

**Közeli infravörös  
spektroszkópia és mikroszkópia**

**Gergely Szilveszter**  
Budapesti Műszaki és Gazdaságtudományi Egyetem  
Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék



Optikai spektroszkópia  
2014. október 29.

black box  
▼  
grey box

NJR  
előadás  
tábla




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**Newton kísérletei I. – a fehéren túl**




Ma  
kísérlet  
más is meg-  
csinálta, de  
é is is ita.



(1704) 2




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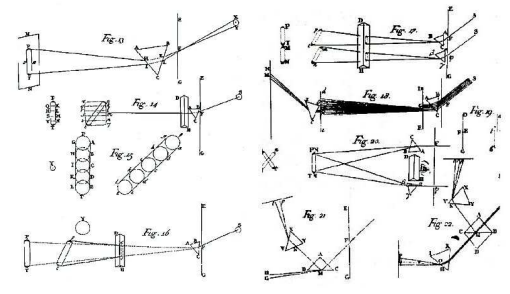
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**Newton kísérletei II. – a fehéren túl**



(1704) 3




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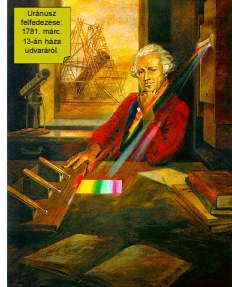
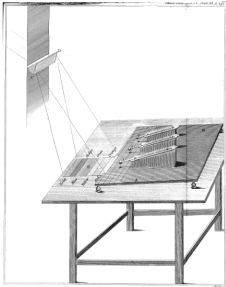
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## Herschel kísérletei – a vörös alatt (no meg az Uránusz és a marslakók...)



1800. február 11.

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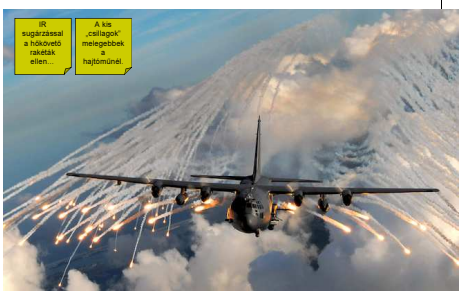
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## IR – hő – energia (egy régi trükk mai kivitelben)



IR sugárzással a hővezető rakéták ellen...  
A kis „csillagok” melegekkel a hajlékony...

An AC-130J gunship fires flares to emit masses of infrared and confuse heat seeking missiles.  
<http://www.wired.com/dangerroom/2012/10/infrared-obscure/>

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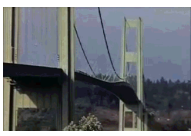
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## Rezgések és hullámok (USA vs. Magyarország)



(Tacoma Narrows Bridge, "Gallopín" Gertie", 1940. november 7.)



BME Schönbusz  
Gaz vs. 4-s  
villamosok

(egy budapesti villamos, "sárga szöcske", 2011)

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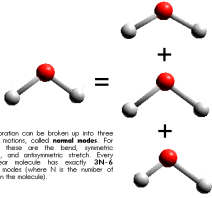
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## A molekulák rezgései

### Theory of Infrared Spectroscopy



- **deformációs rezgés**  
változás a kötési szögben
- **szimm. végértékrezgés**  
változás a kötéshosszban
- **aszimm. végértékrezgés**  
változás a kötéshosszban



A spektrum a molekuláris állapotváltozások összessége:  
kémiai és fizikai „ujjlenyomat”.

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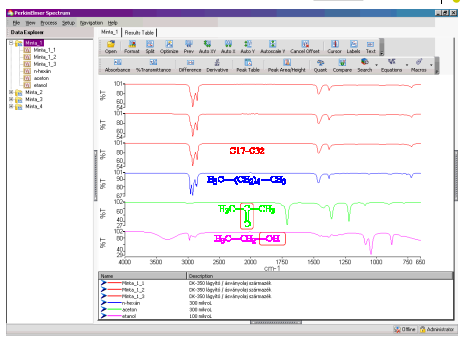
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## Egy gyors példa a (M)IR-re

NIR-IR  
1-100



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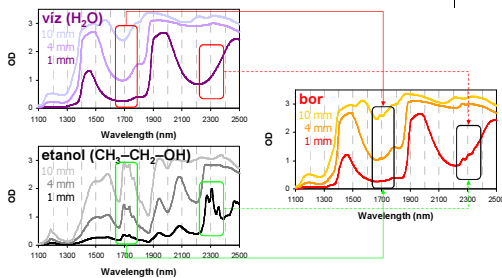
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## És egy másik gyors példa NIR-re



Gergely S. Farkas, K. Forgács, A. Balogh. Quantitative and qualitative differentiations of alcoholic beverages by near infrared spectroscopy  
in Near Infrared Spectroscopy: Proceedings of the 11th International Conference  
Ed by Davies A.M.G., Garrod-Vince A. NIR Publications, Chichester, pp. 569-572 (2004)

