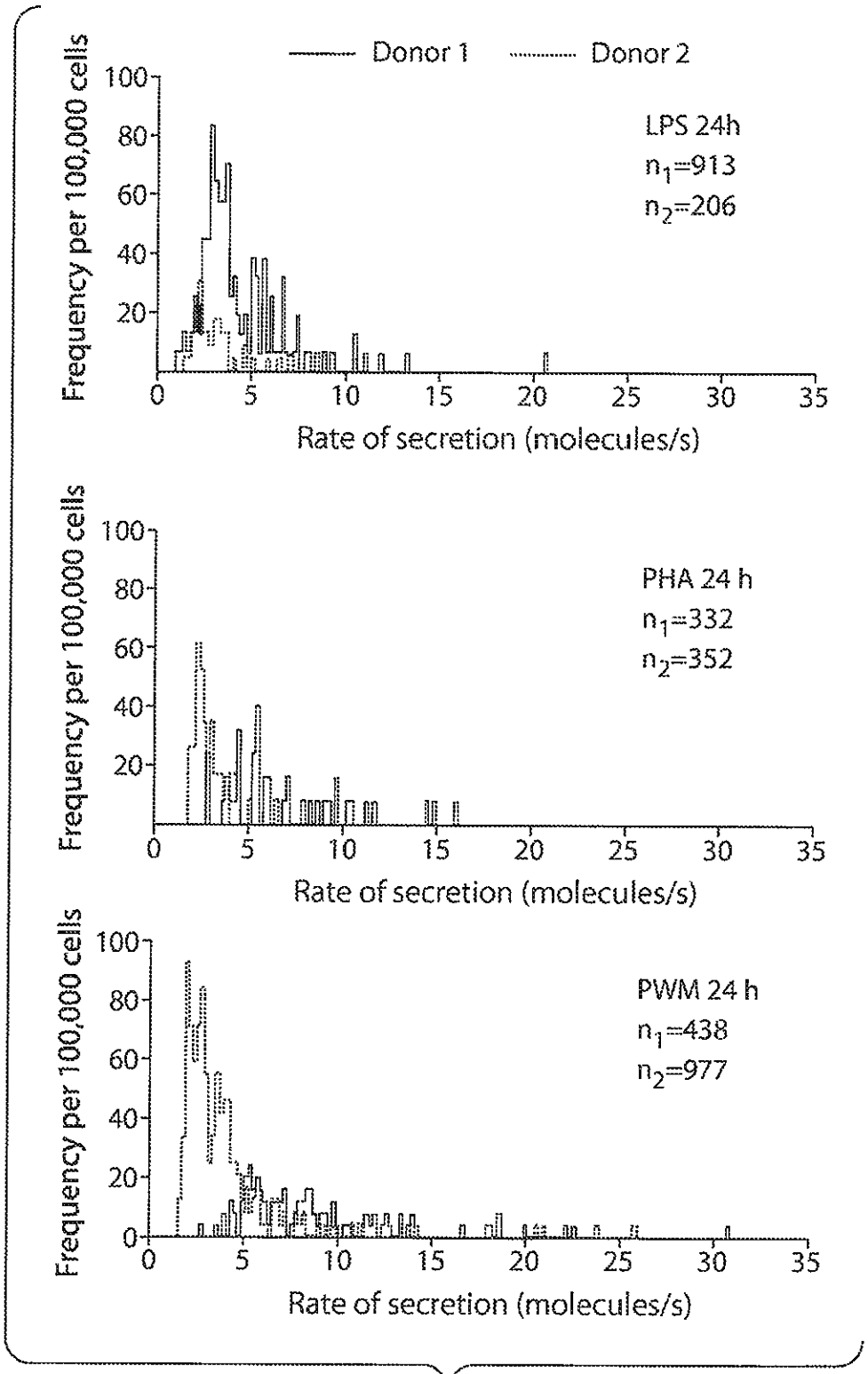

Donor 1
Donor 2
Frequency per 100,000 cells
LPS 24h
n_1=913
n_2=206
Rate of secretion (molecules/s)
PHA 24 h
n_1=332
n_2=352
PWM 24 h
n_1=438
n_2=977

Fig. 6E

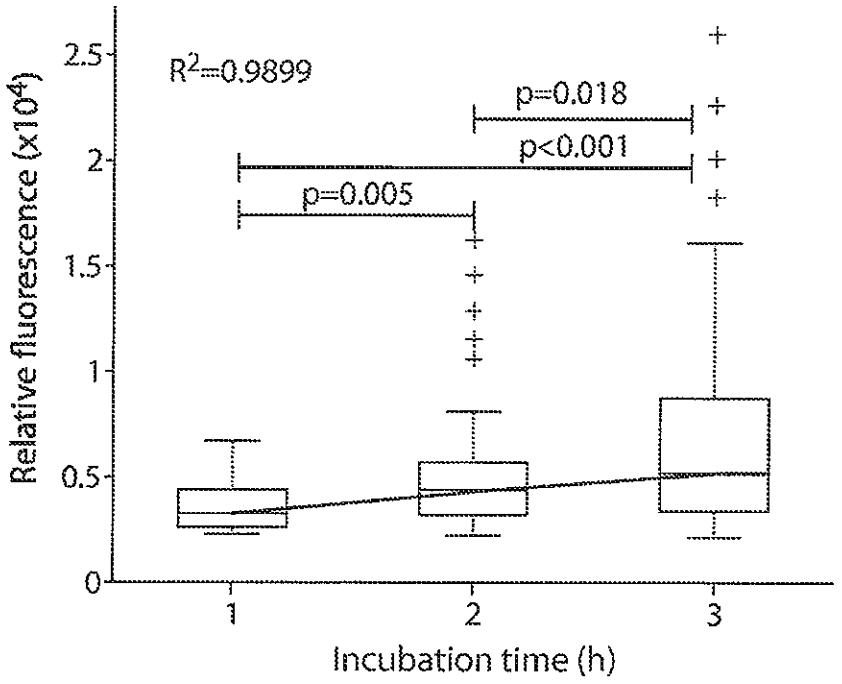

R^2=0.9899
p=0.018
p<0.001
p=0.005
Relative fluorescence (x10^4)
2.5
2
1.5
1
0.5
0
1
2
3
Incubation time (h)

