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# Advanced in situ microscopy for on-line monitoring of animal cell cultures

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Mannheim University of Applied Sciences

Lille University

InVivo BioTech Services GmbH

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Université Lille1 Sciences et Technologies

IN VIVO InVivo BioTech Services GmbH

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In situ microscopy:

Challenge / Hardware

Applications

- Bench top fermenter
- Production environment
- Morphology
- Viability

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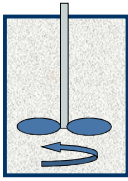
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Monitoring of Animal Cell bioreactors:

- cell density (and viability)
- mostly off line

PAT

Visual data



How can we make cells visible while they are freely "drifting by" in a stirred tank reactor (in situ, at best in real time)?

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ISM design solutions:

- 1) Mechanical sampling device inside the reactor and acquisition of images from « isolated » samples.
- 2) Sampling by optical depth of focus and direct acquisition of images from the moving suspension. (Our solution)

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Main Problem: Blur due to Motion

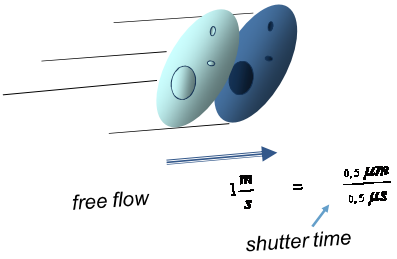


shutter-time: 30ms

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Motion and shutter time



free flow  $\frac{1}{30} \text{ s} = \frac{0.5 \text{ μm}}{0.5 \text{ μs}}$

shutter time

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Even more difficult due to microscopic magnification: Less intensity because the light is „diluted“.

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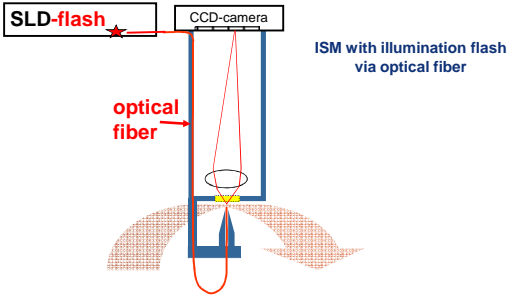
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SLD-flash

CCD-camera

optical fiber

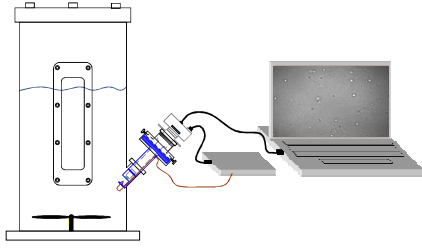
ISM with illumination flash via optical fiber



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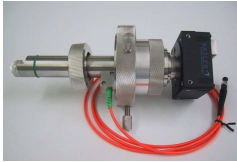
ISM in standard 25 mm port



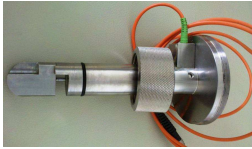
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### Inner and outer tube, CCD camera



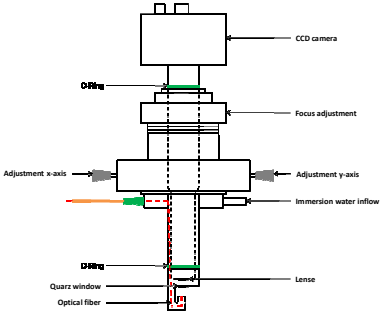
Outer tube – sterilised in situ



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### Inner and outer tube, CCD camera



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- Cell concentration monitoring

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### Bench top single wall bioreactor

- Autoclavable single glass jacket reactor, stainless steel bottom and top (Bioflo 3000, New Brunswick, USA)
- Working volume 4 L
- Hybridoma (BalB/c murine lymphoid cell -DGB02- fused with a murine myeloma -C7021-1; kindly supplied by Diagast SA, Loos, France)
- DMEM + 10% heat-inactivated FCS
- Batch
- Manual counting: Mallassez chamber