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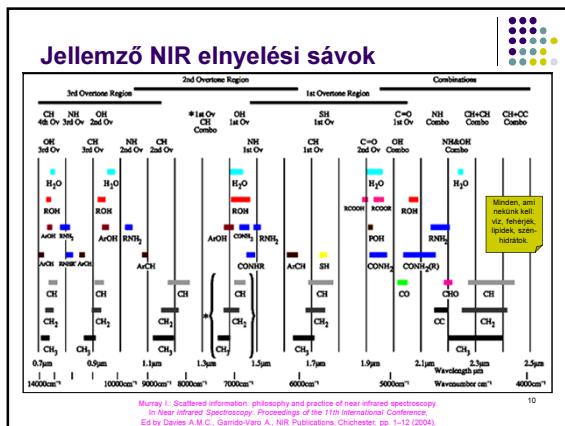
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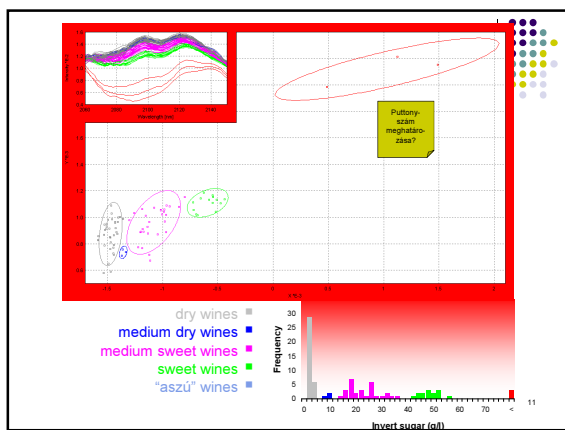
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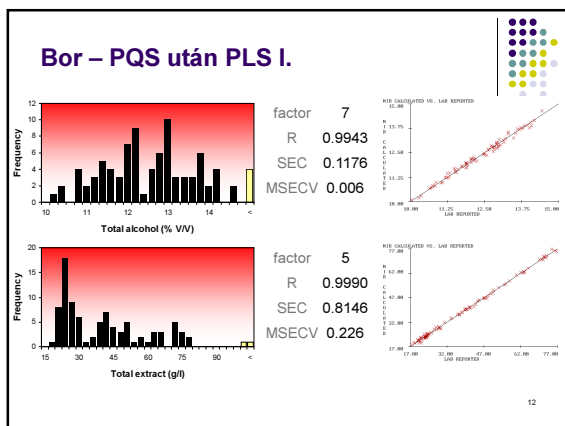
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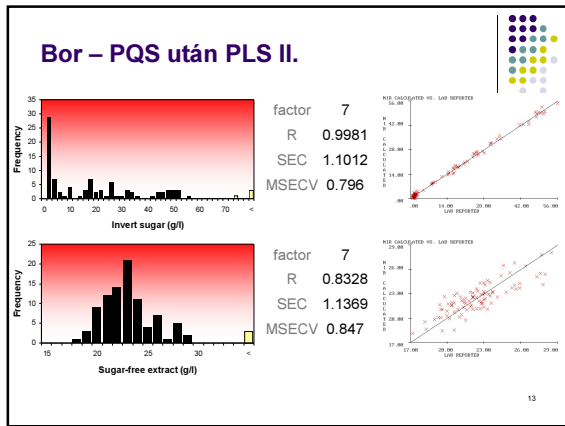
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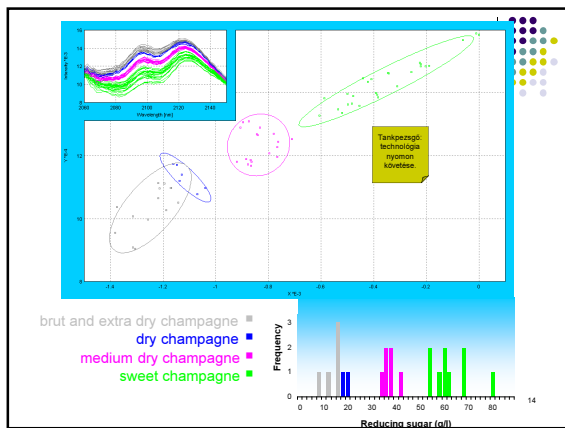
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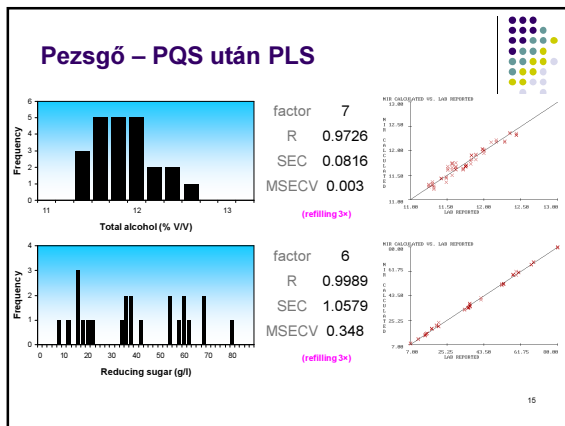
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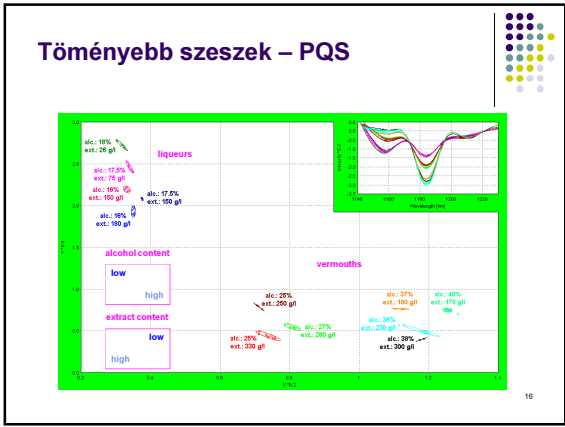
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### Borban az igazság... ... ez nem a reklám helye

QIS (Törleyes, Törley (par))

konferencia poszter (NIR-2003 • 2003. ápr. 6-11.)

Törley-díj (diplomaátadás • 2003. júl. 18.)

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### Borban az igazság... ... ez nem a reklám helye

Vitány (gazda bolt) 2008.06.30

FORRADALOM A BORVIZSGÁLATBAN!

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## Borban az igazság... ... de hogyan ez ide?

### Abstract Near infrared spectroscopy: challenges and opportunities for wine labelling spectroscopy

Olga Czelenka-Mezner,  
Communicating author (olga@iit.it)

CSI, University of Turin, Centre for Technology and Biotechnology in Food, Via Nizza, 100-105  
I-10125 Turin, Italy

**Introduction**  
Near infrared spectroscopy (NIRS) is a powerful analytical tool for wine quality control. It is a non-destructive, rapid, and cost-effective technique that can be used for wine authentication and quality control. In this paper, we present a review of the state-of-the-art in NIRS for wine authentication and quality control. We also discuss the challenges and opportunities for wine labelling spectroscopy.

**Materials and Methods**  
The main purpose of this review is to provide an overview of the state-of-the-art in NIRS for wine authentication and quality control. We focus on the challenges and opportunities for wine labelling spectroscopy. We also discuss the state-of-the-art in NIRS for wine authentication and quality control.

**Results and Discussion**  
NIRS is a powerful analytical tool for wine quality control. It is a non-destructive, rapid, and cost-effective technique that can be used for wine authentication and quality control. In this paper, we present a review of the state-of-the-art in NIRS for wine authentication and quality control. We also discuss the challenges and opportunities for wine labelling spectroscopy.

**Conclusion**  
NIRS is a powerful analytical tool for wine quality control. It is a non-destructive, rapid, and cost-effective technique that can be used for wine authentication and quality control. In this paper, we present a review of the state-of-the-art in NIRS for wine authentication and quality control. We also discuss the challenges and opportunities for wine labelling spectroscopy.

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### Near infrared monitoring of wine fermentation

David Guzzoni<sup>1</sup>, Wini Carter and Robert Outh

<sup>1</sup>The Hirst Institute for Food and Food Packaging, The University of Queensland, St. Louis, Australia  
Corresponding author: david.guzzoni@uq.edu.au

**Introduction**  
The new protein analysis technology (NIRS) has the ability to provide accurate and precise measurements for protein content in food products. This is a significant advantage over traditional methods such as the Kjeldahl method. NIRS is a non-destructive, rapid, and cost-effective technique that can be used for protein authentication and quality control. In this paper, we present a review of the state-of-the-art in NIRS for protein authentication and quality control.