Fig. 7A

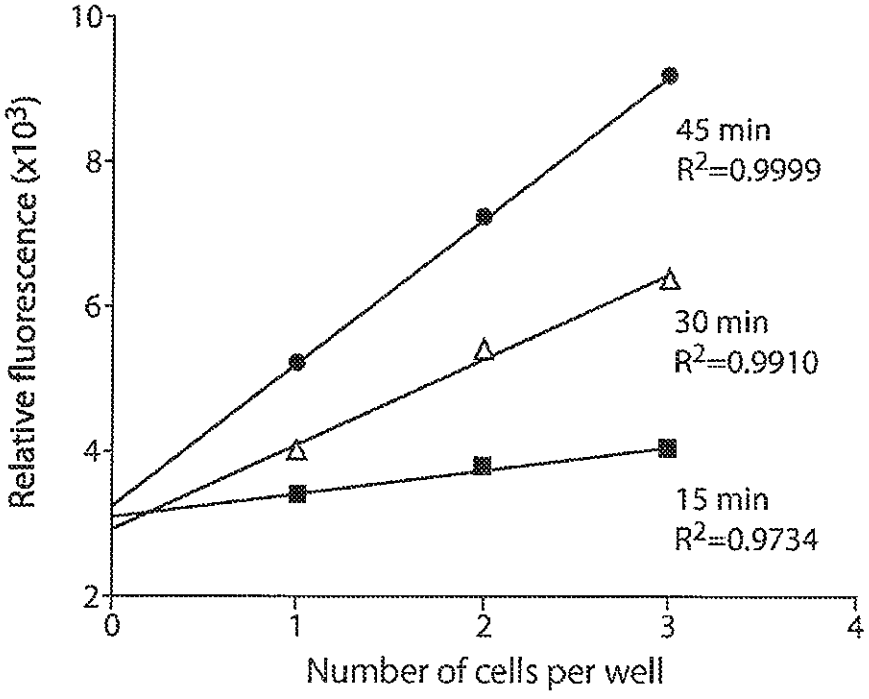

Relative fluorescence (x10^3)
10
8
6
4
2
0
1
2
3
4
Number of cells per well
45 min
R^2=0.9999
30 min
R^2=0.9910
15 min
R^2=0.9734

