Coupling Raman and fluorescence for confocal imaging of biological and pharmaceutical samples

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Nanoparticles for drug targeting and imaging

Anticancer drug targeting
- Drug (DOX), and/or fluorochrome
- Polymer (PEG, ...)
- Biological ligand (FA, ...)
- External magnetic field
- Vascular permeability, biological recognition of cancer cells

Imaging of anticancer drugs/cancer cells
- SPION and/or Au, Ag
- MRI
- SERS and/or fluorescence

Nanoparticles for drug targeting and imaging

Anticancer drug targeting

- Anticancer activity
- Magnetisation without remanence
- Surface plasmons
- Colloidal stability, biocompatibility, drug protection
- Cancer cell targeting, intracellular action

Imaging of anticancer drugs/cancer cells

- response in fluorescence and/or in Raman
- MRI
- SERS
- Increased circulation time in blood
- Cancer cell labelling

Drug (DOX), and/or fluorochrome

SPION and/or Au, Ag

Polymer (PEG, ...)

Biological ligand (FA, ...)

10-20 nm
Analytical approach by confocal spectral imaging

example: subcellular distribution of fluorescent drug MTX

Selection of a treated cancer cell

Multispectral map: 30x30=900 spectra

Spectral acquisition:
- 0.02 sec per spectrum

Statistics over >20 cells

Co-localisation

Vibet et al., DMD, 2007
**Instrumental** (molecular optical spectroscopy)

**Confocal microspectrometer** LabRam (Horiba Jobin Yvon)

Olympus- BX40, obj. x100; x50, x50LWD, x10
Exc. 457, 488 et 514.5 nm (Ar+ laser)
Exc. 632.8 nm (He-Ne laser)
Exc. 785 nm (diode laser)

X-Y-Z motorised plate (0.1 µm / step, 0.5 µm in Z)
Gratings 300 ou 1800 ‘ / mm;
CCD 1024x256 pixels, air-cooled (–70 °C)

**Fast switch between excitation wavelength and between gratings**

**Accessories for live cell manipulation:**

- Thremostated microscopy chamber
- Micro-manipulated femto-injector
  - (tip ext. diameter 0.9±0.3 µm)
How do NP and drugs interact within a live cell?

To kill cancer cells, doxorubicin (DOX) needs to attain nucleus (DNA intercalation, action against the DNA topoisomerase)

DOX-Fe$^{2+}$ adsorbed on SPION (pH-dependant release)

DOX covalently bound to SPION (enzyme-dependent release)

Munnier et al., J. Nanopart. Res 2010
Munnier et al., J. Phys Chem. C. 2010
Shkilnyy et al., Int J Pharm 2008
More specific molecular information from Raman spectra?

- Sensitivity of conventional Raman is low compared to anticancer drug concentration (<1µM)

- SERS (surface-enhanced Raman scattering) is sensitive enough, but needs a noble metal substrate
SERS substrates: plasmonic NP (Ag, Au)

Ag-citrate NPs: aggregate in PBS and in cell
SERS spectroscopy on NP Ag-citrate: analysis of anticancer drug doxorubicine (DOX) released from SPION
How to apply conventional NP-substrates for SERS spectroscopy and imaging of anticancer drug in live cells?

Problems with:
1 – Uncontrolled NP aggregation
2 – Uncontrolled NP distribution
3 – Perturbed cell environment (moderate if few small aggregates/cell)
4 – Limited mapping zones (spots around aggregates)
5 – Uncertain origin of the signal

What about coupling SERS with fluorescence?
Instrumental coupling: Combined band-pass and spectral imaging modes on the same microscope

Video capture
Band-pass confocal microscopy mode
Spectral imaging mode: fluorescence, Raman

MCF-7 cancer cell
Treated with DOX

Most intense DOX fluorescence is in the nucleus

Spectral analysis reveals drug molecular interactions
Instrumental coupling: Combined band-pass and spectral imaging modes on the same microscope

- Video capture
- Band-pass confocal microscopy mode
- Spectral imaging mode: fluorescence, Raman

Bovin embryo incubated with Nile Red fluorochrome

- Fast selection of slice of interest
- Spectral analysis reveals lipidic content of the sample

400x400 points 4 sec per slice

~35000 spectra recorded in 3 min, (SWIFT™ acquisition mode, Horiba Jobin Yvon)
Details of the instrumental innovation

- Combined band-pass and spectral modes for fast confocal 3D imaging

Open inverted microscope
  - dedicated for LifeScience and biomedical applications (cells, tissues...)
  - easy integration of AFM module or injection devices

3 integrated laser sources
  large samples variety

DuoScan™ option
  laser scanning device

Z drive
  (3D scan)

Splitting optics
  (switch between modes)

XploRA™ INV
HORIBA Scientific

spectral confocal mapping module
  compact and rugged

SWIFT™ acquisition mode
  rapid spectra acquisition

band-pass confocal microscopy module
  fast 3D imaging option
Methodological coupling: Simultaneous co-detection SERRS-fluorescence on the same spectral image

Chourpa et al., Chem. Soc. Rev. 2008

Rem 1:
Aggregates ≤ 1 µm are detectable
Rem 2:
SERRS and fluorescence spectral informations are complementary
Details of the methodology

- Few intracellular Ag NPs
- Resonance excitation wavelength to co-detect SERRS (resonance SERS) and fluorescence of the drug

**Diagram:**
- SERRS from the drug fraction adsorbed on the Ag surface
- Fluorescence from non-adsorbed drug molecules
Homogeneous and small size: 10-20 nm
Aggregation-free system
Biocompatible, stealthy coating (PEG₅₀₀₀)
Polymer can carry biological ligands

**Nanotechnology benefits:** use polymer-coated SERS substrates

**Ex:** AgPEG₅₀₀₀, Shkilnyy et al., Analyst 2009

**One-pot synthesis**

\[
\text{AgNO}_3 + \text{NaBH}_4 + \text{PEG-SH} \rightarrow \text{ethanol} \rightarrow \text{N}_2
\]

100 nm

**Intensity contribution, %**

- Ag-citrate
- Ag-citrate + NaCl
- Ag-PEG + NaCl

log₁₀ [HD, nm]

1 10 100 1000
Detail of the methodological approach:
SERS of anticancer drug MTX on NP AgPEG\textsubscript{5000}

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« hot spot-free » SERS is detected, enhancement is ~10\textsuperscript{2} fold lower
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Drugs and ions diffuse through the PEG layer to the Ag surface
Development of novel, polymer-coated NP-substrates for intracellular SERS spectroscopy and spectral imaging

Challenges:
- Selective adsorption/enhancement of chromophores
- Aggregation-free enhancement
- Biological and/or magnetic targeting of NP to cancer cells and selective binding to intracellular compartments
- => Converging to nanocarrier technology
  => composite multifunctional NP
Conclusion and perspectives

- **Performant bio-analytical approaches can be developed**
  by means of confocal imaging, spectral and conventional,
coupling of several techniques (SERS, fluorescence, MRI)
and based on…

- **... multifunctionnal biocompatible NPs, which allow**
specific targeting,
selective and sensitive detection
of anticancer drugs and cancer cells *in vitro et in vivo*
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