**Materials and Methods**  
The main purpose of this review is to provide an overview of the state-of-the-art in NIRS for protein authentication and quality control. We focus on the challenges and opportunities for protein labelling spectroscopy. We also discuss the state-of-the-art in NIRS for protein authentication and quality control.

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FOSS Wine segment brochure  
PIN 1028997, Issue 1 GB, Jan. 2012

## Borban az igazság... ... léptéknövelés

A hordóval  
tartályokkal  
mért is  
beszélve



FOSS Wine segment brochure  
PIN 1028997, Issue 1 GB, Jan. 2012

## Borban az igazság... ... technológia, mintavétel, analitika



FOSS Wine segment brochure  
BWA 10082017, issue 1.08, Jan. 2012

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## PAT (Process Analytical Technology) = a folyamat kézben tartása

A PAT eszközei:

- hagyományos in-line érzékelők (pH, pO<sub>2</sub>, pCO<sub>2</sub>, T szondák)
- a folyamat monitorozó eszközeinek kibővítése egyéb real-time technikákkal (in-/on-line NIR/Raman/IR, in-/on-line HPLC, stb.)
- Korszerű és gyors at-line mérések
- Korszerű adatgyűjtő rendszer (SCADA)
- Kemometriai (MVA) szoftverek (PCA, PLS stb.)

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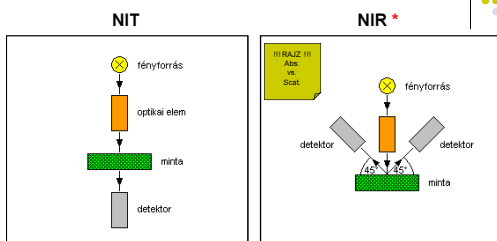
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## Hogyan mérjük? – NIT és NIR



~ 800-1100 nm  
kisebb hullámhossz  
nagyobb energia

~ 1100-2500 nm  
nagyobb hullámhossz  
kisebb energia

(\* ≠ ... UV, VIS, NIR, (M)IR, FIR, ...)

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### Hogyan mérjük? – diszperzió

Beárolt standardok, ha elérhető.

Amplitude

Thermocouple

Reference

Sample

Source

Grating

Chopping Mirror

While the beams alternate, the grating is slowly rotated, passing different frequencies of light to the detector.

IR Tutor Main Menu Help Quit

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### Hogyan mérjük? – FT

ATR: pi-gyémánt (vessző-ak-savúg, 5.7 ósmm)

Michelson Interferometer:  
Mobile mirror  
Beam splitter  
Fixed mirror

Sample

Interferogram

Spectrum

Fourier-Transform

All of the frequencies reach the detector at once, rather than one at a time. The spectrum is obtained by a mathematical calculation (the **Fourier Transformation**) from the data.

IR Tutor Main Menu Help Quit

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### PAT (Process Analytical Technology) = a folyamat közben tartása

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- a folyamat monitorozó eszközeinek kibővítése egyéb real-time technikákkal (**in/on-line NIR/Raman/IR**, in-/on-line HPLC, stb.)
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IR Tutor Main Menu Help Quit

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### ...-line, ...-line, de melyik? I.

Guidance for Industry  
PAT – A Framework for  
Robust Pharmaceutical  
Development, Manufacturing,  
and Quality Assurance

[...] These measurements can be:

- at-line:** Measurement where the sample is removed, isolated from, and analyzed in close proximity to the process stream.
- on-line:** Measurement where the sample is diverted from the manufacturing process, and may be returned to the process stream.
- in-line:** Measurement where the sample is not removed from the process stream, and can be invasive or noninvasive [...]

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### ...-line, ...-line, de melyik? II.

**On-Line Monitoring**

A: In situ Sensor  
B: In situ with sampling bypass  
C: Ex situ with sampling module

Real-time process data due to fast sensor response time  
Near-real-time process data depends on time between sample withdrawal and processing, analyzer response time, and data processing.

**Off-Line Monitoring**

D: Manual sampling → Sample transfer (Time-delay) → QC laboratory (Time-delay)

**At-Line Monitoring**

E: Manual sampling → [Analyzer]

William Whitford W., Julien C.  
Analytical Technology and PAT  
BioProcess International January 2007, 33-41

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### Az in-/on-line eszköze – a száloptika I.

Igen, továbbra is.

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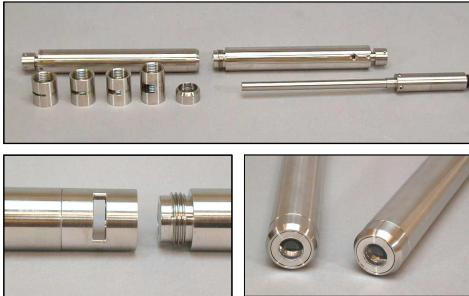
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### Az in-/on-line eszköze – a száloptika II.



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### Az in-/on-line eszköze – a száloptika III.



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### Technológiába illeszthető (egyéb) eszközök I.



Valószínűleg  
keverés  
dohos (fr. t.  
helyett adott  
minőség)

Több-  
csatornás  
ellenőrzés.

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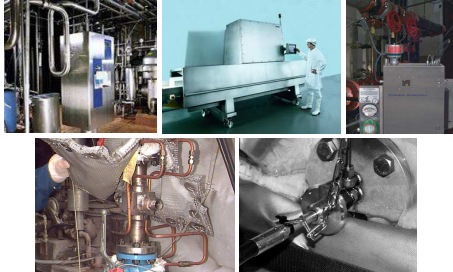
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## Technológiába illeszthető (egyéb) eszközök II.



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## Mobil, hordozható (handheld) eszközök

Pl. Lőr Angélikus  
tevékenység  
fűszerezéssel  
számosételek



NIR

FT-IR

Raman

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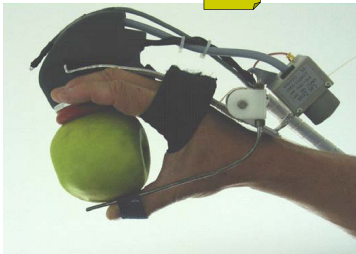
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## Ha egyedül nem megy

Aram dírt  
rakód...