Fig. 7B

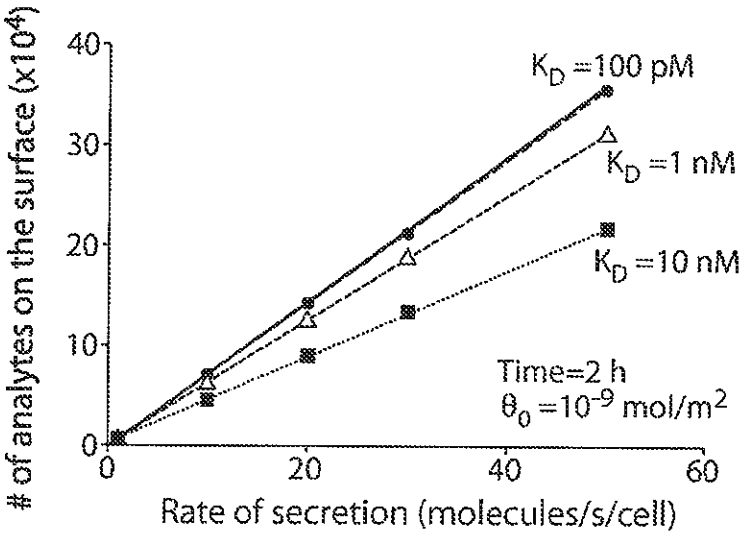


Fig. 8

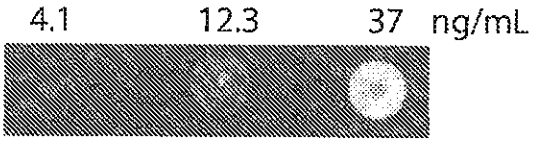


Fig. 9A

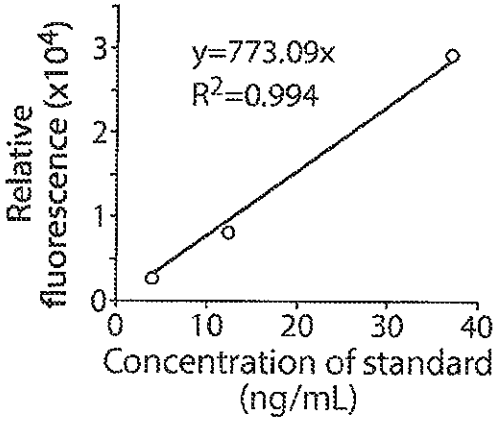


Fig. 9B

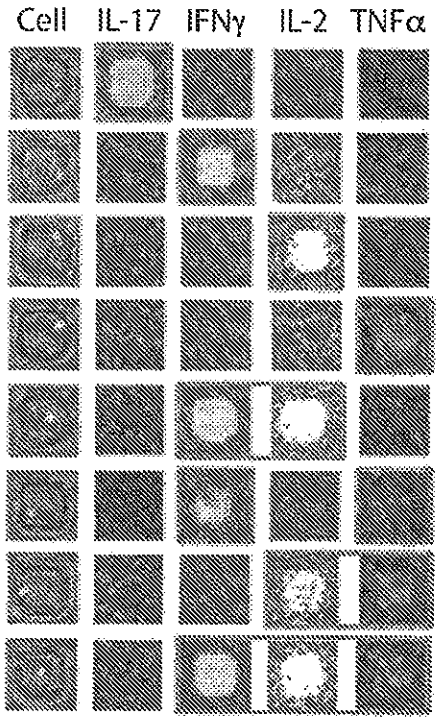


Fig. 10A

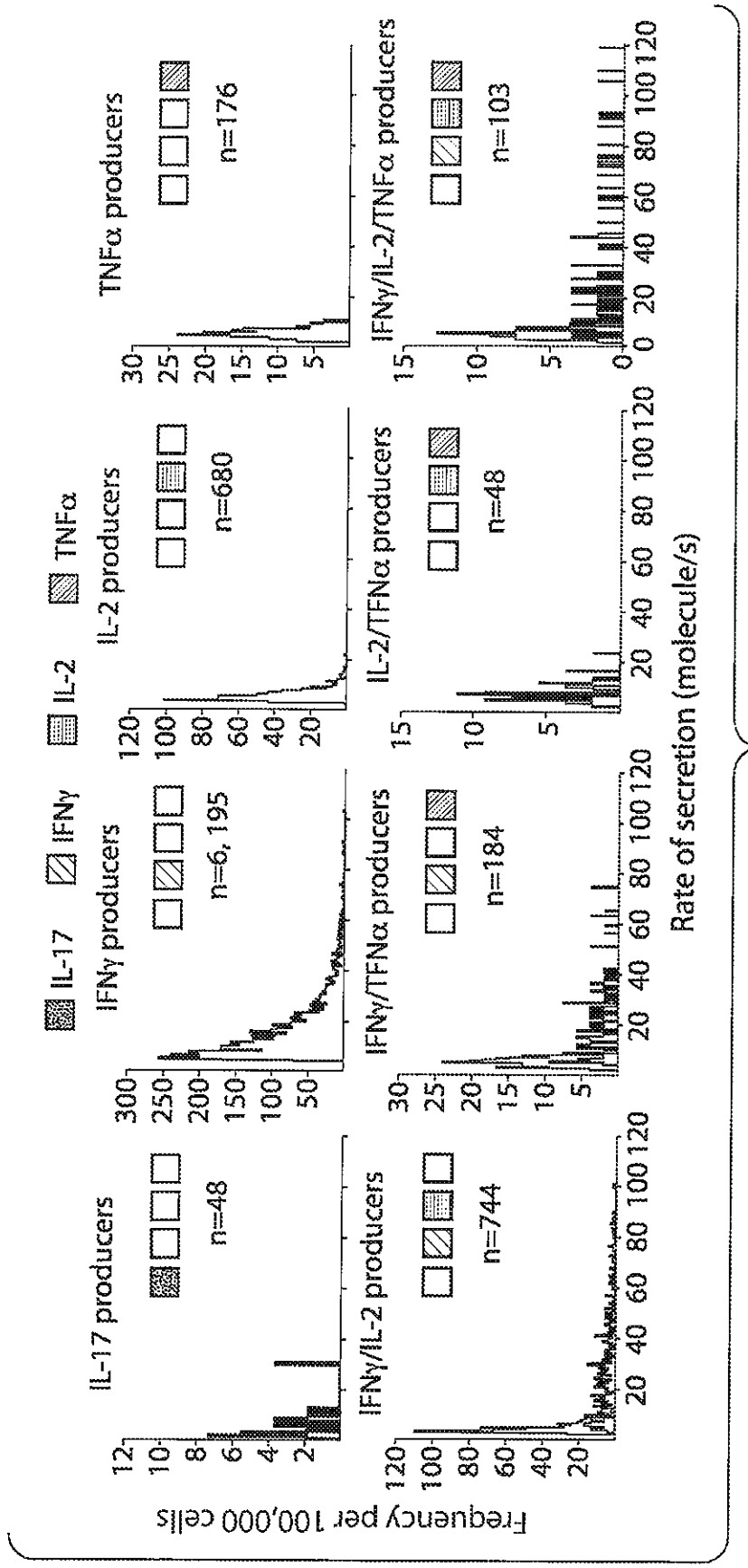

IL-17
IFNγ
IL-2
TNFα
IL-17 producers
n=48
IFNγ producers
n=6, 195
IL-2 producers
n=680
TNFα producers
n=176
IFNγ/IL-2 producers
n=744
IFNγ/TFNα producers
n=184
IL-2/TFNα producers
n=48
IFNγ/IL-2/TNFα producers
n=103
Frequency per 100,000 cells
Rate of secretion (molecule/s)