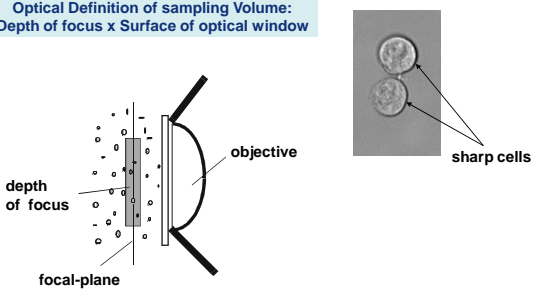


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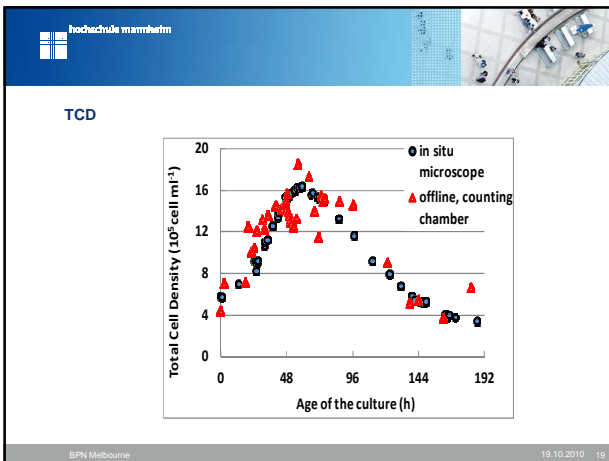
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### Optical Definition of sampling Volume:

Depth of focus x Surface of optical window



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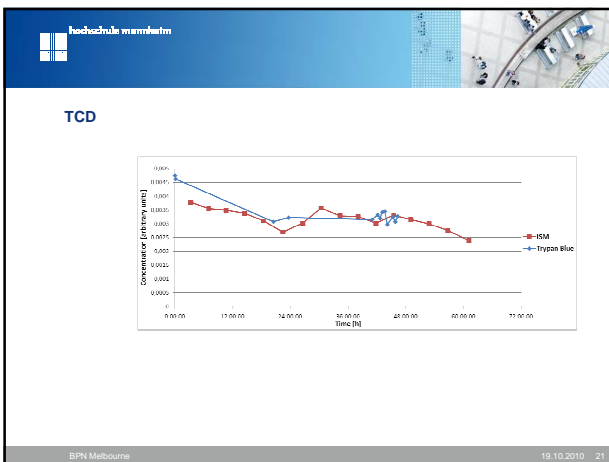


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**Bench top double jacket glass bioreactor**

- Autoclavable custom built autoclavable double glass jacket bench top reactor (HS Mannheim, Germany)
- working volume 0.6 L
- Jurkat (DSMZ ACC 282)
- RPMI 1640 + 10% FBS
- Batch under growth limiting conditions
- Manual counting: Neubauer chamber

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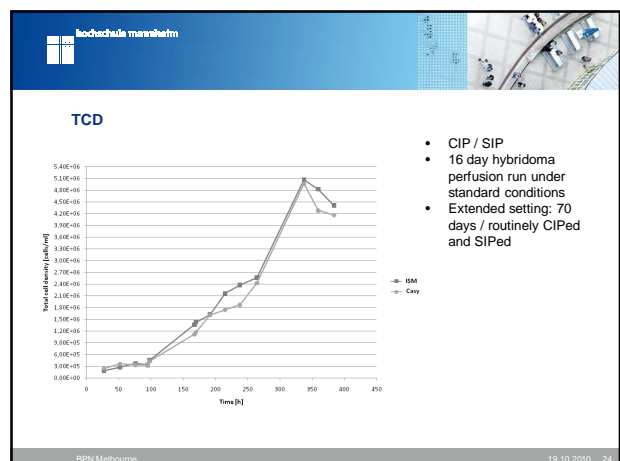
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**Production environment**