A „NIR kesztyű” összetevői:  
NIR – oldható cukrok • hangimpulzus – keménység,  
Vis – érettség (klorofil) • potenciométer – méret

Lee K.A.,  
Review of applications of near infrared spectroscopy to food analysis,  
The NIR Spectrum 2(2), 11-18 (2004)

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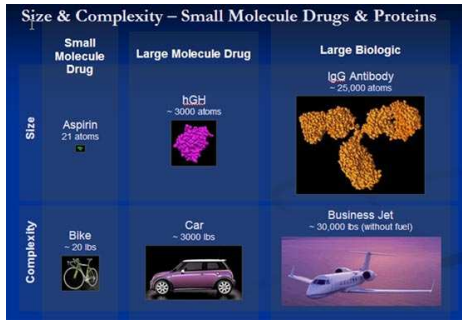
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## Klasszikus API vs. biomolekulák



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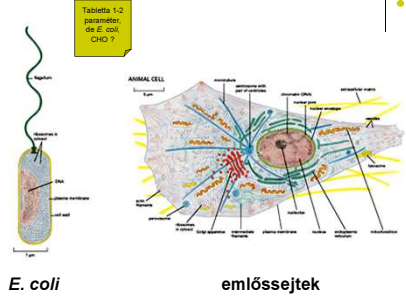
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## Kikkel termeltetünk?



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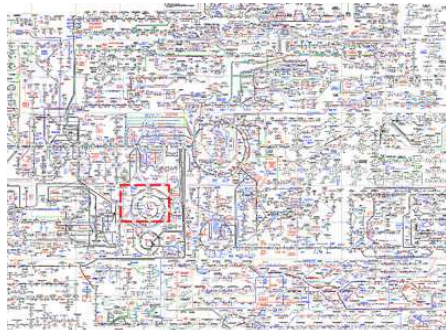
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## Na akkor, modellezzünk...



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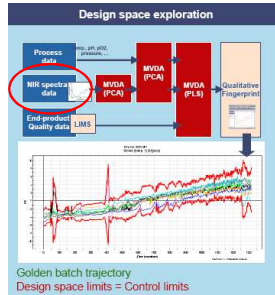
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## A végső cél



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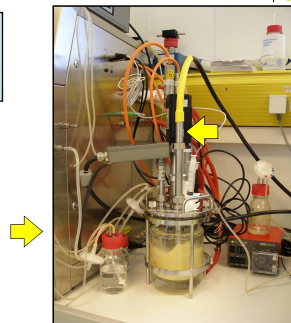
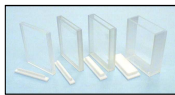
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## Off-line-től a real-time-ig



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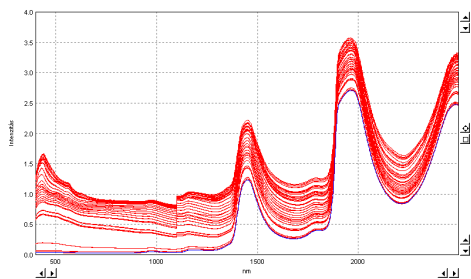
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## Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer



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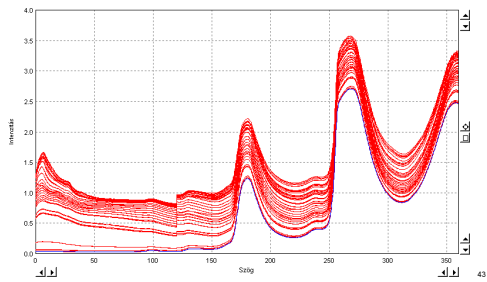
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**



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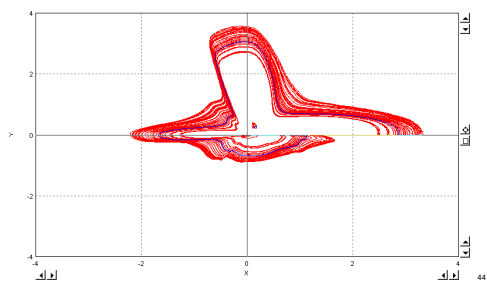
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**



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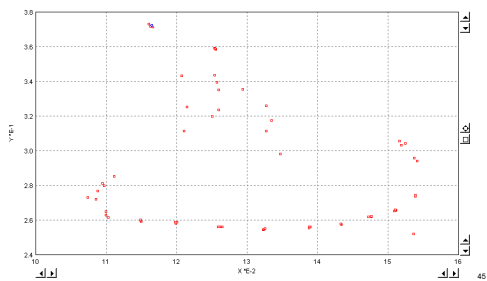
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**



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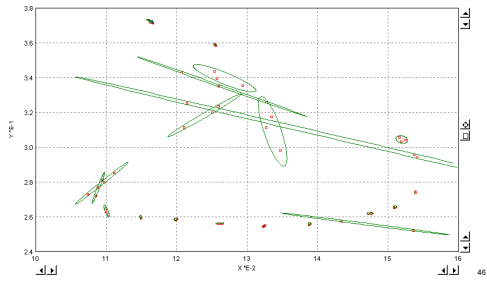
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**




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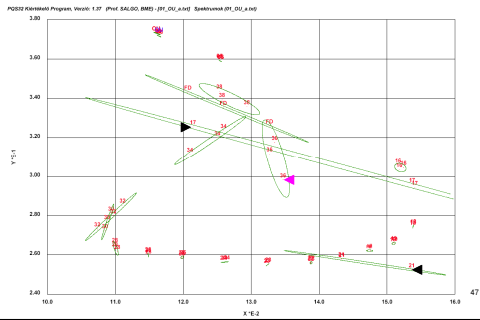
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**




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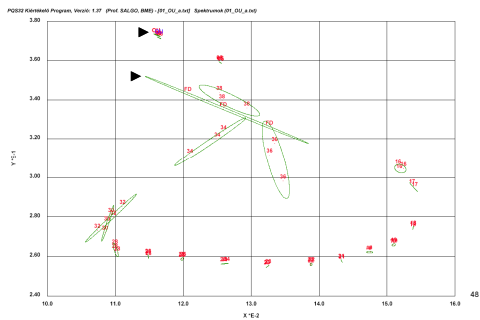
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**




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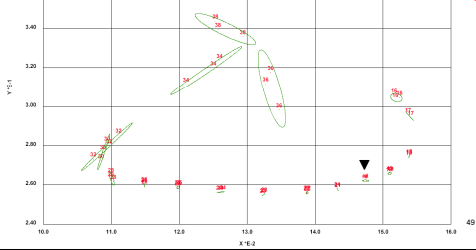
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**




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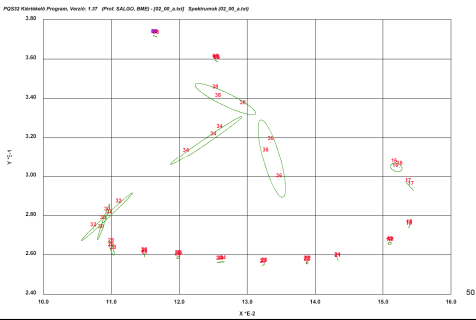
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**