Fig. 10B

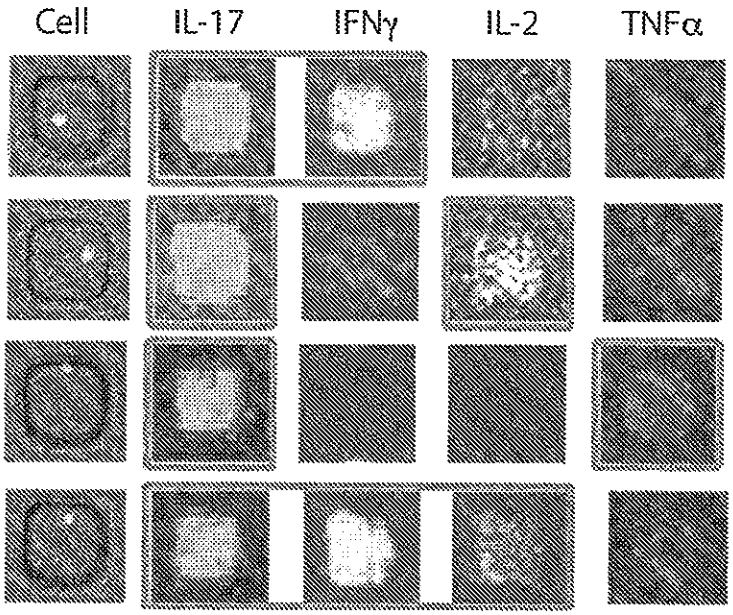


Fig. 11

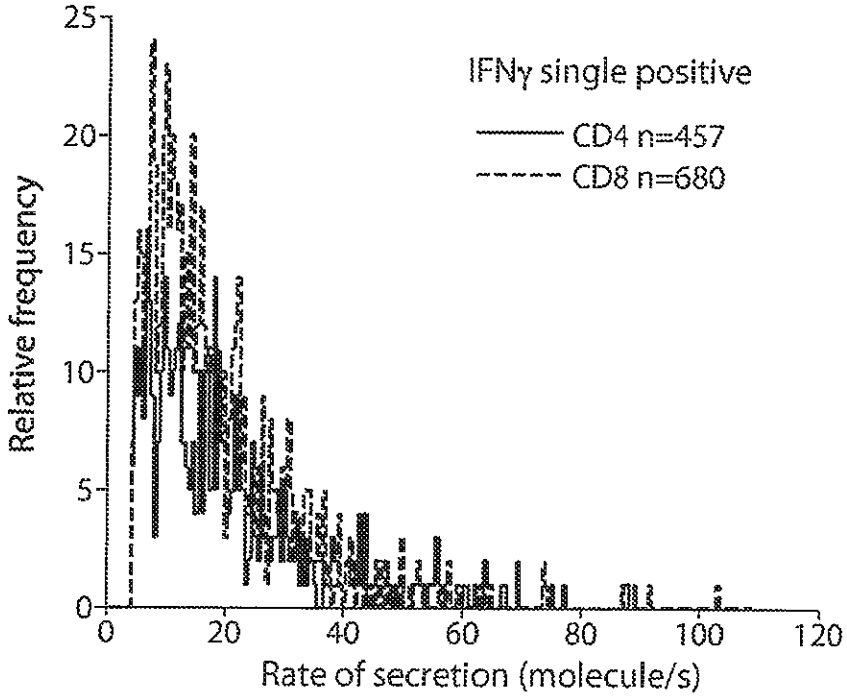


Fig. 12A

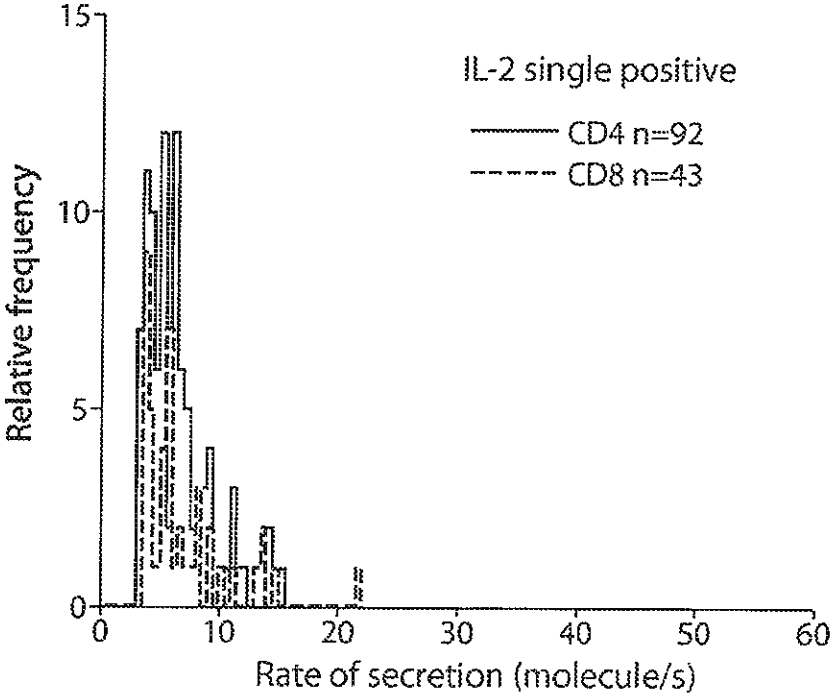


Fig. 12B

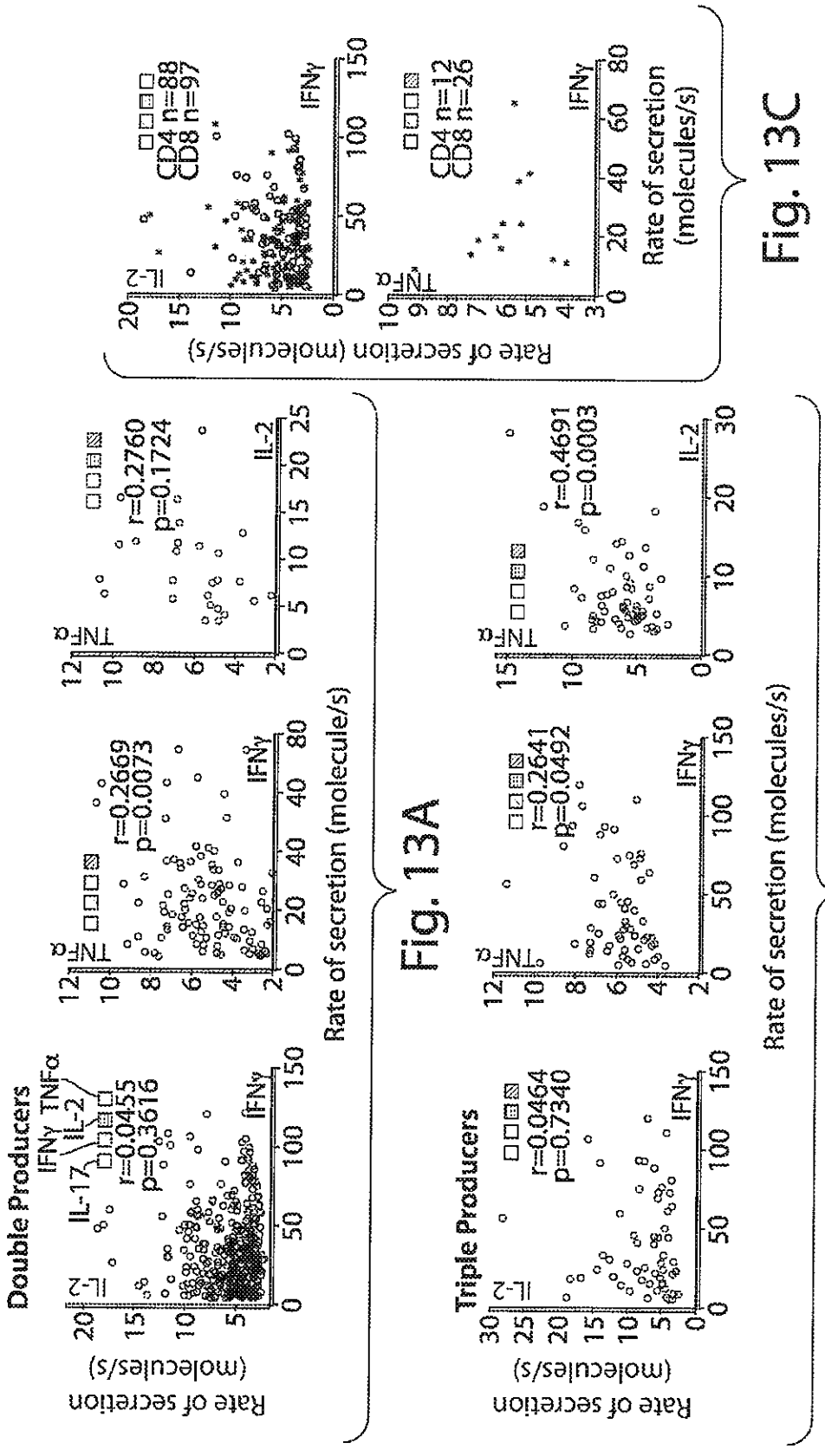


Fig. 13A

Fig. 13B

Fig. 13C

Figure 14.