- Biostat C30 (Sartorius BBI Systems GmbH, Melsungen, Germany)
- Proprietary hybridoma cell (InVivo BioTech Services, Hennigsdorf, Germany)
- Serum free ISF-1 (InVivo BioTech Services; Biochrom AG, Berlin, Germany)
- CASY 1TTC (Schärfe Systems GmbH, Reutlingen, Germany)

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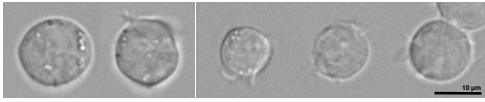
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- On-line cell portraits

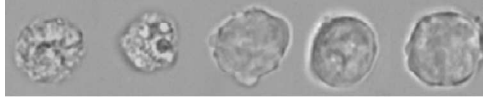
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**Jurkat**



**Hybridoma**



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**Applications**

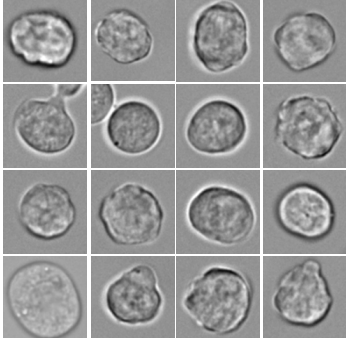
- Bench top fermenter
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**In „real time“:**

**Cell-galleries from within the bioreactor in 10 seconds.**



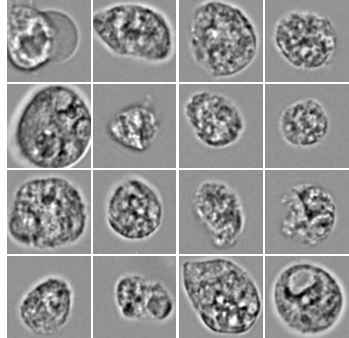
**Hybridoma-culture with high viability (appr. 96%)**

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**In „real time“:**

**Cell-galleries from within the bioreactor in 10 seconds.**



**Hybridoma-culture with very low viability (appr. 20%)**

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Individual ISM cell portraits - and the dispersion of their „grey values“

-from a culture with 85% viability

-from a culture with 25% viability

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• Real time culture monitoring

- ISM
- User Interface
- Morphology data
- TCD estimation
- Viability estimation

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ISM user-interface - real time documentation of a 11 day Jurkat-culture

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Viability (ISM in real time, FACS, ViCell)

- Custom built autoclavable steel bench top reactor (HS Mannheim, Germany)
- working volume 0.6 L
- Jurkat (DSMZ ACC 282)
- RPMI 1640 + 10% FBS
- Batch
- ViCell (Beckman Coulter, Krefeld, Germany)
- Flow cytometer (Partec, Münster, Germany)
- EtOH at 43 h

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Viability (ISM in real time, FACS, ViCell)

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ISM at present:

- Non invasive data acquisition
- No moving or electrical parts inside bioreactor
- Unprecedented frame rate and image quality directly out of suspension
- CIP / SIP / autoclaved bench top reactor
- Working volumes 600 mL – 30 L tested
- Process times up to 16 days, installation times up to 70 days tested
- Portrait galleries / morphology monitoring
- Process monitoring: Cell density and viability in real time

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The slide features a blue header with the logo and name of Hochschule Mannheim. Below this, a list of names is grouped by a vertical bracket on the right. To the right of the names are logos for Hochschule Mannheim, a crest, and SGU (Swiss German University). Further down, logos for Université Lille1 (Sciences et Technologies) and INVIVO (In Vivo Biotech Services GmbH) are shown. At the bottom, the UNSW (University of New South Wales) logo is displayed. The footer contains the text 'BPN Melbourne' and '19.10.2010 37'.

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SGU  
SWISS GERMAN UNIVERSITY

Université  
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INVIVO  
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