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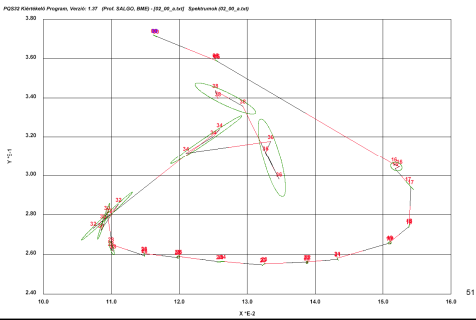
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**Fermentáció (*E. coli*) off-line nyomon követése: PQS módszer**




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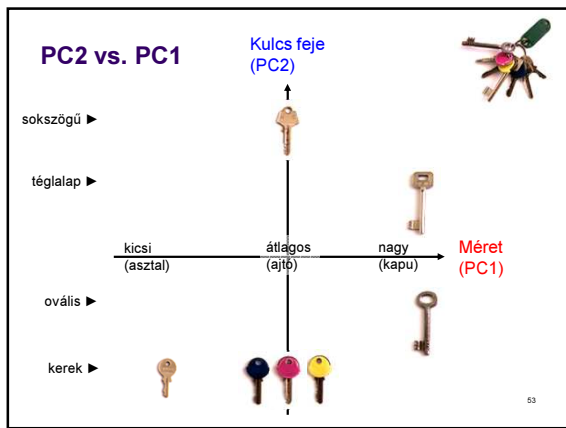
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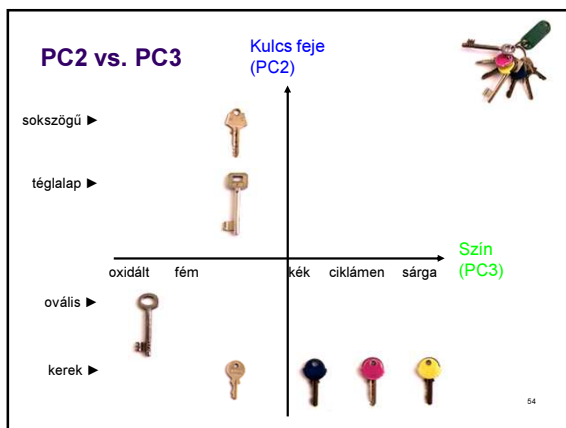
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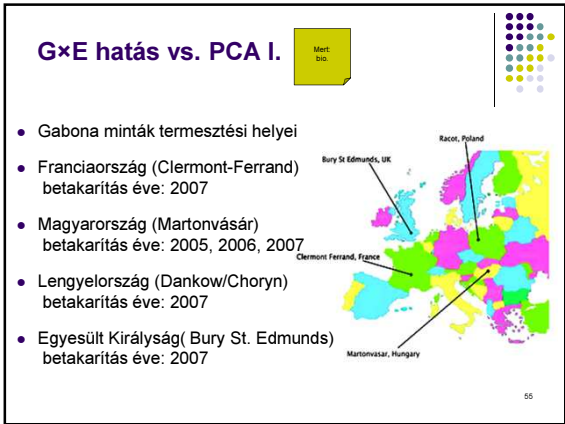
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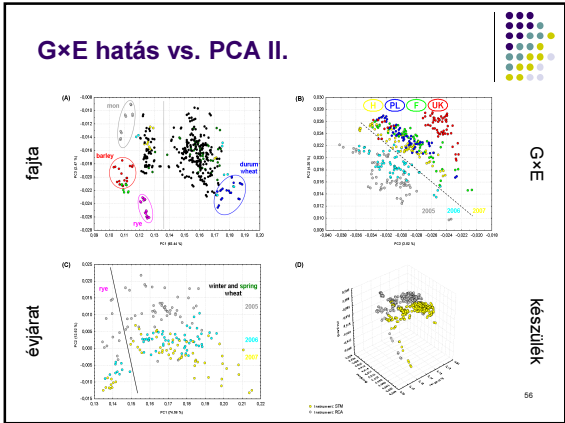
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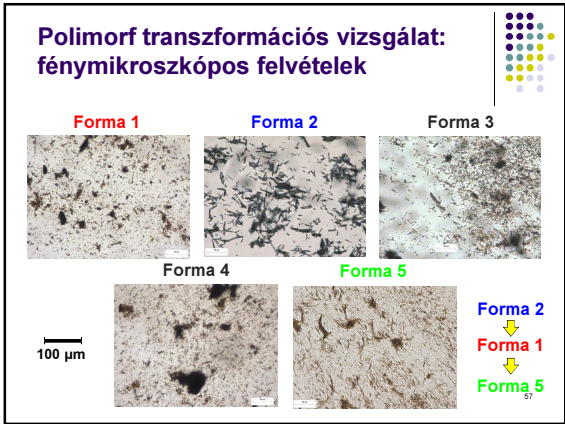
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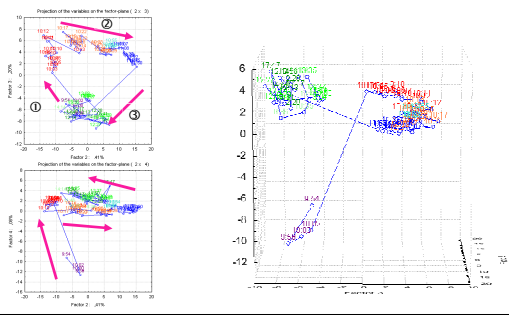
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### Polimorf transformációs vizsgálat: in-line Raman-száloptika (PCA)




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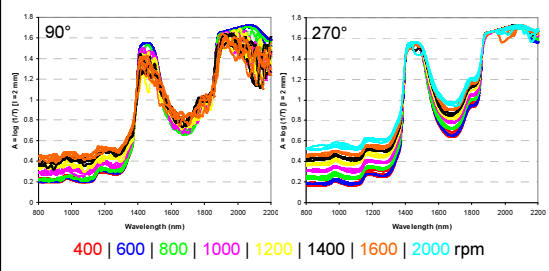
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### Fermentáció (*E. coli*) in-line nyomon követése: kevertetés, fizikai helyzet




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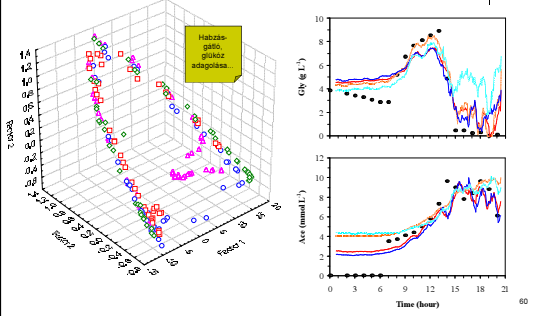
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### Fermentáció (*E. coli*) in-line nyomon követése: diszp. NIR-rel PCA, PLS




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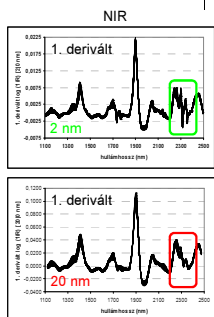
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## API meghatározása – simítás hatása a derivált képzésére