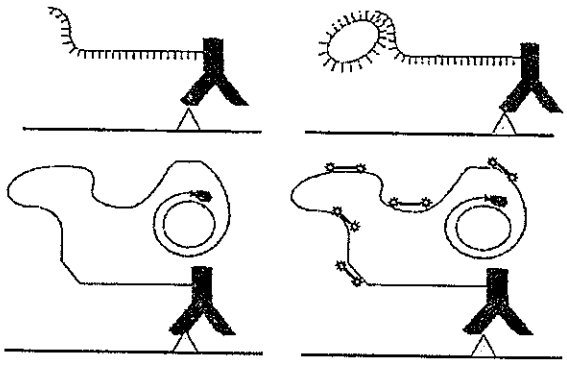


Figure 15.

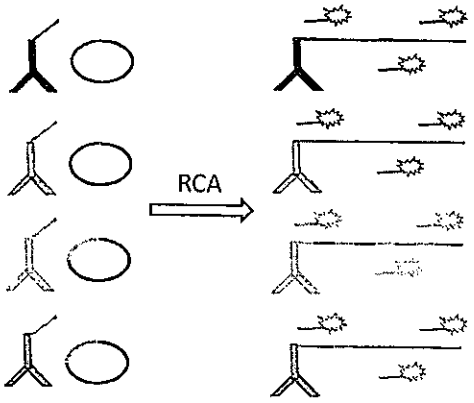
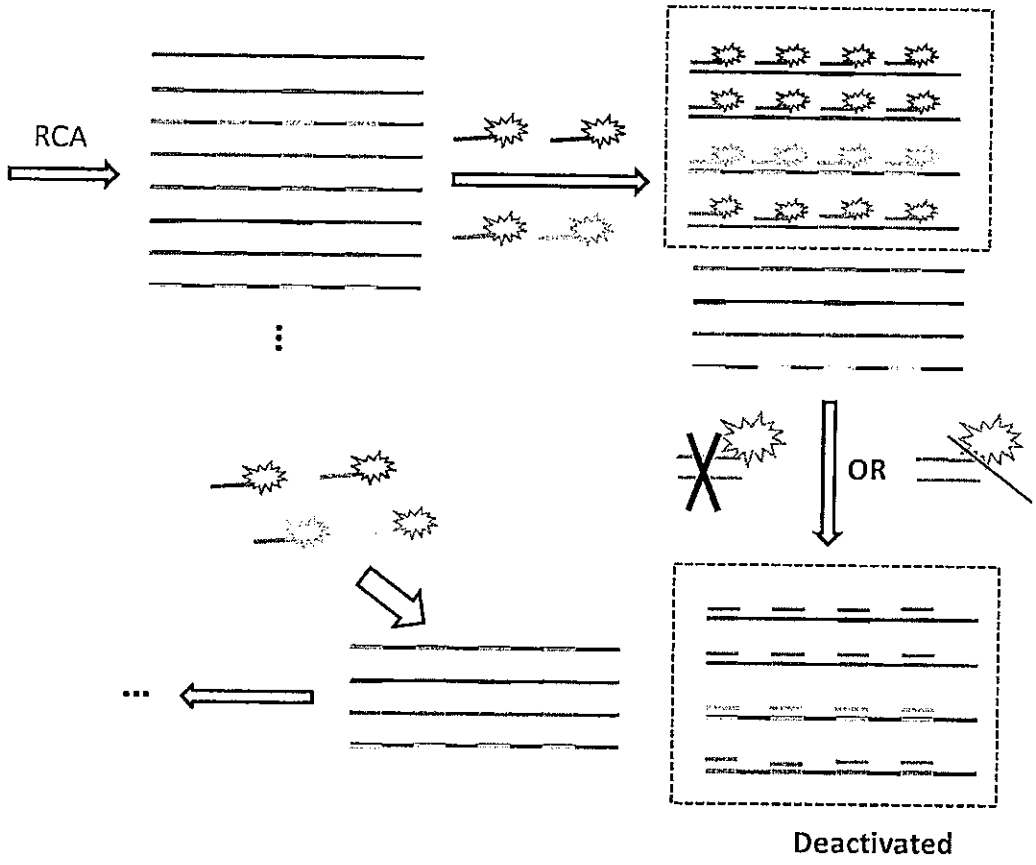
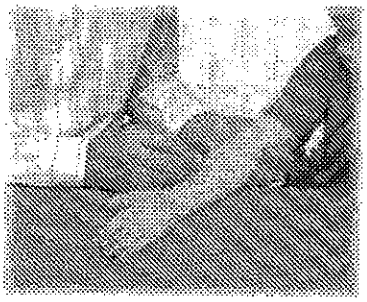


Figure 16.



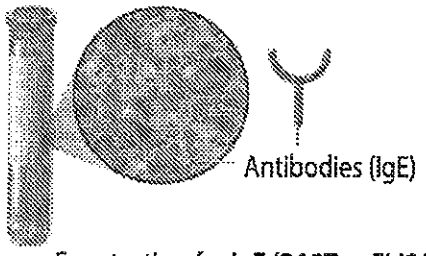
The Problem

Current Allergy Testing



Percutaneous (Skin Prick) Testing

The blood test measures the levels of allergy antibody, or IgE, produced when your blood is mixed with a series of allergens in a laboratory

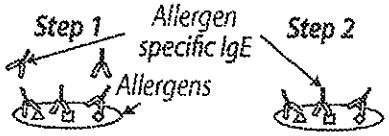


Sera testing for IgE (RAST or ELISA)

Skin testing
- Multiple pin pricks with panel of allergens
- Measures short-term and long-term immune responses
- *Qualitative* assessment of superficial inflammation
- *Traumatic experience* for infants and parents
- Often, *single data point* in child's history

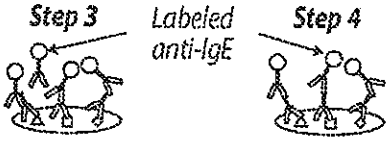
Immunosorbent assays
- Minimally invasive blood draw (5 mL sample)
- Measures serum titers of IgE antibodies
- *Complicated* by pan-specificity of antibodies and elevated levels from other conditions (HIV, skin disease, parasites)
- *Not predictive*—single parameter
- Correlation over time challenging

Food Challenge
- Double blind test with various foods (some that are suspected of inducing a reaction) are given to patient
- Cannot be used on patients with history of severe (anaphylactic) reactions



The serum sample is incubated with an allergen disc. During this step,IgE molecules with specificities for the various allergenic components bind to the allergen disc.