- Az első és második deriváltak képzése a kezeletlen spektrumokból más-más beállításokkal történik:
- Diszperziós NIR esetén: 2, 4, 8, 12, 16, 20 nm-es (1, 3, 5, 7, 9, 11 pontos) kapu [„gap-segment” derivált]
- FT-NIR esetén: 5, 9, 13, 19, 25, 37, 49, 149 pontos kapu [Savitzky-Golay derivált]



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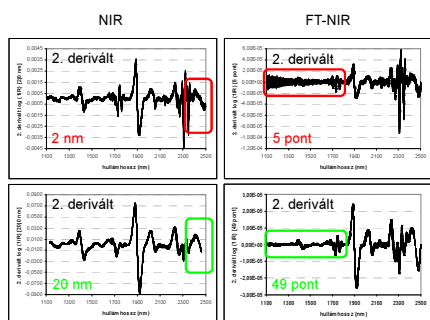
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## API meghatározása – diszperziós vs. FT (pl. detektorok)



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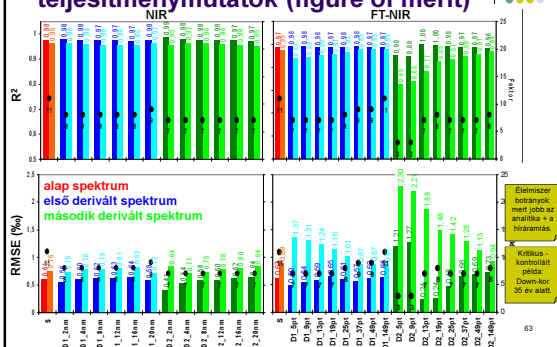
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## API meghatározása – teljesítménymutatók (figure of merit)



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## A feltárás lépései – (IQ)Q = IQ<sup>2</sup>



- **I**dentification – azonosítás
  - Ki vagy te?
    - éles spektrális különbségek keresése
    - korreláció, euklideszi távolság stb.



- **Q**ualification – minősítés
  - Hová tartozol?
    - szubpopulációk definiálása finomabb eltérések alapján
    - főkomponens analízis (PCA), Mahalanobis-távolság stb.



- **Q**uantification – mennyiségi meghatározás
  - Mennyi az annyi?
    - referencia paraméterekkel kalibrációk
    - részleges legkisebb négyzetek (PLS) módszere stb.



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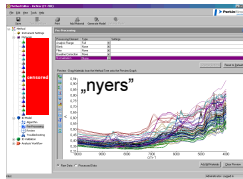
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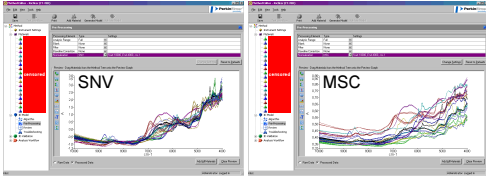
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## Spektrumkönyvtár – anyagazonosítás (NIR)



nyers<sup>o</sup> 18 termék, 27 tétel



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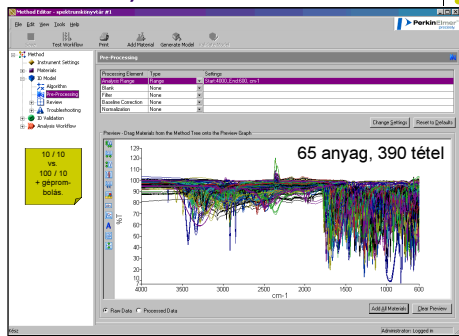
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## Spektrumkönyvtár – anyagazonosítás (analitikai IR)



65 anyag, 390 tétel



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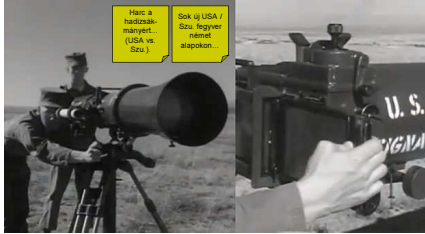
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## Az eszközök fejlődése – (hideg) háborúkon keresztül (hideg háború)



Harc a hadiszakmányért. (USA vs Szov.)

Sok új USA / Szov. fegyver került át azokon.

<http://www.logtech.com/forge-cold-war-camera-with-100-inch-infrared-lens-takes-iso-b-operate/>

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## Az eszközök fejlődése – (hideg) háborúkon keresztül (Korea, Vietnam)



Még ma is megvásárolható az interneten.

[http://www.icollector.com/KOREAN-WAR-ERA-M1-CARBINE-INFRARED-SNIPER-SCOPE\\_5326808](http://www.icollector.com/KOREAN-WAR-ERA-M1-CARBINE-INFRARED-SNIPER-SCOPE_5326808)

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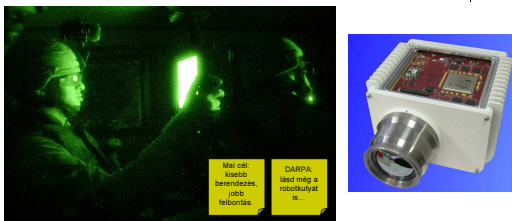
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## Az eszközök fejlődése – (hideg) háborúkon keresztül (pl. Irak)



Más cél: kisebb berendezés, jobb felbontás.

DARPA: tesz meg a robotkutyát is...

<http://www.wired.com/wiredroom/2013/04/star-pa-infrared-camera/>

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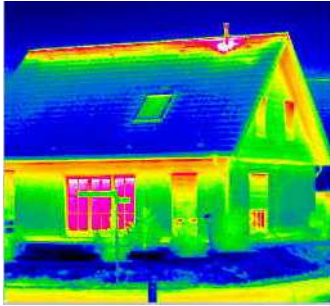
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## Civil alkalmazások – hideg/meleg I.



Energia-  
auditok  
szükséges  
hőszigetítés

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<http://www.novitechhomeenergy.com/services.htm>

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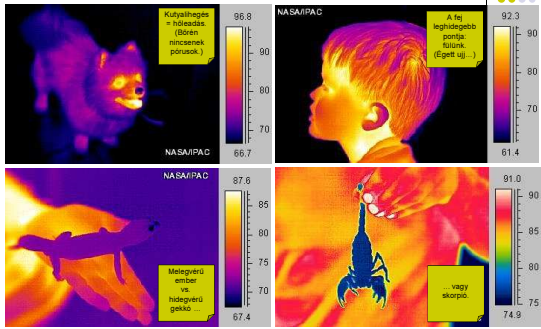
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## Civil alkalmazások – hideg/meleg II.