Proteins non-specifically bound are washed away with a buffer containing detergent. Only specifically bound IgE remains on the disc.



The disc is incubated with labeled anti-human IgE antibody, which binds specifically to the IgE on the disc. The amount of labeled antibody bound is directly proportional to the amount of IgE present on the disc.

Unbound antibody is washed away with buffer containing detergent.



Specific IgE is quantitated either through the reaction of labeled antibody with an enzyme substrate (EIA), or by counting radioactivity (RAST).

Fig. 17-1

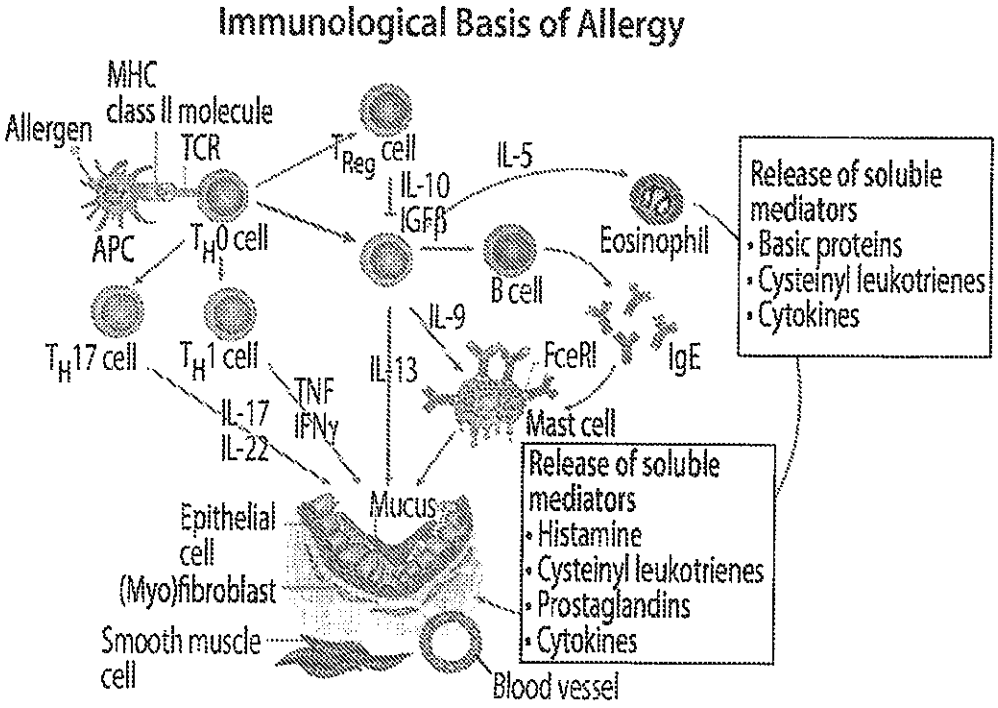
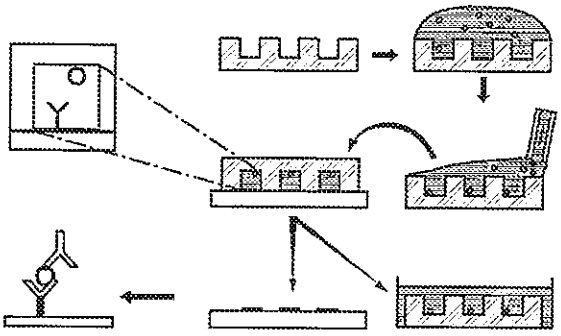

Immunological Basis of Allergy
MHC class II molecule
Allergen
TCR
APC
T_H0 cell
T_{Reg} cell
IL-10
TGFβ
IL-5
Eosinophil
Release of soluble mediators
• Basic proteins
• Cysteinyl leukotrienes
• Cytokines
B cell
IL-9
T_H17 cell
T_H1 cell
IL-13
FcεRI
IgE
TNF
IFNγ
IL-17
IL-22
Mast cell
Release of soluble mediators
• Histamine
• Cysteinyl leukotrienes
• Prostaglandins
• Cytokines
Mucus
Epithelial cell
(Myo)fibroblast
Smooth muscle cell
Blood vessel

Fig. 17-2

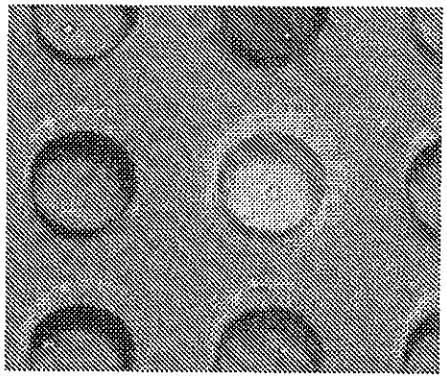
Implementation
Microengraving



Schematic of the Microengraving Method

How it Works

- Cells, suspended in media, are deposited onto a large array of microwells molded on a poly (dimethylsiloxane) slab, and allowed to settle from suspension in the microwells at a density of ~1 cell.well.
- The loaded microwells are then inverted onto a glass slide coated with a specific capture reagent (e.g., anti-cytokine).
- After an incubation period, the microwells are removed and applied to a second glass slide coated with a different capture reagent (e.g., anti-IgG and anti-IgM).
- The resulting microarrays are interrogated with fluorescently labeled reagents for detection and laser-based fluorescence scanners.
- After printing, the cells in the wells can be stained *in situ* for subsequent imaging by immunofluorescence.



Nature Biotechnology, 24, 703-707 (2006)
Clinical Immunology, 129, 10-18 (2008)
Proc. Natl. Acad. Sci. USA, 105, 17902-17907 (2008)
J. Immunol. Methods, in press (2008)

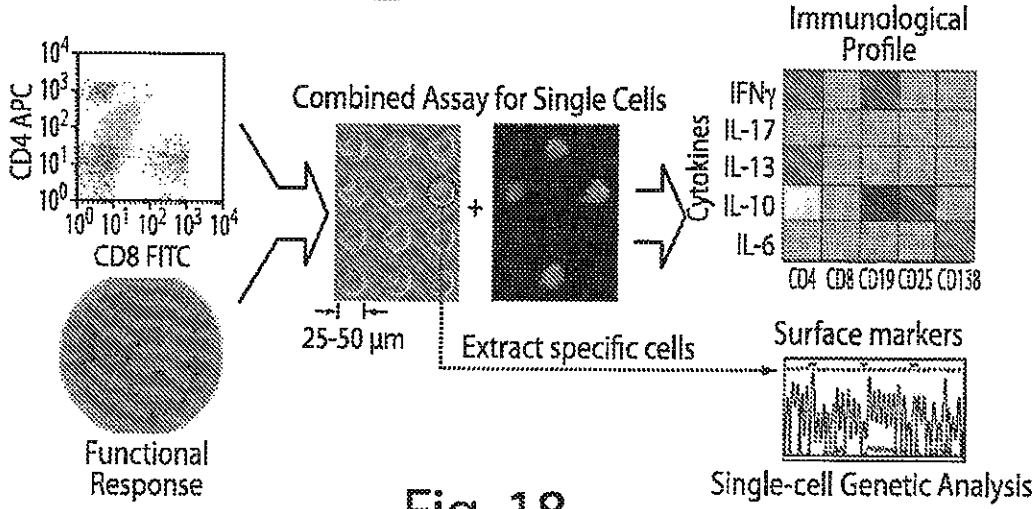
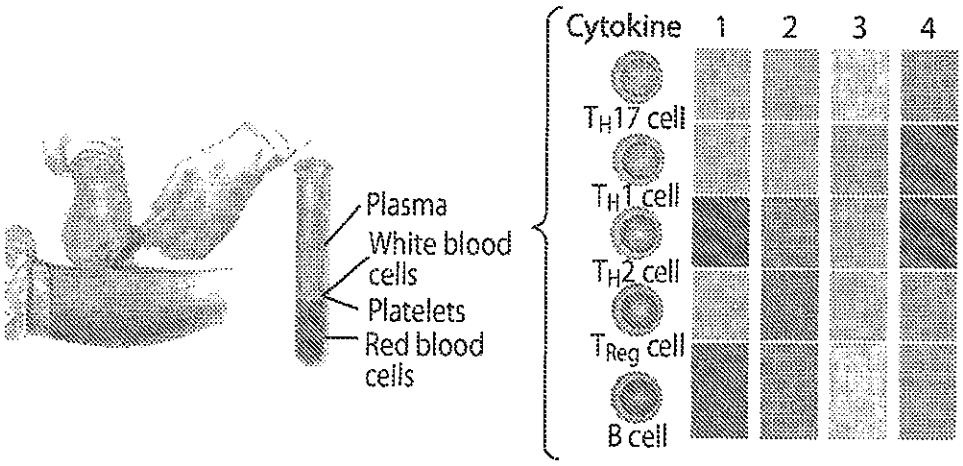


Fig. 18

21/23

The Solution

Profiling the Allergic Response



Elucidating the Unique Immunological Profile of an Allergic Individual

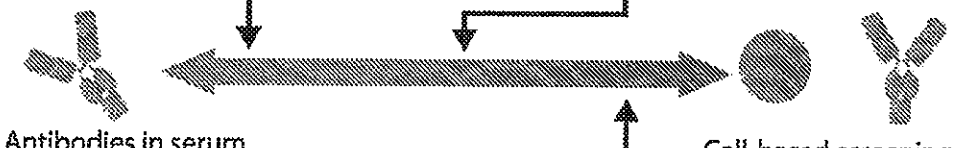
- Whole blood from a patient is drawn, and cells of interest are isolated.
- Cells are stimulated with allergens and are used for microengraving.
- The resulting cellular microarray is read as an "allergy-array" which provides specific data concerning reactivity to the allergen(s) of interest.

Advantages of this Diagnostic:
- Small blood volumes are needed for a comprehensive allergic profile, so pediatric/infant allergy testing is possible.
- Diagnostics are performed over time to assess a patients reactivity over time, as well as potentially measure the efficacy of immunotherapy.
- Data from the "allergy-array" are used to predict cross-reactivity to related allergens.

Specific IgE capture
(Hitachi, Alerchek, Immulite)
- Restricted number of allergens
- Not scalable ($2 / allergen)

ImmunoCAP
(Phadia, Quest Diagnostic)
- High sensitivity
- Small library (< 20 allergens)
- Large volumes (5 mL sample)
- Not definitive - follow-up necessary
- No data on cells

ELISPOT assays
(AID, MAbTech)
- Data on ab-producing cells,
- Tests large allergen library
- No data on types of ab-producing cells
- Low sensitivity and throughput
- ~250,000 cells per allergen



Antibodies in serum

Cell-based screening

Improved Allergy Diagnosis
- Combined assay for cells and IgE/IgG_4
- *High throughput + high content*
- Digital sensitivity
- Low volumes, low costs
- *~10,000 cells per allergen*