[http://coolcool.com/pac/caltech.edu/image\\_gallery/iv\\_zoo/coolwarm.html](http://coolcool.com/pac/caltech.edu/image_gallery/iv_zoo/coolwarm.html)

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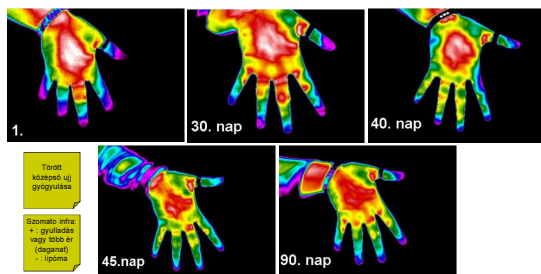
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## Civil alkalmazások – hideg/meleg III.



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[http://vifa.org.hu/2008/07/18/jogszuag\\_kevedes\\_a\\_szomatoinfra\\_berevedezesrol](http://vifa.org.hu/2008/07/18/jogszuag_kevedes_a_szomatoinfra_berevedezesrol)

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### Egér a Marsról I.

Itt már aktív a beta-galactosid. DE rombolás-mechánizmus.

Sokszor csak a szinapsztionon látjuk az Alzheimer-kór állapotát.

Korai észlelés: de terjedés jobb esélyek az Alzheimer-kór állapotát.

CCD camera

Near-infrared light

Golke T.E., Backkal B.J.: Amyloid deposits can be rapidly detected in the brains of living mice using a novel ligand and near-infrared fluorescence imaging. *Nat. Biotechnol.* 23(8), 930-934 (2005)

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### Egér a Marsról II.

30 min 60 min 120 min 240 min

Hintersteiner M. et al.: In vivo detection of amyloid- $\beta$  deposits by near-infrared imaging using an oxazine-derivative probe. *Nat. Biotechnol.* 23(5), 577-580 (2005)

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### Szexálás I.

TOP VIEW

Silkworm Pupa

Array of Light Sources

Tenyésztest az érzékelőkhöz közelebb, hogy mi van a bábban.

Bábot megfigyelni nem lehet, mert akkor elpusztul.

SIDE VIEW

Silkworm Pupa

Array of Light Sources

Electronic Controlling and Processing Unit

Camera

Transparent Support

Sunmohabekajorn S., Chakraborty Kamaladevi C.: Optical penetration-based silkworm pupa gender sensor structure. *Appl. Optics* 51(14), 408-412 (2012)

78

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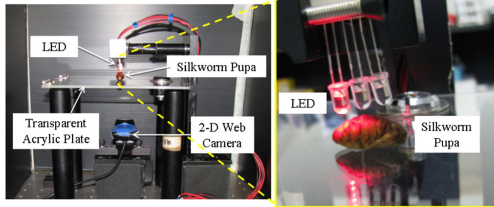
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## Szexálás II.



Sumrédetichajorn S., Chakraborti Kamtongtee C.  
Optical penetration-based silk worm pupa gender sensor structure  
Appl. Optics 51(4), 408-412 (2012)

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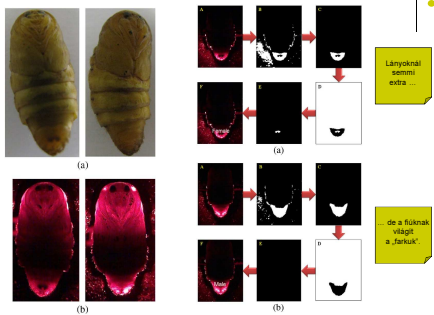
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## Szexálás III.



Sumrédetichajorn S., Chakraborti Kamtongtee C.  
Optical penetration-based silk worm pupa gender sensor structure  
Appl. Optics 51(4), 408-412 (2012)

80

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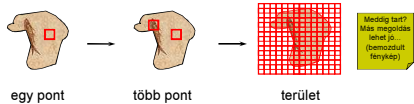
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## Mikro/makroszkópos képalkotás I.

- Pár/tíz mikrométeres nagyságrendek(től)
- Látható kép: vizsgálandó terület megkeresése, majd (N)IR mérés
- Nem, vagy csak kis mintaelőkészítést igényel: kevesebb hiba
- Különböző mérési módok: heterogén minták könnyebb vizsgálata



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### Mikro/makroszkópos képalkotás II.

pont kép

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### Mikro/makroszkópos képalkotás III.

- A 16 elemből álló detektorsor a mintát  $100\ \mu\text{m} \times 6,25\ \mu\text{m}$  vagy  $400\ \mu\text{m} \times 25\ \mu\text{m}$  blokkokban látja (NIR esetén, IR:  $1,56\ \mu\text{m}$ ...)
- 10 lépés másodpercenként, avagy 170 spektrum egy másodperc alatt ( $16\ \text{cm}^{-1}$  mellet)

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### Mikro/makroszkópos képalkotás IV.

- FPA (Focal Plane Array)

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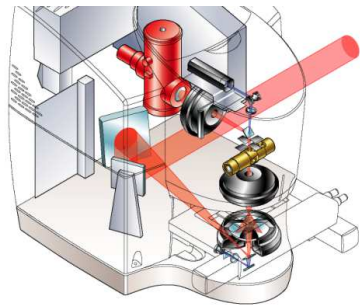
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### Mikro/makroszkópos képalkotás V.



T  
(transzmisszió)

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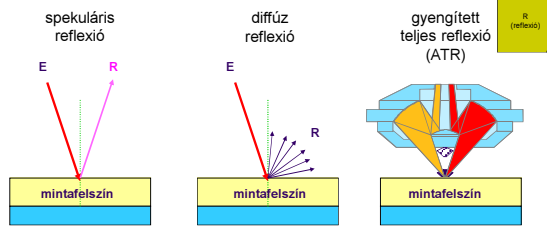
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### Mikro/makroszkópos képalkotás VI.



R  
(reflexió)

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### Mikro/makroszkópos képalkotás VII.



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### Mikro/makroszkópos képalkotás VIII.

Cassegrain objektív

elmozdulás (x,y)      ATR kristály ÉS minta

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### Képalkotás – tablettavizsgálatok

- Technológia nyomon követése I.
  - bevonás

**1390nm bevonat (Opadry Pink): idő előrehaladtával erősödő jel 1390 nm-en**

**2120nm korpusz: idő előrehaladtával gyengülő jel 2120 nm-en**

bevonás ideje

Maurer L., Leuenberger H.  
 Tabletts pulsed imaging and near infrared imaging to monitor the coating process of pharmaceutical tablets.  
 International Journal of Pharmaceutics 370(1-2), 8-16 (2009)

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### Képalkotás – tablettavizsgálatok

- Technológia nyomon követése II.
  - keverés
- Eredetiségvizsgálat

PI halmis Viagra-ban a lek színtipok átlagmértéke adta.