Fig. 19

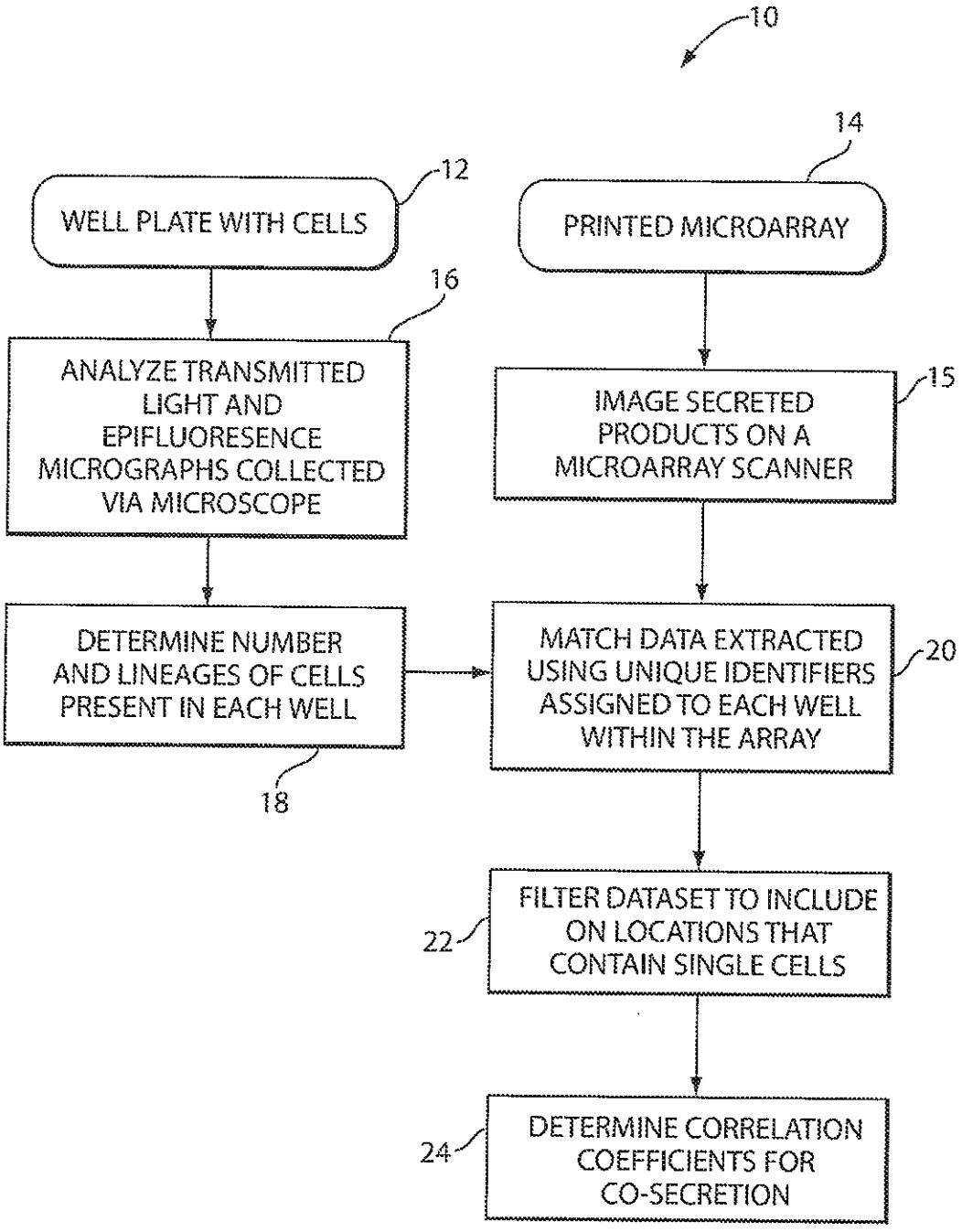

10
14
12
WELL PLATE WITH CELLS
PRINTED MICROARRAY
16
ANALYZE TRANSMITTED LIGHT AND EPIFLUORESENCE MICROGRAPHS COLLECTED VIA MICROSCOPE
15
IMAGE SECRETED PRODUCTS ON A MICROARRAY SCANNER
DETERMINE NUMBER AND LINEAGES OF CELLS PRESENT IN EACH WELL
MATCH DATA EXTRACTED USING UNIQUE IDENTIFIERS ASSIGNED TO EACH WELL WITHIN THE ARRAY
20
18
FILTER DATASET TO INCLUDE ON LOCATIONS THAT CONTAIN SINGLE CELLS
22
DETERMINE CORRELATION COEFFICIENTS FOR CO-SECRETION
24

Fig. 20

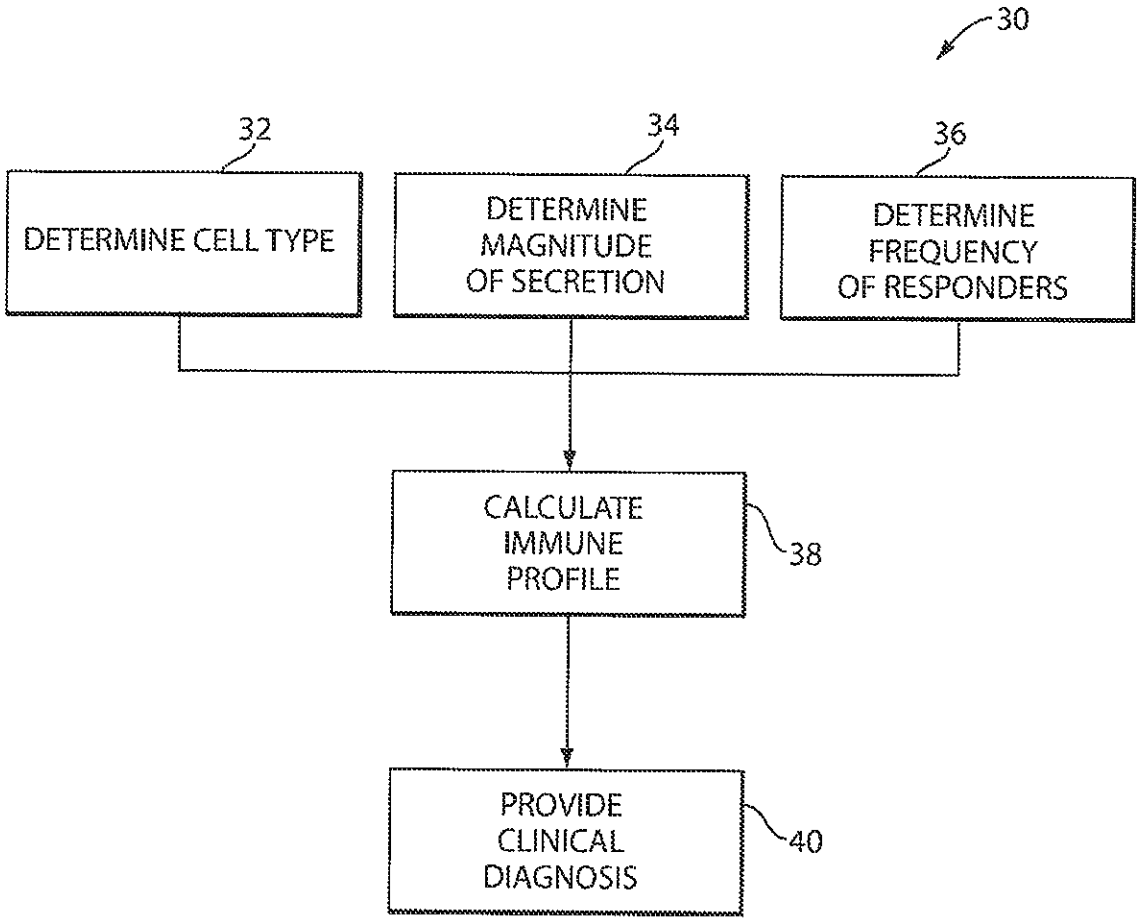

30
32
34
36
DETERMINE CELL TYPE
DETERMINE MAGNITUDE OF SECRETION
DETERMINE FREQUENCY OF RESPONDERS
CALCULATE IMMUNE PROFILE
38
PROVIDE CLINICAL DIAGNOSIS
40

Fig. 21