Hamis      Eredeti

(a) VIS

(b) NIR

Összehasonlítás      Original

Nem kell utalni a "NIR" betűre! (Magyarul a bizonyíték.)

Gendrin C. et al.  
 Content uniformity of pharmaceutical solid dosage forms by near infrared hyperspectral imaging: A feasibility study  
 Talanta 73(4): 733-741 (2007)

Gendrin C. et al.  
 Pharmaceutical applications of vibrational chemical imaging and chemometrics: A review  
 Journal of Pharmaceutical and Biomedical Analysis 48(3): 553-563 (2008)

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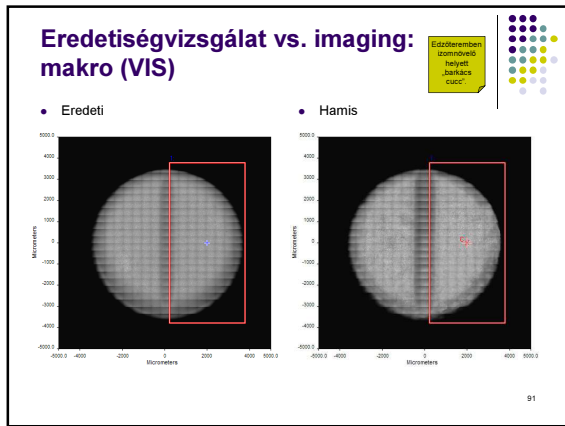
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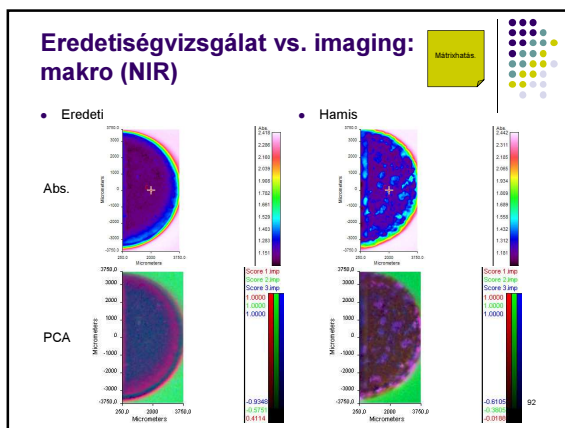
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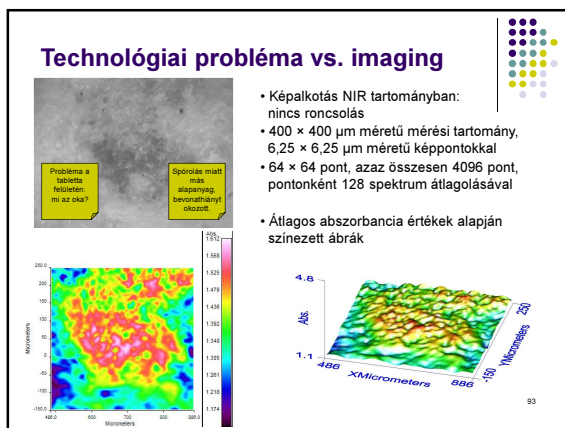
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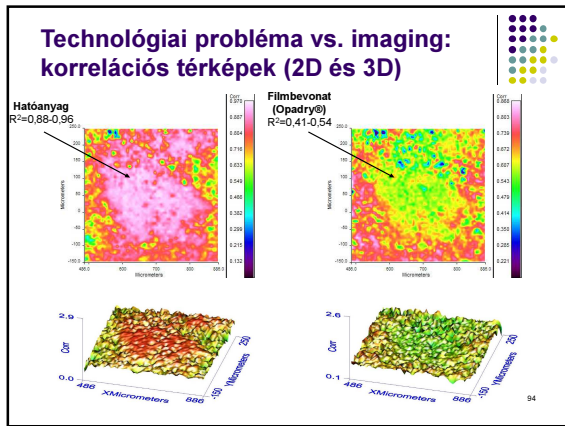
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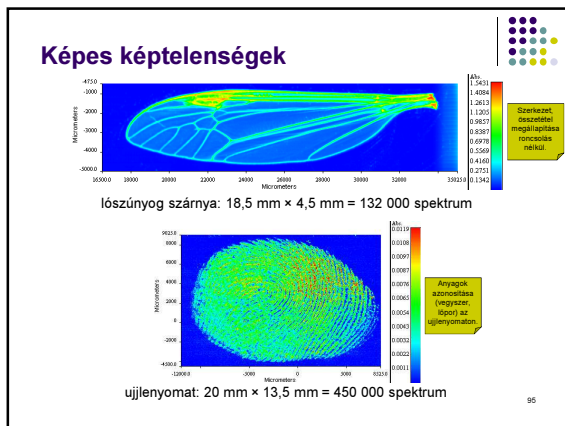
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### A (N)IR módszerek, technológiák előnyei

- Roncsolásmentes (fermentáció, anyagazonosítás, homogenitás, bevonat, hamisítás, visszáru, csomagolóanyag stb.)
- Gyors (jelsorozatok (másod)perces nagyságrendben képezhetők)
- Rengeteg rejtett információ megfejthető (adatbányászat, oknyomozások)
- Gyors visszacsatolás a technológiába, minőségellenőrzésbe, minőségbiztosításba
- FDA „alternatív” módszere
- Költséghatékony, nagy áteresztőképességű
- Tudásintenzív módszerek

A nemindás tudás a tudás kezdete.

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## Köszönetnyilvánítás



Párta László<sup>1</sup>, Ballagi András<sup>1</sup>  
Kiss Violetta<sup>2</sup>, Finta Zoltán<sup>2</sup>  
Horgos József<sup>3,4</sup>, Zelkó Romána<sup>4</sup>  
Jekő József<sup>5</sup>, Csorvássy István<sup>5</sup>  
Lakatos László<sup>6</sup>, Axel Rau<sup>7</sup>  
Izsó Eszter<sup>8</sup>, Tieger Eszter<sup>8</sup>, Lőrincz Áron<sup>8</sup>, Kontsek Endre<sup>8</sup>

<sup>1</sup> Richter Gedeon Nyrt.

<sup>2</sup> sanofi-aventis Zrt.

<sup>3</sup> Hungaropharma Zrt.

<sup>4</sup> SE Egyetemi Gyógyszerészeti, Gyógyszeripari Szervezési Intézet

<sup>5</sup> Nyíregyházi Főiskola, Kémia Intézeti Tanszék / Alkaloida Vegyészeti Gyár Zrt.

<sup>6</sup> PER-FORM Hungária Kft., Analitikai Divízió

<sup>7</sup> PerkinElmer, Rodgau

<sup>8</sup> BME Alkalmazott Biotechnológia és Élelmiszertudományi Tanszék



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